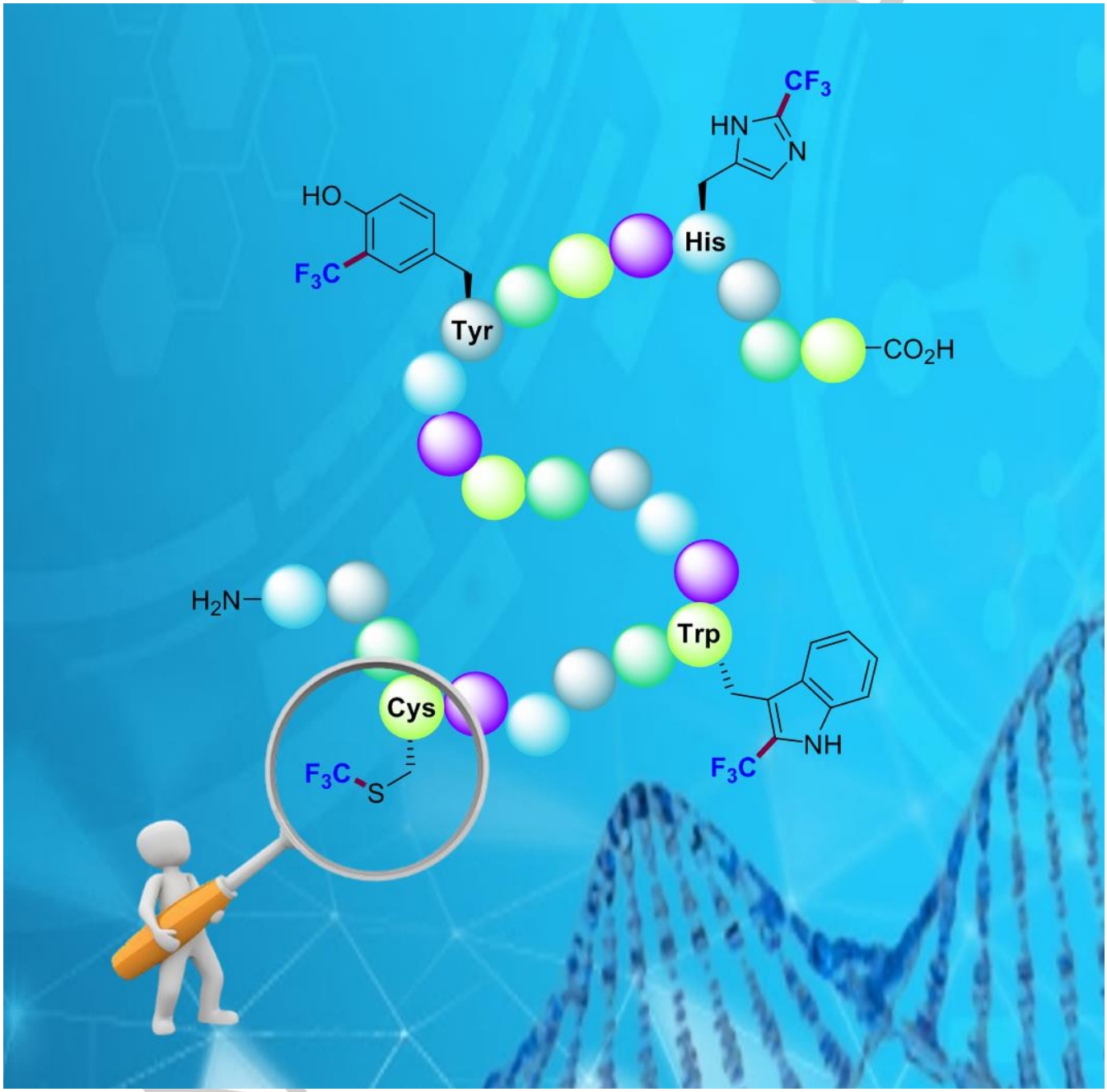
Site-Selective Trifluoromethylation Reactions of Oligopeptides

Itziar Guerrero,[a] and Arkaitz Correa*[a]

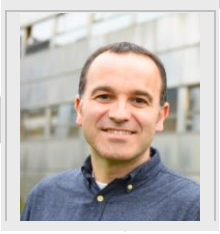
Dedication ((optional))



[a] I. Guerrero, A. Correa
Department of Organic Chemistry I
University of the Basque Country (UPV/EHU)
Joxe Mari Korta R&D Center, Avda. Tolosa 72, 20018 Donostia-San Sebastián (Spain)
E-mail: arkaitz.correa@ehu.eus

Abstract: Site-selective chemical modifications that target proteinogenic amino acid residues complement the methods entailing genetic manipulation, thereby allowing straightforward and rapid access to engineered proteins. The incorporation of the trifluoromethyl group into amino acids within a peptide sequence results in relevant peptidomimetics with unique biomedicinal properties. As a result, the last decade has witnessed the development of a powerful set of protocols toward the selective trifluoromethylation of small-to-medium size peptides and proteins in a late-stage fashion. This minireview seeks to highlight those particularly compelling cases published in the last years.

Itziar Guerrero was born in Donostia in 1993. She received her B.Sc. degree from the University of the Basque Country (UPV/EHU) in 2015. She performed the undegraduate Thesis as ERASMUS student in the University of Strathclyde under the supervision of Prof. Colin Gibson. Later on, she performed the Master Thesis under the supervision of Prof. J. M. Aizpurua at the UPV/EHU. She is currently doing her Ph.D. under the supervision of Dr. Correa at the same university funded by a predoctoral fellowship from the Basque Government.



Arkaitz Correa (Bilbao, 1979) earned his Ph.D. in 2006 under the guidance of Prof. Domínguez at the University of the Basque Country (UPV/EHU). Along that time, he did a short stay with Prof. Feringa at the University of Groningen. Then he conducted several postdoctoral studies at the RWTH Aachen University (Bolm Group) and the ICIQ (Martin Group). In 2014, he started his independent career at the UPV/EHU as a RyC fellow, and he has been recently tenured to Associate Research Professor. He has received the GEQO Young Scientist Award 2019 and the Thieme Chemistry Journal Award 2020.

1. Introduction

Despite their low abundance in naturally occurring molecules, organofluorine compounds have been found widespread applications in pharmaceutical chemistry, agrochemistry, biomedicine and material science.[1] The installation of a fluorine-containing motif into a given molecule typically ushers in a drastic change of its physicochemical properties such as solubility and lipophilicity. As a result, fluorinated compounds commonly exhibit improved metabolic stability, increased bioavailability and cellular membrane permeability.[1] In particular, the trifluoromethyl group stands out among the most privileged moieties of the fluoroalkyl series and a sheer number of drugs and agrochemicals on the worldwide market incorporate such a unique scaffold. The assembly of trifluoromethylated compounds has traditionally entailed the usage of trifluoromethylated synthons upon multi-step and long synthetic sequences. However, with the advent of metal catalysis, the latter have been replaced by more practical transition metal-catalyzed trifluoromethylations of organic halides or organometallic reagents, thus allowing the convenient introduction of the trifluoromethyl group in a modular and late-stage fashion.[2] While conceptually innovative, the use of pre-functionalized compounds represents a serious drawback, which often diminishes the practicality of the methods. In this regard, the last decade has witnessed the outpour of challenging direct trifluoromethylation reactions of otherwise unreactive C–H bonds,[3] thereby spawning a more straightforward and sustainable platform to forge trifluoromethylated compounds.

A variety of trifluoromethylating agents can be utilized in these endeavors, such as electrophilic Umemoto[4] and Togni reagents,[5] nucleophilic Ruppert-Prakash reagent[6] or Langlois reagent,[7] among others (Figure 1). Whereas Langlois reagent is essentially selected to perform radical trifluoromethylation reactions, electrophilic radical trifluoromethyl species can be also generated from Umemoto and Ruppert-Prakash reagents upon reaction with an oxidant or a copper catalyst, respectively, as well as from trifluoromethylsulfonyl chloride (CF_3SO_2Cl) through photoredox catalysis.[8] Despite their common use in academic laboratories, the high price or multi-step synthesis which some of them required have limited the application of some of these CF_3 sources in industrial environments. Accordingly, the development of cost-efficient new trifluoromethyl sources represents a challenging task of capital importance to improve the performance and practicality of existing trifluoromethylation techniques in the context of downstream functionalizations of relevant biomolecules.

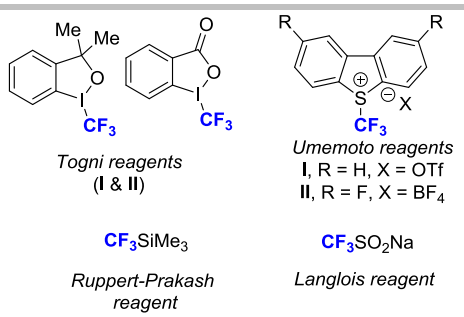


Figure 1. Common trifluoromethylating agents.

The site-selective chemical modification of biomolecules remains an unmet challenge of paramount importance within chemical biology, drug discovery and other disciplines.[9] In this respect, peptides rank among the most important classes of biomolecules due to their wide variety of essential roles in living organisms.[10a,b] Owing to the often improved biological activities and pharmacokinetics of the resulting engineered biomolecules, there is a long-standing interest in the manipulation of oligopeptides and proteins in a tailored manner.[10] In the last years, the development of innovative techniques for the straightforward diversification of amino acid residues in peptide settings has become a prime goal of utmost importance in the burgeoning area of bioconjugation and proteomics.[11] Indeed, the landscape of peptide chemistry is currently experiencing an exponential growth, thereby dramatically expanding the available chemical toolbox and streamlining the rapid assembly of non-proteinogenic α-amino acids and peptides derived thereof.[12] Despite formidable advances in the field, chemoselective diversification of peptides still remains a daunting challenge. With the aim to complement recent reviews on the topic,[12,13] herein we will focus on the latest advances in site-selective late-stage trifluoromethylations of oligopeptides and proteins. The main achievements in the field have been categorized according to the nature of the amino acid residue that undergoes the corresponding fluorination reaction. The objective of this user guide is to offer a rationale assessment of both the advantages and limitations derived from the existing methodologies up to early 2020. Crucial elements of each reactions, including compatibility with other canonical amino acid residues as well as detailed aspects of the underlying mechanism, when applicable, will be commented. The construction of trifluoromethylated peptides based on the use of fluorinated precursors are beyond the scope of this mini-review[14] and only trifluoromethylation protocols occurring within existing peptides or proteins in a late-stage manner will be discussed herein.

