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Preparation of new fluorinated analogs of GABA and development of new inhibitors of antiapoptotic proteins with anticancer activity

Ali Soulieman

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THESE DE DOCTORAT DE

L'UNIVERSITE DE RENNES 1
En Cotutelle Internationale avec
L'Université Libanaise

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Matière, Molécules, Matériaux
Spécialité : Chimie

Par

Ali Soulieman

Preparation of New Fluorinated Analogs of GABA and Development of New Inhibitors of Antiapoptotic Protein with Anticancer Activity

Thèse présentée et soutenue à « Beyrouth », le « 24 Février 2021 »

Unité de recherche : UMR 6226 CNRS, équipe CORInt

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| René Grée | Professeur, Université De Rennes 1 |
| Nicolas Gouault | Associate Professor, Université De Rennes 1 |

Résumé

Ces travaux entrent dans le cadre d'un programme de recherche en collaboration entre l'Université Libanaise de Beyrouth et l'Université de Rennes 1 en France. Cette thèse est divisée en deux parties:

- Chimie du Fluor: synthèse d'hétérocycles azotés portant une chaîne latérale fluorée;
- Chimie médicinale: recherche de nouvelles molécules à activité antitumorale.

La première partie contient trois chapitres : dans le premier on rappelle l'importance des hétérocycles azotés ainsi que de la chimie médicinale, ce qui induit pour la recherche une combinaison gagnante de ces deux thématiques. Dans le second chapitre, nous décrivons la synthèse de nouveaux γ -lactames portant des chaînes latérales *gem*-difluorées ainsi que les essais réalisés pour obtenir des analogues de GABA à partir de ces molécules. Dans le troisième chapitre nous avons fait le design, et synthétisé à partir d'un intermédiaire clé fluoré propargylique, trois séries de nouvelles nitrones portant une chaîne fluorée.

Dans la seconde partie notre objectif était de réinduire l'apoptose au sein de cellules cancéreuses afin d'obtenir de nouveaux agents antitumoraux. Notre étude s'est basée sur la structure révisée de MIM1, un inhibiteur connu de la protéine antiapoptotique Mcl-1. En nous basant sur des études de modélisation moléculaire, nous avons fait le design, puis préparé, 38 analogues originaux qui ont été testés sur les cellules IGROV1. Certains de ces composés ont montré des activités prometteuses dans l'inhibition de l'apoptose sur ces cellules cancéreuses. A partir de ces résultats, nous avons élaboré une première ébauche des relations structure-activité et des études en modélisation moléculaire nous ont permis de proposer un rationnel pour l'activité biologiques de cette nouvelle série d'inhibiteurs de protéines anti-apoptotiques.

Mots-clés: enone *gem*-difluorée, addition (1,4) de Michael, nitrones, réaction en cascade, cyclisation intramoléculaire, dérivés propargyliques *gem*-difluorés, hétérocycles, γ -lactames, GABA, antitumoral, MIM1, Mcl-1, apoptose, cancer.

Abstract

This work is a part of a collaboration program between Lebanese University, Lebanon and University of Rennes 1, France. The thesis is divided into two parts:

- Fluorine chemistry: synthesis of new nitrogenous heterocycles with a fluorinated side chain.
- Medicinal chemistry: research towards new anticancer molecules.

The first part consists of three chapters: in the first, the importance of nitrogen-containing heterocycles, as well as fluorine in medicinal chemistry was highlighted, which ensured their golden combination in pharmaceuticals. In the second chapter the synthesis of new γ -lactams with *gem*-difluorinated side chains was performed, and different attempts were done to obtain GABA analogues starting from the prepared γ -lactams. In the third chapter, three different series of new nitrones bearing a fluorinated chain were designed and synthesized starting from a key fluorinated propargylic intermediate.

In the second part, our goal was to reinduce the proapoptotic properties in cancer cells in order to obtain new antitumor compounds. Our studies were based on the revised structure of MIM1, a known inhibitor of the anti-apoptotic protein Mcl-1. Thus, starting from data obtained through molecular modeling studies, we designed and prepared 38 new analogs which have been screened in IGROV1-R10 cells. Some of these derivatives have demonstrated promising results regarding the inhibition of apoptosis in these cancer cells. From this data, preliminary Structure-Activity Relationships have been obtained and extensive molecular modeling/docking studies allowed us to propose a rationale for the biological activity of this series of new inhibitors of anti-apoptotic proteins.

Key words: *gem*-difluoro enone, 1,4 Michael addition, nitrones, cascade reaction, intramolecular cyclization, *gem*-difluoro propargylic derivatives, heterocycles, γ -lactams, GABA, antitumor, MIM-1, Mcl-1, apoptosis, cancer.

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I dedicate this thesis manuscript very sincerely to my father **Alsheikh Ibrahim Soulieman,** my lovely mother **Thouraya Hijazi,** also to my sisters **Zahraa** and **Sara Soulieman,** grandfather **Hasan Hijazi,** Grandmother **Khadija Hammoud, Fatima Hijazi** my second mother and all my family who have always supported me throughout my studies and always encouraged me to go further.

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Abbreviations

Ar: Aryl

Apaf-1: Apoptotic protease activating factor

Bax: Bcl-2-associated x protein

Bcl-2: B-cell lymphoma 2

Bcl-xL: B-cell lymphoma-extra large

BH: Bcl-2 Homology

Boc: Tert-butoxycarbonyl

t-Bu: Tert-butyl

cat: Catalyst

CDI: Carbonyldiimidazole

COSY: Correlation spectroscopy

DAST: Diethylaminosulfurtrifluoride

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene

DCC: N,N'-Dicyclohexylcarbodiimide

DCM: Dichloromethane

DMAP: 4-Dimethylamino pyridine

DMF: Dimethylformamide

DMSO: Dimethylsulfoxide

DNA: Deoxyribonucleic acid

equiv: Equivalent

ESI: electrospray ionization

et al.: Et alia

HIV: Human Immunodeficiency virus

HRMS: High resolution mass spectrometry

IBX: 2-Iodoxybenzoic acid

IC₅₀: Average concentration inhibitor

IR: Infra-red

LDA: Lithium diisopropyl amide

Mcl-1: Myeloid Cell Leukemia 1

MIM-1: Mcl-1 inhibitor molecule 1

min: Minute

mol: Mole

MOM: Methoxymethyl

MOMP: Mitochondrial outer membrane permeabilization

mp: Melting point

m/z: Mass-to-charge ratio

NMR: Nuclear magnetic resonance

NOESY: Nuclear Overhauser Effect Spectroscopy

Nu: Nucleophile

o: ortho

p: para

Ph: Phenyl

pH: potential hydrogen

pka: Acid dissociation constant

ppm: parts per million

p-TSA: *Para*-toluene sulfonic acid

R: Undefined substituent

R_f: Retention factor

r.t.: Room temperature

T: Temperature

***t*-BuO**: *tert*-Butyl alcohol

THF: Tetrahydrofuran

TLC: Thin layer chromatography

UV: Ultraviolet

Table of Schemes

| | |
|---|----|
| Scheme C1. 1: Fluorination reaction of propargyl and enone derivatives using Deoxo-Fluor™ or DAST..... | 11 |
| Scheme C2. 1: Potential use of β -substituted γ -butyrolactams | 19 |
| Scheme C2. 2: Synthesis of α -methylene- γ -lactams 28 | 20 |
| Scheme C2. 3: Synthesis of Lactam Analogue IK682 33 | 21 |
| Scheme C2. 4: Synthesis of (<i>R</i>)-rolipram 6 | 21 |
| Scheme C2. 5: Synthesis of Pyrrolidin-2-one derivatives 38 | 22 |
| Scheme C2. 6: Synthesis of histone deacetylase inhibitor (HDAC) 41 | 22 |
| Scheme C2. 7: Mechanism of the (4+1) annulation to obtain β -hydroxy- γ -lactams | 23 |
| Scheme C2. 8: Synthesis of (+)-Rolipram 51 | 23 |
| Scheme C2. 9: Preparation of difluorinated GABA 62 and γ -lactams 64 and 65 | 27 |
| Scheme C2. 10: Intramolecular Hydroamination of difluoropropargyl Amides 66 | 27 |
| Scheme C2. 11: Synthesis γ -Fluorophenyl-GABA derivatives and their corresponding γ -lactams..... | 28 |
| Scheme C2. 12: Application for the Synthesis of 3,3-Difluoro-GABA..... | 28 |
| Scheme C2. 13: Retrosynthetic analysis for the preparation of the γ -lactams and GABA with fluorinated side chains in position 4..... | 29 |
| Scheme C2. 14: Synthesis of alkynes 81a-h with the gem-difluoroalkylated chains | 29 |
| Scheme C2. 15: Formation of the double bond in 81'b and 81'h | 30 |
| Scheme C2. 16: Synthesis of nitroester adducts 83a-h with the gem-difluoroalkylated chains in position 4..... | 31 |
| Scheme C2. 17: Synthesis of γ -lactams with the gem-difluoroalkylated chains in position 4 85a-b using Pd(OH) ₂ | 33 |
| Scheme C2. 18: The reduction of the nitro group and the dehalogenation process in 83c-d ... 33 | 33 |
| Scheme C2. 19: Synthesis of γ -lactams with the gem-difluoroalkylated chains in position 4 85a-h using NiCl ₂ .6H ₂ O and NaBH ₄ | 33 |
| Scheme C2. 20: Introduction of R ¹ substituents in position 3..... | 35 |
| Scheme C2. 21: Synthesis of γ -lactams substituted in position 5. | 37 |
| Scheme C2. 22: tentative hydrolysis of γ -lactam to obtain GABA analogue | 39 |
| Scheme C2. 23: Suggested mechanism to afford compound 96 | 39 |
| Scheme C2. 24: Attempts to open the γ -lactam ring using basic and acidic conditions | 39 |

| | |
|---|-----|
| Scheme C3. 1: Top: The spin-trapping mechanism of DMPO 2 to obtain an aminoxyl spin adduct 3 , bottom: the linear nitron PBN 4 | 95 |
| Scheme C3. 2: Mechanistic pathway of the oxidation reaction of the secondary amines 5 to give nitron 1 | 96 |
| Scheme C3. 3: From secondary amines toward nitrones using H ₂ O ₂ or O ₂ | 97 |
| Scheme C3. 4: Decarboxylative oxidation of N-Alkyl- α -amino Acids and some examples where this method was exploited | 98 |
| Scheme C3. 5: Oxidation Mechanism that involves a radical..... | 98 |
| Scheme C3. 6: Mechanism Proposed for the IBX Oxidation of Hydroxylamines | 99 |
| Scheme C3. 7: Baeyer-Villiger type formation of nitrones starting from amines 8 | 99 |
| Scheme C3. 8: Mechanism of the synthesis of nitrones 27 from isoxazolidines 9 | 100 |
| Scheme C3. 9: Condensation reaction between the carbonyl group 28 and <i>N</i> - monosubstituted hydroxylamines 29 | 101 |
| Scheme C3. 10: Synthesis of nitrones from oximes 31 | 101 |
| Scheme C3. 11: Cu-mediated <i>N</i> -vinyl nitron synthesis..... | 102 |
| Scheme C3. 12: Mechanism of the reduction of nitro compounds using Zn/H ⁺ followed by the formation of nitrones | 103 |
| Scheme C3. 13: Suggested mechanism of the formation of <i>N</i> -aryl nitrones 51 | 103 |
| Scheme C3. 14: Preparation of di- and trifluoromethyl nitrones 55a,f and 57a,f | 104 |
| Scheme C3. 15: Synthesis of fluoro-substituted phenyl-tert-butyl nitrones 60 | 104 |
| Scheme C3. 16: Preparation of the aliphatic nitrones 63 | 105 |
| Scheme C3. 17: Formation of enantiopure fluorinated <i>N</i> -oxides 67 using chiral catalyst 65 | 105 |
| Scheme C3. 18: preparation of various cyclic nitrones..... | 106 |
| Scheme C3. 19: Synthesis of <i>gem</i> -difluoro propargylic derivative 77 | 107 |
| Scheme C3. 20: Synthesis of nitro intermediates 80a-e and 81a-e | 108 |
| Scheme C3. 21: Synthesis of nitrones with <i>gem</i> -difluorinated side chains 82a-e and 83a-e | 109 |
| Scheme C3. 22: Mechanism of the formation of dihydropyrroles 90 | 110 |
| Scheme C3. 23: Synthesis of ynol 95 | 112 |
| Scheme C3. 24: Synthesis of nitrones 100 and 102 | 113 |
| Scheme C3. 25: Synthesis of nitrones 105 , 106 , and 109 | 115 |
| Scheme C3. 26: Synthesis of <i>gem</i> -difluorinated propargylic compound 113 | 115 |
| Scheme C3. 27: Synthesis of cyclic intermediate 118a-e | 116 |
| Scheme C3. 28: Formation of nitrones 119a-e | 117 |

| | |
|--|-----|
| Scheme C4. 1: A general strategy to synthesize revised MIM-1 analogues | 181 |
| Scheme C4. 2: Synthesis of thiosemicarbazides 19a-c | 181 |
| Scheme C4. 3: Synthesis of imino-thiosemicarbazides intermediates 21a-f | 182 |
| Scheme C4. 4: Condensation of thiosemicarbazides with α -bromoketones. | 184 |
| Scheme C4. 5: Preparation of α -bromoketones 23h and 23i | 186 |
| Scheme C4. 6: An attempt to obtain intermediate 41 | 186 |
| Scheme C4. 7: Synthesis of Intermediate 47a-l | 187 |

Table of Figures

| | |
|---|-----|
| Figure C1. 1: Structure of fludrocortisone 1 and 5-fluorouracil 2 | 3 |
| Figure C1. 2 (a) Prevalence of fluoro-pharmaceuticals among globally registered drugs (1991–2019). (b) Data for small-molecule drugs over the past five years..... | 4 |
| Figure C1. 3: Fluorine fields of modification | 5 |
| Figure C1. 4: Fluorinated carbonic anhydrase inhibitors showing linear relationship | 6 |
| Figure C1. 5: examples of biomolecules, synthetic and natural drugs containing heterocycles | 7 |
| Figure C1. 6: Some fluoro-functionalized heterocyclic compounds as drugs | 8 |
| Figure C1. 7: Example of Fluorination using electrophilic fluorinating agents..... | 9 |
| Figure C1. 8: A selection of deoxofluorinating reagents. | 10 |
| Figure C1. 9: Fluorinated heterocycles derived from gem-defluorinated propargylic intermediate..... | 12 |
| | |
| Figure C2. 1: γ -lactam analogues of penicillins active as antibiotics. | 18 |
| Figure C2. 2: pharmaceutically active γ -lactams from different sources..... | 19 |
| Figure C2. 3: Overview of synthetic strategies to build the γ -lactam core (X = leaving group). | 20 |
| Figure C2. 4: Summary of the GABA neurotransmitter ²⁴ | 24 |
| Figure C2. 5: Administrative GABA cannot reach CNS due to the BBB. | 25 |
| Figure C2. 6: Mode of action of different synthetic GABA analogues in the CNS..... | 26 |
| Figure C2. 7: Some GABA analogues | 26 |
| Figure C2. 8: analysis of compounds 81h and 81h' | 31 |
| Figure C2. 9: Structure of 85a and 85e by X-Ray Crystallographic analysis | 34 |
| Figure C2. 10: Structure of 87b by X-Ray Crystallographic analysis | 35 |
| Figure C2. 11: ¹ H- ¹⁹ F HOESY experiment (Top), ¹ H- ¹ H NOESY experiment (bottom) for compound 87a | 36 |
| Figure C2. 12: ¹ H- ¹ H NOESY experiment (Top) for compound 90e in CD ₃ CN; ¹ H- ¹ H NOESY experiment (Bottom) for compound 91e in CD ₃ CN..... | 38 |
| | |
| Figure C3. 1: Different reactive sites in nitrones | 94 |
| Figure C3. 2: Precursors of nitrones by oxidative pathway. | 96 |
| Figure C3. 3: Our target nitrones with <i>gem</i> -difluoroalkyl side chains..... | 107 |
| Figure C3. 4: X-Ray structure analysis of 82e | 111 |

| | |
|---|-----|
| Figure C3. 5: Molecular modification in the fluorinated nitrones by having a functional group on the terminal position of the side chain | 112 |
| Figure C3. 6: ¹ H NMR spectra of compounds 100 and 103 | 114 |
| Figure C3. 7: ¹ H- ¹ H NOESY NMR experiment of 118e in CDCl ₃ | 117 |
| Figure C3. 8: X-Ray structure analysis of 119a and 119b | 118 |
| | |
| Figure C4. 1: WHO Coronavirus Disease (COVID-19) Dashboard last updated 28/Oct/2020 | 163 |
| Figure C4. 2: Pathways to cell death in <i>C. elegans</i> and mammals. | 165 |
| Figure C4. 3: Cell's morphological changes during apoptotic process..... | 166 |
| Figure C4. 4: Pathways of apoptosis. The intrinsic pathway triggered by different stress signals and the extrinsic pathway triggered via the binding of death receptors. ^{27b} | 167 |
| Figure C4. 5: The mitochondrial apoptosis pathway | 168 |
| Figure C4. 6: The t(14;18) (q32;q21) translocation where the Bcl-2 is juxtaposed to the immunoglobulin heavy chain locus (IgH) in hallmark of follicular lymphoma. | 169 |
| Figure C4. 7: Classification and protein structure of Bcl-2 family members. | 170 |
| Figure C4. 8: Effect of BH3-mimetics to initiate apoptosis..... | 171 |
| Figure C4. 9: Structures of ABT-737 (1), ABT-263 (2), and ABT-199 (3) BCL-2 inhibitors | 172 |
| Figure C4. 10: Mcl-1 inhibitors derived from 2-carboxylic acid indoles | 174 |
| Figure C4. 11: Non-indole acid Mcl-1 inhibitors..... | 176 |
| Figure C4. 12: Structures of already published MIM1 ⁷⁵ and revised structure. | 178 |
| Figure C4. 13: Binding affinities of MIM1 11 and some analogues with Mcl-1..... | 178 |
| Figure C4. 14: Compound 16 docked onto Mcl-1 (left) and Bcl-xL (right). | 179 |
| Figure C4. 15: Molecular docking of the suggested MIM-1 analogues with meta acid in Mcl-1 protein..... | 180 |
| Figure C4. 16: Envisioned α -bromoketones 23a-u | 185 |
| Figure C4. 17: X-Ray crystallographic structure of compound 31r | 196 |
| Figure C4. 18: Caspase 3 and 7 cleavage monitored by the Incucyte S3 after the exposure of IGROV1-R10 to MIM1 analogues 31f , 31o and 31r , combined or not to siBcl-x _L or siMcl-1. | 198 |
| Figure C4. 19: Cells monitored by the xCELLigence after the exposure of IGROV1-R10 to MIM1 analogues 31y , 31ag and 31ah , combined or not to ABT-737 or S63845..... | 199 |
| Figure C4. 20: Essential molecular modifications. | 200 |

| | |
|---|-----|
| Figure C4. 21: Molecular docking of compounds 31o and 31r in Mcl-1 protein..... | 200 |
| Figure C4. 22: Molecular docking of compound 31y inside Mcl-1 protein | 201 |
| Figure C4. 23: Molecular docking of compounds 31ag and 31ah inside Mcl-1 protein..... | 201 |

Table of Tables

| | |
|---|-----|
| Table C1. 1: Atomic parameters of different atoms including Fluorine. | 4 |
| Table C2. 1: Synthesis of alkynes 81a-h with the gem-difluoroalkylated chains..... | 30 |
| Table C2. 2: Synthesis of nitroester adducts 83a-h with the <i>gem</i> -difluoroalkylated chains in position 4..... | 32 |
| Table C2. 3: Synthesis of γ -lactams with the <i>gem</i> -difluoroalkylated chains in position 4 85a-h using NiCl ₂ .6H ₂ O and NaBH ₄ | 33 |
| Table C2. 4: Yields of the introduction of R ¹ substituents in position 3..... | 35 |
| Table C3. 1: Synthesis of nitro intermediates 80a-e and 81a-e | 108 |
| Table C3. 2: Attempts to optimize the reduction reaction of the nitro group in 80a | 109 |
| Table C3. 3: Synthesis of nitrones with <i>gem</i> -difluorinated side chains 82a-e and 83a-e | 110 |
| Table C3. 4: Synthesis of cyclic intermediate 118a-e | 116 |
| Table C4. 1: Synthesis of imino-thiosemicarbazides intermediates 21a-f | 182 |
| Table C4. 2: Synthesis of Intermediate 47a-l | 187 |
| Table C4. 3: Synthesis of α -bromoketones 23j-u | 189 |
| Table C4. 4: Synthesis of the first series of revised MIM1 analogues..... | 190 |
| Table C4. 5: Biological results of the revised-MIM1 analogues | 196 |

Table of Contents

| | |
|---|-----------|
| CHAPTER I..... | 2 |
| Fluorine and Heterocycles, an Efficient Combination in Medicinal Chemistry | 2 |
| 1. Fluorine | 3 |
| 1.1. Structural effect | 4 |
| 1.2. Impact of fluorine on organic compounds..... | 5 |
| 2. Heterocycles | 6 |
| 3. Strategies to Introduce of fluorine atom into organic molecules. | 8 |
| 3.1. Fluorinating agents | 8 |
| 3.1.1. Electrophilic fluorinating agents | 8 |
| 3.1.2. Nucleophilic fluorinating agents | 9 |
| 3.2. Using gem-difluorinated propargylic derivatives as a building block | 11 |
| 4. References | 13 |
| CHAPTER II..... | 15 |
| Synthesis of New Gem- Difluorinated Gamma Lactams and GABA Analogues | 17 |
| 1. Introduction..... | 18 |
| 1.1. γ -lactams | 18 |
| 1.1.1. Biologically active γ -lactams..... | 18 |
| 1.1.2. Methods to access γ -lactams..... | 20 |
| 1.2. GABA as a neurotransmitter | 24 |
| 1.2.1. Clinical applications | 24 |
| 1.3. Fluorinated γ -lactams and GABA analogues | 27 |
| 2. Results and discussion | 28 |
| 2.1. Retrosynthetic analysis | 28 |

| | | |
|-----------|---|------------|
| 2.2. | Synthesis of γ -lactams with <i>gem</i> -difluorinated side chains..... | 29 |
| 2.2.1. | Synthesis of the fluorinated alkynes 81 | 29 |
| 2.2.2. | Synthesis of difluoro nitroester adducts 83 | 31 |
| 2.2.3. | Synthesis of γ -lactams 84 | 32 |
| 2.2.4. | Synthesis of γ -lactams substituted in position (3) 87a-d..... | 34 |
| 2.2.5. | Synthesis of γ -lactams substituted in position 5..... | 36 |
| 2.3. | Toward the synthesis of GABA analogues with <i>gem</i> -difluorinated side chains 38 | |
| 3. | Conclusion and perspectives | 39 |
| 4. | Experimental Part..... | 41 |
| 4.1. | General Methods..... | 41 |
| 4.2. | Experimental Procedures and Analytical data..... | 42 |
| 5. | References | 89 |
| | CHAPTER III | 93 |
| | Synthesis of Novel Cyclic Nitrones with <i>gem</i>-Difluoroalkyl Side Chains through Cascade Reactions | 93 |
| 1. | Introduction..... | 94 |
| 1.1. | Nitrones | 94 |
| 1.2. | Synthetic methods towards nitrones | 95 |
| 1.2.1. | Oxidative methods..... | 95 |
| 1.2.2. | Non-oxidative methods..... | 100 |
| 1.3. | Fluorinated nitrones | 104 |
| 2. | Objective | 106 |
| 3. | Results and discussion | 107 |
| 3.1. | Synthesis of type <i>A</i> nitrones | 107 |
| 3.2. | Synthesis of type <i>B</i> nitrones | 111 |
| 3.3. | Synthesis of type <i>C</i> nitrones | 115 |

| | | |
|-----------|---|------------|
| 4. | Conclusion and perspectives | 118 |
| 5. | Experimental part | 119 |
| 5.1. | General Methods..... | 119 |
| 5.2. | Experimental Procedures and Analytical data..... | 119 |
| 6. | References | 156 |
| | CHAPTER IV | 162 |
| | Synthesis of New Inhibitors of Anti-Apoptotic Proteins to be Developed as Anti-Cancer Agents | 162 |
| 1. | Introduction..... | 163 |
| 1.1. | Apoptosis | 164 |
| 1.1.1. | The extrinsic pathway, the death receptors pathway | 167 |
| 1.1.2. | The intrinsic pathway; the mitochondrial pathway | 168 |
| 1.2. | The Bcl-2 family..... | 169 |
| 1.2.1. | Bcl-2 Inhibitors..... | 172 |
| 1.2.2. | Mcl-1 protein inhibitors..... | 173 |
| 2. | Objective | 179 |
| 3. | Results and discussion | 180 |
| 3.1. | Synthesis of the revised-MIM1 analogue series..... | 181 |
| 3.1.1. | Synthesis of α -bromoketones 23 | 184 |
| 3.2. | Biological studies | 196 |
| 3.3. | Structure-activity relationship using molecular modeling studies | 199 |
| 4. | Conclusion and perspectives | 202 |
| 5. | Experimental part..... | 203 |
| 5.1. | General Methods..... | 203 |
| 5.2. | Experimental Procedures and Analytical data..... | 203 |
| 6. | References | 240 |

PART I
Fluorine Chemistry

CHAPTER I
**Fluorine and Heterocycles, an Efficient
Combination in Medicinal Chemistry**

1. Fluorine

In recent years, great interest has been shown in the selective fluorination of organic molecules. However, knowledge of the existence of fluorinated compounds is much older. Indeed, it was in 1886 that F₂ element was isolated for the first time by Henri Moissan.¹ This discovery earned him the Nobel Prize in Chemistry in 1906.²

Fluorine has been long considered as an abiotic element limited to military and special materials needs. Its production developed in parallel with the increased necessity to produce fissile U-235, as, uranium hexafluoride (UF₆) was found to possess physicochemical properties to separate uranium isotopes by centrifugation.³ The therapeutic properties of fluorinated organic molecules began to be explored in the early 1954, with the discovery of 9 α -fluoro derivatives of cortisone “fludrocortisone” **1** that demonstrated a remarkable glucocorticoid activity⁴ - which exceeded by a factor of 10.7 that of the parent hormones - and by the subsequent discovery of the antitumor properties of 5-fluorouracil **2** by Heidelberger et al. in 1957 (Figure C1. 1).⁵ These two drugs constituted the fundamental paradigm change in the view of fluorine’s place in biology-related research.

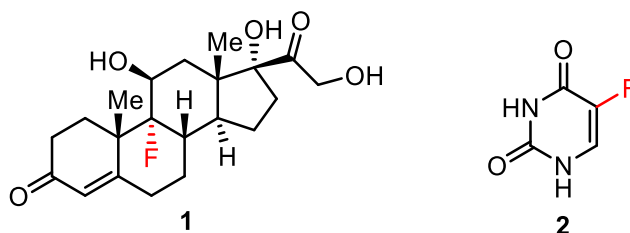


Figure C1. 1: Structure of fludrocortisone **1** and 5-fluorouracil **2**.

Since the early 1980s, fluorine has become a key component not only in medicinal chemistry, but also in agrochemistry. Fast progress in these areas is fuelled by the development of new fluorinating reagents and fluorination processes increasing the range of synthetic fluorinated building blocks amenable to functional group manipulation. The following figure depicts the growth in the number of commercial bioactive fluorinated compounds over the past decades (Figure C1. 2,a). The total number of fluoro-pharmaceuticals (191 drugs) accounted for 18% of the total pharmaceuticals (1072 drugs) and 22% of small-molecule drugs (839 drugs). Whereas the percentages of fluoro-pharmaceuticals among the total number of registered synthetic drugs are extraordinary: 34% (2015), 23% (2016), 25% (2017), 51% (2018), and 43% (2019) (Figure C1. 2,b). The data depicted in the graph strongly suggest a promising future for fluoro-pharmaceuticals.⁶

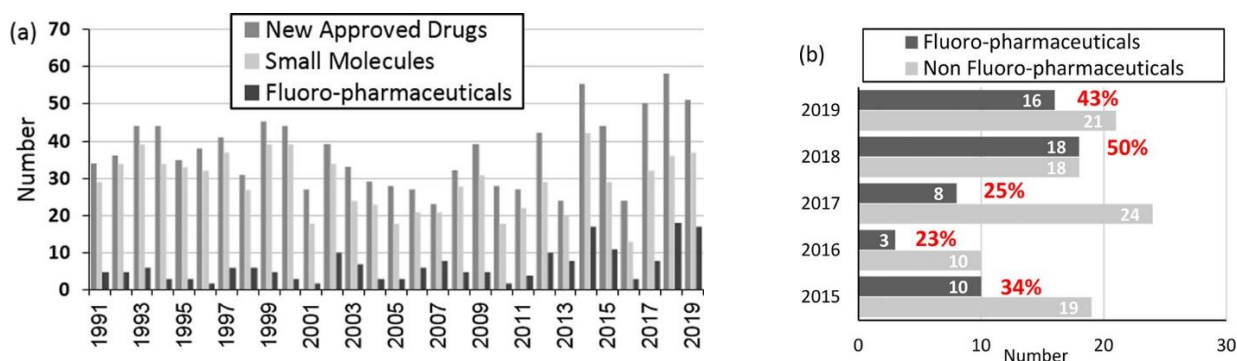


Figure C1. 2 (a) Prevalence of fluoro-pharmaceuticals among globally registered drugs (1991–2019). (b) Data for small-molecule drugs over the past five years.

It seems to be a bit puzzling the presence of the countless number of man-made fluorinated molecules, even though fluorine is rarely found in natural compounds, taking into consideration that it is the 13th most common element on Earth’s crust and significantly more abundant than other halogens. In fact, the number of natural fluorinated compounds is so low that any new discovery on this subject is of great interest.⁷ Actually, the answer can be easily found considering the chemical properties of fluorine and its impact on organic compounds by modifying their physical, chemical and biological properties.

1.1. Structural effect

The fluorine atom has extraordinary characteristics as shown in Table C1. 1.⁸

Table C1. 1: Atomic parameters of different atoms including Fluorine.

| Atom | Ionization Energy (Kcal.mol ⁻¹) | Electronic Affinity (Kcal.mol ⁻¹) | Atomic Polarizability (Å ³) | Van der Waals Radius (Å) | Pauling Electronic Negativity χ_p |
|----------|---|---|---|--------------------------|--|
| H | 313.6 | 17.7 | 0.667 | 1.20 | 2.20 |
| F | 401.8 | 79.5 | 0.557 | 1.47 | 3.98 |
| Cl | 299.0 | 83.3 | 2.18 | 1.75 | 3.16 |
| Br | 272.4 | 72.6 | 3.05 | 1.85 | 2.96 |
| C | 240.5 | 29.0 | 1.76 | 1.70 | 2.55 |
| N | 335.1 | -6.2 | 1.10 | 1.55 | 3.04 |
| O | 314.0 | 33.8 | 0.82 | 1.52 | 3.44 |

Fluorine, the first halogen, is the smallest element in the periodic table after hydrogen. It possess a Van Der Waals radius (1.47 Å) close to that of oxygen and nitrogen, besides being the most electronegative element according to the Pauling scale.⁹ Consequently, a CF group

occupies smaller volume than a methyl or hydroxyl group, similar to that of a CH. From the steric point of view, it is therefore possible to replace a hydrogen atom with a fluorine atom in a biologically active molecule with minimal change in steric hindrance.

Fluorinated compounds have weak intermolecular interactions. This property results from the remarkably high ionization potential and the low polarizability of the fluorine atom.

Carbon-fluorine bonds are much more stable than carbon-hydrogen, carbon-oxygen, or carbon-halogen bonds. The strength of these bonds makes organofluorine compounds often more stable, thus increasing their fields of use. This force increases with the number of fluorine atoms bonded to the carbon.

An excellent overlap of the 2s or 2p orbitals of fluorine with those of carbon leads to the high polarization of the carbon-fluorine bond, which is often described as having ionic character. In turn, this makes the C – F bond stronger, a property widely exploited by medicinal chemists in attempts to block the metabolism of drug candidates.¹⁰

As a consequence of its strong electronegativity and three tightly bound nonbonding electron pairs, fluorine atom exhibits poor polarizability, high inductive attracting character, and being an electron donor by mesomerism.¹¹

1.2. Impact of fluorine on organic compounds

The introduction of fluorine in organic molecules strongly modifies their physical, chemical and biological properties. This topic has been covered in many reviews^{8a,10,12}, but herein it will be discussed briefly (Figure C1. 3).

One of the major effects of fluorination is the modulation of the acidity and basicity of the parent compounds due to its high electron negativity and powerful inductive effect. The introduction of fluorine atom increases the acidity of neighboring hydrogen atoms, carboxylic acid groups, alcohols, and imides.¹³ At the opposite, it induces a strong decrease in the basicity of the amines.¹⁴ This leads to the perturbation of pK_a values which can strongly modify the

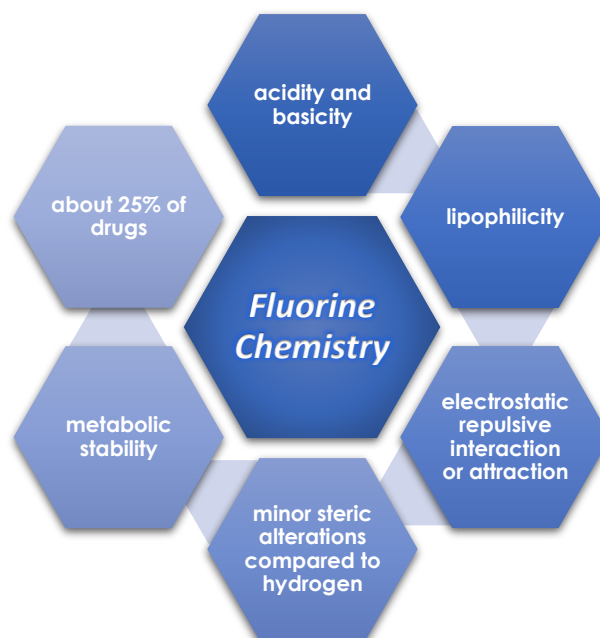


Figure C1. 3: Fluorine fields of modification

binding affinity, the pharmacokinetic properties, and the bioavailability of a pharmaceutical agent.¹⁵ Trifluoromethanesulphonamide was shown to be 160,000 times more potent as inhibitors of carbonic anhydrase and 50,000 times more acidic than methanesulphonamide (Figure C1. 4).¹⁶

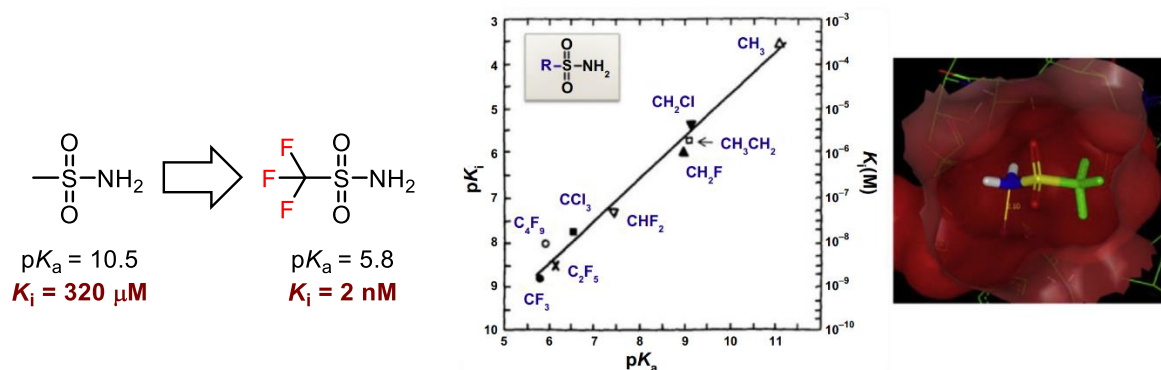


Figure C1. 4: Fluorinated carbonic anhydrase inhibitors showing linear relationship, and much better fitting in the enzyme pocket.

Modulation of lipophilicity can also be effectively done with introduction of fluorine, thus enhancing membrane penetration of drugs and facilitating crossing of molecules into the blood – brain barrier.¹² Moreover, the replacement of hydrogen for fluorine results in minor steric alterations, and the electrostatic repulsive interaction or attraction of fluorine with other functional groups in a molecule leads to significant conformational changes.¹⁷

Another area where fluorine has found considerable use is in attempts to block oxidative metabolism at both aromatic and aliphatic sites.¹⁸

It is noticeably clear that fluorine atom bestows an impact on organic, medicinal, and industrial chemistry. Hence, it may be predicted that the number of fluorine containing drugs on the market will continue to increase. As our understanding of the effects of fluorine substitution expands, further applications in these fields will emerge.

In parallel to that, it must be noticed that most of the fluorinated drugs approved contain heterocycle moiety.

2. Heterocycles

Heterocyclic compounds form the largest and one of the most important classes of organic compounds, with about 55% of organic chemistry publications covering this field. More than 85% of biologically active compounds contain one or more heterocyclic fragments in their structures, and probably about two-thirds of organic compounds belong to this class and

constitutes the most significant compounds for the human being. It was revealed that the total number of unique drugs containing at least one nitrogen heterocycle rises from 613 to 640 (59%) and a total of at least 311 unique oxygen heterocycle-containing pharmaceuticals (27%).

19

They constitute the main structural unit for several natural compounds such as alkaloids, nucleic acids, polyazoles, etc.... In addition, they play a vital role in the metabolism of all living cells. Genetic material DNA is also composed of heterocyclic bases-pyrimidines and purines. Moreover, many heterocyclic compounds, both synthetic and natural, are pharmacologically active and are in clinical use (Figure C1. 5).

Heterocycles are very common in drugs, since they possess hydrogen bond donors and/or acceptors in a rigid framework, and they can therefore effectively interact with target enzymes and receptors in particular via hydrogen bonding formation. Thus, they can enhance binding affinity and improve *in vitro* potency. Heterocycles can modulate lipophilicity of the drug molecules, thus providing desired pharmacokinetic properties and pharmaceutical properties.²⁰

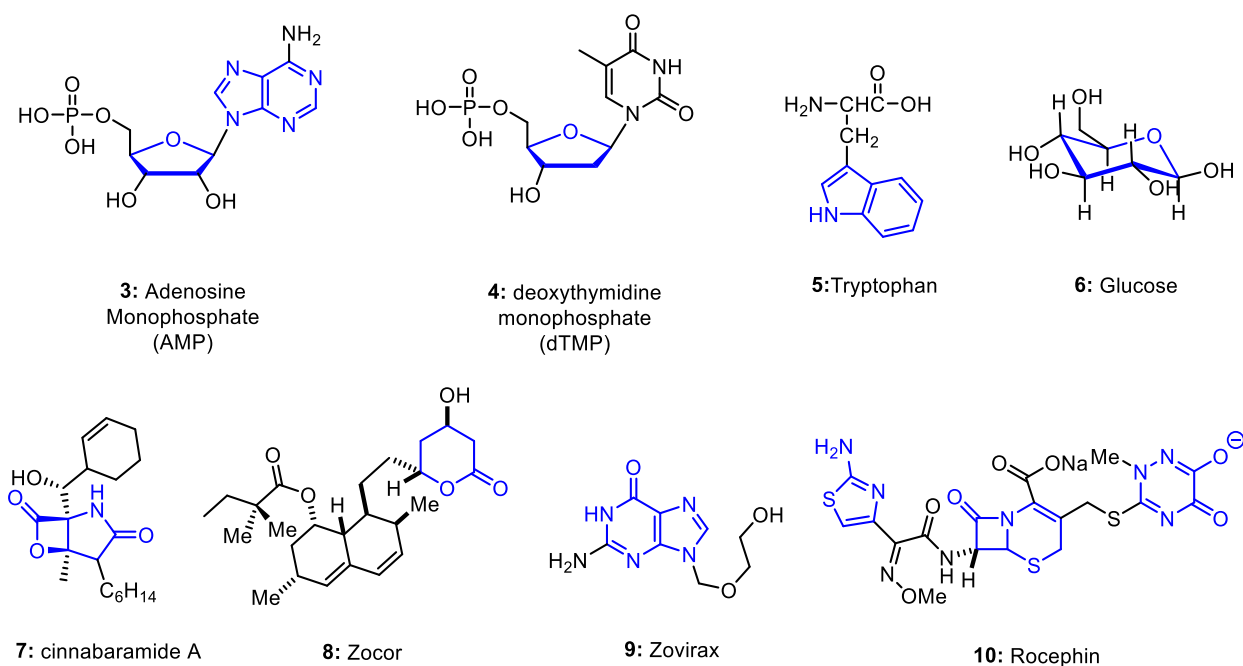


Figure C1. 5: Examples of biomolecules, synthetic and natural drugs containing heterocycles

The predomination of heterocycles in the medicinal field suggests that the fluoro-functionalized heterocyclic compounds should be promising candidates in drug discovery.²¹ Upon checking the fluoro-pharmaceuticals approved since 1954, it was revealed that at least 77% of these drugs contains at least one heterocycle (264 drugs out of 340).⁶ This ensures the golden combination between a fluorine atom and a heterocycle in a given pharmaceutical (Figure C1. 6).

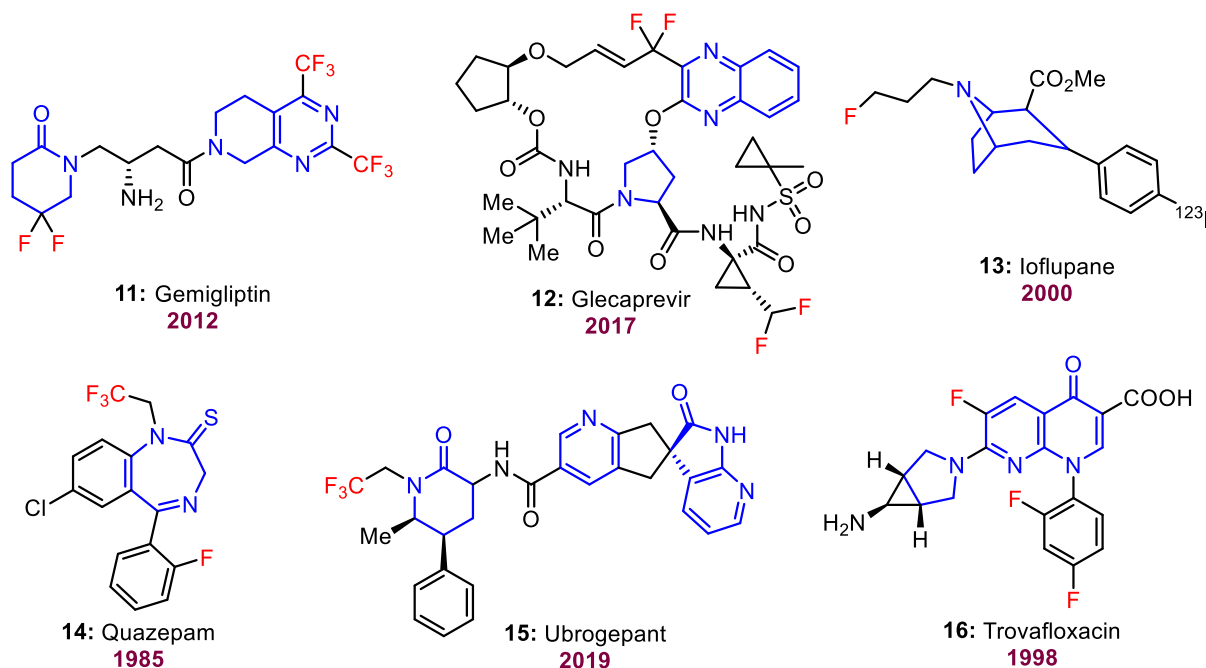


Figure C1. 6: Some fluoro-functionalized heterocyclic compounds as drugs

3. Strategies to Introduce of fluorine atom into organic molecules.

The ability to introduce fluorine atoms into a molecule regio-selectively is of great interest when designing routes to biologically active compounds. The developed methodologies generally rely on the use of fluorinating agents or on the use of readily available fluorinated building-blocks as a fluorine source.

3.1. Fluorinating agents

Fluorinating agents are useful tools to introduce fluorine atoms onto organic molecules. Therefore, several reagents have been developed for this purpose, and are classified in two main categories: nucleophilic and electrophilic depending on the generated reaction active species.

3.1.1. Electrophilic fluorinating agents

Electrophilic fluorinating agents are those where the electron-deficient fluorine atom serves as a reaction active species. Initially, the source of electrophilic fluorine has been fluorine gas, however, in the past decades, NF fluorinating agents have emerged as generally safer, and easier to handle alternatives for electrophilic fluorination. These are either neutral compounds or quaternary ammonium salts. Some representative examples are *N*-fluoro-*o*-benzenedisulfonimide (NFOBS) **17**,²² Selectfluor **18**,²³ and *N*-fluorobenzenesulfonimide (NFSI) **19**.²⁴ A nucleophilic carbon center (electron-rich alkene such as vinyl ether, enolate...)

will react with an electrophilic (N-F) source of fluorine to afford organofluorine compounds (Figure C1. 7).²⁴ Reactions may occur through S_N2 displacement with nucleophilic attack at the fluorine atom, or it may proceed by single-electron transfer pathway.²⁵

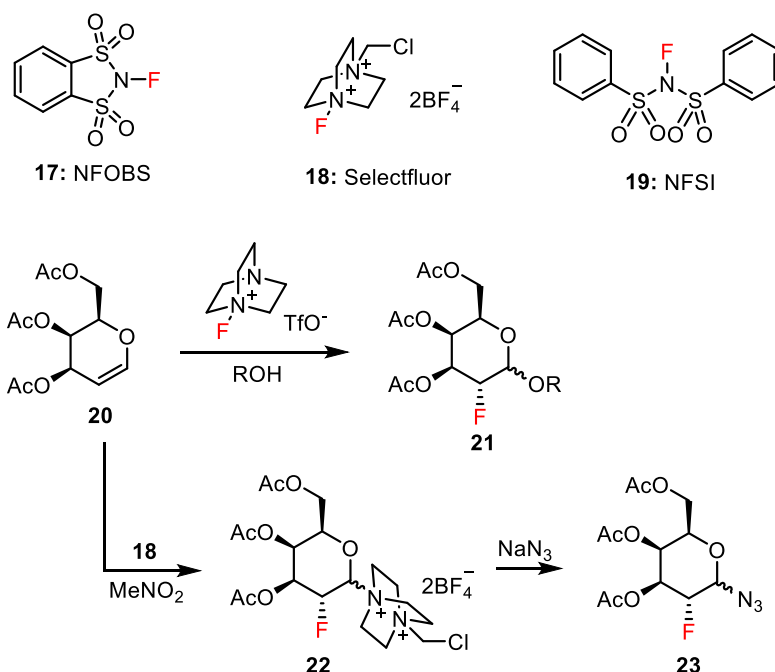


Figure C1. 7: Example of Fluorination using electrophilic fluorinating agents.

3.1.2. Nucleophilic fluorinating agents

Nucleophilic fluorinating reagents allow for the generation of fluoride anion that will serve as a reaction active species. HF is the principal precursor of almost all fluorine-containing compounds. However, its handling causes safety concerns due to its corrosiveness and toxicity. Fluorodeoxygenation at carbon centers commonly requires specialized fluorinating reagents that can accomplish oxygen activation/deoxygenation as well as provide a fluoride source. The first successful reagent was sulfur tetrafluoride.²⁶ However due to its toxicity and volatility new nucleophilic agent was synthesized, such as diethylaminosulfur trifluoride (DAST) **24**,²⁷ which has been used most commonly to fluorinate oxygenated (carbonyl, hydroxyl) or sulfur-containing (thiocarbonyl, sulfide) substrates in laboratories. However, it also has severe drawbacks and must be used with cautions:

- it reacts violently with water
- it is volatile
- it is unstable and begins to decompose into SF₄ and (Et₂N)₂SF₂ from 90 °C, which makes its use on an industrial scale difficult.²⁸
- This is one of the best alcohol fluorination reagents, but dehydration to alkenes is observed for instance with cycloalkanols as a side-reaction.

Beside another fluorinating agents were synthesized such Deoxo-Fluor **25**,²⁹ which is considered safer and a more thermally stable alternative, but is still moisture sensitive and prone to decomposition. This was followed by the development of new nucleophilic agents such as 4-tert-butyl-2,6-dimethylphenylsulfur trifluoride (Fluolead) **26**,³⁰ Yarovenko's reagent **27**,³¹ Ishikawa's reagent **28**,³² TFEDMA **29**,³³ diethylaminodifluorosulfonium tetrafluoroborate (XtalFluor-E) **30**,³⁴ Difluoro(morpholino)sulfonium tetrafluoroborate (XtalFluor-M) **31**,³⁵ *N,N*-Diethyl- α,α -difluoro-3-methylbenzylamine (DFMBA) **32** (Figure C1. 8).³⁶

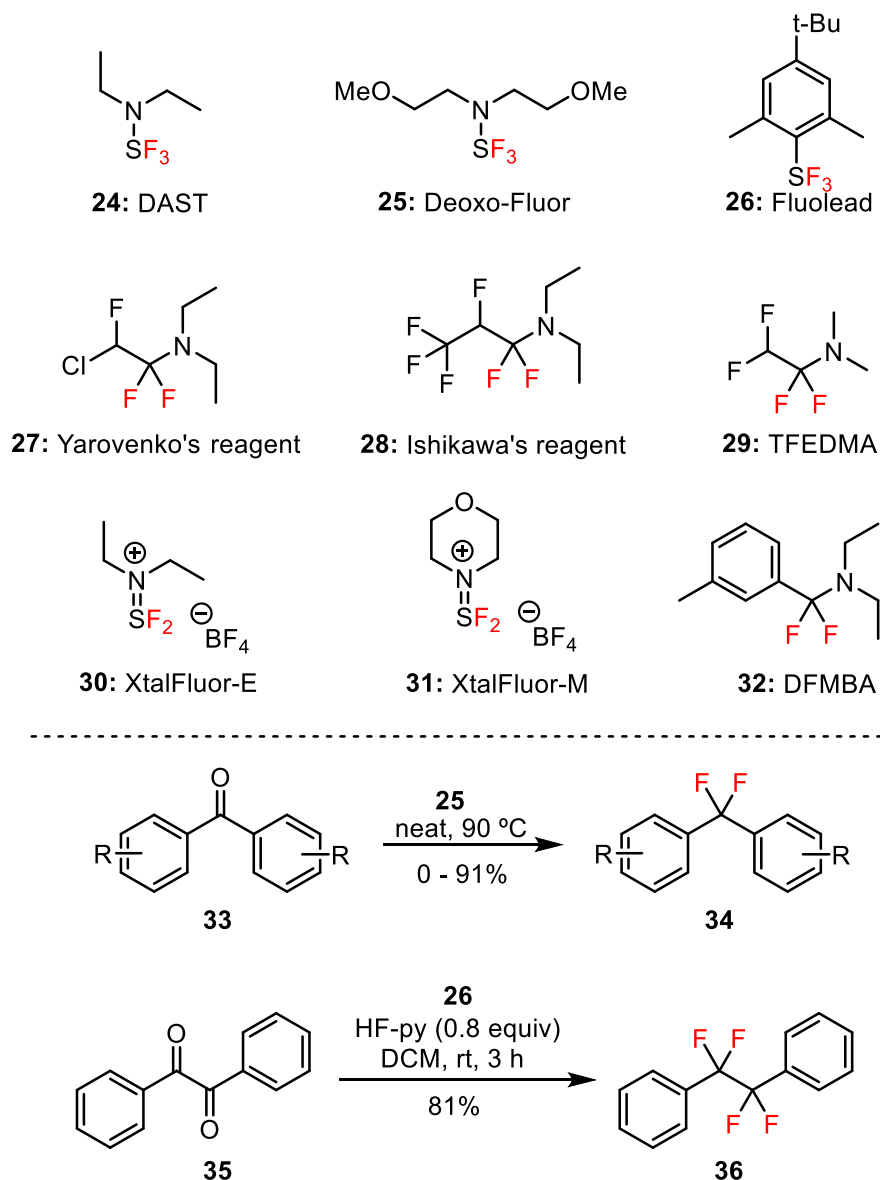


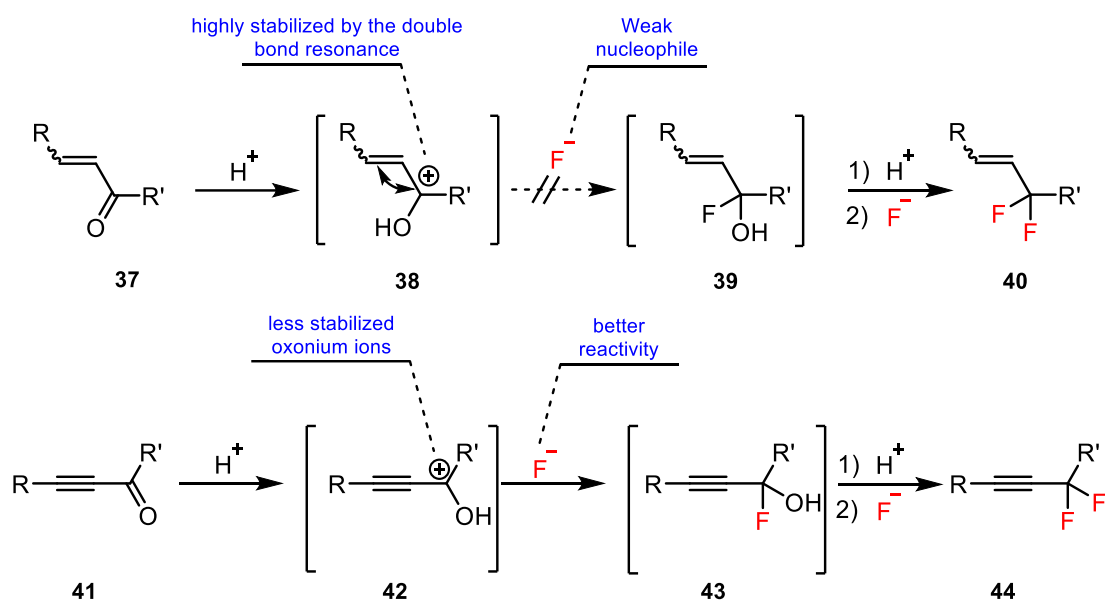
Figure C1. 8: A selection of deoxofluorinating reagents and some applications.

In general, the preparation of fluoro-functionalized heterocyclic compounds falls broadly into two classes. The first involves late stage introduction of fluorine atom, where some problems may occur due to selectivity issues during the fluorination, while the second strategy involves the use of fluorinated building blocks. This strategy played a key role during the

cooperation between the labs in Rennes and Beirut focusing on the preparation and uses of fluorinated propargylic systems as building blocks in synthesis of fluorinated molecules.³⁷

3.2. Using gem-difluorinated propargylic derivatives as a building block

In a previous work done by R. Grée et al., several propargylic derivatives were subjected to monofluorination, using propargylic alcohols and allylic alcohols, and difluorination, using propargylic ketones in presence of DAST or its alternative Deoxo-Fluor™.³⁸ It is worthy to note that, in earlier literature work and using standard molecules, the gem-difluorination of enones occurred only in poor yields except when ultra-high-pressure reaction conditions were used.³⁹ On the contrary, the fluorination of propargylic alcohols or ynones occurs smoothly with moderate to excellent yields.



Scheme C1. 1: Fluorination reaction of propargyl and enone derivatives using Deoxo-Fluor™ or DAST.

As anticipated, the difference in reactivity depends on the stabilities of the carbonium ion intermediates. In such a way, the oxonium ions **38** is highly stabilized by the double bond affording, at best, low yields of the desired difluoro allylic compounds due to the exceptionally low reactivity with the poor nucleophile F^- . Whereas, much higher reactivity was obtained with the less stabilized oxonium ions **42** (Scheme C1. 1).³⁸

Due to this aspect, our laboratories adopted the gem-difluorinated propargylic derivatives as building blocks to access different fluorinated heterocycles. In fact these intermediates have enabled the development of original methods for preparing pyrazolines **45** and pyrrolines **46**,⁴⁰ β -lactams **47** and exoalkylidene β -lactams **48**,⁴¹ chromanones **49**,⁴² tetrahydrofurans,

tetrahydropyrans and their thio-analogues, as well as to pyrrolidines and piperidines **50**,⁴³ benzofurans **51**,⁴⁴ and imidazopyridazines **52**⁴⁵, with fluorine in different positions (Figure C1.9).

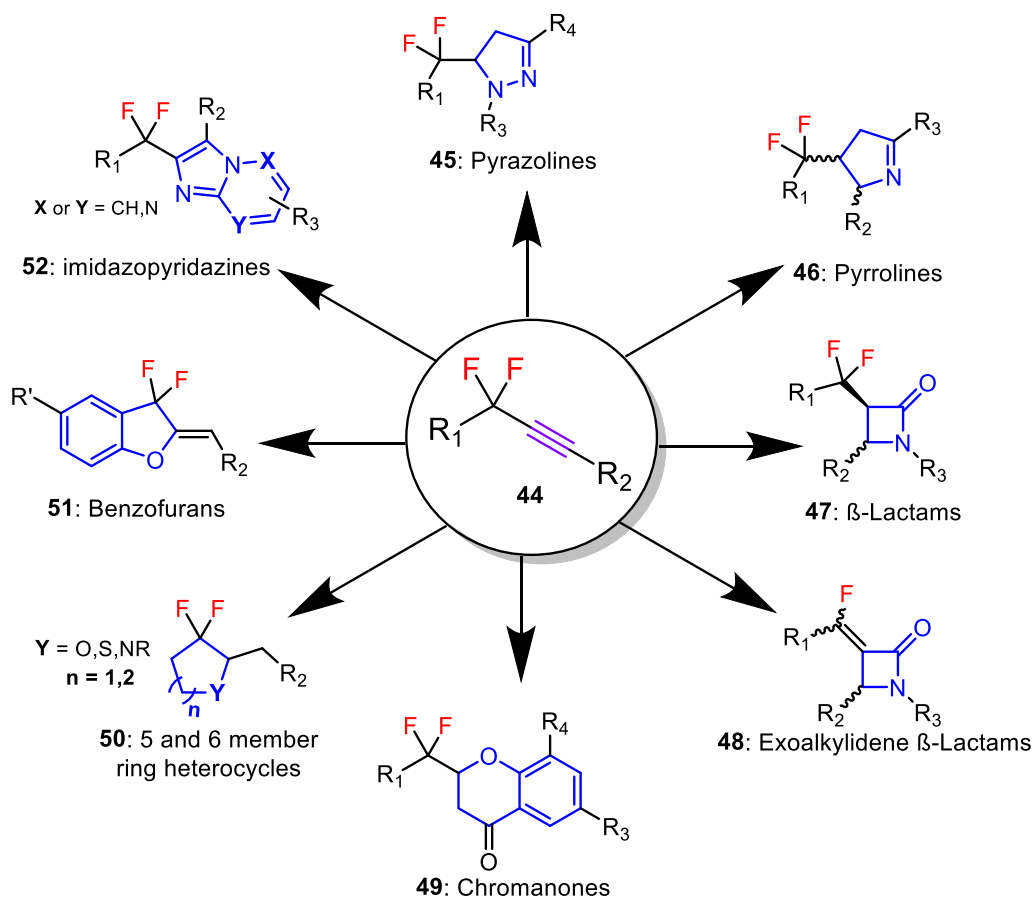


Figure C1. 9: Fluorinated heterocycles derived from gem-defluorinated propargylic intermediate.

These emboldening results due to the use of *gem*-difluorinated propargylic intermediates, have opened the gate toward investigating new fluorinated heterocycles, especially fluorinated nitrones and γ -lactams. The latter has been used as a precursor to study the formation of gamma amino butyric acid (GABA).

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CHAPTER II

Synthesis of New Gem- Difluorinated Gamma Lactams and GABA Analogues

1. Introduction

Oxygen and nitrogen containing-heterocycles are present in various types of molecules with multiple and varied biological activities.¹ The γ -lactams constitute an important classes of heterocycles, as it is the essential unit for various bioactive and synthetic compounds. Another complementary and interesting aspect is that they are considered to be precursors of GABA analogues.

1.1. γ -lactams

The γ -lactam ring, also known as γ -butyrolactam, pyrrolidin-2-one, azolidin-2-one or 2-oxopyrrolidine, is an important structural skeleton of bioactive natural and synthetic products covering a broad range of biological activity.²

The interest in this scaffold started with the increased bacterial resistance toward β -lactams antibiotics. To prevail over this bacterial resistance, it was important to replace the β -lactam core structure with the γ -lactam and trying to preserve the activated amide bond which is important for the anti-bacterial activity. So, in 1986, bicyclic γ -lactams “penem derivatives” were independently reported as antibiotics to mimic the activity of penicillin by Baldwin et al. “compound **3A**”³ and researchers from Eli Lilly “compound **3B**”⁴ (Figure C2. 1)

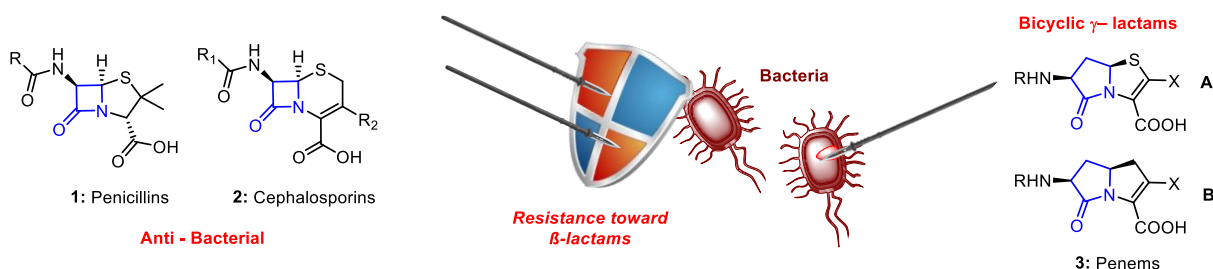


Figure C2. 1: γ -lactam analogues as a new alternative to overcome bacterial resistance.

Compounds **3A** and **3B** did not access the market as anti-bacterial drugs due to their low, albeit detectable, levels of anti-bacterial activity. Yet they had the greatest impact to inspire many chemists to take the lead and design new biologically active compounds possessing a γ -lactam ring and develop many synthetic strategies to access this structural moiety.⁵

1.1.1. Biologically active γ -lactams

A large number of pharmaceutically active γ -lactams have been reported, including antibiotics, anti-inflammatory, cytotoxic and antitumor compounds. Several natural compounds have also been found to possess this structural moiety (Figure C2. 2).⁶ Avastin® or (*R*)-Rolipram **6** was the 8th best-selling drug in the world in 2018⁷ and the 6th in 2019.⁸

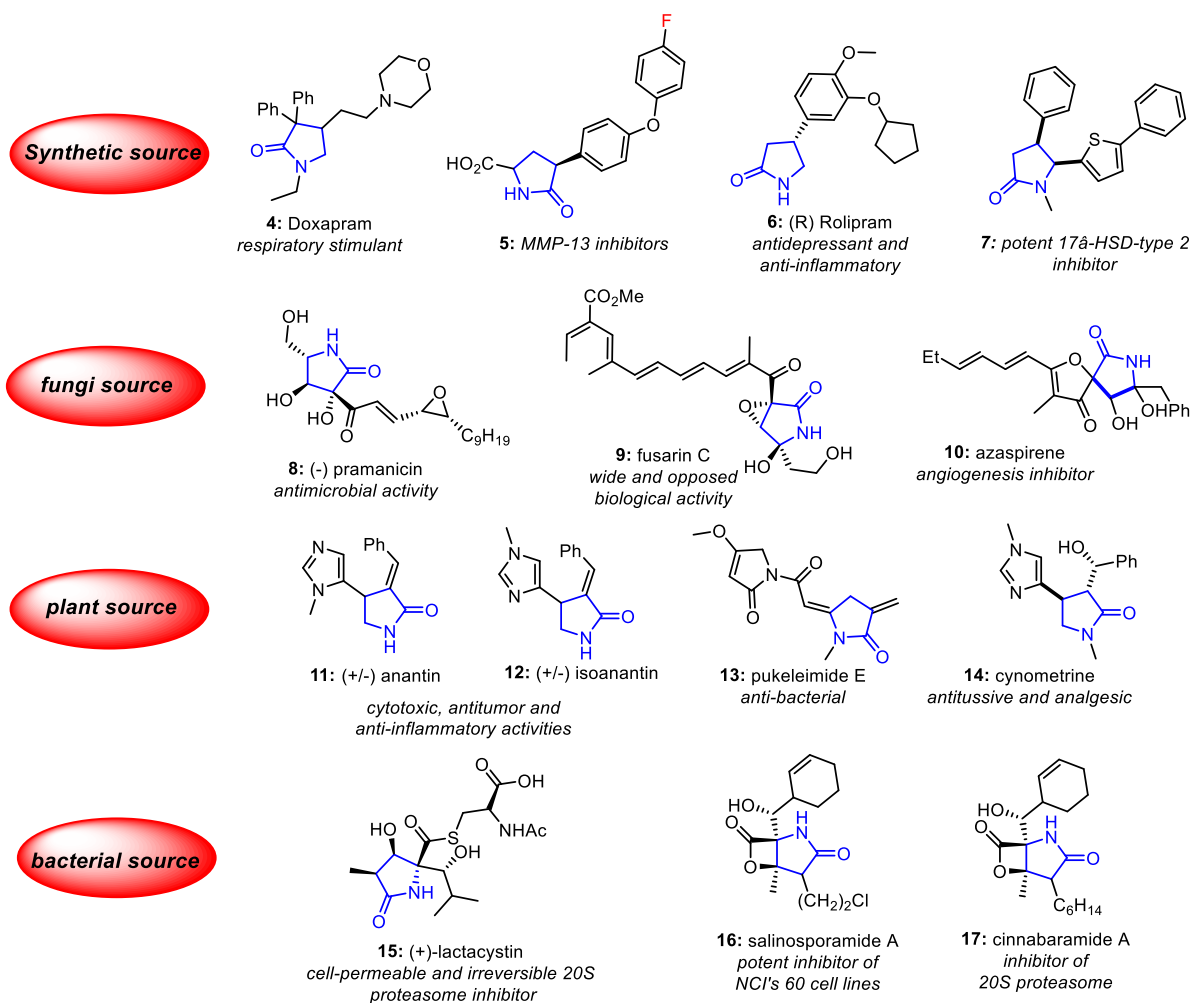
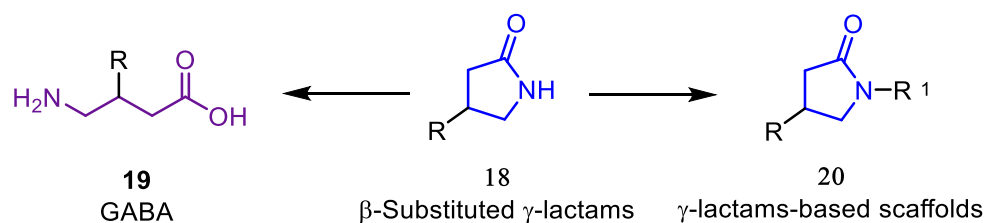


Figure C2. 2: Pharmaceutically active γ -lactams from different sources

γ -Lactams thus represent an important class of compounds both from a synthetic and biological point of view and one can expect an increase in the number of drugs on the market containing a γ -lactam core in the next few years. Another complementary and interesting aspect is that they are considered to be precursors of GABA (Gamma-AminoButyric Acid) analogues (Scheme C2. 1).



Scheme C2. 1: Potential use of β -substituted γ -butyrolactams

1.1.2. Methods to access γ -lactams

Different synthetic methods have been developed to produce this type of heterocycle as shown in Figure C2. 3.

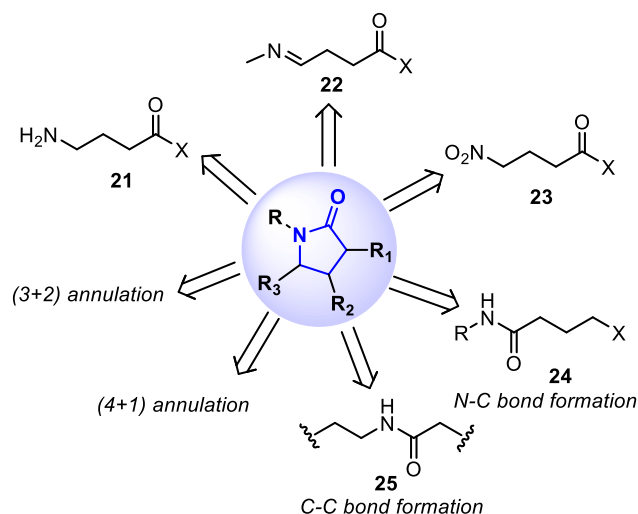
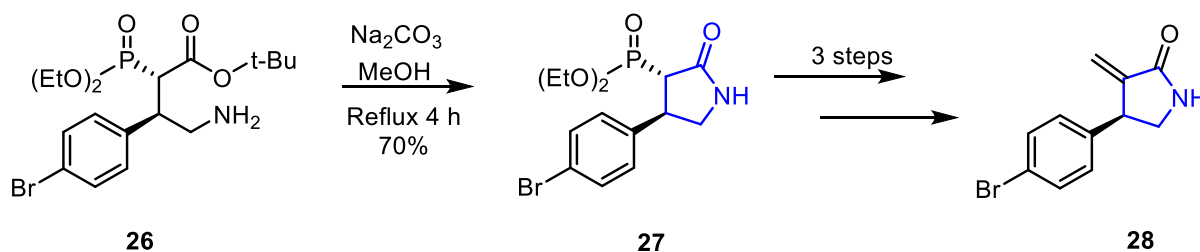


Figure C2. 3: Overview of synthetic strategies to build the γ -lactam core (X = leaving group).

1.1.2.a Amide bond formation from amino acid derivatives

Intramolecular cyclization is due to the attack of the nucleophilic amine functional group to the electrophilic acylhalide, ester, or activated carboxylic acid group. This strategy was employed for instance in the synthesis of compound **28**. In 2008, an intramolecular condensation between the *tert*-butylic ester and the amine group of compound **26** was performed in refluxing methanol and in the presence of sodium carbonate to afford compound **27** in 70% yield. α -Methylene- γ -lactam **28** - a carboplatin analogue- was obtained after three more steps (Scheme C2. 2).⁹

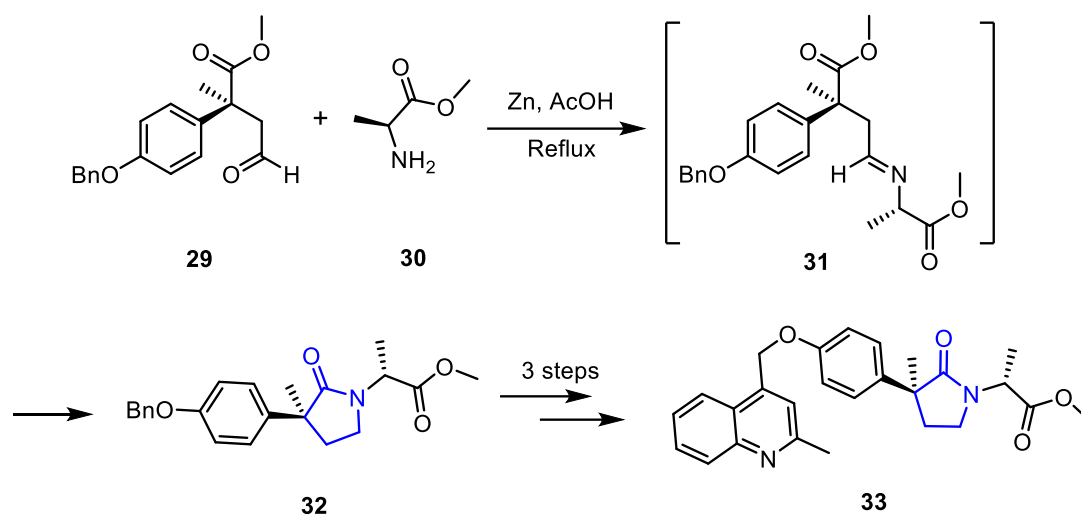


Scheme C2. 2: Synthesis of α -methylene- γ -lactams **28**

1.1.2.b Amide bond formation from an imine

The formation of cyclic amide in this synthetic route is mediated by a reductive amination followed by a cyclization reaction. Where the treatment of **29** with *D*-alanine methyl ester **30** and Zn dust in HOAc at reflux facilitated the formation of imine intermediate **31** and afforded

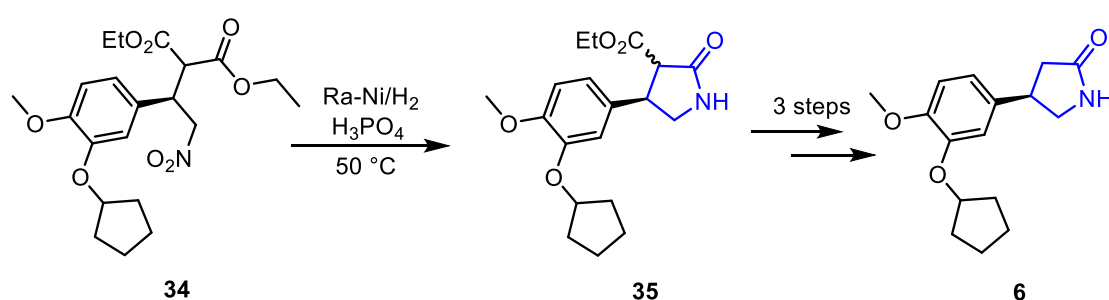
32 after spontaneous cyclization. This strategy was applied to the synthesis of functionalized γ -lactam **33** which resulted in potent and selective TACE inhibitors, exemplified by IK682 (Scheme C2. 3).¹⁰



Scheme C2. 3: Synthesis of Lactam Analogue IK682 **33**

1.1.2.c Amide bond formation from a nitro group

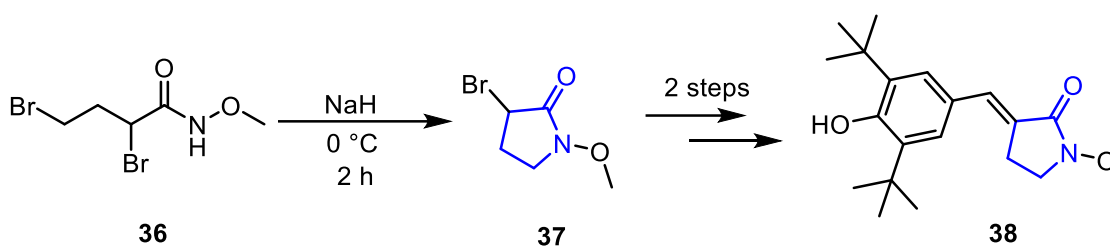
Usually in this type of reactions a reduction step of the nitro group is mandatory to obtain the amine group followed by an intramolecular cyclization of the latter to the carboxylic derivative to obtain the lactam. It was employed during the synthesis of (*R*)-rolipram. The γ -nitroester **34** was reduced and *in situ* cyclized in the presence of Raney® nickel and hydrogen gas to afford γ -lactam **35**. Few more steps permit them to obtain the desired product **6** (Scheme C2. 4).¹¹



Scheme C2. 4: Synthesis of (*R*)-rolipram **6**

1.1.2.d Intramolecular *N*-alkylation of an amide

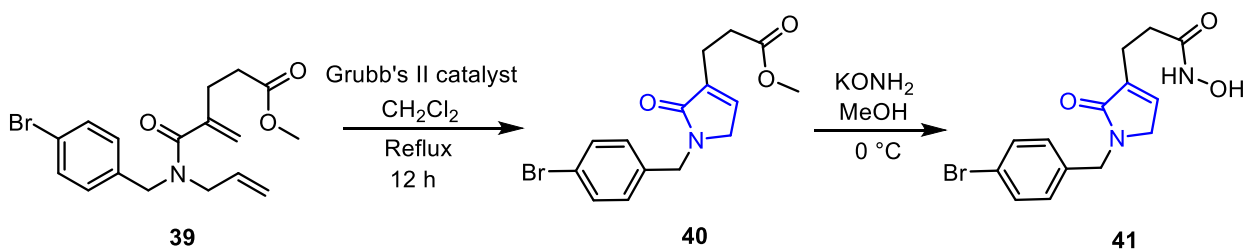
An efficient synthetic route to afford the *N*-methoxy substituted γ -lactams **37** by an intramolecular cyclization of γ -bromo-amide **36** in the presence of sodium hydride as a strong base. This strategy was used to synthesize the compound **38** which showed potent anti-inflammatory activity (Scheme C2. 5).¹²



Scheme C2. 5: Synthesis of Pyrrolidin-2-one derivatives **38**.

1.1.2.e Intramolecular *N*-alkylation of an amide via C-C bond formation

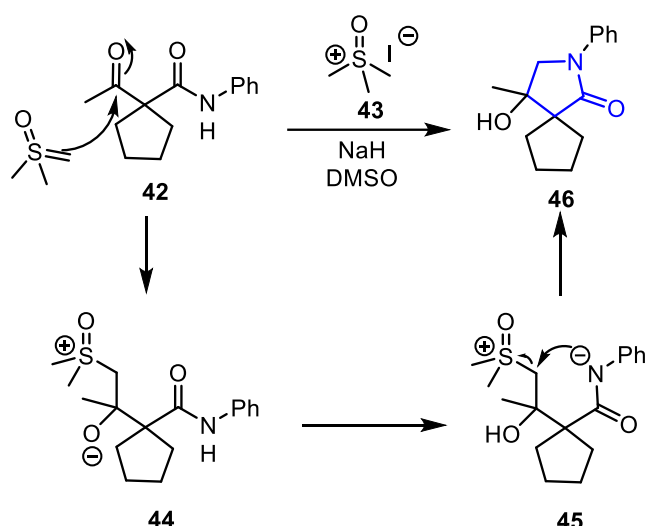
Such approach is one of the most used and efficient pathways since the γ -lactam core structure could be accessed through several types of reactions such as Dieckman condensation of β -keto amide ester,¹³ radical reaction,¹⁴ transition metal-catalyzed reactions,^{5d} intramolecular Michael addition,¹⁵ and ring closing metathesis.¹⁶ Metathesis was used also to synthesize a histone deacetylase inhibitor (HDAC) **41** starting from compound **39** (Scheme C2. 6).¹⁷



Scheme C2. 6: Synthesis of histone deacetylase inhibitor (HDAC) **41**

1.1.2.f (4+1) Annulation

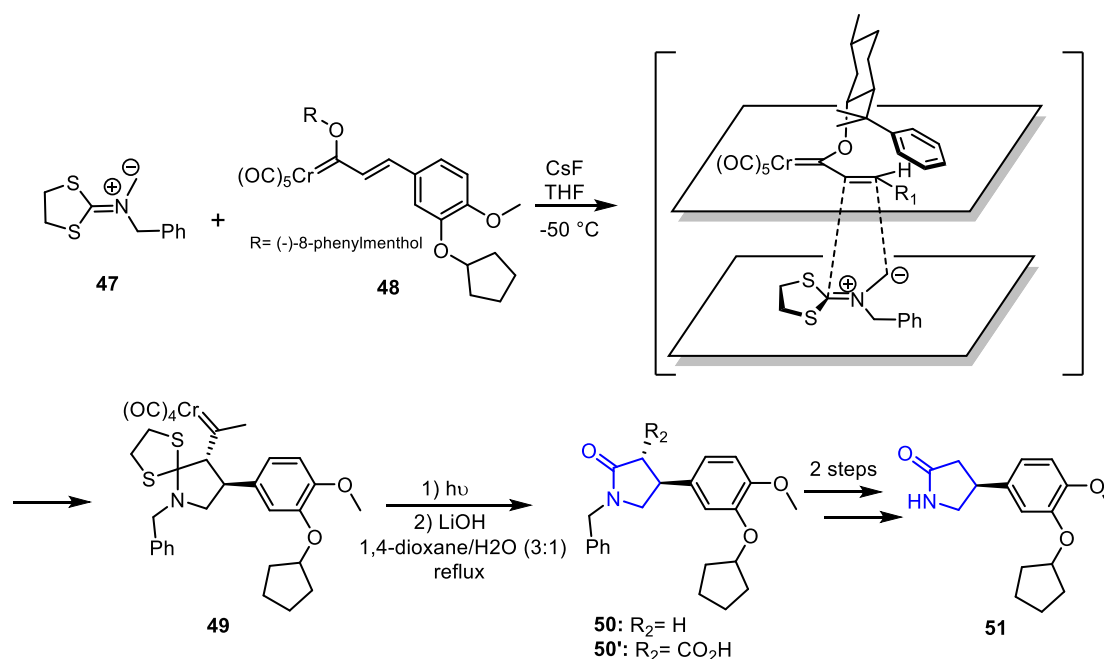
The attack of a sulfur ylide, generated *in situ* from trimethylsulfoxonium iodide **43** in the presence of sodium hydride, on the acetyl group of **42** gives rise to a betaine intermediate **44**, which is in turn followed by facile proton exchange from the carbamate nitrogen to the basic oxide ion to afford the stable species **45**. The final product, γ -lactam **46**, is formed after the intramolecular lactamization of **45** with the elimination of DMSO (Scheme C2. 7).¹⁸



Scheme C2. 7: Mechanism of the (4+1) annulation to obtain β -hydroxy- γ -lactams

1.1.2.g (3+2) Annulation

The anti-inflammatory and antidepressant drug (+)-Rolipram was readily prepared in four steps using a (3+2) cycloaddition between an azomethine ylide **47** and a menthol derived carbene **48** as a key step. The azomethine approached to the olefin from the bottom face because of an effective blocking of the upper face by the phenyl group of the (-)-8-phenylmenthol chiral auxiliary due to a π -stacking interaction between that phenyl group and the alkene double bond. The cycloadduct intermediate **49** was next photochemically oxidized then hydrolysed in basic medium to afford **50** and **50'** in variable proportions. Two more steps allowed the synthesis of (+)-Rolipram **51** (Scheme C2. 8).¹⁹



Scheme C2. 8: Synthesis of (+)-Rolipram **51**

1.2. GABA as a neurotransmitter

In the mammalian brain, GABA is synthesized primarily from glutamate in a reaction that is catalyzed by glutamic acid decarboxylase (GAD) enzymes.²⁰ GABA is loaded into synaptic vesicles by a vesicular neurotransmitter transporter (VGAT)²¹ and is liberated from nerve terminals by calcium-dependent exocytosis. However, nonvesicular forms of GABA secretion (for example, by reverse transporter action) have also been described and might be particularly important during development.²² The effects of GABA can be mediated by the activation of either ionotropic (GABA_A and GABA_C) or metabotropic (GABA_B) receptors, which can be localized either pre- or postsynaptically. GABA signals are terminated by reuptake of the neurotransmitter into nerve terminals and/or into surrounding glial cells by a class of plasma-membrane GABA transporters (GATs); thereafter, GABA is metabolized by a transamination reaction that is catalyzed by GABA transaminase (GABA-T)²³ (Figure C2. 4).

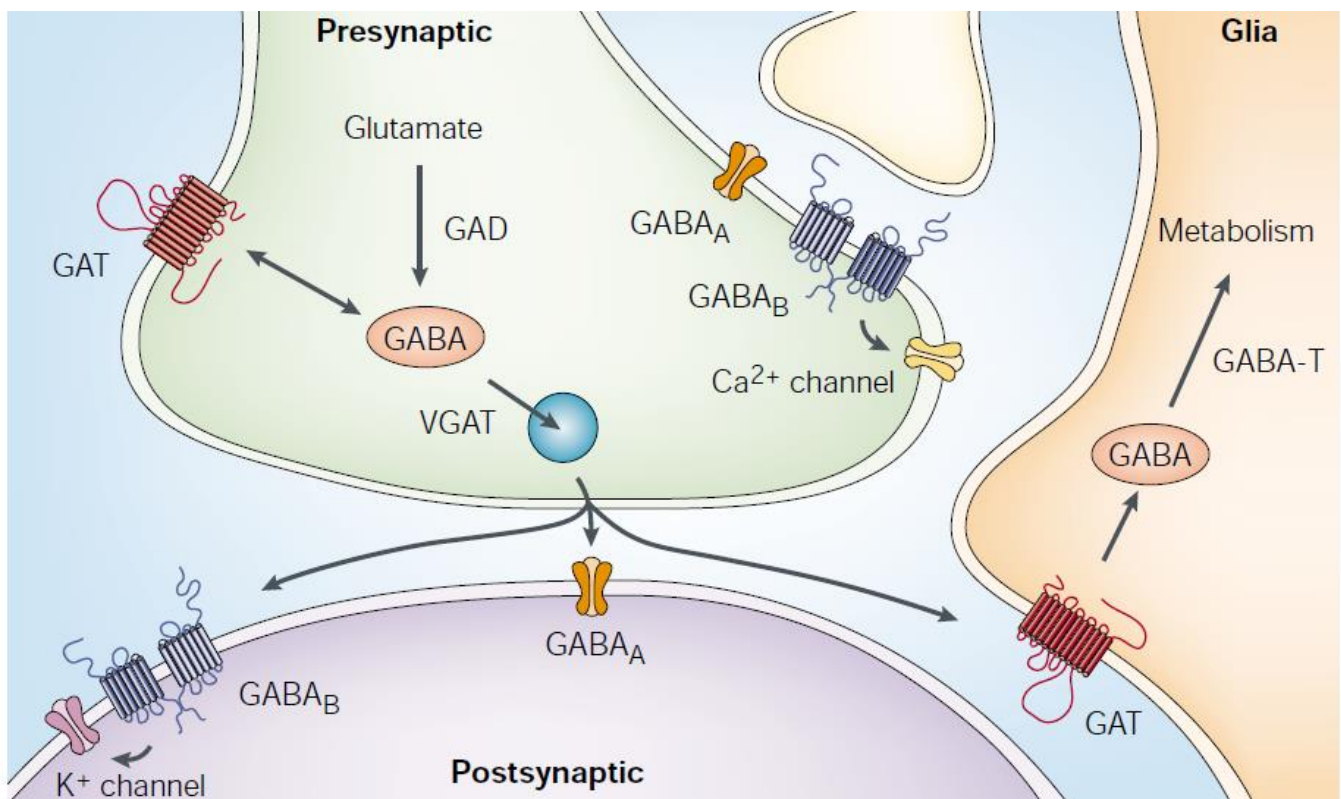


Figure C2. 4: Summary of the GABA neurotransmitter²⁴

1.2.1. Clinical applications

When the concentration of GABA diminishes below a threshold level in the brain, convulsions result²⁵; raising the brain GABA levels terminates the seizure²⁶. A reduction in the concentrations of GABA and of the enzyme GAD has been implicated not only in the

symptoms associated with epilepsy²⁶ but also with several other neurological diseases such as schizophrenia²⁷, Huntington's chorea²⁸, Parkinson's disease²⁹, Alzheimer's disease³⁰, senile dementia³¹, and motion disorders (e.g., multiple sclerosis, action tremors, tardive dyskinesia)³², as well as anxiety³³ and depression³⁴, pain, panic, mania³⁵, alcoholism³⁶ or insomnia.

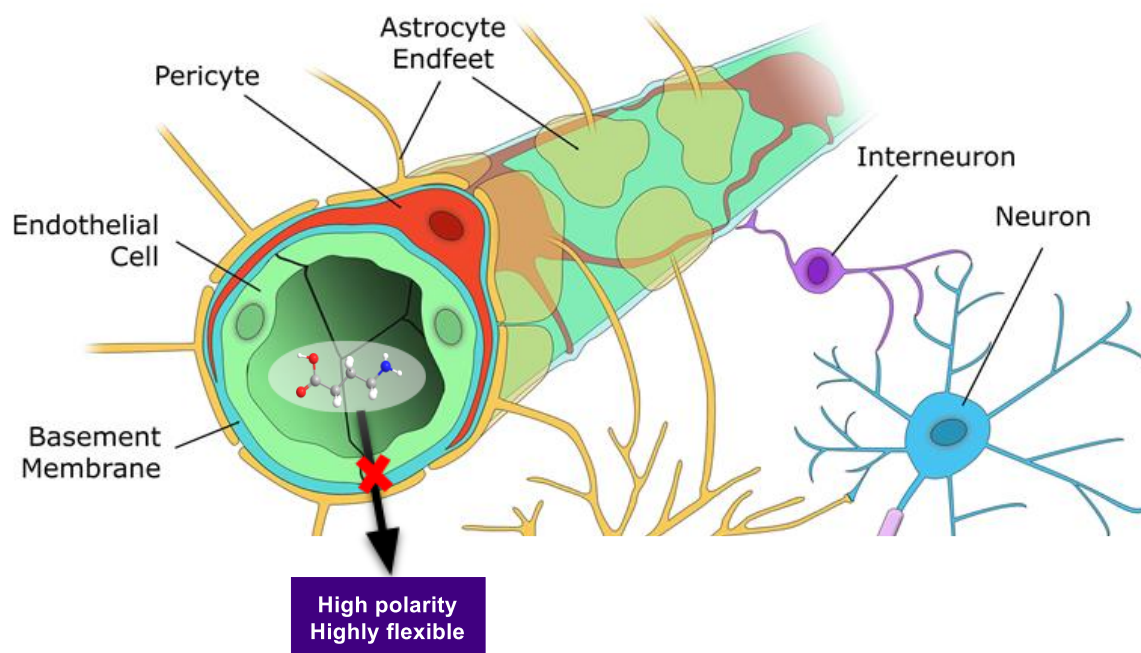


Figure C2. 5: Administrative GABA cannot reach CNS due to the BBB.

However, direct administration of GABA couldn't reach the central nervous system (CNS), due to the presence of blood – brain barrier (BBB), which has a role to isolate and protect nervous tissue of the brain and spinal cord from fluctuations in nutrients, hormones, metabolites, and other blood constituents. Brain endothelial cells (EC) are characterized by the presence of tight junctions (TJs) and lack pinocytotic vacuoles, thus brain ECs fit tightly together and substances cannot pass out readily of the bloodstream³⁷, unless they are lipid – soluble molecules or possess a transportation system³⁸ (Figure C2. 5).

Recently, scientists resort to synthesize new analogues of GABA capable of crossing BBB. Thus the molecule of GABA was modified to increase its lipophilicity and to rigidify its structure. Interestingly those analogues had different mode of actions³⁹.

For example, Tiagabine (TGB) **52** inhibits the re-uptake of GABA from the synaptic cleft by the GABA transporters (GATs), prolonging the action of endogenously released GABA⁴⁰. Vigabatrin (VGB) **53** inhibits the breakdown of endogenous GABA by inhibiting GABA transaminase⁴¹. Gabapentin (GBP) **54** and Pregabalin (PGB) **55** bind to the $\alpha_2\delta_1$ subunit of voltage-dependent Ca^{2+} channels, and may inhibit the release of excitatory neurotransmitters by inhibiting Ca^{2+} current⁴². Interestingly, none of the currently available GABA derived drugs

is an agonist at the GABA-A receptors. The antispastic drug baclofen (Lioresal®) **56** is a GABA-B receptor agonist⁴³, on the other hand (+)-CAMP **57** seems the most selective, as an agonist, for GABA-C receptors⁴⁴ (Figure C2. 6).

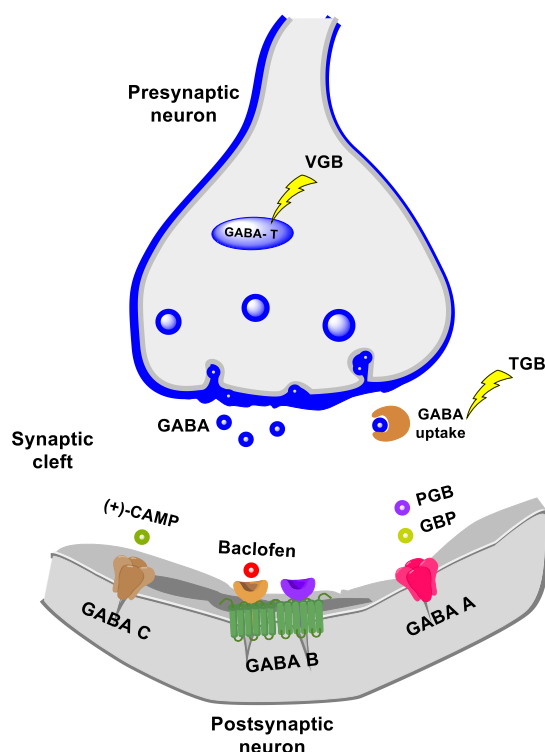


Figure C2. 6: Mode of action of different synthetic GABA analogues in the CNS.

Another new family of GABA analogues are lactam containing molecules such as Levetiracetam (Keppra®) **59**, an anticonvulsant used to treat epilepsy by γ -aminobutyrategic (GABAergic) facilitation⁴⁵ (Figure C2. 7).

