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Synthetic route for ^{18}F labeled triazole-modified oligonucleotides through a new late-stage desulfurative fluorination methodology

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Abstract

This dissertation is thought as a complete collection of all the concepts useful to produce sulfides that can be used in desulfurative halogenation reactions. Particular emphasis is placed on desulfurative fluorination and its possible application for ^{18}F labeling, and the methodology recently developed is described in detail focusing on the challenges encountered in the optimization of the method. A synthetic route for labeled macromolecules is conceptualized, preparing a sulfidoazide probe that can be coupled through “click” triazole linkage. Exploiting highly selective late-stage fluorination the probe could be used for ^{18}F labeling of oligonucleotides acting as a “light bulb" for medical studies through PET imaging. A sustainability assessment of the synthetic route has been performed, following the principles of Green Chemistry, revealing the critical steps and materials. Great attention is placed on the synthesis of thiophenyl (-SPh) sulfide precursors, to have a comprehensive view and explore the possible applications of the method in the field of (bio-)organic chemistry.

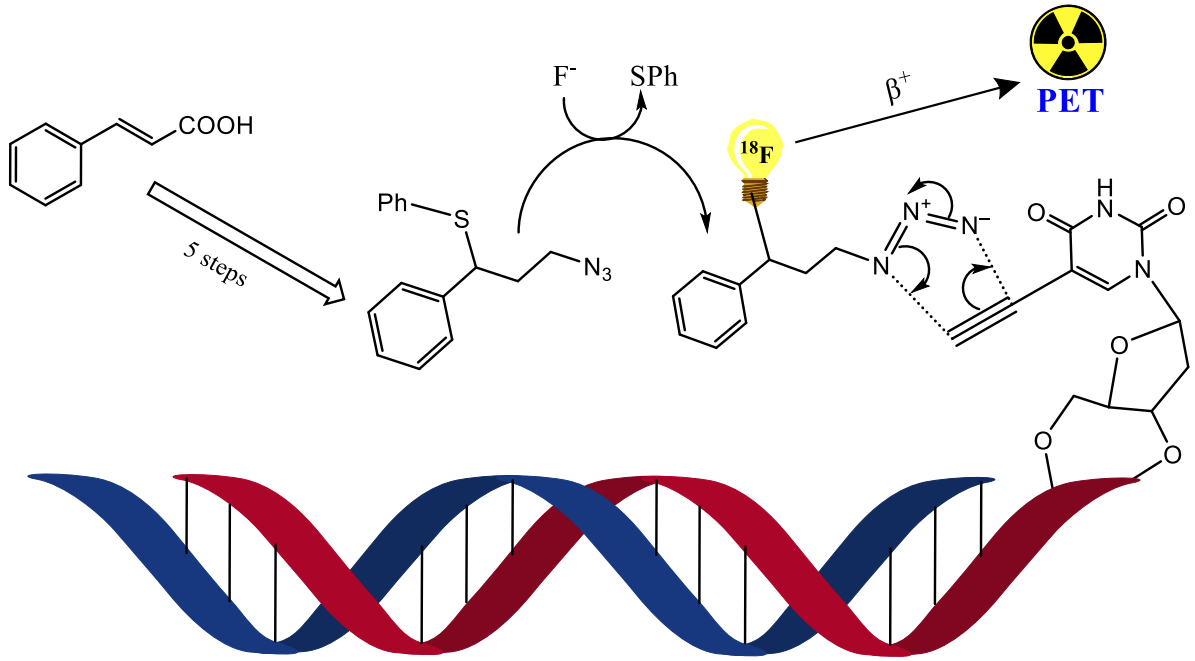


Figure 1: Graphical abstract

Summary

1. Introduction……..1
   1.1. Halogenation…….....2
   1.2. Sulfur Chemistry…….....3
      1.2.1. Synthesis of sulfides…….....3
      1.2.2. Pummerer reactions……...5
      1.2.3. Previous works: desulfurative halogenation……8
   1.3. Fluorine chemistry……...11
      1.3.1. This work: desulfurative fluorination……..11
      1.3.2. Fluorinating agents……..12
      1.3.3. Interest in fluorination in medicinal chemistry……...15
      1.3.4. Fluorine-18 radiochemistry……16
   1.4. Click chemistry for triazole-modified oligonucleotides……19
2. Goals……...22
   2.1. Esterification of cinnamic acids……..22
   2.2. Synthesis of sulfides……23
   2.3. Desulfurative fluorination methodology……23
   2.4. Synthetic route for triazole-modified oligonucleotides……..……23
3. Results and discussion……24
   3.1. Esterification of cinnamic acids……..24
   3.2. Synthesis of sulfides……26
      3.2.1. From α,β-unsaturated compounds……...26
      3.2.2. From secondary and tertiary alcohols……31
   3.3. Desulfurative fluorination methodology……33
   3.4. Synthetic route for triazole-modified oligonucleotides……..37
4. Conclusion……47
5. Experimental section……48
   5.1. Esterification of cinnamic acids……48
   5.2. Synthesis of sulfides…….……..52
      5.2.1. From α,β-unsaturated compounds……..52
      5.2.2. From secondary and tertiary alcolhols……...56
   5.3. Reduction……...57
   5.4. Mesylation……..58
   5.5. Azidation……59
   5.6. Desulfurative fluorination……..60

1. Introduction

This work was done during a curricular internship carried out with the Erasmus+ Traineeship project at the Royal College of Surgeons in Ireland (RCSI) under the supervision of Prof. Mauro Adamo and his research group (MA group from now on). The work was done in the laboratories of RCSI's Department of Pharmaceutical and Medicinal Chemistry (Centre for Synthesis and Chemical Biology, CSCB), and was part of a project involving KelAda Pharmachem, a spin-off co-founded by Prof. Adamo which operates in the laboratories of the University College Dublin (UCD). The project also involved a collaboration with PhD students from the University of Naples (UNINA), who worked as well in the laboratories of RCSI during the past months. The project aims to develop a methodology for desulfurative fluorination based on the previous efforts made by MA group on desulfurative halogenation, which is a type of nucleophilic substitution of sulfides named Pummerer fragmentation with thiophenyl (-SPh) as leaving group. Indeed, the ambition is that this new fluorination methodology could be a valuable approach to introduce an ^{18}F fluorine-tag into biological macromolecules such as peptides and oligonucleotides, obtaining radiotracers for medical studies through positron emission tomography (PET) imaging technique. Recently the topic of click-chemistry gained great attention in the field of bioorganic chemistry, especially for the possibility of performing a simple and traceless triazole ligation for modification of biomolecules. These concepts drove this work towards the conceptualization of a synthetic route for a sulfidoazide which could be then used for the labeling of alkyne-modified oligonucleotides through triazole ligation and finally fluorinated to introduce the ^{18}F fluorine-tag. This synthetic route from the starting material cinnamic acid, schematized in the graphical abstract (*Figure 1*), is described step-by-step in the final chapter, emphasizing all the critical points in terms of safety and in general sustainability, following Green Chemistry principles. A great variety of sulfides has been produced and tested with the methodology to define the scope of fluorination, and the knowledge acquired was used to discuss the possible applications, focusing on the current interest for fluorination, both ^{18}F and ^{19}F.

1.1 Halogenation

Halogenation, particularly bromination and chlorination, is commonly employed in medicinal chemistry to modify drug pharmacological activity, enhance stability, and alter solubility and membrane permeation [(1)]. The interest in fluorination will be discussed in a dedicated chapter since it is also related to ^{18}F labeling. In organic synthesis, Cl^- and Br^- (and I^-) are optimal leaving groups and this feature enables to perform substitution reactions with several nucleophiles, allowing the planning of a broad range of chemical syntheses of drugs [(2)]. The strength of the C-F bond, instead, makes the fluoride anion a poor leaving group. Desulfurative halogenation is now presented as an alternative strategy for preparing optically pure alkyl bromides and chlorides, in contrast with traditional methods like the dehydroxylation (Appel reaction) or halogen exchange in an equilibrium reaction (Finkelstein reaction). In the classical Appel reaction are used tetrahalomethanes as a source of halide and solvent (CCl_4 or CBr_4), and they are very environmentally hazardous reagents, indeed CCl_4 has been phased out by the Montreal Protocol to fight ozone depletion [(3,4)]. Triphenylphosphine (PPh_3) is used as reagent, producing phosphine oxide as coproduct (stoichiometrically) presenting a major drawback in terms of sustainability. Catalytic approaches to regenerate phosphine species have been explored, with a recent publication suggesting the use of oxalyl chloride to convert PPh_3O to its chlorophosphonium salt (CPS) and the use of dimethylcarbonate (DMC) as a greener solvent [(5)]. A major drawback is that oxalyl chloride needs to be continuously added into the mixture since it is consumed during the reaction, being decomposed into CO and CO_2. However, the main limitation of this "dehydration" approach is intrinsically in the scope of the reaction, since the non-oxidative C-O bond activation is not suitable to be performed with polyfunctionalized molecules bearing additional protic groups that would induce side reactions. Instead, the approach proposed earlier by MA group is based on an oxidative C-S bond activation (*Figure 2*), and it is usually compatible with a broad variety of unprotected functional groups, allowing late-stage derivatization. This approach enables the synthesis of a broad range of optically pure bromides and chlorides, and the same considerations could be made for desulfurative fluorination compared to common deoxyfluorination methods.

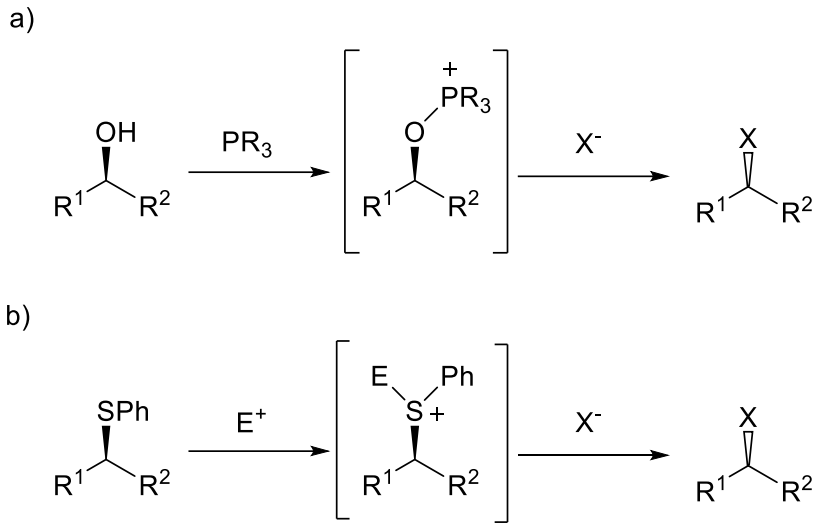


Figure 2: Comparison between the different strategies for enantioselective halogenation. a) Appel reaction, non-oxidative C-O bond activation; b) desulfurative halogenation, oxidative C-S bond activation.

1.2 Sulfur chemistry

1.2.1 Synthesis of sulfides

Sulfa-Michael Addition is the main reaction to produce C-S bonds in organic chemistry. Michael acceptors are typically α,β-unsaturated compounds, while the donors are nucleophilic compounds [(6)]. The term refers generally to the addition of sulfur compounds such as sulfites (-SO_3) and thiols (-SH), and it will be used from now on as a general term (SMA) even though Thia-Michael addition would be the proper term to use in this work. Biologically, this reaction occurs in several modulation mechanisms based on a conjugated system that interacts with a cysteine residue of a protein. A covalent C-S bond is produced, and the reversibility of this interaction suggests its role in regulation of enzymes through reversible covalent inhibition [(7)]. For example, entacapone is a catechol-O-methyltransferase inhibitor used in the treatment of Parkinson's disease, interacting as Michael acceptor with a deprotonated thiol of cysteine unit. The presence of an EWG such as amide group is crucial for the success of nucleophilic β-addition, and it is made evident by the mechanism shown in the *Figure 3*. In the case of entacapone there is an additional EWG (the cyano group), which sensibly increases the rate of thiol addition, but in turn makes the proton in α position much more acidic, enhancing the reversibility of the reaction. This effect has been extensively studied to understand the mechanism of action of drugs, and it is recurrent with cyanoacrylamides and

also with other α-disubstituted acrylates [8]. In chemical synthesis, this factor sets up a limit in the application of SMA for the preparation of sulfides, and consequently in the possible applications of desulfurative fluorination. In this context, the synthesis of a large library of thiophenyl sulfides made in this work ensures a deep understanding of the reaction, focusing on the effect of various substituents and functional groups on α,β-unsaturated compounds.

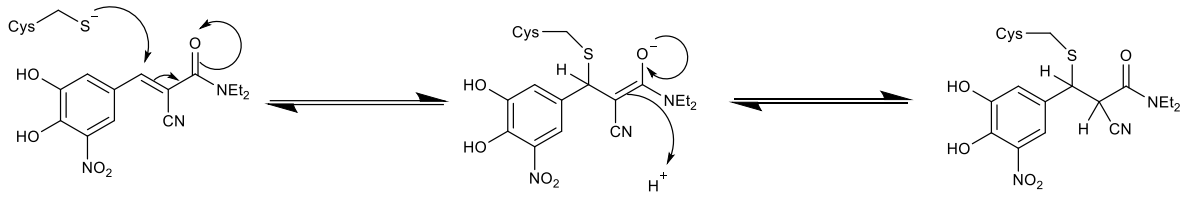


Figure 3: Mechanism of reversible covalent inhibition of entacapone with cysteine residues through Sulfa-Michael Addition.

The SMA can be also performed with high stereocontrol, a possible approach has been developed by MA group and it is based on a Sulfa-Michael Addition (not Thia-Michael) of sodium bisulfite ($NaHSO_3$) to chalcones, converting them into enantiospecific sulfonate salts, which in turn can act as building blocks for chiral sulfides [9]. A direct approach instead is to perform the classic Thia-Michael addition using Chincona alkaloid-based urea or thiourea derivatives as organocatalysts. In particular, following a procedure developed by Fang et al. [10] exploiting an aminothiourea bifunctional catalyst, several chiral sulfides have been prepared at RCSI in the previous works and they are still available in the lab in a stable and pure form, ready to be used as substrates to verify the enantioselectivity of desulfurative fluorination. The reaction is carried out using as substrates cinnamates (3-arylacrylates) that have been activated by conversion to hexafluoroisopropyl esters (HFIP). This very strong EWG ensures an effective nucleophilic addition in the β position, and the method produces chiral sulfides with excellent levels of enantiomeric excess (92-99%).

Another method extensively used at RCSI for the synthesis of sulfides is the substitution of hydroxyl group of alcohols with thiophenyl group using a lewis acid as catalyst. Extensive details on the synthesis of sulfides carried out during the internship through this method and through SMA are discussed in the results section. Obviously, other methodologies are found in the literature to prepare this class of compounds. It is worth to mention one proposed by George Olah's research group, which prepared several sulfides for their desulfurative

fluorination methodology using pyridinium poly(hydrogen fluoride) (PPHF). The method is based on the use of BF_3 as lewis acid, but in this case starting from prochiral ketones instead of alcohols [11].

1.2.2 Pummerer reactions

The sulfides prepared during the internship can be used as substrates for reactions of desulfurative halogenation. These reactions proceed through the cleavage of sp^3 C-S bonds by substitution of a nucleophile, in this case halides, and to understand this kind of substitutions it is important to describe in depth the well-known mechanism of the Pummerer reactions. The first publication related to this topic in 1909 describes the nucleophilic deoxydation of benzyl phenyl sulfoxide induced by an electrophilic activation of the oxygen leaving group [12]. Sulfur chemistry occupies an important role in organic synthesis and comprehends a broad range of different trasformations. The interest in this case is in sulfur atoms with an oxidation state of +IV, related to classes of compounds having C-S bonds such as sulfoxides, sulfonium salts, sulfur ylides and sulfinate salts. The Pummerer reaction on sulfoxides could occur in different types depending on the substituents and on the conditions, the main three are now described:

I. Firstly, the classical Pummerer rearrangement, that provides in two steps the nucleophilic attack on the carbon adjacent to the sulfur atom (benzylic position in the original substrate), after the abstraction of a proton from **2** that generates the key thiocarbonium intermediate **3**. Usually are used anhydrides, which act as both electrophilic activation and nucleophilic source.
II. In the interrupted Pummerer the nucleophilic attack occurs in one single step, with the immediate substitution of the oxygen leaving group OE with the nucleophile, generating a new Nu-S bond.
III. Finally, the Pummerer fragmentation provides the cleavage of C-S bond and subsequent substitution of sulfur with the nucleophile. This will be the starting point for the conceptualization of the desulfurative halogenation methods, in which the nucleophilic species are halides and thiophenyl is the leaving group.

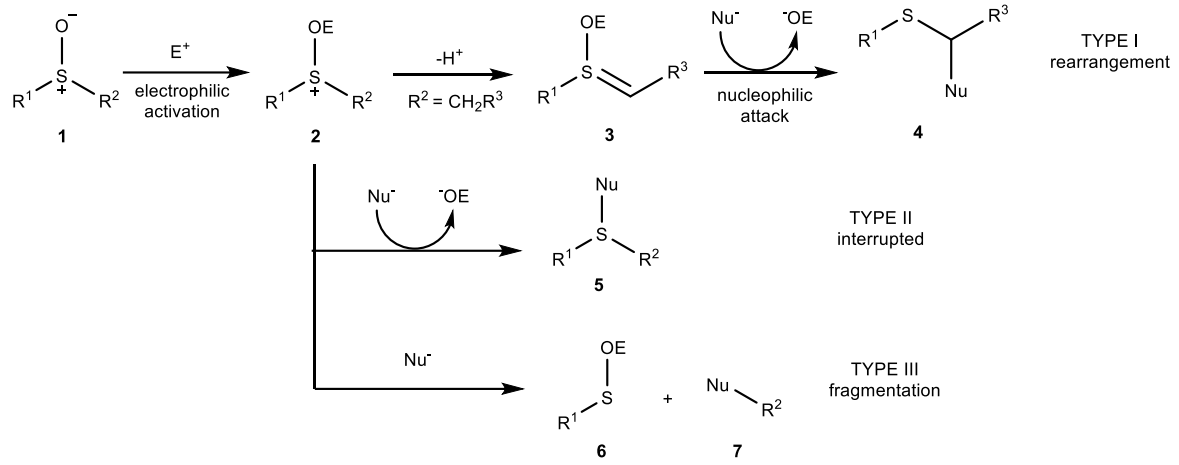


Figure 4: Schematization of Pummerer reactions of sulfoxides, adapted from Maulide et al. [13]

In *Figure 4*, adapted from the review made by Maulide and coworkers [13], the three types of reaction are schematized. Again, this is just a preliminary classification useful to understand the concept of desulfurative halogenation, but other similar transformations could be mentioned such as aromatic and vinylogous Pummerer reactions. For example, it is worth also mentioning a different type of fragmentation, involving the C_α-C_β cleavage, that could possibly occur in the case of substituents that form particularly stable carbocations [14]. From now on, the term "Pummerer fragmentation" will be used only to indicate the kind of Pummerer reactions that include the cleavage of C-S bond (Type III), excluding this last case.

Until now, the Pummerer reactions have been described in this work only on sulfoxides, in which the activation of C-S bond is mediated by the oxygen, with the electrophilic activation of the sulfonium cation **1** and with the final OE leaving group (*Figure 4*). However, it is possible to operate an activation of sulfides (thioethers) mediated directly by electrophilic species such as CH_3^+, NO^+ or halogen X^+ forming an analogous activated sulfonium cation **9** (*Figure 5*). The role of the oxidant/electrophile is crucial for the in-situ formation of sulfonium species, which displays a trigonal pyramidal (TP) molecular geometry due to the presence of the lone pair. These alternative activation methods are reported in a few publications regarding fluoro-Pummerer reactions, and later we will see how the use of NO^+ by Olah's group inspired the development of the new desulfurative fluorination methodology [11]. Even fewer methods are reported for bromo- and chloro-Pummerer reactions, and starting from these few examples an extensive effort from MA group revealed the possibility of developing methods for desulfurative halogenation. A comprehensive study on sulfide

derivatives allowed to have a deeper understanding of the competition between Pummerer rearrangement (type I) and Pummerer fragmentation (Type III), which is driven by the R^2 substituents [15].

