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Využití fluoralkylových hypervalentních sloučenin jódu v C-H funkcionalizaci malých molekul a aromatických aminokyselinových zbytků

Application of fluoroalkyl hypervalent iodine reagents in C-H functionalization of small molecules and aromatic amino acid residues

Disertační práce

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Doctoral thesis

Supervisor: Ing. Petr Beier, Ph.D.

Prague, 2021

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V Praze, 14.09.2021

Podpis

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SUMMARY

The chemistry of fluorolkyl hypervalent iodine reagents has witnessed a great boast in recent years. These compounds are highly attractive as drug candidates, advanced materials and agrochemicals as described in detail in the Introduction. Despite this fact, applications of these reagents in biological studies are rather rare and under developed.

The goal of this thesis is therefore the development of mild and metal-free methods in order to fill this gap. Two ways of application of fluoroalkyl hypervalent iodine reagents in labeling of biologically relevant compounds was explored.

First, the applicability of previously reported parent Togni CF_3 and their analogous tetrafluoroethyl reagents in radical fluoroalkylation of electron-rich substrates such as indole and pyrrole derivatives using sodium ascorbate as reductant was described. This afforded trifluoromethyl or 1,1,2,2-tetrafluoroethyl containing products in moderate to high yields. Next, same reagents were applied for labeling of several peptides and proteins bearing aromatic amino acids in their structure. This way, peptides and proteins containing electron-rich aromatics such as Trp, Phe, Tyr and His were reacted with fluoroalkyl groups with high selectivity toward Trp.

In the second part of the work, a different approach of radical fluoroalkylation of electron-rich substrates or biologically relevant compounds using hypervalent iodine reagents in presence of visible light was investigated. This method offers a more selective way for fluoroalkylation of Trp residues. Such selectivity and mild reaction conditions are hardly achievable with existing methods, therefore our strategies based on hypervalent iodine reagents open up a new approach in the bioconjugation field.

SOUHRN

Chemie fluoralkylových činidel hypervalentního jódu dosáhla v posledních letech velkého uplatnění. Jak je nastíněno v úvodu práce, tyto sloučeniny jsou vysoce atraktivní jako potenciální léčiva, pokročilé materiály a agrochemikálie. Navzdory tomuto širokému využití fluoralkylových činidel hypervalentního jódu, jejich aplikace v biologických studiích není příliš rozvinutá.

Cílem této práce je vývoj nových metod probíhajících za mírných podmínek a bez využití kovů, které by vedly k aplikaci v biologických studiích. V této práci byly prozkoumány dva druhy využití fluoralkylových činidel hypervalentního jódu pro značení biomolekul.

První část práce se zabývá využitím již dříve zmíněných Togniho CF_3 činidel a jejich tetrafluorethylových analogů při radikálových fluoralkylacích elektronově bohatých substrátů jako jsou deriváty indolu a pyrrolu s využitím askorbátu sodného jako redukčního činidla. Tato transformace poskytuje trifluormethylované a 1,1,2,2-tetrafluorethylované produkty ve středně dobrých až vysokých výtěžcích. Tato činidla byla také použita pro značení několika peptidů a proteinů obsahujících aromatické aminokyseliny. Pomocí výše zmíněné reakce byly tyto peptidy a proteiny obsahující elektronově bohaté aromatické řetězce jako Trp, Phe, Tyr a His fluoralkylovány s vysokou selektivitou vůči Trp.

Ve druhé části této práce je popsána radikálová fluoralkylace elektronově bohatých substrátů a biologicky relevantních sloučenin využívající hypervalentní jodová činidla v přítomnosti viditelného světla. Tato metoda poskytuje selektivní fluoralkylaci Trp zbytků. Selektivita této reakce probíhající za mírných podmínek je obtížně dosažitelná pomoci již existujících metod, naše strategie založená na použití fluoralkylových činidel hypervalentního jódu tudíž umožňuje nový přístup k biokonjugacím obecně.

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1. INTRODUCTION

Fluorine is the most abundant halogen on the earth's crust; however, its occurrence in natural organic products is relatively rare, and very few examples of this kind are known. It is frequently found in non-living systems, namely in minerals, such as fluorite (CaF_2), fluoroapatite ($Ca_5(PO_4)_3F$) and cryolith ($Na_3[AlF_6]$), due to the high affinity of fluorine to bond with Lewis acids such as calcium and aluminum.[1]

Organofluorine chemistry has witnessed a great development in the second half of the twentieth century. The incorporation of fluorine has led to the advent of new functional materials with applications in various aspect of our lives. Among them, fire distinguisher agents, Teflon (coating agent), refrigerants, textile membranes, liquid crystals can be mentioned as important examples (Figure 1).

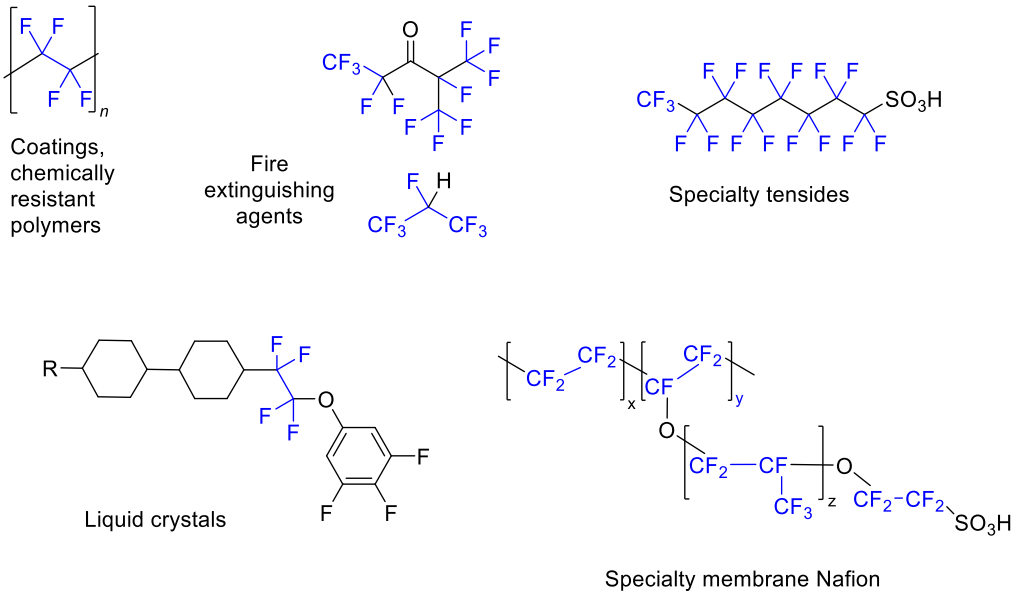


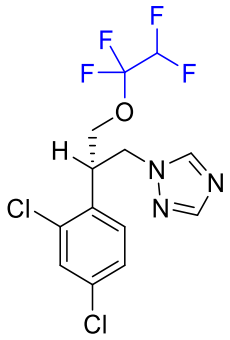
Figure 1 Examples of fluorinated functional materials with important applications.

In general, the incorporation of fluorine atoms into organic compounds positively influences metabolic stability, lipophilicity as well as bioavailability of the resulting compound in comparison to its non-fluorinated counterpart.[2]

Given all these important effects it is not surprising that the development of new methods for straightforward introduction of fluorine atom(s), trifluoromethyl or perfluoroalkyl groups is highly sought-after in the design of new and effective agrochemicals and drug candidates.[3–8]

While many methods have been proposed for the introduction of fluorine containing scaffolds, such as fluoromethyl, difluoromethyl and in general $(CF_2)_n$ ($n > 2$) groups, methods to install 1,1,2,2-tetrafluoroethyl fragments are rather underdeveloped.[9,10]

Recently, attentions has been drawn to the tetrafluoroethylene chemistry. The CF_2CF_2 moiety as a useful building block is currently present in few applied organofluorine materials such as the fungicide Tetraconazole (Figure 2), Nafion or liquid crystalline compounds (Figure 1).



Tetraconazole

Figure 2 Tetrafluoroethyl-containing fungicide.

The goal of this chapter is to introduce applications of fluoroalkyl groups, especially novel 1,1,2,2 tetrafluoroethyl hypervalent iodine reagents (HVI) in transferring the CF_2CF_2 building block to target organic or biologically useful materials.

The focus of this Dissertation is on finding new approaches for selective trifluoromethylation or tetrafluoroethylation of biological compounds and small organic molecules *via* radical pathway, using novel trifluoromethyl and tetrafluoroethyl hypervalent iodine reagents.

The following chapter describes shortly general fluoroalkylation methods.

1.2 Fluoroalkylation

Recently, traditional fluorination methods have lost their popularity among chemists due to the use of harsh reaction conditions, such as the use of highly toxic, explosive and moisture sensitive fluorinating reagents, such as F_2, HF, SbF_3 or SbF_5, which lead usually to unselective and uncontrollable reactions.[11] Instead, new fluoroalkyl reagents appeared, which contain the C-F fragment in their structure, leading to more mild and selective reactions. Unlike in the fluorination resulting in the formation of a C-F bond, in fluoroalkylation process the C-C bond forms. Fluoroalkyl groups, as mimics of short alkyl groups, are very useful building blocks in the development of drug candidates, agrochemicals and advanced materials.[12–17]

It was shown that the incorporation of CF_3 or C_nF_m groups into drug candidates increases antitumor or antivirus activity of the targeted drug by up to 100-fold.[18]

Although the importance of fluorine derivatives in various fields is well known, the challenge is to find methods allowing for selective and straightforward fluoroalkylation, tolerating various functional groups. Therefore, the development of new strategies for functionalization of advanced materials is an important task.

In general, fluoroalkyl transfer can proceed *via* radical, nucleophilic or electrophilic approach using appropriate reagents, as outlined in the next three chapters.

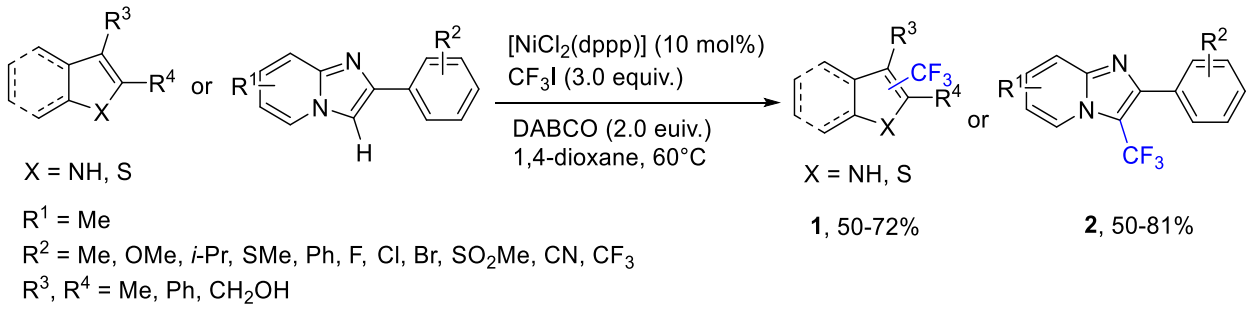
1.2.1 Radical fluoroalkylation

Radical fluoroalkylation can be carried out under thermal, reductive, oxidative, photochemical and electrochemical conditions. Radical trifluoromethylation can be achieved using systems such as zinc[19] or aluminum organometallics, Et_3B[20–22] and Na_2SO_4.[23–27] The above mentioned systems have been successfully used for trifluoromethylations of various nucleophiles, such as olefins.

Several review articles are available highlighting recent advances in radical trifluoromethylation reactions.[28–30]

Fluoroalkyl halides are considered to be the largest source of perfluoroalkyl radicals. Wang and co-workers reported radical trifluoromethylation of a wide scope of heteroarens such as indoles, pyrroles, imidazopyridines and thiophenes using CF_3I as the source of CF_3 radical in presence of

a nickel catalyst at elevated temperature (Scheme 1). Mechanistic investigations revealed the formation of CF_3 radicals. Biologically relevant substrates such as melatonin and zolmitriptan were also tested for trifluoromethylations and afforded 55% and 45% of the trifluoromethylated products (**1** and **2**) respectively.[31]



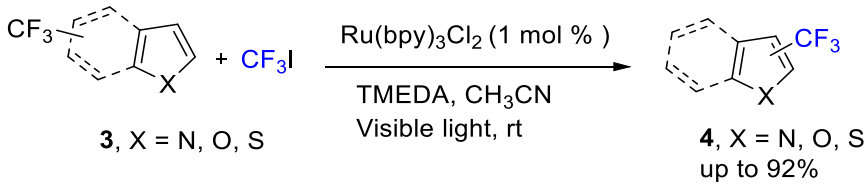
Scheme 1 Radical trifluoromethylation of heteroarenes.

From the environmental and practical point of view, photochemical fluoroalkylations/perfluoroalkylations are preferred over the reactions using reactive and hazardous organic reagents such as peroxides, metal catalyst, etc.

As early as 1912, the light was recognized as abundant, economic and environmental friendly component to trigger chemical processes.[32] Despite the importance of photochemistry as a very important and powerful tool, this field has progressed only in recent years. Advances in photochemistry has mainly been started with the photoredox catalysis.[33–35]

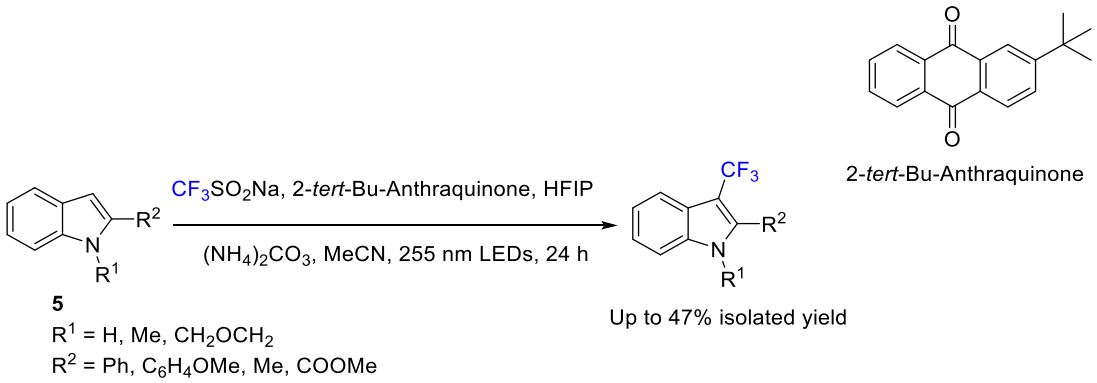
Generally, a photoredox catalyst is a compound, which can absorb the energy of the visible light. As a result, reactive species are formed, which can activate various substrates *via* a single electron transfer (SET) process, leading to reactions under mild condition.[36,37]

In 2012 Cho group employed a photochemical protocol in radical trifluoromethylation of electron-rich aromatics and heteroaromatics **3** promoted by $Ru(bpy)_3Cl_2$ photocatalyst using CF_3I as the source of CF_3 radical and an amine as reductive quencher. The products **4** were isolated in good to excellent yields. The protocol tolerated various functional groups (Scheme 2).[38]



Scheme 2 Visible-light trifluoromethylation of heteroarenes.

Recently, E. Leadbeater and co-workers reported photocatalytic trifluoromethylation of indoles using 2-*tert*-butylanthraquinone as a photocatalyst and trifluoromethylsulfinate (Langlois reagent) as the source of CF_3 radicals (Scheme 3).[39] The reaction was regioselective, as found by both experimental and computational studies. While 1,2-substituted indoles (**5**) afforded trifluoromethylated products in moderate yields, 1- and 1,3-substituted indoles provided low yields of products.

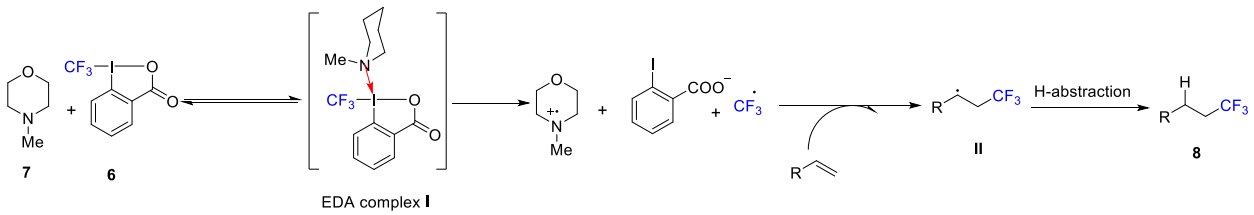


Scheme 3 A photo-induced trifluoromethylation of indoles.

Interesting is fluoroalkylation *via* photochemical approach which does not rely on the use of photoredox catalyst. The chemistry is based on the formation of an EDA complex (also called a charge transfer complex (CT)) which is formed *via* association of an electron acceptor molecule A and a donor D. The individual molecules A and B may not absorb light but the resulting complex can absorb visible light and undergo excitation resulting in the generation of radical species *via* single electron transfer (SET).[40] These complexes can also initiate a reaction upon thermal activation.[41]

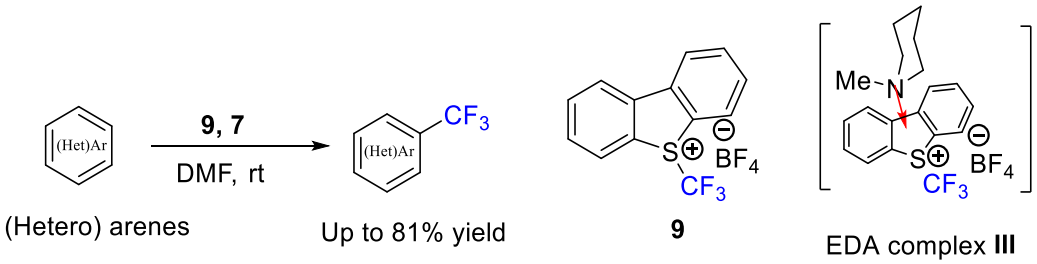
Yu *et al*., synthesized hydrotrifluoromethylated alkenes utilizing an EDA complex which was formed between Togni reagent (**6**) and *N*-methylmorpholine (**7**) in the absence of light. Upon

thermal activation of complex **I**, and further dissociation, CF_3 radical, benzoate ion and radical cation of **7** formed. The resulting CF_3 radical underwent addition to the alkene leading to the formation of alkyl radical **II** which abstracted hydrogen atom from the solvent and yielded hydrotrifluoromethylated alkenes **8** (Scheme 4)[42].



Scheme 4 Mechanism for hydrotrifluoromethylation of alkenes.

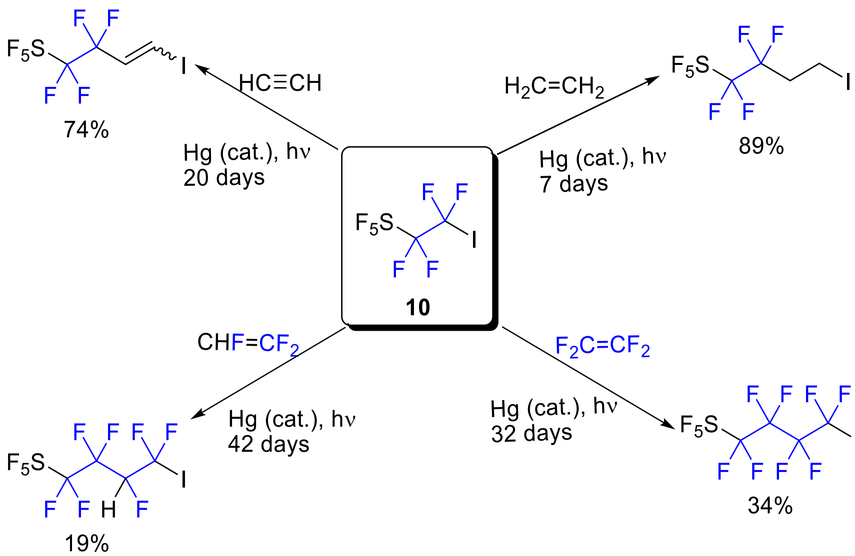
Ma and Yu, investigated the reactivity of EDA complex **III** formed between electron-deficient Umemoto's reagent **9** and **7** in trifluoromethylation of (hetero)arenes.[43] While indole, pyrrole, benzofuran, cyclic enamines and electron-rich benzenes underwent smooth trifluoromethylation, electron-neutral and deficient benzene and also pyridine derivatives were resistant to trifluoromethylation (Scheme 5).



Scheme 5 Trifluoromethylation of various (hetero) arenes based on the formation of EDA complex.

1.2.1.1 Radical tetrafluoroethylation

UV–induced addition of perfluoroalkyl radicals generated from perfluoroalkyl halides **10** to electron-rich substrates, such as alkenes and alkynes was studied extensively.[44–47] Iodoperfluoroalkylation of olefins was carried out using compound **10** and catalytic amount of mercury under irradiation with halogen lamp ($\lambda = 250–800$ nm) (Scheme 6).[48]



Scheme 6 Radical iodofluoroalkylation of olefins using compound **10**.

Fluorine-centered radicals display electrophilic character. The presence of fluorine atom in these radical intermediates has a significant impact on the rate of intramolecular cyclization, as reported by Dolbier and co-workers.[49,50] They observed a significant rate increase of fluorine-containing 5-hexenyl, 6-heptenyl, and 7-octenyl radicals in 5-exo, 6-endo and 6-exo cyclizations (Figure 3).

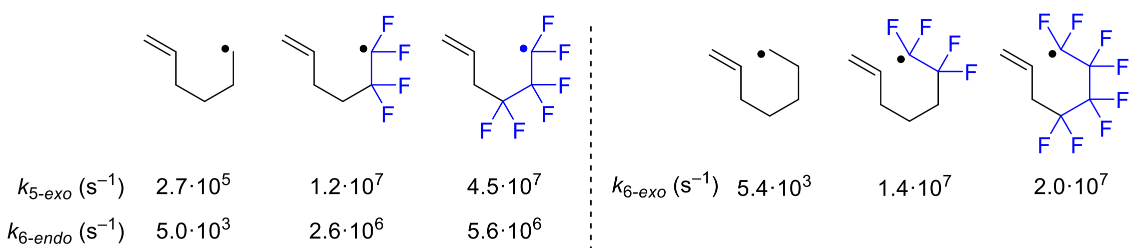
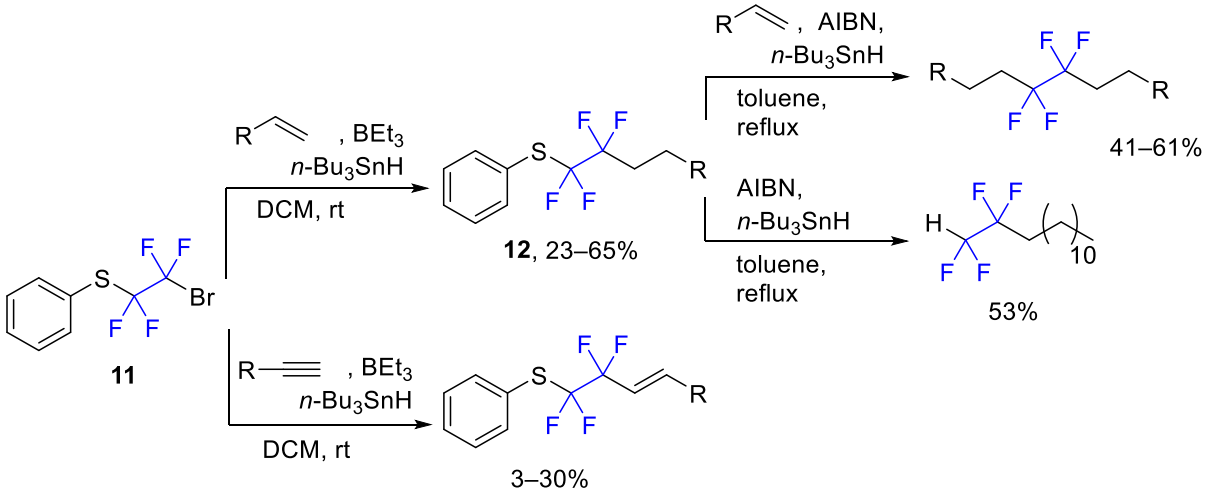


Figure 3 Comparing the effect of fluorine substituent on radical cyclization of hydrocarbons and hydrofluorocarbons given by rate constant.

Chernykh and colleagues have accomplished radical addition of $PhSCF_2CF_2Br$ (**11**) to alkenes and alkynes using *n*-Bu_3SnH/Et_3B system. Various alkenes and alkynes underwent radical tetrafluoroethylation; however, alkenes provided higher yields than alkynes. The resulting tetrafluoroethyl phenyl sulfanyl compounds **12** could undergo further reactions. The phenyl sulfanyl group could be replaced with hydrogen or could undergo a second radical addition to

alkenes, yielding tetrafluoroethylene-linked products. Bromide **11** can therefore be transferred as tetrafluoroethyl and tetrafluoroethylene group to alkenes *via* the formation of radical synthons such as $PhSCF_2CF_2•$, $HCF_2CF_2•$ and $•CF_2CF_2•$ (Scheme 7).[51]

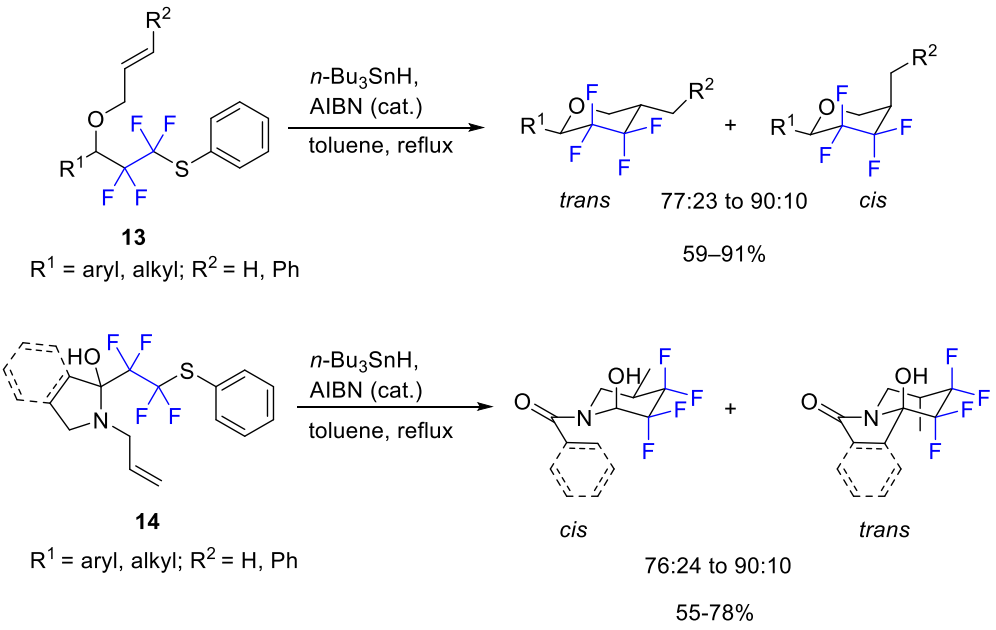


Scheme 7 Radical tetrafluoroethylations of alkenes and alkynes using compound **11**.

Moreover, Burton *et al.* reported radical tetrafluoroethylation of various phosphonites using XCF_2CF_2X (X = Cl, Br, I). 1,2-Dihalotetrafluoroethane generated radical in the form of $[•CF_2CF_2X]$ synthon which can be transferred under thermal and/or photochemical conditions.[52,53]

In addition, $[•CF_2CF_2X]$ and $[•CF_2CF_2•]$ synthons generated from 1,2-dihalotetrafluoroethane were shown to undergo radical tetrafluoroethylation of alkenes and alkynes.[54–57]

O and N-Allyl type compounds (**13** and **14**) containing the CF_2CF_2 building block in their structure, can undergo intramolecular radical cyclization in presence of tributyltin hydride and catalytic amount of AIBN. The reaction afforded *cis* and *trans* products. O-Allyl derivatives yielded tetrahydropyrans[58] with good selectivity of *trans* over *cis* isomer; however, cyclization of N-Allyl derivatives led to the formation of 1-azabicyclic compounds[59] with larger preference to *cis* over *trans* isomer, as illustrated in Scheme 8.



Scheme 8 Intramolecular radical cyclization of O- and N-Allyl compounds **13** and **14** containing the CF_2CF_2 moiety in their structure.

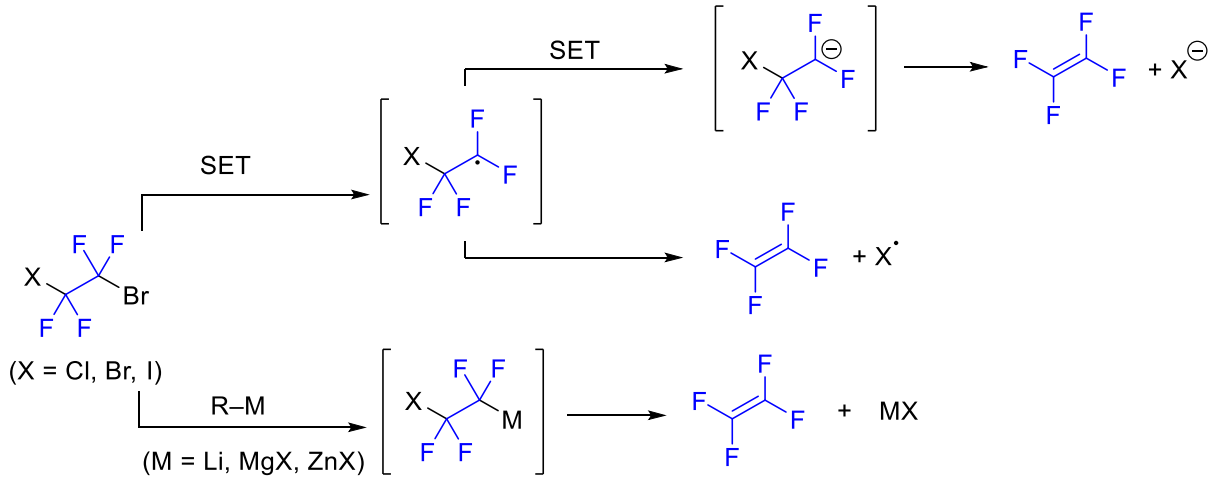
1.2.2 Nucleophilic fluoroalkylation

Nucleophilic fluorination/fluoroalkylation is another synthetic pathway to transfer fluoride or fluoroalkyl group to organic compounds. Transfer of fluoride in nucleophilic way has long been studied; however, the process is not straightforward. Fluoride is a small atom with a large charge density and high affinity for hydrogen bonding to polar solvents. Therefore, this hard anion tend to act as a base rather than nucleophile. Quaternary ammonium fluorides and metal fluorides are mentioned as the main source of fluoride ion. Aside from disadvantages, such as having hydroscopic nature and low solubility in organic solvents, these reagents have been applied in displacement reactions of halides and sulfonate anions in polar aprotic solvents.

In comparison to fluorination, fluoroalkylation and in particular trifluoromethylation has attracted a remarkable attention due to the importance of CF_3 building block in various fields.

Fluoroalkyl organometallic compounds $X(CF_2)_nM$ (X = halogen; M = Li, MgX, ZnX) have been used in nucleophilic fluoroalkylation. However, they display low stability and special attention must be taken into account while working with them.

In XCF_2CF_2M (X = halogen; M = Li, MgX, ZnX) type organometallics, generated from 1,2-dihalotetrafluoroethanes, β-halogen can undergo elimination resulting in the formation of tetrafluoroethylene gas (Scheme 9). Analogous tetrafluoroethyl radical can undergo decomposition to produce tetrafluoroethylene.



Scheme 9 Decomposition pathways of XCF_2CF_2M-type organometallics.

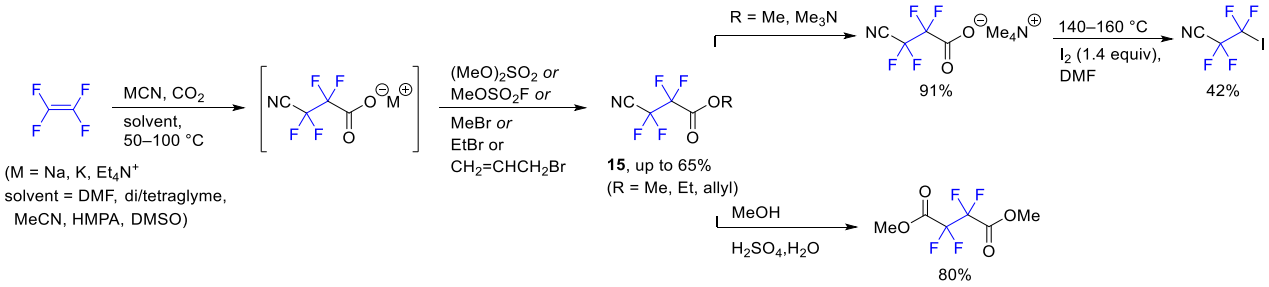
The rest of this chapter describes successful nucleophilic tetrafluoroethyl reagents and examples of their reactivity.

1.2.2.1 Nucleophilic tetrafluoroethylation in the absence of metals

Although organometallic chemistry is the most straightforward synthetic way to produce fluoroalkylated products *via* nucleophilic pathway, fluoroalkylation also can be carried out in the absence of metals. An interesting chemistry was reported by Krespan in 1986 on the reaction of tetrafluoroethylene in the formation of useful intermediates, which can be further converted to various tetrafluoroethyl building blocks.

The reaction of tetrafluoroethylene in the presence of cyanide provided a fluorocarbanion which was then captured by CO_2 to give the corresponding tetrafluoropropionate intermediate. Alkylation of the intermediate was accomplished using various alkylation reagents bearing methyl, ethyl or allyl groups to form **15** (Scheme 10).

Further chemistry followed by converting **15** to interesting materials such as succinate and ICF_2CF_2CN. $ICF_2CF_2N_3$ was also synthesized utilizing the same approach.[60]



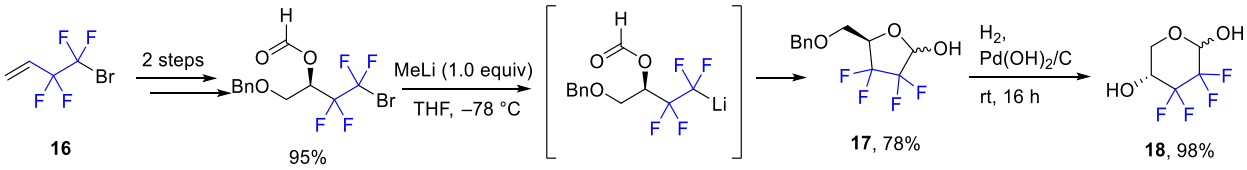
Scheme 10 Nucleophilic addition of fluoro carbanion to CO_2 and further useful chemistry.

1.2.2.2 Organolithium reagents

Recently, fluoroalkyl organolithium compounds have become popular (and more available than organomagnesium reagents) due to their versatility in organic synthesis. Generally, organolithium compounds are highly reactive species, which can be prepared *in situ* and must be used immediately at very low temperatures. Fluoroalkyl organolithium reagents bearing the CF_2CF_2 motif [RCF_2CF_2Li] have been used for tetrafluoroethylation of a variety of electrophiles.

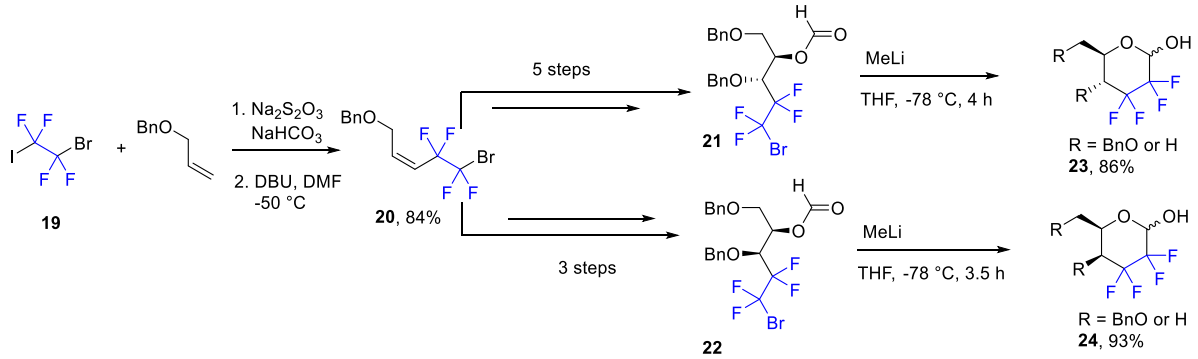
Preparation of monosaccharides and their derivatives, containing tetrafluoroethylene unit is of a particular example. A group of Linclau have reported the use of tetrafluoroethyl organolithium reagents for enantioselective tetrafluoroethylation of sugars.[61]

Commercially available compound **16** was converted to metalated intermediate in a few steps with stoichiometric amount of MeLi. *In situ* generated organolithium specie was then converted to furanose **17** by intermolecular cyclization. Hydrogenation of **17** led to the formation of pyranose **18** in 98% yield (Scheme 11).



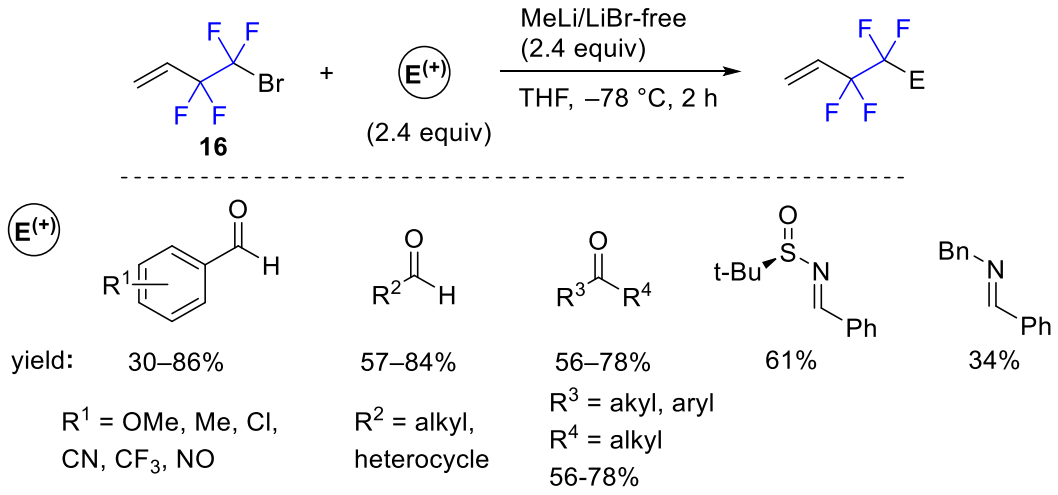
Scheme 11 Synthesis of pentose **17** *via* intermolecular cyclization.

The same procedure was later applied for the synthesis of tetrafluorinated glucose and galactose derivatives. Precursor **20** was obtained from $BrCF_2CF_2I$ (**19**) and allyloxy methyl benzene, and subsequently converted to **21** and **22** through several steps. Bromine-lithium exchange of **21** and **22** and subsequent intermolecular cyclization and further hydrogenation afforded **23** and **24** in high yields (Scheme 12).[62]



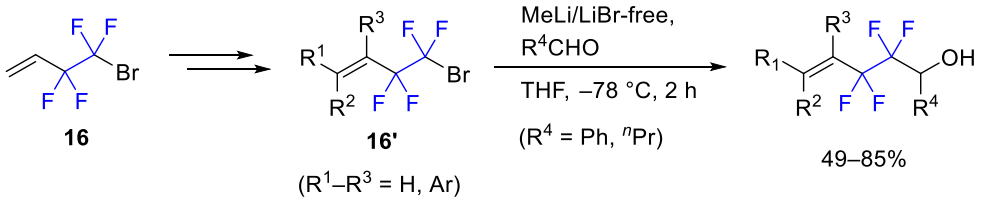
Scheme 12 Synthesis of tetrafluoroethyl derivatives of galactose and glucose.

In another study by Konno *et al*, compound **16** was used in intermolecular addition reactions. It was found that excess of LiBr-free MeLi and electrophile affords higher yields. However, LiBr/MeLi or BuLi afforded lower yields. Other functional groups such as ketones, sulfinyl imine, and imines also reacted well with **16** (Scheme 13).[63]



Scheme 13 Reaction of *in situ* formed organolithium species derived from **16** with various electrophiles.

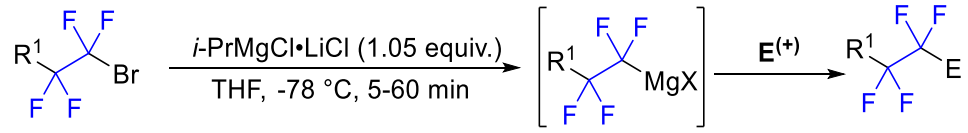
The same group demonstrated in 2017 the synthesis of derivatives of **16** *via* a coupling reaction. **16'** was obtained from **16** through Heck and/or Suzuki–Miyaura couplings. Subsequent metalation of **16'** with benzaldehyde or propanal provided diverse secondary alcohols (Scheme 14).[64]



Scheme 14 Reaction of *in situ* formed organolithium reagents with aldehydes.

1.2.2.3 Organomagnesium reagents

Similar to organolithium reagents, organomagnesium reagents are highly reactive species, which must be *in situ* formed and used at low temperatures. These reagents tend to decompose at high temperatures. Budinská *et al.* reported a protocol using organomagnesium reagents for fluoroalkylation of a wide variety of electrophiles.[65] The reagents were prepared from fluoroalkyl bromides by the treatment with *i*-PrMgCl·LiCl (Turbo Grignard). Bromides bearing OAr, SAr, *N*-heterocyclic and aliphatic substituents which contained useful functional groups, such as ester, halogens, tosyl group or protected aldehydes were metalated, and the resulting reagents were reacted with electrophiles, such as aldehydes, ketones, carbon dioxide, cyclic sulfate and sulfamidate, *N*-sulfonyl imines, nitrone, phosphorochloridate, and an electrophilic azide (Scheme 15).

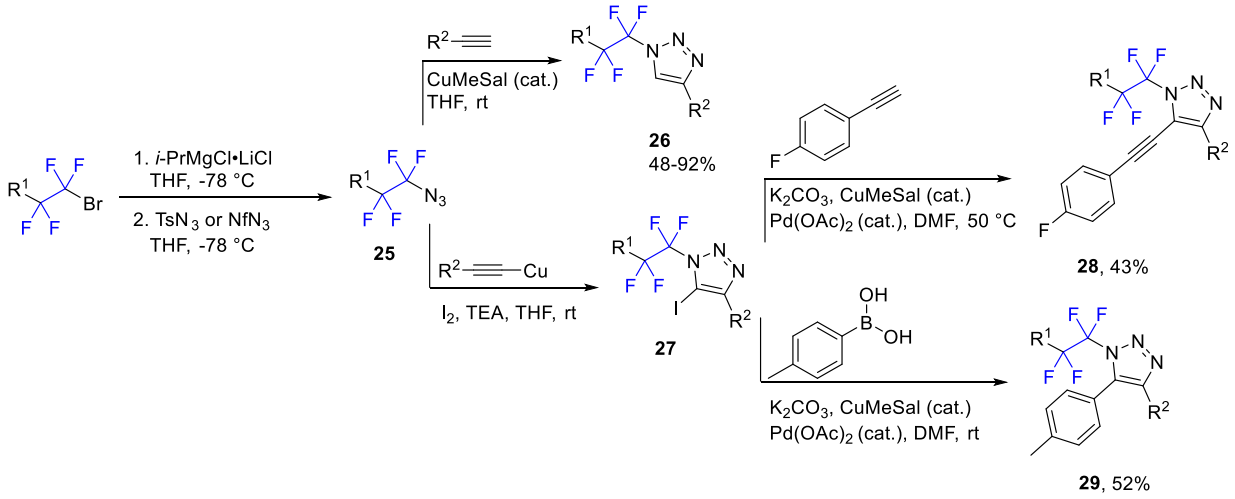


R^1 = ArO, PhS, 1-pyrazoyl, 1-imidazoyl,
Y(CH$_2$)$_2$, PhSO$_2$
(Y = N$_3$, Br, TsO)

Scheme 15 Reaction of *in situ* formed organomagnesium reagents with electrophiles.

In the following study, previously reported organomagnesium reagents were used in the preparation of RCF$_2$CF$_2$–azides (**25**) upon reaction with tosyl azide or nonaflyl azide.[66] The

corresponding azides were then subjected to CuAAC reaction using copper (I) 3-methylsalicylate (CuMeSal) as the catalyst. 4-Substituted 1,2,3-triazoles (**26**) were prepared in good to excellent yields. Intermediate 5-iodotriazoles **27** which were synthesized from **25** and copper acetylide in presence of iodine and a base then underwent Sonogashira or Suzuki–Miyaura cross-coupling reactions to form **28** and **29** (Scheme 16).



Scheme 16 Preparation of azide reagents **25** from organomagnesiums and further reactions.

1.2.2.4 Fluorolkyl silanes

Silicon based reagents have reached a remarkable success in the area of fluoroalkylation, which is due to their good stability, easy handling and mild reaction conditions. In recent years, we have seen an increased interest in $TMSCF_3$ as a nucleophilic source of the trifluoromethyl moiety. $TMSCF_3$, also known as the Ruppert-Prakash reagent was first introduced at 1987 by Ruppert and co-workers, is a robust and most popular nucleophilic trifluoromethylating reagent. Compared to organometallic fluoroalkylating reagents, perfluoroalkyl silanes are rather stable in mildly acidic and aqueous system. Active CF_3^- shows high reactivity toward electrophiles, such as carbonyl compounds. $TMSCF_3$ can serve as a trifluoromethylating or difluoromethylating (in the form of difluorocarbene) reagent.