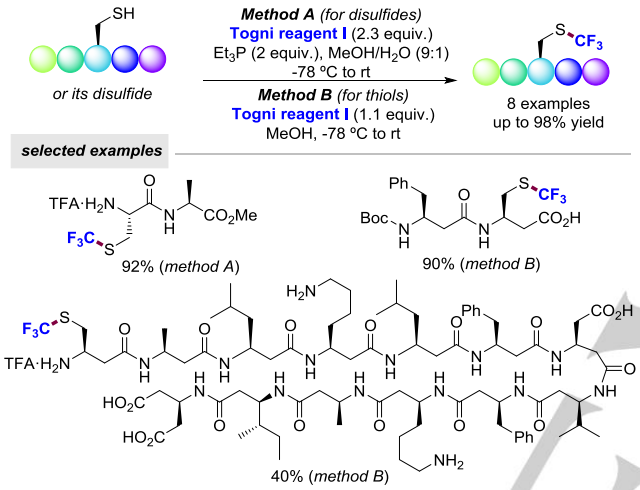
2. Trifluoromethylation of Peptides

The incorporation of the trifluoromethyl group into amino acids within a peptide sequence results in relevant peptidomimetics with unique biomedicinal and pharmaceutical properties.[15] Driven by those outstanding applications, an upsurge of site-selective trifluoromethylations have been lately described to perform the chemoselective modification of peptides and proteins bearing cysteine (Cys), tyrosine (Tyr), histidine (His) and tryptophan (Trp) residues. In the following section, the methods available until early 2020 will be comprehensively analyzed.

2.1. Trifluoromethylation of Cysteine (Cys)

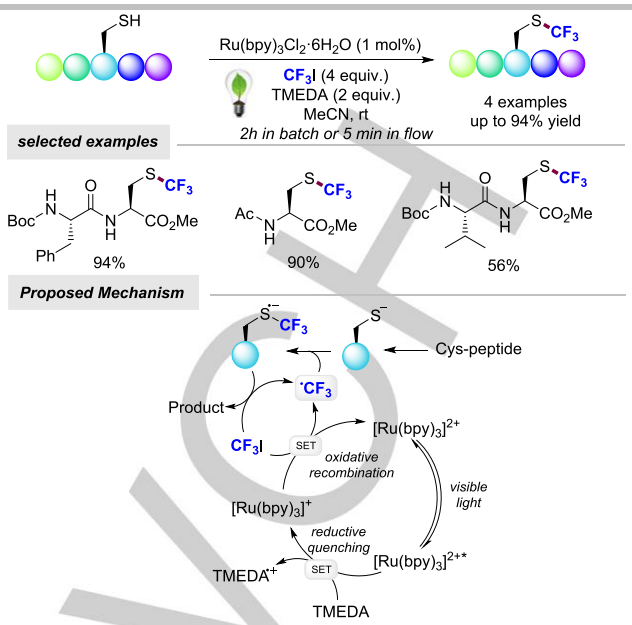
Due to the chemical versatility of its thiol-containing polar, ionizable side chain, Cys occupies a distinguished place in the realm of bioconjugation.[12i] In particular, the substantial nucleophilic character of the thiol motif renders Cys unit an ideal platform for targeted functionalization.[16] Early examples on the *S*-trifluoromethylation of a Cys residue are traced back to 90s, when Soloshonok[17] and Langlois[18] independently utilized CF_3I in liquid ammonia under ultraviolet irradiation and CF_3SO_2Na in combination with *tert*-butyl hydroperoxide (TBHP), respectively, for the assembly of simple trifluoromethylated Cys amino acids. Innovation aside, the latter suffered from serious downsides in terms of operational practicality, and they were only applied to a limited number of simple systems. Later on, Togni and co-workers reported on the synthesis and application of a new family of electrophilic hypervalent iodine reagents for the efficient trifluoromethylation of a vast array of nucleophilic compounds, including simple Cys units.[19] Prompted by the excellent performance of the so-called “Togni reagents”, Togni and Seebach groups unlocked their full synthetic potential and reported jointly on their use for the *S*-trifluoromethylation of more complex α- and β-Cys-containing peptides (of up to 13-residues long).[20a] The exquisite selectivity toward soft nucleophiles (with a high lying highest occupied molecular orbital, HOMO) over hard nucleophiles such as amines or alcohols was harnessed to carry out the process in an aqueous solution of MeOH at cryogenic temperatures (Scheme 1). Remarkably, a high number of amino acid residues such as lysine (Lys), aspartic acid (Asp) or threonine (Thr), among others, were perfectly accommodated and remained intact along the trifluoromethylation reaction. Likewise, the process was applied to unprotected native peptides bearing both free-amino and carboxyl groups at terminal positions of the peptide sequence. Notably, the trifluoromethylation of the cyclic disulfide derivative octreotide[20b] (commercialized by Novartis as

Sandostatin®) of high structural complexity resulted in separable mixtures of ring-opened octapeptides bearing the *S*-trifluoromethylated Cys residues together with the cyclopeptidic octreotide with the trifluoromethylated Trp unit. They further demonstrated that the embedded CF_3 motif could be reduced under Birch reaction conditions, thereby resulting in an overall rapid and efficient Cys/Ala conversion.[20a] The great versatility of hypervalent iodine reagents within the field of bioconjugation was next demonstrated by the selective installation of other perfluoroalkyl groups into simple Cys residues[20c] and Cys-containing complex proteins,[20d] even in the presence of a highly nucleophilic Lys residue.



Scheme 1. *S*-Triluoromethylation of Cys derivatives with Togni reagents.

Building on their successful trifluoromethylation of aromatic thiols upon visible-light induced photocalysis,[21] Noël group developed a convenient technique for the *S*-trifluoromethylation and perfluoroalkylation of Cys residues with CF_3I and other perfluoroalkyl iodide derivatives, respectively.[22] While the process could occur to some extent in the absence of any photocatalyst, the use of $Ru(bpy)_3Cl_2$ resulted in more efficient, cleaner and faster reactions (Scheme 2). Furthermore, the performance of the process in continuous flow notably enhanced the reaction rates, thereby providing higher yields in a shorter reaction time (5 min in flow *vs* 2h in batch). In this respect, the use of micro-reactors enabled a scalable, yet safe, handle of the customarily tedious gaseous CF_3I. The use of a variety of perfluoroalkyl iodides of variable length (C_3 to C_{10}) represented a salient feature of the method due to the higher hydrophobicity of the resulting cysteine derivatives, which may favor their participation in protein interactions within a biological environment.

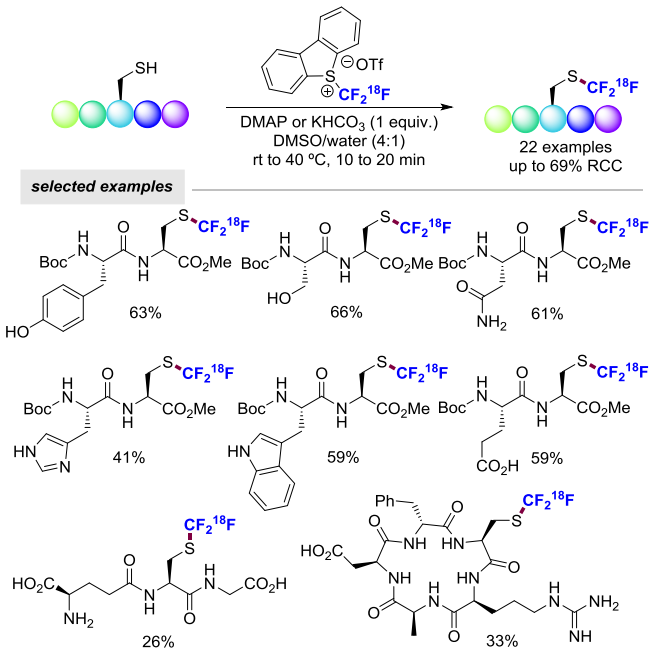


Scheme 2. *S*-Triluoromethylation of Cys derivatives under photoredox catalysis.

The visible-light induced *S*-trifluoromethylation reaction was assumed to proceed through the mechanism disclosed in Scheme 2. Tris(2,2'-bipyridine)ruthenium catalyst was easily excited by an initial blue light absorption and the ensuing photoexcited species $[Ru(bpy)_3]^{2+*}$ was reductively quenched by the organic base TMEDA (sacrificial reducing agent). The so-formed highly reduced $[Ru(bpy)_3]^{+}$ was further oxidized to its ground state upon generation of the transient electrophilic trifluoromethyl radical or its perfluoroalkyl analogue through a Single Electron Transfer (SET) event. The latter eventually reacted with the Cys residue to forge the corresponding S–CF_3 linkage. Based on the obtained quantum yield value, the authors proposed that the formation of the targeted neutral trifloromethylated Cys product could reasonably occur by reaction with the starting fluoroalkyl iodide derivative upon a chain-propagating SET, hence ruling out an alternative chain-terminating SET step with the *in situ* formed $TMEDA^{\bullet+}$.

