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SCIENZE CHIMICHE

CICLO XXIX

COORDINATORE Prof. PIERO BAGLIONI

DIVERSITY-ORIENTED SYNTHESIS OF NOVEL GLYCO- AND PEPTIDOMIMETIC SCAFFOLDS

Settore Scientifico Disciplinare CHIM/06

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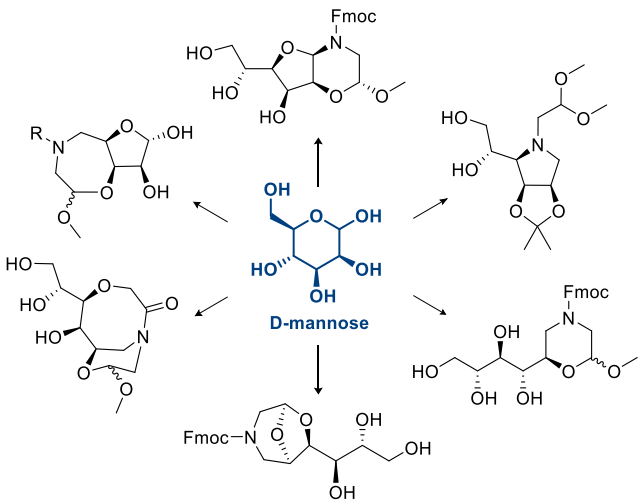
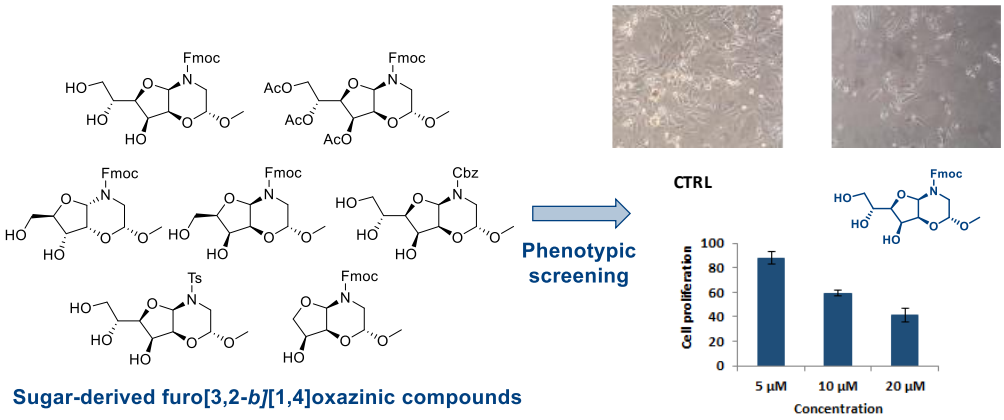
Abstract

After an impressive growth at the end of the last century, the number of new molecular entities launched on the market dramatically decreased in recent years. Thus, there is a need for efficient synthetic processes capable of generating an high number of different molecules, which differ not only for the appendages, but also for the molecular skeleton. In this context, Diversity-Oriented Synthesis (DOS) has proved to be very effective in the achievement of high quality small molecules collections for high-throughput screening, phenotypic assays and chemical genetics studies, leading to the discovery of both new targets and new *lead* compounds.[1]

The aim of this thesis work is to apply the principles of the DOS to obtain densely functionalized molecular scaffolds, potentially able to address crucial interactions in biological systems. For these reasons, carbohydrates and *N*-containing building blocks are taken into account for their abilities of generating glyco- and/or peptidomimetic moieties. In particular, despite their key role in many pathophysiological events and their intrinsic synthetic advantages, carbohydrates remain quite underdeveloped in DOS strategies, because the need of protecting/deprotecting groups contrast with the efficiency criteria of DOS.[2] Neverthless, the exploitation of acetal chemistry, as introduced by glycine-derived amino acetaldehyde, allowed us to generate six novel skeletally different polyhydroxylated nitrogen-containing compounds starting from D-mannose.[3]

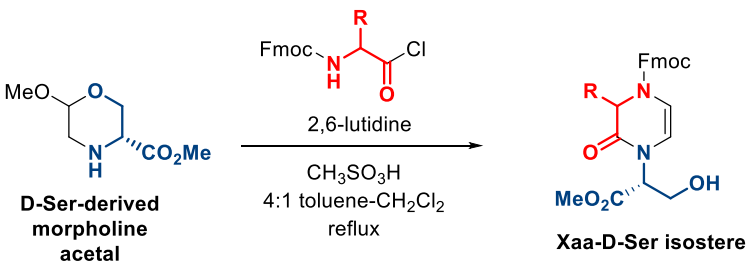


This pool of new compounds was subjected to a phenotypic screening towards the evaluation of the ability in inducing cell growth inhibition in a human metastatic melanoma cell line, performed under the supervision of Dott. Francesca Bianchini at the Department of Clinical and Experimental Biomedical Science of the University of Florence. This cell-based assay, combined with follow up synthesis and further biological studies, led to the discovery of the hexahydro-2*H*-furo[3,2-*b*][1,4]oxazine structure as an active modulator of MDA-MB-231 cell growth, through cytostatic effect.[4]



Sugar-derived furo[3,2-*b*][1,4]oxazinic compounds

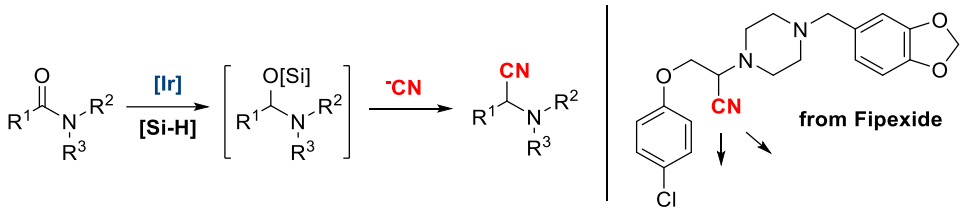
In a second part of this PhD work, *N*-containing compounds were selected as valuable building blocks for the generation of nitrogen-containing heterocyclic scaffolds, through the exploitation of versatile and efficient couple/pair processes. In this context, morpholine acetal, and the related reactivity of the *N*-acyl iminium chemistry, proved to be extremely powerful, giving access to the dipeptide isostere dihydropyrazinone, by a two step one-pot rearrangement of a serine-derived morpholine acetal.[5]



D-Ser-derived morpholine acetal

Xaa-D-Ser isostere

Moreover, α-amino nitriles, with their several different modes of reactivity, are interesting bifunctional building blocks for the generation of different *N*-heterocycles. For this reason, during a secondment activity of this PhD in the group of Prof. Darren J. Dixon at the University of Oxford, the generation of α-amino nitriles from tertiary amides and lactams was studied and developed.[6] Considering the broad presence of amides and lactams in biologically active compounds, the chemoselective reductive cyanation of carboxamide functional groups opens the way to a number of different applications. In particular, this iridium catalyzed methodology was successfully applied in the late stage functionalization of drugs, natural products and proline-containing peptides. The introduction of the α-amino nitrile moiety in complex biologically active molecules may serve as a valuable point of diversification for divergent approaches, thus creating novel different analogues, as proved by the derivatization of cyanated Fipexide drug molecule.



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Part I

Introduction

1

Diversity-Oriented Synthesis as a Tool for Drug Discovery

Despite the massive advantages in chemical biology over the past decades, the number of new molecular entities approved per year still failed to increase.[1]
Although the rational design of ligands remains the "gold standard" in medicinal chemistry, when the biological target is well defined (Figure 1 top), a fundamental challenge has emerged. Many disorders, such as cancer and neurodegenerative diseases, are often associated with complex interactions between transcription factors, proteins and DNA,[2] targets that have been termed "undruggable", due to the difficulty in being applied to traditional drug discovery programs.[3] To exit this impasse, both academia and pharmaceutical companies must apply new approaches. One of the most promising alternative, for the discovery of new targets and new *lead* compounds, is the application of large small molecules libraries in high-throughput screening (HTS), phenotypic assays and chemical genetics studies (Figure 1, bottom).[4] The relevance of this approach is also highlighted by the emergence of international screening initiatives, such as EU-OPENSCREEN[5] or the European Lead Factory.[6]
In these reverse approaches, synthetic chemistry plays a key role in generating high quality chemical libraries at the beginning of the entire process. For this reason, several efforts have been devoted to improve the quality and quantity of small molecules representing a library.

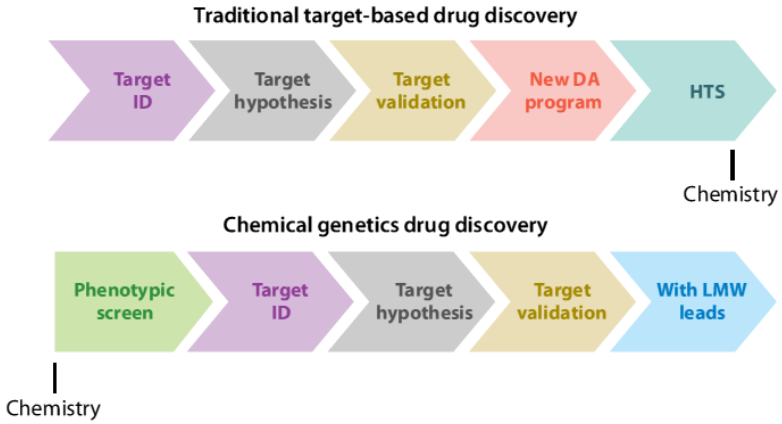


Figure 1: Comparison between conventional target-based drug discovery and chemical genetics drug discovery approaches. Reproduced from ref. 1 with permission from Annual Reviews.