In 2017, Shibata and co-workers described trifluoromethylation of carbonyl compounds by the Ruppert-Prakash reagent using a flow method.[67] Reactions were performed in a simple device consisting of a Pasteur pipette flow reactor packed with Celite/base (Figure 4).

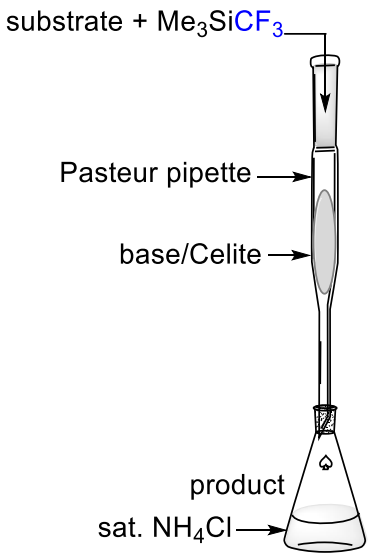
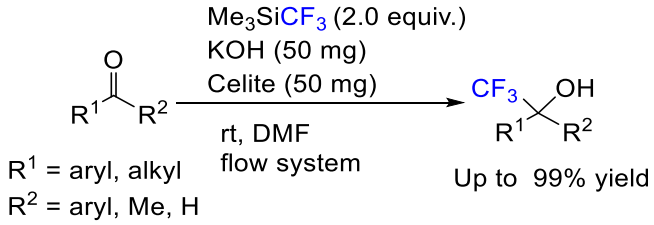


Figure 4 System diagram for flow trifluoromethylation reported by Shibata.

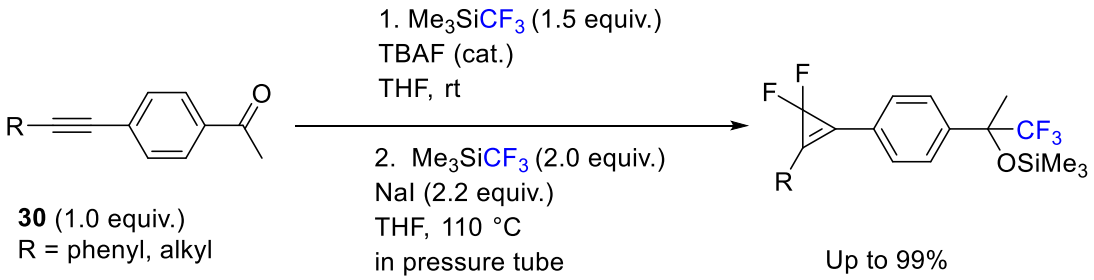
Using this method, diaryl ketones, aryl methyl ketones, containing an enolizable proton, aromatic aldehydes, containing a variety of functional groups, and a cyclic aliphatic ketone were trifluoromethylated in moderate to excellent yields. (Scheme 17).

Moreover, pharmaceuticals, such as Efavirenz were successfully trifluoromethylated using this method.



Scheme 17 Trifluoromethylation of carbonyl compounds by flow method using Ruppert-Prakash reagent.

Hu and co-workers investigated the reactivity of $TMSCF_3$ in both trifluoromethylation and [2 + 1] cycloaddition reaction to **30**. They found that compounds **30** reacted with the CF_3 moiety on the carbonyl side; however, NaI promoted [2 + 1] cycloaddition on the triple bond (Scheme 18).[68]



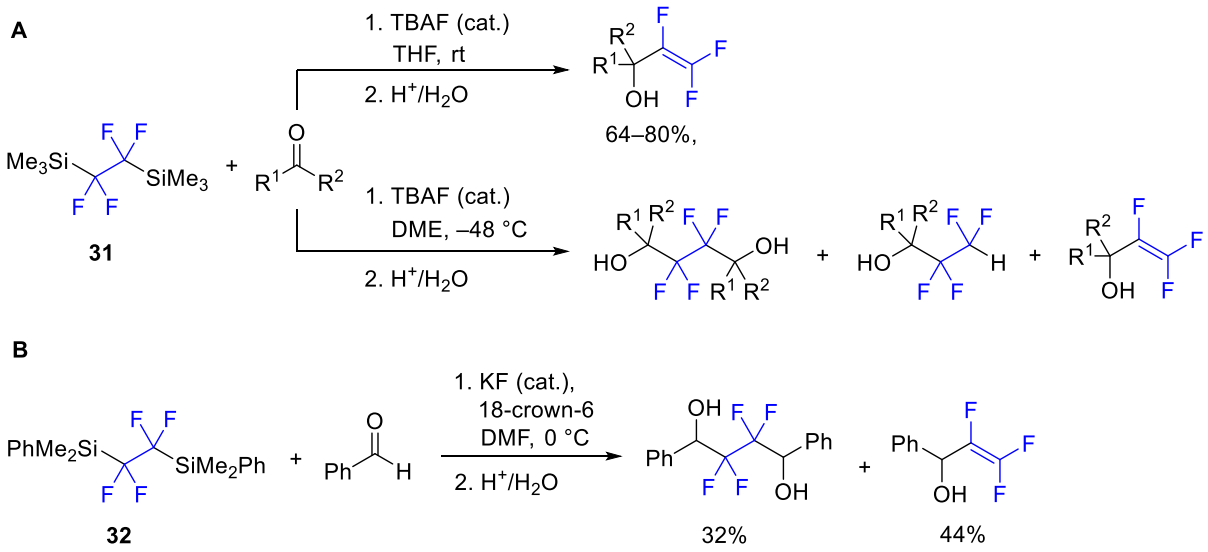
Scheme 18 Trifluoromethylation or [2 + 1] cycloaddition reaction reactions using $TMSCF_3$.

A review article by Liu and Wang provides an extensive coverage on the use of Ruppert-Prakash reagent in organic synthesis.[69]

Tetrafluoroethyl silanes of the general formula $RCF_2CF_2SiMe_3$ are analogous of popular Ruppert-Prakash reagent. Tetrafluoeoethyl silanes have been shown to serve as nucleophilic tetrafluoroethylating reagents to transfer the CF_2CF_2 moiety to various electrophiles.

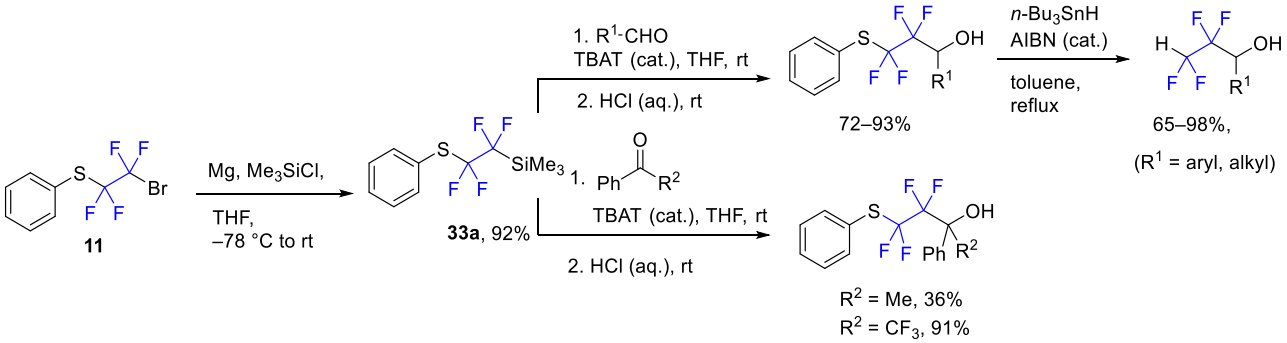
Prakash and co-workers found that the CF_2CF_2 moiety generated from $Me_3SiCF_2CF_2SiMe_3$ (**31**) plays a rule of dianion synthon [$^{-}CF_2CF_2^{-}$] in reactions with carbonyl compounds. They found that higher temperatures, facilitated elimination of Me_3SiF and thus the formation of trifluorovinyl alcohols. However, lower temperatures and excess of electrophile, provided tetrafluoroethyl diols, albeit in lower yields (Scheme 19A).[70]

In a similar study, Fuchikami and Hagiwara developed new conditions for selective preparation of trifluorovynilated product. Using reagent **32**, with slight excess of benzaldehyde, 1,1,2-trifluoro 3-phenyl propene-3-ol was obtained as a sole product. However, 12 equivalents of benzaldehyde led to the mixture of products where the trifluorovinylated product dominated (Scheme 19B).[71]



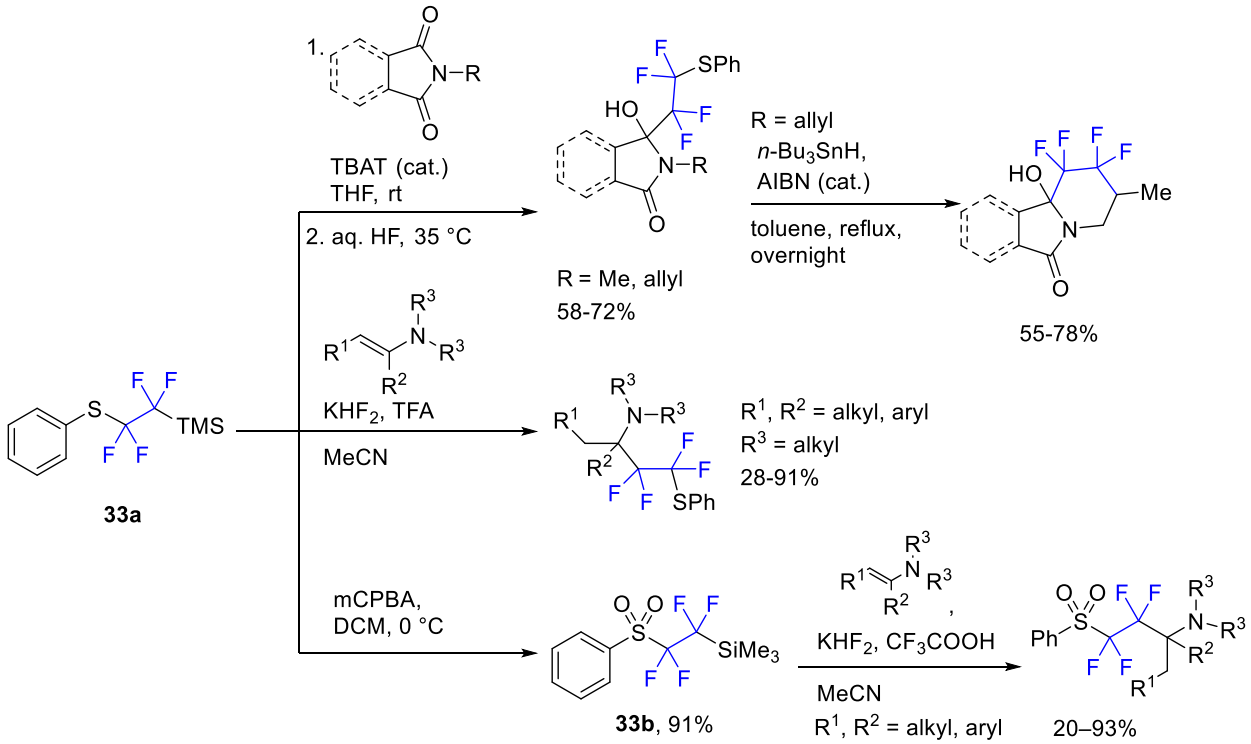
Scheme 19 Reactivity of tetrafluoroethylsilanes **31** and **32** toward carbonyl compounds.

In recent years, an interest has grown in the area of tetrafluoroethylation, therefore new functionalized reagents were developed towards this aim. For instance, Chernykh and co-workers attempted to synthesize new tetrafluoroethyl reagent bearing phenysulfanyl functionality derived from bromide **11**. The resulting reagent **33a** was later tested for tetrafluoroethylation of various aliphatic and aromatic carbonyl compounds. Aliphatic and aromatic aldehydes were transformed to the corresponding alcohols in high yields; however, ketones were less reactive. The reaction with acetophenone led to low yield of the product whereas, cyclohexanone was completely unreactive. Also, substitution of phenysulfanyl group with hydrogen using tributyltin hydride/ AIBN system was successful (Scheme 20).[58]



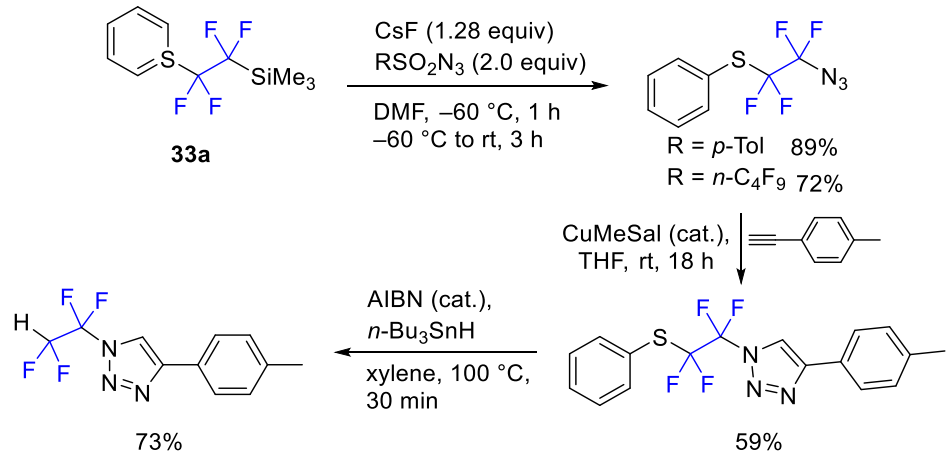
Scheme 20 Nucleophilic tetrafluoroethylation of carbonyl compounds using **33a**.

Similarly, tetrafluoroethylation of cyclic imides and enamines was accomplished using reagent **33a**. It was also possible to perform nucleophilic addition with compound **33b** which is the product of oxidation of reagent **33a**. A range of tetrafluoroethylated amines was prepared in this way (Scheme 21).[72]



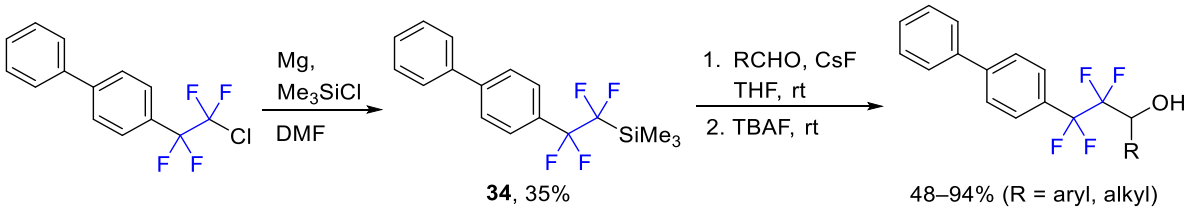
Scheme 21 Nucleophilic addition of silanes **33a** to imides and enamines.

In 2017, Beier group prepared tetrafluoroethylated azides from **33a** using tosyl and nonafyl azide. The resulting azide was successfully used in CuAAC reaction with terminal alkynes. Phenyl sulfanyl group could be substituted with hydrogen using radical condition (Scheme 22).[73]



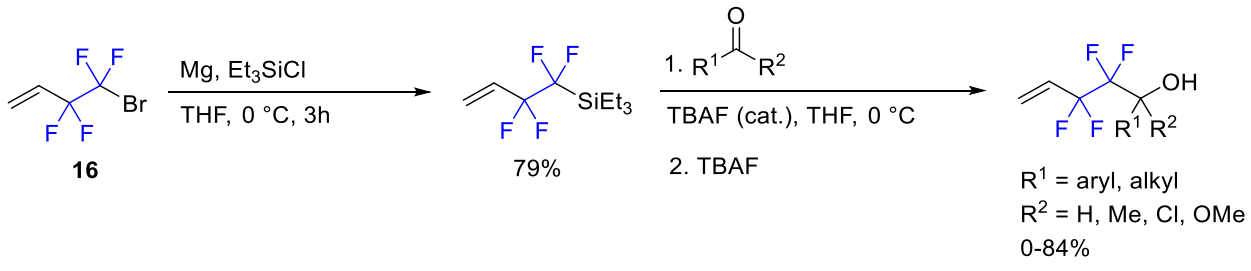
Scheme 22 Synthesis of a tetrafluoroethyl containing azide and subsequent CuAAC reaction with terminal alkynes.

Gouverneur *et al.* applied biphenyl containing silane **34** which was synthesized from related chloride in tetrafluoroethylation of aldehydes. The resulting secondary alcohols formed in moderate to high yields (Scheme 23).[74]



Scheme 23 Reactivity of biphenyl silane **34** with aldehydes.

In analogy to trimethyl silane reagents, Kenno and co-workers synthesized triethyl silane-based reagent derived from bromide **16** which proved to have a lower reactivity than the trimethylsilyl analogue (Scheme 24).

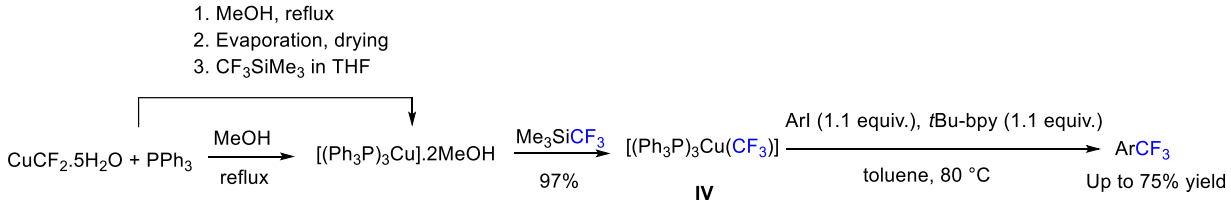


Scheme 24 Reactivity of triethyl silane-based nucleophiles toward carbonyl compounds.

1.2.2.5 Fluoroalkyl oraganocopper reagents

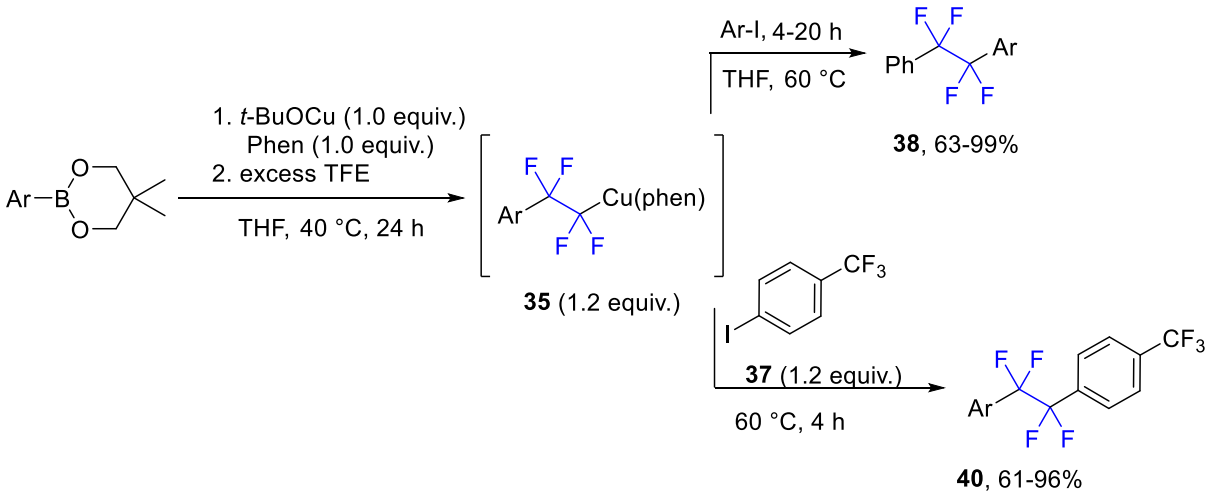
Fluoroalkyl organocopper reagents are another type of nucleophilic fluoroalkylating reagents which attracted a remarkable interest in recent years. These reagents are green and economical and some stable compounds of this kind of reagents are known. Until now, many methods have been introduced for the synthesis of CF_3Cu reagents, but the more appealing ones involved decarboxylation of halodifluoroacetates[75] or the reaction of the Ruppert-Prakash reagent with fluoride ion and CuI.[76]

In 2011, Grushin and co-workers developed a method for trifluoromethylation of a range of haloarenes using an organocopper reagent.[77] The CF_3-copper complex (**IV**) was not only used in trifluoromethylation of aromatic substrates, but it could also serve as a starting material in the synthesis of new Cu-CF_3 compounds (Scheme 25).

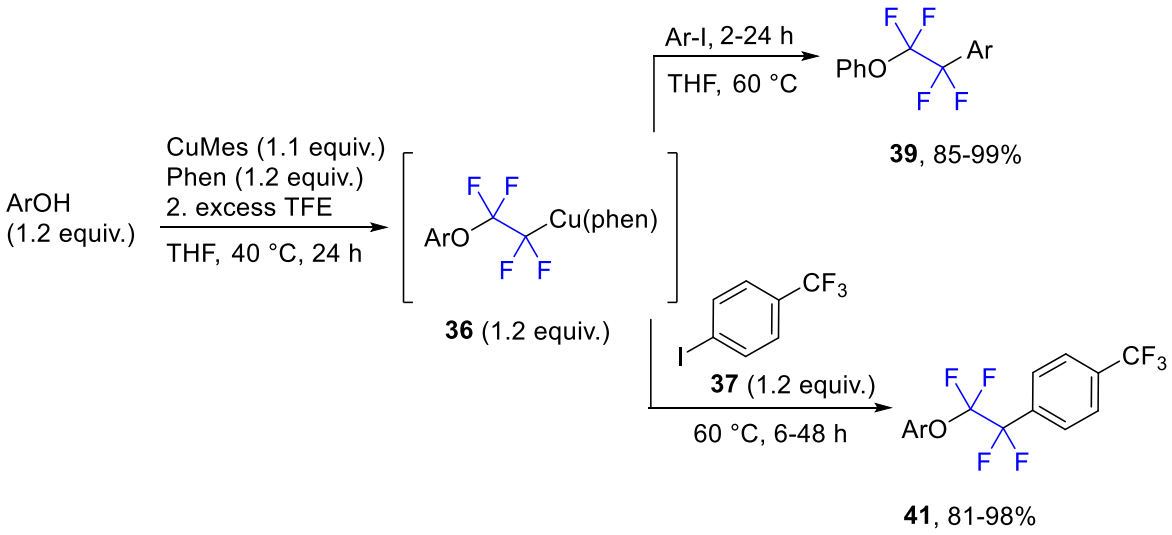


Scheme 25 Synthesis of copper CF_3 complex **IV** and its further reaction in trifluoromethylation of aryl iodides.

Tetrafluoroethyl copper reagents of the general formula [RCF_2CF_2Cu] are analogous to CF_3Cu reagents. Some examples are shown below (Scheme 26 and 27).[78,79] Compound **35** and **36**, which were synthesized from tetrafluoroethylene *via* carbocupration and oxycupration respectively, were further used in coupling reactions. The resulting intermediates could be isolated and used in tetrafluoroethylation of aryl iodides to form **38** and **39** or in one-pot synthesis, **35** and **36** could react with aryl iodides **37** to form **40** and **41**.



Scheme 26 Carbocupration of tetrafluoroethylene and further coupling with aryl iodides.



Scheme 27 Oxycupration of tetrafluoroethylene and further coupling with aryl iodides.

1.2.3 Electrophilic fluoroalkylation

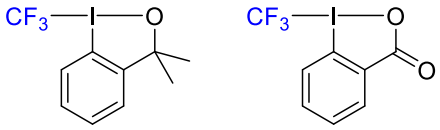
1.2.3.1 Introduction to HVI reagents (structure, synthesis and reactivity)

Unlike other fluoroalkylation methods discussed in previous chapters, electrophilic fluoroalkylation has gained momentum only in recent years.

Until now, several types of electrophilic fluoroalkylation reagents have been developed. The discovery of *S*-(trifluoromethyl)dibenzothiophenium reagents in 1984 by Yagupolski, had a great

influence on chemistry due to the remarkable importance of the trifluoromethyl group in pharmaceuticals, agrochemicals, and functional materials. This influence resulted in the design and synthesis of new prominent reagents, such as Umemoto, Togni, and Shibata reagents. Especially the advent of hypervalent λ^3-iodanes **6** and **42**, which were developed by Togni and co-workers in 2006, has led to significant boost in the field of trifluoromethylation.

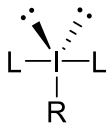
Since their appearance, fluoroalkyl hypervalent λ^3-iodine reagents (HIRs) so-called Togni reagents have been widely used in trifluoromethylation of a diverse array of both carbon-centered and heteroatom-centered nucleophiles, namely sulfides, sulfonates, hydroxyl amines, alcohols, arenes, alkynes, silyl enol ethers, keto derivatives, azoles, etc. Although, depending on reaction conditions, these reagents could operate *via* a radical pathway too, but are still commonly called electrophilic reagents (Scheme 28).



6 **42**

Scheme 28 Structures of first-generation hypervalent λ^3-iodine reagents developed by Togni.

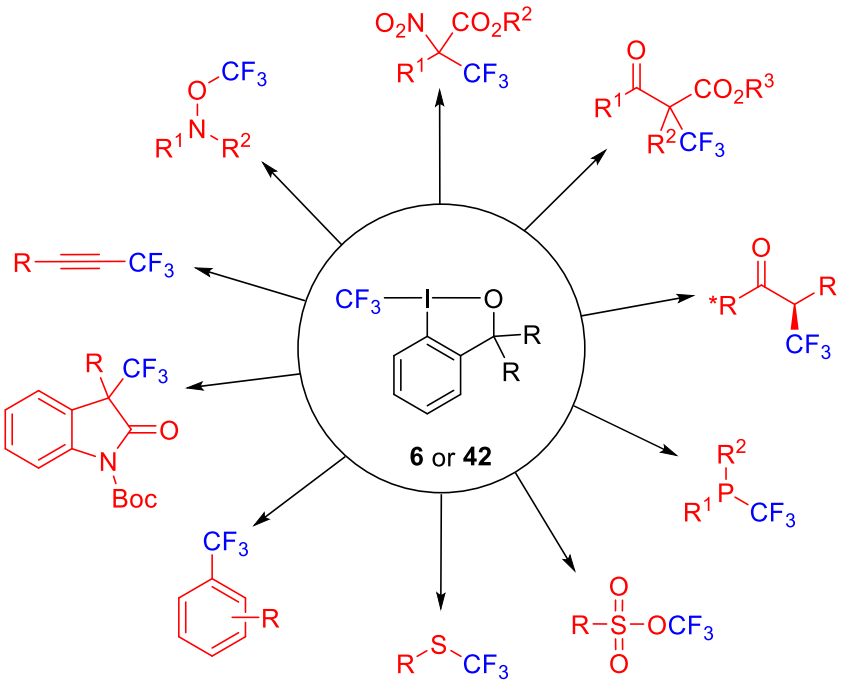
λ^3-Iodanes (10-I-3) possess a trigonal bipyramidal structure. These compounds exhibit a T-shape geometry where two ligands are connected to the iodine atom *via* a 3c-4e bond, which is the key feature in their reactivity. This bond is weaker than a normal covalent bond. Whereas ligands occupy axial positions, the least electronegative groups occupy equatorial position (Figure 5).



λ^3-iodanes

Figure 5 T-shape geometry of λ^3-iodanes.

HIRs have broad applications in organic synthesis. For instance, HIRs possessing trifluoromethyl groups have served in trifluoromethylations of a large array of nucleophiles.[80–82] The general reactivity of these compounds is illustrated in Scheme 29.



Scheme 29 Reactivity of Togni reagents **6** and **42** with various nucleophiles.

Transition-metal-catalyzed trifluoromethylation of alkenes utilizing Togni's reagents **6** and **42** was reviewed by Leroux and co-workers.[83]

Azidation, alkynylation, cyanation, and diazomethylation reactions was accomplished utilizing HIRs bearing different functionalities. These reagents also can be applied as oxidants for substrate activation, such as activation of organic acids, alcohols and C-H activation. A review article by Yang and Chen provided comprehensive details about numerous applications of HIRs using visible-light photoredox catalysis.[84]

Many other electrophilic trifluoromethylation reactions with various electrophilic trifluoromethylating reagents particularly **6** and **42** have been published and reviewed.[41,85–88]

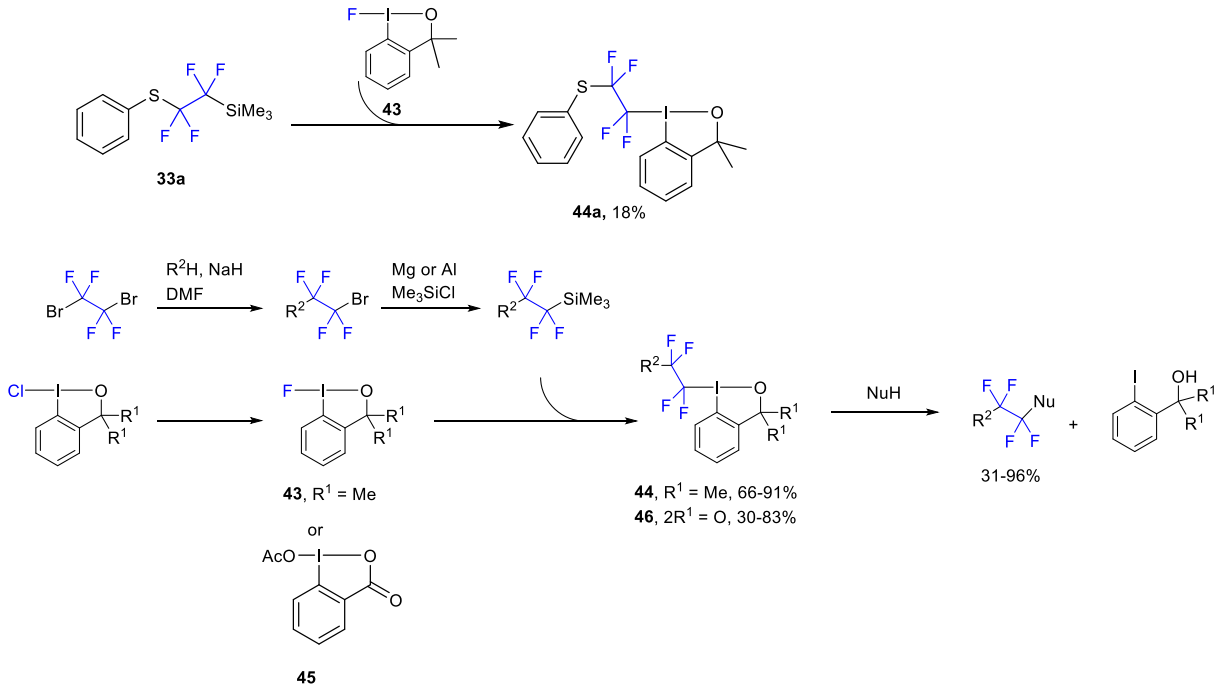
In connection with this thesis, reagents containing the CF_2CF_2 moiety are of our special interest and will be discussed in the next chapter.

1.2.3.2 CF_2CF_2 moiety, synthesis and transfer

In 2016, Matoušek and co-workers expanded the scope of parent hypervalent iodine reagent **6** and **42** to larger fluoroalkyl groups containing the CF_2CF_2 motif.[89]

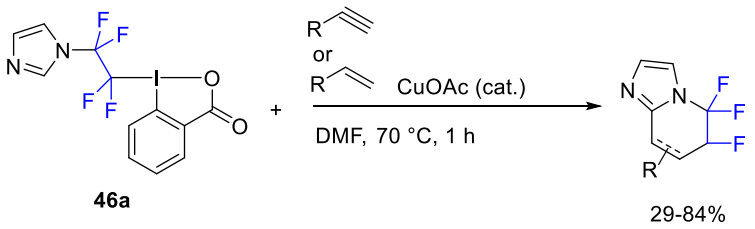
They were successful to prepare reagent **44a** *via* an *umpolung* reaction of silane **33a** with fluoroiodane **43** initiated by potassium fluoride, although in a very low yield (18%) (Scheme 21). Using TBAT (1 mol%) as initiator, increasing the concentration of the reaction, gradual addition of silane in a longer course of time, and performing the reaction at lower temperature, was found to be optimal for the reaction which led to the formation of reagent **44a** in 85% isolated yield. They developed an alternative protocol for the preparation of related reagents, so-called acid-type reagents, which are analogous to **42** by using fluoroiodane **45**.

Using optimum reaction conditions, several ArS, ArO, and *N*-heterocyclic based reagents of both alcohol and acid-type (**44** and **46)** bearing CF_2CF_2 moiety in their structure were prepared. The reagents enable electrophilic tetrafluoroethylation of various nucleophiles. For instance, tetrafluoroethylation of various aromatic and aliphatic thiols, hydroxylamines, a phosphine, as well as C-centered nucleophiles such as β-keto esters, enolates and arenes is reported (Scheme 30).[89]



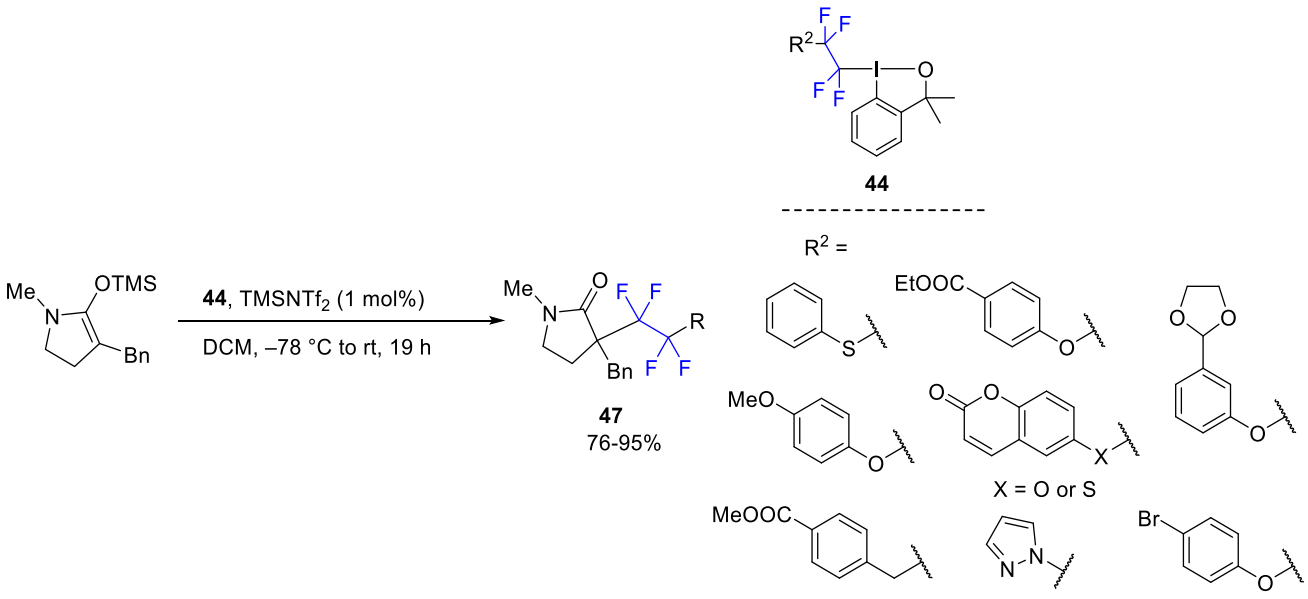
Scheme 30 Synthesis of reagents **44** and **46** and their use in tetrafluoroethylation of nucleophiles.

In 2016, Togni and co-workers employed imidazole reagent **46a** in the synthesis of tetrafluoro-imidazopyridines *via* radical fluoroalkylation cyclization reactions.[90] Using copper (I) acetate as the catalyst, they were able to prepare a range of fluorinated tetra- and dihydroimidazopyridines (Scheme 31). Various functional groups were well tolerated. Mechanistic studies revealed a radical origin of the reaction.



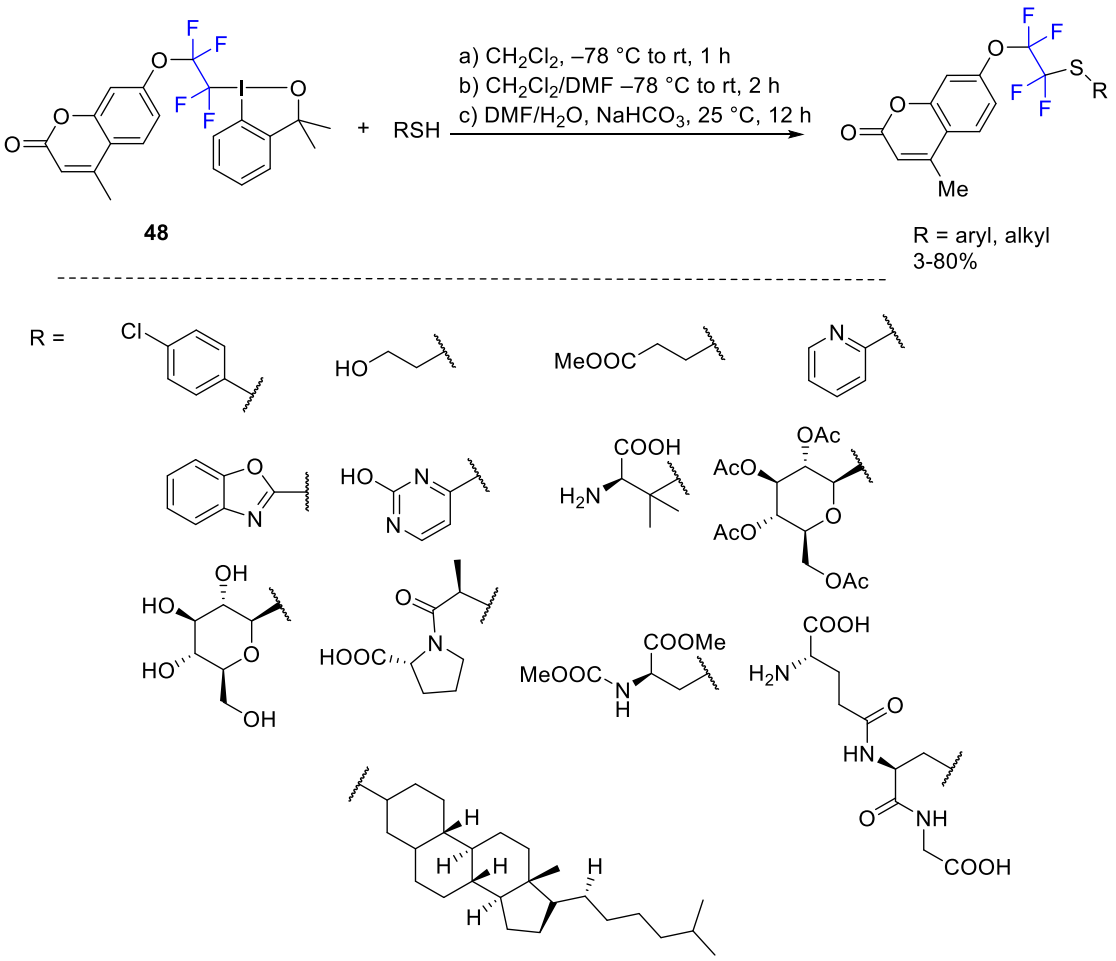
Scheme 31 Synthesis of dihydroimidazopyridines through fluoroalkylation and cyclization using **46a**.

Katayev and co-workers accomplished tetrafluoroethylation of ketene silyl amides (KSAs) derived from lactams. Using reagents **44**, and 3-benzyl-1-methyl-pyrrolidin-2-one in the presence of (Me_3SiNTf_2) as a Lewis-acidic catalyst they were able to prepare a range of α-fluoroalkyl lactams **34** containing the CF_2CF_2 moiety bearing different functionalities on the aromatic ring (Scheme 32).[91]



Scheme 32 Synthesis of α-fluoroalkyl lactams **47** using reagents **44**.

Commare and Togni described the synthesis of a new hypervalent iodine reagent **48** modified with tetrafluoroethoxy coumarin residue which was utilized for tagging of biologically relevant thiols such as captopril (antihypertensive drug), thiocholesterol, 1-thio-β-D-glucose, and thiouracil (Scheme 33).[92]

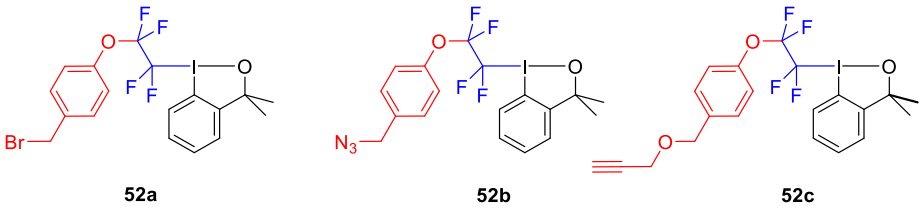


Scheme 33 Tagging of thiols with hypervalent iodine reagent modified with coumarin.

In 2017, Václavík and co-workers published a new class of hypervalent iodine reagents containing a secondary amine group, which allowed late-stage derivatization resulting in the preparation of new reagents containing amide, sulfonamide and tertiary amine. These reagents were then functionalized resulting in the formation of reagents bearing different functionalities, such as halogen, azide, boronate ester, aldehyde, nitrile, sulfonyl fluoride, and alkyne and units, such as

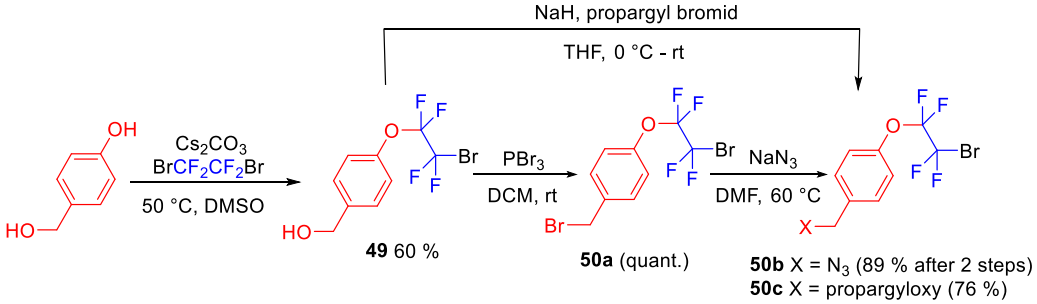
tetraethylene glycol, biotin, and several fluorophores (pyrene, coumarin, fluorescein, and rhodamine).[93]

They synthesized three types of hypervalent iodine reagents (**52a-c**) which could be further functionalized *via* CuACC, SPACC or nucleophilic substitution (Scheme 34).



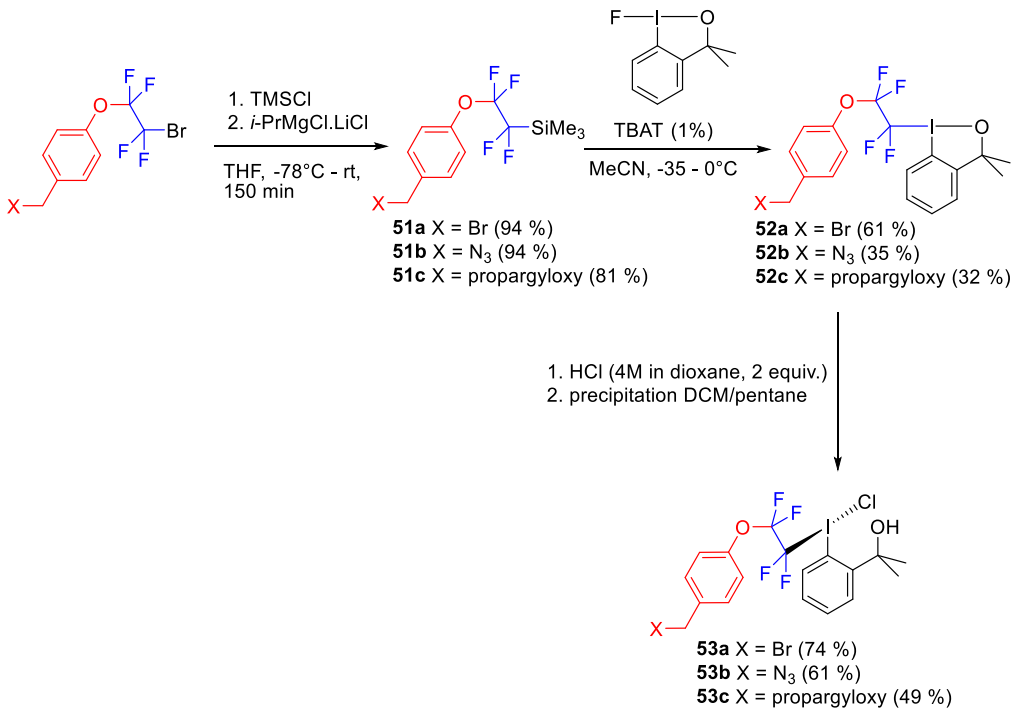
Scheme 34 Precursors of hypervalent iodine reagents made by late-stage modification.

Synthesis started with 4-(hydroxymethyl) phenol. After selective deprotonation of phenolic OH, it was further converted to **49** upon reaction with halon. The free hydroxyl group was then modified to the desired functional group **50a-c** (Scheme 35).



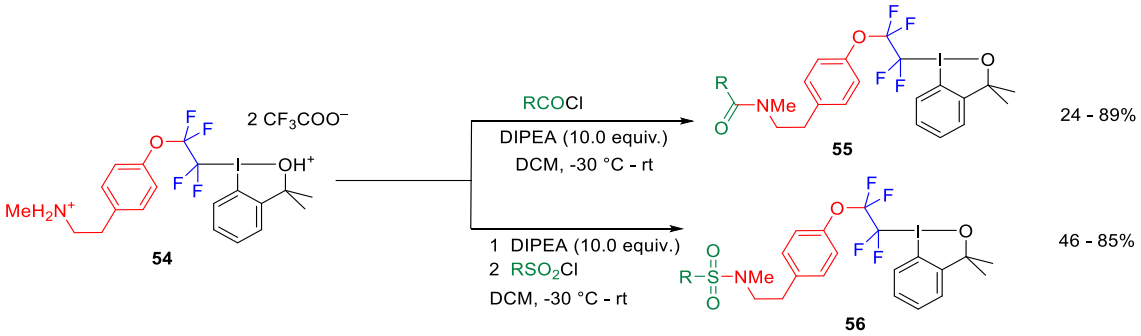
Scheme 35 Synthetic protocol for the preparation of **50a-c**.

