

eman ta zabal zazu



Universidad
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DISEÑO Y EVALUACIÓN DE NUEVOS
DERIVADOS DE AZIRIDINAS FOSFORADAS
COMO AGENTES ANTIPROLIFERATIVOS.

MEMORIA PRESENTADA POR:

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PARA OPTAR AL GRADO DE DOCTOR

Vitoria-Gasteiz, 2021

“La vida no es fácil para ninguno de nosotros. Debemos tener perseverancia y, sobre todo confianza en nosotros mismos. Debemos creer que estamos dotados para algo y que esto debe ser alcanzado.”

Marie Curie

AGRADECIMIENTOS

Quisiera expresar mi más sincero agradecimiento al profesor Francisco Palacios por haber confiado en mí desde un primer momento y por haberme dado la gran oportunidad de formar parte de este maravilloso grupo de investigación de Química Orgánica I.

Con especial cariño quiero dar las gracias a la Dra. Ana María Ochoa de Retana y al Dr. Jesús de los Santos, directores de esta memoria. A la Dra. Ana, quiero agradecerle su gran apoyo en todo momento y su gran optimismo para hacerme ver de una manera positiva las complicaciones que durante estos años iban surgiendo. Al Dr. Jesús de los Santos quiero agradecerle su apoyo decidido y confianza en mí día tras día. Gracias por ser tan paciente conmigo y ayudarme en todo momento, tienes el gran talento de hacer fácil lo difícil.

También quiero dar las gracias a los doctores de la sección departamental; Javier Vicario, Edorta Martínez de Marigorta, Kontxi Alonso, Gloria Rubiales, Begoña Lecea, Yurre Olaizola, Mirari Ayerbe y a Pilar Pérez (técnico de laboratorio), porque de alguna manera siempre me han ayudado cuando lo he necesitado.

Por otro lado, durante estos cuatro años, he tenido la gran suerte de conocer a mis cuatro pequeños farmacéuticos, grandes personas a las que les he cogido un cariño inmenso. Quiero en primer lugar dar las gracias a mi catalana favorita, la gran Dra. Carme Masdeu, a quien adoro y admiro por ser una gran profesora y bellísima persona. Gracias por levantarme cada vez que me he venido abajo, por animarme siempre, ayudarme día tras día y por todos y cada uno de los buenos momentos que me has hecho pasar. A la Dra. María Fuertes, que desde el momento que nos presentaron me dijiste, “para lo que necesites aquí estoy” y así ha sido. Muchísimas gracias por transmitir tu alegría y por estar dispuesta a ayudarme en todo momento. A mi amigo de agobios, Xabier del Corte, alias “pequeña lactamita”, gracias por haber estado siempre ahí, ayudarme, aconsejarme que es lo mejor y ser mi agenda científica. Tenemos que aprender a ser más positivos, porque, aunque sea difícil nosotros mismos vemos que las cosas se consiguen, con esfuerzo, pero se consiguen. Eres un gran amigo y compañero. A Julene, mi amiga de bailete y creadora también de moléculas chiquitinas, gracias por los momentos tan divertidos que me has dado día tras día en el laboratorio, por ofrecerme tu ayuda tanto fuera como dentro de la universidad, por entenderme y en general por ser como eres.

No me olvido del resto de compañeros de laboratorio, tanto de los que ya han terminado como de los que hoy en día están (Asier, Alba, Angela, Xabi, Adrian). Muchísimas gracias por todos los momentos tan divertidos que hemos pasado que de alguna manera han hecho que las horas de laboratorio sean más llevaderas.

A mi cuadrilla de amigos vascos, a mis mañicos y a mis sorianos quiero también darles las gracias, por todos los momentos vividos y porque, aunque no entiendan de mis agobios muchas veces, siempre están dispuestos a animarme, a decirme que tengo que seguir hacia adelante. Gracias por llenar mi vida de grandes momentos.

En lo que respecta a mis padres, a quienes les dedico con mucho cariño esta memoria, me sobran las palabras. Podría llenar cientos de hojas dando las gracias, pero tengo que ser breve. Gracias papá y gracias mamá, por vuestro apoyo, por darme siempre los mejores consejos, y por preocuparos tanto por mí y aguantar mis enfados, sobre todo este último año. Estoy orgulloso de vosotros y sois mi ejemplo a seguir. Os quiero, admiro y adoro. También, agradecer a mis dos chiquitines de cuatro patas, mis ojitos grandes, Dimitri y AK, por todo el cariño y alegría que me dan día tras día.

Finalmente, quiero dar las gracias a mi chica, mi Itzi. Gracias por haber aparecido en mi vida, por estar conmigo en todo momento, estar tan pendiente de mí, hacerme ver siempre el lado bueno de las cosas y sobre todo hacerme vivir momentos tan felices. Te quiero mucho solete mío.

Muchísimas gracias de corazón a todas aquellas personas que durante estos cuatro años han formado parte de mi vida.

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δ	Desplazamiento químico
*	Quiral / Centro de simetría
μM	Micromolar
Å	Amstrong
A549	Adenocarcinoma de pulmón humano
AcOEt	Acetato de etilo
Act	Activador
ADN	Ácido desoxirribonucleico
Alq	Alquilo
Alil	Alilo
Ar	Arilo/Aromático
Arom.	Aromático(s)
AZQ	Diaziquona
Bh	Benzhidril
Bn	Bencilo
Boc	<i>Terc</i> -butoxicarbonil
Bpy	2,2'-bipiridilo
Bu	Butilo
Cal	Caloría(s)
Calcd.	Calculado
Cap.	Capítulo
Cbz	Benziloxicarbonil
CCK-8	Kit de recuento celular
cm	Centímetros
col.	Colaboradores
comp.	Compuesto
COSY	Espectroscopía de correlación homonuclear
Cy	Ciclohexilo
Δ	Calor
d	Doblete
dd	Doble doblete
DFT	Teoría funcional de la densidad
DMF	N, N-dimetilformamida
DMSO	Dimetil sulfóxido
DOX	Doxorrubicina
DPPA	Fosforil azidas
dr	Relación diastereomérica
Ed(s)	Editor(es)
EDA	Diazoacetato de etilo
ee	Exceso enantiomérico
Equiv.	Equivalente(s)
ESI	Ionización de electrospray
esp	α,α',α' -tetrametil-1,3-ácido bencenodipropiónico

Abreviaturas, acrónimos y símbolos

Et	Etilo
G	Energía libre
g	Gramo(s)
GEA	Grupo atractor de electrones
GP	Grupo protector
GS	Grupo saliente
h	Horas(s)
HEK293	Células embrionarias de riñón humano
Hex	Hexilo
HPLC	Cromatografía líquida de alta eficacia
HRMS	Espectroscopía de masas de alta resolución
Hz	Hercios
IC50	Concentración mínima inhibitoria
IR	Infrarrojo
J	Constante de acoplamiento
LDA	Diisopropilamido de litio
LiHMDS	Bis(trimetilsilil) amida de litio
M	Molar
m	Multiplete
m/z	Relación masa/carga
M+	Ion molecular
max	Máxima
Me	Metilo
MeOH	Metanol
Mes	2,4,6-trimetilfenilo (mesitilo)
MHz	Megahercios
mL	Mililitro
ml	Mililitro(s)
mmol	Milimol(es)
mmol	milimol(s)
MRC-5	Fibroblastos derivados de tejido pulmonar
Ms	Mesilo
n	Frecuencia
n.r.	No reportado
NOESY	Espectroscopía de efecto nuclear Overhauser
Nu	Nucleófilo
° C	Grados Celsius
OTf	Triflato
p.f.	Punto de fusión
Pág.	Página(s)
PBS	Tampón fosfato salino
Pent	Pentilo
Pf	Punto de fusión

Ph	Fenilo
Piv	Pivaloilo
ppm	Partes por millón
Pr	Propilo
PTSA	Ácido <i>p</i> -toluensulfónico
Py	Piridina
q	Cuadruplete
Rf	Factor de retención (en cromatografía)
RKO	Carcinoma de colon humano
RMN	Resonancia Magnética Nuclear
Rto.	Rendimiento
s	Singlete
SES	2-trimetilsilil etanosulfonilo
SN2	Sustitución nucleófila bimolecular
t	Triplete
t.a.	Temperatura ambiente
TBS	Terc-butildimetilsililo
TEM	Trietilenomelamina
TEPA	Trietilfosforamida
TFA	Ácido trifluoroacético
TFE	Tetrafluoroetileno
THF	Tetrahidrofurano
TLC	Cromatografía de capa fina
TMSBr	Bromotrimetilsilano
TMSCN	Cianotrimetilsilano
Tol.	Tolilo
Troc	(2,2,2-tricloroetoxi) carbonilo
Ts	Tosilo
UV	Ultravioleta
VANOL	3,3'-difenil-2,2'-bi- (1-naftol)
VAPOL	2,2'-difenil- (4-bifenantrol)
Vol.	Volumen
X	Grupo aniónico estabilizante

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En general, los sistemas heterocíclicos constituyen una familia de compuestos orgánicos de gran importancia desde un punto de vista biológico, debido a la actividad farmacológica que poseen muchos de ellos. Además, tanto por sus aplicaciones como por su gran capacidad de coordinación y donación de electrones, estos compuestos heterocíclicos siguen manteniendo intacto el interés de los investigadores.¹ Los heterociclos de tres miembros, aunque simples, son sistemas versátiles y la literatura continúa proporcionando evidencia de su diversidad, no solo en cuanto a las rutas sintéticas utilizadas para su preparación sino también a su utilidad sintética. Por lo tanto, estos heterociclos de tres miembros se emplean a menudo como intermedios versátiles y selectivos en síntesis orgánica.² Entre los sistemas heterocíclicos de tres miembros, las aziridinas constituyen una clase de moléculas particularmente valiosas y, tanto sus propiedades físicas como las reacciones químicas en las que intervienen han sido objeto de muchas investigaciones teóricas y experimentales.³

¹ a) T. Eicher, S. Hauptmann, A. Speicher. "The chemistry of heterocycles; structure, reactions, synthesis and applications". 2nd ed., Wiley, Weinheim, **2003**, 1-556. b) L. D. Quin, J. A. Tyrell. "Fundamentals of heterocyclic chemistry: importance in nature and in the synthesis of pharmaceuticals". 9th ed., Wiley, New York, **2010**, Cap. 1-2, 1-23. c) J. A. Builla, J. J. Vaquero, J. Barluenga. "Modern heterocyclic chemistry". Wiley, Germany, **2011**, 4, 1-2445. d) P. Arora, V. Arora, H. S. Lamba, D. Wadhwa. "Importance of heterocyclic chemistry: a review". *Int. J. Pharm. Res. Sci.* **2012**, 3, 2947-2954. e) M. Y. Langroudi. "Review of studies focused on heterocyclic compounds containing a heteroatom and aromaticity evaluation methods". *Bull. Env. Pharmacol. Life Sci.* **2014**, 3, 108-115. f) N. M. Aljamali. "Review paper in heterocyclic compounds". *Journal of plastic and polymer technology* **2015**, 1, 49-63. g) S. Sabir, M. I. Alhazza, A. A. Ibrahim. "A review on heterocyclic moieties and their applications". *Catal. Sustain. Energy* **2015**, 2, 99-115. h) A. A. Mulla. "A review: biological importance of heterocyclic compounds". *Der Pharma Chemica* **2017**, 9, 141-147.

² a) A. Weissberger. "Heterocyclic compounds with three and four membered rings". Wiley, New York, **1964**, part two, 1-1119. b) T. J. Mason. "Three membered ring systems in heterocyclic chemistry". Eds. H. Suschitzky, O. M. Cohn, Royal Society of Chemistry, England, **2007**, 3, Cap. 1, 1-49. c) G. Cardillo, L. Gentilucci, A. Tolomelli. "Asymmetric synthesis of three and four membered ring heterocycles in asymmetric synthesis of nitrogen heterocycles". Ed. J. Royer, Wiley, Weinheim, **2009**, part one, 3-50. d) R. Dalpozzo, A. Lattanzi, H. Pellissier. "Applications of chiral three-membered rings for total synthesis: a review". *Curr. Org. Chem.* **2017**, 21, 1143-1191.

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Introducción

Las aziridinas **1** (Figura 1) son dihidro derivados de azirinas, que contienen dos átomos de carbono y un átomo de nitrógeno en un anillo de tres miembros. A estos sistemas heterocíclicos, a menudo se les ha llamado azaciclopropanos o más comúnmente etileniminas.⁴



Figura 1. Estructura de la aziridina.

En 1888, Gabriel describió la primera síntesis de aziridinas a partir del bromhidruro de la 2-bromoetilamina en presencia de óxido de plata.⁵ Las aziridinas son importantes “building blocks” en síntesis orgánica para la preparación de diferentes derivados nitrogenados.⁶ Además, un gran número de químicos sintéticos han dedicado muchos estudios a compuestos que presentan el anillo de aziridina, bien sea por su elevada reactividad o por la elevada tensión de dicho anillo.⁷

1. Propiedades físicas de aziridinas.

La aziridina es un aza heterociclo saturado plano y rígido que describe un triángulo casi equilátero con ángulos de enlace internos C-C-N de 59.9° y C-N-C de 60.6° (Figura 2).⁸ Sin embargo, un ángulo de enlace considerablemente más pequeño en comparación con el ángulo de enlace tetraédrico normal de 109.5° induce la deformación del anillo. Además, el ángulo de enlace periférico H-C-H en el anillo de aziridina es de 115.7°, un valor casi intermedio entre las configuraciones tetraédrica (109.5°) y trigonal (120°) (Figura 2).

⁴ R. K. Bansal. “Three membered heterocyclic compounds with one heteroatom in heterocyclic chemistry”. New age international. 3rd ed., New Delhi, **1999**, Cap. 2, 9-73.

⁵ S. Gabriel. “Über vinylamin und bromathylamin”. *Ber. Dtsch. Chem. Ges.* **1888**, *21*, 2664-2669.

⁶ a) S. Stankovic, M. D'hooghe, S. Catak, H. Eum, M. Waroquier, V. Van Speybroeck, N. De Kimpe, H. J. Ha. “Regioselectivity in the ring opening of non-activated aziridines”. *Chem. Soc. Rev.* **2012**, *41*, 643-665. b) G. Callebaut, T. Meiresonne, N. De Kimpe, S. Mangelinckx. “Synthesis and reactivity of 2-(Carboxymethyl)aziridine derivatives”. *Chem. Rev.* **2014**, *114*, 7954-8015.

⁷ X. Hu. “Nucleophilic ring opening of aziridines”. *Tetrahedron* **2004**, *60*, 2701-2743.

⁸ A. Ebrahimi, F. Deyhimib, H. Roohia. “Nitrogen-inversion in some aziridine type compounds: structural and kinetic investigations by ab initio methods”. *J. Mol. Struct. (Theochem)*. **2001**, *535*, 247-256.

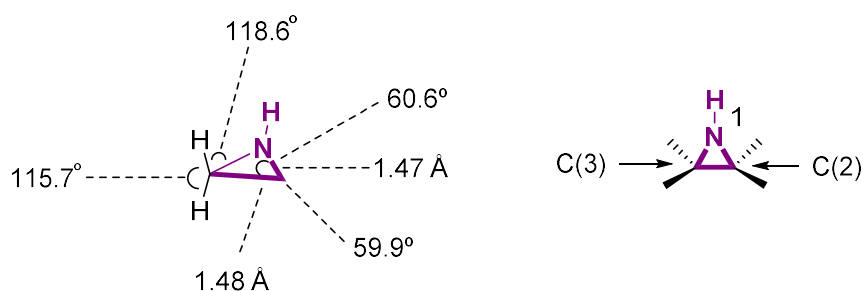


Figura 2. Longitudes y ángulos de enlace en aziridinas.

La longitud de enlace C-C en aziridinas se puede considerar intermedia comparándola con la longitud de un enlace $C_{sp^3}-C_{sp^3}$ (1.54 Å) en un hidrocarburo de cadena abierta y la longitud de un doble enlace $C_{sp^2}-C_{sp^2}$ (1.34 Å), lo que permite la transmisión de la conjugación a través del sistema del anillo.⁹

Es un hecho evidente que en un sistema cíclico hay distorsión en los ángulos de enlace, lo cual es más evidente a medida que disminuye el tamaño del anillo, lo que da lugar a la deformación del ángulo de enlace.¹⁰ La energía necesaria para distorsionar los ángulos de enlace desde su estado de equilibrio se denomina deformación de Baeyer.¹¹ Esta deformación está favorecida por la distorsión del ángulo de enlace desde un ángulo tetraédrico ideal (estimada alrededor de 111 Kcal/mol⁻¹ en aziridinas).¹²

Otra propiedad física atribuida a la geometría del anillo es que el átomo de nitrógeno presenta una barrera energética para la inversión piramidal (AG \approx 17 Kcal/mol) significativamente mayor que la de otras aminas monocíclicas y acíclicas.^{13,14,15}

⁹ V. J. Ram, A. Sethi, M. Nath, R. Pratap. "The chemistry of heterocycles. Nomenclature and chemistry of three- to- five-membered heterocycles". 1st ed., Elsevier, Amsterdam, **2019**, Cap. 3, 19-92.

¹⁰ a) A. Greenberg, J. F. Liebman. *Kinetic and thermodynamic stability* In "Strained organic molecules". Academic Press, New York, **1978**, *38*, Cap. 5, 225-281. b) K. Pihlaja, E. Taskinen. *Some applications of thermochemistry to heterocyclic chemistry* In "Physical methods in heterocyclic chemistry". Ed. A. R. Katritzky, Academic Press, New York, **1974**, *6*, Cap. 5, 199-246.

¹¹ A. Baeyer. "Über polyacetylenverbindungen". *Ber. Dtsch. Chem. Ges.* **1885**, *18*, 2269-2281.

¹² J. B. Sweeney. "Aziridines: epoxides' ugly cousins?". *Chem. Soc. Rev.* **2002**, *31*, 247-258.

¹³ G. Park, S. C. Kim, H. Y. Kang. "Substituents effect on aziridine chemistry: *N*-inversion energy, reactivity and regioselectivity of nucleophilic ring-opening". *Bull. Korean Chem. Soc.* **2005**, *26*, 1339-1343.

¹⁴ L. D. Quin, J. A. Tyrell. "Fundamentals of heterocyclic chemistry: importance in nature and in the synthesis of pharmaceuticals". 9th ed., Wiley, New York, **2010**, Cap. 10, 280-320.

¹⁵ J. A. Deyrup. "The chemistry of heterocyclic compounds in small ring heterocycles, part 1: aziridines, azirines, thiiranes, thiirenes". Ed. A. Hassner, Wiley, New York, **1983**, *42*, Cap. 1, 2-6.

Finalmente, las aziridinas **1** exhiben una basicidad más débil ($pK_a = 7.98$) que las aminas secundarias alifáticas, como por ejemplo la dimetilamina ($pK_a = 10.87$); esto es debido a la presencia de un par de electrones solitario en el átomo de nitrógeno.¹²

2. Aziridinas en productos naturales con actividad biológica.

El término cáncer engloba un grupo de enfermedades que se caracterizan por la pérdida de control de crecimiento, división y propagación de un grupo de células, lo que lleva a un tumor primario que invade y destruye los tejidos adyacentes y puede extenderse a otras regiones del cuerpo a través de un proceso denominado metástasis.

Normalmente, el cáncer está causado por anormalidades del material genético de las células afectadas. La tumorigénesis es un proceso de varios pasos que implica la acumulación de mutaciones sucesivas en oncogenes y genes supresores que desregulan el ciclo celular. Ésta a su vez incluye cambios a pequeña escala en secuencias de ADN, como mutaciones; errores cromosómicos, como translocaciones, deleciones y amplificaciones; así como cambios que afectan a la estructura de la cromatina y están asociados con el control epigenético disfuncional, como la metilación del ADN o la acetilación de histonas.¹⁶ El cáncer no solo es una enfermedad celular, sino también una enfermedad tisular en la que las relaciones normales entre las células epiteliales y sus células estromales subyacentes son alteradas.¹⁷

Actualmente, el objetivo de la mayoría de los fármacos quimioterapéuticos contra el cáncer en uso clínico, es destruir las células tumorales malignas al inhibir alguno de los mecanismos implicados en la división celular. En consecuencia, los compuestos antitumorales desarrollados a través de este enfoque son citostáticos o citotóxicos. Desde la década de 1950, la química ha generado muchos fármacos antitumorales a través de programas de detección *in vitro*, mediante el uso de una variedad de líneas celulares de cáncer. El uso de fármacos antitumorales citotóxicos convencionales se basa en la teoría de que las células que proliferan y se dividen rápidamente son más sensibles a estos compuestos que las células normales.¹⁸ Las interacciones de los agentes citotóxicos con el ADN están mejor definidas hoy en día, y los nuevos compuestos

¹⁶ S. M. Nelson, L. R. Ferguson, W. A. Denny. "DNA and the chromosome-varied targets for chemotherapy". *Cell & Chromosome* **2004**, *3*, 1-26.

¹⁷ M. J. Bissell, D. Radisky. "Putting tumours in context". *Nat. Rev. Cancer* **2001**, *1*, 46-54.

¹⁸ S. Marchini, M. D'Incalci, M. Broggin. "New molecules and strategies in the field of anticancer agents". *Curr. Med. Chem. Anti-cancer agents* **2004**, *4*, 247-262.

que se dirigen a secuencias de bases particulares pueden inhibir los factores de transcripción de manera más específica.

Aunque el ADN continúa siendo un objetivo esencial para la quimioterapia anticancerígena, se ha realizado un gran esfuerzo para descubrir fármacos antitumorales específicamente adecuados para las aberraciones moleculares, las cuales son específicas de las células tumorales.¹⁹ El mecanismo de la mayoría de los fármacos utilizados clínicamente contra el cáncer, consiste en la interferencia con el entrecruzamiento, lo cual puede llevarse a cabo mediante la alquilación del ADN.

Las aziridinas, como potentes agentes alquilantes pueden actuar como fármacos covalentes, que tienen una potencia intrínseca *in vivo* por su capacidad de actuar como agentes de entrecruzamiento del ADN a través de la apertura nucleófila del anillo de los heterociclos de tres miembros.²⁰ Los primeros estudios mostraron que eran necesarias al menos dos unidades de aziridina para una buena actividad citotóxica, que no mejoró mediante la adición de una tercera a cuarta unidad de aziridina, lo que sugiere que la citotoxicidad se debe principalmente a un mecanismo de entrecruzamiento. Los primeros compuestos de esta familia fueron la trietilenomelamina (TEM), la tiotepa, llamada así por ser un análogo de azufre de la trietilfosforamida (TEPA), así como la mitomicina C (Figura 3). Hoy en día, la tiotepa es un agente alquilante aprobado para el tratamiento de cáncer de mama y ovario, así como para el tratamiento de carcinoma de vejiga.²¹ La trietilenomelamina probablemente experimenta reacciones con el ADN, que son similares a la tiotepa, mientras que la mitomicina C requiere una reducción enzimática para activar el anillo de aziridina, antes de que la reacción pueda ocurrir con el ADN, inicialmente para formar un aducto monofuncional.

Otros compuestos antitumorales contienen dos o tres anillos de aziridina unidos a un sistema de benzoquinona, pudiendo actuar como bisalquiladores de ADN y agentes de entrecruzamiento (Figura 4).²¹ Fueron diseñados para cruzar la barrera hematoencefálica debido a su alta lipofilia y baja ionización, y algunos de ellos han sido utilizados en clínica como es el caso de los representados en la figura 4.

¹⁹ D. B. Longley, D. P. Harkin, P. G. Johnston. "5-Fluorouracil; mechanisms of action and clinical strategies". *Nat. Rev. Cancer* **2003**, *3*, 330-338.

²⁰ M. M. Vaidergorn, Z. A. Carneiro, C. D. Lopes, S. de Albuquerque, F. C. C. Reis, S. Mikolaou, J. F. R. e Mello, G. L. Genesi, G. H. G. Trossini, A. Ganesan, F. S. Emeri. "β-Amino alcohols and their respective 2-phenyl-N alkyl aziridines as potential DNA minor groove binders". *Eur. J. Med. Chem.* **2018**, *157*, 657-664.

²¹ C. Avendaño, J. C. Menéndez. *DNA alkylating agents* In "Medicinal chemistry of anticancer drugs". 1st ed., Elsevier, Amsterdam, **2008**, Cap. 5, 139-174.

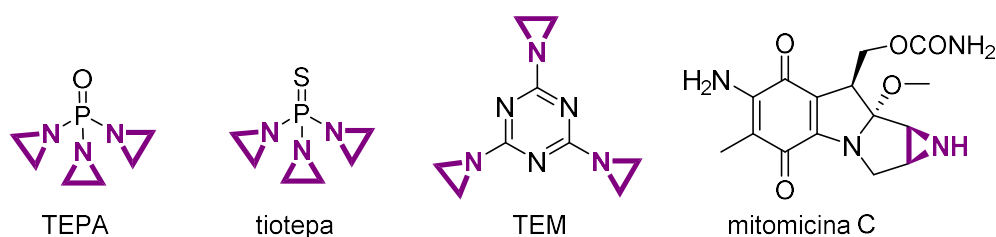


Figura 3. Estructuras de TEPA, tiotepa, TEM y mitomicina C.

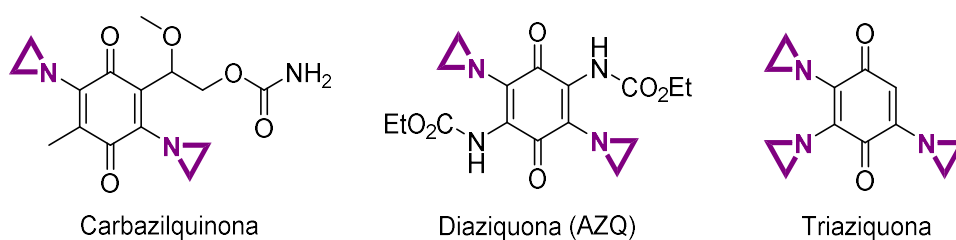


Figura 4. Estructura de la carbazilquinona, AZQ y triaziquona.

El anillo de aziridina se encuentra presente en algunos productos naturales,²² y se ha demostrado que éstos son objetivos sintéticos interesantes con una gran actividad biológica asociada a la reactividad del anillo. Las aziridinas son compuestos heterocíclicos muy valiosos extensamente utilizados como intermedios sintéticos en la síntesis de numerosos fármacos y productos naturales biológicamente activos.^{23,24} Muchos alcaloides presentan el anillo de

²² C. Botuha, F. Chemla, F. Ferreira, A. Pérez-Luna. "Aziridines in natural product synthesis. Heterocycles in natural products synthesis". Eds. K. C. Majumdar, S. K. Chattopadhyay, Wiley, Hoboken, **2011**, 46-61.

²³ a) V. V. Fokin, P. Wu. *Epoxides and aziridines in click chemistry* In "Aziridines and epoxides in organic synthesis". Ed. Yudin AK. 1sted. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, **2006**, 443-477. b) J. B. Sweeney. *Synthesis of aziridines* In "Aziridines and epoxides in organic synthesis". Ed. Yudin AK. 1sted., WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, **2006**, 117-144. c) M. V. Gil, M. J. Arévalo, Ó. López. "Click chemistry – what's in a name? triazole synthesis and beyond". *Synthesis* **2007**, *11*, 1589-1620. d) M. Penkova, S. Nikolova." Application of aziridines for the synthesis of isoquinoline derivatives". *Bul. Chem. Commun.* **2017**, *49*, 105-111.

²⁴ W. McCoull, F. A. Davis. "Recent synthetic applications of chiral aziridines". *Synthesis* **2000**, *10*, 1347-1365.

aziridina y muestran actividades anticancerígenas,²⁵ antibacterianas,²⁶ y/o antimicrobianas²⁷ y antileishmaniales,²⁸ lo que indica que la presencia del anillo de aziridina en productos naturales y sintéticos es esencial para tales actividades. Además, algunos de ellos se comportan como posibles inhibidores de proteasa.²⁹

Las propiedades biológicas de algunos compuestos naturales que contienen el anillo de aziridina, como las azicemicinas,³⁰ miraziridina,³¹ azinomycinas,³² ficelomicinas,³³ FR-900482,³⁴

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- ²⁵ G. A. Prosser, J. N. Copp, S. P. Syddall, E. M. Williams, J. B. Smaill, W. R. Wilson, A. V. Patterson, D. F. Ackerley. "Discovery and evaluation of escherichia coli nitroreductases that activate the anti-cancer prodrug CB1954". *Biochem. Pharmacol.* **2010**, *79*, 678-687.
- ²⁶ A. Keniche, A. Mezrai, J. K. Mulengi. "Synthesis of a novel class of phosphonoaziridines as interesting antibacterial agents". *The Open Conference Proceedings Journal* **2011**, *2*, 28-35.
- ²⁷ A. Giovine, M. Muraglia, M. A. Florio, A. Rosato, F. Corbo, C. Franchini, B. Musio, L. Degennaro, R. Luisi. "Synthesis of functionalized arylaziridines as potential antimicrobial agents". *Molecules* **2014**, *19*, 11505-11519.
- ²⁸ a) C. Schad, U. Baum, B. Frank, U. Dietzel, F. Mattern, C. Gomes, A. P. Sucre, H. Moll, U. Schurig, T. Schirmeistere. "Development of a new antileishmanial aziridine-2,3-dicarboxylate-based inhibitor with high selectivity for parasite cysteine proteases". *Antimicrob. Agents Chemother.* **2016**, *60*, 797-805. b) P. Fey, R. Chartomatsidou, W. Kiefer, J. C. Mottram, C. Kersten, T. Schirmeister. "New aziridine-based inhibitors of cathepsin L-like cysteine proteases with selectivity for the leishmania cysteine protease LmCPB2.8". *Eur. J. Med. Chem.* **2018**, *156*, 587-597.
- ²⁹ a) T. Schirmeier, M. Peric. "Aziridinyl peptides as inhibitors of cysteine proteases: effect of a free carboxylic acid function on inhibition". *Bioorg. Med. Chem.* **2000**, *8*, 1281-1291. b) S. Hanessian, N. Moitessier, D. L. Cantin. "Design and synthesis of MMP inhibitors using *N*-arylsulfonylaziridine hydroxamic acids as constrained scaffolds". *Tetrahedron* **2001**, *57*, 6885-6900. c) R. Vicik, V. Hoerr, M. Glaser, M. Schultheis, E. Hansell, J. H. McKerrow, U. Holzgrabe, C. R. Caffrey, A. P. Sucre, H. Moll, A. Stichb, T. Schirmeistera. "Aziridine-2,3-dicarboxylate inhibitors targeting the major cysteine protease of trypanosoma brucei as lead trypanocidal agents". *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2753-2757.
- ³⁰ T. Tsuchida, H. Linuma, N. Kinoshita, T. Ikeda, T. Sawa, M. Hamada, T. Takeuchi. "Azicemicins A and B, a new antimicrobial agent produced by amycolatopsis. I. Taxonomy, fermentation, isolation, characterization and biological activities". *J. Antibiot.* **1995**, *48*, 217-221.
- ³¹ P. Tabares, B. Degel, N. Schaschke, U. Hentschel, T. Schirmeister. "Identification of the protease inhibitor miraziridine A in the red sea sponge theonella swinhoei". *Pharmacognosy Research* **2012**, *4*, 63-66.
- ³² K. Nagaoka, M. Matsumoto, J. Ono, K. Yokoi, S. Ishizeki, T. Nakashima. "Azinomycins A and B, new antitumor antibiotics. I. Producing organism, fermentation, isolation, and characterization". *J. Antibiot.* **1986**, *39*, 1527-1532.
- ³³ X. He, M. Li, S. Song, X. Wu, J. Zhang, G. Wu, R. Yue, H. Cui, S. Song, C. Ma, F. Lu, H. Zhang. "Ficellomycin: an aziridine alkaloid antibiotic with potential therapeutic capacity". *Appl. Microbiol. Biotechnol.* **2018**, *102*, 4345-4354.
- ³⁴ C. T. Judd, M. R. Williams. "A concise total synthesis of (+)-FR900482 and (+)-FR66979". *J. Org. Chem.* **2004**, *69*, 2825-2830.

Introducción

mitomicinas,³⁵ madurastinas A1 y B1,³⁶ así como la maduropeptina³⁷ son bien conocidas y de gran interés.

Las mitomicinas son una familia de productos naturales que contienen el heterociclo aziridínico en su estructura. La base molecular de la citotoxicidad de las mitomicinas ha sido objeto de una investigación exhaustiva durante muchos años. En 1956 fueron aisladas las mitomicinas A **2** y B **3** (Figura 5) de *Streptomyces caespitosus* por Hata y col.,³⁸ mostrando una gran actividad antibiótica y antitumoral.³⁹ Años más tarde, también de *Streptomyces caespitosus* Wakaki y col.,⁴⁰ aislaron la mitomicina C⁴¹ **4** (Figura 5). Ésta muestra una importante actividad antitumoral debido a la capacidad del alcaloide derivado de aziridina para entrecruzar las hebras de ADN.^{20,42} Sin embargo, existen otras mitomicinas como la J y D aisladas de *Streptomyces fradiae* con propiedades antibióticas,⁴³ así como la mitomicina E obtenida de *S. lavandulae*.⁴⁴ Mientras que las mitomicinas H, G y K se obtuvieron cultivando una cepa de *S. caespitosus* y todas ellas presentan actividad antibacteriana.

³⁵ a) I. Han, H. Kohn. "Aminoaziridinomitosenes: synthesis, structure, and chemistry". *J. Org. Chem.* **1991**, *56*, 4648-4653. b) J. C. Andrez. "Mitomycins syntheses: a recent update". *Beilstein J. Org. Chem.* **2009**, *5*, 1-36.

³⁶ K. I. Harada, K. Tomita, K. Fujii, K. Masuda, Y. Mikami, K. Yazawa, H. Komaki. "Isolation and structural characterization of siderophores, madurastatins, produced by a pathogenic actinomadura madurae". *J. Antibiot.* **2004**, *57*, 125-135.

³⁷ M. Hanada, H. Ohkuma, T. Yonemoto, K. Tomita, M. Ohbayashi, H. Kamei, T. Miyaki, M. Konishi, H. Kawaguchi, S. Forenza. "Maduropeptin, a complex of new macromolecular antitumor antibiotics". *J. Antibiot.* **1991**, *44*, 403-414.

³⁸ T. Hata, T. Hoshi, K. Kanamori, A. Matsumae, Y. Sano, T. Shima, R. Sugawara. "Mitomycin, a new antibiotic from streptomyces. I". *J. Antibiot.* **1956**, *9*, 141-146.

³⁹ J. H. Beijnen, H. Lingeman, H. A. Van Munster, W. J. M. Uderberg. "Mitomycin antitumor agents: a review of their physico-chemical and analytical properties and stability". *J. Pharm. Biomed. Anal.* **1986**, *4*, 275-295.

⁴⁰ S. Wakaki, H. Marumo, K. Tomioka, G. Shimizu, E. Kato, H. Kamada, S. Kudo, Y. Fujimoto. "Isolation of new fractions of antitumor mitomycins". *J. Antibiot.* **1958**, *8*, 228-235.

⁴¹ W. T. Bradner. "Mitomycin C: a clinical update". *Cancer Treat. Rev.* **2001**, *27*, 35-50.

⁴² M. Kasai, M. Kono. "Studies on the chemistry of mitomycins". *Synlett.* **1992**, *10*, 778-790.

⁴³ H. K. Sreenath, R. Joseph. "Effect of temperature, ultraviolet light, mitomycin C and acriflavine on aerial mycelium and glucose isomerase production in *Streptomyces fradiae* SCF5". *Chem. Mikrobiol. Technol. Lebensm.* **1983**, *8*, 78-82.

⁴⁴ N. Sitachitta, N. B. Lopanik, Y. Mao, D. H. Sherman. "Analysis of a parallel branch in the mitomycin biosynthetic pathway involving the mitN-encoded aziridine N-methyltransferase". *J. Biol. Chem.* **2007**, *282*, 20941-20947.

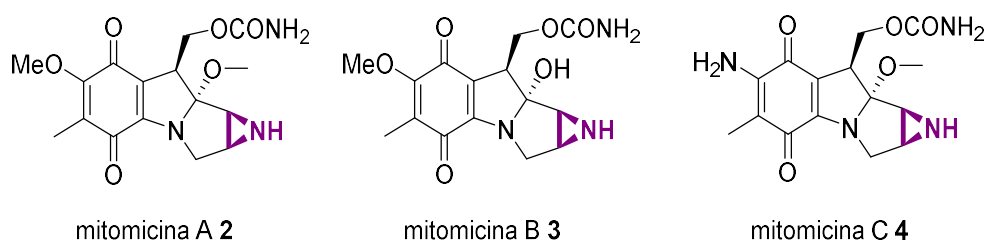


Figura 5. Estructura de las mitomicinas A, B y C.

Estructuralmente relacionados con la familia de los mitomicinas están FR-900482⁴⁵ **5** y FR-66979⁴⁶ **6**, aislados de *Streptomyces sandaensis*⁴⁷ (Figura 6). Ambos muestran una potente actividad citotóxica contra diversas células tumorales *in vitro*. Se ha demostrado que la activación por reducción de FR-900482 y FR-66979 es necesaria para formar cadenas intermedias de ADN, donde el anillo de aziridina es esencial para la actividad biológica.

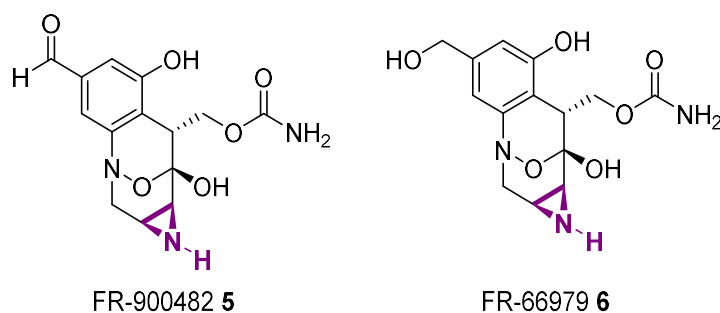


Figura 6. Estructuras de FR-900482 y FR-66979.

En 1975 la (2*S*,3*S*)-dicarboxilaziridina **7** (Figura 7) se aisló de una cepa de *Streptomyces MD 398-A1 (FERM-P 3217)* y se encontró que tenía una moderada actividad antibacteriana contra *Aeromonas Salmonicida*.⁴⁸ A través de estudios posteriores se demostró que actúa como

⁴⁵ I. Uchida, S. Takase, H. Kayakiri, S. Kiyoto, M. Hashimoto. "Structure of FR 900482, a novel antitumor antibiotic from a streptomyces". *J. Am. Chem. Soc.* **1987**, *109*, 4108-4109.

⁴⁶ H. Terano, S. Takase, J. Hosoda, M. Kohsaka. "A new antitumor antibiotic, FR-66979". *J. Antibiot.* **1989**, *42*, 145-148.

⁴⁷ M. Iwami, S. Kiyoto, H. Terano, M. Kohsaka, H. Aoki, H. Imanaka. "A new antitumor antibiotic, FR-900482". *J. Antibiot.* **1987**, *40*, 589-593.

⁴⁸ H. Naganawa, N. Usui, T. Takita, M. Hamada, H. Umezawa. "S-2,3-dicarboxy-aziridine, a new metabolite from a streptomyces". *J. Antibiot.* **1975**, *28*, 828-829.

inhibidor competitivo de la fumarasa, a través de la imitación de un estado de transición carbaniónico.⁴⁹ Asimismo, la ficellomicina **8** (Figura 7), aislada de *Streptomyces ficellus*⁵⁰ en 1976, demostró inhibir el crecimiento de bacterias gram positivas *in vitro* y mostró efectividad *in vivo* contra las infecciones por *Staphylococcus aureus* en ratones. Otros productos naturales que contienen el anillo de aziridina son las azinomicinas A **9** y B **10**, las cuales fueron aisladas de cultivos de *Streptomyces griseofuscus* en 1986. Se descubrió que éstas poseían citotoxicidades *in vitro* muy interesantes,³² así como unas importantes propiedades antitumorales y antibióticas contra bacterias gram positivas y gram negativas, aunque fueron inactivas contra levaduras y hongos (Figura 7). Por otro lado, otro producto natural de gran interés es la miraziridina A **11** (Figura 7), la cual fue aislada de una esponja marina relacionada con *Theonella mirabilis*.⁵¹ Ésta posee tres entidades principales en su estructura: a) un ácido (2*R*,3*R*)-aziridin-2,3-dicarboxílico, b) un ácido (3*S*,4*S*)-4-amino-3-hidroxi-6-metilheptanóico (estatina) y c) un ácido (*E*)-(*S*)-4-amino-7-guanidino-hept-2-enóico.

Varios estudios han demostrado que **11** inhibe la cisteína proteasa catepsina B y que el anillo de aziridina desempeña un papel importante y clave en esta actividad biológica, dando lugar a la inhibición irreversible de las catepsinas B y L. Ésta tiene lugar a través de la alquilación del sitio activo de la cisteína proteasa por la aziridina. Además, quedó demostrado que la miraziridina A preparada por síntesis total, inhibe la papaina-cisteína proteasa (a), la pepsin-aspartil proteasa (b) y la tripsin-serina proteasa (c) (Figura 7).⁵¹

Existen algunos péptidos naturales con anillo de aziridina, como las madurastatinas A1 **12** y B1 **13** (Figura 8), aislados de una cepa patógena de *Actinomadura madurae*.³⁶ El péptido natural de madurastatina A1 consiste en restos de serina y ácido salicílico, así como del anillo aziridínico que es esencial para su actividad biológica, principalmente antibacteriana contra *Micrococcus luteus*. Las azicemicinas A **14** y B **15** (Figura 8) fueron aisladas de *Amycolatopsis sulphurea*^{30,52} y

⁴⁹ J. Greenhut, H. Umezawa, F. B. Rudolph. "Inhibition of fumarase by *S*-2,3-dicarboxyaziridin". *J. Biol. Chem.* **1985**, *260*, 6684-6686.

⁵⁰ A. D. Argoudelis, F. Reusser, H. A. Whaley, L. Baczynskyj, S. A. Mizesak, R. J. Wnuk. "Antibiotics produced by *streptomyces ficellus*". *J. Antibiot.* **1976**, *29*, 1001-1006.

⁵¹ Y. Nakao, M. Fujita, K. Warabi, S. Matsunaga, N. Fusetani. "Miraziridine A, a novel cysteine protease inhibitor from the marine sponge *Theonella* aff. *mirabilis*". *J. Am. Chem. Soc.* **2000**, *122*, 10462-10463.

⁵² a) N. Kinoshita, T. Ikeda, R. Sawa, T. Takahashi, H. Naganawa, T. Sawa, M. Hamada, T. Takeuchi. "Azicemicin A, a new antimicrobial antibiotic from amycolatopsis". *J. Antibiot.* **1993**, *46*, 1772-1774. b) T. Tsuchida, R. Sawa, Y. Takahashi, H. Iinuma, T. Sawa, H. Naganawa, T. Takeuchi. "Azicemicins A and B, new antimicrobial agents produced by amycolatopsis II. Structure determination". *J. Antibiot.* **1995**, *48*, 1148-1152.

mostraron una inhibición moderada del crecimiento de bacterias gram positivas y microbacterias, así como interesantes propiedades fisicoquímicas.

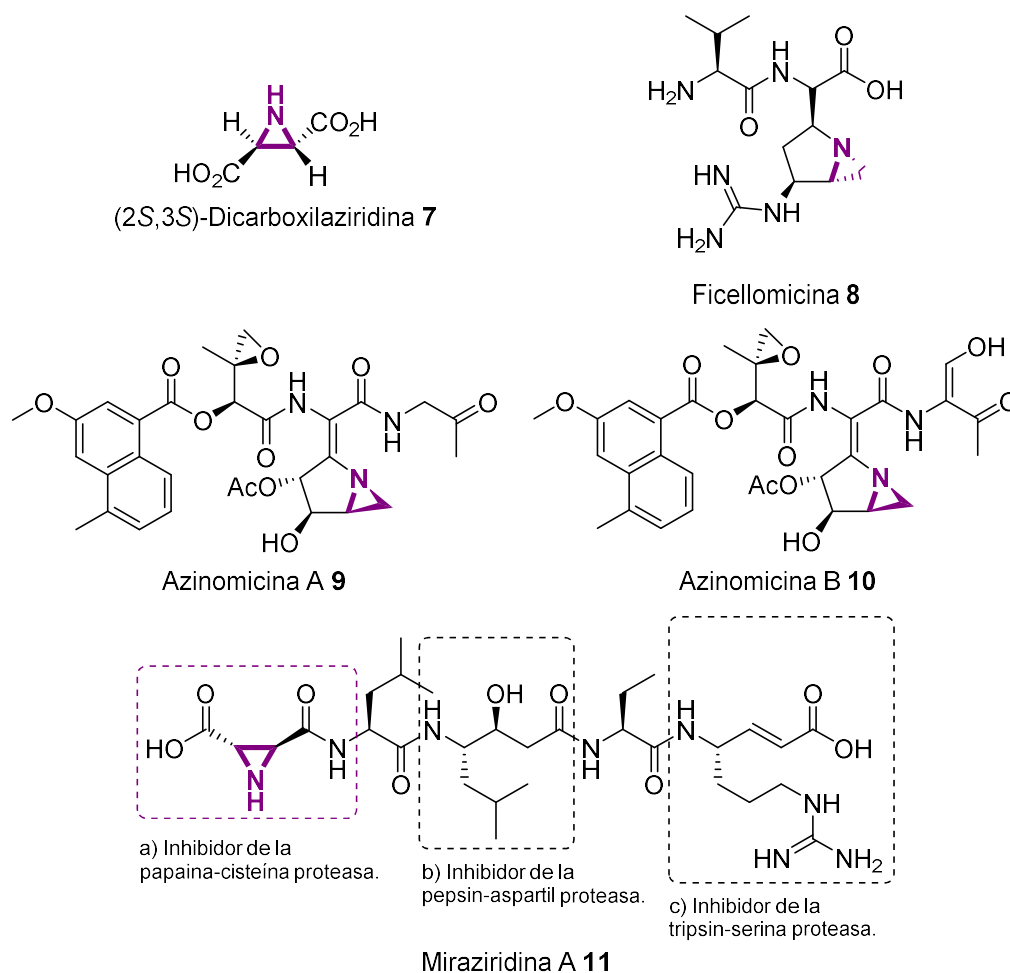


Figura 7. Productos naturales con actividad biológica que presentan el anillo de aziridina.

Finalmente, la maduropeptina **16** (Figura 8), consiste en un complejo (1:1) de una proteína transportadora y un cromóforo aislada de *Actinomadura*.^{37,53} Ésta presenta una actividad *in vitro* contra bacterias gram positivas y una fuerte citotoxicidad contra el melanoma murino B16-F10 y las células tumorales de carcinoma de colon humano.

⁵³ K. Komano, S. Shimamura, Y. Norizuki, D. Zhao, C. Kabuto, I. Sato, M. Hirama. "Total synthesis and structure revision of the (-)-maduropeptin chromophore". *J. Am. Chem. Soc.* **2009**, *131*, 12072-12073.

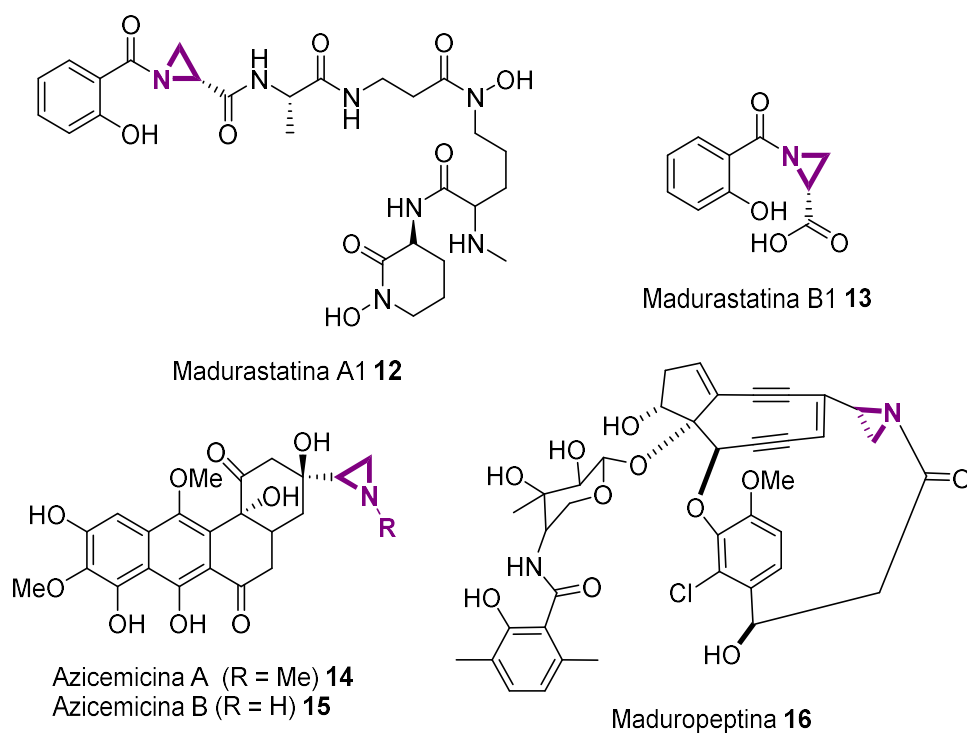


Figura 8. Estructuras de madurastatinas A1 y B1, azicemicinas A y B y maduropeptina.

Los compuestos naturales que contienen el anillo de aziridina tienen un futuro prometedor para el desarrollo de nuevos fármacos contra varias enfermedades, especialmente neoplasias. Por tanto, la incorporación del anillo de aziridina permitirá el desarrollo de nuevos compuestos sintéticos y semisintéticos interesantes con utilidad clínica.⁵⁴

⁵⁴ F. M. D. Ismail, D. O. Levitsky, V. M. Dembitsky. "Aziridine alkaloids as potential therapeutic agents". *Eur. J. Med. Chem.* **2009**, *44*, 3373-3387.

Finalmente, las aziridinas también son intermedios útiles en la síntesis de otros productos naturales tales como kainoides,⁵⁵ esfingosinas,⁵⁶ feldamicina,⁵⁷ (-)-mesembrina,⁵⁸ epicapreomicidina⁵⁹ o actinomicina D,⁶⁰ entre otros (Figura 9).

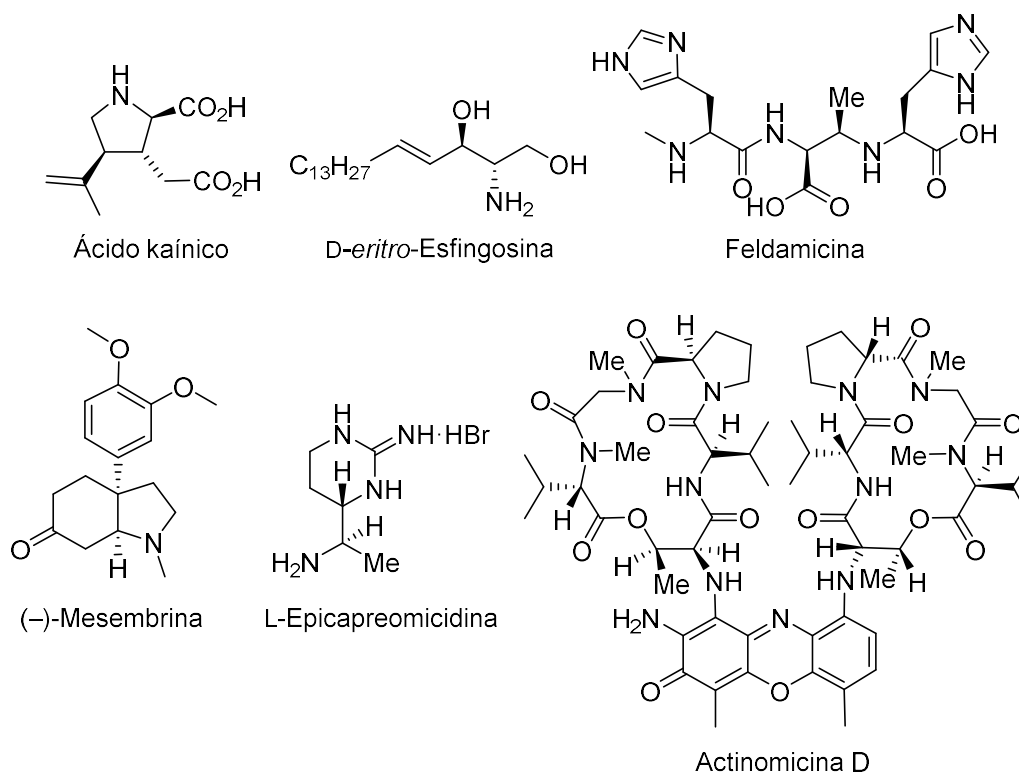


Figura 9. Algunos productos naturales sintetizados a partir de intermedios con estructura de aziridinas.

- ⁵⁵ G. Arena, C. C. Chen, D. Leonori, V. K. Aggarwal. "Concise synthesis of (+)-allo-kainic acid via Mgl₂-mediated tandem aziridine ring opening formal [3+2] cycloaddition". *Org. Lett.* **2013**, *15*, 4250-4253.
- ⁵⁶ B. Olofsson, P. Somfai. "Divergent synthesis of D-erythro-sphingosine, L-threo-sphingosine, and their regioisomers". *J. Org. Chem.* **2003**, *68*, 2514-2517.
- ⁵⁷ K. Imae, H. Kamachi, H. Yamashita, T. Okita, S. Okuyama, T. Tsuno, T. Yamasaki, Y. Sawada, M. Ohbayashi, T. Naito, T. Oki. "Synthesis, stereochemistry and biological properties of the depigmenting agents, melanostatin, feldamycin and analogs". *J. Antibiot.* **1991**, *44*, 76-85.
- ⁵⁸ Z. L. Song, B. M. Wang, Y. Q. Tu, C. A. Fan, S. Y. Zhang. "A general efficient strategy for cis-3a-aryloctahydroindole alkaloids via stereocontrolled ZnBr₂-catalyzed rearrangement of 2,3-aziridino alcohols". *Org. Lett.* **2003**, *5*, 2319-2321.
- ⁵⁹ T. Teshima, K. Konishi, T. Shiba. "Synthesis of L-epicapreomicidine". *Bull. Chem. Soc. Jpn.* **1980**, *53*, 508-511.
- ⁶⁰ T. Tanaka, K. Nakajima, K. Okawa. "Studies on 2-aziridinecarboxylic acid. IV. Total synthesis of actinomycin D (C1) via ring-opening reaction of aziridine". *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1352-1355.

3. Síntesis de aziridinas

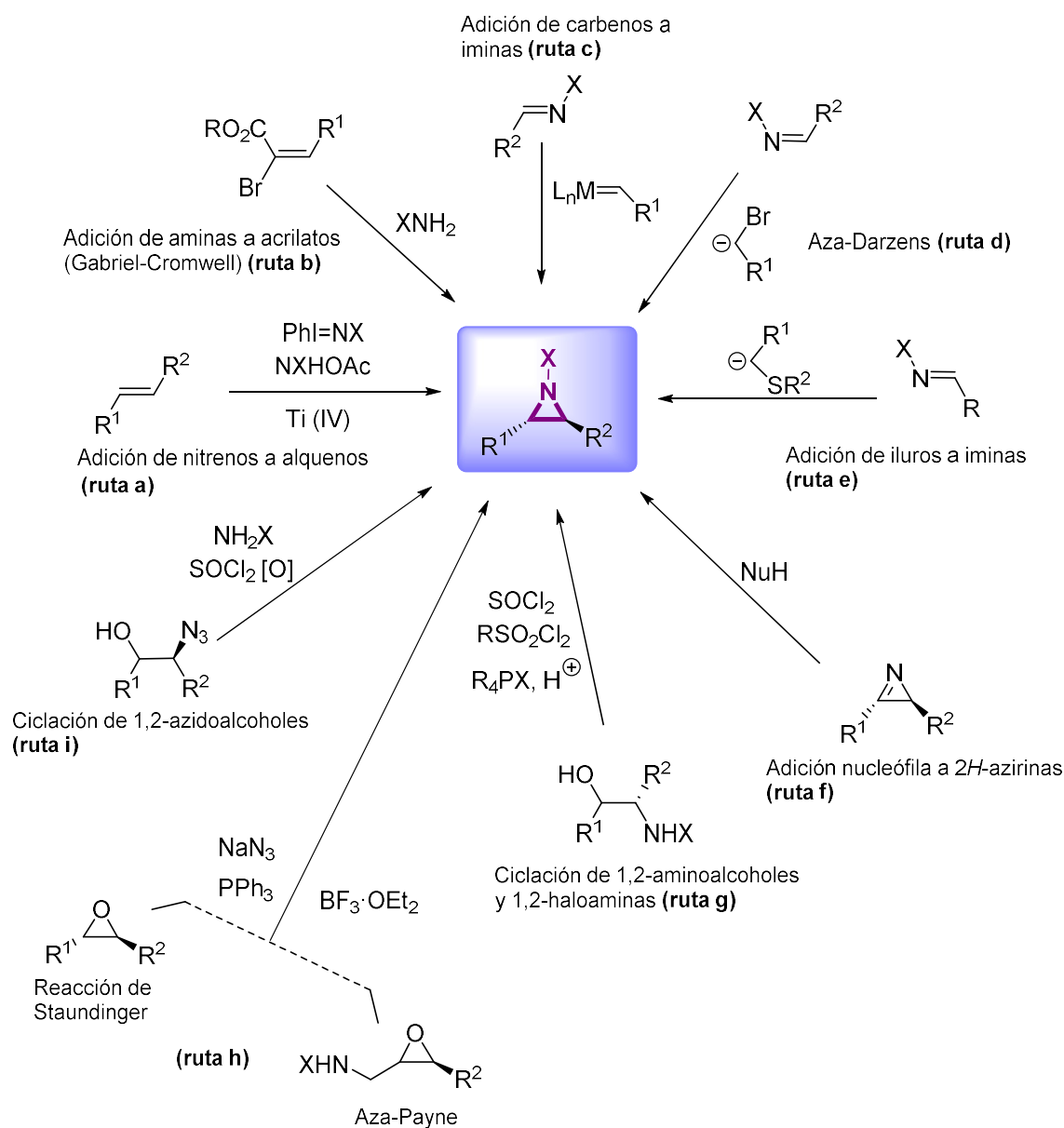
3.1. Estrategias generales para la síntesis de aziridinas.

Como se ha mencionado anteriormente, las aziridinas son intermedios sintéticos en la síntesis de una amplia gama de aminas y heterociclos nitrogenados. Por ello, existe un interés continuo en la búsqueda de metodologías más eficientes y sencillas para acceder a estos heterociclos a partir de una gran variedad de precursores de forma estereocontrolada.

A lo largo de estos últimos años, se han desarrollado una gran variedad de protocolos para la síntesis de aziridinas,^{3c,12,61} como se ilustra en el esquema 1. Los métodos de síntesis que existen en la preparación de aziridinas, pueden trascurrir a través de dos mecanismos principales, adición y ciclación. A su vez, las reacciones de adición se pueden agrupar en varias categorías; la primera implica la adición de nitrenos o equivalentes de nitreno (nitrenoides) a alquenos, también denominadas reacciones $C_2 + N_1$ (**ruta a**), así como procesos de adición-eliminación (**ruta b**), a través de las cuales se han establecido una gran variedad de rutas tanto catalíticas como no catalíticas. Debido a que los alquenos, respecto a las reacciones estereoselectivas en la preparación de aziridinas, son precursores poco eficaces, se han realizado mayores avances mediante reacciones de adición de carbenos o carbenoides a iminas, también denominadas reacciones $(C_1N_1 + C_1)$ (**ruta c**), las cuales suponen un método eficaz para la reacción de aziridinación. Las iminas a su vez, experimentan la reacción de aza-Darzens (**ruta d**), que incluye la adición de diazocompuestos catalizada por un ácido de Lewis o Brønsted a iminas, así como reacciones de aziridinación mediadas con iluros de azufre (**ruta e**). Por otro lado, las 2*H*-azirinas pueden actuar como precursores de las aziridinas mediante reacciones de adición nucleófila al doble enlace C=N de azirinas (**ruta f**). Hoy en día, el desarrollo de nuevas estrategias para la reacción asimétrica de 2*H*-azirinas con el fin de obtener aziridinas quirales con alta enantioselectividad, es un reto importante, el cual requiere una estrategia sintética bien diseñada. Finalmente, los procesos de ciclación intramolecular de derivados de amina implican el cierre de anillo en sustratos tales como 1,2-aminoalcoholes y 1,2-haloaminas (**ruta g**) o equivalentes, como epóxidos (**ruta h**) y 1,2-azidoalcoholes (**ruta i**). Estas rutas sintéticas se vuelven particularmente útiles para la preparación de aziridinas enantiopuras. El estereocontrol

⁶¹ a) A. Padwa, S. S. Murphreeb. "Epoxides and aziridines-a mini review". *Arkivoc.* **2006**, 3, 6-33. b) H. Pellissier. "Recent developments in asymmetric aziridination". *Tetrahedron* **2010**, 66, 1509-1555. c) H. Pellissier. "Recent developments in asymmetric aziridination". *Adv. Synth. Catal.* **2014**, 356, 1899-1935. d) L. Degennaro, P. Trinchera, R. Luisi. "Recent advances in the stereoselective synthesis of aziridines". *Chem. Rev.* **2014**, 114, 7881-7929.

en todas estas estrategias sintéticas (Esquema 1) se puede llevar a cabo mediante el empleo de un agente externo, bien sea, derivado de un catalizador, un ligando o reactivo quiral, o bien mediante un agente interno, derivado de un centro estereogénico preexistente en los productos de partida.⁶²



Esquema 1. Estrategias sintéticas para la síntesis de aziridinas.

⁶² V. Andrushko, N. Andrushko. *Principles, concepts and strategies of stereoselective synthesis* In "Stereoselective synthesis of drugs and natural products". 1st ed., John Wiley & Sons, Inc, 2013, 2, Cap.1, 3-45.

3.2. Adiciones de nitrenos o equivalentes a alquenos (C₂ + N₁).

La aziridinación de alquenos es una estrategia de gran utilidad para acceder a diversos productos nitrogenados e intermedios sintéticos,^{12,24,63} debido a la fácil disponibilidad de los materiales de partida olefínicos y a la naturaleza directa de dicho proceso. En cuanto a la adición de nitrenos y nitrenoides existen dos métodos generales que implican mecanismos de uno o dos pasos. Así, nitrenos y metalonitrenos reaccionan con alquenos mediante una reacción de aziridinación directa, mientras que, por otro lado, los nitrenoides no metálicos reaccionan a través de un proceso de adición-eliminación.

En cuanto a la aziridinación de olefinas, ésta se lleva a cabo utilizando un reactivo de transferencia de nitreno.⁶⁴ En estos últimos años, se ha desarrollado una variedad de reactivos de transferencia de nitrógeno a olefinas, entre los que destacan PhI=NTs, TsNKI, cloramina-T,⁶⁵ bromamina-T⁶⁶ y azidas. El reactivo de aziridinación más clásico es [*p*-(*p*-tolilsulfonyl)imino]feniliodinano PhI=NTs (Ts = tosilo), que fue introducido a principios de la década de los 80 por los grupos de Breslow⁶⁷ y Mansuy.⁶⁸ En los años 90, Evans y Jacobsen describen la aziridinación asimétrica de alquenos catalizada por cobre, utilizando para ello (PhI=NTs) como fuente de nitreno.⁶⁹ Este proceso se ha aplicado con éxito a la síntesis total de una variedad de productos naturales o compuestos biológicamente activos como el potente

⁶³ a) R. S. Atkinson. "3-Acetoxyaminoquinazolinones (QNHOAc) as aziridinating agents: ring opening of *N*-(*Q*)-substituted aziridines". *Tetrahedron* **1999**, *55*, 1519-1559. b) P. Müller, C. Fruit. "Enantioselective catalytic aziridinations and asymmetric nitrene insertions into CH bonds". *Chem. Rev.* **2003**, *103*, 2905-2919.

⁶⁴ T. Uchida, T. Katsuki. "Asymmetric nitrene transfer reactions: sulfimination, aziridination and C-H amination using azide compounds as nitrene precursors". *Chem. Rec.* **2014**, *14*, 117-129.

⁶⁵ M. Nonn, L. Kiss, E. Forro, R. Sillanpaa, F. Fulop. "Synthesis of densely functionalized cispentacin derivatives through selective aziridination and aziridine opening reactions: orthogonally protected di- and triaminocyclopentanecarboxylates". *Tetrahedron* **2014**, *70*, 8511-8519.

⁶⁶ C. M. Sharma, B. Kashyap, P. Phukan. "Aziridination of olefins with bromamine-T in presence of iodine as catalyst". *Indian J. Chem.* **2014**, *53B*, 750-753.

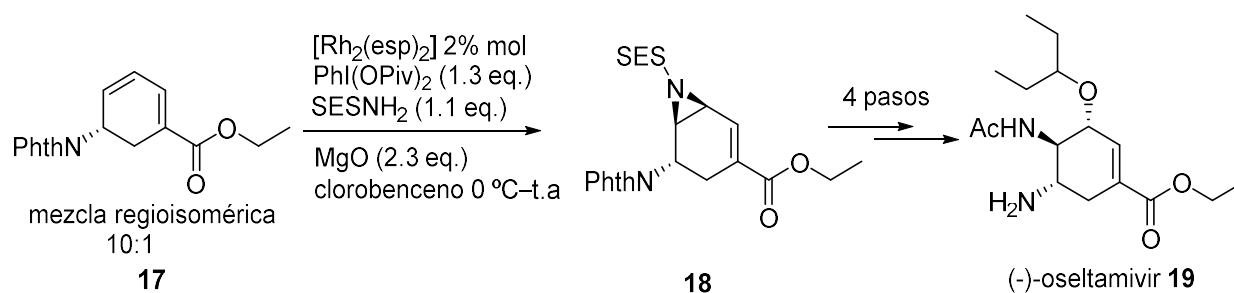
⁶⁷ R. Breslow, S. H. Gellman. "Tosylamidation of cyclohexane by a cytochrome P-450 model". *J. Chem. Soc. Chem. Commun.* **1982**, *24*, 1400-1401.

⁶⁸ D. Mansuy, J. P. Mahy, A. Dureault, G. Bedi, P. Battioni. "Iron- and manganese-porphyrin catalysed aziridination of alkenes by tosyl- and acyl-iminiodobenzene". *J. Chem. Soc. Chem. Commun.* **1984**, *17*, 1161-1163.

⁶⁹ a) D. A. Evans, M. M. Faul, M. T. Bilodeau, B. A. Anderson, D. M. Barnes. "Bis(oxazoline)-copper complexes as chiral catalysts for the enantioselective aziridination of olefins". *J. Am. Chem. Soc.* **1993**, *115*, 5328-5329. b) Z. Li, K. R. Conser, E. N. Jacobsen. "Asymmetric alkene aziridination with readily available chiral diimine-based catalysts". *J. Am. Chem. Soc.* **1993**, *115*, 5326-5327.

agente antineoplásico (+)-agelastatina A.⁷⁰

En la primera década del año 2000, Trost y Zhang emplearon esta metodología para estudiar la aziridinación asimétrica de un dieno quiral **17**,⁷¹ como aparece representado en el esquema 2, con el objeto de desarrollar una síntesis del inhibidor de neuraminidasa, (-)-oseltamivir **19**. En este caso, el uso de catalizadores de cobre no condujo al producto deseado de la reacción de aziridinación regioselectiva a partir del dieno funcionalizado, ya que no se observó selectividad entre los dobles enlaces α , β y γ , δ , formándose una mezcla de productos no separable. Sin embargo, a través del empleo de $\text{PhI}(\text{OPiv})_2$ y SESNH_2 en presencia de un catalizador de rodio $[\text{Rh}_2(\text{esp})_2]$ donde (esp = $\alpha, \alpha', \alpha', \alpha'$ -tetrametil-1,3-ácido bencenodipropiónico) y MgO como base, se obtuvo el producto monoaziridinado **18** de forma regio- y diastereoselectiva, como un único estereoisómero y con un rendimiento del 86% como se observa en el esquema 2. Sucesivas transformaciones en **18** condujeron a (-)-oseltamivir **19**.



Esquema 2. Síntesis de (-)-oseltamivir a través de la aziridinación regioselectiva de un dieno quiral.

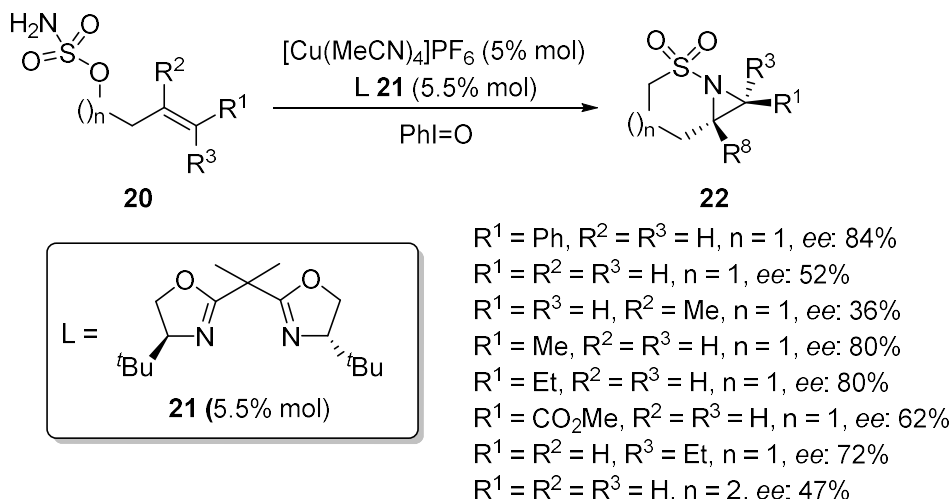
En las últimas dos décadas, se han desarrollado una gran variedad de catalizadores quirales incluyendo catalizadores de cobre, rodio, rutenio y otros complejos metálicos. Hasta la fecha, los sistemas catalíticos quirales más empleados para la aziridinación enantioselectiva mediante transferencia de nitreno a alquenos, se basan en complejos de cobre, usando como ligandos quirales una clase de bisoxazolinas.⁷² En 2007, Dauban y col., utilizaron el ligando de bisoxazolina

⁷⁰ B. M. Trost, G. Dong. "New class of nucleophiles for palladium-catalyzed asymmetric allylic alkylation. Total synthesis of agelastatin A". *J. Am. Chem. Soc.* **2006**, *128*, 6054-6055.

⁷¹ a) B. M. Trost, T. Zhang. "A concise synthesis of (-)-oseltamivir". *Angew. Chem. Int. Ed.* **2008**, *47*, 3759-3761. b) B. M. Trost, T. Zhang. "Development of a concise synthesis of (-)-oseltamivir (Tamiflu)". *Chem. Eur. J.* **2011**, *17*, 3630-3643.

⁷² a) H. A. McManus, P. J. Guiry. "Recent developments in the application of oxazoline-containing ligands in asymmetric catalysis". *Chem. Rev.* **2004**, *104*, 4151-4202. b) G. Desimoni, G. Faita, K. A. Jørgensen.

de Evans **21** para promover la aziridinación intramolecular catalizada por Cu de una amplia gama de sulfamatos insaturados **20**.⁷³ Esta reacción se realizó en presencia de PhI=O y [Cu(MeCN)₄]PF₆, proporcionando las aziridinas quirales **22** correspondientes con rendimientos de hasta el 86% y excesos enantioméricos de hasta el 84%, como se muestra en el esquema 3. En particular, es interesante observar que esta transferencia de nitreno se produjo con el mismo éxito para olefinas alifáticas simples y alquenos deficientes en electrones.



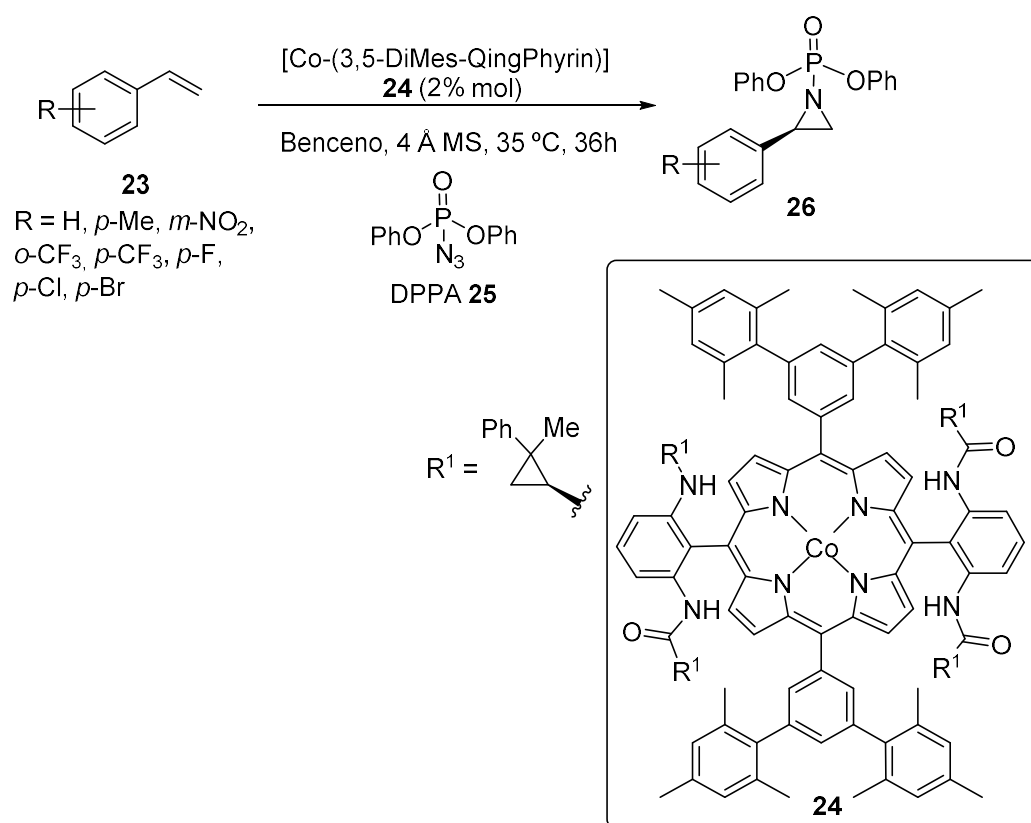
Esquema 3. Aziridinación intramolecular catalizada por Cu de sulfamatos insaturados con PhI=O en presencia del ligando de bisoxazolina de Evans.

Es conocido desde la década de 1960 que la descomposición térmica o fotoquímica de acil azidas da lugar a nitrenos libres, que pueden reaccionar con alquenos para producir aziridinas. De tal forma que, en la segunda década del 2000, en una serie de artículos del grupo de Zhang se describe el empleo de amidoporfirinas simétricas quirales D₂ **24** como catalizadores para la aziridinación asimétrica de olefinas **23** con fosforil azidas (DPPA) **25**⁷⁴ (Esquema 4). Las N-fosforilaziridinas **26** se obtuvieron con altos rendimientos (64-99%) y enantioselectividades moderadas (23-85% ee).

"C₂-Symmetric chiral bis (oxazoline) ligands in asymmetric catalysis". *Chem. Rev.* **2006**, *106*, 3561-3651.

⁷³ A. Estéoule, F. Durán, P. Retailleau, R. H. Dodd, P. Dauban. "Enantioselective intramolecular copper-catalyzed aziridination of sulfamates". *Synthesis* **2007**, *8*, 1251-1260.

⁷⁴ J. Tao, L. M. Jin, X. P. Zhang. "Synthesis of chiral N-phosphoryl aziridines through enantioselective aziridination of alkenes with phosphoryl azide via Co(II)-based metalloradical catalysis". *Beilstein J. Org. Chem.* **2014**, *10*, 1282-1289.



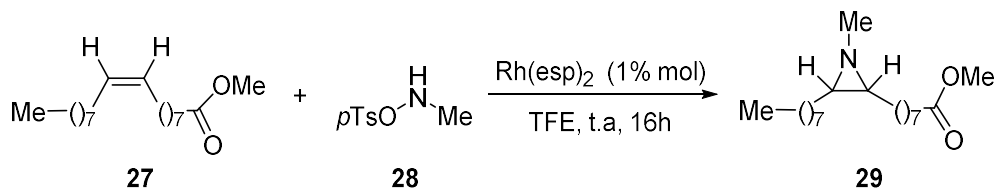
Esquema 4. Aziridinación enantioselectiva de alquenos en presencia de DPPA catalizada por un complejo de Co(II) con un ligando de amidoporfirina simétrica D₂ (3,5-DiMes-QingPhyrin).

Las aziridinas no activadas (*NH/N-Me*) son de especial interés, al estar presentes en muchas moléculas bioactivas naturales, semisintéticas y sintéticas.^{54,75} Asimismo, sirven como building blocks importantes en síntesis orgánica debido a su reactividad intrínseca a través de la apertura del anillo, expansión del anillo o reordenamientos.⁷⁶ A pesar de esto, existen pocos métodos para acceder a aziridinas no activadas directamente a través de olefinas, ya que la mayoría de los procedimientos conocidos están dedicados a sus homólogos activados. En la actualidad, se ha desarrollado un método estereoespecífico, utilizando un catalizador de Rh(II) altamente eficiente, para la preparación de aziridinas no activadas **29**, a partir de olefinas **27** y usando O-

⁷⁵ I. D. G. Watson, L. Yu, A. K. Yudin. "Advances in nitrogen transfer reactions involving aziridines". *Acc. Chem. Res.* **2006**, *39*, 194-206.

⁷⁶ a) R. A. Craig, N. R. O'Connor, A. F. G. Goldberg, B. M. Stoltz. "Stereoselective Lewis acid mediated [3+2] cycloadditions of *N*-H- and *N*-sulfonylaziridines with heterocumulenes". *Chem. Eur. J.* **2014**, *20*, 4806-4813. b) J. R. Vale, F. Siopa, P. S. Branco, C. A. M. Alfonso. "Ring opening of 6-azabicyclo [3.1.0]-hex-3-en-2-ols in water under mild conditions". *Eur. J. Org. Chem.* **2016**, *11*, 2048-2053.

(sulfonil)hidroxilaminas **28** como agentes de aminación (Esquema 5). Las reacciones transcurrieron con una buena quimioselectividad.⁷⁷



Esquema 5. Aziridinación estereoespecífica directa de olefinas no activadas utilizando *O*-(sulfonil)hidroxilaminas como agentes de aminación, para la preparación de aziridinas no activadas.

Por otra parte, en los últimos años se han desarrollado con éxito varios ejemplos de reacción de aziridinación enantioselectiva organocatalítica de olefinas.⁷⁸ De hecho, características importantes como la disponibilidad inmediata y la estabilidad de los catalizadores empleados, su bajo costo y toxicidad, así como su alta tolerancia de grupos funcionales, han confirmado las estrategias organocatalíticas como una herramienta efectiva para desarrollar con éxito importantes retos en síntesis orgánica.⁷⁹

Entre los ejemplos más recientes, se encuentra una metodología para la síntesis de la (–)-alozamizolina **34**, una unidad de aglicona del potente inhibidor de quitinasa alosamidina,⁸⁰ el cual es un derivado de aminociclopentitol con cinco estereocentros contiguos **33**. La estrategia implica una aziridinación enantioselectiva de enonas cíclicas **30**, catalizada por una diamina

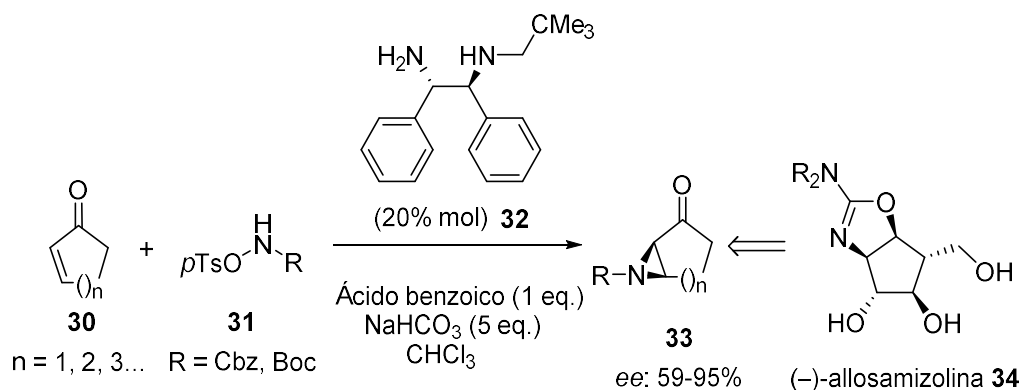
⁷⁷ S. Sabir, C. B. Pandey, A. K. Yadav, B. Tiwari, J. L. Jat. "Direct *N*-H/*N*-Me aziridination of unactivated olefins using *O*-(Sulfonyl)hydroxylamines as aminating agents". *J. Org. Chem.* **2018**, *83*, 12255-12260.

⁷⁸ a) H. Pellissier. "Recent developments in the asymmetric organocatalytic Morita-Baylis-Hillman reaction". *Tetrahedron* **2017**, *73*, 2831-2861. b) E. M. McGarrigle, E. L. Myers, O. Illa, M. A. Shaw, S. L. Riches, V. K. Aggarwal. "Chalcogenides as organocatalysts". *Chem. Rev.* **2007**, *107*, 5841-5883.

⁷⁹ a) F. Vetica, R. M. de Figueiredo, M. Orsini, D. Tofani, T. Gasperi. "Recent advances in organocatalytic cascade reactions toward the formation of quaternary stereocenters". *Synthesis* **2015**, *47*, 2139-2184. b) T. Gasperi, M. Miceli, J. M. Campagne, R. Marcia de Figueiredo. "Non-covalent organocatalyzed domino reactions involving oxindoles: recent advances". *Molecules* **2017**, *22*, 1636-1665.

⁸⁰ S. Sakuda, A. Isogai, S. Matsumoto, A. Suzuki. "Search for microbial insect growth regulators IV allosamidin, a novel chitinase inhibitor". *J. Antibiot.* **1987**, *40*, 296-300.

quiral **32** como se muestra en el esquema 6.⁸¹ Tanto el ácido benzoico como el bicarbonato de sodio son necesarios para evitar la descomposición de los *N*-tosiloxialquilcarbamatos **31**.



Esquema 6. Aziridinación organocatalizada por la diamina quirale a partir de enonas en la síntesis de (-)-allosamizolina.

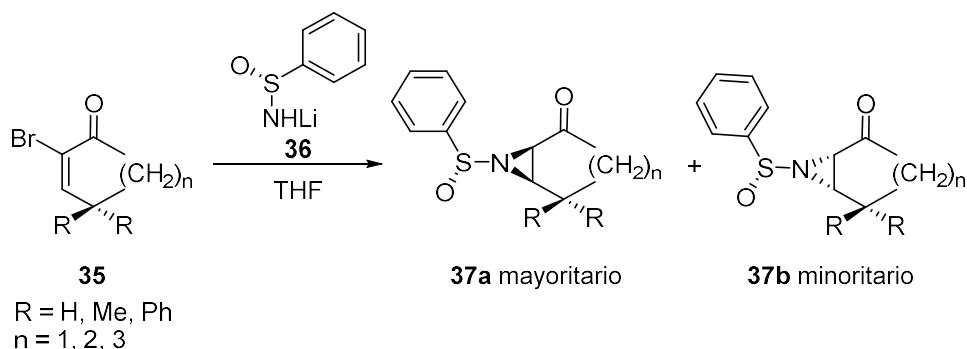
3.3. Aziridinación a través de procesos de adición-eliminación.

La síntesis de Gabriel-Cromwell de aziridinas consta de una adición inicial de Michael seguida de una protonación y cierre del anillo 3-*exo-terc*.⁸² Esto es debido a que esta síntesis lleva consigo la adición nucleofílica formal de un nitreno equivalente a un 2-haloacrilato o un reactivo similar. Fue en el año 2010 cuando Lobo y col.,⁸³ describieron la adición de Michael de la sal de litio de la sulfinamida quirale **36** a las α -bromo enonas cíclicas **35**, siguiendo con la ciclación intramolecular para proporcionar las aziridinas **37a** y **37b** con diastereoselectividades que van del 12 al 65%, como se muestra en el esquema 7.

⁸¹ T. Nemoto, R. Muramoto, T. Ruengsatra, Y. Hamada. "Formal enantioselective synthesis of (-)-allosamizoline using chiral diamine catalyzed asymmetric aziridination of cyclic enones". *Tetrahedron* **2016**, *72*, 1-8.

⁸² G. V. Shustov, O. Krutius, V. N. Voznesensky, I. I. Chervin, A. V. Ereemeev, R. G. Kostyanovskii, F. D. Polyak. "Asymmetric nitrogen-LXXI. Asymmetric synthesis and lactonization of 1-(β -hydroxyalkyl)aziridine-2-carboxylic esters into 4-oxa-1-azabicyclo[4.1.0]heptan-5-ones". *Tetrahedron* **1990**, *46*, 6741-6752.

⁸³ V. D. B. Bonifacio, C. Gonzalez-Bello, H. S. Rzepa, S. Prabhakar, A. M. Lobo. "Chiral aziridination of olefins using a chiral sulfinamide as the nitrogen source". *Synlett*. **2010**, *1*, 145-149.



Esquema 7. Reacción de α -bromo enonas cíclicas con la sal de litio de una sulfonamida quiral.

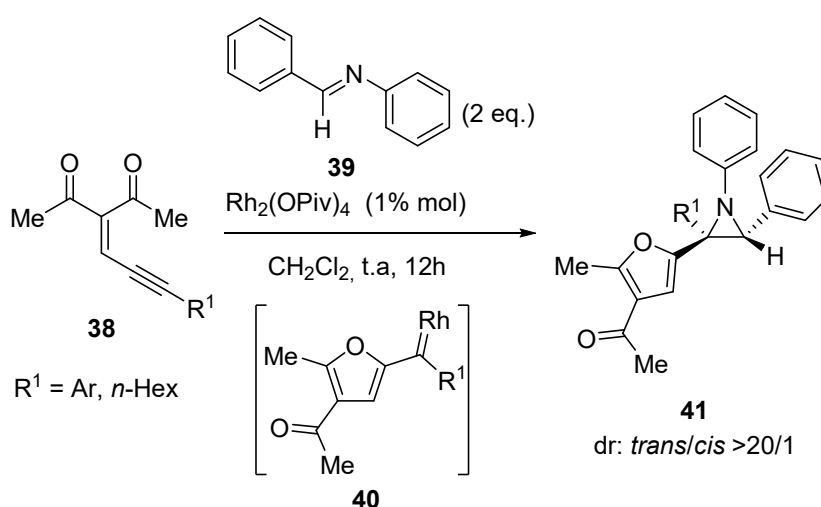
3.4. Adiciones de carbenos o carbenoides a iminas ($\text{C}_1\text{N}_1+\text{C}_1$).

La aziridinación de iminas es un proceso de especial interés, debido a que es considerado un método efectivo y práctico para la síntesis enantioselectiva de aziridinas quirales. Por lo tanto, el desarrollo de reacciones de aziridinación mediante el uso de catalizadores, a partir de precursores aquirales, es muy importante, ya que la mayoría de los métodos para la preparación de aziridinas quirales se basan en el uso de materiales de partida ópticamente activos o cantidades estequiométricas de auxiliares quirales.^{84,85}

- ⁸⁴ a) F. Palacios, A. M. Ochoa de Retana, J. I. Gil, J. M. Ezpeleta. "Simple asymmetric synthesis of 2*H*-azirines derived from phosphine oxides". *J. Org. Chem.* **2000**, *65*, 3213-3217. b) F. Palacios, A. M. Ochoa de Retana, J. I. Gil. "Easy and efficient synthesis of enantiomerically enriched 2*H*-azirines derived from phosphonates". *Tetrahedron Lett.* **2000**, *41*, 5363-5366. c) F. Palacios, D. Aparicio, A. M. Ochoa de Retana, J. M. de los Santos, J. I. Gil, J. M. Alonso. "Asymmetric synthesis of 2*H*-Azirines derived from phosphine oxides using solid-supported amines. Ring opening of azirines with carboxylic acids". *J. Org. Chem.* **2002**, *67*, 7283-7288. d) F. Palacios, D. Aparicio, A. M. Ochoa de Retana, J. M. de los Santos, J. I. Gil, R. L. de Munain. "Asymmetric synthesis of 2*H*-aziridine phosphonates, and α - or β -aminophosphonates from enantiomerically enriched 2*H*-azirines". *Tetrahedron: Asymmetry* **2003**, *14*, 689-700. e) F. Palacios, A. M. Ochoa de Retana, J. M. Alonso. "Reaction of 2*H*-azirine phosphine oxide and phosphonates with nucleophiles. Stereoselective synthesis of functionalized aziridines and α - and β -aminophosphorus derivatives". *J. Org. Chem.* **2005**, *70*, 8895-8901. f) F. Palacios, A. M. Ochoa de Retana, J. M. Alonso. "Regioselective synthesis of fluoroalkylated β -aminophosphorus derivatives and aziridines from phosphorylated oximes and nucleophilic reagents". *J. Org. Chem.* **2006**, *71*, 6141-6148. g) A. Velez del Burgo, A. M. Ochoa de Retana, J. M. de los Santos, F. Palacios. "Reaction of 2*H*-azirine-phosphine oxides and phosphonates with enolates derived from β -keto esters". *J. Org. Chem.* **2016**, *81*, 100-108.
- ⁸⁵ a) F. A. Davis, Y. Wu, H. Yan, W. McCoull, K. R. Prasad. "Asymmetric synthesis of aziridine 2-phosphonates from enantiopure sulfinimines (*N*-sulfinyl imines). Synthesis of α -amino phosphonates". *J. Org. Chem.* **2003**, *68*, 2410-2419. b) Y. Arroyo, A. Meana, M. A. S. Tejedor, I. Alonso, J. L. Garcia Ruano. "2-(*p*-Tolylsulfinyl)benzyl halides as efficient precursors of optically pure *trans*-2,3-disubstituted aziridines". *Chem. Eur. J.* **2010**, *16*, 9874-9883. c) F. Colpaert, S. Mangelinckx, S. De

Las reacciones entre carbenos o equivalentes de carbeno a iminas implican un método de aziridinación útil, donde el mecanismo, con frecuencia, involucra un proceso de eliminación y adición gradual, en lugar de un proceso de formación de enlaces.

Debido a que las eninonas **38**, sirven como buenos sustratos para las reacciones de transferencia de carbeno, se ha desarrollado una metodología sintética para la síntesis diastereoselectiva de aziridinas polisustituidas **41** por reacción de iminas **39** con los carbenoides de rodio **40** (Esquema 8).⁸⁶ La razón de la excelente diastereoselectividad (>20/1) se atribuye a las interacciones π - π stacking entre los dos grupos *cis*-fenilo.



Esquema 8. Aziridinación diastereoselectiva de eninonas e iminas catalizada por rodio.

3.5. Reacción de aza-Darzens.

La primera reacción de aza-Darzens, propuesta por Deyrup⁸⁷ en 1969, es un método conveniente para la preparación de aziridinas funcionalizadas con considerable utilidad sintética,^{88,89} a partir de intermedios sintéticos comunes. La reacción de aza-Darzens implica la

Brabandere, N. De Kimpe. "Asymmetric synthesis of α -chloro- β -amino-*N*-sulfinyl imidates as chiral building blocks". *J. Org. Chem.* **2011**, *76*, 2204-2213.

⁸⁶ H. Luo, K. Chen, H. Jiang, S. Zhu. "A route to polysubstituted aziridines from carbenes and imines through a nondiazo approach". *Org. Lett.* **2016**, *18*, 5208-5211.

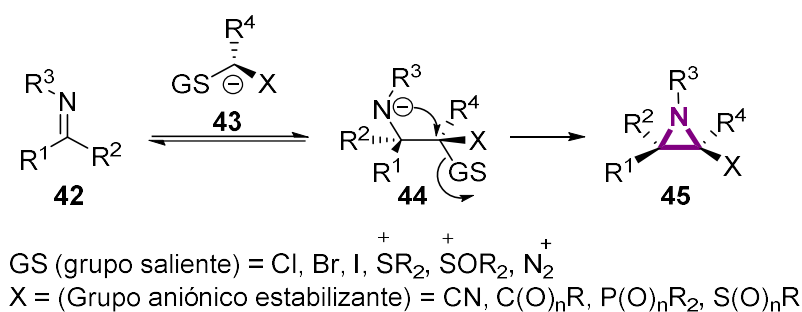
⁸⁷ J. A. Deyrup. "A Darzens aziridine synthesis". *J. Org. Chem.* **1969**, *34*, 2724-2727.

⁸⁸ J. Sweeney. "Aziridine synthesis via nucleophilic attack of carbene equivalents on imines: the aza-Darzens reaction". *Eur. J. Org. Chem.* **2009**, 4911-4919.

⁸⁹ T. M. Sola, I. Churcher, W. Lewis, R. A. Stockman. "Stereo selective aza-Darzens reactions of *tert*-butanesulfinimines: convenient access to chiral aziridines". *Org. Biomol. Chem.* **2011**, *9*, 5034-5035.

Introducción

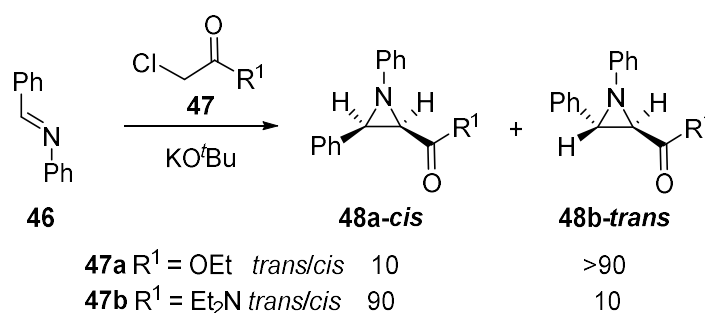
reacción de aniones estabilizados que contienen buenos grupos salientes en la posición α , con iminas, y es análoga a la síntesis de epóxidos de Darzens, empleando en este último caso aldehídos o cetonas, en lugar de iminas. El mecanismo de esta reacción se desarrolla en dos etapas; en la primera, tiene lugar el ataque nucleófilo de carbaniones funcionalizados **43** (proceso reversible) sobre el doble enlace C=N de la imina **42**, mientras que en la segunda etapa ocurre la ciclación 3-*exo-terc* del intermedio **44** para proporcionar la aziridina funcionalizada **45** (proceso irreversible) (esquema 9).⁸⁸



Esquema 9. Mecanismo de la reacción de aza-Darzens.

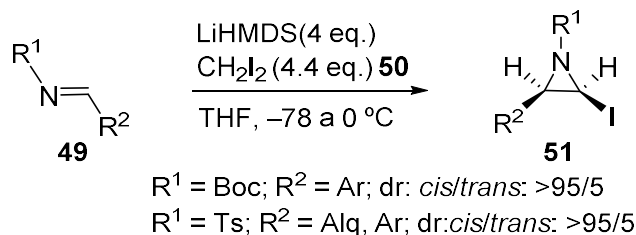
La reacción del cloroacetato de etilo **47a** ($R^1 = \text{OEt}$) con *N*-fenilbenzaldimina **46** en presencia de KO^tBu da lugar a la *trans*-aziridina **48b** con un rendimiento bajo (29%). Sin embargo, cuando se usa la clordietilamina **47b** ($R^1 = \text{Et}_2\text{N}$) se produce la inversión de la diastereoselectividad de la reacción mejorando el rendimiento químico de la misma (Esquema 10).⁸⁷

La variante asimétrica de la reacción de aza-Darzens se ha empleado para acceder a aziridinas enantioenriquecidas con elevadas diastereoselectividades. La selectividad facial de la reacción de aza-Darzens en la etapa de adición inicial puede estar influenciada por el control de reactivos en el componente de la imina o por la elección del nucleófilo. Durante estos últimos años se han descrito varios ejemplos de reacciones aza-Darzens enantio y diastereoselectivas.



Esquema 10. Primera reacción de aza-Darzens descrita por Deyrup en 1969.

Por ejemplo, se han podido sintetizar 2-yodoaziridinas **51** con excelente selectividad *cis* (Esquema 11), a partir de la reacción de *N*-Boc o *N*-tosiliminas **49** con diyodometil litio, generado *in situ* por desprotonación de diyodometano **50** con bis(trimetilsilil) amida de litio (LiHMDS) a -78 °C.⁹⁰ En todos los casos, la reacción con iminas aromáticas es exitosa, mientras que cuando se emplean las *N*-Boc iminas alifáticas no se obtienen los productos esperados.



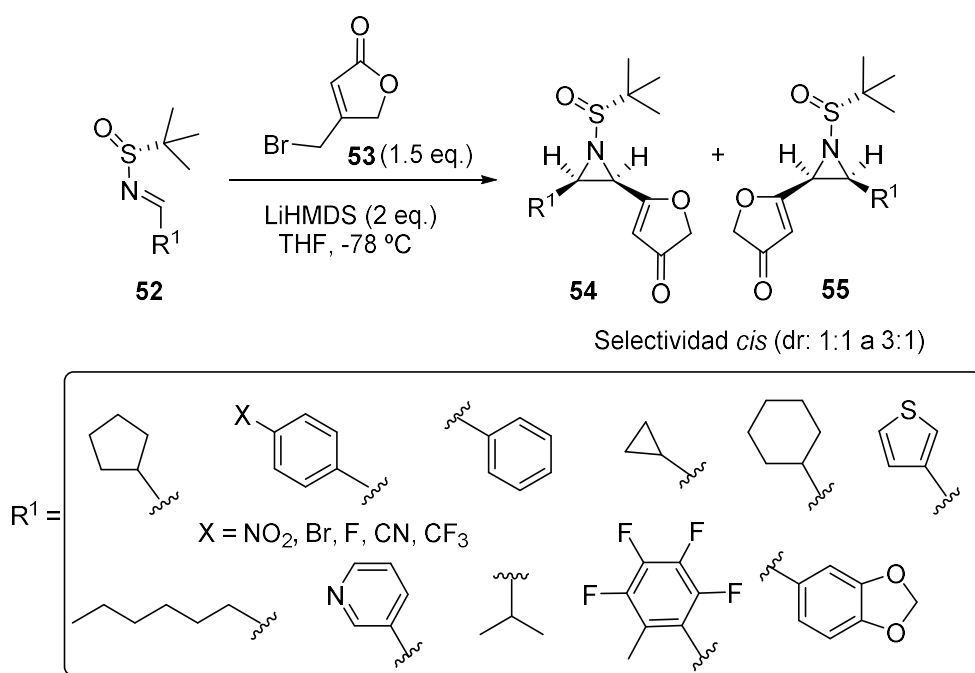
Esquema 11. Aza-Darzens diastereoselectiva para la preparación de *cis* 2-yodoaziridinas.

Recientemente, se ha llevado a cabo la reacción de aza-Darzens viníloga asimétrica.⁹¹ Esta metodología se basa en la reacción de dienolatos sustituidos **53** con un buen grupo saliente en la posición γ , con sulfiminas quirales **52**, en presencia de bis(trimetilsilil)amiduro de litio

⁹⁰ a) J. A. Bull, T. Boulwood, T. A. Taylor. "Highly *cis*-selective synthesis of iodo-aziridines using diiodomethyl lithium and *in situ* generated *N*-Boc-imines". *Chem. Commun.* **2012**, *48*, 12246-12248. b) T. Boulwood, D. P. Affron, A. D. Trowbridge, J. A. Bull. "Synthesis of *cis*-C-Iodo-*N*-tosyl-aziridines using diiodomethyl lithium: reaction optimization, product scope and stability, and a protocol for selection of stationary phase for chromatography". *J. Org. Chem.* **2013**, *78*, 6632-6647. c) T. Boulwood, D. P. Affron, J. A. Bull. "Studies on the synthesis of α -iodoaziridines and improved conditions for the synthesis of alkyl- α -iodoaziridines using CIMgCHI₂". *Tetrahedron* **2015**, *71*, 4949-4959.

⁹¹ I. Chogij, P. Das, M. D. Delost, M. N. Crawford, J. T. Njardarson. "Asymmetric vinylogous aza-Darzens approach to vinyl aziridines". *Org. Lett.* **2018**, *20*, 4942-4945.

(LiHMDS) en THF como disolvente (Esquema 12). La primera etapa de la reacción tiene lugar a través de una adición selectiva *sin*, rindiendo, de esta forma, las correspondientes *cis* vinil aziridinas **54** y **55**, después del desplazamiento del bromuro. Las iminas aromáticas proporcionaron *cis* aziridinas **54** y **55** con rendimientos superiores a las de sus homólogos de alquiliminas **52**. En cuanto a la diastereoselectividad, las alquiliminas **52** producen *cis* aziridinas **54** y **55** en una proporción de 3:1, mientras que en la mayoría de iminas aromáticas esta proporción es de 1:1 a 3:1.



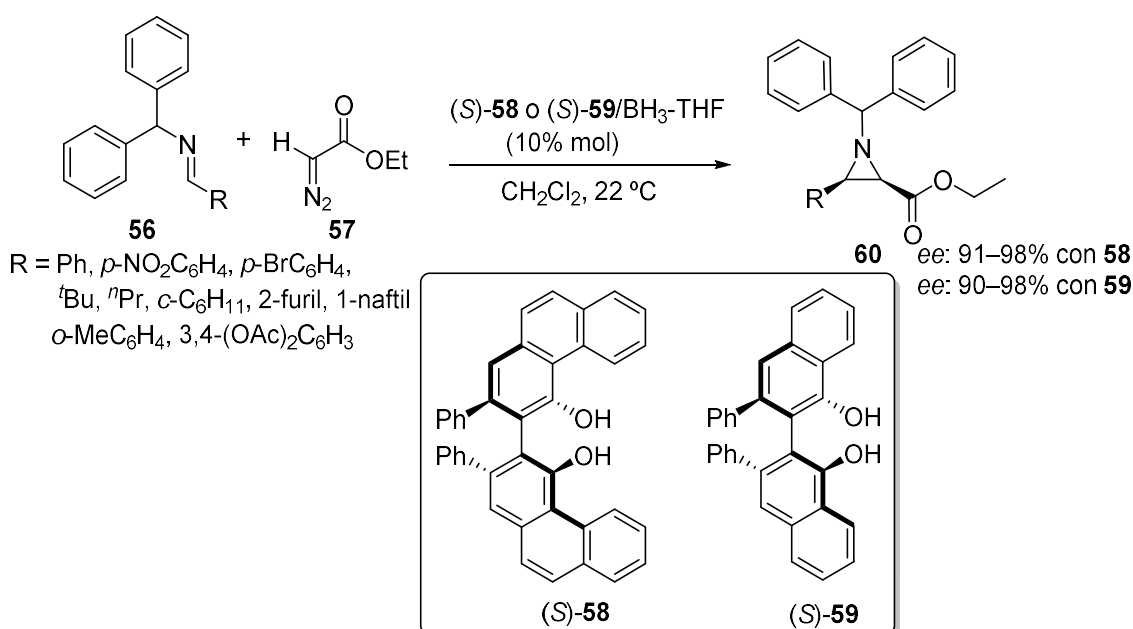
Esquema 12. Síntesis de *cis*-vinil aziridinas mediante la reacción asimétrica viníloga de azarzens.

3.6. Adición de diazo compuestos a iminas activadas.

Hoy en día uno de los métodos más conocidos para la aziridinación, son las reacciones de iminas con carbenoides metálicos, especialmente generados a partir de compuestos α -diazocarbonílicos. Este tipo de reacciones tiene lugar a través de una adición catalizada por un carbeno metálico, mediante un ataque nucleofílico de un diazocompuesto a una imina activada con un ácido de Lewis o Brønsted. A través de estudios cinéticos por efecto isotópico, se ha demostrado que en estas reacciones el paso de formación del enlace C–N en el cierre del anillo

determina las diastereo y enantioselectividades.⁹² Cabe destacar que los complejos más explorados para la aziridinación de iminas catalizada por metales de transición son los complejos de rodio y cobre. Estas reacciones son el resultado del ataque nucleofílico del diazocompuesto a la imina activada, seguido de una ciclación 3-*exo-terc* del intermedio de diazonio.⁹²

Wulff y col., basándose en el uso de ligandos biarilo quirales bifenantrol **58** (VAPOL) y binaftol **59** (VANOL) investigaron la aziridinación enantioselectiva de iminas con diazocompuestos. Estos autores describen la reacción de aziridinación asimétrica de *N*-benzhidril (*N*-Bh) aldimidas **56**, derivadas de aldehídos alifáticos y aromáticos, con diazoacetato de etilo (EDA) **57** mediada por catalizadores quirales preparados a partir de un complejo borano-THF y ligandos VAPOL⁹³ o VANOL^{93b} (Esquema 13).



Esquema 13. Aziridinación asimétrica catalítica mediada por un ácido de boro quiral con ligandos biaril VAPOL y VANOL.

⁹² M. J. Veticatt, A. A. Desai, D. D. Wulff. "Isotope effects and mechanism of the asymmetric borox brønsted acid catalyzed aziridination reaction". *J. Org. Chem.* **2013**, *78*, 5142-5152.

⁹³ a) J. C. Antilla, W. D. Wulff. "Catalytic asymmetric aziridination with a chiral VAPOL-boron Lewis acid". *J. Am. Chem. Soc.* **1999**, *121*, 5099-5100. b) J. C. Antilla, W. D. Wulff. "Catalytic asymmetric aziridination with arylborate catalysts derived from VAPOL and VANOL ligands". *Angew. Chem. Int. Ed.* **2000**, *39*, 4518-4521.

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Las correspondientes *N*-Bh aziridinas **60** se obtuvieron con altos rendimientos y altas inducciones asimétricas de hasta 98% *ee*, favoreciendo la formación de *cis*-aziridinas con alta diastereoselectividad. En algunos casos, se observaron altos rendimientos, así como altas diastereo y enantioselectividades al disminuir la carga del catalizador al 2% mol (Esquema 13). Este proceso catalítico asimétrico fue utilizado por Wulff y col., en la preparación del antibiótico (-)-cloroafenicol,⁹⁴ y en el desarrollo de un agente para el tratamiento de trastornos inmunes e inflamatorios, mediante la síntesis de BIRT-377,⁹⁵ un antagonista de leuointegrina LFA-1.

Por otro lado, Maruoka y col.,⁹⁶ realizan con éxito una reacción de *trans*-aziridinación asimétrica catalítica mediante el empleo de un catalizador ácido de Brønsted quiral **65**, el cual se obtiene por la reacción de un diol quiral **63** y un derivado de ácido boronobenzoico **64**. Este catalizador quiral resulta muy eficiente en la *trans*-aziridinación asimétrica catalítica de *N*-Boc iminas **61** y *N*-fenil-diazoacetamida **62**, para la preparación de *trans*-aziridinas **66** con buenos rendimientos y altas *ee*. Debido a que las *N*-bencil iminas son menos electrófilas y menos reactivas que las *N*-Boc iminas, es necesario variar la acidez del catalizador para mejorar la actividad catalítica sin afectar al entorno quiral. En este caso, el ácido 2-boronobenzoico **64** (R¹ = H) es reemplazado por un ácido 2-boronobenzoico con un grupo electroatractor **64** (R¹ = CF₃) (Esquema 14).

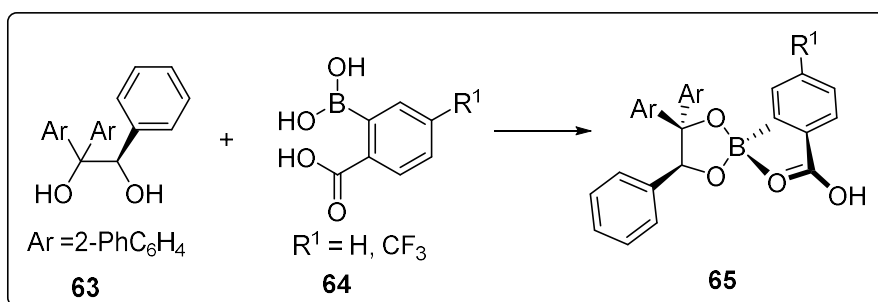
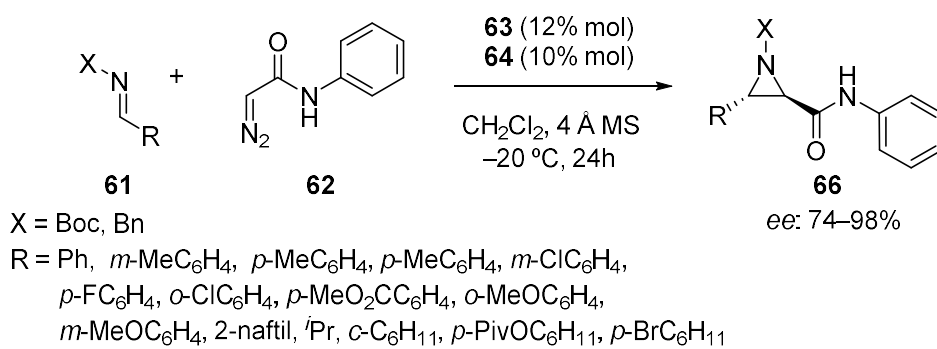
Recientemente, Bew y col.,⁹⁷ han desarrollado la aziridinación multicomponente catalizada por un ácido de Brønsted quiral **70**. Así, el tratamiento de benzaldehído **68** con *o*-terc-butoxianilina **67** y el diazocompuesto **69**, conduce a la correspondiente *N*-aryl *cis*-aziridina **71** con alta enantioselectividad y buen rendimiento (Esquema 15).

⁹⁴ C. Loncaric, W. D. Wulff. "An efficient synthesis of (-)-chloramphenicol via asymmetric catalytic aziridination: a comparison of catalysts prepared from triphenylborate and various linear and vaulted biaryls". *Org. Lett.* **2001**, *3*, 3675-3678.

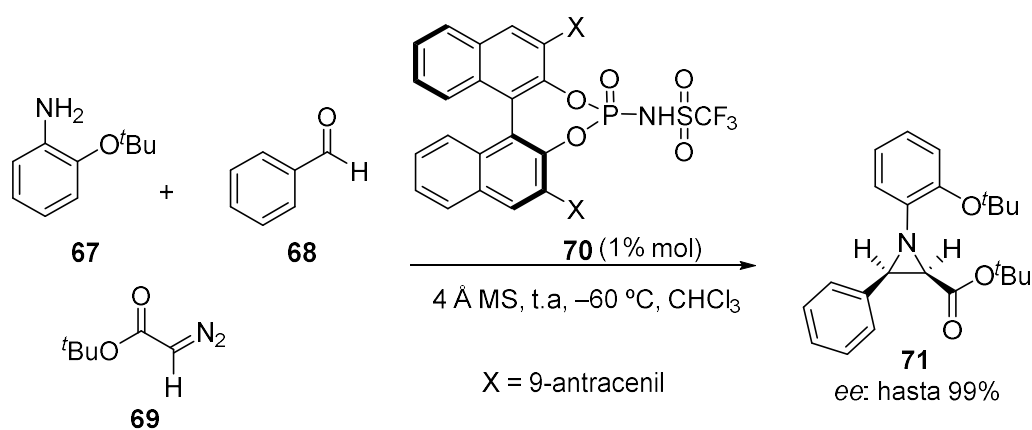
⁹⁵ A. P. Patwardhan, V. R. Pulgam, Y. Zhang, W. D. Wulff. "Highly diastereoselective alkylation of synthesis of LFA-1 antagonist BIRT-377". *Angew. Chem. Int. Ed.* **2005**, *44*, 6169-6172.

⁹⁶ T. Hashimoto, A. Osuna Galvez, K. Maruoka. "In situ assembled boronate ester assisted chiral carboxylic acid catalyzed asymmetric *trans*-aziridinations". *J. Am. Chem. Soc.* **2013**, *135*, 17667-17670.

⁹⁷ S. P. Bew, J. Liddle, D. L. Hughes, P. Pesce, S. M. Thurston. "Chiral Brønsted acid-catalyzed asymmetric synthesis of *N*-aryl-*cis*-aziridine carboxylate esters". *Angew. Chem. Int. Ed.* **2017**, *56*, 5322-5326.



Esquema 14. Síntesis asimétrica catalítica de *trans*-aziridinas mediada por un ácido carboxílico quiral asistido con éster borónico.



Esquema 15. Síntesis asimétrica multicomponente de *N*-aril-*cis*-aziridinas catalizada por un ácido de Brønsted.

3.7. Aziridinación de iminas mediada por iluros de azufre.

Una estrategia directa y eficaz para la síntesis de aziridinas quirales consiste en la aziridinación de iminas mediada por iluro de sulfonio. Esta estrategia se denomina reacción de aza-Corey Chaykovsky,⁹⁸ por analogía con la epoxidación de aldehídos de Corey Chaykovsky, la cual fue desarrollada por los mismos autores. Sin embargo, la aplicación de iluros de azufre enantio-puros en la síntesis asimétrica de aziridinas, desarrollada inicialmente por el grupo de Aggarwal,^{99,100} se ha convertido en una estrategia útil en la aziridinación asimétrica permitiendo un excelente enantiocontrol y buena diastereoselectividad. En el proceso también se pueden emplear iminas quirales, por ejemplo, las sulfiniliminas quirales empleadas en las reacciones con iluros sulfonio¹⁰¹ así como iluros de azufre como auxiliares quirales.¹⁰²

Recientemente, se ha desarrollado una estrategia general para la síntesis diastereoselectiva de aziridinas cuaternarias 2-carboxiladas **74** con rendimientos entre buenos y moderados, así como con excelente diastereoselectividad, mediante la aziridinación de *tert*-butanosulfinil cetimino ésteres **72** usando iluros de azufre **73**¹⁰³ (Esquema 16). Esta misma estrategia se aplicó a la síntesis de espiro-aziridina-oxindoles quirales a través de la reacción aza-Corey-Chaykovsky de *N-tert*-butanosulfinil cetiminas **75** derivadas de isatina,¹⁰⁴ demostrando una excelente selectividad (dr = 98:2 a > 99:1) (Esquema 17).

⁹⁸ E. J. Corey, M. Chaykovsky. "Dimethyloxosulfonium methylide and dimethylsulfonium methylide. Formation and application to organic synthesis". *J. Am. Chem. Soc.* **1965**, *87*, 1353-1364.

⁹⁹ V. K. Aggarwal. "Catalytic asymmetric epoxidation and aziridination mediated by sulfur ylides. Evolution of a project". *Synlett.* **1998**, *4*, 329-336.

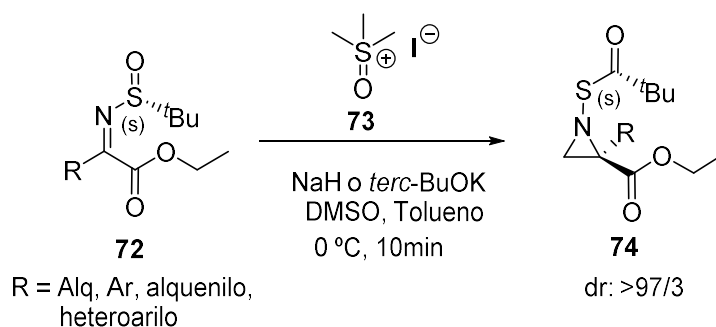
¹⁰⁰ V. K. Aggarwal, A. Thompson, R. V. H. Jones, M. C. H. Standen. "Novel catalytic and asymmetric process for aziridination mediated by sulfur ylides". *J. Org. Chem.* **1996**, *61*, 8368-8369.

¹⁰¹ a) F. A. Davis, P. Zhou, C. H. Liang, R. E. Reddy. "Addition of dimethyloxosulfonium methylide to enantiomerically pure sulfinimines: asymmetric synthesis of 2-substituted aziridines". *Tetrahedron: Asymmetry* **1995**, *6*, 1511-1514. b) J. L. García Ruano, I. Fernández, C. Hamdouchi. "On the reaction of chiral sulfinimines with sulfur ylides: a novel route to the asymmetric aziridination". *Tetrahedron Lett.* **1995**, *36*, 295-298. c) J. L. García Ruano, I. Fernández, M. del Prado Catalina, A. A. Cruz. "Asymmetric aziridination by reaction of chiral *N*-sulfinylimines with sulfur ylides: stereoselectivity improvement by use of *tert*-butylsulfinyl group as chiral auxiliary". *Tetrahedron: Asymmetry* **1996**, *7*, 3407-3414.

¹⁰² a) A. H. Li, L. X. Dai. "Asymmetric ylide reactions: epoxidation, cyclopropanation, aziridination, olefination, and rearrangement". *Chem. Rev.* **1997**, *97*, 2341-2372. b) L. X. Dai, X. L. Hou, Y. G. Zhou. "Stereoselective synthesis of three-membered ring compounds via ylide routes". *Pure Appl. Chem.* **1999**, *71*, 369-376.

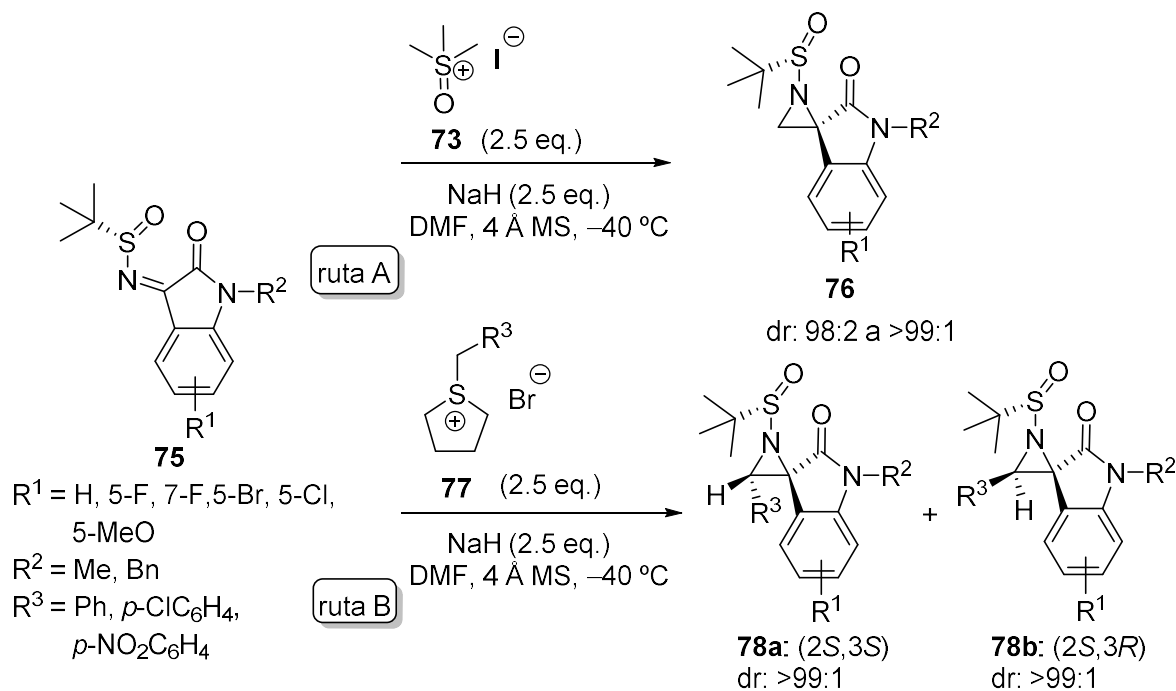
¹⁰³ M. A. Marsini, J. T. Reeves, J. N. Desrosiers, M. A. Herbage, J. Savoie, Z. Li, K. R. Fandrick, C. A. Sader, B. McKibben, D. A. Gao, J. Cui, N. C. Gonnella, H. Lee, X. Wei, F. Roschangar, B. Z. Lu, C. S. Senanayake. "Diastereoselective synthesis of α -quaternary aziridine-2-carboxylates via aza-Corey-Chaykovsky aziridination of *N-tert*-butanesulfinyl ketimino esters". *Org. Lett.* **2015**, *17*, 5614-5617.

¹⁰⁴ S. Hajra, S. M. Aziz, B. Jana, P. Mahish, D. Das. "Synthesis of chiral spiro-aziridine oxindoles via aza-Corey-Chaykovsky reaction of isatin derived *N-tert*-butanesulfinyl ketimines". *Org. Lett.* **2016**, *18*, 532-535.



Esquema 16. Síntesis diastereoselectiva de aziridinas cuaternarias 2-carboxiladas a través de la aziridinación usando iluros de azufre.

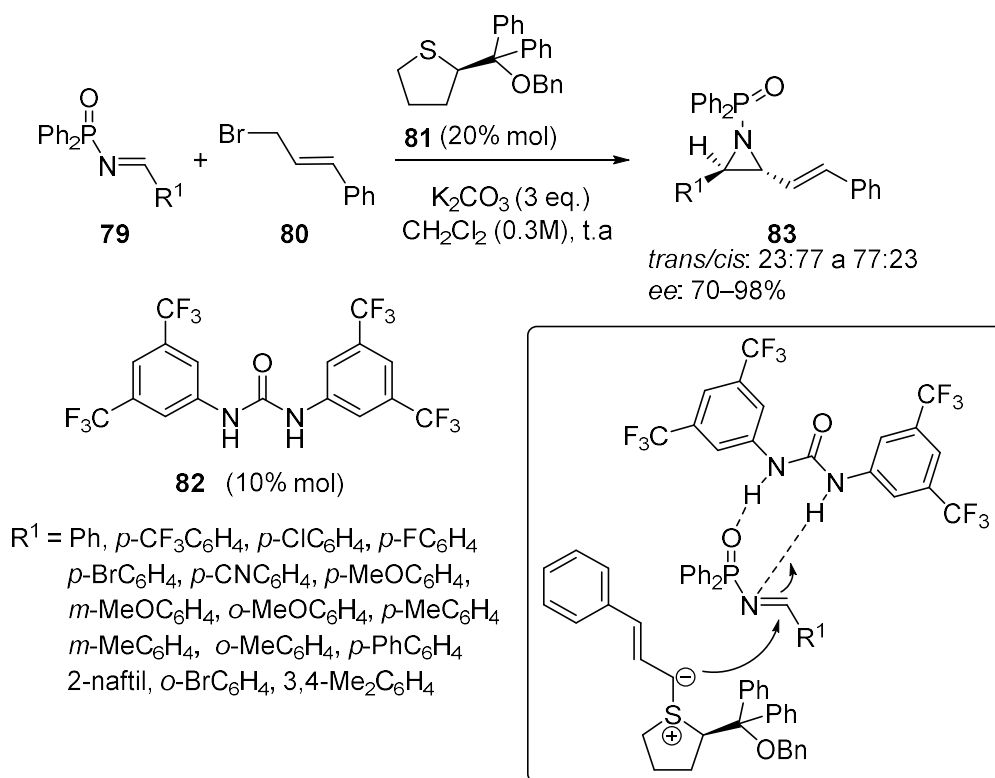
Las *N*-*tert*-butanosulfinil cetiminas **75** reaccionan con iluros de azufre **73** (ruta A), generados a partir de yoduro de trimetil sulfonio e hidruro de sodio, o bromuro de *S*-bencil tetrahidrotiofeno **77** (ruta B) para producir espiro-aziridinas **76** o espiro-aziridina-oxindoles 3-sustituidos **78a** y **78b**, respectivamente, con excelente diastereoselectividad (Esquema 17).



Esquema 17. Síntesis de espiro-aziridina-oxindoles via aza-Corey-Chaykovsky.

Introducción

Una de las rutas para la obtención de aziridinas enantioméricamente puras es la desarrollada recientemente por Chein y col., basada en la síntesis asimétrica de *trans*-aril-cinamyl-aziridinas **83** mediante el empleo de un catalizador dual, basado en el sulfuro quiral **81** y un co-catalizador de urea **82**, como donante de enlaces de hidrógeno (Esquema 18).¹⁰⁵ El uso de un catalizador de tiourea más ácido empeora drásticamente el rendimiento de las aziridinas obtenidas, aunque se obtuvieron excesos enantioméricos muy similares.



Esquema 18. Aziridinación asimétrica mediada por un catalizador dual (iluro de azufre/urea).

3.8. Aziridinación a partir de 2*H*-azirinas.

Las 2*H*-azirinas, descubiertas por Neber en 1932,¹⁰⁶ se han convertido en estructuras que han adquirido gran interés en la síntesis orgánica ya que son el compuesto heterocíclico más

¹⁰⁵ S. H. Wang, R. J. Chein. "(Thiolan-2-yl)diphenylmethyl benzyl ether/*N,N'*-diarylurea cocatalyzed asymmetric aziridination of cinnamyl bromide and aryl aldimine". *Tetrahedron* **2016**, 72, 2607-2615.

¹⁰⁶ W. P. Neber, A. Burgard. "Über den reaktionsverlauf einer neuen art von umlagerung bei ketoximen". *Justus Liebigs Ann. Chem.* **1932**, 493, 281-294.

pequeño y tensionado que posee un átomo de nitrógeno y un doble enlace C–N en un anillo de tres miembros.

Las 2*H*-azirinas sustituidas son compuestos versátiles y se han utilizado para la preparación de diversas aziridinas funcionalizadas sustituidas.^{84a,84c,84d,84e,84f,107,108} La tensión en el anillo, la reactividad de su enlace π , el par de electrones libre del átomo de nitrógeno, la naturaleza del enlace C=N y la labilidad en la rotura de enlaces, hacen que estos compuestos tengan una pronunciada reactividad. Por otro lado, las aziridinas obtenidas en la reacción enantioselectiva de 2*H*-azirinas, también pueden reaccionar con algunos nucleófilos para dar aminas quirales, por lo tanto, las aziridinas son building blocks quirales valiosos en química orgánica.^{61d}

La reactividad más común de las 2*H*-azirinas implica la adición de reactivos nucleófilos al enlace C=N del anillo¹⁰⁹ y como consecuencia de la elevada tensión del heterociclo de tres miembros, el carácter electrófilo del doble enlace C–N es mayor que en el caso de las iminas.

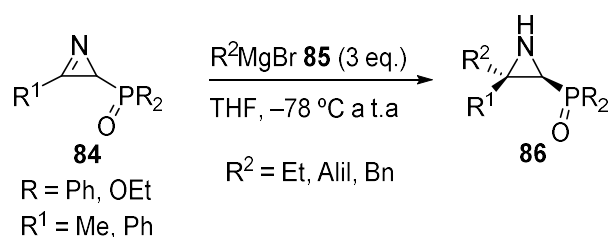
La adición de reactivos de Grignard a 2*H*-azirinas fosforadas proporciona una ruta atractiva para la preparación de aziridinas,^{84e} revelando que éstas se forman por ataque nucleofílico por la cara menos impedida, de una manera similar a lo que ocurre para otros nucleófilos como los hidruros,^{84a} también descrito por otros autores.¹¹⁰ Esta metodología ha sido ampliamente utilizada por nuestro grupo de investigación para la síntesis de *trans*-aziridinas fosforadas funcionalizadas **86** de forma diastereoselectiva, mediante la adición nucleófila de organomagnesianos **85** a 2*H*-azirinas derivadas de óxido de fosfina (R = Ph) y fosfonatos (R = OEt) **84** (Esquema 19).

¹⁰⁷ Para revisiones de 2*H*-azirinas ver: a) F. Palacios, A. M. Ochoa de Retana, E. M. de Marigorta, J. M. de los Santos. "2*H*-Azirines as synthetic tools in organic chemistry". *Eur. J. Org. Chem.* **2001**, *13*, 2401-2414. b) A. F. Khlebnikov, M. S. Novikov. "Recent advances in 2*H*-azirine chemistry". *Tetrahedron* **2013**, *69*, 3363-3401. c) A. F. Khlebnikov, M. S. Novikov, N. V. Rostovskii. "Advances in 2*H*-azirine chemistry: a seven-year update". *Tetrahedron* **2019**, *75*, 2555-2624.

¹⁰⁸ F. Palacios, A. M. Ochoa de Retana, E. M. de Marigorta, J. M. de los Santos. "Preparation, properties and synthetic applications of 2*H*-azirines". *Org. Prep. Proc. Int.* **2002**, *34*, 219-269.

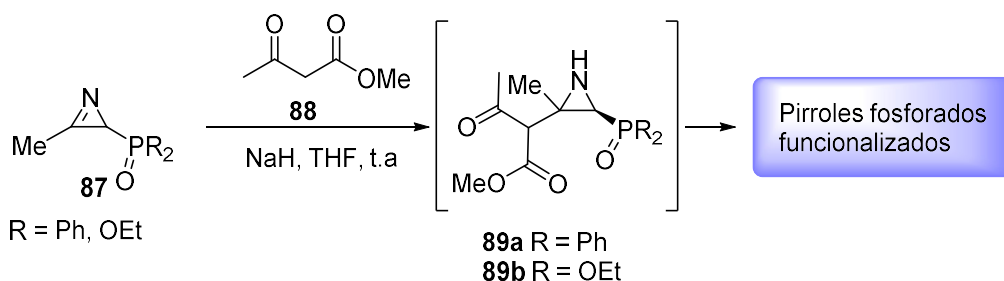
¹⁰⁹ a) T. L. Gilchrist. "Activated 2*H*-azirines as dienophiles and electrophiles". *Aldrichimica Acta* **2001**, *34*, 51-55. b) T. M. V. D. Pinho e Melo, A. M. d'A. R. Gonsalves. "Exploiting 2-halo-2*H*-azirine chemistry". *Curr. Org. Synth.* **2004**, *1*, 275-292.

¹¹⁰ R. M. Carlson, S. Y. Lee "Olefin synthesis via the stereospecific deamination of aziridines". *Tetrahedron Lett.* **1969**, *10*, 4001-4004.



Esquema 19. Adición diastereoselectiva de organomagnesianos a 2H-azirinas fosforadas.

Otro ejemplo, desarrollado por nuestro grupo de investigación, consiste en la formación de aziridinas sustituidas funcionalizadas como intermedios sintéticos para la formación de pirroles fosforados funcionalizados mediante la adición nucleófila de enolatos derivados de β -cetoésteres **88** a 2H-azirinas derivadas de óxido de fosfina (R = Ph) y fosfonato (R = OEt) **87**.^{84g} La reacción de las correspondientes 2H-azirinas **87** con el enolato derivado del β -cetoéster **88** en presencia de NaH y THF a temperatura ambiente permitió obtener las aziridinas derivadas de óxido de difenilfosfina **89a** (R = Ph), así como las aziridinas derivadas de fosfonato **89b** (R = OEt) (Esquema 20).

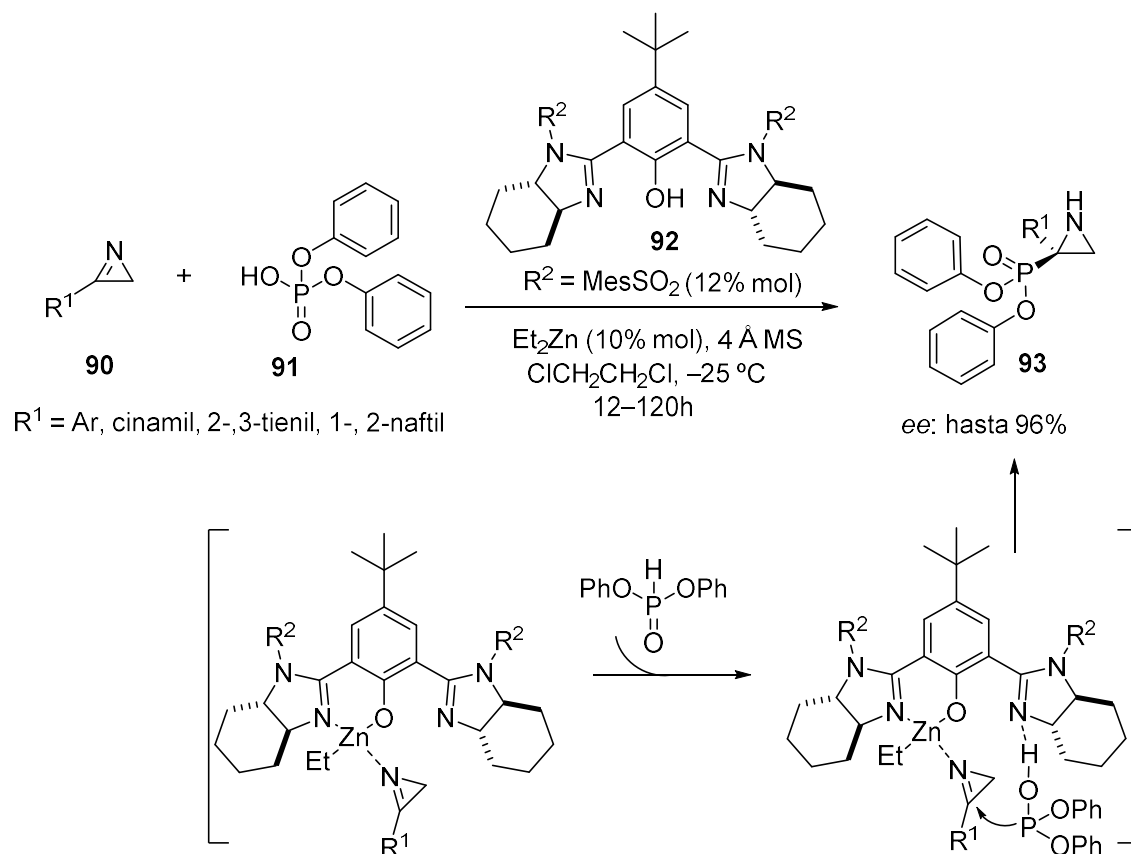


Esquema 20. Adición nucleófila de enolatos derivados de β -cetoésteres a 2H-azirinas-2-fosforadas.

Otra metodología sintética de especial interés, desarrollada a lo largo de estos últimos años para la preparación de aziridinas quirales, consiste en la primera adición nucleófila enantioselectiva de fosfitos a 2H-azirinas.¹¹¹ Para ello, se han empleado diversas 2H-azirinas sustituidas **90** que reaccionan con el fosfito **91** en presencia de catalizadores quirales de Zn

¹¹¹ S. Nakamura, D. Hayama. "Enantioselective reaction of 2H-azirines with phosphite using chiral bis(imidazoline)/zinc (II) catalysts". *Angew. Chem. Int. Ed.* **2017**, 56, 8785-8789.

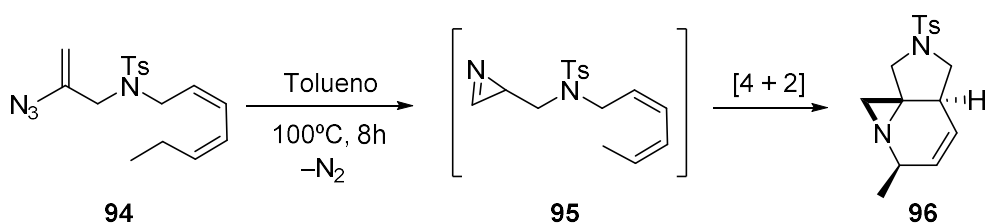
II/bis(imidazolina) **92**, permitiendo el acceso a aziridinas **93** con elevadas enantioselectividades y buenos rendimientos (Esquema 21).



Esquema 21. Adición enantioselectiva de fosfito a 2H-azirinas mediada por un catalizador quiral de Zn II/bis(imidazolina)

Xu y col.,¹¹² llevaron a cabo la primera reacción intramolecular aza-Diels-Alder de 2H-azirinas con un sistema dieno en su estructura, desarrollando una metodología sintética de gran sencillez para la síntesis estereoselectiva de compuestos tricíclicos nitrogenados con un anillo de aziridina fusionado. Por ejemplo, la vinil azida **94** compuesta por un dieno, tras sufrir una descomposición térmica genera una aziridina intermedia **95** que, tras ser sometida a una ciclación intramolecular *endo* [4 + 2] proporciona un núcleo tricíclico *trans*-fusionado **96** con excelente estereoselectividad (Esquema 22).

¹¹² H. D. Xu, H. Zhou, Y. P. Pan, X. T. Ren, H. Wu, M. Han, R. Z. Han, M. H. Shen. "Stereo-selective synthesis of polycycles containing an aziridine group: intramolecular aza-Diels-Alder reactions of unactivated 2H-azirines with unactivated dienes". *Angew. Chem. Int. Ed.* **2016**, *55*, 2540-2544.



Esquema 22. Síntesis del compuesto tricíclico nitrogenado **108** mediante la reacción estereoselectiva de aza-Diels Alder.

3.9. Síntesis de aziridinas mediante reacciones de sustitución intramolecular.

Otra de las rutas versátiles y eficientes para preparar aziridinas quirales está basada en la ciclación S_N2 de 1,2-aminoalcoholes, 1,2-amino haluros, 1,2-acidoalcoholes, 1,2-aminosulfuros o epóxidos. En la síntesis de aziridinas empleando 1,2 aminoalcoholes como precursores, la ciclación se puede llevar a cabo con facilidad cuando el grupo funcional hidroxilo se convierte en el nucleófilo. La reacción de desplazamiento nucleofílico intramolecular, da lugar al anillo de aziridina ya sea por un anión amida o por el par solitario de electrones de la amina.¹¹³

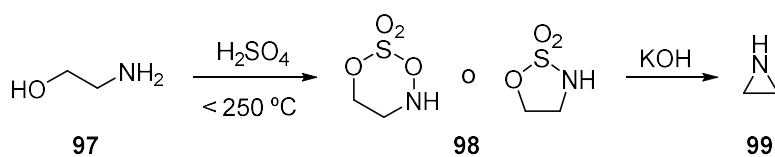
Aunque se han explorado muchos enfoques, la reacción de Wenker,¹¹⁴ basada en la ciclación intramolecular de 1,2-haloaminas o aminoalcoholes vecinales, es una estrategia útil para preparar *NH*-aziridinas estructuralmente diversas.¹¹⁵ Fue en 1935 cuando Wenker demostró que al calentar a elevada temperatura etanolamina **97** en presencia de ácido sulfúrico, se generaba un intermedio de sulfamidato cíclico **98**. La destilación de este intermedio a partir de una base acuosa condujo a la correspondiente aziridina **99** (Esquema 23).

La síntesis de Wenker ha sido desarrollada y mejorada con el paso de los años, aunque para una amplia gama de aminoalcoholes estas condiciones generales de reacción no eran aplicables, ya que proporcionaban mezclas de productos de ciclación y de eliminación cuando el aminoalcohol presenta sustituyentes en la posición α al grupo hidroxilo.

¹¹³ H. M. I. Osborn, J. Sweeney. "The asymmetric synthesis of aziridines". *Tetrahedron: Asymmetry* **1997**, *8*, 1693-1715.

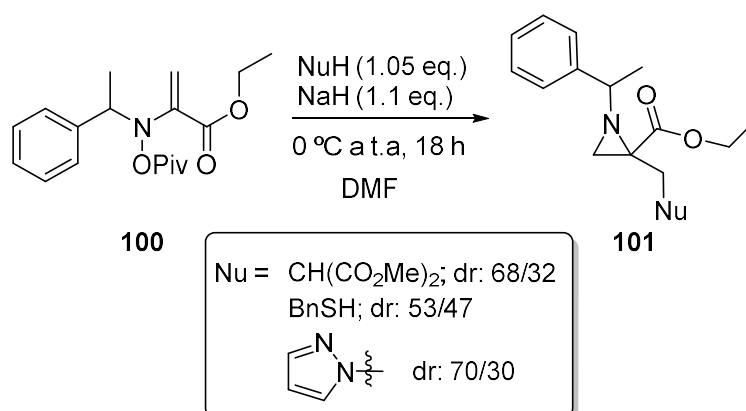
¹¹⁴ H. Wenker. "The preparation of ethylene imine from monoethanolamine". *J. Am. Chem. Soc.* **1935**, *57*, 2328-2328.

¹¹⁵ J. Xu. "A new and expeditious asymmetric synthesis of (*R*)- and (*S*)-2-aminoalkanesulfonic acids from chiral amino alcohols". *Tetrahedron: Asymmetry* **2002**, *13*, 1129-1134.



Esquema 23. Primera reacción de ciclación intramolecular descrita por Wenker para la síntesis de aziridinas.

Beaudegnies y col.,¹¹⁶ desarrollaron una estrategia sintética de cierre de anillo inducida por reacción de Michael para la obtención de aziridinas-2-carboxiladas-2-sustituidas **101**, a partir de un sustrato **100** (Esquema 24). Se sintetizaron una gran variedad de aziridinas **101** a través del empleo de un amplio espectro de reactivos nucleófilos. Además, la utilización de un sustrato quiral hizo que la reacción fuera diastereoselectiva en las condiciones apropiadas.

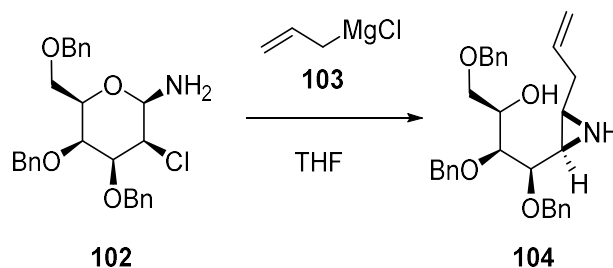


Esquema 24. Síntesis de aziridinas-2-carboxiladas-2-sustituidas a través de una estrategia de cierre de anillo inducida por Michael.

Otra metodología desarrollada recientemente para obtener aziridinas, está basada en la ciclación intramolecular altamente diastereoselectiva de la cloroamina **102** derivada de glicales

¹¹⁶ E. Pietrasiak, G. Schade, M. Baalouch, D. Emery, R. Beaudegnies. "Synthesis of 2-substituted aziridine-2-carboxylic esters via Michael-induced ring-closure strategy". *Synlett*. **2017**, 28, 2115-2120.

(Esquema 25).¹¹⁷ El tratamiento de **102** derivada de D-galactal con bromuro de alilmagnesio **103** proporcionó la aziridina **104**.



Esquema 25. Síntesis de aziridinas quirales mediante ciclación intramolecular a partir de glicales.

4. Reactividad de aziridinas

En estos últimos años, y como consecuencia de sus sorprendentes propiedades químicas, los estudios sobre la síntesis y la reactividad de las aziridinas han sido objeto de numerosos estudios para desarrollar nuevas transformaciones de estos heterociclos. La elevada energía de deformación asociada con el anillo de aziridina promueve una alta reactividad, lo que permite una fácil escisión del enlace C-N. Por lo tanto, las aziridinas pueden sufrir reacciones de apertura de anillo¹¹⁸ con una gran variedad de nucleófilos (carbonados, nitrogenados, oxigenados, azufrados...) de forma esterocontrolada, carbonilación¹¹⁹ o reacciones de cicloadición^{61a,120} con dipolarófilos, proporcionando acceso a una amplia gama de compuestos heterocíclicos de 4 a 11 miembros.

¹¹⁷ R. Thakur, G. K. Rawal, Y. D. Vankar. "Synthesis of chiral aziridines from glycols: application in the synthesis of a piperidine-azepine fused derivative". *Eur. J. Org. Chem.* **2017**, *29*, 4235-4241.

¹¹⁸ a) R. Chawla, A. K. Singh, L. D. S. Yadav. "Organocatalysis in synthesis and reactions of epoxides and aziridines". *RSC Adv.* **2013**, *3*, 11385-11403. b) M. K. Ghorai, D. P. Tiwari, N. Jain. "Lewis acid catalyzed S_N2-type ring opening of N-activated aziridines with electron rich arenes/heteroarenes". *J. Org. Chem.* **2013**, *78*, 7121-7130.

¹¹⁹ P. Davoli, I. Moretti, F. Prati, H. Alper. "Carbonylation of silylated hydroxymethyl aziridines to β -lactams". *J. Org. Chem.* **1999**, *64*, 518-521.

¹²⁰ a) E. A. Ilardi, J. T. Njardarson. "Ring expansions of vinyloxiranes, -thiiranes, and -aziridines: synthetic approaches, challenges, and catalytic success stories". *J. Org. Chem.* **2013**, *78*, 9533-9540. b) G. S. Singh, S. Sudheesh, N. Keroletsw. "Recent applications of aziridine ring expansion reactions in heterocyclic synthesis". *Arkivoc* **2018**, *1*, 50-113.

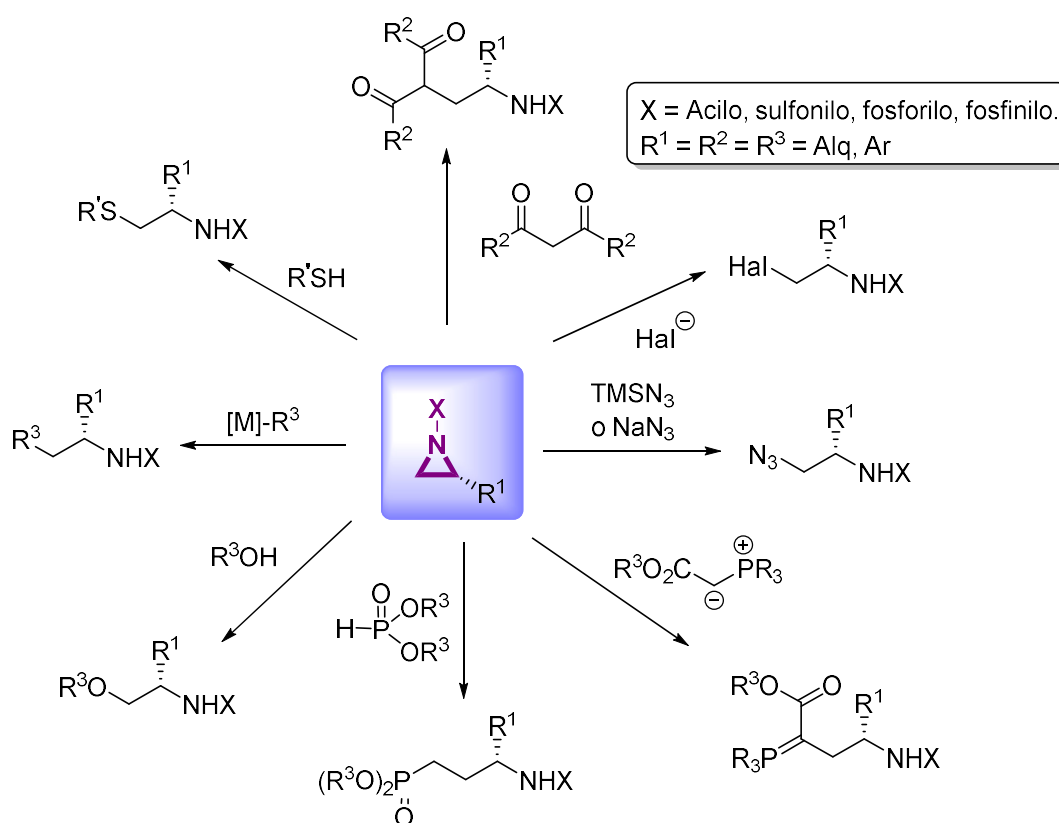
4.1. Reacciones de apertura de anillo de aziridinas mediante el empleo de nucleófilos.

La apertura del anillo de aziridina es una reacción ampliamente estudiada, a partir de la cual se han generado gran cantidad de compuestos orgánicos funcionalizados que no son fácilmente accesibles por otros métodos. La combinación de la tensión de Baeyer inherente al heterociclo de 3 miembros y la electronegatividad del heteroátomo que polariza los enlaces del anillo, hace que las aziridinas puedan sufrir reacciones de escisión en condiciones relativamente suaves. Sin embargo, las reacciones de apertura del anillo en aziridinas presentan una mayor dificultad que las correspondientes reacciones en los epóxidos. Aun así, existen una gran cantidad de ejemplos correspondientes a dicha reactividad, como se describe en el esquema 26. La eficacia de estas reacciones depende en gran medida de la naturaleza de los sustituyentes, del nucleófilo, así como de las condiciones de reacción empleadas.^{7,121} Como se ha mencionado anteriormente, el anillo de aziridina presenta una elevada reactividad como consecuencia de la elevada tensión y como resultado, varios nucleófilos^{121d} reaccionan fácilmente con aziridinas produciendo la apertura del anillo y proporcionando gran variedad de compuestos 1,2-difuncionalizados de forma regio y estereoselectiva.

Otro aspecto de gran importancia es la regioselectividad, tanto el enlace C–N como el enlace C–C del anillo pueden sufrir la escisión, y va a depender en gran medida de los sustituyentes presentes en el anillo aziridínico.^{6a,122} Por lo general, los grupos electroattractores favorecen la escisión del enlace C–N y, por el contrario, la presencia de grupos electrodonores favorecen la escisión del enlace C–C. Además de los efectos electrónicos, el ataque nucleofílico en el anillo también está gobernado por factores estéricos.^{6a}

¹²¹ a) M. Pineschi. "Asymmetric ring-opening of epoxides and aziridines with carbon nucleophiles". *Eur. J. Org. Chem.* **2006**, 4979-4988. b) J. Y. Wang, Y. Hu, D. X. Wang, J. Pan, Z. T. Huang, M. X. Wang. "Unprecedented carbon-carbon bond cleavage in nucleophilic aziridine ring opening reaction, efficient ring transformation of aziridines to imidazolidin-4-ones". *Chem. Commun.* **2009**, 4, 422-424. c) A. T. Matha, M. S. Valle, P. Tercinier, P. Dauban, R. H. Dodd. "Enantiospecific synthesis of a protected equivalent of APTO, the β -amino acid fragment of microsclerodermins C and D, by aziridino- γ -lactone methodology". *Eur. J. Org. Chem.* **2009**, 673-686. d) A. M. Costero, S. Gil, M. Parra, P. Rodríguez. "Unexplored nucleophilic ring opening of aziridines". *Molecules* **2010**, 15, 9135-9144.

¹²² a) P. Lu. "Recent developments in regioselective ring opening of aziridines". *Tetrahedron* **2010**, 66, 2549-2560. b) C. S. Eskesen, P. R. Hansen, A. Kjaer, N. Gillings. "Efficient regioselective ring opening of activated aziridine-2-carboxylates with [18^f]fluoride". *Chemistry Open* **2015**, 4, 65-71.

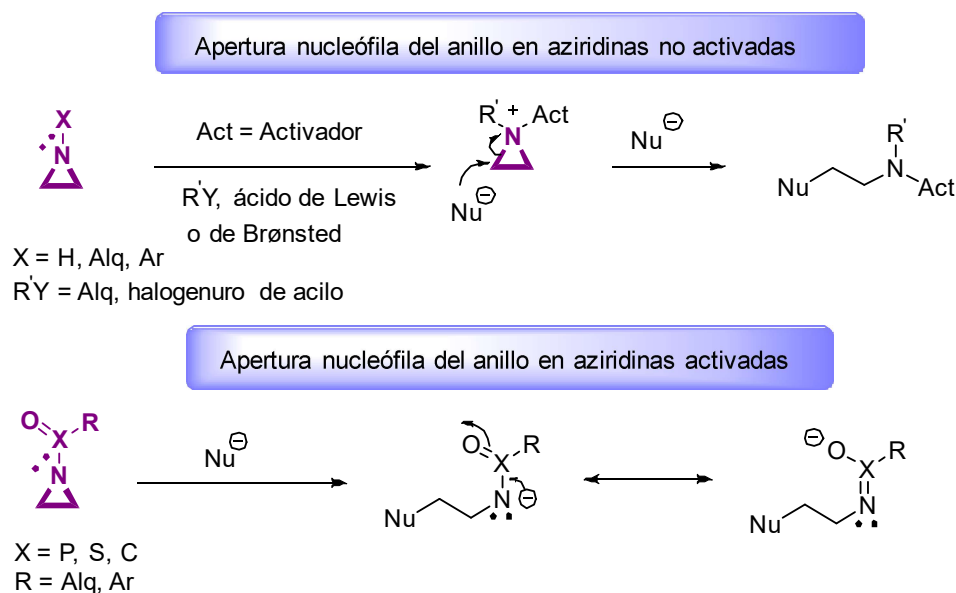


Esquema 26. Esquema general de reacciones de apertura de anillo en aziridinas.

Desde la década de 1960, las aziridinas se han clasificado como “activadas” o “no activadas” en función de si las reacciones de apertura de anillo se producen o no en ausencia de una carga positiva en el nitrógeno;¹²³ esta clasificación está relacionada con la naturaleza del sustituyente en el átomo de nitrógeno. Por lo general, las aziridinas no activadas tienen un átomo de nitrógeno básico. Las *N*-aril, *N*-alquil o *N*-H aziridinas son consideradas no activadas en la reacción de apertura de anillo (Esquema 27). Generalmente, la apertura de anillo en estos casos tiene lugar después de la protonación, cuaternización, o a través de la formación de un aducto de ácido de Lewis. Sin embargo, las aziridinas activadas son capaces de estabilizar conjugativamente y/o inductivamente la carga negativa que se produce en el átomo de nitrógeno como consecuencia del ataque nucleófilo. Por lo tanto, el grupo activador está, a

¹²³ G. E. Ham. “Activated aziridines. I. Reaction of anilines with *O*-ethyl-*N,N*-ethyleneurethan. Mechanism and Hammett ρ -constant”. *J. Org. Chem.* **1964**, *29*, 3052-3055.

menudo, formado por sustituyentes oxigenados, tales como grupos sulfonilo, sulfinilo, fosforilo, fosfinilo o carbonilo (Esquema 27).



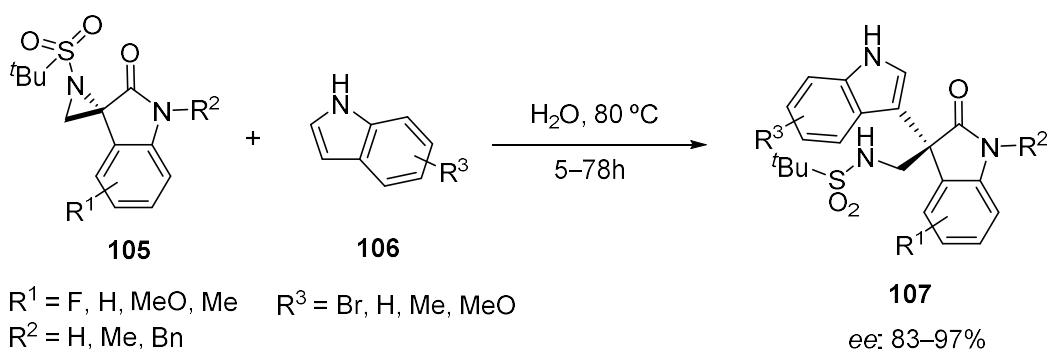
Esquema 27. Apertura nucleófila del anillo en aziridinas no activadas y activadas.

Existe una gran cantidad de bibliografía a cerca de la apertura del anillo tanto de aziridinas activadas como no activadas con nucleófilos de carbono, nitrógeno, azufre y haluro. Entre los nucleófilos carbonados, los más utilizados son arenos, heteroarenos, arinos, nitrilos, ésteres, aldehídos, índoles y compuestos organometálicos, entre otros.¹²⁴

Recientemente, Hajra y col.,¹²⁵ han descrito la primera reacción esterocontrolada de formación de enlaces C–C mediante la apertura del anillo de *N-terc*-butanosulfonil aziridina en espiro aziridina-2-oxindoles quirales **105**, a través del empleo de índoles **106** como nucleófilos. La reacción se lleva a cabo en presencia de agua y sin catalizador, obteniendo rendimientos de buenos a excelentes (Esquema 28). La reacción tiene lugar de forma regio y estereoespecífica dando lugar a 3,3'-bisindoles enantiopuros **107** con retención de la configuración (Esquema 28).

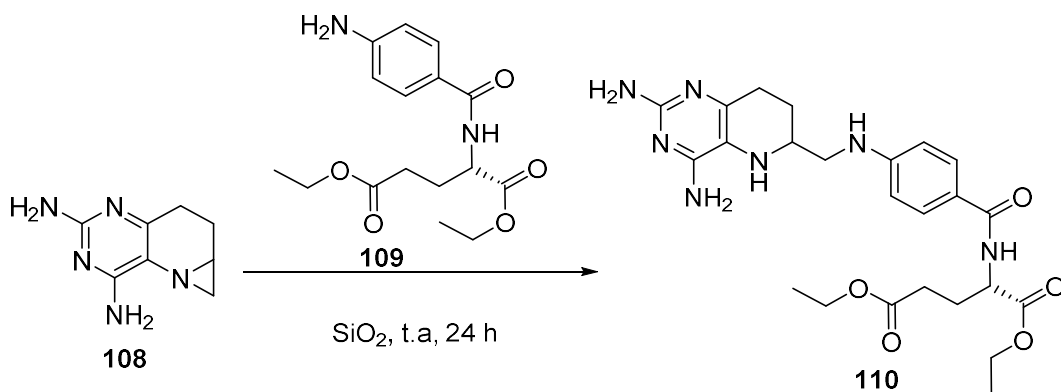
¹²⁴ R. Akhtar, S. A. R. Naqvi, A. F. Zahoor, S. Saleem. "Nucleophilic ring opening reactions of aziridines". *Mol. Divers.* **2018**, *22*, 447-501.

¹²⁵ S. Hajra, S. S. Roy, S. k. M. Aziz, D. Das. "Catalyst-free "on-water" regio- and stereospecific ring-opening of spiroaziridine oxindole: enantiopure synthesis of unsymmetrical 3,3'-bisindoles". *Org. Lett.* **2017**, *19*, 4082-4085.



Esquema 28. Síntesis enantioselectiva de 3,3'-bisindoles asimétricos en presencia de agua.

Entre los nucleófilos nitrogenados más utilizados en las reacciones de apertura de anillo de las aziridinas destacan las aminas y las azidas. Uno de los métodos más eficientes para la construcción del esqueleto de tetrahydrofolatos, compuestos biológicamente activos como inhibidores de metionina sintasa y dihydrofolato reductasa,¹²⁶ consiste en la apertura nucleófila del anillo de una aziridina en el compuesto tricíclico **108**.¹²⁷ El acoplamiento de la amina **109** usando gel de sílice como catalizador y sin disolvente, permite obtener el producto final **110** con un 90% de rendimiento (Esquema 29).

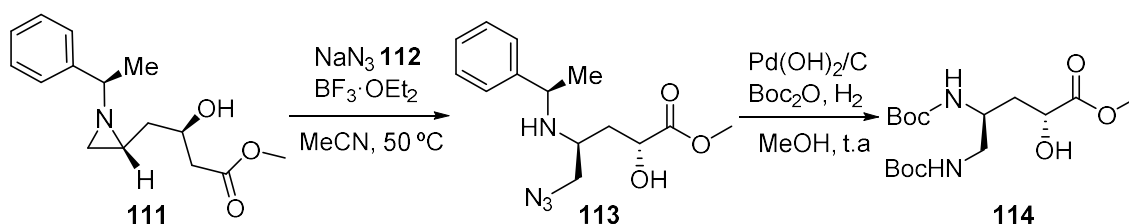


Esquema 29. Síntesis del esqueleto de tetrahydrofolato a través de la apertura de aziridinas.

¹²⁶ C. Tang, Z. L. Zhang, B. Xu, M. Li, J. Y. Liu, J. R. Cui. "Two newly synthesized 5-methyltetrahydrofolate-like compounds inhibit methionine synthase activity accompanied by cell cycle arrest in G1/S phase and apoptosis *in vitro*". *Anticancer Drugs* **2008**, *19*, 697-704.

¹²⁷ S. Zhou, C. Tian, C. Li, Y. Guo, X. Wang, J. Liu, Z. Zhang. "Novel synthesis of 8-deaza-5,6,7,8-tetrahydroaminopterin analogues via an aziridine intermediate". *Molecules* **2012**, *17*, 5604-5614.

Por otro lado, la apertura regioselectiva del anillo de aziridina **111** utilizando como nucleófilo nitrogenado la azida de sodio **112**, permitió la síntesis asimétrica del ácido 3-hidroxi-4,5-diaminopentanoico **114**, fragmento del antibiótico edeína D.¹²⁸ Una vez que se produce la apertura del anillo de aziridina, la hidrogenación catalítica con Pd/C del compuesto **113** en presencia de Boc₂O da lugar a la formación de **114** (Esquema 30).



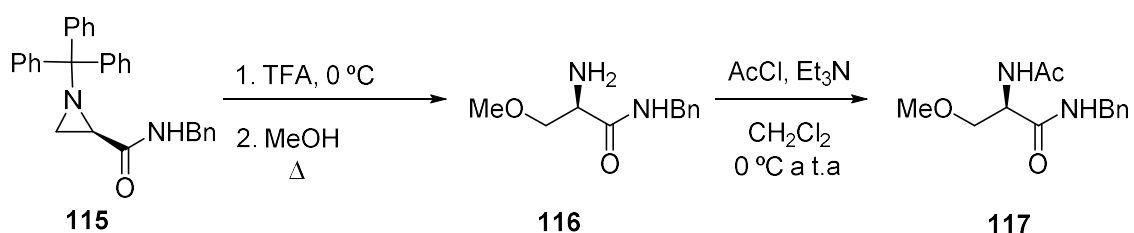
Esquema 30. Síntesis asimétrica regioselectiva de un fragmento del antibiótico edeína D mediante el empleo de azida de sodio.

En general, las aziridinas muestran una menor reactividad con nucleófilos oxigenados. Por ello, la apertura del anillo de aziridinas depende de la activación en el nitrógeno del anillo, bien sea mediante el empleo de grupos electroattractores o mediante el empleo de ácidos de Lewis apropiados en la adición nucleofílica del reactivo oxigenado. Además, las aziridinas quirales con sustituyente carboxamida pueden actuar como building blocks para la síntesis de compuestos biológicamente activos, como en el caso de lacosamida,¹²⁹ un fármaco antiepiléptico de bajo peso molecular. Existen algunos métodos que explican su síntesis, siendo uno de ellos el que aparece representado en el esquema 31, donde el tratamiento de *N*-tritolaziridin-2-carboxamida **115** con una mezcla de ácido trifluoroacético (TFA)/MeOH, tras la apertura del anillo de aziridina por el ataque nucleófilo del metanol, proporciona la amina primaria **116** con un buen rendimiento (75%). Posteriormente la acetilación de **116** dio (*S*)-lacosamina **117** con un buen rendimiento (87%) (Esquema 31).¹³⁰

¹²⁸ H. Mao, H. Jiong, J. Yang, H. J. Ha, J. W. Yang. "Preparation of chiral contiguous epoxyaziridines and their regioselective ring-opening for drug syntheses". *Chem. Eur. J.* **2018**, *24*, 2370-2374.

¹²⁹ T. Söth, H. J. Kupferberg, J. P. Stables, D. Choi, R. H. Harris, H. Kohnf, N. Waltong, H. S. White. "Lacosamide, a novel anti-convulsant drug, shows efficacy with a wide safety margin in rodent models for epilepsy". *Epilepsy Res.* **2007**, *74*, 147-154.

¹³⁰ S. Jarzyński, S. Leśniak, M. Rachwalski. "Synthesis of enantiomerically pure 2-(*N*-aryl, *N*-alkyl-aminomethyl)aziridines: a new class of ligands for highly enantioselective asymmetric synthesis". *Tetrahedron: Asymmetry* **2017**, *28*, 1808-1816.



Esquema 31. Síntesis de (S)-lacosamida a través de la apertura nucleófila de N-tritilaziridin-2-carboxamida.