In particular, during last decades, organic chemists have taken advantage of high-throughput synthesis methods, such as solid-phase techniques,[7] and combinatorial chemistry.[8] Unfortunately, despite the apparent success, these chemistry apparatus have not fulfilled the desired expectations.[9] Automation of discovery processes has proven to be inefficient: in fact, most of the combinatorial libraries are prepared through the functionalization of a common skeleton, so the compounds therein generated possess limited structural diversity and their behaviour in the screening is not much differentiated. Furthermore, combinatorial libraries usually contain flat and simple structures, showing not enough three-dimensional complexity for an efficient interaction with biological macromolecules.[10]

In this context, Diversity-Oriented Synthesis (DOS), which aims to synthesize the largest number of structurally complex small molecules, has revolutionized the construction of libraries for drug discovery issues.[11] DOS has in fact been defined by Spring as "the deliberate, simultaneous and efficient synthesis of more than one target compound in a diversity-driven approach to answer a complex problem",[12] and already proved to be effective for the discovery of non-traditional drug targets.[13] For example, Schreiber and coworkers discovered a potent sonic hedgehog inhibitor, robotnikinin (Figure 2a) from the screening of a DOS library of 2070 amino alcohol-derived macrocycles with the Small Molecule Microarray (SMM) technology.[14] The Spring's group screened a

DOS library of 223 compounds, based on 18 distinct molecular scaffolds, using high-throughput phenotypic assays, thus identifying emmacin (Figure 2b) as a potent antibacterial compound, able to inhibit dihydrofolate reductase.[15]

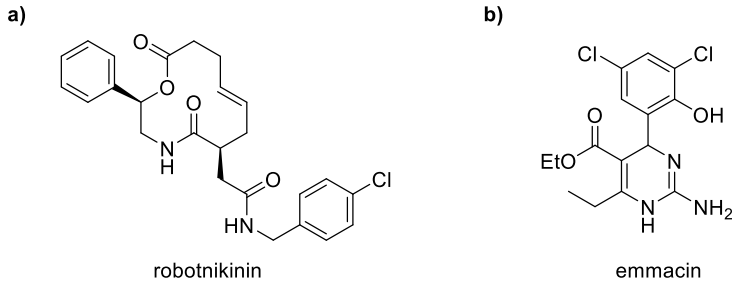


Figure 2: Sonic hedgehog inhibitor, robotnikinin (**a**), and antibacterial compound, emmacin (**b**), discovered from the screening of DOS libraries.

1.1 Molecular Diversity and Chemical Space

Diversity-oriented synthesis, since Schreiber's seminal paper, has evolved and acquired a precise terminology and peculiar synthetic strategies (see Chapter 2). In particular, the concept of molecular diversity has been elaborated and subdivided into four different type of diversity:

1. *Appendage diversity*: diversity resulting from the use of different building blocks or achieved by decorating the functional groups of the scaffold with different appendages. This strategy, already used by combinatorial chemistry, is capable of generating thousands of distinct small molecules in few steps, which, however, cover only a small area of the chemical space.
2. *Functional group diversity*: diversity obtained varying the functional groups present in specific sites of scaffolds, giving them different possibilities of interactions with the biomacromolecule.
3. *Stereochemical diversity*: this type of diversity increases the number of relative orientations of the potential interacting elements of the final molecule. It can best be achieved by using enantio- or diastereoselective reactions that proceed in a general way, overriding specific substrate bias.[16]

4. *Skeletal diversity*: diversity resulting in molecules with distinct molecular shapes, obtained by modifying ring structures and other rigidifying elements. This is the most difficult type of chemical diversity to achieve, but also the most attractive one, considering that the scaffold complexity is a tight requisite for the interactions with target biomacromolecules.[17]

To provide a visual and easily interpretable representation of the relative molecular diversity incorporated into different compound collections, the concept of chemical space proved to be useful.[18] As defined by Dobson, the chemical space is the "the total descriptor space that encompasses all the small carbon-based molecules that could in principle be created".[19] Using this concept, the difference between focused libraries generated by combinatorial chemistry and DOS libraries can be easily observed by mean of graphics. Whereas combinatorial chemistry aims to build a lot of structures whose activity is already known (exploiting retrosynthetic analysis), thus exploring small regions of the chemical space (Figure 3a), DOS uses forward synthetic analysis to explore wide and unknown areas of the chemical space in a much more efficient way (Figure 3b).

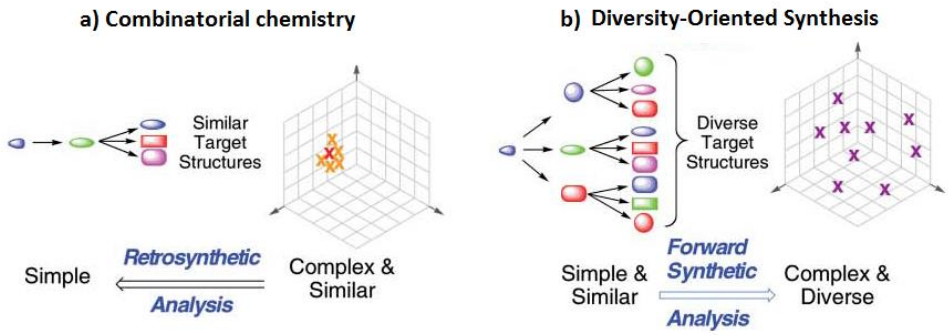


Figure 3: Comparison between combinatorial chemistry (**a**) and DOS strategies (**b**) in terms of interrogated chemical space. Adapted from ref. 4d with permission from the Royal Society of Chemistry.

1.1.1 Chemioinformatic Methods

Chemioinformatic methods play a key role in analyzing quantitatively the diversity of compound collections with the concept of the chemical space.[20] In particular, physicochemical properties are usually analyzed using principal component analysis (PCA),[21] whereas the scaffold diversity can be easily assessed by principal moments of inertia (PMI) analysis.[22]

In details, PCA is a statistical tool used to condense multidimensional chemical properties, such as molecular weight, logP, ring complexity, into single dimensional numerical values, that can be plotted into graphs and superimposed to compare the relative diversity of different molecules collections. Just to give an example, in Figure 4 are reported, in separated plots, the first two principal components obtained from a database containing a random selection of 13506 combinatorial compounds (a), 3287 different natural products (b) and 10968 commercial drugs (c).[23] The first principal component was related to the ratio of aromatic ring atoms towards the total number of heavy atoms and the number of hydrogen bond donors and acceptors. The second principal component was analysed considering the ring fusion degree and the number of carbon-sulfur and carbon-halogen bonds.

Combinatorial compounds cover only a limited area in the diversity space, little over half of which is occupied by natural products and drugs. Much of the area covered by drugs and natural products contain no representative combinatorial compounds, revealing how the current failure of combinatorial libraries may be correlated to the restricted region of chemical space explored, as already discussed above.

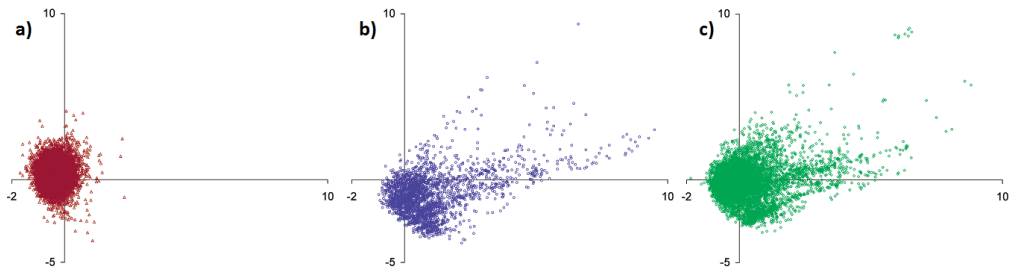


Figure 4: Application of principal components analysis to (**a**) combinatorial compounds (red); (**b**) natural products (blue) and (**c**) commercial drugs (green). Adapted with permission from ref. 23. Copyright (2003) American Chemical Society.

