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Stereo- and Regioselective [3+3] Annulation Reaction Catalyzed by Ytterbium: Synthesis of Bicyclic 1,4-Dihydropyridines

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Abstract: An ytterbium catalyzed formal [3+3] cycloaddition of cyclic enamines and α,β-unsaturated ketones catalyzed is reported. The reaction proceeds with a 'head to tail' regioselectivity through a conjugate addition of the enamine moiety followed by an amine-carbonyl condensation. In addition the use of chiral enamines provided a high degree of stereoselectivity, driven by a possible balance between steric and π-stacking effects. The resulting bicyclic 1,4-dihydropyridines were evaluated as antiproliferative agents against A549 (carcinomic human alveolar basal epithelial cell) and SKOV3 (human ovarian carcinoma) human tumor cell lines. Good toxicities were found for some of the compounds against A549 and SKOV3 cell lines, with best IC_{50} values of 0.89 μM for A549 and 6.69 μM for SKOV3, and a very good selectivity was observed towards MRC5 (non-malignant) cell lines.

Keywords: annulation; γ-lactams; homogeneous catalysis; stereoselective; antiproliferative activity

Introduction

1,4-Dihydropyridine skeleton is a privileged scaffold, that occurs in manifold pharmacologically active substances that have been found to possess a broad range of biological actions.[1] Current marketed drugs including the 1,4-dihydropyridine skeleton are mainly calcium channel modulating agents, very adequate for the treatment of hypertension.[2] However, the biological activity of 1,4-dihydropyridine derivatives is wide-ranging and, thus, they have been reported to show multifarious biological activities such as antidiabetic,[3] anticoagulant,[4] antituberculosis,[5] antioxidant,[6] antidyslipidemic,[6] or multidrug resistance reversal.[7] Remarkably, many calcium channel blockers have demonstrated efficacy as anticancer agents, and a number of 1,4-dihydropyridines have been reported as efficient antiproliferative agents in several studies.[8]

From a synthetic point of view, the annulation of enamines with α,β-unsaturated carbonylic compounds provide a convergent and practical approach for the synthesis of nitrogen heterocycles.[9] These reactions are stepwise processes between two bidentate fragments with complementary reactivity that lead to the formation of a pyridine derivative and, due to their non-concerted mechanism, most of the authors refer to them as 'formal [3+3] cycloadditions'.

In this context, a few decades ago, Hsung developed a formal aza-[3+3] annulation of cyclic enamines and vinyliminium ions, generated from α,β-unsaturated aldehydes, for the construction of piper-

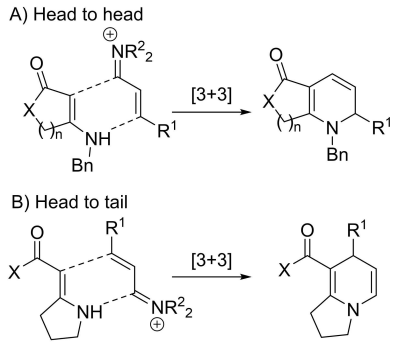
idinyl heterocycles.[10] The process consists on a 'head to head' tandem sequence of Knoevenagel reaction of the enamine and iminium functionalities followed by a pericyclic ring closure of the intermediate 1-azatriene (Scheme 1, A). In addition, a few years later, they reported an unexpected reversal of regiochemistry in a similar reaction, where pyrrolidine-based exocyclic enamines reacted in a 'head to tail' formal [3+3] reaction, by means of an initial conjugate addition of the enamine to the conjugated olefin, followed by a subsequent ring closure through a nucleophilic attack of the enamine nitrogen to the iminium moiety (Scheme 1, B).[11] Remarkably, the reaction proceeds from α,β-unsaturated aldehydes using substoichometric amounts of amine salts, although moderate yields are reported. Moreover, the use of chiral enamines in such reaction has led to a high degree of stereocontrol.[12]

Typical substrates in formal [3+3] cycloaddition reactions are β-enaminones, often cyclic, where the reactivity of the enamine functionality is enhanced due to the presence of the β-carbonyl group (Figure 1).

In the past, we reported the synthesis of 3-amino-1,5-dehydro-1*H*-pyrrol-2-ones through a Brönsted acid catalyzed multicomponent reaction of amines, aldehydes and ethyl pyruvate[13] and, more recently, we have described the enantioselective version if this reaction using chiral phosphoric acids as catalysts.[14] We have also extended this synthetic protocol to the use of phosphorus and fluorine containing aldehydes and pyruvate derivatives[15] and we have described a modified version of this multicomponent process using acetylenedicarboxylates instead of pyruvates.[16] Those substrates are indeed cyclic dehydroaminoacid derivatives and, interestingly, some of those substrates have been identified as p53-MDM2[17] and STAT3[18] inhibitors, which results in a strong antiproliferative activity and, besides, many other pyrrol-2-ones have been described as antitumoral agents.[19]

In our case, we thought that 3-amino-1,5-dehydro-1*H*-pyrrol-2-ones (Figure 1) could be to the same extent, very appropriate substrates for annulation reactions for several reasons. Those molecules have already shown to behave as enamines[15] and, additionally, if substituted substrates are used, the presence of a stereogenic center embedded in a restricted five-membered heterocycle may work as an excellent chiral director, thus inducing a high degree of stereoselectivity in the process.

Taking into account all the considerations mentioned above, herein we report a stereoselective formal [3+3] cycloaddition 3-amino-1,5-dehydro-1*H*-pyrrol-2-ones with conjugated α-ketoesters catalyzed by ytterbium triflate for the synthesis of bicyclic 1,4-dihydropyridines. In addition, due to the potential of 1,4-dihydropyridines as anticancer agents, the evaluation of their antiproliferative activity against lung cancer cells is also described.



Scheme 1. Possible regioselectivies in the formal [3+3] cycloaddition reaction with enamines.

