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# *HETEROCYCLIC ARCHITECTURE FOR THE SYNTHESIS OF NOVEL ANTI-HIV PROTEASE AND ANTI-CANCER COMPOUNDS*

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# ABBREVIATIONS

| | |
|---|---|
| **AcOEt** | Ethyl Acetate |
| **$Ac_2O$** | Acetic anhydride |
| **Bn** | Benzyl- |
| **$CDCl_3$** | Deuterated chloroform |
| **CPME** | Cyclopentyl methyl ether |
| **DPPT** | 1,1'-bis (diphenylphosphino) ferrocene |
| **DEAD** | Diethyl azodicarboxylate |
| **DMF** | *N*,*N*-Dimethylformamide |
| **DMSO** | Dimethyl sulfoxide |
| **EDC** | 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide |
| **EP** | Petroleum Ether |
| **$Et_2O$** | Diethyl ether |
| **$Et_3N$** | Triethylamine |
| **FRET** | Fluorescence resonance energy transfer |
| **GC-MS** | Gas Chromatography Mass Spectrometry |
| **HRMS** | High resolution mass spectrometry |
| **HOBt** | Hydroxybenzotriazole |
| **$IC_{50}$** | Half Maximal Inhibitory Concentration |
| **MeCN** | Acetonitrile |
| **NBS** | N-Bromosuccinimide |
| **NIS** | N-Iodosuccinimide |
| **NMR** | Nuclear Magnetic Resonance |
| **PIs** | HIV Protease inhibitors |
| **Py** | Piridine |
| **rt** | Room Temperature |
| ***t*-AmOK** | Potassium *tert*-Amylate |
| ***t*-BuOK** | Potassium *tert*-Butoxide |
| **TBS** | *tert*-butyldimethylsilyl ether |
| **TBDMS** | *tert*-butylchlorodimethylsilane |
| **TES** | Triethylsilane |
| **TFA** | Trifluoroacetic acid |
| **THF** | Tetrahydrofuran |
| **TLC** | Thin Layer Cromatography |
| **TMS** | Trimethyl silane |
| **$TMSCHF_2$** | Difluoromethyltrimethyl silane |

# CHAPTER 1:
# INTRODUCTION

## *1.1 Heterocycles in medicinal chemistry*

Small-molecule amides or carbamates represent an important class of biologically active molecules, but potential use of these molecules as oral drugs is often compromised by their poor physicochemical and pharmacokinetic properties, largely due to the metabolic instability toward enzymatic degradation and the high polarity. Introduction of heterocyclic moieties in a bioactive molecule can have important effects on physicochemical and pharmacological properties[1]. Thus, this strategy has been widely adopted as a fundamental tactical approach in medicinal chemistry for the design of new drugs, because of their chemical stability and structural rigidity, allowing less entropic energy to be lost upon binding. All these characteristics may lead to compounds with improved potency, selectivity, metabolic stability, and better pharmacokinetic properties, improving bioavailability and aqueous solubility[2], thanks to the number of rotatable bonds[3] and the polarity. Moreover, the possibility of H-bond interactions with target backbone atoms can increase their efficacy[4]. The success of this strategy in identifying new biologically active molecules in distinct therapeutic areas has gained a significant growth and different heterocycles are introduced in biological molecules[5].

Heterocyclic compounds occur widely in nature and many of them are important in biological processes. We find heterocyclic rings in vitamins, coenzymes, porphyrins (like hemoglobin), DNA, RNA. Nitrogen containing heterocyclic rings are the most used molecular fragments in drug design followed by those containing oxygen and sulphur[6,7].

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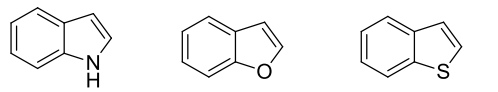
[1] S. Sun, Q. Jia, Z. Zhang, *Bioorganic & Medicinal Chemistry Letters* **2019**, *29*, 2535.
[2] a) G.A. Patani, EJ. LaVoie, *Chem Rev.* **1996**, *96*, 3147. b) NA. Meanwell, *J Med Chem.* **2011**, *54*, 2529.
[3] D.F. Veber, S.R. Johnson, H.Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, *J. Med. Chem.* **2002**, *45*, 2615.
[4] A.K. Ghosh, B.D. Chapsal, I.T. Weber, H. Mitsuya, *Accounts of chemical research* **2008**, *41*, 78.
[5] J. Sangshettia, S.K. Pathan, R.Patil, S.A. Ansari, S. Chhajed, R. Arote, D.B. Shind, *Bioorganic & Medicinal Chemistry* **2019**, *27*, 3979**.**
[6] L. da S.M. Forezi, M.F.C. Cardoso, D.T.G. Gonzaga, F. de C. da Silva, V.F. Ferreira, *Current Topics in Medicinal Chemistry* **2018**, *18*, Issue 17**.**
[7] L.D. Quin, J.A. Tyrell, *The scope of the field of heterocyclic chemistry* in *Fundamentals of Heterocyclic Chemistry*, Wiley, Hoboken, NJ, USA, 1-5, **2010**.

In particular organic scaffolds containing heterocycles, like 1,4-dihydropyridine[8], benzopyran[9], pyranocoumarin[10], isoxazole[11], benzazepinone[12], biphenyltetrazole[13], 4-substituted piperidine[14], indole[15], and others have been described as "privileged structures" since they are capable of binding to many receptors with high affinity[16]. Noteworthy the most know chemicals are based on heteroaryl frameworks[17,18]. To medicinal chemists, the true utility of these "privileged structures" is the ability to synthesize one library based upon one *core* scaffold and screen it against a variety of different receptors, yielding several active compounds[19].

Privileged substructures would appear to be dominated by natural products or natural product derivatives, so construction of heterocyclic moieties, in particular heteroarenes, is of considerable importance in organic chemistry in order to use them as ideal scaffolds for drug development. Furthermore, the substituents attached to the scaffold may be responsible for its specificity. Therefore, right selection and placement of the substituents on the scaffold would be of paramount importance.

A wide range of fused [5,6] ring systems exhibits biological activity. In particular fused [5,6] ring systems, used in the synthetic paths discussed in this work, include indole, benzofuran and benzothiophene (figure 1.1).




*indole* *benzofuran* *benzothiophene*

**Figure 1.1**

[8] K.A. Jacobson, Y.-C. Kim, B.F. King, *J. Auton. Nerv. Syst.* **2000**, *81*, 152.
[9] K.C. Nicolaou, A.J. Roecker, S. Barluenga, J.A. Pfefferkorn, G.Q. Cao, *ChemBioChem*. **2001**, *2*, 460.
[10] K.C. Nicolaou, J.A. Pfefferkorn, S. Barluenga, H.J. Mitchell, A.J. Roecker, G.Q. Cao, *J. Am. Chem. Soc.* **2000**, *122*, 9968.
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[12] A.A. Patchett, R.P. Nargund, *Annu. Rep. Med. Chem.* **2000**,*35*, 289.
[13] J.S. Mason, I. Morize, P.R. Menard, D.L. Cheney, C. Hulme, R.F. Labaudiniere, *J. Med. Chem.* **1999**, *42*, 3251.
[14] C. Zhou, L. Guo, G. Morriello, A. Pasternak, Y. Pan, S.P. Rohrer, E.T. Birzin, S.E.W. Huskey, T. Jacks, K.D. Schleim, K. Cheng, J.M. Schaeffer, A.A. Patchett, L. Yang, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 415.
[15] J.F. Austin, D.W.C. MacMillan, *J. Am. Chem. Soc.* **2002**, *124*, 1172.
[16] D.A. Horton, G.T. Bourne, M.L. Smythe, *Chem. Re*v. **2003**, *103*, 893.
[17] C.A. Rouzer, D. Riendeau, J-P Falgueyret, C.K. Lau, M.J. Gresser, *Biochem Pharmacol* **1991**, *41*, 1365.
[18] T.R. Bosin, E.E. Campaigne, *Drug. Res.* **1977**, *11*, 191.
[19] L.A. Thompson, J.A. Ellman, *Chem. Rev.* **1996**, *96*, 555.

Indole ring is one of the most occurred heterocyclic structures in nature[20]. As a result, it is not surprising that a number of frameworks based upon this type of structure have been used as the core scaffold in combinatorial libraries. In the same way, molecules containing the benzofuryl scaffold possess a wide range of biological activities, as antifungals[21], analgesics[22], antioxidants[23], and it has been appended to other scaffolds to form molecules that have antimitotic[24] or antipsychotic[25] activity. Last but not least, benzothiophenes are also privileged substructures that are closely related to the indole ring. Molecules with this scaffold have antiviral[26] or antimitotic[27] activity.

[20] a) E. Sanders-Bush, S.E. Mayer, in *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th ed.; R.W. Ruddon, Ed.; McGraw-Hill: New York, p 249, **1996**. b) A.L. Smith, G.I. Stevenson, C.J. Swain, J.L. Castro, *Tetrahedron Lett.* **1998**, *39*, 8317. c) H. Wang, A. Ganesan, *Org. Lett* **1999**, *1*, 1647.
[21] R.A. Fecik, K.E. Frank, E.J. Gentry, L.A. Mitscher, M. Shibata, *Pure Appl. Chem.* **1999**, *71*, 559.
[22] P.R. Halfpenny, D.C. Horwell, J. Hughes, J.C. Hunter, D.C. Rees, *J. Med. Chem.* **1990**, *33*, 286.
[23] J. Habermann, S.V. Ley, R. Smits, *J. Chem. Soc., Perkin Trans.* **1999**, *1*, 2421.
[24] P. Wipf, J.T. Reeves, R. Balachandran, K.A. Giuliano, E. Hamel, B.W. Day, *J. Am. Chem. Soc.* **2000**, *122*, 9391.
[25] I. van Wijngaarden, C.G. Kruse, J.A.M. van der Heyden, M.T.M. Tulp, *J. Med. Chem.* **1988**, *31*, 1934.
[26] S.L. Boulware, J.C. Bronstein, E.C. Nordby, P.C. Weber, *Antiviral Res.* **2001**, *51*, 111.
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## *1.2 Heterocycles in HIV inhibitors: background*

For several years, in the laboratory where I did my PhD work, research has been focused on the introduction of heteroaryl structures into molecules with potential activity against the HIV virus. In particular, the study concerned the synthesis of compounds able to block the action of the HIV protease (HIV-Pr), an essential enzyme for the production of mature HIV particles and plays a key role in maintaining infectivity[28]. When a protease inhibitor binds the active site, it prevents cleavage of nascent viral proteins, thereby halting viral replication[29]. The combination therapy of HIV protease inhibitors, reverse transcriptase inhibitors, and/or an integrase inhibitor, referred to as highly active antiretroviral therapy (HAART), is the current most effective AIDS therapy. The AIDS-related mortality has dropped sharply, and AIDS has gradually become a controllable, chronic disease[30]. HIV protease inhibitor is one of the most important components in the combination therapy.

The critical element is the size of the heterocycle moieties relative to the overall molecule; the structure must be a central or crucial component. Detailed knowledge of the structure of HIV protease and its substrate has led to the preparation of specific PIs[31]. In particular, issue of the study is to introduce heterocycle moieties to the central hydroxyethylamine *core* that the structure of HIV-Pr inhibitors mimicking the transition-state for amide hydrolysis. The use of transition-state mimics as non-hydrolysable amide isosteres to replace a specific scissile peptide bond is a well-known approach to overcome one of the major drawbacks in the use of peptides as medical agents, i.e. the rapid degradation by peptidases[32]. A transition state mimic is defined as a functional group that can mimic the tetrahedral transition state of amide bond hydrolysis, but it is not hydrolyzed by the enzyme.

Catalytic machanism of HIV protease is depicted in figure 1.2 and the transition state is circled in red.

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[28] A.G. Tomasselli, R.L. Heinrikson, *Biochim. et Biophys. Acta* **2000**, *1477*, 189–214.
[29] A. Brik, C.H. Wong, *Org. Biomol. Chem*. **2003**, *1*, 5.
[30] Z**.** Lv Z., Y. Chu, Y. Wang, *Research and Palliative Cure* **2015**, *7*, 95.
[31] A.M.J. Wensing, N.M. Van Maarseveen, M. Nijhuis, *Antiviral Res.* **2010**, *85*, 59.
[32] a) E. De Clercq, *J Med Chem.* **1995**., *38*, 2491. b) MG. Bursavich , DH. Rich, *J Med Chem.* **2002**, *45*, 541. c) Y.S. Tsantrizos, *Acc Chem Res.* **2008**, *41*, 1252.

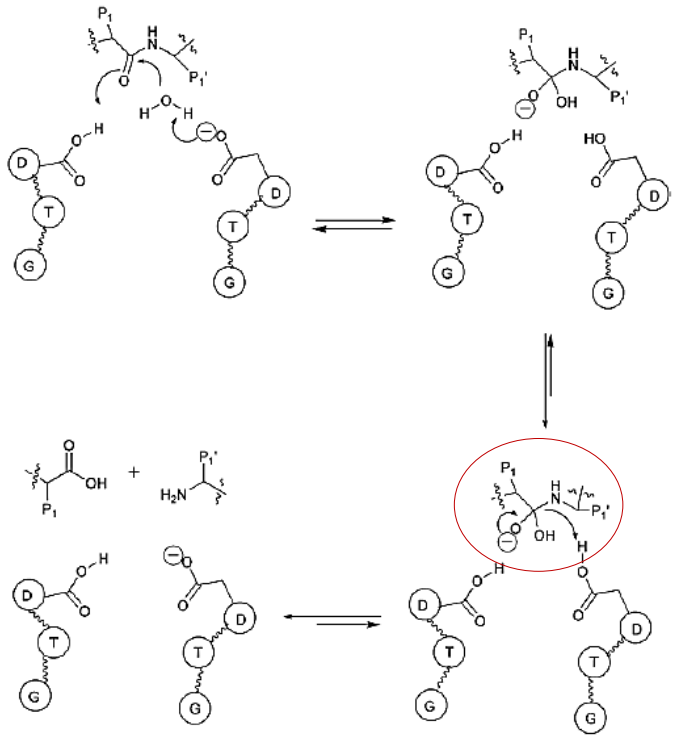
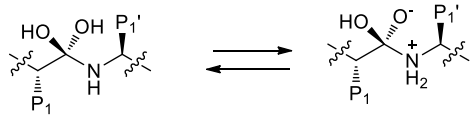


**Figure 1.2** Catalytic mechanism of HIV-Protease

Some transition state mimics that have been successfully used in the design of protease inhibitors are depicted in figure 1.3[1].



Transition-state intermediates of amide hydrolysis

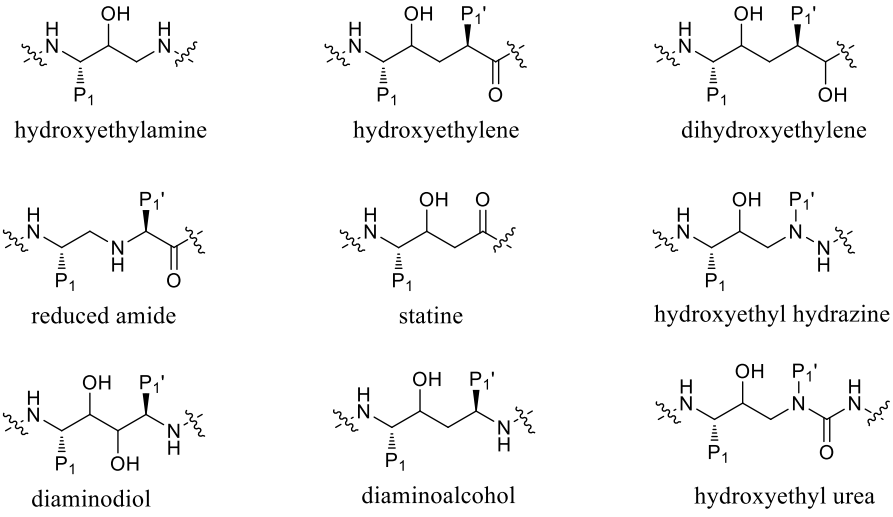
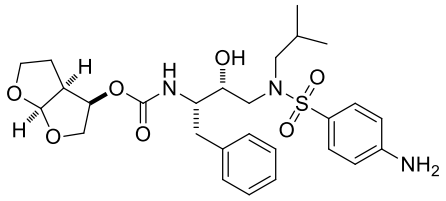



**Figure 1.3** Selected transition-state (TS) mimics

Most of HIV-Pr inhibitors approved by the Food and Drug Administration and commercially available bear a central hydroxyethylamine *core.* Due to the rapid genomic evolution of the HIV, an inevitable consequence in the treatment of the infection is the rise of drug resistance and therefore the dramatic reduction of the commercial inhibitors efficiency. Thus, the emergence of highly mutated viral strains cross-resistant to antivirals prompted to seek novel PIs, desirably with alternative frameworks. For our research the focus was on the synthesis of analogs of the Darunavir (figure 1.4), the last one approved[33]. It is actually the only one active against mutated virus and it shows a double inhibitory activity: inhibition of the enzyme's dimerization and of the proteolytic activity[34].

[33] A.K. Ghosh, Z.L. Dawson, H. Mitsuya, *Bioorg. Med. Chem.* **2007**, *15*, 7576.
[34] a) A.K. Ghosh, K.V. Rao, P.R. Nyalapatla, S. Kovela , M. Brindisi, H.L. Osswald, B.S. Reddy, J. Agniswamy, Y.F. Wang, M. Aoki, S.I. Hattori, I.T. Weber, H. Mitsuya, *Chem Med Chem* **2018**, *13*, 803. b) A.K. Ghosh, J.N. Williams, R.Y. Ho, H.M. Simpson, S.I. Hatton, H. Hayashi, J. Agniswamy, Y.F. Wang, I.T. Weber, *J. Med. Chem.* **2018**, *61*, 9722.

Interestingly, Ghosh showed the potency-enhancing effect of the fused bicyclic tetrahydrofuran (bis-THF) present in the Darunavir structure[35].



**Darunavir**
$IC_{50}$ 1.8 nM

**Figure 1.4**

In fact, protein-ligand X-ray crystal structure indicated that the oxygen atoms of the bicyclic rings of the bis-THF may interact with the backbone atoms. By the structural analysis and comparison of protein–ligand X-ray structures of wild-type and mutant HIV proteases, Ghosh revealed that the active site backbone conformation of mutant proteases is only minimally distorted[36,37].

Thus, an inhibitor which makes not only maximum interactions in the active site of HIV protease, but also extensive hydrogen-bonding interactions with the protein backbone of the wild-type enzyme, will also retain these key interactions and will likely retain potency against the mutant strains, since the mutations cannot easily eliminate the backbone interactions[35]. Our objective is thus focused on designing inhibitors containing heterocyclic moieties that specifically target and maximize these interactions with the backbone. Both extensive hydrogen bonding and hydrophobic interactions with enzyme subsites can limit the protease ability to acquire drug resistance as the geometry of the catalytic site must be conserved to maintain functionality[38].

[35] A.K. Ghosh, B.D. Chapsal, I.T. Weber, H. Mitsuya, *Accounts of Chemical Research* **2008**, *41*, No 1, 78.
[36] L. Hong, X. Zhang, J.A. Hartsuck, J. Tang, *Protein Sci.* **2000**, *9*, 1898.
[37] G.S. Laco, C. Schalk-Hihi, J. Lubkowski, G. Morris, A. Zdanov, A. Olson, J.H. Elder, A. Wlodawer, A. Gustchina, *Biochemistry* **1997**, *36*, 10696.
[38] a) A.K. Ghosh, C.X. Xu, K.V. Rao, A. Baldridge, J. Agniswamy, Y.F. Wang, I.T. Weber, M. Aoki, S.G.P. Miguel, M. Amano, H. Mitsuya, *ChemMedChem* **2010**, *5*, 1850. b) A.K. Ghosh, D.D. Anderson, I.T. Weber, H. Mitsuya, *Angew. Chem.* **2012**, *51*, 1778. c) H. Zhang, Y.F. Wang, C.H. Shen, J. Agniswamy, K.V. Rao, X. Xu, A.K. Ghosh, R.W. Harrison, I.T.J. Weber, *Med. Chem.* **2013**, *56*, 1074.

Another critical issue of current HAART is the poor bioavailability of the PIs[39]. This is responsible for much of the high-dose-related severe side effects and poor compliance issues[40]. Thus, our design of ligands and templates is focused on introduction of cyclic/heterocyclic structures that could extensively interact by strong hydrogen bonding with the backbone atoms in the protease active site and that could improve bioavailability.

Systematic study on Darunavir derivatives started from analogs bearing indole as heteroaryl group, mimicking the aminoindane group of Indinavir[41]. In this investigation the functional group that links indole ring to the central *core* and its position on heterocycle were changed. Different substituted arylsulfonamides were also used in order to evaluate if different electronic properties can have effect on inhibitory activity. These compounds were tested as anti-HIV Pr and results pointed out the general negative effect of the amino linker on the inhibition activity of indole derivatives, whatever the position of the nitrogen on the heterocycle. The best antiviral activity was obtained with compound **A** (figure 1.5). $IC_{50}$ values were obtained by FRET (Fluorescence Resonance Energy Transfer) methodology measuring the initial rates of hydrolysis of the fluorogenic substrate Abz-Thr-Ile-Nle-Phe($NO_2$)-Gln-Arg.

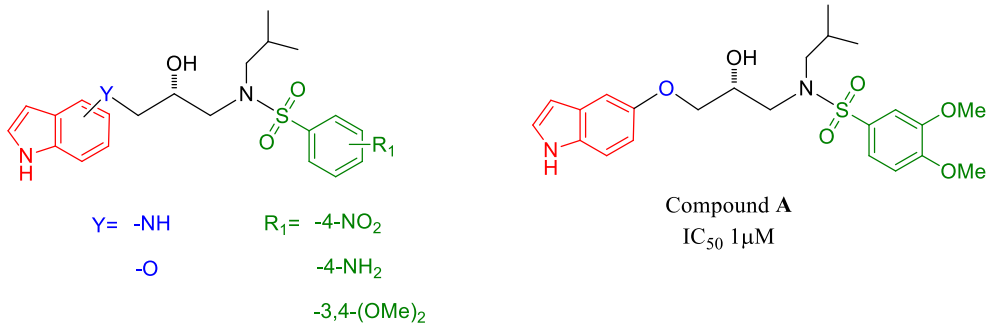
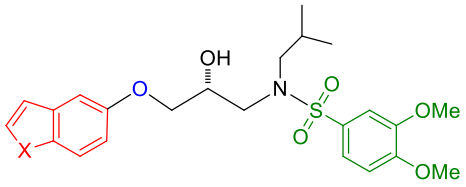



**Figure 1.5** Darunavir analogs synthetized. Compound **A** shows better activity.

Then the study turned to the effect of modification on the indole NH function or the replacement of the heteroaryl group, keeping compound **A** as reference. Thus, nitrogen

[39] a) T. Cihlar, N. Bischofberger, *Med. Chem.* **2000**, *35*, 177. b) K.A. Sepkowitz, *N. Engl. J. Med.* **2001**, *344*, 1764. c) C.W. Flexner, Pharmacology of Drug Interactions of HIV Protease Inhibitors in Protease Inhibitors in AIDS Therapy; R.C. Ogden, C.W. Flexner, Eds. Marcel Dekker: New York, pp 139-160, **2001**.
[40] M.J. Glesby, *Toxicities and Adverse Effects of Protease Inhibitors* in *Protease Inhibitors in AIDS Therapy*; R. C. Ogden, C.W. Flexner, Eds.; Marcel Dekker: New York, 237–256, **2001**.
[41] L. Chiummiento, M. Funicello, P. Lupattelli, F. Tramutola, P. Campaner, *Tetrahedron* **2009**, *65*, 5984.

was protected with methyl or benzyl group and then the heteroatom was switched to oxygen or sulfur in order to check any variation of antiviral activity (figure 1.6). Alkylation at indole nitrogen led to inactive compounds, most likely due to steric reasons, and the replacement of nitrogen with oxygen is not effective; on the contrary the benzothiophene containing inhibitor has shown high potency against wild type HIV protease with $IC_{50}$ = 60 nM, thanks to the lower desolvation penalty to be paid by such hydrophobic moiety[42].

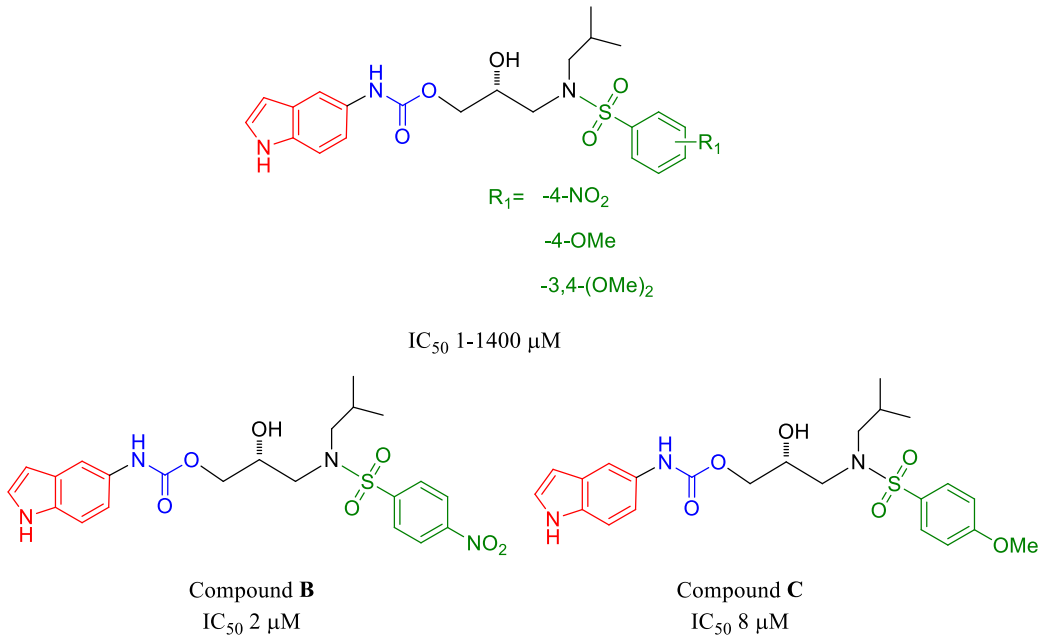



X = NH $IC_{50}$ 1 μM
NMe $IC_{50}$ >1000 μM
NBn $IC_{50}$ >1000 μM
O $IC_{50}$ 130 μM
S $IC_{50}$ 0.06 μM

**Figure 1.6**

Then the effect of a carbamoyl spacer was investigated. Such a moiety was introduced in a reversed way, respect to the reference inhibitor Darunavir. For these compounds (figure 1.7) a general positive effect was noted on the activity, although the biological activity resulted only in the μM range[43] and the best ones were compounds **B** and **C**.

[42] L. Chiummiento, M. Funicello, P. Lupattelli, F. Tramutola, F. Berti, F. Marino-Merlo, *Bioorganic & Medicinal Chemistry Letters* **2012**, *22*, 2948.
[43] C. Bonini, L. Chiummiento, N. Di Blasio, M. Funicello, P. Lupattelli, F. Tramutola, F. Berti, A. Ostric, S. Miertus, V. Frecer, D.X. Kong, *Bioorg Med Chem*. **2014**, *22*, 4792.




Compound **B**
$IC_{50}$ 2 μM

Compound **C**
$IC_{50}$ 8 μM

**Figure 1.7** Carbamoyl spacer between heteroaryl group and central *core.*

For these structures we noted dramatic differences in activity depending on the arylsulfonyl system, the results being somehow in contrast with those obtained for molecules in Figure 1.6. In particular, while 3,4-dimethoxy phenylsulfonyl group was favourable in compound **A**, it failed entirely in the corresponding carbamate. According to computational analysis, carried out at the University of Trieste, the activities of the inhibitors should be determined by the distance between the hydrophobic part of the heteroaryl group and the central carbon atom. For one atom linkers (–O– or –N–), the indole ring lies mostly in a subsite of the enzyme, while for a longer linker as the carbamate one, the heteroaryl group is forced to be accomodated in another subsite. This change has an effect on the interaction of the arylsulfonamide group: shorter linkers match with 3,4-diOMe-phenylsulfonamide group, while longer one better match with either 4-$NO_2$- and 4-OMe-phenylsulfonamide moiety.

In parallel, benzofuryl, indolyl and benzothienyl HIV-Pr inhibitors bearing a carboxyamide spacer were synthesized and the central *core* were modified, with the presence of either H or benzyl as R' substituent (figure 1.8). These inhibitors showed good *in vitro* activity

against native protease, with $IC_{50}$ values in the range of 1–15 nM, denoting a general beneficial effect of carboxyamide moiety. In particular benzofuryl derivatives showed $IC_{50}$ values among the best for such structurally simple inhibitors (compounds **D** and **E**). Docking analysis confirmed the favourable situation of such benzofuryl derivatives in terms of number of interactions in the active site.

These derivatives also showed inhibition activity in mammalian cells, demonstrating their promising potential[44].

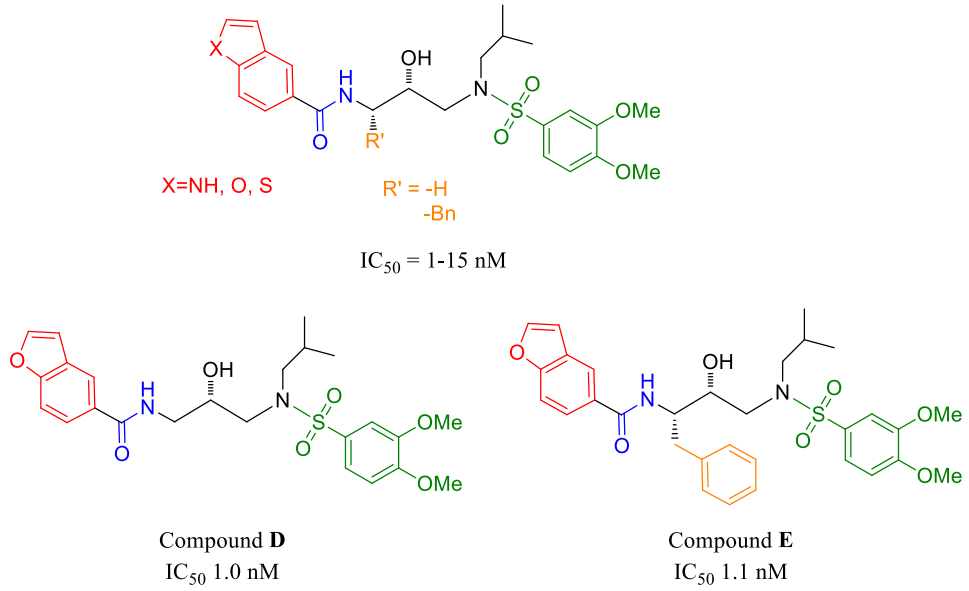



**Figure 1.8** Carboxyamide spacer between heteroaryl group and central *core.*

[44] M. Funicello, L. Chiummiento, F. Tramutola, M.F. Armentano, F. Bisaccia, R. Miglionico, L. Milella, F. Benedetti, F. Berti F., P. Lupattelli, *Bioorganic & Medicinal Chemistry* **2017**, *25*, 4715.

## ***1.3 Heterocycles in biological active compounds: first aims of the project***

Recently, progress in the development of a new class of inhibitors has led to candidates showing clinical promise,[45] especially because there has been growing interest in repurposing PIs for the treatment of cancer.[46] Although the mechanism of antitumor action of such drugs is a matter of debate,[47] early clinical trials employing a PI alone or in combination with radiotherapy have shown promise in treatment of patients with various types of cancer.[48] The use of anti-HIV drugs as cancer treatments is not new. Cancer is a complex disease and it is one of the leading causes of worldwide mortality. Several studies reported that HIV-inhibitors were endowed with anticancer activity; after the success in treatment of HIV-infected patients affected by Kaposi sarcoma with HIV-1 protease inhibitors, the studies were successfully oriented to other solid tumors.[49] Inhibition of tumor-cell invasion and angiogenesis were properties first ascribed to inhibitors of HIV protease.[50] Moreover, PIs have pleiotropic antitumour effects, including inhibition of inflammatory cytokine production, proteasome activity, cell proliferation and survival, and induction of apoptosis. HIV protease inhibitors are thus a new class of anticancer drugs with multiple effects[51].

In particular, literature results showed the HIV protease inhibitors activity against several carcinoma cell lines *in vitro* and *in vivo*, probably due to the presence of the hydroxyethylamine *core*[52]. Specifically, the presence of electron withdrawing group on the sulfonamide ring and the free amino group in the hydroxyethylamine moiety seem to be

[45] P.M. Vadhadiya, M.A. Jean, C. Bouzriba, T. Tremblay, P. Lagüe, S. Fortin, J. Boukouvalas, D. Giguère, *ChemBioChem* **2018**, *19*, 1779.
[46] W.A. Chow, C. Jiang, M. Guan, *Lancet Oncol*. **2009**, *10*, 61.
[47] J.M.W. Wilson, E. Fokas, S.J. Dutton, N. Patel, M.A. Hawkins, C. Eccles, K.Y. Chu, L. Durrant, A.G. Abraham, M. Partridge, M. Woodward, E. O'Neil, T. Maughan, W.G. McKenna, S. Mukherjee, T.B. Brunner, *Radiother. Oncol*. **2016**, *119*, 306.
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[52] V. Esposito, A. Verdina, L. Manente, E.P. Spugnini, R. Viglietti, R. Parrella, P. Pagliano, G. Parrella, R. Galati, A. De Luca, A. Baldi, V. Montesarchio, A. Chirianni, *J Cell Physiol* **2013**, *228*, 640.

important for antitumoral activity (Structure Activity Relationship analysis showed in figure 1.9)[53]

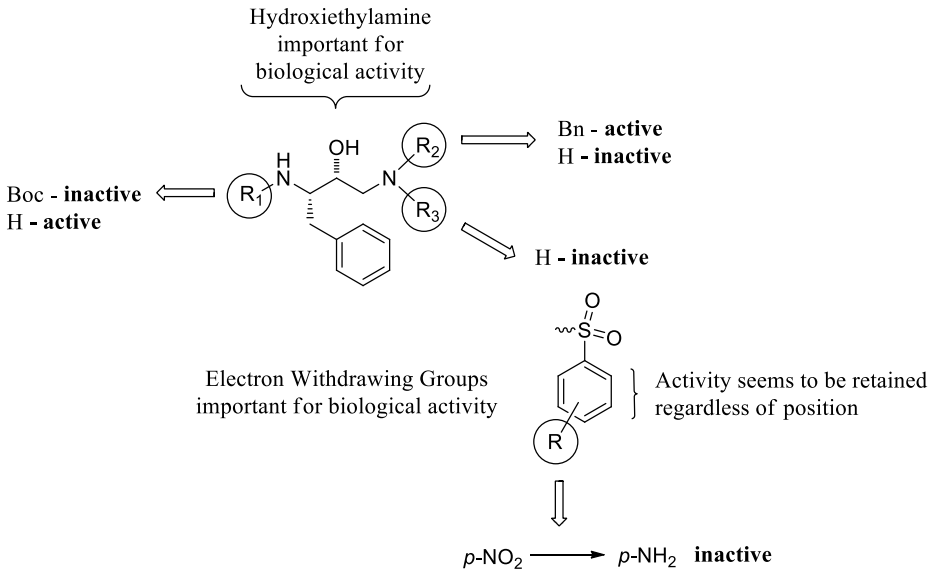



**Figure 1.9** SAR analysis of hydroxyethylamine derivatives

Since development of new drugs for cancer therapy is a lengthy and costly endeavor, the repositioning of an approved drug for cancer therapeutics has many advantages, such as implicit knowledge of its toxicity profile, drug metabolism, pharmacokinetics, and drug interactions. The HIV protease inhibitors possess cellular effects that lead to antitumour activity. Importantly, none is mutually exclusive of another, and the effects are protease-inhibitor dependent[46].

In light on the above, the emergence of drug resistance prompts the scientific community to produce new molecules with lower side effects and higher efficacy, especially if a double biological activity is possible. Sir James W Black, the pharmacologist and 1988 Nobel laureate in medicine, famously said: "most fruitful basis of the discovery of a new drug is to start with an old drug".

Thus, following the concept of targeting the protein backbone and with the aim to design simple non-peptide heteroaryl structures, the present study was focused on the synthesis of novel PIs compounds, bearing hydroxyethylamine *core* with different heteroarenes and

[53] V. Facchinetti, M. Moreth, C.R.B. Gomes, C. O Pessoa, F.A.R. Rodrigues, B.C. Cavalcanti, A.C.A. Oliveira, T.R. Carneiro, I.L. Gama, M.V.N. De Suoza, *Med Chem Res* **2015**, *24*, 533.

sulfonamides, with potentially broad-spectrum activity against drug-resistant HIV variants and anticancer activity. In order to obtain such compounds with double activity we planned a systematic study on the synthesis of new derivatives with general structure reported in figure 1.10, in which the heterocycle is spaced from the central *core* by different functionality, either carboxyamide or carbamoyl function in the same orientation as in Darunavir. The type of heteroaryl group (X = O, NH, S), the sulfonamide moiety and the *core* were modified, with the presence of either H or benzyl as R' substituent. Taking into account our previous results (Figure 1.7, ref. 43), the carboxyamide function was matched with two methoxy groups on sulfonamide moiety, while the carbamoyl functionality was combined with a single substituent on the sulfonamide.

In this work new synthetic paths were studied in order to minimize the steps and to increase the overall yield. Furthermore, in order to prepare a library of compounds, diversity-oriented synthesis was studied to change different functionalities according to needs.

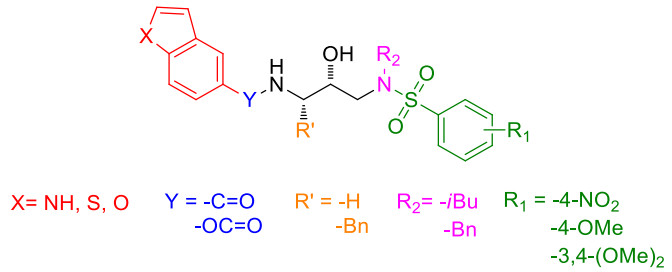
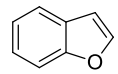



**Figure 1.10** General structure of synthetized compounds

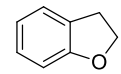
The compounds thus obtained were tested for *in vitro* activity against native protease and for their effects on cellular vitality.

## ***1.4 Heterocycles in biological active compounds: 2,3-dihydrobenzofuran further aims of the project***

Another heterocycle that constitutes the core skeleton of o wide number of biologically active compounds is the 2,3-dihydrobenzofuran ring-system[54], formally derived from benzofuran by hydrogenation of the furan ring (figure 1.11)[55].




Benzofuran




2,3-dihydrobenzofuran

**Figure 1.11**

The 2,3-dihydrobenzofuran scaffold occurs in a wide variety of natural products and synthetic compounds, including approved drugs, attracting considerable attention due to their biological and pharmacological activities, spreading from antiviral to antibacterial, anti-inflammatory, antiangiogenic and antimitotic activity[56]. Figure 1.12 depicts some examples of compound containing 2,3-dihydrobenzofuran moiety and their biological activity[57].

[54] a) M.H. Keylor, B.S. Matsuura, R.J. Stephenson, *Chem. Rev.* **2015**, *115*, 8976. b) K. Chen, M. Pitchakuntla, Y. Jia, *Nat. Prod. Rep.* **2019**, 36, 666.
[55] F. Bertolini, M. Pineschi, *The new Journal for Organic Synthesis* **2009**, *41*, (5), 385.
[56] L. Qin, D.D. Vo, A. Nakhai, C.D. Anderson, M. Efferson, *ACS Combinatorial Science* **2017**, *19*, 370.
[57] a) A.K. Ghosh, Z. Cheng, B. Zhou, *Org Lett.* **2012**, *14 (19)*, 5046. b) D.S. Lee, G.S. Jeong, *Eur J Pharmacol.* **2014**, *728*, 1.

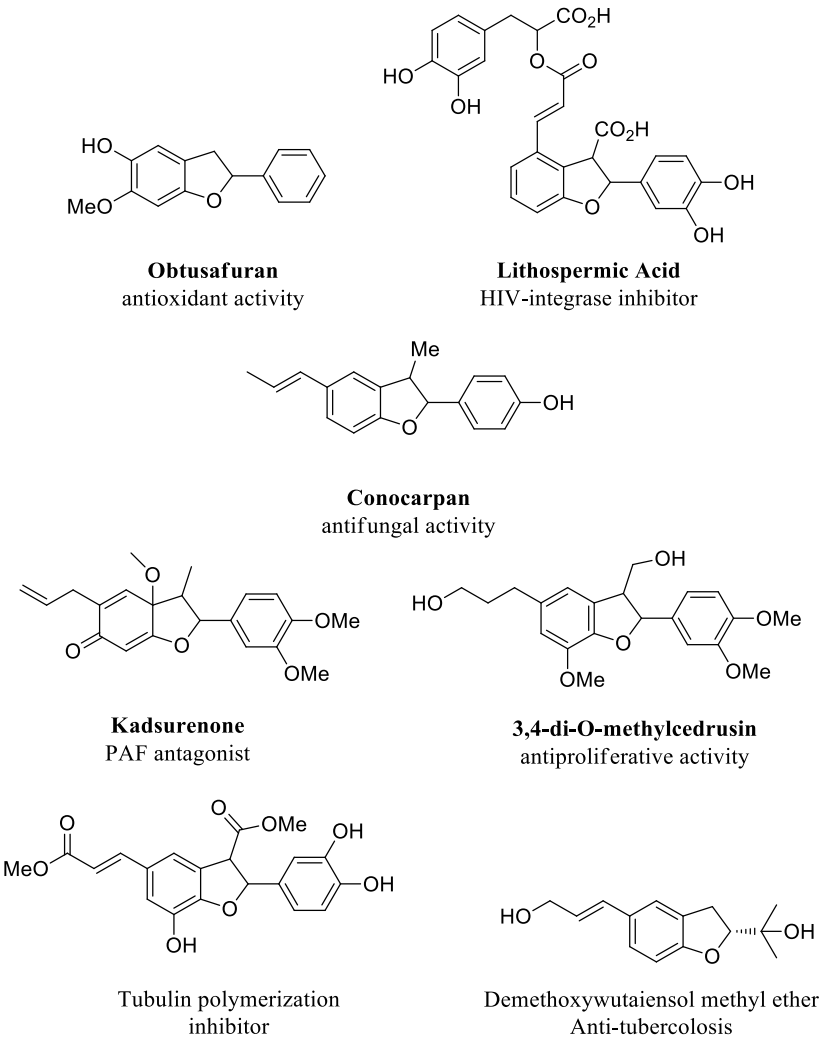



**Figure 1.12** Some compounds based on the 2,3-dihydrobenzofuran scaffold

In particular, the dihydrobenzofuran moiety is present in different oligostilbenes, that represent resveratrol oligomers[58] and have diverse bioactivities[59] (some examples in figure 1.13). These compounds have also been found in plants as a result of infection or stress

[58] M.H. Keylor, B.S. Matsuura, C.R.J. Stephenson, *Chem. Rev.* **2015**,*115*, 8976.
[59] a) T. Ito, T. Tanaka, M. Iiuma, K.-I. Nakaya, Y. Takahashi, R. Sawa, H. Naganawa, V. Cheladurai *Tetrahedron* **2003**, *59*, 1255. b) D.T. Ha, P.T. Long, T.T. Hien, D.T. Tuan, An.N.T. Thuy, N.M. Khoi, H.V. Oanh, T.H. Hung, *Chemistry Central Journal* **2018**,12. c) S. Ficarra, E. Tellone, D. Pirolli, A. Russo, D. Barreca, A. Galtieri, B. Giardina, P. Gavezzotti, S. Riva, M. De Rosa, *J. Name*. **2013**,1. d) N.K. Narayanan, K. Kunimasa, Y. Yamori, M. Mori, H. Mori, *Cancer Medicine* **2015**, *4*(11), 1767.

and are supposed to be formed by oxidative dimerization catalyzed by plant peroxidases and/or phenoloxidases[60].

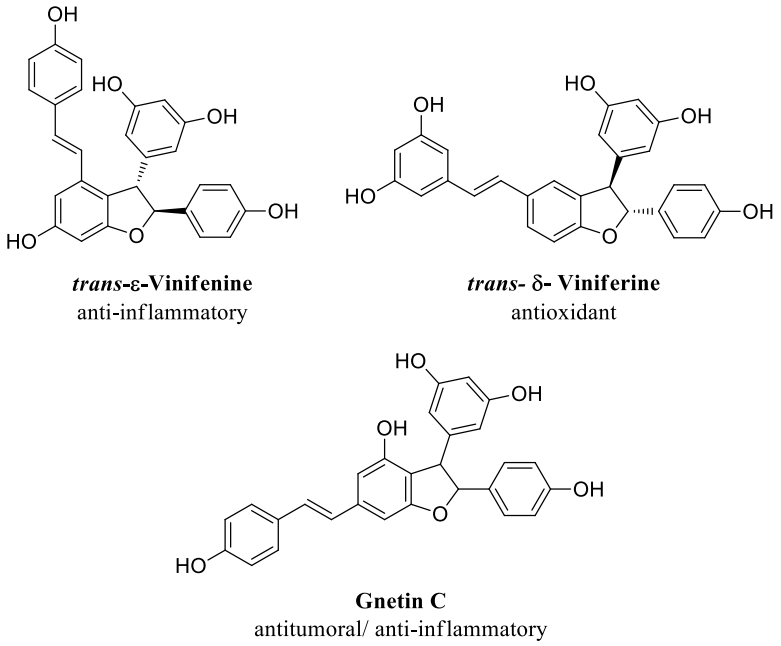



**Figure 1.13** Some examples of Resveratrol oligomers

Thanks to these therapeutic properties, many researchers currently try to synthetize these molecules and to obtain new derivatives with structural changes[61].

There are several approaches to the synthesis of the 2,3-dihydrobenzofuran ring system[62], but a true general method still lacks. Although different synthetic approaches are known for the preparation of 2,3-dihydrobenzofurans, few of them are efficient for the synthesis of 2,3-diaryl-2,3-dihydrobenzofurans. Iron-catalyzed oxidative radical cross-coupling/cyclization between phenols and olefins, promoted either by DDQ[63] or di-*tert*-butylperoxide,[64] allowed the preparation of some derivatives in moderate to good yield. A

[60] R.H. Cichewicz, S.A. Kouzi In *Studies in natural products chemistry*, *Vol. 26*: *Bioactive natural products* (Part G), (Ed.: Attaur-Rahman), Elsevier, Dordrecht, pp 507, **2002**.
[61] B. Salehi, A.P. Mishra, M. Nigam, B. Sener, M. Kilic, M. Sharifi-Rad, P.V.T. Fokou, N. Martins, J. Sharifi –Rad, *Biomedicines* **2018**, *6*, 91.
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[63] Z. Huang, L. Jin, Y. Feng, P. Peng, H. Yi, A. Lei, *Angew. Chem. Int. Ed.* **2013**, *52*, 7151
[64] U. A. Kshirsagar, C. Regev, R. Parnes, D. Pappo, *Org. Lett.* **2013**, *15*, 3174

biomimetic approach was described by Sako[65] in the diastereoselective synthesis of racemic resveratrol *E*-dehydrodimers by oxidative dimerization, while Che et al.[66] reported a ruthenium porphyrin-catalyzed stereoselective intramolecular carbenoid C–H insertion of tosylhydrazones which afforded generally *cis* 2,3-substituted-2,3-dihydrobenzofurans. A programmable, controlled and potentially scalable synthesis of the resveratrol family was described by Snyder, based on the use of a common building block.[67] Even a chemo-enzymatic approach exploiting the laccase-mediated oxidative (homo)coupling of (*E*)-4-styrylphenols was used for the synthesis of 2,3-dihydrobenzofuran scaffolds.[68] Recently several 2,3-diaryl-2,3-dihydrobenzofurans were prepared utilizing Pd catalyzed one-pot multicomponent reactions and ruthenium-catalyzed intramolecular carbenoid C–H insertions, affording to *cis*/*trans* racemic mixtures, which were resolved by HPLC.[69] A simple synthesis of 2,3-dihydrobenzofurans can be achieved by intramolecular dehydration of a molecule containing both a phenol and an alcohol functionality (Mitsunobu type cyclization) (scheme 1.1). [70] The complete inversion of configuration, together with the high yields generally obtained, were the major advantages of such method, especially when substituted chiral hydroxy phenols were employed. Recently we took advantage of this procedure for stereoselective access to *trans* 2,3-diphenyl-2,3-dihydrobenzofurans.[71] Several reports are described on the formation of dihydrobenzofurans by radical cyclization of different precursors[72] and some others by biomimetic couplings and cycloadditions[73] or palladium-catalyzed intramolecular cyclizations.[74]

[65] M. Sako, H. Hosokawa, T. Ito, M. Iinuma, *J. Org. Chem.* **2004**, *69*, 2598
[66] W.-H. Cheung, S.-L. Zheng, W.-Y. Yu, G.-C. Zhou, C.-M. Che, *Org. Lett.* **2003**, *5*, 2535.
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[68] I. Bassanini, I. D'Annessa, M. Costa, D. Monti, G. Colombo, S. Riva, *Org. Biomol. Chem.* **2018**, *16*, 3741.
[69] M. Saleeb, S. Mojica, A. U. Eriksson, C. D. Andersson, A. Gylfe, M. Elofsson, *Eur. J. Med. Chem.* **2018**, *143*, 1077.
[70] F. Bertolini, P. Crotti, V. Di Bussolo, F. Macchia, M. Pineschi *J. Org. Chem* **2007,** *72*, 7761.
[71] T. Laurita, L. Chiummiento, M. Funicello, R. D'Orsi, D. Sallemi, D. Tofani, P. Lupattelli, *European Journal of Organic Chemistry*, **2019**, *27*, 4397.
[72] a) M. Yamashita, Y. Ono, H. Tawada, *Tetrahedron* **2004**, *60*, 2843. b) H. Lin, A. Schall, O. Reiser, *Synlett* **2005**, 2603.
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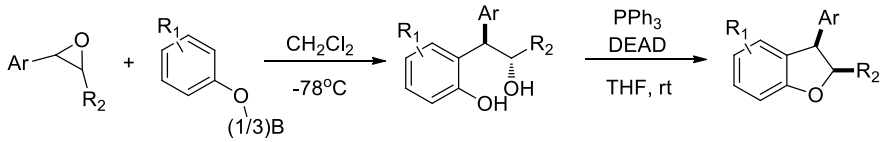
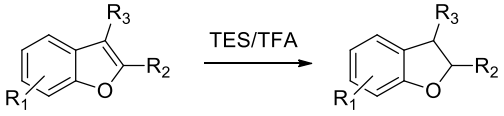
[74] M. Szlosek-Pinaud, P. Diaz, J. Martinez, F. Lamaty, *Tetrahedron* **2007**, *63*, 3340.




**Scheme 1.1**

It is worth noting that the hydrogenation of benzofuran derivatives to the corresponding 2,3-dihydrobenzofurans, which might be a reasonably straightforward method to synthesize these compounds, is difficult to achieve with respect to other heteroaromatic nuclei.[75] In fact, the catalytic hydrogenation of benzofuran, under all conditions, is accompanied by partial cleavage of the furan ring. Only recently, catalytic hydrogenation and triethylsilane-mediated reduction of a particular 2,3-diarylbenzofuran were used in the total synthesis of (±) ε-viniferin[76] and its permethylated analogue.[77] In the light of this findind, another research issue in our laboratory was to study the behaviour of substituted benzofurans in reduction reactions, trying to understand the effect of substituents on the benzene or on the furan ring on reactivity. Among the possible reduction methods, reduction with hydrosilanes was applied in this work (scheme 1.2).




**Scheme 1.2**

[75] E. Baralt, S.J. Smith, J. Hurwitz, I.T. Horvath, R.H. Fish, *J. Am. Chem. Soc.* **1992**, 114, 5187.
[76] A. E. G. Lindgren, C. T. Öberg, J. M. Hillgren, M. Elofsson, *Eur. J. Org. Chem.* **2016**, 426.
[77] J. Zhang, J. Zhang, Y. Kang, J. Shi, C. Yao, *Synlett* **2016**, *27*, 1587.

# CHAPTER 2:
# HIV-PIS AS ANTICANCER THERAPEUTICS

## *2.1 Diversity-oriented synthesis of potential HIV-PIs and antitumor agents*

Due to the rapid genomic evolution of the HIV, an inevitable consequence in the treatment of the infection has been the rise of drug resistance and the dramatic reduction of the commercial inhibitors efficiency. Thus, both the emergence of highly mutated viral strains cross-resistant to antivirals and the occurrence of various debilitating side effects, and not last the high cost of HAART, has prompted to seek novel PIs, with alternative frameworks, which could show possible double biological activity and could be used as anticancer therapeutics.

In the present study new HIV protease inhibitors with simple substituted stereodefined isopropanolamine *core*, containing heteroaryl fragments were synthesized in order to study their broad spectrum activity, both as antitumor agents and as more efficient antiviral compounds, compared to previously analyzed, maximizing interactions with the protein backbone to retain their activity against mutants ones.

General structure of the compounds synthesized is reported in figure 1.10 (see chapter 1, section 1.3):

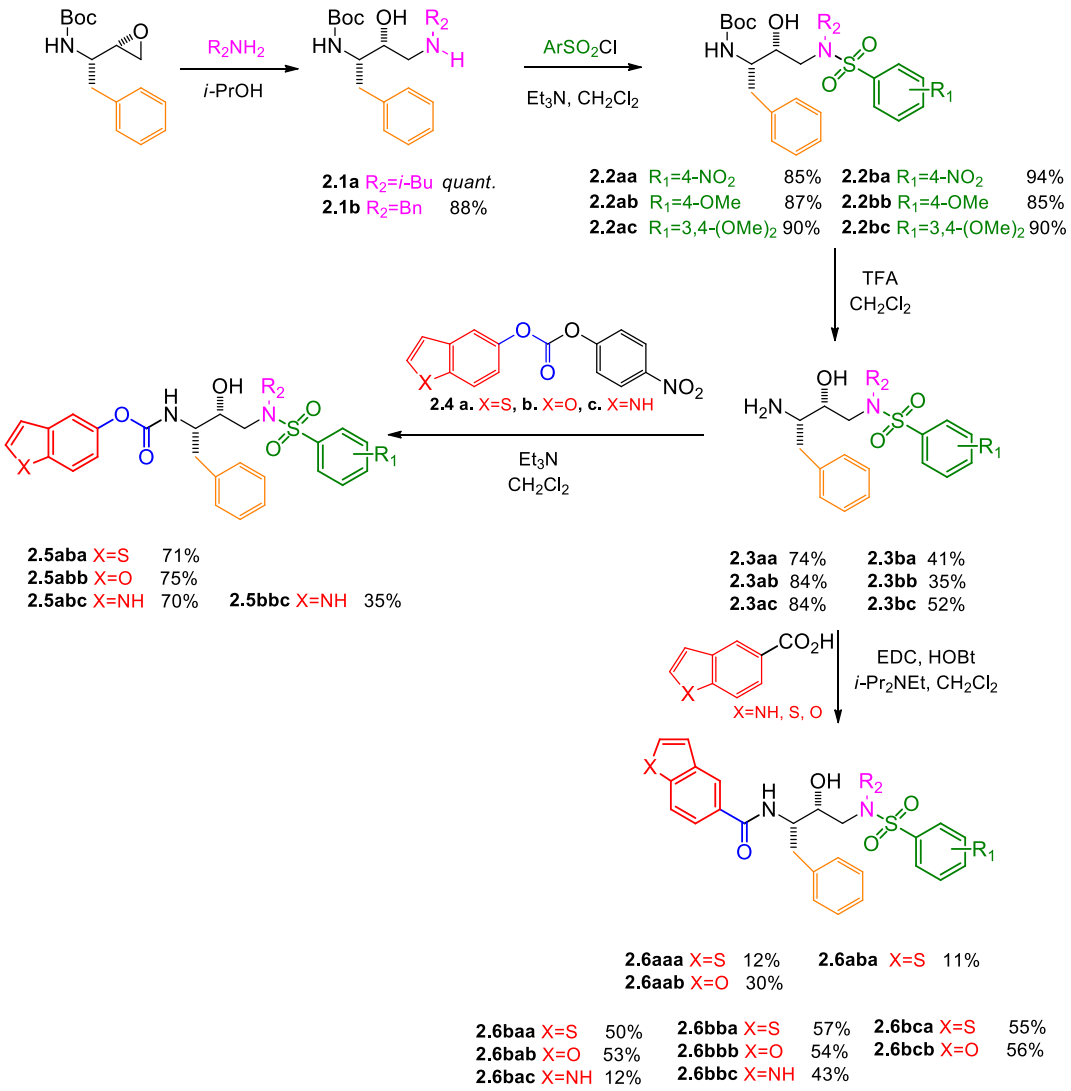
- ~ all compounds bear an hydroxyethylamine *core*;
- ~ the heteroaryl moiety is spaced from the central *core* by different functionalities, either carboxyamide or carbamoyl functions;
- ~ the type of heteroaryl group (X = O, NH, S), the sulfonamide moiety and the *core* were modified, with the presence of H or benzyl as $R^{'}$ substituent;
- ~ it was taken into account that carboxyamide spacer matches with two methoxy groups on sulfonamide moiety, while in the case of carbamoyl spacer only one substituent is required on the sulfonamide.