The Principal Moments of Inertia (PMI) analysis employs normalized shape based descriptors to position minimum energy conformation of each library member in a triangular graph plot, where the vertices represent a perfect rod (acetylene), disc (benzene) and sphere (adamantane), thus describing the chemical space covered by the library with respect to the molecular shape. Greater shape diversity of a library correlates with increased likelihood of the collection to contain molecules capable of interacting with biological targets. Just to give an example, Marcaurelle and coworkers using PMI analysis showed that the use of glycal-derived compounds as building blocks to develop skeletally different bi- and tricyclic pyran-fused products[24] allowed to explore wider chemical space as compared to analogue libraries obtained by the same authors using aldol-[25] or azetidine-derived building blocks (Figure 5).[26]

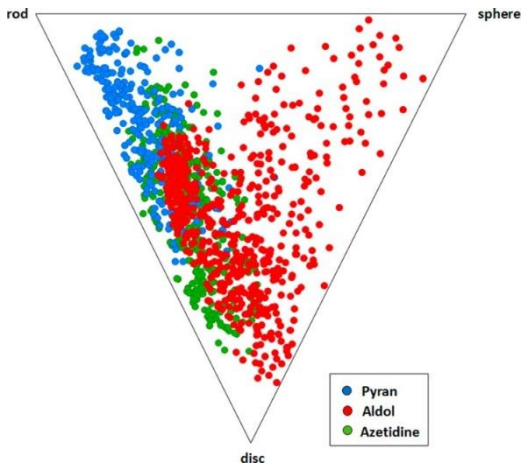


Figure 5: PMI Analysis of pyran-based collection (blue) obtained by Marcaurelle and coworkers, compared to the previously reported aldol- (red) and azetidine- (green) libraries. Reprinted with permission from ref. 24. Copyright (2013) American Chemical Society.

1.2 Biological Applications of DOS Libraries: Getting Insight into Phenotypic Screening Approach[27]

As discussed at the beginning of this chapter, Diversity-Oriented Synthesis (DOS) has revolutionized the construction of libraries for drug discovery issues

and already proved to be effective for the discovery of non-traditional drug targets. In particular, two principal approaches can be followed in order to assess the bioactivity of the resulting libraries generated by DOS strategies. One traditional approach is the application of high-throughput screening looking for activity against several known biological targets.[28] The pharmaceutical industries have employed this strategy for decades and this approach is still renumerative and attractive, as demonstrated by the high number of collaborative public or private screening networks.[5,6]

However, considering that the spectrum of the potential bioactivity of DOS compounds is usually *a priori* unknown and difficult to imagine, a more powerful approach for the discovery of both new ligands and new biological targets is the application of these libraries in phenotypic screening or chemical genetics studies in search of *hit* compounds capable of inducing a desired phenotype. This 'forward pharmacology' process proved to be particularly successful for lead discovery in those complex disorders, such as cancer and neurological or infective pathologies, where multiple targets are involved and/or physiopathological pathways have yet to be discovered (Figure 6).[29]

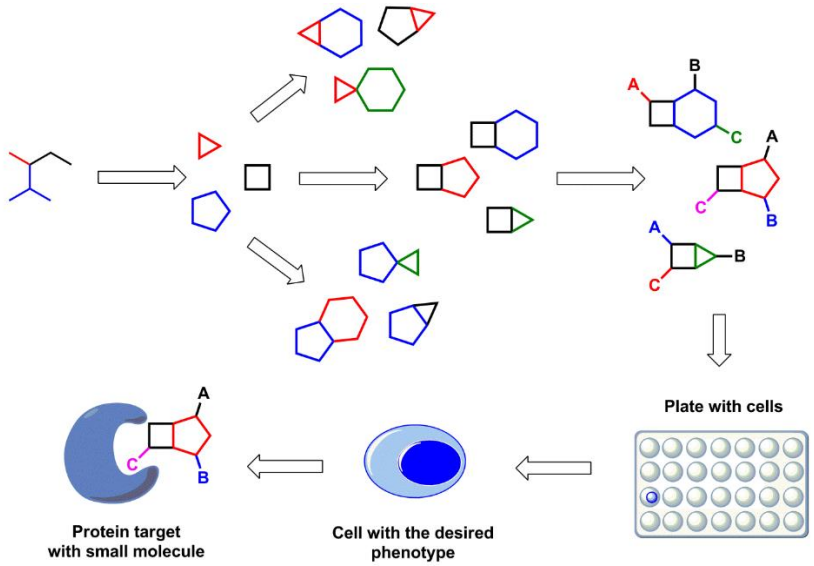
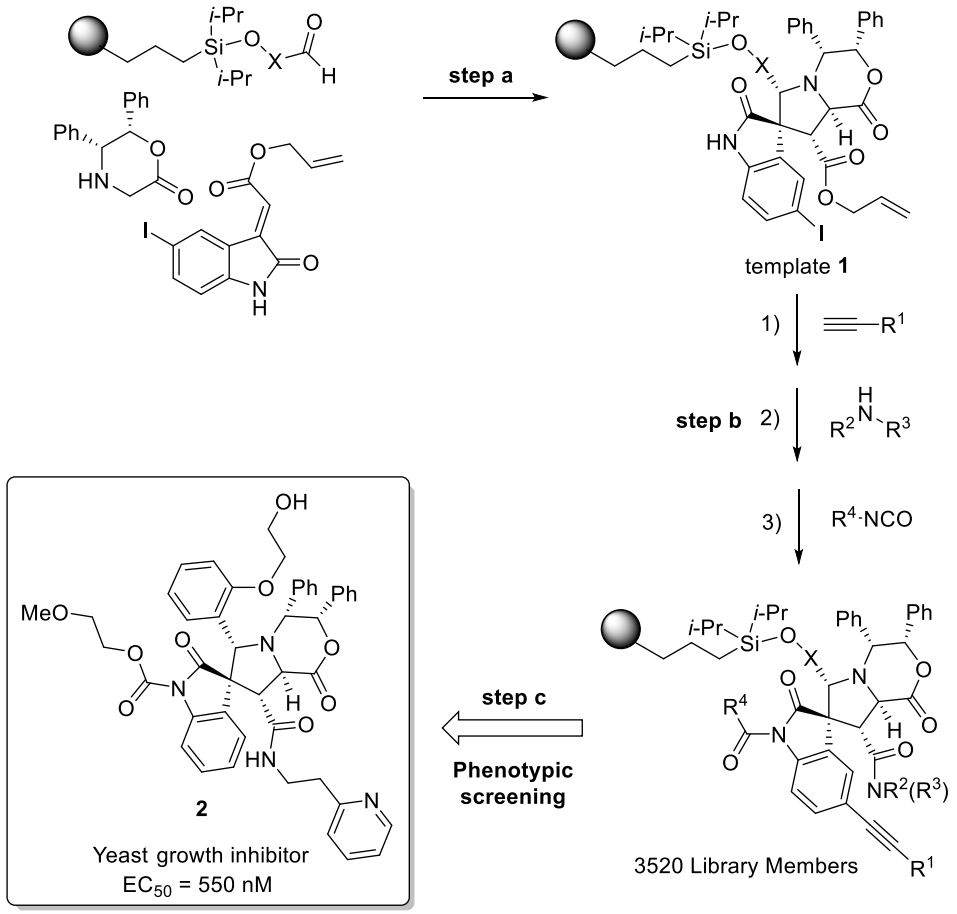


Figure 6: Application of chemical genetics studies on small molecules libraries for the discovery of *hit* compounds and biological targets.

In this context, chemical genetics is one of the best examples of a methodological development in *lead* generation.[4] It uses small molecules to perturb the function of gene products, thus facilitating the dissection of biological processes.

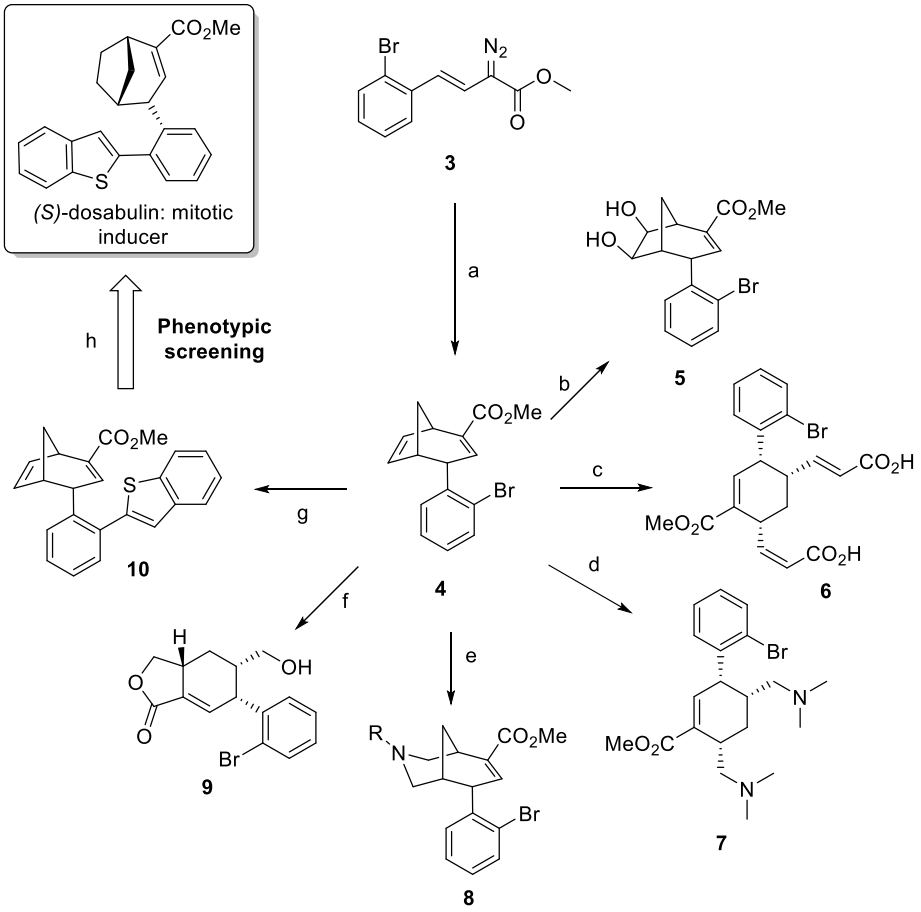
In forward chemical genetics, a small molecule eliciting a desired phenotype is identified, and its protein partner is discovered subsequently following a deconvolution method. These studies (from molecule to protein to phenotype) are exploited when the aim of the investigation is the identification of molecules able to induce a specific biological effect. On the other hand, reverse chemical genetics approaches are fundamental to validate a known target. These studies involve functional small-molecule assays targeted directly to a protein of interest. Once a compound targeting a given protein is identified, the challenge is to discover if the small molecule has an effect in a cellular context. In fact, there is no guarantee that the compounds will affect a protein in a way that results in a functional phenotypic outcome in the cellular context. Also targeting a specific protein may not result in giving the desired therapeutic consequence, whereas forward chemical genetics approach allows to see directly *in vivo* or within complex *in vitro* system if the compound has a remarkable effect. For this reason, phenotypic screening, and the related forward chemical genetic approach, is gaining huge popularity in drug discovery, so that has been claiming that we are approaching "The Renaissance of Phenotypic Research".[30] For sake of clarity, this approach has been always used in pharmaceutical industries, especially in particular fields.[31] For example, nifedipine, nimodipine, and other calcium channel antagonists were found through the screening of large compound collection by looking for molecules able to induce vasodilatation and blood pressure reduction.[32] However, in the last few years phenotypic screenings have evolved and acquired a precise strategy, taking advantage of robotic and miniaturized technologies and the intrinsic molecular diversity of the libraries generated following Diversity-Oriented Synthesis principles.[33]