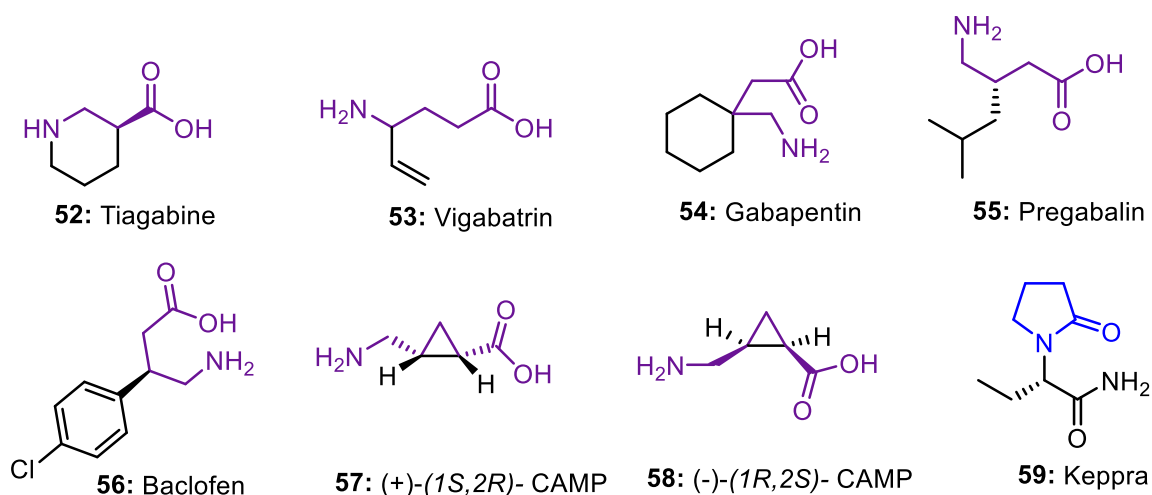
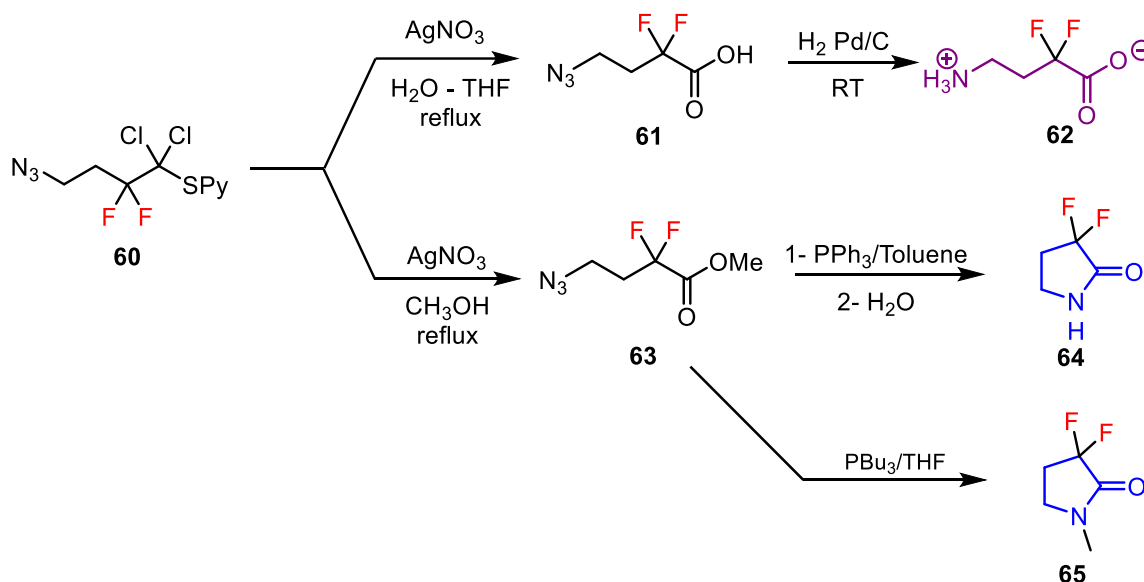


Figure C2. 7: Some GABA analogues

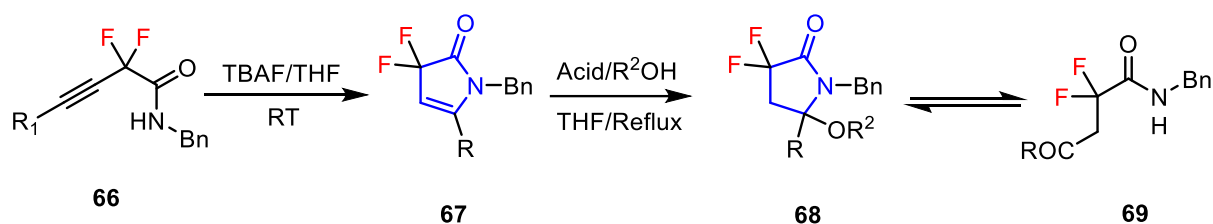
1.3. Fluorinated γ -lactams and GABA analogues

Several attempts were done to synthesize fluorinated γ -lactams and GABA analogues using different synthetic pathways. In 1995, S. Eguchi *et al.* reported a new radical reaction to obtain intermediate **60** which allowed the access to difluorinated GABA analogue **62** and γ -lactams **64** and **65** (Scheme C2. 9).⁴⁶



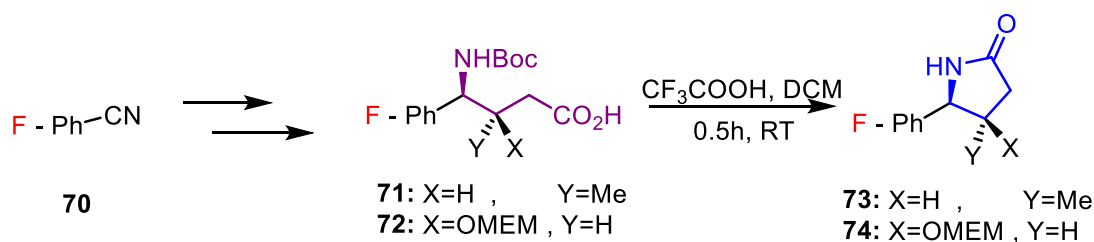
Scheme C2. 9: Preparation of difluorinated GABA **62** and γ -lactams **64** and **65**

Another strategy was developed in 2007 by G. Hammond *et al.* where the treatment of intermediate **66** with base such TBAF allowed the amidic nitrogen activation to make 5-endo dig cyclization and produce the corresponding γ -lactam **67** which can give hemiaminal **68** or ketone **69** when treated with an acid and alcohol (Scheme C2. 10).⁴⁷



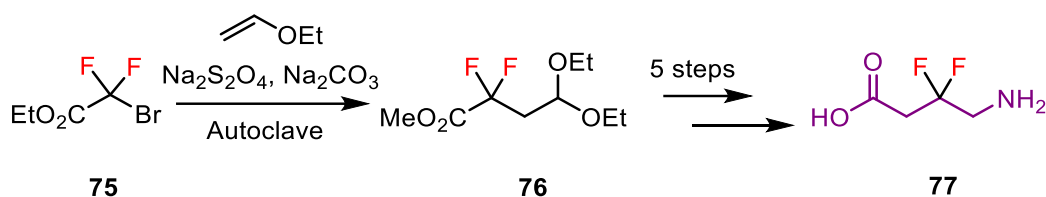
Scheme C2. 10: Intramolecular Hydroamination of difluoropropargyl Amides **66**

In 2009, Biwas *et al.*⁴⁸ were able to obtain *N*-Boc protected γ -fluorophenyl-GABA derivatives **71**, **72** starting from fluorobenzonitrile **70**, which upon deprotection provided the corresponding γ -lactams **73** and **74** (Scheme C2. 11).



Scheme C2. 11: Synthesis γ -Fluorophenyl-GABA derivatives and their corresponding γ -lactams

A further method to obtain difluoro-GABA analogues were developed in 2015 by G. Haufe *et al.* where a radical reaction was done between alkyl 2-bromo-2,2-difluoroacetates **75** with ethyl vinyl ether to afford intermediate **76**, which after 5 steps permitted the access to the desired fluorinated GABA analogue **77** (Scheme C2. 12).⁴⁹



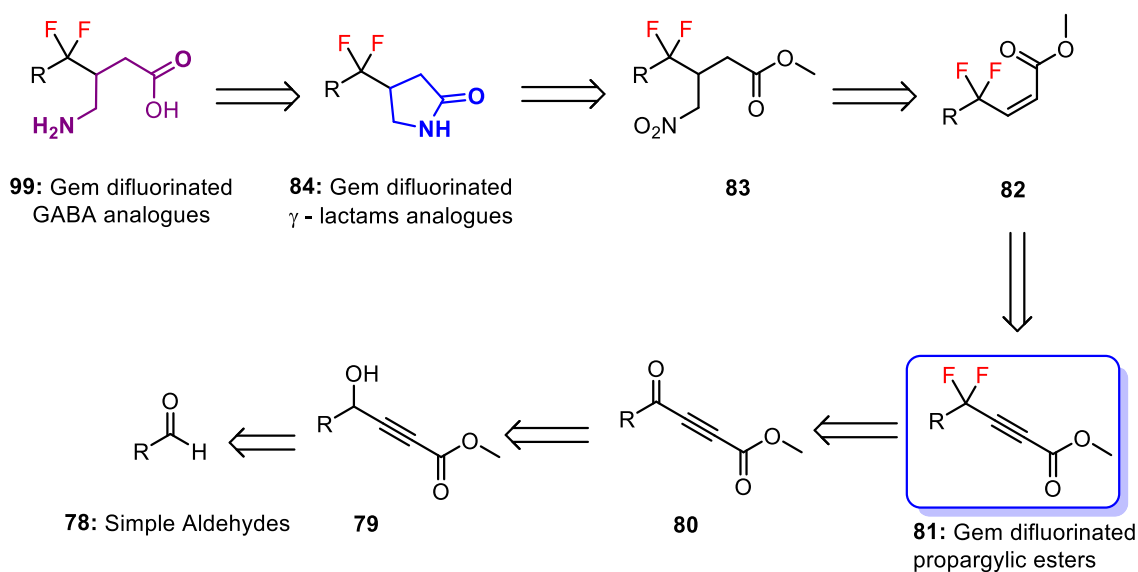
Scheme C2. 12: Application for the Synthesis of 3,3-Difluoro-GABA

2. Results and discussion

Encouraged by the results of previous work carried out in our laboratories, and by the biological and synthetic interest of the cited heterocycles and GABA analogues, we designed a strategy for their preparation in order to create a chemical library around this type of molecules.

2.1. Retrosynthetic analysis

It takes advantage of the easily accessible and versatile *gem*-difluoro propargylic derivatives **81**. The target molecules **99** will be obtained from the ring opening of the fluorinated γ -lactam **84** which is a result of the cyclisation of amino ester intermediates with the desired fluorinated side chains. Later derivatives will be obtained by reduction of corresponding nitro compounds **83**. These intermediates will be obtained by a Michael addition of the nitromethane anion on electrophilic alkenes with the *gem*-difluoro chains **82**. These intermediates will be prepared by reduction of the corresponding *gem*-difluoro propargylic esters **81** (Scheme C2. 13).



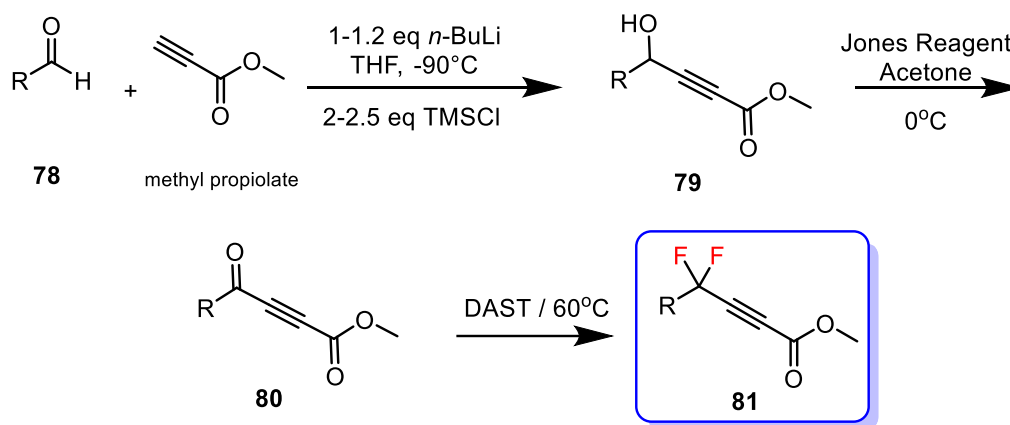
Scheme C2. 13: Retrosynthetic analysis for the preparation of the γ -lactams and GABA with fluorinated side chains in position 4

2.2. Synthesis of γ -lactams with *gem*-difluorinated side chains

2.2.1. Synthesis of the fluorinated alkynes 81

The fluorinated alkynes **81a-h** were prepared in three steps, as indicated in (Scheme C2. 14, Table C2. 1)

The addition of methyl propiolate on various types of aldehydes (aromatic and alkyl) afforded propargylic alcohols **79a-h** in fair to good yields. Subsequent oxidation by Jones reagent gave the ketones **80a-h** also in excellent yields. Then fluorination with Diethylaminosulfur trifluoride (DAST) afforded the target intermediates **81a-h** in satisfying yields, except for **81f** where some decomposition occurred.



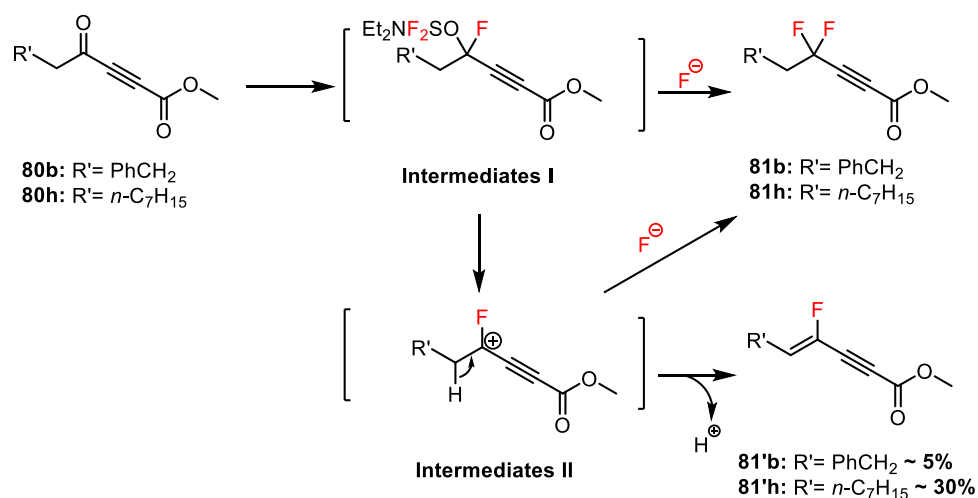
Scheme C2. 14: Synthesis of alkynes **81a-h** with the *gem*-difluoroalkylated chains

Table C2. 1: Synthesis of alkynes **81a-h** with the gem-difluoroalkylated chains

| ENTRY | SM | R | YIELD (%) ^[a] | | |
|-------|-----|---|--------------------------|---------|---------|
| | | | 79 | 80 | 81 |
| 1 | 78a | | 79a(90) | 80a(80) | 81a(65) |
| 2 | 78b | | 79b(69) | 80b(74) | 81b(91) |
| 3 | 78c | | 79c(70) | 80c(79) | 81c(62) |
| 4 | 78d | | 79d(64) | 80d(84) | 81d(76) |
| 5 | 78e | | 79e(81) | 80e(95) | 81e(70) |
| 6 | 78f | | 79f(77) | 80f(93) | 81f(25) |
| 7 | 78g | | 79g(70) | 80g(77) | 81g(79) |
| 8 | 78h | | 79h(60) | 80h(82) | 81h(60) |

^[a] isolated yields

In the entries **81b** and **81h** another byproduct was observed (~ 5% - 30%) due to the loss of one fluorine atom and the formation of a double bond favored by the formation of a conjugated system to afford byproducts **81'b** and **81'h** (Scheme C2. 15).⁵⁰



Scheme C2. 15: Formation of the double bond in **81'b** and **81'h**.

These results were confirmed by the analysis of the crude ^1H NMR (300 MHz, CDCl_3) of **81h**. The presence of **81'h** was confirmed by the existence of double bond with an ethylenic proton found at 5.59 ppm (dt, $^3J_{\text{H-F}} = 32.5$ Hz, $^3J_{\text{H-H}} = 7.9$ Hz, 1H). The deshielded ($\text{H}4'$) shows a strong interaction with a fluorine atom to give a doublet with coupling constant of 32.5 Hz, and interaction with the two hydrogens ($\text{H}3'$) to afford a triplet with coupling constant 7.9 Hz (Figure C2. 8).

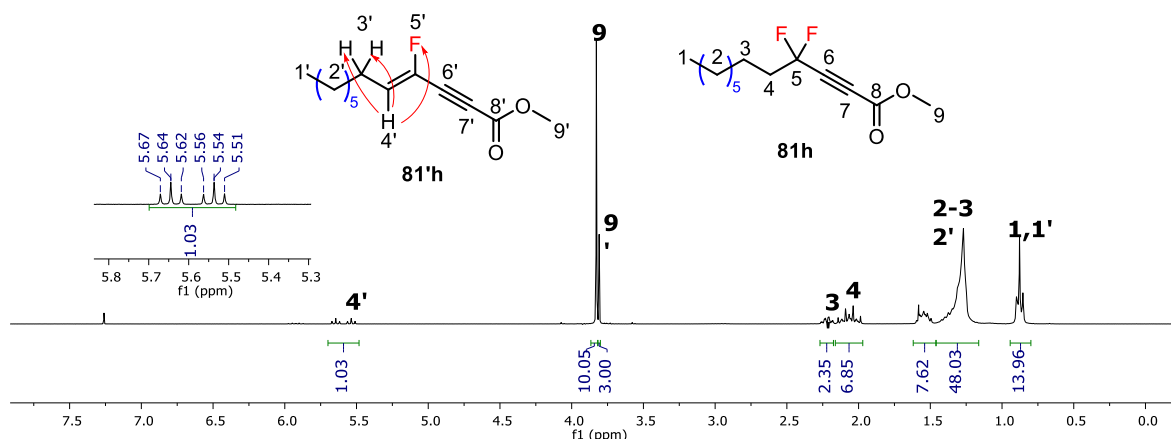
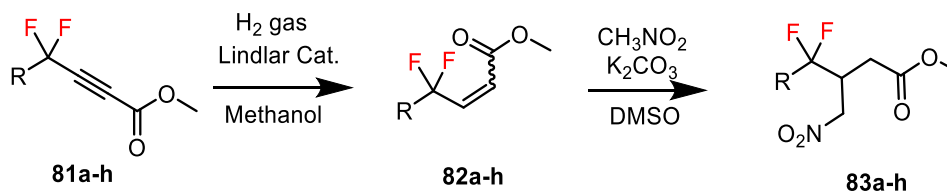


Figure C2. 8: analysis of compounds **81h** and **81'h**'

2.2.2. Synthesis of difluoro nitroester adducts **83**

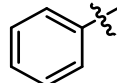
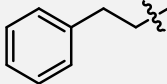
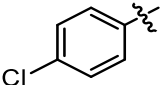
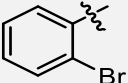
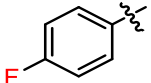
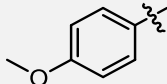
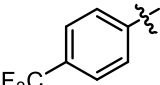
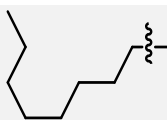
The conversion to the target difluoro nitroester **83** was achieved in two more simple steps (Scheme C2. 16, Table C2. 2). First, the semi-hydrogenation of the triple bond was performed using Lindlar catalyst to give the alkenes **82a-h** in good to excellent yields. However, during these reductions, minor amounts of the (*E*) isomers were obtained in few cases.

On these electrophilic alkenes **82a-h**, the Michael addition of the nitromethane anion was performed in a classical way by using potassium carbonate as the base and DMSO as solvent, affording adducts **83a-h** in good to excellent yields.



Scheme C2. 16: Synthesis of nitroester adducts **83a-h** with the *gem*-difluoroalkylated chains in position 4

Table C2. 2: Synthesis of nitroester adducts **83a-h** with the *gem*-difluoroalkylated chains in position 4

| ENTRY | SM | R | YIELD (%) ^[a] | | |
|-------|-----|---|--------------------------|-----------|---------|
| | | | 82 | Z/E ratio | 83 |
| 1 | 81a |  | 82a(86) | 90/10 | 83a(90) |
| 2 | 81b |  | 82b(65) | 85/15 | 83b(80) |
| 3 | 81c |  | 82c(94) | 93/7 | 83c(88) |
| 4 | 81d |  | 82d(35) ^[b] | 84/16 | 83d(79) |
| 5 | 81e |  | 82e(75) | 75/25 | 83e(87) |
| 6 | 81f |  | 82f(79) | 70/30 | 83f(86) |
| 7 | 81g |  | 82g(80) | 95/5 | 83g(85) |
| 8 | 81h |  | 82h(71) | 83/17 | 83h(70) |

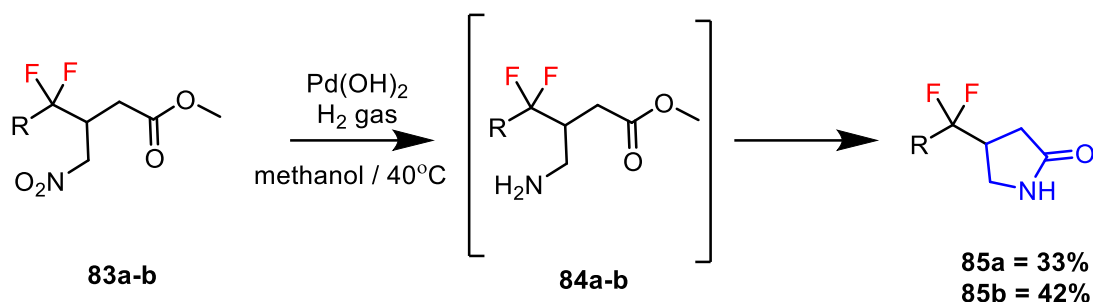
^[a] isolated yields, ^[b] from 40% conversion

2.2.3. Synthesis of γ -lactams **84**

The reduction of the nitro group proved to be a little more challenging, where two catalytic systems were tested.

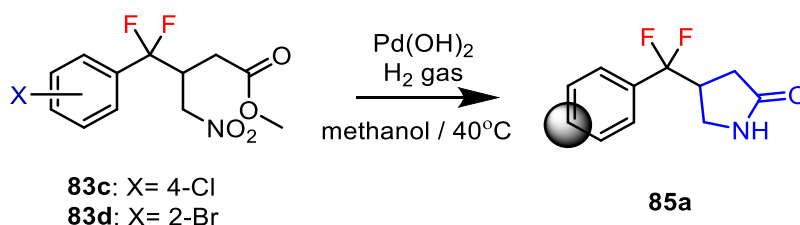
2.2.3.a Using Pd(OH)₂

The first attempt to reduce the nitro group in the difluoro nitroester adducts **83a-b** was accomplished using Pd(OH)₂ under H₂ gas at 40 °C to give the primary amines **84a-b** which cyclized in situ by attacking the ester group to form the targeted γ -lactams **85a-b** in low yields “33% and 43% respectively” (Scheme C2. 17).



Scheme C2. 17: Synthesis of γ -lactams with the *gem*-difluoroalkylated chains in position 4 **85a-b** using Pd(OH)₂

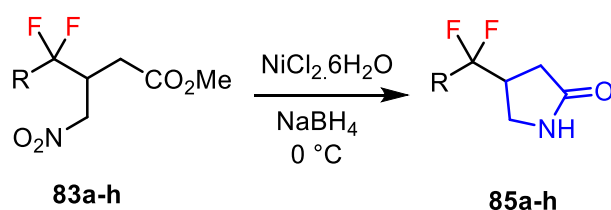
Unfortunately, when starting from compounds **83c** and **83d** bearing the halogen substituents on the aromatic ring, the hydrogenation in the presence of Pd(OH)₂ gave γ -lactam **85a** only (Scheme C2. 18). This dehalogenation process occurs because the Pearlman's catalyst is highly halophilic⁵¹, thus another catalytic system was used to obtain optimized results.



Scheme C2. 18: The reduction of the nitro group and the dehalogenation process in **83c-d**

2.2.3.b Using NiCl₂·6H₂O and NaBH₄

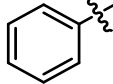
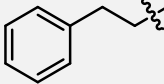
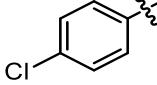
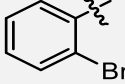
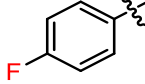
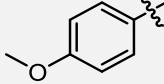
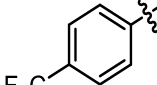
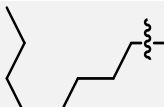
After optimization studies we found that the combination of NiCl₂·6H₂O and NaBH₄,⁵² gave the best results affording the target molecules **85a-h** in excellent yields, except for **85d** where the cleavage of C-Br bond still occurred, affording only **85a** (Scheme C2. 19, Table C2. 3).



Scheme C2. 19: Synthesis of γ -lactams with the *gem*-difluoroalkylated chains in position 4 **85a-h** using NiCl₂·6H₂O and NaBH₄

Table C2. 3: Synthesis of γ -lactams with the *gem*-difluoroalkylated chains in position 4 **85a-h** using NiCl₂·6H₂O and NaBH₄

| YIELD (%) ^[a] | | | |
|--------------------------|----|---|----|
| ENTRY | SM | R | 85 |
| | | | |

| | | | |
|---|-----|---|---------|
| 1 | 83a |  | 85a(80) |
| 2 | 83b |  | 85b(77) |
| 3 | 83c |  | 85c(84) |
| 4 | 83d |  | --- |
| 5 | 83e |  | 85e(90) |
| 6 | 83f |  | 85f(92) |
| 7 | 83g |  | 85g(95) |
| 8 | 83h |  | 85h(84) |

^[a] isolated yields

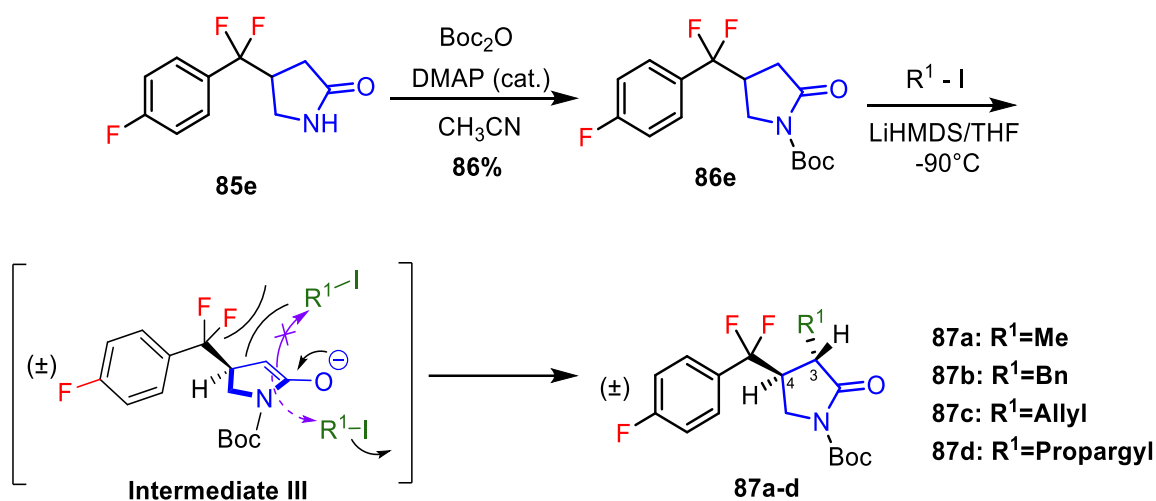
All compounds **79a** to **85h** have spectral (^1H , ^{19}F and ^{13}C NMR) and analytical (HRMS) data in agreement with their structure. In addition, structure of the γ -lactams **85a** and **85e** were confirmed by X-Ray Crystallographic analysis (Figure C2. 9).



Figure C2. 9: Structure of **85a** and **85e** by X-Ray Crystallographic analysis

2.2.4. Synthesis of γ -lactams substituted in position (3) **87a-d**

The next step was the extension of molecular diversity around the γ -lactam scaffold. One obvious way was the introduction of R^1 substituents on the carbon vicinal to CO. Thus, the lactam **85e**, selected as a model, was first protected as *N*-Boc derivative **86e** and then alkylation reactions were performed to introduce various R^1 groups on the desired position. These reactions proved to be completely selective, affording only the *trans* isomers **87a-d** (Scheme C2. 20, Table C2. 4).



Scheme C2. 20: Introduction of R¹ substituents in position 3.

Table C2. 4: Yields of the introduction of R¹ substituents in position 3

| ENTRY | R ¹ | Yields (%) ^[a] |
|-------|----------------|---------------------------|
| 1 | Me | 87a (90) |
| 2 | Bn | 87b (62) |
| 3 | Allyl | 87c (42) |
| 4 | Propargyl | 87d (30) |

^[a] isolated yields

The stereochemistry of compound **87a** was attributed by extensive NMR experiments. In particular strong correlations were found between the fluorine atoms and the proton in position 3 by ¹H-¹⁹F HOESY experiments. Further, NOEs correlations have been observed between the methyl group in position 3 and the proton in position 4 (Figure C2. 11). The structure of **87b** was confirmed by X-Ray crystallographic analysis (Figure C2. 10)

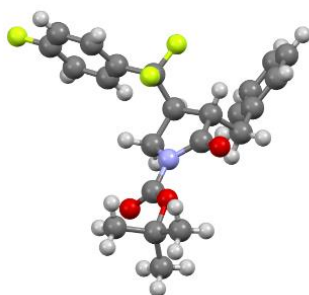


Figure C2. 10: Structure of **87b** by X-Ray Crystallographic analysis

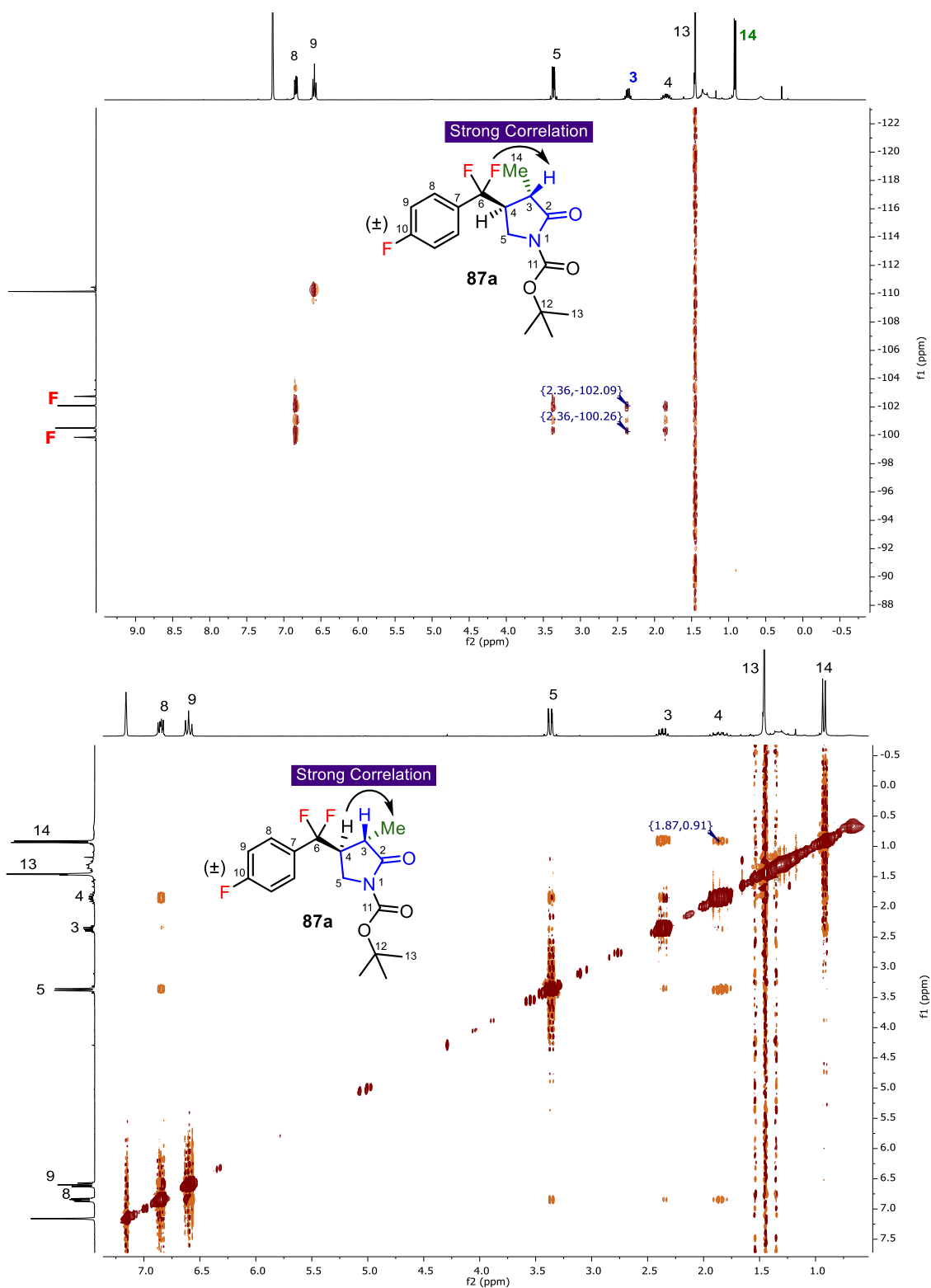
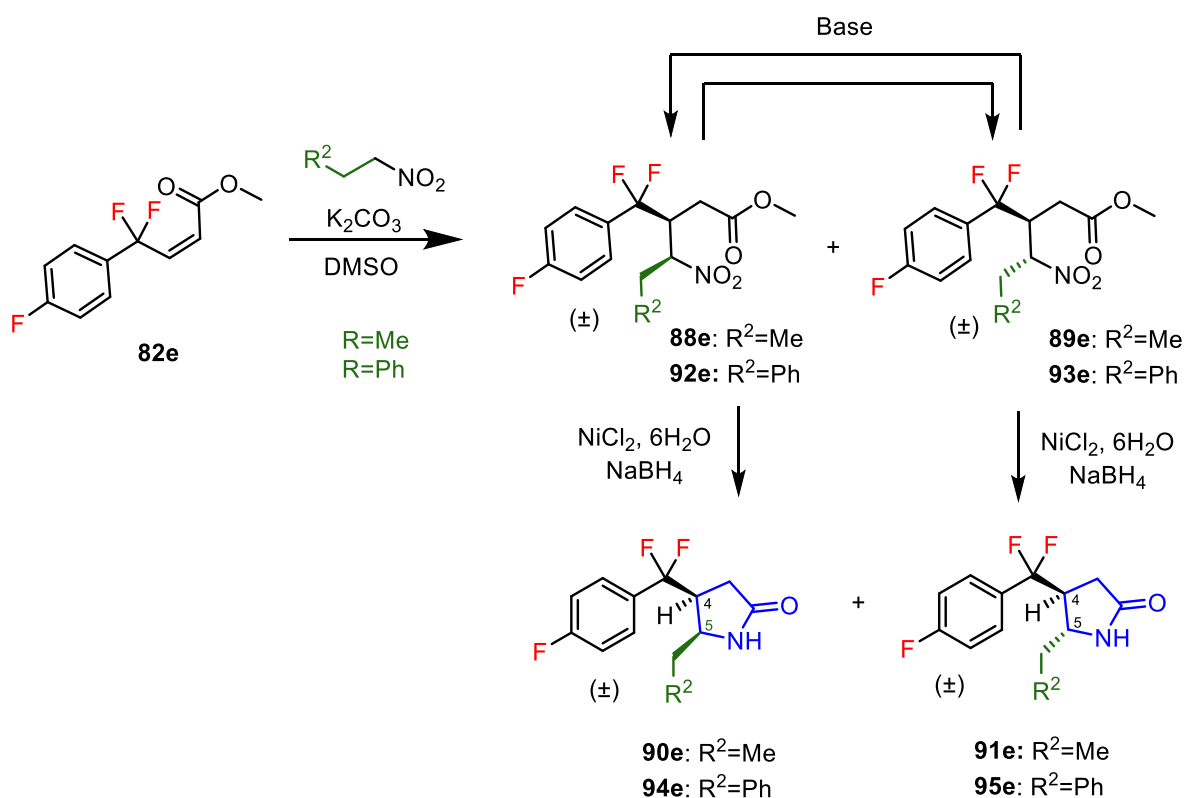


Figure C2. 11: ^1H - ^{19}F HOESY experiment (Top), ^1H - ^1H NOESY experiment (bottom) for compound **87a**

2.2.5. Synthesis of γ -lactams substituted in position 5

To expand the scope of this approach, a second option was to introduce a group $\text{CH}_2\text{-R}_2$ on the carbon vicinal to the nitrogen atom. By using the α - β unsaturated ester **82e** as a

representative model, this could be achieved by the addition of nitroalkyl groups, instead of nitromethane. First the reaction of 1-nitropropane on **82e** gave a 1:1 mixture of adducts **88e** and **89e**, in 66 % overall yield, as shown in Scheme C2. 21. These compounds were separated by chromatography, not surprisingly, there was a slow equilibration between these two stereoisomers under basic conditions. Reduction of the nitro group, under the same conditions as before, afforded stereoselectively in good yields the γ -lactams **90e** and **91e**.



Scheme C2. 21: Synthesis of γ -lactams substituted in position 5.

Their stereochemistry was established by extensive NMR studies. In particular for **91e**, strong NOESY correlations have been observed between the protons of the methine group in position 5 and the neighbouring proton in position 4 of this heterocycle; no such correlations were observed in the case of its diastereoisomer **90e** (Figure C2. 12). The same reactions were performed starting with the 2-nitroethylbenzene, affording a 1:1 inseparable mixture of **92e** and **93e** in 83 % overall yield. After reduction of the nitro groups, the desired γ -lactams **94e** and **95e** could be separated by chromatography and their stereochemistry was established by NMR in the same way as for **90e** and **91e** (Scheme C2. 21).

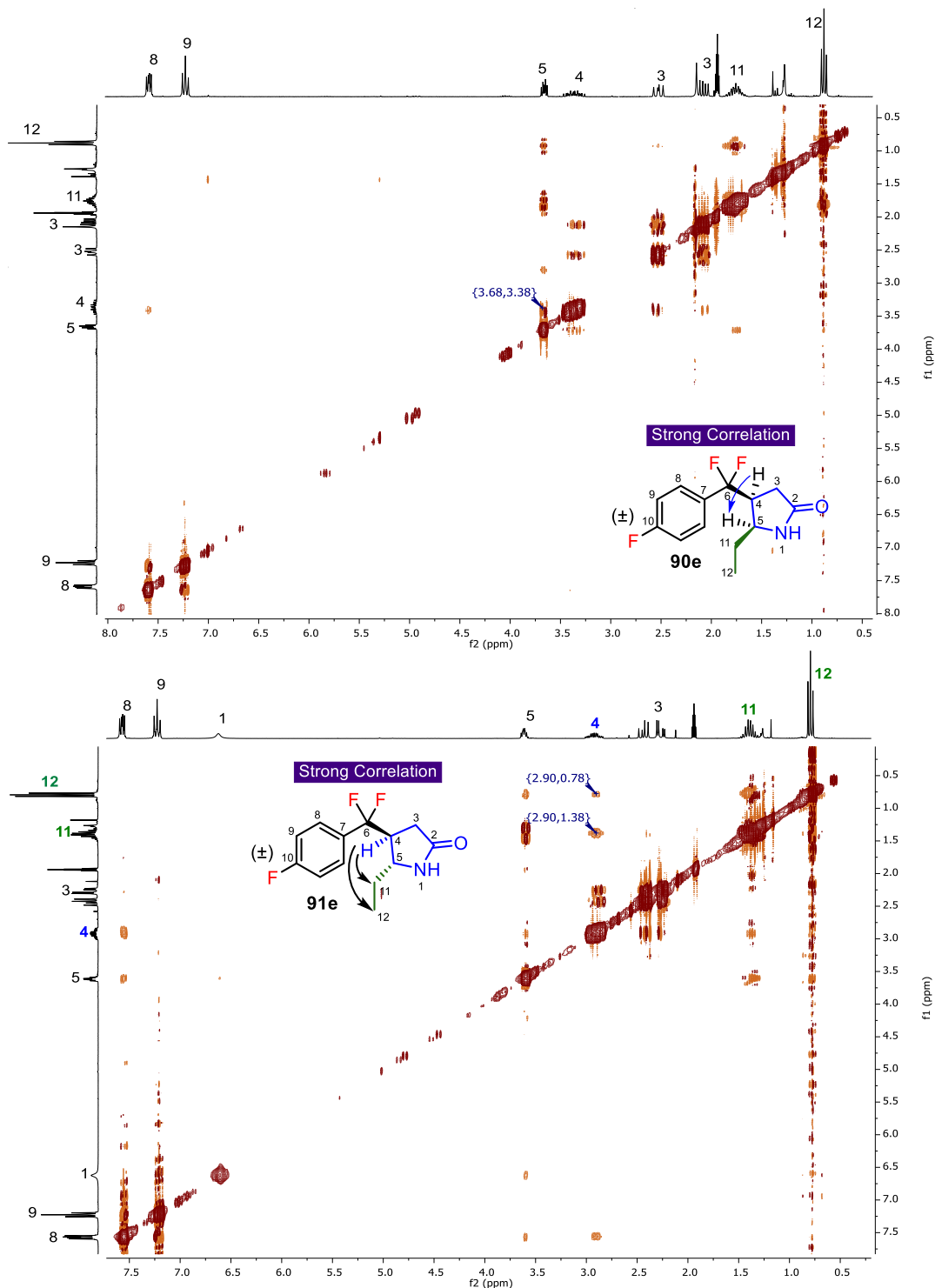
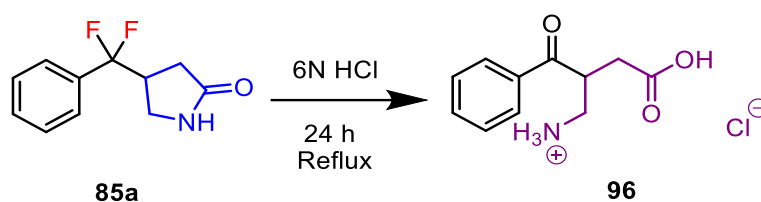


Figure C2. 12: ^1H - ^1H NOESY experiment (Top) for compound **90e** in CD_3CN ; ^1H - ^1H NOESY experiment (Bottom) for compound **91e** in CD_3CN .

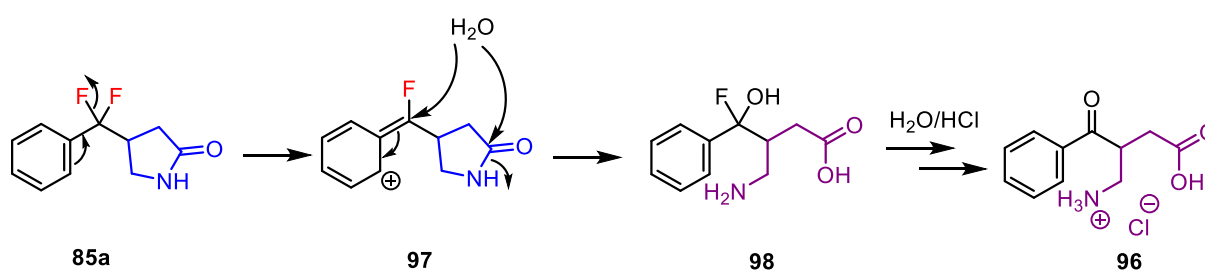
2.3. Toward the synthesis of GABA analogues with *gem*-difluorinated side chains

In order to get difluorinated GABA analogues, γ -Lactam **85a** was treated with 6N HCl under reflux for 24 h. An unexpected GABA analogue **96** was obtained, with the absence of the two fluorine atoms (Scheme C2. 22).



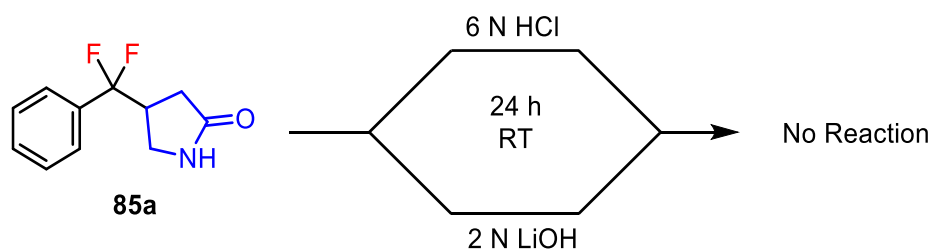
Scheme C2. 22: tentative hydrolysis of γ -lactam to obtain GABA analogue

Another reaction was done with the same reaction conditions but for 4 h instead of 24 h. It revealed that the opening of the γ -lactam group step and the defluorination step were happening at the same time (Scheme C2. 23). So, under these reaction conditions we couldn't get the difluorinated GABA analogues.



Scheme C2. 23: Suggested mechanism to afford compound **96**

Another two attempts were done. Under acidic conditions using 6 N HCl and basic ones using 2 N Lithium hydroxide LiOH at RT for 24 h, unfortunately there was no reaction in both conditions (Scheme C2. 24).



Scheme C2. 24: Attempts to open the γ -lactam ring using basic and acidic conditions

Therefore, other pathways should be tested to open the γ -lactams, such as using γ -lactamase enzymes⁵³.

3. Conclusion and perspectives

In conclusion, we have developed a new method to prepare novel γ -lactams with *gem*-difluorinated side chains.⁵⁴ Such an approach should allow access to chemical libraries of new fluorinated analogues of known bioactive compounds, as well as to molecules designed for their various biological activities. Biological tests regarding their activity in the CNS area will be carried out on some derivatives of this family under the supervision of Dr. Eva Hamade.

The preparation of GABA with *gem*-difluorinated side chains was particularly challenging, where the loss of the fluorine atoms was accompanied by the opening of the γ -lactam ring, so using γ – lactamase enzymes might be an interesting alternative pathway.

4. Experimental Part

4.1. General Methods

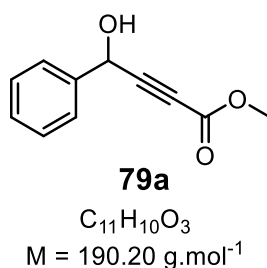
NMR spectra were performed on a Bruker AVANCE 300 or Bruker AVANCE 500 (^1H , 300 MHz or 500 MHz; ^{13}C , 75 MHz or 126 MHz; ^{19}F , 282 MHz or 471 MHz). Solvent peaks were used as reference values with CDCl_3 at 7.26 ppm for ^1H NMR and 77.16 ppm for ^{13}C NMR, with CD_3CN at 1.94 ppm for ^1H NMR and 1.32 and 118.26 ppm for ^{13}C NMR, with $(\text{CD}_3)_2\text{CO}$ at 2.05 ppm for ^1H NMR and 29.84 and 206.26 ppm for ^{13}C NMR, and with C_6D_6 at 7.16 ppm for ^1H NMR and 128.06 ppm for ^{13}C NMR. Chemical shifts δ are given in ppm, and the following abbreviations are used: singlet (s), broad singlet (bs), doublet (d), doublet of doublet (dd), doublet of doublet of doublet (ddd), triplet (t), triplet of doublet (td), quadruplet (q), doublet of quadruplet (dq), and multiplet (m). IR spectra were run on a PerkinElmer Spectrum 16 PC spectrometer. High resolution mass spectra were recorded in the Centre Régional de Mesures Physiques de l'Ouest, Rennes (CRMPO), on a Maxis 4G. Melting points were determined with uncertainty of $\pm 2^\circ\text{C}$ using a KOFER BENCH. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F254 Merck plates), and compounds were visualized under a UVP Mineralight UVGL-58 lamp (254 nm) and with p-anisaldehyde/ Δ . Column chromatography was performed using silica gel 60 (40–63 mm, 230–400 mesh). Solvents were used as received from commercial sources. Reagent were purchased from Sigma-Aldrich, Alfa Aesar and Acros. All products reported showed ^1H , ^{13}C , and ^{19}F NMR spectra in agreement with the assigned structures.

4.2. Experimental Procedures and Analytical data

1. Synthesis of propargylic alcohols:

1.1. Representative procedure: Synthesis of methyl 4-hydroxy-4-phenylbut-2-ynoate (**79a**)

To a solution of methyl propiolate (3.0 mL, 33.9 mmol, 1.2 equiv) in anhydrous THF (50 mL) cooled at -90 °C, was added, dropwise under nitrogen, a 2.5M solution of n-butyllithium (13.5 mL, 1.2 equiv). The reaction mixture was stirred for 30 min at temperature below -80 °C before dropwise addition of benzaldehyde (28.2 mmol) in anhydrous THF (10 mL). After 20 min of stirring at the same temperature, TMSCl (9.0 mL, 2.5 equiv) was added dropwise. The reaction mixture was stirred for additional 1 h at temperature below -80 °C. Then the reaction mixture was allowed to warm to r.t. within 2h. The mixture was then treated with a saturated ammonium chloride solution and extracted with ethyl acetate (3x50mL). The combined organic phases were washed with water, dried over Na₂SO₄ and concentrated *in vacuo*. After purification by chromatography on silica gel, the propargylic alcohol **79a** was obtained as a light yellow oil. 4.84 g (90%).



R_f = 0.5 (Cyclohexane / Ethyl Acetate: 7/3).

¹H NMR (300 MHz, CDCl₃) δ 7.53 – 7.47 (m, 2H), 7.43 – 7.31 (m, 3H), 5.55 (s, 1H), 3.78 (s, 3H), 3.00 (bs, 1H, OH).

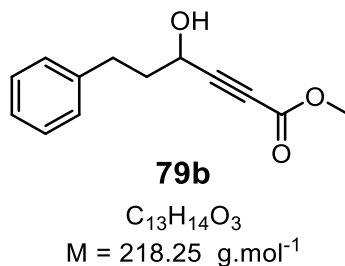
¹³C NMR (75 MHz, CDCl₃) δ 153.9, 138.6, 129.0, 129.0, 126.8, 86.8, 77.6, 64.3, 53.1.

FT-IR (cm⁻¹): 3407, 3064, 3033, 3009, 2954, 2236, 1712, 1493, 1452, 1434, 1245, 1187, 1068, 1001, 942, 918, 841, 749, 696, 665.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₁H₁₀O₃Na) = 213.0522; found: 213.0522 (0 ppm).

1.2. Methyl 4-hydroxy-6-phenylhex-2-ynoate (**79b**):

Starting from 3 g (2.23 mmol) of 3-phenylpropionaldehyde we obtained 3.36 g of **79b** (69%) as a light-yellow oil. Physical data and NMR data are in agreement with literature.⁵⁵

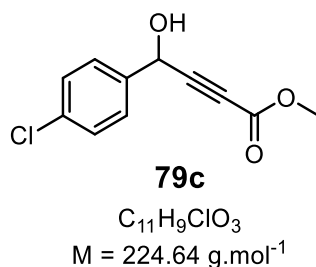


$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.27 – 6.93 (m, 5H), 4.30 (t, $^3J_{H-H} = 6.7 \text{ Hz}$, 1H), 3.60 (s, 3H), 3.16 (s, 1H, OH), 2.64 (t, $^3J_{H-H} = 7.8 \text{ Hz}$, 2H), 1.98 – 1.84 (m, 2H).

$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 154.1, 140.7, 128.5, 128.5, 126.2, 88.4, 76.3, 61.1, 52.9, 38.2, 31.1.

1.3. Methyl 4-(4-chlorophenyl)-4-hydroxybut-2-ynoate (**79c**)

Starting from 3 g (2.13 mmol) of 4-chlorobenzaldehyde we obtained 3.35 g of **79c** (70%) as a light-yellow oil.



R_f = 0.6 (Cyclohexane / Ethyl Acetate: 7/3).

$^1\text{H NMR}$ (500 MHz, CDCl_3) δ 7.44 – 7.38 (m, 2H), 7.35 – 7.32 (m, 2H), 5.51 (s, 1H), 3.77 (s, 3H), 3.51 – 3.20 (bs, 1H, OH).

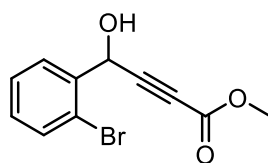
$^{13}\text{C NMR}$ (125 MHz, CDCl_3) δ 154.0, 137.0, 134.8, 129.0, 128.1, 86.4, 77.7, 63.5, 53.2.

FT-IR (cm^{-1}): 3417, 2955, 2238, 1713, 1489, 1434, 1405, 1375, 1245, 1187, 1089, 1074, 1012, 941, 847, 789, 750.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($C_{11}H_9O_3ClNa$) = 247.0132; found: 247.0131 (1 ppm).

1.4. Methyl 4-(2-bromophenyl)-4-hydroxybut-2-ynoate (**79d**)

Starting from 3 g (16.2 mmol) of 2-bromobenzaldehyde we obtained 2.79 g of **79d** (64%) as a light-yellow oil.



79d
C₁₁H₉BrO₃
M = 269.09 g.mol⁻¹

R_f = 0.4 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.70 (dd, ³J_{H-H} = 7.8 Hz, ⁴J_{H-H} = 1.7 Hz, 1H), 7.57 (dd, ³J_{H-H} = 8.0 Hz, ⁴J_{H-H} = 1.3 Hz, 1H), 7.37 (td, ³J_{H-H} = 7.6 Hz, ⁴J_{H-H} = 1.3 Hz, 1H), 7.22 (td, ³J_{H-H} = 7.7, ⁴J_{H-H} = 1.7 Hz, 1H), 5.89 (d, ³J_{H-H} = 4.9 Hz, 1H), 3.78 (s, 3H), 3.07 (d, ³J_{H-H} = 4.9 Hz, 1H, OH).

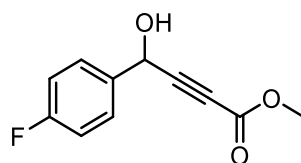
¹³C NMR (75 MHz, CDCl₃) δ 153.8, 137.6, 133.2, 130.6, 128.7, 128.2, 122.6, 85.7, 77.5, 63.9, 53.1.

FT-IR (cm⁻¹): 3450, 3064, 2987, 2954, 2901, 2237, 1712, 1696, 1590, 1570, 1469, 1434, 1245, 1187, 1122, 1074, 1048, 1017, 941, 810, 748, 722.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₁H₉O₃BrNa) = 290.9627; found: 290.9628 (0 ppm).

1.5. Methyl 4-(4-fluorophenyl)-4-hydroxybut-2-ynoate (**79e**)

Starting from 3 g (24.2 mmol) of 4-fluorobenzaldehyde we obtained 4.07 g of **79e** (81%) as a colorless oil.



79e
C₁₁H₉FO₃
M = 208.19 g.mol⁻¹

R_f = 0.2 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.53 – 7.44 (m, 2H), 7.12 – 7.03 (m, 2H), 5.55 (d, ³J_{H-H} = 5.9 Hz, 1H), 3.79 (s, 3H), 2.85 (d, ³J_{H-H} = 5.9 Hz, 1H, OH).

^{13}C NMR (75 MHz, CDCl_3) δ 163.1 (d, $^1J_{\text{C-F}} = 247.9$ Hz), 153.8, 134.5 (d, $^4J_{\text{C-F}} = 3.4$ Hz), 128.7 (d, $^3J_{\text{C-F}} = 8.4$ Hz), 115.9 (d, $^2J_{\text{C-F}} = 21.8$ Hz), 86.3 (d, $^6J_{\text{C-F}} = 0.8$ Hz), 77.8, 63.7 (d, $^5J_{\text{C-F}} = 0.8$ Hz), 53.1.

^{19}F {H} NMR (282 MHz, CDCl_3) δ -112.59 (s).

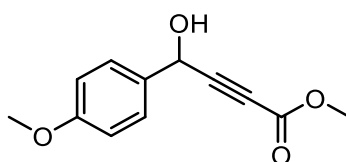
^{19}F NMR (282 MHz, CDCl_3) δ -112.54 – -112.65 (m).

FT-IR (cm^{-1}): 3413, 2987, 2957, 2900, 2238, 1712, 1604, 1507, 1435, 1248, 1222, 1187, 1157, 1099, 1073, 1011, 943, 841, 817, 771, 750.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{11}\text{H}_9\text{O}_3\text{FNa}$) = 231.0428; found: 231.0431 (1 ppm).

1.6. Methyl 4-hydroxy-4-(4-methoxyphenyl)but-2-ynoate (79f)

Starting from 3 g (22.03 mmol) of anisaldehyde we obtained 3.73 g of **79f** (77%) as a light-yellow oil.



79f

$\text{C}_{12}\text{H}_{12}\text{O}_4$

$M = 220.22 \text{ g}\cdot\text{mol}^{-1}$

R_f \equiv 0.4 (Cyclohexane / Ethyl Acetate: 7/3).

^1H NMR (300 MHz, CDCl_3) δ 7.47 – 7.38 (m, 2H), 6.93 – 6.86 (m, 2H), 5.50 (d, $^3J_{\text{H-H}} = 6.1$ Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 2.73 (d, $^3J_{\text{H-H}} = 6.1$ Hz, 1H, OH).

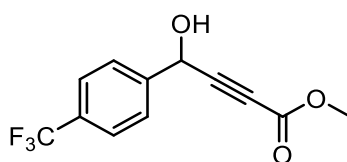
^{13}C NMR (75 MHz, CDCl_3) δ 160.2, 153.9, 130.9, 128.3, 114.3, 86.9, 77.6, 64.0, 55.5, 53.0.

FT-IR (cm^{-1}): 3433, 2956, 2902, 2840, 2235, 1711, 1645, 1609, 1592, 1510, 1434, 1241, 1171, 1112, 1071, 1026, 962, 943, 836, 809, 749.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{12}\text{H}_{12}\text{O}_4\text{Na}$) = 243.0628; found: 243.0631 (1 ppm).

1.7. Methyl 4-hydroxy-4-(4-(trifluoromethyl)phenyl)but-2-ynoate (79g)

Starting from 3 g (17.2 mmol) of 4-(trifluoromethyl)benzaldehyde we obtained 3.11 g of **79g** (70%) as a yellow oil.



79g

$C_{12}H_9F_3O_3$

$M = 258.20 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.2$ (Cyclohexane / Ethyl Acetate: 7/3).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.67 – 7.60 (m, 4H), 5.63 (d, $^3J_{H-H} = 4.5 \text{ Hz}$, 1H), 3.79 (s, 1H), 3.27 (d, $^3J_{H-H} = 4.5 \text{ Hz}$, 1H, OH).

$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 153.8, 142.3 (q, $^5J_{C-F} = 1.2 \text{ Hz}$), 131.2 (q, $^2J_{C-F} = 33.1 \text{ Hz}$), 127.0, 125.9 (q, $^3J_{C-F} = 3.8 \text{ Hz}$), 124.0 (q, $^1J_{C-F} = 272.2 \text{ Hz}$), 85.8, 78.0, 63.6, 53.2.

^{19}F {H} NMR (282 MHz, CDCl_3) δ -62.75 (s).

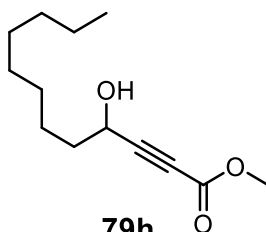
^{19}F NMR (282 MHz, CDCl_3) δ -62.75 (s).

FT - IR (cm^{-1}): 3472, 2960, 2901, 2240, 1720, 1661, 1619, 1582, 1510, 1436, 1412, 1323, 1256, 1167, 1125, 1111, 1064, 1015, 961, 857, 763, 748, 729.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($C_{12}H_9O_3F_3\text{Na}$) = 281.0396; found: 281.0395 (0 ppm).

1.8. Methyl 4-hydroxydodec-2-ynoate (79h)

Starting from 3 g (21.1 mmol) of nonanal we obtained 2.86 g of **79h** (60%) as a yellow oil.



79h

$C_{13}H_{22}O_3$

$M = 226.32 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.5$ (Cyclohexane / Ethyl Acetate: 8/2).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 4.47 (t, $^3J_{H-H} = 6.7 \text{ Hz}$, 1H), 3.77 (s, 3H), 1.79 – 1.70 (m, 2H), 1.54 – 1.17 (m, 13H), 0.86 (t, $^3J_{H-H} = 7.1 \text{ Hz}$, 3H).

$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 154.0, 88.6, 76.3, 62.2, 53.0, 36.9, 31.9, 29.5, 29.3, 29.3, 25.0, 22.8, 14.2.

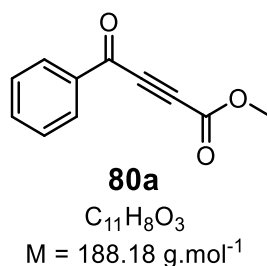
FT-IR (cm⁻¹): 3427, 2953, 2924, 2855, 2236, 1717, 1456, 1434, 1406, 1378, 1334, 1244, 1057, 751.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₃H₂₂O₃Na) = 249.1461; found: 249.1464 (1 ppm).

2. Oxidation to propargylic ketones

2.1. Representative procedure: Synthesis of methyl 4-oxo-4-phenylbut-2-ynoate (**80a**)

To the propargylic alcohol **79a** (2 g, 10.5 mmol) in acetone (20 mL) was added dropwise under magnetic stirring at room temperature, a concentrated (5.4 M) solution of Jones reagent until disappearance of the starting material (TLC analysis). After addition of isopropanol (5.0 equiv), the reaction mixture was filtered, and the filtrate was extracted with ethyl acetate (3x40mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated *in vacuo*. After purification by chromatography on silica gel, the propargylic ketone **80a** was obtained as a light-yellow solid (1.58 g, 80%).



R_f = 0.4 (Cyclohexane / Ethyl Acetate: 8/2). **mp** = 70 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.14 – 8.08 (m, 2H), 7.70 – 7.63 (m, 1H), 7.55 – 7.47 (m, 2H), 3.89 (s, 3H).

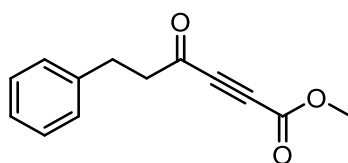
¹³C NMR (75 MHz, CDCl₃) δ 176.2, 152.8, 135.6, 135.3, 129.9, 129.0, 80.2, 80.2, 53.6.

FT-IR (cm⁻¹): 2957, 2926, 1707, 1642, 1594, 1581, 1453, 1432, 1318, 1252, 1177, 1063, 1023, 998, 957, 865, 796, 749, 697, 645.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₁H₈O₃Na) = 211.0366; found: 211.0367 (1 ppm).

2.2. Methyl 4-oxo-6-phenylhex-2-ynoate (**80b**)

Starting from 2 g (9.16 mmol) of methyl 4-hydroxy-6-phenylhex-2-ynoate **79b** we obtained 1.47 g of **80b** (74%) as a light-yellow solid. Physical data and NMR data are in agreement with literature.⁵⁵



80b

C₁₃H₁₂O₃

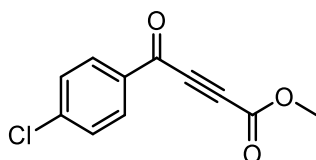
M = 216.24 g.mol⁻¹

¹H NMR (300 MHz, CDCl₃) δ 7.24 – 7.03 (m, 5H), 3.72 (s, 3H), 3.01 – 2.70 (m, 4H).

¹³C NMR (75 MHz, CDCl₃) δ 184.8, 152.6, 139.5, 128.6, 128.3, 126.5, 80.7, 78.1, 53.4, 46.7, 29.2.

2.3. Methyl 4-(4-chlorophenyl)-4-oxobut-2-ynoate (80c)

Starting from 2 g (8.90 mmol) of methyl 4-(4-chlorophenyl)-4-hydroxybut-2-ynoate **79c** we obtained 1.56 g of **80c** (79%) as a yellow solid.



80c

C₁₁H₇ClO₃

M = 222.62 g.mol⁻¹

R_f = 0.6 (Cyclohexane / Ethyl Acetate: 8/2). **mp** = 54 °C.

¹H NMR (500 MHz, CDCl₃) δ 8.04 (d, ³J_{H-H} = 8.0 Hz, 2H), 7.49 (d, ³J_{H-H} = 8.0 Hz, 2H), 3.89 (s, 3H).

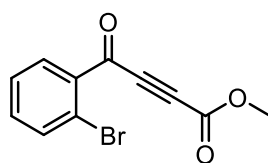
¹³C NMR (126 MHz, CDCl₃) δ 174.9, 152.7, 142.1, 134.1, 131.1, 129.5, 80.5, 79.7, 53.7.

FT-IR (cm⁻¹): 2959, 2923, 1715, 1650, 1584, 1437, 1402, 1259, 1171, 1091, 1065, 1010, 956, 867, 849, 743, 676.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₁H₇O₃ClNa) = 244.9976; found: 244.9979 (1 ppm).

2.4. Methyl 4-(2-bromophenyl)-4-oxobut-2-ynoate (80d):

Starting from 2 g (7.43 mmol) of methyl 4-(2-bromophenyl)-4-hydroxybut-2-ynoate **79d** we obtained 1.67 g of **80d** (84%) as a waxy yellow solid.



80d

$C_{11}H_7BrO_3$

$M = 267.08 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.7 (Cyclohexane / Ethyl Acetate: 8/2).

$^1\text{H NMR}$ (500 MHz, CDCl_3) δ 8.04 (dd, $^3J_{H-H} = 7.7 \text{ Hz}$, $^4J_{H-H} = 1.8 \text{ Hz}$, 1H), 7.71 (dd, $^3J_{H-H} = 7.7 \text{ Hz}$, $^4J_{H-H} = 1.5 \text{ Hz}$, 1H), 7.47 (td, $^3J_{H-H} = 7.5 \text{ Hz}$, $^4J_{H-H} = 1.5 \text{ Hz}$, 1H), 7.43 (td, $^3J_{H-H} = 7.6 \text{ Hz}$, $^4J_{H-H} = 1.8 \text{ Hz}$, 1H), 3.88 (s, 3H).

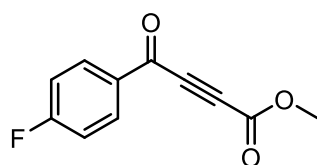
$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 175.2, 152.7, 135.5, 135.3, 134.6, 133.9, 127.7, 121.9, 80.9, 80.5, 53.6.

FT-IR (cm^{-1}): 3074, 3058, 3016, 2958, 2924.6, 848.2, 1717, 1655, 1580, 1561, 1487, 1463, 1435, 1291, 1245, 1227, 1135, 1071, 1026, 954, 866, 788, 734.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($C_{11}H_7O_3\text{BrNa}$) = 288.9471; found = 288.9472 (0 ppm).

2.5. Methyl 4-(4-fluorophenyl)-4-oxobut-2-ynoate (80e)

Starting from 2 g (9.61 mmol) of methyl 4-(4-fluorophenyl)-4-hydroxybut-2-ynoate **79e** we obtained 1.88 g of **80e** (95%) as a yellow solid.



80e

$C_{11}H_7FO_3$

$M = 206.17 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.6 (Cyclohexane / Ethyl Acetate: 8/2). **mp** = 68 °C.

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.18 – 8.10 (m, 2H), 7.24 – 7.15 (m, 2H), 3.89 (s, 3H).

$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 174.5, 167.2 (d, $^1J_{C-F} = 258.7 \text{ Hz}$), 152.7, 132.7 (d, $^3J_{C-F} = 9.9 \text{ Hz}$), 132.2 (d, $^4J_{C-F} = 2.8 \text{ Hz}$), 116.5 (d, $^2J_{C-F} = 22.4 \text{ Hz}$), 80.4, 79.8, 53.6.

^{19}F {H} NMR (282 MHz, CDCl_3) δ -100.80 (s).

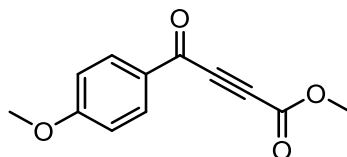
^{19}F NMR (282 MHz, CDCl_3) δ -100.74 to -100.87 (m).

FT-IR (cm⁻¹): 3068, 2958, 2930, 2872, 1717, 1649, 1591, 1504, 1433, 1411, 1297, 1242, 1150, 1107, 1061, 1008, 947, 857, 829, 817, 745.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₁H₇O₃FNa) = 229.0271; found: 229.0271 (0 ppm).

2.6. Methyl 4-(4-methoxyphenyl)-4-oxobut-2-ynoate (80f)

Starting from 2 g (9.08 mmol) of methyl 4-hydroxy-4-(4-methoxyphenyl)but-2-ynoate **79f** we obtained 1.84 g of **80f** (93%) as a colorless solid.



80f
C₁₂H₁₀O₄
M = 218.21 g.mol⁻¹

R_f = 0.3 (Cyclohexane / Ethyl Acetate: 8/2). mp = 71 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.11 – 8.01 (m, 2H), 7.00 – 6.93 (m, 2H), 3.89 (s, 3H), 3.87 (s, 3H).

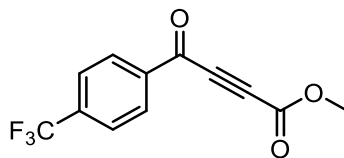
¹³C NMR (75 MHz, CDCl₃) δ 174.5, 165.4, 153.0, 132.4, 129.1, 114.3, 80.5, 79.6, 55.8, 53.5.

FT-IR (cm⁻¹): 3012, 2982, 2955, 2924, 2847, 1713, 1635, 1585, 1506, 1439, 1334, 1320, 1257, 1157, 1117, 1062, 1015, 956, 870, 842, 744.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₂H₁₀O₄Na) = 241.0471; found: 241.0474 (1 ppm).

2.7. Methyl 4-oxo-4-(4-(trifluoromethyl)phenyl)but-2-ynoate (80g)

Starting from 2 g (7.75 mmol) of methyl 4-hydroxy-4-(4-(trifluoromethyl)phenyl)but-2-ynoate **79g** we obtained 1.57 g of **80g** (77%) as a waxy yellow solid.



80g
C₁₂H₇F₃O₃
M = 256.18 g.mol⁻¹

R_f = 0.7 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 8.25 – 8.19 (m, 2H), 7.81 – 7.75 (m, 2H), 3.90 (s, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ 175.1, 152.5, 138.1 (q, $^5J_{\text{C-F}} = 1.2$ Hz), 136.3 (q, $^2J_{\text{C-F}} = 32.9$ Hz), 130.2, 126.1 (q, $^3J_{\text{C-F}} = 3.8$ Hz), 123.4 (q, $^1J_{\text{C-F}} = 273.0$ Hz), 81.1, 79.4, 53.7.

^{19}F { ^1H } NMR (282 MHz, CDCl_3) δ -63.35 (s).

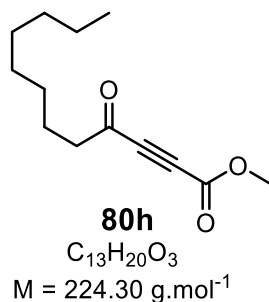
^{19}F NMR (282 MHz, CDCl_3) δ -63.35 (s).

FT-IR (cm^{-1}): 3016, 2964, 2924, 2850, 1719, 1663, 1615, 1582, 1510, 1435, 1411, 1323, 1235, 1189, 1154, 1111, 1062, 1012, 963, 856, 783, 762, 748, 727, 688.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{12}\text{H}_{17}\text{O}_3\text{F}_3\text{Na}$) = 279.0239; found: 279.0236 (1 ppm).

2.8. Methyl 4-oxododec-2-ynoate (80h)

Starting from 2 g (8.83 mmol) of methyl 4-hydroxydodec-2-ynoate **79h** we obtained 1.62 g of **80h** (82%) as a yellow liquid.



R_f = 0.6 (Cyclohexane / Ethyl Acetate: 8/2).

^1H NMR (300 MHz, CDCl_3) δ 3.84 (s, 3H), 2.62 (t, $^3J_{\text{H-H}} = 7.4$ Hz, 2H), 1.73 – 1.61 (m, 2H), 1.36 – 1.16 (m, 10H), 0.86 (t, $^3J_{\text{H-H}} = 7.0$ Hz, 2H).

^{13}C NMR (75 MHz, CDCl_3) δ 186.2, 152.8, 81.1, 77.8, 53.5, 45.4, 31.9, 29.3, 29.1, 28.9, 23.6, 22.7, 14.2.

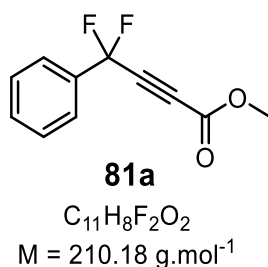
FT-IR (cm^{-1}): 2955, 2925, 2856, 1723, 1687, 1456, 1434, 1403, 1377, 1244, 1134, 1098, 990, 891, 747, 723.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{13}\text{H}_{20}\text{O}_3\text{Na}$) = 247.1305; found: 247.1307 (1 ppm).

3. Synthesis of propargylic fluorides

3.1. Representative procedure: Synthesis of methyl 4,4-difluoro-4-phenylbut-2-ynoate (81a)

To the propargylic ketone **80a** (1 g, 5.31 mmol) one drop of 95% ethanol and DAST (4.2 mL, 31.8 mmol, 6 equiv) were added. The reaction mixture was stirred at 60 °C for 6 h. After coming back to room temperature and hydrolysis, the reaction mixture was extracted with CH₂Cl₂ (3x40mL). The organic layers were separated, washed with water (3x20mL), dried over Na₂SO₄ then filtrated on silica. After purification by chromatography on silica gel, the fluorinated compound **81a** was obtained as a yellow oil (0.73 g, 65%).



R_f = 0.6 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (500 MHz, CDCl₃) δ 7.71 – 7.59 (m, 2H), 7.56 – 7.41 (m, 3H), 3.84 (s, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 152.5, 134.5 (t, ²J_{C-F} = 26.8 Hz), 131.5 (t, J = 1.7 Hz), 128.8, 125.3 (t, ³J_{C-F} = 4.9 Hz), 111.5 (t, ¹J_{C-F} = 235.1 Hz), 78.0 (t, ³J_{C-F} = 5.9 Hz), 77.4 (t, ²J_{C-F} = 44.3 Hz), 53.5.

¹⁹F {¹H} NMR (471 MHz, CDCl₃) δ -79.61 (s).

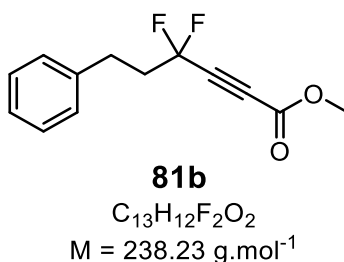
¹⁹F NMR (471 MHz, CDCl₃) δ -79.61 (s).

FT-IR (cm⁻¹): 2960, 2852, 1723, 1454, 1436, 1269, 1243, 1193, 1155, 1038, 1017, 970, 766, 748, 693, 652, 610.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₁H₈O₂F₂Na) = 233.0385; found: 233.0383 (1 ppm).

3.2. Methyl 4,4-difluoro-6-phenylhex-2-ynoate (**81b**)

Starting from 1 g (4.62 mmol) of methyl methyl 4-oxo-6-phenylhex-2-ynoate **80b** we obtained 1.00 g of **81b** (91%) as a yellow oil. Physical data and NMR data are in agreement with literature.⁵⁵

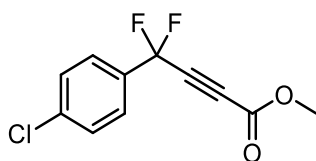


^1H NMR (300 MHz, CDCl_3) δ 7.35 – 7.01 (m, 5H), 3.78 (s, 3H), 2.85 – 2.76 (m, 2H), 2.43 – 2.23 (m, 2H).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -86.55 (s).

3.3. Methyl 4-(4-chlorophenyl)-4,4-difluorobut-2-ynoate (**81c**)

Starting from 1 g (4.49 mmol) of methyl 4-(4-chlorophenyl)-4-oxobut-2-ynoate **80c** we obtained 681 mg of **81c** (62%) as a yellow oil.



81c
 $\text{C}_{11}\text{H}_7\text{ClF}_2\text{O}_2$
 $M = 244.62 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.4 (Cyclohexane / Ethyl Acetate: 9/1).

^1H NMR (500 MHz, CDCl_3) δ 7.59 (d, $^3J_{\text{H-H}} = 8.7 \text{ Hz}$, 2H), 7.45 (d, $^3J_{\text{H-H}} = 8.7 \text{ Hz}$, 2H), 3.84 (s, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 152.3, 137.8, 133.0 (t, $^2J_{\text{C-F}} = 27.5 \text{ Hz}$), 129.3, 126.9 (t, $^3J_{\text{C-F}} = 4.8 \text{ Hz}$), 111.1 (t, $^1J_{\text{C-F}} = 235.5 \text{ Hz}$), 78.5 (t, $^3J_{\text{C-F}} = 5.8 \text{ Hz}$), 77.0 (t, $^2J_{\text{C-F}} = 44.1 \text{ Hz}$), 53.6.

^{19}F {H} NMR (471 MHz, CDCl_3) δ -79.52 (s).

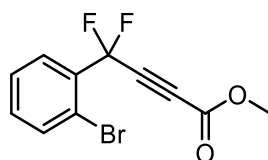
^{19}F NMR (471 MHz, CDCl_3) δ -79.52 (s).

FT-IR (cm^{-1}): 2958, 2926, 2855, 1725, 1603, 1581, 1492, 1435, 1404, 1269, 1243, 1193, 1158, 1092, 1059, 1031, 1011, 970, 870, 829, 748, 720, 693.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{11} \text{H}_7 \text{O}_3 \text{ }^{35}\text{Cl} \text{ Na}$) = 244.99759; found: 244.9979 (1 ppm).

3.4. Methyl 4-(2-bromophenyl)-4,4-difluorobut-2-ynoate (**81d**)

Starting from 1 g (3.74 mmol) of methyl 4-(2-bromophenyl)-4-oxobut-2-ynoate **80d** we obtained 823 mg of **81d** (76%) as a yellow oil.



81d

$C_{11}H_7BrF_2O_2$

$M = 289.08 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.4$ (Cyclohexane / Ethyl Acetate: 9/1).

$^1\text{H NMR}$ (500 MHz, CDCl_3) δ 8.13 – 7.98 (m, 1H), 7.88 – 7.61 (m, 1H), 7.59 – 7.32 (m, 2H), 3.83 (s, 3H).

$^{13}\text{C NMR}$ (126 MHz, CDCl_3) δ 152.5 (t, $^4J_{C-F} = 2.4$ Hz), 135.0, 133.4 (t, $^2J_{C-F} = 26.5$ Hz), 132.6 (t, $^4J_{C-F} = 1.4$ Hz), 127.6, 127.3 (t, $^3J_{C-F} = 7.9$ Hz), 120.8 (t, $^3J_{C-F} = 3.2$ Hz), 110.3 (t, $^1J_{C-F} = 237.4$ Hz), 77.9 (t, $^3J_{C-F} = 6.1$ Hz), 76.7 (t, $^2J_{C-F} = 42.9$ Hz), 53.4.

$^{19}\text{F}\{^1\text{H}\}$ NMR (471 MHz, CDCl_3) δ -83.00 (s).

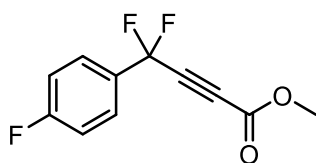
^{19}F NMR (471 MHz, CDCl_3) δ -83.00 (s).

FT-IR (cm^{-1}): 2987, 2957, 2901, 1722, 1591, 1573, 1470, 1435, 1282, 1253, 1234, 1193, 1157, 1130, 1059, 1034, 1014, 969, 872, 760, 747, 722.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($C_{11}H_7O_3BrNa$) = 310.9490; found: 310.9490 (0 ppm).

3.5. Methyl 4,4-difluoro-4-(4-fluorophenyl)but-2-ynoate (**81e**)

Starting from 1 g (4.85 mmol) of methyl 4-(4-fluorophenyl)-4-oxobut-2-ynoate **80e** we obtained 774 mg of **81e** (70%) as a yellow oil.



81e

$C_{11}H_7F_3O_2$

$M = 228.17 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.5$ (Cyclohexane / Ethyl Acetate: 9/1).

$^1\text{H NMR}$ (300 MHz, d_6 acetone) δ 7.86 – 7.78 (m, 2H), 7.42 – 7.33 (m, 2H), 3.86 (s, 3H).

$^{13}\text{C NMR}$ (75 MHz, d_6 acetone) δ 165.4 (dt, $^1J_{C-F} = 250.2$ Hz, $^5J_{C-F} = 2.0$ Hz), 152.6 (t, $^4J_{C-F} = 2.4$ Hz), 131.3 (td, $^2J_{C-F} = 27.5$, $^4J_{C-F} = 3.3$ Hz), 128.8 (dt, $^3J_{C-F} = 9.5$ Hz, $^3J_{C-F} = 4.9$ Hz), 117.1

(d, $^2J_{C-F} = 22.7$ Hz), 112.3 (dd, $^1J_{C-F} = 234.0$ Hz, $^1J_{C-F} = 233.5$ Hz), 79.6 (t, $^3J_{C-F} = 5.9$ Hz), 76.5 (t, $^2J_{C-F} = 43.9$ Hz), 54.0.

^{19}F {H} NMR (282 MHz, CDCl_3) δ -78.42 (d, $^6J_{F-F} = 3.4$ Hz), -108.26 (t, $^6J_{F-F} = 3.4$ Hz).

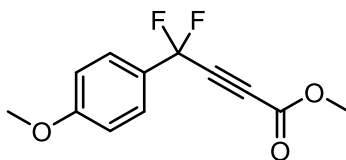
^{19}F NMR (282 MHz, CDCl_3) δ -78.42 (d, $^6J_{F-F} = 3.4$ Hz), -108.19 – -108.33 (m).

FT-IR (cm^{-1}): 2959, 2927, 2854, 1725, 1605, 1512, 1436, 1305, 1269, 1237, 1193, 1159, 1056, 1028, 971, 838.

HRMS (ASAP): calcd. $\text{M}^+(\text{C}_{11}\text{H}_7\text{O}_2\text{F}_3) = 228.0393$; found: 228.0393 (0 ppm).

3.6. Methyl 4,4-difluoro-4-(4-methoxyphenyl)but-2-ynoate (81f)

Starting from 1 g (4.58 mmol) of methyl 4-(4-methoxyphenyl)-4-oxobut-2-ynoate **80f** we obtained 275 mg of **81f** (25%) as a yellow oil.



81f
 $\text{C}_{12}\text{H}_{10}\text{F}_2\text{O}_3$
 $M = 240.21 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.5 (Cyclohexane / Ethyl Acetate: 8/2).

^1H NMR (300 MHz, CDCl_3) δ 7.62 – 7.53 (m, 2H), 6.99 – 6.91 (m, 2H), 3.84 (s, 3H), 3.84 (s, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ 162.0 (t, $^5J_{C-F} = 1.8$ Hz), 152.6 (t, $^4J_{C-F} = 2.3$ Hz), 127.1 (t, $^3J_{C-F} = 4.8$ Hz), 126.7 (t, $^2J_{C-F} = 27.5$ Hz), 114.2 (s), 111.8 (t, $^1J_{C-F} = 234.0$ Hz), 77.9 (t, $^3J_{C-F} = 5.8$ Hz), 77.8 (t, $^2J_{C-F} = 44.7$ Hz), 55.6, 53.5.

^{19}F {H} NMR (282 MHz, CDCl_3) δ -77.38 (s).

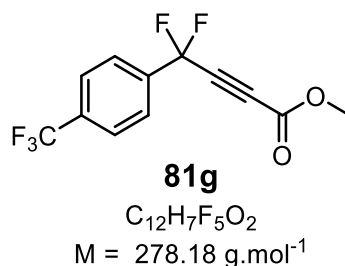
^{19}F NMR (282 MHz, CDCl_3) δ -77.38 (s).

FT-IR (cm^{-1}): 2959, 2933, 2845, 1724, 1611, 1586, 1515, 1461, 1436, 1317, 1276, 1245, 1177, 1153, 1112, 1054, 1020, 969, 941, 870, 833, 748, 716, 613, 595.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{12}\text{H}_{10}\text{O}_3\text{F}_2\text{Na}$) = 263.0490; found: 263.0488 (1 ppm).

3.7. Methyl 4,4-difluoro-4-(4-(trifluoromethyl)phenyl)but-2-ynoate (81g)

Starting from 1 g (3.90 mmol) of methyl 4-oxo-4-(4-(trifluoromethyl)phenyl)but-2-ynoate **80g** we obtained 857 mg of **81g** (79%) as a yellow oil.



R_f = 0.7 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.81 – 7.72 (m, 4H), 3.85 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 152.2 (t, ⁴J_{C-F} = 2.4 Hz), 138.0 (tq, ²J_{C-F} = 27.2 Hz, ⁵J_{C-F} = 1.2 Hz), 133.6 (qt, ²J_{C-F} = 32.8 Hz, ⁵J_{C-F} = 1.7 Hz), 126.1 (q, ³J_{C-F} = 3.8 Hz, 2C), 126.1 (t, ³J_{C-F} = 4.9 Hz, 2C), 123.6 (q, ¹J_{C-F} = 272.6 Hz), 110.8 (t, ¹J_{C-F} = 236.4 Hz), 78.8 (t, ³J_{C-F} = 5.9 Hz), 76.6 (t, ²J_{C-F} = 43.6 Hz), 53.6.

¹⁹F NMR {H} (282 MHz, CDCl₃) δ -63.13 (s), -80.81 (s).

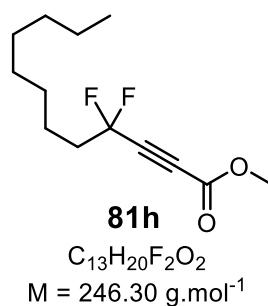
¹⁹F NMR (282 MHz, CDCl₃) δ -63.13 (s), -80.81 (s).

FT-IR (cm⁻¹): 2960, 2928, 2857, 1728, 1623, 1437, 1415, 1378, 1324, 1270, 1247, 1167, 1129, 1067, 1035, 1013, 972, 872, 843, 749, 729.

HRMS (ASAP): calcd. for [M+H]⁺(C₁₂H₈O₂F₅) = 279.0439; found: 279.0439 (0 ppm).

3.8. Methyl 4,4-difluorododec-2-ynoate (**81h**)

Starting from 1 g (4.46 mmol) of methyl 4-oxododec-2-ynoate **80h** we obtained 664 mg of **81h** (60%) as a yellow oil.



R_f = 0.4 (Cyclohexane / Ethyl Acetate: 9/1).

¹H NMR (300 MHz, CDCl₃) δ 3.83 (s, 3H), 2.15 – 1.97 (m, 2H), 1.60 – 1.47 (m, 2H), 1.41 – 1.21 (m, 10H), 0.89 (t, ³J_{H-H} = 6.8 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 152.6 (t, ⁴J_{C-F} = 2.3 Hz), 114.4 (t, ¹J_{C-F} = 235.5 Hz), 77.7 (t, ²J_{C-F} = 42.6 Hz), 76.3 (t, ³J_{C-F} = 6.5 Hz), 53.4, 38.8 (t, ²J_{C-F} = 24.8 Hz), 31.9, 29.3, 29.2, 29.0, 22.8, 22.5 (t, ³J_{C-F} = 3.5 Hz), 14.2.

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -85.94 (s).

¹⁹F NMR (282 MHz, CDCl₃) δ -85.94 (t, ³J_{H-F} = 15.3 Hz).

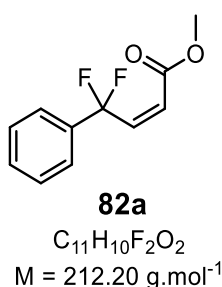
FT-IR (cm⁻¹): 2958, 2928, 2857, 1727, 1459, 1435, 1378, 1314, 1251, 1175, 1125, 1089, 1055, 1012, 984, 938, 748, 644.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₃H₂₀O₂F₂Na) = 269.1324; found: 269.1327 (1 ppm).

4. Synthesis of the alkenes with the *gem*-difluoroalkyl side chains

4.1. Representative procedure: Synthesis of methyl (Z)-4,4-difluoro-4-phenylbut-2-enoate (**82a**)

Methyl 4,4-difluoro-4-phenylbut-2-ynoate **81a** (0.5 g, 2.38 mmol) was stirred with Lindlar catalyst (10%) in methanol (15 mL) under hydrogen gas. The reaction was monitored by TLC and at the end, the reaction mixture was filtrated on celite, and the product was purified by chromatography to obtain the methyl (Z)-4,4-difluoro-4-phenylbut-2-enoate **82a** as a colorless oil (434 mg, 86%).



R_f = 0.4 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (500 MHz, CDCl₃) δ 7.63 – 7.59 (m, 2H), 7.46 – 7.41 (m, 3H), 6.22 (dd, ²J_{H-F} = 25.0, ³J_{H-H} = 12.5 Hz, 1H), 6.13 (dt, ³J_{H-H} = 12.6, ⁴J_{H-F} = 1.3 Hz, 1H), 3.69 (s, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 165.5, 135.9 (t, ²J_{C-F} = 27.1 Hz), 134.7 (t, ²J_{C-F} = 32.3 Hz), 130.4 (t, ⁵J_{C-F} = 1.7 Hz), 128.6 (s, 2C), 125.5 (t, ³J_{C-F} = 5.6 Hz, 2C), 124.9 (t, ³J_{C-F} = 7.1 Hz), 118.6 (t, ¹J_{C-F} = 240.5 Hz), 52.1.

^{19}F {H} NMR (471 MHz, CDCl_3) δ -88.90 (s).

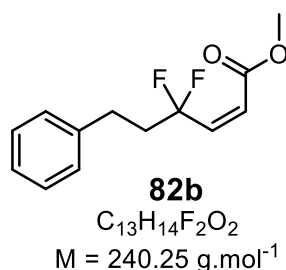
^{19}F NMR (471 MHz, CDCl_3) δ -88.90 (d, $^3J_{\text{H-F}} = 12.5$ Hz).

FT-IR (cm^{-1}): 2988, 2955, 2901, 1734, 1663, 1453, 1437, 1393, 1321, 1260, 1242, 1210, 1182, 1143, 1108, 1085, 1042, 991, 906, 824, 767, 696, 549.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{11}\text{H}_{10}\text{O}_2\text{F}_2\text{Na}$) = 235.05411; found: 235.0540 (0 ppm);
calcd. for $[\text{M}+\text{K}]^+$ ($\text{C}_{11}\text{H}_{10}\text{O}_2\text{F}_2\text{K}$) = 251.0280; found: 251.0279 (1 ppm).

4.2. Methyl (Z)-4,4-difluoro-6-phenylhex-2-enoate (82b)

Starting from 0.5 g (2.10 mmol) of methyl 4,4-difluoro-6-phenylhex-2-ynoate **81b** we obtained 327 mg of **82b** (65%) as a colorless oil.



R_f = 0.3 (Cyclohexane / Ethyl Acetate: 9/1).

^1H NMR (500 MHz, CDCl_3) δ 7.32 – 7.28 (m, 2H), 7.24 – 7.19 (m, 3H), 6.10 – 6.00 (m, 2H), 3.77 (s, 3H), 2.87 – 2.81 (m, 2H), 2.54 – 2.41 (m, 2H).

^{13}C NMR (126 MHz, CDCl_3) δ 165.4, 140.5, 136.4 (t, $^2J_{\text{C-F}} = 30.9$ Hz), 128.7 (2C), 128.5 (2C), 126.4, 125.3 (t, $^3J_{\text{C-F}} = 7.8$ Hz), 120.8 (t, $^1J_{\text{C-F}} = 240.7$ Hz), 52.2, 39.0 (t, $^2J_{\text{C-F}} = 25.5$ Hz), 28.4 (t, $^3J_{\text{C-F}} = 4.4$ Hz).

^{19}F {H} NMR (471 MHz, CDCl_3) δ -93.80 (s).

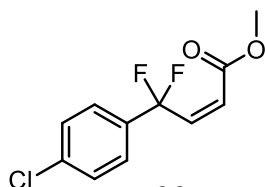
^{19}F NMR (471 MHz, CDCl_3) δ -93.58 to -93.98 (m).

FT-IR (cm^{-1}): 3778, 3457, 3030, 2952, 2856, 2048, 1738, 1636, 1497, 1439, 1400, 1209, 1167, 1132, 1051, 997, 952, 908, 824, 743, 700, 609, 551.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{13}\text{H}_{14}\text{O}_2\text{F}_2\text{Na}$) = 263.0854; found: 263.0854 (0 ppm).

4.3. Methyl (Z)-4-(4-chlorophenyl)-4,4-difluorobut-2-enoate (82c)

Starting from 0.5 g (2.04 mmol) of methyl 4-(4-chlorophenyl)-4,4-difluorobut-2-ynoate **81c** we obtained 474 mg of **82c** (94%) as a colorless oil.



82c

$C_{11}H_9ClF_2O_2$
 $M = 246.64 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.4 (Cyclohexane / Ethyl Acetate: 9/1).

1H NMR (500 MHz, $CDCl_3$) δ 7.57 – 7.53 (m, 2H), 7.43 – 7.37 (m, 2H), 6.20 (dt, $^3J_{H-H} = 12.6$ Hz, $^3J_{H-F} = 11.9$ Hz, 1H), 6.16 (dt, $^3J_{H-H} = 12.6$ Hz, $^4J_{H-F} = 1.1$ Hz, 1H), 3.70 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 165.3, 136.5 (t, $^5J_{C-F} = 2.0$ Hz), 134.7 (t, $^2J_{C-F} = 32.5$ Hz), 134.5 (t, $^2J_{C-F} = 27.7$ Hz), 128.9 (2C), 127.1 (t, $^3J_{C-F} = 5.5$ Hz, 2C), 125.3 (t, $^3J_{C-F} = 7.2$ Hz), 118.2 (t, $^1J_{C-F} = 240.8$ Hz), 52.2.

^{19}F {H} NMR (471 MHz, $CDCl_3$) δ -88.48 (s).

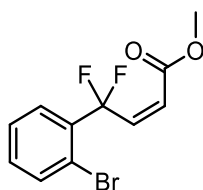
^{19}F NMR (471 MHz, $CDCl_3$) δ -88.48 (d, $^3J_{H-F} = 11.9$ Hz).

FT-IR (cm^{-1}): 2954, 2928, 2853, 1736, 1664, 1603, 1581, 1492, 1437, 1395, 1258, 1209, 1181, 1144, 1092, 1045, 992, 907, 829, 769.

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{11}H_9O_2F_2ClNa$) = 269.0151; found: 269.0152 (0 ppm).

4.4. Methyl (Z)-4-(2-bromophenyl)-4,4-difluorobut-2-enoate (82d)

Starting from 0.5 g (1.73 mmol) of methyl 4-(2-bromophenyl)-4,4-difluorobut-2-ynoate **81d** we obtained 176 mg of **82d** (35% from 40% conversion) as a colorless oil.



82d

$C_{11}H_9BrF_2O_2$
 $M = 291.09 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.4 (Cyclohexane / Ethyl Acetate: 9/1).

1H NMR (300 MHz, $CDCl_3$) δ 7.82 (ddd, $^3J_{H-H} = 7.9$ Hz, $^4J_{H-H} = 1.2$ Hz, $^5J_{H-H} = 0.5$ Hz, 1H), 7.61 (dd, $^3J_{H-H} = 7.9$ Hz, $^4J_{H-H} = 0.9$ Hz, 1H), 7.42 (ddd, $^3J_{H-H} = 8.0$ Hz, $^3J_{H-H} = 7.4$ Hz, $^3J_{H-H} =$

1.2 Hz, 1H), 7.33 – 7.25 (m, 1H), 6.74 (dt, $^3J_{H-H} = 12.6$ Hz, $^3J_{H-F} = 11.7$ Hz, 1H), 6.10 (dt, $^3J_{H-H} = 12.6$ Hz, $^4J_{H-F} = 1.6$ Hz, 1H), 3.59 (s, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ 164.5 (t, $^4J_{C-F} = 1.4$ Hz), 137.4 (t, $^2J_{C-F} = 32.5$ Hz), 136.1 (t, $^2J_{C-F} = 26.6$ Hz), 134.2, 131.6 (t, $^4J_{C-F} = 1.7$ Hz), 128.6 (t, $^3J_{C-F} = 8.3$ Hz), 126.9, 124.8 (t, $^3J_{C-F} = 8.5$ Hz), 120.0 (t, $^3J_{C-F} = 4.2$ Hz), 117.4 (t, $^1J_{C-F} = 241.7$ Hz), 51.9.

^{19}F {H} NMR (282 MHz, CDCl_3) δ -87.30 (s).

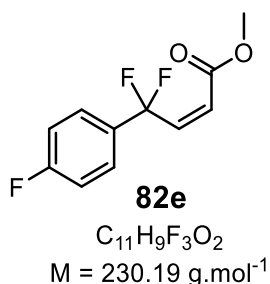
^{19}F NMR (282 MHz, CDCl_3) δ -87.30 (d, $^3J_{H-F} = 11.7$ Hz).

FT-IR (cm^{-1}): 2988, 2953, 2921, 2851, 1734, 1658, 1592, 1572, 1471, 1436, 1395, 1280, 1204, 1147, 1115, 1068, 1032, 1008, 989, 952, 907, 821, 759, 736.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{11}\text{H}_9\text{O}_2\text{F}_2\text{BrNa}$) = 312.9646; found: 312.9647 (0 ppm).
 $[\text{M}+\text{K}]^+$ ($\text{C}_{11}\text{H}_9\text{O}_2\text{F}_2\text{BrK}$) = 328.9386; found: 328.9381 (1 ppm).

4.5. Methyl (E)-4,4-difluoro-4-(4-fluorophenyl)but-2-enoate (**82e**)

Starting from 0.5 g (2.19 mmol) of methyl 4,4-difluoro-4-(4-fluorophenyl)but-2-ynoate **81e** we obtained 379 mg of **82e** (75%) as a pale yellow oil.



R_f = 0.4 (Cyclohexane / Ethyl Acetate: 9/1).

^1H NMR (300 MHz, CDCl_3) δ 7.64 – 7.56 (m, 2H), 7.16 – 7.07 (m, 2H), 6.28 – 6.10 (m, 2H), 3.70 (s, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ 165.4 (t, $^4J_{C-F} = 1.3$ Hz), 163.9 (dt, $^1J_{C-F} = 249.9$ Hz, $^5J_{C-F} = 2.0$ Hz), 134.7 (t, $^2J_{C-F} = 32.5$ Hz), 132.0 (td, $^2J_{C-F} = 27.8$ Hz, $^4J_{C-F} = 3.3$ Hz), 127.9 (dt, $^3J_{C-F} = 8.7$ Hz, $^3J_{C-F} = 5.6$ Hz), 125.1 (t, $^3J_{C-F} = 7.2$ Hz), 118.3 (t, $^1J_{C-F} = 240.4$ Hz), 115.7 (d, $^2J_{C-F} = 22.1$ Hz), 52.2.

^{19}F {H} NMR (282 MHz, CDCl_3) δ -87.68 (d, $^6J_{F-F} = 3.4$ Hz), -110.40 (t, $^6J_{F-F} = 3.4$ Hz).

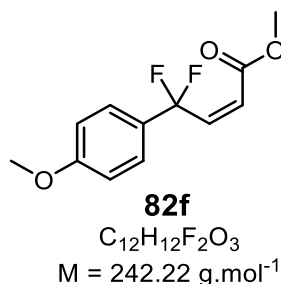
^{19}F NMR (282 MHz, CDCl_3) δ -87.68 (dd, $^3J_{F-H} = 11.1$, $^6J_{F-F} = 3.4$ Hz), -110.33 to -110.46 (m).