The tremendous importance of positron emission tomography (PET) as a molecular imaging technique to visualize biochemical processes *in vivo* has certainly fueled ground-breaking discoveries in the selective ^{18}F-radiolabeling of peptides and proteins.[23] As a result, radiolabeled amino acids stand out as an important class of tumor-specific imaging tracers.[24] In 2018 Davis and Gouverneur jointly designed the elegant synthesis and application of the ^{18}F-labeled Umemoto reagent, which was found

highly useful for tagging a vast array of Cys-containing unmodified peptides of high structural complexity in a radioactive fashion.[25]

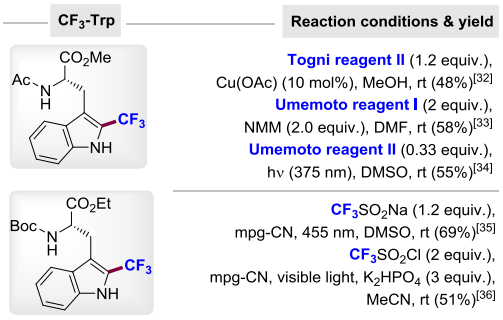


Scheme 3. ^{18}F-Trifluoromethylation of Cys-containing unmodified peptides (RCC = radiochemical conversion).

The radiolabeled reagent was prepared through a halogen exchange ^{18}F-fluorination with ^{18}F-fluoride followed by an oxidative cyclization with oxone and trifluoromethanesulfonic anhydride. Importantly, the ^{18}F-trifluoromethylation process could be accomplished at room temperature in a very short reaction time (10 to 20 min) in the presence of a base such as 4-dimethylaminopyridine (DMAP) or $KHCO_3$. Remarkably, the method was deemed water-tolerant, which represents an added bonus toward its application in protein engineering. The method exhibited an excellent chemoselectivity profile, and boded well with a wide range of Cys-containing oligopeptides bearing Thr, Lys, asparagine (Asn), serine (Ser), arginine (Arg) or glutamic acid (Glu), among others (Scheme 3). The presence of amino acids with aromatic side chains such as His and Trp spawned the target *S*-trifluoromethylated Cys residues as the major products, accompanied with C2-trifluoromethylated products of the corresponding imidazole and indole rings, respectively. Of paramount importance is the successful ^{18}F-radiolabeling of cyclopeptides bearing the biomedicinally relevant Arg-Gly-Asp (RGD) sequence as well as glutathione, extensively used radioligands in PET studies. Preliminary biodistribution experiments evidenced the viability of the resulting ^{18}F-SCF_3 tagged peptides for imaging techniques. In this regard, *in vivo* studies with related ^{18}F-trifluoromethyl Cys residues, prepared from serine-derived cyclic sulfamidates through a nucleophilic ^{18}F-trifluoromethylthiolation followed by a final deprotection reaction, have recently shown high potential as efficient tracers for glioma imaging.[26]

2.2. Trifluoromethylation of Tryptophan (Trp)

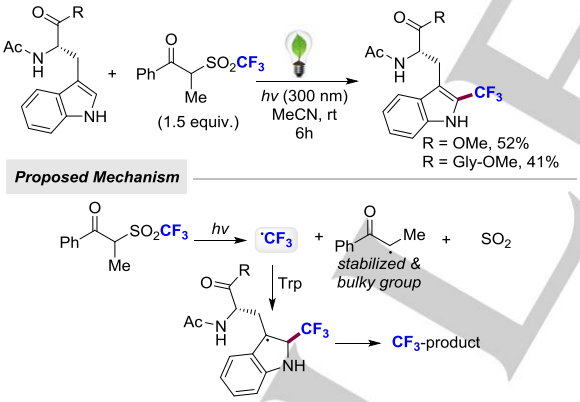
Among the natural proteinogenic amino acids, Trp is the least abundant one with a frequency of about 1.4%;[27] however, around 90% of native proteins contain at least one Trp residue along their primary sequence.[28] Therefore, targeting Trp units represents a promising avenue for bioconjugation of proteins and expanding their functional diversity as biochemical tools and therapeutic agents.[29] The C2-modification of the indole ring of the Trp residue constitutes the most widely explored diversification technique within peptide chemistry to this day.[12a] In fact, a number of chemical transformations performed in simple indole systems, which leveraged its innate chemical reactivity as a highly electron-rich motif, have been lately translated into elegant Trp-modification reactions, including the site-selective C2-trifluoromethylation.[30] The trifluoromethyl radical is a highly electrophilic species with a low-lying singly occupied molecular orbital (SOMO) prone to react with electron-rich motifs such as alkenes or heteroarenes.[8,31] By harnessing the latter reactivity, the C2-trifluoromethylation of simple *N*-protected tryptophan compounds have been achieved upon the use of different trifluoromethyl sources (Scheme 4).



Scheme 4. Trifluoromethylation of simple Trp derivatives.

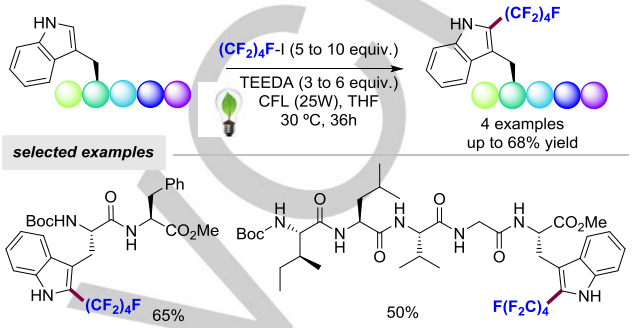
In 2010 Sodeoka and co-workers disclosed an efficient Cu-catalyzed electrophilic trifluoromethylation featuring Togni reagent II as the CF_3 source for the modification of a vast array of indoles, including the Trp derivative Ac-Trp-OMe.[32] More recently, the radical trifluoromethylation of Ac-Trp-OMe has been achieved by using Ummemoto reagents as practical sources for the

production of the corresponding radical trifluoromethyl species by reaction with the electron-donor *N*-methylmorpholine (NMM)[33] or under photo-irradiation conditions.[34] Likewise, the organic semiconductor mesoporous graphitic carbon nitride (mpg-CN) has proven a sustainable, yet highly efficient photocatalyst for the C2-trifluoromethylation of Boc-Trp-OEt with both Langlois reagent[35] and CF_3SO_2Cl.[36] All these pioneering studies in the preparation of the C2-trifluoromethylated Trp unit were mostly focused on the modification of indoles and related heteroarenes, and hence only isolated examples of Trp-based compounds were reported. Likewise, in 2017 Li and co-workers reported a redox-neutral and catalyst-free light-induced innovative protocol to generate CF_3 radicals for the functionalization of a number of (hetero)arenes, including a couple of examples of Trp derivatives.[37] After careful evaluation of different trifluoromethyl compounds, they identified 1-phenyl-2-[(trifluoromethyl)sulfonyl]propan-1-one as the most effective reagent in these endeavors (Scheme 5). Upon light irradiation, the latter was proposed to produce the reactive electrophilic $^{\bullet}CF_3$ species along with a highly stabilized and bulky alkyl radical of comparatively lower reactivity toward the corresponding electron-rich heteroarene. Despite its low-atom economy, the latter non-gaseous trifluoromethyl source may be a rather convenient alternative to handle in pharmaceutical environments.



Scheme 5. Redox-neutral light-induced trifluoromethylation.

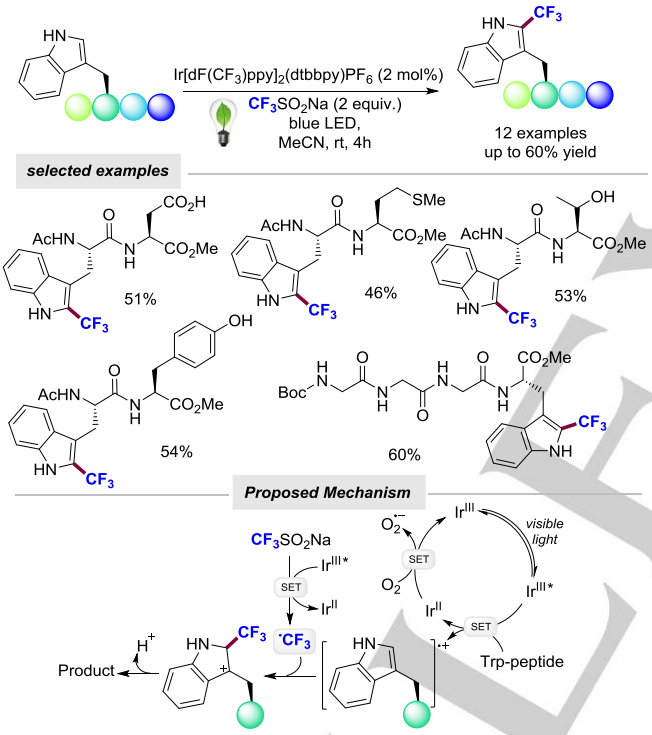
Although not applied toward the installation of the trifluoromethyl group, in 2017 Chen and co-workers devised a C2-selective photochemical perfluoroalkylation of a few Trp-containing peptides (Scheme 6).[38] As highlighted before,[22] the introduction of perfluoroalkyl groups into a given molecule is of great importance because the latter can alter the physicochemical properties of the resulting oligopeptide. The success of the method relied on the use of *N,N,N',N'*-tetraethylethylenediamine (TEEDA), which upon coordination with the starting perfluroalkyl iodide through the formation of halogen bond adducts, enabled the process to occur under low-intensity irradiation (compact fluorescent lamp, UV or even sunlight) in the absence of any expensive photocatalyst, albeit large amount of reagents were required.



Scheme 6. Photochemical perfluoroalkylation of Trp-containing peptides.

