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I. A UNIFIED APPROACH FOR THE SYNTHESIS OF CONFORMATIONALLY RESTRICTED AMINOACIDS
II. SYNTHESIS OF NOVEL HYGROMYCIN *A* ANALOGUES FROM BENZENE

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Part I: A UNIFIED APPROACH FOR THE SYNTHESIS OF CONFORMATIONALLY RESTRICTED AMINOACIDS

1 Proline Analogues and Antihypertensive Drug Derivatives

1.1 Introduction

Among the different ways to develop new drugs, the most successful is the "follow-on"-based strategy, also called analogue-based strategy[1]. It is less expensive, more convenient, and more fruitful than other methodologies (e.g., high-throughput screening, structure-based drug design [2]). In fact, the drug discovery process has become an extremely risky and costly [3], with high rates of failure and attrition at all stages (less than 10% of lead candidates are marketed [4]).

This approach is based on structural modification and further functionalization of the scaffolds in already marketed drugs to produce new drugs bearing better properties such as increased selectivity, potency, and better interactions with target protein [1,2].

Analogue-based strategy has a few advantages over the pioneering drug discovery [1,5]. It is more effective and less risky, since inventing *ex novo* a new drug structure is a difficult challenge due to the attrition rate in pharmaceutical R&D [6,7]. Despite the overall

convenience of this approach, there are some risks, because any modification in a molecule may alter its properties and activity [8].

In general, drug discovery research is aimed at synthetizing molecules that are as "drug-like" as possible [9,10]. Drug-likeness is related to specific physicochemical properties (hydrophobicity, flexibility, molecular size, molecular weight, reactivity, metabolic stability, toxicity, and bioavailability [11]) that make a molecule able to interact with the biophase and exert a pharmacological effect [9].

Lipinki's Rule of 5 (Ro5) [9] summarizes in few points the most important factors for a lead candidate to be more drug-like:

- no more than 5 hydrogen-bond donors;
- no more than 10 hydrogen-bond acceptors;
- molecular mass is less than 500 Da;
- octanol-water partition coefficient (ClogP) that does not exceed 5.

Other important parameters used to evaluate the drug-likeness of a molecule [12] are polar surface area (PSA) [13], lipophilicity ligand efficiency (LLE) [14], the number of rotatable bonds (NRotB), and aromaticity.

1.2 **Ring systems in medicinal chemistry**

1.2.1 **Rings in drug molecules**

Rings are key frameworks in medicinal chemistry. They can be found in a lot of natural products and are the fundamental backbone of most marketed drugs. They offer several advantages in drug development due to their properties, such as molecular rigidity, tridimensionality (aliphatic rings) and flatness (aromatic rings); moreover, they affect polarity, molecular reactivity, solubility, and stability [15].

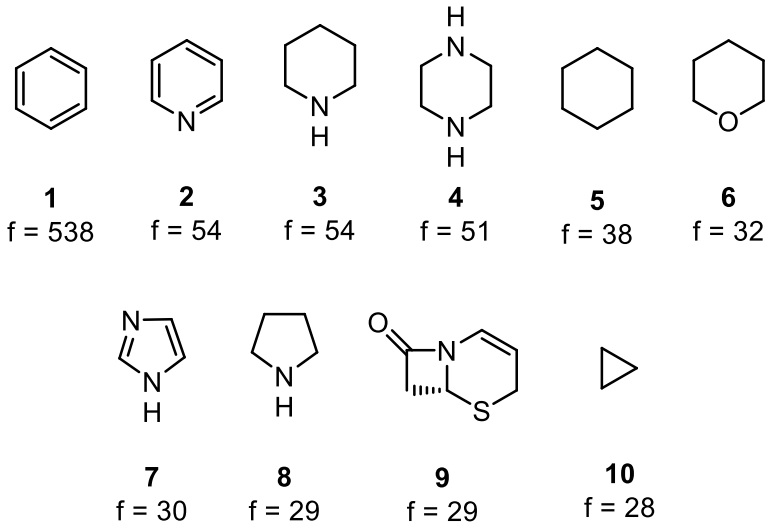


Figure 1*: top 10 most frequently used ring systems from small molecule drugs listed in the FDA Orange Book (Rings in drugs)*

Taylor and co-workers have conducted a thorough analysis on rings in marketed drugs listed in the FDA Orange Book (containing 1175 drugs) [16]; there are 351 ring systems and 1197 ring frameworks in drugs marketed before 2013. The top 10 most frequently ring

systems are shown in **Figure 1**, where f is the frequency of these rings calculated with the algorithm developed by Taylor et al. As we can see, the most common ring is benzene, indicating the relevance of aromatic character in medicinal chemistry.

ChEMBL DrugStore [17] database shows that aromatics are prevalent in marketed drugs, with 76% containing at least one aromatic ring [18]. According to Taylor and co-workers, 40% of investigated drugs do not have any sp^3 carbon atoms in a ring system, and only 5% do not contain any sp^3 carbon at all. This proves that saturated (or partially unsaturated) ring systems are also important in drug design; in fact, as shown in **Figure 1**, while the most common rings are benzene and pyridine, most of the others are saturated rings. While aromatics are fundamental in drug development due to their properties, an excess of aromaticity can cause attrition during drug development [18,19]. For this reason, aromaticity and the ratio saturation/unsaturation must be considered during the drug design and discovery process.

1.2.2 Physicochemical descriptors of aromatic character

Due to the importance of aromatic rings, several physicochemical parameters have been formulated to calculate the aromaticity of marketed drugs and predict the developability of lead candidates [18]; they are also used to investigate the effects of aromaticity on ADMET parameters (Absorption, Distribution, Metabolism, Excretion, and Toxicity) [20,22]. Aromaticity and the number of aromatic rings are essential descriptors to

evaluate the developability of drug candidates, and they are thought to be one of the most important descriptors after lipophilicity [23,24]. The main aromatic descriptors are:

- **Aromatic Indicator (Ar/HA, or AP):** number of atoms in aromatic rings divided by the total number of atoms (excluding hydrogens) [18,20].
- **Aromatic Ring Count (AROM):** number of aromatic rings [25].
- **Aromatic Ratio (ARR):** number of aromatic bonds divided by the total number of bonds (excluding those with hydrogens) [18].
- **Carbo- and Heteroaromatic Rings Count**: relative number of carboaromatics and heteroaromatics and their ratio, which affect physicochemical properties [18].
- **Aliphatic Indicator, or Fraction of sp^3 Carbon (Fsp^3):** first introduced as the number of sp^3 carbon atoms divided by the total number of carbon atoms. It is the opposite of Ar/HA, and it shows an inverse correlation with aromaticity: the higher Fsp^3, the "less aromatic" is the molecule investigated [18,20].
- **Aromatic Atoms minus sp^3 Carbon Atoms (Ar-sp^3)**: unlike Fsp^3, considers compounds with zero aromatic atoms or zero sp^3 atoms [26].

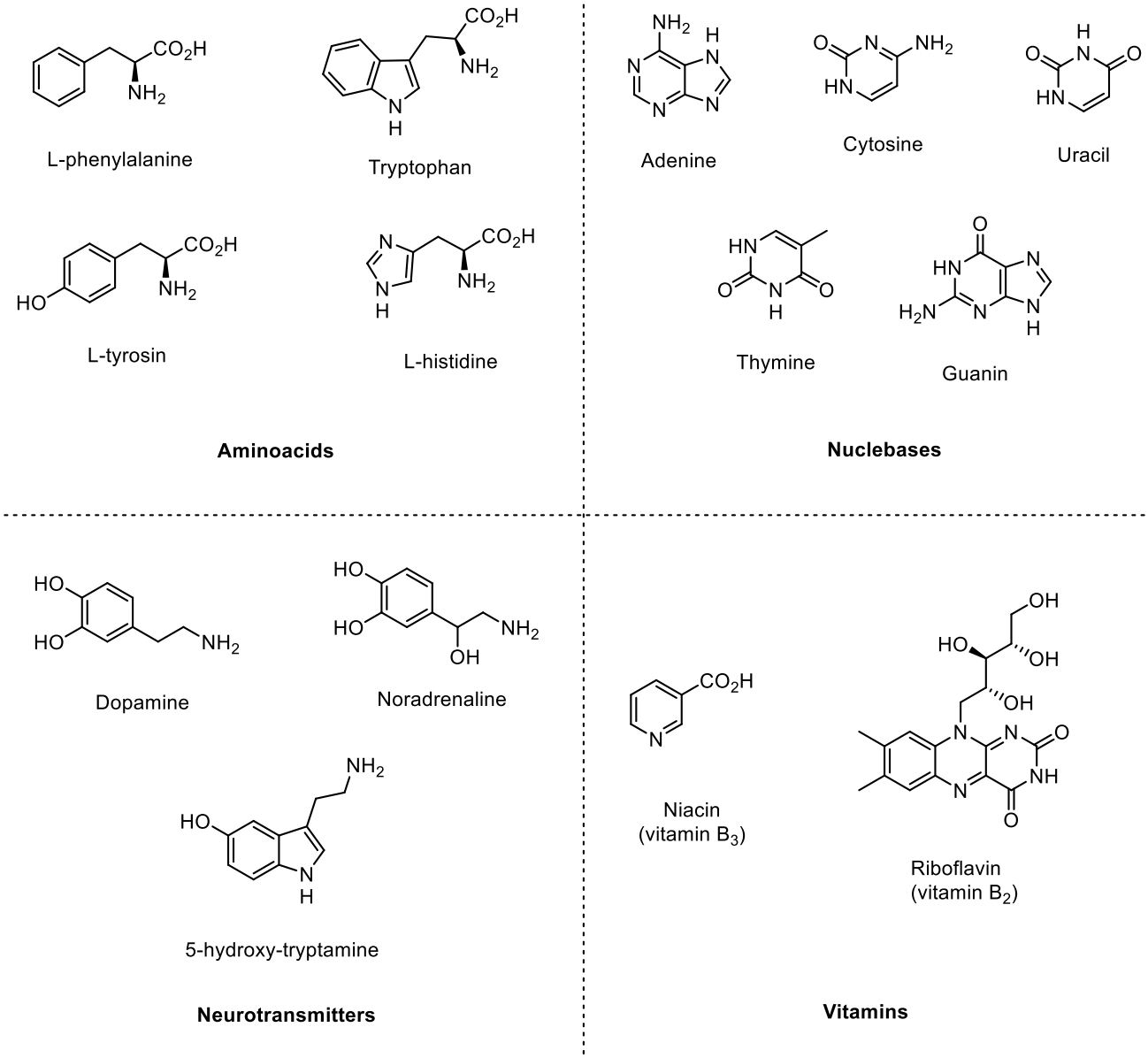


Figure 2: *important biological and physiological products containing aromatic rings*

1.2.3 **Aromatic rings in drug chemistry**

There are several advantages in incorporating aromatic rings in drugs. First, aromatic rings are frequent and common in a variety of natural compounds: amino acids, neurotransmitters, nitrogenous bases in ribonucleic acids, vitamins, signalling molecules, and in several secondary metabolites (**Figure 2**). Many so-called "privileged templates" [27,28] that occur in natural products contain aromatic rings.

Due to their ubiquity in nature, aromatics play a key role in many biological functions, such as molecular recognition, protein binding, receptor inhibitions, neurotransmission. Arenes can interact with the biophase using different kinds of interactions [29,30]. The main interaction modes are arene-arene interactions (**Figure 3**): parallel displaced (**3a**), T-shaped edge-to-face (**3b**), and eclipsed face-to-face, or π-stacking (**3c**) are the main interaction geometries. Substituents also affect the mode of interactions: H-arene interaction are frequent, with T-shaped edge-to face (**3d**-**e**) geometry. We can also have π-cation and π-anion stabilization, S-arene interaction, and hydrogen bonding to π systems. For these reasons, inserting aromatic rings in a drug molecule allows us to exploit these interactions with target proteins in therapy.

Aromatics are versatile substrates: there are several synthetic pathways to modify rings with different functional groups, obtaining a variety of derivatives [19]. Moreover, in recent decades several protocols have been developed to incorporate aromatic rings in a molecular framework by C-C bond formation. Cross-coupling reactions based on

palladium catalysts are extensively used in synthetic chemistry to bind aromatic rings [31,32,33]. Aryne reactivity is used to bind arenes in two different positions, achieving 1,2-disubstituted benzenes [34,35].

Aromatic rings have a plain, rigid structure, whose substituents are projected along well-defined and fixed directions. These frameworks also possess conformational restrictions, avoiding stereochemical implications found in unsaturated rings.

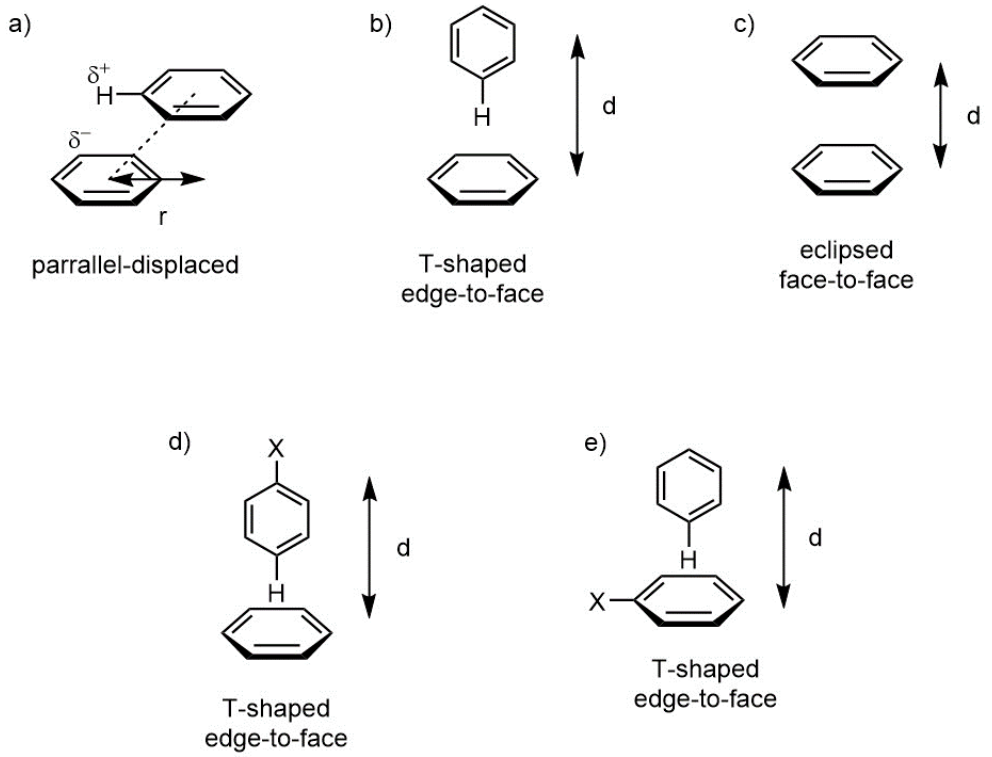


Figure 3: *interaction modes between two aromatic systems: a-c) interaction geometries of benzene dimers; d-e) substituent effects on benzene interactions*

Lastly, aromatic compounds contribute greatly to lipophilicity, increasing the oral availability of drugs. They can be easily absorbed by stomach and gut walls, pass through biological and cell membranes, and interact with proteins and enzymes. In fact, active sites of enzymes are highly hydrophobic spaces, and most of them possess aromatic amino acid functionalities that form strong intermolecular interactions with aromatic rings.

However, in addition to these positive attributes, there are many downsides, especially when a high percentage of aromatic rings is involved [19]. These negative characteristics include poor water solubility, and interference in biological functions via formation of reactive metabolites. An increased number of aromatic rings impact negatively ADMET-parameters; it is also associated with an increase in albumin-binding and inhibition of the hepatic metabolic enzyme CYP450 3A4 [23,36]. An excess of aromaticity may be incorporated in cell membranes, inducing the risk of membrane disruption [19,36].

Other drawbacks are encountered during the drug development process. There is, in fact, an inverse correlation: an excess in aromaticity correlates with attrition and poor developability of candidates [26].

Thorough investigations have been conducted using the aromatic descriptors discussed above. High values of ARR (aromatic ratio) are associated with toxicity attrition during drug development [18]. Aromatic indicator (AP) has been used to estimate water solubility of drugs, suggesting that a high degree of aromatic rings leads to poor solubility

due to their tendency to form dense crystals with poor flexibility and high melting point [37,38]. High values of aromatic ring count (AROM) are associated with low water solubility, CYP450 inhibition, and increase in albumin-binding [23].

Fsp^3 is an important parameter to evaluate the role and importance of aliphatic rings; generally, higher Fsp^3 values increase chances of success. An analysis on molecules taken from GVK BIO database [39] indicates that molecules with high values of Fsp^3 (i.e., less aromatic character) possess higher solubility, low melting point and show less CYP450 inhibition [22,40]. Increasing Fsp^3 has beneficial effects on the development process: marketed drugs have an average Fsp^3 of 0.47 [41]. Molecules with higher Fsp^3 show less CYP450 inhibition, lower melting point, and higher water solubility. Ar-sp^3 is considered a more valuable descriptors than Fsp^3 [18,42] since it considers molecules with either zero sp^3 or aromatic carbons. Higher values of Ar-sp^3 (i.e., higher aromatic character) increase the flatness and structural rigidity, while lower values (i.e., higher aliphatic character) increase three-dimensionality [42].

In conclusion, an excess of aromaticity is likely to induce ADMET-related attrition in the drug discovery process. In general, the higher Fsp^3, the more a candidate can be developed. The molecular rigidity and flatness of aromatic rings have beneficial effects, but a certain level of three-dimensionality and saturation is required for drug success. Thus, carboaliphatic and heteroaliphatic rings play an important role [19].

1.2.4 **The importance of saturated rings**

As previously discussed, increasing the aliphatic character of drug molecules by inclusion of aliphatic rings proves to be a valuable strategy in drug development.

In addition to overcoming the disadvantages and side effects of aromatics, aliphatic rings bring several advantages due to their intrinsic structure. While aromatic compounds are rigid and flat, aliphatic rings are three-dimensional scaffold with higher molecular flexibility and mobility [43,44]; in fact, saturated rings can have more than one possible conformation. Moreover, three-dimensionality increases structural complexity [45,46], allowing chemists to synthetize molecules with complex structures without increasing molecular weight significantly. More complex molecules have the capacity to access greater chemical space and improve protein binding [47]. Switching from sp^2 aromatic carbon atoms to sp^3 introduces an important property, which is stereochemistry [40,43,48], especially in quaternary carbon atoms with four different substituents. Stereochemistry plays an important role in molecular recognition: for example, active sites of enzymes can recognise a single enantiomer or diastereomer of a given substrate.

Saturation, thanks to the conformational complexity and tridimensionality it imparts, is useful to overcome the drawbacks of aromaticity. However, problems may arise from an overabundance of these properties, such as low protein binding due to excessive conformational freedom that leads to unfavourable entropic contribution. For this reason, a valuable solution is the synthesis of three-dimensional substrates whose conformational

freedom is locked or strictly limited (e.g., conformationally restricted sp^2-sp^3 hybrids), to combine the positive characteristics of both aromatic and saturated cyclic frameworks [40].

1.3 Conformational restriction and its relevance in medicinal chemistry

Conformational restriction of a molecule is a "follow-on" approach used in medicinal chemistry to synthetize new drug candidates with high chances of success and improved potency [2,49]. It consists in structural modification of already available marketed drugs to induce rigidity and stiffness, to limit its molecular mobility and ligand flexibility [8]. This strategy is useful to increase binding affinity.

Binding affinity is the difference in Gibbs' free energy ($\Delta G°$) between free and bound state of a drug and its receptor (**Eq. 1**):

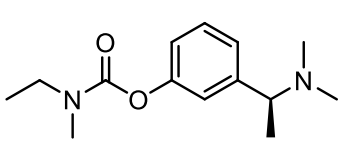
$$\Delta G° = \Delta H° - T\Delta S° \quad \textbf{(Eq. 1)}$$

When a drug binds to its target, binding energy ($\Delta G°$) must compensate for the de-solvation. $\Delta H°$ has a negative contribution and is associated with the energy of intermolecular bonds that are broken (i.e., solvation with water) and those that are formed (e.g., hydrogen-bonding, Van der Waals interactions, hydrophobic interactions) [50]. $\Delta S°$ has a positive contribution and is related to molecular re-organization of solvating water molecules and conformational changes in both drug and receptor before and after the binding [8,51]. When we have formation of non-covalent bonds, enthalpy and entropy contributions are comparable ($\Delta H° \approx \Delta S°$). We observe drug binding when $\Delta G°$ is < 0.

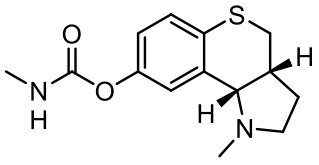
When the binding takes place, the drug loses conformational freedom, motion, translational and rotational flexibility [52,53]. $\Delta S°$ decreases due to loss of degree of motions when two molecules are constrained, thus reducing binding energy [54].

To improve binding efficiency, we must act both on enthalpy and entropy [55]. In the first case, we must improve drug-protein binding by increasing drug lipophilicity, so that interactions with the active site exceed those with water molecules. In the second case, we must reduce the loss of conformational entropy when the bond is formed; to do so, we must modify the structure of drug molecule so that it can complement the binding site in a more efficient way. There are different strategies to minimize loss of entropy: steric hindrance, hybridization, rigidification, and conformational constraint [8,56].

Structural restriction may increase the potency of a drug by stabilizing and "freezing" a particular conformation that suits best the active site of receptor, thus minimizing entropy penalty and increasing selectivity; it is also thought to improve membrane permeability. The insertion of fused rings and spirocyclic systems is a synthetic strategy to lock a particular conformation [57].



11
Rivastigmine
AchE inhibitor
IC_{50} = 1535 nM



12
AchE inhibitor
IC_{50} = 8.11 nM

Figure 4: *application of conformationally restricted molecules in medicinal chemistry*

Conformational restriction has been successfully implemented in several drug synthesis.

An example is shown in **Figure 4.** Rivastigmine (**11**), an acetylcholinesterase inhibitor, is used to treat Alzheimer disease; its structure has been further modified to obtain drug **12**, whose IC_{50} (i.e., the concentration of an inhibitor necessary to inhibit the activity of target protein by 50%) is 190-fold smaller than **11**, making it a more potent drug [2,58]. A possible strategy is exploiting the chemistry of hetero-norbornane or norbornenes derivatives fused with a benzene ring to create conformationally-restricted sp^2-sp^3 hybrids [59]. This synthetic protocol would allow us to fuse together an aromatic ring to an aliphatic ring; using this strategy, we combine the advantages of both aromatic and aliphatic rings while maintaining a good level of tridimensionality and conformational restriction. A valid strategy to obtain this framework is aryne reactivity [34].

1.4 Aryne: properties, synthesis, and applications in medicinal chemistry

1.4.1 Chemical properties of aryne

Arynes are highly reactive species derived from a benzene by removal of two substituents (formally, by abstraction of two hydrogen atoms).

There are three isomers (**Figure 5**): *ortho*-aryne, or 1,2-didehydroaryne, is the most investigated and is involved in a lot of reactions (e.g., pericyclic reactions, nucleophilic aromatic substitutions); *meta*-aryne, or 1,3-didehydroarene; and *para*-aryne, or 1,4-didehydroarene [60,61]. We have considered the chemistry of *ortho*-aryne.

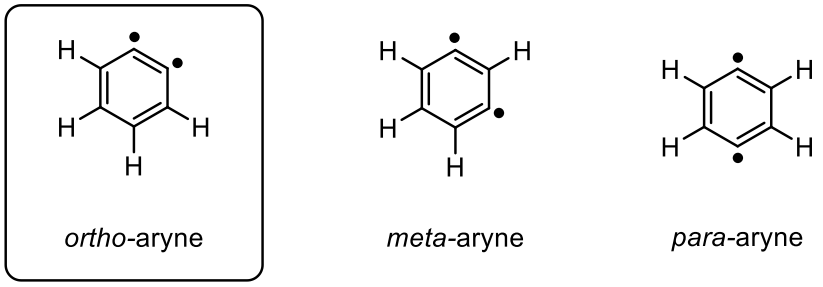


Figure 5: *aryne isomers*

Arynes are generally described as restrained cyclic alkyne. Other structures have been proposed (biradical, cumulene, ylide) [62,63] (**Figure 6**).

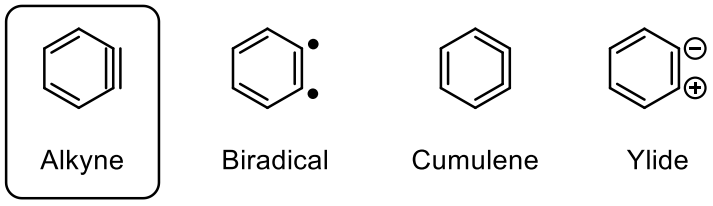
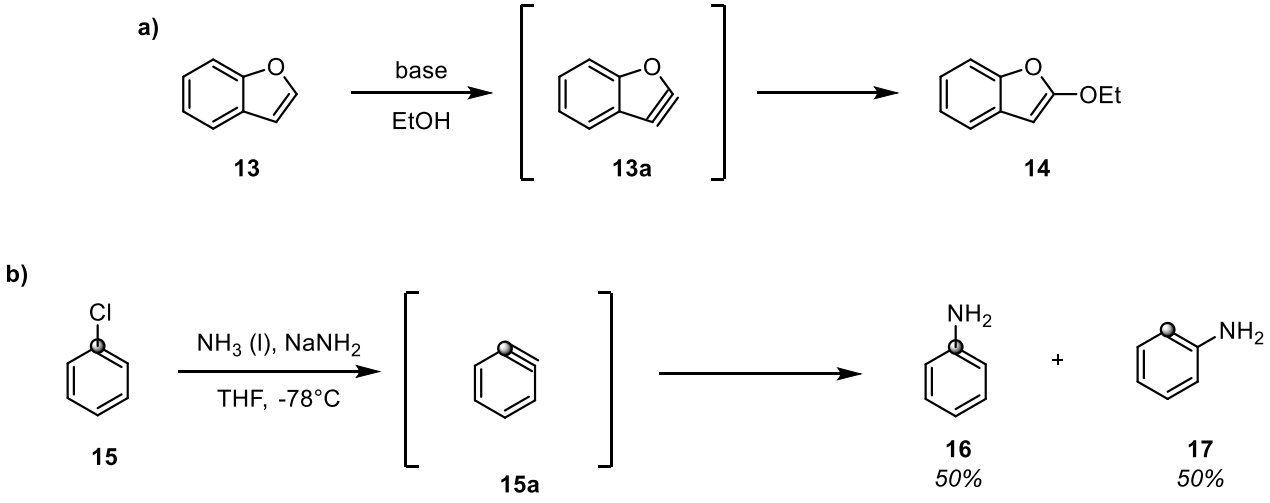


Figure 6: *proposed aryne structures*

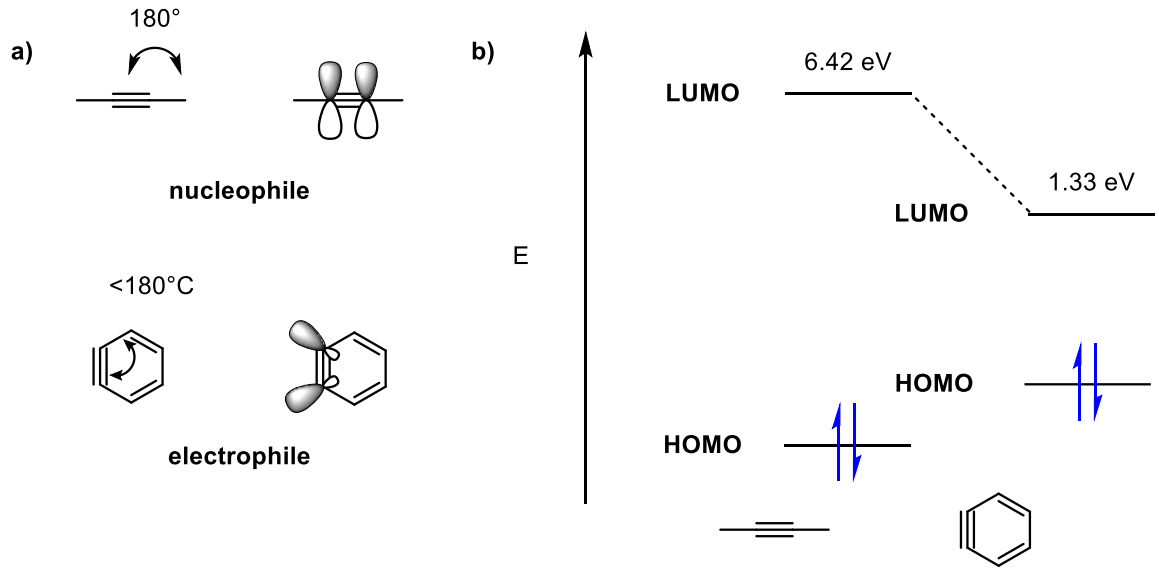
The existence of arynes was postulated by Stoermer and Kahlert in 1902: they observed the formation of 2-ethoxybenzofuran upon treatment of 3-bromobenzofuran with base in ethanol and postulated the formation of a 2,3-didehydrobenzofuran intermediate **13a** [60] (**Scheme 1.a**). Definitive evidence was obtained in 1953 by Roberts et al.: they showed that reaction of ^{14}C-labeled chlorobenzene and sodium amide with liquid ammonia gave equal amounts of two aminated products in *ipso*- and *ortho*-positions [64,65]; the distribution of the products can only derive from a symmetrical intermediate **15a** (**Scheme 1.b**).



Scheme 1: a) *Stoermer & Kahlert experiment;* ***b)*** *Roberts et al. experiment*

The strain on C≡C bond results in reduced overlap of the in-plane p-orbitals and thus lowers the energy of the triple bond, making it weaker than a normal alkyne (**Scheme**

2.a). Geometric constriction significantly reduces the LUMO energy of the benzyne (from 6.42 eV for 2-butyne to 1.33 eV for benzyne), while HOMO energy doesn't change (**Scheme 2.b**). For this reason, benzyne possesses electrophilic properties and easily undergoes reactions with nucleophiles [66].



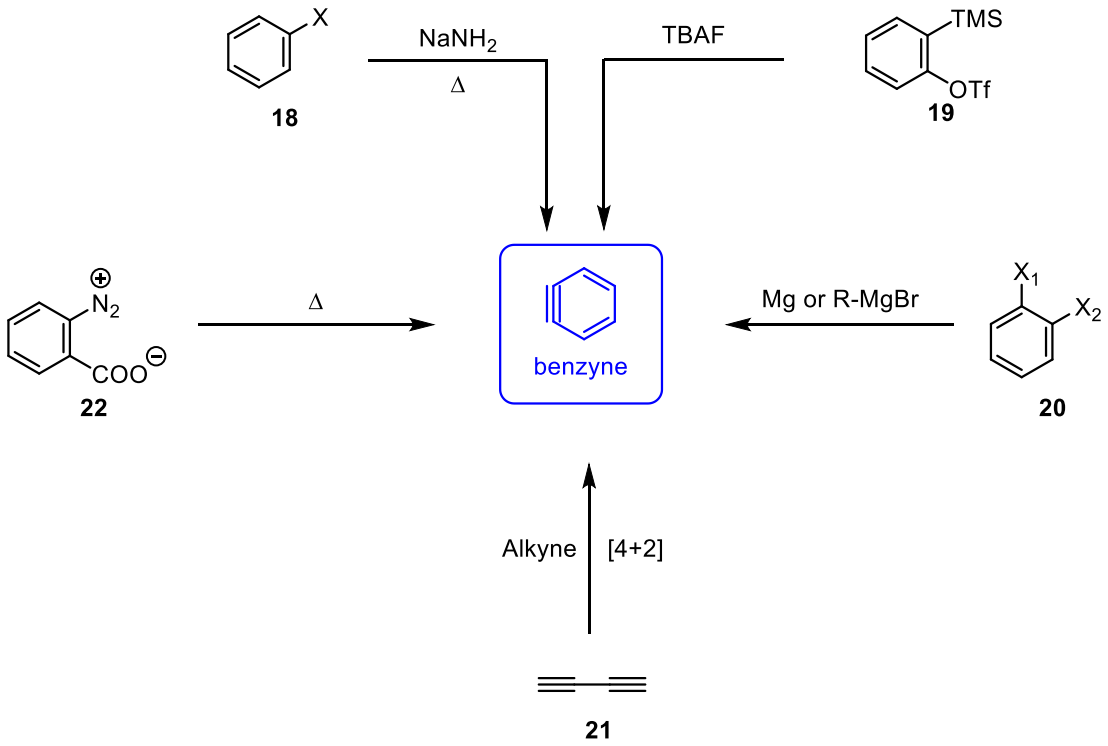
Scheme 2: a) *effects of geometrical constraints on p-orbitals of aryne;* ***b)*** *HOMO-LUMO in aryne*

1.4.2 **Aryne formation**

Several synthetic procedures to generate benzyne have been developed (**Scheme 3**). Due to its extreme reactivity, aryne must be generate *in situ*.

Most of the method employed require metals, strong bases, harsh reaction conditions, and high temperatures, that are not compatible with several functional groups.

The most important benzyne formation reactions are dehydrohalogenation of aryl halides, diazotization of anthranilic acid and fluoride displacement of trimethylsilyl group in trimethylsilyl aryl triflates.

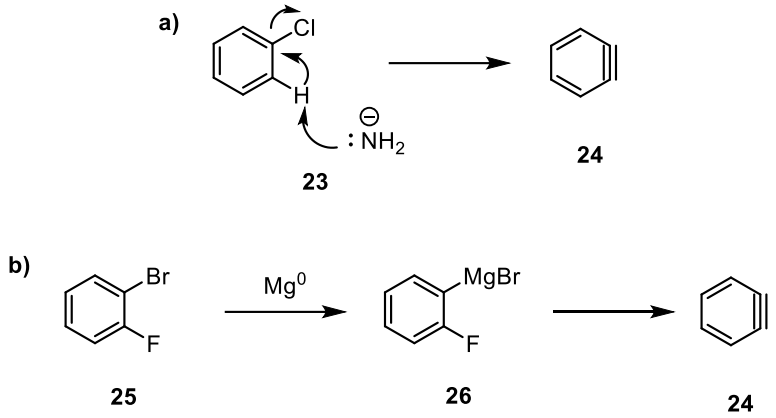


Scheme 3: *synthetic strategies to obtain benzynes*[35]

Dehydrohalogenation of aryl halides

Aryl halides are the typical benzyne precursors; they furnish arynes via dehydrohalogenation, an elimination reaction which removes a hydrogen halide from the substrate molecule. Unfortunately, it requires harsh conditions (elevated reaction

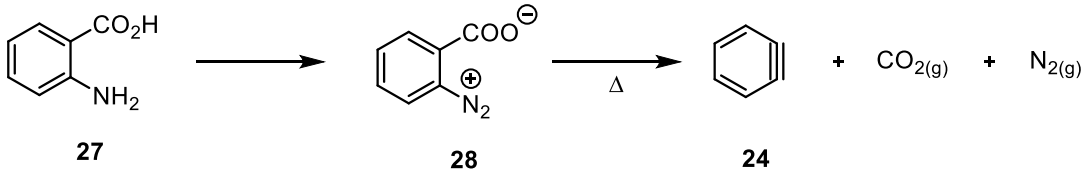
temperatures and a strong base, such as *n*-butyl-lithium or sodium amide) (**Scheme 4.a**) [60,67]. *Ortho*-dihalobenzenes are used under milder conditions: benzyne is generated via dehalogenation of 1,2-disubstituted haloarenes mediated by metals (Mg or Li) or organometallic reagents [68,69] (**Scheme 4.b**).



Scheme 4: *benzyne generation starting from* ***a)*** *aryl halide, and* ***b)*** *1,2-disubstitued haloarenes*

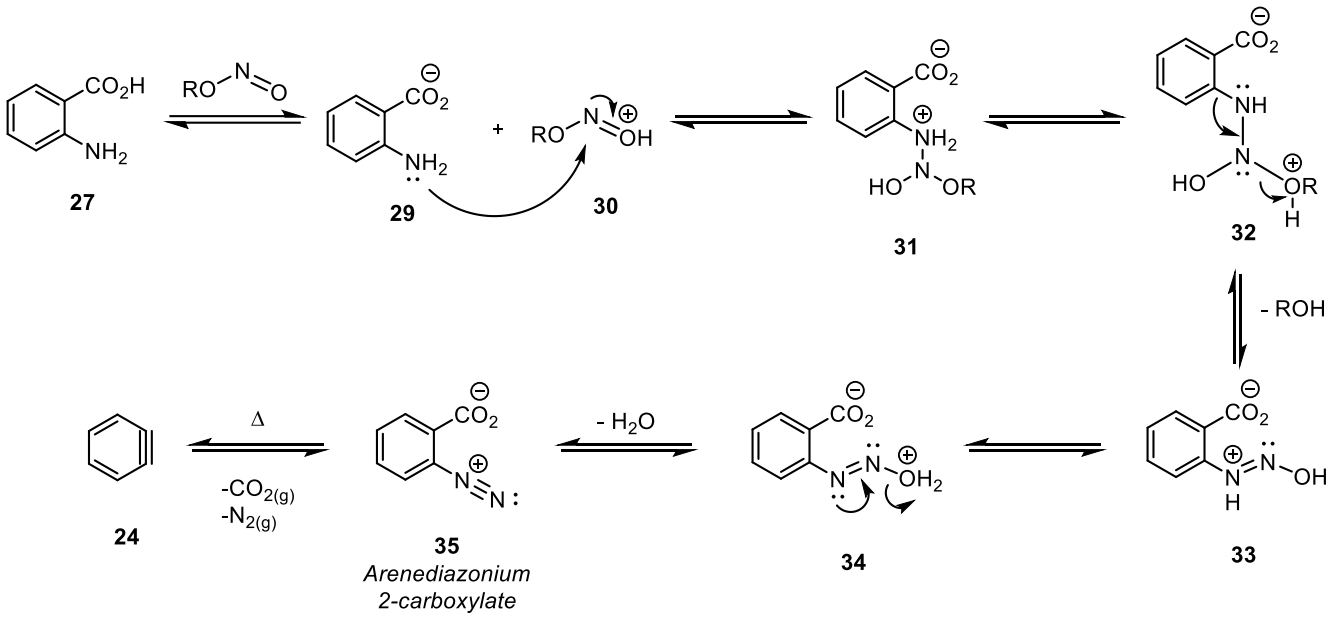
Diazotization of anthranilic acid

Anthranilic acid (**27**), upon treatment with an alkyl nitrite (e.g., pentyl or isoamyl nitrite) generates the zwitterionic arenediazonium 2-carboxylate **28** [70]. This reactive and unstable intermediate decomposes upon heating, inducing decarboxylation and generating benzyne with release of nitrogen and carbon dioxide, the driving force of the process [71] (**Scheme 5**).



Scheme 5: *aryne formation from anthranilic acid via benzenediazonium carboxylate intermediate*

The proposed mechanism of the diazonium salt is shown in **Scheme 6** [71,72].



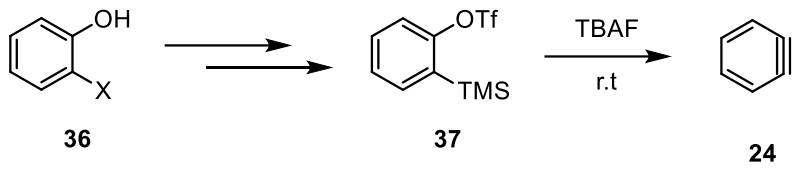
Scheme 6: *mechanism of benzyne formation starting from anthranilic acid **27** via arenediazonium **35***

The use of arenediazonium **35** is convenient and has many advantages: it is generated when heated at controlled temperature; side-products are gases; the precursor **27** is inexpensive. An inconvenient is the extreme reactivity of arenediazonium and its explosive properties [70,71].

Kobayashi's fluoride-induced aryne generation

This strategy, developed by Kobayashi and co-workers [73], involves a fluoride displacement of a trimethylsilyl group in trimethylsilyl aryl triflates. This fluoride-induced 1,2-elimination removes a triflate and forms the aryne. Fluoride source may be tetra-*n*-

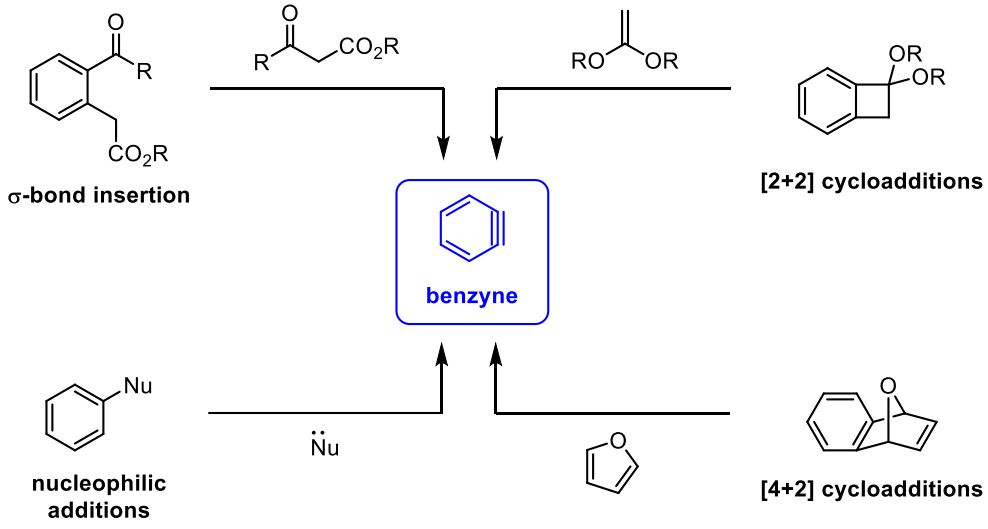
butylammonium fluoride (TBAF). Trimethylsilyl aryl triflate can be prepared from 2-halide phenols (**Scheme 8**). This method requires milder reaction conditions [34,73].



Scheme 8: *Kobayashi's fluoride induced benzyne formation*

1.4.3 **Aryne reactivity**

Due to its geometric constraints and low LUMO energy, aryne are reactive species, even at low temperatures. Due to its electrophilic character, benzyne reacts readily with nucleophiles; given its alkyne nature, it can take part in pericyclic reactions (**Scheme 9**).



Scheme 9: *main reactions involving benzyne ring* [35]

Benzyne must be generated *in situ* so that it can react immediately with the substrate. Aryne chemistry is difficult to control: benzyne may take part in side reactions leading to impurities and by-products formation; it must be trapped, otherwise it dimerizes or trimerizes to biphenylene or triphenylene, respectively (**Figure 7**) [74,75].

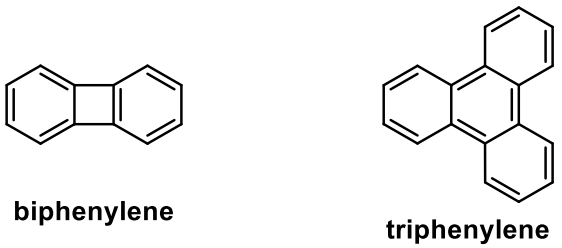


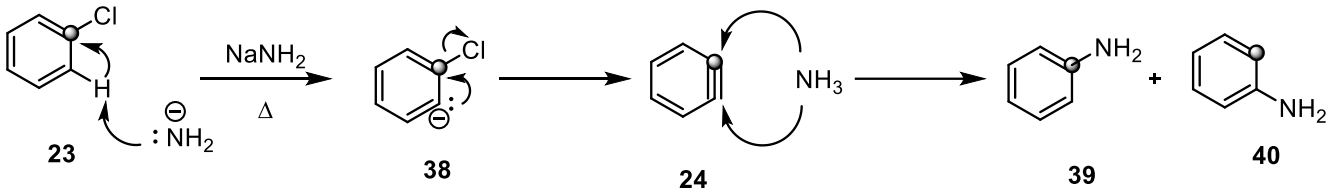
Figure 7: *dimerization and trimerization of benzyne*

Aryne reactivity can be classified in four main classes: 1) nucleophilic aromatic substitutions, 2) pericyclic reactions (notably, [4+2] cycloadditions), 3) multicomponent reactions, and 4) bond-insertion reactions [35,66].

Nucleophilic aromatic substitutions

Nucleophilic aromatic substitution involves displacement of a good leaving group, such as a halide, on an aromatic ring with a nucleophile. Aryne is involved in one of the possible mechanisms, in which aryl halide undergoes dehydrohalogenation by interacting with a strong base (such as, sodium amide). Removal of the acidic hydrogen atom in *ortho*-position by the base is followed by loss of halide ion, generating the benzyne; this

intermediate later undergoes nucleophilic attack equally in *ipso*- and *ortho*-position (**Scheme 10**) [76,77].

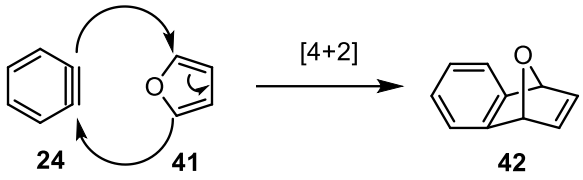


Scheme 10: *benzyne-mediated nucleophilic aromatic substitution*

[4+2] cycloadditions

Diels-Alder cycloaddition is a [4+2] pericyclic reaction between a conjugated diene and a dienophile. Aryne, as a constrained alkyne, can act as a dienophile. It is likely that this aryne cycloadditions proceed by stepwise process, rather than the classical concerted mechanism. Substituents on both the diene and the aryne can heavily influence the reaction mechanism, favouring one over the other.

However, there are significant limitations to the application of aryne [4+2] cycloadditions. Particularly, most of them requires constrained dienes, for examples dienes with bulky substituents, or cyclic ones, such as cyclopentadiene, cyclohexadiene, and furan (**Scheme 11**) [78]. Furan is the less aromatic of all the five-membered aromatic heterocycles, and its conjugated system has a strong diene character [79]. Other heterocycles, such as pyrrole, can act as diene if properly activated [80].



Scheme 11: *[4+2] Diels-Alder cycloaddition between aryne and furan*

We can perform Diels-Alder and Hetero-Diels-Alder reactions with aryne derivatives with cyclic and constrained dienes to build conformationally restrained frameworks (**Figure 8**) in which a carbocycle or heterocycle is fused with a benzene ring. This complex 1,2-disubstituted arenes are difficult to obtain by other conventional methods. For this reason, aryne-based Diels-Alder provides an efficient strategy to build complex molecular scaffolds that may be useful in drug chemistry. All these substrates are basically norbornene and norbornadiene derivatives fused with a benzene ring [81,82].

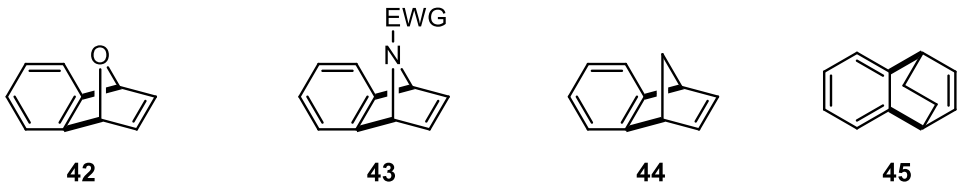
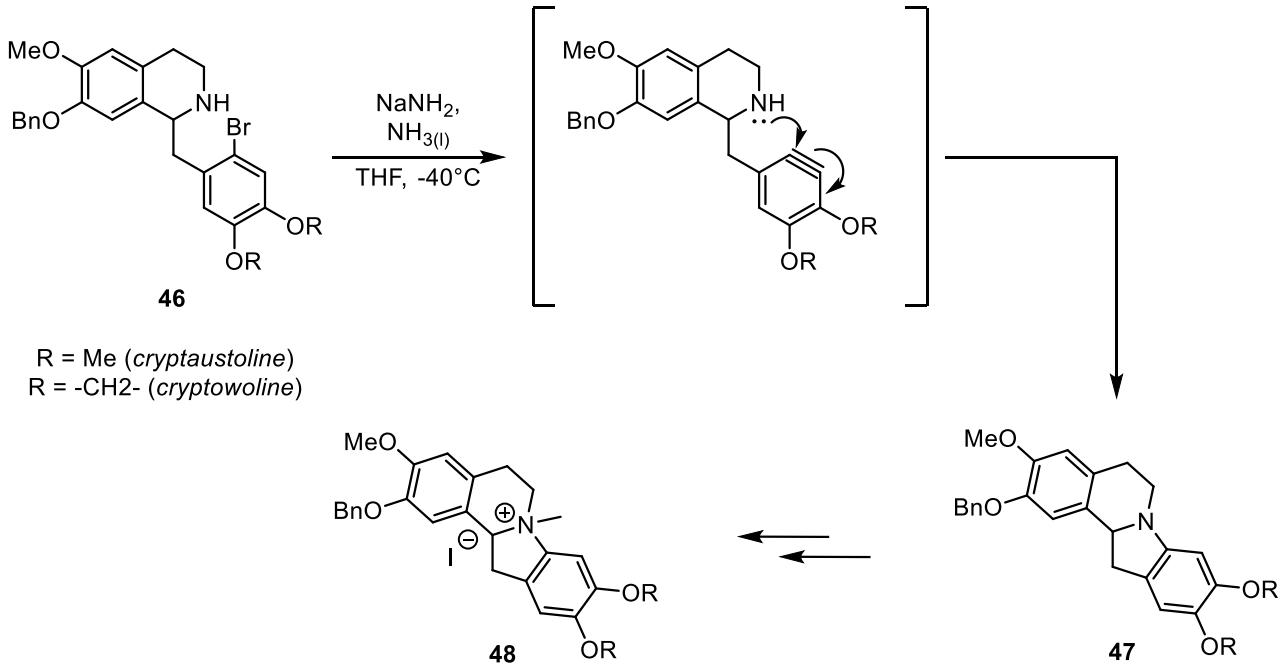


Figure 8: *carbo- and heterocycles fused with benzene ring achieved via aryne [4+2]-cycloaddition*

1.4.4 **Benzyne in total synthesis**

Aryne chemistry has been widely explored to prepare a variety of natural products. More than 75 natural products have been prepared using arynes to generate specific key intermediate.

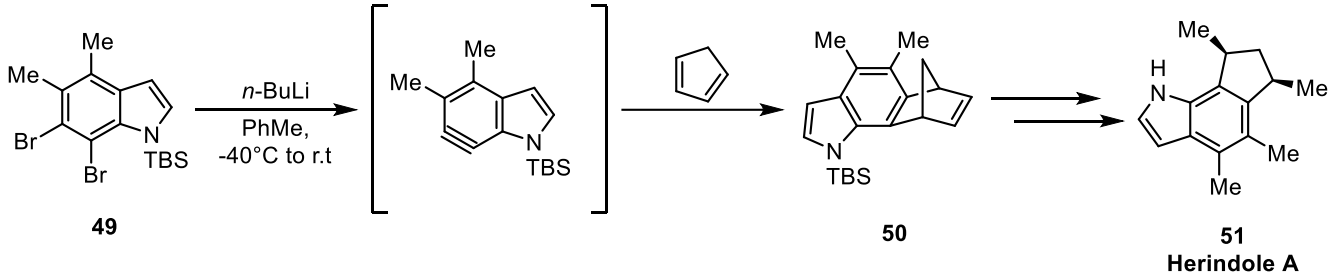


Scheme 12: *total synthesis of (±)-cryptaustoline (R = Me) and (±)-cryptowoline (R = -CH₂-)*

Aryne reactivity has been used to perform intramolecular reactions exploiting spatial proximity of aryl halide with nucleophilic substituents. For example, intramolecular nucleophilic aromatic substitution via benzyne has been widely used in natural product

total synthesis, for example the synthesis of (±)-cryptaustoline and (±)-cryptowoline by Kametani and Ogasawara [83] (**Scheme 12**).

[4+2] cycloadditions with aryne have been commonly used in organic compounds synthesis. For example, it has been applied in the total synthesis of herbindole A by Buszek and coworkers [84]: an indole-derivative undergoes [4+2] cycloaddition with cyclopentadiene to afford a complex tetracyclic framework (**Scheme 13**).



Scheme 13: *total synthesis of herbindole A*

1.5 Antihypertensive ACE-inhibitors

1.5.1 Structure and Activity

Angiotensin-converting-enzyme inhibitors (ACE inhibitors) are used for the treatment of high blood pressure [85, 86].

There are several marketed ACE-inhibitors (**Figure 9**): Captopril (**52**), Enalapril (**53**), Lisinopril (**54**), are the most famous ones, which share a common proline-containing structure.

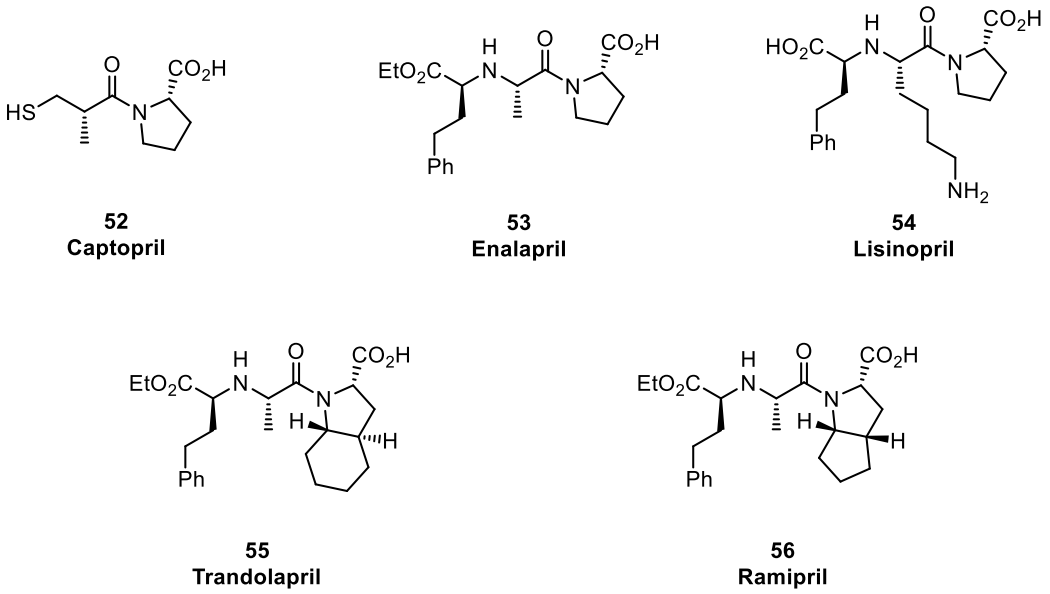


***Figure 9**: Examples of ACE-Inhibitors.*

Captopril (**52**) (Capoten ®; Squibb, 1981) was the first developed oral ACE inhibitor [87]. To overcome its adverse effects, further SAR studies have been performed,

and new derivatives have been developed; the most famous are Enalapril (**53**) and Lisinopril (**54**).

As the name implies, ACE inhibitors block the activity of the angiotensin-converting-enzyme (ACE) involved in the renin-angiotensin-aldosterone system (RAAS) that regulates blood pressure [88]. Renin is an enzyme produced by juxtaglomerular cells in kidneys and secreted into the blood flow; it is a protease that converts angiotensinogen (an α-2-globulin synthetised in the liver) into the decapeptide angiotensin I. Angiotensin I is later converted into the octapeptide angiotensin II via peptide hydrolysis by ACE on the surface of vascular endothelial cells [89]. Angiotensin II is a potent vasoconstrictor which causes narrowing of blood vessels and, consequently, increases blood pressure. It also stimulates aldosterone secretion, a hormone that increases the re-absorption of sodium in renal tubules, hence inducing hypertension.

ACE is the main target for hypertension treatment, because it induces the conversion of vasoconstrictor angiotensin II, but is also involved in bradykinin inactivation, which is a vasodilator [90]. Thus, ACE inhibition decreases angiotensin II and increases bradykinin, resulting in reduced blood pressure. Increased levels of bradykinin stimulate prostaglandin synthesis; this may explain two side effects: cough and angioedema.

ACE is a zinc-dependent dipeptidase[91] containing two catalytic domains: the N domain and the C domain; the latter is the dominant angiotensin-converting site in

controlling blood pressure. It contains two histidine (His) fragments and a glutamate (Glu) that coordinate the zinc co-factor, required for the catalytic activity [92] ACE hydrolyses the peptide by removing a dipeptide from the C terminus; it converts the inactive decapeptide angiotensin I to the active octapeptide angiotensin II via removal of a dipeptide His-Leu [93]. Its mechanism has been investigated by simulation of the ACE in complex with an analogue of angiotensin I, hippruyl-histidyl-leucine (Hip-His-Leu). Other than the three amino acid residues coordinated to Zn^{2+} (His383, His387, Glu411), Glu384 plays an important role because it deprotonates the water molecule bound to the metal centre, producing a nucleophilic Zn-OH which attacks the C=O bond in the peptide chain. There are also two chloride ions outside the active site, which is a unique feature for a metallopeptidase enzyme; Experiments indicated that they are important to maintain the binding structure and the enzyme activity. Unfortunately, the role of the two chloride anions is complicated and still under debate, as well as ACE mechanism is still not completely understood, but only postulated.

Two of the most famous ACE-inhibitors are Lisinopril and Enalapril (pro-drug for Enalaprilat, the actual active ingredient), whose backbones are very close to the Hip-His-Leu analogue [94].

Both enalaprilat and lisinopril interact with the metal cation via the C4-carboxylate group and are further stabilized by additional hydrophobic interactions and hydrogen bonds with amino acid residues in the active site (**Figure 10**). Drug molecules fit perfectly

into the active site; proline ring and the aromatic ring on the side chain binds to the hydrophobic binding sites (S1 and S2'), while the prolinic carboxylate group interacts with the ionic binding site. Since the proline fragment is not hydrolysed, drug binding prevents other molecules (i.e., angiotensin I) to interact with the enzyme, thus inhibiting its activity. Enalapril and lisinopril belongs to the carboxylate class of ACE inhibitors, which differs from the sulfhydryl species (whose main member is captopril) because they bind the metal cation with the sulphide [95].