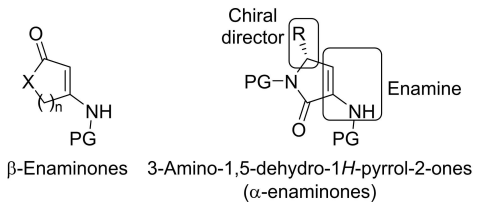


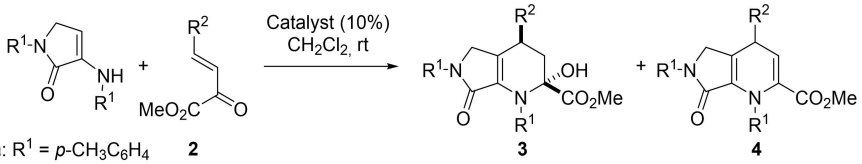
Figure 1. Structure of β-enaminones and 3-amino-1,5-dehydro-1*H*-pyrrol-2-ones.

Results and Discussion

In a first experiment we studied the optimization of the reaction conditions for the annulation reaction of unsubstituted cyclic enamine **1a** (R^1 = *p*-Tolyl) in the presence of a catalytic amount of a Lewis acid (Table 1). As the second partner of the reaction, we chose β,γ-unsaturated α-ketoesters **2**, due to the additional activation of the electrophilic system by the presence of the electron-withdrawing carboxylate group and for our first tests we used β,γ-unsaturated α-ketoester **2a** (R^2 = *p*-$NO_2C_6H_4$) holding a deactivated aromatic group.

Surprisingly, although the reaction did not proceed at all using a Cu (II) salt (Table 1, Entry 1), full conversion of the starting materials was observed after 140 h in the presence of a Cu (I) species and bicyclic tetrahydropyridine **3** was isolated from the reaction. The formation of compound **3** may be explained to arise through a 'head to tail' formal [3+3] cyclization, consisting on an initial conjugate addition of the enamine moiety to β,γ-unsaturated α-ketoester **2a** (R^2 = *p*-$NO_2C_6H_4$), followed by a second nucleophilic attack of the nitrogen to the ketone moiety. The same reaction in the presence of $ZnCl_2$ provided similar results although in lower conversions after 140 h (Table 1, Entry 3). To our surprise, using Ag(OTf) as catalyst, a 30/70 mixture of tetrahydropyridine **3** and bicyclic 1,4-dihydropyridine substrate **4a** (R^2 = *p*-

Table 1. Optimization of the reaction conditions.



1a: R^1 = *p*-$CH_3C_6H_4$ **2** **3** **4**

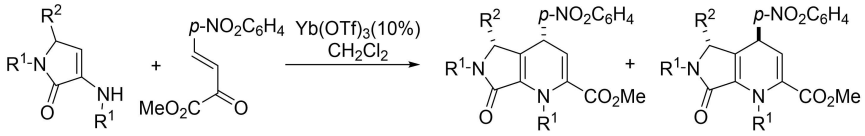
Entry	Cmpd.	Catalyst	R^2	Time (h)	%Conv.	Rate 3/4	%Yield[a]
1	**4a**	$Cu(OTf)_2$	*p*-$NO_2C_6H_4$	140	0	–	n.d.
2	**4a**	$Cu(CH_3CN)_4PF_6$	*p*-$NO_2C_6H_4$	140	100	>99/<1	88.
3	**4a**	$ZnCl_2$	*p*-$NO_2C_6H_4$	140	40	>99/<1	n.d.
4	**4a**	Ag(OTf)	*p*-$NO_2C_6H_4$	120	100	30/70	n.d.
5	**4a**	$NiCl_2(PPh_3)_2$	*p*-$NO_2C_6H_4$	24	100	<1/>99	n.d.
6	**4a**	SmI_2	*p*-$NO_2C_6H_4$	24	100	<1/>99	n.d.
7	**4a**	$In(OTf)_3$	*p*-$NO_2C_6H_4$	24	100	<1/>99	n.d.
8	**4a**	$TiCl_4$	*p*-$NO_2C_6H_4$	24	100	<1/>99	n.d.
9	**4a**	$Sc(OTf)_3$	*p*-$NO_2C_6H_4$	24	100	<1/>99	n.d.
10	**4a**	$YbCl_3$	*p*-$NO_2C_6H_4$	24	30	8/92	n.d.
11	**4a**	$Yb(OTf)_3$	*p*-$NO_2C_6H_4$	1	100	50/50	n.d.
12	**4a**	$Yb(OTf)_3$	*p*-$NO_2C_6H_4$	7	100	<1/>99	84
13	**4b**	$Yb(OTf)_3$	*p*-$CF_3C_6H_4$	7	100	<1/>99	78
14	**4c**	$Yb(OTf)_3$	Ph	14	100	<1/>99	76
15	**4d**	$Yb(OTf)_3$	*p*-Tolyl	14	100	<1/>99	72

[a] Isolated yield.

$NO_2C_6H_4$) was obtained (Table 1, Entry 4). Certainly, compound **4a** is originated by the dehydration of tetrahydropyridine **3**. Next, we tested other Lewis acids as catalyst and, to our delight, complete formation of the formal [3+3] cycloaddition product **4a** was observed after 24 h using $NiCl_2(PPh_3)_2$, SmI_2, $In(OTf)_3$, $TiCl_4$ or $Sc(OTf)_3$ (Table 1, Entries 5–9). The reaction in the presence of $YbCl_3$ provided a mixture 8/92 of **3** and **4a** but in very low conversion after 24 h (Table 1, Entry 10). We attributed this behaviour to the low solubility of $YbCl_3$ in dichloromethane, especially in view of the good result obtained with $Sc(OTf)_3$ if compared with $YbCl_3$ (Table 1, Entry 9 *vs* Entry 10). For this reason, next, we tested $Yb(OTf)_3$ as catalyst in the annulation reaction and a very fast disappearance of the starting materials was observed in this case, obtaining a mixture 50:50 of **3** and **4a** after 1 h (Table 1, Entry 11). However, $Yb(OTf)_3$ was found to be the optimal catalyst for the reaction in dichloromethane at room temperature in 7 h, and bicyclic 1,4-dihydropyridine **4a** (R^2 = *p*-$NO_2C_6H_4$) was isolated as the single product in 84% yield (Table 1, Entry 12). The optimal reaction conditions were next used for the synthesis of several 1,4-dihydropyridines **4**. α-Ketophosphonate **2b** (R^2 = *p*-$CF_3C_6H_4$) bearing a trifluoromethyl substituted deactivated aromatic ring reacted in the same conditions to afford 1,4-dihydropyridine **4b** (Table 1, Entry 13). However, the presence of a simple phenyl substituent in **2c** (R^2 = Ph) or an activated aromatic ring in **2d** (R^2 = *p*-Tolyl) required higher reaction times, and 1,4-dihydropyridines **4c**,**d** were obtained in good yields overnight (Table 1, Entries 14–15).