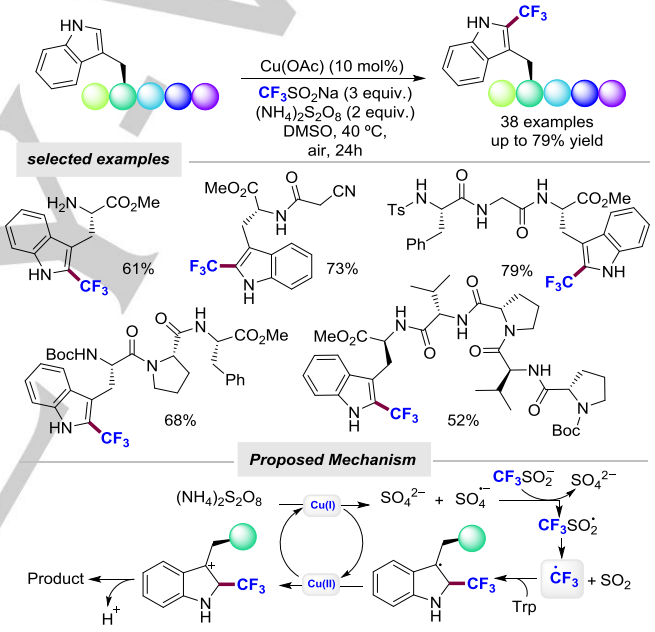
The oxidative decomposition pathway of the sulfinate group, the sulfur congener of a carboxylate, was described by Langlois in the 90s.[7d] However, it took a couple of decades to conceive the so-named Langlois reagent (CF_3SO_2Na) as an easy-to-handle, inexpensive and versatile trifluoromethyl source. The major breakthrough in the field was introduced by Baran and co-workers in 2011, who developed a water-compatible and highly practical C–H trifluoromethylation of numerous heteroarenes showcasing its widespread use in organic synthesis.[39] In that seminal report, the innate chemical reactivity of a variety of nitrogen-containing heterocycles was leveraged toward the ensuing electrophilic radical species derived from the Langlois reagent. Shortly thereafter, the same group reported on the improved yields and selectivity by using zinc sulfinate analogues,[40] which have recently evolved into safe alkyl radical precursors commonly employed by the medicinal chemistry community.[41] Inspired by these fundamental studies, Davis and Gouverneur reported the selective and unprecedented Trp radical trifluoromethylation within native proteins.[42] Based on preliminary residue-specific selectivity assays involving equimolar mixtures of five native amino acid residues (Trp, Phe, Tyr, His and Cys) with the system previously employed by Baran (Langlois reagent/TBHP), they observed the preferential modification of the Trp residue at pH = 6. Remarkably, not even the highly nucleophilic thiol motif within Cys was affected, thus offering new promising perspectives for the challenging site-selective modification of proteins. Accordingly, they succeeded in the trifluoromethylation of biomolecules of high structural complexity containing one Trp unit, such as melittin (a

26-residue peptide) and the protein panthotenate synthetase, two Trp units such as hemoprotein myoglobin, or even the enzyme lysozyme with up to six Trp residues and a high number of Cys units, which remained unaltered. Although apparent adverse effects on the proteins were not detected, the required huge excess amounts of both Langlois reagent and oxidant (200 and up to 25 equivalents, respectively) may raise concerns regarding operational safety and could undermine the atom-economy and sustainability of the process. Related proteins have been trifluoromethylated through laser-initiated processes featuring the use of Langlois reagent in combination with inexpensive H_2O_2.[43] Although of high relevance within the field of preoteomics, site-selectivity was not achieved and the electrophilic $^{\bullet}CF_3$ was found to react with 18 of the 20 common amino acids.



Scheme 7. Ir-catalyzed trifluoromethylation of Trp-containing peptides.

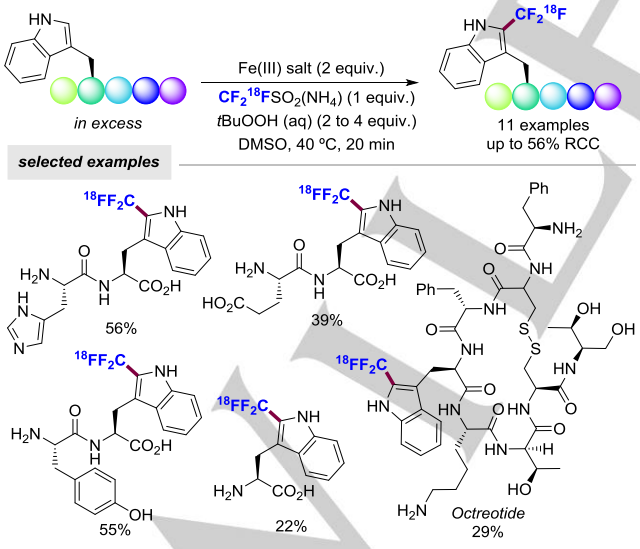
Driven by their experience in photoredox catalysis, Lei and co-workers have recently reported a visible-light induced Ir-catalyzed radical C2-trifluoromethylation of a variety of Trp-containing short peptides with Langlois reagent.[44] Unlike previous examples,[32-36] the method was found applicable for the selective modification of a number of di-, tri- and tetrapeptides bearing amino acids with unprotected alcohols such as Tyr, Thr and Ser, or the carboxyl group such as Asp, among others (Scheme 7). The robustness of the protocol was illustrated by the gram-scale trifluoromethylation of Ac-Trp-OMe and, importantly, an aqueous solvent system could be utilized, albeit higher yields were obtained in neat acetonitrile. Control experiments with radical traps along with electron paramagnetic resonance (EPR) studies supported a radical-radical coupling mechanism. Upon blue LED irradiation, the Ir-based photocatalyst could be excited and further assisted SET events with both the Trp-containing peptide and Langlois reagent to produce a Trp radical cation and $^{\bullet}CF_3$ species, respectively. Subsequent radical-radical coupling would render the corresponding carbocation intermediate, which would eventually furnish the trifluoromethylated compound through a facile deprotonation step. The authors did not entirely rule out an alternate innate radical addition of the transient $^{\bullet}CF_3$ species to the corresponding electron-rich Trp residue.



Scheme 8. Cu-catalyzed trifluoromethylation of Trp-containing peptides.

As part of their interest within the radical diversification of peptides,[45] Correa and co-workers have recently reported a complementary, yet sustainable radical Cu-catalyzed trilfuoromethylation of a myriad of Trp-containing oligopeptides comprising di-, tri-, tetra- and pentapeptides (Scheme 8).[46] This scalable modular coupling was distinguished by its site-specificity, functional group tolerance and full chemoselectivity for Trp residues over other amino acids and heterocyclic units. In fact, the scope could be extended beyond natural peptides and its predictable nature was underpinned by the innate reactivity of the indole scaffold to undergo preferential C–H trifluoromethylation in the presence of other competing sites within medicinally important

heterocycles such as pyridines, 1,2,3-triazoles and tetrahydrofuran. Notably, Trp derivatives housing functional groups such as nitriles as well as biologically relevant cores and active pharmaceuticals, including those derived from fatty acids (palmitic and oleic acid), ibuprofen and aspirine could be accommodated, thereby providing straightforward access to new peptide entities of utmost structural complexity. Whereas the oftentimes oxidizable Met residue remained intact along the process, Cys-containing peptides were found incompatible. Remarkably, HPLC and X-ray analysis verified that the reaction took place with preservation of the α-center chirality of the starting Trp derivatives. The foremost advantage of the method entailed the use of inexpensive and safe $(NH_4)_2S_2O_8$ as the oxidant under an air atmosphere, thus replacing the commonly used TBHP in these endeavors. On the basis of careful control experiments which underpinned the key role of the cost-efficient copper catalyst to obtain the tagged peptides in higher yields, a plausible reaction mechanism was proposed. The Cu(I)-assisted redox decomposition of peroxydisulfate ion would initially deliver the sulfate radical anion $SO_4^{\bullet-}$, which upon reaction with $CF_3SO_2^-$ would afford the active trifluoromethyl radical species. The latter would next undergo innate radical addition at the C2 position of the electron-rich indole motif, and subsequent re-oxidation of the ensuing radical intermediate would afford the targeted trifluoromethylated peptide.



Scheme 9. ^{18}F-trifluoromethylation of Trp-containing native peptides.

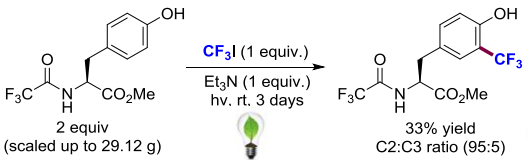
In yet another impressive and elegant display of efficient radiolabeling of peptides,[25] Davis and Gouverneur designed a one-step synthesis of the radiolabeling trifluoromethyl source $[^{18}F]CF_3SO_2NH_4$ and its application to the late-stage ^{18}F-trifluoromethylation of a sheer number of Trp-containing native peptides of high structural complexity[47] and certain Tyr-containing biomolecules (*vida infra*) (Scheme 9). In-depth experimental studies led to the optimal synthesis of the corresponding ^{18}F reagent, which was eventually performed via reaction of 2,2,-difluoro-2-(triphenylphosphonio)acetate (PDFA) with *N*-methylmorpholine·SO_2 (NMM·SO_2) in the presence of $[^{18}F]KF/K_{222}$ in a mixture of propylene carbonate and DMF as solvent at 110 ºC. The resulting radiolabeled trifluoromethyl reagent was purified by reversed phase HPLC and obtained with 99% radiochemical purity. The combination of the latter as the limiting reagent with stoichiometric amounts of an iron(III) salt, either $Fe(NO_3)_3{\cdot}9H_2O$ or $FeCl_3$, and TBHP as oxidant was shown effective in the site-selective and very fast ^{18}F-trifluoromethylation of the Trp unit embedded within a range of oligopeptides, including dipeptides bearing Met, Tyr, His, Lys, Phe, Thr and Asp residues, among others, in radiochemical conversions up to 56%. Importantly, the method boded well for tagging compounds of paramount biological relevance such as endomorphin-1 (a tetrapeptide associated with Alzheimer's disease), melittin or the cyclopeptide octreotide, among others. It is noteworthy that in all cases preferential modification of the Trp residue over other amino acids was observed, albeit the corresponding C2-trifluoromethylated compounds were sometimes accompanied with minor amounts of the corresponding C4 and C7-fluorinated Trp-regioisomers. As an added bonus of the method, they re-optimized several reaction parameters to perform a fully automated radio-synthesis of octreotide labeled within the corresponding Trp unit and further in vivo PET imaging experiments. Without any doubt, this ground-breaking discovery will set the stage for futures advances within the field of radiolabeling of peptides and proteins. In must be emphasized that, unlike some of the existing Trp-diversification techniques,[12] the set of protocols described along this section occur in NH-free Trp-containing compounds and the presence of a directing group into the nitrogen atom of the indole core is not required.