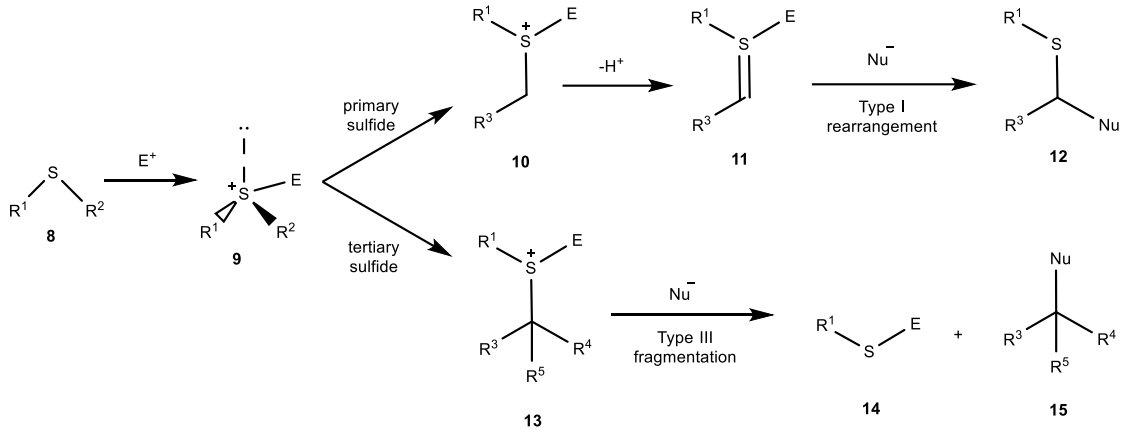


Figure 5: Pummerer reaction of sulfides, showing the competition between rearrangement (Type I) and fragmentation (Type III).

The key is the formation of the thiocarbonium **11** (analogous to **3**) as intermediate, which occurs through proton abstraction in the classical two-step Pummerer rearrangement (Type I). This step is in competition with the related fragmentation (Type III), which instead occurs through the nucleophilic attack on the sulfonium in one step.

The R^2 substituents govern this competition, driving the reaction towards one pathway or another. It is well known that primary alkyl phenyl sulfides like **10** react through the classical rearrangement pathway, with the abstraction of the proton and consequently the nucleophilic attack on **11**. Instead, with tertiary sulfides like **13** the reaction could proceed through the Type III fragmentation pathway, since the absence of hydrogen atom makes intrinsically less likely the formation of the key thiocarbonium intermediate **11**. Then, it was demonstrated that several activated secondary sulfides proceed as well following the Type III fragmentation pathway, a factor that induced the development of desulfurative chlorination and consequently for the bromination and fluorination methods. Obviously, the anionic species affects the reaction as well, indeed the weakly basic chloride is much less prone to abstract the proton compared for example to acetate or triflate.

1.2.3 Previous works: desulfurative halogenation

Desulfurative Chlorination

MA research group developed back in 2017 a mild and rapid method for the nucleophilic chlorination from sulfides [16]. The idea was to find an alternative strategy for the preparation of enantioenriched alkyl chlorides, avoiding the common limitations of the traditional methods such as narrow scope and the use of hazardous reagents with the formation of side products. For comparison, Appel reaction was selected as an example of traditional method for the S_N2 substitution starting from alkyl alcohols, and the two strategies are discussed in the previous chapter. It is well known that primary alkyl phenyl sulfides react with chlorinating agents through H^+ abstraction, obtaining chlorinated sulfides through a classical two-step chloro-Pummerer rearrangement (Type I). The hypothesis was that analogous secondary and tertiary substrates instead provide the desired one-step nucleophilic substitution through Pummerer fragmentation (Type III), with the use of $PhICl_2$ as both oxidant and chloride source and the final displacement of sulfur as leaving group. After initial optimization the reaction was performed in DCM at room temperature with a secondary alkyl phenyl sulfide as substrate, providing the formation of the desired chlorides in 71% yield after silica chromatography. Then, similar secondary and tertiary sulfides activated by the adjacent phenyl ring were also used obtaining chlorides in good yields, and even non-activated tertiary sulfides were suitable substrates. Secondary non-activated sulfides, instead, gave complex mixtures under the same conditions.

Then, chalcone-derived sulfide (secondary) was selected as test substrate for further optimization, to verify if the desulfurative chlorination occurs also with Michael adducts, which are typically sensitive to β-elimination, and the results were promising with all the sulfides previously prepared through SMA. Finally, all the chlorides prepared are summarized in the *Figure 6a* and these will be the typical products that can be prepared with the desulfurative halogenation methodologies. Enantiopure substrates derived from cinnamate demonstrated the high stereospecificity of the reaction, with the proposed mechanism of S_N2 substitution that was rationalized in conclusion and is displayed in the *Figure 6b*. The displacement of chloride from sulfur could occur either before or after the nucleophilic attack, thus the reactive species can be either a trigonal bipyramidal (TBP) sulfurane **17** or a sulfonium cation **18**, which takes on a trigonal piramidal (TP) orientation, basically a distorted tetrahedron due to the high repulsion of the lone pair (similar to NH_3 molecular

geometry). It can be appreciated the inversion of the stereochemistry typical of S_N2 reactions obtaining the enantiomer **19**, even though an alternative S_N1 pathway could also occur through the formation of the carbocation intermediate **20**, which is not chiral, indeed the arrows indicate that the nucleophilic attack occurs in both ways obtaining the racemic chloride **21**. It was found that the quality of $PhICl_2$ was crucial for the success of the experiment, and therefore great attention was put on the choice of the method to generate this reagent, which should be used within 3 weeks of preparation. $PhICl_2$ belongs to a class of hypervalent iodine reagents that have many advantages being non-hazardous, economical, readily available, highly stable, and easy to handle, providing a promising green alternative for application in organic synthesis [17].

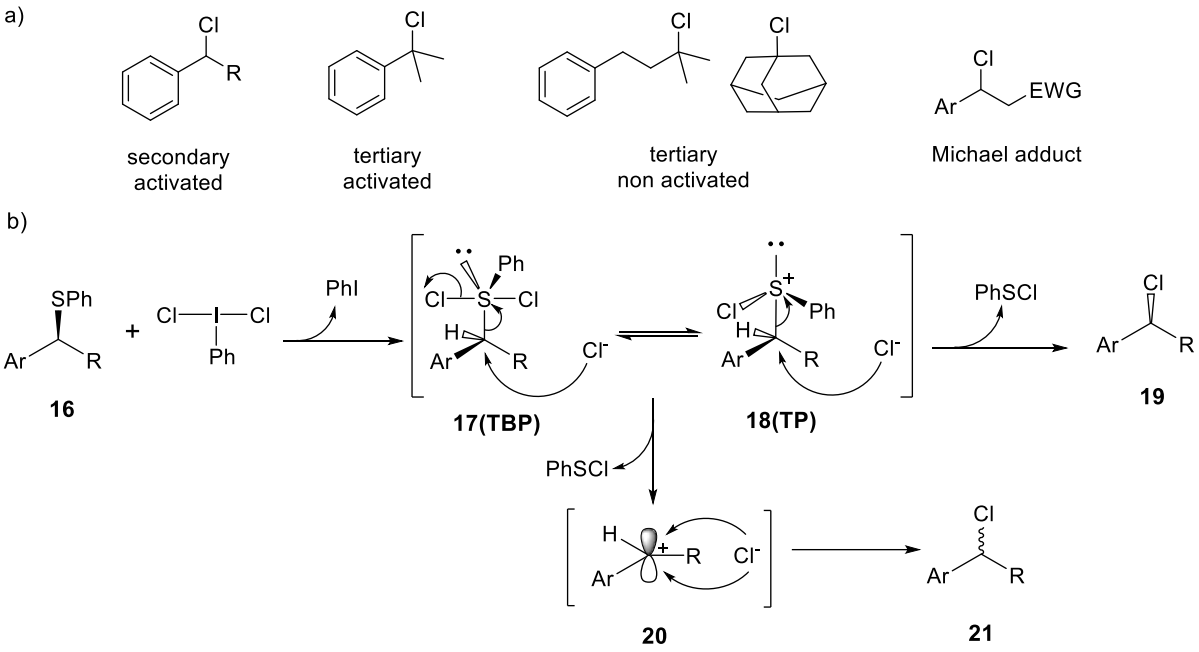


Figure 6: Desulfurative chlorination of sulfides developed by MA group. a) Chlorides prepared with the method from secondary and tertiary sulfides, b) mechanism of the reaction using $PhICl_2$, adapted from [16].

Desulfurative bromination

Dealing with bromination, the limitations in the traditional methods are similar to the ones encountered with chlorination and have been extensively discussed in the related section about halogenation. Thus, a new approach for enantiospecific bromination was of great interest and these considerations drove the research towards the development of a desulfurative bromination methodology [18]. Starting from the concepts of sulfide as leaving

group, it was proposed an analogous bromination methodology using Br_2, a reagent commonly widespread in chemistry laboratories despite its high (acute) inhalation toxicity and corrosiveness. The main issue to be considered is the lower oxidizing capacity of Br_2 and other Br^+ species compared to $PhICl_2$. The reaction proceeds at room temperature, producing alkyl bromides in remarkable yield using different solvents (dichloromethane DCM, toluene, dichloroethane DCE) generally in 15-30 minutes. The desulfurative bromination shows also exceptional levels of chemoselectivity, being compatible with protic functionalities such as carboxylic acid and hydroxyl groups, and enantioselectivity, examined starting from enantioenriched sulfides.

Different species could be generated from the reaction of sulfides with Br_2 (*Figure 7*) and describing them important concepts are highlighted that will be useful to understand desulfurative fluorination, which uses the same oxidant. Mainly, the species reported are analogous to the ones encountered using $PhICl_2$. In particular, the intermediate proposed in the mechanism of the desulfurative bromination is the highly reactive trigonal bipyramidal (TBP) species **23**, which is analogous to chlorinated **17** and is in equilibrium with the trigonal pyramidal (TP) sulfonium cation **24**. Then, the invertive bromide ion attack occurs, producing the final enantioenriched product **25** by S_N2 with displacement of hipobromothioite (PhSBr). Moreover, 2 molecules of PhSBr co-generated from the reaction could then undergo coupling, liberating disulfide PhS-SPh and regenerated Br_2 that enters a new cycle. This recycle mechanism will be important later when describing the desulfurative fluorination, with only 0.5 equivalents of bromine needed as oxidant due to analogous regeneration of Br_2.

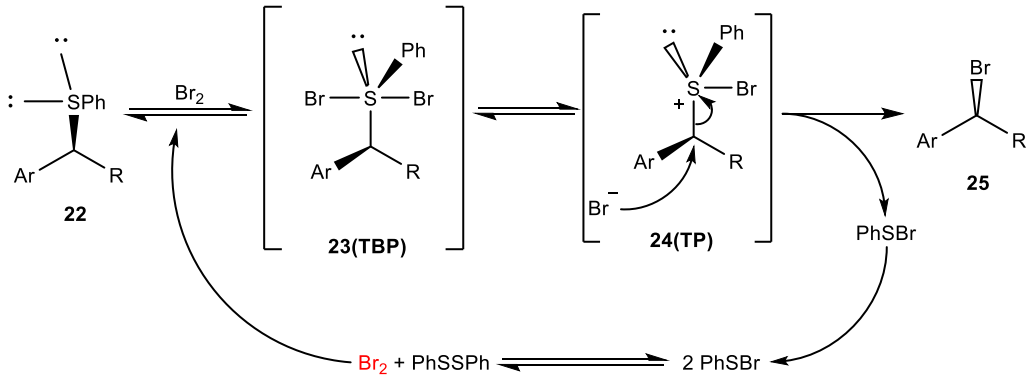


Figure 7: Mechanism of desulfurative bromination of sulfides with regeneration of Br_2, adapted from [18].

Later, the desulfurative bromination was also revised to be carried out ON-water (not IN water), with a significant reduction in the reaction time compared to organic media and

limiting the use of chlorinated solvents [19]. The term on-water indicates that the transformation occurs at the organic side of the interface, being a concept that ensures technical and economic advantages but still not reported by many studies in the literature [20]. It is proposed that water at the interface acts as lewis acid altering the equilibrium between TBP adduct and TP sulfonium species (*Figure 8*), therefore adequate stirring improves the reaction by increasing the surface area.

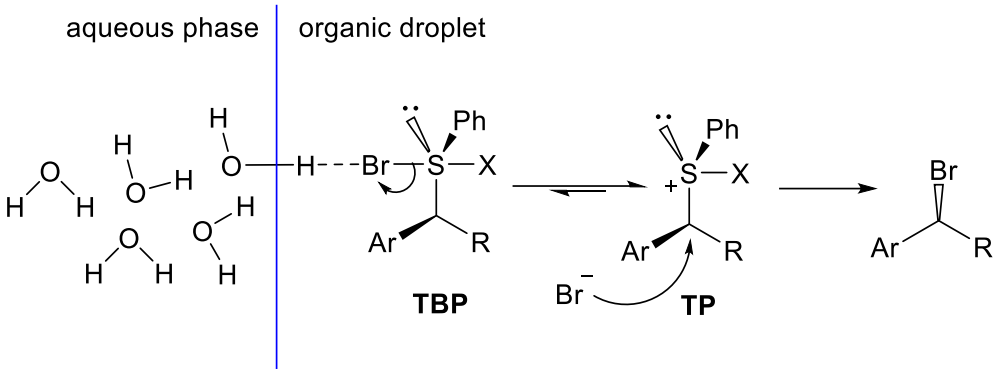


Figure 8: Mechanism of on-water desulfurative bromination, showing the action of water as lewis acid at the interface, adapted from [19].

1.3 Fluorine chemistry

1.3.1 This work: desulfurative fluorination

Starting from the concept of sulfide as leaving group developed in the previous works, an analogous method for desulfurative fluorination has been developed. This concept is not new, since in the field of organic synthesis other fluoro-Pummerer reactions has been already reported [15], for example the "fluorodesulfurization" reaction proposed by Kobayashi back in 1987 [21], which can be defined as a fluoro-Pummerer fragmentation (Type III). In this work methyl fluorosulfonate (FSO_3Me) provides Me^+ for the electrophilic activation, while CsF gave the best results among the fluoride sources examined, even though it could trigger the demethylation of sulfonium cation and consequently the deactivation of reactive species.

Even the name desulfurative fluorination is not new, since a methodology was proposed by George Olah using PPHF (a.k.a. Olah's reagent) together with nitrosonium tetrafluoroborate ($NOBF_4$). The mechanism proposed involves the action of nitrosonium cation (NO^+) as oxidant, providing the electrophilic activation of sulfur and forming the typical sulfonium

species that then undergo S_N2 by nucleophilic fluoride [11]. Similarly, using dithiolanes it is obtained a difluorinated methylene, and other similar procedures have been developed to produce gem-difluorinated compounds [22]. The drawback of the Olah's desulfurative fluorination is the poor chemoselectivity, since $NOBF_4$ is a strong nitrosylating agent that could react with many protic/polar functional groups such as OH, COOH, NH_2. Therefore, a different oxidant should be found to finally develop a methodology that is fast, simple, chemo- and enantioselective allowing the precise preparation of fluorinated compounds for medical studies. The details about the procedure developed by MA group and the optimization of the reaction are discussed in the Results section. In this introduction instead, the focus will be on the state of art of fluorination and on the role of fluorine in medicinal chemistry, emphasizing ^{18}F labeling strategies for macromolecules.

1.3.2 Fluorinating agents

Electrophilic reagents

Fluorinating agents are roughly divided between electrophilic (F^+) and nucleophilic (F^-) reagents, and the focus in this work will be mainly on the latter ones since desulfurative halogenation methods are based on nucleophilic addition. The main electrophilic reagent is F_2, to which it is assigned an EHS (Environment, Health and Safety) score of 2 out of 10 in the GSK's reagent guide for fluorination [23], being an extremely corrosive gas and therefore it is only used by expert practicians for specific purposes. The high atom efficiency of F_2 could lead to considering it a green reagent but the safety concerns and the high energy needed for the production must not be ignored [24]. As described later in the section about radiochemistry, $^{18}F_2$ is the species used for electrophilic radiofluorination and shows some crucial disadvantages compared to nucleophilic $^{18}F^-$. From gaseous F_2 many F^+ reagents are prepared [25], and few of them are listed in the *Figure 9*.

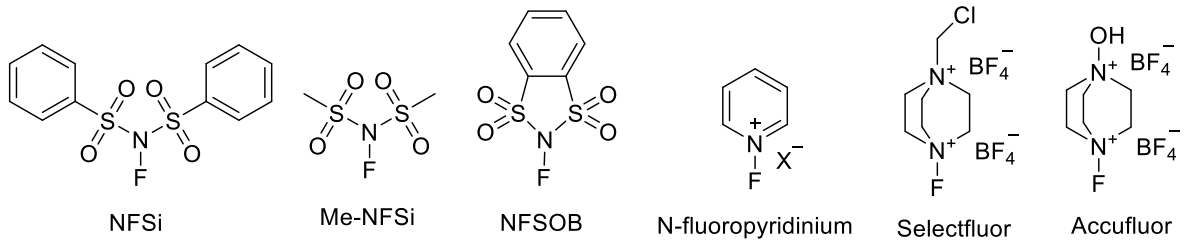


Figure 9: Electrophilic fluorinating agents.

Nucleophilic reagents

About nucleophilic reagents, shown in the *Figure 10*, some are based on the S-F bond and are commonly used for deoxyfluorination. DAST (Diethylaminosulfur trifluoride) is used in a wide variety of processes but presents several drawbacks, with possible decomposition upon heating above 50°C, releasing sulfur tetrafluoride and highly explosive $(NEt_2)_2SF_2$. Then, Bis(2-methoxyethyl)aminosulfur trifluoride (Deoxofluor) was developed as a safer alternative being more thermally stable, even if it still has the same decomposition temperature. Instead, similar reagents such as XtalFluor-M, XtalFluor-E and Fluolead have a significantly higher thermal stability and are easily handled, since they can be used in common glass vessels and do not react violently with water, unlike DAST and Deoxofluor [26]. About fluoride salts, organic ones like TBAF or TMAF are generally the least hazardous reagents for fluorination, but they are only used in SNAr reactions with nitropyridine or nitroarenes that need to be strongly activated [27]. These salts are hygroscopic, and moisture leads to a decrease of F^- reactivity due to hydrogen bonds. $TBAF(tBuOH)_4$ seems to be a more reactive alternative, ensuring good nucleophilicity, low basicity, low hygroscopic character and good solubility in organic solvents [28]. Other F^- salts are formed with highly electropositive inorganic cations such as KF, CsF or NaF. In particular, KF is the most used reagent for ^{18}F labelling, together with Kryptofix 2.2.2 as chelating agent to avoid the hydrogen bonds formed by naked fluoride that reduce the reactivity. $Na^{18}F$ instead is commonly used for skeletal PET imaging [29].

Finally, anhydrous HF is extremely toxic, corrosive and volatile (boiling point 20°C) and it should be avoided its contact with glass, therefore it is stored and used in plastic vessels and require specialized techniques. Instead, HF-amine complexes are liquid at room temperature, therefore are easier to handle compared to HF but also compared to F_2, which is in gaseous phase (bp -188°C) [30]. These complexes, for example PPHF or TEA*3HF, are produced from fluoride anions using an acid catalyst. PPHF (a.k.a. Olah's reagent) is a pyridine-HF complex (30:70) [31,32] and it should not be confused with N-fluoropyridinium salts (which are instead electrophilic reagents) [33].

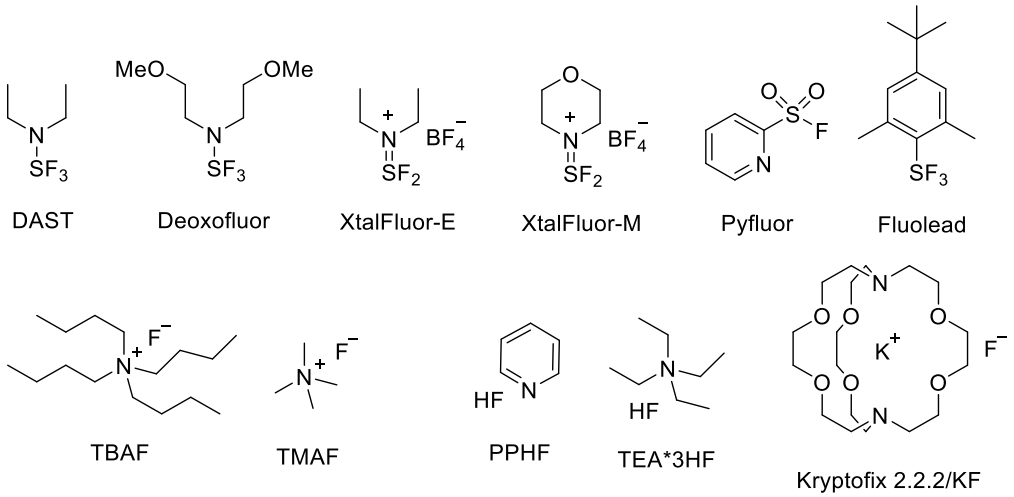


Figure 10: Nucleophilic fluorinating agents.