FT-IR (cm⁻¹): 2988, 2956, 2901, 1735, 1663, 1606, 1512, 1438, 1393, 1305, 1257, 1231, 1210, 1161, 1143, 1099, 1043, 995, 907, 839, 815, 613, 577, 546.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₁H₉O₂F₃Na) = 253.0447; found: 253.0448 (0 ppm).

4.6. Methyl (E)-4,4-difluoro-4-(4-methoxyphenyl)but-2-enoate (82f)

Starting from 0.5 g (2.08 mmol) of methyl 4,4-difluoro-4-(4-methoxyphenyl)but-2-ynoate **81f** we obtained 398 mg of **82f** (79%) as a colorless oil.



R_f = 0.5 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.57 – 7.50 (m, 2H), 6.97 – 6.90 (m, 2H), 6.26 – 6.07 (m, 2H), 3.82 (s, 3H), 3.69 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 165.6, 161.1 (t, ⁵J_{C-F} = 1.7 Hz), 134.9 (t, ²J_{C-F} = 32.9 Hz), 128.1 (t, ²J_{C-F} = 27.6 Hz), 127.1 (t, ³J_{C-F} = 5.5 Hz), 124.5 (t, ³J_{C-F} = 7.0 Hz), 118.8 (t, ¹J_{C-F} = 239.8 Hz), 113.9, 55.4, 52.1.

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -87.25 (s).

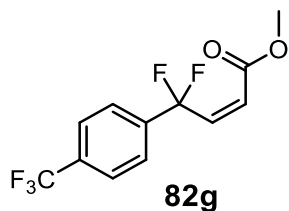
¹⁹F NMR (282 MHz, CDCl₃) δ -87.25 (d, ³J_{F-H} = 11.6 Hz).

FT-IR (cm⁻¹): 3671, 2956, 2912, 2842, 1735, 1662, 1613, 1586, 1515, 1461, 1437, 1392, 1308, 1249, 1209, 1176, 1142, 1110, 1018, 990, 905, 833, 613, 584, 553.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₂H₁₂O₃F₂Na) = 265.0647; found: 265.0649 (1 ppm); [M+K]⁺ (C₁₂H₁₂O₃F₂K) = 281.0386; found: 281.0385 (0 ppm).

4.7. Methyl (E)-4,4-difluoro-4-(4-(trifluoromethyl)phenyl)but-2-enoate (82g)

Starting from 0.5 g (1.80 mmol) of methyl 4,4-difluoro-4-(4-(trifluoromethyl)phenyl)but-2-ynoate **81g** we obtained 403 mg of **82g** (80%) as a yellow oil.



82g
 $C_{12}H_9F_5O_2$
 $M = 280.19 \text{ g}\cdot\text{mol}^{-1}$

$R_f \equiv 0.7$ (Cyclohexane / Ethyl Acetate: 8/2).

$^1\text{H NMR (500 MHz, CDCl}_3)$ δ 7.75 (d, $^3J_{H-H} = 8.3 \text{ Hz}$, 2H), 7.71 (d, $^3J_{H-H} = 8.4 \text{ Hz}$, 2H), 6.37 – 5.95 (m, 2H), 3.70 (s, 3H).

$^{13}\text{C NMR (126 MHz, CDCl}_3)$ δ 165.1, 139.6 (t, $^2J_{C-F} = 27.1 \text{ Hz}$), 134.5 (t, $^2J_{C-F} = 32.2 \text{ Hz}$), 132.5 (q, $^2J_{C-F} = 32.6 \text{ Hz}$), 126.2 (t, $^3J_{C-F} = 5.5 \text{ Hz}$, 2C), 125.8 (t, $^3J_{C-F} = 6.9 \text{ Hz}$), 125.7 (q, $^3J_{C-F} = 3.8 \text{ Hz}$, 2C), 123.8 (q, $^1J_{C-F} = 272.4 \text{ Hz}$), 118.0 (t, $^1J_{C-F} = 241.4 \text{ Hz}$), 52.2.

$^{19}\text{F \{H\} NMR (282 MHz, CDCl}_3)$ δ -62.98 (s), -89.23 (s).

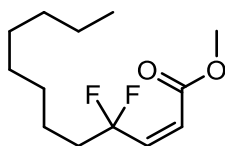
$^{19}\text{F NMR (282 MHz, CDCl}_3)$ δ -62.98 (s), -89.23 (d, $^3J_{F-H} = 11.2 \text{ Hz}$).

$\text{FT-IR (cm}^{-1}\text{)}$: 3001, 2957, 2920, 1737, 1664, 1623, 1438, 1414, 1395, 1324, 1259, 1214.5, 1168, 1126, 1066, 994, 908, 844, 770, 608.

HRMS (ESI) : calcd. for $[\text{M}+\text{Na}]^+$ ($C_{12}H_9O_2F_5\text{Na}$) = 303.0415; found: 303.0414 (0 ppm).

4.8. Methyl (E)-4,4-difluorododec-2-enoate (82h)

Starting from 0.5 g (1.80 mmol) of methyl 4,4-difluorododec-2-ynoate **81h** we obtained 358 mg of **82h** (71%) as a yellow oil.



82h
 $C_{13}H_{22}F_2O_2$
 $M = 248.31 \text{ g}\cdot\text{mol}^{-1}$

$R_f \equiv 0.3$ (Cyclohexane / Ethyl Acetate: 9/1).

$^1\text{H NMR (300 MHz, CDCl}_3)$ δ 6.14 – 5.93 (m, 2H), 3.76 (s, 3H), 1.54 – 1.40 (m, 2H), 1.37 – 1.20 (m, 10H), 0.88 (t, $^3J_{H-H} = 6.9 \text{ Hz}$, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ 165.6 (t, $^4J_{\text{C-F}} = 1.3$ Hz), 136.5 (t, $^2J_{\text{C-F}} = 31.0$ Hz), 124.9 (t, $^3J_{\text{C-F}} = 7.7$ Hz), 121.4 (t, $^1J_{\text{C-F}} = 240.1$ Hz), 52.1, 37.3 (t, $^2J_{\text{C-F}} = 25.4$ Hz), 32.0, 29.5, 29.4, 29.3, 22.8, 22.2 (t, $^3J_{\text{C-F}} = 4.0$ Hz), 14.2.

^{19}F {H} NMR (282 MHz, CDCl_3) δ -93.32 (s).

^{19}F NMR (282 MHz, CDCl_3) δ -93.47 to -93.18 (m).

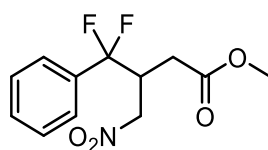
FT-IR (cm^{-1}): 3671, 3649, 2955, 2926, 2856, 1738, 1664, 1459, 1437, 1400, 1379, 1310, 1206, 1171, 1145, 1096, 1054, 994, 934, 908, 824, 751, 723.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{13}\text{H}_{22}\text{O}_2\text{F}_2\text{Na}$) = 271.1480; found: 271.1482 (1 ppm).

5. Nitromethane addition on the alkenes with the *gem*-difluoroalkyl chains

5.1. Representative procedure: Synthesis of methyl 4,4-difluoro-3-(nitromethyl)-4-phenylbutanoate (**83a**)

To methyl (*Z*)-4,4-difluoro-4-phenylbut-2-enoate **82a** (150 mg, 0.7 mmol) was added nitromethane (75 μl , 1.4 mmol) and potassium carbonate (289 mg, 2.1 mmol) in DMSO (3 ml). The reaction mixture was stirred at room temperature and it was monitored by TLC. At the end concentrated NH_4Cl (10 mL) was added and the reaction mixture was extracted with ethyl acetate (3x20mL). The organic layers were separated, washed with water (3x10mL), dried over Na_2SO_4 and concentrated under vacuum. After purification by chromatography on silica gel, methyl 4,4-difluoro-3-(nitromethyl)-4-phenylbutanoate **83a** (174 mg, 90%) was obtained as a yellow oil.



83a

$\text{C}_{12}\text{H}_{13}\text{F}_2\text{NO}_4$
 $M = 273.24 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.4 (Cyclohexane / Ethyl Acetate: 7/3).

^1H NMR (300 MHz, CDCl_3) δ 7.55 – 7.45 (m, 5H), 4.73 (dd, $^2J_{\text{H-H}} = 13.8$ Hz, $^3J_{\text{H-H}} = 5.8$ Hz, 1H), 4.53 (dd, $^2J_{\text{H-H}} = 13.8$ Hz, $^3J_{\text{H-H}} = 6.2$ Hz, 1H), 3.77 – 3.57 (m, 1H), 3.62 (s, 3H), 2.66 (dd, $^2J_{\text{H-H}} = 16.9$ Hz, $^3J_{\text{H-H}} = 5.2$ Hz, 1H), 2.50 (dd, $^2J_{\text{H-H}} = 16.9$, $^3J_{\text{H-H}} = 8.2$ Hz, 1H).

^{13}C NMR (75 MHz, CDCl_3) δ 170.8, 133.9 (t, $^2J_{\text{C-F}} = 25.8$ Hz), 131.0, 129.0 (2C), 125.5 (t, $^3J_{\text{C-F}} = 6.4$ Hz, 2C), 123.7 (dd, $^1J_{\text{C-F}} = 247.4$ Hz), 73.4 (t, $^3J_{\text{C-F}} = 3.4$ Hz), 52.3, 42.6 (t, $^2J_{\text{C-F}} = 27.1$ Hz), 31.4 (dd, $^3J_{\text{C-F}} = 4.2, 3.0$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -98.05 (AB system, $^2J_{\text{F-F}} = 250.8$ Hz), -104.55 (AB system, $^2J_{\text{F-F}} = 250.8$ Hz).

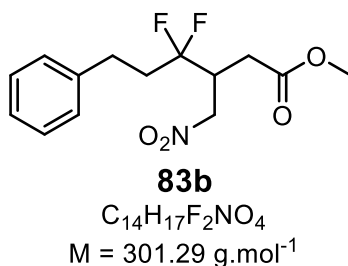
^{19}F NMR (282 MHz, CDCl_3) δ -98.05 (ABX system, dd, $^2J_{\text{F-F}} = 250.8$ Hz, $^3J_{\text{F-H}} = 11.3$ Hz), -104.55 (ABX system, dd, $^2J_{\text{F-F}} = 250.8$ Hz, $^3J_{\text{F-H}} = 17.4$ Hz).

FT-IR (cm^{-1}): 2956, 2928, 1735, 1556, 1452, 1437, 1379, 1320, 1274, 1231, 1213, 1169, 1132, 1049, 982, 953, 923, 894, 765, 697, 632.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{12}\text{H}_{13}\text{NO}_4\text{F}_2\text{Na}$) = 296.0705; found: 296.0706 (0 ppm).

5.2. Methyl 4,4-difluoro-3-(nitromethyl)-6-phenylhexanoate (**83b**)

Starting from 150 mg (0.62 mmol) of methyl 4,4-difluoro-6-phenylhex-2-enoate **82b** we obtained 150 mg of **83b** (80%) as a yellow oil.



R_f = 0.3 (Cyclohexane / Ethyl Acetate: 8/2).

^1H NMR (500 MHz, CDCl_3) δ 7.34 – 7.29 (m, 2H), 7.25 – 7.17 (m, 3H), 4.63 (dd, $^2J_{\text{H-H}} = 14.0$ Hz, $^3J_{\text{H-H}} = 5.9$ Hz, 1H), 4.43 (dd, $^2J_{\text{H-H}} = 14.0$ Hz, $^3J_{\text{H-H}} = 6.1$ Hz, 1H), 3.72 (s, 3H), 3.48 – 3.36 (m, 1H), 2.85 (dd, $J = 8.0$ Hz, 0.4 Hz, 2H), 2.64 (dd, $^2J_{\text{H-H}} = 17.0$ Hz, $^3J_{\text{H-H}} = 5.1$ Hz, 1H), 2.42 (dd, $^2J_{\text{H-H}} = 17.0$ Hz, $^3J_{\text{H-H}} = 8.0$ Hz, 1H), 2.26 – 2.12 (m, 2H).

^{13}C NMR (126 MHz, CDCl_3) δ 170.9, 139.9, 128.8, 128.4, 126.6, 123.6 (t, $^1J_{\text{C-F}} = 246.0$ Hz), 73.5 (t, $^3J_{\text{C-F}} = 4.7$ Hz), 52.4, 40.5 (t, $^2J_{\text{C-F}} = 24.6$ Hz), 36.6 (t, $^2J_{\text{C-F}} = 24.5$ Hz), 31.4 (t, $^3J_{\text{C-F}} = 4.3$ Hz), 27.8 (t, $^3J_{\text{C-F}} = 5.0$ Hz).

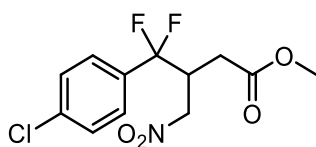
^{19}F {H} NMR (471 MHz, CDCl_3) δ -103.12 (AB system, $^2J_{\text{F-F}} = 249.7$ Hz), -104.86 (AB system, $^2J_{\text{F-F}} = 249.7$ Hz).

FT-IR (cm^{-1}): 3779, 3453, 2050, 1738, 1635, 1563, 1438, 1383, 1210, 1053, 950, 700, 612.

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{14}H_{17}NO_4F_2Na$) = 324.1018; found: 324.1018 (0 ppm).

5.3. Methyl 4-(4-chlorophenyl)-4,4-difluoro-3-(nitromethyl)butanoate (83c)

Starting from 150 mg (0.61 mmol) of methyl 4-(4-chlorophenyl)-4,4-difluorobut-2-enoate **82c** we obtained 164 mg of **83c** (88%) as a yellow oil.



83c

$C_{12}H_{12}ClF_2NO_4$
 $m = 307.68 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.5 (Cyclohexane / Ethyl Acetate: 7/3).

¹H NMR (500 MHz, CDCl₃) δ 7.52 – 7.30 (m, 4H), 4.72 (dd, ²J_{H-H} = 13.9 Hz, ³J_{H-H} = 5.9 Hz, 1H), 4.53 (dd, ³J_{H-H} = 13.9 Hz, 6.0 Hz, 1H), 3.70 – 3.58 (m, 1H), 3.63 (s, 3H), 2.63 (dd, ²J_{H-H} = 17.0 Hz, ³J_{H-H} = 5.2 Hz, 1H), 2.50 (dd, ²J_{H-H} = 17.0 Hz, ³J_{H-H} = 8.2 Hz, 1H).

¹³C NMR (75 MHz, CDCl₃) δ 170.6, 137.3 (t, ⁵J_{C-F} = 2.2 Hz), 132.4 (t, ²J_{C-F} = 26.4 Hz), 129.3, 127.1 (dd, ³J_{C-F} = 6.9 Hz, ³J_{C-F} = 5.9 Hz), 121.8 (dd, ¹J_{C-F} = 248.2 Hz, ¹J_{C-F} = 247.6 Hz), 73.2 (t, ³J_{C-F} = 3.5 Hz), 52.4, 42.5 (t, ²J_{C-F} = 26.9 Hz), 31.3 (dd, ³J_{C-F} = 4.4 Hz, ³J_{C-F} = 2.9 Hz).

¹⁹F {H} NMR (471 MHz, CDCl₃) -97.58 (AB system, ²J_{F-F} = 252.0 Hz), -104.61 (AB system, ²J_{F-F} = 252.0 Hz).

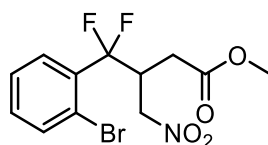
¹⁹F NMR (471 MHz, CDCl₃) δ -97.58 (ABX system, dd, ²J_{F-F} = 252.0 Hz, ³J_{F-H} = 11.0 Hz), -104.61 (ABX system, dd, ²J_{F-F} = 252.0 Hz, ³J_{F-H} = 17.9 Hz).

FT-IR (cm⁻¹): 2957, 2917, 2850, 1735, 1604, 1556, 1494, 1437, 1403, 1379, 1319, 1274, 1230, 1213, 1167, 1092, 1056, 1017, 982, 951, 895, 830, 718.

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{12}H_{12}NO_4F_2ClNa$) = 330.0315; found: 330.0314 (0 ppm).

5.4. Methyl 4-(2-bromophenyl)-4,4-difluoro-3-(nitromethyl)butanoate (83d)

Starting from 150 mg (0.52 mmol) of methyl 4-(2-bromophenyl)-4,4-difluorobut-2-enoate **82d** we obtained 143 mg of **83d** (79%) as a yellow oil.



83d

$C_{12}H_{12}BrF_2NO_4$

$M = 352.13 \text{ g.mol}^{-1}$

R_f = 0.3 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (500 MHz, CDCl₃) δ 7.69 (d, ³J_{H-H} = 7.9 Hz, 1H), 7.55 (dd, ³J_{H-H} = 7.9 Hz, ⁴J_{H-H} = 1.5 Hz, 1H), 7.40 (dd, ³J_{H-H} = 7.9 Hz, ³J_{H-H} = 7.5 Hz, 1H), 7.33 (dd, ³J_{H-H} = 7.9 Hz, ³J_{H-H} = 7.5 Hz, 1H), 4.77 (dd, ²J_{H-H} = 13.8 Hz, ³J_{H-H} = 5.7 Hz, 1H), 4.58 (dd, ²J_{H-H} = 13.8, ³J_{H-H} = 6.0 Hz, 1H), 4.34 – 4.20 (m, 1H), 3.59 (s, 3H), 2.63 (dd, ²J_{H-H} = 16.9 Hz, ³J_{H-H} = 5.5 Hz, 1H), 2.55 (dd, ²J_{H-H} = 16.9 Hz, ³J_{H-H} = 8.0 Hz, 1H).

¹³C NMR (126 MHz, CDCl₃) δ 170.5, 135.4, 132.6 (t, ²J_{C-F} = 25.2 Hz), 132.4, 128.5 (dd, ³J_{C-F} = 10.5 Hz, ³J_{C-F} = 8.6 Hz), 127.6, 121.5 (t, ¹J_{C-F} = 249.5 Hz), 120.3 (t, ³J_{C-F} = 3.9 Hz), 73.2 (t, ³J_{C-F} = 3.7 Hz), 52.3, 40.1 (t, ²J_{C-F} = 24.9 Hz), 31.4 (t, ³J_{C-F} = 3.6 Hz).

¹⁹F {¹H} NMR (471 MHz, CDCl₃) δ -98.62 (AB system, ²J_{F-F} = 254.5 Hz), -102.49 (AB system, ²J_{F-F} = 254.5 Hz).

¹⁹F NMR (471 MHz, CDCl₃) δ -98.62 (ABX system, dd, ²J_{F-F} = 254.5 Hz, ³J_{F-H} = 11.1 Hz), -102.49 (ABX system, dd, ²J_{F-F} = 254.5 Hz, ³J_{F-H} = 17.7 Hz).

FT-IR (cm⁻¹): 2956, 2927, 1735, 1592, 1556, 1472, 1435, 1378, 1283, 1228, 1214, 1164, 1127, 1066, 1048, 1029, 999, 977, 953, 761.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₂H₁₂NO₄F₂BrNa) = 373.9810; found: 373.9809 (0 ppm).

5.5. Methyl 4,4-difluoro-4-(4-fluorophenyl)-3-(nitromethyl)butanoate (83e)

Starting from 150 mg (0.65 mmol) of methyl 4,4-difluoro-4-(4-fluorophenyl)but-2-enoate **82e** we obtained 165 mg of **83e** (87%) as a yellow oil.



83e

$C_{12}H_{12}F_3NO_4$

$M = 291.23 \text{ g.mol}^{-1}$

R_f = 0.3 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.54 – 7.44 (m, 2H), 7.20 – 7.10 (m, 2H), 4.72 (dd, ²J_{H-H} = 13.9 Hz, ³J_{H-H} = 6.0 Hz, 1H), 4.52 (dd, ²J_{H-H} = 13.9 Hz, ³J_{H-H} = 6.0 Hz, 1H), 3.75 – 3.54 (m, 1H), 3.63 (s, 3H), 2.64 (dd, ²J_{H-H} = 17.0 Hz, ³J_{H-H} = 5.3 Hz, 1H), 2.49 (dd, ³J_{H-H} = 16.9 Hz, ³J_{H-H} = 8.1 Hz, 1H).

¹³C NMR (75 MHz, CDCl₃) δ 170.6 (d, ⁴J_{C-F} = 0.5 Hz), 164.1 (dt, ¹J_{C-F} = 251.2 Hz, ⁵J_{C-F} = 2.0 Hz), 130.0 (td, ²J_{C-F} = 26.5, ⁴J_{C-F} = 3.3 Hz), 127.9 (ddd, ³J_{C-F} = 8.8 Hz, ³J_{C-F} = 6.9 Hz, ³J_{C-F} = 6.0 Hz), 121.8 (t, ¹J_{C-F} = 247.7 Hz), 116.2 (d, ²J_{C-F} = 22.1 Hz), 73.3 (t, ³J_{C-F} = 3.5 Hz), 52.4, 42.6 (t, ²J_{C-F} = 27.1 Hz), 31.4 (dd, ³J_{C-F} = 4.4 Hz, ³J_{C-F} = 2.9 Hz).

¹⁹F {H} NMR (282 MHz, CDCl₃) δ -97.00 (ABX system, dd, ²J_{F-F} = 251.8 Hz, ⁶J_{F-F} = 3.0 Hz), -103.58 (ABX system, dd, ²J_{F-F} = 251.8 Hz, ⁶J_{F-F} = 2.2 Hz), -109.09 (t, ⁶J_{F-F} = 2.7 Hz).

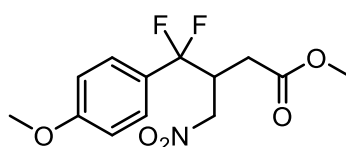
¹⁹F NMR (282 MHz, CDCl₃) δ -97.00 (ABXX' system, ddd, ²J_{F-F} = 251.8 Hz, ³J_{F-H} = 11.4 Hz, ⁶J_{F-F} = 3.0 Hz), -103.58 (ABXX' system, ddd, ²J_{F-F} = 251.8 Hz, ³J_{F-H} = 17.6 Hz, ⁶J_{F-F} = 2.2 Hz), -109.01 to -109.16 (m).

FT-IR (cm⁻¹): 2958, 1736, 1609, 1557, 1514, 1437, 1379.9, 1320, 1299.1, 1274.7, 1229.9, 1162.4, 1104, 1054, 985, 952, 895, 840, 603, 567.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₂H₁₂NO₄F₃Na) = 314.0611; found: 314.0612 (0 ppm).

5.6. Methyl 4,4-difluoro-4-(4-methoxyphenyl)-3-(nitromethyl)butanoate (**83f**)

Starting from 150 mg (0.62 mmol) of methyl 4,4-difluoro-4-(4-methoxyphenyl)but-2-enoate **82f** we obtained 162 mg of **83f** (86%) as a yellow oil.



83f

C₁₃H₁₅F₂NO₅
M = 303.26 g.mol⁻¹

R_f = 0.4 (Cyclohexane / Ethyl Acetate: 7/3).

¹H NMR (300 MHz, CDCl₃) δ 7.41 (d, ³J_{H-H} = 8.9 Hz, 2H), 6.95 (d, ³J_{H-H} = 8.9 Hz, 2H), 4.71 (dd, ²J_{H-H} = 13.8 Hz, ³J_{H-H} = 5.8 Hz, 1H), 4.51 (dd, ²J_{H-H} = 13.8 Hz, ³J_{H-H} = 6.2 Hz, 1H), 3.84 (s, 3H), 3.76 – 3.53 (m, 1H, H₈), 3.63 (s, 3H), 2.66 (dd, ²J_{H-H} = 16.9 Hz, ³J_{H-H} = 5.2 Hz, 1H), 2.48 (dd, ²J_{H-H} = 16.9 Hz, ³J_{H-H} = 8.3 Hz, 1H).

¹³C NMR (75 MHz, CDCl₃) δ 170.7, 161.3 (t, ⁵J_{C-F} = 1.7 Hz), 126.9 (dd, ³J_{C-F} = 6.8 Hz, ³J_{C-F} = 6.0 Hz), 125.8 (t, ²J_{C-F} = 26.4 Hz), 122.1 (dd, ¹J_{C-F} = 247.6 Hz, ¹J_{C-F} = 247.0 Hz), 114.1, 73.4 (t, ³J_{C-F} = 3.5 Hz), 55.4, 52.2, 42.6 (t, ²J_{C-F} = 27.7 Hz), 31.4 (dd, ³J_{C-F} = 4.2 Hz, ³J_{C-F} = 2.9 Hz).

¹⁹F {H} NMR (282 MHz, CDCl₃) δ -96.83 (AB system, ²J_{F-F} = 250.0 Hz), -102.84 (AB system, ²J_{F-F} = 250.0 Hz).

¹⁹F NMR (282 MHz, CDCl₃) δ -96.83 (ABX system, dd, ²J_{F-F} = 250.0 Hz, ³J_{F-H} = 11.6 Hz), -102.84 (ABX system, dd, ²J_{F-F} = 250.0 Hz, ³J_{F-H} = 17.0 Hz).

FT-IR (cm⁻¹): 3671, 2958, 2923, 2846, 1735, 1614, 1557, 1517, 1461, 1437, 1380, 1309, 1253, 1214, 1178, 1118, 1051, 1029, 981, 951, 895, 834, 634, 607, 573.

HRMS (ESI): calcd. For [M+K]⁺ (C₁₃H₁₅NO₅F₂K) = 342.0550; found: 342.0550 (0 ppm).

5.7. Methyl 4,4-difluoro-3-(nitromethyl)-4-(4-(trifluoromethyl)phenyl) butanoate (83g)

Starting from 150 mg (0.54 mmol) of methyl 4,4-difluoro-4-(4-(trifluoromethyl)phenyl)but-2-enoate **82f** we obtained 155 mg of **83f** (85%) as a yellow oil.



83g

C₁₃H₁₂F₅NO₄
M = 341.23 g.mol⁻¹

R_f = 0.3 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.78 – 7.72 (m, 2H), 7.68 – 7.61 (m, 2H), 4.75 (dd, ²J_{H-H} = 14.0 Hz, ³J_{H-H} = 6.0 Hz, 1H), 4.55 (dd, ²J_{H-H} = 13.9 Hz, ³J_{H-H} = 6.0 Hz, 1H), 3.78 – 3.58 (m, 1H), 3.61 (s, 3H), 2.63 (dd, ²J_{H-H} = 17.0 Hz, ³J_{H-H} = 5.4 Hz, 1H), 2.51 (dd, ²J_{H-H} = 17.0, ³J_{H-H} = 8.0 Hz, 1H).

¹³C NMR (126 MHz, CDCl₃) δ 170.4, 137.62 (t, ²J_{C-F} = 26.3 Hz), 133.25 (q, ²J_{C-F} = 33.0 Hz), 126.3 (t, ³J_{C-F} = 12.8 Hz), 126.1 (q, ³J_{C-F} = 3.7 Hz), 123.6 (q, ¹J_{C-F} = 272.5 Hz), 121.5 (t, ¹J_{C-F}

= 248.2 Hz), 73.1 (t, $^3J_{C-F} = 3.5$ Hz), 52.4, 42.4 (t, $^2J_{C-F} = 26.4$ Hz), 31.3 (dd, $^3J_{C-F} = 4.3$ Hz, $^3J_{C-F} = 2.9$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -63.10 (s), -97.95 (AB system, $^2J_{F-F} = 253.5$ Hz), -105.31 (AB system, $^2J_{F-F} = 253.5$ Hz).

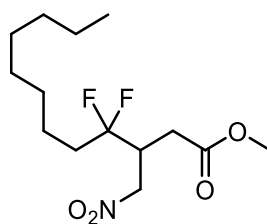
^{19}F NMR (282 MHz, CDCl_3) δ -63.10 (s), -97.95 (AB system, d, $^2J_{F-F} = 253.5$ Hz, $^3J_{F-H} = 10.9$ Hz), -105.31 (AB system, d, $^2J_{F-F} = 253.5$ Hz, $^3J_{F-H} = 18.2$ Hz).

FT-IR (cm^{-1}): 3671, 2959, 2923, 1737, 1624, 1559, 1524, 1438, 1413, 1380, 1323, 1276, 1233, 1216, 1167, 1127, 1067, 1019, 985, 896, 843.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{13}\text{H}_{12}\text{NO}_4\text{F}_5\text{Na}$) = 364.0578; found: 364.0577 (0 ppm).

5.8. Methyl 4,4-difluoro-3-(nitromethyl)dodecanoate (**83h**)

Starting from 150 mg (0.60 mmol) of methyl-4,4-difluorododec-2-enoate **82h** we obtained 131 mg of **83h** (70%) as a yellow oil.



83h

$\text{C}_{14}\text{H}_{25}\text{F}_2\text{NO}_4$
M = 309.35 g.mol $^{-1}$

R_f = 0.3 (Cyclohexane / Ethyl Acetate: 8/2).

^1H NMR (300 MHz, CDCl_3) δ 4.70 (dd, $^2J_{H-H} = 13.9$ Hz, $^3J_{H-H} = 5.8$ Hz, 1H), 4.50 (dd, $^2J_{H-H} = 13.9$ Hz, $^3J_{H-H} = 6.2$ Hz, 1H), 3.72 (s, 3H), 3.46 – 3.24 (m, 1H), 2.71 (dd, $^2J_{H-H} = 16.9$ Hz, $^3J_{H-H} = 5.1$ Hz, 1H), 2.50 (dd, $^2J_{H-H} = 16.9$ Hz, $^3J_{H-H} = 8.1$ Hz, 1H), 1.95 – 1.75 (m, 2H), 1.55 – 1.40 (m, 2H), 1.39 – 1.17 (m, 10H), 0.88 (t, $^3J_{H-H} = 6.8$ Hz, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ 171.1, 124.1 (t, $^1J_{C-F} = 245.7$ Hz), 73.6 (t, $^3J_{C-F} = 4.8$ Hz), 52.5, 40.4 (t, $^2J_{C-F} = 24.9$ Hz), 34.7 (t, $^2J_{C-F} = 24.6$ Hz), 31.9, 31.6 (dd, $^3J_{C-F} = 5.0$ Hz, $^3J_{C-F} = 3.8$ Hz), 29.4, 29.3, 29.2, 22.8, 21.7 (dd, $^3J_{C-F} = 4.8$ Hz, $^3J_{C-F} = 4.2$ Hz), 14.2.

^{19}F NMR {H} (282 MHz, CDCl_3) δ -102.56 (AB system, $^2J_{F-F} = 249.2$ Hz), -104.31 (AB system, $^2J_{F-F} = 249.2$ Hz).

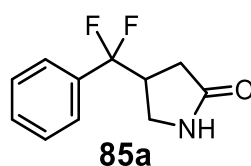
FT-IR (cm⁻¹): 2956, 2926, 2856, 1738, 1558, 1460, 1437, 1379, 1328, 1295, 1200, 1176, 1107, 1071, 993, 928.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₄H₂₅NO₄F₂Na) = 332.1644; found: 332.1644 (0 ppm).

6. Procedure for the reduction of the nitro group

6.1. Representative procedure: Synthesis of 4-(Difluoro(phenyl)methyl)pyrrolidin-2-one (85a)

To a stirred solution of methyl 4,4-difluoro-3-(nitromethyl)-4-phenylbutanoate **83a** (50 mg, 0.18 mmol) in MeOH (2 mL) was added NiCl₂·6H₂O (85 mg, 0.36 mmol) at room temperature. After stirring for 5 min, NaBH₄ (75 mg, 1.98 mmol) was added in four portions. The reaction mixture was stirred for 30 min at room temperature, then NH₄Cl was added and the reaction mixture was extracted with ethyl acetate (3x5mL). The organic layers were separated, washed with water (3x5mL), dried over Na₂SO₄ and concentrated *under vacuum*. The 4-(difluoro(phenyl)methyl)pyrrolidin-2-one **85a** was precipitated and washed by diethyl ether to give a colorless solid (31 mg, 80%).



C₁₁H₁₁F₂NO
M = 211.21 g·mol⁻¹

R_f = 0.3 (DCM / Methanol: 95/5). **mp** = 95 °C .

¹H NMR (300 MHz, CDCl₃) δ 7.45 (bs, 5H), 6.55 (s, 1H, NH), 3.2 (dd, ²J_{H-H} = 9.9 Hz, ³J_{H-H} = 7.0 Hz, 1H), 3.40 (dd, ²J_{H-H} = 9.9 Hz, ³J_{H-H} = 8.8 Hz, 1H), 3.35 – 3.13 (m, 1H), 2.51 (dd, ²J_{H-H} = 17.3 Hz, ³J_{H-H} = 8.1 Hz, 1H), 2.35 (dd, ²J_{H-H} = 17.3 Hz, ³J_{H-H} = 9.6 Hz, 1H).

¹³C NMR (75 MHz, CDCl₃) δ 176.4, 135.4 (t, ²J_{C-F} = 26.4 Hz), 130.5 (t, ⁵J_{C-F} = 1.7 Hz), 128.9 (2C), 125.2 (t, ³J_{C-F} = 6.3 Hz, 2C), 121.9 (t, ¹J_{C-F} = 244.7 Hz), 42.5 (t, ²J_{C-F} = 28.4 Hz), 42.0 (t, ³J_{C-F} = 4.8 Hz), 30.8 (t, ³J_{C-F} = 3.7 Hz).

¹⁹F NMR {H} (282 MHz, CDCl₃) δ -103.06 (AB system, ²J_{F-F} = 248.2 Hz), -104.51 (AB system, ²J_{F-F} = 248.2 Hz).

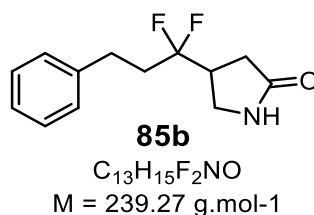
¹⁹F NMR (282 MHz, CDCl₃) δ -103.06 (ABX system, dd, ²J_{F-F} = 248.2 Hz, ³J_{F-H} = 13.3 Hz), -104.51 (ABX system, ²J_{F-F} = 248.2 Hz, ³J_{F-H} = 15.4 Hz).

FT-IR (cm⁻¹): 3177, 3092, 2984, 2958, 2920, 2852, 1683, 1487, 1453, 1359, 1304, 1273, 1142, 1062, 1041, 991, 795, 763, 702, 686.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₁H₁₁NOF₂Na) = 234.0701; found: 234.0699 (1 ppm).

6.2. 4-(1,1-Difluoro-3-phenylpropyl)pyrrolidin-2-one (85b)

Starting from 50 mg (0.166 mmol) methyl 4,4-difluoro-3-(nitromethyl)-6-phenylhexanoate **83b** we obtained 31 mg of **85b** (77%) as a colorless solid.



R_f = 0.3 (DCM / Methanol: 95/5). **mp** = 95 °C.

¹H NMR (500 MHz, CDCl₃) δ 7.36 – 7.27 (m, 2H), 7.25 – 7.17 (m, 3H), 6.43 (s, 1H, NH), 3.51 – 3.44 (m, 2H), 3.03 – 2.89 (m, 1H), 2.87 – 2.81 (m, 2H), 2.49 – 2.36 (m, 2H), 2.20 – 2.06 (m, 2H).

¹³C NMR (126 MHz, CDCl₃) δ 176.4, 140.2, 128.8, 128.4, 126.6, 123.6 (t, ¹J_{C-F} = 243.4 Hz), 41.8 (t, ³J_{C-F} = 5.6 Hz), 40.6 (t, ²J_{C-F} = 25.9 Hz), 37.2 (t, ²J_{C-F} = 25.0 Hz), 30.7 (dd, ³J_{C-F} = 3.2, ³J_{C-F} = 1.6 Hz), 28.0 (t, ³J_{C-F} = 4.7 Hz).

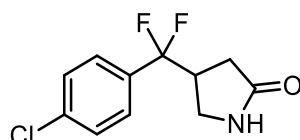
¹⁹F {¹H} NMR (471 MHz, CDCl₃) δ -107.17 (AB system, ²J_{F-F} = 244.7 Hz), -108.06 (AB system, ²J_{F-F} = 244.7 Hz).

FT-IR (cm⁻¹): 3198, 3107, 3086, 3063, 3026, 2966, 292, 2893, 2870, 1664, 1603, 1497, 1454, 1379, 1282, 1197, 1086, 1052, 1036, 930, 905, 741, 698.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₃H₁₅NOF₂Na) = 262.1014; found: 262.1016 (1 ppm).

6.3. 4-((4-Chlorophenyl)difluoromethyl)pyrrolidin-2-one (85c)

Starting from 50 mg (0.163 mmol) of methyl 4-(4-chlorophenyl)-4,4-difluoro-3-(nitromethyl)butanoate **83c** we obtained 33 mg of **85c** (84%) as a colorless solid.



85c

$C_{11}H_{10}ClF_2NO$
 $M = 245.65 \text{ g.mol}^{-1}$

R_f = 0.2 (DCM / Methanol: 95/5). **mp** = 113 °C.

¹H NMR (300 MHz, CDCl₃) δ 7.56 – 7.35 (m, 4H), 6.09 (s, 1H, NH), 3.51 (dd, ²J_{H-H} = 9.9 Hz, ³J_{H-H} = 7.0 Hz, 1H), 3.42 (dd, ²J_{H-H} = 9.9 Hz, ²J_{H-H} = 9.2 Hz, 1H), 3.37 – 3.11 (m, 1H), 2.48 (dd, ²J_{H-H} = 17.4 Hz, ³J_{H-H} = 8.0 Hz, 1H), 2.35 (dd, ²J_{H-H} = 17.2 Hz, ³J_{H-H} = 9.6 Hz, 1H).

¹³C NMR (75 MHz, CDCl₃) δ 176.4, 136.8 (t, ⁵J_{C-F} = 2.1 Hz), 133.8 (t, ²J_{C-F} = 27.0 Hz), 129.2, 126.72 (t, ³J_{C-F} = 6.2 Hz), 121.6 (t, ¹J_{C-F} = 245.0 Hz), 42.4 (t, ¹J_{C-F} = 28.2 Hz), 41.9 (t, ³J_{C-F} = 4.8 Hz), 30.8 (t, ³J_{C-F} = 4.4 Hz).

¹⁹F {H} NMR (282 MHz, CDCl₃) δ -102.76 (AB system, ²J_{F-F} = 249.3 Hz), -104.42 (AB system, ²J_{F-F} = 249.3 Hz).

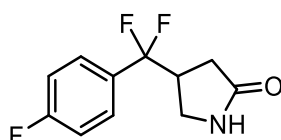
¹⁹F NMR (282 MHz, CDCl₃) δ -102.76 (ABX system, dd, ²J_{F-F} = 249.3 Hz, ³J_{F-H} = 13.1 Hz), -104.42 (ABX system, dd, ²J_{F-F} = 249.3 Hz, ³J_{F-H} = 15.8 Hz).

FT-IR (cm⁻¹): 3116, 2987, 2969, 2923, 1698, 1604, 1493, 1403, 1265, 1093, 1065, 991.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₁H₁₀NOF₂ClNa) = 268.0311; found: 268.0310 (0 ppm).

6.4. 4-(Difluoro(4-fluorophenyl)methyl)pyrrolidin-2-one (85e)

Starting from 50 mg (0.172 mmol) of methyl 4,4-difluoro-4-(4-fluorophenyl)-3-(nitromethyl)butanoate **83e** we obtained 35 mg of **85e** (90%) as a colorless solid.



85e

$C_{11}H_{10}F_3NO$
 $M = 229.20 \text{ g.mol}^{-1}$

R_f = 0.3 (DCM / Methanol: 95/5). **mp** = 128 °C.

¹H NMR (300 MHz, CDCl₃) δ 7.54 – 7.37 (m, 2H), 7.19 – 7.07 (m, 2H), 6.74 (s, 1H, NH), 3.50 (dd, ²J_{H-H} = 10.1 Hz, ³J_{H-H} = 7.0 Hz, 1H), 3.41 (dd, ²J_{H-H} = 10.1 Hz, ³J_{H-H} = 9.7 Hz, 1H),

3.32 – 3.10 (m, 1H), 2.48 (dd, $^2J_{H-H} = 17.3$ Hz, $^3J_{H-H} = 8.0$ Hz, 1H), 2.35 (dd, $^2J_{H-H} = 17.3$ Hz, $^3J_{H-H} = 9.7$ Hz, 1H).

^{13}C NMR (75 MHz, CDCl_3) δ 176.3, 163.9 (dt, $^1J_{C-F} = 250.3$ Hz, $^5J_{C-F} = 2.0$ Hz), 131.4 (td, $^2J_{C-F} = 27.1$ Hz, $^4J_{C-F} = 3.3$ Hz), 127.4 (dt, $^3J_{C-F} = 8.7$ Hz, $^3J_{C-F} = 6.3$ Hz), 121.7 (t, $^1J_{C-F} = 244.9$ Hz), 116.0 (d, $^2J_{C-F} = 22.1$ Hz), 42.5 (t, $^2J_{C-F} = 28.4$ Hz), 42.0 (t, $^3J_{C-F} = 4.9$ Hz), 30.8 (t, $^3J_{C-F} = 3.7$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -101.84 (ABX system, $^2J_{F-F} = 249.2$ Hz, $^6J_{F-F} = 2.5$ Hz), -103.71 (ABX system, $^2J_{F-F} = 249.2$ Hz, $^6J_{F-F} = 2.5$ Hz), -109.98 (t, $^6J_{C-F} = 2.5$ Hz).

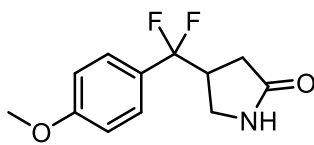
^{19}F NMR (282 MHz, CDCl_3) δ -101.84 (ABXX' system, ddd, $^2J_{F-F} = 249.2$ Hz, $^3J_{F-H} = 12.9$ Hz, $^6J_{F-F} = 2.5$ Hz), -103.71 (ABXX' system, ddd, $^2J_{F-F} = 249.2$ Hz, $^3J_{F-H} = 16.0$ Hz, $^6J_{F-F} = 2.5$ Hz), -109.89 to -110.08 (m).

FT-IR (cm^{-1}): 3190, 3089, 2922, 1682, 1606, 1512, 1304, 1271, 1219, 1148, 1070, 1043, 994, 844, 812, 797, 598.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{11}\text{H}_{10}\text{NOF}_3\text{Na}$) = 252.0607; found: 252.0608 (0 ppm).

6.5. 4-(Difluoro(4-methoxyphenyl)methyl)pyrrolidin-2-one (85f)

Starting from 50 mg (0.165 mmol) of methyl 4,4-difluoro-4-(4-methoxyphenyl)-3-(nitromethyl)butanoate **83f** we obtained 36 mg of **85f** (92%) as a colorless solid.



85f

$\text{C}_{12}\text{H}_{13}\text{F}_2\text{NO}_2$
M = 241.24 g.mol $^{-1}$

R_f = 0.2 (DCM / Methanol: 95/5). **mp** = 141 °C.

^1H NMR (300 MHz, CDCl_3) δ 7.41 – 7.33 (m, 2H), 6.98 (bs, 1H, NH), 6.96 – 6.89 (m, 1H), 3.82 (s, 3H), 3.47 (dd, $^2J_{H-H} = 10.0$ Hz, $^3J_{H-H} = 7.1$ Hz, 1H), 3.38 (t, $^2J_{H-H} = 10.0$ Hz, $^3J_{H-H} = 9.8$ Hz, 1H), 3.31 – 3.09 (m, 1H, H₈), 2.48 (dd, $^2J_{H-H} = 17.3$ Hz, $^3J_{H-H} = 8.0$ Hz, 1H), 2.34 (dd, $^2J_{H-H} = 17.3$ Hz, $^3J_{H-H} = 9.7$ Hz, 1H).

^{13}C NMR (75 MHz, CDCl_3) δ 176.7, 161.0 (t, $^5J_{C-F} = 1.7$ Hz), 127.5 (t, $^2J_{C-F} = 27.0$ Hz), 126.7 (t, $^3J_{C-F} = 6.2$ Hz), 122.1 (t, $^1J_{C-F} = 244.2$ Hz), 114.1, 55.45, 42.4 (t, $^2J_{C-F} = 28.9$ Hz), 42.1 (t, $^3J_{C-F} = 4.7$ Hz), 30.9 (t, $^3J_{C-F} = 3.6$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -101.33 (AB system, $^2J_{\text{F-F}} = 247.5$ Hz), -102.94 (AB system, $^2J_{\text{F-F}} = 247.5$ Hz).

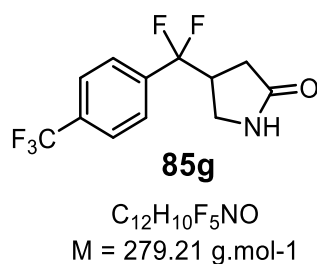
^{19}F NMR (282 MHz, CDCl_3) δ -101.33 (ABX system, dd, $^2J_{\text{F-F}} = 247.5$ Hz, $^3J_{\text{F-H}} = 13.1$ Hz), -102.94 (ABX system, dd, $^2J_{\text{F-F}} = 247.5$ Hz, $^3J_{\text{F-H}} = 15.4$ Hz).

FT-IR (cm^{-1}): 3198, 3107, 2967, 2938, 2902, 1685, 1614, 1516, 1496, 1303, 1249, 1178, 1055, 1024, 1006, 993, 837, 822, 581.

HRMS (ESI): calcd. for $[\text{M}+\text{K}]^+$ ($\text{C}_{12}\text{H}_{13}\text{NO}_2\text{F}_2\text{K}$) = 280.0546; found: 280.0545 (0 ppm).

6.6. 4-(Difluoro(4-(trifluoromethyl)phenyl)methyl)pyrrolidin-2-one (85g)

Starting from 50 mg (0.147 mmol) of methyl 4,4-difluoro-3-(nitromethyl)-4-(4-(trifluoromethyl)phenyl)butanoate **83g** we obtained 39 mg of **85g** (95%) as a colorless solid.



R_f = 0.2 (DCM / Methanol: 95/5). **mp** = 125 °C.

^1H NMR (300 MHz, CD_3CN) δ 7.84 – 7.79 (m, 2H), 7.76 – 7.68 (m, 2H), 6.21 (s, 1H), 3.49 – 3.21 (m, 3H), 2.42 – 2.25 (m, 2H).

^{13}C NMR (75 MHz, CD_3CN) δ 176.2, 140.2 (tq, $^2J_{\text{C-F}} = 26.9$ Hz, $^5J_{\text{C-F}} = 1.3$ Hz), 132.7 (qt, $^2J_{\text{C-F}} = 32.5$ Hz, $^5J_{\text{C-F}} = 1.8$ Hz), 127.2 (t, $^3J_{\text{C-F}} = 6.3$ Hz), 126.8 (q, $^3J_{\text{C-F}} = 3.9$ Hz), 124.9 (q, $^1J_{\text{C-F}} = 271.5$ Hz), 123.1 (t, $^1J_{\text{C-F}} = 243.9$ Hz), 42.4 (t, $^2J_{\text{C-F}} = 27.0$ Hz), 42.1 (t, $^3J_{\text{C-F}} = 5.0$ Hz), 31.0 (t, $^3J_{\text{C-F}} = 3.9$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -63.03 (s), δ -103.38 (AB system, $^2J_{\text{F-F}} = 250.5$ Hz), -105.32 (AB system, $^2J_{\text{F-F}} = 250.5$ Hz).

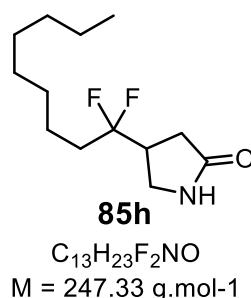
^{19}F NMR (282 MHz, CDCl_3) δ -63.03 (s), δ -103.38 (ABX system, dd, $^2J_{\text{F-F}} = 250.5$ Hz, $^3J_{\text{F-H}} = 12.6$ Hz), -105.32 (ABX system, dd, $^2J_{\text{F-F}} = 250.5$ Hz, $^3J_{\text{F-H}} = 16.0$ Hz).

FT-IR (cm^{-1}): 3393, 3217, 2987, 2902, 1669, 1624, 1494, 1466, 1413, 1384, 1320, 1283, 1267, 1193, 1173, 1117, 1065, 998, 842, 814, 781, 755, 694, 671, 657, 611.

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{12}H_{10}NOF_5Na$) = 302.05747; found: 302.0574 (0 ppm).

6.7. 4-(1,1-Difluorononyl)pyrrolidin-2-one (85h)

Starting from 50 mg (0.162 mmol) of methyl 4,4-difluoro-3-(nitromethyl)dodecanoate **83h** we obtained 34 mg of **85h** (84%) as a colorless solid.



R_f = 0.2 (DCM / Methanol: 95/5). **mp** = 98 °C.

¹H NMR (300 MHz, CDCl₃) δ 6.89 (s, 1H), 3.45 (d, ³J_{H-H} = 7.9 Hz, 2H), 3.05 – 2.79 (m, 1H), 2.40 (d, ³J_{H-H} = 9.1 Hz, 2H), 1.91 – 1.68 (m, 2H), 1.58 – 1.39 (m, 2H), 1.26 (s, 10H), 0.86 (d, ³J_{H-H} = 6.5 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 176.8, 124.2 (t, ¹J_{C-F} = 242.7 Hz), 41.9 (t, ³J_{C-F} = 5.6 Hz), 40.4 (t, ²J_{C-F} = 26.2 Hz), 35.2 (t, ²J_{C-F} = 25.0 Hz), 31.9, 30.9, 29.4, 29.4, 29.2, 22.7, 21.9 (t, ³J_{C-F} = 4.3 Hz), 14.2.

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ δ -106.26 (AB system, ²J_{F-F} = 244.4 Hz), -107.69 (AB system, ²J_{F-F} = 244.4 Hz).

FT-IR (cm⁻¹): 3239, 2958, 2919, 2872, 2854, 1682, 1471, 1393, 1276, 1236, 1222, 1199, 1167, 1133, 1055, 966, 923, 761, 723, 667.

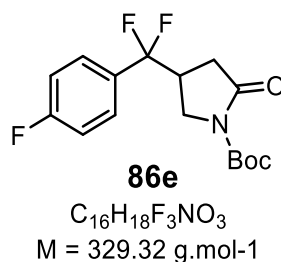
HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{13}H_{23}NOF_2Na$) = 270.1640; found: 270.1643 (1 ppm).

7. Synthesis of the N-Boc protected γ -lactams

7.1. Representative procedure: Synthesis of tert-butyl 4-(difluoro(4-fluorophenyl)methyl)-2-oxopyrrolidine-1-carboxylate (86e)

To a stirred solution of *gem*-difluorinated- γ -lactam **85e** (500 mg, 2.18 mmol) in acetonitrile (15 mL) was added at room temperature di-tert-butyl dicarbonate (Boc₂O) (620 mg, 2.8 mmol) and DMAP (20 mol %). The reaction mixture was stirred for a further 4 h at room temperature,

then concentrated under reduced pressure, and purified by chromatography on silica gel to afford **86e** as a colorless solid (620 mg, 86%).



R_f = 0.5 (Cyclohexane / Ethyl Acetate: 6/4). **mp** = 125 °C.

¹H NMR (300 MHz, CDCl₃) δ 7.50 – 7.41 (m, 2H), 7.19 – 7.09 (m, 2H), 3.85 – 3.73 (m, 2H), 3.14 – 2.92 (m, 1H), 2.68 (dd, ²J_{H-H} = 17.8 Hz, ³J_{H-H} = 8.2 Hz, 1H), 2.56 (dd, ²J_{H-H} = 17.8 Hz, ³J_{H-H} = 9.4 Hz, 1H), 1.51 (s, 9H).

¹³C NMR (75 MHz, CDCl₃) δ 171.2, 164.0 (dt, ¹J_{C-F} = 250.8 Hz, ⁵J_{C-F} = 2.0 Hz), 149.8, 130.9 (td, ²J_{C-F} = 27.0 Hz, ⁴J_{C-F} = 3.3 Hz), 127.4 (dt, ³J_{C-F} = 8.8 Hz, ³J_{C-F} = 6.3 Hz), 121.4 (t, ¹J_{C-F} = 245.4 Hz), 116.2 (d, ²J_{C-F} = 22.1 Hz), 83.6, 45.8 (t, ³J_{C-F} = 4.7 Hz), 38.7 (t, ²J_{C-F} = 28.8 Hz), 33.4 (t, ³J_{C-F} = 3.7 Hz), 28.1.

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -102.40 (ABX system, dd, ²J_{F-F} = 249.7 Hz, ⁶J_{F-F} = 2.0 Hz), -103.48 (ABX system, dd, ²J_{F-F} = 249.7 Hz, ⁶J_{F-F} = 2.0 Hz), -109.67 (t, ⁶J_{F-F} = 2.0 Hz).

¹⁹F NMR (282 MHz, CDCl₃) δ -102.40 (ABXX' system, ddd, ²J_{F-F} = 249.7 Hz, ³J_{F-H} = 13.1 Hz, ⁶J_{F-F} = 2.0 Hz), -103.48 (ABXX' system, ddd, ²J_{F-F} = 249.7 Hz, ³J_{F-H} = 13.9 Hz, ⁶J_{F-F} = 2.0 Hz), -109.59 to -109.76 (m).

FT-IR (cm⁻¹): 2983, 2927, 1771, 1693, 1607, 1514, 1392, 1367, 1298, 1232, 1202, 1178, 1152, 1104, 1068, 1029, 1014, 987, 835, 775.

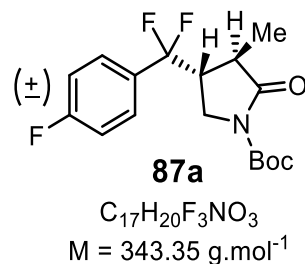
HRMS (ESI): calcd. for [M+Na]⁺ (C₁₆H₁₈NO₃F₃Na) = 352.1131; found: 352.1129 (1 ppm).

8. Alkylation at C₄ position of the γ-lactams

8.1. Representative procedure: Synthesis of tert-butyl 4-(difluoro(4-fluorophenyl)methyl)-3-methyl-2-oxopyrrolidine-1-carboxylate (**87a**)

To a solution of protected γ-Lactam **86e** (120 mg, 0.36 mmol, 1 equiv) in anhydrous THF (10 mL) cooled at -90°C, was added, dropwise under nitrogen, a 1M solution of Lithium

bis(trimethylsilyl)amide (LiHMDS) (380 μ L, 1.05 equiv). The mixture was stirred for 30 min at temperature below -80 $^{\circ}$ C before dropwise addition, of methyl iodide (0.38 mmol, 1.05 equiv). The reaction mixture was stirred for additional 30 min below -80 $^{\circ}$ C and then allowed to warm to r.t. within 2h. The mixture was then treated with water (10 mL), extracted with ethyl acetate (3x10mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. After purification by chromatography on silica gel, the alkylated γ -lactam **87a** was obtained as a white solid 112 mg (90%).



R_f = 0.5 (Cyclohexane / Ethyl Acetate: 6/4). **mp** = 120 $^{\circ}$ C.

^1H NMR (300 MHz, CDCl_3) δ 7.51 – 7.42 (m, 2H), 7.18 – 7.12 (m, 2H), 3.69 (dd, $^2J_{\text{H-H}} = 11.3$ Hz, $^3J_{\text{H-H}} = 8.6$ Hz, 1H), 3.61 (dd, $^2J_{\text{H-H}} = 11.3$ Hz, $^3J_{\text{H-H}} = 8.5$ Hz, 1H), 2.80 – 2.52 (m, 2H), 1.51 (s, 9H), 1.13 (d, $^3J_{\text{H-H}} = 6.6$ Hz, 3H).

^1H NMR (300 MHz, C_6D_6) δ 6.88 – 6.82 (m, 2H), 6.64 – 6.55 (m, 2H), 3.47 – 3.28 (m, 2H), 2.37 (dq, $^3J_{\text{H-H}} = 10.3$ Hz, $^3J_{\text{H-H}} = 7.1$ Hz, 1H), 1.95 – 1.75 (m, 1H), 1.46 (s, 9H), 0.92 (d, $^3J_{\text{H-H}} = 7.1$ Hz, 2H).

^{13}C NMR (75 MHz, CDCl_3) δ 174.1, 164.0 (dt, $^1J_{\text{C-F}} = 250.7$ Hz, $^5J_{\text{C-F}} = 2.0$ Hz), 150.0, 131.2 (td, $^2J_{\text{C-F}} = 27.0$ Hz, $^4J_{\text{C-F}} = 3.4$ Hz), 127.4 (dt, $^3J_{\text{C-F}} = 8.7$ Hz, $^3J_{\text{C-F}} = 6.4$ Hz), 121.6 (t, $^1J_{\text{C-F}} = 245.5$ Hz), 116.2 (d, $^2J_{\text{C-F}} = 22.0$ Hz), 83.6, 47.0 (t, $^2J_{\text{C-F}} = 27.9$ Hz), 44.1 (t, $^3J_{\text{C-F}} = 5.2$ Hz), 39.1, 28.1, 15.8.

^{19}F {H} NMR (376 MHz, CDCl_3) -100.19 (ABX system, dd, $^2J_{\text{F-F}} = 247.9$ Hz, $^6J_{\text{F-F}} = 2.5$ Hz), -102.43 (ABX system, dd, $^2J_{\text{F-F}} = 248.0$ Hz, $^6J_{\text{F-F}} = 2.5$ Hz), -110.16 (t, $^6J_{\text{F-F}} = 2.5$ Hz).

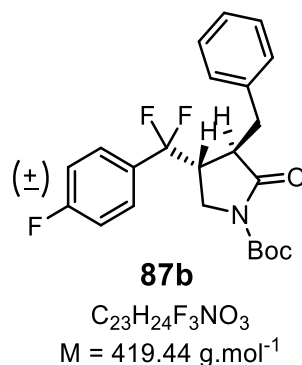
^{19}F NMR (376 MHz, CDCl_3) -100.19 (ABXX' system, ddd, $^2J_{\text{F-F}} = 247.9$ Hz, $^3J_{\text{F-H}} = 12.6$ Hz, $^6J_{\text{F-F}} = 2.5$ Hz), -102.43 (ABXX' system, ddd, $^2J_{\text{F-F}} = 248.0$ Hz, $^3J_{\text{F-H}} = 14.5$ Hz, $^6J_{\text{F-F}} = 2.5$ Hz), -110.10 to -110.21 (m).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+(\text{C}_{17}\text{H}_{20}\text{NO}_3\text{F}_3\text{Na}) = 366.1287$; found: 366.1289 (0 ppm); calcd. for $[\text{M}+\text{K}]^+(\text{C}_{17}\text{H}_{20}\text{NO}_3\text{F}_3\text{K}) = 382.1027$; found: 382.1023 (1 ppm).

The stereochemistry of this molecule has been established through 2D COSY, 2D NOESY (^1H , ^1H) and 2D HOESY (^{19}F , ^1H) experiments.

8.2. Tert-butyl -3-benzyl-4-(difluoro(4-fluorophenyl)methyl)-2-oxopyrrolidine-1-carboxylate (**87b**)

Starting from 120 mg (0.364 mmol) protected γ -lactam **86e** we obtained 94 mg of **87b** (62%) as a colorless solid.



R_f = 0.6 (Cyclohexane / Ethyl Acetate: 6/4). **mp** = 126 °C.

^1H NMR (300 MHz, C_6D_6) δ 7.05 – 6.92 (m, 5H), 6.87 – 6.77 (m, 2H), 6.64 – 6.47 (m, 2H), 3.62 (dd, $^2J_{\text{H-H}} = 11.5 \text{ Hz}$, $^3J_{\text{H-H}} = 6.5 \text{ Hz}$, 1H), 3.18 (dd, $^2J_{\text{H-H}} = 11.5 \text{ Hz}$, $^3J_{\text{H-H}} = 8.7 \text{ Hz}$, 1H), 2.92 (dd, $^2J_{\text{H-H}} = 13.4 \text{ Hz}$, $^3J_{\text{H-H}} = 5.6 \text{ Hz}$, 1H), 2.85 – 2.75 (m, 1H), 2.70 (dd, $^2J_{\text{H-H}} = 13.4$, $^3J_{\text{H-H}} = 5.2 \text{ Hz}$, 1H), 2.51 – 2.31 (m, 1H), 1.42 (s, 9H).

^{13}C NMR (75 MHz, C_6D_6) δ 171.8, 163.9 (dt, $^1J_{\text{C-F}} = 249.6 \text{ Hz}$, $^5J_{\text{C-F}} = 1.9 \text{ Hz}$), 150.6, 137.7, 131.1 (td, $^2J_{\text{C-F}} = 27.0 \text{ Hz}$, $^4J_{\text{C-F}} = 3.2 \text{ Hz}$), 130.0, 128.8, 127.6 (dt, $^3J_{\text{C-F}} = 8.7 \text{ Hz}$, $^1J_{\text{C-F}} = 6.3 \text{ Hz}$), 127.0, 122.3 (t, $^1J_{\text{C-F}} = 245.9 \text{ Hz}$), 115.9 (d, $^2J_{\text{C-F}} = 22.0 \text{ Hz}$), 82.8, 45.9 (dd, $^3J_{\text{C-F}} = 2.9 \text{ Hz}$, $^3J_{\text{C-F}} = 2.5 \text{ Hz}$), 44.1 (t, $^3J_{\text{C-F}} = 5.0 \text{ Hz}$), 41.7 (t, $^2J_{\text{C-F}} = 27.5 \text{ Hz}$), 35.3, 28.0, 27.2.

^{19}F {H} NMR (282 MHz, CDCl_3) δ -100.07 (ABX system, dd, $^2J_{\text{F-F}} = 249.1 \text{ Hz}$, $^6J_{\text{F-F}} = 2.5 \text{ Hz}$), -103.90 (ABX system, dd, $^2J_{\text{F-F}} = 249.1 \text{ Hz}$, $^6J_{\text{F-F}} = 2.5 \text{ Hz}$), -109.93 (t, $^6J_{\text{F-F}} = 2.5 \text{ Hz}$).

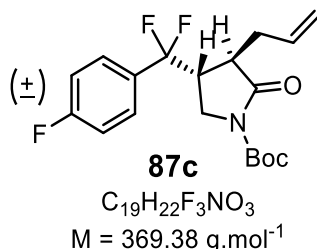
^{19}F NMR (282 MHz, CDCl_3) δ -100.07 (ABXX' system, ddd, $^2J_{\text{F-F}} = 249.1 \text{ Hz}$, $^3J_{\text{F-H}} = 12.8 \text{ Hz}$, $^6J_{\text{F-F}} = 2.5 \text{ Hz}$), -103.90 (ABXX' system, ddd, $^2J_{\text{F-F}} = 249.1 \text{ Hz}$, $^3J_{\text{F-H}} = 16.8 \text{ Hz}$, $^6J_{\text{F-F}} = 2.5 \text{ Hz}$), -109.86 to -110.01 (m).

FT-IR (cm^{-1}): 3674, 2988, 2972, 2923, 2902, 1749, 1708, 1605, 1513, 1455, 1408, 1393, 1365, 1306, 1280, 1262, 1230, 1151, 1103, 1042, 978, 857, 843, 818, 779, 703.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+(\text{C}_{23}\text{H}_{24}\text{NO}_3\text{F}_3\text{Na}) = 442.1600$; found: 442.1600 (0 ppm).

8.3. Tert-butyl (3R,4R)-3-allyl-4-(difluoro(4-fluorophenyl)methyl)-2-oxopyrrolidine-1-carboxylate (87c)

Starting from 120 mg (0.364 mmol) protected γ -lactam **86e** we obtained 57 mg of **87c** (42%) as a colorless solid.



$R_f = 0.7$ (Cyclohexane / Ethyl Acetate: 7/3). $mp = 77 \text{ }^\circ\text{C}$.

$^1\text{H NMR}$ (300 MHz, C_6D_6) δ 6.96 – 6.88 (m, 2H), 6.68 – 6.58 (m, 2H), 5.59 – 5.42 (m, 1H), 4.90 – 4.84 (m, 1H), 4.83 – 4.76 (m, 1H), 3.61 (dd, $^2J_{H-H} = 11.4 \text{ Hz}$, $^3J_{H-H} = 7.4 \text{ Hz}$, 1H), 3.45 (dd, $^2J_{H-H} = 11.4 \text{ Hz}$, $^3J_{H-H} = 8.7 \text{ Hz}$, 1H), 2.63 – 2.51 (m, 1H), 2.51 – 2.29 (m, 1H), 2.04 – 1.87 (m, 1H), 1.43 (s, 9H).

$^{13}\text{C NMR}$ (75 MHz, C_6D_6) δ 171.3, 164.0 (dt, $^1J_{C-F} = 249.8 \text{ Hz}$, $^5J_{C-F} = 1.8 \text{ Hz}$), 150.7, 134.0, 131.4 (td, $^2J_{C-F} = 27.1 \text{ Hz}$, $^4J_{C-F} = 3.2 \text{ Hz}$), 127.6 (dt, $^3J_{C-F} = 8.7 \text{ Hz}$, $^3J_{C-F} = 6.4 \text{ Hz}$), 122.1 (t, $^1J_{C-F} = 245.8 \text{ Hz}$), 118.8, 115.9 (d, $^2J_{C-F} = 22.0 \text{ Hz}$), 82.8, 44.1 (t, $^3J_{C-F} = 5.0 \text{ Hz}$), 43.8 (dd, $^3J_{C-F} = 3.5 \text{ Hz}$, $^3J_{C-F} = 1.7 \text{ Hz}$), 42.1 (t, $^2J_{C-F} = 27.7 \text{ Hz}$), 33.7, 28.0.

$^{19}\text{F}\{^1\text{H}\}$ NMR (282 MHz, $CDCl_3$) δ -100.67 (ABX system, dd, $^2J_{F-F} = 248.7 \text{ Hz}$, $^6J_{F-F} = 2.5 \text{ Hz}$), -103.56 (ABX system, dd, $^2J_{F-F} = 248.7 \text{ Hz}$, $^6J_{F-F} = 2.5 \text{ Hz}$), -109.66 (t, $^6J_{F-F} = 2.5 \text{ Hz}$).

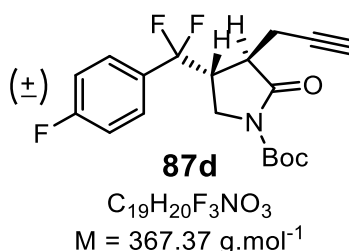
^{19}F NMR (282 MHz, $CDCl_3$) δ -100.67 (ABXX' system, ddd, $^2J_{F-F} = 248.7 \text{ Hz}$, $^3J_{F-H} = 12.2 \text{ Hz}$, $^6J_{F-F} = 2.5 \text{ Hz}$), -103.56 (ABXX' system, ddd, $^2J_{F-F} = 248.7 \text{ Hz}$, $^3J_{F-H} = 14.2 \text{ Hz}$, $^6J_{F-F} = 2.5 \text{ Hz}$), -109.53 to -109.77 (m).

FT-IR (cm^{-1}): 3674, 2986, 2902, 1773, 1693, 1605, 1517, 1403, 1369, 1287, 1260, 1232, 1145, 1102, 1051, 1027, 984, 918, 840, 779.

HRMS (ESI): calcd. for $[M+Na]^+(C_{19}H_{22}NO_3F_3Na) = 392.1444$; found: 392.1447 (1 ppm).

8.4. Tert-butyl-4-(difluoro(4-fluorophenyl)methyl)-2-oxo-3-(prop-2-yn-1-yl)pyrrolidine-1-carboxylate (87d)

Starting from 120 mg (0.364 mmol) protected γ -lactam **86e** we obtained 40 mg of **87d** (30%) as a colorless liquid.



R_f = 0.3 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.51 – 7.44 (m, 2H), 7.18 – 7.09 (m, 2H), 3.73 (dd, ²J_{H-H} = 11.2 Hz, ³J_{H-H} = 9.2 Hz, 1H), 3.65 (dd, ²J_{H-H} = 11.2 Hz, ³J_{H-H} = 8.1 Hz, 1H), 3.35 – 3.08 (m, 1H), 2.87 – 2.73 (m, 2H), 2.23 – 2.11 (m, 1H), 1.98 (t, ⁴J_{H-H} = 2.6 Hz, 1H), 1.50 (s, 9H).

¹³C NMR (75 MHz, CDCl₃) δ 171.5, 164.0 (dt, ¹J_{C-F} = 250.9 Hz, ⁵J_{C-F} = 2.0 Hz), 149.7, 130.9 (td, ²J_{C-F} = 26.9 Hz, ⁴J_{C-F} = 3.3 Hz), 127.4 (dt, ³J_{C-F} = 8.7 Hz, ³J_{C-F} = 6.3 Hz), 121.5 (t, ¹J_{C-F} = 245.5 Hz), 116.1 (d, ²J_{C-F} = 22.1 Hz), 83.7, 79.4, 71.8, 44.2 (t, ³J_{C-F} = 5.1 Hz), 42.7 (t, ³J_{C-F} = 2.6 Hz), 42.0 (t, ²J_{C-F} = 27.9 Hz), 28.0, 19.2.

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -101.44 (ABX system, dd, ²J_{F-F} = 248.8 Hz, ⁶J_{F-F} = 2.6 Hz), -102.46 (ABX system, dd, ²J_{F-F} = 248.8 Hz, ⁶J_{F-F} = 2.6 Hz), -109.43 (t, ⁶J_{F-F} = 2.6 Hz).

¹⁹F NMR (282 MHz, CDCl₃) δ -101.44 (ABXX' system, ddd, ²J_{F-F} = 248.8 Hz, ³J_{F-H} = 14.8 Hz, ⁶J_{F-F} = 2.6 Hz), -102.46 (ABXX' system, ddd, ²J_{F-F} = 248.8 Hz, ³J_{F-H} = 14.2 Hz, ⁶J_{F-F} = 2.6 Hz), -109.33 to -109.53 (m).

FT-IR (cm⁻¹): 2982, 2921, 1788, 1752, 1717, 1609, 1514, 1393, 1367, 1310, 1290, 1235, 1152, 1083, 1045, 998, 840, 777, 627, 603.

HRMS (ESI): calcd. for [M-C₄H₈+Na]⁺(C₁₅H₁₂NO₃F₃Na)= 334.0662; found: 334.0662 (0 ppm).

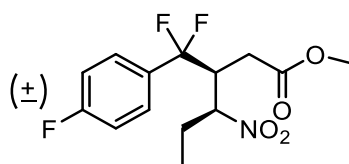
9. Addition of nitroalkanes on the alkenes bearing the *gem*-difluoroalkyl chains

9.1. Representative procedure: Synthesis of methyl-3-(difluoro(4-fluorophenyl)methyl)-4-nitrohexanoate (88e and 89e)

To methyl (Z)-4,4-difluoro-4-phenylbut-2-enoate **82e** (200 mg, 0.87 mmol) was added 1-nitropropane (155 μL, 1.7 mmol) and potassium carbonate (360 mg, 2.6 mmol) in DMSO (5 ml). The reaction mixture was stirred at room temperature and it was controlled using TLC. At the end, saturated NH₄Cl (5 mL) was added and the reaction mixture was extracted with ethyl

acetate (3x10mL). The combined organic layers were washed with water (3x5mL), dried over Na₂SO₄ and concentrated under vacuum. After purification by chromatography on silica gel, Methyl-3-(difluoro(4-fluorophenyl)methyl)-4-nitrohexanoate **88e** and **89e** were obtained as a colorless oil 184 mg (66%, 1:1 mixture of **88e** / **89e**). These diastereoisomers have been separated by chromatography on SiO₂.

9.2. Methyl-3-(difluoro(4-fluorophenyl)methyl)-4-nitrohexanoate (**88e**)



88e

C₁₄H₁₆F₃NO₄
M = 319.28 g.mol⁻¹

Colorless liquid (92 mg, 33%); **R_f** = 0.4 (Cyclohexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.53 – 7.43 (m, 2H), 7.20 – 7.09 (m, 2H), 4.69 (ddd, ³J_{H-H} = 11.3 Hz, ³J_{H-H} = 5.0 Hz, ³J_{H-H} = 3.1 Hz, 1H), 3.70 – 3.53 (m, 1H), 3.57 (s, 3H), 2.69 – 2.50 (m, 2H), 2.12 – 1.74 (m, 2H), 0.96 (t, ³J_{H-H} = 7.3 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 170.8, 164.1 (dt, ¹J_{C-F} = 251.2 Hz, ⁵J_{C-F} = 2.0 Hz), 130.4 (td, ²J_{C-F} = 26.6 Hz, ⁴J_{C-F} = 3.3 Hz), 127.9 (ddd, ³J_{C-F} = 8.8 Hz, ³J_{C-F} = 6.9 Hz, ³J_{C-F} = 6.0 Hz), 122.2 (t, ¹J_{C-F} = 248.6 Hz), 116.1 (d, ²J_{C-F} = 22.1 Hz), 87.6 (t, ³J_{C-F} = 2.1 Hz), 52.4, 46.6 (t, ²J_{C-F} = 26.1 Hz), 29.7 (dd, ³J_{C-F} = 4.3, ³J_{C-F} = 3.0 Hz), 23.2 (t, ⁴J_{C-F} = 1.5 Hz), 10.8.

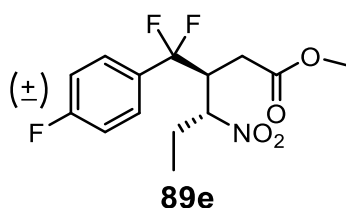
¹⁹F {H} NMR (282 MHz, CDCl₃) δ -95.82 (ABX system, dd, ²J_{F-F} = 251.4 Hz, ⁶J_{F-F} = 3.1 Hz), -101.06 (ABX system, dd, ²J_{F-F} = 251.4 Hz, ⁶J_{F-F} = 2.3 Hz), -109.25 (t, ⁶J_{F-F} = 2.7 Hz).

¹⁹F NMR (282 MHz, CDCl₃) δ -95.82 (ABXX' system, ddd, ²J_{F-F} = 251.4 Hz, ³J_{F-H} = 11.5 Hz, ⁶J_{F-F} = 3.1 Hz), -101.06 (ABXX' system, ddd, ²J_{F-F} = 251.4 Hz, ³J_{F-H} = 18.4 Hz, ⁶J_{F-F} = 2.3 Hz), -109.13 to -109.35 (m).

FT-IR (cm⁻¹): 2954, 1738, 1609, 1554, 1514, 1459, 1438, 1374, 1343, 1314, 1266, 1233, 1196, 1163, 1117, 1104, 1054, 1013, 994, 840, 814.

HRMS (ESI): calcd. for [M+Na]⁺(C₁₄H₁₆NO₄F₃Na) = 342.0924; found: 342.0925 (0 ppm).

9.3. Methyl-3-(difluoro(4-fluorophenyl)methyl)-4-nitrohexanoate (**89e**)



$C_{14}H_{16}F_3NO_4$
 $M = 319.28 \text{ g}\cdot\text{mol}^{-1}$

Colorless liquid (92 mg, 33%); $R_f = 0.3$ (Cyclohexane / Ethyl Acetate: 8/2).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.54 – 7.45 (m, 2H), 7.19 – 7.10 (m, 2H), 4.66 – 4.55 (m, 1H), 3.61 (s, 3H), 3.34 – 3.15 (m, 1H), 2.95 (dd, $^2J_{\text{H-H}} = 17.9 \text{ Hz}$, $^3J_{\text{H-H}} = 4.0 \text{ Hz}$, 1H), 2.69 (dd, $^2J_{\text{H-H}} = 17.9 \text{ Hz}$, $^3J_{\text{H-H}} = 7.2 \text{ Hz}$, 1H), 2.07 (ddq, $^2J_{\text{H-H}} = 14.5$, $^3J_{\text{H-H}} = 10.3$, $^3J_{\text{H-H}} = 7.3 \text{ Hz}$, 1H), 1.75 (dq, $^2J_{\text{H-H}} = 14.7 \text{ Hz}$, $^3J_{\text{H-H}} = 7.3 \text{ Hz}$, $^3J_{\text{H-H}} = 4.8 \text{ Hz}$, 1H), 0.93 (t, $^3J_{\text{H-H}} = 7.3 \text{ Hz}$, 3H).

$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 172.2, 164.1 (dt, $^1J_{\text{C-F}} = 250.9$, $^5J_{\text{C-F}} = 2.0 \text{ Hz}$), 130.5 (td, $^2J_{\text{C-F}} = 26.6$, $^4J_{\text{C-F}} = 3.3 \text{ Hz}$), 128.1 (dt, $^3J_{\text{C-F}} = 8.8$, $^3J_{\text{C-F}} = 6.4 \text{ Hz}$), 122.1 (t, $^1J_{\text{C-F}} = 248.6 \text{ Hz}$), 116.0 (d, $^2J_{\text{C-F}} = 22.1 \text{ Hz}$), 86.7 (dd, $^3J_{\text{C-F}} = 3.8$, $^3J_{\text{C-F}} = 1.4 \text{ Hz}$), 52.4, 46.8 (dd, $^2J_{\text{C-F}} = 27.3 \text{ Hz}$, $^2J_{\text{C-F}} = 26.3 \text{ Hz}$), 28.8 (dd, $^3J_{\text{C-F}} = 3.4 \text{ Hz}$, $^3J_{\text{C-F}} = 2.9 \text{ Hz}$), 10.5.

$^{19}\text{F}\{\text{H}\}$ NMR (282 MHz, CDCl_3) δ -96.86 (ABX system, dd, $^2J_{\text{F-F}} = 253.7 \text{ Hz}$, $^6J_{\text{F-F}} = 2.5 \text{ Hz}$), -101.92 (ABX system, dd, $^2J_{\text{F-F}} = 253.6 \text{ Hz}$, $^6J_{\text{F-F}} = 1.8 \text{ Hz}$), -109.51 (t, $^6J_{\text{F-F}} = 2.6 \text{ Hz}$).

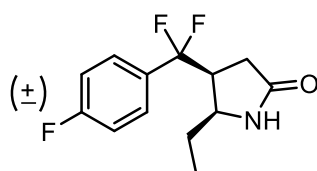
^{19}F NMR (282 MHz, CDCl_3) δ -96.86 (ABXX' system, ddd, $^2J_{\text{F-F}} = 253.7 \text{ Hz}$, $^3J_{\text{F-H}} = 15.1 \text{ Hz}$, $^6J_{\text{F-F}} = 2.5 \text{ Hz}$), -101.92 (ABXX' system, ddd, $^2J_{\text{F-F}} = 253.6 \text{ Hz}$, $^3J_{\text{F-H}} = 17.3 \text{ Hz}$, $^6J_{\text{F-F}} = 1.8 \text{ Hz}$), -109.39 to -109.66 (m).

FT-IR (cm^{-1}): 2973, 2957, 2925, 1739, 1609, 1555, 1514, 1460, 1438, 1413, 1373, 1323, 1299, 1275, 1238, 1196, 1164, 1126, 1104, 1081, 1055, 1005, 841.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+(\text{C}_{14}\text{H}_{16}\text{NO}_4\text{F}_3\text{Na}) = 342.0924$; found: 342.0926 (1 ppm).

9.4. 4-(Difluoro(4-fluorophenyl)methyl)-5-ethylpyrrolidin-2-one (90e)

Starting from 50 mg (0.364 mmol) of methyl 3-(difluoro(4-fluorophenyl)methyl)-4-nitrohexanoate **88e** we obtained 24 mg of **90e** (60%) as a colorless waxy solid.



90e

$C_{13}H_{14}F_3NO$

$M = 257.26 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.5$ (DCM / Methanol: 95/5).

$^1\text{H NMR}$ (300 MHz, CD_3CN) δ 7.65 – 7.54 (m, 2H), 7.28 – 7.18 (m, 2H), 6.53 (bs, 1H), 3.66 (dt, $^3J_{\text{H-H}} = 7.5 \text{ Hz}$, $^3J_{\text{H-H}} = 4.9 \text{ Hz}$, 1H), 3.49 – 3.25 (m, 1H), 2.53 (dd, $^2J_{\text{H-H}} = 16.1$, $^3J_{\text{H-H}} = 12.0 \text{ Hz}$, 1H), 2.07 (dd, $^2J_{\text{H-H}} = 16.1 \text{ Hz}$, $^3J_{\text{H-H}} = 8.4 \text{ Hz}$, 1H), 1.92 – 1.61 (m, 2H), 0.88 (t, $^3J_{\text{H-H}} = 7.5 \text{ Hz}$, 3H).

$^{13}\text{C NMR}$ (75 MHz, CD_3CN) δ 172.6, 164.6 (dt, $^1J_{\text{C-F}} = 247.5 \text{ Hz}$, $^5J_{\text{C-F}} = 2.1 \text{ Hz}$), 133.6 (td, $^2J_{\text{C-F}} = 26.9 \text{ Hz}$, $^4J_{\text{C-F}} = 3.1 \text{ Hz}$), 128.6 (dt, $^3J_{\text{C-F}} = 8.9 \text{ Hz}$, $^3J_{\text{C-F}} = 6.4 \text{ Hz}$), 123.1 (t, $^1J_{\text{C-F}} = 243.9 \text{ Hz}$), 116.7 (d, $^2J_{\text{C-F}} = 22.2 \text{ Hz}$), 61.9 (d, $^3J_{\text{C-F}} = 2.9 \text{ Hz}$), 45.3 (t, $^2J_{\text{C-F}} = 27.2 \text{ Hz}$), 30.75 (dd, $^3J_{\text{C-F}} = 5.9 \text{ Hz}$, $^3J_{\text{C-F}} = 1.6 \text{ Hz}$), 23.1 (t, $^4J_{\text{C-F}} = 2.1 \text{ Hz}$), 10.0 (t, $^5J_{\text{C-F}} = 3.2 \text{ Hz}$).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -98.38 (ABX system, dd, $^2J_{\text{F-F}} = 249.0 \text{ Hz}$, $^6J_{\text{F-F}} = 2.7 \text{ Hz}$), -100.06 (ABX system, dd, $^2J_{\text{F-F}} = 249.0 \text{ Hz}$, $^6J_{\text{F-F}} = 2.7 \text{ Hz}$), -110-19 (t, $^6J_{\text{F-F}} = 2.7 \text{ Hz}$).

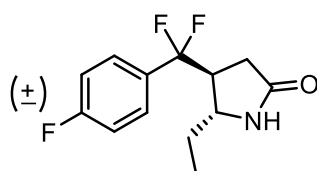
^{19}F NMR (282 MHz, CDCl_3) δ -98.38 (ABXX' system, ddd, $^2J_{\text{F-F}} = 249.0 \text{ Hz}$, $^3J_{\text{F-H}} = 20.8 \text{ Hz}$, $^6J_{\text{F-F}} = 2.7 \text{ Hz}$), -100.06 (ABXX' system, ddd, $^2J_{\text{F-F}} = 249.0 \text{ Hz}$, $^3J_{\text{F-H}} = 10.8 \text{ Hz}$, $^6J_{\text{F-F}} = 2.7 \text{ Hz}$), -110.09 to -110.29 (m).

FT-IR (cm^{-1}): 2971, 2926, 1672, 1607, 1515, 1431, 1397, 1303, 1260, 1238, 1198, 1048, 1028, 1009, 964, 834, 790, 614.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+(\text{C}_{13}\text{H}_{14}\text{NOF}_3\text{Na}) = 280.0922$; found: 280.0923 (0 ppm).

9.5. 4-(Difluoro(4-fluorophenyl)methyl)-5-ethylpyrrolidin-2-one (91e)

Starting from 50 mg (0.364 mmol) methyl 3-(difluoro(4-fluorophenyl)methyl)-4-nitrohexanoate **89e** we obtained 26 mg of **91e** (64%) as a colorless waxy solid.



91e
 $C_{13}H_{14}F_3NO$
 $M = 257.26 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.39 (DCM / Methanol: 95/5).

¹H NMR (300 MHz, CD₃CN) δ 7.61 – 7.53 (m, 2H), 7.30 – 7.17 (m, 2H), 6.62 (bs, 1H), 3.64 – 3.56 (m, 1H), 3.05 – 2.78 (m, 1H), 2.42 (dd, ²J_{H-H} = 17.6 Hz, ³J_{H-H} = 10.0 Hz, 1H), 2.25 (dd, ²J_{H-H} = 17.6 Hz, ³J_{H-H} = 5.4 Hz, 1H), 1.51 – 1.28 (m, 2H), 0.79 (t, ³J_{H-H} = 7.4 Hz, 3H).

¹³C NMR (75 MHz, CD₃CN) δ 175.3, 164.7 (dt, ¹J_{C-F} = 247.6, ⁵J_{C-F} = 2.0 Hz), 132.7 (td, ²J_{C-F} = 27.1 Hz, ⁴J_{C-F} = 3.2 Hz), 128.9 (dt, ³J_{C-F} = 8.9 Hz, ³J_{C-F} = 6.4 Hz), 123.8 (t, ¹J_{C-F} = 244.1 Hz), 116.6 (d, ²J_{C-F} = 22.2 Hz), 55.9 (t, ³J_{C-F} = 3.9 Hz), 47.2 (t, ²J_{C-F} = 26.9 Hz), 31.3 (t, ³J_{C-F} = 3.9 Hz), 30.1, 9.8.

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -101.05 (ABX system, dd, ²J_{F-F} = 248.6 Hz, ⁶J_{F-F} = 2.3 Hz), -104.11 (ABX system, dd, ²J_{F-F} = 248.6 Hz, ⁶J_{F-F} = 2.3 Hz), -109.93 (t, ⁶J_{F-F} = 2.3 Hz).

¹⁹F NMR (282 MHz, CDCl₃) δ -101.05 (ABXX' system, ddd, ²J_{F-F} = 248.6 Hz, ³J_{F-H} = 13.0 Hz, ⁶J_{F-F} = 2.3 Hz), -104.11 (ABXX' system, ddd, ²J_{F-F} = 248.6 Hz, ³J_{F-H} = 17.3 Hz, ⁶J_{F-F} = 2.3 Hz), -109.84 to -110.03 (m).

FT-IR (cm⁻¹): 2968, 2935, 2882, 1697, 1609, 1514, 1314, 1273, 1235, 1163, 1042, 1010, 840.

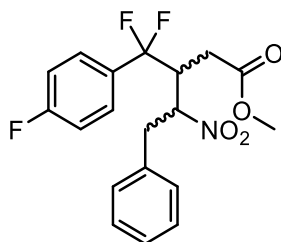
HRMS (ESI): calcd. for [M+Na]⁺(C₁₃H₁₄NOF₃Na) = 280.0920; found: 280.0923 (1 ppm).

The stereochemistry of this molecule has been established through 2D NOESY (¹H, ¹H) experiments: see added spectra.

9.6. Representative procedure: Synthesis of methyl 3-(difluoro(4-fluorophenyl)methyl)-4-nitro-5-phenylpentanoate (92e) and (93e)

To methyl (Z)-4,4-difluoro-4-phenylbut-2-enoate **82e** (200 mg, 0.87 mmol) was added 1-(2-nitroethyl)benzene (262 mg, 1.7 mmol) and potassium carbonate (360 mg, 2.6 mmol) in DMSO (5 ml). The reaction mixture was stirred at room temperature and it was controlled using TLC. At the end saturated NH₄Cl (5 mL) was added and the reaction mixture was extracted with ethyl acetate (3x10mL). The organic layers were separated, washed with water (3x5mL), dried over Na₂SO₄ and concentrated under vacuum. After purification by chromatography on

silica gel, a 1:1 mixture of unseparable diastereoisomers **92e** and **93e** was obtained as a colorless oil 275 mg (83 % overall yield).



92e & 93e

$C_{19}H_{18}F_3NO_4$

$M = 381.35 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.5 (Cyclohexane / Ethyl Acetate: 8/2).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.57 – 7.49 (m, 2H), 7.45 – 7.37 (m, 2H), 7.34 – 7.04 (m, 14H), 5.04 (dt, $^3J_{\text{H-H}} = 10.5 \text{ Hz}$, $^3J_{\text{H-H}} = 4.0 \text{ Hz}$, 1H), 4.91 (ddd, $^3J_{\text{H-H}} = 8.7$, $^3J_{\text{H-H}} = 5.9$, $^3J_{\text{H-H}} = 2.5 \text{ Hz}$, 1H), 3.77 – 3.65 (m, 1H), 3.64 (s, 3H), 3.60 (s, 3H), 3.42 – 3.15 (m, 4H), 3.12 – 2.94 (m, 2H), 2.87 – 2.64 (m, 3H).

$^{19}\text{F}\{\text{H}\}$ NMR (282 MHz, CDCl_3) δ -96.35 (ABX system, dd, $^2J_{\text{F-F}} = 251.3 \text{ Hz}$, $^6J_{\text{F-F}} = 2.8 \text{ Hz}$), -96.70 (A'B'X' system, dd, $^2J_{\text{F-F}} = 253.4 \text{ Hz}$, $^6J_{\text{F-F}} = 2.7 \text{ Hz}$), -99.83 (ABX system, dd, $^2J_{\text{F-F}} = 251.2 \text{ Hz}$, $^6J_{\text{F-F}} = 2.4 \text{ Hz}$), -102.00 (A'B'X' system, dd, $^2J_{\text{F-F}} = 253.3 \text{ Hz}$, $^6J_{\text{F-F}} = 2.7 \text{ Hz}$), -108.94 (t, $^6J_{\text{F-F}} = 2.7 \text{ Hz}$), -109.42 (t, $^6J_{\text{F-F}} = 2.7 \text{ Hz}$).

^{19}F NMR (282 MHz, CDCl_3) δ -96.35 (ABXY system, ddd, $^2J_{\text{F-F}} = 251.3 \text{ Hz}$, $^3J_{\text{F-H}} = 12.2 \text{ Hz}$, $^6J_{\text{F-F}} = 2.8 \text{ Hz}$), -96.70 (A'B'X'Y' system, ddd, $^2J_{\text{F-F}} = 253.4 \text{ Hz}$, $^3J_{\text{F-H}} = 13.7 \text{ Hz}$, $^6J_{\text{F-F}} = 2.7 \text{ Hz}$), -99.83 (ABXY system, ddd, $^2J_{\text{F-F}} = 251.2 \text{ Hz}$, $^3J_{\text{F-H}} = 18.1 \text{ Hz}$, $^6J_{\text{F-F}} = 2.4 \text{ Hz}$), -102.00 (A'B'X'Y' system, ddd, $^2J_{\text{F-F}} = 253.3 \text{ Hz}$, $^3J_{\text{F-H}} = 16.5 \text{ Hz}$, $^6J_{\text{F-F}} = 2.7 \text{ Hz}$), -108.82 to -109.03 (m), -109.27 to -109.58 (m).

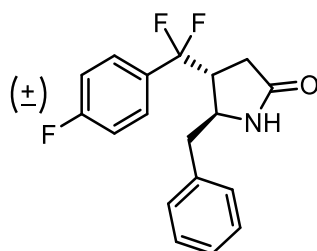
FT-IR (cm^{-1}): 2956, 2922, 1737, 1609, 1555, 1514, 1437, 1371, 1275, 1235, 1197, 1162, 1048, 1001, 840, 699.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+(\text{C}_{19}\text{H}_{18}\text{NO}_4\text{F}_3\text{Na}) = 404.1080$; found: 404.1083 (1 ppm).

9.7. 5-Benzyl-4-(difluoro(4-fluorophenyl)methyl)pyrrolidin-2-one (94e)

Starting from 100 mg (0.262 mmol) of the 1:1 mixture of diastereoisomers **92e** and **93e** we obtained 56 mg of a 1:1 mixture of **94e** and **95e** (67% overall yield) as a colorless oil. These

two diastereoisomers proved to be very difficult to separate but small quantities required for the spectral analyses could be obtained by preparative TLC using (DCM / Methanol: 98/2).



94e

$C_{18}H_{16}F_3NO$
 $M = 319.33 \text{ g.mol}^{-1}$

16e: waxy solid, $R_f = 0.5$ (DCM / Methanol: 95/5).

1H NMR (300 MHz, $CDCl_3$) δ 7.61 – 7.51 (m, 2H), 7.37 – 7.08 (m, 7H), 5.43 (bs, 1H), 3.97 – 3.87 (m, 1H), 3.47 – 3.25 (m, 1H), 3.19 – 3.09 (m, 1H), 2.81 – 2.70 (m, 1H), 2.68 (dd, $^2J_{H-H} = 16.7 \text{ Hz}$, $^3J_{H-H} = 11.5 \text{ Hz}$, 1H), 2.23 (dd, $^2J_{H-H} = 16.7 \text{ Hz}$, $^3J_{H-H} = 8.5 \text{ Hz}$, 1H).

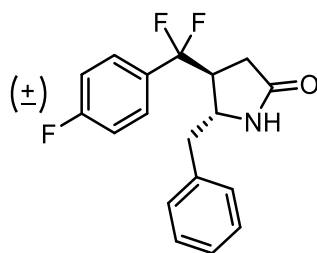
^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 163.9 (dt, $^1J_{C-F} = 250.8 \text{ Hz}$, $^5J_{C-F} = 1.8 \text{ Hz}$), 137.7, 132.3 (td, $^2J_{C-F} = 26.9 \text{ Hz}$, $^4J_{C-F} = 3.3 \text{ Hz}$), 129.3, 129.2, 127.3 (dt, $^3J_{C-F} = 8.7 \text{ Hz}$, $^3J_{C-F} = 6.3 \text{ Hz}$), 127.2, 121.7 (t, $^1J_{C-F} = 245.7 \text{ Hz}$), 116.2 (d, $^2J_{C-F} = 22.1 \text{ Hz}$), 56.6, 46.1 (t, $^2J_{C-F} = 27.6 \text{ Hz}$), 38.1 (t, $^3J_{C-F} = 3.7 \text{ Hz}$), 30.2 (t, $^3J_{C-F} = 4.1 \text{ Hz}$).

^{19}F {H} NMR (282 MHz, $CDCl_3$) δ -97.75 (ABX system, dd, $^2J_{F-F} = 247.2 \text{ Hz}$, $^6J_{F-F} = 2.7 \text{ Hz}$), -98.99 (ABX system, dd, $^2J_{F-F} = 248.9 \text{ Hz}$, $^6J_{F-F} = 2.7 \text{ Hz}$), -109.78 (t, $^6J_{F-F} = 2.7 \text{ Hz}$).

FT-IR (cm^{-1}): 3199, 3101, 3060, 3028, 2956, 2925, 2854, 1688, 1605, 1512, 1454, 1385, 1298, 1258, 1239, 1188, 1161, 1078, 1053, 1031, 989, 839, 777, 699.

HRMS (ESI): calcd. for $[M+Na]^+(C_{18}H_{16}NOF_3Na) = 342.1076$; found: 342.1079 (1 ppm).

9.8. (4S,5R)-5-benzyl-4-(difluoro(4-fluorophenyl)methyl)pyrrolidin-2-one (95e)



95e

$C_{18}H_{16}F_3NO$

$M = 319.33 \text{ g}\cdot\text{mol}^{-1}$

17e: waxy solid; $R_f \approx 0.5$ (DCM / Methanol: 95/5).

1H NMR (300 MHz, CD_3CN) δ 7.51 – 7.41 (m, 2H), 7.34 – 6.95 (m, 7H), 6.15 (bs, 1H), 3.86 (td, $^3J_{H-H} = 6.4$ Hz, $^3J_{H-F} = 3.5$ Hz, 1H), 3.07 – 2.86 (m, 1H), 2.68 (d, $^3J_{H-H} = 6.4$ Hz, 2H), 2.32 (dd, $^2J_{H-H} = 17.6$ Hz, $^3J_{H-H} = 9.3$ Hz, 1H), 2.24 (dd, $^2J_{H-H} = 17.6$ Hz, $^3J_{H-H} = 5.4$ Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5, 163.9 (dt, $^1J_{C-F} = 294.8$ Hz, $^5J_{C-F} = 2.1$ Hz), 136.7, 131.4 (td, $^2J_{C-F} = 27.0$ Hz, $^4J_{C-F} = 3.3$ Hz), 129.2, 129.1, 127.5 (dt, $^3J_{C-F} = 8.7$ Hz, $^3J_{C-F} = 6.3$ Hz), 127.3, 121.9 (t, $^1J_{C-F} = 231.2$ Hz), 116.1 (d, $^2J_{C-F} = 22.1$ Hz), 55.4 (t, $^3J_{C-F} = 3.6$ Hz), 47.5 (t, $^2J_{C-F} = 27.6$ Hz), 43.2, 30.98 (t, $^3J_{C-F} = 3.8$ Hz).