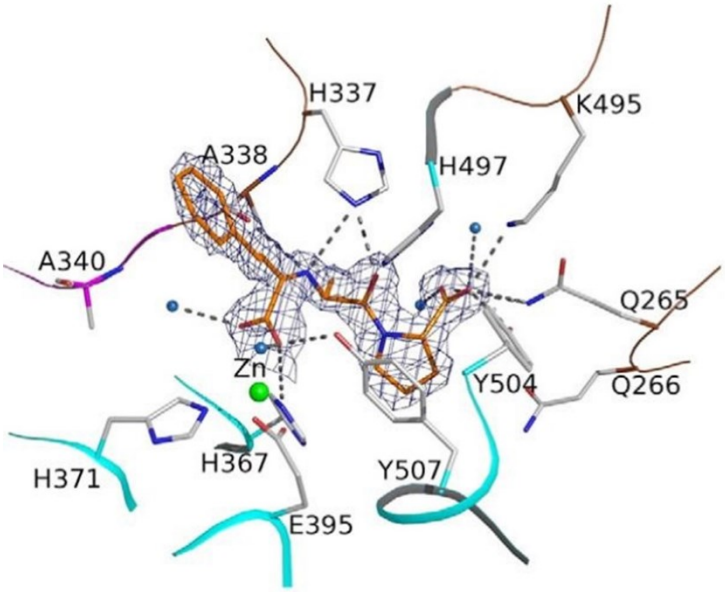


***Figure 10**: Crystal structure of Enalaprilat inside enzyme active site.*

1.5.2 **Enalapril**

Enalapril, or Enalapril maleate (also known as Naprilene® produced by SigmaTau in Italy; Enapren® produced by Merck Sharp & Dohme; Vasotec®), was patented in 1978, and came into medical use in 1984 [96]. It is an essential drug according to the "World Health Organization's List of Essential Medicines".

Like most ACE-inhibitors, Enalapril possesses a L-proline ring. It is a pro-drug activated after hepatic metabolization which transforms Enalapril into the active metabolite, Enalaprilat, via hydrolysis of the ethyl ester. It possesses a carboxylic functional group that binds with the zinc cation in the active site of the enzyme (**Figure 11**).

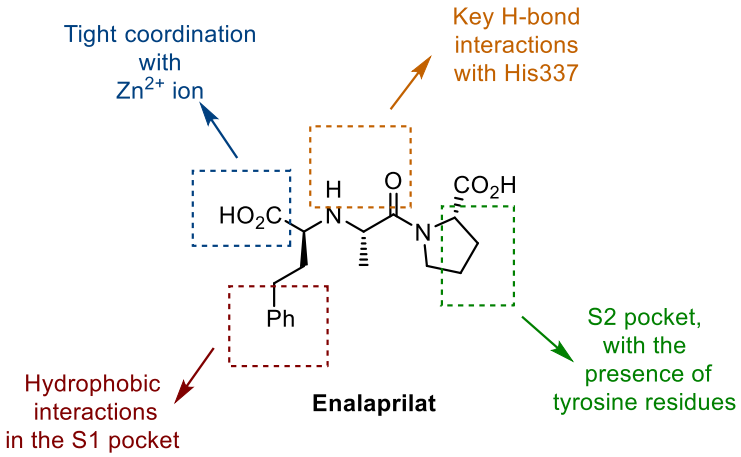


***Figure 11**: Main interactions of Enalaprilat inside enzyme cavity.*

Enalaprilat is the active ingredient developed by Merck & Co. to solve the adverse effects of Captopril due to the sulfhydryl group [97]. However, due to its increased polarity,

Enalaprilat is poorly orally available; for this reason, Merck & Co. researchers further modified its structure by esterification with ethanol to produce Enalapril.

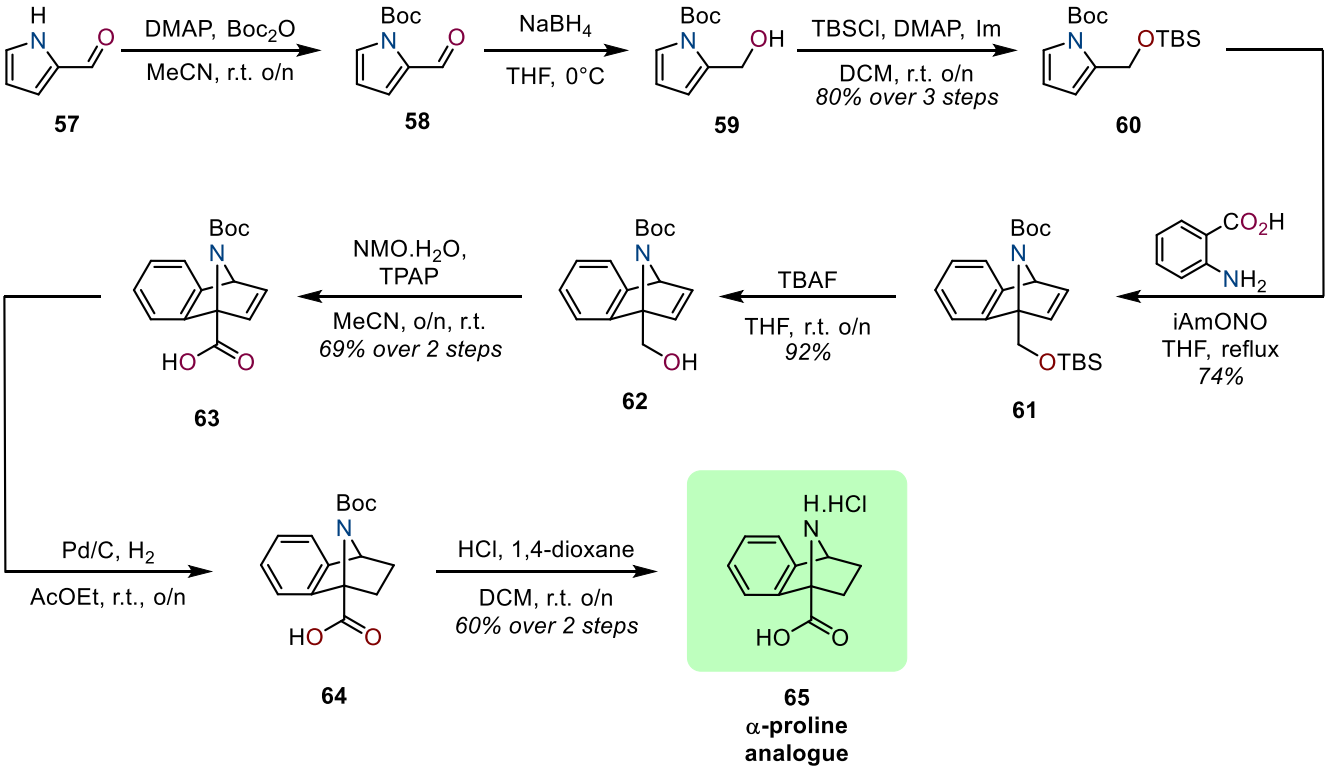
Enalapril is used to treat hypertension and symptomatic heart failure and reduce the worsening of chronic kidney diseases. It is frequently administered with a diuretic, such as furosemide. Enalapril is orally administered, while Enalaprilat can be administered intravenously.

As a part of this project, we decided to synthesize a conformationally-restrained sp^2-sp^3 hybrid analogue in which the proline ring is fused with a benzene ring. The structural constraints may limit conformational freedom, making it more rigid and prone to interact with the enzymatic active site with more efficiency. Moreover, the presence of an aromatic ring should make it more lipophilic, increasing its affinity with the active site, exploiting, for example, π-π stacking and hydrophobic interactions.

1.6 Results and Discussion

1.6.1 Proline Analogues synthesis

We focused on the synthesis of bicyclic conformationally-restricted sp^2-sp^3 hybrids, in particular pyrrolidine derivatives fused with an aromatic ring. The resulting structure is a 7-aza-bicyclclo-[2.2.1]-heptane fused with a benzene; this building block is obtained via Diels-Alder cycloaddition between properly activated pyrroles and benzyne (generated in situ from anthranilic acid and isoamyl nitrite). Starting from 2- and 3-formyl-substituted pyrroles, we have obtained α- and β-amino acid cycloadducts, respectively. The α-amino acid has been used as a substrate to synthetize Enalapril bicyclic analogue.



*Scheme **14**: Synthetis of α-proline analogue.*

1.6.2 **α-Proline Analogue Synthesis**

The total synthesis is shown in **Scheme 14**. The synthetic pathway is based on 8 steps. The key step is the Diels Alder reaction with benzyne; due to its instability, it has to be prepared in situ.

To perform this key reaction, pyrrole-2-carboxaldehyde **57** needs to be modified. The aldehyde is a good electron withdrawing group so it makes the pyrrole too electron-poor to react in the Diels alder reaction. Thus, we decided to reduce the aldehyde to an alcohol. The free alcohol it's still a problem due to its nucleophilic reactivity with benzyne, so we decided to protect it.

The synthesis starts from the commercially available pyrrole-2-carboxaldehyde **57**, which undergoes a Boc protection using Boc_2O and DMAP in catalytic amount. The reaction mixture is stirred at room temperature overnight **58**. Then reduction of the aldehyde using sodium borohydride gives the desired alcohol **59**, which is protected using TBSCl in DCM **60**. These three steps can be performed on a large scale (10g of starting material). Moreover, no chromatography purification is needed for these intermediates: the crude can be used in the next steps without any loss in yield.

The protected pyrrole can be used as diene in the Diels Alder, reacting with benzyne as the dienophile. The reaction system for the cycloaddition step is shown in **Figure 12**.

Protected pyrrole **60** is dissolved in a three-necked flask under nitrogen atmosphere. Then, it's heated up at 100°C using an oil bath. Two dropping funnels are filled with solutions of isoamyl nitrite and anthranilic acid in THF. The two reagents are added dropwise and simultaneously to the refluxing solution. Since benzyne formation is associated with CO_2 and N_2 liberation, the reaction system needs to be equipped with a

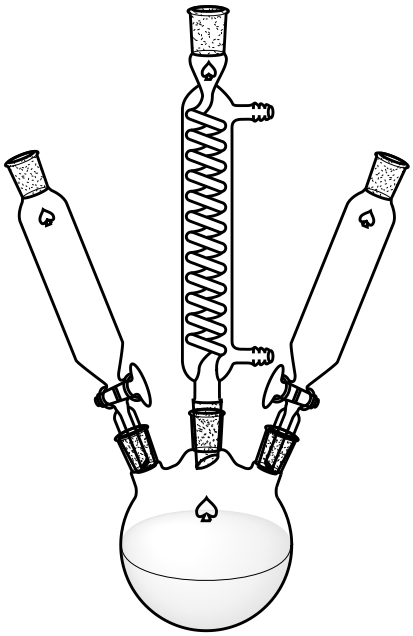


Figure 12: *Reaction system used in the Diels-Alder reaction*

needle vent on the top of the reflux condenser to remove the gases. The additions of the two solutions must be performed in two hours. The desired cycloadduct **61** is obtained after purification by column chromatography in a good yield as a racemic mixture.

Then TBS deprotection using TBAF in THF occurs in an excellent yield to furnish the free alcohol **62** which is converted into the corresponding carboxylic acid using Ley-Griffith oxidation **63**. Catalytic heterogeneous hydrogenation of the double bond in **63** with H_2 and 10% Palladium on carbon affords protected amino acid **64** with excellent yield.

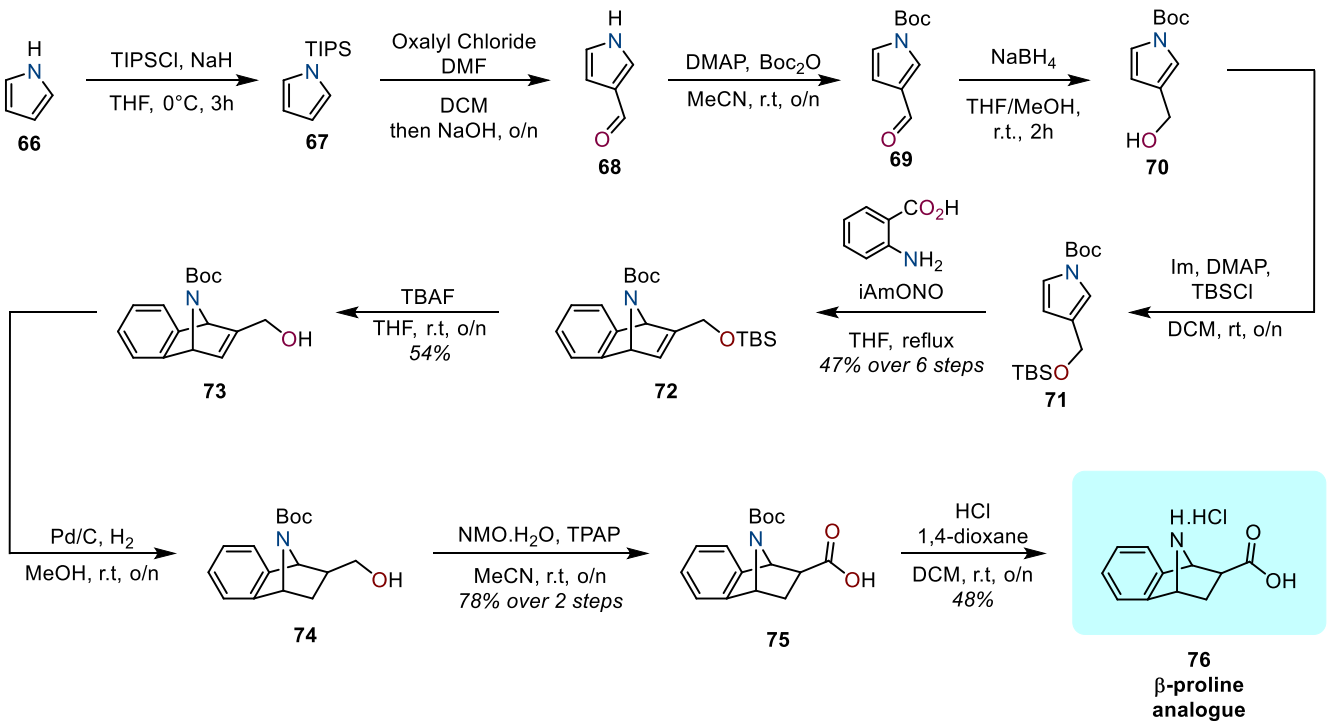
Finally, the compound **64** is treated with a solution of HCl in 1,4-dioxane to remove the Boc group, releasing the free alpha proline analogue **65** as a white solid. This compound is obtained as a hydrochloride salt, so a simple filtration is needed for its purification.

1.6.3 β-Proline Analogue Synthesis

The second compound is an α-proline regioisomer. In this case we have a β-amino acid, that derives from a 3-substituted pyrrole.

The synthetic strategy is like the previous one, with few differences. This time the synthesis didn't start from pyrrole 3-carboxaldehyde, because is too expensive. It was started from pyrrole, which was converted to pyrrole 3-carboxaldehyde following a known procedure.

The total synthesis is shown below (**Scheme 15**). The synthetic pathway is based on 10 steps. Again, the key step is the [4+2] cycloadditions with benzyne.



***Scheme 15**: Total synthesis of β-proline analogue*

At the beginning, pyrrole **66** is protected using sodium hydride and TIPSCl, to obtain the TIPS-protected pyrrole **67**. Formylation can be performed using Vilsmeier-Haack reaction. Since the 2-position of pyrrole is the most nucleophilic one, we chose a hindered protecting group to avoid 2-formylation. Using this strategy, only pyrrole 3-carboxaldehyde **68** will be obtained.

In the first step of the Vilsmeier-Haack reaction, the chloromethylenimminium (also known as Vilsmeier's reagent) is generated reacting DMF and oxalyl chloride. The reaction is performed under nitrogen atmosphere at 0°C. After 30 minutes the Vilsmeier's salt is generated, so the substrate **67** can be added. The resulting mixture is heated up to

40°C. After 2 hours the solvent is removed under vacuum. The crude is suspended in a solution of NaOH and is stirred overnight. With pyrrole 3-carboxaldehyde **68** in hand, the synthetic pathway is the same as the previous one.

Boc protection using Boc_2O and DMAP to obtain compound **69**. Then reduction of the aldehyde with sodium borohydride to have the free alcohol **70** which is protected with TBSCl to obtain the protected pyrrole **71**. Again, all the steps can be scaled up until 10g scale without any column chromatography purification.

Protected diene was used in the Diels Alder reaction keeping same conditions and work up procedure. Cycloadduct **72** is obtained in a good yield, again as a racemic mixture. After the deprotection of the TBS group (TBAF in THF), we tried different oxidations on the allylic alcohol **73**. At first, we tried to obtain the aldehyde, so we perform DMP, Parikh-Doering and Stahl oxidation, but we never obtained the desired α,β-unsaturated aldehyde. Moreover, we also tried a direct oxidation of the alcohol to carboxylic acid using Ley-Griffith oxidation. Unfortunately, we only obtain the degradation of the starting material.

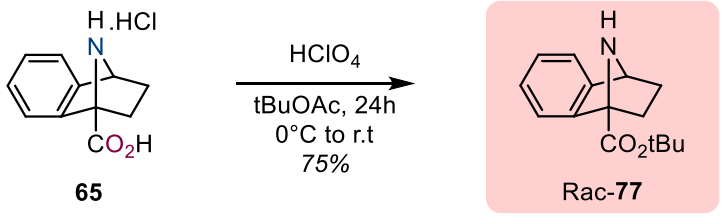
We decided to change strategy: before the oxidation step, we decided to perform the hydrogenation of the double bond using palladium on carbon. After 24h we obtained saturated cycloadduct **74**. The hydrogenation step generates a new stereocenter, so we should obtain a mixture of diastereoisomers. Surprisingly, a single diastereoisomer is

observed. Hydrogenation took place from the bottom face of the norbornene, leading a single isomer. This is probably caused by the steric hindrance of the Boc group on nitrogen. At this point, we performed the Ley-Griffith oxidation on the saturated bicycle **74**, using TPAP and NMO monohydrate. This time we obtained the desired carboxylic acid **75** in a good yield.

The Boc protected cycloadduct undergoes deprotection of the Boc group using HCl in 1,4-dioxane to furnish the free amino acid analogue **76** as a grey solid. Also in this case the final salt was purified with a simple filtration.

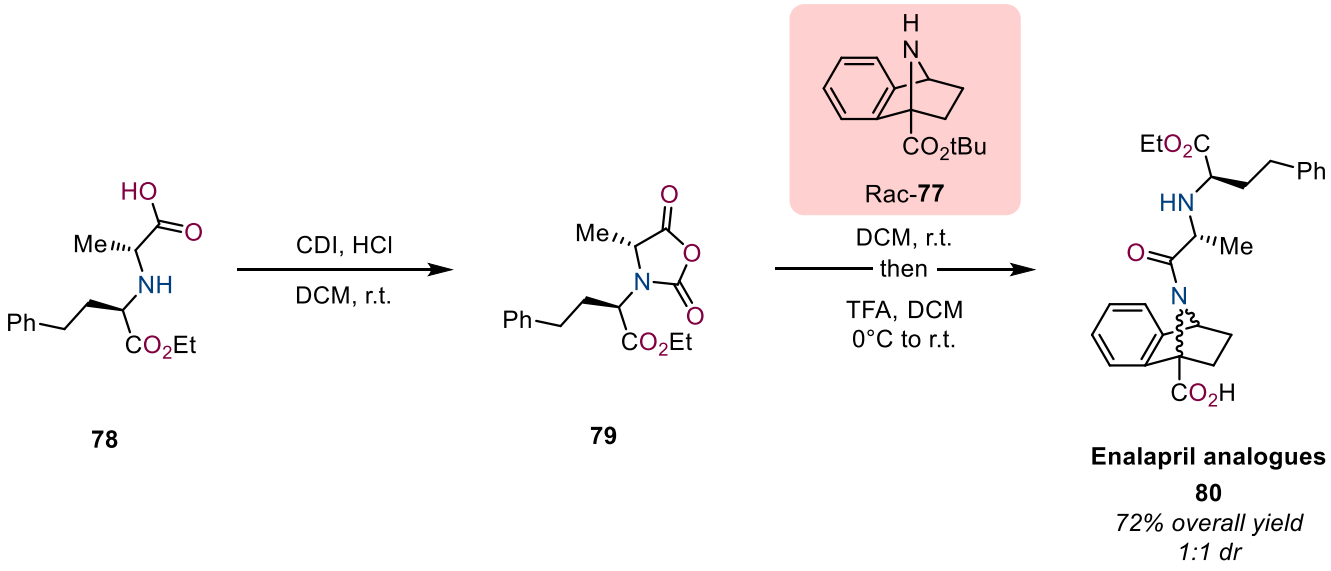
1.6.4 Enalapril Analogue Synthesis

With the bicyclic proline **65** in hand, the next step is the introduction of the side chain N-[(S)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanine **78**. First, the carboxylic group must be adequately protected, so that it won't take part in the following coupling reaction. Treatment with a solution of perchloric acid ($HClO_4$ 65%wt in H_2O) and tert-butyl acetate (*t*BuOAc) leads to the formation of tert-butyl ester **77** via *trans*-esterification (**Scheme 16**).



***Scheme 16**: Tert-butyl protection of carboxylic acid **65**.*

Peptide **78** is activated towards coupling reaction using N,N'-carbonyldiimidazole (CDI). CDI is a widely used coupling agent for amino acids; although its reactivity is less than acyl chloride, it is safer, easier to handle, and prevents hydrochloric acid formation, which can be dangerous and cause side reactions. This reaction is quite fast (around 20 min), and the intermediate **79** is not isolated, nor the reaction mixture is quenched; the tert-Bu-protected cycloadduct **77** diluted in DCM is directly introduced into the reaction flask.



***Scheme 17**: Synthesis of Enalapril analogues.*

After 24h, all the bicyclic proline analogue was consumed (TLC analysis), so we decided to quench the reaction mixture and to isolate the desired products (**Scheme 17**). Derivative **77** is a racemic mixture, so Enalapril analogue is obtained as a mixture of two diastereomers. From the TLC we noted two different closed spots, which were isolated as

the two Enalapril diastereomeric *t*Bu-esters. After a brief optimization, we found that the corresponding *t*Bu deprotection with TFA could be performed *in situ* without quenching the reaction. Finally, after a one-pot reaction, the desired Enalapril bicyclic analogues **80** were separated by simple column chromatography with a total yield of 72% starting from peptide **78**.

2 Phenylalanine Analogues and Anticancer Drug Derivatives

2.1 Peptide based drugs

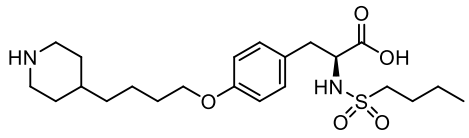
In recent years, peptide-based drugs have received increasing attention. The major interest in peptides is their ability to bind in a specific manner inside their targets; this results in high potencies and low side-effects. This high degree of selectivity in their interactions is the product of evolutionary selection form complementary shapes and sizes from a variety of structural and functional diversity[98].

The importance of peptides was realized in 1922, when insulin was isolated from animal pancreas[99]. Nowadays, isolation of peptides from animal sources is still important.

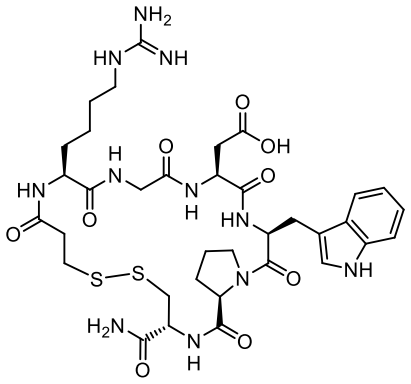
A lot of synthetic strategies have been developed for the synthesis of complicated peptides.

After the success of synthetic human insulin, the market of peptide-base drugs started to increase. In recent years, a lot of FDA (food and drug administration) approved drugs are peptide-based drugs (**Figure 13**).

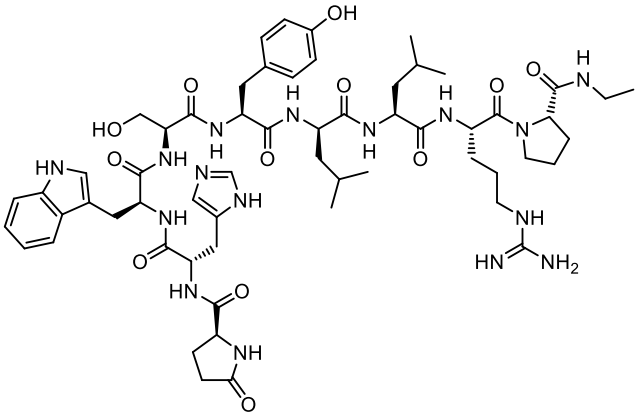
Further advancements are based in the diversification using unnatural amino acids, recombinant synthesis and DELs[100].



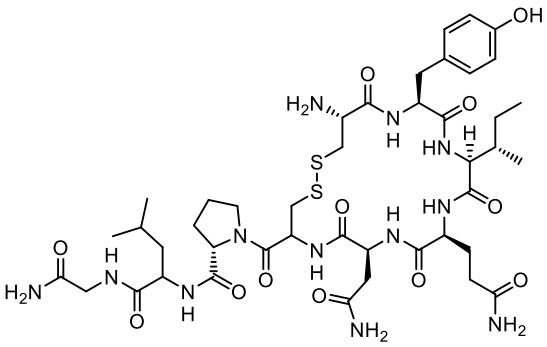
Tirofiban (Aggrastat)
Antiplatet drug



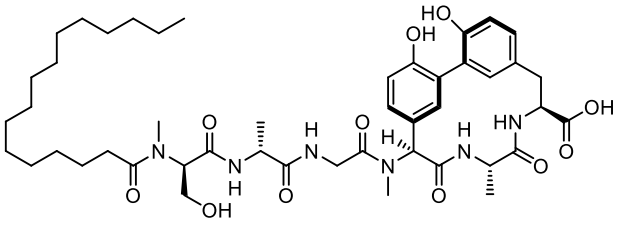
Eptifibatide (Integrilin)
Antiplatet drug



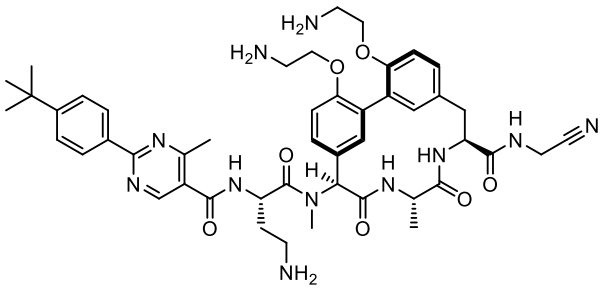
Leuprorelin
hormone used to treat cancer



Oxytocin
hormone



Natural Arylomycin



Synthetic analogues of Arylomycin

***Figure 13**: Examples of peptide-base drugs*

Peptides have short half-life and their metabolites don't accumulate into tissues; this can reduce the risk of metabolic toxicity. Moreover, they have high potency and selectivity.

Despite that, drug discovery for new peptide drugs suffers limitations, caused by structural properties and high manufacturing cost.

The main problems related to peptide-based drugs are:

- Poor metabolic stability
- Poor membrane permeability
- Low oral bioavailability
- Low solubility

The major challenge is to develop orally administered analogues[101].

To overcome all these problems (related to half-life, adsorption, metabolism and bioavailability) structural modifications strategy can be used[102]. So, the development of peptide base drugs is still challenging in the recent years.

However, they found application in anticancer drugs, antihypertensive drugs acting as angiotensin-converting enzyme (ACE) inhibitors, in the treatment of cardiovascular diseases, anti-infective agents and so on.

Probably, due to their importance, the next generation of therapeutics will be based on peptide drugs. In the last decade there was a high number of anticancer and

antimicrobial therapeutics approval; for this reason, the main targets of peptide-based research remain these drug derivatives[103].

2.2 Carfilzomib

Carfilzomib is an anticancer drug, sold under the name of Kyprolis.

It's a tetrapeptide epoxyketone and it's an epoxomicin analogue. Epoxomicin includes two key elements: a peptide portion that binds with high affinity the proteasome and an epoxyketone pharmacophore[104]. Carfilzomib was developed by Onyx Pharmaceuticals and in 2012 was approved by FDA. This drug is used for the treatment of multiple myeloma; it is used in patients who have received at least two prior therapies.

2.2.1 Multiple Myeloma

Multiple myeloma is a disorder in which malignant plasma cells accumulate in the bone marrow and produce an immunoglobulin. Common diseases over multiple myeloma are bacterial infections, anemia, osteolytic lesions and renal insufficiency. Multiple myeloma is responsible for about 1% of all cancer-related deaths in Western countries[105].

2.2.2 Proteasome Inhibitor

Proteasome inhibitors (PIs), exert their antineoplastic activity through targeting the catalytic core of the 26S proteasome, an enzyme complex responsible for the degradation

of proteins[106]. The 26S proteasome is at the hearth of the ubiquitin proteasome pathway and consists of a catalytic 20S core particle and an ATP-dependent 19S cap, or regulatory particle, which recognizes and unfolds proteins that have been tagged for degradation with a string of at least five ubiquitin molecules.

Eukaryotic proteasomes consist of 28 protein subunits[107]. The catalytic core of the proteasome contains chymotrypsin-, trypsin- and caspase-like proteolytic activities and targets proteins in a ubiquitin-dependent manner[108,109] (**Figure 14**). The chymotrypsin-like activity of the proteasome is the rate-limiting step of the proteolytic process and is essential for cell survival, while being highly sensitive to inactivation[110,111].

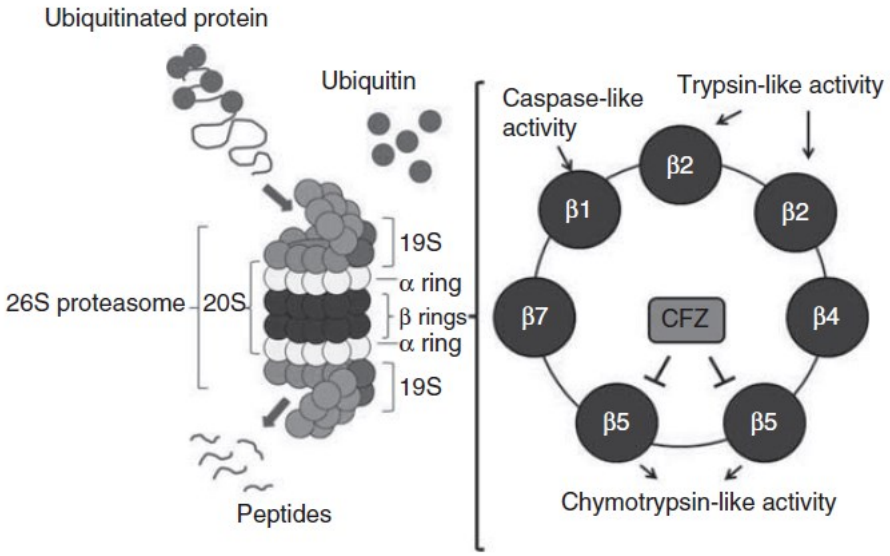


Figure 14: *the 26S proteasome consists of the 20S catalytic subunit and the 19S cap. The 20S catalytic subunit is composed of two β and two α rings; the β ring contains the trypsin-like, chymotrypsin-like and caspase-like proteolytic activities. Carfilzomib binds and inhibits with high affinity and specificity the chymotrypsin activity of the β ring.*[114]

Actively proliferating cells, such as cancer cells, are especially susceptible to inhibition of the constitutive proteosome and immunoproteasome because of the dependence on the regulatory/signalling mechanisms for control of the cell cycle[112,113]. It is believed that inactivation of the chymotrypsin-like active sites is the most detrimental to cell survival, and the majority of proteasome inhibitors are designed to target these sites. As mentioned previously, the 26S proteasome is a critical complex of the ubiquitin-proteasome system (UPS), which coordinates regulation and degradation of intracellular proteins[115]. The UPS is dysregulated in MM, thereby upregulating the activity of the proteasome and resulting in excessive degradation of specific substrates of relevance. A number of processes are affected by proteasome activity that drive tumor progression in MM[116].

2.2.3 **Carfilzomib Development**

In recent years, new and more efficient drugs have become available for the treatment of multiple myeloma (MM). Some of these drugs can be used alone or in combination with other agents. So, the number of therapeutic options its increasing for MM patients as well as their chances to survive.

The new approved drugs include proteasome inhibitors, such as bortezomib and carfilzomib (**Figure 15**).

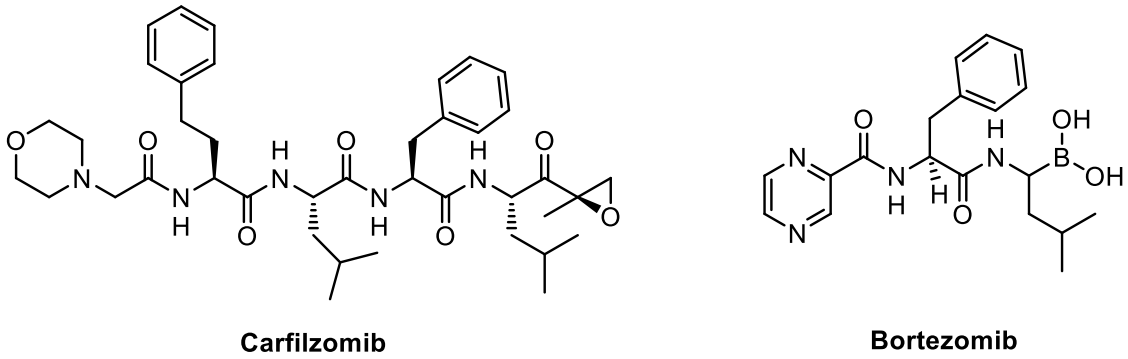
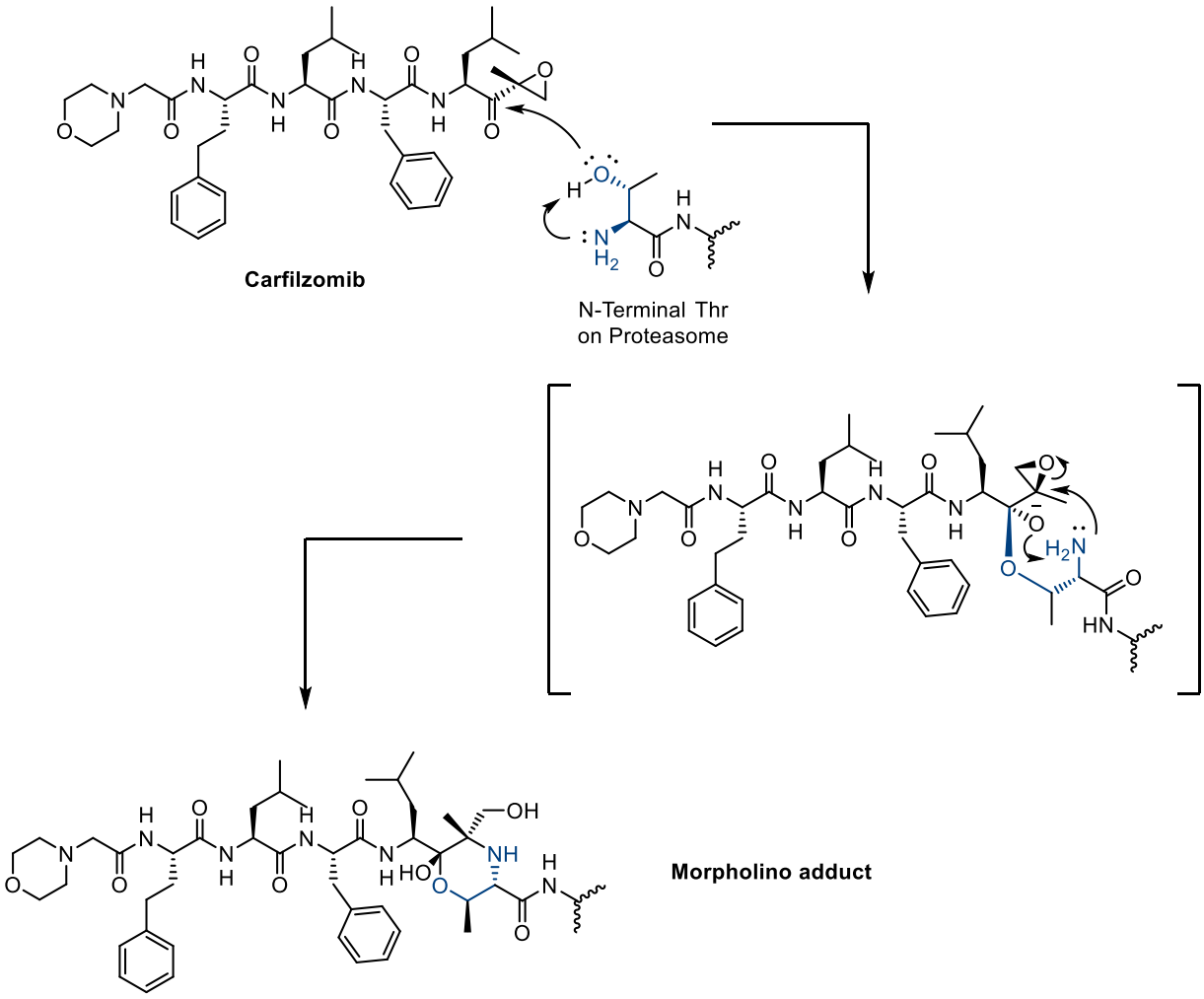


Figure 15: Structures of Carfilzomib and Bortezomib

Bortezomib was the first proteasome inhibitor to enter clinical practice. Bortezomib inactivates the proteasomal active site by a covalent but slowly reversible binding mode. It became useful in the treatment of solid tumors and as prevention of transplant rejection[117,118]. Despite its success, bortezomib causes severe adverse effects, such as neurophaty[119], so it was necessary to find a next generation of proteasome inhibitor.

Epoxomicin is a member of the linear peptide epoxyketone natural product family. It was found in Actinomycetes strain[120]. This natural compound possesses unprecedent selectivity for proteasome inhibitors[121]. This specificity is given by the formation of a six-membered morpholino ring between the amino terminal catalytic Thr-1 of the 20S proteasome and the α'-β'-epoxy ketone pharmacophore of epoxomicin[122] (**Scheme 18)**.



Scheme 18: *Formation of the Morpholine adduct*

This unusual ring formation can be explained by a double nucleophilic attack on the epoxy ketone of epoxomicin. The nucleophiles are the hydroxyl side chain and the free amine of the N-terminal Thr[123]. To improve epoxomicin's anticancer activity, a classical medicinal chemistry approach was used, which led to the development of YU-101[123].

YU-101 shows higher biological activity than epoxomicin and Bortezomib, so in 2003 a new company, Proteolix, was founded, based on YU-101 superior antitumor activity. Due to its poor solubility in water, a modification of this new compound was necessary. To overcome this problem, a morpholine ring was added to the N-terminus of YU-101 (**Figure 16**). This new compound was named Carfilzomib, which shows more efficacy also in patients with acquired resistance to Bortezomib[124].

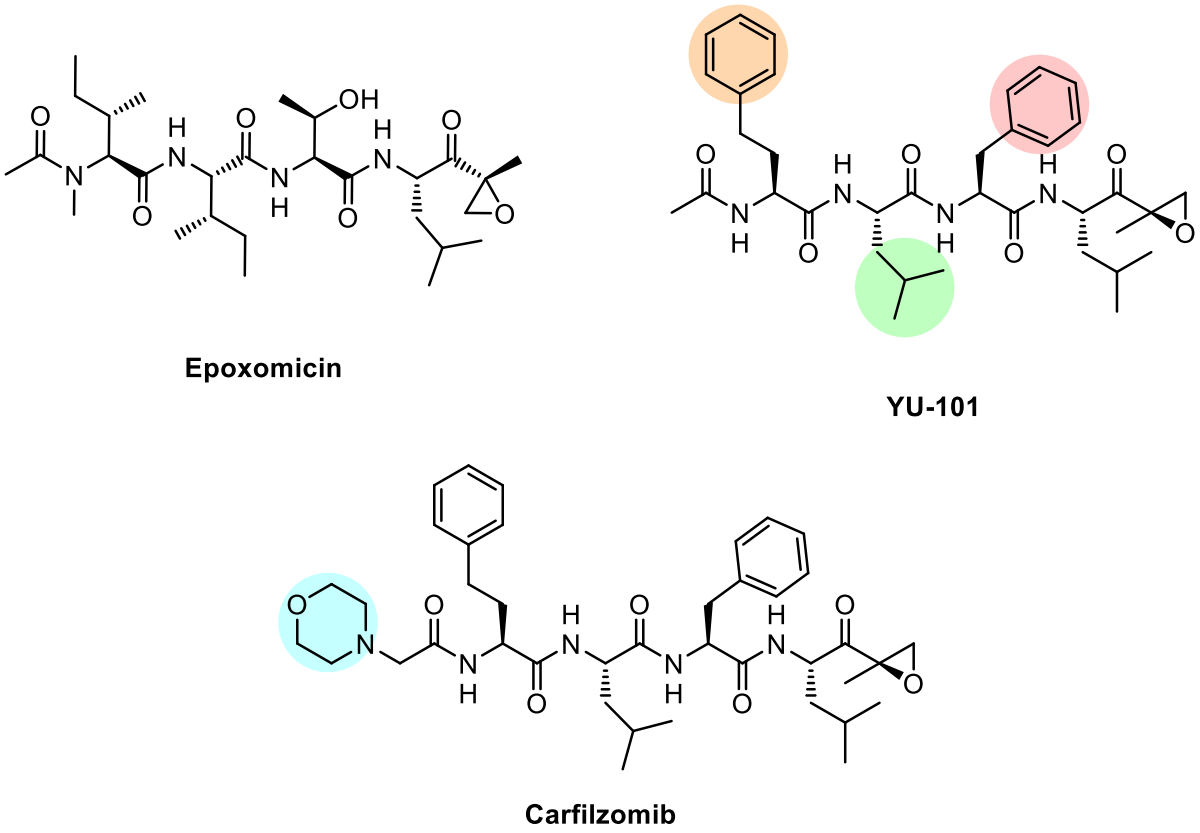


Figure 16: *From Epoxomicin to YU-101 and finally to Carfilzomib*

2.2.4 Interaction of Carfilzomib with Proteasome Specificity Pockets

Carfilzomib forms a covalent adduct with its C-terminal epoxyketone inside the enzyme pocket, modifying Thr1 in each inhibited subunit. With two amino acids residues, a morpholine ring is formed, proteasome inhibition is based on this interaction which is the same of all the epoxyketone inhibitors such as epoxomicin[125]. N-terminal morpholino moiety of carfilzomib is not engaged in any interactions with the protein in any of the catalytic sites.

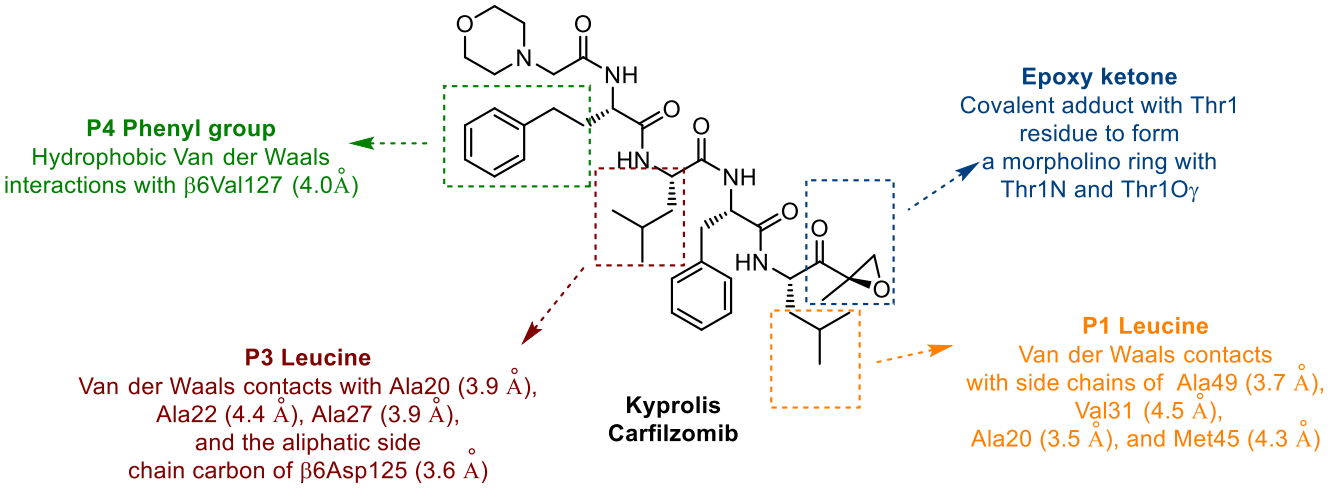


Figure 17: *Carfilzomib interactions inside the enzyme site*

Carfilzomib's **P4** phenyl group is sterically blocked by His 116 from entering the S4 pocket of caspase-like sites (**Figure 17**). Instead, chymotrypsin-like and trypsin-like sites have S4 pockets that make hydrophobic interactions with **P4** phenyl group of carfilzomib. This hydrophobic Van der Waals interactions in the chymotrypsin-like site

are made with the side chain of β6Val127 (4.0Å); while interaction with β2lle126 (3.8 Å) and β3lle127 (4.0 Å) are made in the trypsin-like site (**Figure 18C**).

The **P3** position of carfilzomib is occupied by a leucyl group which make Van der Waals interaction in all of the three catalytic subunits. In chymotrypsin-like sites, the S3 pocket makes hydrophobic Van der Waals interactions with the P3 leucyl with four amino acids side chains: Ala20 (3.9 Å), Ala22 (4.4 Å), Ala27 (3.9 Å) and β6Asp125 (3.6 Å) (**Figure 18C**). In caspase-like site the Van der Waals interactions are made with: Ala27 (3.3 Å), Thr20 (4.0 Å), Thr22 (4.3 Å) and β2Tyr114(3.7 Å). Finally, in trypsin-like site there are only three interactions with: Ala27 (4.3 Å), Ala20 (4.4 Å) and the aliphatic carbon of Glu22.

The **P2** phenylalanine moiety of carfilzomib can be accommodated in all the three active sites, but no interactions are made with the protein.

The **P1** leucyl group of carfilzomib have Van der Waals interactions inside the S1 pocket of chymotrypsin-like subunits. These interactions are established from the P1 leucine and side chains of: Ala49 (3.7 Å), Val31 (4.5 Å), Ala20 (3.5 Å) and Met45 (4.3 Å). The S1 pocket in the caspase-like site is positively charged, this does not favor the hydrophobic P1 leucine group of carfilzomib. Instead, the trypsin-like subunits have a more spacious S1 pocket so two Van der Waals interactions are possible with: Ala49 (3.9 Å) and Ala20 (4.1 Å) (**Figure 18B**).

In conclusion, chymotrypsin-like and trypsin-like subunits have similar interactions with carfilzomib molecule. While, due to electrostatic and steric effects, the caspase-like site seems to unfavour the binding of carfilzomib[126].

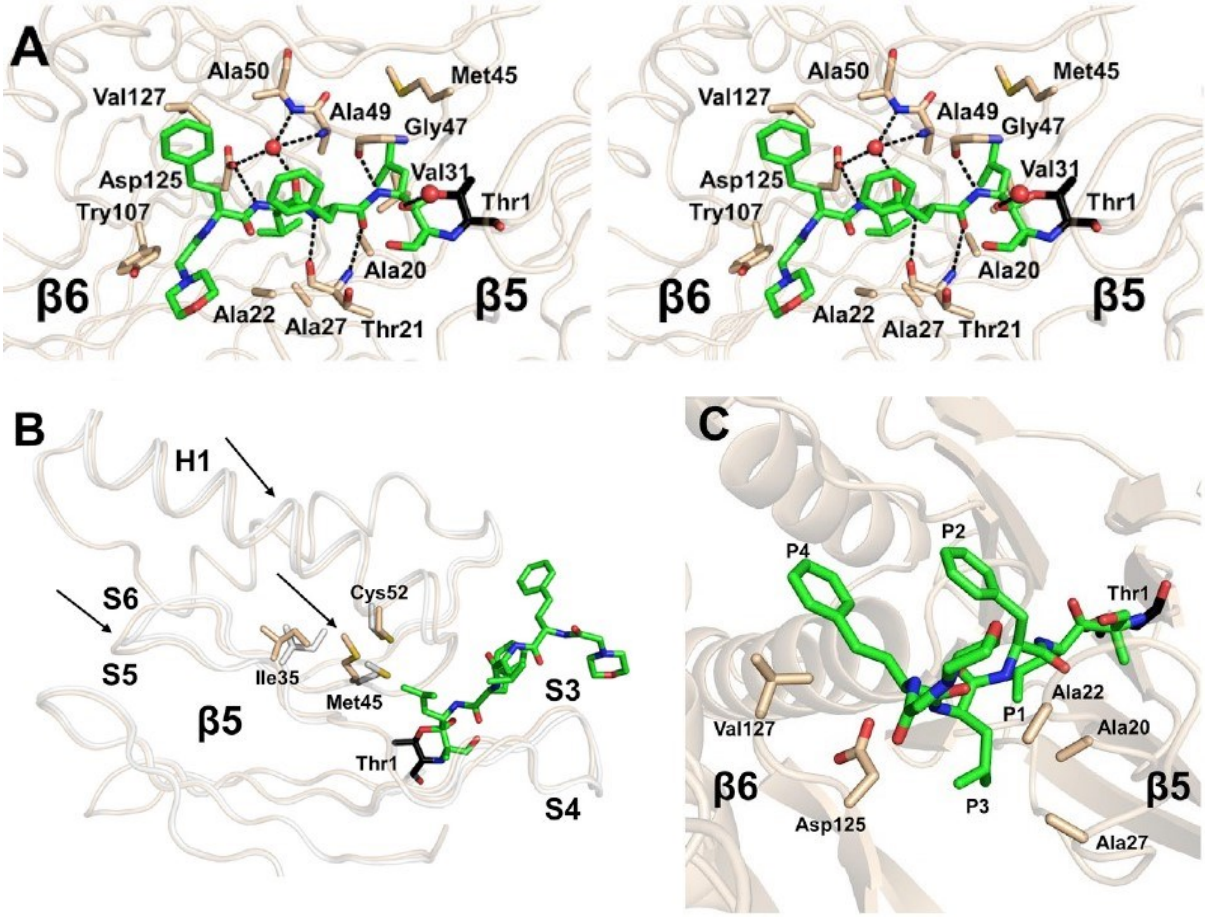


***Figure 18**: (A) Carfilzomib bounded to the chymotrypsin-like site. Carfilzomib is colored green, hydrogen bonds are shown as black dashes lines. Water molecules are red spheres and T1 is colored black. (B) Structural alignment of unbound (white) and carfilzomib bound (wheat) chymotrypsin-like sites. Carfilzomib is colored green. (C) carfilzomib interaction in the P3 and P4 position of the chymotrypsin-like site of the human constitutive proteasome.*

2.2.5 **Carfilzomib Based Therapy**

Initially, Carfilzomib was tested on rats and monkeys, where showed a half-life less than 20 minutes. It was quickly metabolized via peptidase cleavage and epoxide hydrolysis. 31% of the drug undergoes hepatic degradation, while 26% is excreted by kidneys. A lot of side effects were registered, but they can be reduced when Carfilzomib is administered as a 10- or 30- minutes infusion.

In patients with relapse and refractory multiple myeloma, Carfilzomib was administered by intravenous infusion over a period of 2 to 10 minutes at 20mg/m2 for a 28 days cycle. Then, it was given at 27mg/m2 for cycle two and subsequent cycles.

A lot of adverse reactions were registered. The most common are:

- Fatigue
- Anemia
- Diarrhea
- Muscle spasms
- Hypertension
- Upper respiratory tract infection
- Anorexia
- Hypercalcemia

Despite all of these cardiovascular, pulmonary and hepatic adverse reactions, the benefit-risk evaluation for carfilzomib is favourable for the treatment of patients with

multiple myeloma. The patient must have received a previously treatment with lenalidomide, thalidomide, melphalan and other alkylating agents[127].

Due to its importance, Carfilzomib analogue is another focus of this project. The idea was to synthetized conformationally restricted analogue using a constrained phenylalanine derivative. The structural constraints may limit conformational freedom, making it more rigid to interact with the enzyme site with more efficiency.

For this reason, we decided to use derivative **65** and **76** as phenylalanine analogues, since they can be considered also as phenylalanine analogues, other than proline analogues. Moreover, for phenylalanine we decided to synthesize also a γ-analogue (**Figure 19**). The idea was to use all the three analogues: alpha, beta and gamma, whose core is obtained by a Diels-Alder reaction between activated pyrrole or fulvenes and benzyne. The benzyne is generated in situ from anthranilic acid and isoamyl nitrite.

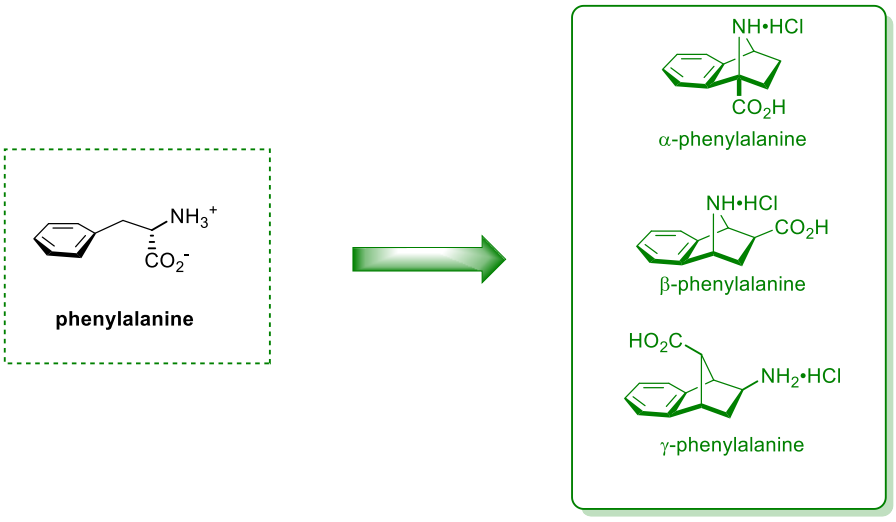


***Figure 19**: Overview of phenylalanine analogues.*

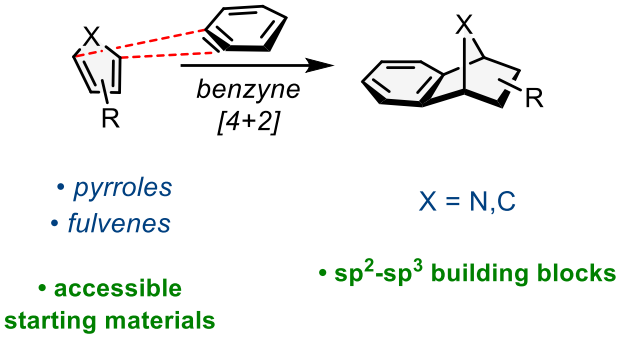


***Figure 20**: General approach to hybrid structures*

From the reaction of 2- and 3-substituted pyrroles we have obtained alpha and beta phenylalanine derivatives and from the reaction of fulvenes with benzyne we are able to obtain gamma phenylalanine analogue (**Figure 20**).

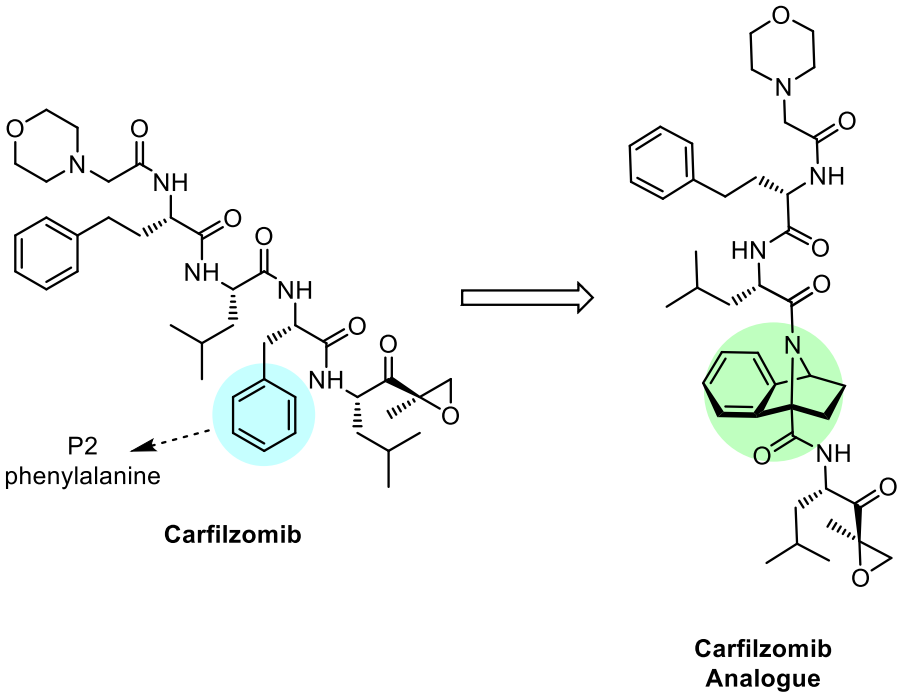


***Figure 21**: From Carfilzomib to our Analogues*

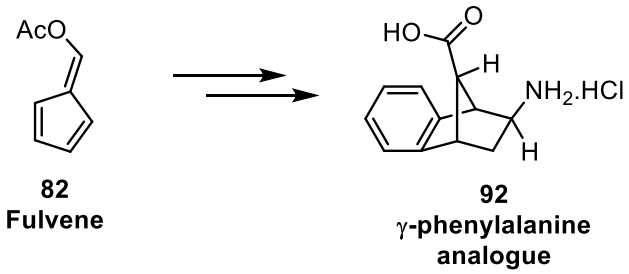
Then the alpha analogue has been used in the total synthesis of Carfilzomib analogue. For the synthetic strategy, we decided to use our analogue to change the phenylalanine (P2), because it has no interactions in the enzyme site. In the constrained drug derivative, we might have some new interactions in one of the three catalytic sites thanks to our new P2 amino acid, increasing the biological activity compared to carfilzomib (**Figure 21**).

2.3 Results and Discussion

2.3.1 γ-phenylalanine analogue synthesis

The synthesis of α- and β-phenylalanine analogues **65** and **76** was reported in schemes **14** and **15**, respectively.

The synthesis of γ-phenylalanine analogue is completely different from the others. The key step is still the Diels Alder reaction using benzyne as dienophile. However, this time we used a fulvene intermediate **82** as diene (**Scheme 19**).