2.4. Trifluoromethylation of Tyrosine (Tyr)

Tyr constitutes a highly abundant non-essential proteinogenic amino acid, which is a prevalent scaffold in a plethora of relevant biologically active compounds such as neurotransmitters and hormones, as well as a versatile precursor to a variety of alkaloids. Accordingly, Tyr-containing derivatives are of widespread use in pharmaceutical, dietary supplements or food additives.[48] Its chemical reactivity is dictated by the electron-rich phenol-

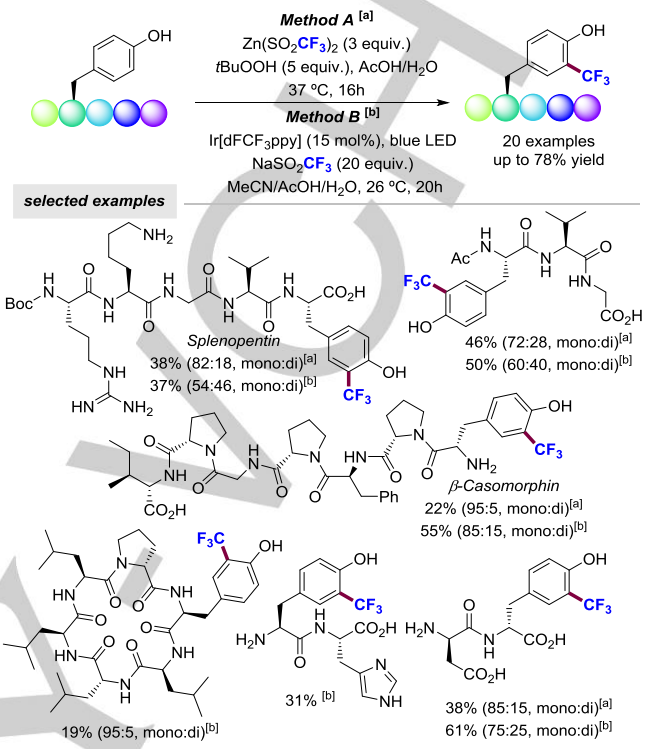
containing aromatic side-chain, which can be tuned by pH control. While the *ortho*-C–H bond can be modified through alkylation, arylation or Mannich-type reactions in acidic or neutral conditions, the modification at the oxygen atom is often achieved under basic environments.[12k] Unlike the trifluoromethylation of the Trp unit, the parent process featuring Tyr-containing peptides has been sparsely explored.

Early reports by Langlois in 1991 demonstrated the feasibility of the radical trifluoromethylation of electron-rich phenol derivatives with large excess of the CF_3SO_2Na/TBHP system and catalytic ammounts of $Cu(OTf)_2$.[7d] However, the very first trifluoromethylation of a Tyr derivative was achieved upon ultraviolet irradiation with CF_3I and triethylamine in MeOH (Scheme 10).[49] Despite the moderate yield and prolonged reaction times, the reaction could be scaled up and performed with almost 30 grams of *N*-trifluoroacetyl-Tyr-OMe as substrate, and the process was favored to occur at C2 over C3 in a 95:5 ratio.



Scheme 10. First C2-trifluoromethylation of a Tyr derivative.

This challenging transformation remained dormant until 2018, when Merck laboratories disclosed a highly reliable radical C–H trifluoromethylation of a vast collection of unprotected Tyr-containing peptides of utmost biological significance with trifluoromethyl sulfinate salts.[50] Slight modifications of the method by Baran featuring the use of $Zn(SO_2CF_3)_2$ and high excess of TBHP in aqueous media led to the selective trifluoromethylation of a variety of unprotected Tyr-containing dipeptides in 35-48% yields (Scheme 11, *method A*). Importantly, potentially reactive residues such as Lys, Asp, Ser and even guanidine-containing Arg, among others, were perfectly accommodated and the trifluoromethylation selectively occurred at the Tyr unit. Conversely, electron-rich Trp unit was shown more reactive, thus being preferentially fluorinated under those conditions, and Cys residue was oxidized to its corresponding disulfide dimer. In order to increase the utility of the method in more intricate settings, careful screening studies allowed the replacement of the chemical oxidant by milder photoredox catalysis, which enabled the use of a large excess of the Langlois reagent for the unprecedented trifluoromethylation of unprotected polypeptides of high structural complexity (Scheme 11, *method B*).

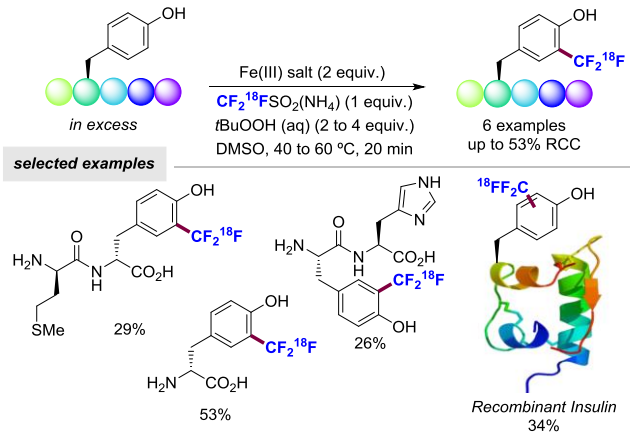


Scheme 11. ^{18}F-trifluoromethylation of Tyr-containing native peptides.

The Ir-based photoredox conditions provided slightly higher yields in the site-selective trifluoromethylation of biomolecules of utmost significance such as cyclopeptides, deltorphin I, angiotensin I and II or β-casomorphin and afforded the mono-trifluoromethylated Tyr derivatives as the major product. The most spectacular application of this labeling technique was the trifluoromethylation of each of the four Tyr residues present in the recombinant human insulin, which comprised two peptide chains of 21 and 30 amino acid length each, linked by three disulfide bonds. Likewise, preliminary studies allowed the installation of other fluoroalkyl motifs at the *ortho* C–H bonds of Tyr units within a cyclopeptide, albeit in low yields, thus paving the way to new opportunities in tagging biomolecules of great significance.

As highlighted before, Davis and Gouverneur have recently reported the synthesis of a novel, yet efficient, radiolabeled trifluoromethylating reagent for the late-stage ^{18}F-trifluoromethylation of electron-rich Trp and Tyr residues embedded into native peptides of great complexity (*vide supra*).[47] As disclosed in Scheme 12, Tyr-containing peptides including His or highly sensitive thioether-containing Met unit could be

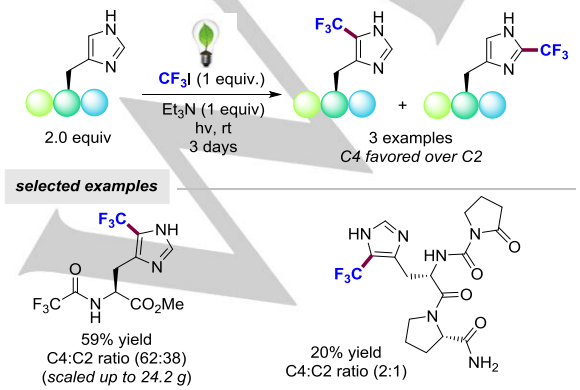
radiolabeled in a late-stage fashion. Importantly, as the method by Parish, Krska and co-workers, unprotected peptides with up to 51 amino acid residues such as recombinant human insulin could undergo the challenging ^{18}F- trifluoromethylation process, thereby enabling the installation of the radiolabeled trifluoromethyl group at the four existing Tyr units. The latter reinforced the potential utility of this radiolabeled trifluoromethyl source within the field of PET-based molecular imaging.



Scheme 12. ^{18}F-Trifluoromethylation of Tyr-containing native peptides.

2.5. Trifluoromethylation of Histidine (His)

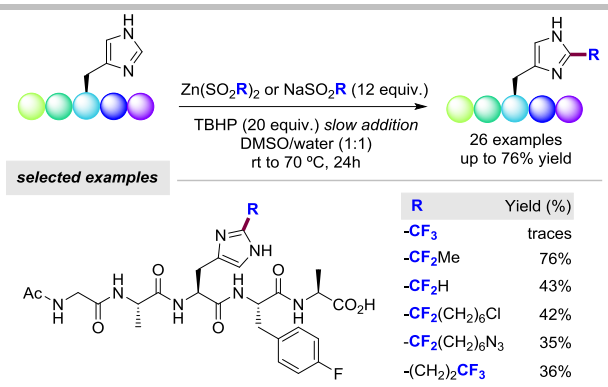
Among the collection of natural amino acids, His is a rather versatile member that plays multiple roles in protein interactions.[51] Given the ionizable nature at physiological conditions of the imidazole group and its high binding ability, His can behave as a powerful hydrogen bond donor or acceptor within a number of enzymatic catalytic reactions in living systems and is prevalent in a vast array of metalloproteins.[52] However, the site-selective modification of such a privileged residue has been overlooked in peptide chemistry and chemoselective modification of His derivatives remain elusive.[12k]



Scheme 13. Early examples of the trifluoromethylation of His derivatives.