From the pioneering work of Schreiber and Tan, about the discovery of an enhancer of latrunculin B (compound **2**, Scheme 1), which induces yeast growth arrest, from a library of a 3250 members based on the tetracyclic template **1** (Scheme 1),[34] several improvement have been made.[35,14,15]



Scheme 1: **(a)** Stereoselective synthesis of the spirooxindole scaffold **1**; **(b**) scaffold decoration by cross-coupling reactions (1), amide formations (2), *N*-acylations (3). (**c**) From this library, compound **2**, a potent enhancer of latrunculin B, was discovered.

Recently, the group of David Spring reported a striking diversity-oriented synthesis combined to a forward chemical genetics study.[36] In this work, they obtained a DOS library of 35 compounds based on 10 distinct molecular scaffolds (some representative examples are shown in Scheme 2) starting from the highly functionalized key intermediate **4** obtained by a cyclopropanation-Cope rearrangement of phenyl diazo ester compound **3.**



Scheme 2: (**a**) Generation of highly functionalized intermediate **4** from phenyldiazoester compound **3** through cyclopropanation and Cope rearrangement. Library development from key building block **4** through (**b**) dihydroxylation, (**c**) ring opening metathesis, (**d**) dihydroxylation/oxidative cleavage + reductive amination, (**e**) dihydroxylation/oxidative cleavage + double reductive amination with primary amines, (**f**) dihydroxylation/oxidative cleavage + esterification, (**g**) Suzuki reactions. (**h**) Identification of dosabulin, partially saturated analogue of compound **10**, as a cell growth inhibitor.

The DOS library was then assayed in a high-content microscopy-based phenotypic screening, finding that a partially saturated analogue of compound **10**, named dosabulin, was able to induce mitotic arrest and cancer cell death by apoptosis.

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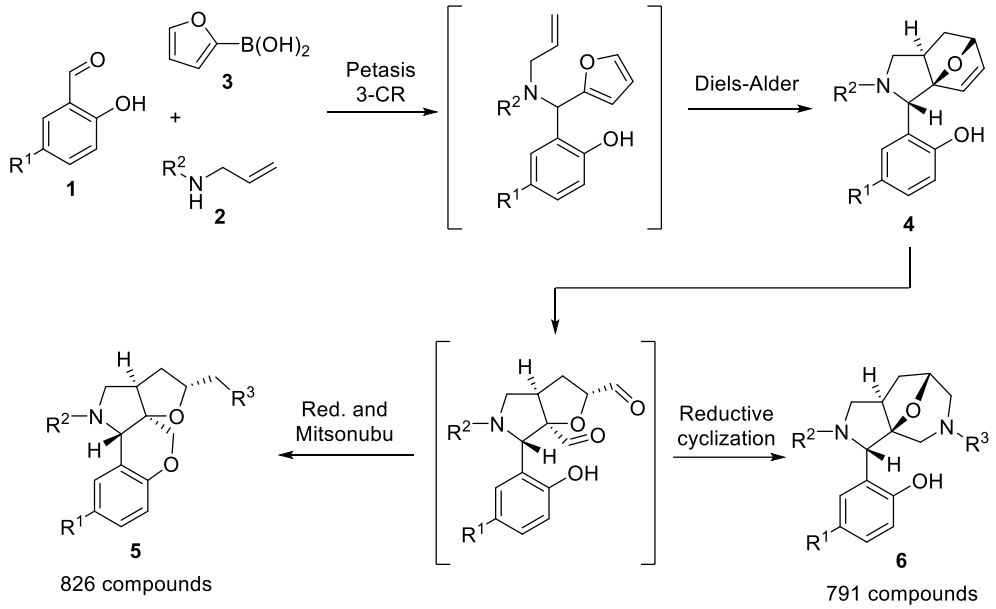
2

DOS Synthetic Strategies

In Diversity Oriented Synthesis, where a small number of compounds are transformed into many distinct structures, synthesis pathways are planned using forward-synthetic analysis. Also, the aim of Diversity-Oriented Synthesis is to develop molecular scaffold possessing the highest structural complexity, using efficient strategies composed of no more than four, five steps.[1] For these reasons, DOS pathways are branched and divergent and make use of complexity-generating reactions and cascade processes, capable of creating sp^3-rich molecular entities, even starting from simple flat building blocks. In this context, Flagstad and coworkers reported the use of the tandem Petasis and Diels–Alder reactions to create the complex key intermediate **4** starting from salicylic aldehyde derivatives **1**, allyl amines **2** and 2-furyl boronic acid **3** (Scheme 1).[2] Then, two different divergent complexity-generating cyclization cascades led to the achievement of sp^3-rich scaffolds **5** and **6**, which have been used for the production of two different libraries composed, overall, of 1617 molecules.

Another powerful approach, for the generation of the highest complexity and diversity, is the exploitation of polyfunctional, stereochemically dense, building blocks or complex intermediate, from the chiral pool, which intrinsically possess potential interacting elements. Particularly relevance has been gaining the biosynthetically inspired divergent approach,[3] the diversity-oriented synthesis of natural-product inspired libraries,[4] and the development of divergent strategies starting from complex natural products precursors.[5]



Scheme 1: Synthetic strategies for the generation of complex scaffolds **5** and **6** for library generation.

The last approach, consisting of building densely functionalized intermediates which contain moieties from natural products proved to be useful to explore a regions of the chemical space with high density of bioactive molecules. Natural products are in fact a source of inspiration for drug design thanks to the intrinsic chemical and structural diversity, the selectivity towards a wide number of targets, and the capability of following Lipinski's rules for drug-like properties.[6] As this approach has been widely used in the following thesis work, different DOS strategies starting from natural products-derived building blocks will be discussed in details in the next paragraphs.

In the last decades, several efforts have been devoted to the development of novel synthetic strategies following DOS efficiency criteria. Different terms have been conceived, such as the twelve-fold branching strategy reported by Dobbins,[7] or the so-called Relay Catalytic Branching Cascade (RCBC) approach.[8] However, one of the most used approach in DOS is still the **build/couple/pair** (**BCP**) strategy.