La reacción de apertura de anillo de aziridinas con nucleófilos azufrados, como tioles, conduce a la formación de tioéteres. Los β -aminosulfuros pueden ser materiales de gran interés para la síntesis de moléculas biológicamente activas como, por ejemplo, aminoácidos,²⁴ alcaloides¹³¹ y heterociclos.¹³² Estas reacciones de apertura en presencia de tioles pueden ocurrir tanto en aziridinas activadas como no activadas. En el caso de aziridinas no activadas, el nitrógeno puede utilizarse como base para extraer un protón tanto del tiofenol como del alquilfenol, formándose un intermedio aziridínico muy lábil. Por lo tanto, el nucleófilo del anión tiol desprotonado ataca generalmente al carbono menos impedido del anillo de aziridina. Un ejemplo de ello, es la apertura del anillo de NH-aziridinas **118** utilizando tioles y tiofenoles en dióxido de azufre líquido¹³³ (Esquema 32), que además de ser el medio de reacción puede utilizarse como ácido de Lewis. Los β -aminosulfuros **119** fueron obtenidos con buenos rendimientos y una excelente β -regioselectividad.

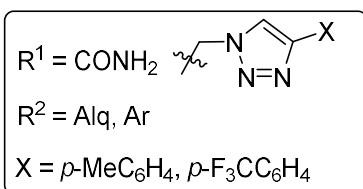
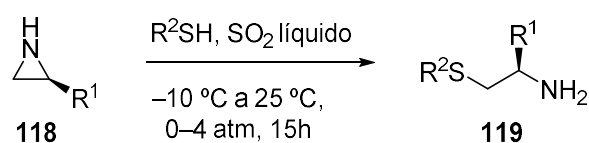
Por el contrario, en el caso de las aziridinas activadas es necesario un ácido de Lewis. Los nucleófilos de azufre se acercan por la cara menos impedida para provocar la apertura del anillo. La primera reacción de apertura de anillo de N-arilsulfonil-2-fenilaziridinas **120** con ariltioamidas **121** catalizada por ZnCl₂ condujo a la formación de amidinas **126**¹³⁴ (Esquema 33).

¹³¹ T. Hudlicky, H. Juna, J. D. Proce, F. Rulin. "Microbial oxidation of chloroaromatics in the enantiodivergent synthesis of pyrrolizidine alkaloids: trihydroxyheliotridanes". *J. Org. Chem.* **1990**, *55*, 4683-4687.

¹³² A. Dureault, I. Tranchepain, J. C. Depezay. "Nucleophilic opening of chiral bis(aziridines): a route to enantiomerically pure α -amino aldehydes or acids and polysubstituted piperidines". *J. Org. Chem.* **1989**, *54*, 5324-5330.

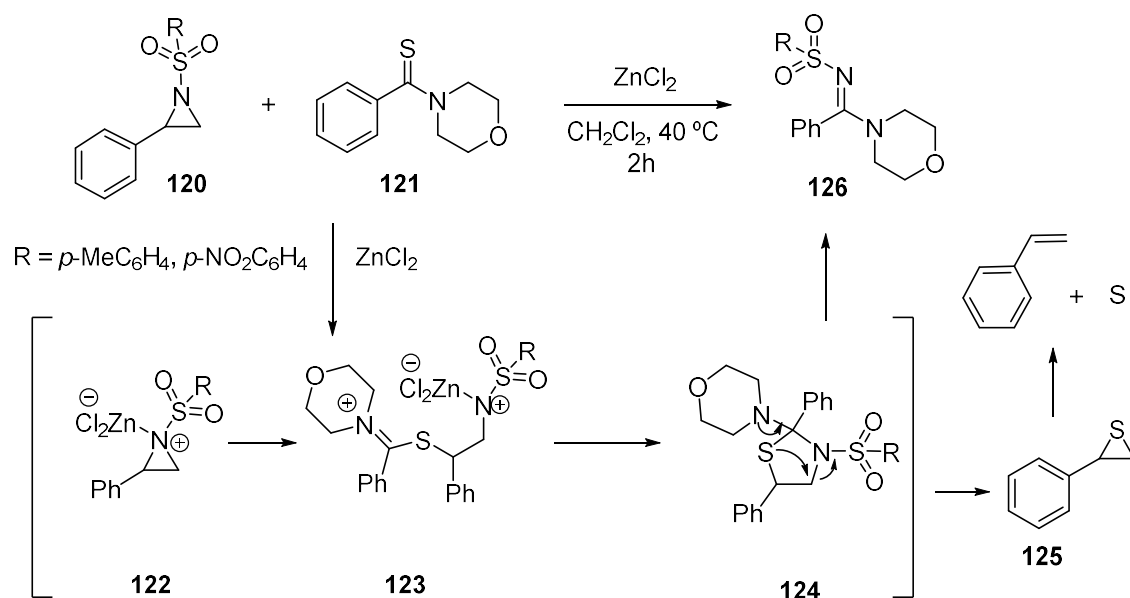
¹³³ J. Luginina, M. Turks. "Regioselective ring opening of N-H-aziridines with sulfur nucleophiles in liquid SO₂". *Synlett.* **2017**, *28*, 939-943.

¹³⁴ K. Hajibabaei, H. Z. Boeini. "Zinc chloride catalyzed ring opening of N-arylsulfonyl aziridines by thioamides: a new approach to the synthesis of amidines". *Synlett.* **2014**, *25*, 2044-2048.



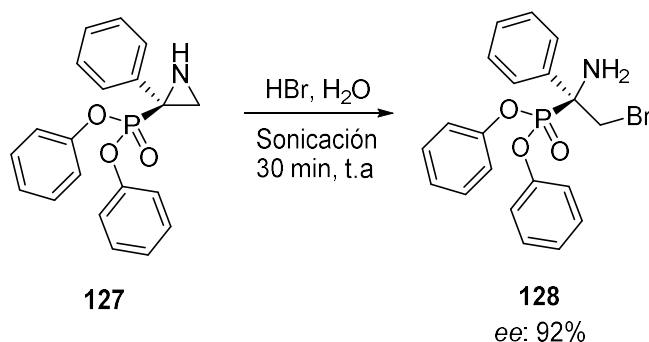
Esquema 32. Apertura regioselectiva de NH-aziridinas con tioles.

El mecanismo de la reacción (Esquema 33), implica en primer lugar la activación de las aziridinas **120** con ZnCl_2 . Seguidamente se produce el ataque nucleófilo al carbono bencílico del intermedio **122** con la posterior ciclación del intermedio resultante **123**, formándose las correspondientes tiazolidinas **124**, que tras la eliminación de una molécula inestable de 2-feniltiirano **125** se transforman en amidinas **126** (Esquema 33).



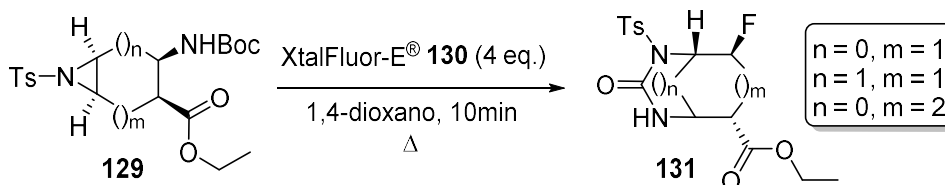
Esquema 33. Síntesis de amidinas mediante apertura nucleófila del anillo de *N*-arilsulfonyl aziridinas por reacción con tioamidas.

Otro de los reactivos nucleófilos utilizados en la apertura del anillo de aziridinas son los halógenos. Así, el tratamiento de la aziridina **127** con bromuro de hidrógeno en agua y bajo condiciones de ultrasonidos conduce al α -amino- β -bromo fosfonato **128** (Esquema 34).¹¹¹



Esquema 34. Síntesis de α -aminofosfonatos mediante la apertura del anillo de aziridinas fosforadas con HBr.

Uno de los retos sintéticos más novedosos en la actualidad, implica la introducción de flúor en una estructura orgánica. Una estrategia desarrollada por Fülöp y col¹³⁵ ha permitido la introducción selectiva de flúor en el esqueleto de un aminociclopentano o ciclohexano carboxilato. Ésta implica la novedosa y eficiente apertura de un anillo de aziridina activado, mediante fluoruro empleando tetrafluoroborato de *N,N*-dietil-*S,S*-difluorosulfiliminio **130** (XtalFluor-E)[®]. La reacción procedió a través de la apertura regioselectiva del anillo de aziridina en **129** con (XtalFluor-E)[®] con la asistencia del grupo vecino de la sulfonamida, lo que proporcionó derivados de diaminoácidos fluorados **131** (Esquema 35). Este método ha sido generalizado para acceder a sustancias fluoradas mono o bicíclicas.



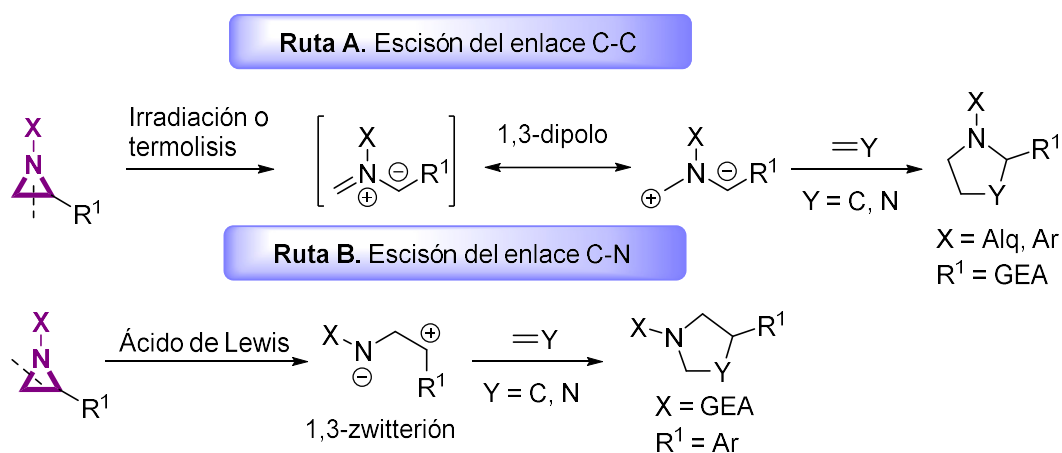
Esquema 35. Síntesis de diaminoácidos fluorados mediante la introducción selectiva de fluor en un esqueleto bicíclico carboxilado.

¹³⁵ M. Nonn, L. Kiss, M. Haukka, S. Fustero, F. Fülöp. "A novel and selective fluoride opening of aziridines by XtalFluor-E. Synthesis of fluorinated diamino acid derivatives". *Org. Lett.* **2015**, *17*, 1074-1077.

4.2 Reacciones de expansión de anillo de aziridinas.

Como se ha observado en el apartado anterior, la química basada en la apertura del anillo de aziridinas se ha empleado para una amplia gama de aplicaciones sintéticas,^{122a,136} y por ello, las aziridinas son intermediarios versátiles para la preparación de una gran variedad de compuestos nitrogenados estructuralmente diversos.²⁴ En cuanto a la tensión del anillo de aziridina, ésta ha facilitado que las aziridinas se utilicen como 1,3-dipolos enmascarados, siempre mediante la escisión del enlace C–N o C–C en distintos tipos de reacciones y de esta manera, poder acceder a diversos compuestos heterocíclicos.

Las aziridinas, tras irradiación o termólisis, se someten a una apertura de anillo electrocíclico a través de la escisión del enlace C–C para dar iluros de azometino, y así, pueden participar en cicloadiciones 1,3-dipolares para dar heterociclos nitrogenados de 5 miembros (ruta A, Esquema 36).¹³⁷ Sin embargo, en presencia de un ácido de Lewis, las aziridinas con sustituyentes electroattractores en el átomo de nitrógeno experimentan la escisión del enlace C–N para generar 1,3-dipolos zwitteriónicos que reaccionan con alquenos, cetonas, aldehídos, nitrilos, alquinos y heterocumulenos (isocianatos, isotiocianatos...), proporcionando cicloaductos 1,3-dipolares formales (ruta B, Esquema 36).



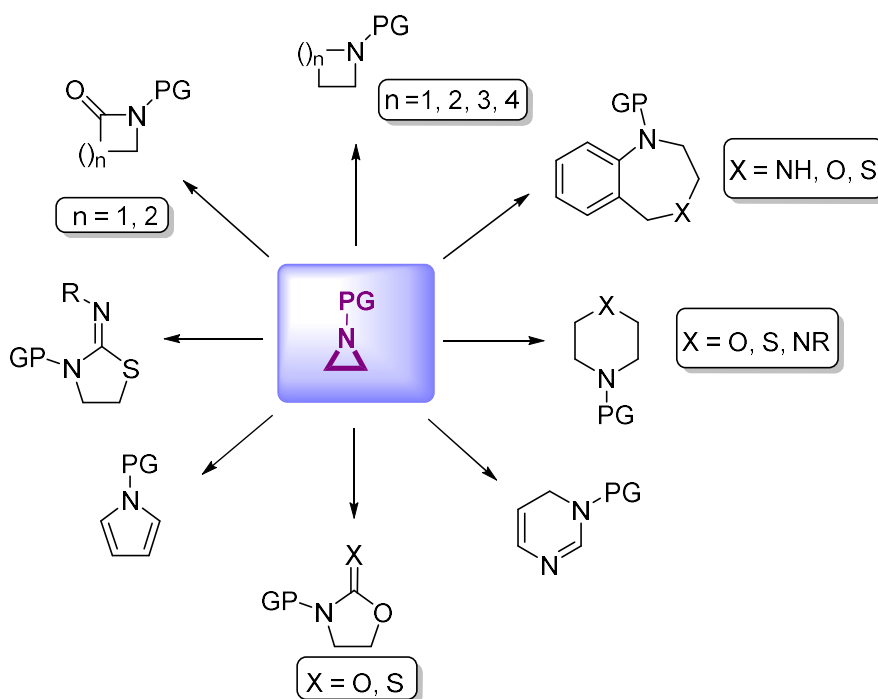
Esquema 36. Reactividad de aziridinas con dipolarófilos mediante la escisión del enlace C–C o C–N.

¹³⁶ S. H. Krake, S. C. Bergmeier. "Inter- and intramolecular reactions of epoxides and aziridines with π -nucleophiles." *Tetrahedron* **2010**, *66*, 7337-7360.

¹³⁷ G. S. Singh, M. D'hooghe, N. De Kimpe. "Synthesis and reactivity of C-heteroatom-substituted aziridines". *Chem. Rev.* **2007**, *107*, 2080–2135.

Introducción

En la mayoría de los estudios que aparecen en la literatura se describe la transformación de aziridinas en heterociclos de 4 a 7 miembros, como, por ejemplo, azetidinas, 2-azetidionas, pirroles, imidazoles, oxazoles, pirimidinas, pirazinas, oxazinas, morfolinas, tiomorfolinas, azepanos y benzodizepinas, entre otros (Esquema 37).



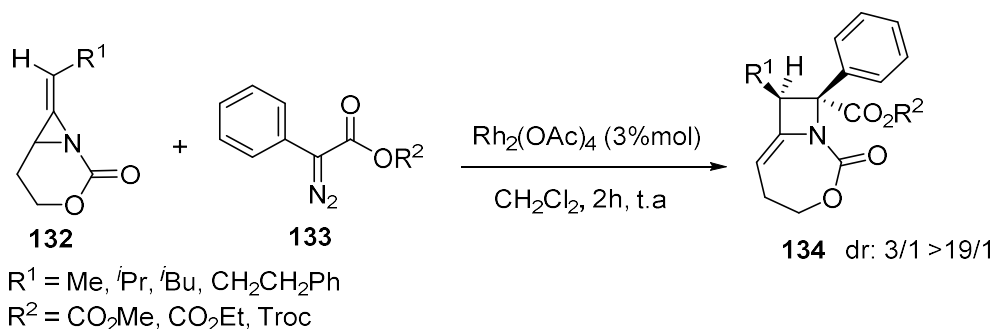
Esquema 37. Síntesis de compuestos heterocíclicos mediante la expansión del anillo en aziridinas.

Las azetidinas¹³⁸ constituyen una clase de compuestos que predominan en varios productos naturales y de síntesis, los cuales exhiben una amplia gama de actividades biológicas. Como consecuencia del difícil acceso a este tipo de heterociclos a partir de anillos de 3 miembros, en la literatura existen pocos ejemplos para este tipo de transformaciones. Un ejemplo de este tipo de transformación, es el desarrollado en 2017 por Schomaker y col.,¹³⁹ basada en la síntesis de metilén azetidinas sustituidas **134** a partir de metilén aziridinas bicíclicas **132** (Esquema 38).

¹³⁸ V. Mehra, I. Lumb, A. Anand, V. Kumar. "Recent advances in synthetic facets of immensely reactive azetidines". *RSC Adv.* **2017**, 7, 45763-45783.

¹³⁹ S. C. Schmid, I. A. Guzei, M. Schomaker. "A stereoselective [3+1] ring expansion for the synthesis of highly substituted methylene azetidines". *Angew. Chem. Int. Ed.* **2017**, 56, 12229-12233.

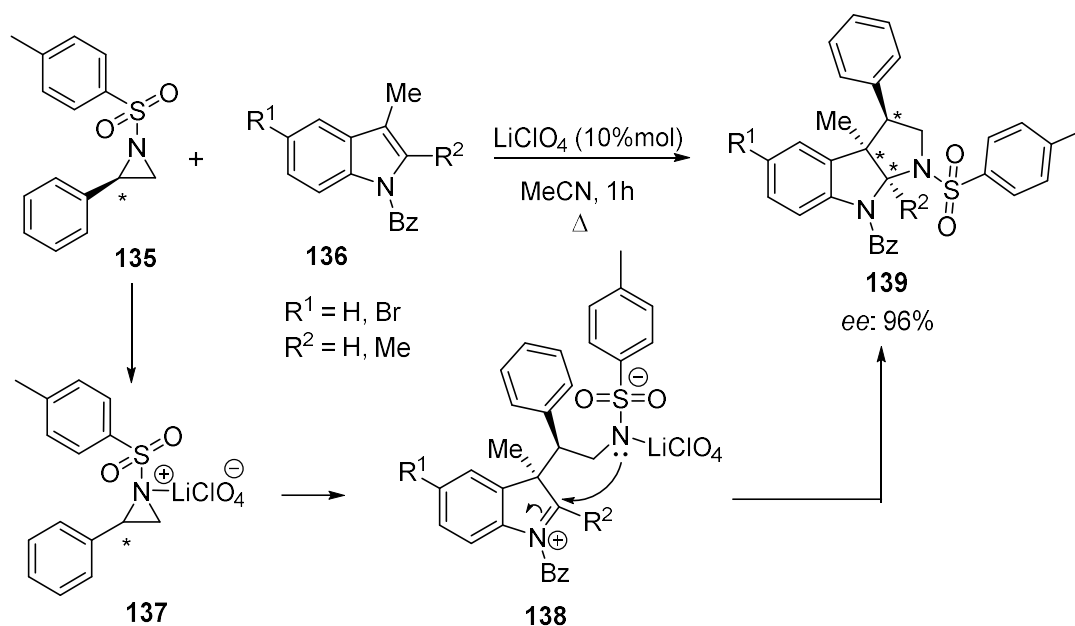
La reacción de carbenoides de rodio, formados a partir de α -diazooésteres **133** con metilen aziridinas **132**, provoca la expansión [3+1] en el anillo de aziridina, obteniendo azetidinas tri y tetrasustituidas **134** con excelente regio y estereoselectividad (Esquema 38). Estos autores proponen que la reacción transcurre a través de un mecanismo tipo iluro, donde tanto la estructura, como la tensión de la aziridina de partida **132**, promueven una cascada de apertura de anillo/ciclación, transfiriendo la quiralidad del sustrato al producto.



Esquema 38. Síntesis estereoselectiva de metilen azetidinas altamente sustituidas mediante la expansión de anillo [3+1] en metilen aziridinas.

Como iluros de azometino enmascarados, las aziridinas son sintones bien conocidos para la síntesis de pirrolidinas, imidazolininas, tiazolininas, oxazolininas y 2,3-dihidrobenzofuranos a través de cicloadiciones [3+2]. Recientemente, se ha desarrollado una ruta sintética *one pot* para acceder a pirrolidinas fusionadas con un anillo de indolina mediante una apertura de anillo de aziridinas activadas **135** seguida de la ciclación con indoles sustituidos en la posición C-3 **136**, así como en otras posiciones (Esquema 39).¹⁴⁰ Los hexahidropirrolo[2,3-*b*]indoles **139** se forman como consecuencia de una apertura de anillo de tipo S_N2 catalizada por el ácido de Lewis LiClO₄. La coordinación del ácido de Lewis con el nitrógeno del anillo de aziridina, genera un intermedio **137** muy reactivo que sufre una adición nucleófila del indol, a través de su posición C-3, generando un ion iminio **138**. Finalmente, **138** da lugar al producto final **139** a través de un ataque nucleófilo intramolecular (Esquema 39).

¹⁴⁰ A. Mal, M. Sayyad, I. A. Wani, M. K. Ghorai. "Domino ring-opening cyclization of activated aziridines with indoles: synthesis of chiral hexahydropyrroloindoles". *J. Org. Chem.* **2017**, *82*, 4-11.



Esquema 39. Síntesis enantioselectiva de hexahidropirrolo[2,3-*b*]indoles quirales catalizada por un ácido de Lewis.

Por otro lado, las imidazolidinas o también conocidas como tetrahydroimidazoles, son heterociclos que presentan una gran variedad de bioactividades tales como, analgésicos, antiinflamatorios, agonistas del receptor adrenérgico, antimicrobianos o anticonvulsivos, entre otras.¹⁴¹

La cicloadición [3+2] de aziridinas con heterocumulenos proporciona un enfoque interesante para la síntesis de heterociclos nitrogenados de 5 miembros.¹⁴² Por ejemplo, se han sintetizado

¹⁴¹ a) V. Sharma, M. S. Y. Khan. "Synthesis of novel tetrahydroimidazole derivatives and studies for their biological properties". *Eur. J. Med. Chem.* **2001**, *36*, 651-658. b) M. C. Caterina, I. A. Perillo, L. Boiani, H. Pezaroglo, H. Cerecetto, M. Gonzalez, A. Salerno. "Imidazolidines as new anti-trypanosoma cruzi agents: biological evaluation and structure-activity relationships". *Bioorg. Med. Chem.* **2008**, *16*, 2226-2234. c) J. K. de A. L. Neves, S. P. S. Botelho, C. M. L. de Melo, V. R. A. Pereira, M. C. A. Lima, I. da Rocha Pitta, M. C. P. A. Albuquerque, S. L. Galdino. "Biological and immunological activity of new imidazolidines against adult worms of *Schistosoma mansoni*". *Parasitol. Res.* **2010**, *107*, 531-538. d) R. J. Gleave, D. S. Walter, P. J. Beswick, E. Fonfria, A. D. Michel, S. A. Roman, S. P. Tang. "Synthesis and biological activity of a series of tetrasubstituted-imidazoles as P2X₇ antagonists". *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4951-4954.

¹⁴² a) A. Okano, S. Oishi, T. Tanaka, N. Fujii, H. Ohno. "Construction of linked nitrogen heterocycles by palladium (0)-catalyzed intramolecular domino cyclization of 2-alkynylaziridines bearing a 2-aminoethyl group via ring expansion with isocyanate". *J. Org. Chem.* **2010**, *75*, 3396-3400. b) A. F. G. Goldberg, N. R. O'Connor, R. A. Craig, B. M. Stoltz. "Lewis acid mediated (3+ 2) cycloadditions of donor-acceptor cyclopropanes with heterocumulenes." *Org. Lett.* **2012**, *14*, 5314-5317. c) T. Jeong, S. Han,

trans-imidazolidin-2-iones **142** por reacción regio y estereoselectiva de *trans*-aziridinas-2-carboxiladas **140** con diferentes isotiocianatos **141** (Esquema 40).¹⁴³

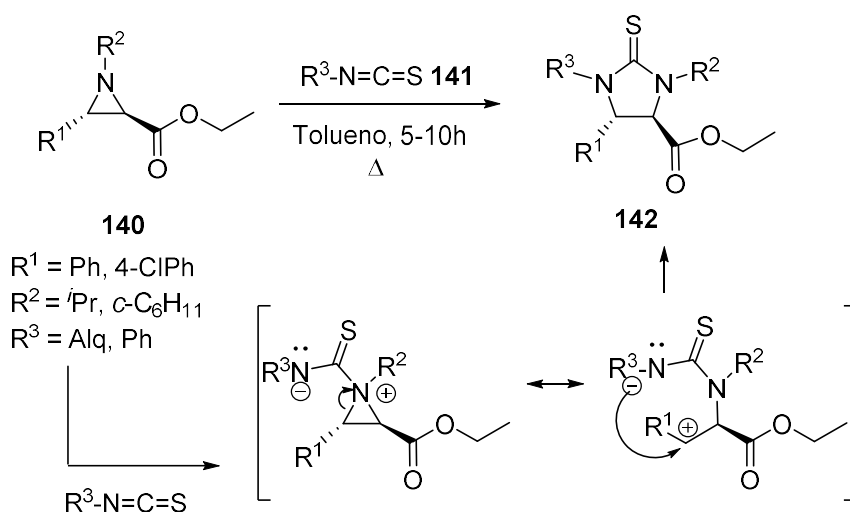
Dentro de los heterociclos de cinco miembros, otro grupo de compuestos importantes son las oxazolin-2-onas, las cuales encuentran gran aplicación tanto en la industria farmacéutica como química. Las oxazolidinonas están consideradas como farmacóforos importantes presentes en gran variedad de compuestos con la finalidad obtener diversas propiedades farmacológicas.¹⁴⁴ Entre las últimas revisiones en la literatura, una de las más destacables hace referencia a la síntesis regio y diastereoselectiva de *trans*-2-hidroximetil-*N*-alquil-1,3-oxazolidin-2-onas **145** a partir de *trans*-aziridinas-2-carboxiladas.¹⁴⁵ Estos últimos compuestos son transformados inicialmente en 2-hidroximetilaziridinas **143** por reducción con LiAlH₄, que en presencia de cloroformiato de metilo **144**, sufren la apertura del anillo y posterior ciclación (Esquema 41).

N. K. Mishra, S. Sharma, S. Y. Lee, J. S. Oh, J. H. Kwak, Y. H. Jung, I. S. Kim. "Rh(III)-catalyzed C–H amidation of indoles with isocyanates". *J. Org. Chem.* **2015**, *80*, 7243-7250. d) T. Jeong, S. H. Lee, N. K. Mishra, U. De, J. Park, P. Dey, J. H. Kwak, Y. H. Jung, H. S. Kim, I. S. Kim. "Synthesis and cytotoxic evaluation of *N*-aroylureas through rhodium (III)-catalyzed C–H functionalization of indolines with isocyanates". *Adv. Synth. Catal.* **2017**, *359*, 2329–2336.

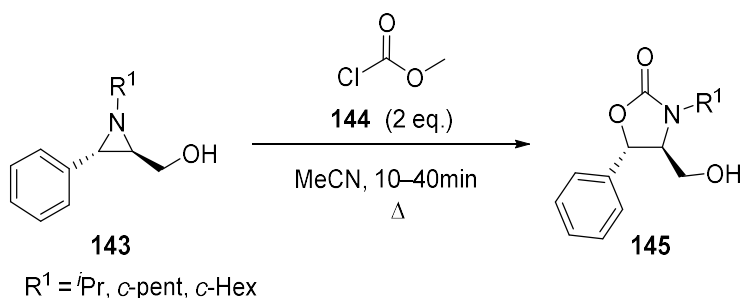
¹⁴³ M. A. Tabarki, R. Besbes. "Selective synthesis of imidazolidine-2-thiones via ring expansion of aziridine-2-carboxylates with isothiocyanates". *Tetrahedron Lett.* **2016**, *57*, 3832-3836.

¹⁴⁴ a) A. Spaulding, K. Takroui, P. Mahalingam, D. C. Cleary, H. D. Cooper, P. Zucchi, W. Tear, B. Koleva, P. J. Beuning, E. B. Hirsch, J. B. Aggen. "Compound design guidelines for evading the efflux and permeation barriers of *escherichia coli* with the oxazolidinone class of antibacterials: test case for a general approach to improving whole cell gram-negative activity". *Bioorg. Med. Chem. Lett.* **2017**, *27*, 5310-5321. b) A. M. Siddiqui, J. A. Sattigeri, K. Javed, S. Shafi, M. Shamim, S. Singhal, Z. M. Malik. "Design, synthesis and biological evaluation of spiropyrimidinetriones oxazolidinone derivatives as antibacterial agents". *Bioorg. Med. Chem. Lett.* **2018**, *28*, 1198-1206.

¹⁴⁵ O. Ouerfelli, M. A. Tabarki, J. Pytkowicz, Y. Arfaoui, T. Brigaud, R. Besbes. "Stereoselective synthesis of 4-hydroxymethyl-1,3-oxazolidin-2-one derivatives from novel 2-hydroxymethylaziridines". *Synth. Commun.* **2018**, *48*, 2242-2252.



Esquema 40. Síntesis estereoselectiva de *trans*-imidazolidin-2-tionas a través de la expansión de anillo de aziridinas-2-carboxiladas con isotiocianatos.

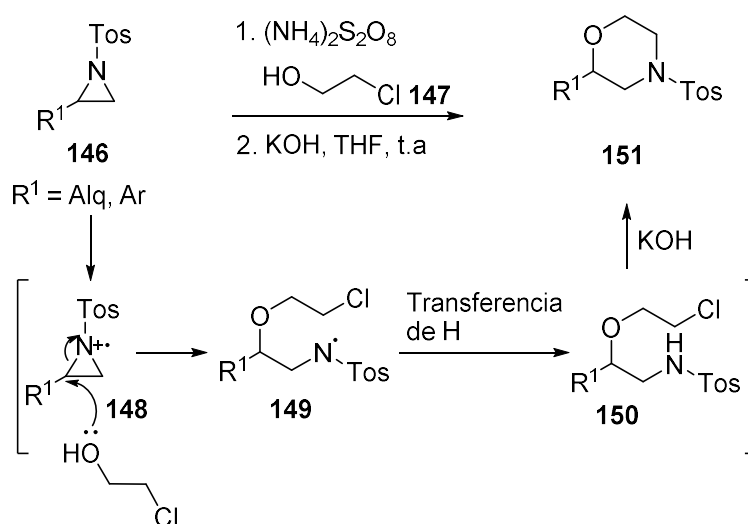


Esquema 41. Síntesis estereoselectiva de *trans*-2-hidroximetil-*N*-alquil-1,3-oxazolidin-2-onas a partir de 2 hidroximetilaziridinas.

A través del empleo de aziridinas como materiales de partida se han sintetizado una gran variedad de heterociclos de 6 miembros, entre los que se encuentran piperidinas, isoquinolinas, pirazinas, morfolinas y pirimidinas entre otros. Xia y col.,¹⁴⁶ desarrollaron una metodología *one-pot* sencilla para el acceso a 2-morfolinas sustituidas **151** y 2,3-morfolinas disustituidas a partir de *N*-tosil-2-arylaziridinas sustituidas **146**. Como oxidante, se ha empleado persulfato de amonio para producir la apertura del anillo de aziridina con 2-cloroetanol **147** (Esquema 42).

¹⁴⁶ H. Sun, B. Huang, R. Lin, C. Yang, W. Xia. "Metal-free *one-pot* synthesis of 2-substituted and 2,3-disubstituted morpholines from aziridines". *Beilstein J. Org. Chem.* **2015**, *11*, 524-529.

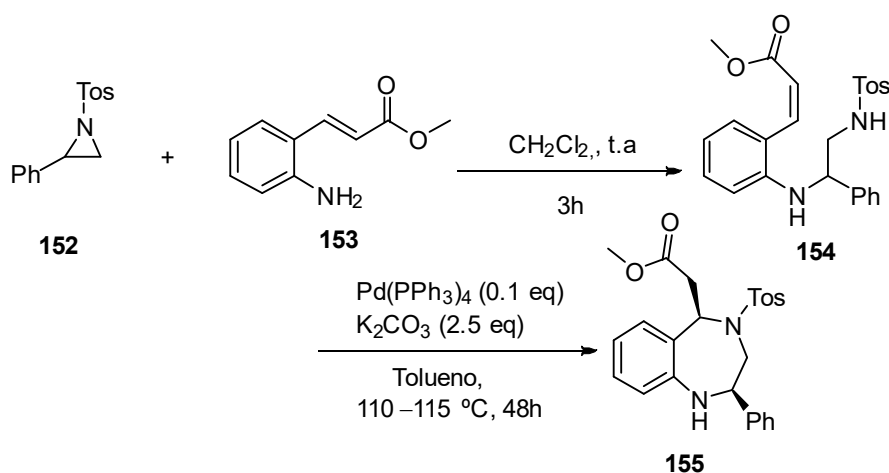
Inicialmente, la aziridina **146** podría participar en la transferencia de un solo electrón con el anión persulfato para producir el catión radical **148**. La apertura concertada del anillo y la adición nucleófila conducen al radical amino intermedio **149**. Posteriormente mediante la abstracción de un átomo de hidrógeno del alcohol, el radical amino **149** se convierte en un cloroamina alcoxi intermedio **150**, que tras la posterior ciclación en presencia de KOH permite obtener las correspondientes morfolinás 2-sustituídas **151** (Esquema 42).



Esquema 42. Síntesis *one pot* de morfolinás 2-sustituídas mediante el empleo de persulfato de amonio como oxidante.

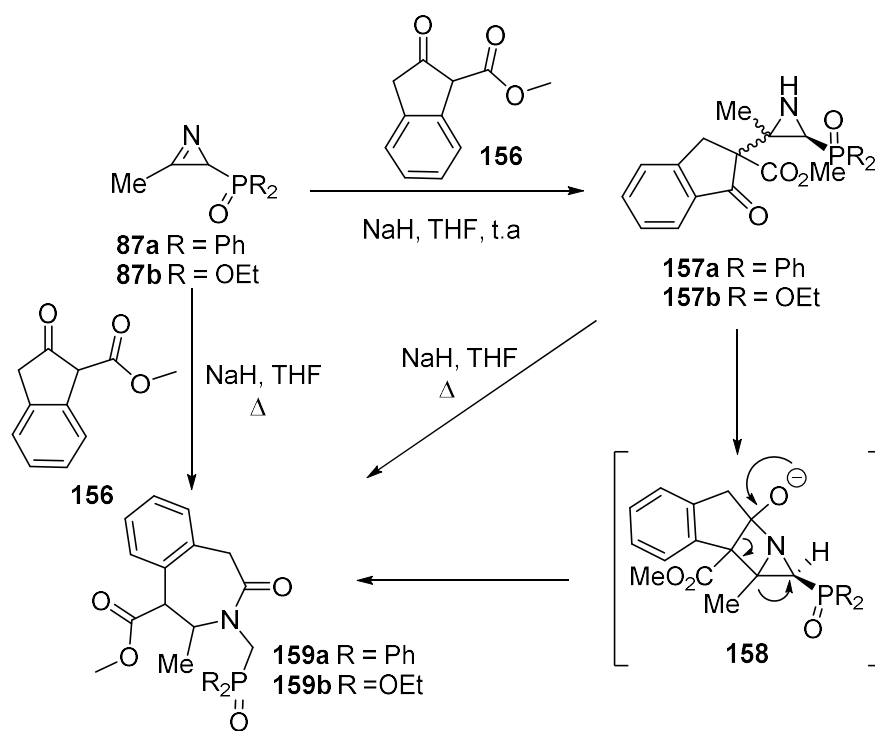
En cuanto a la síntesis de heterociclos de 7-8 miembros a partir de aziridinas podemos decir que no existe mucha información en la literatura. Estos estudios incluyen la síntesis de 2,3,4,5-tetrahydrobenzoxazepinas, 2,3,4,5-tetrahydrobenzodiazepinas, así como de benzoxepanos y sulfamidatos cíclicos. En una revisión de la literatura de 2017, se ha sintetizado la 2,3,4,5-tetrahydrobenzodiazepina **155** mediante la apertura de anillo de la aziridina, a través de la reacción de acrilato de aminofenilo **153** con la aziridina activada **152**. Tras el ataque inicial de **153** a la aziridina, se forma el intermedio **154**, que sufriría una reacción intramolecular aza-Michael catalizada por paladio para dar el derivado de benzodiazepina **155** (Esquema 43).¹⁴⁷

¹⁴⁷ C. K. Shahi, A. Bhattacharyya, Y. Nanaji, M. K. Ghorai. "A stereoselective route to tetrahydrobenzoxazepines and tetrahydrobenzodiazepines via ring-opening and aza-Michael addition of activated aziridines with 2-hydroxyphenyl and 2-aminophenyl acrylates". *J. Org. Chem.* **2017**, *82*, 37-47.



Esquema 43. Síntesis estereoselectiva de 2,3,4,5-tetrahidrobenzodiazepina.

Finalmente, en nuestro grupo de investigación, se ha desarrollado una metodología sintética que permite acceder a 1*H*-benzo[*d*]-azepinas **159a** y **159b** a través de dos rutas. Por un lado, la reacción de las 2*H*-azirinas derivadas de óxido de fosfina **87a** (R = Ph) o fosfonato de dietilo **87b** (R = OEt) con el β-cetoéster **156** y NaH en THF a temperatura ambiente conduce a la formación de las correspondientes aziridinas funcionalizadas derivadas de óxido de fosfina **157a** (R = Ph) o fosfonato **157b** (R = OEt) (Esquema 44). A continuación, el tratamiento térmico de estas aziridinas **157** en presencia de NaH y en THF como disolvente, conduce a la formación de las correspondientes 1*H*-benzo[*d*]-azepinas *N*-sustituidas funcionalizadas **159a** y **159b** con buenos rendimientos (86–88%). Estas 1*H*-benzo[*d*]-azepinas bicíclicas **159** también pueden obtenerse directamente a partir de las correspondientes 2*H*-azirinas fosforadas **87** cuando se tratan con el β-cetoéster **156** en presencia de NaH y a reflujo de THF (Esquema 44).^{84g} La formación de las 1*H*-benzo[*d*]-azepinas funcionalizadas **159** podría explicarse según el mecanismo representado en el esquema 44. El tratamiento térmico de las aziridinas **157** generaría el intermedio policíclico **158** por la adición nucleófila intramolecular del nitrógeno aziridínico sobre el grupo carbonilo en presencia de NaH. La expansión del policiclo fusionado de aziridina-azetidina **158**, implicaría tanto la apertura del enlace C–C del fragmento de ciclopentano-azetidina fusionado, como la simultánea apertura del anillo de aziridina por su enlace C–C, lo que permitiría explicar la formación de los heterociclos bicíclicos **159** (Esquema 44).



Esquema 44. Síntesis de 1H-benzo[d]-azepinas funcionalizadas.

Marco teórico

Las 2H-azirinas constituyen una familia de compuestos con alta reactividad, como consecuencia de una elevada tensión anular que incrementa la reactividad del enlace C=N.¹⁴⁸ Esto hace que las 2H-azirinas sean interesantes building blocks para la construcción de compuestos bicíclicos y presenten una gran versatilidad sintética debido a sus características estructurales. Esto les capacita para actuar como nucleófilos, electrófilos, dienófilos y dipolarófilos en varias reacciones orgánicas, como, por ejemplo, reacciones nucleófilas, electrófilas, ciclaciones de Diels-Alder¹⁴⁹ o 1,3 dipolares,¹⁵⁰ preparación de aminoderivados^{84e} y preparación de aziridinas sustituidas,^{12,24,151} entre otras.

Dentro del grupo de estos heterociclos de tres miembros, presentan especial interés las 2H-azirinas con sustituyentes carboxilados, siendo unos sustratos de partida con un gran potencial en la síntesis de α y β -aminoácidos. En este sentido, la sustitución isostérica del grupo carboxilato por un grupo fosforado en el anillo de azirina (Esquema 45), las convierte en interesantes building blocks para la preparación de ácidos α y β -aminofosfónicos con interesantes propiedades y aplicaciones en distintas áreas como medicina, industria, agricultura, así como su utilidad sintética como intermedios en química orgánica.¹⁵²

De este modo, los análogos fosforados de los aminoácidos (ácidos α ¹⁵³ y β -aminofosfónicos^{152d}) en los cuales el ácido carboxílico es remplazado por un ácido fosfónico,

¹⁴⁸ D. A. Candito, M. Lautens. "Exploiting the chemistry of strained rings: synthesis of indoles via domino reaction of aryl iodides with 2H-azirines". *Org. Lett.* **2010**, *12*, 3312-3315.

¹⁴⁹ A. S. Timén, A. Fischer, P. Somfai. "Stereoselective aza-Diels-Alder reactions with 2H-azirines as dienophiles furnishing highly functionalized tetrahydropyridines". *Chem. Commun.* **2003**, *10*, 1150-1151.

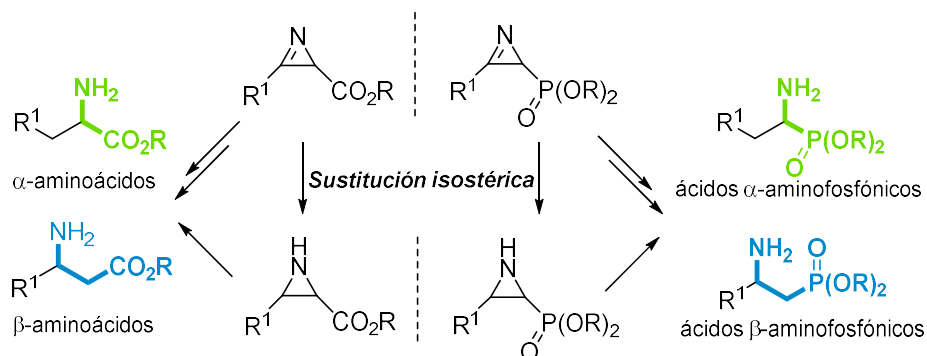
¹⁵⁰ J. O. Mueller, F. G. Schmidt, J. P. Blinco, C. B. Kowolik. "Visible-light-induced click chemistry". *Angew. Chem. Int. Ed.* **2015**, *54*, 10284-10288.

¹⁵¹ D. Tanner. "Chiral aziridines-their synthesis and use in stereoselective transformations". *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 599-619.

¹⁵² a) R. Engel. "Phosphonates as analogues of natural phosphates". *Chem. Rev.* **1977**, *77*, 349-367. b) F. Palacios, C. Alonso, J. M. de los Santos. " β -Phosphono- and phosphinopeptides derived from β -aminophosphonic and phosphinic acids". *Curr. Org. Chem.* **2004**, *8*, 15, 1481-1496. c) K. A. Schug, W. Linder. "Noncovalent binding between guanidinium and anionic groups: focus on biological- and synthetic-based arginine/guanidinium interactions with phosph[on]ate and sulf[on]ate residues". *Chem. Rev.* **2005**, *105*, 67-114. d) F. Palacios, C. Alonso, J. M. de los Santos. "Synthesis of β -aminophosphonates and -phosphinates". *Chem. Rev.* **2005**, *150*, 899-931. e) M. Sienczyk, J. Oleksyszyn. "Irreversible inhibition of serine proteases - design and *in vivo* activity of diaryl α -aminophosphonate derivatives". *Curr. Med. Chem.* **2009**, *16*, 1673-1687.

¹⁵³ a) J. M de los Santos, R. Ignacio, D. Aparicio, F. Palacios. "Michael addition of amine derivatives to conjugate phosphinyl and phosphonyl nitrosoalkenes. Preparation of α -amino phosphine oxide and phosphonate derivatives". *J. Org. Chem.* **2007**, *72*, 5202-5206. b) F. Palacios, J. Vicario, A. Maliszewska,

$P(O)(OH)_2$, o fosfínico, $P(O)(OH)R$ (en el que R puede ser H, un grupo alquilo o arilo), así como un grupo fosfonato, $P(O)(OR)_2$ (en el cual R puede ser un grupo alquilo o arilo), son particularmente atractivos en la preparación de análogos isómeros o bioisómeros de numerosos productos naturales (Figura 10).¹⁵⁴



Esquema 45. Sustitución isostérica del grupo carboxilato por un grupo fosforado en el anillo de 2H-azirina.

La actividad biológica de muchos ácidos aminofosfónicos se conoce desde hace más de un siglo, algunos de los cuales son productos naturales. Desde entonces, la química y biología de este tipo de compuestos forma parte de una rama importante de la química del fósforo.¹⁵⁵ Con frecuencia exhiben propiedades similares, donde los ácidos fosfónicos son reconocidos por enzimas o receptores como falsos sustratos o inhibidores.

- D. Aparicio. "Synthesis of α -phosphorylated α,β -unsaturated imines and their selective reduction to vinylogous and saturated α -aminophosphonates". *J. Org. Chem.* **2007**, *72*, 2682-2685.
- ¹⁵⁴ a) R. L. Hilderbrand. "The role of phosphonates in living systems". 1st Ed. CRC Press: Boca Raton, Florida, **1983**, Cap.6. 1-214. b) P. Kafarski, B. Lejczak. "Biological activity of aminophosphonic acids". *Phosphorus, Sulfur Silicon Relat. Elem.* **1991**, *63*, 193-215. c) P. Kafarski, B. Lejczak. "Aminophosphonic acids of potential medical importance". *Curr. Med. Chem. Anti-Cancer Agents* **2001**, *1*, 301-312. d) R. Engel, J. L. Cohen. "Synthesis of carbon-phosphorus bond". 2nd Ed. CRC Press: Boca Raton, Florida, **2004**, 1-187. e) E. P. Naydenova, P. T. Todorov, K. D. Troev. "Recent synthesis of aminophosphonic acids as potential biological importance". *Amino acids* **2010**, *38*, 23-30. f) F. Orsini, G. Sello, M. Sisti. "Aminophosphonic acids and derivatives. Synthesis and biological applications". *Curr. Med. Chem.* **2010**, *17*, 264-289. g) T. Cytlak, M. Kaźmierczak, M. Skibińska, H. Koroniak. "Latest achievements in the preparation of fluorinated aminophosphonates and aminophosphonic acids". *Phosphorus, Sulfur Silicon Relat. Elem.* **2017**, *192*, 602-620.
- ¹⁵⁵ P. Valery, H. R. Hudson. "Aminophosphonic and aminophosphinic acids: chemistry and biological activity". John Wiley & Sons: Chichester, **2000**, 1-660.

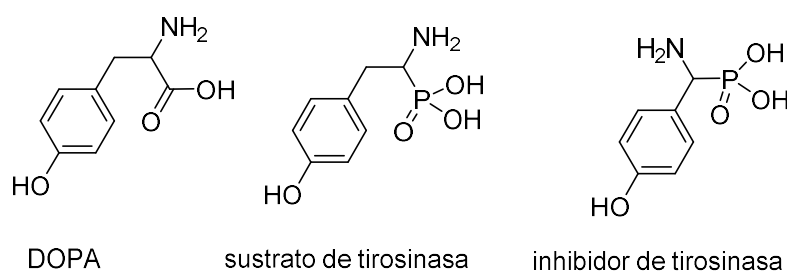


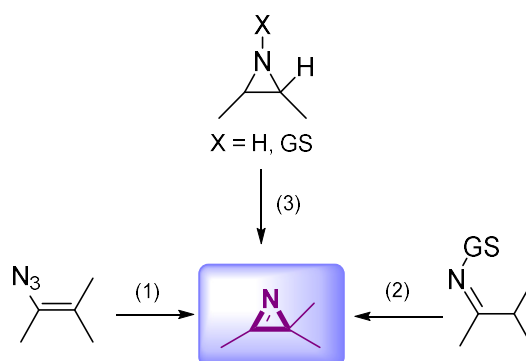
Figura 10. Análogos de DOPA con sustituyentes fosforados.

Generalmente es conocido que los aminofosfonatos poseen una amplia capacidad de influir en los procesos fisiológicos y patológicos con aplicaciones que van desde el campo de la agroquímica a la medicina.^{154b,154c,156} El modo de acción de los aminofosfonatos implica principalmente la inhibición de enzimas de clase y origen diferentes. Esta área de investigación ha sido ampliamente explorada y con frecuencia conduce a prometedores nuevos compuestos con importancia en química médica. Por tanto, la existencia de sustituyentes fosforados en estas estructuras de azirina las convierte en unos derivados con potencial actividad biológica y en precursores de ácidos aminofosfónicos.

Aunque existen varias estrategias para la construcción de 2*H*-azirinas,^{107b,107c} las publicaciones que aparecen en la última década revelan que los tres métodos representados en el esquema 1 son los más versátiles y más utilizados para su preparación (Esquema 46).

1. Termólisis o fotólisis de vinil azidas.
2. Reordenamiento de Neber y procesos relacionados.
3. La oxidación de Swern o eliminación en derivados de aziridina.

¹⁵⁶ a) J. Hiratake, J. Oda. "Aminophosphonic and aminoboronic acids as key elements of a transition state analogue inhibitor of enzymes". *Biosci. Biotechnol. Biochem.* **1997**, *61*, 211-218. b) D. Bonarska, H. Kleszczynska, J. Sarapuk. "Antioxidative activity of some phenoxy and organophosphorous compounds". *Cell Mol. Biol. Lett.* **2002**, *7*, 929-935. c) L. Berlicki, P. Kafarski. "Computer-aided analysis and design of phosphonic and phosphinic enzyme inhibitors as potential drugs and agrochemicals". *Curr. Org. Chem.* **2005**, *9*, 1829-1850. d) S. Vassiliou, M. Xeilari, A. Yiotakis, J. Grembecka, M. Pawelczak, P. Kafarski, A. Mucha. "A synthetic method for diversification of the P1' substituent in phosphinic dipeptides as a tool for exploration of the specificity of the S1' binding pockets of leucine aminopeptidases". *Bioorg. Med. Chem. Lett.* **2007**, *15*, 3187-3200. e) S. Van der Jeught, C. V. Stevens. "Direct phosphorylation of aromatic azaheterocycles". *Chem. Rev.* **2009**, *109*, 2672-2702. f) K. W. Yang, X. Cheng, C. Zhao, C. C. Liu, C. Jia, L. Feng, J. M. Xiao, L. S. Zhou, H. Z. Gao, X. Yang, L. Zhai. "Synthesis and activity study of phosphoramidate dipeptides as potential inhibitors of VanX". *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7224-7227.



Esquema 46. Estrategias sintéticas para la construcción del anillo de 2H-azirina.

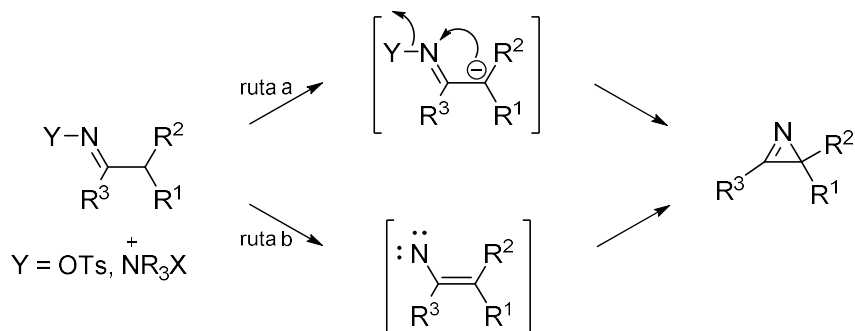
La estrategia de Neber es una de las más empleadas para la preparación de 2H-azirinas funcionalizadas. El mecanismo de reacción (Esquema 47) parece implicar un proceso concertado de desplazamiento nucleófilo interno (ruta a), o bien la formación de un vinilnitreno (ruta b), el cual se genera en medio básico por la pérdida del grupo saliente unido al átomo de nitrógeno imínico de las sulfoniloximas o las sales de hidrazonio en medio básico, que por un proceso electrocíclico evolucionaría a la 2H-azirina.¹⁵⁷

En nuestro grupo de investigación se ha desarrollado un método de preparación de oximas¹⁵⁸ y tosiloximas^{84a,84b,84c,84d,107a,108} fosforadas para su posterior estudio como precursores de las correspondientes 2H-azirinas derivadas de óxidos de fosfina y fosfonatos.¹⁰⁸ Por lo tanto, la estrategia planteada para la síntesis de 2H-azirinas-2-fosforadas que se van a utilizar como productos de partida en esta memoria, aparece representada en el esquema 48. Estas oximas a su vez, se pueden preparar a través de dos rutas diferentes (Esquema 48). La ruta a implica la adición de Michael de hidroxilamina **161** a alenos fosforados **160**, mientras que la ruta b implica la reacción de condensación de hidroxilamina **161** con el correspondiente cetofosfanóxido **163** (R = Ph) o cetofosfonato **163** (R = OEt, OⁱPr). Por último, el tratamiento en medio básico de las

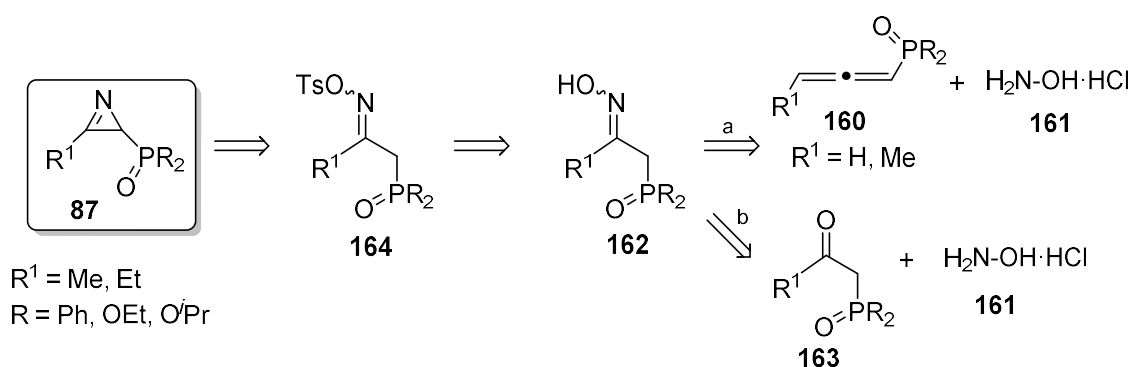
¹⁵⁷ W. H. Pearson, B. W. Lian, S. C. Bergmeier. "Comprehensive heterocyclic chemistry II". Eds. A. R. Katritzky, C. W. Rees, E. F. V. Scriven, Pergamon Press, Oxford, UK, **1996**, 1A, Cap. 1, 1-60.

¹⁵⁸ a) F. Palacios, D. Aparicio, J. M. de los Santos, E. Rodríguez. "An improved and general method for the synthesis of α,β -unsaturated oximes from phosphine oxide allenes". *Tetrahedron Lett.* **1996**, 37, 1289-1292. b) F. Palacios, D. Aparicio, J. M. de los Santos, E. Rodríguez. "An improved and effective method for the preparation of α,β -unsaturated oximes and isoxazole derivatives". *Tetrahedron* **1998**, 54, 599-614. c) F. Palacios, J. M. de los Santos, J. Vicario, C. Alonso. *Phosphorus derivatives of hydroxylamines, oximes and hydroxamic acids* In "PATAI's chemistry of functional groups". Eds. Z. Rappoport, J. F. Liebman, John Wiley & Sons, Ltd.: Chichester, **2011**, 2, Cap. 7, 3-89.

tosilcetoximas fosforadas **164** puede dar lugar a reacciones de cicloeliminación con el fin de obtener las 2*H*-azirinas-2-fosforadas **87** (Esquema 48).



Esquema 47. Mecanismo propuesto para la reacción de Neber.



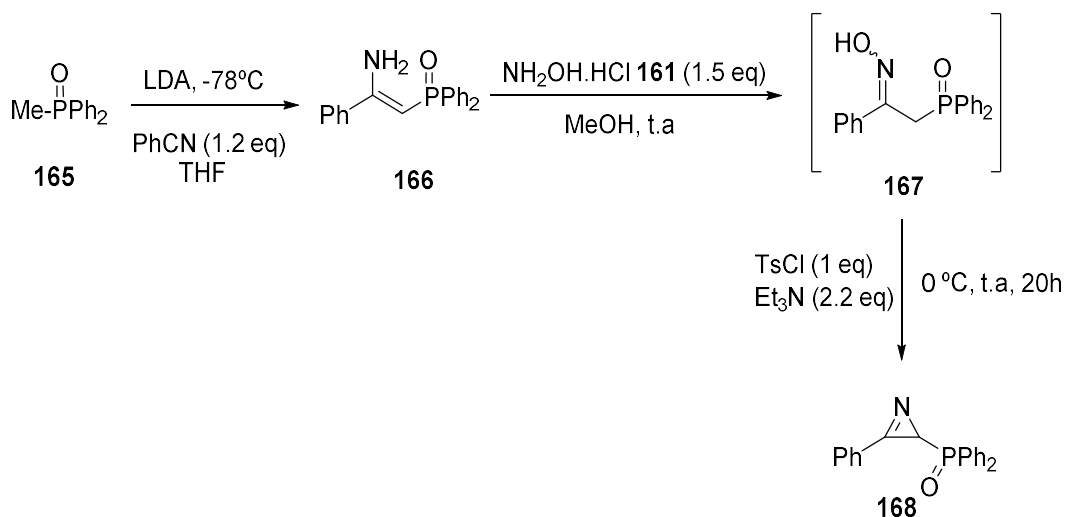
Esquema 48. Esquema retrosintético para la preparación de 2*H*-azirinas fosforadas.

Sin embargo, la 2*H*-azirina-2-fosforada **168** con sustituyente arilo ($R^1 = \text{Ph}$) se preparó *one pot* a partir de la correspondiente oxima **167**. Para llevar a cabo la formación de la oxima **167**, se pensó en una reacción de transaminación de la enamina **166**¹⁵⁹ con hidroxilamina **161**. De esta forma, el tratamiento del óxido de fosfina **165**¹⁶⁰ con LDA seguido de la adición del benzonitrilo, condujo a la enamina **166** intermedia que, a través de una reacción de transaminación con hidroxilamina generó la oxima esperada **167**. Finalmente, esta oxima **167** se

¹⁵⁹ N. Feeder, D. J. Fox, J. A. Medlock, S. Warren. "Synthesis, X-ray crystal structures and Horner-Wittig addition reactions of some protected β -aminophosphine oxides". *J. Chem. Soc., Perkin Trans. 1* **2002**, 1175-1180.

¹⁶⁰ Y. Zhao, T. Feng, G. Li, F. Liu, X. Dai, Z. Dong, X. Qiu. "Synthesis and properties of novel polyimide fibers containing phosphorus groups in the main chain". *RCS Adv.* **2016**, *6*, 42482-42494.

trató con dos equivalentes de Et₃N y cloruro de *p*-toluensulfonilo, proporcionando la 3-fenil-2*H*-azirina-2-fosforada **168** (R = Ph). A través de esta metodología *one pot*, se obtiene un mayor rendimiento, que sintetizando la 2*H*-azirina **168** a partir de la tosilación de la oxima fosforada **167** (Esquema 49).



Esquema 49. Síntesis de la 2*H*-azirina-2-fosforada **168** vía enamina **166**.

Como ya se ha comentado en la introducción, las 2*H*-azirinas son más susceptibles a la adición nucleofílica que las iminas, debido a su elevada tensión anular, por lo que una posibilidad interesante para estos heterociclos nitrogenados de tres miembros consiste en la incorporación de reactivos nucleófilos mediante la adición al doble enlace C-N de la 2*H*-azirina. Más concretamente, las adiciones de nucleófilos más exitosas utilizando 2*H*-azirinas comprenden reducciones en presencia de hidruro de litio y aluminio o borohidruro sódico,^{84b} adiciones enantioselectivas de reactivos organolíticos,¹⁶¹ adiciones de reactivos de Grignard,^{84e} así como adiciones de reactivos nucleófilos nitrogenados, oxigenados, carbonados o azufrados. Mediante estas adiciones nucleófilas se pueden sintetizar una gran variedad de aziridinas funcionalizadas, las cuales pueden dar lugar a la apertura anular conduciendo a los correspondientes ácidos aminofosfónicos, como veremos más adelante.

¹⁶¹ E. Risberg, P. Somfai. "Enantioselective addition of organolithium reagents to a 2*H*-azirine". *Tetrahedron: Asymmetry* **2002**, *13*, 1957-1959.

La estructura de esta tesis doctoral se fundamenta en 4 objetivos principales, alguno de ellos abarca a su vez algunos objetivos secundarios. Por tanto, el marco teórico está estructurado en función de cada uno de los objetivos principales propuestos.

OBJETIVO 1: Adición nucleófila de cianuro al doble enlace C-N de 2H-azirinas fosforadas.

Con la finalidad de explorar la utilidad sintética de las 2H-azirinas fosforadas como intermedios sintéticos, el primer objetivo de esta memoria consiste en el estudio de la adición nucleófila de cianuro a 2H-azirinas fosforadas con el fin de sintetizar nuevas 2-cianoaziridinas derivadas de óxido de fosfina y fosfonato.

En la bibliografía, aparecen algunos ejemplos sobre la adición nucleófila del grupo cianuro al doble enlace C-N de 2H-azirinas. Sin embargo, la utilización de nucleófilos con el grupo nitrilo han sido poco estudiadas y requiere el uso de reactivos tóxicos. El grupo nitrilo es un buen farmacóforo que se encuentra presente en un gran número de productos naturales¹⁶² y juega un papel importante en el desarrollo de nuevos medicamentos.¹⁶³

En 1986, Ghosez y col.,¹⁶⁴ desarrollaron una metodología que permite obtener 3-etoxi-3-cianoaziridinas **170** estables a partir de la reacción de la 3-etoxi-2H-azirina **169** con TMSCN en presencia de cianuro de tetraetilamonio (Et₄NCN) en cantidades catalíticas. Los compuestos **170a** y **170c** fueron sorprendentemente estables, mientras que el compuesto **170b** fue obtenido como una mezcla de estereoisómeros (Esquema 50).

Una síntesis estereoselectiva de *trans*-aziridinas **172** altamente funcionalizadas, la realizan Öhler y col.,¹⁶⁵ mediante la adición de TMSCN a [(2H-azirin-2-il)metil] fosfonatos **171**

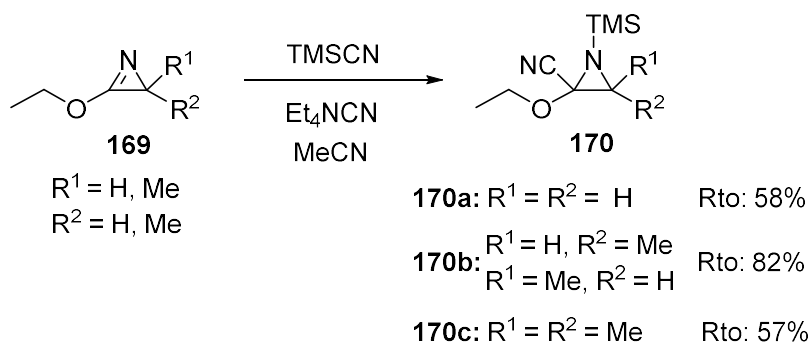
¹⁶² F. F. Fleming. "Nitrile-containing natural products". *Nat. Prod. Rep.* **1999**, *16*, 597-606.

¹⁶³ a) F. F. Fleming, L. Yao, P. C. Ravikumar, L. Funk, B. C. Shook. "Nitrile-containing pharmaceuticals: efficacious roles of the nitrile pharmacophore". *J. Med. Chem.* **2010**, *53*, 7902-7917. b) A. S. Kalgutkar, D. K. Dalvie. "Drug discovery for a new generation of covalent drugs". *Expert Opin. Drug. Dis.* **2012**, *7*, 561-581. c) W. Jiang, L. Hong. "Application of nitrile in drug design". *Chin. J. Org. Chem.* **2012**, *32*, 1643-1652.

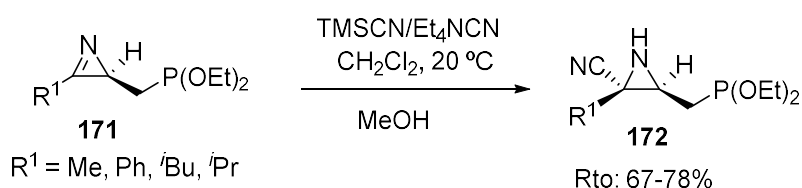
¹⁶⁴ L. Ghosez, F. Sainte, M. Rivera, C. Bernard-Henriet, V. Couverneur. "Synthesis and reactions of 3-alkoxy-2H-azirines". *Recl. Trav. Chim. Pays-Bas* **1986**, *105*, 456-461.

¹⁶⁵ E. Öhler, S. Kanzler. "[[(2H-Azirin-2-yl)methyl]phosphonates: synthesis from allylic α - and γ -hydroxyphosphonates and application to diastereoselective formation of substituted [(aziridin-2-yl)methyl]phosphonates". *Liebigs Ann. Chem.* **1994**, *9*, 867-876.

proporcionando estereoselectivamente las correspondientes *trans*-aziridinas **172** con buenos rendimientos (Esquema 51).



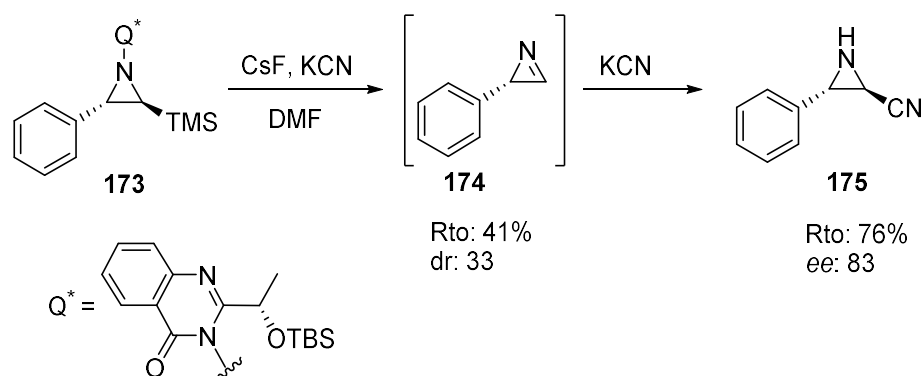
Esquema 50. Síntesis de 3-etoxi-3-cianoaziridinas.



Esquema 51. Síntesis estereoselectiva de *trans*-aziridinas mediante la adición nucleófila de TMSCN .

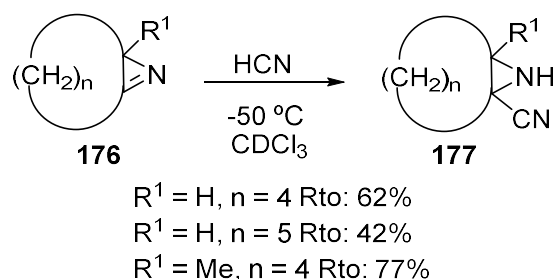
Las Q^* -aziridinas de Atkinson **173**¹⁶⁶ que llevan sustituyentes sililados en el anillo, se eliminan en presencia de fluoruro de cesio (CsF) en DMF para dar azirinas quirales **174**, las cuales reaccionan con cianuro de potasio para dar cianoaziridinas enantioméricamente puras **175** con buenos rendimientos. El ataque nucleófilo del ion cianuro tiene lugar en *anti* con respecto al centro quiral preexistente (Esquema 52).

¹⁶⁶ R. S. Atkinson, M. P. Coogan, I. S. T. Lochrie. "Desilylative elimination of the quinazolinone ring from 1-(4-oxoquinazolin-3-yl)-2-silylaziridines; preparation of an *N*-H aziridine in high enantiomeric excess by *in situ* nucleophilic addition to the derived azirine". *J. Chem. Soc., Perkin Trans. 1*, **1997**, 897-900.



Esquema 52. Síntesis enantioselectiva de NH-cianoaziridinas en presencia de KCN.

Asimismo, 2*H*-azirinas altamente tensionadas **176**¹⁶⁷ reaccionan con cianuro de hidrógeno a -50 °C proporcionando las correspondientes cianoaziridinas **177** con buenos rendimientos (Esquema 53).



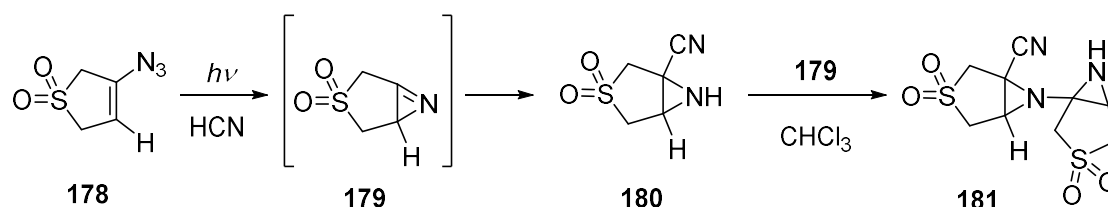
Esquema 53. Adición de HCN a 2*H*-azirinas para la preparación de cianoaziridinas.

Posteriormente, el mismo grupo realizaron la fotólisis de vinil azidas cíclicas **178** a baja temperatura y en presencia de cianuro de hidrógeno, lo que permite la obtención de 2-cianoaziridinas bicíclicas **181** con buenos rendimientos (Esquema 54).¹⁶⁸ Aunque después de la irradiación de la vinil azida **178** a -50 °C en presencia de cianuro de hidrógeno, no es posible aislar el intermedio **179**, si es posible aislar el producto **181** con un rendimiento del 82-86%.

¹⁶⁷ K. Banert, B. Meier. "Synthesis and reactions of highly strained 2,3-bridged 2*H*-azirines". *Angew. Chem. Int. Ed.* **2006**, *45*, 4015-4019.

¹⁶⁸ K. Banert, A. Ihle, A. Kultz, E. Penk, B. Saha, E. U. Würthwein. "Generation of highly strained 2,3-bridged 2*H*-azirines via cycloaddition reactions of 2-azidobuta-1,3-dienes and photolysis of the resulting cyclic vinyl azides". *Tetrahedron* **2013**, *69*, 2501-2508.

La formación de este compuesto **181** se puede explicar por la formación del intermedio **179** seguido del producto de adición de grupo cianuro para dar **180**, el cual se agrega al doble enlace C=N muy reactivo de una segunda molécula de **179**, dando lugar a la formación de **181**.



Esquema 54. Síntesis de 2-cianoaziridinas bicíclicas mediante la adición de HCN a azirinas *ortho*-fusionadas.

La adición nucleófila de NaCN¹⁶⁹ a 2*H*-azirinas **182**, previamente preparadas a partir de precursores de mesiloximas por ciclación promovida por base (Et₃N), se lleva a cabo para la preparación de cianoaziridinas **183**, a través de una técnica de flujo continuo que implica la adición nucleofílica de nitrilo. Para ello, se diseña un proceso extendido que fusiona la corriente de reacción inicial que contiene el producto de 2*H*-azirina **182** con una solución acuosa de cianuro de sodio para generar las cianoaziridinas **183** correspondientes (Esquema 55). Después de pasar a través de una segunda bobina tubular del reactor a temperatura ambiente, las aziridinas **183** se aíslan tras la extracción acuosa y evaporación con buenos rendimientos y alta diastereoselectividad (dr: >19:1).

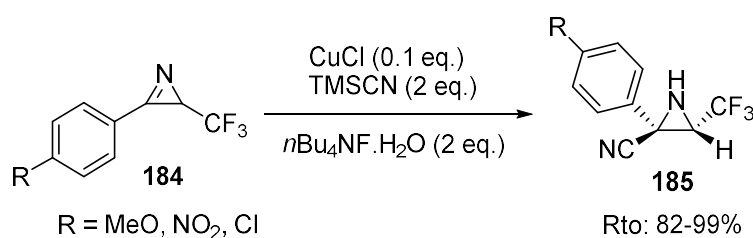
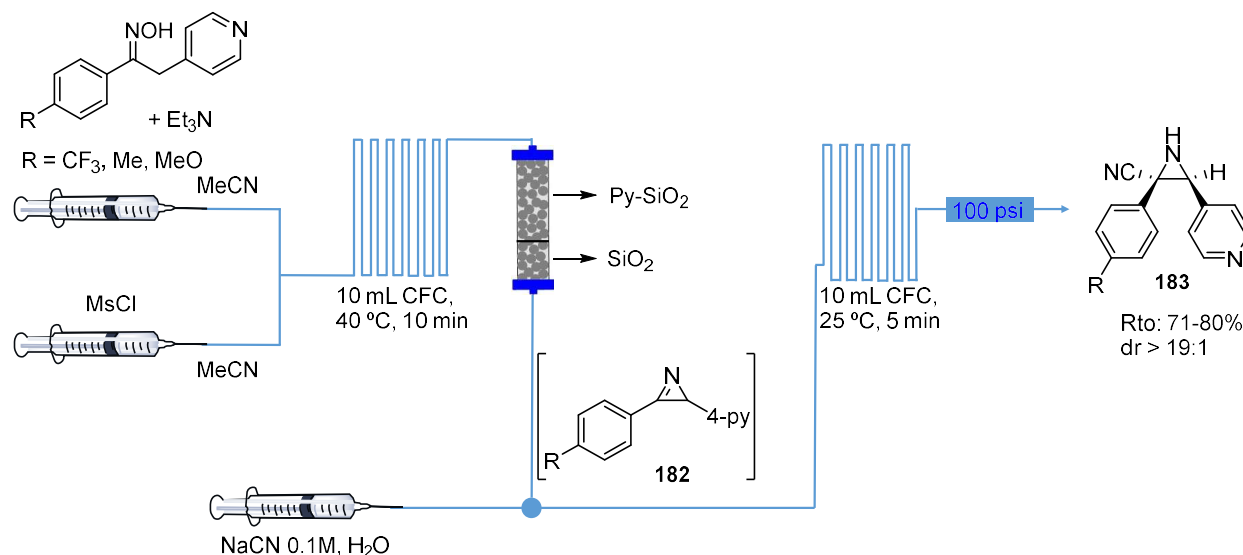
Por otro lado, Liu y col.,¹⁷⁰ realizan la cianación de azirinas **184** que contienen un grupo CF₃ en el átomo de carbono C-3, utilizando TMS-CN como fuente de cianuro y en presencia de un catalizador de Cu(I), lo que proporciona cianoaziridinas **185** con excelentes rendimientos y diastereoselectividades (Esquema 56).

A la vista de estos antecedentes bibliográficos, es posible desarrollar una vía sencilla para la obtención de 3-cianoaziridinas-2-fosforadas. Esta estrategia supone la primera síntesis de 2-

¹⁶⁹ M. Baumann, I. R. Baxendale. "Continuous flow synthesis of 2*H*-azirines and their diastereoselective transformation to aziridines". *Synlett*. **2016**, 27, 159-163.

¹⁷⁰ F. Wang, N. Zhu, P. Chen, J. Ye, G. Liu. "Copper-catalyzed trifluoromethylazidation of alkynes: efficient access to CF₃-substituted azirines and aziridines". *Angew. Chem. Int. Ed.* **2015**, 54, 9356-9360.

cianoaziridinas con sustituyentes fosforados derivados de óxido fosfina o fosfonato en el átomo de carbono C-3 del anillo, mediante adición de TMSCN a 2*H*-azirinas fosforadas.



OBJETIVO 2: Expansión del anillo en cianoaziridinas fosforadas.

Los agentes modificadores del ADN son una clase importante de fármacos usados en quimioterapia. Entre estos compuestos antitumorales, se encuentran aquellos que poseen un anillo de aziridina, y son potentes agentes alquilantes. Debido a su capacidad para actuar como agentes de entrecruzamiento del ADN a través de la apertura del anillo, tienen una potencia *in vivo* intrínseca.²⁰ La mitomicina C y muchos de los derivados de este producto natural se caracterizan por su actividad antitumoral basada en la apertura nucleófila del heterociclo

nitrogenado de 3 miembros, lo que conduce a la alquilación del ADN.¹⁷¹ Además, la mitomicina C es un agente alquilante bioreductor del ADN, es decir, forma entrecruzamientos entre las cadenas en el ADN, así como aductos de ADN monoalquilados.¹⁷² Teniendo en cuenta estos principios, al inicio de la década de los 70, Bicker¹⁷³ desarrolló la síntesis de una gran variedad de derivados de 2-cianoaziridina como posibles agentes carcinostáticos. Así, por ejemplo, la 2-cianoaziridina-1-carbamoil sustituida **186** (Figura 11) mostró actividad contra el sarcoma PIE 2-3 en ratas presentando una baja toxicidad. Este compuesto, aunque mostró una actividad antitumoral poco efectiva en cultivos celulares, fue de especial interés debido a que aumentaba el número de leucocitos.^{173b} Sin embargo, estas cianoaziridinas no mostraron actividad alquilante ni *in vivo* ni *in vitro*.^{173a} Estos resultados sugieren que el grupo ciano reduce la actividad requerida para la alquilación de las bases del ADN, pudiendo reaccionar de manera selectiva con grupos tioles, como por ejemplo, en la cisteína, agotando las reservas de cisteína y glutatión, y permitiendo posteriormente la acumulación de especies reactivas de oxígeno.^{174,175,176} Estudios preclínicos han evaluado la actividad antitumoral y el mecanismo de acción de la cianoaziridina AMP423 **186b** (Figura 11).¹⁷⁴ Se han desarrollado otras cianoaziridinas, como por ejemplo, ciamexón **187** y azimexón **188**¹⁷⁷ (Figura 11), las cuales fueron evaluadas en ensayos clínicos a finales de la década de 1980. Asimismo, imexon **189** (Figura 11), que proviene de la ciclación de la 2-cianoaziridina-1-carboxamida **186a** en presencia de base,^{178,179} es un agente antineoplásico que incrementa el estrés oxidativo y ha sido ampliamente usado, como inhibidor selectivo del

¹⁷¹ M. Tomasz, D. Chowdary, R. Lipman, S. Shimotakahara, D. Veiro, V. Walker, G. L. Verdine. "Reaction of DNA with chemically or enzymatically activated mitomycin C: isolation and structure of the major covalent adduct". *Proc. Natl. Acad. Sci.* **1986**, *83*, 6702-6706.

¹⁷² M. Tomasz, Y. Palom. "The mitomycin bio-reductive antitumor agents: cross-linking and alkylation of DNA as the molecular basis of their activity". *Pharmacol. Ther.* **1997**, *76*, 73-87.

¹⁷³ a) U. Bicker. "N-(2-Cyanoethylene)urea-an asparagine analogous cytostatic compound?". *Exp. Pathol. Jena.* **1975**, *10*, 106-108. b) U. F. Bicker. *Immunopharmacological properties of 2-cyanoaziridine derivatives* In "Immune modulation agents and their mechanisms". Eds. R. L. Fenichel, M. A. Chirigos, Marcel Dekker, New York, **1984**, 447-473.

¹⁷⁴ R. T. Dorr, L. Wisner, B. K. Samulitis, T. H. Landowski, W. A. Remers. "Anti-tumor activity and mechanism of action for a cyanoaziridine-derivative, AMP423". *Cancer Chemother. Pharmacol.* **2012**, *69*, 1039-1049.

¹⁷⁵ W. A. Remers, R. T. Dorr. "Chemistry and pharmacology of imexon and related cyanoaziridines". *Curr. Med. Chem.* **2012**, *19*, 5745-5753.

¹⁷⁶ K. Dvorakova, C. M. Payne, M. E. Tome, M. M. Briehl, T. McClure, R. T. Dorr. "Induction of oxidative stress and apoptosis in myeloma cells by the aziridine-containing agent imexon". *Biochem. Pharmacol.* **2000**, *60*, 749-758.

¹⁷⁷ L. Olsson, U. Bicker. "Effect of the immune modulators BM 12.531 (azimexone) and BM 41.332 on the subsets of T-lymphocytes in mice". *J. Immunopharmacol.* **1981-1982**, *3*, 277-288.

¹⁷⁸ U. Bicker, W. Kampe, W. Steingross. U.S. Patent 4,083,987, Apr 11, **1978**.

¹⁷⁹ B. S. Iyengar, R. T. Dorr, W. A. Remers. "Chemical basis for the biological activity of imexon and related cyanoaziridines". *J. Med. Chem.* **2004**, *47*, 218-223.

crecimiento del mieloma múltiple.^{54,180} Su actividad biológica está estrechamente relacionada con la de las cianoaziridinas, ya que las soluciones de imexón en agua evolucionan lentamente a **186a**. En 1999 Remers y col.,¹⁸¹ describieron la síntesis de una serie de 2-cianoaziridinas-1-carbamoíl sustituidas activas frente a una variedad de tumores sólidos en cultivos celulares. Por ejemplo, el *N*-fenil derivado **186c** (R = Ph, figura 11) fue testado en células tumorales humanas, observándose una actividad similar a la del imexon.

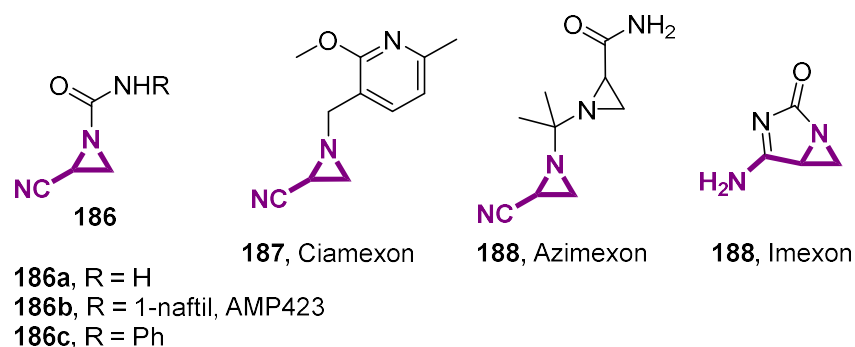


Figura 11. Ejemplos de 2-cianoaziridinas como fármacos antitumorales.

Además, la expansión del anillo en aziridinas con isocianatos como heterocumulenos, es una metodología ampliamente usada en la preparación de una variedad de compuestos nitrogenados de cinco miembros. Por ejemplo, se han descrito reacciones de aziridinas con

¹⁸⁰ S. J. Cohen, M. M. Zalupski, P. Conkling, F. Nugent, W.W. Ma, M. Modiano, R. Pascual, F. C. Lee, L. Wong, E. Hersh. "A phase 2 randomized, double-blind, multicenter trial of imexon plus gemcitabine versus gemcitabine plus placebo in patients with metastatic chemotherapy-naïve pancreatic adenocarcinoma". *Am. J. Clin. Oncol.* **2015**, 1-62.

¹⁸¹ B. S. Iyengar, R. T. Dorr, D. S. Alberts, E. M. Hersh, S. E. Salmon, W. A. Remers. "Novel antitumor 2-cyanoaziridine-1-carboxamides". *J. Med. Chem.* **1999**, 427, 510-514.

isocianatos catalizadas por KI,¹⁸² NaI,¹⁸³ NiI₂,¹⁸⁴ Pd¹⁸⁵, o incluso en ausencia de catalizadores.¹⁸⁶ Estas reacciones dieron lugar a imidazolidinonas o 2-iminooxazolidinas, compuestos de gran interés en la química médica y farmacéutica ya que poseen una amplia gama de actividades biológicas.¹⁸⁷

En la literatura aparecen descritos varios ejemplos relacionados con la síntesis de 2-iminooxazolidinas a partir de la reacción de NH-aziridinas con 2 equivalentes de fenilisocianato. De este modo, Samiri y col.,¹⁸² describen la primera conversión regio y estereoselectiva de NH-cetoaziridinas **190** en 2-iminooxazolidinas **192** utilizando fenilisocianato **191** en presencia de KI (Esquema 57).

Por otro lado, la cicloadición de aziridinas **193** con fenilisocianato **191** en presencia de un catalizador de Niquel condujo a las correspondientes iminooxazolidinas **194** con buenos rendimientos (75-92%). Los mejores resultados se obtienen cuando la reacción se lleva a cabo utilizando el catalizador NiI₂. Un tiempo más largo de reacción permite la isomerización de las iminooxazolidinas **194** a los correspondientes derivados de imidazolidinonas **195** (Esquema 58).¹⁸⁴

¹⁸² H. A. Samimi, B. M. Yamin. "KI-catalyzed ring expansion reaction of *trans*-NH-ketoaziridines to new *trans*-oxazolidines". *Tetrahedron Lett.* **2005**, *46*, 479-482.

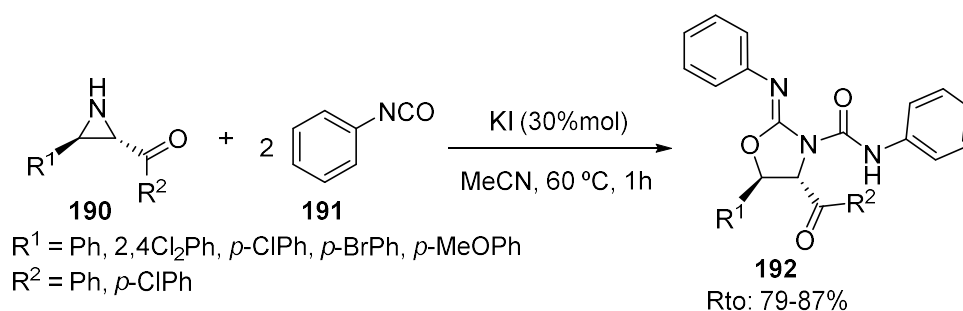
¹⁸³ a) U. K. Nadir, N. Basu. "Regiospecific cycloaddition reaction of 2-aryl and 2-alkyl 1-arenesulfonylaziridines with isocyanates using sodium iodide". *Tetrahedron Lett.* **1992**, *33*, 7949-7952. b) U. K. Nadir, N. Basu. "Reaction of 2,3-disubstituted *N*-arylsulfonylaziridines with isocyanates in presence of sodium iodide - stereospecific conversion of *N*-arylsulfonylaziridines to imidazolidinones". *Tetrahedron Lett.* **1993**, *49*, 7787-7792. c) U. K. Nadir, R. V. Krishna, A. Singh. "A new and facile route for the synthesis of chiral 1,2-diamines and 2,3-diamino acids". *Tetrahedron Lett.* **2005**, *46*, 479-482.

¹⁸⁴ T. Munegumi, I. Azumaya, T. Kato, H. Masu, S. Saito. "[3+2] Cross-coupling reactions of aziridines with isocyanates catalyzed by nickel(II) iodide". *Org. Lett.* **2006**, *8*, 379-382.

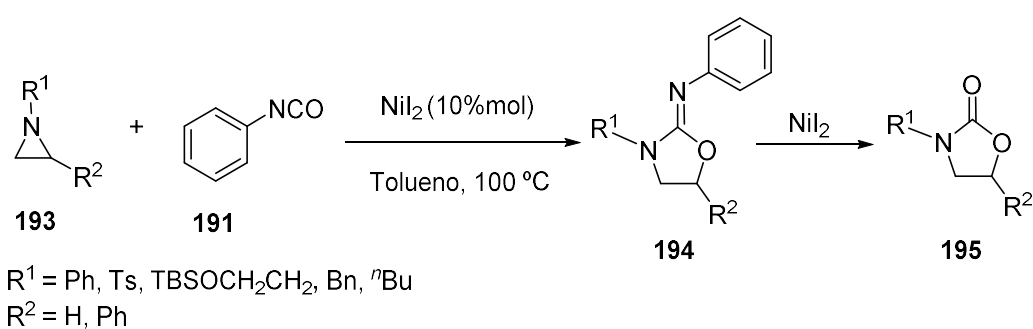
¹⁸⁵ a) D. C. D. Butler, G. A. Inman, H. Alper. "Room temperature ring-opening cyclization reactions of 2-vinylaziridines with isocyanates, carbodiimides, and isothiocyanates catalyzed by [Pd(OAc)₂]/PPh₃". *J. Org. Chem.* **2000**, *65*, 5887-5890. b) B. M. Trost, D. R. Fandrick. "Dynamic kinetic asymmetric cycloadditions of isocyanates to vinylaziridines". *J. Am. Chem. Soc.* **2003**, *125*, 11836-11837. c) C. Dong, H. Alper. "CeCl₃ promoted asymmetric cycloaddition of isocyanates with 2-vinylaziridines". *Tetrahedron: Asymmetry* **2004**, *15*, 1537-1540.

¹⁸⁶ M. A. Tabarki, R. Besbes. "Ring expansion of aziridine-2-carboxylates. An efficient entry to imidazolidin-2-ones and oxazolidin-2-imines". *Tetrahedron Lett.* **2015**, *56*, 1837-1839.

¹⁸⁷ a) S. Ueda, H. Terauchi, A. Yano, M. Ido, M. Matsumoto, M. Kawasaki. "4,5-Disubstituted-1,3-oxazolidin-2-imine derivatives: a new class of orally bioavailable nitric oxide synthase inhibitor". *Bioorg. Med. Chem. Lett.* **2004**, *14*, 313-316. b) K. R. A. Abdellatif, W. A. A. Fadaly, Y. A. Mostafa, D. M. Zaher, H. A. Omar. "Thiohydantoin derivatives incorporating a pyrazole core: design, synthesis and biological evaluation as dual inhibitors of topoisomerase-I and cyclooxygenase-2 with anti-cancer and anti-inflammatory activities". *Bioorg. Chem.* **2019**, *91*, 103-132.



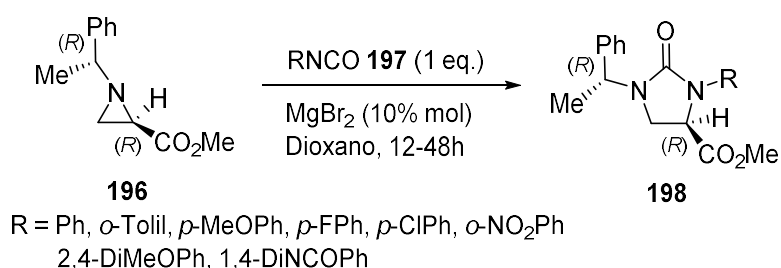
Esquema 57. Síntesis de *trans*-oxazolidinas a partir de *trans*-aróil-3-aziridinas con fenilisocianato.



Esquema 58. Síntesis de iminoxazolidinas mediante reacciones de acoplamiento cruzado de aziridinas catalizadas por NiI_2 .

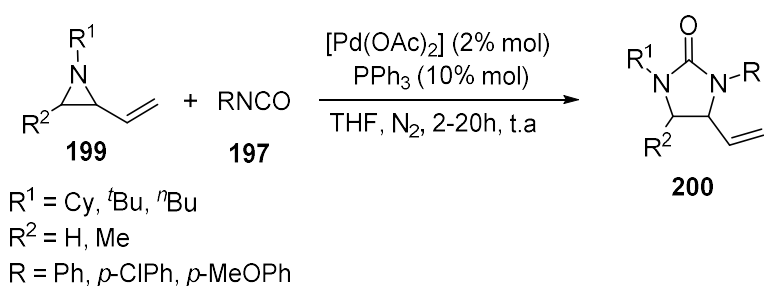
Ha y col.,¹⁸⁸ describen la primera reacción de cicloadición regio y estereoespecífica catalizada por un ácido de Lewis, que involucra aziridinas-2-carboxiladas **196** con isocianatos **197** como un nuevo enfoque estereoespecífico para dar lugar a imidazolidin-2-ona 4-funcionalizadas **198** enantioméricamente puras y con altos rendimientos (65–95%) (Esquema 59). Estas reacciones proceden con retención de la configuración en la posición C-2 de las aziridinas quirales.

¹⁸⁸ M. S. Kim, Y. W. Kim, H. S. Hahm, J. W. Jang, W. K. Lee, H. J. Ha. "Lewis acid-catalyzed stereospecific ring expansion of aziridine-2-carboxylates to imidazolidin-2-ones". *Chem. Commun.* **2005**, 3062-3064.



Esquema 59. Síntesis de imidazolidin-2-onas a partir de aziridinas quirales catalizada por ácidos de Lewis.

Finalmente, en la literatura existen varios ejemplos de reacciones de expansión de anillo catalizadas por Pd. Por ejemplo, es conocido que las reacciones de cicloadición de apertura de anillo de aziridinas sustituidas en las posiciones 1,2 y 1,2,3 con varios heterocumulenos catalizados por $[\text{PdCl}_2(\text{PhCN})_2]$ proceden, tanto regio como estereoselectivamente, para dar los correspondientes heterociclos de anillo de cinco miembros con buenos rendimientos.¹⁸⁹ De este modo, Alper y col^{185a} desarrollan una metodología sintética en la cual, 2-vinilaziridinas **199** experimentan reacciones de cicloadición con isocyanatos **197** en presencia de $\text{Pd}(\text{OAc})_2$ y PPh_3 , a temperatura ambiente y 1 atm de presión, proporcionando regioselectivamente imidazolidinonas **200** con rendimientos de moderados a altos (34–97%) (Esquema 60).



Esquema 60. Síntesis de imidazolidinonas a partir 2-vinilaziridinas catalizada por Pd.

¹⁸⁹ a) J. O. Baeg, H. Alper. "Regiospecific palladium-catalyzed cycloaddition of aziridines and carbodiimides". *J. Org. Chem.* **1992**, *57*, 157-162. b) J. O. Baeg, C. Bensimon, H. Alper. "The first enantiospecific palladium-catalyzed cycloaddition of aziridines and heterocumulenes. Novel synthesis of chiral five-membered ring heterocycles". *J. Am. Chem. Soc.* **1995**, *117*, 4700-4701. c) H. Mass, C. Bensimon, H. Alper. "Ring-opening cycloaddition of aziridines to ketenimines". *J. Org. Chem.* **1998**, *63*, 17-20.

Ante estos antecedentes y siguiendo con nuestros estudios anteriores sobre la preparación de cianoaziridinas fosforadas, se trata de estudiar la preparación de nuevas cianoaziridinas fosforadas *N*-funcionalizadas mediante la reacción de cianoaziridinas inactivadas con isocianatos. Además, se explora la expansión del anillo en imidazolidin-2-onas u oxazolidin-2-iminas tras la reacción con isocianatos.

OBJETIVO 3: Adición de fenoles funcionalizados a 2*H*-azirinas fosforadas.

Una vez establecidas las metodologías sintéticas necesarias para la preparación de cianoaziridinas fosforadas funcionalizadas, por adición de TMSCN a 2*H*-azirinas fosforadas, y continuando con nuestro interés acerca de la reactividad de estos heterociclos de tres miembros, estudiamos la adición nucleófila de fenoles funcionalizados al doble enlace C-N de las 2*H*-azirinas fosforadas.

La historia de las benzoxazinas de pequeño peso molecular se remonta a hace más de 60 años. Holly y Cope¹⁹⁰ describen por primera vez la síntesis de benzoxazinas en 1944. La benzoxazina es una molécula donde un anillo de oxazina (un anillo heterocíclico de seis miembros con un átomo de oxígeno y nitrógeno) está unido a un anillo de benceno. Son posibles varias estructuras isoméricas dependiendo de las posiciones y el grado de oxidación del sistema de anillo. Las dos estructuras isoméricas **201** y **202** (2*H*- y 4*H*-benzo-1,3-oxazina), además de la 3,4-dihidro-2*H*-benzo-1,3-oxazina **203** se ilustran en la figura 12.¹⁹¹

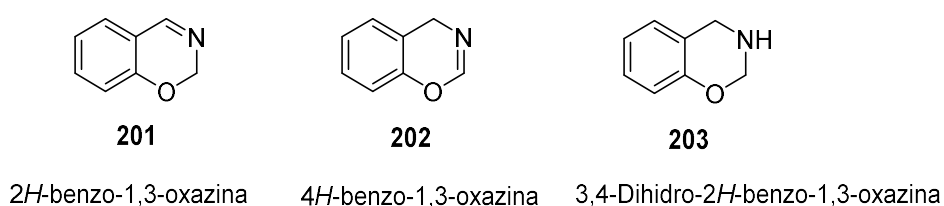


Figura 12. Estructuras químicas de 1,3-benzoxazinas.

¹⁹⁰ F. W. Holly, A. C. Cope. "Condensation products of aldehydes and ketones with *o*-aminobenzyl alcohol and *o*-hydroxy benzylamine." *J. Am. Chem. Soc.* **1944**, *66*, 1875-1879.

¹⁹¹ L. Lázár, F. Fülöp. *1,3-Oxazines and their benzo derivatives*, In "Comprehensive heterocyclic chemistry III". Elsevier Ltd., **2008**, *8*, 373-459.

Los compuestos heterocíclicos derivados de benzoxazina forman un grupo importante de heterociclos benzofusionados con un amplio espectro de actividades biológicas. Además, el núcleo de benzoxazina no solo está presente en muchas moléculas farmacológicamente activas, y productos naturales, sino que también se han utilizado como intermedios para la síntesis de compuestos bioactivos.¹⁹² Varias 1,3-benzoxazinas (Figura 13) muestran interesantes propiedades biológicas y farmacológicas tales como, antiinflamatorias,¹⁹³ analgésicas,¹⁹⁴ como neuroprotectores,¹⁹⁵ actividad antagonista del receptor D2,¹⁹⁶ antivirales,¹⁹⁷ antifúngicas¹⁹⁸ y antitumorales.¹⁹⁹ Además, estos derivados son muy valiosos en la química de los productos naturales debido a la formación de acetal-glucósidos en plantas,²⁰⁰ que actúan como factor de auto resistencia de plantas hacia insectos, plagas, hongos.

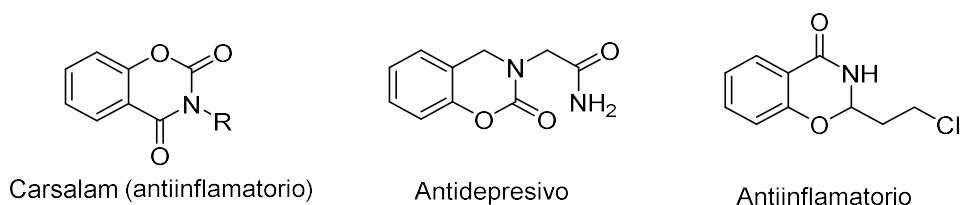
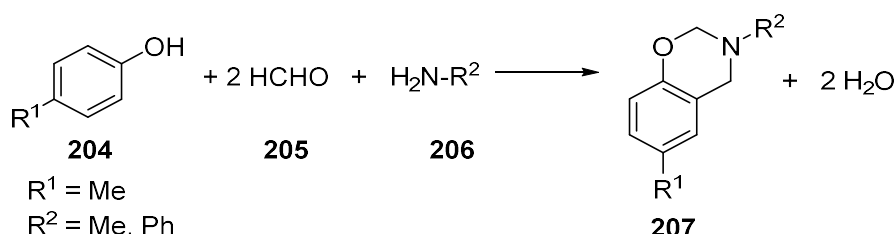


Figura 13. Ejemplos de 1,3-benzoxazinas con actividad farmacológica.

- ¹⁹² A. Váradi, T. C. Palmer, P. R. Notis, G. N. R. Traub, D. Afonin, J. J. Subrath, G. W. Pasternak, C. Hu, I. Sharma, S. Majumdar. "Three-component coupling approach for the synthesis of diverse heterocycles utilizing reactive nitrilium trapping". *Org. Lett.* **2014**, *16*, 1668-1671.
- ¹⁹³ A. Khalaj, M. Abdollahi, A. Kebriaeezadeh, N. Adibpour, Z. Pandi, S. Rasoulamini. "The antinociceptive and antiinflammatory activities and lack of ulcerogenicity of a benzodioxin-4-one and its analog benzoxazine as cyclic acetal-like derivatives of salicylic acid and salicylamide in mice and rats". *Ind. J. Pharmacol.* **2002**, *34*, 184-188.
- ¹⁹⁴ N. Gokhan, H. Erdogan, N. T. Durlu, R. Demirdamar. "Analgesic activity of acylated 2-benzoxazolinone derivatives". *Farmaco* **1999**, *54*, 112-115.
- ¹⁹⁵ K. Kamei, N. Maeda, K. Nomura, M. Shibata, R. K. Ogino, M. Koyama, M. Nakajima, T. Inoue, T. Ohno, T. Tatsuoka. "Synthesis, SAR studies, and evaluation of 1,4-benzoxazepine derivatives as selective 5-HT1A receptor agonists with neuroprotective effect: discovery of Piclozotan". *Bioorg. Med. Chem.* **2006**, *14*, 1978-1992.
- ¹⁹⁶ J. Ilas, P. S. Anderluh, M. S. Dolenc, D. Kikelj. "Recent advances in the synthesis of 2H-1,4-benzoxazin-3-(4H)-ones and 3,4-dihydro-2H-1,4-benzoxazines". *Tetrahedron* **2005**, *61*, 7325-7348.
- ¹⁹⁷ S. Wittmann, I. S. Hofmann, U. Mollmann, D. Ankel-Fuchs, L. Heinisch. "8-Acyloxy-1,3-benzoxazine-2,4-diones as siderophore components of antibiotics". *Arzneim. Forsh.* **2000**, *50*, 752-757.
- ¹⁹⁸ K. Waissner, L. Kubicova, V. Buchta, P. Kubanova, K. Bajerova, L. Jiraskova. "In vitro antifungal activity of 3-phenyl-2H-benzoxazine-2,4(3H)-diones". *Folia Microbiol.* **2002**, *47*, 488-492.
- ¹⁹⁹ M. E. Kuehne, E. A. Konopk. "Dihydro-1,3-oxazines as antitumor agents". *J. Med. Chem.* **1962**, *5*, 257-280.
- ²⁰⁰ D. Sicker, M. Schulz. *Benzoxazinones in plants: occurrence, synthestic access, and biological activity* In "Studies in natural products chemistry". Ed. A. Ur. Rahman, Elsevier, **2002**, *27*, 185-232.

En cuanto a la síntesis de benzoxazinas, los monómeros de dihidro-1,3-benzoxazina **207** se preparan no solo por los métodos tradicionales de condensación de Mannich²⁰¹ de fenoles **204**, aminas **206** y formaldehído **205** (Esquema 61), sino también mediante reacciones de cicloadición.²⁰²



Esquema 61. Síntesis de 1,3-benzoxazinas mediante la reacción de Mannich.

Guo y col^{202b} desarrollaron una cicloadición [3 + 3] promovida por ácido fosfórico **210** de iluros de azometino **209** con quinona monoimina **208** (Esquema 62). La reacción se desarrolla en condiciones suaves para dar una serie de derivados de 2,3-dihidro-benzoxazina **211** con rendimientos entre moderados y excelentes.

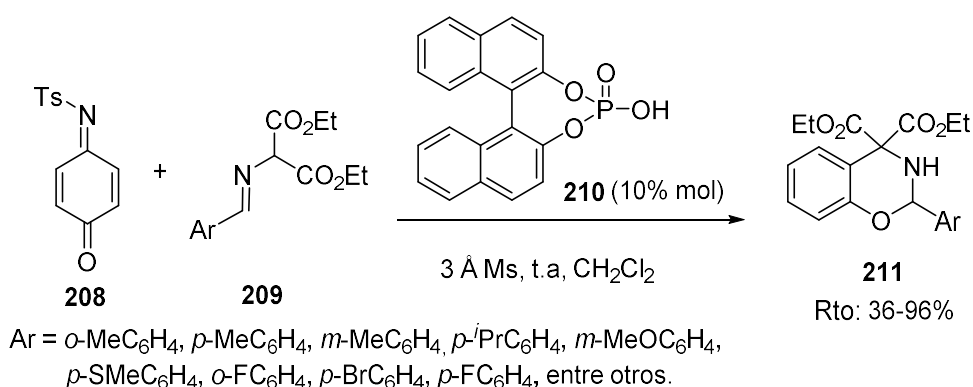
Por otro lado, la síntesis de moléculas híbridas y su evaluación, por sus interesantes aplicaciones como agentes farmacológicos está en constante aumento durante las dos últimas décadas. Las moléculas híbridas²⁰³ se definen, como entidades químicas con dos o más sitios

²⁰¹ a) W. J. Burke. "3,4-Dihydro-1,3-2H-benzoxazines. Reaction of *p*-substituted phenols with *N,N*-dimethylolamines". *J. Am. Chem. Soc.* **1949**, *71*, 609-612. b) J. Wang, H. Wang, J. T. Liu, W. Bin Liu, X. De Shen. "Synthesis, curing kinetics and thermal properties of novel difunctional chiral and achiral benzoxazines with double chiral centers". *J. Therm. Anal. Calorim.* **2013**, *114*, 1255-1264. c) J. Liu, G. Yuan. "The modified-Mannich reaction: conversion of arylboronic acids and subsequent coupling with paraformaldehyde and amines toward the *one-pot* synthesis of Mannich bases and benzoxazines". *Tetrahedron Lett.* **2017**, *58*, 1470-1473.

²⁰² a) H. Sugimoto, S. Nakamura, T. Ohwada. "Generation and application of *o*-quinone methides bearing various substituents on the benzene ring". *Adv. Synth. Catal.* **2007**, *349*, 669-679. b) Y. Wu, G. Qiao, H. Liu, L. Zhang, Z. Sun, Y. Xiao, H. Guo. "Brønsted acid-promoted [3 + 3] cycloaddition of azomethine ylides with quinone monoimine: A practical method towards dihydrobenzoxazine derivatives". *RSC Adv.* **2015**, *5*, 84290-84294. c) L. Zhang, M. Shi, F. Wang. "Practical way for the synthesis of 4*H*-benzo[*e*][1,3]oxazine by anhydride-promoted [4+2] cyclization of *in situ* generated *o*-quinone methides with amides". *Tetrahedron Lett.* **2020**, *61*, 151965-151971.

²⁰³ a) B. Meunier. "Hybrid molecules with a dual mode of action: dream or reality?". *Acc. Chem. Res.* **2008**, *41*, 69-77. b) D. Decker. "Hybrid molecules incorporating natural products: applications in cancer therapy, neurodegenerative disorders and beyond". *Curr. Med. Chem.* **2011**, *18*, 1464-1475. c) Shaveta, S. Mishra, P. Singh. "Hybrid molecules: The privileged scaffolds for various pharmaceuticals". *Eur. J. Med. Chem.* **2016**, *124*, 500-536.

bioactivos que poseen diferentes funciones biológicas y la actividad dual consiste en dos sustancias farmacológicas en una sola molécula y por ello, el nuevo compuesto híbrido sintético es más efectivo farmacológicamente que sus componentes individuales o sus productos metabólicos.²⁰⁴ Por lo tanto, los compuestos híbridos se describen como entidades químicas que combinan al menos dos farmacóforos a través de un enlace covalente.^{203a} En general, dos farmacóforos se pueden unir directamente mediante un enlace covalente estable (por ejemplo, amina, amida) o mediante un conector que también puede agregar una función solubilizante específica al compuesto final.



Esquema 62. Síntesis de derivados de dihidro benzoxazina a través de cicloadición [3+3] promovida por un ácido de Brønsted.

Las moléculas híbridas son más grandes que las dos entidades consistentes individuales, y generalmente son más lipofílicas. Por lo tanto, el conector solubilizante debe ser lo más sencillo posible.²⁰⁵

En comparación con la combinación de múltiples fármacos, las ventajas de las moléculas híbridas pueden establecerse de la siguiente manera: 1) disminución del riesgo de desarrollar resistencia a los medicamentos; y 2) mejorar las características farmacocinéticas y la disminución de los efectos adversos tóxicos en comparación con la administración de múltiples

²⁰⁴ A. Peperidou, D. Kapoukranidou, C. Kontogiorgis, D. HadjipavlouLitina. "Multitarget molecular hybrids of cinnamic acids". *Molecules* **2014**, *19*, 20197-20226.

²⁰⁵ R. Oliveira, D. Miranda, J. Magalhaes, R. Capela, J. M. Perry, M. P. O'Neill, R. Moreira, F. Lopes. "From hybrid compounds to targeted drug delivery in antimalarial therapy". *Bioorg. Med. Chem.* **2015**, *23*, 5120-5130.

agentes. Además de estas ventajas, los compuestos híbridos pueden mejorar el cumplimiento del tratamiento por parte del paciente y reducir el costo del tratamiento.²⁰⁶

Desde un punto de vista sintético, no se han descrito en la bibliografía metodologías para la preparación de híbridos fusionados de azirino[2,1-*b*]benzo[*e*][1,3]oxazina con grupos fosforados derivados de óxido de fosfina o fosfonato. Estas nuevas moléculas híbridas, que pueden retener las propiedades funcionales de las moléculas, se obtienen mediante la conexión del fragmento de 1,3-benzoxazina con el anillo de aziridina, directamente unidos al sustituyente fosforado.

En nuestro caso, la síntesis de nuevos híbridos fusionados de azirino[2,1-*b*]benzo[*e*][1,3]oxazina con grupos fosforados (Figura 14), se lleva a cabo mediante la adición nucleofílica de fenoles funcionalizados a 2*H*-azirinas fosforadas (Figura 14), empleando trietilamina como base. Hasta donde sabemos, este es el primer ejemplo de preparación de azirino[2,1-*b*]benzo[*e*][1,3]oxazinas así como el estudio de la actividad como agentes antiproliferativos.

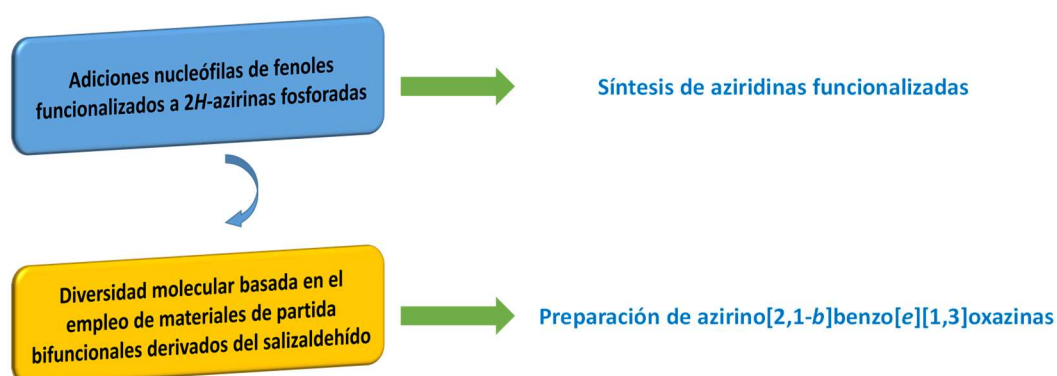


Figura 14. Diversidad molecular basada en reacciones de adición de fenoles a 2*H*-azirinas y reacciones secundarias derivadas de grupos funcionales compatibles con la adición nucleofílica.

OBJETIVO 4: Adición de nucleófilos oxigenados y azufrados a 2*H*-azirinas fosforadas.

Siguiendo con nuestro interés en la preparación de nuevas familias de compuestos fosfonitrogenados, se lleva a cabo la utilización de las 2*H*-azirinas 2-fosforadas como intermedios sintéticos en la preparación de nuevas estructuras acíclicas y cíclicas funcionalizadas

²⁰⁶ E. Kucuksayan, T. Ozben. "Hybrid compounds as multitarget directed anticancer agents". *Curr. Top. Med. Chem.* **2017**, *17*, 907-918.

como derivados aminofosforados o aziridinas fosforadas, mediante el empleo de nucleófilos oxigenados y azufrados.

Es conocida la aplicación de las azirinas carboxiladas como precursores de aminoácidos no protéicos y que forman parte de la estructura de compuestos con actividad biológica como antibióticos naturales y antitumorales. Sin embargo, es conveniente destacar su interés sintético tanto por su reactividad como nucleófilos y electrófilos, como por su utilidad como sintones en la preparación de heterociclos y aminas funcionalizadas.^{107a,107b,108,109b} Por otro lado, la sustitución isostérica de un grupo ácido carboxílico por un grupo fosforado permite acceder a nuevas familias de azirinas y a otros compuestos fosfonitrogenados de gran importancia en química médica, dada la importancia de sustituyentes fosforados en la regulación de funciones biológicas y a su capacidad para incrementar la actividad terapéutica de otros compuestos utilizados como fármacos.^{152a,152b,152c,152d,152e,154c}

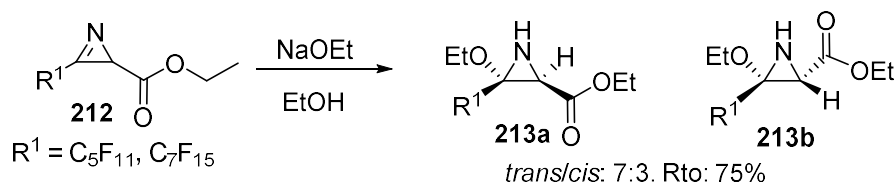
Por todo ello, nos planteamos la posibilidad de acceder a otras aziridinas saturadas que contienen sustituyentes fosforados mediante la adición de reactivos nucleófilos oxigenados y azufrados a 2*H*-azirinas fosforadas.

Existen en la bibliografía ejemplos de adiciones a 2*H*-azirinas, tanto de nucleófilos oxigenados tales como alcoholes alifáticos y aromáticos, así como de nucleófilos azufrados tales como tioles. Es conocido que la presencia de grupos alquilo fluorados en aziridinas y azirinas aumenta la estabilidad del anillo. Un ejemplo que representa la resistencia a la apertura del anillo aparece reflejado en el esquema 63. Mediante la adición de EtOH a la azirina carboxilada **212**, se obtiene una mezcla estereoisomérica *cis/trans* 7:3 de aziridinas **213a** y **213b** con un rendimiento del 75%.²⁰⁷

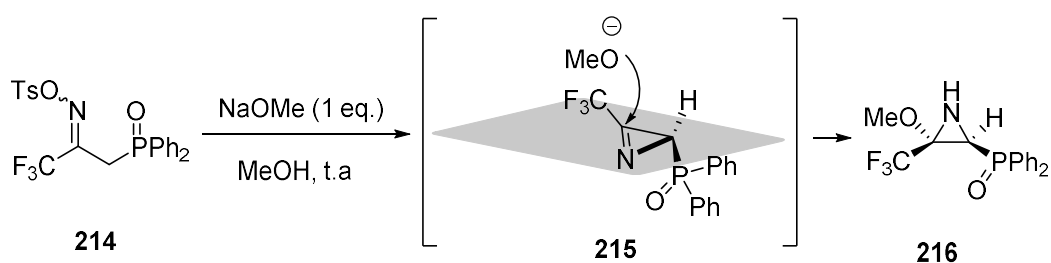
En otro ejemplo representativo, la azirina **215** obtenida mediante la reacción de Neber a partir de la tosiloxima **214**, reacciona con metóxido sódico en MeOH a temperatura ambiente (Esquema 64). El ataque nucleófilo tiene lugar por la cara menos impedida proporcionando

²⁰⁷ a) M. Haddach, R. Pastor, J. G. Riess. "Abnormal stability and reactivity of perfluoroalkyl azirines and aziridines". *Tetrahedron Lett.* **1990**, *31*, 1989-1990. b) M. Haddach, R. Pastor, J. G. Riess. "Synthesis and reactivity of novel synthons of perfluoroalkyls. A typical behavior of F-alkyl azirines and aziridines". *Tetrahedron* **1993**, *49*, 4627-4638. c) P. L. Coe, M. I. Cook. "Reactions of tetrafluoroethene oligomers. Part 16. The synthesis and reactions of perfluoro[2,3-dimethyl-2-(3-methylpentan-3-yl)]-2*H*-azirine: a source of 2,3-bis(trifluoromethyl)aziridine derivatives". *J. Chem. Soc., Perkin Trans. 1.* **2000**, 1537-1539.

diastereoselectivamente la aziridina funcionalizada **216** con un rendimiento del 53% (Esquema 64).^{84e}



Esquema 63. Síntesis diastereoselectiva de *cis/trans*-aziridinas a través de la adición nucleófila de etanol.



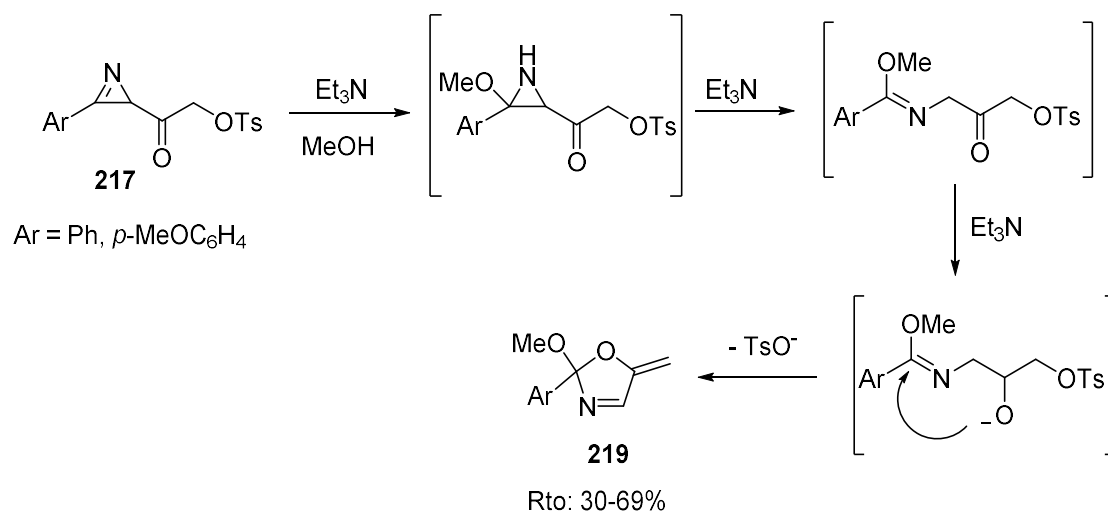
Esquema 64. Síntesis diastereoselectiva de la *trans*-aziridina fluorada **216**.

Cuando se trataron 2-(2-(tosiloxi)acetil) azirinas **217** con Et_3N a reflujo de metanol, se aislaron metilenooxazolinas **218**, que son los productos de la reacción en cascada que implican una apertura del anillo de azirina a través del enlace C2-C3 (Esquema 65).²⁰⁸

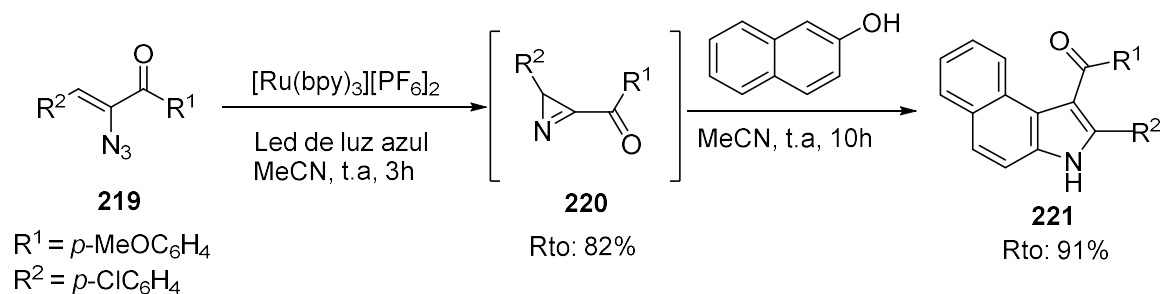
Recientemente, el grupo de Maurya²⁰⁹ ha desarrollado una metodología eficiente para la síntesis de pirroles fusionados **221** (Esquema 66), mediante la adición nucleófila de 2-naftol a 2*H*-azirinas **220** en acetonitrilo, las cuales se obtienen a través de la descomposición fotosensibilizada de las correspondientes α -azidoalconas **219**.

²⁰⁸ P. A. Sakharov, M. S. Novikov, A. F. Khlebnikov. "2-Diazoacetyl-2*H*-azirines: source of a variety of 2*H*-azirine building blocks with orthogonal and domino reactivity". *J. Org. Chem.* **2018**, *83*, 8304-8314.

²⁰⁹ S. Borra, D. Chandrasekhar, U. D. Newar, R. A. Maurya. "Access to 2,3-fused pyrroles via visible light driven coupling of α -azidoalcones with 1/2-naphthols, or 2-hydroxy-1,4-naphthoquinone". *J. Org. Chem.* **2019**, *84*, 1042-1052.



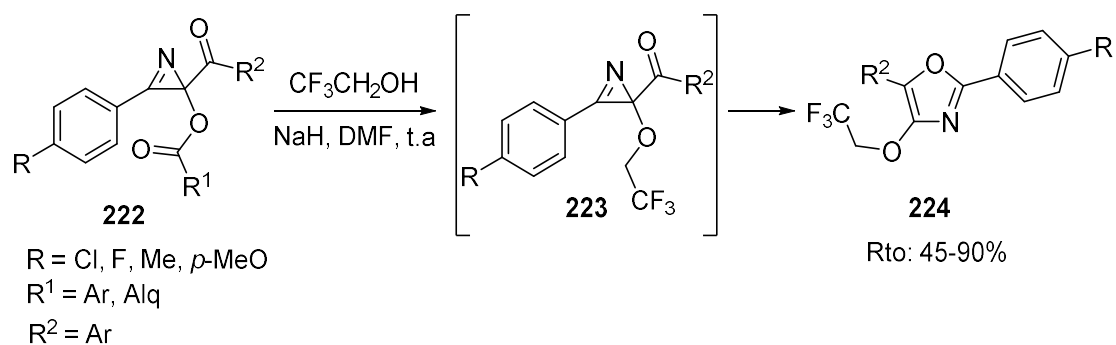
Esquema 65. Síntesis de metilenooxazolinas a partir de 2-(2-(tosiloxi)acetil) azirinas.



Esquema 66. Síntesis de benzoindol a través de la adición nucleófila de 2-naftol a 2*H*-azirinas.

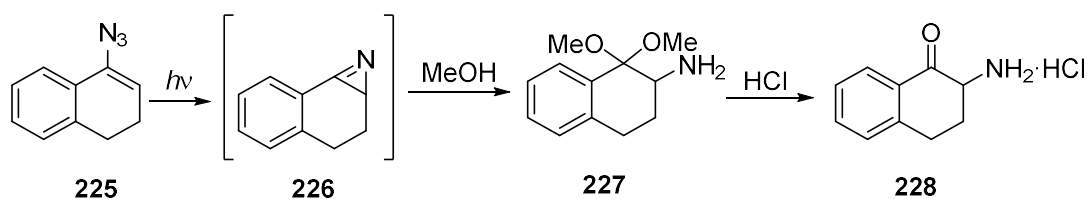
La sustitución nucleófila mediada por NaH del grupo aciloxi en C-2 de azirinas **222** por el grupo 2,2,2-trifluoroetoxietanol, con una expansión posterior del anillo de azirina **223** se empleó para la síntesis de 4-alcoxioxazoles **224** (Esquema 67).²¹⁰

²¹⁰ X. Duan, K. Yang, J. Lu, X. Kong, N. Liu, J. Ma. "Base-mediated cascade substitution-cyclization of 2*H*-azirines: access to highly substituted oxazoles". *Org. Lett.* **2017**, *19*, 3370-3373.



Esquema 67. Síntesis de 4-alcioxazoles a partir de 2-acyloxiazirinas.

El esquema 68, muestra un ejemplo clásico en el que la azirina **226**, obtenida por fotólisis del α -azido alqueno **225**, reacciona con dos equivalentes de MeOH generando el acetal **227**, que con la posterior hidrólisis se convierte en clorhidrato de α -aminocetona **228** (Esquema 68).²¹¹

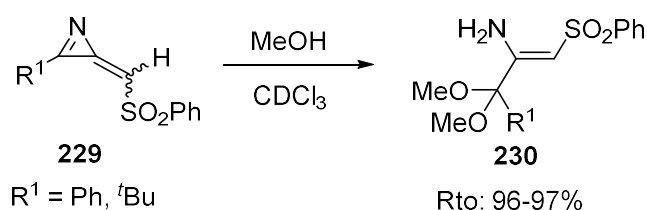


Esquema 68. Síntesis de acetales a partir de azirinas.

Asimismo, también se obtienen acetales de 2-aminoacroleína **Z 230** con alto rendimiento a partir de metileno-2*H*-azirinas **229** mediante una reacción regio controlada en C-3 por adición nucleófila de MeOH (Esquema 69).²¹²

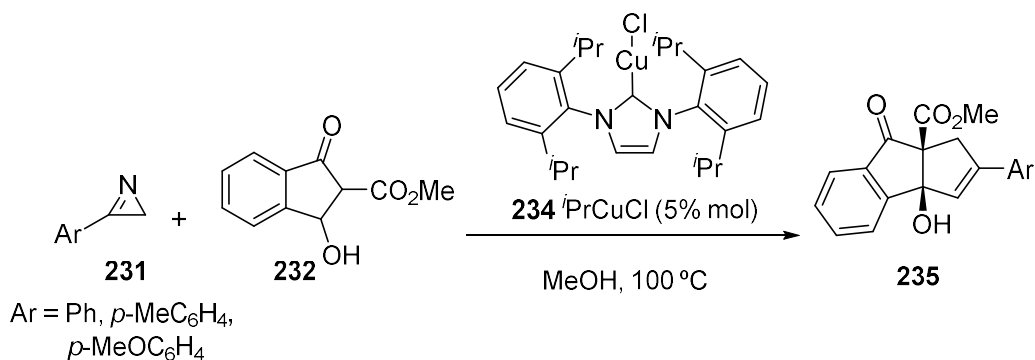
²¹¹ A. Hassner, F. W. Fowler. "Stereochemistry. XXXII. Synthesis and reactions of 1-azirines". *J. Chem. Soc.* **1968**, 90, 2869-2875.

²¹² J. R. Fotsing, K. Banert. "New way to methylene-2*H*-azirines and their use as powerful intermediates for the stereo- and regioselective synthesis of compounds with vinylamine substructure". *Eur. J. Org. Chem.* **2006**, 3617-3625.



Esquema 69. Síntesis de acetales de 2-aminoacroleína mediante adición de MeOH.

Novikov y col.,²¹³ emplean el enol **232** como sustrato de partida para la síntesis de indenos [1,2-*b*] pirroles **234**. La reacción de enol **232** con azirinas **231** en presencia de ⁱPrCuCl **233** proporciona indenopirroles **234** con rendimientos del 56-70% (Esquema 70).

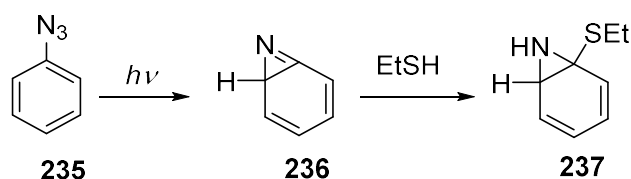


Esquema 70. Síntesis de indenopirroles a partir de 2*H*-azirinas catalizada por Cu(I).

La utilización de tioles como reactivos nucleófilos frente a 2*H*-azirinas también se estudia como procedimiento para la preparación de aziridinas funcionalizadas. A través de nucleófilos azufrados alifáticos, como etanotiol, se sintetizan aziridinas bicíclicas **237** a partir de benzazirinas **236**, obtenidas estas últimas a través de la fotólisis de la vinil azida **235**. (Esquema 71).²¹⁴

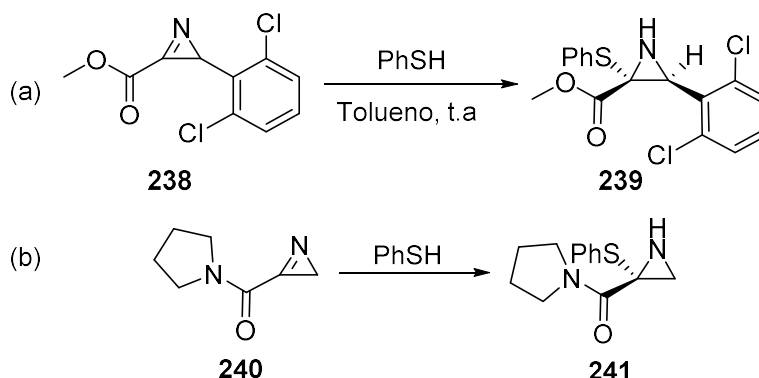
²¹³ P. A. Sakharov, N. V. Rostovskii, A. F. Khlebnikov, M. S. Novikov. "Annulation of five-membered cyclic enols with 3-aryl-2*H*-azirines: Catalytic versus non-catalytic cycloaddition". *Tetrahedron* **2017**, *76*, 4663-4670.

²¹⁴ S. E. Carroll, B. Nay, E. F. V. Striven, R. Suschitzky, D. R. Thomas. "Descomposition of aromatic azides in ethanethiol". *Tetrahedron Lett.* **1977**, *18*, 3175-3178.



Esquema 71. Síntesis de aziridinas mediante adición nucleófila de etanol.

Asimismo, la 2-sulfanilaziridina-2-carboxilada **239** se obtiene a partir de la alquil 2*H*-azirina-3-carboxilada **238** mediante el empleo de tiofenol a temperatura ambiente en tolueno (Esquema 72a).²¹⁵ Por otro lado, la 2*H*-azirina-3-pirrolidincarboxamida **240** se comporta de manera similar proporcionando la aziridina **241** por reacción con tiofenol (Esquema 72b).²¹⁶



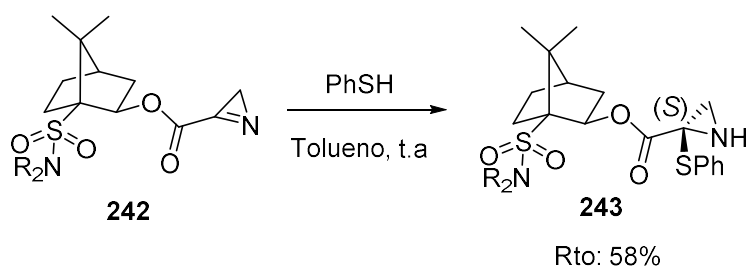
Esquema 72. Adición de tiofenol a 2*H*-azirinas.

Con objeto de obtener aziridinas ópticamente puras **243**, se desarrolla la adición diastereoselectiva de tioles a la 2*H*-azirina 3-carboxilada conteniendo un auxiliar quiral. Así, la 2*H*-azirina 3-carboxilada derivada de (1*R*)-10-(*N,N*-dialquilsulfamoil)isobornilo **242** reacciona con tiofenol en tolueno y a temperatura ambiente para conducir a la correspondiente aziridina enantioméricamente pura con un 58% de rendimiento (Esquema 73).²¹⁷

²¹⁵ M. J. Alves, T. L. Gilchrist, J. H. Sousa. "Reactions of methyl 2-aryl-2*H*-azirine-3-carboxylates with nucleophiles". *J. Chem. Soc., Perkin Trans. 1*. **1999**, 1305-1310.

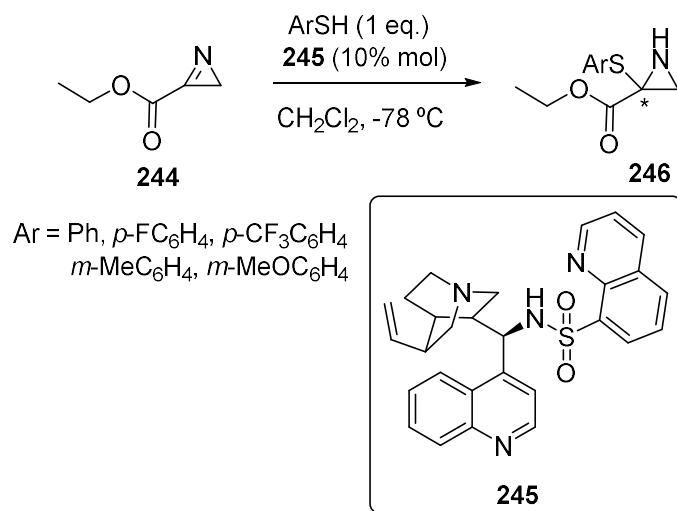
²¹⁶ T. L. Gilchrist, R. Mendonça. "Synthesis and Diels-Alder reactions of 2*H*-azirine-3-carboxamides". *Arkivoc* **2000**, 5, 769-778.

²¹⁷ a) M. J. Alves, J. F. Bickleya, T. L. Gilchrist. "Diastereoselectivity in the addition and cycloaddition reactions of a chiral ester of 2*H*-azirine-3-carboxylic acid". *J. Chem. Soc., Perkin Trans. 1*, **1999**, 1399-



Esquema 73. Síntesis de la aziridina quiral **73** mediante adición de tiofenol a la *2H*-azirina **72**.

Nakamura y col.,²¹⁸ han desarrollado una síntesis altamente enantioselectiva de aziridinas **246** mediante la adición de tioles a *2H*-azirinas **244** catalizada por *N*-sulfonil alcaloides derivados de cinchona **245**. Se emplean tioles con grupos electroattractores, como por ejemplo el *p*-fluorotiofenol (Esquema 74), así como tioles voluminosos y ricos en electrones, lo que les permite acceder a una gran variedad de aziridinas **246** con buenos rendimientos (80-97%) y altas enantioselectividades (72-96% *ee*).

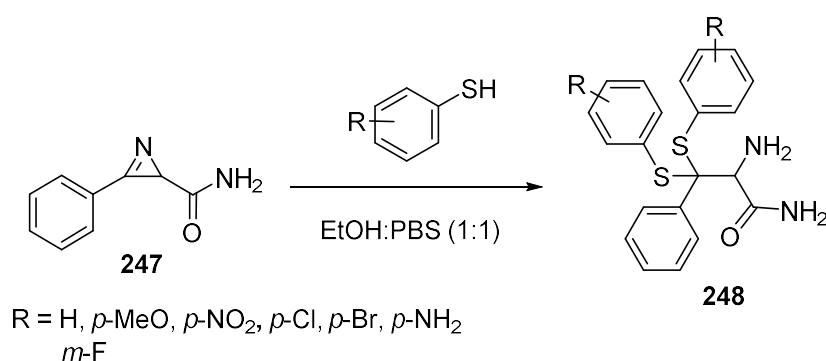


Esquema 74. Síntesis enantioselectiva de aziridinas mediante adición de tioles a *2H*-azirinas catalizada por un alcaloide de cinchona.

1401. b) Y. S. P. Álvares, M. J. Alves, N. G. Azoia, J. F. Bickley, T. L. Gilchrist. "Diastereoselective synthesis of aziridines from (1*R*)-10-(*N,N*-dialkylsulfamoyl)isobornyl *2H*-azirine-3-carboxylates". *J. Chem. Soc., Perkin Trans. 1*, **2002**, 1911-1919.

²¹⁸ S. Nakamura, D. Hayama, M. Miura, T. Hatanaka, Y. Funahashi. "Catalytic enantioselective reaction of *2H*-azirines with thiols using cinchona alkaloid sulfonamide catalysts". *Org. Lett.* **2018**, *20*, 856-859.

Recientemente, se ha explorado la adición nucleófila de tioles a 2*H*-azirinas-2-sustituidas **247**, lo que permite acceder a una amplia gama de tioacetales funcionalizados **248** (Esquema 75).²¹⁹ En general, la reacción tolera una amplia gama de tioles que incluye grupos funcionales como halógeno, nitro, amino o alcohol. Teniendo en cuenta los efectos electrónicos, los tiofenoles con grupos ricos en electrones reaccionan de manera más eficiente y dan los correspondientes tioacetales **248** con rendimientos del 92 al 94%, mientras que tiofenoles con grupos electrodeficientes conducen a tioacetales **248** con rendimientos entre moderados y buenos.



Esquema 75. Adición nucleófila de tioles a 2*H*-azirinas para la preparación de tioacetales.

En nuestro caso, la adición de reactivos nucleófilos como alcoholes y tioles a nuestras 2*H*-azirinas 2-fosforadas, puede conducir a la formación de nuevas familias de compuestos acíclicos y cíclicos funcionalizados como derivados de α o β -aminofosfonatos o aziridinas fosforadas funcionalizadas.

²¹⁹ Y. Chen, W. Yang, J. Wu, W. Sun, T. P. Loh, Y. Jiang. "2*H*-Azirines as potential bifunctional chemical linkers of cysteine residues in bioconjugate technology". *Org. Lett.* **2020**, *22*, 2038-2043.

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En esta memoria se trata de ampliar y optimizar la metodología utilizada por nuestro grupo de investigación, que ha permitido la preparación de una amplia gama de compuestos orgánicos nitrogenados acíclicos y cíclicos fosforados, mediante el empleo de compuestos heterocíclicos de tres eslabones, como son las 2*H*-azirinas.

Como se ha comentado previamente, la estructura de esta tesis doctoral se basa en 4 objetivos fundamentales, algunos de ellos abarcan algunos objetivos secundarios. Cada uno de estos objetivos principales se aborda en cada una de las publicaciones científicas que se incluyen en la memoria.

Una vez ampliadas y optimizadas las metodologías ya utilizadas por el grupo en la preparación de 2*H*-azirinas funcionalizadas en el átomo de carbono C-2 con un sustituyente fosforado, como se ha comentado en la sección anterior, se realiza un estudio de la reactividad de estos sistemas heterocíclicos frente a diferentes nucleófilos con el fin de preparar aziridinas fosforadas funcionalizadas.

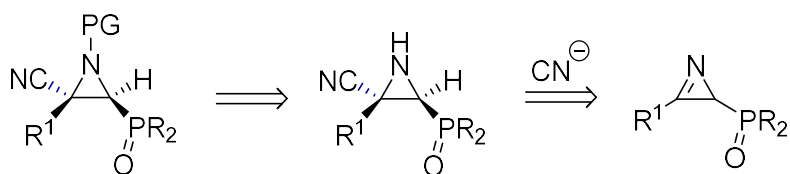
OBJETIVO 1: ESTUDIO DE LA REACTIVIDAD DE 2*H*-AZIRINAS FOSFORADAS FRENTE A NUCLEÓFILOS CARBONADOS COMO EL CIANOTRIMETILSILANO (TMSCN).

Este objetivo se aborda en el manuscrito 1: **“Synthesis and biological evaluation of cyanoaziridine phosphine oxides and phosphonates with antiproliferative activity”**.

Como se ha comentado previamente, el grupo nitrilo es un buen farmacóforo que se encuentra presente en un gran número de productos naturales jugando un importante papel en el desarrollo de nuevos medicamentos. La reacción de cianación de 2*H*-azirinas fosforadas puede conducir a la construcción de cianoaziridinas fósforo-funcionalizadas.

- 1.1. Para ello, se emplea como fuente de nitrilo el cianotrimetilsilano (TMSCN), cuya adición al doble enlace C=N de la 2*H*-azirina de partida, podría conducir a las cianoaziridinas fosforadas esperadas (Esquema 1).
- 1.2. Asimismo, y dentro del objetivo 1, también se aborda la activación del anillo de cianoaziridina mediante reacciones de *N*-acilación y *N*-tosilación (Esquema 1).

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Esquema 1. Esquema retrosintético para la síntesis de 3-cianoaziridinas-2-fosforadas y 3-cianoaziridinas-2-fosforadas N-funcionalizadas.

1.3. Una vez preparadas las correspondientes NH-cianoaziridinas y cianoaziridinas N-funcionalizadas fosforadas, se procede al análisis de su actividad antiproliferativa frente a diferentes líneas celulares de cáncer humano; adenocarcinoma de pulmón humano (A549) y células embrionarias de riñón humano (HEK293). Asimismo, se estudia el efecto citotóxico de los diferentes derivados en células no malignas (MRC-5).

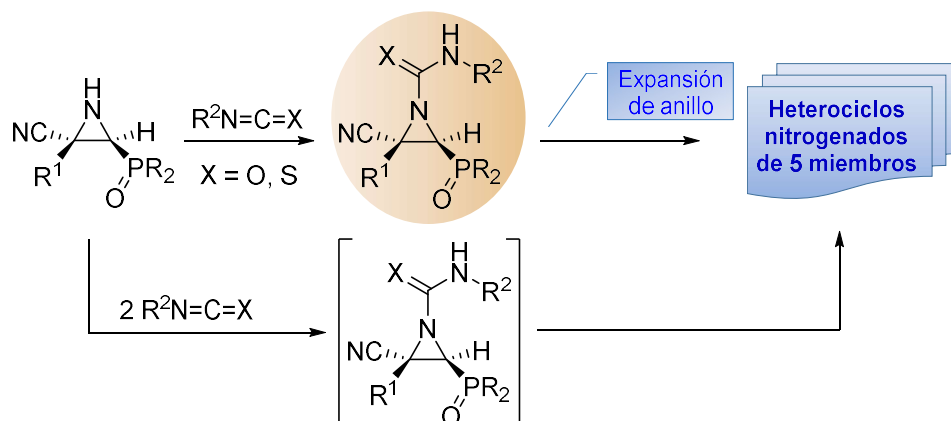
OBJETIVO 2: ESTUDIO DE LA REACTIVIDAD DE 3-CIANOAZIRIDINAS-2-FOSFORADAS FRENTE A ISO(TIO)CIANATOS

Este objetivo se aborda en el manuscrito 2: **“Novel synthesis and antiproliferative activity of phosphorus substituted 4-cyanooxazolines, 2-aminocyanooxazolines, 2-iminocyanooxazolidines, and 2-aminocyanothiazolines by rearrangement of cyanoaziridines”**.

Los avances recientes en la química de las aziridinas incluyen principalmente su aplicación en la síntesis de diversos compuestos heterocíclicos de gran importancia biológica. En los últimos años, se ha dedicado un esfuerzo significativo al estudio de la participación de aziridinas no solo en procesos de expansión de ciclo intramoleculares basados en ataques nucleofílicos, sino, además, como 1,3-dipolos enmascarados en cicloadiciones [3 + 2], con una gran variedad de dipolarófilos, proporcionando diversas rutas para la construcción de una amplia variedad de heterociclos funcionalizados de cinco miembros de gran utilidad en síntesis orgánica. En este tipo de cicloadiciones, el anillo experimenta una apertura seguida de ciclación con una variedad de reactivos, en presencia de un catalizador o sin catalizador.

2.1. Continuando con nuestro interés sobre la reactividad de las aziridinas, en este objetivo 2, se estudia en primer lugar la adición electrófila de iso(tio)cianatos a las cianoaziridinas fosforadas inactivadas, lo que nos permitiría desarrollar una

metodología sintética para la síntesis de nuevas cianoaziridinas fosforadas sustituidas *N*-funcionalizadas (Esquema 2).



Esquema 2. Síntesis de nuevas cianoaziridinas fosforadas *N*-funcionalizadas mediante el acoplamiento de cianoaziridinas inactivadas con iso(tio)cianatos. Expansión del anillo para la preparación de heterociclos nitrogenados de 5 miembros.

- 2.2. Asimismo, dentro de este objetivo 2, también se estudia la expansión del anillo en las cianoaziridinas *N*-funcionalizadas sintetizadas, para la preparación de nuevos heterociclos nitrogenados de 5 miembros, tras la reacción con iso(tio)cianatos.
- 2.3. Tras la síntesis de las nuevas cianoaziridinas fosforadas sustituidas *N*-funcionalizadas, así como de los nuevos heterociclos nitrogenados de 5 miembros sintetizados procedentes de la expansión del anillo, se estudia su actividad antiproliferativa en la línea celular de adenocarcinoma de pulmón humano (A549) y el efecto citotóxico de los diferentes derivados en células no malignas (MRC-5).

OBJETIVO 3: ADICIÓN NUCLEÓFILA DE FENOLES FUNCIONALIZADOS A 2H-AZIRINAS FOSFORADAS.

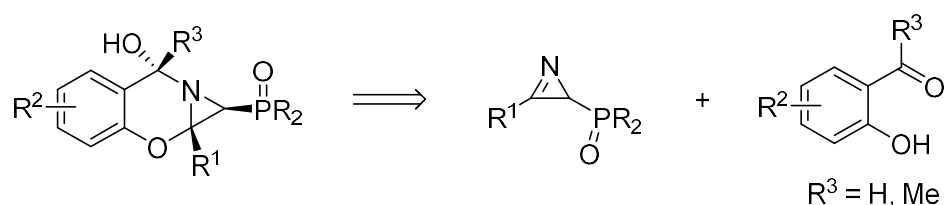
Este objetivo se aborda en el manuscrito 3: **“First synthesis of merged hybrids phosphorylated azirino[2,1-*b*]benzo[*e*][1,3]oxazine derivatives as anticancer agents”**.

El descubrimiento de fármacos basados en moléculas híbridas es una disciplina atractiva y emergente en química médica. La hibridación de moléculas biológicamente activas es una

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herramienta poderosa para el descubrimiento de fármacos que se emplea para tratar una gran variedad de enfermedades; por ejemplo, el cáncer, la malaria, la tuberculosis o el SIDA, entre otras. Por lo tanto, las moléculas híbridas pueden ser buenos candidatos a fármacos específicos, ya que permite proporcionar terapias combinadas en un único agente multifuncional y, hacerlos más específicos y potentes que los tratamientos clásicos convencionales.

- 3.1. Con el fin de obtener nuevos híbridos fusionados de azirino[2,1-*b*]benzo[*e*][1,3]oxazina con grupos fosforados, se estudia la adición nucleofílica de diferentes fenoles funcionalizados, tales como salizaldehídos funcionalizados ($R^3 = H$) o 2-hidroxiacetofenona ($R^3 = Me$), al doble enlace C–N de las 2*H*-azirinas fosforadas (Esquema 3).



Esquema 3. Esquema retrosintético para la síntesis de híbridos fusionados [1,3]benzoxazina-aziridina a partir de 2*H*-azirinas.

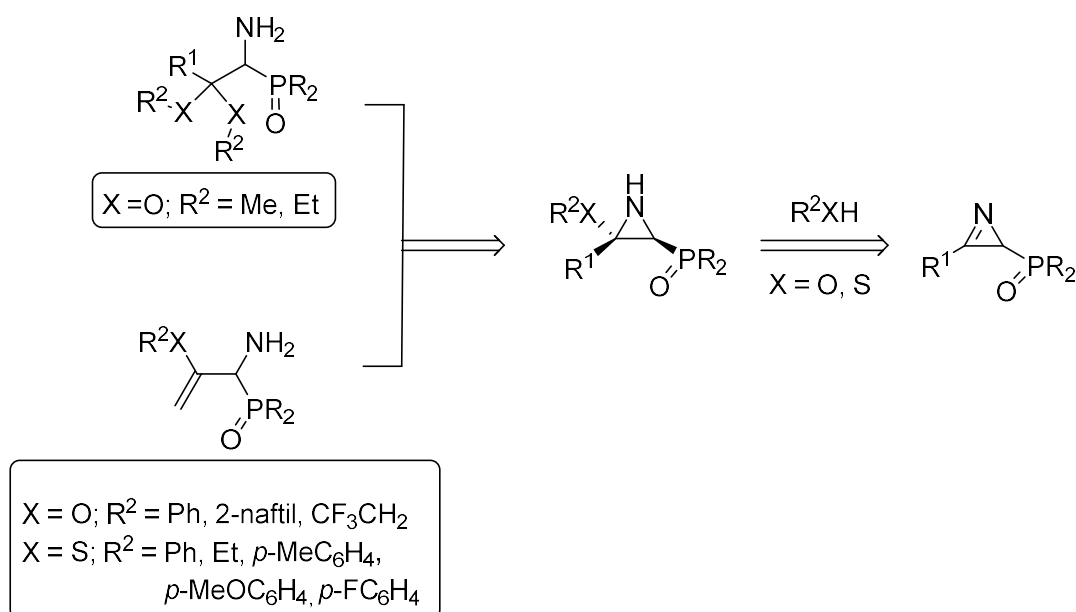
- 3.2. Una vez preparadas las correspondientes azirino[2,1-*b*]benzo[*e*][1,3]oxazinas fosforadas, se procederá al análisis de su actividad antiproliferativa frente a dos líneas diferentes de cáncer humano; adenocarcinoma de pulmón humano (A549) y células embrionarias de riñón humano (HEK293). Asimismo, se estudia el efecto citotóxico de los diferentes compuestos en células no malignas (MRC-5).

OBJETIVO 4: ESTUDIO DE LA REACTIVIDAD DE 2*H*-AZIRINAS FOSFORADAS FRENTE A REACTIVOS NUCLEÓFILOS OXIGENADOS Y AZUFRADOS.

Este objetivo se aborda en el manuscrito 4: “**Synthesis of α -aminophosphonic acid derivatives through the addition of *O*- and *S*-nucleophiles to 2*H*-azirines and their antiproliferative effect on A549 human lung adenocarcinoma cells**”.

Como se ha descrito en el apartado “Marco Teórico”, los α -aminofosfonatos son análogos de aminoácidos, que han encontrado una amplia gama de aplicaciones en las áreas de la química industrial, agrícola y médica debido a sus propiedades biológicas y físicas, así como a su utilidad como intermedios sintéticos.

- 4.1. Se desarrolla una metodología sintética para la preparación de nuevos derivados de α -aminofosfonatos y α -aminofosfanóxidos mediante apertura selectiva del anillo de aziridina, a través del empleo de nucleófilos tanto oxigenados como azufrados. En función de la naturaleza del nucleófilo se pueden obtener distintos compuestos, tales como derivados de α -aminofosforados funcionalizados, derivados de aziridinas, así como α -aminofosfonatos y fosfanóxidos alílicos (Esquema 4).



Esquema 4. Síntesis de derivados de ácidos α -aminofosfónicos mediante la adición de nucleófilos oxigenados y azufrados a 2*H*-azirinas fosforadas.

- 4.2. Asimismo, se estudia la *N*-funcionalización de estos intermedios derivados de fosfonatos y fosfanóxidos a través de la reacción de sulfonilación, para la obtención de los correspondientes *N*-tosil- α -aminofosfonatos y fosfanóxidos, y *N*-tosil- α -aminofosfonatos y fosfanóxidos alílicos.

Objetivos

- 4.3. Una vez preparados los correspondientes derivados α -aminofosforados, se procede al análisis de su actividad antiproliferativa en la línea celular de adenocarcinoma de pulmón humano (A549). También se empleó la línea celular de carcinoma de colon humano (RKO) para evaluar la actividad antiproliferativa de algunos de los derivados α -aminofosforatos. Asimismo, se estudia el efecto citotóxico de los diferentes derivados en células no malignas (MRC-5).

Discusión de los resultados

La numeración de las figuras, esquemas y tablas en los siguientes manuscritos comenzará con el número 1

Manuscrito 1. Synthesis and biological evaluation of cyanoaziridine phosphine oxides and phosphonates with antiproliferative activity.

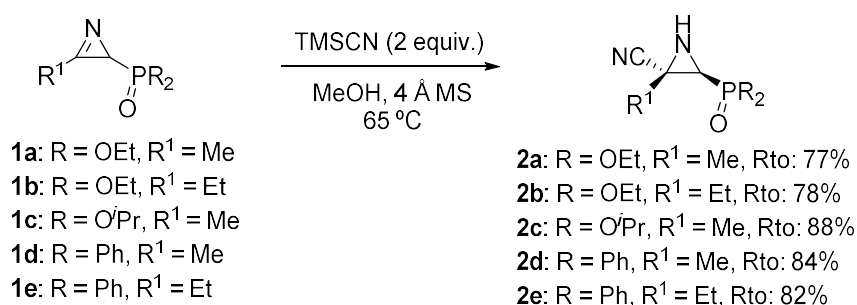
En este trabajo se describe una metodología sintética diastereoselectiva para la preparación de cianoaziridinas sustituidas con grupos fosforados mediante la adición nucleófila de cianotrimetilsilano (TMSCN), como fuente de cianuro, al doble enlace C-N de 2*H*-azirinas derivadas de óxidos de fosfina y fosfonatos. Asimismo, se estudia la actividad antiproliferativa tanto de las *NH*-cianoaziridinas como de las *N*-cianoaziridinas funcionalizadas fosforadas frente a diferentes líneas celulares de cáncer humano; adenocarcinoma de pulmón humano (A549) y células embrionarias de riñón humano (HEK293).

1.1 SÍNTESIS DE CIANOAZIRIDINAS FOSFORADAS

Como se ha puesto de manifiesto en el marco teórico, existen algunos antecedentes de la adición del grupo cianuro a 2*H*-azirinas. Por ejemplo, se ha empleado TMSCN como fuente de cianuro para preparar *N*-trimetilsilil cianoaziridinas. Asimismo, se ha descrito la adición de HCN a 2*H*-azirinas para la preparación de cianoaziridinas. Más recientemente, se han sintetizado cianoaziridinas con excelentes diastereoselectividades mediante una reacción de cianación empleando TMSCN como fuente de cianuro y un catalizador de Cu(I). Con el objeto de evitar la catálisis de metales de transición, así como el empleo de reactivos tóxicos como el HCN, nos propusimos el empleo de TMSCN/MeOH como fuente de cianuro.

Inicialmente se evaluó la reactividad de las 2*H*-azirinas fosforadas frente a TMSCN, y para ello se hizo reaccionar la 2*H*-azirina derivada de fosfonato de etilo **1a** (R = OEt, R¹ = Me) con TMSCN a reflujo de metanol y en presencia de tamiz molecular de 4 Å. Con el fin de asegurarnos una conversión completa, se emplearon 2 equivalentes de la fuente de cianuro. En estas condiciones de reacción, evitando el uso de catalizador, los espectros de ¹H RMN y ³¹P RMN del crudo de reacción revelaron la presencia de dos isómeros en una proporción 43:57. Por ejemplo, los desplazamientos químicos de ambos isómeros en el espectro de ³¹P RMN en CDCl₃, δ_P = 17.7 y 19.5 ppm, son diferentes y están relacionados respectivamente, con el isómero minoritario y mayoritario de la cianoaziridina **2a** (R = OEt, R¹ = Me) (Esquema 1).

Discusión de los resultados



Esquema 1. Adición de TMSCN a 2H-azirinas.

En el espectro de ¹H RMN se observa un singlete a $\delta_H = 1.69$ ppm para el grupo metilo y un doble doblete bien resuelto para el protón correspondiente al grupo metino a $\delta_H = 2.67$ ppm pertenecientes al isómero minoritario de la aziridina **2a**. Del mismo modo, en los datos de espectroscopía de correlación homonuclear (COSY), se observó una señal de cruce entre el NH y el protón metínico ($\delta_H = 1.88$ - 1.96) del anillo de aziridina. Por el contrario, el grupo metilo correspondiente al isómero mayoritario de **2a** se observa como un singlete a $\delta_H = 1.84$ ppm, mientras que el protón metínico resuena a $\delta_H = 2.30$ ppm como un doble doblete. De manera similar, en 2D-COSY se puede observar una señal de cruce entre el protón del NH (1.26-1.38) y el protón metínico del anillo de aziridina. Por todo ello, la presencia de ambos grupos de señales en los espectros de ¹H RMN y ³¹P RMN parece indicar la presencia de dos diastereoisómeros *E* y *Z* para la cianoaziridina **2a**. Debido a que el experimento 1D-NOESY no fue concluyente para confirmar la presencia de ambos diastereoisómeros, se realizó un experimento de ¹H RMN y ³¹P RMN a temperatura variable para el compuesto **2a**, observando que ambos compuestos se equilibran rápidamente a altas temperaturas (> 60 °C), ya que solo se aprecia un único grupo de señales (Figura 1), confirmando la presencia, por tanto, de dos rotámeros. La proporción de ambos rotámeros en el compuesto **2a** se ve afectada por los cambios de disolvente, ya que en disoluciones de CDCl₃, los espectros de ³¹P RMN mostraron dos señales diferentes con una proporción de rotámeros 43:57, mientras que cuando se emplearon disoluciones de **2a** en DMSO-*d*₆ se invirtió la relación de ambas señales mostrando una proporción de rotámeros de 78:22 (Figura 1). Este fenómeno puede atribuirse a las diferentes geometrías en la rotación del enlace C-P, previamente observadas, por ejemplo, en benzofulvenos con un sustituyente

fosfanóxido²²⁰ o en un estudio DFT más reciente, de los rotámeros más estables de la 2-metoxicarbonil aziridina.²²¹

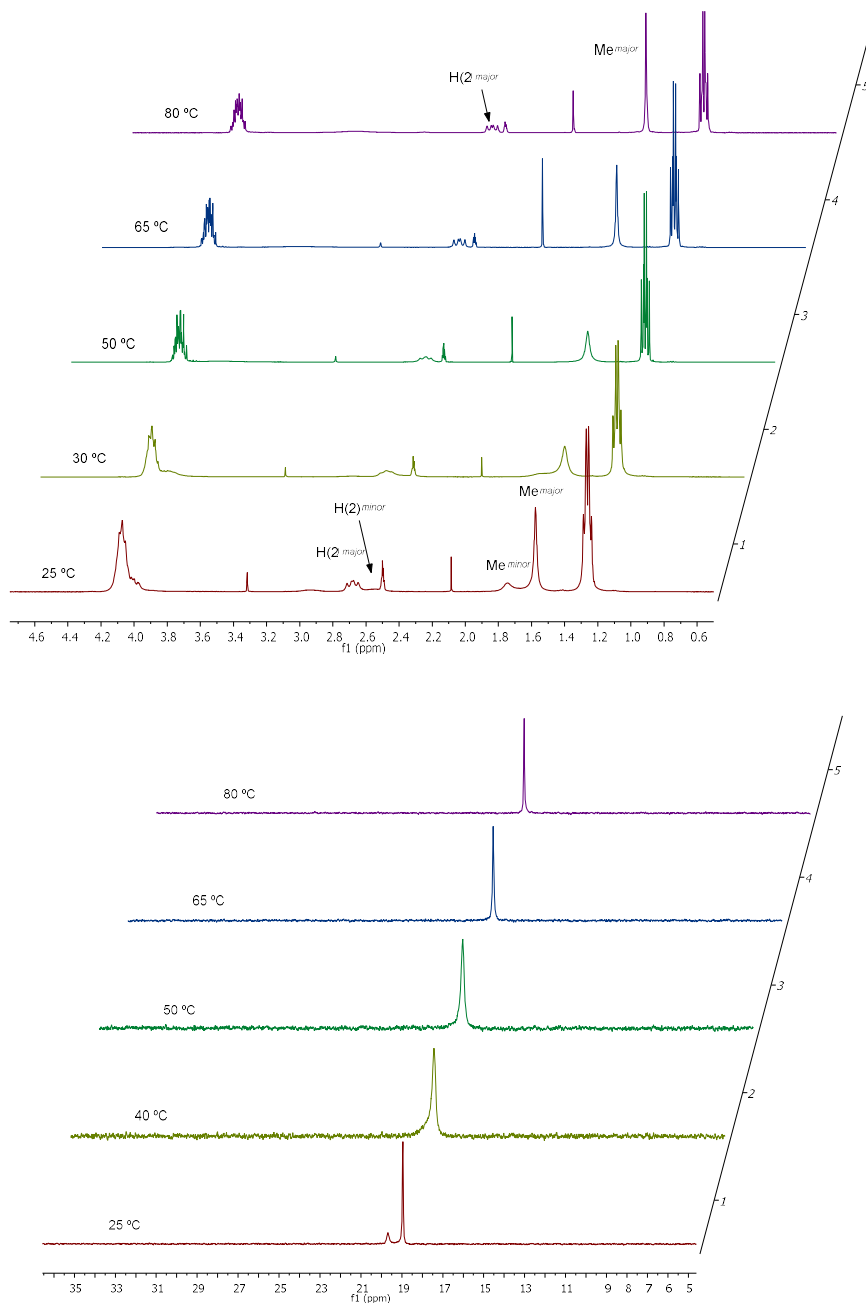


Figura 1. ¹H RMN (arriba) y ³¹P RMN (abajo) de 2a en DMSO-*d*₆ a diferentes temperaturas.

²²⁰ M. Shmittel, S. Kiau. "Thermal and electron transfer-induced reactions of enediynes and enyne-allenes, Part 5. First isolation of two rotamers about the P-C bond in a phosphine oxide". *Liebigs Ann. Recueil* **1977**, 733-736.

²²¹ S. Lopes, I. Reva, R. Fausto. "Infrared spectra and UV-induced photochemistry of methyl aziridine-2-carboxylate isolated in argon and xenón matrices". *Vib. Spectrosc.* **2015**, *81*, 68-82.

Discusión de los resultados

Por otro lado, la ausencia de constante de acoplamiento entre el carbono metílico en C-3 del anillo de aziridina **2a** y el átomo de fósforo del grupo fosfonato en C-2 en ^{13}C -RMN, parece indicar una relación *cis* entre ambos grupos y, por lo tanto, una estereoquímica *E* para el enlace sencillo C-C. Dicha estereoquímica *E* fue finalmente confirmada mediante de la difracción de rayos X (Figura 2).

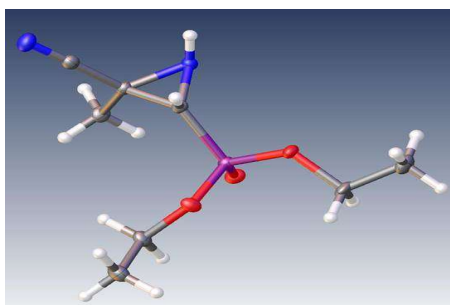


Figura 2. Diagrama ORTEP para la cianoaziridina **2a**.

La cianoaziridina derivada de fosfonato **2a** ($\text{R} = \text{OEt}$, $\text{R}^1 = \text{Me}$) se aisló con un rendimiento del 77% a partir de la 2*H*-azirina **1a**. Además, bajo las mismas condiciones de reacción se pudieron aislar las cianoaziridinas **2b** ($\text{R} = \text{OEt}$, $\text{R}^1 = \text{Et}$) y **2c** ($\text{R} = \text{O}^i\text{Pr}$, $\text{R}^1 = \text{Me}$) con un rendimiento del 78 y 88%, respectivamente, a partir de las 2*H*-azirinas funcionalizadas **1b** y **1c**. Ambas cianoaziridinas se obtuvieron como mezclas de dos rotámeros alrededor del enlace C-P, en unas proporciones de 49:51 para **2b** y 38:62 para **2c** (Esquema 1).

La adición nucleófila del anión cianuro de forma diastereoselectiva ha de provenir del ataque por la cara menos impedida del anillo de 2*H*-azirina. Por lo tanto, el acercamiento del grupo ciano al doble enlace imínico de la 2*H*-azirina, por el lado opuesto al grupo fosforado, parece ser más favorable, debido al elevado ángulo diédrico exocíclico del carbono hibridizado sp^3 y la presencia del grupo fosforado voluminoso (Figura 2).

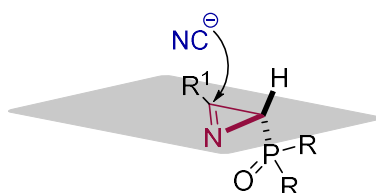
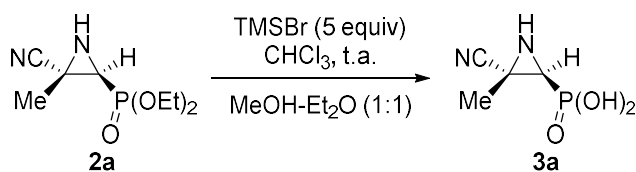


Figura 2. Adición nucleófila diastereoselectiva del grupo ciano a 2*H*-azirinas fosforadas.

Esta metodología se extendió a la adición nucleofílica de TMSCN a 2*H*-azirinas derivadas de óxidos de fosfina **1d-e** (R = Ph) bajo las mismas condiciones de reacción anteriormente mencionadas (Esquema 1). La *E*-cianoaziridina **2d** (R = Ph, R¹ = Me) se obtuvo con buen rendimiento y como un único rotámero (relación de rotámeros 0:100). Sin embargo, la *E*-cianoaziridina **2e** (R = Ph, R¹ = Et) se obtuvo como mezcla de dos rotámeros en una proporción de 19:81 (Esquema 1).

Debido al interés de los ácidos aminofosfónicos en química orgánica y médica,^{152d,222,223} se estudió la hidrólisis del grupo fosfonato en las correspondientes cianoaziridinas **2**, con el objeto de obtener los correspondientes derivados de ácidos aminofosfónicos. Así, la hidrólisis del dietil fosfonato **2a** (R = OEt, R¹ = Me) se llevó a cabo utilizando TMSBr en CHCl₃ con la posterior alcoholisis del silil éster intermedio en una mezcla de MeOH:Et₂O (1:1). De esta manera, se obtuvo la correspondiente cianoaziridina derivada del ácido fosfónico **3a** con un 80% de rendimiento (Esquema 2).