Products of this reaction were later converted to the corresponding silanes **51** in high yields. Eventually, the desired products **52a-c** were synthesized *via umpolung* reactions of silanes using a procedure reported by Matoušek (Scheme 36).[89] However, the final products were not stable and decomposed to 2-(2-iodophenyl)propan-2-ol. This problem was solved by converting the final reagent to the corresponding iodonium salt **53a-c** upon reaction with HCl (Scheme 36).



Scheme 36 Synthesis of reagents **39a-c** and their salt forms **40a-c**.

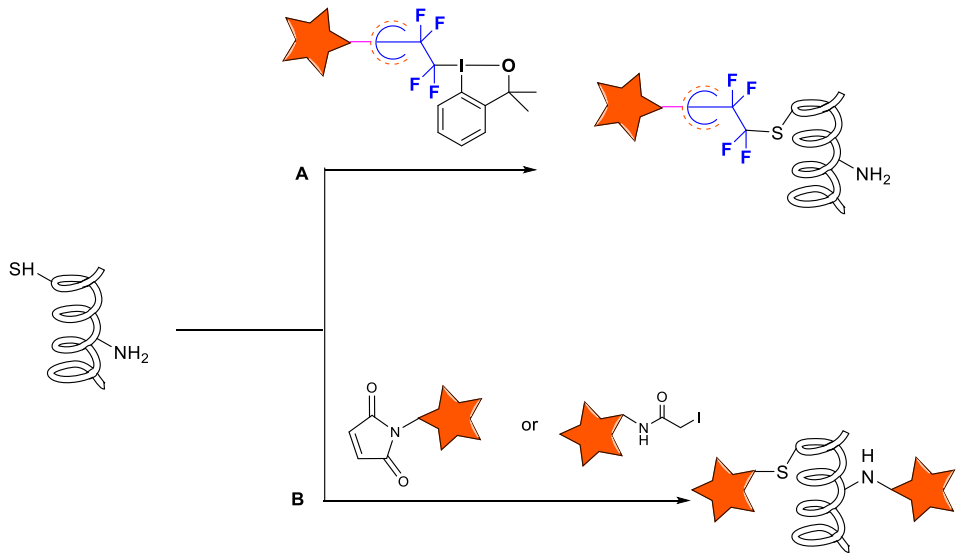
Then, reagent **54** was developed, which allowed the preparation of new potential reagents bearing sulfonamide and amide functionality, compound **55** and **56** respectively (Scheme 37).



Scheme 37 Modification of **54** to amide **55** and sulfonamide **56**.

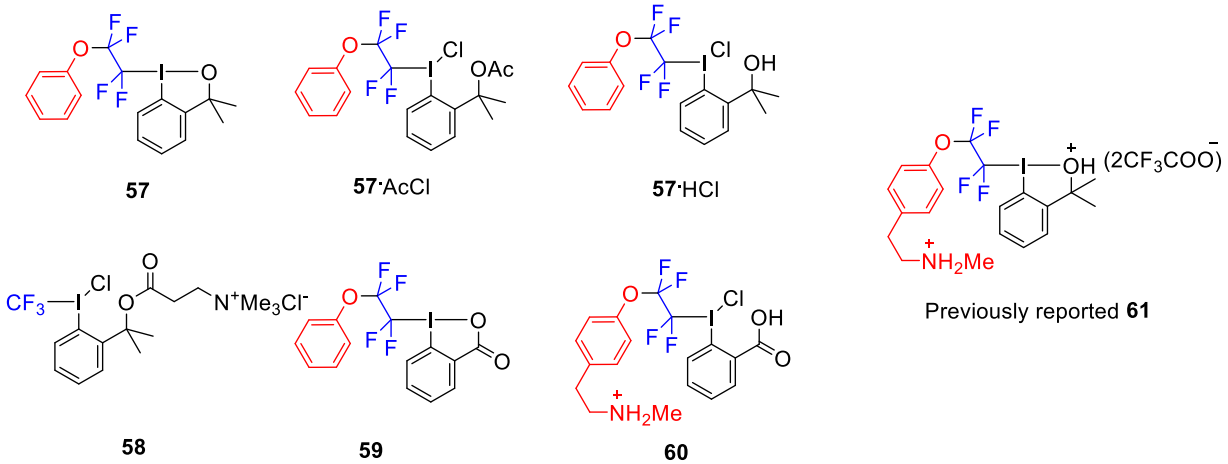
These attractive highly functionalized reagents were applied for labeling of cysteine residues in a selective and irreversible manner. Reagents bearing dansyl and fluorcein moiety were further applied for modification of an enzyme (retroaldolase RA95.5-8 S25C K210M). The enzyme

contained an exposed cysteine and a buried lysine, which is responsible for activity of the enzyme. In comparison to commercially available Atto-565-maleimide and 6-(iodoacetamido)fluorescein which labeled both lysine and cysteine, the novel hypervalent iodine reagents targeted cysteine selectively (Scheme 38).[93]



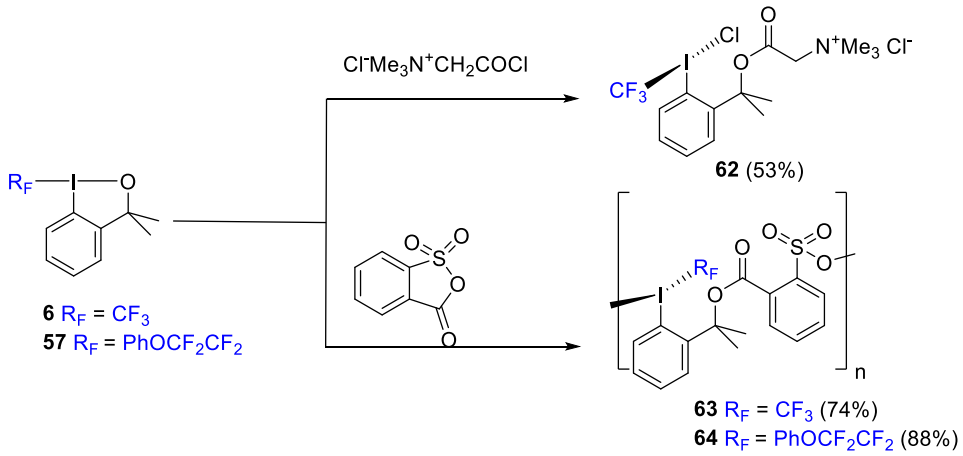
Scheme 38 Comparing the selectivity of **A**: novel highly functionalized hypervalent iodine reagents bearing the CF_2CF_2 functionality with **B**: traditional reagents.

Recently, Klimánková and co-workers reported a new series of water soluble hypervalent iodine reagents **57-60** which were used for tagging of biological thiols (Scheme 39).



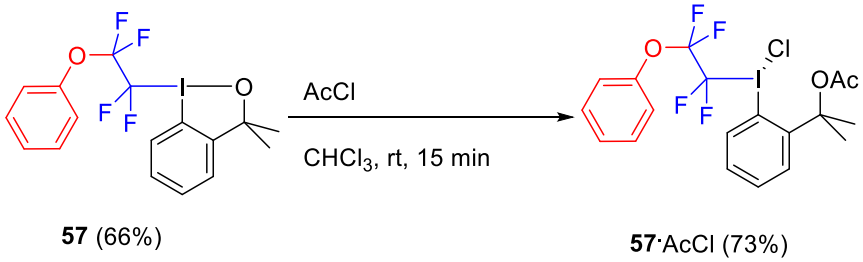
Scheme 39 Structure of open chain water-soluble reagents **57-60** reported by Klimánková and previously reported **61**.

The parent alcohol-type Togni reagent **6** reacted with a charge-carrying betaine chloride to give **62**. To enhance the solubility and stability of the reagents they attempted to introduce negatively charged groups on the reagent. Therefore, **6** and **57** were reacted with 2-sulfobenzoic acid anhydride to give **63** and **64**. Although the resulting reagents were stable they showed poor solubility in water due to the formation of polymeric structures (Scheme 40).



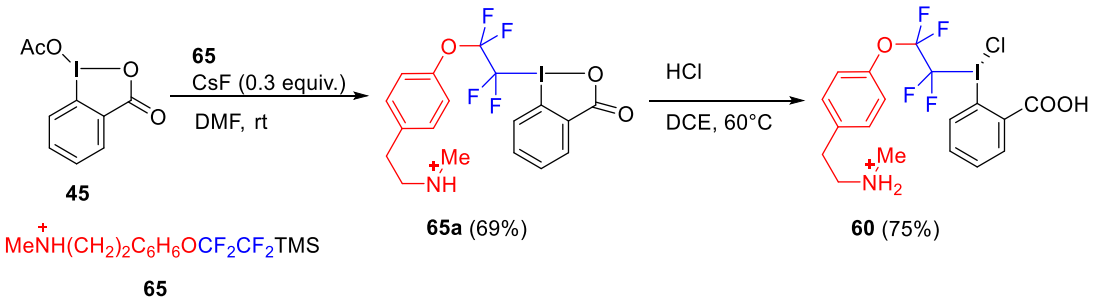
Scheme 40 Synthesis of **62** and polymeric reagents **63** and **64**.

Reagent **57**·AcCl was prepared by the reaction of **57** with acetyl chloride (Scheme 41). This compound also did not show good reactivity toward thiols due to its low solubility in water.



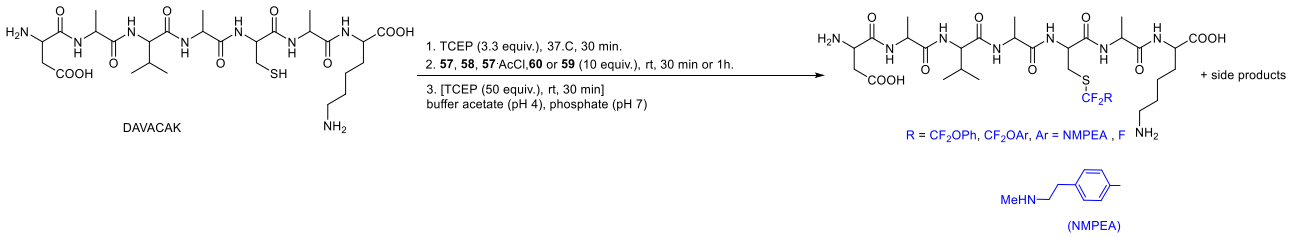
Scheme 41 Synthesis of **57**·AcCl.

Reagent **65a** was synthesized from 1-acetoxy-1,2-benziodoxol-3-(1*H*)-one and the related silane **65** was then converted to **60** in DMF in a relatively high yield (Scheme 42).



Scheme 42 Synthesis of **60**.

Salt **58** was found to be a good reagent for S-fluoroalkylation of cysteine due to its high efficiency over the large range of pH values; however, it showed limited stability in aqueous media. Other reagents, such as **60** and previously reported **61**, showed limited reactivity out of a range of specific pH values.[94] In addition, application of selected reagents for thiol bioconjugation on a heptapeptide containing cysteine was demonstrated. Aside from the fluoroalkylated peptide many other side-products were formed, which was attributed to sulfur oxidation, elimination and the involvement of hypervalent iodine reagent in acylation of nitrogen (Scheme 43).



Scheme 43 Fluoroalkylation of heptapeptide DAVACAK with water-soluble hypervalent iodine reagents.

A review article reported by Václavík and co-workers provides extensive information on the synthesis and application of compounds containing tetrafluoroethyl or tetrafluoroethylene moieties.[95]

1.3. Bioconjugation

Bioconjugation is a burgeoning branch of research on the border of chemistry and biology.[96,97] The establishment of seemingly simple covalent bond between a biomolecule and another

molecule gives access to novel compounds which are not found in nature. The novel bioconjugate contains properties of its individual components as a more efficient construct. The chemically modified proteins found numerous applications, such as antibody-drugs[98] and PEGylated biologics.[99] In accordance with this thesis, bioconjugation of aromatic amino acids will be discussed in the next chapter.

1.3.1 Bioconjugation of aromatic amino acids

In recent years, C-H functionalization of aromatic amino acids have become a popular tool in protein modification. The low abundance of aromatic amino acids on proteins makes them special targets for selective bioconjugation. The process is highly dependent on the intrinsic reactivity of the amino acid and its environment.[100] For instance, targeting histidine and tyrosine residues is pH dependent and modification of tyrosine residues takes place more efficiently at higher pH. Phenyl alanine residues on the other hand are unreactive in most bioconjugation reactions.

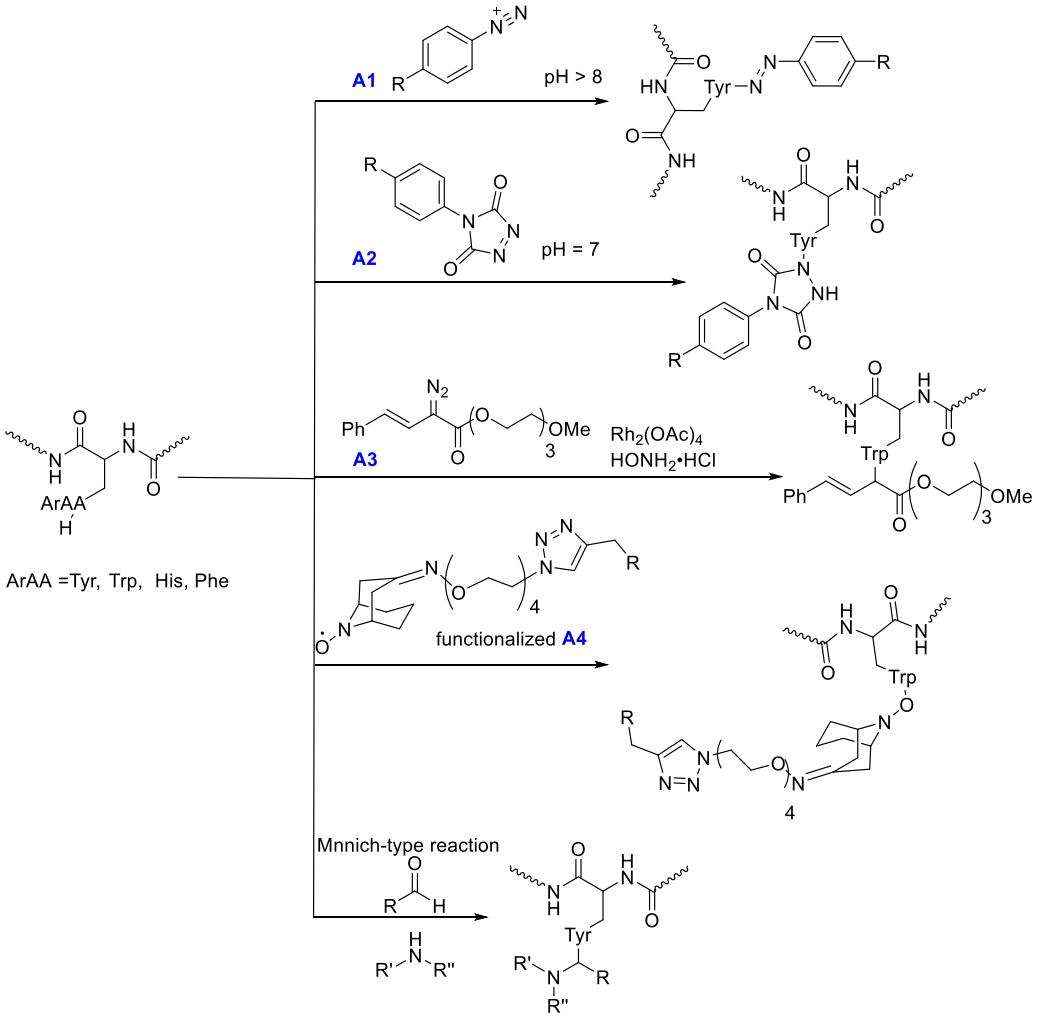
The use of chemical groups that react with specific functional groups is one of the oldest methods in selective modification of amino acids. For instance, highly reactive diazonium salts are utilized for targeting histidine and tyrosine residues in proteins. The diazonium salt **A1** reacted readily with histidine residue at pH 8; however, relatively higher pH was required for the modification of tyrosine residues. This way, tyrosine residues were modified at *ortho*- position of the phenol ring (Scheme 44). This method however, is not applicable for pH sensitive proteins where the preparation of diazonium salts from anilines requires highly acidic conditions. Diazo compounds also undergo cleavage at higher pH by sodium dithionite and sodium borate. These factors together limit the widespread use of diazonium salts in bioconjugations.

Mannich-type reaction provided better conditions for the selective modification with respect to the formation of stronger bonds in comparison to the diazo functionality and the use of milder reaction conditions. Selective modification of tyrosine residue was achieved using the Mannich-type reaction under very mild reaction conditions (pH 6.5, 25–37 °C) and low protein concentration (20–200 mM). However, high incubation times were necessary to achieve a reasonable level of tagging.[101]

Ban and co-workers accomplished selective modification of tyrosine residues using PTAD derivatives **A2** bearing azido, alkyne and ketone groups *via* a tyrosine click chemistry. They demonstrated a good potential of this reaction in PEGylation of proteins such as chymotrypsinogen. Whereas, commonly used methods such as maleimide-type reactions provided less selective approach, tyrosine click approach worked selectively with click reaction at neutral pH. This way, an antibody-drug conjugate with potent HIV neutralization activity was synthesized (Scheme 44).[102]

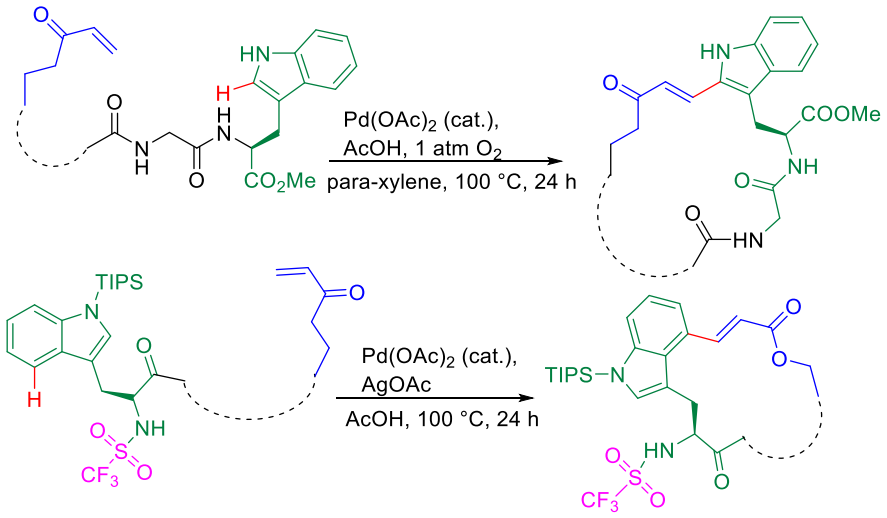
Selective modification of tryptophan residues using metallocarbenoids in aqueous media was reported by Francis and co-workers. A rhodium carbene derived from stabilized vinyl diazo compound **A3** and rhodium catalyst, reacted selectively with nitrogen and C(2) of tryptophan residues (Scheme 44). The authors described successful modification of horse heart myglobin with this method in aqueous media and a small amount of co-solvent.[103]

Seki *et al*. reported a transition-metal-free protocol for selective modification of tryptophan residues under ambient conditions in proteins by using stabilized aminoxy radical **A4**. The method tolerated various functional groups in the structure of protein allowing the protein to maintain its structural integrity and allowed the successful tagging of an antibody without disturbing its reactivity (Scheme 44).[104]



Scheme 44 Selective tagging of aromatic amino acid residues in peptides and proteins.

Wang group conducted transition-metal-catalyzed C-H olefination of tryptophan residues in peptides using a Pd catalyst (Scheme 45).[105] Coupling between alkene functional group present in the backbone of peptide and tryptophan ring at C(2) or C(4) position allowed access to peptide macrocycles.



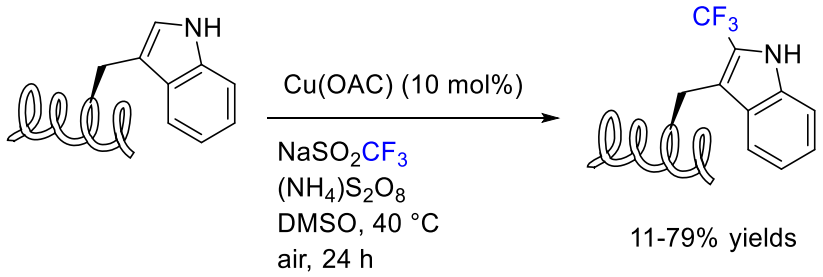
Scheme 45 C-H olefination of tryptophan residues at C(2) and C(4) positions reported by Wang group.

1.3.2 Fluoroalkylation and bioconjugation

As mentioned before, fluorine containing molecule/biomolecules have significant advantages over non-fluorinated counterparts. Particular advantages of this chemistry include 1. Stabilization of biomolecules by increasing its resistance toward oxidative metabolism upon introduction of the fluoroalkyl substituent[106], 2. Increasing membrane permeability, 3. Access to new mechanism-based enzyme inhibitors due to the high electronegativity and relatively small size of fluorine atom, 4. NMR active ^{19}F nucleus allowing a straightforward analysis and mechanistic studies.[107]

Tryptophan is an important and attractive target in biological studies due to its low abundance on protein surface. However, modification of tryptophan residues with reagents containing fluoroalkyl moieties is rather rare and underdeveloped.

Very recently, Correa and co-workers developed a protocol for selective trifluoromethylation of tryptophan residues in several peptides using Langlois reagent in the presence of a copper catalyst. Several oligopeptides containing tryptophan residues underwent trifluoromethylation at C(2) of the indole ring in low to good yields (Scheme 46).[108] However, the protocol was not tested in the presence of other sensitive aromatic amino acids such as tyrosine and histidine.



Scheme 46 C-H trifluoromethylation of tryptophan residues in several oligopeptides reported by Correa.

The same reagent was used in photoredox (iridium-catalyzed) direct trifluoromethylation of tryptophan residues in peptides which allowed the study of fluorinated bioconjugates with ^{19}F NMR. Mechanistic studies revealed that the reaction proceeds *via* a radical-radical cross-coupling pathway. The procedure was not selective to tryptophan and other aromatic amino acids, such as phenyl alanine and tyrosine were also trifluoromethylated, however in lower yields in comparison to tryptophan.[109]

Davis and co-workers accomplished selective radical trifluoromethylation of tryptophan residues in peptides and proteins by using sodium trifluoromethanesulfinate (CF_3SO_2Na) and *tert*-butylhydroperoxide. The procedure allowed the preparation of fluorinated protein constructs for ^{19}F NMR studies.[110]

2. AIMS OF THE PROJECT

This thesis deals with the use of hypervalent iodine reagents (Togni-CF_3 reagents and novel tetrafluoroethyl containing reagents) for the transfer of fluoroalkyl groups into electron-rich aromatics, such as indoles and pyrroles. The main goal of the work is to utilize fluoroalkyl reagents as a tool for selective tagging of tryptophan residues in peptide and proteins.

We reported two new protocols involving free radicals for selective tagging of tryptophan residues in several peptides and proteins. The first protocol deals with targeting tryptophan residues under ambient condition, based on the use of water-soluble biocompatible reductant and the second protocol deals with achieving the same goal by using a light source capable of excitation the hypervalent iodine reagent and electron transfer from electron-rich substrate to the electron-deficient iodine reagent.

3. RESULT AND DISCUSSION

3.1 Preparation of hypervalent iodine reagents

In the first part of the work we prepared HVI reagents needed for our studies. Structures of HVI reagents used in this study are shown in Figure 6. The compounds were prepared in three steps as illustrated in Scheme 47. These reagents are stable and include previously reported **69** and **70**[111] bearing imidazolyl functionality, commercially available Togni reagents **6** and **42**, new reagents **57** and **59** and salts **57**·AcCl, **61'** and **60**. Matoušek and Václavík reported that the reagent **61'** allowed late-stage derivatization resulting in the preparation of new reagents containing amide, sulfonamide and tertiary amine bearing different functionalities such as halogen, azide, boronate ester, aldehyde, nitrile, sulfonyl fluoride, and alkyne and units such as tetraethylene glycol, biotin, and several fluorophores (pyrene, coumarin, fluorescein, and rhodamine) which was discussed in detail in Chapter 1.2.3.2.[93]

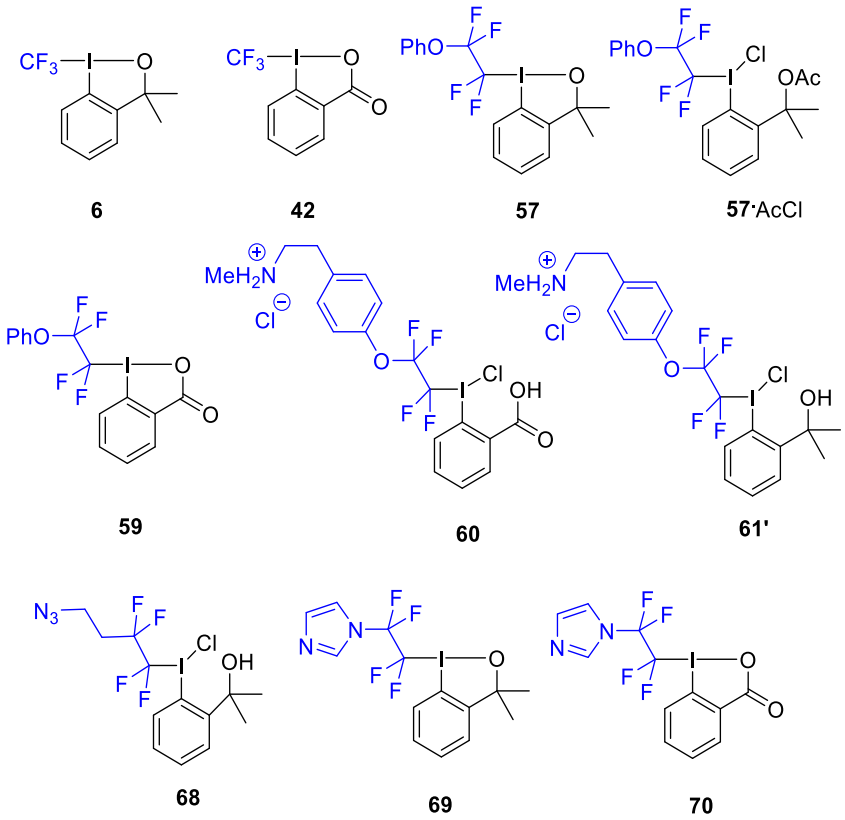
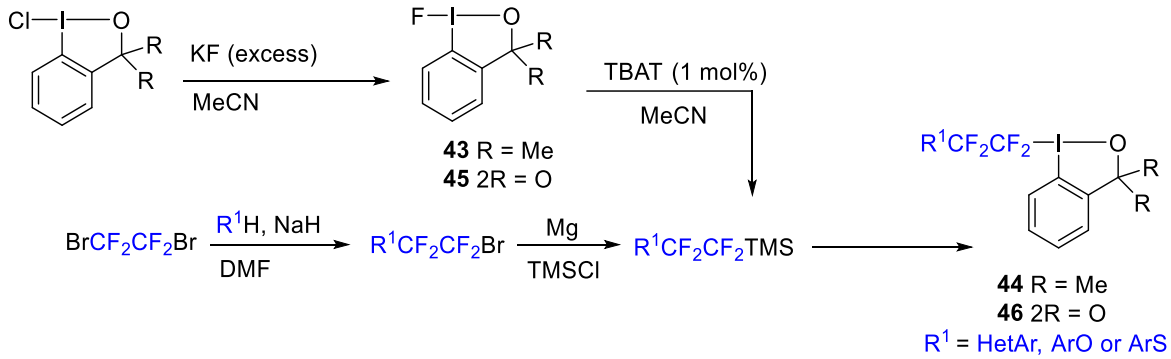


Figure 6 Structures of HVI reagents used in this study.

They showed these reagents essentially share similar reactivity of previously reported parent trifluoromethyl Togni reagents **6** and **42** and can be applied for fluoroalkylation of biological compounds.[93]

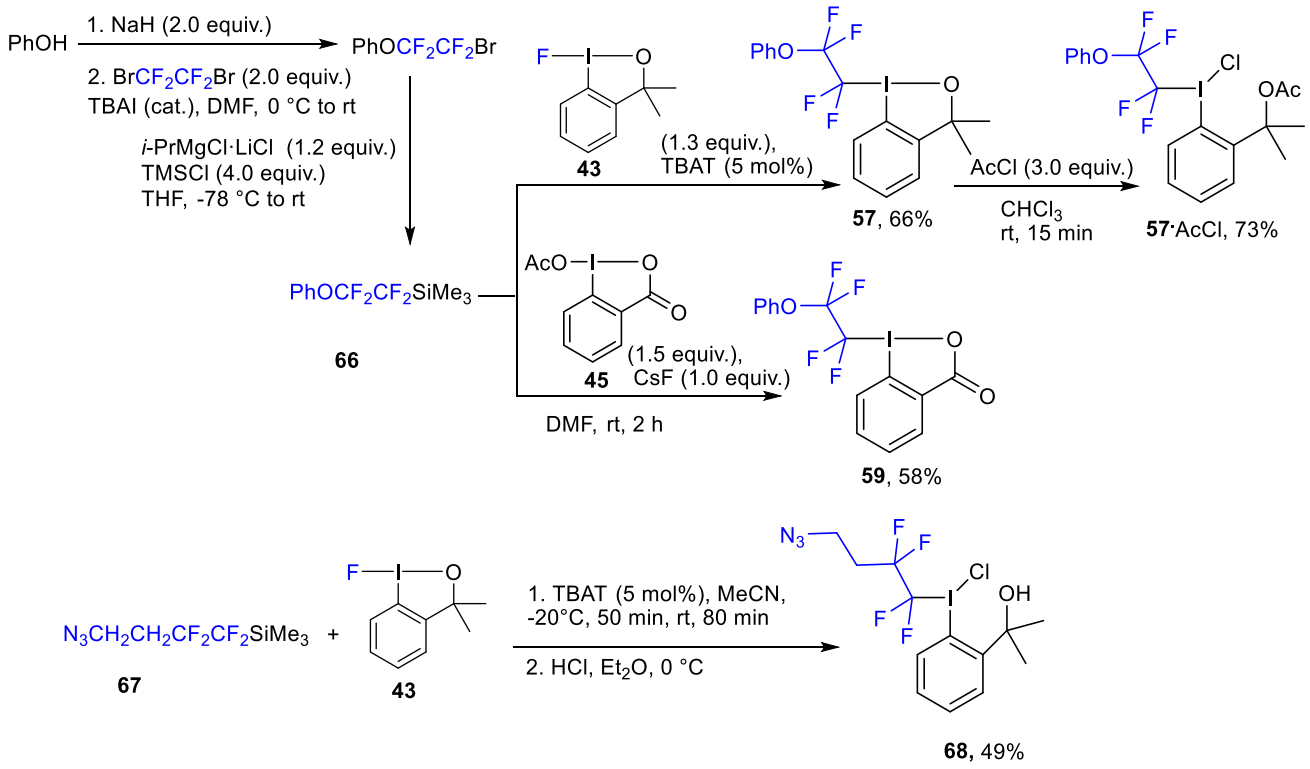


Scheme 47 Synthetic protocol for preparation of tetrafluoroethyl reagents.

Iodanes **57**, **57**·AcCl, **59**, **60** and **68** (Scheme 48) were prepared by *umpolung* reactions of appropriate fluoroalkylsilanes with fluoro- or acetoxyiodanes followed by the reaction with acetyl chloride or protonation with HCl.[112]

Synthesis of **57** and **59** started from phenol, which was deprotonated and further modified with 1,2-dibromo-1,1,2,2-tetrafluoroethane. Further reaction with trimethylsilyl chloride (TMSCl) in the presence of isopropylmagnesium chloride lithium chloride (Turbo Grignard) provided silane **66**, which was further converted to **57** and **59** *via umpolung* reaction. **57**·AcCl was prepared according the procedure reported by Klimánková.[94] Reagent **57** was reacted with acetyl chloride in chloroform affording **57**·AcCl in 73% yield (Scheme 48).

The reagent **68** bearing the azide functionality was prepared *via* the reaction of **43** with 4-azido-1-trimethylsilyl-1,1,2,2-tetrafluorobutane (**67**) in acetonitrile (Scheme 48).



Scheme 48 Structures and synthesis of HVI reagents used in the study.

The described reagents were used in radical fluoroalkylation of small molecules such as indoles, pyrroles and aromatic amino acids, also selective tagging of tryptophan residues in peptides and proteins as discussed in the following chapters.

3.2 Fluoroalkylation of small substrates in the presence of water-soluble reductant

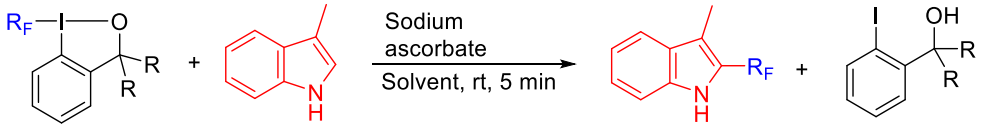
3.2.1 Fluoroalkylation of indoles and pyrroles

To optimize the reaction condition, 3-methylindole (skatole) was used as an electron-rich substrate. This compound has been previously reported to undergo fluorolkylation using **6**, **42** and **69** in the presence of $ZnBr_2$, TMSOTf, $Zn(NTf_2)_2$, $MeReO_3$ or CuOAc (20-80 °C).[89,113,114] We examined **59** in tetrafluoroethylation of skatole in the presence of number of reductants using aqueous methanol or ethanol as solvents (Table 1).

Using NaH_2PO_3, CuOAc, Na_2SO_3, $FeCl_2$ or Et_3B as reductants, led to the formation of product **73a** in low yields (5-23% ^{19}F NMR yield, Table 1, entries 17-21); however, the use of sodium ascorbate provided **73a** in 42% ^{19}F NMR yield (Table 1, entry 1). Further optimization of temperature, reaction time, solvent, the amount of sodium ascorbate and reagent revealed that using a slight excess of **59** in the presence of 50 mol% of sodium ascorbate in aqueous methanol were optimal for the reaction (Table 1). The reaction was finished within 5 minutes under ambient conditions. Further investigations revealed that the order of addition of components was crucial to achieve maximum yield of **73a**. Adding the reagent as the last component to the reaction mixture led to the highest product conversion.

Under optimal reaction conditions (Table 1, entry 11) the scope of radical fluoroalkylation of various indole derivatives was investigated (See part 4.3). While fluoroalkylation of indole derivatives bearing electron-rich groups such as alkyl, cycloalkyl or other electron-rich groups in positions 1, 2 or 3 afforded fluoroalkylated products in positions 2 or 3 in moderate yields, indoles with electron-acceptor groups provided low product yields (Scheme 49).

Table 1 Optimization of reaction condition for radical fluoroalkylation of 3-methylindole with HVI reagents in the presence of sodium ascorbate.

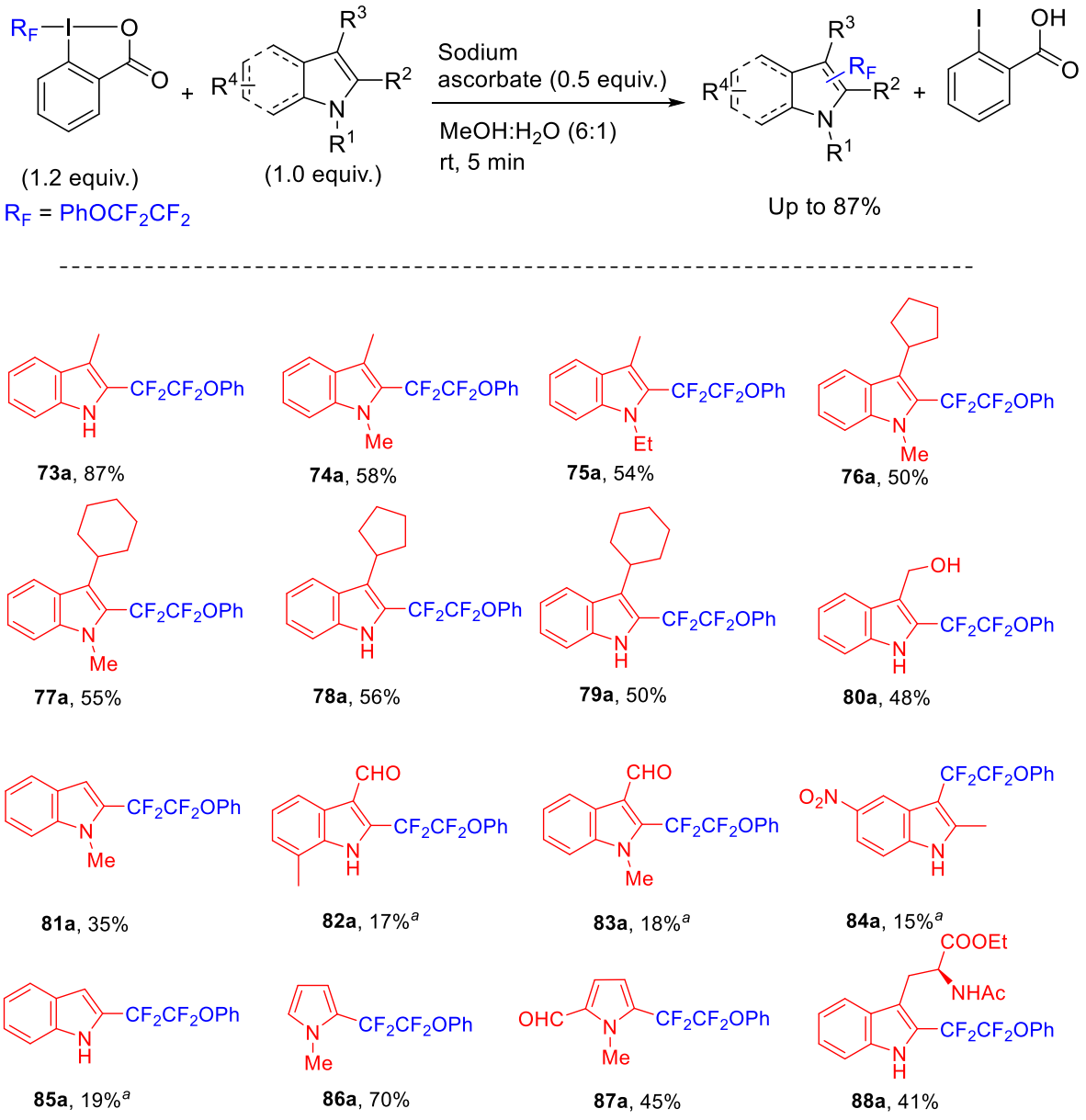


HVI reagent

71 R_F = CF_3
72 R_F = Imidazoyl-CF_2CF_2
73a R_F = $PhOCF_2CF_2$

Entry	HVI reagent (equiv.)	Reductant (equiv.)	Solvent	Yield (%)[a]
1[b]	**59** (1.0)	Sodium ascorbate (0.5)	EtOH	**73a**, 42
2	**59** (1.0)	Sodium ascorbate (0.5)	EtOH/H_2O (1:1)	**73a** ,44
3	**59** (1.2)	Sodium ascorbate (0.5)	Dioxane/H_2O (1:1)	**73a**, 30
4	**59** (1.0)	Sodium ascorbate (0.5)	MeOH/H_2O (6:1)	**73a**, 49
5	**59** (1.2)	Sodium ascorbate (0.5)	MeOH/H_2O (6:1)	**73a**, 53
6	**59** (1.5)	Sodium ascorbate (0.5)	MeOH/H_2O (6:1)	**73a**, 59
7[c]	**59** (1.2)	Sodium ascorbate (0.5)	MeOH/H_2O (6:1)	**73a**, 53
8[d]	**59** (1.2)	Sodium ascorbate (0.5)	MeOH/H_2O (6:1)	**73a**, 32
9	**59** (1.2)	Sodium ascorbate (1.0)	MeOH/H_2O (6:1)	**73a**, 46
10	**59** (1.2)	Sodium ascorbate (0.05)	MeOH/H_2O (6:1)	**73a**, 39
11[e]	**59** (1.2)	Sodium ascorbate (0.5)	MeOH/H_2O (6:1)	**73a**, 98
12[e]	**57** (1.2)	Sodium ascorbate (0.5)	MeOH/H_2O (6:1)	**73a**, 91
13[e]	**6** (1.2)	Sodium ascorbate (0.5)	MeOH/H_2O (6:1)	**71**, 34
14[e]	**42** (1.2)	Sodium ascorbate (0.5)	MeOH/H_2O (6:1)	**71**, 53
15[e]	**69** (1.2)	Sodium ascorbate (0.5)	MeOH/H_2O (6:1)	**72**, 63
16[e]	**70** (1.2)	Sodium ascorbate (0.5)	MeOH/H_2O (6:1)	**72**, 54
17	**59** (1.2)	NaH_2PO_3	MeOH/H_2O (6:1)	**73a**, 5
18	**59** (1.2)	CuOAc	MeOH/H_2O (6:1)	**73a**, 5
19	**59** (1.2)	Na_2SO_3	MeOH/H_2O (6:1)	**73a**, 10
20	**59** (1.2)	$FeCl_2.4H_2O$	MeOH/H_2O (6:1)	**73a**, 23
21	**59** (1.2)	Et_3B	MeOH/H_2O (6:1)	**73a**, 10

[a] ^{19}F NMR yield is reported using (2,2,2-trifluoroethanol) as an internal standard. [b] Reaction time was 1 h. [c] Reaction was conducted at -78 °C. [d] Reaction was conducted at 40 °C. [e] Reaction was done by slow addition (over 5 min) of a solution of HVI reagent in MeOH to the solution of 3-methylindole and sodium ascorbate in aq. MeOH.



Scheme 49 Fluoroalkylation of indole and pyrrole derivatives using **59** in the presence of sodium ascorbate. Isolated yields are presented. [a] ^{19}F NMR yield is shown using (2,2,2-trifluoroethanol) as an internal standard.

3.2.2 Evaluation of reactivity of aromatic amino acids

The transition from harsh reaction conditions such as non-aqueous systems, using transition metal catalysts, high temperature and relatively slow reactions to mainly aqueous condition with non-metal activators which was achieved with ascorbate chemistry, is a highly attractive alternative in

the bioconjugation field. We observed that Togni reagents can undergo single electron transfer (SET) with sodium ascorbate to release a fluoroalkyl radical which can further react with electron-rich substrates such as skatole or tryptophan. Having all this facts in mind we aimed to test the ability of HVI reagents in tagging of biologically relevant compounds containing electron-rich amino acids, such as Trp, Phe, His, and Tyr in their structure. The experiments were done in collaboration with CF plus chemicals and Dr. Petr Novák group.

Trifluoromethylation of aromatic amino acids was evaluated using small excess of **42** (See part 4.4). Under the reaction condition, tryptophan was much more reactive than other amino acids such as tyrosine, histidine and phenylalanine. Performing the reaction under basic condition led to the increasing the reactivity of tyrosine and histidine which were initially less reactive. This finding suggests that under basic conditions, the OH group of tyrosine or imidazolium ring of histidine is deprotonated resulting in the formation of more electron-rich and more reactive substrates (Figures 7-13 and Table 2).

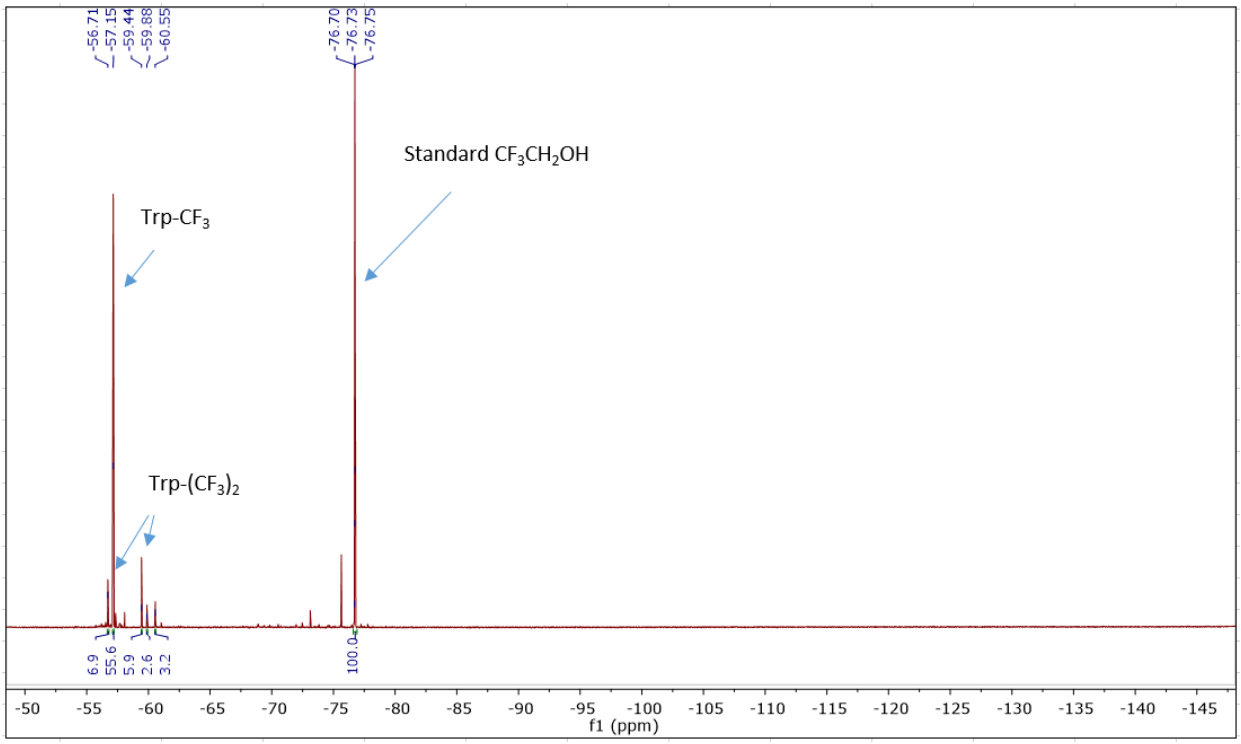


Figure 7 ^{19}F NMR (376 MHz, CD_3OD) of the reaction mixture using *N*-acetyl Trp (MeOH/water).