^{19}F {H} NMR (282 MHz, $CDCl_3$) δ -101.88 (ABX system, dd, $^2J_{F-F} = 247.1$ Hz, $^6J_{F-F} = 2.5$ Hz), -102.97 (ABX system, dd, $^2J_{F-F} = 250.0$ Hz, $^6J_{F-F} = 2.5$ Hz), -109.81 (t, $^6J_{F-F} = 2.5$ Hz).

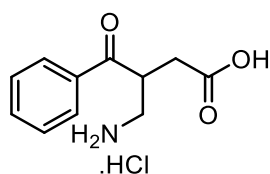
^{19}F NMR (282 MHz, $CDCl_3$) δ -101.88 (ABXX' system, ddd, $^2J_{F-F} = 247.1$ Hz, $^3J_{F-H} = 14.1$ Hz, $^6J_{F-F} = 2.5$ Hz), -102.97 (ABXX' system, ddd, $^2J_{F-F} = 250.0$ Hz, $^3J_{F-H} = 15.5$ Hz, $^6J_{F-F} = 2.5$ Hz), -109.69 to -109.88 (m).

FT-IR (cm^{-1}): 3229, 2924, 2853, 1698, 1608, 1514, 1300, 1271, 1235, 1162, 1056, 840.

HRMS (ESI): calcd. for $[M+Na]^+(C_{18}H_{16}NOF_3Na) = 342.1076$; found: 342.1081 (1 ppm).

10. GABA Analogue formation: 3-(aminomethyl)-4-oxo-4-phenylbutanoic acid hydrochloride (96)

4-(difluoro(phenyl)methyl)pyrrolidin-2-one **85a** (28 mg, 0.13 mmol) was added to 6 N HCl (2 mL), and the resulting mixture was stirred at 110 °C for 18 h. Water then was evaporated using rotavap to obtain a white solid, which was washed three times with acetone to afford unfluorinated GABA analogue **96** (23.5 mg, yield 70%).



96

$C_{11}H_{14}ClNO_3$
M = 243.69 g.mol⁻¹

¹H NMR (300 MHz, D₂O) δ 8.03 (d, ³J_{H-H} = 7.0 Hz, 2H), 7.81 – 7.73 (m, 1H), 7.63 (t, ³J_{H-H} = 7.0 Hz, 2H), 4.41 – 4.27 (m, 1H), 3.45 (dd, ²J_{H-H} = 13.4 Hz, ³J_{H-H} = 7.3 Hz, 1H), 3.21 (dd, ²J_{H-H} = 13.4 Hz, ³J_{H-H} = 5.1 Hz, 1H), 2.89 (dd, ²J_{H-H} = 16.9 Hz, ³J_{H-H} = 5.1 Hz, 2H), 2.73 (dd, ²J_{H-H} = 16.9 Hz, ³J_{H-H} = 5.1 Hz, 2H).

¹³C NMR (75 MHz, D₂O) δ 202.9, 174.8, 135.0, 134.8, 129.3, 128.7, 40.9, 39.7, 34.9.

HRMS (ESI): calcd. for [M+Na]⁺(C₁₁H₁₃NO₃Na) = 230.0788; found: 230.0789 (1 ppm).

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CHAPTER III

Synthesis of Novel Cyclic Nitrones with *gem*-Difluoroalkyl Side Chains through Cascade Reactions

1. Introduction

In an attempt to synthesize the desired *gem*-difluorinated GABA analogues, we encountered several problems as mentioned in the previous chapter, but we could finally prepare new interesting gamma-lactames. As a next step for this research, we considered as a new challenge the synthesis of novel cyclic nitrones with *gem*-difluoroalkyl side chains.

1.1. Nitrones

Structurally, nitrones consist of an imine-*N*-oxide, more specifically a $-C=N^+(O^-)$ functional group like in **1**. They possess different reactive sites (Figure C3. 1), being susceptible to nucleophilic attacks on the carbon of the nitronyl functional group (marked in purple),¹ the potential to act as 1,3-dipoles thus to undergo 1,3-dipolar cycloaddition with alkenes to afford isoxazolidines (marked in red).² Finally the negative charge held by the oxygen can give rise to chelation reactions on metals (assigned in green).³

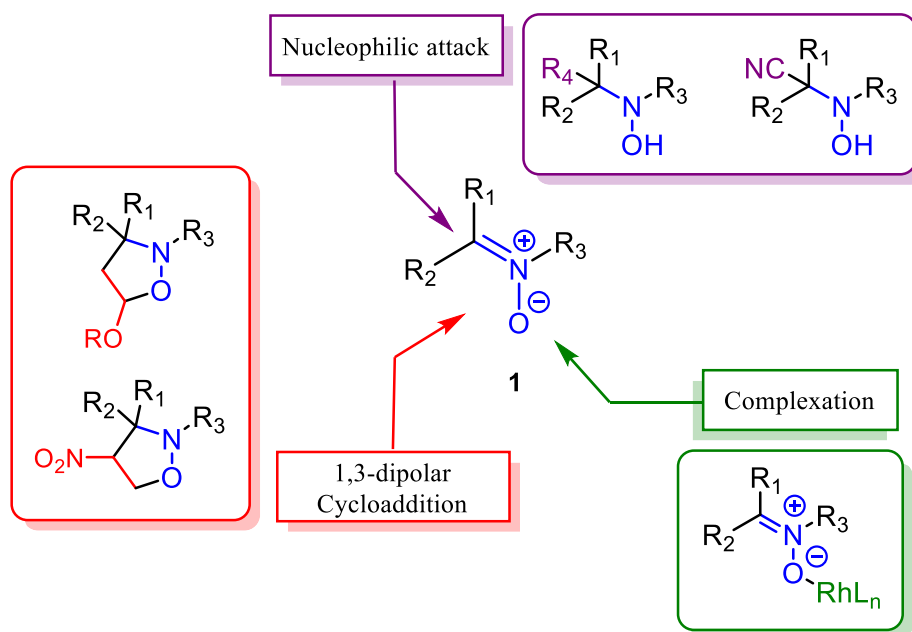
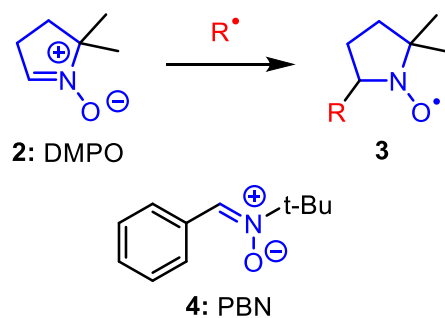


Figure C3. 1: Different reactive sites in nitrones

Nitrones are presently the most frequently used spin traps to characterize free radicals in biological systems. They entail the addition of the free radicals to the carbon-nitrogen double bond, affording more stabilized paramagnetic aminoxyl spin adduct **3** with generally long enough half-lives to be characterized spectroscopically (Scheme C3. 1). Two major categories are extensively studied: cyclic nitrones derived from 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) **2**, and linear nitrones derived from *N*-tert-butyl- α -phenylnitrone (PBN) **4**.



Scheme C3. 1: Top: The spin-trapping mechanism of DMPO **2** to obtain an aminoxyl spin adduct **3**, bottom: the linear nitron PBN **4**

However, due to the variety of radical types to be studied with large differences in physical, chemical and biological properties, there is a constant need to design and develop novel nitrones as spin traps.⁴ Besides, nitrones have been employed also as therapeutic agents in the treatment of diseases related to oxidative stress such as neurodegeneration,⁵ ischemic stroke,⁶ cardiovascular diseases,⁷ cancer,⁸ and other age-related diseases.⁹ In addition, nitrones are valuable intermediates in organic synthesis affording efficiently various types of useful heterocyclic, as well as acyclic molecules.² As a result many synthetic methods were developed to prepare nitrones.

1.2. Synthetic methods towards nitrones

Several methods to synthesize nitrones were encountered in the literature. They can readily be attained by either oxidation reactions, or methods that do not entail oxidations.

1.2.1. Oxidative methods

It comprises an important part in the synthetic routes of nitrones in the literature. The precursors utilized are secondary amines **5**, *N*-alkyl- α -amino acids **6**, *N,N*-disubstituted hydroxylamines **7**, imines **8**, and isoxazolidines **9** (Figure C3. 2).

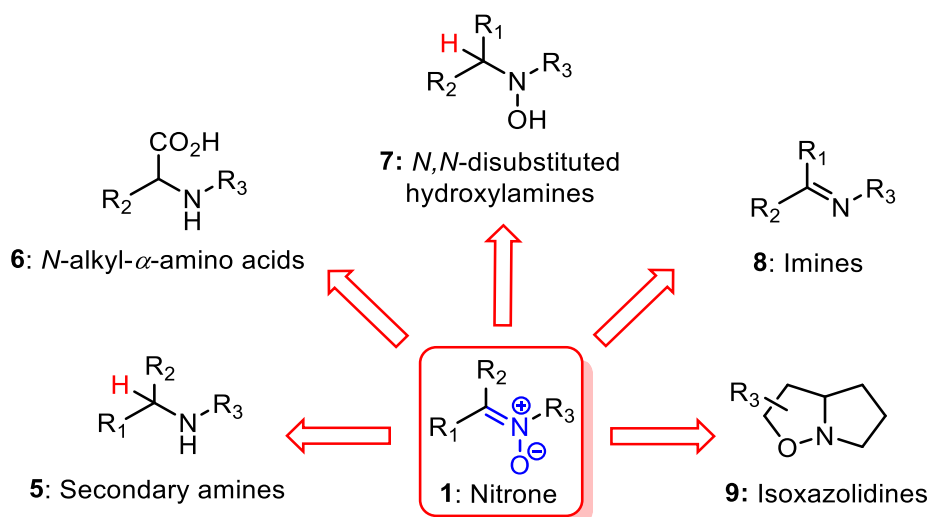
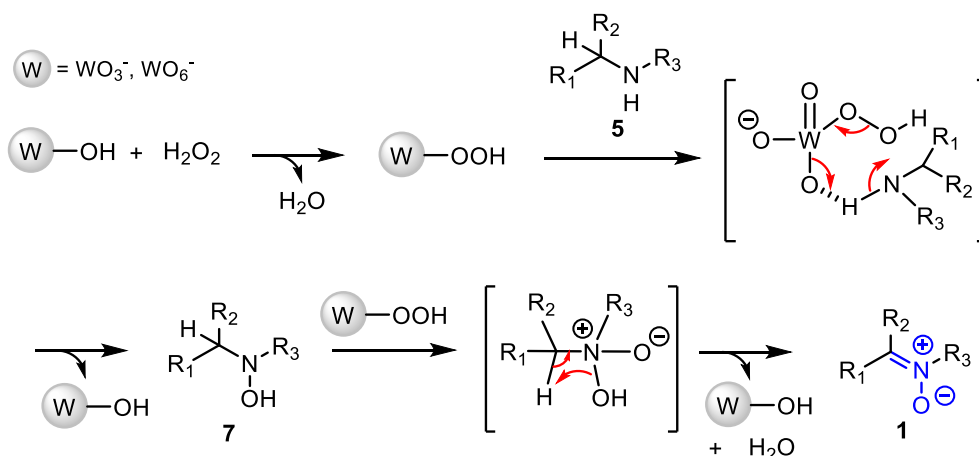


Figure C3. 2: Precursors of nitrones by oxidative pathway.

1.2.1.a Using secondary amines 5

It is the most appropriate method for the preparation of nitrones. It was discovered by Murahashi *et al.* in 1984 by using 30% H_2O_2 solution in the presence of a metal catalyst such as $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ leading to nitrones with high yields (~95%).¹⁰ Its mechanism was then studied in 1990 as shown in Scheme C3. 2 and this method was very useful allowing its usage in preparative scale synthesis.¹¹ Concentrated hydrogen peroxide was later replaced by urea complexed with hydrogen peroxide (UMP) which constitute a safer alternative. Subsequently it was tested with different catalysts such as Na_2MoO_4 and SeO_2 affording comparable results to $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ in several cases.¹²

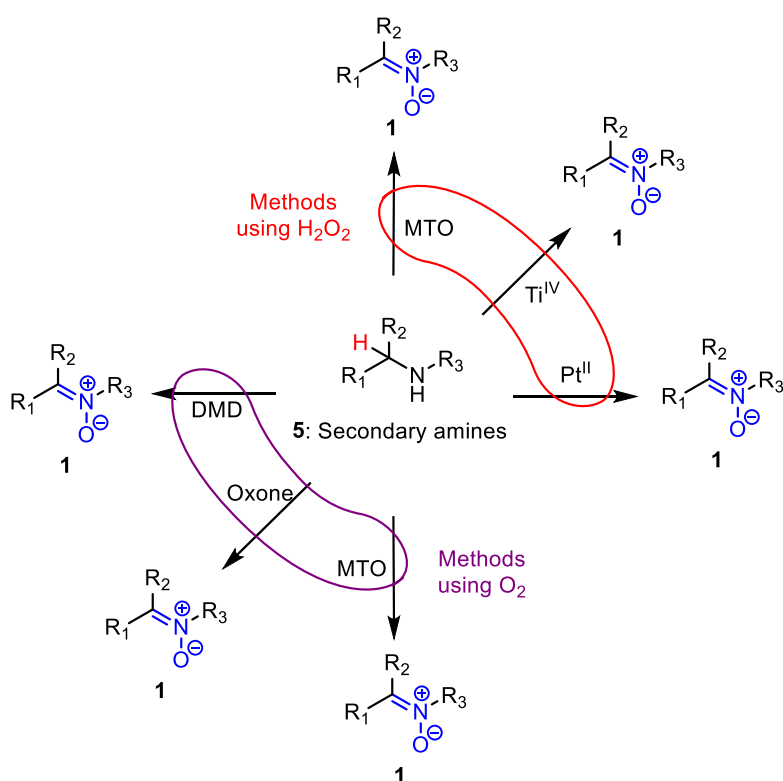


Scheme C3. 2: Mechanistic pathway of the oxidation reaction of the secondary amines **5** to give nitron **1**

Different catalysts have been employed in the oxidation of secondary amines in the presence of H_2O_2 or UMP including methyltrioxorhenium (MeReO_3 , MTO) to reach high yields (up to

90%),¹³ (trialkanolamino)titanium(IV) complexes which can afford nitrones in 99% yield,¹⁴ or by using platinum(II) complex which gave acceptable yields of 55%.

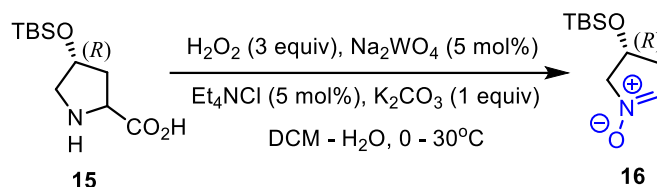
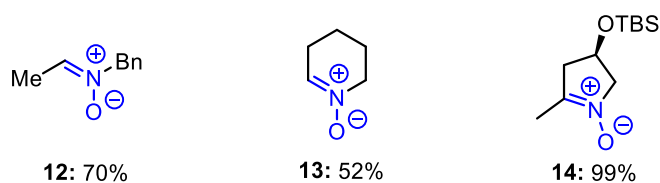
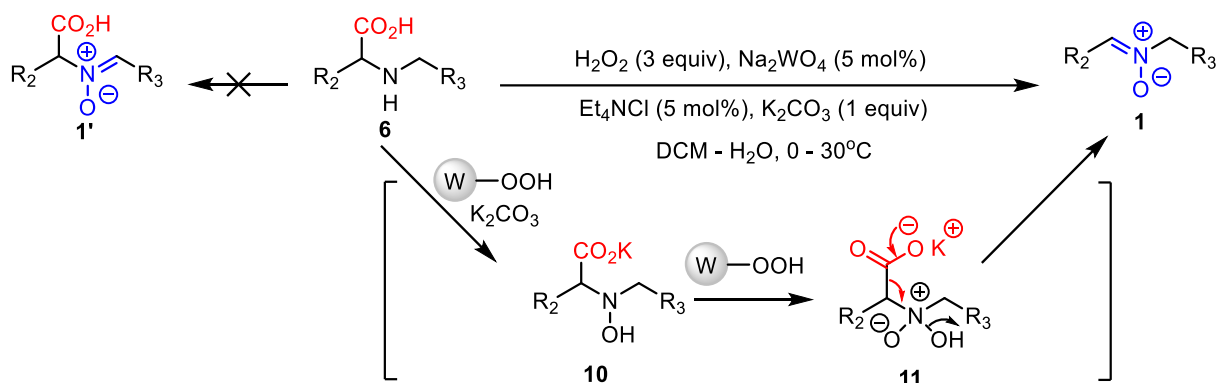
Another alternative to H₂O₂ was the use of molecular oxygen, consequently a new system was developed involving the combination of MTO and O₂ which afforded nitrones in excellent yields.¹⁵ This synthetic tool was environmentally friendlier with simple work-up, hence opening the gate toward developing metal free methodologies, as the use of oxone¹⁶, dimethyldioxirane (DMD),¹⁷ and *C*-Phenyl-*N*-phenylsulfonyloxaziridine (Davis reagent)¹⁸ which was later replaced by a safe, reproducible, and scalable oxidation using *m*-chloroperbenzoic acid (*m*-CPBA).¹⁹ These methods are summarized in Scheme C3. 3.



Scheme C3. 3: From secondary amines toward nitrones using H₂O₂ or O₂

1.2.1.b Using *N*-alkyl- α -amino acids 6

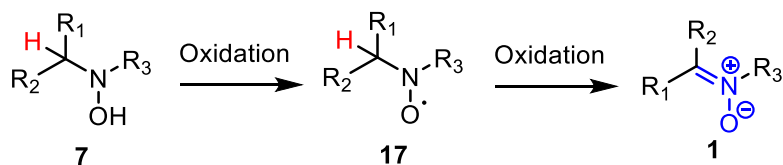
It is an important method involving a decarboxylation step to produce nitrones. However, the oxidation of *N*-alkyl- α -amino acids **6** with hydrogen peroxide did not proceed in the presence of selenium dioxide or sodium tungstate as catalysts. After numerous trials, the researchers found that the use of 1 equiv of base under phase transfer conditions gave good results without the formation of isomeric nitrones **1'** (Scheme C3. 4).²⁰ This method was exploited in the synthesis of the enantiomerically pure (4*R*)-4-((*tert*-butyldimethylsilyl)oxy)-1-pyrroline *N*-oxide **16** which could be prepared from **15** with 70% yield (Scheme C3. 4).²¹



Scheme C3. 4: Decarboxylative oxidation of *N*-Alkyl- α -amino Acids and some examples where this method was exploited

1.2.1.c Using *N,N*-disubstituted hydroxylamines 7

It is a particularly useful, mild, method to obtain nitrones, and this topic was reviewed recently by Matassini *et al.* covering the stoichiometric oxidants of *N,N*-disubstituted hydroxylamines from 1993 to 2017.²² The reaction involves the formation of a nitroxide radical **17**, and it requires the presence of at least one α -hydrogen on the nitroxide function (Scheme C3. 5).

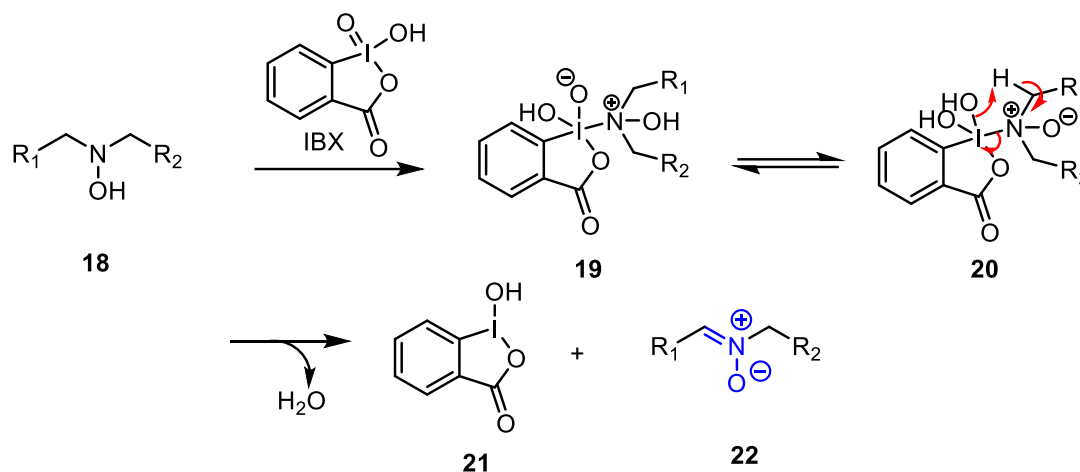


Scheme C3. 5: Oxidation Mechanism that involves a radical.

This strategy was employed by Goti *et al.* who used yellow mercuric oxide (HgO) as an oxidant to obtain the required nitron with a high yield and good regioselectivity.²³ Mercuric salt, due to its toxicity was replaced by Manganese(IV) oxide (MnO₂) as a good alternative.²⁴

Different stoichiometric systems were used, for instance oxidation using 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) at -80 °C for 48h,²⁵ triphenylbismuth carbonate which

gave excellent yields and regioselectivity,²⁶ sodium hypochlorite (NaOCl) which is considered an ecologic and cheap system allowing the large scale production of nitrones,²⁷ and the use of IBX *o*-iodoxybenzoic acid (IBX) which favored the formation of aldonitrones vs ketonitrones due to steric hindrance. The proposed mechanism suggests the coordination of the IBX iodine atom at nitrogen, rather than oxygen, to give adduct **19** (Scheme C3. 6).

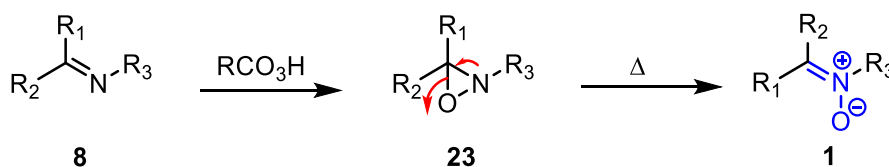


Scheme C3. 6: Mechanism Proposed for the IBX Oxidation of Hydroxylamines

Furthermore, several aerobic catalytic oxidations were reported using palladium black catalyst in 1983,²⁸ tetrapropylammonium perruthenate (TPAP) on its own,²⁹ or combined with *N*-methylmorpholine *N*-oxide (NMO) which are considered as an environmental friendly and mild alternatives.³⁰ More recently, gold nanoparticles supported on silica (Au/SiO₂) were used,³¹ or unsupported nanoporous gold (AuNPore) and both exhibited high efficiency.³²

1.2.1.d Using imines **8**

In 1956, William D. Emmons studied the preparation of oxaziranes **23** by oxidation of the appropriate imine **8** with peracetic acid (RCO₃H), then he noticed that they might undergo rearrangement to get the nitrones **1** (Baeyer-Villiger type).³³



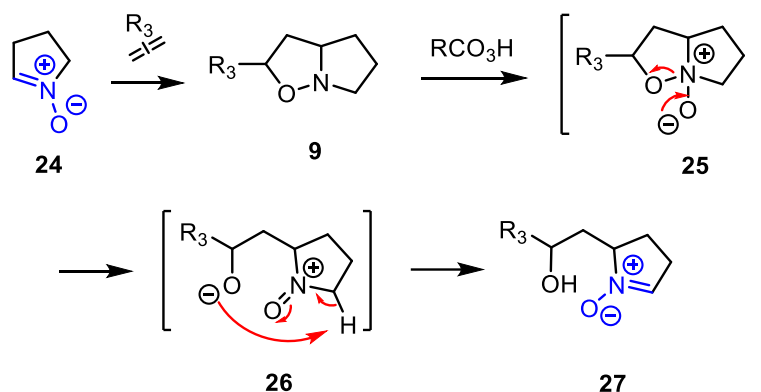
Scheme C3. 7: Baeyer-Villiger type formation of nitrones starting from amines **8**

Subsequently, several groups tried to explore the different factors which impact the oxaziridine:nitrone ratio, and demonstrated that significant amounts of nitrones were formed with imines bearing electron-donating substituents, in addition to the effect of the solvent used and the bulkiness of the substituents on the imine.³⁴

On the other hand, another oxidation reagents could be used to obtain nitrones directly from imines. For example, the use of a solution of dimethyldioxirane (DMD) in dichloromethane-acetone gives nitrones without the apparent formation of oxaziridines **23**,³⁵ as well as the use of graphite oxide catalyst – oxone,³⁶ Nafion supported molybdenum oxychloride – UHP,³⁷ permanganate ion MnO_4^- ,³⁸ and more recently the treatment of aromatic aldehydes and primary amines with UHP – MTO catalyst in one-pot reaction to obtain the corresponding nitrones.³⁹ Similar direct synthesis of nitrones was achieved also using the same catalyst when added to benzylic and cyclic imines.⁴⁰

1.2.1.e Using of isoxazolidines **9**

Isoxazolidines **9**, which are themselves prepared from nitrone **24**, have been reported to undergo oxidative ring opening with peracetic acid or hydrogen peroxide to afford nitrones.⁴¹ This interesting series of transformations apparently proceeds via *N*-oxidation and ring opening to a nitrosonium ion **26**, followed by tautomerization to afford the less substituted aldonitrones regioselectively **27** (Scheme C3. 8).⁴² Moreover chiral nitrones can be prepared selectively.⁴³ Altogether, this is an interesting transformation from a nitrone **24** to a substituted nitrone **27**.



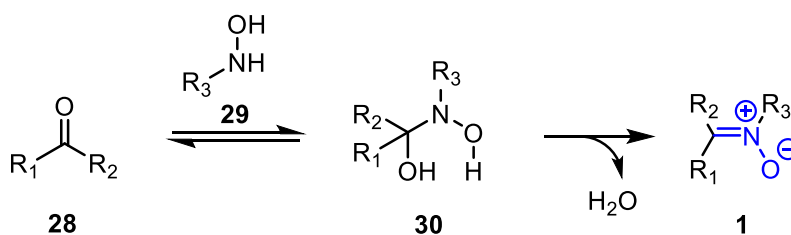
Scheme C3. 8: Mechanism of the synthesis of nitrones **27** from isoxazolidines **9**

1.2.2. Non-oxidative methods

Nitrones can also be synthesized by non-oxidative methods involving *N*-monosubstituted hydroxylamines, oximes, nitro compounds, or nitroso compounds.

1.2.2.a Condensation of *N*-monosubstituted hydroxylamines and carbonyl derivatives

It is the most typical and direct approach to obtain nitrones, the condensation of *N*-monosubstituted hydroxylamines **29** with aldehydes or ketones **28** takes place in two steps: the nucleophilic attack of the R-NH-OH onto the carbonyl group to form the hemiaminal intermediate **30**, followed by elimination of water to form the C=N bond (Scheme C3. 9).⁴⁴



Scheme C3. 9: Condensation reaction between the carbonyl group **28** and *N*-monosubstituted hydroxylamines **29**

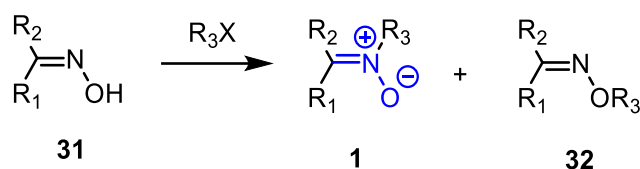
The condensation of *N*-alkyl-, *N*-alkenyl-, and *N*-aryl-substituted hydroxylamines with alkyl aldehydes yielded their corresponding nitrones in the presence of dehydrating reagent such as MgSO_4 ,⁴⁵ or without dehydrating reagent at higher temperature.⁴⁶ It is noteworthy that alkyl ketones showed a sluggish reactivity⁴⁷ and thus Lewis acids as AlCl_3 , MgBr_2 , or ZnCl_2 should be added.⁴⁸

In 2008, F. Lamaty *et al.* developed a new environmentally friendly methodology, where nitrones were synthesized *via* condensation of an equimolar amount of *N*-substituted hydroxylamines and aldehydes, for the first time in a ball-mill under mechanochemical and solvent-free conditions. The nitrones were obtained in pure form without further purification and in high yield.⁴⁹ Another fast and solvent-free method was performed by Rui C. Pinto *et al.* who exploited microwave irradiations to obtain nitrones in very good yields.⁵⁰

More recently, nitrones were obtained upon the treatment of aldehydes with *N*-substituted hydroxylamines efficiently promoted by pyrrolidine in a matter of minutes under very mild conditions in almost quantitative yields after a simple filtration through a short pad of silica gel.⁵¹

1.2.2.b Reaction of oximes

Reaction of oximes **31** with a variety of electrophiles results in the alkylation of the oxime's nitrogen to afford nitrones **1** in principle (Scheme C3. 10).

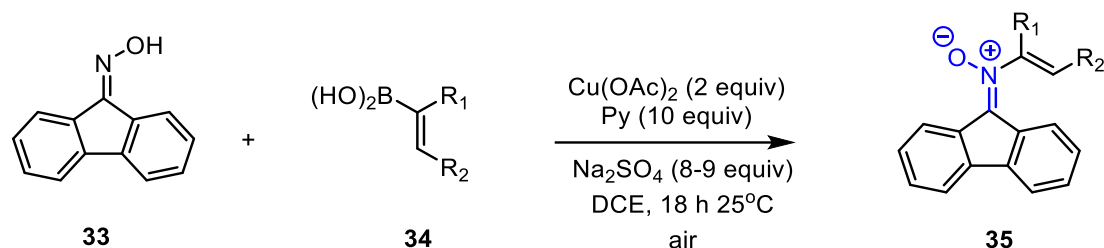


Scheme C3. 10: Synthesis of nitrones from oximes **31**

It is not a much used method due to the competition between the formation of nitron and the *O*-alkyl oximes co-product, and changes in the reaction conditions or variations in the alkyl halides or oximes employed have failed to affect the ratio of *O*- and *N*-alkylation

significantly.^{47,52} To overcome this problem, protection of the oxime's oxygen is possible in the form of *O*-trimethylsilyl oxime followed by the reaction with variety of electrophiles.⁵³

Fluorenone-derived *N*-vinyl nitrones **35** can be prepared in a single-step by copper-mediated coupling of fluorenone oxime **33** and vinyl boronic acids **34** (Scheme C3. 11).⁵⁴ This method was also used for the synthesis of *N*-alkenyl- α,β -unsaturated nitrones.⁵⁵

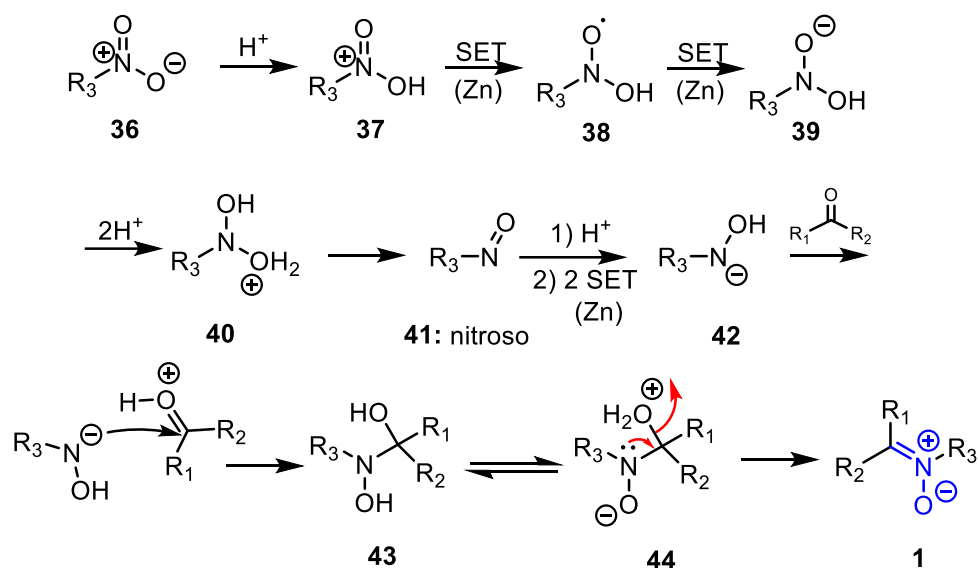


Scheme C3. 11: Cu-mediated *N*-vinyl nitron synthesis

Different catalytic systems have been reported for the synthesis of nitrones starting from oximes including copper-catalyzed conditions,⁵⁶ rhodium-catalyzed reactions,⁵⁷ the Lewis acid-catalyzed reactions,⁵⁸ combination of nickel-based Lewis acid-surfactant-combined catalysts and single-walled carbon nanotubes (SWNT),⁵⁹ and palladium-catalyzed allylic substitution of oximes.⁶⁰

1.2.2.c Using nitro compounds

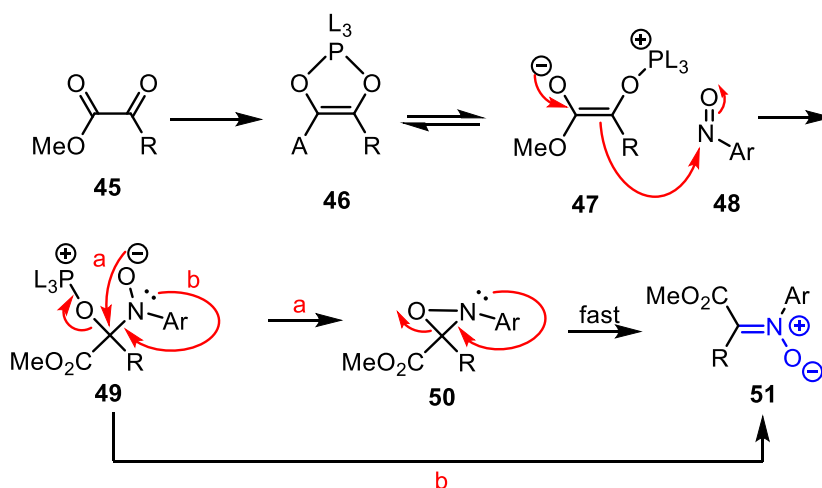
It is one of the most efficient method to synthesize nitrones, where nitro compounds react with aldehydes under reductive conditions. Metals such as zinc powder were used in the presence of aldehyde and NH_4Cl to reduce the nitro group and prepare aromatic, aliphatic, and highly functionalized sugar-derived nitrones through a sequence of single electron transfer (SET)/ protonation reactions (Scheme C3. 12). Different catalytic systems showed the ability to give nitrones, including visible light photoredox catalyst $\text{Ru}(\text{bpy})_3\text{Cl}_2$ and *i*- Pr_2NEt ,⁶¹ the reaction of H_2 using carbon-supported and -decorated platinum nanoparticles,⁶² ultrasmall platinum nanoclusters (PtNCs) encapsulated in amine-functionalized Zr metal-organic framework (MOF),⁶³ and the AuBr_3 -catalyzed cyclization of *o*-(alkynyl)nitrobenzenes under mild conditions.⁶⁴



Scheme C3. 12: Mechanism of the reduction of nitro compounds using Zn/H⁺ followed by the formation of nitrones

1.2.2.d Using nitroso compounds

Taking advantage of the high electrophilicity of N=O in nitroso groups, an umpolung approach toward *N*-aryl nitrone **51** construction utilizing a phosphine-mediated addition of 1,2-dicarbonyls **45** to nitroso compounds **48** was reported by Chavannavar *et al.* Two plausible mechanisms are depicted in Scheme C3. 13, where the nitrone may be formed in a stepwise fashion via a Mitsunobu-like displacement of phosphine oxide to yield oxaziridine **50** followed by ring opening (path a) or directly from zwitterion **49** (path b).⁶⁵

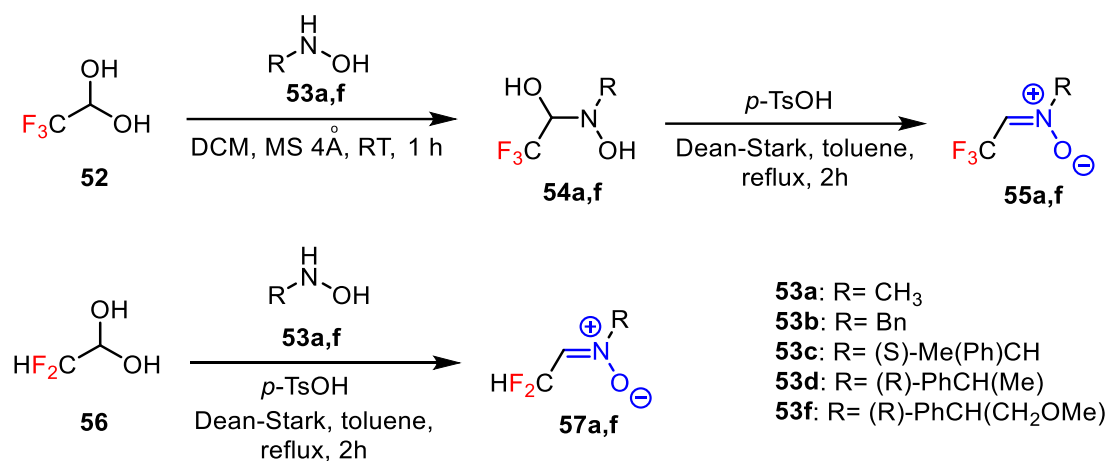


Scheme C3. 13: Suggested mechanism of the formation of *N*-aryl nitrones **51**

1.3. Fluorinated nitrones

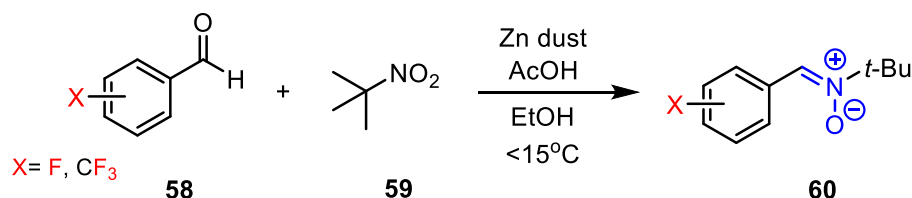
As discussed in detail previously, it is well recognized that introduction of fluorine in organic molecules can be used for a fine tuning of their physical, chemical, and biological properties. It was remarkable to find relatively few fluorinated nitrones described to date in the literature.

N-methyl-*C*-trifluoromethylnitronone **55a** was first reported in 1988 by K. Tanaka *et al.* and was obtained by the dehydration of *N*-methyl-*C*-trifluoromethylnitronone hydrate **54a** under reflux. This fluorinated nitronone was used to investigate different cycloaddition reactions.⁶⁶ This method was adjusted to obtain different analogues of fluorinated nitrones **55a,f** and **57a,f** by treating the corresponding hydroxylamines **53a,f** with fluoral hydrates **52** and **56** in toluene for 2 h, using a Dean–Stark apparatus (Scheme C3. 1).⁶⁷



Scheme C3. 14: Preparation of di- and trifluoromethyl nitrones **55a,f** and **57a,f**

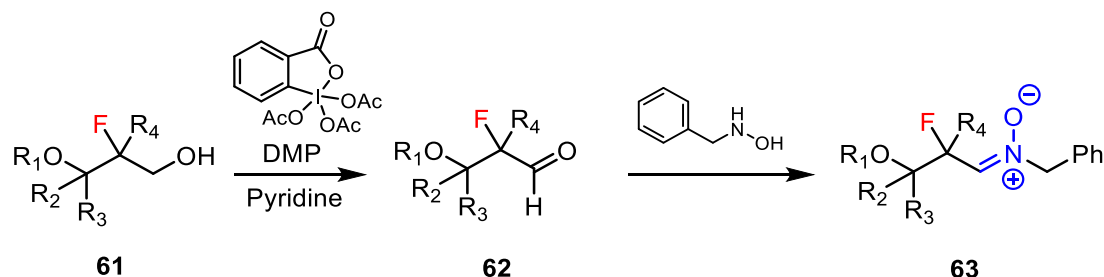
Various fluorinated benzaldehydes were investigated as starting fluorinated building blocks in an order to obtain fluorinated PNB **60**. Reaction was performed by cooling a mixture of 1 equiv of the appropriate aldehyde **58**, 2 equiv of 2-methyl-2-nitropropane **59**, and 3 equiv of activated zinc dust in 95% EtOH to 10–15 °C. Glacial acetic acid was then added dropwise while maintaining the sample temperature below 15 °C (Scheme C3. 15).⁶⁸



Scheme C3. 15: Synthesis of fluoro-substituted phenyl-tert-butyl nitrones **60**

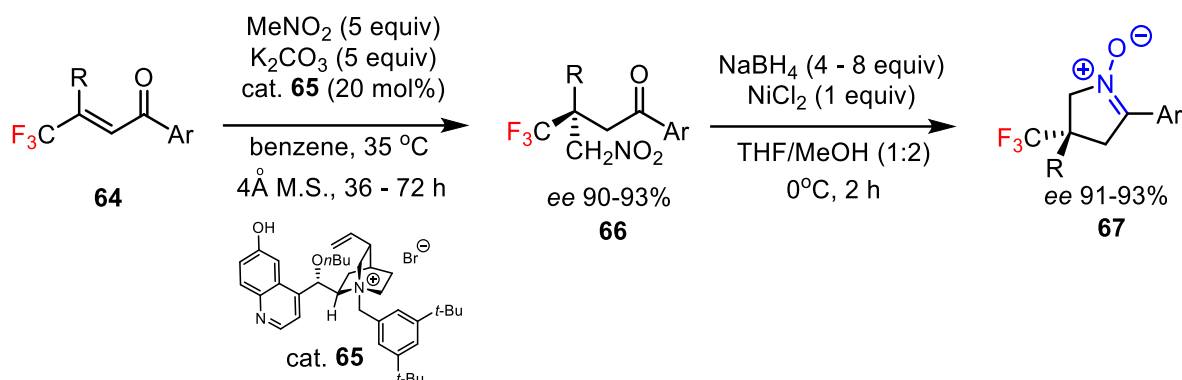
Further attempt to synthesize aliphatic fluorinated nitrones was done by M. Ihara in 1997. The authors approach consisted in oxidation of the alcohol **61** with Dess–Martin periodinane

DMP in the presence of pyridine to afford aldehydes **62**, followed by reaction with *N*-benzylhydroxylamine at room temperature, afforded the desired compounds **63** (Scheme C3. 16).⁶⁹



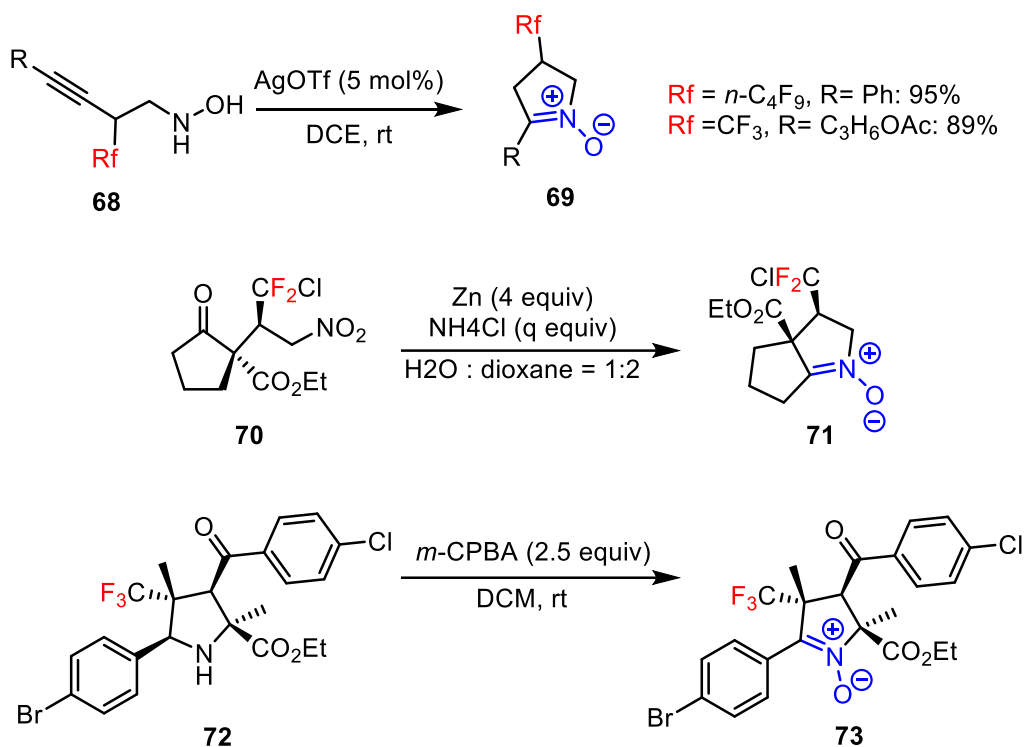
Scheme C3. 16: Preparation of the aliphatic nitrones **63**

Due to the importance of fused nitrogen heterocyclic systems, scientists shed the light on fluorinated cyclic nitrones. Thus, in 2013 H. Kawai *et al.* were able to access trifluoromethyl cyclic-*N*-oxides **67** through enantioselective conjugate addition of nitromethane to β,β -disubstituted enones **64** followed by the reduction of nitro group using NaBH_4 in the presence of NiCl_2 in THF/methanol mixture at 0°C (Scheme C3. 17).⁷⁰



Scheme C3. 17: Formation of enantiopure fluorinated *N*-oxides **67** using chiral catalyst **65**

Few recent articles were reported to access the fluorinated cyclic nitrones moiety including the AgOTf -catalyzed cyclization of *N*-(2-perfluoroalkyl-3-alkynyl)hydroxylamines **68** to give 4-perfluoroalkyl substituted 1-pyrroline *N*-oxides **69**.⁷¹ The use of zinc dust in presence of NH_4Cl and nitro compound **70** in water to obtain the bicyclic fluorinated nitrone **71**,⁷² or the use of *m*-CPBA to oxidize pyrrolidine **72** to afford fluorinated nitrone **73** in 94% yield,⁷³ were also reported.



Scheme C3. 18: preparation of various cyclic nitrones

2. Objective

Fluorinated nitrones have already shown some applications in chemistry and biology. Taking into consideration that ^{19}F NMR could be also a particularly useful tool for monitoring biophysical and biochemical studies, it appeared important to broaden the field of applications of such molecules.⁷⁴ Therefore, the goal of this study is to describe short and efficient syntheses of new cyclic nitrones bearing *gem*-difluoroalkyl side chains in γ -position. More precisely, we will first develop our strategy for the preparation of type **A** model nitrones (Figure C3. 3). Then, we will extend it to molecules **B** with functional groups in terminal position of the alkyl chains. Such groups (ester, azide, alcohol, tosylate, and protected alcohol) could be of much use to adjust the physicochemical and biological properties of these nitrones. Further, these groups could be also employed to graft easily various types of labels eventually required for in depth *in vivo* biological studies. Finally, starting from the same intermediates, we will report the preparation of the bicyclic analogues **C**. All these new nitrones will be prepared in short sequences (2-3 steps) from the easily available type-**D** *gem*-difluoroalkyl propargylic alcohols.⁷⁵

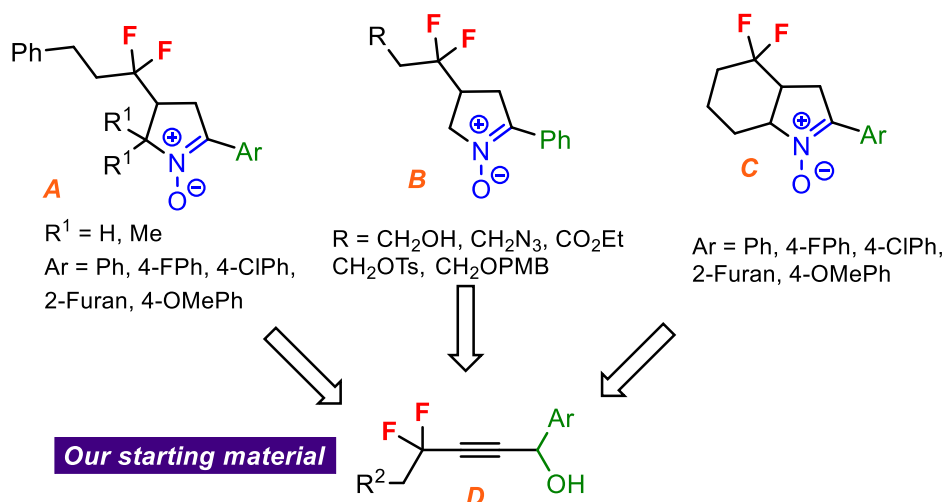


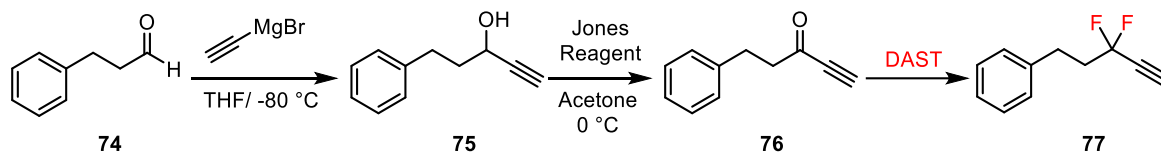
Figure C3. 3: Our target nitrones with *gem*-difluoroalkyl side chains

3. Results and discussion

Inspired by our previous work on γ -lactams with *gem*-difluorinated side chains, we anticipated the importance of the addition of the nitromethane on the α,β -unsaturated system to access different nitrogenous heterocycles. As a result we were able to design a new retrosynthetic pathway to attain our fluorinated nitrones targets.

3.1. Synthesis of type A nitrones

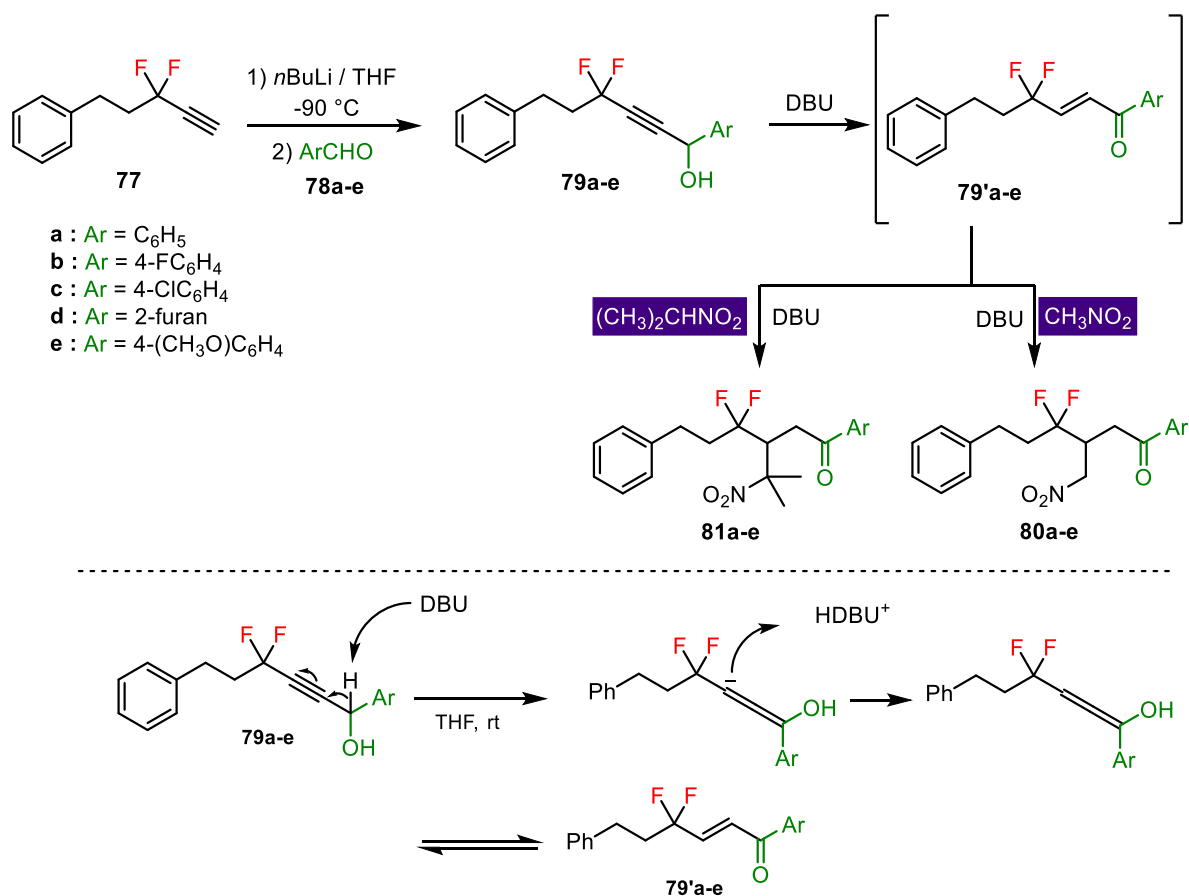
To prepare the first series of nitrones, we started from the known, *gem*-difluoro propargylic derivative **77**.⁷⁵⁻⁷⁶ Grignard addition of ethynylmagnesium bromide onto 3-phenylpropionaldehyde **74** in anhydrous THF at -80°C yielded alcohol **75**, which was readily oxidized to the corresponding propargylic ketone **76** by Jones reagent. On reaction with Diethylaminosulfur trifluoride (DAST) it gave, in fair yields, the *gem*-difluoropropargylic intermediate **77** (Scheme C3. 19).



Scheme C3. 19: Synthesis of *gem*-difluoro propargylic derivative **77**

After reaction with *n*-BuLi at -90°C , followed by addition on different aldehydes **78a-e**, the *gem*-difluorinated alcohols **79a-e** were obtained (Scheme C3. 20, Table C3. 1). The reactions with nitromethane, or 2-nitropropane, in the presence of DBU afforded in fair to good yields the nitro intermediates **80a-e** and **81a-e** respectively. In agreement with previous results, the first step of this domino process is likely the base-mediated isomerization of the propargylic

alcohols into the corresponding (non-isolated) enones **79'a-e** which are trapped *in situ* by the nitroalkane anions to give the desired molecules **80** or **81**.⁷⁷



Scheme C3. 20: Synthesis of nitro intermediates **80a-e** and **81a-e**

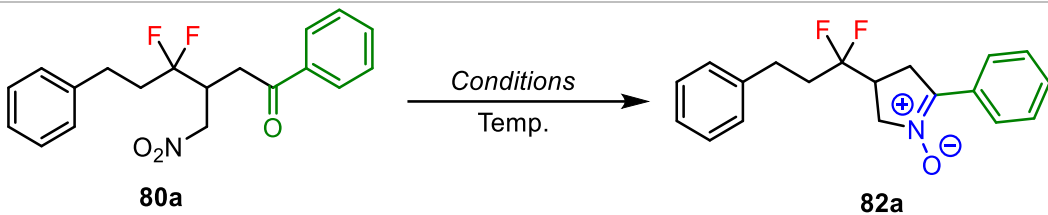
Table C3. 1: Synthesis of nitro intermediates **80a-e** and **81a-e**

| ENTRY | SM | Ar | YIELD (%) ^[a] | | |
|-------|-----|----|--------------------------|---------|---------|
| | | | 79 | 80 | 81 |
| 1 | 78a | | 79a(73) ^[b] | 80a(72) | 81a(69) |
| 2 | 78b | | 79b(81) | 80b(74) | 81b(69) |
| 3 | 78c | | 79c(60) | 80c(69) | 81c(73) |
| 4 | 78d | | 79d(64) | 80d(71) | 81d(72) |
| 5 | 78e | | 79e(67) | 80e(76) | 81e(60) |

^[a] isolated yields, ^[b] Reported previously⁷⁸

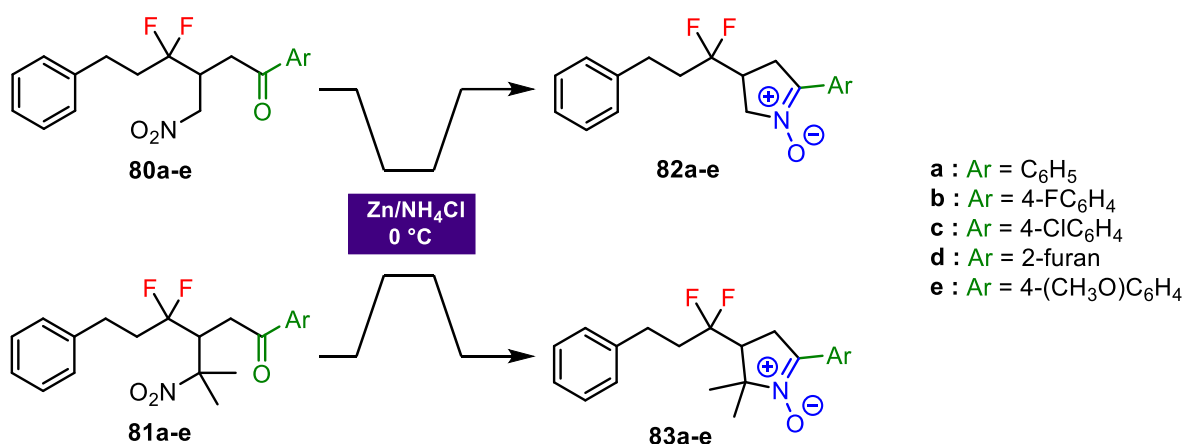
Several attempts were done to reduce the nitro group in **80a** in order to obtain the desired nitrone **82a**, as summarized in Table C3. 2. Upon using palladium hydroxide Pd(OH)₂ and H₂ gas at 40 °C the anticipated nitrone was obtained but unfortunately the yield was very low (15%). Similarly, NiCl₂·6H₂O in the presence of NaBH₄ gave a poor yield (30%) at 0 °C, and even lower yield (10%) was obtained by using ammonium formate NH₄HCO₂ in the presence of Pd/C at 60 °C. However, upon using Zn powder in the presence of NH₄Cl in water and THF at 0 °C, nitrone **82a** was obtained in 65% yield. Hence the latter method was selected for the reduction of the nitro group in the other intermediates.

Table C3. 2: Attempts to optimize the reduction reaction of the nitro group in **80a**



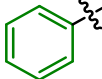
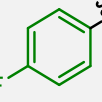
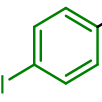
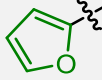
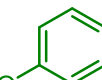
| Entry | Conditions | Temp. °C | Yield % |
|-------|--|----------|---------|
| 1 | Pd(OH) ₂ / H ₂ (g) | 40 | 15 |
| 2 | NiCl ₂ ·6H ₂ O / NaBH ₄ | 0 | 30 |
| 3 | NH ₄ HCO ₂ / Pd/C | 60 | 10 |
| 4 | Zn _(s) / NH ₄ Cl | 0 | 65 |

As a result, different nitrones with *gem*-difluorinated side chains **82a-e** and **83a-e** were successfully synthesized starting from intermediates **80a-e** and **81a-e**, affording our targets in moderate to good yields as shown in Scheme C3. 21 and Table C3. 3.



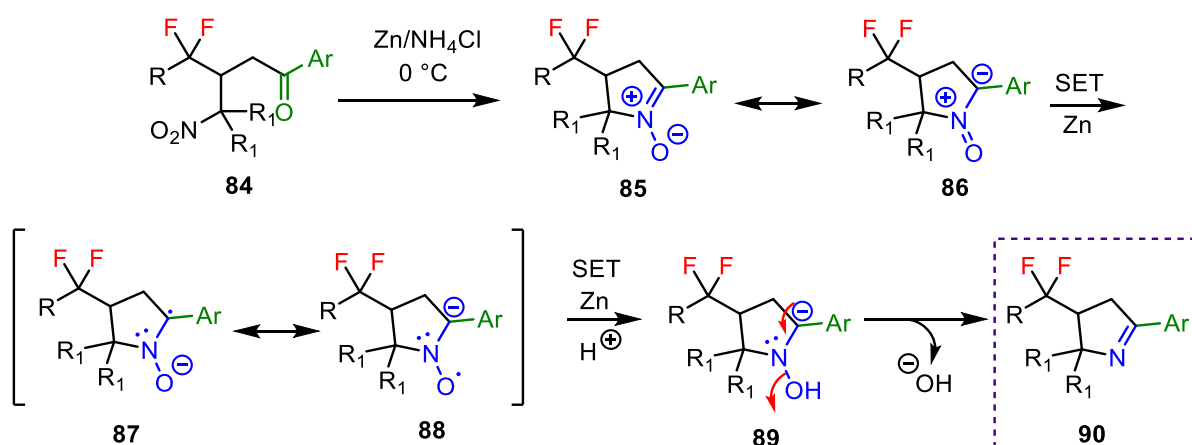
Scheme C3. 21: Synthesis of nitrones with *gem*-difluorinated side chains **82a-e** and **83a-e**

Table C3. 3: Synthesis of nitrones with *gem*-difluorinated side chains **82a-e** and **83a-e**

| ENTRY | Ar | YIELD (%) ^[a] | | | |
|-------|---|--------------------------|-----------------|------------|-----------------|
| | | SM | 82 | SM | 83 |
| 1 |  | 80a | 82a (65) | 81a | 83a (90) |
| 2 |  | 80b | 82b (61) | 81b | 83b (55) |
| 3 |  | 80c | 82c (71) | 81c | 83c (54) |
| 4 |  | 80d | 82d (73) | 81d | 83d (70) |
| 5 |  | 80e | 82e (69) | 81e | 83e (85) |

^[a] isolated yields.

It is noteworthy to keep attention of the reaction's time at this step and keep monitoring it each 5 mins using TLC plate, since in case of prolonged time exposing the reaction mixture to the reaction's reducing conditions, the nitron will be reduced too. Thus the formation of 1-pyrroline **90** will take place through another sequence of single electron transfer (SET)/protonation reactions (Scheme C3. 22).⁷⁹

Scheme C3. 22: Mechanism of the formation of dihydropyrroles **90**

Structures of all the fluorinated nitrones and their intermediates have spectral and analytical data (by NMR spectroscopy and mass spectrometry) in agreement with the proposed. In

addition, the structure of the fluorinated nitronone **82e** was confirmed by X-Ray analysis (Figure C3. 4).⁸⁰

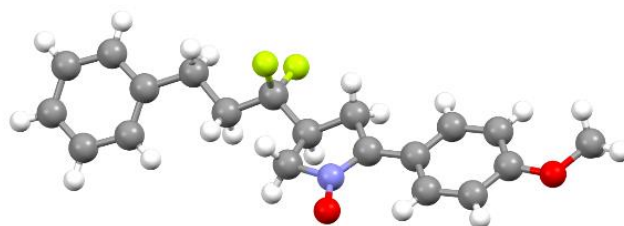


Figure C3. 4: X-Ray structure analysis of **82e**

3.2. Synthesis of type *B* nitronones

Next, we expanded the methodology to compounds having a functional group on the terminal position of the side chain. This could have useful applications in modifying, for instance, the pharmacokinetic parameters (Absorption, Distribution, Metabolism, Excretion) of the nitronones, if used as pharmaceuticals. On the other hand, this could help in changing their physicochemical properties especially if they are used as spin trapping agents. Our plan was to modify the CH₂-Ph group in Type *A* nitronones (Figure C3. 5), by replacing it with PMBO-CH₂ (a protected alcohol group) “*path (a)*” which has different steric and electronic properties compared to benzyl group, “*Path (b)*” on the other hand it will allow the preparation of the nitronone with a free alcohol group. Further, the transformation of alcohol to a good leaving group such as tosyl group should open the door toward S_N2 nucleophilic substitution reactions and thus introduction of other substituents and functions in this position “*path (c)*”; in particular the selection of azide group as an alternative substituent is due to its importance in the formation of different nitrogenous heterocycles mainly upon using it in click reactions “*path (d)*”. Finally, ester group was selected to enable coupling reactions.

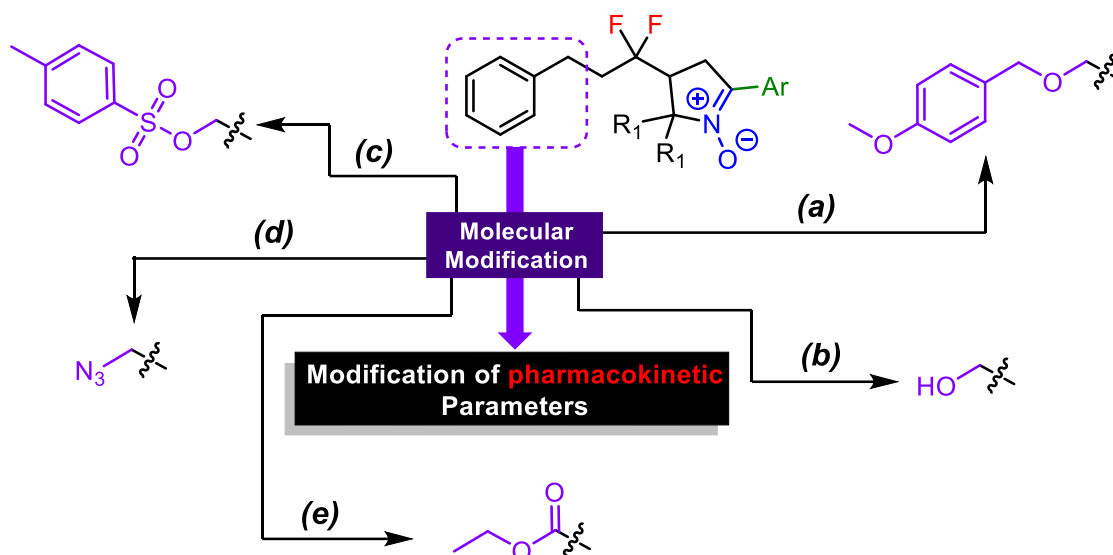
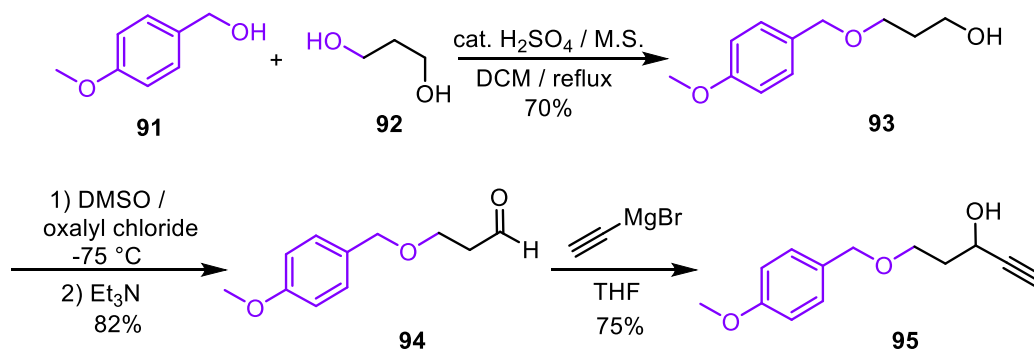


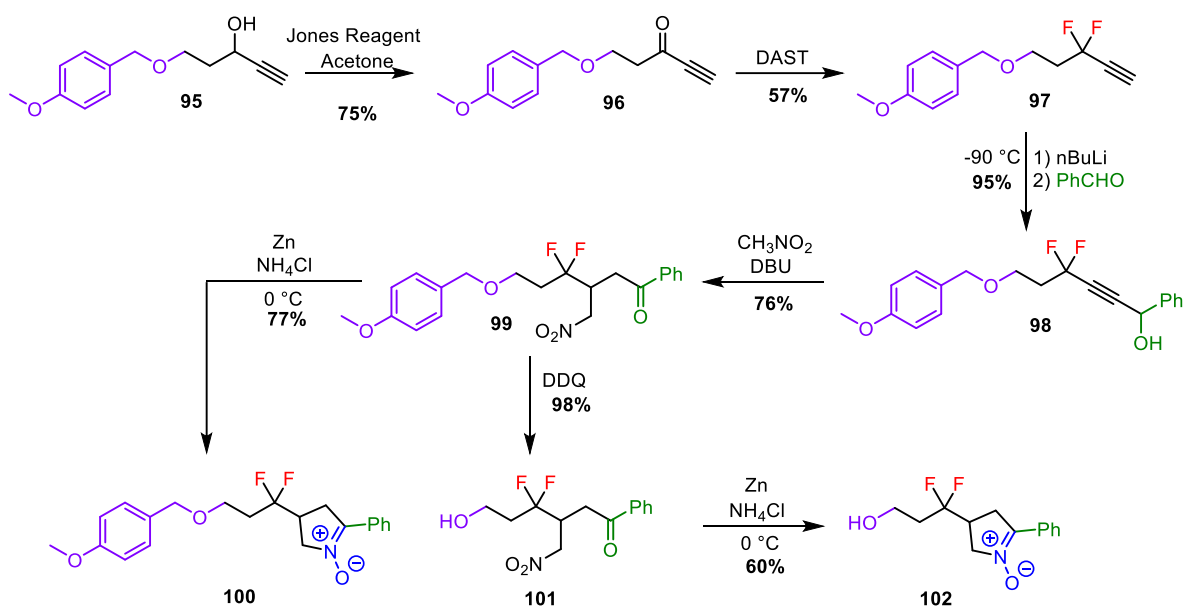
Figure C3. 5: Molecular modification in the fluorinated nitrones by having a functional group on the terminal position of the side chain

Starting from 4-methoxybenzyl alcohol **91** in the presence of 1,3-propanediol **92** and concentrated sulfuric acid in catalytic amount in DCM under reflux, alcohol **93** was obtained in 70% yield. This intermediate was readily oxidized to aldehyde **94** using Swern method with good yield (82%), and finally the addition of ethynylmagnesium bromide solution to **94** in THF at 0 °C gave the known ynol **95** in 75% yield (Scheme C3. 23).⁸¹



Scheme C3. 23: Synthesis of ynol **95**

The ynol **95** was oxidized with Jones reagent at 0°C, the ynone **96** was obtained in 75% yield and it was reacted with Diethylaminosulfurtrifluoride (DAST) to give the *gem*-difluoro propargylic intermediate **97** in 57% yield. The same sequence of reactions as before allowed us to prepare the alcohol **98** in 95% yield using benzaldehyde as a representative example, then the nitromethane adduct **99** with 76% yield and finally the nitrone **100** in 77% yield. On the other hand, the deprotection of **99** afforded the alcohol **101** in excellent yield, and the final reduction gave in 60% yield the nitrone **102** with a free primary alcohol group (Scheme C3. 24).



Scheme C3. 24: Synthesis of nitrones **100** and **102**

The formation of nitrone **100** directly from nitro intermediate **99** was particularly fast, so after 2 mins all starting material was transformed in nitrone **100** and its reduction into 1-pyrroline **103** as a by-product started to occur. The difference between the two products could be mainly detected by ^1H NMR analysis where the proton H^3 in nitrone **100** was found at 8.28 – 8.32 ppm, and it became shielded (moved upfield) in compound **103** at 7.79 – 7.84 ppm (Figure C3. 6).

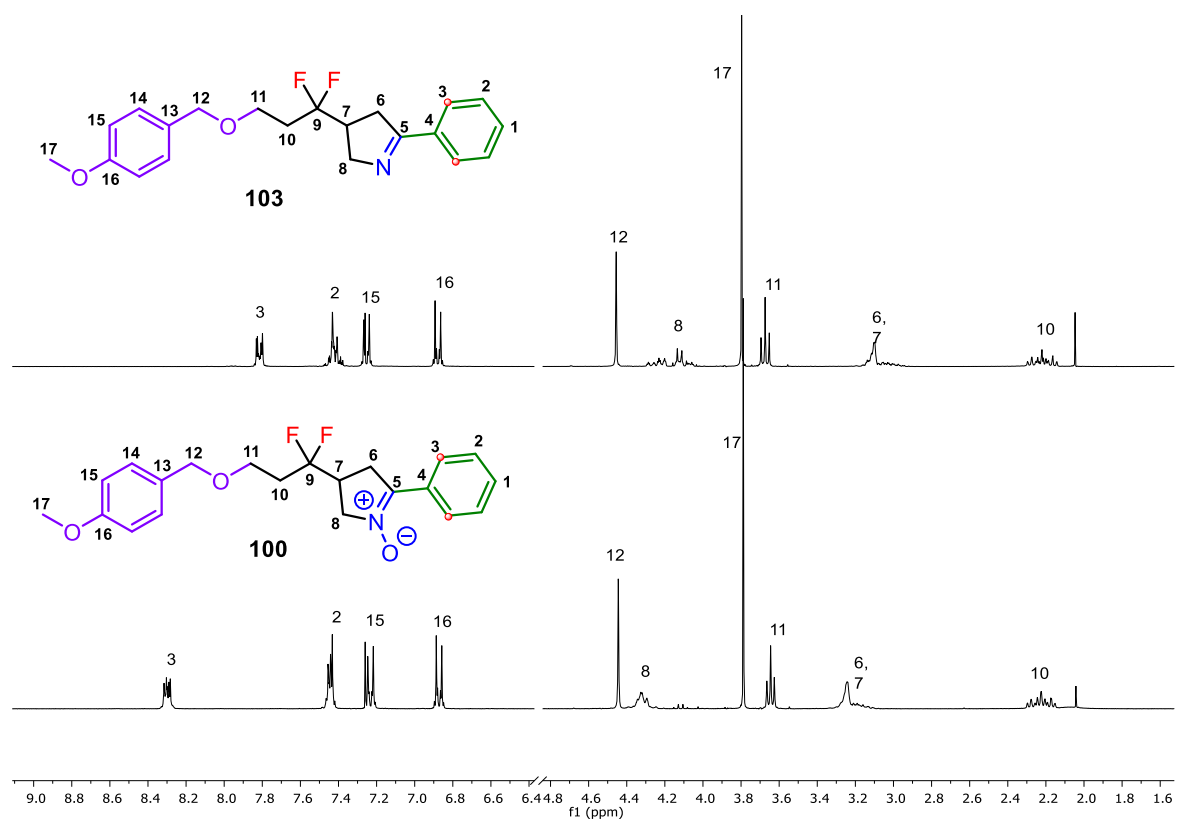
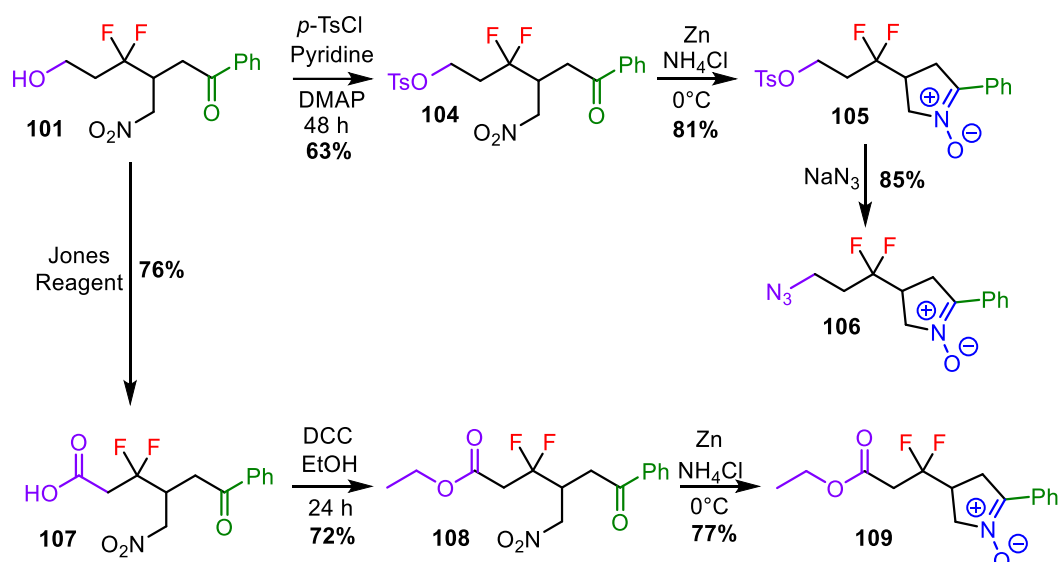


Figure C3. 6: ^1H NMR spectra of compounds **100** and **103**

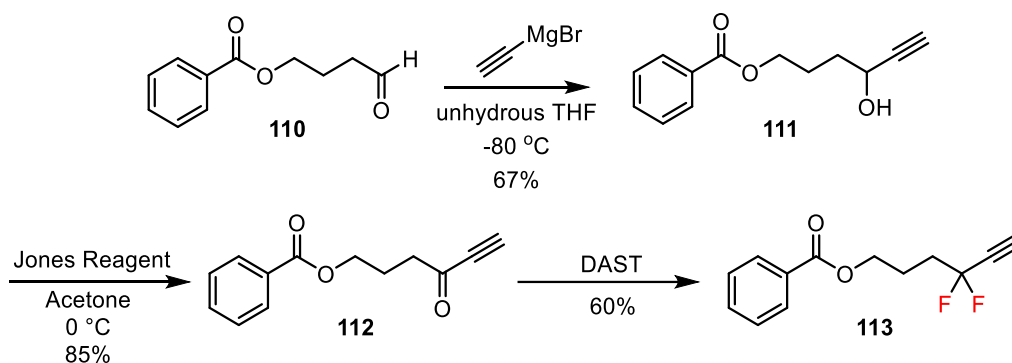
To complete this strategy and extend the range of functional groups in the terminal position of the side chain, alcohol **101** afforded compound **104** upon tosylation reaction. This intermediate was readily reduced to the nitrone **105** bearing a tosylate group on the side chain in 51% overall yield from **101**. Then displacing this tosylate group with sodium azide afforded the nitrone with a terminal azide **106** in 85% yield. On the other hand, alcohol **101** was first transformed into the acid **107** by Jones reagent. After Steglich esterification of this acid, the ester **108** was obtained and the reduction of the nitro group under the same conditions as above gave the nitrone with a terminal ester group **109**, in 42% overall yield from **101** (Scheme C3. 25).



Scheme C3. 25: Synthesis of nitrones **105**, **106**, and **109**

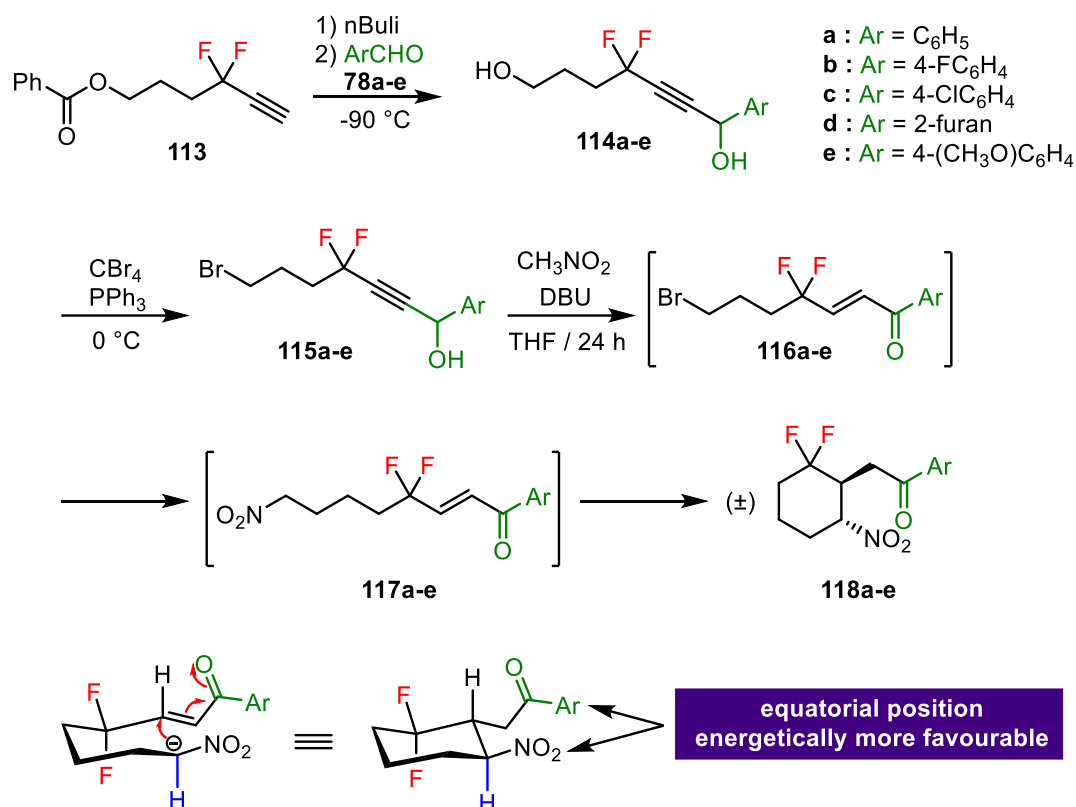
3.3. Synthesis of type C nitrones

To expand the strategy to type-C fused bicyclic nitrones, we started from the known *gem*-difluorinated propargylic compound **113**,⁷⁶ which was the result of the addition of the ethynylmagnesium bromide to the aldehyde **110** in anhydrous THF at -80 °C followed by oxidation to propargyl ketone **112** by Jones reagent. Treatment of this ketone with DAST allowed access to *gem*-difluoropropargylic intermediate **113** with fair yield (Scheme C3. 26).



Scheme C3. 26: Synthesis of *gem*-difluorinated propargylic compound **113**

After the reaction of *gem*-difluoropropargylic intermediate **113** with *n*-BuLi and different aromatic aldehydes, the propargylic alcohols **114a-e** were obtained in good yields (Scheme C3. 27, Table C3. 4). Substitution of the terminal primary alcohol by bromine gave the derivatives **115a-e** in fair to good yields. Then, reaction with nitromethane in the presence of DBU gave directly the cyclohexyl derivatives **118a-e**, as single diastereoisomers (control of the crude reaction mixture using ¹⁹F NMR).



Scheme C3. 27: Synthesis of cyclic intermediate **118a-e**

Table C3. 4: Synthesis of cyclic intermediate **118a-e**

| ENTRY | SM | Ar | YIELD (%) ^[a] | | |
|-------|------------|----|--------------------------|------------------|------------------|
| | | | 114 | 115 | 118 |
| 1 | 78a | | 114a (72) | 115a (72) | 118a (76) |
| 2 | 78b | | 114b (70) | 115b (88) | 118b (66) |
| 3 | 78c | | 114c (86) | 115c (61) | 118c (70) |
| 4 | 78d | | 114d (90) | 115d (51) | 118d (52) |
| 5 | 78e | | 114e (95) | 115e (77) | 118e (49) |

^[a] isolated yields

Based on our previous studies,⁷⁶ in this cascade process, three steps are likely to occur sequentially: isomerization of the propargylic alcohol **115a-e** to the enone **116a-e** followed

nucleophilic substitution of the bromine by the nitromethane anion **117a-e**, and finally intramolecular Michael addition of the nitro anion onto this enone to give **118a-e** as *trans* isomers. During this cyclization, the two functional groups are obtained in the equatorial positions to form likely the most stable isomer (Scheme C3. 27). These stereochemical assignments were confirmed by ^1H - ^1H NOESY NMR experiment as shown in Figure C3. 7 for the analysis of **118e**.

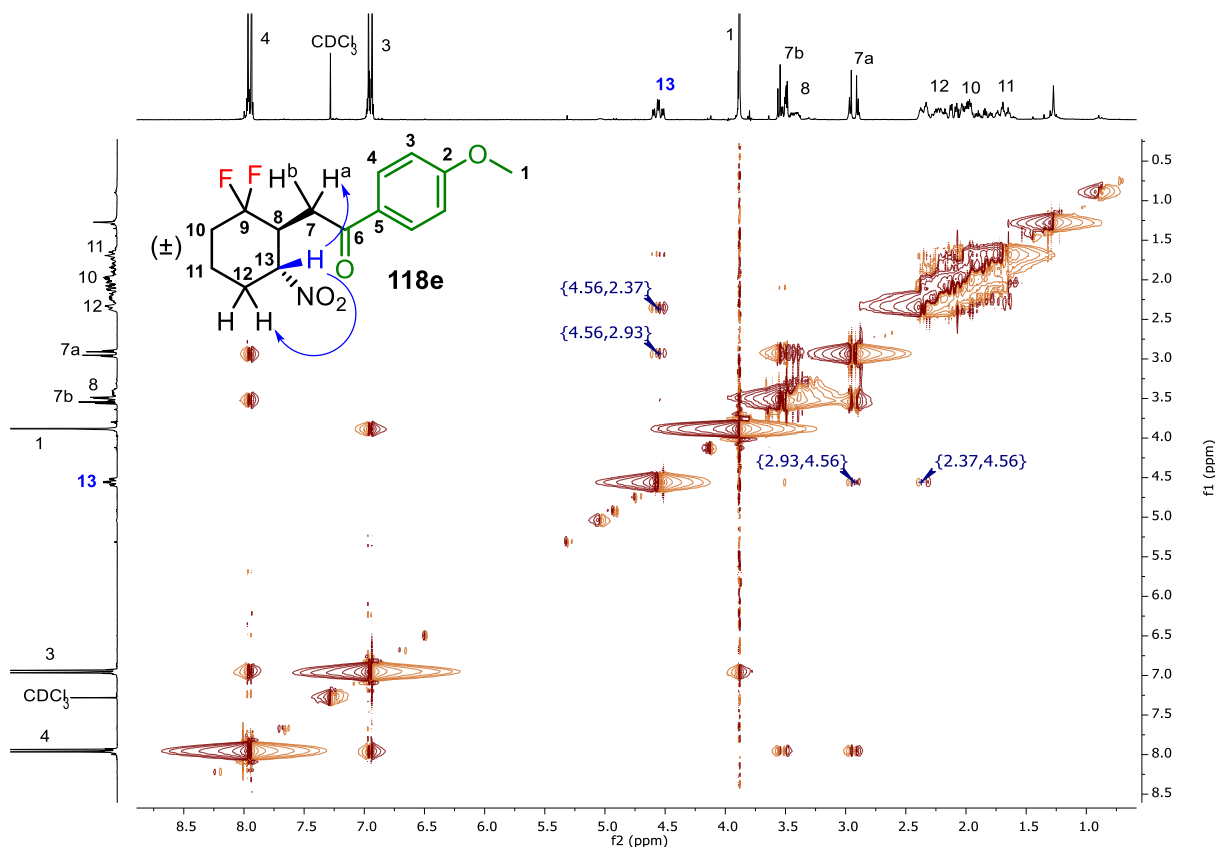
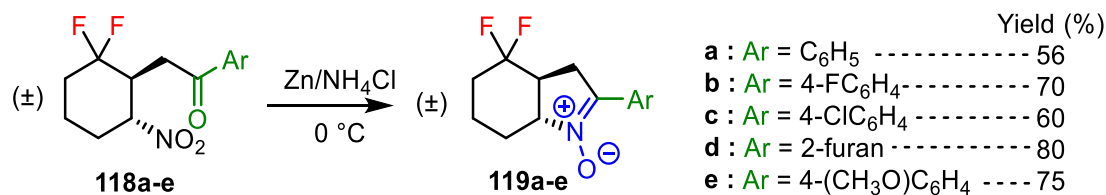


Figure C3. 7: ^1H - ^1H NOESY NMR experiment of **118e** in CDCl_3

In a last step, the desired bicyclic nitrones were obtained using the same reduction procedure as previously to give compounds **119a-e** in good yields (Scheme C3. 28). The structure of **119a** and **119b**, including the *trans* ring junction, were unambiguously established using X-Ray crystallography (Figure C3. 8).⁸²



Scheme C3. 28: Formation of nitrones **119a-e**

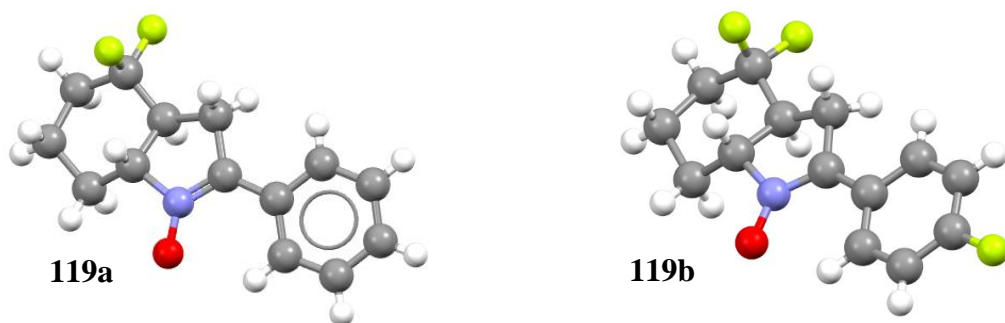


Figure C3. 8: X-Ray structure analysis of **119a** and **119b**.

4. Conclusion and perspectives

Novel mono- and bicyclic nitrones with a CF_2R group in γ position have been efficiently prepared from *gem*-difluoro propargylic alcohols by cascade reactions.⁸³ The key step were the isomerization of propargylic alcohol into enones, followed by the Michael reaction of nitroalkane anions and then a reductive cyclization from this nitro group. Based on this scheme, three groups of nitrones with *gem*-difluoroalkyl chains have been synthesized. Biological properties of these new nitrones will be studied, in order to test in particular their spin trapping activity.

5. Experimental part

5.1. General Methods

NMR spectra were performed on a Bruker AVANCE 300 or Bruker AVANCE 500 (^1H , 300 MHz or 500 MHz; ^{13}C , 75 MHz or 126 MHz; ^{19}F , 282 MHz or 471 MHz). Solvent peaks were used as reference values with CDCl_3 at 7.26 ppm for ^1H NMR and 77.16 ppm for ^{13}C NMR, with CD_3CN at 1.94 ppm for ^1H NMR and 1.32 and 118.26 ppm for ^{13}C NMR, with $(\text{CD}_3)_2\text{CO}$ at 2.05 ppm for ^1H NMR and 29.84 and 206.26 ppm for ^{13}C NMR, and with C_6D_6 at 7.16 ppm for ^1H NMR and 128.06 ppm for ^{13}C NMR. Chemical shifts δ are given in ppm, and the following abbreviations are used: singlet (s), broad singlet (bs), doublet (d), doublet of doublet (dd), doublet of doublet of doublet (ddd), triplet (t), triplet of doublet (td), quadruplet (q), doublet of quadruplet (dq), and multiplet (m). IR spectra were run on a PerkinElmer Spectrum 16 PC spectrometer. High resolution mass spectra were recorded in the Centre Régional de Mesures Physiques de l'Ouest, Rennes (CRMPO), on a Maxis 4G. Melting points were determined with uncertainty of $\pm 2^\circ\text{C}$ using a KOFER BENCH. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F254 Merck plates), and compounds were visualized under a UVP Mineralight UVGL-58 lamp (254 nm) and with *p*-anisaldehyde/ Δ . Column chromatography was performed using silica gel 60 (40–63 mm, 230–400 mesh). Solvents were used as received from commercial sources. Reagents were purchased from Sigma-Aldrich, Alfa Aesar and Acros. All products reported showed ^1H , ^{13}C , and ^{19}F NMR spectra in agreement with the assigned structures.

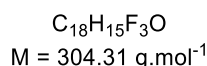
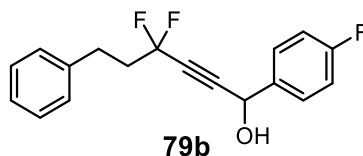
5.2. Experimental Procedures and Analytical data

11. General procedure 1 for the synthesis of gem-difluoropropargylic alcohols **79**

To a solution of *n*-butyllithium 2.5 M (1.44 mL, 3.61 mmol) in anhydrous THF (25 mL) cooled to -90°C , was added under nitrogen, a solution of **77** (500 mg, 2.77 mmol). The mixture was stirred for 30 min at -90°C . Then, aldehyde **78a-e** (1.3 equiv) in anhydrous THF (10 mL) was added at -90°C , stirred for 45 min, then the reaction mixture was allowed to warm to r.t. within 2h. The mixture was then treated with a saturated ammonium chloride solution and extracted with ethyl acetate (3x40 mL). The combined organic phases were washed with water, dried over Na_2SO_4 , and concentrated *in vacuo*. After purification by chromatography on silica gel, the propargylic alcohols **3** were obtained.

11.1. 4,4-difluoro-1-(4-fluorophenyl)-6-phenylhex-2-yn-1-ol 79b

The reaction was performed according to the general procedure 1. After purification by chromatography on silica gel, compound **79b** was obtained as pale-yellow oil, yield 684 mg (81%).



R_f = 0.5 (hexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.50 – 7.37 (m, 2H), 7.32 – 7.10 (m, 5H), 7.09 – 6.97 (m, 2H), 5.44 (t, ³J_{H-H} = 3.9 Hz, 1H), 2.90 – 2.76 (m, 2H), 2.45 – 2.24 (m, 2H), 2.32 – 2.10 (bs, 1H, OH).

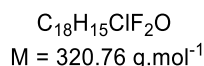
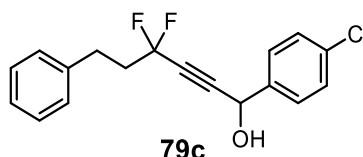
¹³C NMR (75 MHz, CDCl₃) δ 163.1 (d, ¹J_{C-F} = 247.9 Hz), 139.8, 134.9, 128.7, 128.6 (d, ³J_{C-F} = 8.4 Hz), 128.5, 126.6, 115.9 (d, ²J_{C-F} = 21.8 Hz), 114.2 (t, ¹J_{C-F} = 233.6 Hz), 86.8 (t, ³J_{C-F} = 6.7 Hz), 79.6 (t, ²J_{C-F} = 41.0 Hz), 63.6, 41.0 (t, ²J_{C-F} = 26.2 Hz), 29.1 (t, ³J_{C-F} = 4.0 Hz).

¹⁹F {H} NMR (282 MHz, CDCl₃) δ -83.75 (s), -112.77 (s).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₈H₁₅OF₃Na) = 327.0967; found: 327.0970 (1 ppm).

11.2. 1-(4-chlorophenyl)-4,4-difluoro-6-phenylhex-2-yn-1-ol 79c

The reaction was performed according to the general procedure 1. After purification by chromatography on silica gel, compound **79c** was obtained as yellow oil, yield 534 mg (60%).



R_f = 0.2 (hexane / Ethyl Acetate: 9/1).

¹H NMR (300 MHz, CDCl₃) δ 7.48 – 7.35 (m, 4H), 7.35 – 7.27 (m, 2H), 7.26 – 7.16 (m, 3H), 5.49 (bs, 1H), 2.94 – 2.83 (m, 2H), 2.49 – 2.31 (m, 2H), 2.27 (bs, 1H, OH).

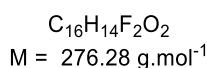
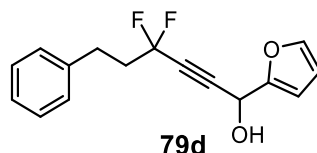
¹³C NMR (75 MHz, CDCl₃) δ 139.8, 137.5, 135.0, 129.2, 128.8, 128.5, 128.1, 126.6, 114.2 (t, ¹J_{C-F} = 233.7 Hz), 86.6 (t, ³J_{C-F} = 6.7 Hz), 79.7 (t, ²J_{C-F} = 41.0 Hz), 63.6, 40.9 (t, ²J_{C-F} = 26.1 Hz), 29.1 (t, ³J_{C-F} = 4.0 Hz).

¹⁹F {H} NMR {H} (282 MHz, CDCl₃) δ -83.81 (s).

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{18}H_{15}OF_2^{35}ClNa$) = 343.0671; 343.0672 (0 ppm).

11.3. 4,4-difluoro-1-(furan-2-yl)-6-phenylhex-2-yn-1-ol 79d

The reaction was performed according to the general procedure 1. After purification by chromatography on silica gel, compound 79d was obtained as black oil, yield 490 mg (64%).



R_f = 0.3 (hexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.41 (dd, $^3J_{H-H} = 1.9$, $^3J_{H-H} = 0.8$ Hz, 1H), 7.33 – 7.24 (m, 2H), 7.23 – 7.14 (m, 3H), 6.44 (dd, $^3J_{H-H} = 3.3$ Hz, $^3J_{H-H} = 0.8$ Hz, 1H), 6.35 (dd, $^3J_{H-H} = 3.3$ Hz, $^3J_{H-H} = 1.9$ Hz, 1H), 5.49 (t, $^5J_{H-F} = 3.9$ Hz, 1H), 2.92 – 2.81 (m, 2H), 2.68 (bs, 1H, OH), 2.46 – 2.27 (m, 2H).