Early reports on the trifluoromethylation of simple imidazoles through photocalysis by Cohen and co-workers[53] lay the foundation for the development of further trifluoromethylation reactions of His-containing compounds.[54] The use of CF_3I in MeOH under UV light irradiation led to the radical trifluoromethylation of His derivatives at the C4 site of the imidazole ring accompanied with significant amounts of the C2 trifluoromethylated isomers. Despite the moderate site-selectivity profile, the protocol showed a remarkable ease for scale-up, and more than 20 grams of *N*-CF_3CO-His-OMe underwent the corresponding photochemical trifluoromethylation to provide a separable mixture of C4 and C2 regioisomers in 59% overall yield.

Driven by the last trends in radical chemistry and with the aim to expand the tagging toolkit of peptides, Noisier, Gopalakrishnan and co-workers have recently disclosed a bio-compatible and late-stage efficient alkylation platform with exquisite selectivity toward the C2-alkylation of an impressive His-containing compound library.[55] The key feature relied on the use of the sulfinate salts developed by Baran as powerful radical precursors under oxidative conditions.[41] Although a huge excess of both sulfinate and oxidant was required, the reaction could be performed in a convenient aqueous environment, which rendered the method applicable in a vast array of highly complex bioactive peptides, some of them selected from the AstraZeneca drug-candidate collection. Whereas the trifluoromethylation reaction occurring at the C2-site of the imidazole ring in the presence of either Langlois reagent or its zinc sulfinate analogue was achieved in low yields, an extensive set of fluorinated motifs could be efficiently installed at the C2-site of His residue in a late-stage fashion (Scheme 14). Noteworthy, when using oligopeptides housing more nucleophilic Trp and Tyr residues, the use of the Langlois reagent favored the preferential trifluoromethylation of the latter residues, thus leaving His residue unreactive. Conversely, by switching to $MeCF_2SO_2Na$, the corresponding difluoroethylation took place selectively at the His unit. Likewise, the reactivity pattern wherein aromatic His residue was selectively modified in the presence of Trp and Tyr residues was also observed when utilizing non-fluorinated alkyl radical precursors.[56] Although the mechanism of these trifluoromethylation reactions are not commented in the original publications, a plausible scenario would likely entail a Minisci-type C–H functionalization step of the imidazole core with the ensuing electrophilic radical species.[57]



Scheme 14. Fluoroalkylation of His-containing unprotected peptides.

3. Conclusions and Outlook

The development of useful synthetic tools to label canonical amino acids within a peptide framework for the ultimate modification of proteins in a late-stage, yet site-selective fashion has recently received increasing attention within the realm of chemical biology. In particular, the incorporation of a trifluoromethyl group represents a challenging goal of capital importance due to the improved metabolic stability, bioavailability and cellular membrane permeability of the resulting trifloromethylated compounds. With the advent of modern C–H functionalization, the last decade has witnessed the upsurge of a set of powerful trifluoromethylation techniques, hence enabling the rapid assembly of a new library of trifluoromethylated peptides and proteins in a sustainable manner. The latter commonly harness the inherent reactivity of the side-chains of certain amino acid residues, such as nucleophilic Cys, Tyr, Trp and His, for generating new peptide and protein analogues housing metabolism blocking fluoroalkyl groups. This review details the currently available toolbox of trifluoromethylation reactions, classified by their site-selectivity pattern with proven applicability to oligopeptides of great structural complexity and medicinal relevance. Despite the advances realized, several challenges need to be addressed in order to improve the practicality of the existing protocols and design other innovative strategies. In principle, an ideal protein bioconjugation manifold should be site-selective and proceed under mild reaction conditions, while preserving the native protein structure. Accordingly, the peptide chemistry landscape clearly demands toward bioorthogonal reactivity and compatibility with the 20 natural existing set of unprotected amino acids under physiological reaction conditions and with full tolerance to aqueous environments.

Emerging trends in this rapidly evolving interdisciplinary field are expected to solve the former and the following issues. First, the existing trifluoromethylation reactions are currently limited to a low number of natural amino acids and hence the installation of the trifluoromethyl group into other residues still represents an unmet challenge. Second, some of the reported protocols are not yet broadly applicable to complex peptides and relied on the use of fully protected short-peptide sequences. Third, huge amount of both trifluoromethylating agents or chemical oxidants as well as Ir-based photoredox catalysts are often required, thus diminishing the atom-economy, sustainability and practicality of the protocol. In this respect, the use of electricity remains an unexplored avenue in these endeavors; an electrochemical setup could avoid the use of chemical oxidants and the generation of byproducts derived thereof as well as the use of often expensive photoredox catalysts. Likewise, as commented above, flow-chemistry has already demonstrated its beneficial effects by shortening reaction times and improving the reaction efficiency, therefore it stands out as another challenging technology to be implemented in future late-stage peptide diversifications. Finally, the downstream ^{18}F-radiolabeling of peptides still remains in its infancy and the cutting-edge methods developed by Davis and Gouverneur would clearly set the stage for future discoveries of paramount significance in biomedicinal chemistry. In summary, we anticipate that efforts along these lines could have a significant impact on this field of expertise, which has not reached yet its full synthetic potential and we hope this review could serve as a practical user guide, while encouraging practitioners in the field to search for innovative trifluoromethylation reactions within fascinating peptide settings in the years to come.

Acknowledgements

We are grateful to MINECO (RTI2018-093721-B-I00) and Basque Government (IT1033-16) for financial support. I. G. thanks Basque Government for a predoctoral fellowship.

Keywords: site-selectivity • trifluoromethylation • radical chemistry • late-stage peptide modification • bioconjugation

[1] a) Y. Zhou, J. Wang, Z. Gu, S. Wang, W. Zhu, J. L. Aceña, V. A. Soloshonok, K. Izawa, H. Liu, *Chem. Rev.* **2016**, *116*, 422-518; b) E. P. Gillis, K. J. Eastman, M. D. Hill, D. J. Donnelly, N. A. Meanwell, *J. Med. Chem.* **2015**, *58*, 8315-8359; c) J. Wang, M. Sánchez-Roselló, J. L. Aceña, C. del Pozo, A. E. Sorochinsky, S. Fustero, V. A. Soloshonok, H. Liu, *Chem. Rev.* **2014**, *114*, 2432-2506; d) S. Purser, P. R. Moore, S. Swallow, V. Gouverneur, *Chem. Soc. Rev.* **2008**, *37*, 320-330; e) K. Müller, C. Faeh, F. Diederich, *Science* **2007**, *317*, 1881-1886; f) P. Jeschke, *ChemBioChem* **2004**, *5*, 570-589.

[2] a) G.-b. Li, C. Zhang, C. Song, Y.-d. Ma, *Beilstein J. Org. Chem.* **2018**, *14*, 155-181; b) A. A. Berger, J.-S. Völler, N. Budisa, B. Koksch, *Acc.*

Chem. Res. **2017**, *50*, 2093-2103; c) T. Liang, C. N. Neumann, T. Ritter, *Angew. Chem. Int. Ed.* **2013**, *52*, 8214-8264; *Angew. Chem.* **2012**, *125*, 8372-8423; d) T. Liu, Q. Shen, *Eur. J. Org. Chem.* **2012**, 6679-6687; e) T. Furuya, A. S. Kamlet, T. Ritter, *Nature* **2011**, *473*, 470-477; f) D. A. Nagib, D. W. C. MacMillan, *Nature* **2011**, *480*, 224-228; g) O. A. Tomashenko, V. V. Grushin, *Chem. Rev.* **2011**, *111*, 4475-4521.

[3] a) C. Alonso, E. Martínez de Marigorta, G. Rubiales, F. Palacios, *Chem. Rev.* **2015**, *115*, 1847-1935; b) H. Liu, Z. Gu, X. Jiang, *Adv. Synth. Catal.* **2013**, *355*, 617-626.

[4] a) A. Prieto, O. Baudoin, D. Bouyssi, N. Monteiro, *Chem. Commun.* **2016**, *52*, 869-881; b) T. Umemoto, *J. Fluorine Chem.* **2014**, *167*, 3-15; c) T. Umemoto, *Chem. Rev.* **1996**, *96*, 1757-177.

[5] J. Charpentier, N. Früh, A. Togni, *Chem. Rev.* **2015**, *115*, 650-682.

[6] a) X. Liu, C. Xu, M. Wang, Q. Liu, *Chem. Rev.* **2015**, *115*, 683-730; b) G. K. S. Prakash, A. K. Yudin, *Chem. Rev.* **1997**, *97*, 757-786; c) I. Ruppert, K. Schlich, W. Volbach, *Tetrahedron Lett.* **1984**, *25*, 2195-2198.

[7] a) Q. Lefebvre, *Synlett* **2017**, *28*, 19-23; b) C. Zhang, *Adv. Synth. Catal.* **2014**, *356*, 2895-2906; c) J.-A. Ma, D. Cahard, *J. Fluorine Chem.* **2007**, *128*, 975-996; d) B. R. Langlois, E. Laurent, N. Roidot, *Tetrahedron Lett.* **1991**, *32*, 7525-7528.

[8] A. Studer, *Angew. Chem. Int. Ed.* **2012**, *51*, 8950-8958; *Angew. Chem.* **2012**, *124*, 9082-9090.

[9] a) J. Ohata, S. C. Martin, Z. T. Ball, *Angew. Chem. Int. Ed.* **2019**, *58*, 6176-6199; *Angew. Chem.* **2019**, *131*, 6238-6264; b) P. G. Isenegger, B. G. Davis, *J. Am. Chem. Soc.* **2019**, *141*, 8005-8013; c) E. M. Sletten, C. R. Bertozzi, *Angew. Chem. Int. Ed.* **2009**, *48*, 6974-6998; *Angew. Chem.* **2009**, *111*, 7108-7133.