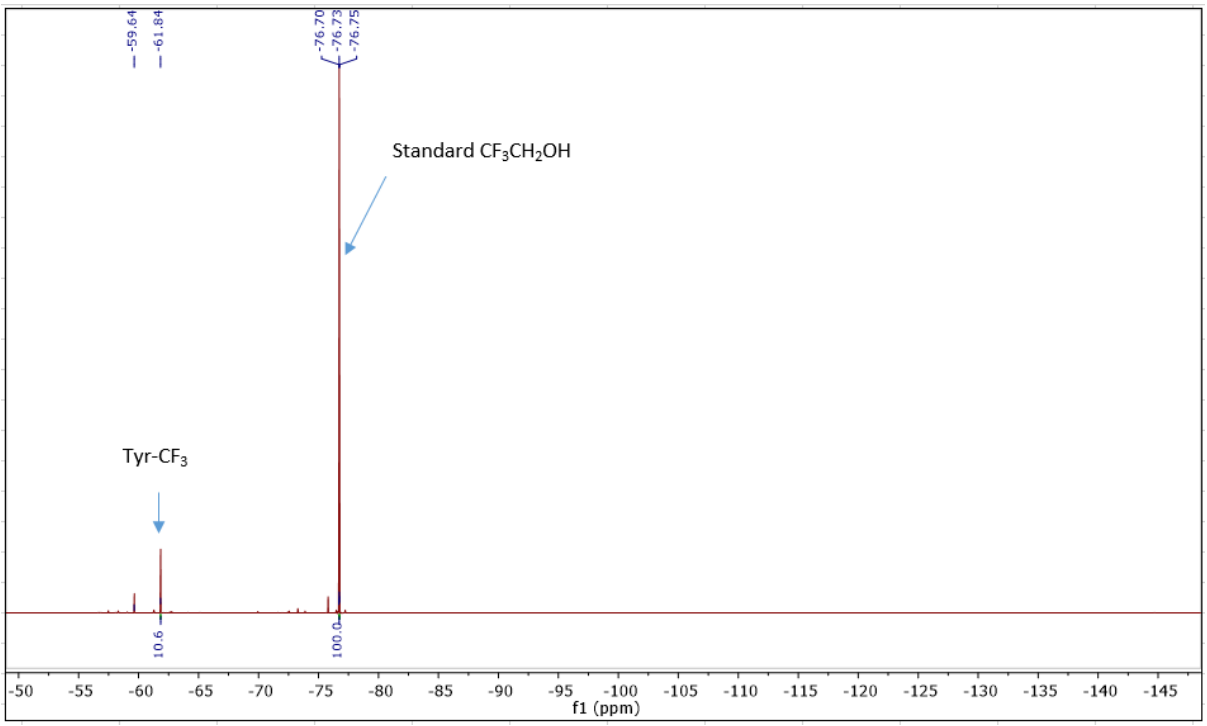


Figure 8 ^{19}F NMR (376 MHz, CD_3OD) of the reaction mixture using *N*-acetyl Tyr (MeOH/water).

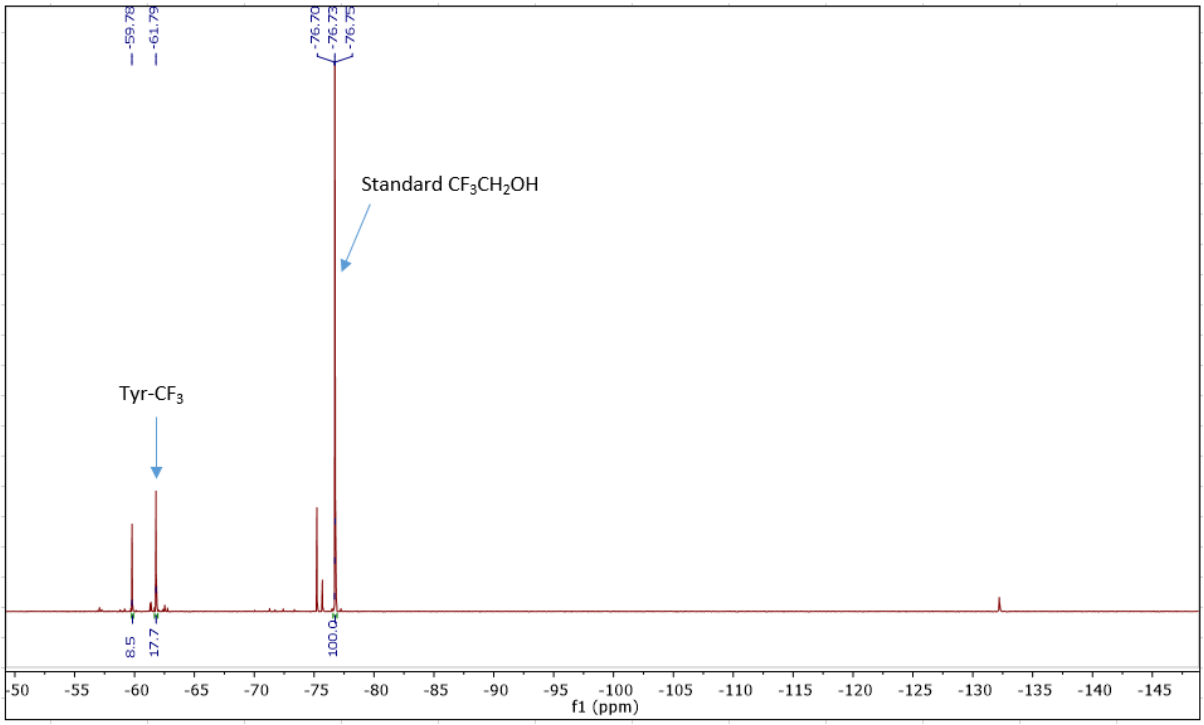


Figure 9 ^{19}F NMR (376 MHz, CD_3OD) of the reaction mixture using *N*-acetyl Tyr (MeOH/buffer pH 9).

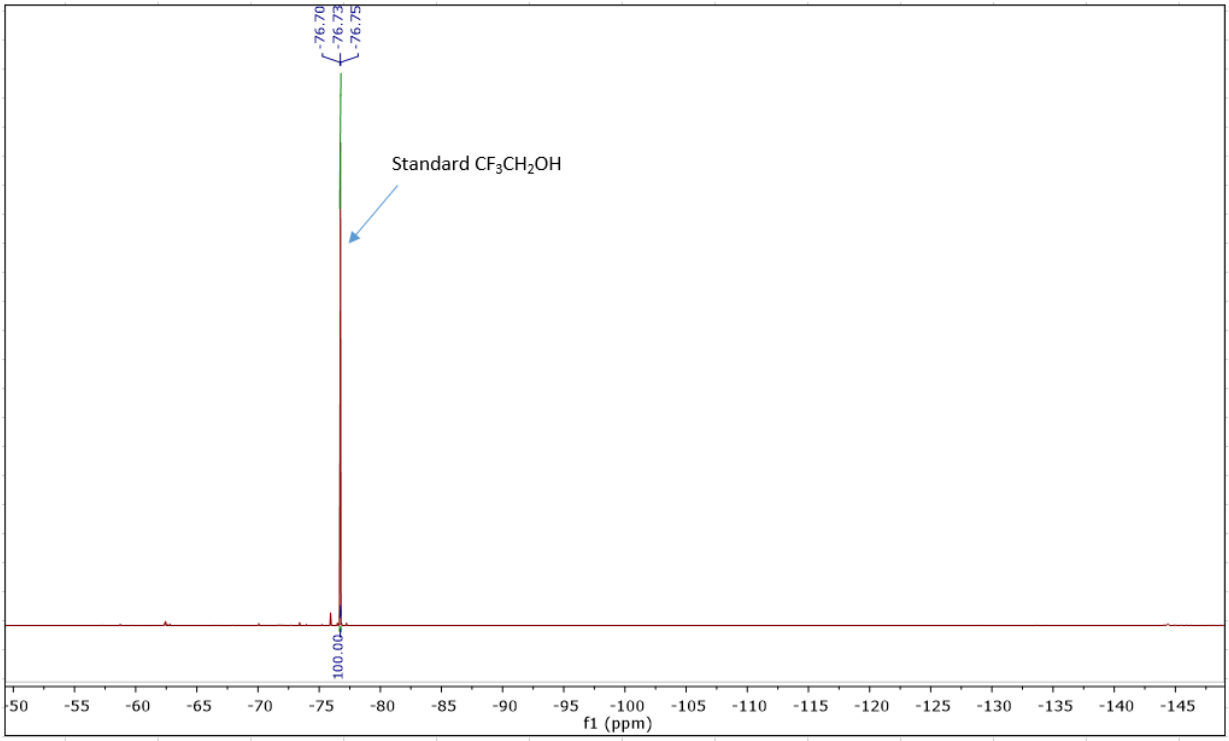


Figure 10 ^{19}F NMR (376 MHz, CD_3OD) of the reaction mixture using Phe ethyl ester (MeOH/buffer pH 5).

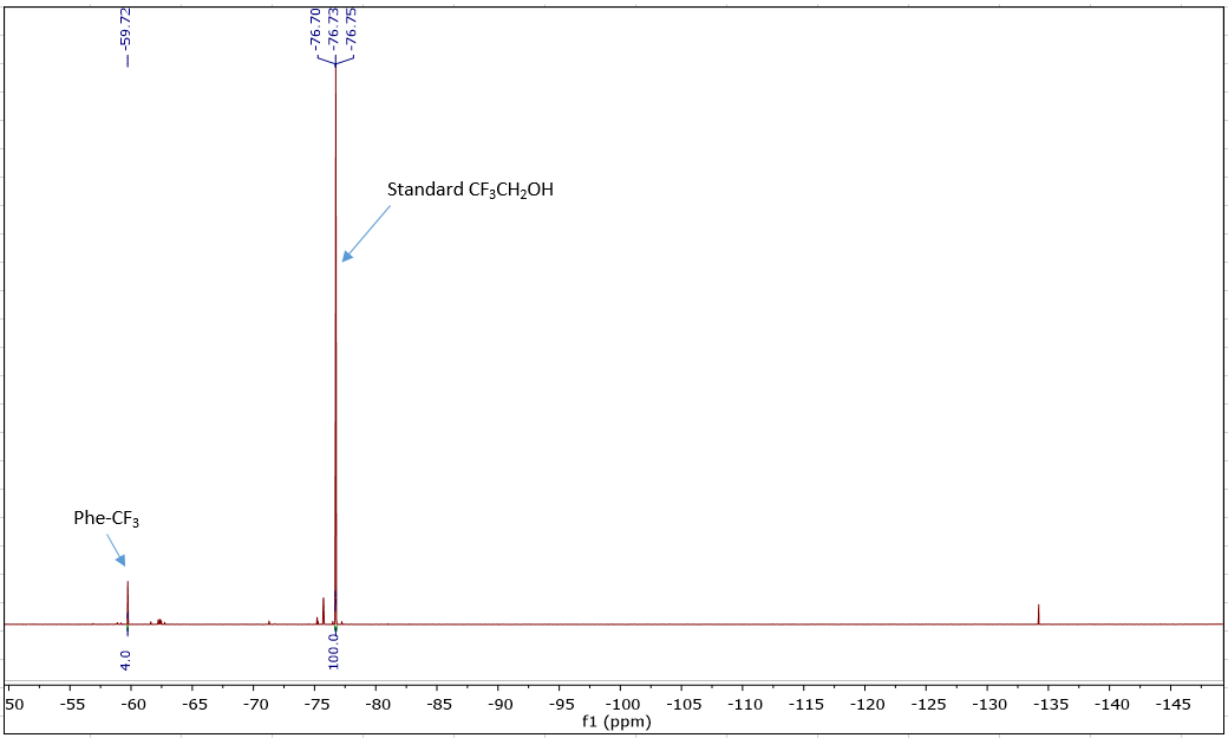


Figure 11 ^{19}F NMR (376 MHz, CD_3OD) of the reaction mixture using Phe ethyl ester (MeOH/buffer pH 9).

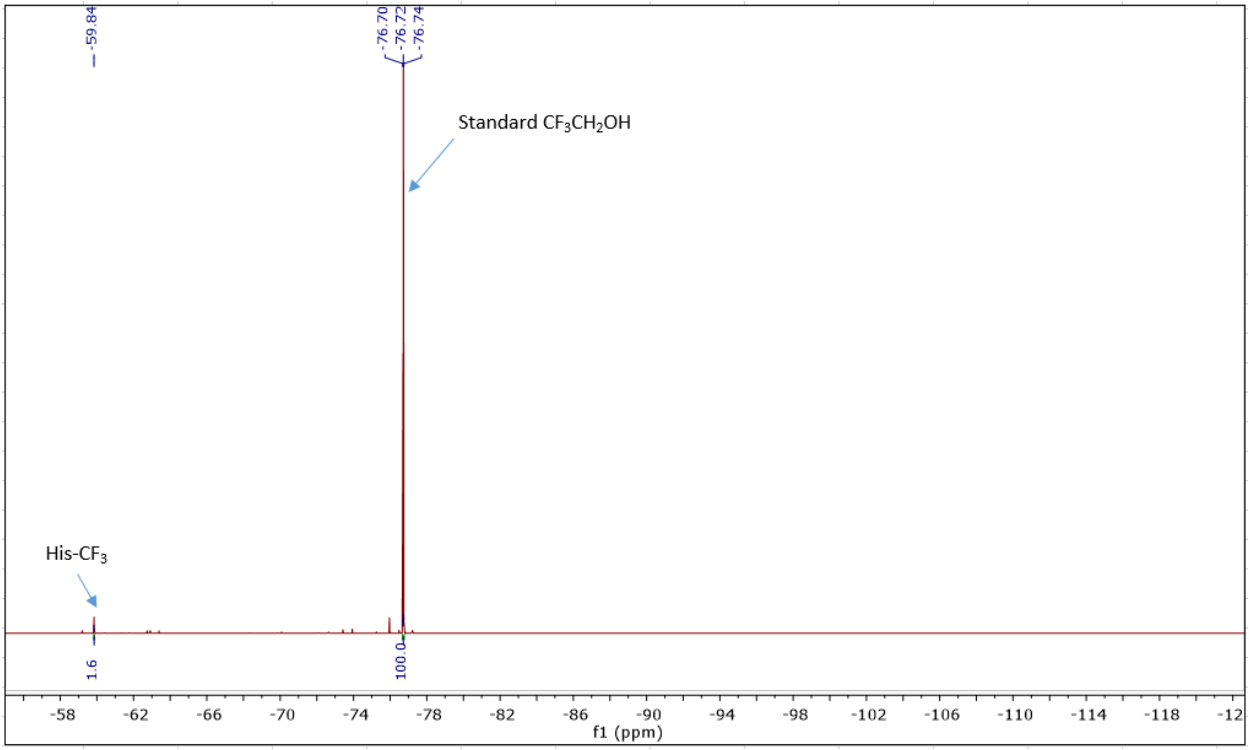


Figure 12 ^{19}F NMR (376 MHz, CD_3OD) of the reaction mixture using His (MeOH/buffer pH 5.

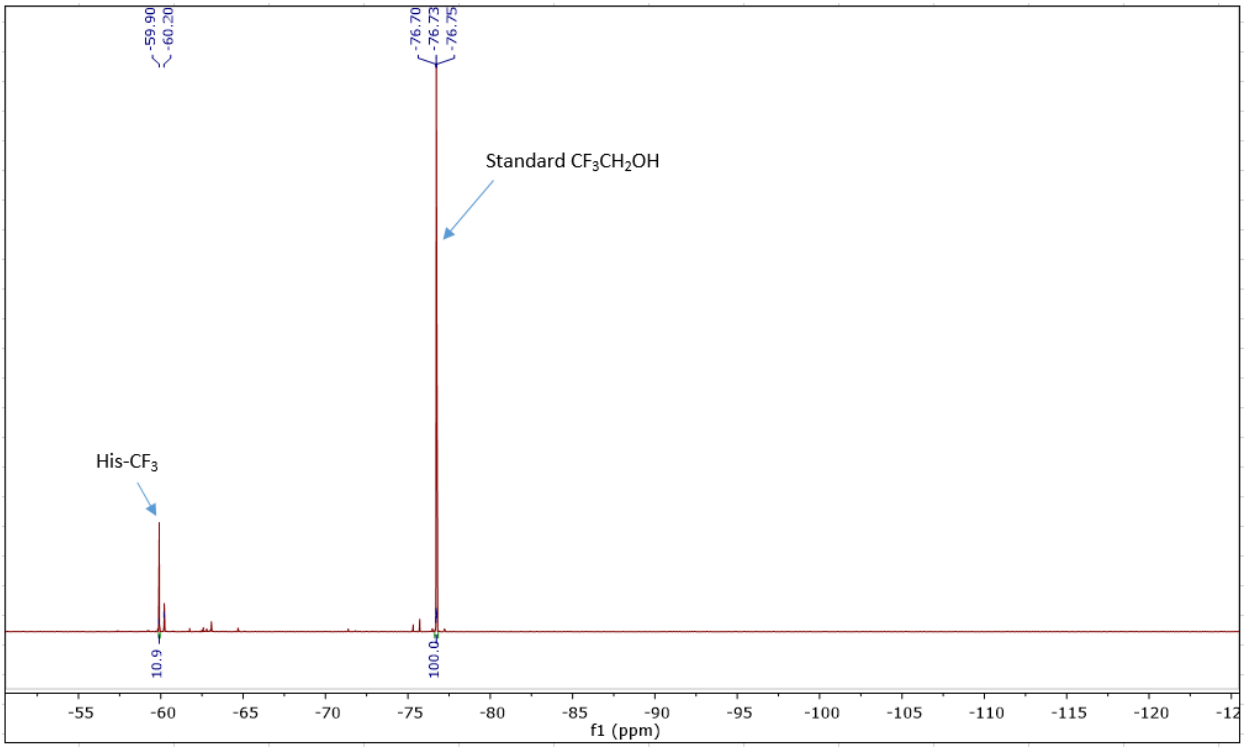
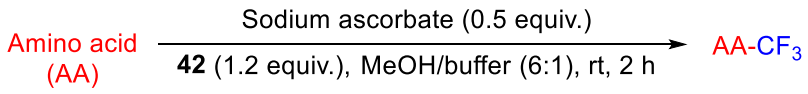


Figure 13 ^{19}F NMR (376 MHz, CD_3OD) of the reaction mixture using His (MeOH/buffer pH 9).

Table 2 Fluoroalkylation of aromatic amino acid derivatives using **42**.



Entry	Amino acid deriv.	pH	Product yield (%)[a]
1	*N*-Ac Trp	[b]	56 + 6[c]
2	*N*-Ac Tyr	[b]	11
3	*N*-Ac Tyr	9	18
4	Phe[d]	5	0
5	Phe[d]	9	4
6	His·HCl	5	2
7	His·HCl	9	11

[a] ^{19}F NMR yields are shown using (trifluoroethanol) as an internal standard. [b] Water instead of buffer was used. [c] Mono- and bis(trifluoromethylation), respectively. [d] Phenylalanine ethyl ester hydrochloride was used.

A competitive trifluoromethylation reaction was evaluated using **6** (10 equiv. to each amino acid) and half the amount of sodium ascorbate (See part 4.5). For this aim, an equimolar mixture of all natural amino acids (5 mM, pH 7.5) was used. The result was analyzed using semiquantitative LCMS, which showed the reactivity of amino acids as follow: Trp >> Cystine > Tyr > Phe > His. Under the given reaction conditions, other amino acids were not reactive. Extracted ion chromatograms revealed that when a large excess of **6** was used, tryptophan underwent mono- and bis(trifluoromethylation, two isomers). Phenylalanine afforded two isomers of mono(trifluoromethylated) products. Cysteine provided trifluoromethylated product probably by the cleavage of S-S bond in presence of trifluoromethyl radicals followed by radical recombination (Figure 14).

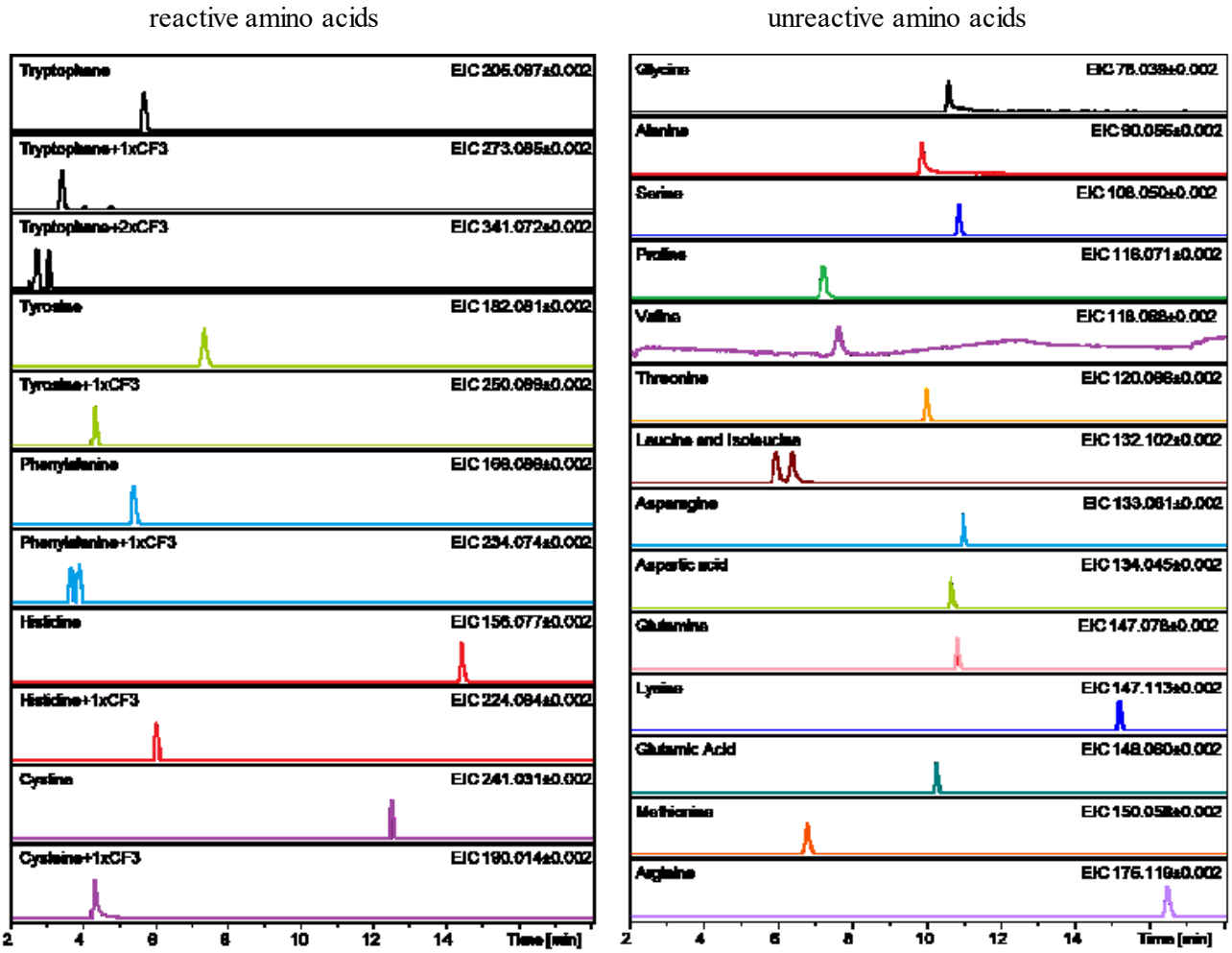


Figure 14 Extracted ion chromatograms of HPLC-MS analysis of a mixture of all natural amino acids in trifluoromethylation with **6** (10 equiv. calculated to each amino acid) and sodium ascorbate (5 equiv. calculated to each amino acid) in buffer (pH 7.5), rt, 15 min.

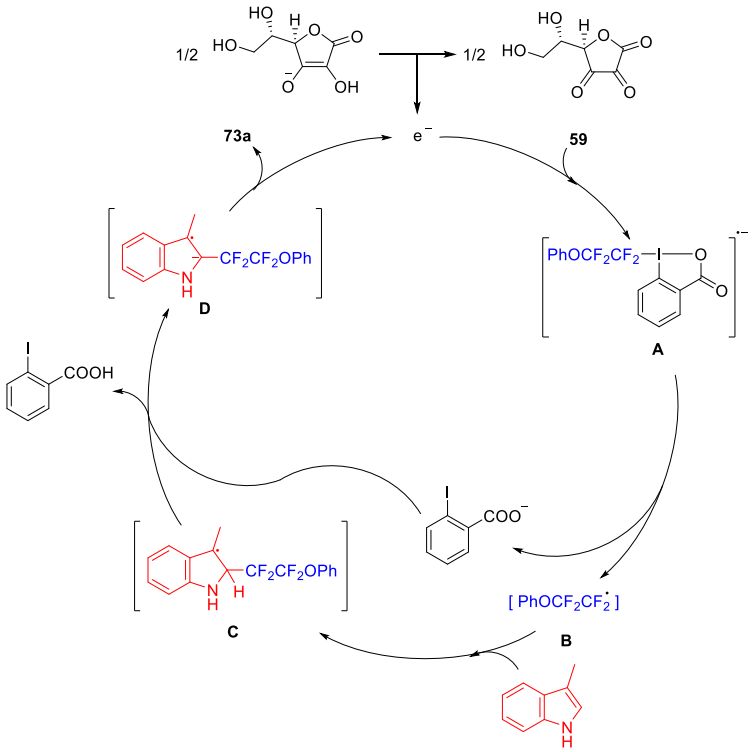
Furthermore, the reactivity of aromatic amino acids toward the reaction with **42** and **70** was evaluated (See part 4.6). MS analysis revealed that tryptophan was much more reactive an afforded mono- and bis(fluoroalkylated) products, while other amino acids were less reactive (Table 3).

Table 3 MS conversions of fluoroalkylated aromatic amino acids in reactions with **42** and **70** in the presence of sodium ascorbate.

Entry	HVI reagent (equiv.)	MS conversion (%)			
		Trp-R_F + Trp-$(R_F)_2$	Tyr-R_F	Phe-R_F	His-R_F
1	**42** (10)	75 + 8	12	2	<1
2	**42** (50)	38 + 62	40	10	7
3	**70** (10)	72 + 14	19	5	0
4	**70** (50)	0 + 100	26	9	0
1	**42** (10)	75 + 8	12	2	<1

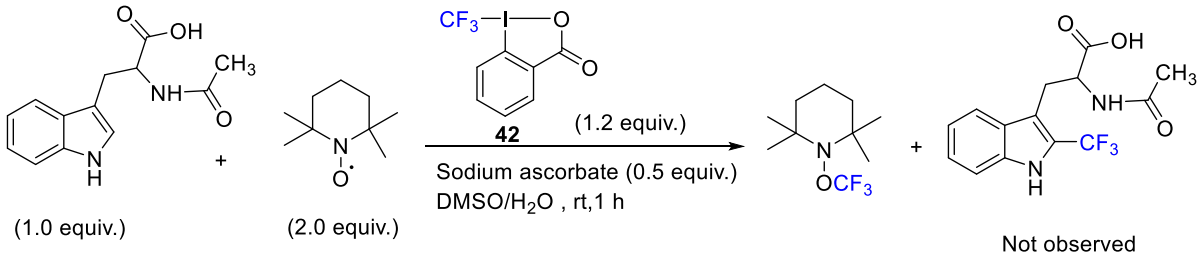
3.3 Mechanism of reductant-induced fluoroalkylation

A likely mechanism for this interesting transformation is shown in Scheme 50. Ascorbate first undergoes a single electron transfer (SET) to Togni reagent **59** resulting in the formation of transient radical anion **A** which further decomposes to 2-iodobenzoate and radical **B**. Radical addition to 3-methyl indole generates stabilized benzylic radical **C** which transfers proton to 2-iodobenzoate to form anion **D**. Intermediate **D** then undergoes SET to form product **73a** closing the catalytic cycle involving electron as a catalyst as proposed by Studer and Curran.[115]



Scheme 50 Proposed reaction mechanism for tetrafluoroethylation of 3-methylindole in the presence of sodium ascorbate and reagent **59**.

A control experiment was conducted to support the idea that fluroalkylation proceeds *via* radical pathway (See part 4.8). For this aim, *N*-acetyl tryptophan reacted with **42** in presence of TEMPO which afforded TEMPO-CF_3 adduct in 43% ^{19}F NMR yield and no trifluoromethylated tryptophan was observed (Figure 15).



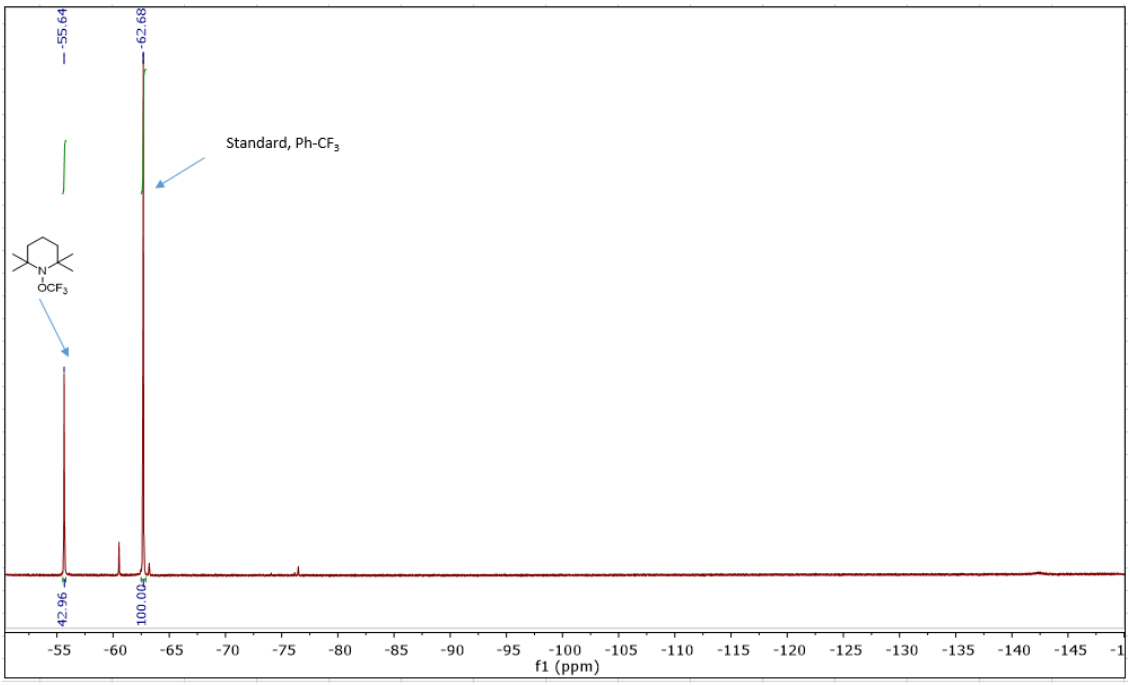


Figure 15 ^{19}F NMR (376 MHz, $CDCl_3$) spectrum of the crude reaction mixture of reaction of *N*-Ac Trp in the presence of **42**, TEMPO and sodium ascorbate.

3.4 Selective fluoroalkylation of aromatic amino acid residues in peptides and proteins using HVI reagents

After observing the reactivity of natural amino acids including aromatic ones we hypothesized that HVI reagents could selectively react with tryptophan residues in peptides and proteins. With the library of HVI reagents in hand, we explored their applicability in forming bioconjugates. Several short peptides containing aromatic amino acids were tested for fluoroalkylation (Figure 16).

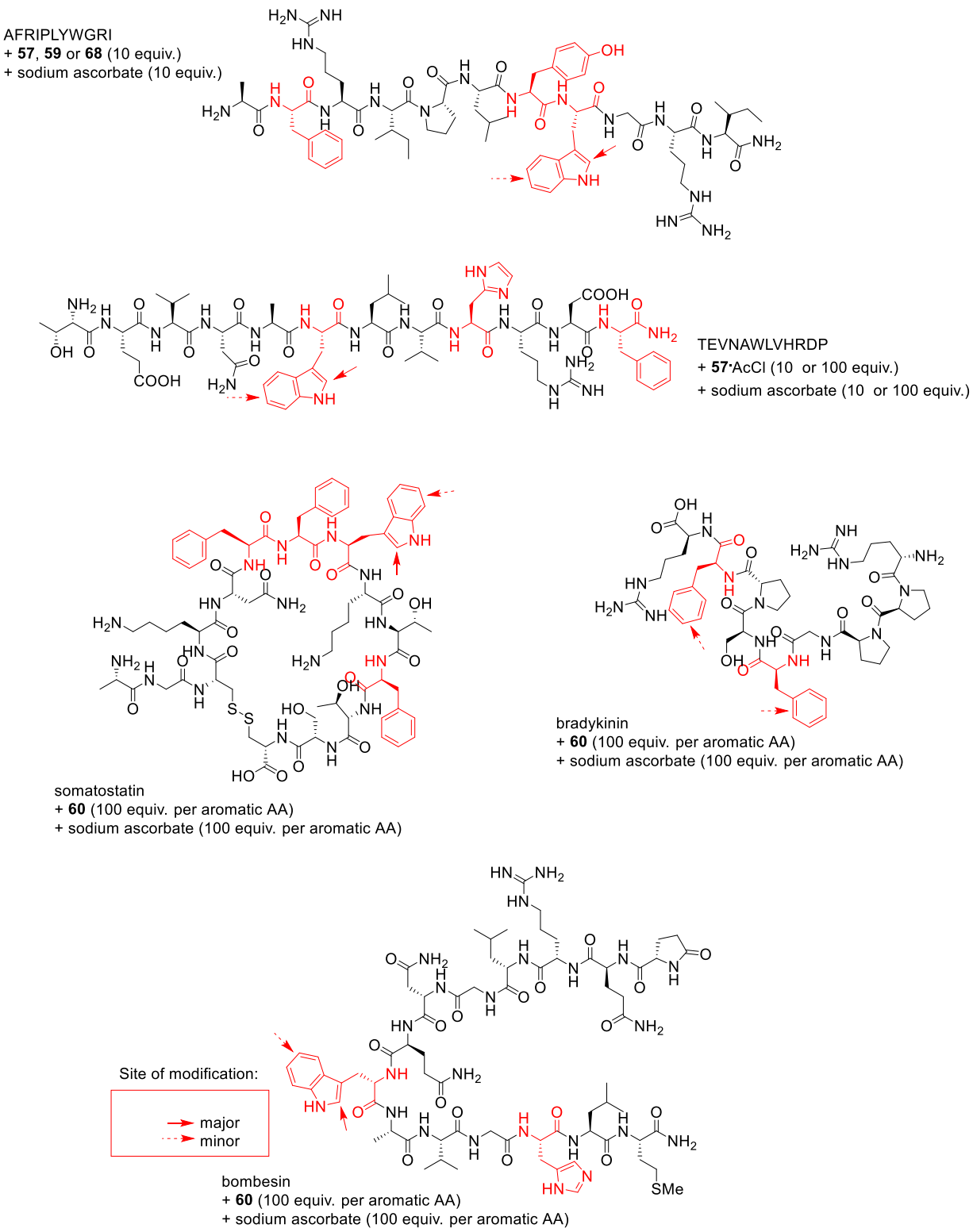


Figure 16 Structures of peptides AFRIPLYWGRI, TEVNAWLVHRDP, bombesin, somatostatin and bradykinin with aromatic amino acid residues highlighted in red. The observed major and minor sites of modification are indicated by red arrows. Sites of trace modification are not shown.

A small peptide, containing reactive amino acids with the amino acid sequence (AFRIPLYWGRI) underwent fluoroalkylation with excess amount of **57**, **59** or **68** (10 equiv.) and sodium ascorbate in aqueous acetonitrile as the solvent (See part 4.7). Peptide AFRI was successfully modified with HVI reagents and afforded mono- and bis(fluoroalkylated) products as the major and minor products respectively (Figures 16 and 17). Subsequent MS/MS analysis revealed that both modifications took place exclusively on Trp residue (Figure 18). However, in all the experiments, formation of oxidized products in small amounts was frequently observed which was minimized either by adding methionine to the reaction mixture or using 50 mol% of sodium ascorbate relative to HVI reagent. The oxidized products are most probably oxindole which was formed from the indole ring of tryptophan.

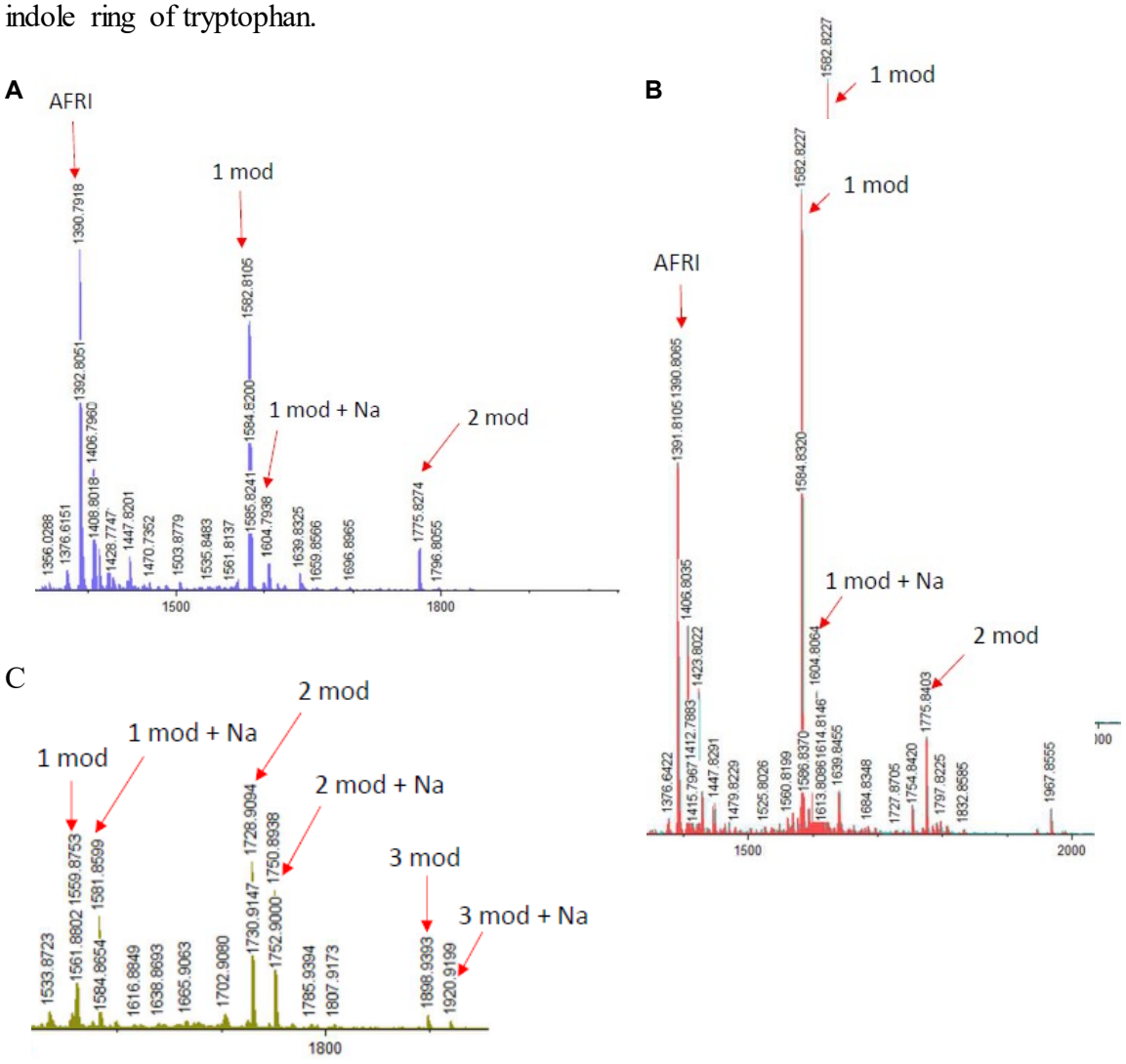


Figure 17 MALDI MS analysis of product mixture of fluoroalkylation of AFRIPLYWGRI with **A**: **57** (10 equiv.), **B**: **59** (10 equiv.), and **C**: **68** (10 equiv.), using sodium ascorbate (10 equiv.), rt, 1 h.

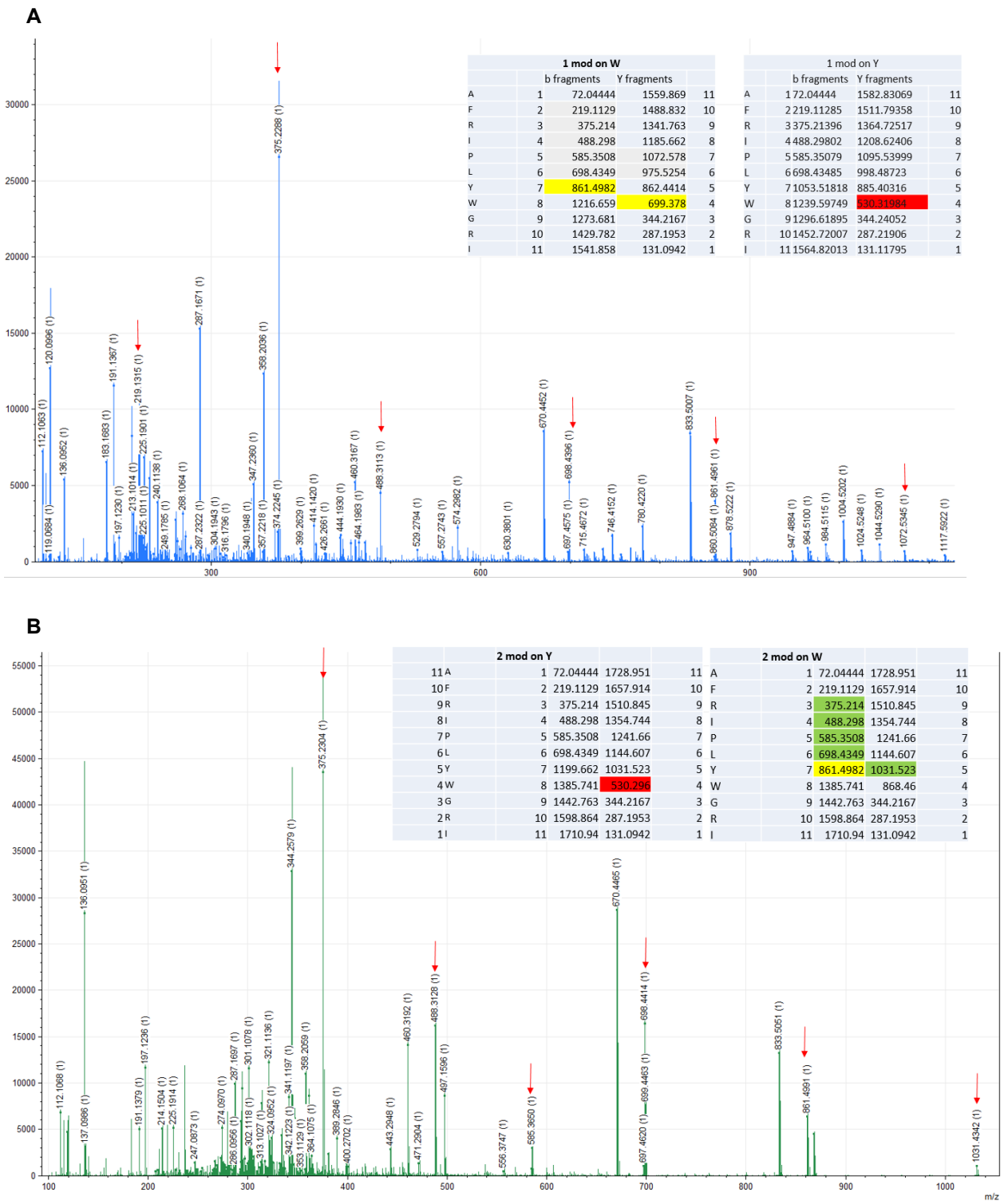


Figure 18 MS/MS analysis of the product of fluoroalkylation of AFRIPLYWGRI with **68**. **A**: confirming mono(fluoroalkylation) of Trp and **B**: confirming bis(fluoroalkylation) of Trp. Red arrows points at fragments that are highlighted in table in right upper corner. Green highlight in the table refers to fragment masses found in the spectrum, yellow refers to fragment masses found in the spectrum that discriminate modification to Trp. Red refers to fragment masses not found in the spectrum.

Another tryptophan containing peptide which was subjected to fluoroalkylation was TEVNAWLVHRDP (See part 4.7). The reaction of this peptide with **57**·AcCl (10 or 100 equiv.) resulted in the formation of mono- and bis(fluoroalkylated) products (Figures 16 and 19). Subsequent MS/MS analysis indicated that both fluoroalkylations took place on Trp residue (Figure 20).