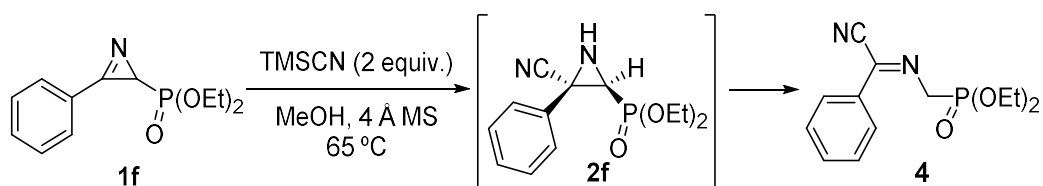


Esquema 2. Hidrólisis del grupo fosfonato en la cianoaziridina **2a**.

Se observó un comportamiento distinto en la adición nucleofílica de TMSCN a la 2*H*-azirina derivada de fosfonato de dietilo funcionalizada con un grupo arilo en C-3 (**1f**, R = OEt, R¹ = Ph, Esquema 3). Bajo las condiciones standard de reacción, la cianoaziridina **2f** no pudo ser aislada, y únicamente se obtuvo la correspondiente cianoimina **4** con un rendimiento de 72%. Este producto parece ser el resultado de la formación inicial de la cianoaziridina **2f** seguido de la apertura de anillo de aziridina a través de la ruptura del enlace C2-C3.

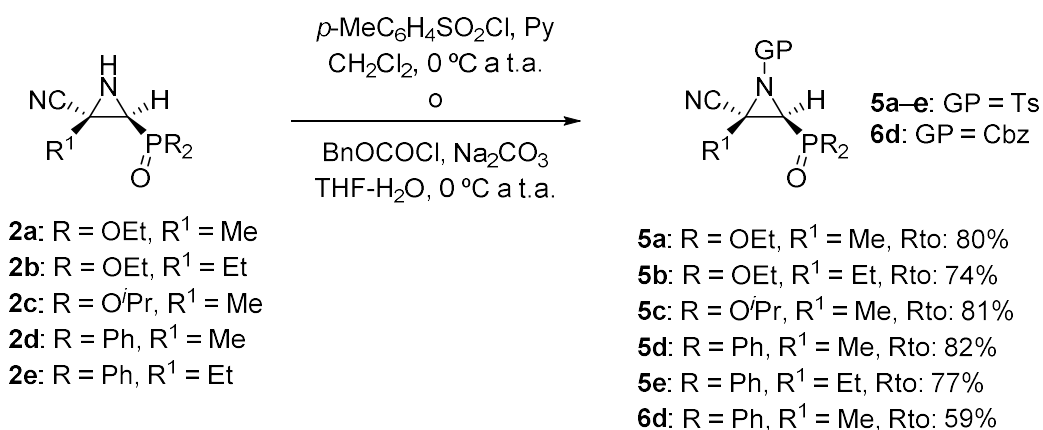
²²² K. Van der Jeught, C. V. Stevens. "Direct phosphorylation of aromatic azaheterocycles". *Chem. Rev.* **2009**, *109*, 2672-2702.

²²³ E. Weglarz-Tomczak, L. Berlicki, M. Pawelczak, B. Nocek, A. Joachimiak, A. Mucha. "A structural insight into the P1-S1 binding mode of diaminoethylphosphonic and phosphinic acids, selective inhibitors of alanine aminopeptidases". *Eur. J. Med. Chem.* **2016**, *117*, 187-196.



Esquema 3. Adición nucleófila de TMSCN a la 2H-azirina con sustituyente arilo.

Las aziridinas activadas que poseen grupos electroattractores en el átomo de nitrógeno de la aziridina, tales como grupos acilo o tosilo, pueden estabilizar la carga negativa sobre el átomo de nitrógeno, incrementando así la electrofilia del compuesto. De esta forma, se procedió a la protección del átomo de nitrógeno de las NH-cianoaziridinas **2** no activadas, empleando para ello grupos protectores como el tosilo (Ts) o benziloxicarbonil (Cbz). La reacción de las cianoaziridinas **2** con cloruro *p*-toluensulfonilo (TsCl) en presencia de piridina, en CH₂Cl₂ y a temperatura ambiente, proporcionó las *N*-Ts-cianoaziridinas **5** deseadas con buenos rendimientos. Por otro lado, la *N*-Cbz cianoaziridina **6d** se obtuvo por tratamiento de **2d** con cloroformiato de bencilo en presencia de Na₂CO₃ como base (Esquema 4).



Esquema 4. Síntesis de *N*-Ts y *N*-Cbz-cianoaziridinas fosforadas.

1.2 ESTUDIO DE LA ACTIVIDAD ANTIPROLIFERATIVA

La actividad antiproliferativa *in vitro* de los nuevos derivados preparados, se estudia frente a diferentes líneas celulares de cáncer humano: adenocarcinoma de pulmón humano (A549) y células embrionarias de riñón humano (HEK293). Con el fin de evaluar la inhibición del

crecimiento celular y las actividades inhibitoras de la proliferación celular como valores de IC₅₀ de todos los compuestos preparados (Tabla 1), se usa el ensayo de CCK-8. Estos resultados se comparan con la actividad antiproliferativa de la doxorubicina (DOX). Además, con el objeto de determinar citotoxicidad selectiva, también se determina la actividad antiproliferativa frente a una línea no cancerígena de células sanas como la MRC-5 (fibroblastos derivados de tejido pulmonar).²²⁴

Respecto a la línea celular A549 *in vitro*, las NH-cianoaziridinas derivadas de fosfonatos **2a-c** (Tabla 1, entradas 2-4), con valores de IC₅₀ entre 1.5 ± 0.84 y 4.3 ± 0.43 μM, muestran actividades similares a las NH-cianoaziridinas derivadas de óxidos de fosfina **2d-e** (Tabla 1, entradas 5-6), con valores de IC₅₀ entre 2.0 ± 0.13 and 3.3 ± 0.23 μM, siendo el compuesto más eficaz la cianoaziridina **2b** (R = OEt, R¹ = Et, GP = H) con un valor de IC₅₀ = 1.5 ± 0.84 μM (Tabla 1, entrada 2). Se puede observar una alta selectividad en la actividad antiproliferativa de las nuevas cianoaziridinas sintetizados **2a-e** (Tabla 1, entradas 2-6) y **3a** (Tabla 1, entrada 7) en la línea celular A549 *versus* la línea celular HEK293.

Tabla 1. Actividad antiproliferativa de las cianoaziridinas **2**, **3**, **5** y **6**.

Entrada	Comp.	R	R ¹	GP	Citotoxicidad IC ₅₀ (μM) ^[a]		
					Pulmón A549	Riñón HEK293	MRC-5
1	DOX	–	–	–	0.48 ± 0.017 ²²⁵	5.68 ± 1.56 ²²⁵	>50 ²²⁶
2	2a	OEt	Me	H	4.3 ± 0.43	>50	>50
3	2b	OEt	Et	H	1.5 ± 0.84	>50	>50
4	2c	O ⁱ Pr	Me	H	3.6 ± 0.08	>50	>50

²²⁴ R. Recio, E. Vengut-Climent, B. Mouillac, H. Orcel, M. L. Lázaro, J. M. Calderón-Montaño, E. Álvarez, N. Khiar, I. Fernández. "Design, synthesis and biological studies of a library of NK1-receptor ligands based on a 5-arylthiosubstituted 2-amino-4,6-diaryl-3-cyano-4H-pyran core: switch from antagonist to agonist effect by chemical modification". *Eur. J. Med. Chem.* **2017**, *138*, 644-660.

²²⁵ R. Gondru, R. Saini, K. Vaarla, S. Singh, N. Sirassu, R. Bavantula, A. K. Saxena. "Synthesis and characterization of chalcone-pyridinium hybrids as potential anti-cancer and anti-microbial agents". *Chemistry* **2018**, *3*, 1424-1431.

²²⁶ R. F. George. "Facile synthesis of simple 2-oxindole-based compounds with promising antiproliferative activity". *Future Med. Chem.* **2018**, *10*, 269-282.

Tabla 1 (Cont.). Actividad antiproliferativa de las cianoaziridinas **2**, **3**, **5** y **6**.

Entrada	Comp.	R	R ¹	GP	Citotoxicidad IC ₅₀ (μM) ^[a]		
					Pulmón	Riñón	MRC-5
					A549	HEK293	
5	2d	Ph	Me	H	2.0 ± 0.13	>50	>50
6	2e	Ph	Et	H	3.3 ± 0.23	>50	>50
7	3a	OH	Me	H	8.5 ± 0.57	>50	>50
8	5a	OEt	Me	Ts	n.r. ^[b]	>50	>50
9	5b	OEt	Et	Ts	4.9 ± 0.38	>50	>50
10	5c	O ⁱ Pr	Me	Ts	3.4 ± 0.36	>50	>50
11	5d	Ph	Me	Ts	3.1 ± 0.38	>50	>50
12	5e	Ph	Et	Ts	2.3 ± 0.96	43.2 ± 0.80	>50
13	6d	Ph	Me	Cbz	8.1 ± 0.73	30.1 ± 4.10	>50

^[a] Los valores de IC₅₀ corresponden a las concentraciones de los compuestos para las cuales se inhibe el crecimiento celular en un 50%. ^[b] No reportado.

A continuación, se estudia el efecto que la sustitución del átomo de nitrógeno en las correspondientes cianoaziridinas produce en la citotoxicidad frente a la línea celular A549. Se observan valores similares de IC₅₀ para las *N*-Tos-cianoaziridinas **5a-e** (Tabla 1, entradas 8-12), siendo el derivado más efectivo la cianoaziridina **5e** (R= Ph, R¹ = Et, GP = Ts) con un valor de IC₅₀ de 2.3 ± 0.96 μM (Tabla 1, entrada 12). Sin embargo, la sustitución del grupo tosilo (Ts) por carbobenzoxi (Cbz) en el nitrógeno de la aziridina muestra una disminución de las propiedades citotóxicas con un valor de IC₅₀ para el derivado **6d** de 8.1 ± 0.73 μM (Tabla 1, entrada 13). Además, las cianoaziridinas activadas **5** y **6** muestran una selectividad muy alta en la línea celular A549 *versus* la línea celular HEK293. Finalmente, el efecto citotóxico de nuestros compuestos en células pulmonares sanas (MRC-5) parece no presentar ningún efecto (Tabla 1).

Manuscrito 2. Novel synthesis and antiproliferative activity of phosphorus substituted 4-cyanooxazolines, 2-aminocyanooxazolines, 2-iminocyanooxazolidines, and 2-aminocyanothiazolines by rearrangement of cyanoaziridines.

Una vez establecida la metodología necesaria para la preparación de aziridinas fosforadas funcionalizadas por adición de TMSCN a 2*H*-azirinas fosforadas, en este segundo manuscrito se describe la síntesis de nuevas cianoaziridinas *N*-funcionalizadas fosforadas mediante la reacción de las *NH*-cianoaziridinas, sintetizadas previamente, con diferentes iso(tio)cianatos. Por otro lado, tras la reacción de las cianoaziridinas con iso(tio)cianatos, se estudia la expansión del anillo de aziridina para la preparación de heterociclos nitrogenados de 5 miembros. Además, se ensaya la actividad antiproliferativa frente a la línea celular de adenocarcinoma de pulmón humano (A549), tanto de las nuevas cianoaziridinas *N*-funcionalizadas, como de los heterociclos nitrogenados de 5 miembros procedentes de la expansión del anillo.

2.1 SÍNTESIS DE NUEVAS CIANOAZIRIDINAS *N*-FUNCIONALIZADAS FOSFORADAS.

En los últimos años, se ha dedicado un esfuerzo significativo al estudio de aziridinas en cicloadiciones formales [3 + 2], proporcionando rutas para la construcción de una amplia variedad de heterociclos funcionalizados de cinco miembros con una amplia gama de actividades biológicas.^{120b,227} Es sabido, que las aziridinas son reactivos útiles para la síntesis de una gran variedad de compuestos nitrogenados y, que el tratamiento con cloruros de acilo^{111,228} o isocianatos^{76a,142a,185a,185b} es una forma eficaz de acceder a distintos sistemas de anillos heterocíclicos. Por ello, inicialmente, se pensó que la adición electrófila de cloruros de acilo o

²²⁷ A. L. Cardoso, T. M. V. D. Pinho e Melo. "Aziridines in formal [3+2] cycloadditions: synthesis of five-membered heterocycles". *Eur. J. Org. Chem.* **2012**, 6479-6501.

²²⁸ a) S. Nakamura, D. Hayama, M. Miura, T. Hatanaka, Y. Funahashi. "Catalytic enantioselective reaction of 2*H*-azirines with thiols using cinchona alkaloid sulfonamide catalysts". *Org. Lett.* **2018**, *20*, 856-859.
b) Q. Peng, D. Guo, J. Bie, J. Wang. "Catalytic enantioselective aza-benzoin reactions of aldehydes with 2*H*-azirines". *Angew. Chem. Int. Ed.* **2018**, *57*, 3767-3771.

iso(tio)cianatos a las NH-cianoaziridinas fosforadas, podría proporcionar un enfoque útil para la construcción de nuevos compuestos nitrogenados de 5 miembros.

Las aziridinas pueden poseer una variedad de grupos unidos a cualquiera de los dos átomos de carbono del anillo, así como distintos grupos protectores en el átomo de nitrógeno. Se ha demostrado que la presencia de estos grupos afecta enormemente a la reactividad de estos sistemas.¹³⁷ Las aziridinas se han utilizado ampliamente como intermedios sintéticos en la obtención de diversos heterociclos, mediante reacciones de expansión del anillo. Concretamente, las oxazolidin-2-onas son importantes compuestos heterocíclicos que han encontrado muchas aplicaciones como auxiliares quirales o compuestos bioactivos.²²⁹

En consecuencia, la transformación de aziridinas en oxazolidin-2-onas ha sido ampliamente estudiada. Esta transformación puede llevarse a cabo a través de reacciones catalizadas por ácidos, condiciones térmicas o incluso condiciones nucleofílicas mediante el empleo de NaI o KI.

La expansión del anillo de aziridinas a oxazolininas, inducida por yoduro, es descubierta por primera vez por Heine en 1959. En ese año es cuando se describe la isomerización de 1-aroylaziridinas a las correspondientes 2-aril-2-oxazolininas en presencia de un exceso de yoduro de sodio en acetona a temperatura ambiente o a reflujo.²³⁰ En la bibliografía se han descrito numerosos ejemplos de la isomerización de acil y benzoil aziridinas a 2-oxazolininas utilizando NaI²³¹, ácidos de Lewis²³² o incluso bases de Lewis.²³³ En nuestro caso, debido a que esta reacción

²²⁹ a) B. Pfeiffer, K. Hauenstein, P. Merz, J. Gertsch, K. H. Altmann. "Synthesis and SAR of C12-C13 oxazoline derivatives of epothilone A". *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3760-3763. b) G. C. Moraski, M. Chang, A. V. Estrada, S. G. Franzblau, U. Möllman, M. J. Miller. "Structure-activity relationship of new anti-tuberculosis agents derived from oxazoline and oxazole benzyl esters". *Eur. J. Med. Chem.* **2010**, *45*, 1703-1716.

²³⁰ H. W. Heine, M. E. Fetter, E. M. Nicholson. "The isomerization of some 1-aroylaziridines. II". *J. Am. Chem. Soc.* **1959**, *81*, 2202-2204.

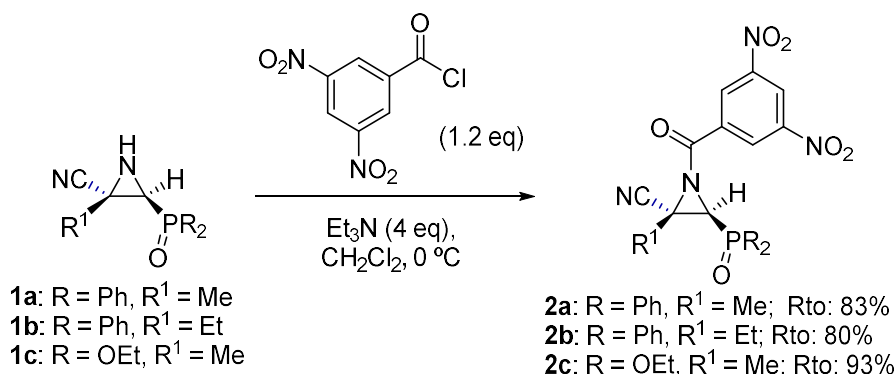
²³¹ a) H. W. Heine, W. G. Kenyon, E. M. Johnson. "The isomerization and dimerization of aziridine derivatives. IV". *J. Am. Chem. Soc.* **1961**, *83*, 2570-2574. b) H. W. Heine, D. C. King, L. A. Portland. "Aziridines. XII. The isomerization of some *cis*- and *trans*-1-*p*-nitrobenzoyl-2,3-substituted aziridines". *J. Org. Chem.* **1966**, *31*, 2662-2665. c) H. W. Heine, M. S. Kaplan. "Aziridines. XVI. Isomerization of some 1-aroyl-aziridines". *J. Org. Chem.* **1967**, *32*, 3069-3074. d) T. A. Foglia, L. M. Gregory, G. Maerker. "Stereochemistry of the isomerization of *N*-acyl-2,3-disubstituted aziridines to Δ^2 -oxazolines". *J. Org. Chem.* **1970**, *35*, 3779-3785.

²³² a) H. Heine, Z. Proctor. "Isomerization of *N*-*p*-ethoxybenzolethylenimine". *J. Org. Chem.* **1958**, *23*, 1554-1556. b) D. Ferraris, W. J. Drury, C. Cox, T. Lectka. "Orthogonal Lewis acids: catalyzed ring opening and rearrangement of acylaziridines". *J. Org. Chem.* **1998**, *63*, 4568-4569.

²³³ a) F. W. Eastwood, P. Perlmutter, Q. Yang. "Preparation of new 2,4-disubstituted oxazoles from *N*-acylaziridines". *J. Chem. Soc., Perkin Trans. 1* **1997**, 35-42. b) F. Bonini, M. Fochi, M. C. Franchini, A. Ricci, L. Thijs, B. Zwanenburg. "Synthesis of ferrocenyl-oxazolines by ring expansion of *N*-ferrocenyl-aziridine-2-carboxylic esters". *Tetrahedron: Asymmetry* **2003**, *14*, 3321-3327.

parece ser una vía sintética eficaz para la síntesis de heterociclos que contienen nitrógeno, nos propusimos inicialmente el empleo de NaI para llevar a cabo de forma regioespecífica la expansión del anillo de nuestras *N*-acil- o *N*-carbamoil- cianoaziridinas fosforadas.

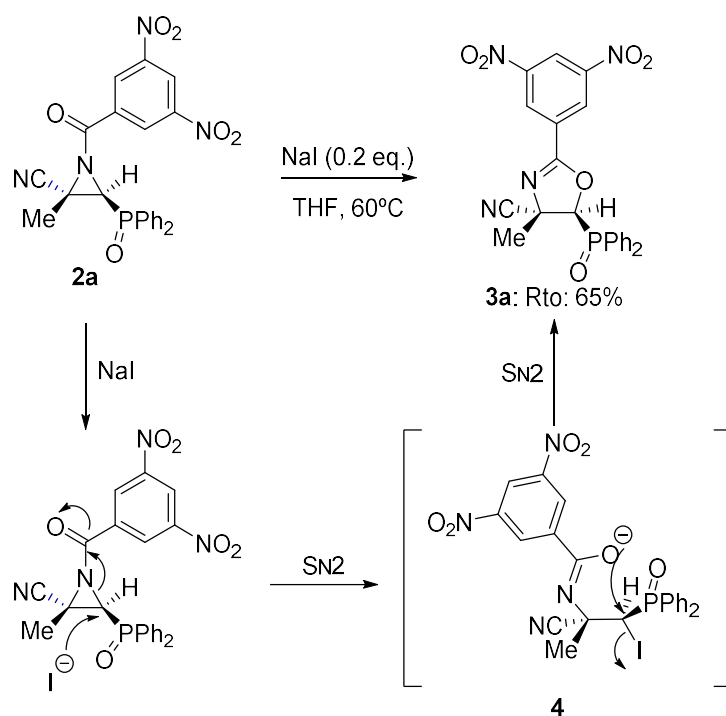
Para ello, teniendo en cuenta que las *N*-acilaziridinas tienen gran importancia en el desarrollo de reacciones de apertura del anillo de aziridina, en primer lugar, se evaluó la *N*-funcionalización de las cianoaziridinas derivadas tanto de óxidos de fosfina **1a** (R = Ph, R¹ = Me) y **1b** (R = Ph, R¹ = Et), como de fosfonato **1c** (R = OEt, R¹ = Me). Así, la reacción de acilación de **1a–c** con cloruro 3,5-dinitrobenzoilo en presencia de Et₃N como base y CH₂Cl₂ como disolvente, permite obtener las correspondientes cianoaziridinas *N*-aciladas derivadas de óxidos de fosfina **2a** (R = Ph, R¹ = Me) y **2b** (R = Ph, R¹ = Et), así como de fosfonato **2c** (R = OEt, R¹ = Me) con buenos rendimientos (80-93%) (Esquema 1).



Esquema 1. Síntesis de cianoaziridinas *N*-aciladas derivadas de óxido de fosfina y fosfonato.

Una vez preparadas las nuevas cianoaziridinas *N*-aciladas **2**, se estudió la reacción de isomerización (reacción de Heine) a las correspondientes 2-oxazolininas **3** (Esquema 2). Inicialmente, se estudió la expansión del anillo en condiciones nucleofílicas empleando NaI como nucleófilo. Así, cuando la aziridina *N*-acilada **2a** (R = Ph, R¹ = Me) reacciona con 0.2 equivalentes de NaI como catalizador a reflujo de THF, conduce de forma regioselectiva a la 4-ciano-oxazolinina derivada de óxido de fosfina **3a** con un 65% de rendimiento (Esquema 2). Por el contrario, cuando la *N*-acilaziridina **2c** derivada de fosfonato reacciona en las mismas condiciones con NaI, en el crudo de reacción se observa la formación de la correspondiente 4-ciano-oxazolinina derivada de fosfonato. Sin embargo, la purificación del crudo de reacción por cromatografía flash en gel de sílice, condujo a mezclas complejas de productos, no siendo posible el aislamiento de dicho derivado.

Aunque, en principio se puede prever el ataque del ion yoduro a cualquiera de los dos átomos de carbono del anillo de aziridina, y que la regioselectividad depende de la naturaleza estereoelectrónica de los sustituyentes, en la bibliografía existen numerosos ejemplos en los que, en la mayoría de las reacciones de este tipo, la apertura del anillo tiene lugar a través de la posición menos impedida.^{24,231c,234} Teniendo en cuenta esto, el mecanismo de reacción más plausible aparece representado en el esquema 2, donde podemos suponer que en una primera etapa tiene lugar el ataque regioespecífico SN2 del ión yoduro sobre el átomo de carbono C-2 menos sustituido, dando como resultado la apertura del anillo de aziridina y la formación del intermedio **4**. A continuación, este intermedio **4** sufre un ataque a través del átomo de oxígeno cargado negativamente en una reacción SN2, produciendo el cierre del anillo y dando lugar a la correspondiente 4-ciano-oxazolina 5-fosforada **3a** con rendimiento moderado (Esquema 2).

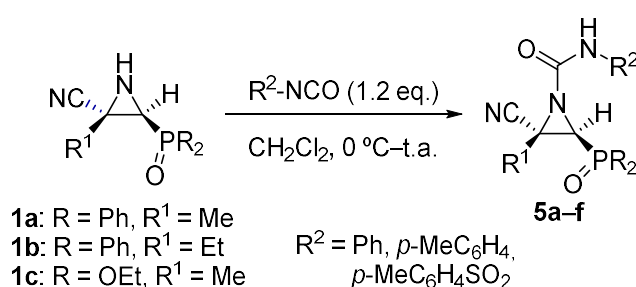


Esquema 2. Expansión del anillo de la *N*-acilcianoaziridina **2a** en presencia de NaI. Síntesis de la 4-ciano oxazolina 5-fosforada **3a**.

²³⁴ a) G. I. Hwang, J. H. Chang, W. K. Lee. "Efficient synthesis of ephedra alkaloid analogs using an enantiomerically pure *N*-[(*R*)-(+)- α -methylbenzyl]aziridine-2-carboxaldehyde". *J. Org. Chem.* **1996**, *61*, 6183-6188. b) G. Cardillo, L. Gentilucci, M. Gianotti, A. Tolomelli. "Microwave-assisted ring expansion of *N*-acetyl 3'-unsubstituted aziridine in the presence of Lewis acids". *Tetrahedron* **2001**, *57*, 2807-2812. c) B. Saha, J. P. Nandy, S. Shukla, I. Siddiqui, J. Iqbal. "Stereoselective synthesis of β -substituted phenylalanine- β -phenylisoserine-derived tripeptides using *N*-cinnamoyl-L-proline as template: synthesis of structural analogues of HIV protease inhibitors". *J. Org. Chem.* **2002**, *67*, 7858-7860.

Es conocido que las aziridinas no protegidas en la posición 1, son compuestos estables en condiciones básicas y pueden reaccionar fácilmente con diversos electrófilos. En nuestro caso, se explora la reacción de *N*-funcionalización de las NH-cianoaziridinas fosforadas **1**, derivadas de óxido de fosfina y fosfonato, mediante la reacción con iso(tio)cianatos aromáticos o alifáticos, para obtener las correspondientes *N*-aril o *N*-alquilcarbamoil cianoaziridinas funcionalizadas.

Inicialmente, para optimizar las condiciones de reacción, se lleva a cabo la adición de fenil isocianato a la cianoaziridina derivada de óxido de fosfina **1a** (R = Ph, R¹ = Me) en CH₂Cl₂ y a 0 °C. Se utilizó 1.2 equivalentes de fenil isocianato con el fin de asegurar una conversión completa. Bajo estas condiciones de reacción, se obtiene la 1-fenilcarbamoil-3-cianoaziridina fosforada derivada de óxido de fosfina **5a** (R = R² = Ph, R¹ = Me) con un rendimiento del 63% (Esquema 3, Tabla 1, entrada 1). Existen algunos ejemplos en la bibliografía de reacciones catalizadas por metales de transición que involucren aziridinas e isocianatos. Así, existen algunos ejemplos de reacciones entre aziridinas e isocianatos en presencia de catalizadores como NiI₂^{184,235} o diferentes ácidos de Lewis.^{76a,185c,188} En nuestro caso, estudiamos la adición de fenil isocianato a la cianoaziridina **1a** catalizada por un ácido de Lewis. De esta forma, se observa un menor tiempo de reacción y un mayor rendimiento para la cianoaziridina **5a** cuando la reacción se lleva a cabo bajo condiciones catalíticas, empleando un 20% mol de Sc(OTf)₃ como catalizador (Esquema 3, Tabla 1, entrada 1). De la misma forma, la cianoaziridina derivada de fosfonato **1c** (R = OEt, R¹ = Me) reacciona con fenil isocianato en CH₂Cl₂ y a 0 °C, sin catalizador, para dar la cianoaziridina *N*-funcionalizada **5b** (R = OEt, R¹ = Me, R² = Ph) con un 98% de rendimiento (Esquema 3, Tabla 1, entrada 2).



Esquema 3. Síntesis de *N*-aril, *N*-alquil y *N*-fenilsulfonilcarbamoil cianoaziridinas fosforadas.

²³⁵ K. Z. Pramod, R. Chopade, J. Louie. "Coupling of vinyl aziridines and phenyl isocyanate". *Tetrahedron Lett.* **2008**, 49, 4306-4309.

Discusión de los resultados

Tal y como aparece reflejado en la tabla 1, esta metodología sintética se puede aplicar a la adición de otros isocianatos aromáticos. Por ejemplo, las cianoaziridinas **1a** y **1c** reaccionan con *p*-tolil isocianato ($R^2 = p\text{-MeC}_6\text{H}_4$), dando lugar a las correspondientes *N*-*p*-tolilcarbamoil cianoaziridinas sustituidas funcionalizadas derivadas de óxido de fosfina **5c** ($R = \text{Ph}$, $R^1 = \text{Me}$, $R^2 = p\text{-MeC}_6\text{H}_4$) y fosfonato **5d** ($R = \text{OEt}$, $R^1 = \text{Me}$, $R^2 = p\text{-MeC}_6\text{H}_4$) con buenos rendimientos (Esquema 3, Tabla 1, entrada 3 y 4, respectivamente).

Tabla 1. *N*-Aril, *N*-alquil y *N*-fenilsulfonilcarbamoil cianoaziridinas fosforadas **5** preparadas.

Entrada	Compuesto	R	R ¹	R ²	Rto (%) ^[a]
1	5a	Ph	Me	Ph	63,71 ^[b]
2	5b	OEt	Me	Ph	98
3	5c	Ph	Me	<i>p</i> -MeC ₆ H ₄	82
4	5d	OEt	Me	<i>p</i> -MeC ₆ H ₄	80
5	5e	Ph	Et	<i>p</i> -MeC ₆ H ₄ SO ₂	86
6	5f	OEt	Me	<i>p</i> -MeC ₆ H ₄ SO ₂	79
7	5g	Ph	Me	Et	86 ^[c]
8	5h	OEt	Me	Et	59 ^[c]
9	5i	Ph	Me	^t Bu	73 ^[c]
10	5j	OEt	Me	^t Bu	75 ^[c]
11	5k	Ph	Et	^t Bu	64 ^[c]

^[a] Rendimiento de los compuestos aislados **5**. ^[b] Rendimiento del compuesto **5a** cuando la reacción se realiza en presencia de Sc(OTf)₃. ^[c] Rendimiento de los compuestos **5g–k** cuando la reacción se realiza en presencia de ZnCl₂.

Asimismo, las cianoaziridinas fosforadas **1** reaccionan también con *p*-toluensulfonil isocianato, bajo las mismas condiciones de reacción (Esquema 3). Cuando la cianoaziridina **1a** reacciona con *p*-toluensulfonil isocianato, en el crudo de reacción se observa únicamente la formación de la cianoaziridina *N*-funcionalizada derivada de óxido de fosfina **5** ($R = \text{Ph}$, $R^1 = \text{Me}$, $R^2 = p\text{-MeC}_6\text{H}_4\text{SO}_2$). Sin embargo, tras la posterior purificación por recristalización, se observa como este derivado **5** se hidroliza rápidamente a la cianoaziridina empleada como sustrato de partida **1a**, observándose ambos conjuntos de señales a través de los espectros de ¹H y ³¹P RMN.

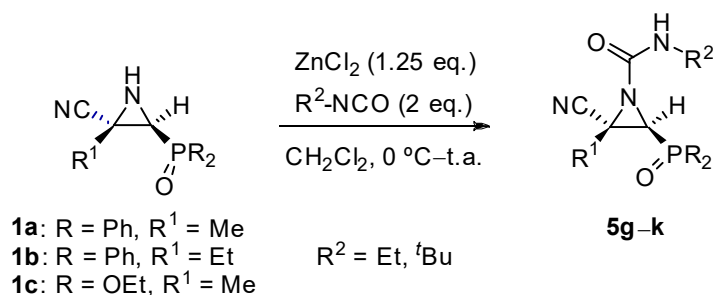
Por el contrario, las correspondientes *N-p*-tosilcarbamoil cianoaziridinas **5e** (R = Ph, R¹ = Et, R² = *p*-MeC₆H₄SO₂) y **5f** (R = OEt, R¹ = Me, R² = *p*-MeC₆H₄SO₂) se obtuvieron con unos rendimientos del 86 y 79%, respectivamente (Esquema 3, Tabla 1, entradas 5 y 6), tras el tratamiento de las correspondientes cianoaziridinas **1b** y **1c** con *p*-tosil isocianato (Esquema 3).

Esta metodología sintética también se puede ampliar al estudio de la reactividad de las cianoaziridinas fosforadas **1** con isocianatos alifáticos, como, por ejemplo, el etil o *tert*-butil isocianato. Inicialmente se estudió la reacción de la cianoaziridina derivada de óxido de fosfina **1a** (R = Ph, R¹ = Me) empleando las mismas condiciones de reacción descritas para la adición de isocianatos aromáticos (1.2 equivalentes de etil isocianato, CH₂Cl₂, 0 °C–t.a.). En este caso, a través de la cromatografía en capa fina (TLC) no se observa evolución de la reacción, permitiendo la recuperación de la cianoaziridina **1a** empleada como sustrato de partida. Ante estos resultados, y dada la menor reactividad de los isocianatos alifáticos en comparación con los aromáticos, pensamos en llevar a cabo la reacción en presencia de un ácido de Lewis. Teniendo en cuenta que, bajo condiciones catalíticas, empleando un 20% mol de Sc(OTf)₃ como catalizador, la adición de fenil isocianato a la cianoaziridina **1a** permite la obtención del derivado **5a** (Tabla 1, entrada 1), nos propusimos estudiar la adición de etil isocianato a la cianoaziridina **1a** empleando un 20% mol de Sc(OTf)₃ en CH₂Cl₂ a temperatura ambiente. En este caso, tras 24 horas de reacción se observa, a través de los espectros de ¹H y ³¹P RMN, un 50% de la correspondiente *N*-etilcarbamoil cianoaziridina **5g**.

A la vista de estos resultados, y teniendo en cuenta una revisión llevada a cabo por el grupo de Stoltz^{76a} basada en el estudio de cicloadiciones [3+2] estereoselectivas de *NH*- y *N*-sulfonilaziridinas con alquil heterocumulenos catalizadas por ácidos de Lewis tales como ZnCl₂ o ZnBr₂, pensamos como alternativa, llevar a cabo la adición de isocianatos alifáticos a las cianoaziridinas fosforadas bajo condiciones estequiométricas utilizando 1.25 equivalentes de ZnCl₂. Por tanto, como se describe en el esquema 4, la cianoaziridina derivada de óxido de fosfina **1a** (R = Ph, R¹ = Me) reacciona con 2 equivalentes de etil isotiocianato y 1,25 equivalentes de ZnCl₂, en CH₂Cl₂ y a temperatura ambiente, observándose la formación de la *N*-etilcarbamoil cianoaziridina **5g** (R = Ph, R¹ = Me, R² = Et) con un 86% de rendimiento (Esquema 4, Tabla 1, entrada 7). De la misma forma, la adición electrófila de etil isocianato a la cianoaziridina derivada de fosfonato **1c** conduce a la aziridina *N*-funcionalizada **5h** (R = OEt, R¹ = Me, R² = Et) (Esquema 4, Tabla 1, entrada 8). El empleo de *tert*-butil isocianato como electrófilo, mediante el uso de ZnCl₂ como ácido de Lewis, conduce a las *N-tert*-butilcarbamoil cianoaziridinas **5i**, **5j** y **5k** respectivamente, con rendimientos moderados (Esquema 4, Tabla 1, entradas 9-11).

Discusión de los resultados

A la vista de estos resultados, también se explora la adición electrófila de isotiocianatos aromáticos a las NH-cianoaziridinas fosforadas **1**. Inicialmente, se lleva a cabo la adición de fenil isotiocianato a la cianoaziridina derivada de óxido de fosfina **1a** (R = Ph, R¹ = Me), usando las mismas condiciones que las usadas previamente para la formación de las N-arilcarbamoil cianoaziridinas **5**.

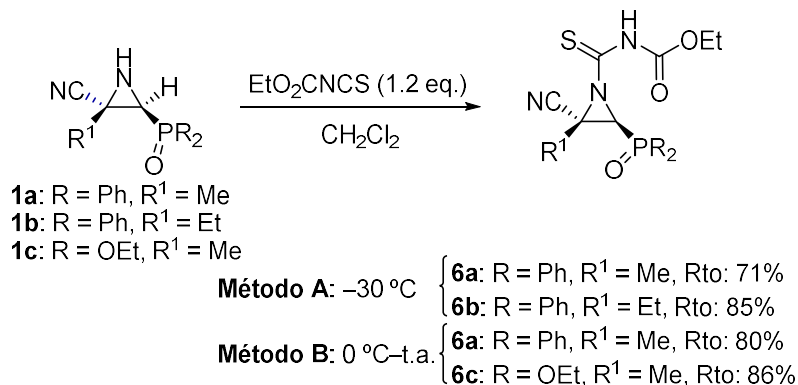


Esquema 4. Adición de isocianatos alifáticos a cianoaziridinas fosforadas.

Bajo estas condiciones de reacción, no se observa la formación de la correspondiente N-tiocarbamoil cianoaziridina, pudiendo recuperar el sustrato de partida sin reaccionar. La adición de fenil isotiocianato a NH-cianoaziridinas fosforadas **1** usando diferentes bases, tales como Et₃N, piridina, NaH, o Cs₂CO₃, así como ácidos de Lewis, tales como ZnCl₂ o Sc(OTf)₃, conduce a similares resultados, obteniendo la cianoaziridina de partida **1** sin reaccionar. El empleo de otros isotiocianatos aromáticos, como, por ejemplo, *p*-metoxi fenilisotiocianato o *p*-nitro fenil isotiocianato tampoco condujo a las N-tiocarbamoil cianoaziridinas esperadas.

Debido a la imposibilidad de obtener las correspondientes N-tiocarbamoil cianoaziridinas funcionalizadas a través de la adición electrófila de isotiocianatos aromáticos a las NH-cianoaziridinas fosforadas **1**, esta metodología sintética se extendió al empleo de isotiocianatos funcionalizados con un grupo electroattractor (CO₂Et), el etoxycarbonil isotiocianato, que reacciona con las cianoaziridinas derivadas de fosfanóxido **1a** (R = Ph, R¹ = Me) o **1b** (R = Ph, R¹ = Et) en CH₂Cl₂ y a -30 °C, para conducir a las correspondientes aziridinas N-tiocarbamoil funcionalizadas **6a** y **6b** con unos rendimientos del 71 y 85%, respectivamente (Método A, Esquema 5). Aumentando la temperatura de la reacción de -30 °C a temperatura ambiente, la cianoaziridina N-funcionalizada **6a** (R = Ph, R¹ = Me) se obtiene con un mayor rendimiento (80%) (Método B, Esquema 5). De la misma forma, la cianoaziridina derivada de fosfonato **1c** (R = OEt, R¹ = Me) también reacciona con el etoxycarbonil isotiocianato en CH₂Cl₂ y a temperatura

ambiente, aislándose la *N*-tiocarbamoil cianoaziridina **6c** (R = OEt, R¹ = Me) con buenos rendimientos (Método B, Esquema 5).



Esquema 5. Adición de etoxicarbonyl isotiocianato a cianoaziridinas fosforadas sustituidas.

Continuando con nuestro interés en la síntesis de nuevos compuestos heterocíclicos fosfonitrogenados de 5 miembros, nos propusimos estudiar la reacción de expansión del anillo de algunos de los derivados de aziridina *N*-carbamoilados fosforados sintetizados, con la finalidad de acceder a nuevos derivados de oxazolina (reacción tipo Heine). Existen varios ejemplos en la literatura de la isomerización del anillo de aziridina con isocianatos e isotiocianatos a aminoxazolinas,²³⁶ aminotiazolinas,²³⁷ imidazolidinonas^{183c,184,235}, e iminooxazolidinas.^{182,184}

Para ello, utilizando las mismas condiciones de reacción que en el caso de las *N*-acil cianoaziridinas **2**, las *N*-arilcarbamoil cianoaziridinas derivadas de óxido de fosfina **5a** (R = R² = Ph) y **5c** (R = Ph, R² = *p*-MeC₆H₄) se hicieron reaccionar con 0.2 equivalentes de NaI a 60 °C en THF, lo que permite la obtención de las correspondientes oxazolinas derivadas de óxido de fosfina **7a** (R = R² = Ph) y **7c** (R = Ph, R² = *p*-MeC₆H₄) con buenos rendimientos (Esquema 6). Según se observa por ¹H y ³¹P RMN del bruto de reacción, las oxazolinas **7** se obtienen como mezcla de dos regioisómeros **7** + **7'**. La proporción de regioisómeros para las oxazolinas derivadas de óxido de fosfina **7a** + **7a'** es de 66:34 mientras que para los derivados **7c** + **7c'** es de 65:35 (Esquema 6).

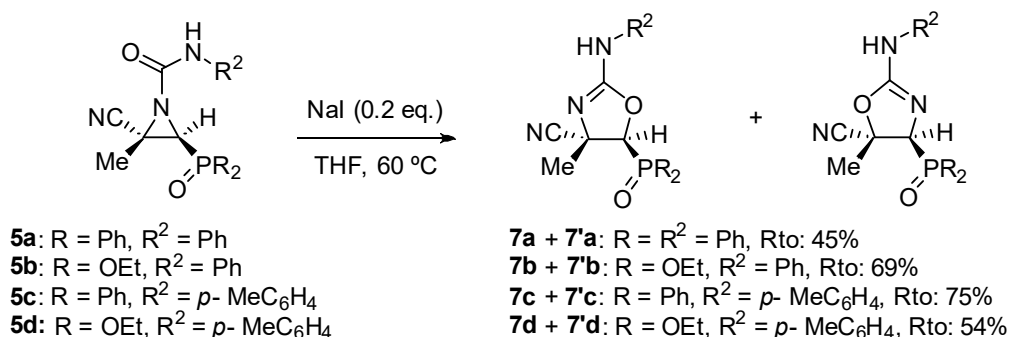
²³⁶ a) N. P. Peet, P. B. Anzeveno. "Synthesis of 2,3-dihydro-5*H*-oxazolo[2,3-*b*]quinazolin-5-ones". *J. Heterocycl. Chem.* **1979**, *16*, 877-880. b) K. S. K. Murthy, D. N. Dhar. "Reaction of chlorosulfonyl isocyanate with aziridines". *J. Heterocycl. Chem.* **1984**, *21*, 1699-1704.

²³⁷ F. G. Weber, H. Liepert, H. Köppel. "Chalcone aziridines. III. Reaction of *trans*-chalcone aziridines with isocyanates, isothiocyanates and isothiocyanic acid". *J. Prakt. Chem.* **1986**, *4*, 612-620.

Discusión de los resultados

Estas oxazolinas **7** fueron purificadas a través de cromatografía flash en gel de sílice, lo que permitió en el caso de los derivados **7a + 7a'** el aislamiento de un único regioisómero, correspondiente al isómero minoritario, como puede observarse a través de los espectros de ^1H , ^{31}P y ^{13}C RMN. Sin embargo, en el caso de los derivados **7c + 7c'** no fue posible la separación de ambos regioisómeros tras la purificación por cromatografía, obteniéndose la mezcla de regioisómeros en una proporción similar a la observada en el crudo de reacción, 65:35.

Esta reacción de expansión de anillo tipo Heine, se amplia también al estudio de las *N*-cianoaziridinas carbamoiladas derivadas de fosfonato de dietilo **5b** ($\text{R} = \text{OEt}$, $\text{R}^2 = \text{Ph}$) y **5d** ($\text{R} = \text{OEt}$, $\text{R}^2 = p\text{-MeC}_6\text{H}_4$), bajo las condiciones de reacción representadas en el esquema 6. En ambos casos y tras 24 horas de reacción a $60\text{ }^\circ\text{C}$ en THF, en los crudos de reacción se observa la formación de las correspondientes oxazolininas derivadas de fosfonato de dietilo **7b + 7'b** y **7d + 7'd** como mezcla de dos regioisómeros, en unas proporciones de 64:36 y 66:34, respectivamente (Esquema 6). La purificación por cromatografía flash en gel de sílice permite la obtención de las oxazolininas **7b + 7'b** (66:34) y **7d + 7'd** (72:28) como mezclas de regioisómeros en unas proporciones prácticamente similares a las observadas en los crudos de reacción, con rendimientos moderados (Esquema 6).



Esquema 6. Expansión del anillo de *N*-cianoaziridinas carbamoiladas catalizada por NaI.

Por el contrario, cuando se estudia la reacción de expansión de anillo catalizada por NaI de las *N*-etil y *N*-terc-butilcarbamoil cianoaziridinas fosforadas **5g** ($\text{R} = \text{Ph}$, $\text{R}^1 = \text{Me}$, $\text{R}^2 = \text{Et}$) y **5k** ($\text{R} = \text{Ph}$, $\text{R}^1 = \text{Et}$, $\text{R}^2 = t\text{Bu}$), no se observa reacción pudiéndose recuperar en ambos casos los sustratos de partida.

La formación de las oxazolininas **7** podría interpretarse a través de una apertura inicial del anillo de aziridina mediante el ataque del yoduro indistintamente al carbono C-2 y C-3 del anillo de

aziridina, seguido del cierre del anillo, dando lugar a las correspondientes oxazolininas como mezcla de dos regioisómeros.

Debido a los grandes esfuerzos llevados a cabo para la síntesis de tiazolininas a partir de aziridinas, nos planteamos la posibilidad de estudiar la reacción de expansión del anillo en las nuevas *N*-tiocarbamoil cianoaziridinas funcionalizadas **6**. Es conocido que a través de la reacción de Heine, se preparan oxazolininas o imidazolininas 2-sustituidas mediante la expansión del anillo de aziridinas o imidoil aziridinas benzoiladas, respectivamente^{231a,231c,238} Algunas aziridinas experimentan reacciones de expansión de anillo en oxazolininas mediante el empleo de ácidos de Lewis²³⁹ y, recientemente, basándose en estos resultados Tepe y col.,²⁴⁰ describen la isomerización de aziridinas a oxazolininas usando BF₃·OEt₂.

Inicialmente, se estudió la apertura del anillo de aziridina en condiciones térmicas. Para ello la *N*-tiocarbamoil cianoaziridina derivada de óxido de fosfina **6a** (R = Ph, R¹ = Me) se calentó a reflujo de CHCl₃. Bajo estas condiciones no se observa reacción, pudiendo recuperar el sustrato de partida **6a**.

A continuación, también se estudió la reacción tipo Heine en condiciones nucleofílicas mediante el empleo de NaI a 60 °C en THF, y al igual que en el caso anterior, tampoco se obtuvieron resultados satisfactorios.

Asimismo, se examinó la conversión de la aziridina en tiazolina bajo condiciones ácidas suaves. Para ello, la reacción de la cianoaziridina *N*-funcionalizada **6a** se expuso tanto a ácidos de Brønsted, tales como el ácido *p*-toluensulfónico (PTSA), como a ácidos de Lewis, tales como el ZnCl₂ o el BF₃·OEt₂. De todos los ácidos usados, solo el empleo de BF₃·OEt₂ condujo a resultados satisfactorios. De esta forma, cuando la *N*-tiocarbamoil cianoaziridina **6a** se trata con 5 equivalentes de BF₃·OEt₂ a -70 °C en THF, se observa la formación de la 2-aminotiazolina **8a** (R = Ph, R¹ = Me) con rendimientos moderados (Esquema 7). Los datos espectroscópicos parecen confirmar la isomerización de la aziridina **6a** en 2-aminotiazolidina **8a**. Así, mientras que en el

²³⁸ a) H. W. Heine, H. S. Bender. "The isomerization of some aziridine derivatives. III. A new synthesis of 2-imidazolines". *J. Org. Chem.* **1960**, *25*, 461-463. b) M. R. Kuszpit, W. D. Wulff, J. J. Tepe. "One-pot synthesis of 2-imidazolines via the ring expansion of imidoil chlorides with aziridines". *J. Org. Chem.* **2011**, *76*, 2913-2919.

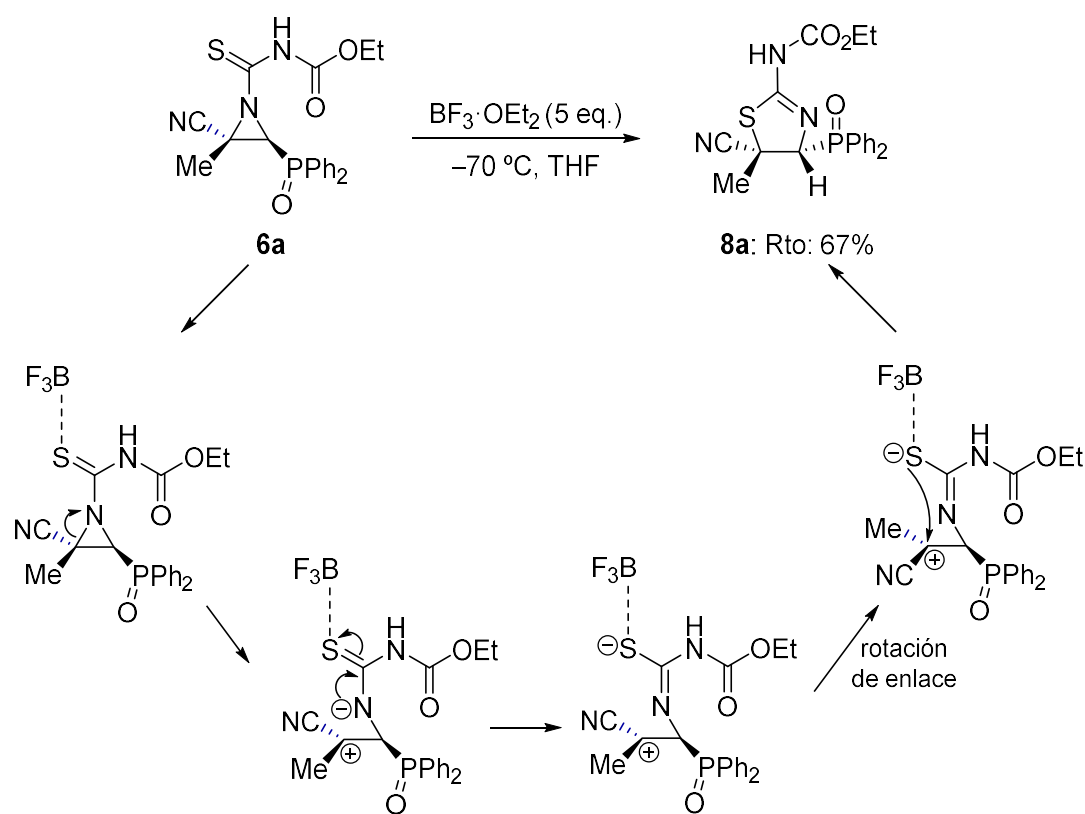
²³⁹ G. Cardillo, L. Gentilucci, A. Tolomelli, C. Tomasini. "Ring expansion of *N*-acyl aziridine-2-imides to oxazoline-4-imides, useful precursors of pure β-hydroxy α-aminoacids". *Tetrahedron Lett.* **1997**, *38*, 6953-6956.

²⁴⁰ M. Shafaat A. Mehedi, J. J. Tepe. "Diastereoselective one-pot synthesis of oxazolines using sulfur ylides and acyl imines". *J. Org. Chem.* **2019**, *84*, 7219-7226.

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espectro de ^1H RMN la aziridina **6a** presenta una señal para el grupo metilo a $\delta_{\text{H}} = 2.0$ ppm y el hidrógeno metínico resuena a $\delta_{\text{H}} = 3.8$ ppm como un doblete bien resuelto ($^2J_{\text{PH}} = 20$ Hz), estas señales en la 2-aminotiazolidina **8a** aparecen a campos más bajo, $\delta_{\text{H}} = 2.11$ y 4.58 ppm como un singlete y un doblete bien resuelto ($^2J_{\text{PH}} = 12.8$ Hz), respectivamente. Por el contrario, también se hizo reaccionar la cianoaziridina *N*-funcionalizada derivada de fosfonato **6c** ($\text{R} = \text{OEt}$, $\text{R}^1 = \text{Me}$) bajo las mismas condiciones de reacción, empleando $\text{BF}_3 \cdot \text{OEt}_2$ como ácido de Lewis.

Sin embargo, en este caso la purificación del crudo de reacción por cromatografía flash en gel de sílice condujo a mezclas complejas de productos.



Esquema 7. Expansión de anillo intramolecular estereoespecífica de *N*-tiocarbamoil cianoaziridinas fosforadas sustituidas mediante el uso de $\text{BF}_3 \cdot \text{OEt}_2$.

Dado que los datos espectrales de ^1H y ^{13}C RMN no fueron concluyentes para asignar la regio- y estereoquímica del producto, se realizó el análisis cristalográfico de rayos X monocristalino del compuesto **8a**. Además de poder verificar la regioquímica del compuesto **8a**, se pudo confirmar

una estereoquímica *sin* entre el grupo nitrilo en posición C-3 y el grupo voluminoso fosforado en C-2 del compuesto **8a** (Figura 1).

El mecanismo más probable que explicaría la formación de **8a** se encuentra representado en el esquema 7. Inicialmente el $\text{BF}_3 \cdot \text{OEt}_2$ se coordinaría con el átomo de azufre de la cianoaziridina **6a** facilitando de esa manera su apertura, a través del enlace N–C3, generando así el carbocatión más estable. El intermedio catiónico generado de la aziridina de estereoquímica *anti* podría isomerizarse, y el cierre del anillo conduciría a la 2-aminotiazolina **8a** como único regio- y estereoisómero.

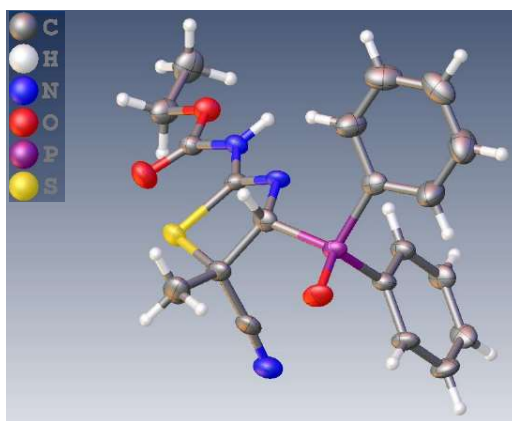


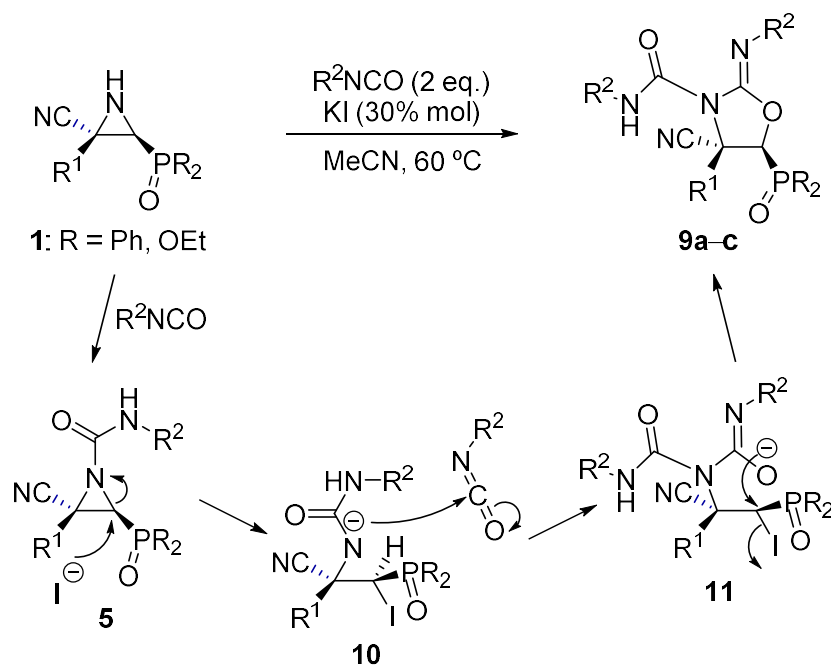
Figura 1. Diagrama ORTEP para la 2-aminotiazolina **8a**.

Siguiendo con nuestro interés en la síntesis de nuevos compuestos heterocíclicos fosfonitrogenados, finalmente se estudió la reacción *one pot* de las cianoaziridinas **1** con isocianatos, con el objeto de obtener nuevos derivados de tipo oxazolina.

Para ello, se hizo reaccionar la cianoaziridina fosforada **1b** ($R = \text{Ph}$, $R^1 = \text{Et}$) con fenil isocianato en acetonitrilo a $60\text{ }^\circ\text{C}$, lo que da lugar a la iminoxazolidina **9a** pero con bajo rendimiento (Esquema 8). La adición de 2 equivalentes de isocianato condujo a **9a** con rendimientos moderados (45%), mientras que cuando la reacción se examina en presencia de KI (30% mol), con 2 equivalentes de isocianato y en acetonitrilo a $60\text{ }^\circ\text{C}$, se obtuvo la correspondiente iminoxazolidina **9a** con un 62% de rendimiento (Esquema 8, Tabla 2, entrada 1). De la misma forma, la cianoaziridina **1a** ($R = \text{Ph}$, $R^1 = \text{Me}$) reacciona con *p*-tolil isocianato en las mismas condiciones de reacción, proporcionando 55% de rendimiento del compuesto **9b** (Esquema 8, Tabla 2, entrada 2). Esta metodología se extendió al empleo de cianoaziridinas derivadas de

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fosfonato. Así, **1c** (R = OEt, R¹ = Me) reacciona con fenil isocianato en presencia de KI en acetonitrilo a 60 °C para dar la iminoxazolidina derivada de fostonato **9c** (Esquema 8, Tabla 2, entrada 3).



Esquema 8. Síntesis de iminoxazolidinas fosforadas a partir de cianoaziridinas e isocianatos catalizada por KI.

Tabla 2. Iminoxazolidinas fosforadas **9** preparadas.

Entrada	Compuesto	R	R ¹	R ²	Rto (%) ^[a]
1	9a	Ph	Et	Ph	62
2	9b	Ph	Me	<i>p</i> -MeC ₆ H ₄	55
3	9c	OEt	Me	Ph	79

^[a] Rendimiento de los compuestos aislados **9**.

Aunque la masa de alta resolución (HRMS) de los compuestos **9**, coincide con la masa teórica para las estructuras propuestas, los datos de ¹H y ¹³C RMN de los compuestos **9** no fueron

concluyentes para asignar la estereoquímica del enlace C4–C5 en las iminoxazolidinas preparadas. Por ello, se intentó realizar el análisis cristalográfico de rayos X con objeto de verificar la estructura del producto propuesta para **9**. Sin embargo, todo intento de obtener cristales adecuados de las iminoxazolidinas **9** para su medida de rayos X, fue inútil.

A falta de confirmar la estereoquímica de los compuestos **9**, una propuesta mecanística para su formación podría indicar la adición inicial de un equivalente de isocianato para dar las *N*-carbamoil cianoaziridinas correspondientes **5** (Esquema 8). A continuación, el ataque regioespecífico S_N2 del ion yoduro sobre el átomo de carbono C-2 menos sustituido en **5**, daría lugar a la apertura del anillo de aziridina obteniéndose un intermedio **10** que atacaría al carbono central del segundo equivalente de isocianato. El intermedio **11** así obtenido, sufriría el cierre del anillo para dar la iminoxazolidinas **9**.

2.2 EVALUACIÓN DE LA ACTIVIDAD BIOLÓGICA.

Se evalúa la citotoxicidad *in vitro* de las nuevas cianoaziridinas *N*-funcionalizadas fosforadas derivadas de óxido de fosfina (R = Ph) y fosfonato de dietilo (R = OEt) **2**, **5**, y **6**, así como de los heterociclos nitrogenados de 5 miembros **3a**, **7**, **8a** y **9** procedentes de la expansión del anillo, evaluando sus actividades antiproliferativas frente a la línea celular de adenocarcinoma de pulmón humano (A549). Con el fin de evaluar la inhibición del crecimiento celular y las actividades inhibitoras de la proliferación celular como valores de IC₅₀ de todos los compuestos preparados (Tabla 3), se usa el ensayo de CCK-8. Estos resultados se comparan con la actividad antiproliferativa de la doxorubicina (DOX).

Respecto a la línea celular A549 *in vitro*, se estudia en primer lugar, el efecto que produce en la citotoxicidad la sustitución del átomo de nitrógeno en las nuevas cianoaziridinas fosforadas *N*-funcionalizadas sustituidas sintetizadas. El resultado más efectivo se observa para la aziridina *N*-benzoilada derivada de óxido de fosfina **2a** (R = Ph, R¹ = Me) con un valor de IC₅₀ = 22.9 ± 1.9 μM (Tabla 3, entrada 2). Sin embargo, las cianoaziridinas *N*-aciladas derivadas de óxido de fosfina (R = Ph) **2b** y fosfonato de dietilo (R = OEt) **2c** (Tabla 3, entradas 3-4), así como las *N*-carbamoil cianoaziridinas **5a-k** (Tabla 3, entradas 6-16) y las *N*-tiocarbamoil cianoaziridinas **6a-c** (Tabla 3, entradas 17-19), no exhiben toxicidad en la línea celular A549.

Asimismo, también se evalúa la citotoxicidad frente a la línea celular A549 de los nuevos heterociclos nitrogenados de 5 miembros procedentes de la expansión del anillo. En primer

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lugar, la 4-ciano-oxazolina **3a**, obtenida a través de la reacción de isomerización tipo Heine, mostró ser un derivado citotóxico con un valor de $IC_{50} = 19.7 \pm 2.8 \mu M$ (Tabla 3, entrada 5). Por el contrario, las oxazolininas **7** derivadas de óxido de fosfina y fosfonato de dietilo (Tabla 3, entradas 20-23), así como la 2-aminotiazolina **8a** (Tabla 3, entrada 24) no mostraron actividad citotóxica en la línea celular A549. Del mismo modo, también se evalúa la citotoxicidad *in vitro* de las iminoxazolidinas derivadas de óxido de fosfina **9a** y **9b**. El valor observado para el IC_{50} del compuesto **9a** ($R = Ph$, $R^1 = Et$) fue de $16.4 \pm 1.5 \mu M$ (Tabla 3, entrada 25), mientras que **9b** muestra un valor de IC_{50} muy similar; $14.8 \pm 1.2 \mu M$ (Tabla 3, entrada 26). Sin embargo, la iminoxazolidina derivada de fosfonato de dietilo **9c** fue el compuesto más eficaz con un valor de $IC_{50} = 6.2 \pm 0.75 \mu M$ (Tabla 3, entrada 27).

Tabla 3. Actividad antiproliferativa de las cianoaziridinas *N*-funcionalizadas fosforadas sustituidas **2**, **5**, y **6**, así como de los heterociclos nitrogenados de 5 miembros procedentes de la expansión de anillo **3a**, **7**, **8a** y **9**.

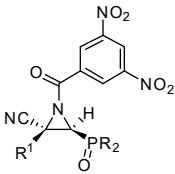
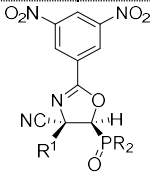
Entrada	Comp.	R	R ¹	R ²	Citotoxicidad IC_{50} (μM) ^[a]	
					Pulmón A549	MRC-5
1	DOX				0.48 ± 0.017 ²²⁵	>50 ²²⁶
						
2	2a	Ph	Me	–	22.9 ± 1.9	>50
3	2b	Ph	Et	–	>50	>50
4	2c	OEt	Me	–	>50	>50
						
5	3a	Ph	Me	–	19.7 ± 2.8	>50

Tabla 3 (Cont.). Actividad antiproliferativa de las cianoaziridinas *N*-funcionalizadas fosforadas sustituidas **2**, **5**, y **6**, así como de los heterociclos nitrogenados de 5 miembros procedentes de la expansión de anillo **3a**, **7**, **8a** y **9**.

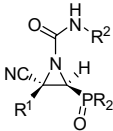
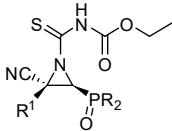
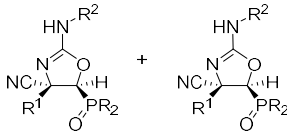
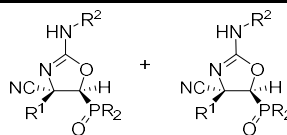
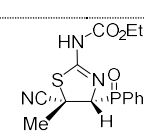
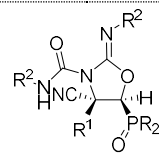
Entrada	Comp.	R	R ¹	R ²	Citotoxicidad IC ₅₀ (μM) ^[a]	
					Pulmón A549	MRC-5
						
6	5a	Ph	Me	Ph	>50	>50
7	5b	OEt	Me	Ph	>50	>50
8	5c	Ph	Me	<i>p</i> -MeC ₆ H ₄	>50	>50
9	5d	OEt	Me	<i>p</i> -MeC ₆ H ₄	>50	>50
10	5e	Ph	Et	<i>p</i> -MeC ₆ H ₄ SO ₂	>50	>50
11	5f	OEt	Me	<i>p</i> -MeC ₆ H ₄ SO ₂	>50	>50
12	5g	Ph	Me	Et	>50	>50
13	5h	OEt	Me	Et	>50	>50
14	5i	Ph	Me	^t Bu	>50	>50
15	5j	OEt	Me	^t Bu	>50	>50
16	5k	Ph	Et	^t Bu	>50	>50
						
17	6a	Ph	Me	–	>50	>50
18	6b	Ph	Et	–	>50	>50
19	6c	OEt	Me	–	>50	>50
						
20	7a + 7a'	Ph	Me	Ph	>50	>50
21	7b + 7b'	OEt	Me	Ph	>50	>50

Tabla 3 (Cont). Actividad antiproliferativa de las cianoaziridinas *N*-funcionalizadas fosforadas sustituidas **2**, **5**, y **6**, así como de los heterociclos nitrogenados de 5 miembros procedentes de la expansión de anillo **3a**, **7**, **8a** y **9**.

Entrada	Comp.	R	R ¹	R ²	Citotoxicidad IC ₅₀ (μM) ^[a]	
					Pulmón A549	MRC-5
						
22	7c + 7c'	Ph	Me	<i>p</i> -MeC ₆ H ₄	>50	>50
23	7d + 7d'	OEt	Me	<i>p</i> -MeC ₆ H ₄	>50	>50
						
24	8a	Ph	Me	–	>50	>50
						
25	9a	Ph	Et	Ph	16.4 ± 1.5	>50
26	9b	Ph	Me	<i>p</i> -MeC ₆ H ₄	14.8 ± 1.2	>50
27	9c	OEt	Me	Ph	6.2 ± 0.7	>50

^[a] Los valores de IC₅₀ corresponden a las concentraciones de los compuestos para las cuales se inhibe el crecimiento celular en un 50%.

Además, con el objeto de determinar citotoxicidad selectiva, también se determina la actividad antiproliferativa frente a una línea no cancerígena de células sanas como la MRC-5 (fibroblastos derivados de tejido pulmonar).²²⁴ En este caso, el efecto citotóxico en células pulmonares sanas (MRC-5), tanto de las nuevas cianoaziridinas *N*-funcionalizadas fosforadas, así como de los heterociclos nitrogenados de 5 miembros procedentes de la expansión de anillo, parece no presentar ningún efecto (Tabla 3).

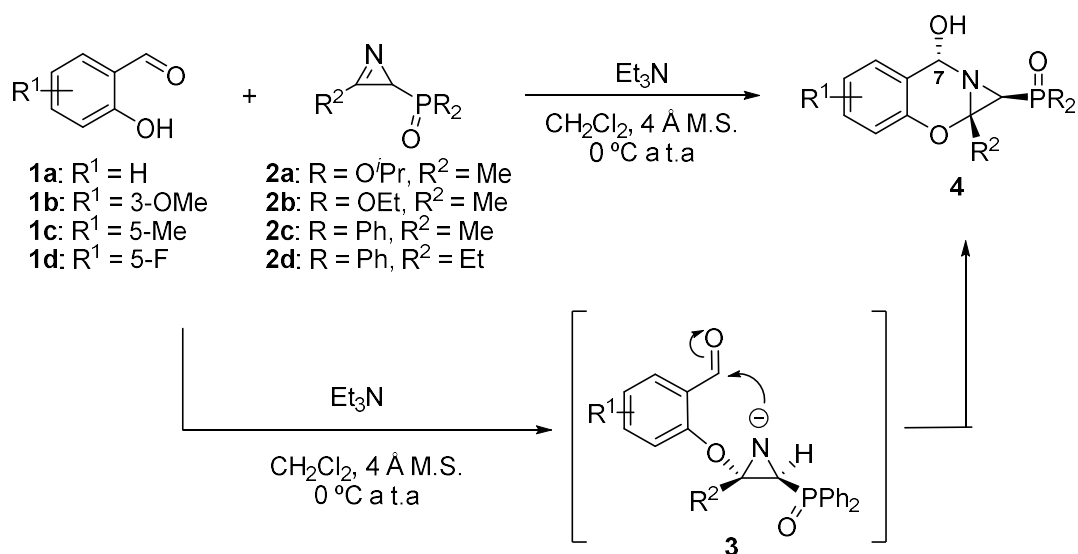
Manuscrito 3. First synthesis of merged hybrids phosphorylated azirino[2,1-*b*]benzo[*e*][1,3]oxazine derivatives as anticancer agents.

En este trabajo se describe una metodología sintética diastereoselectiva para la preparación de azirino[2,1-*b*]benzo[*e*][1,3]oxazinas con sustituyentes fosforados mediante la adición nucleofílica de fenoles funcionalizados al doble enlace C-N de 2*H*-azirinas. También, se estudia la citotoxicidad *in vitro* de las azirino[2,1-*b*]benzo[*e*][1,3]oxazinas fosforadas preparadas ensayando sus actividades antiproliferativas frente a dos líneas diferentes de cáncer humano; adenocarcinoma de pulmón humano (A549) y células embrionarias de riñón humano (HEK293).

2.1. SÍNTESIS DE AZIRINO[2,1-*b*]BENZO[*e*][1,3]OXAZINAS

Inicialmente, se pensó que la adición nucleofílica de salizaldehídos funcionalizados **1** a 2*H*-azirinas fosforadas **2**, proporcionaría una aproximación útil a la síntesis de nuevos derivados de azirino-benzoxazina funcionalizados **4** con un grupo fosforado en C-1 (Esquema 1). En primer lugar, se evaluó la reacción de 2*H*-azirinas con diferentes derivados de salizaldehído **1**. Como se describe en el esquema 1, la adición nucleofílica de salicilaldehído **1a** ($R^1 = H$) a la 2*H*-azirina **2a** ($R = O^iPr$, $R^2 = Me$) se llevó a cabo mediante el empleo de Et_3N como base, en diclorometano y a temperatura ambiente, empleando dos equivalentes de salicilaldehído para asegurar una conversión completa. En estas condiciones de reacción, el derivado de azirino-benzoxazina **4a** se obtuvo como una mezcla de dos isómeros en una proporción de 71:29 (Tabla 1, entrada 1), como lo demuestra la presencia de dos grupos de señales en los espectros de 1H y ^{31}P RMN.

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Esquema 1. Síntesis diastereoselectiva de azirino[2,1-*b*]benzo[*e*]oxazinas.

Tabla 1. Azirino[2,1-*b*]benzo[*e*][1,3]oxazinas derivadas de fosfonato y óxido de fosfina sintetizados.

Entrada	Compuesto	R	R ¹	R ²	Rto(%) ^[a]	Proporción rotámeros ^[b]
1	4a	O ⁱ Pr	H	Me	81	71:29 (81:19) ^[c]
2	4b	OEt	H	Me	76	70:30
3	4c	Ph	H	Me	55	76:24
4	4d	Ph	H	Et	54	81:19
5	4e	Ph	3-OMe	Me	80	76:24
6	4f	Ph	3-OMe	Et	51	74:26
7	4g	Ph	5-Me	Me	84	75:25
8	4h	Ph	5-Me	Et	75	74:26
9	4i	Ph	5-F	Me	96	92:8

^[a] Rendimiento de los compuestos aislados puros **4**. ^[b] Relación de rotámeros calculada por ¹H NMR en solución de CDCl₃. ^[c] Relación de rotámeros calculada por ¹H NMR en solución de DMSO-*d*₆.

En el manuscrito 1, se describe una metodología sintética diastereoselectiva para la preparación de cianoaziridinas fosforadas, las cuales han sido obtenidas como una mezcla de

dos rotámeros. Asumiendo que la mezcla de isómeros obtenida para el compuesto **4a** pudiese ser atribuida a una mezcla de rotámeros alrededor del enlace C-P, se realizaron estudios de ^1H y ^{31}P RMN a temperatura variable para el derivado azirino-benzoxazina **4a** ($\text{R} = \text{O}^i\text{Pr}$, $\text{R}^1 = \text{H}$, $\text{R}^2 = \text{Me}$). Estos estudios apoyan la hipótesis de que la aparición de dos conjuntos de señales en los espectros de ^1H , ^{31}P y ^{13}C RMN se debe a la presencia de dos rotámeros del compuesto **4a**, ya que ambos rotámeros se intercambian por rotación alrededor del enlace C-P. El experimento de ^{31}P RMN a temperatura variable (Figura 1) para el derivado **4a** en $\text{DMSO-}d_6$ muestra una mezcla de dos isómeros en una proporción 81:19 a $18\text{ }^\circ\text{C}$. Sin embargo, esta mezcla tiende a equilibrarse rápidamente a altas temperaturas, observándose a $80\text{ }^\circ\text{C}$ un cambio en la proporción de rotámeros de 62:38.

Resultados similares se obtienen en los estudios de ^1H RMN a temperatura variable (Figura 2). En este caso, se observa una pequeña diferencia en la proporción de rotámeros al cambiar el disolvente de CDCl_3 a $\text{DMSO-}d_6$ (Tabla 1, entrada 1). Como ya se ha mencionado anteriormente, este fenómeno puede deberse a las diferentes geometrías alrededor del átomo de fósforo.^{220,220}

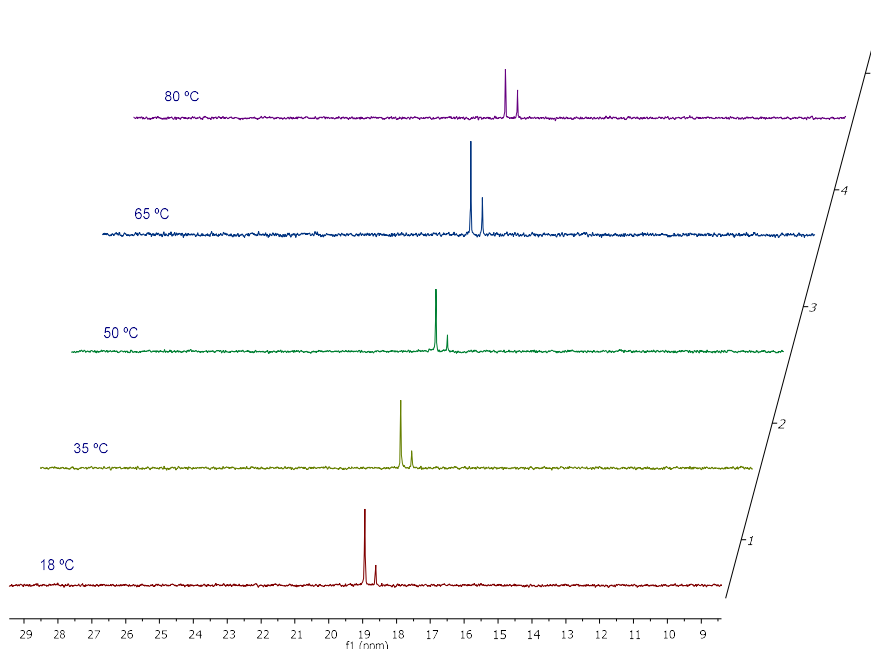


Figura 1. Experimento ^{31}P RMN de temperatura variable para **4a** en $\text{DMSO-}d_6$.

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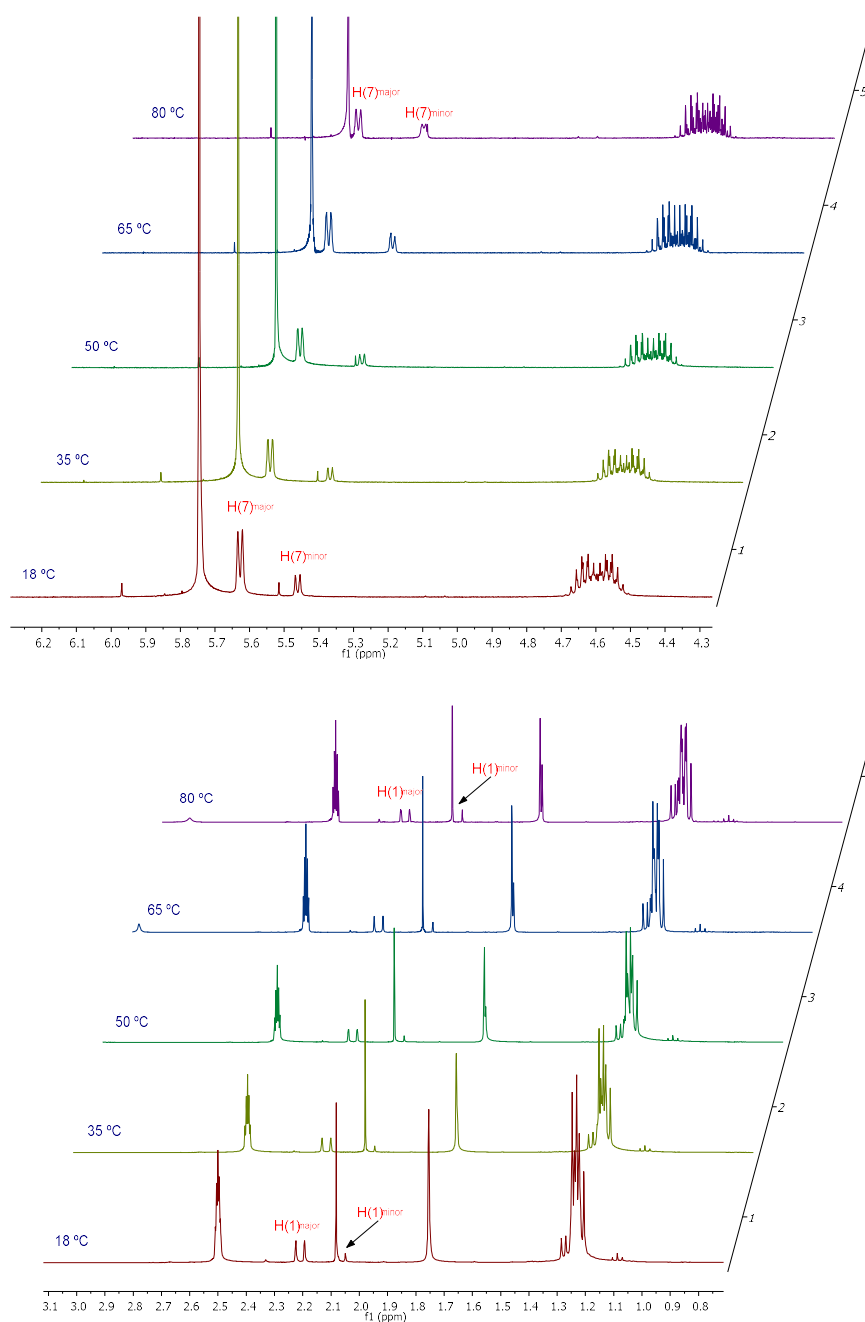


Figura 2. Experimento ^1H RMN de temperatura variable para **4a** en $\text{DMSO-}d_6$.

La ausencia de la constante de acoplamiento observada en el espectro de ^{13}C RMN entre el sustituyente fosforado en C-1 y el grupo metilo en C-1a de la azirino-benzoxazina **4a**, parece indicar una relación *sin* entre ambos grupos. El análisis de difracción de rayos X (Figura 3) no solo estableció la relación *sin* entre el grupo metilo y el átomo de fósforo, sino también la relación *anti* entre estos grupos y el grupo hidroxilo en C-7.

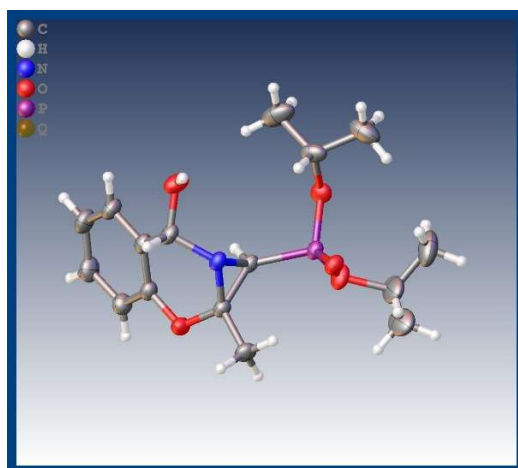


Figura 3. Diagrama ORTEP para el derivado azirino-benzoxazina **4a**.

De la misma forma, la adición de salicilaldehído **1a** a la *2H*-azirina derivada de fosfonato de dietilo **2b** proporciona el derivado de azirino-benzoxazina **4b** ($R = \text{OEt}$, $R^1 = \text{H}$, $R^2 = \text{Me}$) (Esquema 1, Tabla 1, entrada 2).

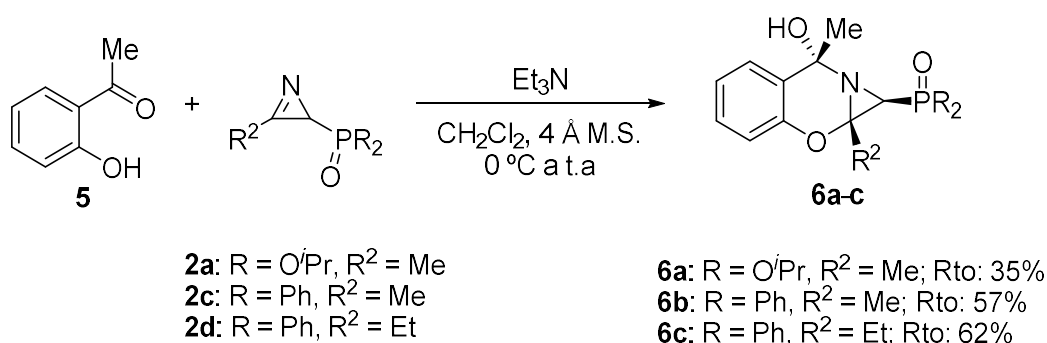
El mecanismo propuesto para la construcción de las azirino[2,1-*b*]benzo[*e*]oxazinas **4** puede explicarse a través de la adición nucleofílica de fenoles funcionalizados **1** al doble enlace C-N de la *2H*-azirina **2**, conduciendo al intermedio **3**. Es de esperar que la adición nucleofílica tenga lugar de forma diastereoselectiva por la cara menos impedida (Esquema 1), así, la aproximación del fenol **1** al doble enlace C-N de la *2H*-azirina **2**, por el lado opuesto al grupo fosforado, parece ser más favorable, debido al elevado ángulo diedro exocíclico del carbono saturado hibridizado sp^3 y a la presencia del grupo fósforado. Después de la adición nucleofílica, la construcción del fragmento tricíclico de azirino-benzoxazina en el derivado **4** tiene lugar mediante un ataque nucleofílo intramolecular diastereoselectivo del fragmento de aziridina al grupo carbonilo del intermedio **3**.

Este proceso, también se extendió a la adición nucleofílica de salicilaldehído **1a** a *2H*-azirinas derivadas de óxido de fosfina **2c-d** ($R = \text{Ph}$) (Esquema 1). Las azirino-benzoxazinas **4c** ($R = \text{Ph}$, $R^1 = \text{H}$, $R^2 = \text{Me}$, Tabla 1, entrada 3) y **4d** ($R = \text{Ph}$, $R^1 = \text{H}$, $R^2 = \text{Et}$, Tabla 1, entrada 4) se obtuvieron con rendimientos moderados y como mezcla de dos rotámeros. Como aparece reflejado en la tabla 1, esta metodología sintética se puede aplicar a una variedad de salicilaldehídos sustituidos funcionalizados **1**. Por ejemplo, las *2H*-azirinas derivadas de óxido de fosfina **2c-d** ($R = \text{Ph}$) reaccionaron con *o*-vanilina **1b** ($R^1 = 3\text{-OMe}$), 5-metilsalicilaldehído **1c** ($R^1 = 5\text{-Me}$), o 5-

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fluorosalicilaldehído **1d** ($R^1 = 5-F$) en presencia de Et_3N , dando lugar a las correspondientes azidinil-benzoxazinas **4e-i** (Tabla 1, entradas 5-9).

Para definir el alcance y las limitaciones del proceso, y así aumentar la diversidad de sustituyentes en nuestros sustratos, esta metodología diastereoselectiva se extendió a la adición de 2-hidroxiacetofenona **5** a las 2*H*-azirinas fosforadas **2**. Para ello, la adición nucleofílica de **5** a las 2*H*-azirinas derivadas de fosfonato **2a** y óxidos de fosfina **2c-d** se llevó a cabo empleando Et_3N como base y en CH_2Cl_2 como disolvente. Bajo estas condiciones de reacción se obtuvieron los derivados **6a-c** con rendimientos moderados (Esquema 2). Estas nuevas azirino-benzoxazinas **6** se obtuvieron como una mezcla de dos rotámeros en una proporción de aproximadamente 85:15.



Esquema 2. Adición de 2-hidroxiacetofenona a 2*H*-azirinas fosforadas.

El análisis de difracción de rayos X para la azirino-benzoxazina **6a** (Figura 4) indica una estereoquímica similar a la observada en el caso de la adición de salizaldehídos funcionalizados. Así, se observa una relación *sin* entre el grupo metilo en C-1a y el grupo fosforado en C-1, así como una relación *anti* entre estos grupos y el grupo hidroxilo en C-7.

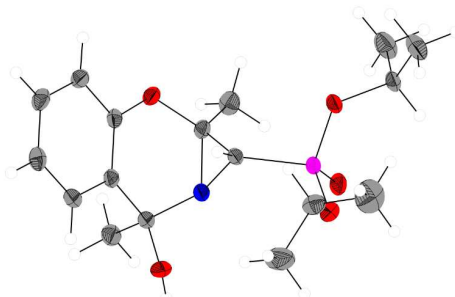


Figura 4. Diagrama ORTEP para el compuesto **6a**.

3.2 ESTUDIO DE LA ACTIVIDAD ANTIPROLIFERATIVA

La actividad antiproliferativa *in vitro* de las azirino[1,2-*b*]benzo[*e*][1,3]oxazinas fosforadas **4** y **6** se estudia frente a diferentes líneas celulares de cáncer humano: adenocarcinoma de pulmón humano (A549) y células embrionarias de riñón humano (HEK293). Con el fin de evaluar la inhibición del crecimiento celular y las actividades inhibitoras de la proliferación celular como valores de IC₅₀ de todos los compuestos preparados (Tabla 2), se usa el ensayo de CCK-8. Estos resultados se comparan con la actividad antiproliferativa de la doxorrubicina (DOX). Además, con el objeto de determinar citotoxicidad selectiva, también se determina la actividad antiproliferativa frente a una línea no cancerígena de células sanas como la MRC-5 (fibroblastos derivados de tejido pulmonar)²²⁴ En general los derivados **4** y **6** presentan una mayor citotoxicidad selectiva en la línea celular de adenocarcinoma de pulmón humano (A549) comparada con la línea celular de riñón embrionario humano (HEK-293).

Las azirino-benzoxazinas derivadas de fosfonato **4a** y **4b** en la línea celular A549 *in vitro* muestran valores diferentes de IC₅₀ entre 3.0 ± 0.29 y 15.8 ± 2.44 μM (Tabla 2, entradas 2-3), siendo la diisopropil azirino-benzoxazina **4a** (R = OⁱPr, R¹ = H, R² = Me) el compuesto más eficaz con un valor de IC₅₀ = 3.0 ± 0.29 μM (Tabla 2, entrada 2). En el caso de las azirino-benzoxazinas derivadas de óxidos de fosfina **4c-i** (Tabla 2, entradas 4-10), se observan unos valores de IC₅₀ entre 1.3 ± 0.22 y 20.7 ± 3.75 μM , donde el compuesto más eficaz fue **4e** (R = Ph, R¹ = 3-OMe, R² = Me, R³ = H) con un valor de IC₅₀ = 1.3 ± 0.22 μM (Tabla 2, entrada 6). Respecto al efecto de la sustitución en C-1a, en general, los derivados con un grupo metilo en C-1a (R² = Me, **4c**, **4e** y **4g**) muestran mejores actividades que aquellos con un grupo etilo (R² = Et, **4d**, **4f** y **4h**), independientemente de la sustitución en el anillo aromático (R¹ = H, 3-OMe, 5-Me). Asimismo, se observa una selectividad muy alta en la actividad antiproliferativa de las de azirino-benzoxazinas **4a-i** (Tabla 2, entradas 2-10) en la línea celular A549 *versus* HEK-293.

También se estudia el efecto de sustitución en C-7 de las correspondientes azirino-benzoxazinas derivadas de fosfonato y óxidos de fosfina **6** en su citotoxicidad en la línea celular A549. A diferencia del efecto de sustitución en C-1a observado para los derivados **4**, los derivados **6** muestran un comportamiento diferente con valores de IC₅₀ entre 3.2 ± 0.27 y 21.1 ± 2.44 μM (Tabla 2, entradas 11-13), siendo la azirino-benzoxazina **6c** (R = Ph, R¹ = H, R² = Et, R³ = Me) la más efectiva con un valor IC₅₀ de 3.2 ± 0.27 μM (Tabla 2, entrada 13).

Tabla 2. Actividad antiproliferativa de azirino[2,1-*b*]benzo[*e*][1,3]oxazinas **4** y **6**.

Entr.	Comp.	R	R ¹	R ²	R ³	Citotoxicidad IC ₅₀ (μM) ^[a]		
						Pulmón	Riñón	MRC-5
						A549	HEK-293	
1	DOX	–	–	–		0.48 ± 0.017 ²²⁵	5.68 ± 1.56 ²²⁵	>50 ²²⁶
2	4a	O ⁱ Pr	H	Me	H	3.0 ± 0.29	>50	>50
3	4b	OEt	H	Me	H	15.8 ± 2.44	n.r. ^[b]	>50
4	4c	Ph	H	Me	H	2.7 ± 0.37	>50	>50
5	4d	Ph	H	Et	H	10.4 ± 0.68	>50	>50
6	4e	Ph	3-OMe	Me	H	1.3 ± 0.22	>50	>50
7	4f	Ph	3-OMe	Et	H	20.7 ± 3.75	>50	>50
8	4g	Ph	5-Me	Me	H	3.2 ± 0.80	>50	>50
9	4h	Ph	5-Me	Et	H	12.9 ± 0.90	>50	>50
10	4i	Ph	5-F	Me	H	19.6 ± 0.24	>50	>50
11	6a	O ⁱ Pr	H	Me	Me	10.3 ± 1.67	>50	>50
12	6b	Ph	H	Me	Me	21.1 ± 2.44	>50	>50
13	6c	Ph	H	Et	Me	3.2 ± 0.27	>50	>50

^[a] Los valores de IC₅₀ corresponden a las concentraciones de los compuestos para las cuales se inhibe el crecimiento celular en un 50%. ^[b] No reportado.

La azirino-benzoxazina derivada de fosfonato de diisopropilo **6a** (R³ = Me) (Tabla 2, entrada 11) resulta menos activa que el derivado correspondiente **4a** (R³ = H) (Tabla 2, entrada 2). Asimismo, como en el caso anterior, se observa una selectividad muy alta en las propiedades citotóxicas de las azirino-benzoxazinas **6** en la línea celular A549 *versus* HEK-293. Con el objetivo de explorar la toxicidad selectiva, se ensayaron los compuestos **4** y **6** en células pulmonares sanas, como los fibroblastos pulmonares no malignos MRC-5. Ninguno de los derivados sintetizados **4** y **6** muestra toxicidad en la línea celular MRC-5 (Tabla 2).

Manuscrito 4. Synthesis of α -aminophosphonic acid derivatives through the addition of *O*- and *S*-nucleophiles to *2H*-azirines and their antiproliferative effect on A549 human lung adenocarcinoma cells.

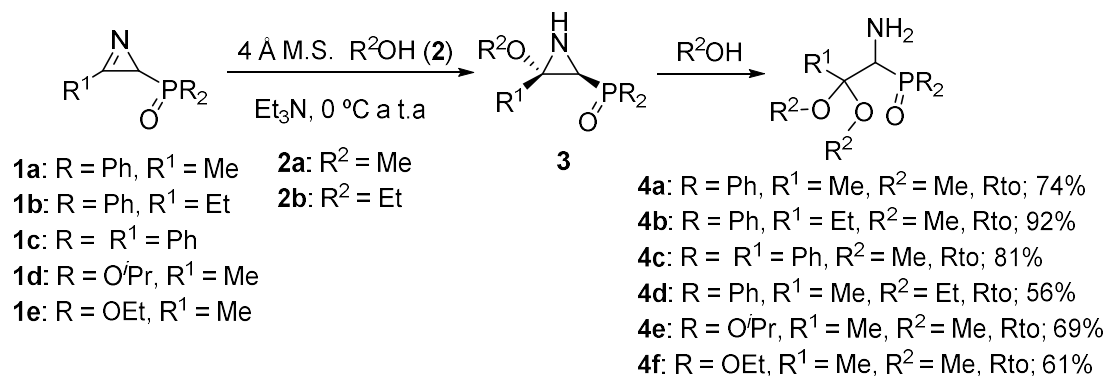
Este trabajo describe una síntesis regioselectiva de α -aminofosfonatos y aminofosfanóxidos mediante la adición de nucleófilos oxigenados y azufrados al doble enlace C–N de *2H*-azirinas fosforadas. En función de la naturaleza del reactivo nucleófilo, se pueden obtener diferentes compuestos α -aminofosforados. Por ejemplo, la adición de alcoholes alifáticos como metanol o etanol, conduce a acetales derivados de α -aminofosfanóxidos y fosfonatos tras la apertura del anillo del intermedio aziridínico a través del enlace N-C3. Por otro lado, la adición de 2,2,2-trifluoroetanol, fenoles, tioles aromáticos y alifáticos a *2H*-azirinas fosforadas, da lugar a la formación de α -aminofosfanóxidos y fosfonatos alílicos. Además, en algunos casos se ha podido aislar la aziridina intermedia antes de la apertura anular. Asimismo, se estudia el efecto citotóxico de los diferentes derivados α -aminofosforados sintetizados en las líneas celulares de adenocarcinoma de pulmón humano (A549), así como en células no malignas (MRC-5).

4.1 SÍNTESIS DE DERIVADOS α -AMINOFOSFORADOS

Inicialmente, se estudia la adición de alcoholes alifáticos, metanol **2a** y etanol **2b**, a *2H*-azirinas **1** derivadas de óxido de fosfina y fosfonato. Para optimizar las condiciones de reacción, inicialmente se llevó a cabo la reacción de la *2H*-azirina **1a** con 2 equivalentes de MeOH y Et₃N como base y en CH₂Cl₂ como disolvente, lo que lleva a la recuperación de la *2H*-azirina de partida **1a**. Como alternativa, se emplea el alcohol alifático **2a** como nucleófilo y disolvente a la vez. Así, se lleva a cabo la adición nucleófila de MeOH **2a** a la *2H*-azirina derivada de óxido de difenilfosfina **1a** (R = Ph, R¹ = Me) empleando Et₃N como base y MeOH como nucleófilo y disolvente (Esquema 1). Bajo estas condiciones de reacción, se puede esperar obtener la correspondiente aziridina funcionalizada **3a**, de la misma forma que se observa en la reacción de metanol con *2H*-azirinas fluoradas.^{84f} Sin embargo, en lugar de la aziridina **3a**, se obtiene el α -aminofosfanóxido funcionalizado **4a** (R = Ph, R¹ = R² = Me) con un rendimiento del 74%, como se puede observar por los dos grupos de señales para grupo metoxilo en ¹H RMN. Bajo las

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mismas condiciones de reacción se pudieron aislar los α -aminofosfanóxidos funcionalizados **4b** (R = Ph, R¹ = Et, R² = Me) y **4c** (R = R¹ = Ph, R² = Me), cuando se usan las correspondientes 2*H*-azirinas fosforadas **1b** y **1c**, respectivamente (Esquema 1).

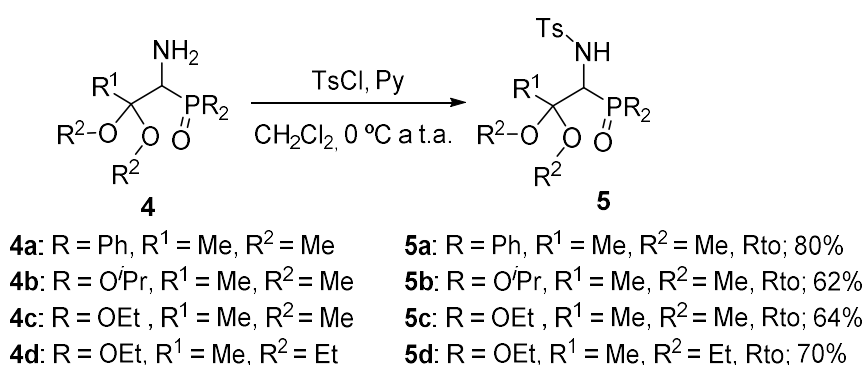


Esquema 1. Adición nucleofílica de MeOH y EtOH a 2*H*-azirinas fosforadas.

Este procedimiento sintético se amplió a la adición de MeOH a 2*H*-azirinas derivadas de fosfonato **1d** (R = OⁱPr) y **1e** (R = OEt), permitiendo así la obtención de los α -aminofosfonatos funcionalizados **4e** (R = OⁱPr, R¹ = R² = Me) y **4f** (R = OEt, R¹ = R² = Me) con rendimientos moderados (Esquema 1). Bajo las condiciones de reacción óptimas, se emplearon otros alcoholes alifáticos en la adición nucleofílica a 2*H*-azirinas **1**. Por ejemplo, la 2*H*-azirina **1a** (R = Ph) reacciona con etanol **2b** en presencia de Et₃N para dar el correspondiente α -aminofosfanóxido funcionalizado **4d** (R = Ph, R¹ = Me, R² = Et) (Esquema 1).

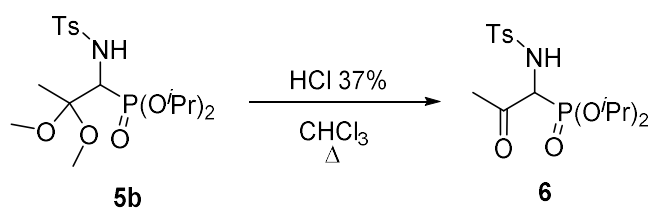
La formación de los correspondientes α -aminofosfanóxidos y fosfonatos funcionalizados **4** se puede explicar mediante la adición nucleofílica inicial de metanol **2a** o etanol **2b** al doble enlace C–N de las 2*H*-azirinas **1**, dando lugar a la aziridina intermedia **3**. El ataque nucleofílico diastereoselectivo es más probable que tenga lugar por la cara menos impedida estéricamente. Por lo tanto, la adición de metanol **2a** al doble enlace C–N de la 2*H*-azirina **1** por el lado opuesto al grupo óxido de fosfina o fosfonato parece ser más favorable, debido al elevado ángulo diédrico exocíclico del carbono saturado hibridizado sp³ y a la presencia del grupo fosforado voluminoso. A continuación, la apertura del anillo para formar los derivados α -aminofosforados **4** tiene lugar con completa selectividad en el enlace N–C3, después del ataque nucleofílico de una segunda molécula de metanol. Como se ha comentado en el marco teórico, este comportamiento se ha observado previamente en la adición de metanol a metilen-2*H*-azirinas, o más recientemente a una 2*H*-azirina sustituida con un grupo arilo.

También se explora la *N*-funcionalización de los derivados α -aminofosforados **4**, utilizando como grupo protector el tosilato. Por lo tanto, la reacción de sulfonilación de los acetales α -aminofosforados **4** se llevó a cabo mediante el empleo de cloruro *p*-toluensulfonilo (TsCl) en presencia de piridina y CH_2Cl_2 a temperatura ambiente, para conducir a los correspondientes *N*-tosilatos **5** con rendimientos de moderados a buenos (Esquema 2). Esta metodología puede llevarse a cabo a través de un proceso *one pot* a partir de las 2*H*-azirinas **1**, de tal forma que, la adición de etanol **2b** a la 2*H*-azirina **1e** en presencia de Et_3N proporciona el compuesto **4**, el cual, sin ser aislado, se somete a la reacción de sulfonilación con cloruro de *p*-toluensulfonilo en presencia de piridina para dar lugar al α -aminofosfonato *N*-tosilado **5d** (Esquema 2).



Esquema 2. *N*-Funcionalización de los α -aminofosfonatos y fosfanóxidos **4**.

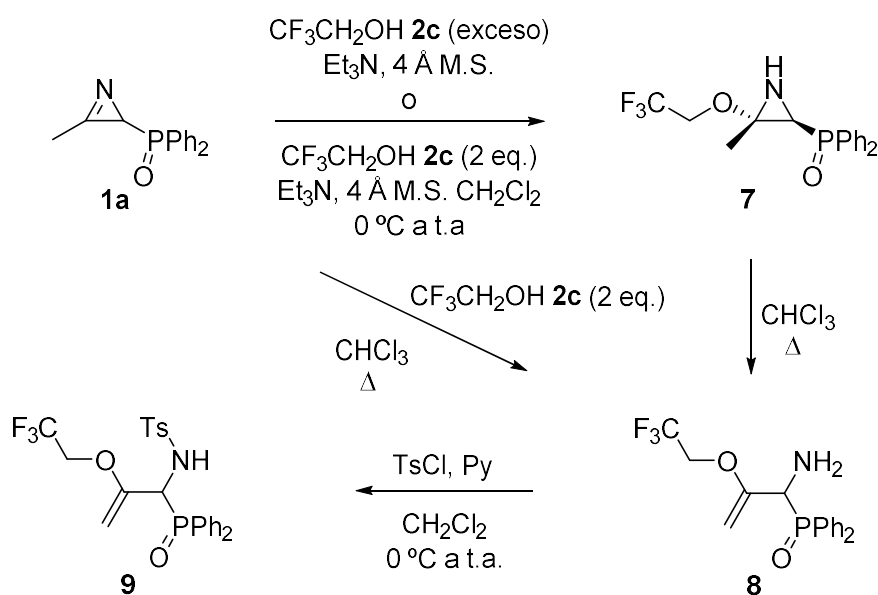
Asimismo, se estudia la hidrólisis del acetal **5b** en condiciones ácidas para la preparación del β -ceto- α -aminofosfonato **6**. El tratamiento de **5b** con una solución de HCl al 37% en cloroformo proporciona a la cetona **6** con un rendimiento del 68% (Esquema 3).



Esquema 3. Reacción de hidrólisis del acetal **5b**.

A continuación, se estudia la reacción de otros alcoholes alifáticos a 2*H*-azirinas **1**, para verificar si este tipo de nucleófilos podrían dar lugar a nuevos derivados α -aminofosforados

funcionalizados. Con este fin, se explora la reacción de la 2*H*-azirina derivada de óxido de fosfina **1a** con 2,2,2-trifluoroetanol **2c**. A diferencia de los derivados α -aminofosforados **4** observados en la reacción de azirinas **1** con metanol o etanol (Esquema 1), al tratar la 2*H*-azirina **1a** con 2,2,2-trifluoroetanol **2c** en las condiciones estándar de reacción (ver esquema 1) o con dos equivalentes de **2c** en presencia de Et₃N como base y CH₂Cl₂ como disolvente, se obtuvo la aziridina **7** con un rendimiento del 91% (Esquema 4). Por otro lado, si la adición de trifluoroetanol **2c** a **1a** se lleva a cabo a reflujo de cloroformo, el derivado α -aminofosfanóxido alílico **8** se forma exclusivamente en lugar de la aziridina **7** (Esquema 4). Los datos espectroscópicos de ¹H y ¹³C RMN concuerdan con la estructura asignada para el compuesto **8**. El mecanismo de la formación del compuesto **8** podría comenzar con la formación inicial de la correspondiente aziridina **7**, como resultado de la adición de trifluoroetanol **2c** al doble enlace C–N de la 2*H*-azirina **1a**. A continuación, la formación del doble enlace C–C y la apertura del anillo a través del enlace N–C3 de la aziridina daría lugar al α -aminofosfanóxido alílico **8**. Este último compuesto resulta ser inestable, y, por tanto, tras tratar **8** con cloruro *p*-toluenosulfonilo en presencia de piridina y CH₂Cl₂ a temperatura ambiente, se transforma en la sulfamida correspondiente **9** con un rendimiento del 90%.

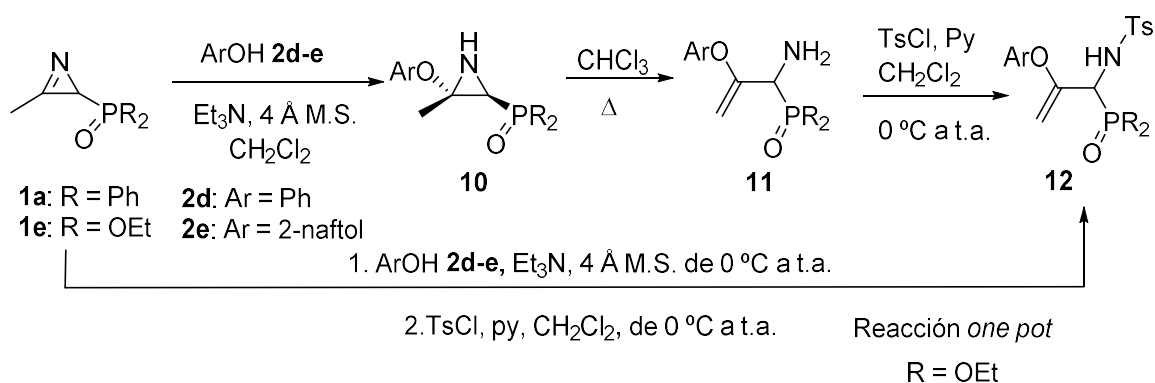


Esquema 4. Adición de 2,2,2-trifluoroetanol a la 2*H*-azirina **1a**.

Con el fin de ampliar el alcance de la adición de reactivos nucleofílicos oxigenados a 2*H*-azirinas **1**, y aumentar la diversidad de sustituyentes en nuestros sustratos, se extiende esta metodología

al estudio de la reactividad de alcoholes aromáticos **2d-e**, a 2*H*-azirinas fosforadas **1**. Así, la adición nucleófila de fenol **2d** a la 2*H*-azirina **1a** se lleva a cabo usando Et₃N como base en CH₂Cl₂, dando lugar a la formación de la aziridina **10a** con un rendimiento moderado (Esquema 5, tabla 1, entrada 1). Por el contrario, la adición de 2-naftol **2d** a la 2*H*-azirina **1a**, bajo las mismas condiciones de reacción, condujo a una mezcla de aziridina **10b** y α-aminofosfanóxido alílico **11b** (Esquema 5, tabla 1, entrada 2). Como consecuencia de la inestabilidad de la aziridina **10b**, la escisión del enlace N–C3 en el anillo de aziridina **10b** se produce rápidamente para dar el correspondiente derivado α-aminofosfanóxido alílico **11b**. Esta observación se puede confirmar, tanto, cuando la aziridina **10a**, o una mezcla de aziridina **10b** y el derivado **11b**, se calienta a reflujo de cloroformo. En estas condiciones de reacción, se obtienen de forma exclusiva los correspondientes α-aminofosfanóxidos alílicos **11a** o **11b**, respectivamente, con buenos rendimientos (Esquema 5, tabla 1, entrada 3 y 4).

Posteriormente, esta metodología se amplía a la adición de alcoholes aromáticos **2d-e** a 2*H*-azirinas derivadas de fosfonato **1e**. En este caso, solo se observaron, directamente en el bruto de reacción, los α-aminofosfonatos alílicos **11**, pero debido a su inestabilidad, no pudieron ser aislados. Por lo tanto, los intermedios derivados de fosfonato **11** se sometieron a una reacción *one pot* de sulfonilación, dando lugar a la formación de *N*-tosil α-aminofosfonatos alílicos **12a-b** (Esquema 5, tabla 1, entradas 5 y 6).



Esquema 5. Adición de fenol o 2-naftol a 2*H*-azirinas fosforadas.

Este proceso representa, hasta donde nosotros sabemos, la primera adición de nucleófilos oxigenados al doble enlace C–N de 2*H*-azirinas con un sustituyente fosforado, para la formación de forma regioselectiva, de derivados α-aminofosforados alílicos.

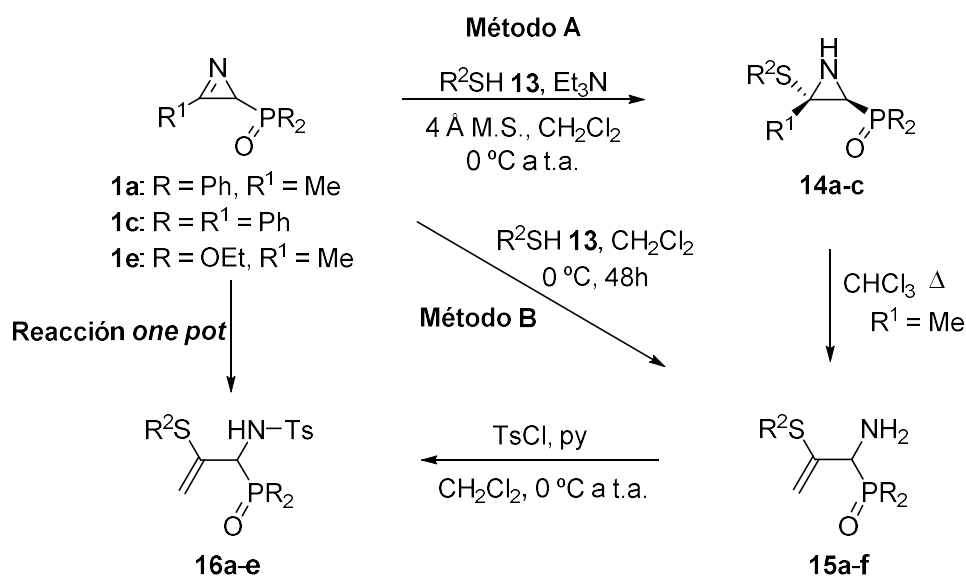
Tabla 1. Aziridinas **10**, α -aminofosfanóxidos **11** y aminofosfonatos **12** sintetizados.

Entrada	Compuesto	R	Ar	Rto(%) ^[a]
1	10a	Ph	Ph	70
2	10b	Ph	2-naftil	^[b]
3	11a	Ph	Ph	93
4	11b	Ph	2-naftil	74
5	12a	OEt	Ph	88 ^[c]
6	12b	OEt	2-naftil	96 ^[c]

^[a] Rendimiento de los compuestos aislados puros **10**, **11** y **12**. ^[b] Compuesto identificado en la mezcla del crudo de reacción. ^[c] Reacción *one pot* a partir de la 2*H*-azirina **1e**.

Finalmente, y con el objeto de ampliar la metodología sintética a otros heteronucleófilos, también se estudia la adición de nucleófilos azufrados como, por ejemplo, tioles y tiofenoles, a las 2*H*-azirinas fosforadas **1**. La adición de tiofenoles o tioles a 2*H*-azirinas **1** proporciona un enfoque útil para la síntesis de nuevos derivados de aziridina **14** o, incluso derivados α -aminofosforados alílicos **15**. Según se representa en el esquema 6, en ensayos iniciales la adición nucleófila de bencenotiol **13a** ($R^2 = \text{Ph}$) a la 2*H*-azirina **1a** ($R = \text{Ph}$, $R^1 = \text{Me}$) se lleva a cabo de manera sencilla usando Et_3N en diclorometano y a temperatura ambiente (método A). Usando estas condiciones de reacción, se obtiene la aziridina **14a** con un rendimiento del 92% (Esquema 6, Tabla 2, entrada 1). Esta aziridina **14a** es muy inestable, y tras la cristalización, en el espectro de ^1H RMN se observan señales correspondientes a la aziridina **14a** y otras de menor intensidad que coinciden con el α -aminofosfanóxido alílico **15a**, formado a través de la apertura del anillo de aziridina a través del enlace N–C3. Tras un breve calentamiento de la mezcla **14a** y **15a** a reflujo de cloroformo, en RMN solo se observan las señales correspondientes al α -aminofosfanóxido alílico **15a** (Esquema 6, Tabla 2, entrada 4). En resultados preliminares,^{84e} solamente se detectó el α -aminofosfanóxido alílico **15a**, y la aziridina intermedia **14a** no se pudo aislar ni detectar en el crudo de reacción a través de RMN.

De forma similar, a partir de la 2*H*-azirina **1a** y 4-metilbencenotiol **13b** ($R^2 = p\text{-MeC}_6\text{H}_4$), se aísla una mezcla de aziridina **14b** (Esquema 6, Tabla2, entrada 2) y α -aminofosfanóxido alílico **15b**, que tras un calentamiento a reflujo de cloroformo proporciona únicamente **15b** (Esquema 6, Tabla2, entrada 5). A continuación, se realiza la adición de bencenotiol **13a** a la 2*H*-azirina **1c** ($R = R^1 = \text{Ph}$) bajo las condiciones estándar de reacción (método A), lo que permite obtener estereoselectivamente la *E*-aziridina **14c** (Esquema 6, Tabla2, entrada 3).



Esquema 6. Adición de tioles alifáticos, aromáticos y *p*-sustituidos a 2*H*-azirinas.

La optimización de las condiciones de reacción, permite llevar a cabo la formación regioselectiva de derivados α -aminofósforados alílicos **15**. Así, cuando la 2*H*-azirina **1a** reacciona con 4-metilbencenotiol **13b** ($R^2 = p\text{-MeC}_6\text{H}_4$) sin base a $0^\circ C$ durante 48 h (método B), solo se observa la formación del derivado **15b** (Esquema 6, Tabla 2, entrada 5). Asimismo, este proceso también es aplicable a otros bencenotioles sustituidos **13**. Por ejemplo, la 2*H*-azirina **1a** ($R = Ph$, $R^1 = Me$) reacciona con 4-fluorobencenotiol **13c** ($R^2 = p\text{-FC}_6\text{H}_4$) o 4-metoxibencenotiol **13d** ($R^2 = p\text{-MeOC}_6\text{H}_4$) durante 48 h a $0^\circ C$, dando los correspondientes α -aminofosfanóxidos alílicos **15c-d** (Esquema 6, Tabla 2, entrada 6-7). Por otro lado, esta metodología también es aplicable a 2*H*-azirinas derivadas de fosfonato ($R = OEt$), dado que la reacción de adición de bencenotiol **13a** a la 2*H*-azirina **1e** ($R = OEt$, $R^1 = Me$) proporciona el α -aminofosfonato alílico **15e** (Esquema 6, Tabla 2, entrada 8). Asimismo, tioles alifáticos como el etanotiol **13e** reacciona con la 2*H*-azirina **1a** dando lugar a la formación del derivado **15f** de forma regioselectiva (Esquema 6, Tabla 2, entrada 9).

Finalmente, se estudia la *N*-protección de los correspondientes α -aminofosfanóxidos y aminofosfonatos alílicos **15**. Para ello, los compuestos **15** se someten a una reacción de sulfonilación utilizando cloruro de tosilo ($TsCl$) en presencia de piridina, CH_2Cl_2 como disolvente y a temperatura ambiente, lo que permite obtener los *N*-tosil α -aminofosfanóxidos y aminofosfonatos alílicos **16a-c** con buenos rendimientos (Esquema 6, Tabla 2, entrada 10-12).

Tabla 2. Aziridinas **14** y α -aminofosfonatos alílicos derivados de óxido de fosfina y fosfonato **15** y **16** obtenidos.

Entrada	Compuesto	R	R ¹	R ²	Rto(%) ^[a]
1	14a	Ph	Me	Ph	92 ^[b]
2	14b	Ph	Me	<i>p</i> -MeC ₆ H ₄	^[c,d]
3	14c	Ph	Ph	Ph	60 ^[b]
4	15a	Ph	–	Ph	91 ^[b]
5	15b	Ph	–	<i>p</i> -MeC ₆ H ₄	89 ^[d]
6	15c	Ph	–	<i>p</i> -FC ₆ H ₄	76 ^[d]
7	15d	Ph	–	<i>p</i> -MeOC ₆ H ₄	70 ^[d]
8	15e	OEt	–	Ph	41 ^[d]
9	15f	Ph	–	Et	64 ^[b]
10	16a	Ph	–	Ph	87
11	16b	Ph	–	<i>p</i> -MeC ₆ H ₄	84
12	16c	OEt	–	Ph	85
13	16d	OEt	–	<i>p</i> -FC ₆ H ₄	85 ^[e]
14	16e	OEt	–	<i>p</i> -MeC ₆ H ₄	73 ^[e]

^[a] Rendimiento de los compuestos purificados aislados **14**, **15**, y **16**. ^[b] Método A. ^[c] Compuesto identificado en la mezcla del bruto de reacción. ^[d] Método B. ^[e] Reacción *one pot* a partir de la 2*H*-azirina **1e**.

Esta metodología puede llevarse a cabo a través de un proceso *one pot* a partir de la 2*H*-azirina **1e** cuando ésta reacciona con bencenotioles *p*-sustituídos **13** a 0 °C durante 48h y a continuación se trata con cloruro de *p*-toluenosulfonilo en presencia de piridina, conduciendo a los correspondientes *N*-tosil α -aminofosfonatos **16d-e** (Esquema 6, Tabla 2, entrada 13-14).

4.2 EVALUACIÓN DE LA ACTIVIDAD BIOLÓGICA

Se evalúa la citotoxicidad *in vitro* de los derivados α -aminofosforados **4** y **5**, β -ceto- α -aminofosfonato **6**, aziridinas **7**, **10** y **14**, así como de los derivados α -aminofosforados alílicos derivados de óxido de fosfina y fosfonato **8**, **9**, **11**, **12**, **15**, y **16**, evaluando sus actividades antiproliferativas frente a la línea celular de adenocarcinoma de pulmón humano (A549). Asimismo, se emplea también la línea celular de carcinoma de colon humano (RKO) para evaluar

la actividad antiproliferativa de algunos de nuestros compuestos. Con el fin de evaluar la inhibición del crecimiento celular y las actividades inhibitoras de la proliferación celular como valores de IC₅₀ de todos los compuestos preparados (Tabla 3), se usa el ensayo de CCK-8. Estos resultados se comparan con la actividad antiproliferativa de la doxorrubicina (DOX). Además, con el objeto de determinar citotoxicidad selectiva, también se determina la actividad antiproliferativa frente a una línea no cancerígena de células sanas como la MRC-5 (fibroblastos derivados de tejido pulmonar).²²⁴

Los derivados α -aminofosforados derivados de óxido de fosfina y fosfonato **4** y **5** presentan un efecto citotóxico cuando se evalúan frente a la línea celular A549 *in vitro*. Por ejemplo, los derivados **4** muestran valores de IC₅₀ entre 1.3 \pm 0.10 y 21.3 \pm 0.22 μ M (Tabla 3, entrada 2-7), siendo el compuesto más efectivo el α -aminofosfonato **4f** con un valor de IC₅₀ de 1.3 \pm 0.10 μ M (Tabla 3, entrada 7). Se observan actividades similares para los *N*-tosil α -aminofosfanóxidos y fosfonatos **5** con valores de IC₅₀ entre 1.7 \pm 0.30 y 8.2 \pm 0.23 μ M (Tabla 3, entrada 8-11), siendo el compuesto más citotóxico el *N*-tosil α -aminofosfonato **5b** (Tabla 3, entrada 9). Por otro lado, la hidrólisis del grupo acetal no parece tener ningún efecto ya que el β -ceto α -aminofosfonato **6** no exhibe ninguna toxicidad hacia la línea celular A549 (Tabla 3, entrada 12).

Tabla 3. Actividad antiproliferativa de los compuestos sintetizados obtenidos de la adición de alcoholes y fenoles a 2*H*-azirinas **1**.

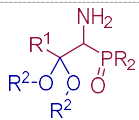
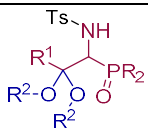
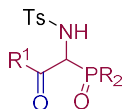
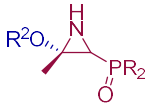
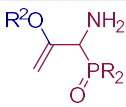
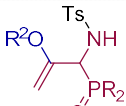
Entrada	Comp.	R	R ¹	R ²	Citotoxicidad IC ₅₀ (μ M) ^[a]	
					Pulmón A549	MRC-5
1	DOX	–	–	–	0.48 \pm 0.017 ²²⁵	>50 ²²⁶
						
2 ^[b]	4a	Ph	Me	Me	4.4 \pm 0.72	>50
3	4b	Ph	Et	Me	21.3 \pm 0.22	>50
4	4c	Ph	Ph	Me	16.1 \pm 2.03	>50
5	4d	Ph	Me	Et	9.6 \pm 1.13	>50
6	4e	O ⁱ Pr	Me	Me	4.6 \pm 0.31	>50
7	4f	OEt	Me	Me	1.3 \pm 0.10	>50

Tabla 3 (Cont). Actividad antiproliferativa de los compuestos sintetizados obtenidos de la adición de alcoholes y fenoles a 2*H*-azirinas **1**.

Entrada	Comp.	R	R ¹	R ²	Citotoxicidad IC ₅₀ (μM) ^[a]	
					Pulmón A549	MRC-5
						
8	5a	Ph	Me	Me	8.2 ± 0.23	>50
9	5b	O ⁱ Pr	Me	Me	1.7 ± 0.30	>50
10	5c	OEt	Me	Me	4.5 ± 0.45	>50
11	5d	OEt	Me	Et	3.7 ± 0.49	>50
						
12	6	O ⁱ Pr	Me	–	>50	^[c]
						
13	7	Ph	–	CH ₂ CF ₃	3.6 ± 0.70	>50
14	10a	Ph	–	Ph	13.3 ± 1.69	>50
						
15	8	Ph	–	CH ₂ CF ₃	>50	>50
16 ^[b]	11a	Ph	–	Ph	1.9 ± 0.13	>50
17	11b	Ph	–	2-Naph	2.7 ± 0.44	33.6 ± 3.73
						
18	9	Ph	–	CH ₂ CF ₃	3.5 ± 0.77	>50
19	12a	OEt	–	Ph	4.8 ± 0.90	>50
20	12b	OEt	–	2-Naph	2.1 ± 0.22	17.5 ± 1.47

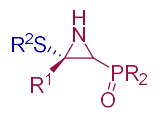
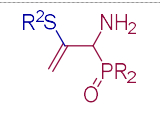
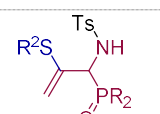
^[a] Los valores de IC₅₀ corresponden a las concentraciones de los compuestos para las cuales se inhibe el crecimiento celular en un 50%. ^[b] El valor de citotoxicidad contra la línea celular de carcinoma de colon humano (RKO) es > 50 μM. ^[c] No determinado.

Con respecto a las aziridinas sintetizadas con sustituyentes oxígenos y azufrados **7**, **10a** y **14c**, el compuesto más citotóxico es el derivado **14c** con un valor $IC_{50} = 1.1 \pm 0.32 \mu M$ (Tabla 4, entrada 2). Por otro lado, respecto a los derivados α -aminofosforados alílicos obtenidos a través de la adición de trifluoroetanol **2c** o alcoholes aromáticos **2d-e**, a parte del derivado α -aminofosfanóxido alílico **8** (Tabla 3, entrada 15) que no exhibe citotoxicidad frente a la línea celular A549; los derivados **11a-b** (Tabla 3, entradas 16-17), el derivado *N*-tosil α -aminofosfanóxido **9** (Tabla 3, entrada 18) y fosfonatos **12a-b** (Tabla 3, entradas 19-20) muestran muy buena citotoxicidad, con valores comprendidos entre $1.9 \pm 0.13 \mu M$ y $4.8 \pm 0.90 \mu M$, siendo los derivados **11a** y **12b** (Tabla 3, entradas 16-20) los que presentan una mayor citotoxicidad.

Posteriormente, se evalúa la citotoxicidad de los derivados α -aminofosforados alílicos **15** y **16**, obtenidos a través de la adición de nucleófilos azufrados. Todos ellos muestran buenos valores de citotoxicidad. Por ejemplo, se obtienen valores de IC_{50} comprendidos entre $0.1 \pm 0.08 \mu M$ y $7.2 \pm 0.49 \mu M$, siendo el derivado **15c** (Tabla 4, entrada 5) el compuesto más efectivo para los derivados α -aminofosforados alílicos **15**. Sin embargo, para los derivados *N*-tosil α -aminofosforados alílicos **16** el compuesto con mayor citotoxicidad es el derivado **16c** con un valor de IC_{50} de $0.2 \pm 0.07 \mu M$ (Tabla 4, entrada 10). Como se ha mencionado anteriormente, también se evalúa la actividad antiproliferativa de algunos de nuestros compuestos en la línea celular de carcinoma de colon humano (RKO). Sin embargo, en este caso algunos compuestos no muestran actividad citotóxica para esta línea celular como por ejemplo el derivado α -aminofosfanóxido **4a** y los α -aminofosfanóxidos alílicos **11a**, **15a**, **15c** y **16c**. El mejor resultado en esta línea celular es el observado para la aziridina **14c** con un valor de $IC_{50} = 9.7 \pm 1.4 \mu M$ (Tabla 4, entrada 2).

Finalmente, la línea celular de pulmón sano, como los fibroblastos pulmonares no malignos MRC-5, se analiza para estudiar la citotoxicidad selectiva.²²⁴ A excepción de algunos derivados α -aminofosforados alílicos, que muestran citotoxicidad moderada, casi todos los derivados α -aminofosforados sintetizados y aziridinas no exhiben toxicidad hacia la línea celular MRC-5. Además, la aziridina **14c** (Tabla 4, entrada 2) que muestra una buena citotoxicidad contra las líneas celulares A549 y RKO, también exhibe una buena citotoxicidad en la línea celular MRC-5.

Tabla 4. Actividad antiproliferativa de compuestos sintetizados obtenidos de la adición de tiofenoles a 2*H*-azirinas **1**.

Entrada	Comp.	R	R ¹	R ²	Citotoxicidad IC ₅₀ (μM) ^[a]	
					Pulmón A549	MRC-5
1	DOX	–	–	–	0.48 ± 0.017 ²²⁵	>50 ²²⁶
						
2 ^[b]	14c	Ph	Ph	Ph	1.1 ± 0.32	4.9 ± 0.49
						
3 ^[c]	15a	Ph	–	Ph	2.6 ± 0.68	15.9 ± 2.79
4	15b	Ph	–	<i>p</i> -MeC ₆ H ₄	5.1 ± 0.77	14.9 ± 1.61
5 ^[c]	15c	Ph	–	<i>p</i> -FC ₆ H ₄	0.1 ± 0.08	>50
6	15d	Ph	–	<i>p</i> -MeOC ₆ H ₄	2.6 ± 0.42	>50
7	15e	OEt	–	Ph	7.2 ± 0.49	>50
						
8	16a	Ph	–	Ph	1.2 ± 0.09	>50
9	16b	Ph	–	<i>p</i> -MeC ₆ H ₄	2.1 ± 0.15	>50
10 ^[c]	16c	OEt	–	Ph	0.2 ± 0.07	24.1 ± 3.55
11	16d	OEt	–	<i>p</i> -FC ₆ H ₄	3.0 ± 0.98	>50
12	16e	OEt	–	<i>p</i> -MeC ₆ H ₄	3.9 ± 0.63	>50

^[a] Los valores de IC₅₀ corresponden a las concentraciones de los compuestos para las cuales se inhibe el crecimiento celular en un 50%. ^[b] El valor de citotoxicidad contra la línea celular de carcinoma de colon humano (RKO) es 9,7 ± 1,4 μM. ^[c] El valor de citotoxicidad contra la línea celular de carcinoma de colon humano (RKO) es > 50 μM.

Conclusiones

La estrategia sintética descrita en esta tesis doctoral abre una nueva área, bien para la preparación de nuevos derivados de cianoaziridinas fosforadas sustituidas o azirino-benzoxazinas funcionalizadas, así como para la preparación de derivados acíclicos, tales como α -aminofosfonatos y fosfanóxidos. Esta estrategia sintética implica la adición al doble enlace C-N de las 2*H*-azirinas fosforadas de diferentes reactivos nucleófilos. Para ello, teniendo en cuenta que las 2*H*-azirinas son heterociclos nitrogenados altamente reactivos, y que han sido ampliamente usados como precursores para la construcción de una amplia gama de compuestos acíclicos y heterocíclicos nitrogenados, inicialmente se han sintetizado una gran variedad de 2*H*-azirinas-2-fosforadas derivadas tanto de óxido de fosfina (R = Ph) como de fosfonatos (R = OEt, OⁱPr) con buenos rendimientos, mediante la reacción de Neber modificada a partir de tosiloximas β -fosforadas. Una vez sintetizadas las 2*H*-azirinas-2-fosforadas se ha estudiado su reactividad frente a una gran variedad de nucleófilos.

Manuscrito 1- Synthesis and biological evaluation of cyanoaziridine phosphine oxides and phosphonates with antiproliferative activity.

- 1.1. Se ha desarrollado una eficiente metodología sintética diastereoselectiva para la preparación de cianoaziridinas-2-fosforadas derivadas de óxido de fosfinas (R = Ph) y fosfonatos (R = OEt, OⁱPr), a través de la adición nucleófila de cianotrimetilsilano (TMSCN) al doble enlace C-N de las 2*H*-azirinas. Mediante el empleo de TMSCN/MeOH como fuente de cianuro, se ha evitado el empleo de reactivos tóxicos como el HCN, así como catálisis usando metales de transición.
- 1.2. Hasta donde sabemos, esta metodología representa el primer ejemplo de adición nucleófila del anión cianuro a 2*H*-azirinas sustituidas con fósforo.
- 1.3. Además, la activación del anillo de cianoaziridina mediante el empleo de grupos electroattractores en el nitrógeno, se puede realizar mediante reacciones de *N*-tosilación o *N*-acilación de las correspondientes *NH*-cianoaziridinas, permitiendo el acceso a *N*-cianoaziridinas funcionalizadas con buenos rendimientos.
- 1.4. Las *NH*-cianoaziridinas fosforadas sintetizadas mostraron un efecto citotóxico *in vitro* muy eficaz, inhibiendo el crecimiento de las líneas celulares derivadas de adenocarcinoma de pulmón humano (A549). Así, tanto las *NH*-cianoaziridinas derivadas de fosfonato **2a–c** como de fosfanóxido **2d–e** presentaron valores comprendidos entre 1.5 ± 0.84 y 4.3 ± 0.43

Conclusiones

μM . Por otro lado, las cianoaziridinas *N*-funcionalizadas **5** y **6** muestran un valor de IC_{50} bastante más moderado, siendo el derivado más activo el compuesto **6d** con un IC_{50} de $30.1 \pm 4.10 \mu\text{M}$.

- 1.5 Se observa una alta selectividad en la actividad antiproliferativa de las cianoaziridinas **2** para la línea de adenocarcinoma de pulmón humano (A549) *versus* la línea celular de cáncer de riñón (HEK293).
- 1.6. Los estudios *in vitro* de citotoxicidad de los derivados de cianoaziridina en una línea celular no cancerosa (células pulmonares de fibroblastos, MRC-5), no parece presentar efecto alguno.

Manuscrito 2. Novel synthesis and antiproliferative activity of phosphorus substituted 4-cyanooxazolines, 2-aminocyanooxazolines, 2-iminocyanooxazolidines, and 2-aminocyanothiazolines by rearrangement of cyanoaziridines.

- 2.1. Se ha estudiado la reactividad de las *NH*-cianoaziridinas fosforadas, preparadas por adición de TMSCN a *2H*-azirinas descritas en el manuscrito 1, frente a electrófilos. Así, la reacción de *N*-acilación empleando cloruro 3,5-dinitrobenzoilo conduce a cianoaziridinas *N*-aciladas derivadas de óxido de fosfinas y fosfonatos. Asimismo, se han obtenido derivados de cianoaziridinas *N*-(tio)carbamoiladas a través de la adición de diferentes iso(tio)cianatos, tanto alifáticos como aromáticos.
- 2.2. Esta metodología constituye, hasta donde nosotros sabemos, el primer ejemplo de *NH*-funcionalización de *NH*-azirinas fosforadas con iso(tio)cianatos para la preparación de *N*-(tio)carbamoil aziridinas.
- 2.3. Por otro lado, se estudia la expansión del anillo de las cianoaziridinas para la preparación de heterociclos nitrogenados de 5 miembros. La reacción de isomerización en presencia de NaI (reacción de Heine) de la cianoaziridina *N*-acilada **2a** condujo de forma regioselectiva a la 4-ciano oxazolina 5-fosforada **3a**.
- 2.4. De igual forma, las cianoaziridinas *N*-carbamoiladas **5** condujeron a una mezcla de oxazolinas regioisoméricas **7** cuando fueron tratadas en las mismas condiciones que las *N*-acil cianoaziridinas **2**.

- 2.5. El estudio de la reacción de expansión del anillo de la *N*-tiocarbamoil cianoaziridinas **6** no condujo a los resultados esperados utilizando, tanto condiciones térmicas como nucleófilas. Por el contrario, la 2-aminotiazolina **8** pudo aislarse con un rendimiento moderado mediante el empleo de condiciones ácidas suaves, utilizando BF₃·OEt₂ como ácido de Lewis.
- 2.6. Finalmente, la expansión del anillo en *N*-carbamoil cianoaziridinas **5** seguido de la inserción de un segundo equivalente de isocianato, da lugar a la formación de iminooxazolidinas derivadas de óxido de fosfina (R = Ph) y fosfonato (R= OEt) **9** regioselectivamente. La reacción implica el tratamiento de las correspondientes *NH*-cianoaziridinas fosforadas con 2 equivalentes de isocianato en presencia de KI.
- 2.7. Se ha evaluado el efecto citotóxico *in vitro*, tanto de las nuevas cianoaziridinas *N*-funcionalizadas fosforadas, como de los heterociclos nitrogenados de 5 miembros procedentes de la expansión de anillo, frente a la línea celular de adenocarcinoma de pulmón humano (A549). A excepción de la *N*-acilaziridina **2a**, cuyo valor de IC₅₀ es de 22.9 ± 1.9 μM, las cianoaziridinas *N*-funcionalizadas **2b–c**, **5** y **6a–c**, no presentaron actividad antiproliferativa frente a la línea celular estudiada. Sin embargo, algunos derivados procedentes de la expansión de anillo exhibieron buenos resultados citotóxicos, como las iminooxazolidinas **9a–c**, cuyos valores de IC₅₀ se encuentran comprendidos entre 16.4 ± 1.5 μM y 6.2 ± 0.7 μM.
- 2.8. Además, el efecto citotóxico de todos nuestros compuestos en fibroblastos pulmonares no malignos (MRC-5) parece no mostrar ningún efecto.

Manuscrito 3- First synthesis of merged hybrids phosphorylated azirino[2,1-*b*]benzo[*e*][1,3]oxazine derivatives as anticancer agents.

- 3.1. En este trabajo, se describe una ruta sintética diastereoselectiva que da acceso a azirino[2,1-*b*]benzo[*e*][1,3]oxacinas con sustituyentes fosforados tales como fosfanóxidos o fosfonatos. Este proceso implica la adición nucleófila de derivados de fenol funcionalizados, como son los derivados de salizaldehído e hidroxiacetofenona, a 2*H*-azirinas-2-fosforadas.

Conclusiones

- 3.2. Esta metodología sintética representa el primer ejemplo de la preparación del núcleo tricíclico de azirino[2,1-*b*]benzo[*e*][1,3] oxazina, lo que hace posible la introducción de una diversidad estructural en las posiciones C-1, C-1a, C-7 y en el anillo aromático del marco tricíclico resultante, en función de la 2*H*-azirina y el derivado de fenol de partida utilizados.
- 3.3. Por otro lado, se evaluó la citotoxicidad *in vitro* de las azirino[2,1-*b*]benzo[*e*][1,3]oxazinas fosforadas testando sus actividades antiproliferativas en dos líneas celulares de cáncer humano diferentes: adenocarcinoma de pulmón humano (A549) y células embrionarias humanas de riñón (HEK-293). Algunas de las benzoxazinas exhibieron muy buen efecto citotóxico inhibiendo el crecimiento de la línea celular tumoral humana A549. Por ejemplo, los mejores resultados se obtuvieron con los compuestos **4a**, **4c** y **4e** con unos valores citotóxicos de IC₅₀ de $3.0 \pm 0.29 \mu\text{M}$, $2.7 \pm 0.37 \mu\text{M}$ y $1.3 \pm 0.22 \mu\text{M}$, respectivamente. Sin embargo, las benzoxazinas sintetizadas no presentaron actividad antiproliferativa frente a la línea de cáncer de riñón (HEK-293).
- 3.4. Además, el efecto citotóxico de las benzoxazinas en fibroblastos pulmonares no malignos (MRC-5) parece no presentar ningún efecto.

Manuscrito 4- Synthesis of α -aminophosphonic acid derivatives through the addition of *O*- and *S*-nucleophiles to 2*H*-azirines and their antiproliferative effect on A549 human lung adenocarcinoma cells.

- 4.1. En este manuscrito, mediante la adición de nucleófilos oxigenados y azufrados al doble enlace C-N de las 2*H*-azirinas y posterior apertura del anillo a través del enlace N-C3 de la aziridina intermedia, se ha desarrollado una atractiva ruta sintética regioselectiva para el acceso a α -aminofosfonatos y fosfanoxidos. Diferentes compuestos α -aminofosforados se pueden obtener en función de la naturaleza del nucleófilo.
- 4.2. La adición de alcoholes alifáticos como el metanol o etanol al doble enlace C-N de las 2*H*-azirinas de partida conduce a α -aminofosfanóxidos y fosfonatos funcionalizado **4**, tras la apertura del enlace N-C3 del anillo de aziridina intermedia.
- 4.3. Por el contrario, la adición de alcoholes aromáticos o 2,2,2-trifluoroetanol, dan lugar a la formación regioselectiva de derivados α -aminofosforados alílicos **8** y **11**. En estas condiciones de reacción, en algunos casos, es posible el aislamiento e incluso la

caracterización de las aziridinas intermedias. Hasta la fecha, este proceso ilustra el primer ejemplo de la formación de derivados α -amino fósforados alílicos, a través de la adición nucleofílica regioselectiva de alcoholes al doble enlace C-N de una 2*H*-azirina fosforada.

- 4.4. Es posible funcionalizar el grupo amino en los derivados α -aminofosforados **4**, **8** y **11**, a través de una reacción de *N*-sulfonación.
- 4.5. Como una extensión a esta metodología, se ha ampliado este proceso a la adición de nucleófilos de azufre, tanto aromáticos como alifáticos, a las 2*H*-azirinas fosforadas. De esta forma, se han preparado nuevas familias de α -aminofosfonatos y fosfanóxidos **15**.
- 4.6. Los α -aminofosfonatos y fosfanóxidos alílicos **8**, **11** y **15** podrían considerarse como moléculas híbridas que introducen dos farmacóforos potenciales, un grupo alilamina y un grupo derivado de un ácido α -aminofosfónico.
- 4.7. Se ha evaluado la eficacia terapéutica de todos los derivados α -aminofósforados y aziridinas sintetizados en la línea celular de cáncer de pulmón humano A549. El mejor efecto citotóxico se observó para el acetal α -aminofosfonato **4f** con un valor de IC_{50} de 1.3 ± 0.10 μ M, el óxido de α -aminofosfina alílico **11a** con un valor de IC_{50} de 1.9 ± 0.13 μ M, así como para **15c** con un valor de IC_{50} de 0.1 ± 0.08 μ M.
- 4.8. Se observó que la línea celular de carcinoma de colon (RKO) no es tan sensible a algunos de los compuestos sintetizados probados.
- 4.9. Además, el efecto citotóxico de casi todos nuestros compuestos en fibroblastos pulmonares no malignos (MRC-5) parece no mostrar ningún efecto.

Anexo: trabajos de investigación

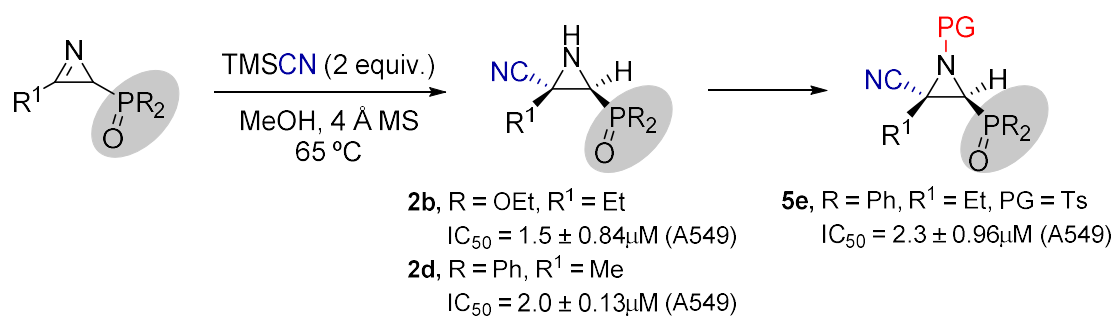
Manuscrito 1

Synthesis and biological evaluation of cyanoaziridine phosphine oxides and phosphonates with antiproliferative activity

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Eur. J. Med. Chem. **2019**, *163*, 736–746.

Agradecimientos a la editorial por otorgar su permiso para reutilizar el material de este artículo en la preparación de esta tesis.

Keywords: 2*H*-azirine, phosphorus substituted cyanoaziridines, antiproliferative effect.

Abstract:

This work reports an efficient diastereoselective synthetic methodology for the preparation of phosphorus substituted cyanoaziridines through the nucleophilic addition of TMSCN, as cyanide source, to the C–N double bond of 2*H*-azirine derivatives. The aziridine ring, in these novel cyanoaziridines, can be activated by simple *N*-tosylation or *N*-acylation. In addition, the cytotoxic effect on cell lines derived from human lung adenocarcinoma (A549) and human embryonic kidney (HEK293) was also screened. *N*-H and *N*-Substituted cyanoaziridines showed excellent activity against the A549 cell line *in vitro*. Moreover, selectivity towards cancer cell (A549) over (HEK293), and non-malignant cells (MCR-5) has been observed.

1. Introduction

A number of innovative technologies have been established in order to covalently tag and modify a biological active molecule in live cells and organisms. Covalent drugs react in a straightforward manner with targets to build covalent bonds and, thereby, they are known to induce additional bioactivities. Consequently, covalent bond formation has become a safe and effective strategy applied not only by nature but also by pharmaceutical industry to improve disease pharmacology. This is mainly due to their remarkably high potency, long-lasting effects, and ligand efficacy, although these favorable features are often linked to a high toxic character.¹ In the history of modern medicine, covalent drugs have been broadly used in many therapies for a wide range of human diseases.² Recently, with the development of targeted covalent inhibitors, new generations of covalent drugs have been explored³ and many modern drugs hold electrophilic moieties acting as “warheads” that capture the active sites by reacting with endogenous nucleophilic functionalities (e.g., thiols and amines). For instance, the electrophilic Michael acceptor acrylamide unit contained in the recently FDA approved drugs osimertinib⁴ and afatinib,⁵ used to treat non-small cell lung carcinoma, works as a covalent warhead for the design of new synthetic kinase inhibitors. Such covalent inhibitors possess numerous advantages, including the high potential for enhanced therapeutic index due to the lower effective dose, prolonged interval of action that may result in less frequent drug dosing, increased biochemical effectiveness, and the potential to inhibit certain drug resistance mechanisms.⁶

In the domain of natural products and related compounds, the aziridine moiety is an illustrative class of warhead, which may react with nucleophilic partners of target enzymes and share a similar reaction mechanism to allow the formation of covalent bonds (Figure 1).⁷

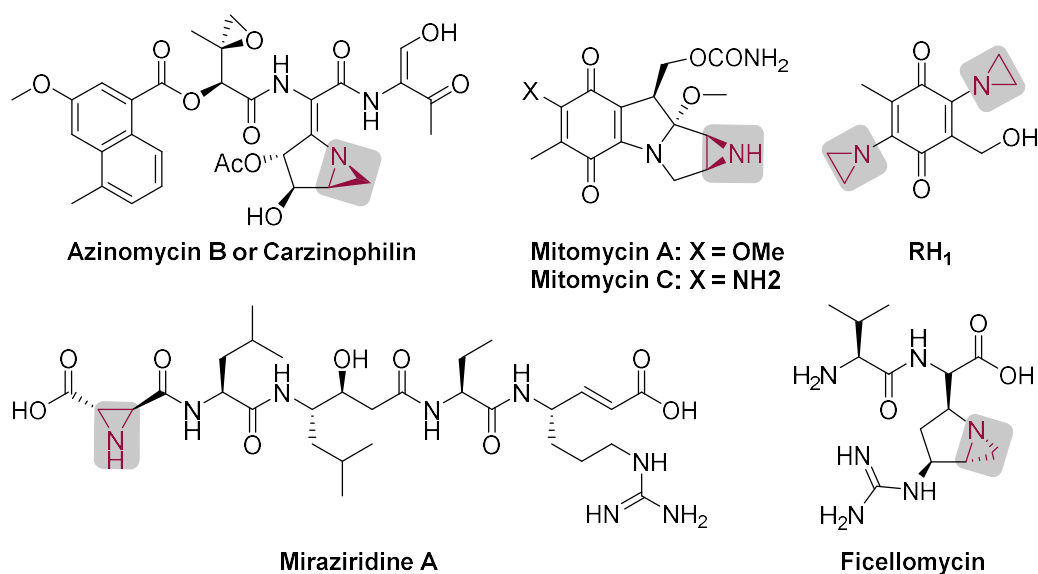


Figure 1. Representative examples of covalent drugs with aziridine warheads. Electrophiles are highlighted.

Moreover, aziridines are valuable building blocks in organic chemistry.⁸ Their widespread application in synthetic organic chemistry arises from the fact that these three-membered nitrogen-containing heterocycles easily undergo regio- and stereoselective ring-opening reactions with an extensive range of nucleophiles.⁹ Aziridines are also important synthetic targets themselves, since they appear in naturally occurring compounds with applications in medicinal chemistry.¹⁰ Therefore, there are several aziridine-containing biologically active natural products, such as azinomycin B¹¹ and maduropeptin,¹² as well as therapeutic agents¹³ including antitumor and antibiotic compounds mitomycin C,¹⁴ azicemicin A,¹⁵ ficellomycin,¹⁶ and madurastatin A1¹⁷ (Figure 1).

Indeed, DNA-modifying agents are a significant class of pharmaceuticals used in conventional chemotherapy. Aziridines, as powerful alkylating agents may act as covalent drugs, having an intrinsic *in vivo* potency by means their capability to act as DNA cross-linking agents *via* nucleophilic ring opening of the three-membered heterocyclic compounds.¹⁸ For instance, mitomycin C (Figure 1) is a member of these drugs and has had the role in the treatment of a

number of cancers, such as bladder, head and neck, gastric, lung, colorectal, and non-small-cell lung cancers.¹⁹ The aziridine core present in these chemotherapy agents is suggested to mostly cause DNA monoalkylation through acid-activation to produce a protonated aziridine that ring opens to dismiss the strain associated with the three-membered ring.²⁰ In addition, from a biological outlook, organophosphorus derivatives are fascinating compounds since it is known that phosphorus substituents may modify the reactivity of heterocycles and control important biological functions.²¹

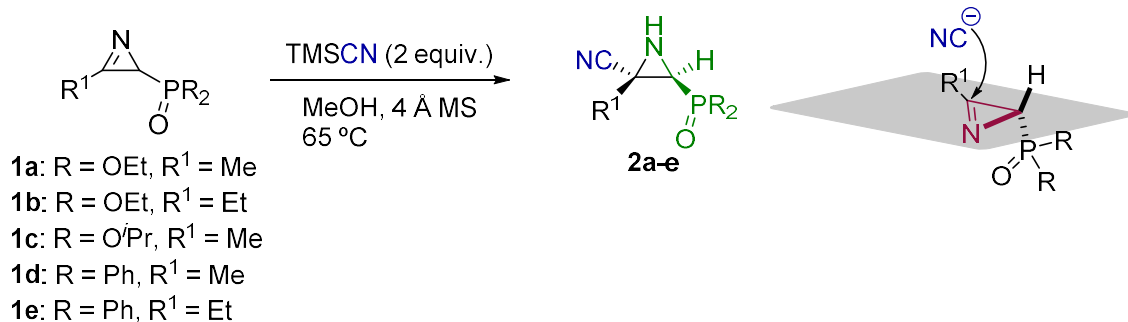
In this context, we have been previously involved in the chemistry of phosphorus containing 2*H*-azirines for the preparation of phosphorylated aziridines.²² In addition, these aziridines have been used as building blocks for the preparation of more intricate compounds, such as pyrroles,^{22a,b} 1*H*-benzo[*d*]azepines,^{22a} oxazoles,^{22f} and α - or β -amino phosphorus acid derivatives,^{22d,e} among others. We are interested in confirming the ability of 2*H*-azirines for the preparation of saturated aziridines containing a phosphine oxide or a phosphonate group. For this reason, here we wish to account the results of the incorporation of a nitrile pharmacophore into the three-membered nitrogen heterocycle, because this group not only occurs in a wide range of natural products,²³ but also plays an important role in the development of pharmaceuticals.²⁴ The cyanation reaction of phosphorus-substituted azirines, may be a new approach for the construction of phosphorus functionalized cyanoaziridines. Besides, these new functionalized cyanoaziridines were tested for antiproliferative activity against different human cancer cell lines. Owing to the potential interest of these new molecules, both in synthetic and medicinal chemistry, this entails an interesting challenge. As far as we know, this is the first example of the preparation of cyanoaziridines containing a phosphorus substituent and their study as antiproliferative agents.

2. Results and discussion

2.1. Chemistry

Only a few reports have appeared regarding the nucleophilic addition of cyanide group to the carbon-nitrogen double bond of strained 2*H*-azirines. The first one, reported by Ghosez *et al.*, make use of trimethylsilyl cyanide (TMSCN) as a cyanide source for the preparation of *N*-TMS-substituted cyanoaziridines. Another two reports described the addition of HCN²⁵ or NaCN,²⁶ this one in a continuous-flow synthesis, to 2*H*-azirines for the preparation of cyanoaziridines. Recently, the cyanation of azirines using TMSCN as the cyanide source and

copper(I) catalyst proceeded smoothly to give cyanoaziridines with excellent diastereoselectivities,²⁷ In order to avoid the use of toxic HCN as reagent and transition-metal catalysis,²⁸ for our purpose we chose the use of TMSCN/MeOH as the cyanide source.²⁹ Initial experiments to assess the reactivity of 2*H*-azirines **1** toward TMSCN were explored. Thus, as outlined in Scheme 1, the addition of TMSCN to 2*H*-azirine-phosphonate **1a** (R = OEt, R¹ = Me) in refluxing methanol was performed. Two equivalents of the cyanide source were used in order to ensure full conversion. Under these reactions conditions, and without the use of any catalyst as reporter previously,²⁷ the crude reaction ¹H NMR and ³¹P NMR spectra revealed the presence of two isomers in a ratio of 43:57. For instance, the chemical shifts of both isomers in CDCl₃ in ³¹P NMR spectra, δ_{P} 17.7 and 19.5 ppm, are remarkably different and are related to minor and major isomers, respectively, corresponding to cyanoaziridine **2a** (R = OEt, R¹ = Me) (Scheme 1, Table 1, entry 1).



Scheme 1. Nucleophilic addition of TMSCN to phosphorus substituted 2*H*-azirines.

In ¹H NMR spectrum, the minor isomer of **2a** gave a singlet at δ_{H} 1.69 for the methyl group and a well-resolved double doublet for the methine proton of the aziridine at δ_{H} 2.67 (³J_{HH} = 11.8 Hz, ²J_{PH} = 13.4 Hz). Likewise, a cross peak between the former signal and the aziridinyl NH proton (δ_{H} 1.88–1.96) was observed in homonuclear correlation spectroscopy (COSY) data (see supplementary data). Conversely, the methyl group corresponding to the major isomer of **2a** showed a singlet at δ_{H} 1.84, since the methine proton of the aziridine ring resonated at δ_{H} 2.30 (³J_{HH} = 9.6 Hz, ²J_{PH} = 13.1 Hz) as a well-resolved double doublet. Similarly, in 2D-COSY a cross peak between the aziridinyl-NH (1.26–1.38) and the methine proton of the aziridine ring was observed. The presence of two sets of signals in ¹H and ¹³C NMR spectra seems to indicate the formation of two diastereoisomers *E*- and *Z*-**2a**. However, since NOESY-1D experiments were

Table 1. Phosphorus substituted cyanoaziridines **2** obtained.

Entry	Compound	R	R ¹	Yield(%) ^[a]	Rotamers ratio ^[b]
1	2a	OEt	Me	77	43:57 (78:22) ^[c]
2	2b	OEt	Et	78	49:51
3	2c	O ⁱ Pr	Me	88	38:62
4	2d	Ph	Me	84	0:100
5	2e	Ph	Et	82	19:81

^[a] Yield of isolated purified compounds **2**. ^[b] Rotamers ratio calculated by ³¹P NMR in CDCl₃ solutions.

^[c] Rotamers ratio calculated by ³¹P NMR in DMSO-*d*₆ solutions.

not conclusive to elucidate the presence of both diastereoisomers, we perform a variable temperature ¹H NMR and ³¹P NMR spectra study for **2a**, showing that both compounds equilibrate immediately at higher temperatures (Figure 2), thus confirming the presence of two rotamers. ¹H NMR spectra of compound **2a** indicated broad signals when recorded at room temperature, while a single set of signals was observed at higher temperatures (> 60 °C). Likewise, variable temperature of ³¹P NMR study for **2a** also showed that both rotamers equilibrate rapidly at higher temperatures (see Figure 2). The ratio of both rotamers are highly impacted by changes in the solvent. Thus, in CDCl₃ solutions, the ³¹P NMR spectra showed two different signals at δ_P 17.7_{minor} and 19.5_{major} ppm in a 43:57 ratio. However, this relation was reversed when the NMR was recorded in DMSO-*d*₆ solutions, and both signals appear in ³¹P NMR spectrum at δ_P 19.0_{major} and 19.7_{minor} ppm (78:22 rotamer ratio). This phenomenon can be attributed to the different geometries around the phosphorus atom,³⁰ as previously observed, for example, in benzofulvene containing phosphine oxide,³¹ or more recently in a DFT study of the most stable rotamers of methyl aziridine-2-carboxylate.³²

The absence of coupling constant between the methyl carbon at C-3 of aziridine ring of **2a** and the phosphorus atom of phosphonate moiety at C(2) in ¹³C NMR spectrum seems to indicate

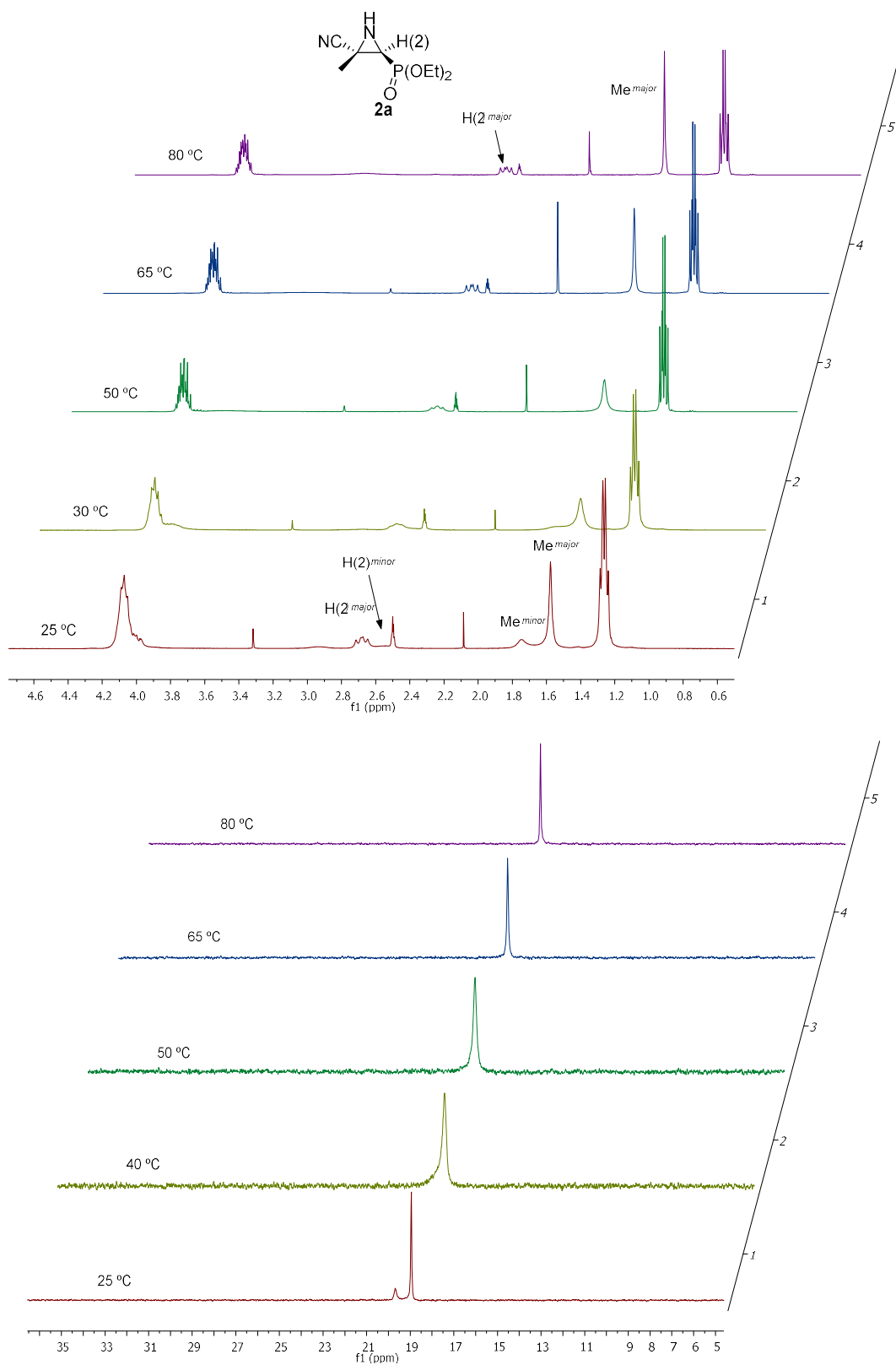


Figure 2. ^1H NMR (top) and ^{31}P NMR (bottom) of **2a** in $\text{DMSO-}d_6$ at different temperatures.

a *cis*-relationship between these two groups, and therefore, an *E*-stereochemistry for the carbon-carbon single bond.^{22d} Finally, the X-ray diffraction (XRD) analysis confirmed the *E*-stereochemistry of the C–C bond in cyanoaziridine phosphonate **2a** (see supplementary data), thus confirming our suspicions.

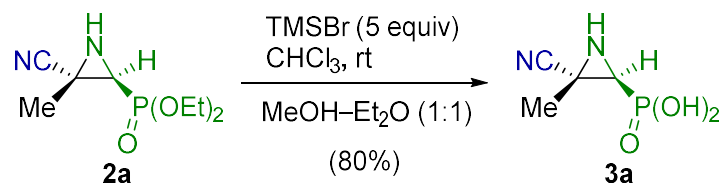
Starting from 2*H*-azirine **1a**, *E*-cyanoaziridine phosphonate **2a** (R = OEt, R¹ = Me) was isolated in 77% yield (Scheme 1, Table 1, entry 1), while the addition of TMSCN to functionalized 2*H*-azirine **1b** and **1c** afforded 78 and 88% of compound *E*-**2b** (R = OEt, R¹ = Et) and *E*-**2c** (R = O^{*i*}Pr, R¹ = Me) respectively, (Scheme 1, Table 1, entries 2 and 3). Similarly, *E*-cyanoaziridine phosphonates **2b** and **2c** were obtained as a mixture of two rotamers around the P–C bond. In order to improve the yields of this process, 2*H*-azirine **1a** was treated with 2 equivalents of TMSCN in the presence of quaternary ammonium salts, such as (Bu₄N)F (1.5 equiv), or even Lewis acid catalysts, for instance, InCl₃. Very good results have been previously observed by using these reagents in the Strecker reaction.^{33,34} Nevertheless, the yields were neither enhanced by using ammonium salts nor Lewis acid catalysts in the nucleophilic addition of TMSCN to 2*H*-azirines **1**, and even we found complex mixtures of products in the crude reaction.

The nucleophilic addition of cyanide anion likely arises diastereoselectively by the side of the less hampered face. Thus, the closeness of the cyanide to the imine carbon-nitrogen double bond of 2*H*-azirine **1** on the opposite site to the phosphine oxide group seems to be more favorable, owing to the high exocyclic dihedral angle of the sp³ hybridized saturated carbon and the presence of the bulky phosphorus group (Scheme 1). This remark has been previously observed in the nucleophilic addition of hydrides,^{22e,g,h} Grignard reagents,^{22c,d} or alcohols^{22c} to phosphorus substituted 2*H*-azirines.

The process was extended to the nucleophilic addition of cyanide anion to 2*H*-azirine-phosphine oxides **1d–e** (R = Ph) under the same reaction conditions (Scheme 1). Thus, 2*H*-azirine **1d** (R = Ph, R¹ = Me) reacted with TMSCN in refluxing methanol, and cyanoaziridine **2d** was obtained in good chemical yield (Table 1, entry 3) as a single rotamer indicated by NMR data (see supplementary data). However, a mixture of two rotamers in a ratio of 19:81 was observed for *E*-cyanoaziridine phosphine oxide **2e** (R = Ph, R¹ = Et) starting from 2*H*-azirine **1d** (Scheme 1, Table 1, entry 5).

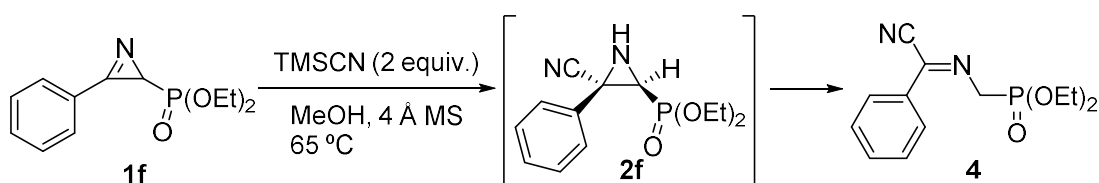
Aminophosphonic acids are of great interest for organic and medicinal chemists,^{35,36} as they show a large variety of biological activities. For this reason, we explore the hydrolysis of phosphonate ester in cyanoaziridine phosphonate **2a** (R = OEt, R¹ = Me). Cleavage of phosphonic

ester group was tested by two different methods. First, by acid hydrolysis of ethyl phosphonate in **2a** using HCl and precipitation in alcohol with propylene oxide. However, under these reaction conditions no cyanoaziridine phosphonic acid **3a** was achieved. Next, we attempted the reaction of dialkyl phosphonate **2a** with TMSBr in dry CHCl₃, and subsequent alcoholysis of the intermediate silyl ester with a mixture of MeOH-Et₂O (1:1). Pure cyanoaziridine phosphonic acid **3a** was obtained in 80% yield by using this hydrolysis method (Scheme 2).



Scheme 2. Synthesis of cyanoaziridine phosphonic acid **3a**.

In order to determine the scope and limitations of the nucleophilic addition of cyanide anion to *2H*-azirines **1**, this procedure was broadened to include the reactivity of *2H*-azirine phosphonate containing an aryl group at C(3) position toward TMSCN. Therefore, *2H*-azirine phosphonate **1f** (R = OEt, R¹ = Ph) was subjected to a refluxing solution of MeOH and 2 equiv of TMSCN. Under these reaction conditions, no cyanoaziridine adduct **2f** was isolated, and the corresponding cyano-imine **4** was obtained instead as a sole reaction product in 72% chemical yield (Scheme 3).