Other methods

Several Palladium-catalyzed methods have been found in the literature, but they are not described because in this work the approach has been that of avoiding heavy transition metals catalysis, preferring organocatalysis instead. A comprehensive review of C-H fluorination that comprehends a broad range of organometallics has been made by Governeur's research group [(34)]. In the same review are also described few interesting methods for fluorination through carbon-centered radicals, for example it has been reported a methodology for fluorination in benzylic position that is also suitable with Fluorine-18. Finally, it is worth to mention a possible biocatalytic route for fluorination, since a fluorinase enzyme has been isolated in a soil bacterium [(35)]. Surprisingly, the reaction catalyzed is very similar to a fluoro-Pummerer fragmentation, with the cofactor S-adenosyl-L-methionine (SAM) as substrate. SAM is a sulfonium cation analogous to the ones encountered previously when describing desulfurative halogenation methods (R_3S^+), and through the action of fluorinase enzyme it occurs the nucleophilic attack and is released the 5'-fluorodeoxyadenosine (5'-FDA) and a methionine residue by fragmentation (*Figure 11a*). The structure of the enzyme-substrate complex can be explored using Protein Data Bank (PDB code 1RQP) [(36)]. In particular, the coordination of C3'-OH with Ser158 residue seems to be important since this residue coordinates also the fluoride anion that provides nucleophilic attack on the C'5 position. In *Figure 11b*, generated using the PDB online viewer and further adapted, it can be appreciated that the position of Ser158 is on the opposite side of the sulfur of SAM, indicating that the fluoride attack occurs in a stereoselective manner, (S_N2-type reaction) on a sulfonium cation with sulfur as leaving

group, just like fluoro-Pummerer fragmentation. A biocatalyst for radiolabeling could be developed from this enzyme providing $^{18}F^-$ in the medium [37] and even a biocatalyst for chlorination if Cl^- is provided [38].

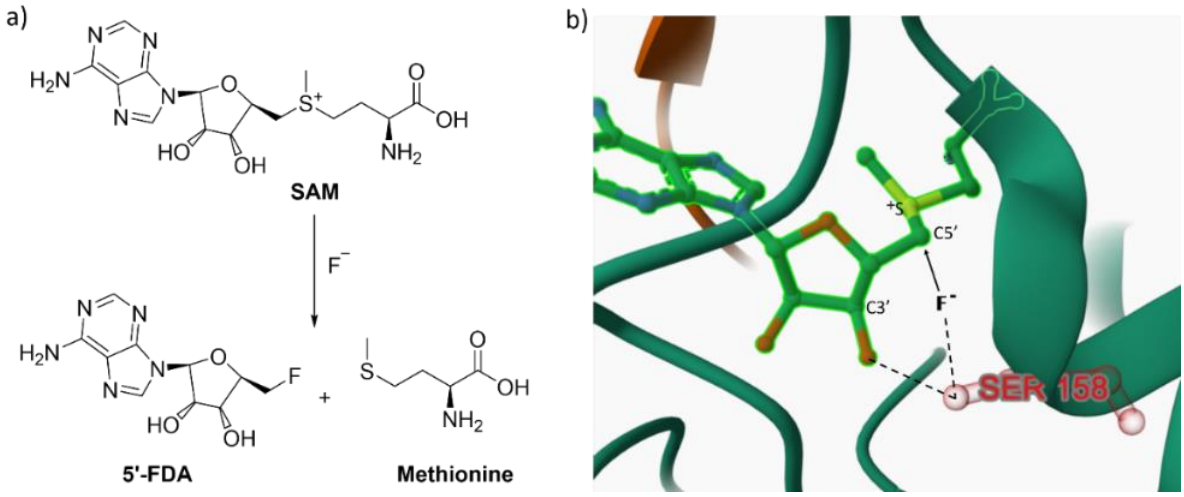


Figure 11: Enzymatic fluorination of SAM. a) Scheme of the reaction, b) coordination of Ser158 residue with fluoride and with C3'-OH (obtained using Protein Data Bank, PDB code 1RQP).

1.3.3 Interest in fluorination in medicinal chemistry

Fluorine possesses unique properties that make it advantageous in medicinal chemistry. It has the smallest Van der Waals radius (excluding H and He), the highest electronegativity, and forms the strongest known bond (C-F) with approximately 108 kcal/mol. These characteristics are exploited in drug design through isosterism, where molecules with similar shape and electronic properties are used interchangeably [39]. Fluorine, often replacing hydrogen, enhances the metabolic stability of molecules, altering physical and biological properties for improved drug activity. For example, in quinolone antibacterial drugs the F substituent in position 6 increases potency by 2- to 17-fold [40]. This strategy is employed not only in drug design but also in medical studies to investigate drug metabolism's role in toxicity. The introduction of fluorine can affect solubility and acidity, modulating the membrane permeability and bioavailability of drugs, with important insights in the planning of drug delivery and optimal administration [39]. The highly polarized C-F bond (1.41 D) influences molecular structure through steric and electronic effects, interacting strongly with other dipolar bonds in various functional groups. For example, it is observed a strong “gauche effect” in 1,2-difluoroethane derivatives, which was extensively studied in GABA (gamma-

aminobutyrric acid) and NMDA (N-methyl-D-aspartate) derivatives to understand the effect on recognition by enzymes [41,42].

1.3.4 Fluorine-18 radiochemistry

Positron emission tomography (PET) is a nuclear imaging technology used in medicine to provide images of the distribution of a radiotracer in a biological system. This technique is widely used to interpret biological functions and interactions of drugs properly labeled with a positron-emitting nuclide. The PET scanner detects the gamma rays generated from the interaction of a positron (β^+) with an electron, creating an image of the distribution of the radiotracer with a resolution of a few millimeters [43]. Fluorine-18 is the most used radioactive isotope for PET in medical research, being a positron-emitting nuclide with high decay ratio (97% β^+ emission, 3% electron capture) with a relatively high half-life (110 min) compared to similar isotopes such as ^{15}O (2 min), ^{13}N (10 min) and ^{11}C (20 min). Due to these favorable properties, several strategies have been developed for ^{18}F labeling employing different fluorination methods including electrophilic and nucleophilic reagents. Fluorine-18 is produced with a cyclotron by proton irradiation of Oxygen-18, a stable isotope with 0.2 % natural abundance, a method named $^{18}O(p,n)^{18}F$ reaction [44]. Stoichiometrically, a high-energy proton interacting with an Oxygen-18 atom will generate a neutron and a Fluorine-18 atom, in a form that depends on the previous state of the ^{18}O source: if is used enriched water the product will be an aqueous solution of $^{18}F^-$ fluoride, while in the case of elemental $^{18}O_2$ the product is gaseous $^{18}F_2$. The choice between these two strategies depends on the chemical reaction that needs to be performed later: F^- is a nucleophilic species while F_2 instead is used for electrophilic fluorination [45]. The two chemical forms have different specific radioactivity (measured in Bq/mol), because the electrophilic $^{18}F_2$ gas needs the addition of $^{19}F_2$ gas as a carrier to extract it from the target, intrinsically reducing the radiochemical yield (RCY). Moreover, F_2 is more difficult to handle being an extremely hazardous gas and it is only used in special vessels by expert practitioners. These drawback makes electrophilic $^{18}F_2$ less suitable for PET tracing, driving the research in nuclear medicine towards methods based on nucleophilic $^{18}F^-$ fluoride labeling [46]. Fluoride anion forms hydrogen bonds in aqueous solution, therefore it should be dehydrated and then placed in polar aprotic organic solvents (dimethylsulfoxide DMSO, dimethylformamide DMF). The solubility and nucleophilicity of F^- in organic solvents are enhanced by the addition of a bulky ammonium cation (e.g.

Tetrabutylammonium or Pyridine) or using a Phase Transfer Catalyst (PTC) such as Kryptofix 2.2.2, that generates a potassium complex with KF that is widely operated for Fluorine-18 labeling [47].

Small radiotracers

Many radiotracers are prepared by nucleophilic substitution with Kryptofix2.2.2/KF, the most known being fluorodeoxyglucose (FDG), which is able to pass the cell membrane and the blood brain barrier (BBB), and it is used to reveal the patterns of glucose metabolization in the organ interested by PET imaging. Other radiotracers prepared by S_N2 for these purposes are fluorothymidine (FLT), fluoromisonidazole (FMISO) and fluoroestradiol (FES) [48,49]. In the CNS Radiotracer Table provided by the NIH [50] are found several other Fluorine-18 labeled ligands that have been advanced for use in Human Studies, and it can be noticed a common pattern in the structure of these molecules since many of them present the fluoroethyloxy group attached to the phenyl ring, for example FET, MNI-444, MNI-659, MK-9470, FEPPA, ISO-1, PSS232. There is a great interest in dopamine and its derivatives for PET brain imaging, targeting dopaminergic receptors in the central nervous system (CNS). In particular, L-dihydroxyphenylalanine (DOPA) is widely used in the clinical treatment of Parkinson's disease, being able to cross the BBB, and consequently there is great interest in the synthesis of ^{18}F labelled derivatives of this drug to be used for diagnosis [51,52]. Some methods available in the literature display insertion of ^{18}F in position 6 (ortho position of the phenyl ring) using proper leaving groups in aromatic substitution reactions [53].

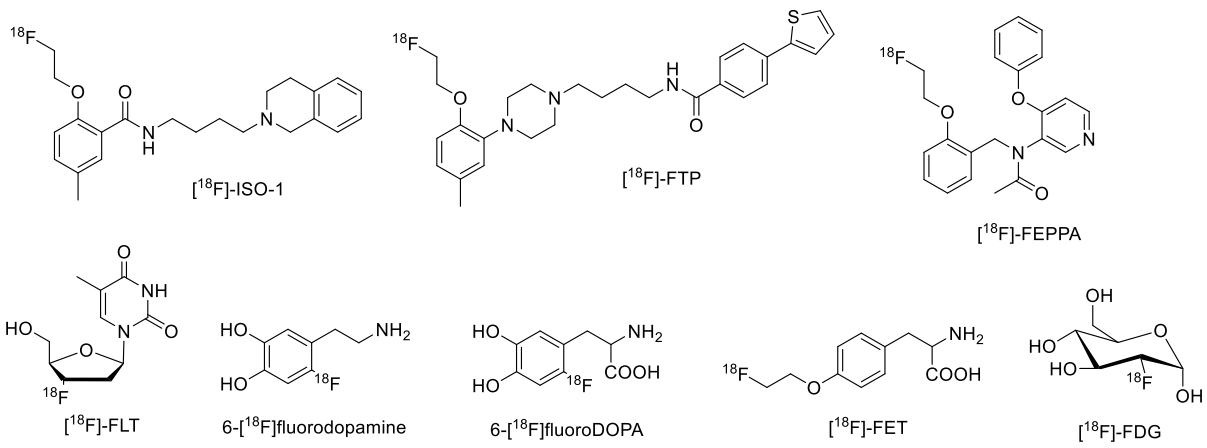


Figure 12: Common [^{18}F] small radiotracers.

Labeling of macromolecules

Until now, only small radiotracers have been presented, but for medical studies a great interest is placed into ^{18}F labeled (bio-) macromolecules, which exhibit very high specific targeting. For peptides and oligonucleotides, the direct strategies for radiolabeling are based on the modification of the biomolecule with some kind of reactive probe/prosthetic group, followed by ^{18}F labeling of the conjugated macromolecule. Few examples are reported, and these methods present several challenges, mainly due to the high selectivity needed and to the fact that biomolecules are sensitive towards high temperatures and acid/basic conditions [(43)].

Indirect labelling is much more variegate, with a broad range of probes that can be labeled with ^{18}F and then coupled with biomolecules. In the *Figure 13* are reported some ^{18}F-labeled prosthetic groups based on amide, ester, or other linkages. The choice of the prosthetic group must be done carefully considering the reactivity of the target biomolecule and the time limitation posed by the ^{18}F half-life. Some interesting strategies employ activated esters, for example N-Succinimidyl 4-fluorobenzoate (SFB) has been used on peptides generating a linkage with free amines [(54)]. Another specific strategy is the use of N-maleoylethyl-4-fluorobenzamide (FBEM) which couples with free thiols such as cysteine residues of proteins [(55)]. The prosthetic groups with azide (N_3) and alkyne functional group are the most interesting for labeling oligonucleotides, through "click" reactions that allow the simple, fast and traceless synthesis of triazole linkages. This topic is introduced in a separate chapter since click chemistry is strictly related to bioorganic chemistry, and a prominent recent field of research regards the synthesis of triazole-modified oligonucleotides that are exploited in medical research as nucleic acids analogous, and a great interest is placed in the ^{18}F fluorination of these compounds to be employed in PET studies [(56)].

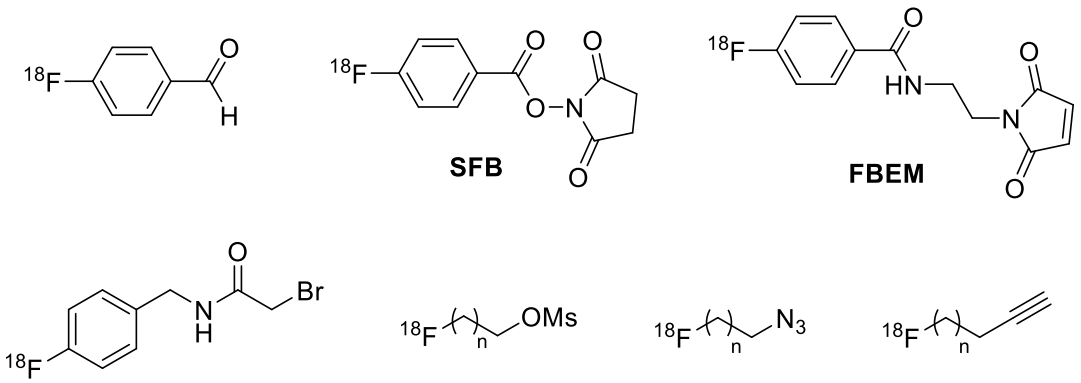


Figure 13: Prosthetic groups for indirect ^{18}F labeling.

1.4 Click chemistry for triazole-modified oligonucleotides

The concept of "click chemistry" was first described by Sharpless in 2001 and it has been revolutionary in the field of (bio-)organic chemistry, being awarded with the 2022 Nobel prize. This class of reactions is characterized by simple conditions, readily available reagents and allows the preparation of stable products without the need for expendious purification steps [57]. The most important "click" reaction is the Huisgen 1,3-dipolar azido-alkyne cycloaddition, in the two versions catalyzed by copper(I) (CuAAC) or ruthenium(II) (RuAAC) ions. The reaction allows the coupling of terminal alkynes with organic azides, obtaining a 1,2,3-triazole linker with different positions of the substituents based on the catalyst used (1,4- for CuAAC, 1,5- for RuAAC). The copper-catalyzed reaction is definitely the most studied for the coupling of biomolecules, due to the availability of the catalyst [58]. The original Sharpless' conditions in H_2O/EtOH used sodium ascorbate to reduce Cu(II) to Cu(I), generally 1% of $CuSO_4$ catalyst is enough to complete the reaction. Other copper reagents such as copper iodide could be used, but with a lower yield and a high amount of DIPEA needed as reducing agent (3 equivalents) and using DMF as solvent, which is much more hazardous compared to the original procedure [59]. Microwave irradiation is a good and simple method to increase the performance of the reaction. The interest for CuAAC in bioorganic chemistry lies in the opportunity to prepare clickable nucleosides, finally obtaining short triazole-modified oligonucleotides and oligodeoxynucleotides (*Figure 14a*) with important biological properties as therapeutic agents. Indeed, the triazole linker is exploited as analogous to the natural phosphodiester bridge of nucleic acids, being compatible with enzymes and/or ribozymes that typically operate with DNA and RNA filaments [60]. A great advantage appears compared to traditional methodologies. The phosphoramidite chemistry cycle (*Figure 14b*) is the main method to prepare synthetically oligonucleotides and oligodeoxynucleotides (generally called ONs from now on) and it has some serious limitations and high cost of operation compared to the quick and easy "click" approach [61].

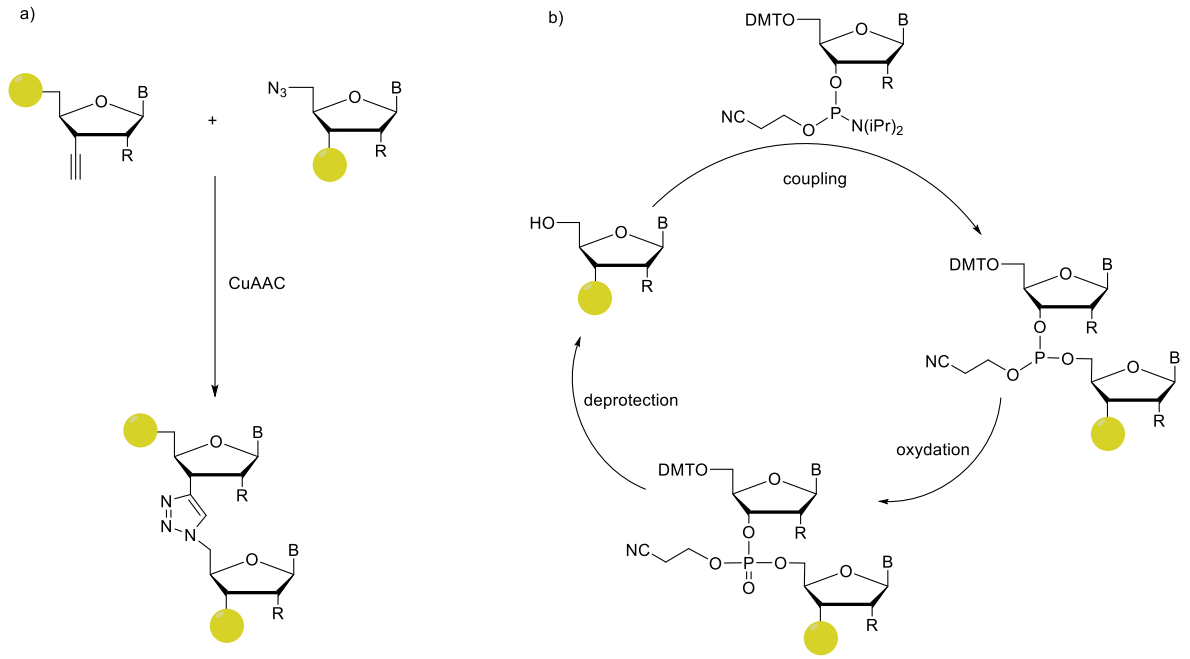


Figure 14: a) Scheme of the copper-catalyzed azide-alkyne cycloaddition; b) phosphoramidite cycle for chemical synthesis of oligonucleotides.

AZT (a.k.a. Zidovudine) is among the most common nucleoside analogs and was found to be a therapeutic agent to treat AIDS, giving a big boost to the development of new methods for the synthesis of similar modified nucleosides bearing different substituents. A strategy for triazole ligation with AZT was proposed by Lazrek et al. [(62)], but through 1,3-dipolar thermal cycloaddition rather than Huisgen cycloaddition. Shortly after that, the introduction of CuAAC will start the "click era" making a great contribution to the research on the field, introducing a new method for ligation towards the preparation of modified ONs as therapeutics [(63)]. The insertion of azide functionality into the nucleoside monomers needs to be carefully planned. The 3'-hydroxyl group of the ribose ring needs to be activated with a proper leaving group such as tosylate, mesylate or triflate (*Figure 15*) while the others need to be protected and then deprotected (not shown in the scheme for simplicity) [(64)].

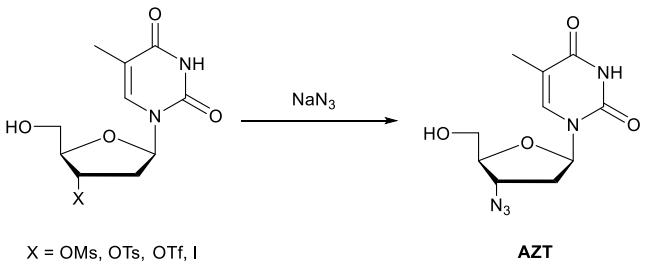


Figure 15: Synthesis of 3'-Azido-2'-deoxythimine (AZT).

Among the various applications of CuAAC, the most interesting approach in the case of fluorine is the ligation for labelling purposes. A good strategy for ONs labelling is the preparation of an alkyne-modified nucleotide residue which can be then coupled with an azido-functionalized label [(65)]. In this context, the use of alkyne-modified nucleosides triphosphates (e.g. dU*TP) are promising in this sense due to a specific feature: the compatibility with the common biochemical methodologies. In fact, these modified nucleosides can be incorporated into DNA strands with the canonical polymerase chain reaction (PCR) procedure and further labelled using any probe bearing the azido terminal group, which results in DNA bearing various triazole modifications (*Figure 16*) [(66)].