The aim is to obtain a library of compounds using synthetic paths with few steps and high yields. Thus, diversity-oriented synthesis was studied to change different functionalities according to need.

### *2.1.1 Synthesis of compounds with benzyl group in the central core*

In particular, when a benzyl group is present in the central *core* ($R^{'}$=Bn, figure 1.10), synthesis starts from commercially available homochiral *N*-Boc protected amino epoxide, according to our previous research[1] (Scheme 2.1). The epoxide was firstly opened with two different amines to afford the monoprotected diaminoalcohols **2.1a-b**. Different arylsulfonyl groups were introduced on secondary amines (**2.2a(a-c)**, **2.2b(a-c)**), and subsequently the *N*-Boc group was efficiently removed by TFA. The crude ammonium trifluoroacetate derivatives were treated with $Et_3N$, affording the free amines (**2.3a(a-c)**, **2.3b(a-c)**). These so obtained compounds have the scaffold of compounds reported in figure 1.9 (see chapter 1, section 1.3). So, starting from such reported SAR analysis, we decided to study the biological activity of **2.3a(a-c)** and **2.3b(a-c)** derivatives. From this common intermediates, we were easily able to achieve two class of compounds: **2.5ab(a-c) and 2.5bbc** where the heteroaryl group is spaced from *core* by a carbamoyl functionality and **2.6a(a-b)(a-b), 2.6b(a-c)(a-c)**, where heteroaryl group is spaced from *core* by carboxyamide functionality; in these last ones, when $R_2$ is a *i*-Bu group, only two sulfonyl groups were used (4-$NO_2$ and 3,4-$(OMe)_2$) because compounds with 4-methoxyphenylsulfonyl group were already tested as antiviral compounds (see figure 1.8 in section 1.2)[1b].

[1] a) C. Bonini, L. Chiummiento, N. Di Blasio, M. Funicello, P. Lupattelli, F. Tramutola, F. Berti, A. Ostric, S. Miertus, V. Frecer, D.-X. Kong, *Bioorg Med Chem*. **2014**, *22*, 4792. b) M. Funicello, L. Chiummiento, F. Tramutola, M.F. Armentano, F. Bisaccia, R. Miglionico, L. Milella, F. Benedetti, F. Berti F., P. Lupattelli, *Bioorganic & Medicinal Chemistry* **2017**, *25*, 4715.



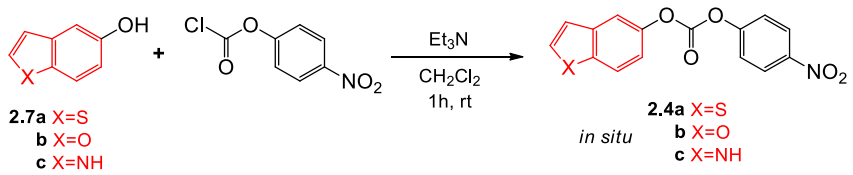

**Scheme 2.1**

**a.** $R_2$=*i*-Bu; **b.** $R_2$=Bn

**a.** $R_1$=4-$NO_2$; **b.** $R_1$=4-OMe; **c.** $R_1$=3,4-$(OMe)_2$

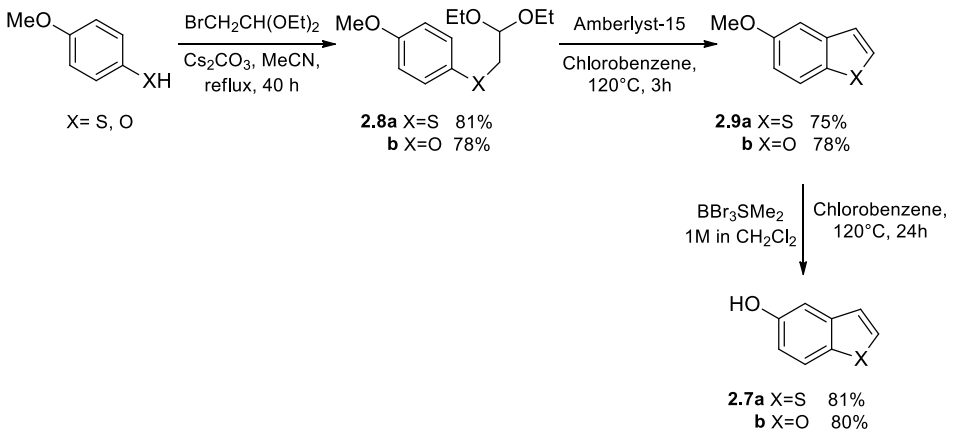
**a.** X=S; **b.** X=O; **c.** X=NH

In the case of carbamoyl functionality, **2.5ab(a-c)** and **2.5bbc** derivatives were obtained by reacting amine **2.3** with the *in situ* preformed carbonates **2.4a-c** of the starting phenols **2.7a-c** (Scheme 2.2)**.**




**Scheme 2.2**

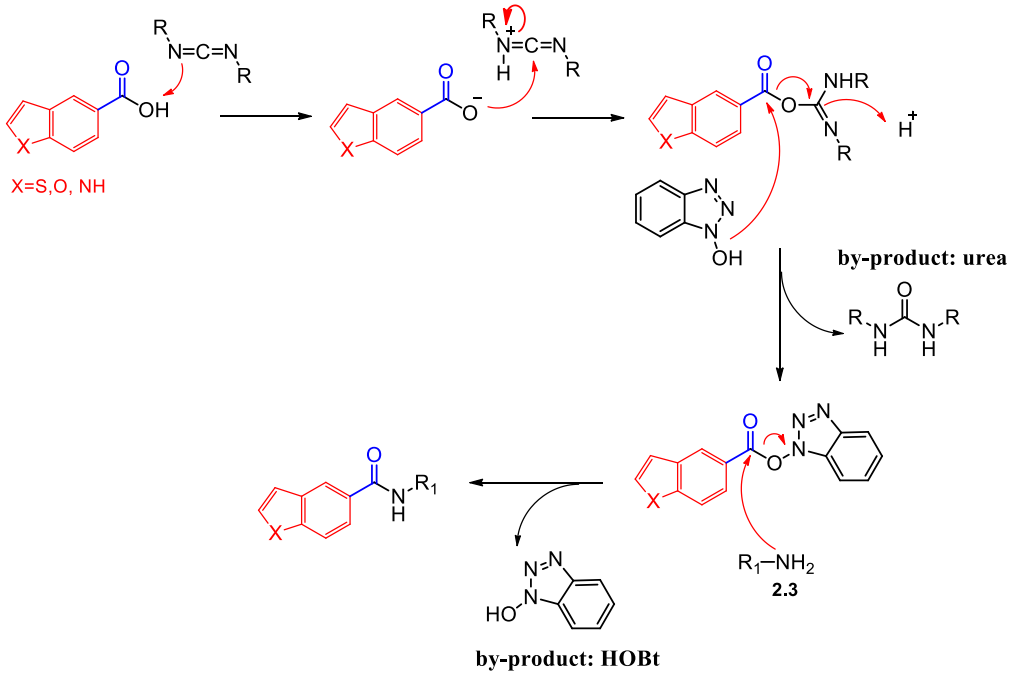
While 5-hydroxyindole (**2.7c**) is cheaply commercially available, 5-hydroxybenzothiophene (**2.7a**) and 5-hydroxybenzofuran (**2.7b**) had to be prepared, due to their high cost. Unfortunately, only few methods are reported in the literature[2]. In particular, those starting from 4-methoxyphenol or 4-methoxythiophenol usually give low overall yield, due to the cyclization step. Hence, we successfully revisited such procedure, using bromoacetaldehyde diethylacetal in the alkylation step and Amberlyst-15[3] in the subsequent cyclization/aromatization step (Scheme 2.3). By these modifications 5-methoxy-heteroarenes **2.9a-b** were obtained in good yield (>60% in two steps). Demethylation of **2.9a-b** afforded to desired 5-hydroxyheteroarenes **2.7a-b** in high yield, making this three steps procedure the more efficient reported so far (>49% yield).




**Scheme 2.3**

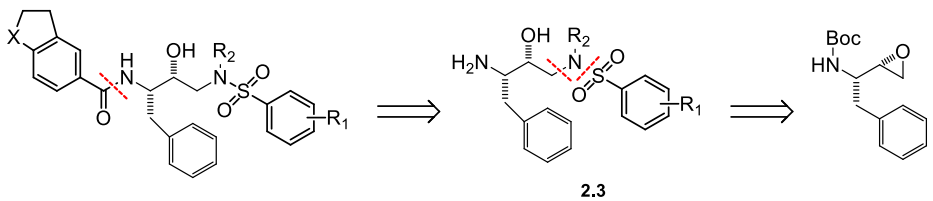
[2] a) J. Bian, X. Li, N. Wang, X. Wu, Q. You, X. Zhang, *Eur. J. Med. Chem.* **2017**, *129*, 27. b) I. Cerminara, L. D'Alessio, M. D'Auria, M. Funicello, A. Guarnaccio, *Helvetica Chimica Acta* **2016**, *99*, 384. c) Y. Yamaguchi, I. Akimoto, K. Motegi, T. Yoshimura, K. Wada, N. Nishizono, K. Oda, *Chem. Pharm. Bull.* **2013**, *61*, 997. d) C. Bonini, G. Cristiani, M. Funicello, L. Viggiani, *Synthetic Communications* **2006**, 1983. e) S. Pérez-Silanes, J. Martínez-Esparza, A.M. Oficialdegui, H. Villanueva, L. Orús, A. Monge, *J. Heter. Chem.* **2001**, *38*, 1025.
[3] J.T. Liu, T.J. Do, C.J. Simmons, J.C. Lynch, W. Gu, Z.-X. Ma, W. Xu, W. Tang, *Org. Biomol. Chem* **2016**, *14*, 8927.

Instead carboxyamide functionality was inserted reacting amines **2.3** with 5-heteroarylcarboxylic acids, previously activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and hydroxybenzotriazole (HOBt) as reported in scheme 2.4.




**Scheme 2.4** Amide bond formation by attivation with EDC and HOBt.

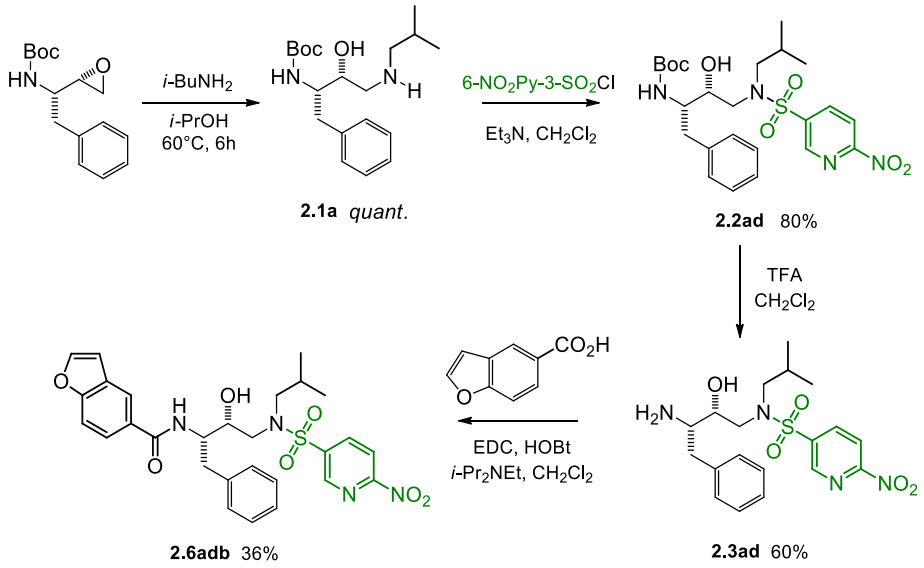
Hence, according to the synthetic path described in scheme 2.1, final amide-containing compounds can be obtained by condensing common intermediate amines **2.3** and desired 5-carboxyarene (scheme 2.5). Amines **2.3** can be prepared through the epoxide opened with amines which were subsequently functionalized to sulfonamides. Thus, changing the amine and the sulfonyl group used, a library of compounds **2.3,** i.e. a library of potential inhibitors, can be obtained.




**Scheme 2.5**

Computational studies, conducted by professor Di Fabio[4], have suggested some changes that could be made on this type of compounds to improve their ADME profile (Absorption, Distribution, Metabolism and Excretion of a pharmaceutical compound). Molecular weight (MW), partition coefficients octanol/water, Polar Surface Area (PSA) and number of heavy atoms (HA) have been evaluated. These criteria, in fact, have effect to drug pharmodynamics and pharmacokinetics, its solubility and exposure to the tissues; hence influence the performance and pharmacological activity. So we decided to introduce heterocycle moiety into sulfonamide portion of the inhibitors structure, changing both sulfonyl group used and the incoming amine.

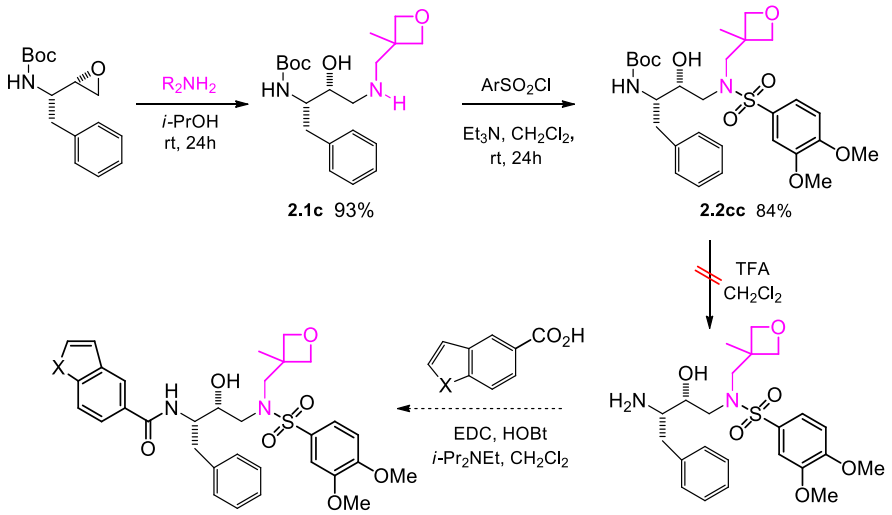
So, with this same synthetic path, a nitropiridyl sulfonyl moiety was introducted to the obtained aminoalcol **2.1a**




**Scheme 2.6**

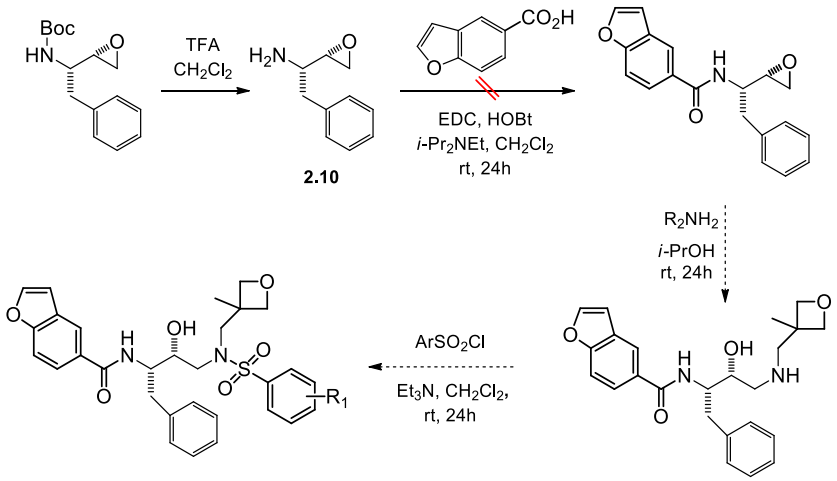
In the same way we looked for the introduction of an heterocycle on amine moiety of the solfonmide. Opening the starting epoxide with (3-methyloxetane-3-yl) methanamine the oxetane ring could give rigidity to the structure and could create H-bond with the active site of the enzyme. But in this case amine **2.3** was not obtained due to the oxetane ring opening occurring in the conditions of Boc-removing.

[4] *Private communication*




**Scheme 2.7**

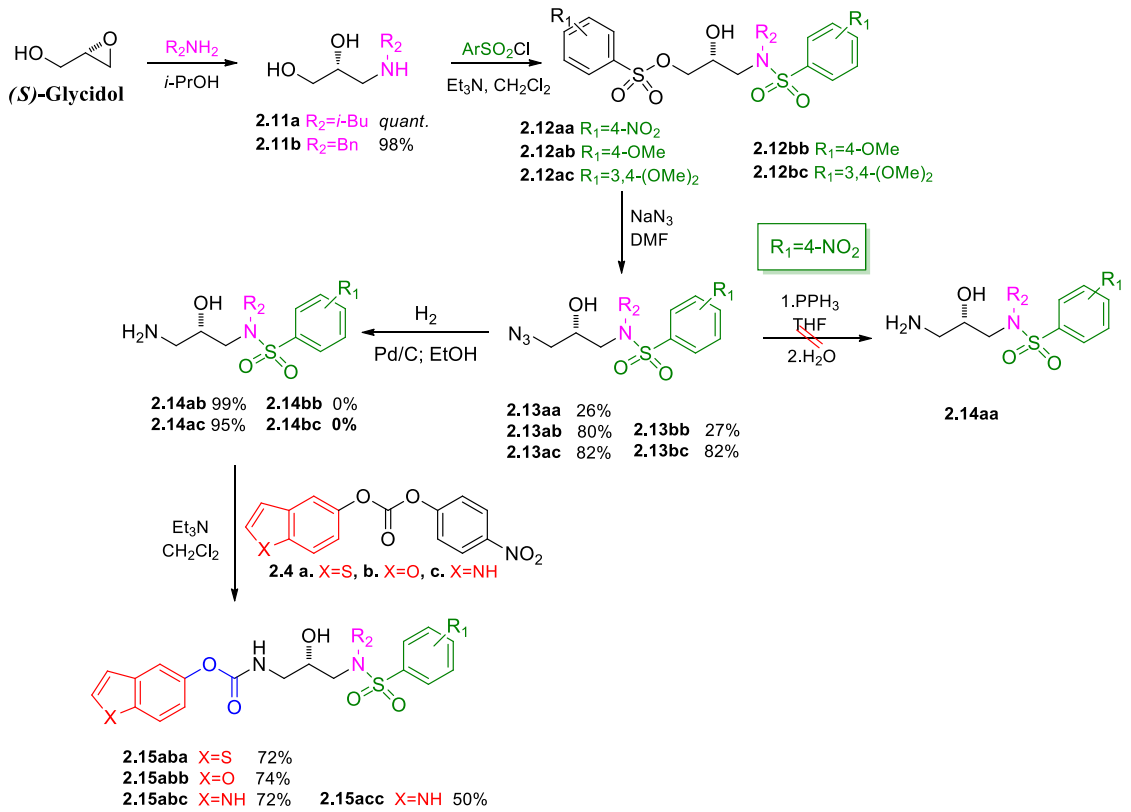
So the strategy was modified as in scheme 2.8, starting with the displacement of Boc group. Attempts to introduce the heteroarene were unsuccessful and only degradation of the substrate was obtained. Another synthetic strategy is necessary in this case.




**Scheme 2.8**

### *2.1.2 Synthesis of compounds with unsubstituted central core*

According to our previous research[1], the synthetic route for the preparation of the simple unsubstituted *core* (R' = H in figure 1.10), started from the commercially available bidentate electrophile (*S*)-glycidol (Scheme 2.9).




**Scheme 2.9**
**a.** $R_2$=*i*-Bu; **b.** $R_2$=Bn
**a.** $R_1$=4-$NO_2$; **b.** $R_1$=4-OMe; **c.** $R_1$=3,4-$(OMe)_2$
**a.** X=S; **b.** X=O; **c.** X=NH

(*S*)-Glycidol was first reacted with two different amines in *iso*propanol to achieve aminodiols **2.11a-b**, which were successfully treated with different arylsulfonyl chlorides and triethylamine in dry $CH_2Cl_2$ to obtain sulfamido-sulfonates **2.12**. These compounds underwent nucleophilic displacement with $NaN_3$, to give the sulfamidyl-azides **2.13**. Azide can be reduced to amine by catalytic hydrogenation to obtain amine **2.14**. In presence of

$NO_2$ group on sulfonamide (**2.13aa**, **2.13ba**) catalytic hydrogenation cannot be used without the concurrent reduction of $NO_2$. So, in this case, Staudinger reaction was tried to reduce selectively the azido group, but it did not afford to desired amine **2.14aa**. Another critical point is that when $R_2$=Bn, catalytic hydrogenation did not occur either on azido group or on benzyl substituent, so final products **2.14 b(b-c)**, cannot be obtained. In the last step, heteroaryl group was introduced on compounds **2.14a(b-c)**, spaced to the central *core* by carbamoyl functionality, since derivatives with carboxyamidyl spacer had been already tested about their biological activity[5].

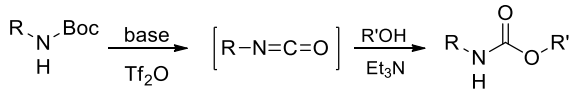
It is clear that this synthetic route, unlike the one seen previously when the central *core* bears a benzyl group , does not guarantee the possibility of varying the residues according to the needs and every single reaction must be analyzed; it is not very simple, therefore, to obtain a library of compounds as seen in the previous case.

[5] M. Funicello, L. Chiummiento, F. Tramutola, M.F. Armentano, F. Bisaccia, R. Miglionico, L. Milella, F. Benedetti, F. Berti, P. Lupattelli, *Bioorganic & Medicinal Chemistry* **2017**, *25*, 4715.

### *2.1.3 Alternative routhes*

#### *2.1.3.1 One-pot synthesis of carbamates*

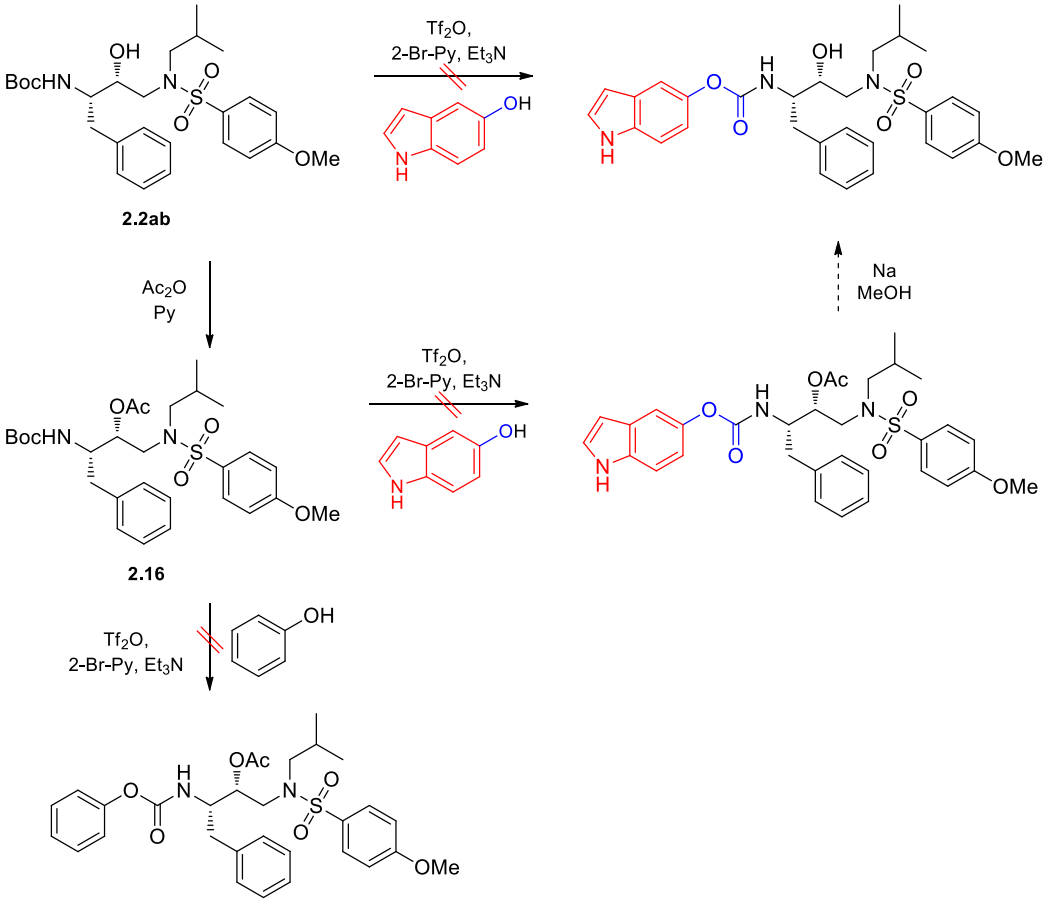
New synthetic paths were studied in order to further reduce synthetic step number and to find a solution to the encountered problems so that the strategy becomes as general, useful and manageable as possible. Firstly a new method to obtain carbamates *one-pot* from Boc-protected amines was tried to eliminate the amine deprotection step. In literature a highly efficient *one-pot* procedure for the synthesis of carbamates has been described: in the presence of 2-bromopyridine and trifluoromethanesulfonyl anhydride from Boc-protected amine an isocyanate intermediate was generated *in situ* for further reaction with an alcohol as nucleophile to afford the desired carbamate (scheme 2.10)[6].




**Scheme 2.10**

This procedure was used on our model substrate **2.2ab** as model using 5-hydroxyindole as nucleophile after the isocyanate intermediate formation. Unfortunately the reaction did not work, probably because of the presence of a free hydroxyl group; thus it was initially protected as acetate to avoid competitive reaction, but also in this case substrate degradation occurred. Then a test with phenol as nucleophile was tried, but also in this case the reaction did not work well (Scheme 2.11).

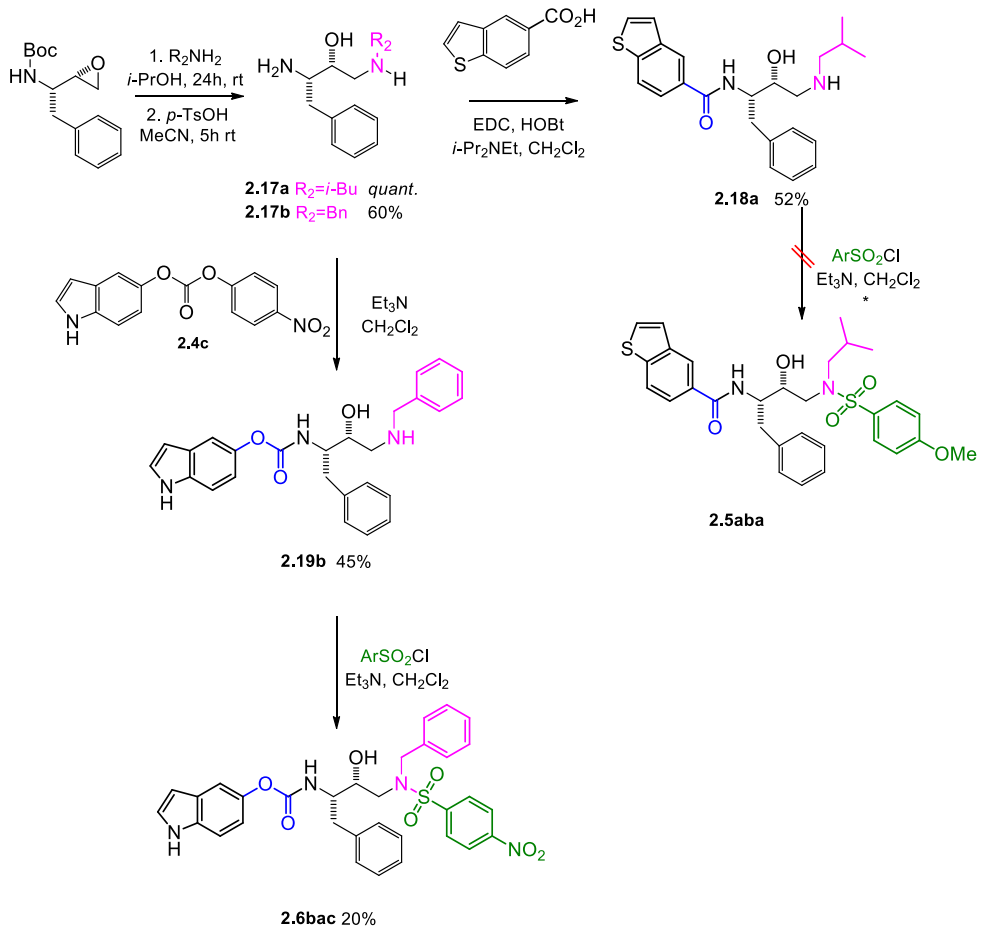
[6] H.K. Kim, A. Lee, *Tetrahedron Letters* **2016**, *57*, 4890.


BocHN
OH
OMe
2.2ab
Tf2O,
2-Br-Py, Et3N
OH
Ac2O
Py
OAc
2.16
Na
MeOH
Tf2O,
2-Br-Py, Et3N
OH
Tf2O,
2-Br-Py, Et3N
OH
OAc
OMe

**Scheme 2.11**

*2.1.3.2 Diversity oriented synthesis*

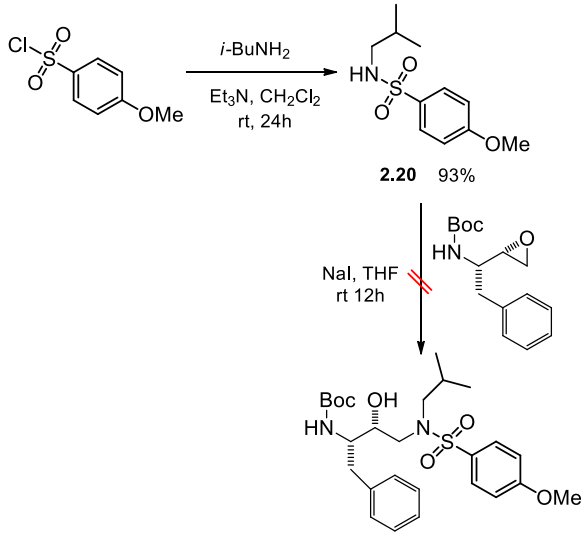
In order to obtain a library of compounds bearing different functionalities according to needs, diversity-oriented synthesis was studied. Taking into account synthetic path described in scheme 2.1, a new strategy was tried introducing first the heteroaryl moiety and then the sulfonamide in order to keep the same heteroaryl group and change only sulfonamide moiety. Thus, after obtaining of intermediates **2.18 and 2.19**, the introduction of the sulfonyl group worked well only with the carbamoyl derivative **2.19b**, while amide **2.18a** was unexpectedly unreactive (Scheme 2.12).




**Scheme 2.12**

***** further tests were carried out by changing the conditions:
**a.** 4-OMePhSO$_2$Cl, Et$_3$N, CH$_2$Cl$_2$, rt, 24h. **b**. 4-OMePhSO$_2$Cl, Et$_3$N, DMF, rt, 24h.
**c.** 4-OMePhSO$_2$Cl, Et$_3$N, AcOEt, rt, 24h. **d.** 4-NO$_2$PhSO$_2$Cl, Et$_3$N, DMF, reflux, 24h.
**e**. 4-NO$_2$PhSO$_2$Cl, Et$_3$N, DMAP, DMF, reflux, 24h.

Thus, a new reaction was studied. Sulfonamide **2.20** was was first prepared[7], and then was used to open the *N*-Boc protected amino epoxide[8]. Unfortunately sulfonamide did not work in reaction (Scheme 2.13).

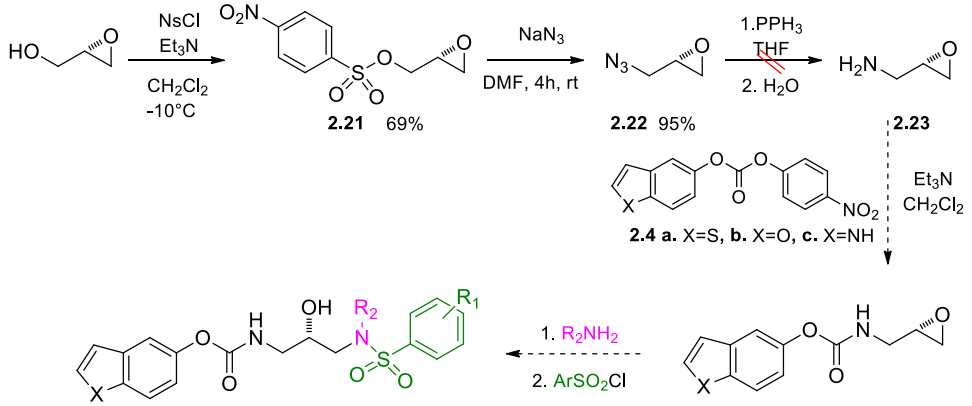



**Scheme 2.13**

[7] Y. Yang, X. Meng, B. Zhu, Y. Jia, X. Cao, S. Huang, *Eur. J. Organic Chemistry* **2019**, *5*, 1166.
[8] M. Flipo, J. Charton, A. Hocine, S. Dassonneville, B. Deprez, R. Deprez-Poulain, *Journal of Medicinal Chemistry* **2009**, *52* (21), 6790.

*2.1.3.3 Different starting material*

Following the problems encountered and illustrated in scheme 2.9, a new synthetic strategy has been studied starting from *S*-glycidol, in order to introduce first the heteroarene and then the amine and/or the desired sulfonyl group by opening the epoxide (scheme 2.14). So we planned a strategy that involves the activation of hydroxy group of the epoxide by the sulfonyl without opening the glycidol itself and a displacement with azide to afford the azidoepoxide **2.22**.




**Scheme 2.14**

Unfortunately reduction of **2.22** has entailed formation of products that are difficult to isolate and characterize, coming from possible intermolecular or intramolecular reactions between the the nucleophile amino group and the electrophile epoxide. So we decided to change starting material.

Since poor results were obtained starting from *S*-glycidol, we turned our attention to other readily available homochiral compounds from chiral pool, which allowed planning a synthetic strategy as versatile as possible. Chiral pool approach represents a concept of asymmetric synthesis in which the elements of chirality of the starting material (or some of them) are retained in the final product. It still stands as powerful tool in asymmetric synthesis, provided the cheap availability of the starting substrates.

Common homochiral starting materials include monosaccharides and amino acids. Among them, *S*-solketal, *S*- and *R*-glyceraldehyde acetonide were chosen for their mutual

interconversion by simple oxidation or reduction and the possibility of alternatively operate at both sides of the molecule.

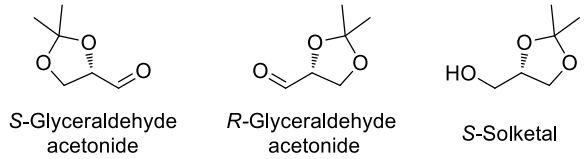
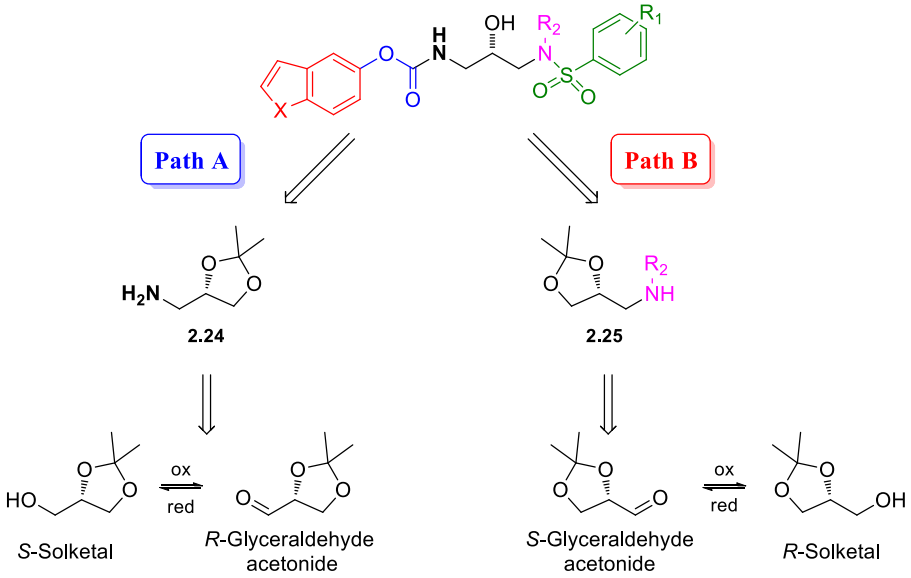



**Figure 2.1**

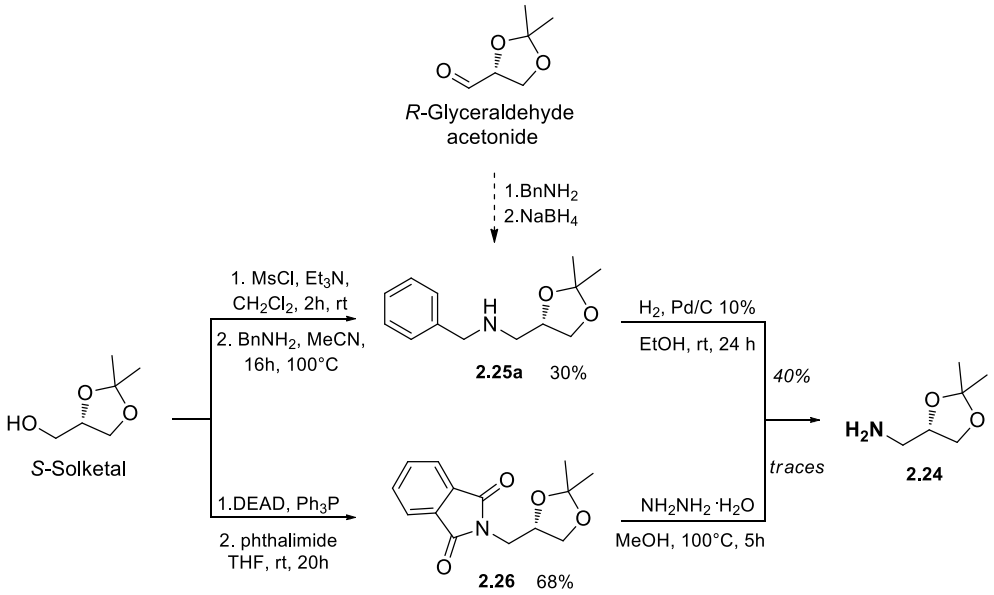
They have been listed to evaluate which could be the most convenient and immediate synthetical strategy; any isomer can be used depending on the side of the molecule on which to operate, as reported in scheme 2.15.




**Scheme 2.15**

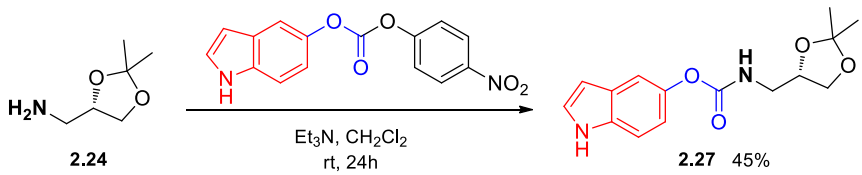
Intermediate **2.24** could be obtained starting from *S*-solketal alternatively by mesylation and subsequent introduction of benzylamine and final reduction or by an initial introduction of phthalimide via Mitsunobu type reaction and subsequent displacement of

phtaloyl group by hydrazine[9]. Alternately **2.24** could be obtained from *R*-glyceraldehyde acetonide, by reductive amination with benzylamine and debenzylation (Scheme 2.16).




**Scheme 2.16**

Once obtained **2.24** intermediate, the heteroarene was introducted as reported in scheme 2.17. These are only preliminary results and subsequent reactions for the introduction of the sulfonamide fragment will be studied.

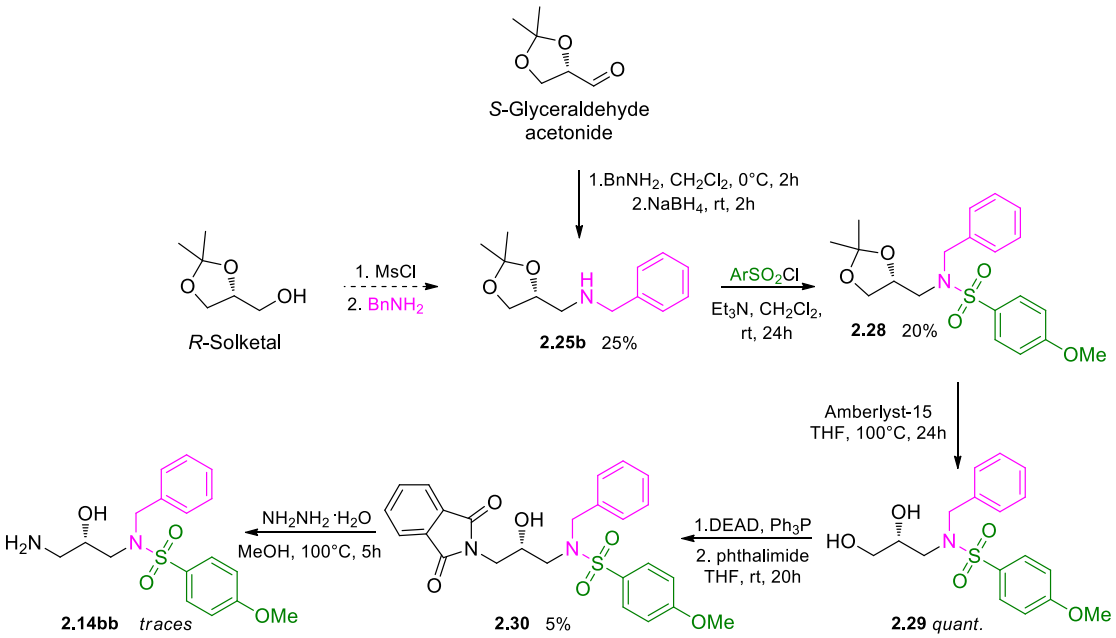



**Scheme 2.17**

In the same way, using the corresponding enantiomers, intermediate **2.24** could be obtained starting from *R*-solketal or from *S*-glyceraldehyde acetonide, as depicted in scheme 2.18. From *S*-glyceraldehyde acetonide the corresponding amine **2.25b** was

[9] M. Goubert, L. Toupet, M.E. Sinibaldi, I. Canet, *Tetrahedron* **2007**, *63*(34), 8255.

generated by reductive amination.[10] Sulfonyl group was introduced to obtain sulfonamide moiety and acetonide was deprotected by reaction with Amberlyst-15 to afford diol **2.29**. The diol was subsequently activated with phthalimide affording the intermediate **2.30**, which was reacted with hydrazine to give **2.14bb** in low yield. Although the way is now open, reactions need to be optimized in order to gain synthetic applicability.




**Scheme 2.18**

[10] C. Guerin, V. Bellosta, G. Guillamot, J. Cossy, *Organic letters* **2011**, *13*(13), 3534.

## ***2.2 Biological assays***

Some of synthesized molecules were tested as antiviral compounds and antitumor agents. In particular, as antiviral, their activity against recombinant protease was studied using FRET (Fluorescence Resonance Energy Transfer) methodology in collaboration with Prof. Berti of University of Trieste. Results are reported as $IC_{50}$ values are the mean of at least three independent experiments. For anticancer activity, in collaboration with Dr. Armentano of the University of Basilicata, cytotoxic activity was studied on human hepatocarcinoma cell lines (HepG 2) compared with healthy hepatocytes (IHH).

### *2.2.1 FRET methodology*

Fluorescence resonance energy transfer (FRET) is a mechanism describing energy transfer between two chromophores[11]. A donor chromophore in its electronic excited state transfers energy to an acceptor chromophore. The efficiency of this energy transfer is inversely proportional to the distance between donor and acceptor, making FRET extremely sensitive to small changes in distance. In particular, in this case, probe is constructed by linking two fluorescent proteins, one cointaing *p*-nitrophenylalanine, fluorescence donator and the other cointaing 2-aminobenzoic acid, fluorescence acceptor, to afford esapeptide Abz-NF*-6. The cleavage of the linker peptide by HIV protease leads to the separation of *p*-$NO_2$Phe residue from Abz residue, quenching FRET between the two chromophores and increasing fluorescence intensity (figure 2.2). This implies that enzyme activity can be associated with measured fluorescence intensity values: how much more the fluorescence is intense, the activity carried out by the enzyme HIV Pr is greater. Conversely, the addition of a protease inhibitor prevents the cleavage of the linker peptide by the protease, allowing FRET from *p*-$NO_2$Phe residue to Abz residue. Thus, HIV protease inhibition can be determined by measuring the FRET signal change generated from the probe. Inhibitory activities are reported as $IC_{50}$ values, corresponding to concentration that inhibits the enzymatic activity by 50%.

[11] P.C. Cheng, In Pawley, James B. (ed.) *Handbook Of Biological Confocal Microscopy* (3rd ed.). New York, NY: Springer. pp. 162–206, **2006**.

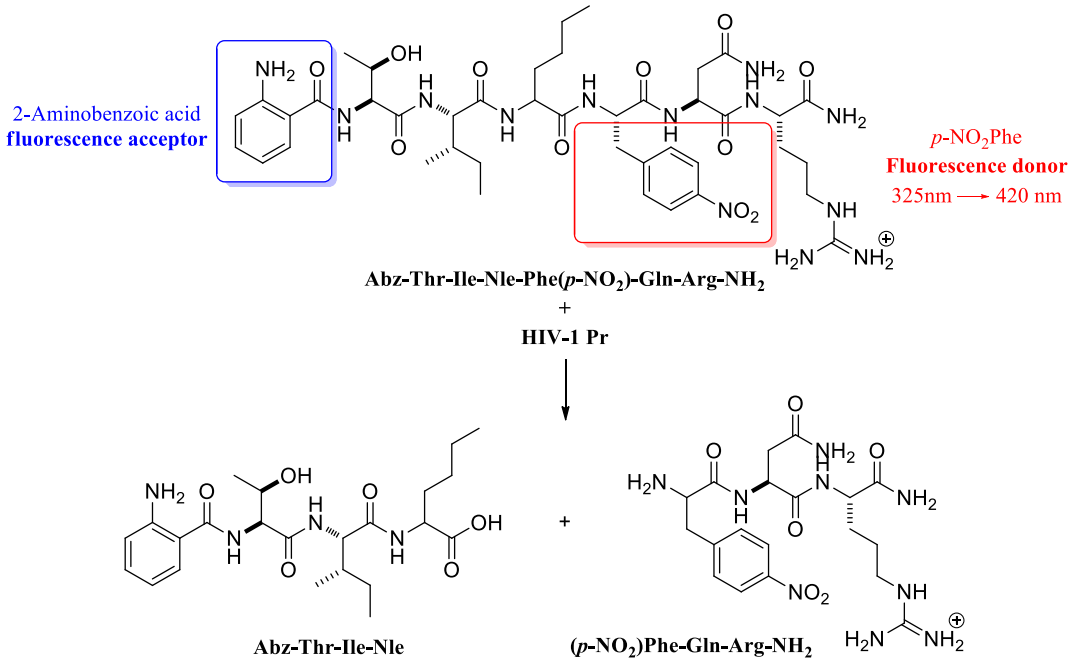



**Figure 2.2**

Once the fluorescence values are measured, the maximum value represents the highest activity of the enzyme, *i.e.* when the inhibitor is absent. Then, increasing concentration of the inhibitor, all the values obtained are interpolated in a straight line that compares the enzymatic activity as a function of the concentration, which is at maximum in the absence of the inhibitor until it is annulled at a certain concentration. From this line it is possible to determine the concentration value necessary to inhibit the activity of the enzyme by 50% ($IC_{50}$).

### *2.2.2 MTT assay*

The MTT assay is a colorimetric assay for assessing cell metabolic activity[12]. The name MTT is related to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; this tetrazolium yellow dye can be reduced in living cells to its formazane, which has a purple color, by cellular oxidoreductase enzymes (figure 2.3)[13]. In particular, this oxidoreductase enzyme activity is proportional to the number of viable cells present because reduction of

[12] J.C. Stockert, R.W. Horobin, L.L. Colombo, A. Blázquez-Castro, *Acta Histochemica* **2018**, *120*, 159.
[13] T. Mosmann, *Journal of Immunological Methods*, **1983**, *65* (1–2), 55.

MTT tetrazolium dyes depends on the cellular metabolic activity due to NAD(P)H flux. Thus tetrazolium dye assays can be used to measure cytotoxicity (loss of viable cells) of potential medicinal agents and toxic materials. MTT assays are usually carried out in the dark since the MTT reagent is sensitive to light.

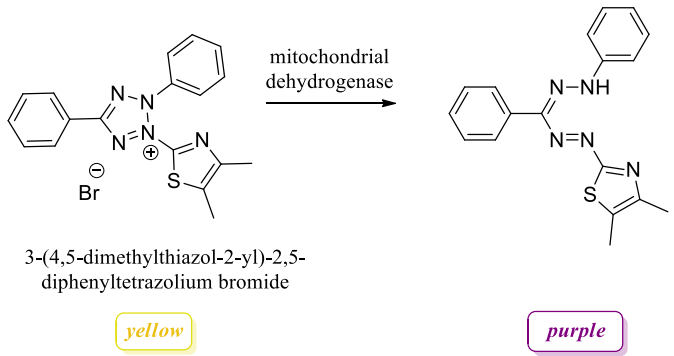



**Figure 2.3**

A solubilization solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength, usually between 500 and 600 nm, by a spectrophotometer. Then, increasing concentration of the drug, the purple coloration decreases gradually, indicating the decrease of the viable cells in which the enzyme dehydrogenase acts. All the values obtained are interpolated in a straight line that compares the cell viability as a function of the drug concentration, which is at maximum in the absence of the drug until all cells die at a certain drug concentration. From this line it is possible to determine the concentration value necessary to inhibit the metabolic activity of 50% of cells ($IC_{50}$).

## ***2.3 Results and discussion***

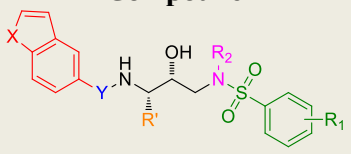
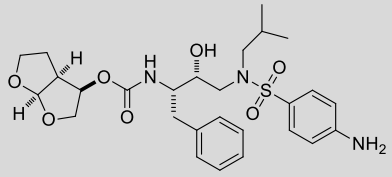
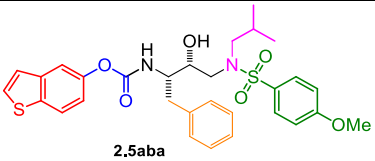
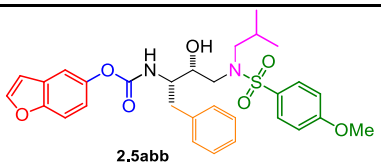
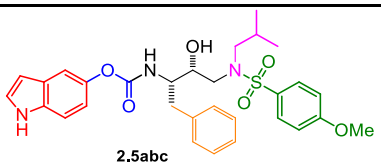
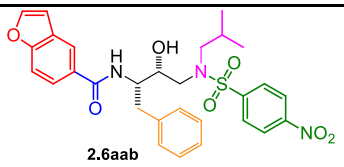
Compounds **2.5ab(a-c)**, **2.5bbc**, **2.6 aab**, **2.6b(a-c)(a-b)**, **2.6adb**, reported in scheme 2.1 were tested by FRET methodology as HIV-protease inhibitors and studies are underway to evaluate their citotoxicity.

Compounds **2.3a(a-d)**, **2.3b(a-c)**, reported in scheme 2.1, were tested as antitumor compounds on human hepatocarcinoma cell lines (HepG 2) and compared with healthy hepatocytes (IHH). Compounds **2.15 ab(a-c)**, reported in scheme 2.9, were tested both as antitumor compounds and as HIV-protease inhibitors.

Compounds **2.14a(b-c)**, **2.14bb** (scheme 2.9), were synthetized and studies to evaluate their cytotoxicity are underway; **2.6 aaa**, **2.6 ab(a-b)**, **2.6 b(a-c)c** (scheme 2.1) and **2.15 acc** (scheme 2.9) were synthetized and studies to evaluate their biological activity as HIV-protease inhibitors and as antitumor compounds are underway.

### *2.3.1 Results on antiviral activity (FRET methodology)*

Biological assay were performed at University of Trieste and results were reported as $IC_{50}$ values in table 2.1; they are the mean of at least three independent experiments. In table 2.1a results obtained when a benzyl group is present in central *core* are reported[14].

| **Compound** | **Inhibition activity $IC_{50}$ (nM)** |
|---|---|
|  | |
|  **Darunavir** | 1.8 ± 0.3 |
|  **2.5aba** | 10.8 ± 1.2 |
|  **2.5abb** | ≤ 0.6* |
|  **2.5abc** | ≤ 0.6* |
|  **2.6aab** | 4.0 ± 0.5 |

[14] a) F. Tramutola, M. F. Armentano, F. Berti, L. Chiummiento, P. Lupattelli, R. D'Orsi, R. Miglionico, L. Milella, F. Bisaccia, M. Funicello, *Bioorganic & Medicinal Chemistry* **2019**, *27*, Issue 9, 1863. b) C. Bonini, L. Chiummiento, M. Funicello, P. Lupattelli, F. Tramutola, Italian Patent n° **102016000115597**.

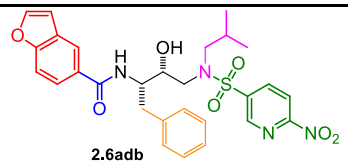
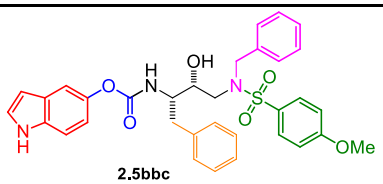
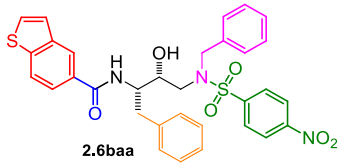
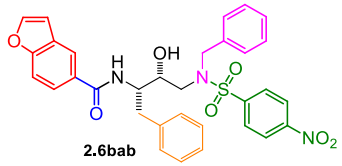
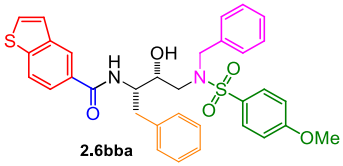
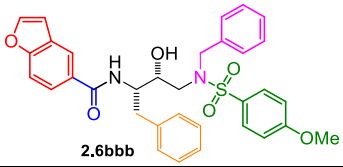
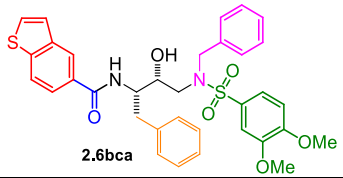
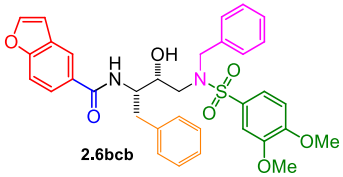
| | |
|---|---|
|  2.6adb | 11 ± 2.4 |
|  2.5bbc | 55 ± 11 |
|  2.6baa | 4.2 ± 0.6 |
|  2.6bab | 47 ± 6 |
|  2.6bba | 2.3 ± 0.4 |
|  2.6bbb | 9.6 ± 0.1 |
|  2.6bca | 6.2 ± 2.1 |
|  2.6bcb | 82 ± 7 |

**Table 2.1**

$IC_{50}$ of HIV-protease inhibitors;

*as these values are very close to one half of the enzyme concentration used in the assay, they should be regarded as estimated upper limits

All the inhibitors proved to be active, with excellent $IC_{50}$ values, in some cases comparable with that of the darunavir, taken as a reference compound due to the structural similarity.

In particular, when $R_2$ is an *i*-Bu, as it can be seen, compounds **2.5abb** and **2.5abc**, in which the heteroaryl group is spaced from the core by carbamoyl functionality are the most powerful inhibitors on the list, their $IC_{50}$ values being less then 0.6 nM. Their activity is therefore higher than that of their corresponding amides, like compound **2.6aab** or those described in our previous work (see figure 1.8)[1]. Furthermore the thesis is confirmed that longer spacer between heteroaryl group and the *core* matches well with 4-OMe-arylsulfonamide moiety. The heteroarenes that have best activity in this case are benzofuran and indole ring. The inhibitor containing pyridine as heterocycle in arylsulfonamide moiety showed good activity but less than the others corresponding methoxyphenylsulfonamides (figure 1.8).

When $R_2$ is a benzyl group, instead, longer spaces as carbamoyl moiety between heteroarene and the *core* decreases the inhibition activity, so the carboxyamide functionality is required. In this case substituent on arylsulfonamide moiety seems not very important for the activity, while the substantial difference relies on the heteroarene bound to the *core*: benzothiophene seems to be the best one, unlike the previuos series in which $R_2$ is a *i*-Bu group.

From the crystallographic analysis[15] it is well know that HIV protease is an aspartyl protease of homodimeric nature with C2 symmetry, consisting of 99 amino acids per monomer. It contains an extended β-sheet region (a rich fold of glycine) known as flap, which constitutes an important part of the enzymatic active site, and the two aspartates, Asp-25 and Asp-25', which are essential for the catalytic activity and lie in the central portion of the enzymatic cavity. In particular, the enzyme has subsites that interacts with the substrate (figure 2.4). Using the standard nomenclature, the subsites S1 and S1'(S2 and S2', etc.) are structurally equivalent. The two S1 subsides are very hydrophobic while the two S2 subsides are mainly hydrophobic except for Asp-29, Asp-29', Asp-30 and Asp-30'.