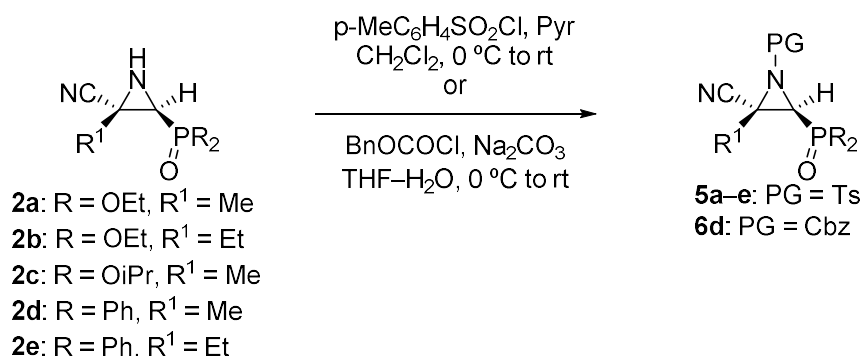
Scheme 3. Nucleophilic addition of TMSCN to aryl-*2H*-azirine **1f**.

The outcome of this conversion may be due to the initial formation of the corresponding cyanoaziridine **2f**, resulting from the addition of cyanide anion to the C–N double bond of *2H*-azirine **1f**. Successive C(2)–C(3) ring opening³⁷ in aziridine **2f** would afford acyclic compound **4** (Scheme 3). This result suggest that the addition of TMSCN to phosphorus substituted *2H*-azirines **1** may be influenced by the nature of substituents at C(3). While aliphatic substituents

at 3-position of **1** undergo functionalized cyanoaziridines **2**, the presence of an aryl group at 3-position of 2*H*-azirine **1** seems to produce an unstable cyanoaziridine **2f**, which evolves to compound **4**, by means an aziridine ring opening.

Activated aziridines bearing electron-withdrawing groups at the *N*-atom, such as acyl³⁸ or tosyl functional groups,³⁹ can stabilize the negative charge of the nitrogen atom increasing their electrophilicity. Therefore, we explore the *N*-functionalization of inactivated cyanoaziridines **2** (Table 2). For this purpose, we assessed the *N*-protection of cyanoaziridines **2** by using the tosyl group as protecting group. Hence, the reaction of cyanoaziridines **2** with *p*-toluenesulfonyl chloride (TsCl) in the presence of pyridine, in methylene chloride (CH₂Cl₂), and at room temperature, afforded the desired *N*-Ts cyanoaziridines **5** in good yields (Table 2, entries 1–5).

Table 2. Phosphorus substituted *N*-Ts **5** and *N*-Cbz-cyanoaziridines **6** obtained.



Entry	Compound	R	R ¹	PG	Yield(%) ^[a]
1	5a	OEt	Me	Ts	90
2	5b	OEt	Et	Ts	74
3	5c	O ⁱ Pr	Me	Ts	81
4	5d	Ph	Me	Ts	82
5	5e	Ph	Et	Ts	77
6	6d	Ph	Me	Cbz	59

^[a] Yield of isolated purified compounds **5** and **6**.

Further exploration revealed that this procedure is also applicable to other functional groups. For instance, Cbz protecting group can also be used to functionalize *N*-H cyanoaziridine **2d**. Thus,

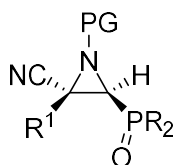
treatment of **2d** with benzyl chloroformate in the presence of a base, such as Na₂CO₃, yielded *N*-Cbz cyano aziridine **6d** in 59% chemical yield (Table 2, entry 6).

This approach represents a practical short diastereoselective route to functionalized cyanoaziridines **2** through nucleophilic addition of TMSCN, as a cyanide source, to phosphorus substituted *2H*-azirines **1**. Additionally, activation of aziridine ring in these phosphorus substituted cyanoaziridines **2**, by adding electron-withdrawing groups at the *N*-atom, can be performed simply by *N*-tosylation (*N*-Ts) or *N*-acylation (*N*-Cbz) of the corresponding *N*-H cyanoaziridine **2**. To the best of our knowledge this represents the first example of cyanide addition to the carbon-nitrogen double bond of a phosphorus substituted *2H*-azirine.

2.2. Biological results

In vitro cytotoxicity of our novel cyanoaziridines was evaluated by testing their antiproliferative activities against different human cancer cell lines: A549 (carcinomic human alveolar basal epithelial cell) and HEK-293 (human embryonic kidney cells). In order to evaluate growth inhibition, cell counting kit (CCK-8) assay was applied. Table 3 shows cell proliferation inhibitory activities, as IC₅₀ values, of all synthesized cyanoaziridines **2**, **3**, **5**, **6** and chemotherapeutic doxorubicin (DOX). Likewise, healthy lung cells, such as MRC-5 non-malignant lung fibroblasts were tested for studying selective cytotoxicity.⁴⁰

Concerning the new *N*-H cyanoaziridines **2** against A549 cell line *in vitro*, cyanoaziridine phosphonates **2a–c** with IC₅₀ values between 1.5 ± 0.84 and 4.3 ± 0.43 μM showed similar activities as the corresponding cyanoaziridine phosphine oxides **2d–e** with IC₅₀ values between 2.0 ± 0.13 and 3.3 ± 0.23 μM, with the most effective compound being cyanoaziridine **2b** (R = OEt, R¹ = Et, PG = H) with an IC₅₀ value of 1.5 ± 0.84 μM. Concerning the effect of the substitution at the phosphonate ester group, in contrast to previous results where it was evidenced that the presence of a bulky group, in general, resulted in an increased activity,⁴¹ no effect has been observed when changing ethyl group for *iso*-propyl group in phosphonate ester moiety (Table 3, compare entries 2 and 4). Even, phosphonic acid derivative **3a** (R = OH, R¹ = Me, PG = H) showed the higher IC₅₀ value of 8.5 ± 0.57 μM (entry 7). Furthermore, very high selectivity was observed in the antiproliferative activity of the novel cyanoaziridine phosphonates **2a–c**, phosphine oxides **2d–e**, and phosphonic acid **3a** against the A549 *versus* HEK293 cell line.

Table 3. Antiproliferative activity of cyanoaziridines **2**, **3**, **5**, and **6**.

Entry	Comp.	R	R ¹	PG	Cytotoxicity IC ₅₀ (μM) ^[a]		
					Lung A549	Kidney HEK293	MRC-5
1	DOX	–	–	–	0.48 ± 0.017 ⁴²	5.68 ± 1.56 ⁴³	>50 ⁴⁴
2	2a	OEt	Me	H	4.3 ± 0.43	>50	>50
3	2b	OEt	Et	H	1.5 ± 0.84	>50	>50
4	2c	O ⁱ Pr	Me	H	3.6 ± 0.08	>50	>50
5	2d	Ph	Me	H	2.0 ± 0.13	>50	>50
6	2e	Ph	Et	H	3.3 ± 0.23	>50	>50
7	3a	OH	Me	H	8.5 ± 0.57	>50	>50
8	5a	OEt	Me	Ts	n.r. ^[b]	>50	>50
9	5b	OEt	Et	Ts	4.9 ± 0.38	>50	>50
10	5c	O ⁱ Pr	Me	Ts	3.4 ± 0.36	>50	>50
11	5d	Ph	Me	Ts	3.1 ± 0.38	>50	>50
12	5e	Ph	Et	Ts	2.3 ± 0.96	43.2±0.80	>50
13	6d	Ph	Me	Cbz	8.1 ± 0.73	30.1±4.10	>50

^[a] The cytotoxicity IC₅₀ values listed are the concentrations corresponding to 50% growth inhibition.

^[b] Not reported.

We next studied the nitrogen-substitution effect of the corresponding cyanoaziridines into their cytotoxicity against A549 cell lines. As in the case of *N*-H cyanoaziridines **2**, the substitution at the phosphonate ester group was no effect into the cytotoxicity, since similar IC₅₀ values have been observed for activated cyanoaziridines **5** (Table 3, entries 8–12), with the most effective derivative being cyanoaziridine **5e** (R = Ph, R¹ = Et, PG = Ts) with an IC₅₀ value of 2.3 ± 0.96 μM (Table 3, entry 12). Varying *N*-Ts by *N*-Cbz in cyanoaziridine derivative **6d**, showed a decreasing of cytotoxic properties (entry 13). Moreover, as before, very high selectivity was detected in cytotoxic properties of the novel *N*-substituted cyanoaziridines **5** and **6** against the A549 *versus* HEK293 cell line. Furthermore, MRC-5 non-malignant lung fibroblasts were tested for studying

selective toxicity,⁴⁰ and none of the prepared phosphorus substituted cyanoaziridines or doxorubicin exhibited any toxicity toward MRC-5 cell line (see Table 3).

3. Conclusions

In summary, we have developed a very efficient new diastereoselective approach to cyanoaziridine phosphine oxides and phosphonates, through the nucleophilic addition of cyanide anion to the carbon-nitrogen double bond of *2H*-azirines. This short alternative avoids the use of toxic HCN as reagent and transition metal catalysis, being replaced by the use of TMSCN/MeOH as the cyanide source. Activation of aziridine ring in cyanoaziridines can be performed by acylation or tosylation of the nitrogen atom of these cyanoaziridines. To the best of our knowledge this method exemplifies the first example of nucleophilic addition of cyanide anion to the carbon-nitrogen double bond of a phosphorus substituted *2H*-azirine. Furthermore, synthesized cyanoaziridines exhibited very good cytotoxic effect inhibiting the growth of human tumor cell lines A549 (carcinomic human alveolar basal epithelial cell), whereas kidney cancer is not so sensitive to the synthesized cyanoaziridines. In addition, cytotoxic effect of our compounds in healthy lung cells, fibroblast lung cells (MRC-5), seems not to present any effect.

4. Experimental section

4.1. Chemistry

4.1.1. General experimental information

Solvents for extraction and chromatography were of technical grade. All solvents used in reactions were freshly distilled and dried over molecular sieves 4 Å before use. All other solvents and reagents were obtained from commercial sources and recrystallized or distilled as necessary or used without further purification. All reactions were performed under an atmosphere of dry nitrogen. Melting points are uncorrected. IR spectra were measured on a Nicolet iS10 Thermo Scientific spectrometer as neat solids. Absorbance frequencies are given at maximum of intensity in cm^{-1} . High-resolution mass spectra (HRMS) were obtained by positive-ion electrospray ionization (ESI) method with a time of flight Q-TOF system. Data are reported in the form m/z (intensity relative to base = 100). ^1H (300, 400 MHz), ^{13}C (75, 100 MHz) and ^{31}P NMR (120, 160 MHz) spectra were recorded on a Varian Unity Plus (300 MHz) or Bruker Avance 400

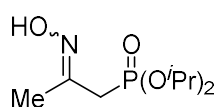
(400 MHz) spectrometers, respectively, in CDCl_3 or $\text{DMSO}-d_6$, as specified below. Chemical shifts (δ_{H}) are reported in parts per million (ppm) with the internal chloroform signal at 7.24 ppm or the internal DMSO signal at 2.50 ppm as standard for ^1H NMR. Chemical shifts (δ_{C} and δ_{P}) are reported in parts per million (ppm) with the internal chloroform signal at 77.0 ppm or the internal DMSO signal at 39.5 ppm as standard for ^{13}C NMR; or the external H_3PO_4 (50%) signal at 0.0 ppm as standard for ^{31}P NMR. All coupling constants (J) values are given in Hz. ^{13}C -NMR spectra were recorded in a broad band decoupled mode, and peak assignments were supported by Distortionless Enhanced Polarization Transfer (DEPT). The data is being reported as (s = singlet, d = doublet, t = triplet, q = quarted, m = multiplet, dd = double doublet, bs = broad singlet). Chromatographic purification was performed as flash chromatography using commercial grades of silica gel finer than 230 mesh with pressure. Analytical thin layer chromatography (TLC) was performed on precoated Merck silica gel 60 F₂₅₄ TLC aluminium plates, and spot visualized with UV light or permanganate stain. 2*H*-Azirines **1** were prepared according to literature procedures.^{22e,h}

4.1.2. Compounds Purity Analysis

All synthesized compounds were analyzed by HPLC to determine their purity. The analyses were performed on Agilent 1260 infinity HPLC system (C-18 column, Hypersil, BDS, 5 μm , 0.4 mm \times 25 mm) at room temperature. All the tested compounds were dissolved in dichloromethane, and 5 μL of the sample was loaded onto the column. Ethanol and heptane were used as mobile phase, and the flow rate was set at 1.0 mL/min. The maximal absorbance at the range of 190–400 nm was used as the detection wavelength. The purity of all the tested cyanoaziridines **2**, **3**, **5**, and **6** is >95%, which meets the purity requirement by the Journal.

4.1.3. Experimental procedure and characterization data for compounds **2–6**.

General procedure and spectral data of functionalized oxime

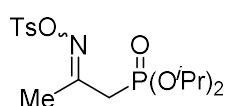


(Z)- and (E)-Diisopropyl (2-(hydroxyimino)propyl)phosphonate.

Hydroxylamine hydrochloride (1.15 g, 16.5 mmol, 1.1 eq) and NaOH (0.66 g, 16.5 mmol, 1 eq) were added to a stirred 0 °C solution of diisopropyl (2-oxopropyl)phosphonate (3.33 g, 15 mmol, 1 eq) in MeOH/H₂O (50:50) (45 mL). The reaction mixture was allowed to reach room temperature and stirred for 2 h. The MeOH layer was

removed by rotary evaporation, and the aqueous layer was extracted twice with AcOEt (20 mL). The organic layers were dried over anhydrous MgSO₄ and filtered. The title compound was very unstable and used without further purification steps (2.38 g, 67% as a yellow oil). IR (neat) ν_{\max} 3253, 3199, 2980, 2927, 1717, 1230, 1103, 1157, 992 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 9.28 (bs, 1H, OH), 4.73–4.61 (m, 2H, OCH), 3.00 (d, ²J_{PH} = 23.5 Hz, 2H, CH₂)_{minor}, 2.69 (d, ²J_{PH} = 21.8 Hz, 2H, CH₂)_{major}, 1.96 (s, 3H, CH₃)_{minor}, 1.95 (s, 3H, CH₃)_{major}, 1.28–1.24 (m, 12H, CH(CH₃)₂) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 150.7 (d, ²J_{PC} = 9.6 Hz, C_{quat})_{major}, 149.2 (d, ²J_{PC} = 9.1 Hz, C_{quat})_{minor}, 70.9 (CH)_{minor}, 70.8 (CH)_{major}, 35.0 (d, ¹J_{PC} = 140.5 Hz, CH₂)_{major}, 28.1 (d, ¹J_{PC} = 138.5 Hz, CH₂)_{minor}, 24.0 (CH₃), 23.9 (CH₃), 23.9 (CH₃), 23.8 (CH₃), 20.7 (CH₃)_{major}, 14.5 (CH₃)_{minor} ppm; ³¹P NMR (120 MHz, CDCl₃) δ 22.8_{major}, 21.6_{minor} ppm; ESI-HRMS (CI) *m/z* calcd. for C₉H₂₁NO₄P ([M+H]⁺) 238.1208, found 238.1205.

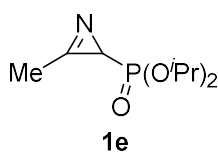
General procedure and spectral data of functionalized tosyloxime



(Z)- and (E)-Diisopropyl 2-((tosyloxy)imino)propylphosphonate. *p*-

Toluenesulfonyl chloride (2.10 g, 11 mmol, 1.1 eq) and pyridine (4.85 mL, 60 mmol, 6 eq) were added to a stirred 0 °C solution of (Z)- and (E)-diisopropyl 2-(hydroxyimino)propylphosphonate (2.37 g, 10 mmol, 1 eq) in dry CH₂Cl₂ (20 mL). The reaction mixture was allowed to reach room temperature and stirred for 15 h. The crude product was diluted with CH₂Cl₂ (20 mL), washed twice with 2M HCl (15 mL) and water (15 mL), and extracted twice with CH₂Cl₂ (10 mL). The organic layers were dried over anhydrous MgSO₄, filtered and the unstable compound was used without further purification steps (3.81 g, 97% as a yellow oil). IR (neat) ν_{\max} 3063, 2977, 2933, 1641, 1600, 1445, 1192, 1179, 986, 805 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.89–7.79 (m, 2H, ArH), 7.38–7.27 (m, 2H, ArH), 4.69–4.54 (m, 2H, OCH), 2.96 (d, ²J_{PH} = 23.7 Hz, 2H, CH₂)_{minor}, 2.71 (d, ²J_{PH} = 21.8 Hz, 2H, CH₂)_{major}, 2.40 (s, 3H, CH₃)_{minor}, 2.39 (s, 3H, CH₃)_{major}, 2.06 (d, ⁴J_{PH} = 2.4 Hz, 3H, CH₃)_{major}, 2.03 (d, ⁴J_{PH} = 2.3 Hz, 3H, CH₃)_{minor}, 1.27–1.12 (m, 12H, CH(CH₃)₂) ppm; ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 161.2 (d, ²J_{PC} = 8.1 Hz, C_{quat})_{major}, 159.2 (d, ²J_{PC} = 8.3 Hz, C_{quat})_{minor}, 144.9 (C_{quat})_{minor}, 144.9 (C_{quat})_{major}, 132.6 (CH), 130.2 (CH), 129.5 (CH), 128.8 (CH), 128.8 (CH), 127.0 (CH), 71.5 (d, ²J_{PC} = 6.8 Hz, CH)_{minor}, 71.4 (d, ²J_{PC} = 6.7 Hz, CH)_{major}, 34.8 (d, ¹J_{PC} = 138.4 Hz, CH₂)_{major}, 30.6 (d, ¹J_{PC} = 136.2 Hz, CH₂)_{minor}, 23.8 (CH₃), 23.8 (CH₃), 23.6 (CH₃), 23.6 (CH₃), 21.6 (CH₃)_{minor}, 16.4 (CH₃)_{major} ppm; ³¹P NMR (160 MHz, CDCl₃) δ 19.5_{major}, 17.8_{minor} ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₆H₂₇NO₆PS ([M+H]⁺) 392.1297, found 392.1291.

General procedure and spectral data of 2*H*-azirine (**1e**)

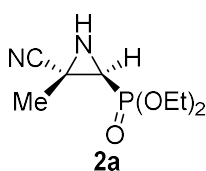


Diisopropyl (3-methyl-2*H*-azirin-2-yl)phosphonate (1e**).** Et₃N (2.23 mL, 16 mmol, 2 eq) was added to a stirred 0 °C solution of (*Z*)- and (*E*)-diisopropyl (2-((tosyloxy)imino)propyl)phosphonate (3.13 g, 8 mmol, 1 eq) in dry CH₂Cl₂ (30 mL). The reaction mixture was allowed to reach room temperature and

stirred for 17 h. The crude product was washed several times with water and the aqueous phase extracted twice with CH₂Cl₂ (10 mL). The combined organic layers were dried over anhydrous MgSO₄, filtered and the solvent was evaporated under vacuum. The resulting unstable 2*H*-azirine **1e** was used without further purification steps (0.97 g, 55% as a yellow oil). IR (neat) ν_{\max} 2972, 2924, 2871, 1787, 1242, 1104, 980 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 4.68–4.58 (m, 2H, OCH), 2.48 (s, 3H, CH₃), 1.65 (d, ²J_{PH} = 39.3 Hz, 1H, CH-P), 1.29–1.22 (m, 12H, CH(CH₃)₂) ppm; ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 161.9 (d, ²J_{PC} = 3.4 Hz, C_{quat}), 70.8, (d, ²J_{PC} = 4.6 Hz, CH), 70.7 (d, ²J_{PC} = 4.5 Hz, CH), 24.0 (CH₃), 23.9 (CH₃), 23.9 (CH₃), 23.8 (CH₃), 23.5 (d, ¹J_{PC} = 215.6 Hz, CH), 13.2 (CH₃) ppm; ³¹P NMR (160 MHz, CDCl₃) δ 21.1 ppm; ESI-HRMS (CI) *m/z* calcd. for C₉H₁₉NO₃P ([M+H]⁺) 220.1103, found 220.1097.

General procedure and spectral data for the reaction of functionalized 2*H*-azirines **1** with trimethylsilyl cyanide (TMSCN)

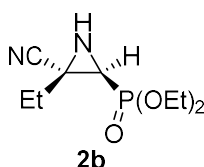
To a 0 °C solution of functionalized 2*H*-azirine **1** (5 mmol) in MeOH (25 mL) was added trimethylsilyl cyanide (1.25 mL, 10 mmol, 2 eq) and 4 Å M.S. The mixture was stirred in refluxing MeOH for 4–12 h until TLC showed the disappearance of 2*H*-azirine **1**. 4 Å M.S. was filtered through a sintered glass vacuum filtration funnel with celite and washed with MeOH. The filtrate was concentrated to dryness in vacuum and the resulting residue was purified by flash-column chromatography.



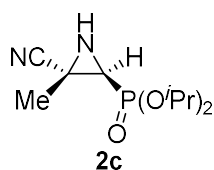
(*E*)-Diethyl ((2*S,3*S**)-3-cyano-3-methylaziridin-2-yl)phosphonate (**2a**).**

(0.83 g, 77%) obtained as a white solid from 2*H*-azirine **1a** (0.96 g, 5 mmol) as described in the general procedure. The crude product was purified by flash-column chromatography (SiO₂, AcOEt/hexane 34:66) gave the title compound **2a**. mp 84–86 °C; IR (neat) ν_{\max} 3198, 2986, 2243, 1454, 1249 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 4.20–4.06 (m, 4H, OCH₂), 2.67 (dd, ³J_{HH} = 11.8 Hz, ²J_{PH} = 13.4 Hz, 1H, CH-P)_{minor}, 2.30 (dd, ³J_{HH} = 9.6 Hz, ²J_{PH} = 13.1 Hz, 1H, CH-P)_{major}, 1.96–1.88 (m, 1H, NH)_{minor}, 1.84 (s, 3H, CH₃)_{major}, 1.69 (s, 3H, CH₃)_{minor}, 1.38–1.26 (m, 7H, CH₃ and NH)_{major} ppm; ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 120.7 (C_{quat})_{minor}, 119.8 (C_{quat})_{major}, 63.2, 63.2, 63.0, 62.9, 62.9, 62.9, 62.8, 62.8 (CH₂), 35.8 (d, ¹J_{PC} = 212.4

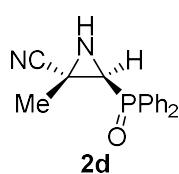
Hz, CH)_{minor}, 35.2 (d, $^1J_{PC}$ = 185.6 Hz, CH)_{major}, 29.4 (C_{quat})_{major}, 27.5 (C_{quat})_{minor}, 17.4 (CH₃), 16.3 (CH₃)_{major}, 16.2 (CH₃)_{minor} ppm; ^{31}P NMR (160 MHz, CDCl₃) δ 19.5_{major}, 17.7_{minor} ppm; ESI-HRMS (CI) m/z calcd. for C₈H₁₆N₂O₃P ([M+H]⁺) 219.0899, found 219.0894.



(E)-Diethyl ((2S*,3S*)-3-cyano-3-ethylaziridin-2-yl)phosphonate (2b). (0.90 g, 78%) obtained as a yellow oil from 2*H*-azirine **1b** (1.03 g, 5 mmol) as described in the general procedure. The crude product was purified by flash-column chromatography (SiO₂, AcOEt/hexane 25:75) to give the title compound **2b**. IR (neat) ν_{max} 3187, 2985, 2975, 2246, 1460, 1230 cm⁻¹; ^1H NMR (400 MHz, CDCl₃) δ 4.18–4.09 (m, 4H, OCH₂), 2.69–2.63 (m, 1H, CH-P)_{minor}, 2.32–2.27 (m, 1H, CH-P)_{major}, 2.08–1.88 (m, 3H, CH₂ and NH_{minor}), 1.33–1.17 (m, 10H, 3CH₃ and NH_{major}) ppm; ^{13}C { ^1H } NMR (100 MHz, CDCl₃) δ 119.7 (C_{quat})_{major}, 118.9 (C_{quat})_{minor}, 63.2 (CH₂), 62.9 (CH₂), 62.7 (CH₂), 35.7 (d, $^1J_{PC}$ = 214.3 Hz, CH)_{major}, 35.5 (C_{quat})_{minor}, 35.3 (d, $^1J_{PC}$ = 185.5 Hz, CH)_{minor}, 33.6 (C_{quat})_{major}, 24.4 (CH₂)_{minor}, 24.2 (CH₂)_{major}, 16.2 (CH₃)_{major}, 16.2 (CH₃)_{minor}, 10.8 (CH₃)_{minor}, 10.2 (CH₃)_{major} ppm; ^{31}P NMR (160 MHz, CDCl₃) δ 19.7_{major}, 18.0_{minor} ppm; ESI-HRMS (CI) m/z calcd. for C₉H₁₈N₂O₃P ([M+H]⁺) 233.1055, found 233.1052.

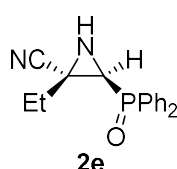


(E)-Diisopropyl ((2S*,3S*)-3-cyano-3-methylaziridin-2-yl)phosphonate (2c). (1.09 g, 88%) obtained as a yellow oil from 2*H*-azirine **1c** (1.10 g, 5 mmol) as described in the general procedure. The crude product was purified by flash-column chromatography (SiO₂, AcOEt/hexane 16:84) to give the title compound **2c**. IR (neat) ν_{max} 3205, 2980, 2236, 1695, 1384, 1368, 1242, 985 cm⁻¹; ^1H NMR (400 MHz, CDCl₃) δ 4.75–4.63 (m, 2H, OCH), 2.63–2.57 (m, 1H, CH-P)_{minor}, 2.25 (dd, $^3J_{\text{HH}}$ = 9.6 Hz, $^2J_{\text{PH}}$ = 12.7 Hz, 1H, CH-P)_{major}, 2.02–1.87 (m, 1H, NH)_{minor}, 1.81 (s, 3H, CH₃)_{major}, 1.67 (s, 3H, CH₃)_{minor}, 1.33–1.21 (m, 13H, CH₃ and NH_{major}) ppm; ^{13}C { ^1H } NMR (100 MHz, CDCl₃) δ 120.9 (C_{quat})_{minor}, 120.0 (C_{quat})_{major}, 72.4 (d, $^2J_{PC}$ = 6.0 Hz, CH)_{minor}, 72.2 (d, $^2J_{PC}$ = 7.0 Hz, CH)_{major}, 36.4 (d, $^1J_{PC}$ = 213.0 Hz, CH)_{minor}, 36.0 (d, $^1J_{PC}$ = 186.0 Hz, CH)_{major}, 29.6 (C_{quat})_{major}, 27.6 (C_{quat})_{minor}, 24.0 (CH₃), 24.0 (CH₃), 23.9 (CH₃), 23.9 (CH₃), 17.3 (CH₃)_{major}, 17.2 (CH₃)_{minor} ppm; ^{31}P NMR (120 MHz, CDCl₃) δ 17.1_{major}, 15.9_{minor} ppm; ESI-HRMS (CI) m/z calcd. for C₁₀H₂₀N₂O₃P ([M+H]⁺) 247.1212, found 247.1208.



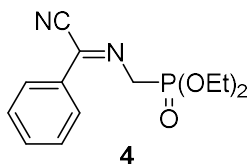
(E)-(2S*,3S*)-3-(Diphenylphosphoryl)-2-methylaziridine-2-carbonitrile (2d). (1.19 g, 84%) obtained as a pale yellow solid from 2*H*-azirine **1d** (1.28 g, 5 mmol) as described in the general procedure. Crystallization from Et₂O gave the title compound **2d**. mp 180–182 °C; IR (neat) ν_{max} 3133, 3054, 2984, 2243, 1436, 1247 cm⁻¹; ^1H NMR (400 MHz, CDCl₃) δ 7.80–7.46 (m, 10H, ArH), 2.75 (dd, $^2J_{\text{PH}}$ = 21.6 Hz,

$^3J_{\text{HH}} = 9.2$ Hz, 1H, CH-P), 1.93 (dd, $^3J_{\text{PH}} = 14.4$ Hz, $^3J_{\text{HH}} = 9.1$ Hz, 1H, NH), 1.72 (s, 3H, CH₃) ppm; ^{13}C { ^1H } NMR (100 MHz, CDCl₃) δ 133.0, 132.8, 130.9, 130.9, 130.8, 129.3, 129.1, 129.1, 128.9 (C_{Ar}), 120.1 (C_{quat}), 38.2 (d, $^1J_{\text{PC}} = 85.8$ Hz, CH), 30.4 (C_{quat}), 16.8 (CH₃) ppm; ^{31}P NMR (160 MHz, CDCl₃) δ 25.1 ppm; ESI-HRMS (CI) m/z calcd. for C₁₆H₁₆N₂OP ([M+H]⁺) 283.1000, found 283.0999.



(E)-(2S*,3S*)-3-(Diphenylphosphoryl)-2-ethylaziridine-2-carbonitrile (2e).

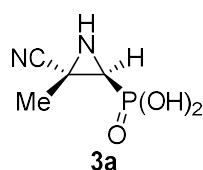
(1.21 g, 81%) obtained as a white solid from 2H-aziridine **1e** (1.35 g, 5 mmol) as described in the general procedure. Crystallization from Et₂O gave the title compound **2e**. mp 176–179 °C; IR (neat) ν_{max} 3222, 3160, 2980, 2237, 1433, 1194 cm⁻¹; ^1H NMR (400 MHz, CDCl₃) δ 7.81–7.46 (m, 10H, ArH), 3.07 (dd, $^2J_{\text{PH}} = 21.9$ Hz, $^3J_{\text{HH}} = 10.8$ Hz, 1H, CH-P)_Z, 2.80 (dd, $^2J_{\text{PH}} = 21.5$ Hz, $^3J_{\text{HH}} = 9.6$ Hz, 1H, CH-P)_E, 2.28–1.88 (m, 3H, NH and CH₂), 1.06 (t, $^3J_{\text{HH}} = 7.2$ Hz, 3H, CH₃)_Z, 0.97 (t, $^3J_{\text{HH}} = 7.4$ Hz, 3H, CH₃)_E ppm; ^{13}C { ^1H } NMR (100 MHz, CDCl₃) δ 133.0, 132.7, 132.5, 131.5, 131.2, 131.1, 130.9, 130.8, 130.4, 129.4, 129.2, 129.1, 129.1, 128.9, 128.8, 128.7 (C_{Ar}), 120.0 (C_{quat})_{minor}, 119.1 (C_{quat})_{major}, 39.5 (d, $^1J_{\text{PC}} = 82.6$ Hz, CH)_{minor}, 38.9 (d, $^1J_{\text{PC}} = 86.3$ Hz, CH)_{major}, 36.6 (C_{quat})_{major}, 35.2 (C_{quat})_{minor}, 23.9 (CH₂)_{minor}, 23.7 (CH₂)_{major}, 11.0 (CH₃)_{major}, 10.7 (CH₃)_{minor} ppm; ^{31}P NMR (160 MHz, CDCl₃) δ 25.0_{major}, 22.5_{minor} ppm; ESI-HRMS (CI) m/z calcd. for C₁₇H₁₈N₂OP ([M+H]⁺) 297.1157, found 297.1155.



Diethyl (((cyanophenylmethylene)amino)methyl)phosphonate (4).

(1.01 g, 72%) obtained as a yellow oil from 2H-aziridine **1f** (1.27 g, 5 mmol) as described in the general procedure. The crude product was purified by flash-column chromatography (SiO₂, AcOEt/hexane 50:50) to give the title compound **4**. IR (neat) ν_{max} 3065, 2982, 2220, 1734, 1449 cm⁻¹; ^1H NMR (300 MHz, CDCl₃) δ 7.99–7.43 (m, 5H, ArH), 4.48 (d, $^2J_{\text{PH}} = 19.2$ Hz, 2H, CH₂), 4.19 (q, $^3J_{\text{HH}} = 7.0$ Hz, 4H, OCH₂), 1.32 (t, $^3J_{\text{HH}} = 7.0$ Hz, 6H, CH₃) ppm; ^{13}C { ^1H } NMR (75 MHz, CDCl₃) δ 145.2 (d, $^3J_{\text{PC}} = 17.9$ Hz, C_{quat}), 133.1 (d, $^4J_{\text{PC}} = 3.6$ Hz, C_{quat}), 132.7 (CH), 129.0 (CH), 127.9 (CH), 109.3 (d, $^4J_{\text{PC}} = 4.4$ Hz, C_{quat}), 62.9 (d, $^2J_{\text{PC}} = 6.6$ Hz, CH₂), 55.9 (d, $^1J_{\text{PC}} = 154.1$ Hz, CH₂), 16.5 (CH₃) ppm; ^{31}P NMR (120 MHz, CDCl₃) δ 19.1 ppm; ESI-HRMS (CI) m/z calcd. for C₁₃H₁₈N₂O₃P ([M+H]⁺) 281.1055, found 281.1051.

General procedure for the hydrolysis of phosphonate



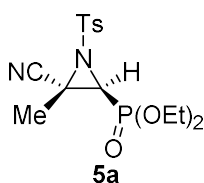
(E)-((2S*,3S*)-3-cyano-3-methylaziridin-2-yl) phosphonic acid (3a).

TMSBr (3.3 mL, 25 mmol, 5 eq) was added to a stirred solution of cyanoaziridine **2a** (1.09 g, 5 mmol, 1 eq) in dry CHCl₃ (21 mL). The reaction mixture was stirred at room temperature until TLC showed the disappearance of starting compound **2a** (8 h). The crude product was subjected to hydrolysis using a mixture of

MeOH/Et₂O (50:50) where the phosphonic acid **3a** recrystallized (0.65 g, 80% as a white solid). mp 216–218 °C (dec); IR (neat) ν_{\max} 3500, 2667, 2303, 1594, 1194, 1157, 1090, 1030, 929 cm⁻¹; ¹H NMR (400 MHz, DMSO) δ 2.30 (d, ²J_{PH} = 13.6 Hz, 1H, CH-P), 1.61 (s, 3H, CH₃) ppm; ¹³C {¹H} NMR (100 MHz, DMSO) δ 121.6 (C_{quat}), 36.5 (d, ¹J_{PC} = 191.6 Hz, CH), 26.8 (C_{quat}), 17.2 (CH₃) ppm; ³¹P NMR (120 MHz, DMSO) δ 13.7 ppm; ESI-HRMS (CI) *m/z* calcd. for C₄H₈N₂O₃P ([M+H]⁺) 163.0273, found 163.0267.

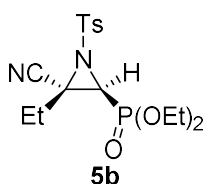
General procedure and spectral data for the *N*-tosyl functionalization of cyanoaziridines

p-Toluenesulfonyl chloride (1g, 5.5 mmol, 1.1 eq) and pyridine (2.4 mL, 30 mmol, 6 eq) were added to a 0 °C solution of cyanoaziridine (5 mmol, 1 eq) in CH₂Cl₂ (25 mL). The reaction mixture was allowed to reach room temperature and stirred until TLC showed the disappearance of starting cyanoaziridine. The crude product was washed twice with a 2M HCl solution (15 mL) and water (15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layers were dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum, and the resulting residue was purified by crystallization or flash-column chromatography.



(E)-Diethyl ((2S*,3S*)-3-cyano-3-methyl-1-tosylaziridin-2-yl)phosphonate (5a). (1.67 g, 90%) obtained as a pale yellow oil from cyanoaziridine **2a** (1.09 g, 5 mmol) after 24 h at room temperature as described in the general procedure. The crude product was purified by flash-column chromatography

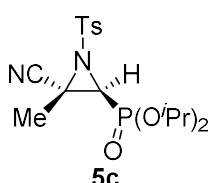
(SiO₂, AcOEt/hexane 50:50) to give the title compound **5a**. IR (neat) ν_{\max} 3205, 2980, 2239, 1603, 1346, 1258, 1163, 1020, 976 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.90 (d, ³J_{HH} = 8.5 Hz, 2H, ArH), 7.36 (d, ³J_{HH} = 8.1 Hz, 2H, ArH), 4.10–3.84 (m, 4H, OCH₂), 3.31 (d, ²J_{PH} = 12.2 Hz, 1H, CH-P), 2.43 (s, 3H, CH₃), 1.74 (s, 3H, CH₃), 1.28 (dt, ³J_{HH} = 7.0 Hz, ⁴J_{PH} = 0.5 Hz, 3H, CH₃), 1.19 (dt, ³J_{HH} = 7.0 Hz, ⁴J_{PH} = 0.6 Hz, 3H, CH₃) ppm; ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 146.1 (C_{quat}), 133.4 (C_{quat}), 129.9 (CH), 128.6 (CH), 115.7 (d, ³J_{PC} = 2.6 Hz, C_{quat}), 63.8 (d, ²J_{PC} = 6.4 Hz, CH₂), 63.3 (d, ²J_{PC} = 6.4 Hz, CH₂), 41.4 (d, ¹J_{PC} = 203.6 Hz, CH), 35.5 (d, ²J_{PC} = 3.3 Hz, C_{quat}), 21.7 (CH₃), 18.6 (CH₃), 16.2 (d, ³J_{PC} = 8.9 Hz, CH₃), 16.2 (d, ³J_{PC} = 9.0 Hz, CH₃) ppm; ³¹P NMR (160 MHz, CDCl₃) δ 12.2 ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₅H₂₂N₂O₅PS ([M+H]⁺) 373.0987, found 373.0988.



(E)-Diethyl ((3S*,3S*)-3-cyano-3-ethyl-1-tosylaziridin-2-yl)phosphonate (5b). (1.42 g, 74%) obtained as a pale yellow oil from cyanoaziridine **2b** (1.16 g, 5 mmol) after 24 h at room temperature as described in the general procedure. The crude product was purified by flash-column chromatography

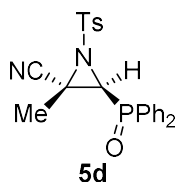
(SiO₂, AcOEt/hexane 50:50) to give the title compound **5b**. IR (neat) ν_{\max} 3284, 2983, 2236, 1596,

1340, 1264, 1014, 979 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.90 (d, $^3J_{\text{HH}} = 8.4$ Hz, 2H, ArH), 7.35 (d, $^3J_{\text{HH}} = 8.0$ Hz, 2H, ArH), 4.11–3.91 (m, 4H, OCH_2), 3.33 (d, $^2J_{\text{PH}} = 12.1$ Hz, 1H, CH-P), 2.43 (s, 3H, CH_3), 2.04–1.91 (m, 2H, CH_2), 1.29 (t, $^3J_{\text{HH}} = 7.0$ Hz, 3H, CH_3), 1.20 (t, $^3J_{\text{HH}} = 7.0$ Hz, 3H, CH_3), 0.92 (t, $^3J_{\text{HH}} = 7.4$ Hz, 3H, CH_3) ppm; ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 146.1 (C_{quat}), 133.1 (C_{quat}), 129.9 (CH), 128.8 (CH), 114.8 (d, $^3J_{\text{PC}} = 2.2$ Hz, C_{quat}), 63.9 (d, $^2J_{\text{PC}} = 6.2$ Hz, CH_2), 63.2 (d, $^2J_{\text{PC}} = 6.5$ Hz, CH_2), 41.4 (d, $^1J_{\text{PC}} = 204.4$ Hz, CH), 41.3 (d, $^2J_{\text{PC}} = 2.4$ Hz, C_{quat}), 25.2 (CH_2), 21.7 (CH_3), 16.2 (d, $^3J_{\text{PC}} = 8.2$ Hz, CH_3), 16.2 (d, $^3J_{\text{PC}} = 8.3$ Hz, CH_3), 9.8 (CH_3) ppm; ^{31}P NMR (160 MHz, CDCl_3) δ 12.4 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_5\text{PS}$ ($[\text{M}+\text{H}]^+$) 387.1144, found 387.1142.



(E)-Diisopropyl ((2S*,*)-3-cyano-3-methyl-1-tosylaziridin-2-yl)phosphonate (5c). (1.62 g, 81%) obtained as a white solid from cyanoaziridine **2c** (1.23 g, 5 mmol) after 24 h at room temperature as described in the general procedure. The crude product was purified by flash-column chromatography (SiO_2 , AcOEt/hexane 50:50) to give the title compound **5c**.

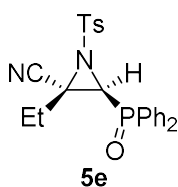
mp 116–117 $^\circ\text{C}$; IR (neat) ν_{max} 3368, 2984, 2936, 2117, 1337, 1252, 1166, 1005, 976 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.92 (d, $^3J_{\text{HH}} = 8.4$ Hz, 2H, ArH), 7.37 (d, $^3J_{\text{HH}} = 8.5$ Hz, 2H, ArH), 4.69–4.55 (m, 2H, OCH), 3.31 (d, $^2J_{\text{PH}} = 12.2$ Hz, 1H, CH-P), 2.45 (s, 3H, CH_3), 1.74 (s, 3H, CH_3), 1.30 (d, $^3J_{\text{HH}} = 6.2$ Hz, 3H, CH_3), 1.27 (d, $^3J_{\text{HH}} = 6.2$ Hz, 3H, CH_3), 1.26 (d, $^3J_{\text{HH}} = 6.2$ Hz, 3H, CH_3), 1.20 (d, $^3J_{\text{HH}} = 6.2$ Hz, 3H, CH_3) ppm; ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 146.0 (C_{quat}), 133.6 (C_{quat}), 129.9 (CH), 128.7 (CH), 115.9 (d, $^3J_{\text{PC}} = 2.3$ Hz, (C_{quat}), 73.0 (d, $^2J_{\text{PC}} = 6.6$ Hz, CH), 72.7 (d, $^2J_{\text{PC}} = 6.5$ Hz, CH), 42.2 (d, $^1J_{\text{PC}} = 204.3$ Hz, CH), 35.3 (d, $^2J_{\text{PC}} = 3.1$ Hz, C_{quat}), 24.0 (d, $^3J_{\text{PC}} = 7.5$ Hz, CH_3), 23.9 (d, $^3J_{\text{PC}} = 7.7$ Hz, CH_3), 23.8 (d, $^3J_{\text{PC}} = 8.2$ Hz, CH_3), 23.8 (d, $^3J_{\text{PC}} = 8.3$ Hz, CH_3), 21.7 (CH_3), 18.5 (CH_3) ppm; ^{31}P NMR (160 MHz, CDCl_3) δ 10.3 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{17}\text{H}_{26}\text{N}_2\text{O}_5\text{PS}$ ($[\text{M}+\text{H}]^+$) 401.1300, found 401.1297.



(E)-(2S*,3S*)-3-(Diphenylphosphoryl)-2-methyl-1-tosylaziridine-2-carbonitrile (5d). (1.78 g, 82%) obtained as a white solid from cyanoaziridine **2d** (1.41 g, 5 mmol) after 15 h at room temperature as described in the general procedure. The crude product was purified by crystallization from Et_2O to give the title compound **5d**. mp 171–173 $^\circ\text{C}$; IR (neat) ν_{max} 3054, 2963, 2244, 1333,

1200, 1160 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.77–7.16 (m, 14H, ArH), 3.76 (d, $^2J_{\text{PH}} = 20.6$ Hz, 1H, CH-P), 2.40 (s, 3H, CH_3), 1.83 (s, 3H, CH_3) ppm; ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 145.6 (C_{quat}), 133.4, 133.1, 133.1, 132.6, 132.6, 131.3, 131.2, 130.9, 130.8, 129.8, 129.3, 129.2, 128.8, 128.7, 128.4 (C_{Ar}), 115.9 (C_{quat}), 45.4 (d, $^1J_{\text{PC}} = 92.0$ Hz, CH), 36.1 (d, $^2J_{\text{PC}} = 3.5$ Hz, C_{quat}), 21.6, 18.4 ppm;

^{31}P NMR (160 MHz, CDCl_3) δ 21.0 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{23}\text{H}_{22}\text{N}_2\text{O}_3\text{PS}$ ($[\text{M}+\text{H}]^+$) 437.1089, found 437.1085.



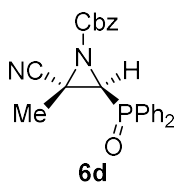
(E)-(2S*,3S*)-3-(Diphenylphosphoryl)-2-ethyl-1-tosylaziridine-2-carbonitrile

(5e). (1.73 g, 77%) obtained as a white solid from cyanoaziridine **2e** (1.48 g, 5 mmol) after 17 h at room temperature as described in the general procedure.

The crude product was purified by crystallization from Et_2O to give the title compound **5e**. mp 170–173 °C; IR (neat) ν_{max} 3054, 2244, 1339, 1160 cm^{-1} ; ^1H

NMR (400 MHz, CDCl_3) δ 7.80–7.20 (m, 14H, ArH), 3.80 (d, $^2J_{\text{PH}} = 20.7$ Hz, 1H, CH-P), 2.41 (s, 3H, CH_3), 2.26–2.20 (m, 1H, CHH), 1.98–1.93 (m, 1H, CHH), 0.90 (t, $^3J_{\text{HH}} = 7.4$ Hz, 3H, CH_3) ppm; ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 145.7 (C_{quat}), 133.2, 133.2, 133.1, 132.7, 132.6, 131.4, 131.3, 131.0, 130.9, 129.8, 129.4, 129.2, 128.8, 128.7, 128.7 (C_{Ar}), 115.1 (C_{quat}), 45.8 (d, $^1J_{\text{PC}} = 92.0$ Hz, CH), 42.1 (d, $^2J_{\text{PC}} = 3.4$ Hz, C_{quat}), 25.0 (CH_2), 21.7 (CH_3), 10.2 (CH_3) ppm; ^{31}P NMR (160 MHz, CDCl_3) δ 20.8 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}_3\text{PS}$ ($[\text{M}+\text{H}]^+$) 451.1245, found 451.1250.

General procedure for the N-Cbz functionalization of cyanoaziridines



Benzyl (E)-(2S*,3S*)-2-cyano-3-(diphenylphosphoryl)-2-methylaziridine-1-carboxylate (6d)

To a 0 °C solution of cyanoaziridine **2d** (1.41 g, 5 mmol, 1 eq) in THF– H_2O (9 mL) were added Na_2CO_3 (0.8 g, 7.5 mmol, 1.5 eq) and benzyl chloroformate (1.20 mL, 8.5 mmol, 1.7 eq). The reaction mixture

was allowed to reach room temperature and stirred for 24 h. A solution of saturated aqueous NH_4Cl (8 mL) and CH_2Cl_2 (8 mL) were then added and the solvent was evaporated under vacuum. The aqueous phase was extracted with CH_2Cl_2 (15 mL) and the organic phase was washed with water (15 mL). The organic layers were dried over anhydrous MgSO_4 , filtered and concentrated to dryness in vacuum, and the resulting residue was purified by crystallization from hexane to afford the title compound **6d** (1.23 g, 59% as a pale yellow solid). mp 146–148 °C; IR (neat) ν_{max} 2971, 2232, 1725, 1437, 1189 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.73–7.21 (m, 15H, ArH), 5.09 (s, 2H, OCH_2), 3.40 (d, $^2J_{\text{PH}} = 21.23$ Hz, 1H, CH-P), 1.74 (s, 3H, CH_3) ppm; ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 158.4 (d, $^3J_{\text{PC}} = 4.4$ Hz, C_{quat}), 134.1 (C_{quat}), 132.8, 132.8, 132.6, 132.6, 131.3, 131.2, 130.8, 130.7, 129.1, 129.0, 128.7, 128.6, 128.5, 128.5, 128.4 (C_{Ar}), 116.9 (C_{quat}), 69.8 (CH_2), 43.5 (d, $^1J_{\text{PC}} = 95.4$ Hz, CH), 35.0 (d, $^2J_{\text{PC}} = 3.4$ Hz, C_{quat}), 16.9 (CH_3) ppm; ^{31}P NMR (160 MHz, CDCl_3) δ 22.1 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{24}\text{H}_{21}\text{N}_2\text{NaO}_3\text{P}$ ($[\text{M}+\text{Na}]^+$) 439.1187, found 439.1195.

4.2. Biology

4.2.1. Materials

Reagents and solvents were used as purchased without further purification. All stock solutions of the investigated compounds were prepared by dissolving the powdered materials in appropriate amounts of DMSO. The final concentration of DMSO never exceeded 10% (v/v) in reactions. The stock solution was stored at 5 °C until it was used.

4.2.2. Cytotoxicity assays.

Cells were cultured according to the supplier's instructions. Cells were seeded in 96-well plates at a density of $2-4 \times 10^3$ cells per well and incubated overnight in 0.1 mL of media supplied with 10% Fetal Bovine Serum (Lonza) in 5% CO₂ incubator at 37 °C. On day 2, drugs were added and samples were incubated for 48 hours. After treatment, 10 µL of cell counting kit-8 was added into each well for additional 2 hours incubation at 37 °C. The absorbance of each well was determined by an Automatic Elisa Reader System at 450 nm wavelength.

Acknowledgments

The authors thank Ministerio de Economía, Industria y Competividad (MINECO, CTQ-2015-67871R) and Departamento de Educación Universidades e Investigación of Gobierno Vasco (GV, IT 992-16) for supporting this work. We also thank SGIker (UPV/EHU) technical support for NMR spectra (MINECO, GV/EJ, and European Social Found).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmech.2018.12.002>.

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Material Complementario – Manuscrito 1

Synthesis and biological evaluation of cyanoaziridine phosphine oxides and phosphonates with antiproliferative activity

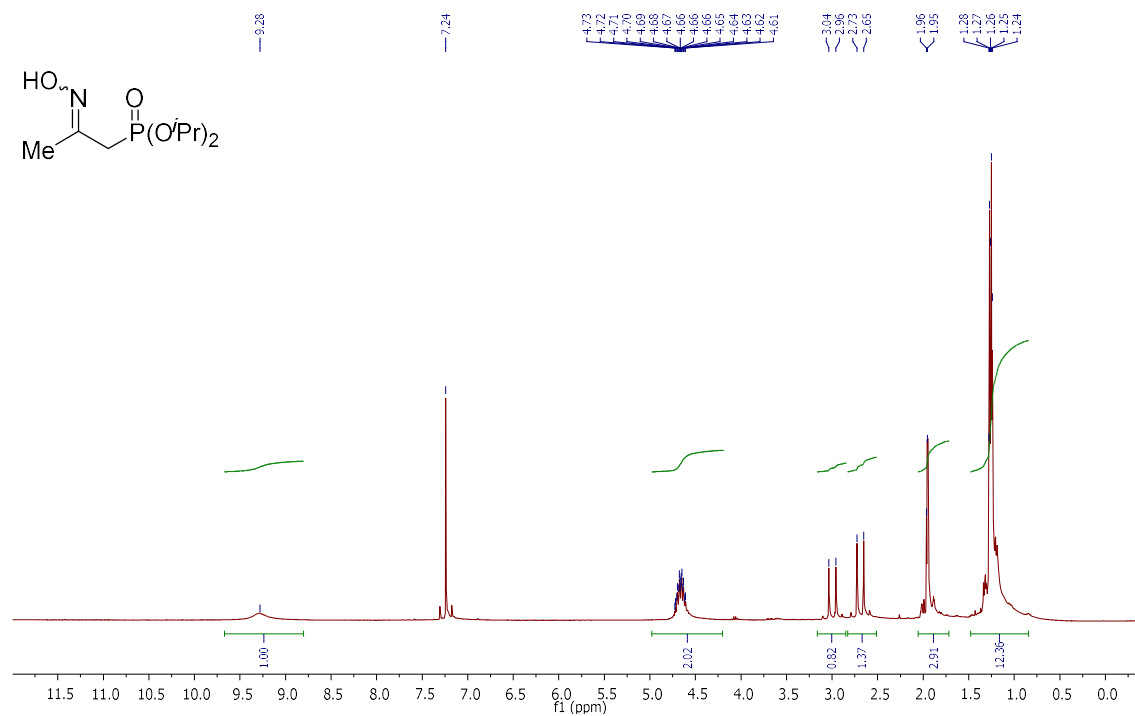
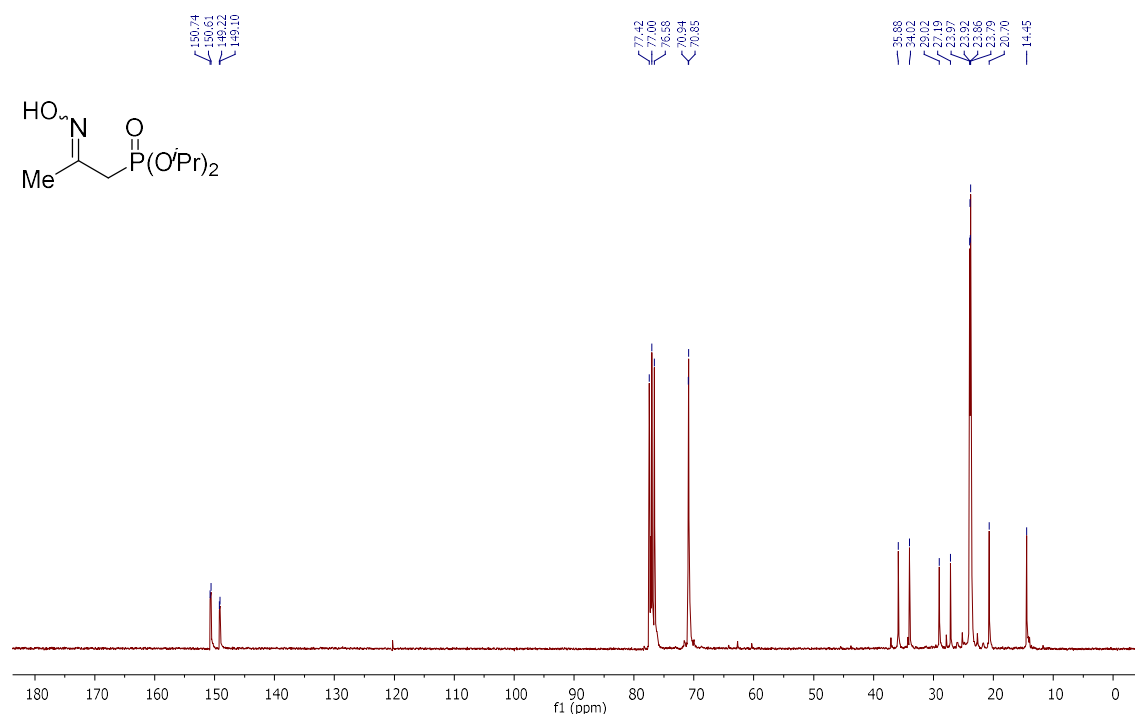
Victor Carramiñana, Ana M. Ochoa de Retana, Ander Vélez del Burgo,
Jesús M. de los Santos*, Francisco Palacios*

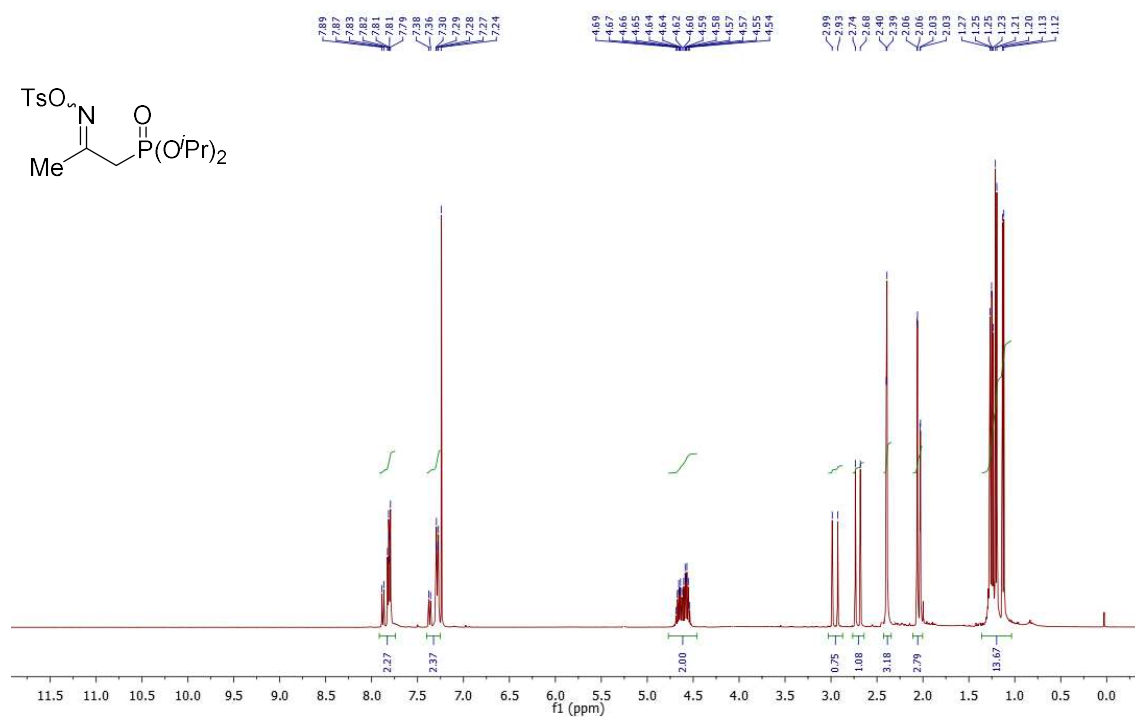
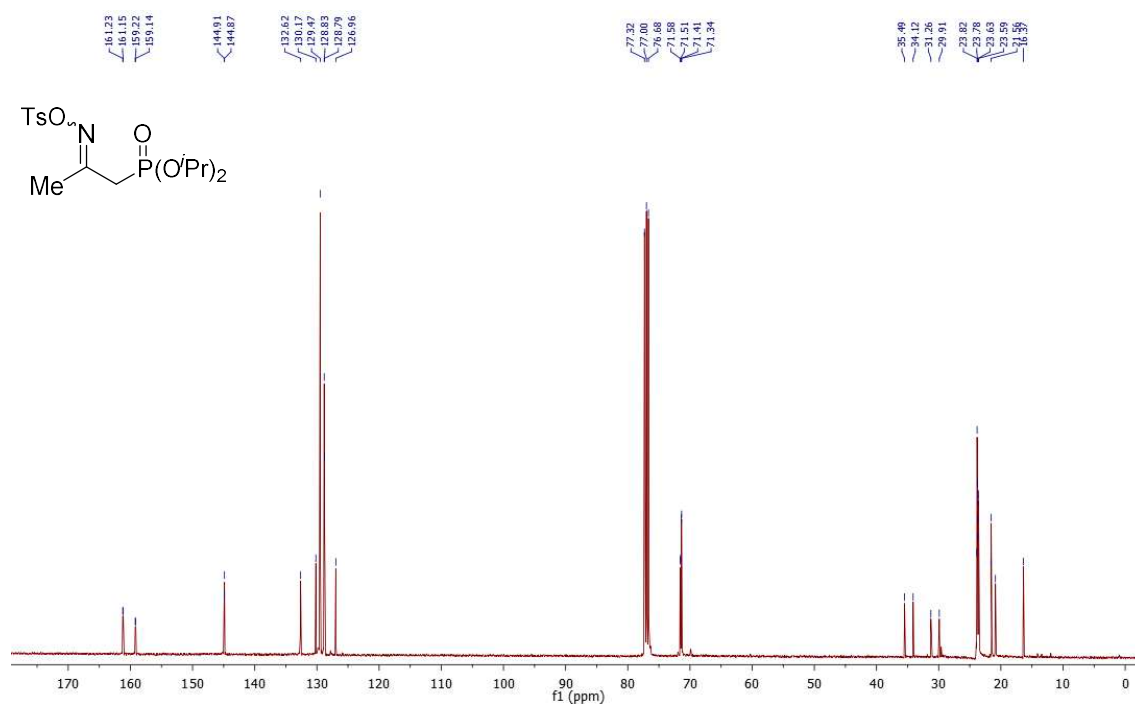
Departamento de Química Orgánica I, Facultad de Farmacia and Centro de Investigaciones y Estudios Avanzados “Lucio Lascaray”, University of the Basque Country (UPV/EHU), Paseo de la Universidad 7, 01006 Vitoria, Spain.

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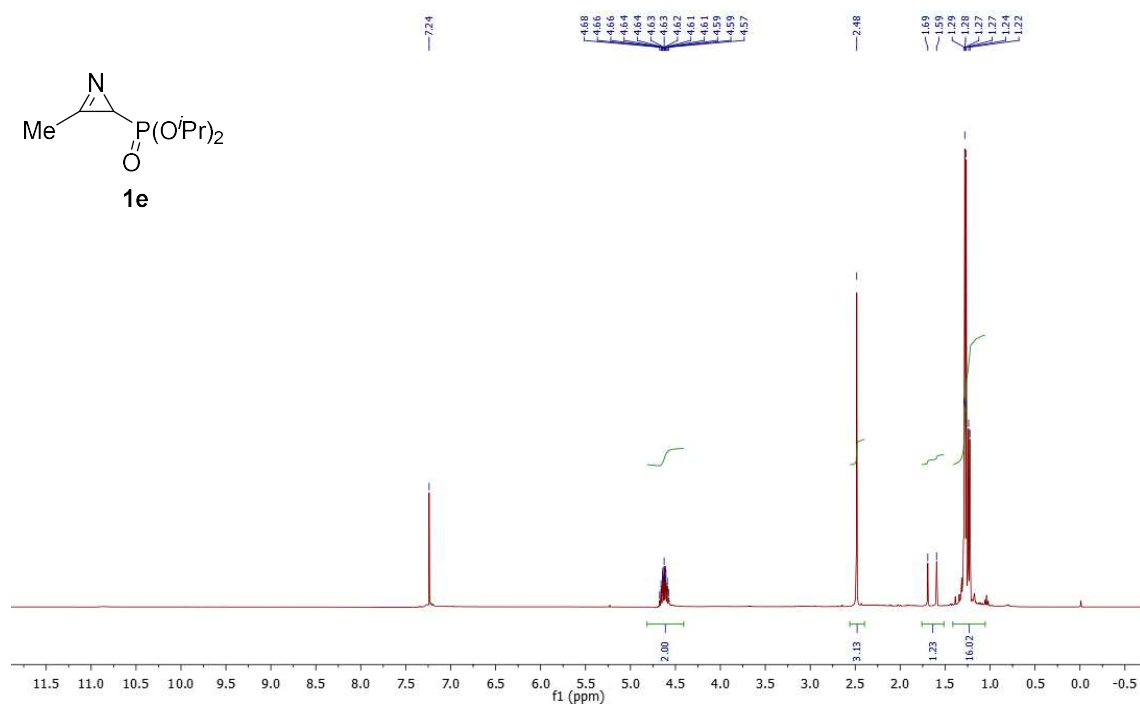
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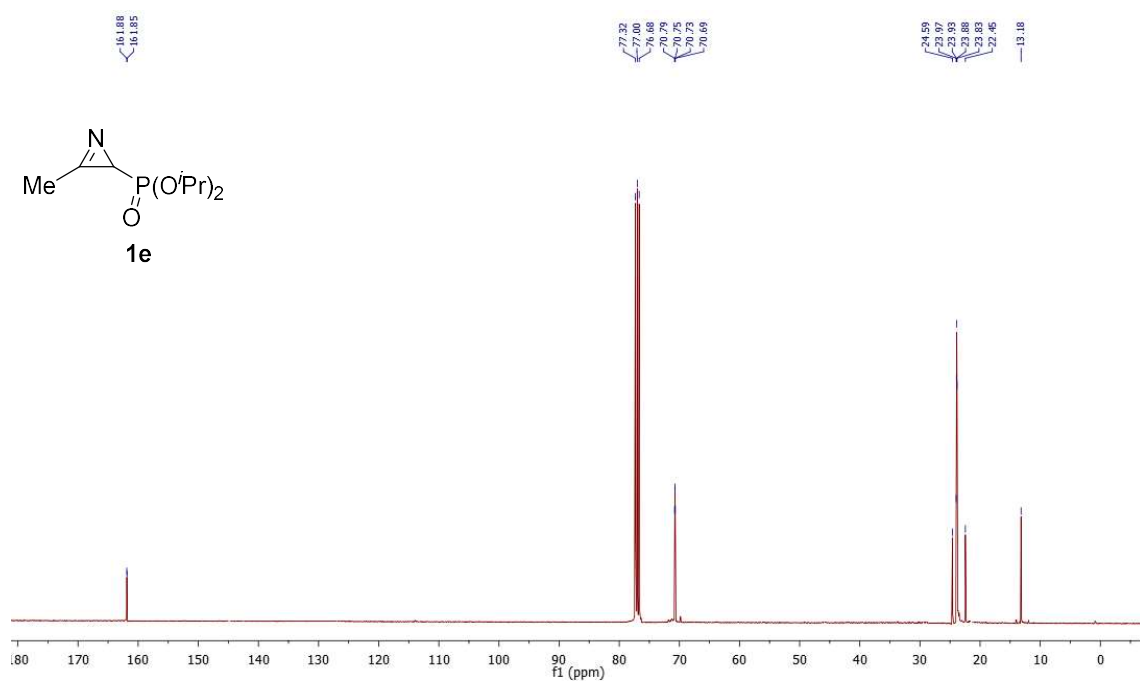
^1H NMR (300 MHz, CDCl_3) of functionalized oxime ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) of functionalized oxime

^1H NMR (400 MHz, CDCl_3) of functionalized tosyloxime ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) of functionalized tosyloxime

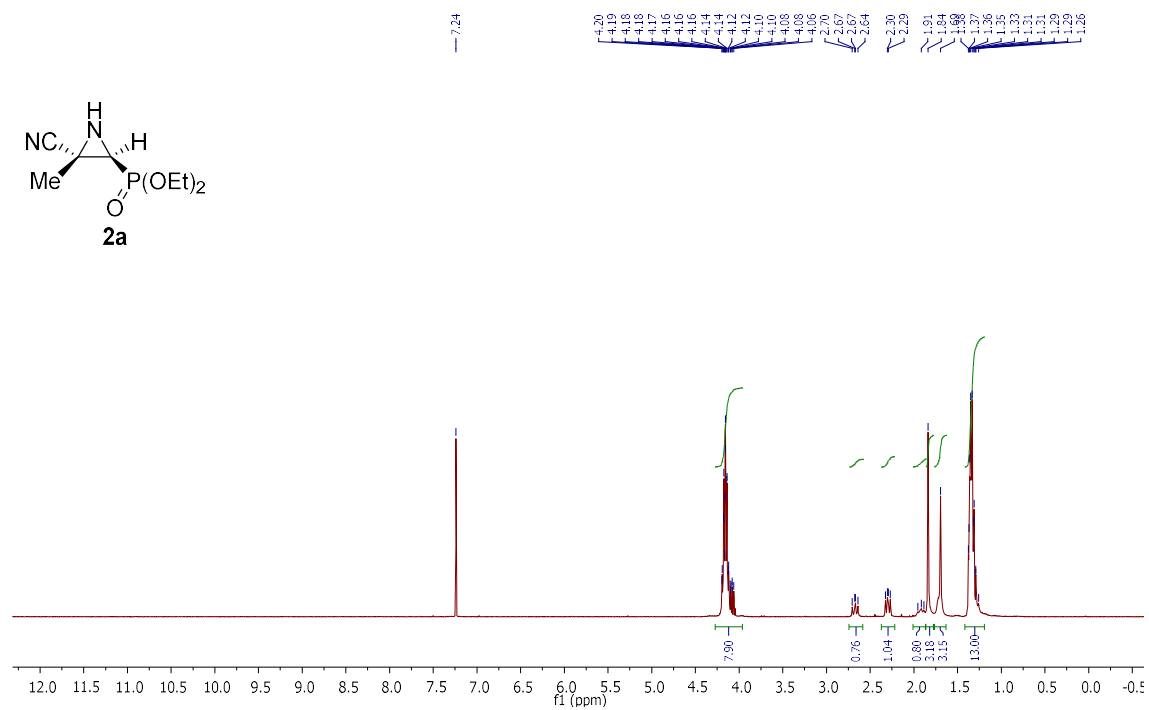
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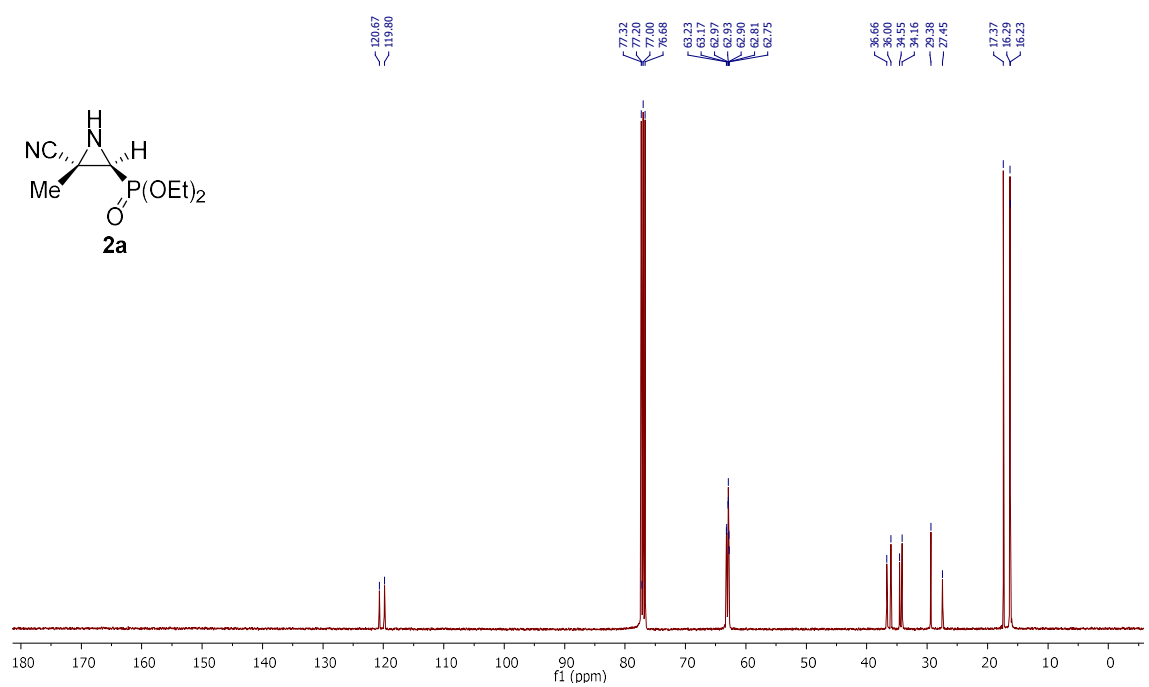
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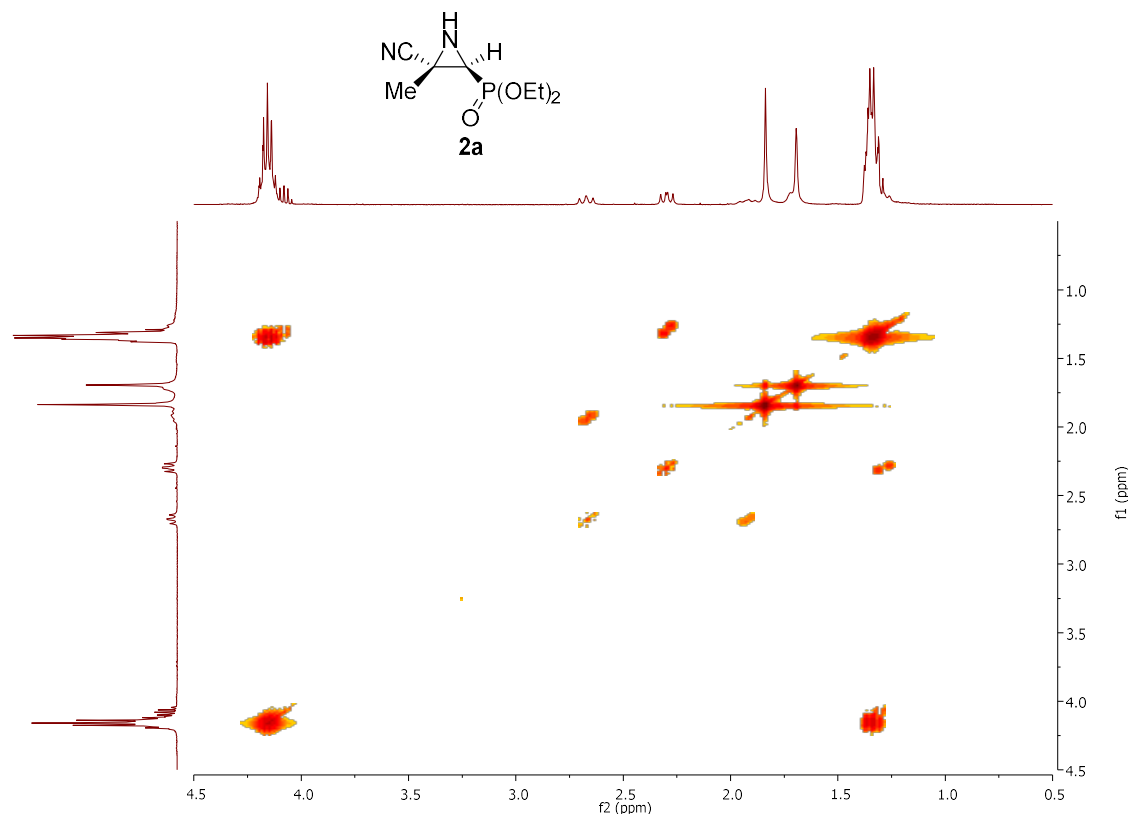
^1H NMR (400 MHz, CDCl_3) of cyanoaziridine **2a**



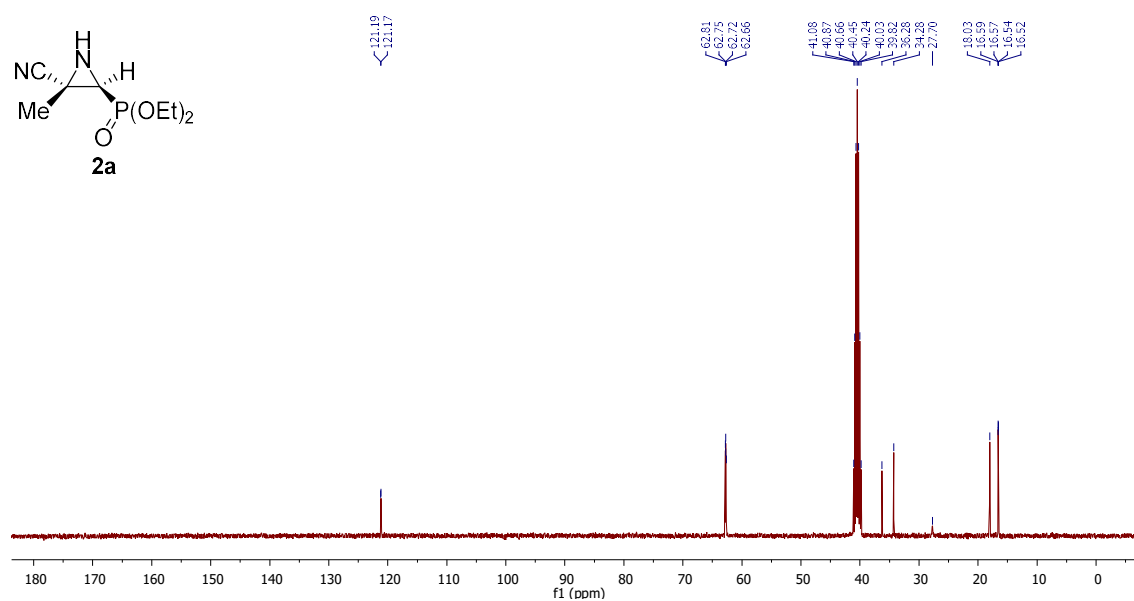
^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) of cyanoaziridine **2a**



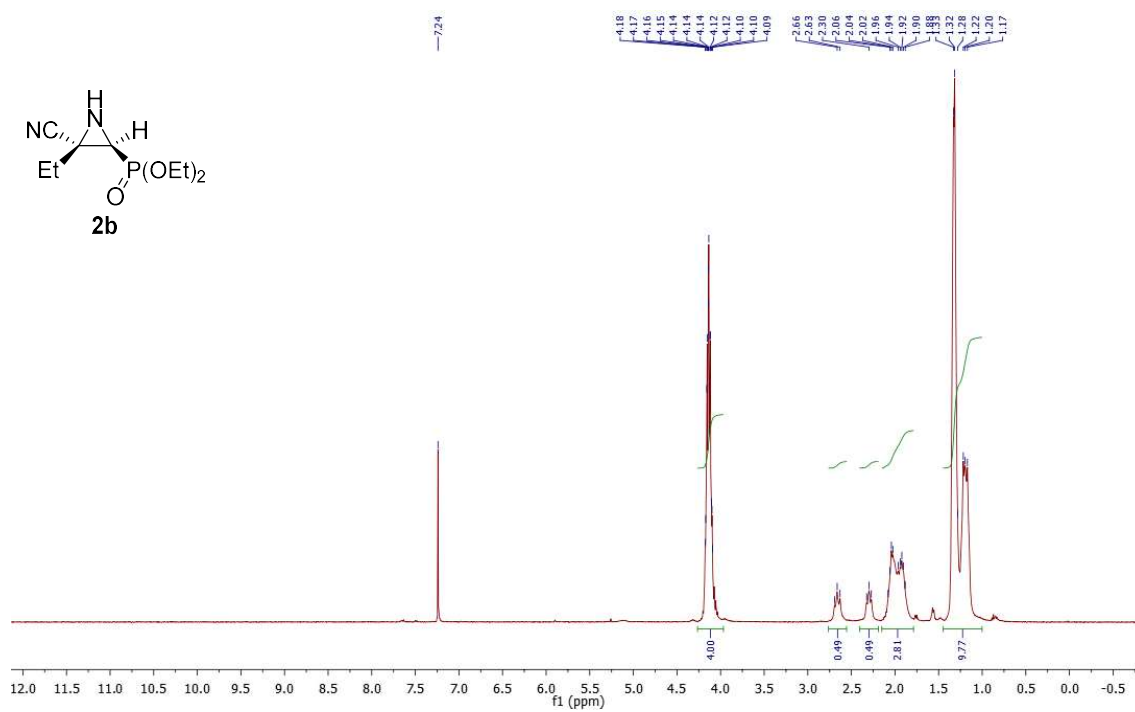
2D-COSY spectra (400 MHz, DMSO-d₆) of cyanoaziridine **2a**



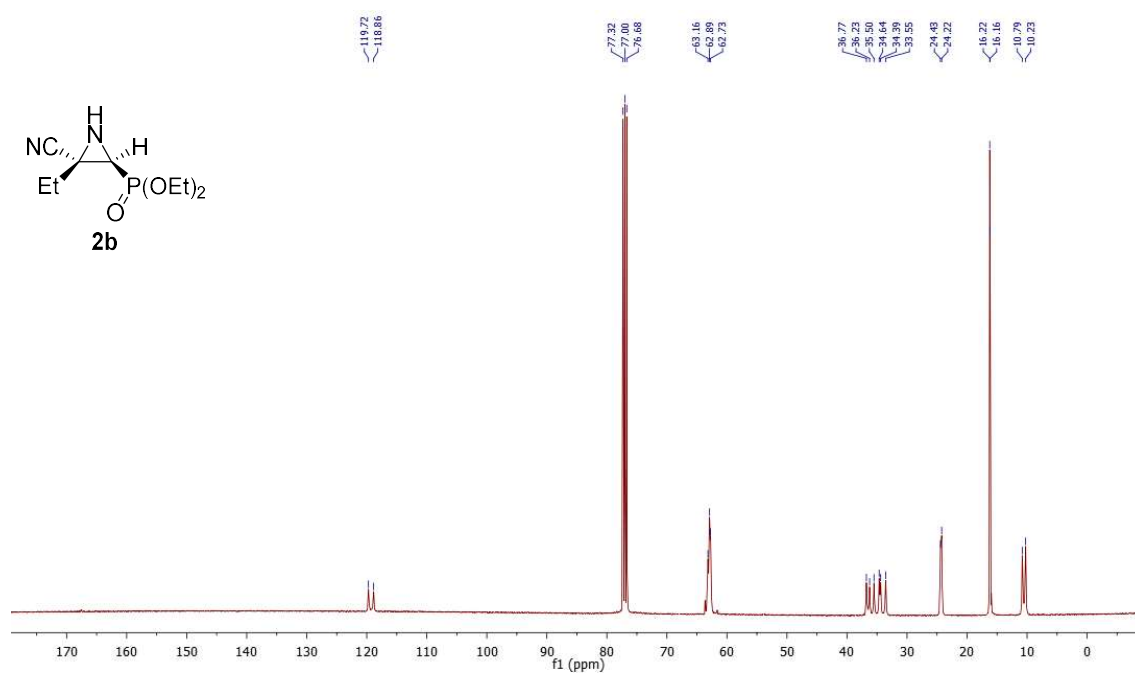
¹³C {¹H} NMR (100 MHz, DMSO-d₆) of cyanoaziridine **2a** at 80 °C



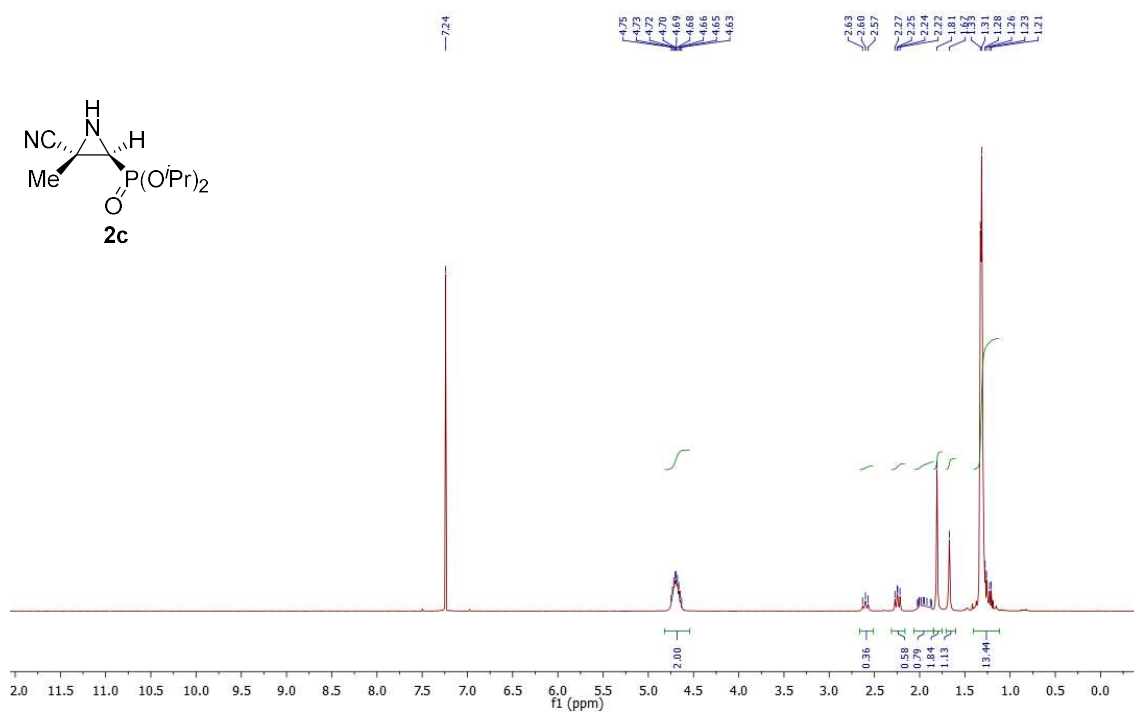
^1H NMR (400 MHz, CDCl_3) of cyanoaziridine **2b**



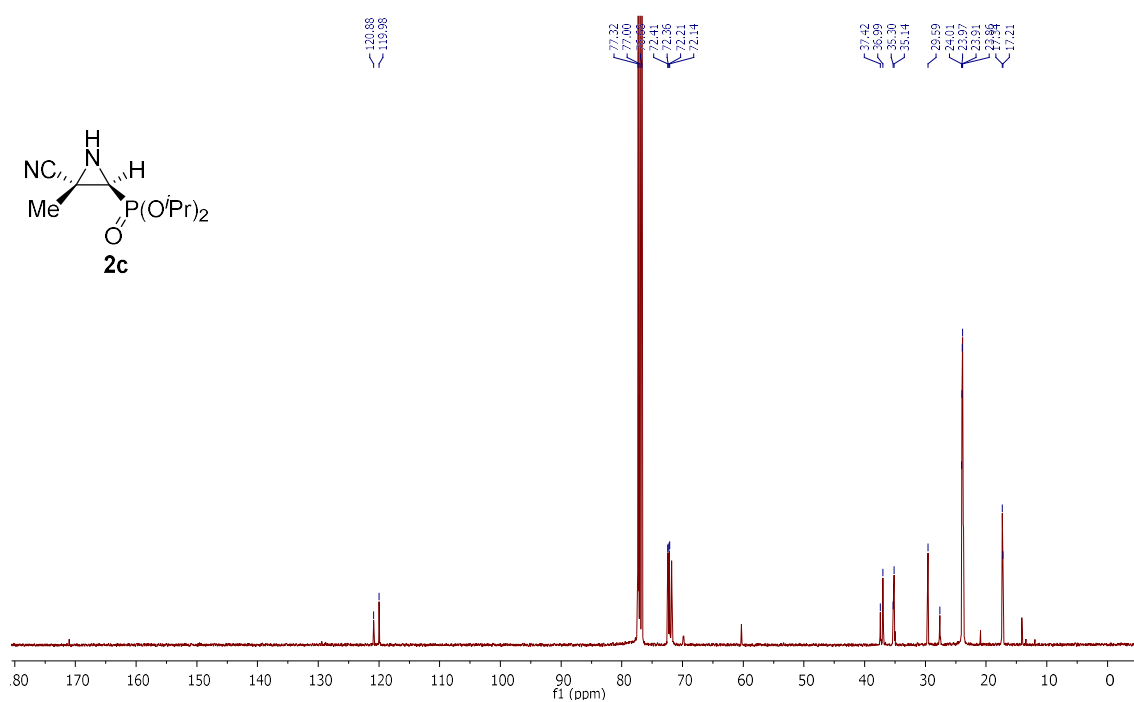
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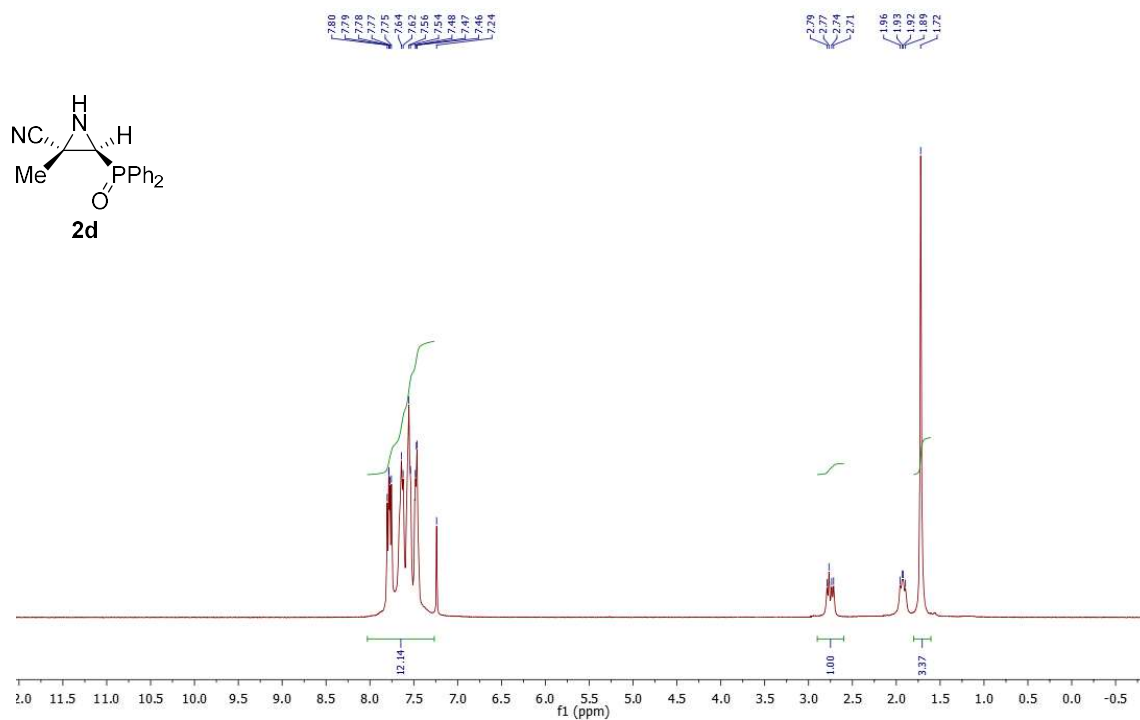
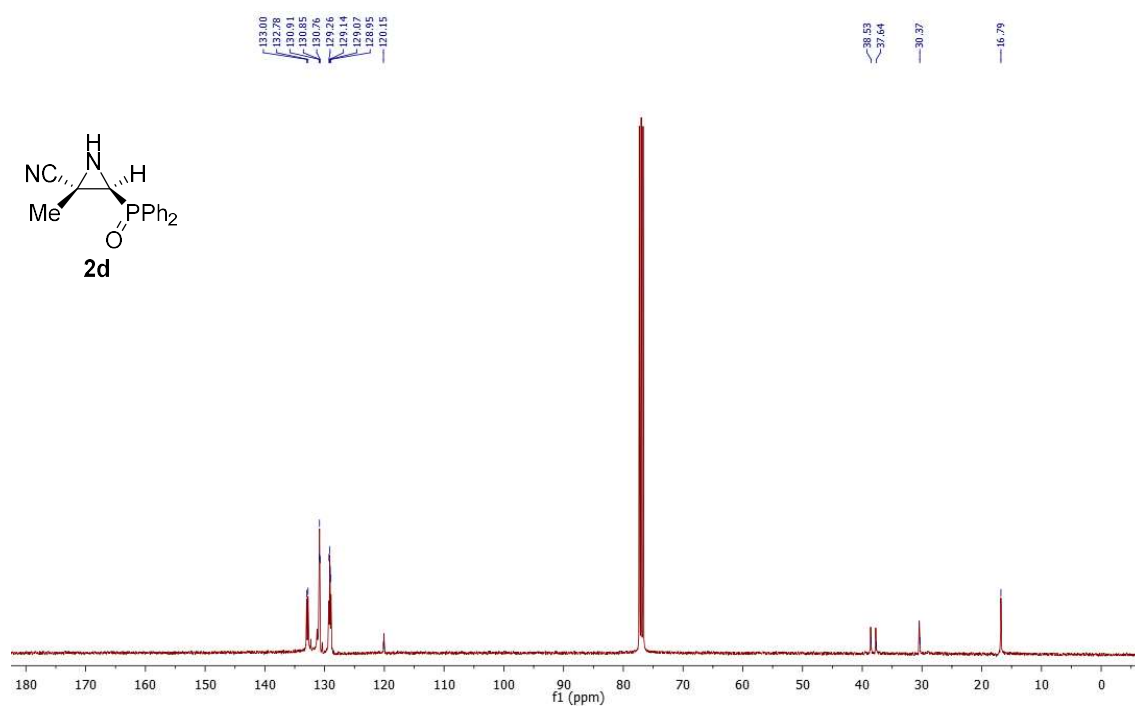


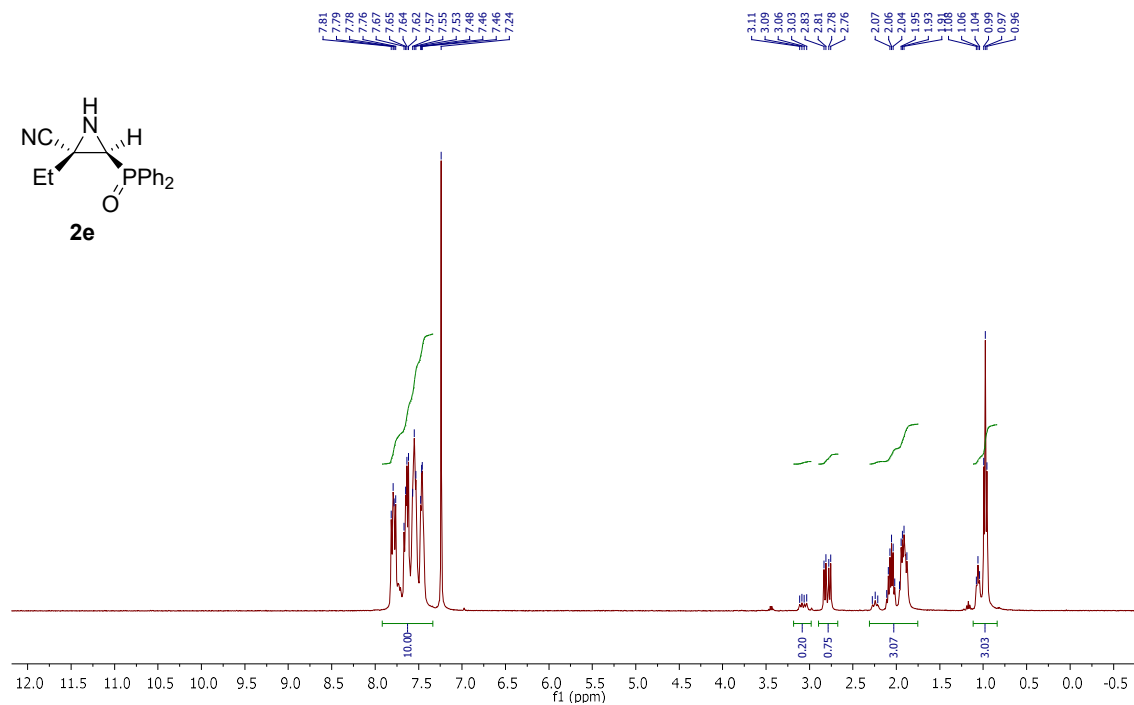
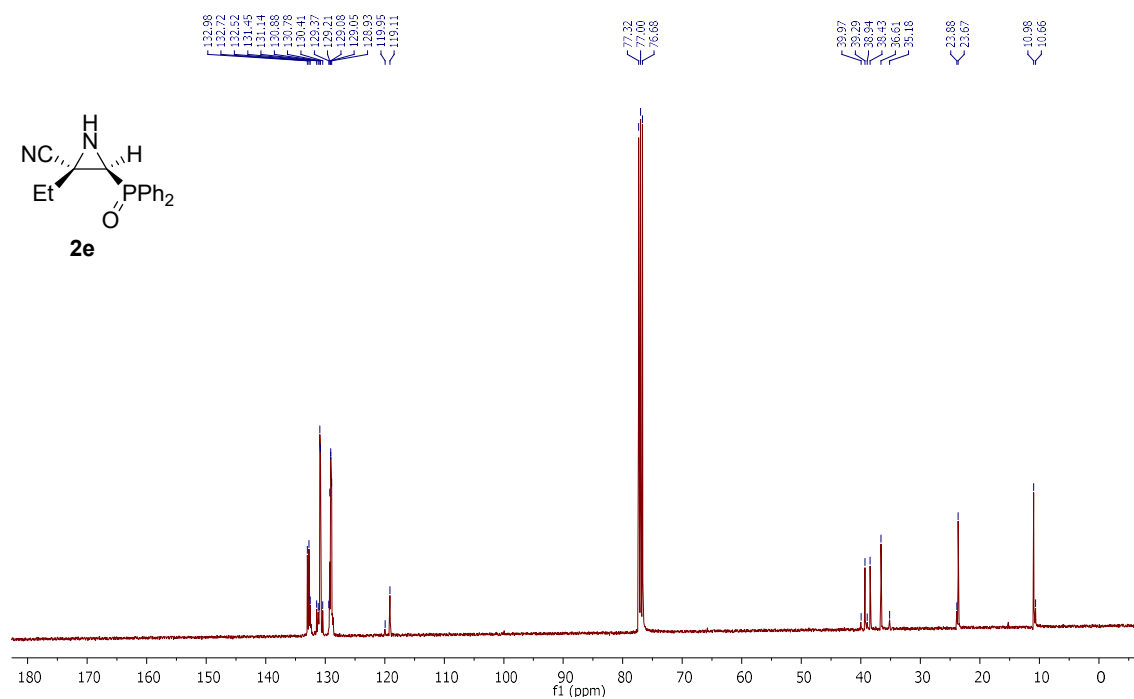
^1H NMR (400 MHz, CDCl_3) of cyanoaziridine **2c**



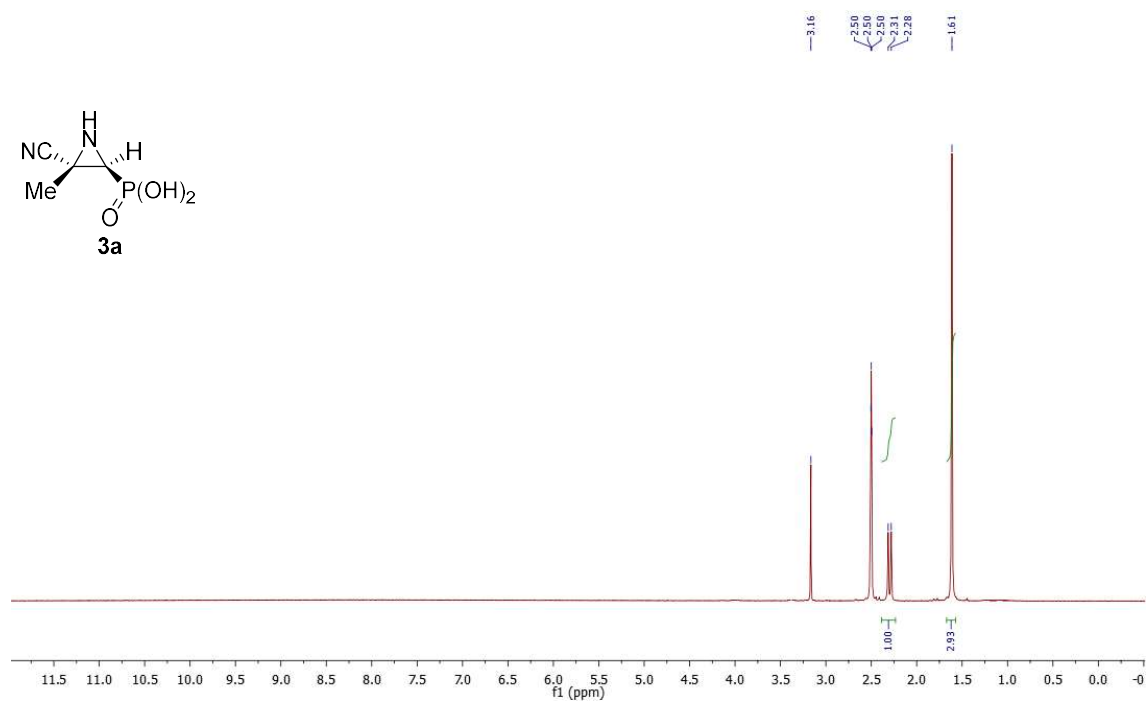
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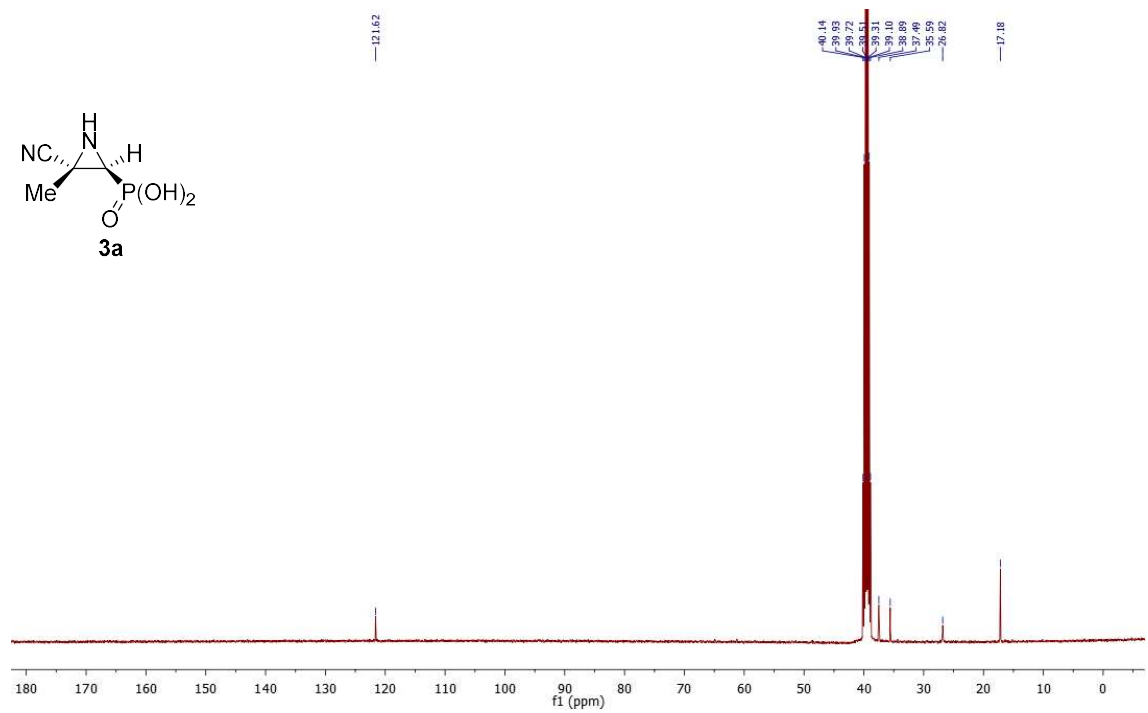
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^1H NMR (400 MHz, CDCl_3) of cyanoaziridine **2e** ^{13}C { ^1H } NMR (100 MHz, CDCl_3) of cyanoaziridine **2e**

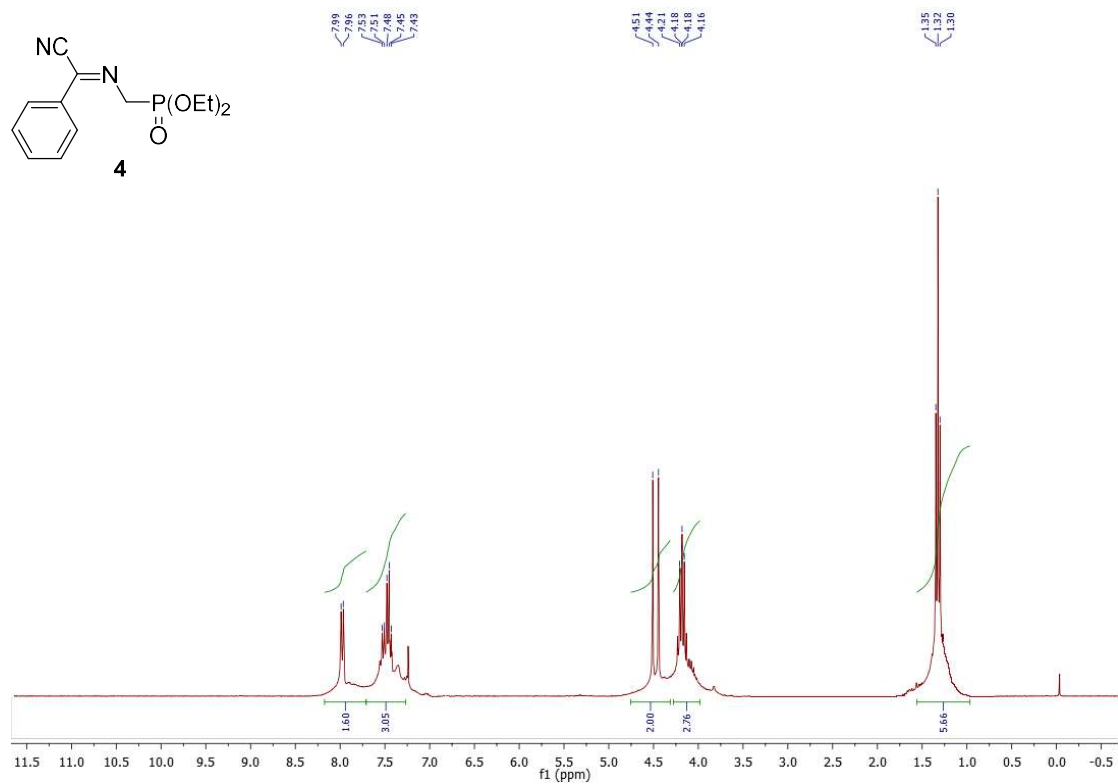
^1H NMR (400 MHz, DMSO) of cyanoaziridine **3a**



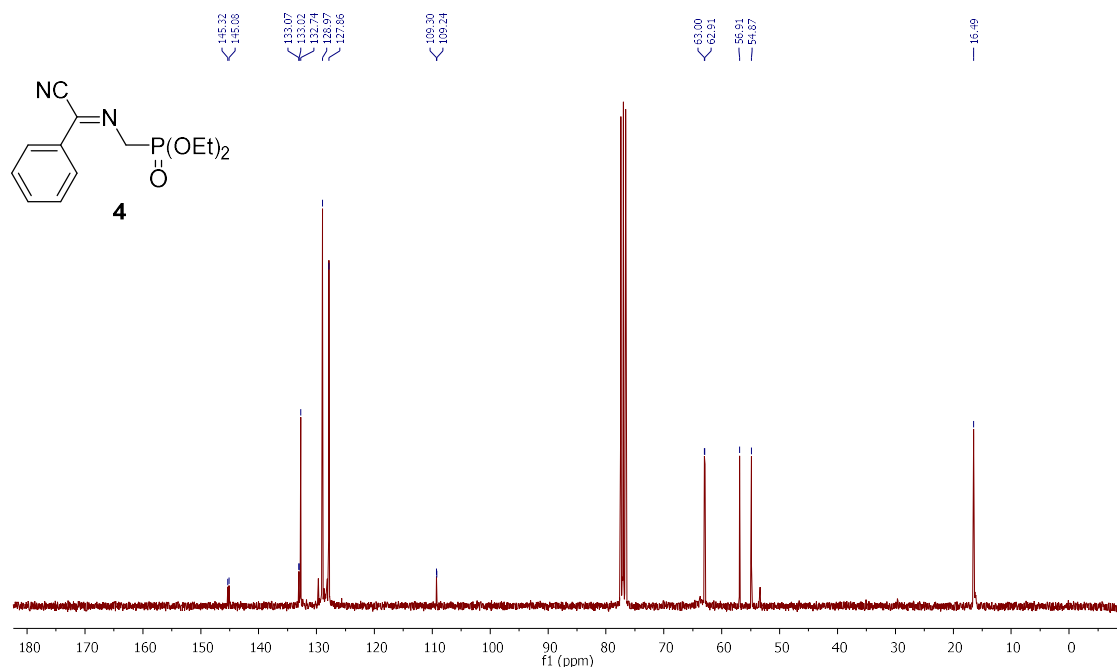
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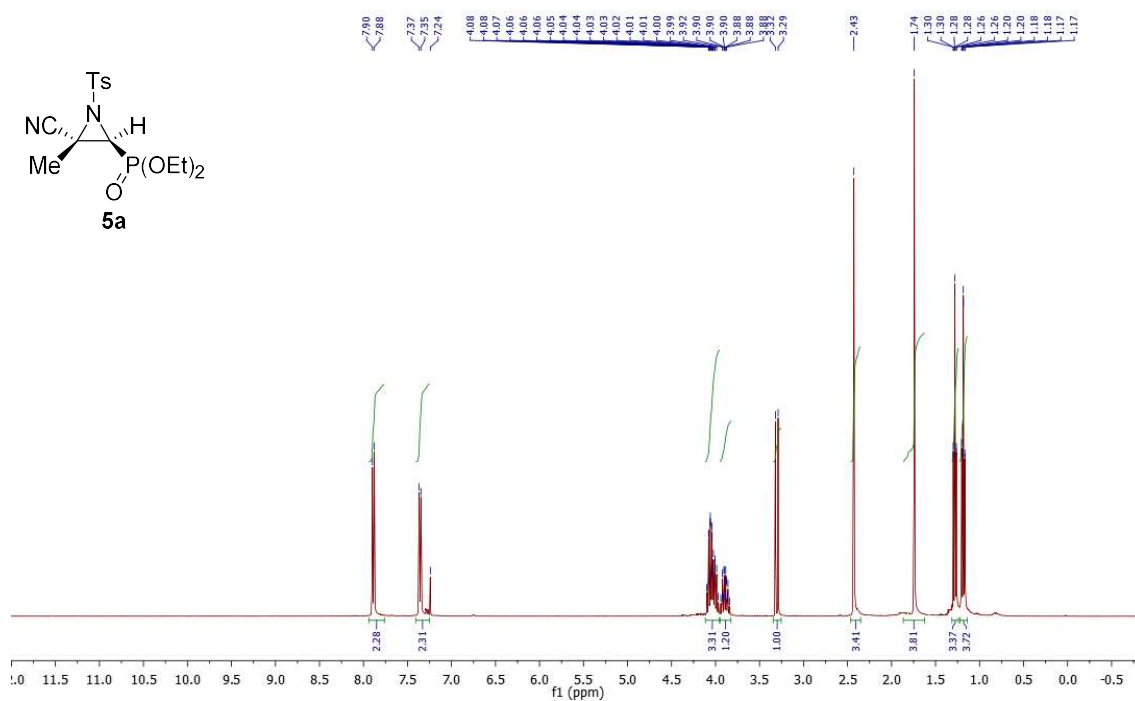
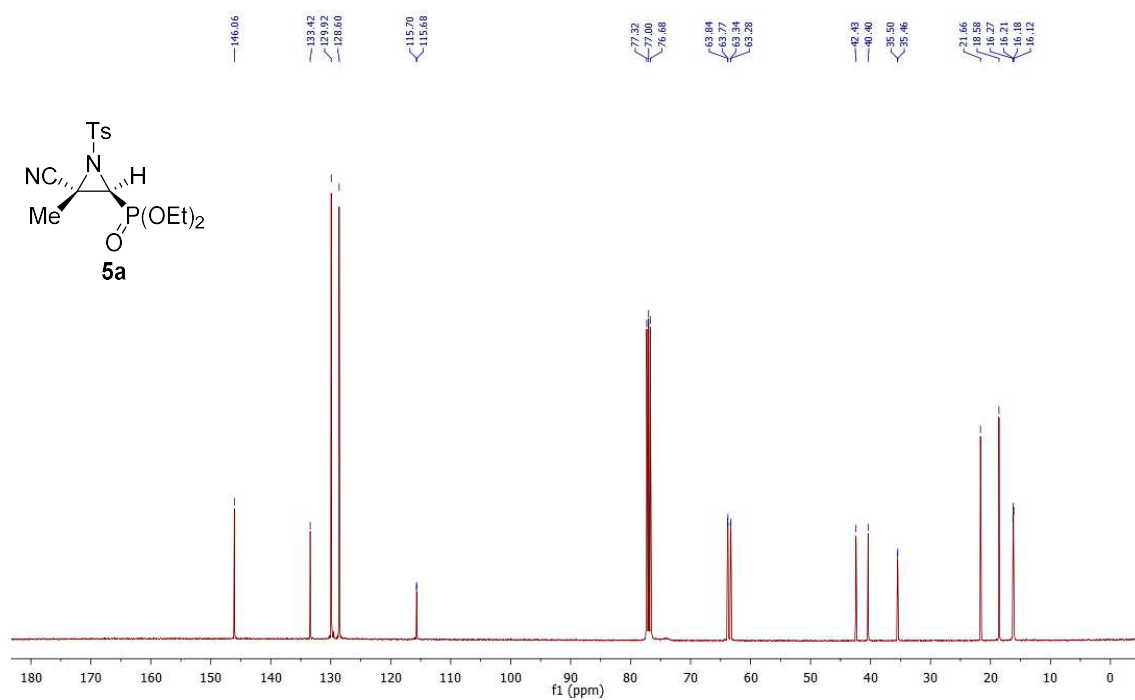


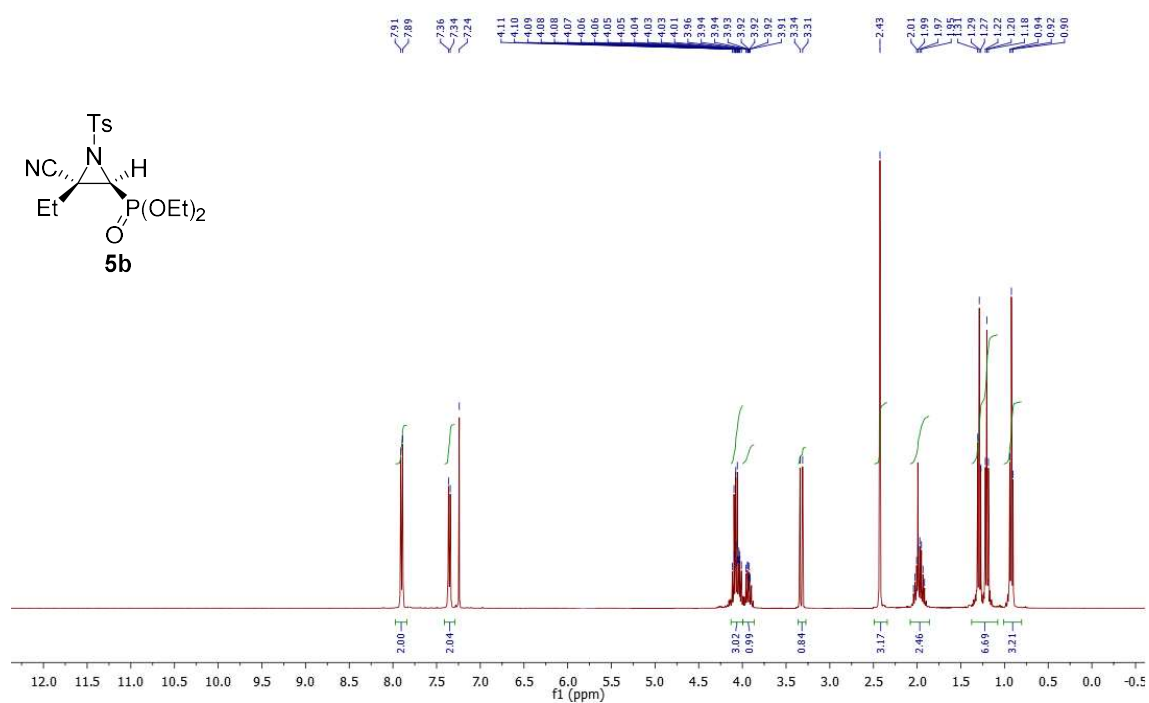
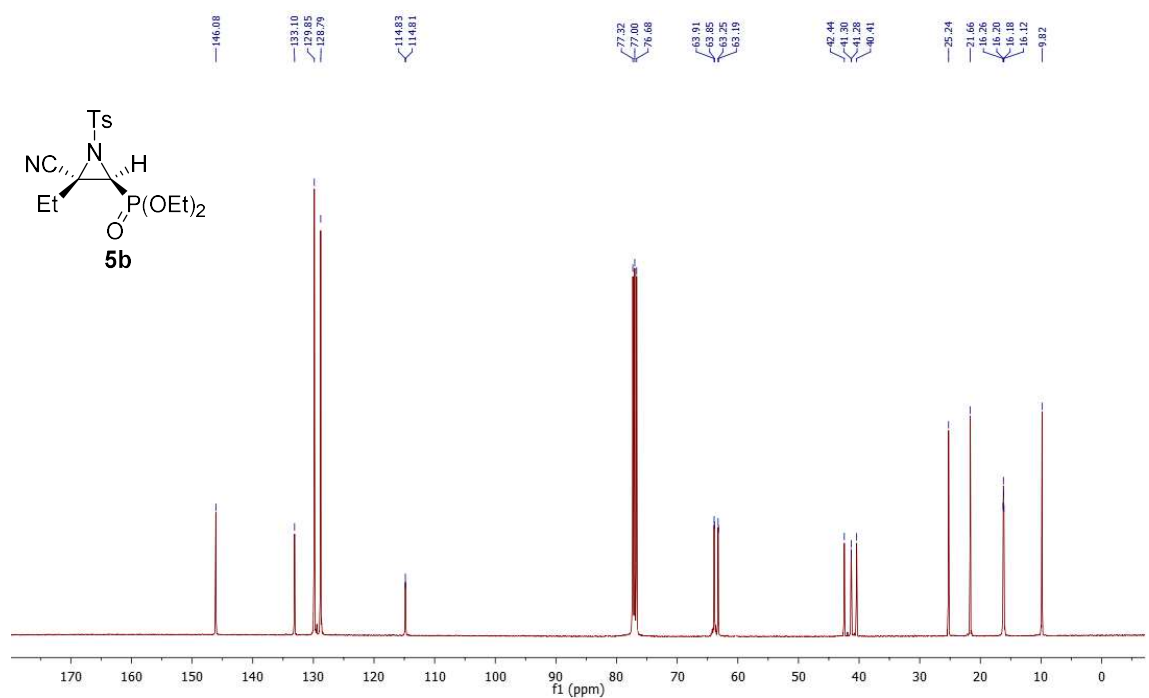
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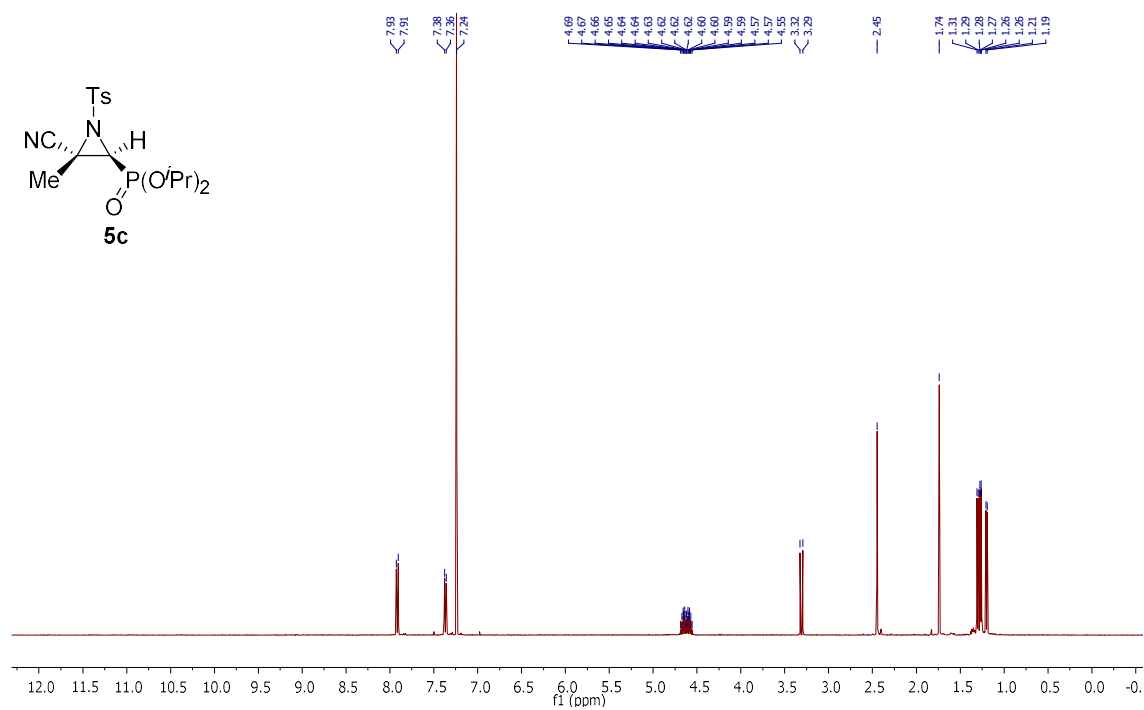
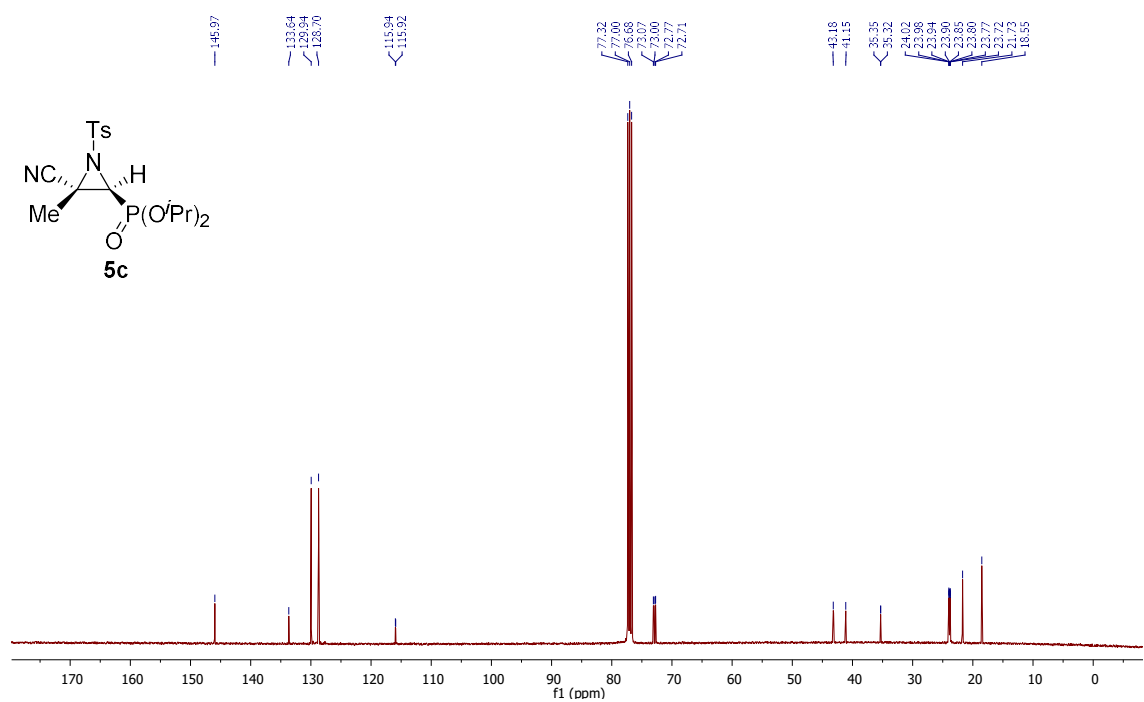


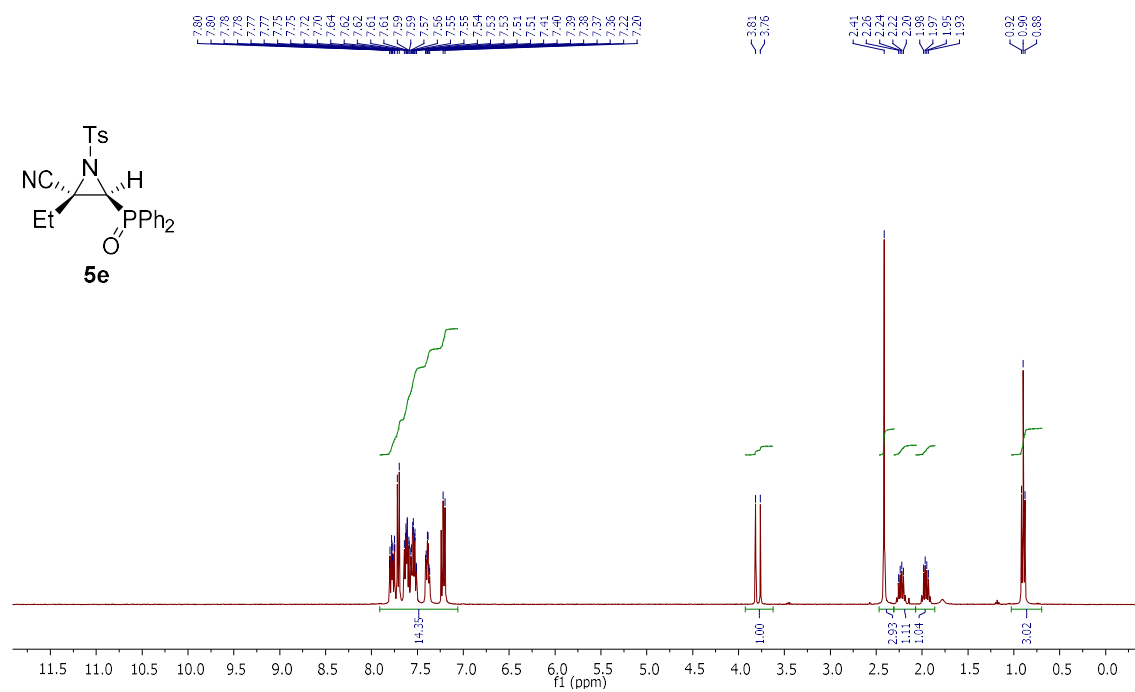
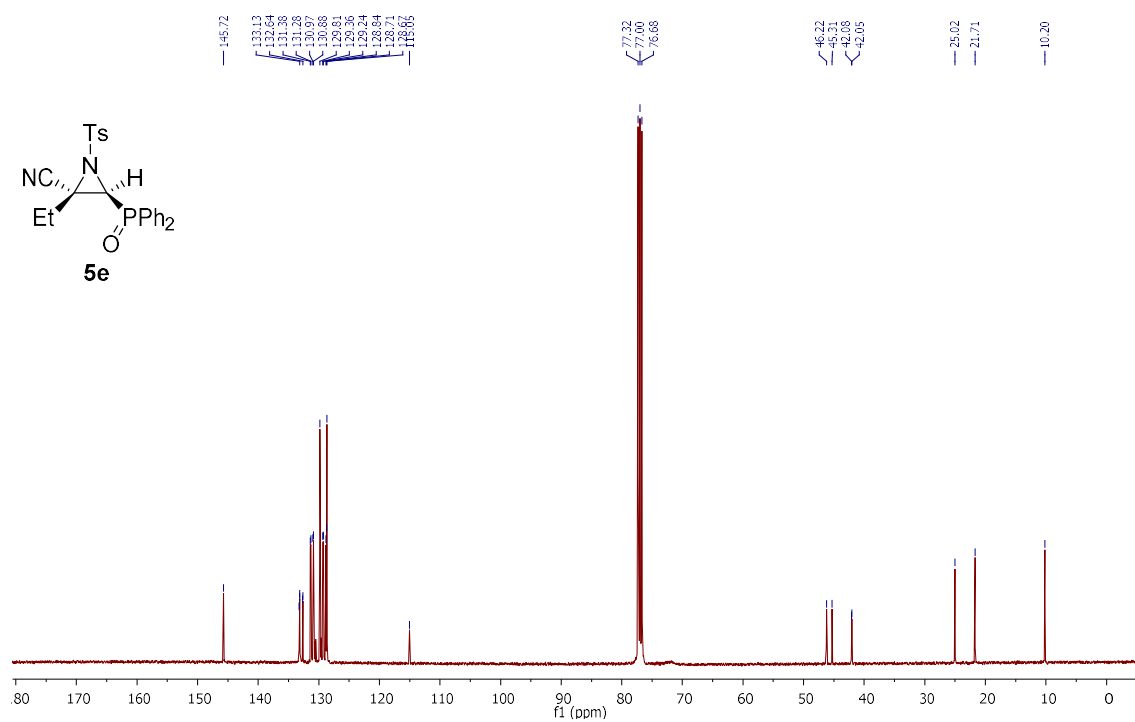
^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) of cyano-imine **4**



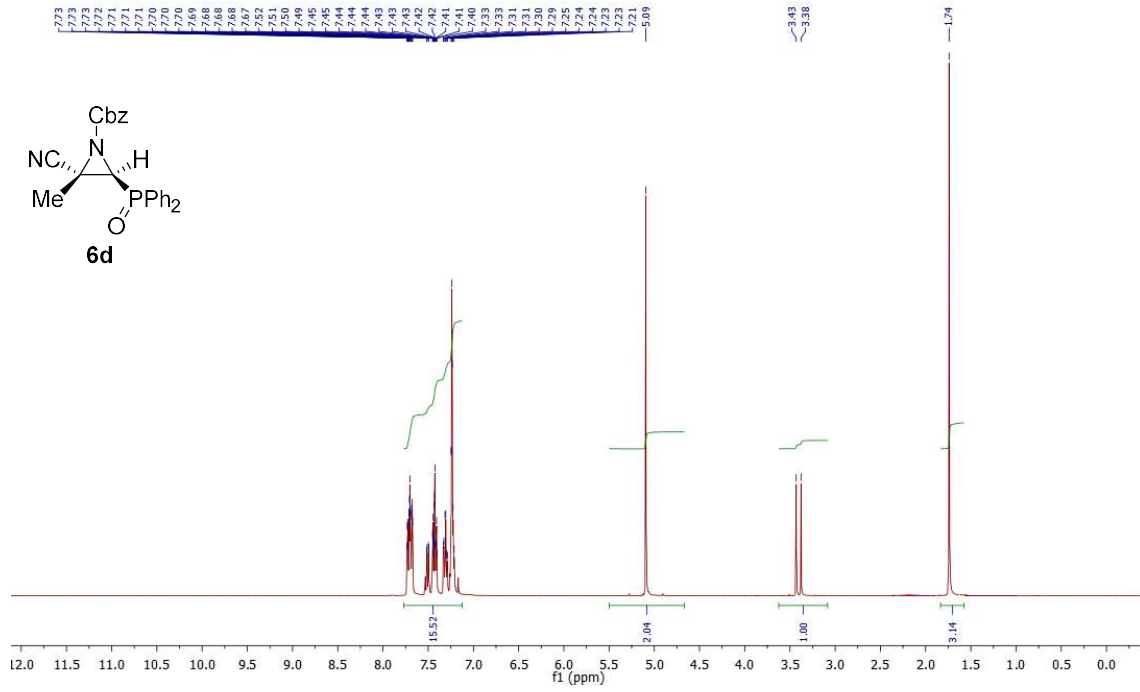
^1H NMR (400 MHz, CDCl_3) of cyanoaziridine **5a** ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) of cyanoaziridine **5a**

^1H NMR (400 MHz, CDCl_3) of cyanoaziridine **5b** ^{13}C { ^1H } NMR (100 MHz, CDCl_3) of cyanoaziridine **5b**

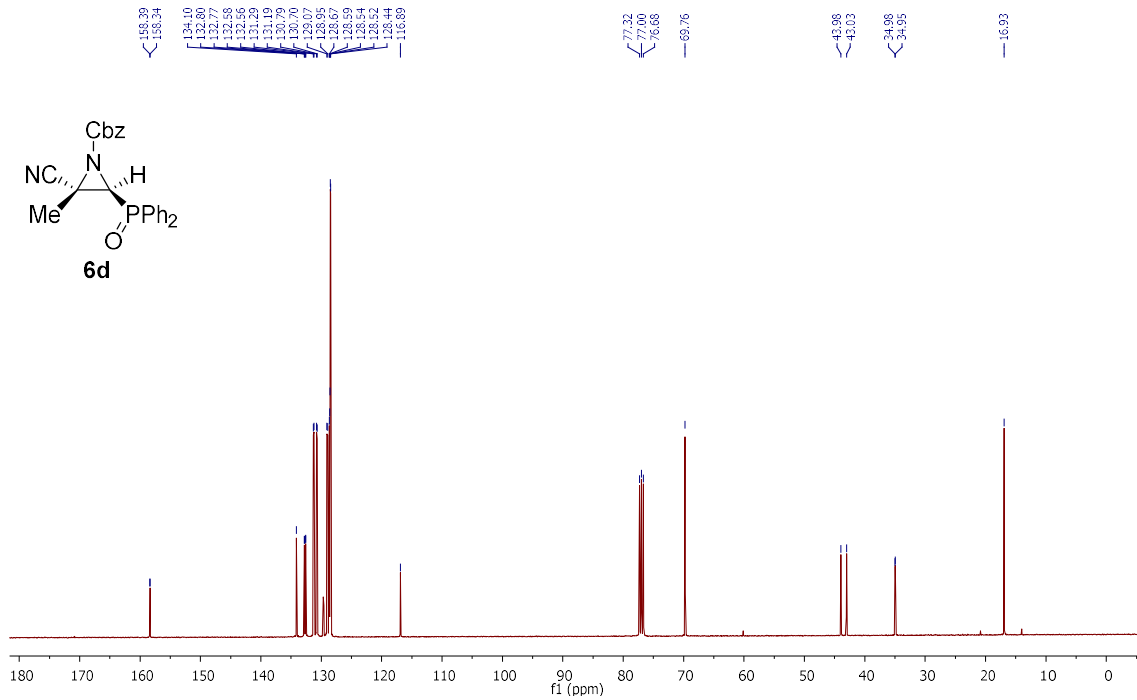
^1H NMR (400 MHz, CDCl_3) of cyanoaziridine **5c** ^{13}C { ^1H } NMR (100 MHz, CDCl_3) of cyanoaziridine **5c**

^1H NMR (400 MHz, CDCl_3) of cyanoaziridine **5e** ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) of cyanoaziridine **5e**

¹H NMR (400 MHz, CDCl₃) of cyanoaziridine **6d**



¹³C {¹H} NMR (100 MHz, CDCl₃) of cyanoaziridine **6d**



Optimized structures for the two (most and less) stable rotamers of compound **2a** after conformational study around the P–C bond.

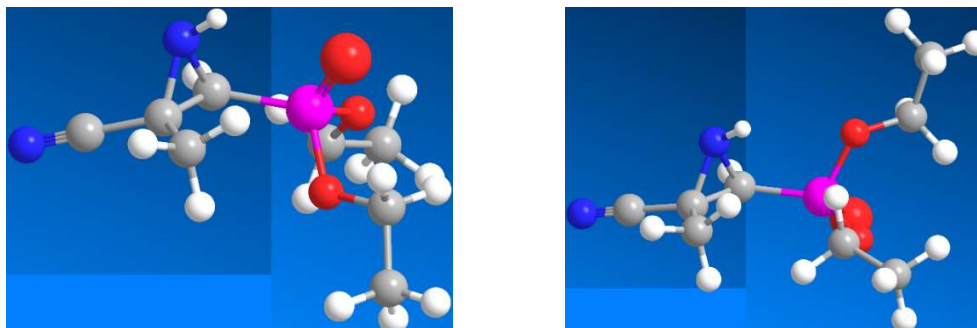


Figure S1. Optimized structures for rotamers I (left) and II (right) of compound **2a**.

ORTEP view and X-ray crystallographic statistics for compounds **2a**

(CCDC deposition number 1875391)

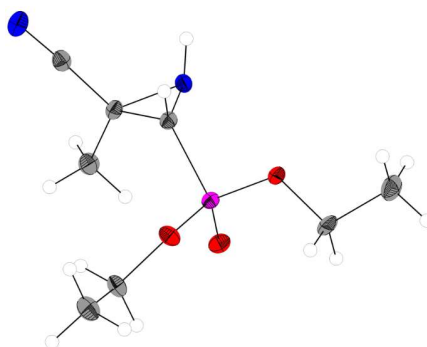


Figure S2. ORTEP diagram of compound **2a**.

Single crystals of $C_8H_{15}N_2O_3P$ [**2a**] were obtained. A suitable crystal was selected and mounted on a SuperNova (Mo), Single source at offset, Eos diffractometer. The crystal was kept at 100.01(10) K during data collection. Using Olex2,¹ the structure was solved with the ShelXS² structure solution program using Direct Methods and refined with the ShelXL³ refinement package using Least Squares minimization.

Table S1. Crystal data and structure refinement for **2a**.

Empirical formula	C ₈ H ₁₅ N ₂ O ₃ P
Formula weight	218.19
Temperature/K	100.01(10)
Crystal system	monoclinic
Space group	P21/n
a/Å	9.2898(2)
b/Å	8.11508(17)
c/Å	14.5232(3)
α/°	90
β/°	94.5823(19)
γ/°	90
Volume/Å ³	1091.36(4)
Z	4
ρ _{calc} /cm ³	1.328
μ/mm ⁻¹	0.238
F(000)	464.0
Crystal size/mm ³	0.520 × 0.283 × 0.245
Radiation	MoKα (λ = 0.71073)
2θ range for data collection/°	5.408 to 51.996
Index ranges	-11 ≤ h ≤ 11, -9 ≤ k ≤ 9, -17 ≤ l ≤ 17
Reflections collected	7697
Independent reflections	2115 [Rint = 0.0310, Rsigma = 0.0304]
Data/restraints/parameters	2115/1/133
Goodness-of-fit on F ²	1.073
R(F) (I > 2σ _I , all)	0.0331, 0.0375
Rw(F ²) ^[a] (I > 2σ _I , all)	0.0816, 0.0849
Largest diff. peak/hole / e Å ⁻³	0.31/-0.45

^[a] Weigh scheme: $1/[\sigma^2(F_o^2) + (0.0400P)^2 + 0.3585P]$ where $P = [\text{Max}(F_o^2, 0) + 2F_c^2]/3$.

^[b] Expression of secondary extinction type SHELXL: $F_c^* = kF_c[1 + 0.001F_c^2\lambda^3/\text{sen}(2\theta)]^{-1/4}$

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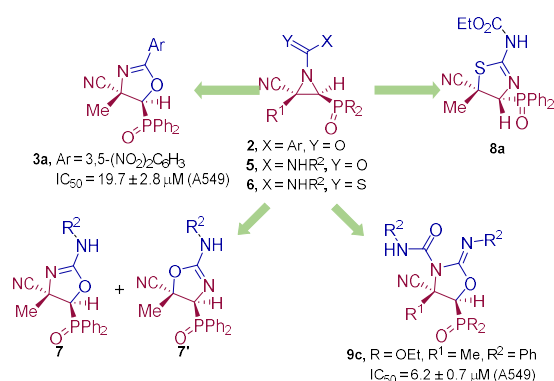
Manuscrito 2

Novel synthesis and antiproliferative activity of phosphorus substituted 4-cyanooxazolines, 2-aminocyanooxazolines, 2-iminocyanooxazolidines, and 2-aminocyanothiazolines by rearrangement of cyanoaziridines

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Artículo pendiente de ser enviado para su publicación.

Keywords: phosphorus substituted cyanoaziridines, 4-cyanooxazolines, 2-aminocyanooxazolines, 2-iminocyanooxazolidines, 2-aminocyanothiazolines, antiproliferative effect

Abstract:

Several phosphorus substituted *N*-acylated **2** and *N*-carbamoylated cyanoaziridines **5** were prepared conveniently in good to high yields. *N*-Acylated cyanoaziridines **2** have been used, after ring expansion, in an efficient synthesis of oxazoline derivatives **3** and in a completely regio-controlled reaction in the presence of NaI. Conversely, *N*-carbamoyl cyanoaziridines **5** react with NaI to afford a regioisomeric mixture of 2-aminocyanooxazolines **7**. Mild acidic conditions can be used for the isomerization of *N*-thiocarbamoyl cyanoaziridine **6a** into 2-aminocyanothiazoline derivative **8a** by using BF₃·OEt₂ as Lewis acid. Likewise, one pot reaction of NH-cyanoaziridines **1** with isocyanates afford 2-iminocyanooxazolidines **9** regioselectively. This synthetic methodology involve the addition of isocyanates to starting cyanoaziridines to afford *N*-carbamoyl cyanoaziridines **5**, which after ring opening, react with a second equivalent of isocyanate to give the final 2-imino cyanooxazolidines **9**. In addition, the cytotoxic effect on cell lines derived from human lung adenocarcinoma (A549) was also screened. 2-Iminooxazolidines **9** exhibited moderate activity against the A549 cell line *in vitro*. Furthermore, selectivity towards cancer cell (A549) over non-malignant cells (MCR-5) has been detected.

1. Introduction

DNA-modifying agents are a significant class of pharmaceuticals used in conventional chemotherapy. Aziridine-based cytostatic compounds, as powerful alkylating agents, have an inherent *in vivo* potency due to their ability to act as DNA cross-linking agents *via* aziridine ring opening.¹ Mitomycin C and many variants of this natural product has been well characterized for their anti-tumor activity based on nucleophilic ring opening of the three-membered nitrogen heterocycle, leading to alkylation of DNA.² Mitomycin C is a conventional DNA cross-linking agent that uses reductive activation of the aziridine moiety to form lethal DNA–DNA cross-links, as well as more often mono-alkylated DNA products.³ Founded on this knowledge, during the early 1970 Bicker^{4,5} developed a variety of 2-cyanoaziridine derivatives as potential carcinostatic agents. For instance, 2-cyanoaziridine-1-carboxamide (**1**, Figure 1) was active against PIE 2-3 sarcoma in Wistar rats, and it had low toxicity. Although, it showed weak antitumor activity in cell cultures, especially interesting was the remark that rather than dropping the number of

leukocytes, it increased them.⁵ However, in contrast with initial findings, these cianoaziridines showed no alkylating activity *in vitro* or *in vivo*.⁴ These results suggest that the cyano group reduces the reactivity required for alkylation of DNA bases and they may selectively react with sulfur moieties in biological thiols such as cysteine, depleting stores of cysteine and glutathione and subsequently allowing the accumulation of cellular reactive oxygen species (ROS).^{6,7,8} Preclinical studies evaluated the antitumor activity and mechanism of action of cyanoaziridine AMP423 **1b** (Figure 1).⁶ Other cyanoaziridines such as ciamexon (**2**) and azimexon (**3**)⁹ (Figure 1) were screened for antitumor activity, receiving brief clinical trials in the late 1980s. Iminopyrrolidone compound imexon (**4**), produced by cyclization of 2-cyanoaziridine-1-carboxamide (**1a**) in the presence of hydroxide ions,^{10,11} is an anti-neoplastic agent that increases oxidative stress in target and it has been extensively used due to its selective growth inhibitory effect against multiple myeloma.¹² The biological activity of imexon is narrowly associated to that of cyanoaziridines since imexon solutions in water slowly revert to **1a**. In 1999 Remers *et al.*¹³ reported the synthesis of a series of 2-cyanoaziridine-1-carboxamides, active against a variety of solid and hematological tumor cells in culture. *N*-Phenyl derivative **1c** (R = Ph, Figure 1) tested in human tumor cells, was found related to imexon in activity.

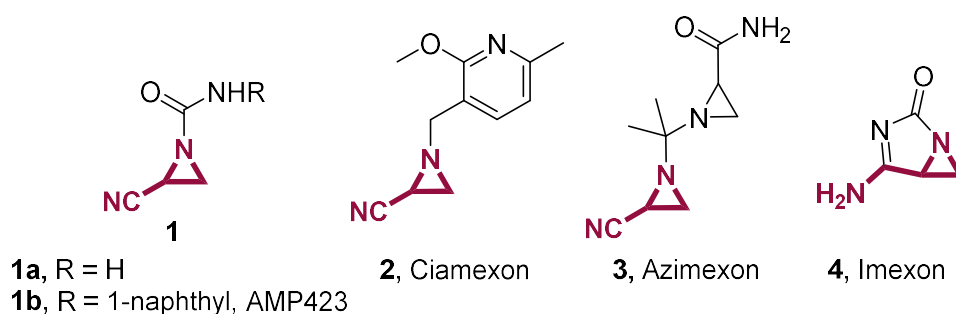


Figure 1. Representative examples of 2-cyanoaziridines as antitumor drugs.

Furthermore, ring-expansion of aziridines with isocyanates has revealed to be a useful pathway for the synthesis of a variety of five-membered nitrogen-containing compounds. For instance, KI,¹⁴ NaI,¹⁵ NiI₂,¹⁶ Pd-catalyzed¹⁷ reactions of aziridines with isocyanates, or even in the absence of catalysts,¹⁸ have been described. All these reactions yielded imidazolidin-2-ones or oxazolidin-2-imines, compounds with a great interest in medicinal and pharmaceutical chemistry since they represent classes of heterocyclic compounds with a broad variety of biological activities.¹⁹

In the last years we have been involved in the chemistry phosphorylated 2*H*-azirines for the preparation of α - or β -aminophosphonic acid derivatives,²⁰ pyrroles,²¹ oxazoles,²² 1*H*-benzo[*d*]azepines,²³ or hybrid molecules such as azirino[2,1-*b*]benzo[*e*][1,3]oxazines,²⁴ among others. Additionally, organophosphorus derivatives are fascinating compounds from a biological point of view, due that these substituents may regulate relevant biological functions modifying the reactivity of heterocyclic systems.²⁵ Recently, we have revealed a diastereoselective method for the preparation of phosphorus substituted cyanoaziridines by means of nucleophilic addition of TMSCN, as cyanide source, to the C–N double bond of 2*H*-azirines. These cyanoaziridines showed very good cytotoxic effect inhibiting the growth of human tumor cell line A549.²⁶

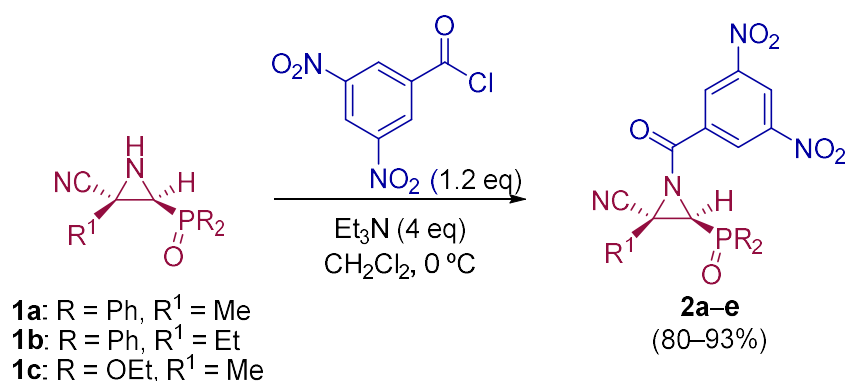
Following our earlier studies on the preparation of phosphorylated cyanoaziridines, here we wish to report the synthesis of structurally new phosphorus substituted *N*-(thio)carbamoyl cyanoaziridines by coupling of inactivated cyanoaziridines with iso(thio)cyanates. Moreover, ring expansion into 2-aminocyanooxazolines, 2-aminocyanothiazolines, or 2-iminocyanooxazolidines under nucleophilic or acidic conditions is also explored. We also focus on their biological activity proving all these new heterocycles for their antiproliferative effect on A549 human lung adenocarcinoma cells.

2. Results and discussion

2.1. Chemistry

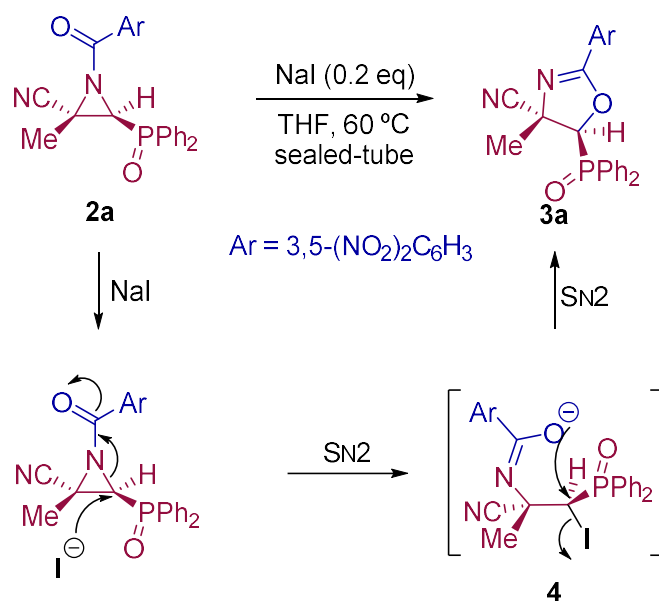
As a continuation of the studies on the synthesis and applications of activated aziridines through *N*-functionalization of inactivated cyanoaziridines,²⁶ and taking into account that *N*-acylaziridines are very important synthons in the development new ring opening aziridine reactions, we initially studied the *N*-acylation reaction of cyanoaziridine phosphine oxides **1a** (R = Ph, R¹ = Me) and **1b** (R = Ph, R¹ = Et), and phosphonates **1c** (R = OEt, R¹ = Me, Scheme 1). Thus, *N*-acylation of cyanoaziridines **1a–c** using 3,5-dinitrobenzoyl chloride in the presence of a base, such as Et₃N, and methylene chloride as the solvent, afforded *N*-acylated cyanoaziridines **2a–c** in good chemical yields (80–93%) (Scheme 1).

We next studied the isomerization reaction (Heine reaction) of the corresponding synthesized phosphorus containing *N*-acylated cyanoaziridines **2** (Scheme 2). For this purpose, we chose nucleophilic conditions,²⁷ and isomerization of benzoyl aziridine **2a** was accomplished



Scheme 1. Synthesis of *N*-acylated cyanoaziridines phosphine oxides and phosphonates **2**.

by the used of sodium iodide method. Indeed, when phosphorus substituted *N*-acylated cyanoaziridine **2a** reacted with 0.2 equivalents of NaI in THF at 60 °C in a sealed-tube; 4-cyanooxazoline derived from phosphine oxide **3a** was obtained in a regioselective way in 65% yield (Scheme 2).



Scheme 2. Ring expansion of cyanoaziridine **2a** to oxazoline **3a**.

Even though the iodide anion may attack both aziridine carbon atoms, and the regioselectivity will be determined by the stereoelectronic nature of substituents, several literature reports describe the aziridine ring opening through the less hindered position.²⁸ Bear

this in mind; the most reasonable mechanism may consider a first step where the iodide anion would attack at the less substituted C2 aziridine carbon in a regioselective manner, followed by the aziridine ring opening affording intermediate **4**. The iodide displacement in the former intermediate may afford phosphorus substituted 4-cyano oxazoline **3a** (Scheme 2).

Inactivated NH-aziridines are very stable compounds in basic conditions and may easily react with electrophiles. Next, we explored the *N*-functionalization of inactivated cyanoaziridines derived from phosphine oxides and phosphonates **1** with aromatic and aliphatic isocyanates for the preparation of functionalized *N*-aryl or *N*-alkylcarbamoyl cyanoaziridines.

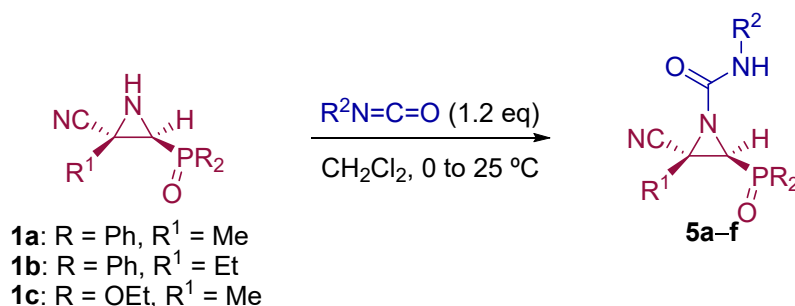
Therefore, the reaction of cyanoaziridines **1** with isocyanates was assessed. Thus, as outlined in Table 1, in an initial experiment the addition of phenyl isocyanate to cyanoaziridine phosphanoxide **1a** (R = Ph, R¹ = Me) was readily achieved in dichloromethane at room temperature. 1.2 Equivalents of the isocyanate component were used in order to ensure full conversion. Under these reactions conditions, the corresponding phosphorus substituted *N*-phenylcarbamoyl cyanoaziridine **5a** (R = R² = Ph, R¹ = Me) was obtained in 63% yield (Table 1, entry 1).

Some examples in the literature describe the use of Lewis acids as transition metal catalysts involving reactions of aziridines and isocyanates^{17a,29} Hence, we explore the reaction of phenyl isocyanate to cyanoaziridine **1a** in the presence of a Lewis acid. Better yields and less reaction times were observed for the synthesis of **5a** using catalytic amounts of Sc(OTf)₃ (20%) (see Table 1, entry 2). In the same way, phosphate derived cyanoaziridine **1c** (R = OEt, R¹ = Me) react with phenyl isocyanate in CH₂Cl₂ at room temperature, without catalyst, to give *N*-functionalized cyanoaziridine **5b** (R = OEt, R¹ = Me, R² = Ph) in very good chemical yield (Table 1, entry 3).

As illustrated in Table 1, this synthetic methodology is tolerant of a variety of functionalized isocyanates with varying substitutions. For instance, cyanoaziridines **1a** and **1c** reacted with *p*-tolyl isocyanate (R² = *p*-MeC₆H₄) to give *N-p*-tolylcarbamoyl cyanoaziridines **5c** and **5d**, respectively (Table 1, entries 4 and 5). Likewise, under the same reaction conditions cyanoaziridine **1a** reacted with *p*-toluenesulfonyl isocyanate. The crude product **5** (R = Ph, R¹ = Me, R² = *p*-MeC₆H₄SO₂) was observed by ¹H and ³¹P NMR, however, any further purification step through crystallization or chromatography gave to the hydrolyzed starting cyanoaziridine **1a**. Conversely, *N-p*-toluenesulfonylcarbamoyl cyanoaziridines **5e** (R = Ph, R¹ = Et, R² = *p*-MeC₆H₄SO₂, Table 1, entry 6) and **5f** (R = Ph, R¹ = Me, R² = *p*-MeC₆H₄SO₂, Table 1, entry 7) were obtained in

86 and 79% yield, respectively by means of treatment of corresponding cyanoaziridines **1b** and **1c** with *p*-toluenesulfonyl isocyanate.

Table 1. Phosphorus substituted *N*-aryl or *N*-alkylcarbamoyl cyanoaziridines **5** obtained.

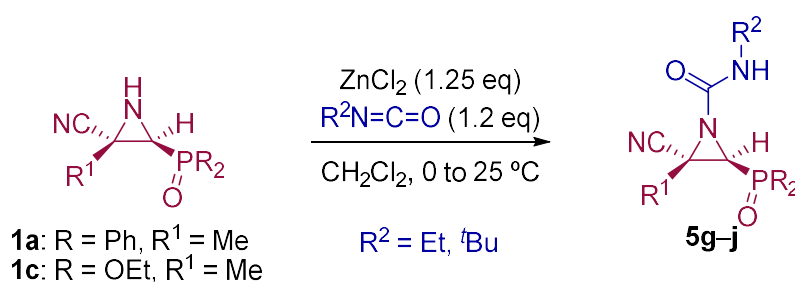


Entry	Compound	R	R ¹	R ²	Yield(%) ^[a]
1	5a	Ph	Me	Ph	63
2	5a	Ph	Me	Ph	71 ^[b]
3	5b	OEt	Me	Ph	98
4	5c	Ph	Me	<i>p</i> -MeC ₆ H ₄	82
5	5d	OEt	Me	<i>p</i> -MeC ₆ H ₄	80
6	5e	Ph	Et	<i>p</i> -MeC ₆ H ₄ -SO ₂	86
7	5f	OEt	Me	<i>p</i> -MeC ₆ H ₄ -SO ₂	79
8	5g	Ph	Me	Et	86 ^[c]
9	5h	OEt	Me	Et	59 ^[c]
10	5i	Ph	Me	^t Bu	73 ^[c]
11	5j	OEt	Me	^t Bu	75 ^[c]

^[a] Yield of isolated purified compounds **5**. ^[b] Reaction conditions: isocyanate (3 eq) and Sc(OTf)₃ (20% mol) in CH₂Cl₂ at 0 °C. ^[c] Reaction conditions: isocyanate (1.2 eq) and ZnCl₂ (1.25 eq) in CH₂Cl₂ at 25 °C. ^[d] Reaction conditions: isocyanate (2 eq) and ZnCl₂ (1.25 eq) in CH₂Cl₂ at 25 °C.

This process was extended to the reactivity of phosphorus substituted cyanoaziridines **1** with alkyl isocyanates, such as ethyl and *tert*-butyl isocyanate. The same reaction conditions used for aromatic isocyanates were employed for the reaction of **1a** with ethyl isocyanate. Nevertheless, no progress were observed on the formation of compound **5g**, and the starting cyanoaziridine **1a** was recovered instead. The presence of a Lewis acid could activate the aziridine ring making more reactive the reaction with alkyl isocyanates. Thus, the presence of 20% mol of Sc(OTf)₃ as Lewis acid, as described before for the synthesis of **5a**, gave only a 50% conversion of *N*-

ethylcarbamoil cyanoaziridine **5g** ($R = \text{Ph}$, $R^1 = \text{Me}$, $R^2 = \text{Et}$) after 24h reaction. However, when two equivalents of ethyl isocyanate react with cyanoaziridine **1a** in the presence of 1.25 equivalents of ZnCl_2 , in CH_2Cl_2 and at room temperature, *N*-functionalized cyanoaziridine **5g** was observed in 86% chemical yield (Scheme 3, Table 1, entry 8). Similarly, the addition of ethyl isocyanate to phosphonate derived cyanoaziridine **1c**, using ZnCl_2 as Lewis acid, gave to the formation of *N*-functionalized aziridine **5h** ($R = \text{OEt}$, $R^1 = \text{Me}$, $R^2 = \text{Et}$) (Scheme 3, Table 1, entry 9). Also the synthesis of *N*-*tert*-butylcarbamoil cyanoaziridines **5i** and **5j** (Scheme 3, Table 1, entries 10 and 11) was achieved in moderate yields, using *tert*-butyl isocyanate as electrophile and ZnCl_2 as Lewis acid.



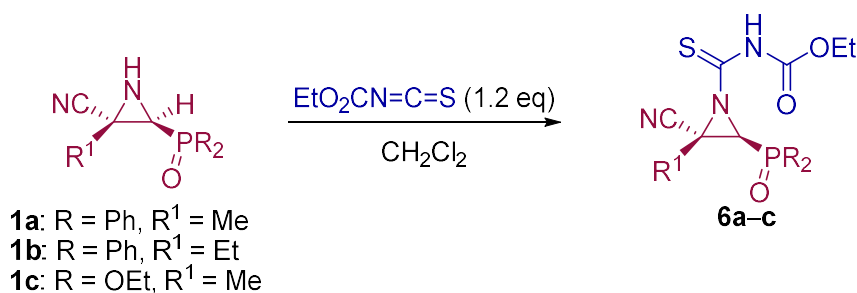
Scheme 3. Synthesis of *N*-alkylcarbamoil cyanoaziridines **5g-j**.

The synthetic procedure for the preparation of *N*-aryl or *N*-alkylcarbamoil cyanoaziridines **5** could be widened to the addition of isothiocyanates to cyanoaziridines **1** (Table 2). Under the same reaction conditions used for the preparation of derivatives **5**, phenyl isothiocyanate, *p*-methoxyphenyl isothiocyanate, or *p*-nitrophenyl isothiocyanate do not react with cyanoaziridine **1a** to yield compounds **6**. Also the use of different bases, such as Et_3N , pyridine, NaH , or CsCO_3 , as well as Lewis acid, such as ZnCl_2 or $\text{Sc}(\text{OTf})_3$, gave to similar results, the formation of *N*-functionalized cyanoaziridines **6** was not observed and the starting compound **1a** was recovered instead.

In order to achieve the synthesis of new derivatives **6** derived from isothiocyanates, we decided to use a more reactive isothiocyanate derivative. Thus, functionalized isothiocyanates with and electron-withdrawing group, such as ethoxycarbonyl isothiocyanate, reacted with cyanoaziridines derived from phosphine oxide **1a** and **1b** in CH_2Cl_2 at -30 °C (Method A). Under these reaction conditions, compounds **6a** and **6b** were attained in 71 and 85% chemical yield, respectively (Table 2, entries 1 and 3). Increasing the reaction temperature to 25 °C (Method B) gave to better yields and the *N*-functionalized cyanoaziridine **6a** was obtained in 80% yield (Table

3, entry 2). Similarly, phosphonate derived cyanoaziridine **1c** reacted with ethoxycarbonyl isothiocyanate in CH₂Cl₂ at room temperature to afford *N*-thiocarbamoyl cyanoaziridine **6c** (R = OEt, R¹ = Me) in good yields (Table 3, entry 4).

Table 2. Phosphorus substituted *N*-ethoxycarbonylthiocarbamoyl cyanoaziridines **6** obtained.



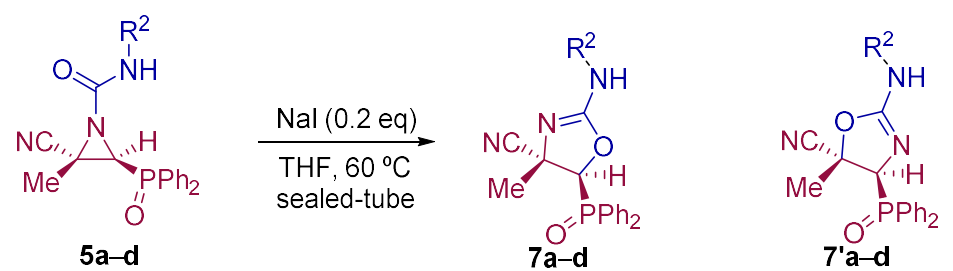
Entry	Compound	R	R ¹	Method ^[a]	Yield(%) ^[b]
1	6a	Ph	Me	A	71
2	6a	Ph	Me	B	80
3	6b	Ph	Et	A	85
4	6c	OEt	Me	B	86

^[a] Reaction conditions. Method A: isothiocyanate (1.2 eq) in CH₂Cl₂ at -30 °C. Method B: isothiocyanate (1.2 eq) in CH₂Cl₂ at 25 °C. ^[b] Yield of isolated purified compounds **6**.

Continuing with our interest in the synthesis of new 5-membered nitrogen containing heterocyclic compounds, next we explore the ring expansion of some *N*-carbamoyl cyanoaziridine derivatives **5**. To this end, and using the same reaction conditions as in the case of *N*-acyl cyanoaziridine **2**, *N*-arylcarbamoyl cyanoaziridines derived from phosphine oxide **5a** (R = R² = Ph) and **5c** (R = Ph, R² = *p*-MeC₆H₄) reacted with 0.2 equivalents of NaI at 60 °C in THF, allowing the preparation of oxazolines **7a** and **7c**, respectively (Table 3, entries 1 and 3). As evidenced by ¹H and ³¹P NMR, oxazolines **7** were obtained as a mixture of two regioisomers **7** and **7'**, in a 66:34 ratio for **7a**, while a 65:35 regioisomers ratio was observed for oxazoline **7c**. Oxazolines **7** were purified by flash-column chromatography, allowing the isolation of a single isomer, corresponding to the minor regioisomer, in the case of **7a+7a'**. However, in the case of regioisomeric oxazolines **7c+7c'** the separation of both regioisomers was not possible, and the same 65:35 regioisomers ratio were obtained after purification by flash-column chromatography. We also tested the ring expansion of *N*-arylcarbamoyl cyanoaziridines derived from phosphonate **5b** and **5d** under the optimal conditions. For instance, phosphonate derived

oxazolines **7b** (R = OEt, R² = Ph) and **7d** (R = OEt, R² = *p*-MeC₆H₄) were obtained as a regioisomeric mixtures after treatment of **5b** and **5d**, respectively, with 0.2 equivalents of NaI at 60 °C in THF (Table 3, entries 2 and 4). Conversely, the NaI catalyzed ring expansion of *N*-alkylcarbamoyl cyanoaziridines **5g** (R = Ph, R¹ = Me, R² = Et) and **5k** (R = Ph, R¹ = Et, R² = ^tBu) to the corresponding oxazolines was not observed, and *N*-functionalized cyanoaziridines **5g** and **5k** were recovered instead.

Table 3. Regioisomeric phosphorus substituted oxazolines derivatives **7** obtained.