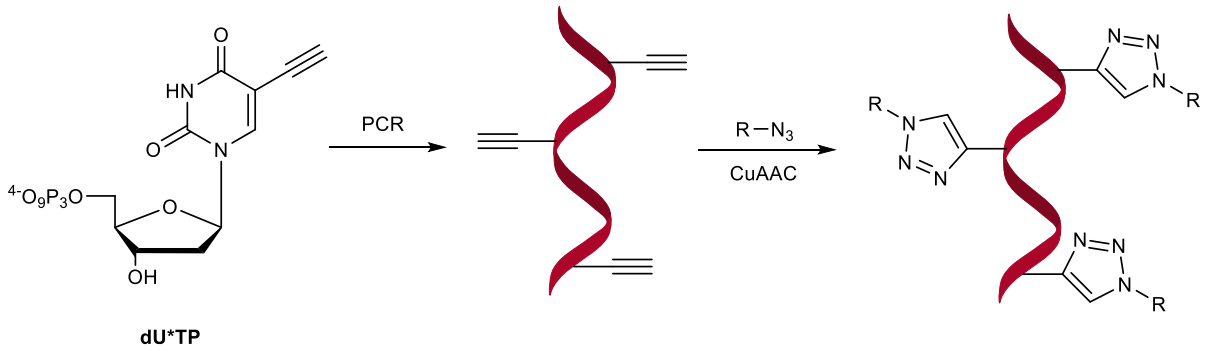


*Figure 16: Labeling of alkyne-modified oligonucleotides prepared by common PCR methodology providing dU*TP in the medium, adapted from [(66)].*

However, the copper ions damage DNA by breaking strands. This problem can be overcome using a Cu(I)-stabilizing ligand, for example tris(benzyltriazolylmethyl)amine (TBTA). Another limitation is that the direct incorporation of azides into synthetic DNA strands is intrinsically difficult, because the azido group reacts through Staudinger reaction with the phosphorus(III) atom of the phosphoramidite group, releasing N_2 and reducing azido to amino group [(60)]. Another drawback of CuAAC is the possible contamination due to the toxicity of copper, which could be a serious concern when performing in-vivo medical studies. Thus, it is worth to mention an alternative copper-free "click" methodology for triazole linkage, in which the alkyne functionality is a strained aza-cyclooctynyl species such as azadibenzocyclooctyne (ADIBO) derivatives [(67)].

Despite the drawbacks emphasized, triazole ligation is a very powerful method for ^{18}F labelling of macromolecules. Starting from these concepts of bio-organic chemistry, one of the main purposes of this work will be the development of a synthetic route for an organic

sulfidoazide ready to be coupled with an alkyne-modified macromolecule and labelled with ^{18}F fluorine-tag through late-stage desulfurative fluorination.

2. Goals

The internship was entirely carried out at RCSI lab with occasional pieces of training performed at KelAda lab. The project described in this work was settled some years before the internship, based on the concepts of sulfide as leaving group defined in the previous works by MA group. The possibility to develop a desulfurative fluorination methodology was first conceptualized and a preliminary screening of electrophilic and nucleophilic fluorine sources was made. PPHF emerged as the best option for this purpose, therefore it was chosen as the reagent to use, together with Br_2 as oxydant to activate the C-S bond. Thus, optimization of the reaction was conducted at KelAda lab, finally defining a methodology that employs $AgNO_3$ as adjuvant. Then, starting from the information acquired, the very first piece of work of the internship was a quick testing of the same methodology with other fluoride sources. Then, an important effort at was put on the synthesis of a large library of sulfides that are then carried to KelAda lab to be tested with the methodology, defining the scope of desulfurative fluorination. It is provided an extensive overview of all the challenges encountered when preparing the compounds bearing the thiophenyl group. An important application of desulfurative fluorination could be the labeling of oligonucleotides through triazole linkage, therefore a synthetic route for a sulfidoazide is developed and each step is described focusing on sustainability aspects.

2.1 Esterification of cinnamic acids

Some cinnamate derivatives were not available in the form of esters, therefore the first piece of work was the esterification of cinnamic acids.

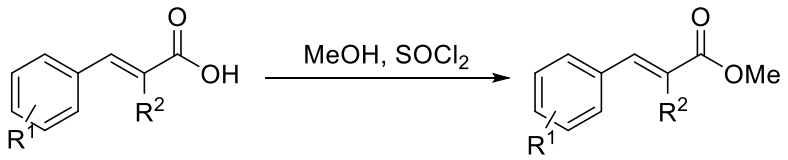


Figure 17: Esterification of cinnamic acids.

2.2 Synthesis of sulfides

For the scope of fluorination it was needed to synthetize each sulfide precursor in 5-10 grams scale. The two reactions performed at RCSI lab using thiophenol started from Michael acceptors (*Figure 18a*) or from secondary and tertiary alcohols (*Figure 18b*).

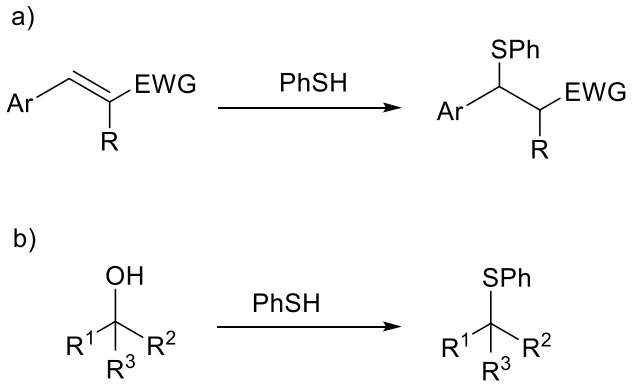


Figure 18: Synthesis of sulfides. a) Sulfa-Michael addition; b) substitution of hydroxyl group.

2.3 Desulfurative fluorination methodology

The optimization of the methodology was conducted at KelAda lab before the start of the internship, and after the preparation the library of sulfides the scope of fluorination was defined. A summary of the results and the concepts useful to understand the method are provided.

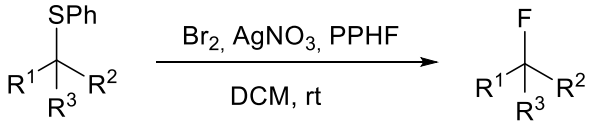


Figure 19: Desulfurative fluorination of sulfides.

2.4 Synthetic route for triazole-modified oligonucleotides

A synthetic route for fluorinated oligonucleotides has been defined starting from cinnamic acid, following the strategy of triazole linkage.

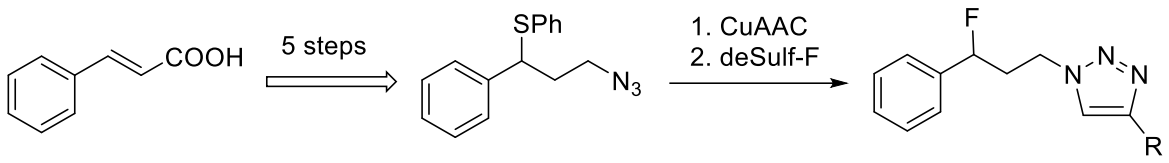
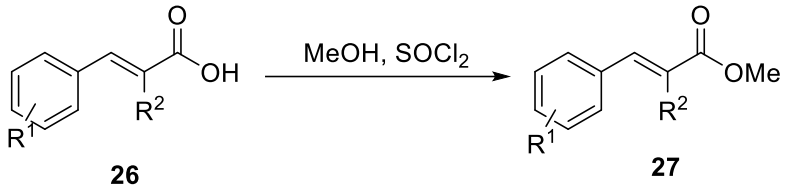


Figure 20: Synthetic route for fluorination of triazole-modified oligonucleotides.

3. Results and discussion

3.1 Esterification of cinnamic acids



26 **27**

Figure 21: Esterification of cinnamic acids

It is possible to perform SMA directly on carboxylic acids following a procedure found in the literature [(68)] using TBAF, obtaining β-sulfidoacids, but this reaction was found to be challenging. The addition to methylesters was much more straightforward and therefore the first piece of work was the preparation of cinnamate esters (*Figure 21*) to later prepare the large variety of β-sulfidoesters needed for the scope of fluorination. The method is now described considering all the cinnamates prepared. A procedure found in the literature [(69)] suggested that using thionyl chloride it was possible to perform esterification of cinnamic acid using methanol in very strong excess (10 equiv), acting both as solvent and reagent. The reaction proceeds through an acyl chloride intermediate that is much more reactive than simple carboxylic group, following the typical mechanism mediated by thionyl chloride. The addition of $SOCl_2$ must be done very carefully at 0°C and strictly under fume hood, being an intrinsically hazardous reagent due to its corrosiveness and due to the production of gaseous byproducts such as SO_2 and HCl. Finally, the reaction was quenched with ice-cold water after total conversion of the substrate was detected by TLC. This last quenching passage was not present in the original procedure and was added to remove residual $SOCl_2$ in excess, improving the safety of the synthesis. With these few simple precautions, the reaction is performed without major challenges obtaining the desired methyl esters with excellent yield

(>80%) after simple work-up by liquid extraction with a green solvent (EtOAc) and an aqueous solution of carbonate salt. The substantial absence of major byproducts allows the avoidance of expendious chromatography procedures as purification method. In some experiments it was observed the formation of a turbid solution, probably due to solubility issues derived from the presence of substituents in the phenyl ring of the substrate (OMe, NO_2, and in less extent also Br and Cl). In those cases, the reaction was additionally heated at 50°C for a few hours to afford complete conversion, usually with appreciable results.

However, using as substrate α-cyano-4-hydroxycinnamic acid **26f** the reaction was very difficult to manage, obtaining very low yield (5-10%) and conversion even after additional heating. The ^{1}H-NMR spectra revealed the absence of major byproducts and the unconverted substrate was recovered by acidification of the aqueous phase with 1M HCl followed by solvent extraction with EtOAc. One possible explanation could be that the presence of cyano group in α position stabilizes the carboxylic group, making it less reactive. Thus, a quick review of the procedures found in Reaxys and SciFinder databases was done looking for different esterification methods. The only experimental method [(70)] found with the substrate **26f** uses H_2SO_4 as acid catalyst (Fischer esterification), which would be the best option in terms of safety and environmental impact. The reaction proceeds at 80°C for 5 hours obtaining the desired ester together with minor side products with a relatively low yield (approximately 50%) after purification by column chromatography, and the same publication describes the preparation of analogous esters following this approach. The Fischer esterification presents some limitations due to the thermal reversibility of the reaction, since it is needed a Dean-Stark apparatus to remove water coproduced, pushing the unfavorable equilibrium towards the product side. Moreover, some substrates could be sensible to hydrolysis from the strong acids used as catalysts. Other examples in the literature perform the esterification of phenyl-substituted α-cyano cinnamic acids using concerning reagents as coupling agents, for example carbodiimides with DMAP as a base (Steglich esterification) [(71)], or triphenylphosphine with DIAD (Mitsunobu reaction) [(72)]. These reagents are extremely hazardous and should be used only for specific purposes, for example carbodiimides such as EDCI or DCC are commonly used for amide coupling in peptide synthesis, as an alternative to benzotriazole derivatives (TBTU, HOAt, HOBt, HBTU, HATU), which are as well very concerning reagents. Comparing all these methods for the esterification, thionyl chloride was confirmed to be the best option, also considering the small scale of operation. With few, simple precautions it is possible to obtain methyl cinnamates with excellent yield without

major side products and without wasteful chromatography purification needed, allowing to perform several reactions in parallel. To summarize, in the *Table 1* are compared the disadvantages and the scores in terms of Clean Chemistry and Greeness of these esterification methods, based on GSK's reagent guide for esterification [23].

Method	Reagent	Clean chemistry	Greeness	Drawbacks
Fischer esterification	H_2SO_4	9	8.3	Reversibility; Side-reactions (hydrolysis)
Acyl chloride conversion	$SOCl_2$	6	5.5	Gaseous byproducts; Corrosiveness
Steglich esterification	DCC + DMAP	3	3.3	Concerning reagent; Urea byproduct (DCU)
Mitsunobu reaction	PPh_3 + DIAD	2	2.5	Concerning reagent; Oxydant carcinogenicity

Table 1: Comparison of different methods for esterification.

Finally, the synthesis of methyl α-cyano-4-hydroxycinnamate **27f** was not improved since this compound was not interesting for the scope of fluorination. This substrate was present in the RCSI's peptide lab since it is commonly used as a matrix for peptides and nucleotides in MALDI mass spectroscopy. Finally, the cinnamate methyl esters prepared are used as substrate to produce a broad range of β-sulfidoesters that are then carried to KelAda lab and tested with desulfurative fluorination.

3.2 Synthesis of sulfides

3.2.1 From α,β-unsaturated compounds

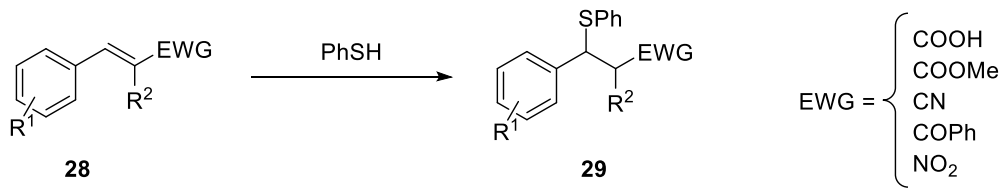


Figure 22: Synthesis of sulfides through Sulfa-Michael Addition.

Methodology

The possible applications of desulfurative fluorination depend on the sulfide precursors that could be prepared. Therefore, it is essential a complete overview of the synthetic methods to prepare such precursors and the relative challenges encountered. Most of the sulfides used to define the scope of desulfurative fluorination were prepared through a Sulfa-Michael Addition (SMA) reaction exploiting the double bond of α,β-unsaturated compounds **28** as acceptors and thiophenol PhSH as donor (*Figure 22*). The presence of an EWG like carbonyl, nitrile, and nitro groups ensures the resonance with the double bond, leaving the β carbon susceptible to nucleophilic addition, obtaining several β-sulfidocompounds **29**. For the SMA the starting materials were mainly cinnamate derivatives (EWG = COOH or COOMe), but also a few cinnamonitriles (CN), and nitrostyrenes (NO_2) have been prepared, obtaining a broad range of (sulfido-) aryl ethyl derivatives bearing different functional groups for further modification. Other substrates were prepared from chalcone, a phenyl-ketone (COPh) which is an important building block in organic chemistry. All the substrates mentioned can be simply detected by UV light being conjugated systems, allowing easy monitoring of the reactions through TLC. For what concerns cinnamate esters, chalcones, and nitrostyrenes, the reaction has some favorable features that allow simple operation, because most of the substrates are converted in solvent-free conditions, at room temperature, and using small amounts of a base that is widely available in chemical laboratories.

Triethylamine is a weak base, needed to deprotonate the thiol functionality, making thiophenol more prone to nucleophilic addition. Thanks to a regeneration mechanism, the amount needed could be extremely low, with 0.1 equivalent moles of Et_3N for each mol of substrate and 1.1 equiv of PhSH. However, some substrates have lower reactivity, therefore the usual conditions may not be sufficient to reach complete conversion after 12-20 hours (as confirmed by TLC monitoring), being necessary one or more additional portions of reagents. In almost all cases performing the reaction with 2 equiv of PhSH and 1 equiv of Et_3N allows to convert completely the substrate in a reasonable time. A good compromise is the use of 1.5 equiv of PhSH and 0.5 equiv of Et_3N, which works well with most of the substrates, avoiding long reaction times or repetition of experiments, considering that the aim was to produce at least 5-10 grams of each sulfide to be used for the scope of desulfurative fluorination. Another way to deal with slow-reacting substrates was to perform the reaction under moderate heating (50-60°C). Due to the stoichiometry, thiophenol should be put in excess to afford total conversion and acting also as “solvent”, even though with some substrates the addition of few

equivalent moles of DCM could be needed due to solubility issues. The sulfides have been purified through flash column chromatography in silica gel, except for chalcone-derived sulfides (sulfidoketones) which can be purified through recrystallization in hot methanol. The separation of unreacted thiophenol is not a difficult task, although presenting some challenges and drawbacks, the first being the high amount of organic solvents needed which is the main environmental impact of the process. Another major problem for purification and in general performing SMA is the strong, repulsive odor of thiophenol, which resembles that of garlic or rotten eggs. This means that all the equipment in contact with PhSH should be properly kept under fume hood and placed in some vessel together with copious amounts of bleach diluted in water and left until thiol is completely oxidized, including the rotary evaporator apparatus that should be also thoroughly cleaned with diluted bleach immediately after each operation.

Before proceeding with purification, the crude (most of the times an oil, due to the presence of PhSH) is analyzed by ^{1}H-NMR to eventually confirm the success of the reaction. A crucial point for chromatography is the choice of the eluent: the mixture must be properly selected and it was usually needed a gradient system to completely separate PhSH without losing the desired product by co-elution, with a significant waste of solvent. Most of the times the eluent used at RCSI was a mixture of petroleum ether (PE) and ethyl acetate (EtOAc), while at KelAda lab it was used pentane instead of PE, with very similar results. Starting with 100% PE, thiophenol is eluted, then gradually increasing the quantity of a more polar solvent such as EtOAc in the mixture the desired product is slowly eluted. A factor involved could be the presence of Et_3N in the crude since this polar compound could interfere with silica chromatography if not removed completely through solvent extraction.

Scope of the SMA

Finally, considering all these small precautions, a broad range of α,β-unsaturated compounds could be easily converted into thiophenyl sulfides through SMA with appreciable yield and in a completely selective manner, affording the β-addition of the nucleophilic thiol with the majority of the substrates tested. From these sulfides could be then obtained compounds with fluorine atom in benzylic position, a feature that is not much diffused in the context of ^{18}F labeling, and investigating this approach could be very interesting. For example, it could be planned the synthesis of dopamine derivatives with ^{18}F fluorine-tag in benzylic position.

The presence of substituents in the phenyl ring and/or in the α position could affect the regioselectivity and the rate of the reaction, and a variety of derivatives were tested in this sense optimizing the synthesis of sulfides and improving our understanding of the method. R^1 substituents are Cl, Br, F, Me, CF_3, OH, OMe, NO_2 in different positions, while only two substrates have R^2 substituents different than H (one is α-CN and the other α-Me). After screening all these substrates, the results gave interesting insights about the reaction, and these concepts are now discussed proposing a few particular cases of cinnamates, being the most used substrates in this work. Being conjugated systems, resonance plays an important role in the mechanism of thiol addition. As described in *Figure 23a*, the presence of strong EDGs such as -OH or -OMe in para position of the phenyl ring increases the delocalization of π electrons of the system, consequently increasing the energy stabilization of the molecule. Performing the reaction with methyl 4-hydroxycinnamate **28h** (R=H) and methyl 4-methoxycinnamate **28i** (R=Me) is a bit challenging, also due to solubility issues since it is formed a viscous solution that hinders adequate stirring, and total conversion does not occur at room temperature after 15 h. In the case of the methoxy group, the further addition of a second portion of reagents was enough to reach complete conversion after a total of 24 h under stirring. With the hydroxyl group, instead, further additions of reagents and heating at 50°C did not improve the reaction, finally obtaining a 60:40 mixture of the desired product and cinnamate substrate after 40 h under stirring. However, repeating the experiment starting the reaction directly at 50°C afforded complete conversion of the substrate into the desired sulfide. Therefore, it was assumed that with similar slow-reacting substrates it could be beneficial to perform the reaction directly under heating.

On the other hand, strong EWG groups in the phenyl ring such as NO_2 affect the regioselectivity of the reaction. In particular, looking at the resonance structure in *Figure 23b* it is evident that the nitro group in para position induces a partial positive charge on the carbon in α position, making it susceptible to nucleophilic attack by thiol. Indeed, performing this reaction it is obtained a 50:50 mixture of α- and β-substituted sulfide, strongly limiting the yield of the desired β-sulfidoester **29j**.

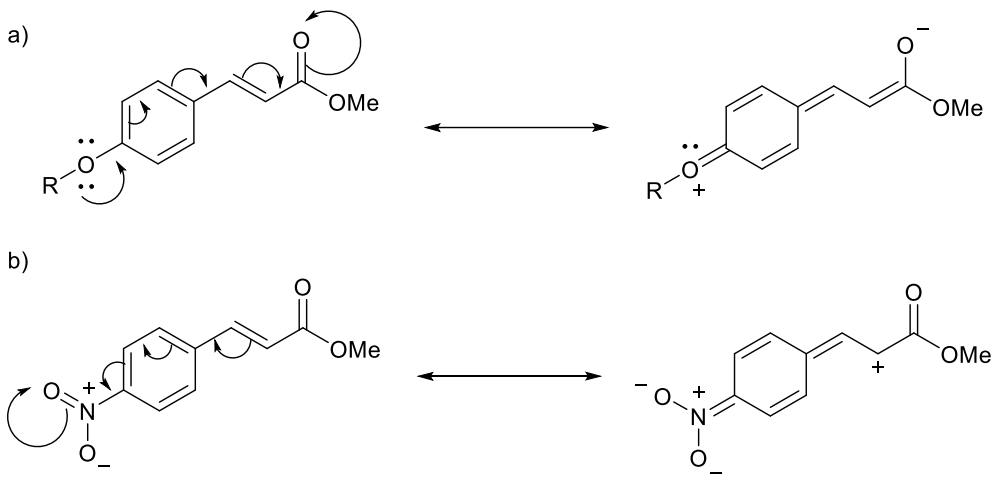


Figure 23: Effect of substituents on the resonance structure of cinnamates. a) EDG, b) EWG.