[15] A. Wlodawer, M. Miller, M. Jaskolski, B.K. Sathyanarayana, E. Baldwin, I.T. Weber, L. Selk, L. Clawson, J. Schneider, S.B.H. Kent, *Science* **1989**, *245*, 616.

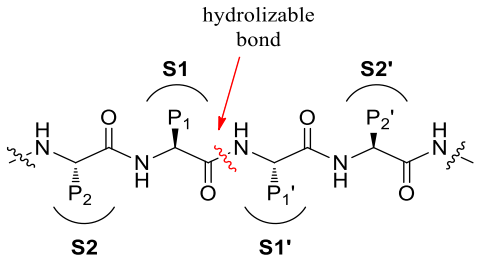



**Figure 2.4**

In order to explain such results, a series of docking runs were made by Prof. Berti of the University of Trieste on the tested inhibitors to evaluate the interaction with the enzyme. The calculation was carried out with Autdock Vina[16]. The best poses were further refined by MD runs, which were carried out with the Gromacs package[17].

Carbomoyl compounds where $R_2$ is an *i*-Bu group were analized first. As outlined in the chapter 1, the same protocol was exploited preliminarly to test the effect of a double substitution at the sulfonamide end of the potential inhibitors with carbamoyl functionality, and the outcome was clearly unfavorable. Data showed that, despite the longer linker connecting the heteroaryl moiety with the core unit of the inhibitors, both **2.5abb** and **2.5abc** can interact very well with the S2 subsite. Moreover, the benzofuran system of **2.5abb** could accept hydrogen bonding from Asp130 either at its side chain or at its backbone NH (figure 2.5). The number of favorable interactions is indeed the same obtained with the corresponding amides already described (compound **E** of figure 1.8), as reported in table 2.1a.

[16] O. Trott, A.J. Olson, *J. Comput. Chem.* **2010**, *31*, 455.
[17] B. Hess, C. Kutzner, D. Van der Spoel, E. Lindahl, *J Chem Theory Comput.* **2008**, *4*, 435.

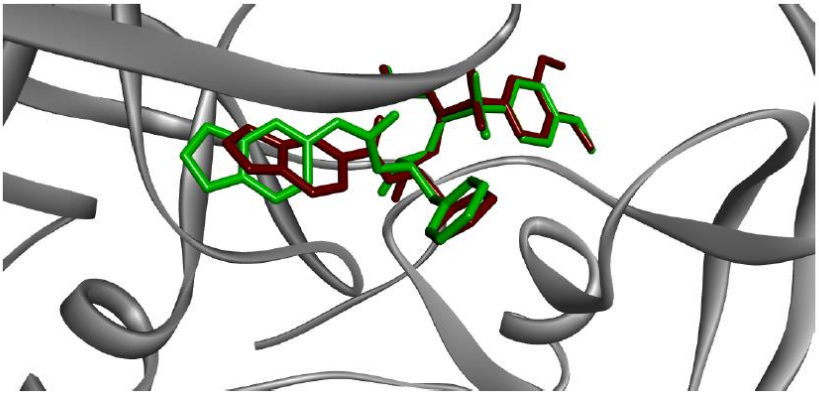


**Figure 2.5**
Overlay of the optimized complex of HIV-protease with compound **2.5abb** (green) with that of corresponding benzofuryl amide, compound **E** of figure 1.8 (red).

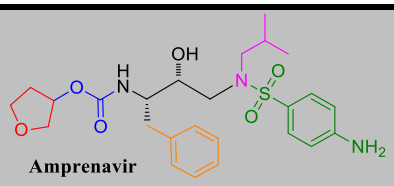
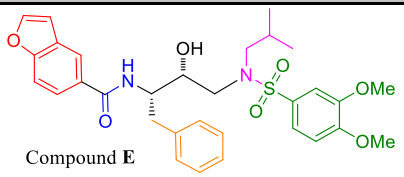
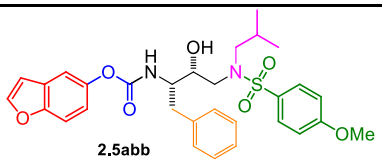
| Inhibitor | S1 | S2 |
|---|---|---|
|  Amprenavir | 4 | 11 |
|  Compound **E** | 2 | 9 |
|  **2.5abb** | 2 | 9 |

**Table 2.1 a**
Number of favorable contacts established by the inhibitors at the S1 and S2 subsites of the protease.

When $R_2$ is a benzyl group and the heteroarene is spaced from *core* by carbamoyl functionality (**2.5bbc**), the activity decreases, so a carboxyamide functionality is necessary.

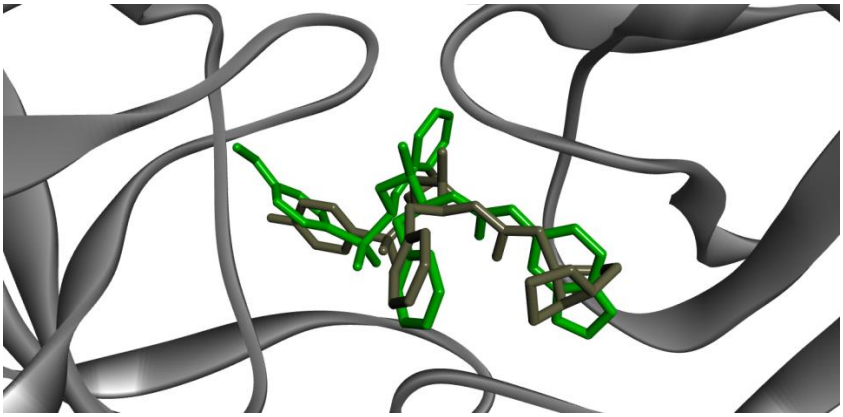


**Figure 2.6**
Overlay of the optimized complex of HIV-protease with Darunavir (grey) and compound **2.5bbc** (green).

The geometry of the two complexes seems to suggest that the crucial point is the different length of the two molecules (figure 2.6). The indolic system is well inserted in S2', although very differently oriented with respect to the corresponding residue of Darunavir, and it still manages to share a hydrogen bond between its indolic NH to AspB30. The rest of the molecule, however, is significantly shifted away from S2, and the methine hydroxide does not interact optimally with the catalytic aspartates. The group in S2 is almost completely off site.

Instead, when $R_2$ is a benzyl group and the heteroarene is spaced from the *core* by carboxyamide functionality, the activity increases. In figure 2.7 overlay of complex of HIV-Pr with Darunavir and benzofuryl compounds **2.6bbb** and **2.6bcb.**

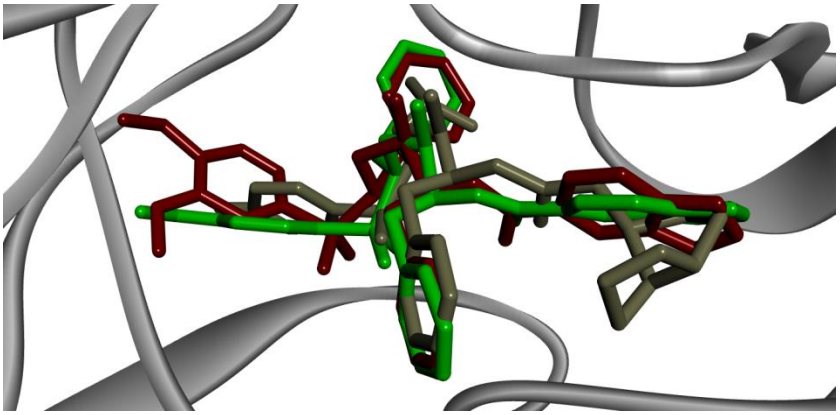


**Figure 2.7**
Overlay of the optimized complex of HIV-protease with Darunavir (grey) and compounds **2.6bbb** (red) and **2.6bcb** (green).

Both compounds **2.6bbb** (red) and **2.6bcb** (green) are able to align the benzyl chains in S1' as that of Darunavir (grey), and those in S1 are well overlapped with the isobutyl chain of Darunavir. Also the benzofuran groups in S2' are practically in the same position and their oxygen is perfectly superimposed with one of the two oxygens of the octane dioxabicycle of Darunavir, and preserves the same interactions. Nothing replaces the second oxygen of Darunavir. However the three groups in S1, S1' and S2' link the two inhibitors well at the site, and consequently also the methine hydroxyl of the transition state analogue is correctly oriented and establishes the usual network of hydrogen bonds with the catalytic aspartates 25 and 25'. The problem of **2.6bcb** is all at the level of S2, because there is not enough space for the two methoxyls and the structure distorts both the inhibitor level and the protein level. It can be observed that the sulfonyl group of **2.6bcb** is in a different position from those of Darunavir and **2.6bbb**, and the aromatic ring is on a different plane and in a different position. In energy terms, the distortion is not very large, and indeed the analogue **2.6bca** with thiophene recovers the order of magnitude of affinity thanks to the already discussed presence of sulfur. For the same reason **2.6bba**, which has the same substituents as **2.6bbb** and thiophene, is the best inhibitor of the series where $R_2$ is benzyl group. The mapping of its interactions is shown in figure 2.8.

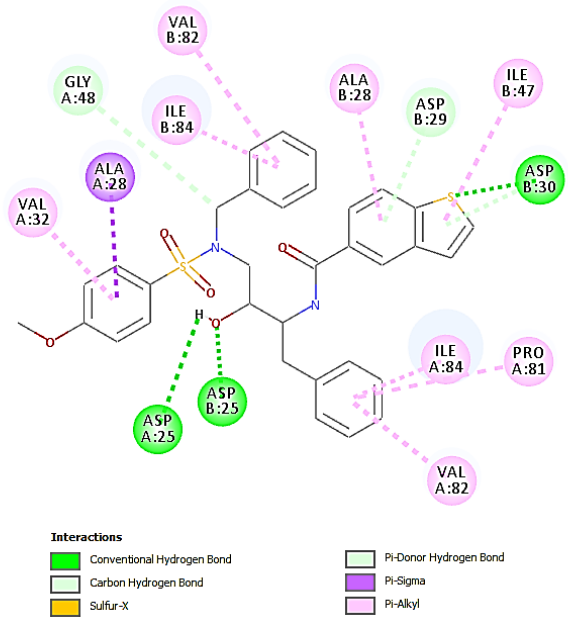



**Figure 2.8**
**2.6bba** inhibitor interactions with the enzyme subsites

It is interesting to note that the analysis identifies specific interactions of the thiophene sulfur with AspB30, which describes both as a hydrogen bond and as a sulfur-oxygen interaction.

When the *p*-nitro aromatic system is found in S2, the situation is delicate. The nitrogroup can accept hydrogen bonds and establish electrostatic interactions with cations at the level of oxygen, and with anions at the level of the nitrogen atom. However, it may also be close to groups that interact unfavorably in electrostatic terms. The best example is, again, the derivative with the thiophene **2.6baa**. In this complex the presence of the nitrogroup is probably favorable due to the formation of the hydrogen bonds, while the electrostatic interaction is overall favorable. As already seen, at the opposite extreme the thiophene interacts favorably with AspB30 (figure 2.9).

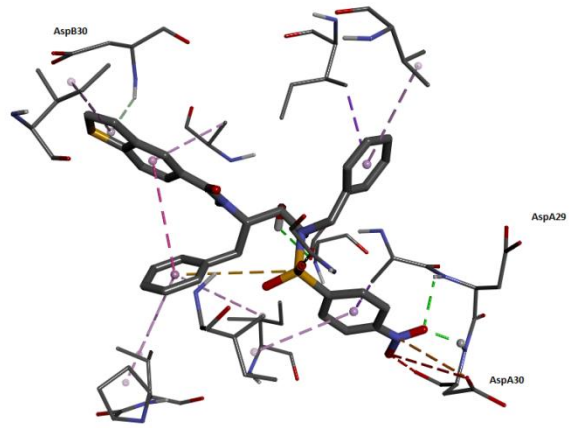



**Figure 2.9**
**2.6baa** interactions with catalytic site. Green: H-Bond.

In **2.6bab**, an oxygenated analogue of **2.6baa**, the interaction with sulfur is lost and the nitroaromatic group moves slightly enough to lose the favorable hydrogen bonds. In **2.6aab** the benzyl in S2 is replaced by an isobutyl and the molecule is arranged better, the electrostatic interactions with the nitrogroup seem to be favorable overall (figure 2.10).

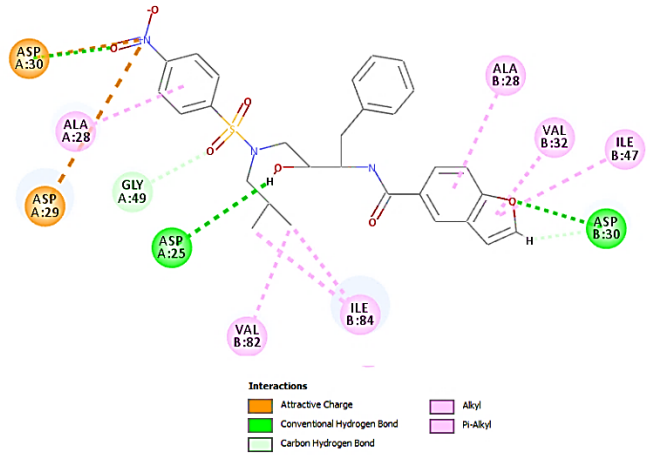
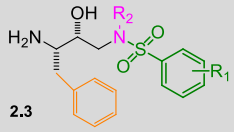
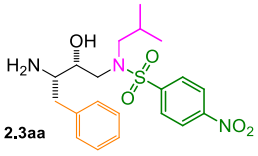
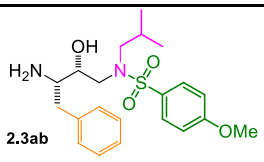
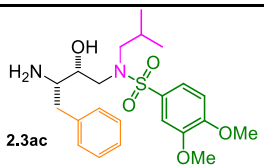
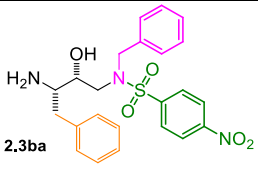



**Figure 2.10**
**2.6aab** interaction with catalytic site.

### *2.3.2 Results on anticancer activity (MTT assay)*

Biological assays were made at laboratory of Prof. Bisaccia, University of Basilicata and cytotoxicity on hepatocarcinoma cell lines (HepG2) was compared to healthy hepatocytes (IHH).

Results on compounds with free amine are reported as $IC_{50}$ values in table 2.2; they are the mean of at least three independent experiments. In table 2.2a results obtained when an heterocycle is linked to unsubstituted central *core* are reported compared to inhibition activity of HIV-protease.

| **Compound**  2.3 | **IHH** **$IC_{50}$ (μM)** | **HepG2** **$IC_{50}$ (μM)** |
|---|---|---|
|  2.3aa | 57.4 | 50.1 |
|  2.3ab | 66.5 | 46.1 |
|  2.3ac | >100 | 85.2 |
|  2.3ba | | >100 |

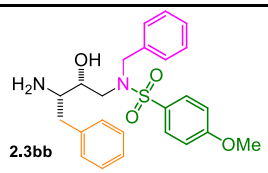
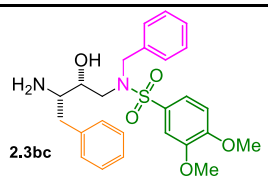
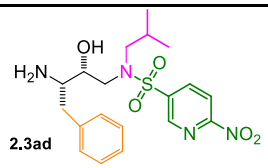
| | | |
|---|---|---|
|  **2.3bb** | 62.7 | 34.2 |
|  **2.3bc** | >100 | 62.7 |
|  **2.3ad** | | 62.4 |

**Table 2.2**
Cytoxic activity of synthesized compounds, without heterocycle.

As it can be see, when the $IC_{50}$ resulted too high, compound was not taken into consideration to evaluate activity on tumor cells. According to reported data, best activity was obtained with compound **2.3bb**, differently from what is reported by Facchinetti[18], where $NO_2$ group on sulfonamide moiety increases the anticancer activity. Anyway, it has been noted that the tumor cell lines analyzed are quite different, probably the cytotoxic activity has different mechanism.

For compounds with heterocyclic moiety cytotoxic activity decreases as expected; this confirm the probably important activity of the free amine on the left side of the molecule. Results are reported in table 2.2a.

[18] V. Facchinetti, M. Moreth, C.R.B. Gomes, C. O Pessoa, F.A.R. Rodrigues, B.C. Cavalcanti, A.C.A. Oliveira, T.R. Carneiro, I.L. Gama, M.V.N. De Suoza, *Med Chem Res* **2015**, *24*, 533.

### *2.3.2 Results on combinated antiviral and anticancer activity*

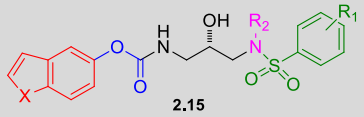
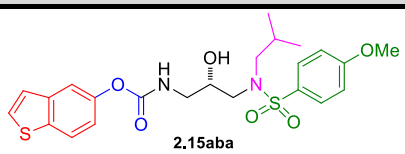
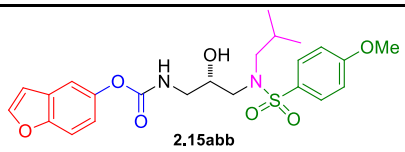
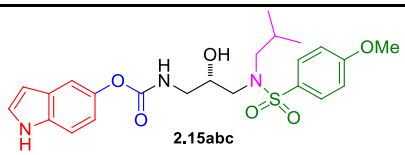
| Compound | Antiviral activity $IC_{50}$ (nM) | IHH $IC_{50}$ (μM) | HepG2 $IC_{50}$ (μM) |
|---|---|---|---|
|  2.15 | | | |
|  2.15aba | 49±7 | | |
|  2.15abb | 15400 ± 500 | | |
|  2.15abc | 13400 ± 220 | 400 | 253 |

**Table 2.2a**
Cytoxic activity of synthetized compounds, with heterocycle.

As it can be seen, these compounds showed very low inhibition activity compared to the correspondent heteroaryl carbamates bearing a benzyl group in the central *core*. Nevertheless the cytotoxic activity of the **2.15abc** derivative is of particular interest. The data show a dose-dependent trend in cell viability following treatment for 24h the cytotoxic activity of the **2.15abc** derivative is of particular interest with this compound. Cytotoxic effects found on HepG 2 are also greater than those found on non-tumor cells, showing a certain selectivity of **2.15abc** towards tumor cells compared to non-tumor cells. Studies are underway to understand its effect on cancer cells and its inhibiting growth mechanism; preliminary tests have shown its probable apoptotic activity, probably due to the proteasoma inhibition.

Also in this case a series of docking runs was made in order to explain such results on antiviral activity. When the benzyl group is removed from the central *core* of the

molecules as in **2.15abb** and **2.15abc**, two main docking solutions are found, but both fail to reach the maximum number of interactions attainable with the amides or with reference inhibitors as Amprenavir. In the first option, the heterocyclic side chain is still found inside S2, with similar interactions, but of course the S1 subsite is completely empty. The second solution is similar to that obtained in our previous work[1] on the corresponding amides (as compound **D**, figure 1.8) lacking of the benzyl group; in fact if benzyl group is present, the S1 site is occupied by the benzyl moiety, as in Amprenavir. This pushes the benzofuryl system completely inside the S2 site, thus allowing to keep many of the interactions observed in Amprenavir. In **2.15** conformation of the inhibitor becomes very different due to lacking a canonical benzyl group and the heteroaryl system is in an intermediate position between S1 and S2. But in this case the connecting chain is too long, and the aromatic system is largely exposed to the solvent outside the catalytic site of the enzyme, without recovering favorable interactions, as it can be clearly seen in the top view of figure 2.11 and in table 2.2b.

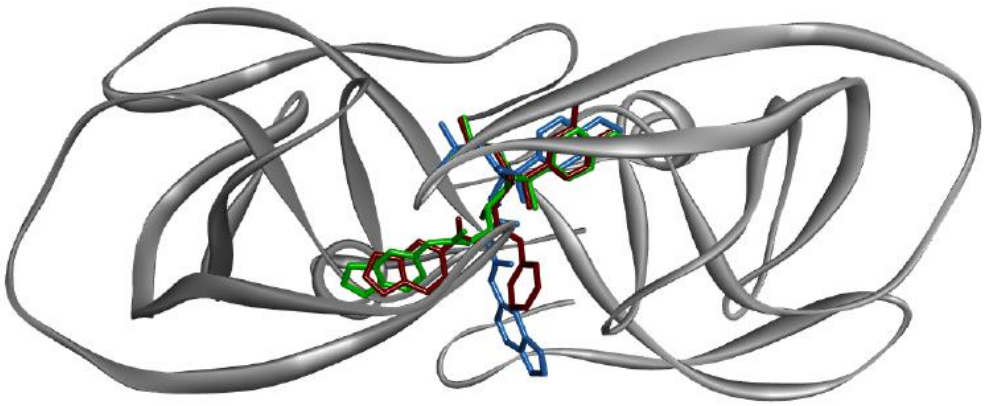


**Figure 2.11**
Top view of the overlay of the best docking solutions for compound **2.15abb, 2.15abc** (green and blue) with the corresponding amide compound **D** (red).

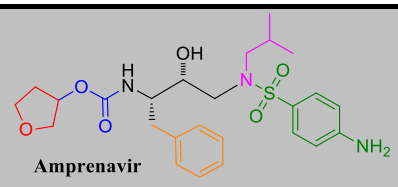
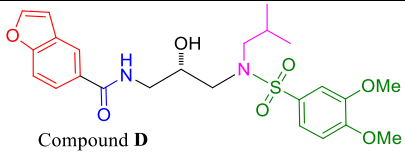
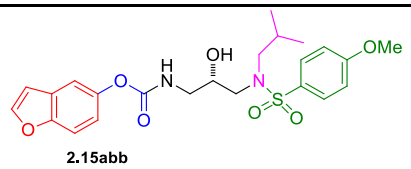
| Inhibitor | S1 | S2 |
|---|---|---|
|  Amprenavir | 4 | 11 |
|  Compound **D** | 11 | 0 |
|  2.15abb | 0 | 8 |

**Table 2.2b**
Number of favorable contacts established by the inhibitors
at the S1 and S2 subsites of the protease

## ***2.4 Experimental section***

### *2.4.1 General*

$CH_2Cl_2$ was dried by distillation over anhydrous $CaCl_2$ in an inert atmosphere; toluene and THF were dried using Na/benzophenone. Dry DMF, *i*-PrOH, 1,4-dioxane, MeCN and chlorobenzene were commercially available.

All reactions in non-aqueous media were conducted under a positive pressure of dry argon in glassware that had been oven dried prior.

All commercially available reagents were used without further purification unless otherwise noted.

Column chromatography was carried out on Merck silica gel 60 (70-230 mesh).

$^{1}H$ and $^{13}C$ NMR spectra were normally carried out in $CDCl_3$ solutions on a Varian Inova 400 MHz with TMS as an internal reference; the chemical shifts are reported in ppm in $\delta$ units. Signal splitting patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint),or multiplet (m), with coupling constants (*J*) in Hertz.

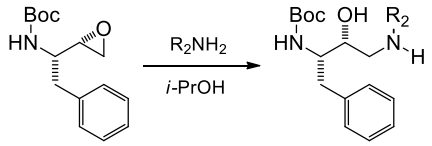
Mass spectra were obtained with a Hewlett–Packard 5971 mass-selective detector on a Hewlett–Packard 5890 gas chromatograph [(OV-1 capillary column between 70 and 250 °C (20 °C $min^{-1}$)]. The optical purity was evaluated by using a polarimeter JASCO Mod Dip-370.

Thin-layer chromatography (TLC) was carried out on precoated silica gel *60* plates (0.2 mm thickness) with the indicated solvents, and the plates were scanned under ultraviolet light at 254 and 365 nm.

Some intermediates were not isolated but exclusively analyzed by GC/MS so their characterization is not completely reported.

### *2.4.2 Sperimental procedures*

Epoxide opening: diaminoalcol




**2.1a** $R_2$=*i*-Bu *quant.*
**2.1b** $R_2$=Bn 88%
**2.1c** $R_2$=3-methyloxetan-3-yl-methan 93%

- (1*R*,2*S*)-(1-Benzyl-2-hydroxy-3-*iso*butylamino-propyl)-carbamic acid *tert*-butyl ester (**2.1a**)

*i*-Bu$NH_2$ (80 mmol) was added to a stirred solution of (2*S*,3*S*)-1,2-epoxy-3-(Boc-amino)-4-phenylbutane (4.0 mmol) in *i*-PrOH (20 mL). The mixture was warmed at 60°C. After 6h the solvent and the excess of *i*-Bu$NH_2$ were removed under reduced pressure. The product **2.1a** was obtained as white solid in quantitative yield.

$[\alpha]\,_D^{20} = +7.0°$ (c : 1.0, $CHCl_3$).

**$^1$H NMR** (500 MHz, $CDCl_3$): δ 7.25 (m, 5H), 4.71 (d, *J* = 8.4 Hz, 1H), 3.80 (m, 1H), 3.48 (dd, *J* = 10.8 Hz, *J* = 6.3 Hz, 1H), 3.02 (d, *J* = 4.7 Hz, 1H), 2.99 (d, *J* = 4.7 Hz, 1H), 2.87 (dd, *J* = 13.7 Hz, *J* = 7.9 Hz, 1H), 2.70 (m, 2H), 2.45 (m, 2H), 1.73 (m, 1H), 1.35 (s, 9H), 0.93 (d, *J* = 6.6 Hz, 6H).

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ 155.9, 137.9, 129.5, 128.4, 126.3, 79.4, 70.6, 57.9, 54.1, 51.4, 36.7, 28.3, 28.3, 20.5, 20.5.

- *tert*-butyl (2*S*,3*R*)-4-(benzylamino)-3-hydroxy-1-phenylbutan-2-ylcarbamate (**2.1b**)

Compound **2.1b** was prepared from a solution of of (2*S*,3*S*)-1,2-epoxy-3-(Boc-amino)-4-phenylbutane (1.6 mmol) and benzylamine (1.5 mmol) in *i*-PrOH (10 mL) that was stirred under reflux for 16 h. The reaction mixture was rotary evaporated, and the crude product was purified by recrystallization in methanol/water (7:3). Compound **2.1b** was obtained as a white solid, yield 88%.

$[\alpha]\,_D^{20} = +2.4°$ (c : 1.0, $CHCl_3$).

**$^1$H NMR** (400 MHz, DMSO-$d_6$): δ 7.27 (m, 10H,), 6.66 (d, *J*= 7.2 Hz, 1H), 6.58 (d, *J* = 7.2 Hz, 1H), 4.78 (m, 1H), 3.73 (m, 1H), 2.97 (dd, $J_1$= 11.0 Hz, $J_2$ = 2.8 Hz, 2H), 2.56 (m, 1H), 2.59 (dd, $J_1$= 9.6 Hz, $J_2$ = 2.4, 1H), 2.48 (m, 1H), 1.23 (s, 9H).

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$): δ 155.2, 140.9, 139.8, 139.7, 129.3, 129.2, 129.1, 128.1, 127.9, 126.5, 125.6, 77.3, 71.9, 55.1, 53.1, 51.9, 36.1, 3.

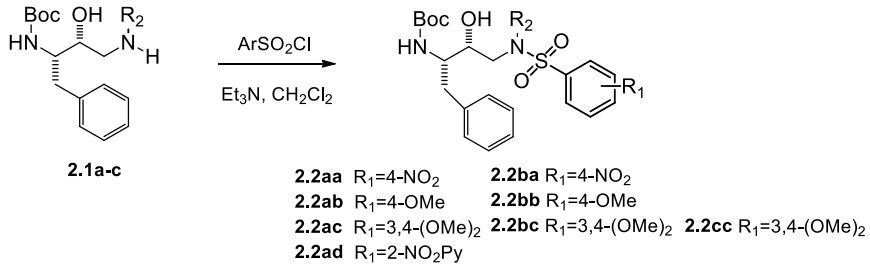
- *tert*-butyl ((2*S*,3*R*)-3-hydroxy-4-(((3-methyloxetan-3-yl)methyl)amino)-1-phenylbutan-2-yl)carbamate (**2.1c**)

(3-methyloxetan-3-yl)methanamine (8.8 mmol) was added to a stirred solution of (2*S*,3*S*)-1,2-epoxy-3-(Boc-amino)-4-phenylbutane (4.0 mmol) in *i*-PrOH (10 mL). The mixture was kept stirring at room temperature to avoid the ring opening. After 6h the solvent were removed under reduced pressure and the crude was purified by column chromatography on silica gel ($CHCl_3$/MeOH 9/1). The product **2.1c** was obtained as a white solid, 93% yield.

**$^1$H NMR** (500 MHz, $CDCl_3$): *δ* 7.30 (m, 5H), 4.60 (m, 6H), 3.87 (bs, 1H), 3.51 (bs, 1H), 3.20-2.80 (m, 6H), 1.35 (m, 12 H).

The obtained intermediate **2.1c** was used in the subsequent reaction.

General procedure for preparation of compounds **2.2**




**2.1a-c**

**2.2aa** $R_1$=4-$NO_2$ **2.2ba** $R_1$=4-$NO_2$
**2.2ab** $R_1$=4-OMe **2.2bb** $R_1$=4-OMe
**2.2ac** $R_1$=3,4-$(OMe)_2$ **2.2bc** $R_1$=3,4-$(OMe)_2$ **2.2cc** $R_1$=3,4-$(OMe)_2$
**2.2ad** $R_1$=2-$NO_2$Py

To a stirred solution of aminoalcohol **2.1** (0.78 mmol) in anhydrous $CH_2Cl_2$ (20 mL), $Et_3N$ (2.02 mmol) and arylsulfonyl chloride (0.93 mmol) were added at room temperature and under Ar atmosphere. After 24 h the reaction was quenched with 5% aqueous $H_2SO_4$

solution. The organic layer was washed adding saturated aqueous $NaHCO_3$ solution and brine. The organic phases collected were dried over $Na_2SO_4$, filtered and concentrated *in vacuo*. The crude was purified by column chromatography on silica gel.

- (1*S*,2*R*)-{1-Benzyl-3-[(4-nitro-benzenesulfonyl)-*iso*butyl-amino]-2-hydroxy-propyl}-carbamic acid *tert*-butyl ester (**2.2aa**)

The crude was purified on silica gel ($CH_2Cl_2$/EtOAc 98/2) and compound **2.2aa** was isolated as awhite solid, yield 89%.

$[\alpha]_D^{20} = +14.8°$ (c : 1.0, $CHCl_3$).

$^1H$ and $^{13}C$ NMR spectra were consistent to literature data[19].

- (1*S*,2*R*)-{1-Benzyl-3-[(4-methoxy-benzenesulfonyl)-*iso*butyl-amino]-2-hydroxy-propyl}-carbamic acid *tert*-butyl ester (**2.2ab**)

The crude was purified on silica gel ($CH_2Cl_2$/EtOAc 95/5) and compound **2.2ab** was isolated as a white solid, yield 87%.

$[\alpha]_D^{20} = +5.9°$ (c : 1.0, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.70 (d, *J* =8.0 Hz, 2H), 7.25 (m, 5H), 6.97 (d, *J* =8.0 Hz, 2H), 4.63 ( bs, 1H), 3.87 (s, 3H), 3.79 (m, 2H), 3.01 (m, 4H), 1.84 (m, 1H), 1.60 (m, 2H), 1.34 (s, 9H), 0.90 (d, *J* = 6.4 Hz, 3H), 0.86 (d, *J* = 6.4 Hz, 3H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 162.9, 137.8, 129.9, 129.5, 129.4, 129.13, 128.4, 126.4, 114.3, 114.2, 79.6, 72.7, 58.6, 55.6, 54.6, 53.7, 35.4, 28.2, 27.1, 20.1, 19.8.

- (1*S*,2*R*)-{1-Benzyl-3-[(3,4-dimethoxy-benzenesulfonyl)-*iso*butyl-amino]-2-hydroxy-propyl}-carbamic acid *tert*-butyl ester (**2.2ac**)

The crude was purified on silica gel ($CH_2Cl_2$/EtOAc 95/5) and compound **2.2ac** was isolated as a white solid, yield 90%.

[19] Z.-H. Yang, X.-G. Bai, L. Zhou, J.-X. Wang, H.-T. Liu, Y.-C. Wanga, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1880.

$[\alpha]_D^{20} = +9.8°$ (c : 1.0, $CHCl_3$).

**$^1$H NMR** (500 MHz, $CDCl_3$): δ 7.41 (dd, *J* = 8.4 Hz, *J* = 2.4 Hz, 1H), 7.28 (m, 6H), 6.95 (d, *J* = 8.4 Hz, 1H), 4.61 (bd, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.80 (bd, 2H), 3.14 (m, 2H), 2.93 (m, 4H), 1.87 (m, 1H), 1.35 (s, 9H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H).

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ 152.7, 149.1, 137.8, 130.1, 129.5, 128.5, 126.4, 121.3, 110.6, 109.9, 72.6, 58.5, 56.3, 56.2, 53.6, 35.5, 28.2, 27.2, 20.1, 19.9.

· (1*S*,2*R*)-{1-Benzyl-3-[(6-nitropyridin-3-sulfonyl)-*iso*butyl-amino]-2-hydroxy-propyl}-carbamic acid *tert*-butyl ester (**2.2ad**)

The crude was purified by column chromatography on silica gel (EP/EtOAc 1/1) and compound **2.2ad** was isolated as a white solid, yield 80%.

$[\alpha]_D^{20} = -2.3°$ (c : 1, $CHCl_3$).

**$^1$H NMR** (500 MHz, $CDCl_3$): δ 8.99 (s, 1H), 8.39 (d, *J* = 9 Hz, 1H), 8.32 (d, *J* = 9 Hz, 1H), 7.32 (m, 3H), 7.19 (m, 2H), 4.59 (bs, 1H), 3.79 (m, 3H), 3.35 (m, 1H), 3.25 (d, J = 15 Hz, 2H), 3.10 (m, 1H), 2.98 (m, 1H), 1.89 (m, 1H), 1.55 (s, 9H), 0.89 (m, 6H).

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ 219.8, 213.9, 208.8, 193.5, 182.4, 154.0, 147.7, 139.3, 137.1, 129.2, 128.8, 126.9, 118.1, 117.5, 80.6, 55.8, 28.2, 22.5, 19.9, 13.2, 2.0, -9.9.

· (1*S*,2*R*)-{1-Benzyl-2-hydroxy-3-[N-benzyl-(4-nitrobenzenesulfonyl)-amino]-propyl}-carbamic acid *tert*-butyl ester (**2.2ba**)

Compound **2.2ba** was isolated as white solid ($CH_2Cl_2$/EtOAc 98/2), yield 94 %.

$[\alpha]_D^{20} = +6.3°$ (c : 1.0, $CHCl_3$).

**$^1$H NMR** (400 MHz, DMSO-$d_6$): δ 8.37 (d, *J* = 9.0 Hz, 2H), 8.10 (d, *J* = 9.0, 2H), 7.33 (m, 5H), 7.21 (m, 2H), 7.13 (m, 3H), 6.63 (d, *J* = 9.0 Hz, 1H), 5.00 (d, *J* = 6.5, 1H), 4.68 (d, *J* = 15.5 Hz, 1H), 4.44 (d, *J* = 15.5 Hz, 1H), 3.46 (m, 2H), 3.35 (m, 1H), 3.10 (dd, $J_1$ = 15.0 Hz, $J_2$ = 9.0 Hz, 1H), 2.89 (dd, $J_1$ = 13.7 Hz, $J_2$ = 3.0 Hz, 1H), 2,42 (dd, $J_1$ = 13.5 Hz, $J_2$ = 11.0 Hz, 1H), 1.20 (s, 9H).

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$): δ 155.2, 149.5, 146.1, 139.3, 136.1, 129.0, 128.5, 128.4, 128.1, 127.8, 127.5, 125.6, 124.3, 77.5, 71.2, 54.9, 50.9, 50.2, 35.2 , 28.1.

- (1*S*,2*R*)-{1-Benzyl-2-hydroxy-3-[N-benzyl-(4-methoxybenzenesulfonyl)-amino]-propyl}-carbamic acid *tert*-butyl ester (**2.2bb**)

Compound **2.2bb** was obtained as white solid ($CH_2Cl_2$/EtOAc 95/5) , yield 85 %.

$[\alpha]_D^{20} = +5.4°$ (c : 1.0, $CHCl_3$).

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$): δ 7.78 (d, *J* = 8.5 Hz, 2H), 7.28 (m, 8H), 7.16–7.08 (m, 4H), 6.60 (d, *J* = 8.8 Hz, 1H), 4.94 (d, *J* = 6.0 Hz, 1H), 4.50 (d, *J* = 15.6 Hz, 1H), 4.37 (d, *J* = 15.6, 1H), 3.85 (s, 3H), 3.48 (m, 2H), 3.35 (m, 1H), 2.93 (m, 2H), 2.45 (dd, *J* = 13.8 Hz, *J* = 10.4 Hz, 1H), 1.22 (s, 9H).

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$): δ 155.2, 139.5, 136.6, 131.6, 129.2, 129.1, 128.2, 128.0, 127.8, 127.2, 125.6, 114.3, 77.4, 72.0, 54.9, 55.6, 54.8, 51.4, 50.7, 35.2, 28.1.

- (1*S*,2*R*)-{1-Benzyl-2-hydroxy-3-[N-Benzyl-(3,4-dimethoxybenzenesulfonyl)-amino]-propyl}-carbamic acid *tert*-butyl ester (**2.2bc**)

Compound **2.2bc** was obtained as a white solid ($CH_2Cl_2$/EtOAc 95/5) , yield 90 %.

$[\alpha]_D^{20} = +6.7°$ (c : 1.0, $CHCl_3$).

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$): δ 7.45 (dd, *J* = 2.0 Hz, *J* = 6.8 MHz, 1H), 7.54 (m, 10H), 6.94 (d, *J* = 8 Hz, 1H), 4.45 (d, *J* = 14, 1H), 4.16 (d, *J* = 27.2 Hz, 1H), 3.95 (s, 3H), 3.89 (s, 3H), 3.64 (d, 1H), 3.34 (d, 1H), 3.18 (d, 1H), 2.8 (m, 2H), 1.32 (s, 9H).

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$): δ 155.2, 149.5, 146.1, 139.3, 136.1, 129.0, 128.5, 128.4, 128.1, 127.8, 127.5, 125.6, 124.3, 77.5, 71.2, 54.9, 50.9, 50.2, 35.2, 28.1.

- *tert*-butyl((2S,3R)-4-(3,4-dimethoxy-N-((3-methyloxetan-3-yl)methyl) phenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamate (**2.2cc**)

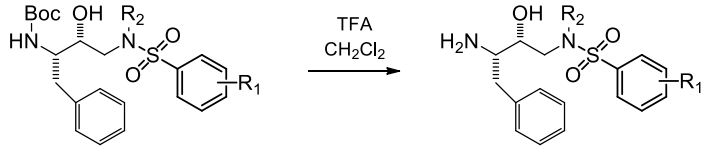
Compound **2.2cc** was obtained as white solid ($CH_2Cl_2$/EtOAc 9/1), yield 84%.

$[\alpha]\,\frac{20}{D}$ =+7.8° (c : 1.0, $CHCl_3$).

**$^1$H NMR** (500 MHz, $CDCl_3$): δ 7.40 (d, *J* = 2.0 Hz, 1H); 7.29-7.20 (m, 6H); 6.93 (s, 1H); 4.51 (m, 2H); 4.35 (m, 1H); 4.25 (s, 1H); 3.95 (s, 3H); 3.92 (s, 3H); 3.7 (bs, 1H); 3.38 (s, 2H); 3.20-2.80 (m, 4H); 1.39 (s, 3H); 1.36(s, 9H).

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ 155.6, 153.0, 150.1, 138.6, 129.2, 128.1, 128.1, 125.9, 118.4, 115.6, 112.2, 84.3, 79.5, 73.5, 58.5, 57.0, 56.1, 51.0, 35.4, 34.6, 28.4, 21.0.

General procedure for preparation of compounds **2.3**




**2.2aa-d**
**2.2ba-c**

**2.3aa-c**
**2.3ba-c**

To a stirred solution of **2.2** (0.78 mmol) in anhydrous $CH_2Cl_2$ (30 mL), trifluoroacetic acid (13 mL) was added at room temperature. After 1 hour the reaction mixture was concentrated and treated with toluene (3 x 20 mL), evaporated *in vacuum*. The crude was treated with $Et_3N$ and purified by chromatography on silica gel ($CHCl_3$/AcOEt 9/1).

- N-(3-Amino-2-hydroxy-4-phenyl-butyl)-N-*iso*butyl-4-nitrobenzenesulfonamide (**2.3aa**)

Compound was obtained as a white solid ($CH_2Cl_2$/EtOAc 9/1), yield 84%.

$^1$H and $^{13}$C NMR spectra were consistent to literature data[19].

- N-(3-Amino-2-hydroxy-4-phenyl-butyl)-N-*iso*butyl-4-methoxybenzenesulfonamide (**2.3ab**)

Compound was obtained as a white solid ($CH_2Cl_2$/EtOAc 9/1), yield 84%.

$^1$H and $^{13}$C NMR spectra were consistent to literature data[19].

- N-(3-Amino-2-hydroxy-4-phenyl-butyl)-N-*iso*butyl-3,4-dimethoxybenzenesulfonamide (**2.3ac**)

Compound was obtained as a white solid ($CH_2Cl_2$/EtOAc 9/1), yield 84%.

$[\alpha]_D^{20}$ = -2.9° (c : 1.0, $CHCl_3$).

**$^1$H NMR** (500 MHz, $CDCl_3$): δ 7.42 (dd, *J* = 2.0 Hz, *J* = 8.5 Hz, 1H), 7.25 (m, 6H), 6.93 (d, *J* = 6.8 Hz, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.71 (bs, 1H), 3.23 (m, 3H), 2.95 (m, 3H), 2.60 (m, 1H), 1.85 (m, 1H), 0.87 (m, 6H).

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ 152.6, 149.1, 138.4, 130.4, 129.3, 128.7, 126.6, 121.3, 110.6, 110.0, 72.6, 58.3, 56.3, 56.2, 55.7, 52.3, 38.6, 27.1, 20.1, 19.9.

- N-(3-Amino-2-hydroxy-4-phenyl-butyl)-N-*iso*butyl-6-nitropyridin-3-sulfonamide (**2.3ad**)

Compound was obtained as a white solid ($CH_2Cl_2$/EtOAc 9/1), yield 60%.

$[\alpha]_D^{20}$ =-10.4° (c : 1.0, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 9.01 (s, 1H), 8.62 (d, *J* = 9 Hz, 1H), 8.27 (d, *J* = 9 Hz, 1H), 7.08 (m, 5H), 4.37 (bs, 1H), 4.23 (m, 1H), 3.55 (m, 2H), 3.23 (m, 2H), 2.98 (m, 2H), 1.75 (m, 1H), 0.85 (m, 6H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 160.2, 144.3, 141.0, 128.5, 127.7, 125.9, 117.8, 79.5, 73.5, 58.5, 57.3, 50.7, 35.4, 25.5, 20.6.

- N-(3-Amino-2-hydroxy-4-phenyl-butyl)-N-benzyl-4-nitrobenzenesulfonamide (**2.3ba**)

Compound was obtained as a white solid ($CH_2Cl_2$/EtOAc 9/1), yield 41%.

$[\alpha]_D^{20}$ = +6.4° (c : 1.0, $CHCl_3$).

**$^1$H NMR** (400 MHz, DMSO-$d_6$): δ 8.34 (d, *J* = 8.6 Hz, 2H), 8,11 (bs, 2H,), 8.06 (d, *J* = 8.6 Hz, 2H), 7.26 (m, 10H), 5.67 (d, *J* = 5.6, 1H), 4.56 (d, *J* = 16.0 Hz, 1H), 4.49 (d, *J* = 16.0 Hz, 1H), 3.96 (bs, 1H), 3.38 (m, 2H), 3.16 (dd, *J*= 14.8 Hz, *J*= 8.8 Hz, 1H), 2.87 (dd, *J* = 14.4 Hz, *J* = 7.2 Hz, 1H), 2.82 (dd, *J* = 14.2 Hz, *J* = 7.6 Hz, 1H).

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$): δ 149.6, 145.3, 136.4, 135.9, 129.3, 128.6, 128.4, 128.1, 127.6, 126.8, 124.4, 67.8, 55.2, 51.5, 49.1, 32.8.

- N-(3-Amino-2-hydroxy-4-phenyl-butyl)-N-benzyl-4-methoxybenzenesulfonamide (**2.3bb**)

Compound was obtained as a white solid ($CH_2Cl_2$/EtOAc 9/1), yield 35%.

$[\alpha]_D^{20}$ =+7.5° (c : 1.0, $CHCl_3$).

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$): δ 7.75 (d, *J* = 8.4 Hz, 2H), 7.20 (m, 10H), 6.99 (d, *J* = 8.8 Hz, 2H), 4.33 (d, *J* = 14.4 Hz, 1H), 4.17 (d, *J* = 14.4Hz, 1H), 4.00 (m, 1H), 3.88 (s, 3H), 3.50 (m, 2H), 3.45 (m, 2H), 2.79 (m, 2H).

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$): δ 136.5, 130.8, 129.3, 128.5, 128.3, 128.0, 127.3, 126.7, 114.4, 68.3, 56.0, 55.7, 51.8, 49.5, 32.5.

- N-(3-Amino-2-hydroxy-4-phenyl-butyl)-N-benzyl-3,4-methoxybenzenesulfonamide (**2.3bc**)

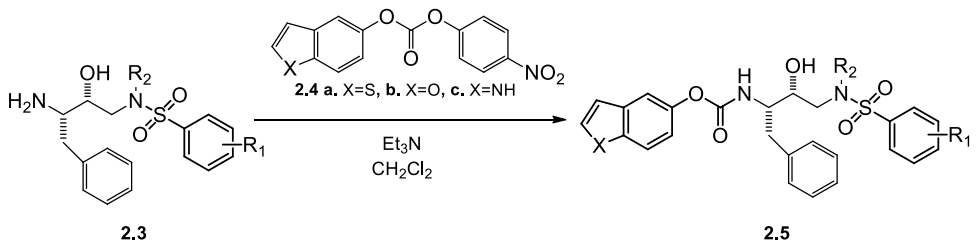
Compound was obtained as a white solid ($CH_2Cl_2$/EtOAc 9/1), yield 52%.

$[\alpha]_D^{20}$ = +8.3° (c : 1.0, $CHCl_3$).

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$): δ 7.41 (dd, *J* = 8 Hz, *J* = 2.1 Hz, 1H), 7.15 (m, 10H), 6.9 (d, *J* =8.4 Hz, 2H), 4.35 (d, *J* = 14.8 Hz, 2H), 4.18 (d, *J* = 14.8 Hz, 2H), 3.98 (s, 1H), 3.92 (s, 3H), 3.82 (s, 3H), 3.56 (s, 1H), 3.25 (m, 2H), 2.82 (m, 2H).

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$): δ 135.9, 130.5, 128.7, 128.2, 127.9, 127.6, 126.9, 126.5, 113.5, 67.9, 56.2, 55.8, 55.3, 51.1, 48.9, 31.8.

General procedure for synthesis of carbamates **2.5**




$Et_3N$ (0.24 mmol) and *p*-nitrophenylchloroformiate (0.24 mmol) were added to a solution of 5-hydroxyheteroarenes **2.7a-c** (0.24 mmol) in anhydrous $CH_2Cl_2$ (2 mL), under Ar atmosphere. The mixture was kept stirring at room temperature for 1 h to afford intermediates **2.4a-c**; then amine **2.3** (0.20 mmol) was added and the mixture was kept stirring for 24h. The solvent was evaporated and the crude compounds were purified by column chromatography on silica gel (EP/EtOAc 8/2), affording to compounds **2.5aba-c** and **2.5bbc.**

- (1*S*,2*R*)-{1-Benzyl-2-hydroxy-3-[*iso*butyl-(4-methoxy-benzenesulfonyl)-amino]propyl}-carbamic acid benzo[b]thiophen-5-yl ester (**2.5aba**)

Following the general procedure the compound **2.5aba** was obtained as a white solid, yield 71%.

$[\alpha]_D^{20}$ =+14.2° (c : 1.0, $CH_2Cl_2$).

**$^1$H NMR** (500 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.5 Hz, 1H), 7.73 (d, *J* = 8.6 Hz, 2H), 7.48 (d, *J* = 5.6 Hz, 1H), 7.42 (bs, 1H), 7.32 (m, 6H), 6.98 (d, *J* = 8.6 Hz, 2H), 6.96 (d, *J* = 7.1 Hz, 1H), 5.40 (bs, 1H), 3.98 (bs, 2H), 3.85 (s, 3H), 3.14 (m, 3H), 3.00 (m, 2H), 2.85 (dd, *J* = 13.4 Hz, *J* = 6.7 Hz, 1H), 1.88 (m, 1H), 1.76 (bs, 1H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.91 (d, *J* = 6.5 Hz, 3H).

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ 163.1, 154.9, 148.0, 140.1, 137.5, 136.6, 129.7, 129.5, 129.4, 128.6, 128.0, 126.6, 123.6, 122.8, 118.6, 115.8, 114.3, 72.5, 58.7, 55.6, 55.3, 53.7, 35.3, 27.2, 20.1, 19.9.

- (1*S*,2*R*)-{1-Benzyl-2-hydroxy-3-[*iso*butyl-(4-methoxy-benzenesulfonyl)-amino]propyl}-carbamic acid benzofuran-5-yl ester (**2.5abb**)

Following the general procedure the compound **2.5abb** was obtained as a white solid, yield 75% .

$[\alpha]_D^{20} =$ +17.5° (c : 1.0, $CH_2Cl_2$).

**$^1$H NMR** (500 MHz, $CDCl_3$): δ 7.65 (d, *J* = 8.7 Hz, 2H), 7.55 (d, *J* = 2.4 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 1H), 7.25 (m, 4H), 7.20 (m, 1H), 7.11 (bs, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.80 (d, *J* = 8.8 Hz, 1H), 6.64 (bs, 1H), 5.14 (d, *J* = 8.3 Hz, 1H), 3.87 (bs, 2H), 3.78 (s, 3H), 3.74 (bs, 1H), 3.09 (dd, *J* = 15.1 Hz, *J* = 8.3 Hz, 1H), 3.01 (m, 2H), 2.92 (m, 2H), 2.75 (dd, *J* = 13.6 Hz, *J* = 6.8 Hz, 1H), 1.79 (m, 1H), 1.18 (bs, 1H), 0.87 (d, *J* = 6.5 Hz, 3H), 0.82 (d, *J* = 6.5 Hz, 1H).

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ 163.1, 155.2, 152.4, 146.4, 146.2, 137.5, 129.7, 129.6, 129.5, 128.7, 127.9, 126.7, 118.1, 114.4, 113.6, 111.6, 106.8, 72.5, 58.9, 55.6, 55.2, 53.8, 35.3, 27.3, 20.2, 19.9.

- (1*S*,2*R*)-{1-Benzyl-2-hydroxy-3-[*iso*butyl-(4-methoxy-benzenesulfonyl)-amino]propyl}-carbamic acid 1H-indol-5-yl ester (**2.5abc**)

Following the general procedure the compound **2.5abc** was obtained as a white solid, yield 70%.

$[\alpha]_D^{20} =$-18.2° (c : 1.0, MeOH).

**$^1$H NMR** (500 MHz, $CDCl_3$, TMS): δ 8.13 (bs, 1H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.36 (m, 4H), 7.20 (m, 3H), 7.13 (m, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.72 (d, *J* = 8.5 Hz, 1H), 6.42 (bs, 1H), 5.09 (bd, *J* = 7.8 Hz, 1H), 3.85 (bs, 2H), 3.78 (s, 3H), 3.04 (m, 3H), 2.90 (m, 2H), 2.76 (dd, *J* = 13.4 Hz, *J* = 6.5 Hz, 1H), 1.80 (m, 1H), 0.86 (d, *J* = 6.5 Hz, 3H), 0.83 (d, *J* = 6.5 Hz, 3H).

**$^{13}$C NMR** (125 MHz, $CDCl_3$): δ 163.0, 155.8, 144.4, 137.5, 133.5, 129.8, 129.6, 129.5, 128.6, 128.1, 126.6, 125.5, 116.2, 114.4, 112.7, 111.2, 102.9, 72.6, 55.6, 55.3, 53.8, 35.3, 27.3, 20.0, 19.8.

- (1*S*,2*R*)-{1-Benzyl-2-hydroxy-3-[N-benzyl-(4-methoxy-benzenesulfonyl)amino]propyl}-carbamic acid 1H-indol-5-yl ester (**2.5bbc**)

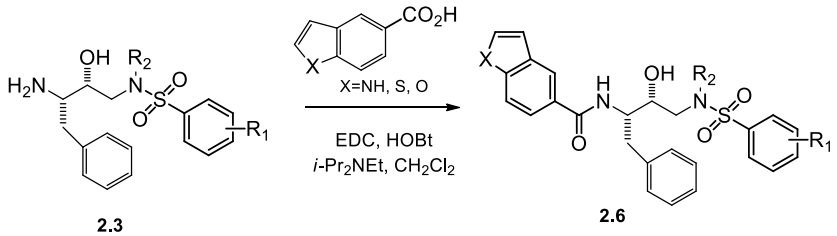
Following the general procedure the compound **2.5bbc** was obtained as a white solid, yield 35%.

$[\alpha]_D^{20}$ =-1.4° (c : 0.5, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.73 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 1H), 7.24 (m, 10H), 7.17 (d, *J* =7.6 Hz, 2H), 7.04 (d, J =7.6 Hz, 2H), 6.97 (m, 3H), 4.22 (s, 2H), 3.90 (s, 2H), 3.88 (s, 3H), 3.28 (m, 1H), 2.55 (m, 1H), 2.36 (s, 2H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 163.8, 154.4, 148.7, 138.6, 136.4, 134.8, 132.0, 128.5, 127.8, 126.1, 124.3, 119.1, 114.6, 111.5, 109.3, 102.4, 73.5, 58.5, 55.8, 53.7, 50.0, 35.4.

General procedure for carboxyamides **2.6**




To a solution of 5-heterobenzoic acid (0.13 mmol), EDCI (0.20mmol), HOBt (0.20 mmol) in anhydrous $CH_2Cl_2$, a solution of amine **2.3** (0.13 mmol) and diisopropylethylamine (0.78 mmol) in anhydrous $CH_2Cl_2$ was added at 0°C under argon atmosphere and it was allowed to stir for 16h at room temperature. The reaction mixture was quenched with water and extracted with $CH_2Cl_2$. The organic layers were dried on $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CH_2Cl_2$/AcOEt 9/1) tu furnish inhibitors **2.6**.

- N-((2*S*,3*R*)-3-hydroxy-4-(N-*iso*butyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)benzo[b]thiophene-5-carboxamide (**2.6aaa**)

Following the general procedure the compound **2.6aaa** was obtained as a white solid, yield 12%.

$[\alpha]_D^{20}$ = +6.8° (c : 0.3, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 8.31 (d, *J* = 8.4 Hz, 2H), 8.09 (d, *J* = 16.0 Hz, 2H), 7.89 (m, 3H), 7.53 (m, 3H), 7.30 (m, 14H), 6.42 (d, *J* = 8.4 Hz, 2H), 5.29 (s, 1H), 4.34 (s, 1H), 4.10 (s, 1H), 3.30 (m, 6H), 1.27 (s, 7H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 168.6, 143.1, 137.4, 129.9, 129.7, 129.3, 128.9, 128.5, 128.2, 126.9, 124.3, 124.2, 122.8, 122.6, 122.1, 72.4, 55.9, 52.6, 35.2, 27.0, 19.9.

- N-((2*S*,3*R*)-3-hydroxy-4-(N-*iso*butyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)benzofuran-5-carboxamide (**2.6aab**)

Following the general procedure the compound **2.6aab** was obtained as a white solid, yield 30%.

$[\alpha]_D^{20}$ =-0.7° (c : 0.5, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 8.24 (d, *J* =8.8 Hz, 2H), 7.87 (d, *J* =8.8 Hz, 2H), 7.82 (s, 1H), 7.64 (s,1H), 7.47 (dd, *J* =16.4 Hz, *J*=8.8 Hz, 2H), 7.24 (m, 5H), 6.75 (s, 1H), 6.37 (d, *J* = 7.6 Hz, 1H), 4.37 (d, *J* = 4 Hz, 1H), 3.97 (m, 1H), 3.07 (m, 5H), 1.82 (m, 1H), 0.83 (m, 6H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 168.7, 156.7, 150.0, 146.5, 144.7, 137.5, 129.3, 129.2, 128.8, 128.5, 127.6, 126.9, 124.4, 124.3, 123.2, 120.7, 111.5, 106.9, 72.4, 60.4, 57.7, 55.3, 52.5, 35.2, 30.9, 26.9, 19.9, 14.2.

- N-((2*S*,3*R*)-3-hydroxy-4-(N-*iso*butyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)benzo[b]thiophene -5-carboxamide (**2.6aba**)

Following the general procedure the compound **2.6aba** was obtained as a white solid, yield 11%.