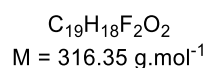
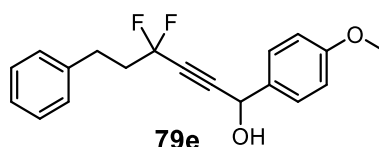
¹³C NMR (75 MHz, CDCl₃) δ 151.4, 143.6, 139.9, 128.7, 128.5, 126.5, 114.2 (t, $^1J_{C-F} = 233.7$ Hz), 110.7, 108.6, 84.7 (t, $^3J_{C-F} = 6.8$ Hz), 78.6 (t, $^2J_{C-F} = 41.1$ Hz), 57.9 (t, $^4J_{C-F} = 1.8$ Hz), 41.0 (t, $^2J_{C-F} = 26.0$ Hz), 29.1 (t, $^3J_{C-F} = 4.0$ Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -84.02 (s).

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{16}H_{14}O_2F_2Na$) = 299.0854; found: 299.0857 (1 ppm).

11.4. 4,4-difluoro-1-(4-methoxyphenyl)-6-phenylhex-2-yn-1-ol 79e

The reaction was performed according to the general procedure 1. After purification by chromatography on silica gel, compound 79d was obtained as pale yellow oil, yield 588 mg (67%).



R_f = 0.2 (Petroleum Ether / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.47 – 7.40 (m, 2H), 7.35 – 7.27 (m, 2H), 7.26 – 7.17 (m, 3H), 6.96 – 6.89 (m, 2H), 5.47 (t, $^5J_{H-F} = 3.9$ Hz, 1H), 3.82 (s, 3H), 2.92 – 2.85 (m, 2H), 2.48 – 2.31 (m, 2H), 2.28 (bs, 1H).

¹³C NMR (75 MHz, CDCl₃) δ 160.1, 139.9, 131.4 (t, ⁵J_{C-F} = 1.6 Hz), 128.7, 128.5, 128.2, 126.5, 114.3, 114.2 (t, ¹J_{C-F} = 233.4 Hz), 87.3 (t, ³J_{C-F} = 6.8 Hz), 79.2 (t, ²J_{C-F} = 40.9 Hz), 63.9 (t, ⁴J_{C-F} = 2.0 Hz), 55.5, 41.0 (t, ²J_{C-F} = 26.2 Hz), 29.1 (t, ³J_{C-F} = 4.0 Hz).

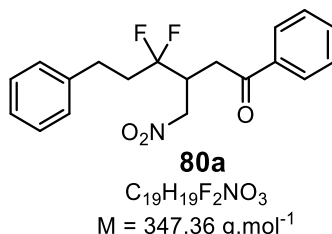
¹⁹F {H} NMR (376 MHz, CDCl₃) δ -83.61 (s).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₉H₁₈O₂F₂Na) = 339.1167; found: 339.1169 (1 ppm).

12. General procedure 2 for the synthesis of gem-difluoronitroketones 4 and 5

12.1. Representative procedure: Synthesis of 4,4-difluoro-3-(nitromethyl)-1,6-diphenylhexan-1-one 80a

To the difluoropropargylic alcohol **79a** (150 mg, 0.52 mmol) was added nitromethane (56 μL, 1.04 mmol) and DBU (310 μL, 2.10 mmol) in THF (3 mL). The reaction mixture was stirred at room temperature overnight. At the end saturated NH₄Cl (10 mL) was added and the reaction mixture was extracted with ethyl acetate (3x20 mL). The organic layers were separated, washed with water (1x10 mL), dried over Na₂SO₄ and concentrated under vacuum. After purification by chromatography on silica gel, **80a** (131 mg, 72%) was obtained as white solid.



R_f = 0.2 (hexane / Ethyl Acetate: 9/1). mp = 102 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.01 – 7.92 (m, 2H), 7.67 – 7.57 (m, 1H), 7.54 – 7.44 (m, 2H), 7.34 – 7.15 (m, 5H), 4.74 (dd, ²J_{H-H} = 13.6 Hz, ³J_{H-H} = 6.1 Hz, 1H), 4.52 (dd, ²J_{H-H} = 13.6 Hz, ²J_{H-H} = 5.5 Hz, 1H), 3.83 – 3.62 (m, 1H), 3.41 (dd, ²J_{H-H} = 18.5 Hz, ²J_{H-H} = 4.6 Hz, 1H), 3.24 (dd, ²J_{H-H} = 18.5 Hz, ²J_{H-H} = 8.1 Hz, 1H), 2.94 – 2.82 (m, 2H), 2.33 – 2.10 (m, 2H).

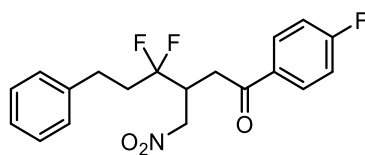
¹³C NMR (75 MHz, CDCl₃) δ 196.1, 140.0, 136.1, 134.0, 129.0, 128.8, 128.4, 128.3, 126.6, 124.1 (t, ¹J_{C-F} = 245.7 Hz), 73.9 (t, ³J_{C-F} = 4.8 Hz), 39.3 (t, ²J_{C-F} = 24.5 Hz), 37.0 (t, ²J_{C-F} = 24.6 Hz), 35.4 (t, ³J_{C-F} = 3.6 Hz), 28.0 (t, ³J_{C-F} = 5.1 Hz).

¹⁹F {H} NMR (282 MHz, CDCl₃) δ -102.95 (AB system, ²J_{F-F} = 248.9 Hz), -104.05 (AB system, ²J_{F-F} = 248.9 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₉H₁₉NO₃F₂Na) = 370.1225; found: 370.1230 (1 ppm).

12.2. 4,4-difluoro-1-(4-fluorophenyl)-3-(nitromethyl)-6-phenylhexan-1-one 80b

The reaction was performed according to the general procedure 2. Yellow solid yield 133 mg (74%) from 150 mg (0.49 mmol) of **79b**.



80b

$C_{19}H_{18}F_3NO_3$
 $M = 365.35 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.4$ (hexane / Ethyl Acetate: 9/1). mp = 129 °C.

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.15 – 7.98 (m, 2H), 7.48 – 7.08 (m, 7H), 4.80 (dd, $^2J_{\text{H-H}} = 13.6$ Hz, $^3J_{\text{H-H}} = 5.9$ Hz, 1H), 4.58 (dd, $^2J_{\text{H-H}} = 13.6$ Hz, $^3J_{\text{H-H}} = 5.6$ Hz, 1H), 3.87 – 3.63 (m, 1H), 3.45 (dd, $^2J_{\text{H-H}} = 18.5$ Hz, $^3J_{\text{H-H}} = 4.6$ Hz, 1H), 3.29 (dd, $^2J_{\text{H-H}} = 18.4$ Hz, $^3J_{\text{H-H}} = 8.0$ Hz, 1H), 3.09 – 2.81 (m, 2H), 2.39 – 2.16 (m, 2H).

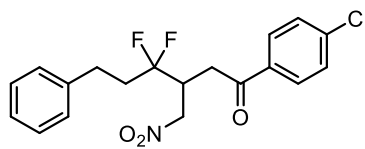
$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 194.5, 166.3 (d, $^1J_{\text{C-F}} = 256.3$ Hz), 139.9, 132.5 (d, $^4J_{\text{C-F}} = 3.0$ Hz), 131.0 (d, $^3J_{\text{C-F}} = 9.5$ Hz), 128.8, 128.4, 124.0 (t, $^1J_{\text{C-F}} = 245.8$ Hz), 116.2 (d, $^2J_{\text{C-F}} = 22.1$ Hz), 73.8 (t, $^3J_{\text{C-F}} = 4.9$ Hz), 39.3 (t, $^2J_{\text{C-F}} = 24.5$ Hz), 36.9 (t, $^2J_{\text{C-F}} = 24.6$ Hz), 35.2 (t, $^3J_{\text{C-F}} = 3.7$ Hz), 28.0 (t, $^3J_{\text{C-F}} = 5.1$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -103.00 (AB system, $^2J_{\text{F-F}} = 248.8$ Hz), -103.52 (s), -139.95 (AB system, $^2J_{\text{F-F}} = 248.8$ Hz).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($C_{19}H_{18}NO_3F_3\text{Na}$) = 388.1131; found: 388.1131 (0 ppm).

12.3. 1-(4-chlorophenyl)-4,4-difluoro-3-(nitromethyl)-6-phenylhexan-1-one 80c

The reaction was performed according to the general procedure 2. White solid, yield 123 mg (69%) from 150 mg (0.47 mmol) of **79c**.



80c

$C_{19}H_{18}ClF_2NO_3$
 $M = 381.80 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.3$ (hexane / Ethyl Acetate: 9/1). mp = 137 °C.

¹H NMR (300 MHz, CDCl₃) δ 7.94 – 7.86 (m, 2H), 7.52 – 7.42 (m, 2H), 7.34 – 7.12 (m, 5H), 4.73 (dd, ²J_{H-H} = 13.6 Hz, ³J_{H-H} = 5.9 Hz, 1H), 4.51 (dd, ²J_{H-H} = 13.6, ³J_{H-H} = 5.6 Hz, 1H), 3.79 – 3.54 (m, 1H), 3.37 (dd, ²J_{H-H} = 18.5 Hz, ³J_{H-H} = 4.6 Hz, 1H), 3.22 (dd, ²J_{H-H} = 18.5 Hz, ³J_{H-H} = 8.0 Hz, 1H), 2.93 – 2.77 (m, 2H), 2.33 – 2.09 (m, 2H).

¹³C NMR (75 MHz, CDCl₃) δ 195.0, 140.6, 139.9, 134.4, 129.7, 129.3, 128.8, 126.6, 124.0 (t, ¹J_{C-F} = 245.7 Hz), 73.8 (t, ³J_{C-F} = 4.9 Hz), 39.3 (t, ²J_{C-F} = 24.5 Hz), 36.9 (t, ²J_{C-F} = 24.7 Hz), 35.3 (t, ³J_{C-F} = 3.6 Hz), 28.0 (dd, ³J_{C-F} = 5.8 Hz, ³J_{C-F} = 5.0 Hz).

¹⁹F {H} NMR (471 MHz, CDCl₃) δ -103.0 (AB system, ²J_{F-F} = 249.2 Hz), -103.6 (AB system, ²J_{F-F} = 249.2 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₉H₁₈NO₃F₂³⁵ClNa) = 404.0835; found: 404.0832 (1 ppm).

12.4. 4,4-difluoro-1-(furan-2-yl)-3-(nitromethyl)-6-phenylhexan-1-one 80d

The reaction was performed according to the general procedure 2. Black solid, yield 130 mg (71%) from 150 mg (0.54 mmol) of **79d**.



80d

C₁₇H₁₇F₂NO₄
M = 337.32 g.mol⁻¹

R_f = 0.4 (hexane / Ethyl Acetate: 8/2). mp = 79 °C.

¹H NMR (300 MHz, CDCl₃) δ 7.68 (dd, ³J_{H-H} = 1.7 Hz, ⁴J_{H-H} = 0.8 Hz, 1H), 7.46 – 7.15 (m, 6H), 6.65 (dd, ³J_{H-H} = 3.6 Hz, ³J_{H-H} = 1.7 Hz, 1H), 4.80 (dd, ²J_{H-H} = 13.7 Hz, ³J_{H-H} = 6.1 Hz, 1H), 4.59 (dd, ²J_{H-H} = 13.7 Hz, ³J_{H-H} = 5.7 Hz, 1H), 3.90 – 3.59 (m, 1H), 3.35 (dd, ²J_{H-H} = 18.1 Hz, ³J_{H-H} = 4.9 Hz, 1H), 3.16 (dd, ²J_{H-H} = 18.1 Hz, ³J_{H-H} = 7.9 Hz, 1H), 3.00 – 2.75 (m, 2H), 2.46 – 2.14 (m, 2H).

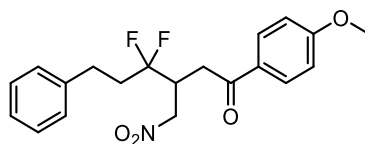
¹³C NMR (75 MHz, CDCl₃) δ 185.1, 152.1, 147.1, 140.0, 128.8, 128.4, 126.5, 124.0 (t, ¹J_{C-F} = 245.8 Hz), 118.0, 112.8, 73.7 (t, ³J_{C-F} = 4.9 Hz), 39.0 (t, ²J_{C-F} = 24.6 Hz), 36.8 (t, ²J_{C-F} = 24.6 Hz), 35.1 (t, ³J_{C-F} = 3.8 Hz), 27.9 (t, ³J_{C-F} = 5.1 Hz).

¹⁹F {H} NMR (282 MHz, CDCl₃) δ -102.94 (AB system, ²J_{F-F} = 248.6 Hz), -104.39 (AB system, ²J_{F-F} = 248.6 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₇H₁₇NO₄F₂Na) = 360.1018; found: 360.1019 (0 ppm).

12.5. 4,4-difluoro-1-(4-methoxyphenyl)-3-(nitromethyl)-6-phenylhexan-1-one 80e

The reaction was performed according to the general procedure 2. White solid, yield 130 mg (76%) from 150 mg (0.47 mmol) of **79e**.



80e

$C_{20}H_{21}F_2NO_4$
 $M = 377.39 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.5 (Petroleum Ether / Ethyl Acetate: 8/2). **mp** = 107 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.03 – 7.87 (m, 2H), 7.42 – 7.10 (m, 5H), 7.01 – 6.91 (m, 2H), 4.73 (dd, ²J_{H-H} = 13.6 Hz, ³J_{H-H} = 6.2 Hz, 1H), 4.52 (dd, ²J_{H-H} = 13.6 Hz, ³J_{H-H} = 5.3 Hz, 1H), 3.88 (s, 3H), 3.80 – 3.62 (m, 1H), 3.35 (dd, ²J_{H-H} = 18.3 Hz, ³J_{H-H} = 4.5 Hz, 1H), 3.18 (dd, ²J_{H-H} = 18.3 Hz, ³J_{H-H} = 8.2 Hz, 1H), 2.94 – 2.81 (m, 2H), 2.32 – 2.09 (m, 2H).

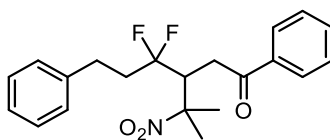
¹³C NMR (75 MHz, CDCl₃) δ 194.5, 164.2, 140.0, 130.6, 129.1, 128.8, 128.4, 126.5, 124.2 (t, ¹J_{C-F} = 245.7 Hz), 114.1, 73.9 (t, ³J_{C-F} = 4.8 Hz), 55.7, 39.4 (t, ²J_{C-F} = 24.3 Hz), 36.9 (t, ²J_{C-F} = 24.6 Hz), 34.9 (dd, ³J_{C-F} = 4.1, ³J_{C-F} = 3.1 Hz), 28.0 (t, ³J_{C-F} = 5.0 Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -102.83 (AB system, ²J_{F-F} = 248.6 Hz), -104.10 (AB system, ²J_{F-F} = 248.6 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₀H₂₁NO₄F₂Na) = 400.1331; found: 400.1333 (0 ppm).

12.6. 4,4-difluoro-3-(2-nitropropan-2-yl)-1,6-diphenylhexan-1-one 81a

The reaction was performed according to the general procedure 2. Yellow solid, yield 136 mg (69%) from 150 mg (0.47 mmol) of **79a**.



81a

$C_{21}H_{23}F_2NO_3$
 $M = 375.42 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.3 (hexane / Ethyl Acetate: 9/1). **mp** = 71 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.10 – 7.94 (m, 2H), 7.68 – 7.59 (m, 1H), 7.58 – 7.42 (m, 2H), 7.32 – 7.07 (m, 5H), 4.40 (ddt, ³J_{H-F} = 25.6 Hz, ³J_{H-F} = 4.7 Hz, ³J_{H-H} = 4.7 Hz, 1H), 3.24 (dd,

$^1J_{H-H} = 19.3$ Hz, $^3J_{H-H} = 4.4$ Hz, 1H), 3.09 (dd, $^1J_{H-H} = 19.2$ Hz, $^3J_{H-H} = 5.2$ Hz, 1H), 2.99 – 2.70 (m, 2H), 2.21 – 1.96 (m, 2H), 1.78 (s, 3H), 1.50 (s, 3H).

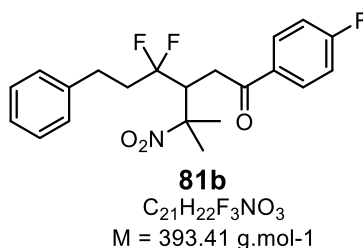
^{13}C NMR (75 MHz, CDCl_3) δ 195.6, 140.2, 135.9, 134.0, 129.0, 128.6, 128.4, 128.3, 126.3, 125.3 (t, $^1J_{C-F} = 247.8$ Hz), 89.2 (d, $^3J_{C-F} = 1.8$ Hz), 45.0 (t, $^2J_{C-F} = 21.6$ Hz), 38.6 (t, $^2J_{C-F} = 24.9$ Hz), 35.8 (t, $^3J_{C-F} = 4.5$ Hz), 28.0 (dd, $^3J_{C-F} = 6.3$ Hz, $^3J_{C-F} = 4.4$ Hz), 27.2 (d, $^4J_{C-F} = 1.5$ Hz), 22.5 (t, $^4J_{C-F} = 3.0$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -100.15 (AB system, $^2J_{F-F} = 247.3$ Hz), -102.16 (AB system, $^2J_{F-F} = 247.3$ Hz).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{21}\text{H}_{23}\text{NO}_3\text{F}_2\text{Na}$) = 398.1538; found: 398.1534 (1 ppm).

12.7. 4,4-difluoro-1-(4-fluorophenyl)-3-(2-nitropropan-2-yl)-6-phenylhexan-1-one 81b

The reaction was performed according to the general procedure 2. pale yellow solid, yield 134 mg (69%) from 150 mg (0.49 mmol) of **79b**.



R_f = 0.4 (hexane / Ethyl Acetate: 9/1). **mp** = 89 °C.

^1H NMR (300 MHz, CDCl_3) δ 8.10 – 7.93 (m, 2H), 7.34 – 7.07 (m, 7H), 4.35 (ddt, $^3J_{H-F} = 25.3$ Hz, $^3J_{H-F} = 4.8$ Hz, $^3J_{H-H} = 4.8$, 1H), 3.20 (dd, $^1J_{H-H} = 19.2$ Hz, $^3J_{H-H} = 4.4$ Hz, 1H), 3.03 (dd, $^1J_{H-H} = 19.2$ Hz, $^1J_{H-H} = 5.1$ Hz, 1H), 2.95 – 2.68 (m, 2H), 2.17 – 1.93 (m, 2H), 1.76 (s, 3H), 1.50 (s, 3H).

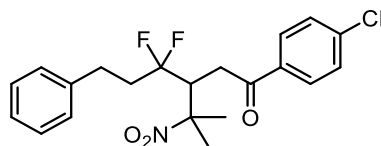
^{13}C NMR (75 MHz, CDCl_3) δ 194.0, 166.3 (d, $^1J_{C-F} = 256.3$ Hz), 140.2, 132.4 (d, $^4J_{C-F} = 3.0$ Hz), 131.0 (d, $^3J_{C-F} = 9.5$ Hz), 128.7, 128.4, 126.4, 125.2 (t, $^1J_{C-F} = 247.8$ Hz), 116.2 (d, $^2J_{C-F} = 22.0$ Hz), 89.2 (d, $^3J_{C-F} = 1.5$ Hz), 45.1 (t, $^2J_{C-F} = 21.7$ Hz), 38.6 (t, $^2J_{C-F} = 24.9$ Hz), 35.7 (t, $^3J_{C-F} = 4.5$ Hz), 28.0 (dd, $^3J_{C-F} = 6.1$ Hz, $^3J_{C-F} = 4.6$ Hz), 27.0, 23.2, 22.7.

^{19}F {H} NMR (282 MHz, CDCl_3) δ -100.05 (AB system, $^2J_{F-F} = 247.5$ Hz), -102.15 (AB system, $^2J_{F-F} = 247.5$ Hz), -103.55 (s).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{21}\text{H}_{22}\text{NO}_3\text{F}_3\text{Na}$) = 416.1444; found: 416.1443 (0 ppm).

12.8. 1-(4-chlorophenyl)-4,4-difluoro-3-(2-nitropropan-2-yl)-6-phenylhexan-1-one 81c

The reaction was performed according to the general procedure 2. Yellow oil, yield 140 mg (73%) from 150 mg (0.47 mmol) of **79c**.



81c

$C_{21}H_{22}ClF_2NO_3$
 $M = 409.86 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.6$ (hexane / Ethyl Acetate: 8/2).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.05 – 7.95 (m, 2H), 7.58 – 7.51 (m, 5H), 7.38 – 7.15 (m, 2H), 4.41 (ddt, $^3J_{\text{H-F}} = 25.3 \text{ Hz}$, $^3J_{\text{H-F}} = 4.8 \text{ Hz}$, $^3J_{\text{H-H}} = 4.8 \text{ Hz}$, 1H), 3.27 (dd, $^2J_{\text{H-H}} = 19.3 \text{ Hz}$, $^3J_{\text{H-H}} = 4.4 \text{ Hz}$, 1H), 3.09 (dd, $^2J_{\text{H-H}} = 19.3 \text{ Hz}$, $^3J_{\text{H-H}} = 5.1 \text{ Hz}$, 1H), 3.02 – 2.74 (m, 2H), 2.24 – 2.01 (m, 2H), 1.83 (s, 3H), 1.56 (s, 3H).

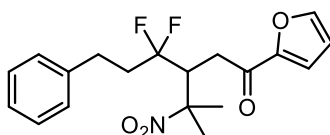
$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 194.5, 140.6, 140.2, 134.2, 129.7, 129.4, 128.7, 128.4, 126.4, 125.2 (t, $^1J_{\text{C-F}} = 248.2 \text{ Hz}$), 89.2 (d, $^3J_{\text{C-F}} = 1.6 \text{ Hz}$), 45.1 (t, $^2J_{\text{C-F}} = 21.6 \text{ Hz}$), 38.6 (t, $^2J_{\text{C-F}} = 24.9 \text{ Hz}$), 35.8 (t, $^3J_{\text{C-F}} = 4.5 \text{ Hz}$), 28.0 (dd, $^3J_{\text{C-F}} = 6.2 \text{ Hz}$, $^3J_{\text{C-F}} = 4.5 \text{ Hz}$), 23.9, 22.8 (t, $^4J_{\text{C-F}} = 3.0 \text{ Hz}$).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -100.0 (AB system, $^2J_{\text{F-F}} = 247.7 \text{ Hz}$), -102.2 (AB system, $^2J_{\text{F-F}} = 247.7 \text{ Hz}$).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{21}\text{H}_{22}\text{NO}_3\text{F}_2^{35}\text{ClNa}$) = 432.1149; found: 432.1150 (0 ppm).

12.9. 4,4-difluoro-1-(furan-2-yl)-3-(2-nitropropan-2-yl)-6-phenylhexan-1-one 81d

The reaction was performed according to the general procedure 2. Yellow oil, yield 117 mg (72%) from 150 mg (0.44 mmol) of **79d**.



81d

$C_{19}H_{21}F_2NO_4$
 $M = 365.38 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.4$ (hexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.69 (dd, ³J_{H-H} = 1.7 Hz, ⁴J_{H-H} = 0.7 Hz, 1H), 7.39 – 7.18 (m, 6H), 6.66 (dd, ³J_{H-H} = 3.6 Hz, ³J_{H-H} = 1.7 Hz, 1H), 4.33 (ddt, ³J_{H-F} = 25.2 Hz, ³J_{H-F} = 4.8 Hz, ³J_{H-H} = 4.8 Hz, 1H), 3.16 (dd, ²J_{H-H} = 19.2 Hz, ³J_{H-H} = 4.5 Hz, 1H), 3.05 (dd, ²J_{H-H} = 19.2 Hz, ³J_{H-H} = 5.2 Hz, 1H), 3.00 – 2.75 (m, 2H), 2.25 – 2.06 (m, 2H), 1.83 (s, 3H), 1.58 (s, 3H).

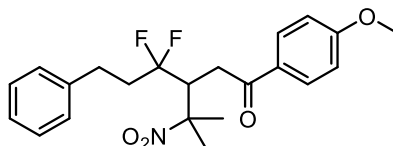
¹³C NMR (75 MHz, CDCl₃) δ 185.0, 152.0, 147.0, 140.3, 128.7, 128.5, 126.4, 125.2 (t, ¹J_{C-F} = 245.3 Hz), 118.0, 112.9, 89.2 (d, ³J_{C-F} = 1.5 Hz), 44.7 (t, ²J_{C-F} = 21.8 Hz), 38.6 (t, ²J_{C-F} = 24.9 Hz), 35.6 (t, ³J_{C-F} = 4.6 Hz), 28.0 (dd, ³J_{C-F} = 6.1 Hz, ³J_{C-F} = 4.6 Hz), 27.3 (d, ⁴J_{C-F} = 1.2 Hz), 22.4 (dd, ⁴J_{C-F} = 2.7, ³J_{C-F} = 0.6 Hz).

¹⁹F {H} NMR (282 MHz, CDCl₃) δ -100.45 (AB system, ²J_{F-F} = 247.3 Hz), -102.24 (AB system, ²J_{F-F} = 247.3 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₉H₂₁NO₄F₂Na) = 388.1331; found 388.1333 (0 ppm).

12.10. 4,4-difluoro-1-(4-methoxyphenyl)-3-(2-nitropropan-2-yl)-6-phenylhexan-1-one 81e

The reaction was performed according to the general procedure 2. Pale yellow solid, yield 115 mg (60%) from 150 mg (0.47 mmol) of **79e**.



81e

C₂₂H₂₅F₂NO₄
M = 405.44 g.mol⁻¹

R_f = 0.5 (Petroleum Ether / Ethyl Acetate: 8/2). **mp** = 111 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.03 – 7.92 (m, 2H), 7.31 – 7.17 (m, 2H), 7.23 – 7.09 (m, 3H), 7.02 – 6.92 (m, 2H), 4.38 (ddt, ³J_{H-F} = 25.7 Hz, ³J_{H-F} = 4.7 Hz, ³J_{H-H} = 4.7 Hz, 1H), 3.89 (s, 3H), 3.16 (dd, ²J_{H-H} = 19.0 Hz, ³J_{H-H} = 4.4 Hz, 1H), 3.01 (dd, ²J_{H-H} = 19.1 Hz, ³J_{H-H} = 5.2 Hz, 1H), 2.98 – 2.67 (m, 2H), 2.20 – 1.94 (m, 2H), 1.76 (s, 3H), 1.49 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 193.9, 164.2, 140.3, 130.6, 129.0, 128.7, 128.5, 126.3, 125.4 (t, ¹J_{C-F} = 247.6 Hz), 114.2, 89.2 (d, ³J_{C-F} = 1.8 Hz), 55.7, 45.0 (t, ²J_{C-F} = 21.5 Hz), 38.6 (t, ²J_{C-F} = 24.9 Hz), 35.4 (dd, ³J_{C-F} = 5.0 Hz, ³J_{C-F} = 3.8 Hz), 28.0 (dd, ³J_{C-F} = 6.3 Hz, ³J_{C-F} = 4.4 Hz), 27.3 (d, ⁴J_{C-F} = 1.4 Hz), 22.5 (t, ⁴J_{C-F} = 2.9 Hz).

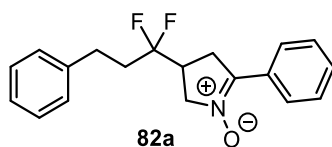
¹⁹F {H} NMR (471 MHz, CDCl₃) δ -100.30 (AB system, ²J_{F-F} = 247.1 Hz), -101.94 (AB system, ²J_{F-F} = 247.1 Hz).

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{22}H_{25}NO_4F_2Na$) = 428.1644; found: 428.1650 (1 ppm).

13. General procedure 3 for the synthesis of difluoronitrones 6 and 7

13.1. Representative procedure: 3-(1,1-difluoro-3-phenylpropyl)-5-phenyl-3,4-dihydro-2H-pyrrole 1-oxide 82a

To the difluoronitro-ketone **80a** (75 mg, 0.22 mmol) was added NH_4Cl (35 mg, 0.65 mmol) in THF (2 ml) and water (1 ml) at 0 °C. The reaction mixture was stirred for 5 mn then Zn powder (212 mg, 3.24 mmol) was added over 15 mn and very slowly. The reaction was monitored using TLC each 5 mn. Prolonged reaction times will lead to the total reduction of the nitro groups into amines. At the end the reaction mixture was filtrated on celite, concentrated under vacuum, and purified by chromatography on silica gel, **82a** (44 mg, 65%) was obtained as yellow solid.



82a
 $C_{19}H_{19}F_2NO$
 $M = 315.36 \text{ g}\cdot\text{mol}^{-1}$

R_f = 0.3 (hexane / Ethyl Acetate: 6/4). **mp** = 114 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.37 – 8.18 (m, 2H), 7.49 – 7.39 (m, 3H), 7.37 – 7.27 (m, 2H), 7.27 – 7.13 (m, 3H), 4.36 (dd, ²*J*_{H-H} = 14.3 Hz, ³*J*_{H-H} = 7.8 Hz, 1H), 4.25 (dd, ²*J*_{H-H} = 14.3 Hz, ³*J*_{H-H} = 9.7 Hz, 1H), 3.25 (d, ³*J*_{H-H} = 8.1 Hz, 2H), 3.12 – 2.93 (m, 1H), 2.93 – 2.79 (m, 2H), 2.32 – 2.09 (m, 2H).

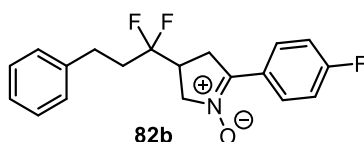
¹³C NMR (75 MHz, CDCl₃) δ 139.9, 139.4, 130.7, 128.8, 128.7, 128.6, 128.3, 127.3, 126.6, 123.3 (t, ¹*J*_{C-F} = 244.0 Hz), 64.2 (dd, ³*J*_{C-F} = 5.5 Hz, ³*J*_{C-F} = 3.8 Hz), 37.0 (t, ²*J*_{C-F} = 24.9 Hz), 36.5 (t, ²*J*_{C-F} = 26.4 Hz), 31.4 (dd, ³*J*_{C-F} = 5.6 Hz, ³*J*_{C-F} = 4.3 Hz), 27.9 (t, ³*J*_{C-F} = 4.7 Hz).

¹⁹F {¹H} NMR (471 MHz, CDCl₃) δ -106.75 (d, *J* = 246.0 Hz), -107.87 (d, *J* = 246.0 Hz).

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{19}H_{19}NOF_2Na$) = 338.1327; found: 338.1330 (1 ppm).

13.2. 3-(1,1-difluoro-3-phenylpropyl)-5-(4-fluorophenyl)-3,4-dihydro-2H-pyrrole 1-oxide 82b

The reaction was performed according to the general procedure 3. Pale yellow solid, yield 42 mg (61%) from 75 mg (0.20 mmol) of **80b**.



82b
 $C_{19}H_{18}F_3NO$
 $M = 333.35 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.1$ (hexane / Ethyl Acetate: 6/4). $mp = 155 \text{ }^\circ\text{C}$.

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.42 – 8.27 (m, 2H), 7.38 – 7.28 (m, 2H), 7.28 – 7.07 (m, 5H), 4.44 – 4.18 (m, 2H), 3.43 – 3.16 (m, 2H), 3.16 – 2.94 (m, 1H), 2.94 – 2.80 (m, 2H), 2.32 – 2.10 (m, 2H).

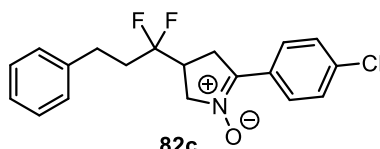
$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 163.7 (d, $^1J_{\text{C-F}} = 252.8 \text{ Hz}$), 140.0, 138.7, 129.8 (d, $^3J_{\text{C-F}} = 8.3 \text{ Hz}$), 128.9, 128.4, 126.7, 125.1, 123.3 (t, $^1J_{\text{C-F}} = 244.1 \text{ Hz}$), 115.8 (d, $^2J_{\text{C-F}} = 21.6 \text{ Hz}$), 64.2, 37.2 (t, $^2J_{\text{C-F}} = 24.9 \text{ Hz}$), 36.5 (t, $^2J_{\text{C-F}} = 26.4 \text{ Hz}$), 31.4, 28.0 (t, $^3J_{\text{C-F}} = 4.6 \text{ Hz}$).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -106.86 (AB system, $^2J_{\text{F-F}} = 246.1 \text{ Hz}$), -107.32 (s), -108.00 (AB system, $^2J_{\text{F-F}} = 246.1 \text{ Hz}$).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($C_{19}H_{18}NOF_3\text{Na}$) = 356.1233; found: 356.1235 (1 ppm).

13.3. 5-(4-chlorophenyl)-3-(1,1-difluoro-3-phenylpropyl)-3,4-dihydro-2H-pyrrole 1-oxide **82c**

The reaction was performed according to the general procedure 3. Pale white solid, yield 52 mg (71%) from 75 mg (0.20 mmol) of **80c**.



82c
 $C_{19}H_{18}ClF_2NO$
 $M = 349.81 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.1$ (Petroleum Ether / Ethyl Acetate: 6/4). $mp = 159 \text{ }^\circ\text{C}$.

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.32 – 8.20 (m, 2H), 7.45 – 7.37 (m, 2H), 7.36 – 7.15 (m, 5H), 4.36 (dd, $^2J_{\text{H-H}} = 14.4 \text{ Hz}$, $^3J_{\text{H-H}} = 7.8 \text{ Hz}$, 1H), 4.24 (dd, $^2J_{\text{H-H}} = 14.4 \text{ Hz}$, $^3J_{\text{H-H}} = 9.7 \text{ Hz}$, 1H), 3.24 (d, $^3J_{\text{H-H}} = 8.1 \text{ Hz}$, 2H), 3.15 – 2.92 (m, 1H), 2.93 – 2.83 (m, 2H), 2.31 – 2.09 (m, 2H).

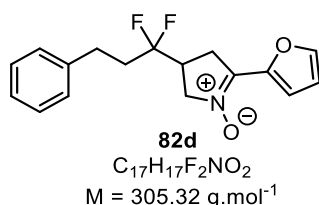
$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 139.9, 138.5, 136.4, 128.9, 128.9, 128.6, 128.4, 127.2, 126.7, 123.3 (t, $^1J_{\text{C-F}} = 244.2 \text{ Hz}$), 64.3 (t, $^3J_{\text{C-F}} = 4.7 \text{ Hz}$), 37.1 (t, $^2J_{\text{C-F}} = 24.9 \text{ Hz}$), 36.5 (t, $^2J_{\text{C-F}} = 26.4 \text{ Hz}$), 31.3 (t, $^3J_{\text{C-F}} = 5.0 \text{ Hz}$), 28.0 (t, $^3J_{\text{C-F}} = 4.7 \text{ Hz}$).

$^{19}\text{F NMR}$ (471 MHz, CDCl_3) δ -106.84 (AB system, $^2J_{\text{F-F}} = 246.3 \text{ Hz}$), -107.86 (AB system, $^2J_{\text{F-F}} = 246.2 \text{ Hz}$).

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{19}H_{18}NOF_3ClNa$) = 372.0937; found: 372.0939 (0 ppm).

13.4. 3-(1,1-difluoro-3-phenylpropyl)-5-(furan-2-yl)-3,4-dihydro-2H-pyrrole 1-oxide 82d

The reaction was performed according to the general procedure 3. Yellow oil, yield 50 mg (73%) from 75 mg (0.22 mmol) of **82d**.



R_f = 0.2 (hexane / Ethyl Acetate: 2/8).

¹H NMR (300 MHz, CDCl₃) δ 7.79 (d, $^3J_{H-H} = 3.2$ Hz, 1H), 7.49 (d, $^3J_{H-H} = 1.1$ Hz, 1H), 7.39 – 7.13 (m, 5H), 6.58 (dd, $^3J_{H-H} = 3.4$ Hz, $^4J_{H-H} = 1.8$ Hz, 1H), 4.36 – 4.06 (m, 2H), 3.38 – 3.14 (m, 2H), 3.15 – 2.92 (m, 1H), 2.92 – 2.77 (m, 2H), 2.33 – 2.07 (m, 2H).

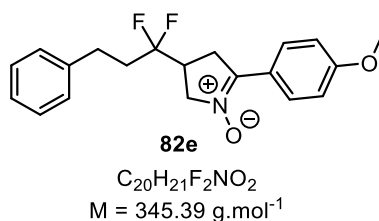
¹³C NMR (75 MHz, CDCl₃) δ 144.9, 143.9, 140.0, 133.5, 128.8, 128.4, 126.6, 123.3 (t, $^1J_{C-F} = 244.1$ Hz), 115.0, 112.6, 62.3 (dd, $^3J_{C-F} = 5.3$ Hz, $^3J_{C-F} = 4.0$ Hz), 37.6 (t, $^2J_{C-F} = 26.4$ Hz), 37.0 (t, $^2J_{C-F} = 24.9$ Hz), 29.3 (t, $^3J_{C-F} = 5.0$ Hz), 28.0 (t, $^3J_{C-F} = 4.7$ Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -106.02 (AB system, $^2J_{F-F} = 246.1$ Hz), -108.38 (AB system, $^2J_{F-F} = 246.1$ Hz).

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{17}H_{17}NO_2F_2Na$) = 328.1120; found: 328.1121 (0 ppm).

13.5. 3-(1,1-difluoro-3-phenylpropyl)-5-(4-methoxyphenyl)-3,4-dihydro-2H-pyrrole 1-oxide 82e

The reaction was performed according to the general procedure 3. Pale yellow solid, yield 55 mg (80%) from 75 mg (0.20 mmol) of **80e**.



R_f = 0.4 (Petroleum Ether / Ethyl Acetate: 6/4). **mp** = 126 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.37 – 8.25 (m, 2H), 7.38 – 7.25 (m, 2H), 7.29 – 7.15 (m, 3H), 6.98 – 6.91 (m, 2H), 4.39 – 4.15 (m, 2H), 3.84 (s, 3H), 3.26 – 3.19 (m, 2H), 3.12 – 2.93 (m, 1H), 2.91 – 2.84 (m, 2H), 2.30 – 2.07 (m, 2H).

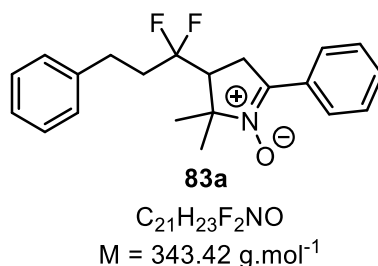
¹³C NMR (75 MHz, CDCl₃) δ 161.3, 140.0, 139.3, 129.3, 128.8, 128.4, 126.6, 123.4 (t, ¹J_{C-F} = 245.2 Hz), 121.6, 113.9, 63.7 (t, ³J_{C-F} = 4.7 Hz), 55.4, 37.1 (t, ²J_{C-F} = 24.9 Hz), 36.5 (t, ²J_{C-F} = 26.4 Hz), 31.4 (t, ³J_{C-F} = 5.0 Hz), 28.0 (t, ³J_{C-F} = 4.7 Hz).

¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -106.9 (AB system, ²J_{F-F} = 246.0 Hz), -107.7 (AB system, ²J_{F-F} = 246.0 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₀H₂₁NO₂F₂Na) = 368.1433; found: 368.1435 (1 ppm).

13.6. 3-(1,1-difluoro-3-phenylpropyl)-2,2-dimethyl-5-phenyl-3,4-dihydro-2H-pyrrole 1-oxide 83a

The reaction was performed according to the general procedure 3. White solid, yield 62 mg (90%) from 75 mg (0.20 mmol) of **81a**.



R_f = 0.4 (hexane / Ethyl Acetate: 6/4). **mp** = 110 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.44 – 8.26 (m, 2H), 7.53 – 7.40 (m, 3H), 7.37 – 7.16 (m, 5H), 3.23 – 3.00 (m, 2H), 3.00 – 2.84 (m, 2H), 2.85 – 2.59 (m, 1H), 2.41 – 2.07 (m, 2H), 1.65 (s, 3H), 1.53 (s, 3H).

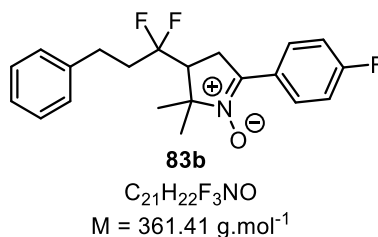
¹³C NMR (75 MHz, CDCl₃) δ 140.2, 136.0, 130.5, 129.4, 128.9, 128.7, 128.5, 127.4, 126.7, 123.7 (t, ¹J_{C-F} = 245.2 Hz), 77.0, 48.5 (t, ²J_{C-F} = 24.1 Hz), 38.9 (t, ²J_{C-F} = 25.1 Hz), 28.5 (dd, ³J_{C-F} = 7.0 Hz, ³J_{C-F} = 4.0 Hz), 28.1 (t, ³J_{C-F} = 5.0 Hz), 27.3 (d, ⁴J_{C-F} = 1.2 Hz), 21.0 (t, ³J_{C-F} = 3.3 Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -102.75 (AB system, ²J_{F-F} = 247.0 Hz), -104.91 (AB system, ²J_{F-F} = 247.0 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₁H₂₃NOF₂Na) = 366.1640; found: 366.1644 (1 ppm).

13.7. 3-(1,1-difluoro-3-phenylpropyl)-5-(4-fluorophenyl)-2,2-dimethyl-3,4-dihydro-2H-pyrrole 1-oxide 83b

The reaction was performed according to the general procedure 3. White solid, yield 38 mg (55%) from 75 mg (0.19 mmol) of **81b**.



R_f = 0.2 (hexane / Ethyl Acetate: 6/4). **mp** = 119 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.46 – 8.29 (m, 2H), 7.39 – 7.27 (m, 2H), 7.30 – 7.18 (m, 3H), 7.19 – 7.04 (m, 2H), 3.17 – 2.98 (m, 2H), 2.99 – 2.83 (m, 2H), 2.73 (m, 1H), 2.38 – 2.12 (m, 2H), 1.64 (s, 3H), 1.53 (s, 3H).

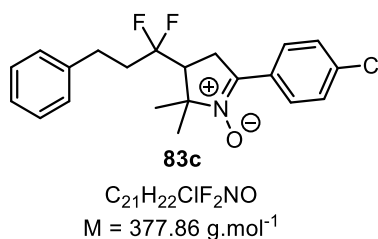
¹³C NMR (75 MHz, CDCl₃) δ 163.4 (d, ¹J_{C-F} = 252.7 Hz), 140.2, 135.1, 129.7 (d, ³J_{C-F} = 8.3 Hz), 128.9, 128.4, 126.7, 125.8 (d, ⁴J_{C-F} = 3.4 Hz), 123.6 (t, ¹J_{C-F} = 245.1 Hz), 115.7 (d, ²J_{C-F} = 21.6 Hz), 76.9, 48.4 (t, ²J_{C-F} = 24.2 Hz), 38.9 (t, ²J_{C-F} = 25.1 Hz), 28.5 (dd, ³J_{C-F} = 7.2, ³J_{C-F} = 4.0 Hz), 28.0 (t, ³J_{C-F} = 5.0 Hz), 27.3, 21.0 (t, ⁴J_{C-F} = 3.6 Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -102.61 (AB system, ²J_{F-F} = 247.3 Hz), -104.92 (AB system, ²J_{F-F} = 247.3 Hz), -108.05 (s).

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₁H₂₂NOF₃Na) = 384.1545; found: 384.1543 (1 ppm).

13.8. 5-(4-chlorophenyl)-3-(1,1-difluoro-3-phenylpropyl)-2,2-dimethyl-3,4-dihydro-2H-pyrrole 1-oxide **83c**

The reaction was performed according to the general procedure 3. Yellow oil, yield 37 mg (54%) from 75 mg (0.18 mmol) of **81c**.



R_f = 0.2 (hexane / Ethyl Acetate: 6/4).

¹H NMR (300 MHz, CDCl₃) δ 8.32 – 8.27 (m, 2H), 7.43 – 7.39 (m, 2H), 7.36 – 7.29 (m, 2H), 7.28 – 7.20 (m, 3H), 3.17 – 3.00 (m, 2H), 2.95 – 2.84 (m, 2H), 2.82 – 2.64 (m, 1H), 2.33 – 2.14 (m, 2H), 1.64 (s, 3H), 1.52 (s, 3H).

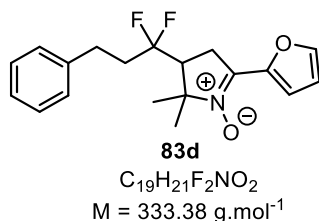
^{13}C NMR (75 MHz, CDCl_3) δ 140.1, 136.0, 135.4, 128.9, 128.7, 128.4, 128.4, 127.8, 126.7, 123.6 (t, $^1J_{\text{F-F}} = 245.3$ Hz), 78.8, 48.4 (t, $^2J_{\text{F-F}} = 24.2$ Hz), 38.9 (t, $^2J_{\text{F-F}} = 25.1$ Hz), 28.4 (dd, $^3J_{\text{F-F}} = 7.1$ Hz, $^3J_{\text{F-F}} = 4.0$ Hz), 28.0 (dd, $^3J_{\text{F-F}} = 5.0$ Hz, $^3J_{\text{F-F}} = 0.1$ Hz), 27.2 (d, $^4J_{\text{F-F}} = 1.6$ Hz), 21.0 (dd, $^3J_{\text{F-F}} = 4.2$ Hz, $^3J_{\text{F-F}} = 3.1$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -102.68 (AB system, $^2J_{\text{F-F}} = 247.3$ Hz), -104.95 (AB system, $^2J_{\text{F-F}} = 247.3$ Hz).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{21}\text{H}_{22}\text{NOF}_2^{35}\text{ClNa}$) = 400.1250; found: 400.1255 (1 ppm).

13.9. 3-(1,1-difluoro-3-phenylpropyl)-5-(furan-2-yl)-2,2-dimethyl-3,4-dihydro-2H-pyrrole 1-oxide 83d

The reaction was performed according to the general procedure 3. Yellow oil, yield 48 mg (70%) from 75 mg (0.21 mmol) of **81d**.



R_f = 0.3 (hexane / Ethyl Acetate: 2/8).

^1H NMR (300 MHz, CDCl_3) δ 7.79 (d, $^3J_{\text{H-H}} = 3.5$ Hz, 1H), 7.48 (dd, $^3J_{\text{H-H}} = 1.8$, $^4J_{\text{H-H}} = 0.8$ Hz, 1H), 7.36 – 7.28 (m, 2H), 7.27 – 7.18 (m, 3H), 6.58 (dd, $^3J_{\text{H-H}} = 3.5$ Hz, $^3J_{\text{H-H}} = 1.8$ Hz, 1H), 3.20 – 2.98 (m, 2H), 2.95 – 2.84 (m, 2H), 2.84 – 2.61 (m, 1H), 2.38 – 2.07 (m, 2H), 1.61 (s, 3H), 1.51 (s, 3H).

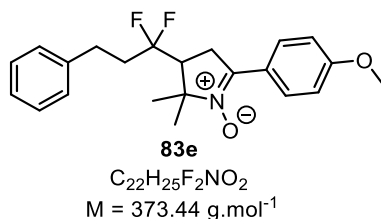
^{13}C NMR (75 MHz, CDCl_3) δ 145.4, 143.5, 140.1, 130.0, 128.8, 128.4, 126.6, 123.6 (dd, $^1J_{\text{C-F}} = 246.0$ Hz, $^1J_{\text{C-F}} = 244.3$ Hz), 114.4, 112.5, 75.6, 49.2 (t, $^2J_{\text{C-F}} = 24.1$ Hz), 38.8 (t, $^2J_{\text{C-F}} = 25.1$ Hz), 28.0 (dd, $^3J_{\text{C-F}} = 5.7$ Hz, $^3J_{\text{C-F}} = 4.3$ Hz), 27.0 (d, $^4J_{\text{C-F}} = 1.6$ Hz), 26.5 (dd, $^3J_{\text{C-F}} = 7.2$ Hz, $^3J_{\text{C-F}} = 4.1$ Hz), 20.9 (dd, $^4J_{\text{C-F}} = 4.6$ Hz, $^4J_{\text{C-F}} = 2.9$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -103.27 (AB system, $^2J_{\text{F-F}} = 246.8$ Hz), -104.94 (AB system, $^2J_{\text{F-F}} = 246.8$ Hz).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{19}\text{H}_{21}\text{NO}_2\text{F}_2\text{Na}$) = 356.1433; found: 356.1436 (1 ppm).

13.10. 3-(1,1-difluoro-3-phenylpropyl)-5-(4-methoxyphenyl)-2,2-dimethyl-3,4-dihydro-2H-pyrrole 1-oxide 83e

The reaction was performed according to the general procedure 3. White solid, yield 59 mg (85%) from 75 mg (0.18 mmol) of **81e**.



R_f = 0.3 (Petroleum Ether / Ethyl Acetate: 7/3). **mp** = 152 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.39 – 8.26 (m, 2H), 7.37 – 7.28 (m, 2H), 7.27 – 7.15 (m, 3H), 6.98 – 6.88 (m, 2H), 3.81 (s, 3H), 3.07 – 2.97 (m, 2H), 2.96 – 2.81 (m, 2H), 2.79 – 2.57 (m, 1H), 2.35 – 2.10 (m, 2H), 1.62 (s, 3H), 1.50 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 160.9, 140.1, 135.7, 129.2, 128.7, 128.3, 126.5, 123.6 (dd, ¹J_{C-F} = 245.4 Hz, ¹J_{C-F} = 244.6 Hz), 122.1, 113.8, 76.2 (d, ³J_{C-F} = 1.1 Hz), 55.3, 48.2 (t, ²J_{C-F} = 24.1 Hz), 38.7 (t, ²J_{C-F} = 25.1 Hz), 28.3 (dd, ³J_{C-F} = 7.0 Hz, ³J_{C-F} = 3.9 Hz), 27.9 (dd, ⁴J_{C-F} = 5.5 Hz, ⁴J_{C-F} = 4.4 Hz), 27.1 (d, ⁴J_{C-F} = 1.5 Hz), 20.8 (dd, ³J_{C-F} = 4.3 Hz, ³J_{C-F} = 2.9 Hz).

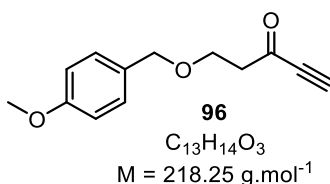
¹⁹F {¹H}synthesis NMR (471 MHz, CDCl₃) δ -102.74 (AB system, ²J_{F-F} = 246.9 Hz), -104.68 (AB system, ²J_{F-F} = 246.9 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₂H₂₅NO₂F₂Na) = 396.1745; found: 396.1745 (0 ppm).

14. Synthesis of type B nitrones

14.1. Synthesis of 5-((4-methoxybenzyl)oxy)pent-1-yn-3-one **96**

To the propargylic alcohol **95** (3 g, 13.62 mmol) in acetone (20 mL) was added dropwise under magnetic stirring at room temperature, a concentrated (5.4 M) solution of Jones reagent until disappearance of the starting material (TLC analysis). After addition of isopropanol (5.0 equiv), the reaction mixture was filtered and the filtrate was extracted with ethyl acetate (3x40mL). The combined organic phases were dried over Na₂SO₄, filtered and concentrated under vacuum. After purification by chromatography on silica gel, 5-((4-methoxybenzyl)oxy)pent-1-yn-3-one **96** was obtained as colorless oil (2.08 g, 70%).



R_f = 0.6 (Petroleum Ether/ Ethyl Acetate: 8/2).

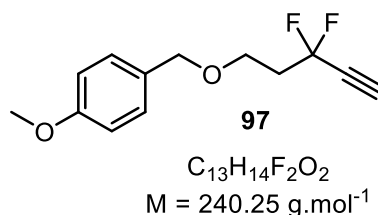
¹H NMR (300 MHz, CDCl₃) δ 7.30 – 7.18 (m, 2H), 6.94 – 6.77 (m, 2H), 4.45 (s, 2H), 3.82 – 3.75 (m, 5H), 3.23 (d, ⁵J_{H-H} = 0.8 Hz, 1H), 2.85 (td, ³J_{H-H} = 6.2 Hz, ⁵J_{H-H} = 0.8 Hz, 2H).

¹³C NMR (75 MHz, CDCl₃) δ 185.3, 159.4, 130.0, 129.5, 113.9, 81.3, 79.1, 73.0, 64.3, 55.4, 45.7.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₃H₁₄O₃Na) = 241.0835; found: 241.0837 (1 ppm).

14.2. Synthesis of 1-(((3,3-difluoropent-4-yn-1-yl)oxy)methyl)-4-methoxybenzene **97**

To the propargylic ketone **96** (2 g, 9.16 mmol) one drop of 95% ethanol and DAST (2.42 mL, 18.32 mmol, 2 equiv) were added. The reaction mixture was stirred at 58 °C for 8 h. After coming back to room temperature and hydrolysis, the reaction mixture was extracted with CH₂Cl₂ (3x40 mL). The organic layers were separated, washed with water (3x20 mL), dried over MgSO₄ then filtrated on silica. After purification by chromatography on silica gel, the 1-(((3,3-difluoropent-4-yn-1-yl)oxy)methyl)-4-methoxybenzene **97** was obtained as a yellow oil (1.25 g, 57%).



R_f = 0.4 (Petroleum Ether/ Ethyl Acetate: 9/1).

¹H NMR (300 MHz, CDCl₃) δ 7.31 – 7.21 (m, 3H), 6.95 – 6.81 (m, 2H), 4.47 (s, 2H), 3.81 (s, 3H), 3.70 (t, ³J_{H-H} = 7.0 Hz, 2H), 2.78 (t, ³J_{H-F} = 5.1 Hz, 1H), 2.41 (tt, ³J_{H-F} = 14.7 Hz, ³J_{H-H} = 6.9 Hz, 2H).

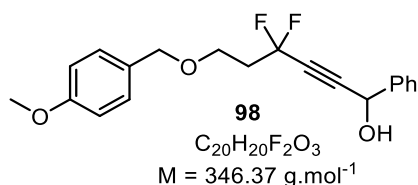
¹³C NMR (75 MHz, CDCl₃) δ 159.4, 130.1, 129.5, 114.0, 113.0 (t, ¹J_{C-F} = 233.3 Hz), 76.4 (t, ²J_{C-F} = 40.5 Hz), 75.7 (t, ³J_{C-F} = 6.8 Hz), 73.0, 63.9 (t, ³J_{C-F} = 4.8 Hz), 55.4, 39.4 (t, ²J_{C-F} = 25.7 Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -82.75 (s).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₃H₁₄O₂F₂Na) = 263.0854; found: 263.0856 (1 ppm).

14.3. Synthesis of 4,4-difluoro-6-((4-methoxybenzyl)oxy)-1-phenylhex-2-yn-1-ol **98**

The reaction was carried out with 1-(((3,3-difluoropent-4-yn-1-yl)oxy)methyl)-4-methoxybenzene **97** (1 g, 4.16 mmol) with Benzaldehyde (550 μ L, 5.41 mmol) according to the general procedure 1. After purification by chromatography on silica gel, **98** was obtained as yellow oil (1.35 g, 95%).



R_f = 0.4 (Petroleum Ether/ Ethyl Acetate: 7.5/2.5).

¹H NMR (300 MHz, CDCl₃) δ 7.55 – 7.34 (m, 5H), 7.29 – 7.19 (m, 2H), 6.94 – 6.81 (m, 2H), 5.48 (bs, 1H), 4.44 (s, 2H), 3.79 (s, 3H), 3.69 (td, ³ $J_{H-H} = 6.8$ Hz, ⁴ $J_{H-F} = 0.6$ Hz, 2H), 2.57 (bs, 1H), 2.42 (tt, ³ $J_{H-F} = 14.0$ Hz, ³ $J_{H-H} = 6.8$ Hz, 2H).

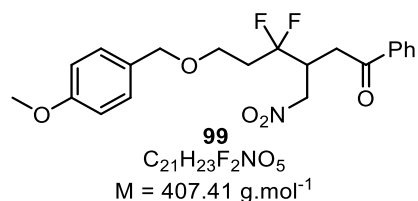
¹³C NMR (75 MHz, CDCl₃) δ 159.4, 139.1 (t, ⁵ $J_{C-F} = 1.6$ Hz), 130.0, 129.5, 129.0, 128.9, 126.7, 114.0, 113.5 (t, ¹ $J_{C-F} = 233.3$ Hz), 87.2 (t, ³ $J_{C-F} = 6.7$ Hz), 79.2 (t, ² $J_{C-F} = 40.5$ Hz), 72.9, 64.2 (t, ⁴ $J_{C-F} = 2.0$ Hz), 63.9 (t, ³ $J_{C-F} = 4.8$ Hz), 55.4, 39.5 (t, ² $J_{C-F} = 26.1$ Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -81.73 (s).

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{20}H_{20}O_3F_2Na$) = 369.1272; found: 369.1272 (0 ppm).

14.4. Synthesis of 4,4-difluoro-6-((4-methoxybenzyl)oxy)-3-(nitromethyl)-1-phenylhexan-1-one **99**

The reaction was carried out with 4,4-difluoro-6-((4-methoxybenzyl)oxy)-1-phenylhex-2-yn-1-ol **98** (1 g, 2.88 mmol) according to the general procedure 2. After purification by chromatography on silica gel, **99** was obtained as a colorless oil (0.89 g, 76%).



R_f = 0.3 (Petroleum Ether/ Ethyl Acetate: 7/3).

¹H NMR (300 MHz, CDCl₃) δ 7.98 – 7.91 (m, 2H), 7.65 – 7.55 (m, 1H), 7.52 – 7.43 (m, 2H), 7.25 – 7.17 (m, 2H), 6.87 – 6.80 (m, 2H), 4.73 (dd, ² $J_{H-H} = 13.4$ Hz, ³ $J_{H-H} = 5.9$ Hz, 1H), 4.59 (dd, ² $J_{H-H} = 13.4$ Hz, ³ $J_{H-H} = 5.0$ Hz, 1H), 4.43 (s, 2H), 3.78 (s, 3H), 3.65 (t, ³ $J_{H-H} = 5.9$ Hz, 2H), 3.43 (dd, ² $J_{H-H} = 18.4$ Hz, ³ $J_{H-H} = 4.0$ Hz, 1H), 3.24 (dd, ² $J_{H-H} = 18.4$ Hz, ³ $J_{H-H} = 8.9$ Hz, 1H), 2.39 – 2.21 (m, 2H).

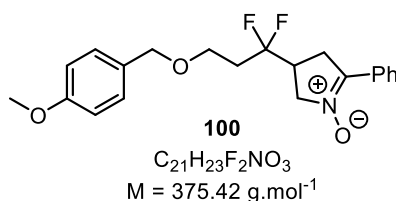
¹³C NMR (75 MHz, CDCl₃) δ 196.3, 159.3, 136.1, 133.7, 129.5, 129.5, 128.8, 128.1, 124.2 (d, ¹J_{C-F} = 244.9 Hz), 113.9, 73.5 (t, ³J_{C-F} = 4.6 Hz), 73.2, 63.4 (t, ³J_{C-F} = 6.3 Hz), 55.3, 39.3 (t, ²J_{C-F} = 24.3 Hz), 35.3 (t, ²J_{C-F} = 25.1 Hz), 34.9 (t, ³J_{C-F} = 3.6 Hz).

¹⁹F {¹H} NMR (471 MHz, CDCl₃) δ -99.53 (AB system, ²J_{F-F} = 253.2 Hz), -100.47 (AB system, ²J_{F-F} = 253.2 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₁H₂₃NO₅F₂Na) = 430.1437; found: 430.1439 (1 ppm).

14.5. Synthesis of 3-(1,1-difluoro-3-((4-methoxybenzyl)oxy)propyl)-5-phenyl-3,4-dihydro-2H-pyrrole 1-oxide 100

The reaction was carried out with 4,4-difluoro-6-((4-methoxybenzyl)oxy)-3-(nitromethyl)-1-phenylhexan-1-one **99** (75 mg, 0.18 mmol) according to the general procedure 3. After purification by chromatography on silica gel, **100** was obtained as colorless oil (53 mg, 77%).



R_f = 0.1 (Petroleum Ether/ Ethyl Acetate: 6/4).

¹H NMR (300 MHz, CDCl₃) δ 8.36 – 8.21 (m, 2H), 7.49 – 7.40 (m, 3H), 7.26 – 7.19 (m, 2H), 6.95 – 6.81 (m, 2H), 4.44 (s, 2H), 4.41 – 4.20 (m, 2H), 3.79 (s, 3H), 3.64 (t, ³J_{H-H} = 5.9 Hz, 2H), 3.36 – 3.05 (m, 3H), 2.33 – 2.13 (m, 2H).

¹³C NMR (75 MHz, CDCl₃) δ 159.5, 139.9, 130.8, 129.7, 129.5, 128.8, 128.6, 127.4, 123.5 (t, ¹J_{C-F} = 243.4 Hz), 114.1, 73.2, 64.4 (dd, ³J_{C-F} = 6.1 Hz, ³J_{C-F} = 3.7 Hz), 63.5 (t, ³J_{C-F} = 6.3 Hz), 55.4, 36.3 (t, ²J_{C-F} = 25.8 Hz), 35.8 (t, ²J_{C-F} = 25.4 Hz), 31.5 (dd, ³J_{C-F} = 6.2, ³J_{C-F} = 4.1 Hz).

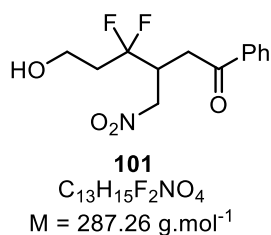
¹⁹F {¹H} NMR (471 MHz, CDCl₃) δ -103.73 (AB system, ²J_{F-F} = 249.2 Hz), -104.83 (AB system, ²J_{F-F} = 249.2 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₁H₂₃NO₃F₂Na) = 398.1538; found: 398.1538 (0 ppm).

14.6. Synthesis of 4,4-difluoro-6-hydroxy-3-(nitromethyl)-1-phenylhexan-1-one 101

To protected nitro compound **99** (0.5 g, 1.22 mmol) in DCM (20 mL) and water (1 mL) was added under magnetic stirring at room temperature, DDQ (420 mg, 1.84 mmol) until disappearance of the starting material (TLC analysis). Then water was added and the reaction mixture was extracted with DCM (3x20 mL). The combined organic phases were dried over

Na₂SO₄, filtered and concentrated in vacuum. After purification by chromatography on silica gel, 4,4-difluoro-6-hydroxy-3-(nitromethyl)-1-phenylhexan-1-one **101** was obtained as yellow oil (345 mg, 98%).



R_f = 0.4 (Petroleum Ether/ Ethyl Acetate: 6/4).

¹H NMR (300 MHz, CDCl₃) δ 7.99 – 7.91 (m, 2H), 7.65 – 7.56 (m, 1H), 7.53 – 7.43 (m, 2H), 4.75 (dd, ²J_{H-H} = 13.5 Hz, ³J_{H-H} = 5.9 Hz, 1H), 4.55 (dd, ²J_{H-H} = 13.5 Hz, ³J_{H-H} = 5.5 Hz, 1H), 3.90 (t, ³J_{H-H} = 5.9 Hz, 2H), 3.87 – 3.69 (m, 1H), 3.45 (dd, ²J_{H-H} = 18.5 Hz, ³J_{H-H} = 4.5 Hz, 1H), 3.25 (dd, ²J_{H-H} = 18.5 Hz, ³J_{H-H} = 8.2 Hz, 1H), 2.23 (tt, ³J_{H-F} = 17.1 Hz, ²J_{H-H} = 5.9 Hz, 2H), 2.08 (bs, 1H).

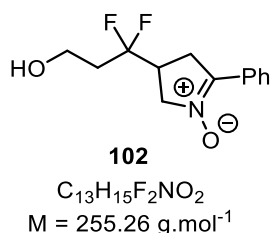
¹³C NMR (75 MHz, CDCl₃) δ 196.5, 136.0, 134.0, 128.9, 128.2, 124.5 (t, J = 245.1 Hz), 73.7 (t, J = 4.9 Hz), 56.5 (t, J = 6.0 Hz), 39.5 (t, J = 24.2 Hz), 37.3 (t, J = 24.1 Hz), 35.2 (dd, J = 4.2, 3.3 Hz).

¹⁹F {H} NMR (471 MHz, CDCl₃) δ -100.56 (AB system, ²J_{F-F} = 252.5 Hz), -101.43 (AB system, ²J_{F-F} = 252.5 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₃H₁₅NO₄F₂Na) = 310.0861; found: 310.0863 (0 ppm).

14.7. Synthesis of 3-(1,1-difluoro-3-hydroxypropyl)-5-phenyl-3,4-dihydro-2H-pyrrole 1-oxide **102**

The reaction was carried out with 4,4-difluoro-6-hydroxy-3-(nitromethyl)-1-phenylhexan-1-one **101** (75 mg, 0.26 mmol) according to the general procedure 3. After purification by chromatography on silica gel, **102** was obtained as white solid (40 mg, 60%).



R_f = 0.1 (Petroleum Ether/ Ethyl Acetate: 2.5/7.5). **mp** = 147 °C.

¹H NMR (300 MHz, CD₃OD) δ 8.37 – 8.27 (m, 2H), 7.56 – 7.42 (m, 3H), 4.46 – 4.18 (m, 2H), 3.78 (t, ³J_{H-H} = 6.3 Hz, 2H), 3.57 – 3.32 (m, 3H), 2.31 – 2.14 (m, 2H).

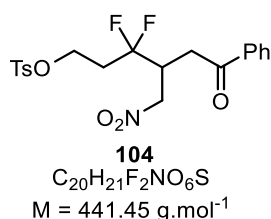
¹³C NMR (75 MHz, CD₃OD) δ 144.8, 132.5, 129.6, 129.5, 129.2, 125.3 (t, ¹J_{C-F} = 242.8 Hz), 65.0 (dd, ³J_{C-F} = 6.1 Hz, ³J_{C-F} = 4.0 Hz), 56.5 (t, ³J_{C-F} = 6.2 Hz), 38.5 (t, ²J_{C-F} = 24.2 Hz), 37.4 (t, ²J_{C-F} = 25.8 Hz), 32.8 (dd, ³J_{C-F} = 6.4 Hz, ³J_{C-F} = 4.1 Hz).

¹⁹F {H} NMR (471 MHz, CD₃OD) δ -105.95 (AB system, ²J_{F-F} = 248.4 Hz), -107.81 (AB system, ²J_{F-F} = 248.4 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₃H₁₅NO₂F₂Na) = 278.0963; found: 278.0961 (1 ppm).

14.8. 3,3-difluoro-4-(nitromethyl)-6-oxo-6-phenylhexyl 4-methylbenzenesulfonate **104**

To 4,4-difluoro-6-hydroxy-3-(nitromethyl)-1-phenylhexan-1-one **101** (150 mg, 0.52 mmol), *p*-toluenesulfonyl chloride (200 mg, 1.04 mmol), pyridine (84 μL, 1.04 mmol), and catalytic amount of DMAP were added and mixed in 5 ml DCM for 48 h. The reaction was monitored by TLC. At the end saturated NH₄Cl (2 mL) was added and the reaction mixture was extracted with DCM (3x10 mL). The organic layers were separated, washed with water (1x10 mL), dried over Na₂SO₄ and concentrated under vacuum. After purification by chromatography on silica gel, **104** (145 mg, 63%) was obtained as white solid.



R_f = 0.3 (Petroleum Ether / Ethyl Acetate: 8/2). **mp** = 89 °C.

¹H NMR (300 MHz, CDCl₃) δ 7.99 – 7.89 (m, 2H), 7.82 – 7.71 (m, 2H), 7.66 – 7.55 (m, 1H), 7.54 – 7.42 (m, 2H), 7.39 – 7.28 (m, 2H), 4.67 (dd, ²J_{H-H} = 13.8 Hz, ³J_{H-H} = 6.2 Hz, 1H), 4.48 (dd, ²J_{H-H} = 13.8 Hz, ³J_{H-H} = 5.2 Hz, 1H), 4.34 – 4.15 (m, 2H), 3.74 – 3.51 (m, 1H), 3.35 (dd, ²J_{H-H} = 18.6 Hz, ³J_{H-H} = 4.5 Hz, 1H), 3.21 (dd, ²J_{H-H} = 18.6 Hz, ³J_{H-H} = 8.1 Hz, 1H), 2.43 (s, 3H), 2.42 – 2.25 (m, 2H).

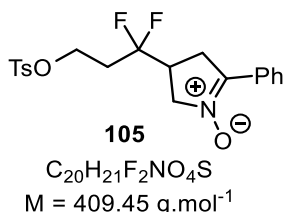
¹³C NMR (75 MHz, CDCl₃) δ 195.8, 145.4, 135.9, 134.1, 132.4, 130.1, 128.9, 128.2, 128.0, 123.1 (t, ¹J_{C-F} = 246.0 Hz), 73.3 (t, ³J_{C-F} = 4.8 Hz), 63.3 (t, ³J_{C-F} = 5.9 Hz), 39.3 (t, ²J_{C-F} = 23.7 Hz), 35.1 (dd, ³J_{C-F} = 4.3 Hz, ³J_{C-F} = 3.0 Hz), 34.5 (t, ²J_{C-F} = 24.8 Hz), 21.7.

¹⁹F {H} NMR (471 MHz, CDCl₃) δ -101.54 (AB system, ²J_{F-F} = 253.2 Hz), -102.91 (AB system, ²J_{F-F} = 253.2 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₀H₂₁NO₆F₂SNa) = 464.0950; found: 464.0956 (1 ppm).

14.9. 3-(1,1-difluoro-3-(tosyloxy)propyl)-5-phenyl-3,4-dihydro-2H-pyrrole 1-oxide **105**

The reaction was carried out with 3,3-difluoro-4-(nitromethyl)-6-oxo-6-phenylhexyl 4-methylbenzenesulfonate **104** (100 mg, 0.23 mmol) according to the general procedure 3. After purification by chromatography on silica gel, **105** was obtained as colorless oil (75 mg, 81%).



R_f = 0.3 (Petroleum Ether/ Ethyl Acetate: 2.5/7.5).

¹H NMR (300 MHz, CDCl₃) δ 8.33 – 8.23 (m, 2H), 7.82 – 7.73 (m, 2H), 7.48 – 7.40 (m, 3H), 7.39 – 7.31 (m, 2H), 4.34 – 4.17 (m, 4H), 3.51 – 2.88 (m, 3H), 2.44 (s, 3H), 2.41 – 2.22 (m, 2H).

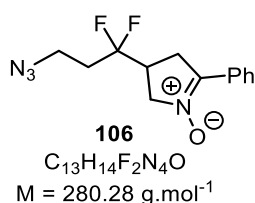
¹³C NMR (75 MHz, CDCl₃) δ 145.5, 139.3, 132.4, 130.8, 130.2, 128.6, 128.0, 127.3, 122.4 (t, ¹J_{C-F} = 244.3 Hz), 63.9 (dd, ³J_{C-F} = 5.7 Hz, ³J_{C-F} = 3.5 Hz), 63.4 (t, ³J_{C-F} = 5.9 Hz), 36.3 (t, ²J_{C-F} = 25.6 Hz), 34.8 (t, ²J_{C-F} = 25.7 Hz), 31.3 (t, ³J_{C-F} = 5.0 Hz), 21.8.

¹⁹F {¹H} NMR (471 MHz, CDCl₃) δ -104.66 (AB system, ²J_{F-F} = 250.8 Hz), -106.45 (AB system, ²J_{F-F} = 250.9 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₀H₂₁NO₄F₂SNa) = 432.1052; found: 432.1058 (2 ppm).

14.10. 3-(3-azido-1,1-difluoropropyl)-5-phenyl-3,4-dihydro-2H-pyrrole 1-oxide **106**

To 3-(1,1-difluoro-3-(tosyloxy)propyl)-5-phenyl-3,4-dihydro-2H-pyrrole 1-oxide **105** (60 mg, 0.15 mmol), sodium azide (15 mg, 0.22 mmol) was added in 2 mL DMF. The reaction was monitored by TLC. At the end saturated NH₄Cl (2 mL) was added and the reaction mixture was extracted with DCM (3x 5 mL). The organic layers were separated, washed with water (1x 5 mL), dried over Na₂SO₄ and concentrated under vacuum. After purification by chromatography on silica gel, **106** (35 mg, 85%) was obtained as colorless oil (75 mg, 81%).



R_f = 0.3 (Petroleum Ether/ Ethyl Acetate: 2.5/7.5). **mp** = 72 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.40 – 8.24 (m, 2H), 7.54 – 7.42 (m, 3H), 4.39 – 4.32 (m, 2H), 3.61 (t, ³J_{H-H} = 6.8 Hz, 2H), 3.44 – 3.02 (m, 3H), 2.30 – 2.11 (m, 2H).

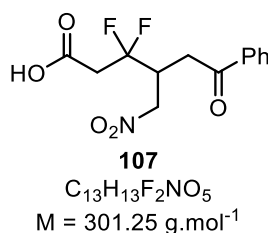
¹³C NMR (75 MHz, CDCl₃) δ 139.5, 130.9, 128.7, 128.6, 127.4, 122.7 (t, ¹J_{C-F} = 244.2 Hz), 64.1 (dd, ³J_{C-F} = 5.5 Hz, ³J_{C-F} = 3.9 Hz), 44.7 (t, ³J_{C-F} = 5.3 Hz), 36.6 (t, ²J_{C-F} = 25.9 Hz), 34.6 (t, ²J_{C-F} = 25.2 Hz), 31.4 (dd, ³J_{C-F} = 5.8 Hz, ³J_{C-F} = 4.4 Hz).

¹⁹F {H} NMR (471 MHz, CDCl₃) δ -101.23 (AB system, ²J_{F-F} = 253.9 Hz), -102.47 (AB system, ²J_{F-F} = 253.9 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₃H₁₄N₄O₂Na) = 303.1027; found: 303.1027 (0 ppm).

14.11. 3,3-difluoro-4-(nitromethyl)-6-oxo-6-phenylhexanoic acid **107**

The reaction was carried out with 4,4-difluoro-6-hydroxy-3-(nitromethyl)-1-phenylhexan-1-one **101** (200 mg, 0.70 mmol) based on the same procedure of the synthesis of compound **96**. After purification by chromatography on silica gel, **107** was obtained as white solid (160 mg, 76%).



R_f = 0.2 (Petroleum Ether/ Ethyl Acetate/methanol: 4.5/5/0.5). **mp** = 130 °C.

¹H NMR (300 MHz, (CD₃)₂CO) δ 8.1 – 8.0 (m, 2H), 7.7 – 7.6 (m, 1H), 7.6 – 7.5 (m, 2H), 4.9 (dd, ²J_{H-H} = 14.2 Hz, ³J_{H-H} = 6.8 Hz, 1H), 4.7 (dd, ²J_{H-H} = 14.2 Hz, ³J_{H-H} = 4.6 Hz, 1H), 4.2 – 4.0 (m, 1H), 3.6 (ddd, ²J_{H-H} = 18.7 Hz, ³J_{H-H} = 3.8 Hz, ⁴J_{H-F} = 0.9 Hz, 1H), 3.5 (dd, ²J_{H-H} = 18.7 Hz, ³J_{H-H} = 9.2 Hz, 1H), 3.4 – 3.1 (m, 2H).

¹³C NMR (75 MHz, (CD₃)₂CO) δ 197.0, 167.9 (dd, ³J_{C-F} = 8.9 Hz, ³J_{C-F} = 4.6 Hz), 137.4, 134.3, 129.5, 129.0, 123.8 (t, ¹J_{C-F} = 245.1 Hz), 74.2 (t, ³J_{C-F} = 4.5 Hz), 40.2 (t, ²J_{C-F} = 26.8 Hz), 39.7 (t, ²J_{C-F} = 23.4 Hz), 36.1 (dd, ³J_{C-F} = 4.9 Hz, ³J_{C-F} = 2.6 Hz).

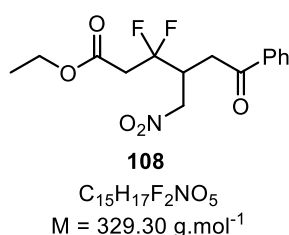
¹⁹F {H} NMR (471 MHz, (CD₃)₂CO) δ -98.03 (AB system, ²J_{F-F} = 254.8 Hz), -100.29 (AB system, ²J_{F-F} = 254.7 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₃H₁₁NO₅F₂Na) = 300.0689; found: 300.0684 (2 ppm).

14.12. ethyl 3,3-difluoro-4-(nitromethyl)-6-oxo-6-phenylhexanoate

108

To 3,3-difluoro-4-(nitromethyl)-6-oxo-6-phenylhexanoic acid **107** (100 mg, 0.33 mmol), N,N'-Dicyclohexylcarbodiimide "DCC" (137 mg, 0.66 mmol), and ethanol (39 μ L, 0.66 mmol), were added and mixed in 4 ml DCM for 24 h. The reaction was monitored by TLC. At the end concentrated NH_4Cl (2 mL) was added and the reaction mixture was extracted with DCM (3x10mL). The organic layers were separated, washed with water (1x10mL), dried over Na_2SO_4 and concentrated under vacuum. After purification by chromatography on silica gel, **108** (78 mg, 72%) was obtained as pale yellow oil.



R_f = 0.6 (Petroleum Ether / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 8.00 – 7.93 (m, 2H), 7.66 – 7.57 (m, 1H), 7.53 – 7.45 (m, 2H), 4.80 (dd, $^2J_{H-H} = 13.6 \text{ Hz}$, $^3J_{H-H} = 5.7 \text{ Hz}$, 1H), 4.62 (dd, $^2J_{H-H} = 13.6 \text{ Hz}$, $^3J_{H-H} = 5.1 \text{ Hz}$, 1H), 4.21 (q, $^3J_{H-H} = 7.1 \text{ Hz}$, 2H), 4.02 – 3.78 (m, 1H), 3.45 (dd, $^2J_{H-H} = 18.4 \text{ Hz}$, $^3J_{H-H} = 4.7 \text{ Hz}$, 1H), 3.34 (dd, $^2J_{H-H} = 18.4 \text{ Hz}$, $^3J_{H-H} = 8.3 \text{ Hz}$, 1H), 3.11 (dd, $^2J_{H-H} = 15.1 \text{ Hz}$, $^3J_{H-H} = 4.1 \text{ Hz}$, 1H), 3.05 (dd, $^2J_{H-H} = 15.1 \text{ Hz}$, $^3J_{H-H} = 3.7 \text{ Hz}$, 1H), 1.29 (t, $^3J_{H-H} = 7.1 \text{ Hz}$, 3H).

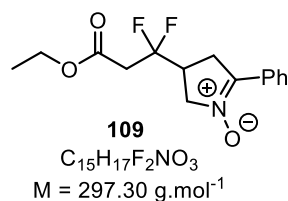
¹³C NMR (75 MHz, CDCl₃) δ 196.1, 166.2 (t, $^3J_{C-F} = 7.4 \text{ Hz}$), 136.1, 134.0, 129.0, 128.3, 121.8 (t, $^1J_{C-F} = 246.9 \text{ Hz}$), 73.6 (t, $^3J_{C-F} = 4.8 \text{ Hz}$), 61.9, 40.7 (t, $^2J_{C-F} = 27.6 \text{ Hz}$), 39.1 (t, $^2J_{C-F} = 23.8 \text{ Hz}$), 34.8 (t, $^3J_{C-F} = 3.7 \text{ Hz}$), 14.1.

¹⁹F NMR (471 MHz, CDCl₃) δ -97.24 (AB system, $^2J_{F-F} = 257.7 \text{ Hz}$), -97.93 (AB system, $^2J_{F-F} = 257.7 \text{ Hz}$).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{15}\text{H}_{17}\text{NO}_5\text{F}_2\text{Na}$) = 352.0967; found: 352.0972 (1 ppm).

14.13. 3-(3-ethoxy-1,1-difluoro-3-oxopropyl)-5-phenyl-3,4-dihydro-2H-pyrrole 1-oxide **109**

The reaction was carried out with ethyl 3,3-difluoro-4-(nitromethyl)-6-oxo-6-phenylhexanoate **108** (60 mg, 0.18 mmol) according to the general procedure 3. After purification by chromatography on silica gel, **109** was obtained as pale yellow oil (42 mg, 77%).



R_f = 0.1 (Petroleum Ether/ Ethyl Acetate: 5/5).

¹H NMR (300 MHz, CDCl₃) δ 8.36 – 8.22 (m, 2H), 7.50 – 7.36 (m, 3H), 4.38 – 4.29 (m, 2H), 4.20 (q, ³J_{H-H} = 7.1 Hz, 2H), 3.61 – 3.15 (m, 3H), 3.10 – 2.85 (m, 2H), 1.28 (t, ³J_{H-H} = 7.1 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 166.3 (t, ³J_{C-F} = 8.0 Hz), 139.3, 130.8, 128.7, 128.6, 127.3, 121.1 (t, ³J_{C-F} = 245.3 Hz), 64.0 (dd, ³J_{C-F} = 5.7 Hz, ³J_{C-F} = 3.5 Hz), 61.8, 40.8 (t, ²J_{C-F} = 28.2 Hz), 35.5 (t, ²J_{C-F} = 25.1 Hz), 31.3 (dd, ³J_{C-F} = 6.1 Hz, ³J_{C-F} = 4.1 Hz), 14.1.

¹⁹F {¹H} NMR (471 MHz, CDCl₃) δ -101.23 (d, AB system, ²J_{F-F} = 253.8 Hz), -102.47 (AB system, ²J_{F-F} = 253.8 Hz).

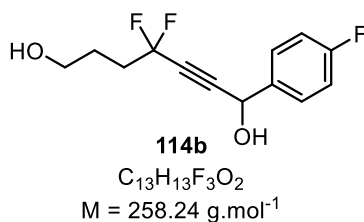
HRMS (ESI): calcd. for [M+Na]⁺ (C₁₅H₁₇NO₃F₂Na) = 320.1069; found: 320.1071 (1 ppm).

15. General procedure 4 for the synthesis of difluoropropargylic alcohols **114**

To a solution of *n*-butyllithium 2.5 M (2.77 mL, 6.92 mmol) in anhydrous THF (40 mL) cooled to -90°C, was added under nitrogen, a solution of **113** (500 mg, 2.10 mmol). The mixture was stirred for 30 min at -90°C. Then, aldehyde **78** (1.3 equiv) in anhydrous THF (10 mL) was added at -90°C, stirred for 45 min, then the reaction mixture was allowed to warm to r.t. within 2h. The mixture was then treated with a saturated NH₄Cl solution and extracted with ethyl acetate (3x40 mL). The combined organic phases were washed with water, dried over Na₂SO₄ and concentrated under vacuum. After purification by chromatography on silica gel, the propargylic alcohols **114** were obtained.

15.1. 4,4-difluoro-1-(4-fluorophenyl)hept-2-yne-1,7-diol **114b**

The reaction was performed according to the general procedure 4. Colorless oil, yield 380 mg (70%).



$R_f = 0.2$ (Hexane / Ethyl Acetate: 7/3).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.54 – 7.39 (m, 2H), 7.12 – 7.01 (m, 2H), 5.48 (t, $^3J_{H-H} = 4.0$ Hz, 1H), 3.68 (t, $^3J_{H-H} = 6.3$ Hz, 2H), 2.38 – 2.02 (m, 3H), 1.95 – 1.63 (m, 2H).

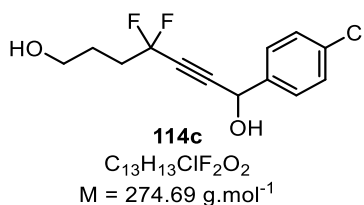
$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 163.0 (d, $^1J_{C-F} = 247.7$ Hz), 135.0 (dt, $^4J_{C-F} = 3.3$ Hz, $^5J_{C-F} = 1.6$ Hz), 128.6 (d, $^3J_{C-F} = 8.5$ Hz), 115.9 (d, $^2J_{C-F} = 21.8$ Hz), 114.9 (t, $^1J_{C-F} = 233.0$ Hz), 86.9 (t, $^3J_{C-F} = 6.8$ Hz), 79.5 (t, $^2J_{C-F} = 41.2$ Hz), 63.5 (t, $^4J_{C-F} = 2.0$ Hz), 61.6, 35.8 (t, $^2J_{C-F} = 26.4$ Hz), 26.0 (t, $^3J_{C-F} = 3.4$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -83.16 (s), -112.91 (s).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($C_{13}H_{13}O_2F_3\text{Na}$) = 281.0760; found: 281.0762 (1 ppm).

15.2. 1-(4-chlorophenyl)-4,4-difluorohept-2-yne-1,7-diol 114c

The reaction was performed according to the general procedure 4. Yellow oil, yield 496 mg (86%).



$R_f = 0.2$ (hexane / Ethyl Acetate: 7/3).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.44 – 7.36 (m, 2H), 7.36 – 7.30 (m, 2H), 5.44 (t, $^3J_{H-H} = 4.0$ Hz, 1H), 3.64 (t, $^3J_{H-H} = 6.3$ Hz, 2H), 3.38 (bs, 1H), 2.35 – 2.05 (m, 2H), 1.91 – 1.61 (m, 2H).

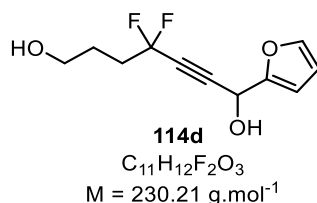
$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 137.6, 134.7, 129.0, 128.1, 114.8 (t, $^1J_{C-F} = 232.9$ Hz), 86.8 (t, $^3J_{C-F} = 6.8$ Hz), 79.4 (t, $^2J_{C-F} = 41.1$ Hz), 63.3 (t, $^4J_{C-F} = 1.9$ Hz), 61.4, 35.7 (t, $^2J_{C-F} = 26.4$ Hz), 25.9 (t, $^3J_{C-F} = 3.5$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -83.07 (s).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($C_{13}H_{13}O_2F_2^{35}\text{ClNa}$) = 297.0464; found: 297.0467 (1 ppm).

15.3. 4,4-difluoro-1-(furan-2-yl)hept-2-yne-1,7-diol 114d

The reaction was performed according to the general procedure 4. Black oil, yield 435 mg (90%).



R_f = 0.1 (hexane / Ethyl Acetate: 7/3).

¹H NMR (300 MHz, CDCl₃) δ 7.43 (dd, ³J_{H-H} = 1.8 Hz, ⁴J_{H-H} = 0.9 Hz, 1H), 6.46 (dt, ³J_{H-H} = 3.3 Hz, ⁴J_{H-H} = 0.8 Hz, 1H), 6.37 (dd, ³J_{H-H} = 3.3 Hz, ³J_{H-H} = 1.9 Hz, 1H), 5.53 (t, ³J_{H-H} = 3.9 Hz, 1H), 3.71 (t, ³J_{H-H} = 6.3 Hz, 2H), 2.33 – 2.04 (m, 3H), 1.94 – 1.75 (m, 2H).

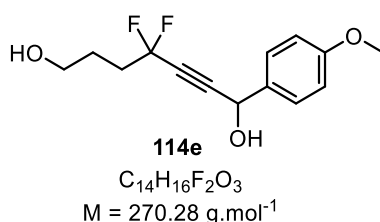
¹³C NMR (75 MHz, CDCl₃) δ 151.5 (t, ⁵J_{C-F} = 1.9 Hz), 143.6, 114.8 (t, ¹J_{C-F} = 233.1 Hz), 110.7, 108.5, 84.7 (t, ³J_{C-F} = 6.8 Hz), 78.5 (t, ²J_{C-F} = 41.3 Hz), 61.6, 57.8 (t, ⁴J_{C-F} = 1.8 Hz), 35.8 (t, ²J_{C-F} = 26.3 Hz), 26.0 (t, ³J_{C-F} = 3.5 Hz).

¹⁹F {H} NMR (282 MHz, CDCl₃) δ -83.55.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₁H₁₂O₃F₂Na) = 253.0647; found: 253.0647 (0 ppm).

15.4. 4,4-difluoro-1-(4-methoxyphenyl)hept-2-yne-1,7-diol 114e

The reaction was performed according to the general procedure 4. Colorless oil, yield 540 mg (95%).



R_f = 0.3 (Petroleum Ether / Ethyl Acetate: 6/4).

¹H NMR (300 MHz, CDCl₃) δ 7.50 – 7.35 (m, 2H), 6.97 – 6.87 (m, 2H), 5.48 (t, ³J_{H-H} = 4.0 Hz, 1H), 3.82 (s, 3H), 3.71 (t, ³J_{H-H} = 6.3 Hz, 2H), 2.32 – 2.06 (m, 2H), 1.90 (bs, 2H, OH), 1.89 – 1.76 (m, 2H).

¹³C NMR (75 MHz, CDCl₃) δ 160.2, 131.4, 128.3, 114.9 (t, ¹J_{C-F} = 232.8 Hz), 114.3, 87.1 (t, ³J_{C-F} = 6.8 Hz), 79.3 (t, ²J_{C-F} = 41.0 Hz), 64.0 (t, ⁴J_{C-F} = 2.0 Hz), 61.7, 55.5, 35.9 (t, ²J_{C-F} = 26.4 Hz), 26.1 (t, ³J_{C-F} = 3.5 Hz).

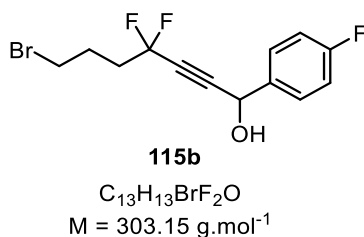
¹⁹F {H} NMR (471 MHz, CDCl₃) δ -83.13 (s).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₄H₁₆O₃F₂Na) = 293.0960; found: 293.0963 (1 ppm).

16. General procedure 5 for the synthesis of bromo- compounds 115

16.1. Representative procedure: 7-bromo-4,4-difluoro-1-(4-fluorophenyl)hept-2-yn-1-ol **115b**

Triphenylphosphine (460 mg, 1.76 mmol) in dichloromethane (5 mL) was added dropwise to a vigorously stirred mixture of alcohol **114b** (350 mg, 1.36 mmol) with carbon tetrabromide (585 mg, 1.76 mmol) in dichloromethane (10 mL) at 0 °C under nitrogen. Petroleum ether was added, and the precipitate was then filtered off. Brominated compound **115b** was separated by column chromatography to obtain a yellow oil (383 mg, 88%).



R_f = 0.6 (Hexane / Ethyl Acetate: 8/2).

¹H NMR (300 MHz, CDCl₃) δ 7.55 – 7.41 (m, 2H), 7.19 – 6.99 (m, 2H), 5.52 (t, ³J_{H-H} = 3.9 Hz, 1H), 3.45 (t, ³J_{H-H} = 6.3 Hz, 2H), 2.69 (bs, 1H), 2.50 – 1.92 (m, 4H).

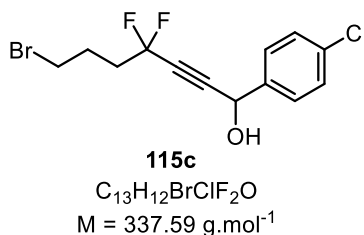
¹³C NMR (75 MHz, CDCl₃) δ 163.1 (d, ¹J_{C-F} = 248.0 Hz), 134.8 (dt, ⁴J_{C-F} = 3.3 Hz, ⁵J_{C-F} = 1.6 Hz), 128.6 (d, ³J_{C-F} = 8.4 Hz), 115.9 (d, ²J_{C-F} = 21.7 Hz), 114.2 (t, ¹J_{C-F} = 233.6 Hz), 86.9 (t, ³J_{C-F} = 6.7 Hz), 79.3 (t, ²J_{C-F} = 41.0 Hz), 63.6 (t, ⁴J_{C-F} = 1.9 Hz), 37.9 (t, ²J_{C-F} = 26.6 Hz), 32.2, 26.2 (t, ³J_{C-F} = 3.7 Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -83.23 (s), -112.63(s).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₃H₁₂OF₃⁷⁹BrNa) = 342.9915; found: 342.9915 (0 ppm).

16.2. 7-bromo-1-(4-chlorophenyl)-4,4-difluorohept-2-yn-1-ol **115c**

The reaction was performed according to the general procedure 5. Yellow oil, yield 262 mg (61%) from 350 mg (1.27 mmol) of **114c**.



R_f = 0.6 (Hexane / Ethyl Acetate: 8/2).

^1H NMR (300 MHz, CDCl_3) δ 7.50 – 7.41 (m, 2H), 7.41 – 7.27 (m, 2H), 5.52 (t, $^3J_{\text{H-H}} = 3.2$ Hz, 1H), 3.45 (t, $^3J_{\text{H-H}} = 6.3$ Hz, 2H), 2.70 (bs, 1H), 2.38 – 2.20 (m, 2H), 2.16 – 1.99 (m, 2H).

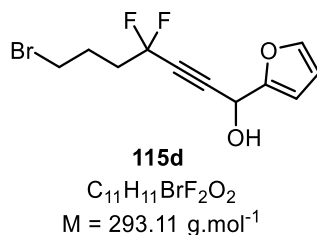
^{13}C NMR (75 MHz, CDCl_3) δ 137.5 (t, $^5J_{\text{C-F}} = 1.5$ Hz), 134.9, 129.2, 128.1, 114.2 (t, $^1J_{\text{C-F}} = 233.6$ Hz), 86.7 (t, $^3J_{\text{C-F}} = 6.7$ Hz), 79.4 (t, $^2J_{\text{C-F}} = 41.0$ Hz), 63.6 (t, $^4J_{\text{C-F}} = 1.9$ Hz), 37.9 (t, $^2J_{\text{C-F}} = 26.6$ Hz), 32.2, 26.1 (t, $^3J_{\text{C-F}} = 3.7$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -83.28 (s).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{13}\text{H}_{12}\text{OF}_2^{35}\text{Cl}^{79}\text{BrNa}$) = 358.9620; found: 358.9621 (0 ppm).

16.3. 7-bromo-4,4-difluoro-1-(furan-2-yl)hept-2-yn-1-ol **115d**

The reaction was performed according to the general procedure 5. Black oil, yield 227 mg (51%) from 350 mg (1.52 mmol) of **114d**.



R_f = 0.5 (Hexane / Ethyl Acetate: 8/2).

^1H NMR (300 MHz, CDCl_3) δ 7.44 (dd, $^3J_{\text{H-H}} = 1.9$ Hz, $^4J_{\text{H-H}} = 0.9$ Hz, 1H), 6.47 (dt, $^3J_{\text{H-H}} = 3.3$ Hz, $^4J_{\text{H-H}} = 0.8$ Hz, 1H), 6.38 (dd, $^3J_{\text{H-H}} = 3.3$ Hz, $^3J_{\text{H-H}} = 1.8$ Hz, 1H), 5.54 (t, $^3J_{\text{H-H}} = 3.9$ Hz, 1H), 3.46 (t, $^3J_{\text{H-H}} = 6.2$ Hz, 2H), 2.81 (bs, 1H), 2.42 – 2.02 (m, 4H).

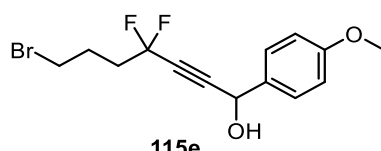
^{13}C NMR (75 MHz, CDCl_3) δ 151.2 (t, $^5J_{\text{C-F}} = 2.0$ Hz), 143.6, 114.1 (t, $^1J_{\text{C-F}} = 233.6$ Hz), 110.7, 108.6, 84.8 (t, $^3J_{\text{C-F}} = 6.9$ Hz), 78.3 (t, $^2J_{\text{C-F}} = 41.1$ Hz), 57.8 (t, $^4J_{\text{C-F}} = 2.0$ Hz), 37.9 (t, $^2J_{\text{C-F}} = 26.5$ Hz), 32.2, 26.1 (t, $^3J_{\text{C-F}} = 3.8$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -83.5 (s).

HRMS (ESI): calcd. for $[\text{M}+\text{H}]^+$ ($\text{C}_{11}\text{H}_{12}\text{O}_2\text{F}_2^{81}\text{Br}$) = 294.9962; found: 294.9962 (0 ppm).

16.4. 7-bromo-4,4-difluoro-1-(4-methoxyphenyl)hept-2-yn-1-ol **115e**

The reaction was performed according to the general procedure 5. Pale yellow oil, yield 332 mg (77%) from 350 mg (1.29 mmol) of **114e**.