With these results in hands, next, we moved our next objective to the study of the stereoselectivity of the formal [3+3] annulation reaction, using substituted cyclic enamines **5a–h** (R^1 = *p*-Tolyl), derived from *p*-toluidine (Table 2). Phenyl substituted cyclic enamine **5a** (R^2 = Ph) reacted in full conversion overnight with β,γ-unsaturated α-ketoesters **2a** (R^3 = *p*-$NO_2C_6H_4$) in the presence of a catalytic amount of $Yb(OTf)_3$ to afford in good yields exclusively the *cis* isomer of bicyclic 1,4-dihydropyridine **6a** (Table 2, Entry 1). The reaction was applied to different aromatic-substituted cyclic enamines with a very high degree of diastereoselectivity, using substrates holding aromatic rings bearing electron withdrawing **5b** (R^2 = *p*-$NO_2C_6H_4$) or fluorine substituents **5c** (R^2 = *p*-FC_6H_4), perfluorophenyl **5d** (R^2 = C_6F_5) or even heteroaromatic **5e** (R^2 = 2-thiophene) substituents (Table 2, Entries 2–5). Interestingly, aliphatic-substituted cyclic enamines afforded exclusively the *trans* isomer, as in the case of cyclohexyl or phosphorylmethyl enamines **5f**,**g** (R^2 = Cy, $CH_2P(O)(OEt)_2$) (Table 2, Entries 6–7). However, trifluoromethyl substituted substrates **5h** (R^2 = CF_3) afforded a mixture 70/30 of *cis* and *trans* isomers (Table 2, Entry 8). These results may suggest a possible balance between steric and π-stacking effects into the determination of the stereoselectivity of the process and, possibly, the low yield obtained in the case of cyclohexyl-substituted substrate **6f** is origi-

Table 2. Stereoselective formal [3 + 3] cycloaddition. Scope of the substituents at cyclic enamines **5**.



5 **2a** ***Cis*-6** ***Trans*-6**

Entry	Cmpd.	R^1	R^2	Time (h)	Temp (°C).	*Cis/trans*	%Yield[a]
1	**6a**	*p*-Tolyl	Ph	14	rt	>99/<1	82
2	**6b**	*p*-Tolyl	*p*-$CF_3C_6H_4$	14	rt	>99/<1	90
3	**6c**	*p*-Tolyl	*p*-FC_6H_4	14	rt	>99/<1	79
4	**6d**	*p*-Tolyl	C_6F_5	14	rt	>99/<1	65
5	**6e**	*p*-Tolyl	2-Thiophene	14	rt	>99/<1	66
6	**6f**	*p*-Tolyl	Cy	24	40	<1/>99	19
7	**6g**	*p*-Tolyl	$CH_2P(O)(OEt)_2$	14	rt	<1/>99	87
8	**6h**	*p*-Tolyl	CF_3	14	40	70/30	72
9	**6i**	*p*-$CH_3OC_6H_4$	Ph	14	rt	>99/<1	76
10	**6j**	*o*-FC_6H_4	Ph	14	rt	>99/<1	84
11	**6k**	*p*-BrC_6H_4	Ph	14	40	70/30	91
12	**6l**	*m*-$CF_3C_6H_4$	Ph	56	40	65/35	94

[a] Isolated yield.

nated from a high steric hindrance between the substituents of the reagents in the transition state.

Next, we explored the effects of the substituents at both nitrogen atoms in the formal [3 + 3] cycloaddition reaction, using phenyl substituted cyclic enamines (R^2 = Ph) and β,γ-unsaturated α-ketoester **2a**, (R^1 = *p*-$NO_2C_6H_4$) again as the electrophilic partner. *para*-Anisidine derived enamine **5** (R^1 = *p*-$CH_3OC_6H_4$) showed very good reactivity and pyridine **6i** was obtained at room temperature in very good yield as a single *cis* isomer (Table 2, Entry 9). A similar result was obtained for *ortho*-fluoroaniline derivative **5** (R^1 = *o*-FC_6H_4) with again very good yield and diastereoselectivity (Table 2, Entry 10). Nevertheless, enamines **5** (R^2 = *p*-BrC_6H_4, *m*-$CF_3C_6H_4$), derived from *para*-bromoaniline and *meta*-trifluoromethylaniline, respectively, afforded the *cis* isomer as the major product together with a significate amount of the *trans* isomer (Table 2, Entries 11–12).

Both diastereoisomers of **6h** were isolated by chromatography and fully characterized by FTIR, NMR and HRMS. In order to determine the regiochemistry and the relative configuration of the two stereocenters in **6h**, nuclear Overhauser effect spectroscopy (NOESY) experiments were performed with its two isomers. The *ortho* protons in the nitro-aromatic ring of both isomers of **6h** showed a 0.9% NOE with the olefinic proton at C-3 and about 5% NOE with the proton the at C-4 of the dihydropyridine ring. This is consistent with both possible configurations. However, the CH proton at the 5-membered ring of the major diastereoisomer of **6h** showed 1.5% NOE with the CH at C-4 of dihydropyridine and no NOE with the aromatic ring. This may suggest a *cis* relative configuration of both protons. On the contrary, the CH proton at the 5-membered ring of the minor diastereoisomer of **6h** showed 1.1% NOE effect with the *ortho* protons of the aromatic ring and no interaction at all with the CH of the 6-membered ring, suggesting in this case a *trans* relative configuration (Figure 2).