[10] a) J. D. Bucheit, L. G. Pamulapati, N. Carter, K. Malloy, D. L. Dixon, E. M. Sisson, *Diabetes Technol. Ther.* **2020**, *22*, 10-18; b) J. B. Hedges, K. S. Ryan, *Chem. Rev.* **2020**, *120*, 3161-3209; c) R. J. Malonis, J. R. Lai, O. Vergnolle, *Chem. Rev.* **2020**, *120*, 3210-3229; d) D. C. Blakemore, L. Castro, I. Churcher, D. C. Rees, A. W. Thomas, D. M. Wilson, A. Wood, A. *Nat. Chem.* **2018**, *10*, 383-394; e) A. Henninot, J. C. Collins, J. M. Nuss, *J. Med. Chem.* **2018**, *61*, 1382-1414; f) F. Albericio, H. G. Kruger, *Future Med. Chem.* **2012**, *4*, 1527-1531.

[11] a) H. R. Reese, C. C. Shanahan, C. Proulx, S. Menegatti, *Acta Biomater.* **2020**, *102*, 35-74; b) C. Zhang, E. V. Vinogradova, A. M. Spokoyny, S. L. Buchwald, B. L. Pentelute, *Angew. Chem. Int. Ed.* **2019**, *58*, 4810-4839; *Angew. Chem.* **2019**, *131*, 4860-4892; c) E. A. Hoyt, P. M. S. D. Cal, B. L. Oliveira, G.J. L. Bernardes, *Nat. Rev. Chem.* **2019**, *3*, 147-171; d) S. Bondalapati, M. Jbara, A. Brik, *Nat. Chem.* **2016**, *8*, 407-418; e) C. D. Spicer, B. G. Davis, *Nat. Commun.* **2014**, *5*, 4750-4753.

[12] For selected reviews, see: a) H. Gruß, N. Sewald, *Chem. Eur. J.* **2020**, *26*, 5328-5340; b) D. G. Rivera, G. M. Ojeda-Carralero, L. Reguera, E. V. Van der Eycken, *Chem. Soc. Rev.* **2020**, *49*, 2039-2059; c) C. Sohrabi, A. Foster, A. Tavassoli, *Nat. Rev. Chem.* **2020**, *4*, 90-101; d) C. Bottecchia, T. Noël, *Chem. Eur. J.* **2019**, *25*, 26-42; e) P. Servatius, L. Junk, U. Kazmaier, *Synlett* **2019**, *30*, 1289-1302; f) M. San Segundo, A. Correa, *Synthesis* **2018**, *50*, 2853-2866; g) T. Brandhofer, O. García Mancheño, *Eur. J. Org. Chem.* **2018**, 6050-6067; h) W. Wang, M. M. Lorion, J. Shah, A. R. Kapdi, L. Ackermann, *Angew. Chem. Int. Ed.* **2018**, *57*, 14700-14717; *Angew. Chem.* **2018**, *130*, 14912-14930; i) S. Mondal, S. Chowdhury, *Adv. Synth. Catal.* **2018**, *360*, 1884-1912; j) L. R. Malins, *Curr. Opin. Chem. Biol.* **2018**, *46*, 25-32; k) J. N. deGruyter, L. R. Malins, P. S. Baran, *Biochemistry* **2017**, *56*, 3863-3873; l) G. He, B. Wang, W. A. Nack, G. Chen, *Acc. Chem. Res.* **2016**, *49*, 635-645; m) A. M. Metz, M. C. Kozlowski, *J. Org. Chem.* **2015**, *80*, 1-7; n) A. F. M. Noisier, M. A. Brimble, *Chem. Rev.* **2014**, *114*, 8775-8806.

[13] W. Zhu, J. Wang, S. Wang, Z. Gu, J. L. Aceña, K. Izawa, H. Liu, V. A. Soloshonok, *J. Fluorine Chem.* **2014**, *167*, 37-54.

[14] For example, see: a) J. Moschner, V. Stulberg, R. Fernandes, S. Huhmann, J. Leppkes, B. Koksch, *Chem. Rev.* **2019**, *119*, 10718-10801; b) J. Simon, J. Pytkowicz, N. Lensen, G. Chaume, T. Brigaud, *J. Org. Chem.* **2016**, *81*, 5381-5392; c) E. N. G. Marsh, *Acc. Chem. Res.* **2014**, *47*, 2878-2886; d) J. L. Aceña, A. E. Sorochinsky, V. A. Soloshonok, *Synthesis* **2012**, *44*, 1591-1602; e) L. Merkela, N. Budisa, *Org. Biomol. Chem.* **2012**, *10*, 7241-7261; f) X.-L. Qiu, F.-L. Qing, *Eur. J. Org. Chem.* **2011**, 3261-3278; g) R. Smits, C. D. Cadicamo, K. Burger, B. Koksch, *Chem. Soc. Rev.* **2008**, *37*, 1727-1739; h) M. R Seyedsayamdost, C. S Yee, J. Stubbe, *Nat. Protoc.* **2007**, *2*, 1225-1235.

[15] a) M. Cheng, C. Guo, M. L. Gross, *Angew. Chem. Int. Ed.* **2020**, *59*, 5880-5889; *Angew. Chem.* **2020**, *132*, 5932-5942; b) N. Budisa, W. Wenger, B. Wiltschi, *Mol. BioSyst.* **2010**, *6*, 1630-1639; c) C. Jäckel, M. Salwiczek, B. Koksch, *Angew. Chem. Int. Ed.* **2006**, *45*, 4198-4203; *Angew. Chem.* **2006**, *118*, 4305-4309; d) M. Zanda, *New J. Chem.* **2004**, *28*, 1401-1411; e) Y. Tang, G. Ghirlanda, W. A. Petka, T. Nakajima, W. F. DeGrado, D. A. Tirrell, *Angew. Chem. Int. Ed.* **2001**, *40*, 1494-1496; *Angew. Chem.* **2001**, *113*, 1542-1544.

[16] a) S. B. Gunnoo, A. Madder, *ChemBioChem* **2016**, *17*, 529-553; b) J. M. Chalker, G. J. L. Bernardes, Y. A. Lin, B. G. Davis, *Chem. Asian J.* **2009**, *4*, 630-640.

[17] V. Soloshonok, V. Kukhar, Y. Pustovit, V. Nazaretian, *Synlett* **1992**, 657-658.

[18] B. Langlois, D. Montègre, N. Roidot, *J. Fluorine Chem.* **1994**, *68*, 63-66.

[19] I. Kieltsch, P. Eisenberger, A. Togni, *Angew. Chem. Int. Ed.* **2007**, *46*, 754-757; *Angew. Chem.* **2007**, *119*, 768-771.

[20] a) S. Capone, I. Kieltsch, O. Flögel, G. Lelais, A. Togni, D. Seebach, *Helv. Chim. Acta* **2008**, *91*, 2035-2056; b) D. Seebach, H. Widmer, S. Capone, R. Ernst, T. Bremi, I. Kieltsch, A. Togni, D. Monna, D. Langenegger, D. Hoyer, *Helv. Chim. Acta* **2009**, *92*, 2577-2586; c) V. Matoušek, J. Václavík, P. Hájek, J. Charpentier, Z. E. Blastik, E. Pietrasiak, A. Budinská, A. Togni, P. Beier, *Chem. Eur. J.* **2016**, *22*, 417-424; d) V. Matoušek, J. Václavík, P. Hájek, J. Charpentier, Z. E. Blastik, E. Pietrasiak, A. Budinská, A. Togni, P. Beier, *Chem. Eur. J.* **2017**, *23*, 6490-6494.

[21] N. J. W. Straathof, B. J. P. Tegelbeckers, V. Hessel, X. Wang, T. Noël, *Chem. Sci.* **2014**, *5*, 4768-4773.

[22] C. Bottecchia, X.-J. Wei, K. P. L. Kuijpers, V. Hessel, T. Noël, *J. Org. Chem.* **2016**, *81*, 7301-7307.

[23] a) J. Rickmeier, T. Ritter, *Angew. Chem. Int. Ed.* **2018**, *57*, 14207-14211; *Angew. Chem.* **2018**, *130*, 14403-14407; b) Z. Yuan, M. B. Nodwell, H. Yang, N. Malik, H. Merkens, F. Bénard, R. E. Martin, P. Schaffer, R. Britton, *Angew. Chem. Int. Ed.* **2018**, *57*, 12733-12736; *Angew. Chem.* **2018**, *130*, 12915-12918; c) K.-J. Langen, N. Galldiks, E. Hattingen, N. J. Shah, *Nat. Rev. Neurol.* **2017**, *13*, 279-289; d) S. Preshlock, M. Tredwell, V. Gouverneur, *Chem. Rev.* **2016**, *116*, 719-766; e) C. N. Neumann, J. M. Hooker, T. Ritter, *Nature* **2016**, *534*, 369-373; f) J. McConathy, W. Yu, N. Jarkas, W. Seo, D. M. Schuster, M. M. Goodman, *Med. Res. Rev.* **2012**, *32*, 868-905; g) J. McConathy, M. M. Goodman, *Cancer Metast. Rev.* **2008**, *27*, 555-573.

[24] a) O. Morris, M. Fairclough, J. Grigg, C. Prenant, A. McMahon, *J. Labelled Compd. Radiopharm.* **2019**, *62*, 4-23; b) I. M. Jackson, P. J. H. Scott, S. Thompson, *Semin. Nucl. Med.* **2017**, *47*, 493-523; c) S. Richter, F. Wuest, *Molecules* **2014**, *19*, 20536-20556.

[25] S. Verhoog, C. W. Kee, Y. Wang, T. Khotavivattana, T. C. Wilson, V. Kersemans, S. Smart, M. Tredwell, B. G. Davis, V. Gouverneur, *J. Am. Chem. Soc.* **2018**, *140*, 1572-1575.

[26] S. Liu, H. Ma, Z. Zhang, L. Lin, G. Yuan, X. Tang, D. Nie, S. Jiang, G. Yang, G. Tang, *Theranostics* **2019**, *9*, 1144-1153.