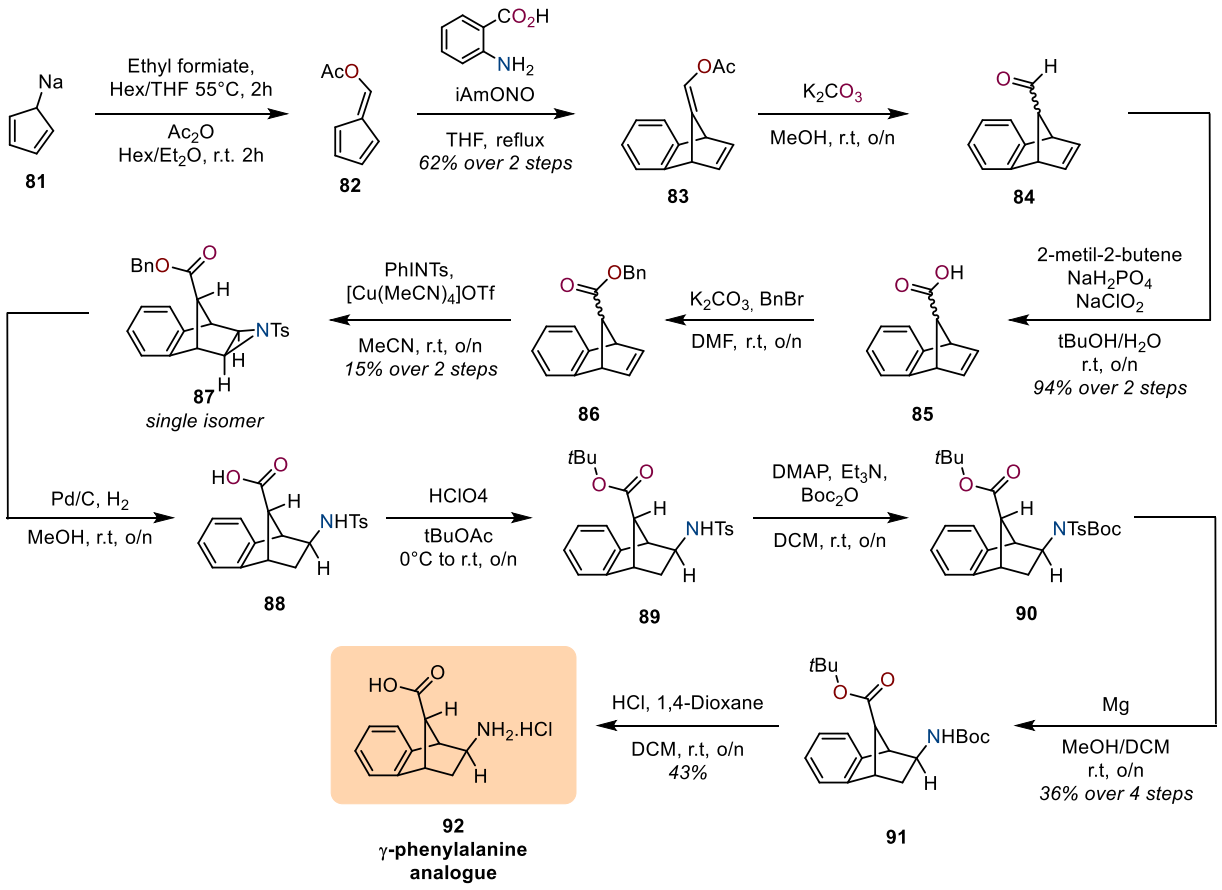


***Scheme 19**: Overview of γ-phenylalanine analogue synthesis*

The total synthesis is shown below (**Scheme 20**). The synthetic pathway is based on 11 steps.

The synthesis starts from the commercially available sodium cyclopentadienide **81** to obtain the acetoxy pentafulvene **82**. This reaction is performed under nitrogen atmosphere. The starting material is added to a mixture of hexane and THF, then ethyl formate is added. After the addition of ethyl formate we have an evolution of CO_2 gas, so

the reaction system needs to be equipped with a needle vent. The mixture is heated up to 55°C using an oil bath.



***Scheme 20**: Total synthesis of γ-phenylalanine analogue*

After 2 hour the mixture is cooled to room temperature and the solvents are removed under reduce pressure. The crude is added to a mixture of hexane and diethyl ether and cooled to 0°C. Acetic anhydride is added dropwise to the solution. The flask is covered with aluminum foils and it is stirred for 2 hours at room temperature. The obtained

product **82** is sensible to temperature and light: for this reason, after the work up the crude product is immediately used in the next step without any further purification. These reactions can be performed on a large scale (6g of starting material).

The instable acetoxy pentafulvene **82** is used in the Diels alder reaction as a diene; conditions, reagents and work up procedures are the same as before.

The cycloadduct **83** is obtained in a good yield, again as a racemic mixture. This compound is stable and can be purified by column chromatography. Hydrolysis occurs using potassium carbonate in methanol, to furnish the desired aldehyde as mixture of inseparable diastereoisomers **84**. Now we can oxidize the aldehyde to the corresponding carboxylic acid **85** using Pinnick Oxidation. Esterification of the carboxylic acid using BnBr occurs to have the corresponding ester **86**. The double bond was aziridinated using Cu-catalysis and an ylide salt (prepare by known procedures) as carbene source.

The aziridination step should generate a mixture of four diastereoisomers, but apparently only one of the two ester diastereoisomers **86** reacts in these conditions. So, we are able to obtain the desired product **87** as a single diasteroisomer and to recover the unreacted reagent as a single diasteroisomer **86**.

Probably the selectivity of this reaction is guided by two effects. The first one is the steric hindrance caused by the benzyl ester: the only reactive diasteroisomer is the one with the benzyl ester oriented towards the aromatic ring. Despite its orientation, the ester might have a coordination with copper, because the aziridination takes place from the

upper face of the olefin. Catalytic heterogeneous hydrogenation with H_2 and 10% Palladium on carbon led to the opening of the aziridine and the deprotection of benzyl group **88**. This time we protect the carboxylic acid with a tert-butyl group using t-butyl acetate and perchloric acid at room temperature **89**. This intermediate was analyzed by 2D-NMR experiments, which confirmed the configuration of the stereocenters (**Figure 22**).

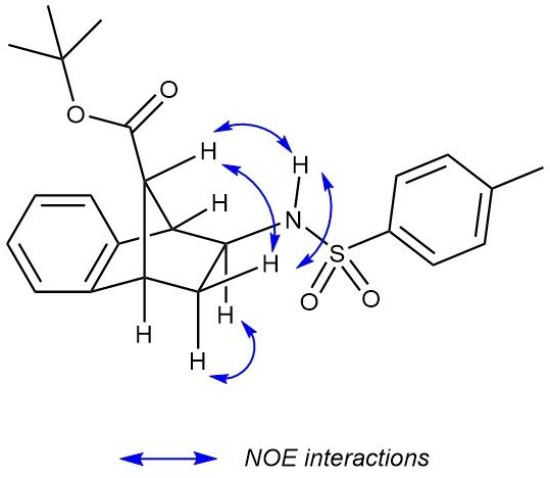
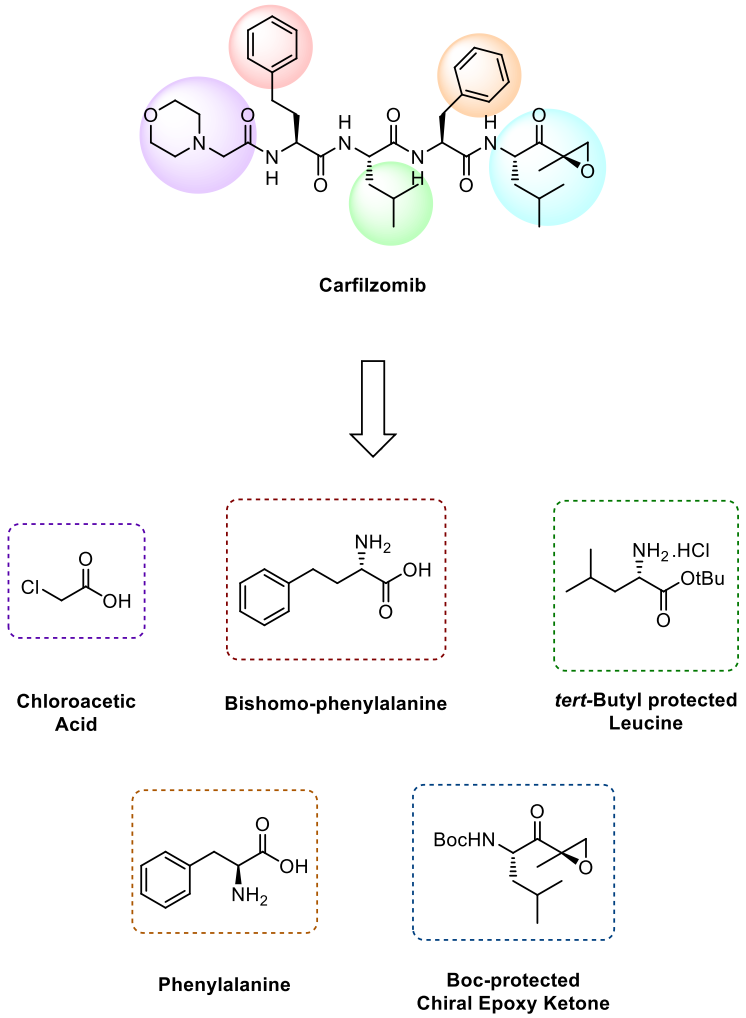


***Figure 22**: NOE interaction in compound **89**. Thanks to NOE experiment, it was possible to confirm the stereochemistry*

Then, we wanted to remove the tosyl group on the nitrogen, so we tried to use magnesium in methanol but we only recovered the starting material. Alternatively, we protected the amine with a Boc group using Boc_2O, DMAP and triethylamine to have the bis-protected amine **90**. This time we were able to remove the tosyl group using the classical conditions (magnesium in methanol, overnight), to obtain the Boc protected amine **91**.

The last step was the deprotection of the Boc and t-Bu protecting groups, with HCl in 1,4-dioxane to obtain the free amino acid **92** as a white solid. The final salt was purified with a simple filtration.

2.3.2 **Carfilzomib Synthesis**

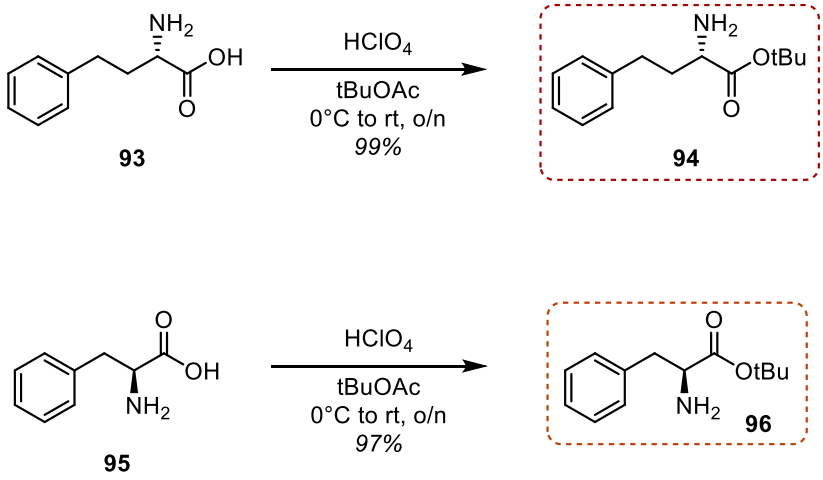


***Scheme 21**: retrosynthetic strategy to Carfilzomib*

At first, we decided to synthesize carfilzomib to optimize reaction conditions. Herein we report our retrosynthetic strategy (**Scheme 21**). We decided to start our synthesis from bishomophenyl alanine, t-butyl protected leucine, phenylalanine and a chiral epoxy ketone. All these reagents are commercially available.

Synthesis of the intermediates

Bishomophenylalanine **93** and phenylalanine **95** are commercially available as free amino acids. The t-Bu protected bishomophenylalanine **94** and phenylalanine **96** were synthetized using t-Butylacetate and perchloric acid (**Scheme 22**). The reactions are quantitative and don't require a purification step.

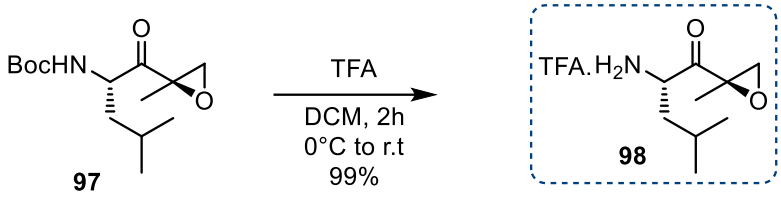


***Scheme 22**: t-butyl protection on Bishomophenylalanine and on Phenylalanine*

The t-Bu protected leucine is commercially available as a hydrochloric acid salt.

We just performed an extraction using sodium bicarbonate, water and DCM, to have the free amine.

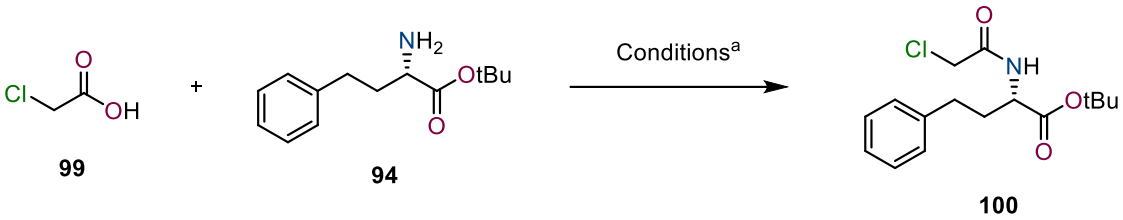
The chiral epoxy ketone **97** is sold as Boc protected amine, so we performed the deprotection of the Boc group using TFA (**Scheme 23**). It's a milder reagent than HCl, so we can avoid the degradation of the epoxy ketone. TFA is added dropwise to the solution of the Boc protected epoxyketone in DCM at 0°C. After 2 hour we obtain the TFA salt of epoxy ketone **98**. Also, in this case no column chromatography was required.



Scheme 23: *Boc deprotection on chiral epoxy ketone*

Amide Couplings Optimization

Initially we decided to perform an optimization of all the coupling steps.



Scheme 24: *Amide coupling between chloroacetic acid **99** and t-butyl protected bishomophenylalanine **94***

Entry	Coupling Reagent	Solvent	Base and additives	Yield
1	PyBOP	DCM	DIPEA, DMAP	94%[c]
2	PyBOP	MeCN	DIPEA, DMAP	48%[b]
3	PyBOP	DCM	DIPEA, DMAP	79%[b]
4	$(COCl)_2$/DMF	DCM	Et_3N	n.d.
5	DCC	DCM	DIPEA,DMAP	26%[b]
6	DCC	DCM	DIPEA, DMAP	80%[c]
7	EDC	DCM	DIPEA,DMAP	26%[b]
8	**EDC**	**DCM**	**DMAP**	**98%[d]**

[a.] All reactions were performed on a 100 mg scale mixing **99** (1.0 equiv.), **94** (1.0 equiv), **coupling reagent** (1.5 equiv.), **base** (5 equiv.), **DMAP** (0.1 equiv.) in DCM/MeCN (0.1M). [b.] The mixture was stirred for 48h. [c.] The mixture was stirred for 24h. [d.] The mixture was stirred for 1h.

Table 1*: Optimization of the first amide coupling*

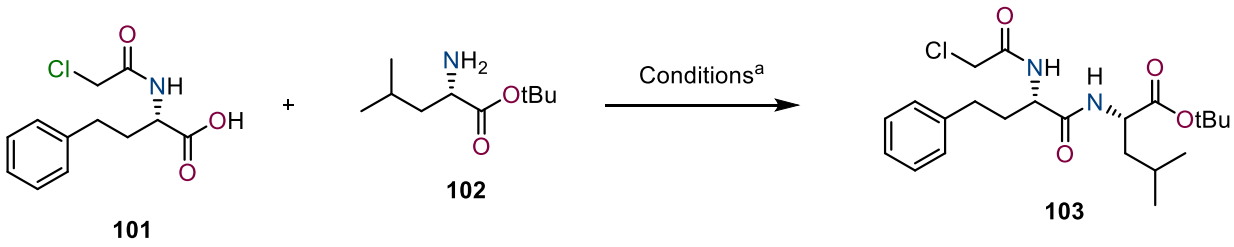
The **first step** (**Scheme 24**) is the coupling of the t-Bu protected bishomophenylalanine **94** with chloroacetic acid **99**. We tried different coupling agents, bases and additives. Also, different reaction times were tested. (**Table 1**)

We decided to start using PyBOP as a coupling agent, because it's one of the most used reagents. We also added DIPEA as base and DMAP as catalyst, using DCM as solvent. We stirred the reaction for 24h (entry 1) and for 48 hour (entry 3). We also tried to run the reaction changing the solvent. We tried to use MeCN, keeping the same conditions (PyBOP, DIPEA, DMAP) stirring the reaction for 24 hour (entry 2). We obtained good results, but PyBOP is a very expensive reagent, so we decided to screen different conditions. We tried to generate the acyl chloride. We treated the chloroacetic acid **99** with oxalyl chloride and a drop of DMF, to generate the corresponding acyl chloride. Then a solution of amine and Et_3N in DCM was added. This time we obtained only degradation. Probably these conditions are too strong for our substrate.

Next, we used DCC to run the reaction, again using DIPEA as base and DMAP as catalyst. We change the reaction time: we stirred the reaction for 24 hour (entry 6) or 48h (entry 5). In the first case we obtain a very good yield (90%) but it wasn't as high as the one obtained with PyBOP (entry 1, 94%). Finally, we tested EDC.HCl, with DIPEA and DMAP. We obtained 26% of yield after 48 hours (entry 7). So, we tried to run the reaction without base, just with catalytic amount of DMAP. We monitored the reaction by TLC and

surprisingly after only 1 hour the reaction reached total conversion (entry 8). A simple column chromatography was needed for the purification step. This time reaction was almost quantitative. At this point we wanted to scale up the synthesis. Applying the optimized conditions (1eq of **99**, 1eq. of **94**, 1.5 eq. of coupling reagent (EDC.HCl) and 0.1eq. of DMAP in DCM) we run the reaction on 500mg and also on a gram scale. In both cases we obtained the desired product **100** in a good yield after only one hour.

The **second coupling** is between the carboxylic acid **101** and the t-Bu protected leucine **102** (which is commercially available) (**Scheme 25**).



Scheme 25**: Second amide coupling of carboxylic acid **101** with t-butyl protected leucine **102

Also, in this case we wanted to perform an optimization. We screened different coupling reactions and different reaction times. At first, we run the reaction on 100mg scale; then, we tried to scale up the synthesis on 1g scale. We tested again DCC and PyBOP as coupling agents, in the presence of DIPEA as base and DMAP as catalyst. In both cases we stirred the reaction for 24 hours on a 100mg scale (entry 1 and 2). With PyBOP we

obtained the desired product in a modest yield. So, we tried to scale up the synthesis on 1g (entry 3), but this time we obtained only degradation.

Entry	Coupling Reagent	Solvent	Base and additives	Yield
1	DCC	DCM	DIPEA, DMAP	25%[b,d]
2	PyBOP	DCM	DIPEA, DMAP	40%[b,d]
3	PyBOP	DCM	DIPEA, DMAP	n.d.[c]
4	$(COCl)_2$/DMF	DCM	Et_3N	n.d.
5	EDC	DCM	DMAP	12%[c]
6	**EDC**	**DCM**	**DMAP**	**70%[b,e]**

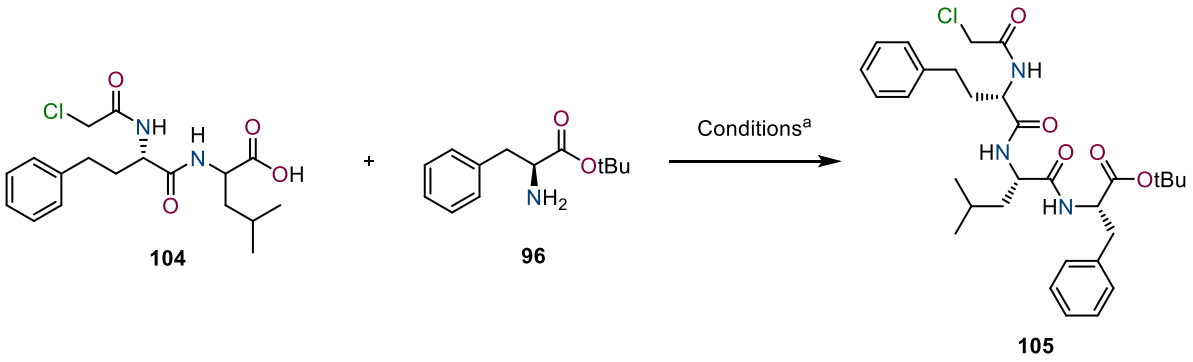
[a.] All reactions were performed mixing **101** (1.0 equiv.), **102** (1.0 equiv), **coupling reagent** (1.5 equiv.), **base** (5 equiv.), **DMAP** (0.25 equiv.) in DCM (0.1M). [b.] 100mg scale [c.]1 g scale. [d.] The mixture was stirred for 24h. [e.] The mixture was stirred for 1h.

Table 2: *Optimization of the second amide coupling*

We tried again to form the acyl chloride (entry 4), but also in this case we only obtained the degradation of the starting material. Finally, we applied the first step optimized conditions. Using 1eq of carboxylic acid **101**, 1eq. of t-Bu protected leucine **102**, 1.5 eq. of coupling reagent (EDC.HCl) and 0.1eq. of DMAP, in DCM, we obtained the desired product in a good yield **103** (entry 6). A simple column chromatography was needed for the purification step.

Then we tried to scale up the reaction using 1g of t-Bu Leucine (entry 5). Unfortunately, we obtained degradation. Probably this reaction step suffers from scalability. We are able to obtain the desired compound performing the reaction only on 100 mg of t-Bu protected leucine.

The **third coupling** is between the carboxylic acid **104** and tBu-protected phenylalanine **96** (**Scheme 26**). Also in this case we decided to screen different conditions.



Scheme 26**: Third amide coupling between carboxylic acid **95** with t-butyl protected phenylalanine **87

We performed all of reactions on a 100 mg scale, using the same solvent at room temperature. Then we tried to scale up the synthesis. At first, we tried to used PyBOP in DCM, using as catalyst DMAP and as base DIPEA. We run the reaction on 100 mg and also on 300 mg scale (entry 1/2 and 3). We stirred the reaction for 24 hours. In both cases we didn't obtain the desired product. This time PyBOP didn't work in this coupling step.

Entry	Coupling Reagent	Solvent	Base and additives	Yield
1	PyBOP	DCM	DIPEA, DMAP	n.d.[c]
2	PyBOP	DCM	DIPEA, DMAP	n.d.[c]
3	PyBOP	DCM	DIPEA, DMAP	n.d.[b]
4	$(COCl)_2$/DMF	DCM	Et_3N	n.d.
5	**EDC**	**DCM**	**DMAP**	**98%**[d]

[a.] All reactions were performed on a 100 mg scale mixing **104** (1.0 equiv.), **96** (1.0 equiv), **coupling reagent** (1.5 equiv.), **base** (5 equiv.), **DMAP** (0.25 equiv.) in DCM (0.2M). [b.] 300 mg scale. [c.] The mixture was stirred for 24h. [d.] The mixture was stirred for 30 minutes.

***Table 3**: Optimization of the third amide coupling*

We moved forward trying to use acyl chloride strategy; again, this are too harsh conditions for this substrate, so we just obtained degradation of our compound (entry 4). Finally, we applied the optimized conditions of the previous steps. Using 1eq of carboxylic acid **104**, 1eq. of t-Bu protected phenylalanine **96**, 1.5 eq. of coupling reagent (EDC.HCl) and 0.1eq. of DMAP, in DCM, we obtained the desired product in a good yield (entry 5). This coupling is faster than the others: in only 30 minutes we obtained our product **105**. A simple column chromatography was required to purify the compound.

Overall Synthesis of Carfilzomib

The total synthesis is shown in **Scheme 27**. The synthetic pathway is based on 8 steps. We have optimized all the coupling steps using the three prepared t-Bu protected amino acids. The synthesis starts with the coupling of chloroacetic acid **99** and t-Bu protected bishomophenylalanine **94**. Using the optimized conditions (EDC.HCl, DMAP, DCM, 1h) the desired product **100** was obtained.

Then, t-butyl ester was deprotected using an excess of TFA. No column chromatography was required after this step. It's necessary to remove all the remaining acid before continuing the synthesis, because further steps can suffer by the presence of TFA. With the carboxylic acid **101** in hand, we performed the second amide coupling. The previously reported optimized conditions were used also for this step. Then, again TFA deprotection of the t-Bu ester led to the corresponding carboxylic acid **104** which was subjected to the third amide coupling.

At this point we performed TFA deprotection on the t-Bu ester **105** to have the corresponding carboxylic acid. We wanted to perform the last coupling using the chiral epoxy ketone, and then we wanted to install the morpholine ring. Surprisingly, we were not able to isolate the desired coupling product with the chiral epoxy ketone installed.

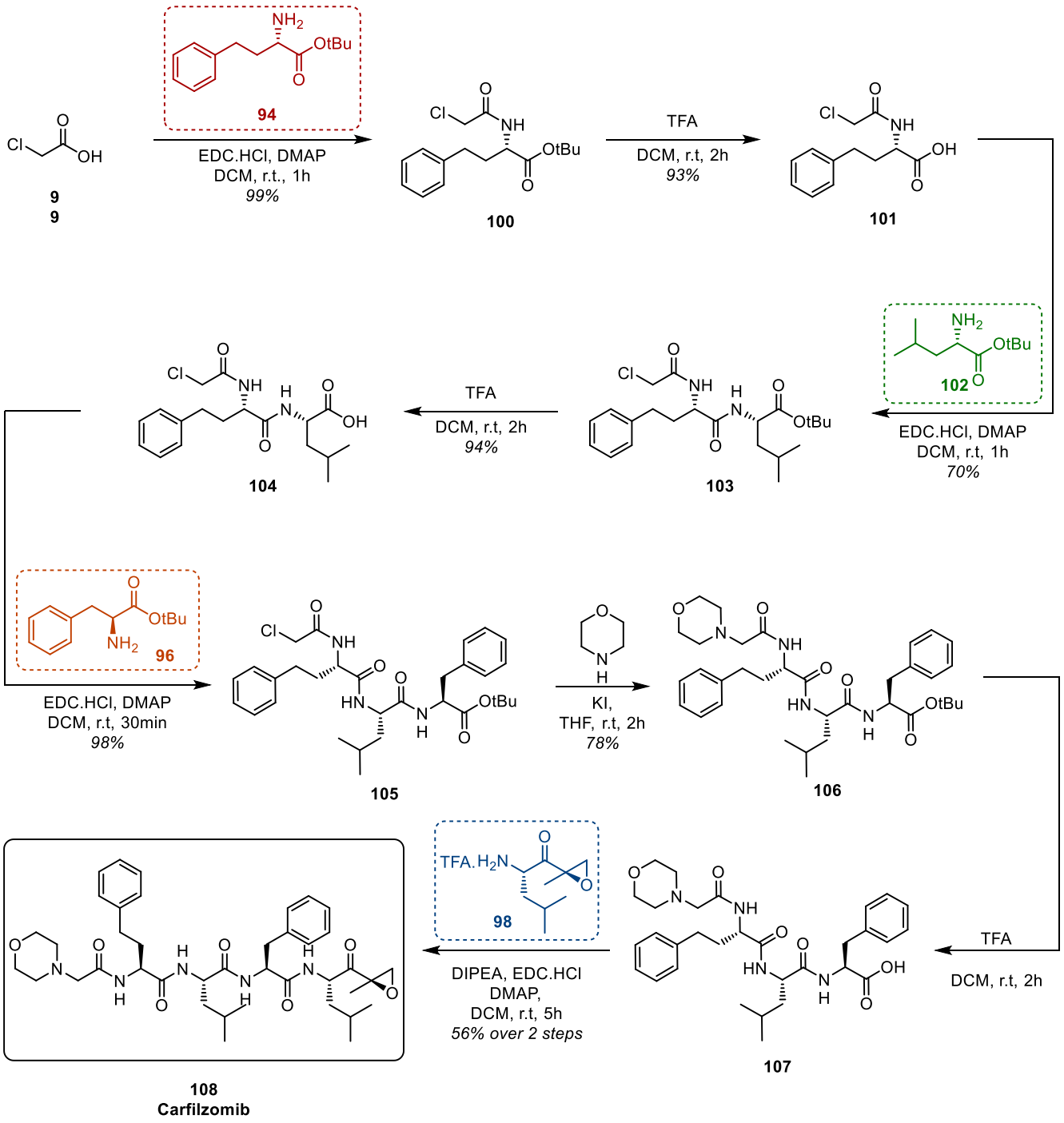
This is probably due to the instability of the epoxy ketone as a free amine. We decided to change completely the order of the reaction, adding this chiral epoxy ketone in

the last step of the synthesis (**Scheme 27**). For this reason, morpholine was installed before the TFA deprotection. We treated our compound **105** with morpholine and KI in THF for 2h to afford the desired compound **106** in a good yield. After the addition of morpholine, our compound became more polar and more stable. Only at this point we performed TFA deprotection of t-Bu group to have the corresponding carboxylic acid **107**.

For the last step we decided to use the TFA salt of the epoxy ketone **98**, instead of the free amine. From ^{1}H-NMR analysis, we noted that TFA salt was stable even after couple of days at -20°C. As for the free amine, after few hours we saw decomposition, even at -20°C.

So, for the coupling step, we used again optimized conditions (EDC.HCl, DMAP and DCM) with the addition of DIPEA to cleave the TFA salt. We added 2eq. of DIPEA to a solution of our TFA salt in DCM. After ten minutes, we added a solution of the carboxylic acid **107** in DCM, followed by EDC.HCl and DMAP. We monitored the reaction by TLC and after 5 hours we obtained full conversion. The desired product **108** was purified by column chromatography with a polar eluent mixture. The carboxylic acid **107** and the final product **108** are unstable so they were kept at -20°C.

The final compound is an anticancer drug used in the treatment of multiple myeloma. Due to its biological activity, a lot of precautions were taken. All glassware was treated with bleach for one night, then it was washed carefully with water and acetone.

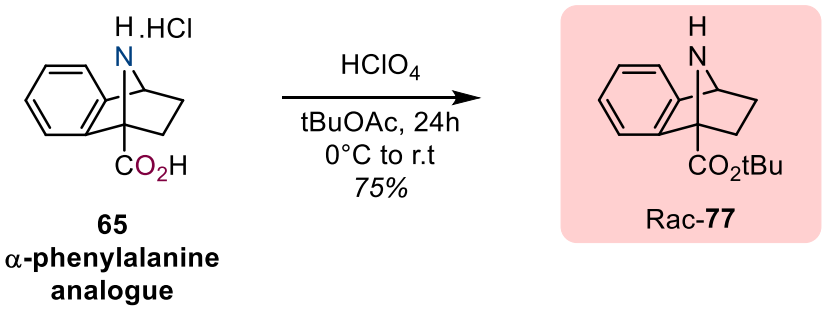


***Scheme 27**: Total synthesis of Carfilzomib*

2.3.3 Carfilzomib Analogue Synthesis

t-butyl Protected Phenylalanine Analogue Synthesis

Alpha phenylalanine analogue was used for the synthesis of carfilzomib analogue. Lateral chain **103** was synthesized with the reported optimized conditions. For the synthesis of carfilzomib analogue, we used derivative **77**, which was prepared from alpha analogue **65 (Scheme 28)**.



***Scheme 28**: t-butyl protection on free amino acid*

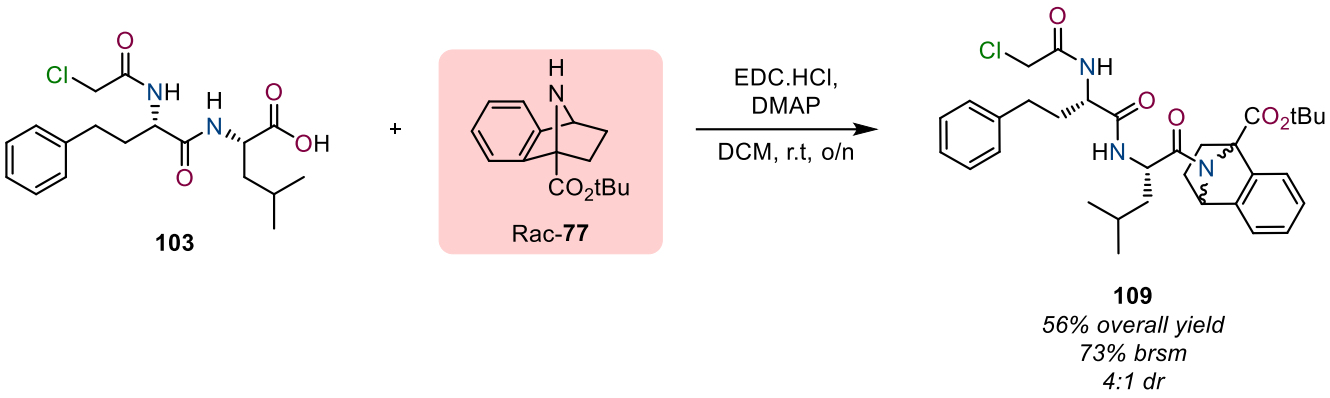
Overall Synthesis of Carfilzomib Analogues

The synthesis of carfilzomib analogues starts from intermediates **103**.

The first step is the coupling of carboxylic acid **103** with t-Bu protected phenylalanine analogue **77** (**Scheme 29**). We decided to apply the optimized conditions also for this step. After 24 hours the reaction wasn't finished. We tried to improve the conversion by adding an excess of carboxylic acid **103**; unfortunately, the conversion was still lower than 100%.

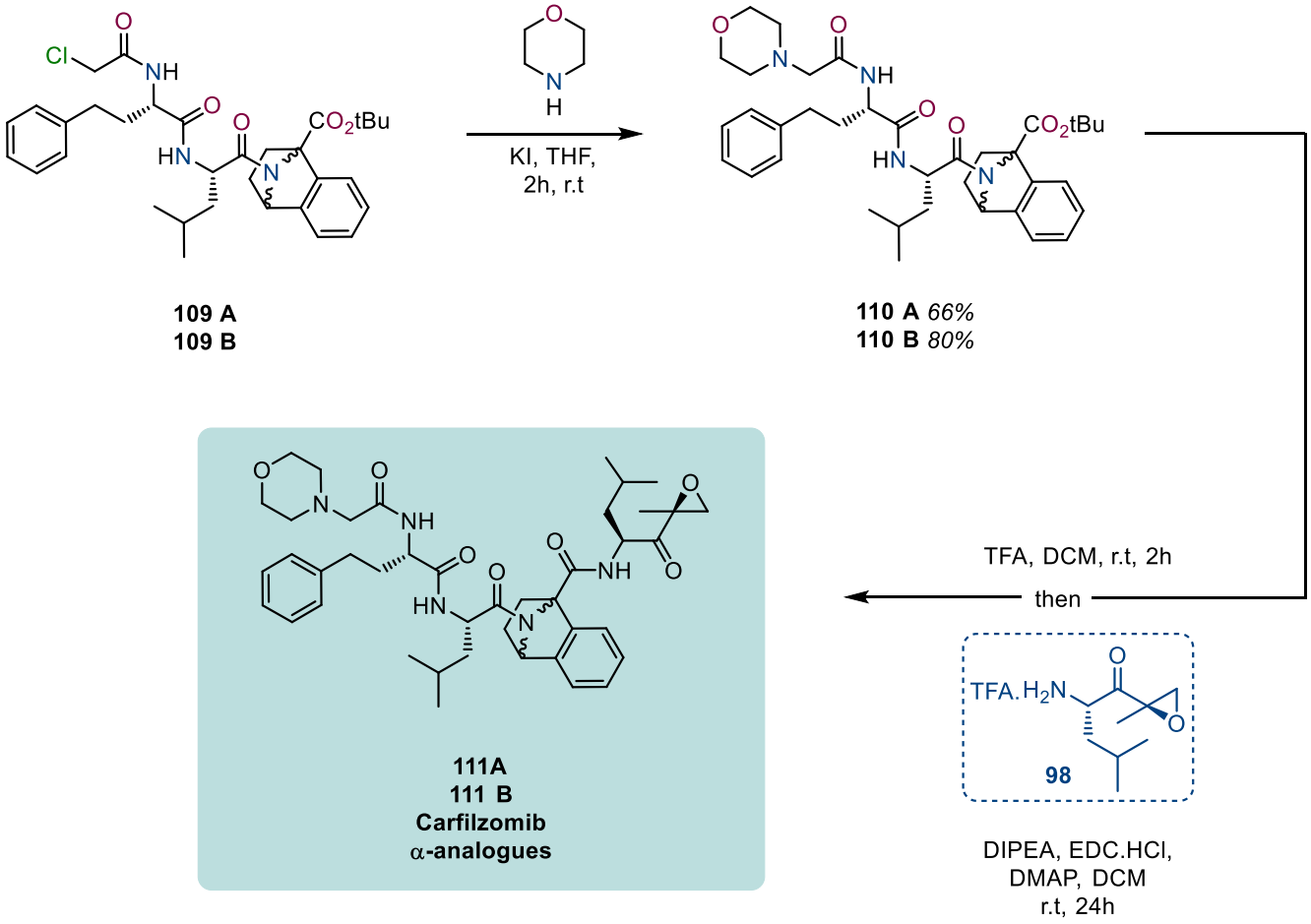
Despite that, we decided to quench the reaction in order to obtain the desired product **109** to continue the synthesis.

Our phenylalanine analogue is a mixture of two enantiomers, so after the coupling step we obtain two diasteroisomers that can be separated by a simple column chromatography. Surprisingly, the diastereomeric ratio of the product is 4:1, despite a partial conversion. This means that the two enantiomers have a different reactivity.



Scheme 29**: Coupling between carboxylic acid **95** with α-phenylalanine analogue **103

Both diastereoisomers are subjected to the same synthetic pathway (**Scheme 30**):



***Scheme 30**: Synthesis endgame*

We treated our compounds **109** with morpholine and KI in THF for 2h to afford the desired compound **110**. Then, t-butyl ester is deprotected using TFA to obtain the corresponding carboxylic acid. Again, all acid traces must be removed before continuing the synthesis, because further steps can suffer by the presence of TFA. This intermediate

proved unstable towards column chromatography conditions. For this reason, the carboxylic acid was directly used in the final step of the reaction without any further purification.

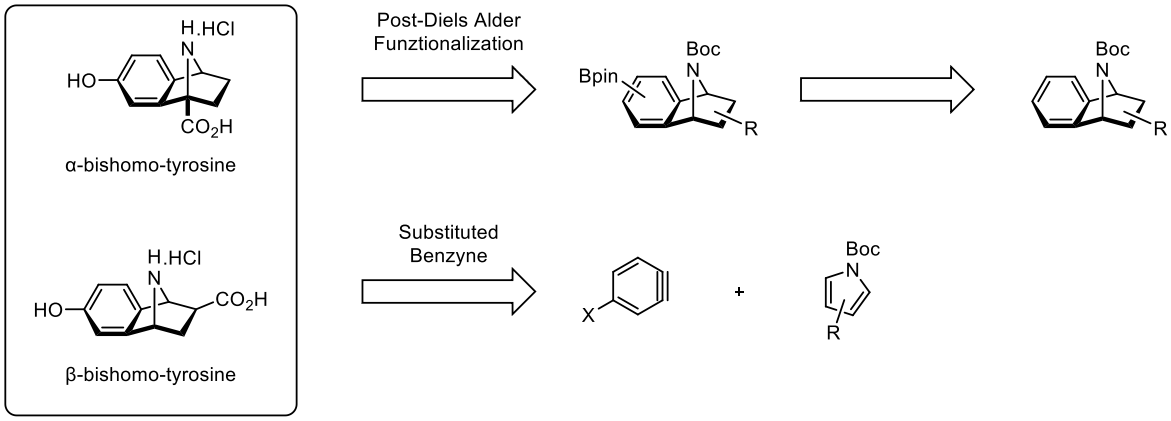
For the last coupling we used the TFA salt of the epoxy ketone **98** and the carboxylic acid. We kept the same optimized conditions, as described before. 2eq. of DIPEA were added to a solution of TFA salt **98** in DCM. After ten minutes, a solution of carboxylic acid in DCM was added, followed by EDC.HCl and DMAP. After 24 hours the reaction was quenched. The crude was very impure, so we run an UPLC-MS to check the presence of our compound. From mass analysis we understood that the desired product was obtained.

Then, we tried to purify the crude by a column chromatography, but we observed degradation of our compound. Moreover, this tetrapeptide proved sensitive to high temperatures. Due to the impossibility to perform a column chromatography, the only purifying method is a preparative HPLC.

We still don't know the right configuration of the two diastereoisomers. We are waiting for an X-Ray analysis to understand the correct stereochemistry.

3 Tyrosine Analogues

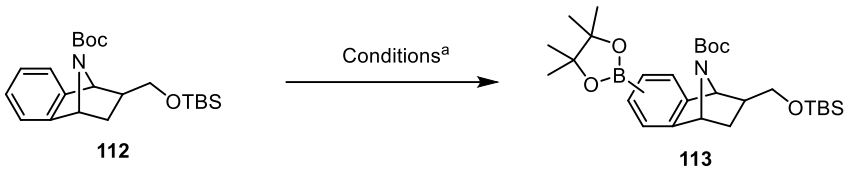
For the synthesis of **Tyrosine analogues**, the idea was to perform a *Post Diels-alder functionalization* on the intermediate of the β-phenylalanine synthesis **112**. Alternatively, a *substituted anthranilic acid* can be used to perform the Diels-Alder reaction (**Scheme 31**).



***Scheme 31**: Tyrosine analogue synthetic strategies overview.*

Following the first idea, we decided to try an Iridium catalyzed borylation on intermediate **112**. Different sources of Boron, different solvents and temperatures were tested but unfortunately the desired product was never obtained (**Table 4**).

Moving on to the second idea, a Diels-Alder reaction using Br-substituted anthranilic acid and 2-substituted pyrrole **60** was performed (**Scheme 32**).



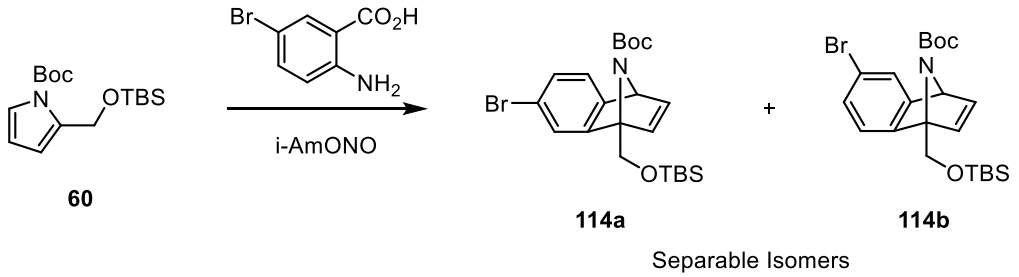
112 **113**

Entry	Catalyst	Ligand	B-source	Solvent	Temperature	yield
1	$[Ir(COD)OMe]_2$	dtbpy	B_2pin_2	THF	80°C	n.d.
2	$[Ir(COD)OMe]_2$	dtpby	HBpin	THF	80°C	n.d.
3	$[Ir(COD)OMe]_2$	dtpby	B_2pin_2	Hex	90°C	n.d.

All the reaction were performed on a 100mg scale adn were stirred for 24h.

***Table 4**: Borylation trials on bicycle **112**.*

The reaction leads to the formation of two isomers that can be separated with a simple column chromatography. An optimization was performed to improve the yield. Different equivalents of anthranilic acid and isoamyl nitrite were used and different temperatures were tested. At 100°C in THF with 8 equivalents of benzyne was possible to obtain 90% of yield (**Entry 6, Table 5**).

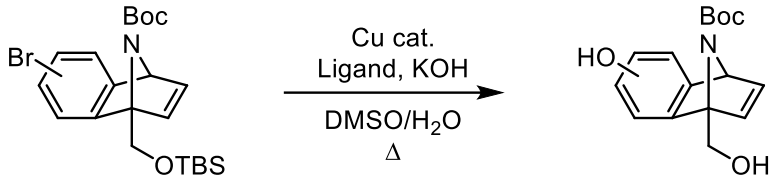


***Scheme 32**: Diels-Alder cycloaddition with Br-substituted anthranilic acid.*

At this point, it was necessary to convert the Br group into a phenol. Again, an optimization was performed (**Table 6**), in this case different Cu sources, ligands and temperatures were tested. Unfortunately, we just obtain degradation or starting material recovery.

Entry	*Solvent*	*Temperature*	*g pyrrole*	*eq acid*	*eq iAmONO*	*yield*
1	THF	80°C	500mg	3	3	45% (64%brsm)
2	THF	80°C	1g	3	3	23% (49% brsm)
3	THF	80°C	500mg	5	5	59% (75% brsm)
4	THF	80°C	1g	5	5	54% (82% brsm)
5	THF	90°C	1g	7	7	78% (83% brsm)
6	**THF**	**100°C**	**1g**	**8**	**8**	**90%**

***Table 5**: Optimization of Diels-Alder cycloaddition with Br-substituted anthranilic acid.*



114 **115**

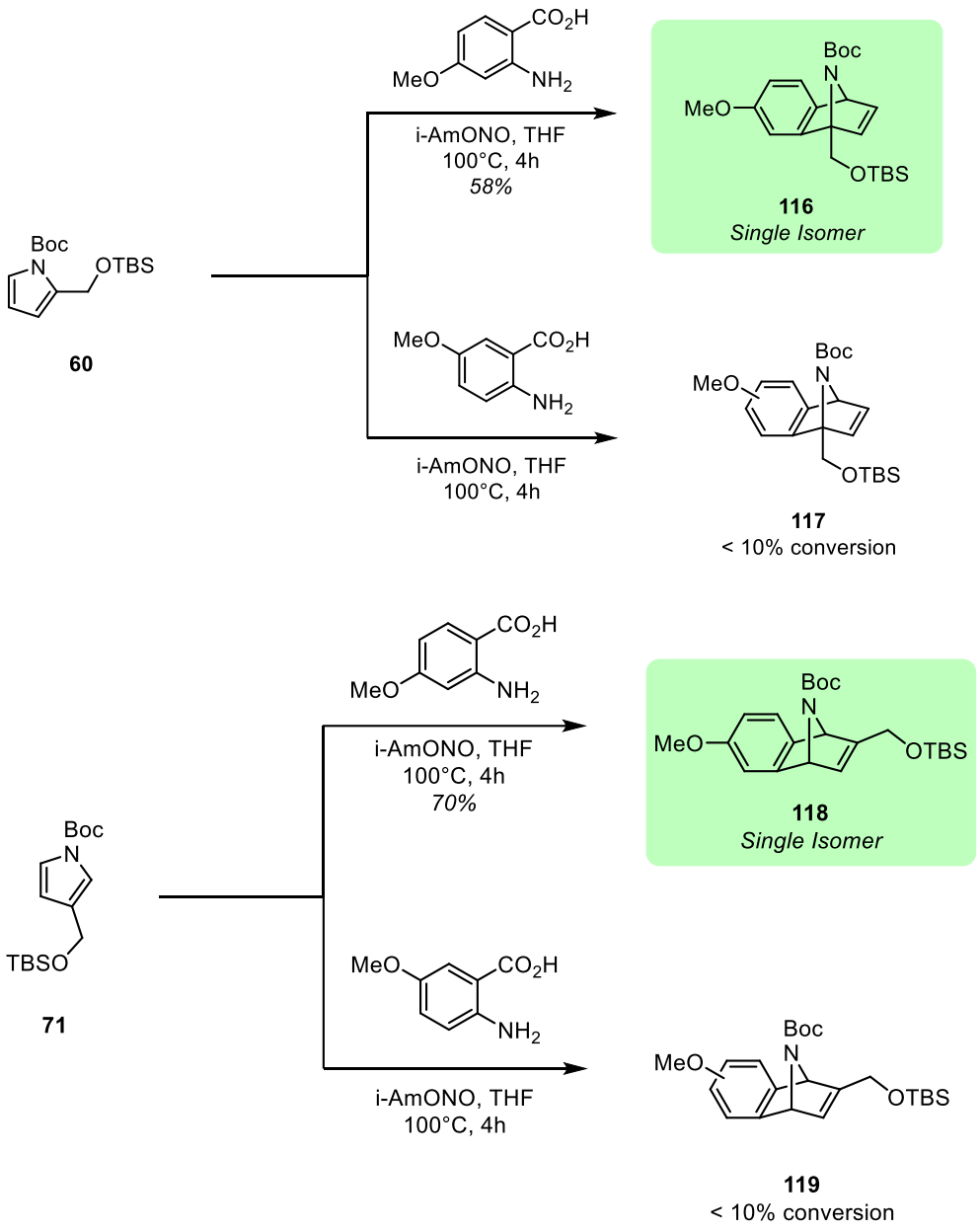
Entry	*Cu catalyst*	*ligand*	*Temperature*	*Yield*
1	CuI	phen	100°C	SM
2	CuI	phen	120°C	Degradation
3	CuSCN	phen	100°C	Degradation
4	CuSCN	bipy	80°C	SM
5	CuSCN	TMEDA	100°C	SM
6	CuBr	phen	100°C	Degradation
7	CuBr	bipy	80°C	Degradation

All reactions were performed on a 100 mg scale. phen = 1,10-phenanthroline; bipy = 2,2'-bipyridyl; TMEDA = N,N,N',N'-tetramethylethylenediamine.

***Table 6**: Optimization of phenol synthesis.*

Due to the problem with the intermediate **114** we decided to change strategy, we moved to the 4- and 5-metoxy substituted anthranilic acid.

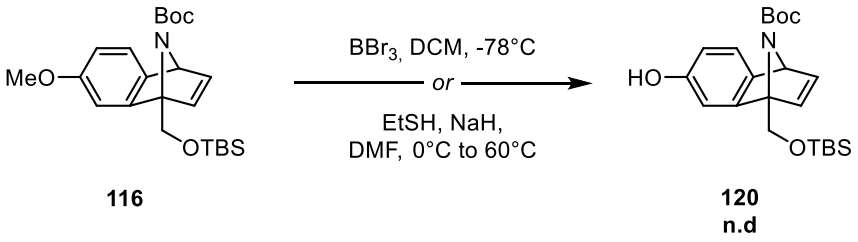
Pyrroles **60** and **71** were tested with 4- and 5-MeO anthranilic acid (**Scheme 33**).



***Scheme 33**: Pyrroles cycloaddition with MeO-substituted anthranilic acids.*

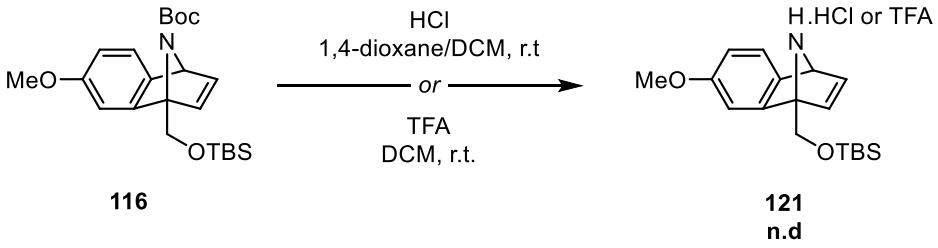
Only 4-MeO anthranilic acid worked in the cycloaddition reaction, leading to the formation of a single isomer.

With cycloadduct **116** we tried different conditions to deprotect the methyl group to obtain the phenol, but unfortunately we only obtained degradation of our compound (**Scheme 34**).



***Scheme 34**: MeO-deprotection trial with BBr_3.*

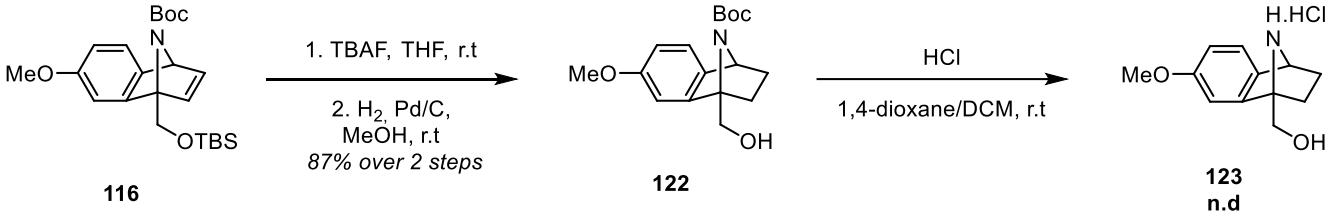
So, we decided to deprotect the Boc group before removing the methyl group. We tried with HCl in dioxane or with TFA in DCM, but the results were disappointing. (**Scheme 35**)



***Scheme 35**: Boc-deprotection trial with HCl on derivative **116**.*

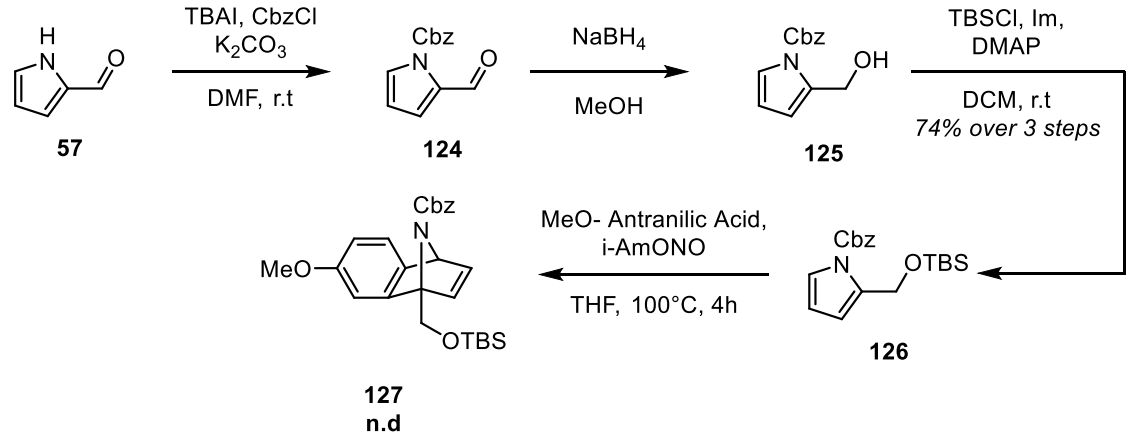
Finally, we thought that maybe the double bond can cause some problems during the methoxy deprotection, so we changed again strategy.

We performed a TBS deprotection using TBAF, then a simple hydrogenation to furnish the desired compound **122**. At this point the deprotection of the Boc group was again performed using HCl in dioxane, but again we only obtain degradation of the starting material (**Scheme 36**).



***Scheme 36**: Final deprotections trial.*

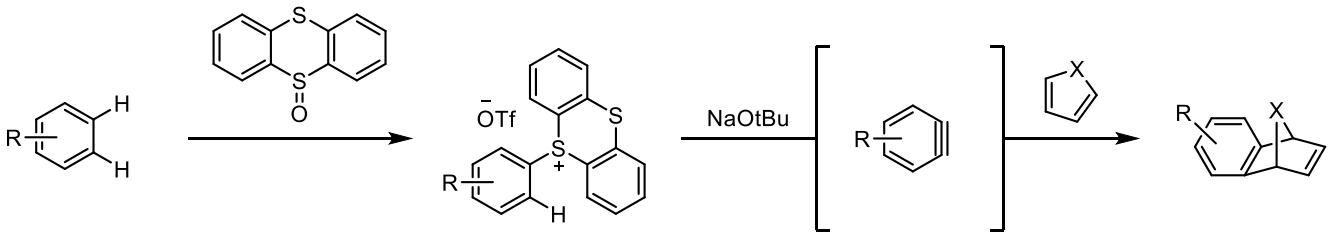
At this point, we tried to restart the synthesis from the beginning changing the pyrrole protecting group (**Scheme 37**).



***Scheme 37**: New strategy using Cbz-protecting group.*

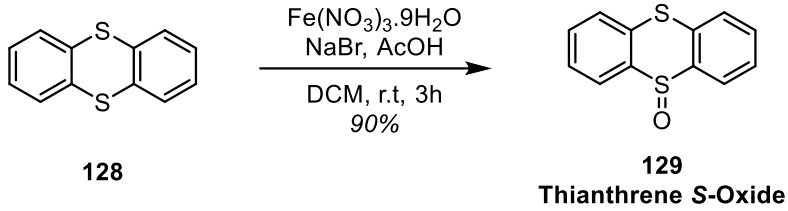
Cbz protection was performed on pyrrole 2-carboxyaldehyde **57**. Then, reduction of the aldehyde with $NaBH_4$ and TBS protection of the corresponding alcohol were performed to have the protected diene **126**. With the protected diene we tried the Diels-Alder cycloaddition using 4-MeO anthranilic acid, but we only obtained degradation of the starting material.

Finally, we completely changed strategy, and we wanted to generate benzyne in another way. Using the thianthrene strategy, the idea was to generate benzyne using a C-H activation (**Scheme 38**).



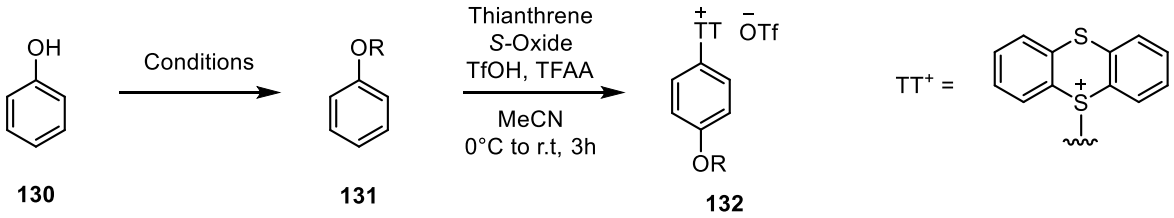
***Scheme 38**: New strategy using C-H activation.*

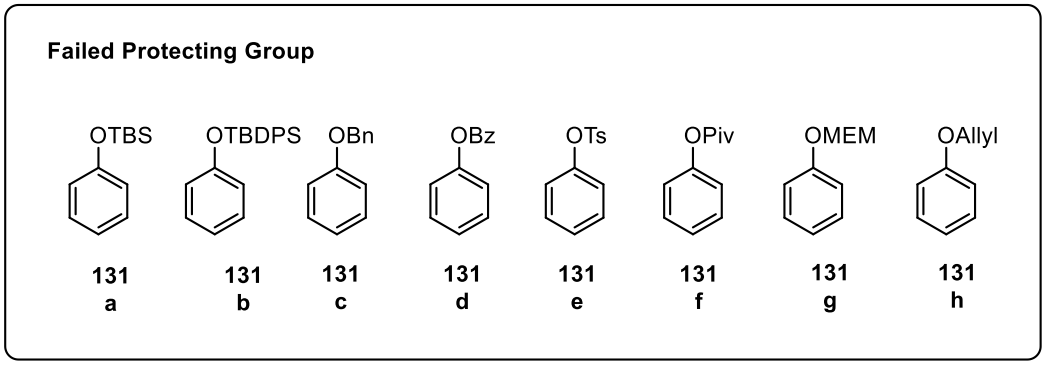
For this strategy, thianthrene S-Oxide **129** was synthetized using ferric nitrate, sodium bromide and acetic acid (**Scheme 39**).