The relative configuration of the other diastereoisomers obtained was established by analogy of their NMR properties with both isomers of **6h**. For example, NOESY experiments suggest a *cis* configuration in perfluorophenyl substituted bicycle **6d**, where NOE is observed between the two protons

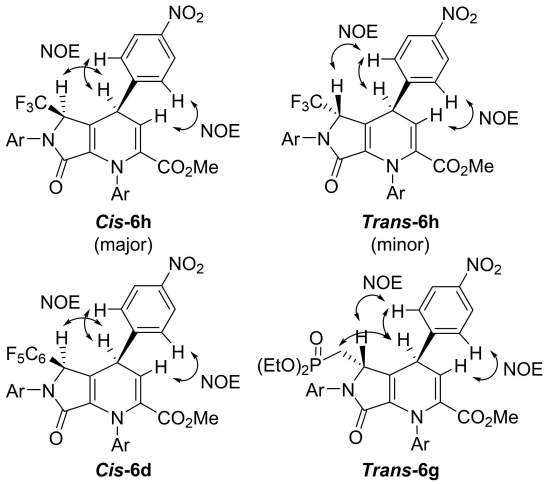


Figure 2. NOE observed in *cis* and *trans* isomers of bicyclic 1,4-dihydropyridines **6**.

configuration in perfluorophenyl substituted bicycle **6d**, where NOE is observed between the two protons at the stereogenic centers. However, a *trans* relative configuration of phosphorated bicycle **6g** may be assigned on the basis of the NOE detected between the methylene group and the CH of the pyridine ring. This proposal is supported by the NOE observed between the CH of the 5-membered ring and the *para*-nitrophenyl group in **6g** (Figure 2). In addition, the absolute configuration of ***trans*-6g** and ***cis*-6i** was unambiguously determined by x-ray diffraction (see Figure 3 and the ESI).

The scope of the reaction was further explored using different substituted β,γ-unsaturated α-ketoesters and α-ketothioesters **2** as bidentate electrophilic substrates (Table 3). The influence of the carboxylic group into the reactivity and the diastereoselectivity of the reaction was first explored. As in the case of methyl carboxylate substituted bicyclic 1,4-dihydropyridine **6a** (R^2 = OMe, R^3 = *p*-$NO_2C_6H_4$), similar substrates derived from benzyl, ethyl or *iso*-propyl carboxylate **6m–o** (R^2 = OBn, OEt, O^{i}Pr, R^3 = *p*-$NO_2C_6H_4$) were obtained in 14 h at room temperature in good yields and as single *cis* diastereoisomers (Table 3, Entries 1–4). However, the replacement of the ester by a thioester group in β,γ-unsaturated α-ketothioester **2** (R^2 = SEt) had a strong influence into the reaction, requiring 24 h in refluxing dichloromethane to obtain bicyclic **6p** in good yield. (Table 3, Entry 5). Nevertheless, a mixture 88/12 of *cis* and *trans* isomers was isolated in this case. We hypothesized that the lower diastereoselectivity and reactivity observed in this case, could be attributed to a lower affinity of thiocarbonyl group to the Lewis acid catalyst, due to its less basicity, and to a higher steric crowding in thioester than in the ester group, in view of the higher Van der Waals radius of sulphur atom versus the oxygen (180 pm vs 152 pm).

Finally, the last point that remains to be addressed in the annulation process is the evaluation of the influence of the substitution in the conjugated double bond of β,γ-unsaturated α-ketoesters **2**. As expected, the presence of other electron poor aromatic substituents at electrophile, such as *para*-trifluoromethylphenyl, yielded bicyclic 1,4-dihydropyridine **6q** (R = *p*-$CF_3C_6H_4$) in very good yield and complete distereoselectivity (Table 3, Entry 6). The reaction also tolerates the presence of a simple phenyl (R^3 = Ph), an electron rich aromatic ring (R^3 = *p*-Tolyl) and even a heteroaromatic substituent (R^3 = 2-thiophene) and, again, very good yields were observed for substrates **6r–u**, that were obtained as a single *cis* isomer (Table 3, Entries 7–10). Other electron withdrawing

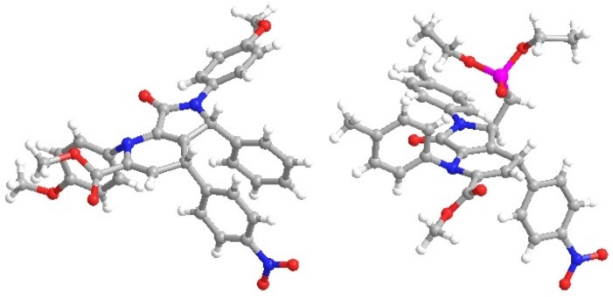
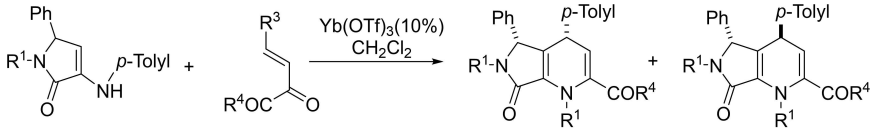


Figure 3. Crystal structure of ***cis*-6i** (left) and **trans-6g** (right) (C, Grey; H, white; N, blue; O, red; P, pink).

Table 3. Stereoselective formal [3 + 3] cycloaddition. Scope of the substituents at α,β-unsaturated carbonyl compound **2**.