$[\alpha]_D^{20}$ =+3.7° (c : 0.2, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 8.09 (s, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 7.55 (m, 2H), 7.30 (m, 6H), 6.94 (d, *J* = 9.2 Hz, 2H), 6.57 (d, *J* = 8.4Hz, 1H), 4.43 (m, 1H), 4.03 (m, 1H), 3.85 (s, 3H), 3.15 (m, 4H), 2.88 (m, 2H), 0.88 (m, 7H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 168.3, 162.9, 142.9, 139.4, 137.8, 130.3, 129.8, 129.43, 129.41, 128.7, 127.9, 126.7, 124.2, 122.6, 122.5, 122.2, 114.3, 72.9, 58.9, 55.6, 54.7, 53.6, 35.0, 27.2, 20.1, 20.0

- N-((2*S*,3*R*)-3-hydroxy-4-(N-*iso*butyl-6-nitropyridin-3-sulfonamido)-1-phenylbutan-2-yl)benzofuran -5-carboxamide (**2.6adb**)

Following the general procedure the compound **2.6adb** was obtained as s white solid, yield 36%.

$[\alpha]_D^{20}$ =-0.5° (c : 0.2, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 8.98 (s, 1H), 8.36 (m, 2H), 7.85 (s, 1H), 7.69 (s, 1H), 7.49 (s, 2H), 7.31 (m, 5H), 6.80 (s, 1H), 6.31 (d, *J* = 7 Hz, 1H), 4.24 (m, 1H), 4.01 (m, 1H), 3.36 (d, *J* = 5.5 Hz, 2H), 3.11 (m, 4H), 1.67 (m, 1H), 0.90 (d, *J* = 6 Hz, 3H), 0.86 (d, *J* = 6 Hz, 3H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 167.2, 160.2, 146.0, 144.3, 141.0, 138.6, 128.8, 128.5, 125.9, 122.7, 120.1, 117.8, 111.6, 105.9, 73.6, 57.4, 57.3, 50.7, 35.5, 25.5, 20.6.

- N-((2*S*,3*R*)-3-hydroxy-4-(N-benzyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)benzo[b]thiophene -5-carboxamide (**2.6baa**)

Following the general procedure the compound **2.6baa** was obtained as a white solid, yield 50%.

$[\alpha]_D^{20}$ =+14.5° (c : 1, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 8.29 (d, *J* = 8.8 Hz, 1H), 7.95 (m, 4H), 7.54 (d, *J* = 6.4 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.75 (m, 12H), 6.07 (d, *J* = 8.0 Hz, 1H), 4.43 (m, 2H), 4.20 (m, 1H), 3.66 (m, 1H), 3.36 (m, 2H), 3.98 (m, 2H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 168.6, 150.0, 144.9, 143.0, 139.4, 137.1, 135.0, 129.8, 129.3, 129.0, 128.8, 128.7, 128.4, 123.3, 126.8, 124.3, 124.1, 122.7, 122.1, 71.7, 54.9, 53.6, 51.5, 35.3.

- N-((2*S*,3*R*)-3-hydroxy-4-(N-benzyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)benzofuran -5-carboxamide (**2.6bab**)

Following the general procedure the compound **2.6bab** was obtained as a white solid, yield 53%.

$[\alpha]_D^{20}$ =+3.12 (c : 0.22, $CHCl_3$).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 8.18 (d, *J* = 8.8 Hz, 2H), 7.98 (d, *J* = 8.8 Hz, 2H), 8.80 (s, 1H), 8.70 (s, 1H), 7.45 (m, 12H), 6.82 (s, 1H), 6.03 (d, *J* = 8.0 Hz, 1H), 4.25 (m, 2H), 4.16 (m, 1H), 3.67 (m, 1H), 3.17 (m, 2H), 3.01 (m, 2H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 168.6, 156.7, 150.0, 146.5, 137.1, 135.0, 129.2, 128.8, 128.7, 128.4, 128.3, 127.6, 126.8, 124.3, 123.1, 120.7, 111.5, 71.7, 55.0, 53.5, 51.5, 35.3.

- N-((2*S*,3*R*)-3-hydroxy-4-(N-benzyl-4-nitrophenylsulfonamido)-1-phenylbutan-2-yl)1H-indol -5-carboxamide (**2.6bac**)

Following the general procedure the compound **2.6bac** was obtained as a white solid, yield 43%.

$[\alpha]_D^{20}$ =+25.8° (c : 1.3, $CHCl_3$).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 8.64 (s, 1H), 7.84 (s, 1H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.40-7.15 (m, 14H), 6.93 (d, *J* = 8.8 Hz, 2H), 6.59 (s, 1H), 6.03 (d, *J* =6 Hz, 1H), 4.25 (m, 2H), 3.84 (s, 3H), 3.50 (m, 1H), 3.35 (dd, *J*= 15 Hz, *J* = 4.4 Hz, 1H), 3.17 (m, 2H), 2.90 (m, 2H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 169.2, 163.0, 137.7, 136.6, 136.0, 129.5, 129.4, 128.8, 128.7, 128.5, 128.0, 127.4, 126.5, 125.7, 120.8, 120.4, 114.4, 111.0, 103.6, 72.1, 55.6, 54.5, 54.2, 52.5, 35.3

- N-((2*S*,3*R*)-3-hydroxy-4-(N-benzyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)benzo[b]thiophene -5-carboxamide (**2.6bba**)

Following the general procedure the compound **2.6bba** was obtained as a white solid, yield 57%.

$[\alpha]_D^{20}$ =+1.45° (c : 0.32, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.98 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.71 (d, *J* = 9.2 Hz, 2H), 7.53 (d, *J* = 5.6 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.37 (d, *J* = 5.2 Hz, 1H), 7.20 (m, 10H), 6.94 (d, *J* = 9.2 Hz, 2H), 6.03 (d, *J* = 8.0 Hz, 1H), 4.25 (m, 3H), 3.86 (s, 3H), 3.50 (m, 1H), 3.34 (m, 1H), 3.08 (m, 2H), 3.98 (m, 1H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 168.1, 163.1, 142.8, 139.3, 137.5, 135.9, 130.1, 129.8, 129.4, 129.4, 128.8, 128.7, 128.5, 128.1, 127.9, 126.6, 124.1, 121.6, 122.5, 122.2, 114.4, 72.0, 55.6, 54.5, 54.2, 52.5, 35.2.

- N-((2*S*,3*R*)-3-hydroxy-4-(N-benzyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)benzofuran -5-carboxamide (**2.6bbb**)

Following the general procedure the compound **2.6bbb** was obtained as a white solid, yield 54%.

$[\alpha]_D^{20}$ =+3.6° (c : 1, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7,79 (s, 1H), 7.70 (m, 1H), 7.47 (m, 2H), 7.18 (m, 10H); 6.95 (d, *J* = 8.4 Hz, 2H), 6.81 (m, 1H), 6.00 (d, *J* = 8 Hz, 1H), 4.25 (m, 3H), 3.87 (s, 3H), 3.6 (m, 1H), 3.55 (m,1H), 3.35 (m, 1H), 3.18 (m, 2H), 2.98 (m, 1H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 168.2, 163.1, 156.6, 146.4, 137.5, 135.9, 129.4, 129.4, 129.3, 128.8, 128.6, 128.5, 128.0, 127.5, 126.5, 123.2, 120.6, 114.6, 114.4, 111.4, 106.9, 72.6, 55.6, 54.5, 54.2, 52.5, 35.2, 31.9.

- N-((2*S*,3*R*)-3-hydroxy-4-(N-benzyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)1H-indol -5-carboxamide (**2.6bbc**)

Following the general procedure the compound **2.6bbc** was obtained as a white solid, yield 12%.

$[\alpha]_D^{20} =$ +10.2° (c : 0.4, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 8.47 (s, 1H) 8.24 (d, *J* = 8.8 Hz, 2H), 7.90(d, *J* = 8.8 Hz, 2H), 7.81 (s, 1H), 7.37-7.17 (m, 14H), 6.59 (s, 1H), 6.04 (d, *J* = 7.2 Hz, 1H), 4.52 (d, *J* = 14.4 Hz, 1H), 4.37 (d, *J* =14.4 Hz, 1H), 4.18 (m, 2H), 3.81-3.24 (m, 2H), 2.95 (m, 2H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): 169.7, 149.9, 145.0, 137.7, 137.2, 135.1, 129.3, 128.8, 128.7, 128.6, 128.4, 128.3, 127.5, 126.8, 125.8, 125.3, 124.3, 120.9, 120.4, 111.1, 103.7, 71.8, 55.1, 53.4, 51.3, 35.6.

- N-((2*S*,3*R*)-3-hydroxy-4-(N-benzyl-3,4-dimethoxyphenylsulfonamido)-1-phenylbutan-2-yl)benzo[b]thiophene -5-carboxamide (**2.6bca**)

Following the general procedure the compound **2.6bca** was obtained as a white solid, yield 55%.

$[\alpha]_D^{20} =$+4.65° (c : 0.1, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.99 (d, *J*=1.6 Hz, 1H), 7.87 (d, *J*=8.8 Hz, 1H), 7.45 (m, 15H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.09 (d, *J* = 8.4 Hz, 1H), 4.13 (m, 3H), 3.98 (s, 3H), 3.79 (s, 3H), 3.58 (m, 1H), 3.38 (m, 1H), 3.06 (m, 2H), 2.98 (m, 1H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 168.1, 152.8, 149.2, 142.8, 139.3, 137.5, 135.9, 130.0, 129.4, 128.8, 128.7, 128.6, 128.1, 128.0, 126.6, 124.2, 122.6, 122.2, 121.2, 110.7, 109.6, 72.0, 56.2, 56.1, 54.4, 54.3, 52.3, 35.2.

- N-((2*S*,3*R*)-3-hydroxy-4-(N-benzyl-3,4-dimethoxyphenylsulfonamido)-1-phenylbutan-2-yl)benzofuran -5-carboxamide (**2.6bcb**)

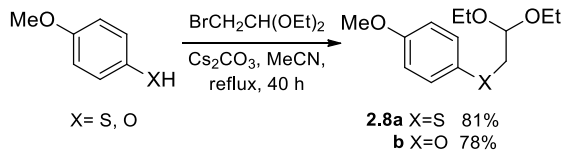
Following the general procedure the compound **2.6bcb** was obtained as a white solid, yield 56%.

$[\alpha]_D^{20}$ =+19.5 (c : 1, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.80 (s, 1H), 7.6 (d, *J* = 2.0 Hz, 1H), 7.43 (m, 3H), 7.20 (m, 12H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.8 (d, *J* = 2.1 Hz, 1H), 6.9 (d, *J* = 7.6Hz, 1H), 3.93 (s, 3H), 3.79 (s, 3H), 3.52 (m, 1H), 3.37 (m, 1H), 3.06 (m, 2H), 2.92 (m, 1H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 168.2, 156.6, 152.8, 130.1, 129.4, 128.9, 128.8, 128.7, 128.5, 128.1, 127.5, 126.6, 123.2, 121.1, 120.7, 111.6, 110.7, 109.6, 106.9, 72.0, 56.2, 56.1, 54.3, 52.4, 35.2, 30.9.

1-(2,2-Diethoxy- ethylsulfanyl)-4-methoxy-benzene (**2.8a)** and 1-(2,2-Diethoxy-ethoxy )-4-methoxy-benzene (**2.8b**)

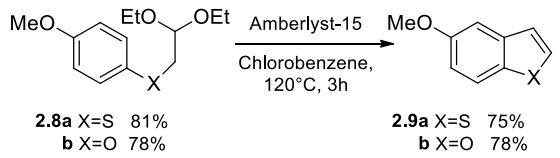



To a mixture of 4-methoxythiophenol (or 4-methoxyphenol) (5 mmol) and $Cs_2CO_3$ (10 mmol) in 30 mL of MeCN was added bromoacetaldehyde diethyl acetal (7.5 mmol) at room temperature. The mixture was stirred at reflux for 40 h before the reaction was completed. After cooling down, water was added and extracted with AcOEt for three times. The combinated organic phase was collected, dried with anhydrous $Na_2SO_4$, filtered, concentrated *in vacuo* and purified on silica gel (EP/AcOEt 9/1) to give the compound **2.8a** as a light and parfumed yellow oil (81%) and **2.8b** (78%) as a yellow oil.

$^1$H and $^{13}$C spectra of compounds **13a-b** were in agreement with literature data.[20,21]

[20] P. Barker, P. Finke, K. Thompson, *Synth. Commun.* **1989**, *19*, 257.
[21] I. Cerminara, L. D'Alessio, M. D'Auria, M. Funicello, A. Guarnaccio, *Helv. Chim. Acta* **2016**, *99*, 384.

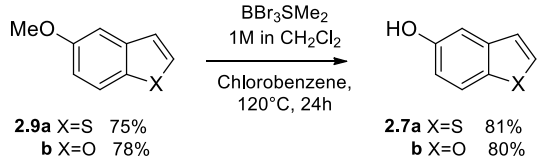
5-methoxybenzothiophene (**2.9a**) 5-methoxybenzofuran (**2.9b**)




To a solution of **2.8a** (or **2.8b**) (3 mmol) in 20 mL of chlorobenzene was added Amberlyst-15 (10 wt%). The mixture was heated at 120°C for 3h. After cooling down to room temperature, the mixture was filtered and the solvent was removed under reduced pressure. The residue were purified on silica gel (EP/$Et_2O$ 8/2) to give compound **2.9a** (or **2.9b**) as a yellow oil with 75% (or 78%) yield.

$^1H$ and $^{13}C$ spectra of compounds were in agreement with literature data[20,21].

5-hydroxybenzothiophene (**2.7a**) and 5-hydroxybenzofuran (**2.7b**)

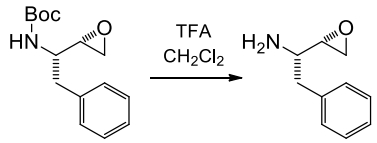



To a solution of **2.9a** (or **2.9b**) (2 mmol) in anhydrous chlorobenzene (20 mL) a solution of $BBr_3SMe_2$ 1M in $CH_2Cl_2$ (4 mmol) was added dropwise at 0°C and under Ar atmosphere. The reaction was kept stirring at reflux for 10h. After cooling down, the reaction was quenched with water and extracted with $CH_2Cl_2$ (three times). The organic layers were collected, dried with anhydrous $Na_2SO_4$, filtered and concentrated *in vacuo*. The crude was purified on silica gel (EP/AcOEt 7/3) to afford compounds **2.7a** (or **2.7b**) with 81% (or 80%) yield.

$^1H$ and $^{13}C$ spectra of compounds **2.7a-b** were in agreement with literature data.[22]

[22] Bonini, C.; Cristiani, G.; Funicello, M.; Viggiani, L. *Synth. Commun.* **2006**, *36*, 1983-1990.

(1S)-1-(oxiran-2-yl)-2-phenylethanamine (**2.10**)



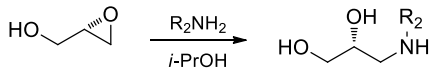

**2.10**

To a stirred solution of (2*S*,3*S*)-1,2-epoxy-3-(Boc-amino)-4-phenylbutane (0.5 mmol) in anhydrous $CH_2Cl_2$ (10 mL), trifluoroacetic acid (3 mL) was added at room temperature. After 1 hour the reaction mixture was concentrated and treated with toluene (3 x 20 mL), evaporated under vacuum.

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.40 (m, 5H), 5.37 (bs, 2H), 4.38 (m, 2H), 4.13 (m, 1H), 3.81 (m, 1H), 3.25 (m, 1H), 3.01 (m, 1H).

Intermediate **2.10** was used in reaction according general procedure for carboxyamides **2.6**.

S-Glycidol ring opening




**2.11a** $R_2$=*i*-Bu *quant.*
**2.11b** $R_2$=Bn 98%

· (2*R*)-3-*iso*butylamino-propane-1,2-diol (**2.11a**)

*i*-$BuNH_2$ (40 mmol) was added to a stirred solution of (S)-glycidol (4.0 mmol) in *i*-PrOH (10 mL) at room temperature. After 24h the solvent and the excess of *i*-$BuNH_2$ were removed under reduced pressure and the aminodiol (**2.11a**) was obtained as an oil in quantitative yield.

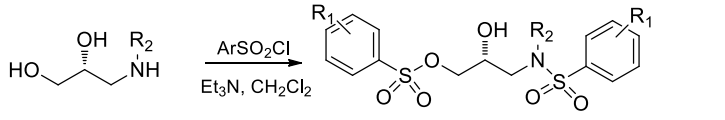
$^1$H and $^{13}$C spectra of compound **2.11a** were consistent to literature data[5].

· (2*R*)-3-(benzylamino)propane-1,2-diol (**2.11b**)

$BnNH_2$ (4.4 mmol) was added to a stirred solution of (S)-glycidol (4.0 mmol) in *i*-PrOH (10 mL) at room temperature. After 24h the solvent and the excess of *i*-$BuNH_2$ were removed under reduced pressure and the aminodiol (**2.11b**) was obtained after pufication on silica gel (EP/AcOEt 7/3) as an oil, 98% yield.

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.33 (m, 4H), 7.29 (m, 1H), 3.82 (m, 2H), 3.81 (m, 1H), 3.65 (bs, 1H), 3.58 (m, 3H), 2.60 (m, 2H), 2.01 (s, 1H).

General procedure for preparation of compounds **2.12**



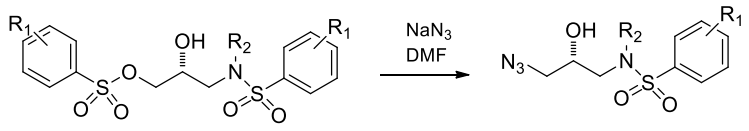

**2.11a-b**

**2.12aa** $R_1$=4-$NO_2$
**2.12ab** $R_1$=4-OMe
**2.12ac** $R_1$=3,4-$(OMe)_2$
**2.12bb** $R_1$=4-OMe
**2.12bc** $R_1$=3,4-$(OMe)_2$

To a stirred solution of aminodiol (**2.11a-b**) (0.78 mmol) in anhydrous $CH_2Cl_2$ (10 mL), $Et_3N$ (2.02 mmol) and arylsulfonyl chloride (1.86 mmol) were added at room temperature

and under Ar atmosphere. After 24h the reaction was quenched by adding quenched by adding $H_2SO_4$ (5%). The mixture was then extracted with $CH_2Cl_2$ and washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was extracted with AcoEt three times, dried over $Na_2SO_4$, filtered and concentrated in vacuo. The crude containing compound was used in the subsequent reaction without any purification.

General procedure for preparation of compounds **2.13**




**2.12aa-c**
**2.12bb-c**

**2.13aa-c**
**2.13bb-c**

The product **2.12** (0.78 mmol) was dissolved in anhydrous DMF (10 mL) and $NaN_3$ (1.60 mmol) was added at room temperarure and under Ar atmosphere. The reaction mixture was warmed at reflux and kept stirring for 4 h. The reaction was then quenched by adding $H_2O$. The mixture was then extracted with $CH_2Cl_2$ (3x30 mL), washed with brine. The organic layer was dried with anhydrous $Na_2SO_4$, filtered and concentrated *in vacuo*. The crude was purified on silica gel.

· (*S*)-N-(3-azido-2-hydroxypropyl)-N-*iso*butyl-4-nitrobenzenesulfonamide (**2.13aa**)

The crude was purified on silica gel ($CH_2Cl_2$/EtOAc 95/5) and compound **2.13aa** was isolated as an yellow oil, yield 26%.

$[\alpha]_D^{20}$ =+7.5° (c : 0.5, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$, TMS): δ 8.09 (d, *J* = 8 Hz, 2H) 8.01 (d, *J* = 8Hz, 2H) 4.69 (s, 1H) 4.02 (s, 1H) 3.57 (d, *J* = 8 Hz, 1H) 3.48 (d, *J* = 8 Hz, 1H) 3.45 (d, *J* = 8 Hz, 1H) 1.59 (s, 1 H) 0.93 (d, *J* = 4 Hz, 3H) 0.91 (d, *J* = 4 Hz, 3H).

· (*S*)-N-(3-azido-2-hydroxypropyl)-N-*iso*butyl-4-methoxybenzenesulfonamide (**2.13ab**)

The crude was purified on silica gel ($CH_2Cl_2$/EtOAc 95/5) and compound **2.13ab** was isolated as a brown oil, yield 80%.

$[\alpha]_D^{20}$ =-8.0° (c : 0.5, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.73 (d, *J* = 6.8 Hz, 2H), 6.90 (d, *J* = 6.8 Hz, 2H), 4.03-3.92 (m, 1H), 3.86 (s, 3H), 3.41-3.23 (m, 3H), 3.18-2.88 (m, 4H), 1.95-1.88 (m, 1H), 0.92 (d, *J* = 5.6 Hz, 3 H), 0.85 (d, *J* = 5.6 Hz, 3H).

· (*S*)-N-(3-azido-2-hydroxypropyl)-N-*iso*butyl-3,4-dimethoxybenzenesulfonamide (**2.13ac**)

The crude was purified on silica gel ($CH_2Cl_2$/EtOAc 95/5) and compound **2.13ac** was isolated as brown oil, yield 82%.

$[\alpha]_D^{20}$ = -12.0° (c : 0.5, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.41 (dd, *J* = 8.8 Hz, *J* = 1.6 Hz, 1H), 7.24 (d, *J* = 1.6 Hz, 1H), 6.95 (d, *J* = 8.8 Hz, 1H), 4.00 (m, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.37 (dd, *J* = 14.0 Hz, *J* = 4.8 Hz, 2H), 3.25 (brs, 1H), 3.17 (dd, *J* = 15.0 Hz, *J*= 8.0 Hz, 1H), 2.97 (m, 2H), 2.85 (dd, *J*= 15.0 Hz, *J* = 8.0 Hz, 1H), 1.85 (m, 1H), 0.93 (d, *J* = 5.6 Hz, 3H), 0.87 (d, *J* = 5.6 Hz, 3H).

· (*S*)-N-(3-azido-2-hydroxypropyl)-N-benzyl-4-methoxybenzenesulfonamide (**2.13bb**)

The crude was purified on florisil ($CH_2Cl_2$/EtOAc 8/2) and compound **2.13bb** was isolated as a brown oil, yield 27%.

$[\alpha]_D^{20}$ =+9.6° (c : 0.5, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.8 Hz, 1H), 7.30(m, 5H), 7.01(d, *J* = 8.8Hz, 1H), 5.15(bs, 1H), 4,37(d, *J* = 2.64 Hz, 2H), 3.90 (s, 3H), 2.09 (m, 2H), 1.33 (m, 2H).

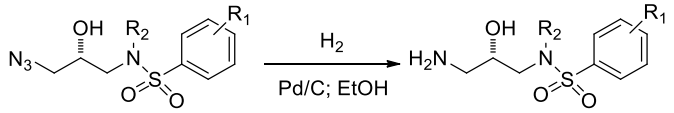
· (*S*)-N-(3-azido-2-hydroxypropyl)-N-benzyl-3,4-dimethoxybenzenesulfonamide (**2.13bc**)

The crude was purified on florisil ($CH_2Cl_2$/EtOAc 8/2) and compound **2.13bc** was isolated as a brown oil, yield 82%.

$[\alpha]_D^{20}$ =+10.5° (c : 0.5, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.45 (s, 1H), 7.33(m, 5H), 7.28 (m, 2H), 4.95(bs, 1H), 4.42 (m, 2H), 3.87 (s, 6H), 3.44 (m, 3H), 1.71 (m, 2H).

General procedure for reduction and preparation of compounds **2.14**




**2.13aa-c**
**2.13bb-c**

**2.14ab-c**

Compound **2.13** (0.50 mmol) was dissolved in EtOH (10 mL) and Pd/C 10% was added. The reaction mixture was stirred under $H_2$ atmosphere at room temperature. After 4h the reaction mixture was fluxed with Ar, filtered on a Celite path and concentrated in vacuo. The crude containing compound **2.14** was used in the subsequent reaction without any purification.

· (2*S*)-*N*-(3-Amino-2-hydroxy-propyl)-*N*-*iso*butyl-4-methoxy-benzenesulfonamide (**2.14ab**)

Compound **2.14ab** was obtained as a brown solid, 99% yield.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 7.76 (d, *J* = 8.4 Hz, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 4.70 (s, 2H), 4.10 (s, 1H), 3.88 (s, 3H), 3.79 (m, 1H), 3.36 (m, 2H), 3.10-2.80 (m, 2H), 2.74 (m, 2H), 1.64 (m, 1H), 0.91 (d, *J* = 6.8 Hz, 3 H), 0.84 (d, *J* = 6.8 Hz, 3H).

- (2*S*)-*N*-(3-Amino-2-hydroxy-propyl)-*N*-*iso*butyl-3,4-dimethoxy-benzenesulfonamide (**2.14ac**)

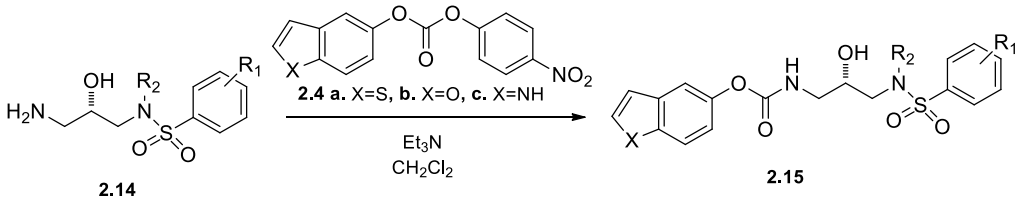
Compound **2.14ac** was obtained as a brown solid, 95% yield.

**$^1$H NMR** (400 MHz, DMSO-$d_6$): $\delta$ 7.96 (s, 2H), 7.39 (d, $J$ = 8.8 Hz, 1H), 7.23 (s, 1H), 7.13 (d, $J$ = 8.8 Hz, 1H), 5.60 (d, $J$ = 5.2 Hz, 1H), 3.83 (bs, 6H), 3.34 (bs, 1H), 3.20 (m, 1H), 2.94 (m, 4H), 2.70 (m, 2H), 1.88 (m, 1H), 0.83 (d, $J$ = 6.4 Hz, 3H), 0.79 (d, $J$ = 6.4 Hz, 3H).

- (2*S*)-*N*-(3-Amino-2-hydroxy-propyl)-*N*-benzyl-4-nitrobenzenesulfonamide (**2.14aa**): Staudinger Reaction

Compound **2.13aa** (0.12 mmol) was dissolved in THF and $PPh_3$ (0.13 mmol) was added. The mixture was kept stirring at room temperature for 2h. Then, 1 ml of $H_2O$ was added and the mixture was kept stirring over night at room temperature. Reaction was extracted with AcOEt and washed with brine. The organic layer was dried with anhydrous $Na_2SO_4$, filtered and concentrated *in vacuo*. The crude was purified on silica gel (EP/AcOEt 7/3), but **2.14aa** has not be found.

General procedure for carbamates **2.15**




$Et_3N$ (0.24 mmol) and *p*-nitrophenylchloroformiate (0.24 mmol) were added to a solution of 5-hydroxyheteroarenes **2.7a-c** (0.24 mmol) in anhydrous $CH_2Cl_2$ (2 mL), under Ar atmosphere. The mixture was kept stirring at room temperature for 1 h to afford intermediates **2.4a-c**; then amine **2.14** (0.20 mmol) was added and the mixture was kept stirring for 24h. The solvent was evaporated and the crude compounds were purified on silica gel ($CHCl_3$/$Et_2O$ 95/5), affording to compounds **2.15**.

- (2*S*)-{2-Hydroxy-3-[*iso*butyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-carbamic acid benzo[b]thiophen-5-yl ester (**2.15aba**)

The product **2.15aba** was obtained as a white solid (72% yield).

$[\alpha]_D^{20}$ =+4.5° (c : 0.5, $CH_2Cl_2$).

**$^1$H NMR** (500 MHz, $CDCl_3$): $\delta$ 7.84 (d, $J$ = 8.6 Hz, 1H), 7.76 (d, $J$ = 8.8 Hz, 2H), 7.59 (d, $J$ = 2.1 Hz, 1H), 7.49 (d, $J$ = 5.4 Hz, 1H), 7.29 (d, $J$ = 5.4 Hz, 1H), 7.13 (dd, $J$= 8.8 Hz, $J$ = 2.1 Hz, 1H), 6.99 (d, $J$ = 8.8 Hz, 2H), 5.81 (bs, 1H), 4.01 (bs, 1H), 3.87 (s, 3H), 3.61 (m, 2H), 3.36 (m, 1H), 3.14 (A of ABX system, $J_{AB}$ = 15.1 Hz, $J_{AX}$ = 4.9 Hz, 1H), 3.05 (B of ABX system, $J_{AB}$ = 15.1 Hz, $J_{BX}$ = 7.2 Hz, 1H), 2.90 (d, $J$ = 7.9 Hz, 2H), 1.89 (m, 1H), 1.63 (bs, 1H), 0.93 (A of AB system, $J_{AB}$ = 6.8 Hz, 3H), 0.92 (B of AB system, $J_{AB}$ = 6.8 Hz, 3H).

**$^{13}$C NMR** (125 MHz, $CDCl_3$): $\delta$ 163.1, 156.3, 148.3, 140.3, 136.7, 129.7, 129.5, 128.1, 123.7, 122.9, 118.7, 115.9, 114.4, 69.9, 59.1, 55.6, 53.2, 44.2, 27.4, 20.1.

- (2*S*)-{2-Hydroxy-3-[*iso*butyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-carbamic acid benzofuran-5-yl ester (**2.15abb**)

The product **2.15abb** was obtained as a white solid (74% yield).

$[\alpha]_D^{20}$ = +10.7° (c : 0.5, $CH_2Cl_2$).

**$^1$H NMR** (500 MHz, $CDCl_3$): $\delta$ 7.75 (d, $J$ = 8.8 Hz, 2H), 7.64 (d, $J$ = 2.0 Hz, 1H), 7.46 (d, $J$ = 8.8 Hz, 1H), 7.35 (d, $J$ = 2.0 Hz, 1H), 7.04 (dd, $J$ = 8.8 Hz, $J$ = 2.0 Hz, 1H), 6.99 (d, $J$ = 8.8 Hz, 2H), 6.74 (brs, 1H), 3.99 (bs, 1H), 3.86 (s, 3H), 3.60 (m, 2H), 3.35 (m, 1H), 3.14 (A of ABX system, $J_{AB}$ = 15.1 Hz, $J_{AX}$ = 4.8 Hz, 1H), 3.04 (B of ABX system, $J_{AB}$ = 15.1 Hz, $J_{BX}$ = 7.3 Hz, 1H), 2.90 (d, $J$ = 7.8 Hz, 2H), 1.88 (m, 1H), 0.93 (A of AB system, $J_{AB}$ = 6.8 Hz, 3H), 0.92 (B of AB system, $J_{AB}$ = 6.8 Hz, 3H).

**$^{13}$C NMR** (125 MHz, $CDCl_3$): $\delta$ 163.1, 156.5, 152.4, 146.5, 146.3, 146.1, 129.6, 129.6, 129.4, 127.9, 118.2, 114.4, 113.7, 111.6, 106.8, 69.9, 59.1, 55.5, 53.1, 44.1, 27.19, 20.1, 19.8.

- (2*S*)-{2-Hydroxy-3-[*iso*butyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-carbamic acid 1H-indol-5-yl ester (**2.15abc**)

The product **2.15abc** was obtained as a white solid (72% yield).

$[\alpha]_D^{20}$ =+32.4° (c : 0.5, $CH_2Cl_2$).

**$^1$H NMR** (500 MHz, $CDCl_3$): $\delta$ 8.32 (bs, 1H), 7.76 (d, $J$ = 8.6 Hz, 2H), 7.37 (bs, 1H), 7.32 (d, $J$ = 8.5 Hz, 1H), 7.22 (bs, 1H), 7.00 (d, $J$ = 8.6 Hz, 2H), 6.95 (d, $J$ = 8.5 Hz, 1H), 6.52 (bs, 1H), 5.76 (bs, 1H), 3.98 (bs, 1H), 3.86 (s, 3H), 3.60 (m, 1H), 3.35 (m, 1H), 3.16 (A of ABX system, $J_{AB}$ = 15.1 Hz, $J_{AX}$ = 4.9 Hz, 1H), 3.04 (B of ABX system, $J_{AB}$ = 15.1 Hz, $J_{AX}$ = 7.4 Hz, 1H), 2.91 (d, $J$ = 7.4 Hz, 2H), 2.18 (s, 3H), 1.89 (m, 1H), 0.94 (A of AB system, $J_{AB}$ = 5.9 Hz, 3H), 0.92 (B of AB system, $J_{AB}$ = 5.9 Hz, 3H).

**$^{13}$C NMR** (125 MHz, $CDCl_3$): $\delta$ 163.1, 157.1, 144.6, 133.6, 129.7, 129.5, 128.1, 125.5, 116.3, 114.4, 112.7, 111.3, 102.9, 70.0, 59.1, 55.6, 53.2, 44.2, 27.4, 20.0.

- (2*S*)-{2-Hydroxy-3-[*iso*butyl-(3,4-dimethoxy-benzenesulfonyl)-amino]-propyl}-carbamic acid 1H-indol-5-yl ester (**2.15acc**)

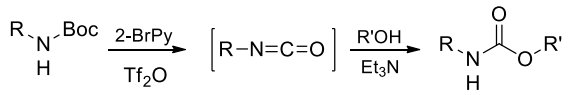
The product **2.15acc** was obtained as a white solid (50% yield).

$[\alpha]_D^{20}$ =-0.2° (c : 0.5, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 8.20 (s, 1H), 7.45 (d, $J$ =9.6 Hz, 1H) 7.36 (dd, $J_1$ =9.6 Hz, $J_2$ = 1.08 Hz, 2H), 7.27 (m, 2H) , 6.96 (d, $J$ =8 Hz, 2H) 6.53 (s, 1H) 5.71 (s, 1H) 3.99 (s, 1H) 3.64 (d, $J$ =1.76, 2H) 3.12(m, 2H) 1.90 (m, 1H) 0.94 (m, 6H).

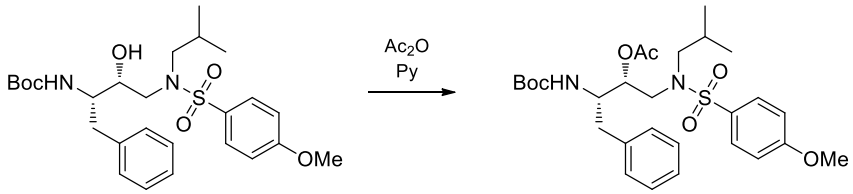
**$^{13}$C NMR** (100 MHz, $CDCl_3$): $\delta$ 152.8, 149.2, 144.7, 133.6, 129.9, 128.1, 125.6, 121.3, 116.3, 112.8, 111.3, 109.9, 103.0, 70.0, 59.0, 56.3, 56.2, 53.2, 44.2, 27.4, 20.1.

General procedure of the one-pot synthesis of carbamates




Boc-protected amine **2.2ab** (1.0 mmol), an 2-Br-pyridine (3.0 mmol) were dissolved in dry dichloromethane (0.05 M). $Tf_2O$ (1.5mmol) was added dropwise over 5 minutes. After stirring for 2 hour at room temperature, alcohol (3.0 mmol) and $Et_3N$ (3.0 mmol) were added to the resulting mixture. After additional stirring for 1 hour, the mixture was diluted with dichloromethane and water, the mixture was extracted with dichloromethane three times and the combined organic layer was washed with brine and water, dried over $Na_2SO_4$, filtered and concentrated under reduced pressure.

- (2*R*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(N-*iso*butyl-4-methoxyphenylsulfonamido)-4-phenylbutan-2-yl acetate (**2.16**)



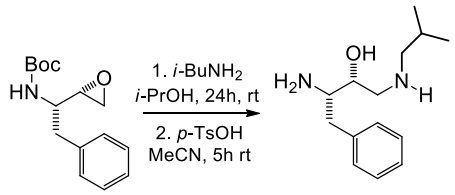

**2.2ab** **2.16**

Compound **2.2ab** was dissolved in pyridine (1 ml) and $Ac_2O$ (1 ml) was added. The mixture was kep stirring for 2h. Then reaction was quenched with HCl 1M three times, extracted with AcOEt three times and washed with saturated $NaHCO_3$ solution, then with brine. Organic phases were dried and concentrated. Compound **2.16** was obtained as yellow oil.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 7.71 (d, $J$ = 8 Hz, 2H), 7.27 (d, $J$ = 8.4 Hz, 2H), 6.96 (m, 3H), 5.17 (bs, 1H), 4.67 (m, 1H), 4.08 (bs, 1H), 3.85 (s, 3H), 3.36 (m, 2H), 2.77 (m, 3H), 2.05 (s, 3H), 1.71 (m, 1H), 1.31 (s, 9H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): $\delta$ 170.6, 162.8, 137.3, 129.5, 129.2, 129.2, 128.4, 126.5, 114.2, 114.1, 73.8, 56.8, 55.6, 52.7, 49.2, 36.7, 28.2, 26.5, 21.0, 20.1, 19.9.

(2*R*,3*S*)-3-amino-1-(*iso*butylamino)-4-phenylbutan-2-ol (**2.17a**)



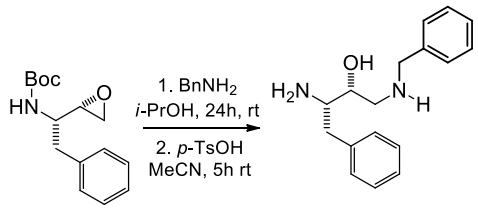

**2.17a**

*i*-$BuNH_2$ (40 mmol) was added to a stirred solution of (2*S*,3*S*)-1,2-epoxy-3-(Boc-amino)-4-phenylbutane (4.0 mmol) in *i*-PrOH (20 mL). The mixture was warmed at 60°C. After 6h the solvent and the excess of *i*-$BuNH_2$ were removed under reduced pressure. The product **2.1a** was obtained as white solid in quantitative yield. Then product **2.1a** (1 mmol) was then dissolved in MeCN (10 ml) and tosic acid monohydrate was added (3 mmol); the resulting mixture was stirred at room temperature for 5 h. The precipitate formed was filtered off and washed with $Et_2O$to give **2.17a** as a white solid, 85% yield.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 7.91 (m, 1H), 7.10 (m, 4H), 4.63 (m, 2H), 3.83 (m, 2H), 2.94 (m, 4H), 2.83 (m, 2H), 2.50 (m, 3H), 0.74 (m, 6H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 138.6, 128.5, 125.9, 79.8, 58.3, 58.3, 51.3, 40.6, 29.1, 20.6.

(2R,3S)-3-amino-1-(benzylamino)-4-phenylbutan-2-ol (**2.17b**)




**2.17b**

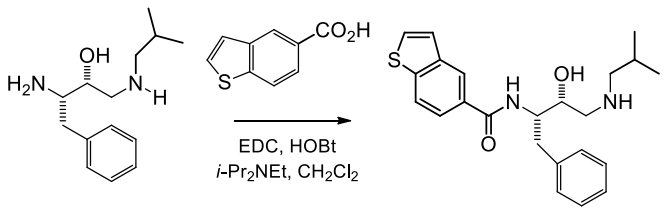
A solution of (2*S*,3*S*)-1,2-epoxy-3-(Boc-amino)-4-phenylbutane (1.6 mmol) and benzylamine (1.5 mmol) in *i*-PrOH (10 mL) was stirred under reflux for 16 h. The reaction mixture was rotary evaporated, and the crude product was purified by recrystallization in methanol/water (7:3) to afford compound **2.1b** as a white solid. Then product **2.1b** (1 mmol) was then dissolved in MeCN (10 ml) and tosic acid monohydrate

was added (3 mmol); the resulting mixture was stirred at room temperature for 5 h. The precipitate formed was filtered off and washed with $Et_2O$ to give **2.17b** as a white solid, 60% yield.

**$^1$H NMR** (400 MHz, DMSO-$d_6$): $\delta$ 9.00 (bs, 1H), 8.89 (bs, 1H), 7.97 (bs, 3H), 7.49 (d, J = 7.6 Hz, 4H), 7.45 (m, 5H), 7.30 (m, 5H), 7.13 (d, J = 7.6 Hz, 4H), 6.11 (bs, 1H), 4.15 (m, 2H), 4.06 (d, J = 10.4 Hz, 1H), 3.53 (m, 1H), 3.13 (m, 1H), 2.86 (m, 3H), 2.29 (s, 6H).

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$): δ 145.3, 137.9, 135.9, 131.3, 130.2, 129.3, 129.1, 128.9, 128.7, 128.1, 127.0, 125.5, 65.7, 54.9, 50.2, 47.3, 33.1, 20.8.

N-((2S,3R)-3-hydroxy-4-(*iso*butylamino)-1-phenylbutan-2-yl)benzo[b]thiophene-5-carboxamide (**2.18a**)



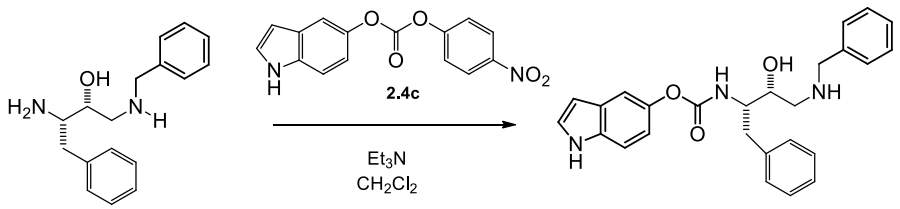

**2.17a** **2.18a**

Compound **2.18a** was obtained according general procedure to obtain carboxyamides **2.6**. It was obtained as s white solid, 50% yield.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 8.12 (s, 1H), 7.83 (m, 3H), 7.61 (d, *J* =8 Hz, 1H), 7.47 (m, 2H), 7.21 (m, 4H), 6.93 (m, 1H), 4.57 (s, 1H), 4.16 (bs, 1H), 3.81 (m, 1H), 3.69 (d, *J* = 14 Hz, 1H), 3.26 (m, 2H), 3.18 (m, 2H), 1.84 (m, 1H), 0.70 (m, 6H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 168.2, 142.8, 140.7, 139.4, 139.2, 137.9, 132.3, 130.4, 129.4, 128.6, 127.7, 126.6, 124.2, 123.9, 122.9, 122.7, 122.6, 122.5, 122.5, 122.3, 73.7, 58.6, 55.1, 50.1, 35.6, 27.1, 19.8, 19.6.

1H-indol-5-yl ((2S,3R)-4-(benzylamino)-3-hydroxy-1-phenylbutan-2-yl)carbamate (**2.19b**)

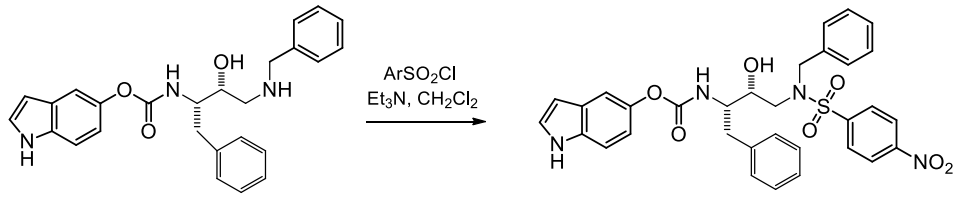



**2.17b** **2.19b**

Compound **2.19b** was obtained according general procedure to obtain carbamates **2.5**. It was obtained only in traces.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 8.72 (s, 1H), 8.28 (m, 1H), 8.21 (d, $J$ = 7.6 Hz, 2H), 7.35 (m, 5H), 7.21 (m, 7H), 6.78 (d, $J$ = 7.2 Hz, 1H), 6.49 (s, 1H), 5.25 (m, 1H), 4.94 (s, 1H), 4.37 (s, 1H), 3.64 (m, 2H), 3.03 (m, 2H), 2.91 (m, 2H).

1H-indol-5-yl ((2S,3R)-4-(N-benzyl-4-nitrophenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl)carbamate (**2.6bac**)



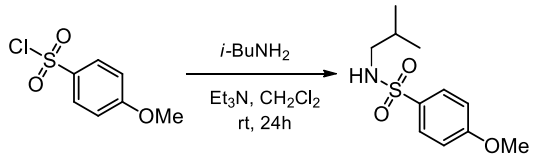

**2.19b** **2.6bac**

To a stirred solution of **2.19b** (0.78 mmol) in anhydrous $CH_2Cl_2$ (10 mL), $Et_3N$ (2.02 mmol) and arylsulfonyl chloride (0.93 mmol) were added at room temperature and under Ar atmosphere. After 24 h the reaction was quenched with 5% aqueous $H_2SO_4$ solution. The organic layer was washed adding saturated aqueous $NaHCO_3$ solution and brine. The organic phases collected were dried over $Na_2SO_4$, filtered and concentrated *in vacuo*. The crude was purified on silica gel. Compound **2.6bac** was obtained in traces.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 8.31 (d, $J$ = 8.8 Hz, 2H), 8.20 (d, J = 19.6 Hz, 1H), 8.09 (s, 1H), 7.97 (d, $J$ = 8.8 Hz, 2H), 7.30 (m, 10H), 7.08 (m, 1H), 6.88 (d, $J$ = 10 Hz, 1H), 6.79

(d, *J* = 10 Hz, 1H), 6.52 (s, 1H), 4.70 (s, 1H), 4.49 (d, *J* = 14 Hz, 1H), 4.36 (d, *J* = 14 Hz, 1H), 3.77(m, 1H), 3.53 (m, 2H), 3.27 (m, 2H), 2.90 (m, 1H).

N-*iso*butyl-4-methoxybenzenesulfonamide (**2.20**)



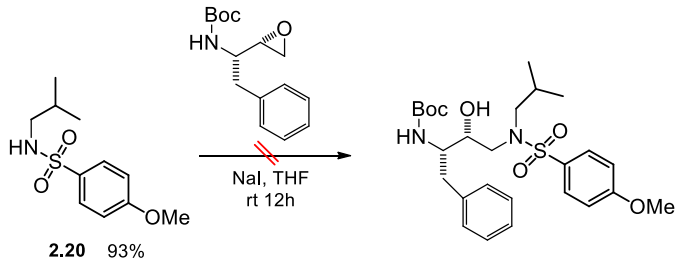

**2.20** 93%

A mixture of sulfonyl chloride (1.0 mmol) and triethylamine (1.0 mmol) in $CH_2Cl_2$ (10 ml) was stirred at room temperature. The primary amine (1.0 mmol) was added dropwise. The reaction mixture was stirred for 4h and monitored by TLC analysis. Upon completion, the reaction mixture was poured into $H_2O$ (10 ml), washed with HCl 1M solution, extracted with $CH_2Cl_2$, and dried over anhydrous $Na_2SO_4$. The crude product was afforded by removing solvent and was purified by a flash chromatography to afford the product as a solid, 92% yield.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 7.79 (d, *J* = 8.4 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 2H), 4.97 (s, 1H), 3.85 (s, 3H), 2.70 (t, *J* = 6.8 Hz, 2H), 1.69 (m, 1H), 0.84 (m, 6H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): $\delta$ 163.8, 136.8, 126.1, 114.6, 55.8, 49.8, 27.7, 20.3.

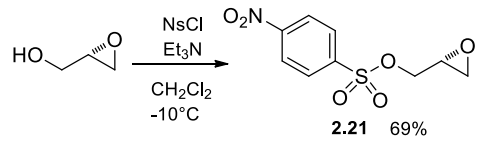
*tert*-butyl((2S,3R)-3-hydroxy-4-(N-*iso*butyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)carbamate




**2.20** 93%

To a suspension of NaH (3 mmol) in THF (8 ml), was added sulfonamide (3 mmol) in THF (8 ml). The reaction mixture was stirred at room temperature for 30 minutes and epoxide (3.3 mmol) was added. The reaction mixture was stirred at room temperature overnight; TLC analysis reveals that substrates didn't react. Reaction mixture was stopped with $H_2O$. THF was evaporated under reduced pressure and the reaction mixture was extracted with ethyl acetate Combinated organic layers were dried on $Na_2SO_4$ and evaporated. NMR analysis revealed starting materials.

(*R*)-oxiran-2-ylmethyl 4-nitrobenzenesulfonate (**2.21**)



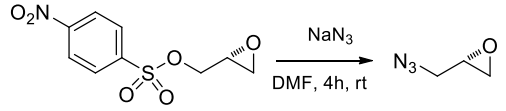

**2.21** 69%

S-Glycidol (6.7 mmol) was dissolved in anhydrous $CH_2Cl_2$ (10 ml) $Et_3N$ (7.4 mmol) and 4-Nitrobenzenesulphonyl chloride (7.4 mmol) were added at -10 ° C with a bath of water and salt and kept stirring for 2h. Reaction was quenched with $H_2O$ and extracted with AcOEt, three times. Organic layers were dried and evaporated. The obtained crude is purified by column chromatography with silica gel ($CHCl_3$/$Et_2O$ 9/1). Product **2.21** was obtained as an amber oil, 70% yield.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 8.25 (d, $J$ = 7.2 Hz, 1H), 7.84 (d, $J$ = 7.2 Hz, 1H), 4.50 (d, $J$ = 1.2 Hz, 1H), 3.22 (d, $J$ =2.4, 1H), 2.85 (d, $J$ =1.2 Hz, 1H), 2.63 (m, 3H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 153.5, 153.7, 130.2, 125.5, 67.9, 51.0, 44.0.

(*S*)-2-(azidomethyl)oxirane (**2.22**)



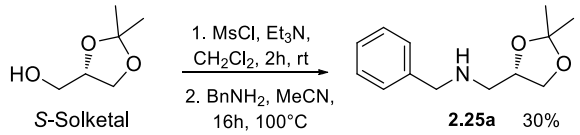

**2.21** 69% **2.22** 95%

Compound **2.21** (0.38 mmol) was dissolved in DMF and MeCN, $NaN_3$ (0.77 mmol) are slowly added. The reaction was kept stirring at room temperature for 3h. After 3h, 0.5

eq of $NaN_3$ are added and kept stirring for 24h at reflux. Reacion was quenched with $H_2O$ and extracted with AcOEt, three times. Organic layers were dried and evaporated. The obtained crude is used in the next reduction reaction, according Staudinger conditions.

(*S*)-N-benzyl-1-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (**2.25a**)

**a.**

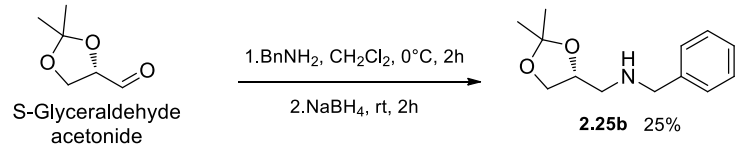



*S*-Solketal **2.25a** 30%

To a mixture of *S*-Solketal (3.80 mmol) and $Et_3N$ in anhydrous $CH_2Cl_2$ (4 ml) at 0 °C under Argon, a solution of Mesylchloride (4.56 mmol) in 2 ml of anhydrous $CH_2Cl_2$ is added dropwise. The reaction was kept stirring for 2h. The reaction was treated with $NaHCO_3$ and extracted with $CH_2Cl_2$. The crude was dried on $Na_2SO_4$, filtered and then the solvent is evaporated under reduced pressure. The crude was then dissolved in MeCN and $BnNH_2$ (15.30 mmol) was added and the mixture was warmed to 100°C overnight. Solvent was evaporated, then AcOEt was added and the mixture was washed with saturated $NaHCO_3$ solution. Organic layer was washed then with brine, dried and evaporated.

(*S*)-N-benzyl-1-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (**2.25b**)

**b.**



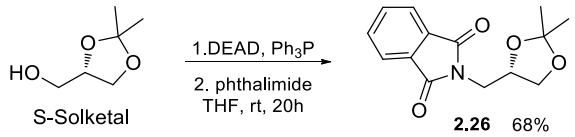

S-Glyceraldehyde acetonide **2.25b** 25%

In a two-necked flask, S-Glyceraldehyde acetonide (1.5 mmol) was dissolved in anhydrous $CH_2Cl_2$. 50 mg of crushed molecular sieves were added for each mmol of substrate (75 mg). Mixture was cooled to 0 ° C and the solution was kept stirring under Ar for 2 h. Then, $BnNH_2$ (1.5 mmol) was added dropwise. Reaction was kept under

vigorous stirring for 2h. In the same flask and without purification the reduction is carried out adding 1.2 eq of $NaBH_4$ (1.8 mmol). After 2h, reaction was quenched with $H_2O$, saturated $NaHCO_3$ solution was added and mixture extracted with AcOEt, then it was washed with brine. Organic phases collected were dried and concentrated.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 7.30 (m, 5H), 4.26 (q, $J$ = 6.0Hz, 1 H), 4.03 (dd, $J_2$ = 8.0 Hz, $J_3$ = 6.5 Hz, 1H), 3.83 (d, $J$ = 13.5 Hz, 1H), 3.82 (d, $J$ = 13.5 Hz, 1H,), 3.68 (dd, $J$ = 8.0 Hz, $J$ = 7.0 Hz, 1H), 2.74 (d, $J$ = 5.5Hz, 2H), 1.89 (s, 1H), 1.40 (s, 3H), 1.35 (s, 3H).

(*S*)-2-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)isoindoline-1,3-dione (**2.26**)

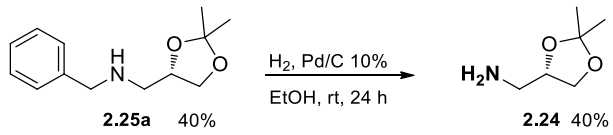



S-Solketal (3.8 mmol) was dissolved in anhydrous THF, $PPh_3$ (4.6 mmol), phthalimide (3.8mmol) and DEAD (4.6 mmol) were added. After about 20h the reaction is stopped and solvent evaporated. The crude was purified on silica gel (AcOEt/EP 6/4) to afford **2.25**.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 7.85(m, 4H), 4.38 (d, $J$ = 5.6 Hz, 1H), 3.92 (m,1H) 3.71(m, 2H), 1.33 (m, 1H), 1.57 (s, 3H).

(*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (**2.24**)

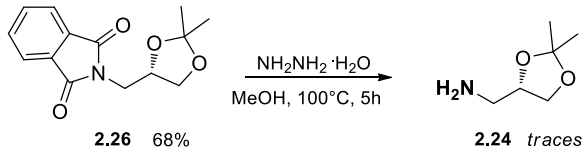
**a.**




Compound **2.25a** (0.18 mmol) is dissolved in EtOH, 10% Pd/C (8 mg) firstly was added and then ammonium formate (1 mmol). The reaction is kept stirring for about

16h. After 16h, the reaction was filtered on celite path and solvent evaporated under reduced pressure.

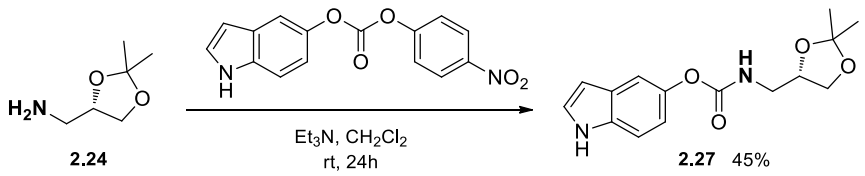
**b.**




Compound **2.26** (2.59 mmol) was dissolved in MeOH and then hydrazine (4.53 mmol) was added. The solution was kept stirring at reflux for 4-5h. Solvent was evaporated, but NMR analysis reveals the product in traces.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 4.12 (dd, *J* = 6.5 Hz, *J* = 4.5Hz, 1H), 4.03 (dd, *J* = 8.0 Hz, *J* = 6.5 Hz, 1H), 3.66 (dd, *J* = 8.0 Hz, *J* = 6.5 Hz, 1H), 2.83 (dd, *J* = 13.0 Hz, *J*= 4.5Hz, 1H), 2.78 (dd, *J* =13.0Hz, *J* = 6.0Hz, 1H), 1.42 (s, 3H), 1.35 (s,3H), 1.26 (s, 2H).

(S)-1H-indol-5-yl ((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)carbamate (**2.27**)



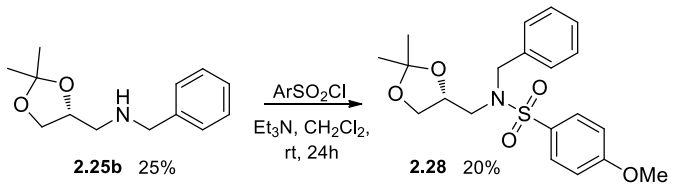

Compound **2.27** was obtained following general procedure to obtain carbamates (see compounds **2.5** or **2.15**). It was isolated by silica gel (EP/AcOEt 7/3).

$[\alpha]_D^{20} = +2.4°$ (c : 1.0, $CHCl_3$).

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 8.33 (s, 1H), 7.36 (s, 1H), 7.28 (d, *J* = 8.4 Hz, 1H), 7.18 (s, 1H), 6.93 (d, *J* = 8.8 Hz, 1H), 6.50 (s, 1H), 5.42 (bs, 1H), 4.30 (m, 1H), 4.12 (m, 1H), 3.74 (t, *J* = 7.2 Hz, 1H), 3.53 (m, 1H), 3.37 (m, 1H), 1.48 (s, 3H), 1.39 (s, 3H)

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 156.0, 144.5, 133.5, 128.1, 125.5, 116.2, 112.7, 111.3, 109.5, 102.7, 74.6, 66.6, 43.4, 26.8, 25.2.