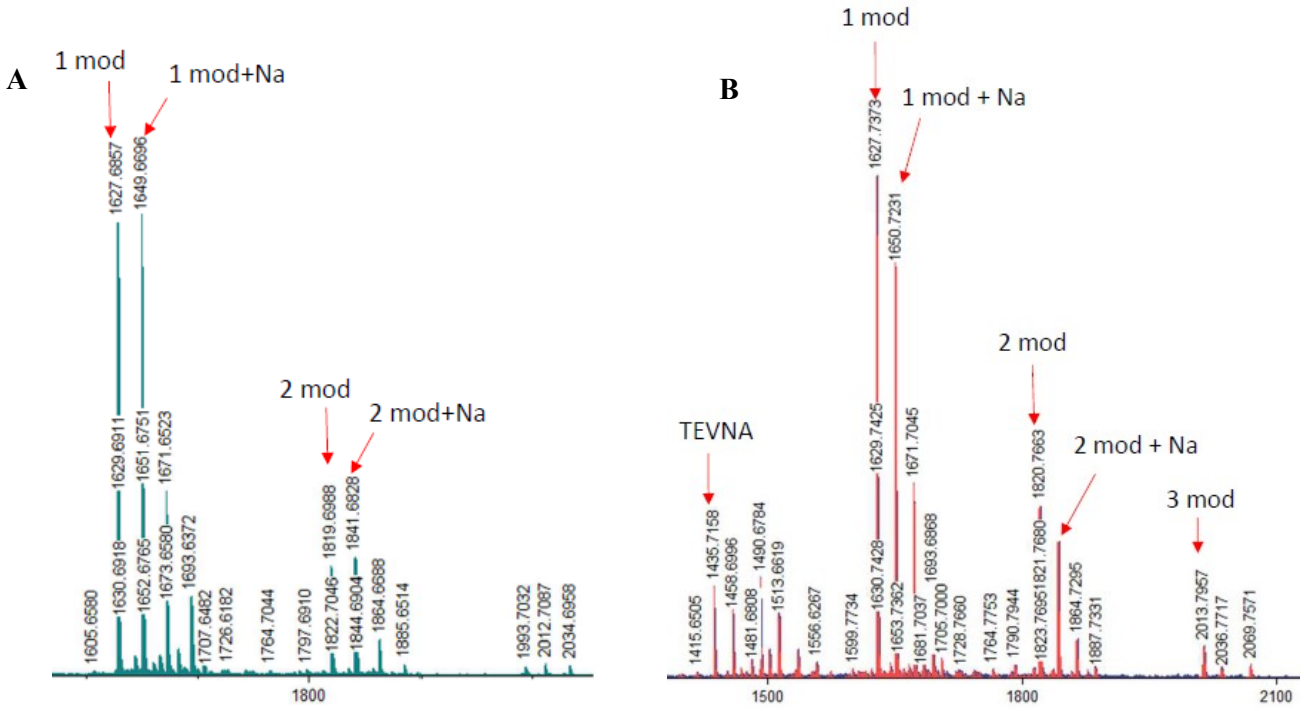


Figure 19 MALDI MS analysis of product mixture of fluoroalkylation of TEVNAWLVHRDP with **57**·AcCl **A**: (10 equiv.) and **B**: (100 equiv.) using sodium ascorbate (10 equiv. and 100 equiv. respectively), rt, 1 h.

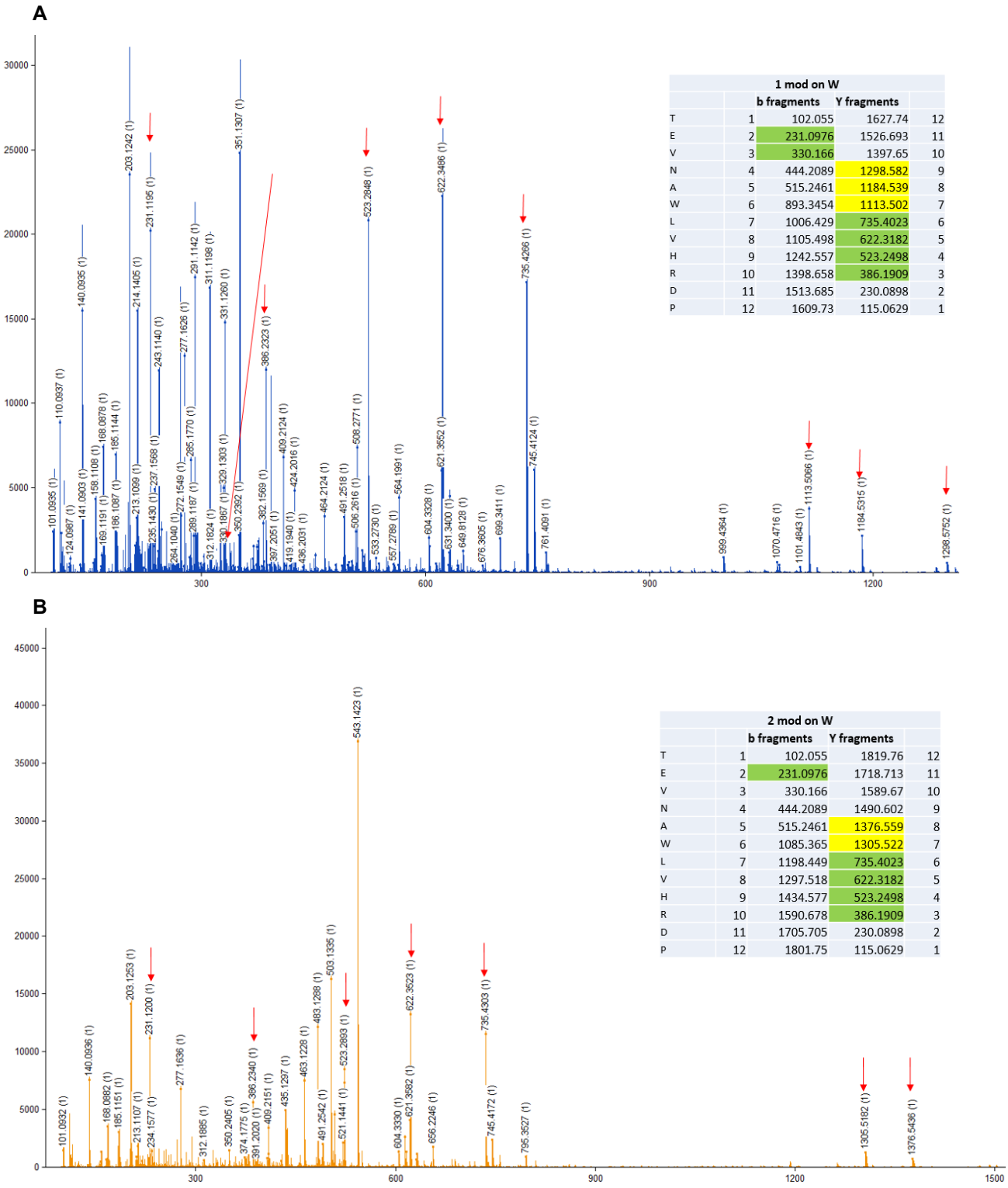


Figure 20: MS/MS analysis of the product of fluoroalkylation of TEVNAWLVHRDP with **57**·AcCl. **A**: confirming mono(fluoroalkylation) and **B**: confirming bis(fluoroalkylation) of Trp. Red arrows points at fragments that are highlighted in table in right upper corner. Green highlight in the table refers to fragment masses found in the spectrum, yellow refers to fragment masses found in the spectrum that discriminate modification to Trp.

Next, bradykinin (RPPGFSPFR) which is a tryptophan-free peptide, was left to react with low amount of **60** (1-16 equiv.) (See part 4.7). This led to only poor conversion of the peptide, therefore, we decided to conduct the reaction with large excess of **60**.

Using 100 equivalents of **60** and 100 equivalents of sodium ascorbate afforded satisfactory level of conversion (Figure 16 and Figure 21). This behavior can be explained by low reactivity of phenylalanine residue and no reactivity of other amino acids present in bradykinin structure. Subsequent MS analysis revealed that phenylalanine was partially fluoroalkylated and traces of bis(fluoroalkylation) was observed (Figure 22).

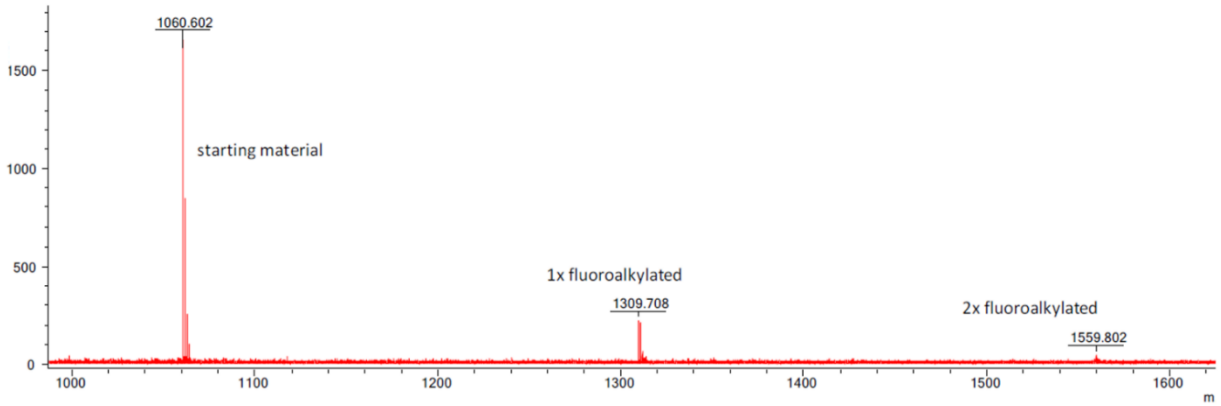


Figure 21 MALDI MS analysis of product mixture of fluoroalkylation of bradykinin with **60** (100 equiv.).

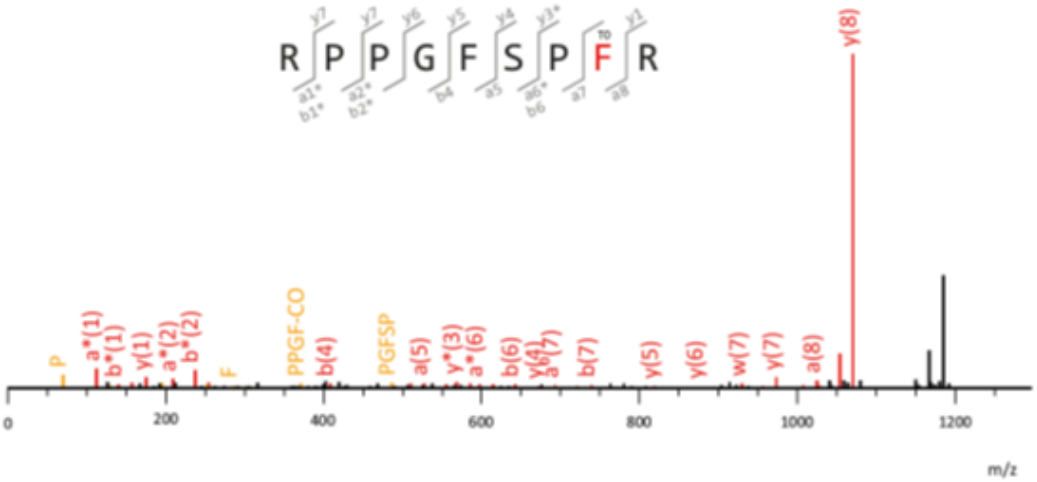


Figure 22 MS/MS analysis of the product of fluoroalkylation of bradykinin with **60** confirming mono(fluoroalkylation) of Phe.

Somatostatin is a hormone regulating peptide and it has the function of inhibiting the secretion of insulin and glucagon. Fluroalkylation of somatostatin with **60** yielded mono(fluoroalkylated) as

the main product, bis(fluoroalkylated) as the minor product and traces of tris(fluoroalkylated) somatostatin (See part 4.7). These products are the results of hydrogen substitution (Figure 16 and Figure 23).

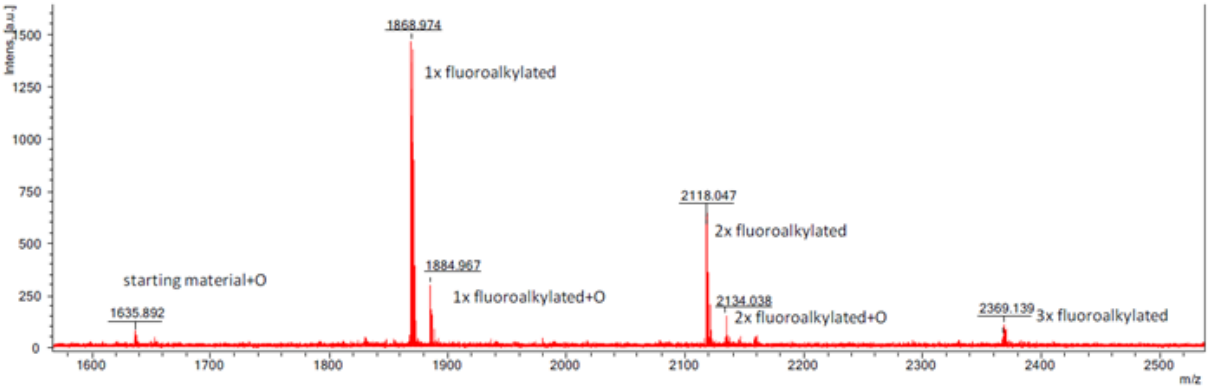


Figure 23 MALDI MS analysis of product mixture of fluoroalkylation of somatostatin with **60** (100 equiv.).

Bombesin which is a peptide consisting of 14-amino acids, simulates gastrin release from G-cells. It is also a second major source of negative feedback signals that stop eating behavior (See part 4.7). Bombesin in reaction with a large excess of **60** afforded mono- and bis(fluoroalkylated) products (Figure 16 and Figure 24) which exclusively took place on tryptophan. However, some traces of tris(fluoroalkylated) and oxidized products were also observed (Figure 25).

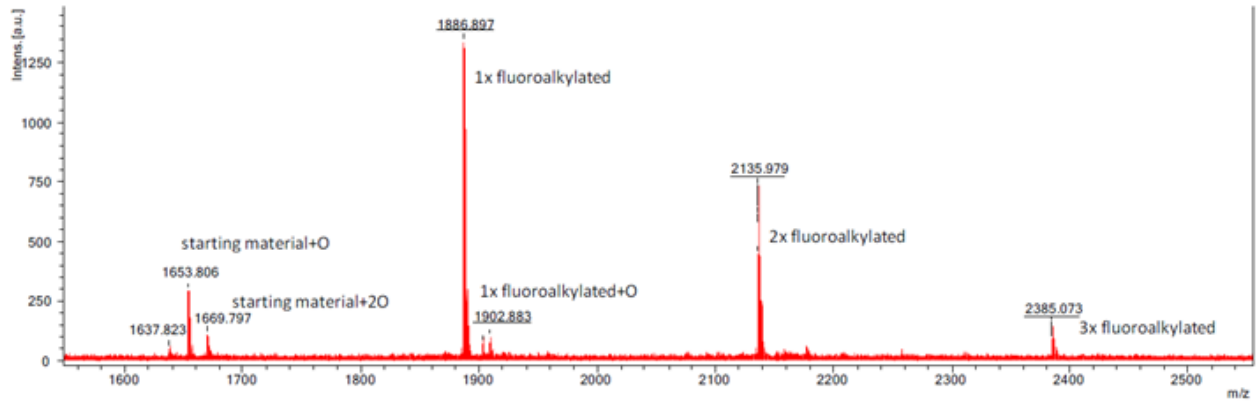


Figure 24 MALDI MS analysis of product mixture of fluoroalkylation of bombesin with **60** (100 equiv.).

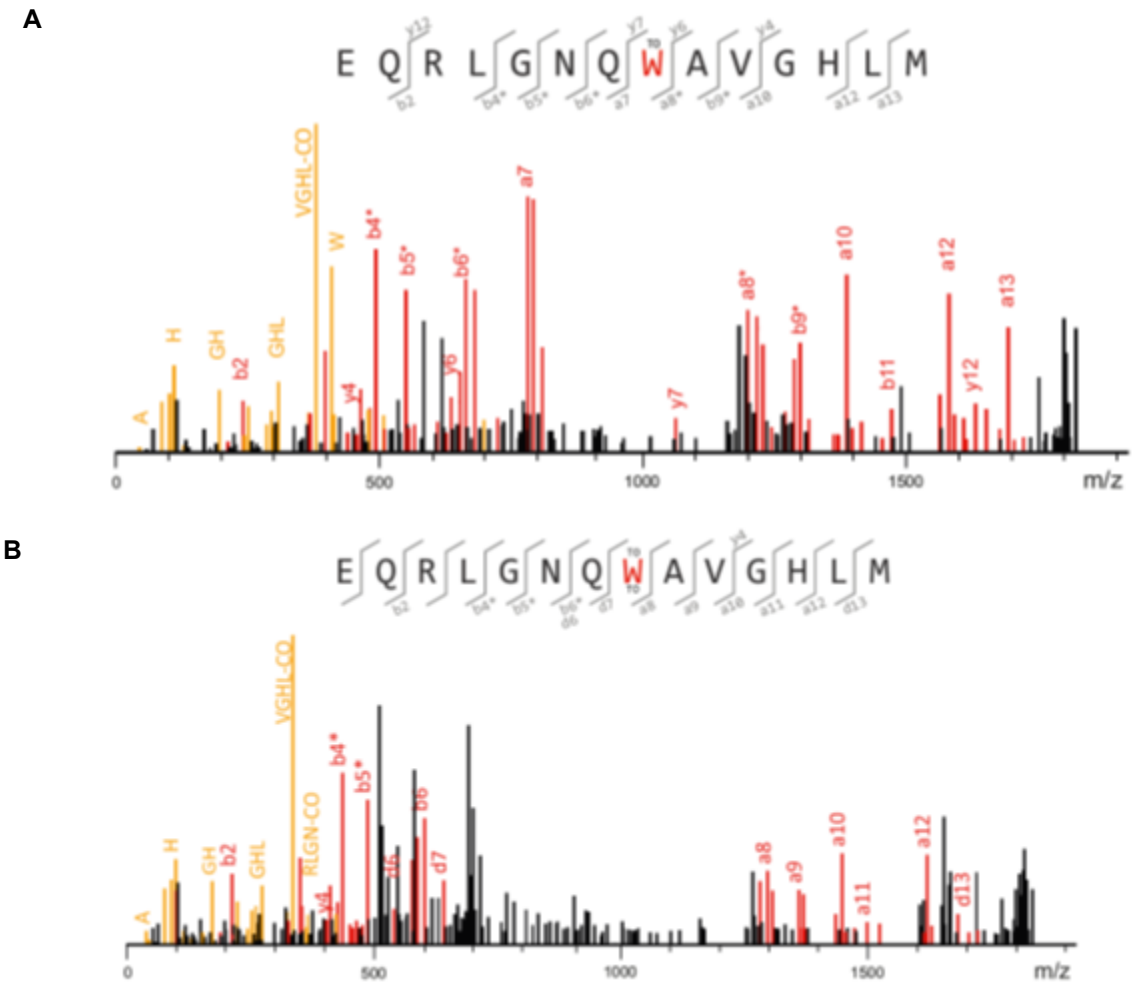


Figure 25 MS/MS analysis of the product of fluoroalkylation of bombesin with **72** confirming **A**: mono(fluoroalkylation) and **B**: bis(fluoroalkylation) of Trp.

Horse heart myoglobin (16.9 kDa) and ubiquitin (8.6 kDa) from bovine erythrocytes were selected to test the potential of HVI reagents for the introduction of a specific biologically relevant tags (See part 4.7). Myoglobin consists of a number of aromatic amino acids such as 7 Phe, 11 His, 2 Tyr and 2 Trp and was subjected to react with 100 equivalents of **68**. The product of the reaction separated by gel filtration and further click reaction with dibenzocyclooctyne-amine (DBCO-amine) was conducted to afford myoglobin containing one and two fluoroalkyl-triazole modifications. A bottom-up analysis revealed that modification predominantly took place on Trp14 residue and to a lesser degree the modification took place on the Trp 7 residue (Figures 26 and 27).

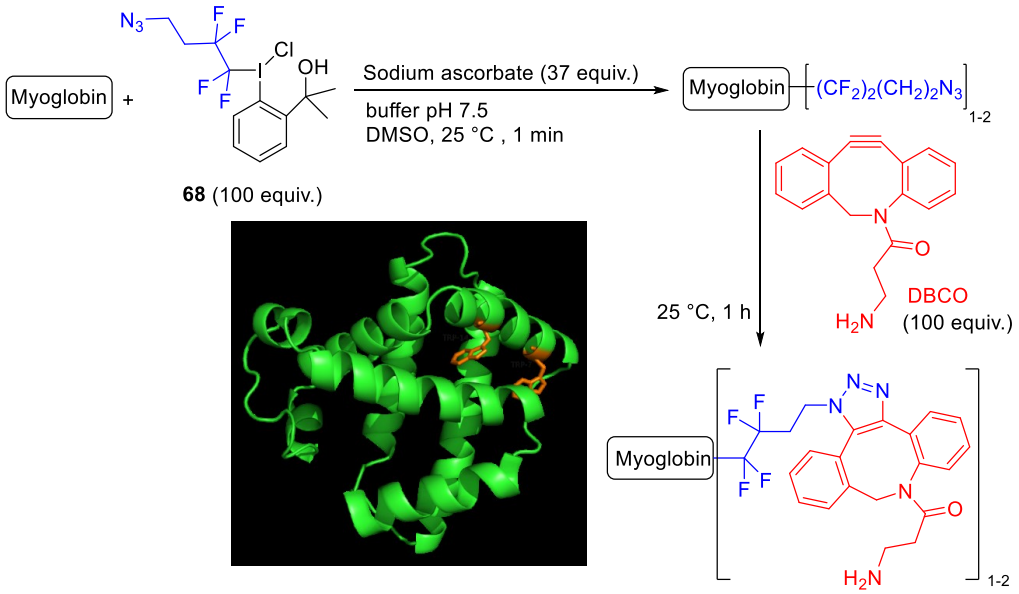


Figure 26 Structure of myoglobin which underwent fluoroalkylation with **68** on highlighted Trp7 and Trp14 residues. A further click reaction with DBCO is shown in a simple reaction.

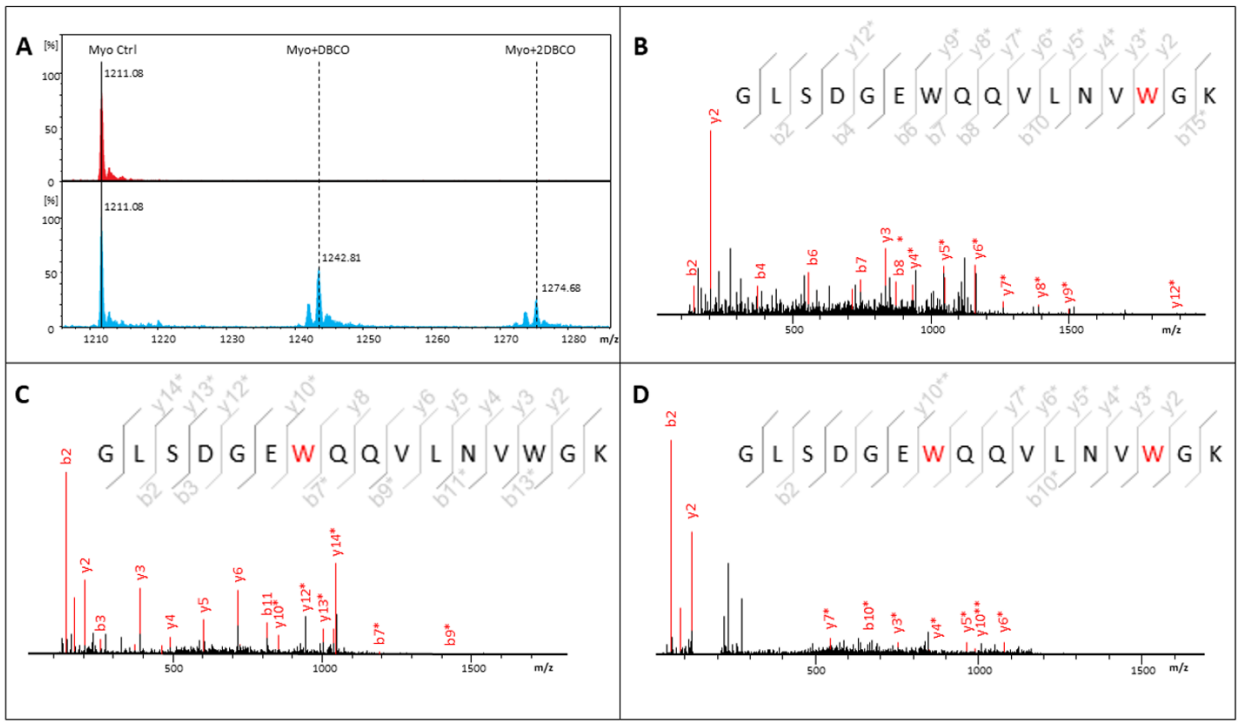


Figure 27 A: ESI-MS spectra comparison of myoglobin control sample (in red) and myoglobin modified by **68** and DBCO (in blue). LC-MS/MS analysis of myoglobin peptide showing **B**: modified Trp14 residue **C:** Trp7 residue and **D**: both Trp7 and Trp14.

With this promising data in hand we decided to carry out a similar experiment for the fluoroalkylation of ubiquitin to see the behavior of other aromatic amino acids (See part 4.7).

Ubiquitin is a Trp-free regulatory protein which is found in most eukaryotes. It contains one His, one Tyr and two Phe residues. Modification of ubiquitin was carried out in 50 µM ammonium bicarbonate buffer (pH 7.5) using 100 equivalents of **68**. Next a click reaction with a DBCO-amine derivative was carried out. Further MS analysis revealed that this protein was mono(fluoroalkylated) (Figure 28A). A top-down analysis confirmed that modification took place on Tyr 59 (Figure 28B).

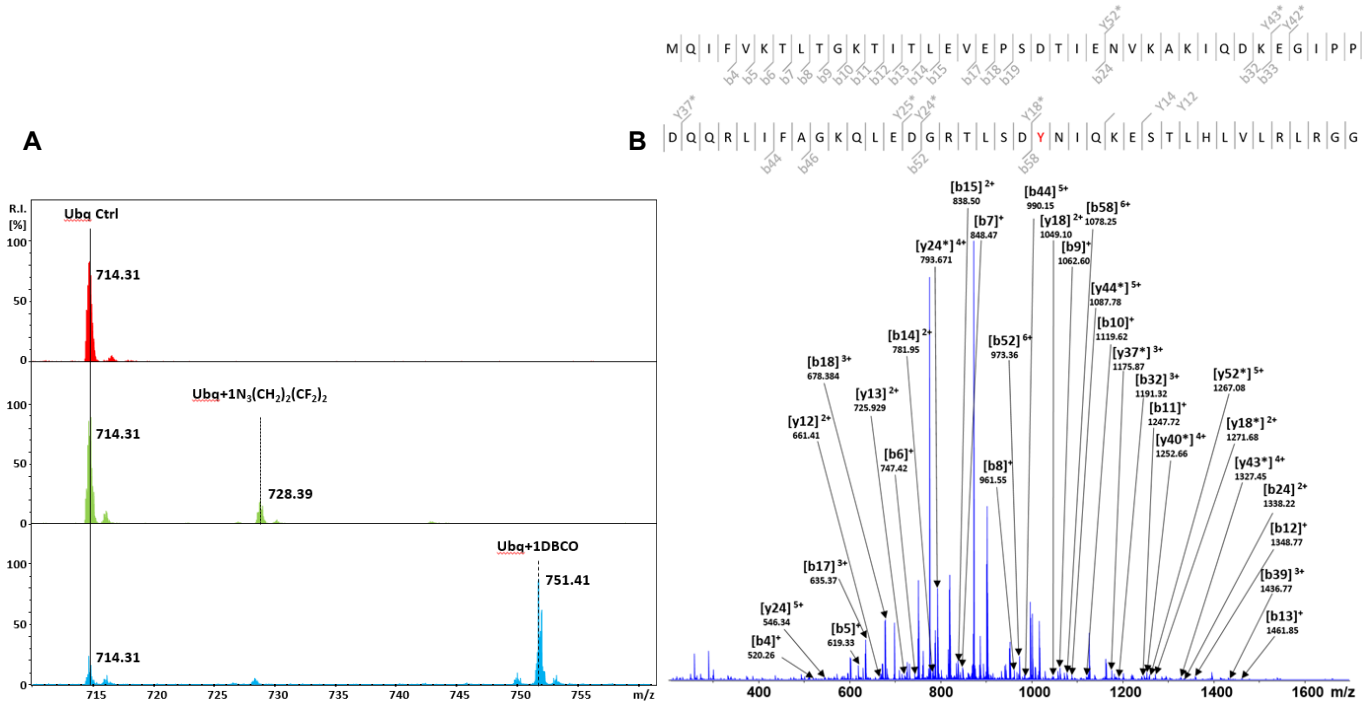


Figure 28 A: ESI-MS spectra comparison of ubiquitin control sample (in red) and modified with **68** (in green) and DBCO (in blue), **B**: MS/MS analysis of ubiquitin showing attachment of the fluoroalkyl chain (modified by **68**) and DBCO to the Tyr 59 residue.

In conclusion, we demonstrated that sodium ascorbate acts as an efficient, mild and biocompatible reductant towards fluoroalkyl-substituted hypervalent iodine reagents for rapid fluoroalkylation of small molecules, such as indoles and pyrroles and aromatic amino acids or aromatic amino acid residues in peptides and proteins with a high selectivity to tryptophan.

The results of our findings was published in the journal *Chemistry—A European Journal*.[116]

3.5 Light promoted fluoroalkylation using HVI reagents

The second part of our studies dealt with finding an alternative approach for fluoroalkylation of electron-rich substrates such as indoles and tryptophan, which could be applicable for selective tagging of Trp residues in biologically relevant substrates.

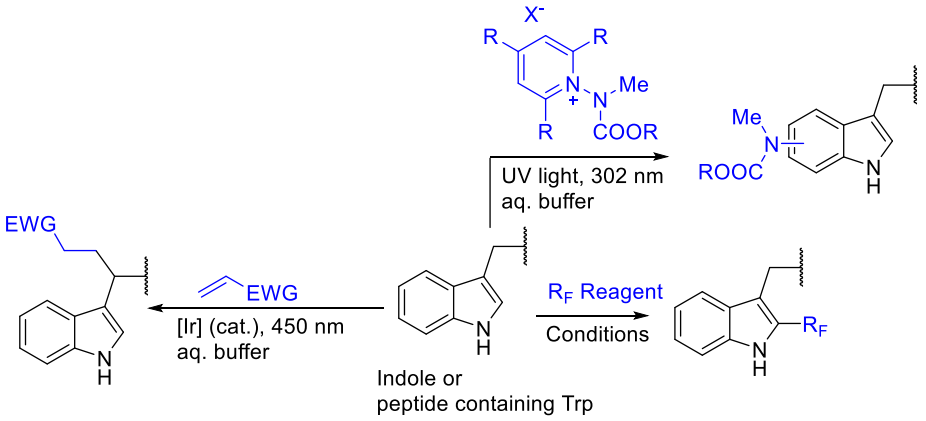
As described in previous chapter fluoroalkylated λ^3-iodanes in combination with sodium ascorbate which is a water-soluble reductant were successfully applied for fluoroalkylation of small electron-rich substrates such as indoles, pyrroles, tryptophan and also tryptophan residues in peptides and proteins.[116]

These promising results led us to speculate whether the reductant might be omitted from the reaction. If we photoexcite the HVI reagent an electron transfer from electron-rich substrate (indole or Trp) to the electron-deficient HVI reagent can take place. For this aim, we decided to use various light sources for the excitation of HVI reagent. This process might allow similar radical process leading to functionalization of indoles or Trp residues in peptides and proteins.

HVI reagents have been utilized in photoredox catalysis. For instance, allyl silanes, [117] vinyl borates, [118] ene carbamates, [119] anilines, [120] or alkenes[121,122] have been trifluoromethylated using Togni reagents in the presence of transition metal photocatalyst.

Visible light promoted trifluoromethylation of electron-rich heterocycles and terminal alkynes using Togni reagent and a photosensitizer such as methylene blue was also studied.[123] All of the given examples used a photosensitizer[124] or metal photocatalyst necessary for light-promoted transformations. Therefore, we aimed to avoid such additives in fluoroalkylation of electron-rich substrates and more importantly in bioconjugation reactions.

In the scheme shown below, methods for fluoroalkylation of indole and Trp derivatives are summarized (Scheme 51)



R_F	R_F Reagent	Conditions
CF_3, C_4F_9	CF_3I, C_4F_9I	amine, THF, compact fluorescent lamp
CF_3	CF_3SO_2Na	[Ir] (cat.), MeCN, blue LED light
CF_3	CF_3-Iodine	methylene blue (cat.), DMF, white LED light
CF_3, CF_2CF_2R	R_F-Iodine	blue LED light, aq. medium, *Our work*

Scheme 51 Reported methods for photochemical fluoroalkylation of indoles and Trp derivatives and our method.

To begin the study, *N*-acetyl tryptophan (**90**) was selected as the model substrates for optimization of the reaction conditions of photo-induced fluoroalkylation. The reaction was examined using a series of λ^3-iodanes (Figure 29) and LED light sources in various solvents (Table 4), (See part 4.9).

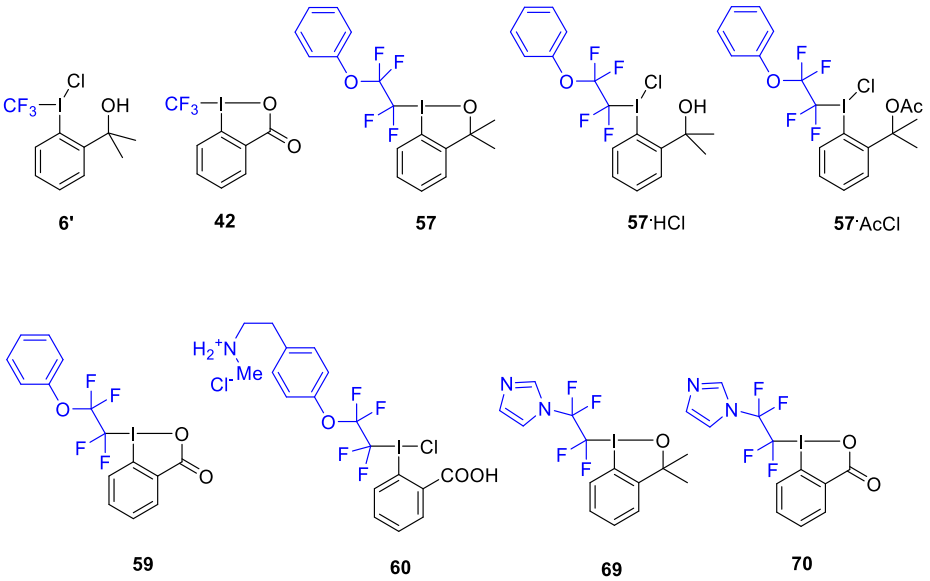


Figure 29 Structures of HVI reagents used for photo-induced fluoroalkylation of *N*-acetyl tryptophan.

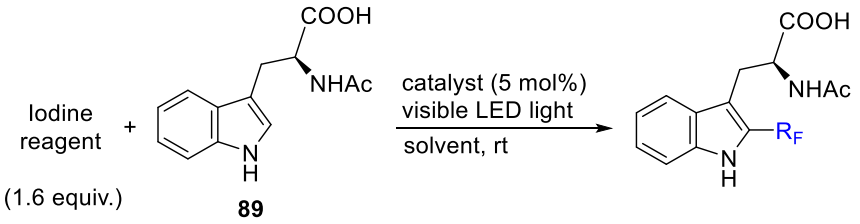
HVI acid-type reagents **59**, **70**, **42**, alcohol-type reagents **57**, **69** and open-chain salt forms **57**·HCl, **57**·AcCl, **6'** and **60** were synthesized or prepared using reported procedures.[89,94,116]

When **59** was applied for tetrafluoroethylation in aqueous DMF in the presence of blue light of λ_{max} 390–410 nm or green light of λ_{max} 515–535 nm, *N*-acetyl tryptophan (c = 0.1 M) was fluoroalkylated in position 2 of the indole ring in low yields (Table 4, entries 1 and 3). However, under identical conditions, with the blue light of 455–475 nm the yield increased (entry 2).

To examine whether the photocatalyst is necessary for transformations, we decided to use photocatalysts, including $Ir(ppy)_3$ (λ_{max} 375 nm), Eosin Y (λ_{max} 539 nm) and methylene blue (λ_{max} 664 nm) with light sources of appropriate wavelengths; however, this did not improve the product yields, suggesting that photocatalyst was not necessary (entries 4-7). Performing the reaction in various solvents we found that methanol, DMF or acetonitrile were optimal solvents (entries 8-11).

Utilizing various HVI reagents to evaluate their reactivity showed that reagents **59**, and salts **57**·HCl and **60** afforded acceptable amount of product (Table 4, entry 12-19).

Table 4 Optimization of the reaction condition for fluoroalkylation of *N*-acetyltryptophan.

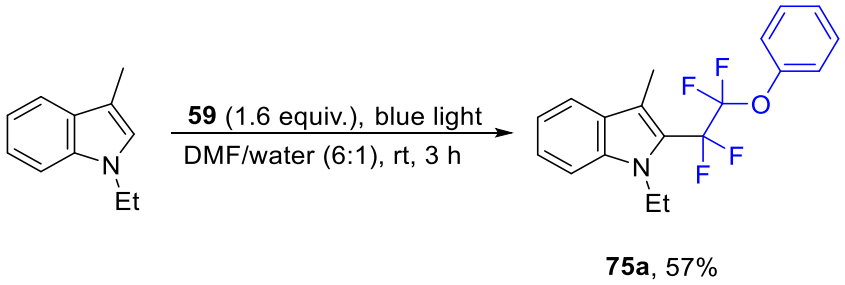


Entry	Reagent	Catalyst	Solvent	LED light (λ_{max})	Time (h)	Yield (%)[a]
1	**59**	none	aq. DMF[b]	blue[c]	5	12
2	**59**	none	aq. DMF[b]	blue[d]	5	30
3	**59**	none	aq. DMF[b]	green[e]	5	18
4	**59**	$Ir(ppy)_3$	aq. DMF[b]	blue[c]	5	31
5	**59**	$Ir(ppy)_3$	aq. DMF[b]	blue[d]	5	21
6	**59**	Eosin Y	aq. DMF[b]	green[e]	5	25
7	**59**	MB[f]	DMF	red[g]	5	25
8	**59**	none	DMF	blue[d]	5	40
9	**59**	none	MeOH	blue[d]	5	47
10	**59**	none	THF	blue[d]	20	20
11	**59**	none	MeCN	blue[d]	5	61
12	**57**·AcCl	none	aq. DMF[b]	blue[d]	3	76
13	**60**	none	aq. DMF[b]	blue[d]	3	45
14	**70**	none	aq. DMF[b]	blue[d]	5	28
15	**69**	none	aq. DMF[b]	blue[d]	5	20
16	**42**	none	aq. DMF[b]	blue[d]	20	12
17	**42**	$Ir(ppy)_3$	aq. DMF[b]	blue[d]	20	10
18	**42**	$Ir(ppy)_3$	aq. DMF[b]	none	20	3
19	**6'**	none	aq. DMF[b]	blue[d]	3	27

[a] ^{19}F NMR yield using 2,2,2-trifluoroethanol as an internal standard. [b] DMF/water (6:1, vol/vol). [c] Blue LED λ_{max} 390–410 nm. [d] Blue LED λ_{max} 455–475 nm. [e] Green LED λ_{max} 515–535 nm. [f] Methylene blue (5 mol %) + TMEDA (2 equiv.). [g] Red LED λ_{max} 648 nm.

Control experiments using **42** as the trifluoromethylating reagent was conducted to evaluate the necessity of the photocatalyst and light source to obtain appreciable amount of the product. These

experiments confirmed that using Ir photocatalyst did not lead to better product yield and the irradiation was necessary for the reaction to start and complete.



75a, 57%

Scheme 52 Preparative photoinduced fluoroalkylation of 1-ethyl 3-methylindole using **59**.

Using **59** for the fluoroalkylation of 1-ethyl-3-methylindole afforded 57% isolated yield of the product (Scheme 52), however alkenes, electron-rich aromatics and amino acids phenylalanine, histidine and tyrosine were resistant to fluoroalkylation (Figure 30). This demonstrated a more selective process in comparison to previously reported radical fluoroalkylation using λ^3-iodanes in the presence of photocatalyst [120,122,123] or sodium ascorbate.[116]

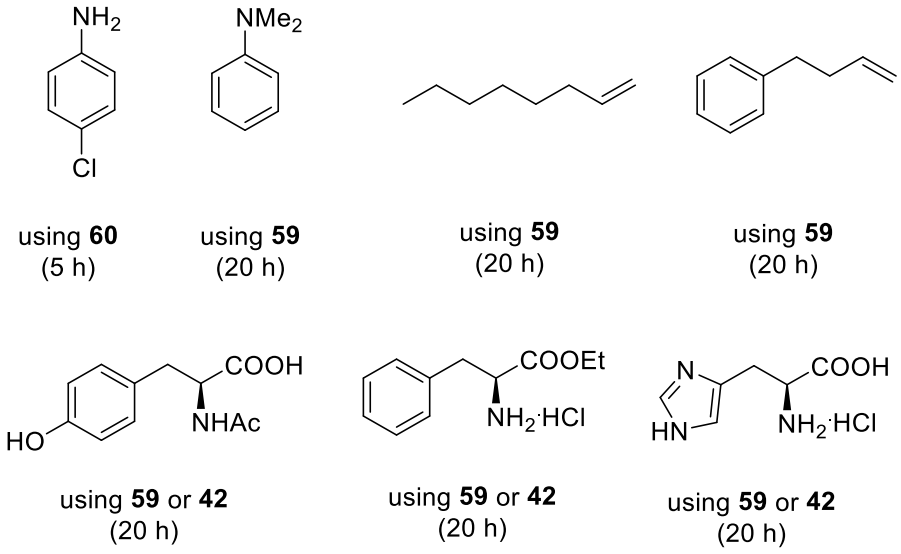


Figure 30 Unreactive substrates toward fluoroalkylation in photoinduced process using blue light of λ_{max} 455–475 nm in presence of reagents **59**, **42** or **60**, in DMF/water (6:1, vol/vol), rt, and ^{19}F NMR monitoring.

3.5.1 MS analysis of competitive reaction between aromatic amino acids in fluoroalkylation

Furthermore, evaluation of an equimolar mixture of aromatic amino acids including *N*-acetyltryptophan, phenylalanine ethyl ester hydrochloride, *N*-acetyltyrosine and histidine hydrochloride using **42** (10 equiv.) in MeOH in the presence of blue light at ambient temperature was performed, (See part 4.10). Analysis of the crude mixture using MS revealed the formation of mono- bis- and tris(trifluoromethylated) tryptophan, however other amino acids were resistant to trifluoromethylation under the reaction condition (Figure 31).

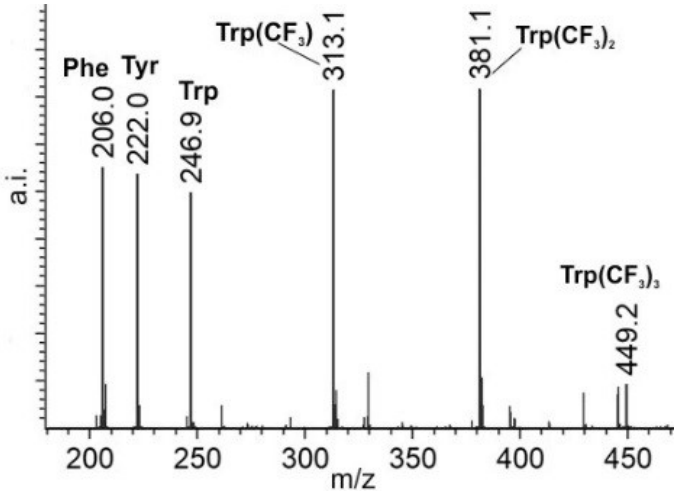


Figure 31 MS (ESI^+) spectrum of the reaction mixture of Tyr, Trp, His and Phe derivatives (0.1 mmol) using **42** (10 equiv.) in aq. MeOH in the presence of blue light λ_{max} 455–475 nm, rt, 3 h.

This finding is in contrast to what we reported in the sodium ascorbate method, where some reactivity of other aromatic amino acids such as phenylalanine, tyrosine and histidine was observed.[116] The main difference between these two methods is that with sodium ascorbate, fluoroalkyl radicals were generated quickly and in large amounts, however in the case of light, fluoroalkyl radicals are generated much more slowly.

3.5.2 ^{19}F NMR study of fluoroalkylation of tripeptide Ac-Cys-Gly-Trp-NH_2

As seen previously, in the absence of light or reductant, tryptophan is unreactive towards fluoroalkylation. On the other hand, it is known that cysteine is reactive under this conditions,[94,125] therefore, we decided to investigate the reactivity and selectivity of the fluorolkylation reaction

toward tryptophan in the presence of cysteine. For this, a tripeptide consisting of Ac-Cys-Gly-Trp-NH_2 (**90**) was evaluated in the reaction with **42** (Table 5), (see part 4.11). In the presence of cysteine, Trp was much less reactive; however, in the absence of light and sodium ascorbate not only cysteine was modified but also tryptophan was fluoroalkylated (Table 5). With this result, we hypothesised that the reaction with cysteine was not purely electrophilic but also free radical species were involved. When sodium ascorbate was used, not only tryptophan but also cysteine was fluoroalkylated to a large degree (entries 2 and 3). Using blue light, cysteine was more reactive; however, tryptophan also was fluoroalkylated to a lesser degree (entry 4). Finally, when an excess of **42** was used, both residues were roughly fluoroalkylated the same (entry 5). These results showed that fluoroalkylation of tryptophan is not selective in the presence of cysteine (Figures 32-36). Modification of the peptide using LED light was confirmed using MALDI MS analysis (Figure 37).