115e

$C_{14}H_{15}BrF_2O_2$

$M = 333.17 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.4$ (Petroleum Ether / Ethyl Acetate: 8/2).

$^1\text{H NMR}$ (300 MHz, Chloroform-*d*) δ 7.46 – 7.39 (m, 2H), 6.96 – 6.89 (m, 2H), 5.49 (t, $^3J_{H-H} = 4.0$ Hz, 1H), 3.82 (s, 3H), 3.46 (t, $^3J_{H-H} = 6.3$ Hz, 2H), 2.36 – 2.07 (m, 5H).

$^{13}\text{C NMR}$ (75 MHz, Chloroform-*d*) δ 160.2, 131.3 (t, $^5J_{C-F} = 1.6$ Hz), 128.3, 114.4, 114.3 (t, $^1J_{C-F} = 233.3$ Hz), 87.4 (t, $^3J_{C-F} = 6.7$ Hz), 79.0 (t, $^2J_{C-F} = 40.8$ Hz), 64.0 (t, $^4J_{C-F} = 2.0$ Hz), 55.5, 38.0 (t, $^2J_{C-F} = 26.7$ Hz), 32.3, 26.2 (t, $^3J_{C-F} = 3.7$ Hz).

^{19}F {H} NMR (471 MHz, CDCl_3) δ -83.08 (s).

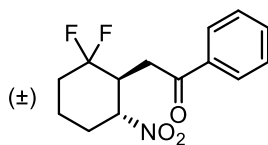
HRMS (ESI): calcd. for $[M+\text{Na}]^+$ ($C_{14}H_{15}O_2F_2^{81}\text{BrNa}$) = 357.0095; found: 357.0095 (0 ppm).

17. General procedure 6 for the synthesis of cyclic nitro compounds

118

17.1. Representative procedure: 2-(2,2-difluoro-6-nitrocyclohexyl)-1-phenylethan-1-one **118a**

To the brominated compound **115a** (200 mg, 0.66 mmol) was added nitromethane (71 μL , 1.31 mmol) and DBU (395 μL , 2.63 mmol) in THF (3 mL). The reaction mixture was stirred at room temperature overnight. At the end saturated NH_4Cl (10 mL) was added and the reaction mixture was extracted with ethyl acetate (3x20 mL). The organic layers were separated, washed with water (1x10 mL), dried over Na_2SO_4 and concentrated under vacuum. After purification by chromatography on silica gel, **118a** (142 mg, 76%) was obtained as white solid.



118a

$C_8H_{10}ArF_2NO_3$

$M = 246.12 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.5$ (Hexane / Ethyl Acetate: 9/1). **mp** = 93 $^\circ\text{C}$.

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.02 – 7.86 (m, 2H), 7.64 – 7.52 (m, 1H), 7.52 – 7.39 (m, 2H), 4.55 (td, $^3J_{H-H} = 11.6$ Hz, $^3J_{H-H} = 4.0$ Hz, 1H), 3.56 (dd, $^2J_{H-H} = 17.8$ Hz, $^3J_{H-H} = 5.6$ Hz, 1H), 3.51 – 3.36 (m, 1H), 2.97 (dd, $^2J_{H-H} = 17.8$ Hz, $^3J_{H-H} = 4.3$ Hz, 1H), 2.43 – 1.61 (m, 6H).

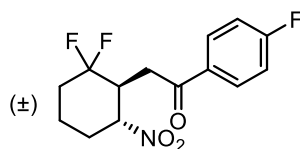
¹³C NMR (75 MHz, CDCl₃) δ 195.8, 136.4, 133.5, 128.8, 128.3, 122.9 (dd, ¹J_{C-F} = 249.5 Hz, ¹J_{C-F} = 242.4 Hz), 87.0 (d, ³J_{C-F} = 8.5 Hz), 42.6 (dd, ²J_{C-F} = 24.4, ²J_{C-F} = 22.2 Hz), 34.0 (t, ³J_{C-F} = 2.4 Hz), 33.2 (dd, ²J_{C-F} = 25.0 Hz, ²J_{C-F} = 22.3 Hz), 30.2 (d, ⁴J_{C-F} = 1.6 Hz), 19.3 (d, ³J_{C-F} = 10.2 Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -95.5 (AB system, ²J_{F-F} = 246.6 Hz), -108.2 (AB system, ²J_{F-F} = 246.6 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₄H₁₅NO₃F₂Na) = 306.0912; found: 306.0914 (1 ppm).

17.2. 2-(2,2-difluoro-6-nitrocyclohexyl)-1-(4-fluorophenyl)ethan-1-one **118b**

The reaction was performed according to the general procedure 6. White solid, yield 124 mg (66%) from 200 mg (0.62 mmol) of **115b**.



118b
C₁₄H₁₄F₃NO₃
M = 301.27 g.mol⁻¹

R_f = 0.5 (Hexane / Ethyl Acetate: 9/1). **mp** = 89 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.04 – 7.90 (m, 2H), 7.19 – 7.00 (m, 2H), 4.53 (td, ³J_{H-H} = 11.6 Hz, ³J_{H-H} = 4.0 Hz, 1H), 3.51 (dd, ²J_{H-H} = 17.6 Hz, ³J_{H-H} = 5.5 Hz, 1H), 3.47 – 3.32 (m, 1H), 2.94 (dd, ²J_{H-H} = 17.6 Hz, ³J_{H-H} = 4.4 Hz, 1H), 2.42 – 1.59 (m, 6H).

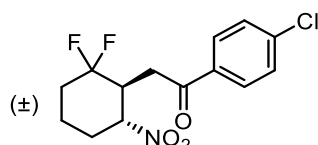
¹³C NMR (75 MHz, CDCl₃) δ 194.2, 166.0 (d, ¹J_{C-F} = 255.3 Hz), 132.8 (d, ⁴J_{C-F} = 3.1 Hz), 131.0 (d, ³J_{C-F} = 9.5 Hz), 122.9 (dd, ¹J_{C-F} = 249.4 Hz, ¹J_{C-F} = 242.5 Hz), 115.9 (d, ²J_{C-F} = 22.0 Hz), 86.9 (d, ³J_{C-F} = 8.4 Hz), 42.7 (dd, ²J_{C-F} = 24.4 Hz, ²J_{C-F} = 22.2 Hz), 33.9 (t, ³J_{C-F} = 2.4 Hz), 33.2 (dd, ²J_{C-F} = 24.9 Hz, ²J_{C-F} = 22.2 Hz), 30.1 (d, ⁴J_{C-F} = 1.7 Hz), 19.2 (d, ³J_{C-F} = 10.2 Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -95.51 (d, ²J_{F-F} = 246.3 Hz), -104.61 (s), -108.22 (d, ²J_{F-F} = 246.4 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₄H₁₄NO₃F₃Na) = 324.0818; found: 324.0822 (1 ppm).

17.3. 1-(4-chlorophenyl)-2-(2,2-difluoro-6-nitrocyclohexyl)ethan-1-one **118c**

The reaction was performed according to the general procedure 6. White solid, yield 132 mg (70%) from 200 mg (0.59 mmol) of **115c**.



118c

$C_{14}H_{14}ClF_2NO_3$

$M = 317.72 \text{ g.mol}^{-1}$

$R_f = 0.5$ (Hexane / Ethyl Acetate: 9/1). $mp = 98 \text{ }^\circ\text{C}$.

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.91 – 7.83 (m, 2H), 7.47 – 7.39 (m, 2H), 4.52 (td, $^3J_{H-H} = 11.7$ Hz, $^3J_{H-H} = 4.1$ Hz, 1H), 3.50 (dd, $^2J_{H-H} = 17.6$ Hz, $^3J_{H-H} = 5.5$ Hz, 1H), 3.46 – 3.32 (m, 1H), 2.94 (dd, $^2J_{H-H} = 17.6$ Hz, $^3J_{H-H} = 4.3$ Hz, 1H), 2.40 – 1.62 (m, 6H).

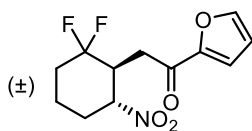
$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 194.7, 140.0, 134.7, 129.7, 129.1, 122.9 (dd, $^1J_{C-F} = 249.4$ Hz, $^1J_{C-F} = 242.4$ Hz), 86.9 (d, $^3J_{C-F} = 8.4$ Hz), 42.7 (dd, $^2J_{C-F} = 24.4$ Hz, $^2J_{C-F} = 22.2$ Hz), 33.9 (t, $^3J_{C-F} = 2.4$ Hz), 33.2 (dd, $^2J_{C-F} = 25.0$ Hz, $^2J_{C-F} = 22.2$ Hz), 30.2 (d, $^4J_{C-F} = 1.6$ Hz), 19.2 (d, $^3J_{C-F} = 10.3$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -95.43 (AB system, $^2J_{F-F} = 246.4$ Hz), -108.22 (AB system, $^2J_{F-F} = 246.4$ Hz).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($C_{14}H_{14}NO_3F_2^{35}\text{ClNa}$) = 340.0522; found: 340.0520 (1 ppm).

17.4. 2-(2,2-difluoro-6-nitrocyclohexyl)-1-(furan-2-yl)ethan-1-one 118d

The reaction was performed according to the general procedure 6. White solid, yield 97 mg (52%) from 200 mg (0.68 mmol) of **115d**.



118d

$C_{12}H_{13}F_2NO_4$

$273.24 \text{ g.mol}^{-1}$

$R_f = 0.3$ (Hexane / Ethyl Acetate: 9/1). $mp = 98 \text{ }^\circ\text{C}$.

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.58 (dd, $^3J_{H-H} = 1.7$ Hz, $^4J_{H-H} = 0.8$ Hz, 1H), 7.22 (dd, $^3J_{H-H} = 3.6$ Hz, $^4J_{H-H} = 0.8$ Hz, 1H), 6.54 (dd, $^3J_{H-H} = 3.6$ Hz, $^3J_{H-H} = 1.7$ Hz, 1H), 4.54 (td, $^3J_{H-H} = 11.5$ Hz, $^3J_{H-H} = 4.0$ Hz, 1H), 3.49 – 3.19 (m, 2H), 2.89 – 2.76 (m, 1H), 2.40 – 1.54 (m, 6H).

$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 184.8, 152.1, 146.7, 122.8 (dd, $^1J_{C-F} = 249.4$ Hz, $^1J_{C-F} = 242.5$ Hz), 117.6, 112.6, 86.9 (d, $^3J_{C-F} = 8.4$ Hz), 42.2 (dd, $^2J_{C-F} = 24.5$ Hz, $^2J_{C-F} = 22.3$ Hz), 33.6 (t,

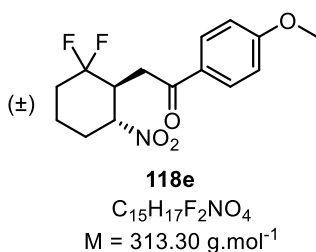
$^3J_{C-F} = 2.5$ Hz), 33.2 (dd, $^2J_{C-F} = 25.0$ Hz, $^2J_{C-F} = 22.3$ Hz), 30.3 (d, $^4J_{C-F} = 1.5$ Hz), 19.3 (d, $^3J_{C-F} = 10.2$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -95.46 (AB system, $^2J_{F-F} = 246.8$ Hz), -108.40 (d, $^2J_{F-F} = 246.8$ Hz).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{12}\text{H}_{13}\text{NO}_4\text{F}_2\text{Na}$) = 296.0705; found: 296.0708 (1 ppm).

17.5. 2-(2,2-difluoro-6-nitrocyclohexyl)-1-(4-methoxyphenyl)ethan-1-one **118e**

The reaction was performed according to the general procedure 6. White solid, yield 92 mg (49%) from 200 mg (0.60 mmol) of **115e**.



R_f = 0.4 (Petroleum Ether / Ethyl Acetate: 8/2). **mp** = 125 °C.

^1H NMR (300 MHz, CDCl_3) δ = 7.97 – 7.89 (m, 2H), 6.99 – 6.86 (m, 2H), 4.61 – 4.45 (m, 1H), 3.86 (s, 3H), 3.58 – 3.29 (m, 2H), 2.42 – 1.52 (m, 6H).

^{13}C NMR (75 MHz, CDCl_3) δ 194.2, 163.8, 130.6, 129.4, 122.9 (dd, $^1J_{C-F} = 249.7$ Hz, $^1J_{C-F} = 249.5$ Hz), 113.9, 87.0 (d, $^3J_{C-F} = 8.4$ Hz), 55.6, 42.6 (dd, $^2J_{C-F} = 24.4$ Hz, $^2J_{C-F} = 22.2$ Hz), 33.5 (t, $^3J_{C-F} = 2.3$ Hz), 33.2 (dd, $^2J_{C-F} = 25.1$ Hz, $^2J_{C-F} = 22.4$ Hz), 30.1 (d, $^4J_{C-F} = 1.6$ Hz), 19.3 (dd, $^3J_{C-F} = 10.2$ Hz, $^3J_{C-F} = 0.5$ Hz).

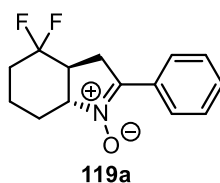
^{19}F {H} NMR (471 MHz, CDCl_3) δ -95.60 (AB system, $^2J_{F-F} = 246.2$ Hz), -108.21 (AB system, $^2J_{F-F} = 246.6$ Hz).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{15}\text{H}_{17}\text{NO}_4\text{F}_2\text{Na}$) = 336.1018; found: 336.1022 (1 ppm).

18. Synthesis of fused cyclic nitrones **119**

18.1. Synthesis of 4,4-difluoro-2-phenyl-3a,4,5,6,7,7a-hexahydro-3H-indole 1-oxide **119a**

According to the general procedure 3. White solid, yield 37 mg (56%) from 75 mg (0.26 mmol) of **118a**.



119a

$C_{14}H_{15}F_2NO$

$M = 251.28 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.2$ (Hexane / Ethyl Acetate: 4/6). $mp = 206 \text{ }^\circ\text{C}$.

^1H NMR (300 MHz, CDCl_3) δ 8.38 – 8.18 (m, 2H), 7.52 – 7.33 (m, 3H), 3.97 (t, $^3J_{H-H} = 11.9$ Hz, 1H), 3.23 – 3.01 (m, 2H), 2.64 – 2.49 (m, 1H), 2.45 – 2.17 (m, 2H), 2.16 – 1.45 (m, 4H).

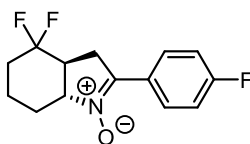
^{13}C NMR (75 MHz, CDCl_3) δ 140.1, 130.5, 129.3, 128.7, 127.2, 122.3 (dd, $^1J_{C-F} = 246.9$ Hz, $^1J_{C-F} = 245.3$ Hz), 73.4 (d, $^3J_{C-F} = 9.0$ Hz), 46.9 (dd, $^2J_{C-F} = 25.5$ Hz, $^2J_{C-F} = 22.6$ Hz), 34.2 (dd, $^2J_{C-F} = 25.0$ Hz, $^2J_{C-F} = 21.9$ Hz), 27.8, 26.5 (d, $^4J_{C-F} = 1.6$ Hz), 21.2 (d, $^3J_{C-F} = 9.5$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -99.88 (AB system, $^2J_{F-F} = 234.0$ Hz), -113.99 (AB system, $^2J_{F-F} = 234.0$ Hz).

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($C_{14}H_{15}NOF_2Na$) = 274.1013; found: 274.1013 (0 ppm).

18.2. 4,4-difluoro-2-(4-fluorophenyl)-3a,4,5,6,7,7a-hexahydro-3H-indole 1-oxide 119b

According to the general procedure 3. Pale yellow solid, yield 47 mg (70%) from 75 mg (0.25 mmol) of **118b**.



119b

$C_{14}H_{14}F_3NO$

$M = 269.27 \text{ g}\cdot\text{mol}^{-1}$

$R_f = 0.1$ (Hexane / Ethyl Acetate: 4/6). $mp = 216 \text{ }^\circ\text{C}$.

^1H NMR (300 MHz, CDCl_3) δ 8.41 – 8.27 (m, 2H), 7.19 – 7.06 (m, 2H), 3.95 (t, $^3J_{H-H} = 11.9$ Hz, 1H), 3.24 – 2.92 (m, 2H), 2.63 – 2.49 (m, 1H), 2.45 – 2.13 (m, 2H), 2.10 – 1.43 (m, 4H).

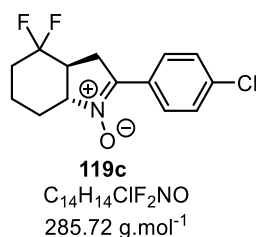
^{13}C NMR (75 MHz, CDCl_3) δ 163.4 (d, $^1J_{C-F} = 252.8$ Hz), 139.1, 129.5 (d, $^3J_{C-F} = 8.3$ Hz), 125.8 (d, $^4J_{C-F} = 3.3$ Hz), 122.3 (dd, $^1J_{C-F} = 246.9$ Hz, $^1J_{C-F} = 245.4$ Hz), 115.8 (d, $^2J_{C-F} = 21.7$ Hz), 73.3 (d, $^3J_{C-F} = 8.9$ Hz), 46.8 (dd, $^2J_{C-F} = 25.5$ Hz, $^2J_{C-F} = 22.6$ Hz), 34.2 (dd, $^2J_{C-F} = 25.0$ Hz, $^2J_{C-F} = 21.9$ Hz), 27.8, 26.5 (d, $^4J_{C-F} = 1.7$ Hz), 21.1 (d, $^3J_{C-F} = 9.6$ Hz).

^{19}F {H} NMR (282 MHz, CDCl_3) δ -99.90 (AB system, $^2J_{F-F} = 234.4$ Hz), -107.81 (s), -114.08 (AB system, $^2J_{F-F} = 234.4$ Hz).

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{14}H_{14}NOF_3Na$) = 292.0919; found: 292.0918 (1 ppm).

18.3. 2-(4-chlorophenyl)-4,4-difluoro-3a,4,5,6,7,7a-hexahydro-3H-indole 1-oxide 119c

According to the general procedure 3. Yellow solid, yield 40 mg (60%) from 75 mg (0.24 mmol) of **25c**.



R_f = 0.2 (Hexane / Ethyl Acetate: 4/6). **mp** = 208 °C.

¹H NMR (300 MHz, CDCl₃) δ 8.40 – 8.18 (m, 2H), 7.47 – 7.30 (m, 2H), 3.95 (t, ³J_{H-H} = 12.0 Hz, 1H), 3.22 – 2.97 (m, 2H), 2.64 – 2.49 (m, 1H), 2.43 – 1.97 (m, 3H), 1.90 – 1.46 (m, 3H).

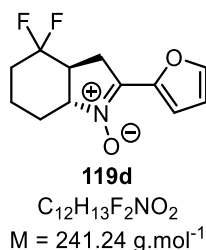
¹³C NMR (75 MHz, CDCl₃) δ 139.0, 136.0, 128.9, 128.4, 127.8, 122.2 (dd, ¹J_{C-F} = 246.9 Hz, ¹J_{C-F} = 245.4 Hz), 73.5 (d, ³J_{C-F} = 9.0 Hz), 46.8 (dd, ²J_{C-F} = 25.5 Hz, ²J_{C-F} = 22.6 Hz), 34.2 (dd, ²J_{C-F} = 25.0 Hz, ²J_{C-F} = 21.9 Hz), 27.6, 26.4 (d, ⁴J_{C-F} = 1.6 Hz), 21.1 (d, ³J_{C-F} = 9.5 Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -99.90 (AB system, ²J_{F-F} = 234.2 Hz), -113.97 (AB system, ²J_{F-F} = 234.2 Hz).

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{14}H_{14}NOF_2^{35}ClNa$) = 308.0624; found: 308.0627 (1 ppm).

18.4. 4,4-difluoro-2-(furan-2-yl)-3a,4,5,6,7,7a-hexahydro-3H-indole 1-oxide 119d

According to the general procedure 3. Yellow solid, yield 53 mg (80%) from 75 mg (0.27 mmol) of **25d**.



R_f = 0.1 (Hexane / Ethyl Acetate: 4/6). **mp** = 213 °C.

¹H NMR (300 MHz, CDCl₃) δ 7.78 (d, ³J_{H-H} = 3.5 Hz, 1H), 7.49 (d, ³J_{H-H} = 1.7 Hz, 1H), 6.58 (dd, ³J_{H-H} = 3.5 Hz, ³J_{H-H} = 1.8 Hz, 1H), 3.88 (t, ³J_{H-H} = 12.1 Hz, 1H), 3.21 (dd, ²J_{H-H} = 16.1 Hz, ³J_{H-H} = 6.6 Hz, 1H), 3.03 (ddd, ²J_{H-H} = 16.1 Hz, ³J_{H-H} = 11.1 Hz, ⁴J_{H-F} = 2.8 Hz, 1H), 2.62 – 2.48 (m, 1H), 2.46 – 2.00 (m, 3H), 1.94 – 1.40 (m, 3H).

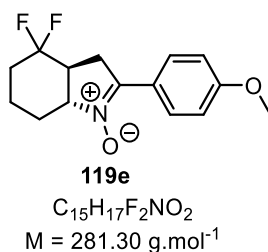
¹³C NMR (75 MHz, CDCl₃) δ 145.4, 143.7, 133.7, 122.2 (dd, ¹J_{C-F} = 247.0 Hz, ¹J_{C-F} = 245.4 Hz), 114.7, 112.7, 72.0 (d, ³J_{C-F} = 9.1 Hz), 47.6 (dd, ²J_{C-F} = 25.5 Hz, ²J_{C-F} = 22.6 Hz), 34.2 (dd, ²J_{C-F} = 25.1 Hz, ²J_{C-F} = 21.9 Hz), 26.3 (d, ³J_{C-F} = 1.6 Hz), 26.0 (d, ⁴J_{C-F} = 1.0 Hz), 21.2 (d, ³J_{C-F} = 9.6 Hz).

¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ -99.8 (AB system, ²J_{F-F} = 234.7 Hz), -113.7 (AB system, ²J_{F-F} = 234.7 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₂H₁₃NO₂F₂Na) = 264.0807; found: 264.0810 (1 ppm).

18.5. 4,4-difluoro-2-(4-methoxyphenyl)-3a,4,5,6,7,7a-hexahydro-3H-indole 1-oxide 119e

According to the general procedure 3. White solid, yield 51 mg (75%) from 75 mg (0.24 mmol) of **118e**.



R_f = 0.1 (Petroleum Ether / Ethyl Acetate: 5/5). **mp** = 201 °C.

¹H NMR (500 MHz, CDCl₃) δ 8.44 – 8.25 (m, 2H), 7.06 – 6.78 (m, 2H), 4.05 – 3.78 (m, 1H), 3.86 (s, 3H), 3.33 – 2.97 (m, 2H), 2.75 – 2.48 (m, 1H), 2.40 – 2.03 (m, 3H), 1.92 – 1.49 (m, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 161.3, 140.8, 129.4, 127.4, 122.4 (t, ¹J_{C-F} = 246.2 Hz), 114.0, 73.1, 55.5, 47.0 (t, ²J_{C-F} = 23.8 Hz), 34.3 (dd, ²J_{C-F} = 25.2 Hz, ²J_{C-F} = 21.8 Hz), 27.7, 26.6, 21.2 (d, ³J_{C-F} = 9.5 Hz).

¹⁹F {¹H} NMR (471 MHz, CDCl₃) δ -99.85 (AB system, ²J_{F-F} = 234.0 Hz), -113.96 (AB system, ²J_{F-F} = 234.0 Hz).

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₅H₁₇NO₂F₂Na) = 304.1120; found: 304.1123 (1 ppm).

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PART II
Medicinal Chemistry

CHAPTER IV
**Synthesis of New Inhibitors of Anti-
Apoptotic Proteins to be Developed as
Anti-Cancer Agents**

1. Introduction

Since the first cases of Coronavirus 2019 (COVID-19) were reported in China in early December and the virus subsequently spread to the whole world (over than 219 countries), leading to a lockdown in many of them with more than 44 million confirmed cases including 1.16 million deaths as reported by World Health Organization (WHO) by the end of October 2020 (Figure C4. 1).¹ This pandemic had a wide effects not only on the global economy and health but also on society, hospitality and medical care mainly on cancer care.²

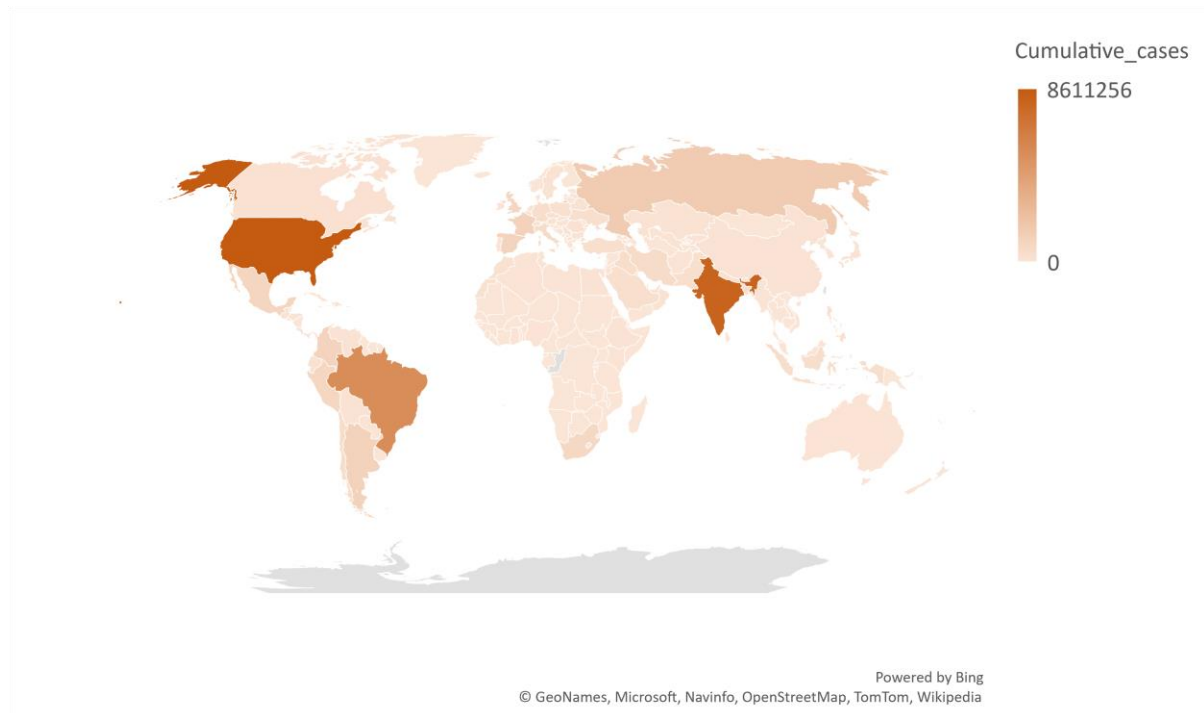


Figure C4. 1: WHO Coronavirus Disease (COVID-19) Dashboard last updated 28/Oct/2020

Despite advances in the field of anticancer strategies over the last decade, cancer malignancies remain responsible for 8.7 million death in 2015 worldwide being the second leading cause of death behind cardiovascular diseases.³ The GLOBOCAN 2018 database reported increased global cancer to 9.6 million deaths with 18.1 million new cases in 2018. This is anticipated to escalate at 70% by 2030.⁴ The war on cancer is becoming more challenging with the current situation, especially with the exponential increase of COVID-19 cases which triggers scientist to find fast and efficient cure for both Covid-19 and cancer malignancies.⁵

Cancer is a disease involving the abnormal accumulation of cells resulting from an imbalance of proliferation and programmed cell death such as the phenomenon of apoptosis.⁶ It involves complex networks of protein-protein interaction that dictate survival or cell death, so these interactions are substantial for the regulation of control points of cellular homeostasis.⁷

In this context, the phenomenon of apoptosis will be discussed briefly, Bcl-2 family of proteins and especially Mcl-1 and their role in apoptosis will be described and distinguished, then MIM-1 compound which showed an interesting interaction with the binding groove on the surface of Mcl-1 as well as a promising bioactivity will be revised and represented. Indeed, it has been an interesting starting molecule to our research group for the design and development of several new series of analogs.

1.1. Apoptosis

A genetically controlled normal physiological process of programmed cell death (PCD) is responsible for modulating the cellular population of multicellular organisms both quantitatively and qualitatively during embryonic development and tissue homeostasis. Moreover, apoptosis is essential for maintaining the health of the body by eliminating old, unnecessary and unhealthy cells.⁷

The term “apoptosis” was first described by Kerr and Wyllie in 1972,⁸ where they defined the morphological and biochemical bases of this inherently programmed phenomenon, in addition they showed that it can be initiated or inhibited by a variety of environmental stimuli, both physiological and pathological. However, some concepts of apoptosis were evident many years before yet under different illustrations.⁹

Thus, several studies of the nematode *Caenorhabditis elegans* have greatly contributed to a better understanding of the mechanisms of apoptosis. In fact, a *C. elegans* hermaphrodite generates 1090 somatic cell nuclei during its development, 131 of which gradually undergo apoptosis leaving 959 cells by the end of embryonic development.¹⁰

Genetic studies have identified 11 genes that define a genetic pathway for programmed cell death in *C. elegans*,¹¹ four genes among them were found to be critical in the apoptotic regulation, so called *egl-1*, *ced-3*, *ced-4* and *ced-9*. *Ced-3* and *ced-4* have been identified as essential in the occurrence of cell death (pro-apoptotic). Mutagenesis work has shown that if one of these genes is inactivated, then cells destined for death survive and differentiate into neurons.¹² The activity of both *ced-3* and *ced-4* is regulated by a third gene *ced-9* which has an antagonist function generated by its protein and thereby protects cells that should survive from any accidental activation of the death program. In addition to the presence of a fourth gene acting as apoptosis inducer called *egl-1*.¹³ Relying on the fact that these genes and their products are activated or inhibited will determine whether a cell will stay alive or will undergo apoptosis.

The mechanism of apoptosis is remarkably conserved between *C. elegans* and mammals (Figure C4. 2), albeit with the expected greater complexity in mammals. It became clear that they encode components of a universal and highly conserved death machinery: CED-9 and Bcl-2 proved to be functional and structural homologs,¹⁴ and their survival function is opposed either by close relatives such as Bax,¹⁵ or by distant cousins such as mammalian Bik (also known as Nbk) and nematode EGL-1.¹⁶ The execution phase was illuminated when CED-3 proved to belong to a family of cysteine proteases, now called caspases (c-asp-ases: cysteine dependent aspartate-specific protease), whose sequential activation and cleavage of key target proteins dismantles the cell.¹⁷ The long-mysterious CED-4 and its mammalian homolog Apaf-1¹⁸ are now recognized to be adapters for facilitating the autocatalysis that initiates the proteolytic cascade.¹⁷

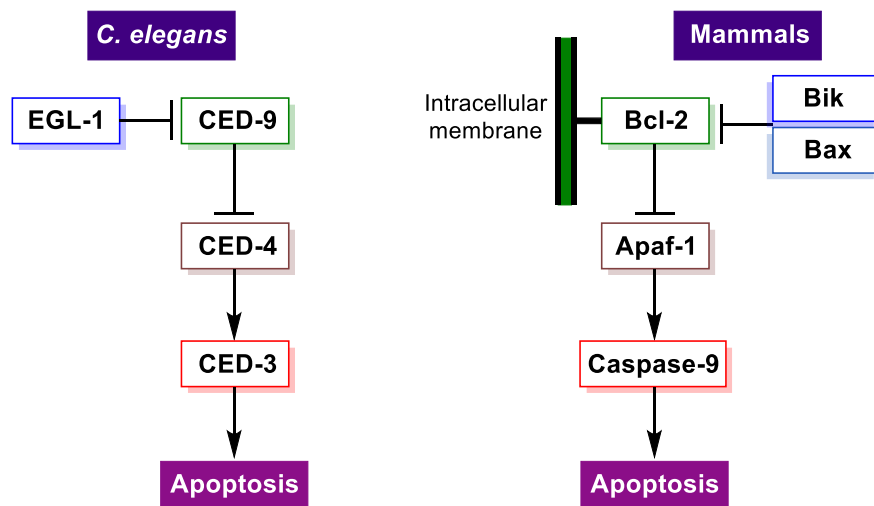


Figure C4. 2: Pathways to cell death in *C. elegans* and mammals.

This shows that apoptosis is an active, complex, and just like cell differentiation and proliferation.¹⁹

Apoptotic cells disappear "silently", leaving no trace of inflammatory response.²⁰ Disruption of the regulation of this physiological process is implicated in several pathological conditions such as cancer, autoimmunity and degenerative disorders.²¹ Thus, an excess of apoptotic cell death may be incriminated in neurodegenerative diseases (Alzheimer, Parkinson ...) ²² and AIDS.²³

Cells undergoing programmed death exhibit particular morphological characteristics, recognized even before the term apoptosis was introduced.⁸ They shrink, exhibit condensation of nuclear chromatin and cytoplasm, membrane protuberances, and DNA cleavage into fragments of approximately 180 base pairs, called nucleosomes.⁸ Eventually, the plasma membrane undergoes blebbing, thus cells are fragmented into vesicles or apoptotic bodies

which are then engulfed by macrophages (Figure C4. 3). The execution of apoptosis involves many intracellular organelles, membrane receptors, proteins, however, mitochondria is the best studied.²⁴

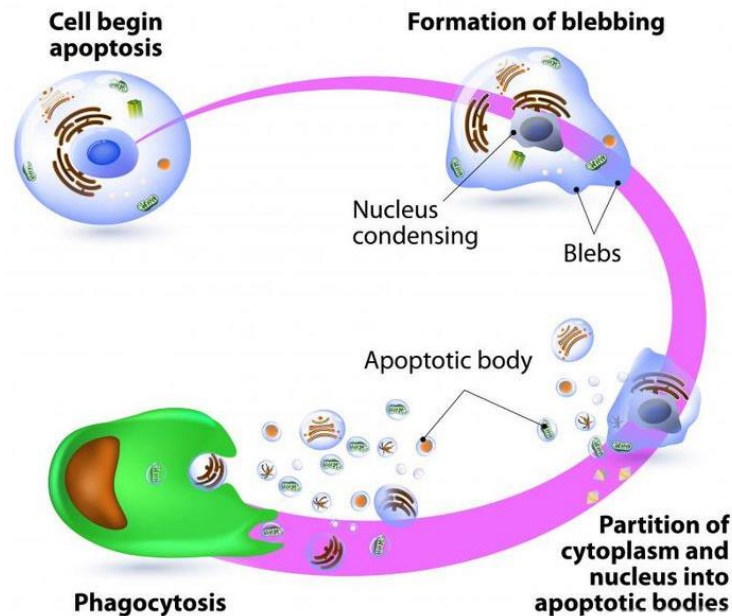


Figure C4. 3: Cell's morphological changes during apoptotic process

Most of the morphological changes associated with apoptosis are caused by caspases, which are the most specific proteases, recognizing at least four contiguous amino acids. They are generally divided into two categories: the initiator caspases, which consist of caspase-2, caspase-8, caspase-9, and caspase-10, and the effector caspases, including caspase-3, caspase-6, and caspase-7. An initiator caspase is characterized by an extended N-terminal prodomain of > 90 amino acids, whereas an effector caspase contains only 20–30 residues in its prodomain.²⁵

Caspases exist in all cells as catalytically inactive zymogens (pro-caspases). The activation of a first contingency of so-called initiating caspases leads to the sequential activation of the effector caspases, in particular caspase-3, within a proteolytic cascade. The activation process can be initiated by various types of intra- and extra-cellular signals.²⁶

Two pathways have been described as a focal point for caspase activation. The extrinsic apoptotic pathway is activated by extracellular ligands (FasL) binding to cell-surface death receptors: TNF receptors (Tumor Necrosis Factor) and Fas receptors (First Apoptosis Signal, also known as Apo-1 or CD95). On the other hand, the intrinsic pathway is activated by various cellular stresses such as: deprivation of growth factors, hypoxia, DNA damage and activation of oncogenes, which lead to signal transduction from stress to mitochondria (Figure C4. 4).²⁷ This pathway is mediated by proteins belonging to the family of Bcl-2.

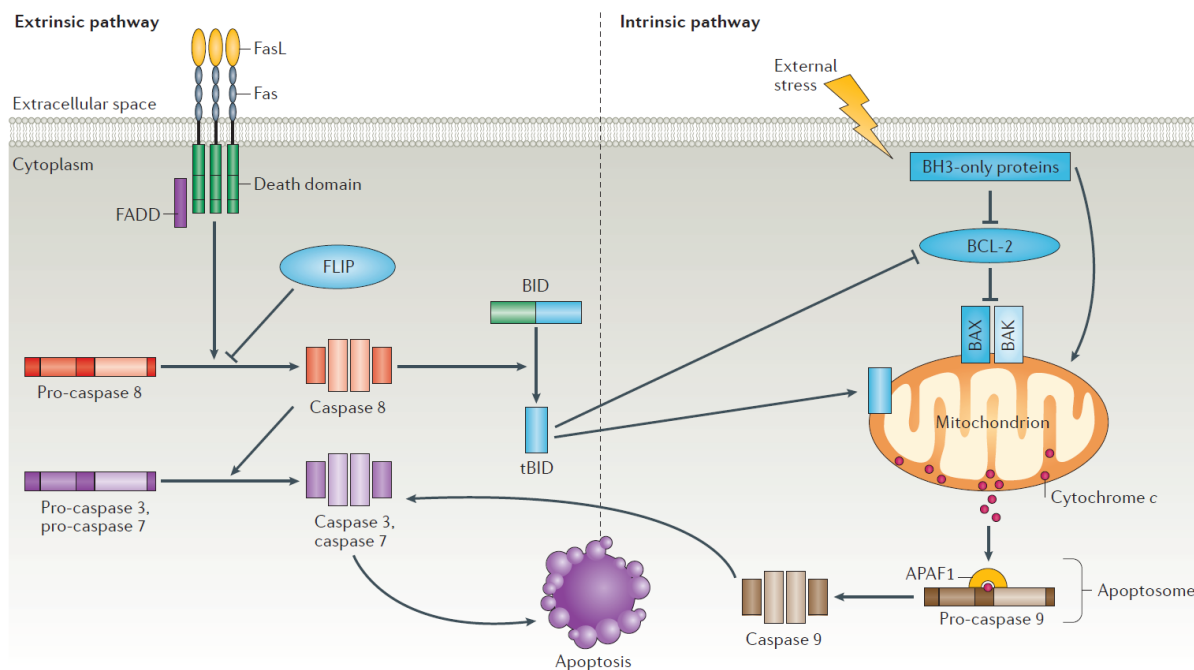


Figure C4. 4: Pathways of apoptosis. The intrinsic pathway triggered by different stress signals and the extrinsic pathway triggered via the binding of death receptors.^{27b}

1.1.1. The extrinsic pathway, the death receptors pathway

In the death receptor pathway, caspase-8 is the key initiator caspase (Figure C4. 4). Death receptors are members of the tumor necrosis factor (TNF) receptor super-family and comprise a subfamily that is characterized by the intracellular death domain (DD), the classical death receptors are FAS and TNFR1.²⁸ The most prominent death ligands are CD95-ligand/Fas ligand, TNF, and TNF-related apoptosis inducing ligand (TRAIL). Upon ligand binding, receptors oligomerize and their death domains attract the intracellular adaptor protein FADD (Fas-associated death domain protein), which, in turn, recruits the inactive proform of caspase-8 or caspase-10 via their death-effector domain (DED). This proteolytic step is mediated by the adaptor protein Fas-associated death domain protein (FADD) and can be inhibited by FADD-like apoptosis regulator (also known as FLIP), a catalytically inactive homologue of caspase 8.²⁹ Subsequently, caspase 8 cleaves and produces large and small caspase-8 subunits that activates caspase 3 and caspase 7, which in turn induce the degradative phase of apoptosis.³⁰

An alternative pathway of Fas-induced cell death involves crosstalk between the extrinsic and the intrinsic apoptotic pathways. This crosstalk is mediated by the activation of the Bcl-2 pro-apoptotic protein BH3-interacting domain death agonist (also known as BID),³¹ which is cleaved by caspase 8 following Fas ligation. The cleaved form of BID is then targeted to the mitochondria and ultimately induces the intrinsic apoptotic pathway (Figure C4. 4).³²

1.1.2. The intrinsic pathway; the mitochondrial pathway

The intrinsic pathway (*via mitochondria*) plays a key role in regulating cell death in response to various stimuli (Figure C4. 5). To initiate apoptosis, cellular stress or damage signals (step 1) typically unleash pro-apoptotic proteins (BH3-only ‘activators’ of apoptosis) via their upregulation (such as Bcl-2-interacting mediator of cell death (BIM) or p53-upregulated modulator of apoptosis (PUMA)) or cleavage (BH3-interacting domain death agonist (BID) to form the active, truncated form (tBID)) (step 2).³³

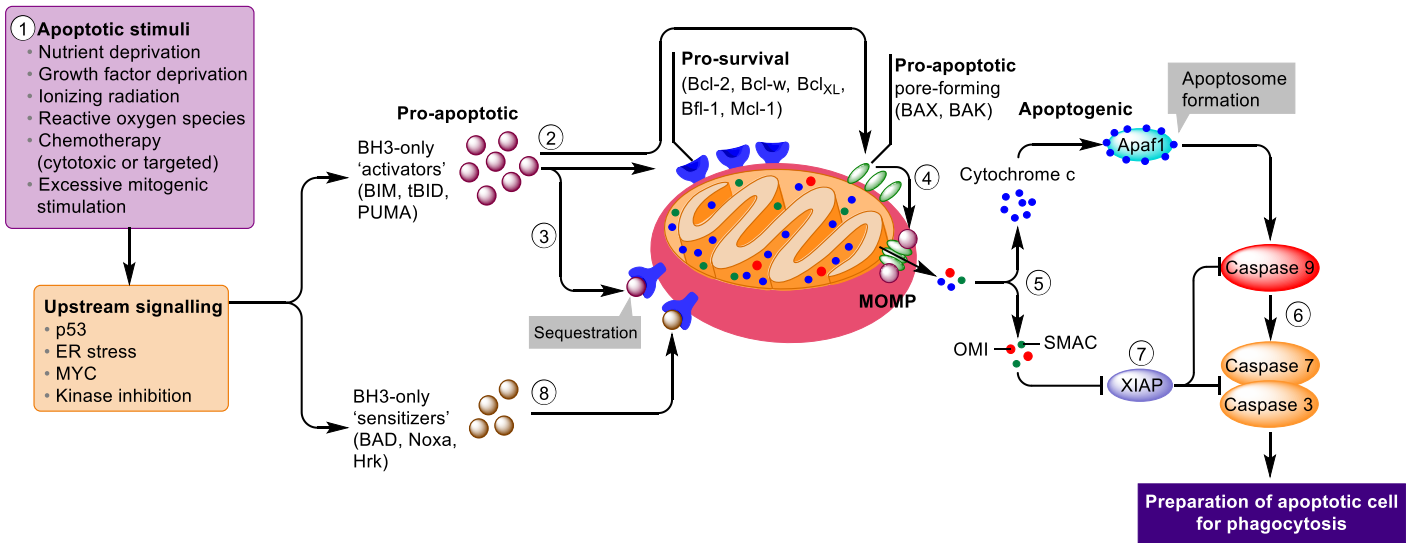


Figure C4. 5: The mitochondrial apoptosis pathway

These pro-apoptotic activator proteins either can be bound and sequestered by pro-survival proteins such as Bcl-2, B cell lymphoma extra large (Bcl-_{xL}) or myeloid cell leukaemia 1 (Mcl-1) (step 3) or, when these pro-survival proteins are saturated or absent, can activate Bcl-2-associated X protein (BAX) and/or Bcl-2 antagonist/killer (BAK) (step 4).³⁴ Activated BAX or BAK oligomerizes and forms pores to cause mitochondrial outer membrane permeabilization (MOMP), resulting in the release of apoptogenic molecules including second mitochondria-derived activator of caspases (SMAC), serine protease OMI and cytochrome c from the mitochondrial intermembrane space.³⁵

Cytochrome c binds apoptotic protease-activating factor 1 (Apaf1) in the cytosol to form the apoptosome (step 5), which serves as a platform for the activation of caspase 9, which then activates the effector caspases 3 and 7 (step 6) to dismantle the cell and prepare it for phagocytosis. Caspase activation can be blocked by the X-linked inhibitor of apoptosis protein (XIAP) (step 7), which in turn is inhibited by the released SMAC and OMI proteins from the mitochondria (step 5).³⁶

Upstream damage or stress signalling can also activate BH3-only ‘sensitizer’ proteins that do not efficiently activate BAX and BAK but inhibit the activity of pro-survival Bcl-2 family proteins to release any sequestered BH3-only activators, which trigger MOMP (step 8).^{21b,37}

Hence, it is clearly demonstrated that the key to the regulation and execution of intrinsic apoptosis is the Bcl-2 family of proteins, which includes both pro-apoptotic and pro-survival (anti-apoptotic) members. The careful modulation of the balance between these two groups of Bcl-2 proteins can largely determine cell fate decisions between life and death.

1.2. The Bcl-2 family

BCL-2 family proteins was identified in human B cell follicular lymphoma in which the chromosomal translocation t(14;18) (q32;q21) induces Bcl-2 gene overexpression (Figure C4.6).³⁸

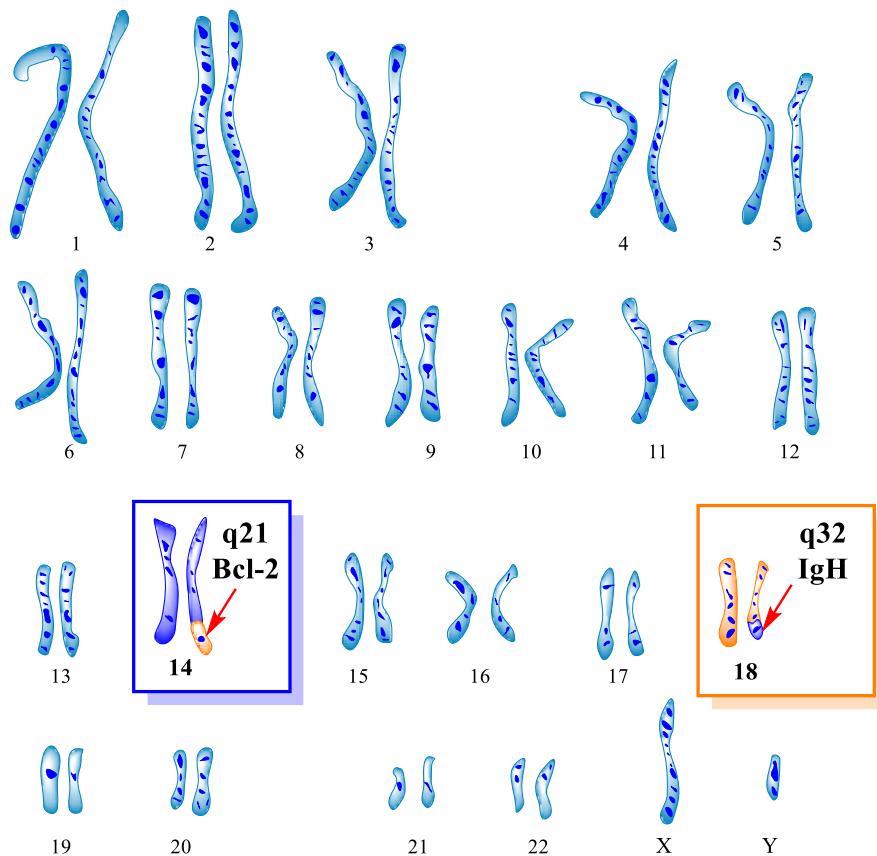


Figure C4. 6: The t(14;18) (q32;q21) translocation where the Bcl-2 is juxtaposed to the immunoglobulin heavy chain locus (IgH) in hallmark of follicular lymphoma.

Members of BCL-2 family are characterized by their domain of homology (BH1- BH4) and their functional activities. Based on these functional and structural homologies, the Bcl-2 family was subdivided into three groups based on the regions of BH domains and the function.

- Anti-apoptotic members containing up to four homology domains (BH1-BH4), this subfamily includes in addition to the Bcl-2, other proteins such as Bcl-x_L, Mcl-1, B-cell lymphoma-w (Bcl-w), Bcl-2-related protein A1/Bcl-2-related isolated from fetal liver-11 (A1/Bfl-1) and, in humans, Bcl-2 family protein resembling Boo (Bcl-B).³⁹ In these proteins, the BH1-3 domains are close together and constitute a hydrophobic pocket forming a receptor domain, while the BH4 domain will stabilize the structure by masking the exposure of certain residues. This hydrophobic pocket will be able to dimerize with apoptotic proteins *via* their homology domain BH3. The resultant is sequestration of pro-apoptotic proteins as an inactive heteromer (Figure C4. 7).⁴⁰

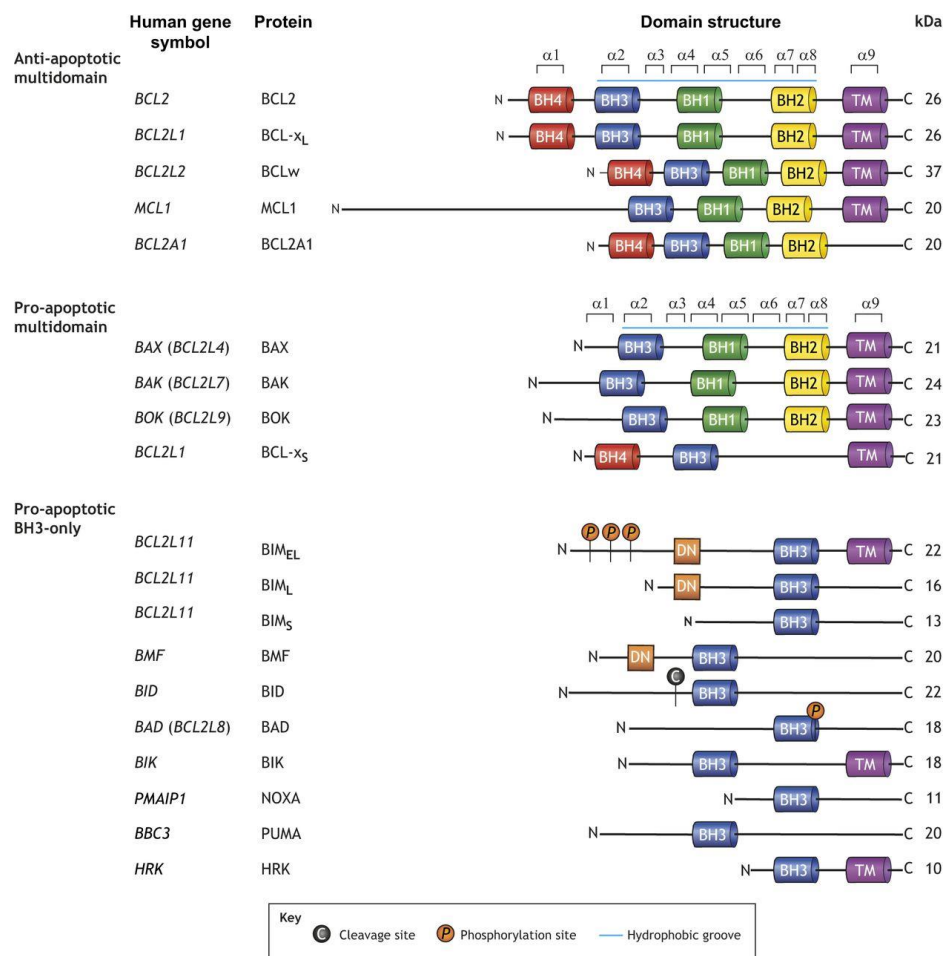


Figure C4. 7: Classification and protein structure of Bcl-2 family members.

- The multi-domain pro-apoptotic proteins (pro-apoptotic effectors) that possess the BH1-BH2-BH3 domains (BAX type) and therefore a strong homology with Bcl-2 (Figure C4. 7). We find in particular BAX, BAK, and Bcl-2 related ovarian killer (BOK).⁴¹ These executioner proteins oligomerize to form pores and permeabilize the outer mitochondrial membrane, thereby releasing components of the intermembrane space that activate the final effector caspases of apoptosis.⁴²

- The pro-apoptotic proteins (pro-apoptotic *activators*) called "BH3-only" because they have only one domain of homology with the other members. Members of this family include the proteins, BIM, Bcl2-modifying factor (BMF), hara-kiri (HRK), (Bcl2-interacting killer) BIK / BLK / NBK, Bcl2 antagonist of cell death (BAD), BID, NOXA (Latin for damage) and PUMA (Figure C4. 7). This family is known to sense cellular stress and activate pro-apoptotic effectors (either directly or indirectly).⁴²

The Bcl-2 network consists of both anti- and pro-apoptotic members and has a crucial function in shifting the balance in favour of either an anti- or pro-apoptotic outcome. Bcl-2 family anti-apoptotic proteins have been demonstrated as overexpressed in human cancers⁴³ and are frequently associated with resistance to conventional chemotherapies as well as targeted therapies.⁴⁴ Oncological scientific community thus focused its attention on these possible targets in order to identify selective pharmacological inhibitors. For this purpose, one approach is to mimic the BH3 domain of the pro-apoptotic proteins, such molecules being thus called "BH3-mimetics" (Figure C4. 8). Numerous studies previously showed that the inhibition of the expression or activity of these targets can lead to apoptotic cell death on its own in cancer cells,⁴⁵ as well as to sensitize them to various anti-tumor agents.⁴⁶

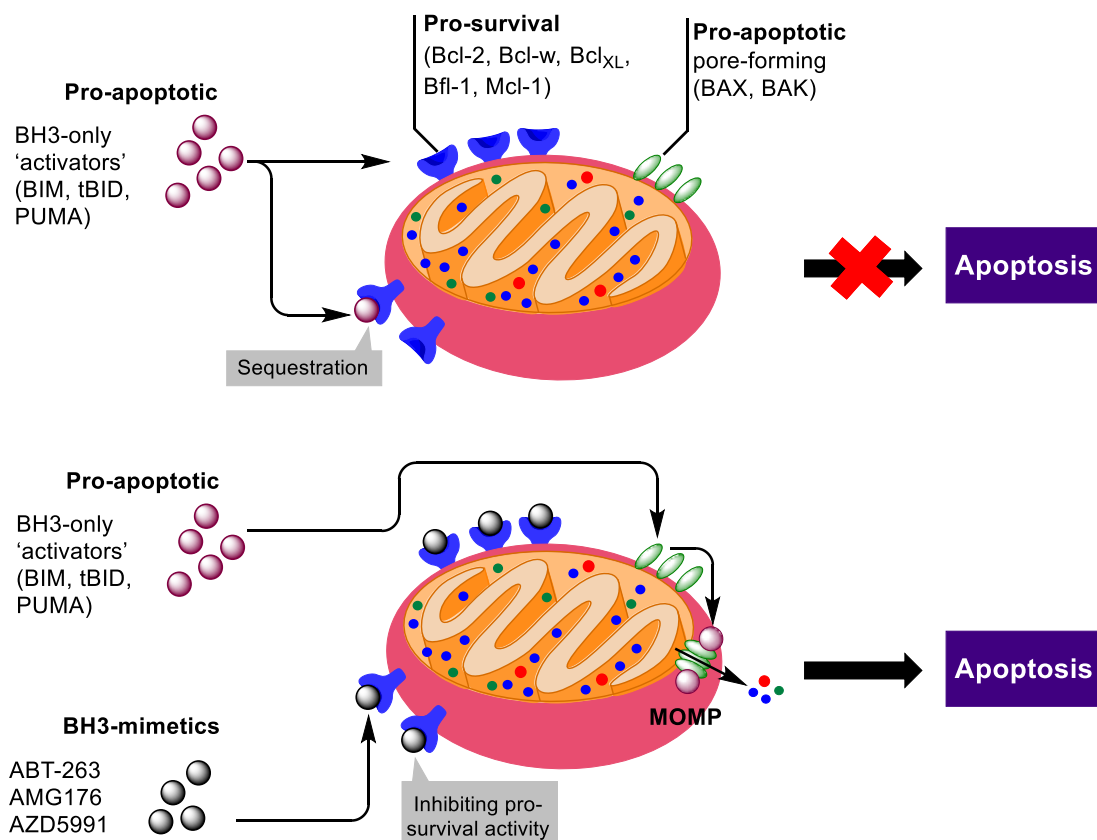


Figure C4. 8: Effect of BH3-mimetics to initiate apoptosis

1.2.1. Bcl-2 Inhibitors

The BH3-mimetic concept has prompted the design of numerous small BH3 peptides or organic molecules thus binding to the pro-survival proteins with high affinity and inhibiting their activity. Clinical trials have been performed within the last decade with ABT-737 (Figure C4. 9: **1**) directed against Bcl-2, Bcl-w, and Bcl-x_L with remarkably high binding affinity toward the latter ($K_i < 0.5$ nM)⁴⁷ but with much lower affinity to Mcl-1 or Bcl2A1 ($K_i > 20$ μ M).⁴⁸ The potential of ABT-737 as an anticancer agent has further been demonstrated in a set of cancer cells including lung cancer cell lines.⁴⁹ Different studies were performed and investigated the effect of ABT-737 in small cell line cancer (SCLC), and identified an essential role of Mcl-1 and its high expression levels in determining resistance to ABT-737 which sequester the pro-apoptotic Bcl-2 proteins previously displaced from Bcl-2, Bcl-w, and Bcl-x_L.⁵⁰

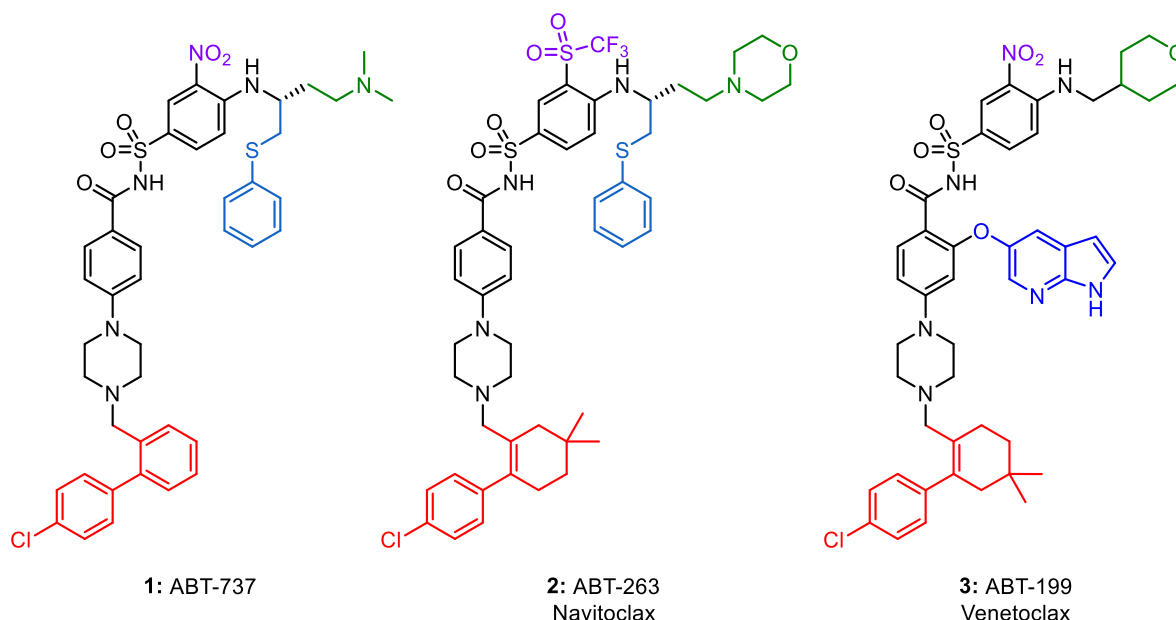


Figure C4. 9: Structures of ABT-737 (**1**), ABT-263 (**2**), and ABT-199 (**3**) BCL-2 inhibitors

Unfortunately, ABT-737 was not bioavailable after oral administration, as a result ABT-263 “Navitoclax” (Figure C4. 9: **2**) was developed by Abbott Laboratories “now AbbVie”, which is orally bio-available with similar binding affinity towards Bcl-2 ($K_i < 1$ nM), Bcl-w ($K_i < 1$ nM), and Bcl-x_L ($K_i < 0.5$ nM), and low binding affinity towards Mcl-1 ($K_i > 550$ nM) and Bcl2A1 ($K_i > 354$ nM).⁵¹ In the clinic, navitoclax displayed excellent pharmacology and showed promising signals of efficacy in hematologic malignancies.⁵² However, a mechanism-based side-effect of Navitoclax emerged, thrombocytopenia, which was attributed to Bcl-x_L inhibition.⁵³ Analysis of Bcl-x_L knockout mice showed that this antiapoptotic protein is required for the longevity of platelets thus acting as a molecular clock in platelets.⁵⁴

Thus, an effort was initiated to dial out Bcl-x_L inhibition, while retaining potent Bcl-2 inhibition, hence creating a highly potent, orally bioavailable and Bcl-2–selective inhibitor (EC₅₀ = 3 nM), ABT-199/Venetoclax (Figure C4. 9: 3).⁵⁵ Leading to its approval by the Food and Drug Administration (FDA) for cancer treatment of patients with chronic lymphocytic leukemia and acute myeloid leukemia.⁵⁶

The effectiveness of these agents and others in several cancers is often limited by chemoresistance, which has most commonly been ascribed to high expression levels of other pro-survival Bcl-2 family members, particularly Mcl-1.^{55a,57} Mcl-1-directed therapeutic strategies are particularly promising since Mcl-1 is within one of the most frequently amplified chromosomal regions in human cancers making it a high priority therapeutic target.⁵⁸

1.2.2. Mcl-1 protein inhibitors

Mcl-1 (Myeloid Cell Leukemia-1) is an anti-apoptotic member of the Bcl-2 family originally identified in the ML-1 myeloid leukemia cells more than two decades ago.⁵⁹ Mcl-1 protein is overexpressed and expanded in numerous cancers as it plays a vital role in promoting the survival of several tumor types by allowing them to evade apoptosis, including lung, breast, prostate, pancreatic, ovarian, and cervical cancers, as well as melanoma and leukemia.⁶⁰

One important aspect to bear in mind when considering Mcl-1 inhibitors for clinical applications is the key pro-survival role of Mcl-1 in many normal tissues. It has shown to be substantial for the development and survival of diverse cell types: for example, the development of B and T-lymphocytes^{60a} and for neural development. It is also particularly prevalent in the regulation of macrophage apoptosis,⁶¹ and is essential for the survival of haematopoietic stem cells.⁶²

Mcl-1 has a high homology with Bcl-2 with respect to the presence of the homology domains (BH) and the transmembrane domain (TM) at the C-terminal level. A number of residues in the binding groove differentiate Mcl-1 from its homologues,^{60h} in which its groove appears more electropositive than other pro-survival proteins (Bcl-x_L groove for example is almost completely uncharged).⁶³ The presence of the TM domain allows Mcl-1 to localize at different intracellular membranes, especially in the outer mitochondrial membrane. This would explain the important role of Mcl-1 in controlling events in the mitochondria during apoptosis. In addition, Mcl-1 was also detected at other cellular compartments distinct from the mitochondria.⁶⁴

Over the past two decades, many papers and patent applications have described Mcl-1 inhibitors, yet the literature is tainted with molecules that exert their phenotypic effects through other mechanisms rather than Mcl-1 inhibition.⁶⁵ However, more recently a series of publications have begun to describe Mcl-1 inhibitors, and some molecules are even under clinical evaluation.

1.2.2.a Mcl-1 inhibitors derived from 2-carboxylic acid indoles

➤ A-1210477

Researchers at AbbVie published a series of Mcl-1 inhibitors derived from indole-2-carboxylic acid which have been obtained by high-throughput screening and structure-guided design that resulted in the discovery of A-1210477 (Figure C4. 10, 4).⁶⁶ A-1210477 revealed a strong binding affinity toward Mcl-1 ($K_i = 0.45$ nM), with good selectivity over the other Bcl-2 family proteins investigated: Bcl-2 ($K_i = 0.132$ μ M) and Bcl-x_L ($K_i = 0.660$ μ M) that can disrupt the interactions of Mcl-1 with BIM and NOXA. Further, it penetrates living cells, and acts via on-target mechanism.^{66c} This compound is therefore the first BH3 mimetic targeting selectively Mcl-1, however no *in vivo* activity was shown for A-1210477, which is likely due to the low micromolar potency of this compound in cell viability assays.

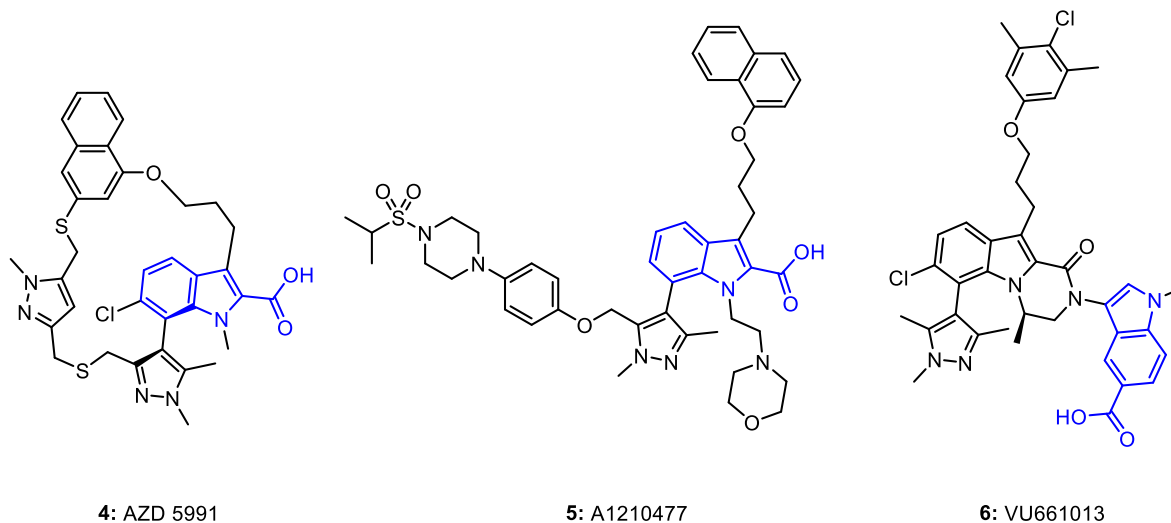


Figure C4. 10: Mcl-1 inhibitors derived from 2-carboxylic acid indoles

➤ VU661013

With the intention of improving potency and reducing the plasma protein binding properties of different Mcl-1 inhibitors Ramsey *et. al* discovered VU661013 (Figure C4. 10, 5).⁶⁷ VU661013 has very high binding affinity for Mcl-1 ($K_i = 0.097 \pm 0.030$ nM), with more than 7000 folds lower affinity for Bcl-2 (0.73 μ M) and Bcl-x_L ($K_i > 40$ μ M). The combination of VU661013 with venetoclax showed enhanced apoptotic activity in the *in vitro* studies which

was then translated in the *in vivo* studies. Nevertheless, a drop-off in binding affinity for Mcl-1 proteins in mice was seen (mouse Mcl-1 $K_i = 7.37 \pm 0.06$ nM), but this is caused by the differences in the amino acid sequences in the ligand binding pocket in mouse vs. human Mcl-1 which was seen in other selective Mcl-1 inhibitors.⁶⁸ Recently, a new study showed that it inhibit cell survival in estrogen receptor-positive breast cancer when used in combination with navitoclax.⁶⁹

➤ AZD5991

The structure-based optimization of a series of indole-2-carboxylic acids was recently disclosed by the discovery of the potent and selective macrocyclic Mcl-1 inhibitor, AZD5991 achieved at AstraZeneca (Figure C4. 10, **6**).⁷⁰ AZD5991 displays the hallmarks of a true Mcl-1 inhibitor with sub-nanomolar-binding affinity ($K_i = 0.2$ nM), and high selectivity versus other Bcl-2 family proteins: Bcl-2 ($K_i = 6.8$ μ M), Bcl-x_L ($K_i = 18$ μ M), Bcl-w (25 μ M), and Bfl-1 ($K_i = 12$ μ M). The mechanistic pathway of the inhibition of Mcl-1 by AZD5991 was achieved by the rapid disruption of the Mcl-1:BAK complex, thus activating the mitochondrial apoptotic pathway. In addition to that, AZD5991 showed cytotoxic activity in Mcl-1-dependent but not Mcl-1-independent cell lines, this remarkable cytotoxic activity of AZD5991 in *in vitro* and *in vivo* models of multiple myeloma (MM) and acute myeloid Leukemia (AML) at tolerated doses supported selection of AZD5991 as a clinical candidate for the treatment of patients with haematological malignancies as a single agent “phase I” or in combination with venetoclax “phase II” (ClinicalTrials.gov identifier NCT03218683).

1.2.2.b Non-indole acid Mcl-1 inhibitors

➤ AMG-176 and AMG-397

A team at Amgen performed a series of optimizations to a series of spiromacrocyclic molecules using structure-based drug design and conformational restriction to discover AMG-176 (Figure C4. 11: **7**).⁷¹ AMG-176 has potent and highly selective affinity toward Mcl-1 ($K_i = 60$ pM) vs Bcl-2 ($K_i = 0.95$ μ M) and Bcl-x_L ($K_i = 0.7$ μ M). A notable feature of this class of Mcl-1 inhibitors is their rapid induction of apoptosis in hematologic cancer cell lines, providing clear rationale for testing the clinical molecule AMG-176 with discontinuous dosing strategies. As a result it was the first Mcl-1 inhibitor to enter clinical development and it is evaluated in MM and AML combination with Azacitidine (NCT02675452), and also evaluated in MM, Non-Hodgkin's Lymphoma (NHL), and Diffuse Large B-cell Lymphoma (DLBCL) in combination with venetoclax (NCT03797261). Although AMG-176 was administered orally in preclinical studies, intravenous infusion is used in the clinic.

Recently, AMG-176 was subjected to different modifications due to the observation of two conformations in AMG-176 bound to Mcl-1 with a difference of 2.8 kcal/mol in energy. Thus after different refinements in potency and pharmacokinetics properties of AMG-176 culminated in the discovery of AMG 397, the first Mcl-1 inhibitor dosed orally in the clinic. It exhibited picomolar affinities for MCL1 ($K_i = 15$ pM), selectively competing for binding to the BH3-binding groove of Mcl-1 with pro-apoptotic Bcl-2 family members (e.g. BIM). Currently it is in clinical trials, administering AMG-397 orally once daily for two consecutive days followed by five days break at a weekly interval in patients with MM, AML, DLBCL and NHL (NCT03465540). The chemical structure of AMG-397 has not been released yet.

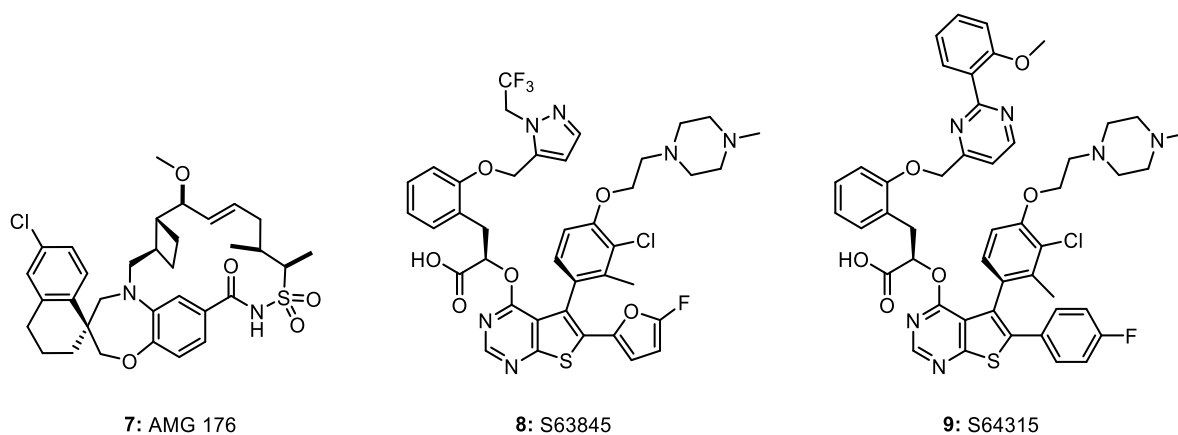


Figure C4. 11: Non-indole acid Mcl-1 inhibitors

➤ S63845 and S64315/MIK665

A team at Servier and Vernalis had designed a new potent selective Mcl-1 inhibitor S63845 with an *in vivo* study that was reported in the literature in 2016 (Figure C4. 11: **8**).⁷² S63845 showed high binding affinity toward Mcl-1 (with a dissociation constant $K_d = 0.19$ nM), with no appreciable binding to BCL-2 ($K_i > 10$ μ M) or Bcl-x_L ($K_i > 10$ μ M). For comparison, A-1210477 has an approximately 20-fold lower affinity for human Mcl-1. It has a broad therapeutic applicability of Mcl-1 inhibition across panels of multiple myeloma, lymphomas, leukaemias and primary AML cells. Also they showed that the therapeutic potential of Mcl-1 inhibitors extends to several solid tumour models, some of which demonstrated sensitivity to S63845 monotherapy, while many others were only killed when treated with a combination of S63845 and inhibitors of oncogenic kinases.

S63845 was not selected for clinical evaluation and instead a phase 1 clinical trial was launched with the Mcl-1 inhibitor S64315/MIK665 (Figure C4. 11: **9**) which showed much important potency to Mcl-1 ($K_i = 26$ pM) with very low affinity toward Bcl-2 and Bcl-x_L (58 μ M and 237 μ M respectively, in the FP assay).⁷³ These trials are in progress at “phase I” in

patients with relapsed and/or refractory MM, lymphoma, AML and Myelodysplastic Syndrome (MDS) (NCT02979366 and NCT02992483). Moreover, S64315/MIK665 is being tested in combination with venetoclax in patients with AML (NCT03672695).

➤ MIM1 inhibitor

Among Mcl-1 inhibitors, MIM1 “Mcl-1 Inhibitor Molecule 1” was identified from a broad screening of 71296 small molecules which were then decreased into 64 potent and selective Mcl-1 inhibitors to find those that could displace a fluorescently labeled Mcl-1 SAHB_A (stabilized α -helix of Bcl-2 domain)⁷⁴ from the BH3-binding domain of Mcl-1⁷⁵ and not displace a fluorescently labeled BAD-BH3 peptide from the BH3-binding domain of Bcl-x_L. Despite a modest Mcl-1 inhibitor activity with IC₅₀ value of 4.8 μ M, MIM1 was the first small molecule to exhibit selectivity for Mcl-1 over Bcl-x_L (IC₅₀ > 50 μ M).

However, several questions remain. Especially, later studies have indicated that the effects of MIM1 could be cell-line dependent.⁷⁶ Moreover, the first structure-activity relationships provided evidence of specific binding but did not shed much light on those aspects of MIM1 that are truly responsible for the observed inhibition.⁷⁷ However, cells devoid of the intrinsic apoptosis machinery are unaffected by MIM1, suggesting its cell activity is on-target. Given the modest activity of MIM1, it is envisaged that a structure–activity relationship (SAR) campaign is presently underway toward its optimization.⁷⁸

Therefore, a program started with Dr. Assaad Nasr El Dine under the supervision of Dr. René Grée around this structure to design new and more potent analogues. Indeed, MIM1 attracted their attention since they had already performed several studies on polyphenol-type derivatives as inhibitors of the anti-apoptotic protein Bcl-x_L.⁷⁹ During this study, which I participated in later, it was realized that the proposed MIM1 structure was not correct. Thus, extensive NMR, X-Ray crystallography and various physicochemical analyses were performed to revise the structure of MIM1 molecule from **10** to **11** (Figure C4. 12). The mechanism will be discussed later.

between Mcl-1 and Bcl-x_L, it was found that the global shape of the grooves (more open in Mcl-1 and narrower for Bcl-x_L) appears to play an important role. This topological property forces ligands to be more desolvated than for Mcl-1, and hydrophilic groups, such as pyridines, are more penalized within Bcl-x_L binding site (Figure C4. 14). This was confirmed by molecular dynamics, showing that **16** is more solvated in Mcl-1 binding site than is Bcl-x_L, and explaining why **16** is selective for Mcl-1 despite a similar binding mode in both proteins.

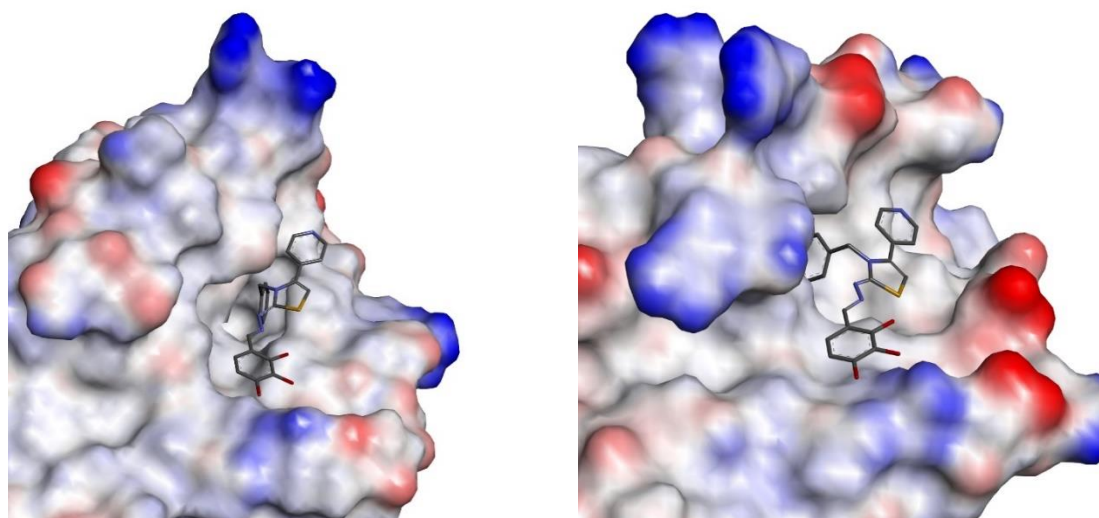


Figure C4. 14: Compound **16** docked onto Mcl-1 (left) and Bcl-x_L (right).

2. Objective

The development of compounds capable of restoring functional apoptotic pathways within cancer cells is a very promising area of research. Our program aims to develop novel apoptosis inducers usable in the treatment of cancers. We mainly target an important anti-apoptotic protein Mcl-1 that was demonstrated to play a key role in the mechanisms of apoptosis of tumor cells and also to likely play a role in the migratory and invasive capacities of tumor cells.⁸⁰

The analysis of the docking of the revised MIM1 analogues, done by Assaad *et al.*, in the proteins (extensive studies performed by Dr N. Levoine, Bioprojet, Rennes) indicated that the triphenol part played a key role in the mode of binding to these anti-apoptotic proteins. This triphenolic motif, however, has clearly limits in terms of "drugability" for corresponding derivatives. Therefore, it seems appropriate to design, synthesize and test novel molecules, derived from the previous ones.

For the new series, the binding to the target proteins would be done more conventionally by electrostatic attraction between arginine 263 (particularly important in Mcl-1) and a carboxylic acid carried by the potential inhibitor. Starting from this hypothesis and making a first analysis

by molecular modeling / docking, it appeared that compounds, similar to those prepared previously, but in which the three phenols were replaced by an acid in *meta* in particular (the *para* position is also possible, but probably less favorable) could have very attractive properties (Figure C4. 15).

Indeed, the acid can bind to both R_263 and N_260 while the two arms of these molecules can be inserted into the P2-P3 pockets. Naturally, here again, the modulations of the nature of the heteroaromatic/aromatics on the two arms of these molecules have a great potential for optimizing the filling in the two pockets P2, and P3 of the protein.

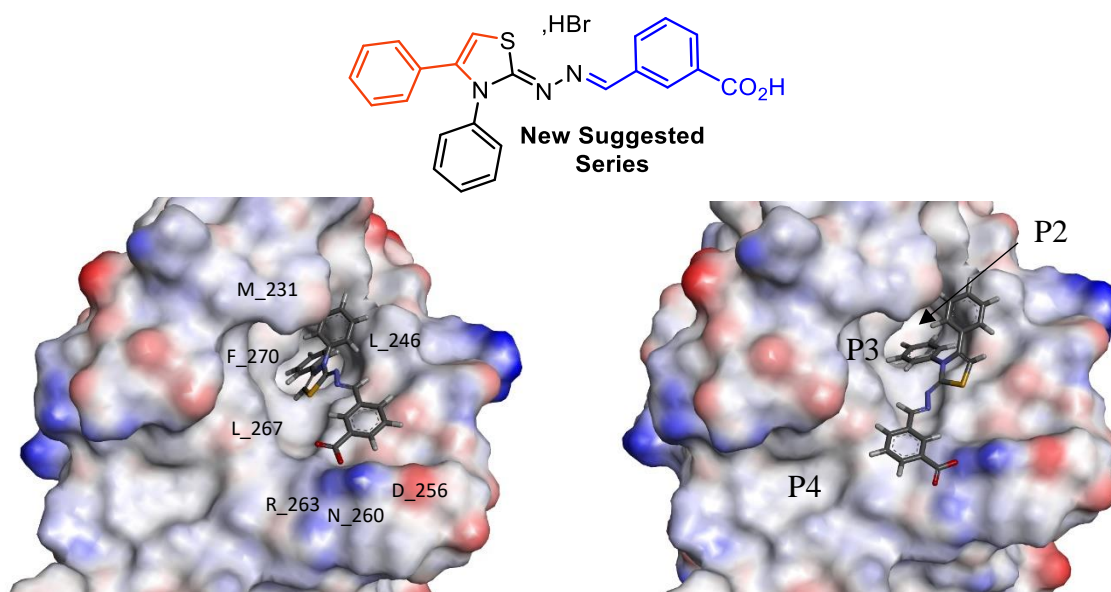
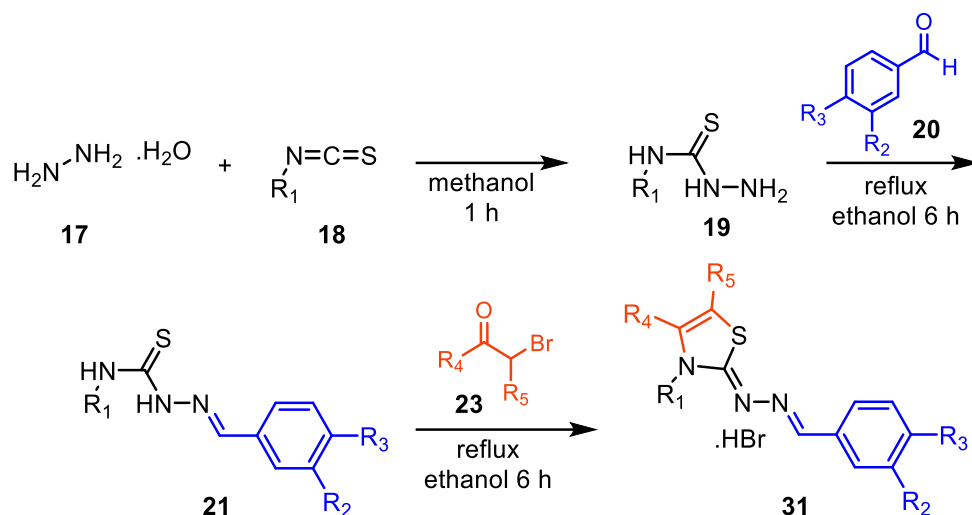


Figure C4. 15: Molecular docking of the suggested MIM-1 analogues with meta acid in Mcl-1 protein.

3. Results and discussion

Relying on the previous results, a new series of the revised MIM1 analogues was synthesized to obtain optimized and potent Mcl-1 inhibition.

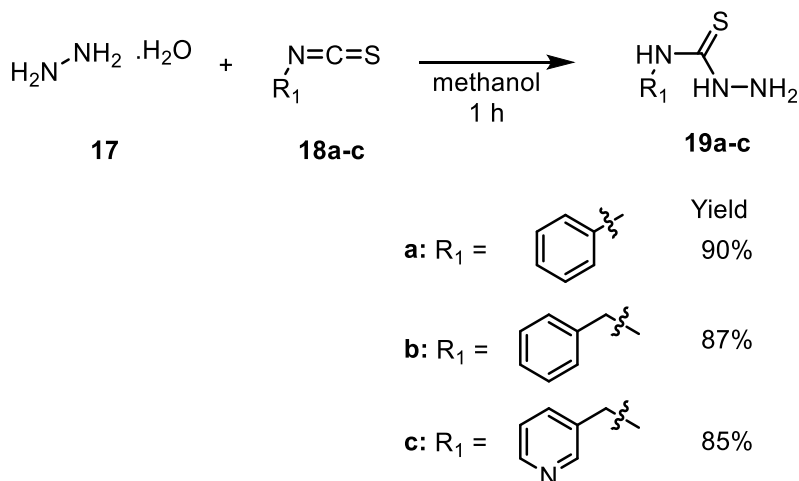
The strategy envisaged for the synthesis of the new MIM-1 analogues is presented in Scheme C4. 1 based on the classical method reported by L. D. Walensky *et al.*⁷⁵ and patent application (WO2013142281). It shows how it is possible to modulate easily the R₁, R₂, R₃ and R₄ groups.



Scheme C4. 1: A general strategy to synthesize revised MIM-1 analogues

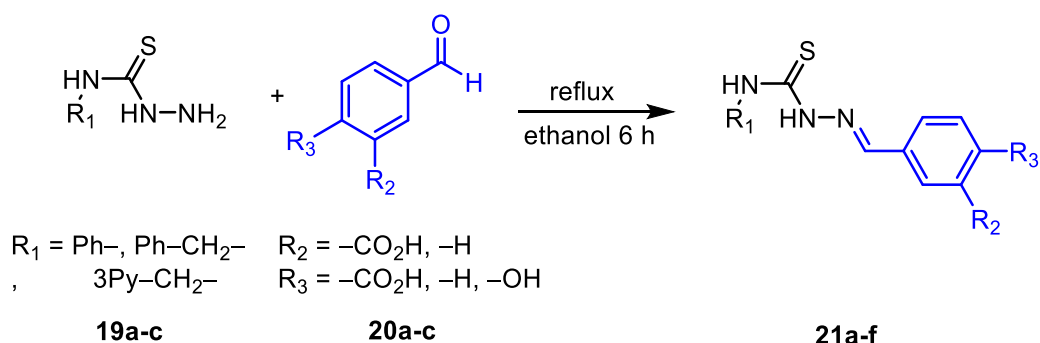
3.1. Synthesis of the revised-MIM1 analogue series

With the above consideration in mind we started the synthesis of a first series of the revised MIM1 analogues bearing an acid in *meta* and *para* positions instead of the trihydroxybenzene moiety. First, the appropriate isothiocyanate **18a-c** reacted with hydrazine hydrate **17** in methanol to give the desired thiosemicarbazides **19a-c** with excellent yields (Scheme C4. 2).



Scheme C4. 2: Synthesis of thiosemicarbazides **19a-c**.

The condensation between these thiosemicarbazides **19a-c** and an aromatic aldehyde **20a-c** led to the six imino-thiosemicarbazides intermediates **21a-f** with good yields (Scheme C4. 3, Table C4. 1).



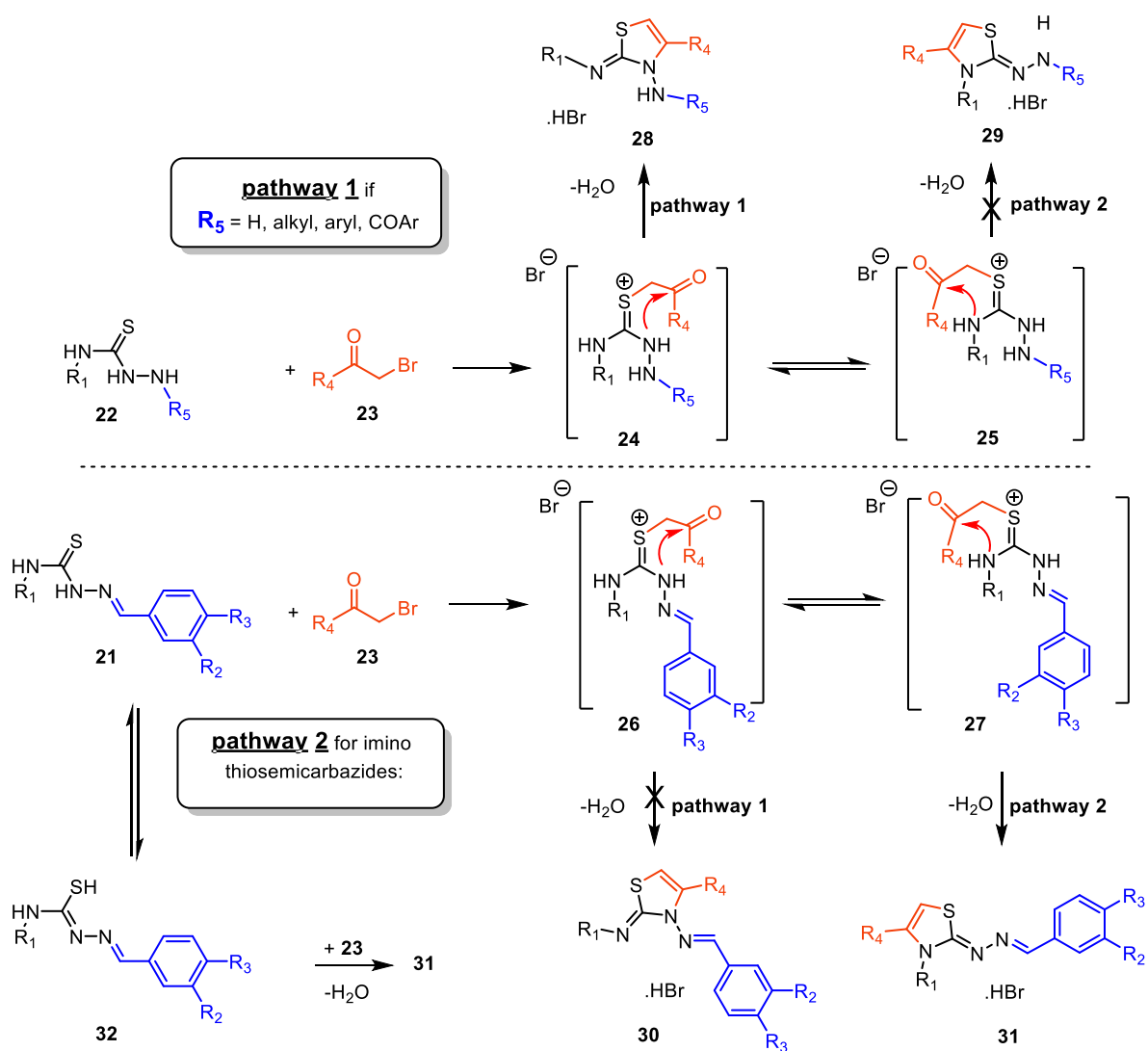
Scheme C4. 3: Synthesis of imino-thiosemicarbazides intermediates **21a-f**

Table C4. 1: Synthesis of imino-thiosemicarbazides intermediates **21a-f**

| Entry | SM 19 | SM 20 | Intermediate 21 | Yield ^[a] |
|-------|-------|-------|-----------------|----------------------|
| 1 | 19a | | | 95 |
| 2 | 19a | | | 86 |
| 3 | 19b | | | 81 |
| 4 | 19b | | | 85 |
| 5 | 19a | | | 65 |
| 6 | 19c | | | 77 |

^[a] isolated yields

The analysis of the literature indicates that the reaction of thiosemicarbazides with α -halogeno ketones has been extensively studied.⁸¹ Careful analysis of corresponding data shows that two types of heterocycles have been obtained and the heterocyclization process is strongly depending upon the structure of the starting thiosemicarbazides, as indicated in (Scheme C4.4). In a first step, the sulfur in molecules **21/22** displaces the bromide from **23** to afford sulfonium ion intermediates **24-27**. For these compounds, two forms are possible, **24/26** and **25/27**, and for each of them the intramolecular cyclisation by a nitrogen atom, followed by water and HBr elimination, will lead to a different structure: a thiazol-3(2H)-yl)imino core **28/30** if the nitrogen of the hydrazone group is concerned by the cyclization (pathway 1) or a [2(3*H*)-thiazolylidene]hydrazone **29/31** if the terminal nitrogen is involved (pathway 2). Careful analysis of literature data indicates that the pathway 1 is observed for molecules **22** in which the amino groups have $R_5 = H$, alkyl or aryl, and also when this group is protected (R_5 being COAr for instance) to give type **6** molecules.⁸² However, when an imino-thiosemicarbazide such as **21** is involved as starting material, the pathway 2 (through **27**), is favored affording compounds-type **31**. Alternatively it is possible to start the reaction from **32**, the tautomeric form of **21** with a conjugated diazadienyl thiol structure, leading also to **32**.⁸³

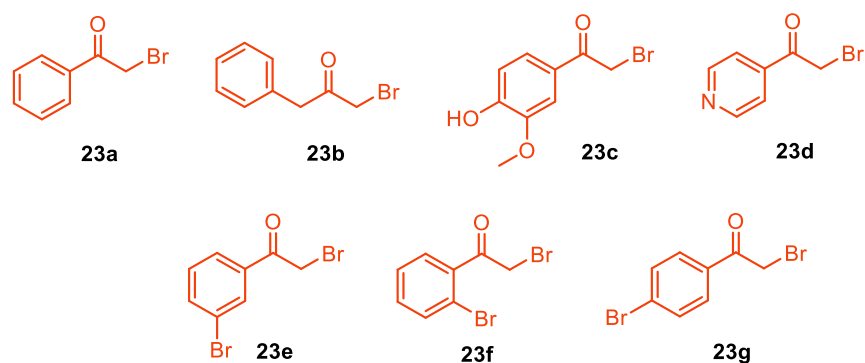


Scheme C4. 4: Condensation of thiosemicarbazides with α -bromoketones.

3.1.1. Synthesis of α -bromoketones 23

Diverse α -bromoketones **23a-u** were envisioned, some of them were commercially available and others needed further synthesis (Figure C4. 16).