(*R*)-N-benzyl-N-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-4-methoxybenzenesulfonamide (**2.28**)

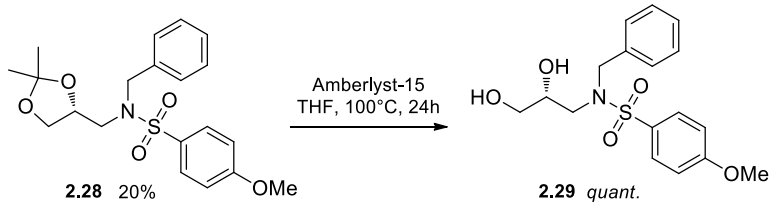



Compound **2.28** was obtained as previously described procedure after purification on silica gel (EP/AcOEt 8/2).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): *δ* 7.76 (d, *J* = 8.8 Hz, 2H) 7.25 (m, *J* = 5.6 Hz, 4H), 6.97 (d, *J* = 8.8 Hz, 2H), 4.37(d, *J* = 5.6 Hz, 2H), 4.02 ( m, 1H) 3.82 (d, *J* = 2.4Hz, 1H), 3.79 (d, *J* = 6.4Hz, 1H), 3.46 (d, *J* = 2.4 Hz, 1H,), 3.30 (d,1H, *J* = 6.4 Hz, 1H), 1.24 (m, 6H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 162.9, 136.4, 131.3, 129.4, 128.6, 128.5, 127.9, 114.3, 109.4, 77.3, 67.5, 55.6, 53.1, 50.6, 26.7.

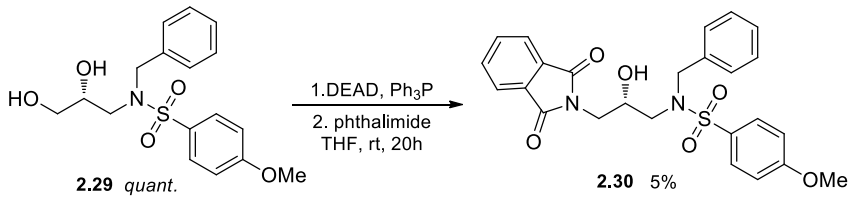
(*R*)-N-benzyl-N-(2,3-dihydroxypropyl)-4-methoxybenzenesulfonamide (**2.29**)




Compound **2.28** was dissolved in THF, Amberlyst-15 was added and the mixture was warmed to 100°C overnight. Then mixture was filtered and evaporated. Product **2.28** was obtained in quantitative yield.

**$^1$H NMR** (400 MHz, $CDCl_3$): $\delta$ 7.79 (d, $J$ = 8 Hz, 2H) 7.77 (d, $J$ = 8 Hz, 2H) 7.31 (t, $J$ = 2 Hz, 2H), 7.02 (m, 2H), 7.00 (t, $J$ = 2 Hz, 1H), 4.68 (s, 1H) 4.68 (s, 1H) 4.24 (d, $J$ = 1.68 Hz, 1H), 4.2 ( d, $J$ = 1.68 Hz,1H) 3.89 (s, 3H) 3.64 (m, 3H).

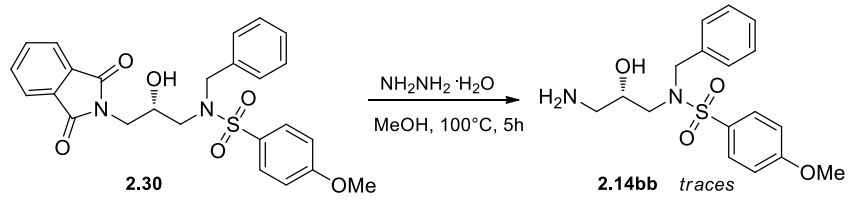
(*S*)-N-benzyl-N-(3-(1,3-dioxoisoindolin-2-yl)-2-hydroxypropyl)-4-methoxybenzenesulfonamide (**2.30**)




**2.29** *quant.* **2.30** 5%

Compound **2.27**(0.25 mmol) was dissolved in anhydrous THF, $PPh_3$ (0.29 mmol), phthalimide (0.25 mmol) and DEAD (0.29 mmol) were added. After 20h solvent was evaporated under reduced pressure. Product 2.29 was obtained after gel chromatography on silica gel (AcOEt/EP 6/4). Crystallization with AcOEt and Pentane to eliminate phosphinoxide was carried out. Due to te poor yield characterization was not reported.

(*S*)-N-(3-amino-2-hydroxypropyl)-N-benzyl-4-methoxybenzenesulfonamide (**2.14bb**)




**2.30** **2.14bb** *traces*

Crude compound **2.30** (0.01 mmol) was dissolved in MeOH and idrazine (0.02 mmol) was added. Reaction mixture was kept stirring for 5 h. Solvent was evaporated, but NMR analysis reveals the product in traces.

## *2.5 Conclusions*

Compounds containing heterocycles, and in particular heteroarenes, with an hydroxyethylamine *core* were synthetized, using a synthetic path with few steps and high yields. In the case of the unsubstituted *core*, according usually strategy, synthesis starts from *S*-glycidol, but some problems have been found if moieties of the molecules have to be changed. Thus, new synthetic paths were studied in order to further minimize the steps. In particular, good results have been achieved using different building block of the chiral pool, like *S*-solketal or *R*-glyceraldehyde acetonide, but reactions have to be studied and optimized to increase the overall yield. In the case of presence of benzyl group in the *core* it is to be highlighted that this synthetic pathway appears very solid, high yielding and general, irrespective on the N-alkyl group, the sulfonamide or the type of heteroaryl moiety chosen, except for oxethane used, for which particular attention is required.

Synthetized compounds have been tested by FRET methodology an inhibitors of the HIV-protease and results showed that:

- for antiviral activity, benzyl group in the *core* has general beneficial effect.
- If $R_2$ moiety, linked to S2 subsite of the enzyme, is isobutyl, carbamoyl functionality to space the heteroarene to the *core* is preferable to the carboxyamide; interactions with the one substitutent on sulfonamide moiety seem to be favorable; benzofuryl and indolic derivatives showed inhibition activity among the best for such structurally simple inhibitors. Probably due to the greater number of interactions with the aminoacid residues in the subsite respect to the benzothiophene. The presence of carbamate function makes these new compounds metabolically as stable as the most currently used inhibitors. In particular, benzofuran is apparently the heterocycle which confers greater metabolic stability.
- If $R_2$ moiety is a benzyl group, carboxyamidic functionality works better then carbamoyl one; the substantial difference is made by the heteroarene bound to the *core*: benzothiophene seems to be the best one, instead substituent on arylsulfonamide moiety seems not very important for the activity.

Docking analysis allowed to identify the favorable situation of such derivatives in terms of number of interactions in the active site, supporting the experimental results.

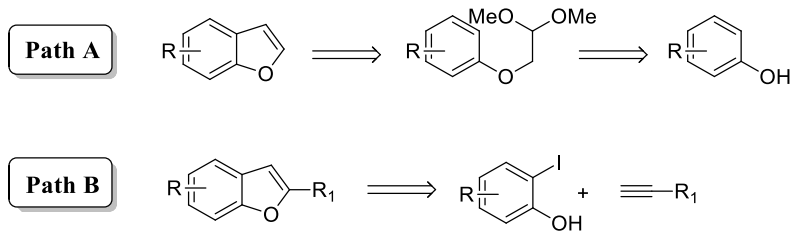
Some of this compounds have been also tested on human hepatocarcinoma cell lines, both final compounds and also free amines obtained before the addition of the heterocycle to the *core*. Good results have been obtained, expecially for compound without heteroaryl residue linked to the *core*, but studies to understand the mechanism of cytotoxicity and which group of the molecule is important for biological activity are still underway. Anywhere, the easy access of common intermediates represents an open door to different target molecules with pharmacological activities even beyond the inhibition of HIV-protease.

# CHAPTER 3:
# 2,3-DIHYDROBENZOFURANS

## *3.1 Synthesis of benzofuran ring*

As said previously, 2,3-dihydrobenzofuran ring should be obtained with some difficulty by the hydrogenation of benzofuran, so understanding of the substituted benzofuran ring reactivity in the hydrogenation reactions sould be very interesting. Most synthetic approaches towards benzofurans are based on the generation of the O–C2 or the C2–C3 bonds, in the crucial ring closing step, via intramolecular cyclization of an already appropriately functionalized precursor.[1]

In this work benzofuran ring was obtained by two different strategies depending of the different substituents present on the ring: **Path A** in which benzofuran ring was obtained by preparation and cyclization of aryloxyacetaldehyde acetals[2]; **Path B** in which benzofuran ring was obtained by Sonogashira cross-coupling and contemporary cyclization (scheme 3.1):

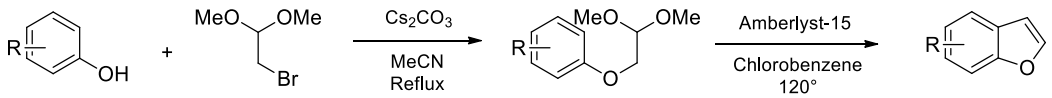



**Scheme 3.1**

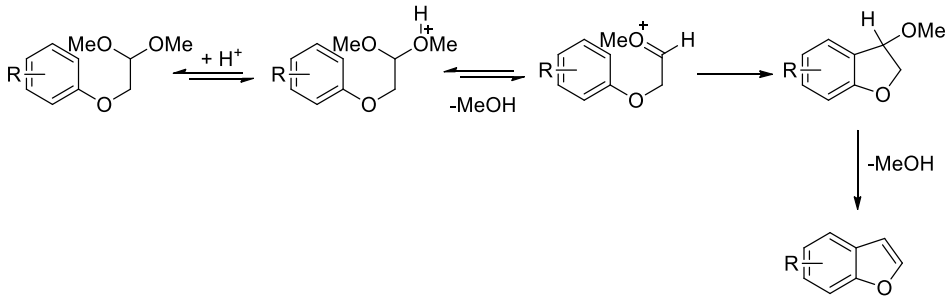
**Path A** consists of two steps as shown in Scheme 3.2. In the first step substituted phenol was alkylated by nucleophilic substitution with bromoacetaldehyde dimethyl acetal under basic conditions. Second step consists of a cyclization and aromatization process, typically conducted with polyphosphoric acid in toluene as reported in literature from 1935[3]. These conditions represent critic point in terms of yields obtained and different intermediate species including

[1] a) M. M. Heravi, V. Zadsirjan; H. Hamidi; P. Hajiabbas Tabar Amiri *RSC Adv.* **2017**, *7*, 24470. b) M. M. Heravi, V. Zadsirjan *Adv. Heter. Chem.* **2015**, *117*, 261.
[2] P. Barker, P. Finke, K. Thompson, *Synth. Commun.* **1989**, *19*, 257.
[3] a) L.F. Fieser, R.G. Kennelly, *J. Am. Chem. Soc.* **1935**, *57*, 1611. b) C. Bonini, G. Cristiani, M. Funicello, L. Viggiani, *Synth. Commun.* **2006**, *36*, 1983.

non-aromatic compounds were produced. So the procedure was changed, using Amberlyst-15 for cyclodehydratation process[4], obtaining desidered benzofuran with high yield. The mechanism proposed for such reaction consists of an electrophilic aromatic substitution followed by elimination of MeOH which allowed to aromatization (scheme 3.3)[5].

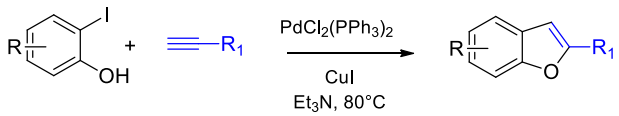



**Scheme 3.2** Benzofuran synthesis according Path A




**Scheme 3.3** Proposed mechanism of cyclization and aromatization process

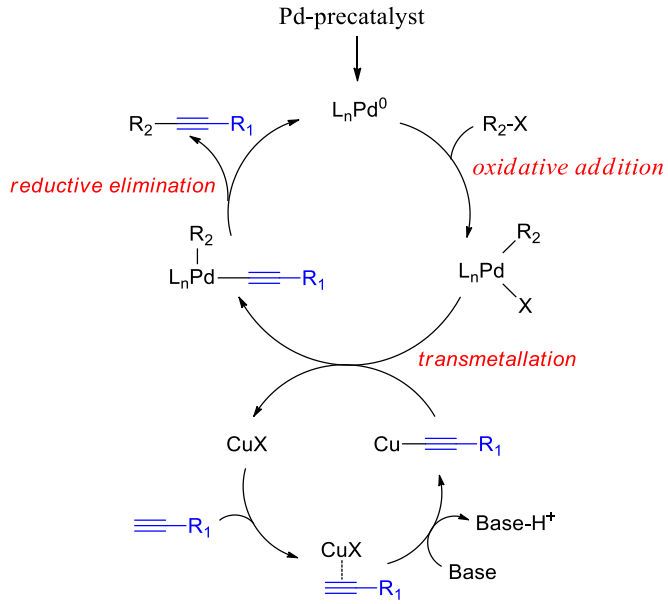
According **Path B** benzofuran ring was alternatively obtained by contemporary Sonogashira cross-coupling and cyclization. As reported in scheme 3.4, substituted 2-iodophenol reacts with a terminal alkyne in a cross-coupling reaction using palladium as catalyst and copper as co-catalyst under basic conditions by triethylamine that acts also as solvent, and then intramolecular cyclization occurs.




**Scheme 3.4**

[4] a) I. Kim, S.-H. Lee, S. Lee, *Tetrahedron Lett.* **2008**, *49*, 6579. *b*) I. Kim, J. Choi , *Org. Biomol. Chem.* **2009**, *7*, 2788. *c*) J. H. Lee, M. Kim, I. Kim, *J. Org. Chem.* **2014**, *79*, 6153.
[5] I. Cerminara, L. D'Alessio, M. D'Auria, M. Funicello, A. Guarnaccio, *Helv. Chim. Acta* **2016**, *99*, 384.

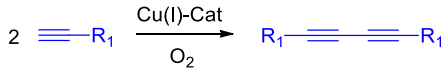
Mechanism of C-C bond formation according to Sonogashira reaction is reported in scheme 3.5 and revolves around a palladium cycle and a copper cycle; palladium precatalyst species is activated under reaction conditions to form a reactive $Pd^0$-compound. The exact identity of the catalytic species depends strongly upon reaction conditions; with simple phosphines, such as $PPh_3$ (n=2), it was demonstrated that monoligated species (n=1) are formed[6]. Furthermore, some evidences suggest the formation of anionic palladium species, $[L_2Pd^0Cl]^-$, which could be the real catalysts in the presence of anions and halides[7]. The active $Pd^0$ catalyst is involved in the oxidative addition step with the aryl halide substrate to produce $Pd^{II}$ species. This complex reacts with copper acetylide in a transmetallation step, regenerating the copper catalyst. The last one step is a reductive elimination where the substrate motifs need to be in close vicinity, so the product is expelled from the complex and the active Pd catalytic species is regenerated. The copper cycle is not entirely well described: it is suggested that the presence of a base results in the formation of a π-alkyne complex; this increases the acidity of the terminal proton and leads to the formation of copper acetylide, upon deprotonation. Acetylide is then involved in the transmetallation reaction with palladium intermediate.




**Scheme 3.5** Sonogashira cross-coupling

[6] J.P. Stambuli, M. Buhl, J.F. Hartwig, *J. Am. Chem. Soc.* **2002**, *124*, 9346.
[7] C. Amatore, A. Jutand, *Acc. Chem. Res.* **2000**, *33*, 314.

The presence of copper as catalyst can result in the formation of alkyne dimers; this leads to the the so-called "Glaser coupling" reaction, which is an undesired formation of homocoupling products of acetylene derivatives upon oxidation (scheme 3.6)[8]. As a result, if the Sonogashira reaction is performed with a copper co-catalyst, it is necessary to run the reaction in an inert atmosphere to avoid the unwanted dimerization.



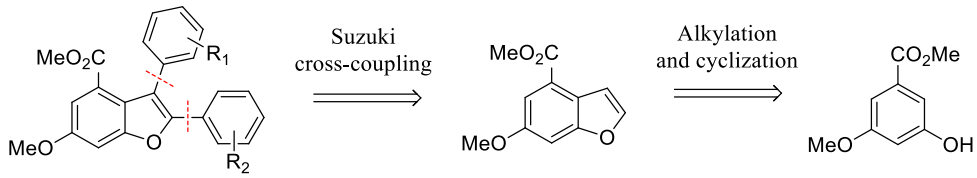

**Scheme 3.6** Glaser coupling

Thus, synthetised benzofuran were used in reduction reaction in order to study how the substituents and their position could affect such reactions.

[8] a) V.P.W Bohm, W.A. Herrmann, *European Journal of Organic Chemistry* **2000**, *200*, 3679. b) D. Mery, K. Heuze, D. Astruc, *Chem. Commun.* **2003**, *15*, 1934.

## *3.2 Synthesis of substituted benzofurans*

### *3.2.1 Synthesis of 2,4,6-substituted benzofurans and 2,3,4,6-substituted benzofurans*



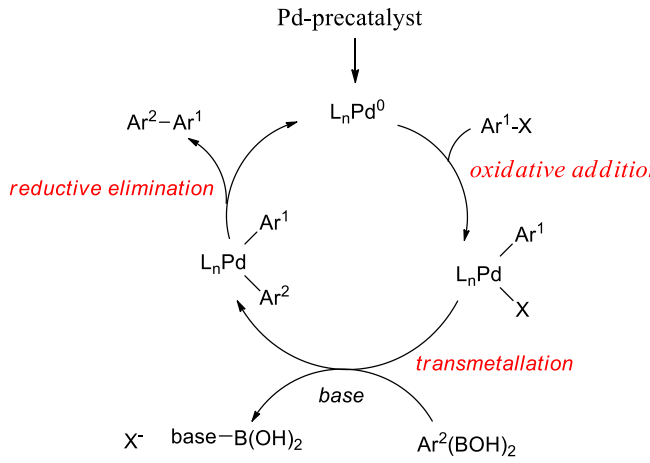

**3.1** **3.2**

**Scheme 3.7**

As reported in scheme 3.7, 2,4,6-trisubstituted benzofurans and 2,3,4,6-tetrasubstituted benzofurans were obtained by Suzuki cross-coupling, once obtained benzofuran core (**3.1**), according **Path A**.

In the Suzuki cross-coupling reaction a carbon-carbon single bond is formed by coupling an organoboron species with a halide using a palladium catalyst and a base[9]. The general scheme for the Suzuki reaction is shown in scheme 3.9. The first step is the oxidative addition of palladium to the halide to form the organopalladium species. Transmetallation with boronic acid, activated by the presence of the base, forms another organopalladium species where the two coupling partners are present. Reductive elimination of the desired coupling product restores the original palladium catalyst, which completes the catalytic cycle. The Suzuki coupling takes place in the presence of a base and for a long time the role of the base was not fully understood; Le Duc and coworkers[10] investigated the role of the base in the reaction mechanism for the Suzuki coupling and they found that the base has three roles: formation of the palladium complex [$Ar^1Pd(base)L_n$], formation of the trialkyl borate and the acceleration of the reductive elimination step by reaction with the palladium complex.

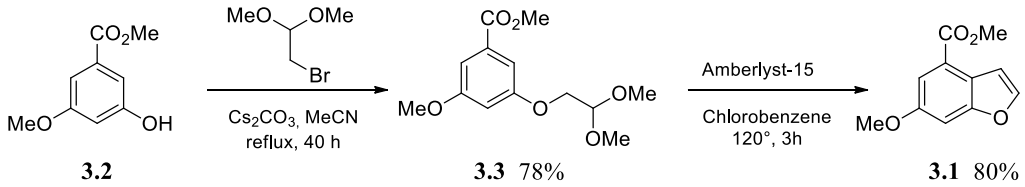
[9] a) N. Miyaura, K. Yamada, A. Suzuki, *Tetrahedron Letters* 1979, *20*, 3437. b) N. Miyaura, A. Suzuki, *Chem. Comm.*, 866, **1979**. c) N. Miyaura, A. Suzuki, *Chemical Reviews.* **1995**, *95*, 2457.
[10] C. Amatore, A. Jutand, G. Le Duc, *Chemistry: A European Journal* **2011**, *17* (8), 2492.




**Scheme 3.8** Suzuki cross-coupling Reaction

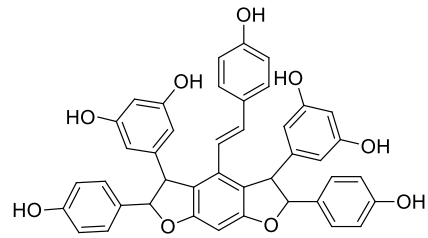
The synthesis of this substituted benzofuran starts from monoprotected resorcinol derivative (**3.2**) that is commercially available, or conveniently from methyl-3,5-dihydroxybenzoate.

The benzofuran core **3.1** was synthesized from **3.2** by alkylation with bromoacetaldehyde dimethylacetal and subsequent cyclodehydration using Amberlyst-15, according to **Path A** previously described (Scheme 3.9)




**Scheme 3.9**

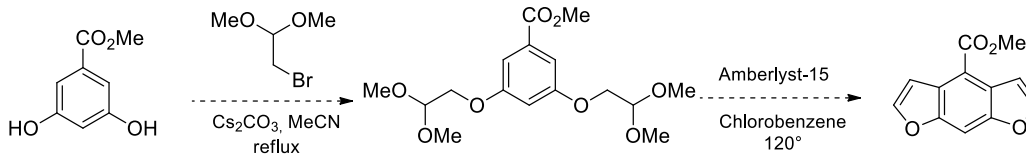
This sequence was tried also on methyl-3,5-dihydroxybenzoate, in order to obtain a double furan ring fused with benzene, as reported in scheme 3.10. This moiety is present in the structure of Gnetin H, a natural *viniferin* with important biological activities (figure 3.1).



**Gnetin H**

**Figure 3.1**

Unfortunately double alkylation reaction did not occur, even after several attempts:

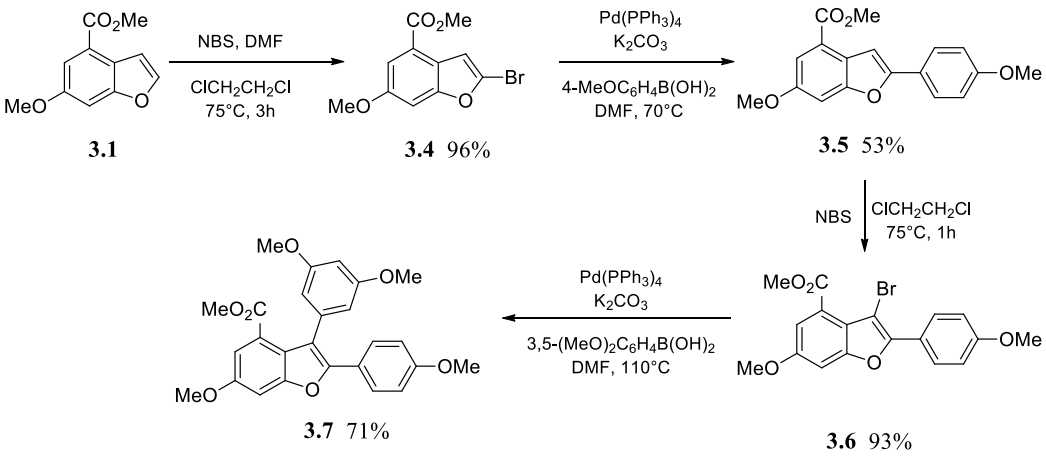



**Scheme 3.10**

Once obtained the benzofuran core (**3.1**), it was brominated using NBS, DMF in dichloroethane (Scheme 3.11). Although it is known that electrophilic substitution on benzo[*b*]furan occurs at C-2 preferentially,[11] the direct bromination is not generally used,[12] and 2-bromobenzofurans are usually prepared by lithiation and quenching with electrophilic bromination reagents[13]. Probably the carboxylate ester substituent on the 4-position deactivated the 3-position for bromination, thus leading to the formation of 2-bromobenzofuran **3.4** as the unique product. Pd-catalyzed cross-coupling of 2-bromobenzofuran with 4-methoxyphenyl boronic acid afforded product **3.5**. A second bromination afforded to 3-Bromobenzofuran **3.6** in high yield. The second cross-coupling with 3,5-dimethoxyphenyl boronic acid occurred at higher temperature to yield the 2,3-diarylbenzofuran **3.7**.

The 2,4,6-substituted benzofuran (**3.5**) and the 2,3,4,6-substituted benzofuran (**3.7**) have been then subjected to reduction reaction (See section 3.4).

[11] J. A. Joule; K. Mills *In Heterocyclic Chemistry*, Fifth Ed., Wiley, Blackwell Publishing Ltd., 2010, p 371
[12] S. G. Newman; V. Aureggi; C. S. Bryan; M. Lautens *Chem. Comm.* **2009**, 5236
[13] L. Lu, H. Yan, P. Sun, Y. Zhu, H. Yang, D. Liu, G. Rong, J. Mao, Eur. *J. Org. Chem.* **2013**, 1644.

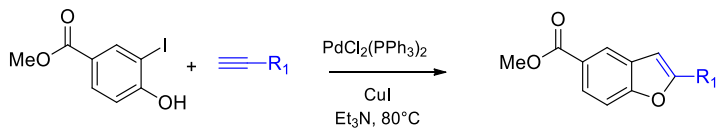



**3.1** **3.4** 96% **3.5** 53% **3.6** 93% **3.7** 71%

**Scheme 3.11**

### *3.2.2 Synthesis of 2,5-substituted benzofurans*

To obtain 2,5-substituted benzofurans the **Path B** was used (scheme 3.12). Methyl-4-hydroxy-3-iodobenzoate was reacted with two different terminal alkynes to obtain the products reported in table 3.1.




**Scheme 3.12**

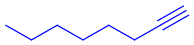
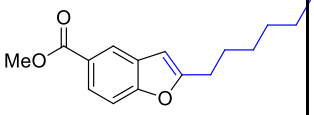
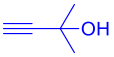
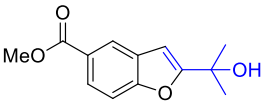
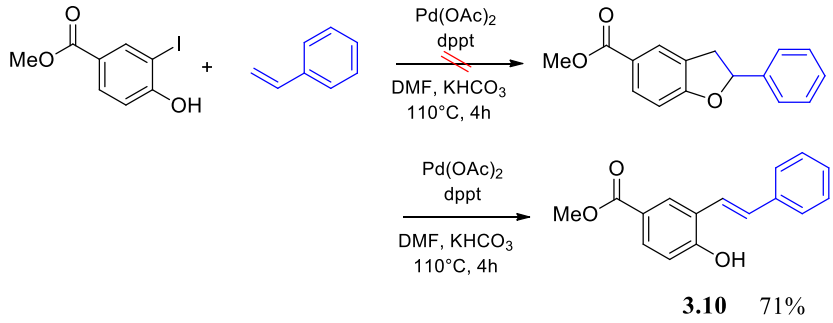
| $R_1$ | Alkyne | Product |
|---|---|---|
|  |  **1-Octine** |  **3.8** 72% |
|  |  **2-Methyl-3-butyn-2-olo** |  **3.9** 80% |

**Table 3.1**

2,5-substituted benzofurans, **3.8** and **3.9** were then used in reduction reaction (see section 3.4). Furthermore compound **3.9** was also acetylated and studied in reduction reaction (see section 3.4, table 3.2 entry 15).

Another cross-coupling reaction, following Larock conditions[14], was tried using methyl-4-hydroxy-3-iodobenzoate with styrene in order to obtain methyl-2-phenyl-2,3-dihydrobenzofuran-5-carboxylate. The reaction did not afford the desired cyclized product, but the stilbene derivative one (scheme 3.13).

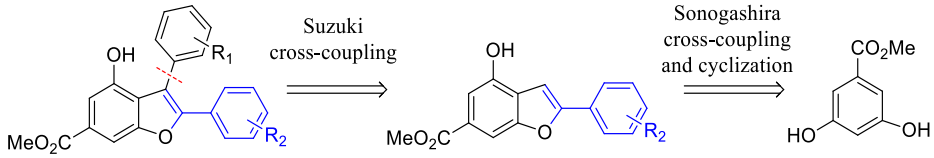
[14] X. Pan, T.D. Bannister, *Org. Lett.* **2014**, *16*, 6124.




**3.10** 71%

**Scheme 3.13** Larock annulation reaction. dppt = 1,1'-bis (diphenylphosphino) ferrocene

### *3.2.3 Synthesis of 2,3,4,6-substituted benzofurans*

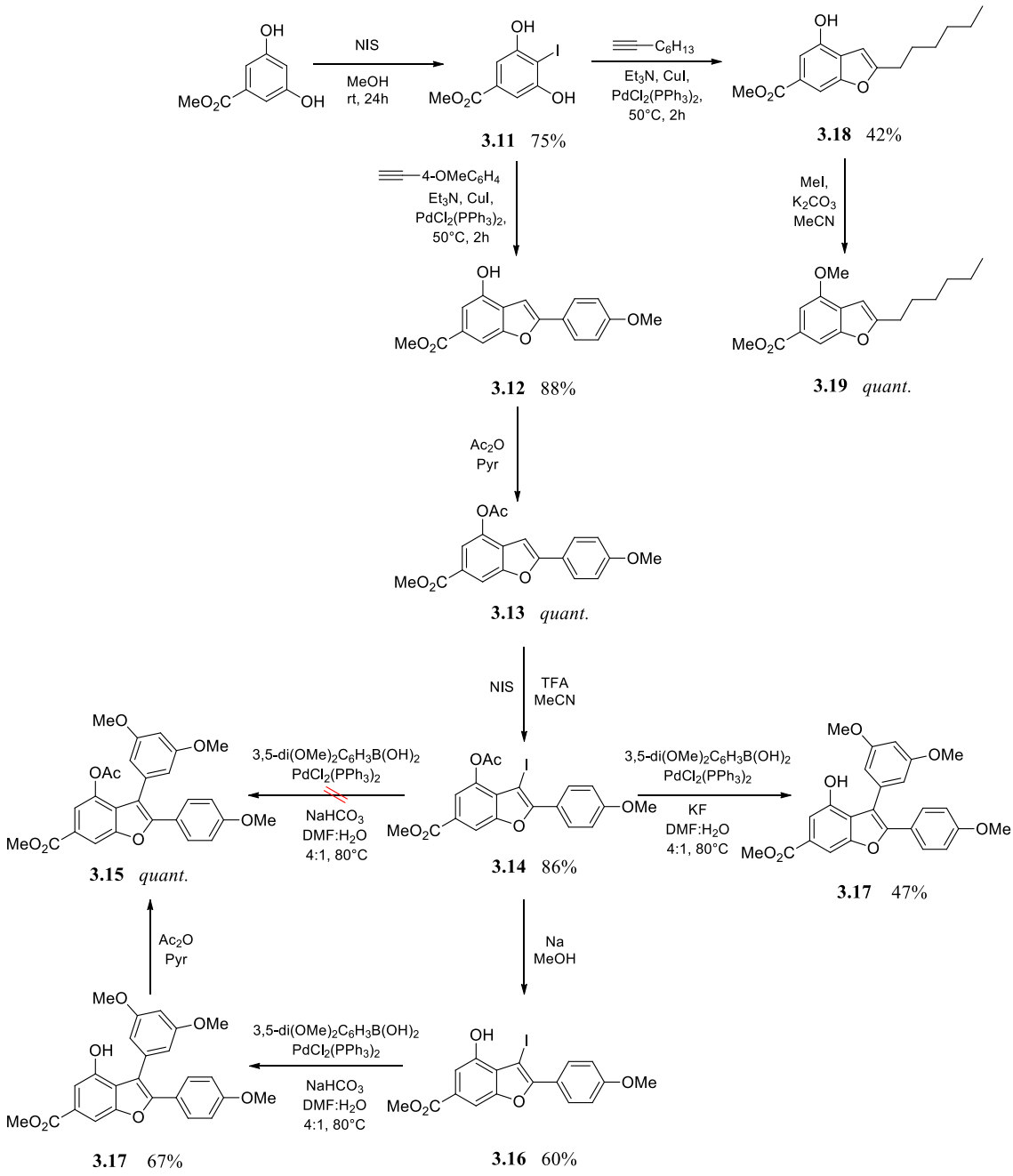



**Scheme 3.14**

As reported in scheme 3.14 to obtain 2,3,4,6-substituted benzofuran, in which the ester group is in position C6 instead of C4 as in compound **3.7** of scheme 3.11, the key reactions are the last Suzuki cross-coupling and a Sonogashira reaction and cyclization to obtain the 2-substituted benzofuryl intermediate. Synthetic path is detailed in scheme 3.15.

Methyl-3,5-dihydrobenzofuran was efficiently iodinated and used in the Sonogashira cross-coupling and cyclization to afford compound **3.12**. Protection of hydroxyl group and the subsequent iodination gave rise compound **3.14**, which was reacted in Suzuki cross-coupling. Unfortunately the desired product **3.15** was not obtained, probably due to the presence of acetyl group which interferes with the base in the reaction mixture. So substrate **3.14** was deacetylated to afford product **3.16** and coupled with 3,5-dimethoxyboronic acid in Suzuki reaction to afford **3.17**. Final acetylation furnished product **3.15**. Changing the base from $NaHCO_3$ to KF, Suzuki reaction on **3.14** afforded the desired coupling product with simultaneous deacetylation.

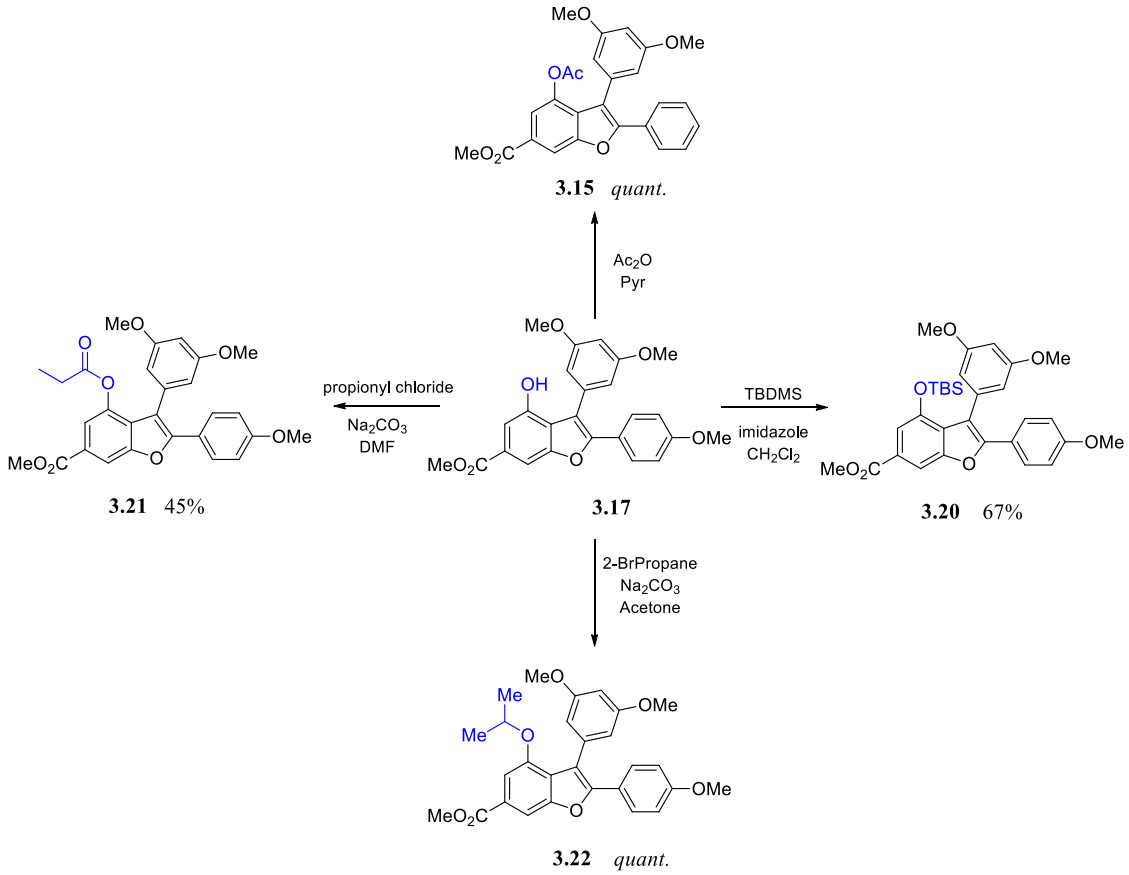
Substrates **3.12**, **3.15**, **3.17** and **3.19** have been subjected to reduction (see section 3.4).


NIS
MeOH
rt, 24h
3.11 75%
C6H13
Et3N, CuI,
PdCl2(PPh3)2,
50°C, 2h
3.18 42%
4-OMeC6H4
Et3N, CuI,
PdCl2(PPh3)2,
50°C, 2h
3.12 88%
MeI,
K2CO3
MeCN
3.19 quant.
Ac2O
Pyr
3.13 quant.
NIS
TFA
MeCN
3,5-di(OMe)2C6H3B(OH)2
PdCl2(PPh3)2
NaHCO3
DMF:H2O
4:1, 80°C
3.15 quant.
3.14 86%
3,5-di(OMe)2C6H3B(OH)2
PdCl2(PPh3)2
KF
DMF:H2O
4:1, 80°C
3.17 47%
Ac2O
Pyr
Na
MeOH
3,5-di(OMe)2C6H3B(OH)2
PdCl2(PPh3)2
NaHCO3
DMF:H2O
4:1, 80°C
3.17 67%
3.16 60%

**Scheme 3.15**

Furtheremore compound **3.17** was protected with different protecting groups. It was used acetyl, *tert*-butyldimethylsilyl ether, isopropyl, and propionyl esther as protecting groups (Scheme 3.16).

These substrates have been subjected to reduction (see section 3.4, table 3.2)




**Scheme 3.16**

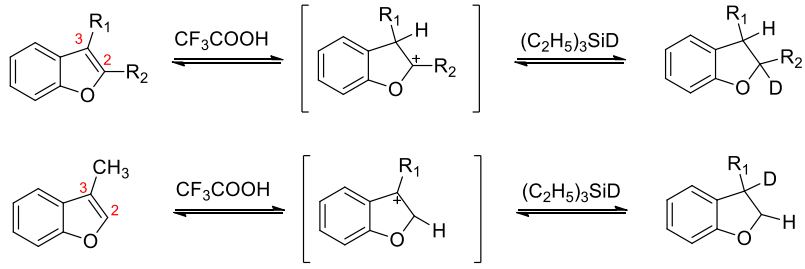
## *3.3 Reduction of substituted benzofurans*

Several methods are known to reduce benzofuran to 2,3-dihydrobenzofuran[15]. In this work the ionic hydrogenation was the method of choice, consisting in the successive addition of a proton and a hydride ion. In particular, trifluoroacetic acid (TFA) was used as proton donor and triethylsilane (TES) as hydride donor[16]. This combination constitutes a powerful, yet selective, tool for the hydrogenation of many functional groups. Many acetals, alcohols, aldehydes, alkenes, cyclopropanes, hemiacetals, ketones, thiophenes and some alkynes, dihydropyridines, disulfides, enamines, ethers, furans, imidazolones, imines, indoles, *O*-acyl oximes, sulfides, thiopyrans, *p*-quinones, and α,β-unsaturated ketones are reduced by this reagent system. Functionalities which are generally unaffected by these reductive conditions include most amide, arene, carboxylate, ester, ether, halide, nitrile, nitro, sulfonate, and phenolic and primary aliphatic hydroxy groups[17]. The reactions are sometimes run without solvent, but most often a solvent such as $MeNO_2$, $CCl_4$, $CHCl_3$, or, preferably, $CH_2Cl_2$ is used. Reactions are performed at room temperature and are worked up by careful addition of a base, such as sodium bicarbonate.[18] It has been demonstrated that heteroaromatic compounds readily undergo ionic hydrogenation[19].

Triethylsilane is an example of an organosilicon that acts as a hydride donor and is frequently used as a synthetic reagent due to its availability, good physical properties and low cost[20]. Proposed mechanism consists of the initial protonation of the substrate by the TFA and the subsequent donation of hydride to the cation formed by TES. In particular, in benzofuran ring, the regioselectivity of protonation was established in experiments with hydrogenating pairs in which one of the substances contained deuterium. Using triethyldeuterosilane $(C_2H_5)_3SiD$, if benzofuran has substituents in 2 and 3 positions, the proton added to C3, while in the case of 3-methylbenzofuran proton added to C2 (scheme 3.17).

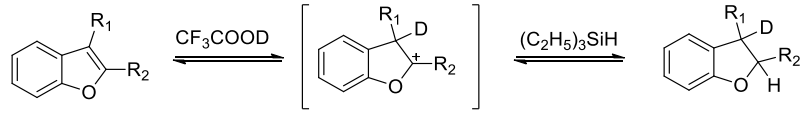
---

[15] a) T.J. Donohoe, C.R. Jones, C. Winter, *Comprehensive Organic Synthesis II* **2014**, *8*, 803. b) J. Zhang, J. Zhang, Y. Kang, J. Shi, C. Yao, *Synlett* **2016**, *27*, 1587.
[16] D.N. Kursanov, Z. N. Parnes , *The Chemical Society Russian Chemical Reviews*, *38*, Number 10, **1969**.
[17] a) D.N. Kursanov, Z.N. Parnes, M.I. Kalinkin, N.M. Loim, *Ionic Hydrogenation and Related Reactions*; Harwood: Chur, Switzerland, **1985**.
[18] a) F.A. Carey, H.S. Tremper, *JACS* **1968**, *90*, 2578. b) H. Mayr, N. Basso, G. Hagen, *JACS* **1992**, *114*, 3060.
[19] a) Z. N. Parnes, G.I. Bolesova, A.I. Belen'kH, D.N. Kursanov, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk* **1973**, 1918. b) E.A. Karakhanov, E.A. Dem'yanova, L.N. Borodina, E.A. Viktorova, *Dold. Akad. Nauk SSSR* **1974**, *214*, 584.
[20] J. L. Fry, *Encyclopedia of Reagents, for Organic Synthesis,*, 1-9, **2001**.



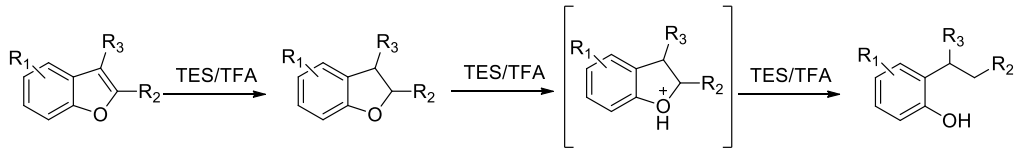

**Scheme 3.17**

The formation of monodeuterated 2,3-dihydrobenzofurans with a fixed position of the label during the ionic hydrogenation of benzofurans constitutes evidence for the absence of 1,2-hydride and methyl shifts in the intermediately formed carbonium ions. This mechanism was then confirmed using deuterotrifluoroacetic and triethylsilane as hydrogenating pairs[21].




**Scheme 3.18**

Although examples of reduction of benzofuran ring to 2,3-dihydrobenzofuran are reported in literature[22], in some cases the obtained product is the over-reducted open chain product (Scheme 3.19), or in other cases the reaction does not occur at all.




**Scheme 3.19**

[21] L.N. Borisova, S.G. Rozenberg, N.F. Kucherova, V.A. Zagorevskii, *Chem Heterocycl Compd.* **1981**, *17*, 869.
[22] C.K. Lau, P.C. Belanger C. Dufrsne, J. Scheigetz, M. Therien, B. Fitzsimmons, R.N. Young, A.W. Ford-Hutchinson, D. Riendeau, D. Denis, J. Guay, S. Charleson, H. Piechuta, C.S. McFalane, S.H. Lee Chiu, D. Eline, R.F. Alvaro, G. Miwa, J.L. Walsh, *J. Mrd. Chem*. **1992**, *35*, 129.

The present work is an extension of a work already started for years in the laboratory where I worked, whose principal aims was the synthesis of the permethylated Gnetin C, a *viniferin* which contains 2,3-dihydrobenzofuran core (figure 3.2). Gnetin C has inhibitory properties of melanogenesis, anti-inflammatory, anti-angiogenic, antibacterial and anti-cancer properties[23]. Its methylated form can increase its permeability, its metabolic stability and its bioavailability.

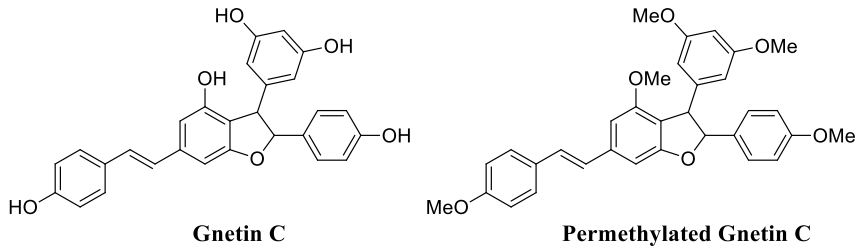
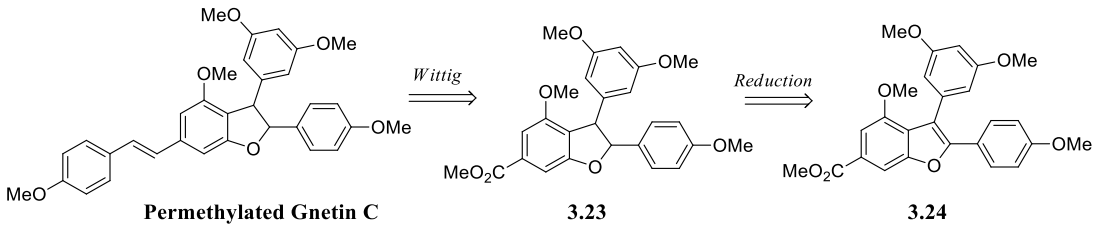



**Figure 3.2**

Retrosynthetic path, as reported in scheme 3.20, provides reduction of substrate **3.24** to obtain the 2,3-dihydrobenzofuran core **3.23**.



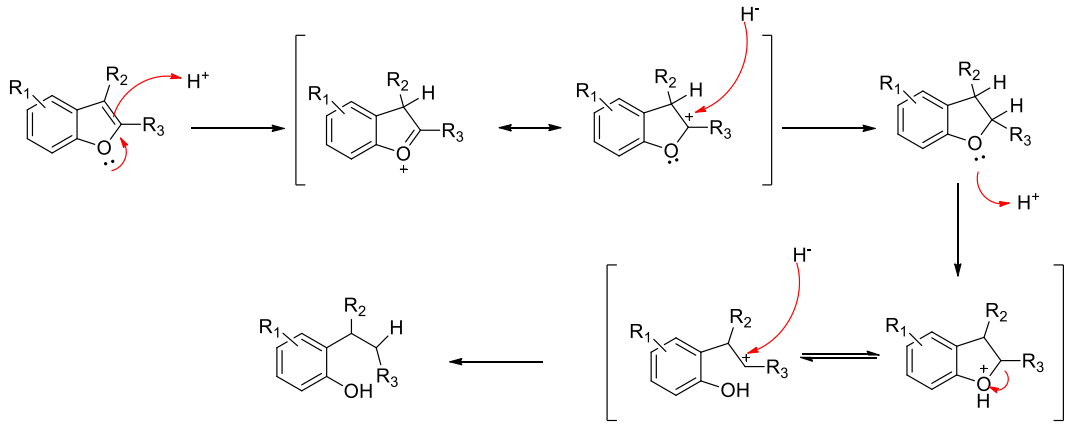

**Scheme 3.20** Retrosynthetic path of Permethylated Gnetin C

[23] a) C. Kloypan, R. Jeenapongsa, P. Sri-In, S. Chanta, D. Dokpuang, S. Tip-Pyang, N. Surapinit, *Phytother. Res.* **2012**, 26, 1564. b) M. Yanagihara, M. Yoshimatsu, A. Inoue, T. Kanno, T. Tatefuji, K. Hashimoto, *Biol. Pharm. Bull.* **2012**, *35*, 993.

## *3.4 Results and discussion: reduction of substituted benzofurans*[24]

In this section all attempts to reduce benzofuran are reported. Benzofuran rings are synthetized as previously descripted according **Path A** or **Path B** only changing substituents. Not all benzofuran compounds have been synthesized in this work, but all attempts are reported for the rationalization of the results. Some intermediates were analyzed on GC-MS, so products conversion is reported; if products were isolated, the yield% is reported.

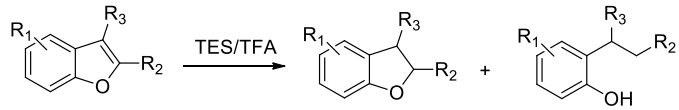
Previuosly was studied the reactivity of 2-substituted benzofuran (table 3.2a, **entries 1-2**; $R_1$, $R_3$=H) to note that if $R_2$ is an alkyl group, reaction affords to 2,3-dihydrobenzofuran, instead if $R_2$ is an aryl group, the reaction proceeds to over reduction and the furan ring opening. These results can be explain with the supposed reduction mechanism (see also section 3.3), where the first step is a protonation by TFA, so carbocation has been formed in C2, and then reduction occurs with hydride donation by TES (scheme 3.21). Subsequently a new protonation can occur on oxigen atom, so, in the case of $R_3$ = Ph, stabilized benzyl carbocation can be formed by furyl ring opening and over-reduction occurs.



**Scheme 3.21**

[24] L. Chiummiento et al. *manuscript in preparation.*

**2-substituted benzofurans ($R_1$, $R_3$ =H)**




**3.25 a-b** **3.26 a-b** **3.27 a-b**

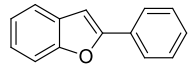
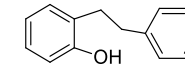
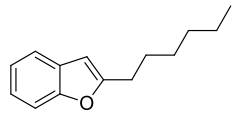
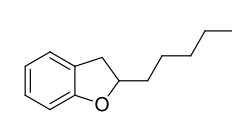
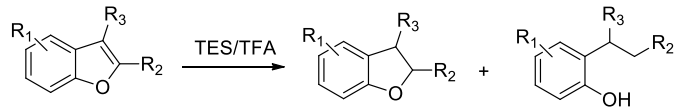
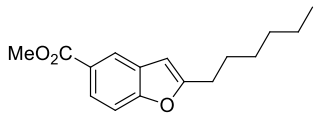
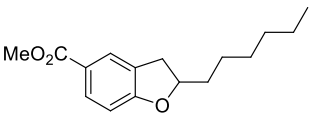
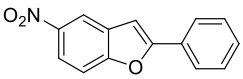
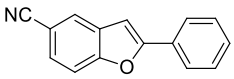
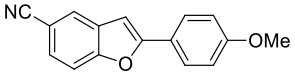
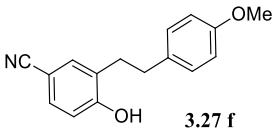
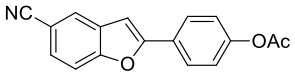
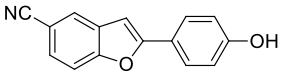
| **Entry** | Benzofuran | Time | Products ratio **Start. : 3.26 : 3.27** | Product **(Yield %)** |
|---|---|---|---|---|
| **1** |  **3.25 a** | 18 h | 0 : 0 : 100 |  **3.27 a (100%)** |
| **2** |  **3.25 b** | 6 h | 40 : 60 : 0 |  **3.26 b (60%)** |

**Table 3.2a**

Then the reaction on 2-5-disubstituted benzofurans was performed (table 3.2b, **entries 1-13**, $R_3$=H).

| **2,5-substituted benzofurans ($R_3$ =H)** | | | | |
|---|---|---|---|---|
|  <br>**3.8; 3.9; 3.25 d-o** **3.26 c-m** **3.27 c-m** | | | | |
| **Entry** | Benzofuran | Time | Products ratio<br>**Start. : 3.26 : 3.27** | Product<br>**(Yield %)** |
| **1** |  <br>**3.8** | 24h | 0 : 100 : 0 |  <br>**3.26 c**<br>**(100%)** |
| **2** |  <br>**3.25 d** | 21h | 90 : 10 : 0 | |
| **3** |  <br>**3.25 e** | 20 h | 80 : 20 : 0 | |
| **4** |  <br>**3.25 f** | 3h | 0 : 0 : 100 |  <br>**3.27 f**<br>**(100%)** |
| **5** |  <br>**3.25 g** | 24 h | 60 : 0 : 0 |  <br>**3.25 h**<br>**(40%)** |

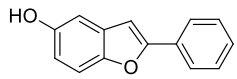
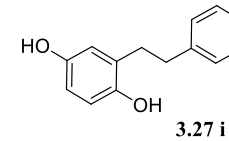
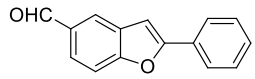
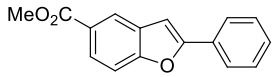
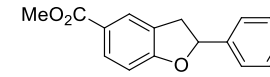
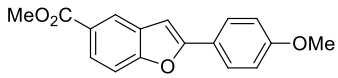
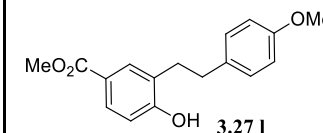
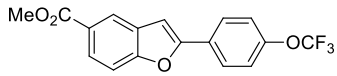
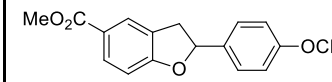
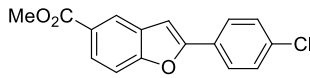
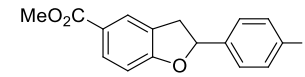
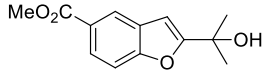
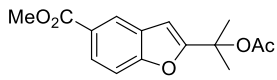
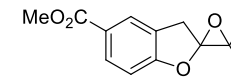
| | | | | |
|---|---|---|---|---|
| **6** |  **3.25 i** | 17 h | 0 : 0 : 100 |  **3.27 i (100%)** |
| **7** |  **3.25 j** | 24 h | | *degradation* |
| **8** |  **3.25 k** | 26 h | 58 : 42 : 0 |  **3.26 k (42%)** |
| **9** |  **3.25 l** | 72 h | 0 : 0 : 100 |  **3.27 l (100%)** |
| **10** |  **3.25 m** | 24h + 24h, 40°C | 20 : 50 : 30 |  **3.26 m** |
| **11** |  **3.25 n** | 24 h | 6 : 25 : 69 |  **3.27 n** |
| **12** |  **3.9** | 24 h | | *degradation* |
| **13** |  **3.25 o** | 16 h | |  |

**Table 3.2 b**

Entry **1** is in line with entry **2** of table 3.2a, in which an alkyl substituents at C-2 leads to 2,3-dihydrobenzofuran (**3.26c**); when a phenyl group is present at C-2 and an electron withdrawing group is at C-5 (**entries 2-3**), the oxygen lone pair is less available to protonation, due to the delocalization with the electron withdrawing group in *para* position, so reduction occurs very slowly and over reduction does not occur (figure 3.3, **A**). Instead, if a electron donating group is present on phenyl at C-2 (**entry 4**), its effect of stabilizating the carbocation predominates and over-reduction occurs (figure 3.3, **B**).

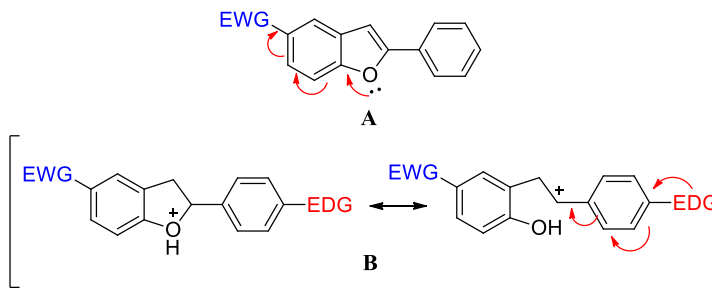


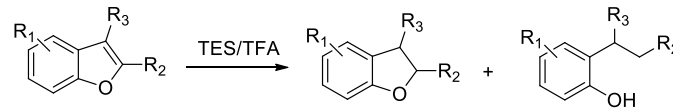

**Figure 3.3**

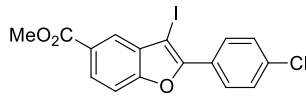
If 4-acetoxyphenyl group is present at C-2, its behaviour reflected, more or less, that of unsubstitued phenyl derivative **3.25e.** The only product observed was the deacetylated derivative **3.25h**. No reduced product was observed, despite the electron donating nature of OH group. This suggested a likely transesterification reaction leading to the intermediate trifluoroacetoxy derivative, which was rapidly hydrolized on silica gel (**entry 5**). An hydroxylic group at C-5 led to over reduction (**entry 6**); while an aldehyde group on benzene ring was not tolerated, leading the formation of many degradation products (**entry 7**).

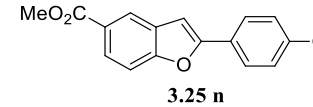
This trend was confirmed for substrates bearing an ester at C-5 (**entries 8-13**). If unsubstituted phenyl group is present at C-2, reduction occured slowly and the delocalization of oxigen lone pair did not allow further protonation(**entry 8**). On the other hand, if a *p*-methoxyl substituent is present on the phenyl group at C-2, over-reduction occured (**entry 9**). Switching to -$OCF_3$ group, in which fluoro atoms decrease the electron releasing effect of oxygen, reduction occured slowly, giving rise to an interesting 50% yield of **3.26m**. The presence of *p*–Cl group, which is considered a weak electron withdrawing group, made the substrate **3.25n** reactive toward reduction and the over-reduction occurred with long reaction time (**entry 11**). In this case the double nature of the substituent (EWG by inductive effect, EDG by mesomeric effect) can play

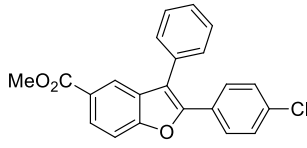
a role which is hard to be fully rationalized. If an alcholic group was present at C-2 (**entry 12**) degradation of the substrate occurred, due to the formation of side products as elimination products; if this alcoholic group is protected as acetyl ester, deprotection and intramolecular cyclization occurred (**entry 13**).