***Scheme 39**: Thianthrene-S-Oxide synthesis.*

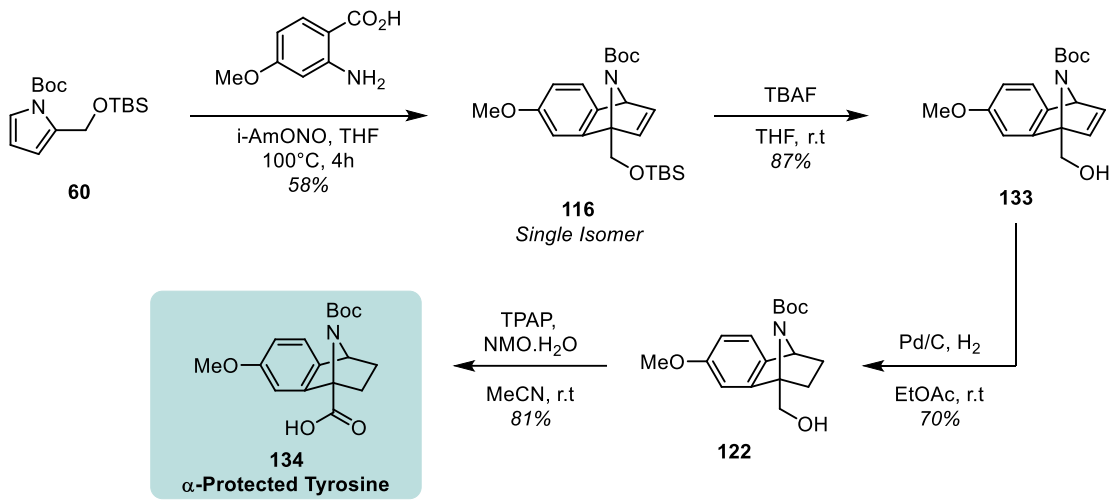
For this new strategy we needed to start from a protected phenol derivative. Then, C-H activation step can occur, using the thianthrene S-Oxide **129**, triflic acid and trifluoroacetic anhydride in MeCN at 0°C. Unfortunately, none of the protecting groups used on the phenol could survive to the CH activation conditions (**Scheme 40**).





***Scheme 40**: All the protecting groups tried in the C-H activation strategy.*

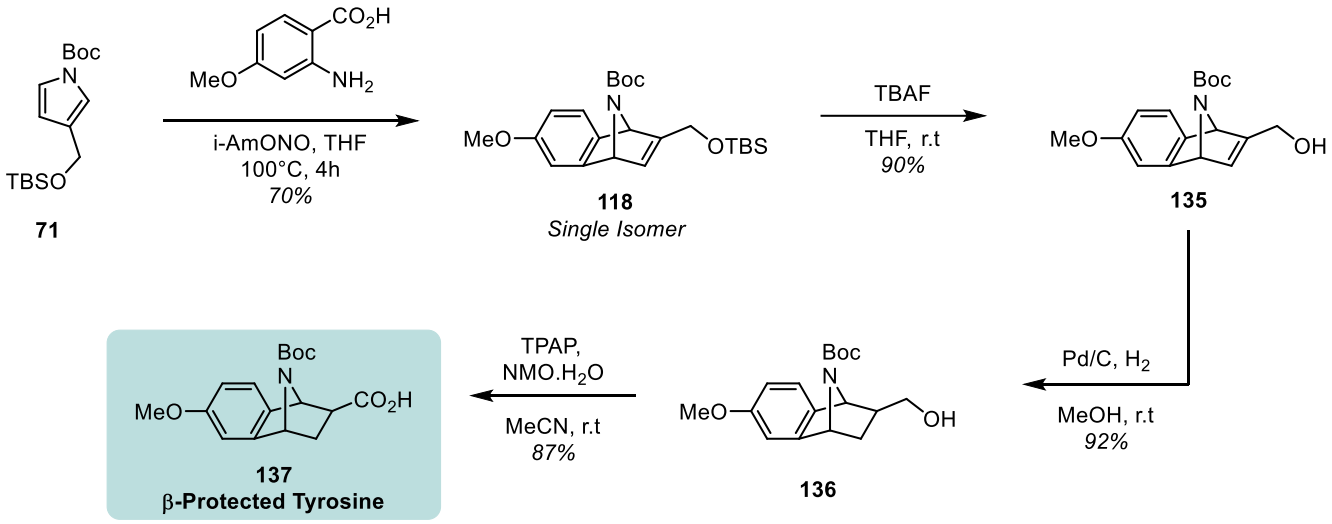
In the end, we decided to leave the thianthrene strategy and to synthetize a protected tyrosine using the previous strategy (**Scheme 41**).



***Scheme 41**: Synthesis of protected α-tyrosine derivative.*

Starting from the protected 2-substituted pyrrole **60**, the cycloaddition reaction was performed using 4-MeO anthranilic acid. The corresponding cycloadduct **116** undergoes a TBS deprotection using TBAF to have the free alcohol **133**, then hydrogenation furnish the desired saturated product **122**. Finally, carboxylic acid **134** was obtained using Ley oxidation. In this way, we synthetized a protected α-tyrosine analogue (**Scheme 41**).

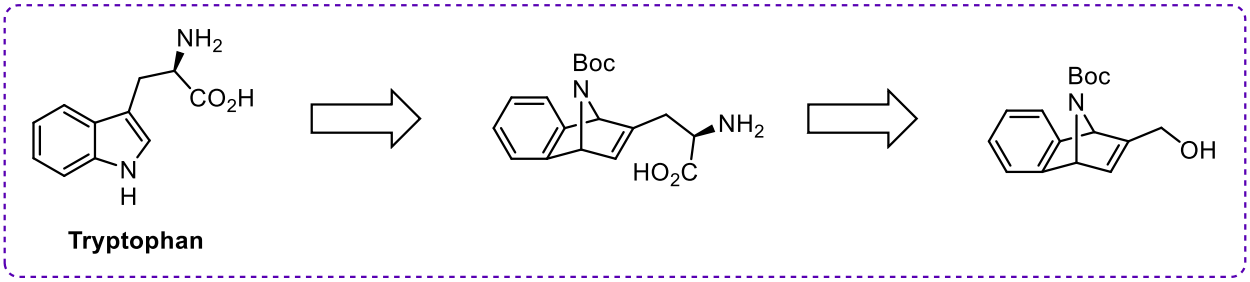
The same reaction pathway was performed on the protected 3-substituted pyrrole **71** to obtain the protected β-tyrosine analogue (Scheme **42**).



***Scheme 42**: Synthesis of protected β-tyrosine derivative.*

4 Tryptophan Analogues

The last conformational restricted ammino acid that we wanted to synthesize is a tryptophan analogue. We decided to use one common intermediate **73** of the β-phenylalanine pathway (Scheme **43**).



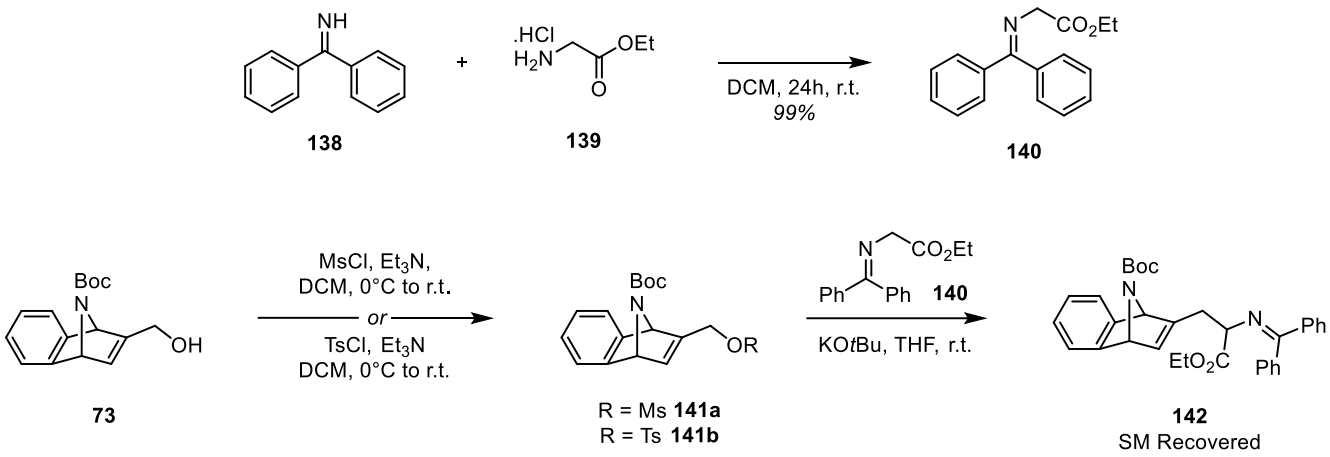
***Scheme 43**: Overview of tryptophan analogue synthesis.*

The first idea was to subject the activated primary alcohol **73** to a nucleophilic substitution using a protected glycine (**Scheme 44**).

So, we protected the commercially available glycine **139** using benzophenone imine to have the desired protected glycine **140**. We decided to use this protecting group because it is the most used in literature for this kind of alkylation reactions.

Mesyl chloride and tosyl chloride were used to activate alcohol. The corresponding intermediates were used in the alkylation step without further purification.

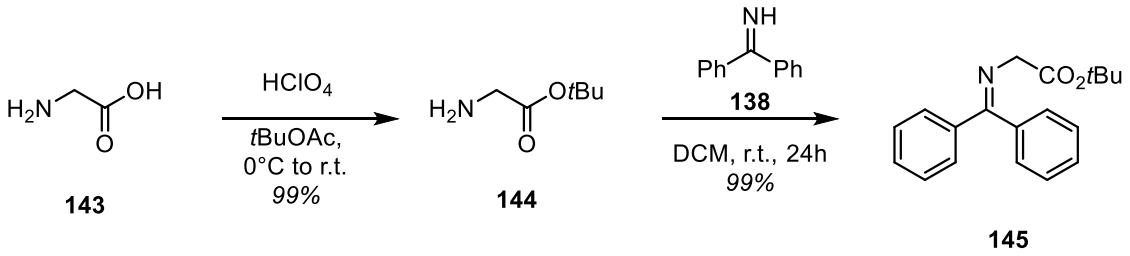
The protected glycine was added to intermediate **141**, potassium tert-butoxide was used as base and THF as solvent and the reaction was stirred for 24h. Unfortunately, we only recovered the starting material.



***Scheme 44**: First alkylation trial.*

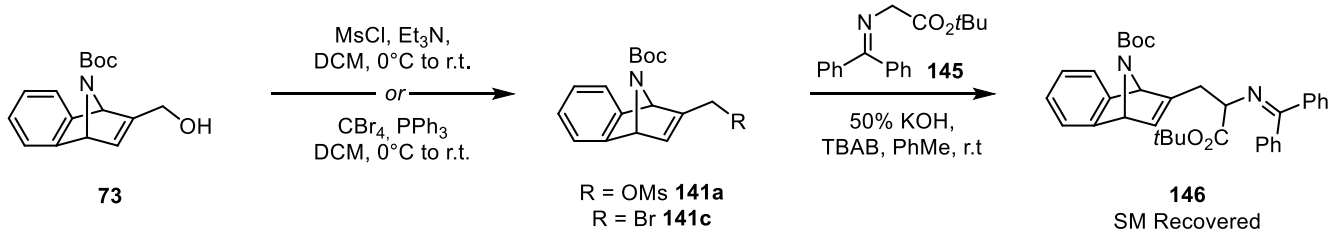
We changed glycine protecting group, using glycine tert-butyl ester to have all acid sensitive protecting groups. In this way, we can avoid some deprotection steps, to have a more straightforward synthesis.

So, we performed on glycine a simple tert-butyl esterification and then again we protected the nitrogen using the benzophenone imine group (**Scheme 45**).



***Scheme 45**: Tert-butyl protection on glycine.*

Again, we performed the mesylation of the primary alcohol; on the same time, we tried an Appel reaction to install a bromide group. On both intermediate **141**, we performed the nucleophilic substitution using the new protected glycine **145**. For this reaction we used a solution of potassium hydroxide as base and a phase transfer catalyst (TBAB), unfortunately we only recovered the starting material (**Scheme 46**).



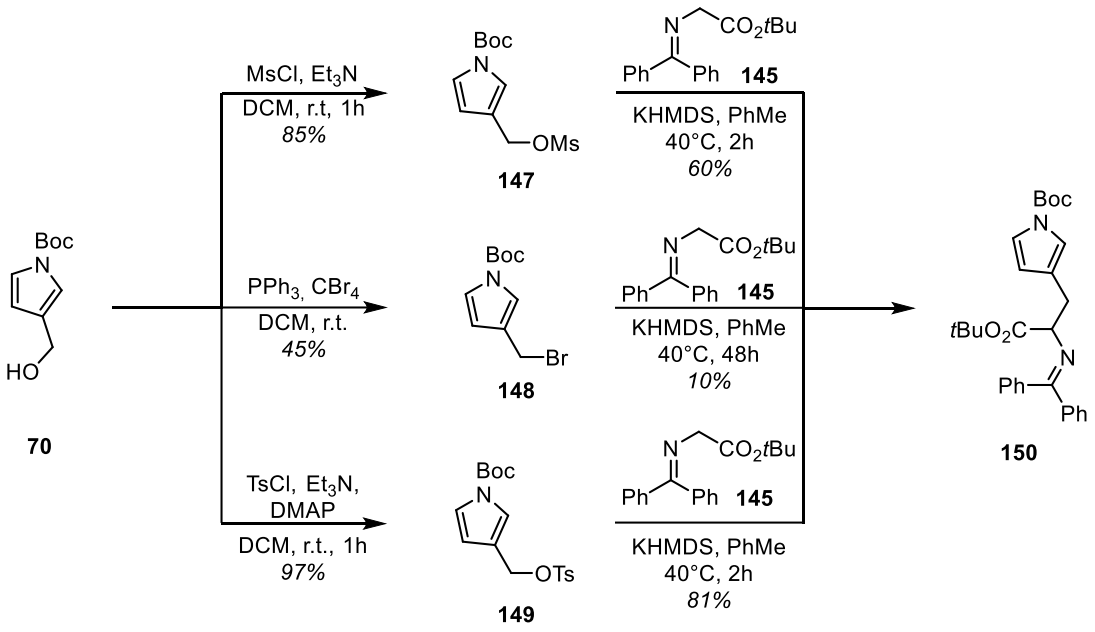
73

R = OMs **141a**
R = Br **141c**

146
SM Recovered

***Scheme 46**: Second alkylation trial with PTC.*

We chose to restart the synthesis from pyrrole **66** and to perform the nucleophilic substitution before the Diels-Alder cycloaddition. A TIPS protection was performed to furnish the desired product, then Vilsmeier-Haack reaction occurs to have 3-carboxyaldehyde. Boc protection of nitrogen and final reduction of the aldehyde to alcohol using sodium borohydride gave desired product. At this point, we tried different reaction on the free alcohol of pyrrole **70** (**Scheme 47**).



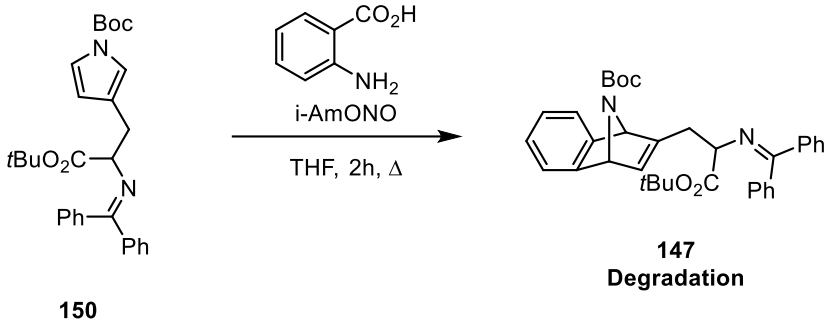
***Scheme 47**: Strategies using pyrrole **70**.*

We performed the activation of the alcohol using mesyl **147** and tosyl **149** group and we also performed an Appel reaction to install the bromide group **148**.

All of these intermediates were used in a nucleophilic substitution using the t-Butyl protecting glycine **145**, potassium bis(trimethylsilyl)amide (KHMDS) as a base, in toluene at 40°C. The reaction was monitored by TLC and all reactions lead to the desired product **150**. They differ only in reaction time and yield, as reported in **Scheme 47**.

Now it only remains to perform the Diels-Alder reaction to have the cycloadduct and finally the deprotection step in acid conditions to have the tryptophan analogue.

Unfortunately, the cycloaddition using the substituted pyrrole **150** never occured. We tested different temperatures (100°C, 80°C and 60°C) but we only obtained the degradation of the starting material (**Scheme 48**).



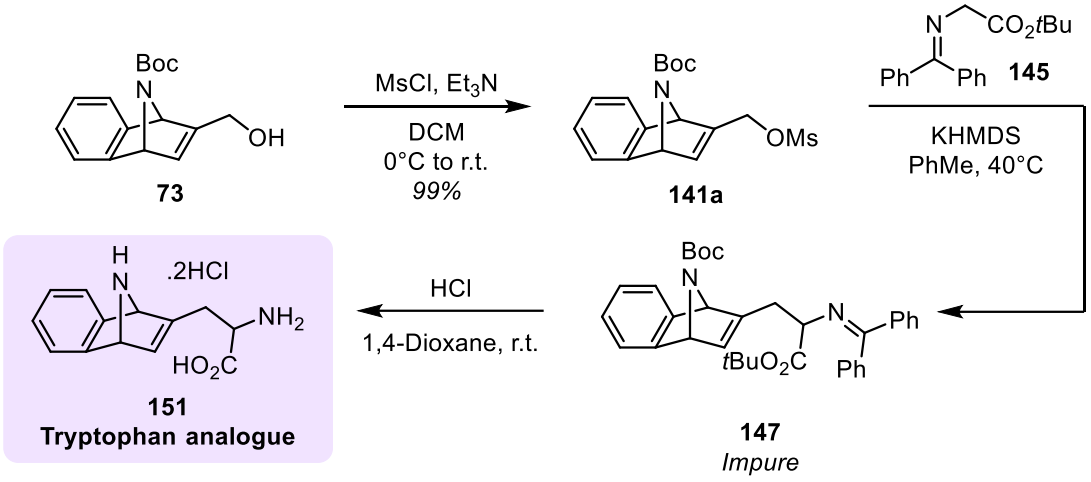
Scheme 48**: Diels-Alder cycloaddition using alkylated pyrrole **150.

We turned again on mesylated intermediate **141a** this time using KHMDS as base. After 24h at 40°C we obtained the desired product as a mixture of two diasteroisomers.

The two isomers **147** and also an impurity run together in TLC, we tried to purify the crude by a simple column chromatography, but it wasn't possible to obtain the desired product pure.

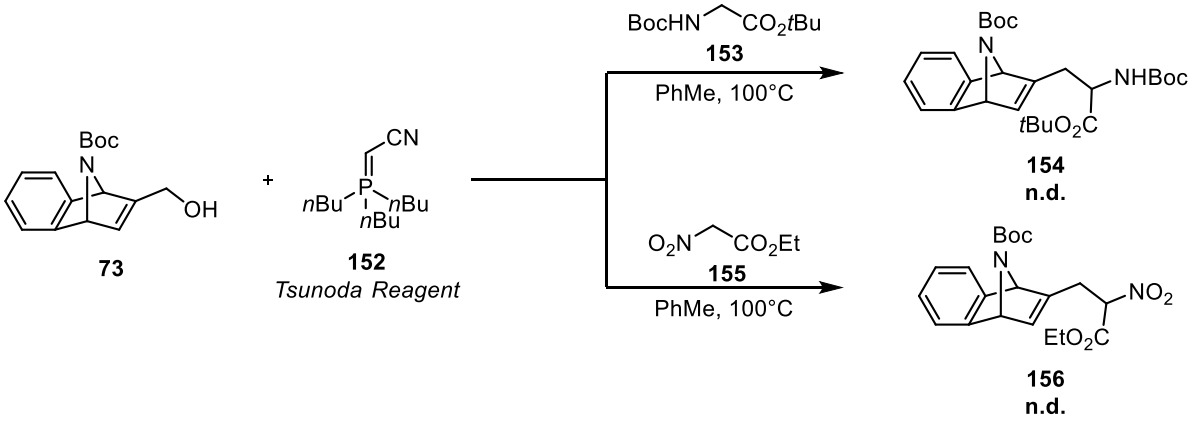
We tried to remove all the protecting group in acid conditions using HCl in Dioxane; we tried to purify in this way the final product by simple filtration. Unfortunately, also in this case the desired product **151** wasn't enough pure (**Scheme 49**).

***Scheme 43**: Overview of tryptophan analogue synthesis.*



***Scheme 49**: Last alkylation strategy.*

As the last trial we used the Tsunoda reagent to perform a Mitsunobu like reaction. The cycloadduct **73** was reacted with Boc protected glycine **153**; we also decided to try this new strategy using ethyl nitroacetate **155**. We choose this compound for the acidity of its protons. Both reactions were heated at 100°C and they were stirred for 24h. In both cases, we only obtained the degradation of the starting material (**Scheme 50**).



***Scheme 50**: Strategy based on Tsunoda reagent.*

5 Conclusions Part I

In this work, we developed an innovative synthetic protocol for the synthesis of **conformationally-restricted sp^2-sp^3 hybrids**. The key reaction was the Diels-Alder cycloaddition of **benzyne** with different dienes. This strategy was powerful for the synthesis of constrained amino acids derivatives.

At first, we focused our attention on *proline analogues*. Starting from 2- and 3-substituted pyrroles we have been able to synthesize respectively α- and β-amino acid derivates. **Enalapril,** an ACE-inhibitor, was selected as a drug to develop our new hybrid strategy. Using our α-derivative, we were able to obtain two different diastereomers, which can be separated by simple column chromatography.

We moved to *phenylalanine*, which is a common motif in a lot of natural compounds and drugs. This time we wanted to use again α– and β-derivatives; moreover, acetoxy pentafulvene was used to obtain a further γ-phenylalanine analogue.

We selected **Carfilzomib** to apply again our hybrid strategy and to obtain a Carfilzomib restricted analogue.

At first, we optimized a protocol to synthesize this important drug. After that, we decided to apply the optimized conditions for the synthesis of Carfilzomib analogue using our phenylalanine derivatives. We decided to use α-phenylalanine derivative because it is the easiest to synthesize. The total synthesis of Carfilzomib analogues has been successful;

we obtained two diasteroisomers that can be separated by a simple column chromatography. The final compounds will be subjected to biological testing. Hopefully, the conformationally restricted analogues will fit better in enzyme active site leading to an increased biological activity. Further investigations will be performed using the β- and γ-phenylalanine analogues in the synthesis of other Carfilzomib derivatives.

In the end, we tried to obtain constrained derivatives of *tyrosine* and *tryptophan*. In the first case, we tried different strategies with different substitutions patterns to obtain the phenolic hydroxyl group. We were able to use Methoxy-substituted anthranilic acid, but unfortunately, we were not able to remove methyl group, so we just obtained two protected tyrosine analogues.

As for tryptophan, despite our efforts we were not able to obtain a bicyclic derivative, because of different impurities in the final reaction steps. Further investigation will be performed to find alternative synthetic possibilities.

Part II: SYNTHESIS OF HYGROMYCIN A ANALOGUES FROM BENZENE

6 Introduction

6.1 Hygromycin A

6.1.1 Antibacterial activity

Antibiotics are used to treat or prevent bacterial infections. They saved the lives of many patients and represent one of the major advances in medicine. Since the discovery of penicillin by Sir Alexander Fleming in 1928, the modern era of antibiotics has begun, and with it the problem of antibiotic resistance. It has been attributed to the over-use and misuse of these medications. Increasing resistance to antibiotics has made the clinical treatment of bacterial infections problematic, threatening global public health[128]. As a guideline, the World Health Organization has published a list of the most dangerous drug-resistant bacteria that requires the development of new treatments. This list includes various Gram-positive bacteria such as Methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus faecium, and β-lactamase-resistant Streptococcus pneumoniae, which pose significant health risks[129]. For these reasons, a new class of antibiotics with activity against resistant Gram-positive pathogens is needed.

Hygromycin A (**Figure 23**) is a potent inhibitor of *Gram-positive* bacteria and some *Gram-negative* bacteria[130] and is a promising target for the development of new derivatives with antibacterial activity.

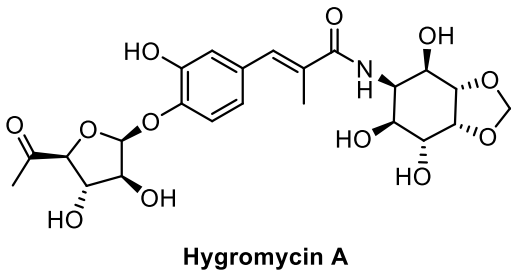


***Figure 23**: Hygromycin A structure.*

The isolation of Hygromycin A as a metabolite excreted by Streptomyces hygroscopius was reported in 1953 [131]. Its unique chemical structure consists of furanose, a cinnamic acid and an aminocyclitol moiety. Hygromycin A blocks protein synthesis; moreover, it was shown to inhibit the ribosomal peptidyl transferase activity [132]. Hygromycin A represents a promising starting point for the creation of new antibiotics for the treatment of drug-resistant pathogen infections [133]. The potential of Hygromycin A and its analogues has led to the study of its biosynthesis with the long-term objective to synthesize new antibiotics [134].

6.1.2 Activity against Lyme disease

Lyme disease is the most common vector-borne disease in the United States. It is caused by the bacterium *Borrelia burgdorferi* and rarely, *Borrelia mayonii*. It is transmitted to humans through the bite of infected blacklegged ticks [135]. Different antibiotics have been developed to treat this infection; unfortunately, these antibiotics can also harm 'good' bacteria that comprise the microbiome of our digestive, reproductive, respiratory, and central nervous systems. Recently, it has been published a rediscovery of Hygromycin A for Lyme disease treatment, for its antibiotic activity. For this reason, we decided to focus on the synthesis of different Hygromycin A analogues.

6.1.3 Structure modifications

Structure-activity relationships (SAR) studies about sugar, aromatic ring, enamide and aminocyclitol portions show that the aminocyclitol is essential for the biological activity of this antibiotic while the furanoside unit is not. These important features were discovered during a program to develop new Hygromycin A analogues by introducing various modifications on all key moieties.

The vinylic methyl group of Hygromycin A was replaced by other alkyl and halogen substituents. For a methyl-like substituent the activity was retained, while in the presence of a hydrogen as substituent or with larger groups the biological activity is lower. Replacement with halogens does not offer considerable improvement [136].

To improve the antibacterial properties, the sugar moiety was replaced by an acyclic sugar surrogate which would also alleviate the C4" epimerization problems (red-coloured squares, **Figure 24**). Early works demonstrated the biological activity retention of allyl ether analogue [137], which has been the guideline for the exploration of new compounds containing an allyl ether substituent at C(4') and various substituents in the other aromatic positions [138].

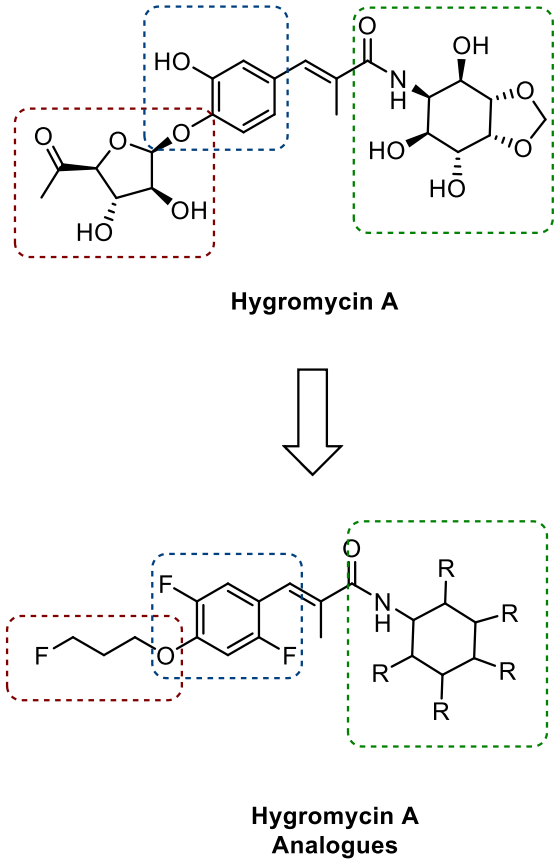


***Figure 24**: Structure modifications on Hygromycin A.*

The SAR results showed that the 2'-position is very sensitive to steric factors, since chloro and methoxy-substitution completely removes activity. Position 3', on the other hand, allows for variations, and encouraging results arised from fluorine replacement, leading to the development of diflourinated analogues. The 3',5'-disubstituted analogues showed a loss of activity while the best result was obtained with 2,5-difluorinated analogue (blue-coloured squares, **Figure 24**). A variety of other ether, amino and carbon analogues in C(4') were prepared to explore SAR in the aryl region. Analogues possessing small lipophilic substituents (less than five methylene groups) maintain comparable activity, in contrast analogues bearing longer chains or hydroxyl groups are less potent.

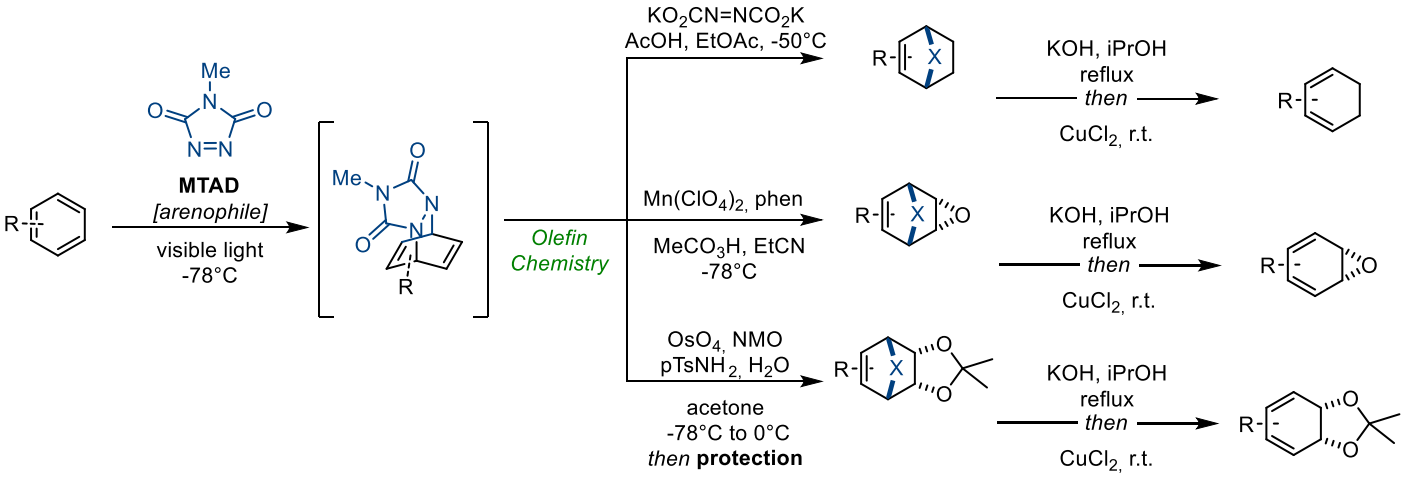
Aminocyclitols are a class of natural compounds possessing a variety of biological activities that find application in organic and medicinal chemistry. They are often structural subunits of antibiotics[139] and glycosidase inhibitors[140]. Structurally, aminocyclitols are cycloalkanes containing at least one amino group and three additional hydroxy groups on the ring atoms [141]. The aminocyclitol moiety of Hygromycin A has been modified as well (green-coloured squares, **Figure 24**): studies suggest that methylation of the 2, 5, 6 hydroxyl groups and the absence or substitution of methylenedioxy ring causes loss in potency. These changes led to the discovery that 1 can support the alkylation in 2 and 6 positions, while 5-hydroxyl is critical to maintain antibacterial activity. In this case the activity of analogues that were alkylated in the 6-position was noticeably worse than the corresponding 2-derivatives [142]. However, in most cases, even small changes in

aminocyclitol moiety of Hygromycin A or its analogues led to great variation of antibacterial activity underlining the importance of this moiety for its biological properties.

6.2 **Arenophile-mediated dearomatization**

Aminocyclitols are key intermediates of various natural products, and much effort has been devoted to the total synthesis of natural aminocyclitols and their analogues. The most significant synthetic methods for the synthesis of aminocyclitols often make use of carbohydrate derivatives as chiral pool starting materials for enantioselective synthesis. In general, a wide range of synthetic methods have acquired importance for the synthesis of aminocyclitols. In this work, we propose an innovative synthetic method, which involves an arenophile-mediated dearomatization strategy.

Dearomatization of aromatic hydrocarbons is used synthetically to convert simple arenes into unsaturated compounds of fundamental importance for the synthesis of natural products, pharmaceuticals, polymers, agrochemicals, and other fine chemicals.



***Scheme 51**: Overview of arenophile-mediated dearomatization strategy. Conditions for dearomative reduction, epoxidation and dihydroxylation are reported, together with cycloreversion conditions.*

In the last century, several examples of dearomatization have revolutionized and expanded the chemistry of aromatic rings, previously considered completely inert to many common chemical transformations.

Arenophile-mediated dearomative photocatalytic reactions have been developed based on the studies conducted previously by Sheridan's group which reported the first visible-light-mediated para-cycloaddition reaction between naphthalene and N-methyl-1,2,4-triazoline-3,5-dione (MTAD) in 1984 [143]. The cyclic azodicarbonyl MTAD, is a photoactivable 2π-compounds used to perform dearomatizion of arenes in a [4+2] fashion in analogy to thermal cycloadditions [144]. Mechanistic studies showed that MTAD, when is photoexcited, undergoes para-cycloaddition with arenes resulting in a formation of bycicle and isolation of π-bond, which can be potentially then used in olefin chemistry. Subsequent cycloreversion of the arenophile moiety affords different 1,3-cyclohexadienes that are not synthetically accessible using existing dearomatization reactions (**Scheme 51**) [145].

Exploration of olefin chemistry for the arenophile-mediated dearomative functionalization strategy led to the development of dearomative cis-hydroxylation strategy[146], dearomative reduction applied to mono and polynuclear arens[147], and dearomative synthesis of arene oxides and oxepines [148]. These dearomative functionalizations of arenes and its derivatives give access to diverse and elaborate small molecules that can then be used for the synthesis of more complex natural compounds, including aminocyclitols.

6.3 Results and Discussion

6.3.1 General overview and lateral chain synthesis

Following the arynophile-mediated dearomatization strategy, the project overview is reported in **Figure 25**. Starting from benzene, dearomative functionalizations are performed, followed by a cycloreversion reaction to obtain a variety of 1,3-cyclohexadiene derivatives. Then, divergent functionalizations can be performed to generate a multitude of aminocyclitols. Ultimately, to obtain all Hygromycin A analogues, a simple amide coupling is performed, using the carboxylic acid lateral chain.

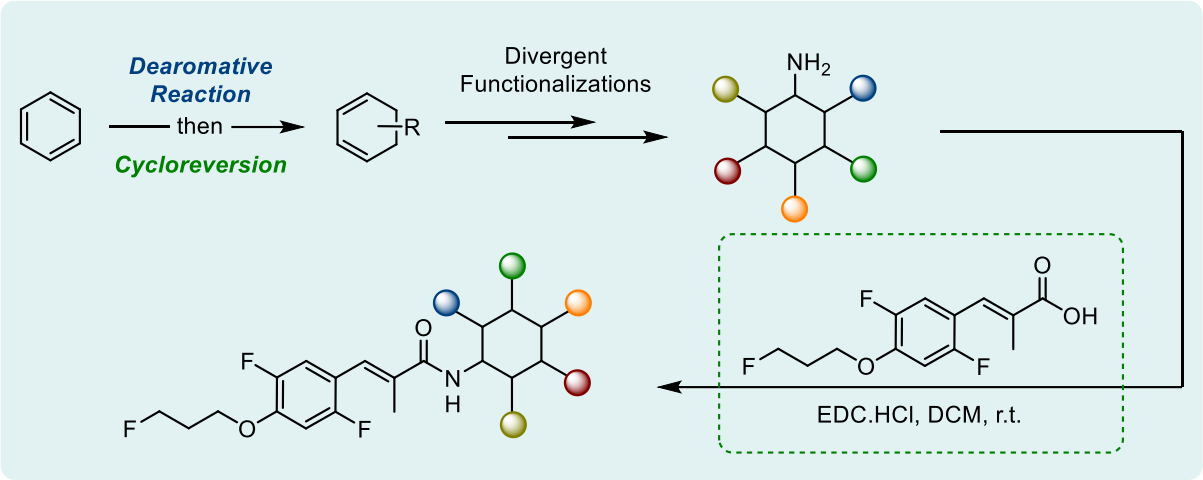
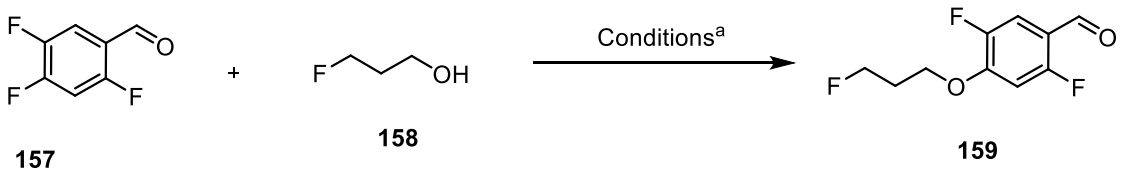


***Figure 25**: Overview of Hygromycin A analogues synthesis.*

The synthesis of lateral chain **162** was optimized, starting from commercially available 2,4,5-trifluorobenzaldehyde **157**. Initially, a nucleophilic aromatic substitution S_nAr was performed using 3-fluoro-1-propanol, to install the alkylic lateral chain.



157 **158** **159**

Entry	Alcohol eq.	Base eq.	Temperature (°C)	Yield
1	2	2	80	42%
2	3	3	80	45%
3	4	4	80	50%
4	6	2	90	82%
5	**8**	**2**	**90**	**97%**

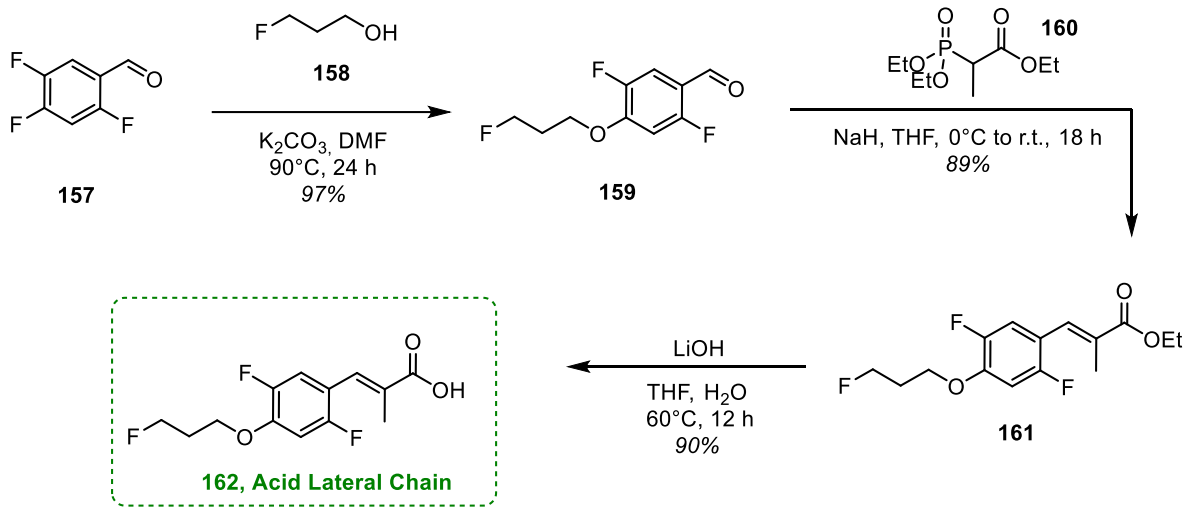
[a.] All reactions were performed on a 1 g scale using 1 eq. of **157** in DMF (0.4M) for 24h and K_2CO_3 as base.

***Table** 7: Optimization of nucleophilic aromatic substitution.*

K_2CO_3 was selected as the base, and DMF was used as solvent. A brief optimization was performed, which is reported in **Table 7**.

The most important parameter was alcohol equivalents, which was varied from 2 to 10 equivalents. Temperature was also varied, while base equivalents and reaction concentration were not changed. The best conditions are reported in entry **5**, using 8 eq. of nucleophile, 2 eq. of base, in DMF (0.4M) at 90°C for 24 h, affording the desired aldehyde product **159** with a 97% of yield.

With alkyl-substituted difluorobenzaldehyde **159** in hand, we turned our attention on the HWE reaction to install the unsaturated ester moiety. In this reaction, commercially available phosphonate **160** was used. NaH was selected as a base, performing the reaction in THF. The desired olefin **161** was obtained with high yield after a simple column chromatography purification. Finally, the desired carboxylic acid **162** was obtained with a simple hydrolysis of the ethyl ester.

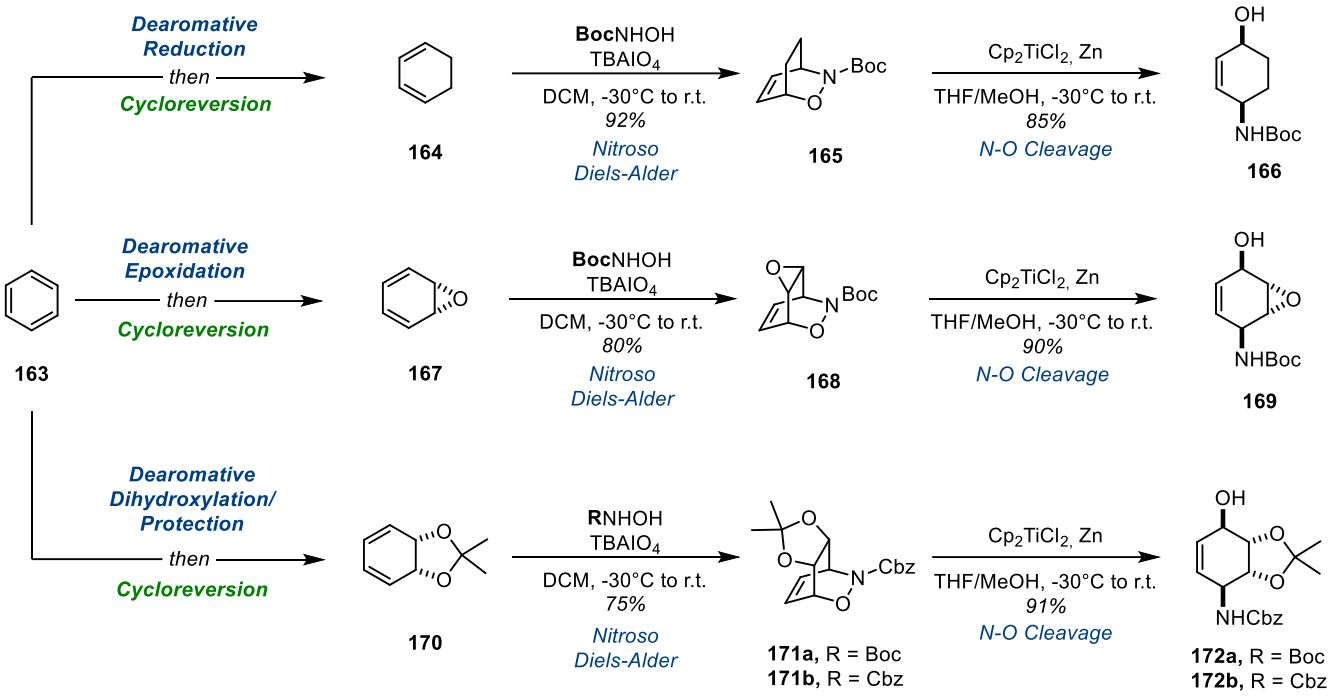


***Scheme 52**: Overview of lateral chain synthesis.*

We found that using LiOH in THF/H_2O mixture at 60°C afforded hydrolysis product with high conversion and high yields. The final optimized synthesis is reported in **Scheme 52**.

6.3.2 **Dearomative reduction products**

With the carboxylic acid **162** in hand, we focused our attention on the synthesis of the aminocyclitol fragment starting from benzene. In **Scheme 53** is reported the general strategy. We decided to employ three different dearomative strategies: a formal dearomative reduction, a dearomative epoxidation and a dearomative dihydroxylation.

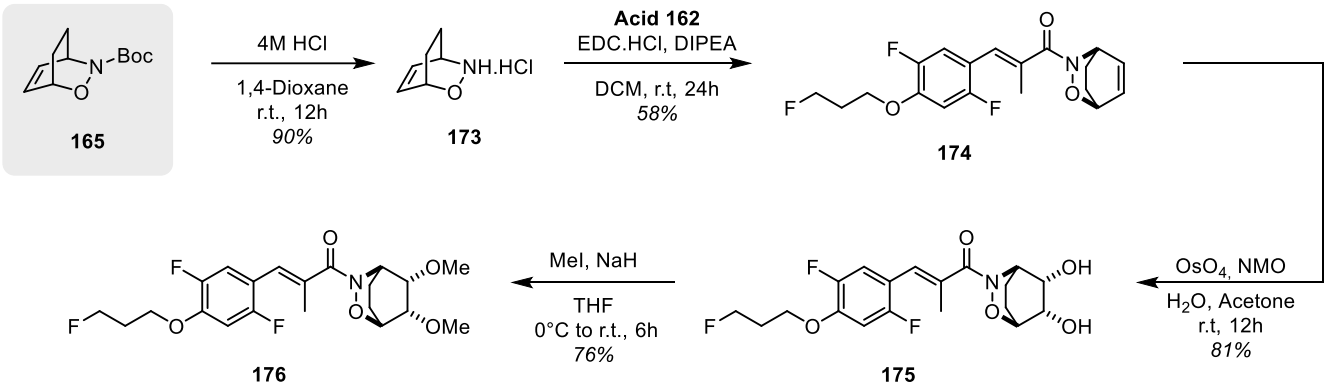


***Scheme 53**: Overview of cyclohexadiene-derivatives synthesis.*

In all cases, the corresponding intermediates were subjected to cycloreversion using optimized procedures reported in literature. Three different cyclohexadiene intermediates, **164**, **167** and **170**, were obtained.

Once we had cyclohexadiene derivatives in hand, we wanted to introduce an amine functionality, for the final amide coupling. We decided to perform a nitroso Diels-Alder reaction using a protected hydroxylamine derivatives. The nitroso group was generated in situ using tetrabutylammonium periodate as the oxidant, and the corresponding cycloadducts were obtained. At this point, the olefin could be used for further functionalizations; alternatively, the N-O bond was cleaved using mild conditions to obtain the ring-opened cyclohexene derivatives. Starting from these crucial intermediates, a wide range of aminocyclitols were obtained.

We decided to start from the simplest intermediate **165** (**Scheme 54**). The first goal was to obtain a free amine, to try the desired amide coupling with acid **162**.

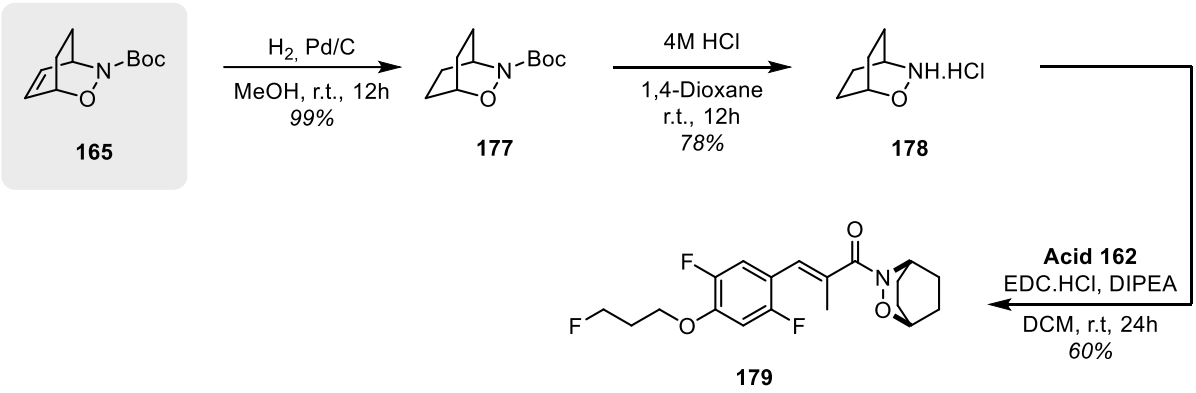


***Scheme 54**: Functionalization of intermediate **165** starting from Boc-deprotection.*

For this reason, we tried at first a simple Boc-deprotection using a 4M solution of HCl in Dioxane. We obtained the desired HCl salt of amine **173**. At this point we tried

amide coupling reaction, using carboxylic acid **162** and EDC.HCl as coupling agent, together with DIPEA as base. After 24 hours we obtained the desired amide **174**, which constitutes the first Hygromycin A analogue. Moreover, is **the first bicyclic analogue** ever synthesized in literature.

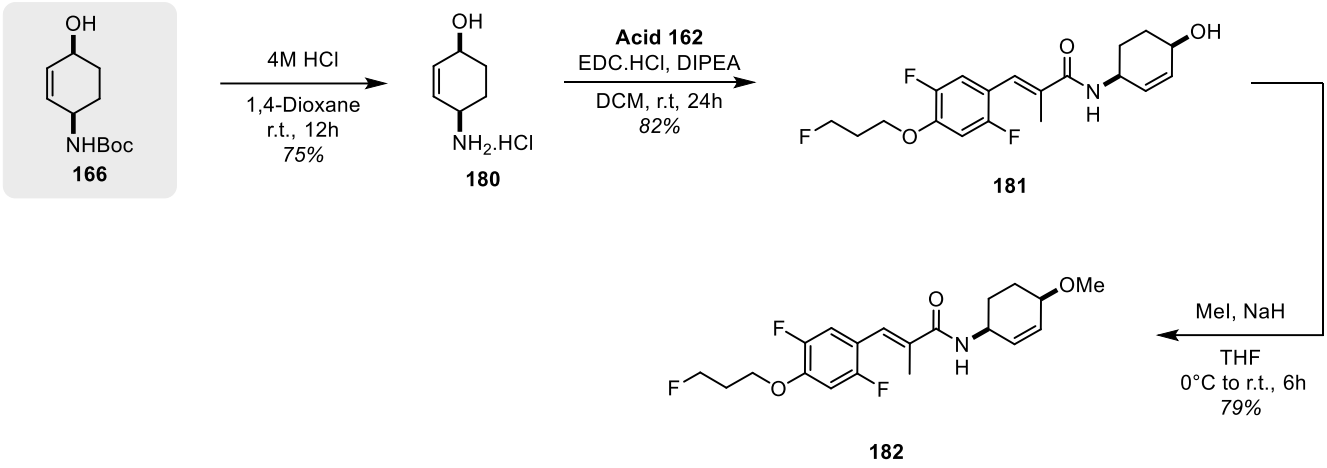
With amide **174** in hand, we wanted to functionalize further the olefin functionality. We opted for a dihydroxylation reaction which furnished the desired diol **175** with high yields. As a last step, the two hydroxyl groups were methylated using MeI and NaH in THF, affording the demethylated amide analogue **176**.



***Scheme 55**: Functionalization of intermediate **165** starting from hydrogenation.*

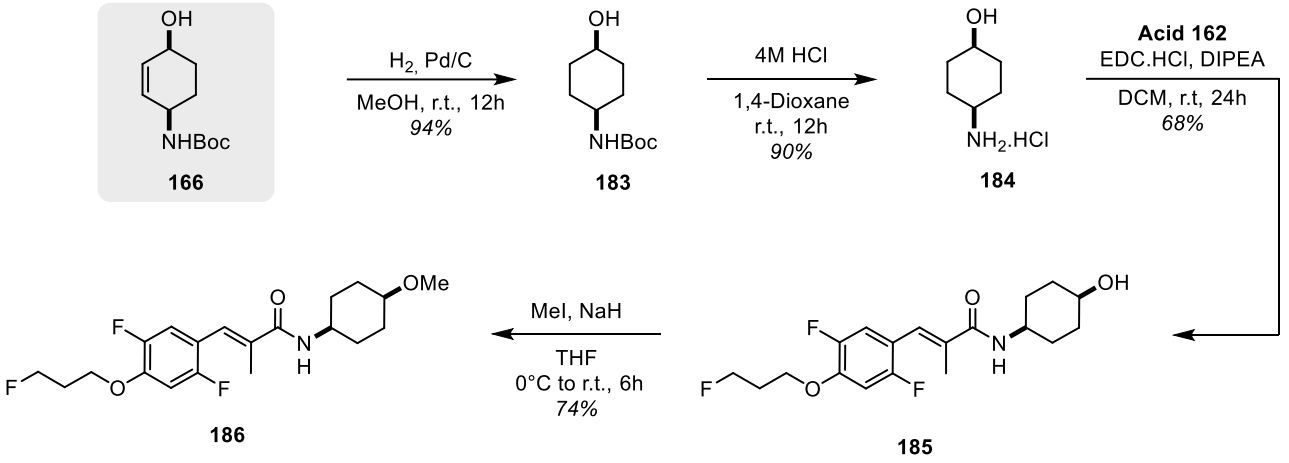
We were able to use intermediate **165** again to obtain a simpler amide analogue. We decided to simply reduce the double bond with a hydrogenation reaction, affording the saturated amine **177 (Scheme 55).** At this point, we performed the same steps as described before: Boc-deprotection, followed by amide coupling. The corresponding saturated amide **179** was obtained with high yield.

Next, we turned our attention to the ring-opened intermediate **166**, which is obtained after N-O cleavage. In the first case, as reported in **Scheme 56**, we used the same reaction steps (Boc-deprotection, amide coupling and methylation) to obtain a new Hygromycin A analogue **181** and the corresponding methylated analogue **182**.



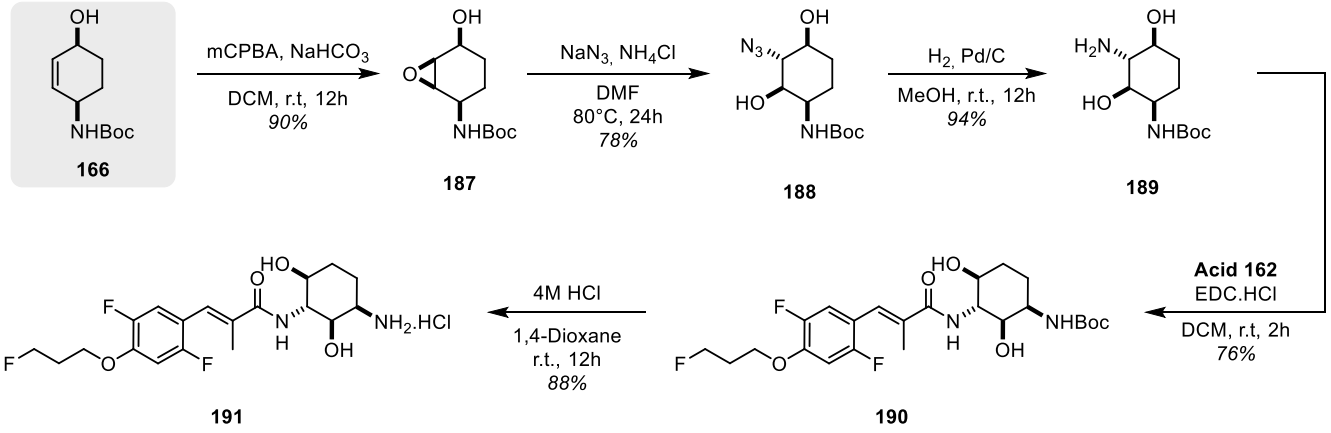
***Scheme 56**: Functionalization of intermediate **166** starting from Boc-deprotection.*

Then, we decided to perform a hydrogenation on derivative **166** to obtain saturated Boc-protected amine **183**. At this point, in just 2 steps we arrived at amide **185**, which was methylated using the same conditions to afford amide **186** (**Scheme 57**).



***Scheme 57**: Functionalization of intermediate **166** starting from hydrogenation.*

Finally, we decided to use olefin functionality in intermediate **166** with epoxidation (**Scheme 58**). *m*CPBA epoxidation was performed, with the resulting epoxide stereochemistry guided by the allylic alcohol.



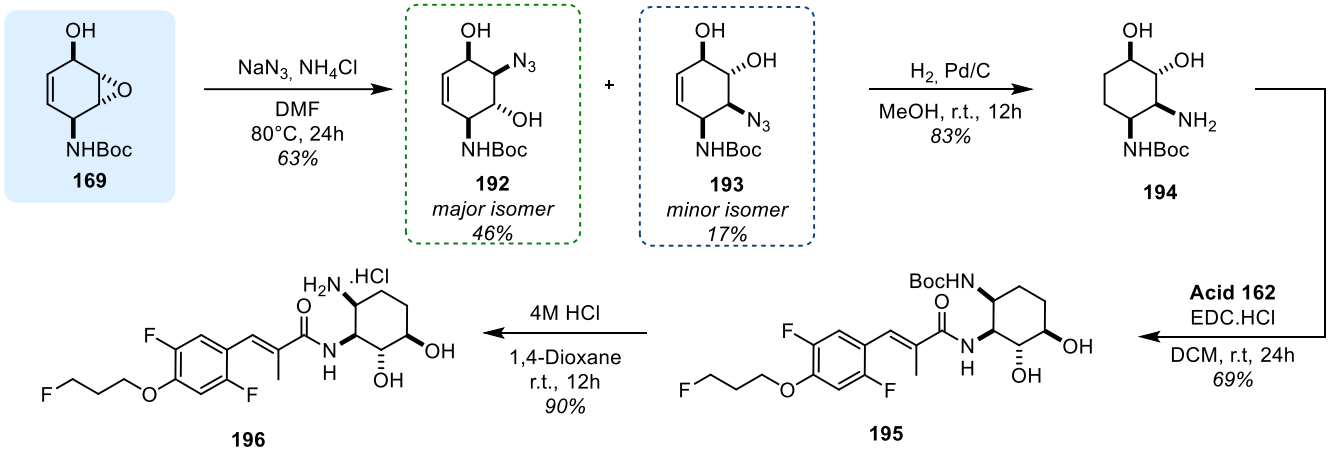
***Scheme 58**: Functionalization of intermediate **166** starting from epoxidation.*

Next, we wanted to open the epoxide to have a further functionality on that aminocyclitol. We decided to use an **azide** nucleophile: we found that treating epoxide **187** with NaN_3 and NH_4Cl at 80°C we had epoxide opening, obtaining a single regioisomer (probably due to the presence of the bulky Boc-protected amine on one side).