Commercially Available



Synthetic

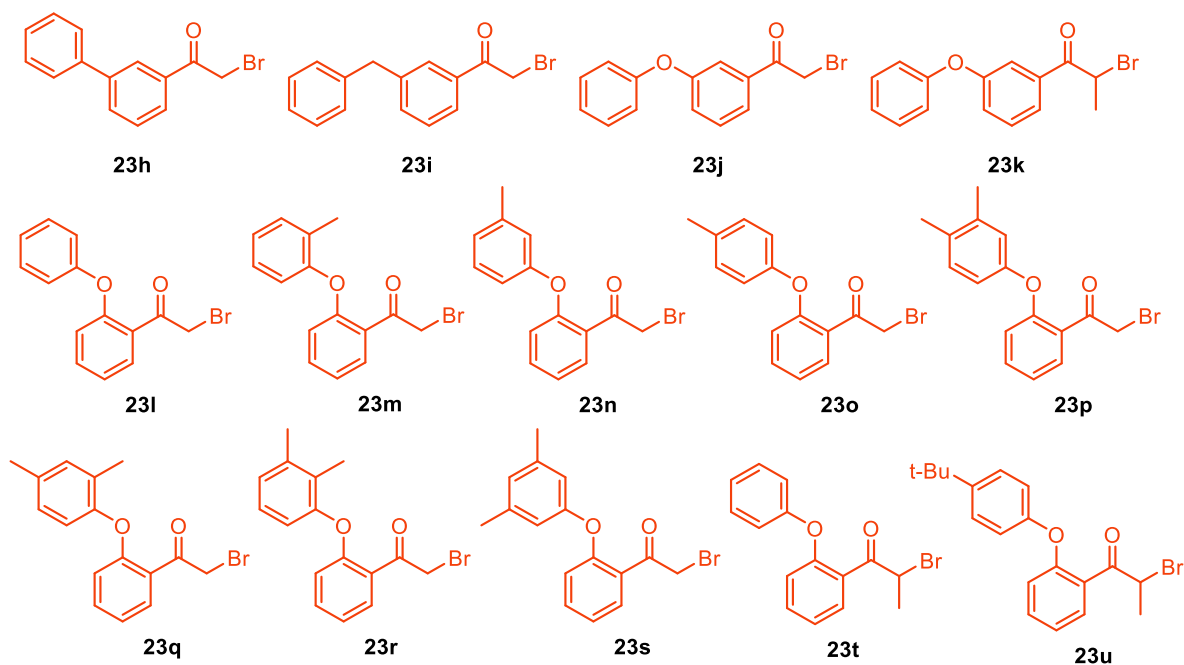
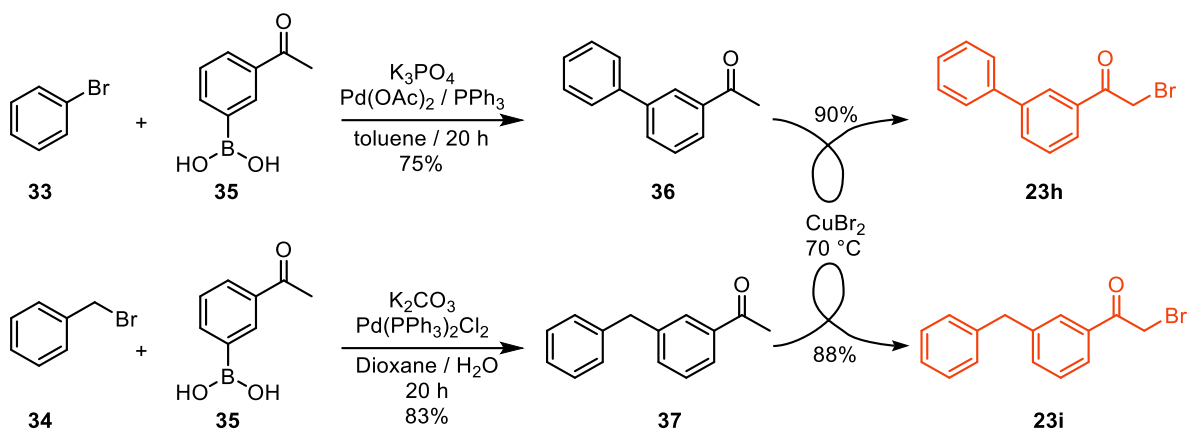


Figure C4. 16: Envisioned α -bromoketones **23a-u**

3.1.1.a Preparation of α -bromoketones **23h** and **23i**

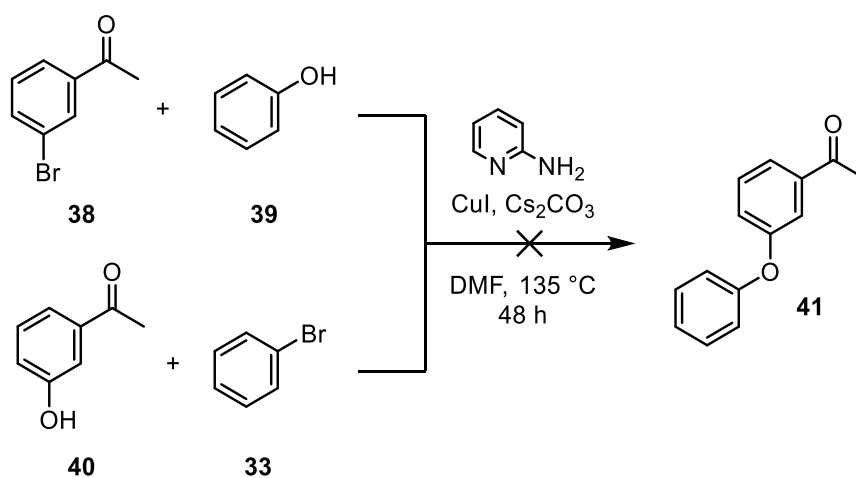
The synthesis starts with a Suzuki coupling reaction between bromobenzene **33** or benzyl bromide **34** with (3-acetylphenyl)-boronic acid **35** to obtain acetyl derivatives **36** and **37** in 75% and 83% yields, respectively.⁸⁴ The reaction with CuBr_2 at 70 °C leads to the formation of the desired α -Bromoketones **32c** and **32d** in excellent yields (Scheme C4. 5).



Scheme C4. 5: Preparation of α -bromoketones **23h** and **23i**

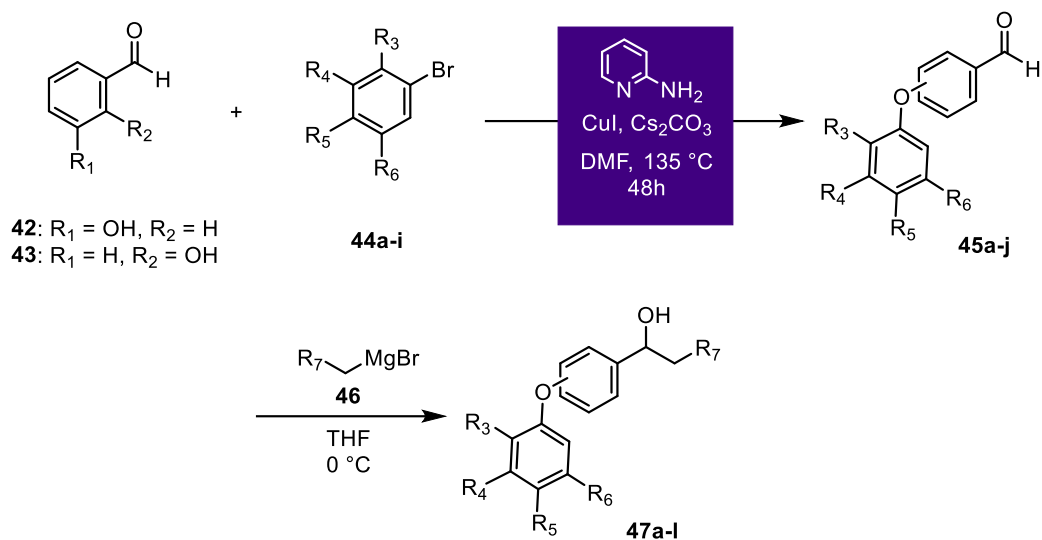
3.1.1.b Preparation of α -bromoketones **23j-u**

In a first attempt to synthesize phenoxyphenyl- α -Bromoketones **23j-k**, Ullmann coupling reaction was performed between 3-bromoacetophenone **38** and phenol **39** in presence of 10 mol% CuI, 20 mol% 2-aminopyridine, and Cesium carbonate in DMF at 135 °C for 48 h, unfortunately this reaction didn't give the desired compound **41**, instead aldolization reaction occurred. The same result was obtained when using 3-hydroxyacetophenone **40** and bromobenzene **33** (Scheme C4. 6).



Scheme C4. 6: An attempt to obtain intermediate **41**

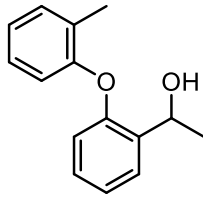
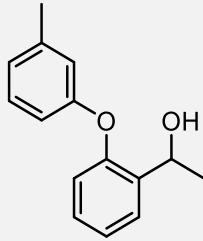
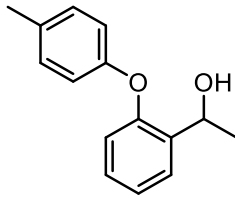
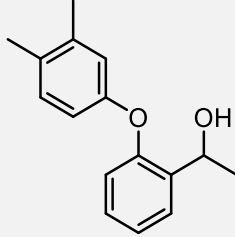
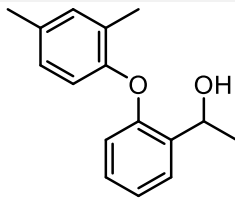
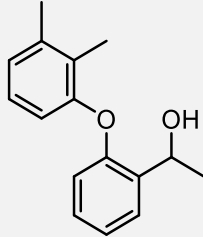
Consequently, hydroxybenzaldehydes **42** and **43** were used in the presence of substituted-bromobenzene **44a-i** under the same previous conditions to afford compounds **45a-j** (Scheme C4. 7). The polarities of the starting material **42** and **43** were remarkably close to aldehydes **45a-j** posing a problem during their purification, thus the synthetic sequence continued by adding Grignard reagent **46** to aldehydes **45a-j** to afford compounds **47a-l**. The results are summarized in Scheme C4. 7 and Table C4. 2.

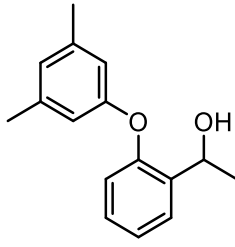
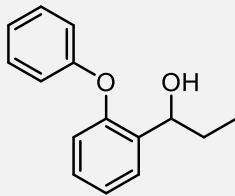
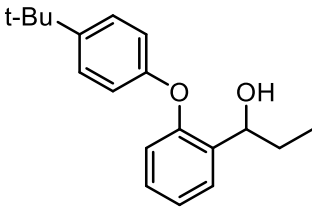


Scheme C4. 7: Synthesis of Intermediate **47a-l**

Table C4. 2: Synthesis of Intermediate **47a-l**

| Entry | Aldehyde | R ₇ | Alcohol 47 | Yield ^[a] |
|-------|----------|-----------------|------------|----------------------|
| 1 | 42 | H | 47a | 48% |
| 2 | 42 | CH ₃ | 47b | 50% |
| 3 | 43 | H | 47c | 41% |

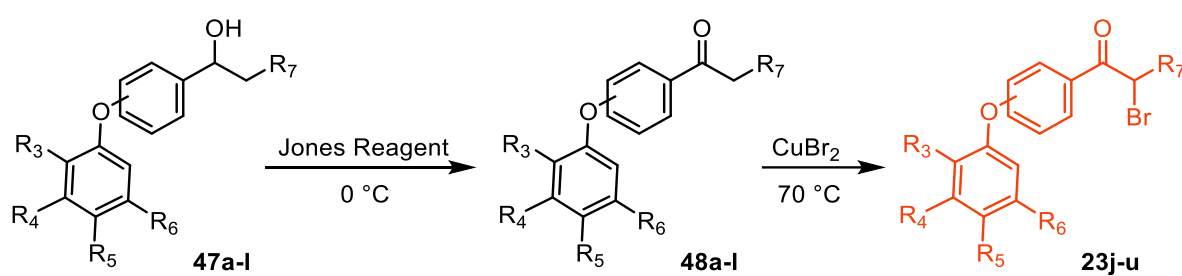
| | | | | |
|---|----|---|---|-----|
| 4 | 43 | H |  47d | 57% |
| 5 | 43 | H |  47e | 42% |
| 6 | 43 | H |  47f | 47% |
| 7 | 43 | H |  47g | 53% |
| 8 | 43 | H |  47h | 39% |
| 9 | 43 | H |  47i | 38% |

| | | | | |
|----|----|-----------------|--|-----|
| 10 | 43 | H |  | 51% |
| 11 | 43 | CH ₃ |  | 53% |
| 12 | 43 | CH ₃ |  | 32% |

^[a] overall yield of both steps to obtain 45 and 47

The same sequence of reactions as before allowed us to prepare ketones **48a-l** and subsequently α -bromoketones **23j-u** in good yields (Table C4. 3).

Table C4. 3: Synthesis of α -bromoketones **23j-u**



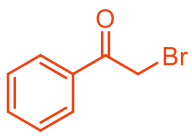
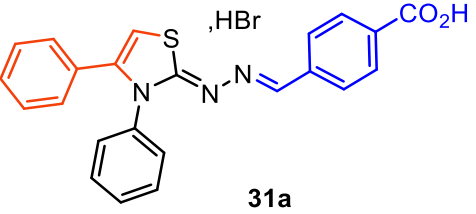
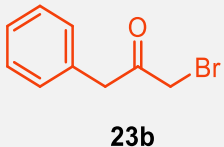
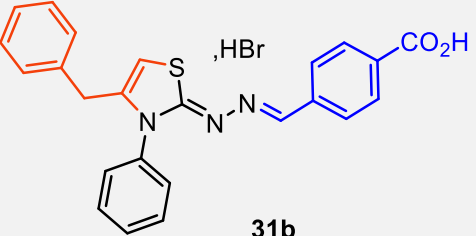
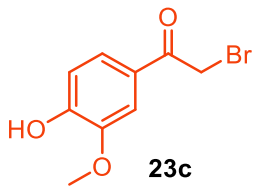
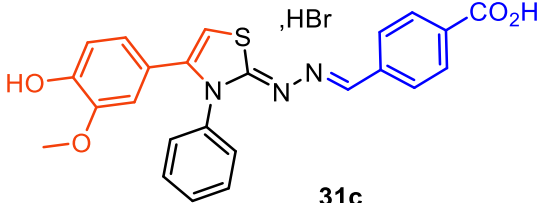
| Entry | SM | Ketone | yield ^[a] | |
|-------|------------|-----------------|----------------------|------------------------|
| | | | | α -Bromoketones |
| 1 | 47a | 48a (92) | | 23j (84) |
| 2 | 47b | 48b (95) | | 23k (75) |
| 3 | 47c | 48c (98) | | 23l (85) |
| 4 | 47d | 48d (91) | | 23m (79) |
| 5 | 47e | 48e (89) | | 23n (71) |

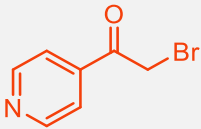
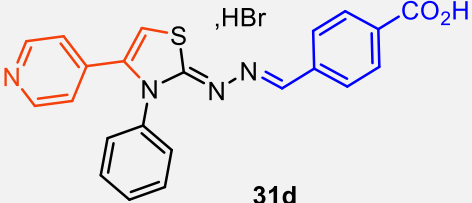
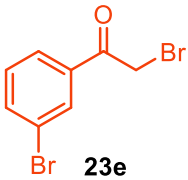
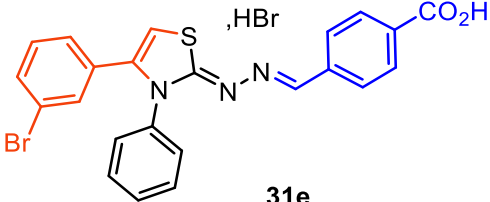
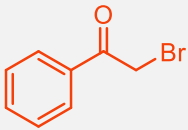
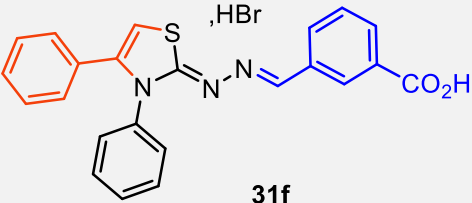
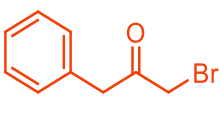
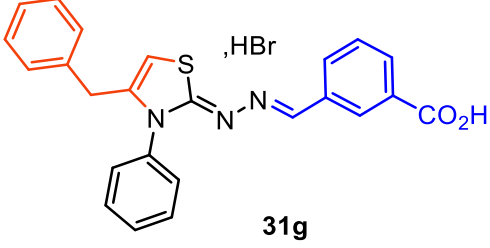
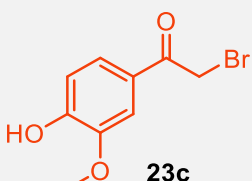
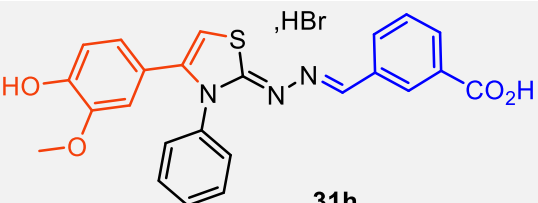
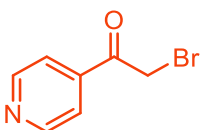
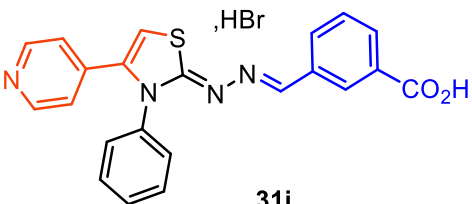
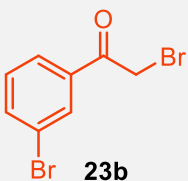
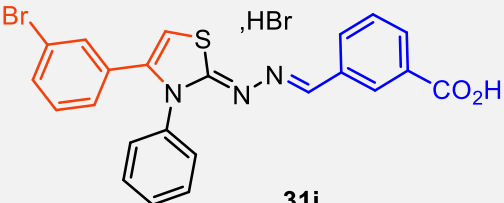
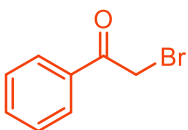
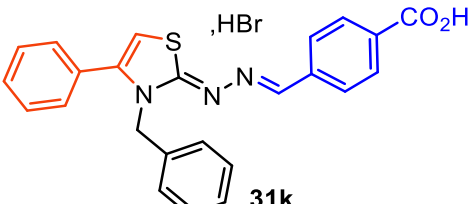
| | | | |
|----|-----|----------|----------|
| 6 | 47f | 48f (92) | 23o (80) |
| 7 | 47g | 48g (87) | 23p (90) |
| 8 | 47h | 48h (81) | 23q (73) |
| 9 | 47i | 48i (95) | 23r (82) |
| 10 | 47j | 48j (87) | 23s (74) |
| 11 | 47k | 48k (91) | 23t (71) |
| 12 | 47l | 48l (97) | 23u (76) |

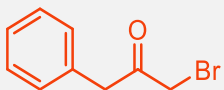
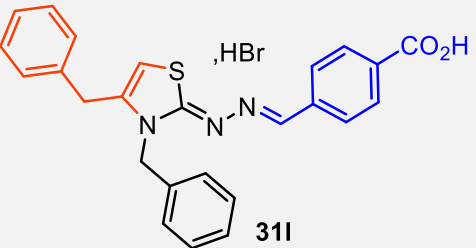
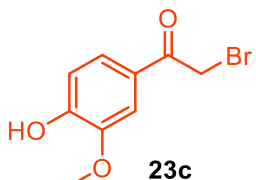
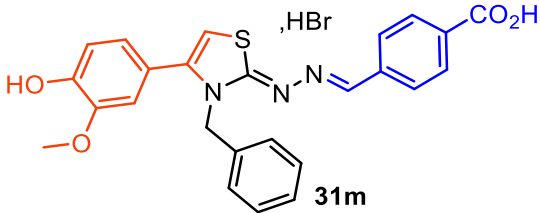
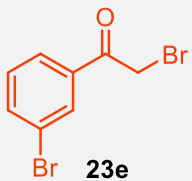
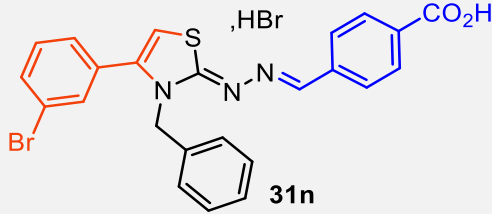
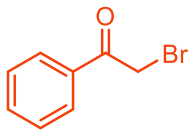
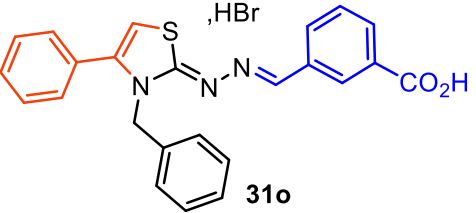
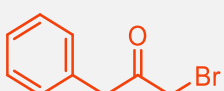
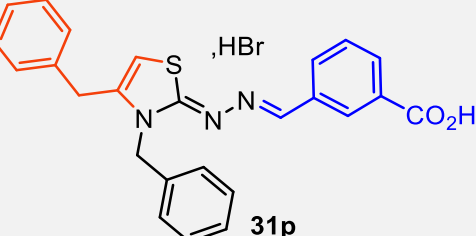
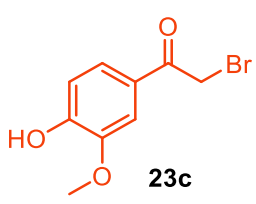
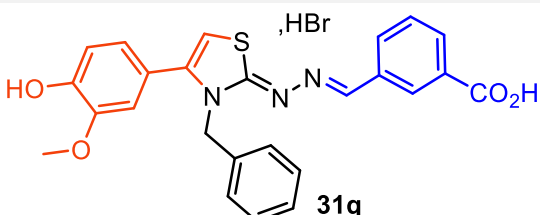
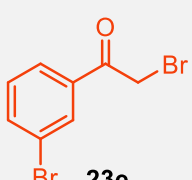
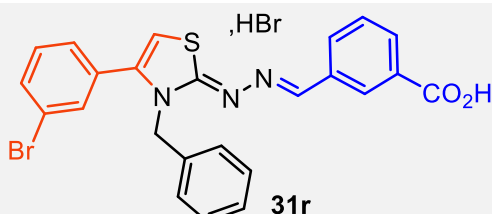
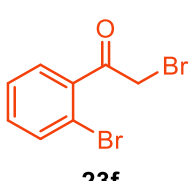
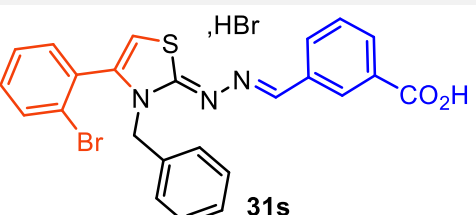
^[a] isolated yields

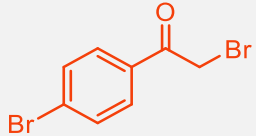
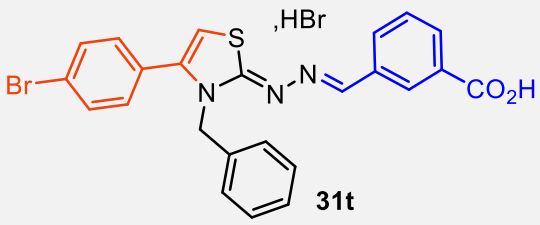
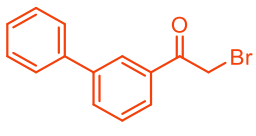
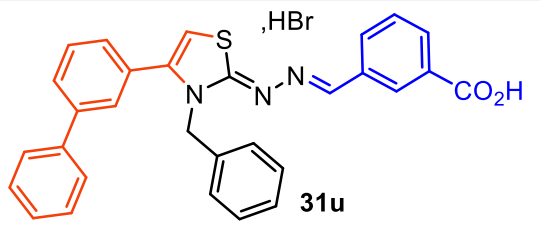
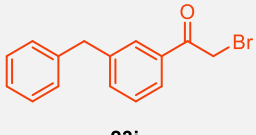
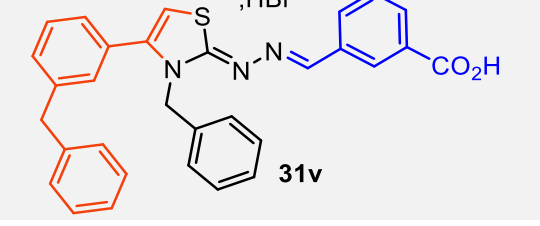
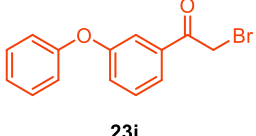
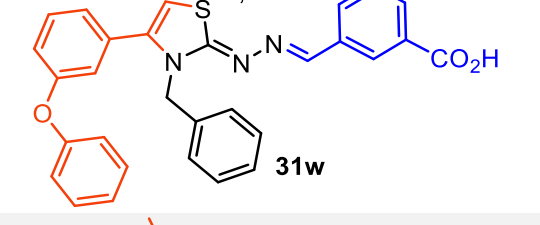
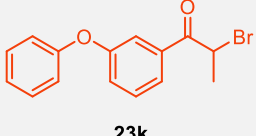
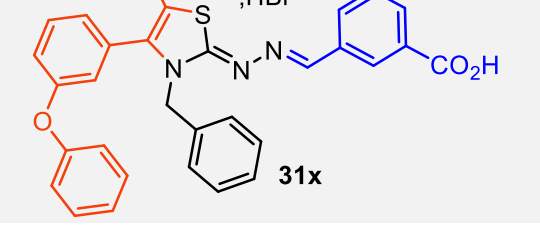
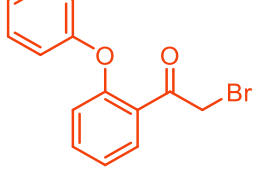
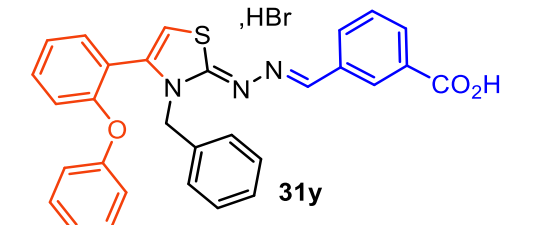
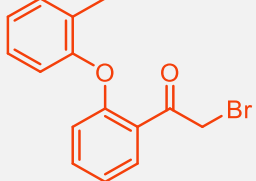
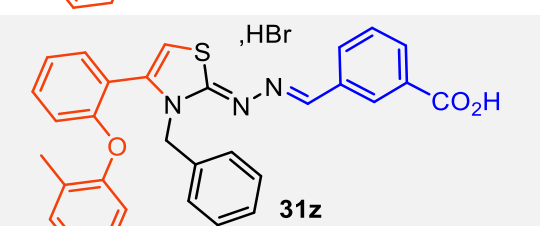
Based on the previous mechanistic study, revised-MIM1 analogues **31a-al**, were prepared by the reaction between intermediates **21a-f** and α -bromoketones **23a-u**, and the results are summarized in Table C4. 4.

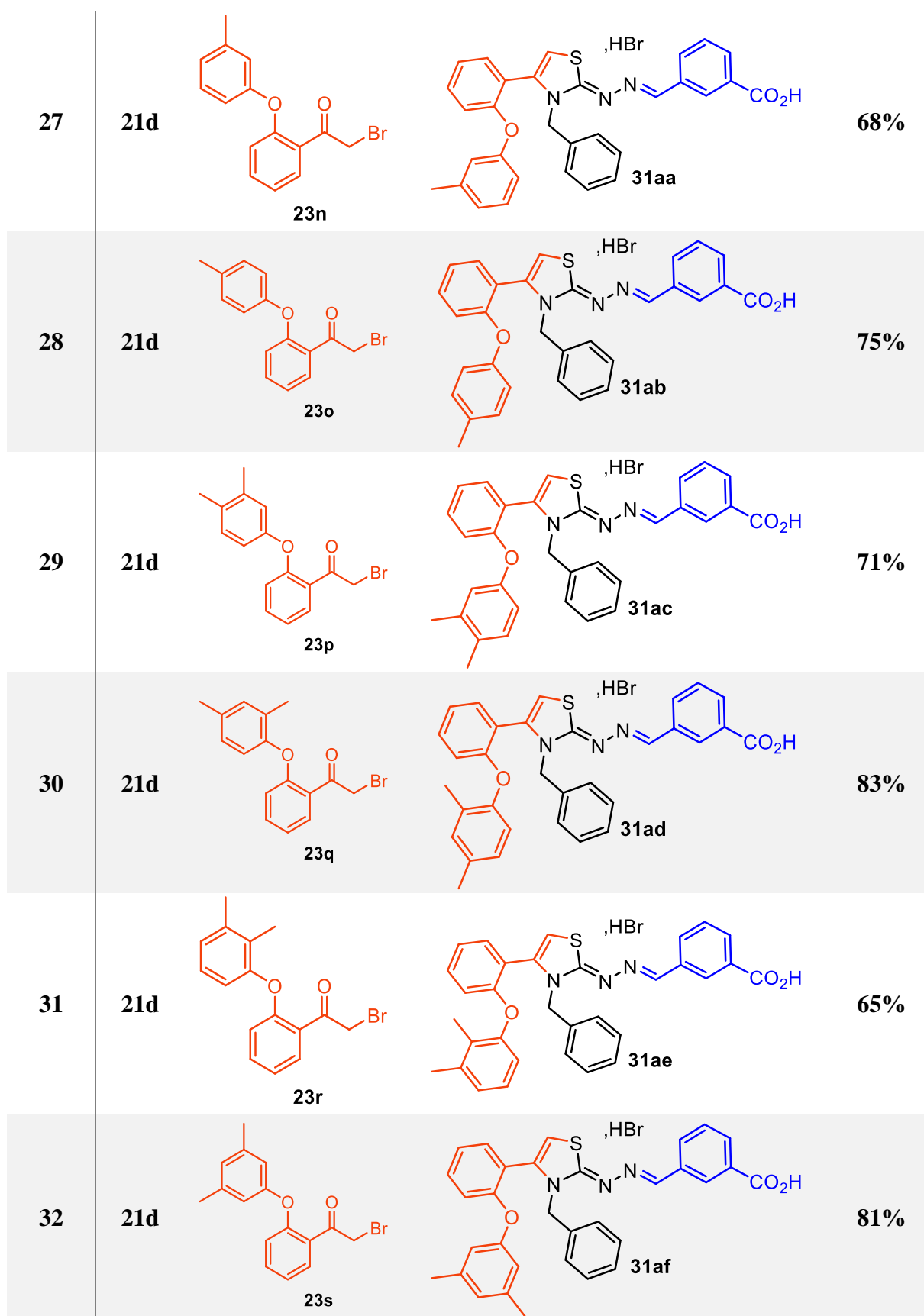
Table C4. 4: Synthesis of the first series of revised MIM1 analogues

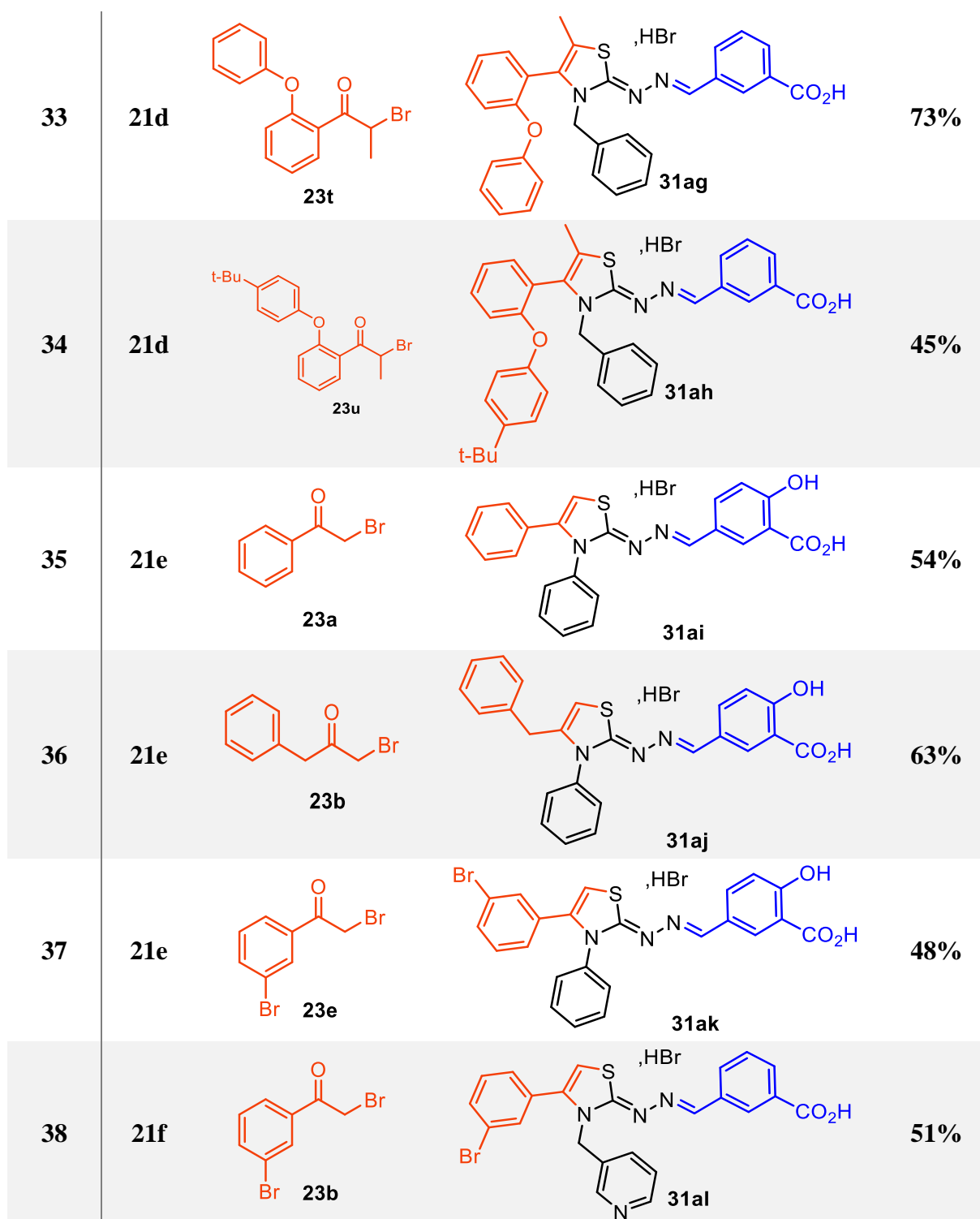
| Entry | SM 21 | SM 23 | Analogues 31 | yield ^[a] |
|-------|-------|--|---|----------------------|
| 1 | 21a |  23a |  31a | 65% |
| 2 | 21a |  23b |  31b | 92% |
| 3 | 21a |  23c |  31c | 90% |

| | | | | |
|----|-----|---|--|-----|
| 4 | 21a |  |  | 55% |
| 5 | 21a |  |  | 88% |
| 6 | 21b |  |  | 88% |
| 7 | 21b |  |  | 86% |
| 8 | 21b |  |  | 93% |
| 9 | 21b |  |  | 40% |
| 10 | 21b |  |  | 65% |
| 11 | 21c |  |  | 95% |

| | | | | |
|----|-----|--|---|-----|
| 12 | 21c |  23b |  31l | 89% |
| 13 | 21c |  23c |  31m | 78% |
| 14 | 21c |  23e |  31n | 87% |
| 15 | 21d |  23a |  31o | 90% |
| 16 | 21d |  23b |  31p | 51% |
| 17 | 21d |  23c |  31q | 47% |
| 18 | 21d |  23e |  31r | 71% |
| 19 | 21d |  23f |  31s | 79% |

| | | | | |
|----|-----|---|--|-----|
| 20 | 21d |  <p>23g</p> |  <p>31t</p> | 76% |
| 21 | 21d |  <p>23h</p> |  <p>31u</p> | 90% |
| 22 | 21d |  <p>23i</p> |  <p>31v</p> | 79% |
| 23 | 21d |  <p>23j</p> |  <p>31w</p> | 53% |
| 24 | 21d |  <p>23k</p> |  <p>31x</p> | 85% |
| 25 | 21d |  <p>23l</p> |  <p>31y</p> | 62% |
| 26 | 21d |  <p>23m</p> |  <p>31z</p> | 69% |





^[a] isolated yields

The structures of these analogues were confirmed by NMR and mass spectrometry, along with the structure of compound **31r** which was further confirmed by X-Ray crystallographic analysis (Figure C4. 17).

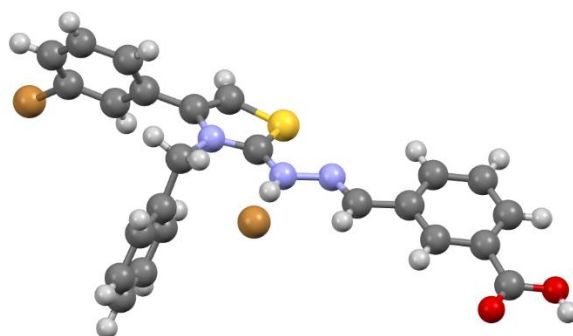


Figure C4. 17: X-Ray crystallographic structure of compound **31r**

3.2. Biological studies

Biological tests were performed at the ImpedanCELL platform (Caen) to these MIM1 analogues. The biological activity of these molecules was evaluated in an ovarian cancer cell line IGROV1-R10 whose survival depends on both Bcl-x_L and Mcl-1.⁸⁵ Whereas neither Bcl-x_L inhibition nor Mcl-1 inhibition led to cell death, their concomitant inhibition induces a massive apoptotic cell death.

Several molecules showed apoptotic activity in IGROV1-R10 cells, *via* Bcl-x_L inhibition (Mcl-1 inhibitors) or a combination with Mcl-1 inhibition and Bcl-x_L inhibition (dual inhibitors) at 10 μM or 25 μM. The Biological results are summarized in Table C4. 5.

Table C4. 5: Biological results of the revised-MIM1 analogues

| Entry | Compound | Biological Activity 25 μM | | Biological Activity 10 μM | |
|-------|----------|---------------------------|--------------------|---------------------------|--------------------|
| | | Mcl-1 | Bcl-x _L | Mcl-1 | Bcl-x _L |
| 1 | 31a | - | - | - | - |
| 2 | 31b | - | - | - | - |
| 3 | 31c | - | - | - | - |
| 4 | 31d | - | - | - | - |
| 5 | 31e | - | - | - | - |
| 6 | 31f | - | - | - | - |
| 7 | 31g | - | - | + | + |
| 8 | 31h | - | - | - | - |
| 9 | 31i | - | - | - | - |
| 10 | 31j | - | - | - | - |
| 11 | 31k | - | - | - | - |

| | | | | | |
|----|------|------|------|-----|---|
| 12 | 31l | - | - | - | - |
| 13 | 31m | - | - | - | - |
| 14 | 31n | - | - | - | - |
| 15 | 31o | + | - | - | - |
| 16 | 31p | + | - | - | - |
| 17 | 31q | ++ | ++ | - | - |
| 18 | 31r | ++ | ++ | ++ | - |
| 19 | 31s | + | + | - | - |
| 20 | 31t | ++ | ++ | + | + |
| 21 | 31u | + | + | - | - |
| 22 | 31v | + | + | - | - |
| 23 | 31w | + | + | - | - |
| 24 | 31x | - | - | - | - |
| 25 | 31y | +++ | +++ | +++ | - |
| 26 | 31z | + | - | - | - |
| 27 | 31aa | + | - | - | - |
| 28 | 31ab | + | - | - | - |
| 29 | 31ac | - | - | - | - |
| 30 | 31ad | - | - | - | - |
| 31 | 31ae | - | - | - | - |
| 32 | 31af | ++ | - | - | - |
| 33 | 31ag | ++++ | ++++ | + | + |
| 34 | 31ah | ++++ | ++++ | - | - |
| 35 | 31ai | - | - | - | - |
| 36 | 31aj | - | - | - | - |
| 37 | 31ak | - | - | - | - |
| 38 | 31al | - | - | - | - |

(-): No activity / (+): Low activity / (++): Active / (+++): good activity / (++++): very good activity

Some biological tests were carried out based on a fluorescent probe whose intensity increases by binding to cleaved caspase 3 and 7, witnesses of apoptosis. Fluorescence intensity monitoring is performed using the Incucyte S3.

By comparison with the curves obtained using MIM1 analogues **31** or siRNAs used alone (Figure C4. 18), **31f** has no specific effect and its fluorescence peak can be attributed to the inhibition of Bcl-x_L by RNA interference (less specific and more damaging to the cell than pharmacological molecules). **31o** molecule has a weak activity on Mcl-1 with a stronger activation of caspases in combination with siRNA Mcl-1 compared when used alone and molecule **31r** has only weak activity on Mcl-1.

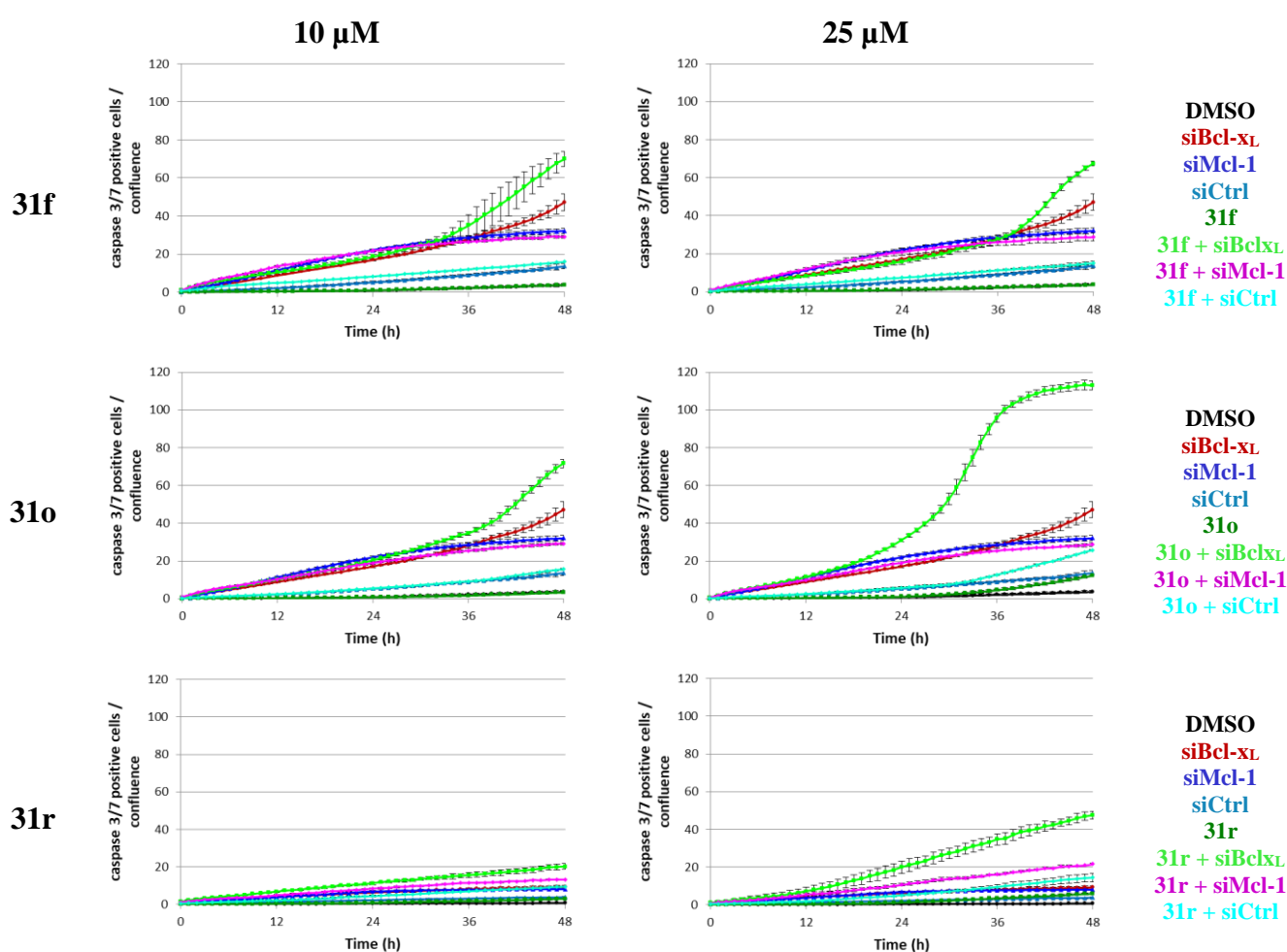


Figure C4. 18: Caspase 3 and 7 cleavage monitored by the Incucyte S3 after the exposure of IGROV1-R10 to MIM1 analogues **31f**, **31o** and **31r**, combined or not to siBcl-x_L or siMcl-1.

The RNA interference technology was then stopped in favour of pharmacological molecules (S63845 to inhibit Mcl-1 and ABT-737 to inhibit Bcl-x_L). The Incucyte technique with a Caspase 3/7 probe has been abandoned to study the effect of molecules by xCELLigence which measures impedance in culture wells. This integrative parameter takes into account the strength of the adhesion of the cells, their spreading, their number ... It does not make it possible to

characterize cell death directly, but our results tend to show that the fall of the curves is directly correlated (in the present case) to cell death by apoptosis.

As a result, molecule **31y** showed good specific activity toward Mcl-1 protein visible from 10 μM . At 25 μM the effect is not increased but the selectivity toward Mcl-1 protein decreased by targeting also Bcl-x_L. Compound **31ag** gave a weak bispecific activity at 10 μM but powerful activity at 25 μM , similar to compound **31ah** which also presents a strong bispecific inhibition at 25 μM (Figure C4. 19).

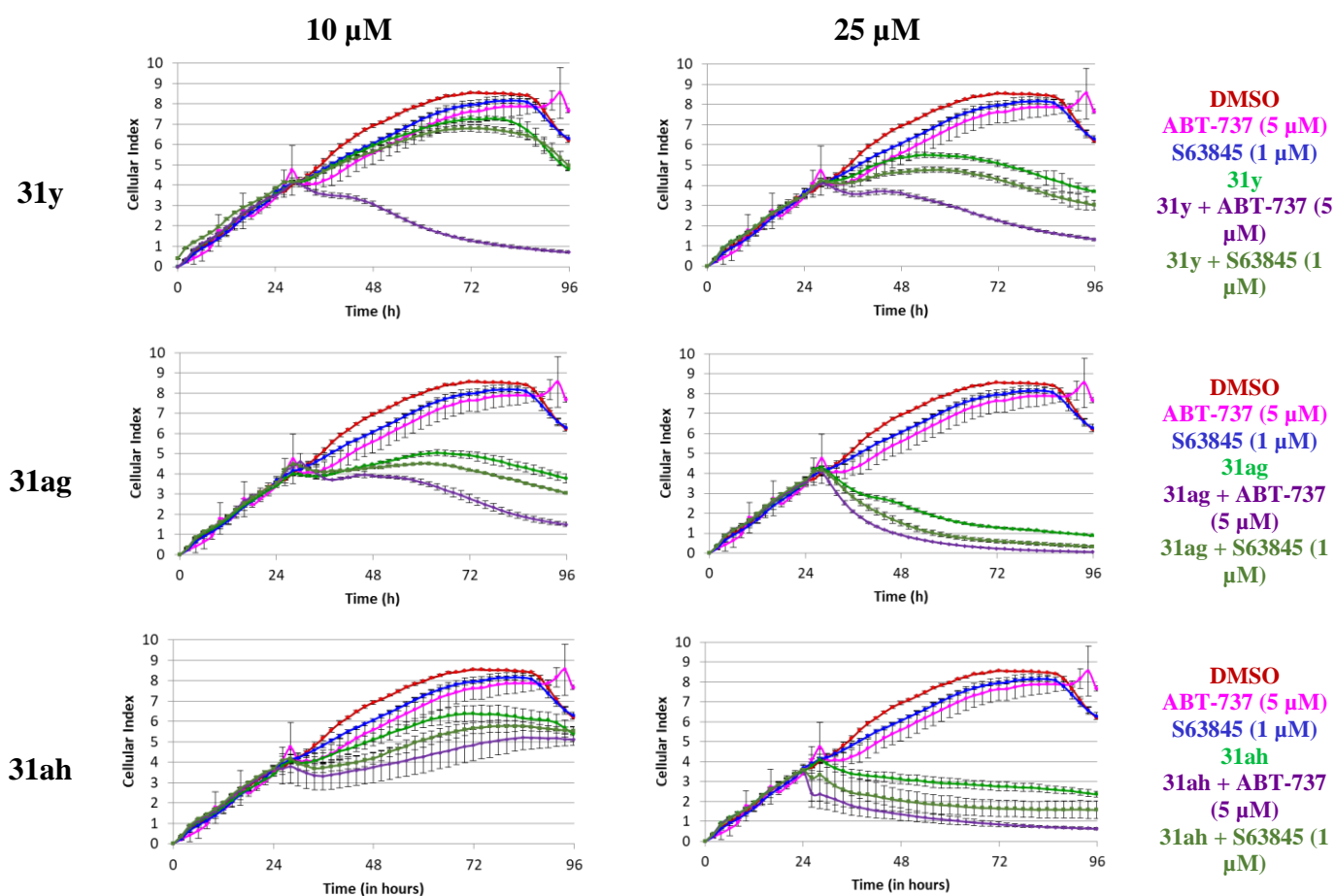


Figure C4. 19: Cells monitored by the xCELLigence after the exposure of IGROV1-R10 to MIM1 analogues **31y**, **31ag** and **31ah**, combined or not to ABT-737 or S63845.

3.3. Structure-activity relationship using molecular modeling studies

Based on the biological activities, we were able to understand the structure-activity relationship of the MIM1 analogues.

The *meta*-acid is essential in the binding to the Mcl-1 and Bcl-x_L proteins, thus neither the acid in *para* position nor the acid in *meta* position along with an alcohol in *para* position were active (Figure C4. 20). In addition to that no biological activity was observed when R₁ was

either a phenyl or pyridine groups, thus a benzyl group at R₁ is highly essential to have a biological activity (Figure C4. 20). It was also noticed that the best biological activities were achieved by modifying R₄ and R₇.

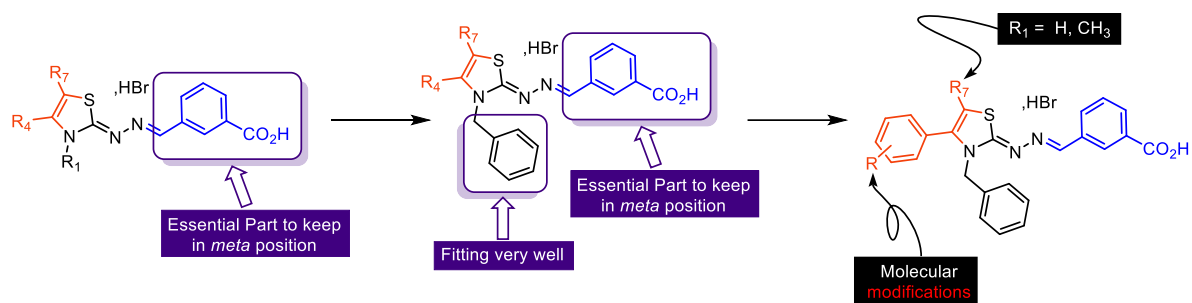


Figure C4. 20: Essential molecular modifications.

As a result, there was a remarkable increase in the biological activity between **31o** and **31r** showing how the bromine in position *meta* is filling better the P2 pocket, affording a more potent derivative (Figure C4. 21).

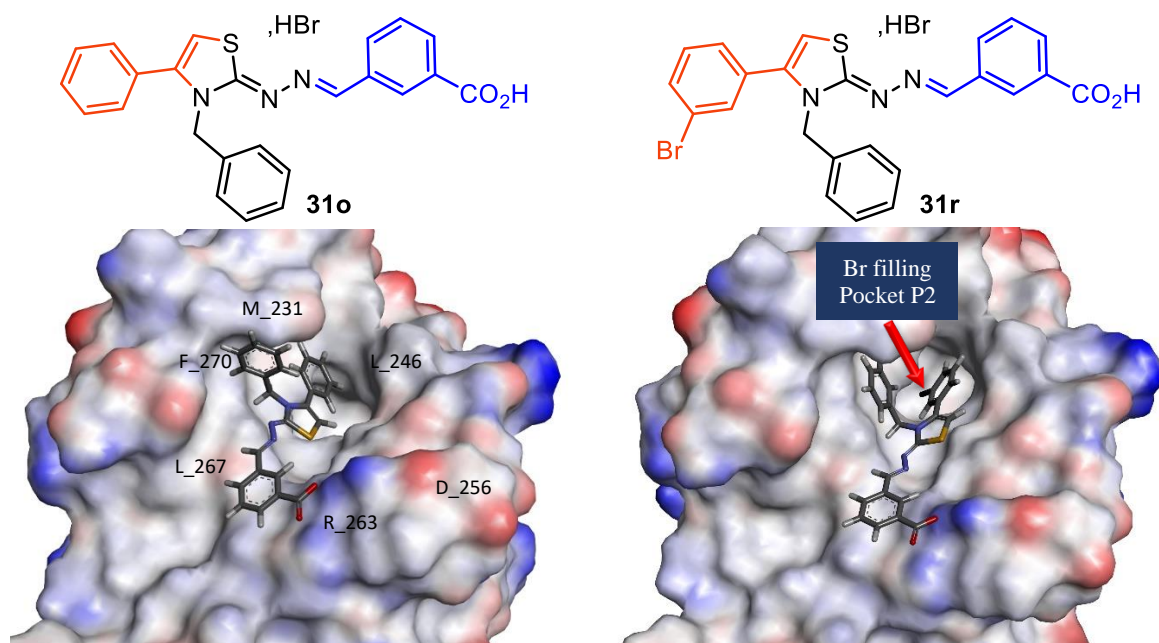


Figure C4. 21: Molecular docking of compounds **31o** and **31r** in Mcl-1 protein

On the other hand, when the *meta*-Br was substituted with a phenoxy group in *ortho* position **31y**, a more selective and potent derivative was obtained, caused by a flip of the ligand in the binding site thus filling better pocket P2. Thus, the thiazole cycle is located under M₂₃₁ and buried around F₂₇₀, V₂₄₉ and L₂₆₇, within a cavity that extends up to L₁₈₆, L₂₉₀, L₂₄₆ and I₂₉₄. The strong hydrophobicity of the cavity explains the preference for hydrophobic substituents of the thiazole which are engulfed deeply inside pocket P2 (Figure C4. 22).

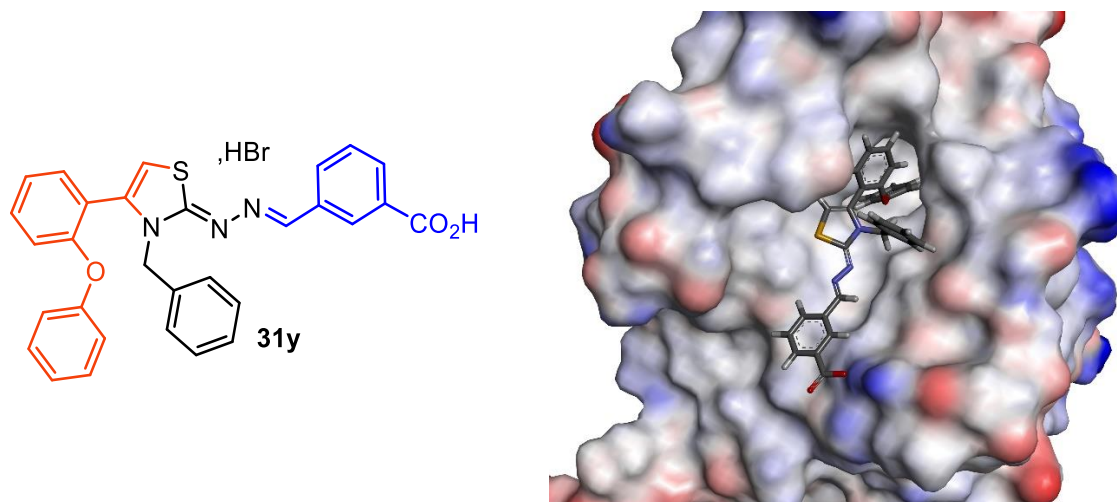


Figure C4. 22: Molecular docking of compound **31y** inside Mcl-1 protein

Surprisingly, when R7 was a methyl group, as in **31ag** and **31ah**, a strong dual inhibition of both Mcl-1 and Bcl-x_L proteins was observed at 25 μL. This could be explained by molecular modeling which shows how the phenoxy group fits better inside pocket P2 and the methyl group lay under M_231 (Figure C4. 23). These results are still under studies to understand well the loss of selectivity toward Mcl-1 protein.

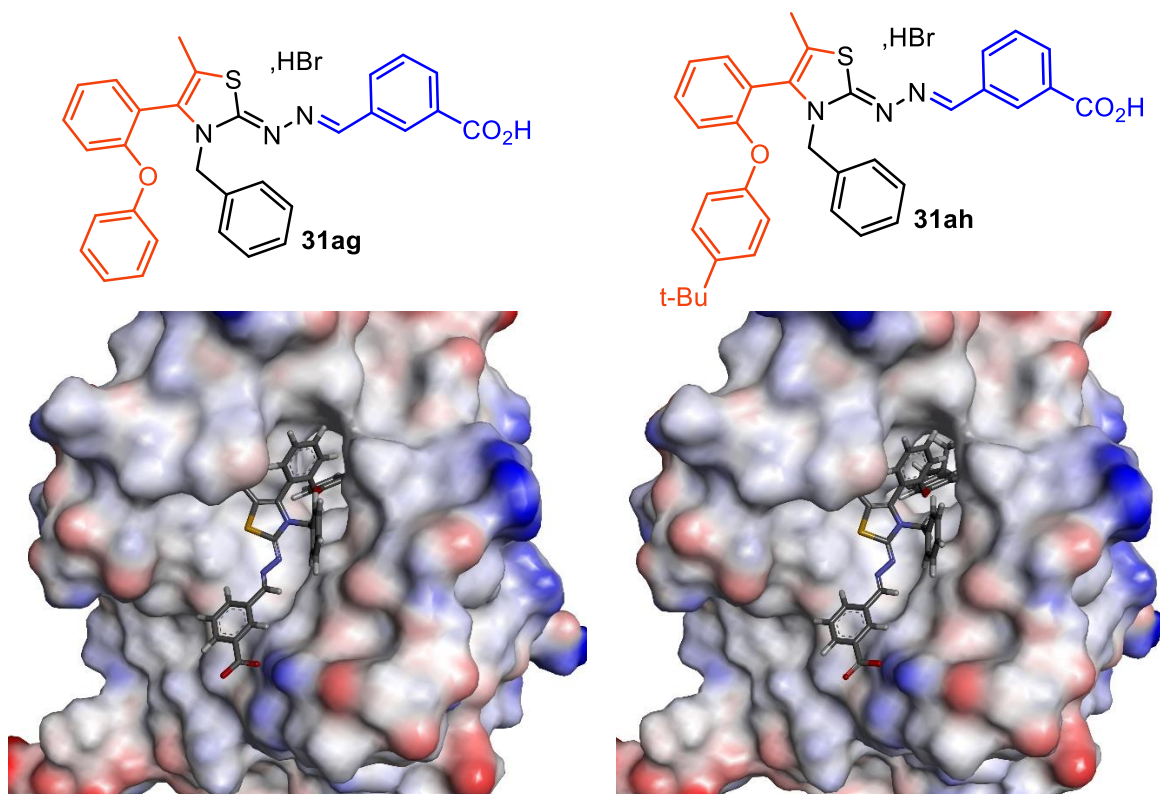
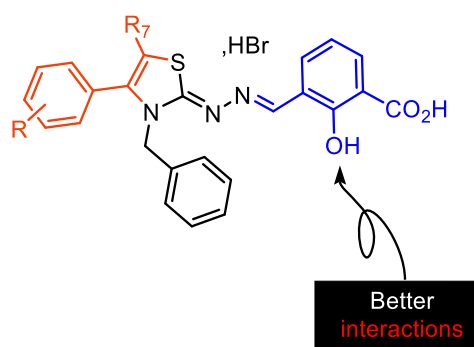


Figure C4. 23: Molecular docking of compounds **31ag** and **31ah** inside Mcl-1 protein

4. Conclusion and perspectives

In conclusion, after revision of the structure of MIM-1, we have prepared a chemical library of designed analogues of this molecule. Detailed biological studies using the ovarian cancer cell lines, IGROV1-R10 allowed us to discover derivatives which are potent dual inhibitors of Bcl-x_L and Mcl-1. All these data gave us a preliminary Structure-Activity relationship for these new MIM1 analogues. Extensive computational studies allowed us to propose a rationale to this SAR, highlighting the role of the *meta*-acid moiety to anchor these molecules to the anti-apoptotic proteins and the position of the aromatic/benzyl groups inside the hydrophobic pockets of these proteins.

Additional analysis is needed to better understand the binding mode of compounds **31ag** and **31ah** in Mcl-1 and Bcl-x_L. Moreover, it would be noteworthy to study the effect of an *ortho*-alcohol group combined with a *meta*-acid to increase the binding affinity toward R₂₆₃ and D₂₅₆.



5. Experimental part

5.1. General Methods

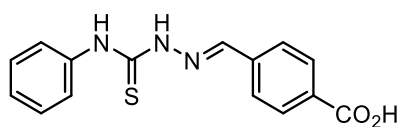
NMR spectra were performed on a Bruker AVANCE 300 or Bruker AVANCE 500 (^1H , 300 MHz or 500 MHz; ^{13}C , 75 MHz or 126 MHz; ^{19}F , 282 MHz or 471 MHz). Solvent peaks were used as reference values with CDCl_3 at 7.26 ppm for ^1H NMR and 77.16 ppm for ^{13}C NMR, with CD_3CN at 1.94 ppm for ^1H NMR and 1.32 and 118.26 ppm for ^{13}C NMR, with $\text{DMSO-}d_6$ at 2.5 ppm for ^1H NMR and 39.52 for ^{13}C NMR. Chemical shifts δ are given in ppm, and the following abbreviations are used: singlet (s), broad singlet (bs), doublet (d), doublet of doublet (dd), doublet of doublet of doublet (ddd), triplet (t), triplet of doublet (td), quadruplet (q), doublet of quadruplet (dq), and multiplet (m). High resolution mass spectra were recorded in the Centre Régional de Mesures Physiques de l'Ouest, Rennes (CRMPO), on a Maxis 4G. Melting points were determined with uncertainty of $\pm 2^\circ\text{C}$ using a KOFER BENCH. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F254 Merck plates), and compounds were visualized under a UVP Mineralight UVGL-58 lamp (254 nm) and with *p*-anisaldehyde/ Δ . Column chromatography was performed using silica gel 60 (40–63 mm, 230–400 mesh). Solvents were used as received from commercial sources. Reagents were purchased from Sigma-Aldrich, Alfa Aesar and Acros. All products reported showed ^1H and ^{13}C spectra in agreement with the assigned structures.

5.2. Experimental Procedures and Analytical data

19. General procedure 1: Synthesis of imino-thiosemicarbazides intermediates 21a-f

19.1. Representative procedure: synthesis of (E)-4-((2-(phenylcarbamothioyl)hydrazono)methyl)benzoic acid 21a

A methanolic (20 ml) solution of N-phenylhydrazinecarbothioamide **19a** (2 g, 11.96 mmol) was added to a solution of 4-formylbenzoic acid **20a** (2.7 g, 17.9 mmol) in methanol (35 mL) and the reaction mixture was refluxed for 6h. The product formed was filtered, washed with methanol, and dried under vacuum to obtain (E)-4-((2-(phenylcarbamothioyl)hydrazono)methyl)benzoic acid **21a** as yellow solid (3.4 g, 95%).



21a

Chemical Formula: C₁₅H₁₃N₃O₂S

Molecular Weight: 299.35

mp > 260 °C

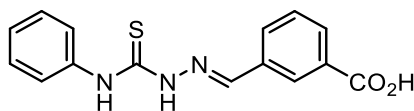
¹H NMR (300 MHz, DMSO-*d*₆) δ 11.93 (s, 1H), 10.21 (s, 1H), 8.20 (s, 1H), 8.03 (d, ³*J*_{H-H} = 8.5 Hz, 2H), 7.96 (d, ³*J*_{H-H} = 8.5 Hz, 2H), 7.59 – 7.50 (m, 2H), 7.44 – 7.32 (m, 1H), 7.25 – 7.17 (m, 1H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 176.4, 167.1, 141.7, 139.1, 138.2, 131.7, 129.6, 128.2, 127.7, 126.2, 125.6.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₅H₁₃N₃O₂NaS) = 322.0621; found: 322.0621 (0 ppm).

19.2. (E)-3-((2-(phenylcarbamothioyl)hydrazono)methyl)benzoic acid **21b**

According to the general procedure 1, yellow solid was obtained, yield 3.1 g (86%) from 2 g (11.9 mmol) of **19a** and 2.7 g (17.9 mmol) **20b**.



21b

Chemical Formula: C₁₅H₁₃N₃O₂S

Molecular Weight: 299.35

mp > 260 °C

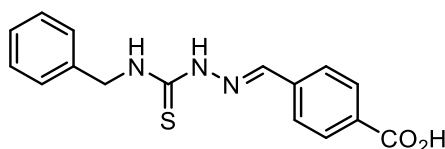
¹H NMR (500 MHz, DMSO-*d*₆) δ 13.16 (bs, 1H), 11.88 (s, 1H), 10.23 (s, 1H), 8.32 – 8.20 (m, 3H), 7.97 (dt, ³*J*_{H-H} = 7.7 Hz, ⁴*J*_{H-H} = 1.5 Hz, 1H), 7.60 – 7.52 (m, 3H), 7.43 – 7.32 (m, 2H), 7.27 – 7.17 (m, 1H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 176.3, 167.1, 142.2, 139.1, 134.6, 131.4, 131.3, 130.6, 129.0, 128.7, 128.1, 126.2, 125.5.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₅H₁₃N₃O₂NaS) = 322.0621; found: 322.0619 (0 ppm).

19.3. (E)-4-((2-(benzylcarbamothioyl)hydrazono)methyl)benzoic acid **21c**

According to the general procedure 1, white solid was obtained, yield 2.8 g (81%) from 2 g (11.0 mmol) of **19b** and 2.7 g (17.9 mmol) **20a**.



21c

Chemical Formula: C₁₆H₁₅N₃O₂S

Molecular Weight: 313.38

mp = 235 °C

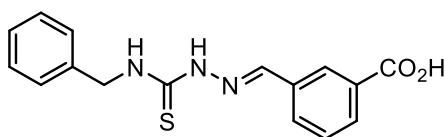
¹H NMR (300 MHz, DMSO-*d*₆) δ 11.75 (s, 1H), 9.22 (t, ³*J*_{H-H} = 6.3 Hz, 1H), 7.94 (s, 4H), 7.40 – 7.19 (m, 5H), 4.85 (d, ³*J*_{H-H} = 6.3 Hz, 2H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 177.8, 167.0, 141.0, 139.4, 138.4, 131.5, 129.6, 128.2, 127.3, 127.3, 126.8, 46.7.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₆H₁₅N₃O₂NaS) = 336.0777; found: 336.0779 (0 ppm).

19.4. (E)-3-((2-(benzylcarbamothioyl)hydrazono)methyl)benzoic acid **21d**

According to the general procedure 1, white solid was obtained, yield 2.9 g (85%) from 2 g (11.0 mmol) of **19b** and 2.7 g (17.9 mmol) **20b**.



21d

Chemical Formula: C₁₆H₁₅N₃O₂S

Molecular Weight: 313.38

mp = 235 °C

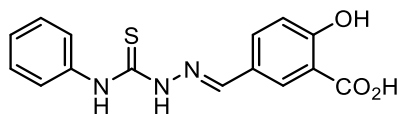
¹H NMR (300 MHz, DMSO-*d*₆) δ 11.65 (s, 1H), 9.17 (t, ³*J*_{H-H} = 6.2 Hz, 1H), 8.23 (t, ⁴*J*_{H-H} = 1.7 Hz, 1H), 8.18 (s, 1H), 8.14 (dt, ³*J*_{H-H} = 7.8 Hz, ⁴*J*_{H-H} = 1.5 Hz, 1H), 7.95 (dt, ³*J*_{H-H} = 7.8 Hz, ⁴*J*_{H-H} = 1.5 Hz, 1H), 7.54 (t, ³*J*_{H-H} = 7.8 Hz, 1H), 7.42 – 7.14 (m, 5H), 4.87 (d, ³*J*_{H-H} = 6.2 Hz, 2H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 177.7, 167.1, 141.7, 139.5, 134.7, 131.5, 131.1, 130.5, 129.0, 128.4, 128.2, 127.2, 126.8, 46.7.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₆H₁₅N₃O₂NaS) = 336.0777; found: 336.0778 (0 ppm).

19.5. (E)-2-hydroxy-5-((2-(phenylcarbamothioyl)hydrazono)methyl)benzoic acid **21e**

According to the general procedure 1, white solid was obtained, yield 1.2 g (65%) from 1 g (5.98 mmol) of **19a** and 1.5 g (9.0 mmol) **20c**.



21e

Chemical Formula: C₁₅H₁₃N₃O₃S

Molecular Weight: 315.35

mp > 260 °C

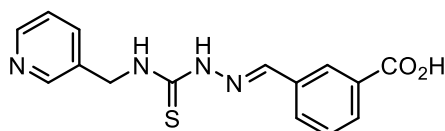
¹H NMR (300 MHz, DMSO-*d*₆) δ 12.09 (s, 1H), 8.50 (d, ⁴*J*_{H-H} = 1.8 Hz, 1H), 7.99 (s, 1H), 7.41 – 7.22 (m, 5H), 7.03 (dd, ³*J*_{H-H} = 8.4 Hz, ⁴*J*_{H-H} = 0.8 Hz, 1H), 6.95 (tt, ³*J*_{H-H} = 7.8, ⁴*J*_{H-H} = 1.2 Hz, 1H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 179.0, 171.3, 164.8, 142.5, 139.0, 135.2, 129.1, 128.7, 127.7, 124.7, 123.9, 114.4, 113.2.

HRMS (ESI): calcd. for [M+Na]⁺ (C₁₅H₁₃N₃O₃NaS) = 338.3368; found: 338.3368 (0 ppm).

19.6. (E)-3-((2-((pyridin-3-ylmethyl)carbamothioyl)hydrazono)methyl)benzoic acid **21f**

According to the general procedure 1, yellow solid was obtained, yield 664 mg (77%) from 500 mg (2.7 mmol) of **19c** and 616 mg (4.1 mmol) **20b**.



21f

Chemical Formula: C₁₅H₁₄N₄O₂S

Molecular Weight: 314.36

mp = 215 °C

¹H NMR (300 MHz, DMSO-*d*₆) δ 11.70 (s, 1H), 8.56 (dd, ⁴*J*_{H-H} = 1.9 Hz, ⁴*J*_{H-H} = 1.5 Hz, 1H), 8.47 (dt, ³*J*_{H-H} = 4.7 Hz, ⁴*J*_{H-H} = 1.9 Hz, 1H), 8.25 (t, ⁴*J*_{H-H} = 1.7 Hz, 1H), 7.98 (s, 1H), 7.87 (dd, ³*J*_{H-H} = 7.9 Hz, ⁴*J*_{H-H} = 1.7 Hz, 1H), 7.72 (dd, ³*J*_{H-H} = 8 Hz, ⁴*J*_{H-H} = 1.9 Hz, 1H), 7.61 (d, ³*J*_{H-H} = 7.9 Hz, 1H), 7.47 (ddd, ³*J*_{H-H} = 7.9 Hz, ⁴*J*_{H-H} = 1.7 Hz, ⁴*J*_{H-H} = 1.5 Hz, 1H), 7.36 (dd, ³*J*_{H-H} = 8.0 Hz, ³*J*_{H-H} = 4.7 Hz, 1H), 4.51 (s, 2H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 179.0, 168.2, 149.8, 148.6, 142.5, 135.5, 135.2, 134.9, 132.3, 129.7, 129.2, 127.7, 126.5, 12.7, 48.7.

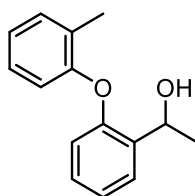
HRMS (ESI): calcd. for [M+Na]⁺ (C₁₅H₁₄N₄O₂NaS) = 337.0735; found: 337.0736 (1 ppm).

20. General procedure 2: Synthesis of alcohols 47d-j

In an ace pressure tube, the corresponding hydroxybenzaldehyde **42** or **43** (500 mg, 4.1 mmol) was added, followed by the addition of the desired substituted-bromobenzene **44a-i** (8.2 mmol), Cs₂CO₃ (2.7 g, 8.2 mmol), pyridin-2-amine (77 mg, 0.8 mmol), and CuI (156 mg, 0.8 mmol). 5 mL DMF was then added and the mixture was left to stir for 48 h at 135 °C. At the end saturated NH₄Cl (10 mL) was added and the reaction mixture was extracted with ethyl acetate (3x20 mL). The organic layers were separated, washed with water (1x10 mL), dried over Na₂SO₄ and concentrated under vacuum. A fast silica column was done, where the product **45a-j** and the hydroxybenzaldehyde have the same polarity. Consequently, the next step was to add Grignard reagent (1.5 equiv) to the crude mixture in THF and stir them under nitrogen gas at 0 °C. The reaction was monitored by TLC, then saturated NH₄Cl (10 mL) was added and the reaction mixture was extracted with ethyl acetate (3x20 mL). The organic layers were separated, washed with water (1x10 mL), dried over Na₂SO₄ and concentrated under vacuum. After purification by chromatography on silica gel alcohols **47a-l** were obtained.

20.1. 1-(2-(o-tolyloxy)phenyl)ethan-1-ol **47d**

The reaction was performed according to the general procedure 2. Colorless oil yield 532 mg (57%).



47d

Chemical Formula: C₁₅H₁₆O₂

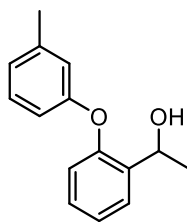
Molecular Weight: 228.29

¹H NMR (300 MHz, CDCl₃) δ 7.54 – 7.48 (m, 1H), 7.29 – 7.25 (m, 1H), 7.22 – 7.04 (m, 4H), 6.85 (dd, ³J_{H-H} = 8.0 Hz, ⁴J_{H-H} = 1.4 Hz, 1H), 6.68 – 6.58 (m, 1H), 5.25 (q, ³J_{H-H} = 6.5 Hz, 1H), 2.37 (bs, 1H), 2.27 (s, 3H), 1.58 (d, ³J_{H-H} = 6.5 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 154.5, 154.4, 135.3, 131.6, 129.7, 128.5, 127.4, 126.6, 124.1, 123.1, 119.2, 116.5, 66.2, 23.8, 16.3.

20.2. 1-(2-(m-tolyloxy)phenyl)ethan-1-ol **47e**

The reaction was performed according to the general procedure 2. Pale yellow oil yield 393 mg (42%).



47e

Chemical Formula: C₁₅H₁₆O₂

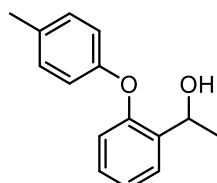
Molecular Weight: 228.29

¹H NMR (300 MHz, CDCl₃) δ 7.57 – 7.48 (m, 1H), 7.25 – 7.09 (m, 3H), 6.96 – 6.70 (m, 4H), 5.19 (q, ³J_{H-H} = 6.5 Hz, 1H), 2.33 (s, 3H), 1.97 (bs, 1H), 1.53 (d, ³J_{H-H} = 6.5 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 157.3, 154.0, 140.2, 136.6, 129.7, 128.5, 126.7, 124.2, 123.9, 119.2, 118.9, 115.5, 66.0, 23.8, 21.5.

20.3. 1-(2-(p-tolyloxy)phenyl)ethan-1-ol 47f

The reaction was performed according to the general procedure 2. colorless oil yield 439 mg (47%).



47f

Chemical Formula: C₁₅H₁₆O₂

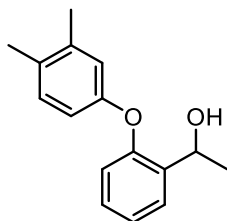
Molecular Weight: 228.29

¹H NMR (300 MHz, CDCl₃) δ 7.55 – 7.46 (m, 1H), 7.23 – 7.05 (m, 4H), 6.93 – 6.74 (m, 3H), 5.21 (q, ³J_{H-H} = 6.5 Hz, 1H), 2.34 (s, 3H), 1.54 (d, ³J_{H-H} = 6.5 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 154.9, 154.4, 136.3, 133.0, 130.4, 128.5, 126.7, 123.6, 118.7, 118.3, 66.0, 23.8, 20.8.

20.4. 1-(2-(3,4-dimethylphenoxy)phenyl)ethan-1-ol 47g

The reaction was performed according to the general procedure 2. colorless oil yield 526 mg (53%).



47g

Chemical Formula: C₁₆H₁₈O₂

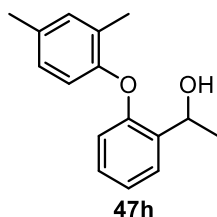
Molecular Weight: 242.32

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.53 – 7.44 (m, 1H), 7.23 – 7.02 (m, 3H), 6.87 – 6.75 (m, 2H), 6.72 (dd, $^3J_{\text{H-H}} = 8.1$ Hz, $^4J_{\text{H-H}} = 2.7$ Hz, 1H), 5.20 (q, $^3J_{\text{H-H}} = 6.5$ Hz, 1H), 2.24 (s, 6H), 1.54 (d, $^3J_{\text{H-H}} = 6.5$ Hz, 3H).

$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 155.0, 154.5, 138.5, 136.2, 131.8, 130.8, 128.5, 126.6, 123.5, 120.1, 118.3, 116.0, 66.1, 23.7, 20.1, 19.1.

20.5. 1-(2-(2,4-dimethylphenoxy)phenyl)ethan-1-ol 47h

The reaction was performed according to the general procedure 2. colorless oil yield 387 mg (39%).



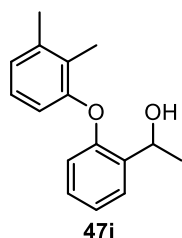
Chemical Formula: $\text{C}_{16}\text{H}_{18}\text{O}_2$
Molecular Weight: 242.32

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.52 – 7.47 (m, 1H), 7.18 – 6.94 (m, 4H), 6.78 (d, $^3J_{\text{H-H}} = 8.2$ Hz, 1H), 6.61 (dd, $^3J_{\text{H-H}} = 8.2$ Hz, $^4J_{\text{H-H}} = 1.3$ Hz, 1H), 5.27 (q, $^3J_{\text{H-H}} = 6.5$ Hz, 1H), 2.58 (bs, 1H), 2.34 (s, 3H), 2.22 (s, 3H), 1.59 (d, $^3J_{\text{H-H}} = 6.5$ Hz, 3H).

$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 154.8, 151.9, 135.0, 133.7, 132.2, 129.5, 128.3, 127.8, 126.5, 122.6, 119.5, 115.8, 66.1, 23.7, 20.8, 16.2.

20.6. 1-(2-(2,3-dimethylphenoxy)phenyl)ethan-1-ol 47i

The reaction was performed according to the general procedure 2. Pale yellow oil yield 377 mg (38%).



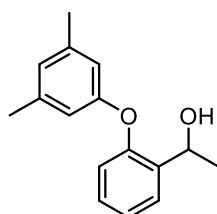
Chemical Formula: $\text{C}_{16}\text{H}_{18}\text{O}_2$
Molecular Weight: 242.32

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.54 – 7.47 (m, 1H), 7.20 – 6.94 (m, 4H), 6.75 – 6.69 (m, 1H), 6.63 – 6.57 (m, 1H), 5.26 (q, $^3J_{\text{H-H}} = 6.5$ Hz, 1H), 2.43 (bs, 1H), 2.34 (s, 3H), 2.18 (s, 3H), 1.59 (d, $^3J_{\text{H-H}} = 6.5$ Hz, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ 154.9, 154.2, 139.1, 135.1, 128.5, 128.4, 126.6, 126.5, 125.7, 122.8, 117.1, 116.4, 66.3, 23.7, 20.2, 12.3.

20.7. 1-(2-(3,5-dimethylphenoxy)phenyl)ethan-1-ol **47j**

The reaction was performed according to the general procedure 2. Pale yellow oil yield 506 mg (51%).



47j

Chemical Formula: $\text{C}_{16}\text{H}_{18}\text{O}_2$

Molecular Weight: 242.32

^1H NMR (300 MHz, CDCl_3) δ 7.54 – 7.48 (m, 1H), 7.25 – 7.10 (m, 2H), 6.87 – 6.83 (m, 1H), 6.79 – 6.71 (m, 1H), 6.65 – 6.57 (m, 2H), 5.19 (q, $^3J_{\text{H-H}} = 6.5$ Hz, 1H), 2.29 (s, 6H), 2.18 (bs, 1H), 1.53 (d, $^3J_{\text{H-H}} = 6.5$ Hz, 3H).

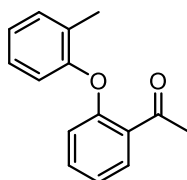
^{13}C NMR (75 MHz, CDCl_3) δ 157.2, 154.1, 139.8, 136.5, 128.5, 126.7, 125.2, 123.8, 118.9, 116.2, 66.0, 23.8, 21.4.

21. General procedure 3: Synthesis of Ketones **47a-l**

To the obtained alcohols **47a-l** (1.24 mmol) in acetone (10 mL) was added dropwise under magnetic stirring at room temperature, a concentrated (5.4 M) solution of Jones reagent until disappearance of the starting material (TLC analysis). After addition of isopropanol (5.0 equiv), the reaction mixture was filtered, and the filtrate was extracted with ethyl acetate (3x40mL). The combined organic phases were dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. After purification by chromatography on silica gel, the propargylic ketones **48a-l** were obtained.

21.1. 1-(3-(*o*-tolylloxy)phenyl)ethan-1-one **48d**

The reaction was performed according to the general procedure 3. Pale yellow oil yield 255 mg (91%) starting from **47d** 283 mg (1.24 mmol).



48d

Chemical Formula: C₁₅H₁₄O₂

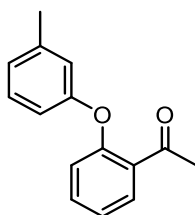
Molecular Weight: 226.28

¹H NMR (300 MHz, CDCl₃) δ 7.85 (ddd, ³J_{H-H} = 7.7 Hz, ⁴J_{H-H} = 1.8 Hz, ⁵J_{H-H} = 0.4 Hz, 1H), 7.37 (ddd, ³J_{H-H} = 8.3 Hz, ³J_{H-H} = 7.3 Hz, ⁵J_{H-H} = 1.8 Hz, 1H), 7.33 – 7.27 (m, 1H), 7.24 – 7.16 (m, 1H), 7.15 – 7.07 (m, 2H), 6.90 (dd, ³J_{H-H} = 8.0 Hz, ⁴J_{H-H} = 1.4 Hz, 1H), 6.69 (dd, ³J_{H-H} = 8.3, ⁴J_{H-H} = 1.0 Hz, 1H), 2.70 (s, 3H), 2.27 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 199.3, 157.2, 153.8, 133.8, 131.8, 130.7, 130.0, 129.4, 127.6, 124.8, 122.6, 119.7, 117.1, 32.0, 16.4.

21.2. 1-(2-(m-tolylloxy)phenyl)ethan-1-one 48e

The reaction was performed according to the general procedure 3. Pale yellow oil yield 250 mg (89%) starting from **47d** 283 mg (1.24 mmol).



48e

Chemical Formula: C₁₅H₁₄O₂

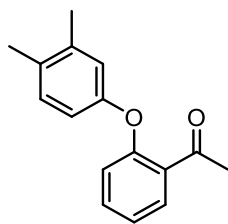
Molecular Weight: 226.28

¹H NMR (300 MHz, CDCl₃) δ 7.84 (dd, ³J_{H-H} = 7.8 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.42 (ddd, ³J_{H-H} = 8.3 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.25 (t, ³J_{H-H} = 7.7 Hz, 1H), 7.16 (ddd, ³J_{H-H} = 7.8 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.1 Hz, 1H), 6.96 (ddq, ³J_{H-H} = 7.7 Hz, ⁴J_{H-H} = 1.5 Hz, ⁵J_{H-H} = 0.8 Hz, 1H), 6.90 (dd, ³J_{H-H} = 8.3 Hz, ⁴J_{H-H} = 1.1 Hz, 1H), 6.86 – 6.78 (m, 2H), 2.64 (s, 3H), 2.35 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 199.2, 156.7, 156.5, 140.5, 133.8, 130.6, 130.5, 129.9, 124.8, 123.4, 119.7, 119.4, 116.0, 31.7, 21.5.

21.3. 1-(2-(3,4-dimethylphenoxy)phenyl)ethan-1-one 48g

The reaction was performed according to the general procedure 3. colorless oil yield 259 mg (87%) starting from **47i** 300 mg (1.24 mmol).



48g

Chemical Formula: C₁₆H₁₆O₂

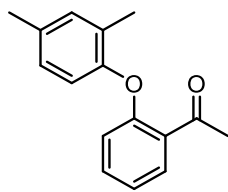
Molecular Weight: 240.30

¹H NMR (300 MHz, CDCl₃) δ 7.83 (ddd, ³J_{H-H} = 7.8 Hz, ⁴J_{H-H} = 1.8 Hz, ⁵J_{H-H} = 0.4 Hz, 1H), 7.40 (ddd, ³J_{H-H} = 8.3 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.18 – 7.07 (m, 2H), 6.88 (dd, ³J_{H-H} = 8.3 Hz, ⁴J_{H-H} = 1.1 Hz, 1H), 6.84 – 6.81 (m, 1H), 6.76 (dd, ³J_{H-H} = 8.1 Hz, ⁴J_{H-H} = 2.7 Hz, 1H), 2.66 (s, 3H), 2.25 (s, 6H).

¹³C NMR (75 MHz, CDCl₃) δ 199.4, 157.2, 154.2, 138.7, 133.7, 132.4, 131.0, 130.6, 130.2, 123.1, 120.5, 118.9, 116.5, 31.8, 20.1, 19.2.

21.4. 1-(2-(2,4-dimethylphenoxy)phenyl)ethan-1-one 48h

The reaction was performed according to the general procedure 3. colorless oil yield 241 mg (81%) starting from **47i** 300 mg (1.24 mmol).



48h

Chemical Formula: C₁₆H₁₆O₂

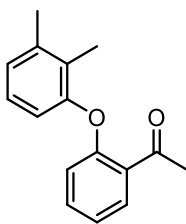
Molecular Weight: 240.30

¹H NMR (300 MHz, CDCl₃) δ 7.83 (dd, ³J_{H-H} = 7.8, ⁴J_{H-H} = 1.9 Hz, 1H), 7.34 (ddd, ³J_{H-H} = 8.4, ³J_{H-H} = 7.3, ⁴J_{H-H} = 1.8 Hz, 1H), 7.12 – 6.96 (m, 3H), 6.81 (d, ³J_{H-H} = 8.2 Hz, 1H), 6.66 (dd, ³J_{H-H} = 8.4, ³J_{H-H} = 1.1 Hz, 1H), 2.71 (s, 3H), 2.33 (s, 3H), 2.21 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 199.5, 157.7, 151.3, 134.5, 133.8, 132.5, 130.7, 129.9, 129.1, 128.1, 122.3, 120.0, 116.6, 32.1, 20.9, 16.3.

21.5. 1-(2-(2,3-dimethylphenoxy)phenyl)ethan-1-one 48i

The reaction was performed according to the general procedure 3. colorless oil yield 283 mg (95%) starting from **47i** 300 mg (1.24 mmol).



48i

Chemical Formula: C₁₆H₁₆O₂

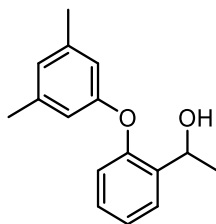
Molecular Weight: 240.30

¹H NMR (300 MHz, CDCl₃) δ 7.85 (dd, ³J_{H-H} = 7.8 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.35 (ddd, ³J_{H-H} = 8.3 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.14 – 7.00 (m, 3H), 6.77 (d, ³J_{H-H} = 7.8 Hz, 1H), 6.66 (dd, ³J_{H-H} = 8.3 Hz, ⁴J_{H-H} = 1.1 Hz, 1H), 2.71 (s, 3H), 2.35 (s, 3H), 2.18 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 199.3, 157.6, 153.5, 139.3, 133.8, 130.7, 129.2, 128.8, 126.7, 126.3, 122.4, 117.6, 117.0, 32.0, 20.2, 12.4.

21.6. 1-(2-(3,5-dimethylphenoxy)phenyl)ethan-1-one **48j**

The reaction was performed according to the general procedure 3. colorless oil yield 259 mg (87%) starting from **47j** 300 mg (1.24 mmol).



48j

Chemical Formula: C₁₆H₁₈O₂

Molecular Weight: 242.32

¹H NMR (300 MHz, CDCl₃) δ 7.84 (dd, ³J_{H-H} = 7.8 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.42 (ddd, ³J_{H-H} = 8.3 Hz, ³J_{H-H} = 7.2 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.15 (ddd, ³J_{H-H} = 7.8 Hz, ³J_{H-H} = 7.2 Hz, ⁴J_{H-H} = 1.1 Hz, 1H), 6.91 (dd, ³J_{H-H} = 8.3 Hz, ⁴J_{H-H} = 1.1 Hz, 1H), 6.83 – 6.74 (m, 1H), 6.64 (s, 2H), 2.64 (s, 3H), 2.30 (s, 3H), 2.30 (s, 3H).

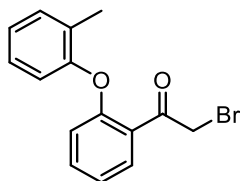
¹³C NMR (75 MHz, CDCl₃) δ 199.3, 156.8, 156.5, 140.1, 133.7, 130.6, 130.4, 125.8, 123.3, 119.5, 116.7, 31.8, 21.4.

22. General procedure 4: Synthesis of bromoketones **23j-u**

To the obtained ketones **48a-l** (0.90 mmol) in ethyl acetate (2 mL) was added CuBr₂ (200 mg, 0.90 mmol) under magnetic stirring at 70 °C and monitored by TLC. The reaction mixture was purified by chromatography on silica gel under the bromoketones **23j-u** were obtained.

22.1. 2-bromo-1-(2-(o-tolyloxy)phenyl)ethan-1-one **23m**

The reaction was performed according to the general procedure 4. Yellow oil yield 217 mg (79%) starting from **48d** 205 mg (0.90 mmol).



23m

Chemical Formula: C₁₅H₁₃BrO₂

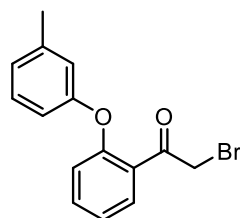
Molecular Weight: 305.17

¹H NMR (300 MHz, CDCl₃) δ 7.93 (ddd, ³J_{H-H} = 7.8 Hz, ⁴J_{H-H} = 1.8 Hz, ⁵J_{H-H} = 0.4 Hz, 1H), 7.40 (ddd, ³J_{H-H} = 8.4 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.34 – 7.29 (m, 1H), 7.25 – 7.20 (m, 1H), 7.18 (dd, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.5 Hz, 1H), 7.12 (ddd, ³J_{H-H} = 7.8 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.1 Hz, 1H), 6.98 (dd, ³J_{H-H} = 7.8 Hz, ⁴J_{H-H} = 1.5 Hz, 1H), 4.71 (s, 2H), 2.26 (s, 4H).

¹³C NMR (75 MHz, CDCl₃) δ 192.1, 157.4, 152.9, 134.8, 132.0, 131.9, 130.5, 127.8, 125.7, 125.5, 122.7, 120.6, 116.2, 37.4, 16.4.

22.2. 2-bromo-1-(2-(m-tolylloxy)phenyl)ethan-1-one **23n**

The reaction was performed according to the general procedure 4. Yellow oil yield 217 mg (79%) starting from **48e** 205 mg (0.90 mmol).



23n

Chemical Formula: C₁₅H₁₃BrO₂

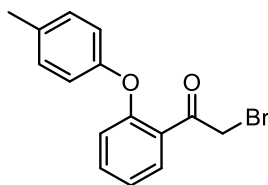
Molecular Weight: 305.17

¹H NMR (300 MHz, CDCl₃) δ 7.92 (ddd, ³J_{H-H} = 7.8 Hz, ⁴J_{H-H} = 1.8 Hz, ⁵J_{H-H} = 0.4 Hz, 1H), 7.45 (ddd, ³J_{H-H} = 8.3 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.34 – 7.23 (m, 1H), 7.16 (ddd, ³J_{H-H} = 7.8 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.1 Hz, 1H), 7.05 – 7.00 (m, 1H), 6.93 – 6.84 (m, 3H), 4.65 (s, 2H), 2.37 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 192.1, 157.1, 155.4, 140.7, 134.7, 131.7, 130.0, 126.6, 125.7, 123.3, 120.5, 118.1, 116.9, 37.4, 21.5.

22.3. 2-bromo-1-(2-(p-tolylloxy)phenyl)ethan-1-one **23o**

The reaction was performed according to the general procedure 4. Yellow oil yield 220 mg (80%) starting from **48f** 205 mg (0.90 mmol).



23o

Chemical Formula: C₁₅H₁₃BrO₂

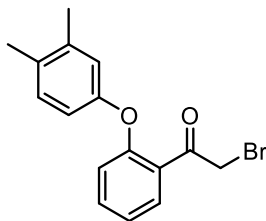
Molecular Weight: 305.17

¹H NMR (300 MHz, CDCl₃) δ 7.91 (ddd, ³J_{H-H} = 7.9 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.43 (ddd, ³J_{H-H} = 8.4 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.23 – 7.18 (m, 2H), 7.14 (ddd, ³J_{H-H} = 7.9 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.0 Hz, 1H), 7.02 – 6.96 (m, 2H), 6.83 (dd, ³J_{H-H} = 8.4 Hz, ⁴J_{H-H} = 1.0 Hz, 2H), 4.66 (s, 2H), 2.37 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 192.2, 157.5, 153.0, 134.7, 134.6, 131.7, 130.8, 126.4, 123.1, 120.0, 117.6, 37.4, 20.9.

22.4. 2-bromo-1-(2-(3,4-dimethylphenoxy)phenyl)ethan-1-one 23p

The reaction was performed according to the general procedure 4. Yellow oil yield 258 mg (90%) starting from **48g** 215 mg (0.90 mmol).



23p

Chemical Formula: C₁₆H₁₅BrO₂

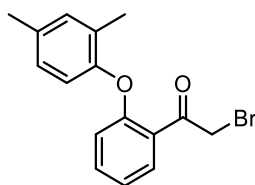
Molecular Weight: 319.20

¹H NMR (300 MHz, CDCl₃) δ 7.91 (ddd, ³J_{H-H} = 7.8 Hz, ⁴J_{H-H} = 1.9 Hz, ⁵J_{H-H} = 0.4 Hz, 1H), 7.43 (ddd, ³J_{H-H} = 8.4 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.19 – 7.09 (m, 2H), 6.91 – 6.79 (m, 3H), 4.66 (s, 2H), 2.27 (s, 6H).

¹³C NMR (75 MHz, CDCl₃) δ 192.1, 157.6, 153.2, 138.9, 134.6, 133.3, 131.7, 131.1, 126.4, 123.0, 121.2, 117.7, 117.2, 37.5, 20.1, 19.2.

22.5. 2-bromo-1-(2-(2,4-dimethylphenoxy)phenyl)ethan-1-one 23q

The reaction was performed according to the general procedure 4. Yellow oil yield 210 mg (73%) starting from **48h** 215 mg (0.90 mmol).



23q

Chemical Formula: C₁₆H₁₅BrO₂

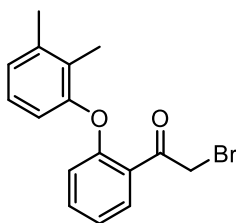
Molecular Weight: 319.20

¹H NMR (300 MHz, CDCl₃) δ 7.92 (dd, ³J_{H-H} = 7.8 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.38 (ddd, ³J_{H-H} = 8.4 Hz, ³J_{H-H} = 7.2 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.14 – 7.07 (m, 2H), 7.09 – 6.99 (m, 1H), 6.88 (d, ³J_{H-H} = 8.4 Hz, 1H), 6.63 (dd, ³J_{H-H} = 8.4 Hz, ⁴J_{H-H} = 1.0 Hz, 1H), 4.71 (s, 2H), 2.35 (s, 3H), 2.21 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 192.1, 157.7, 150.5, 135.2, 134.7, 132.6, 131.8, 130.2, 128.3, 125.4, 122.4, 120.6, 115.8, 37.5, 20.9, 16.3.

22.6. 2-bromo-1-(2-(2,3-dimethylphenoxy)phenyl)ethan-1-one 23r

The reaction was performed according to the general procedure 4. Yellow oil yield 236 mg (82%) starting from **48i** 215 mg (0.90 mmol).



23r

Chemical Formula: C₁₆H₁₅BrO₂

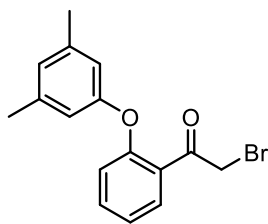
Molecular Weight: 319.20

¹H NMR (300 MHz, CDCl₃) δ 7.93 (dd, ³J_{H-H} = 7.9, ⁴J_{H-H} = 1.8 Hz, 1H), 7.39 (ddd, ³J_{H-H} = 8.3, ³J_{H-H} = 7.3, ⁴J_{H-H} = 1.8 Hz, 1H), 7.17 – 7.00 (m, 3H), 6.84 (dd, ³J_{H-H} = 7.9, ⁴J_{H-H} = 1.1 Hz, 1H), 6.63 (dd, ³J_{H-H} = 8.3, ⁴J_{H-H} = 1.1 Hz, 1H), 4.71 (s, 2H), 2.35 (s, 3H), 2.17 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 192.1, 157.7, 152.8, 139.5, 134.7, 131.8, 129.2, 126.9, 126.9, 125.5, 122.5, 118.3, 116.2, 37.4, 20.2, 12.5.