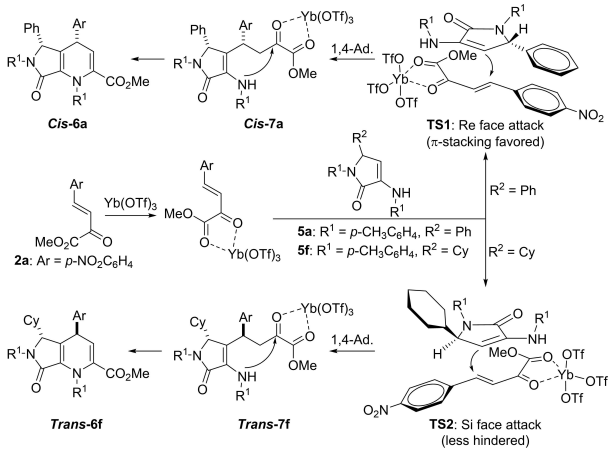


Entry	Cmpd.	R^3	R^4	Time (h)	Temp (°C).	*Cis/trans*	%Yield[a]
1	**6a**	*p*-$NO_2C_6H_4$	OMe	14	rt	>99/<1	82
2	**6m**	*p*-$NO_2C_6H_4$	OBn	14	rt	>99/<1	68
3	**6n**	*p*-$NO_2C_6H_4$	OEt	14	rt	>99/<1	79
4	**6o**	*p*-$NO_2C_6H_4$	O^{i}Pr	14	rt	>99/<1	76
5	**6p**	*p*-$NO_2C_6H_4$	SEt	24	40	88:12	85
6	**6q**	*p*-$CF_3C_6H_4$	O^{i}Pr	14	rt	>99/<1	89
7	**6r**	Ph	O^{i}Pr	14	rt	>99/<1	84
8	**6s**	*p*-Tolyl	OMe	14	rt	>99/<1	63
9	**6t**	*p*-Tolyl	O^{i}Pr	14	rt	>99/<1	76
10	**6u**	2-Thiophene	O^{i}Pr	14	rt	>99/<1	67
11	**6v**	CO_2Et	O^{i}Pr	14	rt	>99/<1	86
12	**6w**	CF_3	O^{i}Pr	14	rt	>99/<1	84
13	**6x**	CH_3	O^{i}Pr	24	rt	85/15	65

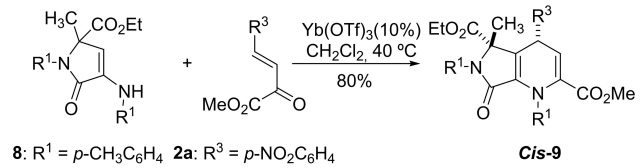
[a] Isolated yield.

substituents such as ethyl carboxylate or trifluoromethyl groups were successfully used in β,γ-unsaturated α-ketoesters **2** ($R^3 = CO_2Et$, CF_3), leading to the formation of substrates **6v**,**w**, exclusively in a *cis* configuration (Table 3, Entries 11–12). To end with the scope, aliphatic substituted bicyclic 1,4-dihydropyridine **6x** ($R^3 = CH_3$) was obtained in good yield under reflux after 24 h as a mixture *cis*/*trans* in a rate 85/15 (Table 3, Entry 13).

The exclusive formation of the *cis* isomer of aromatic substituted bicyclic dihydropyridines **6a–e** may be explained by a π-stacking effect between the aromatic group in cyclic enamine substrate **5a–e** and the *para*-nitrophenyl substituent of β,γ-unsaturated α-ketoester **2a** that would push both substituents to the same orientation. On the contrary, the steric repulsion between the tetrahedral aliphatic groups of cyclic enamines **5f–h** and the aromatic substituent on β,γ-unsaturated α-ketoester **2a** may push both substituents to the opposite orientation, explaining the exclusive formation of the *trans* diastereoisomer (Scheme 2). It should be noted that a thermodynamic equilibrium between the two diastereoisomers is discarded in this case since the separation of both isomers of **6h** and subsequent heating of each pure diastereoisomer, neat or in the presence of a base, an acid or $Yb(OTf)_3$ did not provide mixtures of diastereoisomers.



Scheme 2. Proposed pathway for the formation of either *cis* or *trans* isomers of bicyclic 1,4-dihydropyridines **6**.

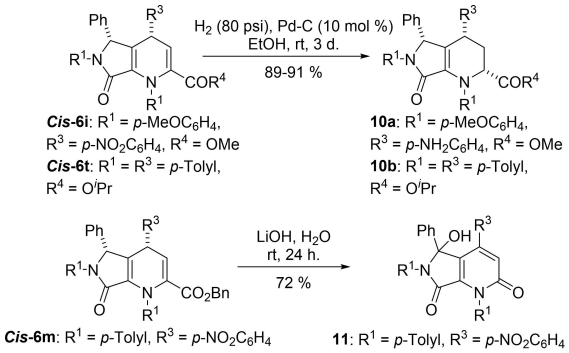


Scheme 3. Stereoselective formal [3+3] cycloaddition of disubstituted cyclic enamine **8**.

In addition, the formal [3+3] cycloaddition of disubstituted cyclic enamine **8** with **2a** in the presence of $Yb(OTf)_3$ under refluxing dichloromethane yields in 24 h exclusively bicyclic dihydropyridine ***cis*-9** (Scheme 3). The fact that in this case a *cis* relative orientation is observed between the carboxyl and the aromatic substituent may be also explained by the prevalence of the π-stacking effect over the steric interaction, thus giving support to our proposed mechanism.

In order to underscore the usefulness of our bicyclic 1,4-dihydropyridine substrates, next we studied some synthetic applications of compounds **6**. First, the hydrogenation of the 1,4-dihydropyridine ring was achieved through the treatment of substrates ***cis*-6i** and ***cis*-6s** under hydrogen atmosphere in the presence of a catalytic amount of palladium on carbon. Under these conditions tetrahydropyridines **10a**,**b** were obtained in high yield and full diastereoselectivity (Scheme 4). As expected, NOESY experiments revealed a relative *cis* configuration of the new stereocenter with respect to the other two chiral carbons (See ESI), which is in agreement with a *syn* addition of hydrogen that, in our case, approaches to the olefinic bond from the opposite face to the substituent at the stereogenic carbons. Remarkably, under those reaction conditions, in the case of *p*-nitrophenyl substituted substrate ***cis*-6i**, the reduction of the nitro to an amino group was also observed.

Moreover, we attempted the hydrolysis of the ester substituent at the 1,4-dihydropyridine ring. However, under the conventional conditions for basic or acidic hydrolyses of esters we failed to obtain the carboxylic acid. The hydrogenolysis of benzylic esters also proved to be unfeasible but, curiously, the treatment of benzylester with lithium hydroxide afforded bicyclic 2-pyridone derivative **11** where apparently the hydrolysis of the ester group is followed by a decarboxylation, a subsequent oxidation and a hydroxylation of the γ-lactam ring (Scheme 4).



Scheme 4. Synthetic applications of bicyclic 1,4-dihydropyridines **6**.