The result is different when the substituent in para position is CF_3, which is still a strong EWG but does not affect conjugation, with a significant increase in the rate of the reaction thanks to the strong inductive effect that pulls electron cloud towards the substituent in the phenyl ring, making β carbon more reactive. In the synthesis of **29e** it was reached complete conversion in just 30 minutes, while most of the α,β-unsaturated compounds are not totally converted in less than 10 hours.

The thiol addition to α-substituted cinnamates seems to be challenging. The substrate α-cyano-4-hydroxycinnamate (*Figure 24*) did not react with the common SMA procedure. Therefore, it was worth testing it with a different procedure [68] already exploited in the previous works by MA group and in this work to prepare a few sulfidoacids. This method allows the direct conversion of different cinnamic acids to relative Michael adducts using TBAF (a stronger base than Et_3N) at 50°C in 24 h or less. However, this α-substituted cinnamate was still not converted, and a possible explanation could be found considering the reversibility of the reaction, which has already been discussed in the introduction when dealing with covalent inhibition mechanisms involving cysteine residues. More in detail, the presence of the additional EWG is expected to boost the rate of SMA, but in turn it probably makes the adjacent proton (circled in red in *Figure 24*) more acid, triggering the reversible elimination and making impossible the isolation of the Michael adduct as described with cyanoacrylamides [8]. Another reason for the low reactivity of this substrate could be the additional delocalization of the conjugated system induced by the hydroxyl group, as observed before.

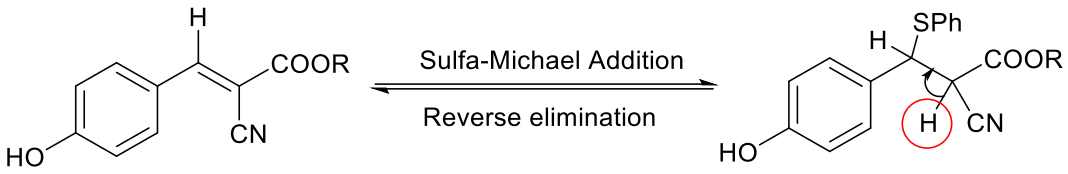


Figure 24: Sulfa-Michael Addition on α-cyano-4-hydroxycinnamate, highlighting the reversibility of the reaction.

Instead, in the case of α-methylcinnamate it was obtained a 55:45 mixture of the substrate and the desired product (i.e. 55% conversion), but it is not totally clear the role of the additional methyl group in the interference with β-addition. These two α-substituted substrates were not needed for the scope of fluorination, but they were still tested to better understand the potentiality of sulfides preparation and confirm theoretical concepts. Moreover, a quick experiment made using cinnamoyl alcohol as substrate revealed that thiol addition to the double bond does not occur without an adjacent strong EWG, also confirming the absence of side reactions of thiol with free hydroxyl groups when a weak base as Et_3N is used. Thiophenol instead reacts with secondary and tertiary alcohols in the presence of a lewis acid, and this other approach to prepare sulfides is now described in detail.

3.2.2 From secondary and tertiary alcohols

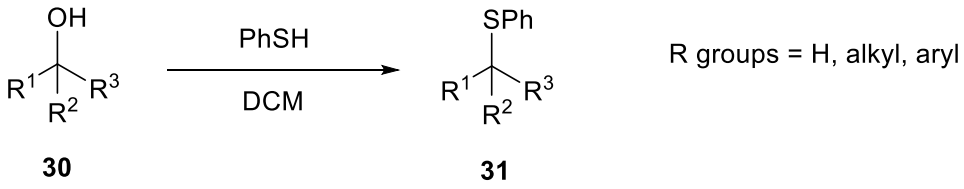


Figure 25: Synthesis of sulfides through substitution of hydroxyl group.

Another powerful method to prepare sulfides is the substitution of hydroxyl groups of activated alkyl alcohols with thiophenol (dehydroxy-thioetherification, *Figure 25*), a strategy that significantly broadens the possibilities to finally obtain a range of alkyl fluorides, following the same approach of the previous works for enantiopure alkyl chlorides and bromides. The activating agent needed in this case is a Lewis acid and it was initially used ZnI_2, which activates secondary and tertiary alcohols triggering the nucleophilic attack by thiol through an S_N1 mechanism. However, when bleach was used to remove the strong odor

of thiophenol from the aqueous phase, a red-brownish precipitate was formed, and the nature of this precipitate was not understood exactly due to the complexity of the mixture. One possible explanation could be the formation of triiodide anion I_3^-, which typically has a red color in solution. $ZnBr_2$ and $ZnCl_2$ are probably suitable alternatives, the latter being commonly used in undergraduate laboratory teaching experiments to differentiate alcohols [73,74]. To avoid additional filtrating and neutralization procedures, BF_3*Et_2O was later chosen as an alternative to ZnI_2, based on the reagents available at RCSI lab.

The main substrates used were secondary and tertiary alkyl alcohols, reminding that primary sulfides are not interesting for fluorination due to the intrinsic mechanism of fluoro-Pummerer fragmentation. The alcohols were first tested on a small scale to confirm the possibility of preparing the desired sulfides with this method. In particular, the uncertainty was about the substrates with hydroxyl group not in benzylic position, but in the end this approach worked well and the sulfides **31d**, **31f** and **31g** were prepared accordingly, together with the other sulfides indicated in *Figure 26*. The only detail to be noted is that in the case of sulfide **31e,** derived from secondary alcohol **30e** (1-phenyl-1-butanol), it was recorded by HNMR the presence of the elimination product 1-phenylbutane (approx. 5 %), which was not a big deal since it was easily removed later by flash column chromatography together with thiophenol in excess. The same happened with the sulfide **31d**. It was assumed that this side reaction could occur due to the action of zinc, following a reductive elimination mechanism similar to Clemmensen reduction. Thus, using a different Lewis acid such as BF_3*Et_2O this kind of byproduct should be avoided. Finally, with this method the sulfides indicated have been synthesized, and other similar alkyl/aryl derivatives were already available in pure form at KelAda lab. This method expands the applications of fluorination, with more secondary and tertiary sulfides that can be prepared. Furthermore, it could be a valuable approach for the synthesis of sulfidoaminoacids which could be then exploited for labeling of peptides. For example, threonine residues (the only common aminoacid with secondary hydroxyl group) could be converted into a modified derivative bearing thiophenyl group, and with other non-common aminoacids this possibility could be further expanded.

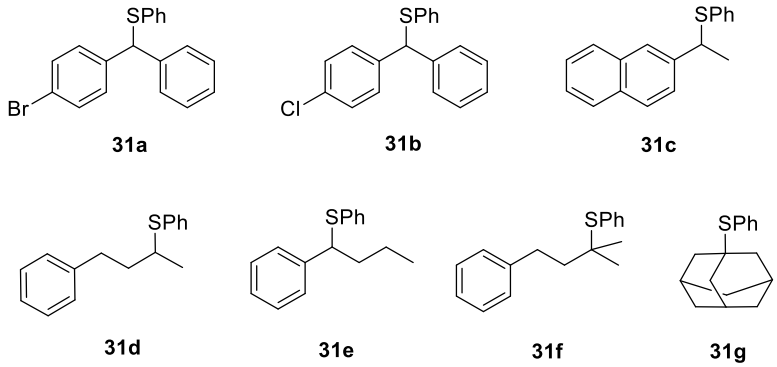


Figure 26: Sulfides prepared through substitution of hydroxyl group.

3.3 Desulfurative fluorination methodology

The desulfurative fluorination developed by Olah's and coworkers [(11)], previously described in the introduction, presents great limitations due to the presence of nitrosonium cation NO^+, which could act as nitrosylating agent. Therefore, a different oxidant should be found, allowing a direct fluorination of poly-functionalized molecules such as peptides and oligonucleotides. A preliminary methodology was conceptualized in the previous works, and after optimization at KelAda lab all the procedures were captured in the final version of the PTC patent application submitted by Prof. Adamo on behalf of RCSI in July 2023. The intellectual property is cited in the bibliography of this thesis, and the patent will not be published before July 2024. Nevertheless, it is still possible to describe all the procedures, since this thesis is going to be in an embargo status (i.e. not publicly available online) for the period of at least 1 year. The previous works from MA group suggested Br_2 as a promising option, since Br^+ is able to selectively oxidize sulfides. Then, bromide Br^- is free to react as nucleophile with the reactive TP/TBP sulfonium species generated (as seen in the desulfurative bromination), therefore it should be removed/trapped in some way to avoid the formation of undesired bromide. This issue was crucial in the optimization of the method, since the brominated compound was the main undesired product of the reaction, together with the elimination product (*Figure 27*).

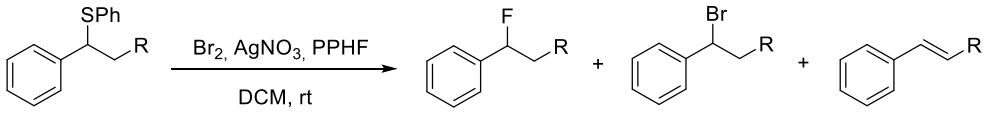


Figure 27: Optimization of the reaction, showing the desired fluorinated product and the two main byproducts obtained (brominated product and elimination product).

Optimization of the reaction made at KelAda lab with cinnamate-derived sulfide (R=COOMe) revealed that the subsequent addition of bromine, PPHF and silver nitrate to the sulfide gives approximately a 50:50 mixture of brominated:fluorinated product. Instead, a better approach is the addition of Br_2 to a solution of $AgNO_3$ dissolved in DCM, leading to the formation of a brick-orange solution. Then, PPHF is added dropwise, and the solution is left under vigorous stirring, observing a gradual change of the color to light yellow and the formation of a white precipitate. After 3-7 minutes a solution of sulfide dissolved in DCM is added dropwise and finally the desired compound is obtained, usually within additional 10 minutes under stirring at room temperature, with a gradual color change from light yellow to white milk/cream. The premixing time is crucial for the selectivity of the reaction: adding the sulfide after 3-4 minutes is obtained a mixture of the three products (F, Br, elim) while 7 minutes of premixing usually produce exclusively the desired fluoride.

The whole mechanism known/inferred is now described and schematized in the *Figure 28*, emphasizing the dissociation of Br_2 into bromonium Br^+ (in solution with nitrate) and bromide Br^- (in the form of silver salt). This silver salt (AgBr) is observed as a white precipitate, while the presence of $BrNO_3$ is suggested by the formation of an orange-brick solution. Lown and Joshua [75] reported the formation of these products from Br_2 and $AgNO_3$, using this mixture to carry out addition of bromonium and nitrate to double bonds, producing brominated alkyl nitrates but also bromo-pyridinium salts due to the use of pyridine as solvent. Similarly, bromonium and HF could react, with in-situ formation of an interhalogen species (BrF), as reported in the literature [76]. It must be kept in mind that the definition of the actual species reacting is not straightforward. Gaseous BrF can be prepared only at -80°C and cannot be isolated since disproportion rapidly occurs. Moreover, it needs to be accounted the coexistence of HF, F^- and H_2F^+, due to the dissociation of hydrofluoric acid, indeed it was observed that the water from reaction quench was slightly acidic (pH 4-5), due to the release of H^+. When the starting material is added to the mixture, the C-S bond is activated by the electrophilic bromonium cation, generating the trigonal pyramidal (TP) intermediate. This sulfur(IV) species is highly reactive towards invertive nucleophilic attack (S_N2) and is quickly converted into the desired fluoride and hipobromothioite (PhSBr), following the mechanism of Pummerer fragmentation (Type III) widely discussed in the introduction. As stated before, only 0.5 equivalents of Br_2 are needed for the reaction, therefore it is probable that a regeneration mechanism occurs, similarly to what has been observed in desulfurative bromination. In that case, bromine needs to be added in excess, but this regeneration

mechanism has been observed by in-line NMR experiments, involving the dismutation of the coproduct PhSBr, with the formation of Br_2 and disulphide PhS-SPh or similar species. In the case of fluorination, an additional regeneration mechanism could occur, since BrF commonly disproportionates into Br_2 + BrF_3, eventually producing new sources of electrophilic bromonium and nucleophilic fluoride [77].

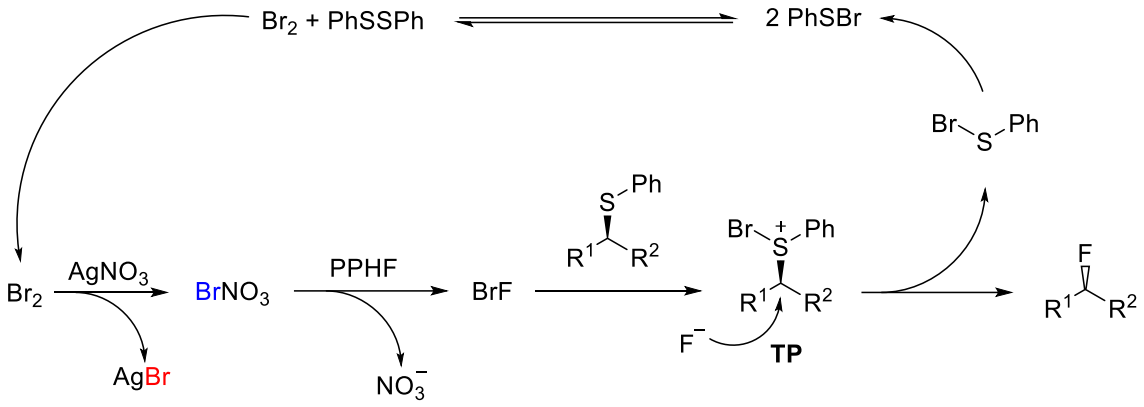


Figure 28: Proposed mechanism of the reaction, considering the regeneration of bromine.

After the reaction has been optimized using simple cinnamate-derived sulfide, a list of several thiophenyl-compounds bearing different substituents was defined. Most of the sulfides were prepared in 5-10 grams scale at RCSI lab during the internship and the results of these synthesis are discussed in the next chapter. These sulfides were then carried to KelAda lab to be tested with the same procedure to define the scope of desulfurative fluorination. They were tested in the form of racemates to verify the chemoselectivity of the reaction, demonstrating that it is compatible with a broad range of functional groups such as COOR, OH, NH_2, NO_2, CN, and the resulting fluorides obtained are summarized in the *Figure 29*. As expected, the substrates suitable are secondary and tertiary sulfides similarly to the ones encountered in the previous works, and they had been synthesized at RCSI mainly by SMA on cinnamates, which can be further modified, but also from other Michael acceptors such as chalcones, cinnamonitriles, nitrostyrenes, and secondary and tertiary alcohols. The substrates for the last two entries have been prepared with another procedure developed at KelAda, specifically used to prepare long chain functionalized secondary sulfides.

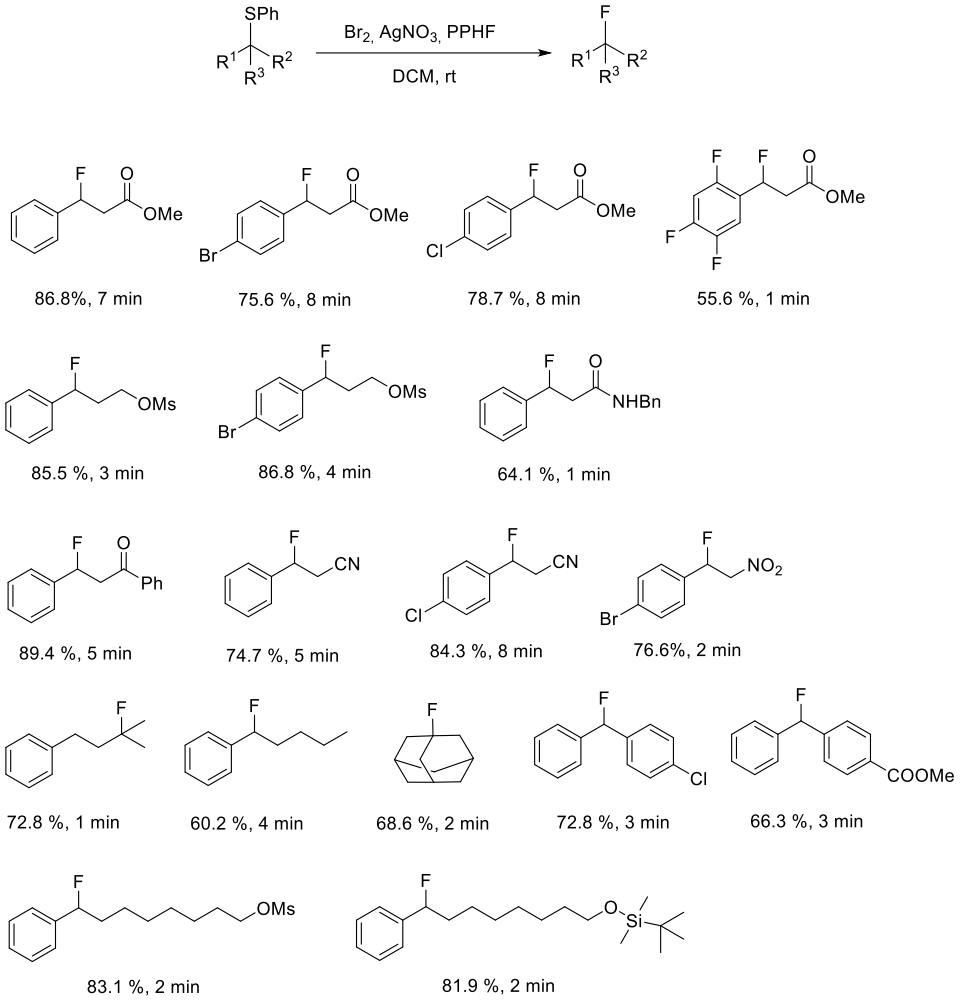


Figure 29: Scope of the desulfurative fluorination.

The reaction was also tested with pure chiral sulfides derived from chalcone and cinnamate, which were available in a stable and pure form at RCSI, being prepared during the previous works by MA group using the selective aminothiourea catalyst. As expected, HPLC demonstrated that fluoro-Pummerer fragmentation produces C-F bonds through a S_N2 type mechanism (i.e. with inversion of stereocenter) with high level of stereocontrol, a feature that could be very interesting for the synthesis of drugs. Finally, the concept of desulfurative fluorination has been successfully developed, defining a fast, simple method to prepare alkylfluorides with excellent values of yield and selectivity. These features make the methodology suitable for ^{18}F fluorination, thus it can be exploited for medical studies in PET imaging. Specific experiments are needed to verify the compatibility of desulfurative fluorination with radiochemical methods, due to the possible different reactivity of Fluorine-18 compared to Fluorine-19, but the premises seem to be very promising.

3.4 Synthetic route for triazole-modified oligonucleotides

It is well-known that the sulfides prepared at RCSI are stable in several reaction conditions, allowing further modification of the compound without affecting the thiophenyl group needed for late stage desulfurative fluorination. Thus, a broad range of synthetic routes could be conceptualized to prepare drugs or any other thiophenyl-compound which could be also converted easily due to the chemoselectivity of the fluorination method. Several transformations have been performed in the previous works to prepare functionalized sulfides, but the concept of triazole linkage has not yet been explored. The strategy chosen is to prepare an azide probe to be exploited for labeling of modified biomolecules. For example, this type of probe could be coupled with dU*TP, an alkyne-modified nucleoside well known in the field of biological chemistry, thanks to its compatibility with PCR methodology. The azide functionality is usually inserted through nucleophilic substitution of proper leaving groups such as halides, -OTf, -OTs and -OMs using sodium azide or in less extent lithium azide. The preparation of mesylates and tosylates was already defined as suitable with thiophenyl moiety, therefore the setting of a synthetic route exploiting one of these leaving groups seemed to be a reasonable option. Thus, the whole synthetic route for fluorinated macromolecules is now described step by step (*Figure 30*) starting from cinnamic acid **32**. Every step is analyzed in detail, focusing on the challenges encountered and emphasizing the different possibile strategies to perform the synthesis of specific compounds.