The azide group was then reduced with simple hydrogenation, to have a free amine group. At this point, coupling with acid **162** was performed in just 2 hours, and we obtained amide **190**. Finally, Boc-deprotection was done to obtain amide **191**, with an amine on the aminocyclitol moiety.

6.3.3 **Dearomative epoxidation products**

Next, we focused our attention on intermediate **169**. With an epoxide in hand, our first idea was to open that epoxide with the conditions already discussed (NaN_3 and NH_4Cl at 80°C) (**Scheme 59**).

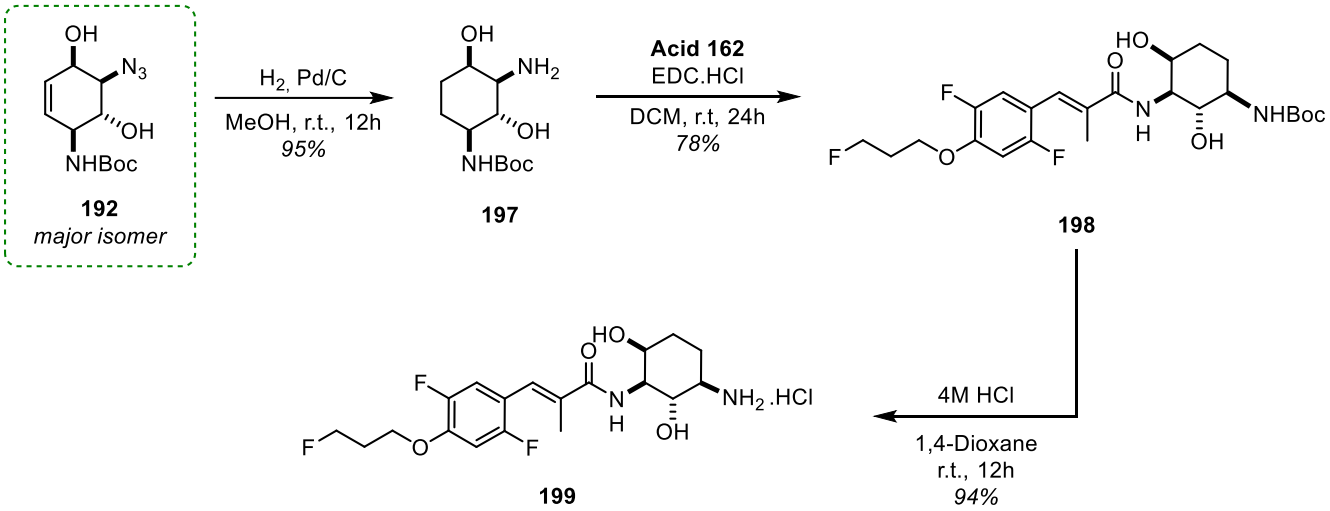


***Scheme 59**: Functionalization of intermediate **169** starting from epoxide opening.*

We tried the opening reaction, and this time we obtained both regioisomers. This is due to the different relative stereochemistry of the epoxide. At first, we took minor isomer **193** and we tried the same reaction steps. Azide group was hydrogenated, and the corresponding amine **194** was used in the coupling reaction to obtain amide **195**. Again, Boc-deprotection furnished amide **196**.

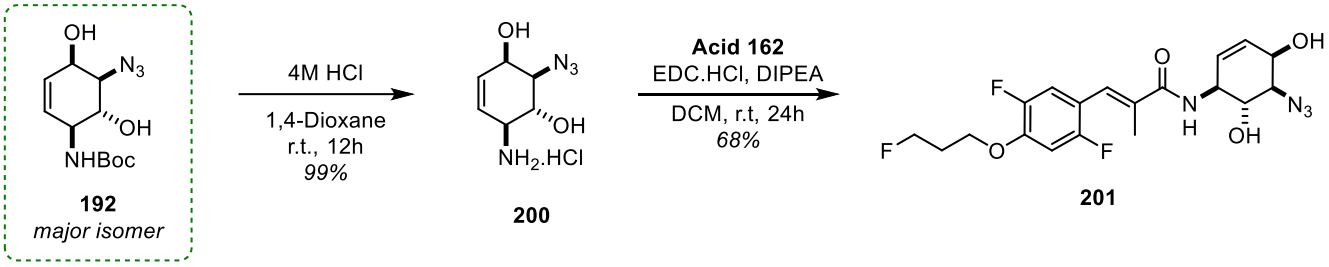
Then, we turned our attention to the major isomer. This time, we wanted to demonstrate that we can use both Nitrogen sources for the coupling reaction (azide group and Boc-protected amine).

For this reason, we started performing the same synthetic sequence, made up by hydrogenation, amide coupling and Boc-deprotection (**Scheme 60**).



***Scheme 60**: Functionalization of major isomer **192** starting from azide reduction.*

We were able to obtaine amides **198** and **199**. Next, we decided to keep the azide group, and to use the other amine functionality (**Scheme 61**).

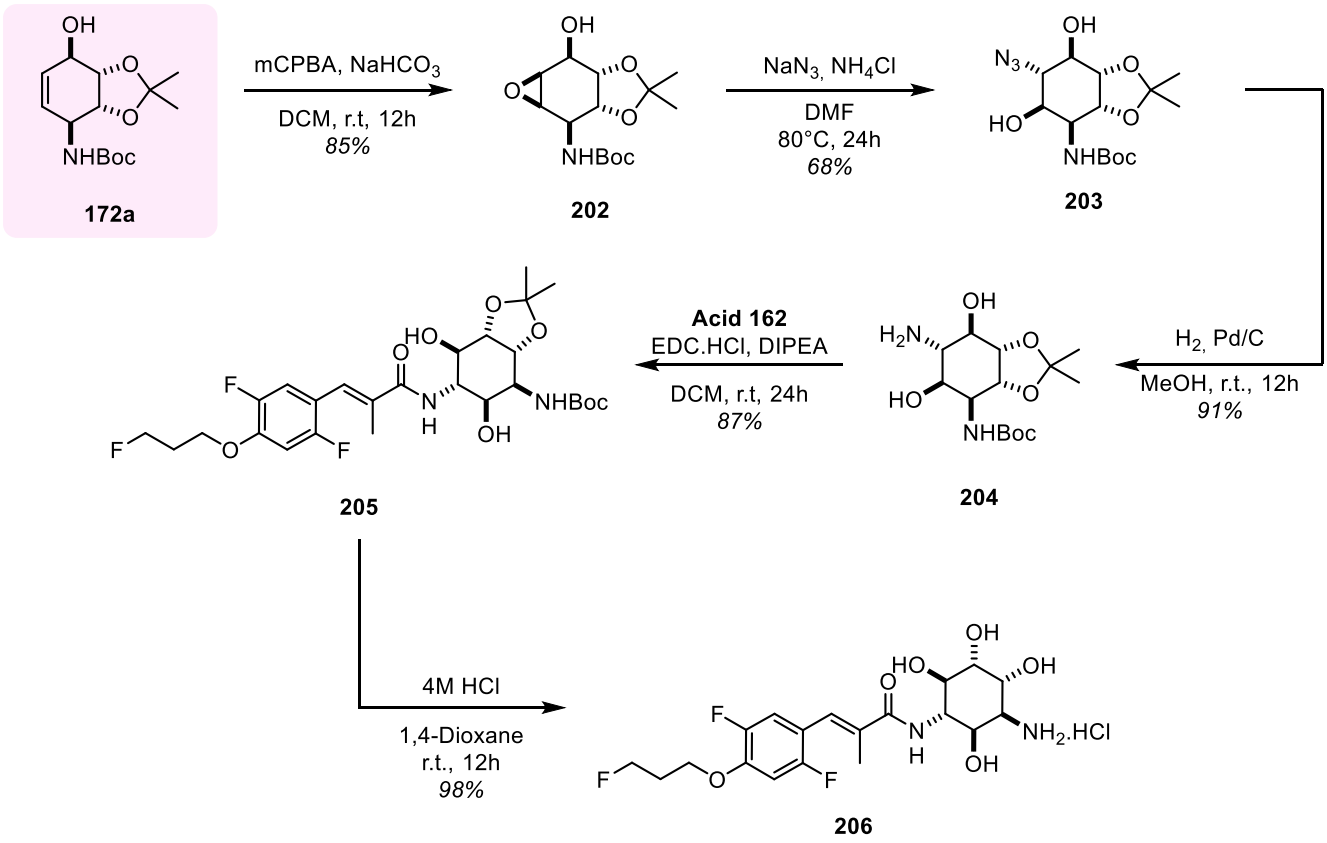


***Scheme 61**: Functionalization of major isomer **192** starting from Boc-deprotection.*

We performed a Boc-deprotetion on major isomer **192**, obtaining amine **200**. Again, we performed coupling with acid lateral chain, and we were able to obtain the desired amide **201**. In this case, we obtained an Hygromycin A analogue with an azide functionality.

6.3.4 **Dearomative dihydroxylation products**

Finally, we focused on the most substituted cyclohexadiene derivative. We started from intermediate **172a**, and we wanted to epoxidize the double bond, to have another functionality (**Scheme 63**).

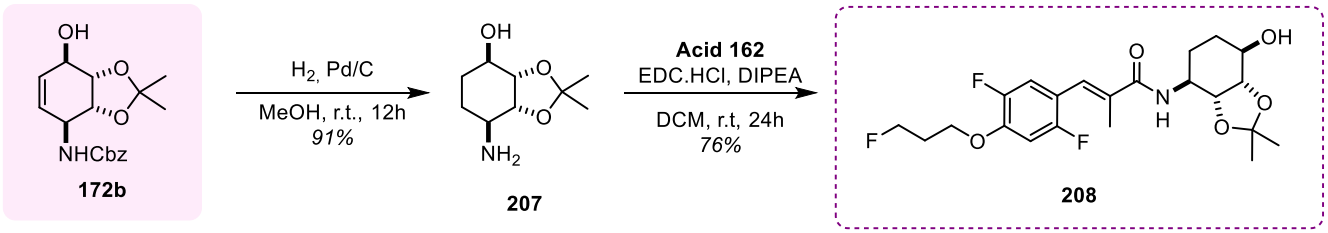


***Scheme 63**: Functionalization of intermediate **172a** starting from epoxidation.*

*m*CPBA epoxidation was performed, and the desired epoxide **202** was obtained in high yield. Then, we used the same already described synthetic pathway, performing epoxide opening, reduction and amide synthesis, to afford amide **205**. At this point, treating this intermediate with HCl in Dioxane we have both Boc and Acetal deprotection, obtaining analogue **206**. In this first reaction scheme, we used a Boc-protecting group because we had a concomitant protecting group deprotection only in the last step.

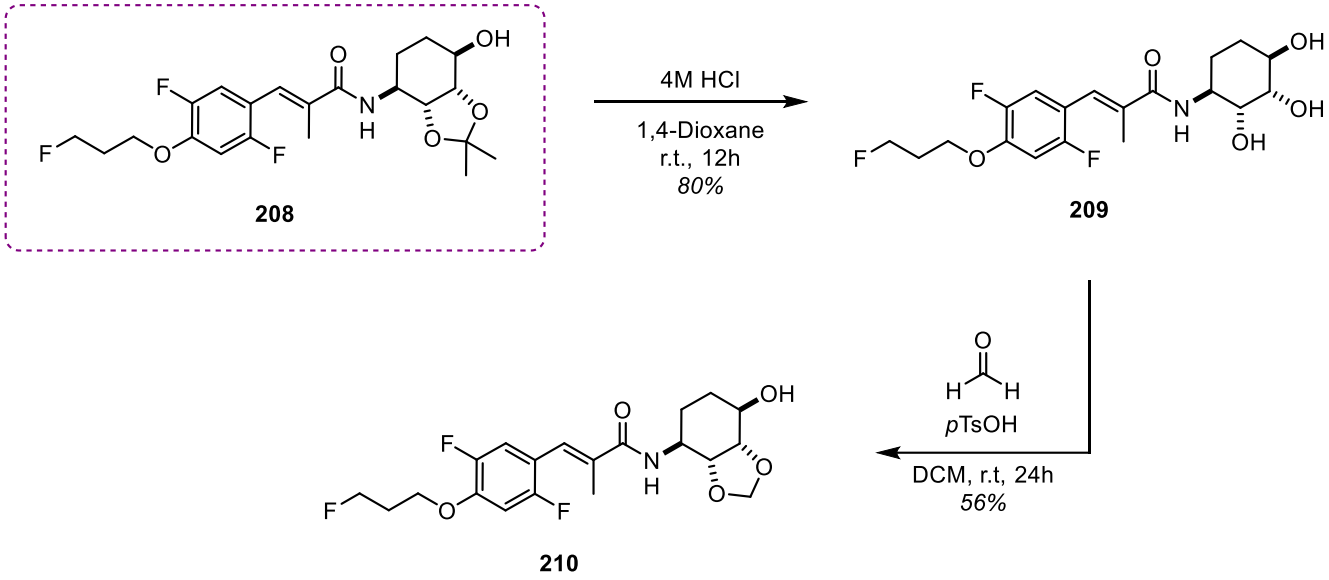
In the following synthesis, we decided to change the protecting group using Cbz group. In all these cases we wanted to use only Nitrogen functionality for the amide coupling, so we kept the acetal protecting group for polarity reasons. With a Boc-group, before amide coupling, we should have used acidic conditions for the deprotection, obtaining in most cases an amine with multiple free hydroxyl groups. These compounds proved to be really challenging to manipulate because of their polarity.

For all these reasons, we turned our attention to intermediate **172b**.



***Scheme 64**: Functionalization of intermediate **172b** starting from Cbz-deprotection.*

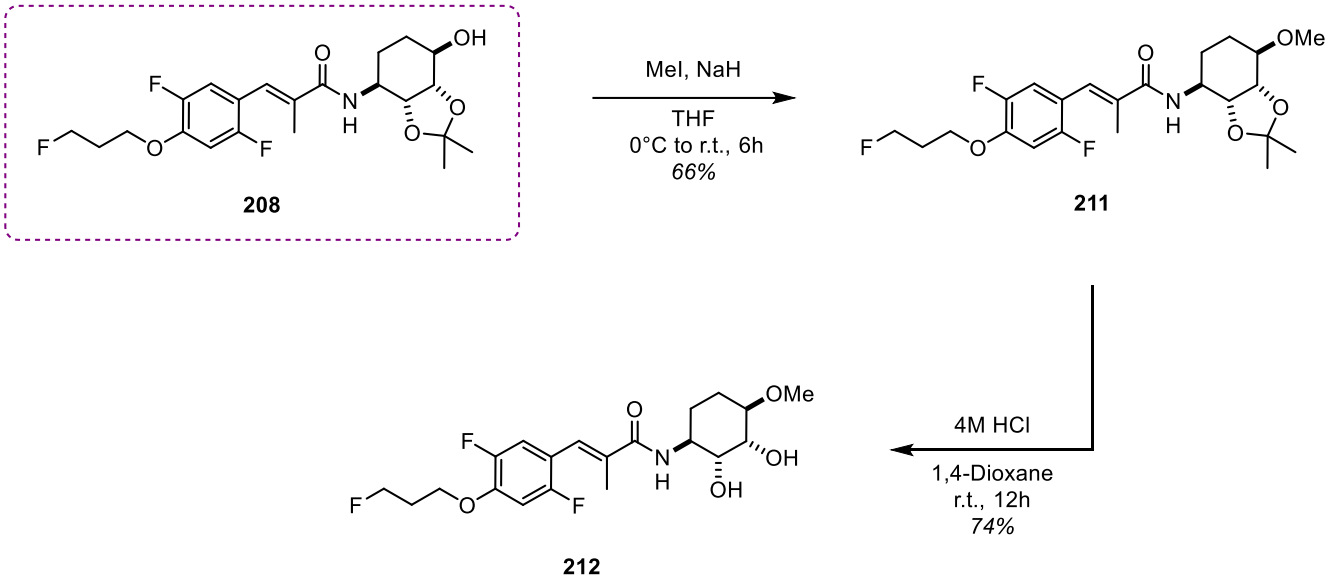
At first, we tried a simple Cbz-deprotection using hydrogenation conditions (**Scheme 64**).



***Scheme 65**: Functionalization of amide **208** starting from Acetal-deprotection.*

Obviously, the double bond is also reduced, obtaining free saturated amine **207**. Using the standard coupling conditions, amide **208** was obtained. This intermediate was used for different purposes (**Scheme 65**).

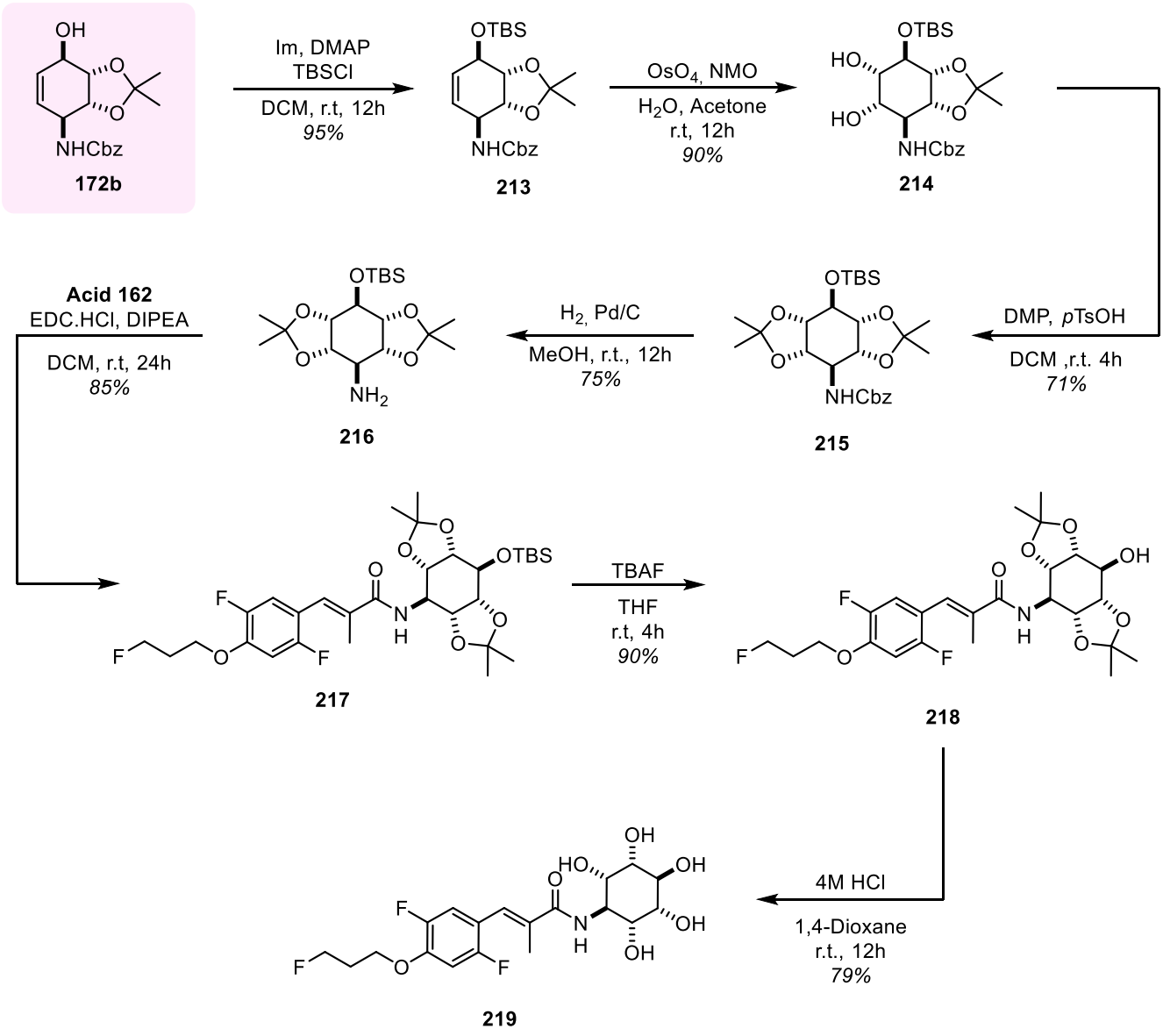
At first, acetal group was deprotected using acidic conditions, affording triol analogue **209**. Then, a different protection of diol was tried using formaldehyde in acid conditions, to obtain the methylenedioxy group (which is present in Hygromycin A) in analogue **210**.



***Scheme 66**: Functionalization of amide **208** starting from methylation.*

In another synthetic sequence, intermediate **208** was at first methylated using the standard conditions to obtain amide **211**. Finally, acetal group was removed in acid conditions to afford analogue **212**. In this case, we demonstrated that we could have different protections at different hydroxyl groups (**Scheme 66**).

In the last synthetic sequence, we decided to use intermediate **172b** to perform a dihydroxylation of the double bond (**Scheme 67**).



***Scheme 67**: Functionalization of intermediate **172b** performing a dihydroxylation strategy.*

At first, free alcohol of **172b** was protected as a TBS ether. Then, dihydroxylation was performed to obtain diol **214**, which was protected again as an acetal. Cbz group was

deprotected with hydrogenation to obtain free amine **216**, which was used for the coupling reaction to afford protected amide **217**. At this point we could deprotect selectively the TBS group, using TBAF in THF, affording analogue **218**. Moreover, with HCl, we removed both the acetals group, to have analogue **219** with five free hydroxyl groups.

7 Conclusions Part II

In this work, **25 different Hygromycin A analogues** were synthesized starting from benzene. Arenophile-mediated dearomatization strategy was used to obtain crucial 1,3-cyclohexadiene derivatives, which were functionalized using a very divergent strategy. Different aminocyclitols were obtained, and an amide coupling was performed with acid lateral chain, whose synthesis was optimized starting from readily available materials.

A great variety of functional groups were obtained in the aminocyclitol moiety: free and protected alcohols, free and protected amines, olefins, methylene fragments, azide. All the final analogues will be tested for their antibacterial activity; moreover, activity against Lyme disease will be tested as well.

Part III: Experimental Section

For the experimental part of this thesis, the following materials and instrument have been used. For the characterization of the compounds:

- NMR Bruker 400,200 MHz spectrometer;
- FTIR Perkin-Elmer Paragon 1000 PC spectrophotometer

For the chromatographic separations:

- Kieselgel 60 Merck (230-400 mesh) for the silica gel column chromatographies;
- Silica Gel GF-254 Merck (0.25 mm) for TLCs;
- Fluorescent Lamp at 254 and 366 nm;
- Vanillin, Ninidrin and $KMnO_4$ as a TLC stain;

For the melting point analyses:

- Melting Point Apparatus Fisher-Johns

For those reactions involving anhydrous conditions, inert atmosphere or air-sensitive reagents, they were conducted under nitrogen or argon atmosphere in oven-dried glassware.

The solvents used were dried by distillation using drying agents under Argon atmosphere. Tetrahydrofuran was distilled over Sodium and Benzophenone, while

Dichloromethane was distilled over Calcium Hydride. All the other solvents were used as commercially available.

Chemical shift (δ) values are reported in parts per million (ppm) from the tetramethylsilane (TMS) used as internal standard. Coupling constants (J) are expressed in Hz. The following terminology was used to report the spectra: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet.

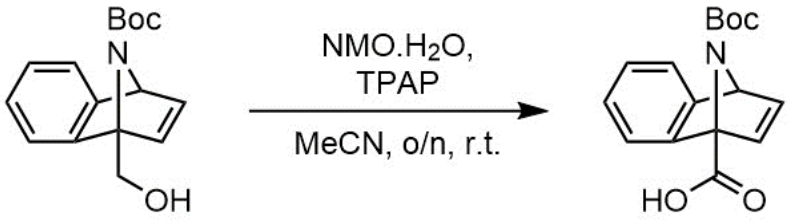
Abbreviations Used:

- DCM = dichloromethane
- THF = tetrahydrofuran
- Hex = hexane
- EtOAc = ethyl acetate
- MeCN = acetonitrile
- MeOH = methanol
- DMF = dimethylformamide
- Et_2O = diethyl ether
- tBuOH = *tert*-butyl alcohol
- tBuOAc = *tert*-butyl acetate
- MtBE = *tert*-butyl methyl ether
- Boc_2O = di-*tert*-butyl dicarbonate
- DMAP = 4-dimethylaminopyridine
- Im = imidazole
- $NaBH_4$ = sodium borohydride
- TBSCl = tert-butyl-dimethylsilyl chloride
- TIPSCl = triisoproprylsilyl chloride
- TBAF = tetra-n-butylammonium fluoride
- TPAP = tetrapropylammonium perruthenate

- TFA = trifluoroacetic acid
- NMO = N-methylmorpholine N-oxide
- DMP = Dess-Martin periodinane
- DMSO = dimethyl sulfoxide
- NMO = N-methylmorpholine N-oxide
- HCl = hydrochloric acid
- $HClO_4$ = perchloric acid
- Pd/C = palladium on carbon
- Na_2SO_4 = sodium sulphate
- $NaHCO_3$ = sodium bicarbonate
- Boc = di-*tert*-butyl dicarbonyl group
- TBS = *tert*-butyldimethylsilyl
- TIPS = triisopropylsilyl
- r.t. = room temperature
- o/n = over night
- R_f = retention factor
- TLC = thin layer chromatography
- HPLC = high-performance liquid chromatography
- UPLC-MS = ultra-performance liquid chromatography-mass spectrometry

8 Synthesis of Proline Analogues

8.1 α-proline Analogue



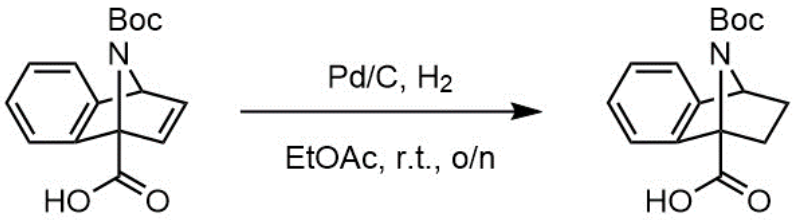
9-(tert-butoxycarbonyl)-1,4-epiminonaphthalene-1(4H)-carboxylic acid: Tert-butyl (1S,4S)-1-(hydroxymethyl)-1,4-dihydro-1,4-epiminonaphthalene-9-carboxylate was prepared starting from pyrrole 2-carboxaldehyde according to a known literature procedure [59]. To a solution of alcohol (1.00 g, 3.66 mmol, 1.0 eq.) and N-methyl morpholine N-oxide (NMO) monohydrate (2.49 g, 18.3 mmol,5.0 equiv.) in 37 ml of acetonitrile (0.1M), TPAP (129 mg, 0.366 mmol, 0.1 eq.) is added and the mixture is stirred at room temperature for 12 hours. On completion the reaction is quenched with an excess of 2-propanol. The solvent is evaporated, and the residue is filtered over a pad of silica using ethyl acetate containing 1% of acetic acid. The filtrate is concentrated in vacuo and the crude product is subjected to column chromatography on silica gel (Hexane:Ethyl Acetate = 1:1) to afford carboxylic acid (0.80 g, 76%) as a colorless oil.

R_f 0.40 (SiO_2, Hex:EtOAc 7:3, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (dd, *J* = 5.4, 3.0 Hz, 1H), 7.24 - 7.19 (m, 1H), 7.09 (d, *J* = 5.4 Hz, 1H), 6.99 - 6.89 (m, 3H), 5.49 (d, *J* = 2.7 Hz, 1H), 1.35 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 147.3, 146.9, 125.9, 125.6, 121.1, 121.0, 83.7, 67.9, 60.5, 28.3, 28.0, 14.2.

IR (ATR, neat, cm^{-1}) 2976, 1701, 1368, 1346, 1318, 1295, 1249, 1154, 1098.



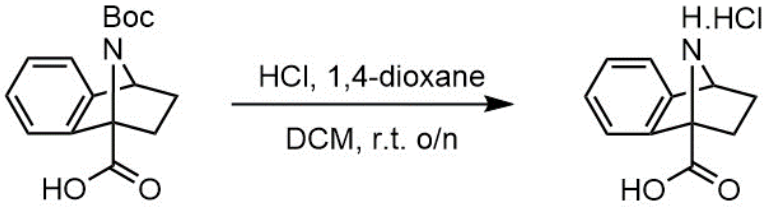
9-(tert-butoxycarbonyl)-3,4-dihydro-1,4-epiminonaphthalene-1(2H)-carboxylic acid: Unsaturated carboxylic acid (750 mg, 2.61 mmol, 1.0 eq.) was solubilized in 25 mL of MeOH (0.1M). The mixture was purged with Ar, then Pd/C (10% Pd on C, 278 mg, 0.10 eq.) was added to the mixture. The flask was filled with H_2 gas using a double balloon, and the reaction mixture was stirred for 18 h at room temperature under an atmosphere of H_2. Once complete, the mixture was filtered over a pad of celite, using MeOH as the eluent. The solvent was evaporated under reduced pressure, and the resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 7:3) to afford pure saturated carboxylic acid (700 mg, 93%) as a colorless oil.

R_f 0.33 (SiO_2, Hex:EtOAc 7:3, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.18 (bs, 1H), 7.51 - 7.46 (m, 1H), 7.21 - 7.17 (m, 1H), 7.16 - 7.11 (m, 2H), 5.15 (d, *J* = 4.5 Hz, 1H), 2.49 - 2.41 (m, 1H), 2.25 - 2.14 (m, 1H), 1.69 - 1.60 (m, 1H), 1.35 - 1.26 (m, 10H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.5, 143.6, 143.3, 127.3, 127.0, 120.1, 119.6, 82.5, 63.3, 32.1, 28.4, 28.0, 27.1.

IR (ATR, neat, cm^{-1}) 3049, 1705, 1366, 1325, 1250, 1157, 1120, 900, 840.



3,4-dihydro-1,4-epiminonaphthalene-1(2H)-carboxylic acid hydrochloride: Boc-protected derivative (500 mg, 1.73 mmol, 1.0 eq.) was solubilized in 5 mL of DCM (0.15 M). The mixture was purged with Ar, then HCl (4M in Dioxane, 8.64 mL, 20 eq.) was added to the mixture. The reaction mixture was stirred for 18 h at room temperature under an atmosphere of Ar. Once complete, the mixture was filtered under vacuum, using DCM as the eluent. The corresponding hydrochloride salt was obtained (250 mg, 76%) as a white solid.

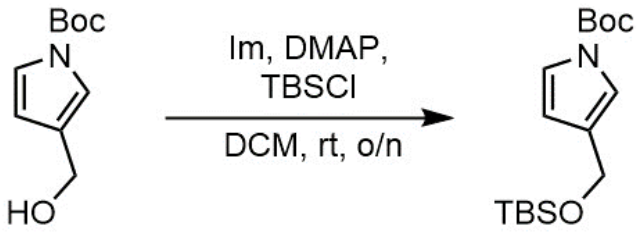
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 - 7.53 (m, 1H), 7.49 - 7.44 (m, 1H), 7.43 - 7.38 (m, 2H), 5.24 (m, 1H), 2.50 - 2.40 (m, 2H), 1.94 - 1.84 (m, 1H), 1.72 - 1.62 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 138.7, 137.8, 129.3, 129.0, 121.4, 120.2, 74.9, 60.9, 28.1, 25.1.

IR (ATR, neat, cm^{-1}) 3395, 2853, 1733, 1593, 1459, 1407, 1360, 1321, 1250.

m.p. 194-196 °C.

8.2 β-proline Analogue



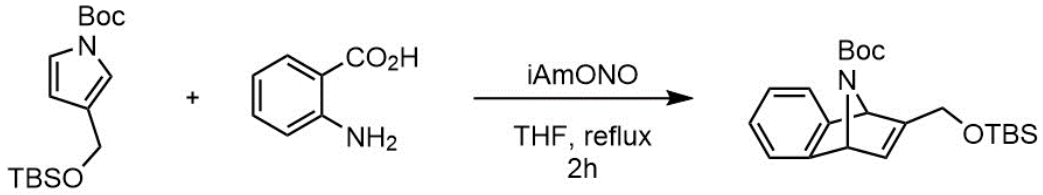
tert-butyl 3-(((tert-butyldimethylsilyl)oxy)methyl)-1H-pyrrole-1-carboxylate: Tert-butyl 3-(hydroxymethyl)-1H-pyrrole-1-carboxylate was prepared according to a known literature procedure [149]. To a solution of alcohol (5.00 g, 25.4 mmol, 1.0 eq.) and imidazole (3.40 g, 50.7 mmol, 2.0 eq.) in 60 mL of DCM (0.4 M) was added *tert*-butylchlorodimethylsilane (5.73 g, 38.0 mmol, 1.5 eq.) at room temperature. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. The reaction mixture was extracted with DCM, quenched with sat. aq. $NaHCO_3$ solution and washed with water. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 95:5) to afford protected alcohol (6.40 g, 81%) as a yellow liquid.

R_f 0.69 (SiO_2, Hex:EtOAc 95:5, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (t, *J* = 2.6 Hz, 1H), 7.04 (m, 1H), 6.09 (dd, *J* = 3.3, 1.7 Hz, 1H), 4.49 (m, 2H), 1.49 (s, 9H), 0.83 (s, 9H), 0.00 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.9, 127.8, 120.3, 117.0, 111.4, 83.4, 59.0, 28.0, 26.0, 18.4, -5.2.

IR (ATR, neat, cm^{-1}) 1742, 1474, 1409, 1347, 1246, 1157, 1064, 971, 835, 770.



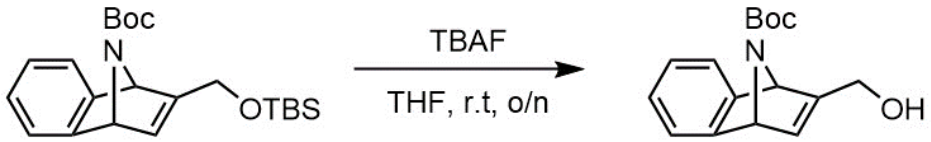
tert-butyl-2-(((tert-butyldimethylsilyl)oxy)methyl)-1,4-dihydro-1,4-epiminonaphthalene-9 carboxylate: Solutions of anthranilic acid (6.60 g, 48.2 mmol, 3.0 eq.) in tetrahydrofuran (96 mL, 0.5 M) and isopentyl nitrite (7.01 mL, 52.2 mmol, 3.25 eq.) in tetrahydrofuran (104 mL, 0.5 M) were added dropwise and simultaneously to a refluxing solution of tert-butyl 3-(((tert-butyldimethylsilyl)oxy)methyl)-1H-pyrrole-1-carboxylate (5.00 g, 16.1 mmol, 1.0 eq.) in tetrahydrofuran (16 mL, 1 M) over a period of about 2 h. The solution was refluxed for further 2 h, it was cooled to room temperature, aqueous sodium bicarbonate was added. The organic solvent was evaporated, and the aqueous phase was extracted with Et_2O. The organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 95:5 to 9:1) to afford the corresponding cycloadduct (3.90 g, 63%) as a yellow oil.

R_f 0.52 (SiO_2, Hex:EtOAc 9:1, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 - 7.20 (m, 2H), 6.97 - 6.91 (m, 2H), 6.56 (s, 1H), 5.44 (s, 1H), 5.30 (s, 1H), 4.40 - 4.27 (m, 2H), 1.36 (s, 9H), 0.88 (s, 9H), 0.00 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 155.2, 148.8, 148.1, 129.5, 125.1, 124.7, 120.9, 120.6, 114.4, 80.5, 66.8, 60.8, 30.9, 28.2, 25.9, 18.4, -5.4.

IR (ATR, neat, cm^{-1}) 1707, 1455, 1366, 1329, 1250, 1164, 1075, 835, 761.



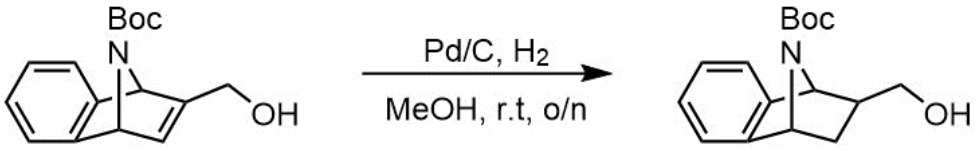
tert-butyl 2-(hydroxymethyl)-1,4-dihydro-1,4-epiminonaphthalene-9-carboxylate: To a solution of protected alcohol (2.50 g, 6.45 mmol, 1.0 eq.) in 55 mL of anhydrous THF (0.1 M) under Ar, was added tetra n-butylammonium fluoride (TBAF, 1M in THF, 9.67 mL, 9.67 mmol, 1.5 eq.) dropwise at room temperature and the mixture was stirred for 6 h. Once complete, the mixture was extracted with EtOAc and washed with water. The organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 95:5 to 9:1) to afford the free alcohol (1.65 g, 94%) as a yellow oil.

R_f 0.46 (SiO_2, Hex:EtOAc 6:4, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 - 7.21 (m, 1H), 7.19 - 7.14 (m, 1H), 6.93 - 6.85 (m, 2H), 6.58 (s, 1H), 5.40 (s, 1H), 5.32 (s, 1H), 4.24 (m, 2H), 1.69 (br s, 1H), 1.30 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 155.4, 148.6, 148.0, 129.5, 125.3, 125.0, 120.7, 120.2, 114.6, 80.8, 77.2, 66.9, 60.2, 28.2.

IR (ATR, neat, cm^{-1}) 3403, 1701, 1684, 1366, 1327, 1250, 1157, 857, 792.



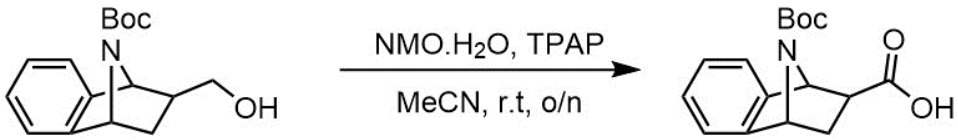
tert-butyl 2-(hydroxymethyl)-1,2,3,4-tetrahydro-1,4-epiminonaphthalene-9-carboxylate: Unsaturated alcohol (1.00 g, 3.66 mmol, 1.0 eq.) was solubilized in 35 mL of MeOH (0.1M). The mixture was purged with Ar, then Pd/C (10% Pd on C, 388 mg, 0.10 eq.) was added to the mixture. The flask was filled with H_2 gas using a double balloon, and the reaction mixture was stirred for 18 h at room temperature under an atmosphere of H_2. Once complete, the mixture was filtered over a pad of celite, using MeOH as the eluent. The solvent was evaporated under reduced pressure, and the resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 7:3) to afford pure saturated alcohol (0.92 g, 92%) as a colorless oil and as a single isomer.

R_f 0.33 (SiO_2, Hex:EtOAc 7:3, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 - 7.20 (m, 1H), 7.17 - 7.11 (m, 1H), 7.10 - 7.03 (m, 2H), 5.12 - 5.05 (m, 1H), 4.97 (d, J = 4.7 Hz, 1H), 3.15 (q, J = 4.4, 4.3 Hz, 1H), 2.66 - 2.54 (m, 2H), 2.20 (ddd, J = 11.5, 9.5, 4.8 Hz, 1H), 1.93 (br s, 1H), 1.30 (s, 9H), 0.65 (dd, J = 11.5, 3.7 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 155.3, 145.3, 141.2, 126.9, 126.3, 121.7, 119.4, 80.2, 64.1, 63.2, 61.2, 41.0, 30.6, 28.2.

IR (ATR, neat, cm^{-1}) 3432, 1700, 1675, 1364, 1254, 1162, 1090, 1051, 1023, 905.



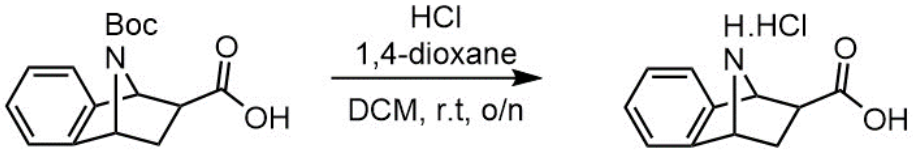
9-(tert-butoxycarbonyl)-1,2,3,4-tetrahydro-1,4-epiminonaphthalene-2-carboxylic acid: Alcohol (450 mg, 1.63 mmol, 1.0 eq.) and N-methyl morpholine N-oxide monohydrate (2.23 g, 16.3 mmol, 10.0 equiv.) are dissolved in 15 ml of acetonitrile (0.1 M). TPAP (57 mg, 0.16 mmol, 0.1 eq.) is added and the mixture is stirred at room temperature. On completion the reaction is quenched with an excess of 2-propanol. The solvent is evaporated, and the residue is filtered over a pad of silica using ethyl acetate containing 1% of acetic acid. The filtrate is concentrated in vacuo and the resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 8:2 to 7:3) to afford pure saturated alcohol (400 mg, 85%) as a pale yellow oil.

R_f 0.43 (SiO_2, Hex:EtOAc 7:3, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.19 - 7.17 (m, 1H), 7.15 - 7.09 (m, 2H), 7.06 - 7.02 (m, 1H), 5.22 (d, J = 4.6 Hz, 1H), 5.06 (d, J = 4.4 Hz, 1H), 3.35 - 3.30 (m, 1H), 2.30 - 2.23 (m, 1H), 1.59 (dd, J = 12.1, 4.0 Hz, 1H), 1.32 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 176.7, 154.8, 144.9, 140.6, 127.4, 126.5, 121.6, 119.5, 80.8, 62.8, 61.6, 44.4, 30.5, 28.2.

IR (ATR, neat, cm^{-1}) 3070, 1675, 1520, 1480, 1420, 1385, 1162, 920, 830.



1,2,3,4-tetrahydro-1,4-epiminonaphthalene-2-carboxylic acid hydrochloride: Boc-protected derivative (250 mg, 0.86 mmol, 1.0 eq.) was solubilized in 3 mL of DCM (0.15 M). The mixture was purged with Ar, then HCl (4M in Dioxane, 4.32 mL, 20 eq.) was added to the mixture. The reaction mixture was stirred for 18 h at room temperature under an atmosphere of Ar. Once complete, the mixture was filtered under vacuum, using DCM as the eluent. The corresponding hydrochloride salt was obtained (125 mg, 76%) as a white solid.

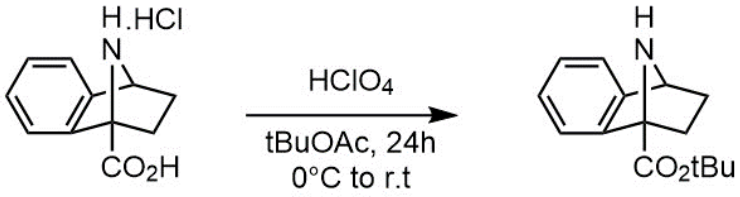
^{1}H NMR (400 MHz, D_2O) δ 7.45 -7.39 (m, 2H), 7.38 - 7.30 (m, 2H), 5.39 (d, *J* = 4.8 Hz, 1H), 5.27 (d, *J* = 4.6 Hz, 1H), 3.72 (ddd, *J* = 10.1, 4.9, 4.1 Hz, 1H), 2.52 (ddd, *J* = 13.4, 10.1, 4.7 Hz, 1H), 1.83 (dd, *J* = 13.4, 4.1 Hz, 1H).

^{13}C NMR (101 MHz, D_2O) δ 173.3, 139.1, 135.2, 129.7, 128.8, 122.9, 121.1, 62.5, 62.2, 41.9, 27.5.

IR (ATR, neat, cm^{-1}) 3090, 1700, 1614, 1347, 1213, 1161, 854, 766.

m.p. 214-215 °C.

8.3 **Enalapril Analogues**



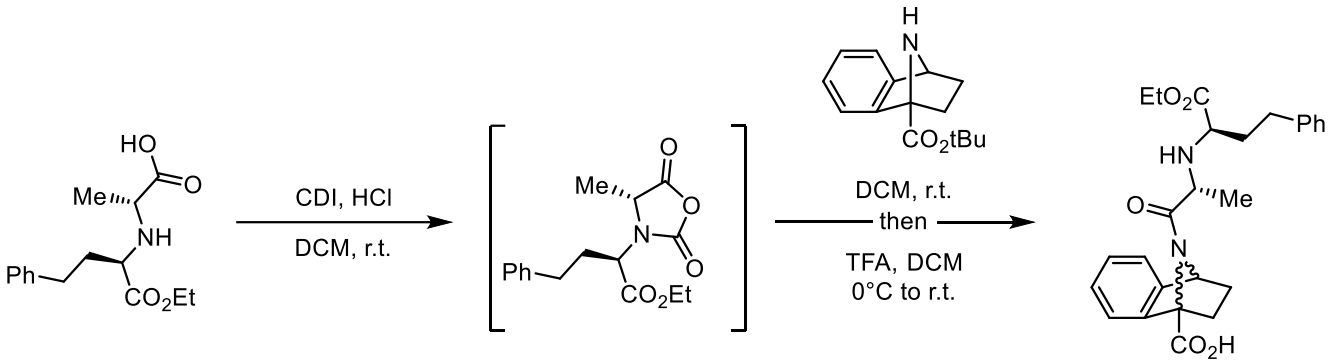
tert-butyl-3,4-dihydro-1,4-epiminonaphthalene-1(2H)-carboxylate: Free amino acid (250 mg, 1.11 mmol, 1.0 eq.) was solubilized in 11 mL of *t*BuOAc (0.1M). Under stirring, 0.14 mL (1.44 mmol, 1.3 eq.) of $HClO_4$ (65% in H_2O) were added slowly at 0 °C. The mixture was then stirred at room temperature for 24 h. After adjusting the pH-value to 6 by addition of solid $NaHCO_3$, the excess of $NaHCO_3$ was filtered off and the solution was concentrated under reduced pressure. The residue was dissolved in 30% aqueous NH_3 and the solution extracted three times with M*t*BE. The combined organic phases were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 7:3) to afford *t*-butyl ester (150 mg, 55%) as a colorless liquid.

R_f 0.48 (SiO_2, Hex:EtOAc 3:7, UV-Vis, Ninidrin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 - 7.25 (m, 1H), 7.17 - 7.12 (m, 1H), 7.09 - 7.03 (m, 2H), 4.48 (d, J = 3.8 Hz, 1H), 2.37 (s, 1H), 2.18 - 2.07 (m, 2H), 1.55 - 1.44 (m, 10H), 1.25 - 1.17 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 148.1, 146.8, 126.7, 126.1, 119.4, 118.9, 81.9, 72.9, 60.4, 30.4, 28.2, 24.5.

IR (ATR, neat, cm^{-1}) 2976, 1733, 1457, 1368, 1323, 1263, 1161, 929, 843.



9-(((R)-1-ethoxy-1-oxo-4-phenylbutan-2-yl)-D-alanyl)-3,4-dihydro-1,4-epiminonaphthalene-1(2H)-carboxylic acid:N-[(S)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanine (344 mg, 279.34 g/mol, 1.23 mmol, 3.0 eq.) is solubilized in anhydrous DCM (2.50 mL, 0.5 M). CDI (200 mg, 162.15 g/mol, 1.23 mmol, 3.0 eq.) is added, followed by addition of HCl (4 M in anhydrous 1,4-dioxane, 53 µL, 36.45 g/mol, 0.21 mmol, 0.5 eq.). The mixture is stirred for 10 minutes at room temperature, then *tert*-butyl ester analogue (100 mg, 245.32 g/mol, 0.41 mmol, 1.0 eq.) is added as a solution of DCM (205 µL, 2 M). The mixture is stirred for 48 hours at room temperature, monitoring with TLC. Once complete, TFA (630 µL, 1.49 g/mL, 935 mg, 114.02 g/mol, 8.2 mmol, 20 eq.) is added at 0 °C, and the reaction is stirred for further 2 hours at room temperature. The solvent is then removed under reduced pressure, and the crude product is purified via column chromatography on silica gel (DCM:MeOH = 98:2 to 95:5) to afford Enalapril bicyclic analogues as a 1:1 mixture of separable diastereomers (72%).

Isomer A

R_f 0.50 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (dd, J = 5.4, 3.2 Hz, 1H), 7.24 (dd, J = 5.8, 2.9 Hz, 1H), 7.18 – 7.00 (m, 7H), 5.49 (d, J = 4.3 Hz, 1H), 4.24 – 4.13 (m, 2H), 3.62 (q, J = 7.0 Hz, 1H), 3.47 (dd, J = 8.6, 4.4 Hz, 1H), 2.61 – 2.52 (m, 3H), 2.46 – 2.36 (m, 1H), 2.22 – 2.08 (m, 2H), 1.81 (ddd, J = 11.8, 8.6, 3.5 Hz, 1H), 1.47 (d, J = 7.0 Hz, 3H), 1.42 (ddd, J = 11.0, 8.6, 3.1 Hz, 1H), 1.22 (t, J = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7, 164.8, 162.2, 143.6, 142.1, 140.4, 128.6, 128.2, 127.5, 127.2, 126.3, 119.8, 119.5, 66.8, 61.5, 60.6, 59.8, 58.2, 32.6, 32.6, 30.2, 27.5, 20.4, 14.2

IR (ATR, neat, cm^{-1}) 3021, 1738, 1659, 1447, 1320, 1250, 1204, 1124, 1088, 1035, 935, 861, 757, 701

m.p. 170 – 172 °C.

Isomer B

R_f 0.35 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.29 (m, 1H), 7.26 – 7.19 (m, 3H), 7.16 – 7.10 (m, 5H), 5.47 (d, J = 3.7 Hz, 1H), 4.22 – 4.17 (m, 2H), 3.68 (q, J = 7.1 Hz, 1H), 3.54 (dd, J = 9.1, 5.4 Hz, 1H), 2.74 – 2.51 (m, 4H), 2.46 – 2.38 (m, 1H), 2.18 – 2.15

(m, 2H), 1.43 – 1.41 (m, 1H), 1.37 – 1.35 (m, 1H), 1.23 (t, J = 7.2 Hz, 3H), 1.04 (d, J = 7.0 Hz, 3H).

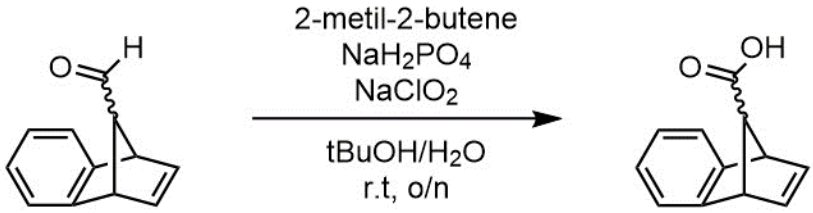
^{13}C NMR (101 MHz, $CDCl_3$) δ 169.6, 165.6, 165.4, 144.6, 143.8, 140.5, 128.7, 128.7, 128.6, 128.3, 127.4, 127.1, 126.4, 120.4, 118.5, 67.7, 61.5, 61.1, 60.0, 59.6, 33.0, 32.6, 30.2, 26.1, 18.8, 14.2.

IR (ATR, neat, cm^{-1}) 3024, 1736, 1664, 1452, 1210, 1030, 867, 759.

m.p. 166 – 168 °C.

9 Synthesis of Phenylalanine Analogues

9.1 γ-Phenylalanine Analogue



1,4-dihydro-1,4-methanonaphthalene-9-carboxylic acid: To a solution of aldehyde[150] (5.00 g, 29.4 mmol, 1.0 eq.) in 500 mL of *t*BuOH (0.05 M) and 500 mL of H_2O (0.05 M) at room temperature, 2-methyl-2-butene (94 mL, 881 mmol, 30 eq.) was added, together with $NaH_2PO_4.2H_2O$ (68.8 g, 441 mmol, 15 eq.) and $NaClO_2$ (26.6 g, 294 mmol, 10 eq.). The reaction mixture was stirred for 12 h at room temperature. The reaction was quenched with the addition of sat. $NaHCO_3$ solution. The aqueous phase was extracted with DCM, and the organic layers were combined. Then, 1M NaOH was added, and the aqueous phase was washed with DCM. Finally, the aqueous layer was acidified with 1M HCl, and it was extracted with DCM. The organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to afford carboxylic acid (4.50 g, 82%) as a white solid and as a 3/2 mixture of inseparable isomers.

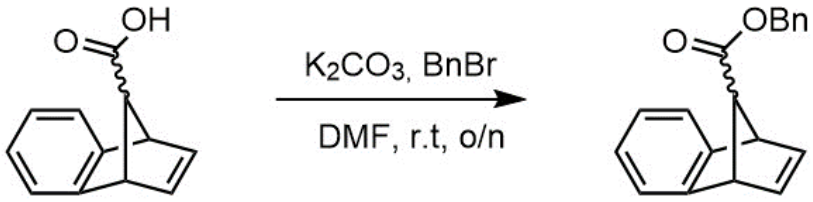
R_f 0.29 (SiO_2, Hex:EtOAc 8:2, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.79 (brs, 1H), 7.10 - 7.07 (m, 2H), 7.03 - 6.99 (m, 2H), 6.64 - 6.61 (m, 2H), 3.53 - 3.51 (m, 2H), 2.74 - 2.72 (m, 1H), 2.05 - 2.02 (m, 2H), 1.15 - 1.11 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 178.0, 146.7, 126.1, 120.6, 61.9, 45.3, 25.2.

IR (ATR, neat, cm^{-1}) 3051, 1698, 1414, 1258, 1174, 949, 915, 837.

m.p. 153 – 156 °C.



Benzyl-1,4-dihydro-1,4-methanonaphthalene-9-carboxylic acid: To a solution of carboxylic acid (2.00 g, 10.7 mmol, 1.0 eq.) in 30 mL of DMF (0.35 M) at room temperature, K_2CO_3 (2.97 g, 21.5 mmol, 2 eq.) was added, together with benzyl bromide (1.53 mL, 12.9 mmol, 1.2 eq.). The reaction mixture was stirred for 18 h at room temperature. The reaction was quenched with the addition of water, and the aqueous phase was extracted with Et_2O. The organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 95:5) to afford benzyl ester (2.60 g, 88%) as a white solid and as a mixture of isomers.

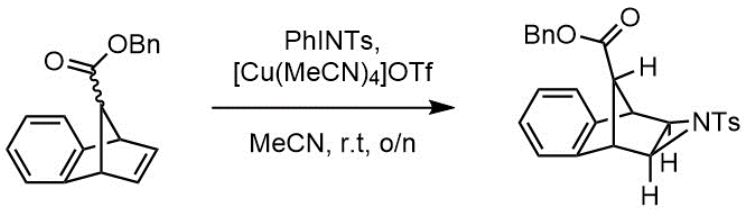
R_f 0.32 (SiO_2, Hex:EtOAc 95:5, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 - 7.21 (m, 5H), 7.14 - 7.09 (m, 2H), 6.87 - 6.83 (m, 2H), 6.65 - 6.63 (m, 2H), 5.01 (s, 2H), 4.13 - 4.11 (m, 2H), 3.27 - 3.25 (m, 1H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 149.8, 141.0, 136.1, 128.6, 128.2, 128.0, 124.9, 121.8, 80.8, 66.3, 52.0.

IR (ATR, neat, cm^{-1}) 1724, 1694, 1450, 1213, 1180, 1012, 937, 890, 743, 699.

m.p. 84 – 88 °C.



benzyl-1-tosyl-1a,2,7,7a-tetrahydro-1H-2,7-methanonaphtho[2,3-b]azirine-8-carboxylate: To a solution of olefin (1.00 g, 4.99 mmol, 1.0 eq.) in 50 mL of MeCN (0.10 M) at room temperature, PhINTs (2.24 g, 5.99 mmol, 1.2 eq.) was added, together with Cu catalyst (188 mg, 0.49 mmol, 0.1 eq.). The reaction mixture was stirred for 18 h at room temperature. Once complete, it was filtered over a pad of silica gel using EtOAc as the eluent, and the solvent was evaporated. The resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 8:2) to afford aziridinated bicycle (0.70 g, 38%) as a light yellow solid and as a single isomer.

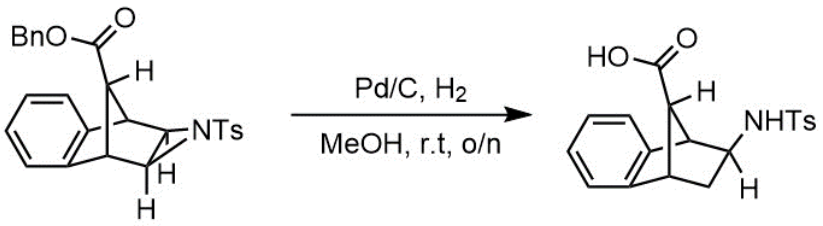
R_f 0.24 (SiO_2, Hex:EtOAc 8:2, UV-Vis, Vanillin, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 - 7.72 (m, 2H), 7.33 - 7.31 (m, 2H), 7.17 - 7.13 (m, 5H), 7.04 - 7.01 (m, 2H), 6.90 - 6.87 (m, 2H), 4.75 (s, 2H), 3.71 (s, 2H), 3.23 (d, J = 1.8 Hz, 1H), 3.13 (s, 2H), 2.36 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 144.8, 143.9, 135.6, 135.1, 131.5, 130.4, 129.9, 128.4, 128.0, 127.9, 127.7, 127.1, 123.6, 66.2, 55.4, 46.1, 46.0, 21.7.

IR (ATR, neat, cm^{-1}) 1726, 1597, 1347, 1327, 1213, 1194, 1153, 1086, 1016, 990, 889, 725, 695.

m.p. 120 – 123 °C.