2.1 Build/Couple/Pair (BCP) Strategy

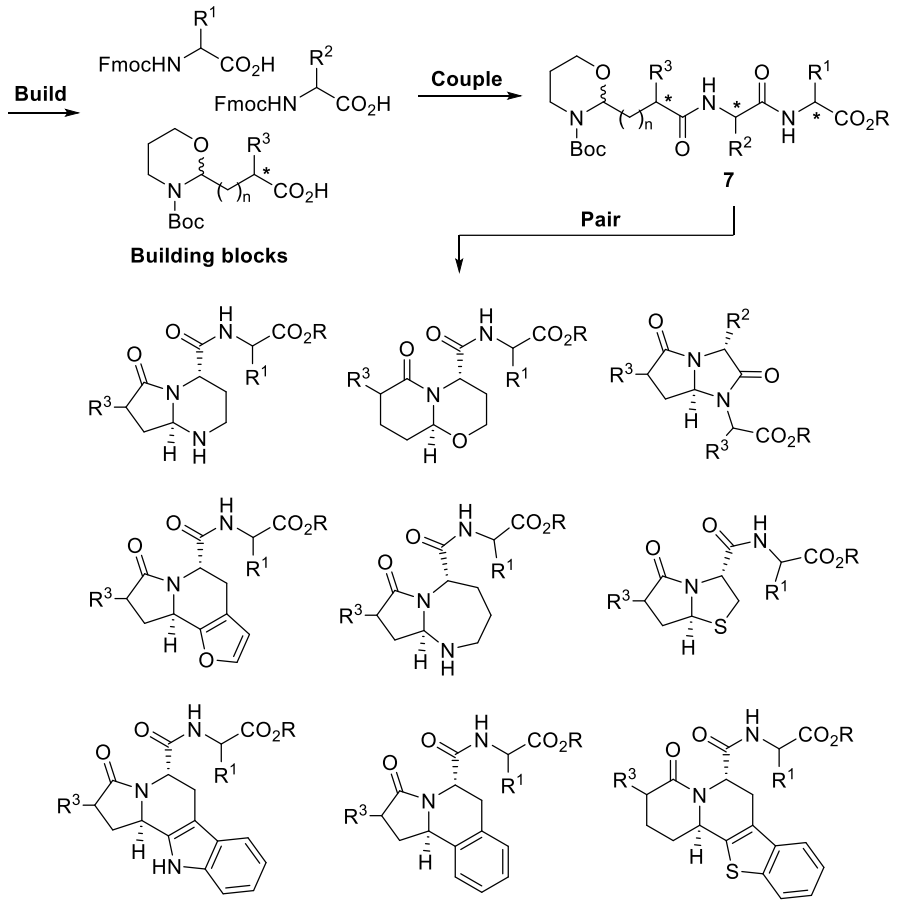
In the build/couple/pair strategy,[9] different building blocks containing orthogonal sets of functional groups are coupled together possibly with complete control of the stereochemical outcomes. The resulting multipotent, polyfunctional intermediates are then submitted to different intramolecular functional group pairing reactions.[10] For this purpose, functional groups used in the subsequent pairing reactions should be strategically positioned to allow as many ring-closing modes as possible, and the chemoselectivity, such as the functional group preferences of different transition metals, should be exploited to achieve as many different skeletons as possible. All the process should count no more than five steps, ideally with high yields and complete selectivity.

In this context, functional group pairings are usually subdivided into three categories:

1. *polar/polar*, such as for example the reaction between an amine and an ester group to form a lactam.
2. *nonpolar/nonpolar*, such as for example the alkene/alkene ring-closing metathesis to generate a cycloalkene;
3. *polar/nonpolar*, such as for example the use of a cycloacetalization reaction between an alcohol and an alkyne function.

In scheme 2 it is shown, as a representative example, the BCP strategy reported by Nielsen and coworkers,[11] where, starting from the modified tripeptide **7**, a variety of bi- and tricyclic heterocyclic scaffolds was generated exploiting different polar-polar couplings (each passed through a nucleophilic addition to an iminium ion).

In particular, among all the possible building blocks that have been exploited during last decades in diversity oriented synthesis, carbohydrates and nitrogen-containing building blocks will be taken into account in the next paragraphs, as this thesis work is mainly focused in the generation and/or application of these precursors.



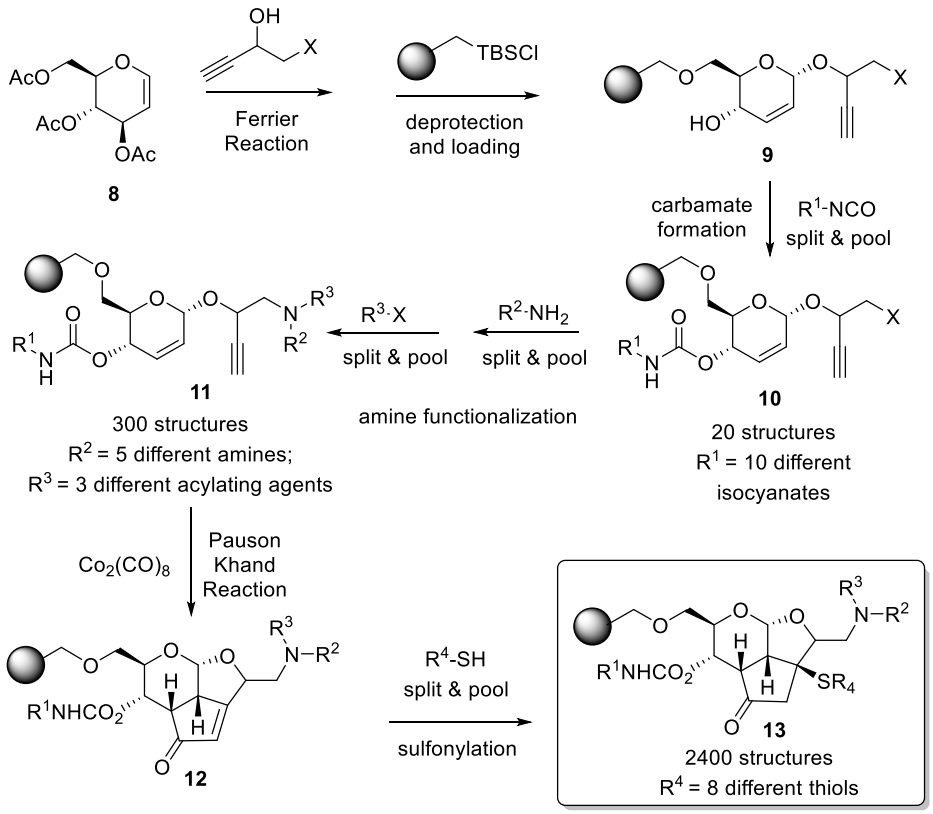
Scheme 2: Build/Couple/Pair strategy, exploiting the polar/polar functional group pairing of the modified tripeptide intermediate **7**.

2.2 Carbohydrates in Diversity-Oriented Synthesis[12]

Carbohydrates are attractive building blocks for the generation of high-quality small molecule collections, thanks to their structural bias and the high density of polar functional groups, which offer many possibilities in scaffold decoration and further chemical manipulation. In addition, it has been proved by chemioinformatic analysis that natural products have a greater number of oxygen atoms than the corresponding combinatorial products, thereby highlighting the significance of synthetizing oxygen-rich small molecules for increasing the chance of identifying new *hit* compounds.[13] This difference in the

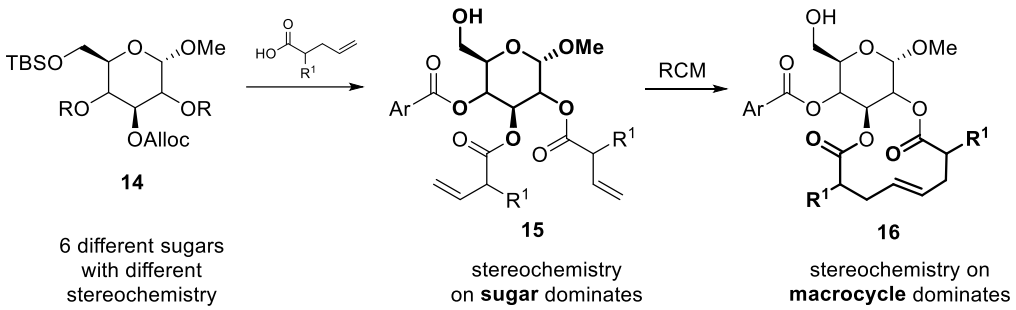
number of oxygen atoms could in fact have important consequences in the interactions with biomacromolecules, considering that hydroxyl groups play an important role acting both as donors and acceptors groups in hydrogen-bonding interactions.

However, only a limited area in the world of pharmacopeia is covered by sugar-derived compounds and many important carbohydrate-protein interactions have yet to be discovered. Also, carbohydrates remain one of the most underused and unexplored groups of chiral compounds in diversity-generating synthetic strategies, even if some contributions on the application of carbohydrates or their derivatives in DOS have recently appeared in the literature. Most relevant examples of these strategies employ D-glucose,[14] D-mannitol,[15] D-ribose,[16] D-xylose,[17] and are related to the achievement of appendage diversity around a common scaffold. In this context, Schreiber and coworkers reported the split-and-pool synthesis of a library of 2400 different tricyclic compounds **13** starting from the D-glucal **9** (Scheme 3).[18]



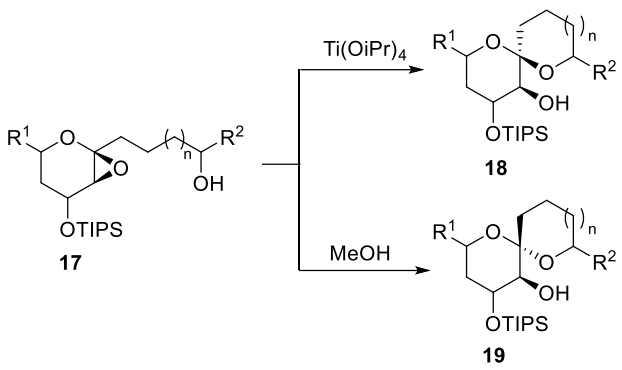
Scheme 3: Split and pool library synthesis of 2400 tricyclic compounds **13**.