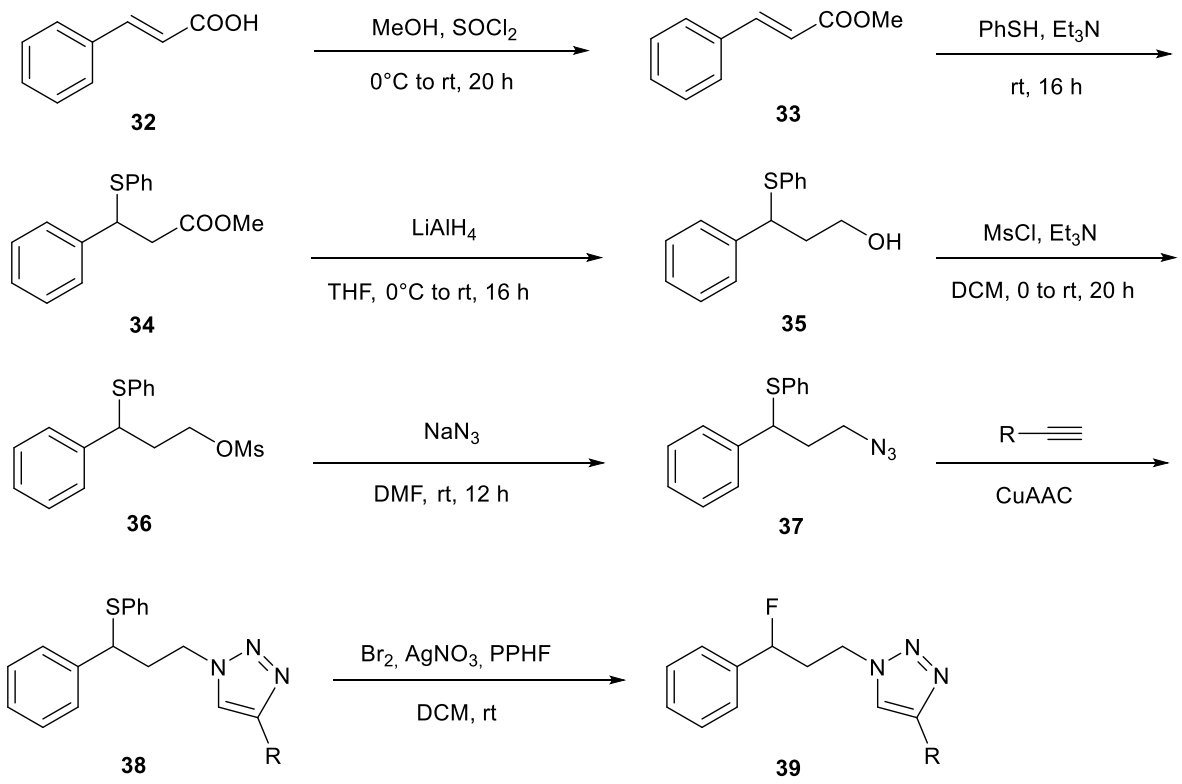


Figure 30: Synthetic route for fluorinated triazole-modified oligonucleotides.

Starting material

Being widely available at RCSI lab, the synthesis of cinnamic acid **32** (and methyl ester **33**) was not an issue. However, it was explored the possibility to synthetize other cinnamate derivatives following literature procedures, which was interesting to eventually prepare a couple of aryl derivatives poorly available in the market. Thus, a quick review of the current methods for the preparation of cinnamates is presented, which could be also useful to provide a more comprehensive view of the whole synthetic route, eventually exploring the possibilities of improvement. Cinnamic acid **32** could be extracted directly from the bark of trees belonging to the *Cinnamomum* species, together with cinnamaldehyde **40**. However, being secondary metabolites, they are usually found in low concentrations [(78)]. Cinnamaldehyde could be used to produce sulfidoaldehyde **41** which is then reduced to sulfidoalcohol **35** (a weak reducing agent such as $NaBH_4$ is probably enough). In the end, this approach opens up the possibility of obtaining a valuable product from a natural source, as schematized in *Figure 31*.

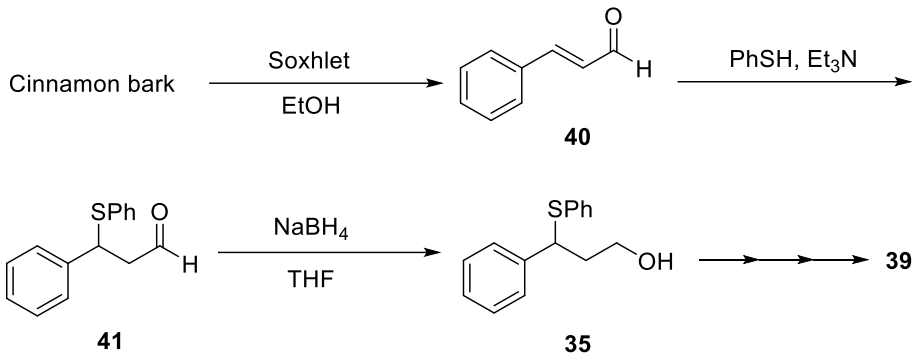


*Figure 31: Alternative pathway for sulfidoalcohol **35**, starting from natural sources.*

About chemical synthesis, a broad range of processes are found in the literature to prepare cinnamates. From these methods three classes of reactions have been selected:

a) The Knoevenagel condensation (Doebner modification).
b) The Wittig reaction with related modifications (Horner-Wadsworth-Emmons reaction, Still-Gennari reaction).
c) The cross-coupling reactions, with transition metal catalysts such as Palladium (classic Heck and oxidative Heck reaction).

In the end, none of these reactions have been performed since cinnamate was already available at RCSI lab in high quantity in the form of ester, and in the same way many others aryl derivatives were available in the market in the form of methyl esters. The esterification process already described is relatively simple and provides pure compounds without the need of wasteful chromatography operations, allowing to carry out several reactions in parallel. Thus, it was not an issue in the context of this synthetic route, and it was not needed an alternative pathway. Eventually, the investigation of these methods could be useful to prepare aryl derivatives of cinnamate that are not available in the market, starting from corresponding benzaldehyde or halobenzene derivatives.

Early stage: (esterification + Sulfa-Michael Addition)

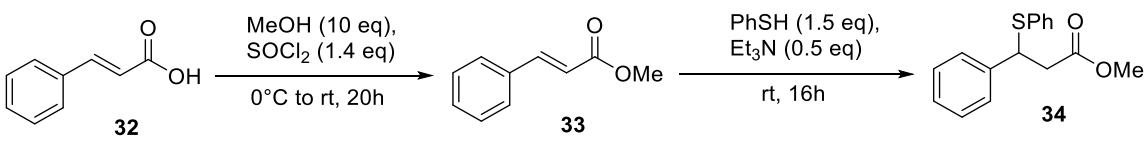


Figure 32: First two steps of the synthetic route.

The synthesis of cinnamate esters and β-sulfidoesters (*Figure 32*) have already been discussed in detail previously in the dedicated chapters. Therefore, this small paragraph is only provided to describe the typical ^{1}H-NMR spectra of a sulfidoester, comparing it to the spectra of the corresponding cinnamate ester**.** Typically, when performing SMA the presence in the crude of the desired β-sulfidoesters (like **34)** was confirmed through ^{1}HNMR before proceeding with chromatography. The key is the proton H_A in β-position, which usually appears at values of chemical shift that range from 4.5 to 5.2 ppm, with a clear triplet multiplicity due to the coupling exclusively with the two equivalent protons H_B in α position, which instead appear at values of chemical shift that range from 2.6 and 3 ppm. Instead, the presence of unconverted starting material (cinnamate ester, like **33**) could be revealed by the two peaks with doublet multiplicity generated by the two protons bounded to sp^2 carbons of the double bond, which appear at values of chemical shift around 7.6-8 ppm for hydrogen in β position H_A and much lower values (around 6.5 ppm) for the hydrogen in α position H_B. Usually, the other peaks in this zone of the spectra, generated by the hydrogens bounded to sp^2 carbons of the two aromatic rings (totally 10 H without considering substituents) are found in an intermediate range of values, between 7.4 and 6.9 ppm, therefore the detection of unconverted substrate is usually straightforward. For comparison, the ^{1}H-NMR spectra of a cinnamate ester **27e** and the related β-sulfidoester are provided in the *Figure 33*. It cannot be appreciated in this figure but the spectra of cinnamate ester appears clean, demonstrating that simple solvent extraction is enough to obtain a pure compound, avoiding chromatography.

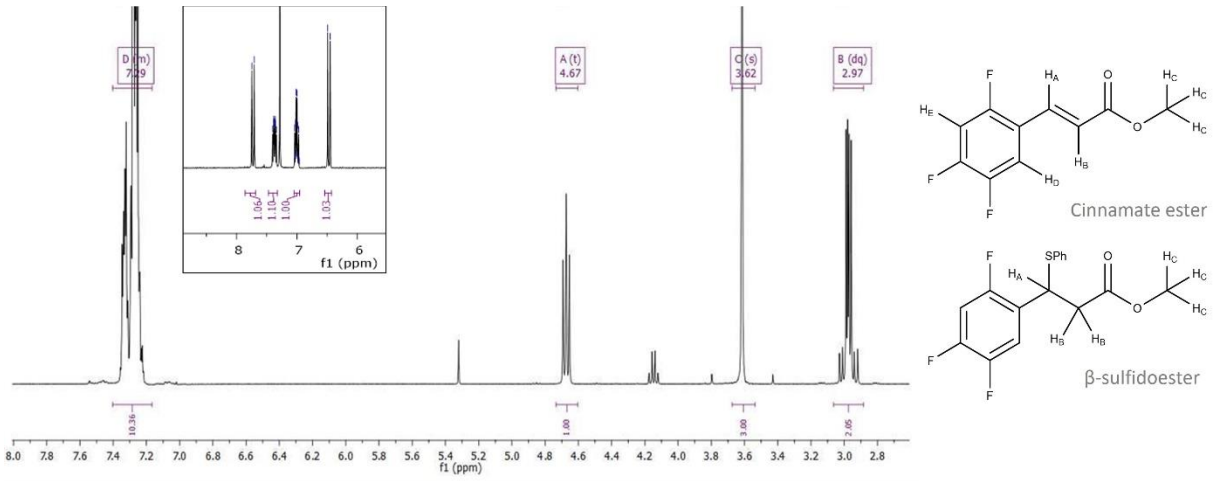


Figure 33: ^{1}H-NMR spectra of β-sulfidoester. For comparison, in the small panel is provided a detail of the spectra of the corresponding cinnamate ester, showing the peaks generated by the hydrogens in the aromatic region (from left to right: H_A, H_D, $CDCl_3$, H_E, H_B).

Reduction

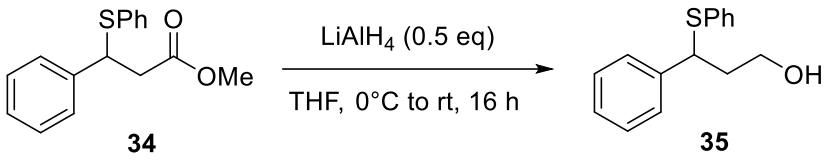


Figure 34: Reduction of β-sulfidoester using hydride.

As stressed before, β-sulfidoesters are more straightforward to prepare compared to β-sulfidoacids. Moreover, the cinnamate-derived sulfide **34** (*Figure 34*) was extensively used as test substrate to optimize the desulfurative fluorination, therefore it was needed in greater quantity than any other sulfide (at least 30g). Thus, it seemed natural to develop the synthetic route starting from this sulfidoester, considering that each intermediate of the route should be then tested as well for the scope of fluorination. The reduction of **34** was already made in the previous works and it is done using $LiAlH_4$, which is a strong reducing agent. The reaction of hydrides is strongly exothermic, therefore the addition of the reagent is carried out drop by drop carefully after cooling the reaction mixture in an ice bath and the mixture should be diluted with an appropriate amount of solvent, usually tetrahydrofuran (THF) or diethylether (Et_2O), which must be totally dry since hydrides react violently with water. Surprisingly, only 0.5 equivalents of hydride are needed to complete the reaction, which is carefully quenched with saturated aqueous NH_4Cl and then extracted with Et_2O, obtaining primary (sulfido-) alcohol **35** without major byproducts present in the crude. Indeed, it is supposed that alluminate salts formed as byproducts are separated in the aqueous phase, leaving the desired product substantially pure as a yellow-orange oil after solvent evaporation. However, the degree of purity of the samples required to be tested for fluorination is extremely high and no trace of byproducts or residual solvents must be present, therefore a purification through flash column chromatography was made in a couple of experiments, finally obtaining a white solid. For the experiments in which **35** was an intermediate the mixture was properly extracted, the solvent removed in vacuo, and the crude was used for the next step (mesylation) without further purification. Notice that in this case the use of EtOAc as green solvent for the reaction and for the extraction was not suitable. $LiAlH_4$ reagents are usually found dissolved in appropriate solvents such as THF or Et_2O, which are both hazardous “red” solvents.

Mesylation

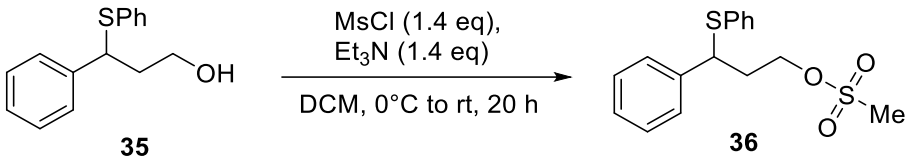


Figure 35: Mesylation of sulfidoalcohol with methansulfonyl chloride.

The activation of hydroxyl group of sulfidoalcohol is done by converting it into methanesulphonate using an excess of triethylamine (*Figure 35*). An analogous procedure is reported in the previous works for the conversion into tosylate but requires an additional 0.1 equivalents of DMAP. The procedure starts with addition of mesyl chloride (MsCl) to a solution of the sulfidoalcohol **35** in dry DCM at 0°C under stirring, and then triethylamine is added dropwise. The reaction was completed without major byproducts even when the substrate was not pure, confirming the expectations set in the previous step and opening up the possibility of performing this synthetic route with few chromatography purifications, a feature that avoids the waste of high amounts of solvents usually hazardous such as DCM or PE. In this case, the unconverted MsCl (used in excess) seems to be more persistent than the alluminate salts of the previous step, being easily detected by ^{1}H-NMR as a singlet peak at 3.7 ppm. Comparing the values of the integrals it is revealed that unconverted mesyl chloride is present in minor quantities in the crude (<10%) and it is removed almost completely after chromatography (<1%). Finally, in the *Figure 36* the ^{1}H-NMR spectra of pure mesylate **36** is provided and compared with the spectra of the crude (solvent extraction only), detecting the disappearance of the signal generated by mesyl chloride.

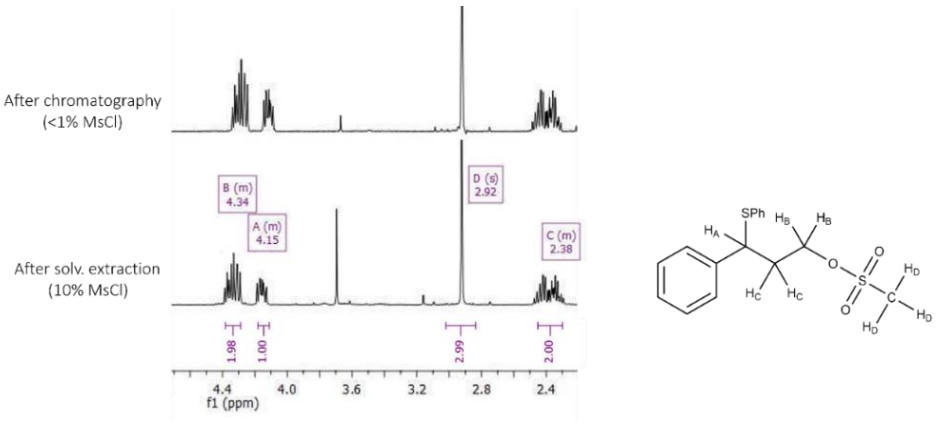


Figure 36:[1] H-NMR spectra of sulfidomesylate, showing the disappearance of mesyl chloride (3.7 ppm) from the crude after chromatography purification.

Azidation

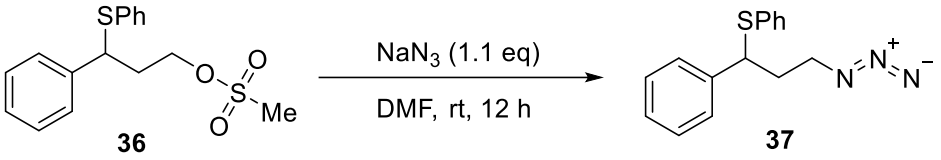


Figure 37: Azidation of sulfidomesylate.

The preparation of sulfidoazide (*Figure 37*) was performed following a procedure found in the literature that was used as proof of concept for the definition/planning of the route [79]. The synthesis of organic azides exploiting mesylate as leaving group is reported in several publications, and in the reference procedure the crude mesylate synthetized in the previous step was quenched with ice cold water, extracted with EtOAc and then used for the azidation without further purification. Based on these observations, the first experiment was done in a small-scale (1 mmol) to test the reaction using a sample of **36** that was not purified by chromatography in any of the previous two steps (reduction and mesylation), and it was reached complete conversion obtaining the desired sulfidoazide **37** without major byproducts. Finally, the reaction was successfully scaled up obtaining few grams of the desired compound that could be used for several tests with proper alkyno-modified biomolecules. Again, for the scope of fluorination the purity of the substrates was extremely important, therefore after extraction with EtOAc the crude was purified by flash chromatography with PE:EtOAc as gradient eluent system obtaining pure sulfidoazide with a final yield of 70%. A major drawback of this reaction regards the hazards of sodium azide (and N_3 salts in general), which could produce toxic gas hydrazoic acid (HN_3) in contact with acids. In addition, NaN_3 could react explosively in contact with metals and demonstrates severe toxicity in aquatic environments, therefore it is strictly forbidden the discharge into drain pipes unless it is previously diluted with copious amount of water.

Late stage (cycloaddition + fluorination)

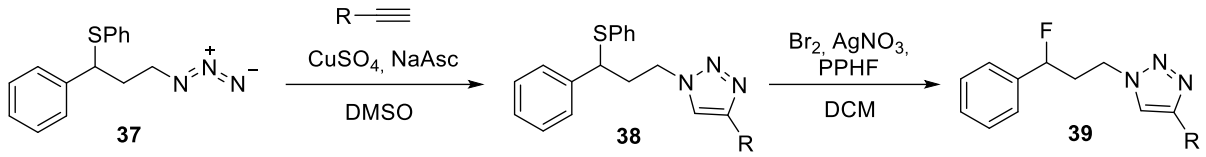


Figure 38: Final steps of the synthesis, coupling the probe through triazole ligation and late-stage fluorination.

Finally, in the last two steps are operated the triazole linkage and the fluorination of the probe (*Figure 38*). The selectivity of desulfurative fluorination allows to operate the fluorination in the very last step, obtaining the tagged biomolecule **39** with the highest RCY possible. The coupling could be made following the classic procedure using $CuSO_4$ and sodium ascorbate (NaAsc) in DMSO, obtaining the triazole linkage usually in a reasonable time (a couple of hours). A comprehensive assessment of the whole synthetic route is now presented, focusing on the sustainability of the process. These last two steps (cycloaddition + fluorination) are excluded from this assessment, since they will be performed at KelAda lab only after the scope of the fluorination has been completed, using alkyne-modified nucleoside dU*TP both protected and unprotected. This probe could be also exploited for the labeling of any alkyne-modified peptides, for example using non-common aminoacid HPG (homopropargylglycine) which is cell-permeable and is randomly incorporated in place of methionine residues during translation [80].

Sustainability Assessment

The PMI-LCA tool from ACS GCI Pharmaceuticals Roundtable [81], designed for large-scale pharmaceutical processes (results are normalized on kg of material per kg of product), is used to evaluate the environmental impact of sulfidoazide synthesis. Being adaptable to lab-scale experiments, it is applied using experimental data obtained during the internship, focusing on material contributions to Process Mass Intensity, and a preliminary assessment reveals that purification steps significantly impact the overall PMI. In particular, purification by chromatography is by far the most impactful part of the process in terms of kg of material consumed, with petroleum ether as the main solvent used in copious amounts, therefore it is excluded from this assessment. The graph in *Figure 39* highlights the other main contributors

being water, used in all steps for extraction, and EtOAc, which was always the first choice for the organic phase, except for the steps of mesylation (DCM) and reduction (Et_2O). Other contributions include solvents for the reaction mixture (THF, DCM, DMF), and reagents in solvent-free reactions (MeOH, thiophenol). Other materials of the route include triethylamine, thionyl chloride, mesyl chloride, and cinnamic acid. $LiAlH_4$ and sodium azide have minimal contribution to the PMI being used in relatively small amounts.