As it has been addressed above 1,4-dihydropyridines have been reported as efficient antiproliferative agents.[8] For this reason and, in order to verify the biological activity of our substrates, *in vitro* cytotoxicity of the bicyclcic 1,4-dihydropyridine derivatives was evaluated by testing their antiproliferative activities against several human cancer cell lines. Cell counting kit (CCK-8) assay was used for the evaluation of growth inhibition. Moreover, non-malignant MRC5 lung fibroblasts were tested for studying selective toxicity[20] and chemotherapeutic doxorubicin was used as reference value.

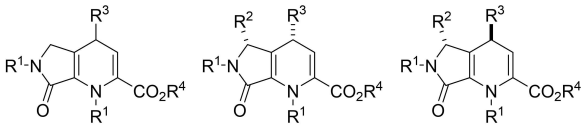
First, the cytotoxicity of 1,4-dihydropyridines **4a–d**, that were prepared from unsubstituted γ-lactams, was evaluated. However, only substrate **4a**, bearing a *para*-nitrophenyl substituent at the 6-membered ring, showed some activity against A549 cell line, with an IC_{50} value of 44.83 ± 2.57 μM. In addition, substrate **4a** proved to have no adverse effect on non-malignant cells (Table 4, Entry 1).

Then, using substrate **4a** as template, we studied the effect of the introduction of substituents at the lactam core into the biological activity of our substrates. Substrate ***cis*-6d** ($R^2 = C_6F_5$) with a perfluorophenyl group at the lactam ring showed good IC_{50} values of 2.04 ± 0.68 and 9.05 ± 1.39 μM on A549 and SKOV3 cell lines, respectively, although some toxicity on non-malignant cells was also obtained in this case (Table 4, Entry 2).

Following with the biological study, next, we evaluated the influence of the amine substituent into the antiproliferative activity of 1,4-dihydropyridines **6**. *p*-Bromophenyl substituted substrate ***cis*-6k** ($R^1 = p$-BrC_6H_4) showed an IC_{50} value of 36.51 ± 5.14 μM on A549 cell line and no toxicity on SKOV3 or MRC5 cell lines (Table 4, Entry 3). Interestingly, both isomers of *m*-trifluorophenyl substituted lactam ***cis*-6l** and ***trans*-6l** ($R^1 = m$-$CF_3C_6H_4$) showed a good toxicity against A549 cell line with IC_{50} values of 7.79 ± 1.59 and 7.33 ± 0.37 μM, respectively, and with a very good selectivity towards SKOV3 and MRC5 cell lines (Table 4, Entries 4–5). It is well known that the introduction of fluorine atoms in the structure of organic compounds, very often it leads to increased or new activities.[21]

The next step in the SAR study was the evaluation of the influence of the ester group. To our delight, *iso*-propyl ester ***cis*-6o** ($R^4 = O^iPr$) proved to be far superior than other derivatives producing IC_{50} values of 10.12 ± 1.03 and 10.71 ± 0.87 μM against A549 and SKOV3 cell lines, respectively, with also a very good selectivity towards non-malignant cells (Table 4, Entry 6). In the last part of the SAR study, we evaluated the influence of the substituent at the 1,4-dihydropyridine ring into the antiproliferative activity of the substrates. For this study we chose *iso*-propyl ester ***cis*-6o** as the headliner and compared its activity with differently substituted bicyclic 1,4-dihydropyridines **6**. Switching the *p*-nitrophenyl by a *p*-trifluoromethylphenyl group at the 1,4-dihydropyridine ring had a slightly positive effect into the activity against A549 cell line and compound ***cis*-6q** ($R^3 = p$-$CF_3C_6H_4$) presented an IC_{50} value of 8.44 ± 0.79 μM. However, the presence of such substituent led to a complete loss of the toxicity in SKOV3 cell line. Compound ***cis*-6q** proved to be very selective against A549 cell line, since an IC_{50} value over 50 μM was observed in non-malignant cells (Table 4, Entry 7). A simple phenyl substituent at the same position in ***cis*-6r** ($R^3 = Ph$) did not prove to be superior to *p*-trifluoromethylphenyl group (Table 4 Entry 8 *vs* Entry 7). Thiophene derivative ***cis*-6u** ($R^3 = 2$-thiophene) showed a strong anti-

Table 4. Antiproliferative activity of selected derivatives **4** and **6**.



4 ***Cis*-6** ***Trans*-6**

Entry	Cmpd.	R^1	R^2	R^3	R^4	IC_{50}(μM) A549	SKOV3	MRC5
1	**4a**	*p*-Tolyl	–	*p*-$NO_2C_6H_4$	OMe	44.83 ± 2.57	> 50	> 50
2	***Cis*-6d**	*p*-Tolyl	Ph	*p*-$NO_2C_6H_4$	OMe	2.04 ± 0.68	9.05 ± 1.39	20.16 ± 0.71
3	***Cis*-6k**	*p*-BrC_6H_4	Ph	*p*-$NO_2C_6H_4$	OMe	36.51 ± 5.14	> 50	> 50
4	***Cis*-6l**	*m*-$CF_3C_6H_4$	Ph	*p*-$NO_2C_6H_4$	OMe	7.79 ± 1.59	> 50	> 50
5	***Trans*-6l**	*m*-$CF_3C_6H_4$	Ph	*p*-$NO_2C_6H_4$	OMe	7.33 ± 0.37	> 50	> 50
6	***Cis*-6o**	*p*-Tolyl	Ph	*p*-$NO_2C_6H_4$	O^iPr	10.12 ± 1.03	10.71 ± 0.87	> 50
7	***Cis*-6q**	*p*-Tolyl	Ph	*p*-$CF_3C_6H_4$	O^iPr	> 50	8.44 ± 0.79	> 50
8	***Cis*-6r**	*p*-Tolyl	Ph	Ph	O^iPr	30.69 ± 1.38	> 50	> 50
9	***Cis*-6u**	*p*-Tolyl	Ph	2-Thiophene	O^iPr	0.89 ± 0.27	11.90 ± 1.20	32.02 ± 3.43
10	***Cis*-6w**	*p*-Tolyl	Ph	CF_3	O^iPr	5.07 ± 0.74	6.69 ± 0.13	15.97 ± 1.11

proliferative activity against A549 cell line and moderate activity against SKOV3 cell line, with IC_{50} values of 0.89 ± 0.27 and 11.90 ± 1.20 μM, respectively. Although compound ***cis*-6u** presented some toxicity against non-malignant cells, with IC_{50} value of 32.02 ± 3.43 μM, its selectivity proved to be far superior against SKOV3 cell line and, particularly, against A549 cell line (Table 4, Entry 9).