Entry	Compound	R	R ²	Yield (%) ^[a]	Ratio ^[b]
1	7a+7'a	Ph	Ph	45	66:34
2	7b+7'b	OEt	Ph	69	64:36
3	7c+7'c	Ph	<i>p</i> -MeC ₆ H ₄	75	65:35
4	7d+7'd	OEt	<i>p</i> -MeC ₆ H ₄	54	66:34

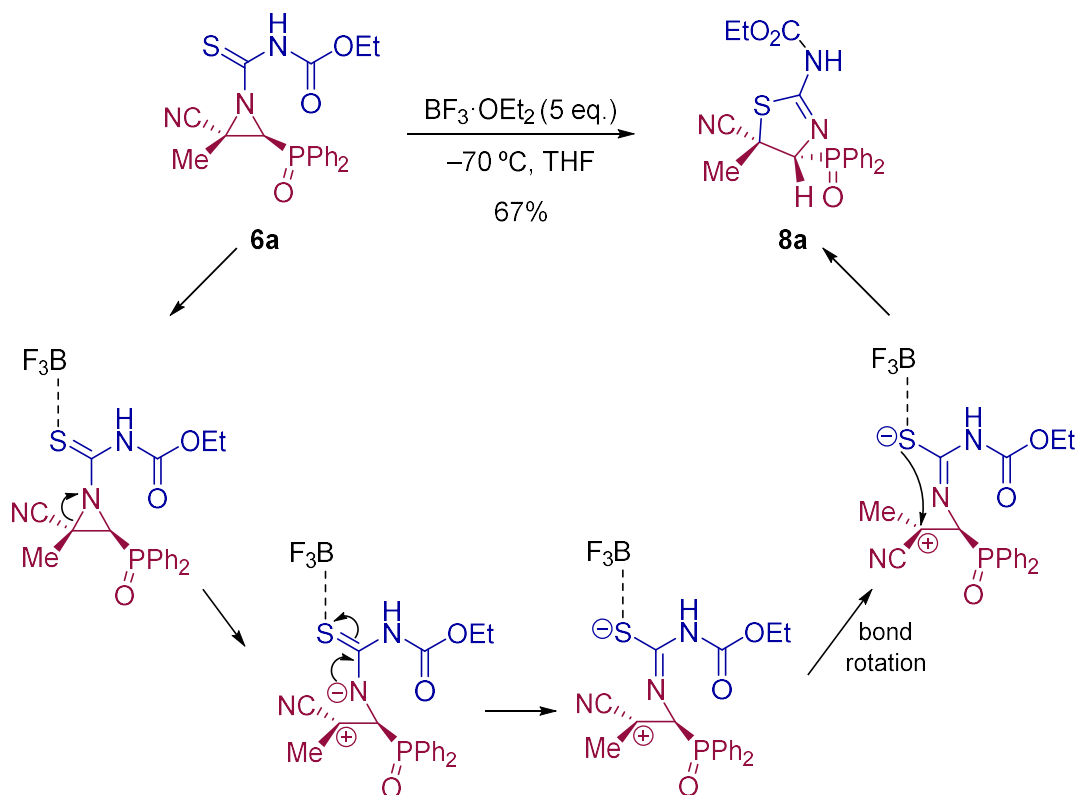
^[a] Yield of isolated purified oxazolines **7**. ^[b] Regioisomeric ratio was determined by crude ³¹P NMR spectra.

A rational mechanism for the formation of oxazoline derivatives **7** can be explained *via* initial aziridine ring opening in **5** by indiscriminately iodide attack to either aziridine carbons C2 or C3. Subsequent ring closure by iodide displacement would afford a mixture of regioisomeric oxazolines **7** and **7'**.

Several efforts has been carried out in for the synthesis of thiazoline derivatives starting from aziridines. It is known that 2-substituted oxazolines or imidazolines can be prepared by ring expansion of aziridines or benzoylated imidoyl aziridines, respectively,^{27b,d,30} through the Heine reaction. For instance, aziridines undergo ring expansion reactions into oxazolines with Lewis acids³¹ and, recently, based on these results, Tepe *et al.*³² describe the isomerization of aziridines to oxazolines using BF₃·OEt₂. For this reason, we explore the ring expansion of functionalized *N*-thiocarbamoyl cyanoaziridine **6a**.

Initially, we studied the aziridine ring opening under thermal conditions. Thus, *N*-thiocarbamoyl cyanoaziridine derived from phosphine oxide **6a** was heated under CHCl_3 refluxing. Under these conditions, no reaction was observed, and the unreacted starting substrate was recovered. Next, the Heine-type reaction was also studied under nucleophilic conditions by using NaI at 60°C in THF, and as in the previous case, no satisfactory results was attained.

Likewise, the conversion of aziridine to thiazoline under mild acidic conditions was examined. *N*-functionalized cyanoaziridine **6a** was treated with both Brønsted acids, such as *p*-toluenesulfonic acid (PTSA), and Lewis acids, such as ZnCl_2 or $\text{BF}_3\cdot\text{OEt}_2$. Only the use of $\text{BF}_3\cdot\text{OEt}_2$ gave to satisfactory results. Hence, when *N*-thiocarbamoyl cyanoaziridine **6a** reacted in the presence of 5 equivalents of $\text{BF}_3\cdot\text{OEt}_2$ at -70°C in THF, the formation of 2-aminothiazoline phosphine oxide **8a** was detected (Scheme 4). Spectroscopic data confirmed the isomerization of aziridine **6a** into 2-aminothiazoline **8a**. While the ^1H NMR spectrum of **6a** shows a signal for the methyl group at $\delta_{\text{H}} = 2.0$ ppm and the methine hydrogen resonates at $\delta_{\text{H}} = 3.8$ ppm as a well-



Scheme 4. Stereospecific ring expansion of *N*-thiocarbamoyl cyanoaziridine **6a** to 2-aminothiazoline **8a**.

resolved doublet ($^2J_{\text{PH}} = 20$ Hz), in 2-aminothiazolidine **8a** these signals appear at lower fields, $\delta_{\text{H}} = 2.11$ and 4.58 ppm as a singlet and a well-resolved doublet ($^2J_{\text{PH}} = 12.8$ Hz), respectively.

Since ^1H and ^{13}C NMR were not irrefutable to assign the regio- and stereochemistry of compound **8a**, conclusively, the X-ray diffraction analysis not only established the regiochemistry of compound **8a**, but also the *syn*-relationship between the cyano group at C3 position and the phosphorus moiety at C2 position of **8a** (Figure 2).

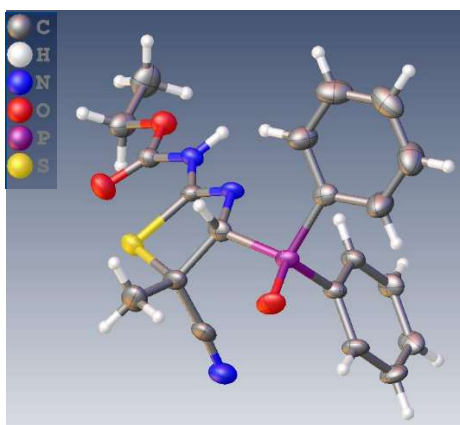


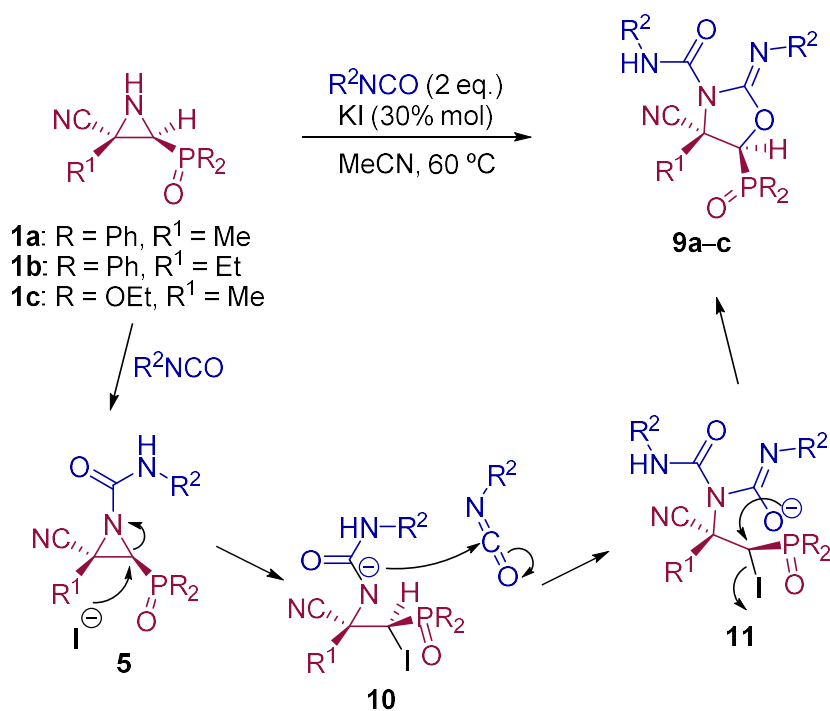
Figure 2. ORTEP diagram of compound **8a**.

A reasonable mechanism that would explain the formation of **8a** is exemplified in Scheme 4. First, $\text{BF}_3 \cdot \text{OEt}_2$ would coordinate with the sulfur atom of cyanoaziridine **6a** thus, assisting the ring opening reaction, through the N–C3 bond, with the concomitant generation of the most stable carbocation. The cationic intermediate coming from the aziridine with an *E*-stereochemistry would isomerize, and the ring closure would lead to 2-aminothiazoline **8a** as the only regio- and stereoisomer.

Continuing with our interest in the synthesis of new nitrogen-containing heterocyclic compounds, finally we examined the one pot reaction of cyanoaziridines **1** with isocyanates, in order to obtain new oxazoline derivatives.

For this purpose, phosphorus substituted cyanoaziridine **1b** ($\text{R} = \text{Ph}$, $\text{R}^1 = \text{Et}$) reacted with phenyl isocyanate in acetonitrile at 60 °C, giving to the formation of iminoxazolidine **9a** in low yield (Scheme 5). The addition of 2 equivalents of isocyanate led to **9a** in moderate yields (45%), whereas, when the reaction is examined in the presence of KI (30% mol) using 2 equivalents of phenyl isocyanate in acetonitrile at 60 °C, the corresponding iminoxazolidine **9a** was obtained

in 62% yield (Scheme 5, Table 4, entry 1). Similarly, cyanoaziridine **1a** (R = Ph, R¹ = Me) reacts with *p*-tolenesulfonyl isocyanate using the same reaction conditions, providing 55% yield of iminoxazolidine **9b** (Scheme 5, Table 4, entry 2). This synthetic methodology was extended to the use of cyanoaziridines derived from phosphonate. Thus, **1c** (R = OEt, R¹ = Me) reacts with phenyl isocyanate in the presence of KI in acetonitrile at 60 °C to give iminoxazolidine **9c** (Scheme 5, Table 4, entry 3).



Scheme 5. One pot procedure to the preparation of iminoxazolidines **9** through one pot reaction from cyanoaziridines **1**.

Table 4. Preparation of iminoxazolidines **9**.

Entry	Compound	R	R ¹	R ²	Yield (%) ^[a]
1	9a	Ph	Et	Ph	62
2	9b	Ph	Me	<i>p</i> -MeC ₆ H ₄	55
3	9d	OEt	Me	Ph	79

^[a] Yield of isolated purified compounds **9**.

Although high-resolution mass spectrometry (HRMS) of compounds **9** matches the theoretical mass for suggested structures, ^1H and ^{13}C NMR spectral data of compound **9** were not conclusive to assign the stereochemistry of the C4–C5 bond in iminoxazolidines **9**. Furthermore, all attempts to obtain crystals of iminoxazolidines **9** in order to confirm their suggested structure for X-ray measurement was unsuccessful.

Although the stereochemistry of compounds **9** remain to be known, a reliable mechanism for the formation of **9** would indicate the addition of an equivalent of isocyanate to cyanoaziridine **1** to afford the corresponding *N*-carbamoyl cyanoaziridines **5** (Scheme 5). Then, the regiospecific attack of iodide ion at the less substitute carbon atom (C2) in aziridines **5**, would give to the ring opening, affording intermediates **10**. The former intermediates would attack the carbon center of a second isocyanate equivalent followed by ring closure to yield iminoxazolidines **9**.

2.2. Biological results

In vitro cytotoxicity of our novel *N*-functionalized cyanoaziridines **2**, **5** and **6**, derived from phosphine oxide (R = Ph) and phosphonate (R = OEt), as well as, five-membered nitrogen-containing heterocycles **3a**, **7**, **8a** and **9** was evaluated by testing their antiproliferative activities against the human cancer cell line A549 (carcinomic human alveolar basal epithelial cells). In order to evaluate growth inhibition, cell counting kit (CCK-8) assay was applied. Cell proliferation inhibitory activities as IC_{50} values for all synthesized compounds and chemotherapeutic doxorubicin (DOX) are displayed in Table 5. Likewise, healthy lung cells, such as MRC-5 non-malignant lung fibroblasts were tested for studying selective cytotoxicity.³³

We first examined the nitrogen-substitution effect of the corresponding cyanoaziridines into their cytotoxicity against A549 cell lines. The best result was observed for *N*-acylated cyanoaziridine **2a** derived from phosphine oxide with an IC_{50} value of $22.9 \pm 1.9 \mu\text{M}$ (Table 5, entry 2). However, *N*-acylated cyanoaziridines derived from phosphine oxide **2b** and phosphonate **2c** (Table 5, entries 3 and 4), as well as *N*-carbamoyl cyanoaziridines **5a–k** (Table 5, entries 6–16) and *N*-thiocarbamoyl cyanoaziridines **6a–c** (Table 5, entries 17–19) did not exhibit any toxicity toward A549 cell line.

Concerning the new 5-membered nitrogen-containing heterocycles derived from ring expansion of *N*-functionalized cyanoaziridines against A549 cell line *in vitro*, oxazoline derivative **3a** showed a IC_{50} value of $19.7 \pm 2.8 \mu\text{M}$ (Table 6, entry 5). Conversely, neither regioisomeric

oxazolines **7+7'** (Table 5, entries 20–23) nor 2-aminothiazoline derivative **8a** (Table 5, entry 24) displayed any cytotoxicity against the same cell line. Finally, we studied the cytotoxicity effect in iminoxazolidines **9a–c** against A549 cell lines. All of them showed cytotoxicity. For instance, IC₅₀ values between 6.2 ± 0.7 and 16.4 ± 1.5 μM was observed, being iminoxazolidine **9c** (Table 5, entry 27) the most effective compound with an IC₅₀ value of 6.2 ± 0.7 μM.

Tabla 5. Antiproliferative activity of synthesized *N*-functionalized cyanoaziridines **2**, **5** and **6** and 5-membered nitrogen-containing heterocycles **3a**, **7**, **8a** and **9**.

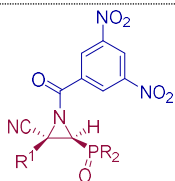
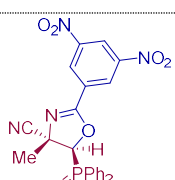
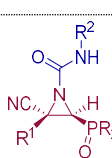
Entry	Comp.	R	R ¹	R ²	Cytotoxicity IC ₅₀ (μM) ^[a]	
					Lung A549	MRC-5
1	DOX				0.48 ± 0.017 ³⁴	>50 ³⁵
						
2	2a	Ph	Me	–	22.9 ± 1.9	>50
3	2b	Ph	Et	–	>50	>50
4	2c	OEt	Me	–	>50	>50
						
5	3a	Ph	Me	–	19.7 ± 2.8	>50
						
6	5a	Ph	Me	Ph	>50	>50
7	5b	OEt	Me	Ph	>50	>50
8	5c	Ph	Me	<i>p</i> -MeC ₆ H ₄	>50	>50

Tabla 5 (cont.). Antiproliferative activity of synthesized *N*-functionalized cyanoaziridines **2**, **5** and **6** and 5-membered nitrogen-containing heterocycles **3a**, **7**, **8a** and **9**.

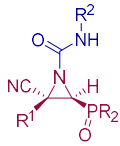
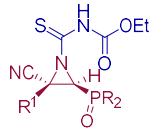
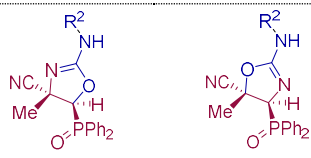
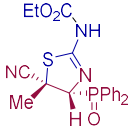

Entry	Comp.	R	R ¹	R ²	Cytotoxicity IC ₅₀ (μM) ^[a]	
					Lung A549	MRC-5
						
9	5d	OEt	Me	<i>p</i> -MeC ₆ H ₄	>50	>50
10	5e	Ph	Et	<i>p</i> -MeC ₆ H ₄ SO ₂	>50	>50
11	5f	OEt	Me	<i>p</i> -MeC ₆ H ₄ SO ₂	>50	>50
12	5g	Ph	Me	Et	>50	>50
13	5h	OEt	Me	Et	>50	>50
14	5i	Ph	Me	^t Bu	>50	>50
15	5j	OEt	Me	^t Bu	>50	>50
16	5k	Ph	Et	^t Bu	>50	>50
						
17	6a	Ph	Me	–	>50	>50
18	6b	Ph	Et	–	>50	>50
19	6c	OEt	Me	–	>50	>50
						
20	7a +7a'	Ph	Me	Ph	>50	>50
21	7b +7b'	OEt	Me	Ph	>50	>50
22	7c +7c'	Ph	Me	<i>p</i> -MeC ₆ H ₄	>50	>50
23	7d +7d'	OEt	Me	<i>p</i> -MeC ₆ H ₄	>50	>50

Table 5 (cont.). Antiproliferative activity of synthesized *N*-functionalized cyanoaziridines **2**, **5** and **6** and 5-membered nitrogen-containing heterocycles **3a**, **7**, **8a** and **9**.

Entry	Comp.	R	R ¹	R ²	Cytotoxicity IC ₅₀ (μM) ^[a]	
					Lung A549	MRC-5
						
24	8a	Ph	Me	–	>50	>50
						
25	9a	Ph	Et	Ph	16.4 ± 1.5	>50
26	9b	Ph	Me	<i>p</i> -MeC ₆ H ₄	14.8 ± 1.2	>50
27	9c	OEt	Me	Ph	6.2 ± 0.7	>50

^[a] The cytotoxicity IC₅₀ values listed are the concentrations corresponding to 50% growth inhibition.

Furthermore, MRC-5 non-malignant lung fibroblasts were tested for studying selective toxicity,³³ and none of the synthesized phosphorus substituted *N*-functionalized cyanoaziridines, 5-membered nitrogen-containing heterocycles or doxorubicin exhibited any toxicity toward MRC-5 cell line (see Table 5).

3. Conclusions

In summary, we herein report the activation of *NH*-cyanoaziridines with phosphorus substituents by *N*-acylation or *N*-carbamoylation reactions. As far as we know, this methodology constitutes the first example of *N*-functionalization of phosphorus substituted *NH*-cyanoaziridines with iso(thio)cyanates for the preparation of *N*-(thio)carbamoyl cyanoaziridines derived from phosphine oxide and phosphonate. Furthermore, we examine the ring expansion reaction of synthesized cyanoaziridines for the preparation of 5-membered nitrogen-containing heterocycles. For instance, *N*-benzoylated cyanoaziridine **2a** regioselectively isomerize (Heine type reaction) to 4-cyanooxazoline **3a** in the presence of NaI. However, when *N*-carbamoyl cyanoaziridines **5** react in the same reaction conditions, 2-aminocyanooxazolines **7** are achieved as a mixture of regioisomers. The Heine-type reaction of *N*-thiocarbamoyl cyanoaziridine **6a** was

performed using mild acidic conditions ($\text{BF}_3 \cdot \text{OEt}_2$ as Lewis acid), since neither thermal nor nucleophilic conditions gave to the corresponding 2-aminocyanothiazoline **8a**. We also examine the one pot reaction of cyanoaziridines **1** with isocyanates. The ring expansion reaction of *N*-carbamoyl cyanoaziridines **5**, *in situ* prepared by reaction of cyanoaziridines **1** with isocyanates, followed by the insertion of a second equivalent of isocyanate, afford 2-iminocyanooxazolidines **9** in a regioselective way. Additionally, we have evaluated the cytotoxic effect of all the synthesized compounds inhibiting the growth of human tumor cell lines A549 (carcinomic human alveolar basal epithelial cells). Within the *N*-acylated and *N*-(thio)carbamoylated cyanoaziridines, only compound **2a** exhibit moderate cytotoxic effect with an IC_{50} of $22.9 \pm 1.9 \mu\text{M}$. Concerning the 5-membered nitrogen-containing heterocycles, 4-cyanooxazoline **3a** show a IC_{50} value of $19.7 \pm 2.8 \mu\text{M}$, since 2-iminooxazolidines **9** exhibit IC_{50} values between 6.2 ± 0.7 and $16.4 \pm 1.5 \mu\text{M}$. In addition, cytotoxic effect of our compounds in healthy lung cells, fibroblast lung cells (MRC-5), seems not to present any effect.

4. Experimental section

4.1. Chemistry

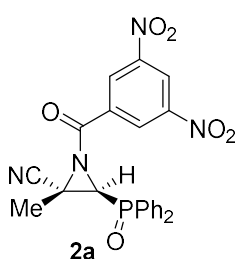
4.1.1. General experimental information

Solvents for extraction and chromatography were of technical grade. All solvents used in reactions were freshly distilled and dried over molecular sieves 4 \AA before use. All other solvents and reagents were obtained from commercial sources and recrystallized or distilled as necessary or used without further purification. All reactions were performed under an atmosphere of dry nitrogen. Melting points are uncorrected. IR spectra were measured on a Nicolet iS10 Termo Scientific spectrometer as neat solids. Absorbance frequencies are given at maximum of intensity in cm^{-1} . High-resolution mass spectra (HRMS) were obtained by positive-ion electrospray ionization (ESI) method with a time of flight Q-TOF system. Data are reported in the form m/z (intensity relative to base = 100). ^1H (300, 400 MHz), ^{13}C (75, 100 MHz) and ^{31}P NMR (120, 160 MHz) spectra were recorded on a Varian Unity Plus (300 MHz) or Bruker Avance 400 (400 MHz) spectrometers, respectively, in CDCl_3 or $\text{DMSO}-d_6$, as specified below. Chemical shifts (δ_{H}) are reported in parts per million (ppm) with the internal chloroform signal at 7.24 ppm or the internal DMSO signal at 2.50 ppm as standard for ^1H NMR. Chemical shifts (δ_{C} and δ_{P}) are reported in parts per million (ppm) with the internal chloroform signal at 77.0 ppm or the

internal DMSO signal at 39.5 ppm as standard for ^{13}C NMR; or the external H_3PO_4 (50%) signal at 0.0 ppm as standard for ^{31}P NMR. All coupling constants (J) values are given in Hz. ^{13}C -NMR spectra were recorded in a broad band decoupled mode, and peak assignments were supported by Distortionless Enhanced Polarization Transfer (DEPT). The data is being reported as (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, bs = broad singlet). Chromatographic purification was performed as flash chromatography using commercial grades of silica gel finer than 230 mesh with pressure. Analytical thin layer chromatography (TLC) was performed on precoated Merck silica gel 60 F₂₅₄ TLC aluminium plates, and spot visualized with UV light or permanganate stain. Cyanoaziridines **1** were prepared according to literature procedures.²⁶

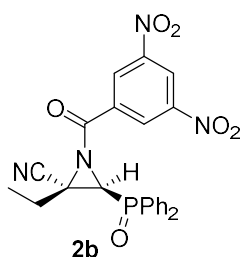
4.1.2. Experimental procedure and characterization data for compounds 2–9.

4.1.2.1. General procedure and spectral data for the addition of 3,5-dinitrobenzoyl chloride to functionalized cyanoaziridines. 3,5-Dinitrobenzoyl chloride (1.4 g, 6 mmol, 1.2 eq) and Et_3N (2.8 mL, 20 mmol, 4 eq) were added to a 0 °C solution of cyanoaziridine (5 mmol, 1 eq) in CH_2Cl_2 (25 mL). The reaction mixture was stirred at 0 °C until TLC showed the disappearance of starting cyanoaziridine. The crude product was washed three times with a saturated NaCl solution (15 mL) and water (15 mL) and extracted with CH_2Cl_2 (15 mL). The organic layers were dried over anhydrous MgSO_4 , filtered and concentrated to dryness in vacuum, and the resulting residue was purified by crystallization from Et_2O /pentane or washed with pentane.

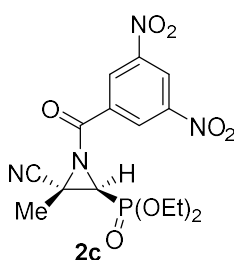


(E)-(2S*,3S*)-1-(3,5-dinitrobenzoyl)-3-(diphenylphosphoryl)-2-methylaziridine-2-carbonitrile (2a). (1.97 g, 83%) obtained as a grey solid from cyanoaziridine **1a** (1.41 g, 5 mmol) after 24 h at 0 °C as described in the general procedure. The crude product was purified by crystallization from Et_2O /pentane (50:50) to give the title compound **2a**. mp 120–122 °C; IR (neat) ν_{max} 3067, 2948, 2237, 1699, 1546, 1346, 1252, 1210, 1152 cm^{-1} ;

^1H NMR (300 MHz, CDCl_3) δ 9.23 (t, $^4J_{\text{HH}} = 2.0$ Hz, 1H, ArH), 9.08 (d, $^4J_{\text{HH}} = 2.0$ Hz, 2H, ArH), 7.85–7.48 (m, 10H, ArH), 3.86 (d, $^2J_{\text{PH}} = 20.5$ Hz, 1H, CH-P), 2.17 (s, 3H, CH_3) ppm; ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) δ 171.8 (d, $^3J_{\text{PC}} = 3.0$ Hz, C=O), 149.0 (C_{quat}), 135.1 (C_{quat}), 133.5, 133.5, 133.4, 133.3, 131.3, 131.1, 131.1, 131.0, 129.7, 129.5, 129.4, 129.2, 129.0 (C_{Ar}), 120.4 (C_{quat}), 116.6 (CN), 43.4 (d, $^1J_{\text{PC}} = 90.7$ Hz, CH-P), 37.0 (d, $^2J_{\text{PC}} = 2.5$ Hz, C_{quat}), 16.9 (CH_3) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 21.9 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{23}\text{H}_{18}\text{N}_4\text{O}_6\text{P}$ ($[\text{M}+\text{H}]^+$) 477.0964, found 477.0971.

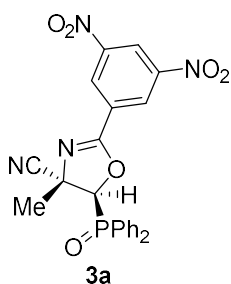


(E)-(2S*,3S*)-1-(3,5-dinitrobenzoyl)-3-(diphenylphosphoryl)-2-ethylaziridine-2-carbonitrile (2b). (1.95 g, 80%) obtained as a grey solid from cyanoaziridine **1b** (1.48 g, 5 mmol) after 24 h at 0 °C as described in the general procedure. The crude product was purified by crystallization from Et₂O/pentane (50:50) to give the title compound **2b**. mp 201–203 °C; IR (neat) ν_{\max} 3101, 2884, 2237, 1710, 1630, 1544, 1460, 1441, 1344, 1294, 1202 1147, 1122 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 9.24 (t, ⁴J_{HH} = 2.1 Hz, 1H, ArH), 9.11 (d, ⁴J_{HH} = 2.1 Hz, 2H, ArH), 7.90–7.47 (m, 10H, ArH), 3.90 (d, ²J_{PH} = 20.4 Hz, 1H, CH-P), 2.61–2.46 (m, 2H, CH₂), 1.08 (t, ³J_{HH} = 7.4 Hz, 3H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 172.4 (d, ³J_{PC} = 3.3 Hz, C=O), 148.9 (C_{quat}), 135.1 (C_{quat}), 133.5, 133.4, 133.3, 133.2, 131.2, 131.2, 131.1, 131.0, 129.6, 129.5, 129.3, 129.2, 129.1, 123.4 (C_{Ar}), 115.6 (CN), 43.6 (d, ¹J_{PC} = 90.6 Hz, CH-P), 43.2 (d, ²J_{PC} = 2.6 Hz, C_{quat}), 24.2 (CH₂), 10.5 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 21.4 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₄H₂₀N₄O₆P ([M+H]⁺) 491.1120, found 491.1135.



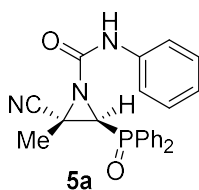
Diethyl (E)-((2S*,3S*)-3-cyano-1-(3,5-dinitrobenzoyl)-3-methylaziridin-2-yl)phosphonate (2c). (1.92 g, 93%) obtained as a brown oil from cyanoaziridine **1c** (1.09 g, 5 mmol) after 24 h at 0 °C as described in the general procedure. The crude product was washed with pentane to give the title compound **2c**. Rf: 0.5 (AcOEt); IR (neat) ν_{\max} 3112, 2984, 2246, 1710, 1627, 1552, 1344, 1294, 1255, 1041, 1022 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 9.25 (t, ⁴J_{HH} = 2.1 Hz, 1H, ArH), 9.05 (d, ⁴J_{HH} = 2.1 Hz, 2H, ArH), 4.30–4.17 (m, 4H, OCH₂CH₃), 3.37 (d, ²J_{PH} = 11.9 Hz, 1H, CH-P), 2.09 (s, 3H, CH₃), 1.42–1.34 (m, 6H, OCH₂CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 171.4 (d, ³J_{PC} = 5.0 Hz, C=O), 149.1 (C_{quat}), 134.8 (C_{quat}), 128.9, 123.4 (C_{Ar}), 116.3 (³J_{PC} = 2.1 Hz, CN), 64.2 (d, ²J_{PC} = 6.1 Hz, OCH₂), 63.7 (d, ²J_{PC} = 6.5 Hz, OCH₂) 40.4 (d, ¹J_{PC} = 202.3 Hz, CH-P), 35.8 (d, ²J_{PC} = 2.7 Hz, C_{quat}), 17.6 (CH₃), 16.5 (d, ³J_{PC} = 5.9 Hz, OCH₂CH₃), 16.4 (d, ³J_{PC} = 5.9 Hz, OCH₂CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 12.3 ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₅H₁₈N₄O₈P ([M+H]⁺) 413.0862, found 413.0857.

4.1.1.2. General procedure and spectral data for compound 3a. To a stirred solution of *N*-functionalized cyanoaziridine **2a** (5 mmol, 1 eq) in THF (15 mL), NaI (0.02 g, 1 mmol, 0.2 eq) was added dropwise. The mixture was heated at 60 °C for 24h until TLC showed the disappearance of starting cyanoaziridine. The reaction mixture was concentrated to dryness in vacuum to remove THF. The crude product was washed three times with water (15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layer was dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum. The crude product was purified by flash column chromatography.

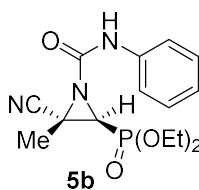


(E)-(4S*,5S*)-2-(3,5-dinitrophenyl)-5-(diphenylphosphoryl)-4-methyl-4,5-dihydrooxazole-4-carbonitrile (3a). (1.56 g, 65%) obtained as a yellow solid from cyanoaziridine **2a** (2.38 g, 5 mmol) after 24 h at heating in THF as described in the general procedure. The crude product was purified by flash column chromatography (SiO₂, AcOEt/hexane 25:75) to give the title compound **3a**. mp 129–131 °C; IR (neat) ν_{\max} 3103, 2934, 2243, 1655, 1546, 1438, 1352, 1197, 1119 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 9.19 (t, ⁴J_{HH} = 2.1 Hz, 1H, ArH), 8.99 (d, ⁴J_{HH} = 2.1 Hz, 2H, ArH), 7.98–7.52 (m, 10H, ArH), 5.60 (d, ²J_{PH} = 6.7 Hz, 1H, CH-P), 1.80 (s, 3H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 161.7 (d, ³J_{PC} = 5.6 Hz, C_{quat}), 148.8, 133.8, 133.8, 133.4, 133.3, 131.4, 131.3, 131.2, 131.1, 129.9, 129.7, 129.4, 129.3, 128.8, 122.2 (C_{Ar}), 119.8 (d, ³J_{PC} = 8.9 Hz, CN), 108.1 (C_{quat}), 83.2 (d, ¹J_{PC} = 76.7 Hz, CH-P), 68.3 (d, ²J_{PC} = 2.0 Hz, C_{quat}), 23.0 (d, ³J_{PC} = 5.2 Hz, CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 22.5 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₃H₁₈N₄O₆P ([M+H]⁺) 477.0964, found 477.0965.

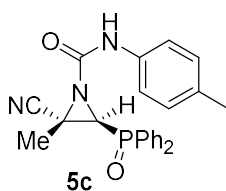
4.1.2.3. General procedures and spectral data for the addition of isocyanates to functionalized cyanoaziridines 1. Method A. To a 0 °C solution of cyanoaziridine (5 mmol, 1 eq) in CH₂Cl₂ (25 mL) was added dropwise the corresponding isocyanate (6 mmol, 1.2 eq). The reaction mixture was allowed to reach room temperature and stirred for 6–24h. The crude products were concentrated to dryness in vacuum and were purified by crystallization. **Method B.** To a 0 °C solution of cyanoaziridine (5 mmol, 1 eq) in CH₂Cl₂ (25 mL) was added dropwise phenyl isocyanate (15 mmol, 3 eq), and Sc(OTf)₃ (0.49 g, 1 mmol, 0.2 eq). The reaction mixture was stirred at 0 °C for 5 h until TLC showed the disappearance of starting cyanoaziridine. The reaction mixture was washed with water (3 x 15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layer was dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum. The crude product was purified by crystallization from Et₂O. **Method C.** To a solution of cyanoaziridine (5 mmol, 1 eq) in CH₂Cl₂ (25 mL) was added dropwise the corresponding aliphatic isocyanate (10 mmol, 2 eq), and ZnCl₂ (0.85 g, 6.25 mmol, 1.25 eq). The reaction mixture was stirred at room temperature for 5–48h until TLC showed the disappearance of starting cyanoaziridine. The reaction mixture was washed with saturated NH₄Cl (1 x 15 mL) and water (3 x 15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layers were dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum. The crude product was purified by crystallization.



(E)-(2S*,3S*)-2-cyano-3-(diphenylphosphoryl)-2-methyl-N-phenylaziridine-1-carboxamide (5a). (1.27 g, 63%) obtained as a white solid from cyanoaziridine **1a** (1.41 g, 5 mmol) and phenylisocyanate (0.65 mL, 6 mmol, 1.2 eq) as described in the general procedure (**method A**). The crude product was purified by crystallization from Et₂O to give the title compound **5a**. (1.42 g, 71%) obtained as an orange pale solid from cyanoaziridine **1a** (1.41 g, 5 mmol), phenylisocyanate (0.65 mL, 6 mmol, 1.2 eq) and Sc(OTf)₃ (1 mmol, 0.49 g) as described in the general procedure (**method B**). The crude product was purified by crystallization from Et₂O to give the title compound **5a**. mp 179–181 °C; IR (neat) ν_{\max} 3220, 3053, 2926, 2256, 1710, 1596, 1544, 1435, 1252, 1202, 1127 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 9.11 (bs, 1H, NH), 7.88–7.07 (m, 15H, ArH), 3.76 (d, ²J_{PH} = 23.1 Hz, 1H, CH-P), 1.92 (s, 3H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 157.2 (C=O), 137.8 (C_{quat}), 133.1, 133.1, 133.1, 133.0, 131.6, 131.4, 131.2, 131.2, 131.0, 130.1, 130.0, 129.5, 129.4, 129.3, 129.2, 129.1, 124.6, 120.1 (C_{Ar}), 117.1 (CN), 41.3 (d, ¹J_{PC} = 102.1 Hz, CH-P), 37.4 (C_{quat}), 17.9 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 23.4 ppm; ESI-HRMS (CI) m/z calcd. for C₂₃H₂₁N₃O₂P ([M+H]⁺) 402.1371, found 402.1374.

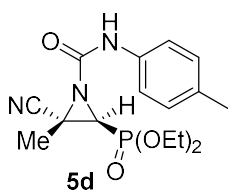


Diethyl (E)-((2S*,3S*)-3-cyano-3-methyl-1-(phenylcarbamoyl)aziridin-2-yl)phosphonate (5b). (1.65 g, 98%) obtained as a white solid from cyanoaziridine **1c** (1.09 g, 5 mmol) and phenylisocyanate (0.65 mL, 6 mmol, 1.2 eq) as described in the general procedure (**method A**). The crude product was purified by crystallization from CH₂Cl₂/pentane to give the title compound **5b**. mp 137–139 °C; IR (neat) ν_{\max} 3253, 3065, 2984, 2240, 1716, 1607, 1544, 1499, 1444, 1249, 1044, 1024 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.30 (bs, 1H, NH), 7.48–7.09 (m, 5H, ArH), 4.25–4.15 (m, 4H, OCH₂CH₃), 3.29 (d, ²J_{PH} = 13.6 Hz, 1H, CH-P), 1.92 (s, 3H, CH₃), 1.40–1.32 (m, 6H, OCH₂CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 156.6 (C=O), 137.3 (C_{quat}), 129.2, 124.8, 120.0 (C_{Ar}), 116.9 (CN), 63.9 (d, ²J_{PC} = 5.4 Hz, OCH₂), 63.3 (d, ²J_{PC} = 6.0 Hz, OCH₂) 38.7 (d, ¹J_{PC} = 207.4 Hz, CH-P), 36.0 (C_{quat}), 18.2 (CH₃), 16.6 (d, ³J_{PC} = 4.9 Hz, OCH₂CH₃), 16.5 (d, ³J_{PC} = 4.7 Hz, OCH₂CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 14.6 ppm; ESI-HRMS (CI) m/z calcd. for C₁₅H₂₁N₃O₄P ([M+H]⁺) 338.1270, found 338.1264.



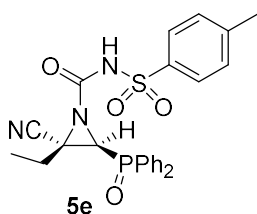
(E)-(2S*,3S*)-2-cyano-3-(diphenylphosphoryl)-2-methyl-N-(p-tolyl)aziridine-1-carboxamide (5c). (1.70 g, 82%) obtained as a yellow solid from cyanoaziridine **1a** (1.41 g, 5 mmol) and *p*-tolyl isocyanate (0.76 mL, 6 mmol, 1.2 eq) as described in the general procedure (**method A**). The crude product was purified by crystallization from Et₂O to give the title

compound **5c**. mp 193–195 °C; IR (neat) ν_{\max} 3237, 3056, 2917, 2254, 1710, 1599, 1546, 1539, 1408, 1249, 1197 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 9.17 (bs, 1H, NH), 7.86–7.06 (m, 14H, ArH), 3.76 (d, $^2J_{\text{PH}} = 23.0$ Hz, 1H, CH-P), 2.28 (s, 3H, CH_3), 1.92 (s, 3H, CH_3) ppm; ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) δ 157.1 (d, $^3J_{\text{PC}} = 4.2$ Hz, C=O), 135.2 (C_{quat}), 134.2 (C_{quat}), 133.0, 133.0, 131.3, 131.2, 131.1, 131.0, 129.5, 129.4, 129.3, 129.1, 120.2, 120.0, (C_{Ar}), 117.1 (CN), 41.2 (d, $^1J_{\text{PC}} = 100.5$ Hz, CH-P), 37.3 (d, $^2J_{\text{PC}} = 3.1$ Hz, C_{quat}), 21.0 (CH_3), 17.9 (CH_3) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 23.6 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{24}\text{H}_{23}\text{N}_3\text{O}_2\text{P}$ ($[\text{M}+\text{H}]^+$) 416.1528, found 416.1532.



Diethyl(E)-(2S*,3S*)-3-cyano-3-methyl-1-(p-tolylcarbamoyl)aziridin-2-yl)phosphonate (5d). (1.40 g, 80%) obtained as a pale yellow solid from cyanoaziridine **1c** (1.09 g, 5 mmol) and *p*-tolyl isocyanate (0.76 mL, 6 mmol, 1.2 eq) as described in the general procedure (**method A**). The crude product was purified by crystallization from CH_2Cl_2 /pentane to give the

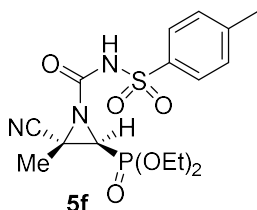
title compound **5d**. mp 140–142 °C; IR (neat) ν_{\max} 3256, 3040, 2987, 2240, 1710, 1607, 1538, 1444, 1516, 1321, 1247, 1039 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 8.15 (bs, 1H, NH), 7.33 (d, $^3J_{\text{HH}} = 7.9$ Hz, 2H, ArH), 7.09 (d, $^3J_{\text{HH}} = 7.9$ Hz, 2H, ArH), 4.24–4.14 (m, 4H, OCH_2CH_3), 3.28 (d, $^2J_{\text{PH}} = 13.5$ Hz, 1H, CH-P), 2.28 (s, 3H, CH_3), 1.91 (s, 3H, CH_3), 1.39–1.32 (m, 6H, OCH_2CH_3) ppm; ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) δ 156.6 (d, $^3J_{\text{PC}} = 5.9$ Hz, C=O), 134.9 (C_{quat}), 134.3 (C_{quat}), 129.5, 120.1 (C_{Ar}), 116.9 (CN), 63.9 (d, $^2J_{\text{PC}} = 6.2$ Hz, OCH_2), 63.2 (d, $^2J_{\text{PC}} = 6.5$ Hz, OCH_2), 38.4 (d, $^1J_{\text{PC}} = 206.8$ Hz, CH-P), 36.0 (d, $^2J_{\text{PC}} = 2.2$ Hz, C_{quat}), 20.9 (CH_3), 18.1 (CH_3), 16.5 (d, $^3J_{\text{PC}} = 6.0$ Hz, OCH_2CH_3), 16.4 (d, $^3J_{\text{PC}} = 6.0$ Hz, OCH_2CH_3) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 14.8 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{16}\text{H}_{23}\text{N}_3\text{O}_4\text{P}$ ($[\text{M}+\text{H}]^+$) 352.1426, found 352.1419.



(E)-(2S*,3S*)-2-cyano-3-(diphenylphosphoryl)-2-ethyl-N-tosylaziridine-1-carboxamide (5e). (2.12 g, 86%) obtained as a white solid from cyanoaziridine **1b** (1.48 g, 5 mmol) and *p*-toluenesulfonyl isocyanate (0.92 mL, 6 mmol, 1.2 eq) as described in the general procedure (**method A**). The crude product was purified by crystallization

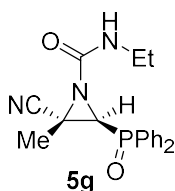
from Et_2O to give the title compound **5e**. mp 201–203 °C; IR (neat) ν_{\max} 3257, 2931, 2245, 1743, 1605, 1444, 1360, 1242, 1124, 1094 cm^{-1} ; ^1H NMR (400 MHz, MeOD) δ 7.92–7.38 (m, 14H, ArH), 3.39 (d, $^2J_{\text{PH}} = 23.4$ Hz, 1H, CH-P), 1.97 (m, 2H, CH_2), 2.44 (s, 3H, CH_3), 1.02 (t, $^3J_{\text{HH}} = 7.4$ Hz, 3H, CH_3) ppm; ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, MeOD) δ 153.5 (C=O), 146.0 (C_{quat}), 137.8 (C_{quat}), 134.2, 134.2, 133.9, 133.9, 132.2, 132.1, 132.1, 132.0, 130.5, 130.4, 130.3, 130.2, 130.0, 129.0 (C_{Ar}), 120.4 (CN), 39.5 (d, $^1J_{\text{PC}} = 101.6$ Hz, CH-P), 36.6 (C_{quat}), 25.2 (CH_2), 21.5 (CH_3), 11.1 (CH_3) ppm; ^{31}P NMR

(120 MHz, MeOD) δ 27.2 ppm; ESI-HRMS (CI) m/z calcd. for $C_{25}H_{25}N_3O_4PS$ ($[M+H]^+$) 494.1303, found 494.1292.



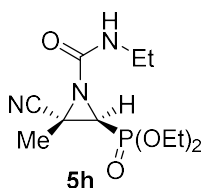
Diethyl(*E*)-(2*S,3*S**)-3-cyano-3-methyl-1-(tosylcarbamoyl)aziridin-2-yl)phosphonate (5f).** (1.64 g, 79%) obtained as a waxy white solid from cyanoaziridine **1c** (1.09 g, 5 mmol) and *p*-toluenesulfonyl isocyanate (0.92 mL, 6 mmol, 1.2 eq) as described in the general procedure (**method A**). The crude product was purified by crystallization from

CH_2Cl_2 /pentane to give the title compound **5f**. Rf: 0.4 (AcOEt). IR (neat) ν_{max} 3248, 3092, 2992, 2237, 1738, 1649, 1596, 1446, 1335, 1247, 1160, 1047, 1027 cm^{-1} ; 1H NMR (300 MHz, $CDCl_3$) δ 10.50 (bs, 1H, NH), 7.89–7.78 (m, 4H, ArH), 4.24–4.10 (m, 4H, OCH_2CH_3), 3.04 (d, $^2J_{PH} = 13.2$ Hz, 1H, CH-P), 2.38 (s, 3H, CH_3), 1.83 (s, 3H, CH_3), 1.37–1.28 (m, 6H, OCH_2CH_3) ppm; ^{13}C $\{^1H\}$ NMR (75 MHz, $CDCl_3$) δ 155.3 (d, $^3J_{PC} = 6.6$ Hz, C=O), 145.3 (C_{quat}), 135.1 (C_{quat}), 129.8, 129.7, 128.6, 128.1, 126.5 (C_{Ar}), 116.2 (CN), 64.3 (d, $^2J_{PC} = 6.0$ Hz, OCH_2), 63.8 (d, $^2J_{PC} = 6.4$ Hz, OCH_2), 38.9 (d, $^1J_{PC} = 206.6$ Hz, CH-P), 36.3 (d, $^2J_{PC} = 3.0$ Hz, C_{quat}), 21.8 (CH_3), 17.7 (CH_3), 16.5 (d, $^3J_{PC} = 6.3$ Hz, OCH_2CH_3), 16.4 (d, $^3J_{PC} = 6.2$ Hz, OCH_2CH_3) ppm; ^{31}P NMR (120 MHz) δ 13.3 ppm; ESI-HRMS (CI) m/z calcd. for $C_{16}H_{23}N_3O_6PS$ ($[M+H]^+$) 416.1045, found 416.1038.



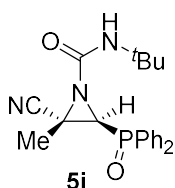
(*E*)-(2*S,3*S**)-2-cyano-3-(diphenylphosphoryl)-*N*-ethyl-2-methylaziridine-1-carboxamide (5g).** (1.51 g, 86%) obtained as a pale pink solid from cyanoaziridine **1a** (1.41 g, 5 mmol) and ethyl isocyanate (0.79 mL, 10 mmol, 2 eq) as described in the general procedure (**method C**). The crude product was purified by crystallization from Et_2O /pentane 50:50 to give the title compound

5g. mp 182–184 °C; IR (neat) ν_{max} 3253, 3053, 2976, 2240, 1702, 1544, 1438, 1283, 1258, 1191, 1124 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ 7.84–7.46 (m, 10H, ArH), 5.98 (t, $^3J_{HH} = 5.9$ Hz, 1H, NH), 3.65 (d, $^2J_{PH} = 22.1$ Hz, 1H, CH-P), 3.37–3.18 (m, 2H, $NHCH_2CH_3$), 1.85 (s, 3H, CH_3), 1.13 (t, $^3J_{HH} = 7.3$ Hz, $NHCH_2CH_3$) ppm; ^{13}C $\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 159.1 (d, $^3J_{PC} = 4.6$ Hz, C=O), 133.0, 132.9, 132.8, 132.8, 131.6, 131.4, 131.3, 131.1, 131.1, 130.6, 130.3, 129.3, 129.2, 129.1, 129.0 (C_{Ar}), 117.1 (CN), 41.6 (d, $^1J_{PC} = 100.1$ Hz, CH-P), 36.7 (d, $^2J_{PC} = 3.4$ Hz, C_{quat}), 36.4 ($NHCH_2CH_3$), 18.1 (CH_3), 15.1 ($NHCH_2CH_3$) ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ 23.1 ppm; ESI-HRMS (CI) m/z calcd. for $C_{19}H_{21}N_3O_2P$ ($[M+H]^+$) 354.1371, found 354.1372.



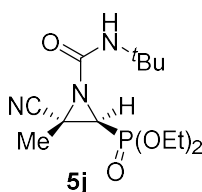
Diethyl(*E*)-(2*S,3*S**)-3-cyano-1-(ethylcarbamoyl)-3-methylaziridin-2-yl)phosphonate (5h).** (0.85 g, 59%) obtained as a waxy solid from cyanoaziridine **1c** (1.09 g, 5 mmol) and ethyl isocyanate (0.79 mL, 10 mmol, 2 eq) as described in the general procedure (**method C**). The crude product was purified by crystallization from Et₂O/pentane 50:50 to give the title

compound **5h**; Rf: 0.3 (AcOEt); IR (neat) ν_{\max} 3281, 3062, 2987, 2243, 1699, 1541, 1455, 1385, 1371, 1252, 1160, 1044, 1039 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 6.03 (bs, 1H, NH), 4.19–4.09 (m, 4H, OCH₂CH₃), 3.36–3.18 (m, 2H, NHCH₂CH₃), 3.12 (d, ²J_{PH} = 13.8 Hz, 1H, CH-P), 1.79 (s, 3H, CH₃), 1.34–1.28 (m, 6H, OCH₂CH₃) 1.13 (t, ³J_{HH} = 7.2 Hz, NHCH₂CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 158.8 (d, ³J_{PC} = 6.4 Hz, C=O), 116.9 (CN), 63.7 (d, ²J_{PC} = 5.7 Hz, OCH₂), 63.0 (d, ²J_{PC} = 6.2 Hz, OCH₂), 38.6 (d, ¹J_{PC} = 207.0 Hz, CH-P), 36.2 (NHCH₂CH₃), 35.3 (d, ²J_{PC} = 3.2 Hz, C_{quat}), 18.2 (CH₃), 16.4 (d, ³J_{PC} = 5.2 Hz, OCH₂CH₃), 16.4 (d, ³J_{PC} = 5.3 Hz, OCH₂CH₃), 15.0 (NHCH₂CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 15.1 ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₁H₂₁N₃O₄P ([M+H]⁺) 290.1270, found 290.1275.



(*E*)-(2*S,3*S**)-*N*-(*tert*-Butyl)-2-cyano-3-(diphenylphosphoryl)-2-methylaziridine-1-carboxamide (5i).** (1.39 g, 73%) obtained as a white solid from cyanoaziridine **1a** (1.41 g, 5 mmol) and *tert*-butyl isocyanate (1.14 mL, 10 mmol, 2 eq) as described in the general procedure (**method C**). The crude product was purified by crystallization from Et₂O/pentane 50:50 to give the

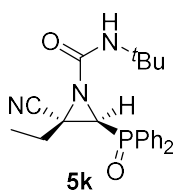
title compound **5i**. mp 166–168 °C; IR (neat) ν_{\max} 3259, 3056, 2976, 2237, 1707, 1541, 1452, 1441, 1369, 1285, 1208, 1127 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.82–7.48 (m, 10H, ArH), 5.53 (s, 1H, NH), 3.62 (d, ²J_{PH} = 22.1 Hz, 1H, CH-P), 1.85 (s, 3H, CH₃), 1.33 (s, 9H, C(CH₃)₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 157.4 (d, ³J_{PC} = 4.6 Hz, C=O), 132.9, 132.9, 132.8, 132.8, 131.9, 131.6, 131.4, 131.3, 131.1, 131.0, 130.5, 130.2, 129.3, 129.2, 129.1, 129.0 (C_{Ar}), 117.0 (CN), 52.1 (C(CH₃)₃), 41.2 (d, ¹J_{PC} = 99.9 Hz, CH-P), 36.5 (d, ²J_{PC} = 3.5 Hz, C_{quat}), 28.7 (C(CH₃)₃), 18.1 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 23.1 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₁H₂₅N₃O₂P ([M+H]⁺) 382.1684, found 382.1687.



Diethyl(*E*)-(2*S,3*S**)-1-(*tert*-butylcarbamoyl)-3-cyano-3-methylaziridin-2-yl)phosphonate (5j).** (1.19 g, 75%) obtained as a white solid from cyanoaziridine **1c** (1.09 g, 5 mmol) and *tert*-butyl isocyanate (1.14 mL, 10 mmol, 2 eq) as described in the general procedure (**method C**). The crude product was purified by flash column chromatography (SiO₂, AcOEt/hexane

20:80) to give the title compound **5j**. mp 96–98 °C; IR (neat) ν_{\max} 3284, 3051, 2979, 2246, 1705,

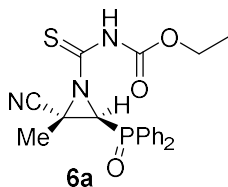
1538, 1477, 1457, 1394, 1369, 1260, 1160, 1038 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 5.45 (bs, 1H, NH), 4.21–4.11 (m, 4H, OCH_2CH_3), 3.13 (d, $^2J_{\text{PH}} = 13.6$ Hz, 1H, CH-P), 1.81 (s, 3H, CH_3), 1.36–1.30 (m, 15H, $\text{C}(\text{CH}_3)_3 + \text{OCH}_2\text{CH}_3$) ppm; ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) δ 157.1 (d, $^3J_{\text{PC}} = 6.7$ Hz, C=O), 116.9 (d, $^3J_{\text{PC}} = 2.4$ Hz, CN), 63.7 (d, $^2J_{\text{PC}} = 6.1$ Hz, OCH_2), 63.0 (d, $^2J_{\text{PC}} = 6.5$ Hz, OCH_2), 52.1 ($\text{C}(\text{CH}_3)_3$), 38.3 (d, $^1J_{\text{PC}} = 207.1$ Hz, CH-P), 35.3 (d, $^2J_{\text{PC}} = 3.4$ Hz, C_{quat}), 28.7 ($\text{C}(\text{CH}_3)_3$), 18.4 (CH_3), 16.5 (d, $^3J_{\text{PC}} = 5.9$ Hz, OCH_2CH_3), 16.4 (d, $^3J_{\text{PC}} = 5.6$ Hz, OCH_2CH_3) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 15.5 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{13}\text{H}_{24}\text{N}_3\text{NaO}_4\text{P}$ ($[\text{M}+\text{Na}]^+$) 340.1402, found 340.1400.



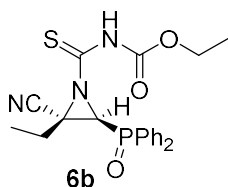
(E)-(2S*,3S*)-N-(tert-butyl)-2-cyano-3-(diphenylphosphoryl)-2-ethylaziridine-1-carboxamide (5k). (1.27 g, 64%) obtained as a white solid from cyanoaziridine **1b** (1.48 g, 5 mmol) and *tert*-butyl isocyanate (1.14 mL, 10 mmol, 2 eq) as described in the general procedure (**method C**). The crude product was purified by flash column chromatography (SiO_2 , AcOEt/hexane

50:50) to give the title compound **5k**. mp 197–199 °C; IR (neat) ν_{max} 3262, 2976, 2240, 1718, 1499, 1457, 1438, 1369, 1274, 1199, 1122 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.83–7.45 (m, 10H, ArH), 5.43 (s, 1H, NH), 3.67 (d, $^2J_{\text{PH}} = 22.2$ Hz, 1H, CH-P), 2.34–2.24 (m, 1H, CH_2CH_3), 2.01–1.99 (m, 1H, CH_2CH_3), 1.33 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.09 (t, $^3J_{\text{HH}} = 1.9$ Hz, 3H, CH_2CH_3) ppm; ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 157.5 (C=O), 132.9, 132.8, 131.3, 131.2, 131.1, 131.0, 129.3, 129.2, 129.1, 129.0 (C_{Ar}), 116.1 (CN), 52.0 ($\text{C}(\text{CH}_3)_3$), 42.3 (d, $^2J_{\text{PC}} = 1.7$ Hz, C_{quat}), 41.9 (d, $^1J_{\text{PC}} = 117.2$ Hz, CH-P), 28.7 ($\text{C}(\text{CH}_3)_3$), 24.6 (CH_2CH_3), 10.8 (CH_2CH_3) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 22.8 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{22}\text{H}_{27}\text{N}_3\text{O}_2\text{P}$ ($[\text{M}+\text{H}]^+$) 396.1841, found 396.1847.

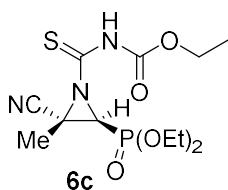
4.1.2.4. General procedures and spectral data for the addition of ethoxycarbonyl isothiocyanate to functionalized cyanoaziridines 1. Method A. To a -30 °C solution of cyanoaziridine **1** (5 mmol, 1 eq) in CH_2Cl_2 (25 mL) was added dropwise ethoxycarbonyl isothiocyanate (6 mmol, 1.2 eq). The reaction mixture was stirred at -30 °C for 6-8h until TLC showed the disappearance of starting cyanoaziridine. The crude products were concentrated to dryness in vacuum and were purified by crystallization. **Method B.** To a 0 °C solution of cyanoaziridine **1** (5 mmol, 1 eq) in CH_2Cl_2 (25 mL) was added dropwise ethoxycarbonyl isothiocyanate (6 mmol, 1.2 eq). The reaction mixture was allowed to reach room temperature and stirred 6-24h. The crude product was concentrated to dryness in vacuum and was purified by crystallization.



Ethyl-(E)-((2S*,3S*)-2-cyano-3-(diphenylphosphoryl)-2-methylaziridine-1-carbonothioyl)carbamate (6a). (1.47 g, 71%) obtained as an orange solid from cyanoaziridine **1a** (1.41 g, 5 mmol) and ethoxycarbonyl isothiocyanate (0.71 mL, 6 mmol, 1.2 eq) as described in the general procedure (**method A**). The crude product was purified by crystallization from Et₂O to give the title compound **6a**. (1.66 g, 80%) obtained as an orange solid from cyanoaziridine **1a** (1.41g, 5 mmol) and ethoxycarbonyl isothiocyanate (0.71 mL, 6 mmol, 1.2 eq) as described in the general procedure (**method B**). The crude product was purified by crystallization from Et₂O to give the title compound **6a**. mp 156–158 °C; IR (neat) ν_{\max} 3406, 3147, 2984, 2254, 1771, 1593, 1491, 1438, 1383, 1233, 1152, 1122, 1041 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 9.43 (bs, 1H, NH), 7.94–7.42 (m, 10H, ArH), 4.17 (q, ³J_{HH} = 7.1 Hz, 2H, OCH₂), 3.92 (d, ²J_{PH} = 20.0 Hz, 1H, CH-P), 1.97 (s, 3H, CH₃). 1.23 (t, ³J_{HH} = 7.1 Hz, 3H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 190.5 (d, ³J_{PC} = 4.6 Hz, C=S), 149.0 (C=O), 133.0, 133.0, 132.8, 132.8, 131.8, 131.7, 131.5, 131.2, 131.1, 130.9, 130.1, 129.4, 129.3, 129.1, 128.8, 128.6 (C_{Ar}), 116.5 (CN), 63.0 (CH₂), 48.8 (d, ¹J_{PC} = 94.5 Hz, CH-P), 42.4 (d, ²J_{PC} = 3.2 Hz, C_{quat}), 18.6 (CH₃), 14.2 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 22.7 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₀H₂₁N₃O₃PS ([M+H]⁺) 414.1041, found 414.1041.



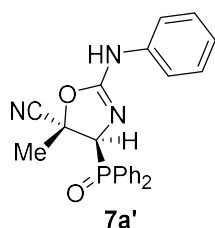
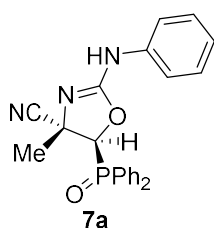
Ethyl-(E)-((2S*,3S*)-2-cyano-3-(diphenylphosphoryl)-2-ethylaziridine-1-carbonothioyl)carbamate (6b). (1.82 g, 85%) obtained as a pale yellow solid from cyanoaziridine **1b** (1.48 g, 5 mmol) and ethoxycarbonyl isothiocyanate (0.71 mL, 6 mmol, 1.2 eq) as described in the general procedure (**method A**). The crude product was purified by crystallization from Et₂O to give the title compound **6b**. mp 179–181 °C; IR (neat) ν_{\max} 3409, 3062, 2981, 2254, 2237, 1752, 1541, 1438, 1230, 1197, 1163, 1044 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.96 (bs, 1H, NH), 7.97–7.44 (m, 10H, ArH), 4.20 (q, ³J_{HH} = 7.2 Hz, 2H, OCH₂), 3.96 (d, ²J_{PH} = 20.2 Hz, 1H, CH-P), 2.59–2.50 (m, 1H, CH₂), 2.37–2.28 (m, 1H, CH₂). 1.27 (t, ³J_{HH} = 7.2 Hz, 3H, CH₃), 0.99 (t, ³J_{HH} = 7.5 Hz, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 190.1 (d, ³J_{PC} = 4.4 Hz, C=S), 149.0 (C=O), 133.1, 133.0, 132.8, 132.8, 131.8, 131.6, 131.5, 131.4, 131.3, 131.2, 130.1, 129.9, 129.3, 129.2, 128.9, 128.7 (C_{Ar}), 115.4 (CN), 63.2 (OCH₂), 48.8 (d, ¹J_{PC} = 94.4 Hz, CH-P), 48.0 (d, ²J_{PC} = 3.1 Hz, C_{quat}), 24.6 (CH₂), 14.3 (CH₃), 10.4 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 21.8 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₁H₂₃N₃O₃PS ([M+H]⁺) 428.1198, found 428.1204.



Ethyl-(*E*)-((2*S,3*S**)-2-cyano-3-(diethoxyphosphoryl)-2-methylaziridine-1-carbonothioyl)carbamate (**6c**).** (1.50 g, 86%) obtained as an orange solid from cyanoaziridine **1c** (1.09 g, 5 mmol) and ethoxycarbonyl isothiocyanate (0.71 mL, 6 mmol, 1.2 eq) as described in the general procedure (**method B**). The crude product was purified by crystallization from Et₂O/pentane to give the title compound **6c**. mp 116–118 °C; IR (neat) ν_{\max} 3395, 3162, 2987, 2251, 1774, 1491, 1385, 1241, 1158, 1041 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 9.03 (bs, 1H, NH), 4.30–4.14 (m, 6H, OCH₂CH₃ + CH₂CH₃), 3.41 (d, ²J_{PH} = 12.3 Hz, 1H, CH-P), 1.98 (s, 3H, CH₃). 1.38–1.27 (m, 9H, OCH₂CH₃ + CH₂CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 190.3 (d, ³J_{PC} = 6.7 Hz, C=S), 148.7 (C=O), 116.4 (d, ³J_{PC} = 2.3 Hz, CN), 64.2 (d, ²J_{PC} = 5.8 Hz, OCH₂CH₃), 63.3 (d, ²J_{PC} = 6.9 Hz, OCH₂CH₃), 63.2 (CH₂), 45.8 (d, ¹J_{PC} = 201.7 Hz, CH-P), 41.8 (d, ²J_{PC} = 3.2 Hz, C_{quat}), 18.6 (CH₃), 16.5 (d, ³J_{PC} = 4.0 Hz, OCH₂CH₃), 16.4 (d, ³J_{PC} = 6.0 Hz, OCH₂CH₃), 14.2 (CH₂CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 13.3 ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₂H₂₁N₃O₅PS ([M+H]⁺) 350.0940, found 350.0932.

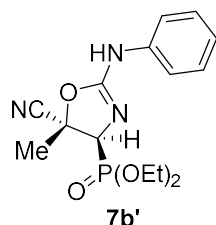
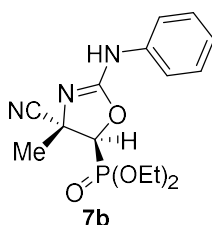
4.1.2.5. General procedure and spectral data for the reaction of NaI with *N*-carbamoyl cyanoaziridines **7**.

To a stirred solution of *N*-functionalized cyanoaziridine **5** (5 mmol, 1eq) in THF (15 mL), NaI (0.02 g, 1 mmol, 0.2 eq) was added dropwise. The mixture was heated at 60 °C for 24h until TLC showed the disappearance of starting cyanoaziridine. NaI was filtered through a sintered glass vacuum filtration funnel with celite and washed with THF. The filtrate was concentrated to dryness in vacuum and the resulting residue was purified by flash-column chromatography.



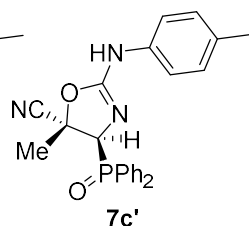
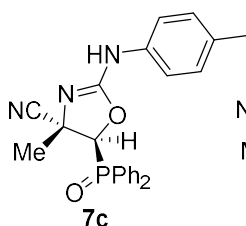
(4*S,5*S**)-5-(Diphenylphosphoryl)-4-methyl-2-(phenylamino)-4,5-dihydrooxazole-4-carbonitrile (**7a**) and (4*S**,5*S**)-4-(Diphenylphosphoryl)-5-methyl-2-(phenylamino)-4,5-dihydrooxazole-5-carbonitrile (**7a'**).** (0.90 g, 45%) obtained as a yellow solid from *N*-functionalized cyanoaziridine **5a** (2.00 g, 5 mmol) as described in the general procedure. The crude product was purified by flash column chromatography (SiO₂, AcOEt/hexane 50:50) to give the minor regioisomer. mp 117–119 °C; IR (neat) ν_{\max} 3420, 3057, 2981, 2237, 1674, 1438, 1402, 1199, 1122 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.95–7.20 (m, 15H, ArH), 4.23 (bs, 1H, NH), 3.00 (d, ²J_{PH} = 16.2 Hz, 1H, CH-P), 1.76 (s, 3H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 164.6 (d, ³J_{PC} = 5.9 Hz, C=N), 132.9, 132.1, 131.9, 131.2, 131.1, 130.4, 130.0, 129.4, 129.2, 129.1, 128.9, 126.8

(C_{Ar}), 127.6 (CN), 51.8 (d, ¹J_{PC} = 95.5 Hz, CH-P), 29.8 (C_{quat}), 12.2 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 21.0 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₃H₂₁N₃O₂P ([M+H]⁺) 402.1371, found 402.1368.



(E)-Diethyl (4S*,5S*)-4-cyano-4-methyl-2-(phenylamino)-4,5-dihydrooxazol-5-yl)phosphonate (7b) and (E)-Diethyl (4S*,5S*)-5-cyano-5-methyl-2-(phenylamino)-4,5-dihydrooxazol-4-yl)phosphonate (7b'). (1.16 g, 69%) obtained as a waxy white solid from

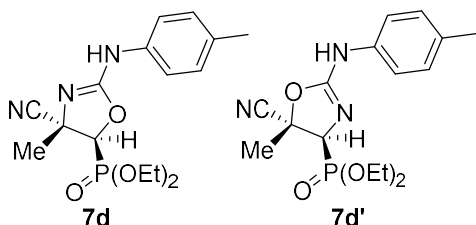
N-functionalized cyanoaziridine **5b** (1.68 g, 5 mmol) as described in the general procedure. The crude product was purified by flash column chromatography (SiO₂, AcOEt) to give the title compound **7** as a mixture of two regioisomers **7b + 7b'**. Rf: 0.1 (AcOEt); IR (neat) ν_{\max} 3370, 3061, 2990, 2240, 1666, 1499, 1402, 1255, 1158, 1052, 1019 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.10 (bs, 1H, NH), 7.52–7.12 (m, 11H, ArH + NH), 4.32–4.20 (m, 8H, OCH₂), 2.65 (d, ²J_{PH} = 11.0 Hz, 1H, CH-P)_{major}, 2.62 (d, ²J_{PH} = 11.1 Hz, 1H, CH-P)_{minor}, 1.89 (s, 3H, CH₃)_{major}, 1.84 (s, 3H, CH₃)_{minor}, 1.40–1.33 (m, 12H, OCH₂CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 166.5 (d, ³J_{PC} = 3.7 Hz, C=N)_{minor}, 161.8 (d, ³J_{PC} = 4.5 Hz, C=N)_{major}, 132.4 (C_{quat}), 130.9 (C_{quat}), 130.3, 129.7, 129.3, 128.7, 127.7, 127.0, 126.7 (C_{Ar}), 121.6 (CN)_{minor}, 121.5 (CN)_{major}, 64.3 (d, ²J_{PC} = 6.0 Hz, OCH₂)_{minor}, 64.0 (d, ²J_{PC} = 6.0 Hz, OCH₂)_{minor}, 63.3 (d, ²J_{PC} = 6.2 Hz, OCH₂)_{major}, 48.4 (d, ¹J_{PC} = 201.8 Hz, CH-P)_{major}, 47.8 (d, ¹J_{PC} = 201.3 Hz, CH-P)_{minor}, 47.5 (d, ²J_{PC} = 3.4 Hz C_{quat})_{major}, 46.0 (d, ²J_{PC} = 3.00 Hz C_{quat})_{minor}, 16.6, 16.5, 16.5, 16.4 (OCH₂CH₃), 12.5 (CH₃)_{major}, 12.1 (CH₃)_{minor} ppm; ³¹P NMR (120 MHz, CDCl₃) δ 13.8_{major}, 13.3_{minor} ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₅H₂₁N₃O₄P ([M+H]⁺) 338.1270, found 338.1254.



(E)-(4S*,5S*)-5-(Diphenylphosphoryl)-4-methyl-2-(p-tolylamino)-4,5-dihydrooxazole-4-carbonitrile (7c) and (E)-(4S*,5S*)-4-(Diphenylphosphoryl)-5-methyl-2-(p-tolylamino)-4,5-dihydrooxazole-5-carbonitrile (7c'). (1.56 g, 75%) obtained as a white solid from

N-functionalized cyanoaziridine **5c** (2.07 g, 5 mmol) as described in the general procedure. The crude product was purified by flash column chromatography (AcOEt) to give the title compound **7** as a mixture of two regioisomers **7c + 7c'**. mp 128–130 °C; IR (neat) ν_{\max} 3425, 3059, 2959, 2235, 1617, 1516, 1438, 1405, 1197, 1119 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.01 (bs, 1H, NH), 7.96–7.01 (m, 29H, ArH + NH), 2.98 (d, ²J_{PH} = 15.5 Hz, 1H, CH-P)_{minor}, 2.98 (d, ²J_{PH} = 16.7 Hz, 1H, CH-P)_{major}, 2.37 (s, 3H, CH₃)_{major}, 2.33 (s, 3H, CH₃)_{minor}, 1.80 (s, 3H, CH₃)_{major}, 1.68 (s, 3H, CH₃)_{minor} ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 166.8 (C=N)_{minor}, 162.1 (C=N)_{major}, 140.0 (C_{quat}), 138.8 (C_{quat}), 132.9, 132.0, 131.9, 131.3, 131.2, 131.1, 131.0, 130.4, 130.0, 129.7, 129.3, 129.2,

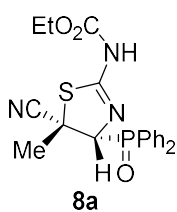
129.0, 128.9, 128.2, 127.5, 126.8, 126.4 (C_{Ar}), 121.5 (d, $^3J_{PC} = 3.8$ Hz, CN), 51.5 (d, $^1J_{PC} = 95.9$ Hz, CH-P)_{major}, 51.6 (d, $^1J_{PC} = 95.2$ Hz, CH-P)_{minor}, 48.8 (d, $^2J_{PC} = 3.1$ Hz C_{quat})_{major}, 47.1 (C_{quat}), 21.3 (CH_3), 12.3 (CH_3)_{major}, 11.9 (CH_3)_{minor} ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ 21.0_{major}, 20.8_{minor} ppm; ESI-HRMS (CI) m/z calcd. for $C_{24}H_{23}N_3O_2P$ ($[M+H]^+$) 416.1528, found 416.1544.



(E)-Diethyl (4S*,5S*)-4-cyano-4-methyl-2-(p-tolylamino)-4,5-dihydroazazol-5-yl)phosphonate (7d) and (E)-Diethyl (4S*,5S*)-5-cyano-5-methyl-2-(p-tolylamino)-4,5-dihydroazazol-5-yl)phosphonate (7d'). (0.95 g, 54%) obtained as a waxy white solid

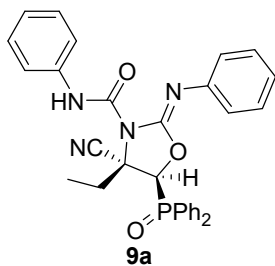
from *N*-functionalized cyanoaziridine **5d** (1.75 g, 5 mmol) as described in the general procedure. The crude product was purified by flash column chromatography (SiO_2 , AcOEt) to give the title compound **7d + 7d'** as a mixture of two regioisomers. Rf: 0.1 (AcOEt); IR (neat) ν_{max} 3356, 3037, 2987, 2237, 1671, 1516, 1444, 1402, 1321, 1260, 1160, 1127, 1049, 1024 cm^{-1} ; 1H NMR (300 MHz, $CDCl_3$) δ 8.05 (bs, 1H, NH)_{minor}, 7.49 (bs, 1H, NH)_{major}, 7.31–7.00 (m, 8H, ArH), 4.33–4.21 (m, 8H, OCH_2), 2.62 (d, $^2J_{PH} = 11.1$ Hz, 1H, CH-P)_{major}, 2.60 (d, $^2J_{PH} = 11.3$ Hz, 1H, CH-P)_{minor}, 2.37 (s, 3H, CH_3)_{major}, 2.33 (s, 3H, CH_3)_{minor}, 1.89 (s, 3H, CH_3)_{major}, 1.85 (s, 3H, CH_3)_{minor}, 1.41–1.33 (m, 12H, OCH_2CH_3) ppm; ^{13}C $\{^1H\}$ NMR (75 MHz, $CDCl_3$) δ 166.7 (d, $^3J_{PC} = 3.0$ Hz, C=N)_{minor}, 162.1 (d, $^1J_{PC} = 3.7$ Hz, C=N)_{major}, 140.0 (C_{quat}), 138.9 (C_{quat}), 131.0, 130.6, 130.1, 129.7, 128.2, 126.9, 126.5 (C_{Ar}), 127.6_{major} (CN), 121.3 (d, $^3J_{PC} = 5.5$ Hz, CN), 64.3_{minor} (d, $^2J_{PC} = 6.0$ Hz, OCH_2), 64.0 (d, $^2J_{PC} = 5.9$ Hz, OCH_2)_{minor}, 63.3 (d, $^2J_{PC} = 6.2$ Hz, OCH_2)_{major}, 48.4 (d, $^1J_{PC} = 201.8$ Hz, CH-P)_{major}, 47.9 (d, $^1J_{PC} = 201.8$ Hz, CH-P)_{minor}, 47.5 (d, $^2J_{PC} = 3.2$ Hz, C_{quat})_{major}, 46.0 (C_{quat})_{minor}, 21.3 (CH_3), 16.6, 16.6, 16.5, 16.5 (OCH_2CH_3), 12.5 (CH_3)_{major}, 12.2 (CH_3)_{minor} ppm; ^{31}P NMR (120 MHz, $CDCl_3$) δ 13.9_{major}, 13.4_{minor} ppm; ESI-HRMS (CI) m/z calcd. for $C_{16}H_{23}N_3O_4P$ ($[M+H]^+$) 352.1426, found 352.1426.

4.1.2.6. General procedure and spectral data for compound 8a. To a -70 °C solution of **6a** (5 mmol, 1 eq) in THF (25 mL) was added dropwise boron trifluoride diethyl etherate (25 mmol, 5 eq). The reaction mixture was stirred at -70 °C for 24h until TLC showed the disappearance of starting *N*-functionalized cyanoaziridine. The crude product was washed three times with water (15 mL) and extracted with CH_2Cl_2 (15 mL). The organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated to dryness in vacuum, and the resulting residue was purified by flash column chromatography.



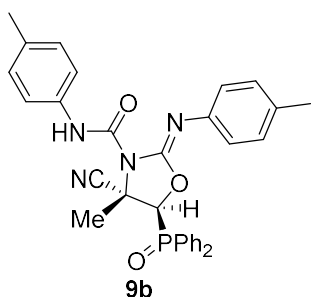
Ethyl-(Z)-((4R*,5S*)-5-cyano-4-(diphenylphosphoryl)-5-methyl-4,5-dihydrothiazol-2-yl)carbamate (8a). (1.39 g, 67%) obtained as a pale yellow solid from *N*-functionalized cyanoaziridine **6a** (2.06 g, 5 mmol) and boron trifluoride diethyl etherate (3.1 mL, 25 mmol, 5 eq) as described in the general procedure. The crude product was purified by flash column chromatography (SiO₂, AcOEt/hexane 50:50) to give the title compound **8a**. mp 208–210 °C; IR (neat) ν_{\max} 3145, 3065, 2937, 2254, 2232, 1724, 1624, 1507, 1438, 1244, 1174, 1113, 1094 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.06–7.40 (m, 11H, ArH + NH), 4.58 (d, ²J_{PH} = 12.8 Hz, 1H, CH-P), 4.22–4.16 (m, 2H, CH₂), 2.11 (s, 3H, CH₃), 1.25 (t, ³J_{HH} = 7.1 Hz, 3H, CH₂CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 157.1 (d, ³J_{PC} = 19.4 Hz, C=N), 152.8 (C=O), 134.0 (C_{quat}), 133.3, 133.2, 132.7, 132.6, 132.5, 132.4, 132.4, 131.3, 131.2, 129.2, 128.9, 128.7, 128.2, 128.1, 127.9 (C_{Ar}), 118.8 (d, ³J_{PC} = 7.1 Hz, CN), 76.6 (d, ¹J_{PC} = 82.7 Hz, CH-P), 63.1 (CH₂), 52.0 (d, ²J_{PC} = 1.3 Hz, C_{quat}), 26.4 (CH₃), 14.4 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 26.1 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₀H₂₁N₃O₃PS ([M+H]⁺) 414.1041, found 414.1046.

4.1.2.7. General procedure and spectral data for the reaction of cyanoaziridines 1 and isocyanates in the presence of KI. A mixture of corresponding isocyanate (2 mmol, 2 eq), KI (0.25 g, 0.3 mmol) and cyanoaziridine (1 mmol, 1 eq) in CH₃CN (15 mL) was stirred at 60 °C until TLC showed the disappearance of starting cyanoaziridine. After reaction completion, the solvent was evaporated under reduced pressure and the crude product was washed three times with water (15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layers were dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum, and the resulting residue was purified by crystallization or by flash column chromatography.



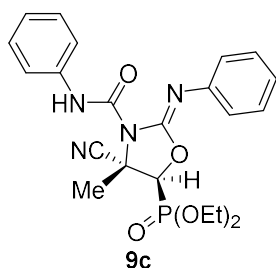
(E)-((4S*,5S*)-4-cyano-5-(diphenylphosphoryl)-4-ethyl-N-phenyl-2-(phenylimino)oxazolidine-3-carboxamide (9a). (1.66 g, 62%) obtained as a white solid from cyanoaziridine **5a** (1.48 g, 5 mmol) and phenyl isocyanate (1.09 mL, 10 mmol, 2 eq) as described in the general procedure. The crude product was purified by flash column chromatography (SiO₂, AcOEt/hexane 40:60) to give the title compound **9a**. mp 208–210 °C; IR (neat) ν_{\max} 3267, 3062, 2973, 2246, 1779, 1560, 1502, 1435, 1383, 1316, 1260, 1225, 1119 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.01–7.07 (m, 21H, ArH + NH), 3.32 (d, ²J_{PH} = 18.7 Hz, 1H, CH-P), 2.46–2.27 (m, 2H, CH₂), 1.05 (t, ³J_{HH} = 7.4 Hz, 3H, CH₂CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 164.6 (d, ³J_{PC} = 6.0 Hz, C=N), 157.9 (C=O), 138.0 (C_{quat}), 133.1, 133.0, 132.2, 132.1, 131.9, 131.8, 131.7, 129.0, 126.9, 124.2, 119.2 (C_{Ar}), 121.6 (CN), 55.1 (d, ¹J_{PC}

= 96.5 Hz, CH-P), 54.5 (C_{quat}), 18.9 (CH₂), 10.3 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 24.1 ppm; ESI-HRMS (CI) *m/z* calcd. for C₃₁H₂₈N₄O₃P ([M+H]⁺) 535.1899, found 535.1899.



(E)-((4S*,5S*)-4-cyano-5-(diphenylphosphoryl)-4-methyl-N-(p-tolyl)-2-(p-tolylimino)oxazolidine-3-carboxamide (9b). (1.51 g, 55%) obtained as a white solid from cyanoaziridine **5c** (1.41 g, 5 mmol) and *p*-tolyl isocyanate (1.26 mL, 10 mmol, 2 eq) as described in the general procedure. The crude product was purified by flash column chromatography (SiO₂, AcOEt/hexane 50:50) to give the title compound **9b**. mp 220–222 °C; IR (neat) ν_{\max} 3259, 3040, 2926,

2246, 1777, 1613, 1596, 1513, 1438, 1391, 1241, 1172, 1191, 1155 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.03–7.01(m, 19H, ArH + NH), 3.21 (d, ²J_{PH} = 18.2 Hz, 1H, CH-P), 2.33 (s, 3H, CH₃), 2.29 (s, 3H, CH₃), 1.91 (s, 3H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 164.6 (d, ³J_{PC} = 6.0 Hz, C=N), 158.1 (C=O), 139.4 (C_{quat}), 135.4 (C_{quat}), 133.8 (C_{quat}), 133.1, 132.9, 132.9, 132.0, 131.9, 131.8, 130.0, 129.7, 129.5, 129.3, 129.2, 128.9, 126.7, 120.9, 120.3, 119.2, (C_{Ar} + CN), 53.9 (d, ¹J_{PC} = 92.0 Hz, CH-P), 49.1 (d, ²J_{PC} = 3.8 Hz, C_{quat}), 21.3 (CH₃), 21.0 (CH₃), 12.6 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 23.7_{major}, 25.0_{minor} ppm; ESI-HRMS (CI) *m/z* calcd. for C₃₂H₃₀N₄O₃P ([M+H]⁺) 549.2056, found 549.2056.



Diethyl (E)-((4S*,5S*)-4-cyano-4-methyl-3-(phenylcarbamoyl)-2-(phenylimino)oxazolidin-5-yl)phosphonate (9c). (1.80 g, 79%) obtained as a pale yellow solid from cyanoaziridine **1c** (1.09 g, 5 mmol) and phenyl isocyanate (1.09 mL, 10 mmol, 2 eq) as described in the general procedure. The crude product was purified by crystallization from Et₂O to give the title compound **9c**. mp 169–171 °C; IR (neat) ν_{\max}

3231, 3140, 2985, 2249, 1716, 1610, 1580, 1499, 1488, 1313, 1249, 1194, 1052, 1024 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.47–7.02 (m, 10H, ArH), 6.58 (d, 1H, NH), 4.19–4.02 (m, 4H, OCH₂), 3.98 (d, ²J_{PH} = 12.4 Hz, 1H, CH-P), 1.98 (s, 3H, CH₃), 1.24–1.16 (m, 3H, OCH₂CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 177.3 (d, ²J_{PC} = 15.9 Hz, C=N), 158.5 (C=O), 151.2 (d, ³J_{PC} = 12.4 Hz, C_{quat}), 145.2 (C_{quat}), 134.5 (C_{quat}), 129.5, 129.3, 129.1, 128.6, 128.5, 127.9, 127.7, 124.4, 123.7, 123.5 (C_{Ar}), 120.1 (CN), 80.3 (d, ²J_{PC} = 6.1 Hz, C_{quat}), 64.4 (d, ²J_{PC} = 6.8 Hz, OCH₂), 63.7 (d, ²J_{PC} = 6.7 Hz, OCH₂), 52.0 (d, ¹J_{PC} = 156.6 Hz, CH-P), 19.2 (CH₃), 16.3 (d, ²J_{PC} = 5.8 Hz, OCH₂CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 15.1_{major}, 15.5_{minor} ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₂H₂₆N₄O₅P ([M+H]⁺) 457.1641, found 457.1629.

4.2. Biology

4.2.1. Materials

Reagents and solvents were used as purchased without further purification. All stock solutions of the investigated compounds were prepared by dissolving the powdered materials in appropriate amounts of DMSO. The final concentration of DMSO never exceeded 10% (v/v) in reactions. The stock solution was stored at 5 °C until it was used.

4.2.2. Cytotoxicity assays.

Cells were cultured according to the supplier's instructions. Cells were seeded in 96-well plates at a density of $2-4 \times 10^3$ cells per well and incubated overnight in 0.1 mL of media supplied with 10% Fetal Bovine Serum (Lonza) in 5% CO₂ incubator at 37 °C. On day 2, drugs were added and samples were incubated for 48 hours. After treatment, 10 µL of cell counting kit-8 was added into each well for additional 2 hours incubation at 37 °C. The absorbance of each well was determined by an Automatic Elisa Reader System at 450 nm wavelength.

Acknowledgments

The authors thank Ministerio de Ciencia, Innovación y Universidades (MCIU), Agencia Estatal de Investigación (AEI) y Fondo Europeo de Desarrollo Regional (FEDER) (RTI2018-101818-B-I00, UE) and Gobierno Vasco (GV), (IT 992-16) for supporting this work. We also thank technical and human support provided by SGIker (UPV/EHU/ ERDF, EU).

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Material Complementario – Manuscrito 2

Novel synthesis and antiproliferative activity of phosphorus substituted 4-cyanooxazolines, 2-aminocyanooxazolines, 2-iminocyanooxazolidines, and 2-aminocyanothiazolines by rearrangement of cyanoaziridines

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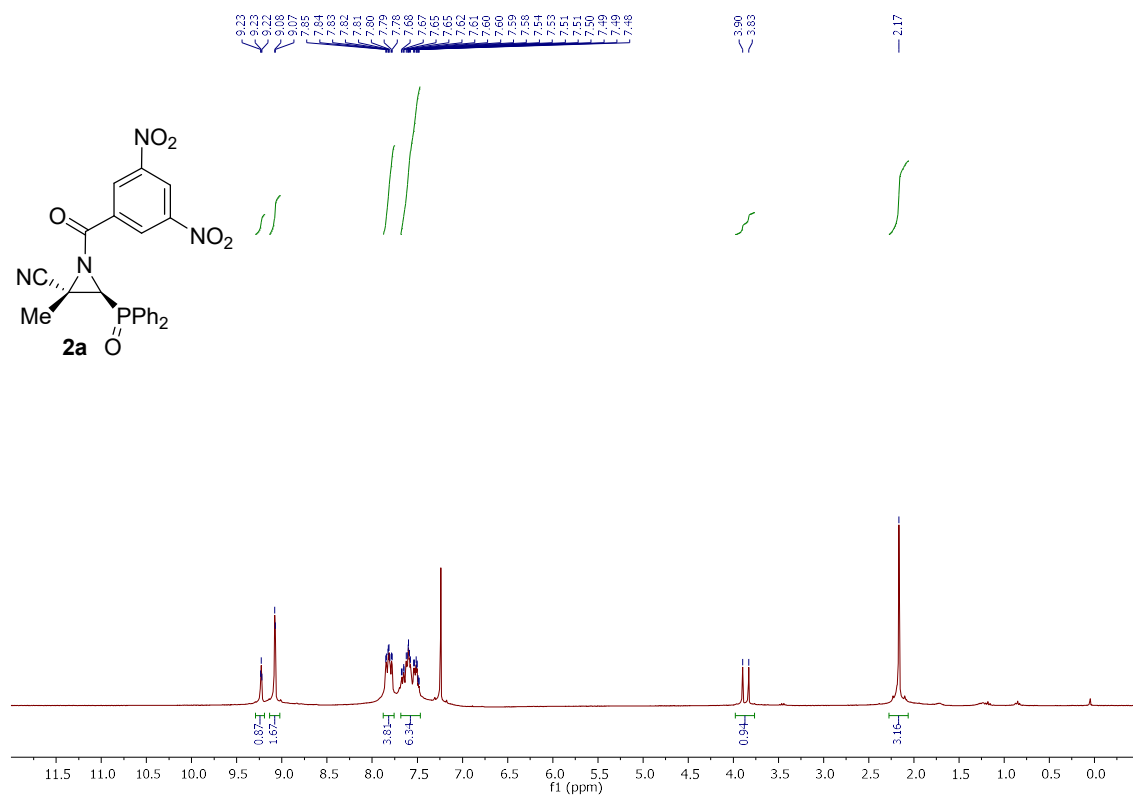
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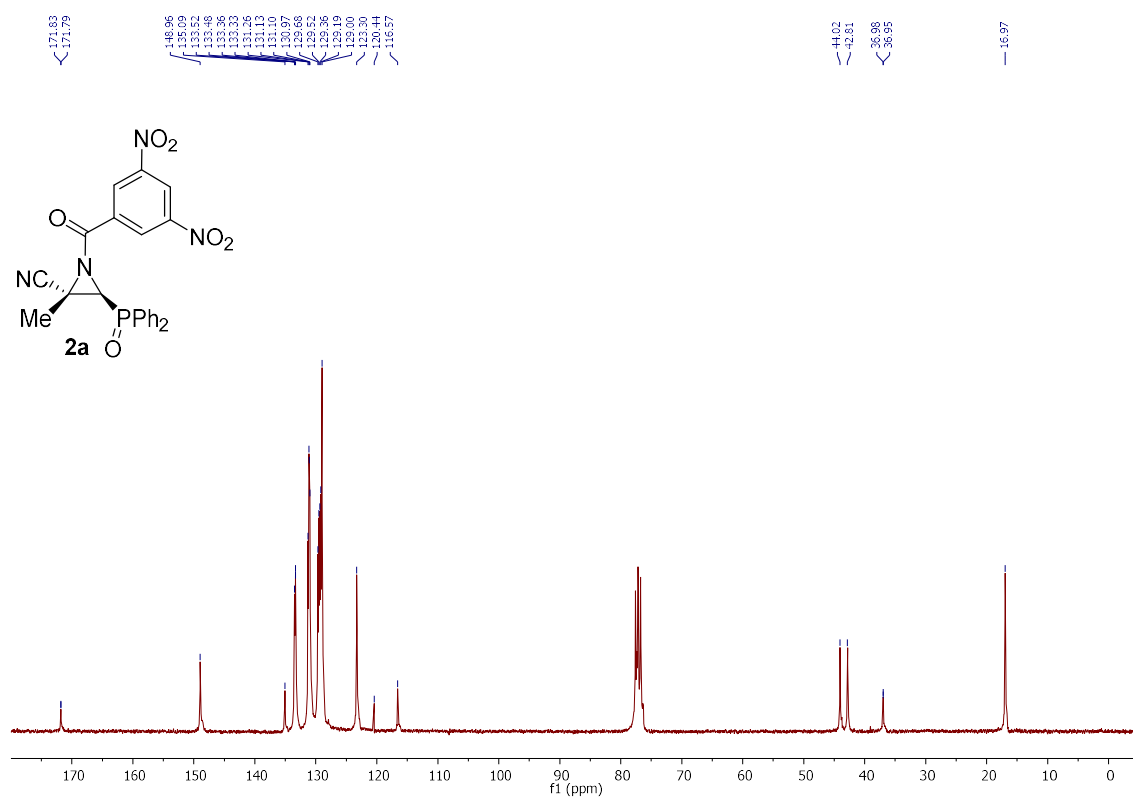
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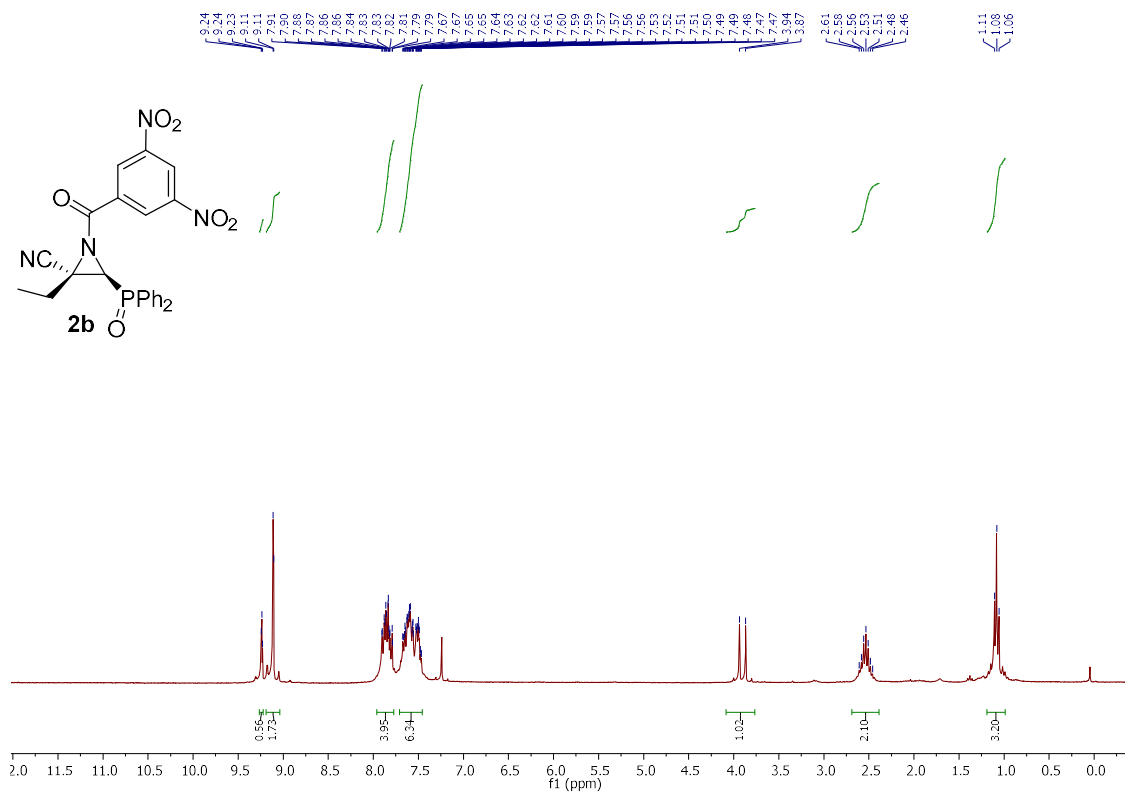
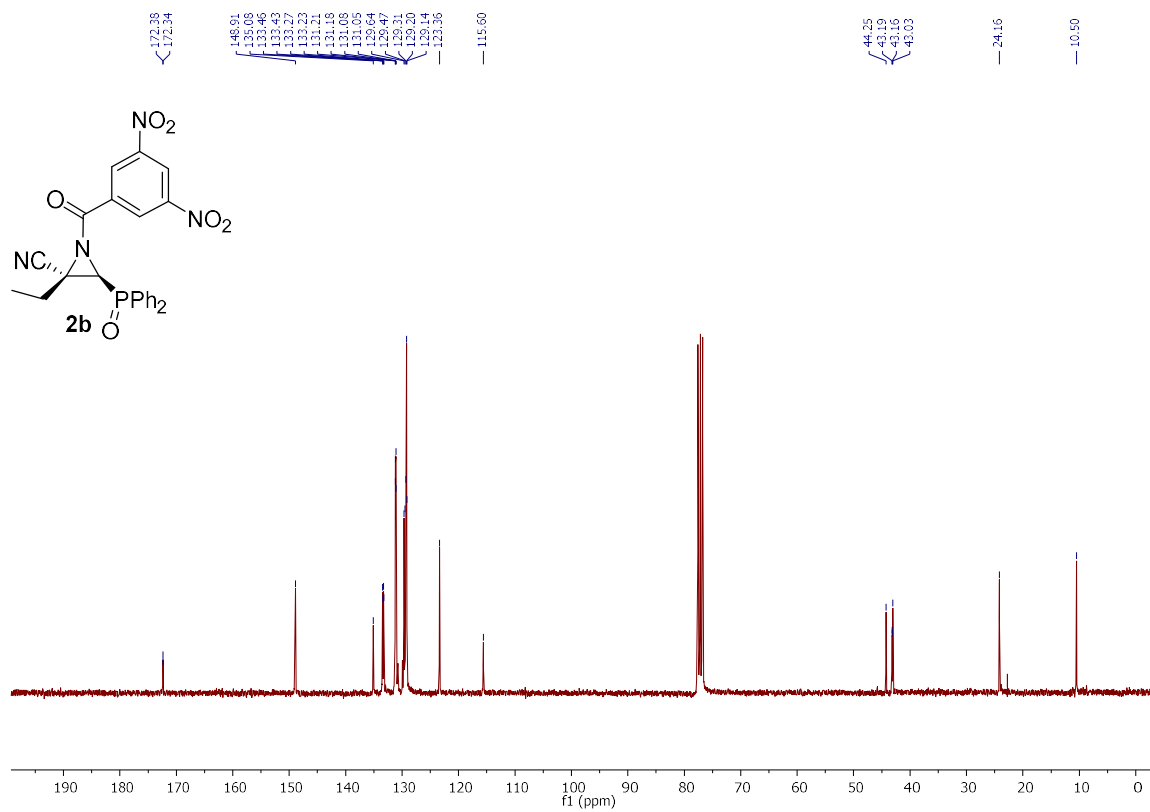
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¹ H and ¹³ C NMR spectra of cyanooxazoline 3a	Page 253
¹ H and ¹³ C NMR spectra of <i>N</i> -functionalized cyanoaziridines 5a–k	Page 254
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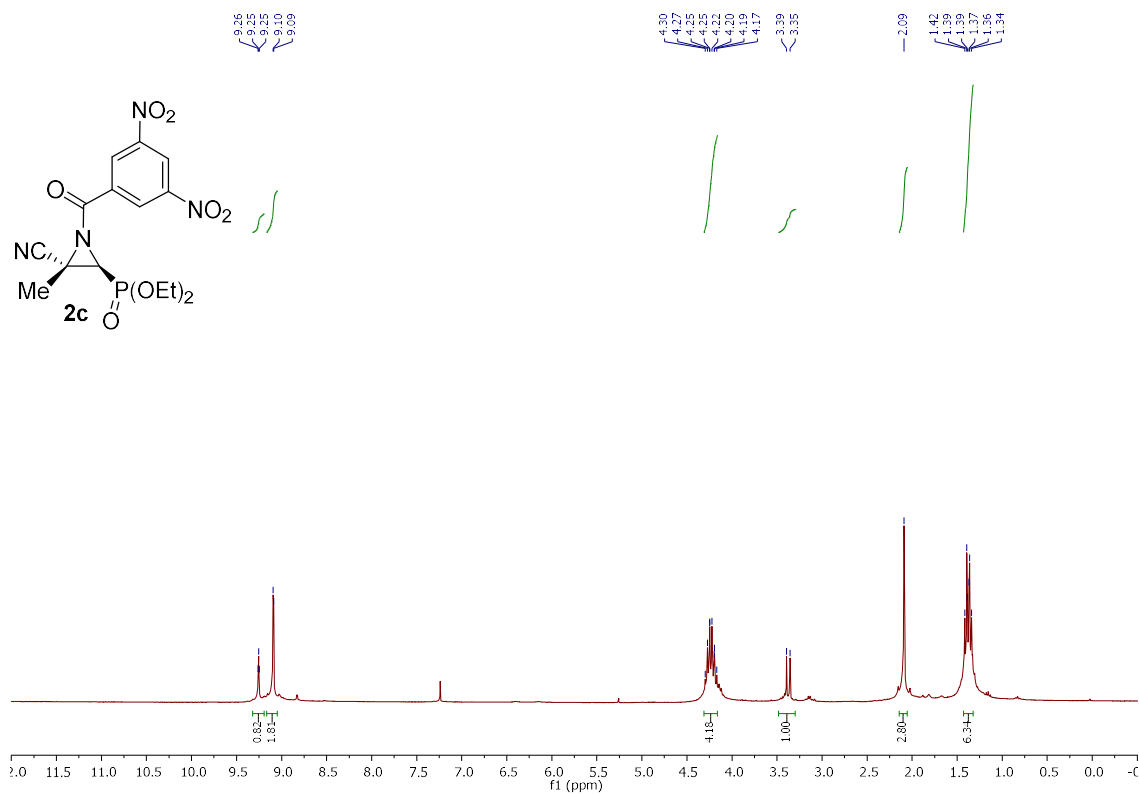
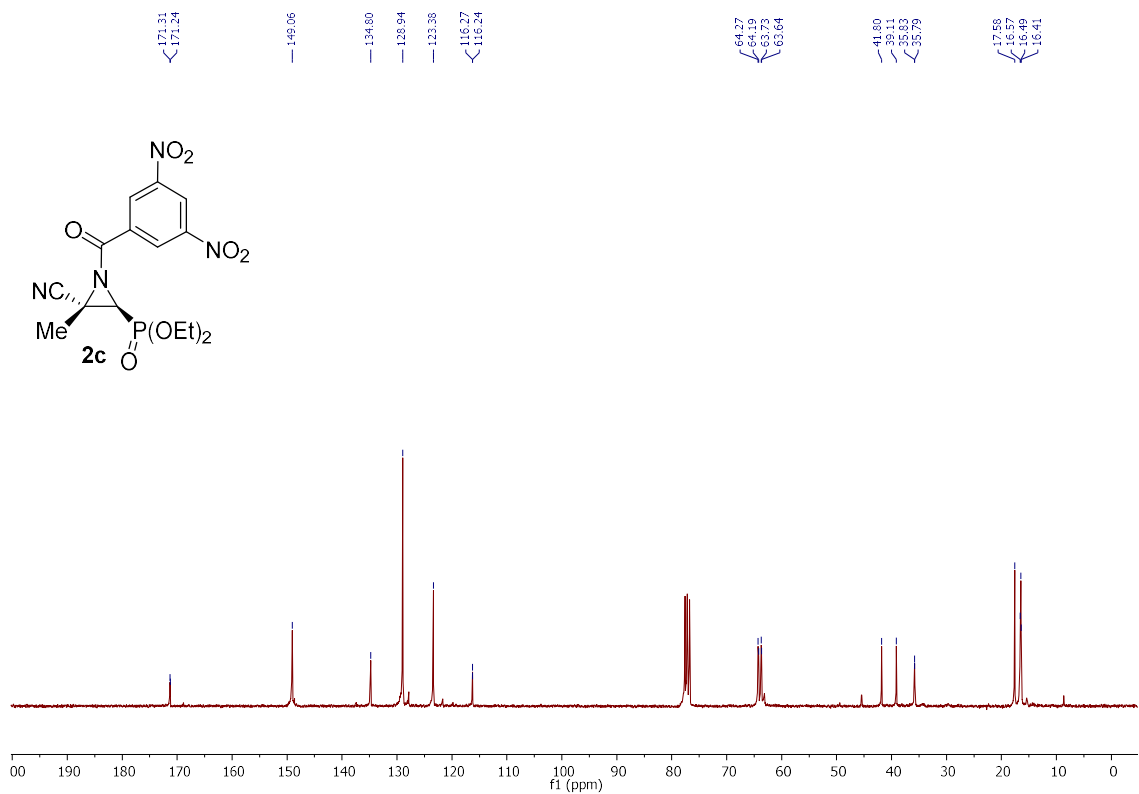
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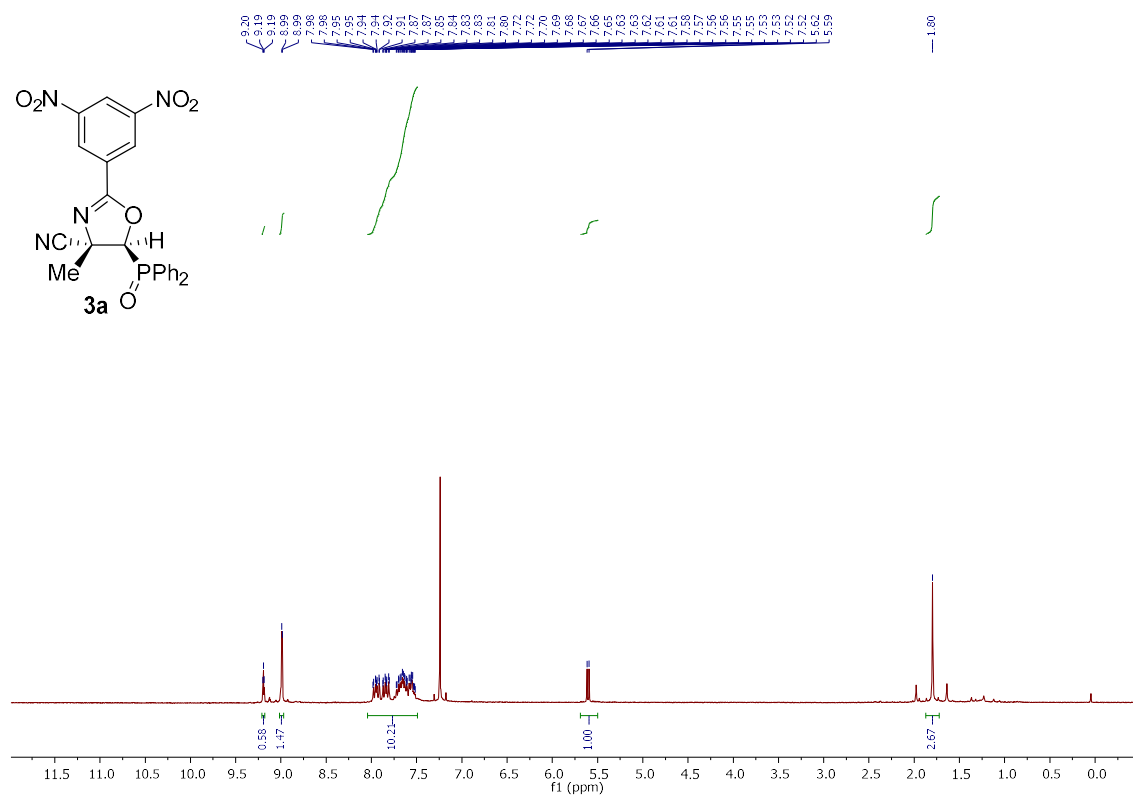
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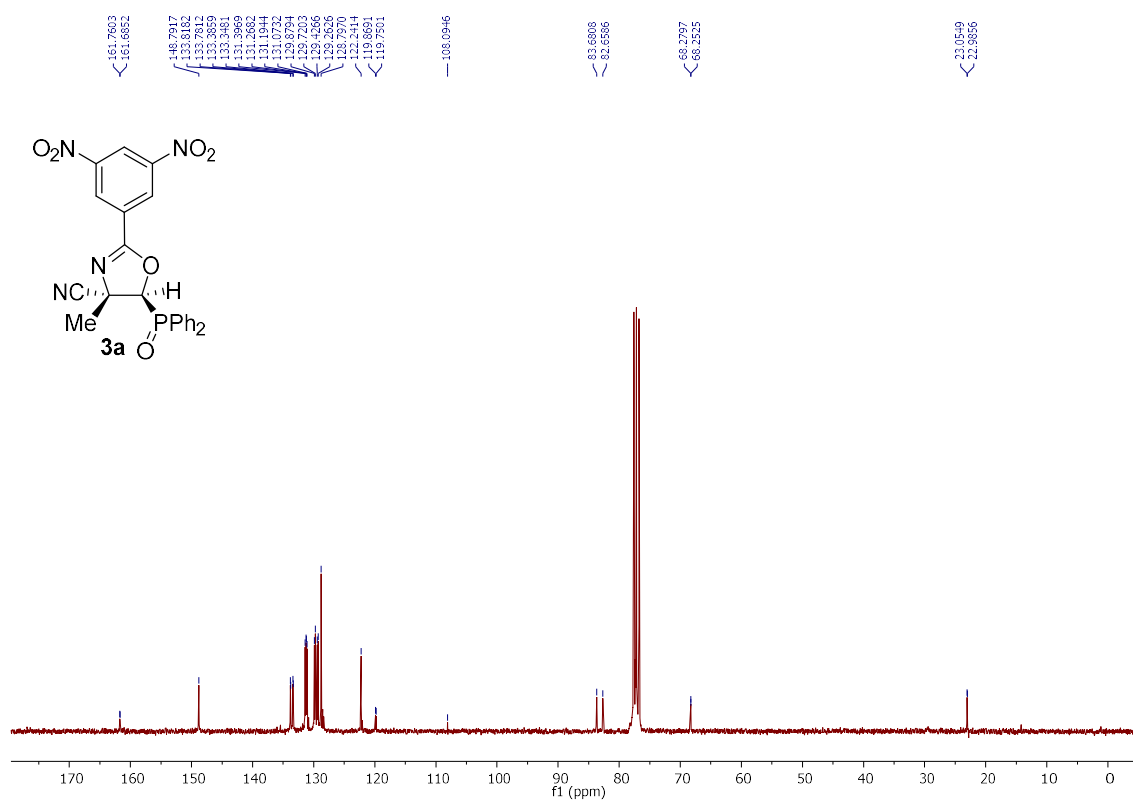
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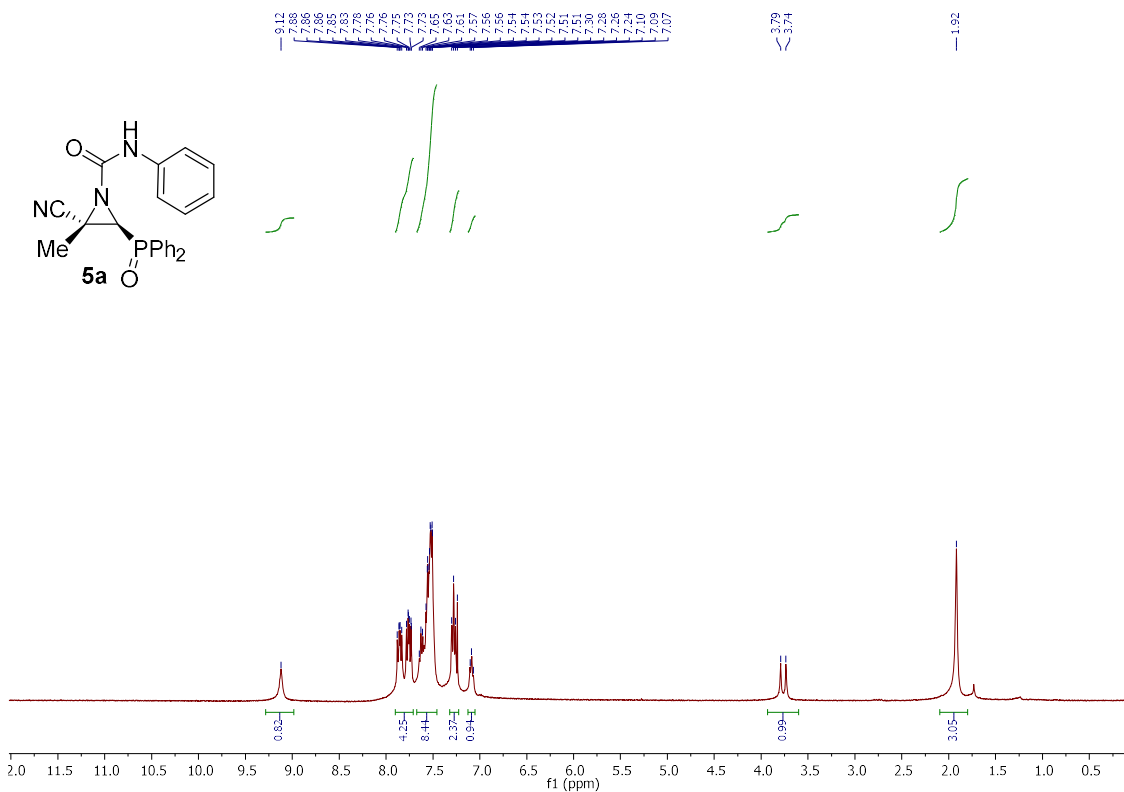
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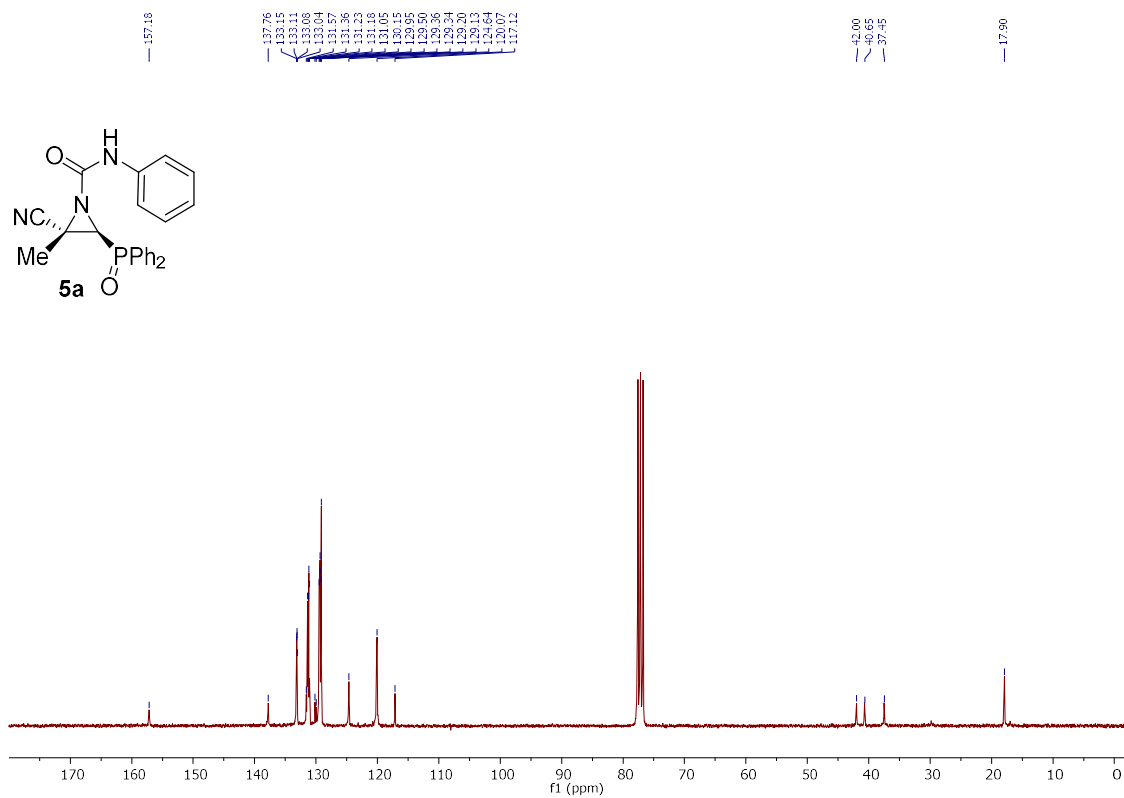
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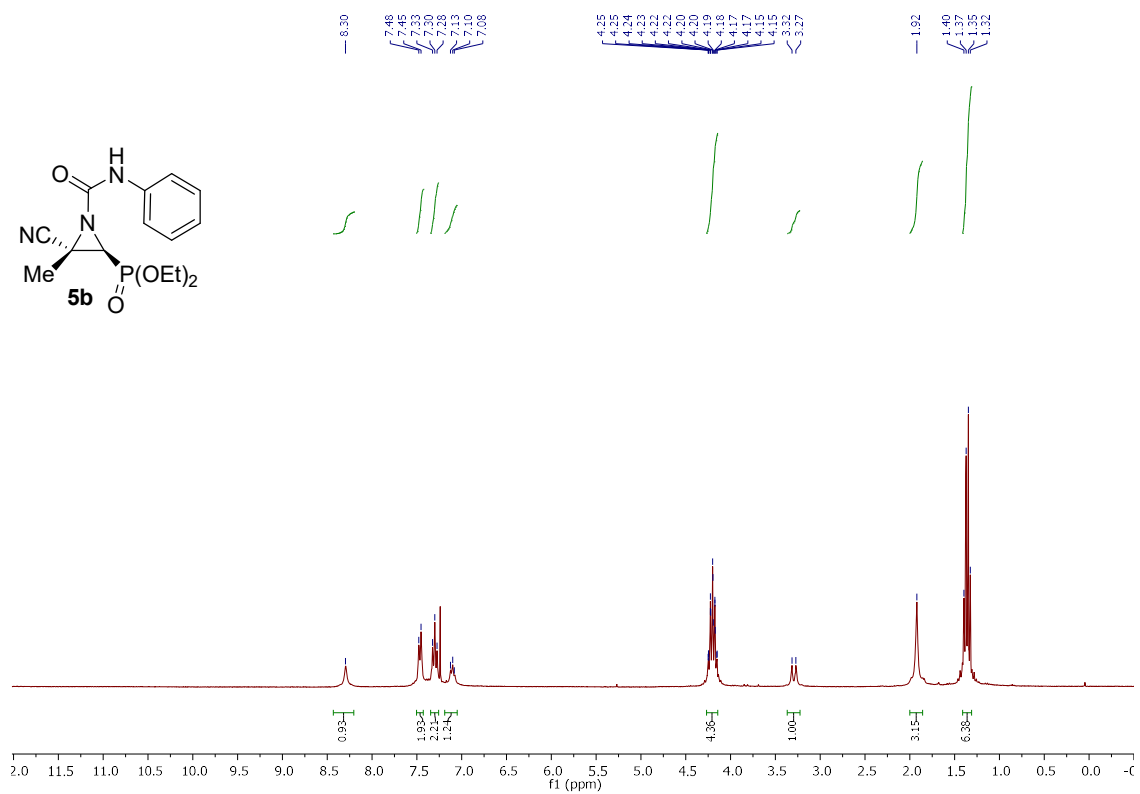
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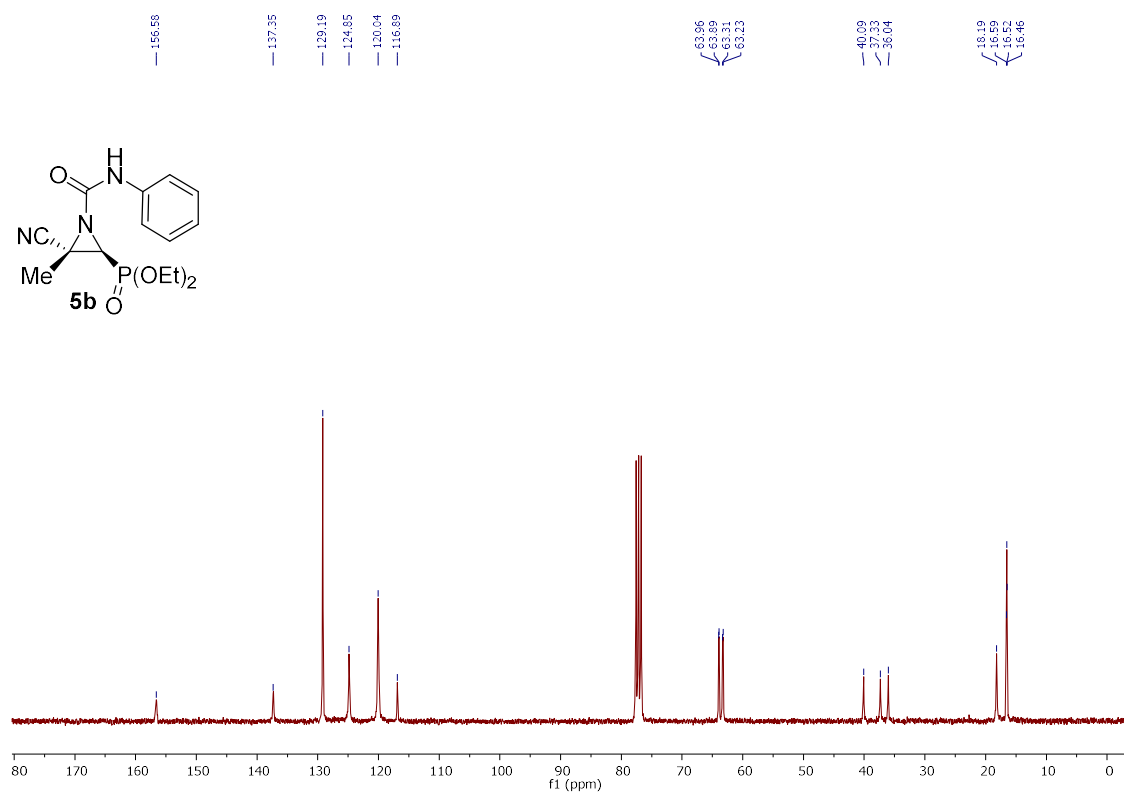
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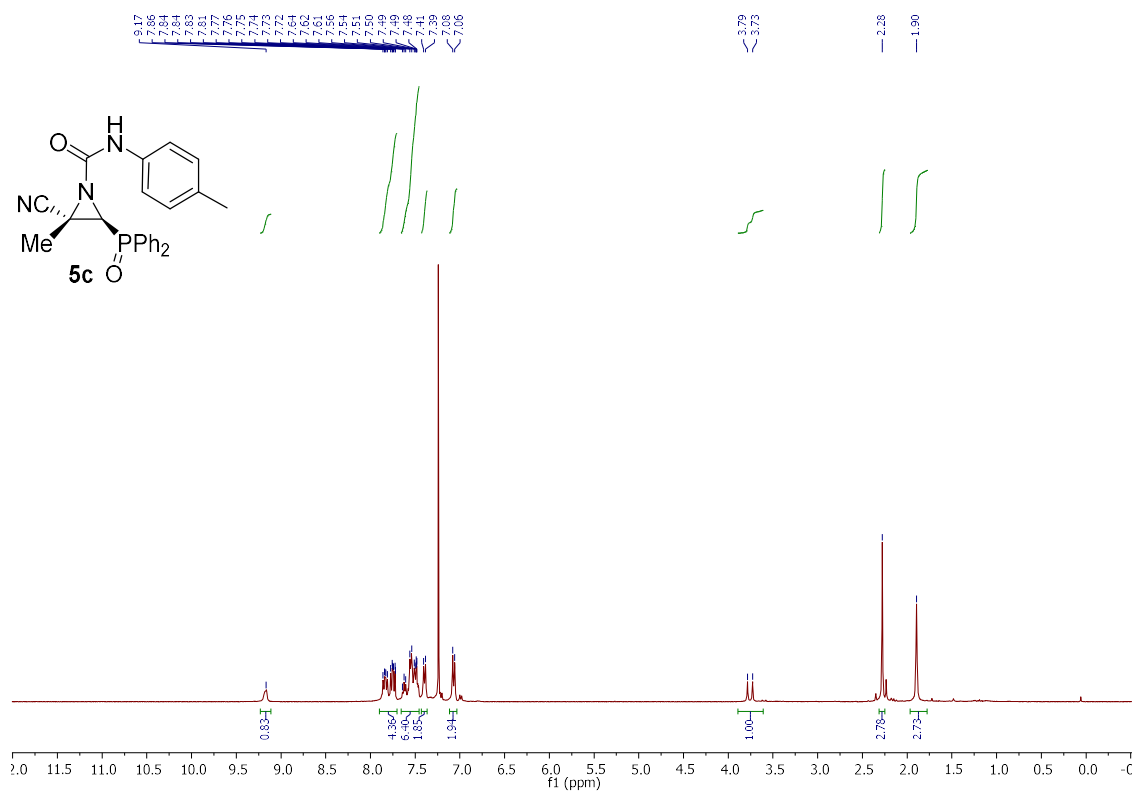
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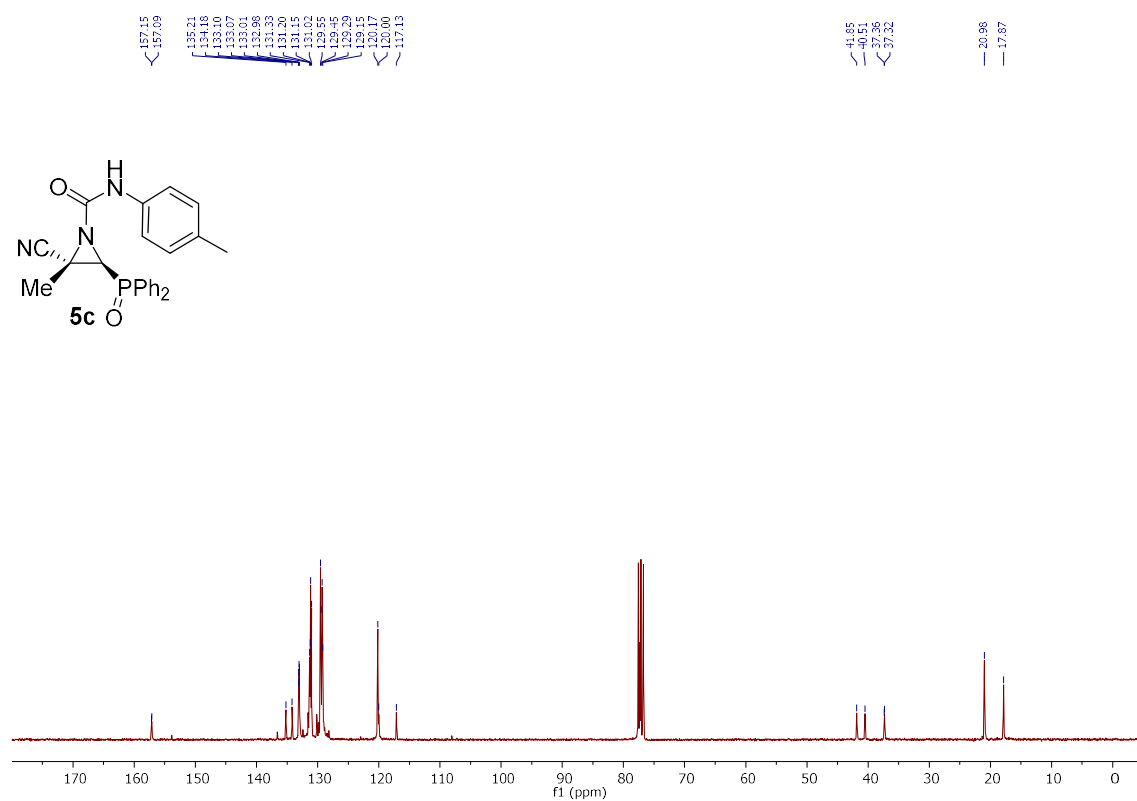
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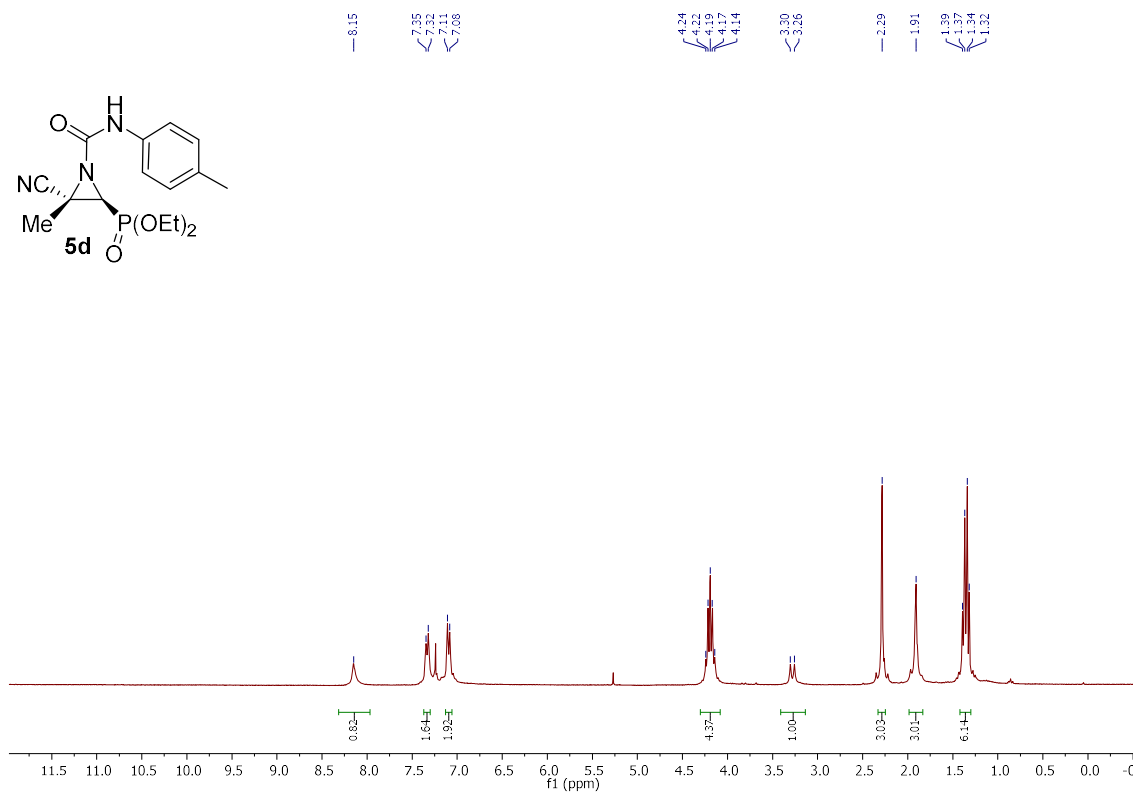
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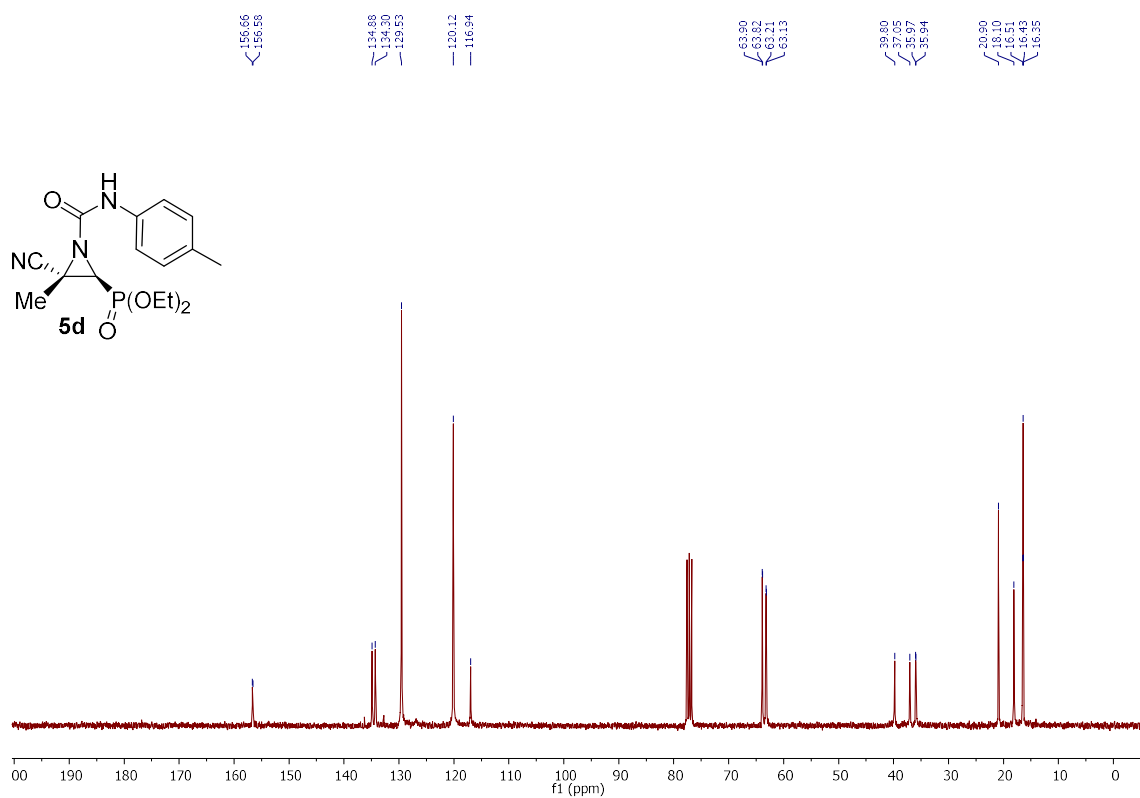
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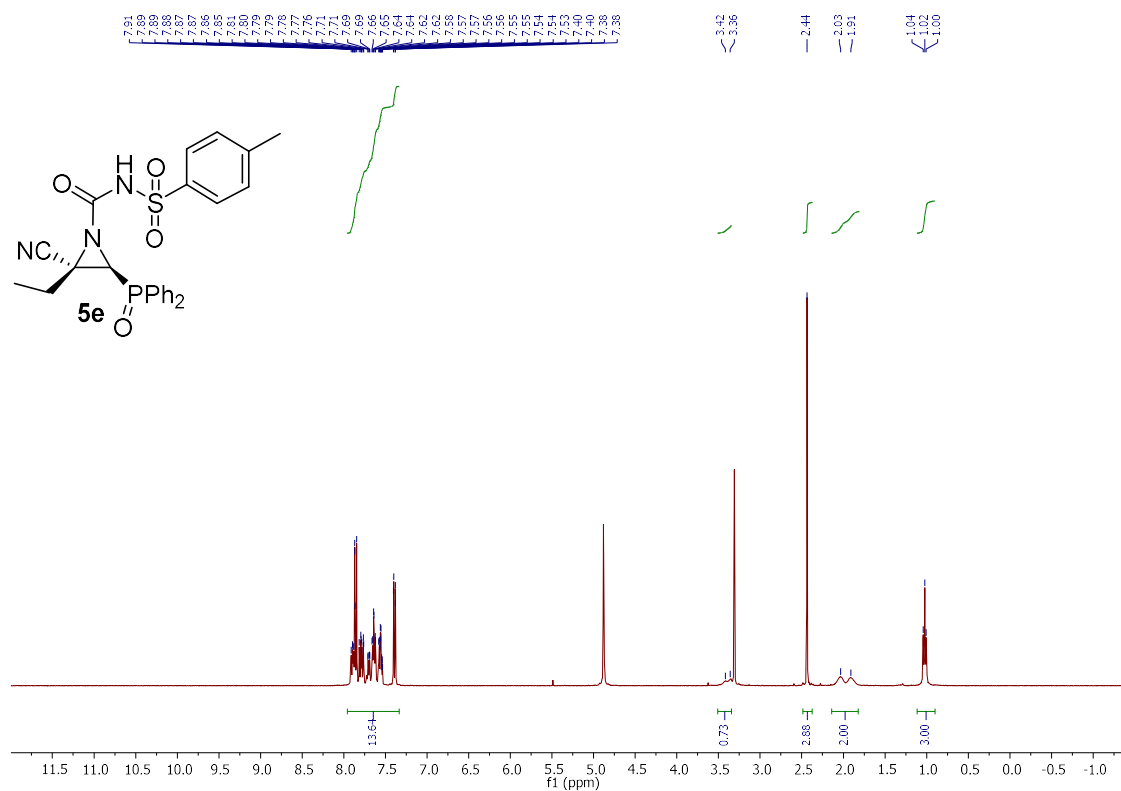
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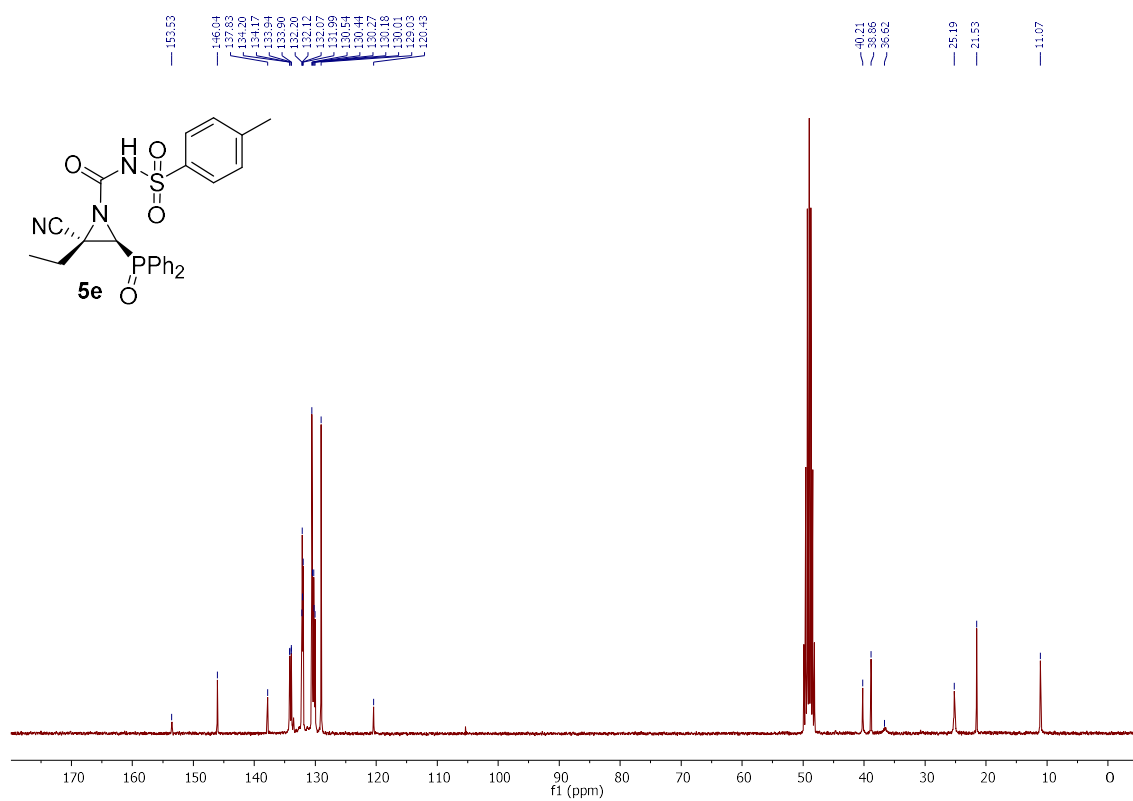
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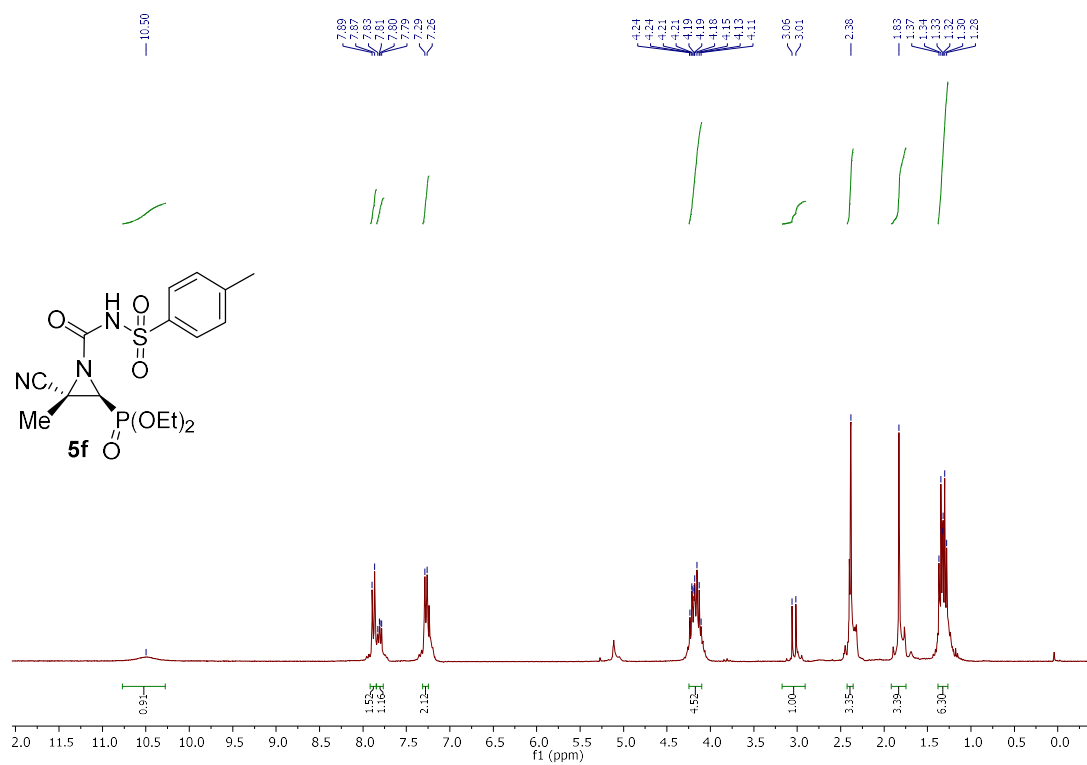
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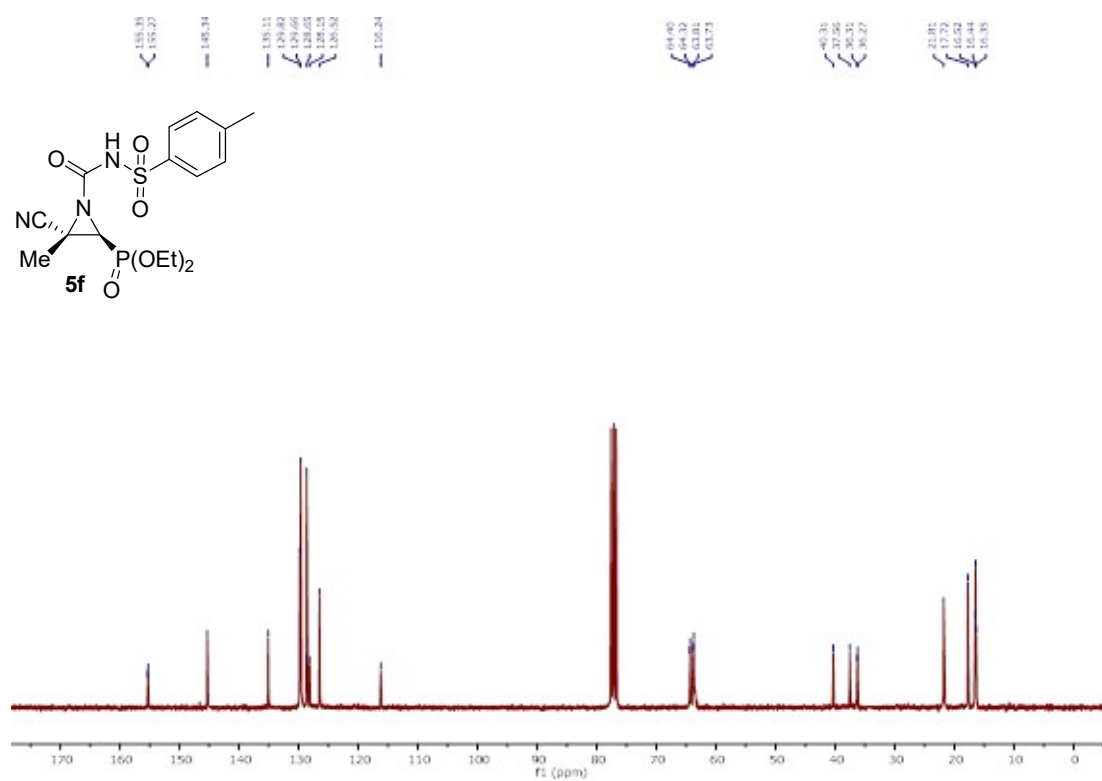
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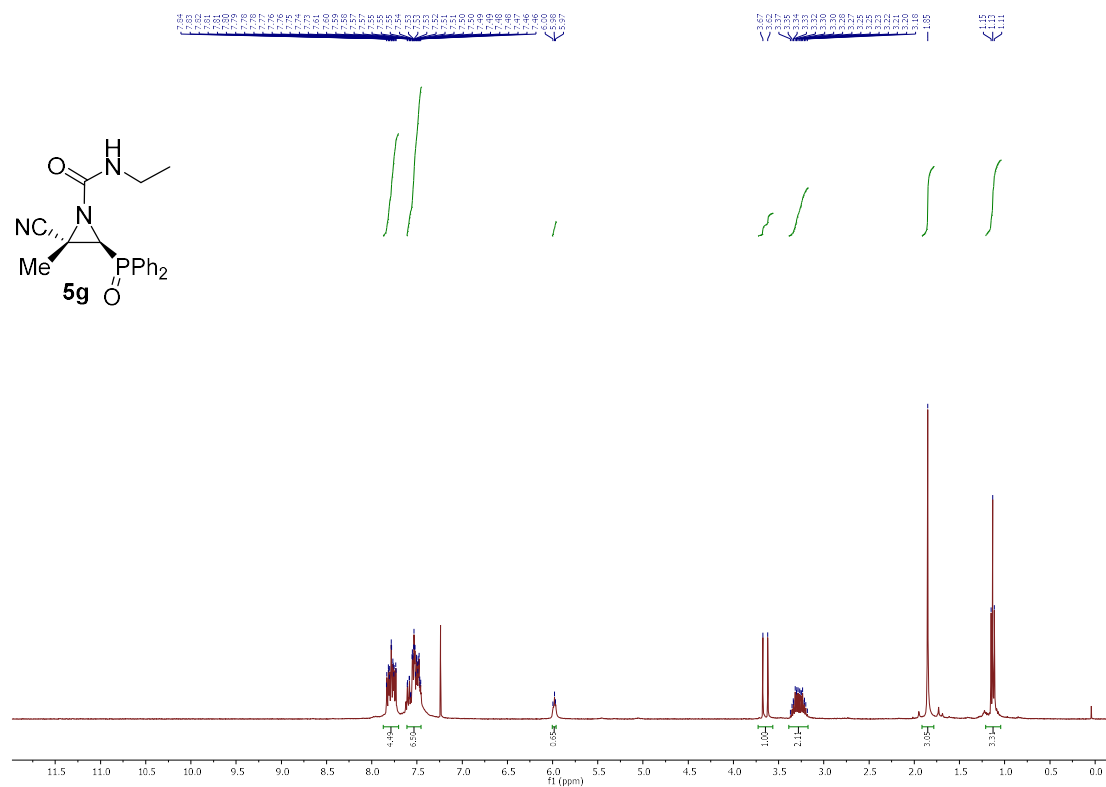
^1H NMR (300 MHz, CDCl_3) of compound **5f**.



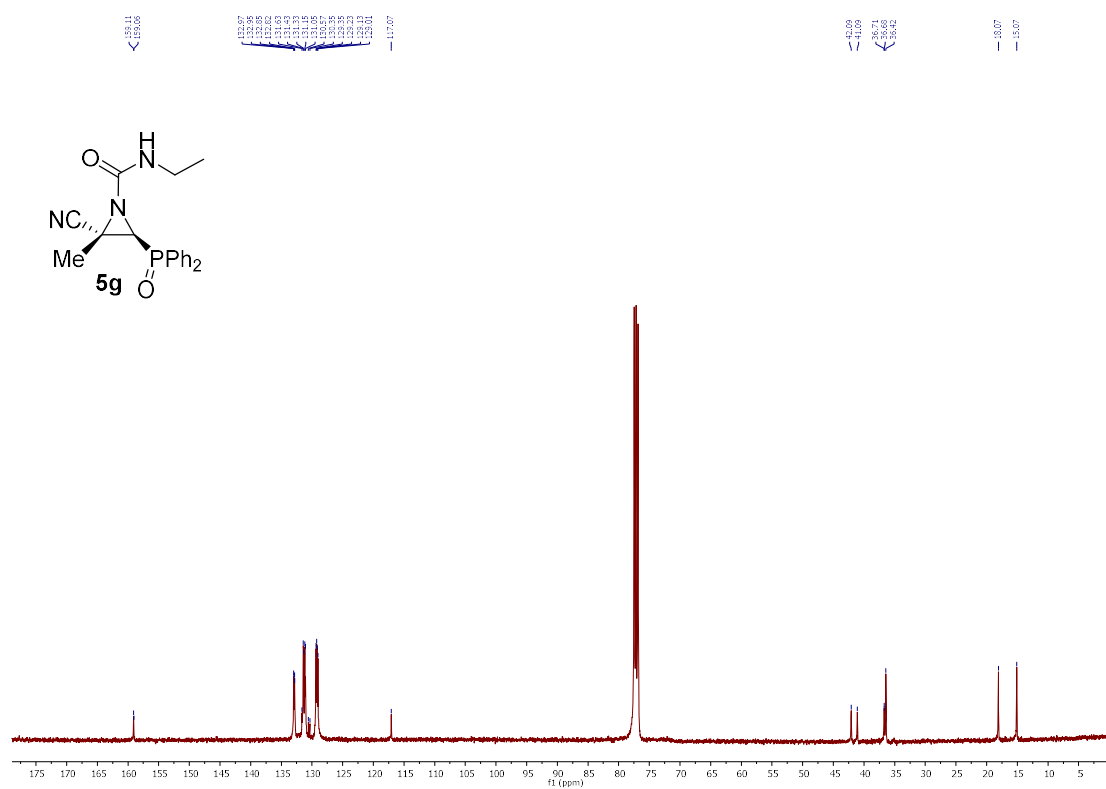
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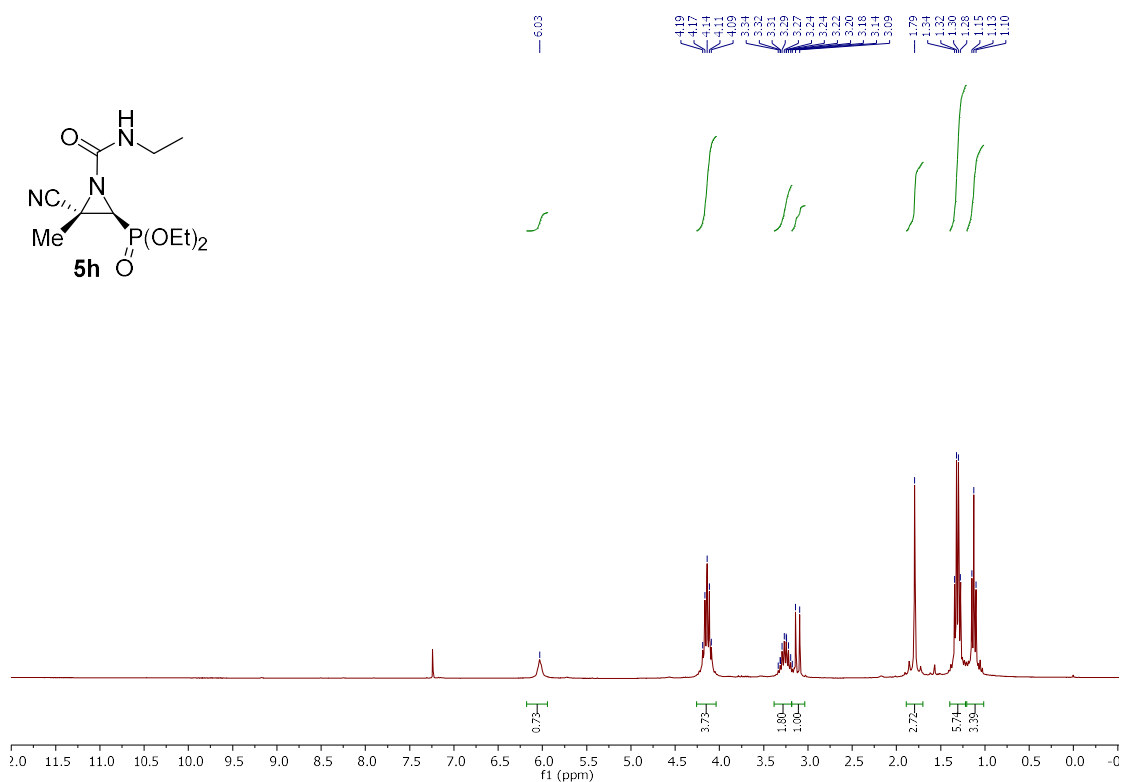
^1H NMR (400 MHz, CDCl_3) of compound **5g**.