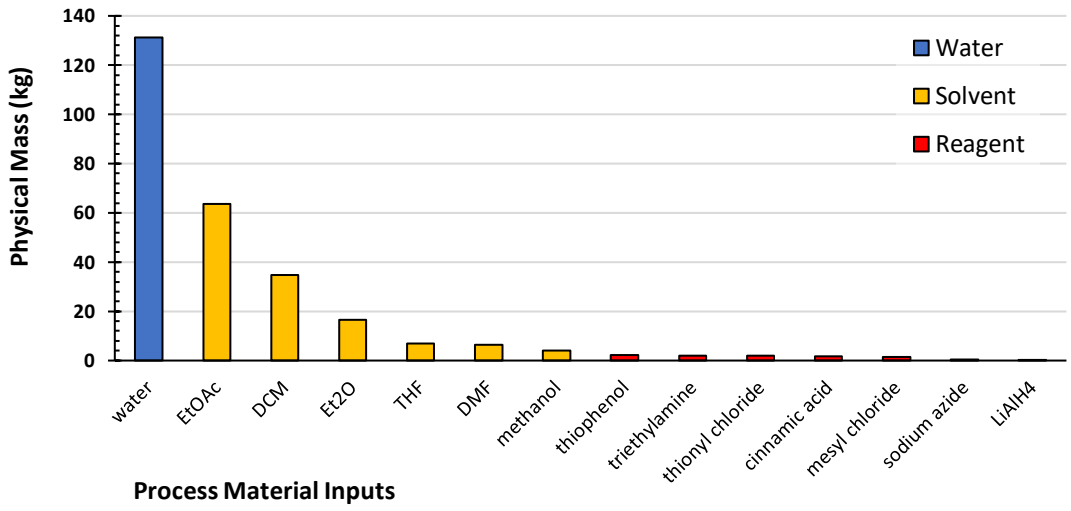


*Figure 39: Breakdown of the PMI of the synthesis of sulfidoazide **37**, without considering the chromatography procedures.*

The tool provides also results for six life-cycle impact assessment (LCIA) indicators (mass net, energy, global warming potential, acidification, eutrophication, water) based on data acquired from ecoinvent database [82], and these results are interesting since it is considered the life cycle of each solvent used. However, for reagents and raw materials ("reagent" class, in red) the impact can be severely underestimated since they are generally processed by the software with the only distinction between bulk and fine chemicals (based on iGAL, innovation Green Aspiration Level) [83]. For what concerns the impact assessment, contribution analysis are provided by the tool, revealing the most impactful steps and materials for each of the six LCIA categories. As an example, in *Figure 40* is displayed a breakdown of acidification potential for each material used in the process (again, excluding chromatography), highlighting the strong impact of DCM in this category, despite being used in lower amount than EtOAc.

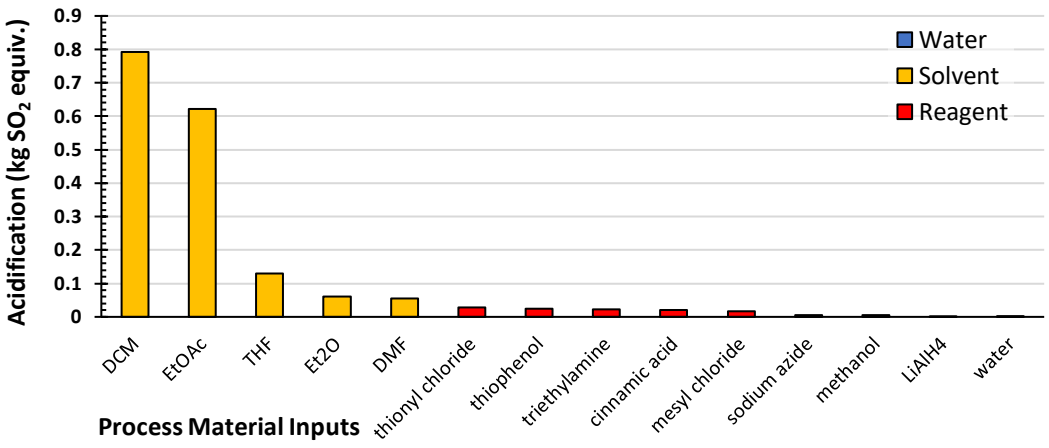


*Figure 40: Breakdown of acidification potential for each material used in the synthesis of sulfidoazide **37**, without considering chromatography procedures.*

Another way to obtain information about sustainability of chemical processes is using the CHEM21 tool, as well provided by ACS GCI [81]. This powerful tool is used with experimental data results of SMA and fluorination procedures to assess these reactions in terms of green metrics. Considering only the reaction (without work-up), the SMA reveals generally good results, and it is shown as example an experiment performed in 10g scale using methyl cinnamate **33** as substrate. In this case were used 2 equivalents of PhSH and the final yield was 83%, but in other cases were reached higher values (up to 95% yield) and less equivalents of thiophenol (1.1-1.5 equiv). The main feature of SMA is the 100% atom economy (AE) of the reaction, being an addition, which in turn ensures high reaction mass efficiency (RME), varying from 60% in this example to almost 90% in the best cases. The purified β-sulfidoester **34** is then converted into fluoride **42a** with the methodology developed, and the results are plotted in the same tool to assess fluorination. The lower AE (and RME) is intrinsic in the fact that is a substitution/fragmentation reaction, but a correlation of the two gives a new metric called optimum efficiency with a high value of 87%. The OE (=RME/AE) allows for a direct comparison of different reaction types, something that is not always possible with AE and RME since certain types of chemistries are intrinsically more atom efficient, as seen before in the case of addition [84].

4. Conclusion

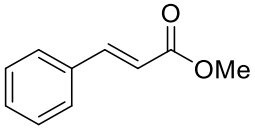
The methodology for desulfurative fluorination has been successfully developed at KelAda, enabling the production of enantiospecific C-F bonds in a rapid, simple, and efficient manner applicable to organic synthesis. A large library of sulfides has been synthesized to explore the method's scope, confirming the high chemoselectivity of the “desulfurative” approach. Extensive investigation into sulfide synthesis, particularly through Sulfa-Michael Addition on cinnamates, revealed numerous possibilities for applying this innovative fluorination technique. The features of this method support its potential application in the ^{18}F labeling of (bio-)macromolecules. A dedicated synthetic route for a sulfidoazide probe has been developed accordingly, and the method is proved to be valuable for the derivatization of oligonucleotides and peptides through the favorable features of "click" triazole ligation. Each step of the process has been meticulously analyzed following the principles of green chemistry, highlighting critical stages and materials used. A comprehensive sustainability assessment has confirmed the initial assumption regarding the significant impact of solvents. In this context, the efforts to eliminate two chromatography steps represent a crucial initiative in waste reduction, with encouraging results. Through a simple series of steps modified oligonucleotides can be obtained, offering several applications in medical studies by leveraging the advantageous features of triazole linkage as an analog of the classic phosphate linkage of DNA and RNA. The traditional scientific approach of mimicking the chemistry of biological systems persists in modernity, as researchers continuously strive to enhance these features and discover novel applications in related fields, with the aim to make human society prosper without in turn damaging the environment. Doing like nature, better than nature, in harmony with nature. Let’s do it.

5. Experimental section

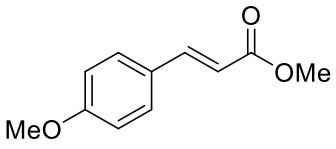
5.1 Esterification of cinnamic acids

General procedure for cinnamate esters:

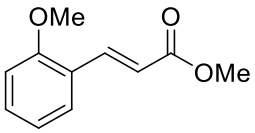
To a round-bottom flask fitted with magnetic stirrer was added cinnamic acid (1 equiv) and dissolved in methanol (10 equiv). The reaction mixture was cooled in an ice bath and thionyl chloride (1.4 equiv) was added dropwise. The mixture was left under stirring at 0°C for 1.5 h, then left under stirring at room temperature until consumption of the cinnamate starting material was confirmed by TLC (ca. 15-20 h). After consumption of starting material was confirmed by TLC, the reaction mixture was poured into ice-cold water to quench residual $SOCl_2$ and extracted in a separatory funnel with EtOAc (3x20 mL). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ and brine (20 mL), before drying over sodium sulfate and filtered. The mixture was concentrated *in vacuo* and crude residue analysed by ^{1}H NMR spectroscopy.



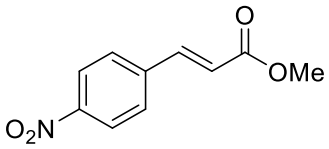
33 Methyl cinnamate: prepared from cinnamic acid (0.913 g, 6.16 mmol, 1 eq) according to the general procedure. After work-up, yellowish crystals were obtained (0.860 g, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 16.0 Hz, 1H), 7.53 (dd, J = 6.6, 2.9 Hz, 2H), 7.43 – 7.34 (m, 3H), 6.45 (d, J = 16.0 Hz, 1H), 3.81 (s, 3H).



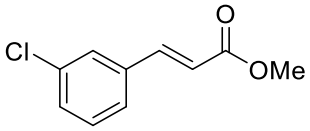
27a Methyl 3-(4-methoxyphenyl)acrylate: prepared from 4-methoxycinnamic acid (4.63 g, 26.01 mmol, 1 eq) according to the general procedure. After work-up, white crystals were obtained (4.86g, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 7.65 (d, J = 16.0 Hz, 1H), 7.48 (m, 2H), 6.91 (m, 2H), 6.31 (d, J = 16.0, 1H), 3.84 (s, 3H), 3.79 (s, 3H).



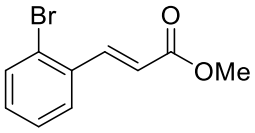
<u>27b</u> Methyl 3-(2-methoxyphenyl)acrylate: prepared from 2-methoxycinnamic acid (1.85 g, 10.4 mmol, 1 eq) according to the general procedure. The reaction was stirred at room temperature for 17h and then additionally heated at 60°C for 2h. After work-up, a brown oil was obtained (1.596 g, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 16.2 Hz, 1H), 7.50 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.39 – 7.31 (m, 1H), 6.99 – 6.89 (m, 2H), 6.53 (d, *J* = 16.2 Hz, 1H), 3.89 (s, 3H), 3.80 (s, 3H).



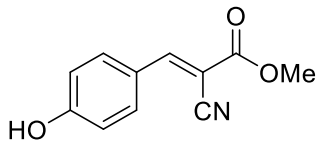
<u>27c</u> Methyl 3-(4-nitrophenyl)acrylate: prepared from 4-nitrocinnamic acid (5.0 g, 25.9 mmol, 1 eq) according to the general procedure, the reaction was stirred at room temperature for 16h and then was additionally heated at 60°C for 6h. After work-up, yellow crystals were obtained (4.8 g, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 8.26 (m, 2H), 7.72 (d, J = 16 Hz, 1H), 7.68 (m, 2H), 6.57 (d, J = 16.0 Hz, 1H), 3.64 (s, 3H).



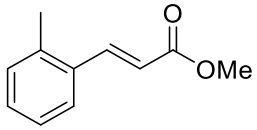
<u>27d</u> Methyl 3-(3-chlorophenyl)acrylate: prepared from 3-chlorocinnamic acid (6.15 g, 33.7 mmol, 1 eq) according to the general procedure, the reaction was stirred at room temperature for 18h and then additionally heated at 60°C for 2h. After work-up, white crystals were obtained (6.4 g, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 7.58 (d, J = 16 Hz, 1H), 7.45 (brt, 1H), 7.31 (m, 3H), 6.40 (d, J = 16.0 Hz, 1H), 3.79 (s, 3H).



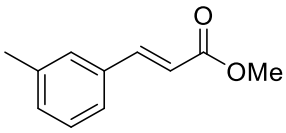
<u>27e</u> Methyl 3-(2-bromophenyl)acrylate: prepared from 2-bromocinnamic acid (2.82 g, 12.44 mmol, 1 eq) according to the general procedure, the reaction was stirred for 16h and then additionally heated at 60°C for 2h. After work-up, a yellow oil was obtained (2.56 g, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 8.05 (d, J = 16.0 Hz, 1H), 7.61 (m, 2H), 7.33 (t, J = 7.5, 1H), 7.23 (td, J = 8.0 Hz, 1.5 Hz, 1H), 6.39 (d, J = 16.0, 1H), 3.83 (s, 3H).



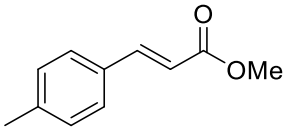
27f Methyl (E)-2-cyano-3-(4-hydroxyphenyl)acrylate: prepared from α-ciano-4-hydroxycinnamic acid (4.65 g, 24.6 mmol, 1 eq) according to the general procedure. After work-up, yellow crystals were obtained (0.281 g, 6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.97 (d, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 8.7 Hz, 2H), 3.92 (s, 3H). The aqueous phase was then acidified with HCl 1M (50 mL, pH 5) and extracted with EtOAc (2 x 20 mL) to recover the unconverted starting material (2.494 g recovered). ^{1}H NMR (400 MHz, MeOD) δ 8.19 (s, 1H), 7.96 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 2H).



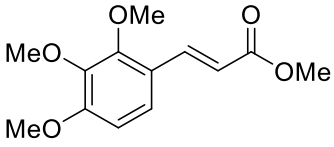
27g Methyl 3-(o-tolyl)acrylate: prepared from 2-methylcinnamic acid (10 g, 61.6 mmol, 1 eq) according to the general procedure. After work-up and solvent evaporation, a yellow oil was obtained (10 g, 96.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 16.0 Hz, 1H), 7.57 (m, 1H), 7.30 (m, 1H), 7.24 (t, J = 7 Hz, 2H), 6.39 (d, *J* = 16.0 Hz, 1H), 3.84 (s, 3H).



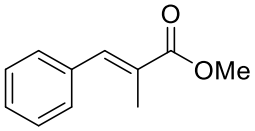
27h Methyl 3-(m-tolyl)acrylate: prepared from 3-methylcinnamic acid (1.5 g, 9.25 mmol, 1 eq) according to the general procedure. After work-up, a white oil was obtained (1.41 g, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 7.68 (d, J = 16.0 Hz, 1H), 7.33 (m, 2H), 7.27 (t, J = 7.5 Hz, 1H), 7.20 (m, 1H), 6.43 (d, J = 16.0, 1H), 3.80 (s, 3H).



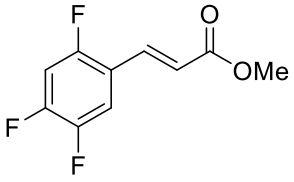
27i Methyl 3-(p-tolyl)acrylate: prepared from 4-methylcinnamic acid (4.6 g, 28.38 mmol, 1 eq) according to the general procedure. After work-up, white crystals were obtained (4.48 g, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 15.9 Hz, 1H), 7.42 (d, *J* = 7.9 Hz, 2H), 7.19 (d, *J* = 7.8 Hz, 2H), 6.40 (d, *J* = 16.0 Hz, 1H), 3.80 (s, 3H), 2.38 (s, 3H).



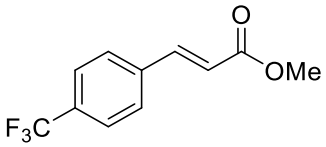
27j Methyl (E)-3-(2,3,4-trimethoxyphenyl)acrylate: prepared from 2,3,4-trimethoxycinammic acid (1.4 g, 5.94 mmol, 1 eq) according to the general procedure. After work-up, a white oil was obtained (1.1, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.89 (dd, J = 2.0 Hz, 16.0 Hz, 1H) 7.26 (dd, J = 9.0 Hz, 2.0 Hz, 1H), 6.70 (dd, J = 9.0 Hz, 2.0 Hz), 6.43 (dd, J = 16.00 Hz, 2.0 Hz), 3.92 (s, 3H), 3.89 (s, 3H), 3.87 (s, 3H), 3.80 (s, 3H).



27k Methyl 2-methyl-3-phenylacrylate: prepared from α-methylcinnamic acid (4.6 g, 28.38 mmol, 1 eq) according to the general procedure. After work-up, white crystals were obtained (4 g, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.40 (d, *J* = 4.3 Hz, 4H), 7.33 (dq, *J* = 8.7, 4.3 Hz, 1H), 3.82 (s, 3H), 2.12 (d, *J* = 1.1 Hz, 3H).



27l Methyl 3-(2,4,5-trifluorophenyl)acrylate: prepared from 2,4,5-trifluorocinnamic acid (3.1 g, 14.7 mmol, 1.0 eq) according to the general procedure. After work-up, a white solid was obtained. (3g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 17.0 Hz, 1H), 7.37 (m, 1H), 7.00 (m, 1H), 6.47 (d, J = 17.0 Hz, 1H), 3.84 (s, 3H).



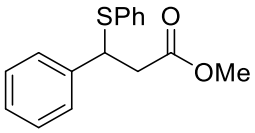
27m Methyl (E)-3-(4-(trifluoromethyl)phenyl)acrylate: prepared from 4-trifluoromethylcinnamic acid (15 g, 69.4 mmol, 1 eq) according to the general procedure. After work-up, a white solid was obtained (15 g, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 16.1 Hz, 1H), 7.67 – 7.59 (m, 4H), 6.51 (d, *J* = 16.0 Hz, 1H), 3.83 (s, 3H).

5.2 Synthesis of sulfides

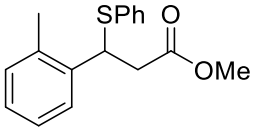
5.2.1 From α,β-unsaturated compounds

General procedure for β-sulfidoesters:

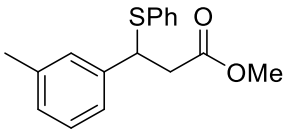
To a 100 mL round bottom flask was added cinnamate ester (1.0 equiv) followed by thiophenol (2.0 equiv.) and triethylamine (1.0 equiv) in one portion and the reaction mixture was stirred at rt until consumption of the cinnamate was observed by TLC (12-24 h). After consumption of cinnamate was confirmed by TLC, water (20 mL) was added to quench the reaction. The mixture was then extracted with EtOAc or DCM (2 x 20 mL), the collected organic phase was washed with brine (1 x 20 mL), dried over sodium sulfate, concentrated *in vacuo*. The residue obtained (oil or crystals) was analyzed by ^{1}H NMR. The crude product was purified by flash chromatography using a gradient elution system with petroleum ether and EtOAc (PE 100% → PE:EtOAc = 8:2).



34 Methyl 3-phenyl-3-(phenylthio)propanoate: prepared from methyl cinnamate (10 g, 61 mmol, 1 eq) according to the general procedure. After flash chromatography, a yellowish oil was obtained (13.8 g, 83% yield). ^{1}H NMR (400 MHz, MeOD) δ 7.36 – 7.13 (m, 10H), 4.64 (t, *J* = 7.8 Hz, 1H), 3.55 (s, 3H), 2.93 (d, *J* = 7.8 Hz, 2H).

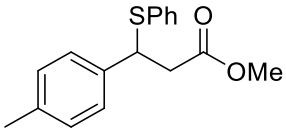


29a Methyl 3-(phenylthio)-3-(o-tolyl)propanoate: prepared from methyl 2-methylcinnamate (2 g, 11.3 mmol, 1 eq) according to the general procedure. After flash chromatography, a yellowish oil was obtained (1 g, 31% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (dt, *J* = 8.5, 3.7 Hz, 2H), 7.26 (s, 3H), 7.18 – 7.09 (m, 4H), 4.87 (t, *J* = 7.7 Hz, 1H), 3.57 (s, 3H), 2.98 (d, *J* = 7.7 Hz, 2H), 2.41 (s, 3H).

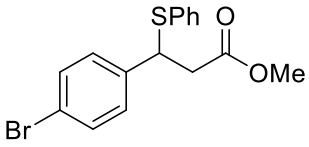


29b Methyl 3-(phenylthio)-3-(m-tolyl)propanoate: prepared from methyl 3-methylcinnamate (1.4 g, 7.94 mmol, 1 eq) according to the general procedure. To consume the starting material, additional 1 equivalent of triethylamine was added to the

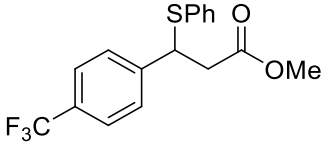
mixture and additional stirring for 24h at room temperature. After flash chromatography a yellow oil was obtained (1.08 g, 90 % yield) ^{1}H NMR (400 MHz, $CDCl_3$): 7.49 (m, 1H), 7.32 (m, 3H), 7.23 (m, 3H), 7.15 (t, J = 7.0 Hz, 1H) 4.62 (t, J = 7.6 Hz, 1H), 3.57 (s, 3H), 2.93 (m, 2H).



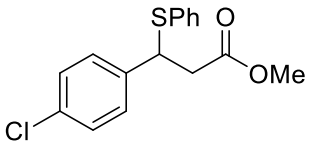
29c Methyl 3-(phenylthio)-3-(p-tolyl)propanoate: prepared from methyl 4-methylcinnamate (2 g, 11.3 mmol, 1 eq) according to the general procedure. After flash chromatography, a yellowish oil was obtained (2.9 g, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.28 (m, 2H), 7.28 – 7.21 (m, 3H), 7.11 (dd, *J* = 30.4, 8.0 Hz, 4H), 4.63 (t, *J* = 7.7 Hz, 1H), 3.58 (s, 3H), 3.03 – 2.79 (m, 2H), 2.31 (s, 3H).