Despite the fact that the introduction of a trifluoromethyl group resulted in an good cytotoxic power in ***cis*-6w** ($R^3 = CF_3$) with IC_{50} values of 5.07 ± 0.74 and 6.69 ± 0.13 μM in A549 and SKOV3 cell lines, respectively, this substrate showed a moderate toxicity in MRC5 cell line (Table 4, Entry 10).

Conclusion

In conclusion we report a stereoselective formal [3 + 3] cycloaddition of cyclic enamines and conjugated α-ketoesters catalyzed by ytterbium triflate that affords bicyclic 1,4-dihydropyridines. The generality of the reaction has been put into manifest with a scope comprising 27 substrates. We were able to detect the intermediate of the sterpwise process, resulting from the intitial conjugate addition of the enamine to the α,β-unsaturated ketone, which allowed to determine the reaction mechanism. The results obtained may suggest a possible balance between steric and π-stacking effects into the determination of the stereoselectivity of the reaction. Some synthetic transformations are also reported, including a catalytic hydrogenation that generates a third stereogenic center in a diastereoselective fashion.

In addition, some of the obtained bicyclic 1,4-dihydropyridines showed *in vitro* cytotoxicity inhibiting the growth of human tumor cell lines A549 (carcinomic human alveolar basal epithelial cell) and SKOV3 (human ovarian carcinoma). Thiophene derived substrate ***cis*-6u** showed a good toxicity against A549 and SKOV3 cell lines with IC_{50} of 0.89 ± 0.27 and 11.90 ± 1.20 μM, respectively and a very good selectivity towards MRC5 cell lines.

Experimental Section

Representative procedure for the formal [3 + 3] annulation of γ-lactams 1, 5 or 8 and α,β-unsaturated ketoesters 2. $Yb(OTf)_3$ (62.0 mg, 0.1 mmol) and α,β-unsaturated ketoester **2** (1.1 mmol) were added to a solution of γ-lactam (1 mmol) in CH_2Cl_2 (10 mL) and the reaction mixture was stirred at 40 °C or room temperature for several hours (See Tables 1 and 3 and Supporting Information). The resulting mixture was washed with a saturated solution of $NaHCO_3$ (2×5 mL) and H_2O (2×5 mL) and the combined organic phases were dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by crystallization in diethyl ether, DCM:hexane or by column chromatography (Hexanes /AcOEt) to afford compounds **3**, **4**, **6** and **9**.

Supporting Information Available

Full experimental details, characterization and copies of ^{1}H, ^{13}C, ^{31}P and ^{19}F NMR spectra for new compounds **2**, **3**, **4**, **6**, **9**, **10** and **11** and cif file and thermal ellipsoid plot for **6g**.

Accession Codes

CCDC 1970306 (compound ***trans*-6g**) and CCDC 1581117 (compound ***cis*-6i**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data request/cif or by emailing data request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336033.

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References

[1] Reviews: a) N. Edraki, A. R. Mehdipour, M. Khoshneviszadeh, R. Miri, *Drug Discovery Today* **2009**, *14*, 1058–1066; b) G. Swarnalatha, G. Prasanthi, N. Sirisha, C. Madhusudhana Chetty, *Int. J. Chemtech Res.* **2012**, *3*, 75–89; c) S. A. Khedkar, P. B. Auti, *Mini-Rev. Med. Chem.* **2014**, *14*, 282–290.

[2] a) D. J. Triggle, *Cell. Mol. Neurobiol.* **2003**, *23*, 293–303; b) K. S. Elmslie, *J. Neurosci. Res.* **2004**, *75*: 733–741; c) W. J. Elliott, C. V. S. Ram *J. Clin. Hypertens.* **2011**, *13*, 687–689; d) A. Siddiq, I. Mukhtar, S. G. Baig, *World. J. Pharm. Sci.* **2019**, *8*, 1674–1687.

[3] a) A. K. Ogawa, C. A. Willoughby, R. Bergeron, K. P. Ellsworth, W. M. Geissler, R. W. Myers, J. Yao, G. Harris, K. T. Chapman, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3405–3408; b) R. Gómez-Pliego, J. Gómez-Zamudio, B. Velasco-Bejarano, M. Ibarra-Barajas, R. Villalobos-Molina, *J. Pharmacol. Sci.* **2013**, *122*, 184–192; c) K. Ošiņa, E. Rostoka, J. Sokolovska, N. Paramonova, E. Bisenieks, G. Duburs, N. Sjakste, T. Sjakste, *Cell Biochem. Funct.* **2016**, *34*, 3–6; d) E. Praveenkumar, N. Gurrapu, P. K. Kolluri, V. Yerragunta, B. R. Kunduru, N. J. P. Subhashini, *Bioorg. Chem.* **2019**, *90*, 103056–103064.

[4] R. S. Kumar, A. Idhayadhulla, A. J. Abdul Nasser, J. Selvin, *Eur. J. Med. Chem.* **2011**, *46*, 804–810.