If a substituent is introducted at C-3, reduction does not occur easly probably because of the difficulty of the first protonation (table 3.2c, **entries 1-5**). In the case of 3-iodo derivative **3.25p** dealogenation occured first leading to **3.25n** as the major product, with some traces of reduced product **3.26n**. In general observing results obtained in entries **2-5**, has to be noted that if position 3 is substituted, reduction does not occur easly. If it occurs, the substituent on the phenyl at C-2 drives the reaction, confirming the trend described in table 3.2b: With *p*-$OCH_3$ on the phenyl at C-2 the reduction occurred slowly and the main product was the open chain derivative **3.27u** (**entry 5**).

<table>
<tr><th colspan="5">2,3,5-substituted benzofurans</th></tr>
<tr><td colspan="5"> 

3.25 p-u 3.26 3.27 u</td></tr>
<tr><th>Entry</th><th>Benzofuran</th><th>Time</th><th>Products ratio<br>Start. : 3.26 : 3.27</th><th>Product<br>(Yield %)</th></tr>
<tr><td>1</td><td> 

3.25 p</td><td>24 h</td><td>0 : traces : 0</td><td> 

3.25 n</td></tr>
<tr><td>2</td><td> 

3.25 r</td><td></td><td></td><td>No product</td></tr>
</table>

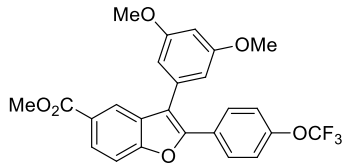
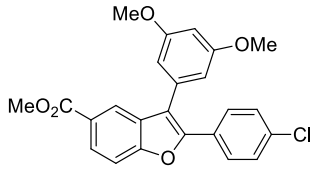
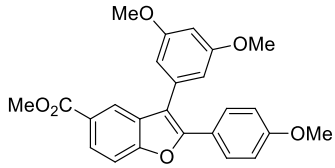
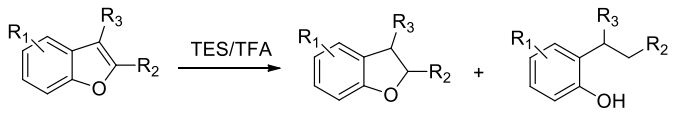
| | | | | |
|---|---|---|---|---|
| **3** |  **3.25 s** | | | *No product* |
| **4** |  **3.25 t** | | | *No product* |
| **5** |  **3.25 u** | 48 h | 74 : 5 : 21 | |

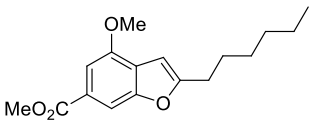
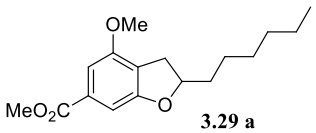
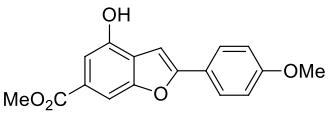
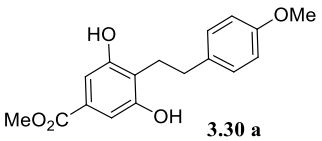
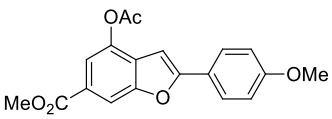
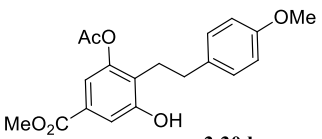
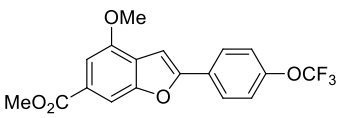
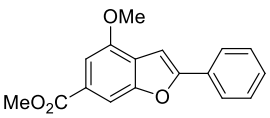
**Table 3.2 c**

Then 2,4,6-substituted benzofurans were studied ($R_1$=5-OR, 6-$CO_2$Me; $R_3$=H; table 3.2d, **entries 1-8**). If $R_2$ is an alkyl group , as previously described, 2,3-dihydrobenzofuran was obtained (**entry 1**). With this substrate, substituents at C-4 and C-6 did not affect the reactivity because charge delocalization do not occur in these positions. Indeed, the reaction was driven by the substituent on phenyl at C-2, so the trend described previuosly was confirmed. Thus, if *p*-EDG group, which stabilizes benzylic carbocation, was present, over-reduction occured (**entry 2**). In the case of 4-OAc derivative **3.28b** the conversion was low and the only product detected was **3.30b** (**entry 3**). In this case, the presence of acetyl group can sterically clutter up the first protonation. On the other hand, the presence of *p*–$OCF_3$ blocks the electron donating effect as an unsubstituted phenyl (**entries 4-5**). Then we tried to understand if the esther functionality could have an important role in reduction reaction; changing it with bromine or with hydrogen, over reduction products were obtained (**entries 6-7**). Furtheremore, when positions of ester and ether functionalities were reversed over-reduction products were obtained.

**2,4,6-substituted benzofurans ($R_1$=5-OR, 6-$CO_2$Me; $R_3$=H)**




**3.5; 3.12; 3.19; 3.28 a-f** → **3.29 a-g** + **3.30 a-g**

| **Entry** | Benzofuran | Time | Products ratio **Start. : 3.29 : 3.30** | Product **(Yield %)** |
|---|---|---|---|---|
| **1** |  **3.19** | 3 h | 0 : 100 : 0 |  **3.29 a (95%)** |
| **2** |  **3.12** | 2 h | 0 : 0 : 100 |  **3.30 a (100%)** |
| **3** |  **3.28 b** | 4h | 67: 0 : 33 |  **3.30 b (33%)** |
| **4** |  **3.28 c** | | | *No product* |
| **5** |  **3.28 d** | | | *No product* |

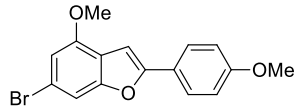
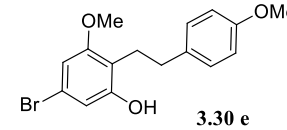
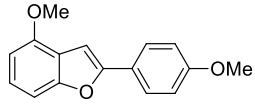
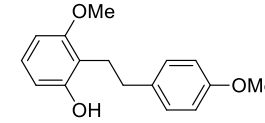
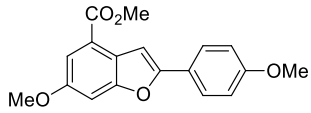
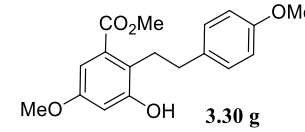
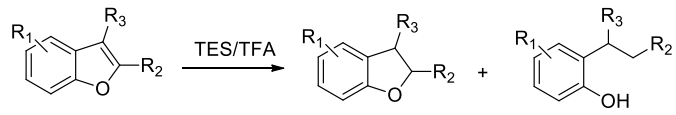
| | | | | |
|---|---|---|---|---|
| **6** |  **3.28 e** | 1 h | 0 : 0 : 100 |  **3.30 e (100%)** |
| **7** |  **3.28 f** | 3 h | 0 : 0 : 100 |  **3.30 f (100%)** |
| **8** |  **3.5** | 2 h | 0 : 0 : 100 |  **3.30 g (100%)** |

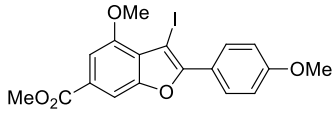
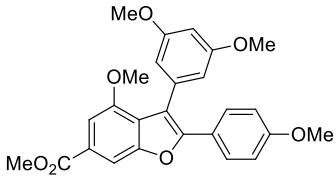
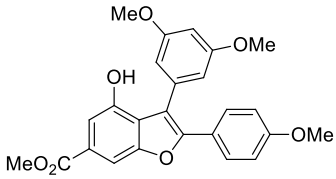
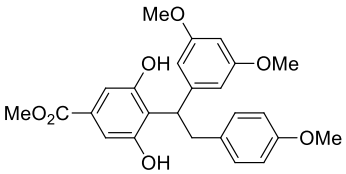
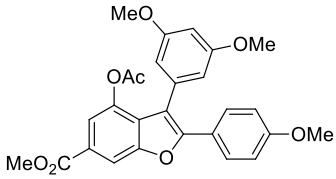
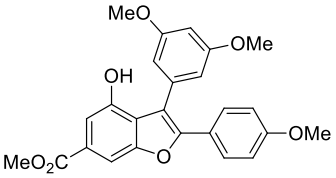
**Table 3.2d**

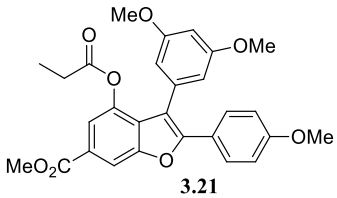
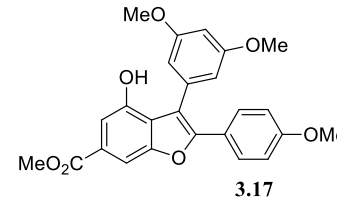
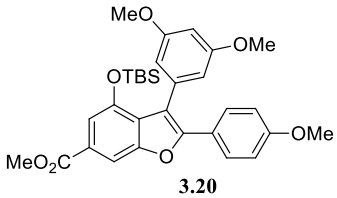
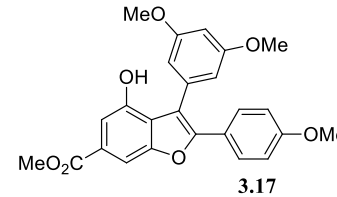
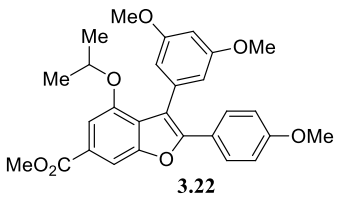
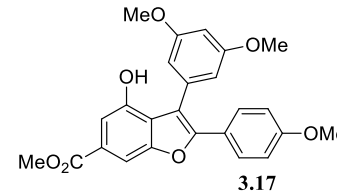
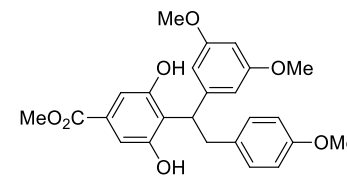
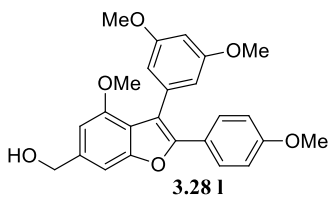
Finally, 2,3,4,6-substitued benzofurans were studied (table 3.2e, **entries 1-11**). In the case of **3.28h** and **3.24** (**entry 1** and **2**), in which $R_3$ is an iodine atom or an aryl substituent only over-reduction occurred, although in these last examples the protonation should be more difficult so reduction should be slow. This implies that the trend of reduction was driven by $R_2$ substituent. In the case of **3.17** (**entry 3**), which bears a free OH at C-4, low conversion was obtained, the over-reduction product **3.30** being the only product observed. In the cases of acetyl (**3.15**), propionyl (**3.21**), tributylsilyl (**3.20**) protected derivatives (**entries 4-6**), only deprotected compound **3.17** was slowly obtained. Only *i*-propyl group was removed fast enough to undergo subsequent reduction (and over-reduction), affording **3.30i** in 25% yield after 24h (**entry 7**). Changing ester functionality at C-6 with hydroxymethyl moiety and its protected derivatives (**entries 8-10**), degradation occured, probably due to the greater reactivity of such moieties than that of benzofuran core. In this case of 4-methoxycarbonyl-6-methoxy derivative **3.7** (**entry 11**), the desired 2,3-dihydrobenzofuran **3.29o** was obtained in modest yield, probably due to the difficulty in accessing of further hydride.

**2,3,4,6-substituted benzofurans**




**3.7; 3.15; 3.17; 3.20-3.22; 3.28 h-n** — **3.29 h-l** — **3.30 h-l**

| **Entry** | Benzofuran | Time | Products ratio **Start. : 3.29 : 3.30** | Product **(Yield %)** |
|---|---|---|---|---|
| **1** |  **3.28 h** | 3 h | 0 : 0 : 100 | |
| **2** |  **3.24** | | 0 : 0 : 100 | |
| **3** |  **3.17** | 24 h | 50 : 0 : 50 |  **3.30 i (48%)** |
| **4** |  **3.15** | 48 h + 24 h 50°C | 0 : 0 : 0 |  **3.17 (75%)** |

| | | | | |
|---|---|---|---|---|
| **5** |  **3.21** | 48 h | 0 : 0 : 0 |  **3.17 (100%)** |
| **6** |  **3.20** | 24 h | 50 : 0 : 0 |  **3.17 (50%)** |
| **7** |  **3.22** | 24 h | 15 : 0 : 25 |  **3.17 (60%)**  **3.30 i (25%)** |
| **8** |  **3.28 l** | 24h | | *degradation* |

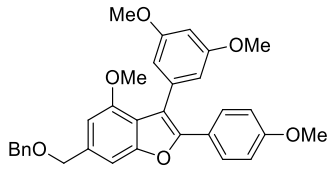
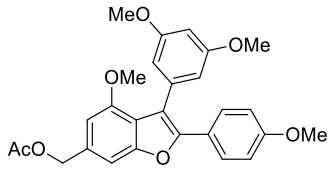
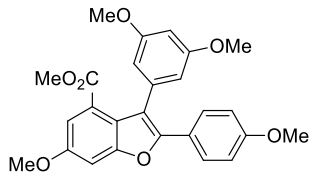
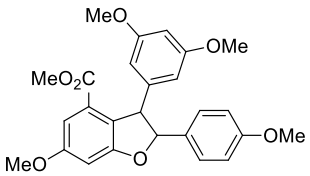
| | | | | |
|---|---|---|---|---|
| **9** |  **3.28 m** | 1 h | | *degradation* |
| **10** |  **3.28 n** | 1 h | | *degradation* |
| **11** |  **3.7** | 2 h | 70 : 0 : 30 |  **3.29 o (25%)** |

**Table 3.2e**

## *3.5 Experimental section*

### *3.5.1 General*

$CH_2Cl_2$ was dried by distillation over anhydrous $CaCl_2$ in an inert atmosphere; toluene and THF were dried using Na/benzophenone. Dry DMF, *i*-PrOH, 1,4-dioxane, MeCN and chlorobenzene were commercially available.

All reactions in non-aqueous media were conducted under a positive pressure of dry argon in glassware that had been oven dried prior.

All commercially available reagents were used without further purification unless otherwise noted.

Column chromatography was carried out on Merck silica gel 60 (70-230 mesh).

$^{1}H$ and $^{13}C$ NMR spectra were normally carried out in $CDCl_3$ solutions on a Varian Inova 400 MHz with TMS as an internal reference; the chemical shifts are reported in ppm in $\delta$ units. Signal splitting patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint),or multiplet (m), with coupling constants (*J*) in Hertz.

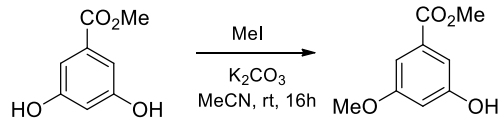
GC-MS: Hewlett–Packard GC/MS6890-5973.

Thin-layer chromatography (TLC) was carried out on precoated silica gel *60* plates (0.2 mm thickness) with the indicated solvents, and the plates were scanned under ultraviolet light at 254 and 365 nm.

Some intermediates were not isolated but exclusively analyzed by GC/MS so their characterization is not completely reported.

### *3.5.2 Synthesis of products*

Methyl-3-methoxy-5-hydroxy benzoate (**3.2**)



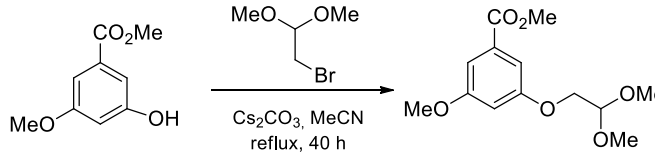

**3.2** 45%

Methyl-3,5-dihydroxy benzoate (0.300 g, 1.8 mmol) was dissolved in MeCN (10 ml) and then $K_2CO_3$ and MeI were added to solution. The mixture was stirred for 16 h. The reaction was quenched with $NH_4Cl$ solution, extracted with $CH_2Cl_2$ for three times, washed with brine and then dried on anhydrous $Na_2SO_4$. The mixture was then filtered and evaporated in vacuum. Crude mixture was purified on silica gel (EP/THF 85/15; $R_f$ 0.4) to obtain **3.2** as a white solid.

**GC-MS**: *m/z* 182.1 ($M^+$, 100%), 151.1 (95%), 123.1 (45%), 108.1 (25%), 93.1 (15%), 69.1 (10%).

Product data are reported in literature[25].

Methyl-3-(2,2-dimethoxyethyloxy)-5-methoxy benzoate (**3.3**)



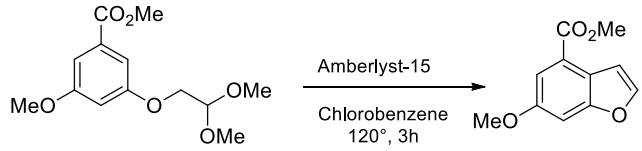

**3.2** **3.3** 78%

To a mixture of compound **3.2** (0.116 g, 0.6 mmol) and $Cs_2CO_3$ (2 equiv., 0.417 g) in 10 ml of MeCN was added 2-bromo-1,1-dimethoxyethane (1.5 equiv, 0.116 ml) at room temperature. The mixture was stirred at reflux for 40 h before the reaction was completed. After cooling down, water was added and extracted with ethyl acetate for three times. The combined organic phase was collected, concentrated and purified on flash column to give methoxybenzoate **3.3** as a colorless oil.

**GC-MS***: m/z* 270 ($M^+$, 15% ), 239.1 (11%), 207.1 (10%), 75.1 (100%).

Product data coincide with those reported in literature[25].

Methyl-6-methoxybenzofuran-4-carboxylate (**3.1**)



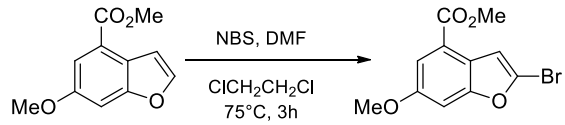

**3.3** **3.1** 80%

To a solution of methoxybenzoate **3.3** (0.120 g, 0.45 mmol) in 10 mL of chlorobenzene was added Amberlyst-15 (12 mg, 10 wt%). The mixture was heated at 120 °C for 3 h. After cooling down to room temperature, the mixture was filtered and the valotile solvent was removed under reduced pressure to give a yellow residue. The crude was purified on silica gel (EP/$Et_2O$ 8/2; $R_f$ 0.8). Product **3.1** was obtained as a white solid.

**GC-MS***: m/z* 206.1 ($M^+$, 100%), 191.0 (45%), 175.0 (50%), 161.0 (10%), 147.0 (20%), 132.0 (24%).

Product data coincide with those reported in literature[25].

Methyl 2-bromo-6-methoxybenzofuran-4-carbozylate (**3.4**)




**3.1** **3.4** 96%

To a solution of **3.1** (0.050 g, 0.24 mmol) in 3 ml of 1, 2-dichloroethane was added NBS (0.065 g, 0.36 mmol) and 0.01 ml DMF. The mixture was stirred at 70° C for 3 h before all the substrate was converted to the product. Saturated $Na_2S_2O_3$ solution was added to quench the reaction and extracted with ethyl acetate. The combined organic phase was collected,

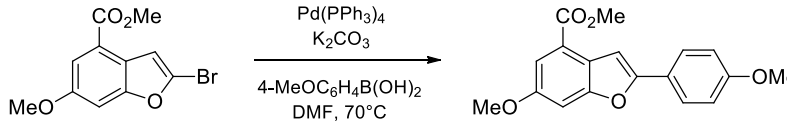
[25] J.T. Liu, T.J. Do, C. J. Simmons, J.C. Lynch, W. Gu, Z.-X. Ma, W. Xuc, W. Tang, *Org. Biomol. Chem.* **2016**, *14*, 8927.

concentrated and purified on silica gel (*n*-hexane/AcOEt 9/1; $R_f$ 0.85) to obtain **3.4** as a white solid.

**GC-MS**: *m/z* 283.9 ($M^+$, 100%), 270.9 (51%), 252.9 (30%), 240.9 (20%), 224.9 (14%), 221.9 (10%), 198.9 (5%), 103.0 (25%), 75.1 (15%).

Product data coincide with those reported in literature[25]

Methyl 6-methoxy-2-(4-methoxyphenyl)benzofuran-4-carboxylate (**3.5**)



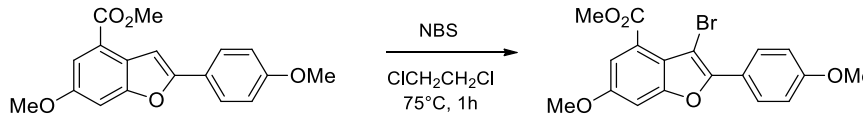

**3.4** **3.5** 53%

To a 50 mL flask was added **3.4** (0.050 g, 0.18 mmol), (4-methoxyphenyl)boronic acid (0.052 g, 0.36 mmol), $K_2CO_3$ powder (0.118 g, 0.85 mmol) and DMF (3 mL). The mixture was degassed three times before $Pd(PPh_3)_4$ (0.004 mmol) was added. The flask was degassed again and refilled with argon. The mixture was heated at 70 ° C overnight. Water was added and extracted with ethyl acetate three times. The organic phase was collected and washed with brine. The organic layer was dried with $Na_2SO_4$, concentrated and purified on silica gel (EP/AcOEt 9/1; $R_f$ 0.6) to obtain **3.5** as a white solid.

**GC-MS**: *m/z* 312.3 ($M^+$, 100%), 297.1 (100%), 281.1 (10%), 254.0 (7%), 223.1 (6%), 207.0 (4%), 156.0 (15%), 139.0 (10%), 119.0 (25%), 32.0 (15%).

Product data coincide with those reported in literature[25].

3-Bromo-6-methoxy-2-(4-methoxyphenyl)-4-methylbenzofuran (**3.6**)



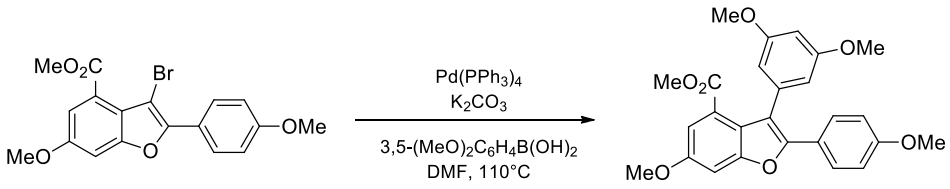

**3.5** **3.6** 93%

To a solution of **3.5** (0.010 g, 0.03 mmol) in 3 mL of 1, 2-dichloroethane was added NBS (0.009 mg, 0.05 mmol). The mixture was stirred at 70° C for 1 hour before the reaction was completed. Saturated $Na_2S_2O_3$ solution was added to quench the reaction and extracted with ethyl acetate for three times. The combined organic phase was collected, dried on $Na_2SO_4$ concentrated to give bromide **3.6** as a white solid.

**GC-MS**: *m/z* 390 ($M^+$, ), 280.9 (15%), 206.9 (30%), 157.0 (15%), 143.1 (35%), 135.0 (30%), 128.0 (20%), 119.1 (28%), 105.1 (25%), 81.2 (28%), 69.0 (27%), 55.0 (25%), 43.1 (100%), 32.1 (80%).

Product data coincide with those reported in literature[25].

3-(3,5-dimethoxyphenyl)-6-methoxy-2-(4-methoxyphenyl)-4-methylbenzofuran (**3.7**)




**3.6** **3.7** 71%

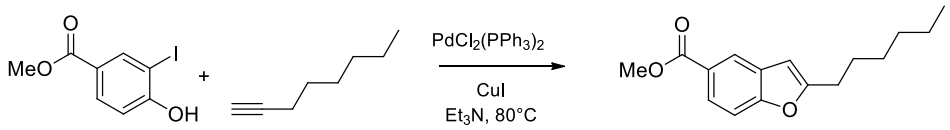
To a 50 mL flask was added bromide **3.6** (0.0010 g, 0.026 mmol), (3,5-dimethoxyphenyl)boronic acid (0.015 g, 0.078 mmol), $K_2CO_3$ power (0.018 g, 0.13 mmol) and DMF (3 ml). The mixture was degassed three times before $Pd(PPh_3)_4$ (0.05 equiv) was added. The flask was degassed again and refilled with argon. Then the mixture was heated at 110° C for 4 h. Water was added and the aqueous solution was extracted with ethyl acetate three times. The organic phases were combined and washed with brine. Then the organic layer was dried

with $Na_2SO_4$, concentrated and purified on silica gel (*n*-hexane/$Et_2O$ 8/2; $R_f$ 0.4) to give **3.7** as a light yellow oil.

**GC-MS**: *m/z* 448 ($M^+$, 100%), 433.0 (45%), 208.0 (10%), 180.0 (5%).

Product data coincide with those reported in literature[25].

Methyl 2-hexylbenzofuran-5-carboxylate (**3.8**)




**3.8** 72%

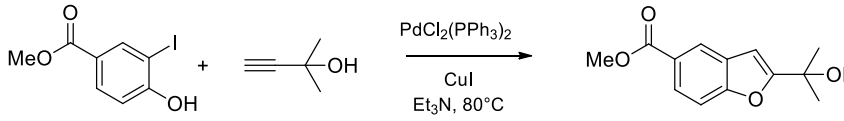
Methyl 4-hydroxy-3-iodobenzoate (0.280 g, 1 mmol) was dissolved, under Ar atmpsphere, in $Et_3N$; then CuI (0.07 mmol), $PdCl_2(PPh_3)_2$ (0.06 mmol) and 1-octine (0.22 ml, 1.5 mmol) were added to solution. The mixture was warmed to 80° C and kept stirring for 2 hours. Reaction was quenched with saturated $NH_4Cl$ solution, extracted with AcOEt and washed with brine. Organic phases were collected, dried on $Na_2SO_4$, concentrated and the crude was purified on silica gel (*n*-hexane/$Et_2O$ 8/2; $R_f$ 0.7). Compound **3.8** was obtained as colorless oil.

**GC-MS:** *m/z* 260.1 ($M^+$, 45%), 229.1 (17%), 189.1 (100%), 166.1 (10%), 159.0 (8%), 130.1 (25%), 95.1 (35%).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 8.21 (s, 1H), 7.93 (d, *J* = 8 Hz, 1H), 7.41 (d, *J* = 8 Hz, 1H), 6.43 (s, 1H), 3.93 (s, 3H), 2.77 (t, *J*=7.6 Hz, 2H), 1.74 (m, 2H), 1.32 (m, 6H), 0.89 (t, *J*=6.4 Hz, 3H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 167.5, 161.4, 157.3, 129.0, 124.9, 124.7, 122.6, 110.5, 102.2, 52.0, 31.5, 28.8, 28.4, 27.5, 22.5, 14.0.

Methyl 2-(2-hydroxypropan-2-yl)benzofuran-5-carboxylate (**3.9**)




**3.9** 80%

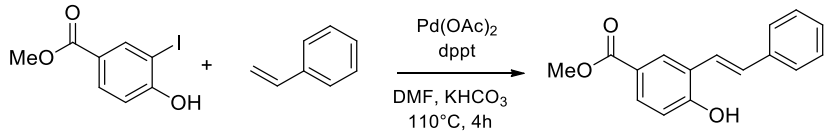
Methyl 4-hydroxy-3-iodobenzoate (0.280 g, 1 mmol) was dissolved, under Ar atmosphere, in $Et_3N$; then CuI (0.07 mmol), $PdCl_2(PPh_3)_2$ (0.06 mmol) and 2-methyl-3-butyn-2-ol (0.15 ml, 1.5 mmol) were added to the solution. The mixture was warmed to 80° C and kept stirring overnight. Reaction was quenched with saturated $NH_4Cl$ solution, extracted with AcOEt and washed with brine. Organic phases were collected, dried on $Na_2SO_4$, concentrated and the crude was purified on silica gel (EP/$Et_2O$ 6/4; $R_f$ 0.6). Compound **3.9** was obtained as a colorless oil.

**GC-MS**: *m/z* 234.1 ($M^+$, 27%), 219.0 (100%), 203.1 (15%), 160.0 (7%), 145.0 (6%), 105.1 (5%), 43.1 (13%).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.46 (d, *J* = 8.8 Hz, 1H), 6.63 (s, 1H), 3.93 (s, 3H), 1.68 (s, 6H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 167.3, 157.3, 128.9, 128.8, 125.0, 123.4, 111.0, 100.8, 69.2, 60.4, 52.1, 28.7, 14.2.

(E)-Methyl 4-hydroxy-3-styrylbenzoate (**3.10**)




**3.10** 71%

Methyl 4-hydroxy-3-iodobenzoate (0.667 g, 2.4 mmol), styrene (0.250 g, 2.4 mmol), $Pd(OAc)_2$ (0.05 equiv), 1,1'bis(diphenilphosphino)ferrocene (0.05 equiv), and $KHCO_3$ (1 g, 7.2 mmol) were added to dry DMF (5 ml). The solution was heated for 4 h at 110 °C, after which time the reaction was complete as determined by GC-MS analysis. Water was added and the mixture was

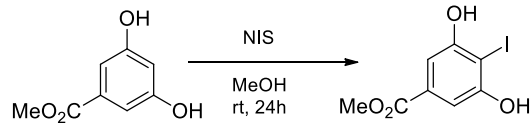
extracted with AcOEt. The organic extracts were combinated, dried and purified on silica gel (EP/$Et_2O$ 1/1; $R_f$ 0.4). Compound **3.10** was obrained as a white solid.

**GC-MS:** *m/z* 254.1 ($M^+$, 100%), 223.1 (55%), 194.1 (20%), 177.1 (15%), 165.1 (45%), 152.1 (25%), 111.4 (13%).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ. 8.28 (s, 1H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.55 (m, 5H), 7.30 (d, *J* = 12.8, 1H), 7.22 (d, *J* = 12.8, 1H), 6.00 (s, 1H), 3.95 (s, 3H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 167.1, 157.0, 137.2, 131.2, 130.3, 129.2, 128.7, 127.9, 126.7, 124.7, 122.9, 121.9, 120.8, 115.7, 114.9, 52.1.

Methyl 3,5-dihydroxy-4-iodobenzoate (**3.11**)

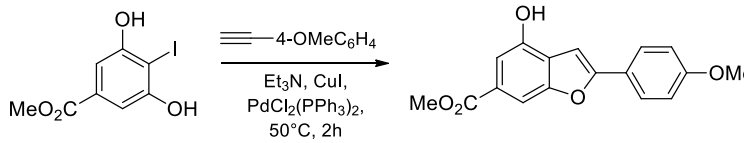



**3.11** 75%

Methyl 3,5-dihydroxybenzoate (2.000 g, 0.01 mol) was dissolved in MeOH (15 ml) and NIS (2.700 g, 0.012 mol) was added. The mixture was stirred for 24 h at room temperature. Reaction was quenched with $Na_2SO_3$ solution, extracted with AcOEt three times and washed with $H_2O$ and brine. Organic phases were collected, dried and concentrated. The crude was purified by column chromatography on silica gel (*n*-hexane/$Et_2O$ 1/1; $R_f$ 0.4). Product **3.11** was obtained as a dark yellow solid.

**$^1$H NMR** (400 MHz, $CDCl_3$): δ. 7.20 (s, 2H), 5.53 (s, 2H), 3.91 (s, 3H).

Methyl 4-hydroxy-2-(4-methoxyphenyl)benzofuran-6-carboxylate (**3.12**)

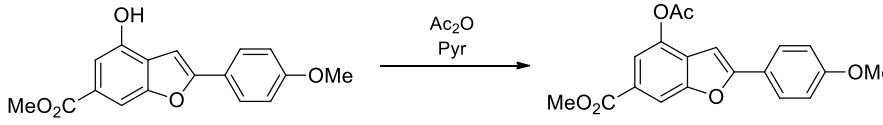



**3.11** **3.12** 88%

Methyl 3,5-dihydroxy-4-iodobenzoate (0.250 g, 0.85 mmol), was dissolved in $Et_3N$ (3 ml) under Ar atmosphere; CuI (0.004 g, 0.02 mmol), $PdCl_2(PPh_3)_2$ (0.014 g, 0.02 mmol) and 4-methoxyphenyl acetilene (0.330 g, 2.5 mmol) were added to solution and warmed to 50°C for 2 hours. After this time, reaction was quenched with saturated solution of $NH_4Cl$, extracted with AcOEt three times and washed with brine, dried and concentrated. The crude was purified on silica gel (n-hexane/AcOEt 7/3; $R_f$ 0.4). The product **3.12** was obtained after purification and recrystallization from $Et_2O$ and *n*-hexane as a white solid.

**$^1$H NMR** (400 MHz, $CDCl_3$, TMS): δ. 7.82 (m, 3H), 7.35 (s, 1H), 7.01 (m, 3H), 5.31 (bs, 1H), 3.95 (s, 3H), 3.89 (s, 3H)[26].

Methyl 4-acetoxy-2-(4-methoxyphenyl)benzofuran-6-carboxylate (**3.13**)




**3.12** **3.13** *quant.*

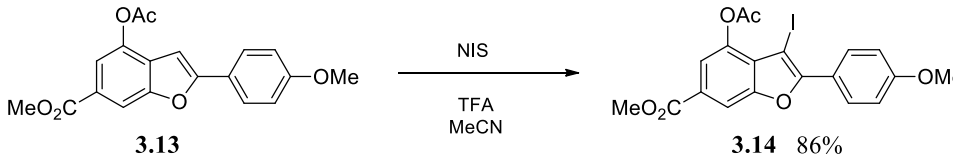
Compound **3.12** was dissolved in pyridine (1 ml) and $Ac_2O$ (1 ml) was added; the mixture was kept stirring for 1 hour, then was quenched with HCl 1M three times, extracted with AcOEt three times and washed with saturated $NaHCO_3$ solution, then with brine. Organic phases were dried and concentrated. Compound **3.13** was obtained as a yellow oil.

**$^1$H NMR** (400 MHz, $CDCl_3$): δ. 8.09 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.69 (s, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.80 (s, 1H), 3.94 (s, 3H), 3.87 (s, 3H), 2.42 (s, 3H).

[26] L. Chiummiento, M. Funicello, M. T. Lopardo, P. Lupattelli, S. Choppin, F. Colobert *Eur. J. Org. Chem.* **2012**, 188.

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 168.8, 166.5, 160.8, 159.2, 155.1, 142.5, 127.6, 127.0, 125.8, 122.1, 116.9, 144.4, 110.6, 97.0, 70.5, 69.3, 63.0, 55.4, 52.3, 21.0.

Methyl 4-acetoxy-3-iodo-2-(4-methoxyphenyl)benzofuran-6-carboxylate (**3.14**)



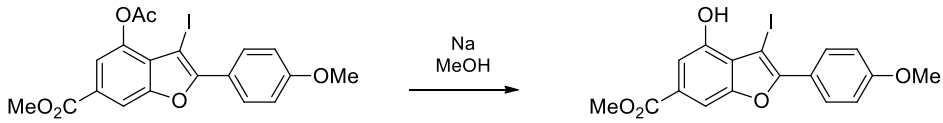

**3.13** **3.14** 86%

Compound **3.13** (0.030 g, 0.09 mmol) was dissolved in 3 ml di MeCN, NIS (0.022 g, 0.1 mmol) and TFA (0.003 ml, 0.03 mmol) were added and the mixture was kept stirring for 4 hours. Then, reaction was quenched with saturated $Na_2SO_3$ solution, extracted with AcOEt and washed with brine; organic phases were dried and concentrated. Compound **3.14** was obtained as a yellow oil after purification on silica gel (*n*-hexane/AcOEt 7/3; $R_f$ 0.4).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ. 8.13 (s, 1h), 8.07 (d, *J* = 8.8 Hz, 2H), 7.69 (s, 1H), 7.03 (d, *J* = 8.8 Hz, 2H), 3.96 (s, 3H), 3.90 (s, 3H), 2.49 (s, 3H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 166.1, 161., 157.1, 154.5, 143.1, 129.8, 127.6, 126.7, 121.6, 118.5, 114.0, 111.0, 70.5, 69.3, 63.0, 55.4, 52.4, 47.2, 29.6.

Methyl 4-hydroxy-3-iodo-2-(4-methoxyphenyl)benzofuran-6-carboxylate (**3.16**)



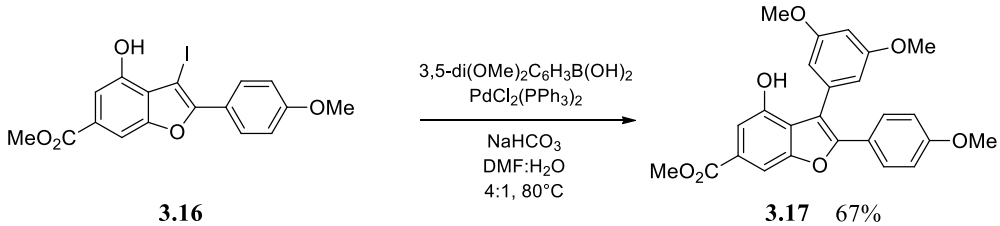

**3.14** **3.16** 60%

Compound **3.14** was dissolved in MeOH (2 ml), using a trace of Na. The mixture after 2 hours was diluited with $H_2O$ and extracted with AcOEt. Compound **3.16** was obtained after purification on silica gel (EP/AcOEt 7/3; $R_f$ 0.3).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ. 8.03 (d, *J* = 8.8 Hz, 2H), 7.84 (s, 1h), 7.44 (s, 1H), 7.03 (d, *J* = 8.8 Hz, 2H), 6.38 (bs, 1H), 3.95 (s, 3H), 3.89 (s, 3H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 166.7, 160.8, 155.1, 154.5, 149.6, 129.4, 127.4, 121.7, 114.3, 114.1, 110.7, 105.9, 60.4, 55.4, 52.3, 51.8, 29.7.

Methyl 3-(3,5-dimethoxyphenyl)-4-hydroxy-2-(4-methoxyphenyl)benzofuran-6-carboxylate (**3.17**)



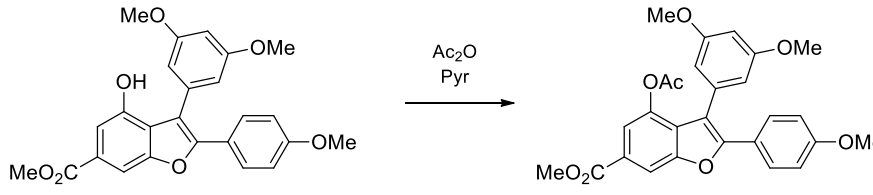

**3.16** **3.17** 67%

Compound **3.16** (0.064 g, 0.15 mmol) was dissolved in a mixture of DMF/$H_2O$ 4/1 (5 ml). Then, 3,5-dimethoxy phenyl boronic acid (0.038 g, 0.21 mmol), $NaHCO_3$ (0.018 g, 0.21 mmol) and $PdCl_2(PPh_3)_2$ (0.005 g, 0.007 mmol) were added and the mixture was stirred at 80° C overnight. The raction was quenched with saturated $NH_4Cl$ solution, extracted with AcOEt three times, washed with brine three times. Then organic phases were collected, dried and concentrated. The crude was purified on silica gel (EP/AcOEt 7/3; $R_f$ 0.2) to obtain **3.18** as a white solid.

**$^1$H NMR** (400 MHz, $CDCl_3$): δ. 7.83 (s, 1h), 7.55 (d, *J* = 8.8 Hz, 2H), 7.37 (s, 1H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.67 (s, 2H), 6.57 (s, 1H), 5.40 (bs, 1H), 3.94 (s, 3H), 3.82 (m, 9H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 167.1, 161.9, 160.1, 154.0, 152.1, 149.7, 134.6, 128.2, 127.1, 122.3, 121.7, 114.0, 113.7, 110.0, 107.5, 105.6, 101.0, 55.5, 55.3, 52.21.

Methyl4-acetoxy-3-(3,5-dimethoxyphenyl)-2-(4-methoxyphenyl)benzofuran-6-carboxylate (**3.15**)



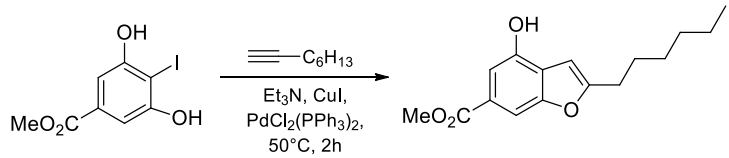

**3.17** **3.15** *quant.*

Compound **3.17** was dissolved in pyridine (1 ml) and $Ac_2O$ (1 ml) was added; the mixture was kept stirring for 1 hour,then was quenched with solution HCl 1M three times, extracted with AcOEt three times and washed with saturated $NaHCO_3$ solution, then with brine. Organic phases collected were dried and concentrated. Compound **3.15** was obtained as a yellow oil.

**$^1$H NMR** (400 MHz, $CDCl_3$): δ. 8.19 (s,1H), 7.58 (s, 1H), 7.48 (d, *J* = 7.2Hz, 2H), 6.83 (d, *J* = 7.2 Hz, 2H), 6.55 (s, 2H), 6.53 (s, 1H), 3.94 (s, 3H), 3.81 (s, 3H), 3.78 (s, 6H), 1.73 (s, 3H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 169.4, 160.9, 160.3, 154.1, 154.1, 143.0, 134.3, 128.6, 127.2, 127.0, 126.2, 122.1, 118.0, 114.4, 114.0, 110.8, 108.3, 100.4, 55.5, 55.3, 52.3, 19.9.

Methyl 2-hexyl-4-hydroxybenzofuran-6-carboxylate (**3.18**)



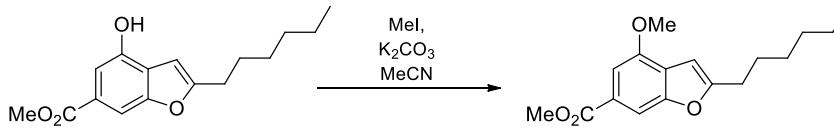

**3.11** **3.18** 42%

Methyl 3,5-dihydroxy-4-iodobenzoate (0.050 g, 0.17 mmol), was dissolved in $Et_3N$ (1 ml) under Ar atmosphere; CuI (0.001 g, 0.005 mmol), $PdCl_2(PPh_3)_2$ (0.03 g, 0.007 mmol) and 1-octine (0.037 ml, 0.26 mmol) were added to solution and warmed to 50°C overnight. Reaction was quenched with saturated solution of $NH_4Cl$, extracted with AcOEt three times and washed with

brine, dried and concentrated. The crude was purified on silica gel (EP/$Et_2O$ 6/4; $R_f$ 0.5). The product **3.18** was obtained as a white solid.

**GC-MS**: *m/z* 276.1 ($M^+$, 45%), 245.0 (15%), 205.0 (100%), 181.0 (10%), 146.0 (17%), 95.1 (9%), 32.1 (8%).

Methyl 2-hexyl-4-methoxybenzofuran-6-carboxylate (**3.19**)




**3.18** **3.19** *quant.*

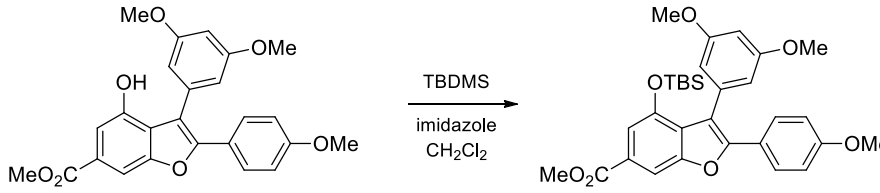
Compound 3.19 (0.018 g, 0.06 mmol) was dissolved in MeCN (3 ml); $K_2CO_3$ (0.008 g, 0.06 mmol) and MeI (0.03 ml, 0.07 mmol) were added and the mixture was kept stirring overnight at room temperature. Reaction was quenched with saturated $NH_4Cl$ solution, extracted with AcOEt three times and then washed with brine. The organic layers were collected, dried and concentrated to obtain compound **3.19** as a white solid.

**GC-MS**: *m/z* 290.1 ($M^+$, 70%), 259.1 (20%), 219.0 (100%), 204.0 (15%), 195.0 (16%), 160.0 (14%), 146.0 (12%).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ. 7.78 (s,1H), 7.32 (s, 1H), 6.51 (s, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 2.77 (t, *J* = 7.2 Hz, 2H), 1.74 (m, 2H). 1.31 (m, 6H), 0.91 (m, 3H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 161.7, 152.1, 129.4, 114.3, 106.4, 104.0, 99.5, 55.6, 52.1, 43.4, 31.4, 29.3, 28.7, 27.4, 22.6, 19.9, 14.2.

Methyl 4-((tert-butyldimethylsilyl)oxy)-3-(3,5-dimethoxyphenyl)-2-(4-methoxyphenyl) benzofuran-6-carboxylate (**3.20**)



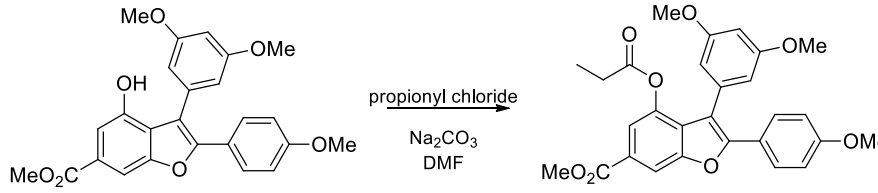

**3.17** **3.20** 67%

Compound **3.17** (0.027 g, 0.06 mmol) wass dissolved in anhydrous $CH_2Cl_2$ (3 ml), *tert*-butylclorodimethylsilane (0.01 ml, 0.07 mmol) and imidazole (0.008 g, 0.12 mmol) were added and the mixture was kept stirring overnight. At the end of reaction, the mixture was quenched with saturated $NH_4Cl$ solution, extracted with $CH_2Cl_2$ three times and washed with brine. Organic phases were collected, dried and concentrated. Product **3.20** was obtained as white solid after purification on silica gel (EP/$Et_2O$ 6/4; $R_f$ 0.8).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.84 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.32 (s, 1H), 6.81 (d, *J* = 8.4 Hz, 2H), 6.58 (s, 2H), 6.49 (s, 1H), 3.94 (s, 3H), 3.80 (s, 3H), 3.76 (s, 6H), 0.74 (s, 9H), 0.06 (s, 6H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 160.8, 159.8, 154.5, 152.4, 149.7, 135.3, 128.4, 126.1, 125.8, 122.8, 115.8, 113.9, 113.0, 108.9, 106.1, 100.0, 55.4, 55.3, 52.2, 31.9, 29.7, 29.4, 25.6, 22.7, 18.2, 14.1, -4.4.

Methyl3-(3,5-dimethoxyphenyl)-2-(4-methoxyphenyl)-4-(propionyloxy)benzofuran-6-carboxylate(**3.21**)

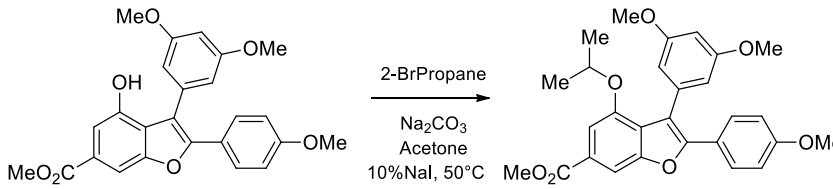



**3.17** **3.21** 45%

Compound **3.17** (0.007 g, 0.02 mmol) wass dissolved in anhydrous $CH_2Cl_2$ (2 ml), propionyl chloride (0.002 ml, 0.024 mmol) and $Na_2CO_3$ (0.004 g, 0.04 mmol) were added and the mixture was kept stirring overnight. At the end of reaction, the mixture was quenched with HCl 2M, extracted with $CH_2Cl_2$ three times and washed with saturated $NaHCO_3$ solution and then with brine. Organic phases were collected, dried and concentrated. Product **3.21** was obtained as white solid after purification on silica gel ($CHCl_3$; $R_f$ 0.6).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.57 (m, 3H), 6.85 (d, *J* = 8.8 Hz, 2H), 6.54 (d, *J* = 8.8 Hz, 2H), 5.34 (s, 1H), 3.96 (s, 3H), 3.82 (s, 3H), 3.79 (s, 6H), 2.33 (m, 2H), 1.99 (m, 3H).

Methyl 3-(3,5-dimethoxyphenyl)-4-isopropoxy-2-(4-methoxyphenyl)benzofuran-6-carboxylate (**3.22**)




**3.17** **3.22** *quant.*

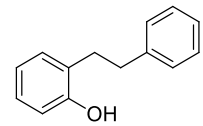
Compound **3.17** (0.013 g, 0.03 mmol) wass dissolved in anhydrous $CH_2Cl_2$ (2 ml), 2-bromopropane (0.005 ml, 0.05 mmol) and $Na_2CO_3$ (0.009 g, 0.09 mmol) were added and the mixture was kept stirring overnight. Since reaction didn't work NaI (10%) was added and the mixture was warmed to 50°C. At the end of reaction, the mixture was quenched with saturated $NH_4Cl$ 2M, extracted with AcOEt three times and washed with brine. Organic phases were collected, dried and concentrated. Product **3.22** was obtained as a white solid and has the same $R_f$ of the starting material.

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.83 (s, 1H), 7.56 (d, *J* = 9.2 Hz, 2H), 7.37 (s, 1H), 6.85 (d, *J* = 9.2 Hz, 2H), 6.67 (s, 2H), 6.58 (s, 1H), 3.95 (s, 3H), 3.82 (s, 3H), 3.81 (s, 6H), 2.18 (s,1H), 1.26 (s, 6H).

### *3.5.3 General Procedure for reduction reactions*

In a round flask, under Ar atmosphere, substrate containing benzofuran ring was solubilized in TFA (6 equiv.) and then TES (6 equiv.) was added to the mixture. The mixture was added for time and at temperature reported in table 3.1. Reaction was monitored every hour by GC-MS. The reaction was quenched with a solution of $NaHCO_3$ at 0°C until the effervescence disappears, then the mixture was extracted with AcOEt three times, washed with brine and dried on anhydrous $Na_2SO_4$. Mixture was filtered and then evaporated in vacuum. The crude was analyzed by GC-MS or NMR and the purified on silica gel.

2-Phenethylphenol (**3.27 a**)

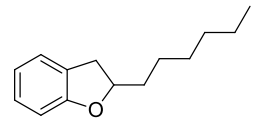



**3.27 a**
**(100%)**

**GC-MS**: *m/z* 198.1 ($M^+$, 40%), 107.1 (100%), 91.1 (35%), 77.1 (25%), 63.0 (6%), 43.1 (10%).

**$^1$H NMR** (400 MHz, $CDCl_3$), δ 7.3 (m, 5H), 7.10 (m, 2H), 6.81 (t, *J* = 8 Hz, 1H),6.78 (d, *J* = 8 Hz, 1H), 4.60 (bs, 1H), 2.85 (s, 4H).

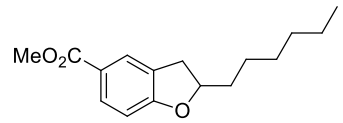
2-hexyl-2,3-dihydrobenzofuran (**3.26 b**)




**3.26 b**
**(60%)**

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.14 (m, 2H), 6.80 (m, 2H), 4.76 (m, 1H), 3.24 (m, 1H), 2.84 (m, 1H), 1.25 (m, 8H),0.81 (s, 3H).

Methyl 2-hexyl-2,3-dihydrobenzofuran-5-carboxylate (**3.26 c**)



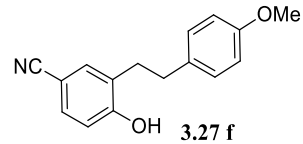

**3.26 c**
**(100%)**

**GC-MS**: *m/z* 262.1 (75%), 231.1 (25%), 191.1 (26%), 178.0 (70%), 165.0 (100%), 147.0 (30%), 131.1 (25%), 105.1 (29%).

**$^{1}$H NMR** (400 MHz, $CDCl_3$), δ 7.85 (m, 2H), 6.74 (d, *J*=8.4, 2H), 4.85 (q, *J*= 7.6 Hz, 1H), 3.86 (s, 3H), 3.28 (dd, $J_1$=8.8 Hz, $J_2$=8.8 Hz, 1H), 2.86 (dd, $J_1$=7.6, $J_2$=7.6 Hz, 1H), 1.82 (m, 1H), 1.68 (m, 1H), 1.30 (m, 8H), 0.89 (m, 3H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 167.0, 163.7, 131.0, 127.4, 126.7, 122.2, 108.9, 84.7, 51.7, 36.0, 34.7, 31.7, 29.1, 25.2, 22.5, 14.0.

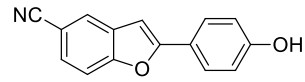
4-hydroxy-3-(4-methoxyphenethyl)benzonitrile (**3.27 f**)




**3.27 f**
**(100%)**

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 7.39 (m, 2H), 7.06 (d, *J* = 8.0 Hz, 2H), 6.82 (m, 3H), 3.80 (s, 3H), 2.87 (m, 4H).

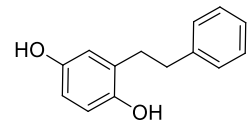
2-(4-hydroxyphenyl)benzofuran-5-carbonitrile (**3.25 h**)




**3.25 h**
**(40%)**

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.92 (d, *J* = 8 Hz, 1H), 7.88 (d, *J* = 8 Hz 7.03, 2H), 7.24 (s, 1H), 7.22 (d, *J* = 8 Hz, 2H,), 7.03 (s, 1H).

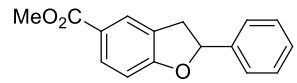
2-Phenethylbenzene-1,4-diol (**3.27 i**)




**3.27 i**
**(100%)**

**$^1$H NMR** (400 MHz, $CDCl_3$, TMS), δ 7.2-7.5 (m, 5H), 6.5-7.0 (m, 3H), 2.85 (m, 4H).

Methyl 2-phenyl-2,3-dihydrobenzofuran-5-carboxylate (**3.26 k**)

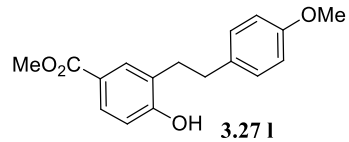



**3.26 k**
**(42%)**

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.93 (d, *J* = 8.4 Hz, 1H), 7.90 (s ,1H), 7.52 (m, 1H), 7.39 (m, 4H), 6.87 (d, *J* = 8.4 Hz, 1H), 5.84 (dd, *J* = 9.2 Hz, *J* = 9.6 Hz, 1H), 3.88 (s, 3H), 3.64(dd, *J* = 16 Hz, *J* = 9.6 Hz, 1H), 3.27(dd, *J* = 16 Hz, *J* = 9.2 Hz, 1H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 29.7, 30.9, 52.1, 101.5, 111.0, 123.3, 125.0, 126.0, 128.7, 128.8, 129.2, 129.9, 157.4, 167.3.

Methyl 4-hydroxy-3-(4-methoxyphenethyl)benzoate (**3.27 l**)

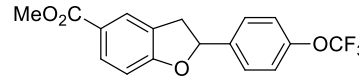



**3.27 l**
**(100%)**

**$^{1}$H NMR** (400MHz, $CDCl_3$): δ 7.85 (d, *J* = 2.0 Hz, 1H), 7.79 (dd , *J*= 8.4 Hz, *J*=2.0 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 2H), 6.83 (d, *J* = 8.4 Hz, 2H,), 6.77 (d, *J* = 8.4 Hz, 1H), 5.38 (bs, 1H), 3.89(s, 3H), 3.83 (s, 3H), 2.88 (m, 4H).

**$^{13}$C NMR** (100MHz, $CDCl_3$): δ 167.0, 157.7, 133.6, 132.2, 129.5, 129.3, 129.2, 127.9, 115.2, 113.9, 55.3, 51.9, 35.1, 32.4.

Methyl 2-(4-(trifluoromethoxy)phenyl)-2,3-dihydrobenzofuran-5-carboxylate (**3.26 m**)



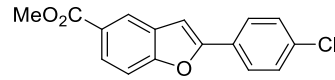

**3.26 m**
**(50%)**

**GC-MS**: *m/z* 338.1 ($M^+$, 100%), 307.0 (53%), 253.1 (8%), 165.1 (25%), 145 (3%), 111 (1%), 69 (10%).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 7.95 (d, *J* = 8.4 Hz, 1H), 7.90 (s, 1H), 7.42 (d, *J* = 8.8 Hz, 2H), 7.25 (d, *J* = 7.6 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 2H), 5.86 (s, 1H), 3.89 (s, 3H).

**$^{13}$C NMR** (400 MHz, $CDCl_3$): δ 166.8, 163.4, 149.0, 140, 131.3, 129.7, 127.1, 126.7, 126.1, 124.7, 123.2, 121.6, 121.1, 119.1, 109.1, 84, 51.9.

Methyl 2-(4-chlorophenyl)-benzofuran-5-carboxylate (**3.25 n**)



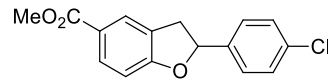

**3.25 n**

**GC-MS:** *m/z* 286($M^+$, 100%), 255 (66%), 227 (29%), 199 (11%), 163 (20%), 127 (13%), 100 (7%).

**$^1$H NMR** (400 MHz, $CDCl_3$) : δ 8.31 (s, 1H), 8.02 (d, *J* = 8.4Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 8 Hz, 2H), 7,05 (s, 1H), 3.94 (s, 3H).