Carbohydrates are also particularly useful in the development of DOS strategies towards stereochemical diversity, considering the intrinsic variety of the four/five stereogenic centers of the sugar template. In this context, Kim and coworkers described a systematic and quantitative method to measure the role of stereochemistry and conformational constraints in modulating protein interactions.[19] They reported the synthesis of a collection of 122 carbohydrate-derived monocyclic precursors **15** (Scheme 4), having different stereochemistry and different alkene-based appendages, in order to provide a variety of 12-membered macrocyclic compounds **16** through a ring-closing metathesis reaction. The effects of the stereochemistry of both monocyclic and bicyclic compounds towards bioactivity were assessed in a multidimensional chemical genetics approach, studying the perturbation of four cellular events, namely DNA synthesis, esterase activity, mitochondrial membrane potential and the intracellular reducing activity, finding that the stereochemistry within the carbohydrate ring played a dominant role in the monocyclic compounds **15**, whereas in the bicyclic compounds **16** the stereochemistry on the macrocycle dominated.



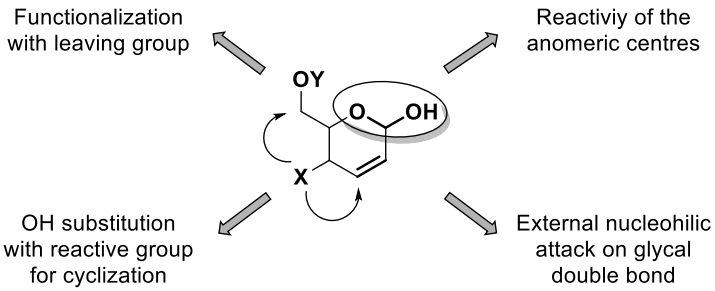
Scheme 4: Synthesis of monocyclic compounds **15** and bicyclic compounds **16** and role of the stereochemistry towards biological activity.

In addition, several stereochemically-divergent synthesis have been proposed during these last years starting from carbohydrate-derived building blocks, as the generation of anomeric spiroketals **18** and **19** from alkylpyran **17** developed by Tan and co-workers (Scheme 5).[20]



Scheme 5: Stereochemically divergent synthesis of spiroketals **18** and **19**.

On the other hand, only few papers accounted for the exploitation of structural rearrangement of carbohydrate-based compounds to develop skeletal diversity around such moieties. The need of protecting/deprotecting groups, often required in sugar chemistry, contrast with the efficiency criteria of DOS, where the synthetic efforts, that are not directed through a validate target, must be restrained. This is nevertheless the most promising one, because it is able to develop novel carbohydrate-derived scaffold having enhanced structural complexity, but still retaining the high-value sugar functionalities, such as stereocentres, polyhydroxylated chains and conformational constraints. For these reasons, some efforts have been direct to the exploitation of different sugar moiety, such as glycal double bond, anomeric carbon reactivity and OH-functionalization/substitution (Scheme 6).

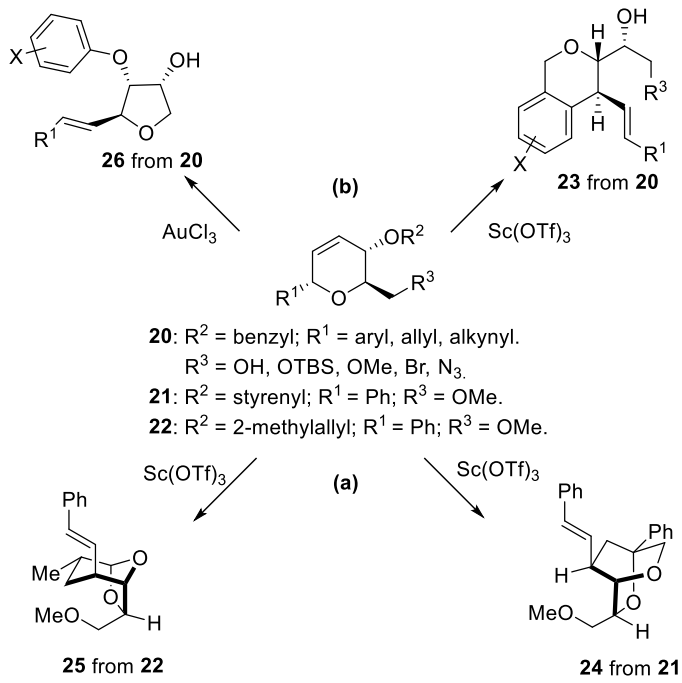


Scheme 6: Strategic exploitation of different sugar moieties, for the achievement of carbohydrate-derived scaffold.

Diverse and complex skeletons, according to DOS principles, can be achieved in two ways:[21] using different reagents on the same substrate (*reagent-based* or *differentiating* approach) or pre-encoding the transformation in different

starting materials and subjecting them to a common set of reaction conditions (*substrate-based* or *folding* approach).

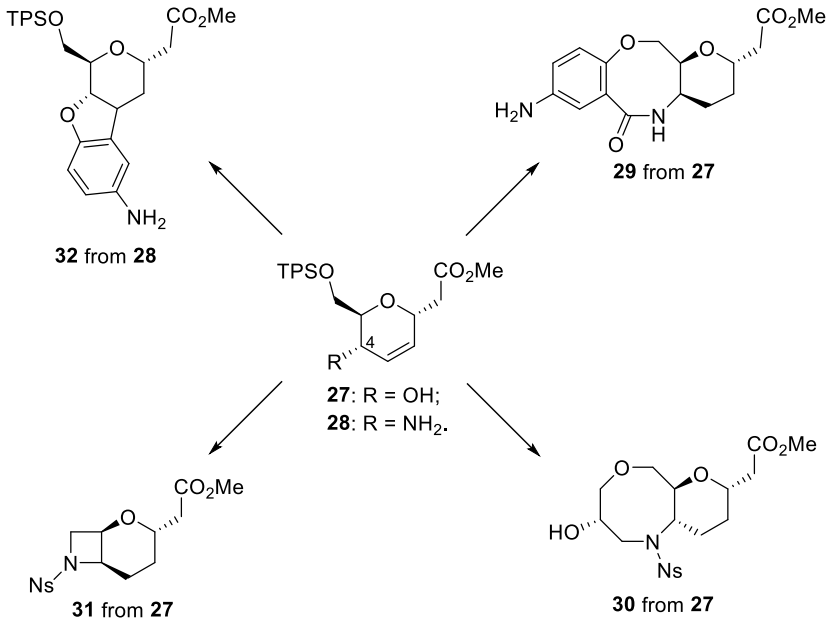
In Scheme 7 is reported the substrate-based (a) and reagent-based approaches (b) exploited by Porco and co-workers to achieve skeletal diversity using glycal-derived compounds.[22] Starting from different glycal-derived dihydropyrans **20**-**22**, easily obtained from tri-*O*-acetyl-D-glucal, three skeletally different scaffolds **23**-**25** were achieved simply by changing the nature of C-1 and C-6 substituents (Scheme 7a). In addition, the same aryl ether C-glycosides **20** gave the highly substituted tetrahydrofuran **26** by changing the reaction conditions (Scheme 7b).



Scheme 7: (**a**) Substrate-based and (**b**) reagent-based approaches for the achievement of skeletally different compounds from dihydropyrans **20**-**22**.

Glycals and glycal-like compounds were exploited widely in recent years also by other authors.[23] Just to give an example, Marcaurelle reported the skeletal diversity-oriented synthesis of different bi- and tricyclic pyran-fused products starting from glycal derivatives **27**-**28**, exploiting three step processes consisting in functionalizing C-4 with appropriate counterparts, able to undergo

intramolecular cyclization with the internal double bond or with the substituted primary hydroxylic function (Scheme 8).[24]



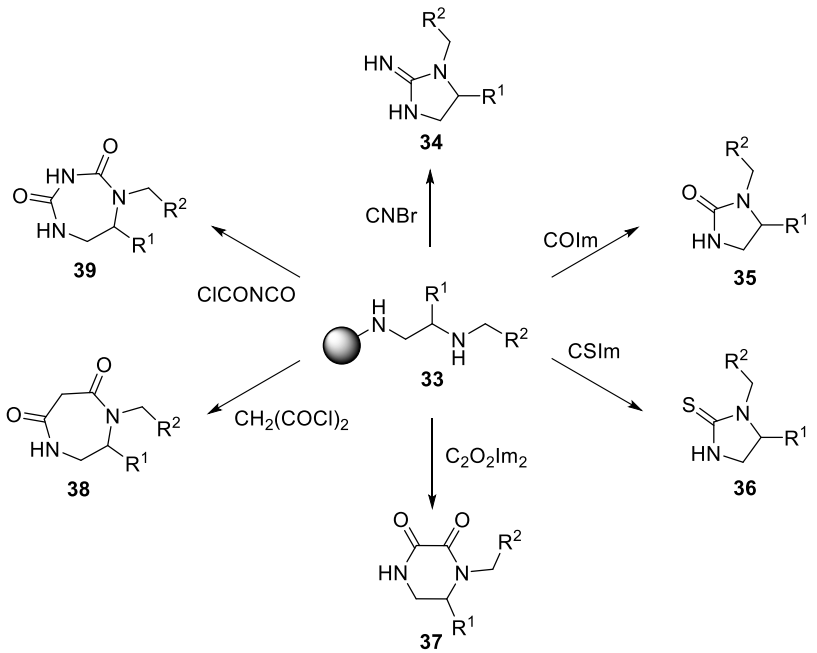
Scheme 8: Skeletal diversity from glycal derivatives **27** and **28**.