2-((4-methylphenyl)sulfonamido)-1,2,3,4-tetrahydro-1,4-methanonaphthalene-9-carboxylic acid: Unsaturated carboxylic acid (450 mg, 1.01 mmol, 1.0 eq.) was solubilized in 10 mL of EtOAc (0.1M). The mixture was purged with Ar, then Pd/C (10% Pd on C, 106 mg, 0.10 eq.) was added to the mixture. The flask was filled with H_2 gas using a double balloon, and the reaction mixture was stirred for 24 h at room temperature under an atmosphere of H_2. Once complete, the mixture was filtered over a pad of celite, using EtOAc as the eluent. The solvent was evaporated under reduced pressure, and the ring-opened carboxylic acid was obtained (340 mg, 94%) as a white foam.

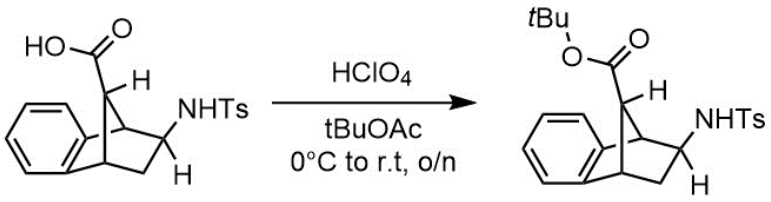
R_f 0.44 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 7.76 - 7.74 (m, 2H), 7.40 - 7.38 (m, 2H), 7.13 - 6.92 (m, 4H), 3.50 - 3.49 (d, J = 3.7 Hz, 1H), 3.37 (s, 1H), 3.18 - 3.17 (m, 1H), 3.08 (dd, *J*= 8.1, 3.7 Hz, 1H), 2.44 (s, 3H), 1.70 (dt, *J*= 12.8, 3.7, 3.7 Hz, 1H), 1.58 (dd, *J*= 12.8, 8.1 Hz, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 173.9, 145.8, 143.5, 142.3, 137.9, 129.4, 126.8, 126.7, 126.2, 121.9, 121.2, 60.6, 55.0, 52.4, 45.2, 36.6, 20.1.

IR (ATR, neat, cm^{-1}) 1701, 1687, 1342, 1159, 1087, 993, 894, 814, 777, 723.

m.p. 173 – 177 °C.



tert-butyl(1R,2S,4S,9S)-2-((4-methylphenyl)sulfonamido)-1,2,3,4-tetrahydro-1,4-methanonaphthalene-9-carboxylate: a solution of carboxylic acid (500 mg, 1.40 mmol, 1.0 eq.) in 14 mL *t*BuOAc (0.1M) was prepared. Under stirring, 0.2 mL (1.82 mmol, 1.3 eq.) of $HClO_4$ (65% in H_2O) were added slowly at 0 °C. The mixture was then stirred at room temperature for 24 h. After adjusting the pH-value to 6 by addition of solid $NaHCO_3$, the excess of $NaHCO_3$ was filtered off and the solution was concentrated under reduced pressure. The residue was dissolved in 30% aqueous NH_3 and the solution extracted three times with M*t*BE. The combined organic phases were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 7:3) to afford *t*-butyl ester (318 mg, 55%) as a white solid.

R_f 0.38 (SiO_2, Hex:EtOAc 7:3, UV-Vis, Vanillin).

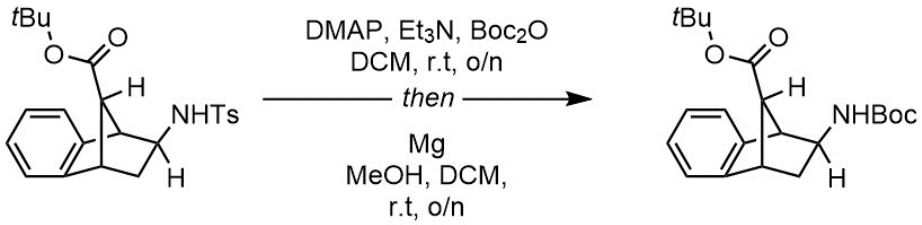
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.3 Hz, 2H), 7.18 - 7.07 (m, 4H), 5.38 (d, J = 6.5 Hz, 1H), 3.50 - 3.46 (m, 2H), 3.28 - 3.24 (m, 1H),

3.06 - 3.04 (m, 1H), 2.44 (s, 3H), 1.78 - 1.73 (m, 1H), 1.69 - 1.64 (m, 1H), 1.14 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 145.7, 143.7, 142.2, 137.2, 129.9, 127.2, 126.9, 126.4, 122.4, 121.5, 80.5, 62.1, 54.9, 52.7, 45.7, 37.6, 27.6, 21.6.

IR (ATR, neat, cm^{-1}) 3250, 2970, 1715, 1446, 1334, 1148, 1088, 813.

m.p. 132 – 134 °C.



tert-butyl (1R,2S,4S,9S)-2-((tert-butoxycarbonyl)amino)-1,2,3,4-tetrahydro-1,4-methanonaphthalene-9-carboxylate: Tosyl protected amino acid (1.74g, 4.21mmol, 1.0 eq.) was solubilized in 42 mL of DCM (0.1M). Triethylamine was added to the mixture (1.76 mL, 12.60 mmol, 3.0 eq.), followed by Boc anhydride (3.40 mL, 14.70 mmol, 3.5 eq.). The mixture was stirred for 12 h, and the solvent was evaporated under reduced pressure. The resulting residue was solubilized in a mixture of 10 mL of MeOH and 20 mL of DCM (0.1M). Magnesium turnings (1.06g, 43.60 mmol, 20 eq.) were added, and the mixture was sonicated for 1 hour. It was stirred at room temperature for 24 hours, then saturated NH_4Cl was added. The aqueous phase was extracted with DCM, the organic phases were reunited, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 9:1 to 8:2) to afford detosylated product (1.00 g, 67%) as a white solid.

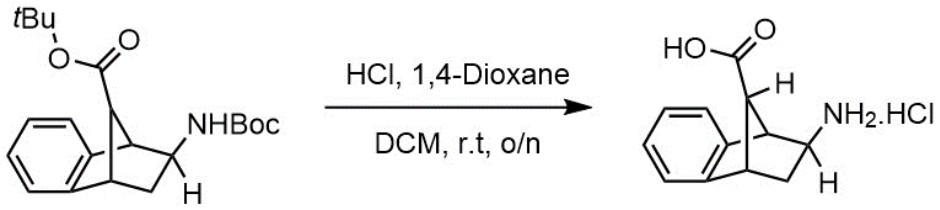
R_f 0.36 (SiO_2, Hex:EtOAc 9:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 - 7.18 (m, 1H), 7.10 - 6.98 (m, 3H), 4.68 (d, J = 6.5 Hz, 1H), 3.57 - 3.45 (m, 3H), 2.89 (s, 1H), 1.86 - 1.81 (m, 1H), 1.51 - 1.38 (m, 10H), 1.08 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2, 145.7, 142.7, 126.6, 126.3, 122.6, 121.3, 80.5, 62.3, 52.8, 52.5, 45.8, 37.5, 28.4, 27.7.

IR (ATR, neat, cm^{-1}) 3373, 2978, 1692, 1520, 1360, 1237, 1155, 730.

m.p. 128 °C.



(1R,2S,4S,9S)-2-amino-1,2,3,4-tetrahydro-1,4-methanonaphthalene-9-carboxylic acid hydrochloride: Boc-protected derivative (850 mg, 2.36 mmol, 1.0 eq.) was solubilized in 20 mL of DCM (0.15 M). The mixture was purged with Ar, then HCl (4M in Dioxane, 11.8 mL, 20 eq.) was added to the mixture. The reaction mixture was stirred for 18 h at room temperature under an atmosphere of Ar. Once complete, the mixture was filtered under vacuum, using DCM as the eluent. The corresponding hydrochloride salt was obtained (243 mg, 43%) as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 7.32 - 7.29 (m, 1H), 7.26 - 7.23 (m, 1H), 7.19 - 7.12 (m, 2H), 3.74 (s, 1H), 3.70 - 3.68 (m, 1H), 3.35 (t, J = 1.6 Hz, 1H), 3.22 (dd, J = 8.0, 3.9 Hz, 1H), 1.96 - 1.83 (m, 2H).

^{13}C NMR (101 MHz, D_2O) δ 175.2, 144.8, 141.0, 127.9, 127.1, 122.8, 122.1, 66.5, 60.3, 52.1, 49.8, 45.5, 34.0.

IR (ATR, neat, cm^{-1}) 2929, 1725, 1528, 1222, 1185, 920, 823, 697

m.p. Decomposition after 250°C

9.2 Carfilzomib Analogues

Amide Couplings

To a solution of amine (1.0 eq.) in DCM (0.1M) the carboxylic acid (1.0eq.) is added. Then EDC.HCl (1.5 eq.) and DMAP (0.1 eq) are added. The reaction is stirred until full conversion of the reagents. Once complete, the solvent is evaporated under reduced pressure to obtain the crude product, which is purified via flash column chromatography on silica gel.

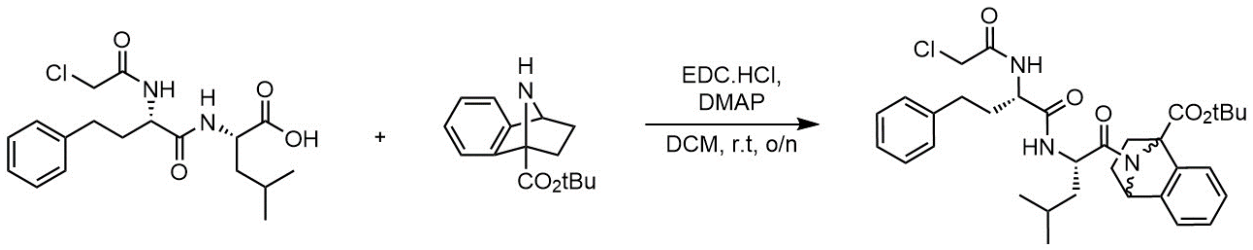
In Carfilzomib synthesis, the characterization data of all the intermediates is consistent with that reported in literature[151].

tert-Butyl esters synthesis

A solution of carboxylic acid (1.0 eq.) in *t*BuOAc (0.1M) was prepared. Under stirring, HClO4 (65% in H_2O, 1.3eq.) were added slowly at 0 °C. The mixture was then stirred at room temperature for 24 h. After adjusting the pH-value to 6 by addition of solid $NaHCO_3$, the excess of $NaHCO_3$ was filtered off and the solution was concentrated under reduced pressure. The residue was dissolved in 30% aqueous NH_3 and the solution extracted three times with M*t*BE. The combined organic phases were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product can be used without further purification.

Boc-deprotection of chiral epoxy ketone

To a solution of epoxyketone fragment (1.0 eq.) in DCM (0.2 M), was added trifluoroacetic acid (1.0 M) dropwise at 0°C. The reaction mixture was then warmed to room temperature and stirred for 2 h. The volatiles were evaporated and the crude salt was used in the next step without further purification[152].



tert-butyl 9-(((S)-2-(2-chloroacetamido)-4-phenylbutanoyl)-L-leucyl)-3,4-dihydro-1,4-epiminonaphthalene-1(2H)-carboxylate: To a solution of t-Bu protected phenylalanine analogue (400 mg, 1.63 mmol, 1.0 eq.) in 8 mL DCM (0.2 M) the carboxylic acid (842 mg, 2.28 mmol, 1.4 eq.) was added. Then EDC.HCl (469mg, 2.45mmol, 1.5 eq.) and DMAP (50mg, 0.40mmol, 0.25eq.) were added to the mixture. The reaction was then stirred at room temperature for 24 h.

The solvent was removed under reduce pressure and the crude was purified by flash column chromatography on silica gel (Hexane:Ethyl Acetate = 7:3) to afford the two

diastereomeric products (isomer A 94 mg, 13%, light-yellow oil, and isomer B 390 mg, 43%, white solid, *dr* 1:4).

Isomer A

R_f 0.38 (SiO_2, Hex:EtOAc 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 - 7.06 (m, 5H), 7.02 - 6.98 (m, 3H), 6.93 - 6.83 (m, 2H), 6.74 (d, J = 9.0 Hz, 1H), 5.36 (d, J = 4.3 Hz, 1H), 4.78 - 4.73 (m, 1H), 4.31 - 4.26 (m, 1H), 3.91 - 3.80 (m, 2H), 2.55 - 2.26 (m, 4H), 2.08 - 1.98 (m, 1H), 1.86 - 1.77 (m, 1H), 1.62 - 1.48 (m, 13H), 1.38 - 1.31 (m, 1H), 0.90 (dd, J = 13.2, 6.2 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 169.9, 167.6, 165.9, 143.8, 143.5, 140.7, 128.5, 128.2, 126.9, 126.1, 122.0, 121.3, 118.9, 81.9, 71.2, 62.6, 53.0, 49.2, 48.4, 42.3, 42.2, 34.7, 32.1, 31.4, 30.9, 28.4, 28.0, 24.7, 22.9, 22.4.

IR (ATR, neat, cm^{-1}) 3280, 2955, 1733, 1640, 1524, 1259, 1162, 1121, 1080, 730.

Isomer B

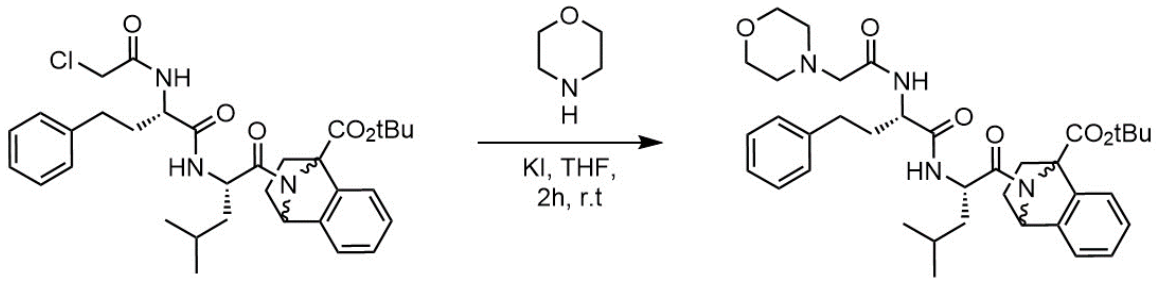
R_f 0.25 (SiO_2, Hex:EtOAc 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 - 7.06 (m, 11H), 5.34 - 5.28 (m, 1H), 4.79 - 4.70 (m, 1H), 4.51 - 4.38 (m, 1H), 3.99 - 3.93 (m, 2H), 2.68 - 2.54 (m, 2H), 2.41 - 2.32 (m, 1H), 2.20 - 2.06 (m, 2H), 2.00 - 1.91 (m, 1H), 1.58 - 1.33 (m, 14H), 0.90 - 0.74 (m, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 171.4, 170.4, 170.4, 167.5, 167.4, 166.0, 165.9, 144.1, 142.8, 142.7, 140.6, 140.4, 128.6, 128.4, 128.4, 128.4, 128.3, 127.3, 126.3, 119.2, 119.2, 119.1, 82.0, 82.0, 72.5, 72.5, 62.9, 62.8, 53.2, 53.1, 49.0, 48.9, 42.5, 42.4, 42.3, 41.9, 34.4, 34.1, 31.6, 31.5, 30.3, 30.1, 28.0, 27.6, 27.4, 24.7, 24.6, 22.9, 22.74, 22.3, 22.2

IR (ATR, neat, cm^{-1}) 3280, 2955, 1733, 1640, 1524, 1259, 1162, 1121, 1080, 730.

m.p. 71-72 °C.



tert-butyl 9-(((S)-2-(2-morpholinoacetamido)-4-phenylbutanoyl)-L-leucyl)-3,4-dihydro-1,4-epiminonaphthalene-1(2H)-carboxylate: A solution of tBu ester (138mg, 0,23 mmol, 1eq.) in 2.31mL THF (0.1M) was prepared. Then morpholine (0.08mL, 0.93mmol, 4eq.) and potassium iodide (KI, 80mg, 0.46mmol, 2eq.) were added. The reaction was stirred at room temperature for 2 hours. Then, the solvent was reduced under pressure. The crude was purified via flash column chromatography (Hexane:Ethyl Acetate 3:7) to afford the morpholine adduct as colorless oil (100mg, 66%).

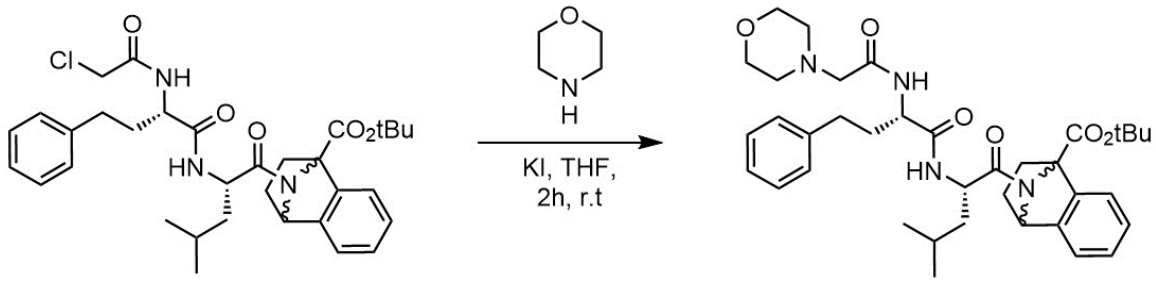
Due to the presence of rotamers, peaks of all rotamers were reported in both ^{1}H- and ^{13}C-NMR.

R_f 0.41 (SiO_2, Hex:EtOAc 3:7, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 - 7.61 (m, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.21 - 6.99 (m, 14H), 6.61 (d, J = 8.7 Hz, 1H), 6.56 (d, J = 8.1 Hz, 1H), 5.34 (d, J = 4.3 Hz, 1H), 5.21 (s, 1H), 4.73 - 4.68 (m, 1H), 4.50 - 4.44 (m, 1H), 4.41 - 4.35 (m, 2H), 3.66 - 3.63 (m, 4H), 3.60 (t, J = 4.6 Hz, 4H), 2.91 - 2.89 (m, 2H), 2.85 (d, J = 4.5 Hz, 2H), 2.62 - 2.23 (m, 15H), 2.17 - 2.02 (m, 2H), 1.96 - 1.75 (m, 2H), 1.64 - 1.32 (m, 24H), 0.90 (dd, J = 15.7, 6.3 Hz, 6H), 0.84 (t, J = 6.6 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 171.2, 170.9, 170.4, 169.9, 169.8, 167.5, 143.9, 143.7, 141.0, 140.9, 128.5, 128.4, 128.3, 127.0, 126.9, 126.1, 121.4, 119.0, 81.8, 81.8, 71.2, 66.9, 66.9, 62.7, 61.9, 61.8, 53.8, 53.7, 53.5, 52.3, 52.2, 51.5, 49.2, 42.4, 41.3, 34.5, 34.3, 31.9, 31.8, 31.7, 28.5, 28.0, 24.9, 24.7, 23.0, 22.8, 22.5, 22.0.

IR (ATR, neat, cm^{-1}) 3276, 2955, 1729, 1643, 1509, 1263, 1114, 1013, 864, 745.



tert-butyl 9-(((S)-2-(2-morpholinoacetamido)-4-phenylbutanoyl)-L-leucyl)-3,4-dihydro-1,4-epiminonaphthalene-1(2H)-carboxylate: A solution of tBu ester (400mg, 0.67mmol, 1eq.) in 6.71mL THF (0.1M) was prepared. Then morpholine (0.23mL, 2.68mmol, 4eq.) and potassium iodide (KI, 223mg, 1.34mmol, 2eq.) were added. The reaction was stirred at room temperature for 2 hours. Then, the solvent was reduced under pressure. The crude was purified via flash column chromatography (Hexane:Ethyl Acetate 3:7 to 2:8) to afford the morpholine adduct as colorless oil (350mg, 80%).

Due to the presence of rotamers, peaks of all rotamers were reported in both ^{1}H- and ^{13}C-NMR.

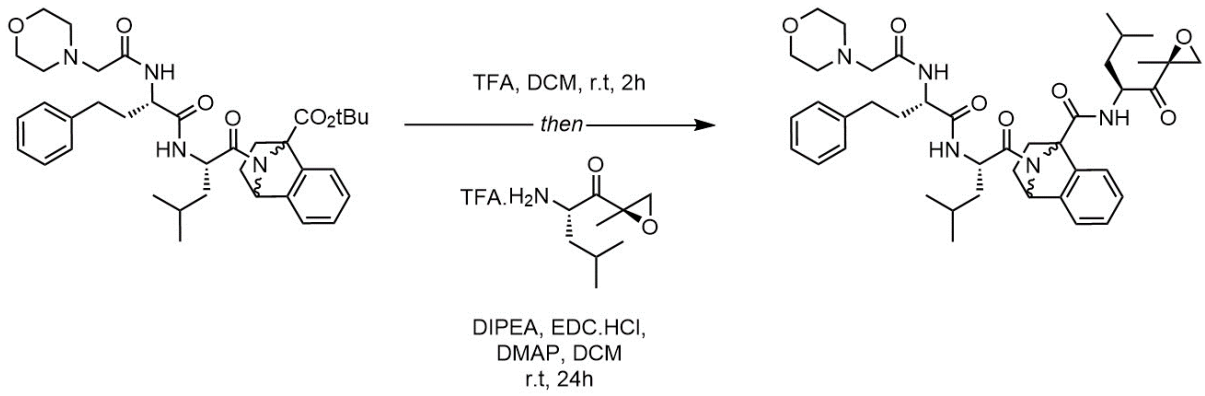
R_f 0.27 (SiO_2, Hex:EtOAc 3:7, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 7.31 - 7.29 (m, 1H), 7.24 - 7.10 (m, 8H), 5.48 (d, J = 2.6 Hz, 1H), 4.70 - 4.63 (m, 1H), 4.48 - 4.41 (m, 1H), 3.70 - 3.67 (m, 4H), 3.26 - 3.24 (m, 2H), 3.03 - 2.93 (m, 2H), 2.62 - 2.57 (m, 2H), 2.52 - 2.42 (m, 4H), 2.33 - 2.26 (m, 2H), 2.12 - 1.89 (m, 2H), 1.63 - 1.54 (m, 1H), 1.49 - 1.45 (m, 9H), 1.40 - 1.30 (m, 2H), 0.88 - 0.81 (m, 6H),

^{13}C NMR (101 MHz, CD_3OD) δ 172.6, 172.5, 172.2, 172.1, 172.0, 171.6, 170.9, 170.8, 168.1, 168.0, 144.0, 143.9, 143.7, 143.3, 143.3, 141.1, 141.0, 140.9, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.1, 127.0, 126.9, 126.8, 126.5, 125.8, 125.7, 125.5, 120.2, 119.2, 118.9, 118.6, 118.5, 81.6, 72.6, 72.4, 66.6, 66.5, 62.9, 62.8, 61.1, 53.4, 53.3, 53.2, 52.5, 52.4, 52.3, 49.1, 40.8, 40.7, 34.2, 31.6, 31.5, 29.7, 29.6, 27.2, 27.0, 26.9, 26.8, 24.5, 24.4, 21.7, 21.7, 21.6, 21.1, 21.0.

IR (ATR, neat, cm^{-1}) 3276, 2955, 1729, 1643, 1509, 1263, 1114, 1013, 864, 745.

m.p. 72 °C.

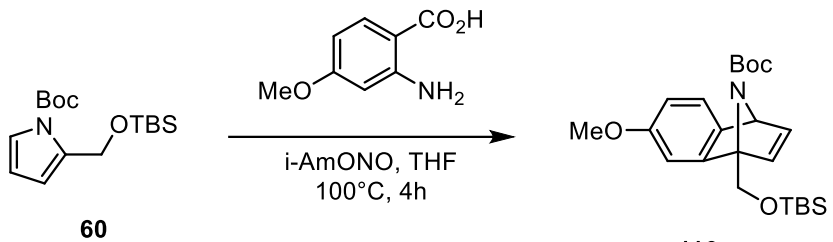


N-((S)-4-methyl-1-((S)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)-9-(((S)-2-(2-morpholinoacetamido)-4-phenylbutanoyl)-L-leucyl)-3,4-dihydro-1,4-epiminonaphthalene-1(2H)-carboxamide: To a solution of tBu ester (100mg, 0.15mmol, 1eq.) in 3.9 mL DCM (0.04M), TFA (0.77mL, 0.15mmol, 0.20M) was added dropwise. The reaction was left stirring for 2h then the solvent was removed under reduce pressure keeping attention at rotavapor bath temperature (15°C). To remove all the TFA traces the crude was dried under vacuum and the carboxylic acid was used directly in the final coupling without further purification. To a solution of the chiral epoxy ketone (50mg, 0.17mmol, 1eq.) in 1.0mL DCM (0.2M) was added DIPEA (0.06mL, 0.34mmol, 2eq.). The mixture was left stirring for 10minute, after that the carboxylic acid was added. Finally, EDC.HCl (40mg, 0.25mmol, 1.5eq.) and DMAP (5.17mg, 0.04mmol, 0.25eq.) were added. The reaction mixture was left stirring at room temperature for 24 hours. DIPEA is quenched with an aqueous solution of citric acid and the mixture is extracted

three times using DCM. The combined organic phases were dried over Na_2SO_4 and filtered. The solvent is removed under reduced pressure, keeping attention at rotavapor bath temperature (15°C). The crude will be purified by preparative HPLC.

The same procedure was applied on both isomers (A and B).

10 Synthesis of Tyrosine Analogues



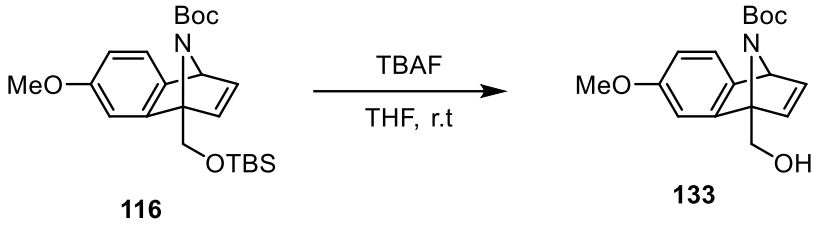
tert-butyl -1-(((tert-butyldimethylsilyl)oxy)methyl)-7-methoxy-1,4-dihydro-1,4-epiminonaphthalene-9-carboxylate: Solutions of methoxy-anthranilic acid (3.0 eq.) in tetrahydrofuran (0.5 M) and isopentyl nitrite (3.25 eq.) in tetrahydrofuran (0.5 M) were added dropwise and simultaneously to a refluxing solution of tert-butyl 2-(((tert-butyldimethylsilyl)oxy)methyl)-1H-pyrrole-1-carboxylate (1.0 eq.) in tetrahydrofuran (1 M) over a period of about 2 h. The solution was refluxed for further 2 h, it was cooled to room temperature, aqueous sodium bicarbonate was added. The organic solvent was evaporated, and the aqueous phase was extracted with Et_2O. The organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 95:5 to 9:1) to afford the corresponding cycloadduct (58%) as a yellow oil.

R_f 0.36 (SiO_2, Hex:EtOAc 9:1, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 - 7.08 (m, 1H), 6.94 - 6.69 (m, 3H), 6.28 – 6.21 (m, 1H), 5.22 – 5.20 (m, 1H), 4.59 – 4.56 (m, 1H), 4.36 (t, J = 9.1 Hz, 1H), 3.58 (s, 3H), 1.16 (s, 9H), 0.78 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 157.1, 154.7, 151.4, 144.9, 142.2, 115.4, 114.6, 109.7, 107.3, 80.5, 76.5, 69.3, 67.5, 61.5, 55.5, 28.2, 25.9, -5.3, -5.4.

IR (ATR, neat, cm^{-1}) 1725, 1537, 1412, 1373, 1320, 1240, 1134, 1006, 970, 831, 758.



116 **133**

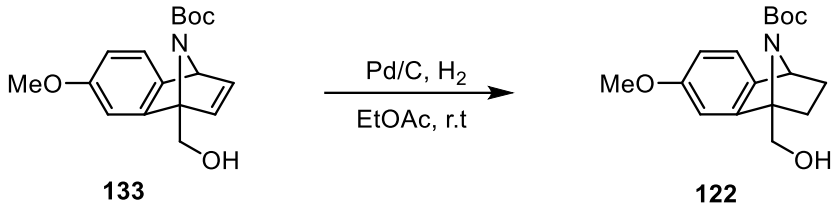
tert-butyl-1-(hydroxymethyl)-7-methoxy-1,4-dihydro-1,4 epiminonaphthalene-9-carboxylate: To a solution of protected alcohol (1.0 eq.) in anhydrous THF (0.1 M) under Ar, was added tetra n-butylammonium fluoride (TBAF, 1M in THF, 1.5 eq.) dropwise at room temperature and the mixture was stirred for 6 h. Once complete, the mixture was extracted with EtOAc and washed with water. The organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 95:5 to 9:1) to afford the free alcohol (87%) as a yellow oil.

R_f 0.31 (SiO_2, Hex:EtOAc 8:2, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (d, J = 7.9 Hz, 1H), 6.94 (dd, J = 5.5, 2.5 Hz, 1H), 6.81 (d, J = 2.3 Hz, 1H), 6.76 (d, J = 5.6 Hz, 1H), 6.36 (dd, J = 7.8, 2.3 Hz, 1H), 5.39 (d, J = 2.5 Hz, 1H), 4.47 (s, 2H), 3.69 (s, 3H), 1.34 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 157.5, 150.0, 144.4, 143.7, 140.4, 120.7, 109.5, 107.8, 81.5, 78.7, 66.6, 59.0, 55.6, 28.2.

IR (ATR, neat, cm^{-1}) 3391, 1707, 1664, 1528, 1455, 1396, 1343, 1230, 1189, 956, 867.



133 122

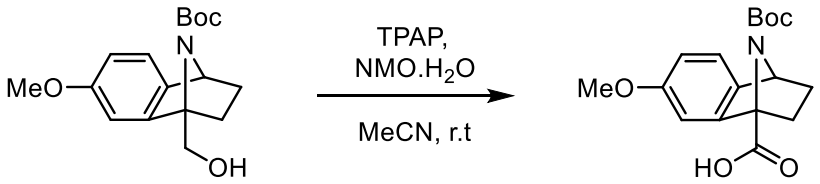
tert-butyl -1-(hydroxymethyl)-7-methoxy-1,2,3,4-tetrahydro-1,4-epiminonaphthalene-9-carboxylate: Unsaturated alcohol (1.0 eq.) was solubilized in EtOAc (0.1M). The mixture was purged with Ar, then Pd/C (10% Pd on C, 0.10 eq.) was added to the mixture. The flask was filled with H_2 gas using a double balloon, and the reaction mixture was stirred for 18 h at room temperature under an atmosphere of H_2. Once complete, the mixture was filtered over a pad of celite, using EtOAc as the eluent. The solvent was evaporated under reduced pressure, and the resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 7:3) to afford pure saturated alcohol (70%) as a colorless oil.

R_f 0.32 (SiO_2, Hex:EtOAc 8:2, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (dd, J = 8.1, 4.7 Hz, 1H), 6.75 (dd, J = 5.3, 2.3 Hz, 1H), 6.59 (ddd, J = 8.0, 5.5, 2.6 Hz, 1H), 5.02 (d, J = 3.9 Hz, 1H), 4.40 – 4.26 (m, 2H), 3.69 (s, 3H), 2.08 – 2.01 (m, 2H), 1.33 (s, 9H), 1.25 – 1.10 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 158.7, 146.6, 137.3, 137.1, 119.9, 111.2, 105.9, 80.8, 73.1, 61.5, 60.0, 60.0, 55.5, 28.3.

IR (ATR, neat, cm^{-1}) 3418, 1726, 1665, 1520, 1391, 1264, 1122, 1088, 1021, 917.



122 **134**

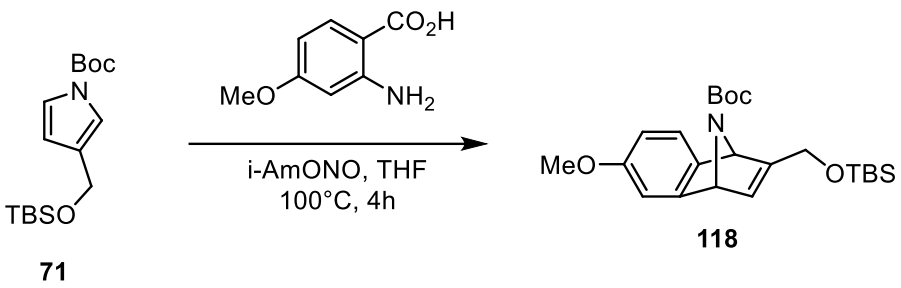
9-(tert-butoxycarbonyl)-7-methoxy-3,4-dihydro-1,4-epiminonaphthalene-1(2H)-carboxylic acid: Alcohol (1.0 eq.) and N-methyl morpholine N-oxide monohydrate (10.0 equiv.) are dissolved in acetonitrile (0.1 M). TPAP (0.1 eq.) is added, and the mixture is stirred at room temperature. On completion the reaction is quenched with an excess of 2-propanol. The solvent is evaporated, and the residue is filtered over a pad of silica using ethyl acetate containing 1% of acetic acid. The filtrate is concentrated in vacuo and the resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 8:2 to 7:3) to afford pure saturated alcohol (81%) as a pale yellow oil.

R_f 0.35 (SiO_2, Hex:EtOAc 7:3, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 6.76 (m, 2H), 6.65 (ddd, J = 8.2, 5.8, 2.4 Hz, 1H), 5.12 (d, J = 4.6 Hz, 1H), 2.46 – 2.38 (m, 1H), 2.18 – 2.08 (m, 1H), 1.78 – 1.67 (m, 1H), 1.40 – 1.25 (m, 10H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 159.3, 159.0, 144.8, 135.6, 121.1, 120.2, 111.1, 106.7, 83.2, 63.3, 62.9, 55.6, 55.5, 29.7, 28.1.

IR (ATR, neat, cm^{-1}) 3047, 1697, 1578, 1386, 1318, 1240, 1177, 1132, 1012, 908, 798.



71 118

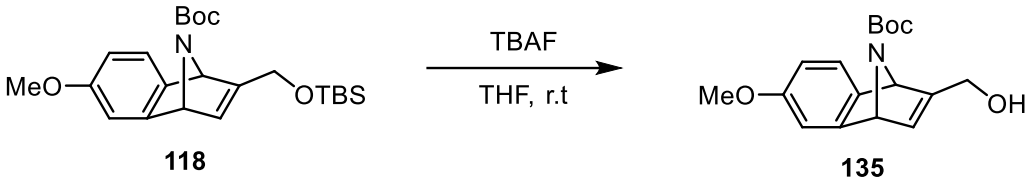
tert-butyl -2-(((tert-butyldimethylsilyl)oxy)methyl)-6-methoxy-1,4-dihydro-1,4-epiminonaphthalene-9-carboxylate: Solutions of methoxy-anthranilic acid (3.0 eq.) in tetrahydrofuran (0.5 M) and isopentyl nitrite (3.25 eq.) in tetrahydrofuran (0.5 M) were added dropwise and simultaneously to a refluxing solution of tert-butyl 3-(((tert-butyldimethylsilyl)oxy)methyl)-1H-pyrrole-1-carboxylate (1.0 eq.) in tetrahydrofuran (M) over a period of about 2 h. The solution was refluxed for further 2 h, it was cooled to room temperature, aqueous sodium bicarbonate was added. The organic solvent was evaporated, and the aqueous phase was extracted with Et_2O. The organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 95:5 to 9:1) to afford the corresponding cycloadduct (70%) as a yellow oil.

R_f 0.43 (SiO_2, Hex:EtOAc 9:1, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 – 7.08 (m, 1H), 6.94 – 6.86 (m, 1H), 6.58 – 6.46 (m, 1H), 6.39 (ddd, J = 7.9, 6.9, 2.3 Hz, 1H), 5.46 – 5.32 (m, 1H), 5.27 – 5.24 (m, 1H), 4.39 – 4.25 (m, 2H), 3.74 (s, 3H), 1.36 (s, 9H), 0.87 (s, 9H), 0.00 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 157.5, 155.1, 150.7, 140.6, 139.7, 120.7, 114.7, 109.5, 107.4, 80.4, 66.9, 60.7, 55.5, 28.2, 25.9, 18.4, -5.4.

IR (ATR, neat, cm^{-1}) 1710, 1560, 1461, 1329, 1267, 1144, 1069, 945, 839, 745.



118 **135**

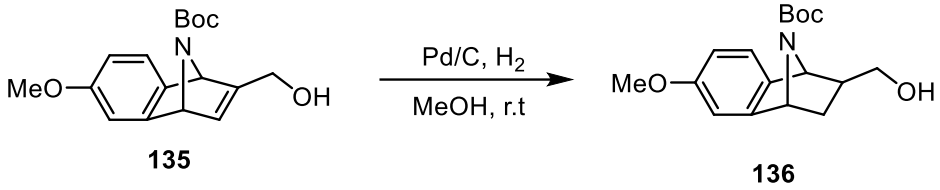
tert-butyl -2-(hydroxymethyl)-6-methoxy-1,4-dihydro-1,4-epiminonaphthalene-9-carboxylate: To a solution of protected alcohol (1.0 eq.) in anhydrous THF (0.1 M) under Ar, was added tetra n-butylammonium fluoride (TBAF, 1M in THF, 1.5 eq.) dropwise at room temperature and the mixture was stirred for 6 h. Once complete, the mixture was extracted with EtOAc and washed with water. The organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 95:5 to 9:1) to afford the free alcohol (90%) as a yellow oil.

R_f 0.41 (SiO_2, Hex:EtOAc 8:2, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 – 7.05 (m, 1H), 6.89 – 6.78 (m, 1H), 6.54 – 6.51 (m, 1H), 6.33 (ddd, J = 7.8, 5.5, 2.3 Hz, 1H), 5.33 (s, 1H), 5.25 (s, 1H), 4.26 – 4.15 (m, 2H), 3.66 (s, 3H), 1.76 (br s, 1H), 1.28 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 157.6, 155.3, 149.7, 140.2, 139.4, 115.5, 114.7, 110.1, 107.5, 80.8, 66.8, 60.2, 60.1, 55.6, 28.2.

IR (ATR, neat, cm^{-1}) 3283, 1698, 1624, 1417, 1393, 1334, 1239, 1151, 1080, 889, 790.



135 **136**

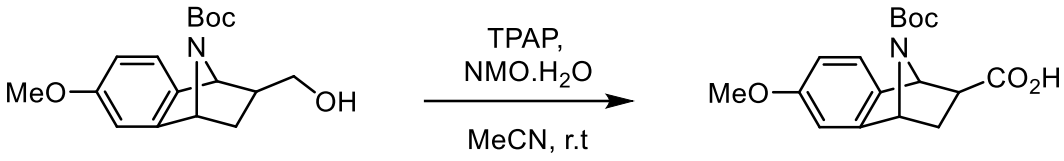
tert-butyl -2-(hydroxymethyl)-6-methoxy-1,2,3,4-tetrahydro-1,4-epiminonaphthalene-9-carboxylate: Unsaturated alcohol (1.0 eq.) was solubilized in MeOH (0.1M). The mixture was purged with Ar, then Pd/C (10% Pd on C, 0.10 eq.) was added to the mixture. The flask was filled with H_2 gas using a double balloon, and the reaction mixture was stirred for 18 h at room temperature under an atmosphere of H_2. Once complete, the mixture was filtered over a pad of celite, using MeOH as the eluent. The solvent was evaporated under reduced pressure, and the resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 8:2) to afford pure saturated alcohol (92%) as a colorless oil.

R_f 0.33 (SiO_2, Hex:EtOAc 8:2, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (dd, J = 35.5, 8.0 Hz, 1H), 6.81 (dd, J = 37.8, 2.3 Hz, 1H), 6.60 (ddd, J = 8.1, 4.4, 2.6 Hz, 1H), 5.06 – 4.94 (m, 2H), 3.71 (s, 3H), 3.20 – 3.18 (m, 1H), 2.66 – 2.60 (m, 2H), 2.24 – 2.15 (m, 1H), 1.32 (s, 9H), 0.65 (d, J = 11.7 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 158.9, 155.3, 155.3, 146.9, 142.9, 137.7, 133.5, 122.3, 108.8, 106.5, 80.1, 64.2, 64.1, 55.5, 55.4, 28.2.

IR (ATR, neat, cm^{-1}) 3402, 1711, 1665, 1490, 1361, 1255, 1132, 1070, 1028, 925.



136 **137**

9-(tert-butoxycarbonyl)-6-methoxy-1,2,3,4-tetrahydro-1,4-epiminonaphthalene-2-carboxylic acid: Alcohol (1.0 eq.) and N-methyl morpholine N-oxide monohydrate (10.0 equiv.) are dissolved in acetonitrile (0.1 M). TPAP (0.1 eq.) is added, and the mixture is stirred at room temperature. On completion the reaction is quenched with an excess of 2-propanol. The solvent is evaporated, and the residue is filtered over a pad of silica using ethyl acetate containing 1% of acetic acid. The filtrate is concentrated in vacuo and the resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 8:2 to 7:3) to afford pure saturated alcohol (87%) as a pale yellow oil.

R_f 0.43 (SiO_2, Hex:EtOAc 7:3, UV-Vis, $KMnO_4$).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.05 (dd, J = 15.8, 8.1 Hz, 1H), 6.78 – 6.74 (m, 1H), 6.58 (ddd, J = 26.3, 8.1, 2.3 Hz, 1H), 5.21 – 5.14 (m, 1H), 5.04 – 4.96 (m, 1H), 3.69 (d, J = 15.1 Hz, 3H), 3.34 – 3.28 (m, 1H), 2.28 – 2.18 (m, 1H), 1.58 (dt, J = 12.0, 4.5 Hz, 1H), 1.32 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 176.6, 159.2, 154.8, 142.2, 132.9, 122.4, 120.2, 111.9, 111.2, 108.7, 106.3, 80.7, 55.5, 55.4, 28.2.

IR (ATR, neat, cm^{-1}) 3078, 1679, 1502, 1466, 1400, 1385, 1213, 1142, 980, 877.

11 Synthesis of Hygromycin A Analogues

11.1 Lateral Chain Synthesis

2,5-difluoro-4-(3-fluoropropoxy)benzaldehyde: 3-fluoropropanol (9.76 g, 125 mmol, 10.0 eq.) and potassium carbonate (3.45 g, 12.5 mmol, 2.0 eq.) are added to a stirring solution of 2,4,5-trifluorobenzaldehyde (2.00 g, 12.5 mmol, 1.0 eq.) in DMF (0.5M, 25 mL). The reaction mixture is heated at 80 °C overnight. Upon completion, monitored by TLC, the reaction was warmed at room temperature and H_2O is added. The aqueous phase was extracted with Et_2O, the organic phases were reunited, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 95:5 to 8:2) to afford the desired product (2.65 g, 97%) as a light-yellow solid.

R_f 0.24 (SiO_2, Hex:EtOAc 9:1, UV-Vis, Vanillin).

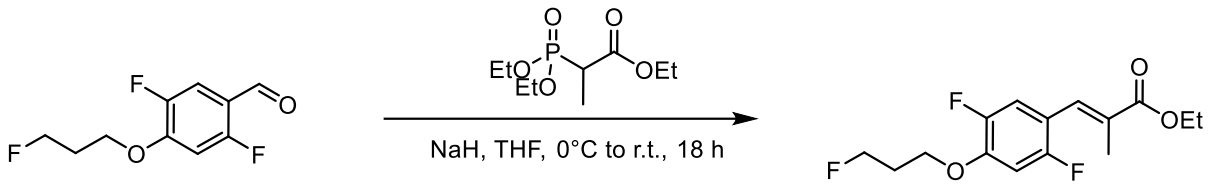
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.11 (d, J = 2.9 Hz, 1H), 7.46 (dd, J = 10.7, 6.5 Hz, 1H), 6.72 (dd, J = 11.5, 6.4 Hz, 1H), 4.68 (t, J = 5.6 Hz, 1H), 4.56 (t, J = 5.7 Hz, 1H), 4.18 (t, J = 6.1 Hz, 2H), 2.21 (dp, J = 26.3, 5.9 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 184.89 (dd, J = 6.1, 1.5 Hz), 161.86 (dd, J = 256.0, 2.0 Hz), 155.70 – 145.55 (m), 118.89 – 113.61 (m), 113.76 (dd, J = 20.7, 4.0 Hz), 101.88 (dd, J = 27.2, 1.8 Hz), 79.96 (d, J = 165.1 Hz), 65.44 (d, J = 4.7 Hz), 29.90 (d, J = 20.1 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -123.7 (d, J = 14.9 Hz), -138.2 (d, J = 14.4 Hz), -223.1.

IR (ATR, neat, cm^{-1}) 2980, 1671, 1619, 1500, 1436, 1349, 1313, 1291, 1209, 1164, 1105, 1082

m.p. 42 °C.



ethyl (E)-3-(2,5-difluoro-4-(3-fluoropropoxy)phenyl)-2-methylacrylate: To a solution of sodium hydride (264 mg, 11.0 mmol, 1.2 eq.) in THF (0.55M, 20 mL) is added triethylphosphonopropionate (2.62 g, 11.0 mmol, 1.2 eq.) dropwise at 0 °C. The reaction mixture is stirred 30 minutes and then aldehyde (2.00 g, 9.17 mmol, 1 eq.) in THF (2M, 4 mL) is added. The solution is allowed to warm to room temperature, and it is stirred overnight. The reaction is quenched with saturated aqueous NH_4Cl solution and extracted with ethyl acetate. The combined organic layers are washed with brine and dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude is purified by flash chromatography on silica gel (Hex/EtOAc = 9:1 to 7:3) to afford the desired product as a white solid (2.47 g, 89%).

R_f 0.35 (SiO_2, Hex:EtOAc 9:1, UV-Vis, Vanillin).

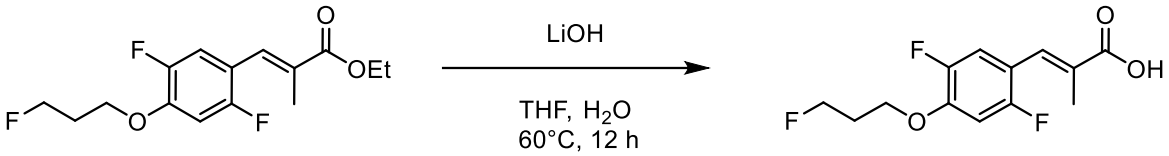
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (dd, J = 2.1, 1.1 Hz, 1H), 7.10 (dd, J = 11.7, 6.9 Hz, 1H), 6.72 (dd, J = 11.1, 7.1 Hz, 1H), 4.69 (t, J = 5.7 Hz, 1H), 4.57 (t, J = 5.7 Hz, 1H), 4.24 (q, J = 7.1 Hz, 2H), 4.14 (t, J = 6.1 Hz, 2H), 2.19 (dp, J = 26.2, 5.9 Hz, 2H), 2.02 (t, J = 1.4 Hz, 3H), 1.31 (t, J = 7.2 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.97, 156.53 (dd, J = 247.3, 2.3 Hz), 151.57 – 144.80 (m), 130.06 (d, J = 1.1 Hz), 129.80 (dd, J = 3.3, 1.5 Hz), 116.58 (dd, J = 21.1, 4.7 Hz), 115.58 (dd, J = 15.9, 6.6 Hz), 102.42 (dd, J = 28.4, 2.2 Hz), 80.17 (d, J = 165.0 Hz), 65.13 (d, J = 5.0 Hz), 60.96, 30.14 (d, J = 20.2 Hz), 16.38 – 12.00 (m).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.4 (d, J = 14.5 Hz), -139.7 (d, J = 14.3 Hz), -222.8.

IR (ATR, neat, cm^{-1})

m.p. 50 °C.



(E)-3-(2,5-difluoro-4-(3-fluoropropoxy)phenyl)-2-methylacrylic acid: Ethyl ester (1.00 g, 3.31 mmol, 1.0 eq.) is solubilized in THF (0.25M, 13 mL) and a solution of LiOH in water (396 mg, 16.5 mmol, 5.0 eq.) is added. The mixture is stirred at 60 °C overnight, then it is quenched with 1M HCl. The organic phase is separated, and the aqueous phase is extracted with ethyl acetate. The combinate organic phases are dried over Na_2SO_4, filtered, and evaporated to obtain the crude carboxylic acid, which is purified by flash chromatography on silica gel (Hex/EtOAc = 8:2 to 3:7) to afford a white solid (0.82 g, 90%).

R_f 0.35 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.68 (s, 1H), 7.79 – 7.75 (m, 1H), 7.18 (dd, J = 11.6, 6.9 Hz, 1H), 6.76 (dd, J = 11.1, 7.0 Hz, 1H), 4.73 (t, J = 5.7 Hz, 1H), 4.61 (t, J = 5.6 Hz, 1H), 4.18 (t, J = 6.1 Hz, 2H), 2.23 (dp, J = 26.3, 5.9 Hz, 2H), 2.08 (d, J = 2.7 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.77, 156.82 (dd, J = 248.3, 2.3 Hz), 151.63 – 144.86 (m), 132.29 (dd, J = 3.6, 1.5 Hz), 128.79, 116.63 (dd, J = 21.2, 4.6 Hz), 115.27 (dd, J = 15.5, 6.7 Hz), 102.47 (dd, J = 28.3, 2.2 Hz), 80.20 (d, J = 165.1 Hz), 65.18 (d, J = 4.8 Hz), 30.16 (d, J = 20.0 Hz), 13.88 (d, J = 1.4 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -114.7 (d, J = 16.1 Hz), -139.4 (d, J = 14.2 Hz), -222.8.

IR (ATR, neat, cm^{-1}) 2959, 2920, 2644, 1681, 1623, 1513, 1272, 1125, 1045, 1013

m.p. 123 - 127 °C.

11.2 General Synthetic Procedures

General Procedure A for Nitroso Diels-Alder reaction

To a stirring solution of diene (1.0 eq.) in dichloromethane (0.4M) is added protected hydroxylamine (1.5 eq.), then the mixture is cooled in at – 20 °C and tetrabutylammonium (meta)periodate (1.0 eq.) in dichloromethane (0.78M) is added dropwise under N_2 atmosphere. The reaction mixture is stirred at room temperature for 15 hours and then it is quenched with saturated aqueous $Na_2S_2O_3$ solution, and the aqueous fraction is extracted with dichloromethane. The reunited organic phases are dried over Na_2SO_4, filtered, and evaporated under reduced pressure, then the crude is purified by flash chromatography on silica gel to afford the corresponding cycloadduct.

General Procedure B for N-O bond cleavage

A clean, flame-dried, round-bottom flask equipped with a stir bar is filled with N2. Titanocene dichloride (2.5 eq.) and activated zinc (5.0 eq.), taken from glovebox and put together in the flame dried flask, are dissolved in well degassed tetrahydrofuran (0.08M) with N_2 and sonicator; then this solution is stirred at room temperature under N_2 for 45 minutes. The reaction mixture changed colour from dark red to olive green. The reaction mixture was cooled to -30 °C and a degassed methanol solution (0.1M,) of substrate (1.0 eq.) is canulated dropwise. The reaction mixture is stirred for 45 min-1h while the bath temperature was kept between -10 and -30 °C, then the solution was warmed to room

temperature and quenched with potassium carbonate. The mixture, collected with ethyl acetate, is first filtered through a celite pad to remove all the titanium and zinc residues. The pad is washed well with ethyl acetate and to a filtered solution is added water; if necessary is employed a second filtration and then the aqueous phase is extracted with ethyl acetate, the combined organics phases is dried over Na_2SO_4, filtered, and brought to dryness. The crude material is purified by flash chromatography on silica gel to afford the desired product.

General Procedure C for Boc and Acetal deprotection

Boc-protected or acetal-protected derivative (1.0 eq.) is solubilized in DCM (0.15 M). The mixture was purged with Ar, then HCl (4M in Dioxane, 20 eq.) is added to the mixture. The reaction mixture is stirred for 18 h at room temperature under an atmosphere of Ar. Once complete, the mixture is filtered under, using DCM as the eluent. The corresponding product is dried under air (or Ar, if sensitive).

General Procedure D for Hydrogenation

Unsaturated substrate or azide substrate (1.0 eq.) is solubilized in MeOH (0.1M). The mixture is purged with Ar, then Pd/C (10% Pd on C, 0.10 eq.) is added to the mixture. The flask is filled with H_2 gas using a double balloon, and the reaction mixture is stirred

for 24 h at room temperature under an atmosphere of H_2. Once complete, the mixture is filtered over a pad of celite, using MeOH as the eluent. The solvent is evaporated under reduced pressure, to obtain the corresponding reduced product.

General Procedure E for Epoxidation

Unsaturated substrate (1.0 eq.) is solubilized in DCM (0.1M), then the mixture is cooled to 0°C. $NaHCO_3$ (5.0 eq.) is added, followed by *m*CPBA (3.0 eq.). The mixture is warmed to room temperature, and it is stirred until olefin completion. A saturated solution of $Na_2S_2O_3$ is added, the organic phase is separated, and the aqueous phase is extracted with DCM. The reunited organic phases are dried over Na_2SO_4, filtered, and evaporated under reduced pressure, then the crude is purified by flash chromatography on silica gel to afford the corresponding epoxide product.

General Procedure F for Epoxide Opening

NaN_3 (10.0 eq.) and NH_4Cl (2.0 eq.) are added to a solution of epoxide (1.0 eq.) in DMF (0.2M) and the resultant solution is stirred at 80°C for 24 h. The reaction mixture is then washed with saturated solution of Na_2CO_3, and it is extracted with Et_2O. The combined organic extracts were washed sequentially with H_2O and brine, then dried over

Na_2SO_4 and concentrated in vacuo. The corresponding ring-opened product is purified by column chromatography.

General Procedure G for Alcohol Methylation

Alcohol substrate (1.0 eq.) is solubilized in dry THF (0.1M), and the resulting solution is cooled to 0°C. NaH (1.5 eq.) is added portionwise at 0°C, then MeI (1.2 eq.) is added dropwise after 30 minutes. The resultant solution is stirred at room temperature until completion. The reaction mixture is then quenched with saturated solution of NH_4Cl, and it is extracted with ethyl acetate. The combined organic extracts were washed sequentially with H_2O and brine, then dried over Na_2SO_4 and concentrated in vacuo. The corresponding Methylated product is purified by column chromatography.

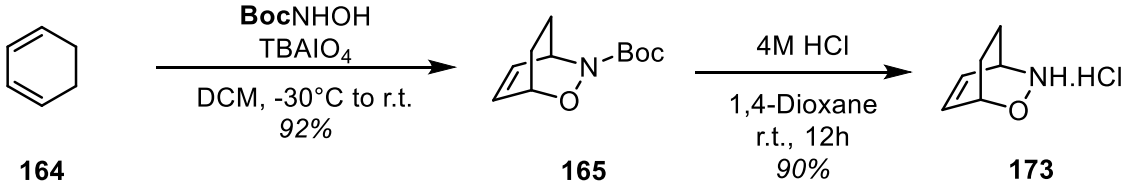
General Procedure H for Amide coupling

To a solution of amine (1.0 eq.) in DCM (0.1M) the carboxylic acid (1.0eq.) is added. Then, EDC.HCl (1.5 eq.) and DMAP (0.1 eq) are added. The reaction is stirred until full conversion of the reagents. Once complete, the solvent is evaporated under reduced pressure to obtain the crude product, which is purified via flash column chromatography on silica gel.

General Procedure I for Amide coupling

To a solution of amine (1.0 eq.) in DCM (0.1M) the carboxylic acid (1.0eq.) is added. Then, EDC.HCl (1.5 eq.), DIPEA (2.5 eq.) and DMAP (0.1 eq) are added. The reaction is stirred until full conversion of the reagents. Once complete, a 1M solution of HCl is added to quench the base, the organic phase is separated, and the aqueous phase is extracted with DCM. The reunited organic phases are dried over Na_2SO_4, filtered, and evaporated under reduced pressure, then the crude is purified by flash chromatography on silica gel.

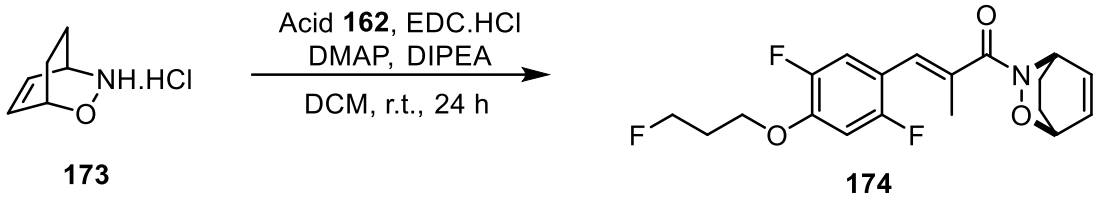
11.3 **Dearomative reduction-derived analogues**



164 **165** **173**

Amine **173** was synthesized starting from 1,3-cyclohexadiene. Initially, following **General Procedure A**, bicycle **165** was obtained, which was Boc-deprotected following **General procedure C**.

Analytical and spectroscopic characterization of product are consistent with those reported in literature [153].



173 **174**

Amide **174** was synthesized following **General Procedure I**, starting from amine **173**. The product was obtained as a colorless oil (58%).

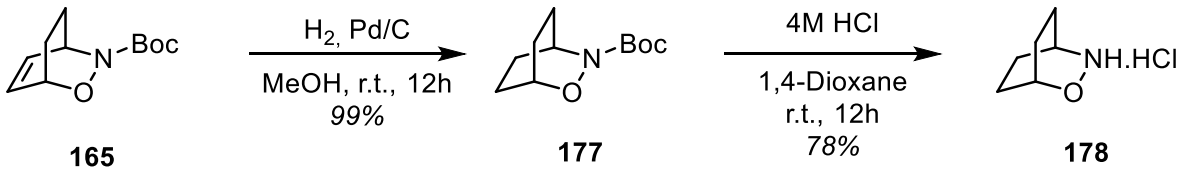
R_f 0.31 (SiO_2, Hex:EtOAc 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (dd, J = 11.7, 7.0 Hz, 1H), 6.75 – 6.64 (m, 2H), 6.64 – 6.57 (m, 1H), 6.54 (ddd, J = 8.0, 5.6, 1.7 Hz, 1H), 5.10 (s, 1H), 4.81 (t, J = 4.7 Hz, 1H), 4.69 (t, J = 5.7 Hz, 1H), 4.57 (t, J = 5.7 Hz, 1H), 4.12 (t, J = 6.2 Hz, 2H), 2.30 – 2.12 (m, 4H), 1.99 (d, J = 2.8 Hz, 3H), 1.58 – 1.42 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.23 (dd, J = 245.9, 2.5 Hz), 151.57 – 144.78 (m), 134.06, 131.82, 130.97, 118.90 – 114.20 (m), 115.69 (dd, J = 16.4, 6.6 Hz), 102.50 (dd, J = 28.4, 2.2 Hz), 80.25 (d, J = 164.9 Hz), 65.16 (d, J = 4.8 Hz), 32.71 – 27.51 (m), 15.75 (d, J = 1.5 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -116.5, -139.8 (d, J = 68.3 Hz), -222.8 (d, J = 17.4 Hz).