**$^{13}$C NMR** (400 MHz, $CDCl_3$): δ 167.2, 157.4, 156.2, 134.8, 133.7, 129.1, 129.1, 128.3, 126.2, 125.5, 123.4, 111.0,101.9, 52.1.

Methyl 2-(4-chlorophenyl)-2,3-dihydrobenzofuran-5-carboxylate (**3.28 n**)



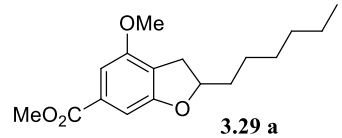

**3.28 n**

**GC-MS**: *m/z* 288 (M+, 100%), 270 (11%), 257 (58%), 194 (17%), 165 (41%), 111 (14%), 82.2 (10%)

**$^1$H NMR** (400 MHz, $CDCl_3$) : δ 7.92 (d, *J* = 8.4 Hz, 1H), 7.88 (s, 1H), 7.33 (m, 4H), 6.87 (d, *J* = 8.4 Hz, 1H), 5.82 (m, 1H), 3.88 (s, 3H), 3.66 (dd, $J_1$= 15.6 Hz, $J_2$= 10 Hz, 1H), 3.18 (dd, $J_1$= 15.6 Hz, $J_2$= 7.6 Hz, 1H).

**$^{13}$C NMR** (400 MHz, $CDCl_3$): δ 166.9, 163.4, 138.8, 134.1, 131.4, 128.9, 127.1, 126.7, 126.6, 123.2, 109.1, 54.39, 51.9, 37.7.

Methyl 2-hexyl-4-methoxy-2,3-dihydrobenzofuran-6-carboxylate (**3.29 a**)



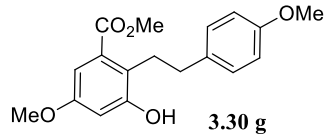

**3.29 a**
**(95%)**

**GC-MS**: *m/z* 292.1 (90%), 261.1 (25%), 221.0 (45%), 208.0 (35%), 195.0 (100%), 189.0 (20%), 182.0 (37%), 175.0 (23%), 163.0 (20%), 148.0 (18%), 135.0 (18%), 121.0 (19%), 105.0 (21%), 91.0 (17%), 77.0 (18%), 59.0 (19%), 41.1 (18%).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 7.09 (d, *J* = 10.4 Hz, 1H), 4.83 (m, 1H), 3.89 (s, 3H), 3.87(s, 3H), 3.23 (dd, *J* = 9.6 Hz, *J* = 9.2 Hz, 1H), 2.79 (dd, *J* = 7.6, *J* = 7.6 Hz, 1H), 1.80 (m, 1H), 1.64 (m, 3H), 1.36 (m, 6H), 0.88 (m, 3H).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 167.1, 160.9, 156.0, 131.3, 119.4, 104.4, 104.1, 84.5, 55.6, 52.1, 36.2, 32.9, 31.7, 29.1, 25.2, 22.6, 14.1.

Methyl 3-hydroxy-5-methoxy-2-(4-methoxyphenethyl)benzoate (**3.30 g**)

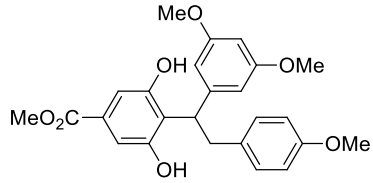



**3.30 g**
**(100%)**

**GC-MS**: *m/z* 316.1 ($M^+$, 30%), 195.0 (100%), 121.1 (75%).

**$^{1}$H NMR** (400 MHz, $CDCl_3$) : δ 7.14 (d, *J* = 8 Hz, 2H), 6.99 (s, 1H), 6.84 (d, *J* = 8 Hz, 2H), 6.51 (s, 1H), 3.88 (s, 3H), 3.79 (s, 6H), 3.09 (t, *J* = 7.6 Hz, 2H), 2.82 (t, *J* = 7.6 Hz, 2H).

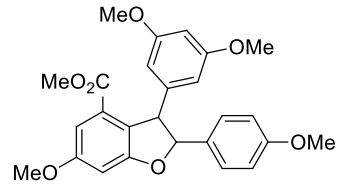
Methyl 4-(1-(3,5-dimethoxyphenyl)-2-(4-methoxyphenyl)ethyl)-3,5-dihydroxybenzoate (**3.30 i**)




**3.30 i**
**(48%)**

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 6.96 (m, 4H), 6.69 (d, *J* = 7.6 Hz, 1H, 2H), 6.56 (s, 2H), 6.34 (s, 1H), 4.92 (s,2H), 4.69 (s, 1H), 3.85 (s, 3H), 3.74 (s, 9H).

3-(3,5-dimethoxyphenyl)-6-methoxy-2-(4-methoxyphenyl)-4-methyl-2,3-dihydrobenzofuran (**3.29 o**)

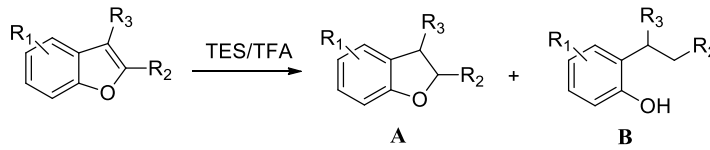



**3.29 o**

**GC-MS**: *m/z* 450.2 (100%), 435.2 (27%), 418.1 (17%), 391.2 (26%), 331.1 (10%), 281.0 (19%), 207.1 (26%), 135.0 (10%).

## *3.6 Conclusions*

Different substituted benzofurans were synthetized according to two synthetic path: **Path A**, where benzofuran core was obtained by alkylation and subsequently cyclization and aromatization and **Path B**, where cyclization step occurs by Sonogashira cross-coupling reaction. Then, the reactivity of this synthetized benzofurans was studied during the ionic hydrogenation by TES/TFA, in order to understand the substituent effect on benzofuran ring during the reduction reaction. It can be noted that in this condition of hydrogenation (scheme 3.22):




**Scheme 3.22**

- If $R_2$ is an alkyl group reduction occurs and 2,3-dihydrobenzofuran, **A**, is obtained, regardless of substituents $R_3$ and $R_1$; instead if $R_2$ is an aryl group, and $R_1$ and $R_3$ are H, the product of over reduction, **B**, is obtained, because of the benzyl carbocation stabilization by mesomeric effect.
- If $R_1$ is electron withdrawing group in position 5 of benzofuran ring, compound **A** was obtained, instead if $R_2$ contains an electron donating group, a further protonation is possible and the furan ring is opened to afford compound **B**.
- If protected alcoholic functionality is present, deprotection occurs.
- If $R_3 \neq H$, protonation can have difficulty to occur and the reaction driver is the substituent $R_2$, so if it is EDG group over reduction to compound **B** occurs.
- Also in 2,4,6-substituted benzofuran the reaction trend is determinated by $R_2$, and when also substituent $R_3$ is present, there is more difficulty acces to protonation and reduction is slower.

# APPENDIX

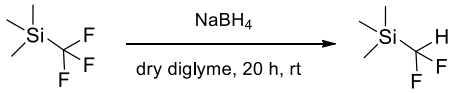
## *A.1 Difluoromethylation of thioamides*

Incorporation of fluorine into bioactive molecules is of special interest to the pharmaceutical industries due to the ability of fluorine to increase metabolic stability and bioavailability of these compounds, lowering susceptibility to P450 cytochrome oxidation. Moreover fluorine increases lipophilicity of hydroxy or thiol groups and has the capability of H-bonding interactions[1]. Major classes of organofluorine compounds with biological acivity include trifluoromethyl- and difluoromethyl groups. In particular, difluoromethyl functional group ($CF_2H$) has strong lipophilic and electron-withdrawing properties that can significantly enhance the physiological activity of organic molecules, this moiety proved to be an essential donor in lipophilic hydrogen bonding, allowing it to serve as a bioisoster of thiol and alchols[2]. So the applications of $CF_2H$-containing compounds in the fields of drugs have attracted great attention of many research groups. Therefore, the development of effective and general methodologies for the incorporation of this group via nucleofilic[3], electrophilic[4] and radical pathways[5] has become one of the hotspots in the field of organic chemistry. In particular the exploration of practical, inexpensive and simply handled difluoromethylating agents is of particular interest. Recently, new difluoromethylation reagents and methods that were able to efficiently incorporate the difluoromethyl group under mild conditions have been developed rapidly[6]. Hu and co-workers demonstrated the effectiveness of employing $TMSCHF_2$ in difluoromethylation under nucleophilic regime of carbonyls (ketones and aldehydes) and imines[7]. Remarkably, it manifests a series of

[1] T. Zhu, Z. Zhang, J. Tao, K. Zhao, T. Loh, *Organic Letter* **2019**, *21*, 6155.
[2] a) J. Hu, *J. Fluorine Chem.* **2009**, *130*, 1130. b) C. Ni, M. Hu, J. Hu, *Chem. Rev* **2015**, *115*, 765. c) M. C. Belhomme, T. Besset, T. Poisson, X. Pannecoucke, *Chem.- Eur. J.* **2015**, *21*, 12836. d) J. Rong, C. Ni, J. Hu, *Asian J. Org. Chem.* **2017**, *6*, 139.
[3] a) Y. Gu, X. B. Leng, Q. Shen, *Nat. Commun.* **2014**, *5*, 5405 b) X.L. Jiang, Z. H. Chen, X. H. Xu, F.L. Qing, *Org. Chem. Front.* **2014**, *1*, 774. c) D.L. Chang, Y. Gu, Q. Shen, *Chem. Eur. J.* **2015**, *21*, 6074.
[4] a) C.S. Thomoson, W.R. Dolbier, *J. Org. Chem.* **2013**, *78*, 8904. b) K. Aikawa, K. Maruyama, K. Honda, K. Mikami, *Org. Lett.* **2015**, *17*, 4882.
[5] a) S. Zhang, L. Li, J. Zhang, M. Xue, K. Xu, *Chem. Sci.* **2019**, *10*, 3181. b) P. Xiong, H.-H. Xu, J. Song, H.-C. Xu, *J. Am. Chem. Soc.* **2018**, *140*, 2460. c) P. Dai, X. Yu, P. Teng, W.H. Zhang, C. Deng, *Org. Lett.* **2018**, *20*, 6901.
[6] Wang, Weiqiang & Yu, Qinwei & Zhang, Qian & Li, Jiangwei & Hui, Feng & Yang, Jianming & Jian; *Recent Progress on Difluoromethylation Methods*, *Chinese Journal of Organic Chemistry* **2018**, *38*, 1569.
[7] Y. Zhao, W. Huang, J. Zheng and J. Hu, *Org. Lett.* **2011**, *13*, 5342.

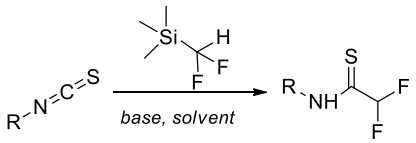
positive features including easy manipulability and the protecting TMS group is advantageously cleaved during the activation event.

Difluoromethyltrimethylsilane is commercially available or obtained by reduction from trifluoromethyltrimethylsilane, note as *Ruppert–Prakash reagent* (scheme A1.1)[8].




**Scheme A1.1**

In this work, in collaboration with the Department of Pharmaceutical Chemistry of University of Wien, the $CHF_2$- moiety was introduced by nucleophilic addition to an electrophilic carbon. Pace group, in fact, showed as the nucleophilic addition of functionalized organometallic reagents to isocyanates and isothiocyanates costitutes a versatile, direct, one-pot and high yielding approach to introduce this moiety and represents an attractive tool for preparation of amide-type compounds[9]. Working in this direction, difluoromethyltrimethylsilane was used as nucleophilic reagent and different isothiocyanates as reactive electrophilic reagents to obtain unprecedented α,α-difluorothiamides (scheme A1.2). This is a new method to introduce this group in order to replace method that needs expensive and toxic elements or fluorinating agent.



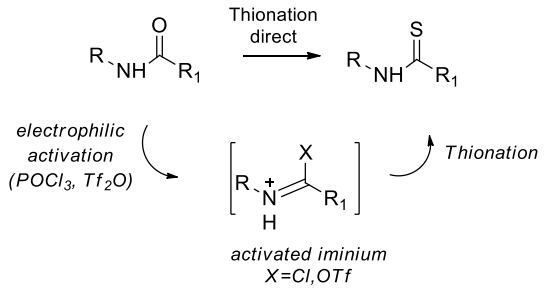

**Scheme A1.2**

Products of this type of reaction are thioamides, a pivotal class of organic molecules with attractive features, like stability, crystallizability, absence of unpleasant smell; moreover the pronounced resonance stabilization, arising from the donation of the non-bonding nitrogen lone pair, is increased by the high polarizability of the sulfur atom. The combination of their chemical reactivity (feasibility of nucleophilic additions of reducing

[8] a) P.S. Fier, J.F. Hartwig, *J. Am. Chem. Soc.* **2012**, *134*, 5524. b) Tyutyunov, S. Boyko, S. Igoumnov, *Fluorine Notes* **2011**, *74*, 1-2.
[9] V. Pace, S. Monticelli, K. de la Vega-Hernandez, L. Castoldi, *Org. Biomol. Chem* **2016**, *14*, 7848.

agents or organometallics to electrophilic carbon) and their physical properties made them highly valuable scaffolds[10].

Normally this functionality is obtained by direct thionation as reported in scheme A1.3.

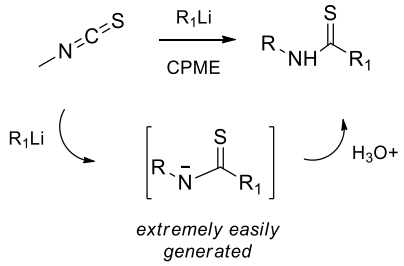



**Scheme A1.3**

This reaction has a lot of disadvantages like long reaction times, problematic work-up and highly contaminating[11] because of the use of toxic sulfurating agents[12]. Furthermore, thionation procedures may not be stainghtforward towards the formal replacement of oxygen with sulfur becouse it requires harsh reaction conditions (high temperature, prolonged reaction time, tedious work up)[13], so the electrophilic activation of oxoamide functionality is often necessary prior to the treatment with sulfurating agents. With nucleophilic addition, there are some advantages: high-yields regardless of the nature of the two reacting partners used; obtainment of the desired thioamides as the exclusive reaction products: simple work-up and purification procedures, mild conditions, simple method, broad scope, non malodorant and eco-friendly reactions, using cyclopentyl methyl ether as reaction medium. These advantages make this reaction a robust and reliable method to access both simple and complex thioamides, including enantiopure ones, avoiding noxiuos and unpleasant-smelling sulfurating agents (scheme A1.4)[11]. This renders the protocol highly attractive, also for sustainability aspect.

---

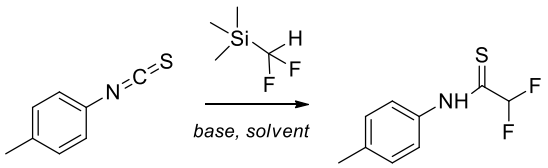
[10] a) M. Iwata, R. Yazaki, I. H. Chen, D. Sureshkumar, N. Kumagai, M. Shibasaki, *J. Am. Chem. Soc.* **2011**, *133*, 5554. b) H. Wang, L. Wang, J. Shang, X. Li, H. Wang, J. Gui, A. Lei, *Chem. Commun.* **2012**, *48*, 76.
[11] V. Pace, L. Castoldi, S. Monticelli, S. Safaranek, A. Roller, T. Langer, W. Holzer, *Chem. Eur. J.* **2015**, *21*, 18966.
[12] a) F. Shibahara, R. Sugiura, T. Murai, *Org. Lett.* **2009**, *11*, 3064. b) T. Ozturk, E. Ertas, O. Mert, *Chem. Rev.* **2007**, *107*, 5210.
[13] a) T. B. Nguyen, M. Q. Tran, L. Ermolenko, A. Al-Mourabit, *Org. Lett.* **2014**, *16*, 310. b) T. Guntreddi, R. Vanjari, K. N. Singh, *Org. Lett.* **2014**, *16*, 3624.


R1Li
CPME
S
R
NH
R1
R1Li
S
R
N
R1
H3O+
extremely easily
generated

**Scheme A1.4**

## *A.2 Methodological study* [14]

In this work, conditions of nucleophilic addition of difluoromethyltrimethylsilane to an isothiocyanate were studied, using *p*-tolylisothiocianate as model of reaction changing base and solvent used (scheme A2.1):




**A1**

**Scheme A2.1**

Conditions used are reported in table A2.1 and the better one seems to be entry 3.

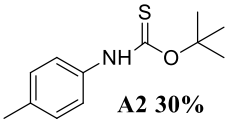
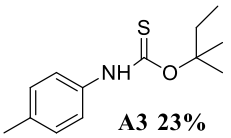
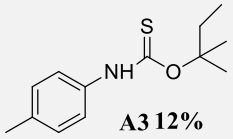
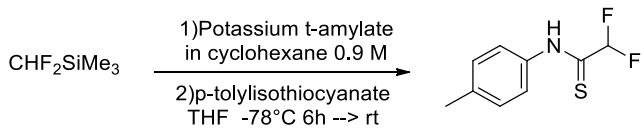
| Entry | $CHF_2SiMe_3$ | Base | Conditions | Yield | By-product |
|---|---|---|---|---|---|
| **1** | 2 eq | *t*-BuOK in THF 0.8 M<br>2 eq | THF -78°C<br>6h → rt | **61%** |  **A2 30%** |
| **2** | 2 eq | Potassium *t*-amylate in cyclohexane 0.9 M<br>2 eq | THF -78°C<br>6h → rt | **67%** |  **A3 23%** |
| **3** | **2 eq** | **Potassium *t*-amylate in cyclohexane 0.9 M**<br>**1.2 eq** | **THF -78°C**<br>**6h → rt** | **75%** |  **A3 12%** |
| **4** | 2 eq | Potassium *t*-amylate in cyclohexane 0.9 M<br>1.2 eq | DMF:THF 3:2<br>6h → rt | ***No reaction*** | |

**Table A2.1**

[14] M. Miele, R. D'Orsi, S. Vellaisamy, W. Holzer, V. Pace, *Chem. Commun*. **2019**, *55*, 12960.

Firstly, potassium *tert*-butoxide was used as base in THF[15] (table A2.1, entry **1**), but this conditions led to formation of byproduct due to the attack of *t*-BuO anion on the isothiocyanate. Thus, the base was changed with potassium *t*-amylate, a more sterically hindered alkoxide (table A2.1, entry **2**). Then base amount was lowered, so desired thioamide was obtained in high yield as the major product together with minimal amount of thiocarbamate (table A2.1, entry **3**). At least solvent used was changed; the reaction did not work in this case (table A2.1, entry **4**).

At least procedure was changed, used firstly nucleophilic agent in presence of the base, and in a second step isothiocyanate was added to mixture (scheme A2.2), first after 30 minutes (table A2.2, entry ), then after 5 minutes (table A2.2, entry) and last after 1 minute (table A2.2, entry).




**Scheme A2.2**

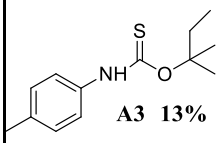
| Entry | $CHF_2SiMe_3$ | *t*-Amylate | isothiocyanate | Conversion |
|---|---|---|---|---|
| **1** | 1.5 eq | 1.2 eq | Added after 30’ | **No** |
| **2** | 1.5 eq | 1.2 eq | Added after 5’ | **8%** |
| **3** | **1.5 eq** | **1.2 eq** | **Added after 1’** | **18%** |

**Table A2.2**

A small amount of the product was obtained only in the last case. This proves the instability of generated carbanion, so it’s necessary to add difluoromethyl trimethyl silane and the base to a isothiocyanate solution, like previuosly reported in scheme A2.1. This

[15] E. Obijalska, G. Utecht, M.K. Kowalski, G. Mloston, M. Rachwalski; *Tetrahedron Letters* **2015**, *56*, 4701.

same procedure was used to study the better reaction time and temperature (table A2.3, entries **1-5**) and the best solvent to use (table A2.3, entries **6-10**). Results proved that reaction depends on the solvent used. It is also worth noting that, decreasing the nucleophile loading to 1.5 equivalents, complete suppression of the undesired product was achieved, thus allowing to prepare **A1** with full chemocontrol. The reaction is quite fast, reaching completion within 1 h at 0 °C; moreover, the use of a small excess (0.3 equiv) of $TMSCHF_2$ compared to *t*-AmOK guarantees the nucleophile generation event to proceed quantitatively (table A2.3, entry **3**).

| **Entry** | **$CHF_2SiMe_3$** | **Base** | **Conditions** | **Yield** | **By-product** |
|---|---|---|---|---|---|
| **1** | 1.5 eq | Potassium *t*-amylate 1.2 eq | THF -78°C 1 minute | **68%** | |
| **2** | 1.5 eq | Potassium *t*-amylate 1.2 eq | THF 0°C 5 minutes | 75% | |
| **3** | **1.5 eq** | **Potassium *t*-amylate 1.2 eq** | **THF 0°C 1 hour** | **89%** | |
| **4** | 1.5 eq | Potassium *t*-amylate 1.2 eq | THF rt 1 hour | **68%** |  **A3 13%** |
| **5** | 1.5 eq | Potassium *t*-amylate 1.2 eq | THF -15°C 1 hour | **20%** | |
| **6** | 1.5 eq | Potassium *t*-amylate 1.2 eq | $Et_2O$ 0°C 1 hour | **No reaction** | |

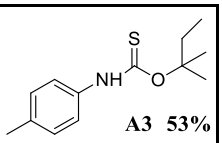
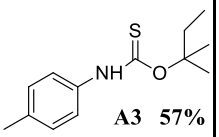
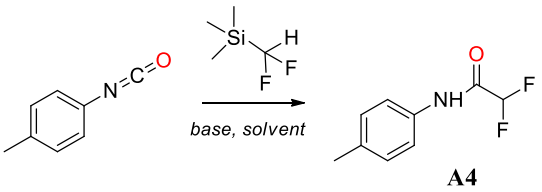
| | | | | | |
|---|---|---|---|---|---|
| **7** | 1.5 eq | Potassium *t*-amylate 1.2 eq | CPME 0°C 1 hour | **36%** |  **A3 53%** |
| **8** | 1.5 eq | Potassium *t*-amylate 1.2 eq | 1,4-dioxane 0°C 1 hour | **21%** | |
| **9** | 1.5 eq | Potassium *t*-amylate 1.2 eq | toluene 0°C 1 hour | **31%** |  **A3 57%** |
| **10** | 1.5 eq | Potassium *t*-amylate 1.2 eq | 2-MeTHF 0°C 1 hour | **85%** | |

**Table A2.3**

Then same study was applied to *p*-tolylisocyanate (scheme A2.3) and results are reported in table A2.4. In every case a by-product was obtained.




**A4**

**Scheme A2.3**

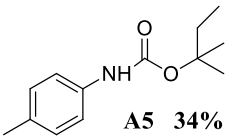
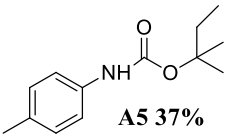
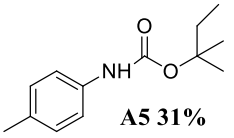
| Entry | $CHF_2SiMe_3$ | *t*-Amylate | Conditions | Yield | By-product |
|---|---|---|---|---|---|
| **1** | 1.5 eq | 1.2 eq | THF rt<br>30 min | **56%** |  **A5 34%** |
| **2** | 1.5 eq | 1.2 eq | THF 0°C<br>30 min | **61%** |  **A5 37%** |
| **3** | 1.5 eq | 1.2 eq | THF rt<br>10 min | **43%** |  **A5 31%** |

**Table A2.4**

Moreover, applying this reaction to different electrophiles, in same cases desired product was not obtained (table A2.5). This has proved that reaction works differently depending on the electrophile used.

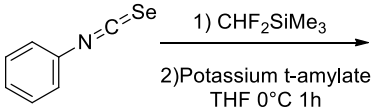
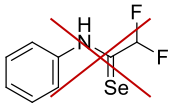
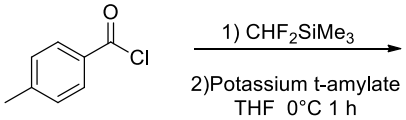
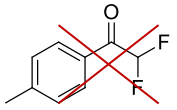
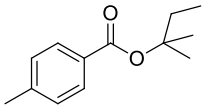
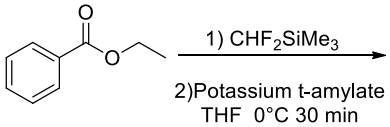
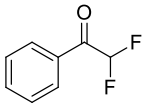
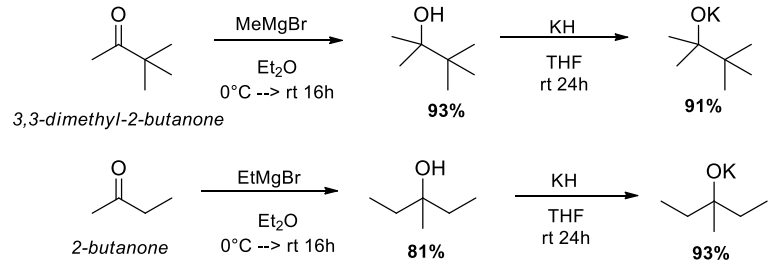
| Entry | Reaction | Desidered product | Obtained |
|---|---|---|---|
| **1** |  |  | **No reaction** |
| **2** |  |  |  |
| **3** |  |  | **47%** |

**Table A2.5**

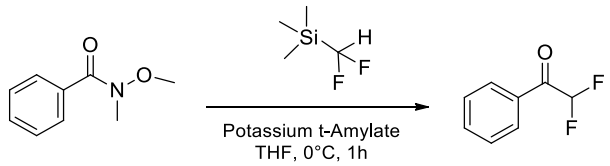
Another optimization was tried synthesizing new sterically cluttered bases to eliminate the amount of by-productderived from addition of the alkoxide to the electrophile (scheme A2.4). These bases were used in reactions with *p*-tolylisothiocyanate and *p*-tolylisocianate

at condition reported in table A2.3, entry **3**, but in no case the base worked in the reaction and desired product was not obtained.




**Scheme A2.4**[16]

This study was applied to Weinreb amide, that afford to difluorinated ketone (scheme A2.5), with condition reported in table A2.6. The best one is entry **4**.




**Scheme A2.5**

| Entry | $CHF_2SiMe_3$ | *t*-Amylate | Time | Yield |
|---|---|---|---|---|
| **1** | 1.5 eq | 1.2 eq | 1 h | **48%** |
| **2** | 1.5 eq | 1.2 eq | 2 h | **49%** |
| **3** | 1.5 eq | 1.2 eq | 3 h | **51%** |
| **4** | 1.5 eq | 1.2 eq | 4 h | **62%** |
| **5** | 1.5 eq | 1.2 eq | 5 h | **60%** |
| **6** | 1.5 eq | 1.2 eq | 6 h | **56%** |
| **7** | 1.5 eq | 1.2 eq | 7 h | **54%** |

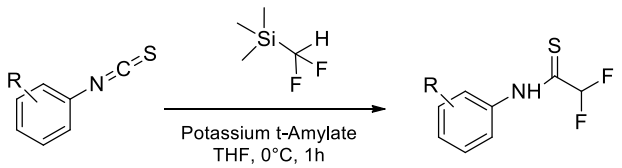
**Table A2.6**

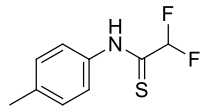
[16] a) N.J. Lawrence, C.A. Davies, M. Gray, *Org. Lett. 6*, 4957-4960, **2004**. b) Charles Allan BROWN, *Synthesis* **1974**, *6*, 427.

## ***A.3 Fluorinated thioamides***

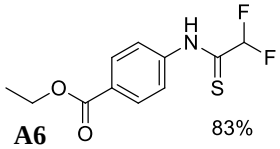
Once the optimal condition for nucleofilic addition of difluoromethyl trimethylsilane to isothiocyanate were established (table A2.3, entry **3**), the reaction of variously functionalized commercially available isothiocyanates with difluoromethyltrimethylsilane afforded a wide range of thioamides in high yields, to use as scaffolds in medicinal synthesis or as precursors of biologically active molecules. Only in a few cases the reaction did not work (inactive isothiocyanates reported in table A3.2). Susceptible electrophilic functionalities such as ester (**A6**) nitrile (**A7, A8**) and nitro (**A9**) remained completely untouched during reaction. Moreover, nitrogen-containing groups did not interfere with the transformation, as deducted in the case of azide (**A10**) and diazo (**A11**). Halogenes are tolerated (**A12-A15**). Electron donating groups (**A16-A18**) resulted in clean reactions of comparable efficiency. Simple aromatic or with steric hindrance work well in reaction (**A19-23**). Stereochemistry is mantained (A**24**). The nature of the isothiocyanate does not influence the effectiveness of the technique: also aliphatic compounds undergo the difluoromethylation giving the corresponding thioamides in high yields (**A25-A28**). This implies an excellent versatility of the method used, which renders it applicable to the synthesis of thiamides ranging from the simplest one to sterically hindered and complex one. Preservation of enantiopurity when using chiral isothiocyanates showcases its potential. Products are reported in table A3.1.

**Reaction**

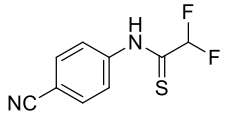


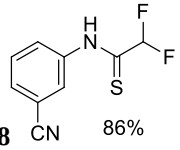

**A1** 90%




**A6** 83%




**A7** 93%

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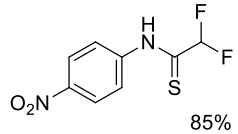


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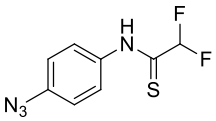
**A9**

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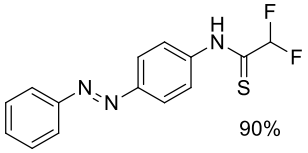


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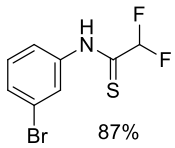


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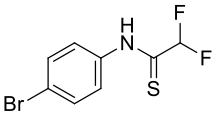


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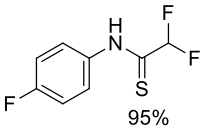


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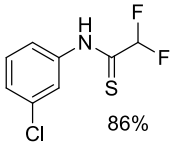


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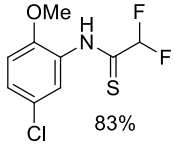


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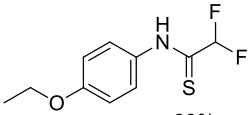


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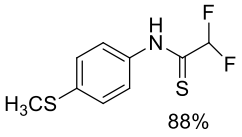


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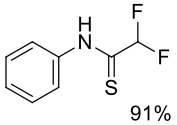


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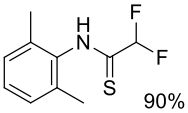


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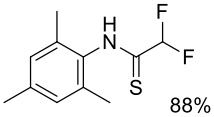


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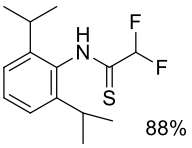


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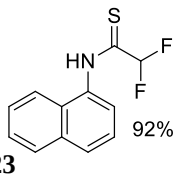


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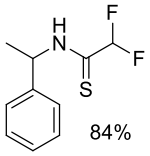


84%
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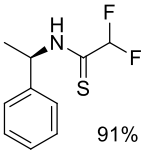


91%
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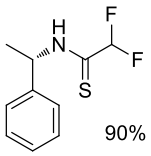


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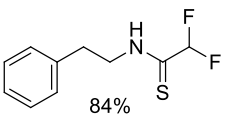



84%
**A25**

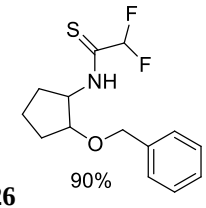
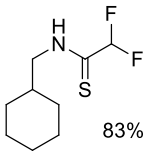
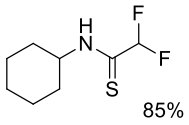
| | | |
|---|---|---|
|  90% **A26** |  83% **A27** |  85% **A28** |

**Table A3.1**

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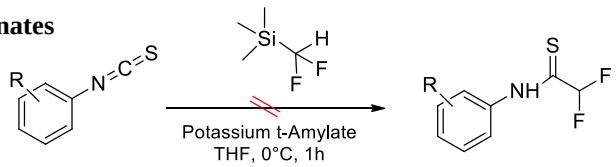
<tr><td colspan="4">Inactive isothiocyanates<br> 
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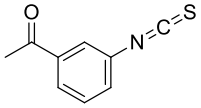
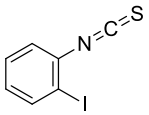
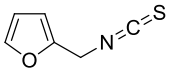
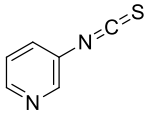
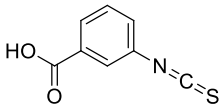
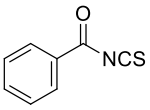
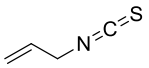
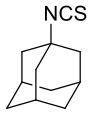
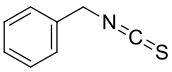
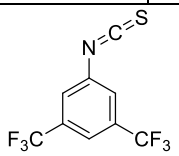
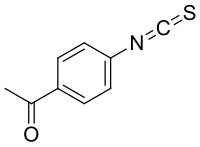
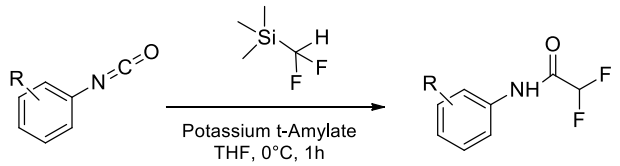
<tr><td>  <br>3-Acetylphenyl isothiocyanate</td><td>  <br>2-iodophenyl isothiocyanate</td><td>  <br>Furfuryl isothiocyanate</td><td>  <br>3-Pyridyl isothiocyanate</td></tr>
<tr><td>  <br>3-Carboxyphenyl isothiocyanate</td><td>  <br>Benzoyl isothiocyanate</td><td>  <br>Allyl isothiocyanate</td><td>  <br>Adamantyl isothiocyanate</td></tr>
<tr><td>  <br>Benzyl isothiocyanate</td><td>  <br>3,5-bis( trifluoromethyl) phenyl isothiocyanate</td><td>  <br>4-Acetylphenyl isothiocyanate</td></tr>
</table>

**Table A3.2**

## ***A.4 Fluorinated amides***

The success of the procedure prompted us to evaluate the reactivity of oxo-analogues isocyanates, to obtain α,α-difluoromethyl amides, synthetically and structurally relevant scaffolds[17] (table A4.1).

| **Reaction**   | | | |
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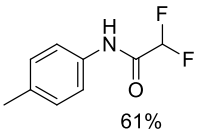
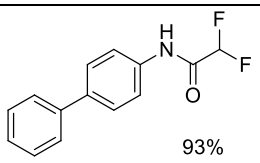
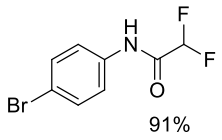
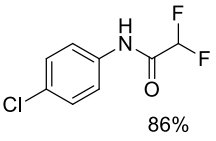
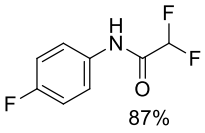
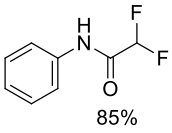
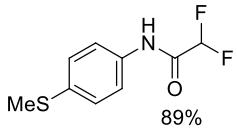
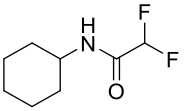
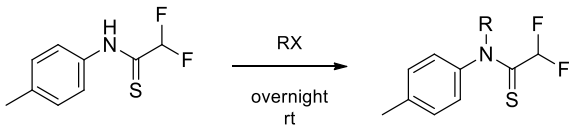
|  61% **A4** |  93% **A29** |  91% **A30** |  86% **A31** |
|  87% **A32** |  85% **A33** |  89% **A34** |  **A35** |

[17] a) P.J. Czerwiński, B. Furman, *Chem. Commun.* **2019**, *55*, 9436. b) C.R. Jones, P.K. Baruah, A.L. Thompson, S. Scheiner, M.D. Smith, *J. Am. Chem. Soc.* **2012**, *134*, 12064.

## ***A.5 Applications***

### *A.5.1 Alkylation-Arylation*

Once obtained these thioamides, they were used for some applications to study their reactivity. 2,2-difluoro-N-(*p*-tolyl)ethanethioamide was reacted with alkyl and aryl halides to study its alkylation reaction. Results are reported in table A5.1




**Scheme A5.1**

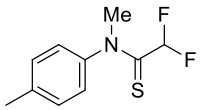
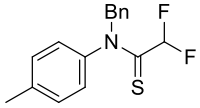
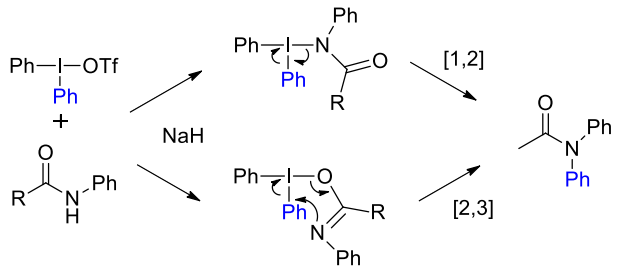
| Entry | RX | Product | Conversion |
|---|---|---|---|
| **1** | MeI |  | **35%** |
| **2** | Benzylbromide |  | ***No*** |

**Table A5.1**

Unfortunately, methylated product (table A5.1, entry **1**) was lost in column during purification process and the yield could not be calculated; thus conversion obtained through GC-MS is reported.

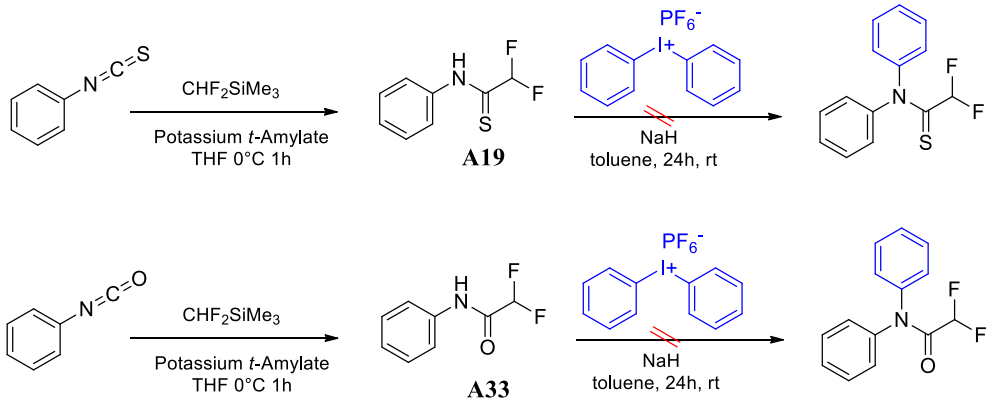
Furtheremore aryl amides are found in a range of natural and synthetic products. Arylation of secondary amides has been achieved with diaryliodonium salt, allowing synthesis of tertiary amides with highly congested aryl moiety according to the mechanism reported in scheme A5.2[18].

[18] F. Tinnis, E. Stridfeldt , H. Lundeberg, H. Adolfsson, B. Olofsson, *Org. Lett.* **2015**, *17*, 2688.




**Scheme A5.2**

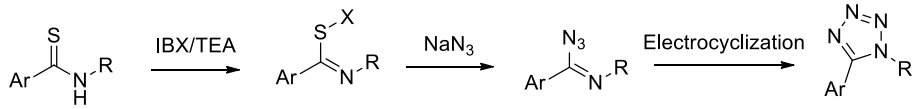
So, synthetized 2,2-difluoro-N-phenylethanethioamide (**A19**) and 2,2-difluoro-N-phenylethaneamide (**A33**) with nucleofilic addition of difluoromethyltrimethylsilane to phenyliso(tio)cyanate, products were used as substrates for this reaction, as reported in scheme A5.3. Desired products were not obtained probably due to the presence of difluorinated moiety that changes the electron density.




**Scheme A5.3**

### *A.5.2 Heterocycles synthesis*

Azoles are of paramount importance in medicinal chemistry and pharmaceutical industry and find wide applications in different fields. Different synthetic methods are reported in the literature, but the most efficient one uses *o*-iodoxybenzoic acid (IBX) as desulfuring agent, replacement with sodium azide and subsequent electrocyclization of azide (Scheme A5.4)[19]




**Scheme A5.4**

Difluorothioamides synthetized were used in this reaction, according conditions reported in table A5.2. Entries **1** and **2** used sodium azide as nucleophilic agent, but the reaction did not afford desired product; in entry **3** diethylaluminium azide was used as nucleophilic agent, prepared *in situ* by nucleophilic substitution on diethylaluminium chloride with sodium azide[20]. Syntethic usefulness of diethylaluminium azide arises from the combination of two features, high nucleophilicity of the azide anion and the high Lewis acidity of alluminum; this particular characteristic makes it a reliable reagent when the attack of azide needs an activated substrate. In this case the substrate did not work with the use of sodium azide alone, so activation with diethylaluminium azide was attempted (scheme A5.4). But even in this case desired product was not obtained.

[19] P.S Chaudhari, S.P. Pathare, K.G. Akamanchi *J. Org. Chem.* **2012**, *77*, 3716.
[20] S. Monticelli, V. Pace, *Aust. J. Chem.* **2015**, *68*, 703.

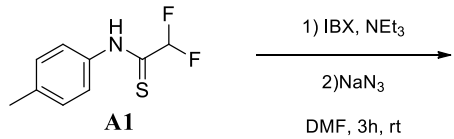
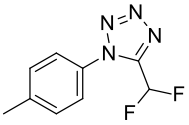
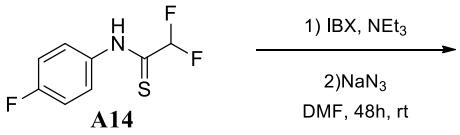
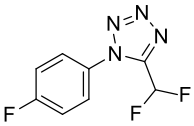
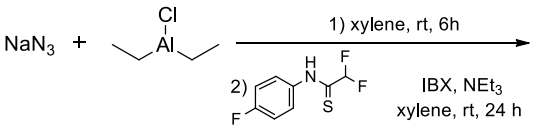
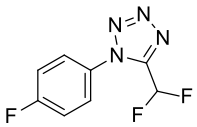
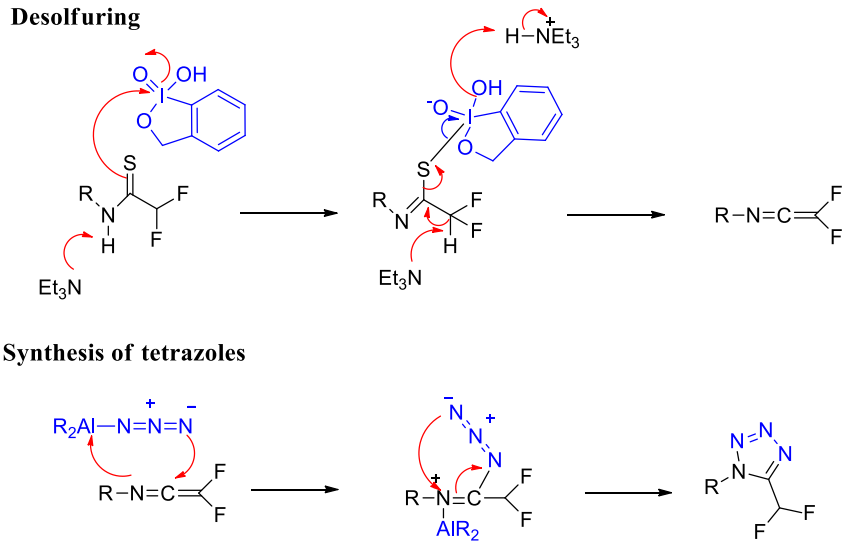
| Entry | Reaction | Desidered product |
|---|---|---|
| **1** |  |  |
| **2** |  |  |
| **3** |  |  |

**Table A5.2**

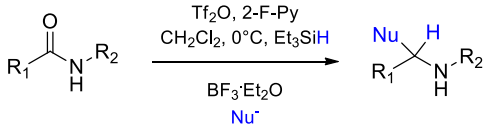
From literature data the hypotetized mechanism for cyclization of difluorothioamides synthetized is reported in scheme A5.5.




**Scheme A5.5.** Hypotetized mechanism

### *A.5.3 One-pot preparation of secondary amines from secondary amides*

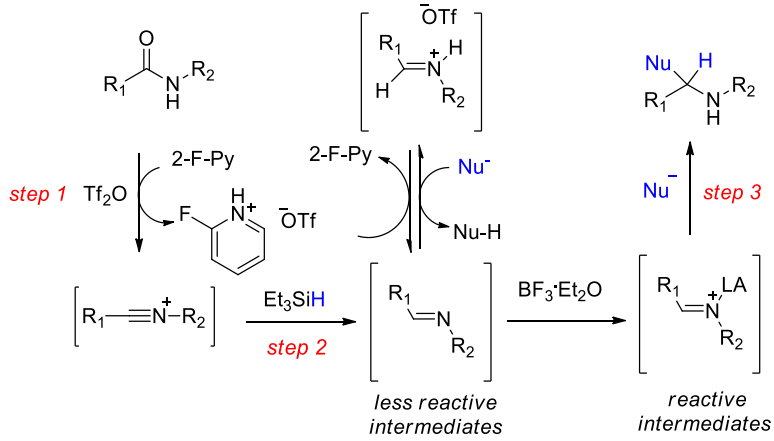
Direct transformation of amides is an emerging area in organic synthesis, for example secondary amides constitute a class of versatile synthetic intermediates and they can be transformed into useful functional group, such as amines; amino group infact plays a pivotal role for bioactivity of pharmaceuticals[21]. Secondary amines can be obtained by *in situ* amide activation with trifluoromethanesulfonic anhydride ($Tf_2O$)/ 2-fluoropyridine, partial reduction with triethylsilane[22] and addition of C-nucleophiles, as reported in scheme A5.6.




**Scheme A5.6**

Plausible mechanism of this kind of reaction is depicted in scheme A5.7, with formation of nitrinium ion intermediate, reducted with $Et_3SiH$ to give imine; a proton exchange can occur between the protonated 2-F-pyridine more basic imine, resulting in iminium ion, that consumes nucleophile by proton exchange to yield back imine. The employment of Lewis acid is necessary to convert imine into the reactive chelating species, subjected to nucleophilic addition to yield desired amine[23].

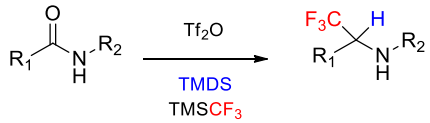
[21] R.N. Salvatore, C.H. Yoon, K.W. Jung, *Tetrahedron* **2001**, *57*, 7785.
[22] D.N. Kursanov, Z.N. Parnes, N.M. Loim, *Synthesis* **1974**, *9*, 633.
[23] P.Q. Huang, Y.H. Huang, K.J. Xiao, Y. Wang, X.E. Xia, *J. Org. Chem.* **2015**, *80*, 2861.




**Scheme A5.7**

So, amide **A35** was reacted in one-pot reductive functionalization of secondary amide with organometallic reagent as nucleophile agent, but surprisingly secondary amine was not obtained (Table A5.3, entry 1).

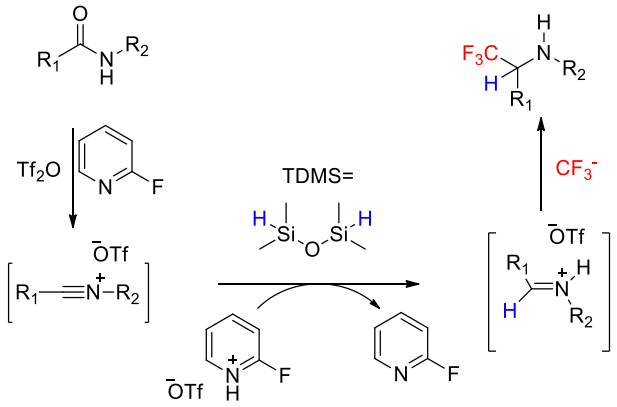
Furthermore, introduction of trifluoromethyl group into amines constitutes a good strategy to obtain quite attractive motif in medicinal chemistry. Starting from secondary amides, *α*-trifluoromethylamines can be obtained with one-pot method, consisting of in situ activation of amides with triflic anhydride, partial reduction with 1,1,3,3-tetramethyldisiloxane (TDMS) and nucleophilic trifluoromethylation with trifluoromethyltrimethylsilane ($TMSCF_3$)[24] as reported in scheme A5.8.




**Scheme A5.8**

Mechanism of this reaction provides *in situ* activation of amide, mild reduction by siloxane to form protonated imine as reactive intermediate and at least nucleophilic trifluoromethylation to iminium ion (scheme A5.9)[25].

[24] H. Chen, J.L. Ye, P.Q. Huang, *Org. Chem. Front.* **2018**, *5*, 943.
[25] a) P.Q. Huang, Q.W. Lang, X.N. Hu, *J. Org. Chem.* **2016**, *81*, 10227. b) P.Q. Huang, Q.W. Lang, A.E. Wang, J.F. Zheng, *Chem Commun.* **2015**, *51*, 1096.




**Scheme A5.19**

Product **A35** was reacted in *one-pot* reductive trifluoromethylation of amides using $KHF_2$, MeCN as additives and DMF as solvent, but, despite this reaction exhibits good functional group tolerance, even in this case desired *α*-trifluoromethylamine was not obtained and provided to unidentified side products (Table A5.3, entry 2).

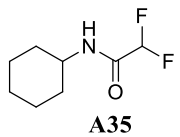
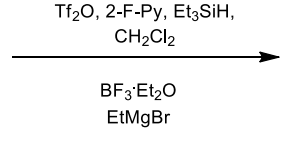
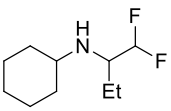
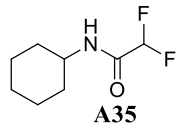
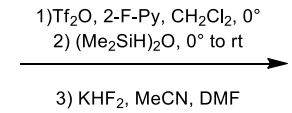
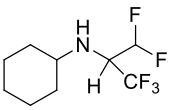
| Entry | Reaction | Desired product |
|---|---|---|
| **1**[23] |  **A35**  $Tf_2O$, 2-F-Py, $Et_3SiH$, $CH_2Cl_2$ / $BF_3{\cdot}Et_2O$ EtMgBr |  |
| **2**[24] |  **A35**  1)$Tf_2O$, 2-F-Py, $CH_2Cl_2$, 0° 2) $(Me_2SiH)_2O$, 0° to rt / 3) $KHF_2$, MeCN, DMF $TMSCF_3$, -78° to rt, 18h |  |

**Table A5.3**

## ***A.6 Experimental section***

### *A.6.1 Instrumentation and General Analytical Methods*

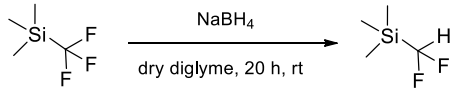
Melting Points were determined on a Reichert-Kofler hot-stage microscope and are uncorrected. Mass spectra were obtained on a Shimadzu QP 1000 instrument (EI, 70 eV) and on a Bruker maXis 4G instrument (ESI-TOF, HRMS). $^{1}H$, $^{13}C$, $^{15}N$ and $^{19}F$ NMR spectra were recorded on a Bruker Avance III 400 spectrometer (400 MHz for $^{1}H$, 100 MHz for $^{13}C$, 40 MHz for $^{15}N$, 376 MHz for $^{19}F$) at 297 K using a directly detecting broadband observe (BBFO) probe. The centre of the solvent signal was used as an internal standard which was related to TMS with δ 7.26 ppm (1H in CDCl3), δ 77.00 ppm ($^{13}C$ in $CDCl_3$). $^{15}N$ spectra (gsHMBC) were referenced against neat, external nitromethane, $^{19}F$ NMR spectra by absolute referencing via Ξ ratio. Spin-spin coupling constants (J) are given in Hz.

All the reactions were carried out under inert atmosphere of argon. THF was distilled over Na/benzophenone. Chemicals were purchased from Sigma-Aldrich, Acros, Alfa Aesar and TCI Europe. Solutions were evaporated under reduced pressure with a rotary evaporator.

TLC was carried out on aluminium sheets precoated with silica gel 60F254 (Merchery-Nagel, Merk); the spots were visualised under UV light (λ = 254 nm).

### *A.6.2 Sperimental Procedures*

#### *A.6.2.1 Synthesis of difluoromethyltrimethyl silane*




**Scheme A1.1**

To anydrous diglyme, sodium borohydride was added at -10°C. Then, trifluoromethyltrimethylsilane was added dropwise. Reaction mixture was stirred 20 h at room temperature. Difluoromethyltrimethylsilane was isolated by distillation (b.p. 65-66°C) as a colorless liquid. NMR analysis corresponding with literature data.

#### *A.6.2.2 General procedure A for synthesis of α,α-difluorothioamides.*

Difluoromethyltrimethylsilane (1.5 equiv) was added under an inert atmosphere to a solution of isothiocyanate (1 equiv) in dry THF (10 mL) and the mixture was cooled down to 0°C. Then potassium *tert*-pentoxide solution (0.9 M in THF, 1.2 equiv) was added dropwise over a period of 30 minutes. Then the mixture was stirred at 0°C for 1 hour and then quenched with aqueous $NH_4Cl$ solution. The reaction mixture was then exhaustively extracted with $Et_2O$, washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated in vacuo. The resulting crude compounds were purified through column chromatography as reported in **A6.3**.

#### *A.6.2.3 General procedure B for synthesis of α,α-difluoroamides.*

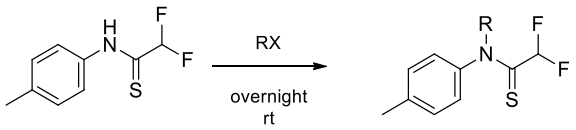
Difluoromethyltrimethylsilane (1.5 equiv) was added under an inert atmosphere to a solution of isocyanate (1 equiv) in dry THF (10 ml) and the mixture was cooled down to 0°C. Then potassium tert-pentoxide solution (0.9 M in THF, 1.2 equiv) was added dropwise over a period of 30 minutes. Then the mixture was stirred at 0°C for 1 hour and then quenched with aqueous $NH_4Cl$ solution. The reaction mixture was then exhaustively

extracted with $Et_2O$, washed with brine, dried over anhydrous $Na_2SO4$ and concentrated in vacuo. The resulting crude compounds were purified through column chromatography as reported **A6.4**.

*A.6.2.4 General procedure for synthesis of 2,2-difluoro-1-phenyl ethanone.*

Difluoromethyltrimethylsilane (1.5 equiv) was added under inert atmosphere to a solution of *N*-methoxy-*N*-methylbenzamide (1 equiv) in dry THF and the mixture was cooled down to 0°C. Then potassium *tert*-pentoxide solution 0.9 M in THF (1.2 equiv) was added dropwise over a period of 30 minutes. After adding, the mixture was stirred at 0°C for time reported in table A2.5 and then quenched with aqueous $NH_4Cl$. Reaction mixture was extracted with $Et_2O$, washed with brine, dried over $Na_2SO_4$ and concentrated *in vacuo*. The resulting crude compounds were analysed by NMR or GCMS analysis, corresponding with literature data.

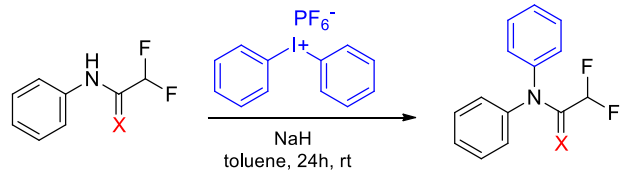
*A.6.2.5 Procedure for alkylation reactions*




**Scheme A5.1**

Difluoromethyltrimethylsilane (1.5 equiv) was added under inert atmosphere to a solution of 1-isothiocyanato-4-methylbenzene (1 equiv) in dry THF and the mixture was cooled down to 0°C. Then potassium *tert*-pentoxide solution 0.9 M in THF (1.2 equiv) was added dropwise over a period of 30 minutes. After adding, the mixture was stirred at 0°C for 1 hour and then RX (3 equiv) was added dropwise and kept stirred overnight. Then the reaction mixture was quenched with aqueous $NH_4Cl$, extracted with $Et_2O$, washed with brine, dried over $Na_2SO_4$ and concentrated *in vacuo*. The resulting crude compounds were purified through column chromatography.

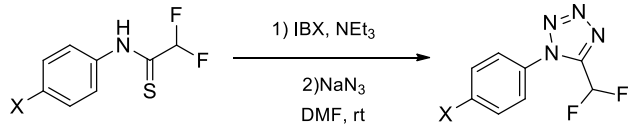
### *A.6.2.6 Procedure for arylation reactions*




(Thio)amide, diaryliodonium salt and NaH were added in a round flask under inert atmosphere; anhydrous toluene (10 mL) was added and stirring was started. The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated and the crude reaction cecked by NMR and GCMS without any work-up.