22.7. 2-bromo-1-(2-(3,5-dimethylphenoxy)phenyl)ethan-1-one 23s

The reaction was performed according to the general procedure 4. Yellow oil yield 213 mg (74%) starting from **48j** 215 mg (0.90 mmol).



23s

Chemical Formula: C₁₆H₁₅BrO₂

Molecular Weight: 319.20

¹H NMR (300 MHz, CDCl₃) δ 7.92 (ddd, ³J_{H-H} = 7.8 Hz, ⁴J_{H-H} = 1.9 Hz, ⁵J_{H-H} = 0.4 Hz, 1H), 7.45 (ddd, ³J_{H-H} = 8.3 Hz, ³J_{H-H} = 7.3 Hz, ⁴J_{H-H} = 1.8 Hz, 1H), 7.16 (ddd, ³J_{H-H} = 7.8 Hz, ³J_{H-H} = 7.3 Hz, ³J_{H-H} = 1.0 Hz, 1H), 6.92 – 6.81 (m, 2H), 6.75 – 6.62 (m, 2H), 4.66 (s, 2H), 2.32 (dd, ³J_{H-H} = 0.6 Hz, ³J_{H-H} = 0.6 Hz, 6H).

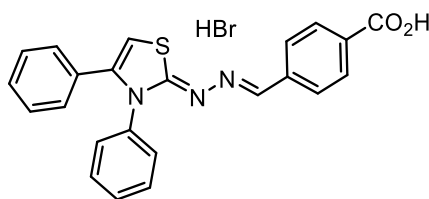
¹³C NMR (75 MHz, CDCl₃) δ 192.0, 157.2, 155.4, 140.3, 134.7, 134.6, 131.7, 126.6, 123.2, 118.2, 117.5, 37.5, 21.4.

23. General procedure 5: Synthesis of the revised-MIM1 analogues

A mixture of the appropriate intermediate **21a-f** (0.4 mmol) and bromoketone **23a-u** (0.8 mmol) in ethanol (10 mL) was refluxed for 6 h. The mixture was concentrated and washed using acetone several times.

23.1. 4-((E)-(((E)-3,4-diphenylthiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide **31a**

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 125 mg (65%).



31a

Chemical Formula: C₂₃H₁₈BrN₃O₂S

Molecular Weight: 480.38

mp = 264 °C

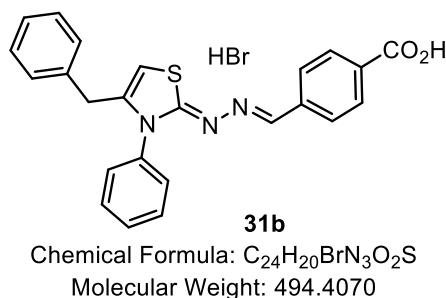
¹H NMR (300 MHz, DMSO-*d*₆) δ 8.21 (s, 1H), 7.95 (d, ³J_{H-H} = 8.4 Hz, 2H), 7.78 (d, ³J_{H-H} = 8.4 Hz, 2H), 7.42 – 7.10 (m, 10H), 6.70 (s, 1H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 171.0, 167.1, 150.5, 140.3, 138.9, 136.9, 131.4, 130.2, 129.8, 129.2, 128.8, 128.6, 128.3, 127.0, 103.1.

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{23}H_{17}N_3O_2NaS$) = 422.0939; found: 422.0939 (0 ppm).

23.2. 4-((E)-(((E)-4-benzyl-3-phenylthiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31b

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 182 mg (92%).



mp > 260 °C

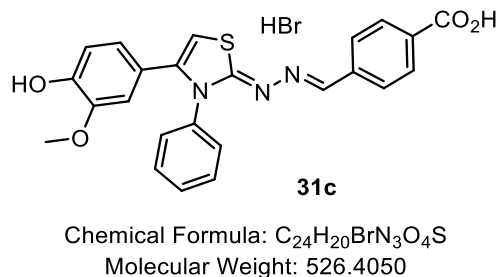
1H NMR (500 MHz, DMSO- d_6) δ 8.22 (s, 1H), 7.96 (d, $^3J_{H-H}$ = 8.4 Hz, 2H), 7.75 (d, $^3J_{H-H}$ = 8.4 Hz, 2H), 7.63 – 7.42 (m, 3H), 7.42 – 7.01 (m, 5H), 7.06 – 6.83 (m, 2H), 6.44 (s, 1H), 3.62 (s, 2H).

^{13}C NMR (126 MHz, DMSO- d_6) δ 171.1, 167.0, 149.9, 139.8, 138.5, 136.0, 135.4, 131.5, 129.8, 129.6, 129.5, 128.7, 128.6, 128.3, 127.0, 126.7, 102.3, 33.8.

HRMS (ESI): calcd. for $[M-H]^-$ ($C_{24}H_{18}N_3O_2S$) = 412.1125; found: 412.1127 (0 ppm).

23.3. 4-((E)-(((E)-4-(4-hydroxy-3-methoxyphenyl)-3-phenylthiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31c

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 190 mg (90%).



mp = 178 °C

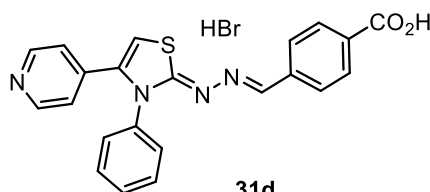
1H NMR (500 MHz, DMSO- d_6) δ 8.29 (s, 1H), 7.98 (d, $^3J_{H-H}$ = 8.4 Hz, 2H), 7.79 (d, $^3J_{H-H}$ = 8.4 Hz, 2H), 7.64 – 7.30 (m, 5H), 6.83 (s, 1H), 6.76 – 6.44 (m, 3H), 3.49 (s, 3H).

^{13}C NMR (126 MHz, DMSO- d_6) δ 170.7, 167.0, 150.3, 147.2, 146.9, 141.0, 138.4, 136.5, 131.6, 129.8, 129.4, 129.0, 128.9, 127.1, 121.9, 120.6, 115.2, 112.7, 102.3, 55.4.

HRMS (ESI): calcd. for $[\text{M}+\text{Na}]^+$ ($\text{C}_{24}\text{H}_{19}\text{N}_3\text{O}_4\text{NaS}$) = 468.0988; found: 468.0988 (0 ppm).

23.4. 4-((E)-(((E)-3-phenyl-4-(pyridin-4-yl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31d

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 106 mg (55%).



31d

Chemical Formula: $\text{C}_{22}\text{H}_{17}\text{BrN}_4\text{O}_2\text{S}$
Molecular Weight: 481.3680

mp pyrolysis at 215 °C

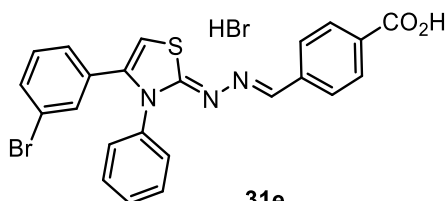
^1H NMR (500 MHz, DMSO- d_6) δ 8.81 (s, 2H), 8.30 (s, 1H), 7.97 (d, $^3J_{\text{H-H}} = 8.4$ Hz, 2H), 7.81 (d, $^3J_{\text{H-H}} = 8.4$ Hz, 2H), 7.70 (d, $J = 6.5$ Hz, 2H), 7.63 (s, 1H), 7.53 – 7.37 (m, 5H).

^{13}C NMR (126 MHz, DMSO- d_6) δ 170.3, 167.0, 152.0, 145.3, 142.3, 138.7, 136.6, 135.4, 131.5, 129.8, 129.6, 128.8, 128.4, 127.1, 124.4, 113.2.

HRMS (ESI): calcd. for $[\text{M}+\text{H}]^+$ ($\text{C}_{22}\text{H}_{17}\text{N}_4\text{O}_2\text{S}$) = 401.1067; found: 401.1070 (1 ppm).

23.5. 4-((E)-(((E)-4-(3-bromophenyl)-3-phenylthiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31e

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 197 mg (88%).



31e

Chemical Formula: $\text{C}_{23}\text{H}_{17}\text{Br}_2\text{N}_3\text{O}_2\text{S}$
Molecular Weight: 559.2760

mp > 260 °C

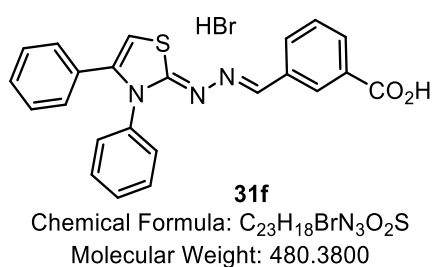
^1H NMR (500 MHz, DMSO- d_6) δ 8.28 (s, 1H), 7.97 (d, $^3J_{\text{H-H}} = 8.3$ Hz, 2H), 7.79 (d, $^3J_{\text{H-H}} = 8.3$ Hz, 2H), 7.51 – 7.33 (m, 6H), 7.24 – 7.05 (m, 2H), 7.00 (s, 1H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 170.7, 167.0, 150.7, 138.7, 138.6, 136.4, 132.2, 131.6, 131.5, 131.2, 130.3, 129.8, 129.4, 129.0, 128.7, 127.7, 127.1, 121.4, 104.8.

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₃H₁₆N₃O₂⁷⁹BrNaS) = 500.0038; found: 500.0035 (1 ppm).

23.6. 3-((E)-(((E)-3,4-diphenylthiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31f

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 169 mg (88%).



mp > 260 °C

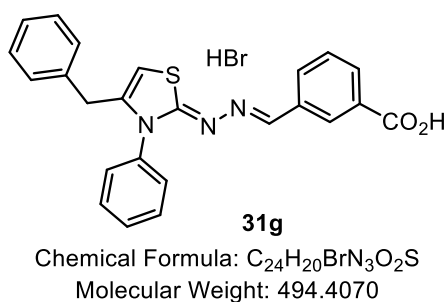
¹H NMR (500 MHz, DMSO-*d*₆) δ 8.32 (s, 1H), 8.28 (s, 1H), 7.96 (d, ³J_{H-H} = 7.7 Hz, 1H), 7.92 (d, ³J_{H-H} = 7.8 Hz, 1H), 7.57 (t, ³J_{H-H} = 7.7 Hz, 1H), 7.46 – 7.34 (m, 5H), 7.32 – 7.14 (m, 5H), 6.91 (s, 1H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 170.4, 167.0, 150.8, 140.6, 136.3, 134.9, 131.4, 130.7, 129.9, 129.4, 129.3, 129.0, 128.9, 128.8, 128.7, 128.3, 127.6, 103.9.

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₃H₁₇N₃O₂NaS) = 422.0933; found: 422.0932 (0 ppm).

23.7. 3-((E)-(((Z)-4-benzyl-3-phenylthiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31g

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 170 mg (86%).



mp = 146 °C

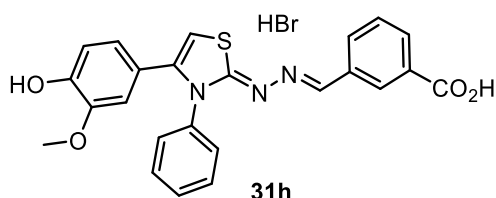
¹H NMR (300 MHz, Methanol-*d*₄) δ 8.36 – 8.32 (m, 1H), 8.30 (s, 1H), 8.13 (dt, ³*J*_{H-H} = 7.8, ⁴*J*_{H-H} = 1.5 Hz, 1H), 8.02 – 7.90 (m, 1H), 7.73 – 7.54 (m, 4H), 7.45 – 7.38 (m, 2H), 7.26 – 7.19 (m, 3H), 6.98 – 6.91 (m, 3H), 3.77 (s, 2H).

¹³C NMR (75 MHz, Methanol-*d*₄) δ 171.8, 168.7, 152.3, 144.1, 136.2, 134.6, 134.3, 133.5, 133.0, 132.9, 132.8, 132.0, 130.4, 129.9, 129.8, 129.8, 129.2, 128.3, 108.1, 35.1.

HRMS (ESI): calcd. for [M+H]⁺ (C₂₄H₂₀N₃O₂S) = 414.1270; found: 414.1269 (0 ppm).

**23.8. 3-((E)-(((E)-4-(4-hydroxy-3-methoxyphenyl)-3-phenylthiazol-2(3H)-ylidene)hydrazineylidene)methyl)benzoic acid hydrobromide
31h**

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 196 mg (93%).



Chemical Formula: C₂₄H₂₀BrN₃O₄S
Molecular Weight: 526.4050

mp = 232 °C

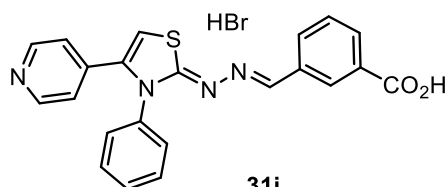
¹H NMR (500 MHz, DMSO-*d*₆) δ 8.34 (s, 1H), 8.28 (s, 1H), 7.97 (d, ³*J*_{H-H} = 7.7 Hz, 1H), 7.92 (d, ³*J*_{H-H} = 7.9 Hz, 1H), 7.58 (t, ³*J*_{H-H} = 7.7 Hz, 1H), 7.51 – 7.24 (m, 5H), 6.88 (s, 1H), 6.77 – 6.53 (m, 3H), 3.49 (s, 3H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 170.2, 167.0, 150.6, 147.3, 146.9, 141.2, 136.2, 134.6, 131.5, 131.5, 130.8, 129.6, 129.3, 129.3, 128.9, 127.6, 122.0, 120.4, 115.2, 112.8, 102.9, 55.4.

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₄H₁₉N₃O₄NaS) = 468.0988; found: 468.0985 (1 ppm).

23.9. 3-((E)-(((E)-3-phenyl-4-(pyridin-4-yl)thiazol-2(3H)-ylidene)hydrazineylidene)methyl)benzoic acid hydrobromide 31i

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 77 mg (40%).



31i

Chemical Formula: C₂₂H₁₇BrN₄O₂S

Molecular Weight: 481.3680

mp pyrolysis at 240 °C

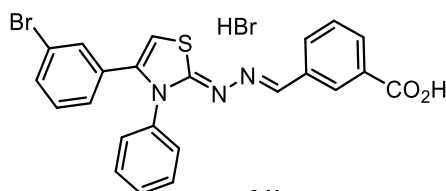
¹H NMR (500 MHz, DMSO-*d*₆) δ 8.79 (s, 1H), 8.78 (s, 1H), 8.32 (s, 1H), 8.30 (s, 1H), 7.94 (t, ³*J*_{H-H} = 8.3 Hz, 2H), 7.69 (d, ³*J*_{H-H} = 5.9 Hz, 2H), 7.59 (s, 1H), 7.56 (t, ³*J*_{H-H} = 7.7 Hz, 1H), 7.51 – 7.35 (m, 5H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 169.8, 167.0, 152.2, 145.2, 142.4, 136.7, 135.4, 135.2, 131.5, 131.4, 130.5, 129.5, 129.2, 128.8, 128.4, 127.6, 124.3, 112.9.

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₂H₁₆N₄O₂NaS) = 423.0886; found: 423.0884 (0 ppm).

23.10. 3-((E)-(((E)-4-(3-bromophenyl)-3-phenylthiazol-2(3H)-ylidene)hydrazineylidene)methyl)benzoic acid hydrobromide **31j**

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 145 mg (65%).



31j

Chemical Formula: C₂₃H₁₇Br₂N₃O₂S

Molecular Weight: 559.2760

mp = 211 °C

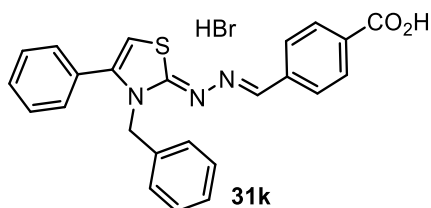
¹H NMR (500 MHz, DMSO-*d*₆) δ 8.30 (s, 1H), 8.27 (s, 1H), 7.95 (d, ³*J*_{H-H} = 7.7 Hz, 1H), 7.92 (d, ³*J*_{H-H} = 7.4 Hz, 1H), 7.56 (t, ³*J*_{H-H} = 7.7 Hz, 1H), 7.52 – 7.31 (m, 7H), 7.26 – 7.10 (m, 2H), 6.98 (s, 1H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 170.3, 167.0, 150.9, 138.7, 136.4, 135.0, 132.2, 131.6, 131.4, 131.2, 130.5, 130.4, 129.4, 129.2, 128.9, 128.8, 127.6, 127.6, 121.4, 104.6.

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₃H₁₆N₃O₂⁷⁹BrNaS) = 500.0038; found: 500.0036 (1 ppm).

23.11. 4-((E)-(((E)-3-benzyl-4-phenylthiazol-2(3H)-ylidene)hydrazineylidene)methyl)benzoic acid hydrobromide **31k**

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 187 mg (95%).



Chemical Formula: $C_{24}H_{20}BrN_3O_2S$
Molecular Weight: 494.4070

mp > 260 °C

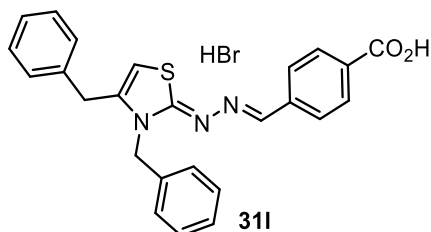
1H NMR (500 MHz, DMSO- d_6) δ 8.34 (s, 1H), 7.97 (d, $J = 8.0$ Hz, 2H), 7.81 (d, $^3J_{H-H} = 8.0$ Hz, 2H), 7.57 – 7.09 (m, 8H), 6.98 (d, $^3J_{H-H} = 7.2$ Hz, 2H), 6.65 (s, 1H), 5.12 (s, 2H).

^{13}C NMR (126 MHz, DMSO- d_6) δ 170.5, 167.0, 149.7, 140.6, 139.0, 136.4, 131.1, 130.1, 129.8, 129.5, 129.0, 128.8, 128.6, 127.3, 126.9, 126.3, 102.3, 48.5.

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{24}H_{19}N_3O_2NaS$) = 436.1090; found: 436.1084 (1 ppm).

23.12. 4-((E)-(((E)-3,4-dibenzylthiazol-2(3H)-ylidene)hydrazineylidene)methyl)benzoic acid hydrobromide 311

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 181 mg (89%).



Chemical Formula: $C_{25}H_{22}BrN_3O_2S$
Molecular Weight: 508.4340

mp = 227 °C

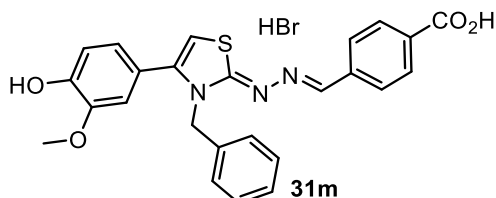
1H NMR (500 MHz, DMSO- d_6) δ 8.27 (s, 1H), 7.95 (d, $^3J_{H-H} = 8.4$ Hz, 2H), 7.77 (d, $^3J_{H-H} = 8.4$ Hz, 2H), 7.38 – 7.31 (m, 4H), 7.30 – 7.23 (m, 2H), 7.21 – 7.15 (m, 4H), 6.08 (s, 1H), 5.13 (s, 2H), 3.78 (s, 2H).

^{13}C NMR (126 MHz, DMSO- d_6) δ 171.1, 167.1, 149.1, 139.3, 139.2, 136.4, 136.2, 131.0, 129.8, 128.8, 128.8, 128.7, 127.5, 127.0, 126.8, 126.2, 100.7, 47.4, 33.2.

HRMS (ESI): calcd. for $[M-H]^-$ ($C_{25}H_{20}N_3O_2S$) = 426.1281; found: 426.1277 (1 ppm).

**23.13. 4-((E)-(((E)-3-benzyl-4-(4-hydroxy-3-methoxyphenyl)thiazol-2(3H)-ylidene)hydrazineylidene)methyl)benzoic acid hydrobromide
31m**

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 169 mg (78%).



Chemical Formula: $C_{25}H_{22}BrN_3O_4S$
Molecular Weight: 540.4320

mp > 260 °C

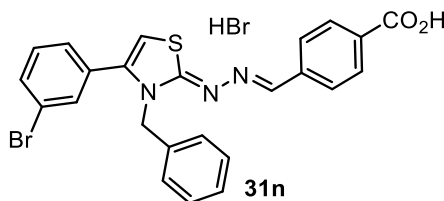
1H NMR (500 MHz, DMSO- d_6) δ 8.34 (s, 1H), 7.98 (d, $^3J_{H-H}$ = 8.4 Hz, 2H), 7.81 (d, $^3J_{H-H}$ = 8.4 Hz, 2H), 7.31 (t, $^3J_{H-H}$ = 7.5 Hz, 2H), 7.24 (t, $^3J_{H-H}$ = 7.3 Hz, 1H), 7.06 (d, $^3J_{H-H}$ = 7.3 Hz, 2H), 6.88 – 6.69 (m, 3H), 6.60 (s, 1H), 5.12 (s, 2H), 3.50 (s, 3H).

^{13}C NMR (126 MHz, DMSO- d_6) δ 170.4, 167.0, 149.5, 147.8, 147.3, 141.2, 138.9, 136.5, 131.2, 129.8, 128.7, 127.3, 126.9, 126.2, 122.1, 120.6, 115.5, 112.7, 101.6, 55.2, 49.0.

HRMS (ESI): calcd. for $[M-H]^-$ ($C_{25}H_{20}N_3O_4S$) = 458.118; found: 458.118 (1 ppm).

23.14. 4-((E)-(((E)-3-benzyl-4-(3-bromophenyl)thiazol-2(3H)-ylidene)hydrazineylidene)methyl)benzoic acid hydrobromide 31n

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 200 mg (87%).



Chemical Formula: $C_{24}H_{19}Br_2N_3O_2S$
Molecular Weight: 573.3030

mp = 246 °C

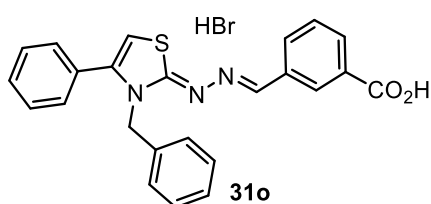
1H NMR (500 MHz, DMSO- d_6) δ 8.34 (s, 1H), 7.97 (d, $^3J_{H-H}$ = 8.4 Hz, 2H), 7.81 (d, $^3J_{H-H}$ = 8.4 Hz, 2H), 7.66 – 7.61 (m, 1H), 7.52 – 7.50 (m, 1H), 7.36 (d, $^3J_{H-H}$ = 5.1 Hz, 2H), 7.31 – 7.20 (m, 3H), 7.00 (d, $^3J_{H-H}$ = 7.3 Hz, 2H), 6.71 (s, 1H), 5.10 (s, 2H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 170.6, 167.1, 149.9, 139.1, 138.9, 136.4, 132.3, 132.3, 131.5, 131.1, 130.8, 129.8, 128.7, 128.0, 127.4, 126.9, 126.3, 121.8, 103.3, 48.7.

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₄H₁₈N₃O₂⁷⁹BrNaS) = 514.0195; found: 514.0195 (0 ppm).

23.15. 3-((E)-(((E)-3-benzyl-4-phenylthiazol-2(3H)-ylidene)hydrazineylidene)methyl)benzoic acid hydrobromide **31o**

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 178 mg (90%).



Chemical Formula: C₂₄H₂₀BrN₃O₂S
Molecular Weight: 494.4070

mp > 260 °C

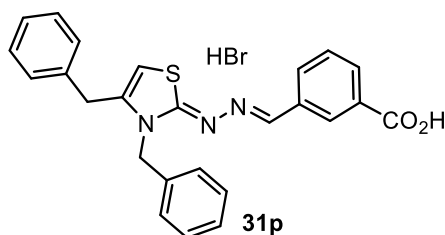
¹H NMR (300 MHz, DMSO-*d*₆) δ 8.34 (s, 1H), 8.29 (s, 1H), 7.93 (dd, ³J_{H-H} = 7.7, ⁴J_{H-H} = 1.6 Hz, 2H), 7.55 (t, ³J_{H-H} = 7.7 Hz, 1H), 7.50 – 7.14 (m, 9H), 6.98 (d, J = 6.7 Hz, 2H), 6.58 (s, 1H), 5.10 (s, 2H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.2, 167.1, 149.9, 140.5, 136.6, 135.5, 131.4, 131.2, 130.2, 130.1, 129.5, 129.2, 129.0, 128.8, 128.6, 127.5, 127.3, 126.3, 101.9, 48.4.

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₄H₁₉N₃O₂NaS) = 414.1271; found: 414.1274 (1 ppm).

23.16. 3-((E)-(((Z)-3,4-dibenzylthiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide **31p**

The reaction was performed according to the general procedure 5. Fade green solid was obtained with 103 mg (51%).



Chemical Formula: C₂₅H₂₂BrN₃O₂S
Molecular Weight: 508.4340

mp = 241 °C

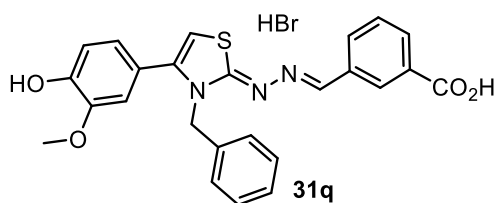
¹H NMR (300 MHz, Methanol-*d*₄) δ 8.41 – 8.34 (m, 2H), 8.18 – 8.08 (m, 1H), 8.05 – 7.94 (m, 1H), 7.66 – 7.54 (m, 1H), 7.47 – 7.23 (m, 6H), 7.23 – 7.17 (m, 2H), 7.16 – 7.08 (m, 2H), 6.74 (s, 1H), 5.48 (s, 2H), 3.95 (s, 2H).

¹³C NMR (75 MHz, Methanol-*d*₄) δ 171.4, 168.7, 152.1, 144.1, 136.0, 134.6, 134.1, 133.5, 133.0, 132.9, 130.5, 130.4, 130.2, 130.0, 129.9, 129.6, 128.7, 126.7, 108.8, 51.0, 34.6.

HRMS (ESI): calcd. for [M+Na]⁺ (C₂₅H₂₁N₃O₂NaS) = 450.1246; found: 450.1242 (1 ppm).

23.17. 3-((E)-(((Z)-3-benzyl-4-(4-hydroxy-3-methoxyphenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31q

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 102 mg (47%).



Chemical Formula: C₂₅H₂₂BrN₃O₄S
Molecular Weight: 540.4320

mp = 175 °C

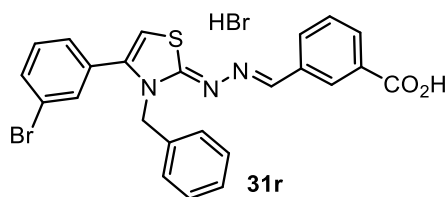
¹H NMR (300 MHz, Methanol-*d*₄) δ 8.49 – 8.34 (m, 2H), 8.15 (dt, ³J_{H-H} = 7.8, ⁴J_{H-H} = 1.5 Hz, 1H), 8.02 (dt, ³J_{H-H} = 7.8, ⁴J_{H-H} = 1.5 Hz, 1H), 7.62 (t, ³J_{H-H} = 7.8 Hz, 1H), 7.48 – 7.27 (m, 3H), 7.17 – 7.02 (m, 3H), 6.84 (s, 2H), 6.71 (s, 1H), 5.35 (s, 2H), 3.55 (s, 3H).

¹³C NMR (75 MHz, Methanol-*d*₄) δ 170.7, 168.8, 152.1, 150.3, 149.0, 145.3, 135.0, 134.6, 133.5, 133.1, 132.9, 132.9, 130.4, 129.9, 129.5, 127.0, 124.5, 120.1, 116.6, 114.0, 108.8, 56.2, 52.1.

HRMS (ESI): calcd. for [M-H]⁻ (C₂₅H₂₀N₃O₄S) = 45.118; found: 45.118 (1 ppm).

23.18. 3-((E)-(((Z)-3-benzyl-4-(3-bromophenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31r

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 163 mg (71%).



Chemical Formula: $C_{24}H_{19}Br_2N_3O_2S$

Molecular Weight: 573.3030

mp = 235 °C

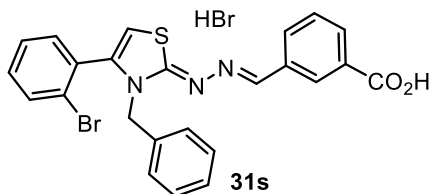
1H NMR (500 MHz, DMSO- d_6) δ 8.36 (s, 1H), 8.30 (d, $^4J_{H-H}$ = 1.8 Hz, 1H), 7.94 (dd, $^3J_{H-H}$ = 7.7, $^4J_{H-H}$ = 1.8 Hz, 2H), 7.68 – 7.61 (m, 1H), 7.56 (t, $^3J_{H-H}$ = 7.7 Hz, 1H), 7.54 – 7.50 (m, 1H), 7.43 – 7.19 (m, 5H), 7.03 – 6.98 (m, 2H), 6.69 (s, 1H), 5.11 (s, 2H).

^{13}C NMR (126 MHz, DMSO- d_6) δ 170.1, 167.1, 150.1, 138.8, 136.5, 135.5, 132.4, 132.3, 131.5, 131.3, 131.2, 130.8, 130.2, 129.2, 128.7, 128.0, 127.5, 127.4, 126.3, 121.8, 103.0, 48.6.

HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{24}H_{18}N_3O_2^{79}BrNaS$) = 514.0195; found: 514.0193 (0 ppm).

23.19. 3-((E)-(((Z)-3-benzyl-4-(2-bromophenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31s

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 181 mg (79%).



Chemical Formula: $C_{24}H_{19}Br_2N_3O_2S$

Molecular Weight: 573.3030

1H NMR (300 MHz, DMSO- d_6) δ 8.35 (s, 1H), 8.29 (t, $^4J_{H-H}$ = 1.7 Hz, 1H), 7.94 (d, $^4J_{H-H}$ = 1.7 Hz, 1H), 7.92 (d, $^4J_{H-H}$ = 1.7 Hz, 1H), 7.74 (dd, $^3J_{H-H}$ = 7.7, $^4J_{H-H}$ = 1.7 Hz, 1H), 7.55 (t, $^3J_{H-H}$ = 7.7 Hz, 1H), 7.39 (pd, $^3J_{H-H}$ = 7.4, $^4J_{H-H}$ = 1.7 Hz, 2H), 7.30 – 7.15 (m, 4H), 6.96 – 6.89 (m, 2H), 6.60 (s, 1H), 5.19 (d, $^2J_{H-H}$ = 16.2 Hz, 1H), 4.63 (d, $^2J_{H-H}$ = 16.2 Hz, 1H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 169.6, 167.2, 150.0, 138.3, 136.2, 136.1, 135.6, 132.9, 132.1, 131.5, 131.3, 131.0, 130.6, 130.3, 129.3, 128.6, 128.0, 127.6, 126.8, 124.3, 103.3, 48.3.

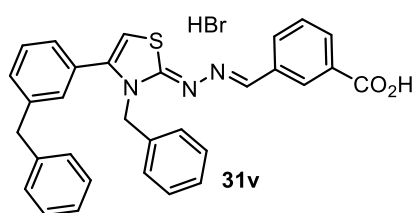
HRMS (ESI): calcd. for $[M+Na]^+$ ($C_{24}H_{18}N_3O_2^{79}BrNaS$) = 514.0195; found: 514.0194 (0 ppm).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.3, 167.2, 150.1, 140.6, 140.5, 139.1, 136.6, 135.4, 131.4, 131.3, 130.7, 130.3, 129.6, 129.3, 129.1, 128.8, 128.0, 128.0, 127.8, 127.5, 127.4, 127.1, 126.8, 126.3, 102.6, 49.0.

HRMS (ESI): calcd. for [M+Na]⁺ (C₃₀H₂₃N₃O₂NaS) = 512.1409; found: 512.1409 (0 ppm).

23.22. 3-((E)-(((Z)-3-benzyl-4-(3-benzylphenyl)thiazol-2(3H)-ylidene)hydrazone)methyl)benzoic acid hydrobromide 31v

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 185 mg (79%).



Chemical Formula: C₃₁H₂₆BrN₃O₂S
Molecular Weight: 584.5320

mp = 221 °C

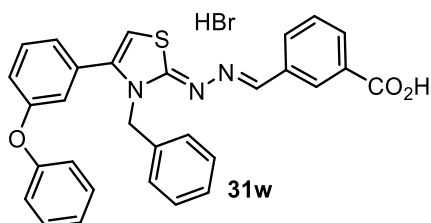
¹H NMR (300 MHz, DMSO-*d*₆) δ 8.41 (s, 1H), 8.31 (t, ⁴J_{H-H} = 1.7 Hz, 1H), 8.01 – 7.91 (m, 2H), 7.56 (t, ³J_{H-H} = 7.7 Hz, 1H), 7.40 – 7.06 (m, 12H), 6.99 – 6.89 (m, 2H), 6.70 (s, 1H), 5.14 (s, 2H), 3.87 (s, 2H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.0, 167.0, 150.0, 141.9, 140.8, 136.0, 135.0, 131.4, 131.3, 130.4, 129.9, 129.9, 129.3, 129.2, 128.9, 128.6, 128.6, 128.5, 127.6, 127.4, 127.3, 126.7, 126.3, 126.1, 103.0, 48.9, 40.8.

HRMS (ESI): calcd. for [M+Na]⁺ (C₃₁H₂₆N₃O₂NaS) = 504.1740; found: 504.1736 (1 ppm).

23.23. 3-((E)-(((Z)-3-benzyl-4-(3-phenoxyphenyl)thiazol-2(3H)-ylidene)hydrazone)methyl)benzoic acid hydrobromide 31w

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 124 mg (53%).



Chemical Formula: C₃₀H₂₄BrN₃O₃S
Molecular Weight: 586.5040

mp = 215 °C

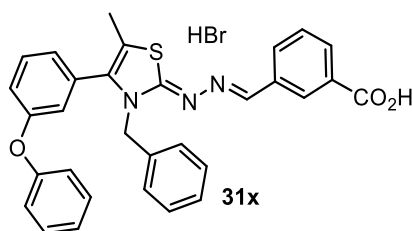
¹H NMR (300 MHz, DMSO-*d*₆) δ 8.39 (s, 1H), 8.30 (t, ⁴*J*_{H-H} = 1.8 Hz, 1H), 7.99 – 7.90 (m, 2H), 7.56 (t, ³*J*_{H-H} = 7.7 Hz, 1H), 7.43 (t, ³*J*_{H-H} = 7.9 Hz, 1H), 7.36 (t, ³*J*_{H-H} = 7.9 Hz, 2H), 7.31 – 7.20 (m, 3H), 7.19 – 7.02 (m, 3H), 7.01 – 6.85 (m, 5H), 6.72 (s, 1H), 5.16 (s, 2H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.0, 167.0, 156.7, 156.1, 150.0, 139.8, 136.1, 135.1, 131.6, 131.4, 131.2, 130.5, 130.3, 130.1, 129.2, 128.6, 127.5, 127.4, 126.3, 124.0, 123.8, 119.6, 118.9, 118.7, 103.2, 48.8.

HRMS (ESI): calcd. for [M+Na]⁺ (C₃₀H₂₃N₃O₃NaS) = 528.1352; found: 528.1348 (1 ppm).

23.24. 3-((E)-(((Z)-3-benzyl-5-methyl-4-(3-phenoxyphenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31x

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 204 mg (85%).



Chemical Formula: C₃₁H₂₆BrN₃O₃S
Molecular Weight: 600.5310

mp = 258 °C

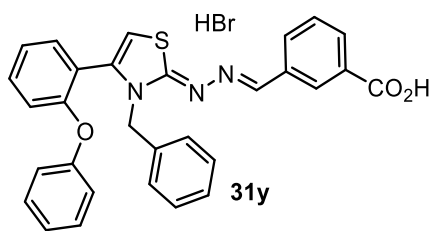
¹H NMR (300 MHz, DMSO-*d*₆) δ 8.37 (s, 1H), 8.30 (t, ⁴*J*_{H-H} = 1.4 Hz, 1H), 7.93 (tt, ³*J*_{H-H} = 9.0, ⁴*J*_{H-H} = 1.4 Hz, 2H), 7.55 (t, ³*J*_{H-H} = 7.7 Hz, 1H), 7.48 – 7.42 (t, ³*J*_{H-H} = 7.7 Hz, 1H), 7.39 – 7.32 (m, 2H), 7.29 – 7.17 (m, 3H), 7.16 – 7.13 (m, 1H), 7.12 – 7.04 (m, 2H), 6.98 – 6.90 (m, 2H), 6.90 – 6.84 (m, 2H), 6.76 (t, *J* = 2.0 Hz, 1H), 5.04 (s, 2H), 2.02 (s, 3H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 168.4, 167.1, 156.8, 156.2, 149.6, 135.9, 135.1, 134.8, 131.5, 130.7, 130.4, 130.4, 130.2, 129.3, 128.7, 127.5, 127.4, 126.4, 125.4, 123.9, 120.2, 119.8, 118.9, 118.8, 113.7, 49.1, 12.3.

HRMS (ESI): calcd. for [M+Na]⁺ (C₃₁H₂₅N₃O₃NaS) = 542.1508; found: 542.1505 (1 ppm).

23.25. 3-((E)-(((Z)-3-benzyl-4-(2-phenoxyphenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31y

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 145 mg (62%).



Chemical Formula: C₃₀H₂₄BrN₃O₃S
Molecular Weight: 586.5040

mp = 228 °C

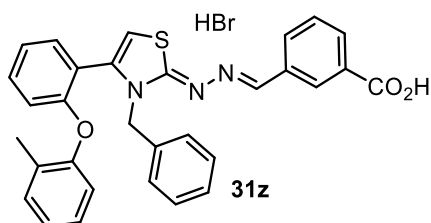
¹H NMR (300 MHz, DMSO-*d*₆) δ 8.35 (s, 1H), 8.27 (s, 1H), 7.92 (t, ³*J*_{H-H} = 6.6 Hz, 2H), 7.64 – 6.80 (m, 15H), 6.63 (s, 1H), 5.12 (s, 2H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 169.6, 167.1, 155.7, 155.1, 149.8, 136.6, 136.1, 135.2, 132.5, 132.0, 131.4, 131.3, 130.3, 130.2, 129.3, 128.6, 127.5, 127.5, 126.7, 124.3, 123.6, 120.9, 119.1, 118.0, 103.9, 48.6.

HRMS (ESI): calcd. for [M+Na]⁺ (C₃₀H₂₄N₃O₃S) = 506.1533; found: 506.1536 (1 ppm).

23.26. 3-((E)-(((Z)-3-benzyl-4-(2-(o-tolyloxy)phenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31z

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 165 mg (69%).



Chemical Formula: C₃₁H₂₆BrN₃O₃S
Molecular Weight: 600.5310

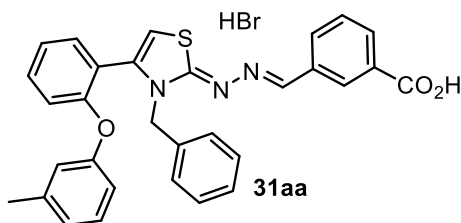
mp = 213 °C

¹H NMR (300 MHz, DMSO-*d*₆) δ 8.29 (s, 1H), 8.25 (t, ⁴*J*_{H-H} = 1.7 Hz, 1H), 7.89 (ddt, ³*J*_{H-H} = 9.8 Hz, ³*J*_{H-H} = 8.0 Hz, ⁴*J*_{H-H} = 1.4 Hz, 2H), 7.53 (t, ³*J*_{H-H} = 7.7 Hz, 1H), 7.39 (ddd, ³*J*_{H-H} = 8.4 Hz, ³*J*_{H-H} = 7.4 Hz, ⁴*J*_{H-H} = 1.7 Hz, 1H), 7.33 – 7.24 (m, 2H), 7.24 – 7.13 (m, 4H), 7.07 (tt, ³*J*_{H-H} = 7.4 Hz, ⁴*J*_{H-H} = 1.2 Hz, 2H), 7.00 – 6.94 (m, 2H), 6.72 (dd, ³*J*_{H-H} = 8.0, ³*J*_{H-H} = 1.3 Hz, 1H), 6.66 (dd, ³*J*_{H-H} = 8.3 Hz, ⁴*J*_{H-H} = 1.0 Hz, 1H), 6.55 (s, 1H), 5.09 (s, 2H), 2.07 (s, 3H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.1, 167.5, 155.5, 153.6, 150.0, 136.8, 136.5, 135.6, 132.7, 132.2, 132.0, 131.7, 131.6, 130.6, 129.6, 129.4, 128.9, 128.0, 127.8, 127.7, 126.9, 125.0, 123.4, 120.5, 119.6, 116.8, 103.5, 48.7, 16.0.

23.27. 3-((E)-(((Z)-3-benzyl-4-(2-(m-tolyloxy)phenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31aa

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 163 mg (68%).



Chemical Formula: $C_{31}H_{26}BrN_3O_3S$
Molecular Weight: 600.5310

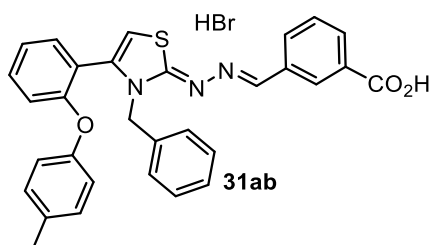
mp = 201 °C

1H NMR (300 MHz, DMSO- d_6) δ 8.28 (s, 1H), 8.25 (t, $^4J_{H-H}$ = 1.7 Hz, 1H), 7.91 (dt, $^3J_{H-H}$ = 7.8 Hz, $^4J_{H-H}$ = 1.6 Hz, 1H), 7.87 (dt, $^3J_{H-H}$ = 7.8 Hz, $^3J_{H-H}$ = 1.3 Hz, 1H), 7.52 (t, $^4J_{H-H}$ = 7.7 Hz, 1H), 7.48 – 7.36 (m, 1H), 7.30 – 7.17 (m, 5H), 7.10 (td, $^4J_{H-H}$ = 7.5 Hz, $^4J_{H-H}$ = 1.1 Hz, 1H), 6.99 – 6.89 (m, 3H), 6.84 (dd, $^4J_{H-H}$ = 8.3 Hz, $^4J_{H-H}$ = 1.1 Hz, 1H), 6.71 – 6.63 (m, 2H), 6.53 (s, 1H), 5.06 (s, 2H), 2.22 (s, 3H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 170.1, 167.5, 155.8, 155.4, 150.0, 140.4, 136.9, 136.5, 135.6, 132.7, 132.2, 131.7, 131.6, 130.6, 130.2, 129.6, 128.9, 127.8, 127.6, 126.9, 125.3, 123.8, 121.2, 119.9, 118.1, 116.4, 103.7, 48.7, 21.2.

23.28. 3-((E)-(((Z)-3-benzyl-4-(2-(p-tolyloxy)phenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31ab

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 180 mg (75%).



Chemical Formula: $C_{31}H_{26}BrN_3O_3S$
Molecular Weight: 600.5310

mp = 212 °C

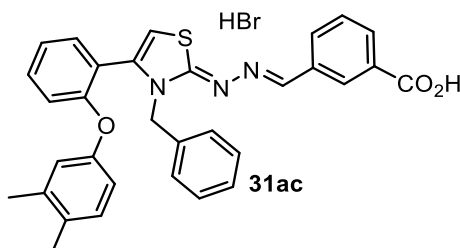
1H NMR (300 MHz, DMSO- d_6) δ 8.31 (s, 1H), 8.25 (t, $^4J_{H-H}$ = 1.8 Hz, 1H), 7.92 (dt, $^3J_{H-H}$ = 7.9, $^4J_{H-H}$ = 1.5 Hz, 1H), 7.87 (dt, $^3J_{H-H}$ = 7.9, $^4J_{H-H}$ = 1.5 Hz, 1H), 7.52 (t, $^3J_{H-H}$ = 7.8 Hz, 1H),

7.38 (td, $^3J_{H-H} = 7.8$, $^4J_{H-H} = 1.8$ Hz, 1H), 7.28 – 7.15 (m, 4H), 7.12 (d, $^3J_{H-H} = 8.3$ Hz, 2H), 7.06 (t, $^3J_{H-H} = 7.5$ Hz, 1H), 6.98 – 6.91 (m, 2H), 6.81 – 6.71 (m, 3H), 6.63 (s, 1H), 5.10 (s, 2H), 2.22 (s, 3H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 169.9, 167.5, 155.9, 153.4, 150.1, 137.2, 136.1, 135.3, 133.9, 132.7, 132.3, 131.7, 131.7, 130.9, 130.8, 129.6, 128.9, 127.9, 127.8, 126.9, 123.5, 120.6, 119.6, 117.5, 104.6, 49.0, 20.7.

23.29. 3-((E)-(((Z)-3-benzyl-4-(2-(3,4-dimethylphenoxy)phenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31ac

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 175 mg (71%).



Chemical Formula: $\text{C}_{32}\text{H}_{28}\text{BrN}_3\text{O}_3\text{S}$
Molecular Weight: 614.5580

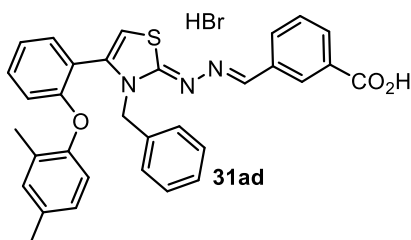
mp = 181 °C

^1H NMR (300 MHz DMSO- d_6) δ 8.28 (s, 1H), 8.25 (t, $J = 1.7$ Hz, 1H), 7.91 (dt, $J = 8.2$, 1.7 Hz, 1H), 7.87 (dt, $J = 8.0$, 1.2 Hz, 2H), 7.52 (t, $J = 7.7$ Hz, 1H), 7.39 (ddd, $J = 8.5$, 7.4, 1.8 Hz, 1H), 7.25 (dd, $J = 7.6$, 1.7 Hz, 1H), 7.23 – 7.16 (m, 3H), 7.09 (d, $J = 8.3$ Hz, 1H), 7.05 (dd, $J = 7.5$, 1.1 Hz, 1H), 6.99 – 6.93 (m, 2H), 6.77 (dd, $J = 8.4$, 1.1 Hz, 1H), 6.67 (d, $J = 2.6$ Hz, 1H), 6.61 (dd, $J = 8.1$, 2.7 Hz, 1H), 6.53 (s, 1H), 5.06 (s, 2H), 2.14 (s, 3H), 2.13 (s, 3H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 170.1, 167.6, 156.0, 153.5, 151.5, 150.0, 138.8, 137.0, 136.6, 135.7, 132.7, 132.1, 131.7, 131.6, 131.2, 130.6, 129.6, 128.9, 127.8, 127.6, 126.9, 123.4, 120.8, 120.8, 117.4, 116.9, 103.5, 48.7, 19.8, 19.0.

23.30. 3-((E)-(((Z)-3-benzyl-4-(2-(2,4-dimethylphenoxy)phenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31ad

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 204 mg (83%).



Chemical Formula: C₃₂H₂₈BrN₃O₃S
Molecular Weight: 614.5580

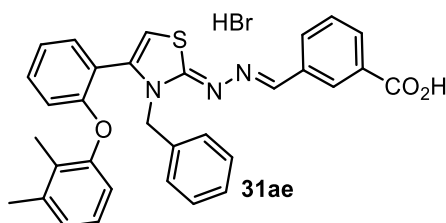
mp = 215 °C

¹H NMR (300 MHz, DMSO-*d*₆) δ 8.29 (s, 1H), 8.25 (t, ⁴*J*_{H-H} = 1.8 Hz, 1H), 7.91 (dt, ³*J*_{H-H} = 7.7 Hz, ⁴*J*_{H-H} = 1.5 Hz, 1H), 7.88 (dt, ³*J*_{H-H} = 7.7 Hz, ⁴*J*_{H-H} = 1.1 Hz, 1H), 7.52 (t, ³*J*_{H-H} = 7.7 Hz, 1H), 7.35 (ddd, ³*J*_{H-H} = 8.5 Hz, ³*J*_{H-H} = 7.4 Hz, ⁴*J*_{H-H} = 1.8 Hz, 1H), 7.26 (dd, ³*J*_{H-H} = 7.6 Hz, ⁴*J*_{H-H} = 1.8 Hz, 1H), 7.24 – 7.15 (m, 4H), 7.08 – 6.94 (m, 5H), 6.63 (d, ³*J*_{H-H} = 8.2 Hz, 1H), 6.58 (dd, ³*J*_{H-H} = 8.3 Hz, ⁴*J*_{H-H} = 1.0 Hz, 1H), 6.57 (s, 1H), 5.10 (s, 2H), 2.21 (s, 3H), 2.01 (s, 3H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.1, 167.5, 155.9, 151.1, 150.0, 137.0, 136.5, 135.6, 134.3, 132.7, 132.5, 132.2, 131.7, 131.6, 130.6, 129.6, 129.3, 128.9, 128.4, 127.8, 127.7, 126.9, 122.9, 120.0, 120.0, 116.1, 103.6, 48.7, 20.7, 16.0.

23.31. 3-((E)-(((Z)-3-benzyl-4-(2-(2,3-dimethylphenoxy)phenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31ae

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 160 mg (65%).



Chemical Formula: C₃₂H₂₈BrN₃O₃S
Molecular Weight: 614.5580

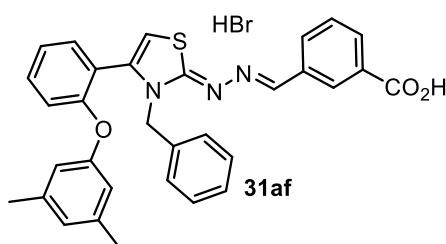
mp = 220 °C

¹H NMR (300 MHz, DMSO-*d*₆) δ 8.30 (s, 1H), 8.25 (t, ⁴*J*_{H-H} = 1.8 Hz, 1H), 7.91 (dt, ³*J*_{H-H} = 7.8 Hz, ⁴*J*_{H-H} = 1.5 Hz, 1H), 7.52 (t, ³*J*_{H-H} = 7.7 Hz, 1H), 7.36 (ddd, ³*J*_{H-H} = 8.6 Hz, ³*J*_{H-H} = 7.5 Hz, ⁴*J*_{H-H} = 1.8 Hz, 1H), 7.27 (dd, ³*J*_{H-H} = 7.6 Hz, ⁴*J*_{H-H} = 1.7 Hz, 1H), 7.25 – 7.15 (m, 3H), 7.09 – 7.01 (m, 2H), 7.02 – 6.92 (m, 3H), 6.59 (s, 1H), 6.63 – 6.54 (m, 2H), 5.10 (s, 2H), 2.20 (s, 3H), 1.96 (s, 3H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.0, 167.5, 155.9, 153.3, 150.0, 139.2, 137.0, 136.4, 135.5, 132.6, 132.2, 131.7, 131.6, 130.6, 129.6, 128.9, 128.2, 127.8, 127.7, 127.0, 126.9, 126.5, 123.0, 120.1, 117.6, 116.4, 103.8, 48.8, 20.0, 12.2.

23.32. 3-((E)-(((Z)-3-benzyl-4-(2-(3,5-dimethylphenoxy)phenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31af

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 199 mg (81%).



Chemical Formula: C₃₂H₂₈BrN₃O₃S
Molecular Weight: 614.5580

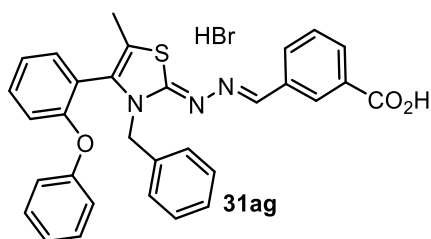
mp = 199 °C

¹H NMR (300 MHz, DMSO-*d*₆) δ 8.28 (s, 1H), 8.25 (t, ⁴J_{H-H} = 1.7 Hz, 1H), 7.89 (ddt, ³J_{H-H} = 10.6 Hz, ³J_{H-H} = 8.0 Hz, ⁴J_{H-H} = 1.4 Hz, 2H), 7.88 (dt, ³J_{H-H} = 8.0 Hz, ⁴J_{H-H} = 1.4 Hz, 1H), 7.52 (t, ³J_{H-H} = 7.7 Hz, 1H), 7.44 – 7.35 (m, 1H), 7.29 – 7.17 (m, 4H), 7.09 (td, ³J_{H-H} = 7.5 Hz, ⁴J_{H-H} = 1.1 Hz, 1H), 6.98 – 6.93 (m, 2H), 6.83 (dd, ³J_{H-H} = 8.3 Hz, ⁴J_{H-H} = 1.1 Hz, 1H), 6.77 – 6.74 (m, 1H), 6.52 (s, 1H), 6.51 – 6.43 (m, 2H), 5.04 (s, 2H), 2.18 (s, 6H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.1, 167.6, 155.7, 155.6, 150.0, 140.0, 136.9, 136.6, 135.7, 132.7, 132.2, 131.7, 131.6, 130.6, 129.6, 128.9, 127.8, 127.6, 126.9, 126.2, 123.6, 121.1, 118.0, 117.1, 103.5, 48.7, 21.2.

23.33. 3-((E)-(((Z)-3-benzyl-5-methyl-4-(2-phenoxyphenyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31ag

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 175 mg (73%).



Chemical Formula: C₃₁H₂₆BrN₃O₃S
Molecular Weight: 600.5310

mp > 260 °C

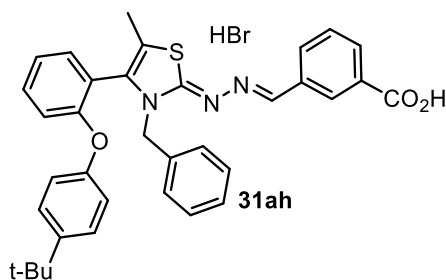
¹H NMR (300 MHz, DMSO-*d*₆) δ 8.38 (s, 1H), 8.29 (t, ⁴*J*_{H-H} = 1.8 Hz, 1H), 7.93 (t, ³*J*_{H-H} = 8.3 Hz, 2H), 7.55 (t, ³*J*_{H-H} = 7.7 Hz, 1H), 7.52 – 7.43 (m, 1H), 7.40 – 6.73 (m, 13H), 5.22 (d, ²*J*_{H-H} = 16.4 Hz, 1H), 4.93 (d, ²*J*_{H-H} = 16.4 Hz, 1H), 1.97 (s, 3H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 168.1, 167.0, 155.6, 155.4, 149.3, 135.9, 135.1, 132.9, 131.9, 131.5, 131.4, 131.3, 130.3, 130.0, 129.2, 128.5, 127.4, 127.3, 126.6, 124.1, 123.6, 119.4, 118.8, 118.3, 114.5, 48.9, 12.3.

HRMS (ESI): calcd. For [M+H]⁺(C₃₁H₂₆N₃O₃S) = 520.1690; found: 520.1695 (1 ppm).

23.34. 3-((E)-(((Z)-3-benzyl-4-(2-(4-(tert-butyl)phenoxy)phenyl)thiazol-2(3H)-ylidene)hydrazone)methyl)benzoic acid hydrobromide 31ah

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 118 mg (45%).



Chemical Formula: C₃₅H₃₄BrN₃O₃S
Molecular Weight: 656.6390

mp = 241 °C

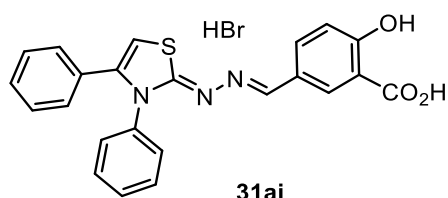
¹H NMR (300 MHz, DMSO-*d*₆) δ 8.33 – 8.25 (m, 2H), 7.92 (d, ³*J*_{H-H} = 7.5 Hz, 1H), 7.88 (d, ³*J*_{H-H} = 7.9 Hz, 1H), 7.53 (t, ³*J*_{H-H} = 7.7 Hz, 1H), 7.43 (td, ³*J*_{H-H} = 7.7 Hz, ⁴*J*_{H-H} = 1.8 Hz, 1H), 7.32 (d, ³*J*_{H-H} = 8.7 Hz, 2H), 7.26 – 7.15 (m, 3H), 7.11 (t, ³*J*_{H-H} = 7.5 Hz, 1H), 6.94 (dd, ³*J*_{H-H} = 6.6 Hz, ⁴*J*_{H-H} = 2.9 Hz, 2H), 6.87 (d, ³*J*_{H-H} = 8.3 Hz, 1H), 6.74 (d, ³*J*_{H-H} = 8.7 Hz, 2H), 5.14 (d, ²*J*_{H-H} = 16.2 Hz, 1H), 4.86 (d, ²*J*_{H-H} = 16.2 Hz, 1H), 1.94 (s, 3H), 1.21 (s, 9H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 168.5, 167.3, 156.0, 153.4, 149.5, 146.8, 136.2, 135.4, 133.1, 132.1, 131.7, 131.6, 130.5, 129.5, 128.7, 127.7, 127.5, 126.9, 126.8, 123.6, 119.4, 118.7, 118.3, 114.5, 49.0, 34.3, 31.4, 12.5.

HRMS (ESI): calcd. for [M+H]⁺ (C₃₅H₃₄N₃O₃S) = 576.2315; found: 576.2317 (0 ppm).

**23.35. 5-((E)-(((Z)-3,4-diphenylthiazol-2(3H)-ylidene)hydrazono)methyl)-2-hydroxybenzoic acid hydrobromide
31ai**

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 107 mg (54%).



Chemical Formula: C₂₃H₁₈BrN₃O₃S
Molecular Weight: 496.3790

mp = 239 °C

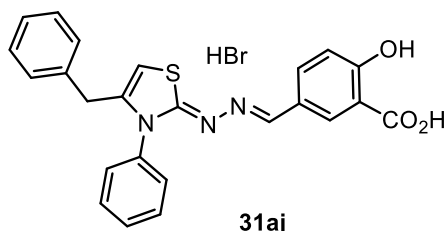
¹H NMR (300 MHz, DMSO-*d*₆) δ 8.19 (s, 1H), 8.10 (d, ⁴J_{H-H} = 2.2 Hz, 1H), 7.88 (dd, ³J_{H-H} = 8.7, ⁴J_{H-H} = 2.2 Hz, 1H), 7.46 – 7.30 (m, 5H), 7.30 – 7.14 (m, 5H), 7.04 (d, ³J_{H-H} = 8.7 Hz, 1H), 6.78 (s, 1H).

¹³C NMR (75 MHz, DMSO-*d*₆) δ 171.5, 169.5, 162.4, 150.6, 140.2, 136.8, 133.6, 130.2, 130.2, 129.5, 129.2, 128.7, 128.7, 128.5, 128.3, 126.1, 117.9, 113.4, 102.8.

HRMS (ESI): calcd. for [M-H]⁻ (C₂₃H₁₆N₃O₃S) = 414.0918; found: 414.0921 (1 ppm).

**23.36. 5-((E)-(((Z)-4-benzyl-3-phenylthiazol-2(3H)-ylidene)hydrazono)methyl)-2-hydroxybenzoic acid hydrobromide
31aj**

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 129 mg (63%).



Chemical Formula: $C_{24}H_{20}BrN_3O_3S$
Molecular Weight: 510.4060

mp = 223 °C

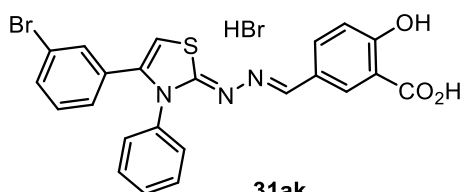
1H NMR (300 MHz, Methanol- d_4) δ 8.18 (s, 1H), 8.16 (d, $^4J_{H-H}$ = 2.2 Hz, 1H), 7.90 (dd, $^3J_{H-H}$ = 8.7, $^4J_{H-H}$ = 2.2 Hz, 1H), 7.73 – 7.56 (m, 3H), 7.43 – 7.36 (m, 2H), 7.27 – 7.17 (m, 3H), 7.03 (d, $^3J_{H-H}$ = 8.7 Hz, 1H), 7.00 – 6.89 (m, 2H), 6.90 (s, 1H), 3.75 (s, 2H).

^{13}C NMR (75 MHz, Methanol- d_4) δ 172.8, 171.3, 165.9, 152.3, 143.9, 136.3, 134.9, 134.3, 132.9, 132.3, 132.0, 129.8, 129.8, 129.3, 128.3, 125.4, 119.4, 114.4, 107.8, 35.1.

HRMS (ESI): calcd. for $[M-H]^-$ ($C_{24}H_{18}N_3O_3S$) = 428.1075; found: 428.1077 (1 ppm).

23.37. 5-((E)-(((Z)-4-(3-bromophenyl)-3-phenylthiazol-2(3H)-ylidene)hydrazono)methyl)-2-hydroxybenzoic acid hydrobromide
31ak

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 110 mg (48%).



Chemical Formula: $C_{23}H_{17}Br_2N_3O_3S$
Molecular Weight: 575.2750

mp > 260 °C

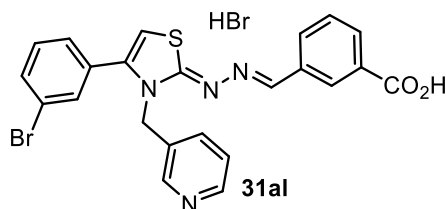
1H NMR (300 MHz, DMSO- d_6) δ 11.43 (bs, 1H), 8.18 (s, 1H), 8.09 (d, $^4J_{H-H}$ = 2.2 Hz, 1H), 7.88 (dd, $^3J_{H-H}$ = 8.7, $^4J_{H-H}$ = 2.2 Hz, 1H), 7.49 – 7.29 (m, 7H), 7.23 – 7.12 (m, 2H), 7.03 (d, $^3J_{H-H}$ = 8.7 Hz, 1H), 6.86 (s, 1H).

^{13}C NMR (75 MHz, DMSO- d_6) δ 171.5, 169.3, 162.3, 150.8, 138.3, 136.8, 133.6, 132.5, 131.4, 131.0, 130.3, 129.5, 129.2, 128.7, 128.5, 127.4, 126.2, 121.3, 117.9, 113.3, 103.7.

HRMS (ESI): calcd. for $[M-H]^-$ ($C_{23}H_{15}N_3O_3^{79}BrS$) = 492.0023; found: 492.0021 (0 ppm).

23.38. 3-((E)-(((Z)-4-(3-bromophenyl)-3-(pyridin-3-ylmethyl)thiazol-2(3H)-ylidene)hydrazono)methyl)benzoic acid hydrobromide 31al

The reaction was performed according to the general procedure 5. Yellow solid was obtained with 117 mg (51%).



Chemical Formula: $C_{23}H_{18}Br_2N_4O_2S$
Molecular Weight: 574.2910

mp = 238 °C

1H NMR (300 MHz, Methanol- d_4) δ 8.79 (d, $^3J_{H-H}$ = 5.7 Hz, 1H), 8.72 (d, $^4J_{H-H}$ = 2.0 Hz, 1H), 8.38 (dt, $^3J_{H-H}$ = 8.3, $^4J_{H-H}$ = 1.7 Hz, 1H), 8.35 (t, $^4J_{H-H}$ = 1.7 Hz, 1H), 8.31 (s, 1H), 8.10 – 8.00 (m, 2H), 7.95 (d, $^3J_{H-H}$ = 8.0 Hz, 1H), 7.74 – 7.61 (m, 1H), 7.55 (s, 1H), 7.52 (t, $^3J_{H-H}$ = 8.0 Hz, 1H), 7.44 – 7.36 (m, 2H), 6.52 (s, 1H), 5.31 (s, 2H).

^{13}C NMR (75 MHz, Methanol- d_4) δ 171.7, 169.3, 152.7, 146.4, 142.3, 142.2, 139.8, 139.0, 136.9, 134.1, 133.5, 133.3, 132.6, 132.4, 132.1, 131.9, 130.0, 129.5, 129.3, 128.6, 123.9, 104.6, 47.4.

HRMS (ESI): calcd. for $[M-H]^-$ ($C_{23}H_{16}N_4O_2^{79}BrS$) = 491.0182; found: 491.0181 (0 ppm).

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General Conclusion

This thesis is divided into two independent parts,

This work is a part of a collaboration program between Lebanese University, Lebanon and University of Rennes 1, France. The thesis is divided into two parts:

- Fluorine chemistry: synthesis of new nitrogenous heterocycles with a fluorinated side chain.
- Medicinal chemistry: research towards new anticancer molecules.

The first part consists of three chapters: in the first, the importance of fluorine was highlighted along with heterocycles, which suggests that the fluoro-functionalized heterocyclic compounds should be a promising candidate in drug discovery. In the second chapter, we have developed a flexible synthesis toward new γ -lactams with *gem*-fluorinated side-chains in position 4 of the heterocycle. Further, the increase in molecular diversity by adding other substituents in positions 3 and 5 of the γ -lactam has been demonstrated. Molecules of this type appear of interest in various areas of bioorganic or medicinal chemistry and such studies will be performed in a near future. The preparation of GABA with *gem*-difluorinated side chains starting from the prepared γ -lactams was particularly challenging, so alternative pathways should be used in future. In the third chapter, novel mono- and bicyclic nitrones with a CF_2R group in γ position have been efficiently prepared from *gem*-difluoro propargylic alcohols.

In the second part, our goal was to reinduce the proapoptotic properties in cancer cells to obtain new antitumor compounds. So, after the revision of the structure of MIM1, we have prepared a chemical library of designed analogues of this molecule. Detailed biological studies using ovarian cancer cell lines, IGROV1-R10 allowed us to discover derivatives which are potent dual inhibitors of Bcl-x_L and Mcl-1. All these data gave us a preliminary Structure-Activity relationship for these new MIM1 analogues. Extensive computational studies allowed us to propose a rationale to this SAR, highlighting the role of the *meta*-acid to anchor these molecules to the anti-apoptotic proteins and the position of the aromatic/benzyl groups inside the hydrophobic pockets of these proteins. Based on these data, new molecules are under active study in our groups and results will be reported in due course.

Résumé en Français

Ce travail fait partie d'un programme de collaboration entre l'Université Libanaise, Liban et l'Université de Rennes 1, France. La thèse est divisée en deux parties :

- Chimie du fluor : synthèse de nouveaux hétérocycles azotés à chaîne latérale fluorée.
- Chimie médicinale : recherche de nouvelles molécules anticancéreuses.

1. Chimie du fluor

La première partie se compose de trois chapitres :

1.1. Fluor et hétérocycles, une combinaison efficace en chimie médicinale

Dans le premier, l'importance du fluor a été soulignée avec les hétérocycles, ce qui suggère que les composés hétérocycliques fluoro-fonctionnalisés devraient être un candidat prometteur dans la découverte de médicaments. La chimie des composés organofluorés joue un rôle extrêmement important dans de nombreux domaines, allant des matériaux à la chimie bioorganique et à la chimie médicinale. Il est bien connu que l'introduction de fluor dans les molécules organiques modifie fortement leurs propriétés physiques, chimiques et biologiques. Environ 28 % des médicaments et 30 à 40 % des produits commercialisés en agrochimie sont considérés comme contenant au moins un atome de fluor.¹

Compte tenu du très grand intérêt pour les molécules portant les motifs CF_2H et CF_2R , notre laboratoire est impliqué depuis plusieurs années dans un projet visant à développer de nouvelles stratégies de synthèse de composés organofluorés, en particulier de molécules gem-difluorées. Nous proposons de développer une nouvelle méthode de préparation de gamma-lactames gem-difluorés et de nitrones cycliques, qui sont des analogues de produits bioactifs. La présence de fluor, dans nos analogues conçus, devrait augmenter la lipophilie et favoriser l'absorption de ces composés (Figure 1).

¹ Wang, J.; Sánchez-Roselló, M.; Aceña, J. L.; del Pozo, C.; Sorochinsky, A. E.; Fustero, S.; Soloshonok, V. A.; Liu, H. *Chem. Rev* **2014**, *114*, 2432

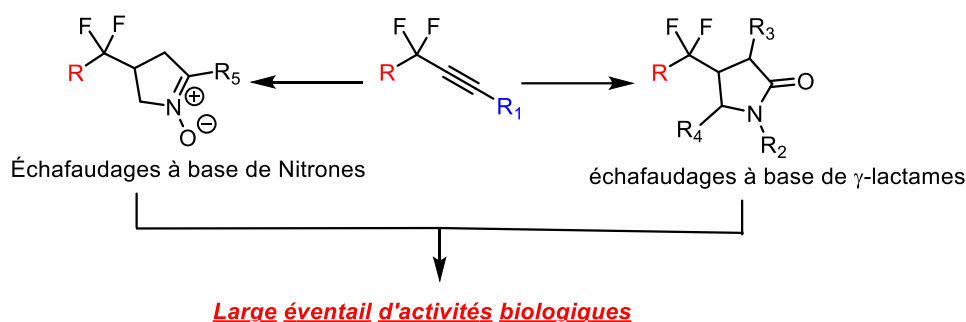
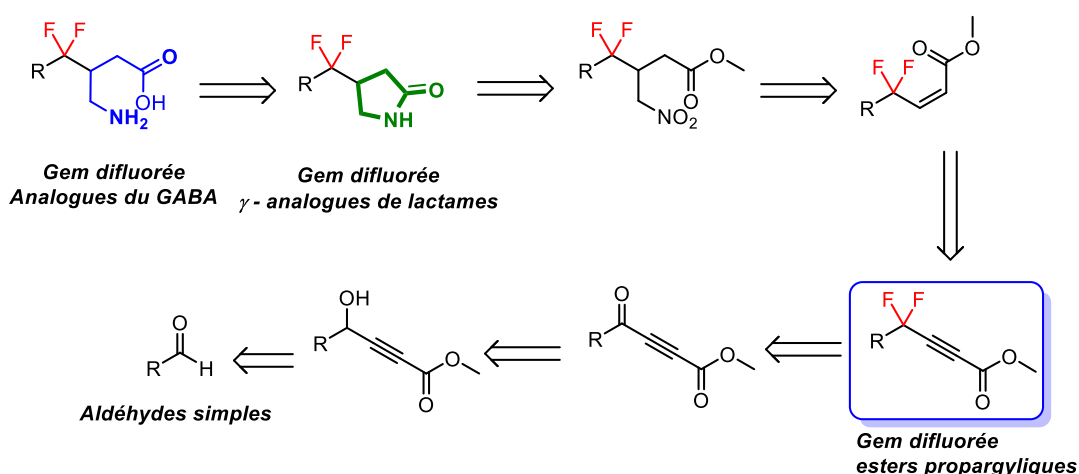


Figure 1: Utilisation potentielle de nitrones et de γ -lactames substitués

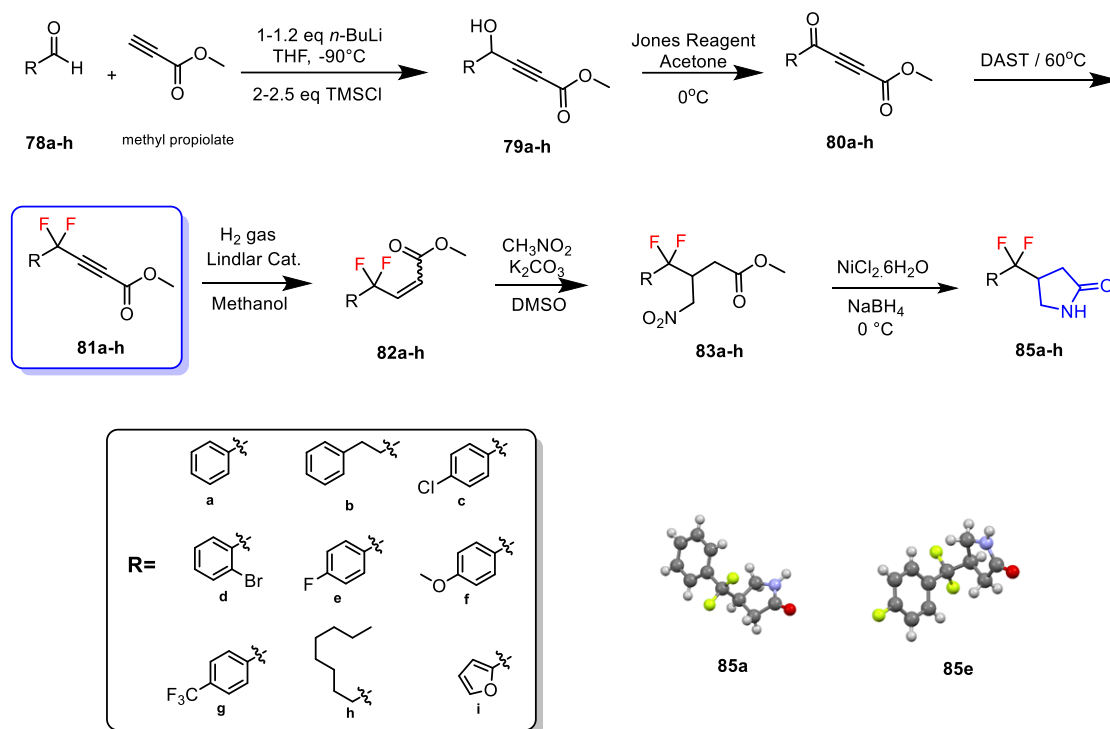
1.2. Synthèse de nouvelles γ -lactamines gem-difluorées et analogues du GABA

La première étape consistait à développer une synthèse courte et efficace de gamma-lactames avec des chaînes gem-difluorées en position 4 de l'hétérocycle comme le montre la rétrosynthèse (Scheme 1).



Scheme 1: Analyse rétrosynthétique pour la préparation de gem - analogues GABA difluorés.

Cela a été fait avec succès en permettant la préparation de la nouvelle famille ciblée de gamma-lactames avec des chaînes gem-difluorées en position 4 à partir d'aldéhydes aryliques et alkyliques. Les structures des γ -lactames fluorés **85a** et **85e** ont été confirmées par analyse cristallographique aux rayons X (Scheme 2).

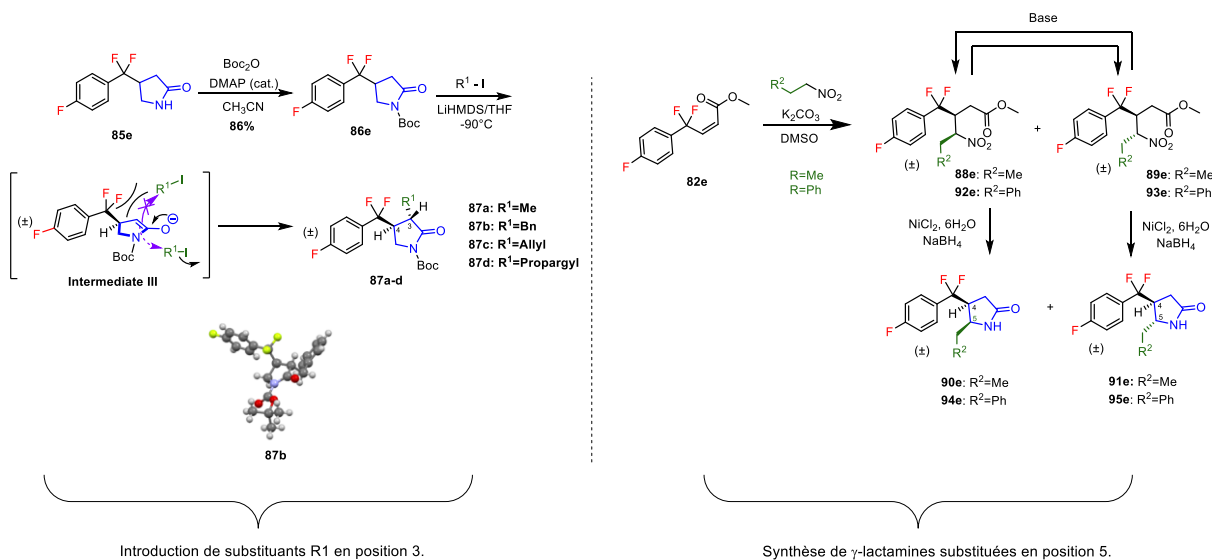


Scheme 2: Synthèse des analogues gem –difluorés – lactame **85** et de la structure aux rayons X de **85a** et **85e**

L'étape suivante était l'extension de la diversité moléculaire autour de l'échafaudage γ -lactame. Une voie évidente a été l'introduction de substituants R_1 sur le carbone vicinal du CO. Ainsi, le lactame **85e**, choisi comme modèle, a d'abord été protégé en tant que dérivé N-Boc puis des réactions d'alkylation ont été effectuées pour introduire divers groupes R_1 sur la position souhaitée (Scheme 3). Ces réactions se sont avérées complètement sélectives, ne donnant que les isomères trans **87a-d**. D'autre part, la deuxième option consistait à introduire un groupe $\text{CH}_2\text{-R}_2$ sur le carbone voisin de l'atome d'azote. En utilisant l'ester insaturé α - β **82e** comme modèle représentatif, cela pourrait être réalisé par l'ajout de groupes nitroalkyle, au lieu de nitrométhane, suivi de la réduction du groupe nitro pour obtenir **90e**, **91e** et **94e**, **95e** comme indiqué dans le Scheme 3. Ces résultats ont été publiés dans Synlett.²

En revanche, les premiers tests d'ouverture de ces lactames en acides aminés fluorés, analogues du GABA, ont échoué.

² Soulieman, A.; Gouault, N.; Roisnel, T.; Justaud, F.; Boustie, J.; Grée, R.; Hachem, A. *Synlett* **2019**, *30*, 2258-2262.



Scheme 3: Synthèse d'analogues *gem* – difluorés γ – lactame substitués en positions 3 et 5 et la radiographie de **87b**.

1.3. Synthèse de nouvelles nitrones cycliques avec des chaînes latérales *gem*-difluoroalkyle par des réactions en cascade

Dans le troisième chapitre, nous nous sommes concentrés sur le développement de synthèses courtes et efficaces de nouvelles nitrones portant des chaînes latérales *gem*-difluoroalkyle en position . Plus précisément, nous avons d'abord développé notre stratégie de préparation de nitrones modèles de type **A** (Figure 2). Ensuite, nous l'avons étendu aux molécules **B** avec des groupes fonctionnels en position terminale des chaînes alkyles. De tels groupements (esters, amine et alcool) pourraient être d'une grande utilité pour ajuster les propriétés physico-chimiques et biologiques de ces nitrones. En outre, ces groupes pourraient également être utilisés pour greffer facilement divers types de marqueurs éventuellement requis pour des études biologiques *in vivo* approfondies. Enfin, à partir des mêmes intermédiaires, nous avons préparé des analogues bicycliques **C**. Toutes ces nouvelles nitrones ont été préparées en séquences courtes (2-3 étapes) à partir de l'alcool propargylique *gem*-difluoroalkyle facilement disponible de type **D** (Figure 2).

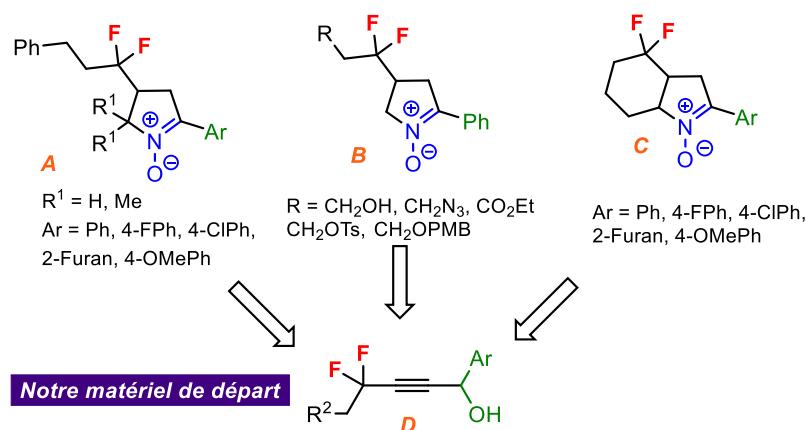


Figure 2: Notre cible nitrones à chaînes latérales *gem*-difluoroalkyle

De nouvelles nitrones mono- et bicycliques avec un groupe CF_2R en position ont été préparées efficacement à partir d'alcools *gem*-difluoro propargyliques par des réactions en cascade.³

2. Chimie médicinale : recherche de nouvelles molécules anticancéreuses

Le développement de composés capables de restaurer des voies apoptotiques fonctionnelles au sein des cellules cancéreuses constitue un axe de recherche très prometteur. Notre programme vise à développer de nouveaux inducteurs d'apoptose utilisables dans le traitement des cancers. Nous nous concentrons sur deux protéines anti-apoptotiques très importantes, à savoir Bcl-xL et Mcl-1, qui jouent un rôle clé dans les mécanismes d'apoptose des cellules tumorales et sont également susceptibles de jouer un rôle dans les capacités migratoires et invasives des cellules tumorales. Dans les travaux précédents, développés dans la thèse d'Assaad Nasr El Dine, plusieurs séries d'analogues d'un inhibiteur connu (MIM-1) de la protéine anti-apoptotique Mcl-1 ont été préparées. Plus de 50 analogues ont été testés sur trois variétés de cellules cancéreuses (sein, ovaire et mélanome). Un certain nombre de ces composés ont montré des activités prometteuses dans ces domaines (Figure 3).

³ Soulieman, A.; Ibrahim, R.; Barakat, Z.; Gouault, N.; Roisnel, T.; Boustie, J.; Grée, R.; Hachem, A. *Eur. J. Org. Chem.* **2020**, 5741-5751.

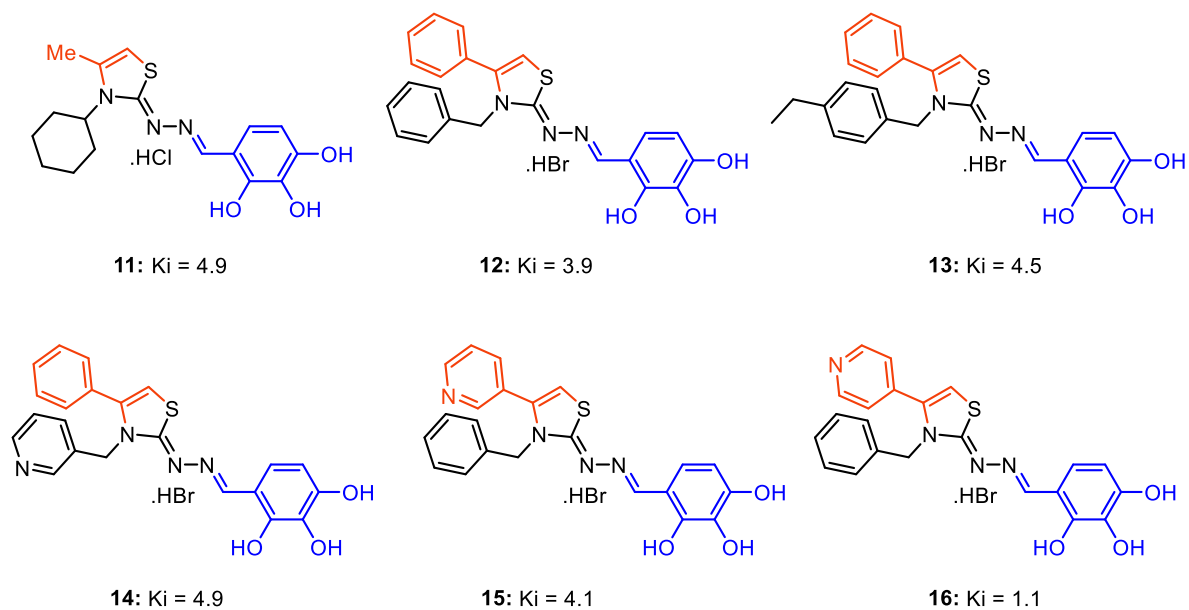


Figure 3: Affinités de liaison de MIM1 **11** et de certains analogues avec Mcl-1.

Sur la base des données de modélisation moléculaire, nous proposons dans cette section de préparer de nouvelles séries de molécules obtenues par des modifications structurales de la partie tri phénolique et remplacées par l'acide méta ou para-benzoïque qui se sont avérés être les plus actifs (Figure 4).

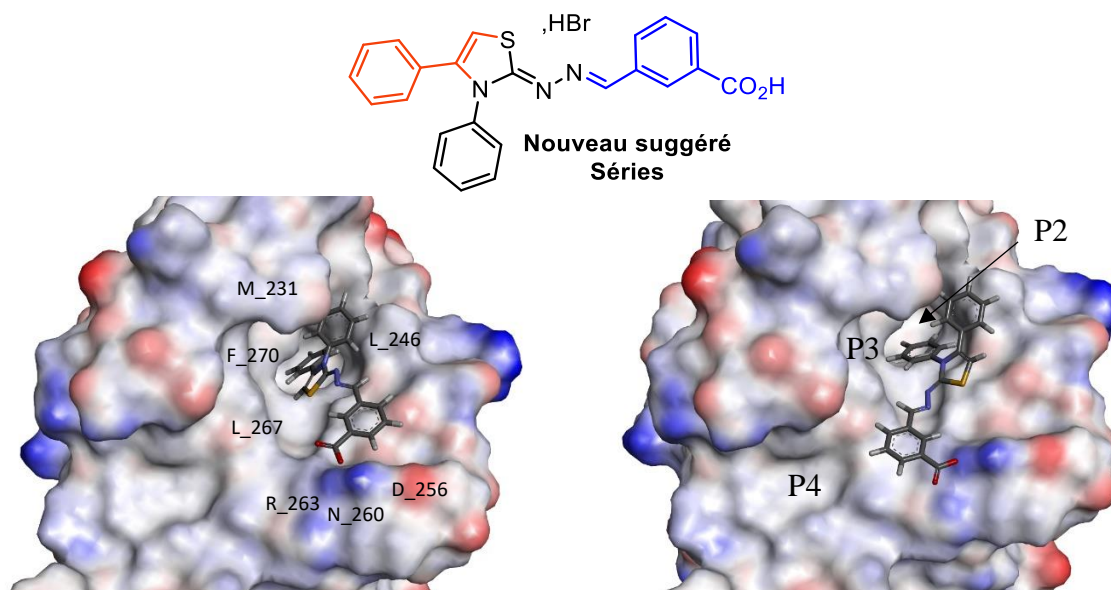
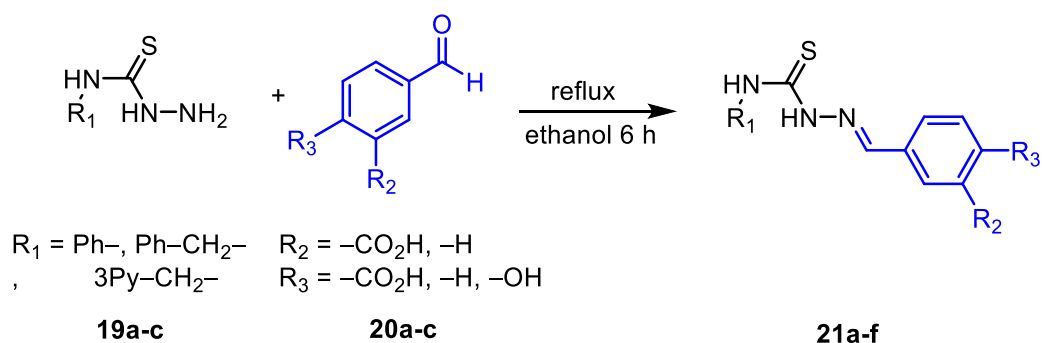


Figure 4: Amarrage moléculaire des analogues MIM-1 suggérés avec un méta-acide dans la protéine Mcl-1.

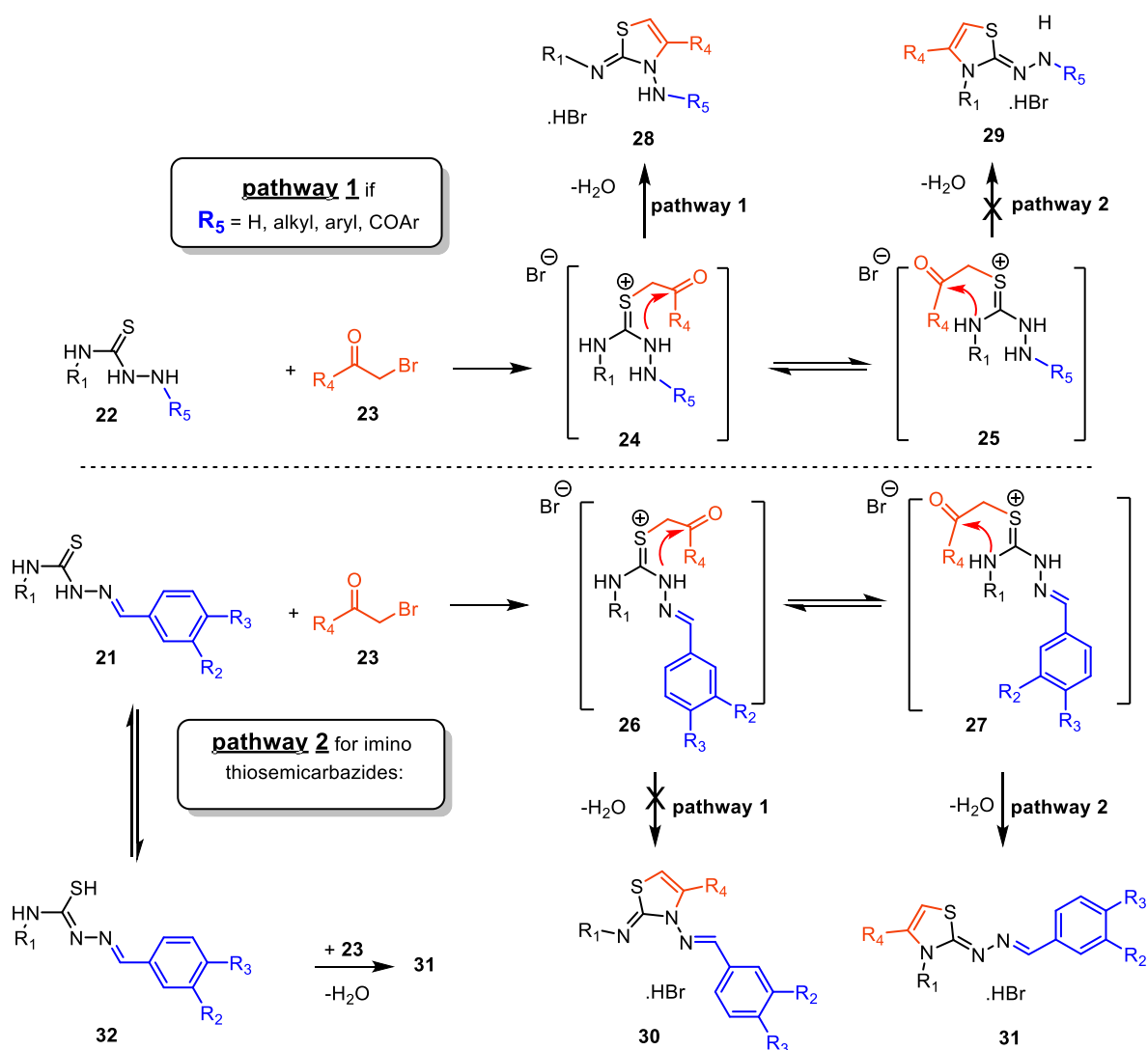
Ainsi, nous avons travaillé sur le développement d'une méthode de synthèse appropriée des composés originaux dont la conception avait été faite par modélisation moléculaire.

Cette première étape a été réalisée avec succès et une dizaine de composés ont été préparés et soumis à des tests biologiques d'inhibition de la protéine anti-apoptotique Mcl-1 (Scheme 4).



Scheme 4: Synthèse des intermédiaires imino-thiosemicarbazides **21a-f**.

De manière surprenante cependant, dans la dernière étape, la condensation entre ces thiosemi-carbazides avec les bromocétone, n'a pas donné les produits attendus **30** mais les dérivés régioisomères **31** (Scheme 5).



Scheme 5: Condensation de thiosemicarbazides avec des α -bromocétone.

Ceci a été établi par l'analyse cristallographique aux rayons X du composé **31r**, puis confirmé par des expériences de RMN 2D (Figure 5).

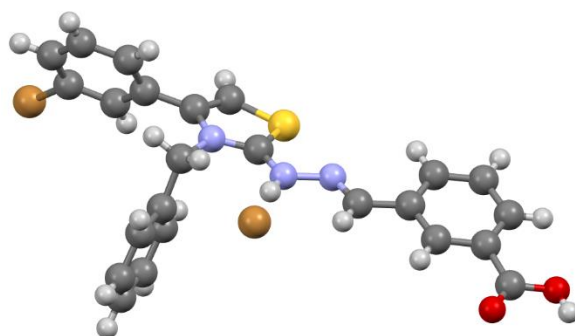


Figure 5: Structure cristallographique aux rayons X du composé **31r**

Des études biologiques détaillées utilisant les lignées cellulaires du cancer de l'ovaire, IGROV1-R10, nous ont permis de découvrir des dérivés qui sont de puissants inhibiteurs doubles de Bcl-xL et Mcl-1. Toutes ces données nous ont donné une relation Structure-Activité préliminaire pour ces nouveaux analogues de MIM1. Des études computationnelles approfondies nous ont permis de proposer une justification à ce SAR, mettant en évidence le rôle de la fraction méta-acide pour ancrer ces molécules aux protéines anti-apoptotiques et la position des groupes aromatiques/benzyle à l'intérieur des poches hydrophobes de ces protéines.

Titre : Préparation de nouveaux analogues de GABA fluorés et développement de nouveaux inhibiteurs de protéines antiapoptotiques à visée anticancéreuse

Mots clés : Fluor, Nitrones, γ -Lactames, inhibiteurs de Mcl-1, Apoptose, Cancer

Résumé : Cette thèse est divisée en deux parties: chimie du Fluor et chimie médicinale. La première partie consacrée à la synthèse d'hétérocycles azotés portant une chaîne latérale fluorée contient trois chapitres : dans le premier on rappelle l'importance des hétérocycles azotés ainsi que de la chimie du fluor en chimie médicinale. Dans le second chapitre, nous décrivons la synthèse de nouveaux γ -lactames portant des chaînes latérales gem-difluorées ainsi que les essais réalisés pour obtenir des analogues de GABA. Le troisième chapitre est consacré à la synthèse à partir d'un intermédiaire propargylique fluoré, de trois séries de nouvelles nitrones portant une chaîne fluorée.

Dans la seconde partie dédiée à la recherche de nouvelles molécules à activité antitumorale, notre objectif était de réinduire l'apoptose au sein de cellules cancéreuses en se basant sur la structure révisée de MIM1, un inhibiteur connu de la protéine antiapoptotique Mcl-1. A partir d'une étude de modélisation moléculaire, nous avons désigné, puis préparé 38 analogues originaux qui ont été testés sur les cellules IGROV1. Certains de ces composés induisent une activité apoptotique de cellules cancéreuses prometteuse. Une étude des relations structure-activité et un travail de modélisation moléculaire ont été réalisés et nous ont permis de rationaliser les résultats obtenus.

Title : Preparation of new fluorinated analogs of GABA and development of new inhibitors of antiapoptotic proteins with anticancer activity

Keywords : Fluorine, Nitrones, γ -Lactams, Mcl-1 inhibitors, Apoptosis, Cancer

Abstract : The thesis is divided into two parts: Fluorine Chemistry and Medicinal Chemistry. The first part devoted to the synthesis of nitrogen heterocycles bearing a fluorinated side chain contains three chapters: in the first one we recall the importance of nitrogen heterocycles as well as fluorine chemistry in medicinal chemistry. In the second chapter, we describe the synthesis of new γ -lactams bearing gem- difluorinated side chains as well as the tests carried out to obtain GABA analogs. The third chapter is devoted to the synthesis from a fluorinated propargyl intermediate of three series of new nitrones bearing a fluorinated chain.

In the second part dedicated to the search for novel molecules with anti-tumor activity, our objective was to reinduce apoptosis in cancer cells based on the revised structure of MIM1, a known inhibitor of the anti-apoptotic protein Mcl-1. Based on a molecular modeling study, we designed and prepared 38 original analogues that were tested on IGROV1 cells. Some of these compounds induce promising apoptotic activity in cancer cells. A study of structureactivity relationships and molecular modeling work were performed and allowed us to rationalize the results obtained.