2.3 Nitrogen-containing Building Blocks in Diversity-Oriented Synthesis

Nitrogen-containing building blocks are extremely interesting for the development of biologically active scaffolds, considering the wide presence of *N*-heterocycles and nitrogen-containing functional groups in natural products and drugs.[25] Not surprisingly, within the top 100 most frequently used ring systems in the FDA drugs, as analysed by Taylor and coworkers,[26] more than sixty contain at least one nitrogen atom. Amino moieties and nitrogen heterocycles play in fact a relevant role in biological system, thanks to the ability to act as hydrogen bond donor and acceptor or to be involved into electrostatic interactions. In addition, nitrogen functionalities represent a versatile point of diversification for appendage diversity and scaffold decoration (i.e. exploiting urea formation, alkylation, acylation, sulfonylation, reductive amination).[27] For these reasons and for their wide synthetic

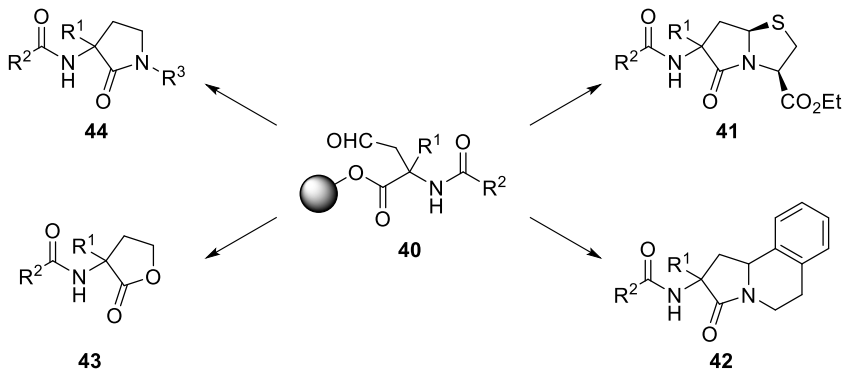
potentials, *N*-containing building blocks have attracted considerable attention in Diversity-Oriented Synthesis.

In particular, amino acids and derivatives has been exploited from the beginning, thanks to the intrinsic chemical diversity of the side chains and their versatility in generating *N*-heterocycles. In this field, several DOS strategies from natural amino acids have been reported,[28] especially using proline derivatives.[29] Just to give an example, Nefzi and coworkers reported the application of a resin-bound amino acid-derived ethylendiamines **33** for the generation of skeletally different diaza- and triazacyclic compounds **34**-**39** (Scheme 9).[30]



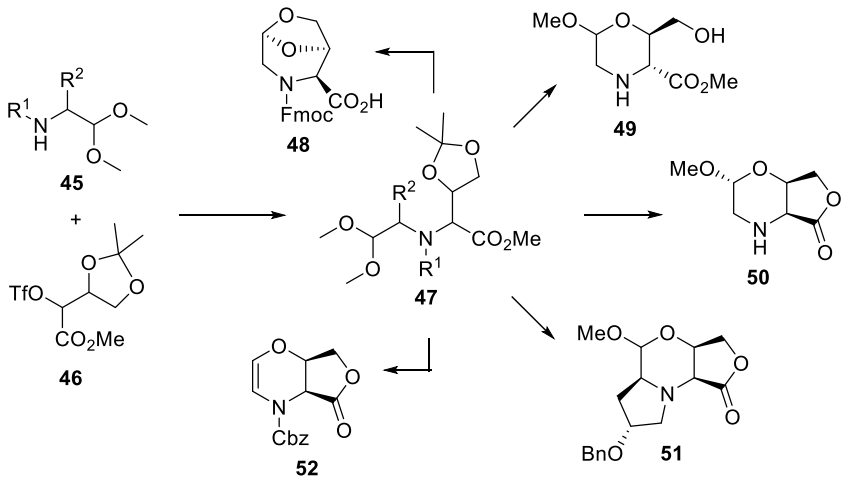
Scheme 9: Diversity-Oriented Synthesis of different *N*-heterocycles starting from amino acid derived ethylendiamines **33**.

Amino aldehydes have been also used to generate complex cyclic scaffolds. As shown in Scheme 10, a wide variety of substituted lactam-containing peptidomimetics **41**-**44** have been achieved from resin-bound aldehyde intermediate **40** through chemical manipulations and cyclitive cleavage process.[31]



Scheme 10: Peptidomimetic lactam-containing scaffolds generated from resin-bound amino aldehyde intermediate **40**.

The contribution of our research group in this field consisted of build/couple/pair strategies for the generation of peptidomimetic libraries starting from amino acid and sugar derivatives.[32] The combination of amino acetal **45** and L-ascorbic acid derivative **46** into the polyfunctional coupling intermediates **47** allowed to generate a high degree of chemical diversity around the morpholine ring (compounds **48**-**52** in Scheme 11).[33]

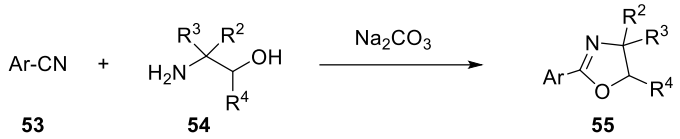


Scheme 11: Skeletal diversity resulting from the coupling intermediate **47** obtained starting from amino acetal **45** and L-ascorbic acid derivative **46**.

In the context of useful *N*-containing building blocks for divergent synthesis, nitrile-containing compounds have a key role for the generation of *N*-heterocycles and related scaffolds. Their interest in library development for drug discovery issue is not solely related to their synthetic potentials, but also

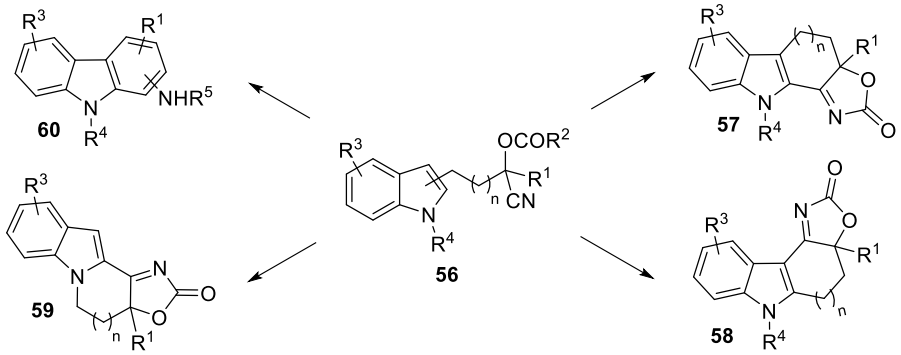
for the intrinsic physicochemical properties of nitriles. In fact, nitrile functional group is found in many bioactive natural products and pharmaceutical compounds and can be involved into hydrogen-bonding or π-π interactions, due to its high polarity and the characteristic linear geometry.[34]

Furthermore, the nitrile group is a valuable and versatile precursor to a wide range of functional groups including amines, amides, carbonyl compounds and carboxylic acid derivatives as well as five- and six-membered ring heterocycles.[35] These features have been exploited since the beginning in combinatorial chemistry, in particular for the achievement of heterocyclic libraries with wide appendage diversity, taking advantage of the key role of nitriles in multicomponent reaction.[36] In scheme 12 is shown, as a representative example, the synthesis of structurally diverse 2-aryl/heteroaryloxazolines **55** from aryl nitriles **53** and aminoalcohols **54**.[37]



Scheme 12: Synthesis of 2-aryl/heteroaryloxazolines **55** from aryl nitriles **53** and aminoalcohols **54**.

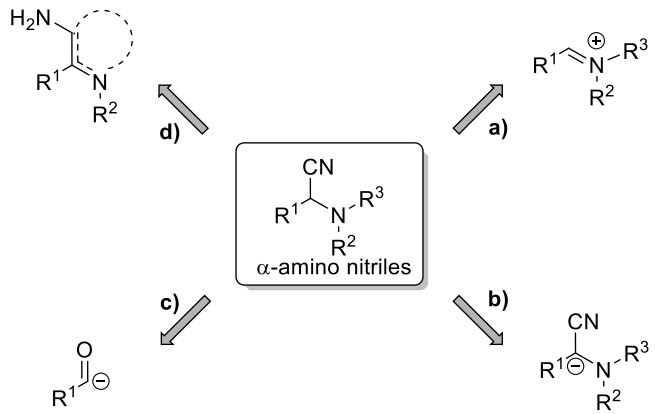
Moreover, nitrile-derived compounds proved to be valuable intermediates even in the achievement of skeletal diversity. Starting from cyanohydrin derivative **56**, Wang and coworkers reported the synthesis of carbazoles **57** and **58**, tetrahydropyrido[1,2-*a*]-indole **59** and carbazolamines **60**, exploiting the Pd-catalyzed intramolecular C−H addition of indoles to nitrile (Scheme 13).[38]



Scheme 13: Synthesis of different polycyclic scaffolds from intermediate **56**.