29d Methyl 3-(4-bromophenyl)-3-(phenylthio)propanoate: prepared from methyl 4-bromocinnamate (2 g, 8.3 mmol, 1 eq) according to the general procedure. After flash chromatography, a yellowish oil was obtained (2.75 g, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.4 Hz, 2H), 7.32 – 7.21 (m, 5H), 7.11 (d, *J* = 8.4 Hz, 2H), 4.58 (dd, *J* = 8.5, 7.0 Hz, 1H), 3.60 (s, 3H), 2.91 (qd, *J* = 15.9, 7.7 Hz, 2H).

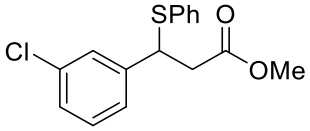


29e Methyl 3-(phenylthio)-3-(4-(trifluoromethyl)phenyl)propanoate: prepared from methyl 4-trifluoromethylcinnamate (10 g, 43.4 mmol, 1 eq) according to the general procedure. The reaction was extremely fast and it was completed in less than 1 hour. After flash chromatography using silica gel and a gradient elution system (PE 100% → PE:AcOEt (8:2)) a white solid was obtained (13g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, J = 8 Hz, 2H), 7.36 (d, J = 8 Hz, 2H), 7.32-7.27 (ovl, 5H), 4.69 (dd, 1H), 3.63 (s, 3H), 2.99 (m, 2H).

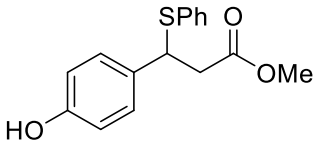


29f Methyl 3-(4-chlorophenyl)-3-(phenylthio)propanoate: prepared from methyl 4-chlorocinnamate (2 g, 10.2 mmol, 1 eq) according to the general

procedure. After flash chromatography, a yellowish oil was obtained (2.5 g, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.27 (m, 2H), 7.27 – 7.20 (m, 5H), 7.16 (d, *J* = 8.5 Hz, 2H), 4.60 (dd, *J* = 8.5, 7.0 Hz, 1H), 3.60 (s, 3H), 2.91 (qd, *J* = 15.9, 7.7 Hz, 2H).



29g Methyl 3-(3-chlorophenyl)-3-(phenylthio)propanoate: prepared from methyl 3-chlorocinnamate (2.20 g, 11.2 mmol, 1 eq) according to the general procedure. To consume the starting material, additional 1 equivalent of triethylamine was added to the mixture and additional stirring for 24h at room temperature. After flash chromatography a yellow oil was obtained (1.7 g, 50 % yield) ^{1}H NMR (400 MHz, $CDCl_3$): 7.31-7.24 (m, 6H), 7.19 (m, 2H), 7.10 (m, 1H), 4.59 (t, J = 7.5 Hz, 1H), 3.61 (s, 3H), 2.92 (m, 2H).

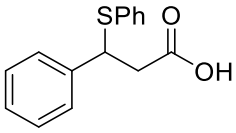


29h Methyl 3-(4-hydroxyphenyl)-3-(phenylthio)propanoate: prepared from methyl 3-(4-hydroxyphenyl)acrylate (3g, 16.8 mmol, 1 equiv) according to the general procedure. The reaction was stirred at 50°C for 24h. To consume the starting material, additional 1 equivalent of triethylamine was added to the mixture and additional stirring for 24h at room temperature. After work up, the crude was monitored by H NMR which revealed that there was the 10% of the not consumed starting material. The reaction was kept again in the same conditions, and finally the crude was purified by flash chromatography using silica gel and a gradient elution system (PE 100% → PE:AcOEt = 7:3) to obtain a white solid (3.2 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (m, 1H), 7.24 (m, 2H), 7.12 (m, 2H), 6.72 (m, 2H), 4.60 (dd, 1H), 3.59 (s, 3H), 2.90 (m, 2H).

General procedure for β-sulfidoacids:

To a mixture of cinnamic acid (1.0 equiv) and thiophenol (1.5 equiv), TBAF·3H_2O (0.2 equiv) was added and the mixture was stirred at 50°C in solventless condition for 24 h. The reaction mixture was diluted with water (30 mL) and then extracted twice with dichlorometane (2 x 30 mL). Then, the organic phase was washed twice with brine solution (2 x 30 mL). The organic phase was dried with sodium sulfate, filtered and the solvent was removed in vacuo. The

crude mixture was purified by flash column chromatography on silica gel using a mixture of Petroleum ether and Ethyl Acetate as eluent solvents.

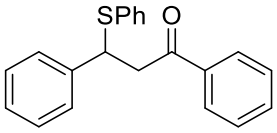


29i 3-phenyl-3-(phenylthio)propanoic acid: prepared from cinnamic acid (3.0 g, 20.2 mmol, 1 eq) according to the general procedure. To consume the starting material, an additional 1 equivalent of $TBAF{\cdot}3H_2O$ was added and the reaction was stirred for additional 24h. After flash chromatography using silica gel and a gradient elution system (PE 100% → PE:AcOEt 6:4) white crystals were obtained (2.0 g, 40 % yield) ^1H NMR (400 MHz, $CDCl_3$): 7.32 (ovl, 2H), 7.26 (ovl, 8H), 4.63 (t, J = 7.5 Hz, 1H), 3.00 (m, 2H).

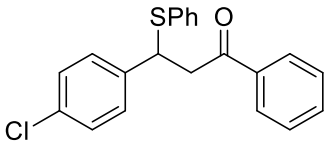
General procedure for sulfidophenylketones:

To a stirred solution of chalcone (1 equiv) in CH_2Cl_2 were added thiophenol (1.1 equiv) and triethylamine (0.1 equiv) and the reaction mixture was stirred at rt until complete consumption of the starting material was observed by TLC analysis (1 h).

The solvent was removed in vacuo and the crude reaction mixture was purified by recrystallization from hot MeOH (ca. 100 mL).



29j 1,3-diphenyl-3-(phenylthio)propan-1-one: prepared from Chalcone (5 g, 24.0 mmol, 1 eq) according to the general procedure. After recrystallization from hot MeOH (ca. 500 mL) a white solid was obtained (4.5 g, 60% yield)

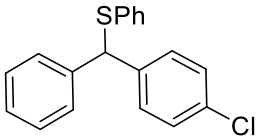


29k 3-(4-chlorophenyl)-1-phenyl-3-(phenylthio)propan-1-one: prepared from Chlorochalcone (5 g, 20.6 mmol, 1 eq) according to the general procedure. After recrystallization from hot MeOH (ca. 500 mL) a white solid was obtained (3.0 g, 50% yield).

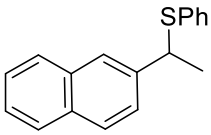
5.2.2 From secondary and tertiary alcohols

General procedure for aryl/alkyl sulfides:

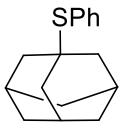
ZnI_2 (0.5 equiv) was placed in a Schlenk tube and carefully dried under vacuum with a heatgun. Under N2 atmosphere, dry CH2Cl2 (10 mL) and the alcohol (3.0 mmol, 1 equiv) were added to the solid. Thiophenol (1.2 equiv) was added to the obtained suspension and the mixture was stirred at rt until complete consumption of the starting material, as observed by TLC analysis (2-7 h). The reaction mixure was quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (2 x 10 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo to yield the crude product which was purified by flash column chromatography on silica gel.



31a ((4-chlorophenyl)(phenyl)methyl)(phenyl)sulfane: Prepared from (4-chlorophenyl)(phenyl)methanol (5.5 g, 25.2 mmol, 1.0 eq) according to the general procedure. After flash chromatography using silica gel and a gradient elution system (PE 100% → PE:AcOEt 8:2) a white solid was obtained (5.9 g, 75 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.10 (m, 14H), 5.42 (s, 1H).

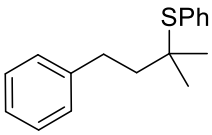


31c (1-(naphthalen-2-yl)ethyl)(phenyl)sulfane: Prepared from 1-(naphthalen-2-yl)ethanol (5.5 g, 31.9 mmol, 1.0 eq) according to the general procedure. After flash chromatography using silica gel and a gradient elution system (PE 100% → PE:AcOEt 8:2) a white solid was obtained (6.1 g, 72 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (m, 2H), 7.64 (m, 1H), 7.44 (d, J = 7 Hz, 1H), 7.23 (m, 2H), 7.34 (m, 2H) 7.20 (m, 2H), 7.08 (m, 3H), 4.40 (q, 1H), 1.62 (d, J = , 3H).



31g adamantan-1-yl(phenyl)sulfane: prepared from adamantan-1-ol according to the general procedure. After flash chromatography using silica gel and a gradient elution system (PEt 100% → PEt:AcOEt = 9:1) a white solid was obtained (4.5 g, 70%). ^{1}H NMR

(400 MHz, $CDCl_3$): 7.55 (m, 2H), 7.50 (m, 2H), 7.32 (m, 3H), 2.00 (ovl, 3H), 1.81 (ovl, 6H), 1.62 (q, 7H).

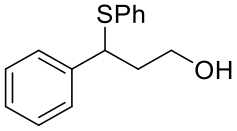


31f (2-methyl-4-phenylbutan-2-yl)(phenyl)sulfane: prepared from 2-methyl-4-phenylbutan-2-ol according to the general procedure. After flash chromatography using silica gel and a gradient elution system (PEt 100% → PEt:AcOEt = 9:1) a yellowish oil was obtained (2 g, 64%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.55 (m, 2H), 7.34 (m, 3H), 7.26 (m, 2H), 7.18 (m, 3H), 2.82 (m, 2H), 1.77 (m, 2H), 1.31 (s, 6H). ^{13}C NMR: 142.4, 137.5, 132.2, 128.7, 128.5, 128.4, 128.37, 125.7, 49.2, 44.2, 31.3, 28.9.

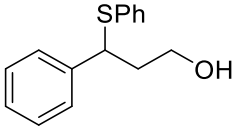
5.3 Reduction

General procedure for sulfidoalcohols:

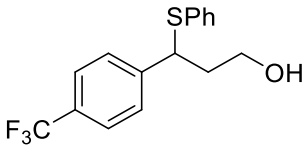
To a stirred solution of previously synthesized β-sulphidoester (2.0 mmol) in dry THF (4.0 mL) was added $LiAlH_4$ (0.5 mL, 2.0 M solution in THF, 1.0 mmol, 0.5 equiv) dropwise with stirring at 0 °C. The reaction mixture was warmed to rt and stirred until consumption of the starting material was confirmed by TLC. The solution was carefully quenched with sat. aq. NH_4Cl (20 mL) and extracted with Et_2O (3 x 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent removed *in vacuo* to obtain the corresponding alcohol, which was used without further purification.



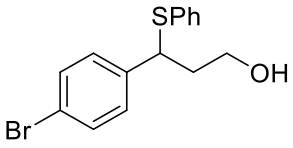
35 3-phenyl-3-(phenylthio)propan-1-ol: prepared from methyl 3-phenyl-3-(phenylthio)propanoate (5 g, 18.36 mmol) according to the general procedure. After work-up, an orange solid was obtained (4.1 g, impure). ^{1}H NMR in $CDCl_3$ was recorded to confirm the presence of the product and this crude was used for the next step without further purification.



35 **3-phenyl-3-(phenylthio)propan-1-ol:** prepared from 3-phenyl-3-(phenylthio)propanoate (6.0 g, 22.03 mmol, 1 eq) according to the general procedure. After flash chromatography using silica gel and a gradient elution system (DCM 100% → DCM:Methanol 95:5) a white solid was obtained (4.3 g, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.22 (m, 2H), 4.39 (dd, 1H), 3.77 (m, 1H), 3.61 (m, 1H), 2.26 (m, 1H), 2.17 (m, 1H), 1.72 (bs, 1H).



3-(phenylthio)-3-(4-(trifluoromethyl)phenyl)propan-1-ol: prepared from methyl 3-(phenylthio)-3-(2,4,5-trifluorophenyl)propanoate. (7.0 g, 20.5 mmol, 1 eq) according to the general procedure. After flash chromatography using silica gel and a gradient elution system (PE:AcOEt 95:5 → PE:AcOEt 6:4) to obtain a white solid (3.3 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, J = 8.3, 2H), 7.34 (d, J = 8.3, 2H), 7.21 (ovl, 5H), 4.41 (t, J = 7.8, 1H), 3.81 (m, 1H), 3.61 (m, 1H), 2.24 (m, 1H), 2.13 (m, 1H).



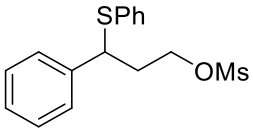
3-(4-bromophenyl)-3-(phenylthio)propan-1-ol: prepared from methyl 3-(4-bromophenyl)-3-(phenylthio)propanoate (5.0 g, 14.2 mmol, 1 eq) according to the general procedure. After work-up a white solid was obtained (3.6 g, impure). ^{1}H NMR in $CDCl_3$ was recorded to confirm the presence of the product and this crude was used for the next step without further purification.

5.4 Mesylation

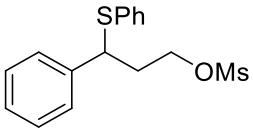
General procedure for sulfidomesylates:

To a stirred solution of the sulfidoalcohol (1.5 mmol) and methanesulphonyl chloride (1.4 equiv) in CH_2Cl_2 (5 mL) was added dropwise triethylamine (1.4 equiv) at 0 °C. The reaction mixture was allowed to reach rt and stirred until consumption of the starting material was observed by TLC analysis. The reaction mixture was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with H_2O (3 x 20 mL),

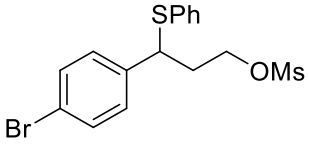
brine and dried over Na_2SO_4. Finally, the solvent was removed in vacuo and the crude obtained need further purification.



<u>36</u> 3-phenyl-3-(phenylthio)propyl methanesulfonate: prepared from 3-phenyl-3-(phenylthio)propan-1-ol (impure, 4.1 g, 16.8 mmol) according to the general procedure. After work-up, an orange-brown oil was obtained (5.34 g, impure). ^{1}HNMR in $CDCl_3$ was recorded to confirm the presence of the product and this crude was used in the next step without further purification.



<u>36</u> 3-phenyl-3-(phenylthio)propyl methanesulfonate: prepared from 3-phenyl-3-(phenylthio)propan-1-ol (7.4 g, 30.3 mmol, 1 eq) according to the general procedure. After flash chromatography using silica gel and a gradient elution system (PE:AcOEt = 95:5 → PE:AcOEt = 6:4) an orange-brown oil was obtained (7.5 g, 77 % yield). ^{1}H NMR (400 MHz, $CDCl_3$). 7.24 (ovl, 10H), 4.31 (ovl, 1H), 4.31 – 4.13 (m, 2H), 2.89 (s,3H), 2.39 – 2.32 (m, 2H).



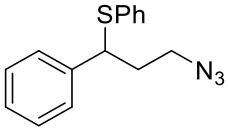
3-(4-bromophenyl)-3-(phenylthio)propyl methanesulfonate: prepared from 3-(4-bromophenyl)-3-(phenylthio)propan-1-ol (3.6g, 11.1 mmol, 1 eq) according to the general procedure. After flash chromatography using silica gel and a gradient elution system (PE 100% → PE:AcOEt = 9:1) an orange-brown oil was obtained (3.9 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.40 (m, 2H), 7.23 (ovl, 5H), 7.10 (m, 2H), 4.35-4.15 (m, 2H), 4.25 (t, *J* = 7.5 Hz, 1H), 2.36 – 2.27 (m, 2H).

5.5 Azidation

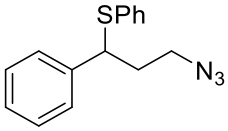
General procedure for sulfidoazides:

The sulfidomesylate previously prepared (1 equiv) was dissolved in DMF (5 mL) and placed in a 100 mL round bottom flask with a magnet under N_2 atmosphere. Then NaN3 was added in one portion into the mixture under N_2 atmosphere. The mixture was stirred at rt and

monitored by TLC until complete conversion (18h) and then was quenched by being poured into ice-cold water. The mixture was extracted with EtOAc (3x30 mL), then the combined organic layers were washed with water (3x30 mL) and brine (3x30 mL) and dried over $MgSO_4$ overnight. After solvent evaporation under vacuum, a yellow oil was obtained, which was analyzed by HNMR. The crude product was purified by flash chromatography using a gradient elution system with petroleum ether and EtOAc.



37 (3-azido-1-phenylpropyl)(phenyl)sulfane: prepared from 3-phenyl-3-(phenylthio)propyl methanesulfonate (impure, 0.35 g, 1 mmol, 1 equiv) according to the general procedure. After work-up, a yellow oil was obtained, which was analyzed by HNMR confirming the presence of the desired product and the absence of major byproducts.



37 (3-azido-1-phenylpropyl)(phenyl)sulfane: prepared from 3-phenyl-3-(phenylthio)propyl methanesulfonate (4.0 g, 12.4 mmol, 1 eq) according to the general procedure. After flash chromatography using silica gel and a gradient elution system (PE 100% → PE:AcOEt 95:5) a yellow oil was obtained (2.3 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.21 (m, 10H), 4.25 (dd, 1H), 3.41 (m, 1H), 3.22 (m, 1H), 2.22 (m, 1H), 2.13 (m, 1H).

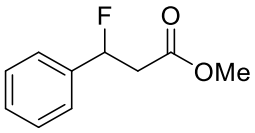
5.6 Desulfurative fluorination

General Procedure for fluorides:

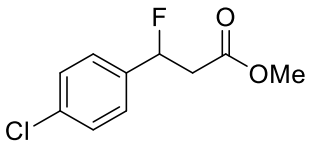
In a 15 mL plastic falcon vial, $AgNO_3$ (0.4 equiv) was added together with two small magnets. Then, $AgNO_3$ was dissolved into 750 μL of dry dichloromethane under N_2 atmosphere. Under vigorous stirring ensured by the two magnets and at room temperature, bromine (0.2 equiv) was added via syringe. The reaction mixture turned from colorless to dark orange. After that, PPHF (0.1 mL) is added in an excess amount via syringe to the mixture whose color changed from dark orange to turbid light orange within 10-15 minutes. The β-sulfidoester (0.2 mmol, 1 equiv) is separately dissolved in dry dichlorometane and

added to the reaction mixture via syringe. Then, within 8 minutes, the reaction color changed from turbid light orange to white milk.

The reaction mixture was then diluted with water and extracted three times with fresh dichloromethane. The organic phase was directly transferred to a round bottom flask by using a funnel enriched with cotton and Na_2SO_4. The solvent was removed in vacuo and the ^1H NMR and ^{19}F NMR were collected to verify the presence of the fluorinated product. The yield was determined by HNMR spectroscopy using 1,3,5-Trimethoxybenzene (0.2 mL) as internal standard (-6.1 ppm).



42a Methyl 3-fluoro-3-phenylpropanoate: Prepared from methyl 3-phenyl-3-(phenylthio) propanoate (54.4 mg, 0.2 mmol 1.0 equiv) according to the general procedure. After 7 minutes the title compound was obtained in 86.6% yield.
Benzyl Fluoride C–H Shift: ^1H-NMR (400 MHz, $CDCl_3$): δ = 5.93 (ddd, J = 46.9, 9.1,4.1 Hz). Benzylic Fluoride Shift: ^{19}F NMR (376 MHz, $CDCl_3$) δ -173.12 (ddd, J = 46.5, 32.6, 13.6 Hz). The spectral data are in accordance with those reported in literature (https://doi.org/10.1021/ja410815u J. Am. Chem. Soc. 2013, 135, 46, 17494–17500)



42b Methyl 3-(4-chlorophenyl)-3-fluoropropanoate: prepared from methyl 3-(4-chlorophenyl)-3-(phenylthio) propanoate (61.2 mg, 0.2 mmol 1.0 equiv) according to the general procedure. After 8 minutes the title compound was obtained in 78.7% yield. ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, J = 8.3 Hz, 2H), 7.30 (dd, J = 4.6, 4.1 Hz, 2H), 5.89 (ddd, J = 46.6, 8.8, 4.5 Hz, 1H), 3.73 (s, 3H), 3.02 (ddd, J = 16.0, 13.7, 8.8 Hz, 1H), 2.78 (ddd, J = 30.9, 16.0, 4.5 Hz, 1H). ^{19}F NMR (376 MHz, $CDCl_3$) δ -173.41 – -173.73 (m).
Benzyl Fluoride C–H Shift: 1H-NMR (400 MHz, $CDCl_3$): δ = 5.89 (ddd, J = 46.6, 8.7, 4.4 Hz). Benzylic Fluoride Shift: ^{19}F-NMR (282 MHz, $CDCl_3$): δ = –173.5 (m).

Bibliography

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