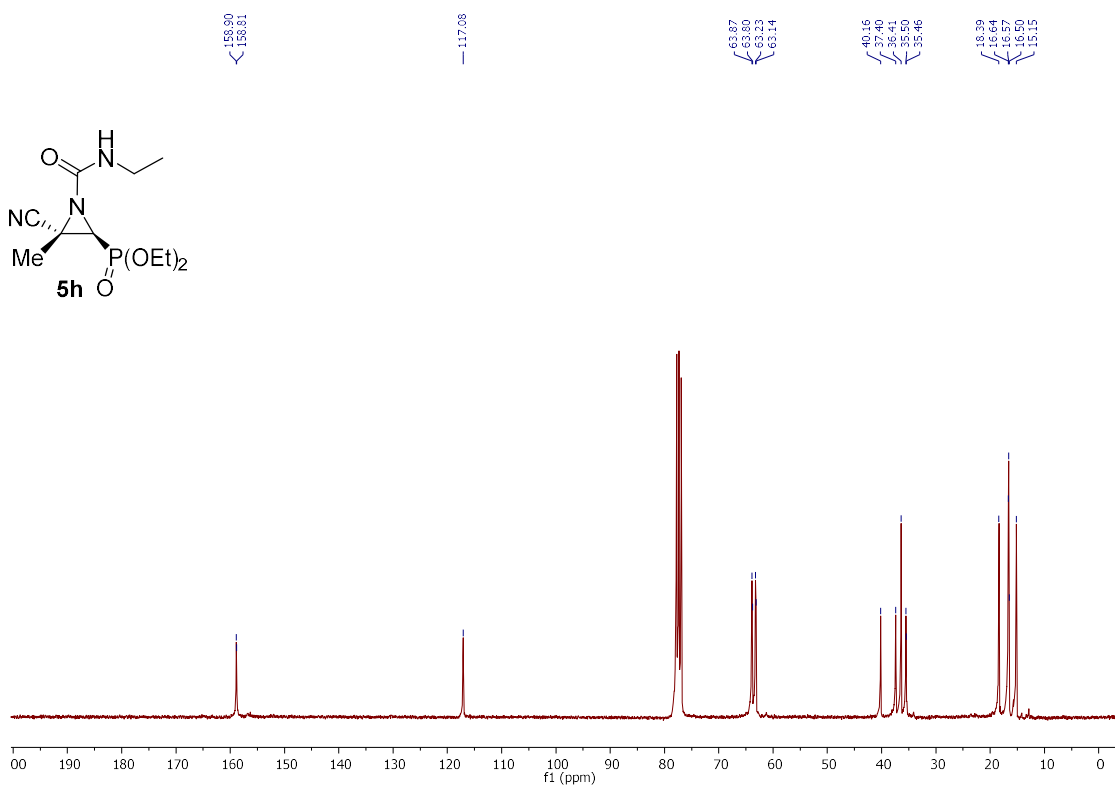
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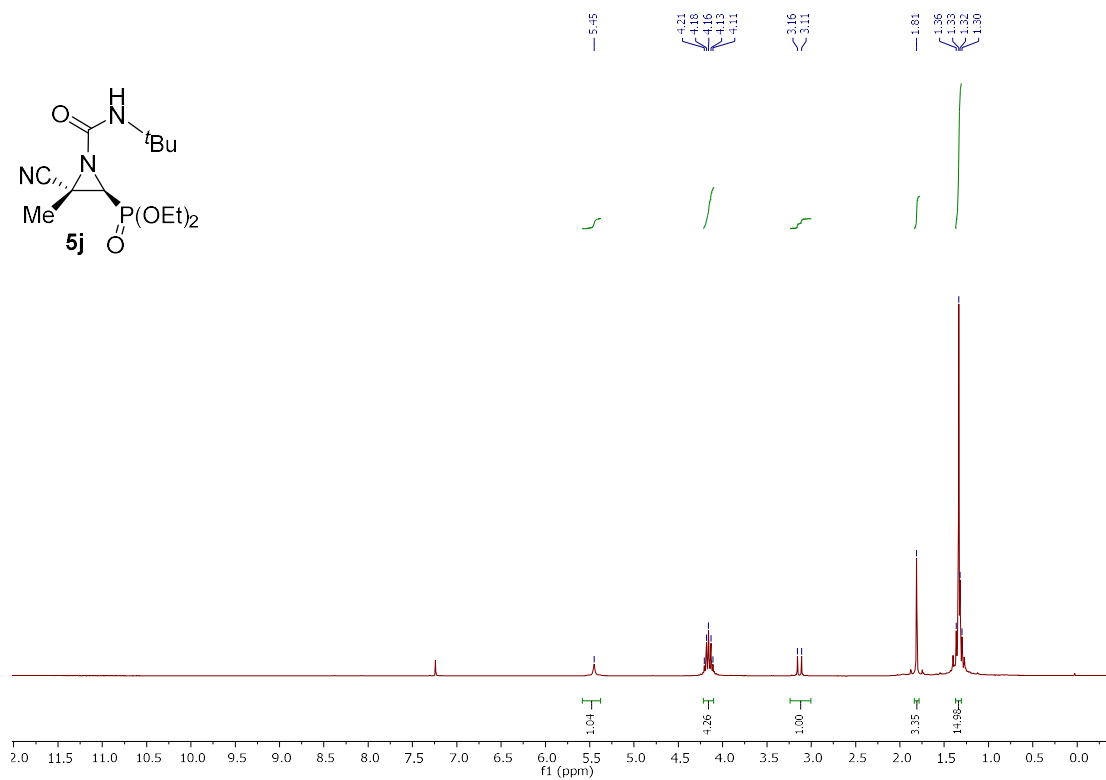
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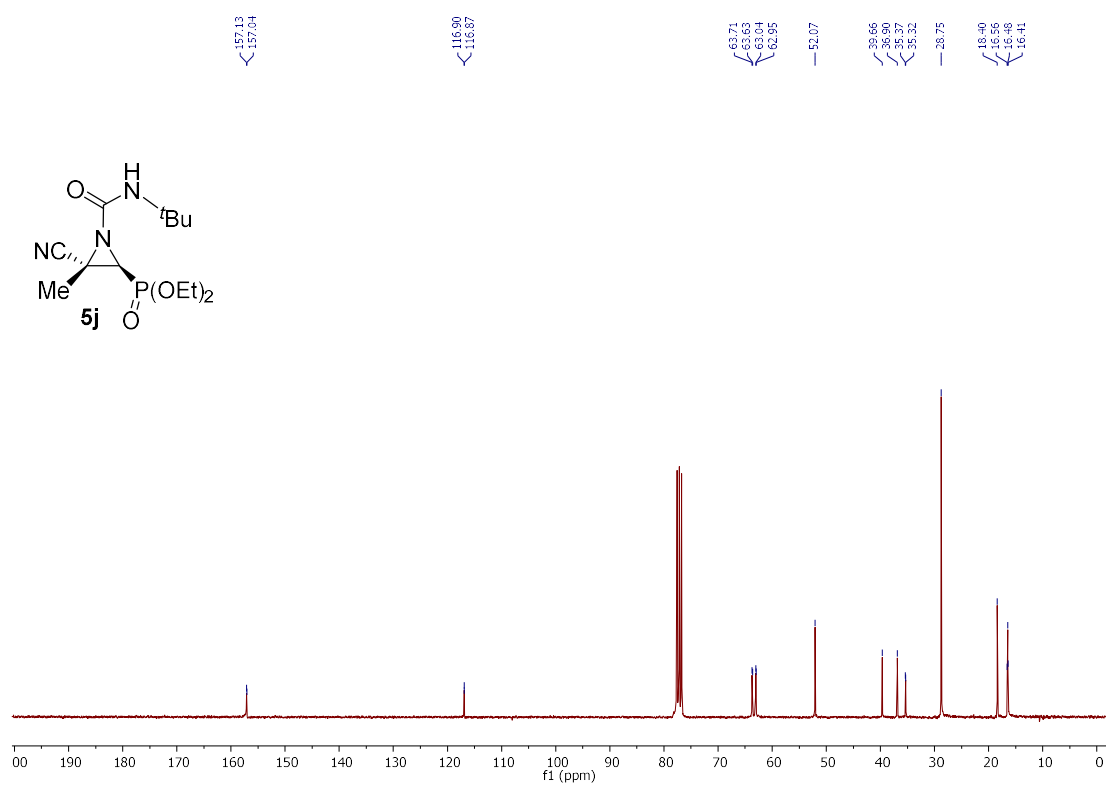
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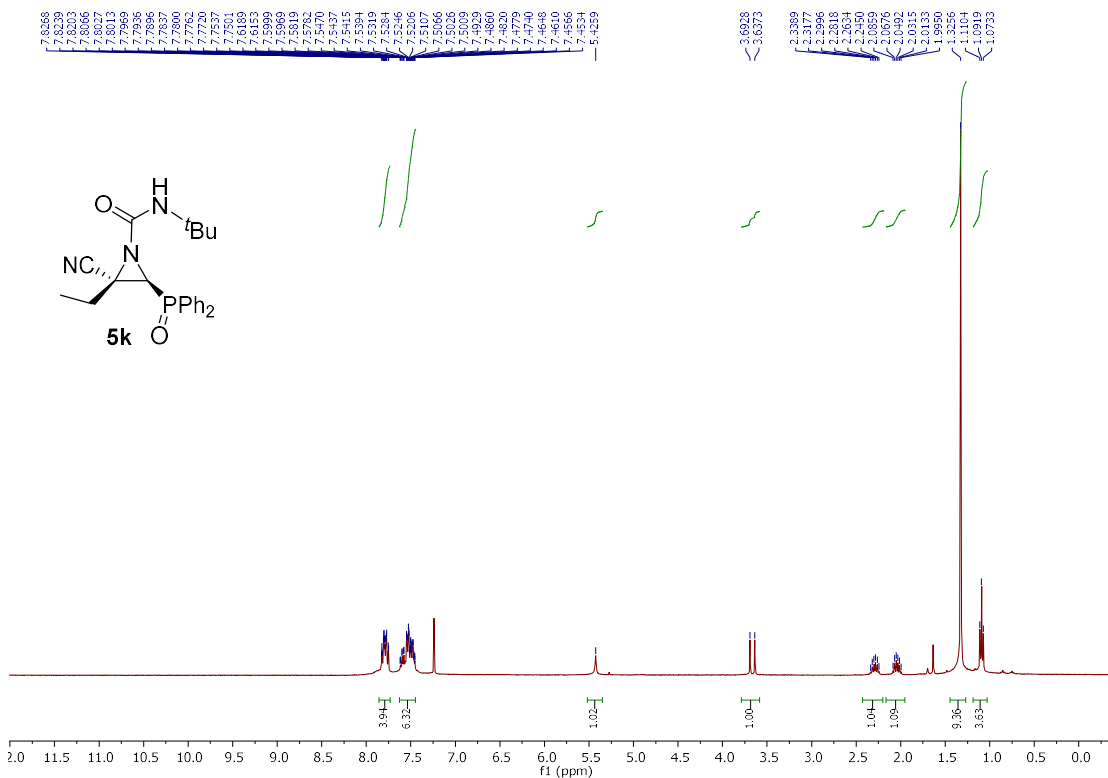
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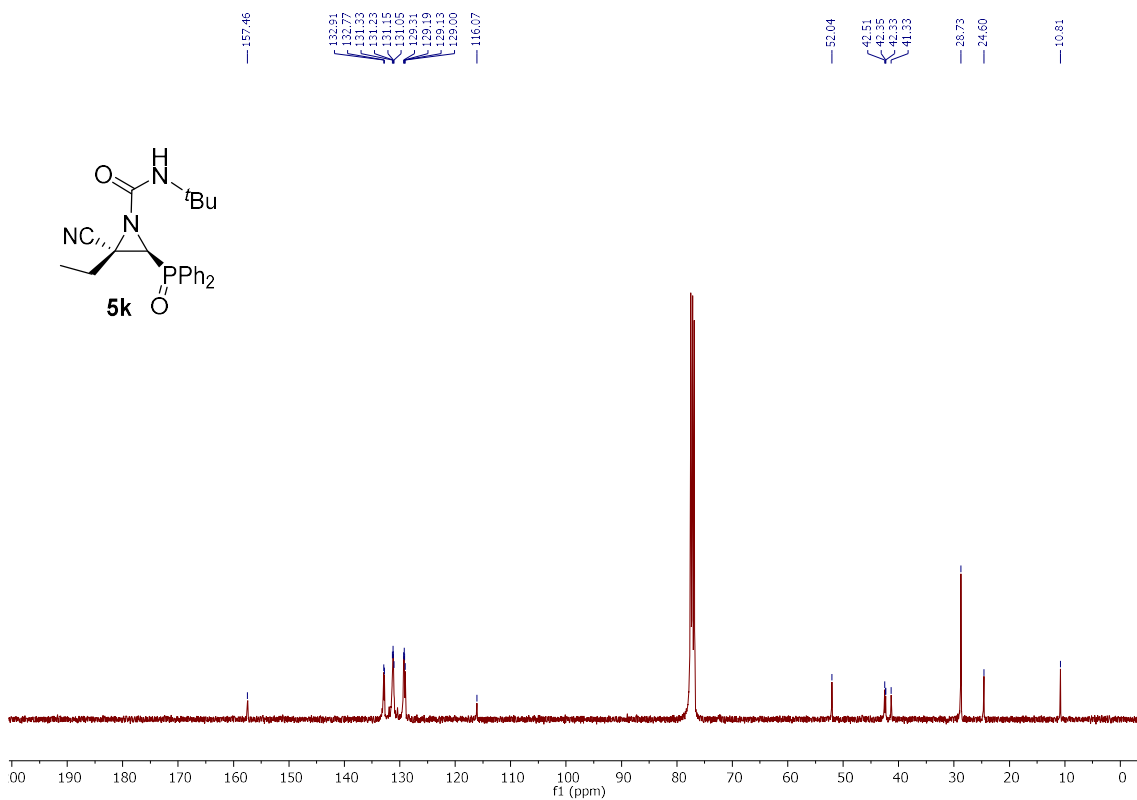
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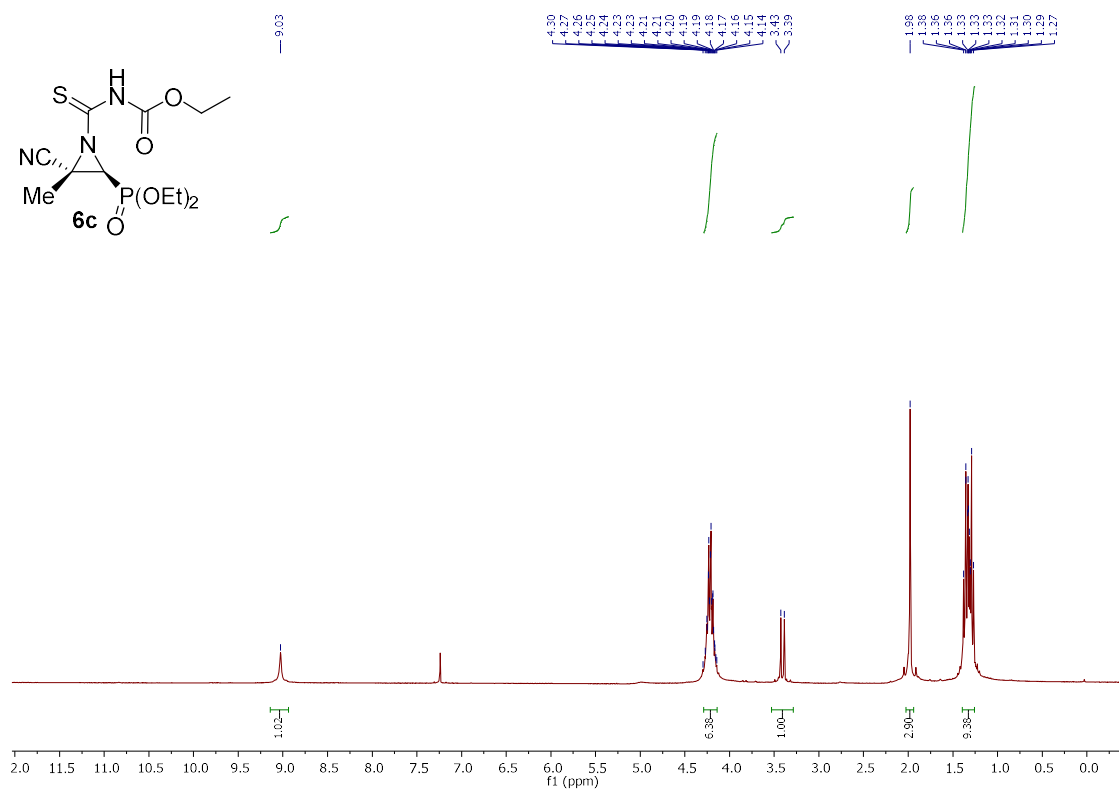
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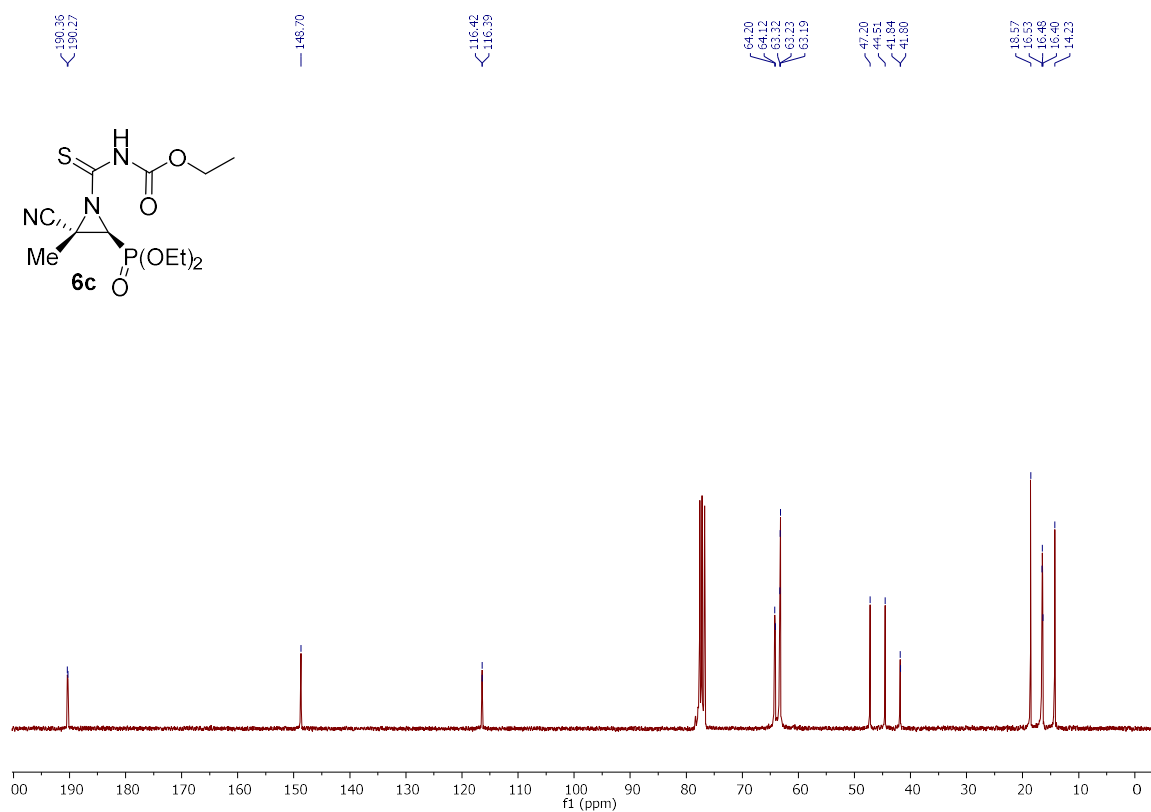
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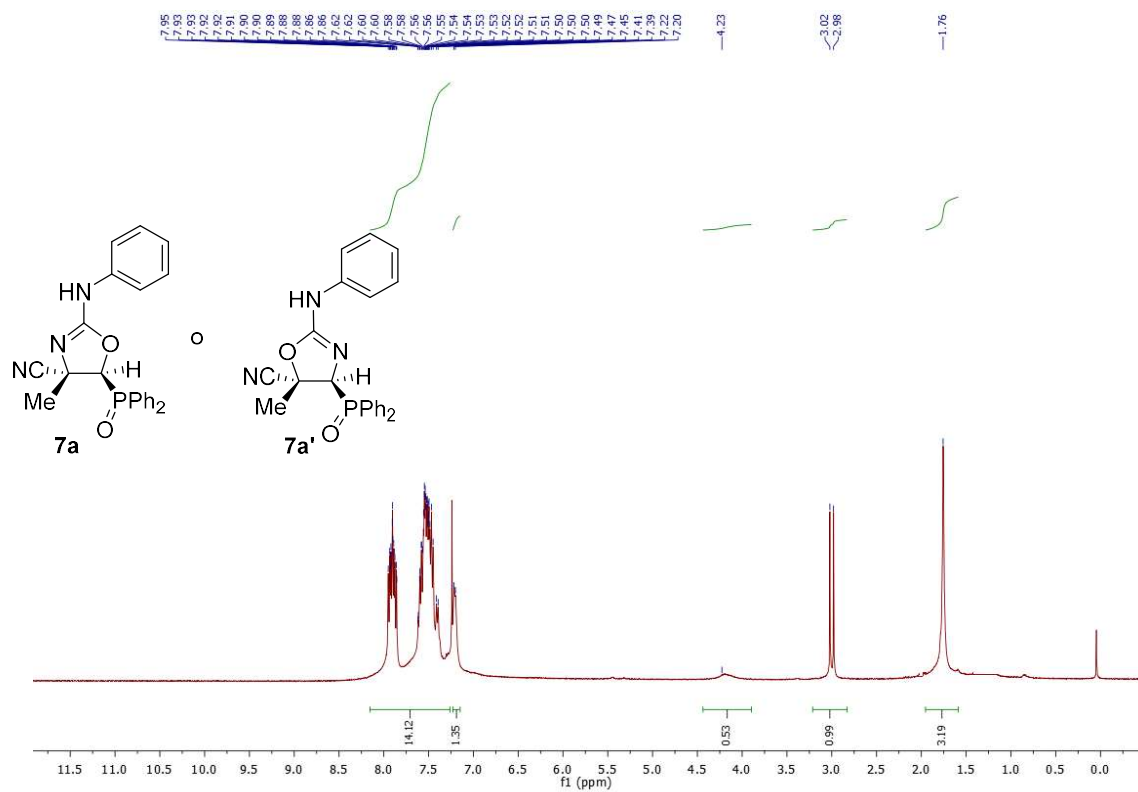
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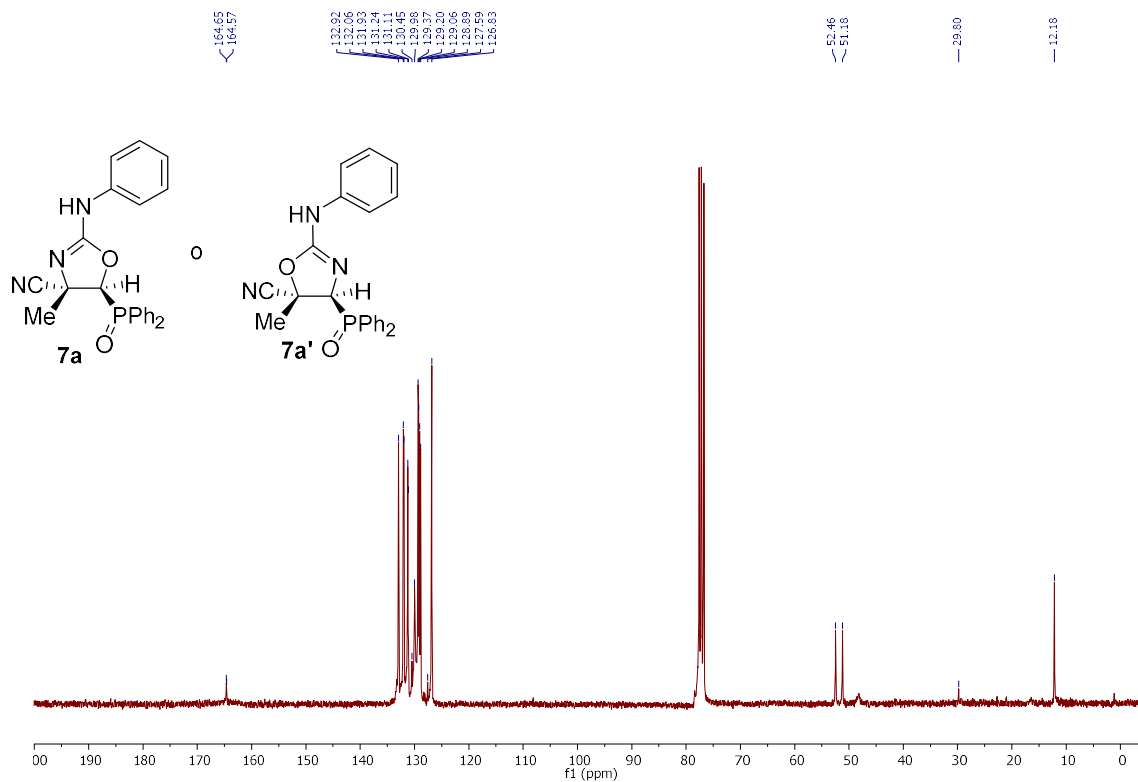
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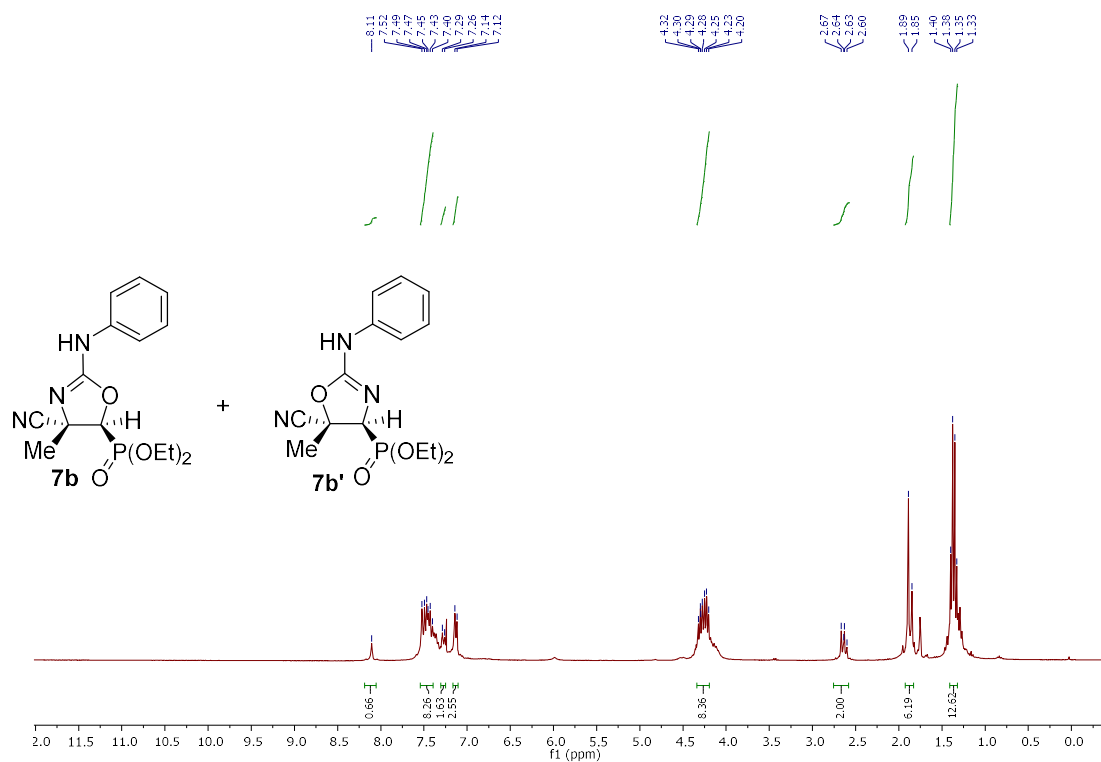
^1H NMR (400 MHz, CDCl_3) of compound **7a** or **7a'**.



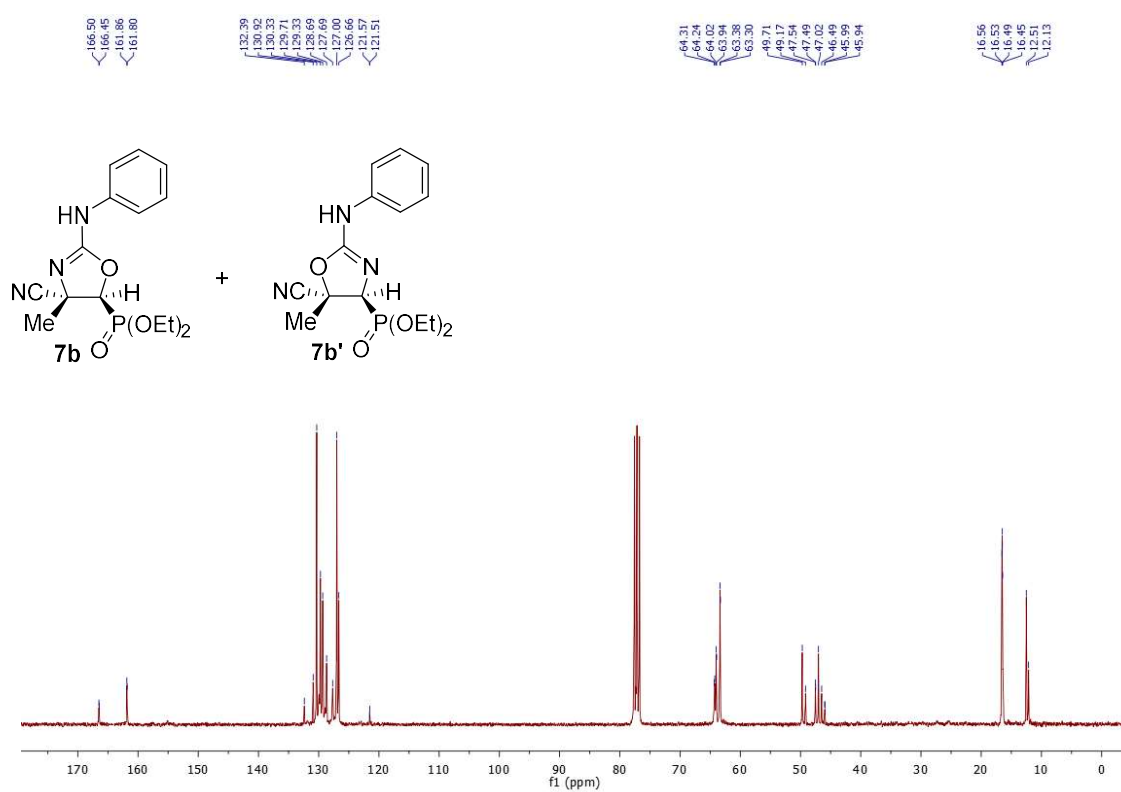
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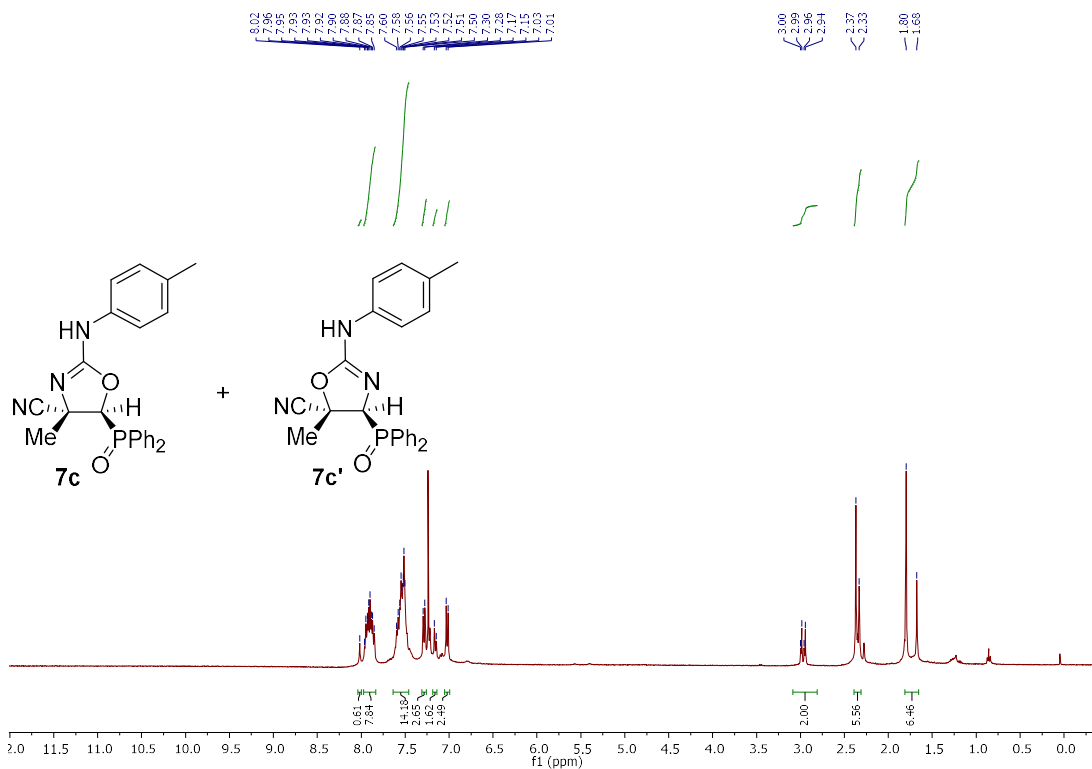
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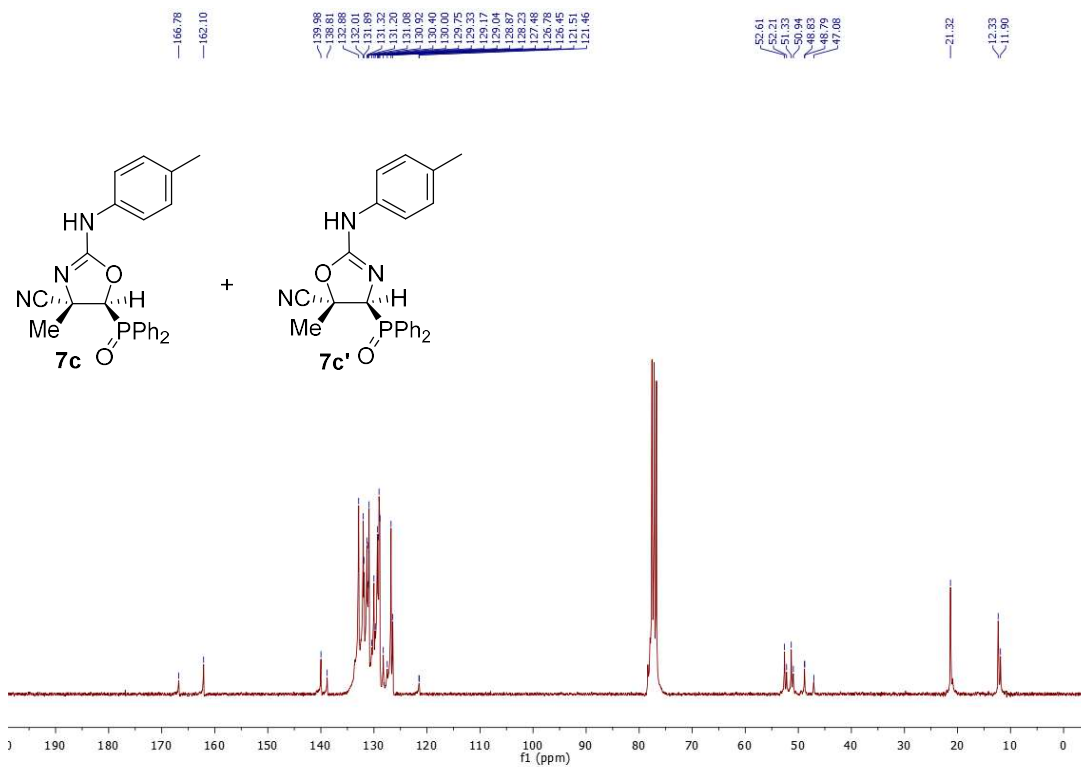
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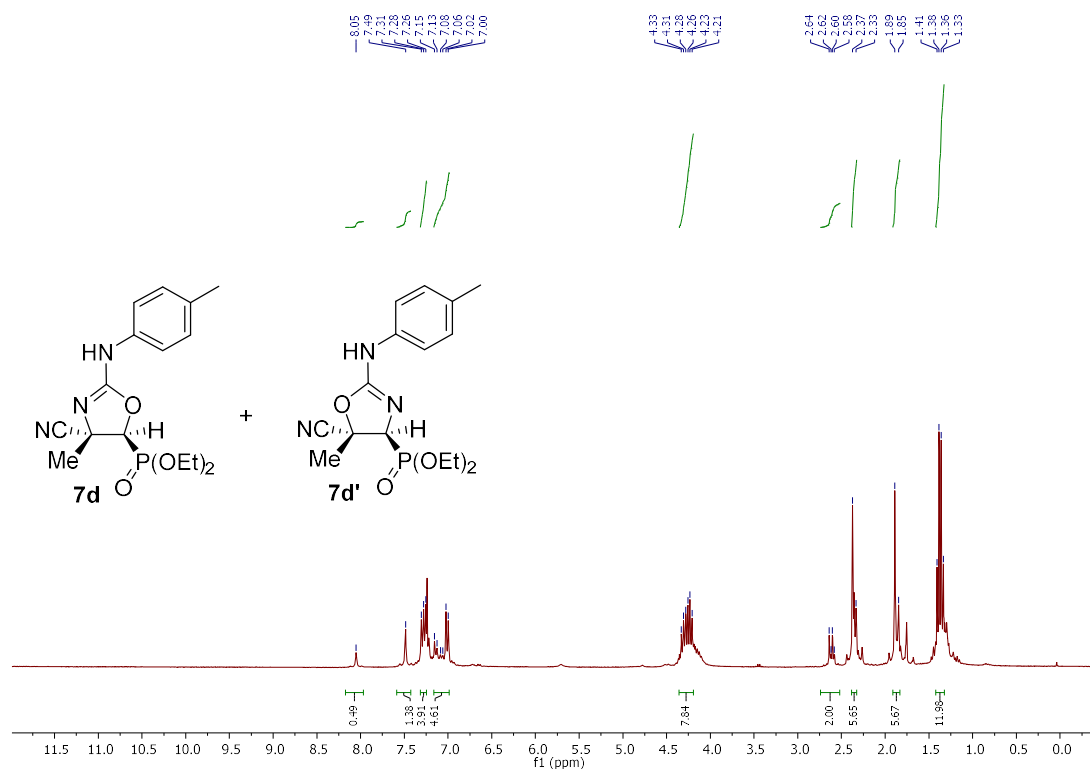
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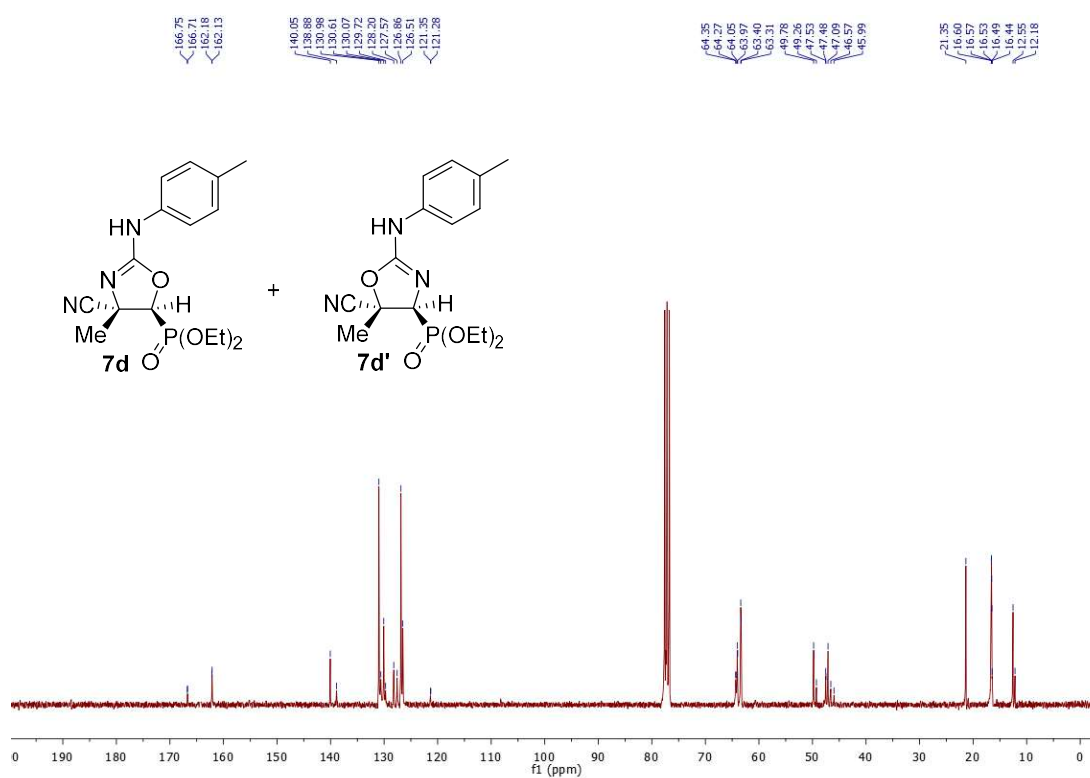
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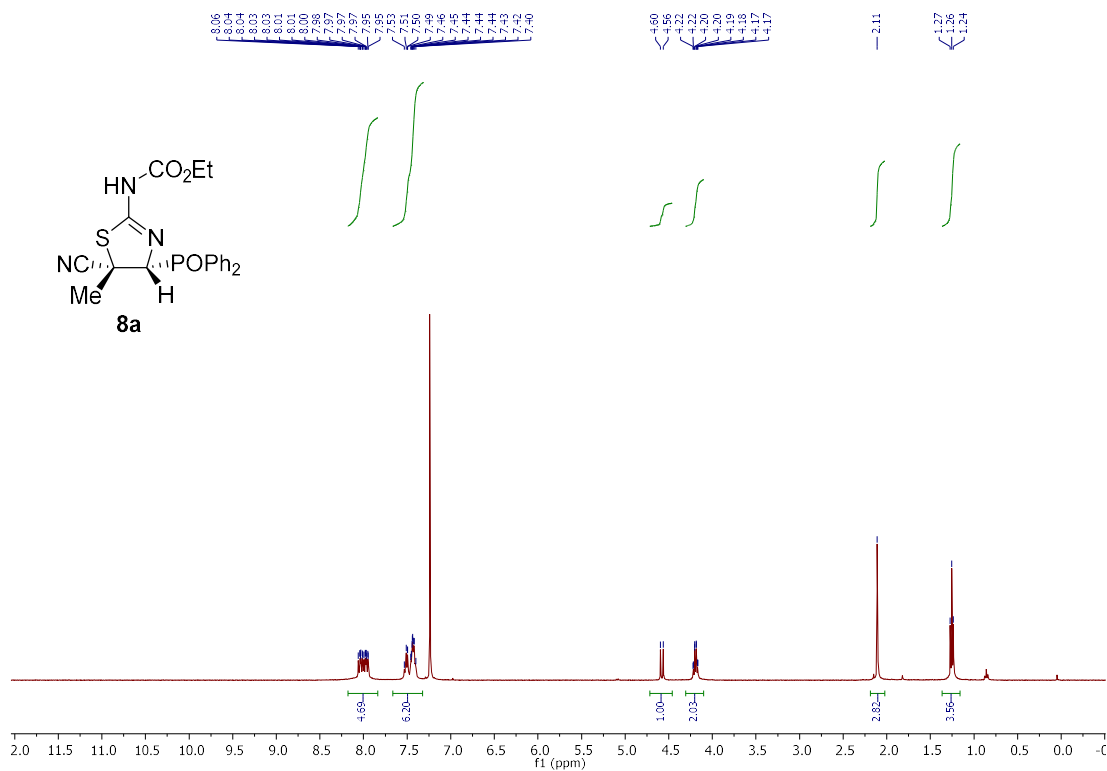
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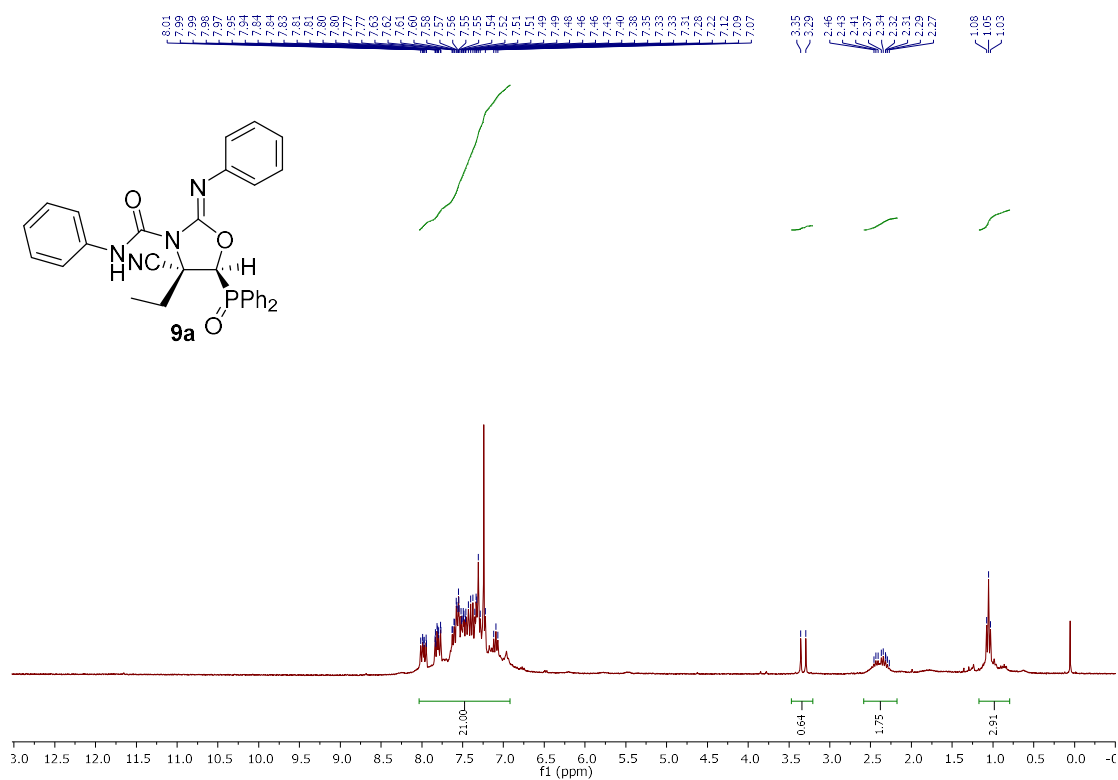
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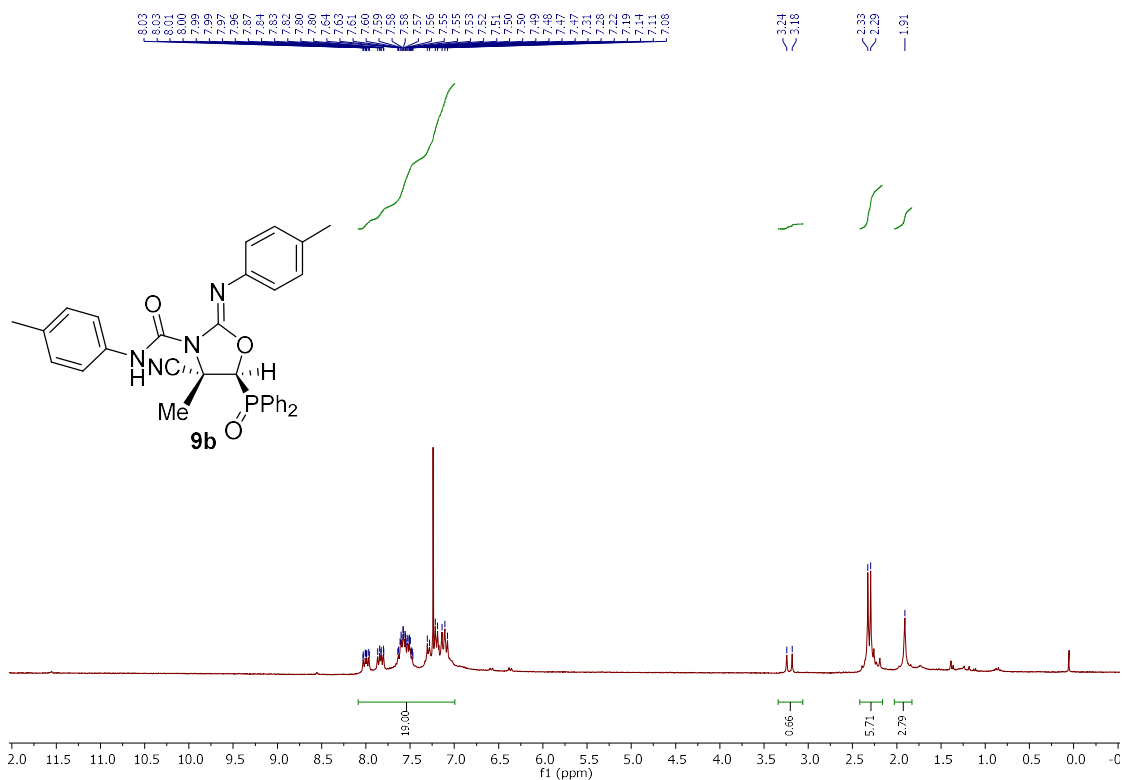
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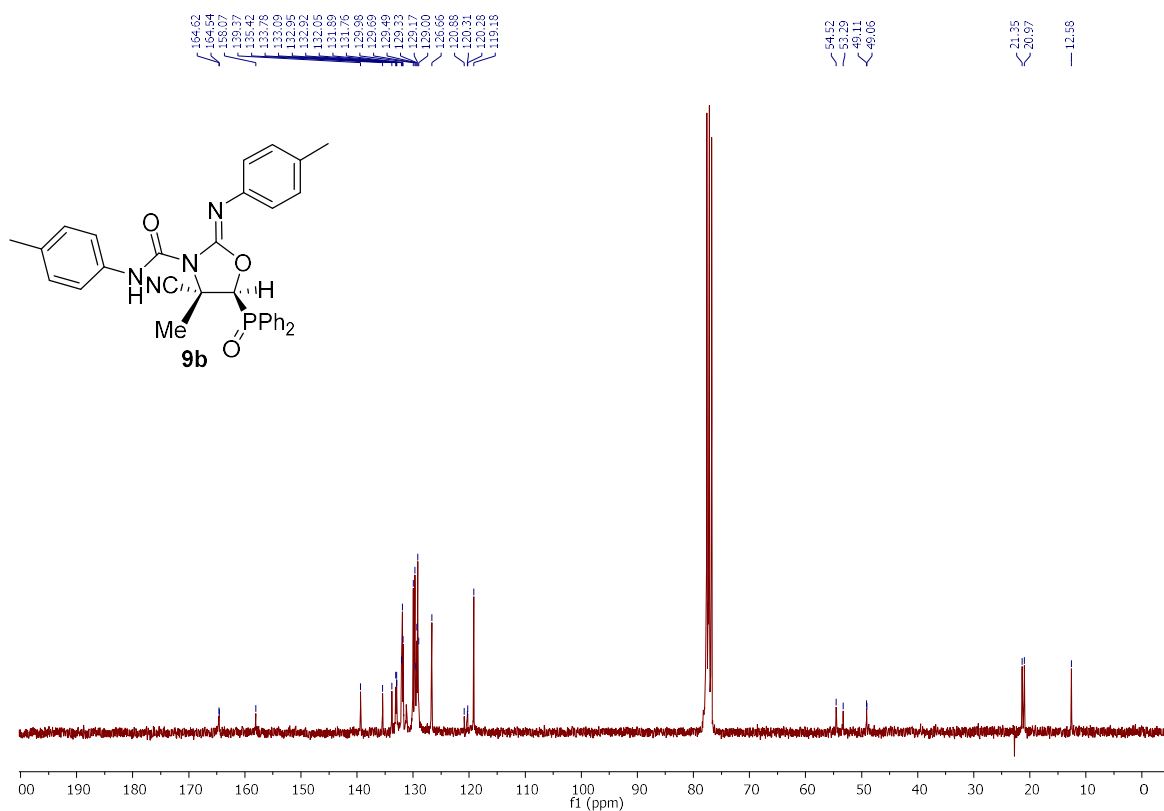
^1H NMR (300 MHz, CDCl_3) of compound **9a**.

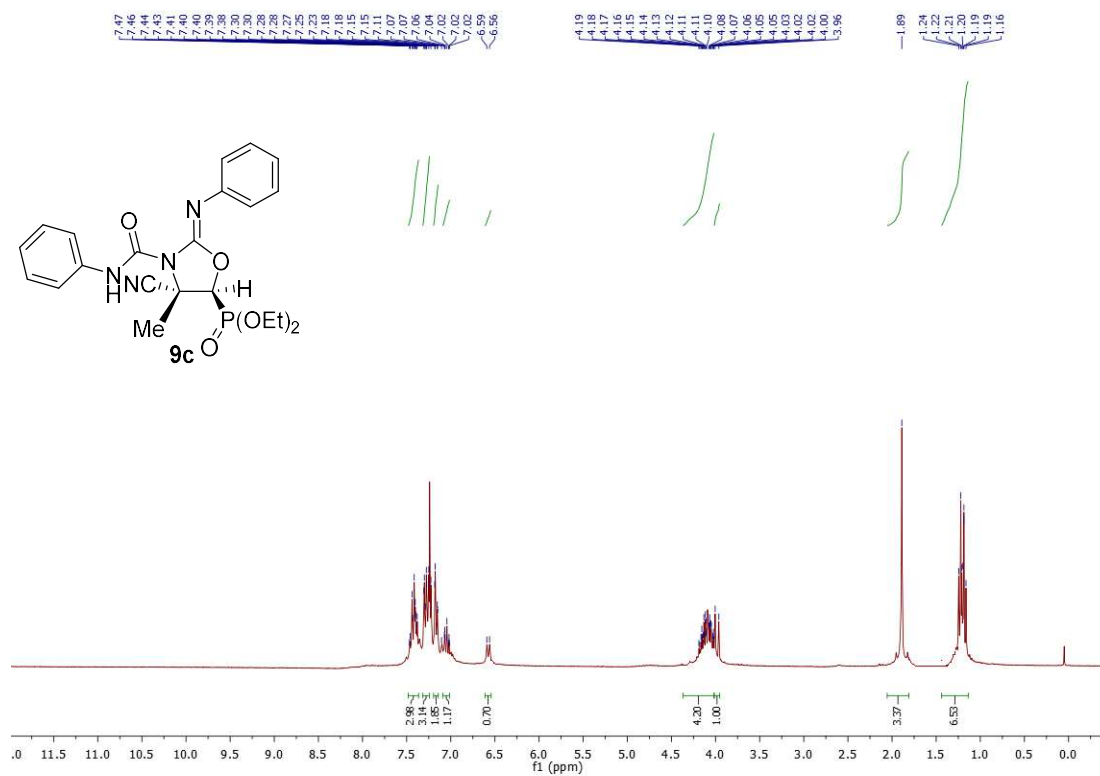
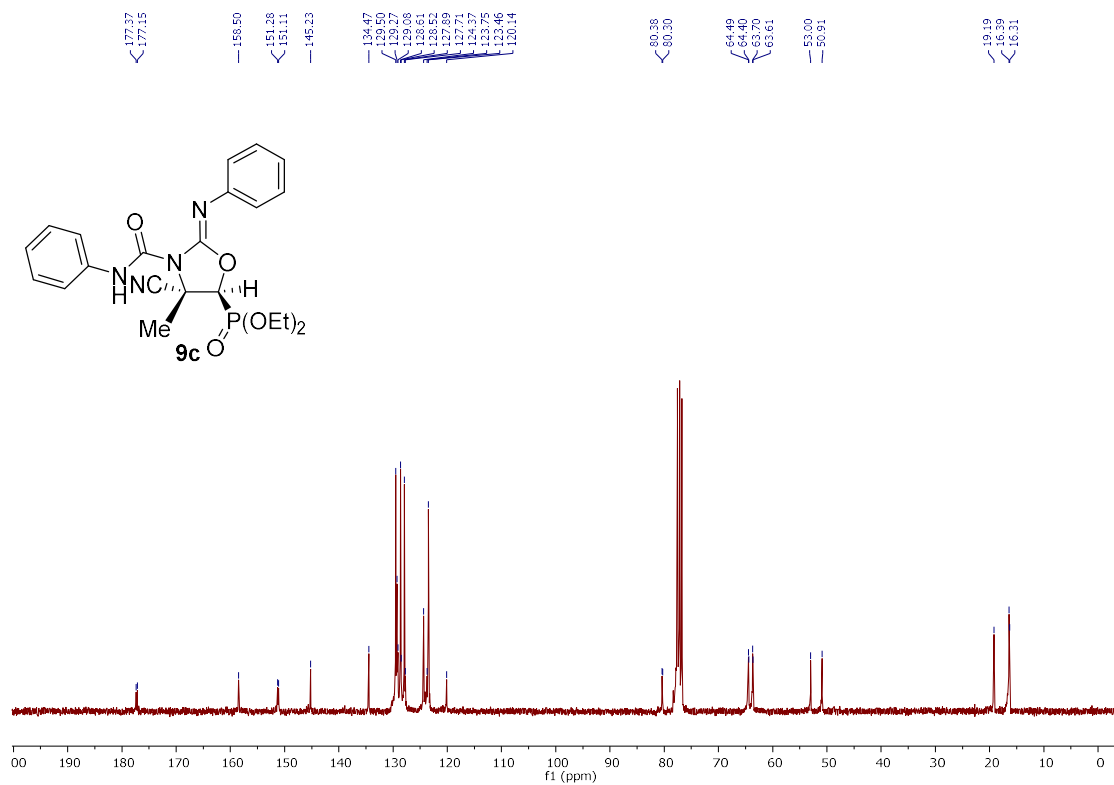


^1H NMR (300 MHz, CDCl_3) of compound **9b**.



^{13}C NMR (75 MHz, CDCl_3) of compound **9b**.



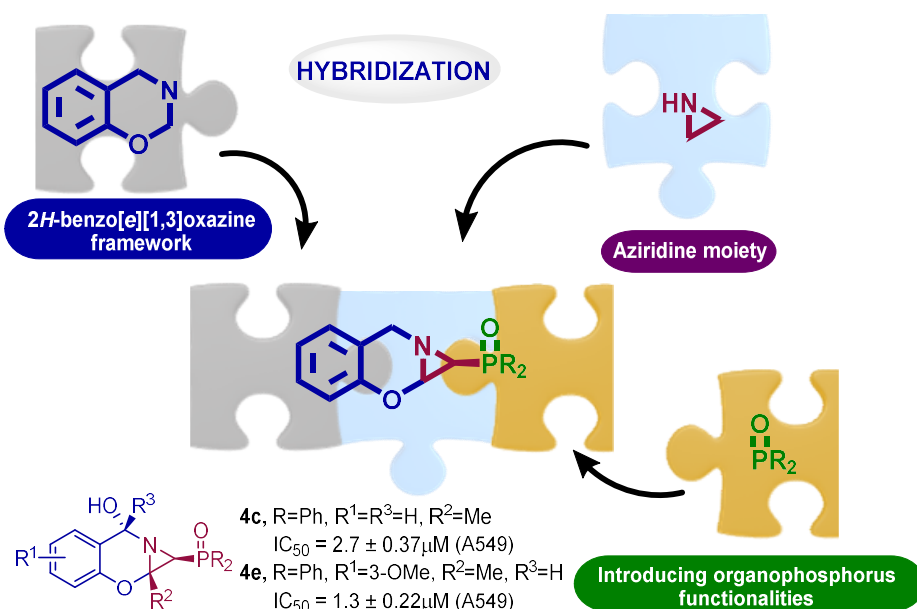
^1H NMR (300 MHz, CDCl_3) of compound **9c**. ^{13}C NMR (75 MHz, CDCl_3) of compound **9c**.

First synthesis of merged hybrids phosphorylated azirino[2,1-*b*]benzo[*e*][1,3]oxazine derivatives as anticancer agents.

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Eur. J. Med. Chem. **2020**, *185*, 111771.

Agradezco a la editorial por otorgar su permiso para reutilizar el material de este artículo en la preparación de esta tesis.

Keywords: antiproliferative effect, aziridines, 2*H*-azirines, merged hybrid compounds, phosphorus substituted azirino[2,1-*b*]benzo[*e*][1,3]oxazines.

Abstract:

This work describes a straightforward diastereoselective synthetic access to azirino[2,1-*b*]benzo[*e*][1,3]oxazines containing phosphorus substituents such as phosphonate or phosphine oxide, by means of nucleophilic addition of functionalized phenols to the C–N double bond of 2*H*-azirine derivatives. In addition, the cytotoxic effect on cell lines derived from human lung adenocarcinoma (A549) and human embryonic kidney (HEK293) was also screened. Some azirino[2,1-*b*]benzo[*e*][1,3]oxazines **4** and **6** exhibited very good activity against the A549 cell line *in vitro*. Furthermore, selectivity towards cancer cell (A549) over (HEK293), and non-malignant cells (MCR-5) has been detected.

1. Introduction

In the increasing field of irreversible inhibitor drug discovery, there is a new revival of the interest in covalent binding drugs owing to the FDA approval of safe and efficient covalent therapeutics and a better understanding of the profits of the covalent binding mechanism.^{1,2} These facts are making irreversible inhibitors a robust comeback in cancer therapeutics.^{3,4} Covalent inhibitors own many advantages over reversible inhibitors, including less frequent drug dosing due to the prolonged interval of action, improved biochemical efficiency, the potential to prevent determined drug resistance mechanisms, and a lower effective dose may result in high potential for greater therapeutic index.⁵ In recent times, with the growth of targeted covalent inhibitors, many new drugs contain electrophilic moieties acting as “warheads”. Several molecules with a diversity of electrophilic warheads, including ketone, ester, nitrile, α,β -unsaturated carbonyl, epoxide, or aziridine functionalities have been recognized as covalent inhibitors.⁶ Therefore, obtaining a scaffold with an appropriate platform for insertion of an electrophilic moiety at the right trajectory relative to the nucleophilic partner is being used as a further challenge.

The aziridine three-membered ring is a structural component found in a wide range of biologically active agents, natural products, and related compounds. Aziridine-based inhibitors displayed high selectivity for parasite protozoan cysteine protease,⁷ while aziridine-2,3-dicarboxylate was used as trypanocidal agents targeting the major cysteine protease of

Trypanosoma brucei.⁸ In addition, a biotinylated dibenzylaziridine-2,3-dicarboxylate was designed by Leippe *et al.*⁹ as an irreversible cysteine protease inhibitor to target the malaria parasite *Plasmodium falciparum*. The antitumor and antibiotic properties of some of these compounds, including azinomycin B,^{10,11} mitomycin C,^{12,13} maduropeptin,¹⁴ and FR-900482,¹⁵ a close cousin of mitomycin C since they share a similar structural analogy, are widely known. The therapeutic applications of some others are more varied.¹⁶ For instance, ficellomycin¹⁷ displays high *in vivo* activity against Gram-positive bacteria, as well as, multidrug resistant strains of *Staphylococcus aureus*; however, azicemycin^{18,19} shows inhibitory activities toward Gram-negative bacteria and mycobacterias (Figure 1).

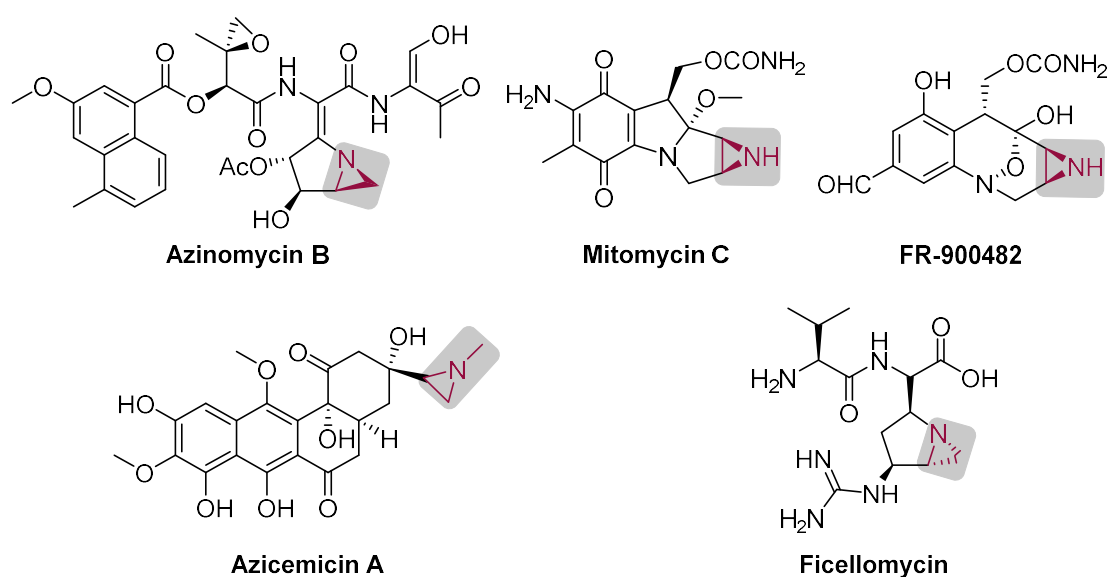


Figure 1. Representative examples of covalent drugs with aziridine warheads. Electrophiles are highlighted.

Mitomycins are a class of pharmaceuticals with very potent antibacterial and anti-cancer activity. For instance, mitomycin C (Figure 1) has been in extensive clinical use for more than 20 years for its activity against bladder tumors, stomach, breast, esophagus, colorectal, and non-small-cell lung cancers.²⁰ This class of chemotherapy agents are the classical bioreductive drugs integrating a quinone moiety for reductive activation *in vivo* and subsequent electrophilic sites for DNA alkylation and covalent cross-linking. The aziridine ring present in these chemotherapeutics seems to cause DNA monoalkylation *via* acid-activation to produce a protonated aziridine that ring opens relieving the strain related to the three-membered ring.^{21,22} Therefore, aziridines are potent alkylating agents which may act as covalent drugs through their

capability to act as DNA cross-linking agents *via* nucleophilic ring opening of the three-membered heterocycle.²³

To overcome the drastic side effects related to a single drug, the development of hybrid molecules introducing two or more potentially pharmacophores to concurrently modulate several targets of multifactorial diseases, have been recognized as a popular approach for multidrug therapy.²⁴ Whereas aziridine compounds are valuable as reactive building blocks on their own,^{25,26,27} several aziridine-containing products have proved to possess biological activity, essentially owing to the presence of the aziridine moiety. Moreover, from a biological viewpoint, organophosphorus compounds are fascinating compounds as it is known that phosphorus substituents may modify the reactivity of heterocycles and control important biological functions,²⁸ including new antileishmanial²⁹ and antiproliferative agents.^{30,31} Strategies for incorporation of organophosphorus functionalities in adequate synthons have been extensively applied in the synthesis of phosphorylated azaheterocycles,^{32,33} aminophosphonates,³⁴ or phosphinates.³⁵ Likewise, the benzoxazine framework is present in many active compounds of biological relevance, such as anti-malarials,³⁶ anti-mycobacterials,³⁷ anti-inflammatory,³⁸ as well as potent inhibitors of methionyl-tRNA synthetase (MRS)³⁹ or DNA-PK enzyme with antiproliferative activity.⁴⁰

Considering these facts, here we wish to account the synthesis of new [1,3]benzoxazine-aziridine merged hybrids with phosphorus containing groups such as phosphine oxide or phosphonate, through nucleophilic addition of functionalized salicylaldehydes to 2*H*-azirines. These new hybrid molecules, which may retain the functional properties of the parent molecules, are obtained by overlapping of [1,3]benzoxazine and aziridine frameworks, directly connected to the phosphorus substituent (Figure 2). Besides, these new hybrid molecules were

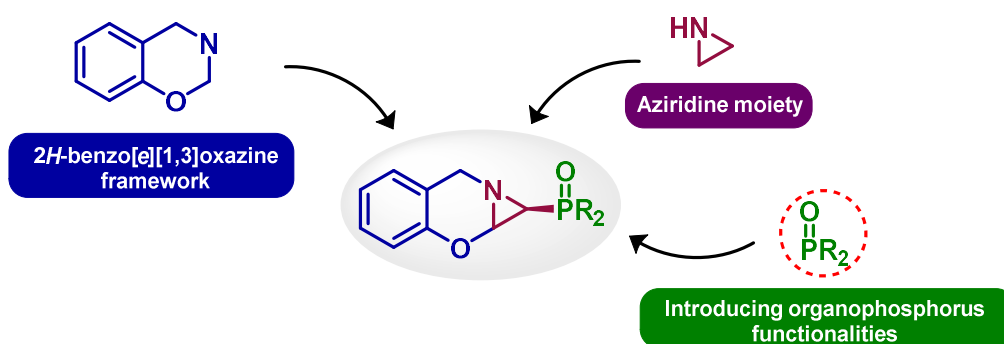


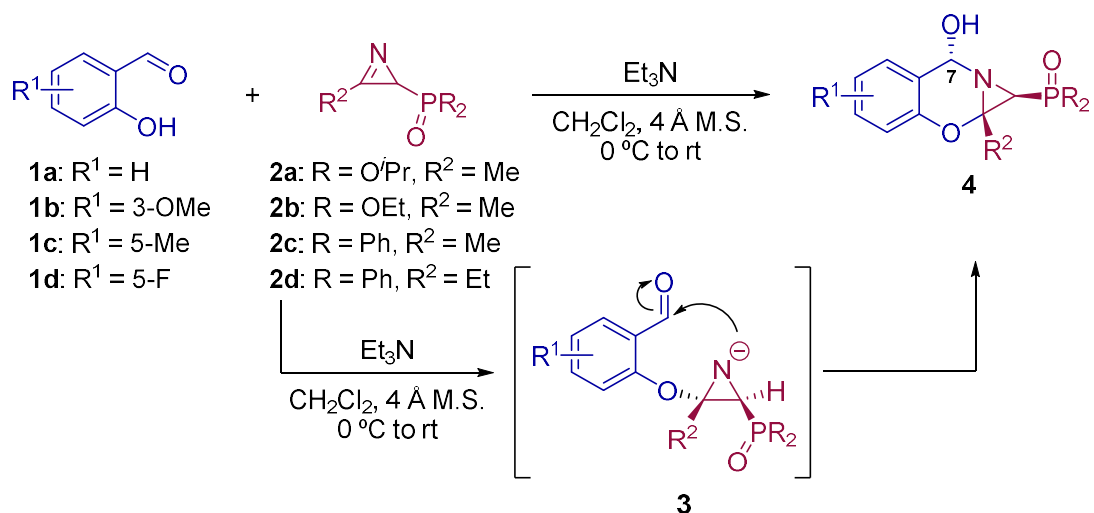
Figure 2. Phosphorylated azirino[2,1-*b*]benzo[e][1,3]oxazines as new merged hybrid molecules.

tested for antiproliferative activity against different human cancer cell lines. Owing to the inherent interest of these new hybrids, both in synthetic and medicinal chemistry, this entails an interesting challenge. As far as we know, this is the first example of the preparation of azirino[2,1-*b*]benzo[*e*][1,3]oxazines and their study as antiproliferative agents.

2. Results and discussion

2.1. Chemistry

At first, we anticipated that nucleophilic addition of functionalized salicylaldehydes **1** to phosphorus substituted 2*H*-azirines **2**, would provide a useful approach to the synthesis of functionalized azirino-benzoxazine derivatives **4** with phosphorus substituents at C-1 (Scheme 1). Therefore, the reaction of 2*H*-azirines **2** with salicylaldehyde derivatives **1** was assessed. Thus, as outlined in Scheme 1, in an initial experiment the nucleophilic addition of salicylaldehyde **1a** ($R^1 = H$) to 2*H*-azirine-phosphate **2a** ($R^2 = Me$, $R = O^iPr$) was readily achieved using Et_3N in dichloromethane at room temperature. Two equivalents of the salicylaldehyde component were used in order to ensure full conversion. Under these reaction conditions, the azirino-benzoxazine derivative **4a** was apparently obtained as a mixture of two isomers in a ratio of 71:29 (Table 1, entry 1), as evidenced by the presence of two sets of signals in 1H and ^{31}P NMR spectra.



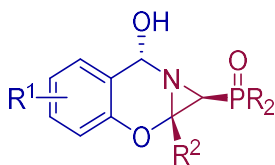
Scheme 1. Synthesis of azirino[2,1-*b*]benzo[*e*][1,3]oxazine phosphonates and phosphine oxides.

We have recently reported a diastereoselective synthetic methodology for the preparation of phosphorus substituted cyanoaziridines.⁴¹ These compounds have been obtained as a mixture of two rotamers attributed to the different geometries around the phosphorus atom. Assuming we could obtain a mixture of rotamers around the P–C bond of azirino-benzoxazine derivative **4a**, as before, we achieve variable-temperature ¹H NMR and ³¹P NMR studies for **4a** (R = OⁱPr, R¹ = H, R² = Me). These studies supported the hypothesis that the doubling of signals in ¹H, ³¹P, and ¹³C NMR spectra was due to the presence of two rotamers of compound **4a**, since both rotamers interchange by rotation around the P–C bond (see supplementary data). Variable-temperature ³¹P NMR study for **4a** in DMSO-*d*₆ showed a mixture of two isomers in a ratio of 81:19 at 18 °C. However, this mixture tend to equilibrate at higher temperatures since at 80 °C the ratio of rotamers changed to 62:38. Similar results has been observed for variable-temperature ¹H NMR study (see supplementary data). A slight difference in rotamers ratio can be detected by changing the solvent from CDCl₃ to DMSO-*d*₆ (see Table 1, entry 1). The fact that two rotamers were observed may be connected to the different geometries around the phosphorus atom, as previously observed in a benzofulvene-phosphine oxide,⁴² or more recently in a DFT study of the most stable rotamer of an aziridine-2-carboxylate.⁴³

The absence of coupling constant observed in ¹³C NMR spectrum between the di(*iso*-propyl) phosphonate substituent at C–1 and the methyl group at C–1a of azirino-benzoxazine **4a**, appears to indicate a *syn*-relationship between both groups.⁴⁴ Conclusively, the X-ray diffraction analysis not only established the *syn*-relationship between the methyl group and the phosphorus atom, but also the *anti*-relationship between those groups and the hydroxyl group at C–7 (see supplementary data).

Starting from 2*H*-azirine-phosphonate **2a** and salicylaldehyde **1a**, azirino-benzoxazine derivative **4a** (R = OⁱPr, R¹ = H, R² = Me) was isolated in 81% yield (Scheme 1, Table 1, entry 1), while the addition of salicylaldehyde **1a** to functionalized 2*H*-azirine-phosphonate **2b** provide 76% of azirino-benzoxazine derivative **4b** (R = OEt, R¹ = H, R² = Me) (Scheme 1, Table 1, entry 2).

A reasonable mechanism for the construction of heterocycles **4** can be explained through initial nucleophilic addition of functionalized phenol **1** at the carbon-nitrogen double bond of 2*H*-azirine **2** to give intermediate **3**. This nucleophilic addition is expected to arise in a diastereoselective way through the less hindered face. Hence, the nearness of phenol group of functionalized salicylaldehyde **1** to the C–N double bond of 2*H*-azirine **2** on the other side of

Table 1. Azirino[2,1-*b*]benzo[*e*][1,3]oxazine phosphonates and phosphine oxides **4** obtained.

Entry	Compound	R	R ¹	R ²	Yield(%) ^[a]	Rotamers ratio ^[b]
1	4a	O ⁱ Pr	H	Me	81	71:29 (81:19) ^[c]
2	4b	OEt	H	Me	76	70:30
3	4c	Ph	H	Me	55	76:24
4	4d	Ph	H	Et	54	81:19
5	4e	Ph	3-OMe	Me	80	76:24
6	4f	Ph	3-OMe	Et	51	74:26
7	4g	Ph	5-Me	Me	84	75:25
8	4h	Ph	5-Me	Et	75	74:26
9	4i	Ph	5-F	Me	96	92:8

^[a] Yield of isolated purified compounds **4**. ^[b] Rotamers ratio calculated by ¹H NMR in CDCl₃ solutions.

^[c] Rotamers ratio calculated by ¹H NMR in DMSO-*d*₆ solutions.

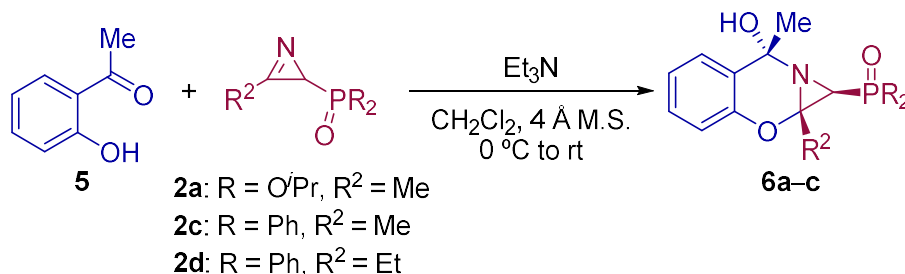
the phosphonate group appears to be more favorable, due to the high exocyclic dihedral angle of the sp³ hybridized saturated carbon and the presence of the large phosphorus group. We have previously observed this remark in the nucleophilic addition of Grignard reagents,^{44,45} hydrides,⁴⁶ alcohols,⁴⁵ or cyanide anion⁴¹ to *2H*-azirines. After the nucleophilic addition, the construction of tricyclic azirino-benzoxazine framework in derivative **4** takes place through a diastereoselective intramolecular nucleophilic attack of the aziridine moiety to the carbonyl group of intermediate **3**, in a similar way to the previously reported for the reaction of *2H*-azirine with enolates derived from β-keto esters.⁴⁷

This process was extended to the nucleophilic addition of salicylaldehyde **1a** to *2H*-azirine-phosphine oxides **2c,d** (R = Ph) under the same reaction conditions (Scheme 1). Azirino-benzoxazines **4c** (R = Ph, R¹ = H, R² = Me, Table 1, entry 3) and **4d** (R = Ph, R¹ = H, R² = Et, Table 1, entry 4) were obtained in moderate chemical yields and as a mixture of two rotamers. Further investigation of the process revealed that this strategy is also suitable to other salicylaldehyde derivatives **1**. As illustrated in Table 1, the method is tolerant of a variety of functionalized

salicylaldehydes **1** with varying substitutions. For instance, 2*H*-azirine-phosphine oxides **2c,d** (R = Ph) reacted with *o*-vanillin **1b** (R¹ = OMe), 5-methylsalicylaldehyde **1c** (R¹ = Me), or 5-fluorosalicylaldehyde **1d** (R¹ = F) in the presence of Et₃N, producing the corresponding azidiny-benzoxazines **4e-i** (see Table 1, entries 5–9).

In order to define the scope and limitations of the nucleophilic addition of functionalized phenols to 2*H*-azirines **2** and increase the diversity of substituents in our substrates, this diastereoselective approach was extended to include the reactivity of 2-hydroxyacetophenone **5** toward phosphorus substituted 2*H*-azirines **2**. For this purpose, the nucleophilic addition of **5** to 2*H*-azirine phosphonate **2a** and phosphine oxides **2c,d** were performed using Et₃N as the base in CH₂Cl₂ to yield derivatives **6a-c** in moderate yields (Table 2). These new azirino-benzoxazines **6** were obtained, as before, as a mixture of two rotamers in a ratio around 85:15. A similar configuration to that found in the case of salicylaldehydes was observed for azirino-benzoxazine **6a** and was unambiguously proved by X-ray diffraction analysis (see supplementary data).

Table 2. Azirino[2,1-*b*]benzo[*e*][1,3]oxazine phosphonate and phosphine oxides **6** obtained.



Entry	Compound	R	R ²	Yield(%) ^[a]	Rotamers ratio ^[b]
1	6a	<i>O</i> ^{<i>i</i>} Pr	Me	35	85:15 (~100:0) ^[c]
2	6b	Ph	Me	57	85:15
3	6c	Ph	Et	62	87:13

^[a] Yield of isolated purified compounds **6**. ^[b] Rotamers ratio calculated by ¹H NMR in CDCl₃ solutions. ^[c] Rotamers ratio calculated by ¹H NMR in DMSO-*d*₆ solutions.

This methodology describes a straightforward diastereoselective route to functionalized azirino[2,1-*b*]benzo[*e*][1,3]oxazine phosphonate and phosphine oxides **4** and **6** by means nucleophilic addition of functionalized phenol, such as salicylaldehyde derivatives **1** and 2-

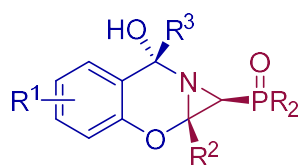
hydroxyacetophenone **5**, to phosphorus substituted 2*H*-azirines **2**. To our knowledge, this approach represents the first example of an azirino[2,1-*b*]benzo[*e*][1,3]oxazine.

2.2. Biological results

In vitro cytotoxicity of our newly prepared phosphorylated azirino[2,1-*b*]benzo[*e*][1,3]oxazines **4** and **6** was assessed by testing their antiproliferative activities against two different human cancer cell lines: A549 (carcinomic human alveolar basal epithelial cells) and HEK-293 (human embryonic kidney cells). Cell counting kit (CCK-8) assay was applied to evaluate growth inhibition. Table 3 displays cell proliferation inhibitory activities as IC₅₀ values of all synthesized compounds and chemotherapeutic doxorubicin (DOX).

As reported in table 3, tested compounds exhibited a wide spectrum of antiproliferative activity against the cancer cell lines tested in culture. In general, compounds **4** and **6** presented a greater selective cytotoxicity in the human lung adenocarcinoma cell line (A549) than in the human embryonic kidney cell line (HEK-293). Likewise, healthy lung cells, such as MRC-5 non-malignant lung fibroblasts were tested for studying selective cytotoxicity,⁴⁸ and as outlined in table 3, none of the synthesized compounds **4** and **6** exhibited any toxicity toward MRC-5 cells.

Regarding the new azirino-benzoxazines **4** against A549 cell line *in vitro*, diisopropyl **4a** and diethyl azirino-benzoxazine phosphonate **4b** showed dissimilar IC₅₀ values between 3.0 ± 0.29 and 15.8 ± 2.44 μM with the most effective compound being diisopropyl azirino-benzoxazine **4a** (R = O^{*i*}Pr, R¹ = R³ = H, R² = Me) (Table 3, compare entries 1 and 2). As previously reported,⁴⁹ the presence of a bulky group at the phosphonate ester moiety give rise to an increased activity. Azirino-benzoxazine phosphine oxides **4c–i** (Table 3, entries 3–10) showed IC₅₀ values between 1.3 ± 0.22 and 20.7 ± 3.75 μM with the most effective derivative being azirino-benzoxazine phosphine oxide **4e** (R = Ph, R¹ = 3-OMe, R² = Me, R³ = H) with an IC₅₀ value of 1.3 ± 0.22 μM . Concerning the effect of the substitution at C–1a, in general, compounds with a methyl group at C–1a (R² = Me, **4c**, **4e**, and **4g**) showed better activities than those with an ethyl group (R² = Et, **4d**, **4f**, and **4h**), independently of the substitution at the aromatic ring (R¹ = H, 3-OMe, 5-Me). Furthermore, very high selectivity has been noted in the antiproliferative activity of the novel azirino-benzoxazine phosphonates **4a,b** and phosphine oxides **4c–i** against the A549 *versus* HEK-293 cell line.

Table 3. Antiproliferative activity of azirino[2,1-*b*]benzo[*e*][1,3]oxazines **4** and **6**.

Entry	Comp.	R	R ¹	R ²	R ³	Cytotoxicity IC ₅₀ (μM) ^[a]		
						Lung A549	Kidney HEK-293	MRC-5
1	DOX	–	–	–		0.48 ± 0.017 ⁵⁰	5.68 ± 1.56 ⁵¹	>50 ⁵²
2	4a	<i>O</i> ⁱ Pr	H	Me	H	3.0 ± 0.29	>50	>50
3	4b	OE _t	H	Me	H	15.8 ± 2.44	n.r. ^[b]	>50
4	4c	Ph	H	Me	H	2.7 ± 0.37	>50	>50
5	4d	Ph	H	Et	H	10.4 ± 0.68	>50	>50
6	4e	Ph	3-OMe	Me	H	1.3 ± 0.22	>50	>50
7	4f	Ph	3-OMe	Et	H	20.7 ± 3.75	>50	>50
8	4g	Ph	5-Me	Me	H	3.2 ± 0.80	>50	>50
9	4h	Ph	5-Me	Et	H	12.9 ± 0.90	>50	>50
10	4i	Ph	5-F	Me	H	19.6 ± 0.24	>50	>50
11	6a	<i>O</i> ⁱ Pr	H	Me	Me	10.3 ± 1.67	>50	>50
12	6b	Ph	H	Me	Me	21.1 ± 2.44	>50	>50
13	6c	Ph	H	Et	Me	3.2 ± 0.27	>50	>50

^[a] The cytotoxicity IC₅₀ values listed are the concentrations corresponding to 50% growth inhibition. ^[b] Not reported.

Afterwards, we studied the C–7 substitution effect of the corresponding azirino-benzoxazine phosphonate and phosphine oxides **6** (Table 3, entries 11–13) into their cytotoxicity against A549 cell lines. However, unlike the C–1a substitution effect observed in derivatives **4**, compounds **6** showed different behavior with IC₅₀ values between 3.2 ± 0.27 and 21.1 ± 2.44 μM, being azirino-benzoxazine **6c** (R = Ph, R¹ = H, R² = Et, R³ = Me) the most effective derivative with an IC₅₀ value of 3.2 ± 0.27 μM (Table 3, entry 13). Diisopropyl azirino-benzoxazine phosphonate **6a** (R³ = Me, Table 3, entry 11) resulted less active than the corresponding derivative **4a** (R³ = H, Table 3, entry 1). Likewise, as before, very high selectivity was detected in cytotoxic properties of the novel azirino-benzoxazines **6** against A549 *versus* HEK293 cell line. Additionally, MRC-5 non-malignant lung fibroblasts were tested for exploring selective toxicity,⁴⁸

and none of the synthesized phosphorylated azirino-benzoxazines **4** o **6** or doxorubicin exhibited any toxicity toward MRC-5 cell line (see Table 3).

3. Conclusions

In conclusion, the development of a new straightforward diastereoselective approach to azirino[2,1-*b*]benzo[*e*][1,3]oxazines containing phosphorus substituents such as phosphonate or phosphine oxide, through the nucleophilic addition of functionalized phenol derivatives to the carbon-nitrogen double bond of 2*H*-azirines are reported. As far as we know, this method represents the first example of the preparation of the tricyclic azirino[2,1-*b*]benzo[*e*][1,3]oxazine core, making possible the introduction of structural diversity at positions C-1, C-1a, C-7, and the aromatic ring of the resulting tricyclic framework depending on the starting 2*H*-azirine and phenol derivative.

The therapeutic efficiency of all the synthesized benzo-oxazines was evaluated against two different human cancer cell lines A549 and HEK-293. Some of the newly prepared benzo-oxazines exhibited very good cytotoxic effect inhibiting the growth of human tumor cell line A549. The best cytotoxic effects were observed for azirino[2,1-*b*]benzo[*e*][1,3]oxazine phosphine oxides **4e** with an IC₅₀ value of $1.3 \pm 0.22 \mu\text{M}$, as well as for **4c** with an IC₅₀ value of $2.7 \pm 0.37 \mu\text{M}$, and for azirino[2,1-*b*]benzo[*e*][1,3]oxazine phosphonate **4a** with an IC₅₀ value of $3.0 \pm 0.29 \mu\text{M}$. Whereas kidney cancer is not so sensitive to the synthesized benzo-oxazines. Additionally, cytotoxic effect of our compounds in non-malignant lung fibroblasts (MRC-5) seems not to present any effect. These preliminary results suggested that some of these new benzo-oxazines exhibited a significant antiproliferative effect by inhibiting the growth of human tumor cell lines.

4. Experimental section

4.1. Chemistry

4.1.1. General experimental information

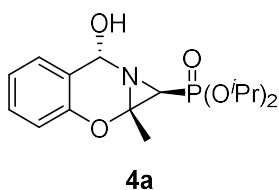
Solvents for extraction and chromatography were of technical grade. All solvents used in reactions were freshly distilled and dried over molecular sieves 4 Å before use. All other solvents and reagents were obtained from commercial sources and recrystallized or distilled as necessary or used without further purification. All reactions were performed under an atmosphere of dry nitrogen. Melting points are uncorrected. IR spectra were measured on a Nicolet iS10 Thermo Scientific spectrometer as neat solids. Absorbance frequencies are given at maximum of intensity in cm^{-1} . High-resolution mass spectra (HRMS) were obtained by positive-ion electrospray ionization (ESI) method with a time of flight Q-TOF system. Data are reported in the form m/z (intensity relative to base = 100). ^1H (300, 400 MHz), ^{13}C (75, 100 MHz), ^{19}F (282 MHz), and ^{31}P NMR (120, 160 MHz) spectra were recorded on a Varian Unity Plus (300 MHz) or Bruker Avance 400 (400 MHz) spectrometers, respectively, in CDCl_3 or $\text{DMSO}-d_6$, as specified below. Chemical shifts (δ_{H}) are reported in parts per million (ppm) with the internal chloroform signal at 7.24 ppm or the internal DMSO signal at 2.50 ppm as standard for ^1H NMR. Chemical shifts (δ_{C} and δ_{P}) are reported in parts per million (ppm) with the internal chloroform signal at 77.0 ppm or the internal DMSO signal at 39.5 ppm as standard for ^{13}C NMR; the external fluorotrichloromethane (CFCl_3) signal at 0.0 ppm as standard for ^{19}F NMR; or the external H_3PO_4 (50%) signal at 0.0 ppm as standard for ^{31}P NMR. All coupling constants (J) values are given in Hz. ^{19}F and ^{13}C NMR spectra were recorded in a broad band decoupled mode from hydrogen nuclei. Distortionless Enhanced Polarization Transfer (DEPT) supported peak assignments for ^{13}C NMR. The data is being reported as (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, bs = broad singlet). Chromatographic purification was performed as flash chromatography using commercial grades of silica gel finer than 230 mesh with pressure. Analytical thin layer chromatography (TLC) was performed on precoated Merck silica gel 60 F₂₅₄ TLC aluminium plates, and spot visualized with UV light or permanganate stain. 2*H*-Azirines **2** were prepared according to literature procedures.^{41,46a,c}

4.1.2. Compounds Purity Analysis

All synthesized compounds were analyzed by HPLC to determine their purity. The analyses were performed on Agilent 1260 infinity HPLC system (C-18 column, Hypersil, BDS, 5 μ m, 0.4 mm \times 25 mm) at room temperature. All the tested compounds were dissolved in dichloromethane, and 5 μ L of the sample was loaded onto the column. Ethanol and heptane were used as mobile phase, and the flow rate was set at 1.0 mL/min. The maximal absorbance at the range of 190–400 nm was used as the detection wavelength. The purity of all the tested azirino-benzoxazine derivatives **4** and **6** is >95%, which meets the purity requirement by the Journal.

4.1.3. Experimental procedure and characterization data for compounds **4** and **6**.

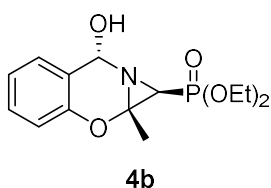
4.1.3.1. General procedure and spectral data for the preparation of functionalized azirino[2,1-*b*]benzo[*e*][1,3]oxazine derivatives **4.** To a 0 °C solution of 2*H*-azirine (5 mmol, 1 eq) **2** in CH₂Cl₂ (25 mL) and 4 Å M.S., was added dropwise functionalized salicylaldehyde **1** (10 mmol, 2 eq) and Et₃N (1.39 mL, 10 mmol, 2 eq) under a nitrogen atmosphere. The reaction mixture was allowed to reach room temperature and stirred at the same temperature until TLC showed the disappearance of 2*H*-azirine **2** (6–8 h). 4 Å M.S. was filtered through a sintered glass vacuum filtration funnel with celite and washed with CH₂Cl₂. The reaction mixture was washed with water (3 \times 15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layers were dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum. The crude product was purified by crystallization to afford the corresponding azirino-benzoxazine derivatives **4**.



Diisopropyl ((1*S,1*aS**,7*S**)-7-hydroxy-1*a*-methyl-1,1*a*-dihydro-7*H*-azirino[2,1-*b*]benzo[*e*][1,3]oxazin-1-yl)phosphonate (**4a**).** (1.38 g, 81%) as a pale yellow solid from 2*H*-azirine **2a** (1.10 g, 5 mmol, 1 eq) and salicylaldehyde **1a** (1.06 mL, 10 mmol, 2 eq) as described in the general procedure. The crude product was purified by crystallization

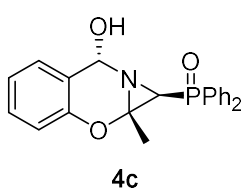
from hexane/Et₂O (50:50) to afford the title compound **4a**. mp 110–112 °C; IR (neat) ν_{max} , 3288, 2974, 2933, 1464, 1236, 983 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.50–6.79 (m, 4H, ArH), 5.81 (d, ³J_{HH} = 4.3 Hz, 1H, CHOH)_{major}, 5.64 (bs, 1H, CHOH)_{minor}, 4.97 (bs, 1H, OH)_{major}, 4.76–4.66 (m, 2H, OCH), 4.11 (s, 1H, OH)_{minor}, 2.53 (d, ²J_{PH} = 11.9 Hz, 1H, CH-P)_{major}, 2.24 (d, ²J_{PH} = 12.7 Hz, 1H, CH-P)_{minor}, 1.87 (s, 3H, CH₃)_{minor}, 1.85 (s, 3H, CH₃)_{major}, 1.33–1.27 (m, 12H, CH(CH₃)₂) ppm; ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 148.8_{minor}, 148.5_{major}, 130.8_{minor}, 129.8_{major}, 129.0_{minor}, 127.3_{major},

123.2_{minor}, 123.1_{major}, 120.8_{major}, 119.7_{minor}, 117.4_{minor}, 116.5_{major} (C_{Ar}), 81.0 (d, $^3J_{PC} = 7.3$ Hz, CH)_{minor}, 76.8 (d, $^3J_{PC} = 5.0$ Hz, CH)_{major}, 73.5 (d, $^2J_{PC} = 3.8$ Hz, C_{quat})_{major}, 73.2 (d, $^2J_{PC} = 4.6$ Hz, C_{quat})_{minor}, 71.4 (d, $^2J_{PC} = 6.4$ Hz, CH)_{major}, 71.1 (d, $^2J_{PC} = 6.4$ Hz, CH)_{minor}, 33.6 (d, $^1J_{PC} = 210.5$ Hz, CH)_{minor}, 30.3 (d, $^1J_{PC} = 210.6$ Hz, CH)_{major}, 24.2 (CH₃), 24.2 (CH₃), 24.1 (CH₃), 24.1 (CH₃), 20.3_{minor}, 20.0_{major} ppm; ^{31}P NMR (120 MHz, CDCl₃) δ 19.5_{major}, 18.8_{minor} ppm; ESI-HRMS (CI) m/z calcd. for C₁₆H₂₅NO₅P ([M+H]⁺) 342.1470, found 342.1471.



Diethyl ((1S*,1aS*,7S*)-7-hydroxy-1a-methyl-1,1a-dihydro-7H-azirino[2,1-b]benzo[e][1,3]oxazin-1-yl)phosphonate. (4b). (1.18 g, 76%) as a pale yellow solid from 2H-azirine **2b** (1.00 g, 5 mmol, 1 eq) and salicylaldehyde **1a** (1.06 mL, 10 mmol, 2 eq) as described in the general procedure. The crude product was purified by crystallization

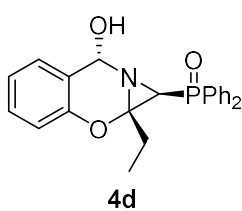
from Et₂O to afford the title compound **4b**. mp 134–136 °C; IR (neat) ν_{max} 3423, 2981, 2934, 1613, 1466, 1238, 1024, 974 cm⁻¹; ^1H NMR (400 MHz, CDCl₃) δ 7.52–6.83 (m, 4H, ArH), 5.84 (d, $^3J_{\text{HH}} = 4.5$ Hz, 1H, CHOH)_{major}, 5.70 (bs, 1H, CHOH)_{minor}, 4.89 (d, $^3J_{\text{HH}} = 4.8$, 1H, OH)_{major}, 4.22–4.12 (m, 4H, OCH₂), 4.01 (d, $^3J_{\text{HH}} = 3.5$, 1H, OH)_{minor}, 2.61 (d, $^2J_{\text{PH}} = 12.4$ Hz, 1H, CH-P)_{major}, 2.31 (d, $^2J_{\text{PH}} = 13.1$ Hz, 1H, CH-P)_{minor}, 1.89 (s, 3H, CH₃), 1.34–1.30 (m, 6H, CH₂(CH₃)₂) ppm; ^{13}C { ^1H } NMR (75 MHz, CDCl₃) δ 148.4_{minor}, 148.1_{major}, 130.4_{minor}, 129.6_{major}, 128.8_{minor}, 127.1_{major}, 123.0_{minor}, 122.9_{major}, 120.4_{major}, 119.4_{minor}, 117.1_{minor}, 116.2_{major} (C_{Ar}), 80.5 (d, $^3J_{PC} = 7.1$ Hz, CH)_{minor}, 76.3 (d, $^3J_{PC} = 4.7$ Hz, CH)_{major}, 73.3 (d, $^2J_{PC} = 4.0$ Hz, C_{quat})_{major}, 72.9 (d, $^2J_{PC} = 4.7$ Hz, C_{quat})_{minor}, 62.6 (d, $^2J_{PC} = 6.2$ Hz, CH₂)_{major}, 62.4 (d, $^2J_{PC} = 3.0$ Hz, CH₂)_{minor}, 62.3 (d, $^2J_{PC} = 2.5$ Hz, CH₂)_{minor}, 62.1 (d, $^2J_{PC} = 6.4$ Hz, CH₂)_{major}, 32.5 (d, $^1J_{PC} = 209.8$ Hz, CH)_{minor}, 29.2 (d, $^1J_{PC} = 209.0$ Hz, CH)_{major}, 20.2 (CH₃)_{minor}, 19.9 (CH₃)_{major}, 16.3 (CH₃), 16.2 (CH₃) ppm; ^{31}P NMR (120 MHz, CDCl₃) δ 21.4_{major}, 20.8_{minor} ppm; ESI-HRMS (CI) m/z calcd. for C₁₄H₂₁NO₅P ([M+H]⁺) 314.1157, found 314.1164.



((1S*,1aS*,7S*)-7-Hydroxy-1a-methyl-1,1a-dihydro-7H-azirino[2,1-b]benzo[e][1,3]oxazin-1-yl)diphenylphosphine oxide (4c). (1.03 g, 55%) obtained as a white solid from 2H-azirine **2c** (1.27 g, 5 mmol, 1 eq) and salicylaldehyde **1a** (1.06 mL, 10 mmol, 2 eq) as described in the general procedure. The crude product was purified by crystallization from

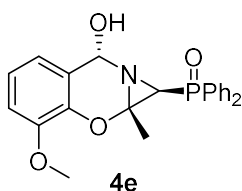
hexane/CH₂Cl₂ (50:50) to afford the title compound **4c**. mp 161–163 °C; IR (neat) ν_{max} 3243, 2930, 1587, 1482, 1461, 1438, 1176, 1119, 973, 735 cm⁻¹; ^1H NMR (400 MHz, DMSO) δ 7.83–6.74 (m, 14H, ArH), 5.72 (d, $^3J_{\text{HH}} = 4.9$ Hz, 1H, CHOH)_{major}, 5.37 (d, $^3J_{\text{HH}} = 5.3$ Hz, 1H, CHOH)_{minor}, 2.96 (d, $^2J_{\text{PH}} = 23.1$ Hz, 1H, CH-P)_{minor}, 2.93 (d, $^2J_{\text{PH}} = 19.8$ Hz, 1H, CH-P)_{major}, 1.80 (s, 3H, CH₃)_{minor}, 1.67 (s, 3H, CH₃)_{major} ppm; ^{13}C { ^1H } NMR (100 MHz, DMSO) δ 148.3_{minor}, 148.2_{major}, 134.2, 133.6, 133.2,

133.2, 132.8, 132.6, 132.2, 132.0, 131.9, 131.8, 131.8, 131.7, 131.7, 131.5, 131.4, 131.0, 130.9, 130.6, 130.5, 130.5, 130.4, 130.0, 130.0, 129.6, 129.0, 128.9, 128.8, 128.8, 128.7, 128.6, 128.3, 128.2, 127.1, 122.8_{major}, 122.7_{minor}, 121.1_{major}, 121.0_{minor}, 116.4_{minor}, 116.0_{major} (C_{Ar}), 79.4 (d, ³J_{PC} = 5.0 Hz, CH)_{minor}, 75.7 (d, ³J_{PC} = 3.8 Hz, CH)_{major}, 73.7 (d, ²J_{PC} = 5.4 Hz, C_{quat})_{minor}, 73.4 (d, ²J_{PC} = 4.7 Hz, C_{quat})_{major}, 35.2 (d, ¹J_{PC} = 106.3 Hz, CH)_{minor}, 32.4 (d, ¹J_{PC} = 108.2 Hz, CH)_{major}, 20.2 (CH₃)_{minor}, 19.8 (CH₃)_{major} ppm; ³¹P NMR (160 MHz, DMSO) δ 22.1_{major}, 21.9_{minor} ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₂H₂₁NO₃P ([M+H]⁺) 378.1259, found 378.1252.



((1S*,1aS*,7S*)-1a-Ethyl-7-hydroxy-1,1a-dihydro-7H-azirino[2,1-b]benzo[e][1,3]oxazin-1-yl)diphenylphosphine oxide (4d). (1.05 g, 54%) as a white solid from 2*H*-azirine **2d** (1.34 g, 5 mmol, 1 eq) and salicylaldehyde **1a** (1.06 mL, 10 mmol, 2 eq) as described in the general procedure. The crude product was purified by crystallization from

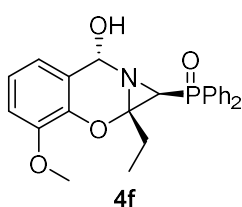
hexane/Et₂O (50:50) to afford the title compound **4d**. mp 133–135 °C; IR (neat) ν_{\max} 3227, 3060, 2971, 2939, 1673, 1464, 1438, 1182, 1122, 732 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.85–6.80 (m, 14H, ArH), 5.86 (bs, 1H, OH), 5.74 (s, 1H, CHOH)_{major}, 5.54 (s, 1H, CHOH)_{minor}, 3.06 (d, ²J_{PH} = 21.6 Hz, 1H, CH-P)_{major}, 2.70 (d, ²J_{PH} = 22.6 Hz, 1H, CH-P)_{minor}, 2.24–2.06 (m, 2H, CH₂), 1.11 (t, ³J_{HH} = 7.36 Hz, 3H, CH₃)_{minor}, 1.03 (t, ³J_{HH} = 7.35 Hz, 3H, CH₃)_{major} ppm; ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 148.9_{minor}, 148.6_{major}, 132.9, 132.7, 132.7, 132.1, 132.0, 132.0, 131.8, 131.8, 131.7, 131.7, 131.6, 131.2, 131.1, 131.1, 131.1, 131.0, 131.0, 130.5, 129.5, 129.0, 128.8, 128.7, 128.7, 128.7, 128.6, 128.5, 128.4, 128.3, 127.6, 122.9_{minor}, 122.7_{major}, 120.5_{major}, 119.1_{minor}, 117.2_{minor}, 116.1_{major} (C_{Ar}), 80.1 (d, ³J_{PC} = 4.9 Hz, CH)_{minor}, 77.4 (d, ²J_{PC} = 4.3 Hz, C_{quat})_{major}, 77.4 (d, ²J_{PC} = 4.3 Hz, C_{quat})_{minor}, 76.7 (d, ³J_{PC} = 3.4 Hz, CH)_{major}, 36.3 (d, ¹J_{PC} = 106.7 Hz, CH)_{minor}, 33.5 (d, ¹J_{PC} = 107.5 Hz, CH)_{major}, 26.8 (CH₂)_{major}, 9.1 (CH₃)_{major}, 9.1 (CH₃)_{minor} ppm; ³¹P NMR (120 MHz, CDCl₃) δ 25.0_{major}, 23.8_{minor} ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₃H₂₃NO₃P ([M+H]⁺) 392.1416, found 392.1410.



((1S*,1aS*,7S*)-7-Hydroxy-3-methoxy-1a-methyl-1,1a-dihydro-7H-azirino[2,1-b] benzo[e][1,3]oxazin-1-yl)diphenylphosphine oxide (4e). (1.63 g, 80%) as a white solid from 2*H*-azirine **2c** (1.27 g, 5 mmol, 1 eq) and *o*-vanillin **1b** (1.52 gr, 10 mmol, 2 eq) as described in the general procedure. The crude product was purified by crystallization from

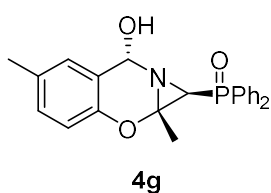
hexane/CH₂Cl₂ (50:50) to afford the title compound **4e**. mp 173–175 °C; IR (neat) ν_{\max} 3436, 3056, 2980, 2934, 1587, 1485, 1438, 1269, 1226, 1124, 1063 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.84–6.74 (m, 13H, ArH), 5.73 (d, ³J_{HH} = 4.0 Hz, 1H, CHOH)_{major}, 5.47 (d, ³J_{HH} = 3.0 Hz 1H, CHOH)_{minor}, 4.76 (d, ³J_{HH} = 4.2 Hz, 1H, OH)_{major}, 3.96 (d, ³J_{HH} = 3.2 Hz, 1H, OH)_{minor}, 3.83 (s, 3H, OCH₃)_{minor}, 3.81

(s, 3H, OCH₃)_{major}, 3.01 (d, ²J_{PH} = 21.7 Hz, 1H, CH-P)_{major}, 2.68 (d, ²J_{PH} = 22.2 Hz, 1H, CH-P)_{minor}, 1.95 (s, 3H, CH₃)_{minor}, 1.93 (s, 3H, CH₃)_{major} ppm; ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 148.3minor, 147.7major, 138.0minor, 137.7major, 132.6, 132.1, 132.1, 131.9, 131.9, 131.8, 131.7, 131.5, 131.3, 131.2, 131.2, 131.2, 128.9, 128.7, 128.6, 128.4, 128.3, 122.7, 122.3, 121.1, 120.3, 120.1, 118.9, 112.9, 111.8 (C_{Ar}), 80.5 (d, ³J_{PC} = 4.8 Hz, CH)_{minor}, 76.4 (d, ³J_{PC} = 3.4 Hz, CH)_{major}, 74.5 (d, ³J_{PC} = 4.6 Hz, C_{quat})_{major}, 74.2 (d, ³J_{PC} = 5.3 Hz, C_{quat})_{minor}, 56.2 (OCH₃)_{minor}, 56.1 (OCH₃)_{major}, 36.4 (d, ¹J_{PC} = 106.4 Hz, CH)_{minor}, 33.1 (d, ¹J_{PC} = 107.56 Hz, CH)_{major}, 20.5 (CH₃)_{minor}, 20.2 (CH₃)_{major} ppm; ³¹P NMR (160 MHz, CDCl₃) δ 25.5_{major}, 24.1_{minor} ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₃H₂₃NO₄P ([M+H]⁺) 408.1365, found 408.1352.



((1S*,1aS*,7S*)-1a-Ethyl-7-hydroxy-3-methoxy-1,1a-dihydro-7H-azirino[2,1-b]benzo[e][1,3]oxazin-1-yl) diphenylphosphine oxide (4f).

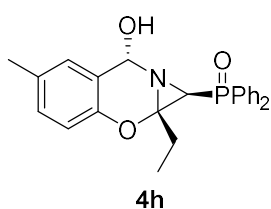
(1.08 g, 51%) as a yellow solid from 2*H*-azirine **2d** (1.34 g, 5 mmol, 1 eq) and *o*-vanillin **1b** (1.52 gr, 10 mmol, 2 eq) as described in the general procedure. The crude product was purified by crystallization from hexane/Et₂O (50:50) to afford the title compound **4f**. mp 145–147 °C; IR (neat) *v*_{max} 3240, 2971, 2930, 1594, 1482, 1438, 1270, 1163, 1059, 739 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.83–6.75 (m, 13H, ArH), 5.71 (d, ³J_{HH} = 3.2 Hz, 1H, CHOH)_{major}, 5.48 (bs, 1H, CHOH)_{minor}, 5.36 (d, ³J_{HH} = 3.9 Hz, 1H, OH)_{major}, 4.18 (bs, 1H, OH)_{minor}, 3.82 (s, 3H, OCH₃)_{minor}, 3.80 (s, 3H, OCH₃)_{major}, 3.07 (d, ²J_{PH} = 21.6 Hz, 1H, CH-P)_{major}, 2.72 (d, ²J_{PH} = 22.7 Hz, 1H, CH-P)_{minor}, 2.25–2.14 (m, 2H, CH₂), 1.13 (t, ³J_{HH} = 7.3 Hz, 3H, CH₃)_{minor}, 1.07 (t, ¹J_{HH} = 7.3 Hz, 3H, CH₃)_{major} ppm; ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 148.5_{minor}, 148.0_{major}, 138.6_{minor}, 138.2_{major}, 132.9, 132.9, 132.2, 132.1, 132.1, 132.0, 131.9, 131.9, 131.8, 131.8, 131.7, 131.4, 131.3, 131.3, 131.2, 131.2, 129.0, 128.9, 128.8, 128.8, 128.7, 128.7, 128.5, 128.4, 122.6_{minor}, 122.2_{major}, 121.3_{major}, 120.5_{minor}, 120.2_{minor}, 119.2_{major}, 113.1_{minor}, 112.2_{major} (C_{Ar}), 80.2 (d, ³J_{PC} = 4.9 Hz, CH)_{minor}, 78.0 (d, ³J_{PC} = 4.4 Hz, C_{quat})_{major}, 77.7 (d, ³J_{PC} = 5.2 Hz, C_{quat})_{minor}, 76.9 (d, ³J_{PC} = 3.5 Hz, CH)_{major}, 56.4 (OCH₃)_{major}, 56.4 (OCH₃)_{minor}, 37.1 (d, ¹J_{PC} = 106.8 Hz, CH)_{minor}, 34.0 (d, ¹J_{PC} = 107.4 Hz, CH)_{major}, 26.9 (CH₂)_{major}, 26.8 (CH₂)_{minor}, 9.5 (CH₃)_{major}, 9.2 (CH₃)_{minor}, ppm; ³¹P NMR (120 MHz, CDCl₃) δ 24.9_{major}, 23.7_{minor} ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₄H₂₅NO₄P ([M+H]⁺) 422.1521, found 422.1508.



((1S*,1aS*,7S*)-7-Hydroxy-1a,5-dimethyl-1,1a-dihydro-7H-azirino[2,1-b]benzo[e][1,3]oxazin-1-yl)diphenylphosphine oxide (4g).

(1.65 g, 84%) as a yellow solid from 2*H*-azirine **2c** (1.27 g, 5 mmol, 1 eq) and 2-hydroxy-5-methylbenzaldehyde **1c** (1.36 g, 10 mmol, 2 eq) as described in the general procedure. The crude product was purified by

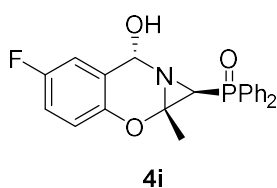
crystallization from hexane/CH₂Cl₂ (50:50) to afford the title compound **4g**. mp 168–170 °C; IR (neat) ν_{\max} 3242, 3056, 2867, 1496, 1438, 1435, 1177, 1069, 724 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.83–6.69 (m, 13H, ArH), 5.75 (s, 1H, CHOH)_{major}, 5.48 (s, 1H, CHOH)_{minor}, 5.23 (bs, 1H, OH), 2.98 (d, ²J_{PH} = 21.8 Hz, 1H, CH-P)_{major}, 2.64 (d, ²J_{PH} = 22.3 Hz, 1H, CH-P)_{minor}, 2.25 (s, 3H, CH₃)_{major}, 2.23 (s, 3H, CH₃)_{minor}, 1.88 (s, 3H, CH₃)_{minor}, 1.83 (s, 3H, CH₃)_{major} ppm; ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 146.4_{minor}, 146.3_{major}, 132.9, 132.5, 132.2, 132.2, 132.1, 132.0, 131.9, 131.9, 131.8, 131.4, 131.4, 131.3, 131.2, 130.4, 129.3, 129.0, 128.9, 128.8, 128.6, 128.5, 127.7, 120.2_{major}, 118.8_{minor}, 117.1_{minor}, 116.1_{major} (C_{Ar}), 80.8 (d, ³J_{PC} = 4.6 Hz, CH)_{minor}, 76.7 (d, ³J_{PC} = 3.6 Hz, CH)_{major}, 74.2 (d, ²J_{PC} = 4.4 Hz, C_{quat})_{major}, 74.0 (d, ²J_{PC} = 5.4 Hz, C_{quat})_{minor}, 35.8 (d, ¹J_{PC} = 106.5 Hz, CH)_{minor}, 32.8 (d, ¹J_{PC} = 107.6 Hz, CH)_{major}, 20.9 (CH₃)_{major}, 20.8 (CH₃)_{minor}, 20.6 (CH₃)_{minor}, 20.1 (CH₃)_{major} ppm; ³¹P NMR (120 MHz, CDCl₃) δ 25.7_{major}, 24.3_{minor} ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₃H₂₃NO₃P ([M+H]⁺) 392.1416, found 392.1403.



((1S*,1aS*,7S*)-1a-Ethyl-7-hydroxy-5-dimethyl-1,1a-dihydro-7H-azirino[2,1-b]benzo[e][1,3]oxazin-1-yl)diphenylphosphine oxide (4h**).**

(1.57 g, 75%) as a pale yellow solid from 2*H*-azirine **2d** (1.34 g, 5 mmol, 1 eq) and 2-hydroxy-5-methylbenzaldehyde **1c** (1.36 g, 10 mmol, 2 eq) as described in the general procedure. The crude product was purified

by crystallization from hexane/Et₂O (50:50) to afford the title compound **4h**. mp 122–124 °C; IR (neat) ν_{\max} 3243, 3060, 2977, 1619, 1590, 1495, 1438, 1175, 1119, 730, 701 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.83–6.71 (m, 13H, ArH), 5.73 (d, ³J_{HH} = 3.9 Hz, 1H, CHOH)_{major}, 5.48 (bs, 1H, CHOH)_{minor}, 5.21 (d, ³J_{HH} = 3.9 Hz, 1H, OH)_{major}, 3.89 (d, ³J_{HH} = 2.4 Hz, 1H, OH)_{minor}, 3.03 (d, ²J_{PH} = 21.8 Hz, 1H, CH-P)_{major}, 2.69 (d, ²J_{PH} = 22.7 Hz, 1H, CH-P)_{minor}, 2.25 (s, 3H, CH₃)_{major}, 2.23 (s, 3H, CH₃)_{minor}, 2.25–2.06 (m, 2H, CH₂), 1.11 (t, ³J_{HH} = 7.4 Hz, 3H, CH₃)_{minor}, 1.03 (t, ³J_{HH} = 7.4 Hz, 3H, CH₃)_{major} ppm; ¹³C {¹H} NMR (75 MHz, DMSO) δ 146.4_{minor}, 146.1_{major}, 134.4, 133.3, 133.0, 131.9, 131.8, 131.6, 131.5, 131.4, 131.3, 130.9, 130.8, 130.6, 130.5, 130.1, 129.1, 128.9, 128.8, 128.7, 128.6, 128.6, 128.3, 128.1, 127.1, 120.7, 120.5, 119.9, 116.3, 115.9, (C_{Ar}), 79.2 (d, ³J_{PC} = 5.0 Hz, CH)_{minor}, 77.3 (d, ²J_{PC} = 5.2 Hz, C_{quat})_{minor}, 76.8 (d, ²J_{PC} = 4.5 Hz, C_{quat})_{major}, 76.2 (d, ³J_{PC} = 3.5 Hz, CH)_{major}, 35.6 (d, ¹J_{PC} = 106.0 Hz, CH)_{minor}, 33.0 (d, ¹J_{PC} = 108.4 Hz, CH)_{major}, 26.3 (CH₂), 20.4 (CH₃)_{major}, 20.2 (CH₃)_{minor}, 9.3 (CH₃)_{minor}, 9.2 (CH₃)_{major} ppm; ³¹P NMR (120 MHz, CDCl₃) δ 25.1_{major}, 23.9_{minor} ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₄H₂₅NO₃P ([M+H]⁺) 406.1572, found 406.1561.

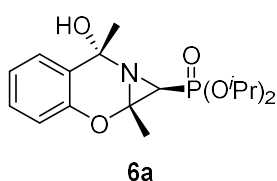


4i

((1S*,1aS*,7S*)-5-Fluoro-7-hydroxy-1a-methyl-1,1a-dihydro-7H-azirino[2,1-b]benzo[e][1,3]oxazin-1-yl) diphenylphosphine oxide (4i).

(1.90 g, 96%) as a white solid from 2*H*-azirine **2c** (1.27 g, 5 mmol, 1 eq) and 5-fluorosalicylaldehyde **1d** (0.84 g, 10 mmol, 1.2 eq) as described in the general procedure. The crude product was purified by crystallization from hexane/CH₂Cl₂ (50:50) to afford the title compound **4i**. mp 169–171 °C; IR (neat) ν_{\max} 3417, 2930, 2857, 1483, 1432, 1182, 1141, 732, 691 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.87–6.74 (m, 13H, ArH), 5.92 (d, ³J_{HH} = 4.3 Hz, 1H, CHOH)_{major}, 5.69 (d, ³J_{HH} = 4.0 Hz, 1H, OH)_{major}, 5.47 (bs, 1H, CHOH)_{minor}, 4.77 (bs, 1H, OH)_{minor}, 2.91 (d, ²J_{PH} = 21.7 Hz, 1H, CH-P)_{major}, 2.60 (d, ²J_{PH} = 22.0 Hz, 1H, CH-P)_{minor}, 1.85 (s, 3H, CH₃)_{minor}, 1.83 (s, 3H, CH₃)_{major} ppm; ¹³C {¹H} NMR (75 MHz, DMSO) δ 159.1, 155.1, 144.6_{minor}, 144.4_{major}, 134.3, 133.0, 133.0, 131.9, 131.7, 131.5, 131.4, 131.0, 130.9, 130.6, 130.5, 128.8, 128.8, 128.7, 128.3, 128.2, 122.0, 119.9, 117.9, 116.6, 116.3, 113.3, 113.0, (C_{Ar}), 79.3 (d, ³J_{PC} = 4.7 Hz, CH)_{minor}, 75.7 (d, ³J_{PC} = 3.0 Hz, CH)_{major}, 74.0 (d, ²J_{PC} = 5.3 Hz, C_{quat})_{minor}, 73.6 (d, ²J_{PC} = 4.3 Hz, C_{quat})_{major}, 35.2 (d, ¹J_{PC} = 105.5 Hz, CH)_{minor}, 32.2 (d, ¹J_{PC} = 108.0 Hz, CH)_{major}, 20.1 (CH₃)_{minor}, 19.7 (CH₃)_{major} ppm; ³¹P NMR (120 MHz, CDCl₃) δ 25.9_{major}, 24.5_{minor} ppm; ¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -119.8 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₂H₂₀FNO₃P ([M+H]⁺) 396.1165, found 396.1153.

4.1.3.2. General procedure and spectral data for the reaction of functionalized 2*H*-azirines **2 with 2-hydroxyacetophenone **5**.** To a 0 °C solution of 2*H*-azirine (5 mmol, 1 eq) **2** in CH₂Cl₂ (25 mL) and 4 Å M.S., was added dropwise 2-hydroxyacetophenone **5** (1.20 mL, 10 mmol, 2 eq) and Et₃N (2.09 mL, 15 mmol, 3 eq) under a nitrogen atmosphere. The reaction mixture was allowed to reach room temperature and stirred at the same temperature until TLC showed the disappearance of 2*H*-azirine **2** (72–120 h). 4 Å M.S. was filtered through a sintered glass vacuum filtration funnel with celite and washed with CH₂Cl₂. The reaction mixture was washed with water (3×15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layers were dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum. The crude product was purified by crystallization or flash-column chromatography to afford the corresponding azirino-benzoxazine derivatives **6**.

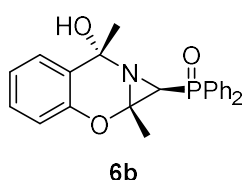


6a

Diisopropyl ((1S*,1aS*,7S*)-7-hydroxy-1a,7-dimethyl-1,1a-dihydro-7H-azirino[2,1-b]benzo[e][1,3]oxazin-1-yl)phosphonate (6a).

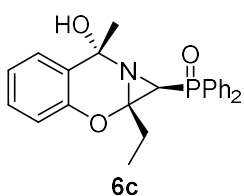
(0.63 g, 35%) as a white solid from 2*H*-azirine **2a** (1.27 g, 5 mmol, 1 eq) as described in the general procedure after stirring at room temperature for 120 h. The crude product was purified by flash-column chromatography (SiO₂,

AcOEt/hexane 50:50) to afford the title compound **6a**. mp 122–124 °C; IR (neat) ν_{\max} 3331, 2981, 2934, 1612, 1587, 1374, 1280, 1228, 1011, 986 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.48–7.46 (m, 1H, ArH), 7.23–7.21 (m, 1H, ArH), 7.06–6.99 (m, 1H, ArH), 6.83–6.81 (m, 1H, ArH), 4.74–4.68 (m, 2H, CH), 3.27 (d, $^3J_{\text{PH}} = 5.7$ Hz, 1H, OH)_{major}, 2.48 (bs, 1H, OH)_{minor}, 2.38 (d, $^2J_{\text{PH}} = 12.3$ Hz, 1H, CH-P)_{major}, 2.14 (d, $^2J_{\text{PH}} = 12.5$ Hz, 1H, CH-P)_{minor}, 1.90 (s, 3H, CH_3)_{minor}, 1.84 (s, 3H, CH_3)_{major}, 1.83 (s, 3H, CH_3)_{minor}, 1.63 (s, 3H, CH_3)_{major}, 1.34–1.28 (m, 12H, CH_3) ppm; ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 148.1_{minor}, 147.5_{major}, 130.4, 129.5, 126.6, 126.3, 126.2, 125.8, 123.2, 123.0, 117.4, 116.5 (C_{Ar}), 83.5 (d, $^3J_{\text{PC}} = 7.0$ Hz, C_{quat})_{minor}, 83.5 (d, $^3J_{\text{PC}} = 4.9$ Hz, C_{quat})_{major}, 73.7 (d, $^2J_{\text{PC}} = 4.4$ Hz, C_{quat}), 71.4 (d, $^2J_{\text{PC}} = 6.4$ Hz, OCH), 71.1 (d, $^2J_{\text{PC}} = 6.7$ Hz, OCH), 32.0 (CH_3), 31.4 (d, $^1J_{\text{PC}} = 211.5$ Hz, CH), 24.2 (d, $^3J_{\text{PC}} = 3.4$ Hz, CH_3), 24.1 (d, $^3J_{\text{PC}} = 4.4$ Hz, CH_3), 24.1 (d, $^3J_{\text{PC}} = 3.0$ Hz, CH_3), 23.9 (d, $^3J_{\text{PC}} = 5.5$ Hz, CH_3), 20.3 (CH_3)_{minor}, 20.0 (CH_3)_{major} ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 19.6_{major}, 19.1_{minor} ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{17}\text{H}_{27}\text{NO}_5\text{P}$ ($[\text{M}+\text{H}]^+$) 356.1627, found 356.1628.



((1S*,1aS*,7S*)-7-Hydroxy-1a,7-dimethyl-1,1a-dihydro-7H-azirino[2,1-b]benzo[e][1,3]oxazin-1-yl)diphenylphosphine oxide (6b**)**. (1.11 g, 57%) as a white solid from 2H-azirine **2c** (1.27 g, 5 mmol, 1 eq) as described in the general procedure after stirring at room temperature for 96 h. The crude product was purified by crystallization from Et_2O and washed

several times with pentane to afford the title compound **6b**. mp 163–165 °C; IR (neat) ν_{\max} 3250, 2977, 2930, 1613, 1594, 1435, 1185, 1157, 729 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.76–6.82 (m, 14H, ArH), 2.79 (d, $^2J_{\text{PH}} = 21.5$ Hz, 1H, CH-P)_{major}, 2.56 (bs, 1H, OH), 2.46 (d, $^2J_{\text{PH}} = 23.0$ Hz, 1H, CH-P)_{minor}, 2.00 (s, 3H, CH_3)_{minor}, 1.90 (s, 3H, CH_3)_{major}, 1.61 (s, 3H, CH_3)_{major}, 1.20 (s, 3H, CH_3)_{minor} ppm; ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$) δ 148.1_{minor}, 147.4_{major}, 132.8, 132.4, 132.4, 132.3, 132.1, 131.5, 131.4, 131.2, 131.1, 131.0, 130.7, 130.3, 129.4, 129.2, 129.0, 128.9, 128.8, 128.7, 128.7, 128.6, 126.6, 126.3, 125.5, 123.3, 123.0, 122.4, 117.3, 116.4 (C_{Ar}), 82.7 (d, $^3J_{\text{PC}} = 3.5$ Hz, C_{quat}), 74.0 (d, $^2J_{\text{PC}} = 4.7$ Hz, C_{quat})_{major}, 73.6 (d, $^2J_{\text{PC}} = 5.7$ Hz, C_{quat})_{minor}, 35.7 (d, $^1J_{\text{PC}} = 107.1$ Hz, CH)_{minor}, 33.3 (d, $^1J_{\text{PC}} = 108.8$ Hz, CH)_{major}, 32.2 (CH_3), 20.5 (CH_3)_{minor}, 20.1 (CH_3)_{major} ppm; ^{31}P NMR (160 MHz, CDCl_3) δ 25.2_{major}, 24.2_{minor} ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{23}\text{H}_{23}\text{NO}_3\text{P}$ ($[\text{M}+\text{H}]^+$) 392.1416, found 392.1403.



((1S*,1aS*,7S*)-1a-Ethyl-7-hydroxy-7-methyl-1,1a-dihydro-7H-azirino[2,1-b]benzo[e][1,3]oxazin-1-yl)diphenylphosphine oxide (6c**)**. (1.25 g, 62%) as a yellow solid from 2H-azirine **2d** (1.34 g, 5 mmol, 1 eq) as described in the general procedure after stirring at room temperature for 72 h. The crude product was purified by crystallization from Et_2O and

washed several times with pentane to afford the title compound **6c**. mp 147–179 °C; IR (neat) ν_{\max} 3253, 2974, 2924, 1619, 1587, 1442, 1163, 910, 729, 698 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.79–6.85 (m, 14H, ArH), 2.87 (d, $^2J_{\text{PH}} = 21.7$ Hz, 1H, CH-P)_{major}, 2.74 (bs, 1H, OH), 2.53 (d, $^2J_{\text{PH}} = 23.1$ Hz, 1H, CH-P)_{minor}, 2.32–2.12 (m, 2H, CH₂), 1.64 (s, 3H, CH₃), 1.22–1.12 (m, 2H, CH₃) ppm; ^{13}C { ^1H } NMR (100 MHz, CDCl_3) δ 148.4_{minor}, 147.7_{major}, 133.2, 132.5, 132.2, 132.2, 132.1, 132.1, 132.1, 131.8, 131.7, 131.5, 131.3, 131.2, 129.6, 128.9, 128.7, 128.6, 128.5, 126.4, 124.9, 123.2, 116.8 (C_{Ar}), 82.9 (d, $^3J_{\text{PC}} = 3.4$ Hz, C_{quat}), 78.2 (d, $^2J_{\text{PC}} = 4.7$ Hz, C_{quat}), 34.7 (d, $^1J_{\text{PC}} = 108.2$ Hz, CH), 32.9 (CH₃), 26.9 (CH₂), 9.2 (CH₃) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 25.0_{major}, 23.9_{minor} ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{24}\text{H}_{25}\text{NO}_3\text{P}$ ([M+H]⁺) 406.1572, found 406.1558.

4.2. Biology

4.2.1. Materials

Reagents and solvents were used as purchased without further purification. All stock solutions of the investigated compounds were prepared by dissolving the powdered materials in appropriate amounts of DMSO. The final concentration of DMSO never exceeded 10% (v/v) in reactions. The stock solution was stored at 5 °C until it was used.

4.2.2. Cytotoxicity assays.

Cells were cultured according to the supplier's instructions. Cells were seeded in 96-well plates at a density of 2–4 × 10³ cells per well and incubated overnight in 0.1 mL of media supplied with 10% Fetal Bovine Serum (Lonza) in 5% CO₂ incubator at 37 °C. On day 2, drugs were added and samples were incubated for 48 hours. After treatment, 10 μL of cell counting kit-8 was added into each well for additional 2 hours incubation at 37 °C. The absorbance of each well was determined by an Automatic Elisa Reader System at 450 nm wavelength.

Declaration of competing interest

None.

Acknowledgments

The authors thank Ministerio de Ciencia, Innovación y Universidades (MCIU); Agencia Estatal de Investigación (AEI) y Fondo Europeo de Desarrollo Regional (FEDER; RTI2018-101818-B-I00, UE), and Gobierno Vasco (GV, IT 992-16) for supporting this work. We also thank for technical and human support provided by SGIker (UPV/EHU/ ERDF, EU).

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Material complementario – Manuscrito 3

First synthesis of merged hybrids phosphorylated azirino[2,1-*b*] benzo[*e*][1,3]oxazine derivatives as anticancer agents.

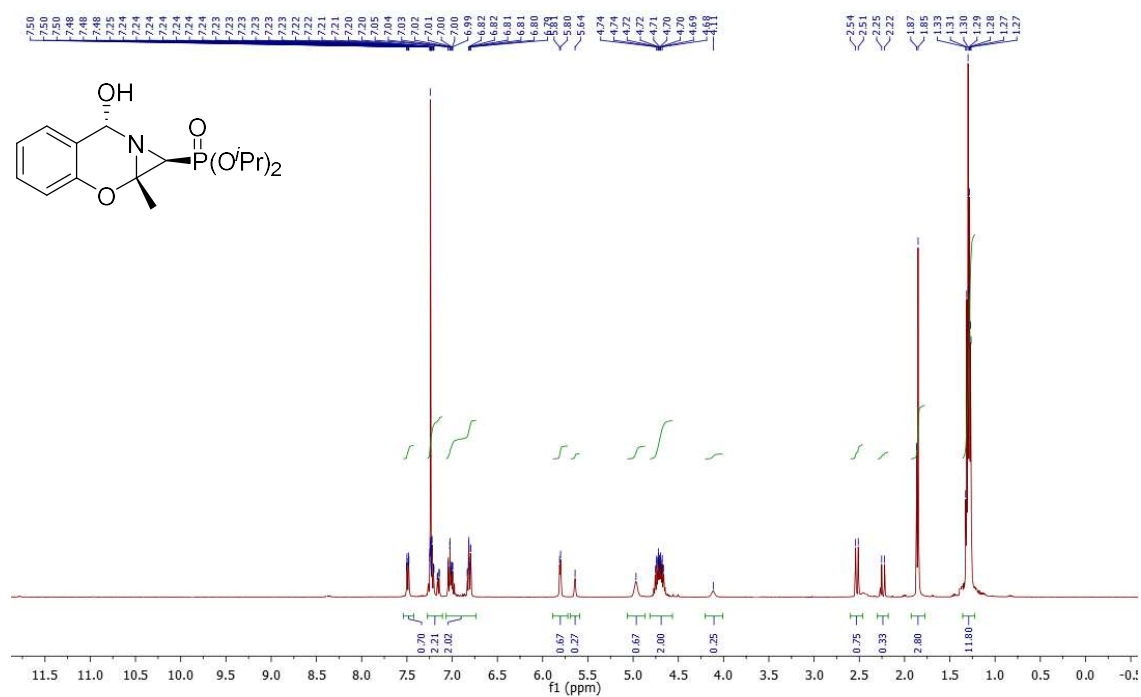
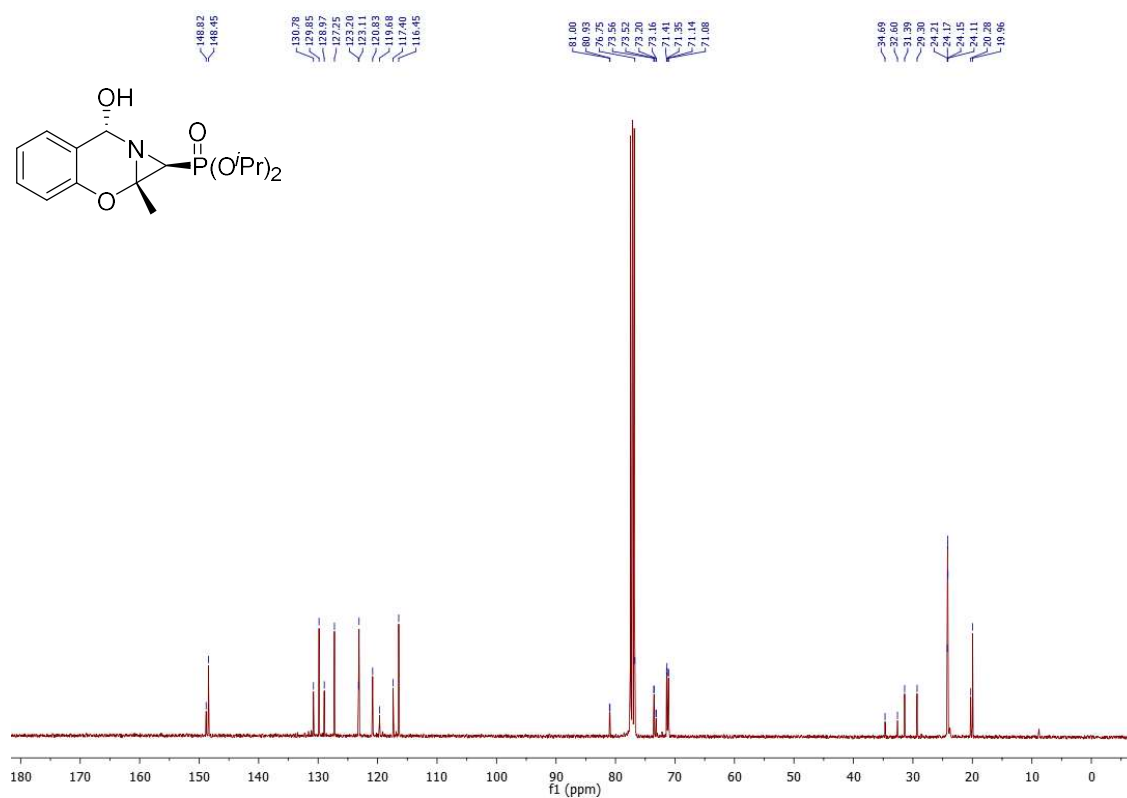
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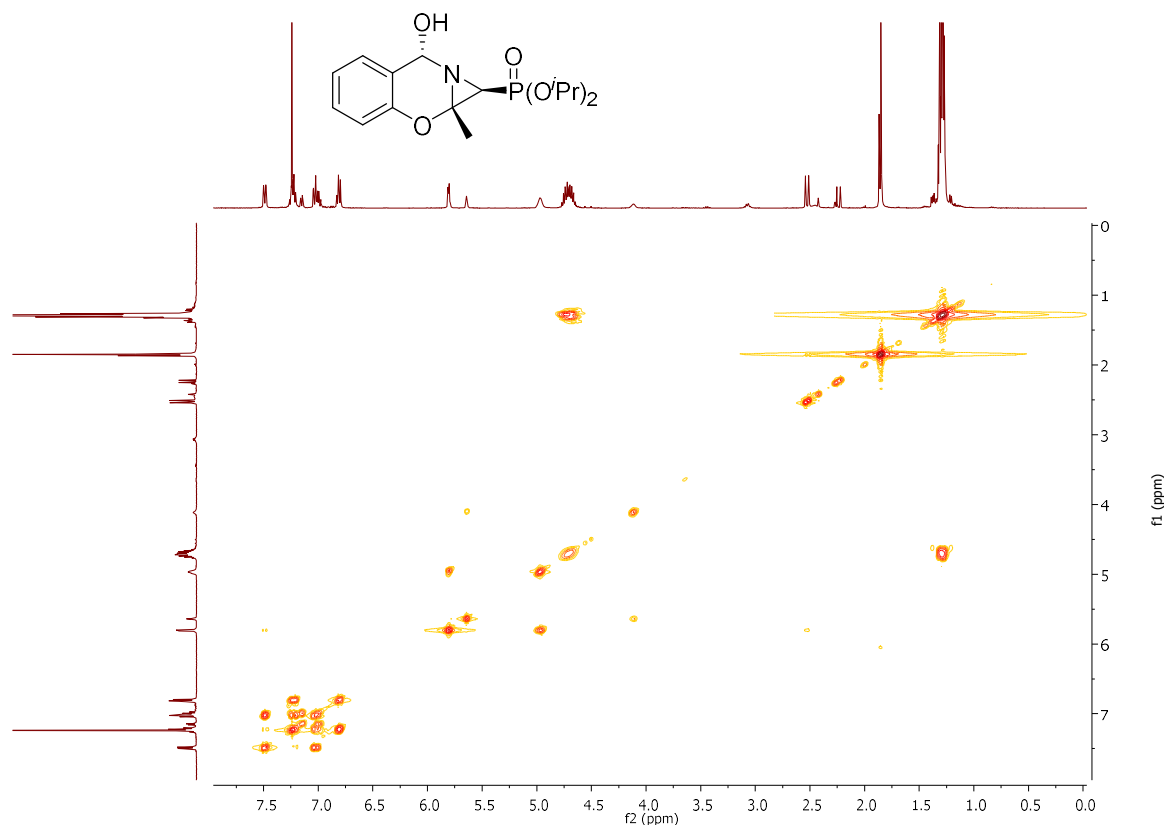
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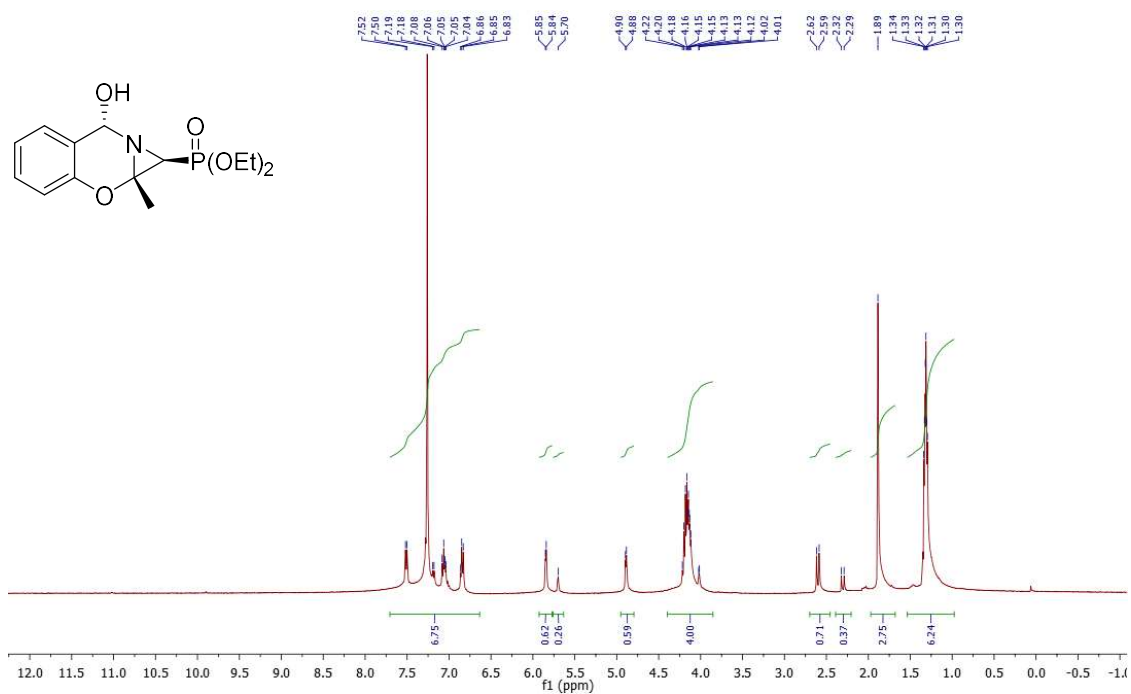
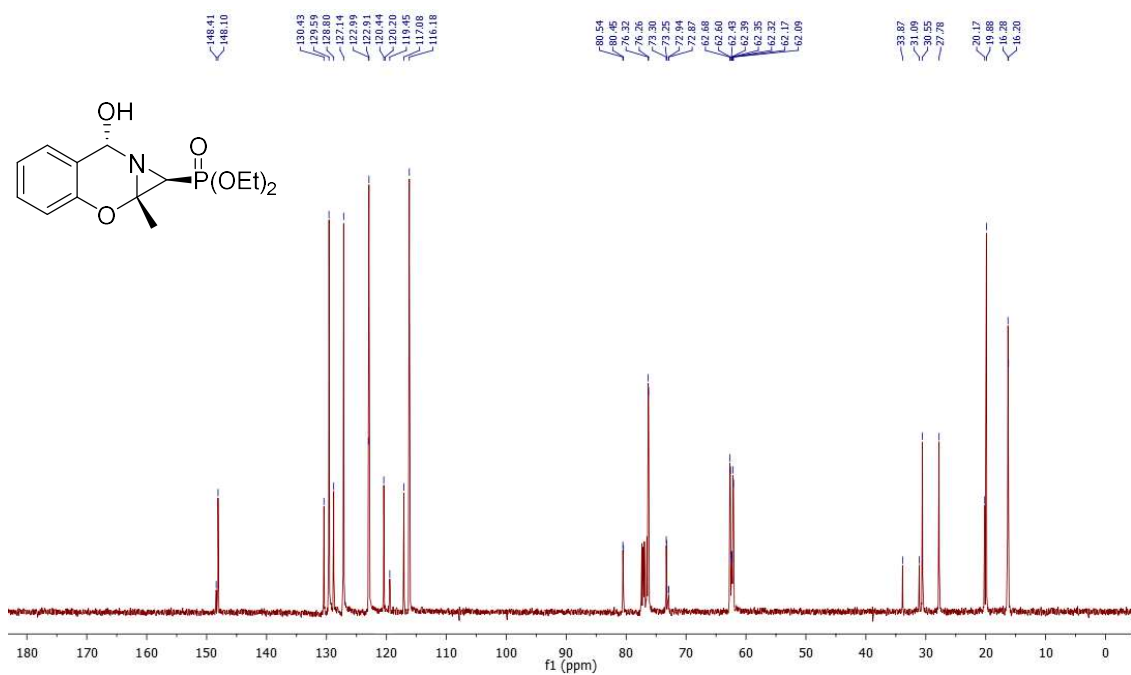
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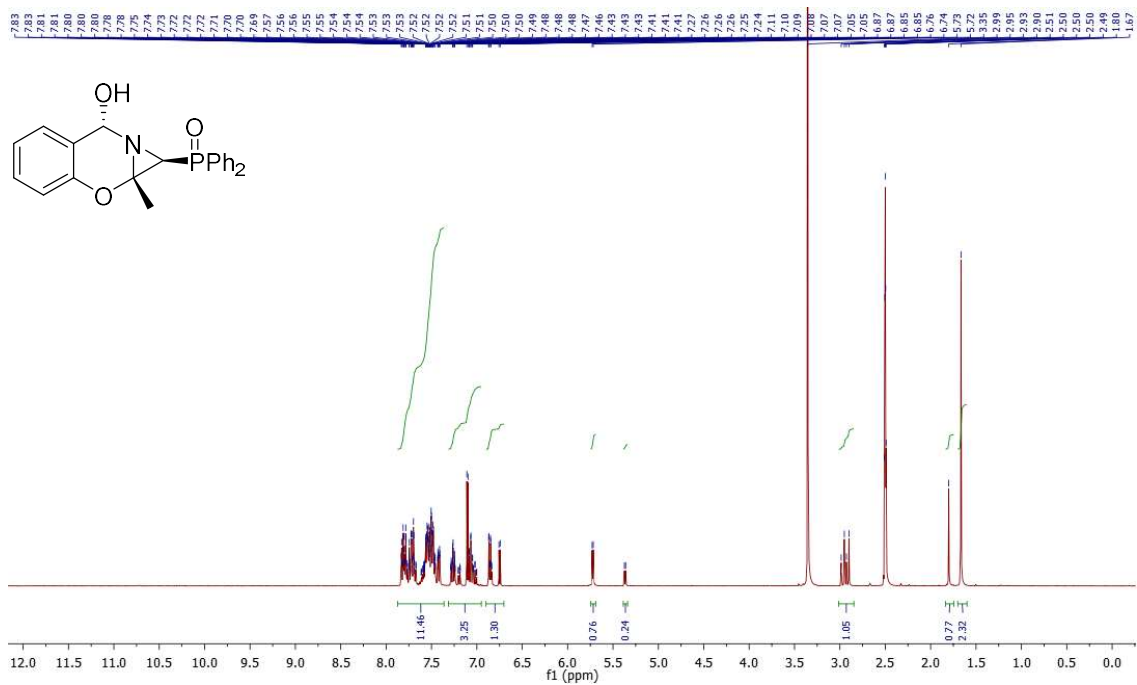
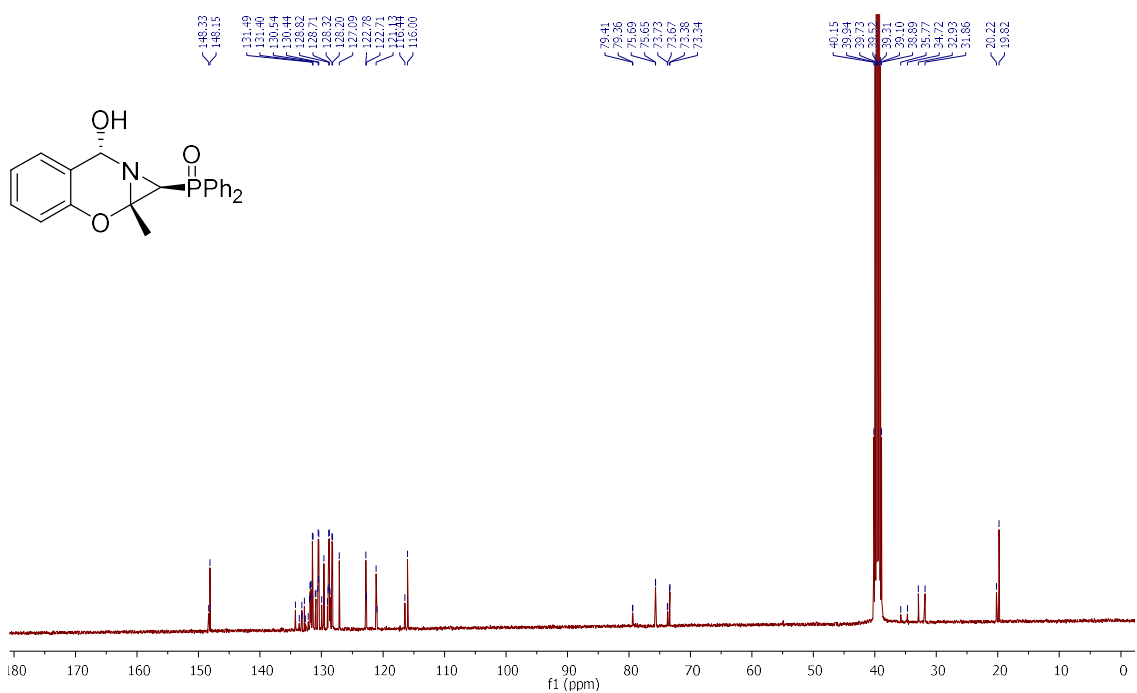
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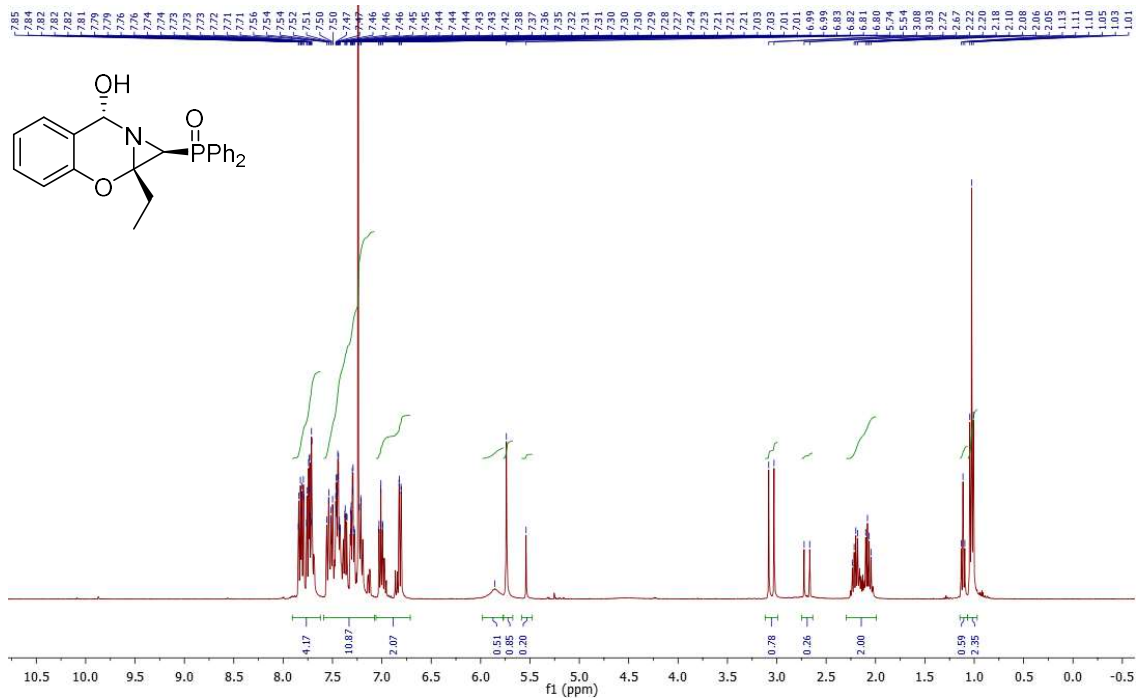
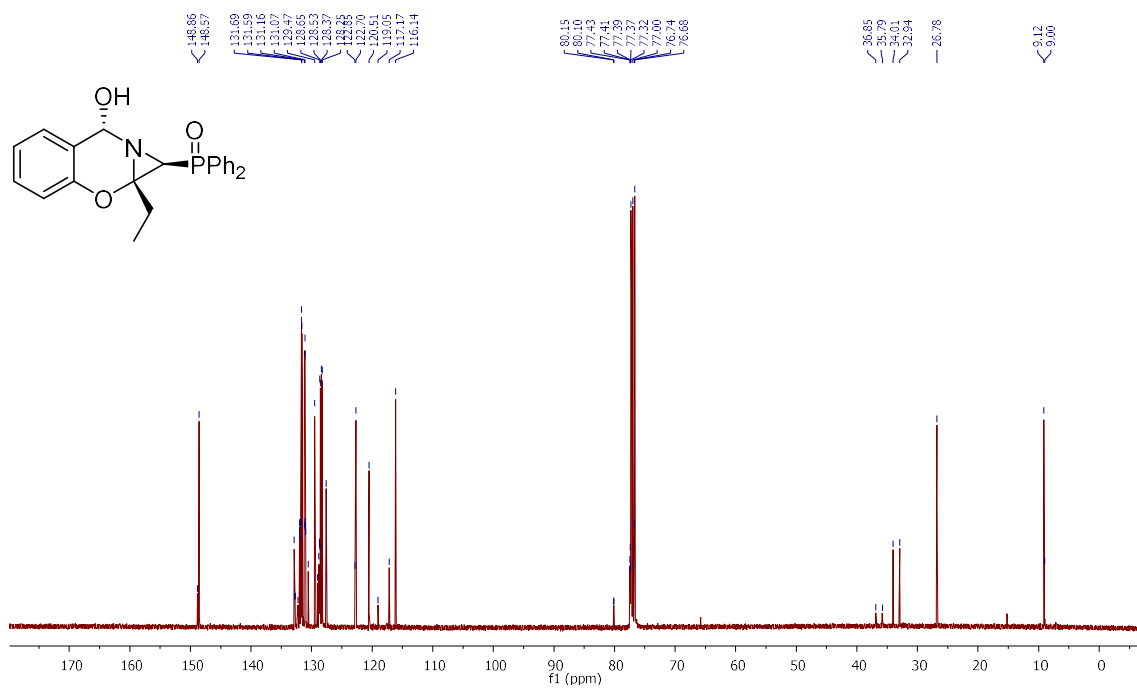
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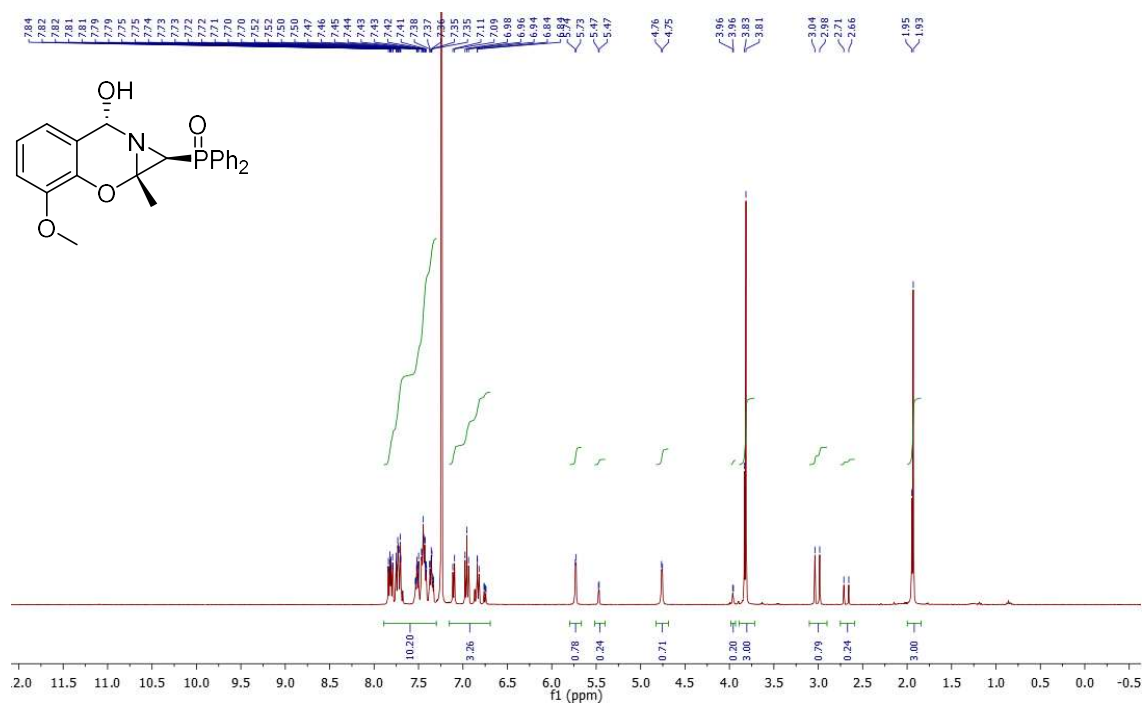
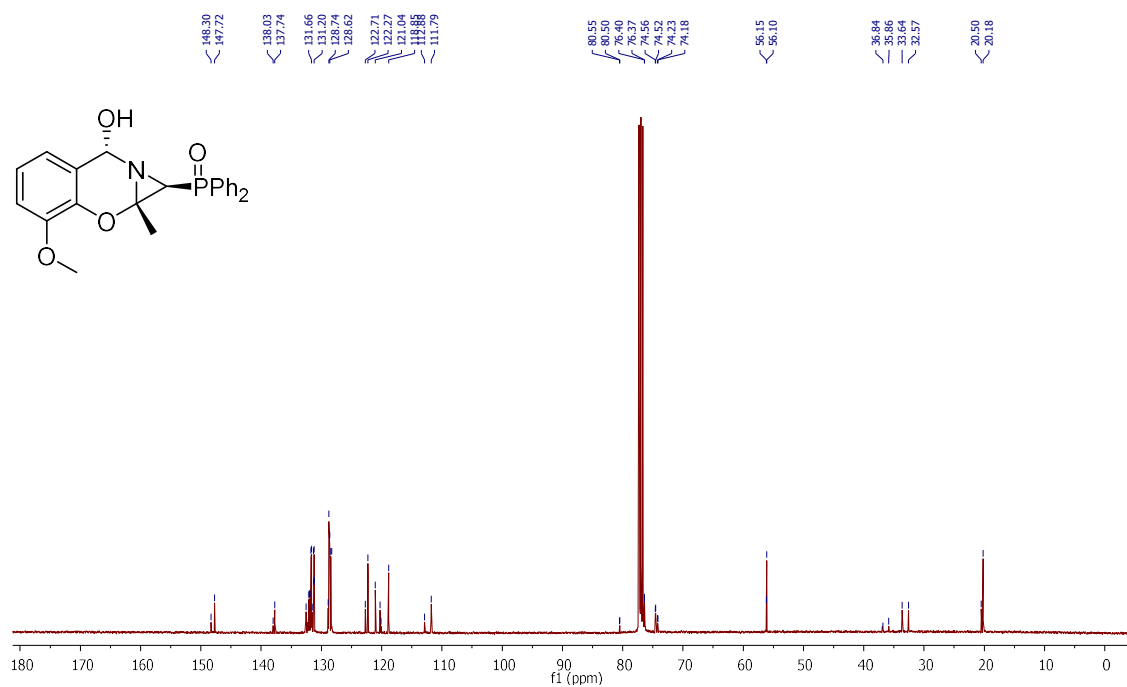
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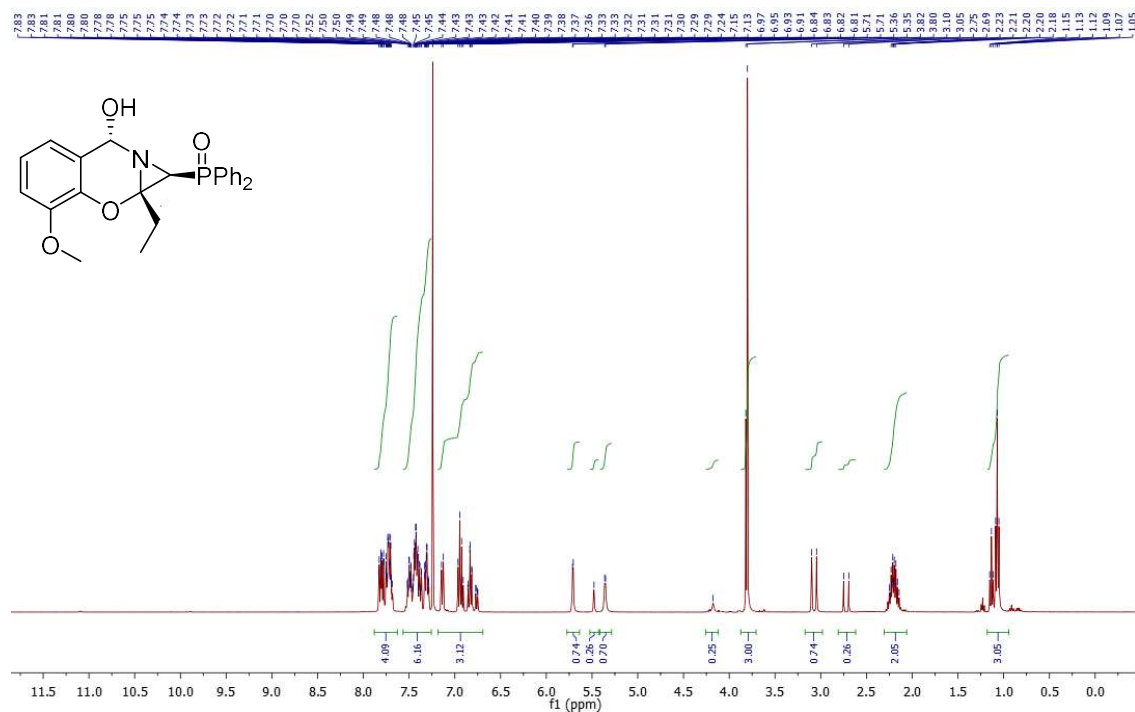
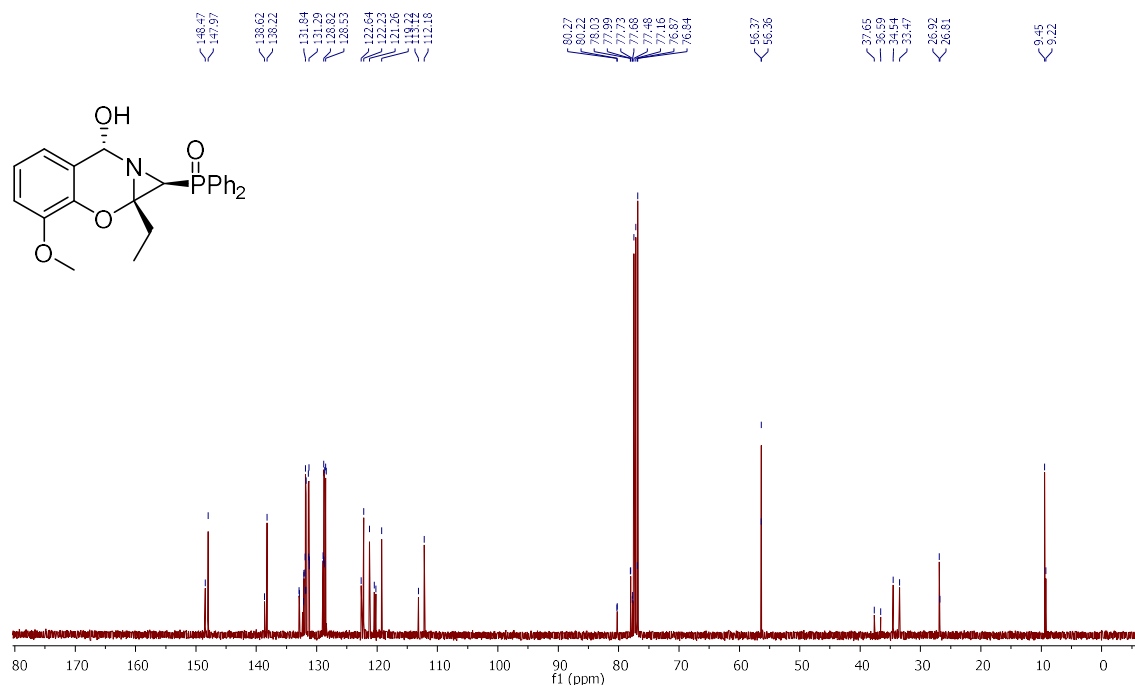


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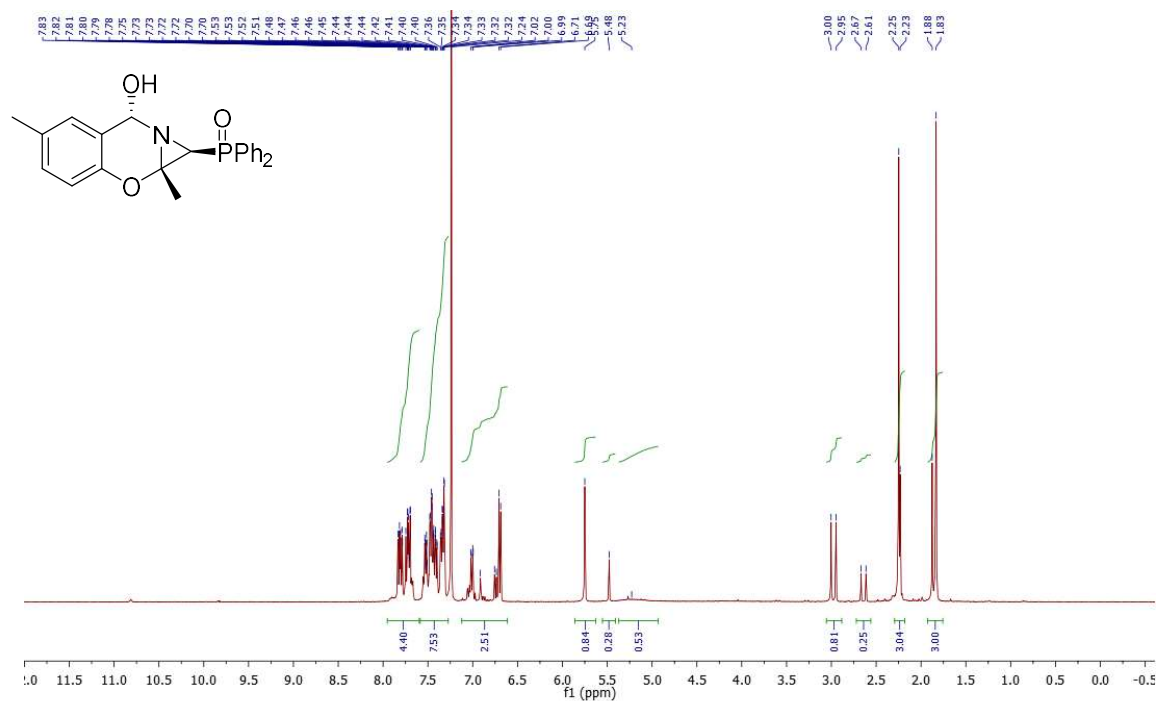
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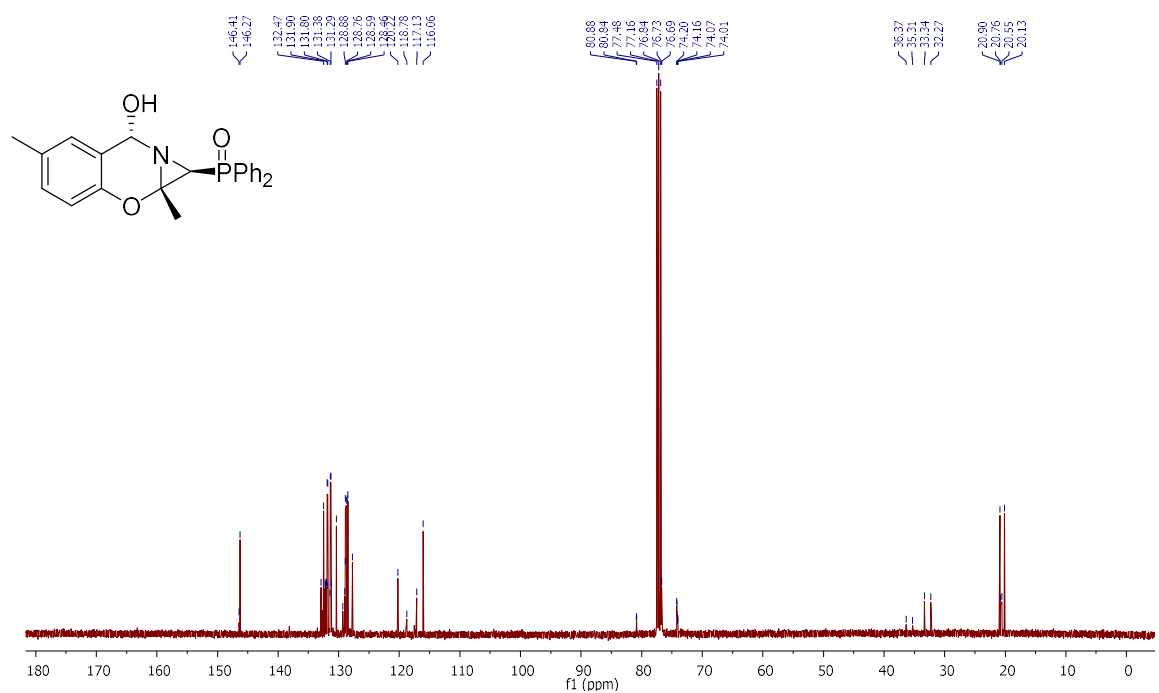
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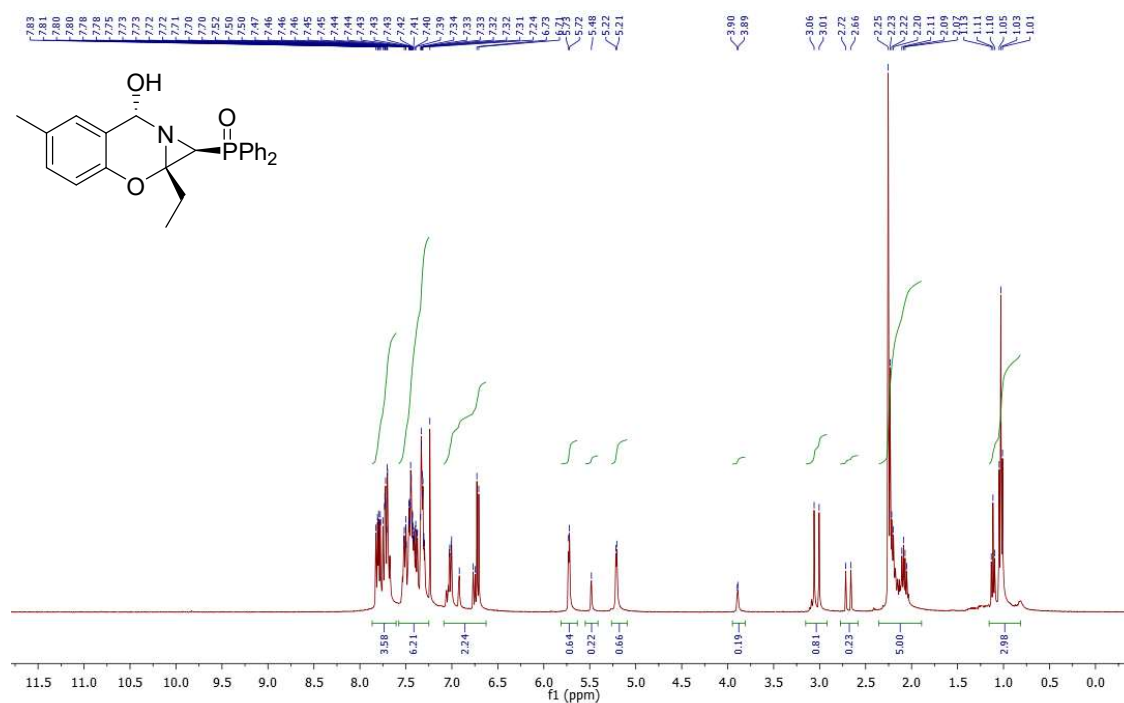
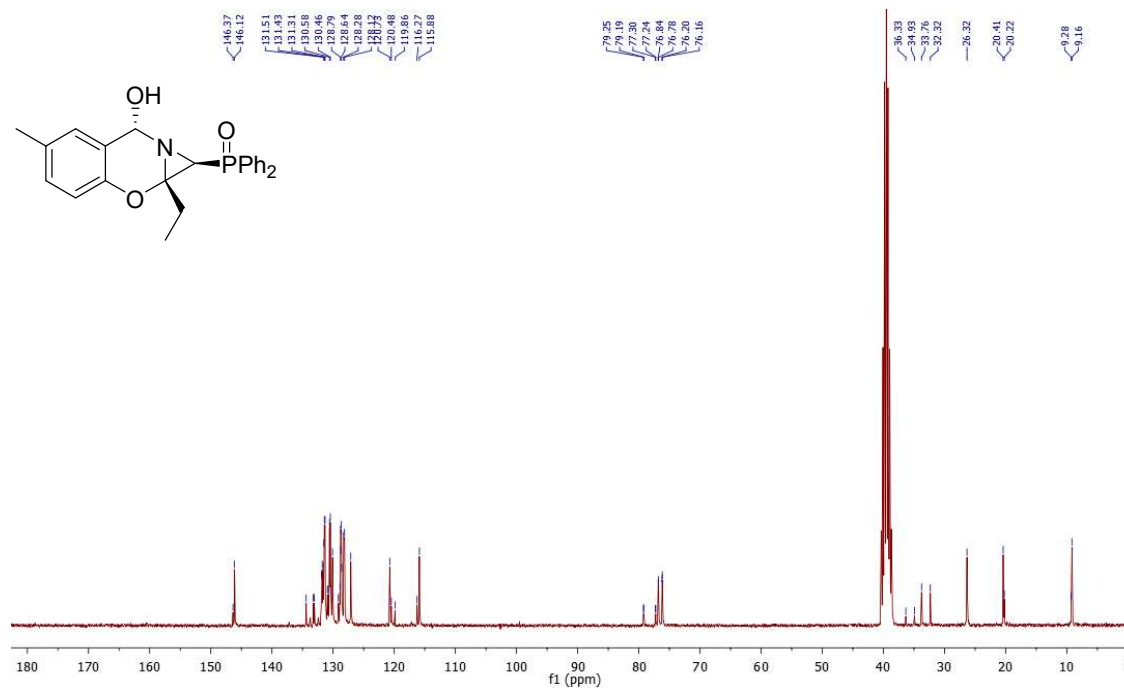
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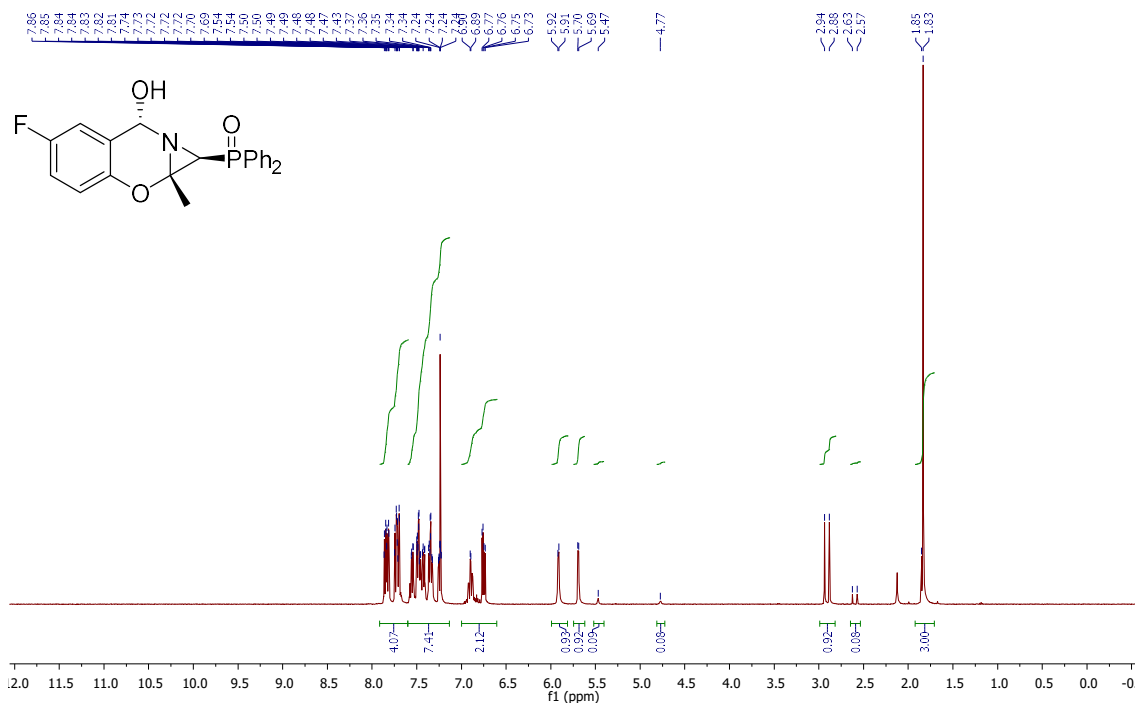
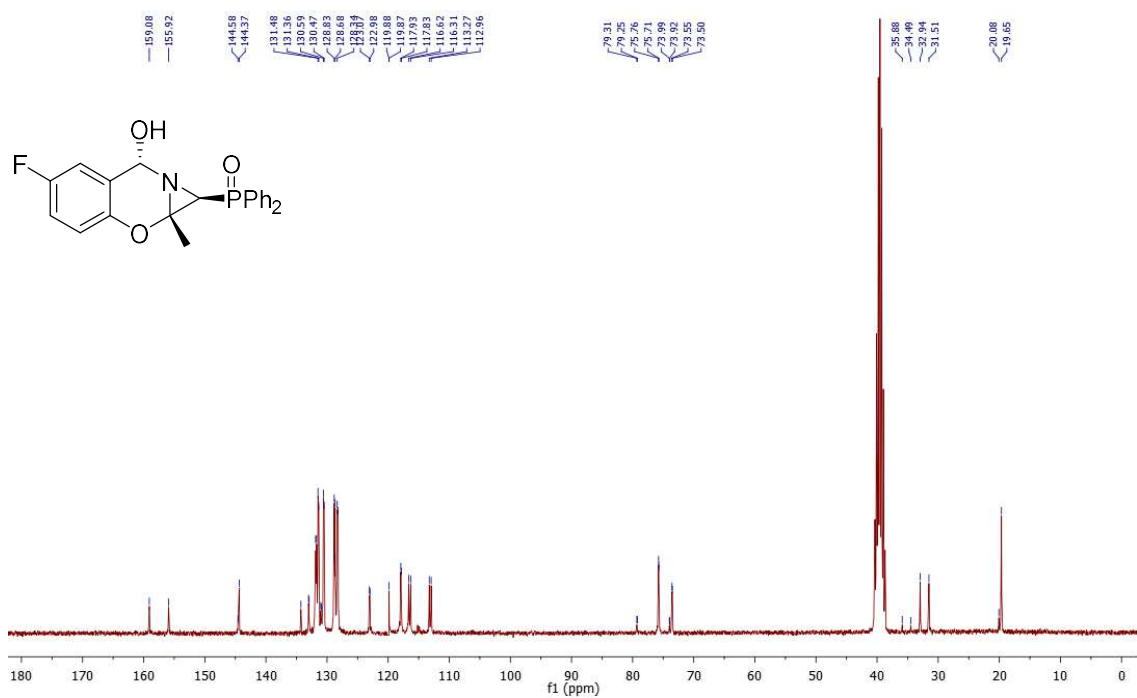
^1H NMR (400 MHz, CDCl_3) of aziridinyl fused benzoxazine **4g**



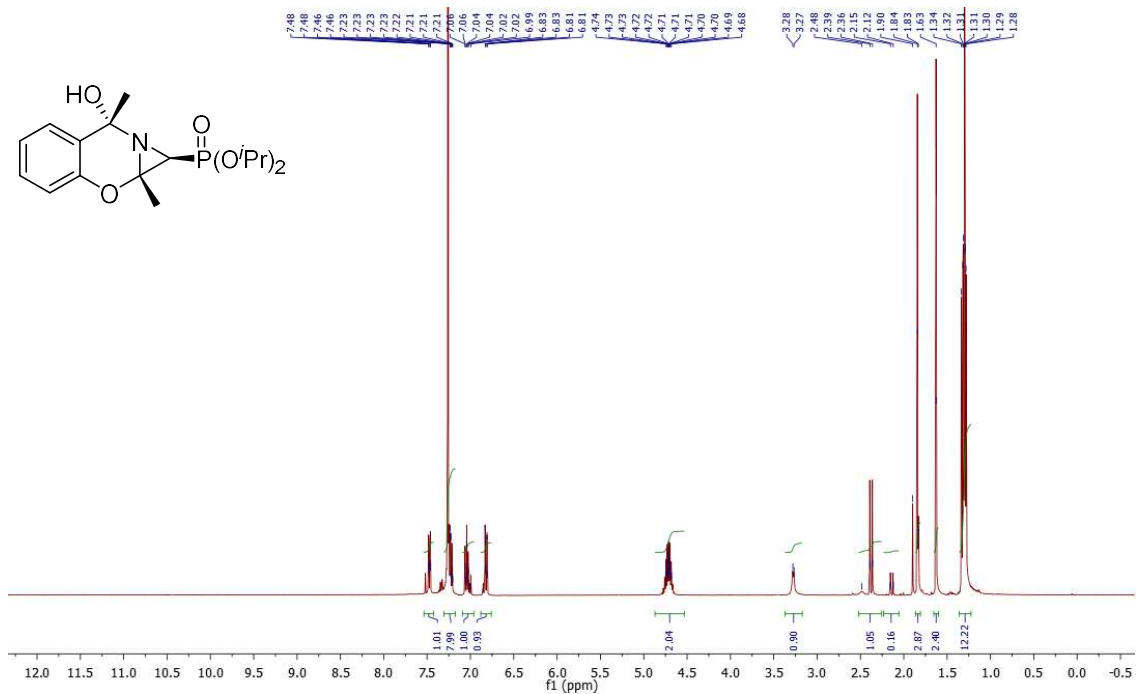
^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) of aziridinyl fused benzoxazine **4g**



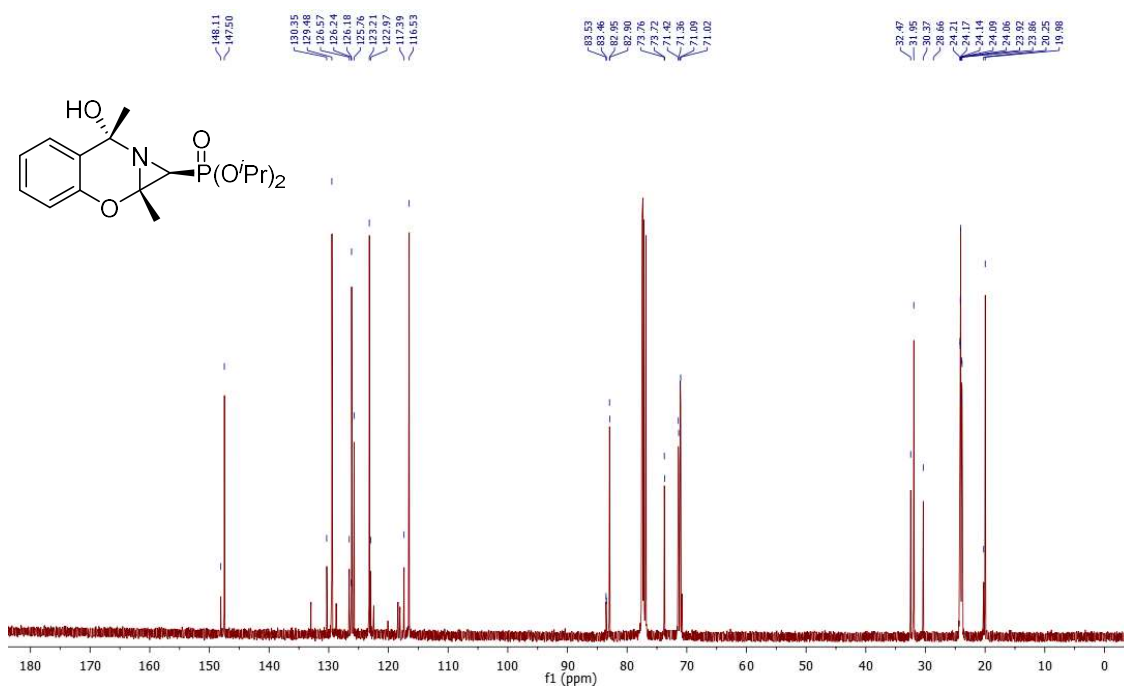
^1H NMR (400 MHz, CDCl_3) of aziridiny fused benzoxazine **4h** ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, DMSO) of aziridiny fused benzoxazine **4h**

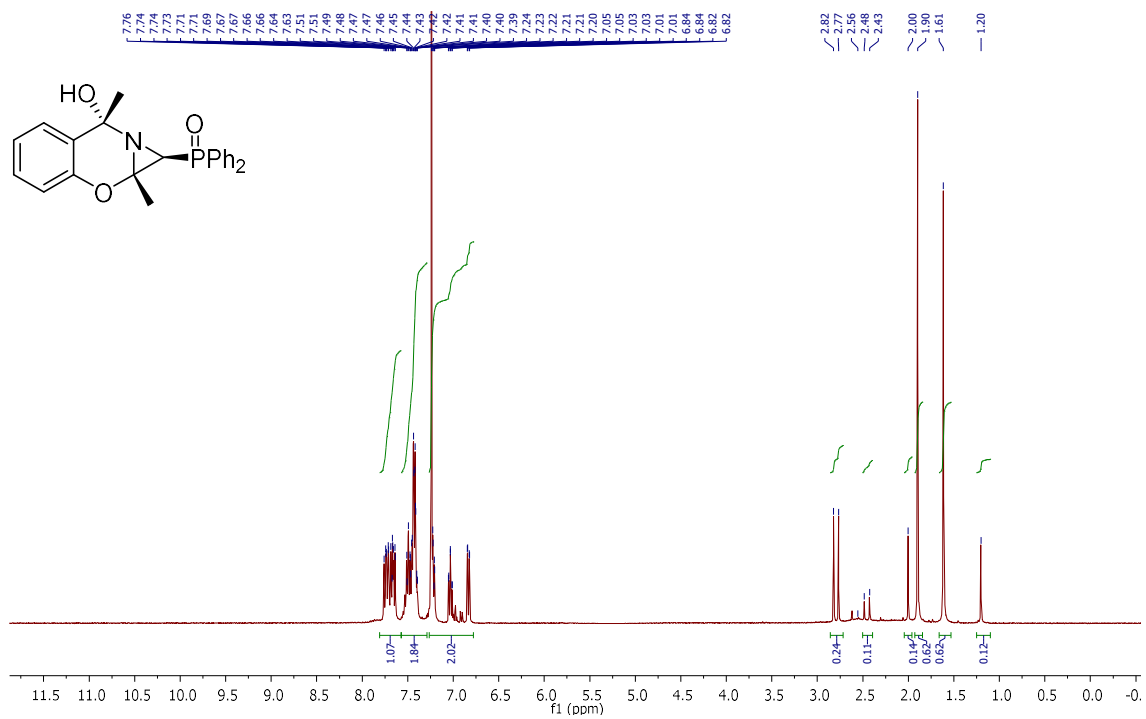
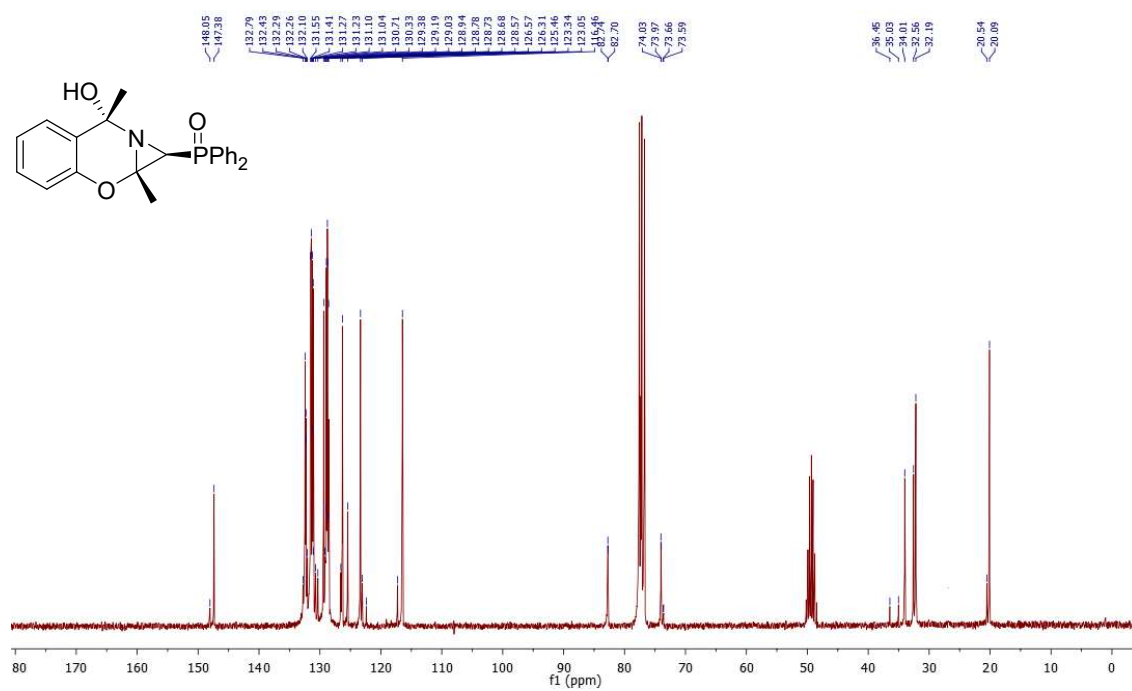
^1H NMR (400 MHz, CDCl_3) of aziridiny fused benzoxazine **4i** ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, DMSO) of aziridiny fused benzoxazine **4i**

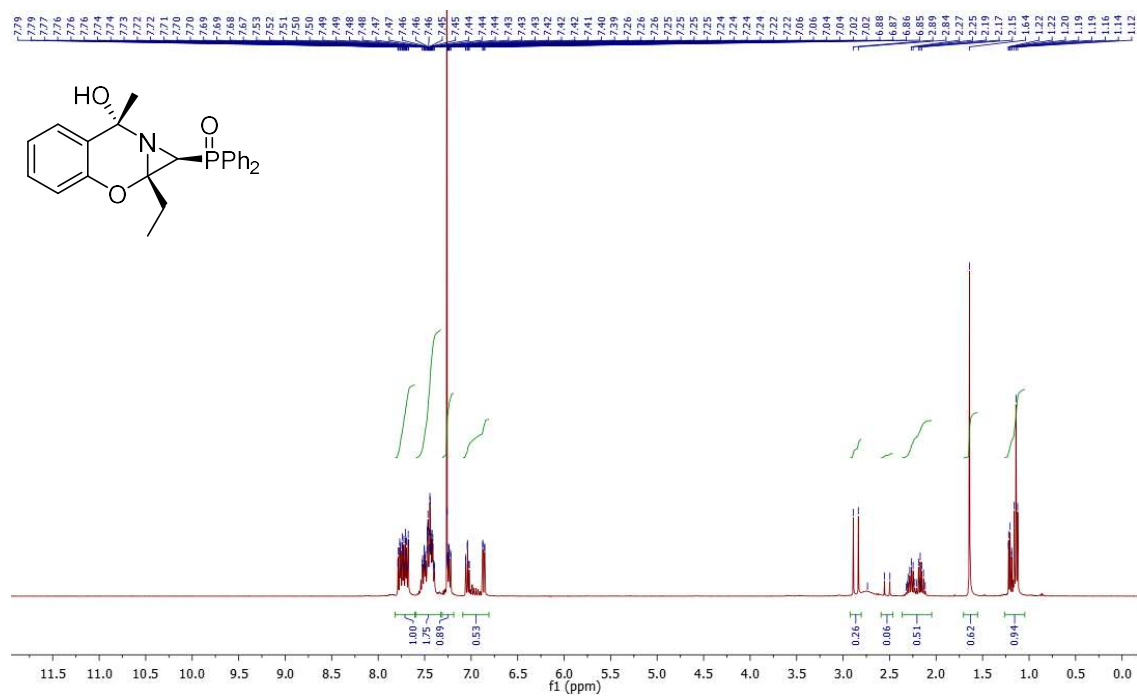
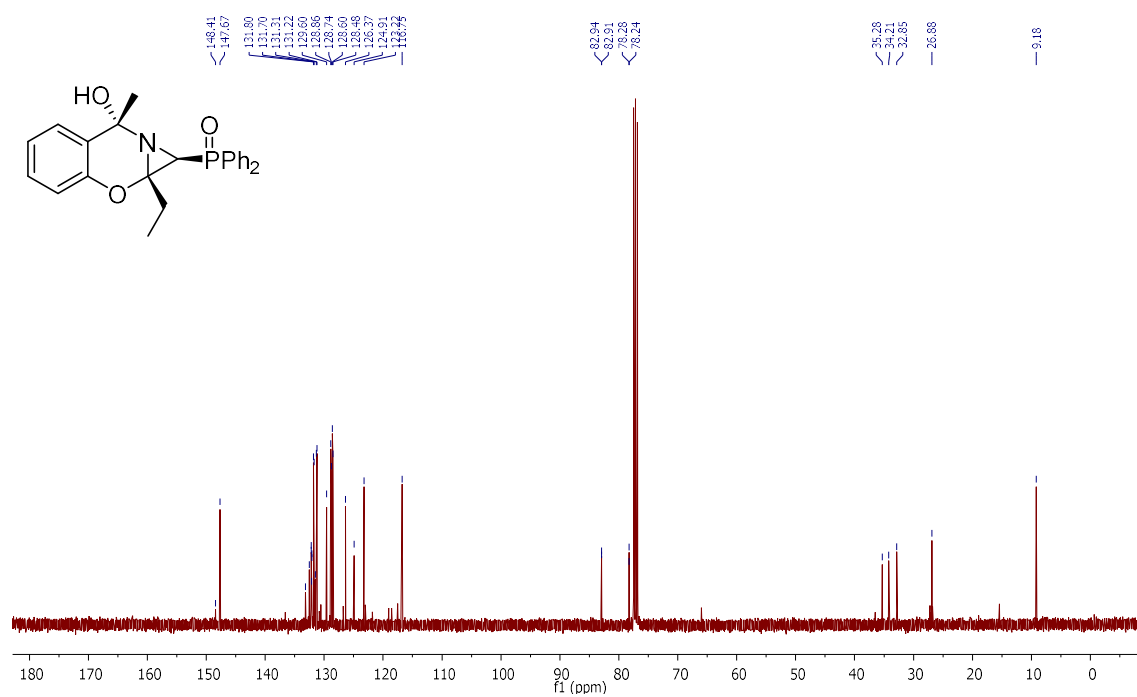
^1H NMR (400 MHz, CDCl_3) of aziridine derivative **6a**



^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) of aziridine derivative **6a**



¹H NMR (400 MHz, CDCl₃) of aziridine derivative **6b**¹³C {¹H} NMR (75 MHz, CDCl₃ + CD₃OD) of aziridine derivative **6b**

^1H NMR (400 MHz, CDCl_3) of aziridine derivative **6c** ^{13}C { ^1H } NMR (100 MHz, CDCl_3) of aziridine derivative **6c**

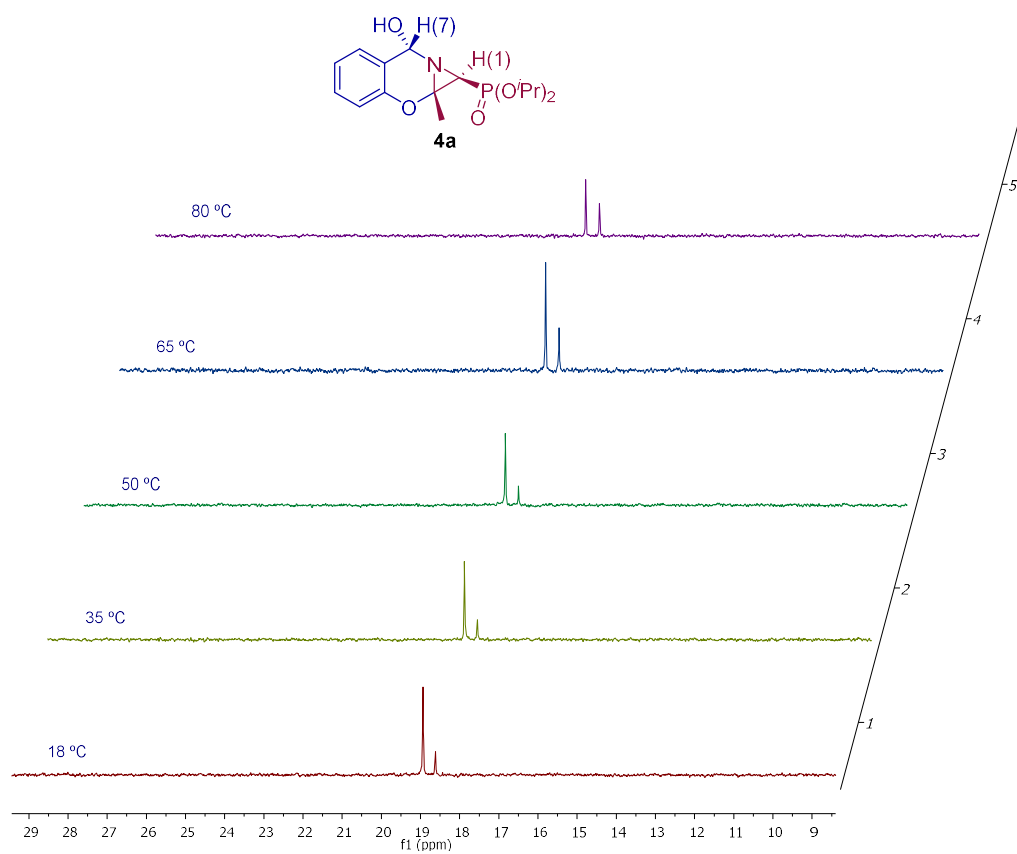


Figure S1. Variable-temperature ^{31}P NMR study for **4a** in $\text{DMSO-}d_6$.