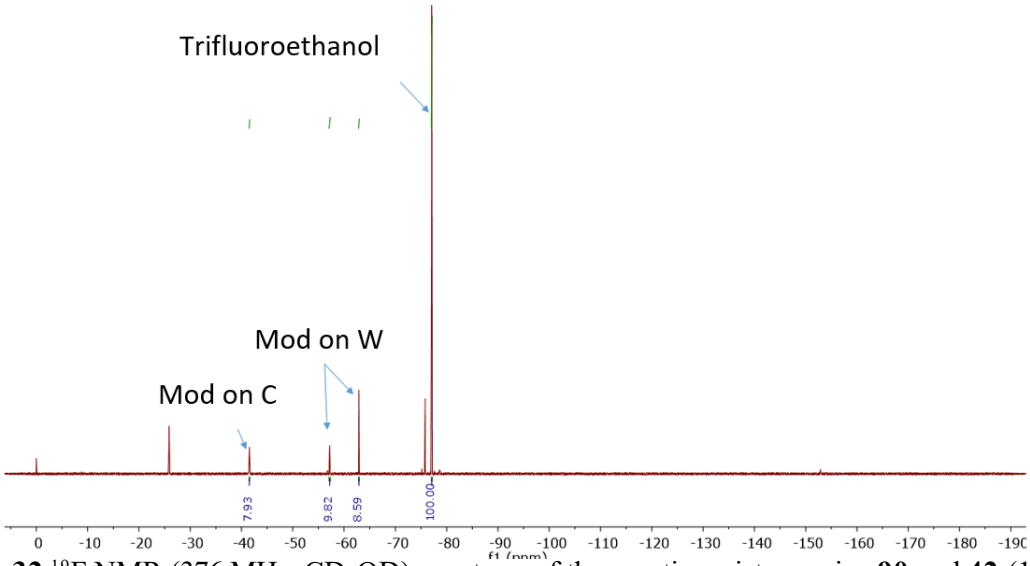


Figure 32 ^{19}F NMR (376 MHz, CD_3OD) spectrum of the reaction mixture using **90** and **42** (1.2 equiv.) in the absence of light.

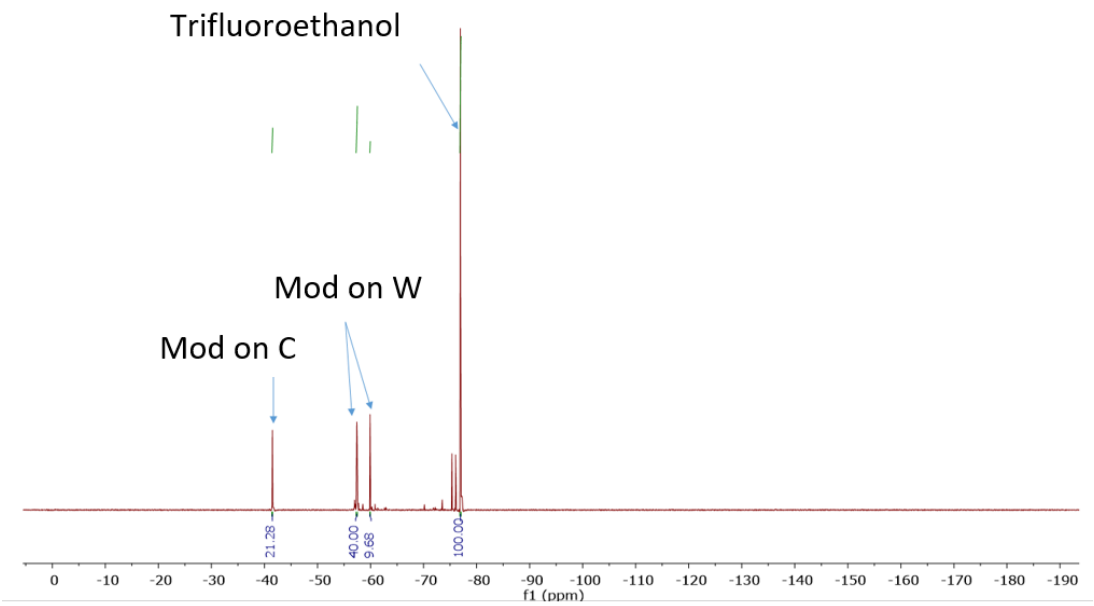


Figure 33 ^{19}F NMR (376 MHz, CD_3OD) spectrum of the reaction mixture using **90** and **42** (1.2 equiv.) in the presence of sodium ascorbate (0.5 equiv.).

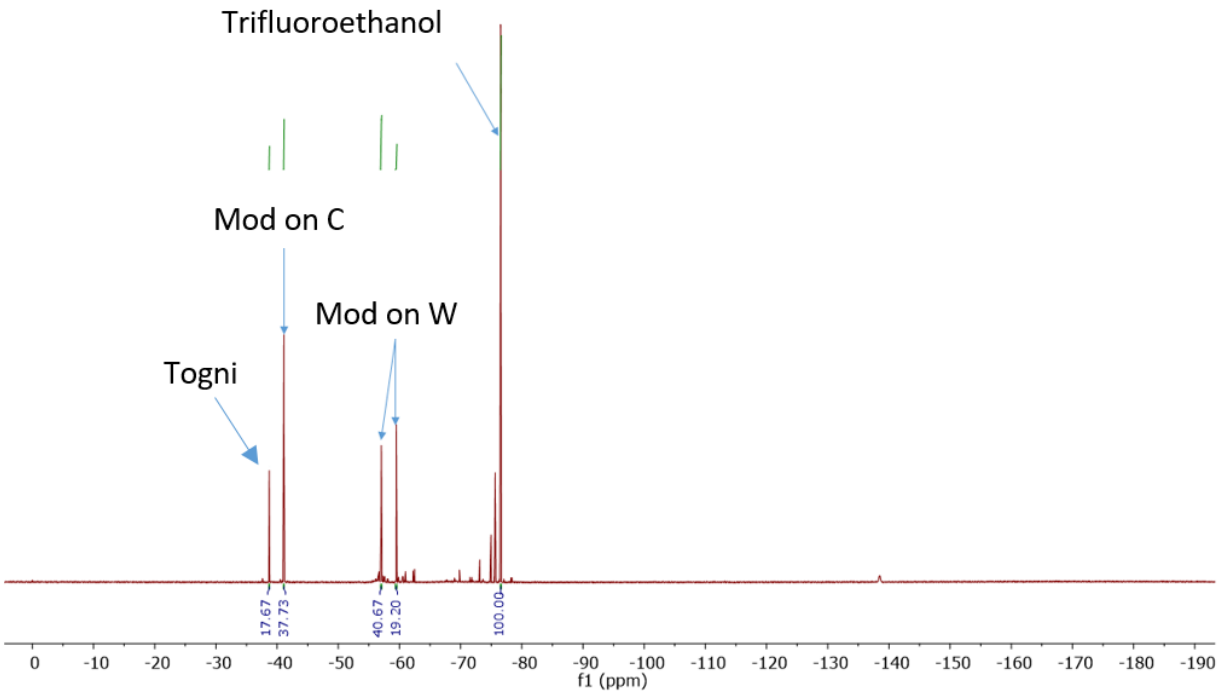


Figure 34 ^{19}F NMR (376 MHz, CD_3OD) spectrum of the reaction mixture using **90** and **42** (3.0 equiv.) in the presence of sodium ascorbate (0.5 equiv.).

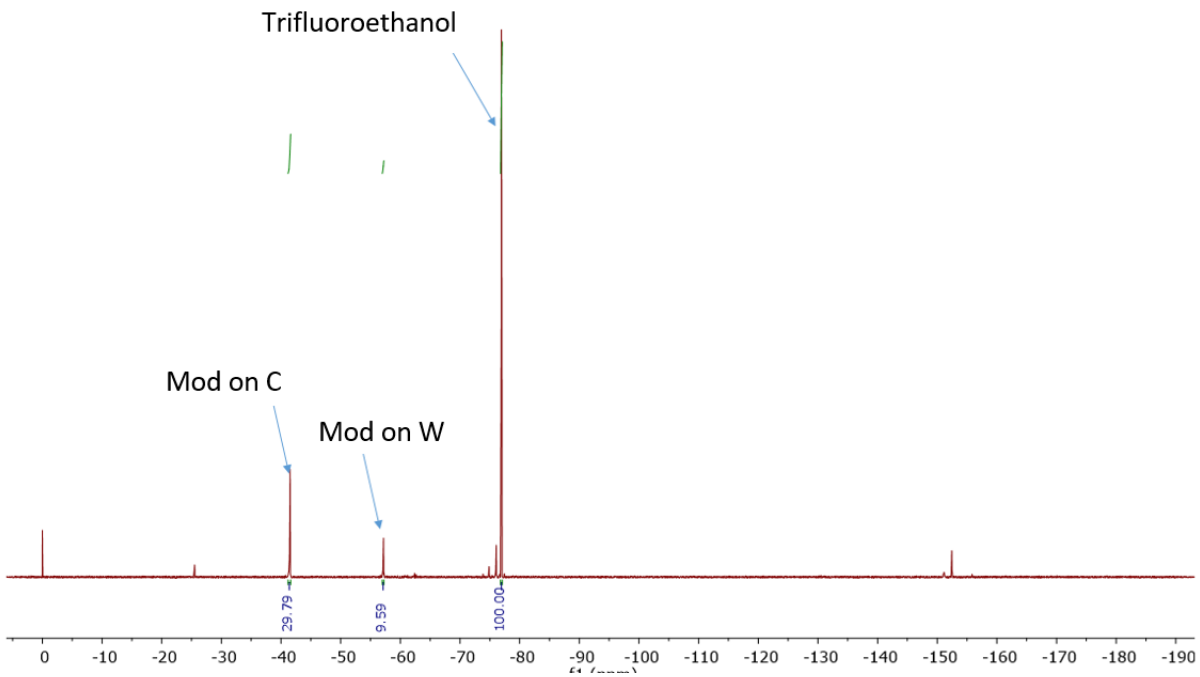


Figure 35 ^{19}F NMR (376 MHz, CD_3OD) spectrum of the reaction mixture using **90** and **42** (1.2 equiv.) in the presence blue LED light.

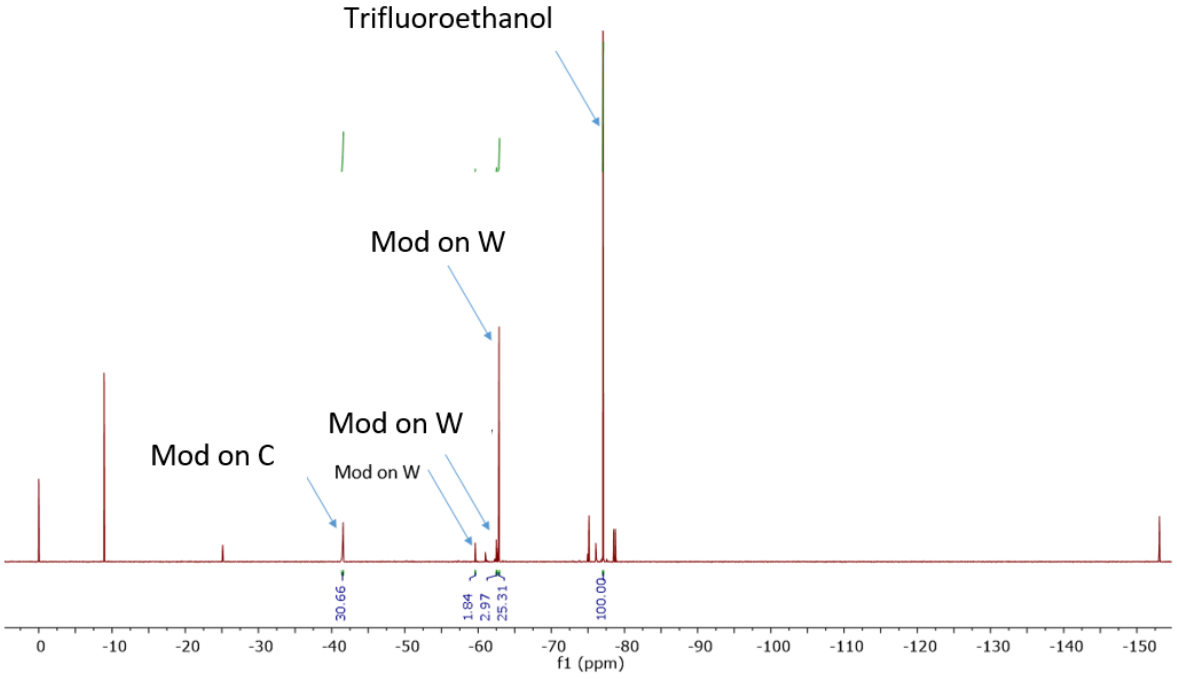


Figure 36 ^{19}F NMR (376 MHz, CD_3OD) spectrum of the reaction mixture using **90** and **42** (3.0 equiv.) in the presence blue LED light.

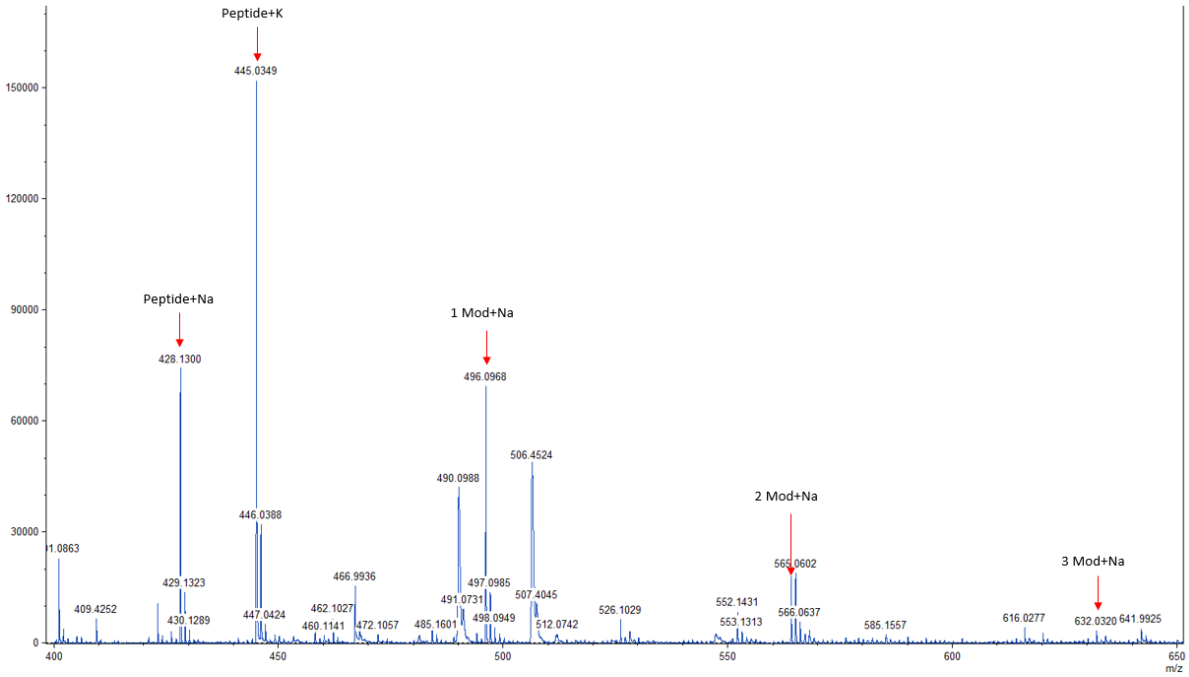
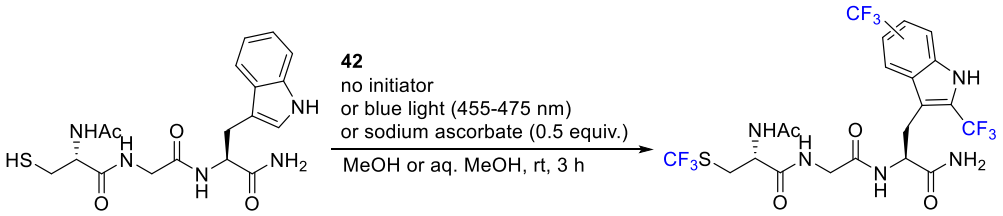


Figure 37 MALDI MS analysis of the product mixture of fluoroalkylation of **90** with **42** (3.0 equiv.) in the presence blue LED light.

Table 5 Screening the reactivity of **90** using **42**.



90

Entry	**42** (equiv.)	Initiator	Modification	
			Cys (%)[a]	Trp (%)[a]
1	1.2	none	8	19
2	1.2	sodium ascorbate	21	49
3	3.0	sodium ascorbate	38	60
4	1.2	blue light	30	10
5	3.0	blue light	30	30

[a] ^{19}F NMR yield using 2,2,2-trifluoroethanol as an internal standard.

3.5.3 Photo-induced bioconjugation utilizing λ^3 iodanes

Next, iodanes shown in Figure 26 were used in the preparation of fluoroalkylated bioconjugates in the presence of visible light at ambient temperature.

At first, undecapeptide with the amino acid sequence AFRIPLYWGRI was left to react with 10 equivalent of **59** in aqueous acetonitrile at ambient temperature and in the presence of blue light (λ_{max} 455–475 nm) for 3 hours. This led to the formation of the mixture of mono- and traces of bis(fluoroalkylated) peptide which was confirmed by MALDI MS analysis. To localize the site of modification, MS/MS analysis was conducted and confirmed that both modifications took place on Trp residue. Using 100 equivalents of **59** afforded mono- bis- and traces of tris(fluoroalkylated) peptide on Trp residue (Figure 38). We found out that the conversion can be increased not only by using a large excess of reagent but also a more concentrated reaction mixture (See part 4.11).

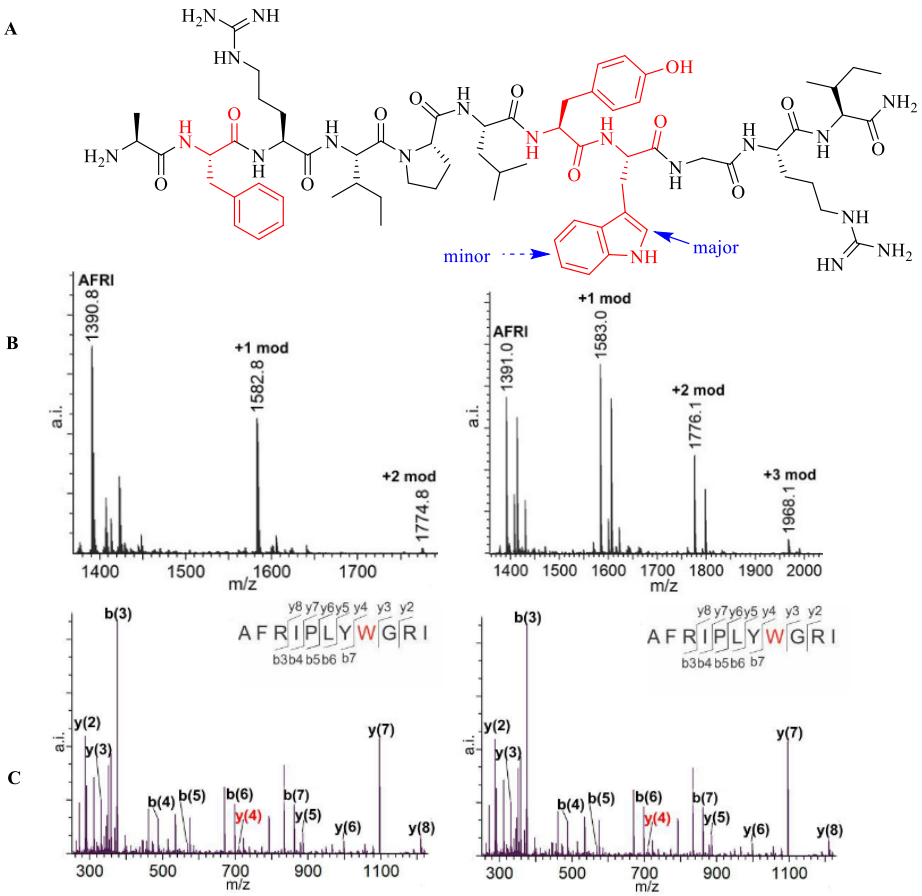


Figure 38 A: Photoinduced fluoroalkylation of AFRIPLYWGRI using **59** (10 and 100 equiv.) in the presence of blue LED light. Aromatic residues are highlighted in red and sites of major and minor fluoroalkylation are shown with blue arrows. **B**: MALDI MS spectra of the product mixture using **59** (10 equiv.) (left) and 100 equiv. (right). **C**: MS/MS analysis for the mono(fluoroalkylated) product (left) and bis(fluoroalkylated) product (right).

Dodecapeptide TEVNAWLVHRDP was successfully modified with **59** and afforded mono- and bis(fluoroalkylated) products as the major and minor products respectively. MS/MS analysis indicated the formation of fluoroalkylated TEVNAWLVHRDP on Trp residue (Figure 39), (See part 4.11).

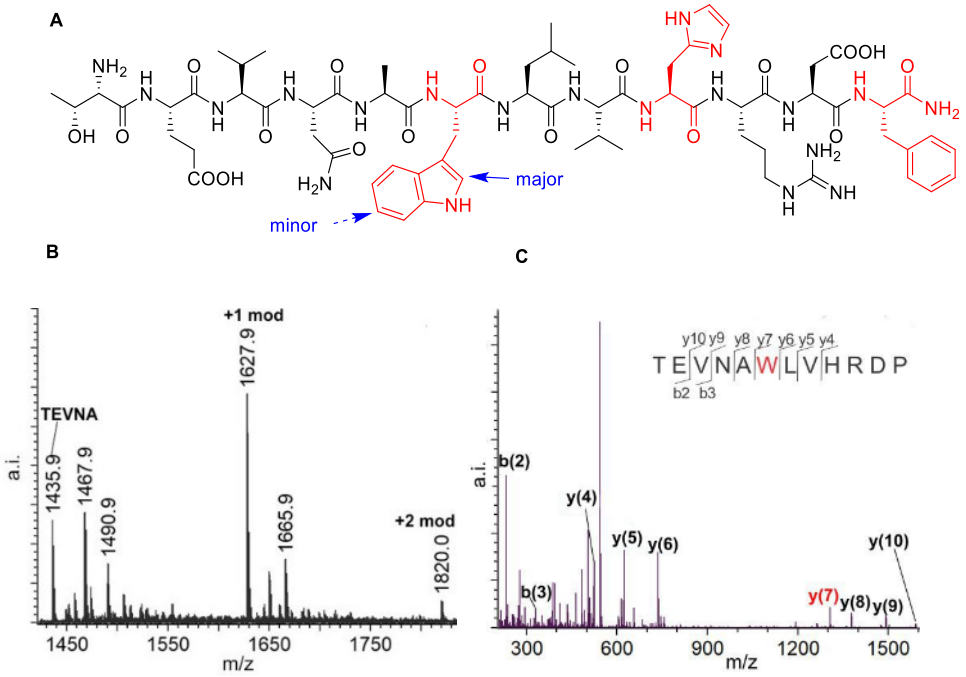


Figure 39 Photo-induced fluoroalkylation of peptide TEVNAWLVHRDP using **59** (10 equiv.), in the presence of blue LED light, rt, 3 h. **A**: Aromatic residues are highlighted in red and sites of major and minor fluoroalkylation are shown with blue arrows. **B**: MALDI MS spectra of the product mixture using **59** (10 equiv.). **C**: MS/MS analysis for the mono(fluoroalkylated) product confirming fluoroalkylation on Trp.

Somatostatin is a cyclic tetradecapeptide, produced particularly in nervous and gastrointestinal system tissues, having a variety of functions, such as inhibiting the secretion of other hormones, acting as a local regulator or a neurotransmitter (Figure 40A). Modification of somatostatin was carried out using reagents **59** which afforded appreciable conversion of somatostatin to mono- (as major) and bis(fluoroalkylated) (as minor) products (Figure 40B). The cyclic structure of peptide prevented to localize the site of modification by MS/MS analysis (See part 4.11).

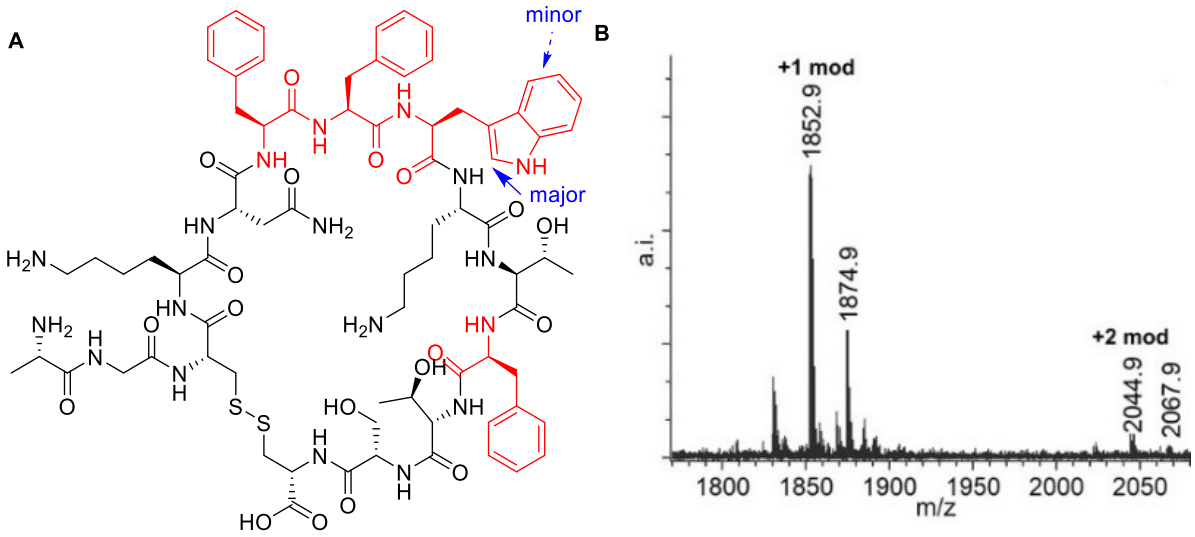


Figure 40 Photo-induced fluoroalkylation of somatostatin using **59** (10 equiv.), in the presence of blue LED light, rt, 3 h. **A**: Aromatic residues are highlighted in red and sites of major and minor fluoroalkylation are shown with blue arrows. **B**: MALDI MS spectra of the product mixture using **59** (10 equiv.).

Bradykinin, which is a potent vasodilator peptide, with the amino acid sequence (RPPGFSPFR), has multiple physiological actions in human body, such as regulation of blood vessel tone and renal function. Modification of bradykinin with a large excess of **57** (100 equiv.) yielded mono(fluoroalkylated) conjugate. In the case of **59**, the conversion was better, also small signal of bis(fluoroalkylated) conjugate was observed; however, a large amount of peptide was left unreacted. Subsequent MS/MS analysis confirmed the formation of fluoroalkylated bradykinin on Phe residue (Figure 41), (See part 4.11).

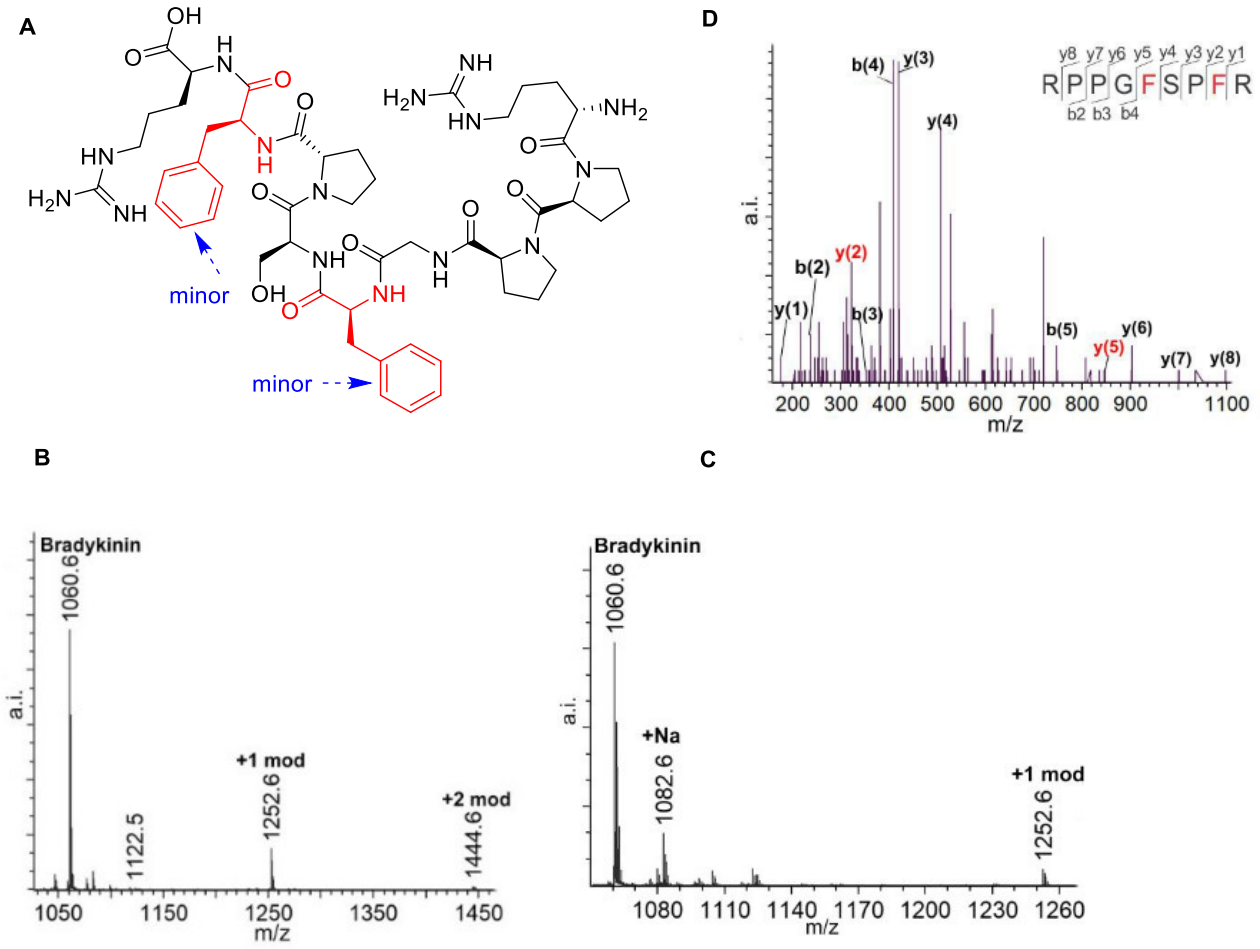


Figure 41 Photo-induced fluoroalkylation of bradykinin using **59** or **57** (100 equiv.), in the presence of blue LED light, rt, 3 h. **A**: Aromatic residues are highlighted in red and sites of major and minor fluoroalkylation are shown with blue arrows. **B**: MALDI MS spectra of the product mixture using **59** (100 equiv.). **C**: MALDI MS spectra of the product mixture using **57** (100 equiv.). **D**: MS/MS analysis for the mono(fluoroalkylated) product confirming fluoroalkylation on Phe.

Next, fluoroalkylation of peptide bombesin was investigated. Bombesin in reaction with 10 equivalents of **59**, afforded mono(fluoroalkylated) bombesin (Figure 42B). Subsequent MS/MS analysis showed the formation of mono(fluoroalkylated) bombesin on Trp residue (Figure 42C), (See part 4.11).

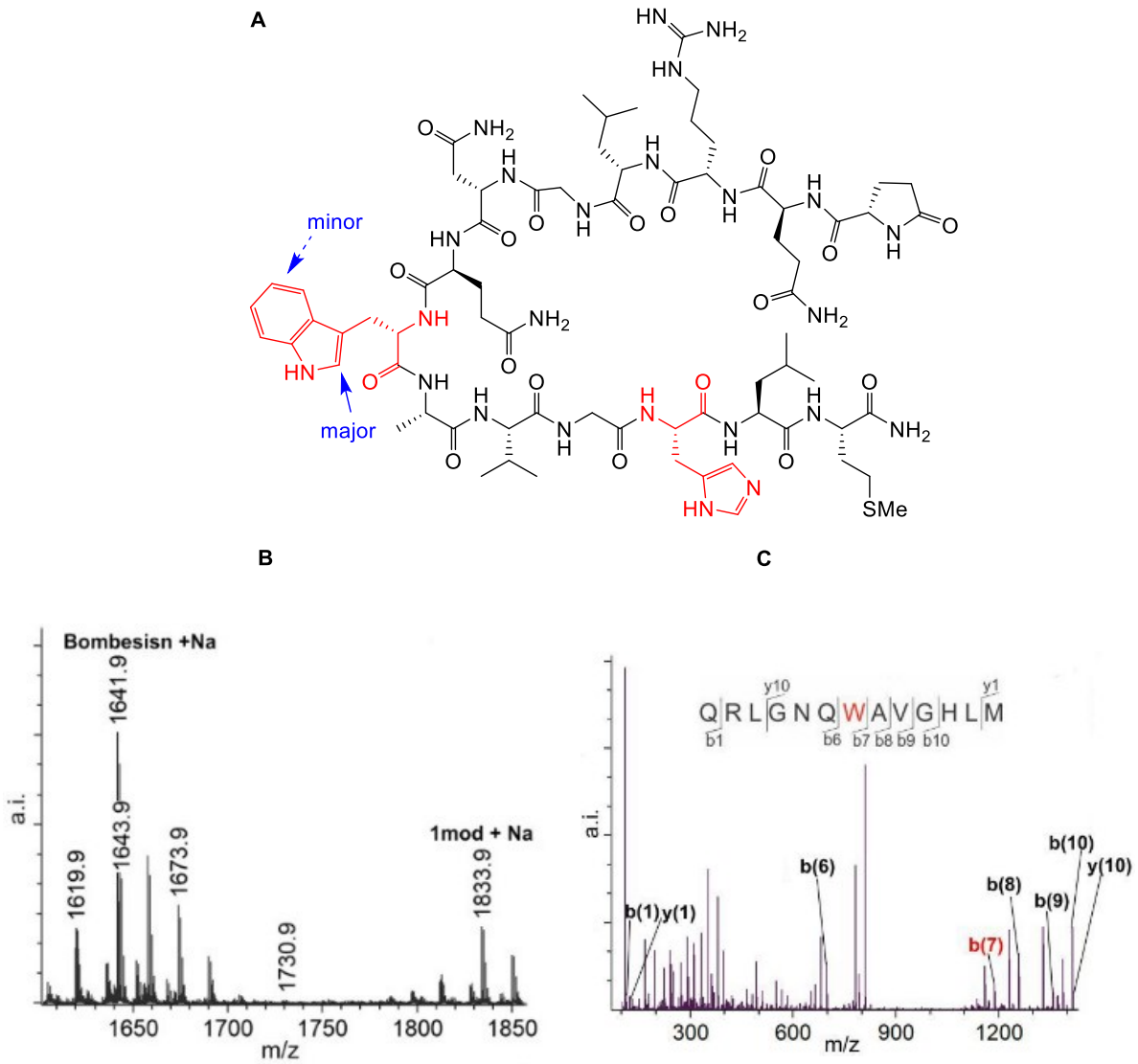


Figure 42 Photo-induced fluoroalkylation of bombesin using **59** (10 equiv.), in the presence of blue LED light, rt, 3 h. **A**: Aromatic residues are highlighted in red and sites of major and minor fluoroalkylation are shown with blue arrows. **B**: MALDI MS spectra of the product mixture using **59** (10 equiv.). **C**: MS/MS analysis for the mono(fluoroalkylated) product confirming fluoroalkylation on Trp.

3.6 Photochemical and spectroscopic investigations

As mentioned previously, fluoroalkylation of *N*-acetyl tryptophan (**89**) and tryptophan residues in peptides proceeds upon irradiation with blue light. The components **59** and **89** are colourless compounds, therefore they should not react upon irradiation with visible light. This fact brought a question: If the light is absorbed, which component is the chromophore and how does the excitation lead to the product formation? A series of experiments in collaboration with Dr. Tomáš Slanina at IOCB Prague were performed to answer these questions. We found out that tryptophan derivatives

has only minimal absorption upon light excitation; however, the solution of **59** in MeOH absorbs the light and therefore is the only chromophore (Figure 43), (See part 4.12).

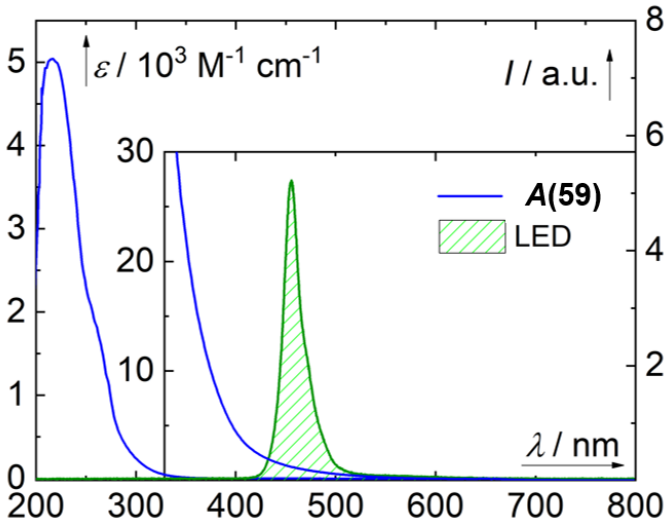


Figure 43 The blue solid line is the absorption spectra of **59** (c = 1-100 mM in MeOH). The inlet determines the zoomed spectrum at in region 330-800 nm. Emission spectrum of the LED used for irradiation (λ_{irr} = 455 nm, green line, right y-axis).

Furthermore, we observed that the absorption spectrum of the mixture of **59** and **89** almost matches the sum of absorption spectra of individual components (Figure 44).

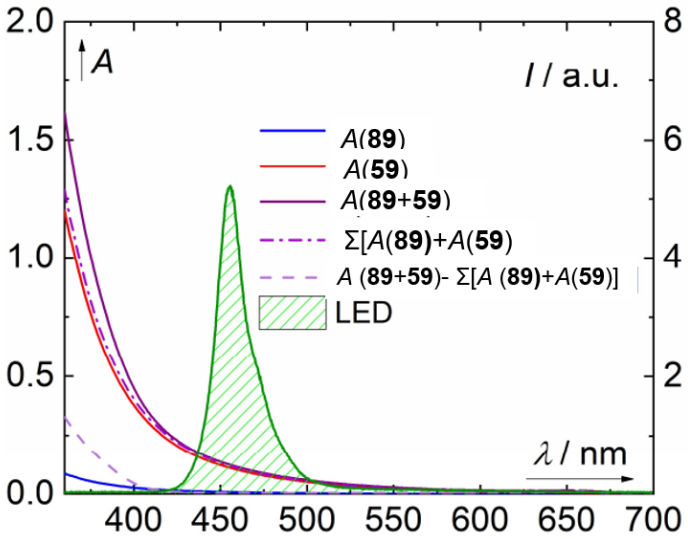


Figure 44 Absorption spectra of **59** (red solid line) **89** (blue solid line) and their equimolar mixture (violet solid line; c = 0.1 M in MeOH). Sum spectrum of **1** and **2** (violet dash-dotted line) and difference spectrum between the spectrum of a mixture of **1** and **2** and a sum spectrum of **59** and **89** (violet dashed line). Emission spectrum of LED used for irradiation (λ_{irr} = 470 nm, green line).

Also, when we compared the ^{1}H and ^{19}F NMR spectra of the mixture of **89** and **59** with individual components, no new signals were observed (Figures 45 and 46) suggesting that the formation of an electron-donor-acceptor (EDA) complex[126] is improbable under the reaction condition (See part 4.12). Furthermore, binding free energy of **89** and **59** is 36 kJ/mol (calculated at the BMK/6-31+g* level with GD3 dispersion correction) which is in contrast to the EDA complex formation observed between *N*-methyl morpholine and **59**.[42]

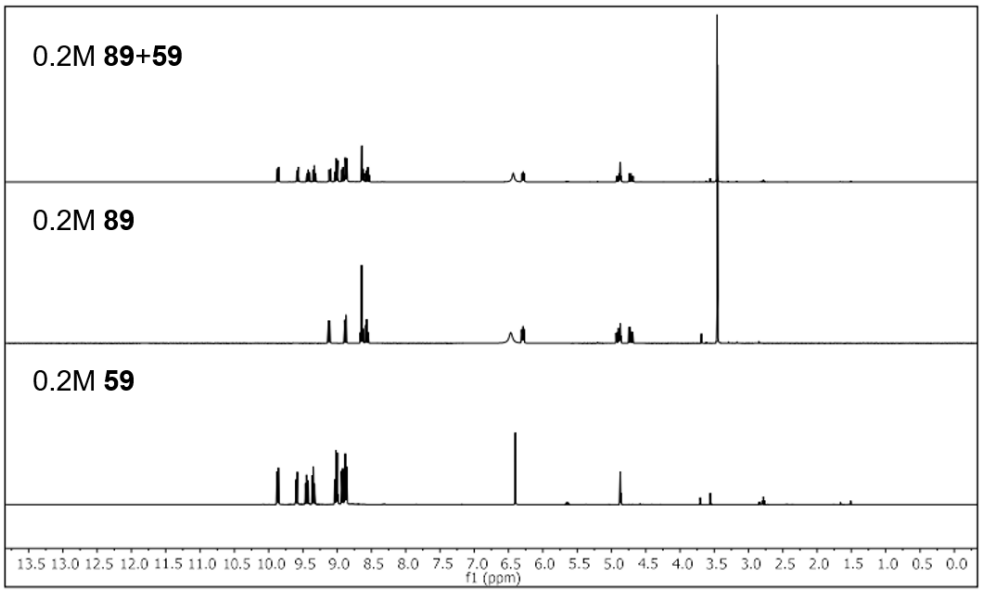


Figure 45 ^{1}H NMR (400 MHz, $CDCl_3$) of a mixture of **89** and **59** showing that no new signals were observed upon mixing the constituents.

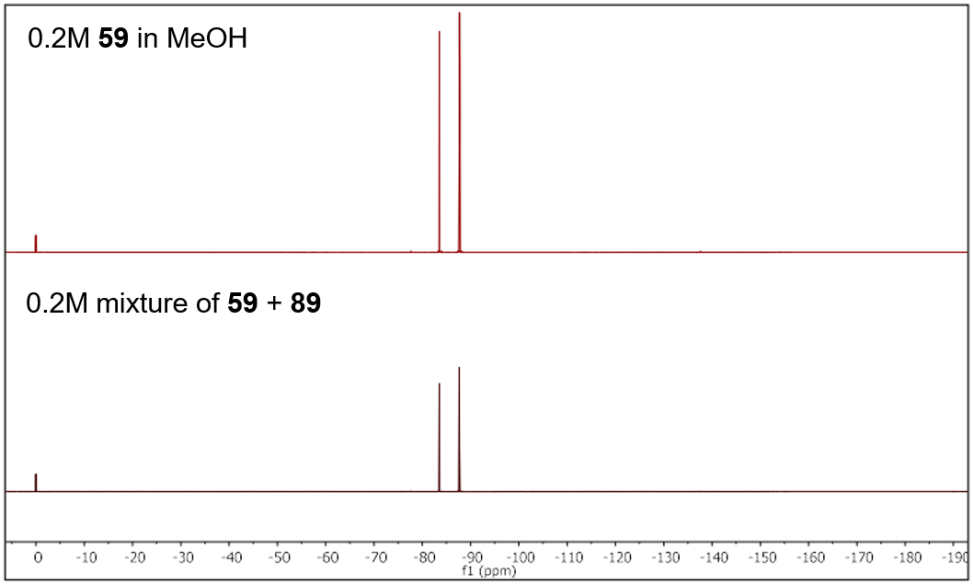


Figure 46 ^{19}F NMR (376 MHz, $CDCl_3$) of a mixture of **89** and **59** showing that no new signals were observed upon mixing the constituents.

Testing the photoreactivity of **59** (Figure 47) revealed that this compound is relatively photostable (monitored by ^{19}F NMR, Figure 48). We observed a completely different result for the analogous reaction with **89** (Figure 47A). A strong absorption peak was observed at ~380 nm with a shoulder that goes to ~600 nm. This happened partially by the polychromatic light of the diode-array spectrometer (induction period) and partially by the LED light after a significant visible absorption. However, this process is not productive and leads to polymerization and decomposition of **89**.

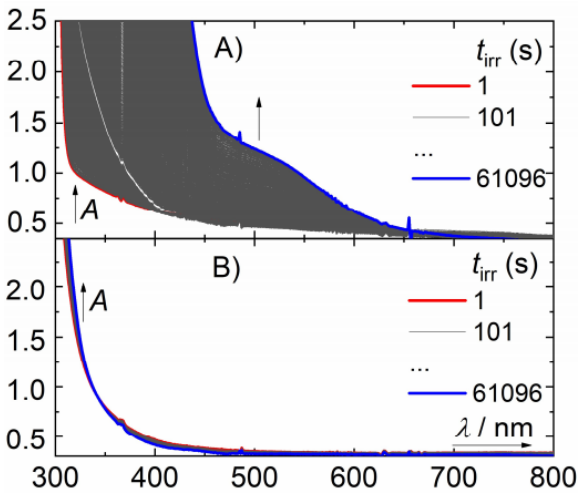


Figure 47 Absorption spectra of **89** (A) and **59** (B) (c = 0.1 M in MeOH) irradiated by LED (λ_{irr} = 470 nm) for 17 h (spectra taken every 100 s).