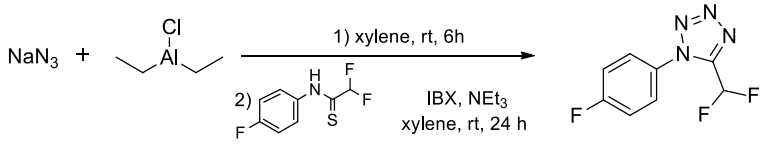
### *A.6.2.7 Procedure for tetrazoles synthesis*

a) Table A5.2, entry 1-2[19]




Thioamide was added to a stirred solution of IBX (1 equiv) and TEA (3 equiv) in DMF (5 mL) for 5 minutes. Then, $NaN_3$ was slowly added to the reaction mixture with continued stirring at room temperature. The reaction mixture was quenched with a saturated solution of $NaHCO_3$. The acqueous layer was extracted with $Et_2O$ (three times) and washed with brine five times. The combined organic layers were dried over $Na_2SO_4$ and evaporated to afford the crude product, purified on silica (Hexane/EtOAc 8/2).

b) Table A5.2, entry 3[20]




Diethylaluminium azide ($Et_2AlN_3$) was prepared in situ from sodium azide (4.3 equiv) and diethylaluminium chloride (4.3 equiv, 0.9 M in toluene) in xylene (3 mL) under inert

atmosphere stirred at 0°C for 15 minutes and then the heterogeneous mixture was warmed to room temperature and stirred for 6 hours (during the formation of the reagent a suspension of sodium chloride was formed). After this period, thioamide (**A14**, 1 equiv), IBX (1 equiv) and TEA (3 equiv) were added to the suspension of $Et_2AlN_3$ and the resulting mixture was stirred for 24h at room temperature. The reaction mixture was the cooled to 0°C and added a solution of 15% aq NaOH containing sodium nitrite (solution pH 13.5). The pH value was then adjusted to 1.5 with 6N HCl and the mixture was exhaustively extracted with ethyl acetate. The solvents were removed under reduced pressure to afford the crude product, which was re-dissolved in ethyl acetate and extracted with aq $K_2CO_3$ (10%) to the aqueous phase as the potassium salt (pH 11). The combined basic aq phases were cooled to 0°C and carefully treated with 6N HCl to adjust the pH value to 2.5. The product was then extracted with AcOEt, the combined organic phases were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The crude product was analyzed by NMR and GCMS.

*A.6.2.8 Procedure for secondary amine synthesis from secondary amide*

a) Table A5.3, entry 1[23]

To a solution of secondary amide (**A35**, 1 equiv) in $CH_2Cl_2$ (0.7 mL, 0.25 M), 2-fluoropyridine (1.1 equiv) and trifluoromethanesulfonic anhydride (1.1 equiv) was successively added dropwise at 0°C under Ar atmosphere and the reaction was stirred for 30 min. Then, 1,1,3,3-tetramethyldidiloxane [$(Me_2SiH)_2O$] (0.7 equiv) was added dropwise at 0°C and the mixture was stirred for 30 min; the mixture was allowed to warm up to room temperature and stirred for 5 h. $CH_2Cl_2$ was evaporated under reduced pressure. To the residue $KHF_2$ (2.5 equiv) was added. Then MeCN (0.05 M) and DMF (3 equiv) was added at -78°C under Ar atmosphere; the mixture was warmed up to room temperature and stirred for 10 min. Again the mixture was cooled to -78°C and $TMSCF_3$ ( 3 equiv) was added and stirred for 48h at room temperature. The reaction was quenched with a saturated aqueous $Na_2CO_3$ and stirred for 5 min, diluting with water and extracted with diethyl ether:hexane ( 1:3, 3 times). The combinated organic phases were dried over $Na_2SO_4$,

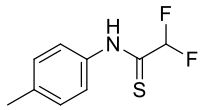
filtered and concentrated under reduced pressure. The residue was analyzed by NMR and GCMS.

b) Table A5.3, entry 2[24]

To a solution of secondary amide (**A35**, 1 equiv) in $CH_2Cl_2$ (0.7 mL, 0.25 M), 2-fluoropyridine (1.2 equiv) and trifluoromethanesulfonic anhydride (1.2 equiv) was successively added dropwise at 0°C under Ar atmosphere and the reaction was stirred for 30 min. To the resulting mixture, triethylsilane (1.1 equiv) was added dropwise at 0°C and the mixture was stirred for 10 min; the mixture was allowed to warm up to room temperature and stirred for 5 h. After being cooled to 0°C, $BF_3{\cdot}Et_2O$ (1.5 equiv) was added and the mixture was stirred for 30 min. EtMgBr (4 equiv) was added dropwise to the resultant mixture at 0°C. Then, the mixture was warmed slowly to room temperature and stirred for 48 h. The reaction was quenched with a saturated aqueous solution of ammonium chloride and extracted with $CH_2Cl_2$ 3 times. The combined organic phases were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was analyzed by NMR and GCMS.

### *A.6.3 Characterization of the α,α-difluorothioamides*

2,2-difluoro-N-(4-methylphenyl)ethanethioamide (**A1**)



**A1** 90%

By following general procedure A, starting from 1-isothiocyanato-4-methylbenzene (0.149 g, 1 mmol, 1.0 equiv), compound **A1** was obtained in 90% yield (0.181 g) as brown oil after column chromatography on silica gel (n-hexane:ethyl acetate 9:1).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.22 (br s, 1H, NH), 7.69 (m, 2H, Ph H-2,6), 7.25 (m, 2H, Ph H-3,5), 6.28 (t, $^{2}J_{H,F}$ = 56.6 Hz, 1H, $CHF_2$), 2.38 (s, 3H, $CH_3$).

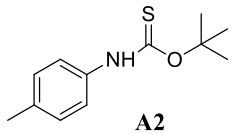
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 186.4 (t, $^{2}J_{C,F}$ =20.2 Hz, C=S), 137.8 (Ph C-4), 134.3 (Ph C-1), 129.7 (Ph C-3,5), 122.6 (Ph C-2,6), 112.8 (t, $^{1}J_{C,F}$ = 258.0 Hz, $CHF_2$), 21.2 ($CH_3$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -289.9 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.1 (dd, $^{2}J_{H,F}$ = 56.6 Hz, $^{n}J_{H,F}$ = 3.4 Hz, $CHF_2$ ).

**HRMS** (ESI), *m/z*: calcd. for $C_9H_9F_2NNaS$: 224.0316 $[M+Na]^+$; found: 224.0310.

O-*tert*-butyl *p*-tolylcarbamothioate (**A2**)



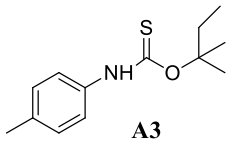
**A2**

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 7.92 (m, 2H, Ph H-2,6), 7.39 (m, 2H, Ph H-3,5), 6.63 (br s, 1H, NH), 2.25 (s, 3H, $CH_3$), 1.19 (s, 9H, $CH_3$).

**$^{13}$C NMR** (100 MHz, CDCl3): δ 150.3 (C=S), 142.6 (Ph C-4), 130.8 (Ph C-2,6), 124.7 (Ph C-1), 117.3 (Ph C-3,5), 83.7 ($C(CH_3)_2$), 25.7 (3C, $CH_3$), 20.6 ($CH_3$).

**HRMS** (ESI), *m/z*: calcd. for $C_{12}H_{17}NOS^{+}$: 224.1013 $[M+H]^{+}$; found: 294.1312.

O-tert-pentyl p-tolylcarbamothioate (**A3**)



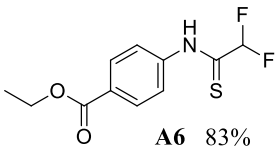

**A3**

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 7.97 (m, 2H, Ph H-2,6), 7.42 (m, 2H, Ph H-3,5), 6.69 (br s, 1H, NH), 2.25 (s, 3H, $CH_3$), 1.84 (q, $^{3}J$ =7.5 Hz, 2H, $CH_2$), 1.49 (s, 6H, $CH_3$), 0.93 (t, $^{3}J$=7.5 Hz, 3H, $CH_3$).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 166.3 (CO), 152.1 (C=S), 142.6 (Ph C-4), 130.8 (Ph C-2,6), 124.7 (Ph C-1), 117.3 (Ph C-3,5), 83.7 ($C(CH_3)_2$), 33.6 ($CH_2$), 25.7 (2C, $CH_3$), 21.6 ($CH_3$), 14.3 ($CH_3$).

**HRMS** (ESI), *m/z*: calcd. for $C_{13}H_{19}NOS^{+}$: 237.1417 $[M+H]^{+}$; found: 237.1619.

Ethyl 4-[(2,2-difluoroethanethioyl)amino]benzoate (**A6**)




**A6** 83%

By following general procedure A, starting from ethyl 4-isothiocyanatobenzoate (0.207 g, 1 mmol, 1.0 equiv), compound **A6** was obtained in 83% yield (0.215 g) as yellow solid (mp 71-74°C) after column chromatography on silica gel (*n*-hexane:ethyl acetate 9:1).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.33 (br s, 1H, NH), 8.13 (m, 2H, Ph H-2,6), 7.99 (m, 2H, Ph H-3,5), 6.27 (t, $^{2}J_{H,F}$ = 56.5 Hz, 1H, $CHF_2$), 4.39 (q, $^{3}J$ =7.1 Hz, 2H, $OCH_2$), 1.41 (t, $^{3}J$ = 7.1 Hz, 3H, $CH_3$).

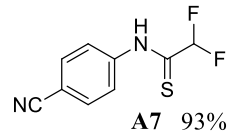
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 165.5 (CO), 140.6 (Ph C-4), 130.8 (Ph C-2,6), 129.2 (Ph C-1), 121.7 (Ph C-3,5), 112.9 (t, $^1J_{C,F}$ = 258.3 Hz, $CHF_2$), 61.2 ($OCH_2$), 14.3 ($CH_3$), C=S was not found.

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -291.0 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -115.9 (dd, $^2J_{H,F}$ = 56.5 Hz, $^nJ_{H,F}$ = 3.4 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_{11}H_{12}F_2NO_2S^+$: 260.0551 $[M+H]^+$; found: 260.0546.

*N*-(4-cyanophenyl)-2,2-difluoroethanethioamide (**A7**)




**A7** 93%

By following general procedure A, starting from 4-isothiocyanatobenzonitrile (0.160 g, 1 mmol, 1.0 equiv), compound **A7** was obtained in 93% yield (0.197 g) as pale yellow solid (mp 155-158 °C) after column chromatography on silica gel (*n*-hexane:ethyl acetate 9:1).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 9.33 (br s, 1H, NH), 8.07 (m, 2H, Ph H-2,6), 7.74 (m, 2H, Ph H-3,5), 6.27 (t, $^2J_{H,F}$ = 56.4 Hz, 1H, $CHF_2$).

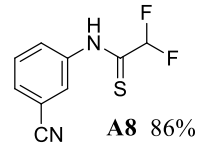
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 187.4 (t, $^2J_{C,F}$ =20.3 Hz, C=S), 140.6 (Ph C-1), 133.3 (Ph C-3,5), 122.4 (Ph C-2,6), 118.0 (CN), 112.8 (t, $^1J_{C,F}$ = 258.8 Hz, $CHF_2$), 110.7 (Ph C-4).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -292.4 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -115.9 (dd, $^2J_{H,F}$ = 56.4 Hz, $^nJ_{H,F}$ = 3.4 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_9H_6F_2N_2NaS$: 235.0112 $[M+Na]^+$; found: 235.0109.

*N*-(3-cyanophenyl)-2,2-difluoroethanethioamide (**A8**)




**A8** 86%

By following general procedure A, starting from 3-isothiocyanatobenzonitrile (0.160 g, 1 mmol, 1.0 equiv), compound **A8** was obtained in 86% yield (0.182 g) as yellow solid (mp 112-115°C) after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.34 (br s, 1H, NH), 8.31 (m, 1H, Ph H-2), 8.02 (m, 1H, Ph H-6), 7.62 (m, 1H, Ph H-4), 7.57 (m, 1H, Ph H-5), 6.28 (t, $^{2}J_{H,F}$ = 56.3 Hz, 1H, $CHF_2$).

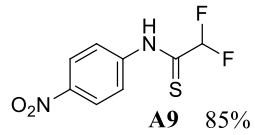
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 187.8 (t, $^{2}J_{C,F}$ =20.8 Hz, C=S), 137.7 (Ph C-1), 130.9 (Ph C-4), 130.2 (Ph C-5), 126.9 (Ph C-6), 125.8 (Ph C-2), 117.7 (CN), 113.4 (Ph C-3), 112.7 (t, $^{1}J_{C,F}$ = 258.4 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -294.1 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.1 (dd, $^{2}J_{H,F}$ = 56.3 Hz, $^{n}J_{H,F}$ = 3.2 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_9H_6F_2N_2NaS$: 235.0112 $[M+Na]^+$; found: 235.0111.

2,2-difluoro-*N*-(4-nitrophenyl)ethanethioamide (**A9**)




**A9** 85%

By following general procedure A, starting from 1-isothiocyanato-4-nitrobenzene (0.180 g, 1 mmol, 1.0 equiv), compound **A9** was obtained in 85% yield (0.197 g) as brown solid (mp 60-63°C) after column chromatography on silica gel (*n*-hexane:ethyl acetate 7:3).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.41 (br s, 1H, NH), 8.33 (m, 2H, Ph H-3,5), 8.14 (m, 2H, Ph H-2,6), 6.29 (t, $^{2}J_{H,F}$ = 56.3 Hz, 1H, $CHF_2$).

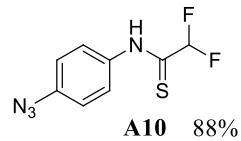
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 187.6 (t, $^2J_{C,F}$ =20.6 Hz, C=S), 145.7 (Ph C-4), 142.2 (Ph C-1), 125.0 (Ph C-3,5), 122.2 (Ph C-2,6), 112.8 (t, $^1J_{C,F}$ = 258.8 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -293.0 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -115.9 (dd, $^2J_{H,F}$ = 56.3 Hz, $^nJ_{H,F}$ = 3.2 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_6F_2N_2NaO_2S$: 255.0010 $[M+Na]^+$; found: 255.0009.

*N*-(4-azidophenyl)-2,2-difluoroethanethioamide (**A10**)



**A10** 88%

By following general procedure A, starting from 1-isothiocyanato-4-azidobenzene (0.176 g, 1 mmol, 1.0 equiv), compound **A10** was obtained in 88% yield (0.201 g) as yellow oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 9.25 (br s, 1H, NH), 7.84 (m, 2H, Ph C-2,6), 7.10 (m, 2H, Ph C-3,5), 6.28 (t, $^2J_{H,F}$ = 56.5 Hz, 1H, $CHF_2$).

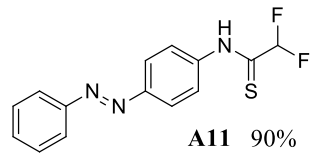
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 186.6 (t, $^2J_{C,F}$ =20.4 Hz, C=S), 133.6 (Ph C-1), 139.1 (Ph C-4), 124.2 (Ph C-2,6), 119.6 (Ph C-3,5), 112.8 (t, $^1J_{C,F}$ = 258.1 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -291.7 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.1 (dd, $^2J_{H,F}$ = 56.5 Hz, $^nJ_{H,F}$ = 3.3 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_7F_2N_4S^+$: 229.0354 $[M+H]^+$; found: 229.0342.

2,2-difluoro-*N*-{4-[(*E*)-phenyldiazenyl]phenyl}ethanethioamide (**A11**)



**A11** 90%

By following general procedure A, starting from 1-(4-isothiocyanatophenyl)-2-phenyldiazene (0.239 g, 1 mmol, 1.0 equiv), compound **A11** was obtained in 90% yield (0.262 g) as orange solid (mp 83-86°C) after column chromatography on silica gel (*n*-hexane:diethyl ether 7:3).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.37 (br s, 1H, NH), 8.08 (m, 2H, Ph1 H-2,6), 8.02 (m, 2H, Ph1 H-3,5), 7.93 (m, 2H, Ph2 H-2,6), 7.54 (m, 2H, Ph H-3,5), 7.51 (m, 1H, Ph2 H-4), 6.30 (t, $^{2}J_{H,F}$ = 56.5 Hz, 1H, $CHF_2$).

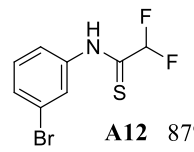
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 186.7 (C=S), 152.6 (Ph2 C-1), 151.0 (Ph1 C-4), 138.9 (Ph1 C-1), 131.4 (Ph2 C-4), 129.2 (Ph2 C-3,5), 123.9 (Ph1 C-3,5), 123.0 (Ph2 C-2,6), 122.7 (Ph1 C-2,6), 112.9 (t, $^{1}J_{C,F}$ = 258.3 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -290.6 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -115.9 (dd, $^{2}J_{H,F}$ = 56.5 Hz, $^{n}J_{H,F}$ = 3.4 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_{14}H_{12}F_2N_3S^+$: 292.0715 $[M+H]^+$; found: 292.0706.

*N*-(3-bromophenyl)-2,2-difluoroethanethioamide (**A12**)



**A12** 87%

By following general procedure A, starting from 1-isothiocyanato-3-bromobenzene (0.213 g, 1 mmol, 1.0 equiv), compound **A12** was obtained in 87% (0.230 g) as yellow oil after column chromatography on silica gel (*n*-heptane:ethyl acetate 8:2).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.21 (br s, 1H, NH), 8.10 (m, 1H, Ph H-2), 7.76 (m, 1H, Ph H-6), 7.47 (m, 1H, Ph H-4), 7.32 (m, 1H, Ph H-5), 6.27 (t, $^{2}J_{H,F}$ = 56.5 Hz, 1H, $CHF_2$).

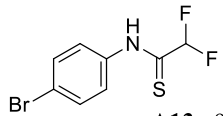
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 187.0 (C=S), 138.0 (Ph C-1), 130.6 (Ph C-4), 130.5 (Ph C-5), 125.5 (Ph C-2), 122.6 (Ph C-3), 121.3 (Ph C-6), 112.8 (t, $^{1}J_{C,F}$ = 258.3 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -292.9 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.1 (dd, $^{2}J_{H,F}$ = 56.5 Hz, $^{n}J_{H,F}$ = 3.3 Hz, $CHF_2$ ).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_7BrF_2NS^+$: 265.9445 $[M+H]^+$; found: 265.9421.

*N*-(4-bromophenyl)-2,2-difluoroethanethioamide (**A13**)




**A13** 91%

By following general procedure A, starting from 1-isothiocyanato-4-bromobenzene (0.214 g, 1 mmol, 1.0 equiv), compound **A13** was obtained in 91% yield (0.242 g) as yellow oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 9:1).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.20 (br s, 1H, NH), 7.75 (m, 2H, Ph H-2,6), 7.57 (m, 2H, Ph H-3,5), 6.27 (t, $^{2}J_{H,F}$ = 56.5 Hz, 1H, $CHF_2$).

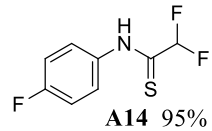
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 186.9 (t, $^{2}J_{C,F}$ =20.3 Hz, C=S), 135.9 (Ph C-1), 132.3 (Ph C-3,5), 124.1 (Ph C-2,6), 120.6 (Ph C-4), 112.8 (t, $^{1}J_{C,F}$ = 258.2 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -292.3 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.1 (dd, $^{2}J_{H,F}$ = 56.5 Hz, $^{2n}J_{H,F}$ = 3.3 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_7BrF_2NS^+$: 265.9445 $[M+H]^+$; found: 265.9438.

2,2-difluoro-*N*-(4-fluorophenyl)ethanethioamide (**A14**)




**A14** 95%

By following general procedure A, starting from 1-isothiocyanato-4-fluorobenzene (0.153 g, 1 mmol, 1.0 equiv), compound **A14** was obtained in 95% yield (0.195 g) as yellow oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 9.26 (br s, 1H, NH), 7.79 (m, 2H, Ph H-2,6), 7.14 (m, 2H, Ph H-3,5), 6.28 (t, $^2J_{H,F}$ = 56.5 Hz, 1H, $CHF_2$).

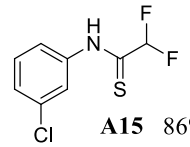
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 187.1 (t, $^2J_{C,F}$ = 20.4 Hz, C=S), 161.1 (d, $^1J_{C,F}$ = 248.7 Hz, Ph C-4), 132.8 (d, $^4J_{C,F}$ = 3.0 Hz, Ph C-1), 124.9 (d, $^3J_{C,F}$ = 8.4 Hz, Ph C-2,6), 116.1 (d, $^2J_{C,F}$ = 23.0 Hz, Ph C-3,5), 112.8 (t, $^1J_{C,F}$ = 258.0 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -308.6 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.2 (dd, $^2J_{H,F}$ = 56.5 Hz, $^nJ_{H,F}$ = 3.2 Hz, $CHF_2$), -112.4 (m, Ph-F).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_7F_3NS^+$: 206.0246 $[M+H]^+$; found: 206.0233.

*N*-(3-chlorophenyl)-2,2-difluoroethanethioamide (**A15**)




**A15** 86%

By following general procedure A, starting from 1-isothiocyanato-3-chlorobenzene (0.169 g, 1 mmol, 1.0 equiv), compound **A15** was obtained in 86% yield (0.190 g) as yellow oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.21 (br s, 1H, NH), 7.98 (m, 1H, Ph H-2), 7.69 (m, 1H, Ph H-6), 7.38 (m, 1H, Ph H-5), 7.31 (m, 1H, Ph H-4), 6.27 (t, $^{2}J_{H,F}$ = 56.5 Hz, 1H, $CHF_2$).

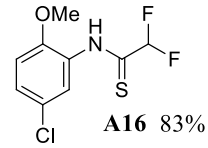
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 187.1 (t, $^{2}J_{C,F}$ = 20.8 Hz, C=S), 137.9 (Ph C-1), 134.9 (Ph C-3), 130.2 (Ph C-5), 127.7 (Ph C-4), 122.7 (Ph C-2), 120.7 (Ph C-6), 112.8 (t, $^{1}J_{C,F}$ = 258.4 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -292.5 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.1 (dd, $^{2}J_{H,F}$ = 56.4 Hz, $^{n}J_{H,F}$ = 3.3 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_6ClF_2NNaS$: 243.9770 $[M+Na]^+$; found: 243.9767.

*N*-(5-chloro-2-methoxyphenyl)-2,2-difluoroethanethioamide (**A16**)




**A16** 83%

By following general procedure A, starting from ethyl 4-cloro-2-isothiocyanato-1-methoxybenzoate (0.199 g, 1 mmol, 1.0 equiv), compound **A16** was obtained in 83% yield (0.208 g) as brown solid (mp 70-72°C) after column chromatography on silica gel (*n*-hexane:ethyl acetate 7:3).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.99 (br s, 1H, NH), 9.25 (d, $^{4}J_{H,H}$ =2.5 Hz, 1H, Ph H-6), 7.22 (dd, $^{3}J_{H,H}$ = 8.8 Hz, $^{4}J_{H,H}$ =2.5 Hz, 1H, Ph H-4), 6.90 (d, $^{3}J_{H,H}$ = 8.8 Hz, 1H, Ph H-3), 6.23 (t, $^{2}J_{H,F}$ = 56.6 Hz, 1H, $CHF_2$), 3.95 (s, 3H, $OCH_3$).

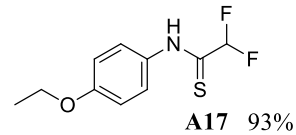
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 185.1 (t, $^{2}J_{C,F}$ =20.4 Hz, C=S), 148.0 (Ph C-2), 127.6 (Ph C-1), 126.8 (Ph C-4), 125.6 (Ph C-5), 120.4 (Ph C-6), 113.0 (t, $^{1}J_{C,F}$ = 258.4 Hz, $CHF_2$), 111.2 (Ph C-3), 56.4 ($OCH_3$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -299.5 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -115.8 (dd, $^{2}J_{H,F}$ = 56.6 Hz, $^{2n}J_{H,F}$ = 3.4 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_9H_9ClF_2NOS^+$: 252.0056 $[M+H]^+$; found: 252.0050.

*N*-(4-ethoxyphenyl)-2,2-difluoroethanethioamide (**A17**)




**A17** 93%

By following general procedure A, starting from 1-isothiocyanato-4-ethoxybenzene (0.179 g, 1 mmol, 1.0 equiv), compound **A17** was obtained in 93% yield (0.214 g) as brown oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.20 (br s, 1H, NH), 7.72 (m, 2H, Ph H-2,6), 6.94 (m, 2H, Ph H-3,5), 6.28 (t, $^{2}J_{H,F}$ = 56.6 Hz, 1H, $CHF_2$), 4.06 (q, $^{3}J$ = 7.0 Hz, 2H, $OCH_2$), 1.43 (t, $^{3}J$ = 7.0 Hz, 3H, $CH_3$).

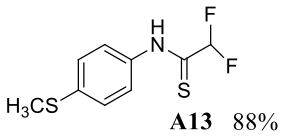
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 186.0 (t, $^{2}J_{C,F}$ =20.3 Hz, C=S), 158.0 (Ph C-4), 129.6 (Ph C-1), 124.3 (Ph C-2,6), 114.8 (Ph C-3,5), 112.8 (t, $^{1}J_{C,F}$ = 257.8 Hz, $CHF_2$), 63.8 ($OCH_2$), 14.7 ($CH_3$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -290.5 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.1 (dd, $^2J_{H,F}$ = 56.6 Hz, $^nJ_{H,F}$ = 3.4 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_{10}H_{11}F_2NNaOS$: 254.0422 $[M+Na]^+$; found: 224.0420.

2,2-difluoro-*N*-[4-(methylsulfanyl)phenyl]ethanethioamide (**A18**)




**A13** 88%

By following general procedure A, starting from 1-isothiocyanato-4-methylsulfanylbenzene (0.181 g, 1 mmol, 1.0 equiv), compound **A18** was obtained in 88% yield (0.209 g) as yellow oil after column chromatography on silica gel (heptane:ethyl acetate 8:2).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 9.22 (br s, 1H, NH), 7.77 (m, 2H, Ph H-2,6), 7.30 (m, 2H, Ph H-3,5), 6.27 (t, $^2J_{H,F}$ = 56.5 Hz, 1H, $CHF_2$), 2.51 (s, 3H, $SCH_3$).

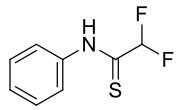
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 186.1 (t, $^2J_{C,F}$ =20.5 Hz, C=S), 138.4 (Ph C-4), 133.9 (Ph C-1), 126.7 (Ph C-3,5), 123.0 (Ph C-2,6), 112.8 (t, $^1J_{C,F}$ = 258.1 Hz, $CHF_2$), 15.7($SCH_3$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -290.6 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.0 (dd, $^2J_{H,F}$ = 56.5 Hz, $^nJ_{H,F}$ = 3.5 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_9H_{10}F_2NS_2$: 234.0217 $[M+H]^+$; found: 234.0205.

2,2-difluoro-*N*-phenylethanethioamide (**A19**)

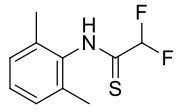


**A19** 91%

By following general procedure A, starting from isothiocyanatobenzene (0.135 g, 1 mmol, 1.0 equiv), compound **A19** was obtained in 91% yield (0.170 g) as yellow oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 85:15).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.26 (br s, 1H, NH), 7.83 (m, 2H, Ph H-2,6), 7.45 (m, 2H, Ph H-3,5), 7.33 (m, 1H, Ph H-4) 6.28 (t, $^{2}J_{H,F}$ = 56.5 Hz, 1H, $CHF_2$).
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 186.7 (t, $^{2}J_{C,F}$ =20.5 Hz, C=S), 136.8 (Ph C-1), 129.2 (Ph C-3,5), 127.7 (Ph C-4), 122.6 (Ph C-2,6), 112.9 (t, $^{1}J_{C,F}$ = 258.2 Hz, $CHF_2$).
**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -290.0 (NH).
**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.1 (dd, $^{2}J_{H,F}$ = 56.5 Hz, $^{n}J_{H,F}$ = 3.3 Hz, $CHF_2$).
**HRMS** (ESI), *m/z*: calcd. for $C_8H_8F_2NS^+$: 188.0340 $[M+H]^+$; found: 188.0342.

*N*-(2,6-dimethylphenyl)-2,2-difluoroethanethioamide (**A20**)



**A20** 90%

By following general procedure A, starting from 2-isothiocyanato-1,3-dimethylbenzene (0.163 g, 1 mmol, 1.0 equiv), compound **A20** was obtained in 90% yield (0.194 g) as brown oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 9:1).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 8.93 (br s, 1H, NH), 7.24 (m, 1H, Ph H-4), 7.15 (d, $^{3}J$ = 7.5 Hz, 2H, Ph H-3,5), 6.36 (t, $^{2}J_{H,F}$ = 56.2 Hz, 1H, $CHF_2$), 2.23 (s, 6H, $CH_3$).

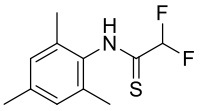
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 189.7 (t, $^{2}J_{C,F}$ =20.9 Hz, C=S), 135.2 (Ph C-2,6), 133.6 (Ph C-1), 129.0 (Ph C-4), 128.6 (Ph C-3,5), 112.8 (t, $^{1}J_{C,F}$ = 256.8 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -295.8 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.1 (dd, $^2J_{H,F}$ = 56.2 Hz, $^nJ_{H,F}$ = 2.7 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_{10}H_{11}F_2NNaS$: 238.0472 $[M+Na]^+$; found: 238.0475.

2,2-difluoro-*N*-mesitylethanethioamide (**A21**)




**A21** 88%

By following general procedure A, starting from 2-isothiocyanato-1,3,5-trimethylbenzene (0.177 g, 1 mmol, 1.0 equiv), compound **A21** was obtained in 88% yield (0.202 g) as brown oil after column chromatography on silica gel (*n*-heptane:ethyl acetate 9:1).

**$^1$H NMR** (400 MHz, $C_6D_6$): δ 7.83 (br s, 1H, NH), 6.64 (s, 2H, Ph H-3,5), 5.98 (t, $^2J_{H,F}$ = 56.3 Hz, 1H, $CHF_2$), 2.05 (s, 3H, Ph 4-$CH_3$), 1.92 (s, 6H, Ph 2,6-$CH_3$).

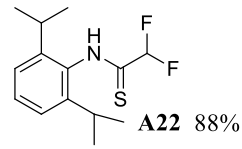
**$^{13}$C NMR** (100 MHz, $C_6D_6$): δ 189.8 (t, $^2J_{C,F}$ = 20.9 Hz, C=S), 138.3 (Ph C-4), 135.0 (Ph C-2,6), 132.0 (Ph C-1), 129.3 (Ph C-3,5), 113.6 (t, $^1J_{C,F}$ = 256.9 Hz, $CHF_2$), 21.0 (Ph 4-$CH_3$), 17.5 (Ph 2,6-$CH_3$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -232.2 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -115.7 (dd, $^2J_{H,F}$ = 56.3 Hz, $^nJ_{H,F}$ = 2.3 Hz, $CHF_2$ ).

**HRMS** (ESI), *m/z*: calcd. for $C_{11}H_{13}F_2NNaS$: 252.0629 $[M+Na]^+$; found: 252.0631.

*N*-(2,6-diisopropylphenyl)-2,2-difluoroethanethioamide (**A22**)




**A22** 88%

By following general procedure A, starting from 1,3-diisopropyl-2-isothiocyanatobenzene (0.149 g, 1 mmol, 1.0 equiv), compound **A22** was obtained in 88% yield (0.238 g) as pale yellow solid (mp 96-99°C) after column chromatography on silica gel (*n*-hexane:DCM 8:2).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 8.88 (br s, 1H, NH), 7.25 (d, $^{3}J$ = 7.8 Hz, 2H, Ph H-3,5), 7.41 (t, $^{3}J$ = 7.8 Hz, 1H, Ph H-4), 6.39 (t, $^{2}J_{H,F}$ = 56.2 Hz, 1H, $CHF_2$), 2.92 (sept., $^{3}J$ = 6.85 Hz, 2H, C<u>H</u>$(CH_3)_2$), 1.24 (d, $^{3}J$ = 6.8 Hz, 6H, $CH_3$), 1.19 (d, $^{3}J$ = 6.9 Hz, 6H, $CH_3$).

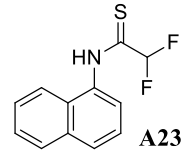
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 191.1 (t, $^{2}J_{C,F}$ =20.8 Hz, C=S), 145.6 (Ph C-2,6), 129.8 (Ph C-4), 130.9 (Ph C-1), 124.1 (Ph C-3,5), 112.9 (t, $^{1}J_{C,F}$ = 257.0 Hz, $CHF_2$), 28.7 ($CH(CH_3)_2$), 24.2 ($CH_3$), 23.2 ($CH_3$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -297.9 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -116.2 (dd, $^{2}J_{H,F}$ = 56.2 Hz, $^{n}J_{H,F}$ = 2.6 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_{14}H_{19}F_2NNaS$: 294.1098 $[M+Na]^+$; found: 294.1103.

2,2-difluoro-*N*-(1-naphthyl)ethanethioamide (**A23**)




**A23** 92%

By following general procedure A, starting from 1-isothiocyanatonaphthalene (0.185 g, 1 mmol, 1.0 equiv), compound **A23** was obtained in 92% yield (0.218 g) as brown oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 9.52 (br s, 1H, NH), 7.93 (m, 2H, H-4,5), 7.91 (m, 1H, H-2), 7.80 (m, 1H, H-8), 7.58 (m, 1H, H-7), 7.57 (m, 1H, H-6), 7.55 (m, 1H, H-3), 6.45 (t, $^{2}J_{H,F}$ = 56.3 Hz, 1H, $CHF_2$).

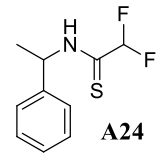
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 189.6 (t, $^{2}J_{C,F}$ = 20.7 Hz, C=S), 134.3 (napht C-1), 131.9 (napht C-4a), 129.0 (napht C-5), 128.9 (napht C-4), 128.0 (Ph C-9), 127.3 (napht C-7), 126.7 (napht C-6), 125.3 (napht C-3), 123.7 (napht C-2), 120.8 (napht C-8), 113.0 (t, $^{1}J_{C,F}$ = 257.4 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -297.2 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -115.9 (dd, $^{2}J_{H,F}$ = 56.3 Hz, $^{n}J_{H,F}$ = 3.0 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_{12}H_9F_2NNaS$: 260.0316 $[M+Na]^+$; found: 260.0318.

2,2-difluoro-N-(1-phenylethyl)ethanethioamide (**A24**)




**A24** 84%

By following general procedure A, starting from (1-isothiocyanatoethyl)benzene (0.163 g, 1 mmol, 1.0 equiv), compound **A24** was obtained in 91% yield (0.196 g) as pale yellow oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 7.99 (br s, 1H, NH), 7.39 (m, 2H, Ph H-3,5), 7.34 (m, 2H, Ph H-2,6), 7.33 (m, 1H, Ph H-4), 6.18 (t, $^{2}J_{H,F}$ = 56.3 Hz, 1H, $CHF_2$), 5.66 (m, 1H, NCH), 1.66 (d, $^{3}J$ = 6.9 Hz, 3H, $CH_3$).

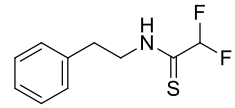
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 188.3 (t, $^2J_{C,F}$ = 21.0 Hz, C=S), 140.1 (Ph C-1), 129.0 (Ph C-3,5), 128.3 (Ph C-4), 126.5 (Ph C-2,6), 112.1 (t, $^1J_{C,F}$ = 256.0 Hz, $CHF_2$), 54.0 (NCH), 19.8 ($CH_3$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -281.6 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -117.2 (dd, $^2J_{H,F}$ = 56.3 Hz, $^nJ_{H,F}$ = 2.4 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_{10}H_{11}F_2NNaS$: 238.0467 $[M+Na]^+$; found: 238.0474.

2,2-difluoro-*N*-(2-phenylethyl)ethanethioamide (**A25**)




**A25** 84%

By following general procedure A, starting from (2-isothiocyanatoethyl)benzene (0.163 g, 1 mmol, 1.0 equiv), compound **A25** was obtained in 84% yield (0.181 g) as orange oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 9:1).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.88 (br s, 1H, NH), 7.35 (m, 2H, Ph H-3,5), 7.28 (m, 1H, Ph H-4), 7.22 (m, 2H, Ph H-2,6), 6.15 (t, $^2J_{H,F}$ = 56.2 Hz, 1H, $CHF_2$), 3.96 (m, 2H, $CH_2NH$), 3.01 (t, $^3J$ = 7.1 Hz, $CH_2Ph$).

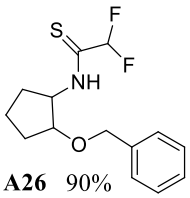
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 189.7 (t, $^2J_{C,F}$ = 21.0 Hz, C=S), 137.4 (Ph C-1), 129.0 (Ph C-3,5), 128.6 (Ph C-2,6), 127.1 (Ph C-4), 112.0 (t, $^1J_{C,F}$ = 255.7 Hz, $CHF_2$), 46.0 ($CH_2NH$), 33.4 ($CH_2Ph$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -297.5 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -117.4 (dd, $^2J_{H,F}$ = 56.2 Hz, $^nJ_{H,F}$ = 2.7 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_{10}H_{12}F_2NS^+$: 216.0653 $[M+H]^+$; found: 216.0650.

*N*-[2-(benzyloxy)cyclopentyl]-2,2-difluoroethanethioamide (**A26**)




**A26** 90%

By following general procedure A, starting from (2-isothiocyanatocyclopentyloxy) methylbenzene (0.233 g, 1 mmol, 1.0 equiv), compound **A26** was obtained in 90% yield (0.257 g) as yellow oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 7.67 (br s, 1H, NH), 7.34 (m, 4H, Ph H-2,3,5,6), 7.29 (m, 1H, Ph H-4), 6.16 (t, $^{2}J_{H,F}$ = 56.4 Hz, 1H, $CHF_2$), 4.75 (m, 1H, H-1), 4.66 (A-part of an AB-system, $^{2}J_{AB}$= 12.2 Hz, 1H, $OCH_2$), 4.66 (B-part of an AB-system, $^{2}J_{AB}$= 12.2 Hz, 1H, $OCH_2$), 3.94 (m, 1H, H-2), 2.39 (m, 1H, H-5), 1.92 (m, 2H, H-4,3), 1.83 (m, 1H, H-3), 1.75 (m, 1H, H-4), 1.53 (m, 1H, H-5).

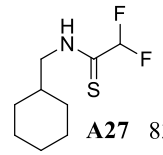
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 188.9 (t, $^{2}J_{C,F}$ =21.0 Hz, C=S), 138.2 (Ph C-1), 128.4 (Ph C-3,5), 127.69 (Ph C-4) 127.66 (Ph C-2,6), 112.1 (t, $^{1}J_{C,F}$ = 256.0 Hz, $CHF_2$), 83.4 (C-2), 71.3 ($OCH_2$), 60.9 (C-1), 30.7 (C-3), 29.5 (C-5), 21.7 (C-4).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -287.3 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -117.1 (d, $^{2}J_{H,F}$ = 56.4 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_{14}H_{17}F_2NNaOS$: 308.0891 $[M+Na]^+$; found: 308.0889.

*N*-(cyclohexylmethyl)-2,2-difluoroethanethioamide (**A27**)




**A27** 83%

By following general procedure A, starting from (isothiocyanatomethyl)cyclohexane (0.155 g, 1 mmol, 1.0 equiv), compound **A27** was obtained in 83% yield (0.172 g) as yellow oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 9:1).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 7.91 (br s, 1H, NH), 6.19 (t, $^{2}J_{H,F}$ = 56.4 Hz, 1H, $CHF_2$), 3.55 (t, $^{3}J$ = 6.2 Hz, $NCH_2$), 1.76 (m, 4H, H-2,3,5,6), 1.74 (m, 1H, H-1), 1.69 (m, 1H, H-4), 1.26 (m, 2H, H-3,5), 1.19 (m, 1H, H-4), 1.03 (m, 2H, H-2,6).

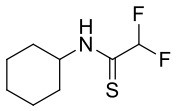
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 189.6 (t, $^{2}J_{C,F}$ = 21.0 Hz, C=S), 112.1 (t, $^{1}J_{C,F}$ = 255.7 Hz, $CHF_2$), 51.2 ($NCH_2$), 36.6 (C-1), 30.9 (C-2,6), 26.1 (C-4), 25.6 (C-3,5).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -297.3 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -117.2 (dd, $^{2}J_{H,F}$ = 56.4 Hz, $^{n}J_{H,F}$ = 2.7 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_9H_{15}F_2NNaS$: 230.0785 $[M+Na]^+$; found: 230.0778.

*N*-cyclohexyl-2,2-difluoroethanethioamide (**A28**)




**A28** 85%

By following general procedure A, starting from isothiocyanatocyclohexane (0.141 g, 1 mmol, 1.0 equiv), compound **A28** was obtained in 85% yield (0.164 g) as brown oil after column chromatography on silica gel (*n*-hexane:ethyl acetate 9:1).

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 7.68 (br s, 1H, NH), 6.15 (t, $^{2}J_{H,F}$ = 56.4 Hz, 1H, $CHF_2$), 4.33 (m, 1H, H-1), 2.10 (m, 2H, H-2,6), 1.78 (m, 2H, H-3,5), 1.68 (m, 1H, H-4), 1.43 (m, 2H, H-3,5), 1.30 (m, 2H, H-2,6), 1.25 (m, 1H, H-4).

**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 187.9 (t, $^{2}J_{C,F}$ =20.9 Hz, C=S), 112.1 (t, $^{1}J_{C,F}$ = 255.9 Hz, $CHF_2$), 53.6 (C-1), 31.0 (C-2,6), 25.3 (C-4), 24.5 (C-3,5).

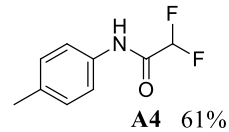
**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -281.1 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -117.5 (dd, $^{2}J_{H,F}$ = 56.4 Hz, $^{n}J_{H,F}$ = 2.5 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_{13}F_2NNaS$: 216.0629 $[M+Na]^+$; found: 216.0622.

### *A.6.4 Characterization of the α,α-difluoroamides*

2,2-difluoro-*N*-(4-methylphenyl)acetamide (**A4**)



**A4** 61%

By following general procedure B, starting from 1-isocyanato-4-methylbenzene (0.133 g, 1.0 mmol, 1.0 equiv), compound **A4** was obtained in 87% yield (0.162 g) as pale yellow solid (mp 106-108 °C) after column chromatography on silica gel (*n*-hexane:ethyl acetate 9:1).

**$^{1}$H NMR** (400 MHz, $CDCl_3$) δ: 7.83 (br s, 1H, NH), 7.45 (m, 2H, Ph H-2,6), 7.18 (m, 2H, Ph H-3,5), 6.01 (t, $^{2}J_{H,F}$ = 54.4 Hz, 1H, $CHF_2$), 2.34 (s, 3H, $CH_3$).

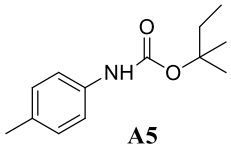
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ: 160.1 (C=0), 135.7 (Ph C-4), 133.0 (Ph C-1), 129.8 (Ph C-3,5), 120.3 (Ph C-2,6), 108.6 (t, $^{1}J_{C,F}$ = 254.2 Hz, $CHF_2$), 20.9 ($CH_3$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$) δ: -259.4 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$) δ: -125.5 (dd, $^{2}J_{H,F}$ = 54.4 Hz, $^{n}J_{H,F}$ = 2.3 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_9H_9F_2NNaO$: 208.0544 $[M+Na]^+$; found: 208.0562.

*tert*-pentyl p-tolylcarbamate (**A5**)



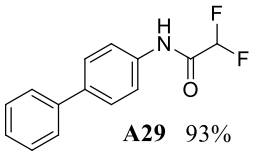
**A5**

**$^{1}$H NMR** (400 MHz, $CDCl_3$): δ 7.85 (m, 2H, Ph H-2,6), 7.36 (m, 2H, Ph H-3,5), 6.72 (br s, 1H, NH), 2.28 (s, 3H, $CH_3$), 1.76 (q, $^{3}J$ =7.5 Hz, 2H, $CH_2$), 1.42 (s, 6H, CH3), 0.98 (t, $^{3}J$ =7.5 Hz, 3H, $CH_3$).

**13C NMR** (100 MHz, CDCl3): δ 162.6 (CO), 142.6 (Ph C-4), 128.9 (Ph C-2,6), 123.5 (Ph C-1), 116.8 (Ph C-3,5), 83.7 ($C(CH_3)_2$), 33.6 ($CH_2$), 21.3 ($CH_3$), 25.7 (2C, CH3), 8.2 (CH3).

**HRMS** (ESI), *m/z*: calcd. for $C_{13}H_{19}NO_2^+$: 221.3105 $[M+H]^+$; found: 221.3098.

*N*-(biphenyl-4-yl)-2,2-difluoroacetamide (**A29**)




**A29** 93%

By following general procedure A, starting from 4-isothiocyanato-4-phenylbenzene (0.195 g, 1 mmol, 1.0 equiv), compound **A29** was obtained in 93% yield (0.460 g) as pale yellow solid (mp 177-180°C) after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^1$H NMR** (400 MHz, $CDCl_3$): δ 7.92 (br s, 1H, NH), 7.66 (m, 2H, Ph1 C-3,5), 7.62 (m, 2H, Ph1 C-2,6), 7.58 (m, 2H, Ph2 C-2,6), 7.45 (m, 2H, Ph2 C-3,5), 7.35 (m, 1H, Ph2 C-4), 6.05 (t, $^2J_{H,F}$ = 54.4 Hz, 1H, $CHF_2$).

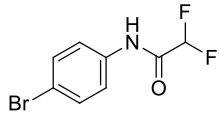
**$^{13}$C NMR** (100 MHz, $CDCl_3$): δ 160.2 (m, C=O), 140.1 (Ph2 C-1), 138.8 (Ph1 C-1), 134.8 (Ph1 C-4), 127.9 (Ph1 C-2,6), 128.9 (Ph2 C-3,5), 127.5 (Ph2 C-4), 126.9 (Ph2 C-2,6), 120.6 (Ph1 C-3,5), 108.6 (t, $^1J_{C,F}$ = 254.8 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$): δ -295.5 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$): δ -125.4 (dd, $^2J_{H,F}$ = 54.4 Hz, $^nJ_{H,F}$ = 2.4 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_{14}H_{11}F_2NNaO$: 270.0701 $[M+Na]^+$; found: 270.0696.

*N*-(4-bromophenyl)-2,2-difluoroacetamide (**A30**)




**A30** 91%

By following general procedure B, starting from 1-bromo-4-isocyanatobenzene (0.198 g, 1.0 mmol, 1.0 equiv), compound **A30** was obtained in 91% yield (0.227 g) as brown solid (mp 114-116 °C) after column chromatography on silica gel (*n*-hexane:ethyl acetate 9:1).

**$^{1}$H NMR** (400 MHz, $CDCl_3$) δ: 7.93 (br s, 1H, NH), 7.49 (‘s’, 4H, Ph H-2,3,5,6), 6.01 (t, $^{2}J_{H,F}$ = 54.2 Hz, 1H, $CHF_2$).

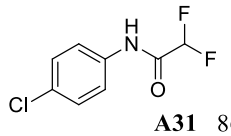
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ: 160.3 (t, $^{2}J_{C,F}$ = 24.6 Hz, C=O), 134.7 (Ph C-1), 132.3 (Ph C-3,5), 121.8 (Ph C-2,6), 118.7 (Ph C-4), 108.4 (t, $^{1}J_{C,F}$ = 254.3 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$) δ: -260.0 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$) δ: -125.6 (dd, $^{2}J_{H,F}$ = 54.2 Hz, $^{n}J_{H,F}$ = 1.2 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_6BrF_2NNaO$: 271.9493 $[M+Na]^+$; found: 271.9488.

*N*-(4-chlorophenyl)-2,2-difluoroacetamide (**A31**)




**A31** 86%

By following general procedure B, starting from 1-chloro-4-isocyanatobenzene (0.153 g, 1.0 mmol, 1.0 equiv), compound **A31** was obtained in 86% yield (0.177 g) as yellow solid (mp 101-103 °C ) after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^{1}$H NMR** (400 MHz, $CDCl_3$) δ: 7.91 (br s, 1H, NH), 7.53 (m, 2H, Ph H-2,6), 7.34 (m, 2H, Ph H-3,5), 6.01 (t, $^{2}J_{H,F}$ = 54.3 Hz, 1H, $CHF_2$).

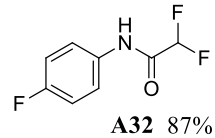
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ: 160.3 (t, $^2J_{C,F}$ = 24.6 Hz, C=O), 134.2 (Ph C-1), 131.1 (Ph C-4), 129.4 (Ph C-3,5), 121.5 (Ph C-2,6), 108.4 (t, $^1J_{C,F}$ = 254.3 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$) δ: -260.3 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$) δ: -125.5 (dd, $^2J_{H,F}$ = 54.3 Hz, $^nJ_{H,F}$ = 2.4 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_6ClF_2NNaO$: 227.9998 $[M+Na]^+$; found: 227.9999.

2,2-difluoro-*N*-(4-fluorophenyl)acetamide (**A32**)




**A32** 87%

By following general procedure B, starting from 1-fluoro-4-isocyanatobenzene (0.137 g, 1.0 mmol, 1.0 equiv), compound **A32** was obtained in 87% yield (0.164 g) as white crystals (mp 91-93 °C) after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^1$H NMR** (400 MHz, $CDCl_3$) δ: 7.86 (br s, 1H, NH), 7.55 (m, 2H, Ph H-2,6), 7.08 (m, 2H, Ph H-3,5), 6.02 (t, $^2J_{H,F}$ = 54.3 Hz, 1H, $CHF_2$).

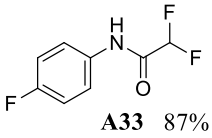
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ: 160.3 (t, $^2J_{C,F}$ =24.4 Hz, C=O), 160.2 (d, $^1J_{C,F}$ = 245.8 Hz, Ph C-4), 131.6 (d, $^4J_{C,F}$ = 3.0 Hz, Ph C-1), 122.2 (d, $^3J_{C,F}$ = 8.1 Hz, Ph C-2,6), 116.1 ($^2J_{C,F}$ = 22.8 Hz, Ph C-3,5), 108.5 (t, $^1J_{C,F}$ = 254.3 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$) δ: -261.1 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$) δ: -125.5 (dd, $^2J_{H,F}$ = 54.3 Hz, $^nJ_{H,F}$ = 2.3 Hz, $CHF_2$), -115.6 (m, Ph 4-F).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_6F_3NNaO$: 212.0294 $[M+Na]^+$; found: 212.0294.

2,2-difluoro-*N*-phenylacetamide (**A33**)



**A33** 87%

By following general procedure B, starting from isocyanatobenzene (0.119 g, 1.0 mmol, 1.0 equiv), compound **A33** was obtained in 85% yield (0.145 g) as brown solid (mp 54-56 °C) after column chromatography on silica gel (*n*-hexane:ethyl acetate 9:1).

**$^1$H NMR** (400 MHz, $CDCl_3$) δ: 7.94 (br s, 1H, NH), 7.58 (m, 2H, Ph H-2,6), 7.38 (m, 2H, Ph H-3,5), 7.21 (m, 1H, Ph H-4), 6.02 (t, $^2J_{H,F}$ = 54.4 Hz, 1H, $CHF_2$).

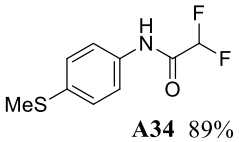
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ: 160.3 (t, $^2J_{C,F}$ =24.5 Hz, C=O), 135.6 (Ph C-1), 129.3 (Ph C-3,5), 125.8 (Ph C-4), 120.3 (Ph C-2,6), 108.5 (t, $^1J_{C,F}$ = 254.2 Hz, $CHF_2$).

**$^{15}$N NMR** (40 MHz, $CDCl_3$) δ: -258.7 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$) δ: -125.5 (dd, $^2J_{H,F}$ = 54.4 Hz, $^nJ_{H,F}$ = 2.3 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_7F_2NNaO$: 194.0388 $[M+Na]^+$; found: 194.0386.

2,2-difluoro-*N*-[4-(methylsulfanyl)phenyl]acetamide (**A34**)



**A34** 89%

By following general procedure B, starting from 1-isocyanato-4-(methylsulfanyl)benzene (0.165 g, 1.0 mmol, 1.0 equiv), compound **A34** was obtained in 89% yield (0.193 g) as brown solid (mp 110-121 °C ) after column chromatography on silica gel (*n*-hexane:ethyl acetate 8:2).

**$^{1}$H NMR** (400 MHz, $CDCl_3$) δ: 7.94 (br s, 1H, NH), 7.50 (m, 2H, Ph H-2,6), 7.25 (m, 2H, Ph H-3,5), 6.01 (t, $^{2}J_{H,F}$ = 54.3 Hz, 1H, $CHF_2$), 2.48 (s, 3H, $CH_3$).

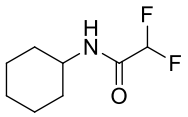
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ: 160.2 (t, $^{2}J_{C,F}$ =24.4 Hz, C=O), 135.8 (Ph C-4), 132.9 (Ph C-1), 127.5 (Ph C-3,5), 120.9 (Ph C-2,6), 108.5 (t, $^{1}J_{C,F}$ = 254.1 Hz, $CHF_2$), 16.1 ($CH_3$) .

**$^{15}$N NMR** (40 MHz, $CDCl_3$) δ: -259.4 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$) δ: -125.5 (d, $^{2}J_{H,F}$ = 54.3Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_9H_9F_2NNaOS$: 240.0265 $[M+Na]^+$; found: 240.0262.

<u>*N*-cyclohexyl-2,2-difluoroacetamide</u> (**A35**)




**A35** 83%

By following general procedure B, starting from isocyanatocyclohexane (0.125 g, 1.0 mmol, 1.0 equiv), compound **A35** was obtained in 83% yield (0.147 g) as volatile white solid (mp 65-67 °C) after column chromatography on silica gel (*n*-hexane:ethyl acetate 9:1).

**$^{1}$H NMR** (400 MHz, $CDCl_3$) δ: 6.14 (br s, 1H, NH), 5.86 (t, $^{2}J_{H,F}$ = 54.5 Hz, 1H, $CHF_2$), 3.80 (m, 1H, cyclohexyl H-1), 1.95 (m, 2H, cyclohexyl H-2,6 ), 1.74 (m, 2H, cyclohexyl H-3,5), 1.64 (m, 1H, cyclohexyl H-4), 1.38 (m, 2H, cyclohexyl H-3,5), 1.21 (m, 2H, cyclohexyl H-2,6), 1.19 (m, 2H, cyclohexyl H-4).

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ: 161.6 (t, $^2J_{C,F}$ = 24.3 Hz, C=O), 108.5 (t, $^1J_{C,F}$ = 252.7 Hz, $CHF_2$), 48.4 (cyclohexyl C-1), 32.6 (cyclohexyl C-2,6), 25.3 (cyclohexyl C-4), 24.6 (cyclohexyl C-3,5).

**$^{15}$N NMR** (40 MHz, $CDCl_3$) δ: -256.7 (NH).

**$^{19}$F NMR** (376 MHz, $CDCl_3$) δ: -126.2 (dd, $^2J_{H,F}$ = 54.5 Hz, $^nJ_{H,F}$ = 2.0 Hz, $CHF_2$).

**HRMS** (ESI), *m/z*: calcd. for $C_8H_{13}F_2NNaO$: 200.0857 $[M+Na]^+$; found: 200.0858.

## ***A.7 Conclusions***

A new reaction was studied to obtain α,α-difluoro(thio)amides, unprecedented scaffolds, by nucleophilic addition to iso(thio)cyanates of difluoromethyltrimethylsilane, activating with potassium *tert*-amylate. The high-yielding transfer of the fluorinated nucleophile takes place under full chemocontrol. Several functionalities (*e.g.* ester, nitrile, nitro, azido groups) can be accommodated across the isothiocyanate core thus allowing to manifest a wide scope. High yields, direct procedure, high chemoselectivity, broad functional group tolerance are advantages of this unprecedent method used to obtain thioamides. Thioamides represent a stable and reactive motif and difluoromethyl moiety has some advantageous pharmaceutical features like H-bond donator because of relatively acidic proton, weakly acidity, it acts as more lipophilic isoster of –OH, -SH[26].

However, the well-known instability of F-containing carbanions[27] posed important risks on the success of the technique. In fact, to make the strategy productive, two key requirements had to be fulfilled:

1) the conditions to generate the $CHF_2$-carbanion must ensure its limited chemical integrity;

2) the reactive isothiocyanate had to be fully preserved during the carbanion generation event.

The strategy is modular and adaptable to the synthesis of analogous α,α-difluoromethyl oxoamide derivatives by simply switching to isocyanates as the starting materials.

Reactivity of (thio)amides obtained was investigated to afford products with biological activity, but preliminary studies did not afford desired products.

[26] a) Y. Zafrani, G. Sod-Moriah, D. Yeffet, A. Berliner, D. Amir, D. Marciano, S. Elias, S. Katalan, N. Ashkenazi, M. Madmon, E. Gershonov and S. Saphier, *J. Med. Chem.* **2019**, *62*, 5628. b) Y. Zafrani, D. Yeffet, G. Sod-Moriah, A. Berliner, D. Amir, D. Marciano, E. Gershonov and S. Saphier, *J. Med. Chem.* **2017**, *60*, 797.

[27] J. Hu, W. Zhang, F. Wang, *Chem. Commun.* **2009**, 7465. b) A.D. Dilman, V.V. Levin, *Acc. Chem. Res.* **2018**, *51*, 1272.

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