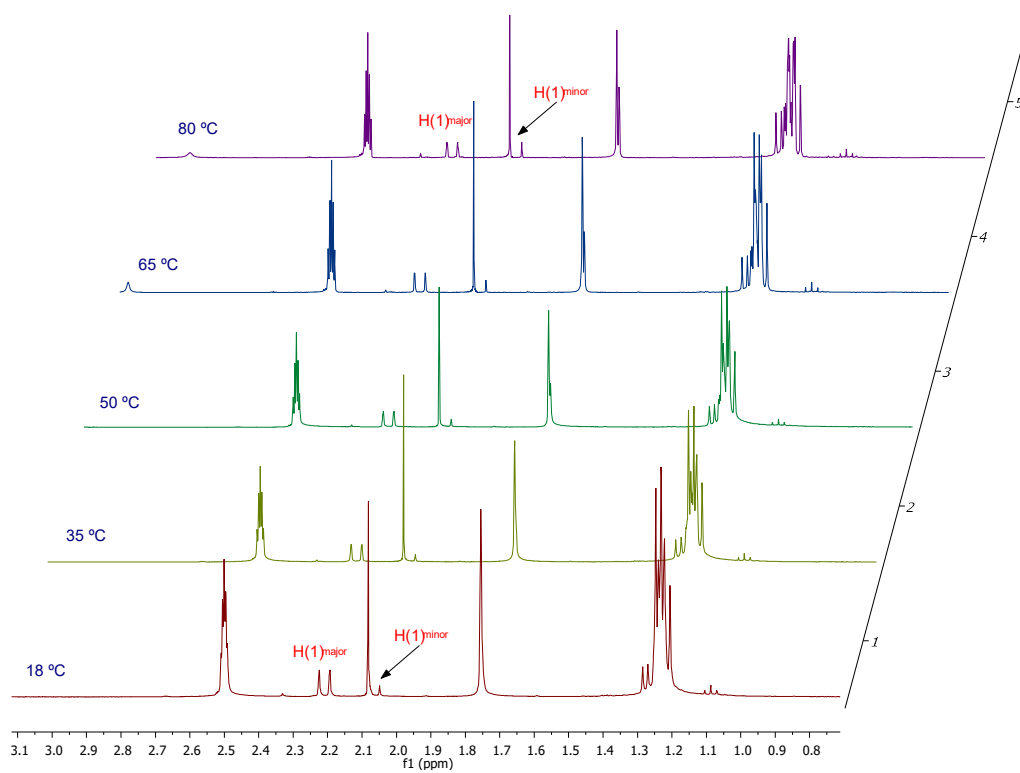
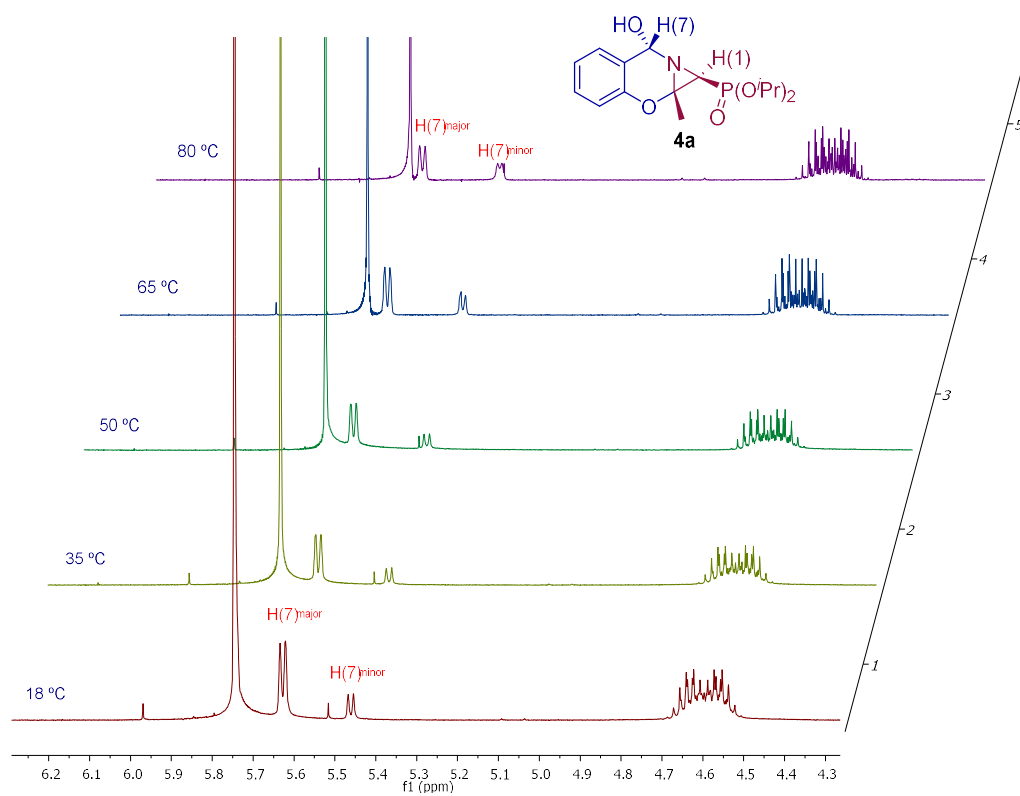


Figure S2. Variable-temperature ^1H NMR study for **4a** in $\text{DMSO-}d_6$.

ORTEP view and X-ray crystallographic statistics for compounds 4a

(CCDC deposition number 1942125)

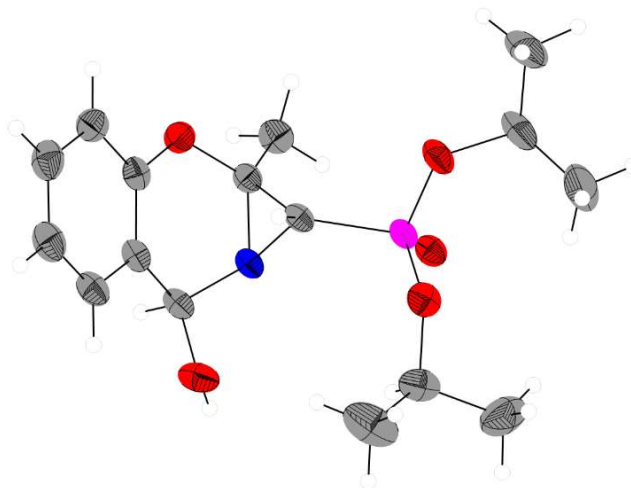


Figure S3. ORTEP diagram of compound **4a**.

Single crystals of $C_{16}H_{24}NO_5P$ [**4a**] were obtained. A suitable crystal was selected and mounted on a SuperNova, Single source at offset/far, Atlas diffractometer. The crystal was kept at 150.01(10) K during data collection. Using Olex2,¹ the structure was solved with the ShelXS² structure solution program using Direct Methods and refined with the ShelXL³ refinement package using Least Squares minimization.

Table S1. Crystal data and structure refinement for **4a**.

Empirical formula	C ₁₆ H ₂₄ NO ₅ P
Formula weight	341.33
Temperature/K	150.01(10)
Crystal system	monoclinic
Space group	P2 ₁ /n
a/Å	10.97315(10)
b/Å	12.38357(8)
c/Å	13.37350(12)
α/°	90.0
β/°	102.4220(9)
γ/°	90.0
Volume/Å ³	1774.74(3)
Z	4
ρ _{calc} /cm ³	1.277
μ/mm ⁻¹	1.583
F(000)	728.0
Crystal size/mm ³	0.604 × 0.439 × 0.381
Radiation	CuKα (λ = 1.54184)
2θ range for data collection/°	10.918 to 137.99
Index ranges	-11 ≤ h ≤ 13, -14 ≤ k ≤ 14, -16 ≤ l ≤ 13
Reflections collected	24219
Independent reflections	3262 [R _{int} = 0.0294, R _{sigma} = 0.0148]
Data/restraints/parameters	3262/0/214
Goodness-of-fit on F ²	1.039
Final R indexes [I >= 2σ (I)]	R ₁ = 0.0448, wR ₂ = 0.1153
Final R indexes [all data]	R ₁ = 0.0460, wR ₂ = 0.1162
Largest diff. peak/hole / e Å ⁻³	0.74/-0.59

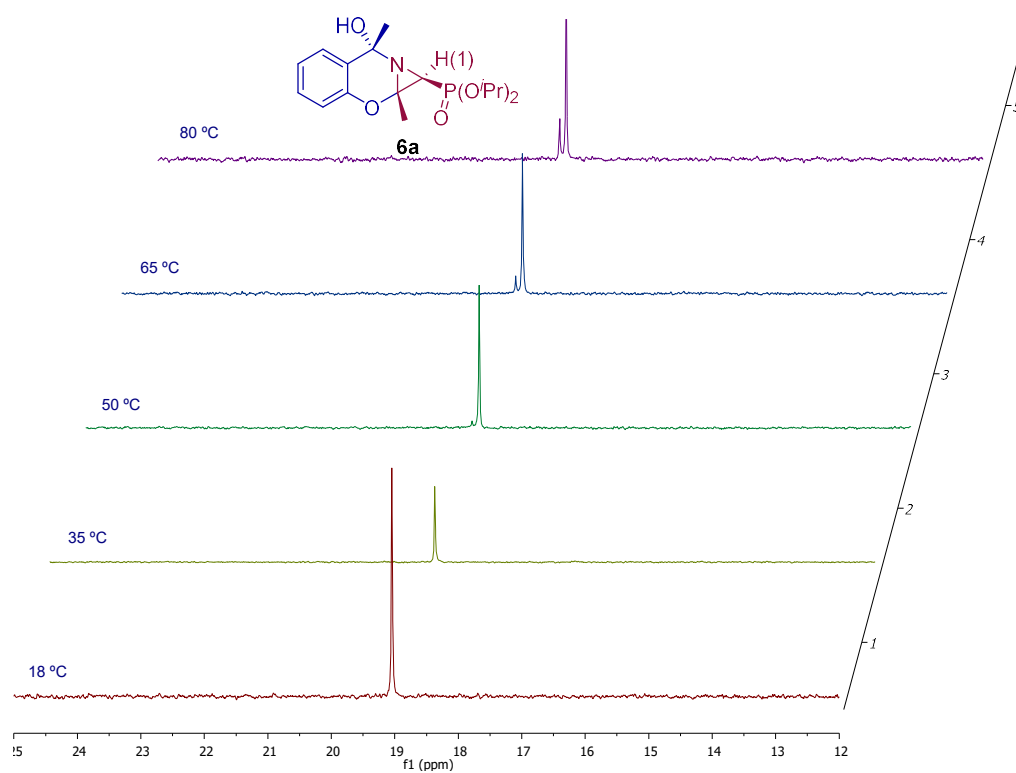


Figure S4. Variable-temperature ^{31}P NMR study for **6a** in $\text{DMSO-}d_6$.

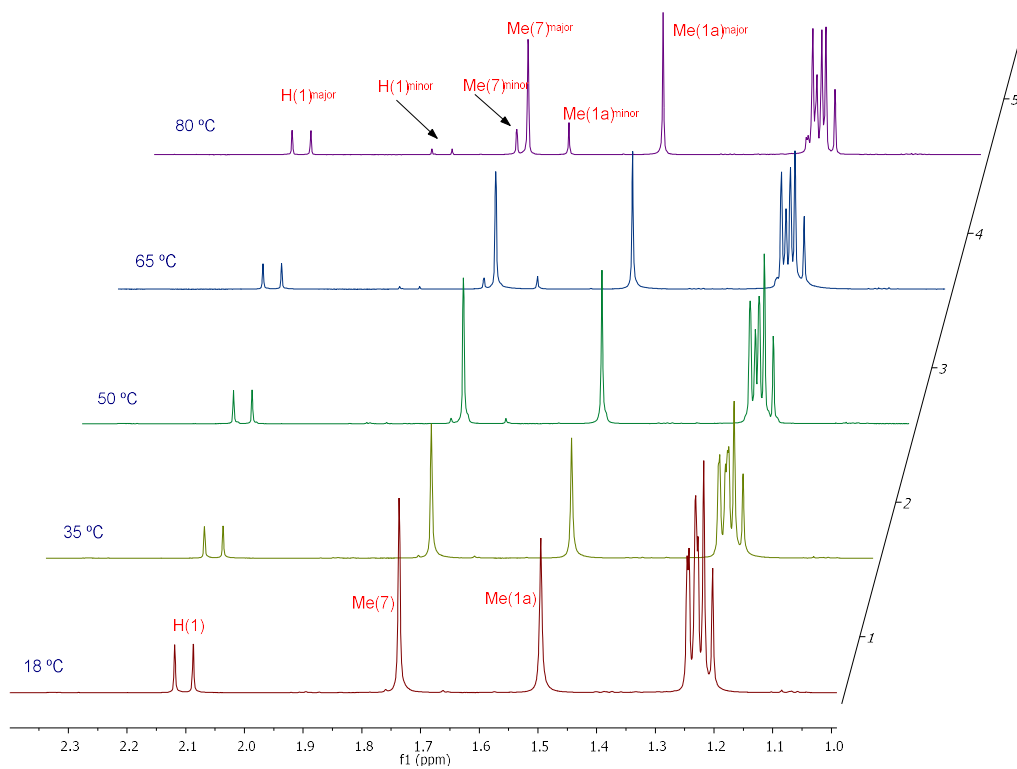
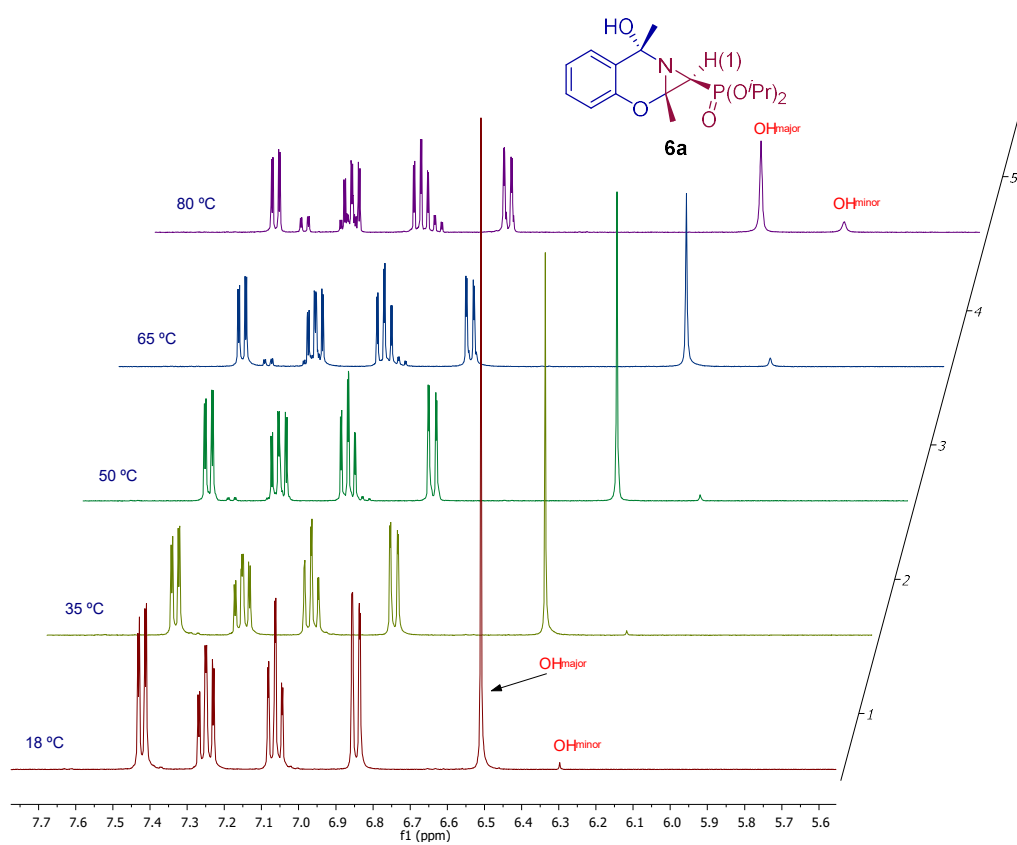


Figure S5. Variable-temperature ¹H NMR study for **6a** in DMSO-*d*₆.

ORTEP view and X-ray crystallographic statistics for compounds 6a

(CCDC deposition number 1942241)

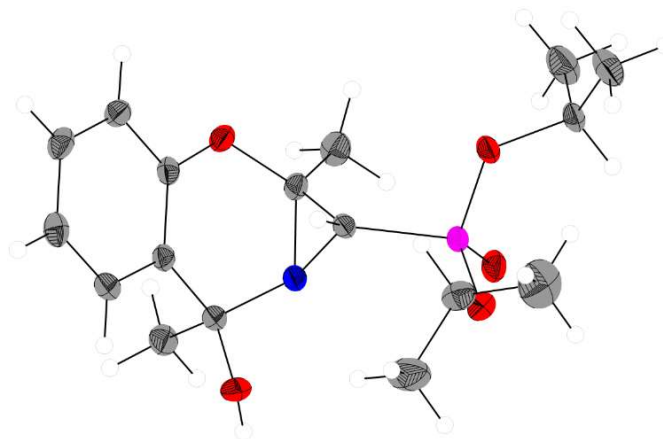


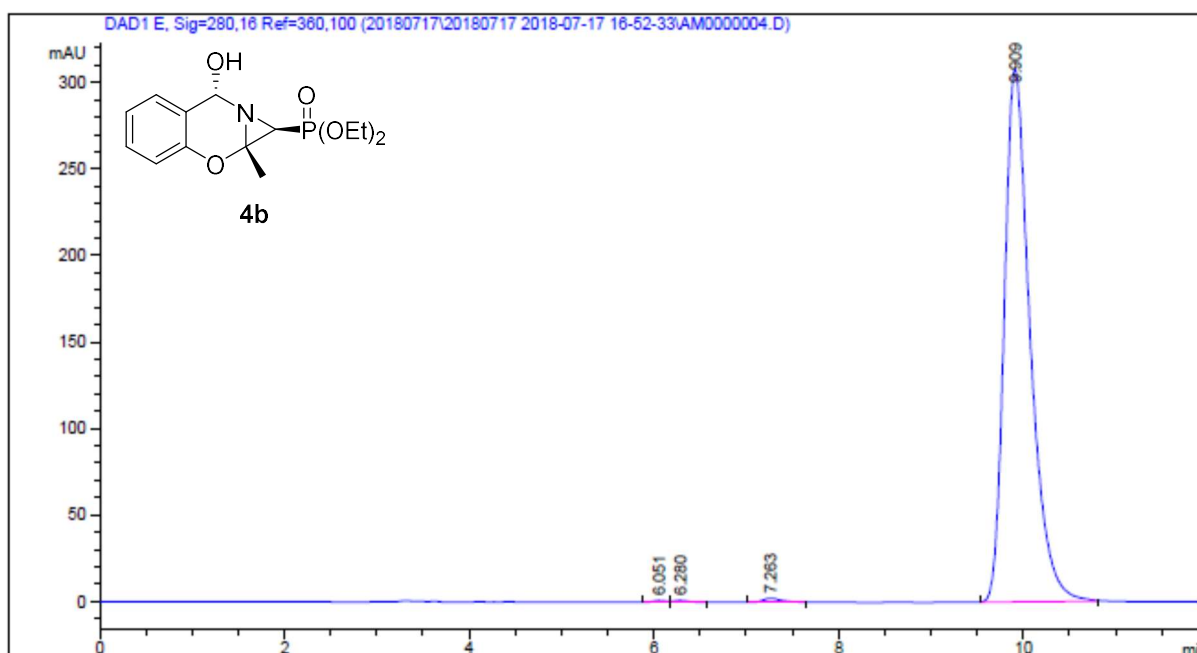
Figure S6. ORTEP diagram of compound **6a**.

Single crystals of $C_{17}H_{26}NO_5P$ [**6a**] were obtained. A suitable crystal was selected and mounted on a SuperNova, Single source at offset/far, Atlas diffractometer. The crystal was kept at 150.01(10) K during data collection. Using Olex2,¹ the structure was solved with the ShelXS² structure solution program using Direct Methods and refined with the ShelXL³ refinement package using Least Squares minimization.

Table S2. Crystal data and structure refinement for **6a**.

Empirical formula	C ₁₇ H ₂₆ NO ₅ P
Formula weight	355.36
Temperature/K	150.01(10)
Crystal system	monoclinic
Space group	P2 ₁ /n
a/Å	10.06027(12)
b/Å	11.42994(13)
c/Å	15.9603(2)
α/°	90.0
β/°	96.0461(12)
γ/°	90.0
Volume/Å ³	1825.04(4)
Z	4
ρ _{calc} /cm ³	1.293
μ/mm ⁻¹	1.543
F(000)	760.0
Crystal size/mm ³	0.31 × 0.184 × 0.11
Radiation	CuKα (λ = 1.54184)
2θ range for data collection/°	9.536 to 137.98
Index ranges	-11 ≤ h ≤ 12, -13 ≤ k ≤ 13, -19 ≤ l ≤ 19
Reflections collected	19882
Independent reflections	3387 [R _{int} = 0.0375, R _{sigma} = 0.0244]
Data/restraints/parameters	3387/0/224
Goodness-of-fit on F ²	1.040
Final R indexes [I ≥ 2σ (I)]	R ₁ = 0.0330, wR ₂ = 0.0839
Final R indexes [all data]	R ₁ = 0.0386, wR ₂ = 0.0878
Largest diff. peak/hole / e Å ⁻³	0.34/-0.33

HPLC Chromatograms for some representative compounds



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                          Area Percent Report
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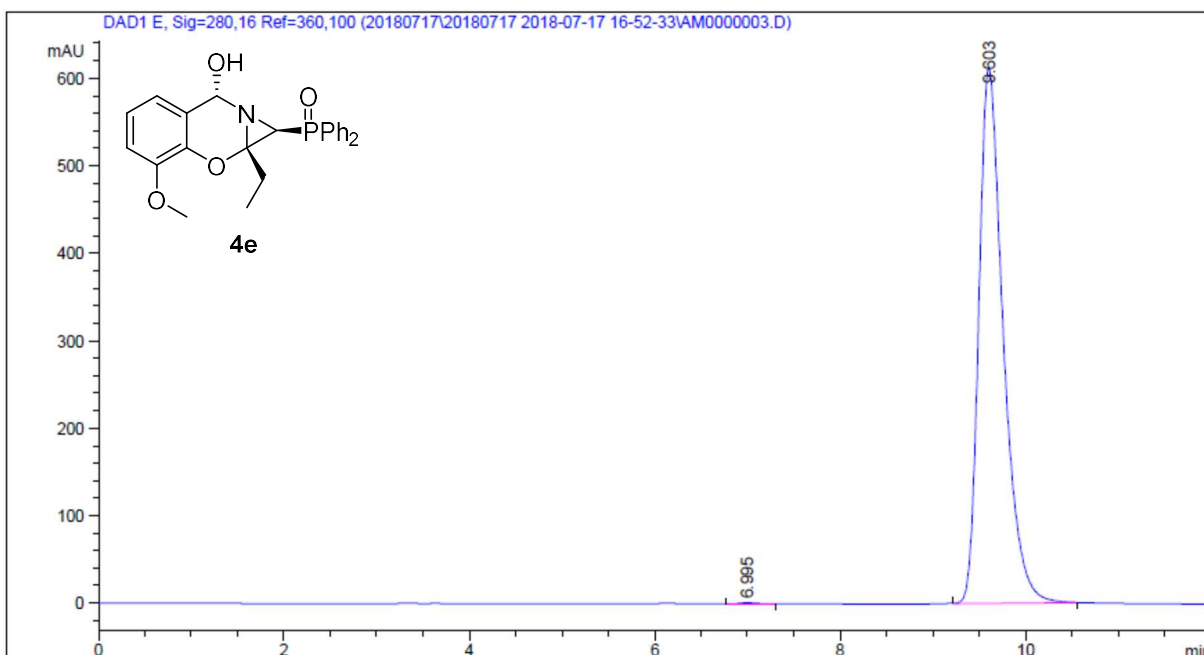
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Dilution           :      1.0000
Use Multiplier & Dilution Factor with ISTDs
  
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Signal 1: DAD1 E, Sig=280,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.051	BV	0.1434	10.42839	1.11219	0.1731
2	6.280	VB	0.1736	13.05122	1.12327	0.2166
3	7.263	BB	0.2226	33.49171	2.23810	0.5558
4	9.909	BB	0.2920	5969.05371	308.06888	99.0546

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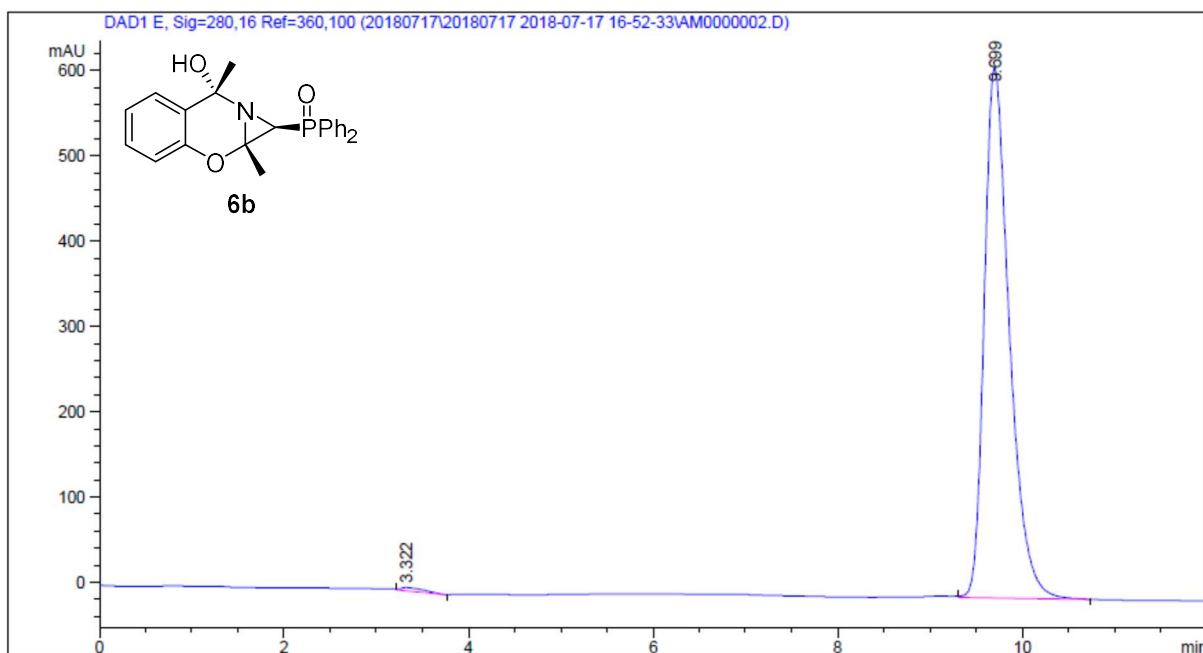


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 Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 E, Sig=280,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.995	BB	0.1954	17.47686	1.34639	0.1561
2	9.603	BB	0.2752	1.11807e4	611.89722	99.8439
Totals :				1.11981e4	613.24361	



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                          Area Percent Report
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Sorted By      :      Signal
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Dilution      :      1.0000
Use Multiplier & Dilution Factor with ISTDs
  
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Signal 1: DAD1 E, Sig=280,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.322	BV	0.2416	68.14645	3.75792	0.5765
2	9.699	BB	0.2845	1.17530e4	621.78510	99.4235

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Totals :                1.18211e4  625.54301
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References

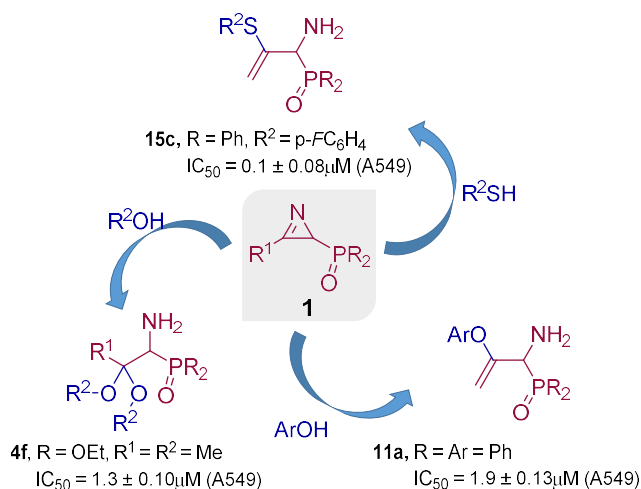
- ¹ O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J. A. K. Howard, H. Puschmann, *J. Appl. Cryst.* **2009**, *42*, 339–341.
- ² G. M. Sheldrick, *Acta Cryst.* **2008**, *A64*, 112–122.
- ³ G. M. Sheldrick, *Acta Cryst.* **2015**, *C71*, 3–8.

Synthesis of α -aminophosphonic acid derivatives through the addition of *O*- and *S*-nucleophiles to 2*H*-azirines and their antiproliferative effect on A549 human lung adenocarcinoma cells.

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Molecules **2020**, *25*, 332.

Agradecimientos a la editorial por otorgar su permiso para reutilizar el material de este artículo en la preparación de esta tesis.

Keywords: allylic α -aminophosphorus compounds; α -aminophosphine oxide or phosphonate acetals; antiproliferative effect; aziridines; 2*H*-azirines.

Abstract:

This work reports a straightforward regioselective synthetic methodology to α -aminophosphine oxide and phosphonates through the addition of oxygen and sulfur nucleophiles to the C–N double bond of 2*H*-azirine derivatives. Determined by the nucleophile nature, different α -aminophosphorus compounds might be obtained. For instance, aliphatic alcohols such as methanol or ethanol afford α -aminophosphine oxide and phosphonate acetals after N–C3 ring opening of the intermediate aziridine. However, addition of 2,2,2-trifluoroethanol, phenols, substituted benzenethiols or ethanethiol to 2*H*-azirine phosphine oxides or phosphonates yields allylic α -aminophosphine oxides and phosphonates with good to high general yields. In some cases, the intermediate aziridine attained by the nucleophilic addition of *O*- or *S*-nucleophiles to the starting 2*H*-azirine may be isolated and characterized before ring opening. Additionally, the cytotoxic effect on cell lines derived from human lung adenocarcinoma (A549) and non-malignant cells (MCR-5) was also screened. Some α -aminophosphorus derivatives exhibited very good activity against the A549 cell line *in vitro*. Furthermore, selectivity towards cancer cell (A549) over non-malignant cells (MCR-5) has been detected in almost all compounds tested.

1. Introduction

α -Aminophosphonic acids are structural bioisosters of amino acids displaying a wide range of biological properties and applications in many areas ranging from agrochemistry to medicine.¹ Some of their varied applications cover antitumor agents,² potent antibiotics,³ as antibacterial agents,⁴ antiviral,⁵ and enzyme inhibitors⁶ such as renin,^{6b,7} or HIV protease,⁸ among others. Joined with their structural similarity to natural carboxylic acids, the intriguing properties of α -aminophosphonic acids also stem from the fact that the tetrahedral geometry of phosphonic acid functionality resembles in a stable manner the high-energy transition state of peptide bond hydrolysis.⁹ The last-mentioned feature is directly responsible for the biological activity of α -aminophosphonic acids, mostly as enzyme inhibitors involved in peptide metabolism.

Moreover, it is well-known that allylic amines¹⁰ are key structural features in variety of natural products and pharmaceuticals, such as the calcium channel blocker flunarizine (**I**),¹¹ effective in the prophylaxis of migraine, and the antifungal drugs naftifine (**II**)¹² and terbinafine

(III),^{12a,13} and have been recognized as an important class of organic compounds owing to their use as valuable intermediates vital for molecular complexity buildup¹⁴ (Figure 1).

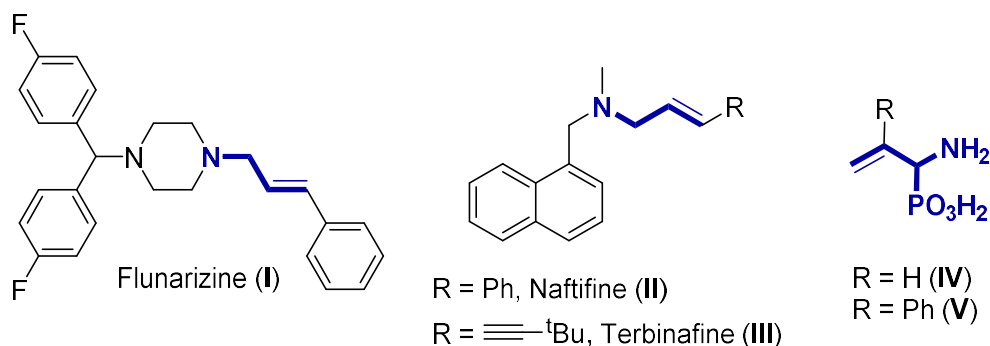


Figure 1. Representative examples of drugs containing allylic amine or allylic α -aminophosphonic acid functionalities.

To overcome the drastic side effects related to a single drug, hybrid molecules modulate several targets of multifactorial diseases, and have been established as a popular approach for multidrug therapy.¹⁵ Within this class of drugs, hybrids molecules introducing two potentially pharmacophores, including allylic amine moieties and α -aminophosphonic acid functional groups, such as allylic α -aminophosphonic acid derivatives (IV and V), have attracted scarce attention since only a few examples have been reported in the literature. For instance, (1-amino-2-propenyl)phosphonic acid (IV) inhibit alanine racemase and D-alanine:D-alanine ligase,¹⁶ while α -aminophosphonic acid analogue (V) of the natural phenylalanine bearing a methylidene at the β -position acts as an inhibitor of phenylalanine ammonia-lyases (PAL)¹⁷ (Figure 1).

There is a new renewal of the interest in covalent binding therapeutics due to the FDA support of efficient and innocuous covalent drugs and a better understanding of the benefits of the covalent binding mechanism.¹⁸ Numerous new drugs contain electrophilic moieties acting as “warheads”, and many molecules with a variety of electrophilic warheads, including epoxide, ketone, nitrile, ester, α,β -unsaturated carbonyl, or aziridine functionalities have been recognized as covalent inhibitors.¹⁹ Aziridines as powerful alkylating agents, may act as covalent drugs by means of their ability to act as DNA cross-linking agents through nucleophilic ring opening of the three-membered nitrogen heterocycle.²⁰

In this sense, we have been previously involved in the synthesis of phosphorus-substituted aziridines *via* nucleophilic addition to 2*H*-azirines.²¹ Moreover, these aziridines are valuable building blocks for the preparation of more complex products, such as 1*H*-benzo[*d*]azepines,^{21a}

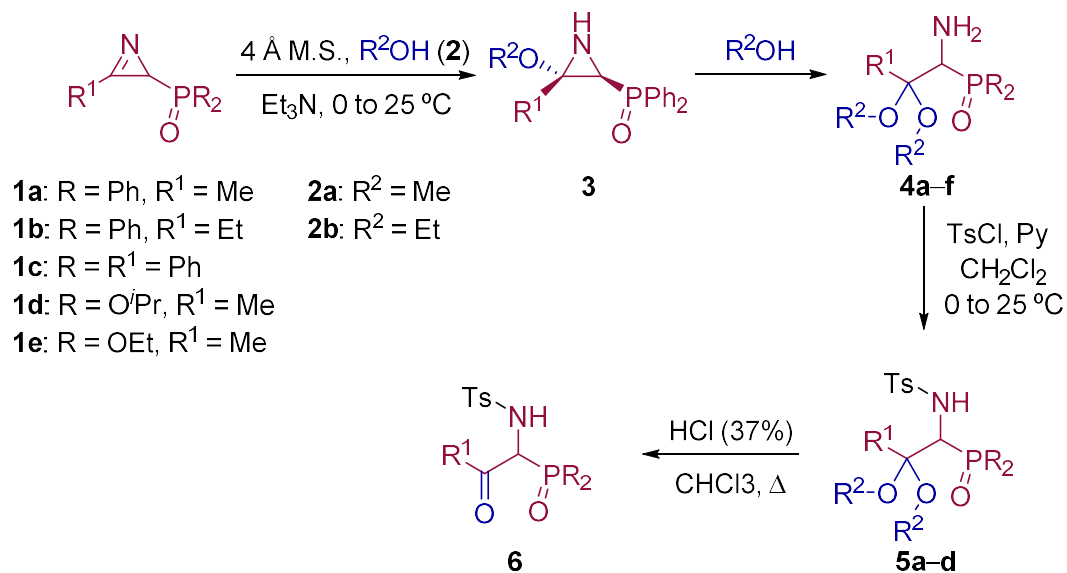
pyrroles,^{21a,b} oxazoles,^{21f} and α - or β -aminophosphorus acid derivatives,^{21d,e} among others. More recently, we have disclosed a diastereoselective approach to cyanoaziridines²² and hybrid molecules, such as azirino[2,1-*b*]benzo[*e*][1,3]oxazines,²³ through nucleophilic addition of cyanide anion or functionalized phenols, respectively, to the C–N double bond of phosphorus substituted 2*H*-azirines. We were intrigued about the possibility of accessing other saturated aziridines containing phosphorus substituents by means of the addition of oxygen and sulfur nucleophilic reagents to 2*H*-azirines. For this aim, here we wish to account the results of the incorporation of aliphatic alcohols, phenols, thiols and benzenethiols into the three-membered nitrogen heterocycle, since these nucleophilic additions to 2*H*-azirines may be a new approach for the construction of substituted aziridines containing phosphorus substituents or even, more complex ring opening compounds. Furthermore, all these new functionalized acyclic and heterocyclic compounds were proved for antiproliferative activity against human cancer cell lines. This strategy entails a stimulating challenge due to the inherent interest of these new molecules, both in synthetic and medicinal chemistry.

2. Results

2.1. Chemistry

First, we studied the addition of aliphatic alcohols **2a–b** to 2*H*-azirine phosphine oxides and phosphonates **1**. Initially, we tested the reaction of 2*H*-azirine phosphine oxide **1a** with two equivalents of methanol (**2a**) in the presence of triethylamine as the base and using methylene chloride as the solvent. Since no reaction was observed using these conditions, the reaction of 2*H*-azirines **1** with aliphatic alcohol **2** as nucleophilic reagent and as the solvent, all at once, was assessed. Therefore, as outlined in Scheme 1, in an initial experiment the nucleophilic addition of methanol (**2a**) to 2*H*-azirine-phosphine oxide **1a** (R = Ph, R¹ = Me) was readily achieved using Et₃N at 25 °C and MeOH as the nucleophile and solvent. Under these reaction conditions, we anticipated to obtain the desired aziridine **3a**, as previously observed in the reaction of fluoroalkylated 2*H*-azirines with methanol.^{21c} Conversely, rather than aziridine **3a**, functionalized α -aminophosphine oxide dimethyl acetal (**4a**, R = Ph, R¹ = R² = Me, Table 1, entry 1) in 74% yield was attained, as evidenced by the two sets of signals for the methoxy group which appeared as singlets in ¹H NMR (see supplementary data). Starting from 2*H*-azirine-phosphine oxide **1b**, α -aminophosphine oxide dimethyl acetal (**4b**, R = Ph, R¹ = Et, R² = Me) was isolated in 92% yield (Scheme 1, Table 1, entry 2), while the addition of methanol (**2a**) to

functionalized 2*H*-azirine-phosphine oxide **1c** furnished 81% of α -aminophosphine oxide dimethyl acetal (**4c**, R = R¹ = Ph, R² = Me) (Scheme 1, Table 1, entry 3).



Scheme 1. Synthesis of α -aminophosphine oxides and phosphonates **4–6**. All synthesized compounds are racemic.

Table 1. α -Aminophosphine oxides and phosphonates **4**, **5** and **6** obtained.

Entry	Compound	R	R ¹	R ²	Yield(%) ^[1]
1	4a	Ph	Me	Me	74
2	4b	Ph	Et	Me	92
3	4c	Ph	Ph	Me	81
4	4d	Ph	Me	Et	56
5	4e	O ⁱ Pr	Me	Me	69
6	4f	OEt	Me	Me	61
7	5a	Ph	Me	Me	80
8	5b	O ⁱ Pr	Me	Me	62
9	5c	OEt	Me	Me	64
10	5d	OEt	Me	Et	70 ^[2]
11	6	O ⁱ Pr	Me	–	68

^[1] Yield of isolated purified compounds **4**, **5** and **6**. ^[2] One pot reaction from 2*H*-azirine **1e**.

A rational mechanism for the formation of α -aminophosphine oxide acetals **4** can be explained *via* initial nucleophilic addition of methanol (**2a**) at the carbon-nitrogen double bond of *2H*-azirine **1** to give aziridine intermediate **3**. As reported previously,^{21c–e,g,h–23} this nucleophilic addition is likely to arise in a diastereoselective way through the less hindered face. Subsequent ring opening to form α -aminophosphine oxide acetals **4** occurs with complete site selectivity at N–C3 bond, after nucleophilic attack of a second molecule of methanol. This behavior has been previously observed in the addition of methanol to methylene-*2H*-azirines,²⁴ or more recently to an aryl substituted *2H*-azirine.²⁵

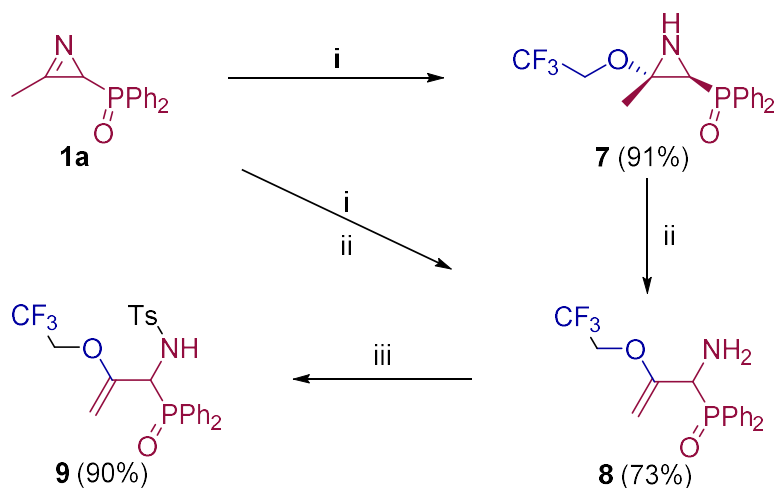
This synthetic procedure could be broadened to the nucleophilic addition of methanol (**2a**) to *2H*-azirine-phosphonates **1d** (R = OⁱPr) and **1e** (R = OEt) under the same reaction conditions (Scheme 1). α -Aminophosphonate dimethyl acetals **4e** (R = OⁱPr, R¹ = R² = Me, Table 1, entry 5) and **4f** (R = OEt, R¹ = R² = Me, Table 1, entry 6) were attained in moderate yields. Next, we tested other aliphatic alcohols in the nucleophilic addition to *2H*-azirines **1**, under the optimal reaction conditions. For instance, *2H*-azirine **1a** (R = Ph) reacted with ethanol (**2b**) in the presence of Et₃N, producing the corresponding α -aminophosphine oxide diethyl acetal **4d** (see Table 1, entry 4).

We also explored the *N*-functionalization of α -aminophosphine oxide and phosphonate acetals **4** using the tosyl group as protecting group. Hence, sulfonylation of compounds **4** were achieved by treatment with *p*-toluenesulfonyl chloride (TsCl) in the presence of pyridine, in methylene chloride (CH₂Cl₂) at 25 °C. The corresponding *N*-tosylates **5** were obtained in moderate to good yields (Table 1, entries 7–9). This process might be performed in a one-pot operation from *2H*-azirines **1** that would be appealing from an atom-economic alternative for carbon-heteroatom bond construction. Therefore, addition of ethanol (**2b**) to *2H*-azirine **1e** in the presence of triethylamine afforded compound **4**, which, without isolation, was subjected to sulfonylation conditions to yield α -aminophosphonate diethyl acetal **5d** (Table 1, entry 10).

In addition, we studied deacetalization reaction of compounds **5** under acidic conditions in order to get β -keto- α -aminophosphonates **6** (Scheme 1). Treatment of α -aminophosphonate dimethyl acetal **5b** with a solution of 37% HCl in chloroform gave ketone **6** in 68% yield (Table 1, entry 11).

Reaction of other aliphatic alcohols to *2H*-azirines **1** was also studied to check if these nucleophiles could give a new entry to functionalized α -aminophosphorus derivatives. For this aim, we explored the reaction of *2H*-azirine phosphine oxide **1a** with 2,2,2-trifluoroethanol (**2c**). However, unlike the α -aminophosphine oxide acetals **4** observed in the reaction of azirines **1**

with methanol or ethanol, when 2*H*-azirine **1a** was treated, even with the standard conditions (see scheme 1) or with two equivalents of trifluoroethanol (**2c**) in the presence of a base such as Et₃N and CH₂Cl₂ as the solvent, aziridine **7** was obtained in very good yield (Scheme 2).



Reagents and reaction conditions: (i) CF₃CH₂OH (**2c**, excess), Et₃N, 4 Å M.S. or CF₃CH₂OH (**2c**, 2eq), Et₃N, 4 Å M.S., CH₂Cl₂, 0 to 25 °C; (ii) CHCl₃, Δ; (iii) TsCl, Py, CH₂Cl₂, 0 to 25 °C.

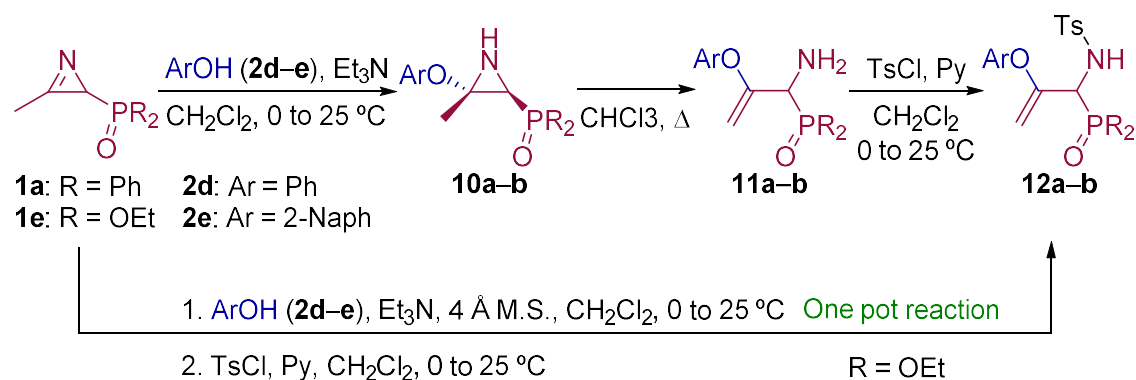
Scheme 2. Addition of 2,2,2-trifluoroethanol to 2*H*-azirine **1a**. All synthesized compounds are racemic.

However, if the addition of trifluoroethanol (**2c**) to **1a** was kept at refluxing chloroform, [1-amino-2-(2,2,2-trifluoroethoxy)allyl]diphenyl phosphine oxide **8** was exclusively obtained instead of aziridine **7** (Scheme 2). Spectroscopic data were in agreement with the assigned structure for compound **8** (see characterization data for compound **8**). The outcome of this conversion may be due to the initial formation of the corresponding aziridine **7**, resulting from the addition of trifluoroethanol (**2c**) to the imine bond of 2*H*-azirine **1a**. Subsequent C–C double bond formation and ring opening through N–C3 bond of aziridine afforded allylic α-aminophosphine oxide **8**. The former compound turned out to be unstable and therefore it was converted into the sulfonamide derivative **9** in 90% chemical yield by treatment with *p*-toluenesulfonyl chloride in the presence of pyridine (Scheme 2).

In order to limit the scope of the addition of *O*-nucleophilic reagents to 2*H*-azirines **1** and increase the diversity of substituents in our substrates, this methodology was extended to include the reactivity of phenols **2d–e** toward phosphorus substituted 2*H*-azirines **1**. For this

purpose, the nucleophilic addition of phenol (**2d**) to 2*H*-azirine phosphine oxide **1a** was performed using Et₃N as the base in CH₂Cl₂ to yield aziridine **10a** in moderate yield (Table 2, entry 1). Conversely, the addition of 2-naphthol (**2d**) to 2*H*-azirine **1a**, in the same reaction conditions, yielded a mixture of aziridine **10b** and allylic α-aminophosphine oxide **11b** (Table 2, entry 2). Aziridine **10b** seemed to be very unstable and cleavage of the C3–N bond in the three-membered ring of **10b** promptly occurs to give allylic α-aminophosphine oxide **11b**. This observation was further confirmed when aziridine **10a**, or a mixture of aziridine **10b** and derivative **11b** was heated at refluxing chloroform. Under these reaction conditions, allylic α-aminophosphine oxide **11a** or **11b**, respectively, was obtained in good yields (Table 2, entry 3 and 4). We then extended the scope of the nucleophilic addition of phenols (**2d–e**) to 2*H*-azirine phosphonate **1e**. In this case, only allylic α-aminophosphonates **11** were directly observed in the crude NMR, but owing to their instability, they could not be isolated. Hence, intermediates **11**

Table 2. Aziridines **10** and allyl α-aminophosphine oxides and phosphonates **11** and **12** obtained^[1].



Entry	Compound	R	Ar	Yield(%) ^[2]
1	10a	Ph	Ph	70
2	10b	Ph	2-Naph	^[3]
3	11a	Ph	Ph	93
4	11b	Ph	2-Naph	74
5	12a	OEt	Ph	88 ^[4]
6	12b	OEt	2-Naph	67 ^[4]

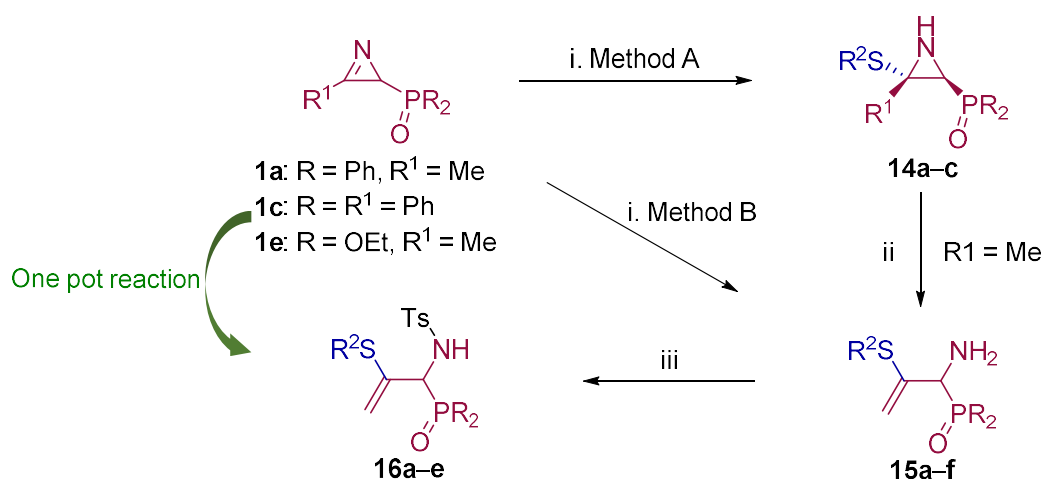
^[1] All synthesized compounds are racemic. ^[2] Yield of isolated purified compounds **10**, **11**, and **12**. ^[3] Identified compound in the crude reaction mixture. ^[4] One pot reaction from 2*H*-azirine **1e**.

derived from phosphonates were submitted to sulfonylation reaction in a one-pot procedure giving to the formation of allylic *N*-tosyl α -aminophosphonates **12a–b** (Table 2, entries 5 and 6).

As far as we know, this regioselective process represents the first example of the synthesis of an allylic α -aminophosphorus derivative through the addition of oxygen nucleophiles to the carbon-nitrogen double bond of a phosphorus substituted *2H*-azirines.

Finally, in order to authenticate the potential of our synthetic methodology, we investigated the nucleophilic addition of sulfur nucleophiles to our phosphorus substituted *2H*-azirines **1**. We anticipated that nucleophilic addition of thiophenols and thiols to *2H*-azirines **1**, would supply a useful approach to the synthesis of aziridine derivatives **14** or even allylic α -aminophosphorus compounds **15**. Thus, as outlined in Scheme 3, in an initial experiment the nucleophilic addition of benzenethiol (**13a**) ($R^2 = \text{Ph}$) to *2H*-azirine phosphine oxide **1a** ($R = \text{Ph}$, $R^1 = \text{Me}$) was readily attained using Et_3N in dichloromethane at 25 °C (method A). Under these reaction conditions, aziridine derivative **14a** was achieved in 92% yield (Table 3, entry 1). This aziridine **14a** was very unstable since after crystallization the ^1H NMR spectrum showed different signals corresponding to aziridine **14a** and minor ones matching to the allylic α -aminophosphine oxide **15a**, formed through C3–N bond cleavage of aziridine ring. After a brief heating of the **14a** and **15a** compounds mixture in refluxing chloroform, only allylic α -aminophosphine oxide **15a** was observed by NMR (Table 3, entry 4). In our previous results,^{21d} both trapping of aziridine intermediate nor detection in crude NMR could be accomplished, and only the allylic α -aminophosphine oxide **15a** was observed instead. Similarly, starting from *2H*-azirine **1a** and 4-methylbenzenethiol (**13b**) ($R^2 = p\text{-MeC}_6\text{H}_4$), a mixture of aziridine **14b** (method A, Table 3, entry 2) and allylic α -aminophosphine oxide **15b** was isolated, which afforded **15b** after heating in refluxing chloroform. Next, we carried out the addition of benzenethiol (**13a**) to *2H*-azirine phosphine oxide **1c** ($R = R^1 = \text{Ph}$) avoiding the C–C double bond formation and confirming the reaction mechanism. Thus, reaction of *2H*-azirine **1c** with benzenethiol (**13a**) in the standard reaction conditions (method A) allowed us to get *E*-aziridine derivative **14c** stereoselectively (Scheme 3, Table 3, entry 2).

Optimization of the reaction conditions, allow us to achieve the successful regioselective formation of allylic α -aminophosphorus derivatives **15**. Therefore, when *2H*-azirine **1a** reacted with 4-methylbenzenethiol (**13b**) ($R^2 = p\text{-MeC}_6\text{H}_4$) without base at 0 °C for 48h (method B), only the formation of derivative **15b** was observed in 89% chemical yield (Scheme 3, Table 3, entry



Reagents and reaction conditions: (i) Method A: R_2SH (**13**), Et_3N , 4 Å M.S., CH_2Cl_2 , 0 to 25 °C, Method B: R_2SH (**13**), CH_2Cl_2 , 0 °C, 48h; (ii) $CHCl_3$, Δ ; (iii) $TsCl$, Py , CH_2Cl_2 , 0 °C.

13a: $R^2 = Ph$; **13b:** $R^2 = p\text{-MeC}_6\text{H}_4$; **13c:** $R^2 = p\text{-FC}_6\text{H}_4$; **13d:** $R^2 = p\text{-MeOC}_6\text{H}_4$; **13e:** $R^2 = Et$

Scheme 3. Synthesis of azidines **14** and allylic α -aminophosphine oxides and phosphonates **15** and **16**. All synthesized compounds are racemic.

5). Further scrutiny of the synthetic approach revealed that this process is also suitable to other substituted benzenethiols **13**. For instance, as outlined in Scheme 3, 2*H*-azirine phosphine oxide **1a** (R = Ph, R¹ = Me) reacted with 4-fluorobenzenethiol (**13c**) ($R^2 = p\text{-FC}_6\text{H}_4$) or 4-methoxybenzenethiol (**13d**) ($R^2 = p\text{-MeOC}_6\text{H}_4$) for 48h at 0 °C, giving the corresponding allylic α -aminophosphine oxides **15c–d** (see Table 3, entries 6–7). This method also accommodates other 2*H*-azirines with phosphonate substitution, given that addition reaction of benzenethiol (**13a**) to 2*H*-azirine **1e** (R = OEt, R¹ = Me) afforded allylic α -aminophosphonate **15e** in moderate yield (Table 3, entry 8). Likewise, aliphatic thiols such as ethanethiol (**13e**) satisfactorily reacted with 2*H*-azirine **1a** giving to the formation of derivative **15f** in a regioselective fashion (Table 3, entry 9).

Finally, we also examined the *N*-protection of allylic α -aminophosphine oxides and phosphonates **15**. As before, for this aim we used the tosyl group as *N*-protecting group. Then, compounds **15** were subjected to sulfonylation reaction using the standard conditions already used formerly ($TsCl$ in the presence of pyridine, CH_2Cl_2 as the solvent, and at 25 °C), and allylic *N*-tosyl α -aminophosphine oxides and phosphonates **16a–c** were attained in good yields (Scheme 3, Table 3, entries 10–12). The process might be performed in a one-pot procedure from 2*H*-azirine **1e** when it reacts with *p*-substituted benzenethiols **13** at 0 °C for 48 h and

subsequent treatment with *p*-toluenesulfonyl chloride in the presence of pyridine, yielding allylic *N*-tosyl α -aminophosphonates **16d–e** (Table 3, entries 13–14).

This approach represents a practical short regioselective route to allylic α -aminophosphine oxides and phosphonates **15** *via* addition reaction of sulfur nucleophiles to phosphorus substituted 2*H*-azirines **1**. Moreover, *N*-functionalization by adding electron-withdrawing groups can be performed by *N*-tosylation of the corresponding derivatives **15**.

Table 3. Aziridines **14** and allylic α -aminophosphine oxides and phosphonates **15** and **16** obtained.

Entry	Compound	R	R ¹	R ²	Yield(%) ^[1]
1	14a	Ph	Me	Ph	92 ^[2]
2	14b	Ph	Me	<i>p</i> -MeC ₆ H ₄	^[3,4]
3	14c	Ph	Ph	Ph	60 ^[2]
4	15a	Ph	–	Ph	91 ^[2]
5	15b	Ph	–	<i>p</i> -MeC ₆ H ₄	89 ^[4]
6	15c	Ph	–	<i>p</i> -FC ₆ H ₄	76 ^[4]
7	15d	Ph	–	<i>p</i> -MeOC ₆ H ₄	70 ^[4]
8	15e	OEt	–	Ph	41 ^[4]
9	15f	Ph	–	Et	64 ^[2]
10	16a	Ph	–	Ph	87
11	16b	Ph	–	<i>p</i> -MeC ₆ H ₄	84
12	16c	OEt	–	Ph	85
13	16d	OEt	–	<i>p</i> -FC ₆ H ₄	85 ^[5]
14	16e	OEt	–	<i>p</i> -MeC ₆ H ₄	73 ^[5]

^[1] Yield of isolated purified compounds **14**, **15**, and **16**. ^[2] Using method A. ^[3] Identified compound in the crude reaction mixture. ^[4] Using method B. ^[5] One pot reaction from 2*H*-azirine **1e**.

2.2. Biological results

The cytotoxicity of the new α -aminophosphine oxide and phosphonate acetals **4** and **5**, β -keto- α -aminophosphonate **6**, aziridines **7**, **10** and **14**, and allylic α -aminophosphine oxides and phosphonates **8**, **9**, **11**, **12**, **15** and **16** was investigated *in vitro* by checking their antiproliferative

activities against the human cancer cell line A549 (carcinomic human alveolar basal epithelial cells). Human colon carcinoma cell line (RKO) was also used to test the antiproliferative activity of some of our compounds. In order to assess growth inhibition, cell counting kit (CCK-8) assay was employed. Cell proliferation inhibitory activities as IC_{50} values for all synthesized compounds and chemotherapeutic doxorubicin (DOX) are displayed in Tables 4 and 5. Likewise, healthy lung cells, such as MRC-5 non-malignant lung fibroblasts were tested for studying selective cytotoxicity.²⁶

Primary **4** and secondary α -aminophosphine oxides and phosphonate acetals **5** demonstrated cytotoxic effect when evaluated against A549 cell line *in vitro* (Table 4, entries 2–11). For instance, compounds **4** showed IC_{50} values between 1.3 ± 0.10 and 21.3 ± 0.22 μ M, with the most effective compound being α -aminophosphonate dimethyl acetal **4f** (Table 4, entry 7) with an IC_{50} value of 1.3 ± 0.10 μ M. Similar activities was observed for secondary α -aminophosphine oxides and phosphonate acetals **5** with IC_{50} values between 1.7 ± 0.30 and 8.2 ± 0.23 μ M, with the most cytotoxic compound being *N*-tosyl α -aminophosphonate dimethyl acetal **5b** (Table 4, entry 9). The hydrolysis of acetal group seemed not to have any effect since β -keto- α -aminophosphonate **6** do not exhibited any toxicity toward A549 (Table 4, entry 12).

Concerning allylic α -aminophosphorus derivatives obtained from the addition of trifluoroethanol (**2c**) or phenols (**2d–e**), besides allylic α -aminophosphine oxide **8** (Table 4, entry 15) which do not exhibited any toxicity effect toward A549, derivatives **11a–b** even allylic *N*-tosyl α -aminophosphine oxide **9** and phosphonates **12a–b** displayed very good cytotoxicity (Table 4, entries 16–17, 18, 19–20, respectively).

Regarding the new oxygen and sulfur-containing aziridine derivatives **7**, **10a**, (Table 4) and **14c** (Table 5) against A549 cell line *in vitro*, diphenyl [3-phenyl-3-(phenylthio)aziridin-2-yl]phosphine oxide (**14c**) was the most cytotoxic compound with an IC_{50} value of 1.1 ± 0.32 μ M (Table 5, entry 2).

We next studied allylic α -aminophosphorus derivatives with sulfur substituents **15** and **16** into their cytotoxicity against A549 cell line (Table 5). All of them showed good cytotoxicity. For instance, IC_{50} values between 0.1 ± 0.08 and 7.2 ± 0.49 μ M was observed, being allylic α -aminophosphine oxide **15c** (Table 5, entry 5) the most effective compound for primary allylic α -aminophosphorus derivatives **15**. However, for allylic *N*-tosyl α -aminophosphorus derivatives **16**, the most cytotoxic compound with an IC_{50} value of 0.2 ± 0.07 μ M was derivative **16c** (Table 5, entry 10).

Table 4. Antiproliferative activity of synthesized compounds obtained from the addition of alcohols and phenols to 2*H*-azirines **1**.

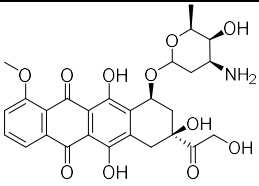
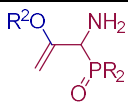
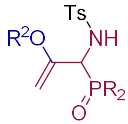
Entry	Comp.	R	R ¹	R ²	Cytotoxicity IC ₅₀ (μM) ^[1]	
					Lung A549	MRC-5
1	 Doxorubicin	–	–	–	0.48 ± 0.017 ²⁷	>50 ²⁸
2 ^[2]	4a	Ph	Me	Me	4.4 ± 0.72	>50
3	4b	Ph	Et	Me	21.3 ± 0.22	>50
4	4c	Ph	Ph	Me	16.1 ± 2.03	>50
5	4d	Ph	Me	Et	9.6 ± 1.13	>50
6	4e	O ⁱ Pr	Me	Me	4.6 ± 0.31	>50
7	4f	OEt	Me	Me	1.3 ± 0.10	>50
8	5a	Ph	Me	Me	8.2 ± 0.23	>50
9	5b	O ⁱ Pr	Me	Me	1.7 ± 0.30	>50
10	5c	OEt	Me	Me	4.5 ± 0.45	>50
11	5d	OEt	Me	Et	3.7 ± 0.49	>50
12	6	O ⁱ Pr	Me	–	>50	^[3]
13	7	Ph	–	CH ₂ CF ₃	3.6 ± 0.70	>50
14	10a	Ph	–	Ph	13.3 ± 1.69	>50

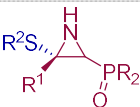
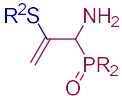
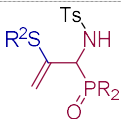
Table 4 (cont.). Antiproliferative activity of synthesized compounds obtained from the addition of alcohols and phenols to 2*H*-azirines **1**.

Entry	Comp.	R	R ¹	R ²	Cytotoxicity IC ₅₀ (μM) ^[1]	
					Lung A549	MRC-5
						
15	8	Ph	–	CH ₂ CF ₃	>50	>50
16 ^[2]	11a	Ph	–	Ph	1.9 ± 0.13	>50
17	11b	Ph	–	2-Naph	2.7 ± 0.44	33.6 ± 3.73
						
18	9	Ph	–	CH ₂ CF ₃	3.5 ± 0.77	>50
19	12a	OEt	–	Ph	4.8 ± 0.90	>50
20	12b	OEt	–	2-Naph	2.1 ± 0.22	17.5 ± 1.47

^[1] The cytotoxicity IC₅₀ values listed are the concentrations corresponding to 50% growth inhibition. ^[2] The cytotoxicity value against human colon carcinoma cell line (RKO) is >50 μM. ^[3] Not determined.

Some of our synthesized compounds were tested as antiproliferative agents toward RKO cell line. For instance, α-aminophosphine oxide acetal **4a**, allylic α-aminophosphine oxide **11a** (Table 4, entries 2, 16), and allylic α-aminophosphine oxides **15a**, **15c**, and **16c** (Table 5, entries 3, 5, and 10) do not exhibited any toxicity toward RKO. However, good cytotoxicity effect was observed for aziridine phosphine oxide **14c**, with an IC₅₀ value of 9.7 ± 1.4 μM (Table 5, entry 2). Additionally, MRC-5 non-malignant lung fibroblasts were tested to explore selective toxicity [²⁶]. Except for some allylic α-aminophosphorus derivatives, which displayed moderate cytotoxicity, nearly all the synthesized α-aminophosphorus derivatives, aziridines, and doxorubicin did not exhibit toxicity toward MRC-5 cell line (see Tables 4 and 5). Additionally, aziridine **14c**, (Table 5, entry 2) which showed good cytotoxicity against A549 and RKO cell lines, also exhibited good cytotoxicity toward MRC-5 cell line.

Table 5. Antiproliferative activity of synthesized compounds obtained from the addition of thiophenols to 2*H*-azirines **1**.

Entry	Comp.	R	R ¹	R ²	Cytotoxicity IC ₅₀ (μM) ^[1]	
					Lung A549	MRC-5
1	DOX	–	–	–	0.48 ± 0.017 ²⁷	>50 ²⁸
						
2 ^[2]	14c	Ph	Ph	Ph	1.1 ± 0.32	4.9 ± 0.49
						
3 ^[3]	15a	Ph	–	Ph	2.6 ± 0.68	15.9 ± 2.79
4	15b	Ph	–	<i>p</i> -MeC ₆ H ₄	5.1 ± 0.77	14.9 ± 1.61
5 ^[3]	15c	Ph	–	<i>p</i> -FC ₆ H ₄	0.1 ± 0.08	>50
6	15d	Ph	–	<i>p</i> -MeOC ₆ H ₄	2.6 ± 0.42	>50
7	15e	OEt	–	Ph	7.2 ± 0.49	>50
						
8	16a	Ph	–	Ph	1.2 ± 0.09	>50
9	16b	Ph	–	<i>p</i> -MeC ₆ H ₄	2.1 ± 0.15	>50
10 ^[3]	16c	OEt	–	Ph	0.2 ± 0.07	24.1 ± 3.55
11	16d	OEt	–	<i>p</i> -FC ₆ H ₄	3.0 ± 0.98	>50
12	16e	OEt	–	<i>p</i> -MeC ₆ H ₄	3.9 ± 0.63	>50

^[1] The cytotoxicity IC₅₀ values listed are the concentrations corresponding to 50% growth inhibition. ^[2] The cytotoxicity value against human colon carcinoma cell line (RKO) is 9.7 ± 1.4 μM.

^[3] The cytotoxicity value against human colon carcinoma cell line (RKO) is >50 μM.

3. Conclusions

To sum up, we have developed a very efficient new approach to α-aminophosphine oxide and phosphonate acetals **4**, through the nucleophilic addition of methanol or ethanol to the carbon-nitrogen double bond of 2*H*-azirine and subsequent ring opening through the N–C3 bond. Conversely, addition of *O*-nucleophiles such as 2,2,2-trifluoroethanol or even phenols to phosphorylated 2*H*-azirines, gave to the regioselective formation of allylic α-aminophosphorus

derivatives **8** and **11**. Initially aziridine intermediate formation, following carbon-carbon double bond construction and ring opening by means of the N–C3 aziridine bond occurred to afford compounds **8** and **11**. Under these reaction conditions, in some cases, aziridine intermediates **7** and **10** can be isolated and characterized. To the best of our knowledge, this process exemplifies the first example of a regioselective nucleophilic addition of oxygen nucleophiles to the carbon-nitrogen double bond of a phosphorus substituted 2*H*-azirine with the formation of allylic α -aminophosphorus derivatives. Furthermore, *N*-functionalization of α -aminophosphine oxide and phosphonate acetals **4** or allylic α -aminophosphorus derivatives **8** and **11** were assessed by sulfonylation reaction.

As an extension of our previous results, we have broadened this process through the addition of sulfur nucleophiles to phosphorylated 2*H*-azirines, with the synthesis of novel sulfur-containing allylic α -aminophosphine oxides and phosphonates **15**.

Oxygen and sulfur containing α -allylic phosphine oxides and phosphonates, here synthesized, might be regarded as new hybrid molecules introducing two potential pharmacophores, allylic amine and α -aminophosphonic acid moieties. These new hybrid molecules may retain the functional properties of the parent molecules. Moreover, the therapeutic efficiency of all the synthesized α -aminophosphorus derivatives and aziridines was evaluated against the human cancer cell line A549. The best cytotoxic effect was observed for α -aminophosphonate acetal **4f** with an IC_{50} value of $1.3 \pm 0.10 \mu\text{M}$, allylic α -aminophosphine oxide **11a** with a IC_{50} value of $1.9 \pm 0.13 \mu\text{M}$, as well as for **15c** with an IC_{50} value of $0.1 \pm 0.08 \mu\text{M}$. Whereas, colon carcinoma cell line (RKO) is not so sensitive to some of the tested synthesized compounds. In addition, cytotoxic effect of almost all of our compounds in non-malignant lung fibroblasts (MRC-5) seems not to exhibit any effect.

4. Experimental section

4.1. Chemistry

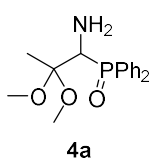
4.1.1. General experimental information

Solvents for extraction and chromatography were of technical grade. All solvents used in reactions were freshly distilled and dried over molecular sieves 4 Å before use. All other solvents and reagents were obtained from commercial sources and recrystallized or distilled as necessary

or used without further purification. All reactions were performed under an atmosphere of dry nitrogen. Melting points were determined with an Electrothermal IA9100 Digital Melting Point Apparatus and are uncorrected. IR spectra were measured on a Nicolet iS10 Thermo Scientific spectrometer as neat solids. Absorbance frequencies are given at maximum of intensity in cm^{-1} . High-resolution mass spectra (HRMS) were obtained by positive-ion electrospray ionization (ESI) method with a time of flight Q-TOF system. Data are reported in the form m/z (intensity relative to base = 100). ^1H (300, 400 MHz), ^{13}C (75, 100 MHz), ^{19}F (282 MHz), and ^{31}P NMR (120, 160 MHz) spectra were recorded on a Varian VXR 300 MHz or Bruker Avance 400 MHz spectrometers, respectively, in CDCl_3 or $\text{DMSO}-d_6$, as specified below at 25 °C. Chemical shifts (δ_{H}) are reported in parts per million (ppm) with the internal chloroform signal at 7.24 ppm as standard for ^1H NMR. Chemical shifts (δ_{C} and δ_{P}) are reported in parts per million (ppm) with the internal chloroform signal at 77.0 ppm as standard for ^{13}C NMR; the external fluorotrichloromethane (CFCl_3) signal at 0.0 ppm as standard for ^{19}F NMR; or the external H_3PO_4 (50%) signal at 0.0 ppm as standard for ^{31}P NMR. All coupling constants (J) values are given in Hz. ^{19}F and ^{13}C NMR spectra were recorded in a broadband decoupled mode from hydrogen nuclei. Distortionless Enhanced Polarization Transfer (DEPT) supported peak assignments for ^{13}C NMR. The data is being reported as (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, bs = broad singlet). Chromatographic purification was performed as flash chromatography using commercial grades of silica gel finer than 230 mesh with pressure. Analytical thin layer chromatography (TLC) was performed on precoated Merck silica gel 60 F₂₅₄ TLC aluminium plates, and spot visualized with UV light or permanganate stain. 2*H*-Azirines **1** were prepared according to literature procedures.^{21e,h,22,29}

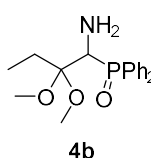
4.1.2. Experimental procedure and characterization data for compounds **4**, **5** and **6**.

*4.1.2.1. General procedure and spectral data for the addition of aliphatic alcohols to functionalized 2*H*-azirines **1**.* To a 0 °C solution of 2*H*-azirine **1** (5 mmol, 1 eq) in aliphatic alcohol **2a–b** (25 mL) and 4 Å M.S., Et_3N (1.4 mL, 10 mmol, 2 eq) was added dropwise. The reaction mixture was allowed to reach 25 °C and stirred until TLC showed the disappearance of starting 2*H*-azirine **1** (24 h). 4 Å M.S. was filtered through a sintered glass vacuum filtration funnel with celite and washed with alcohol. The filtrate was concentrated to dryness in vacuum and the resulting residue was diluted with CH_2Cl_2 (15 mL). The organic phase was washed with water (3 × 15 mL) and extracted with CH_2Cl_2 . The organic layer was dried over anhydrous MgSO_4 , filtered and concentrated to dryness in vacuum. The crude products **4** were purified by crystallization or by flash-column chromatography.



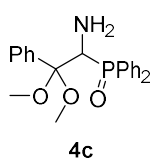
4a

(1-Amino-2,2-dimethoxypropyl) diphenylphosphine oxide (4a). (1.18 g, 74%) obtained as a yellow solid from 2*H*-azirine **1a** (1.28 g, 5 mmol) using MeOH as described in the general procedure. The crude product was purified by crystallization from Et₂O to afford the title compound **4a**. mp 125–127 °C; IR (neat) ν_{\max} 3386, 3056, 2990, 2942, 2832, 1442, 1385, 1179, 1116, 1040, 723, 701 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.80–7.65 (m, 4H, ArH), 7.39–7.21 (m, 6H, ArH), 3.67 (d, ²*J*_{PH} = 5.5 Hz, 1H, CH–P), 3.03 (s, 3H, OCH₃), 2.64 (s, 3H, OCH₃), 1.47 (bs, 2H, NH₂), 1.32 (s, 3H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 133.0 (d, ¹*J*_{PC} = 97.6 Hz, C_{quat}), 133.0 (d, ¹*J*_{PC} = 97.9 Hz, C_{quat}), 131.4, 131.3, 131.1, 131.0, 130.9, 130.9, 128.4 (d, *J*_{PC} = 11.3 Hz), 127.6 (d, *J*_{PC} = 11.8 Hz) (C_{Ar}), 102.5 (d, ²*J*_{PC} = 4.9 Hz, C_{quat}), 54.0 (d, ¹*J*_{PC} = 75.6 Hz, CH–P), 48.1 (OCH₃), 47.6 (OCH₃), 18.4 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 30.9 ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₇H₂₂NNaO₃P ([M + Na]⁺) 342.1235, found 342.1230.



4b

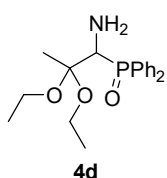
(1-Amino-2,2-dimethoxybutyl)diphenylphosphine oxide (4b). (1.53 g, 92%) obtained as a yellow solid from 2*H*-azirine **1b** (1.35 g, 5 mmol) using MeOH as described in the general procedure. The crude product was purified by crystallization from Et₂O to afford the title compound **4b**. mp 96–97 °C; IR (neat) ν_{\max} 3322, 3060, 2939, 2822, 1442, 1182, 1159, 1097, 1046 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.91–7.81 (m, 4H, ArH), 7.45–7.37 (m, 6H, ArH), 3.84 (d, ²*J*_{PH} = 6.6 Hz, 1H, CH–P), 3.10 (s, 3H, OCH₃), 2.97 (s, 3H, OCH₃), 2.01–1.81 (m, 2H, CH₂), 1.74 (bs, 2H, NH₂), 0.98 (t, ³*J*_{HH} = 7.5 Hz, 3H, CH₃) ppm; ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 133.5 (d, ¹*J*_{PC} = 97.0 Hz, C_{quat}), 133.2 (d, ¹*J*_{PC} = 97.2 Hz, C_{quat}), 131.4, 131.4, 131.3, 131.0, 131.0, 128.5 (d, *J*_{PC} = 11.2 Hz), 127.7 (d, *J*_{PC} = 11.9 Hz) (C_{Ar}), 103.2 (d, ²*J*_{PC} = 3.3 Hz, C_{quat}), 55.5 (d, ¹*J*_{PC} = 75.4 Hz, CH–P), 49.3 (OCH₃), 48.5 (OCH₃), 26.1 (CH₂), 8.8 (CH₃) ppm; ³¹P NMR (160 MHz, CDCl₃) δ 30.3 ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₈H₂₄NNaO₃P ([M + Na]⁺) 356.1391, found 356.1386.



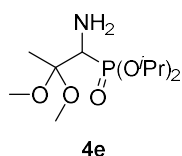
4c

(1-Amino-2,2-dimethoxy-2-phenylethyl)diphenylphosphine oxide (4c). (1.54 g, 81%) obtained as a yellow solid from 2*H*-azirine **1c** (1.59 g, 5 mmol) using MeOH as described in the general procedure. The crude product was purified by flash-column chromatography (SiO₂, AcOEt/hexane 50:50) to afford the title compound **4c**. mp 113–115 °C; IR (neat) ν_{\max} 3462, 3060, 2984, 2939, 1448, 1438, 1372, 1242, 1116, 1097, 1046 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.91–7.20 (m, 15H, ArH), 4.12 (d, ²*J*_{PH} = 8.6 Hz, 1H, CH–P), 3.23 (s, 3H, OCH₃), 3.15 (s, 3H, OCH₃), 1.68 (bs, 2H, NH₂) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 137.2, 134.6, 133.3, 132.9, 131.6, 131.3, 131.2, 131.0, 130.9, 130.9, 130.8, 128.0, 127.9, 127.9, 127.8, 127.8, 127.6, 127.5 (C_{Ar}), 103.7 (d, ²*J*_{PC} = 3.8 Hz, C_{quat}), 56.7 (d, ¹*J*_{PC} = 78.2 Hz, CH–P), 49.8

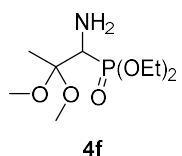
(OCH₃), 48.8 (OCH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 27.8 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₂H₂₄NNaO₃P ([M + Na]⁺) 404.1391, found 404.1386.



(1-Amino-2,2-diethoxypropyl)diphenylphosphine oxide (4d). (0.97 g, 56%) obtained as a yellow solid from 2*H*-azirine **1a** (1.28 g, 5 mmol) using EtOH as described in the general procedure. The crude product was purified by flash-column chromatography (SiO₂, AcOEt) to afford the title compound **4d**. mp 126–128 °C; IR (neat) ν_{\max} 3386, 3326, 2974, 2927, 2889, 1438, 1385, 1182, 1120, 1068, 1049, 951, 723, 695 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.90–7.76 (m, 4H, ArH), 7.44–7.34 (m, 6H, ArH), 3.80 (d, ²*J*_{PH} = 5.0 Hz, 1H, CH-P), 3.48–3.38 (m, 2H, CH₂), 3.24–3.00 (m, 2H, CH₂), 1.59 (bs, 2H, NH₂), 1.49 (s, 3H, CH₃), 1.12 (t, ³*J*_{HH} = 7.0 Hz, 3H, CH₃), 0.51 (t, ³*J*_{HH} = 7.0 Hz, 3H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 133.5 (d, ¹*J*_{PC} = 97.8 Hz, C_{quat}), 133.5 (d, ¹*J*_{PC} = 97.8 Hz, C_{quat}), 131.4, 131.3, 131.0, 130.9, 130.8, 128.5 (d, *J*_{PC} = 11.1 Hz), 127.6 (d, *J*_{PC} = 11.9 Hz) (C_{Ar}), 102.4 (d, ²*J*_{PC} = 4.3 Hz, C_{quat}), 56.2 (OCH₂), 55.1 (OCH₂), 54.63 (d, ¹*J*_{PC} = 75.5 Hz, CH-P), 19.5 (CH₃), 15.2 (CH₃), 14.2 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 31.1 ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₉H₂₆NNaO₃P ([M + Na]⁺) 370.1548, found 370.1543.



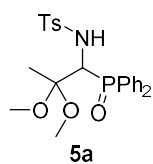
Diisopropyl (1-amino-2,2-dimethoxypropyl)phosphonate (4e). (0.98 g, 69%) obtained as a yellow oil from 2*H*-azirine **1d** (1.10 g, 5 mmol) using MeOH as described in the general procedure. The crude product was purified by flash-column chromatography (SiO₂, AcOEt) to afford the title compound **4e**. IR (neat) ν_{\max} 3300, 2971, 2933, 1467, 1381, 1239, 983 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 4.68–4.58 (m, 2H, OCH), 3.19–3.16 (m, 1H, CH-P), 3.15 (s, 3H, OCH₃), 3.08 (s, 3H, OCH₃), 1.57 (bs, 2H, NH₂), 1.36 (s, 3H, CH₃), 1.25–1.21 (m, 12H, CH(CH₃)₂) ppm; ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 101.8 (d, ²*J*_{PC} = 7.8 Hz, C_{quat}), 70.8 (d, ²*J*_{PC} = 6.8 Hz, OCH), 70.2 (d, ²*J*_{PC} = 7.2 Hz, OCH), 53.0 (d, ¹*J*_{PC} = 153.4 Hz), 48.4 (OCH₃), 47.5 (OCH₃), 24.2 (d, ³*J*_{PC} = 2.7 Hz, CH₃), 24.0 (d, ³*J*_{PC} = 3.4 Hz, CH₃), 23.7 (d, ³*J*_{PC} = 5.2 Hz, CH₃), 23.5 (d, ³*J*_{PC} = 5.8 Hz, CH₃), 17.7 (CH₃) ppm; ³¹P NMR (160 MHz, CDCl₃) δ 23.8 ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₀H₂₃NO₄P ([M-OMe]⁺) 252.1370, found 252.1361.



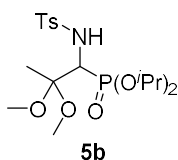
Diethyl (1-amino-2,2-dimethoxypropyl)phosphonate (4f). (0.78 g, 61%) obtained as a brown oil from 2*H*-azirine **1e** (0.96 g, 5 mmol) using MeOH as described in the general procedure. The crude product was purified by flash-column chromatography (SiO₂, AcOEt/MeOH 99:1) to afford the title compound **4f**. IR (neat) ν_{\max} 3466, 3319, 2928, 2945, 2905, 1650, 1454, 1236, 1022 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 4.18–4.05 (m, 4H, OCH₂), 3.32 (d, ²*J*_{PH} = 17.6 Hz, 1H, CH-P), 3.24 (s, 3H, OCH₃), 3.16 (s, 3H, OCH₃), 1.61 (bs, 2H, NH₂), 1.44 (s, 3H, CH₃), 1.31 (dt, ³*J*_{HH} = 7.0 Hz, ³*J*_{PH} = 0.5 Hz, 3H,

CH₃), 1.30 (dt, ³J_{HH} = 7.1 Hz, ³J_{PH} = 0.5 Hz, 3H, CH₃) ppm; ¹³C {1H} NMR (100 MHz, CDCl₃) δ 101.8 (d, ²J_{PC} = 8.1 Hz, C_{quat}), 62.5 (d, ²J_{PC} = 6.6 Hz, OCH₂), 61.8 (d, ²J_{PC} = 6.8 Hz, OCH₂), 52.7 (d, ¹J_{PC} = 152.0 Hz, CH-P), 48.8 (OCH₃), 47.8 (OCH₃), 17.9 (CH₃), 16.5 (d, ³J_{PC} = 4.9 Hz, CH₃), 16.4 (d, ³J_{PC} = 4.9 Hz, CH₃) ppm; ³¹P NMR (160 MHz, CDCl₃) δ 25.9 ppm; ESI-HRMS (CI) *m/z* calcd. for C₈H₁₉NO₄P ([M-OMe]⁺) 224.1057, found 224.1052.

4.1.2.2. *General procedure and spectral data for the N-tosyl functionalization of α-aminophosphine oxide and phosphonate acetals 4.* *p*-Toluenesulfonyl chloride (1 g, 5.5 mmol, 1.1 eq) and pyridine (2.42 mL, 30 mmol, 6 eq) were added to a 0 °C solution of α-aminophosphine oxide or phosphonate acetal **4** (5 mmol, 1 eq) in CH₂Cl₂ (25 mL). The reaction mixture was allowed to reach 25 °C and stirred until TLC showed the disappearance of starting compound **4**. The crude product was washed twice with a 2M HCl solution (15 mL) and water (15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layers were dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum. The crude products **5** were purified by crystallization or by flash-column chromatography.

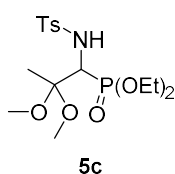


N-[1-(Diphenylphosphoryl)-2,2-dimethoxypropyl]-4-methylbenzenesulfonamide (5a). (1.89 g, 80%) obtained as a yellow solid from α-aminophosphine oxide **4a** (1.60 g, 5 mmol) after 24 h at 25 °C as described in the general procedure. The crude product was purified by crystallization from Et₂O/CH₂Cl₂ 50:50 to afford the title compound **5a**. mp 205–207 °C; IR (neat) *v*_{max} 3440, 2990, 2939, 2885, 1445, 1331, 1182, 1157, 1119, 1097, 1046 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.92–7.06 (m, 14H, ArH), 4.80 (t, ³J_{PH} = 18.1 Hz, ³J_{HH} = 9.1 Hz, 1H, CH-P), 2.92 (s, 3H, OCH₃), 2.60 (s, 3H, OCH₃), 2.31 (s, 3H, CH₃), 1.40 (s, 3H, CH₃), ppm; ¹³C {1H} NMR (100 MHz, CDCl₃) δ 142.2, 139.7, 133.8, 132.8, 132.0, 131.6, 131.3, 131.2, 131.2, 131.1, 131.1, 131.0, 130.9, 128.8, 128.5, 128.4, 127.8, 127.7, 126.5 (C_{Ar}), 102.4 (d, ²J_{PC} = 7.1 Hz, C_{quat}), 55.6 (d, ²J_{PC} = 74.1 Hz, CH-P), 48.3 (OCH₃), 48.0 (OCH₃), 21.4 (CH₃), 19.0 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 31.5 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₄H₂₈NNaO₅PS ([M+Na]⁺) 496.1323, found 496.1318.



Diisopropyl [2,2-dimethoxy-1-((4-methylphenyl)sulfonamido)propyl]phosphonate (5b). (1.36 g, 62%) obtained as a white solid from α-aminophosphonate **4e** (1.42 g, 5 mmol) after 24 h at 25 °C as described in the general procedure. The crude product was purified by flash-column chromatography (SiO₂, AcOEt/hexane 16:84) and crystallization from Et₂O to afford the title compound **5b**. mp 137–139 °C; IR (neat) *v*_{max} 3161, 2923, 1590, 1378, 1328, 1176, 989 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.73 (d, ³J_{HH} = 8.3 Hz, 2H, ArH), 7.24 (d, ³J_{HH} = 8.0 Hz, 2H, ArH), 5.04 (dd,

$^3J_{\text{HH}} = 9.1$ Hz, $^3J_{\text{PH}} = 6.7$ Hz, 1H, NH), 4.76–4.64 (m, 2H, OCH), 4.10 (dd, $^3J_{\text{HH}} = 9.1$ Hz, $^2J_{\text{PH}} = 21.4$ Hz, 1H, CH-P), 3.14 (s, 3H, OCH₃), 2.88 (s, 3H, OCH₃), 2.38 (s, 3H, CH₃), 1.36 (s, 3H, CH₃), 1.32–1.29 (m, 12H, CH(CH₃)₂) ppm; ^{13}C { ^1H } NMR (75 MHz, CDCl₃) δ 142.6, 139.5, 129.0, 126.9 (C_{Ar}), 101.3 (d, $^2J_{\text{PC}} = 12.0$ Hz, C_{quat}), 71.9 (d, $^2J_{\text{PC}} = 7.0$ Hz, OCH), 71.8 (d, $^2J_{\text{PC}} = 7.2$ Hz, OCH), 55.0 (d, $^1J_{\text{PC}} = 154.1$ Hz, CH-P), 49.1 (OCH₃), 48.1 (OCH₃), 23.8 (CH₃), 23.7 (CH₃), 23.6 (CH₃), 23.5 (CH₃), 21.5 (CH₃), 18.4 (CH₃) ppm; ^{31}P NMR (120 MHz, CDCl₃) δ 18.3 ppm; ESI-HRMS (CI) m/z calcd. for C₁₇H₂₉NO₆PS ([M–OMe]⁺) 406.1459, found 406.1450.

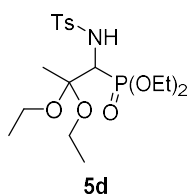


Diethyl [2,2-dimethoxy-1-((4-methylphenyl)sulfonamido)propyl]phosphonate (5c). (1.31 g, 64%) obtained as a pale yellow oil from α -aminophosphonate **4f** (1.28 g, 5 mmol) after 3 h at 25 °C as described in the general procedure. The crude product was purified by flash-column chromatography (SiO₂, AcOEt/hexane 20:80) to afford the title compound **5c**. IR (neat) ν_{max} 3174,

2987, 1331, 1239, 1220, 1157, 995 cm⁻¹; ^1H NMR (400 MHz, CDCl₃) δ 7.74 (d, $^3J_{\text{HH}} = 8.4$ Hz, 2H, ArH), 7.21 (d, $^3J_{\text{HH}} = 8.0$ Hz, 2H, ArH), 5.98 (dd, $^3J_{\text{HH}} = 9.4$ Hz, $^3J_{\text{PH}} = 5.2$ Hz, 1H, NH), 4.15–4.00 (m, 5H, CH-P and OCH₂), 3.14 (s, 3H, OCH₃), 2.86 (s, 3H, OCH₃), 2.36 (s, 3H, CH₃), 1.36 (s, 3H, CH₃), 1.26 (t, $^3J_{\text{HH}} = 7.1$ Hz, 3H, CH₃), 1.24 (t, $^3J_{\text{HH}} = 7.1$ Hz, 3H, CH₃) ppm; ^{13}C { ^1H } NMR (75 MHz, CDCl₃) δ 142.3, 139.7, 128.8, 126.7 (C_{Ar}), 101.3 (d, $^2J_{\text{PC}} = 12.7$ Hz, C_{quat}), 62.9 (d, $^2J_{\text{PC}} = 6.6$ Hz, OCH₂), 62.9 (d, $^2J_{\text{PC}} = 7.1$ Hz, OCH₂), 54.2 (d, $^1J_{\text{PC}} = 153.6$ Hz, CH-P), 49.0 (OCH₃), 48.0 (OCH₃), 21.4 (CH₃), 18.4 (CH₃), 16.2 (CH₃), 16.2 (CH₃) ppm; ^{31}P NMR (120 MHz, CDCl₃) δ 20.3 ppm; ESI-HRMS (CI) m/z calcd. for C₁₅H₂₅NO₆PS ([M–OMe]⁺) 378.1146, found 378.1133.

4.1.2.3. One pot procedure for the synthesis of *N*-tosyl- α -aminophosphonate acetal **5d.** To a 0 °C solution of 2*H*-azirine **1e** (0.96 g, 5 mmol) in EtOH (25 mL) and 4 Å M.S., Et₃N (1.4 mL, 10 mmol, 2 eq) was added dropwise. The reaction mixture was allowed to reach 25 °C and stirred for 24 h until TLC showed the disappearance of starting 2*H*-azirine **1e**. 4 Å M.S. was filtered through a sintered glass vacuum filtration funnel with celite and washed with EtOH. The filtrate was concentrated to dryness in vacuum and the resulting residue was diluted with CH₂Cl₂ (15 mL). The organic phase was washed with water (3 × 15 mL) and extracted with CH₂Cl₂. The organic layer was dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum. To a 0 °C solution of the crude product **4** in CH₂Cl₂ (25 mL) was directly added *p*-toluenesulfonyl chloride (1 g, 5.5 mmol, 1.1 eq) and pyridine (2.42 mL, 30 mmol, 6 eq). The reaction mixture was allowed to reach 25 °C and stirred for 24 h. The crude product was washed twice with a 2M HCl solution (15 mL) and water (15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layer was

dried over anhydrous MgSO_4 , filtered and concentrated to dryness in vacuum. The crude product **5d** was purified by crystallization from Et_2O .



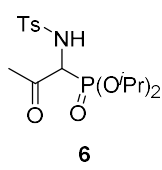
Diethyl [2,2-diethoxy-1-((4-methylphenyl)sulfonamido)propyl]phosphonate (5d). (1.53 g, 70%) obtained as a white solid. mp 126–128 °C; IR

(neat) ν_{max} 3434, 3189, 2979, 2931, 2885, 1560, 1474, 1463, 1391, 1330, 1241, 1158, 1136, 1088, 1052, 1013, 975, 950, 890 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3)

δ 7.76 (d, $^3J_{\text{HH}} = 7.7$ Hz, 2H, ArH), 7.21 (d, $^3J_{\text{HH}} = 7.9$ Hz, 2H, ArH), 5.62 (dd, $^3J_{\text{HH}}$

= 9.5 Hz, $^3J_{\text{PH}} = 5.3$ Hz, 1H, NH), 4.18–3.94 (m, 5H, CH-P and OCH_2), 3.50–3.20 (m, 4H, OCH_2), 2.35 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.25–1.19 (m, 6H, CH_3), 1.10 (t, $^3J_{\text{HH}} = 7.1$ Hz, 3H, CH_3), 0.88 (t, $^3J_{\text{HH}} = 7.1$ Hz, 3H, CH_3) ppm; ^{13}C { ^1H } NMR (75 MHz, CDCl_3) δ 142.6, 139.5, 129.0, 126.8, 120.3 (C_{Ar}), 100.9 (d, $^2J_{\text{PC}} = 11.7$ Hz, C_{quat}), 62.7 (d, $^2J_{\text{PC}} = 7.1$ Hz, OCH_2), 62.7 (d, $^2J_{\text{PC}} = 6.6$ Hz, OCH_2), 56.9 (OCH_2), 55.1 (d, $^1J_{\text{PC}} = 153.6$ Hz, CH-P), 56.0 (OCH_2), 21.4 (CH_3), 19.7 (CH_3), 16.3 (d, $^3J_{\text{PC}} = 3.1$ Hz, CH_3), 16.2 (d, $^3J_{\text{PC}} = 3.0$ Hz, CH_3), 15.1 (CH_3), 14.7 (CH_3) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 20.8 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{18}\text{H}_{32}\text{NNaO}_7\text{PS}$ ($[\text{M} + \text{Na}]^+$) 460.1535, found 460.1549.

4.1.2.4. General procedure and spectral data of β -keto- α -aminophosphine oxide 6. To a stirred solution of α -aminophosphonate acetal **5b** (0.87 g, 2 mmol) in CHCl_3 (10 mL), a 37% solution of HCl (5 drops) was added dropwise. The mixture was refluxed for 5 h and was allowed to reach 25 °C. The crude product was washed twice with water (5 mL). The organic layer was dried over anhydrous MgSO_4 , filtered and concentrated to dryness in vacuum, and the resulting residue was purified by crystallization from Et_2O /hexane 50:50 to afford the title compound **6**.



Diisopropyl [1-((4-methylphenyl)sulfonamido)-2-oxopropyl]phosphonate (6).

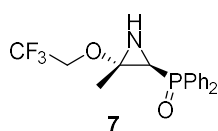
(0.53 g, 68%) as a white solid. mp 121–122 °C; IR (neat) ν_{max} 3136, 2977, 1717, 1328, 1230, 995 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.67 (d, $^3J_{\text{HH}} = 8.3$ Hz, 2H, ArH),

7.26 (d, $^3J_{\text{HH}} = 8.0$ Hz, 2H, ArH), 5.55 (dd, $^3J_{\text{HH}} = 9.3$ Hz, $^3J_{\text{PH}} = 2.0$ Hz, 1H, NH),

4.79–4.71 (m, 2H, OCH), 4.70–4.62 (m, 2H, OCH), 4.41 (dd, $^3J_{\text{HH}} = 9.3$ Hz, $^2J_{\text{PH}} = 25.2$ Hz, 1H, CH-P), 2.39 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 1.34–1.25 (m, 12H, $\text{CH}(\text{CH}_3)_2$) ppm; ^{13}C { ^1H } NMR (100 MHz, CDCl_3) δ 199.8 (C=O), 144.2, 136.0, 129.8, 127.4 (C_{Ar}), 73.4 (d, $^2J_{\text{PC}} = 7.2$ Hz, OCH), 73.3 (d, $^2J_{\text{PC}} = 7.2$ Hz, OCH), 61.5 (d, $^1J_{\text{PC}} = 143.2$ Hz, CH-P), 28.8 (CH_3), 24.0 (d, $^3J_{\text{PC}} = 3.6$ Hz, CH_3), 23.9 (d, $^3J_{\text{PC}} = 3.8$ Hz, CH_3), 23.7 (d, $^3J_{\text{PC}} = 5.2$ Hz, CH_3), 23.6 (d, $^3J_{\text{PC}} = 5.4$ Hz, CH_3), 21.6 (CH_3) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 11.8 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{16}\text{H}_{27}\text{NO}_6\text{PS}$ ($[\text{M} + \text{H}]^+$) 392.1297, found 392.1293.

4.1.3. Experimental procedure and characterization data for compounds **7**, **8** and **9**

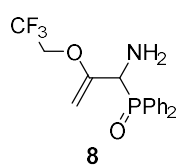
4.1.3.1. *General procedure and spectral data for the addition of 2,2,2-trifluoroethanol (2c) to functionalized 2H-azirines 1.* To a 0 °C solution of 2H-azirine **1a** (1.28 g, 5 mmol, 1 eq) in CH₂Cl₂ (25 mL) was added dropwise 2,2,2-trifluoroethanol (**2c**) (0.73 mL, 10 mmol, 2 eq), Et₃N (3.15 mL, 22.5 mmol, 4.5 eq), and 4 Å M.S. The reaction mixture was allowed to reach 25 °C and stirred at the same temperature for 24 h. 4 Å M.S. was then filtered through a sintered glass vacuum filtration funnel with celite and washed with CH₂Cl₂. The reaction mixture was washed with water (3 × 15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layers were dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum. The crude product was purified by crystallization in Et₂O.



[(2S*,3S*)-3-Methyl-3-(2,2,2-trifluoroethoxy)aziridin-2-yl]diphenylphosphine oxide (7). (1.62 g, 91%) as a yellow solid. mp 98–100 °C; IR (neat) ν_{\max}

3439, 3248, 2972, 2941, 1635, 1590, 1438, 1394, 1359, 1280, 1252, 1169, 1122, 1080, 745, 726, 694 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.78–7.38 (m, 10H, ArH), 3.88 (dq, ²J_{HH} = 1.5 Hz, ³J_{HF} = 8.7 Hz, 2H, CH₂), 2.53 (dd, ³J_{HH} = 9.4 Hz, ²J_{PH} = 21.5 Hz, 1H, CH-P), 1.84 (dd, ³J_{HH} = 9.7 Hz, ³J_{PH} = 18.0 Hz, 1H, NH), 1.69 (s, 3H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 132.5, 132.4, 132.3, 132.2, 131.2, 130.9, 130.9, 130.8, 130.7, 129.0, 128.8, 128.7 (C_{Ar}), 125.4 (d, ¹J_{CF} = 277.8 Hz, CF₃), 71.6 (C_{quat}), 61.8 (q, ²J_{CF} = 33.8 Hz, CH₂), 38.0 (d, ¹J_{PC} = 89.9 Hz), 17.0 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 25.2 ppm; ¹⁹F NMR (282 MHz, CDCl₃) δ -74.6, -74.7, -74.7 ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₇H₁₈F₃NO₂P ([M+H]⁺) 356.1027, found 356.1014.

4.1.3.2. *General procedure and spectral data of allylic α -aminophosphine oxide 8.* A solution of aziridine **7** (1.78 g, 5 mmol, 1 eq) was stirred in refluxing CHCl₃ (11 mL) for 15 h until TLC showed the disappearance of aziridine **7**. The crude product was purified by flash-column chromatography (SiO₂, AcOEt/hexane 50:50) to afford the title compound **8**.

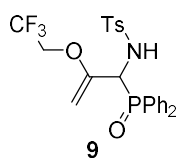


[1-Amino-2-(2,2,2-trifluoroethoxy)allyl]diphenylphosphine oxide (8). (1.30 g, 73%) as a yellow oil. IR (neat) ν_{\max} 3387, 3314, 3059, 2940, 1638, 1591, 1438,

1288, 1169, 1119, 1102, 975, 910, 827, 730, 694 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.90–7.37 (m, 10H, ArH), 4.45 (t, ²J_{HH} = 3.5 Hz, 1H, =CH₂), 4.20 (d, ²J_{PH} = 8.7 Hz, 1H, CH-P), 4.10 (t, ²J_{HH} = 3.2 Hz, 1H, =CH₂), 3.92–3.80 (m, 1H, CH₂), 3.64–3.52 (m, 1H, CH₂), 1.95 (bs, 2H, NH₂) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 158.6 (d, ²J_{PC} = 1.9 Hz, =C-O), 132.0, 132.0, 131.9, 131.9, 131.6, 131.5, 131.5, 131.4 (C_{Ar}), 122.7 (q, ¹J_{CF} = 277.6 Hz, CF₃), 86.8 (d, ³J_{PC} = 6.2 Hz, =CH₂), 64.5 (q, ²J_{CF} = 36.2 Hz, CH₂), 55.4 (d, ¹J_{PC} = 71.9 Hz) ppm; ³¹P NMR (120 MHz, CDCl₃)

δ 30.2 ppm; ^{19}F NMR (282 MHz, CDCl_3) δ -73.9, -74.0, -74.0 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{17}\text{H}_{17}\text{F}_3\text{NNaO}_2\text{P}$ ($[\text{M} + \text{Na}]^+$) 378.0847, found 378.0851.

4.1.3.3. *General procedure and spectral data of allylic N-tosyl α -aminophosphine oxide 9.* *p*-Toluenesulfonyl chloride (1 g, 5.5 mmol, 1.1 eq) and pyridine (2.4 mL, 30 mmol, 6 eq) were added to a 0 °C solution of **8** (1.78 g, 5 mmol, 1 eq) in CH_2Cl_2 (25 mL). The reaction mixture was allowed to reach 25 °C and stirred for 24 h. The crude product was washed twice with a 2M HCl solution (15 mL) and water (15 mL) and extracted with CH_2Cl_2 (15 mL). The organic layer was dried over anhydrous MgSO_4 , filtered and concentrated to dryness in vacuum. The crude product was purified by crystallization from Et_2O to afford the title compound **9**.



***N*-[1-(Diphenylphosphoryl)-2-(2,2,2-trifluoroethoxy)allyl]-4-methyl-benzene**

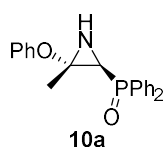
sulfonamide (9). (2.29 g, 90%) obtained as a pale yellow solid. mp 201–203 °C;

IR (neat) ν_{max} 3412, 3062, 2942, 2879, 1652, 1596, 1444, 1338, 1285, 1160, 910, 733 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.85–7.72 (m, 4H, ArH), 7.67 (d, $^3J_{\text{HH}} = 8.3$

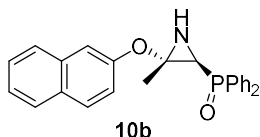
Hz, 2H, ArH), 7.56–7.38 (m, 6H, ArH), 7.11 (d, $^3J_{\text{HH}} = 8.4$ Hz, 2H, ArH), 7.07 (d, $^3J_{\text{HH}} = 9.8$ Hz, 1H, NH), 4.80 (t, $^2J_{\text{PH}} = 11.1$ Hz, 1H, CH-P), 4.48 (t, $^2J_{\text{HH}} = 3.8$ Hz, 1H, =CH₂), 3.67 (t, $^2J_{\text{HH}} = 3.6$ Hz, 1H, =CH₂), 3.42–3.33 (m, 1H, CH₂), 3.00–2.91 (m, 1H, CH₂), 2.34 (s, 3H, CH₃) ppm; ^{13}C { ^1H } NMR (100 MHz, CDCl_3) δ 153.4, 143.2, 137.5, 132.4, 132.3, 131.6, 131.5, 130.0, 129.8, 129.0, 128.8, 128.7, 128.4, 128.2, 127.6 (C_{Ar}), 122.5 (q, $^1J_{\text{CF}} = 277.5$ Hz, CF₃), 89.0 (d, $^3J_{\text{PC}} = 6.1$ Hz, =CH₂), 64.2 (q, $^2J_{\text{CF}} = 35.6$ Hz, CH₂), 55.5 (d, $^1J_{\text{PC}} = 73.5$ Hz, CH-P), 21.5 (CH₃) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 29.6 ppm; ^{19}F NMR (282 MHz, CDCl_3) δ -73.9, -73.9, -73.9 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{24}\text{H}_{24}\text{F}_3\text{NO}_4\text{PS}$ ($[\text{M} + \text{H}]^+$) 510.1116, found 510.1117.

4.1.4. Experimental procedure and characterization data for compounds **10**, **11** and **12**.

4.1.4.1. *General procedure and spectral data for the addition of phenols (2d–e) to functionalized 2H-azirines 1.* To a 0 °C solution of 2H-azirine **1** (5 mmol, 1 eq) in CH_2Cl_2 (25 mL), the corresponding phenols (**2d–e**) (10 mmol, 2 eq) and Et_3N (1.4 mL, 10 mmol, 2 eq) was added dropwise. The reaction mixture was allowed to reach 25 °C and stirred at the same temperature for 24 h. The reaction mixture was washed with water (3 \times 15 mL) and extracted with CH_2Cl_2 (15 mL). The organic layers was dried over anhydrous MgSO_4 , filtered and concentrated to dryness in vacuum. The crude products were purified by crystallization or by flash-column chromatography.

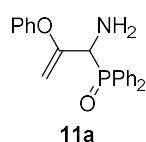


[(2S*,3S*)-3-Methyl-3-phenoxyaziridin-2-yl]diphenylphosphine oxide (10a). (1.22 g, 70%) obtained as a pale yellow solid from 2*H*-azirine **1a** (1.28 g, 5 mmol) and phenol (**2d**) (0.88 g, 10 mmol) as described in the general procedure. The crude product was purified by crystallization from Et₂O to afford the title compound **10a**. mp 124–126 °C; IR (neat) ν_{\max} 3203, 3059, 2990, 1593, 1488, 1438, 1391, 1349, 1224, 1191, 1122, 733, 691 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.85–7.42 (m, 10H, ArH), 7.07–6.63 (m, 5H, ArH), 2.62 (dd, ³J_{HH} = 10.0 Hz, ²J_{PH} = 22.0 Hz, 1H, CH-P), 2.06 (dd, ³J_{HH} = 10.2 Hz, ³J_{PH} = 18.2 Hz, 1H, NH), 1.87 (s, 3H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 154.9 (OC_{Ar}), 132.5, 132.4, 132.2, 131.2, 131.1, 131.0, 130.9, 129.2, 129.0, 128.8, 128.7, 121.7, 116.7 (C_{Ar}), 70.3 (C_{quat}), 37.9 (d, ¹J_{PC} = 88.7 Hz, CH-P), 16.9 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 25.1 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₁H₂₁NO₂P ([M+H]⁺) 350.1310, found 350.1302.

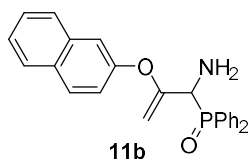


[(2S*,3S*)-3-Methyl-3-(naphthalen-2-yloxy)aziridin-2-yl]diphenylphosphine oxide (10b). Obtained as a pale yellow solid from 2*H*-azirine **1a** (1.28 g, 5 mmol) and 2-naphthol (**2e**) (1.44 g, 10 mmol) as described in the general procedure. The crude product was purified by crystallization from Et₂O to afford title compound **10b**. This product was identified only by ¹H NMR, since cleavage of C3–N bond in the three-membered ring of **10b** promptly occurs to give a mixture of aziridine **10b** and allyl α -aminophosphine oxide **11b**. ¹H NMR (300 MHz, CDCl₃) δ 7.92–6.83 (m, 17H, ArH), 2.77 (d, ²J_{PH} = 20.0 Hz, 1H, CH-P), 2.23 (bs, 1H, NH), 2.02 (s, 3H, CH₃) ppm.

4.1.4.2. General procedure for the preparation of allylic α -aminophosphine oxides 11. A solution of aziridine **10** (5 mmol, 1 eq) was stirred in refluxing CHCl₃ (11 mL) for 8 h until TLC showed the disappearance of aziridine **10**. The crude product was concentrated to dryness in vacuum to afford the title compound **11**.



(1-Amino-2-phenoxyallyl)diphenylphosphine oxide (11a). (1.62 g, 93%) obtained as an orange oil from aziridine **10a** (1.75 g, 5 mmol) as described in the general procedure. IR (neat) ν_{\max} 3389, 3060, 2933, 2860, 1638, 1587, 1489, 1442, 1264, 1220, 1182, 1122, 910, 742 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.91–6.55 (m, 15H, ArH), 4.36 (t, *J* = 2.8 Hz, 1H, =CH₂), 4.30 (d, ²J_{PH} = 8.6 Hz, 1H, CH-P), 3.94 (t, *J* = 2.3 Hz, 1H, =CH₂), 2.21 (bs, 2H, NH₂) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 159.8 (d, ²J_{PC} = 2.6 Hz, =C_{quat}), 153.8 (OC_{Ar}), 131.5, 131.5, 131.4, 131.4, 131.3, 131.2, 130.3, 130.0, 129.1, 128.2, 128.0, 128.0, 127.8, 124.1, 120.5, 116.2, 115.4 (C_{Ar}), 90.5 (d, ³J_{PC} = 6.6 Hz, =CH₂), 55.1 (d, ¹J_{PC} = 73.1 Hz, CH-P) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 30.2 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₁H₂₀NNaO₂P ([M + Na]⁺) 372.1129, found 372.1134.

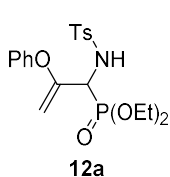


[1-Amino-2-(naphthalen-2-yloxy)allyl]diphenylphosphine oxide (11b).

(1.48 g, 74%) obtained as an orange oil from aziridine **10b** (2.00 gr, 5mmol) as described in the general procedure. IR (neat) ν_{\max} 3382, 3069, 2923, 1635, 1597, 1508, 1438, 1249, 1211, 1179, 1125, 907, 745, 698 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 8.04–6.53 (m, 17H, ArH), 4.55 (t, $J = 2.9$ Hz, 1H, =CH₂), 4.46 (d, $^2J_{\text{PH}} = 8.4$ Hz, 1H, CH-P), 4.14 (t, $J = 2.5$ Hz, 1H, =CH₂), 2.11 (bs, 2H, NH₂) ppm; ^{13}C { ^1H } NMR (75 MHz, CDCl_3) δ 160.0 (d, $^2J_{\text{PC}} = 2.6$ Hz, =C_{quat}), 151.7 (OC_{Ar}), 133.9, 131.9, 131.8, 131.7, 131.6, 130.6, 129.4, 128.5, 128.4, 128.3, 128.2, 127.5, 127.1, 126.2, 125.1, 120.9, 117.1, 111.6, 109.4 (C_{Ar}), 91.5 (d, $^3J_{\text{PC}} = 6.6$ Hz, =CH₂), 55.5 (d, $^1J_{\text{PC}} = 72.3$ Hz, CH-P) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 30.5 ppm; ESI-HRMS (CI) m/z calcd. for C₂₅H₂₂NNaO₂P ([M + Na]⁺) 422.1286, found 422.1291.

4.1.4.3. One pot procedure for the synthesis of N-tosyl allyl amines **12 derived from phosphonate.**

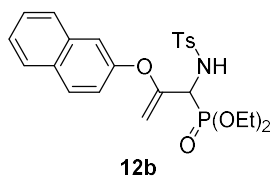
To a 0 °C solution of 2H-azirine **1e** (0.96 g, 5 mmol) in CH₂Cl₂ (25 mL), the corresponding alcohol (**2d–e**) (10 mmol, 2 eq) and Et₃N (1.4 mL, 10 mmol, 2 eq) was added dropwise. The reaction mixture was allowed to reach 25 °C and stirred for 24 h until TLC showed the disappearance of starting 2H-azirine **1e**. The reaction mixture was washed with water (3 × 15 mL) and extracted with CH₂Cl₂. The organic layer was dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum. Without any further purification step, to a 0 °C solution of crude products **11** in CH₂Cl₂ (25 mL) was directly added *p*-toluenesulfonyl chloride (1 g, 5.5 mmol, 1.1 eq) and pyridine (2.42 mL, 30 mmol, 6 eq). The reaction mixture was allowed to reach 25 °C and stirred for 24 h. The crude product was washed twice with a 2M HCl solution (15 mL) and water (15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layer was dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum. The crude products **12** were purified by crystallization from Et₂O.



Diethyl [1-((4-methylphenyl)sulfonamido)-2-phenoxyallyl]phosphonate

(12a). (1.93 g, 88%) obtained as a white solid from phenol (**2d**) (0.88 g, 10 mmol) following the general procedure described above. mp 117–119 °C; IR (neat) ν_{\max} 3270, 3123, 2984, 2934, 2915, 2881, 1643, 1593, 1494, 1452, 1391, 1344, 1241, 1091, 1044, 1013, 972 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.79 (d, $^3J_{\text{HH}} = 8.3$ Hz, 2H, ArH), 7.25–7.04 (m, 5H, ArH), 6.61 (d, $^3J_{\text{HH}} = 8.5$ Hz, 2H, ArH), 6.09 (dd, $^3J_{\text{HH}} = 10.1$ Hz, $^3J_{\text{PH}} = 3.1$ Hz, 1H, NH), 4.44 (dd, $^3J_{\text{HH}} = 10.1$ Hz, $^2J_{\text{PH}} = 23.9$ Hz, 1H, CH-P), 4.31 (t, $J = 3.2$ Hz, 1H, =CH₂), 4.24–4.09 (m, 4H, OCH₂), 3.83 (t, $J = 2.5$ Hz, 1H, =CH₂), 2.40 (s, 3H, CH₃), 1.29 (q, $^3J_{\text{HH}} = 7.0$ Hz, 3H, CH₃), 1.28 (q, $^3J_{\text{HH}} = 7.0$ Hz, 3H, CH₃) ppm; ^{13}C { ^1H } NMR (100 MHz, CDCl_3) δ 155.1 (d, $^2J_{\text{PC}} = 2.7$ Hz, =C_{quat}), 154.0 (OC_{Ar}), 143.4 (C_{quat}Ar), 137.9 (d, $^4J_{\text{PC}} = 1.6$ Hz, C_{quat}Ar), 129.4, 129.4, 127.5, 124.7,

121.0 (C_{Ar}), 91.6 (d, ³J_{PC} = 8.8 Hz, =CH₂), 63.8 (d, ²J_{PC} = 6.7 Hz, OCH₂), 63.7 (d, ²J_{PC} = 6.9 Hz, OCH₂), 54.1 (d, ¹J_{PC} = 157.4 Hz, CH-P), 21.4 (CH₃), 16.3 (d, ³J_{PC} = 6.1 Hz, CH₃), 16.3 (d, ³J_{PC} = 6.2 Hz, CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 18.0 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₀H₂₆NO₆PS ([M + H]⁺) 440.1297, found 440.1304.



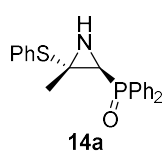
Diethyl [1-((4-methylphenyl)sulfonamido)-2-(naphthalen-2-yloxy)-allyl]phosphonate (12b). (1.64 g, 67%) obtained as a grey solid from 2-naphthol (**2e**) (1.44 g, 10 mmol) following the general procedure described above. mp 147–149 °C; IR (neat) ν_{max} 3425, 3065, 2981,

2926, 1599, 1380, 1191, 1177, 816, 714, 664 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.87 (d, ³J_{HH} = 8.1 Hz, 2H, ArH), 7.81–7.39 (m, 5H, ArH), 7.31 (d, ³J_{HH} = 8.2 Hz, 2H, ArH), 7.07 (d, *J* = 1.8 Hz, 1H, ArH), 6.85 (dd, *J* = 2.2 Hz, *J* = 8.9 Hz, 1H, ArH), 6.17 (dd, ³J_{HH} = 10.0 Hz, ³J_{PH} = 3.3 Hz, 1H, NH), 4.54 (dd, ³J_{HH} = 10.1 Hz, ²J_{PH} = 23.8 Hz, 1H, CH-P), 4.41 (t, *J* = 3.1 Hz, 1H, =CH₂), 4.33–4.16 (m, 4H, OCH₂), 3.93 (t, *J* = 2.6 Hz, 1H, =CH₂), 2.46 (s, 3H, CH₃), 1.35 (q, ³J_{HH} = 7.2 Hz, 6H, CH₃) ppm; ¹³C {1H} NMR (75 MHz, CDCl₃) δ 155.1 (d, ²J_{PC} = 2.9 Hz, =C_{quat}), 151.5 (OC_{Ar}), 143.5 (C_{quat}Ar), 138.0 (C_{quat}Ar), 133.9 (C_{quat}Ar), 130.9 (C_{quat}Ar), 129.5, 129.4, 127.7, 127.6, 127.2, 126.4, 125.3, 120.9, 117.6 (C_{Ar}), 92.1 (d, ³J_{PC} = 9.1 Hz, =CH₂), 63.9 (d, ²J_{PC} = 6.7 Hz, OCH₂), 63.8 (d, ²J_{PC} = 7.1 Hz, OCH₂), 54.2 (d, ¹J_{PC} = 157.5 Hz, CH-P), 21.5 (CH₃), 16.4 (d, ³J_{PC} = 5.6 Hz, CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 18.1 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₄H₂₉NO₆PS ([M + H]⁺) 490.1453, found 490.1469.

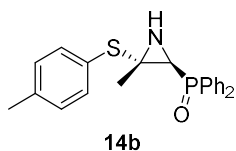
4.1.5. Experimental procedure and characterization data for compounds **14**, **15** and **16**.

4.1.5.1. General procedure and spectral data for the addition of thiophenols and thiols to 2H-azirines 1. Method A: To a 0 °C solution of 2H-azirine **1** (5 mmol, 1 eq) in CH₂Cl₂ (25 mL) was added dropwise thiophenol or thiol (5.5 mmol, 1.1 eq), Et₃N (1.40 mL, 10 mmol, 2 eq), and 4 Å M.S. The reaction mixture was allowed to reach 25 °C and stirred at the same temperature for 24 h. 4 Å M.S. was then filtered through a sintered glass vacuum filtration funnel with celite and washed with CH₂Cl₂. The reaction mixture was washed with water (3 × 15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layers were dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum to give aziridine **14**. In the case of R¹ = Me, aziridines **14**, allylic α-aminophosphine oxides or phosphonates **15**, or a mixture of both compounds can be obtained. When aziridines **14** or the mixture is obtained in the reaction crude, stirring of this crude in refluxing CHCl₃ afford allylic α-aminophosphine oxides or phosphonates **15**. The crude products **14** or **15** were purified by crystallization or by flash-column chromatography. **Method B:** To a 0 °C solution of 2H-azirine **1** (5 mmol, 1 eq) in CH₂Cl₂ (25 mL) was added dropwise the corresponding *p*-substituted benzenethiol (5.5 mmol, 1.1 eq). The reaction mixture was stirred

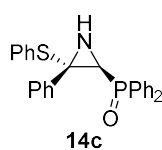
at 0 °C for 48 h until TLC showed the disappearance of starting compound **1**. The reaction mixture was concentrated to dryness in vacuum to afford allylic α -aminophosphine oxides or phosphonates **15**.



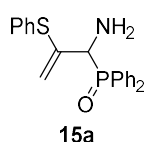
[(2S*,3S*)-3-Methyl-3-(phenylthio)aziridin-2-yl]diphenylphosphine oxide (14a). (1.68 g, 92%) as a white solid from 2*H*-azirine **1a** (1.28 g, 5 mmol) and benzenethiol (**13a**) (0.56 mL, 5.5 mmol) as described in the general procedure (method A). The crude product was purified by crystallization from Et₂O to afford the title compound **14a**. This product was identified only by ¹H and ³¹P NMR, since cleavage of C3–N bond in three-membered ring of **14a** promptly occurs to give allylic α -aminophosphine oxide **15a**. ¹H NMR (400 MHz, CDCl₃) δ 7.68–7.21 (m, 15H, ArH), 2.60 (dd, ³J_{HH} = 8.4 Hz, ²J_{PH} = 23.2 Hz, 1H, CH-P), 1.95 (dd, ³J_{HH} = 8.8 Hz, ³J_{PH} = 15.0 Hz, 1H, NH), 1.76 (s, 3H, CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 26.8 ppm.



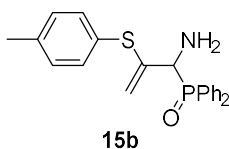
Diphenyl [(2S*,3S*)-3-methyl-3-(*p*-tolylthio)aziridin-2-yl]phosphine oxide (14b). From 2*H*-azirine **1a** (1.28 g, 5 mmol) and 4-methylbenzenethiol (**13b**) (0.68 g, 5.5 mmol) as described in the general procedure (method B). This product was identified only by ¹H and ³¹P NMR in a mixture of aziridine **14b** and allylic α -aminophosphine oxide **15b**, since cleavage of C3–N bond in three-membered ring of **14b** promptly occurs to give allylic α -aminophosphine oxide **15b**. ¹H NMR (300 MHz, CDCl₃) δ 8.08–7.43 (m, 28H, ArH)_{mixture}, 2.78 (d, ²J_{PH} = 23.4 Hz, 1H, CH-P), 2.48 (s, 3H, CH₃), 1.90 (s, 3H, CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 26.8 ppm.



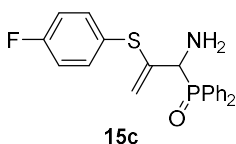
Diphenyl [(2S*,3S*)-3-phenyl-3-(phenylthio)aziridin-2-yl]phosphine oxide (14c). (1.29 g, 60%) as a white solid from 2*H*-azirine **1c** (1.59 g, 5 mmol) and benzenethiol (**13a**) (0.56 mL, 5.5 mmol) as described in the general procedure (method A). The crude product was purified by crystallization from Et₂O/CH₂Cl₂ 50:50 to afford the title compound **14c**, whose data are in agreement with those reported previously.^{21d}



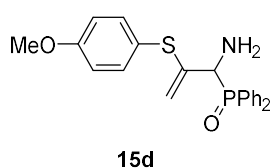
[1-Amino-2-(phenylthio)allyl]diphenylphosphine oxide (15a). Following the general procedure described above (method A), aziridine intermediate **14a** was stirred in refluxing CHCl₃ (11mL) for 8 h. The crude product was concentrated to dryness in vacuum to afford the title compound **15a**, whose data are in agreement with those reported previously.^{21d}



[1-Amino-2-(*p*-tolylthio)allyl]diphenylphosphine oxide (15b). (1.69 g, 89%) as a yellow oil from 2*H*-azirine **1a** (1.28 g, 5 mmol) and 4-methylbenzenethiol (**13b**) (0.68 g, 5.5 mmol) as described in the general procedure (method B). IR (neat) ν_{\max} 3389, 3060, 3022, 2987, 2930, 1676, 1635, 1590, 1489, 1442, 1242, 1185, 1119, 1106, 910, 729, 694 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.96–7.06 (m, 14H, ArH), 5.56 (d, $^2J_{\text{HH}} = 3.3$ Hz, 1H, =CH₂), 4.88 (d, $^2J_{\text{HH}} = 3.2$ Hz, 1H, =CH₂), 4.13 (d, $^2J_{\text{PH}} = 6.0$ Hz, 1H, CH-P), 2.29 (s, 3H, CH₃), 2.21 (bs, 2H, NH₂) ppm; ^{13}C { ^1H } NMR (100 MHz, CDCl_3) δ 144.2 (=C_{quat}), 138.4, 133.6, 131.8, 131.6, 131.5, 131.5, 131.4, 129.9, 129.6, 128.5, 128.4, 128.1, 127.9 (C_{Ar}), 115.1 (d, $^3J_{\text{PC}} = 6.6$ Hz, =CH₂), 55.8 (d, $^1J_{\text{PC}} = 72.8$ Hz, CH-P), 21.0 (CH₃) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 30.9 ppm; ESI-HRMS (CI) m/z calcd. for C₂₂H₂₃NOPS ([M + H]⁺) 380.1238, found 380.1225.

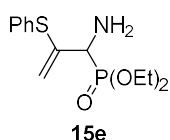


[1-Amino-2-(4-fluorophenylthio)allyl]diphenylphosphine oxide (15c). (1.46 g, 76%) as an orange oil from 2*H*-azirine **1a** (1.28 g, 5 mmol) and 4-fluorobenzenethiol (**13c**) (0.59 mL, 5.5 mmol) as described in the general procedure (method B). R_f = 0.15 (AcOEt); IR (neat) ν_{\max} 3381, 3301, 3065, 2914, 1593, 1495, 1437, 1226, 1185, 1157, 1122, 834, 726, 694 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 7.86–6.86 (m, 14H, ArH), 5.50 (d, $^2J_{\text{HH}} = 3.3$ Hz, 1H, =CH₂), 4.76 (d, $^2J_{\text{HH}} = 3.1$ Hz, 1H, =CH₂), 4.11 (d, $^2J_{\text{PH}} = 6.3$ Hz, 1H, CH-P), 2.13 (bs, 2H, NH₂) ppm; ^{13}C { ^1H } NMR (75 MHz, CDCl_3) δ 164.2 (C_{Ar}-F), 160.9 (C_{quat}), 144.1 (=C_{quat}), 135.7, 135.6, 131.8, 131.7, 131.7, 131.6, 131.6, 131.5, 131.4, 131.4, 131.3, 130.2, 128.4, 128.3, 128.0, 127.9, 126.8, 126.7 (C_{Ar}), 116.4, 116.1, 115.0 (d, $^3J_{\text{PC}} = 6.8$ Hz, =CH₂), 55.9 (d, $^1J_{\text{PC}} = 72.5$ Hz, CH-P) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 30.6 ppm; ^{19}F NMR (282 MHz, CDCl_3) δ -112.6 ppm; ESI-HRMS (CI) m/z calcd. for C₂₁H₂₀FNOPS ([M + H]⁺) 384.0987, found 384.0988.

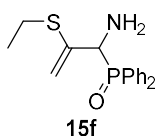


[1-Amino-2-(4-methoxyphenylthio)allyl]diphenylphosphine oxide (15d). (1.37 g, 70%) as a yellow oil from 2*H*-azirine **1a** (1.28 g, 5 mmol) and 4-methoxybenzenethiol (**13d**) (0.77 g, 5.5 mmol) as described in the general procedure (method B). IR (neat) ν_{\max} 3381, 3053, 2965, 2940, 2837, 1596, 1574, 1491, 1463, 1438, 1288, 1247, 1180, 1113, 1102, 1030, 830, 725, 694 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.90–7.34 (m, 10H, ArH), 7.09 (d, $^3J_{\text{HH}} = 8.9$ Hz, 2H, ArH), 6.79 (d, $^3J_{\text{HH}} = 8.8$ Hz, 2H, ArH), 5.48 (d, $^2J_{\text{HH}} = 3.3$ Hz, 1H, =CH₂), 4.71 (d, $^2J_{\text{HH}} = 2.9$ Hz, 1H, =CH₂), 4.11 (d, $^2J_{\text{PH}} = 5.9$ Hz, 1H, CH-P), 3.75 (s, 3H, OCH₃), 1.95 (bs, 2H, NH₂) ppm; ^{13}C { ^1H } NMR (100 MHz, CDCl_3) δ 160.1 (C_{Ar}-O), 145.3 (=C_{quat}), 136.0, 132.6, 131.9, 131.9, 131.7, 131.7, 131.6, 131.6, 131.5, 128.6, 128.5, 128.1, 128.0, 121.7, 114.8 (C_{Ar}), 113.3 (d, $^3J_{\text{PC}} = 6.7$ Hz, =CH₂), 55.8 (d, $^1J_{\text{PC}} =$

72.8 Hz, CH-P), 55.3 (OCH₃)ppm; ³¹P NMR (120 MHz, CDCl₃) δ 30.9 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₂H₂₃NO₂PS ([M + H]⁺) 396.1187, found 396.1183.

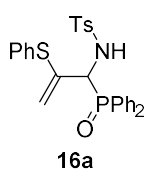


Diethyl [1-amino-2-(phenylthio)allyl]phosphonate (15e). (0.62 g, 41%) as a yellow oil from 2*H*-azirine **1e** (0.96 g, 5 mmol) and benzenethiol (**13a**) (0.56 mL, 5.5 mmol) as described in the general procedure (method B). The crude product was purified by flash-column chromatography (SiO₂, AcOEt) to afford the title compound **15e**, whose data are in agreement with those reported previously.^{21d}



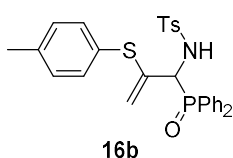
[1-Amino-2-(ethylthio)allyl]diphenylphosphine oxide (15f). (1.02 g, 64%) as a pale yellow oil from 2*H*-azirine **1a** (1.28 g, 5 mmol) and ethanethiol (**13e**) (0.40 mL, 5.5 mmol) as described in the general procedure (method A). The crude product was purified by flash-column chromatography (SiO₂, AcOEt/methanol 95:5) to afford the title compound **15f**. IR (neat) ν_{\max} 3414, 3060, 2977, 1682, 1631, 1590, 1435, 1246, 1188, 1122, 739, 698 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.91–7.383 (m, 10H, ArH), 5.46 (d, ²J_{HH} = 3.3 Hz, 1H, =CH₂), 4.84 (d, ²J_{HH} = 2.4 Hz, 1H, =CH₂), 4.07 (d, ²J_{PH} = 6.1 Hz, 1H, CH-P), 2.57 (q, ³J_{HH} = 7.4 Hz, 2H, CH₂), 2.26 (bs, 2H, NH₂), 1.09 (q, ³J_{HH} = 7.4 Hz, 3H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 143.5 (=C_{quat}), 131.8, 131.7, 131.6, 131.6, 131.5, 131.5, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8 (C_{Ar}), 109.7 (d, ³J_{PC} = 7.2 Hz, =CH₂), 57.3 (d, ¹J_{PC} = 73.2 Hz, CH-P), 25.8 (CH₂), 12.7 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 30.9 ppm; ESI-HRMS (CI) *m/z* calcd. for C₁₇H₂₁NOPS ([M + H]⁺) 318.1081, found 318.1071.

4.1.5.2. General procedure and spectral data for the *N*-tosyl functionalization of allylic α -aminophosphine oxides and phosphonates **15.** *p*-Toluenesulfonyl chloride (1 g, 5.5 mmol, 1.1 eq) and pyridine (2.4 mL, 30 mmol, 6 eq) were added to a 0 °C solution of derivative **15** (5 mmol, 1 eq) in CH₂Cl₂ (25 mL). The reaction mixture was allowed to reach 25 °C and stirred for 24 h. The crude product was washed twice with a 2M HCl solution (15 mL) and water (15 mL) and extracted with CH₂Cl₂ (15 mL). The organic layer was dried over anhydrous MgSO₄, filtered and concentrated to dryness in vacuum. The crude product was purified by crystallization or by flash-column chromatography to afford *N*-tosyl allylic α -aminophosphine oxides and phosphonates **16**.



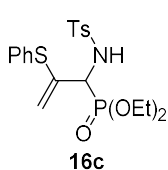
***N*-[1-(Diphenylphosphoryl)-2-(phenylthio)allyl]-4-methylbenzenesulfo-namide (16a).** (2.26 g, 87%) obtained as a white solid from allylic α -aminophosphine oxide **15a** (1.83 g, 5 mmol) as described in the general procedure. The crude product was purified by flash-column chromatography (SiO₂, AcOEt/hexane 10:20) to

afford the title compound **16a**. mp 199–201 °C; IR (neat) ν_{\max} 3431, 3356, 3062, 2920, 2876, 2743, 1602, 1460, 1438, 1335, 1191, 1163, 1122, 1094, 1066, 911, 730 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.93–7.10 (m, 17H, ArH), 6.72 (d, $^3J_{\text{HH}} = 6.9$ Hz, 2H, ArH), 5.54 (d, $^2J_{\text{HH}} = 2.5$ Hz, 1H, =CH₂), 4.83 (t, $^2J_{\text{PH}} = 10.1$ Hz, 1H, CH-P), 4.33 (d, $^2J_{\text{HH}} = 2.7$ Hz, 1H, =CH₂), 2.37 (s, 3H, CH₃) ppm; ^{13}C {1H} NMR (100 MHz, CDCl_3) δ 142.7 (=C_{quat}), 139.4, 138.5, 134.4, 132.2, 132.1, 131.5, 131.4, 130.5, 129.1, 129.0, 128.7, 128.7, 128.6, 128.2, 128.1, 127.7 (C_{Ar}), 115.2 (d, $^3J_{\text{PC}} = 6.6$ Hz, =CH₂), 56.4 (d, $^1J_{\text{PC}} = 74.1$ Hz, CH-P), 21.5 (CH₃) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 30.8 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{28}\text{H}_{27}\text{NO}_3\text{PS}_2$ ([M + H]⁺) 520.1170, found 520.1174.



N-[1-(Diphenylphosphoryl)-2-(p-tolylthio)allyl]-4-methylbenzenesulfonamide (16b). (2.24 g, 84%) obtained as an orange solid from allylic α -aminophosphine oxide **15b** (1.90 g, 5 mmol) as described in the general procedure. The crude product was purified by crystallization from

$\text{Et}_2\text{O}/\text{CH}_2\text{Cl}_2$ 50:50 to afford the title compound **16b**. mp 180–182 °C; IR (neat) ν_{\max} 3428, 3059, 2926, 2870, 1599, 1438, 1333, 1191, 1160, 911, 739 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.86–7.41 (m, 12H, ArH), 7.13 (d, $^3J_{\text{HH}} = 8.5$ Hz, 2H, ArH), 6.99 (d, $^3J_{\text{HH}} = 7.8$ Hz, 2H, ArH), 6.60 (d, $^3J_{\text{PH}} = 8.0$ Hz, 2H, ArH), 5.37 (d, $^2J_{\text{HH}} = 2.7$ Hz, 1H, =CH₂), 4.82 (t, $^2J_{\text{PH}} = 10.0$ Hz, 1H, CH-P), 4.26 (d, $^2J_{\text{HH}} = 2.5$ Hz, 1H, =CH₂), 2.38 (s, 3H, CH₃), 2.28 (s, 3H, CH₃) ppm; ^{13}C {1H} NMR (75 MHz, CDCl_3) δ 142.9 (=C_{quat}), 140.3 (C_{quat}Ar), 139.0 (C_{quat}Ar), 138.1 (C_{quat}Ar), 134.6, 132.3, 132.2, 132.2, 132.1, 131.5, 131.4, 129.9, 129.1, 128.8, 128.6, 128.2, 128.0, 127.7, 126.6 (C_{Ar}), 114.0 (d, $^3J_{\text{PC}} = 7.0$ Hz, =CH₂), 56.4 (d, $^1J_{\text{PC}} = 73.8$ Hz, CH-P), 21.5 (CH₃), 21.2 (CH₃) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 31.2 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{29}\text{H}_{29}\text{NO}_3\text{PS}_2$ ([M + H]⁺) 534.1326, found 534.1329.

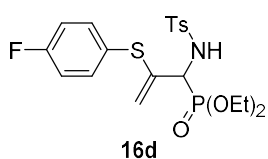


Diethyl [1-((4-methylphenyl)sulfonamido)-2-(phenylthio)allyl]phosphonate (16c). (1.93 g, 85%) obtained as a pale yellow solid from allylic α -aminophosphonate **15e** (1.51 g, 5 mmol) as described in the general procedure.

The crude product was purified by flash-column chromatography (SiO_2 , $\text{AcOEt}/\text{hexane}$ 17:83) to afford the title compound **16c**. mp 99–101 °C; IR (neat) ν_{\max} 3126, 2990, 2927, 1600, 1480, 1438, 1337, 1242, 1166, 1055, 1027, 910, 726 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 7.75 (d, $^3J_{\text{HH}} = 8.2$ Hz, 2H, ArH), 7.29–7.11 (m, 7H, ArH), 6.82 (dd, $^3J_{\text{PH}} = 4.4$ Hz, $^3J_{\text{HH}} = 9.8$ Hz, 1H, NH), 5.45 (d, $^2J_{\text{HH}} = 3.9$ Hz, 1H, =CH₂), 4.65 (d, $^2J_{\text{HH}} = 3.7$ Hz, 1H, =CH₂), 4.36 (dd, $^3J_{\text{HH}} = 9.9$ Hz, $^2J_{\text{PH}} = 24.4$ Hz, 1H, CH-P), 4.25–4.08 (m, 4H, OCH₂), 2.41 (s, 3H, CH₃), 1.31 (q, $^3J_{\text{HH}} = 7.0$ Hz, 6H, CH₃) ppm; ^{13}C {1H} NMR (75 MHz, CDCl_3) δ 143.1 (=C_{quat}), 139.8 (C_{quat}Ar), 138.1 (C_{quat}Ar), 134.1, 131.0, 129.2, 129.1, 128.6, 127.5 (C_{Ar}), 115.4 (d, $^3J_{\text{PC}} = 9.1$ Hz, =CH₂), 64.1 (d, $^2J_{\text{PC}} = 7.0$ Hz, OCH₂), 63.8 (d, $^2J_{\text{PC}} = 7.0$ Hz, OCH₂), 55.0 (d, $^1J_{\text{PC}} = 157.6$ Hz, CH-P), 21.4 (CH₃), 16.6 (d, $^3J_{\text{PC}} = 5.8$ Hz, CH₃) ppm;

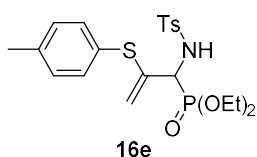
^{31}P NMR (120 MHz, CDCl_3) δ 18.4 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{20}\text{H}_{27}\text{NO}_5\text{PS}_2$ ($[\text{M} + \text{H}]^+$) 456.1068, found 456.1071.

4.1.5.3. *One pot procedure for the synthesis of N-tosyl allylic α -aminophosphonates 16d–e.* To a 0 °C solution of 2*H*-azirine **1e** (0.96 g, 5 mmol) in CH_2Cl_2 (25 mL) was added dropwise the corresponding *p*-substituted benzenethiol (5.5 mmol, 1.1 eq). The reaction mixture was stirred at 0 °C for 48 h until TLC showed the disappearance of starting compound **1e**. The reaction mixture was concentrated to dryness in vacuum to afford derivatives **15**. Without any further purification step, to a 0 °C solution of crude products **15** in CH_2Cl_2 (25 mL) was directly added *p*-toluenesulfonyl chloride (1 g, 5.5 mmol, 1.1 eq) and pyridine (2.42 mL, 30 mmol, 6 eq). The reaction mixture was allowed to reach 25 °C and stirred for 24 h. The crude product was washed twice with a 2M HCl solution (15 mL) and water (15 mL) and extracted with CH_2Cl_2 (15 mL). The organic layer was dried over anhydrous MgSO_4 , filtered and concentrated to dryness in vacuum. The crude products **16d–e** were purified by flash-column chromatography.



Diethyl [2-((4-fluorophenyl)thio)-1-((4-methylphenyl)sulfonamido)allyl]phosphonate (16d). (2.01 g, 85%) obtained as a pale yellow solid from 2*H*-azirine **1e** (0.96 g, 5 mmol) and 4-fluorobenzenethiol (**13c**) (0.59 mL, 5.5 mmol) in a one pot reaction as described in the general

procedure. The crude product was purified by flash-column chromatography (SiO_2 , AcOEt/hexane 10:30) to afford the title compound **16d**. mp 117–119 °C; IR (neat) ν_{max} 3370, 3161, 2984, 2927, 1594, 1492, 1239, 1166, 1052, 1030, 907, 739 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 8.02 (d, $^3J_{\text{HH}} = 8.0$ Hz, 2H, ArH), 7.54–7.21 (m, 4H, ArH), 7.52 (d, $^3J_{\text{HH}} = 8.1$ Hz, 2H, ArH), 6.99 (dd, $^3J_{\text{HH}} = 9.8$ Hz, $^3J_{\text{PH}} = 4.5$ Hz, 1H, NH), 5.70 (d, $^2J_{\text{HH}} = 4.3$ Hz, 1H, =CH₂), 4.87 (d, $^2J_{\text{HH}} = 3.6$ Hz, 1H, =CH₂), 4.62 (dd, $^3J_{\text{HH}} = 9.8$ Hz, $^2J_{\text{PH}} = 24.4$ Hz, 1H, CH-P), 4.53–4.31 (m, 4H, OCH₂), 2.70 (s, 3H, CH₃), 1.61–1.55 (m, 6H, CH₃) ppm; ^{13}C { ^1H } NMR (75 MHz, CDCl_3) δ 164.7 (C_{Ar-F}), 161.4 (C_{quat}), 143.2 (=C_{quat}), 140.3, 138.1, 136.6, 136.5, 129.2, 127.5, 116.5, 116.3 (C_{Ar}), 115.0 (d, $^3J_{\text{PC}} = 9.1$ Hz, =CH₂), 64.2 (d, $^2J_{\text{PC}} = 7.0$ Hz, OCH₂), 63.8 (d, $^2J_{\text{PC}} = 7.0$ Hz, OCH₂), 54.9 (d, $^1J_{\text{PC}} = 157.5$ Hz, CH-P), 21.4 (CH₃), 16.4 (CH₃), 16.3 (CH₃) ppm; ^{31}P NMR (120 MHz, CDCl_3) δ 18.3 ppm; ^{19}F NMR (282 MHz, CDCl_3) δ -112.1 ppm; ESI-HRMS (CI) m/z calcd. for $\text{C}_{20}\text{H}_{26}\text{FNO}_5\text{PS}_2$ ($[\text{M} + \text{H}]^+$) 474.0974, found 474.0976.