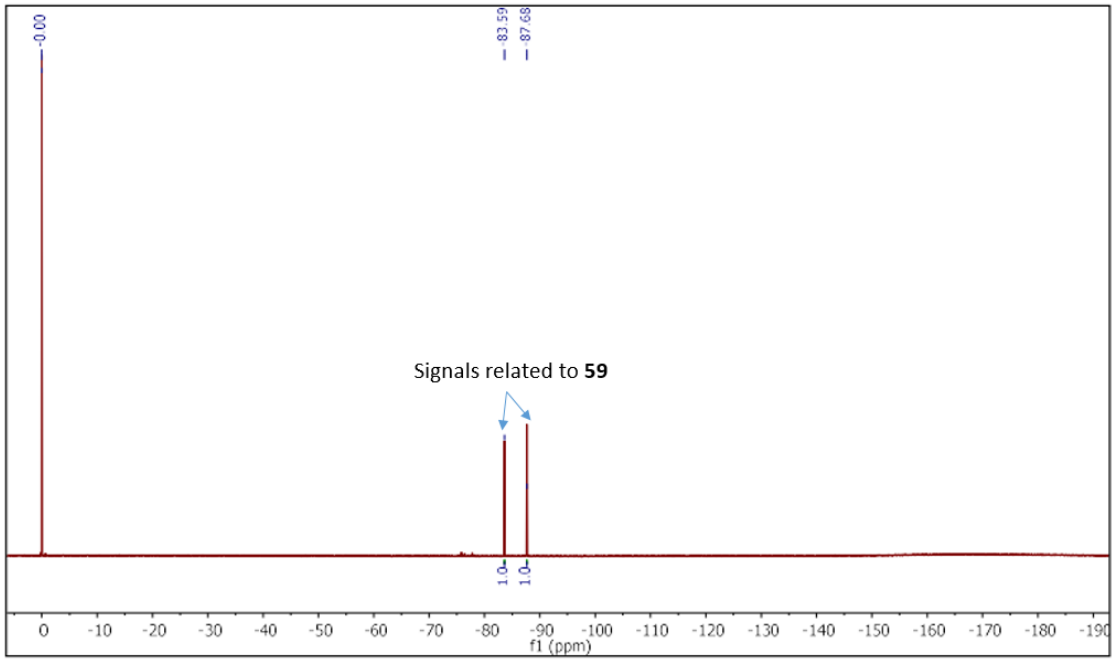


Figure 48 ^{19}F NMR (376 MHz, CD_3OD) of **59** after irradiation with blue LED light (455–475 nm) for 17 h.

Next, to mimic the real reaction conditions, the absorption spectra of the mixture of **59** and **89** (*c* = 0.1 M in MeOH) were measured (Figure 49). The formation of the product was indicated by ^{19}F NMR (Figure 50). We observed a visible absorption shoulder which is gradually formed over the course of irradiation. This characteristic behaviour does not contribute to the product formation and corresponds to the photoinduced decomposition of **89**.

The irradiation was interrupted for a defined time to see if the growth of the visible light absorption is a photoinduced process. The growth of the absorbance was stopped and was restored after the light source turned on again indicating that the process is photoinduced. The hysteresis of the curve might be caused by limited diffusion in the reaction cuvette and by dark processes initiated by light (e.g. radical-chain reaction), (See part 4.12).

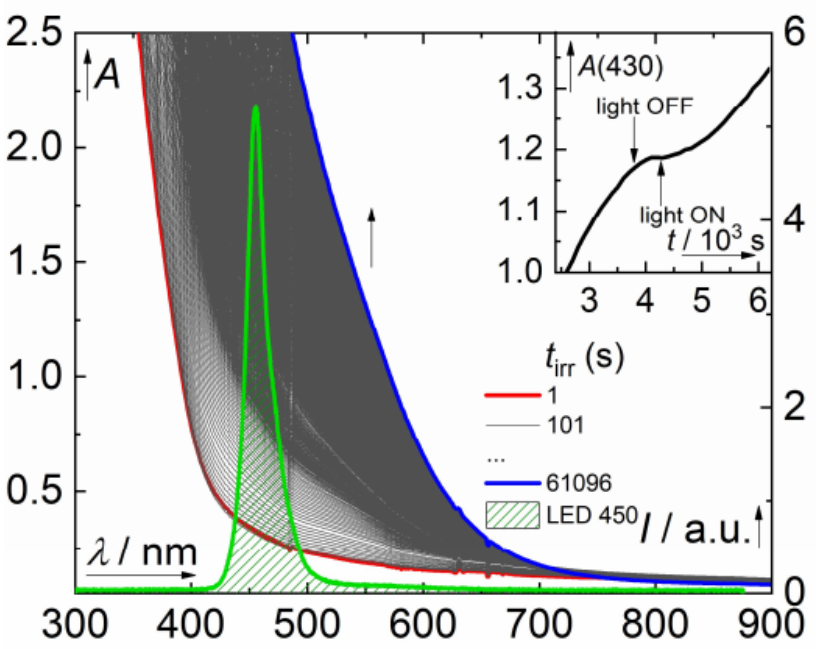


Figure 49 Absorption spectra of mixture of **59** and **89** (c = 0.1 M in MeOH) irradiated by LED (λ_{irr} = 470 nm) for 17 h (spectra taken every 100 s). Emission spectrum of the LED used for irradiation (green line). Development of absorption of the mixture at 430 nm (insert) with interrupted irradiation (indicated by arrows).

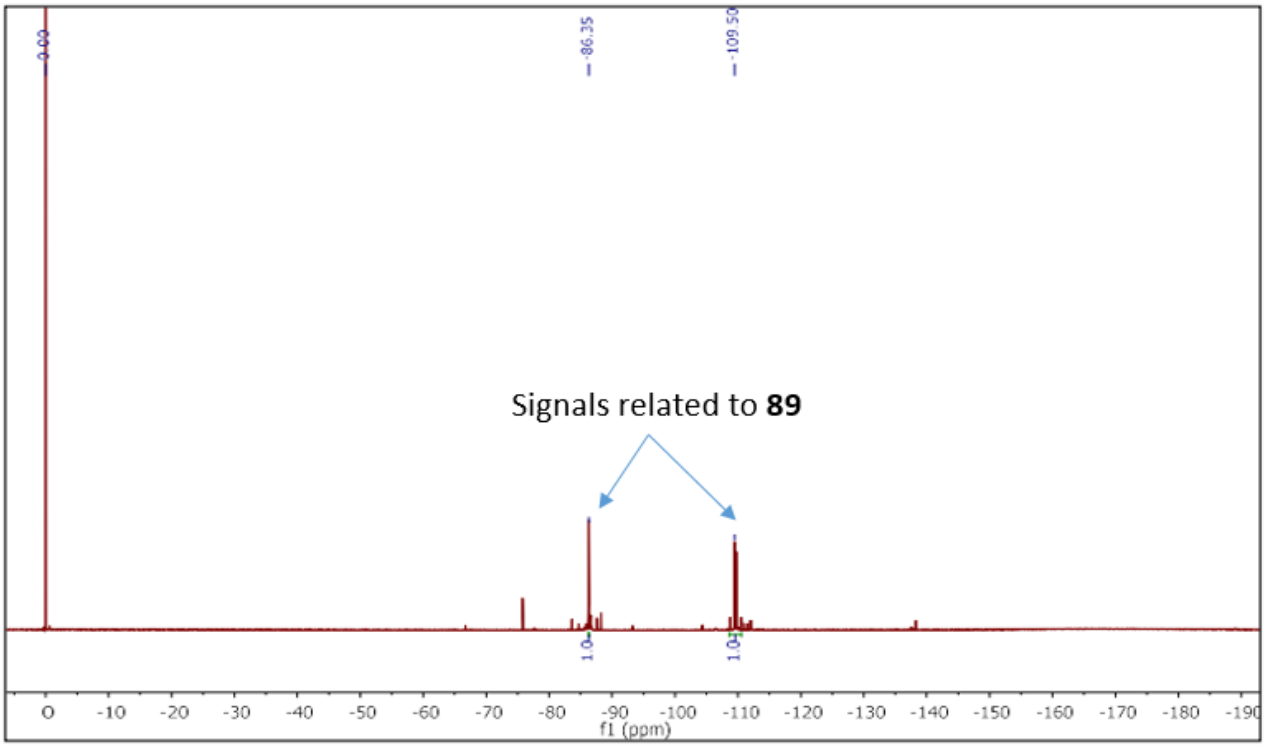


Figure 50 ^{19}F NMR (376MHz, CD_3OD) of the reaction of **89** with **59** in MeOH in the presence of blue LED light (455–475 nm).

Compound **59** was excited at 455 nm by luminescence quenching to see if it interacts with **89**. No emission was detected at this excitation wavelength; however, when **89** was excited with 368 nm the emissive state was found with weak luminescence at 470 nm whose lifetime and intensity were quenched by the presence of **59** (Figure 51). The observed dynamic quenching[127] originating from the photoinduced electron transfer between **89** and **59** was not relevant for the real reaction condition as the emissive state is not accessible by the excitation wavelength (See part 4.12).

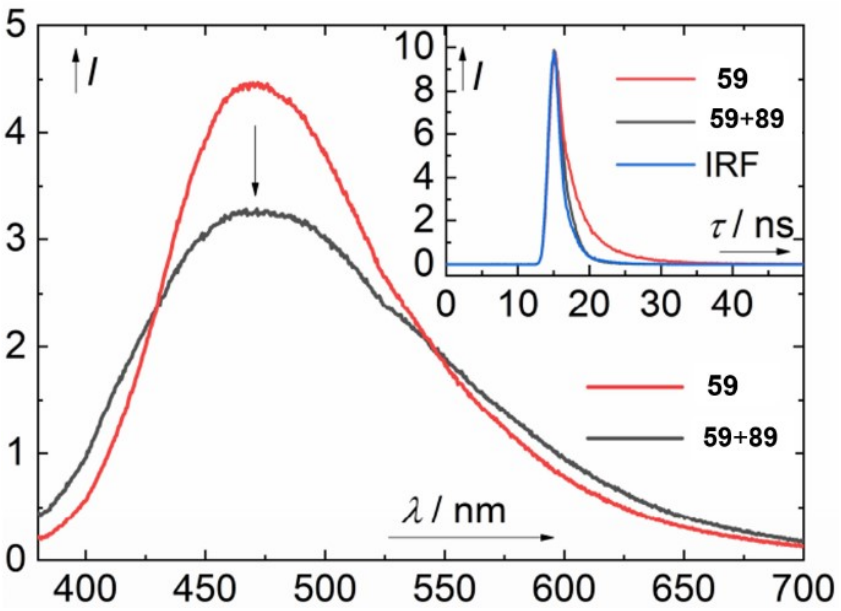


Figure 51 Luminescence spectra of **89** and the mixture of **59** and **89** (c = 0.1 M in EtOH, λ_{exc} = 368 nm). Luminescence lifetimes (insert, TCSPC analysis) of **59** and the mixture of **59** and **89** (c = 0.1 M in EtOH, λ_{exc} = 368 nm) together with the internal response function (IRF).

The view on the **59** photoreactivity was complemented by *ab initio* calculations, this investigation was performed by Dr. Petr Slavíček and his student Josef Filgas.[128] The calculations was done using a model molecule, with CF_3 substituent representing the fluoroalkyl group. For more information see the paper.[128]

3.7 Mechanism of light-induced fluoroalkylation

Based on photochemical and spectroscopic investigations as well as quantum chemical calculations a plausible mechanism was suggested as follow: Compound **59** dissociates upon irradiation by 455 nm LED light leading to the formation of fluoroalkyl radical. The resulting fluoroalkyl radical reacts with electron-rich substrate **89** to produces adduct **A** which is oxidized with another equivalent of **59** and re-aromatizes by deprotonation to give **B**.

Compound **59** is reduced to generate 2-iodobenzoic acid and another equivalent of perfluoroalkyl radical (Figure 52).

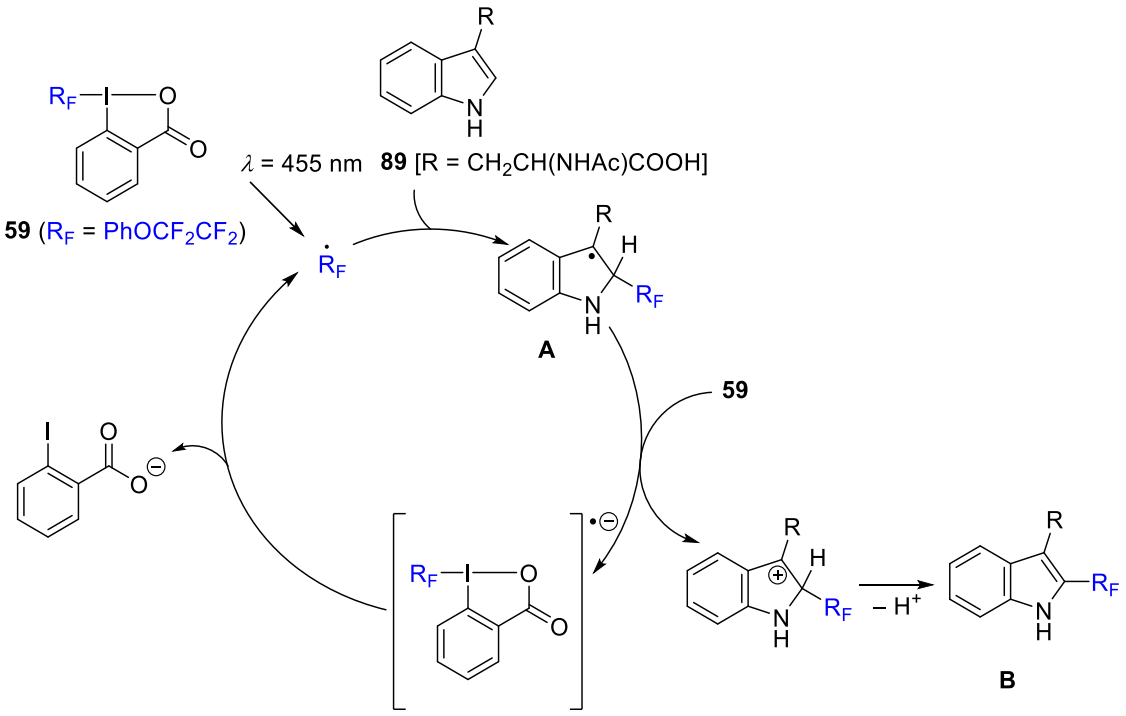


Figure 52 Proposed reaction mechanism for light-induced fluoroalkylation.

In conclusion, visible light driven fluoroalkylation of indoles, tryptophan and tryptophan-containing peptides was achieved using various fluoroalkylated hypervalent iodine reagents under biocompatible conditions. This process did not require any catalyst or additive and was selective to Trp versus other aromatic amino acids.

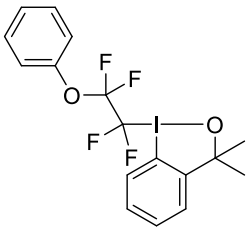
The results of our project with light-induced fluoroalkylation was published in *ChemPhotoChem* journal.[128]

4. Experimental part

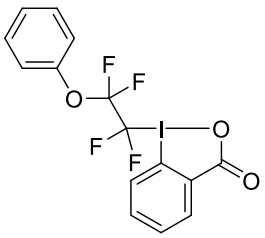
4.1 General remarks

Reactions with air-sensitive materials were carried out under argon atmosphere using standard Schlenk techniques. Solvents were dried by activated molecular sieves (3 Å) and stored under argon. All commercially available chemicals were used as received unless stated otherwise. Flash column chromatography was performed using silica gel 60 (0.040–0.063 mm). Automated flash column chromatography was performed on Teledyne ISCO CombiFlash Rf+ Lumen Automated Flash Chromatography System with UV/Vis detection. TLC analyses were done using TLC silica gel 60 F254 aluminum sheets, which were visualized under UV (254/366 nm) or using the $KMnO_4$ and phosphomolybdic acid stain solution. ^{1}H, ^{13}C, and ^{19}F NMR spectra were measured on Bruker Avance III™ HD 400 MHz at ambient temperature using 5 mm diameter NMR tubes. ^{13}C spectra were proton or proton and fluorine decoupled. The chemical shift values (δ) are reported in ppm relative to internal Me_4Si (0 ppm for ^{1}H and ^{13}C NMR) or residual solvents and internal $CFCl_3$ (0 ppm for ^{19}F NMR). Structural elucidation was aided by additional acquisition of ^{13}C APT and/or various 2D spectra (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC). GC-MS spectra were recorded on Agilent 7890A GC (column HP-5MS, 30 m × 0.25 mm × 0.25 μm, 5% phenyl methylpolysiloxane) coupled with 5975C quadrupole mass selective electron impact (EI) detector (70 eV). High resolution MS spectra (HRMS) were recorded on an LTQ Orbitrap XL using electrospray ionization (ESI), on a Waters Micromass AutoSpec Ultima or Agilent 7890A GC coupled with Waters GCT Premier orthogonal acceleration timeof-flight detector using electron impact (EI) ionization, and on a Bruker solariX 94 ESI/MALDI-FT-ICR using dual ESI/MALDI ionization. UPLC-MS analyses were performed on Acquity UPLC Instrument. MALDI-MS spectra of modified peptides were recorded using UltrafleXtreme™MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) by dried-droplet method 4:1, steel sampling plate. 3-Cyclohexyl-1*H*-indole,[129] 3-cyclopentyl-1*H*-indole,[129] 3-cyclohexyl-1-methyl-1*H*-indole,[130] 3-cyclopentyl-1-methyl-1*H*-indole,[130] 1-ethyl-3-methyl-1*H*-indole,[131] 1,3-dimethyl-1*H*-indole[131] were synthetized following literature procedures. As light sources were used LED diodes (3 W) of appropriate wavelengths (blue λ_{max} 390–410 nm, blue λ_{max} 455–475 nm, green λ_{max} 515–535 nm, red λ_{max} 648 nm).The light was carried through a glass rod directly to the reaction mixture.

4.2 Preparation of reagents

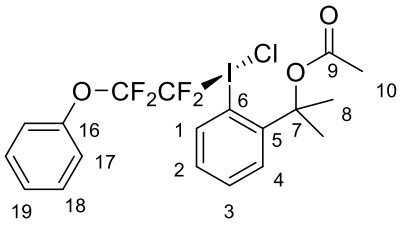


3,3-Dimethyl-1-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1,3-dihydro-1λ^3-benzo[*d*][1,2]iodaoxole (57): In a round bottom flask under argon, 1-fluoro-3,3-dimethyl-1,3-dihydro-1λ^3-benzo[*d*][1,2]iodaoxole (4.19 g, 14.9 mmol, 1.3 equiv.) and TBAT (0.30 g, 0.55 mmol, 5 mol%) were dissolved in dry MeCN (30 mL). The solution was cooled to -35 °C. A solution of $PhOCF_2CF_2TMS$ (3.00 g, 11.26 mmol, 1 equiv.) in MeCN (9 ml) was added dropwise over 20 min, then the mixture was left to warm up to room temperature over 45 min while being stirred. The solvent was removed under reduced pressure; the product was redissolved in cyclohexane and filtered over activated alumina. The filtrate was evaporated to dryness, the product was redissolved in Et_2O (10 ml) and the solution was cooled to 0 °C. 1.4 M solution of HCl in Et_2O (15.7 ml, 2 equiv.) was added. The formed precipitate was filtered and washed with pentane (30 ml). Ice-cold saturated solution of $NaHCO_3$ (30 ml) was added and the product was extracted to Et_2O (30 ml). Drying ($MgSO_4$), solvent removal and further drying in vacuum afforded pure product as a white solid: Yield (3.35 g, 66%); ^{1}H NMR (400MHz, CD_3OD): δ 1.51 (s, 6H), 7.26–7.29 (m, 2H, $C_{Ar}H$), 7.34–7.38 (m, 1H, $C_{Ar}H$), 7.44–7.49 (m, 2H, $C_{Ar}H$), 7.50–7.58 (m, 2H, $C_{Ar}H$), 7.62 (ddd, J_{HH} = 7.8, 6.9, 0.9 Hz, 1H, $C_{Ar}H$), 7.82 (dq, J_{HH} = 8.4, 1.2 Hz, 1H, $C_{Ar}H$); ^{19}F NMR (376 MHz, CD_3OD): δ –83.72 (t, $^3J_{FF}$ = 4.8 Hz, 2F, CF_2), –95.69 (t, $^3J_{FF}$ = 4.8 Hz, 2F, CF_2); ^{13}C {^{1}H, ^{19}F} NMR (101 MHz, CD_3OD): δ 31.01, 77.47, 111.16, 112.45, 118.53, 122.70, 128.19, 129.08, 130.24, 131.01, 131.09, 132.07, 150.09, 151.14; HRMS (ESI$^+$): *m/z* calcd for $C_{17}H_{16}F_4IO_2$ $[M+H]^+$ 455.0125, found 455.0123.

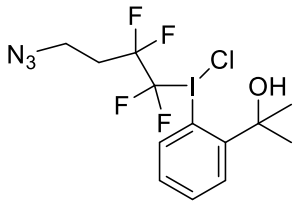


1-(1,1,2,2-Tetrafluoro-2-phenoxyethyl)-1λ^3-benzo[*d*][1,2]iodaoxol-3(1*H*)-one (59): A Schlenk flask under argon was charged with CsF (7.11 g, 23.6 mmol, 1 equiv.), 3-oxo-1λ^3-benzo[*d*][1,2]iodaoxol-1(3*H*)-yl acetate (10.877 g, 35 mmol, 1.5 equiv.) and dry DMF (42 ml). The mixture was stirred for 5 min and a solution of $PhOCF_2CF_2TMS$ (6.97 g, 26.25 mmol, 1 equiv.) in dry DMF (82 ml) was added. The reaction mixture was stirred for 2 h and then filtered. EtOAc (50 ml) was added to the filtrate and the mixture was washed with brine (3 × 40 ml), 1M

aqueous $NaHCO_3$ (3 × 40 ml), 1M aqueous LiCl (3 × 40 ml), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by trituration with a mixture of Et_2O/pentane (1:5), decantation and drying under vacuum to afford pure product (6.70 g, 58% yield) as a pale yellow solid; Yield: (6.70 g, 58% yield), ^{1}H NMR (401 MHz, CD_3OD): δ 7.33–7.41 (m, 3H, $C_{Ar}H$), 7.46–7.52 (m, 2H, $C_{Ar}H$), 7.83 (td, J_{HH} = 7.4, 0.9 Hz, 1H, $C_{Ar}H$), 7.90–7.94 (m, 1H, $C_{Ar}H$), 8.09 (dd, J_{HH} = 8.4, 0.9 Hz, 1H, $C_{Ar}H$), 8.34 (dd, J_{HH} = 7.6, 1.8 Hz, 1H, $C_{Ar}H$); ^{19}F NMR (376 MHz, CD_3OD): δ –83.57 (t, $^3J_{FF}$ = 6.2 Hz, 2F, CF_2), –87.61 (t, $^3J_{FF}$ = 6.2 Hz, 2F, CF_2); ^{13}C {^{1}H} NMR (101 MHz, CD_3OD): δ 111.05 (t, J_{CF} = 40.7 Hz), 116.08, 117.63 (t, J_{CF} = 25.7 Hz), 122.75, 128.63, 130.44 (t, J_{CF} = 6.0 Hz), 131.23, 132.73, 133.60, 134.22, 136.79, 149.71, 169.46; HRMS (ESI$^+$): *m/z* calcd for $C_{15}H_{10}F_4IO_3$ [M+H]$^+$ 440.9605, found 440.9602.



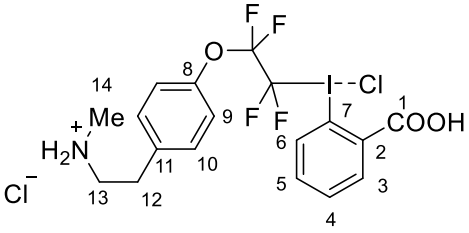
2-(2-(Chloro(1,1,2,2-tetrafluoro-2-phenoxyethyl)-λ^3-iodaneyl)phenyl)propan-2-yl acetate (57·AcCl): Iodane **57** (227 mg, 0.5 mmol) was dissolved in dry $CHCl_3$ (1 ml) under argon atmosphere and acetyl chloride (0.1 ml, 1.5 mmol, 3 equiv.) was added in one portion. The mixture was stirred for 15 minutes at rt. After removing volatiles under reduced pressure, trituration with Et_2O, and solvent removal gave pure product. Yield: 194 mg (73%), white particles; ^{1}H NMR (401 MHz, $CDCl_3$): δ 2.09 (s, 6H, C(8)H_3), 2.22 (s, 3H, C(10)H_3), 7.20 (d, $^3J_{HH}$ = 8.1 Hz, 2H, C(17)H), 7.29–7,36 (m, 2H, C(3)H and C(19)H), 7.40 (t, $^3J_{HH}$ = 7.8 Hz, 2H, C(18)H), 7.67–7.73 (m, 2H, C(2)H and C(4)H), 8.37 (d, $^3J_{HH}$ = 8.0 Hz, 1H, C(1)H); ^{19}F NMR (377.28 MHz, $CDCl_3$): δ –87.78 (br s, 2F, CF_2I), –84.25 (t, $^3J_{FF}$ = 6.8 Hz, 2F, CF_2O); ^{13}C {^{1}H} NMR (101 MHz, $CDCl_3$): δ 22.94 (C(10)H_3), 28.72 (C(8)H_3), 82.16 (C(7)), 112.86 (tt, $^1J_{CF}$ = 345.1 Hz, $^2J_{CF}$ = 40.7 Hz, ICF_2), 114.36 (C(6)), 116.48 (tt, $^1J_{CF}$ = 277.2 Hz, $^2J_{CF}$ = 25.8 Hz, OCF_2), 121.46 (C(17)H), 127.32 (C(19)H), 129.61 (C(4)H), 129.99 (C(18)H), 130.95 (C(3)H), 133.18 (C(2)H), 141.84 (C(1)), 145.62 (C(5)), 148.18 (C(16)), 169.44 (C(9)); HRMS (ESI$^+$) *m/z* calcd for $C_{19}H_{18}O_3ClF_4INa$ [M+Na]$^+$ 554.9818, found 554.9811.



2-(2-((4-Azido-1,1,2,2-tetrafluorobutyl)chloro-λ^3-iodanyl)phenyl)propan-2-ol (68): 1-Fluoro-3,3-dimethyl-1,3-dihydro-1λ^3-benzo[*d*][1,2]iodaoxole (1.83 g, 6.41 mmol, 1.3 equiv.) was dissolved in MeCN (15 ml) and to the resulting solution was added TBAT (133 mg, 0.247 mmol, 5 mol%) The reaction mixture was cooled to -20 °C and a solution of 4-azido-1-trimethylsilyl-1,1,2,2-tetrafluorobutane (1.5 g of 80% purity, 4.93 mmol, 1 equiv.) in MeCN (10 ml) was slowly introduced to the reaction mixture within 50 min. After the addition was complete, the reaction mixture was gradually warmed to room temperature within 80 min. The resulting brownish solution was evaporated to dryness under reduced pressure and the resulting viscous oil was redissolved in cyclohexane (35 ml). The solution was filtered through a pad of alumina (activated by heatgun drying in vacuo) and evaporated to dryness under reduced pressure. The resulting liquid was dissolved in a mixture of Et_2O (3 ml) and pentane (7 ml), the solution was cooled to 0 °C and HCl in Et_2O (3.3 ml of 3M solution, 9.86 mmol, 2 equiv) was slowly added. The resulting white solid was filtered, washed with pentane and dried in vacuo. Yield: 0.97 g (49%). ^{1}H NMR (401 MHz, $CDCl_3$): δ 1.72 (s, 6H, CH_3), 2.30–2.43 (m, 2H, CH_2CF_2), 3.59 (t, J = 7.0 Hz, 2H, CH_2N_3), 4.56 (br s, 1H, OH), 7.25–7.29 (m, 1H, $C_{Ar}H$), 7.59–7.66 (m, 2H, $C_{Ar}H$), 8.13 (d, J = 8.1 Hz, 1H, $C_{Ar}H$); ^{19}F NMR (377 MHz, $CDCl_3$) δ –80.6 (s, 2F, CF_2), –106.4 (t, J = 18.2 Hz, 2F, CF_2); ^{13}C {^{1}H} NMR (100.8 Hz, $CDCl_3$) δ 29.6 (t, $^2J_{CF}$ = 22.4 Hz, CH_2CF_2), 31.9 (CH_3), 43.4 (t, $^3J_{CF}$ = 3.8 Hz, CH_2N_3), 74.2 (C-OH), 112.8 (C-I), 112.0–119.3 (m, CF_2), 129.8, 130.1, 132.8, 139.2, 147.3; HRMS (ESI$^+$): *m/z* calcd for $C_{13}H_{15}ON_3F_4I$ $[M]^+$ 432.01904, found 432.01924.

***Tert*-butyl mthyl(4-(1,1,2,2-tetrafluoro-2-(3-oxo-1 λ^3-benzo[*d*][1,2]iodaoxol-1(3*H*)-yl)ethoxy)phenethyl)carbamate (60')**: CsF (68 mg, 0.45 mmol) and 3-oxo-1λ^3-benzo[*d*][1,2]iodaoxol-1(3*H*)-yl acetate (918 mg, 3 mmol) were mixed with dry DMF (2.5 ml) under argon atmosphere. To the stirring suspension, a solution of *t*-butyl methyl(4-(1,1,2,2-tetrafluoro-2-(trimethylsilyl)ethoxy)-phenethyl)carbamate (635 mg, 1.5 mmol) in dry DMF (5 ml) was added dropwise. After 2 h the reaction mixture was diluted with EtOAc (50 ml), washed with water (10 ml), 1 M $NaHCO_3$ (2 ×

10 ml), 1 M LiCl (2 × 10 ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by filtration through alumina (20 g) eluting impurities first with Et_2O (150 ml), then product with MeOH (75 ml). Solvent evaporation afforded **60'**as a colorless oil. Yield: 604 mg (69%); ^{1}H NMR (401 MHz, $CDCl_3$): δ 1.34–1.39 (br s, 9H, C(17)H_3), 2.76–2.83 (br m, 5H, C(12)H_2 and C(14)H_3), 3.41 (t, $^3J_{HH}$ = 7.2 Hz, 2H, C(13)H_2), 7.12 (d, $^3J_{HH}$ = 8.2 Hz, 2H, C(9)H or C(10)H), 7.16–7.25 (br m, 2H, C(9)H or C(10)H), 7.66–7.80 (m, 2H, C(4)H and C(5)H), 7.90 (d, $^3J_{HH}$ = 8.1 Hz, 1H, C(6)H), 8.43 (dd, $^3J_{HH}$ = 7.3 Hz, $^4J_{HH}$ = 2.1 Hz, 1H, C(3)H); ^{19}F NMR (377.28 MHz, $CDCl_3$): δ –89.5 (br s, 2F, CF_2), –84.4 (br s, 2F, CF_2); ^{13}C {^{1}H} NMR (101 MHz, $CDCl_3$): δ 28.2 (C(17)), 33.3 and 33.7 (C(12)), 34.1 and 34.6 (C(14)), 49.9 and 50.4 (C(13)), 79.3 (C(16)), 110.5 (tt, $^1J_{CF}$ = 335.4 Hz, $^2J_{CF}$ = 40.0 Hz, CF_2), 114.8 (C(7)), 117.2 (tt, $^1J_{CF}$ = 277.9 Hz, $^2J_{CF}$ = 25.6 Hz, CF_2), 121.4 (br s, C(9) or C(10), 128.1 (t, $^4J_{CF}$ = 5.7 Hz, C(6), 130.3 (C(9) or C(10), 131.5 (C(2)), 132.3 (C(4)), 133.7 (C(3)), 135.2 (C(5)), 138.8 (m, C(11)), 146.4 (C(8)), 155.4 (C(15)), 165.9 (C(1)); HRMS (ESI$^+$): *m/z* calcd for $C_{23}H_{24}F_4INO_5Na$ $[M+Na]^+$ 620.0528, found 620.0530.

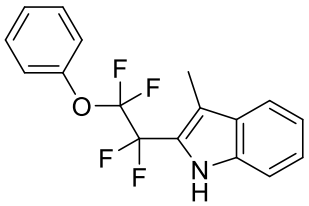


2-(4-(2-((2-Carboxyphenyl)chloro-λ^3-iodaneyl)-1,1,2,2-tetrafluoroethoxy)phenyl)-*N*-methylethan-1-aminium chloride (60): The intermediate iodane **60'** (2.00 g, 3.4 mmol) was dissolved in 1,2-dichloroethane (68 ml) in a round bottom flask. HCl (4 M in dioxane, 34 mmol, 8.5 ml) was added. The mixture was stirred 1 h at 60 °C. The solvent was removed under reduced pressure and product triturated with Et_2O to afford pure product. Yield: 1.46 g (75%); m.p. 120–123 °C; white solid; ^{1}H NMR (401.00 MHz, DMSO-*d*$_6$): δ 2.54 (t, $^3J_{HH}$ = 5.3 Hz, 3H, C(14)H_3), 2.98–3.01 (br m, 2H, C(12)H_2, 3.06–3.21 (br m, 2H, C(13)H_2), 7.25 (d, $^3J_{HH}$ = 8.0 Hz, 2H, C(9)H), 7.37 (d, $^3J_{HH}$ = 8.0 Hz, 2H, C(10)H), 7.70 (t, $^3J_{HH}$ = 7.6 Hz, 1H, C(5)H), 7.82 (t, $^3J_{HH}$ = 7.5 Hz, 1H, C(4)H), 8.24 (d, $^3J_{HH}$ = 7.6 Hz, 1H, C(6)H), 8.49 (d, $^3J_{HH}$ = 7.6 Hz, 1H, C(3)H), 9.17 (s, 2H, NH_2); ^{19}F NMR (377.28 MHz, DMSO-*d*$_6$): δ –88.5 (br s, 2F, CF_2), –82.9 (t, $^3J_{FF}$ = 6.6 Hz, 2F, CF_2); ^{13}C {^{1}H} NMR (101 MHz, DMSO-*d*$_6$): δ 31.1 (C(12)), 32.8 (C(14)), 49.3 (C(13)), 112.2 (tt, $^1J_{CF}$ = 341.8 Hz, $^2J_{CF}$ = 41.2 Hz, CF_2), 116.9 (tt, $^1J_{CF}$ = 275.5 Hz, $^2J_{CF}$ = 26.9 Hz, CF_2), 119.7 (C(7)), 121.5 (br s, C(9)), 130.8 (C(10)), 131.1 (C(2)), 132.2 (C(6)), 133.1 (C(4)), 135.5 (C(5)), 137.0 (m, C(8)),

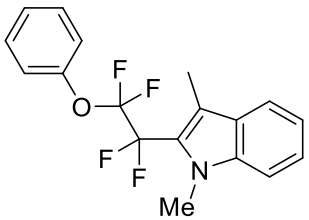
140.0 (C(3)), 147.1 (C(11)), 165.5 (C(1)); HRMS (ESI$^+$): *m/z* calcd for $C_{18}H_{17}F_4INO_3$, $[M+H]^+$ 498.0184, found 498.0183.

4.3 Fluoroalkylation of small molecules, general procedure

Substrate (indole or pyrrole derivative) (0.5 mmol, 1 equiv.) was dissolved in MeOH (2.14 ml) and the solution was degassed with argon. A solution of sodium ascorbate (0.25 mmol, 0.5 eq.) in water (0.72 ml) was added, followed by dropwise addition (over 5 min) of a solution of **59** (0.6 mmol, 1.2 equiv.) in MeOH (2.14 ml). The reaction mixture was stirred at room temperature for 5 min., MeOH was removed under reduced pressure, water (15 ml) was added and the product was extracted with DCM (15 ml). The organic phase was washed with water (3 × 15 ml), brine (3 × 15 ml), aqueous saturated $NaHCO_3$ (3 × 15 ml), dried over $MgSO_4$ and concentrated under reduced pressure. Purification by chromatography (silica gel) provided pure product.

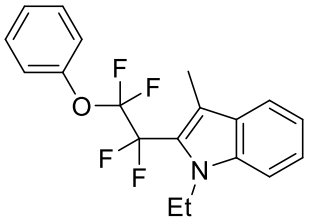


3-Methyl-2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indole (73a): Purified by flash chromatography (cyclohexane/DCM, 3:1). Yield: 140 mg (87%); pale yellow oil; R_f = 0.27 (cyclohexane/DCM, 3:1); ^{1}H NMR (400 MHz, CD_3OD): δ 2.53 (t, J_{HH} = 2.2 Hz, 3H, CH_3), 7.14–7.20 (m, 3H, $C_{Ar}H$), 7.27–7.33 (m, 2H, $C_{Ar}H$), 7.38–7.44 (m, 2H, $C_{Ar}H$), 7.48–7.51 (m, 1H, $C_{Ar}H$), 7.68 (m, 1H, $C_{Ar}H$); ^{19}F NMR (376 MHz, CD_3OD): δ –86.48 (t, $^3J_{FF}$ = 6.4 Hz, 2F, CF_2), –110.84 (tt, $^3J_{FF}$ = 5.9, 2.4 Hz, 2F, CF_2); ^{13}C {^{1}H, ^{19}F} NMR (126 MHz, CD_3OD): δ 8.86 (3H, CH_3), 112.68, 114.71, 115.04, 119.41, 120.34, 120.44, 122.54, 122.66, 124.75, 127.57, 129.42, 130.76, 131.71, 150.48; HRMS (ESI$^+$) *m/z* calcd for $C_{17}H_{13}F_4NO$ $[M]^+$ 323.0933, found 323.0935.

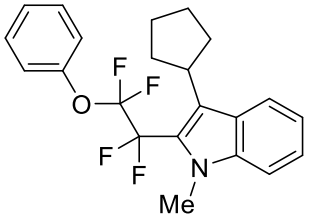


1,3-Dimethyl-2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indole (74a): Purified by flash chromatography (cyclohexane/DCM, 6:1). Yield: 97 mg (58%); pink oil; R_f = 0.25 (cyclohexane/DCM, 6:1); ^{1}H NMR (500 MHz, CD_3OD): δ 2.36 (t, J_{HH} = 3.0 Hz, 3H, CH_3), 3.77 (t, J_{HH} = 1.6 Hz, 3H, N-Me), 7.00–7.05 (m, 3H, $C_{Ar}H$), 7.15–7.18 (m, 1H, $C_{Ar}H$), 7.22 (ddd, J_{HH} = 8.3, 6.9, 1.1 Hz, 1H, $C_{Ar}H$), 7.24–7.28 (m, 2H, $C_{Ar}H$), 7.33 (dt, J_{HH} = 8.4, 0.9 Hz, 1H, $C_{Ar}H$), 7.53

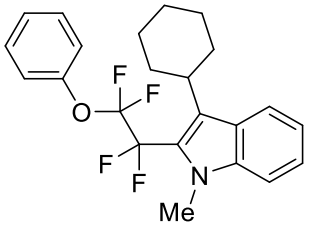
(dt, J_{HH} = 8.0, 1.0 Hz, 1H, $C_{Ar}H$); ^{19}F NMR (376 MHz, CD_3OD): δ –86.24 (t, $^3J_{FF}$ = 6.9 Hz, 2F, CF_2), –106.46 to –106.51 (m, 2F, CF_2); ^{13}C {^1H, ^{19}F} NMR (126 MHz, CD_3OD): δ 9.37 (CH_3), 32.11 (N-Me), 110.77, 115.61, 117.11, 119.56, 120.62, 120.84, 122.64, 123.38, 125.37, 127.78, 128.72, 130.91, 139.76, 150.35; HRMS (ESI$^+$) *m/z* calcd for $C_{18}H_{15}F_4NO$ $[M+H]^+$ 338.1162, found 338.1163.



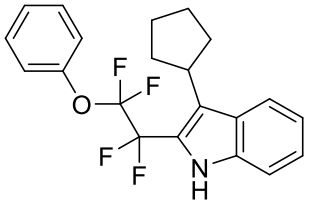
1-Ethyl-3-methyl-2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indole (75a): Purified by flash chromatography (cyclohexane/DCM, 6:1). Yield: 94 mg (54%); white solid; R_f = 0.30 (cyclohexane/DCM, 6:1); ^1H NMR (400 MHz, CD_3OD): δ 1.26 (t, J_{HH} = 7.1 Hz, 3H, CH_3), 2.36 (t, J_{HH} = 2.9 Hz, 3H, CH_3), 4.29 (q, J_{HH} = 7.3 Hz, 2H, CH_2), 6.98–7.05 (m, 3H, $C_{Ar}H$), 7.14–7.29 (m, 4H, $C_{Ar}H$), 7.34 (dt, J_{HH} = 8.1, 1.0 Hz, 1H, $C_{Ar}H$), 7.54 (dt, J_{HH} = 8.1, 1.0 Hz, 1H, $C_{Ar}H$); ^{19}F NMR (376 MHz, CD_3OD): δ –86.30 (t, $^3J_{FF}$ = 7.1 Hz, 2F, CF_2), –106.50 (td, J_{FF} = 7.0, 3.5 Hz, 2F, CF_2); ^{13}C {^1H, ^{19}F} NMR (126 MHz, CD_3OD): δ 9.42 (CH_3), 15.68 (CH_3), 41.17 (N-CH_2), 111.05, 115.72, 117.05, 119.50, 120.83, 120.85, 122.59, 122.66, 125.32, 127.79, 129.14, 130.91, 138.58, 150.33; HRMS (ESI$^+$) *m/z* calcd for $C_{19}H_{17}F_4NO$ $[M]^+$ 351.1246, found 351.1244.



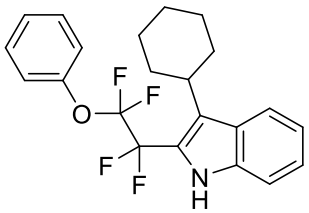
3-Cyclopentyl-1-methyl-2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indole (76a): Purified by flash chromatography (cyclohexane/DCM, 30:1), followed by purification by manual column in pure pentane. Yield: 96.6 mg (50%); white oil; R_f = 0.3 (cyclohexane/DCM, 30:1); ^1H NMR (400 MHz, CD_3OD): δ 1.70–1.80 (m, 2H), 1.92–2.13 (m, 6H), 3.60–3.67 (m, 1H), 3.84 (t, J_{HH} = 2.0 Hz, 3H), 7.06–7.12 (m, 3H, $C_{Ar}H$), 7.23–7.31 (m, 2H, $C_{Ar}H$), 7.31–7.37 (m, 2H, $C_{Ar}H$), 7.44 (dt, J_{HH} = 8.4, 1.0 Hz, 1H, $C_{Ar}H$), 7.74 (dt, J_{HH} = 8.2, 1.0 Hz, 1H, $C_{Ar}H$); ^{19}F NMR (376 MHz, CD_3OD): δ –86.44 (t, $^3J_{FF}$ = 7.4 Hz, 2F, CF_2), –104.63 to 104.67 (m, 2F, CF_2); ^{13}C {^1H, ^{19}F} NMR (101 MHz, CD_3OD): δ 27.66, 32.22, 34.32, 38.19, 111.34, 115.62, 119.36, 120.32, 122.50, 122.66, 123.19, 124.92, 125.31, 126.10, 127.78, 130.89, 140.54, 150.34; HRMS (ESI$^+$) *m/z* calcd for $C_{22}H_{21}F_4NO$ $[M]^+$ 391.1559, found 391.1556.



3-Cyclohexyl-1-methyl-2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indole (77a): Purified by flash chromatography (cyclohexane/DCM, 30:1), followed by purification by normal column chromatography (cyclohexane); Yield: 111 mg (55%); white oil; R_f = 0.30 (cyclohexane/DCM, 30:1); ^{1}H NMR (400 MHz, DMSO): δ 1.30–1.44 (m, 3H), 1.68–1.83 (m, 5H), 2.02 (m, 2H), 3.08–3.14 (m, 1H), 3.85 (t, J_{HH} = 2.0 Hz, 3H, CH_3), 7.11 (ddd, J_{HH} = 8.0, 6.9, 1.0 Hz, 1H, $C_{Ar}H$), 7.19–7.23 (m, 2H, $C_{Ar}H$), 7.29–7.37 (m, 2H, $C_{Ar}H$), 7.43–7.48 (m, 2H), 7.58 (dt, J_{HH} = 8.6, 0.9 Hz, 1H, $C_{Ar}H$), 7.92 (dt, J_{HH} = 8.2, 1.0 Hz, 1H, $C_{Ar}H$); ^{19}F NMR (376 MHz, DMSO): δ –84.90 (t, $^3J_{FF}$ = 68.1 Hz, 2F, CF_2), –106.68 (t, $^3J_{FF}$ = 6.2 Hz, 2F, CF_2); ^{13}C {^{1}H} NMR (101 MHz, DMSO): δ 35.01, 35.78, 36.17, 41.29 (t, J = 6.0 Hz), 41.77, 45.53 (t, J = 5.3 Hz), 102.35, 128.89, 129.76, 130.60, 131.63, 133.27, 134.12, 134.81, 136.46, 139.70, 139.73, 148.03, 157.77; HRMS (ESI$^+$) *m/z* calcd for $C_{23}H_{23}F_4NO$ [M]$^+$ 405.1716, found 405.1713.