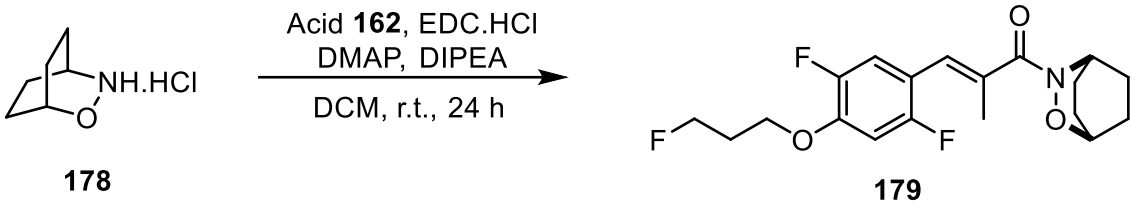
IR (ATR, neat, cm^{-1}) 3058, 2963, 2937, 1626, 1507, 1423, 1332, 1285, 1172, 1112, 1045, 1015.



165 **177** **178**

Amine **178** was synthesized starting from intermediate **165**. Hydrogenation was performed following **General Procedure D**, then Boc deprotection was performed using **General Procedure C**.

Analytical and spectroscopic characterization of product are consistent with those reported in literature [154].



178 **179**

Amide **179** was synthesized following **General Procedure I**, starting from amine **178**. The product was obtained as a light-yellow solid (60%).

R_f 0.29 (SiO_2, Hex:EtOAc 7:3, UV-Vis, Vanillin).

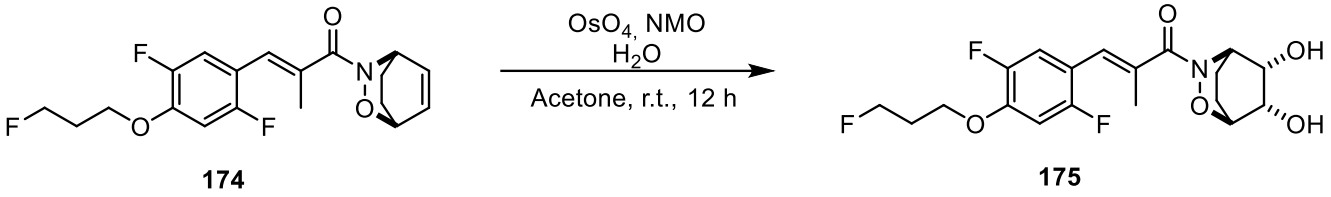
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 – 7.75 (m, 1H), 7.06 (t, J = 9.2 Hz, 1H), 6.68 (dd, J = 11.0, 7.1 Hz, 1H), 4.67 (t, J = 5.7 Hz, 1H), 4.56 (t, J = 5.7 Hz, 1H), 4.48 – 4.15 (m, 2H), 4.11 (t, J = 6.1 Hz, 2H), 2.17 (dp, J = 26.2, 5.9 Hz, 6H), 2.02 (t, J = 1.4 Hz, 3H), 1.87 – 1.64 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.18 (d, J = 246.0 Hz), 148.17 (d, J = 241.7 Hz), 134.01, 118.78 – 114.23 (m), 80.24 (d, J = 164.9 Hz), 65.15 (d, J = 5.0 Hz), 42.25 (d, J = 5.1 Hz), 30.16 (d, J = 20.1 Hz), 25.03, 15.97 (d, J = 1.5 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -116.7 (d, J = 265.4 Hz), -139.9 (d, J = 202.3 Hz), -222.8.

IR (ATR, neat, cm^{-1}) 2948, 2864, 1623, 1507, 1446, 1330, 1300, 1174, 1110.

m.p. 91 °C.



174 **175**

Amide **174** (1.0 eq.), NMO (N-methylmorpholineN-oxide, 1.2 eq.), acetone (0.2M), pure water (20 eq.) were mixed and stirred. The mixture was cooled to 0°C and a solution of OsO_4 (0.2M in MeCN, 0.025 eq.) was added and the mixture was stirred at room temperature. The completion of the reaction was confirmed, and a saturated solution of $Na_2S_2O_3$ was added and the mixture was stirred at room temperature for 2h. Acetone was removed under reduced pressure, and the aqueous phase was extracted three times with ethyl Acetate. The organic phase was dried over Na_2SO_4 and it was evaporated under reduced pressure to afford the crude diol, which was purified by silica gel column chromatography (Hex:AcOEt 3:7) to afford a colorless oil (81%).

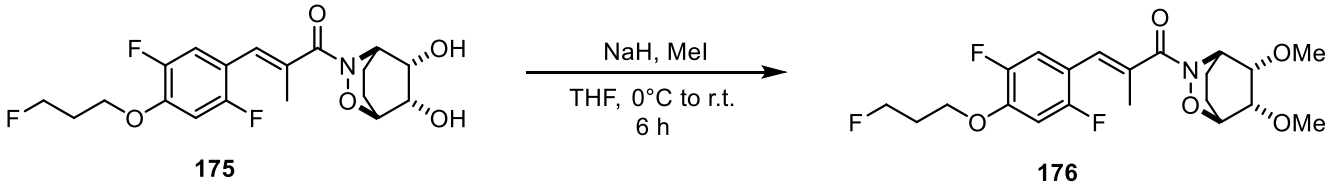
R_f 0.33 (SiO_2, Hex:EtOAc 3:7, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD3OD) δ 7.08 (dd, J = 11.7, 7.0 Hz, 1H), 6.87 (dd, J = 11.4, 7.1 Hz, 1H), 6.62 – 6.51 (m, 1H), 4.58 (t, J = 5.8 Hz, 1H), 4.46 (t, J = 5.8 Hz, 1H), 4.28 – 4.24 (m, 1H), 4.12 – 4.03 (m, 5H), 2.15 – 2.02 (m, 4H), 1.92 (s, 3H), 1.85 – 1.72 (m, 2H).

^{13}C NMR (101 MHz, CD_3OD) δ 156.3 (d, J = 244.6 Hz), 149.5 – 147.0 (m), 133.1, 124.9 – 122.6 (m), 116.0 (dd, J = 21.3, 4.9 Hz), 115.1 – 114.2 (m), 102.3 (dd, J = 29.1, 2.1 Hz), 80.8, 79.2, 75.8, 65.1 (d, J = 5.5 Hz), 64.6, 30.0, 29.8, 18.3, 14.7 (d, J = 1.7 Hz).

^{19}F NMR (376 MHz, CD_3OD) δ -118.3, -141.5, -224.2.

IR (ATR, neat, cm^{-1}) 3402, 2894, 1627, 1547, 1421, 1363, 1306, 1174, 1090, 954, 802.



175 **176**

Amide **176** was synthesized following **General Procedure G**, starting from the corresponding diol **175**. The product was obtained as a light-yellow oil (76%).

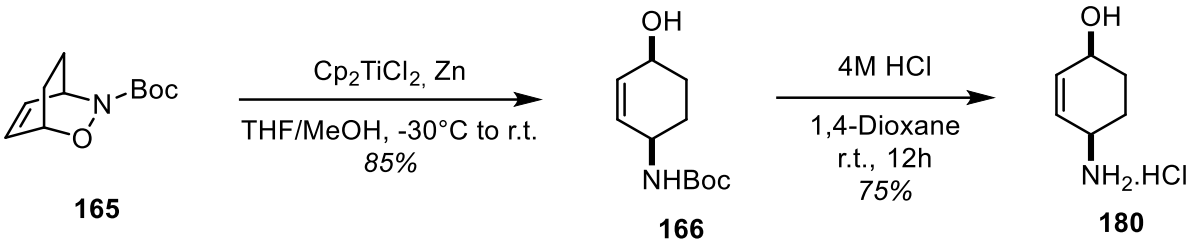
R_f 0.28 (SiO_2, Hex:EtOAc 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (ddd, J = 11.6, 7.0, 4.5 Hz, 1H), 6.70 – 6.64 (m, 2H), 4.68 – 4.49 (m, 3), 4.29 – 4.21 (m, 1H), 4.08 (t, J = 6.1 Hz, 2H), 3.88 – 3.81 (m, 2H), 3.43 – 3.40 (m, 6H), 2.21 – 1.75 (m, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.0, 157.9 – 155.0 (m), 149.4 – 146.9 (m), 133.4, 130.1 – 129.8 (m), 124.3, 116.7 – 115.5 (m), 102.5 (dt, J = 28.4, 2.4 Hz), 81.0, 79.4, 74.9, 73.1, 65.2 (d, J = 5.0 Hz), 61.0, 59.0, 58.4, 30.15 (d, J = 20.3 Hz), 19.1, 15.9 (d, J = 1.7 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -116.6 (d, J = 229.3 Hz), -139.8 (d, J = 52.6 Hz), -222.8.

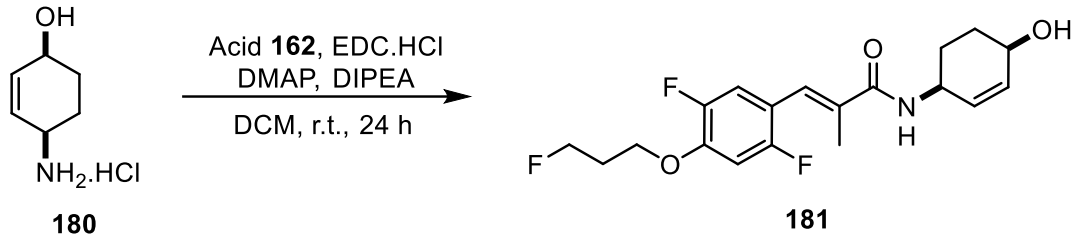
IR (ATR, neat, cm^{-1}) 2933, 1701, 1625, 1513, 1344, 1267, 1174, 1110, 946.



165 **166** **180**

Amine **180** was synthesized starting from intermediate **165.** Initially, following **General Procedure B** for N-O cleavage, amine **166** was obtained, which was Boc-deprotected following **General procedure C**.

Analytical and spectroscopic characterization of product are consistent with those reported in literature [155].



180 **181**

Amide **181** was synthesized following **General Procedure I**, starting from amine **180**. The product was obtained as a light-yellow oil (82%).

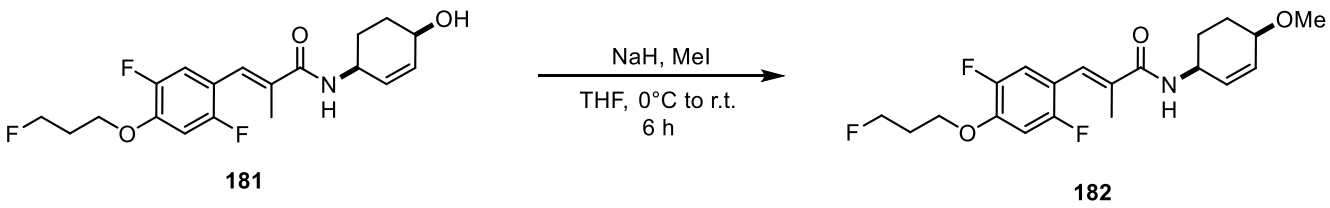
R_f 0.46 (SiO_2, Hex:EtOAc 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (s, 1H), 7.06 (dd, J = 11.8, 7.0 Hz, 1H), 6.66 (dd, J = 11.0, 7.1 Hz, 1H), 6.09 (dt, J = 8.7, 4.0 Hz, 1H), 5.81 (ddd, J = 10.1, 3.9, 2.2 Hz, 1H), 4.81 (dd, J = 9.0, 3.5 Hz, 1H), 4.66 (t, J = 5.6 Hz, 1H), 4.54 (t, J = 5.6 Hz, 1H), 4.25 – 4.17 (m, 1H), 4.09 (t, J = 6.0 Hz, 2H), 2.23 – 2.03 (m, 6H), 2.01 – 1.83 (m, 2H), 1.66 – 1.55 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 156.3 (d, J = 246.6 Hz), 149.4 – 147.0 (m), 134.4, 127.3, 126.5, 123.5, 116.7 (dd, J = 21.1, 4.9 Hz), 116.1 (dd, J = 16.1, 6.8 Hz), 102.9 (d, J = 28.2 Hz), 80.3 (d, J = 165.1 Hz), 75.1, 65.1 (d, J = 5.0 Hz), 63.5, 30.2 (d, J = 20.1 Hz), 26.0, 21.7, 15.2 (d, J = 1.4 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.8 (d, J = 14.1 Hz), -139.9 (d, J = 14.8 Hz), -222.8.

IR (ATR, neat, cm^{-1}) 3332, 1606, 1513, 1423, 1326, 1174, 1043, 879.



181 **182**

Amide **182** was synthesized following **General Procedure G**, starting from the corresponding alcohol **181**. The product was obtained as a white solid (79%).

R_f 0.20 (SiO_2, Hex:EtOAc 7:3, UV-Vis, Vanillin).

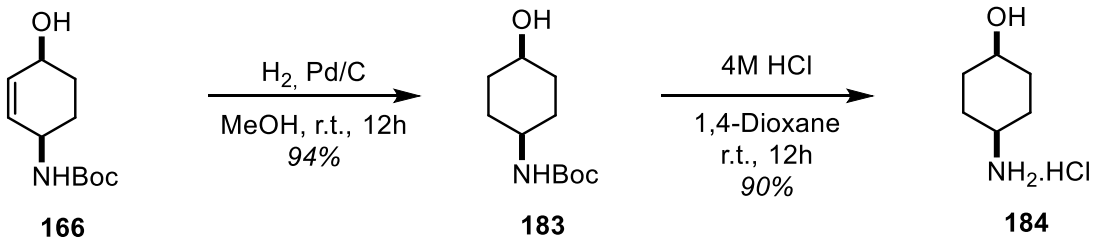
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (t, J = 1.6 Hz, 1H), 6.96 (dd, J = 11.6, 6.9 Hz, 1H), 6.66 (dd, J = 11.0, 7.1 Hz, 1H), 5.91 (ddd, J = 10.0, 3.5, 1.9 Hz, 1H), 5.73 (ddd, J = 10.1, 3.2, 1.2 Hz, 1H), 4.64 (t, J = 5.7 Hz, 1H), 4.54 – 4.47 (m, 2H), 4.08 (t, J = 6.1 Hz, 2H), 3.66 – 3.62 (m, 1H), 3.31 (s, 3H), 2.15 (dp, J = 26.2, 5.9 Hz, 6H), 1.93 (t, J = 1.4 Hz, 3H), 1.84 – 1.63 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2, 156.2 (d, J = 246.2 Hz), 149.4 – 147.0 (m), 133.9, 131.0, 130.6, 125.3, 116.6 (dd, J = 21.0, 5.0 Hz), 115.6 (dd, J = 16.5, 6.7 Hz), 102.5 (d, J = 28.3 Hz), 80.2 (d, J = 164.9 Hz), 73.1, 65.1 (d, J = 5.0 Hz), 56.3, 45.1, 30.1 (d, J = 20.2 Hz), 25.3, 24.9, 14.5 (d, J = 1.8 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.6, -139.7, -222.7.

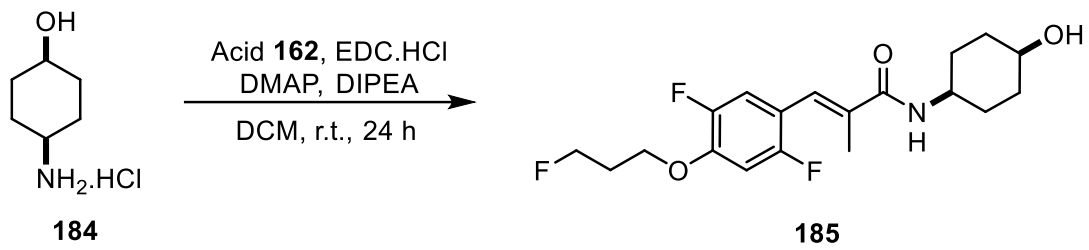
IR (ATR, neat, cm^{-1}) 3287, 2948, 1606, 1509, 1334, 1174, 1095, 1047, 872.

m.p. 56 °C.



Amine **184** was synthesized starting from intermediate **166.** Initially, following **General Procedure D** for hydrogenation, amine **183** was obtained, which was Boc-deprotected following **General procedure C**.

Analytical and spectroscopic characterization of product are consistent with those reported in literature [156].



184 **185**

Amide **185** was synthesized following **General Procedure I**, starting from amine **184**. The product was obtained as a light-yellow solid (68%).

R_f 0.15 (SiO_2, Hex:EtOAc 3:7, UV-Vis, Vanillin).

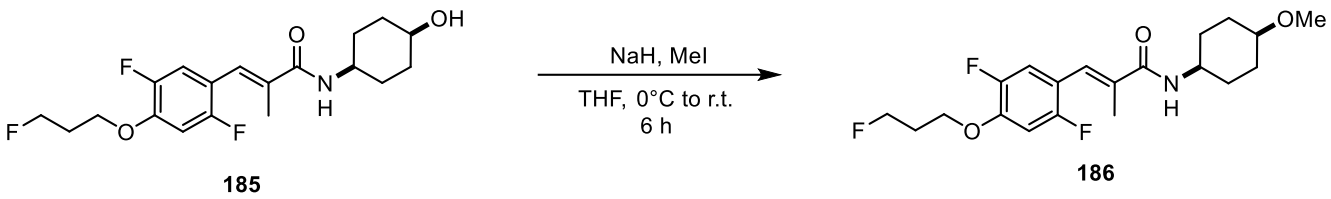
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.96 (dd, J = 11.6, 6.9 Hz, 1H), 6.65 (dd, J = 11.0, 7.1 Hz, 1H), 5.90 (d, J = 8.0 Hz, 1H), 4.64 (t, J = 5.7 Hz, 1H), 4.53 (t, J = 5.7 Hz, 1H), 4.08 (t, J = 6.1 Hz, 2H), 3.88 – 3.86 (m, 2H), 2.21 – 2.07 (m, 3H), 1.94 (t, J = 1.4 Hz, 3H), 1.68 – 1.63 (m, 8H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.3, 156.2 (d, J = 246.0 Hz), 149.4 – 147.0 (m), 134.3, 125.0, 116.6 (dd, J = 21.0, 5.0 Hz), 115.6 (dd, J = 16.5, 6.7 Hz), 102.5 (d, J = 28.4 Hz), 80.3 (d, J = 164.9 Hz), 66.0, 65.1 (d, J = 4.8 Hz), 47.1, 31.3, 30.1 (d, J = 20.1 Hz), 27.2, 14.5 (d, J = 1.8 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.7, -139.7, -222.7.

IR (ATR, neat, cm^{-1}) 3585, 3406, 2926, 1658, 1613, 1513, 1252, 1218, 1182, 1032.

m.p. 91 - 92 °C.



185 186

Amide **186** was synthesized following **General Procedure G**, starting from the corresponding alcohol **185**. The product was obtained as a colorless oil (74%).

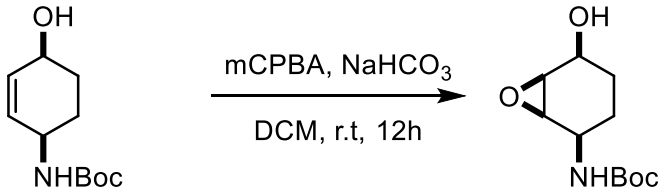
R_f 0.38 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (t, J = 1.6 Hz, 1H), 7.00 (dd, J = 11.6, 6.9 Hz, 1H), 6.70 (dd, J = 11.0, 7.1 Hz, 1H), 5.93 (d, J = 8.0 Hz, 1H), 4.68 (t, J = 5.7 Hz, 1H), 4.56 (t, J = 5.7 Hz, 1H), 4.12 (t, J = 6.1 Hz, 2H), 3.94 – 3.87 (m, 1H), 3.37 – 3.34 (m, 1H), 3.28 (s, 3H), 2.18 (dp, J = 26.2, 5.9 Hz, 2H), 1.97 (t, J = 1.5 Hz, 3H), 1.86 – 1.51 (m, 8H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.1, 156.1 (d, J = 246.0 Hz), 149.4 – 147.0 (m), 134.4, 124.8, 116.6 (dd, J = 21.0, 5.0 Hz), 115.7 (dd, J = 16.5, 6.7 Hz), 102.5 (d, J = 28.4 Hz), 80.2 (d, J = 164.8 Hz), 74.5, 65.1 (d, J = 4.8 Hz), 55.5, 47.4, 31.3, 30.1 (d, J = 20.1 Hz), 28.0, 27.3, 14.5 (d, J = 1.8 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.8 (d, J = 15.9 Hz), -139.8 (d, J = 15.9 Hz), -222.7.

IR (ATR, neat, cm^{-1}) 2933, 1621, 1509, 1472, 1259, 1174, 1088, 1047.



166 **187**

Epoxide **187** was synthesized following **General Procedure E**, starting from olefin **166**. The product was obtained as a white solid (90%).

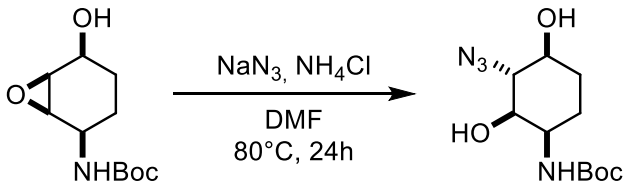
R_f 0.28 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14 (d, J = 8.9 Hz, 1H), 4.03 – 3.99 (m, 2H), 3.46 – 3.42 (m, 2H), 2.72 (d, J = 8.6 Hz, 1H), 1.59 – 1.47 (m, 4H), 1.44 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 155.3, 79.6, 65.3, 57.0, 56.8, 45.1, 28.4, 27.3, 24.7.

IR (ATR, neat, cm^{-1}) 3459, 2870, 1654, 1502, 1244, 1159, 1069, 998, 887.

m.p. 107 - 109 °C.



187 **188**

Azide-substituted cyclohexane **188** was synthesized following **General Procedure F**, starting from epoxide **187**. The product was obtained as a white solid (78%).

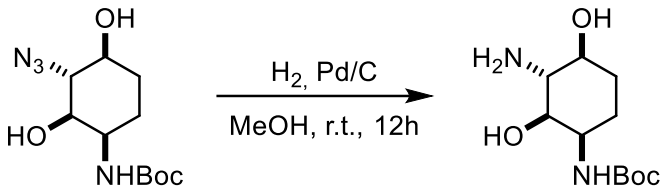
R_f 0.43 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.81 (s, 1H), 3.93 – 3.71 (m, 2H), 3.61 – 3.52 (m, 1H), 3.49 – 3.36 (m, 2H), 2.91 (s, 1H), 1.80 – 1.68 (m, 2H), 1.52 – 1.38 (m, 2H), 1.32 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 157.0, 80.5, 73.1, 70.4, 67.1, 50.1, 28.4, 27.1, 23.6.

IR (ATR, neat, cm^{-1}) 3365, 2094, 1688, 1524, 1364, 1252, 1159, 1047, 991.

m.p. 116 - 117 °C.



188 **189**

Amine **189** was synthesized following **General Procedure D**, starting from azide **188**. The product was obtained as a light-yellow solid (94%).

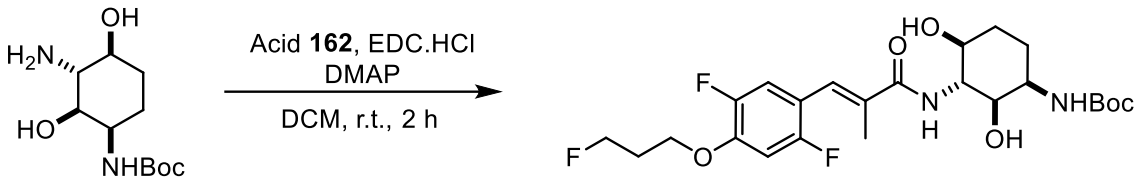
R_f 0.32 (SiO_2, EtOAc.MeOH 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 3.76 – 3.72 (m, 1H), 3.29 (dd, J = 10.0, 4.4 Hz, 1H), 3.22 – 3.14 (m, 2H), 2.60 (t, J = 9.5 Hz, 1H), 1.79 – 1.75 (m, 1H), 1.63 – 1.58 (m, 1H), 1.42 – 1.31 (m, 11 H).

^{13}C NMR (101 MHz, CD_3OD) δ 157.3, 79.0, 72.8, 72.4, 56.6, 50.6, 27.3, 25.0.

IR (ATR, neat, cm^{-1}) 3354, 3280, 2866, 1703, 1505, 1394, 1244, 1155, 1039, 849.

m.p. 172 °C.



189 **190**

Amide **190** was synthesized following **General Procedure H**, starting from amine **189**. The product was obtained as a white solid (76%).

R_f 0.35 (SiO_2, Hex:EtOAc 3:7, UV-Vis, Vanillin).

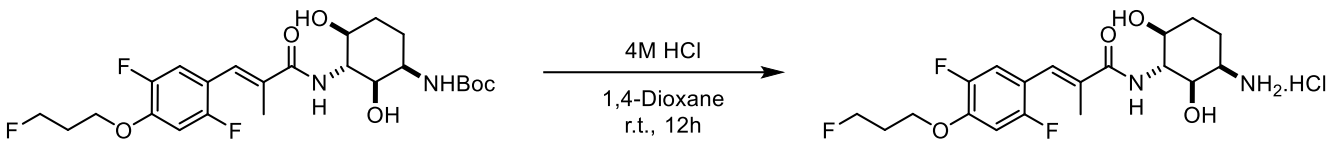
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (dd, J = 11.5, 6.9 Hz, 1H), 6.66 (dd, J = 11.0, 7.0 Hz, 1H), 6.41 (s, 1H), 5.09 – 4.83 (m, 2H), 4.65 (t, J = 5.6 Hz, 1H), 4.56 – 4.46 (m, 2H), 4.09 (t, J = 6.1 Hz, 2H), 3.99 – 3.83 (m, 2H), 3.63 – 3.50 (m, 2H), 2.15 (dp, J = 26.3, 5.9 Hz, 2H), 1.97 (s, 3H), 1.91 – 1.79 (m, 2H), 1.66 – 1.50 (m, 2H), 1.38 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 156.3 (d, J = 249.0 Hz), 149.4 – 147.0 (m), 133.0, 126.8, 116.7 (dd, J = 21.0, 4.8 Hz), 115.4 (dd, J = 16.4, 6.6 Hz), 102.6 (d, J = 28.4 Hz),81.1, 80.0 (d, J = 117.4 Hz), 72.9, 72.3, 65.2 (d, J = 4.8 Hz), 58.2, 30.2 (d, J = 20.2 Hz), 29.7, 28.4, 24.6, 22.7, 14.5 (d, J = 1.9 Hz), 14.1.

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.2 (d, J = 14.0 Hz), -139.6 (d, J = 17.7 Hz), -222.8.

IR (ATR, neat, cm^{-1}) 3429, 3295, 2926, 1722, 1632, 1513, 1319, 1241, 1162, 1043.

m.p. 110 °C.



190 **191**

Amide **191** was synthesized following **General Procedure C**, starting from Boc-protected amide **190**. The product was obtained as a white solid (88%).

R_f 0.25 (SiO_2, EtOAc:MeOH 8:2, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 7.15 (t, J = 1.5 Hz, 1H), 7.09 (dd, J = 11.7, 7.0 Hz, 1H), 6.89 (dd, J = 11.3, 7.1 Hz, 1H), 4.60 (t, J = 5.8 Hz, 1H), 4.48 (t, J = 5.8 Hz, 1H), 4.09 (t, J = 6.1 Hz, 2H), 4.02 (t, J = 9.4 Hz, 1H), 3.84 (dd, J = 9.8, 4.4 Hz, 1H), 3.62 (td, J = 9.5, 9.3, 4.1, 3.9 Hz, 1H), 3.55 – 3.51 (m, 1H), 2.10 (dp, J = 25.4, 6.0 Hz, 2H), 1.99 – 1.62 (m, 7H).

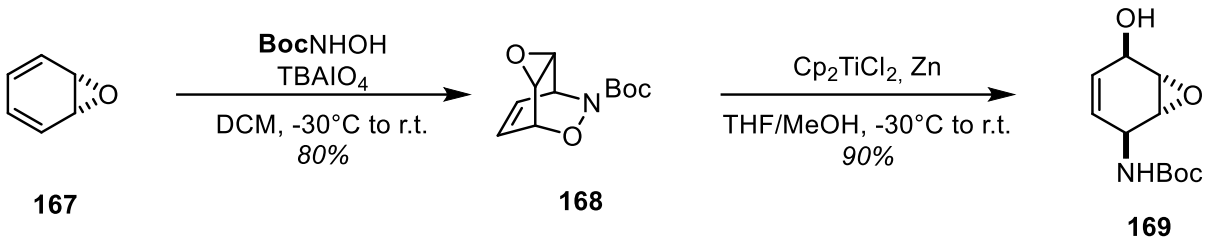
^{13}C NMR (101 MHz, CD_3OD) δ 172.0, 156.3 (d, J = 244.9 Hz), 149.4 – 147.0 (m), 134.1, 125.5, 116.3 (dd, J = 21.0, 5.0 Hz), 115.4 (dd, J = 16.8, 6.7 Hz), 102.4 (d, J = 29.0 Hz), 80.0 (d, J = 163.7 Hz), 69.6, 68.9, 65.2 (d, J = 5.5 Hz), 56.1, 51.7, 29.9 (d, J = 20.2 Hz), 26.9, 22.2, 13.7 (d, J = 2.1 Hz).

^{19}F NMR (376 MHz, CD_3OD) δ -117.1 (J = 14.7 Hz), -141.6 (J = 13.7 Hz), -224.1.

IR (ATR, neat, cm^{-1}) 3335, 2912, 1709, 1631, 1500, 1374, 1223, 1160, 1041, 965.

m.p. 148 – 150 °C.

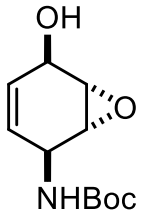
11.4 **Dearomative epoxidation-derived analogues**



167 **168** **169**

Amine **169** was synthesized starting from benzene oxide **167**. Initially, following **General Procedure A** for Nitroso Diels-Alder reaction, bicycle **168** was obtained, which was subjected to **General procedure B** for N-O cleavage, affording intermediate **169**.

Analytical and spectroscopic characterization of product **168** is consistent with the one reported in literature. Characterization for epoxide **169** is reported below.



169

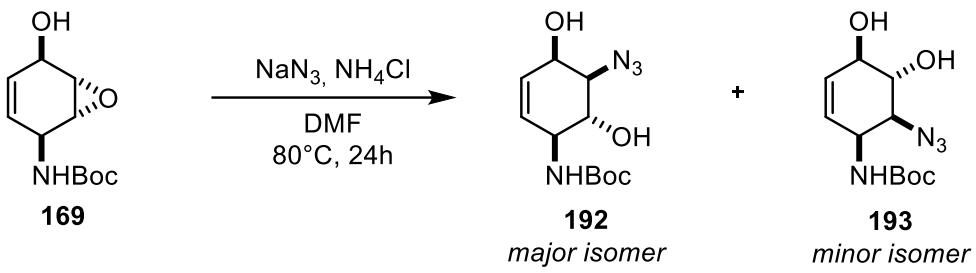
R_f 0.20 (SiO_2, Hex:EtOAc 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 5.72 (ddt, J = 10.2, 4.6, 1.6 Hz, 1H), 5.54 (ddd, J = 10.5, 4.7, 1.9 Hz, 1H), 4.36 – 4.26 (m, 2H), 3.20 (dq, J = 3.2, 1.6 Hz, 1H), 3.16 (dt, J = 3.1, 1.8 Hz, 1H), 1.46 (s, 9H).

^{13}C NMR (101 MHz, CD_3OD) δ 157.7, 127.8, 125.5, 80.7, 63.0, 53.9, 53.3, 45.4, 28.7.

IR (ATR, neat, cm^{-1}) 3287, 2974, 2903, 2564, 2415, 1681, 1550, 1423, 1367, 1252, 1155, 1043, 1017, 872, 726.

m.p. 119 – 120 °C.



Amine **169** was subjected to epoxide opening following **General Procedure F**, affording two different regioisomers. The products were obtained as white solids (63% total yield).

Major Isomer

R_f 0.47 (SiO_2, Hex:EtOAc 4:6, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 – 5.79 (m, 1H), 5.59 (dd, J = 9.9, 2.3 Hz, 1H), 5.10 (d, J = 8.1 Hz, 1H), 4.26 (t, J = 4.4 Hz, 1H), 4.19 – 4.02 (m, 2H), 3.93 – 3.89 (m, 1H), 3.48 (dd, J = 10.2, 3.8 Hz, 1H), 3.02 (s, 1H), 1.39 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 157.0, 130.4, 127.9, 80.8, 72.1, 66.0, 65.2, 55.0, 28.3.

IR (ATR, neat, cm^{-1}) 3336, 2098, 1684, 1517, 1364, 1241, 1162, 1047, 995, 756.

m.p. 77 - 79 °C.

Minor Isomer

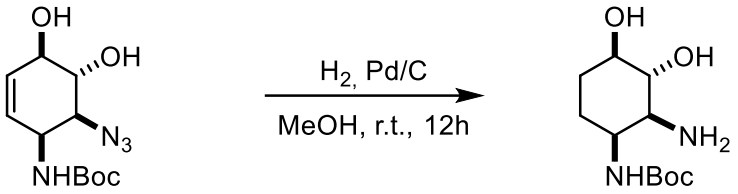
R_f 0.26 (SiO_2, Hex:EtOAc 4:6, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 – 5.71 (m, 2H), 4.99 – 4.96 (m, 1H), 4.70 – 4.40 (m, 2H), 4.18 – 4.16 (m, 1H), 4.07 – 3.85 (m, 1H), 3.75 – 3.55 (m, 2H), 1.49 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 155.5, 131.2, 126.0, 80.3, 72.2, 72.1, 63.2, 48.2, 28.3.

IR (ATR, neat, cm^{-1}) 3305, 2898, 1670, 1532, 1421, 1341, 1148, 1065, 995, 814.

m.p. 70 °C.



193 **194**

Amine **194** was synthesized following **General Procedure D**, starting from azide **193**. The product was obtained as a light-yellow solid (83%).

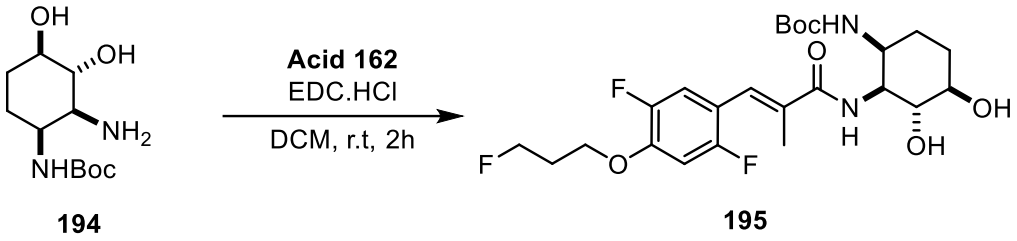
R_f 0.38 (SiO_2, EtOAc.MeOH 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) 5.43 (s, 1H), 3.87 – 3.68 (m, 1H), 3.68 – 3.20 (m, 6H), 2.72 (dd, J = 10.2, 4.1 Hz, 1H), 1.86 – 1.74 (m, 2H), 1.51 – 1.32 (m, 11H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.4, 79.6, 75.6, 73.1, 55.7, 50.8, 28.4, 27.3, 26.7.

IR (ATR, neat, cm^{-1}) 3369, 3280, 3160, 2858, 1695, 1595, 1509, 1364, 1174, 1080, 887.

m.p. 126 °C



194 195

Amide **195** was synthesized following **General Procedure H**, starting from amine **194**. The product was obtained as a white solid (69%).

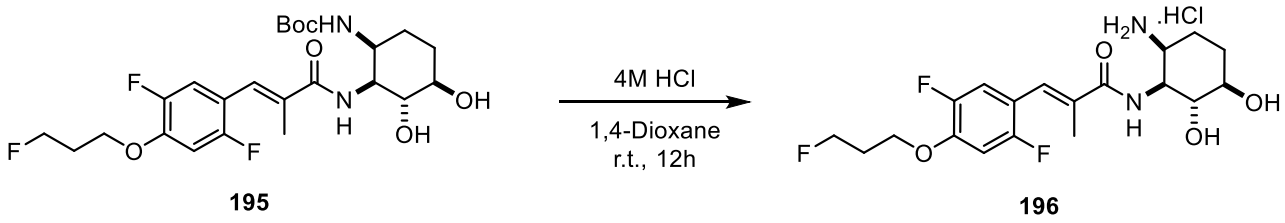
R_f 0.35 (SiO_2, Hex:EtOAc 3:7, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 8.02 (m, 1H), 7.33 (s, 1H), 6.98 (dd, J = 11.6, 6.9 Hz, 1H), 6.66 (dd, J = 10.9, 7.0 Hz, 1H), 5.00 – 4.85 (m, 2H), 4.65 (t, J = 5.7 Hz, 1H), 4.54 (t, J = 5.7 Hz, 1H), 4.10 – 4.01 (m, 3H), 3.89 – 3.85 (m, 1H), 3.56 – 3.42 (m, 2H), 3.16 (br s, 1H), 2.15 (dp, J = 26.3, 5.9 Hz, 2H), 2.01 – 1.90 (m, 3H), 1.78 – 1.67 (m, 2H), 1.41 – 1.33 (m, 10H), 1.22 – 1.17 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 156.3 (d, J = 248.1 Hz), 149.4 – 147.0 (m), 132.3, 127.5, 116.7 (dd, J = 21.0, 4.9 Hz), 115.7 (dd, J = 16.4, 6.6 Hz), 102.6 (d, J = 28.4 Hz), 80.3 (d, J = 165.6 Hz), 74.5, 65.2 (d, J = 4.9 Hz), 60.4, 57.8, 50.1, 30.2 (d, J = 20.1 Hz), 28.3, 26.9, 26.1, 14.2.

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.0 (d, J = 14.2 Hz), -139.8 (d, J = 15.8 Hz), -222.8.

IR (ATR, neat, cm^{-1}) 3352, 2951, 1694, 1533, 1425, 1346, 1293, 1171, 1040, 882.



Amide **196** was synthesized following **General Procedure C**, starting from Boc-protected amide **195**. The product was obtained as a white solid (90%).

R_f 0.32 (SiO_2, EtOAc:MeOH 8:2, UV-Vis, Vanillin).

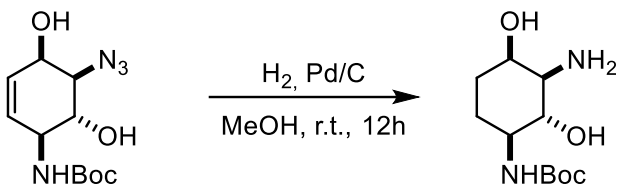
^{1}H NMR (400 MHz, CD_3OD) δ 7.40 (t, J = 1.6 Hz, 1H), 7.21 (dd, J = 11.7, 7.0 Hz, 1H), 7.03 (dd, J = 11.4, 7.1 Hz, 1H), 4.71 (t, J = 5.8 Hz, 1H), 4.59 (t, J = 5.8 Hz, 1H), 4.47 – 4.44 (m, 1H), 4.22 (t, J = 6.1 Hz, 2H), 3.95 – 3.90 (m, 2H), 3.71 (dt, J = 11.6, 3.7 Hz, 1H), 3.34 – 3.32 (m, 1H), 2.21 (dp, J = 25.4, 6.0 Hz, 2H), 2.07 (d, J = 1.4 Hz, 3H), 2.04 – 1.93 (m, 2H), 1.88 – 1.83 (m, 1H), 1.75 – 1.70 (m, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 169.9, 156.5 (d, J = 245 Hz), 149.5 – 147.1 (m), 132.5, 126.8, 116.2 (dd, J = 21.2, 4.8 Hz), 115.1 (dd, J = 16.7, 6.7 Hz), 102.4 (d, J = 28.9 Hz), 80.0 (d, J = 163.8 Hz), 69.6, 68.4, 65.2 (d, J = 5.4 Hz), 51.8, 49.4, 29.9 (d, J = 20.1 Hz), 25.9, 19.6, 12.9 (d, J = 1.8 Hz).

^{19}F NMR (376 MHz, CD_3OD) δ -117.4 (d, J = 15.3), -141.6, -224.4.

IR (ATR, neat, cm^{-1}) 3425, 2950, 2913, 1680, 1543, 1468, 1290, 1159, 1079, 978, 872.

m.p. 221 °C.



192 **197**

Amine **197** was synthesized following **General Procedure D**, starting from azide **192**. The product was obtained as a light-yellow solid (95%).

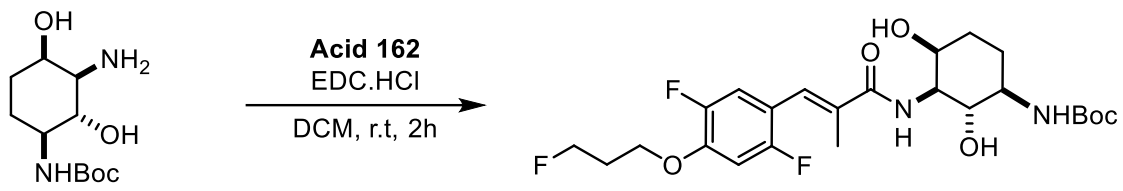
R_f 0.36 (SiO_2, EtOAc.MeOH 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 4.01 – 3.99 (m, 1H), 3.39 – 3.30 (m, 3H), 2.57 (d, J = 9.0 Hz), 1.84 – 1.79 (m, 1H), 1.75 – 1.54 (m, 3H), 1.46 (s, 9H).

^{13}C NMR (101 MHz, CD_3OD) δ 157.1, 78.6, 73.2, 68.0, 58.1, 54.7, 29.7, 27.4, 25.4.

IR (ATR, neat, cm^{-1}) 3317, 2974, 2482, 1707, 1520, 1412, 1364, 1162, 1095, 1013.

m.p. 210 °C.



197 **198**

Amide **198** was synthesized following **General Procedure H**, starting from amine **197**. The product was obtained as a white solid (78%).

R_f 0.35 (SiO_2, Hex:EtOAc 3:7, UV-Vis, Vanillin).

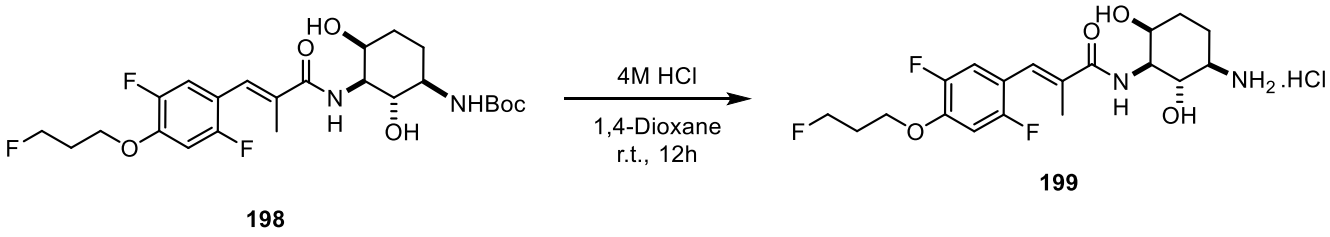
^{1}H NMR (400 MHz, Acetone-*d*6) δ 7.13 (s, 1H), 7.06 (dd, J = 11.9, 7.0 Hz, 1H), 6.98 (d, J = 7.8 Hz, 1H), 6.91 (dd, J = 11.4, 7.2 Hz, 1H), 5.87 – 5.78 (m, 1H), 4.58 (t, J = 5.8 Hz, 1H), 4.47 (t, J = 5.8 Hz, 1H), 4.32 – 4.22 (m, 1H), 4.12 (t, J = 6.2 Hz, 2H), 3.99 – 3.91 (m, 2H), 3.80 – 3.75 (m, 1H), 3.65 – 3.59 (m, 1H), 2.09 (dp, J = 25.4, 6.1 Hz, 2H), 1.90 (s, 3H), 1.70 – 1.62 (m, 3H), 1.56 – 1.48 (m, 1H), 1.27 (s, 9H).

^{13}C NMR (101 MHz, Acetone-*d*6) δ 168.9, 157.5 – 155.0 (m), 149.3 – 146.9 (m), 134.6, 124.7, 116.6 (dd, J = 21.0, 5.1 Hz), 115.8 (dd, J = 16.8, 6.8 Hz), 102.6 (d, J = 29.0 Hz), 80.3 (d, J = 163.1 Hz), 77.9, 71.8, 68.1, 65.3 (d, J = 5.6 Hz), 58.1, 55.7, 27.8, 25.4, 13.9 (d, J = 1.7 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -116.9 (d, J = 14.6 Hz), -141.0, -222.3.

IR (ATR, neat, cm^{-1}) 3350, 1651, 1617, 1517, 1390, 1315, 1282, 1162, 1043, 943.

m.p. 110 °C.



198 **199**

Amide **199** was synthesized following **General Procedure C**, starting from Boc-protected amide **198**. The product was obtained as a white solid (94%).

R_f 0.23 (SiO_2, EtOAc.MeOH 7:3, UV-Vis, Vanillin).

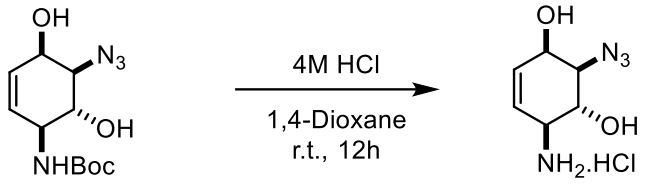
^{1}H NMR (400 MHz, CD_3OD) δ 7.18 (t, J = 1.5 Hz, 1H), 7.09 (dd, J = 11.7, 6.9 Hz, 1H), 6.90 (dd, J = 11.4, 7.2 Hz, 1H), 4.60 (t, J = 5.8 Hz, 1H), 4.48 (t, J = 5.8 Hz, 1H), 4.10 (t, J = 6.1 Hz, 2H), 4.00 – 3.98 (m, 1H), 3.84 – 3.73 (m, 2H), 3.03 – 2.97 (m, 1H), 2.10 (dp, J = 25.4, 6.0 Hz, 2H), 1.97 (s, 3H), 1.87 – 1.79 (m, 3H), 1.66 – 1.57 (m, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 170.6, 156.3 (d, J = 244.8 Hz), 149.5 – 147.0 (m), 133.8, 125.6, 116.3 (dd, J = 21.2, 4.9 Hz), 115.4 (dd, J = 16.8, 6.6 Hz), 102.4 (d, J = 28.8 Hz), 80.0 (d, J = 163.8 Hz), 69.1, 67.6, 66.8, 65.2 (d, J = 5.5 Hz), 57.1, 55.0, 30.0, 29.8, 28.9, 22.6, 13.4 (d, J = 1.9 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -117.3, -141.6, -224.2.

IR (ATR, neat, cm^{-1}) 3317, 2922, 1722, 1625, 1513, 1334, 1267, 1170, 1043.

m.p. 136 - 138 °C.



192 **200**

Amine **200** was synthesized following **General Procedure C**, starting from Boc-protected amine **192**. The product was obtained as a white solid (99%).

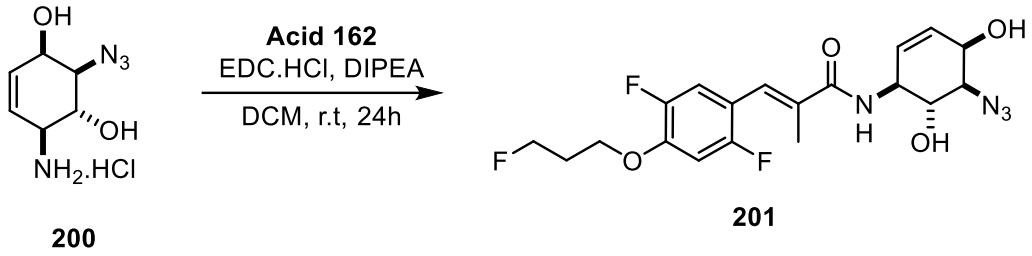
R_f 0.17 (SiO_2, EtOAc.MeOH 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 6.07 (ddd, J = 10.0, 5.0, 2.5 Hz, 1H), 5.78 (dd, J = 10.0, 2.1 Hz, 1H), 4.42 – 4.40 (m, 1H), 3.98 (dd, J = 10.5, 8.5 Hz, 1H), 3.76 – 3.73 (m, 1H), 3.52 (dd, J = 10.5, 4.0 Hz, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 131.7, 124.1, 67.6, 65.7, 64.6, 54.5.

IR (ATR, neat, cm^{-1}) 3459, 3403, 2113, 1617, 1517, 1401, 1308, 1267, 1084, 957.

m.p. 230 °C.



200 **201**

Amide **201** was synthesized following **General Procedure I**, starting from amine **200**. The product was obtained as a white solid (68%).

R_f 0.41 (SiO_2, Hex:EtOAc 3:7, UV-Vis, Vanillin).

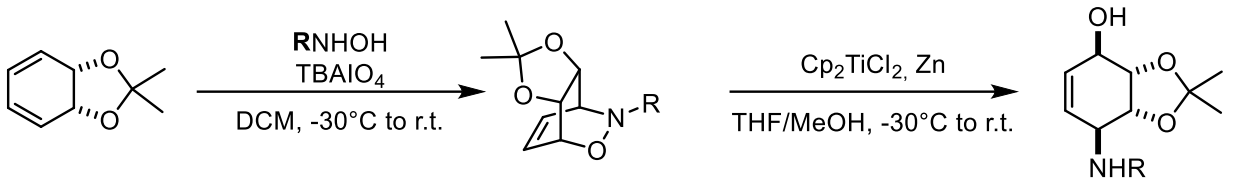
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, J = 1.6 Hz, 1H), 7.05 (dd, J = 11.5, 6.9 Hz, 1H), 6.74 (dd, J = 11.0, 7.0 Hz, 1H), 6.64 (d, J = 8.0 Hz, 1H), 5.97 (ddd, J = 9.9, 4.9, 2.4 Hz, 1H), 5.72 (dd, J = 10.0, 2.6 Hz, 1H), 4.74 (t, J = 5.6 Hz, 1H), 4.64 – 4.56 (m, 3H), 4.42 – 4.37 (m, 1H), 4.20 – 4.08 (m, 3H), 3.65 (dd, J = 9.9, 3.8 Hz, 1H), 3.24 (d, J = 4.7 Hz, 1H), 2.23 (dp, J = 26.3, 5.9 Hz, 2H), 2.04 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 156.3 (d, J = 246.6 Hz), 149.5 – 147.0 (m), 132.7, 129.6, 128.5, 126.8, 116.6 (dd, J = 21.1, 4.8 Hz), 115.3 (dd, J = 16.3, 6.7 Hz), 102.5 (d, J = 28.3 Hz), 80.2 (d, J = 164.9 Hz), 71.6, 66.1, 65.1 (d, J = 1.4 Hz), 54.3, 30.2 (d, J = 20.2), 14.5 (d, J = 1.7 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.4, -139.4, -222.8.

IR (ATR, neat, cm^{-1}) 3402,1689, 1533, 1401, 1336, 1291, 1150, 1060, 860.

11.5 **Dearomative dihydroxylation-derived analogues**

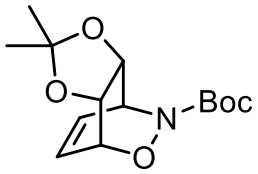


170

171a, R=Boc, *75%*
171b, R=Cbz, *82%*

172a, R=Boc, *94%*
172b, R=Cbz, *89%*

Amine **172** was synthesized starting from protected diol **170**. Initially, following **General Procedure A** for Nitroso Diels-Alder reaction, bicycle **171** was obtained, which was subjected to **General procedure B** for N-O cleavage, affording intermediate **172**. Two different Hydroxylamine derivatives were used, with Cbz and Boc protecting groups.



171a

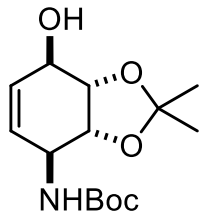
R_f 0.40 (SiO_2, Hex:EtOAc 8:2, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.49 – 6.36 (m, 2H), 4.98 (ddd, J = 5.8, 4.0, 2.1 Hz, 1H), 4.87 (ddd, J = 5.8, 4.3, 1.8 Hz, 1H), 4.57 – 4.46 (m, 2H), 1.45 (s, 9H), 1.31 (s, 3H), 1.30 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 130.5, 129.6, 111.0, 82.6, 73.4, 72.9, 71.2, 53.2, 28.2, 25.7, 25.5.

IR (ATR, neat, cm^{-1}) 2981, 2933, 1707, 1371, 1252, 1207, 991, 834, 726.

m.p. 130 - 132 °C.



172a

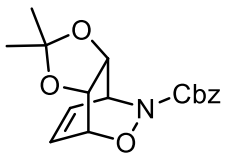
R_f 0.39 (SiO_2, Hex:EtOAc 3:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 5.77 (dt, J = 9.9, 2.6 Hz, 1H), 5.61 (dt, J = 9.8, 2.7 Hz, 1H), 4.13 (dq, J = 4.4, 2.4 Hz, 1H), 4.11 – 4.03 (m, 2H), 3.97 (d, J = 5.9 Hz, 1H), 1.45 (m, 12H), 1.34 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 157.9, 132.7, 130.9, 110.1, 81.1, 80.4, 78.1, 71.0, 52.5, 28.7, 27.6, 25.1.

IR (ATR, neat, cm^{-1}) 3518, 3488, 3384, 2929, 1684, 1513, 1297, 1084, 730.

m.p. 109 - 110 °C.



171b

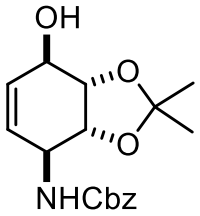
R_f 0.36 (SiO_2, Hex:EtOAc 8:2, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.17 (m, 5H), 6.34 – 6.23 (m, 2H), 5.11 – 4.96 (m, 2H), 4.93 (ddd, J = 5.9, 4.0, 2.1 Hz, 1H), 4.76 (ddd, J = 5.9, 4.3, 1.9 Hz, 1H), 4.43 – 4.36 (m, 2H), 1.17 (s, 3H), 1.16 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 157.9, 135.6, 130.6, 129.5, 128.6, 128.5, 128.4, 128.1, 127.0, 111.0, 73.1, 72.5, 71.3, 68.2, 53.0, 25.6, 25.4.

IR (ATR, neat, cm^{-1}) 2989, 2942, 1716, 1444, 1379, 1310, 1243, 1203, 1066.

m.p. 68 – 71 °C.



172b

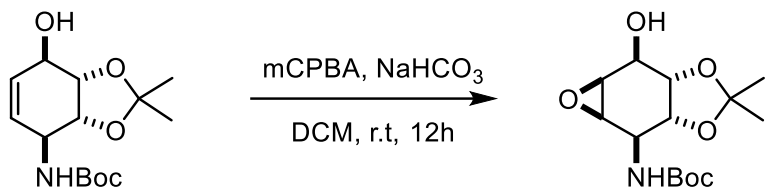
R_f 0.34 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.18 (m, 5H), 5.83 (ddd, J = 9.9, 3.3, 2.1 Hz, 1H), 5.70 (ddd, J = 9.9, 3.8, 1.7 Hz, 1H), 5.30 (d, J = 8.2 Hz, 1H), 5.06 – 4.95 (m, 2H), 4.19 – 4.08 (m, 3H), 4.04 (ddt, J = 10.0, 4.6, 2.1 Hz, 1H), 2.85 (d, J = 4.4 Hz, 1H), 1.35 (s, 3H), 1.24 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.1, 136.3, 131.3, 129.7, 128.6, 128.3, 109.3, 79.3, 69.1, 67.1, 51.3, 27.0, 24.8.

IR (ATR, neat, cm^{-1}) 3322, 2983, 2935, 2901, 1697, 1526, 1254, 1213, 1060.

m.p. 125 °C.



172a **202**

Epoxide **202** was synthesized following **General Procedure E**, starting from olefin **172a**. The product was obtained as a white solid (85%).

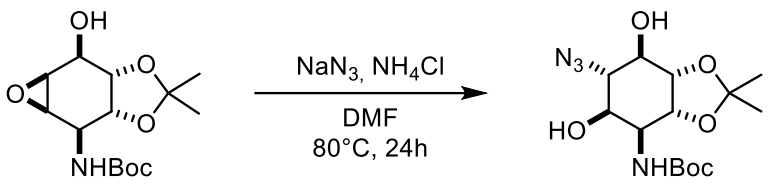
R_f 0.45 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.17 – 5.13 (m, 1H), 4.17 – 4.10 (m, 2H), 4.06 – 3.98 (m, 2H), 3.46 – 3.32 (m, 3H), 1.46 (s, 12H), 1.30 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 155.5, 108.3, 80.2, 78.5, 75.5, 71.5, 55.2, 54.5, 51.9, 28.3, 26.9, 24.1.

IR (ATR, neat, cm^{-1}) 3350, 2981, 1684, 1520, 1237, 1166, 1058, 943.

m.p. 91 °C.



202 **203**

Azide-substituted cyclohexane **203** was synthesized following **General Procedure F**, starting from epoxide **202**. The product was obtained as a white solid (68%).

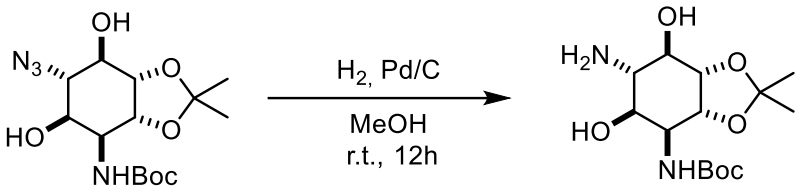
R_f 0.50 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 4.08 (t, J = 5.6 Hz, 1H), 3.93 (dd, J = 7.8, 6.0 Hz, 1H), 3.86 (s, 1H), 3.60 (dd, J = 7.2, 4.1 Hz, 1H), 3.38 (dd, J = 9.4, 7.8 Hz, 1H), 3.25 (dd, J = 9.4, 7.2 Hz, 1H), 1.32 (s, 12H), 1.18 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 156.8, 109.1, 79.2, 78.1, 76.0, 73.7, 69.8, 66.7, 51.6, 27.3, 26.8, 24.4.