Diethyl [1-((4-methylphenyl)sulfonamido)-2-(*p*-tolylthio)allyl]phosphonate (16e). (1.71 g, 73%) obtained as a pale yellow solid from 2*H*-azirine **1e** (0.96 g, 5 mmol) and 4-methylbenzenethiol (**13b**) (0.68 g, 5.5 mmol) in a one pot reaction as described in the general procedure. The

crude product was purified by flash-column chromatography (SiO₂, AcOEt/hexane 10:20) to afford the title compound **16e**. mp 168–170 °C; IR (neat) ν_{\max} 3128, 2979, 2934, 2867, 1596, 1491, 1446, 1341, 1247, 1149, 1011, 961, 905, 819 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.73 (d, ³J_{HH} = 8.3 Hz, 2H, ArH), 7.31–6.98 (m, 6H, ArH), 6.34 (dd, ³J_{HH} = 9.8 Hz, ³J_{PH} = 4.5 Hz, 1H, NH), 5.32 (d, ²J_{HH} = 4.1 Hz, 1H, =CH₂), 4.55 (d, ²J_{HH} = 2.7 Hz, 1H, =CH₂), 4.35 (dd, ³J_{HH} = 9.9 Hz, ²J_{PH} = 24.2 Hz, 1H, CH-P), 4.24–4.03 (m, 4H, OCH₂), 2.41 (s, 3H, CH₃), 2.31 (s, 3H, CH₃), 1.30 (t, ³J_{HH} = 7.0 Hz, 6H, CH₃) ppm; ¹³C {¹H} NMR (75 MHz, CDCl₃) δ 143.2 (=C_{quat}), 140.6 (C_{quat}), 139.0 (C_{quat}), 138.0 (C_{quat}), 134.6, 130.0, 129.2, 127.6, 127.1 (C_{quat}) (C_{Ar}), 114.2 (d, ³J_{PC} = 9.2 Hz, =CH₂), 64.1 (d, ²J_{PC} = 7.3 Hz, OCH₂), 63.9 (d, ²J_{PC} = 6.9 Hz, OCH₂), 55.1 (d, ¹J_{PC} = 157.4 Hz, CH-P), 21.5 (CH₃), 21.2 (CH₃), 16.4 (CH₃), 16.3 (CH₃) ppm; ³¹P NMR (120 MHz, CDCl₃) δ 18.5 ppm; ESI-HRMS (CI) *m/z* calcd. for C₂₁H₂₉NO₅PS₂ ([M + H]⁺) 470.1225, found 470.1229.

4.2. Biology

4.2.1. Materials

Reagents and solvents were used as purchased without further purification. All stock solutions of the investigated compounds were prepared by dissolving the powdered materials in appropriate amounts of DMSO. The final concentration of DMSO never exceeded 5% (v/v) in reactions. The stock solution was stored at 5 °C until it was used.

4.2.2. Cytotoxicity assays.

Cells were cultured according to the supplier's instructions. Cells were seeded in 96-well plates at a density of 2–4 x 10³ cells per well and incubated overnight in 0.1 mL of media supplied with 10% Fetal Bovine Serum (Lonza) in 5% CO₂ incubator at 37 °C. On day 2, compounds were added and samples were incubated for 48 hours. After treatment, 10 μ L of cell counting kit-8 was added into each well for additional 2 hours incubation at 37 °C. The absorbance of each well was determined by an Automatic Elisa Reader System at 450 nm wavelength.

Supplementary Materials

The following are available online, ^1H and ^{13}C NMR spectra of compounds **4–12**, **14–16**.

Funding

Financial support by the Ministerio de Ciencia, Innovación y Universidades (MCIU), Agencia Estatal de Investigación (AEI) y Fondo Europeo de Desarrollo Regional (FEDER) (RTI2018-101818-B-I00, UE), and Gobierno Vasco (GV), (IT 992-16) is gratefully acknowledged.

Acknowledgments

The authors thank technical and human support provided by SGIker (UPV/EHU/ ERDF, EU).

Conflicts of Interest

The authors declare no conflict of interest.

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Material complementario – Manuscrito 4

Synthesis of α -aminophosphonic acid derivatives through the addition of *O*- and *S*-nucleophiles to 2*H*-azirines and their antiproliferative effect on A549 human lung adenocarcinoma cells.

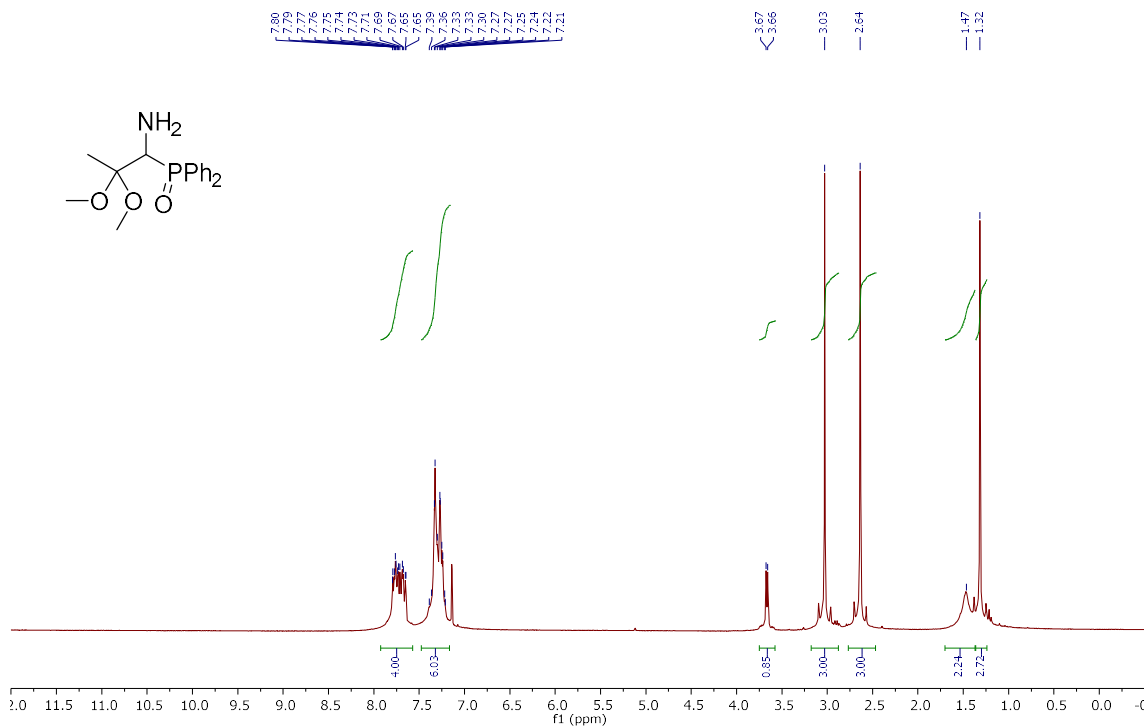
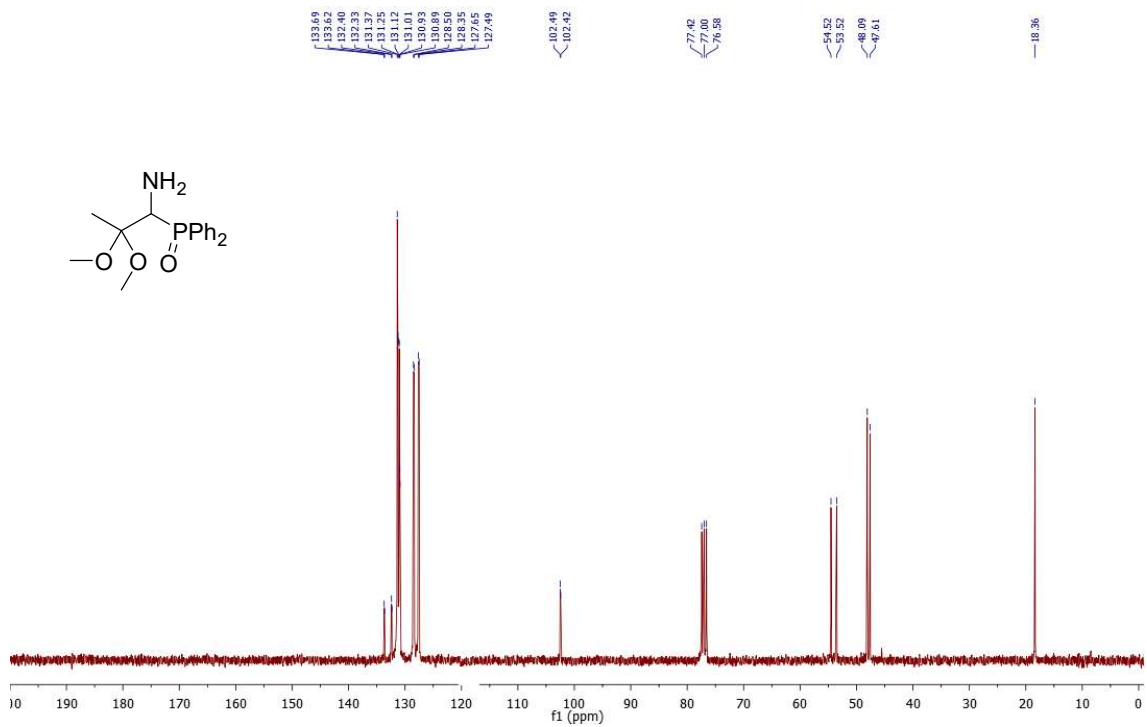
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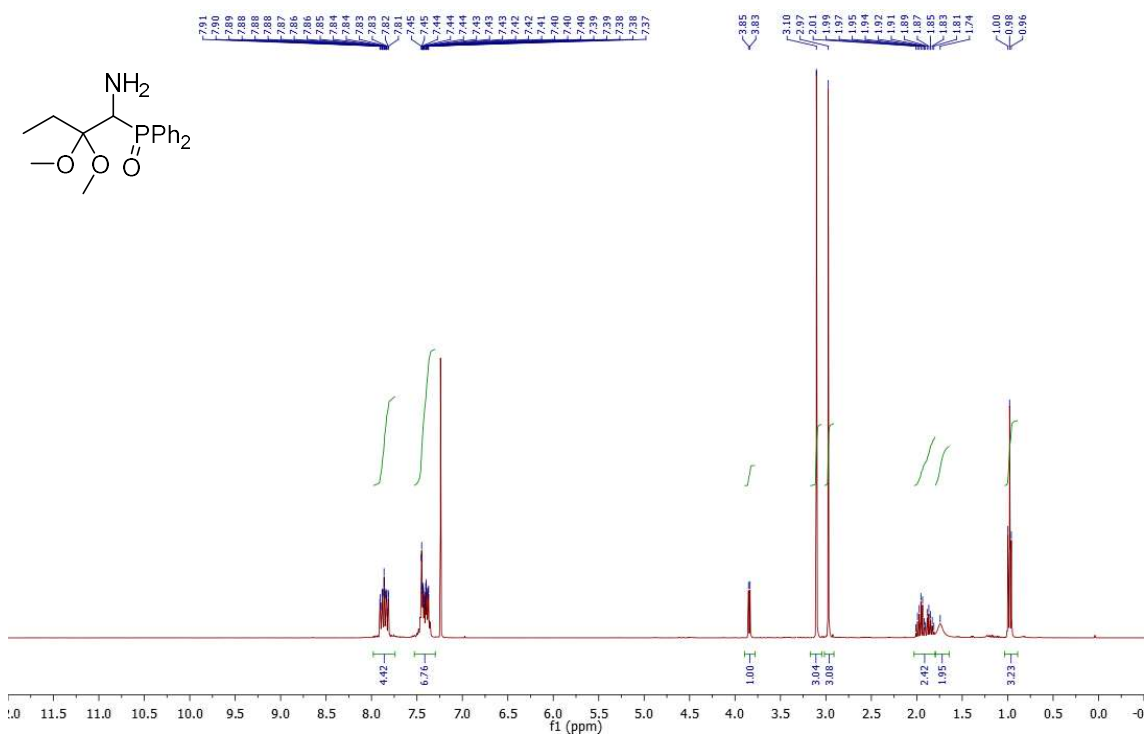
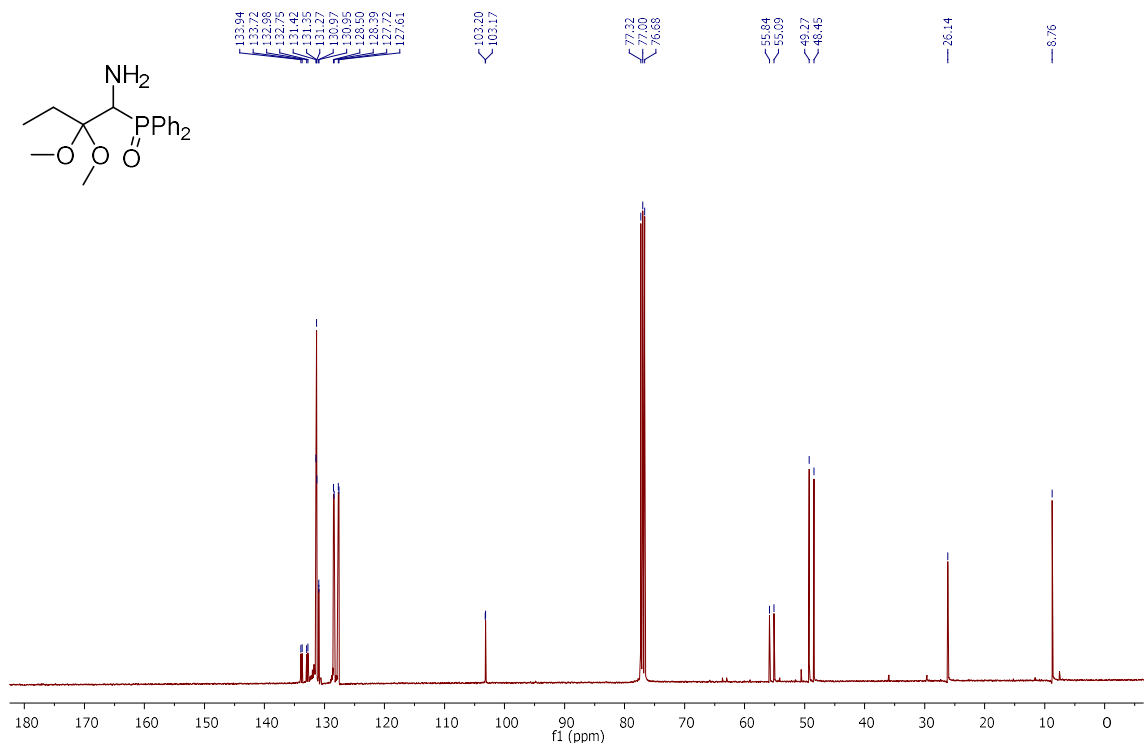
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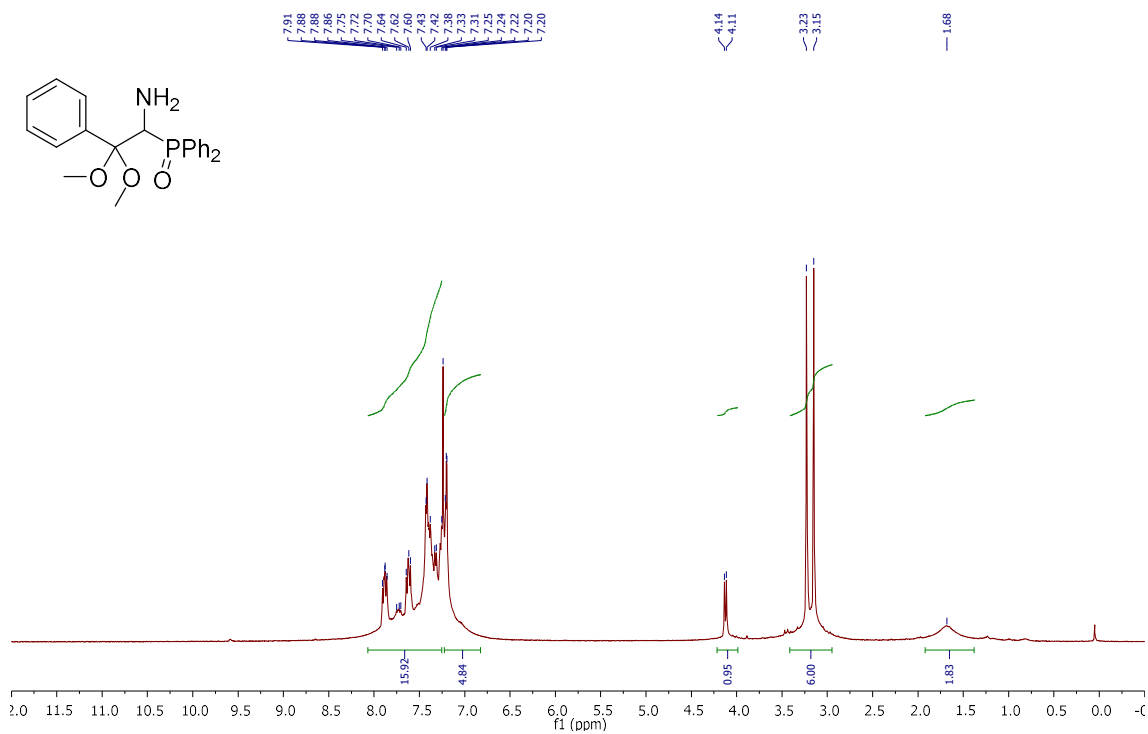
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¹ H and ¹³ C NMR spectra of allylic <i>N</i> -tosyl-α-aminophosphine oxides and phosphonates 16a–e	398

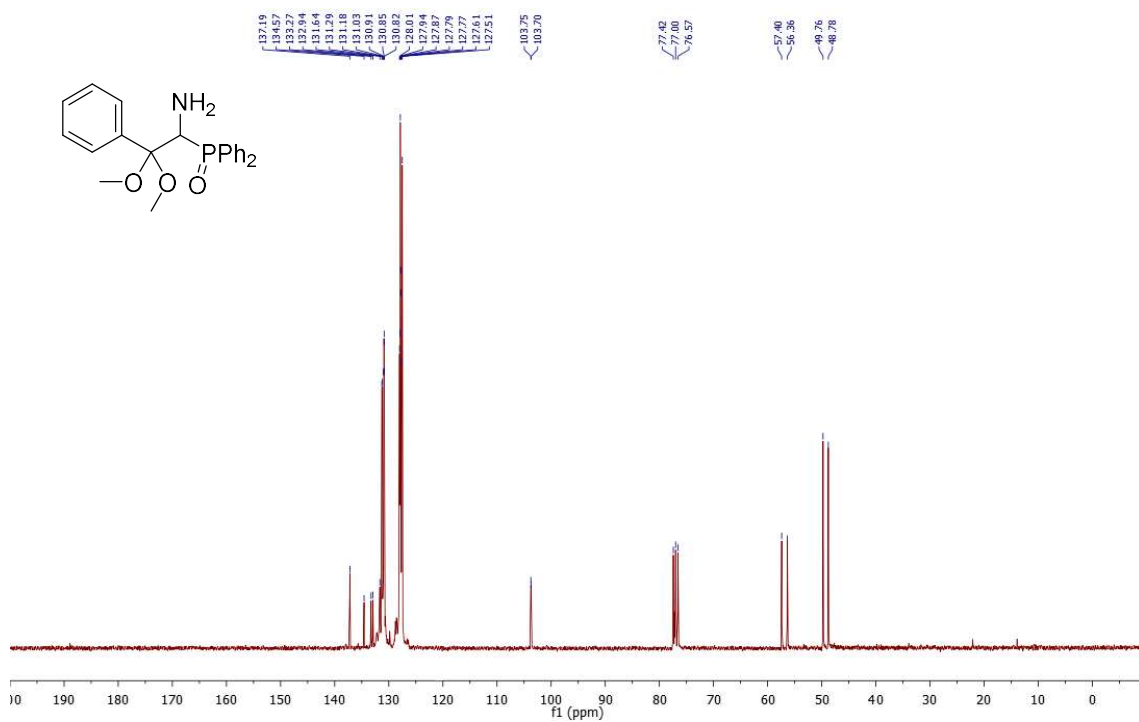
^1H NMR (300 MHz, CDCl_3) of compound **4a** ^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **4a**

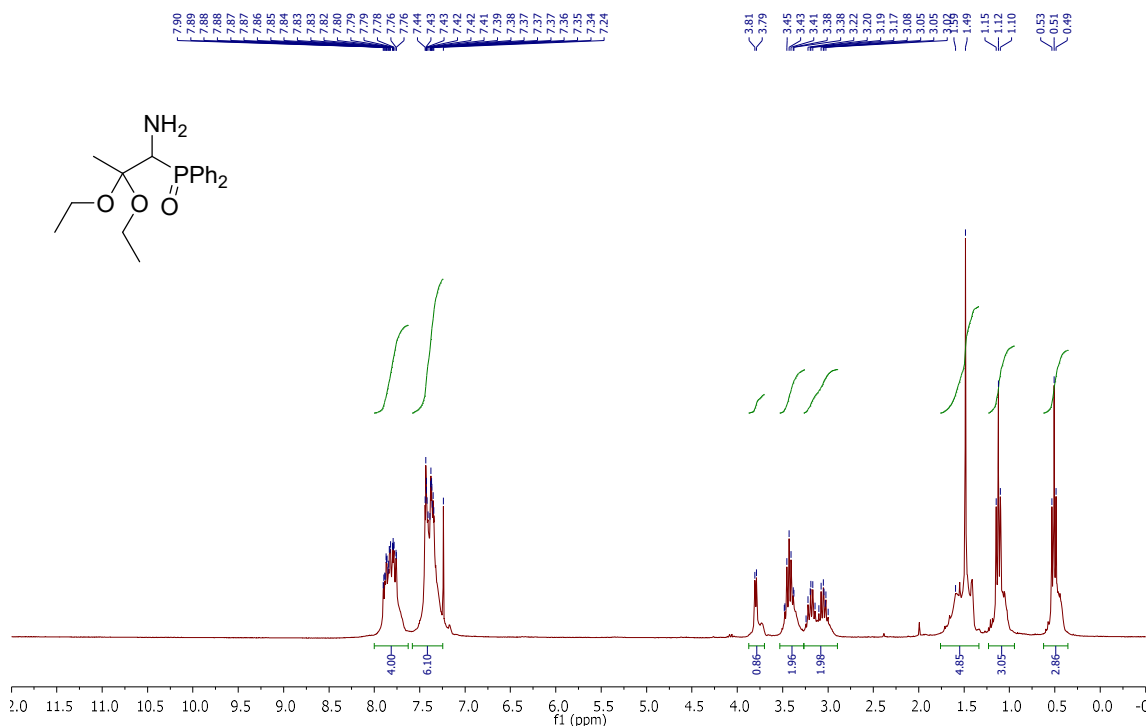
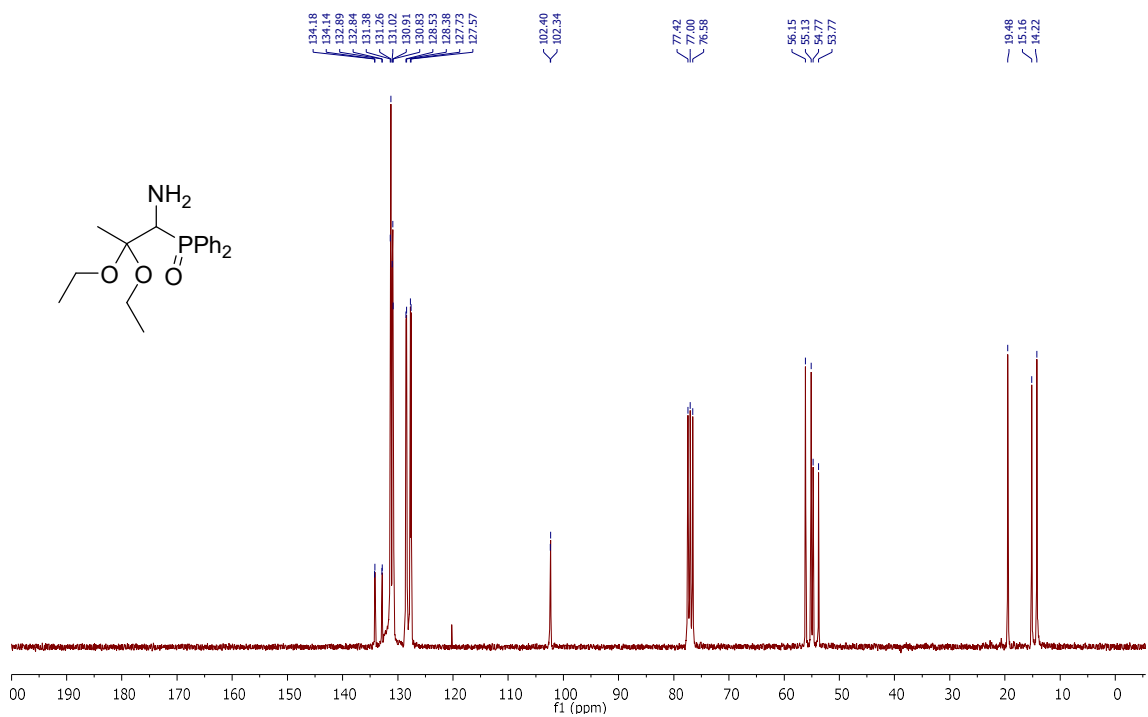
^1H NMR (400 MHz, CDCl_3) of compound **4b** ^{13}C { ^1H } NMR (100 MHz, CDCl_3) of compound **4b**

^1H NMR (400 MHz, CDCl_3) of compound **4c**

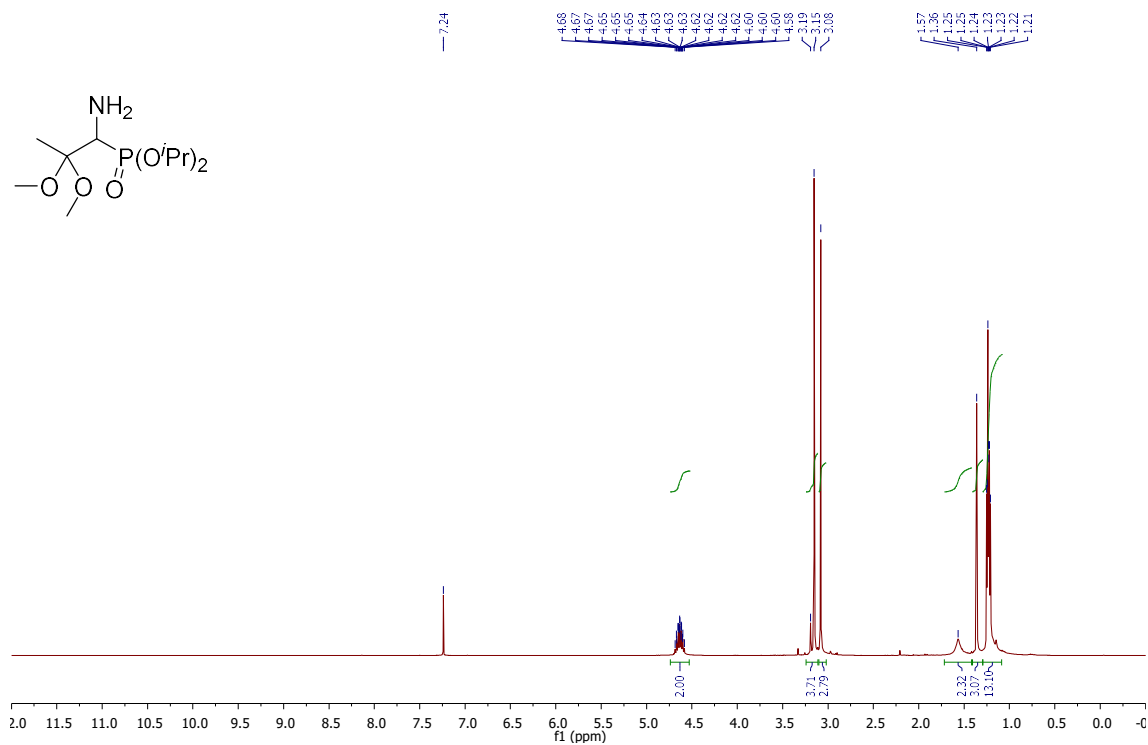


^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **4c**

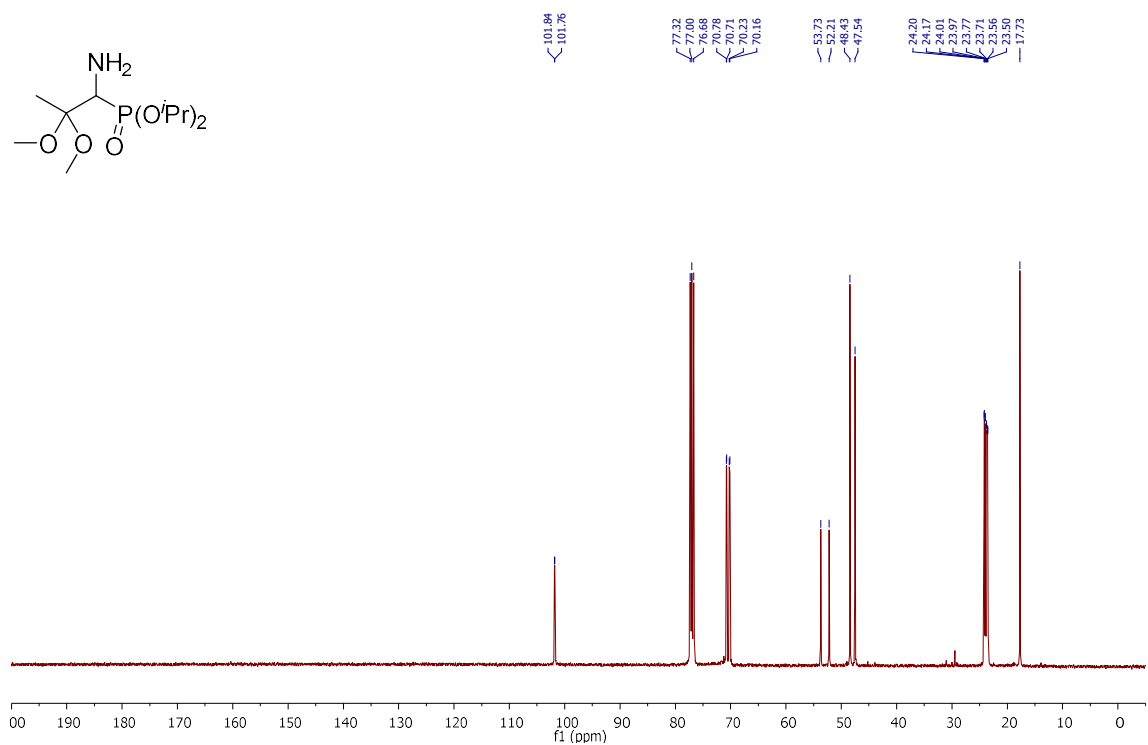


^1H NMR (300 Hz, CDCl_3) of compound **4d** ^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **4d**

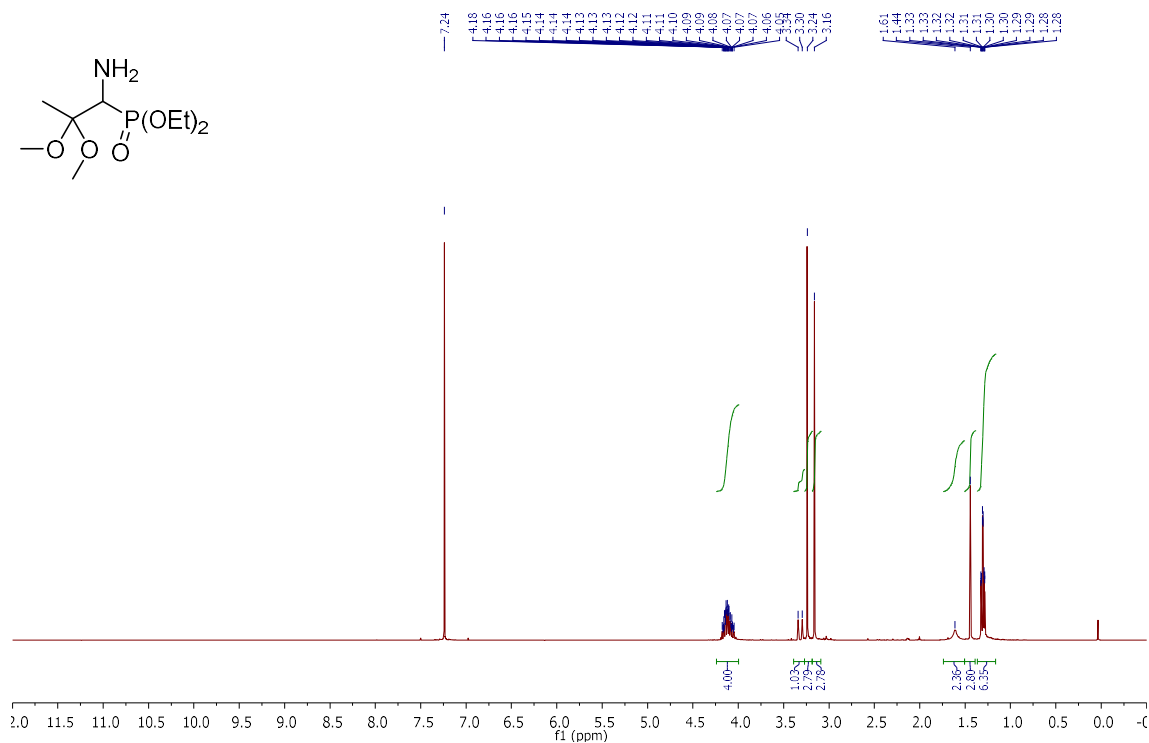
^1H NMR (400 MHz, CDCl_3) of compound **4e**



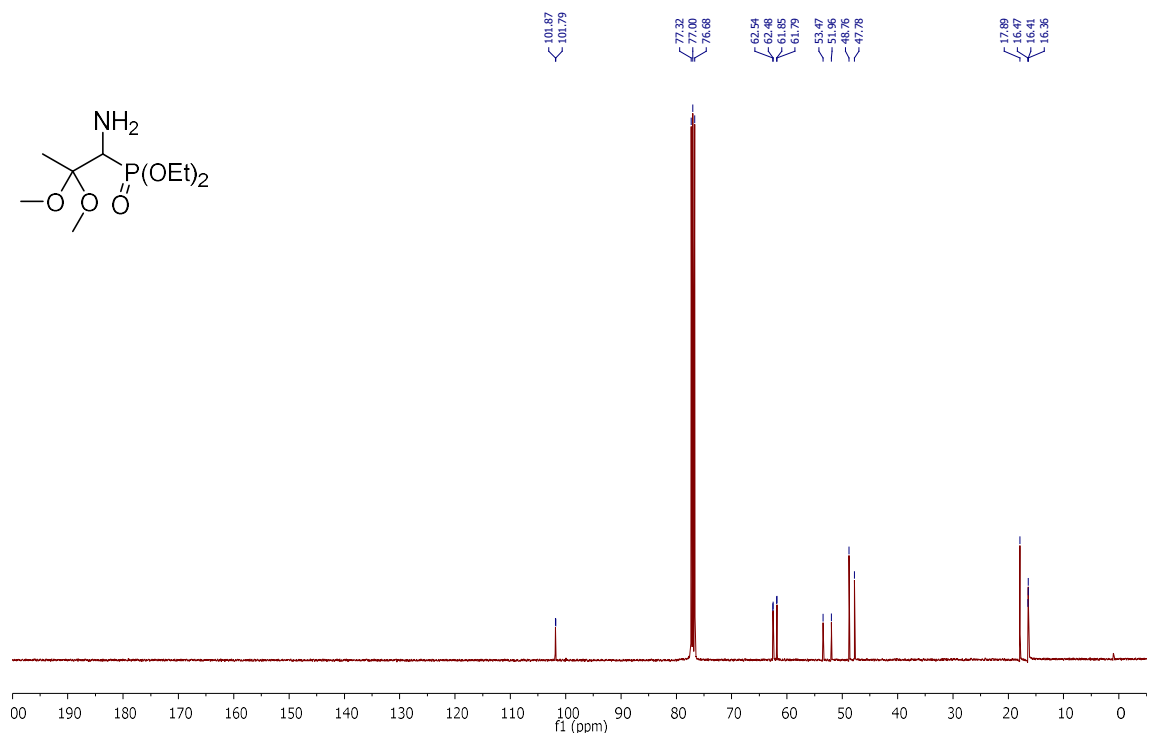
^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) of compound **4e**

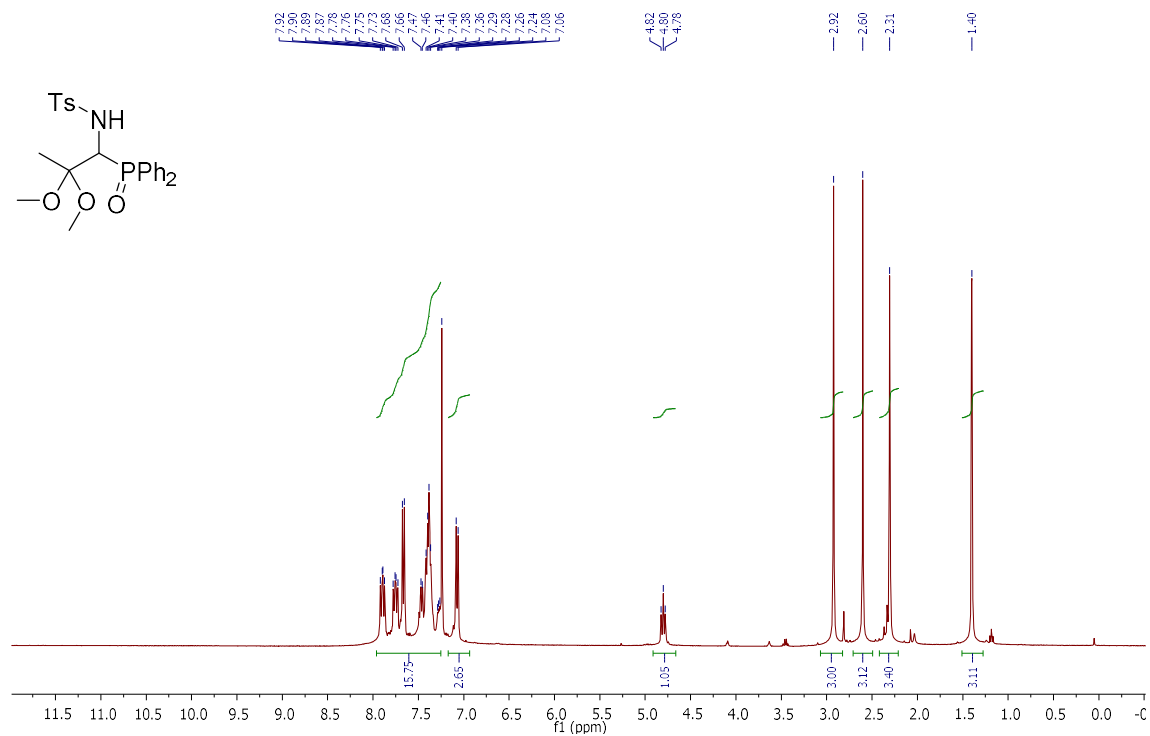
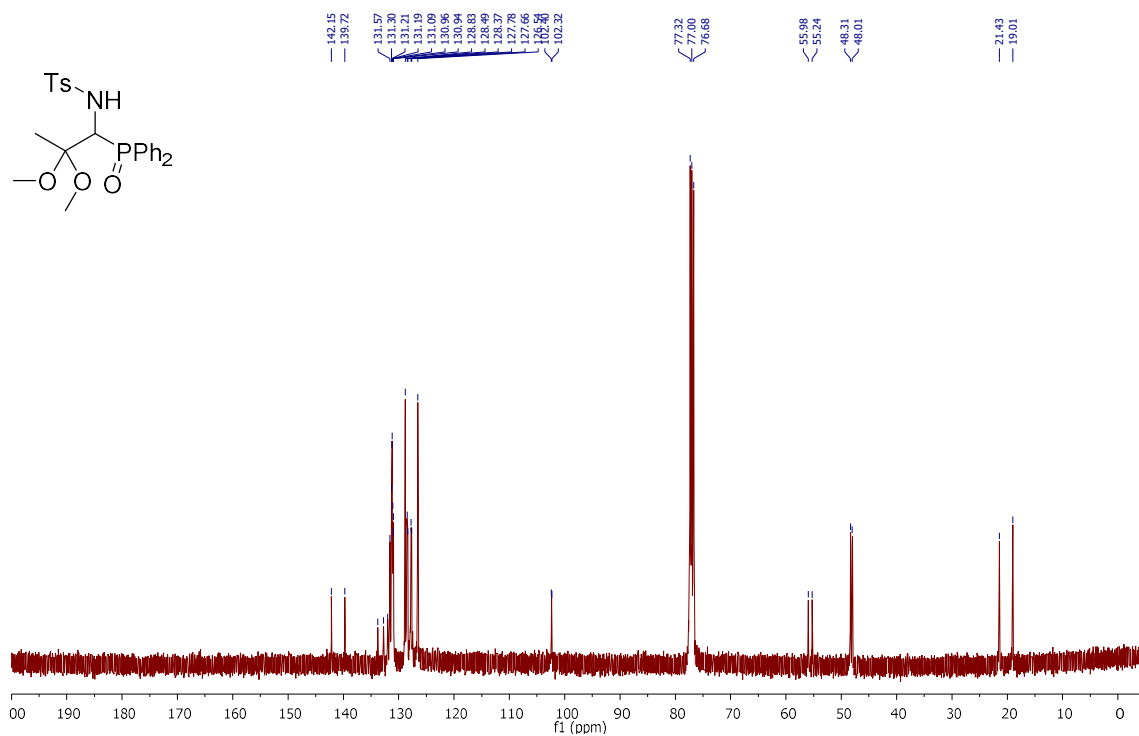


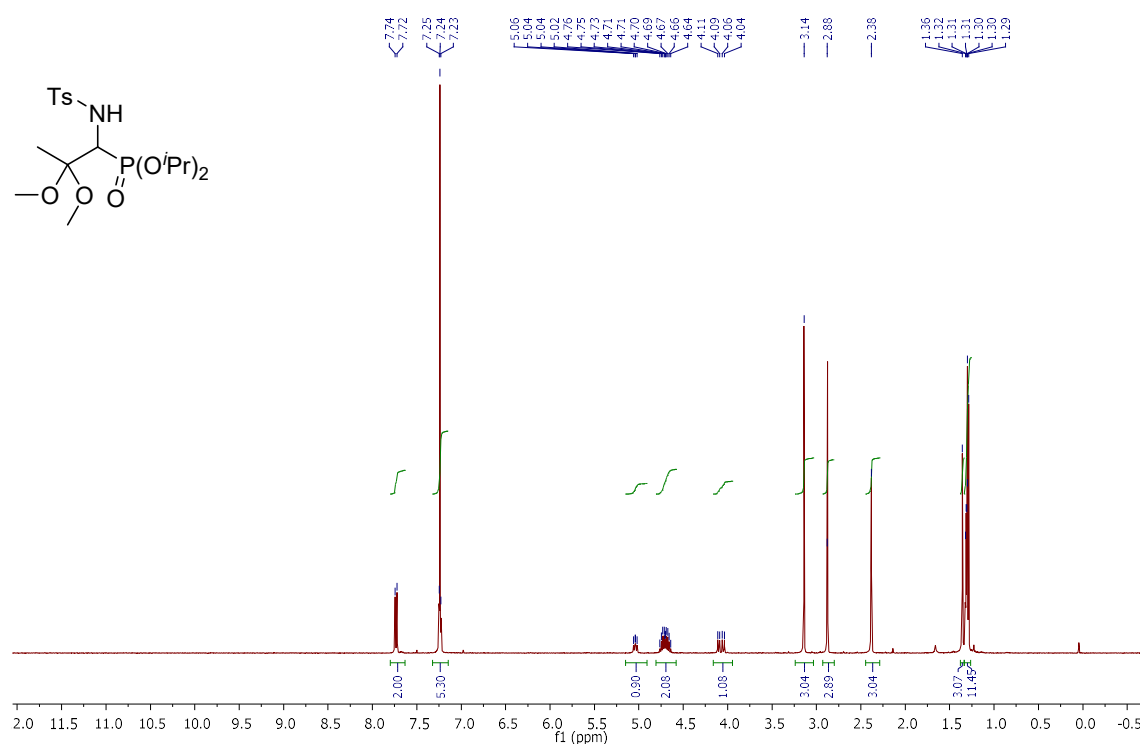
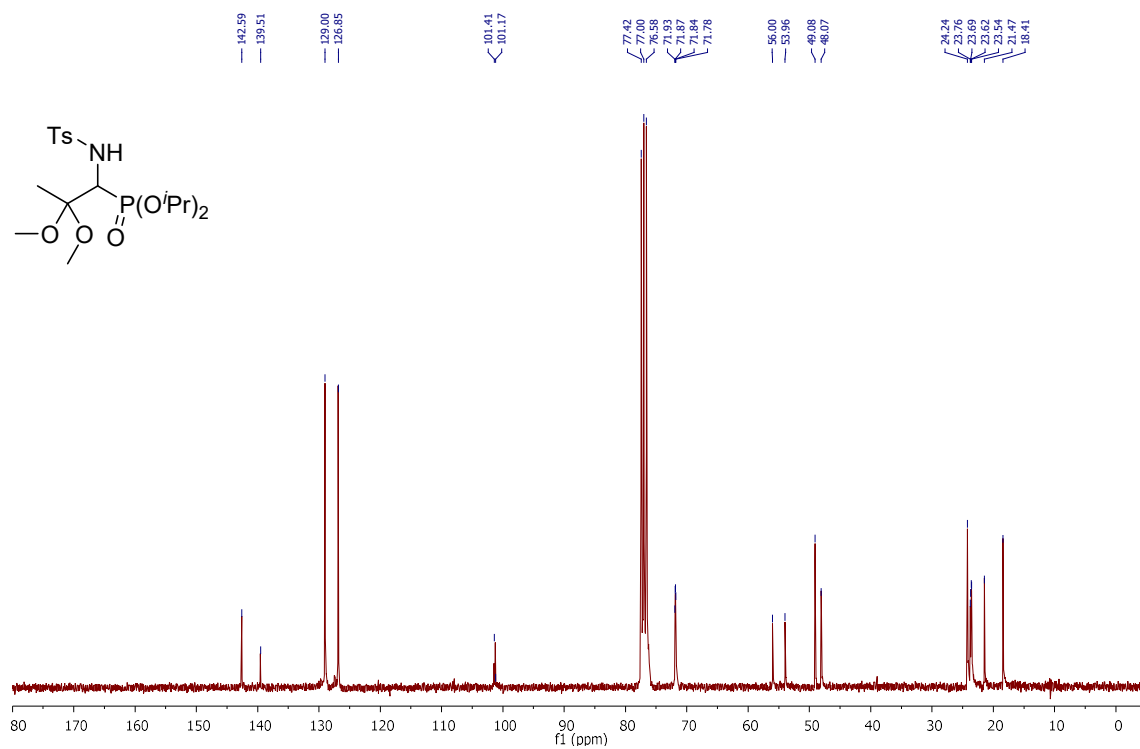
^1H NMR (400 MHz, CDCl_3) of compound **4f**

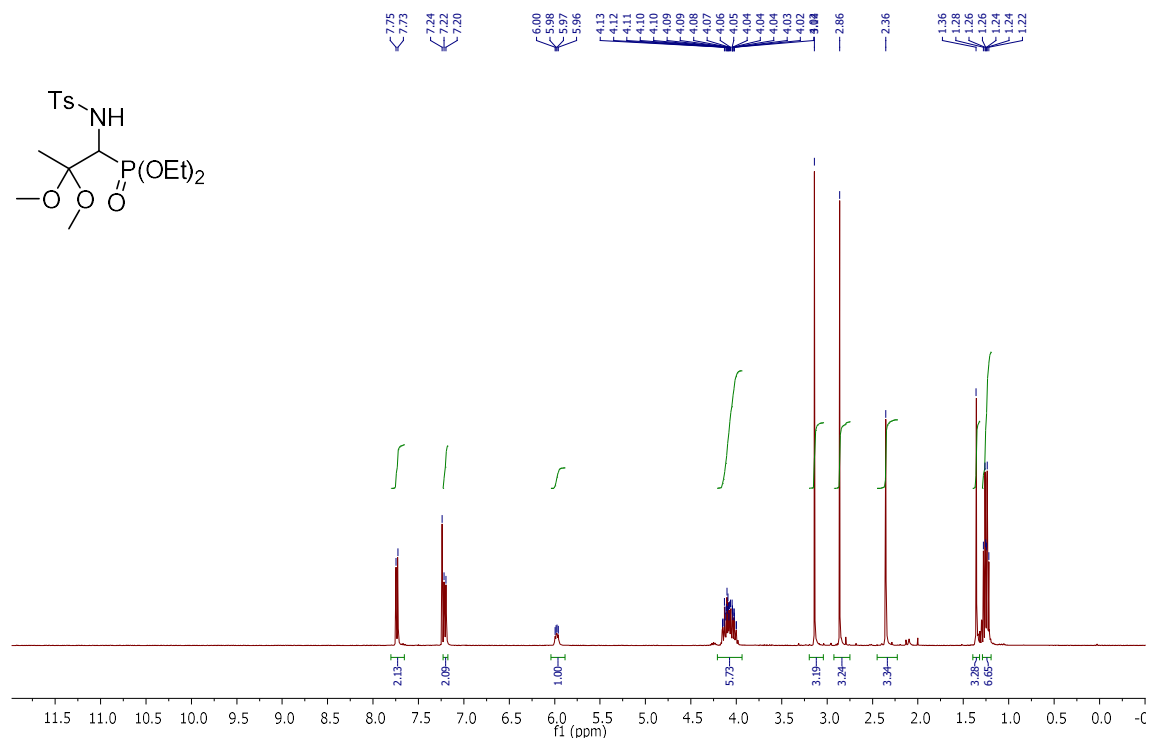
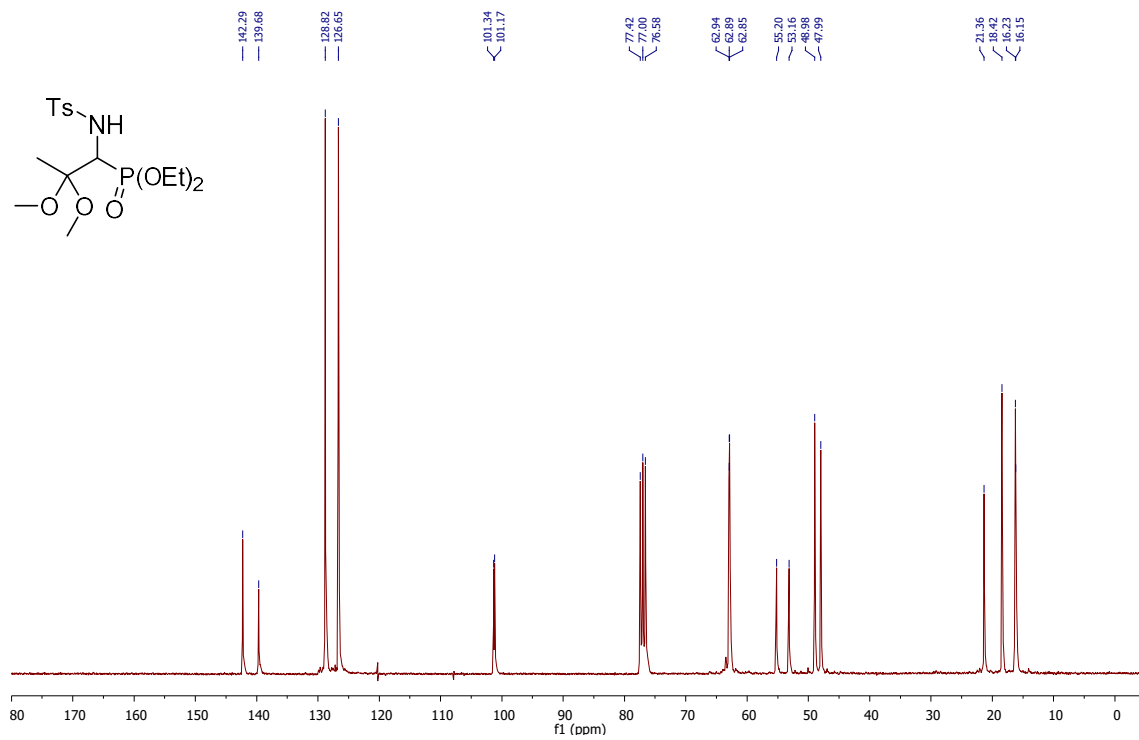


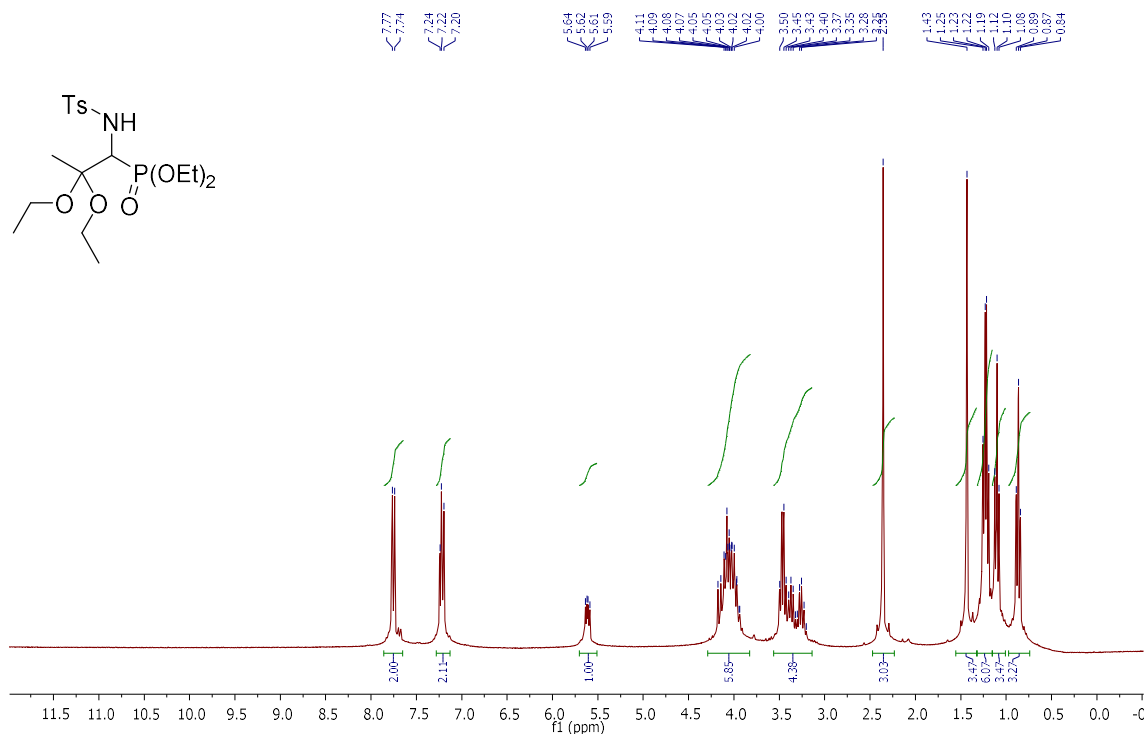
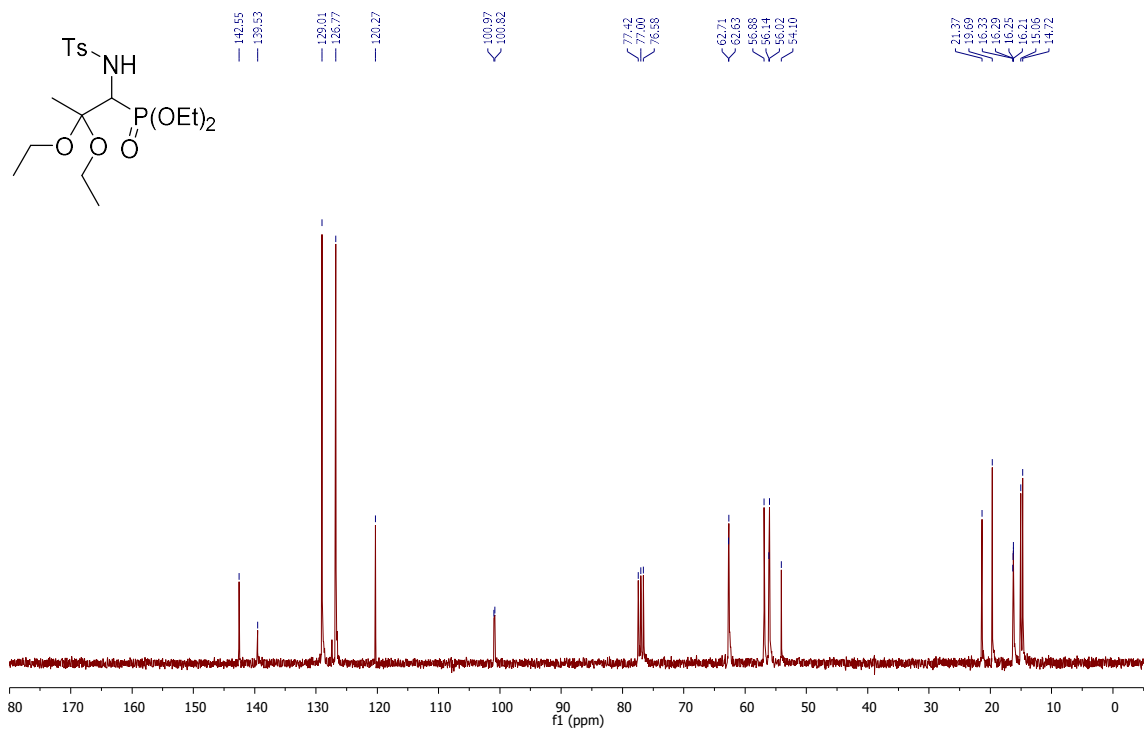
^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) of compound **4f**

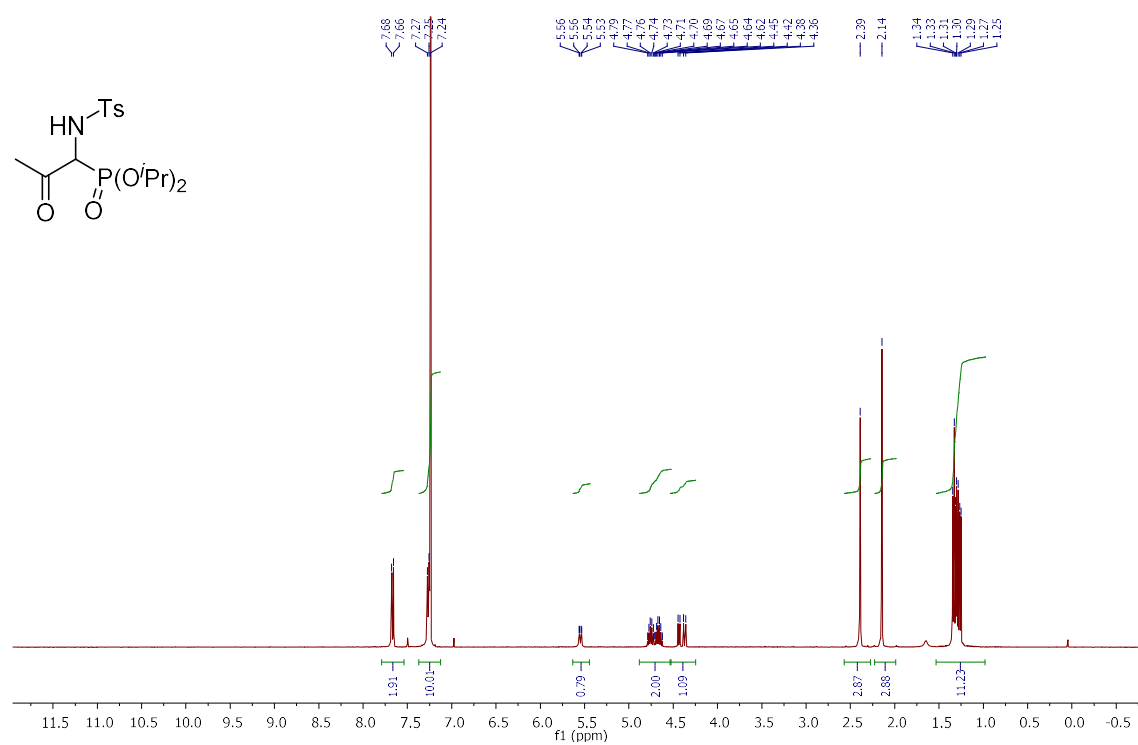
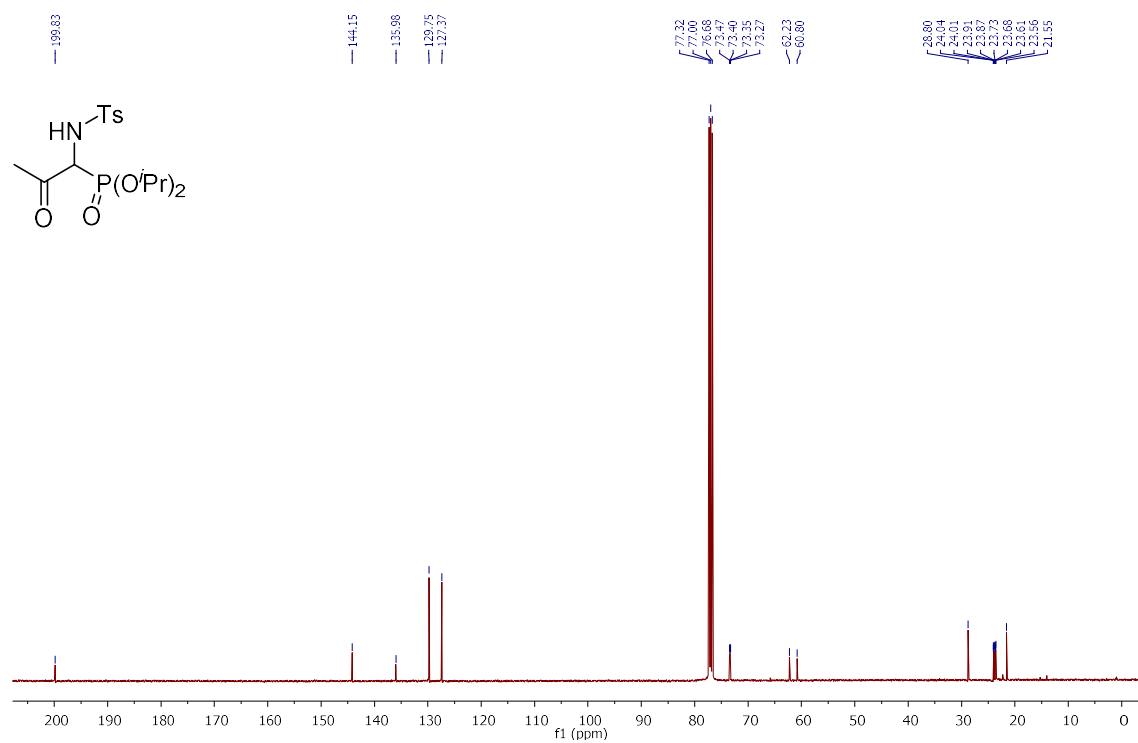


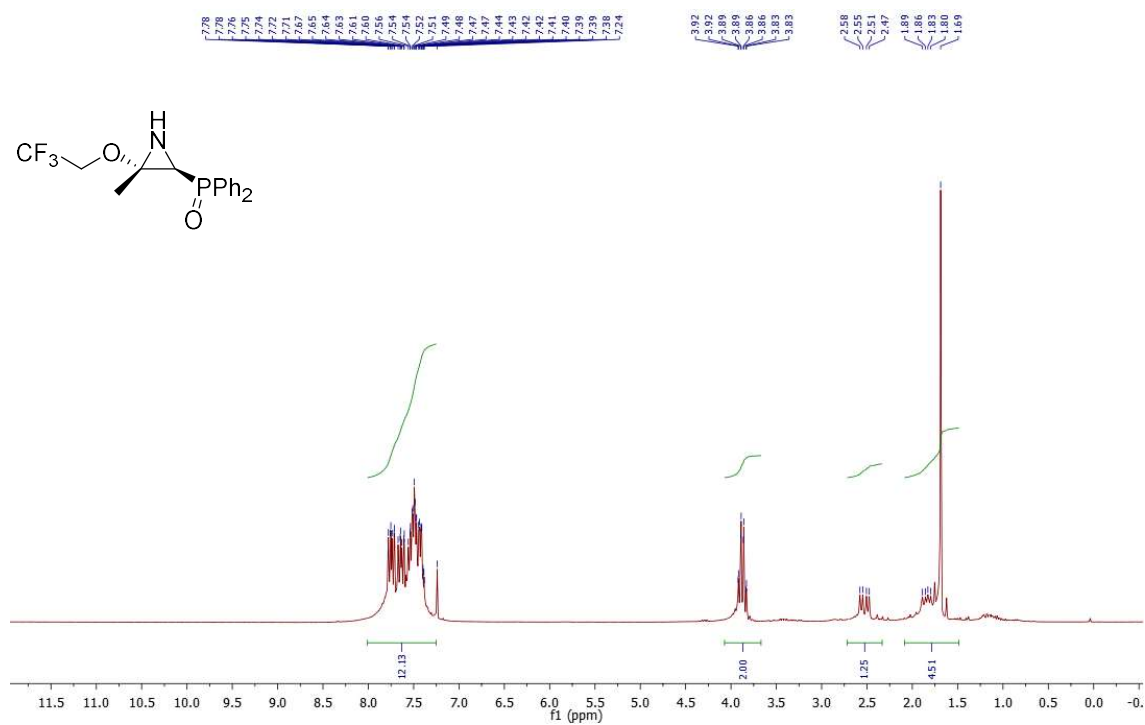
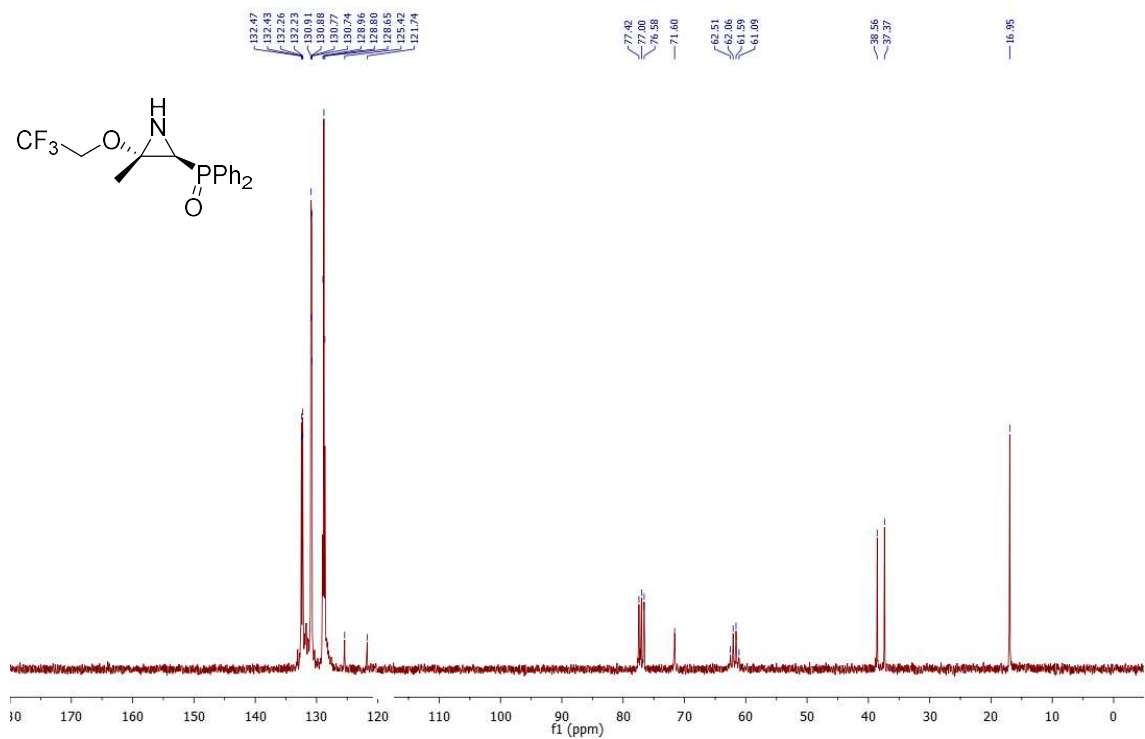
^1H NMR (400 MHz, CDCl_3) of compound **5a** ^{13}C { ^1H } NMR (100 MHz, CDCl_3) of compound **5a**

^1H NMR (400 MHz, CDCl_3) of compound **5b** ^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **5b**

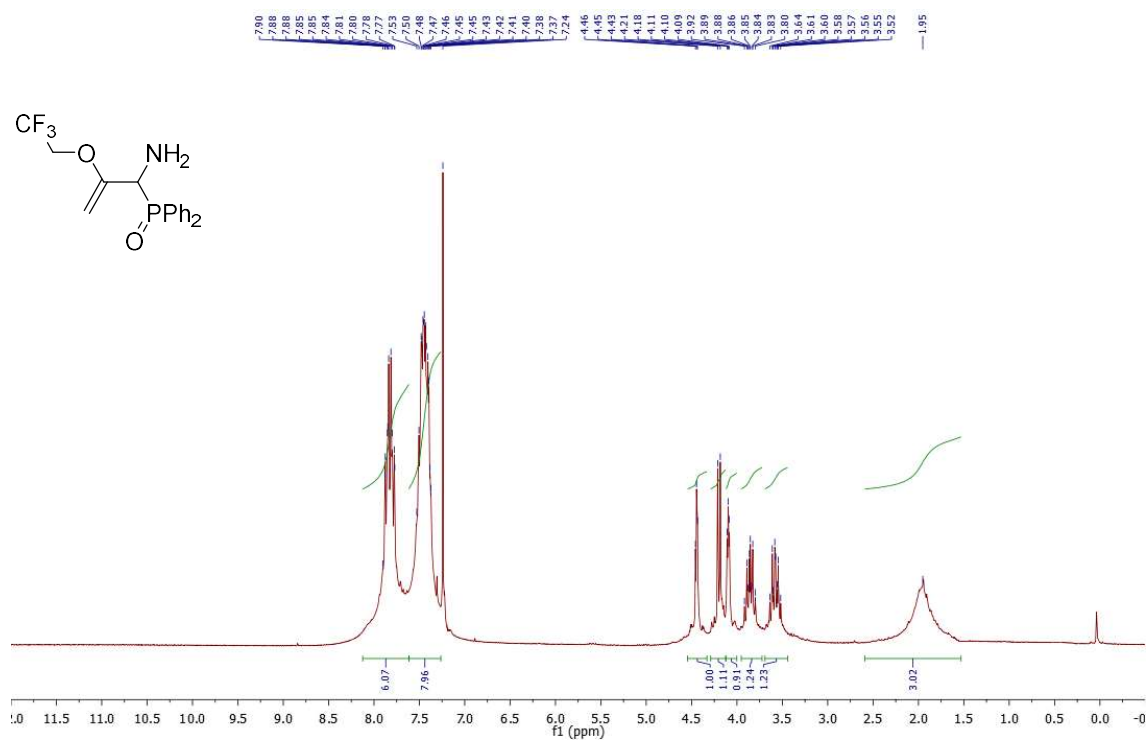
^1H NMR (400 MHz, CDCl_3) of compound **5c** ^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **5c**

^1H NMR (300 Hz, CDCl_3) of compound **5d** ^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **5d**

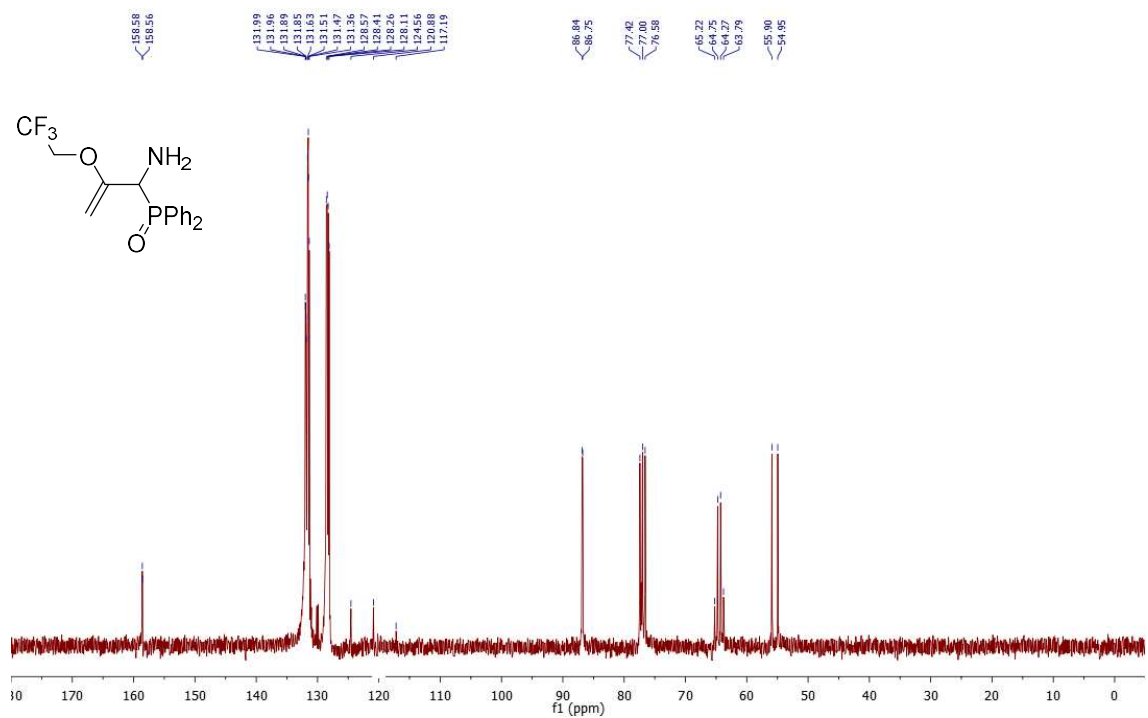
^1H NMR (400 MHz, CDCl_3) of compound 6 ^{13}C { ^1H } NMR (100 MHz, CDCl_3) of compound 6

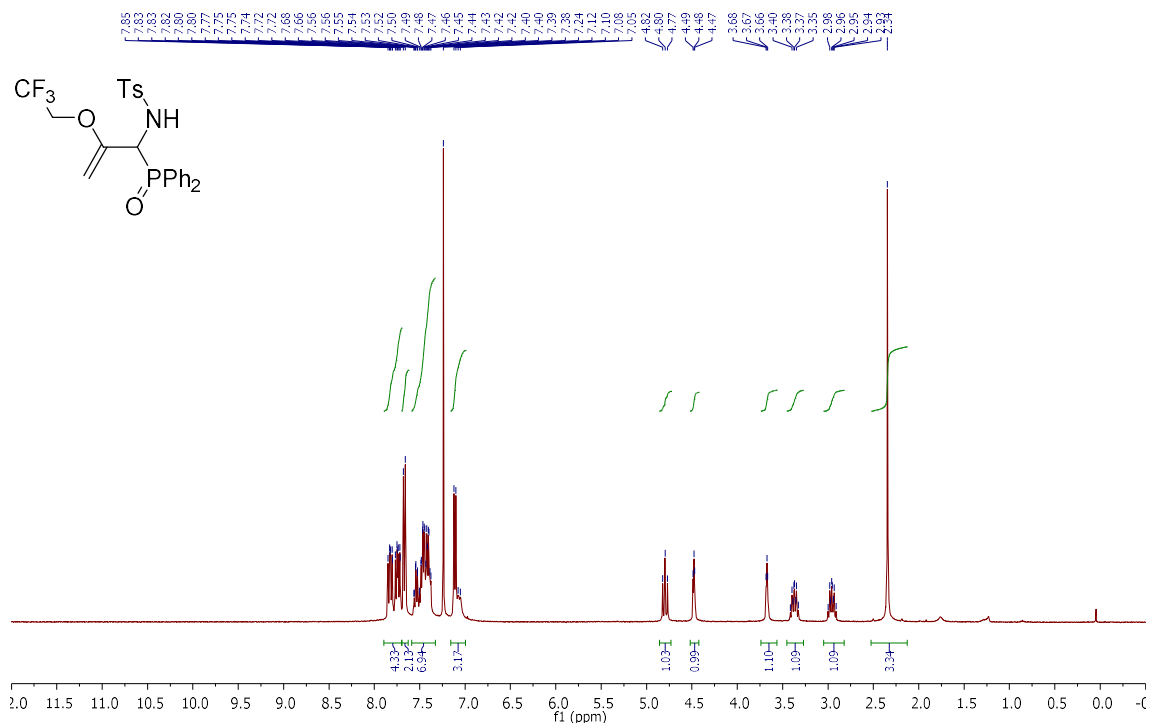
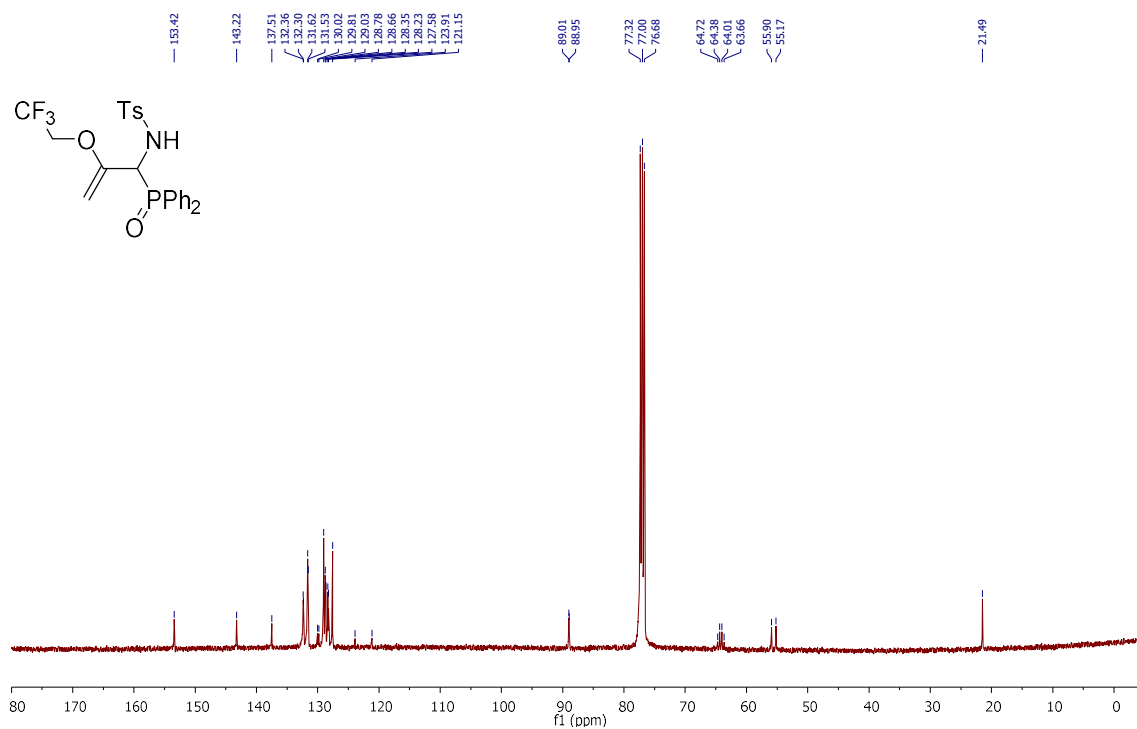
^1H NMR (300 Hz, CDCl_3) of compound **7** ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) of compound **7**

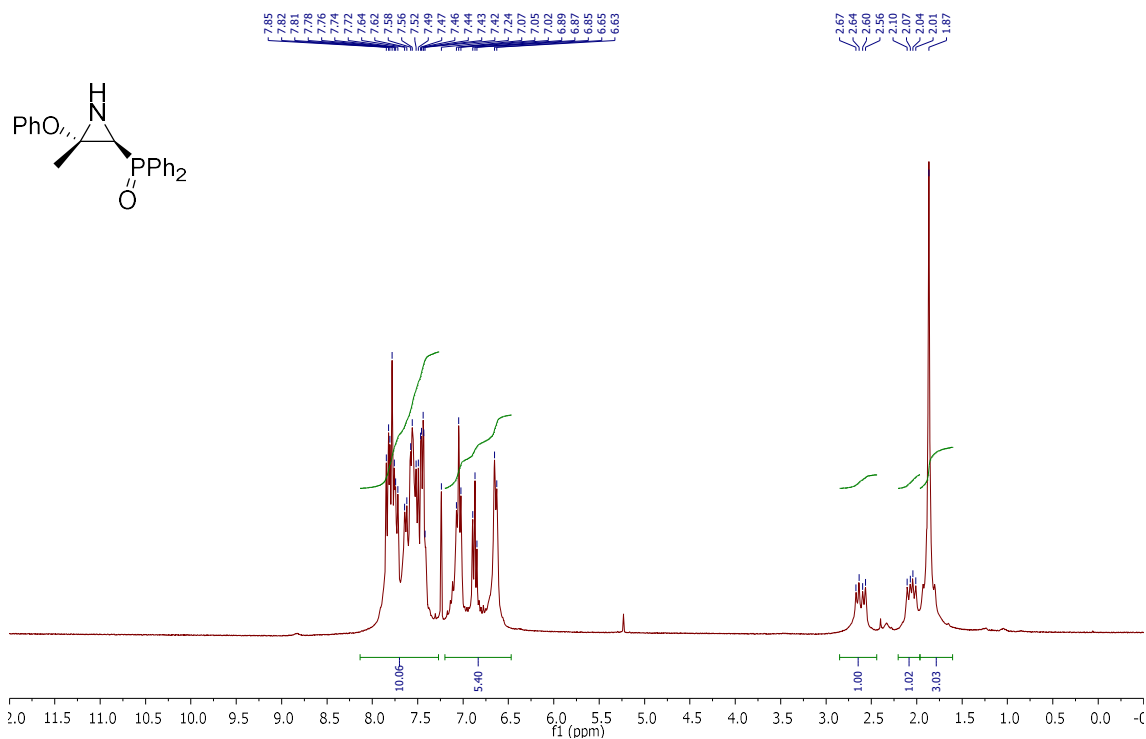
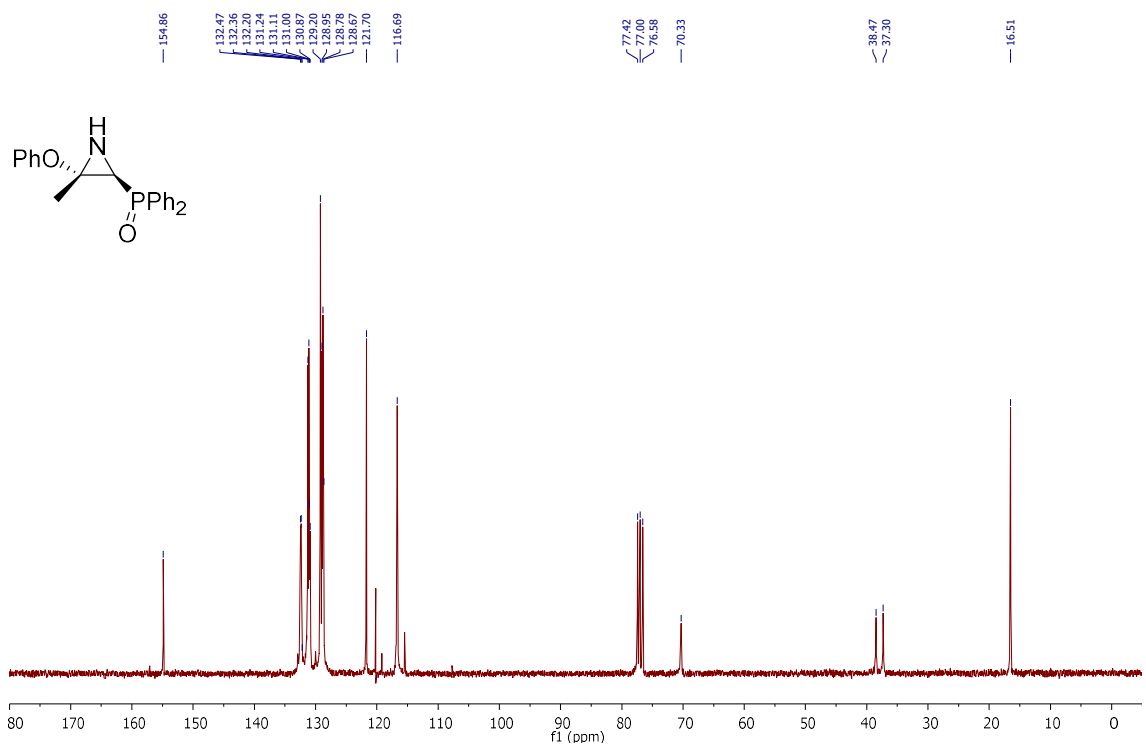
^1H NMR (300 Hz, CDCl_3) of compound **8**



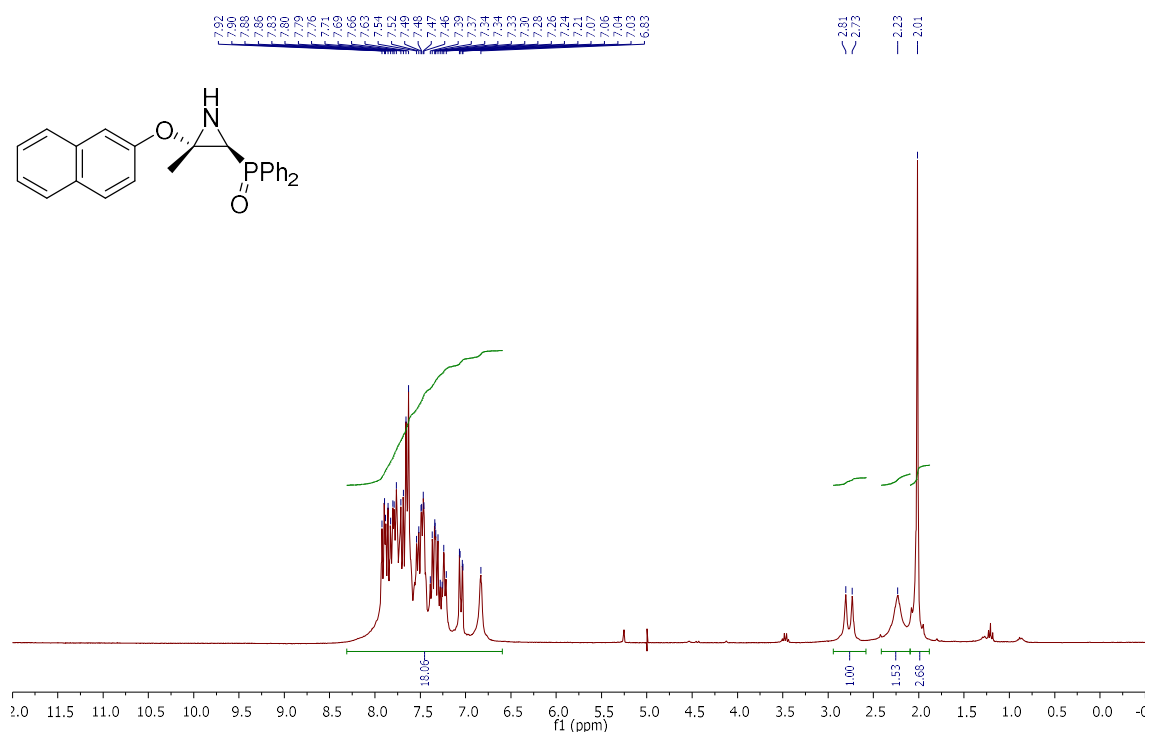
^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) of compound **8**

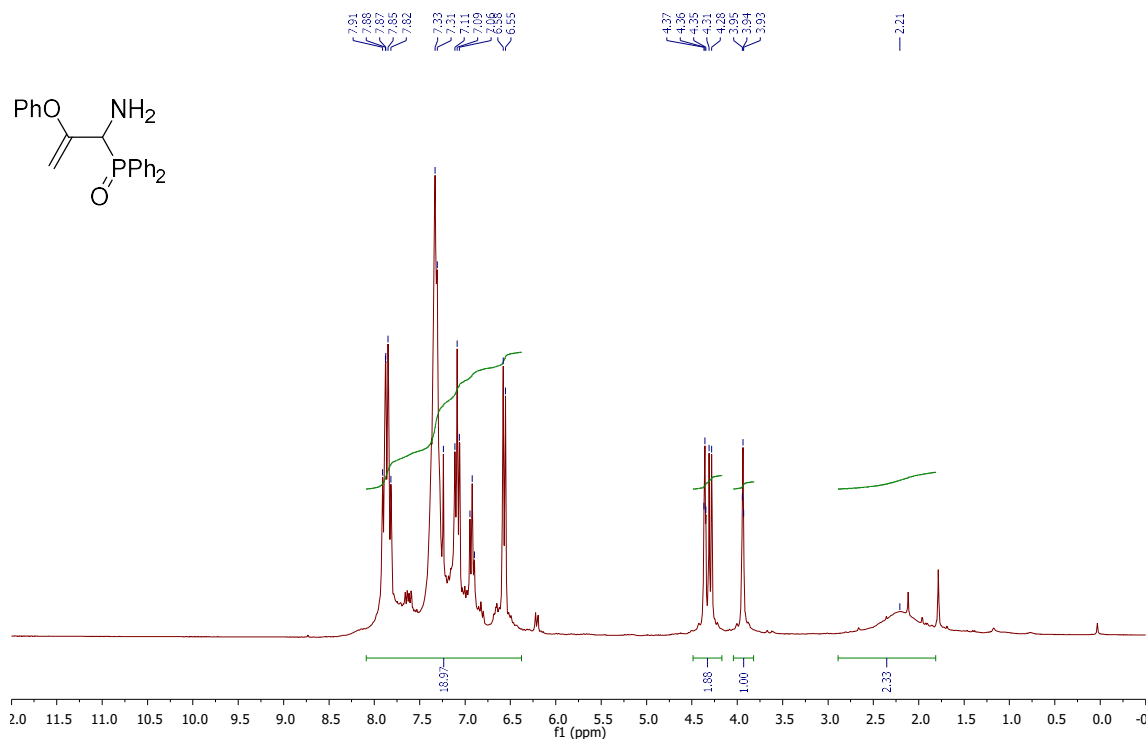
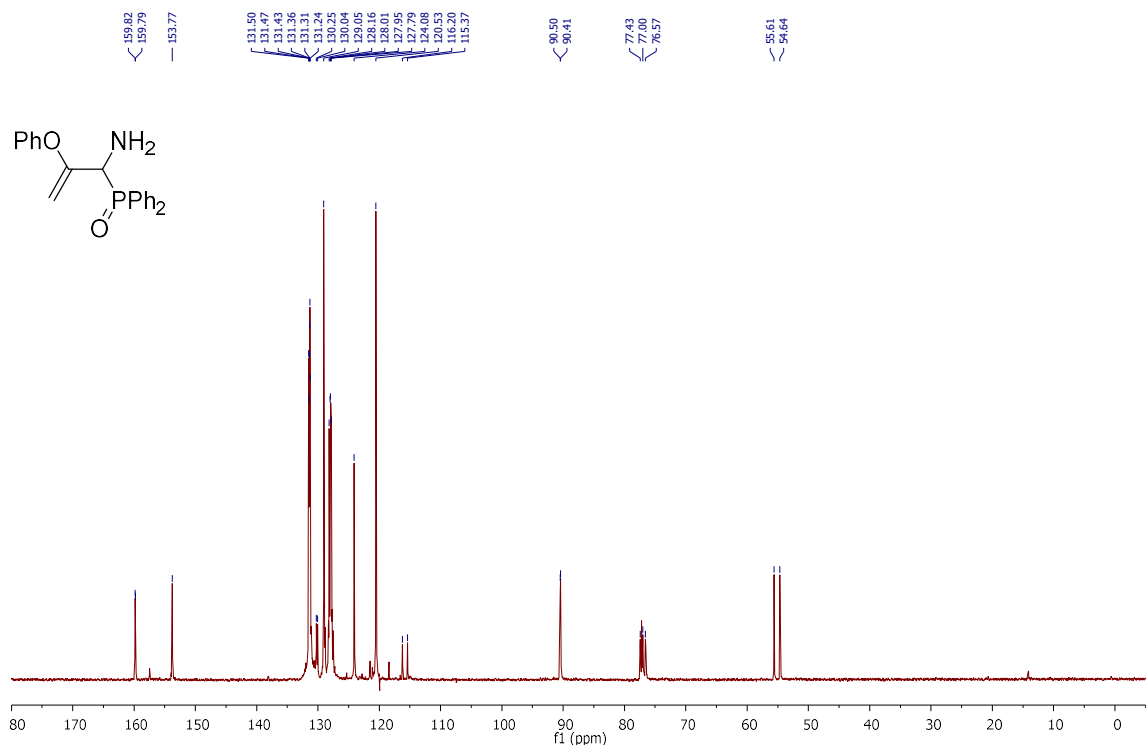


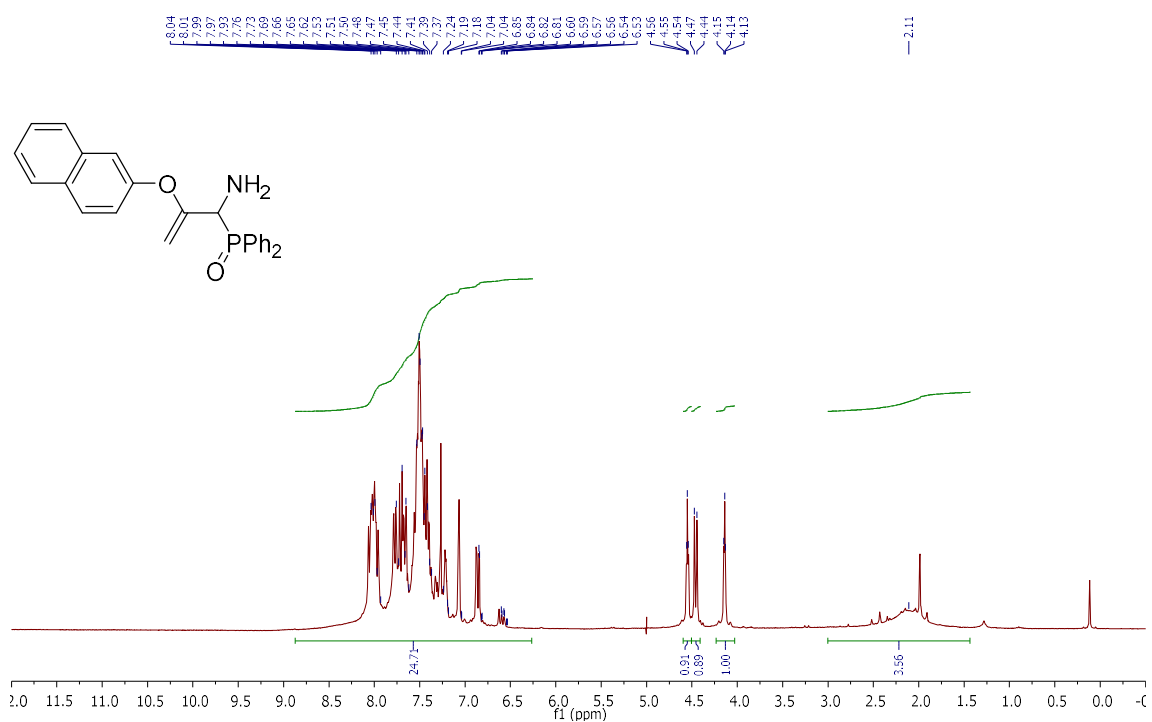
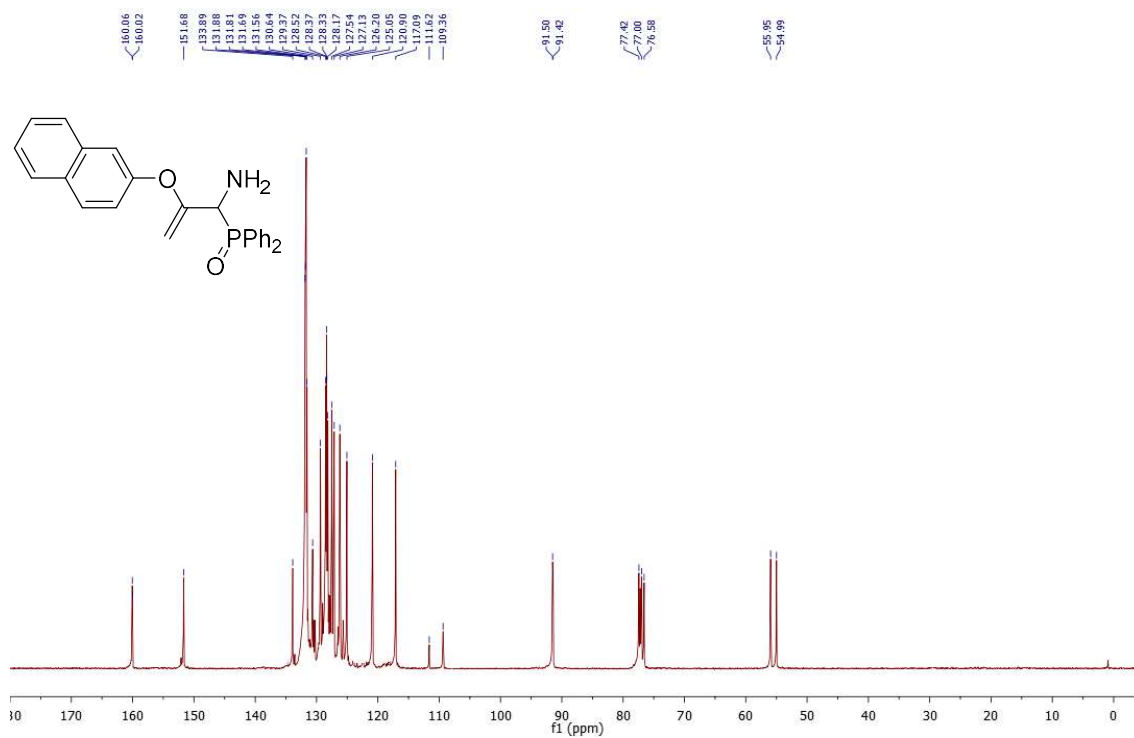
^1H NMR (400 Hz, CDCl_3) of compound **9** ^{13}C { ^1H } NMR (100 MHz, CDCl_3) of compound **9**

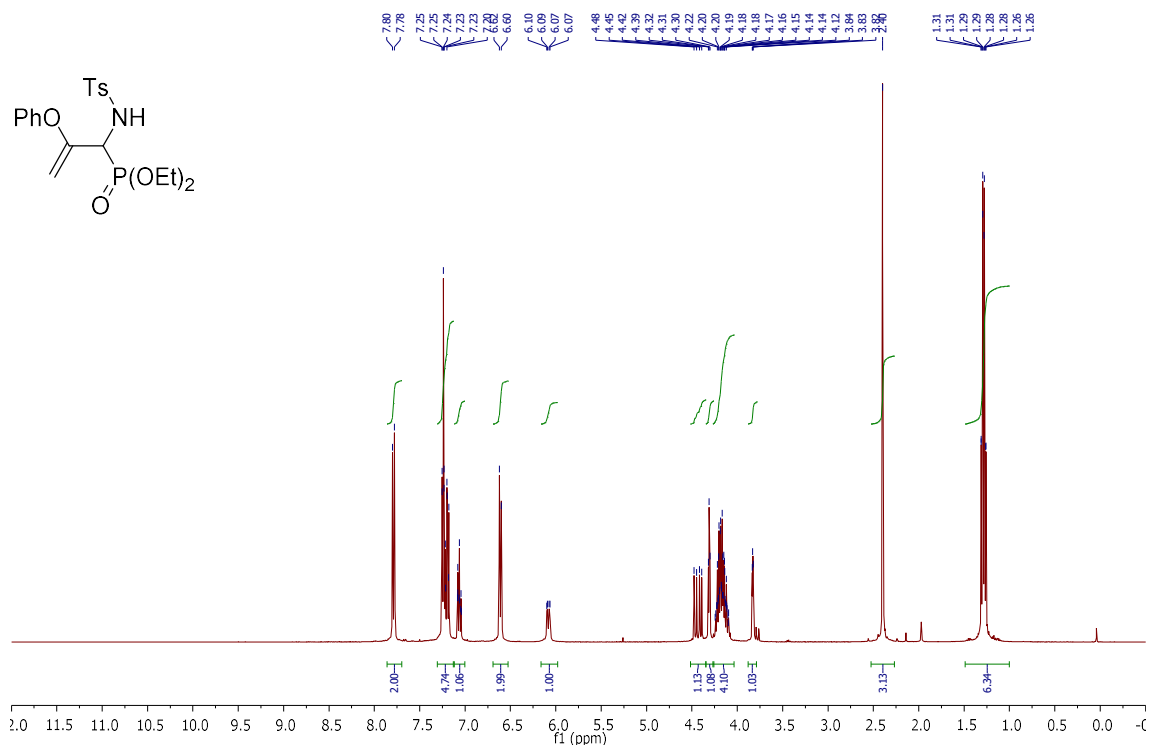
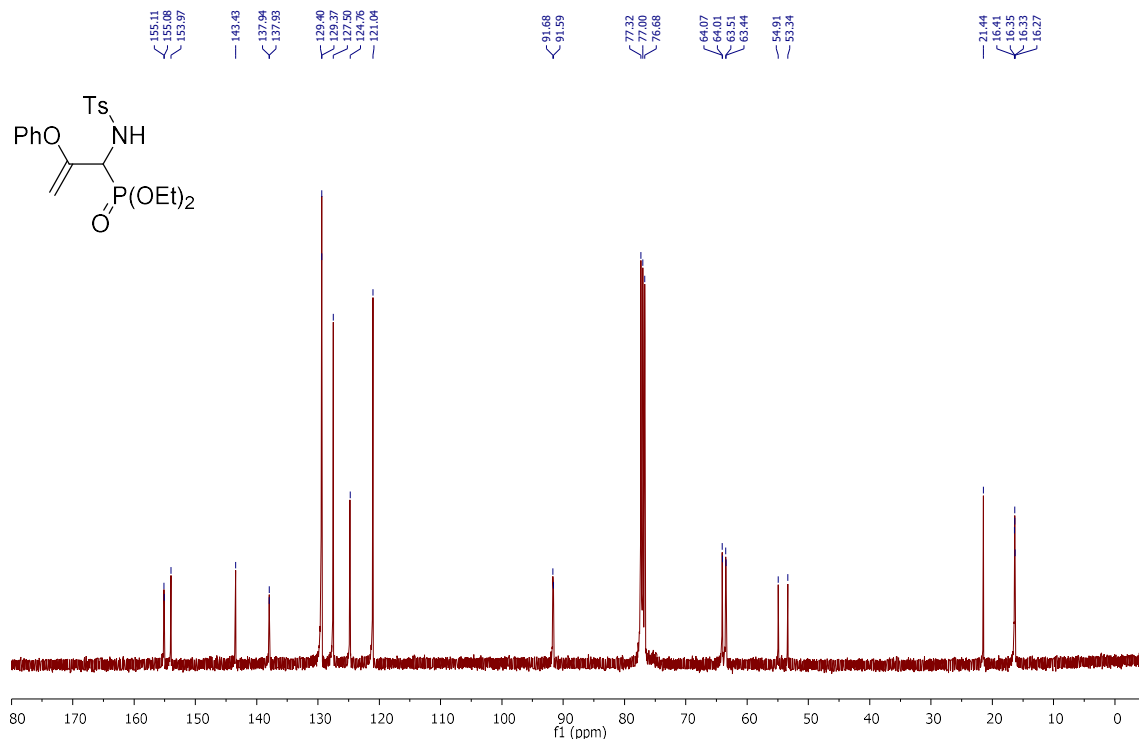
^1H NMR (300 Hz, CDCl_3) of compound **10a** ^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **10a**

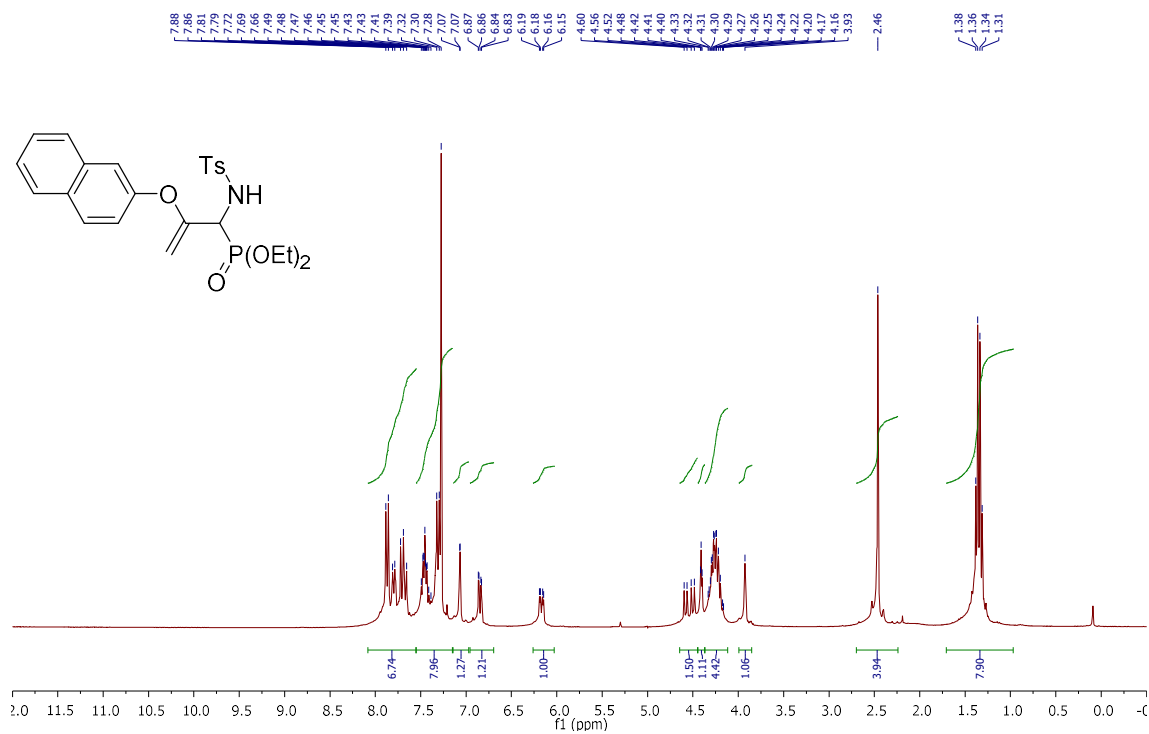
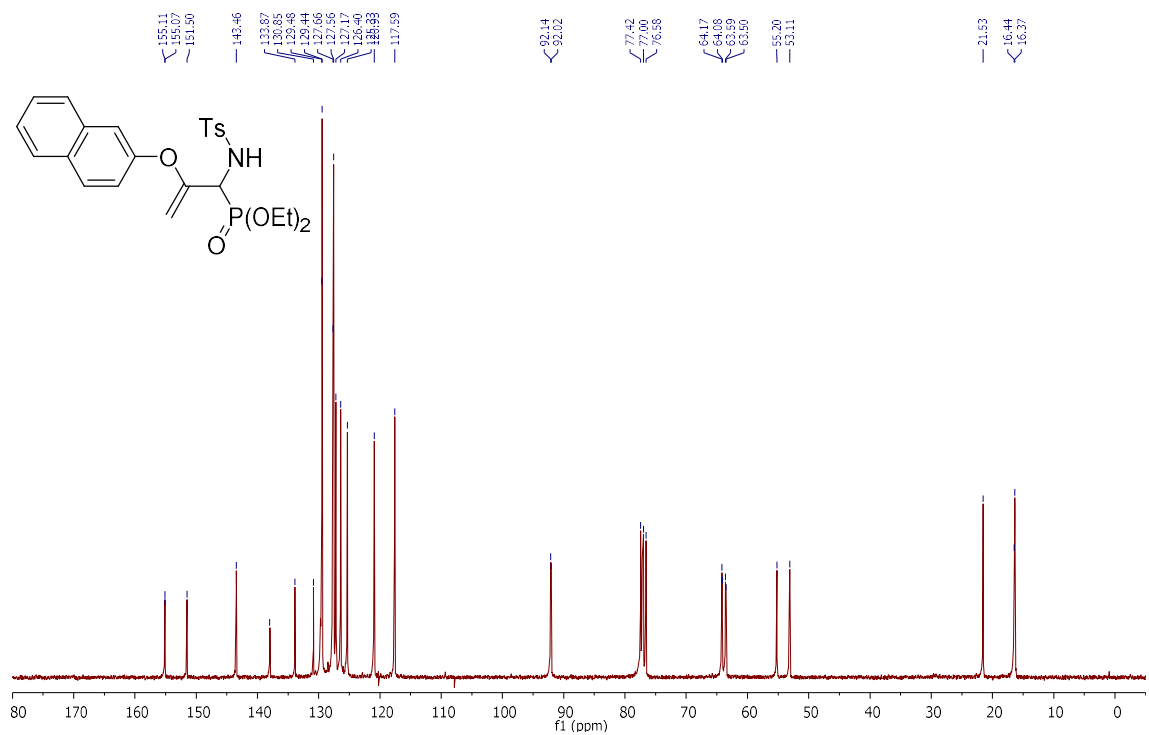
^1H NMR (300 Hz, CDCl_3) of compound **10b**



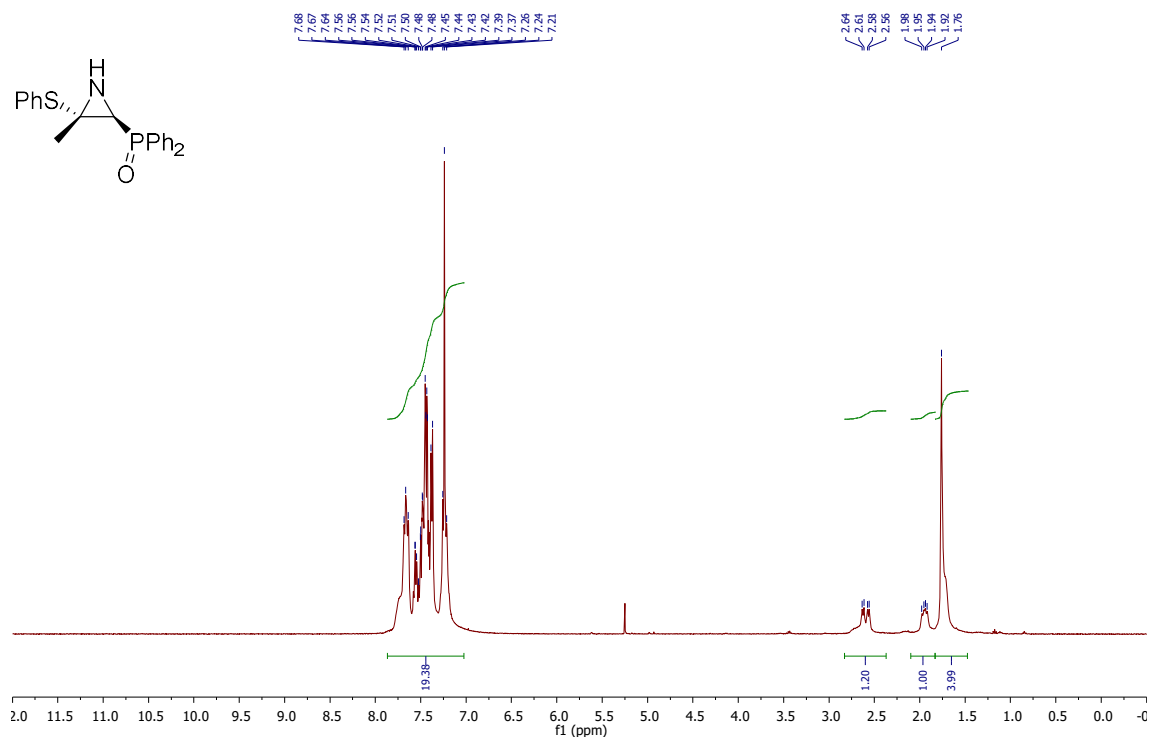
^1H NMR (300 Hz, CDCl_3) of compound **11a** ^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **11a**

^1H NMR (300 Hz, CDCl_3) of compound **11b** ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) of compound **11b**

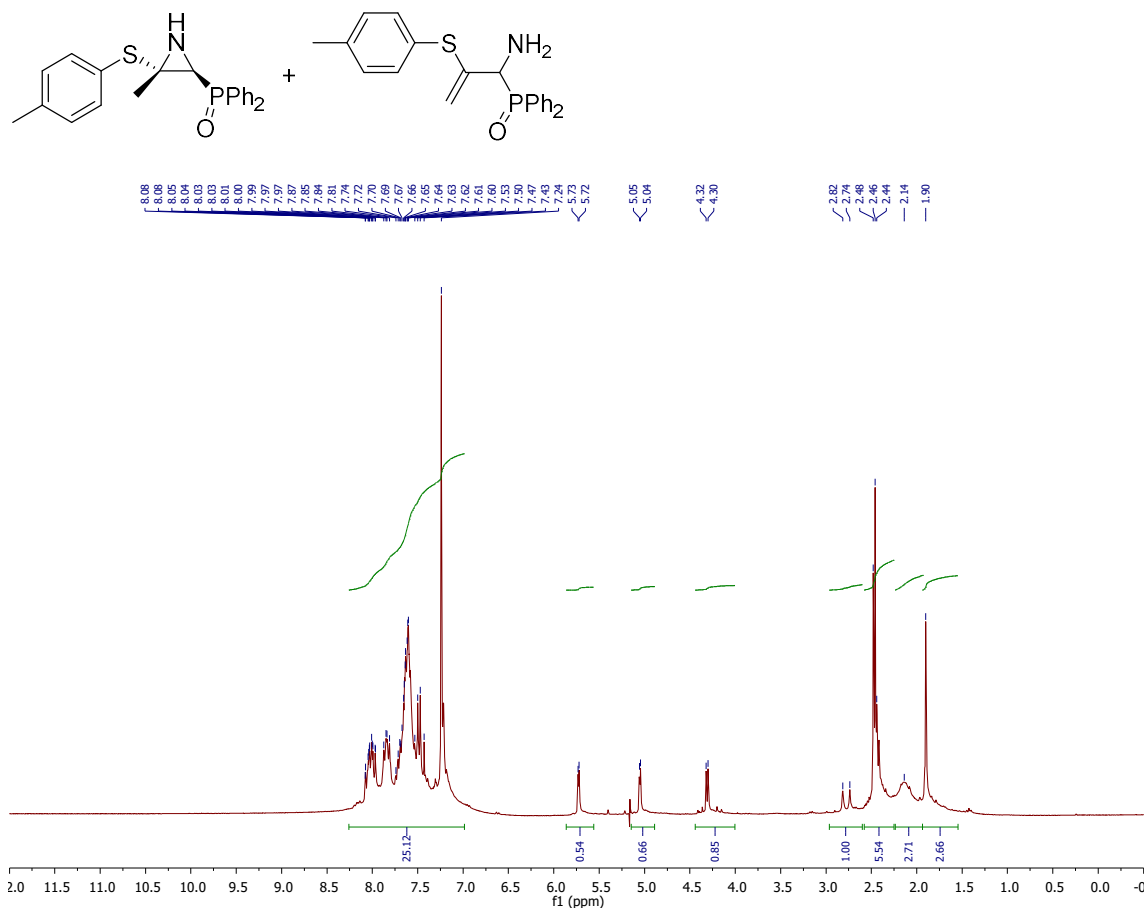
^1H NMR (400 Hz, CDCl_3) of compound **12a** ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) of compound **12a**

^1H NMR (300 Hz, CDCl_3) of compound **12b** ^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **12b**

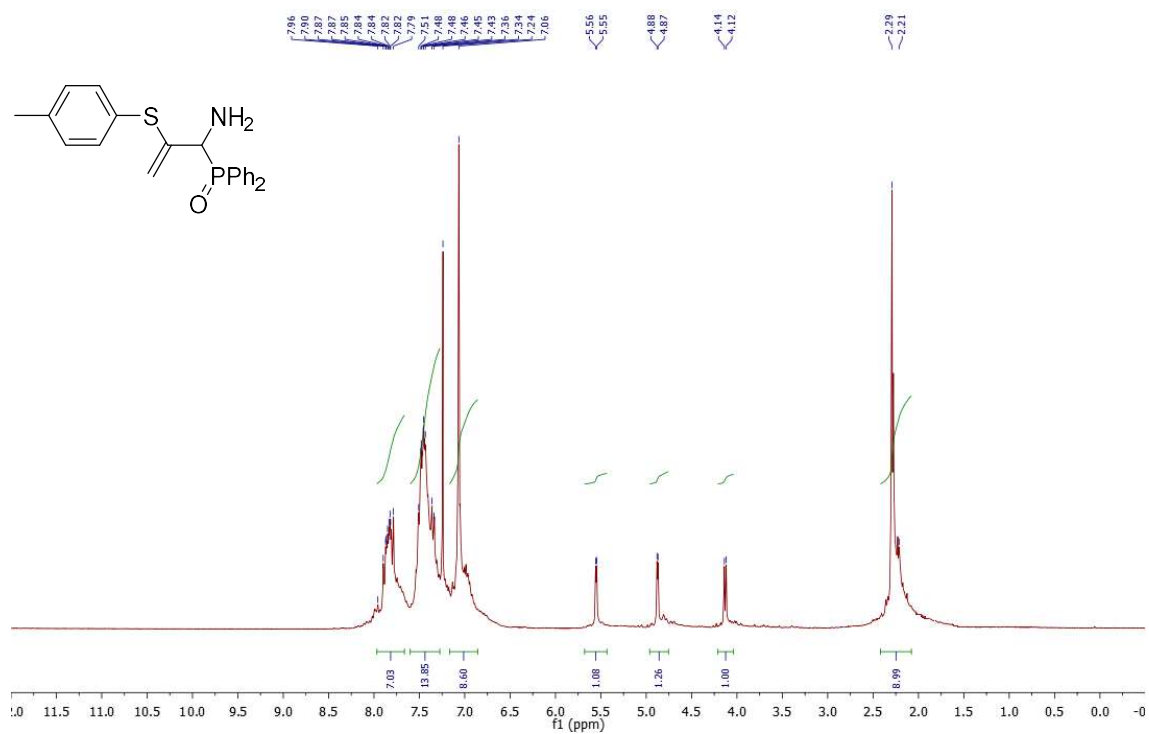
^1H NMR (400 Hz, CDCl_3) of compound **14a**



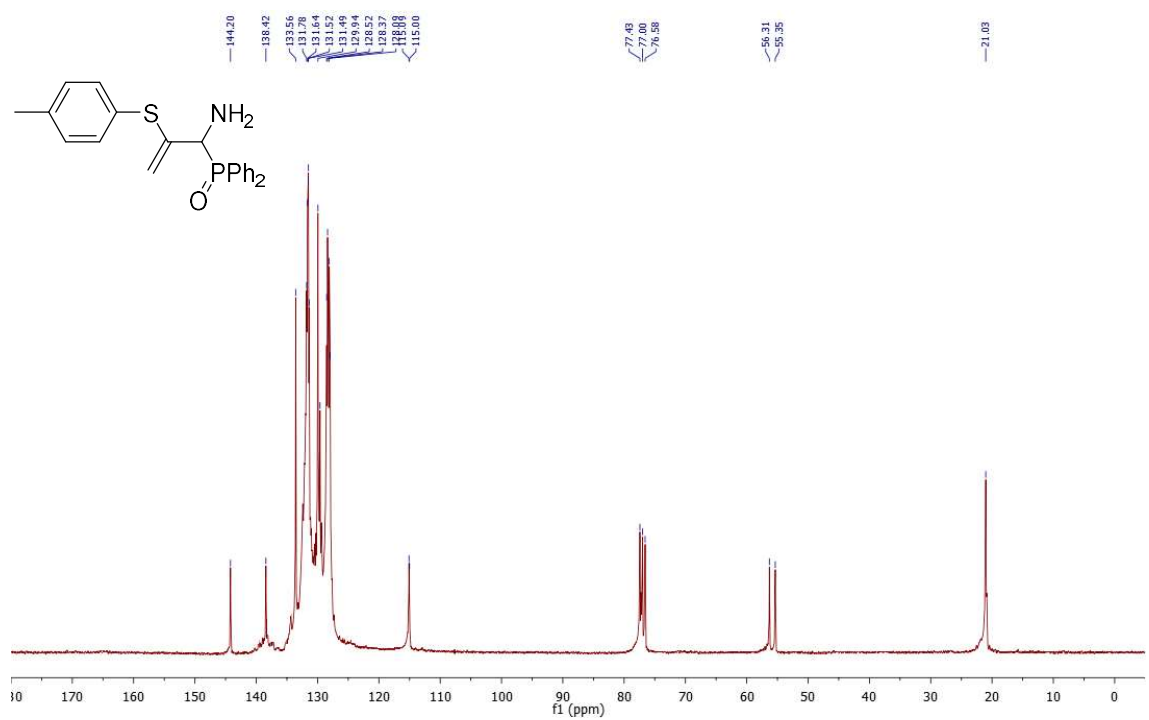
^1H NMR (300 Hz, CDCl_3) of a crude mixture of **14b** + **15b**.

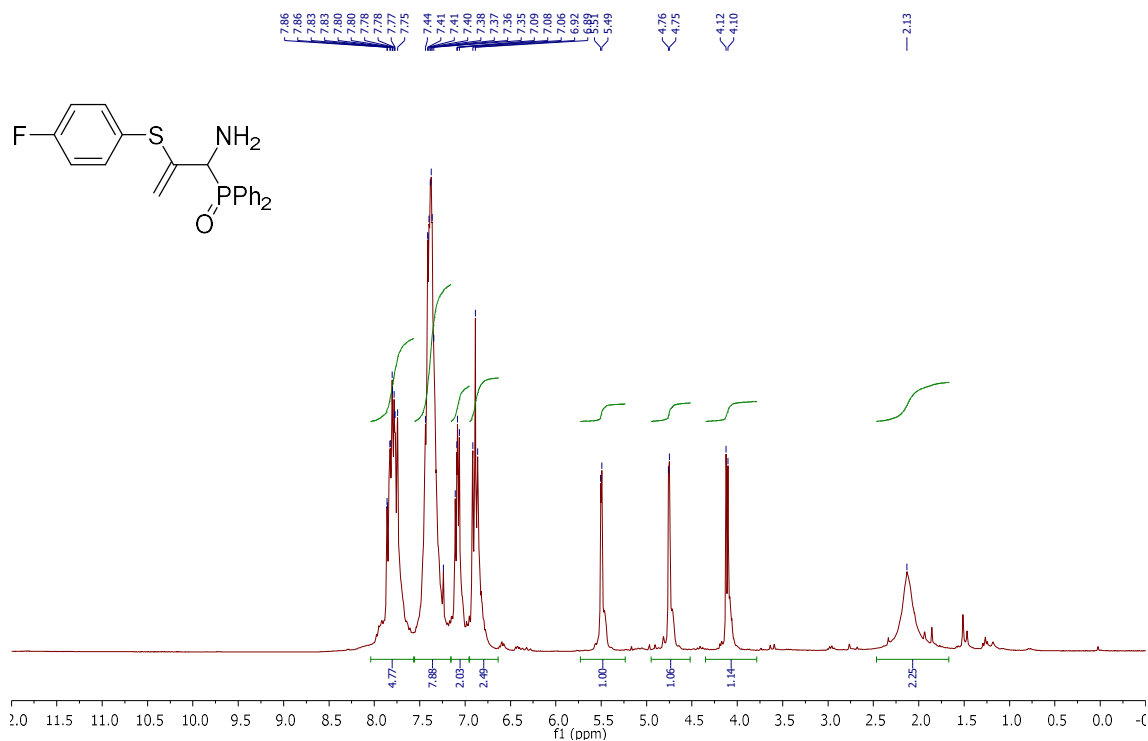
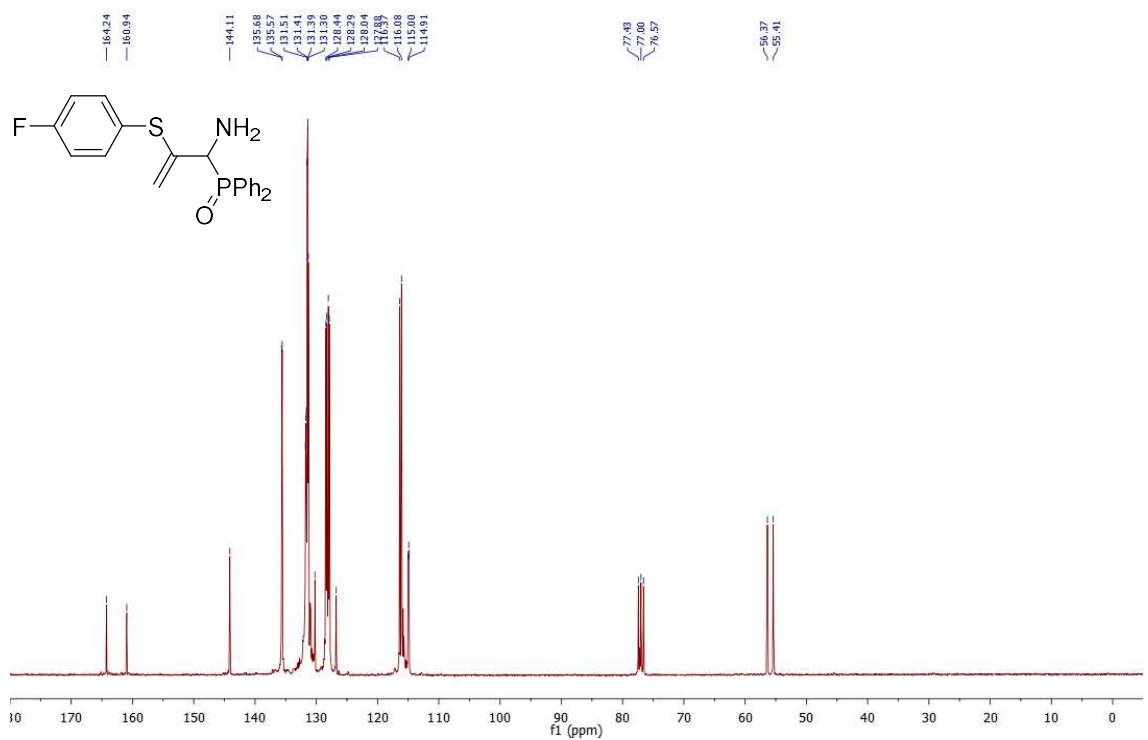


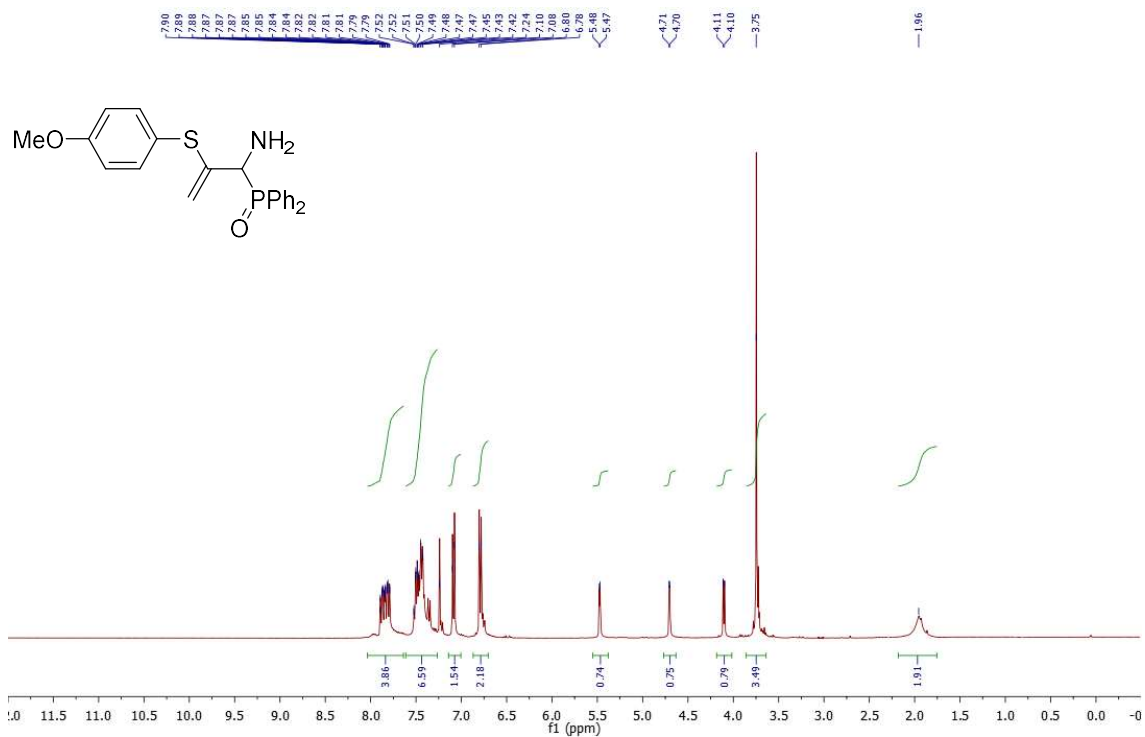
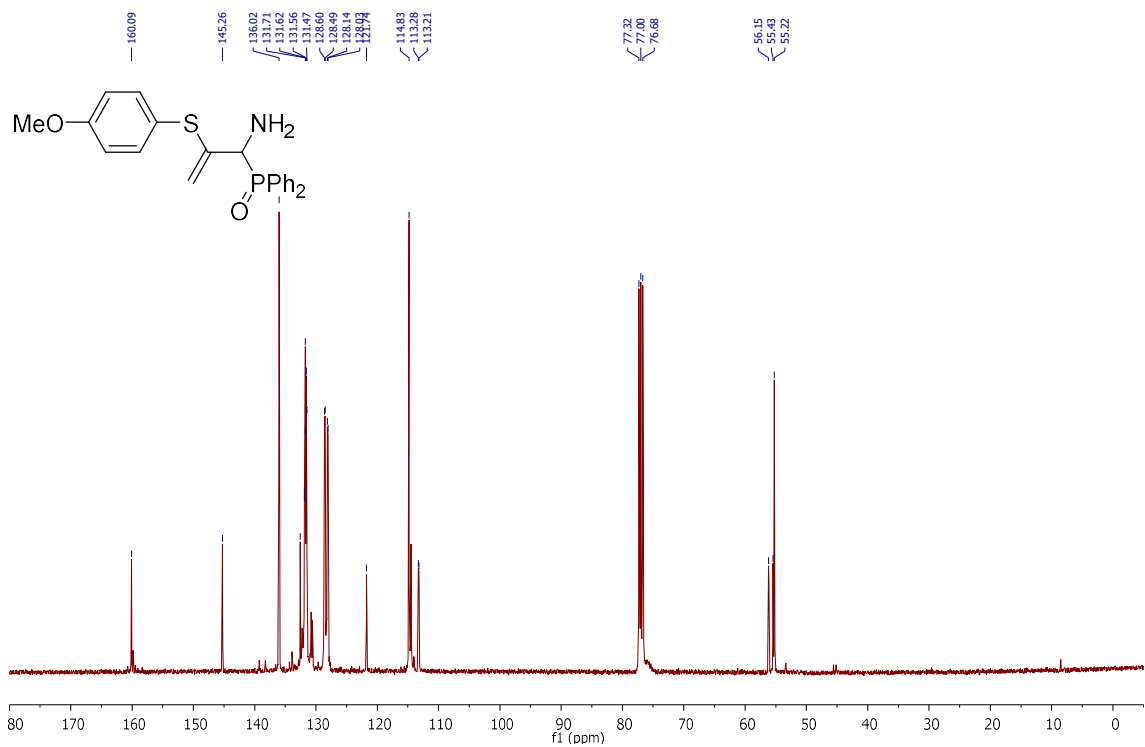
^1H NMR (300 Hz, CDCl_3) of compound **15b**



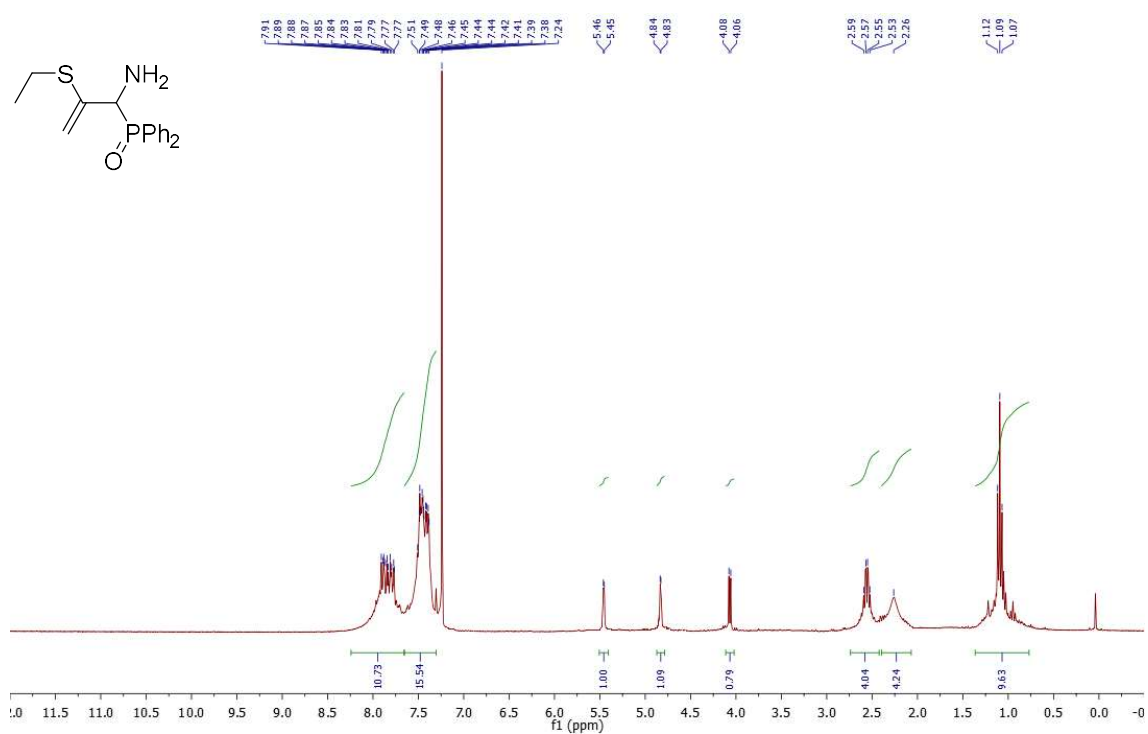
^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) of compound **15b**



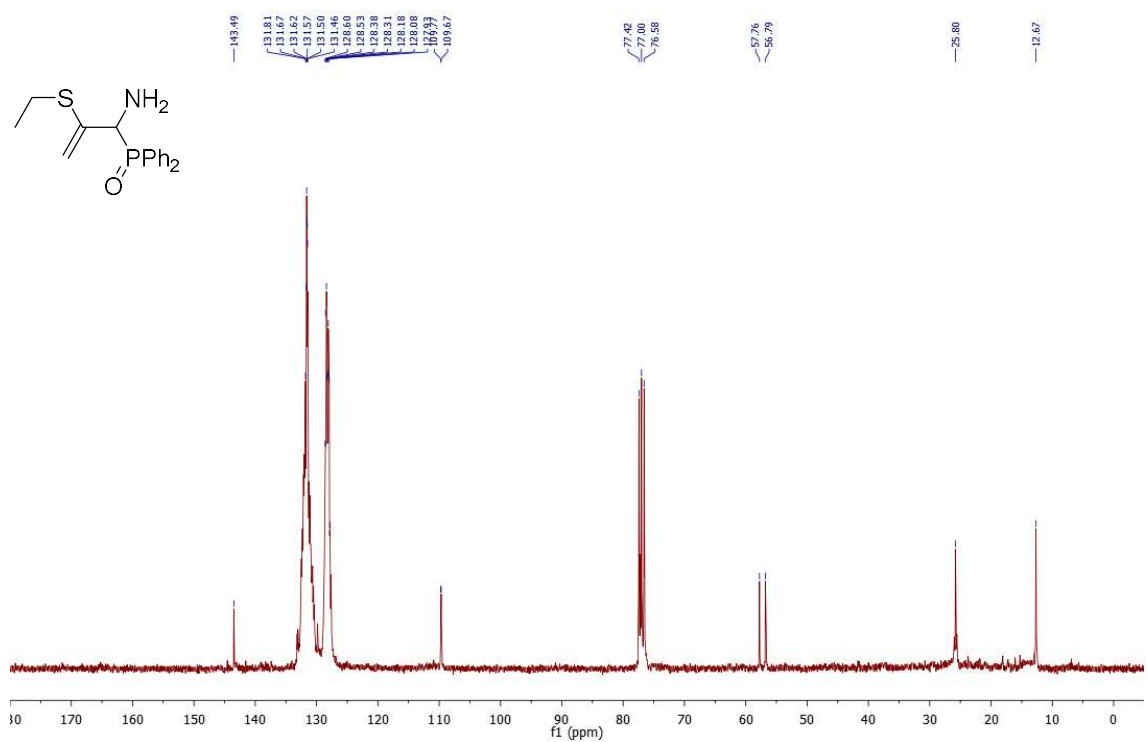
^1H NMR (300 Hz, CDCl_3) of compound **15c** ^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **15c**

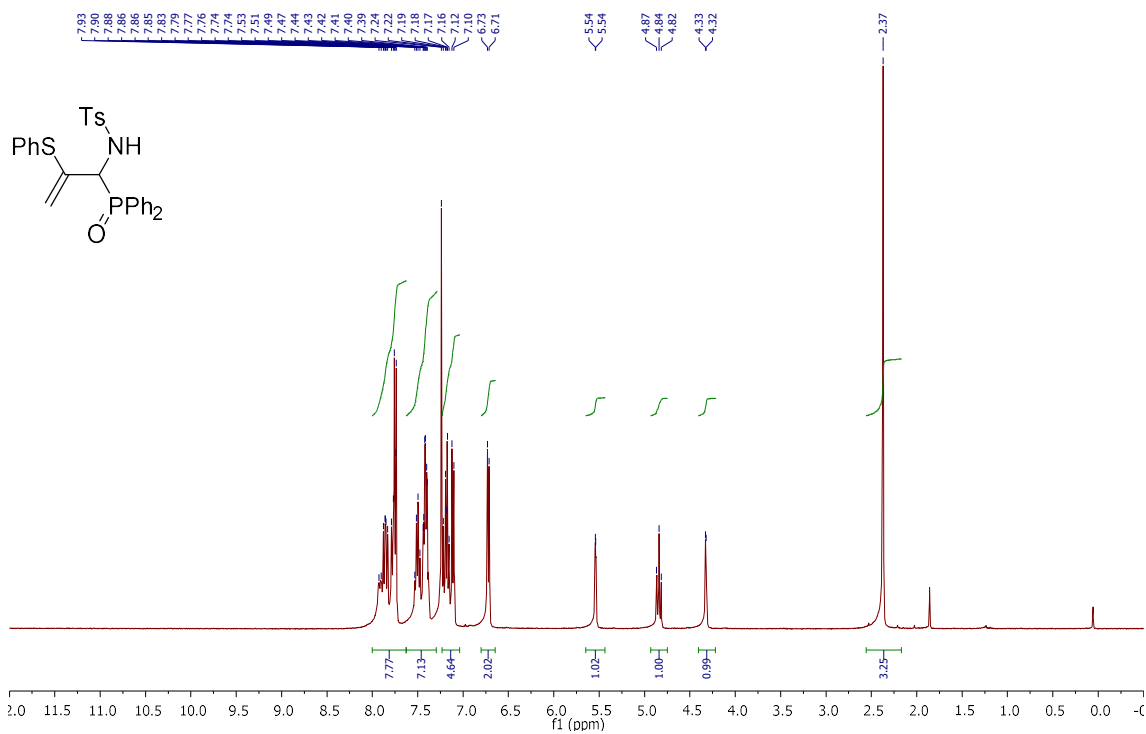
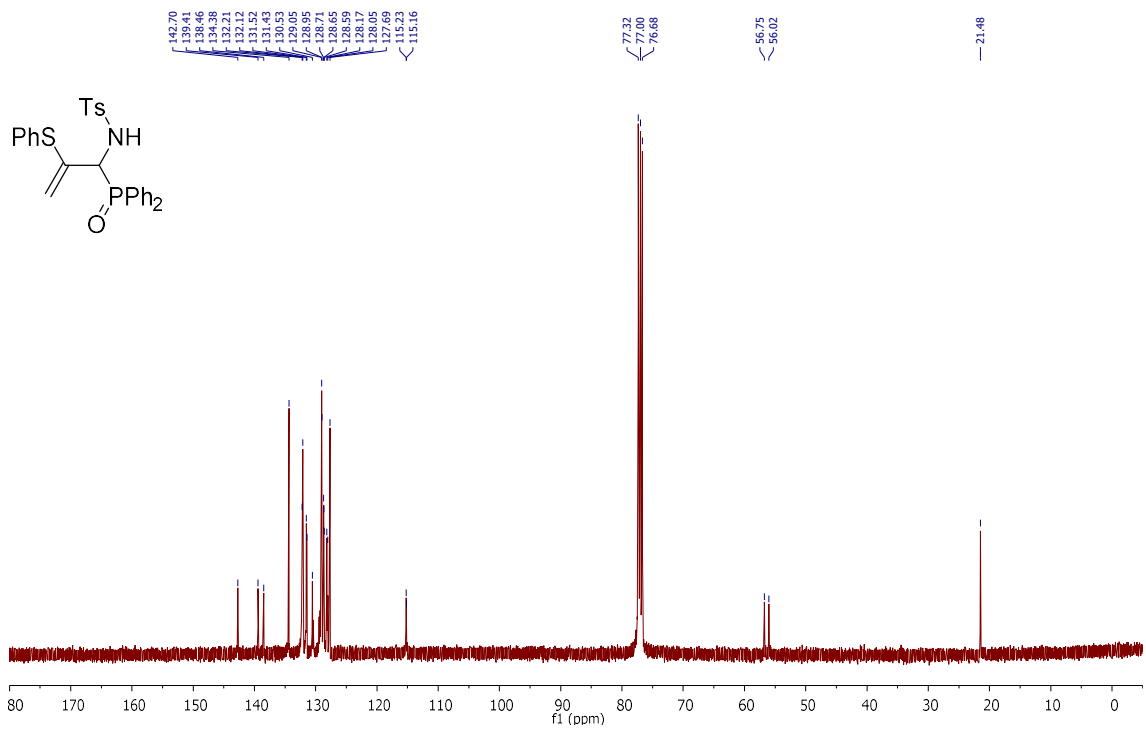
^1H NMR (400 Hz, CDCl_3) of compound **15d** ^{13}C { ^1H } NMR (100 MHz, CDCl_3) of compound **15d**

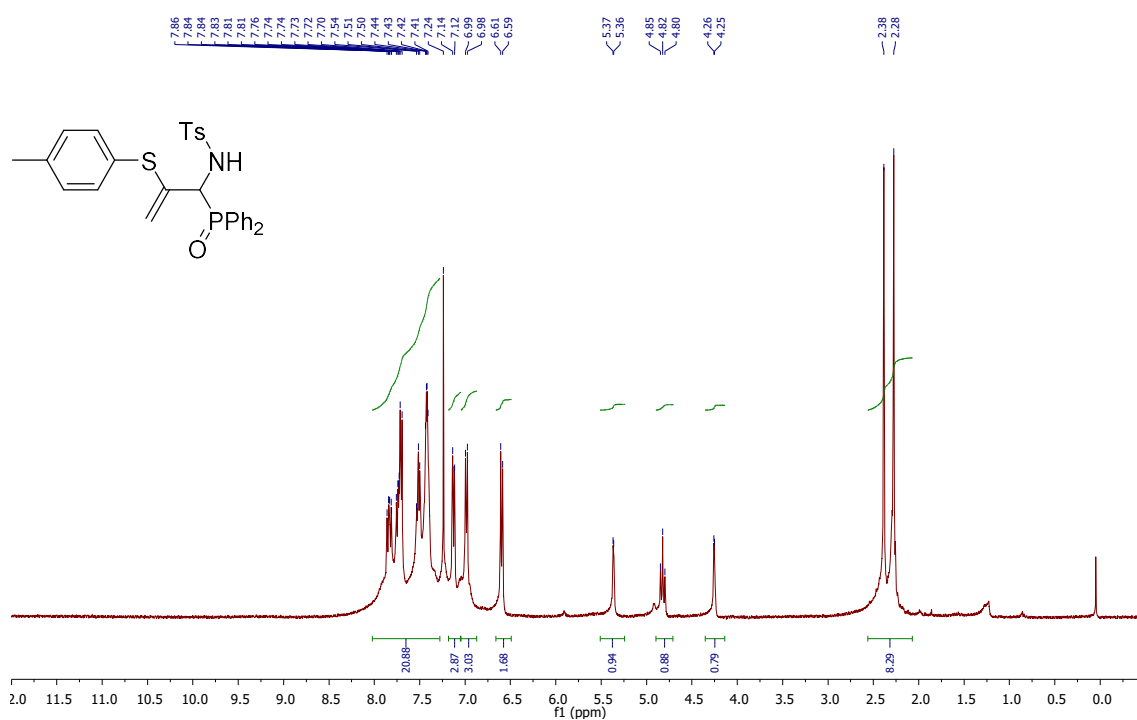
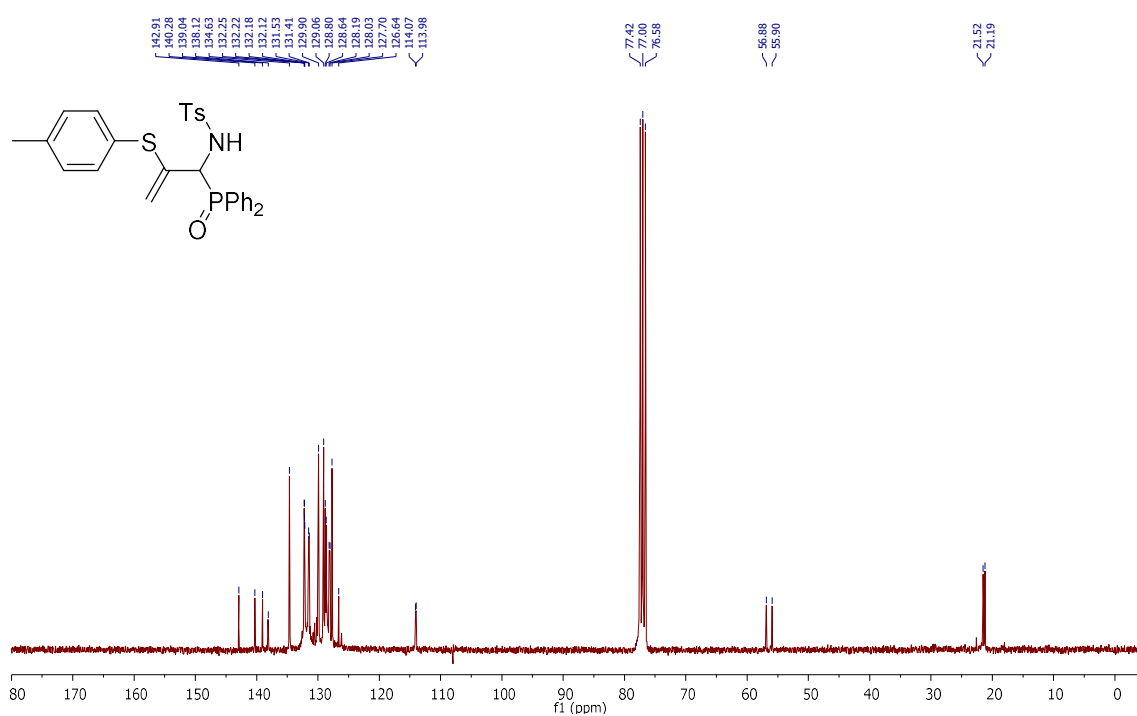
^1H NMR (300 Hz, CDCl_3) of compound **15f**

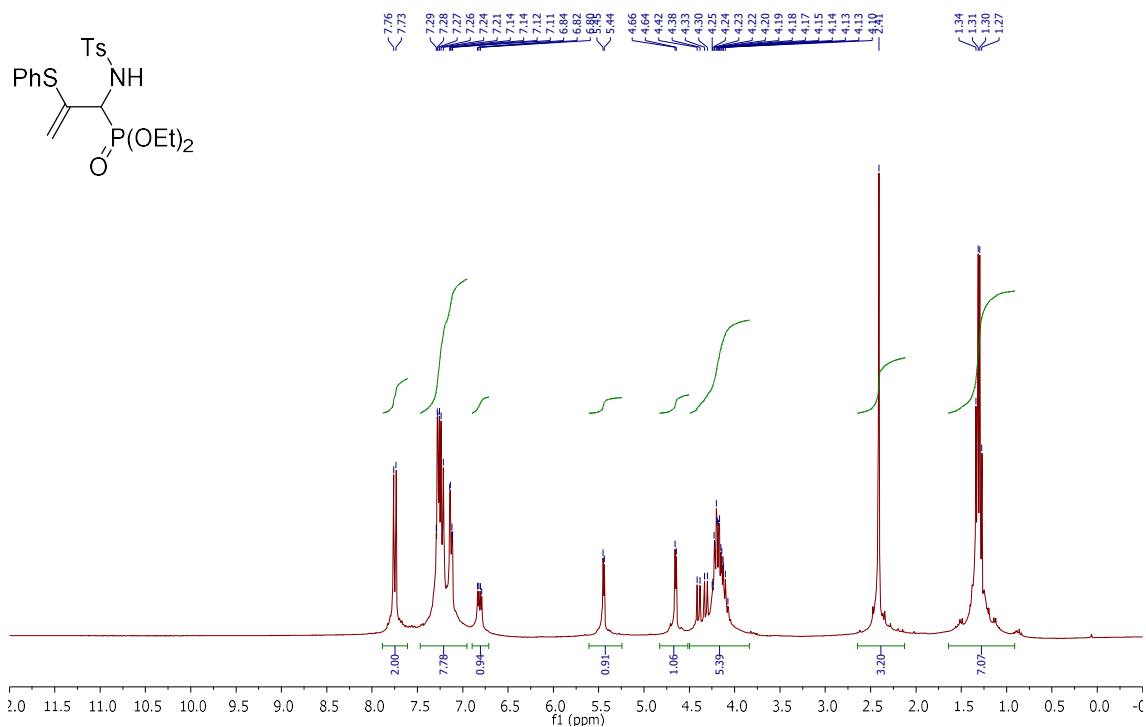
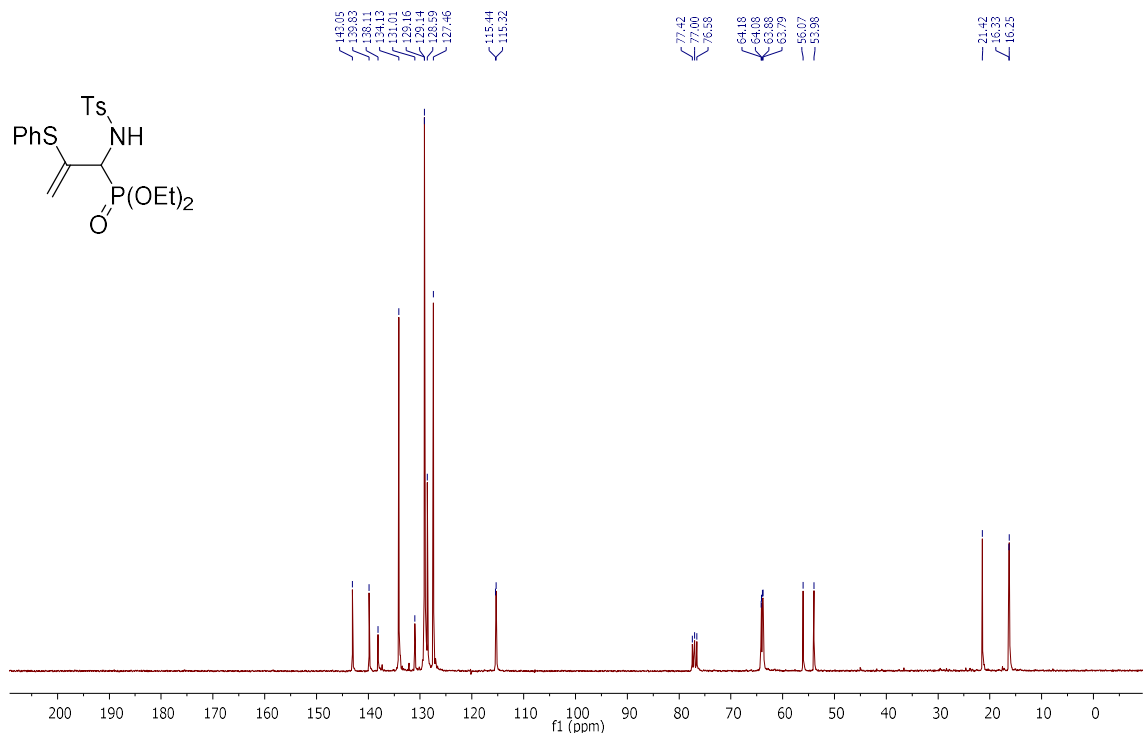


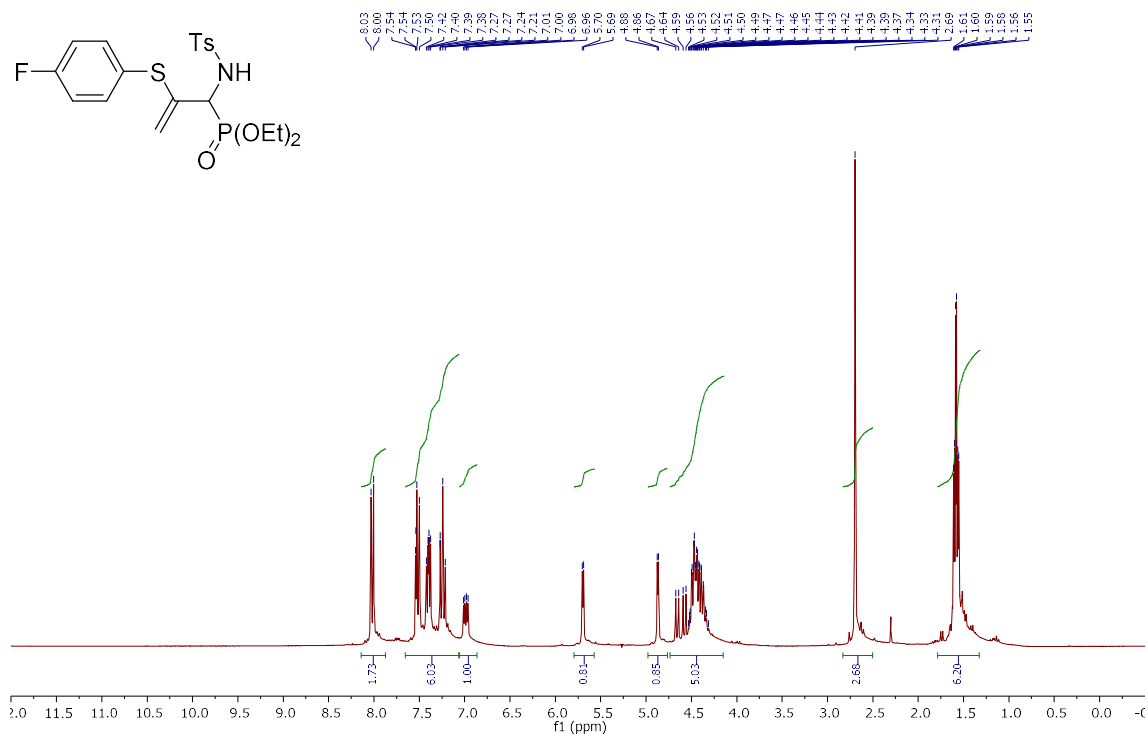
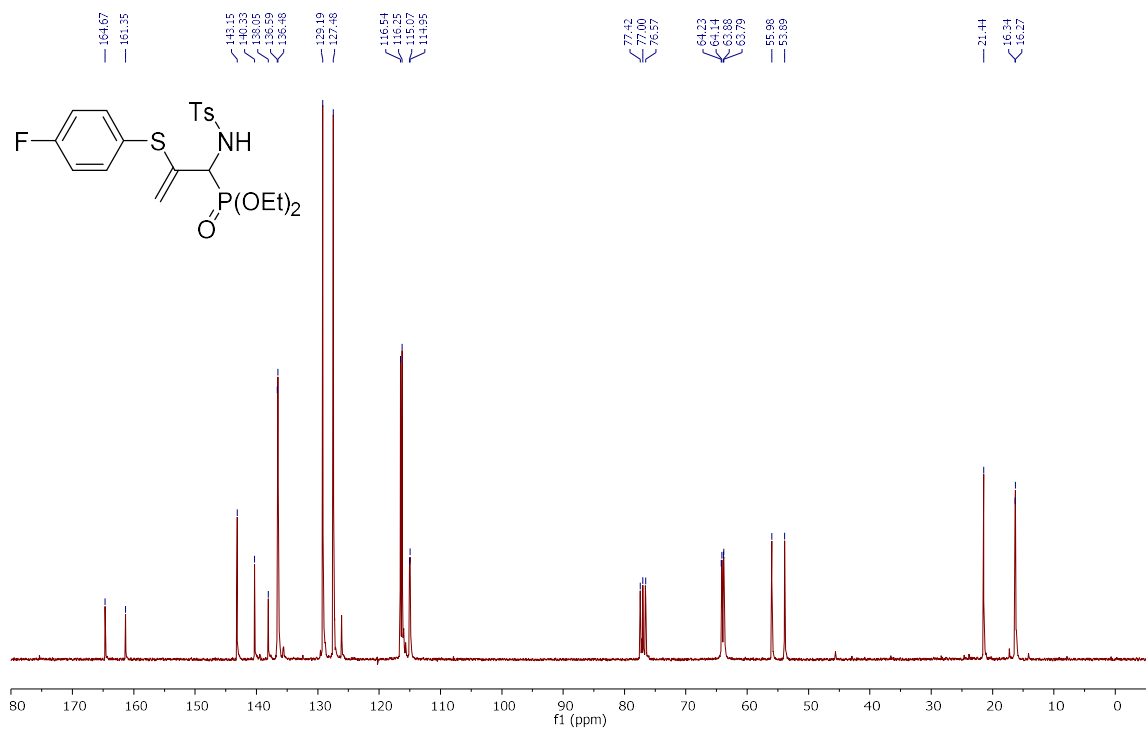
^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **15f**

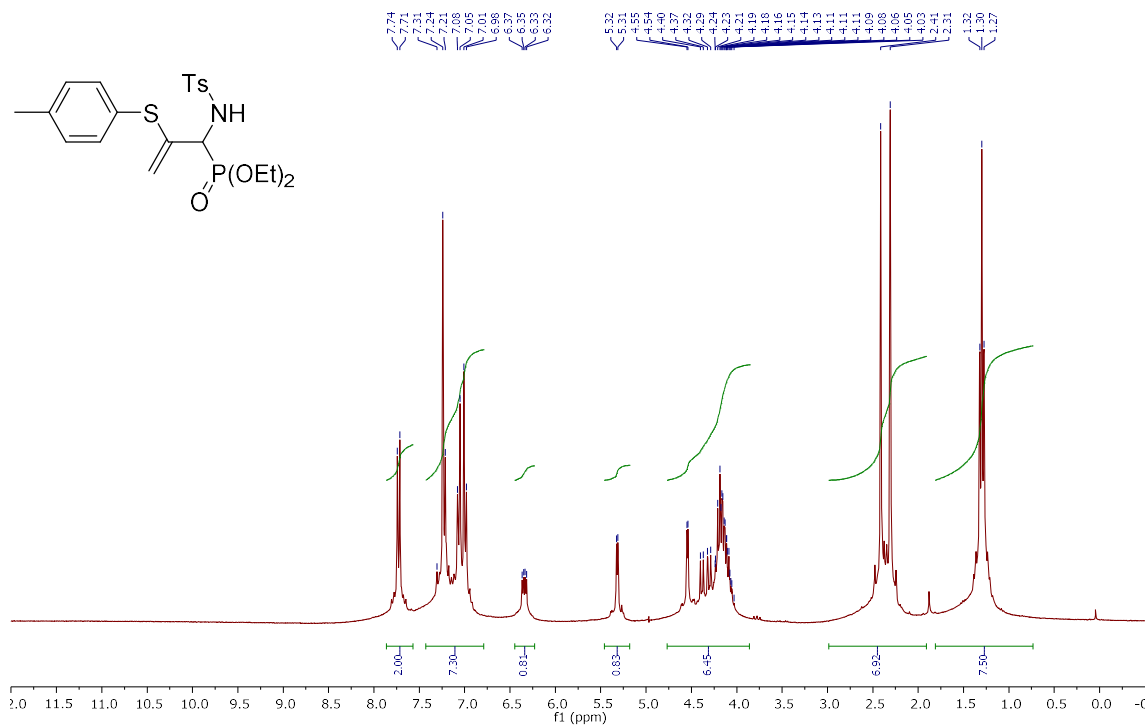


^1H NMR (400 Hz, CDCl_3) of compound **16a** ^{13}C { ^1H } NMR (100 MHz, CDCl_3) of compound **16a**

^1H NMR (400 Hz, CDCl_3) of compound **16b** ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) of compound **16b**

^1H NMR (300 Hz, CDCl_3) of compound **16c** ^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **16c**

^1H NMR (300 Hz, CDCl_3) of compound **16d** ^{13}C { ^1H } NMR (75 MHz, CDCl_3) of compound **16d**

^1H NMR (300 Hz, CDCl_3) of compound **16e** ^{13}C $\{^1\text{H}\}$ NMR (75 MHz, CDCl_3) of compound **16e**