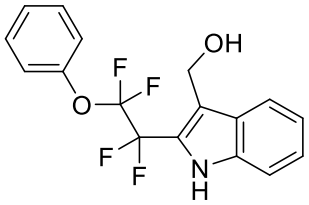


3-Cyclopentyl-2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indole (78a): Purified by flash chromatography (cyclohexane/DCM, 30:1), followed by purification by column chromatography (cyclohexane); Yield: 105.9 mg (56%); white oil; R_f = 0.30 (cyclohexane/DCM, 30:1); ^{1}H NMR (400 MHz CD_3OD): δ 1.74–1.79 (m, 2H), 1.93-2.12 (m, 6H), 3.57–3.62 (m, 1H), 7.05 (ddd, J_{HH} = 8.1, 7.0, 1.1 Hz, 1H, $C_{Ar}H$), 7.10–714 (m, 2H, $C_{Ar}H$), 7.19–7.28 (m, 2H, $C_{Ar}H$), 7.33–7.39 (m, 2H, $C_{Ar}H$), 7.45 (dt, J_{HH} = 8.3, 1.0 Hz, 1H, $C_{Ar}H$), 7.72 (dt, J_{HH} = 8.2, 1.0 Hz, 1H, NH); ^{19}F NMR (376 MHz, CD_3OD): δ –86.68 (t, $^3J_{FF}$ = 6.5 Hz, 2F, CF_2), –109.72 (t, $^3J_{FF}$ = 6.5 Hz, 2F, CF_2); ^{13}C {^{1}H} NMR (101 MHz, CD_3OD): δ 27.58, 34.21, 38.03, 113.28, 115.11 (t, J_{CF} = 38.4 Hz), 119.27 (t, J_{CF} = 37.0), 120.00, 122.17, 122.39, 122.78, 122.85 (t, J_{CF} = 2.84 Hz), 124.34, 126.84, 127.65, 130.80, 138.50, 150.52; HRMS (ESI$^+$) *m/z* calcd for $C_{21}H_{19}F_4NO$ [M]$^+$ 377.1403, found 377.1401.

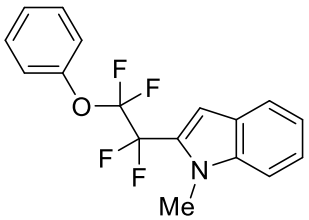


3-Cyclohexyl-2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indole (79a): Purified by flash chromatography (cyclohexane/DCM, 30:1), followed by purification by column chromatography (cyclohexane). Yield: 97.7 mg (50%); white oil; R_f = 0.30 (cyclohexane/DCM, 30:1);

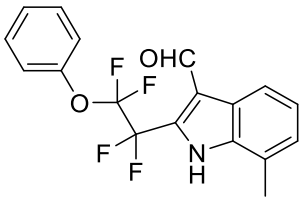
^{1}H NMR (400 MHz, CD_3OD): δ 1.38-1.47 (m, 3H), 1.80–1.89 (m, 5H), 2.04–2.13 (m, 2H), 3.16 (td, J_{HH} = 12.3, 3.4 Hz, 1H), 7.06 (ddd, J_{HH} = 8.2, 7.0, 1.1 Hz, 2H, $C_{Ar}H$), 7.12–7.16 (m, 1H, $C_{Ar}H$), 7.20 (ddd, J_{HH} = 8.2, 7.00, 1.1 Hz, 1H, $C_{Ar}H$), 7.26–7.30 (m, 1H, $C_{Ar}H$), 7.36–7.41 (m, 2H, $C_{Ar}H$), 7.43 (dt, J_{HH} = 8.3, 1.0 Hz, 1H, $C_{Ar}H$), 7.87 (dt, J_{HH} = 8.2, 1.0 Hz, 1H, $C_{Ar}H$); ^{19}F NMR (376 MHz, CD_3OD): δ –86.76 (t, $^{3}J_{FF}$ = 6.6 Hz, 2F, CF_2), –109.60 (t, $^{3}J_{FF}$ = 6.7 Hz, 2F, CF_2); ^{13}C {^{1}H} NMR (101 MHz, CD_3OD): δ 27.41, 27.97, 28.28, 34.09, 37.68, 113.12, 119.29 (t, J_{CF} = 37.4), 119.95, 121.46 (t, J_{CF} = 29.1 Hz), 122.74, 123.02, 124.25, 124.77, 127.39, 127.66, 130.84, 138.40, 150.53; HRMS (ESI$^+$) *m/z* calcd for $C_{22}H_{21}F_4NO$ [M]$^+$ 391.1559, found 391.1558.



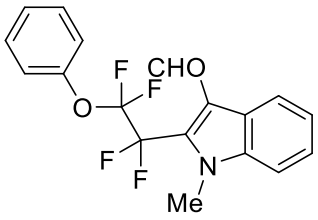
(2-(1,1,2,2-Tetrafluoro-2-phenoxyethyl)-1*H*-indol-3-yl) methanol (80a): Purified by flash chromatography (cyclohexane/DCM, 6:1). Yield: 81 mg (48%); colorless oil; R_f = 0.30; ^{1}H NMR (400 MHz, CD_3OD): δ 4.87 (s, CH_2), 7.03–7.07 (m, 3H, $C_{Ar}H$), 7.14–7.19 (m, 2H, $C_{Ar}H$), 7.25–7.29 (m, 2H, $C_{Ar}H$), 7.36 (dt, J_{HH} = 8.3, 1.0 Hz, 1H, $C_{Ar}H$), 7.76 (dt, J_{HH} = 8.1, 1.0 Hz, 1H, $C_{Ar}H$); ^{19}F NMR (377 MHz, CD_3OD): δ –86.66 (t, $^{3}J_{FF}$ = 6.4 Hz, 2F, CF_2), –109.97 (t, $^{3}J_{FF}$ = 6.3 Hz, 2F, CF_2); ^{13}C {^{1}H} NMR (101 MHz, CD_3OD): δ 55.21, 112.85, 114.73 (tt, J_{CF} = 252.2, 38.6 Hz), 118.40 (t, J_{CF} = 2.75 Hz), 119.07 (tt, J_{CF} =252.5, 36.5 Hz), 121.08, 121.32, 122.69, 123.60 (t, J_{CF} = 28.3 Hz), 125.01, 127.68, 128.64, 130.81, 137.89, 150.46; HRMS (ESI$^+$) *m/z* calcd for $C_{17}H_{13}F_4NO_2Na$ [M+Na]$^+$ 362.0778, found 362.0774.



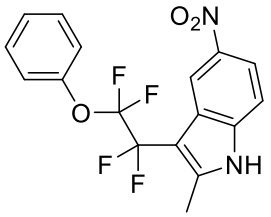
1-Methyl-2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indole (81a): Purified by flash chromatography (cyclohexane/DCM, 20:1), followed by purification by column chromatography (pentane). Yield: 56.5 mg (35%); colorless oil; R_f = 0.35 (cyclohexane/DCM, 20:1); ^{1}H NMR (500 MHz, CD_3OD): δ 3.93 (s, 3H, CH_3), 7.00 (s, 1H, $C_{Ar}H$), 7.13–7.21 (m, 3H, $C_{Ar}H$), 7.28–7.36 (m, 2H, $C_{Ar}H$), 7.38–7.43 (m, 2H, $C_{Ar}H$), 7.49–7.50 (m, 1H, $C_{Ar}H$), 7.66 (dd, J_{HH} = 8.0, 1.1 Hz, 1H, $C_{Ar}H$); ^{19}F NMR (376 MHz, CD_3OD): δ –84.90 (t, $^{3}J_{FF}$ = 6.1 Hz, 2F, CF_2), –106.68 (t, $^{3}J_{FF}$ = 6.1 Hz, 2F, CF_2); ^{13}C {^{1}H, ^{19}F} NMR (126 MHz, CD_3OD): δ 31.92, 107.28, 111.02, 114.49, 119.01, 121.58, 122.74, 122.83, 125.29, 127.45, 127.85, 130.88, 130.95, 140.47, 150.37; HRMS (ESI$^+$) *m/z* calcd for $C_{17}H_{13}F_4NO$ [M]$^+$: 323.0933; found: 323.0932.



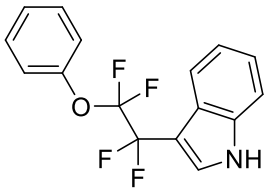
7-Methyl-2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indole-3-carbaldehyde (82a): Not isolated. ^{19}F NMR yield: 17%; ^{19}F NMR (376 MHz, acetone-d_6): δ –87.84 (t, $^3J_{FF}$ = 6.5 Hz, CF_2, 2F), –109.96 (t, $^3J_{FF}$ = 6.5 Hz, CF_2, 2F).



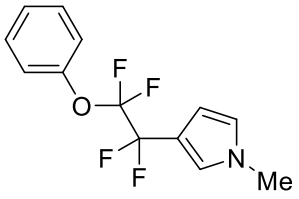
1-Methyl-2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indole-3-carbaldehyde (83a): Not isolated. ^{19}F NMR yield: 18%; ^{19}F NMR (376 MHz, acetone-d_6): δ – 87.08 (t, J_{FF} = 7.2 Hz, CF_2, 2F), –104.84 (s).



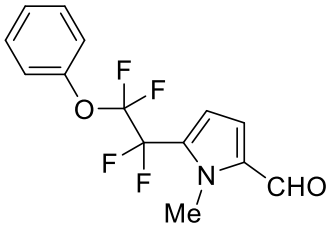
2-Methyl-5-nitro-3-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indole (84a): Not isolated. ^{19}F NMR yield: 15%; ^{19}F NMR (376 MHz, acetone-d_6): δ –88.61 (t, $^3J_{FF}$ = 6.4 Hz, CF_2, 2F), –108.93 (t, $^3J_{FF}$ = 6.4 Hz, CF_2, 2F).



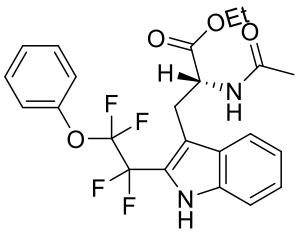
2-(1,1,2,2-Tetrafluoro-2-phenoxyethyl)-1*H*-indole (85a): Not isolated. ^{19}F NMR yield: 19%; ^{19}F NMR (376 MHz, acetone-d_6): δ –88.15 (t, $^3J_{FF}$ = 6.4 Hz, CF_2, 2F), –112.09 (t, $^3J_{FF}$ = 6.4 Hz, CF_2, 2F).



1-Methyl-2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-pyrrole (86a): Purified by flash chromatography (cyclohexane); Yield: 95.5 mg (70%); pale yellow oil; R_f = 0.3 (cyclohexane); ^{1}H NMR (400 MHz, CD_3OD): δ 5.91 (t, J_{HH} = 1.3 Hz, 3H, CH_3), 8.18–8.20 (m, 1H), 8.68–8.70 (m, 1H), 9.02 (dq, J_{HH} = 2.8, 1.2 Hz, 1H), 9.31–9.35 (m, 2H), 9.39–9.43 (m, 1H); 9.51–9.57 (m, 2H); ^{19}F NMR (376 MHz, CD_3OD): δ –84.95 (t, $^3J_{FF}$ = 6.5 Hz, 2F, CF_2), –104.92 (t, $^3J_{FF}$ = 6.5 Hz, 2F, CF_2); ^{13}C {^{1}H} NMR (101 MHz, CD_3OD): δ 55.18, 127.30, 133.45, 137.88, 139.65, 141.58, 146.76, 147.95, 150.02, 169.16, 225.34; HRMS (ESI$^+$) *m/z* calcd for $C_{13}H_{12}F_4NO[M+H]^+$ 274.0855, found 274.0856.



1-Methyl-5-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-pyrrole-2-carbaldehyde (87a): Purified by flash chromatography (cyclohexane); Yield: 70.1 mg (45%); pale yellow oil; $R_f = 0.30$; ^{1}H NMR (400 MHz, CD_3OD): δ 4.14 (t, $J = 1.2$ Hz, 3H), 6.75 (dt, $J_{HH} = 4.3$, 0.7 Hz, 1H, $C_{Ar}H$), 7.09 (dt, $J_{HH} = 4.3$, 1.0 Hz, 1H, $C_{Ar}H$), 7.19–7.22 (m, 2H, $C_{Ar}H$), 7.31–7.35 (m, 1H, $C_{Ar}H$), 7.41–7.46 (m, 2H, $C_{Ar}H$), 9.70 (s, 1H, CHO); ^{19}F NMR (376 MHz, CD_3OD): δ –85.07 (t, $^3J_{FF} = 6.1$ Hz, 2F, CF_2), –107.78 (t, $^3J_{FF} = 6.1$ Hz, 2F, CF_2); ^{13}C {^{1}H} NMR (101 MHz, CD_3OD): δ 34.80 (t, $J_{CF} = 4.2$ Hz), 113.67 (tt, $J_{CF} = 38.5$, 251.6 Hz), 114.50 (t, $J_{CF} = 5.7$ Hz), 118.76 (tt, $J_{CF} = 35.5$, 274.1 Hz), 122.58, 123.50, 127.95, 130.28 (t, $J_{CF} = 27.7$ Hz), 130.99, 136.42, 150.20 (t, $J_{CF} = 1.8$ Hz), 182.72; HRMS (ESI$^+$) *m/z* calcd for $C_{14}H_{11}F_4NO$ $[M]^+$ 301.0726, found 301.0725.



Ethyl (*S*)-2-acetamido-3-(2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indol-3-yl)propanoate (88a): Purified by column chromatography (cyclohexane/DCM, 4-6:1); Yield: 95.5 mg (41%); pale brown oil; $R_f = 0.30$ (cyclohexane/DCM, 4:1); ^{1}H NMR (400 MHz, CD_3OD): δ 0.97 (t, $J = 7.1$ Hz, 3H), 1.24 (t, $J = 7.1$ Hz, 2H), 1.92 (d, $J_{HH} = 0.7$ Hz, 2H), 2.01 (s, 1H), 3.34-3.51 (m, 2H), 3.90-4.01 (m, 2H), 4.76 (td, $J_{HH} = 7.7$, 2.3, 1H), 7.11–7.18 (m, 3H, $C_{Ar}H$), 7.24–7.29 (m, 2H, $C_{Ar}H$), 7.34–7.39 (m, 2H, $C_{Ar}H$), 7.47 (dt, $J_{HH} = 8.3$, 0.9 Hz, 1H, $C_{Ar}H$), 7.70 (dt, $J_{HH} = 8.1$, 1.0 Hz, 1H, $C_{Ar}H$); ^{19}F NMR (376 MHz, CD_3OD): δ –86.35 (ddd, $J_{FF} = 29.0$, 8.5, 5.0 Hz, CF_2), –108.82 to –110.93 (m, CF_2); ^{13}C {^{1}H} NMR (101 MHz, CD_3OD): δ 14.00, 14.44, 20.85, 22.33, 27.89 (d, $J_{CF} = 3.8$ Hz), 55.42, 61.50, 62.20, 112.97, 114.36 (t, $J_{CF} = 2.8$ Hz), 120.61, 120.88, 122.70, 125,00 127.69, 128.91, 130.79, 137.80, 150.36, 172.96, 173.44; HRMS (ESI$^+$) *m/z* calcd for $C_{23}H_{22}F_4N_2O_4$ $[M]^+$ 466.1516, found 466.1520.

4.4 Evaluation of reactivity of aromatic amino acids with 42 (Table 2)

To a solution of a derivative of amino acid (0.1 mmol) (*N*-acetyl tryptophan, *N*-acetyl tyrosine, phenylalanine ethyl ester hydrochloride or histidine hydrochloride monohydrate) in MeOH (0.2

ml) was added a solution sodium ascorbate (0.05 mmol, 0.5 equiv.) in water or buffer of pH 5 (phosphate) of pH 9 (carbonate/acetate) (0.15 ml). A solution of **42** (0.12 mmol, 1.2 equiv.) in MeOH (0.65 ml) was added dropwise over 2 min. After stirring at rt for 2 h, CF_3CH_2OH (0.1 mmol) and CD_3OD were added and the resulting mixture was analyzed by ^{19}F NMR to determine NMR yield.

4.5 Competitive experiment with a mixture of natural amino acids and 6

A stock solution of 20 proteinogenic amino acid standards was dissolved in 50mM ammonium bicarbonate buffer (pH 7.5) to reach 2 mM concentration. Reagent **6** (10 equiv. calculated to each amino acid, 30mM in DMSO) was added, followed by the addition of sodium ascorbate (5 equiv. calculated to each amino acid, 283mM in water). After stirring at room temperature for 15 min, semiquantitative HPLC-MS was performed and extracted ion chromatograms are shown in Figure 14.

4.6 Competitive experiment with a mixture aromatic amino acids and 42 or 70 (Table 3)

A stock solution of a mixture of Trp, Tyr, Phe and His was dissolved in 50mM ammonium bicarbonate buffer (pH 7.5) to reach 2 mM concentration. Freshly prepared solution of ascorbic acid in water (280mM, 5 or 25 equiv. to total amino acid content) and **42** or **70** in DMSO (120mM, 10 or 50 equiv. to total amino acid content) were added. The reaction mixture was incubated 5 min at room temperature. The labeling reaction was quenched by adding acetic acid to 1% final concentration. The reaction products were injected onto the HILIC column (Imtakt, Intrada amino acid, 2.1x150 mm) according to the manufacturer instruction and separated in 15 min gradient from 20% Buffer A (100mM ammonium acetate), 80% Buffer B (0.1% formic acid in AcN) to 100% Buffer A. Eluate was on-line analyzed by Q-TOF mass spectrometer (Bruker Daltonics, Maxis II) and data were interpreted using vendor software (Bruker Daltonics, Data Analysis 5.0).

4.7 Fluoroalkylation of peptides and proteins in the presence of sodium ascorbate

Fluoroalkylation of AFRIPLYWGRI

A solution of peptide AFRIPLYWGRI (1 mg) in MeCN (0.7 ml) containing 1% formic acid and water (0.3 ml) was prepared. Solution of **57**, **59** or **68** (50 mM) in MeCN and a solution of sodium ascorbate (50 mM) in water were prepared. Solution of the peptide (45 µl) was diluted with water (0.4 ml) containing methionine (20 mM) and to 0.1 ml of this solution 1.46 µl (10 equiv.) of ascorbate solution was added. Finally, the solution of **59** (1.46 µl, 10 equiv.) was added and the mixture was vortexed for a few seconds. After 1 h at ambient temperature, the mixture was analyzed by MALDI MS,

Fluoroalkylation of TEVNAWLVHRDP

A solution of peptide TEVNAWLVHRDP (1 mg) in MeCN (0.7 ml) containing 1% formic acid and water (0.3 ml) was prepared. Solution of **57**·AcCl (50 mM) in MeCN and a solution of sodium ascorbate (50 mM) in water were prepared. The solution of the peptide (45 µl) was diluted with water (0.2 ml) containing methionine (20 mM) and to 0.1 ml of this solution 1.46 µl (10 equiv.) of ascorbate solution was added. Finally, the solution of **57**·AcCl (1.46 µl, 10 equiv.) was added and the mixture was vortexed for a few seconds. After 1 h at ambient temperature, the mixture was analyzed by MALDI MS. In a separate experiment, the peptide solution (45 µl) was diluted with water (0.4 ml) containing methionine (20 mM) and to 0.1 ml of this solution 14.6 µl (100 equiv.) of ascorbate solution was added. Finally, the solution of **57**·AcCl (14.6 µl, 100 equiv.) was added and the mixture was vortexed for a few seconds. After 1 h at ambient temperature, the mixture was analyzed by MALDI MS.

Fluoroalkylation of bradykinin

Bradykinin (1 equiv. as a 1 mg/ml degassed solution in 20% v/v MeCN/50 mM pH 7 HEPES buffer) was mixed with sodium ascorbate (100 equiv. calculated to molar amount of aromatic residues, 20 mM solution in water) and solution of **60** was added (100 equiv. calculated to molar amount of aromatic resides, 8.7 mM in 50% v/v MeCN/H_2O). The mixture was shaken for 15 min at 25 °C. MALDI MS analysis indicated partial formation of mono(fluoroalkylated) bradykinin

and traces of bis(fluoroalkylated) bradykinin. MS/MS analysis revealed that Phe residue was fluoroalkylated.

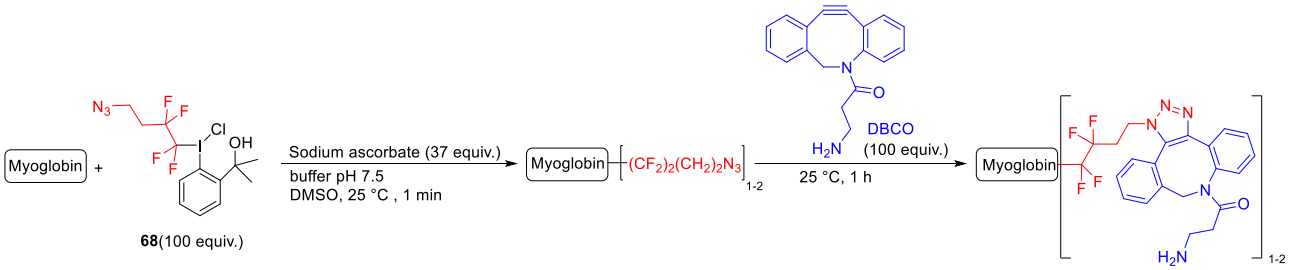
Fluoroalkylation of somatostatin

Somatostatin (1.0 equiv. as 1 mg/ml degassed solution in 20% v/v MeCN/50 mM pH 7 HEPES buffer) was mixed with sodium ascorbate (100 equiv. calculated to molar amount of aromatic residues, 20 mM solution in water) and solution of **60** was added (100 equiv. calculated to molar amount of aromatic resides, 8.7 mM in 50% v/v MeCN/H_2O). The mixture was shaken for 15 min at 25 °C. MALDI MS analysis indicated formation of mono(fluoroalkylated), bis(fluoroalkylated), tris(fluoroalkylated) somatostatin, and traces of mono(fluoroalkylated) oxidized somatostatin.

Fluoroalkylation of bombesin

Bombesin (1.0 equiv. as 1 mg/ml degassed solution in 20% v/v MeCN/50 mM pH 7 HEPES buffer) was mixed with sodium ascorbate (100 equiv. calculated to molar amount of aromatic residues, 20 mM solution in water) and a solution of **60** was added (100 equiv. calculated to molar amount of aromatic resides, 8.7 mM in 50% v/v MeCN/H_2O). The mixture was shaken for 15 min at 25 °C. MALDI MS analysis indicated formation of mono(fluoroalkylated) and bis(fluoroalkylated) bombesin, traces of tris(fluoroalkylated) and mono(fluoroalkylated) oxidized bombesin. MS/MS analysis revealed that only the positions of first and second modification were on the Trp residue.

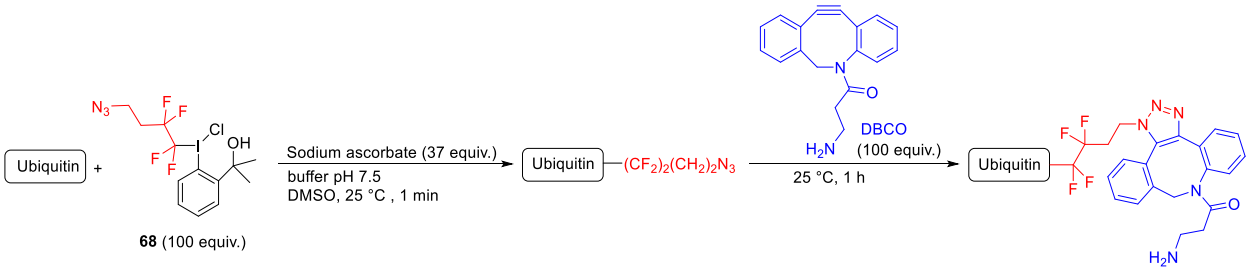
Fluoroalkylation of myoglobin



Myoglobin from horse heart (Myo) in water was transferred to 50mM ammonium bicarbonate buffer (pH 7.5) using a micro BioSpin 6 column (Biorad) and diluted in the same buffer to reach

31 µM concentration (0.5 mg/ml). Freshly prepared solution of ascorbic acid in water and **68** in DMSO were added in 37× and 100× molar excess to protein. The reaction mixture was incubated 1 min at 25°C and transferred to 50mM ammonium bicarbonate buffer (pH 7.5) using a micro BioSpin 6 column in order to remove side-products. The modified protein was mixed with dibenzocyclooctyne-amine (DBCO) in molar excess 1:100. The reaction mixture was incubated for 1 h at 25°C in dark. The output of the reaction was analyzed using direct infusion MS analysis. The modified amino acids were identified using bottom-up approach. Protein sample (5 µg) was subjected to trypsin digestion for 6 h at 37°C (enzyme:protein ratio 1:20, w/w). The resulting peptide mixture was dried using SpeedVac and re-suspended in water containing 2% acetonitrile and 0.1% TFA. ESI-MS analysis was performed for myoglobin control sample and modified myoglobin by **68** and DBCO. LC-MS/MS analysis of myoglobin peptide 1-16 showed modified Trp14 residue, Trp7 residue and both Trp7 and Trp14 residues.

Fluoroalkylation of ubiquitin



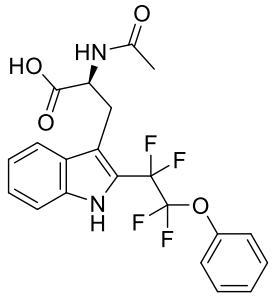
Ubiquitin from bovine erythrocytes (Ubq) in water was transferred to 50mM ammonium bicarbonate buffer (pH 7.5) using a micro BioSpin 6 column (Biorad) and diluted in same buffer to reach 58 µM concentration (0.5 mg/ml). Freshly prepared solution of ascorbic acid in water and **68** in DMSO were added in 37× and 100× molar excess to protein. The reaction mixture was incubated 1 min at 25°C and transferred to 50mM ammonium bicarbonate buffer (pH 7.5) using a micro BioSpin 6 column in order to remove side-products. The modified protein was mixed with dibenzocyclooctyne-amine (DBCO) in molar excess 1:100. The reaction mixture was then incubated 1 h at 25°C in dark. The output of the reaction was analyzed using direct infusion mass spectrometric analysis. The modified amino acids were identified using a top-down MS analysis. The mixture was desalted using Peptide Microtrap in the off-line holder (MichromBioresources) according manufacturer instruction, and eluted in 50 µl of 80% acetonitrile/5% acetic acid.

4.8 Trifluoromethylation in the presence of TEMPO

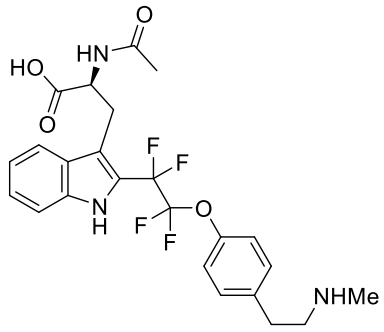
N-Acetyl tryptophan (0.1 mmol) and TEMPO (0.2 mmol) were dissolved in MeOH (0.2 ml). A solution of sodium ascorbate (0.05 mmol) in water (0.15 ml) was added, followed by the addition of **42** (0.12 mmol) in MeOH (0.65 ml). The reaction mixture was stirred at rt for 1 h, solvent was evaporated and internal standard $PhCF_3$ (0.1 mmol) and $CDCl_3$ were added. ^{19}F NMR analysis showed the formation of TEMPO-CF_3 adduct (–55.5 ppm)[132] in 43% yield, and no signal corresponding to trifluoromethylated tryptophan (–57.5 ppm).

4.9 Light-induced fluoroalkylation of small molecules

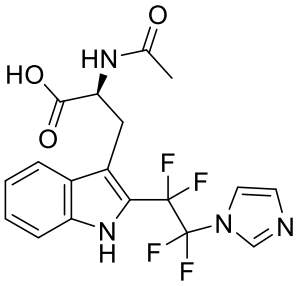
Substrtrate (*N*-acetyl-L-tryptophan, L-histidine monohydrochloride monohydrate, *N*-acetyl tyrosine, L-phenylalanine ethyl ester hydrochloride, 1-ethyl-3-methylindole, 1-decene, 4-phenyl-1-butene, 4-chloroaniline and *N,N*-dimethylaniline) (0.1 mmol, 1 equiv.) and reagent shown in Figure 29 (0.16 mmol, 1.6 equiv.) were dissolved in DMF/water (6:1, v/v) (1 ml). The mixture was irradiated with blue LED light (λ_{max} 455–475 nm) and stirred at room temperature for 3-20 h. 2,2,2-Trifluoroethanol (0.1 or 0.15 mmol, 1 or 1.5 equiv.) was added and the crude mixture was analyzed by ^{19}F NMR. For L-histidine monohydrochloride monohydrate, *N*-acetyl tyrosine, L-phenylalanine ethyl ester hydrochloride, 1-decene, 4-phenyl-1-butene, 4-chloroaniline and *N,N*-dimethylaniline no fluoroalkylated products were observed.



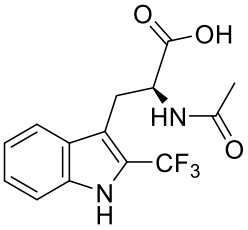
(*S*)-2-Acetamido-3-(2-(1,1,2,2-tetrafluoro-2-phenoxyethyl)-1*H*-indol-3-yl)propanoic acid: using **59** (5 h) 30% ^{19}F NMR yield (Table 4, entry 2) or in MeCN 61% ^{19}F NMR yield (Table 4, entry 11); using **57**·HCl (3 h) 76% ^{19}F NMR yield (Table 1, entry 12) ^{19}F NMR (376 MHz, CD_3OD): δ –87.93 to –88.02 (m, 2F, CF_2), –110.40 to –112.14 (m, 2F, CF_2).



(*S*)-2-(4-(2-(3-(2-Acetamido-2-carboxyethyl)-1*H*-indol-2-yl)-1,1,2,2-tetrafluoroethoxy)phenyl)-*N*-methylethan-1-aminium: using **60** (3 h) 45% ^{19}F NMR yield (Table 4, entry 13); ^{19}F NMR (376 MHz, acetone-d_6): δ –86.94 to –87.02 (m, 2F, CF_2), –109.15 to –111.40 (m, 2F, CF_2).



(*S*)-2-Acetamido-3-(2-(1,1,2,2-tetrafluoro-2-(1*H*-imidazol-1-yl)ethyl)-1*H*-indol-3-yl)propanoic acid: using **70** (5 h) 28% ^{19}F NMR yield (Table 4, entry 14); using **69** (5 h) 20% ^{19}F NMR yield (Table 4, entry 15); ^{19}F NMR (376 MHz, acetone-d_6): δ –96.32 (br s, CF_2, 2F), –108.46 to –109.98 (m, 2F, CF_2).



(*S*)-2-Acetamido-3-(2-(trifluoromethyl)-1*H*-indol-3-yl)propanoic acid: using **42** (20 h) 12% ^{19}F NMR yield (Table 4, entry 16); using **6'** (3 h) 27% ^{19}F NMR yield (Table 4, entry 19); ^{19}F NMR (376 MHz, acetone-d_6): δ –57.55 (s, CF_3).

4.10 Competitive experiment with a mixture of aromatic amino acids using LED light

A mixture of *N*-acetyltryptophan, *N*-acetyltyrosine, phenylalanine ethyl ester hydrochloride and histidine hydrochloride (0.1 mmol of each) was dissolved in MeOH/H_2O (10:1, v/v, 3 ml) and the solution was analyzed by ESI MS (Q-Tof micro). The reagent **42** (1 mmol, 10 equiv.) was added and the mixture was irradiated with blue LEDs light (455–475 nm) for 3 h followed by analysis by ESI MS.

4.11 Light-induced fluoroalkylation of peptides

Fluoroalkylation of tripeptide Ac-Cys-Gly-Trp-NH_2 (90) using LED light (Table 5)

Peptide **90** (0.05 mmol, 1 equiv.) was placed in a round bottom flask and was dissolved in MeOH (0.2 ml). **42** (1.2 equiv., 0.06 mmol) was dissolved in MeOH (0.3 ml) and was added to the peptide solution. The mixture was reacted for 3 h and then analyzed by ^{19}F NMR using (2,2,2-trifluoroethanol, 0.05 mmol) as an internal standard.

In a separate experiment, tripeptide **90** (0.05 mmol, 1 equiv.) was placed in a round bottom flask and was dissolved in MeOH (0.2 ml). Sodium ascorbate (0.025 mmol, 0.5 equiv.) was dissolved in water (70 µl) and was added to the solution of peptide. **42** (1.2 or 3 equiv., 0.06 or 0.15 mmol) was dissolved in MeOH (0.22 ml) and was added to the mixture of peptide and sodium ascorbate. The mixture was reacted for 3 h and then analyzed by ^{19}F NMR using (2,2,2-trifluoroethanol, 0.05 mmol) as an internal standard.

In a separate experiment, tripeptide **90** (0.05 mmol, 1 equiv.) was placed in a round bottom flask and was dissolved in MeOH (0.2 ml). **42** (1.2 or 3 equiv., 0.06 or 0.15 mmol) was dissolved in MeOH (0.3 ml) and was added to the solution of peptide. The mixture was irradiated by blue LEDs light (455–475 nm) for 3 h and then analyzed by ^{19}F NMR using (2,2,2-trifluoroethanol, 0.05 mmol) as an internal standard and analyzed by MALDI MS.

Light-induced fluoroalkylation of AFRIPLYWGRI

A solution of peptide AFRIPLYWGRI (1 mg) in MeCN (0.7 ml) containing 1% formic acid and water (0.3 ml) was prepared. A solution of **59** (50 mM) in MeCN was prepared. The peptide solution (45 µl) was diluted with water (0.2 ml) containing methionine (20 mM). To 0.1 ml of this solution, a solution of **59** (10 or 100 equiv. calculated to molar amount of aromatic amino acid residues) was added and the mixture was irradiated by blue LED light (455–475 nm) for 3 h followed by analysis by MALDI MS and MS/MS. MALDI MS analysis indicated the formation of mono(fluoroalkylated) peptide and traces of bis- and tris(fluoroalkylated) peptide. MS/MS analysis revealed that Trp residue was fluoroalkylated.

Light-induced fluoroalkylation of TEVNAWLVHRDP

A solution of peptide TEVNAWLVHRDP (1 mg) in MeCN (0.7 ml) containing 1% formic acid and water (0.3 ml) was prepared. A solution of **59** (50 mM) in MeCN was prepared. The peptide solution (45 µl) was diluted with water (0.4 ml - method A or 0.2 ml - method B) containing methionine (20 mM). To 0.1 ml of this solution, the solution of **59** (10 equiv. calculated to molar amount of aromatic residues) was added. The mixture was vortexed for a few seconds and then irradiated by blue LEDs light (455–475 nm) for 3 h followed by analysis by MALDI MS and MS/MS. MALDI MS analysis indicated the formation of mono(fluoroalkylated) peptide and traces of bis(fluoroalkylated) peptide. MS/MS analysis revealed that Trp residue was fluoroalkylated.

Light-induced fluoroalkylation of somatostatin

A solution of somatostatin (1 mg/ml) in MeCN (0.7 ml) containing 1% formic acid and water (0.3 ml) was prepared. The peptide solution (45 µl) was diluted with water (0.2 ml), containing methionine (20 mM), and to 0.1 ml of this solution, a solution of **59** (10 equiv. calculated to molar amount of aromatic residues) was added. The mixture was vortexed for a few seconds and then irradiated by blue LED light (455–475 nm) for 3 h followed by analysis by MALDI MS which indicated the formation of mono- and bis(fluoroalkylated) somatostatin.

Light-induced fluoroalkylation of bradykinin

A solution of bradykinin (1 mg/ml) in MeCN (0.7 ml) containing 1% formic acid and water (0.3 ml) was prepared. The peptide solution (45 µl) was diluted with water (0.2 ml), containing methionine (20 mM). To 0.1 ml of this solution, the solution of **59** or **57** was added (100 equiv. calculated to molar amount of aromatic residues). The mixture was vortexed for a few seconds and then irradiated by blue LEDs light (455–475 nm) for 3 h, followed by analysis by MALDI MS and MS/MS. MALDI MS analysis indicated the formation of mono(fluoroalkylated) and traces of bis(fluoroalkylated) bradykinin. MS/MS analysis revealed that Phe residue was fluoroalkylated.

Light-induced Fluoroalkylation of bombesin

A solution of bombesin (1 mg/ml) in MeCN (0.7 ml) containing 1% formic acid and water (0.3 ml) was prepared. The peptide solution (45 µl) was diluted with water (0.2 ml), containing methionine (20 mM). To 0.1 ml of this solution, the solution of **59** was added (10 equiv. calculated to molar amount of aromatic residues). The mixture was vortexed for a few seconds and then irradiated by blue LED light (455–475 nm) for 3 h, followed by analysis by MALDI MS and MS/MS. MALDI MS analysis indicated the formation of mono(fluoroalkylated) bombesin MS/MS analysis revealed that Trp residue was fluoroalkylated.

4.12 Photochemical and spectroscopic investigations

Absorption spectra of 59 and 89

Absorption spectra were obtained on a diode-array UV-vis spectrometer measuring in a range 190–1100 nm with matched 1.0 cm quartz cells.

NMR spectra of a mixture of 59 and 89

A solution of **59** (0.2 mmol) in $CDCl_3$ and a solution of **89** (0.2 mmol) in $CDCl_3$ were analyzed by ^{1}H and ^{19}F NMR, mixed and the mixture was again analyzed by NMR confirming that no new signals (corresponding to EDA complex) were observed upon mixing.

Photostability of 59 and 89

A solution of **59** or **89** (c = 0.1 M) in MeOH (3.0 mL) in a matched 1.0 cm quartz PTFE screw-cap fluorescence cuvette equipped with a stir bar was irradiated with a light source (32 LEDs emitting at the selected wavelength: λ_{max} = 450 nm; the bandwidth at half height ~20 nm). The reaction progress was monitored by UV-vis spectrometry using a diode-array spectrophotometer in a kinetic mode ^{19}F NMR spectra of the solution of **59** in CD_3OD showed a good photostability upon irradiation for 17 h with blue LED light (455–475 nm).

Monitoring of the reaction of 59 and 89

A solution of **59** and **89** (c = 0.1 M) in MeOH (3.0 mL) in a matched 1.0 cm quartz PTFE screw-cap fluorescence cuvette equipped with a stir bar was irradiated with a light source (32 LEDs emitting at the selected wavelength: λ_{max} = 450 nm; the bandwidth at half height ~20 nm). The reaction progress was monitored by UV-vis spectrometry using a diode-array spectrophotometer in a kinetic mode.

N-Acetyltryptophan (**89**) (0.1 mmol) and **59** (0.16 mmol) were dissolved in CD_3OD (1 ml). The solution was irradiated with blue LED light (455–475 nm) for 3 h at room temperature. ^{19}F NMR indicated formation of the product.

Luminescence measurements

Luminescence spectra were recorded on a diode-array automated combined luminescence and UV-vis spectrometer in 1.0 cm quartz fluorescence cuvettes at 23 ± 1 °C. The sample concentration was set to keep the absorbance below 0.5 at λ_{irr} and a correction on self-absorption was applied for each spectrum. Emission spectra were also corrected using standard correction files. Each sample was measured 3–5 times, and the spectra were averaged. Fresh samples of **59** were used for each measurement.

Luminescence lifetimes were measured on a time-correlated single photon counting spectrophotometer equipped with a pulsed nanosecond LED (λ_{exc} = 368 nm). The data obtained were deconvolved from the measured decay curves of the sample and the instrumental response function (IRF). The lifetime of **59** in absence of **89** was determined by TCSPC to be (2.54 ± 0.05) ns. The lifetime was considerably quenched by addition of **89** to (0.95 ± 0.03) ns.

5. CONCLUSIONS

The main aim of this work was to explore the possibility of employing fluoroalkylated hypervalent iodine reagents in bioconjugation studies. This was evaluated using two different approaches on biologically relevant targets, such as amino acids, peptides and proteins, for example bradykinin, somatostatin, horse heart myoglobin and other.

The first conceptually new method was the functionalization of electron-rich substrates or tryptophan residues in peptides and proteins by fluoroalkylated λ^3 iodane reagents in the presence of water-soluble reductant under biological conditions. This method employed sodium ascorbate as a reductant for rapid generation of fluoroalkyl radicals.

Radical fluoroalkylation of electron-rich aromatics employing fluoroalkylated cyclic λ^3 iodanes and their salts (**6**, **42**, **57**, **57**·AcCl, **59**, **60**, **61'**, **68**, **69**, **70**) in the presence of sodium ascorbate was investigated.

Reagent **59** reacted with various indole and pyrrole derivatives to furnish the corresponding tetrafluoroethylated products. However, the reactivity of substrates bearing electron-donating groups in positions one, two or three was higher than reacting substrates with electron-withdrawing groups, which was in agreement with the electron-deficient nature of the fluoroalkyl hypervalent iodine reagents. It was found that the order of addition of components was crucial in order to achieve high conversions. Reagent **59** decomposed in the presence of sodium ascorbate and high yield of fluoroalkylated 3-methylindole was observed when **59** was added as the last component to the reaction mixture.

The unstable and highly reactive fluoroalkyl radicals were shown to react within seconds with peptides and proteins containing aromatic amino acids. It was found that at least 10 or 100 equivalents of the reagent was required in order to achieve high conversions. Furthermore, the reaction efficiency was concentration dependant. The fluoroalkyl radicals reacted with all aromatic amino acids present in the reaction mixture; however, a preferential reactivity toward tryptophan was observed. The general reactivity of aromatic amino acids was observed as follow: Trp >> Cys > Tyr > Phe > His.

The second part of the Dissertation focused on the finding an alternative method which could be more selective in tagging of biologically relevant compounds and would not require an additive.

In this study, visible light was employed for fluoroalkylation of tryptophan and tryptophan-containing peptides in aqueous media. This was achieved by photoexcitation of fluoroalkylated cyclic λ^3-iodanes and their acyclic salts (**6'**, **42**, **57**, **57**·HCl, **57**·AcCl, **59**, **60**, **69**, **70**). Similar to sodium ascorbate initiated fluoroalkylation, electron-rich substrates were more reactive; however, with light-induced fluoroalkylation, the method was more selective toward tryptophan residues, which was due to the gradual formation of fluoroalkyl radicals. This method did not require any additives or catalysts and the reaction proceeded only with the reagent and the substrates dissolved in aqueous media in the presence of a visible light source. Several tryptophan-containing peptides such as AFRIPLYWGRI, TEVNAWLVHRDP, bombesin, somatostatin were tested for fluoroalkylation which afforded appreciable amount of tagged product. Tryptophan-free peptide such as bradykinin was less reactive and afforded traces of the fluoroalkylated peptide.

However, the procedure was not selective toward tryptophan when cysteine was present in the reaction mixture. This was tested using tripeptide **Ac-Cys-Gly-Trp-NH_2** and reagent **42**. Both tryptophan and cysteine were fluoroalkylated to a high degree. Spectroscopic and photochemical investigations, as well as quantum calculations reveled that the process proceeded *via* the formation of fluoroalkyl radicals from the excited state of the reagent.

In summary, two different methods of transferring of the trifluoromethyl or tetrafluoroethylene building blocks to electron-rich substrates were developed. Importantly, the use of electrophilic fluoroalkyl hypervalent iodine reagents opened a new and biocompatible pathway of labeling biologically relevant molecules. The ascorbate chemistry can be regarded as a versatile and straightforward method for fast C-H functionalization of electron-rich small molecules and biomolecules with aromatic or heteroaromatic especially indole-containing moieties. Visible light driven fluoroalkylation as an alternative method provided a more selective means of fluoroalkylation biomolecules having aromatic amino acids, especially tryptophan residues.

Remarkably, both used protocols are transition-metal free, and operate in aqueous media at ambient temperature which is highly attractive in the fields of chemical biology and biochemistry.

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7. LIST OF ABBREVIATIONS

3c-4e	3-centered, 4-electron
Ac	acetyl
Bn	benzyl
Bu	butyl
CuAAC	copper-catalyzed azide-alkyne cycloaddition
Cy	cyclohexyl
Cys	cysteine
DBCO-amine	dibenzocyclooctyne amine
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DIPEA	*N,N*-diisopropylethylamine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
Et	ethyl
GC	gas chromatography
His	histidine
HVI	λ^3-hypervalent iodine
Im	imidazole
MALDI	matrix-assisted laser desorption/ionization
mCPBA	*meta*-chloroperoxybenzoic acid

Me	methyl
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NMM	*N*-methylmorpholine
PEG	polyethylene glycol
Ph	phenyl
Phe	phenylalanine
R_F	perfluoroalkyl
rt	room temperature
SET	single electron transfer
TBAT	tetrabutylammonium triphenyldifluorosilicate
TEMPO	2,2,6,6-tetramethylpiperidine 1-oxyl
THF	tetrahydrofuran
TMS	trimethylsilyl
Trp	tryptophan
Tyr	tyrosine
R	alkyl group
MeCN	acetonitrile
TMEDA	tetramethylethylenediamine
HFIP	hexafluoroisopropyl alcohol
EDA	electron donor-acceptor

AIBN	azobisisobutyronitrile
HMPA	hexamethylphosphoramide
E^+	electrophile
Pr	Propyl
TEA	triethylamine
MeSal	methylsalisilate
TBAF	tetra-*n*-butylammonium fluoride
DME	dimethoxyethane
Cat	catalyst
Aq	aqueous
p-Tol	*Para*-tolyl
Phen	phenanthrene
TMSNTf2	*N*-trimethylsilyl-bis(trifluoromethanesulfonyl)imide
TCEP	tris(2-carboxyethyl)phosphine
ArAA	aromatic amino acid
HPLC	high-performance liquid chromatography
Mod	modification
HPLC	high-performance liquid chromatography
Mod	modification
ESI-MS	electrospray ionisation mass spectrometry
Equiv.	equivalent

LED	light emitting diode
SPAAC	strain-promoted azide–alkyne cycloaddition

8. PUBLICATIONS AND CONFERENCES

Publications:

K. Rahimidashaghoul, I. Klimánková, M. Hubálek, M. Korecký, M. Chvojka, D. Pokorný, V. Matoušek, L. Fojtík, D. Kavan, Z. Kukačka, P. Novák, P. Beier, *Chemistry – A European Journal* **2019**, *25*, 15779–15785.

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ESOC2019/0528, Vienna /Austria from July 14-18, 2019.

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