[5] a) M. Zandhaghighi, F. Hadizadeh, S. Soleimanpour, Z. Meshkat, S. A. Rezaee, M. Derakhshan, K. Ghazvini, *J. Infect. Dev. Ctries.* **2017**, *11*, 453–458; b) F. Lentz, N. Reiling, G. Spengler, A. Kincses, A. Csonka, J. Molnár, A. Hilgeroth, *Molecules* **2019**, ***24***, 2873–2884.
[6] A. Kumar, R. A. Maurya, S. Sharma, M. Kumar, G. Bhatia, *Eur. J. Med. Chem.* **2010**, *45*, 501–509.
[7] a) M. Kawase, A. Shah, H. Gaveriy, N. Motohashi, H. Sakagami, A. Varga, J. Molnar, *Bioorg. Med. Chem.* **2002**, *10*, 1051–1055; b) O. Firuzi, K. Javidnia, E. Mansourabadi, L. Saso, A. R. Mehdipour, R. Miri, *Arch. Pharmacal Res.* **2013**, *36*, 1392–1402.
[8] Selected examples: a) Y. S. Lee, M. M. Sayeed, R. D. Wurster, *Mol. Chem. Neuropathol.* **1994**, *22*, 81–95; b) J. Yoshida, T. Ishibashi, M. Nishio, *Eur. J. Pharmacol.* **2003**, *472*, 23–31; c) H. Engi, H. Sakagami, M. Kawase, A. Parecha, D. Manvar, H. Kothari, P. Adlakha, A. Shan, N. I. O. Motohashi, J. Molnar *In Vivo* **2006**, *20*, 637–644; d) F. Foroughinia, K. Javidnia, Z. Amirghofran, A. R. Mehdipour, R. Mir, *J. Pharm. Pharmacol.* **2008**, *60*, 1481–1489; e) L. Bazargan, S. Fouladdel, A. Shafiee, M. Amini, S. M. Ghaffari, E. Azizi, *Cell Biol. Toxicol.* **2008**, *24*, 165–174; f) K. Sirisha, G. Achaiah, V. M. Reddy, *Arch. Pharm. Chem. Life Sci.* **2010**, *343*, 342–352; g) H. A. S. Abbas, W. A. El Sayed, N. M. Fathy, *Eur. J. Med. Chem.* **2010**, *45*, 973–982; h) P. Anaikutti, P. Makam, *Bioorg. Chem.* **2020**, *105*, 104379–104389; i) N. Deswal, A. Shrivastava, M. Summon Hossain, P. Gahlyan, R. Bawa, R. D. Gupta, R. Kumar, *ChemistrySelect* **2021**, *6*, 717–725.
[9] Some reviews: a) J. P. A. Harrity, O. Provoost, *Org. Biomol. Chem.* **2005**, *3*, 1349–1358; b) R. P. Hsung, A. V. Kurdyumov, N. Sydorenko, *Eur. J. Org. Chem.* **2005**, 23–44; c) G. S. Buchanan, J. B. Feltenberger, R. P. Hsung, *Curr. Org. Chem.* **2010**, *7*, 363–401; d) X. Xu, M. P. Doyle, *Acc. Chem. Res.* **2014**, *47*, 1396–1405.
[10] R. P. Hsung, L.-L. Wei, H. M. Sklenicka, C. J. Douglas, M. J. McLaughlin, J. A. Mulder, L. J. Yao, *Org. Lett.* **1999**, *1*, 509–512.
[11] G. S. Buchanan, H. Dai, R. P. Hsung, A. I. gerasyuto, C. M. Schneinebeck, *Org. Lett.* **2011**, *13*, 4402–4405.
[12] H. M. Sklenicka, R. P. Hsung, M. J. McLaughlin, L.-L. Wei, A. I. Gerasyuto, W. B. Brennessel, *J. Am. Chem. Soc.* **2002**, *124*, 10435–10442.
[13] F. Palacios, J. Vicario, D. Aparicio, *Eur. J. Org. Chem.* **2006**, 2843–2850.
[14] X. Del Corte, A. Maestro, E. Martinez de Marigorta, J. Vicario, F. Palacios, *Org. Lett.* **2018**, *20*, 317–320.
[15] X. Del Corte, A. Lopez-Francés, A. Maestro, E. Martínez de Marigorta, F. Palacios, J. Vicario, *J. Org. Chem.* **2020**, *85*, 14369–14383.
[16] X. Del Corte, E. Martínez de Marigorta, F. Palacios, J. Vicario, *Molecules* **2019**, *24*, 2951–2961.
[17] C. Zhuang, Z. Miao, L. Zhu, G. Dong, Z. Guo, S. Wang, Y. Zhang, Y. Wu, J. Yao, C. Sheng, W. Zhang, *J. Med. Chem.* **2012**, *55*, 9630–9642.
[18] W. Huang, Z. Dong, F. Wang, H. Peng, J.-Y. Liu, J.-T. Zhang, *ACS Chem. Biol.* **2014**, *9*, 1188–1196.
[19] Some examples: a) V. O. Koz′minykh, N. M. Igidov, S. S. Zykova, V. E. Kolla, N. S. Shuklina, T. Odegova, *Pharm. Chem. J.* **2002**, *36*, 188–191; b) S. S. Tarnavsky, G. G. Dubinina, S. M. Golovach, S. M. Yarmoluk, *Biopolym. Cell.* **2003**, *19*, 548–552; c) C. Sánchez, L. Zhu, A. F. Braña, A. P. Salas, J. Rohr, C. Méndez, J. A. Salas, *Proc. Nat. Acad. Sci.* **2005**, *102*, 461–466; d) W. Cao, Z. Wang, X. Han, J. Liu, W. Wang, *Bioorg. Med. Chem. Lett.* **2019**, *29*, 126665–126670; e) N. Joksimović, J. Petronijević, N. Janković, D. Baskić, S. Popović, D. Todorović, S. Matić, G. A. Bogdanović, M. Vraneš, A. Tot, Z. Bugarčić, *Bioorg. Chem.* **2019**, *88*, 102954–102968; f) D. Yang, C. Huang, H. Liao, H. Zhang, S. Wu, Q. Zhu, Z.-Z. Zhou, *ACS Omega* **2019**, *4*, 17556–17560; g) X. del Corte, A. López-Francés, A. Maestro, I. Villate-Beitia, M. Sainz-Ramos, E. Martínez de Marigorta, J. L. Pedraz, F. Palacios, J. Vicario, *Pharmaceuticals* **2021**, *14*, 782–803.
[20] R. Recio, E. Vengut-Climent, B. Mouillac, H. Orcel, M. López-Lázaro, J. M. Calderón-Montaño, E. Alvarez, N. Khiar, I. Fernández, *Eur. J. Med. Chem.* **2017**, *138*, 644–660.
[21] a) K. Müller, C. Faeh, F. Diederich, *Science* **2007**, *317*, 1881–1886; b) P. Shah, A. D. Westwell, *J. Enzyme Inhib. Med. Chem.* **2007**, *22*, 527–540; c) J. P. Bégué, D. Bonnet-Delpon, *Bioorganic and Medicinal Chemistry of Fluorine* Wiley: Hoboken, NJ, 2007 pp 1–365.