IR (ATR, neat, cm^{-1}) 3444, 3373, 2985, 2105, 1688, 1520, 1364, 1244, 1155, 1073.

m.p. 181 °C.



203 **204**

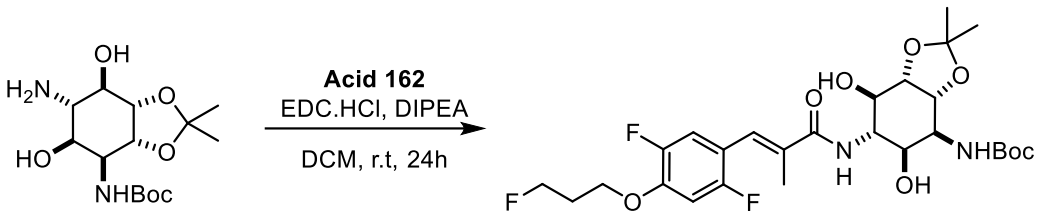
Amine **204** was synthesized following **General Procedure D**, starting from azide **203**. The product was obtained as a light-yellow oil (91%).

R_f 0.78 (SiO_2, EtOAc.MeOH 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (br s, 1H), 4.31 – 3.37 (m, 8H), 2.72 (t, J = 8.6 Hz), 1.40 (s, 3H), 1.36 (s, 9H), 1.26 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.5, 109.2, 80.1, 78.8, 74.5, 71.0, 54.9, 53.4, 28.4, 28.0, 25.8.

IR (ATR, neat, cm^{-1}) 3343, 2981, 2933, 1684, 1509, 1367, 1244, 1162, 1043, 864.



204 **205**

Amide **205** was synthesized following **General Procedure H**, starting from amine **204**. The product was obtained as a colorless oil (87%).

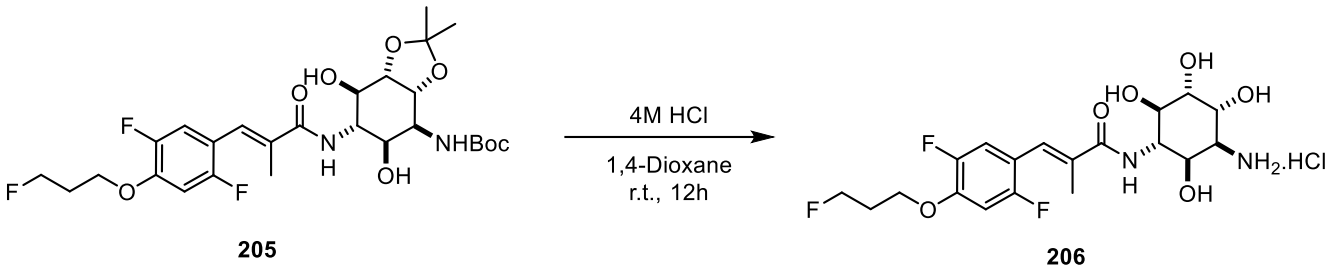
R_f 0.33 (SiO_2, Hex:EtOAc 4:6, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (d, J = 2.8 Hz, 1H), 7.00 – 6.84 (m, 2H), 6.65 (dd, J = 11.0, 7.0 Hz, 1H), 5.30 (br s, 1H), 5.08 (s, 1H), 4.65 (t, J = 5.7 Hz, 1H), 4.58 – 4.49 (m, 2H), 4.37 – 4.18 (m, 3H), 4.11 – 4.00 (m, 4H), 3.91 – 3.82 (m, 1H), 2.15 (dp, J = 26.2, 5.9 Hz, 2H), 1.93 (s, 3H), 1.51 (s, 3H), 1.36 (s, 9H), 1.32 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.3, 157.5 – 155.8 (m), 149.4 – 147.0 (m), 132.2, 127.1, 116.6 (dd, J = 20.9, 4.9 Hz), 115.3 (dd, J = 16.3, 6.7 Hz), 109.6, 102.5 (d, J = 28.2 Hz), 81.0 – 79.4 (m), 78.4, 75.5, 72.6, 70.0, 65.15 (d, J = 4.8 Hz), 54.9, 51.3, 30.2 (d, J = 20.1 Hz), 28.4, 27.9, 25.7, 14.30 (d, J = 1.8 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.0, -139.5, -222.8.

IR (ATR, neat, cm^{-1}) 3332, 2981, 2933, 1692, 1513, 1338, 1263, 1162, 1047, 872.



205 **206**

Amide **206** was synthesized following **General Procedure C**, starting from Boc-protected and acetal-protected amide **205**. The product was obtained as a white solid (98%).

R_f 0.19 (SiO_2, EtOAc:MeOH 7:3, UV-Vis, Vanillin).

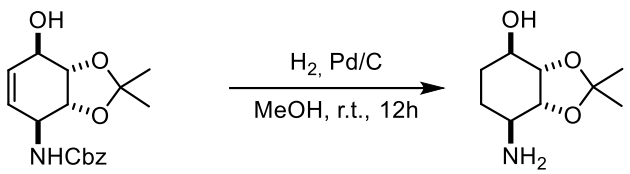
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (t, J = 1.5 Hz, 1H), 7.07 (dd, J = 11.7, 6.9 Hz, 1H), 6.91 (dd, J = 11.4, 7.1 Hz, 1H), 4.59 (t, J = 5.8 Hz, 1H), 4.47 (t, J = 5.8 Hz, 1H), 4.30 – 4.24 (m, 1H), 4.16 – 4.05 (m, 4H), 4.05 – 3.99 (m, 1H), 3.98 – 3.94 (m, 1H), 3.47 (dd, J = 10.2, 3.3 Hz, 1H), 2.10 (dp, J = 25.4, 6.1 Hz, 6H), 1.91 (t, J = 1.4 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.6, 156.4 (d, J = 244.7 Hz), 149.5 – 147.1 (m), 132.7, 126.3, 116.2 (dd, J = 21.2, 4.8 Hz), 115.0 (dd, J = 16.6, 6.8 Hz), 102.4 (d, J = 28.8 Hz), 80.0 (d, J = 163.8 Hz), 73.2, 71.2, 69.4, 65.2 (d, J = 5.4 Hz), 52.7, 51.8, 29.9 (d, J = 20.2 Hz), 13.0 (d, J = 1.7 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -117.5, -141.5, -224.3.

IR (ATR, neat, cm^{-1}) 33393164, 2926, 1654, 1628, 1509, 1423, 1338, 1233, 1174, 1043, 909.

m.p. 215 - 217 °C.



172b **207**

Amine **207** was synthesized following **General Procedure D**, starting from Cbz-protected amine **172b**. The product was obtained as a white solid (91%).

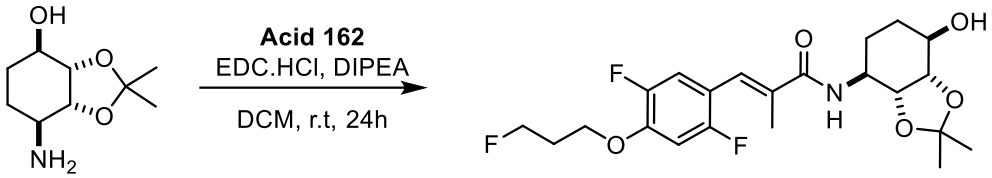
R_f 0.32 (SiO_2, CH_2Cl_2:MeOH 9:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 4.36 – 3.62 (m, 3H), 2.89 (s, 1H), 1.64 (dd, J = 47.9, 28.9 Hz, 4H), 1.41 (d, J = 48.3 Hz, 6H).

^{13}C NMR (101 MHz, CD_3OD) δ 109.6, 81.3, 79.8, 68.9, 52.4, 28.5, 27.8, 26.4, 25.9.

IR (ATR, neat, cm^{-1}) 2981, 2935, 2871, 1373, 1215, 1062, 1043, 1011.

m.p. 128 °C.



207 **208**

Amide **208** was synthesized following **General Procedure I**, starting from amine **207**. The product was obtained as a white solid (76%).

R_f 0.23 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin).

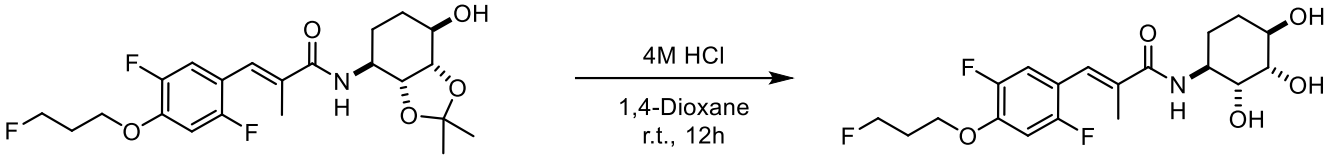
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (s, 1H), 7.00 (dd, J = 11.6, 6.9 Hz, 1H), 6.69 (dd, J = 11.0, 7.0 Hz, 1H), 6.51 (d, J = 8.3 Hz, 1H), 4.69 (t, J = 5.7 Hz, 1H), 4.58 (t, J = 5.7 Hz, 1H), 4.20 (tt, J = 7.9, 3.6 Hz, 1H), 4.16 – 4.03 (m, 5H), 3.35 – 2.96 (m, 1H), 2.19 (dp, J = 26.2, 5.8 Hz, 2H), 2.00 – 1.95 (m, 3H), 1.89 (tdd, J = 12.1, 10.2, 9.5, 4.6 Hz, 2H), 1.65 (tdt, J = 15.0, 10.7, 4.7 Hz, 2H), 1.48 (s, 3H), 1.32 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.6, 156.2 (dd, J = 246.2, 2.4 Hz), 151.6 – 144.8 (m), 133.8, 125.6, 116.6 (dd, J = 20.9, 4.9 Hz), 115.6 (dd, J = 16.6, 6.6 Hz), 108.9, 102.5 (dd, J = 28.5, 2.2 Hz), 83.2 – 74.4 (m), 67.8, 65.14(d, J = 4.9 Hz), 48.9, 30.1 (d, J = 20.0 Hz), 25.8 (d, J = 14.6 Hz), 14.4 (d, J = 1.8 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.5 (d, J = 14.3 Hz), -139.7 (d, J = 15.2 Hz), -222.7.

IR (ATR, neat, cm^{-1}) 3313, 2976, 2931, 1612, 1511, 1315, 1213, 1172, 1097, 1049.

m.p. 80 - 83 °C.



208 **209**

Amide **209** was synthesized following **General Procedure C**, starting from Boc-protected and acetal-protected amide **208**. The product was obtained as a white solid (80%).

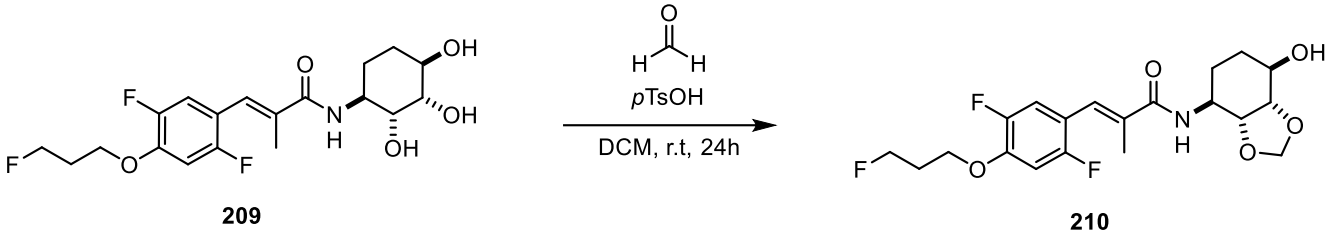
R_f 0.39 (SiO_2, CH_2Cl_2:MeOH 9:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 7.20 – 7.08 (m, 2H), 6.94 (dd, J = 11.3, 7.1 Hz, 1H), 4.64 (t, J = 5.8 Hz, 1H), 4.52 (t, J = 5.8 Hz, 1H), 4.12 (dt, J = 14.5, 7.4 Hz, 3H), 3.89 (dtd, J = 12.2, 9.7, 8.4, 2.9 Hz, 3H), 2.14 (dp, J = 25.0, 5.8 Hz, 2H), 2.00 (t, J = 1.4 Hz, 3H), 1.93 – 1.55 (m, 4H).

^{13}C NMR (101 MHz, CD_3OD) δ 171.8, 156.3 (dd, J = 245.2, 2.2 Hz), 151.5 – 144.8 (m), 132.9, 126.2 (d, J = 2.2 Hz), 116.2 (dd, J = 21.2, 4.8 Hz), 115.0 (dd, J = 16.5, 6.7 Hz), 102.4 (dd, J = 29.0, 2.0 Hz), 80.1 (d, J = 163.5 Hz), 75.2 – 67.4 (m), 65.2 (d, J = 5.4 Hz), 50.5, 29.8 (d, J = 20.1 Hz), 25.7, 24.4, 13.7 (d, J = 1.9 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -117.2 (d, J = 16.2 Hz), -141.60(d, J = 15.9 Hz), -224.1.

IR (ATR, neat, cm^{-1}) 3289, 2927, 1626, 1511, 1338, 1172, 1144, 1112, 1093, 1047.



Amide **209** (1.0 eq.) was solubilized in DCM (0.1M), then pTsOH (0.1 eq.) was added, followed by the addition of paraformaldehyde (30.0 eq.). The reaction was stirred at room temperature for 24 hours, then it was filtered over a celite plug, washing with DCM. The organic solvent was evaporated, and the crude was purified by silica gel column chromatography. The product was obtained as a white solid (56%).

R_f 0.34 (SiO_2, Hex:EtOAc 3:7, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (t, J = 1.6 Hz, 1H), 6.98 (dd, J = 11.6, 6.9 Hz, 1H), 6.67 (dd, J = 11.0, 7.0 Hz, 1H), 6.10 (d, J = 8.1 Hz, 1H), 5.14 (d, J = 0.8 Hz, 1H), 4.90 (d, J = 0.8 Hz, 1H), 4.66 (t, J = 5.7 Hz, 1H), 4.54 (t, J = 5.7 Hz, 1H), 4.19 – 4.00 (m, 4H), 3.93 (dd, J = 5.3, 4.1 Hz, 1H), 2.15 (dp, J = 26.3, 5.9 Hz, 2H), 1.96 (s, 3H), 1.91 – 1.83 (m, 1H), 1.67 – 1.57 (m, 3H).

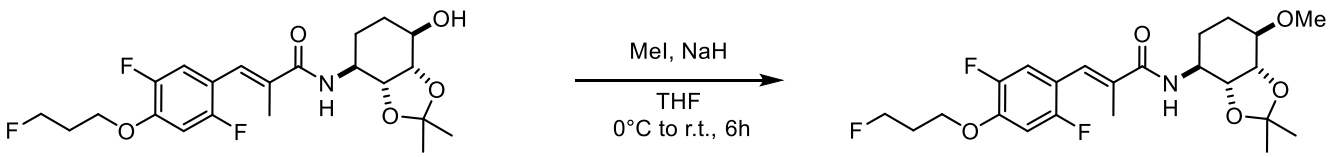
^{13}C NMR (101 MHz, $CDCl_3$) δ 168.7, 156.2 (d, J = 244.0 Hz), 149.5 – 147.1 (m), 133.9, 125.5, 116.7 (dd, J = 20.9, 4.9 Hz), 115.6 (dd, J = 16.5, 6.7 Hz), 102.6 (dd, J =

28.4, 2.2 Hz), 94.6, 80.3 (d, J = 165.0 Hz), 78.6, 67.6, 65.2 (d, J = 4.8 Hz), 47.7, 30.2 (d, J = 20.2 Hz), 26.2, 23.1, 14.6 (d, J = 1.8 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.6 (d, J = 14.2 Hz), -139.6 (d, J = 14.8 Hz), -222.8.

IR (ATR, neat, cm^{-1}) 3309, 2922, 1610, 1513, 1319, 1203, 1170, 1103, 1047, 905.

m.p. 98 °C.



208 **211**

Amide **211** was synthesized following **General Procedure G**, starting from the corresponding alcohol **208**. The product was obtained as a colorless oil (66%).

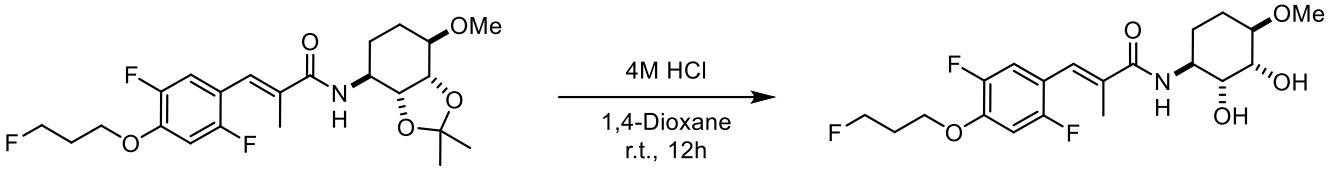
R_f 0.31 (SiO_2, Hex:EtOAc 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (t, J = 1.6 Hz, 1H), 6.89 (dd, J = 11.6, 6.9 Hz, 1H), 6.58 (dd, J = 11.0, 7.1 Hz, 1H), 6.25 (d, J = 8.7 Hz, 1H), 4.57 (t, J = 5.7 Hz, 1H), 4.45 (t, J = 5.7 Hz, 1H), 4.17 – 4.05 (m, 2H), 4.01 (t, J = 6.1 Hz, 2H), 3.95 (t, J = 6.1 Hz, 1H), 3.45 (q, J = 3.5 Hz, 1H), 3.27 (s, 3H), 2.07 (dp, J = 26.3, 5.9 Hz, 2H), 1.86 (t, J = 1.4 Hz, 3H), 1.83 – 1.62 (m, 3H), 1.42 – 1.32 (m, 4H), 1.21 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2, 156.2 (d, J = 246.2 Hz), 149.5 – 147.0 (m), 134.0, 125.4, 116.7 (dd, J = 20.9, 4.9 Hz), 115.8 (dd, J = 16.5, 6.7 Hz), 108.9, 102.6 (dd, J = 28.4, 2.2 Hz), 80.2 (d, J = 165.0 Hz), 75.5, 65.2 (d, J = 4.9 Hz), 57.1, 48.1, 30.2 (d, J = 20.1 Hz), 27.6, 25.6, 22.6, 21.9, 14.5 (d, J = 1.7 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.6 (d, J = 14.3 Hz), -139.7 (d, J = 14.3 Hz), -222.8.

IR (ATR, neat, cm^{-1}) 2929, 1654, 1617, 1513, 1472, 1382, 1330, 1207, 1174, 1051.



211 **212**

Amide **212** was synthesized following **General Procedure C**, starting from acetal-protected amide **211**. The product was obtained as a colorless oil (74%).

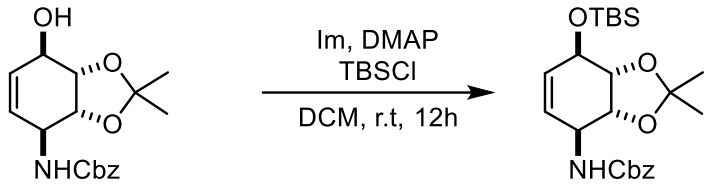
R_f 0.29 (SiO_2, Hex:EtOAc 2:8, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (t, J = 1.6 Hz, 1H), 6.95 (dd, J = 11.6, 6.9 Hz, 1H), 6.64 (dd, J = 11.0, 7.1 Hz, 1H), 6.13 (d, J = 7.6 Hz, 1H), 4.64 (t, J = 5.7 Hz, 1H), 4.53 (t, J = 5.7 Hz, 1H), 4.16 – 3.97 (m, 4H), 3.78 (br s, 1H), 3.67 (dd, J = 9.7, 3.1 Hz, 1H), 3.51 – 3.45 (m, 1H), 3.27 (s, 3H), 2.14 (dp, J = 26.3, 5.9 Hz, 2H), 1.94 (t, J = 1.4 Hz, 3H), 1.86 – 1.63 (m, 2H), 1.56 – 1.42 (m, 1H), 1.29 – 1.14 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 156.2 (d, J = 246.5 Hz), 149.5 – 147.0 (m), 133.4, 126.1, 116.7 (dd, J = 21.0, 4.9 Hz), 115.5 (dd, J = 16.5, 6.7 Hz), 102.5 (dd, J = 28.4, 2.2 Hz), 80.2 (d, J = 164.9 Hz), 78.5, 73.2, 70.7, 65.1 (d, J = 5.0 Hz), 56.6, 50.4, 30.2 (d, J = 20.1 Hz), 25.1, 22.7, 14.5 (d, J = 1.8 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.4 (d, J = 15.9 Hz), -139.7 (d, J = 13.7 Hz), -222.7.

IR (ATR, neat, cm^{-1}) 3358, 2929, 1513, 1423, 1174, 1099, 1051, 879.



172b **213**

To a solution of alcohol **172b** (1.0 eq.) and imidazole (2.0 eq.) in DCM (0.4 M) was added tert-butylchlorodimethylsilane (1.5 eq.) at room temperature. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. The reaction mixture was extracted with DCM, quenched with sat. aq. $NaHCO_3$ solution and washed with water. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 95:5) to afford protected alcohol (95%) as a white solid.

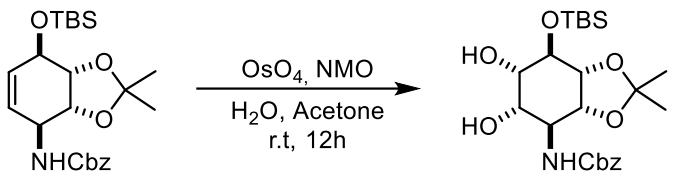
R_f 0.43 (SiO_2, Hex:EtOAc 9:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.16 (m, 5H), 5.93 – 5.86 (m, 2H), 5.42 (d, J = 9.1 Hz, 1H), 5.01 – 4.95 (m, 2H), 4.26 – 4.13 (m, 3H), 4.07 (q, J = 2.4 Hz, 1H), 1.26 (s, 3H), 1.18 (s, 3H), 0.75 (s, 9H), 0.00 (s, 3H), -0.03 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 155.8, 136.5, 132.3, 130.6, 128.4, 128.0, 108.4, 78.8, 67.3, 66.7, 48.3, 26.5, 25.7, 24.5, 17.9, -4.7, -4.9.

IR (ATR, neat, cm^{-1}) 2952, 1684, 1539, 1252, 1110, 1058, 883, 834, 775.

m.p. 92 °C.



213 **214**

Amine **213** (1.0 eq.), NMO (N-methylmorpholineN-oxide, 1.2 eq.), acetone (0.2M), pure water (20 eq.) were mixed and stirred. The mixture was cooled to 0°C and a solution of OsO_4 (0.2M in MeCN, 0.025 eq.) was added and the mixture was stirred at room temperature. The completion of the reaction was confirmed, and a saturated solution of $Na_2S_2O_3$ was added and the mixture was stirred at room temperature for 2h. Acetone was removed under reduced pressure, and the aqueous phase was extracted three times with ethyl Acetate. The organic phase was dried over Na_2SO_4 and it was evaporated under reduced pressure to afford the crude diol, which was purified by silica gel column chromatography (Hex:AcOEt 7:3) to afford a white solid (90%).

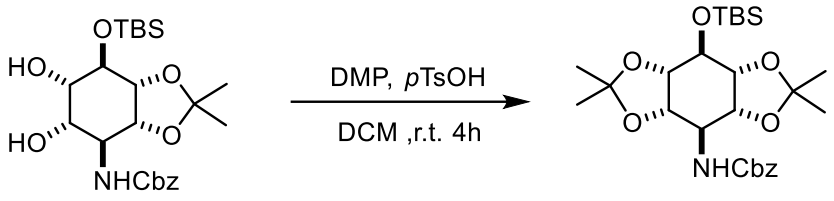
R_f 0.40 (SiO_2, Hex:EtOAc 7:3, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 – 7.11 (m, 5H), 5.79 (d, J = 9.2 Hz, 1H), 4.94 (s, 2H), 4.09 – 3.91 (m, 3H), 3.85 – 3.71 (m, 3H), 3.21 (br s, 1H), 2.78 (br s, 1H), 1.35 (s, 3H), 1.19 (s, 3H), 0.75 (s, 9H), 0.00 (s, 3H), -0.02 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.4, 136.4, 128.5, 128.1, 128.1, 109.4, 73.7, 71.6, 69.8, 66.9, 53.1, 28.1, 26.1, 25.7, 17.9, -4.7, -4.9.

IR (ATR, neat, cm^{-1}) 3414, 2929, 1688, 1520, 1457, 1364, 1248, 1114, 1058.

m.p. 116 °C.



214 **215**

To a solution of diol **214** (1.0 eq.) and pTsOH (0.1 eq.) in DCM (0.1 M) was added DMP (6.0 eq.) at room temperature. The reaction mixture was stirred for 12 h. 1M NaOH was added, and the reaction mixture was extracted with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified via flash column chromatography on silica gel (Hexane:Ethyl Acetate = 95:5) to afford protected alcohol (71%) as a colourless liquid.

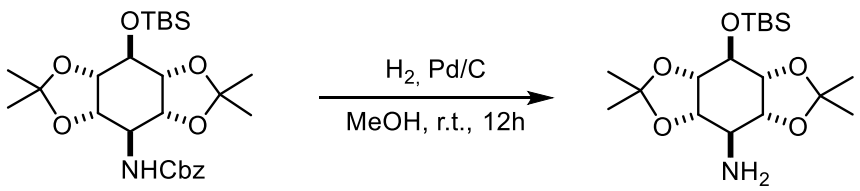
R_f 0.32 (SiO_2, Hex:EtOAc 9:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.14 (m, 5H), 5.26 – 5.12 (m, 1H), 5.04 – 4.89 (m, 2H), 4.30 – 4.15 (m, 3H), 4.05 (dd, J = 9.3, 7.5 Hz, 1H), 3.77 (dd, J = 6.8, 2.7 Hz, 1H), 3.73 – 3.64 (m, 1H), 1.33 (s, 3H), 1.30 (s, 3H), 1.17 (s, 3H), 1.16 (s, 3H), 0.78 (s, 9H), 0.00 (s, 3H), -0.01 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.3, 136.4, 128.4, 128.2, 128.0, 109.2, 108.8, 74.3, 74.0, 71.6, 66.9, 51.5, 27.1, 25.9, 25.9, 24.4, 23.6, 18.3, -4.4, -4.7.

IR (ATR, neat, cm^{-1}) 2929, 1718, 1524, 1371, 1259, 1207, 1133, 1080, 984.

m.p. 139 - 141 °C.



215 **216**

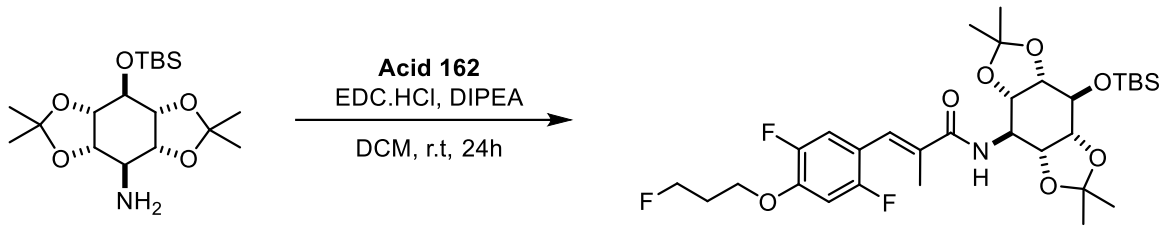
Amine **216** was synthesized following **General Procedure D**, starting from Cbz-protected amine **215**. The product was obtained as a light-yellow oil (75%).

R_f 0.34 (SiO_2, Hex:EtOAc 1:1, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 – 4.23 (m, 2H), 4.19 (t, J = 7.6 Hz, 1H), 3.95 (dd, J = 9.7, 7.8 Hz, 1H), 3.67 (dd, J = 7.5, 2.2 Hz, 1H), 2.79 – 2.47 (m, 3H), 1.33 (s, 3H), 1.32 (s, 3H), 1.19 (m, 6H), 0.80 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 109.1, 108.3, 75.6, 72.1, 52.2, 27.3, 25.9, 25.9, 24.4, 23.5, 18.3, -4.3, -4.8.

IR (ATR, neat, cm^{-1}) 3312, 2929, 1723, 1544, 1370, 1253, 1202, 1170, 1131, 1068, 987.



216 **217**

Amide **217** was synthesized following **General Procedure I**, starting from amine **216**. The product was obtained as a colorless oil (85%).

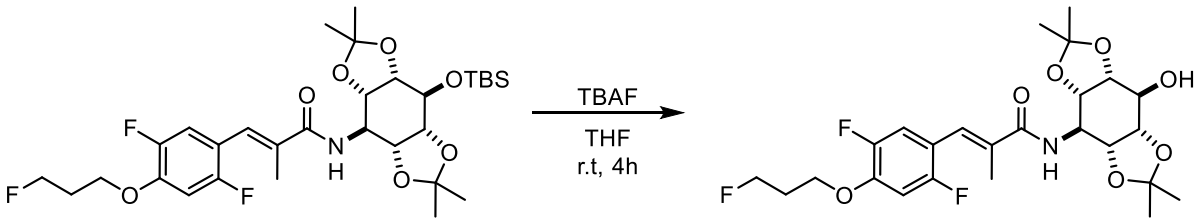
R_f 0.32 (SiO_2, Hex:EtOAc 8:2, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, J = 2.0 Hz, 1H), 6.91 (dd, J = 11.6, 6.9 Hz, 1H), 6.59 (dd, J = 11.0, 7.1 Hz, 1H), 6.05 (d, J = 8.5 Hz, 1H), 4.58 (t, J = 5.7 Hz, 1H), 4.46 (t, J = 5.7 Hz, 1H), 4.30 (dd, J = 7.7, 3.5 Hz, 1H), 4.24 – 4.20 (m, 2H), 4.14 (dd, J = 9.7, 7.4 Hz, 1H), 4.04 – 4.00 (m, 3H), 3.80 (dd, J = 6.9, 2.6 Hz, 1H), 2.06 (dp, J = 26.2, 5.8 Hz, 2H), 1.91 (d, J = 1.5 Hz, 3H), 1.34 (s, 3H), 1.32 (s, 3H), 1.17 (s, 6H), 0.78 (s, 9H), 0.00 (s, 3H), -0.01 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.9, 156.2 (dd, J = 246.3, 2.3 Hz), 149.5 – 147.1 (m), 134.0, 125.7, 116.7 (dd, J = 21.0, 5.0 Hz), 115.8 (dd, J = 16.6, 6.7 Hz), 109.3, 108.9, 102.6 (dd, J = 28.4, 2.2 Hz), 80.2 (d, J = 165.0 Hz), 74.0, 73.9, 71.9, 67.8, 65.2 (d, J = 4.9 Hz), 50.0, 30.2 (d, J = 20.1 Hz), 27.2, 26.0, 25.9, 24.6, 23.5, 18.4, 14.7 (d, J = 1.8 Hz), -4.4, -4.8.

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.4 (d, J = 15.0 Hz), -139.7 (d, J = 14.2 Hz), -222.8.

IR (ATR, neat, cm^{-1}) 2985, 1662, 1628, 1513, 1263, 1051, 980, 875, 730.



217 **218**

Amide **217** (1.0 eq.) was solubilized in THF (0.25 M), and a solution of TBAF (1.0 M in THF, 1.5 eq.) was added dropwise. The mixture was stirred at room temperature for 4 h, then the organic solvent was evaporated. The crude was purified by silica gel column chromatography to afford the product as a white solid (90%).

R_f 0.32 (SiO_2, Hex:EtOAc 3:7, UV-Vis, Vanillin).

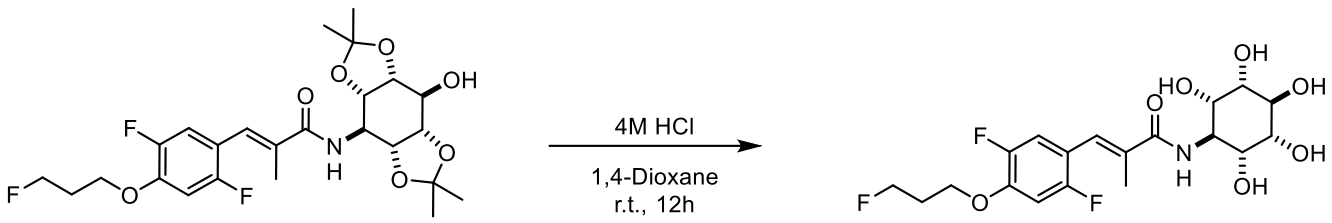
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (s, 1H), 6.99 (dd, J = 11.6, 6.9 Hz, 1H), 6.67 (dd, J = 11.0, 7.1 Hz, 1H), 6.29 (d, J = 8.4 Hz, 1H), 4.65 (t, J = 5.6 Hz, 1H), 4.53 (t, J = 5.7 Hz, 1H), 4.50 – 4.44 (m, 2H), 4.31 – 4.28 (m, 2H), 4.25 – 4.21 (m, 1H), 4.09 (t, J = 6.1 Hz, 2H), 3.83 – 3.81 (m, 1H), 2.15 (dp, J = 26.3, 5.9 Hz, 2H), 1.98 (d, J = 1.5 Hz, 3H), 1.42 (s, 3H), 1.41 (s, 3H), 1.28 (s, 3H), 1.26 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.7, 156.2 (dd, J = 246.2, 2.3 Hz), 149.5 – 147.0 (m), 133.6, 126.1, 116.7 (dd, J = 20.9, 5.0 Hz), 115.7 (dd, J = 16.6, 6.7 Hz), 109.5, 109.3, 102.6 (dd, J = 28.4, 2.2 Hz), 80.2 (d, J = 164.9 Hz), 76.6, 75.3, 74.3, 70.8, 65.2 (d, J = 4.8 Hz), 49.9, 30.2 (d, J = 20.1 Hz), 27.0, 25.8, 24.4, 23.4,14.5 (d, J = 1.9 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ -115.4 (d, J = 16.3 Hz), -139.7 (d, J = 14.7 Hz), -222.8.

IR (ATR, neat, cm^{-1}) 3367, 2935, 1682, 1563, 1412, 1342, 1253, 1098, 960, 867, 736.

m.p. 110 °C.



218 **219**

Amide **219** was synthesized following **General Procedure C**, starting from acetal-protected amide **218**. The product was obtained as a white solid (79%).

R_f 0.27 (SiO_2, CH_2Cl_2:MeOH 8:2, UV-Vis, Vanillin).

^{1}H NMR (400 MHz, CD_3OD) δ 7.21 (s, 1H), 7.09 (dd, J = 11.7, 6.9 Hz, 1H), 6.91 (dd, J = 11.4, 7.1 Hz, 1H), 4.62 (t, J = 5.8 Hz, 1H), 4.50 (t, J = 5.8 Hz, 1H), 4.27 (t, J = 4.8 Hz, 1H), 4.12 (t, J = 6.1 Hz, 2H), 4.06 – 3.98 (m, 3H), 3.83 – 3.73 (m, 1H), 2.12 (dp, J = 25.4, 6.0 Hz, 2H), 1.95 (d, J = 1.6 Hz, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 155.9 (dd, J = 245.2, 2.2 Hz), 149.5 – 147.0 (m), 133.4, 125.7 (d, J = 2.2 Hz), 116.3 (dd, J = 21.2, 4.8 Hz), 115.3 (dd, J = 16.5, 6.7 Hz), 102.4 (dd, J = 29.0, 2.0 Hz), 80.0 (d, J = 163.5 Hz), 75.2 – 67.4 (m), 65.1 (d, J = 5.4 Hz), 29.9 (d, J = 20.1 Hz), 13.1 (d, J = 1.9 Hz).

^{19}F NMR (376 MHz, CD_3OD) δ -113.4 (d, J = 265.4 Hz), -137.7 (d, J = 202.3 Hz), -220.3.

IR (ATR, neat, cm^{-1}) 3293, 1724, 1638, 1496, 1455, 1315, 1246, 1194, 1174, 928, 842.

m.p. 148 - 151 °C.

12 References

1. Zhao, H.; Guo, Z., *Drug Discov. Today*, **2009**, *9-10*, 516-22.
2. Fang, Z.; Song, Y.; Zhan, P.; Zhang, Q.; Liu, X., *Future Med. Chem.*, **2014**, *8*, 885-901.
3. Schmid, E.F.; Smith, D.A., *Drug Discov. Today*, **2007**, *12*, 998–1006.
4. C.M.R International, 2006/7 Pharmaceutical R&D Factbook.
5. Garattini, S., *J. of Nephrol.*, **1997**, *10*, 283-94.
6. Zambrowicz, B.P.; Sands, A.T, *Nat. Rev. Drug Discov.*, **2003**, *2*, 38–51.
7. DiMasi, J.A.; Paquette, C., *Pharmacoeconomics*, **2004**, *22*, 1–14.
8. Wipf, P.; Skoda, E.M.; Mann, A. The Practice of Medicinal Chemistry (Fourth Edition), Academic Press, **2015**, 279-299.
9. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J., *Adv. Drug Deliv. Rev.*, **2001**, *46*, 3−26.
10. Sadowski, J.; Kubinyi, H., *J. Med. Chem.*, **1998**, *41*, 3325−3329.
11. Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D., *J. Med. Chem.*, **2002**, *45*, 2615−2623.
12. Danishuddin and Khan, A.U., *Drug Discov. Today*, **2016**, *21*, 1291–1302.
13. Martin, Y.C., *J. Med. Chem.*, **2005**, *48*, 3164–3170.
14. Abad-Zapatero, C., *Expert Opin. Drug Discov.*, **2007**, *2*, 469–488.
15. Dalvie, D.; Sajiv, N.; Kang, P.; Loi, C.-M., *Royal Society of Chemistry Cambridge*, **2010**, 275−327.
16. FDA Home Page. http://www.fda.gov.
17. Gaulton, A.; Bellis, L. J.; Bento, A. P.; Chambers, J.; Davies, M.; Hersey, A.; Light, Y.; McGlinchey, S.; Michalovich, D.; Al-Lazikani, B.; Overington, J. P., *Nucleic Acid Res.,* **2012**, *40*, D1100−D1107.
18. Ritchie, T.J.; MacDonald, S.J.F., *J. Med. Chem.*, **2014**, *57 (17),* 7206-7215.
19. Ward. S.E.; Beswick, P., *Expert Opin. Drug Discov.*, **2014**, *9*, 995-1003.
20. Yan, A.; Gasteiger, J., *J. Chem. Inf. Comput. Sci.*, **2003**, *43*, 429−434.
22. Yang, Y.; Engkvist, O.; Llinas, A.; Chen, H., *J. Med. Chem.*, **2012**, *55*, 3667−3677.
23, Ritchie, T. J.; Macdonald, S. J. F., *Drug Discov. Today*, **2009**, *14*, 1011−1020.
24. Young, R. J.; Green, D. V. S.; Luscombe, C. N.; Hill, A. P., *Drug Discov. Today*, **2011**, *16*, 822−830.
25. Huuskonen, J.; Livingstone, D.J.; Manallack, D.T., *Environ. Res. Lett.*, **2008**, *19*, 191−212.
26. Leeson, P. D.; St-Gallay, S. A.; Wenlock, M. C., *Med.l Chem. Comm.*, **2011**, *2*, 91−105.

27. Kombarov, R.; Altieri, A.; Genis, D.; Kirpichenok, M.; Kochubey, V.; Rakitina, N.; Titarenko, Z., *Mol. Divers.*, **2010**, *1*, 193-200.
28. DeSimone, R.W.; Currie, K.S.; Mitchell, S.A.; Darrow, J.W.; Pippin, D.A., *Comb. Chem.* **2004**, *5*, 473-94.
29. Salonen, L.M.; Ellermann, M.; Diderich, F., *Angew. Chem., Int. Ed*., **2011**, *50*, 4715−4989.
30. Meyer, E.A.; Castellano, R.K; Diederich, F., *Angew. Chem., Int. Ed*., **2003**, *11*, 1210-1250.
31. King, A.O.; Yasuda, N., *Top. Organomet. Chem.*, **2004**, *6*, 205–245.
32. Johansson Seechurn, Carin C. C.; Kitching, Matthew O.; Colacot, Thomas J.; Snieckus, V., *Angew. Chem., Int. Ed. Engl*., **2012**, *51*, 5062–5085.
33. Corbet, Jean-Pierre; Mignani, G., *Chem. Rev.*, **2006**, *7*, 2651–2710.
34. Tony Roy; Avishek Guin; Akkattu T. Biju., *Modern Aryne Chemistry First Edition*, **2021**, *WILEY-VCH*.
35. Tadross, P. M.; Stoltz, B. M., *Chem. Rev.*, **2012**, *112*, 3550-77.
36. Edwards, P. J.; Sturino, C., *Curr. Med. Chem.*, **2011**, *18*, 3116-35.
37. Delaney, J. S., *J. Chem. Inf. Comput. Sci*, **2004**, *44*, 1000−1005.
38. Lamanna, C.; Bellini, M.; Padova, A.; Westerberg, G.; Maccari, L., *J. Med. Chem.,* **2008**, *51*, 2891−2897.
39. GVK Bio Web site: http://www.gvkbio.com/Informatics.html.
40. Lovering, F., *Med. Chem. Comm.*, **2013**, *3*, 515−519.
41. Kombo, D. C; Tallapragada, K.; Jain, R.; Chewning, J.; Mazurov, A. A.; Speake, J. D.; Hauser, T. A.; Toler, S*., J. Chem. Inf.*, **2013**, *53*, 327-342.
42. Nakashima, S.; Yamamoto, K.; Arai, Y.; Ikeda, Y., *Chem. Pharm. Bull.,* **2013**, *61*, 1228−1238.
43. Marson, Charles M., *Adv. in Heterocycl. Chem*., **2016**, *20*.
44. Hung, A. W.; Ramek, A.; Wang, Y.; Kaya, T.; Wilson, J. A.; Clemons, P. A.; Young, D. W., *Proc. Natl. Acad. Sci. U.S.A.*, **2011**, *108*, 6799–6804.
45. Schuffenhauer, A.; Floersheim, P.; Acklin, P.; Jacoby, E., *J. Chem. Inf. Comput. Sci.,* **2003**, *43*, 391–405.
46. Schuffenhauer, A.; Brown, N.; Selzer, P.; Ertl, P.; Jacoby, E., *J. Chem. Inf. Comput. Sci*., **2006**, *46*, 525–535.
47. Burke, M. D.; Schreiber, S. L., *Angew. Chem., Int. Ed*., **2004**, *43*, 46–58.
48. Marson, Charles M., *Chem. Soc. Rev*., **2011**, *40*, 5514-5533.

49. Valle G.; Kazmierski, W. M; Crisma, M., *Int. J. Pept. Protein Res.,* **1992**, *40*, 222–232.
50. Williams, D. H.; Westwell, M. S., *Chem. Soc. Rev.*, **1998**, *27*, 57-64.
51. Mark S. Searle; Dudley H. Williams., *J. Am. Chem. Soc.*, **1992** *114*, 10690-10697.
52. P. R. Andrews; D. J. Craik; J. L. Martin., *J. Med. Chem.*, **1984**, *27* (12), 1648-1657.
53. Robert B. Raffa; Frank Porreca., *Life Sci.*, **1989**, *44*, 245-258.
54. Page, M. I., *Angew. Chem. Int. Ed. Engl*., **1977**, *16*, 449-59.
55. Velazquez-Campoy, A.; Luque, I.; Freire, E., *Thermochim. Acta*, **2001**, *380*, 217-227.
56. Chang, C. E.; Chen, W.; Gilson, M. K., *Proc. Natl. Acad. Sci. U.S.A.*, **2007**, *104(5),* 1534–1539.
57. Zheng, Y.; Tice, C. M.; Singh, S. B., *Bioorg. Med. Chem. Lett.*, **2014**, *24*, 3673-3682.
58. Bolognesi ML, Bartolini M, Cavalli A, Andrisano V, Rosini M, Minarini A, Melchiorre C., *J. Med. Chem*., **2004**, *47*, 5945-5952.
59. Xu, M.; Tu, J.; Franzini, R. M., *Chem. Comm.*, **2017**, *53*, 6271-6274.
60. Wenk, H. H.; Winkler, M.; Sander, W., *Angew. Chem. Int. Ed*., **2003**, *42 (5),* 502–528.
61. Leopold, D. G., Amy E. S. Miller, and W. C. Lineberger., *J. Am. Chem. Soc*., **1986**, *108 (7),* 1379-1384.
62. Anslyn, E. V.; Dougherty, D. A., *University Science Books*, **2006**, *612*.
63. W. E. Bachmann and H. T. Clarke., *J. Am. Chem. Soc.*, **1927**, *49 (8),* 2089-2098.
64. John D. Roberts, Howard E. Simmons Jr., L. A. Carlsmith, and C. Wheaton Vaughan, *J. Am. Chem. Soc*., **1953**, *75 (13)*, 3290-3291.
65. Roberts, J.; Semenow, D. A., *J. Am. Chem. Soc*., **1956**, *78 (3),* 601-611.
66. Gampe, C. M.; Carreira, E. M., *Angew. Chem. Int. Ed. Engl*., **2012**, *51 (16),* 3766–3778.
67. Tsugio Kitamura., *Australian J. Chem*., **2010**, *63(7)*, 987-1001.
68. Greg W. Ebert, Deborah R. Pfennig, Scott D. Suchan, Thomas A. Donovan., *Tetrahedron Lett*., **1993**, *34*, 2279-2282.
69. Takashi Matsumoto, Takamitsu Hosoya, Miyoko Katsuki, Keisuke Suzuki., *Tetrahedron Lett*., ***1991***, *32*, 6735-6736.
70. Logullo, Francis M.; Seitz, Arnold M.; Friedman, Lester, *Org. Synth*., **1968**, *48*, 12.
71. Lester. Friedman and Francis M. Logullo., *J. Am. Chem. Soc.,* **1963**, *85 (10),* 1549-1549.
72. Imon Jyoti Dutta, T. Punniyamurthy., Summer Research Fellowship Programme of India's Science Academies.

73. Himeshima, Yoshio; Sonoda, Takaaki; Kobayashi, Hiroshi., *Chem. Lett.,* **1983***, 12(8),* 1211–1214.
74. Heaney, H.; Millar, I. T., *Org. Synth*., **1973**, *40*, 1120.
75. Campbell, C.D.; C.W. Rees., *J. Am. Chem. Soc.,* **1969**, *5*, 742–747.
76. Panar, M., California Institute of Technology (Ph.D. Thesis), 4–5.
77. H., Lowry, Thomas., Mechanism and theory in organic chemistry. *Richardson, Kathleen Schueller (3rd ed.).* New York: Harper & Row. pp. 643.
78. Wittig, G.; Liselotte Pohmer., *Angew. Chem*., **1955**, *67*, 348.
79. Horner, K. E.; Karadakov, P. B., *J. Org. Chem.*, **2013**, *78 (16),* 8037-8043.
80. Kamimura, A.; Nakano, T. *J. Org. Chem.*, **2010***;75(9):*3133-6.
81. Regis Leung-Toung, Yanzhou Liu, Joseph M. Muchowski, and Yu-Lin Wu. *J. Org. Chem***., 1998**, *63* (10), 3235-3250.
82. Cifuentes-Pagano, E.; Saha, J.; Csányi, G.; Ghouleh, I. A.; Sahoo, S.; Rodríguez, A.; Wipf, P.; Pagano, P. J.; Skoda, E. M. Bridged. *Med. Chem. Comm.,* **2013**, *4(7)*1085-1092.
83. Kametani, T.; Ogasawara, K. *J. Chem. Soc.,* **1967**, 2208-2212.
84. Buszek, K. R.; Brown, N.; Kuo, D. Concise. *Org. Lett.*, **2009**, *11 (1)*, 201–204.
85. Aronow, W. S. *Brocklehurst's Textbook of Geriatric Medicine and Gerontology. Elsevier*. **2010**, 327–337.
86. Acharya, K.R.; Sturrock, E.D.; Riodan, J.K.; Ehlers, M.R., *Nat. Rev. Drug Discov*., **2003**, *2 (11),* 891–902.
87. Cushman, D. W.; Ondetti, M. A., *Hypertension*, **1991**, *17 (4),* 589–592.
88. Lappin, S. L.; Fountain, J. H. *Physiology, Renin-Angiotensin System*. NCBI. NIH, **2019**.
89. Ng, K. K.; Vane, J. R., *Nature*, **1967**, *216 (5117),* 762–6.
90. Rocha e Silva M.; Beraldo, W. T.; Rosenfeld, G., *Am. J. Physiol*., **1949**, *156 (2),* 261–73.
91. Bhaskar J. Bhuyan; Govindasamy Mugesh., *Org. Biomol. Chem*., **2011**, *9*, 5185-5192.
92. Bünning, P., *Clin. Exp. Hypertension*, **1983**, *5 (7–8),* 1263–1275.
93. Natesh, R.; Schwager, S.; Sturrock, E.; Schwager, S. L. U.; Acharya, K.R., *Nature*, **2003**, *421*, 551–554.
94. Natesh, R.; Schwager, S. L.; Evans, H. R.; Sturrock, E. D.; Acharya, K. R., *Biochem.* **2004**, *43(27),* 8718-8724.
95. Krapcho, C.; Turk, C.; Cushman, D. W.; Powell, J. R.; DeForrest, J. M.; Spitzmiller, E.R.; Karanewsky, D. S.; Duggan, M.; Rovnyak, G., *J. Med. Chem.,* **1988**, *31 (6),* 1148-1160.

96. Fischer, J.; Ganellin, C.R., Analogue-based Drug Discovery. *John Wiley & Sons*. **2006,** 467
97. Li, J.J. Chapter 1: History of Drug Discovery. Drug Discovery: Practices, Processes, and Perspectives. *John Wiley & Sons*, **2013**.
98. Craik D.J., Fairlie D.P., Liras S., Price D., *Chem. Biol. Drug. Des.,* **2013**, *81*, 136–147
99. Henninot A., Collins J.C., Nuss J.M., *J. Med. Chem.*, **2018**, *61*, 4, 1382–1414
100. Sharma K., Sharma K.K., Sharma A., Jain R., *Drug Discov. Today* ,**2023**,*28*,103464
101. Brown, T.D., Whitehead,K., Mitragotri, S. . *Nat. Rev. Mater*., **2020**, *5*, 127–148
102. Goodwin, D., Simerska, P., Toth I., *Curr. Med. Chem*., **2012**, *19*, 4451–4461
103. (FDA (2022) Drugs@FDA: FDA-Approved Drugs. www.fda.gov/drugs/development-approval-process-drugs/new-drugs-fda-cders-new-molecularentities-and-new-therapeutic-biological-products [Accessed November 30,2022].)
104. Sin N, Kim KB, Elofsson M, et al., *Bioorg. Med. Chem. Lett.*, **1999,** *9(15)*, 2283-8
105. Bataille R., Harousseau, J.L, *N. Engl. J. Med*, **1997**,*336*,1657-1664
106. Jain S., Diefenbach C., Zain J., O'Connor, D.O, *Core Evid.*, **2011**, *6*, 43-57
107. Akopian T.N., Castillo V., Goldberg A.L., Kisselev, A.F., *Mol. Cell*., **1999**, *4*, 395-402
108. Grune,T., Jung T., Catalgol B., *Mol.Aspects Med*., **2009**, *30*, 191-296
109. Stewart A.K., Vallone M., Molineaux C.J., Kunkel L.A., Gerecitano J.F., Orlowski R.Z., O'Connor, O.A., *Clin. Cancer Re.s*, **2009**, *15*, 7085-7091)
110. Bennet M.K., Kirk, C.J., *Discov. Devel.*, **2008**, *11*, 616-625
111. Rock K.L., Gramm C., Dick L., Hwang D., Goldberg A.L., Rothstein L., *Cell*, **1994**, *78*, 761-771
112. Chauhan, D., Catley, L., Li, G., Podar, K., Hideshima, T., Velankar, M., Mitsiades, C., Mitsiades, N., Yasui, H., Letai, A., et al., *Cancer Cell,* **2005***, 8*, 407–419.
113. Jullig, M., Zhang, W.V., Ferreira, A., and Stott, N.S., *Apoptosis*, **2006**, *11*, 627–641
114. Andreu-Vierya C., Berenson, J.R, *Expert Opin. Biol. Ther*.,**2014**,*14*, 1685-1699
115. Hideshima, T., & Anderson, K. C., *Seminars in Hematology*, **2012**, *49(3)*, 223–227.
116. Gandolfi S., Laubach J.P., Hideshima T., Chauhan D., Anderson K.C., Richardson P.G., *Cancer Metastasis Rev.,* **2017**, *36*, 561-584
117. Nina J., Bailey H.H., Kolesar J.M., Eickhoff J., Reid J.M., Ames, M.M., Mcgovern R.M., Alberti D:, Marnocha R, Schelman W.R:, Deming, D.A, *Invest. New Drugs*, **2014**, *32*, 323-329

118. Jeroudi A., Achkar K., Gaber A.O., Patel S.J., Abdellatif A., Raghavan,R., *J. Trasplant*., **2010**, *698594*
119. Cavaletti, G., Jakubowiak A.J., *Leuk. Lymphoma*, **2010**, *51*, 1178-1187
120. Hanada M., Sugarawara K., Kaneta K., TOda S., Yamamoto H., Oki, T., *J. Antibiot.*, **1992**, *45*, 1746-1752
121. Sin N., Elofsson M., Meng L., Auth H., Kwok B.H.B., Crews, C.M., *Bioorg. Med. Chem. Lett.,***1999,** *9,* 2283-2288
122. Groll M., Kim K.B., Kairies N., Huber R., Crews, C.M., *J. Am. Chem. Soc*., **2000**, *122*, 1237-1238
123. Kim K.B., Crews, C.M., *Nat. Prod. Rep*., **2013**, *30,* 600
124. Siegel D., Martin T., Wang M., Andrzej V., Jakubowiak J., Lonial S., Trudel S., Kukreti V., Bahlis N., Alsina M., Chanan-Kha A., Jagannath, S., *Blood*, **2012**, *120*, 2817-2825
125. Groll, M., Huber R., Potts B.C.M., *J. Am. Chem. So*c, **2006**, *128*, 5136-5141
126. Harshbarger W., Miller C., Diedrich C., Sacchettini, J., *Structure*, **2015**, *23*, 418-424
127. Deisseroth A., Kaminskas E., Herndon, T.M., *Clin. Cancer Res*., **2013**, *19*
128. Ventola, C. L., *PubMed*, **2015**, *40(4),* 277–283.
129. Jubeh, B.; Breijyeh, Z.; Karaman, R. *Molecules,* **2020**, *25*(12), 2888.
130. Mann, R. L.; Gale, R. M.; Van Abeele, R. F. *Antibiotics and Chemotherapy,* **1953**, *3*, 1279–1282.
131. Pittenger, R. C.; Wolfe, R. N.; Hoehn, M. M.; Marks, P. N.; Daily, W. A.; McGuire, J. M. *Antibiotics and Chemotherapy,* **1953**, *3*, 1268–1278.
132. Guerrero, M. D.; Modolell, J. *Eur. J. Biochem.,* **1980**, *107*, 409–414.
133. Palaniappan, N.; Ayers, S.; Gupta, S.; Habib, E. E.; Reynolds, K. A.; *Chem. Biol.,* **2006**, *13*(7), 753–764.
134. Habib, E. E.; Scarsdale, J. N.; Reynolds, K. A. *Antimicrobial Agents and Chemotherapy,* **2003**, *47*(7), 2065–2071.
135. Leimer, L., *Cell*, **2021**, *184*, 5405–5418.
136. Hecker, S. J.; Minich, M. L.; Werner, K. *Bioorg. Med. Chem. Lett.,* **1992**, *2*(6), 533–536.
137. Jaynes, B. H.; Elliott, N. C.; Schicho, D. L. *J. Antibiotics,* **1992**, *45*, 1705–1707.
138. Hecker, S. J.; Cooper, C. B.; Blair, K. T.; Lilley, S. C.; Minich, M. L.; Werner, K. *Bioorg. Med. Chem. Lett.,* **1993**, *3*, 289–294.
139. Ferrier, R. J.; Blattner, R.; Clinch, K.; Furneaux, R. H.; Gardiner, J. M.; Tyler, P. C.;Wightman, R. H.; Williams, N. R. *Carbohydrate Chem.,* **1996**, *28*, 251–262.

140. el Ashry, E. S.; Rashed, N.; Shobier, A. H. *Pharmazie*, **2000**, *55*, 403–415.
141. Delgado, A. *Eur. J. Org.Chem.,* **2008**, *23*, 3893–3906.
142. Díaz, L. E.; Delgado, A. *Curr. Med. Chem.,* **2010**, *17*, 2393-2418.
143. Kjell, D. P.; Sheridan, R. S. *J. Am. Chem. Soc.,* **1984**, *106*, 5368–5370.
144. Southgate, E. H.; Pospech, J.; Fu, J.; Holycross, D. R.; Sarlah, D. *Nature Chem.,* **2016**, *8*(10), 922–928.
145. Okumura, M.; Huynh, S. M. N.; Pospech, J.; Sarlah, D. *Angew. Chem.,* **2016**, *55*(51), 15910–15914.
146. Moretti, R. A.; Du Bois, J.; Stack, T. D. P. *Org. Lett*., **2016**, *18*, 2528−2531.
147. Cha, J. K.; Kim, N. S. *Chem. Rev.,* **1995**, *95*(6), 1761–1795.
148. Siddiqi, Z. R.; Wertjes, W. C.; Sarlah, D. *J. Am. Chem. Soc.,* **2020**, *142*(22), 10125–10131.
149. Davies H.M, Matasi J.J., Ahmed G., *J. Org. Chem.,* **1996**, *61*, 2305-2313.
150. Warrener R.N, Collin G.J, Foley P.J, *Molecules*, **2001**, *6*, 194-202.
151. Wang Z., Wang X., Wang P., Zhao J., *J. Am. Chem. Soc.*, **2021**, *143*, 10374-10381.
152. Zhang W., Wang X., Yang L., Miao H., Yang X., Lei M., Zhu Y., *Bioorg.Med.Chem.,* **2021**, *40,* 116-182.
153. Noguchi, H.; Aoyama, T.; Shioiri, T. *Heterocycles,* **2002**, *58*(1)*,* 471-504.
154. Nelsen, S. F.; Thompson-Colon, J.; Kirste, B.; Rosenhouse, A.; Kaftory, M. *J. Am. Chem. Soc.,* **1987**,*109*(23), 7128–7136.
155. King, B. S.*, J. Am. Chem. Soc.,* **1999**, *121*, 6769-6770.
156. Miller, J. M., *J. Org. Chem*. **2009**, *74*, 448–451.
157. Noguchi, H., *Heterocycles*, **2002**, *58*, 471-504.