[27] D. Gilis, S. Massar, N. Cerf, M. Rooman, *Genome Biology* **2001**, *2*, research0049.1- 0049.12.

[28] K. Gevaert, P. Van Damme, L. Martens, J. Vandekerckhove, *Anal. Biochem.* **2005**, *345*, 18-29.

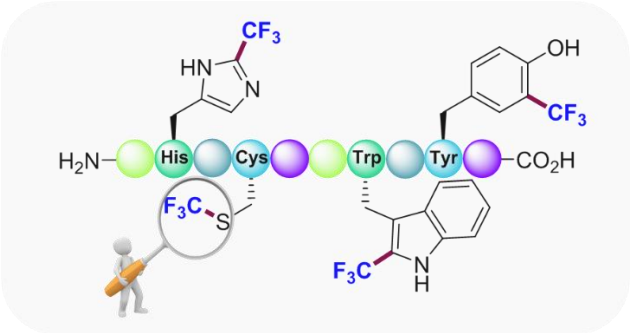
[29] a) Y. Seki, T. Ishiyama, D. Sasaki, J. Abe, Y. Sohma, K. Oisaki, M. Kanai, *J. Am. Chem. Soc.* **2016**, *138*, 10798-10801; b) O. Konievab, A. Wagner, *Chem. Soc. Rev.* **2015**, *44*, 5495-5551; c) N. Stephanopoulos, M. B Francis, *Nat. Chem. Biol.* **2011**, *7*, 876-884.

[30] For selected reactions with simple indoles or heteroarenes, see: a) I. Abdiaj, C. Bottecchia, J. Alcazar, T. Noël, *Synthesis* **2017**, *49*, 4978-4985; b) D. Wang, G.-J. Deng, S. Chen, H. Gong, *Green Chem.* **2016**, *18*, 5967-5970; c) J. C. Fennewald, B. H. Lipshutz, *Green. Chem.* **2014**, *16*, 1097-1100; d) N. J. W. Straathof, H. P. L. Gemoets, X. Wang, J. C. Schouten, V, Hessel, T. Noël, *ChemSusChem* **2014**, *7*, 1612-1617; e) L. Chu, F.-L. Qing, *J. Am. Chem. Soc.* **2012**, *134*, 1298-1304; f) N. Iqbal, S. Choi, E. Ko, E. J. Cho, *Tetrahedron Lett.* **2012**, *53*, 2005-2008; g) X. Mu, S. Chen, X. Zhen, G. Liu, *Chem. Eur. J.* **2011**, *17*, 6039-6042; h) V. M.

Muzalevskiy, A. V. Shastin, E. S. Balenkova, G. Haufe, V. G. Nenajdenko, *Synthesis* **2009**, *23*, 3905-3929.
[31] W. R. Dolbier, *Chem. Rev.* **1996**, *96*, 1557-1584.
[32] R. Shimizu, H. Egami, T. Nagi, J. Chae, Y. Hamashima, M. Sodeoka, *Tetrahedron Lett.* **2010**, *51*, 5947-5949.
[33] Y. Cheng, X. Yuan, J. Ma, S. Yu, *Chem. Eur. J.* **2015**, *21*, 8355-8359.
[34] H. Egami, Y. Ito, T. Ide, S. Masuda, Y. Hamashima, *Synthesis* **2018**, *50*, 2948-2953.
[35] I. Ghosh, J. Khamrai, A. Savateev, N. Shlapakov, M. Antonietti, B. König, *Science* **2019**, *365*, 360-366.
[36] M. Baar, S. Blechert, *Chem. Eur. J.* **2015**, *21*, 526-530.
[37] P. Liu, W. Liu, C.-J. Li, *J. Am. Chem. Soc.* **2017**, *139*, 14315-14321.
[38] Y. Wang, J. Wang, G.-X. Li, G. He, G. Chen, *Org. Lett.* **2017**, *19*, 1442-1445.
[39] Y. Ji, T. Brueckl, R. D. Baxter, Y. Fujiwara, I. B. Seiple, S. Su, D. G. Blackmond, P. S. Baran, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 14411-14415.
[40] a) A. G. O'Brien, A. Maruyama, Y. Inokuma, M. Fujita, P. S. Baran, D. G. Blackmond, *Angew. Chem. Int. Ed.* **2014**, *53*, 11868-11871; *Angew. Chem.* **2014**, *126,* 12062-12065; b) Y. Fujiwara, J. A. Dixon, F. O'Hara, E. D. Funder, D. D. Dixon, R. A. Rodriguez, R. D. Baxter, B. Herlé, N. Sach, M. R. Collins, I. Ishihara, P. S. Baran, *Nature* **2012**, *492*, 95-99.
[41] a) J. M. Smith, J. A. Dixon, J. N. deGruyter, P. S. Baran, *J. Med. Chem.* **2019**, *62*, 2256-2264; b) F. O'Hara, R. D Baxter, A. G O'Brien, M. R Collins, J. A Dixon, Y. Fujiwara, Y. Ishihara, P. S Baran, *Nat. Protoc.* **2013**, *8*, 1042-1047.
[42] M. Imiołek, G. Karunanithy, W.-L. Ng, A. J. Baldwin, V. Gouverneur, B. G. Davis, *J. Am. Chem. Soc.* **2018**, *140*, 1568-1571.
[43] M. Cheng, B. Zhang, W. Cui, M. L. Gross, *Angew. Chem. Int. Ed.* **2017**, *56*, 14007-14010; *Angew. Chem.* **2017**, *129*, 14195-14198.
[44] B. Ding, Y. Weng, Y. Liu, C. Song, L. Yin, J. Yuan, Y. Ren, A. Lei, C.-W. Chiang, *Eur. J. Org. Chem.* **2019**, 7596-7605.
[45] a) M. San Segundo, A. Correa, *Chem. Sci.* **2019**, *10*, 8872-8879; b) M. San Segundo, A. Correa, *ChemSusChem* **2018**, *11*, 3893-3898; c) M. San Segundo, I. Guerrero, A. Correa, *Org. Lett.* **2017**, *19*, 5288-5291.
[46] I. Guerrero, A. Correa, *Org. Lett.* **2020**, *22*, 1574-1579.
[47] C. W. Kee, O. Tack, F. Guibbal, T. C. Wilson, P. G. Isenegger, M. Imiołek, S. Verhoog, M. Tilby, G. Boscutti, S. Ashworth, J. Chupin, R. Kashani, A. W. J. Poh, J. K. Sosabowski, S. Macholl, C. Plisson, B. Cornelissen, M. C. Willis, J. Passchier, B. G. Davis, V. Gouverneur, *J. Am. Chem. Soc.* **2020**, *142*, 1180-1185.
[48] a) J. Lee, M. Ju, O. H. Cho, Y. Kim, K. T. Nam, *Adv. Sci.* **2019**, *6*, 1801255; b) M. I. Chávez-Béjar, J. L. Báez-Viveros, A. Martínez, F. Bolívar, G. Gosset, *Process Biochem.* **2012**, *47*, 1017-1026.
[49] K. L. Kirk, M. Nishida, S. Fujii, H. Kimoto, *J. Fluorine Chem.* **1992**, *59*, 197-202.
[50] N. Ichiishi, J. P. Caldwell, M. Lin, W. Zhong, X. Zhu, E. Streckfuss, H.-Y. Kim, C. A. Parish, S. W. Krska, *Chem. Sci.* **2018**, *9*, 4168-4175.
[51] a) F. Agostini, J.-S. Vçller, B. Koksch, C. G. Acevedo-Rocha, V. Kubyshkin, N. Budisa, *Angew. Chem. Int. Ed.* **2017**, *56*, 9680-9703; *Angew. Chem.* **2017**, *129*, 9810-9835; b) S.-M. Liao, Q.-S. Du, J.-Z. Meng, Z.-W. Pang, R.-B. Huang, *Chem. Cent. J.* **2013**, *7*, 44-55.
[52] a) T. Dudev, C. Lim, *Chem. Rev.* **2014**, *114*, 538-556; b) Y. Lu, N. Yeung, N. Sieracki, N. M. Marshall, N. M. *Nature* **2009**, *460*, 855-862.
[53] H. Kimoto, S. Fujii, L. A. Cohen, *J. Org. Chem.* **1982**, *47*, 2867-2872.
[54] a) V. M. Labroo, R. B. Labroo, L. A. Cohen, *Tetrahedron Lett.* **1990**, *31*, 5705-570; b) H. Kimoto, S. Fujii, L. A. Cohen, *J. Org. Chem.* **1984**, *49*, 1060-1064.
[55] A. F. M. Noisier, M. J. Johansson, L. Knerr, M. A. Hayes, W. J. Drury III, E. Valeur, L. R. Malins, R. Gopalakrishnan, *Angew. Chem. Int. Ed.* **2019**, *58*, 19096-19102; *Angew. Chem.* **2019**, *131,* 19272-19278.
[56] For an elegant His-specific oligopeptide modification via visible-light assisted C2-alkylation with non-fluorinated C4-alkyl-1,4-dihydropyridine reagents, see: X. Chen, F. Ye, X. Luo, X. Liu, J. Zhao, S. Wang, Q. Zhou, G. Chen, P. Wang. *J. Am. Chem. Soc.* **2019**, *141*, 18230-18237.
[57] For a recent comprehensive review, see: R. S. J. Proctor, R. J. Phipps, *Angew. Chem. Int. Ed.* **2019**, *58*, 13666-13699; *Angew. Chem.* **2019**, *131,* 13802-13837.

Entry for the Table of Contents

Insert graphic for Table of Contents here. ((Please ensure your graphic is in **one** of following formats))



Site-selective trifluoromethylation reactions within a peptide framework poses a challenging task of capital synthetic relevance with profound implications in proteomics, chemical biology and drug discovery. This minireview summarizes the most recent advances in the introduction of metabolism-blocking trifluromethyl group in a late-stage fashion as an enabling tool for the modification of oligopeptides and ultimate engineering of proteins.