In this context, bifunctional α-amino nitriles, possess particularly interesting features for the generation of skeletally different *N*-heterocycles, thanks to their several different modes of reactivity.[39]

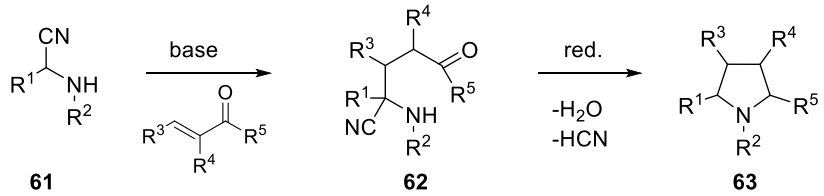
In particular, α-amino nitriles can act as iminium ions (Scheme 14a), when the cyanide ion is trapped with nucleophiles, such as hydride or organometallic reagents, giving access to the corresponding enamine,[40] or, after hydrolysis, to the corresponding carbonyl compound.[41] Also, when these compounds possess an hydrogen atom in α-position, they can be deprotonated generating α-amino carbanions (Scheme 14b), that can react with nucleophiles, such as α,β-unsaturated carbonyl compounds, aldehydes, alkynes or epoxide moieties.



Scheme 14. Different reactivity of α-amino nitriles.

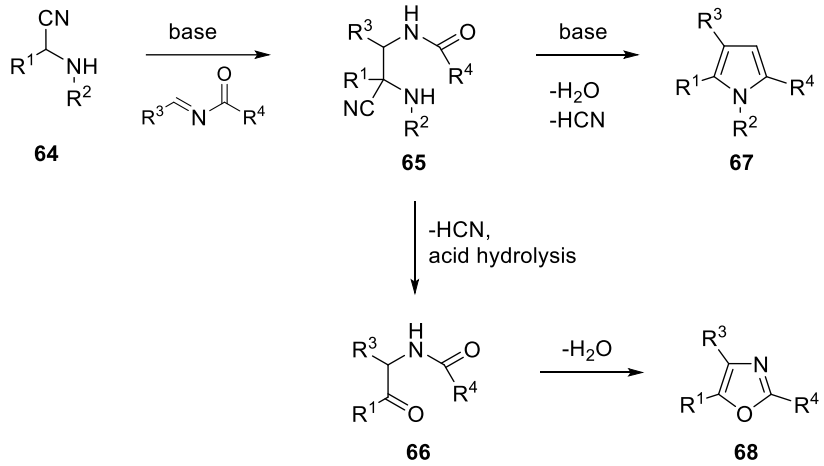
Deprotonated α-amino nitriles represent also synthetic equivalents of acyl anions, useful synthons for the synthesis of 1,4-dicarbonyl compounds and their subsequent derivatives (Scheme 14c).[42] Finally, the electrophilicity of the nitrile group, linked together with the nucleophilic amine function, creates a unique condition for the achievement of novel methods of intramolecular cyclization into *N*-heterocycles (Scheme 14d).

Just to give some examples, Meyer and Opatz exploited the reactivity of α-amino nitriles as α-amino carbanions, reporting the vinylogous addition of compounds **61** to different α,β-unsaturated carbonyl compounds, thus achieving, after reduction and elimination processes, the synthesis of a variety of polysubstituted pyrrolidines **63** (Scheme 15). [43]



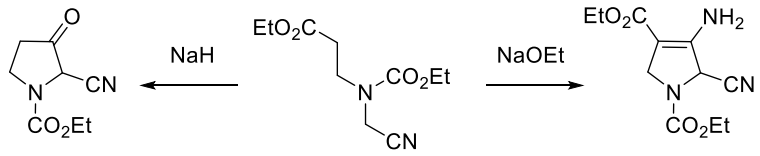
Scheme 15: Synthesis of substituted pyrrolidines **63** from α-amino nitrile compound **61**.

In an analogue way, Kison and coworkers synthesized tetrasubstitued imidazoles **67** and trisubstituted oxazoles **68**, starting from α-(acylamino)imines **65** and α-(acylamino)ketones **66**, obtained by a coupling reaction between deprotonated α-amino nitrile **64** and *N*-acylimines (Scheme 16).[44]



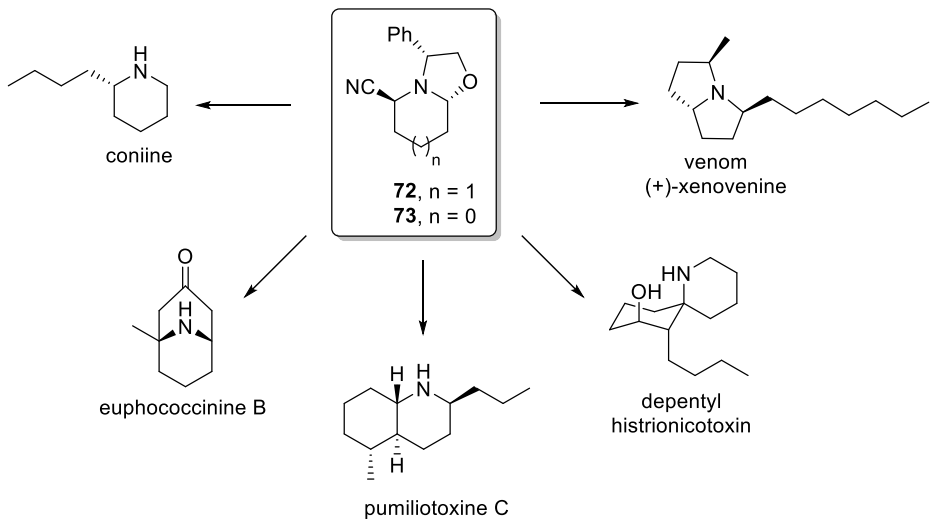
Scheme 16: Synthesis of imidazoles **67** and oxazoles **68** by 1,2 addition of deprotonated α-amino nitrile **64** and *N*-acylimines**.**

Furthermore, when α-amino nitrile moiety is present in intermediates containing other functional groups, different ways of cyclization can be achieved in a divergent fashion. For example, starting from aminonitrile ester **69**, different reaction conditions led to the Dieckmann-type cycloadduct **70** or the cyclic enamino ester **71** (Scheme 17), in agreement with the reagent-based approach.[45]



Scheme 17: Different ways of cyclization of aminonitrile ester **69**.

In conclusion, in scheme 18 is shown the potentiality of α-amino nitrile containing precursors for the synthesis of natural product compounds. In fact, as reviewed by Husson,[46] the *N*-cyanomethyloxazolidine system contained in oxazolopyridine **72** and oxazolopyrroline **73**, where the nitrogen atom is an integral part of both α-aminonitrile and α-aminoether functions, has been exploited in the generation of several natural products bearing piperidine and pyrrolidine rings. In details, (*S*)-(+)-coniine has been achieved from **72** in just three steps,[47] whereas the homotropan alkaloid euphococcinine,[48] and (-)-pumiliotoxine-C in, respectively, four and five steps.[49] In addition, the substitution of α-hydrogen atom with a propanal chain allowed to obtain the depentyl histrionicotoxin alkaloid.[50] Finally, the synthesis of venom (+)-xenovenine has been reported in three steps from compound **73**.[51]



Scheme 18: Natural products obtained from cyanomethyloxazolidinic precursors **72** and **73**.

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3

Work Overview

The screening of small molecule libraries represents a powerful approach for *lead* discovery, especially in those complex disorders, such as cancer and infectious diseases, where the lack of validated targets makes conventional drug discovery programs difficult to succeed. Accordingly, this thesis work aims to apply the principles of Diversity Oriented Synthesis in the generation of novel molecular scaffolds for library development. In particular, with the purpose of creating novel structure potentially able to address crucial interactions in biological systems, carbohydrates and *N*-containing building blocks are taken into account for their abilities of generating glyco- and/or peptidomimetics moieties.

In a first work, the build/couple/pair strategy has been applied to D-mannose and glycine-derived amino acetaldehyde, as a preliminary case study of the exploitation of a monosaccharide with an amino acid building block, generating six novel skeletally different polyhydroxylated nitrogen-containing scaffolds. Then, a phenotypic screening towards the evaluation of the ability of inducing cell growth inhibition in a human metastatic melanoma cell line, has been envisioned as a first biological output of these compounds. This cell-based assay, combined with follow up synthesis and further biological studies, allowed for the selection of the hexahydro-2*H*-furo[3,2-*b*][1,4]oxazine structure as an interesting scaffold for the development of MDA-MB-231 cell growth modulators.

In a second part of this PhD work, *N*-containing compounds have been selected as valuable building blocks for the generation of nitrogen-containing heterocyclic scaffolds, through the exploitation of versatile couple/pair processes, as required by DOS efficiency criteria. In this context, morpholine acetal and the related reactivity of the *N*-acyl iminium chemistry, proved to be extremely powerful for the achievement of peptidomimetic scaffolds. In particular, rearrangement processes of serine-derived morpholine acetal, together with amino acid chlorides, allowed for the achievement of the uncommon dihydropyrazinone heterocycle, an interesting dipeptide isostere. The concept of obtaining skeletal diversity by using same reagents in a different manner is extremely powerful in expanding the access to chemical diversity. In this context, nitrile-derived compounds, and in particular α-amino nitriles, with their several different modes of reactivity, proved to be valuable building blocks for the generation of skeletally different *N*-heterocycles. Considering the broad presence of amides and lactams in biologically active compounds, we envisioned to develop a methodology for the selective reductive cyanation of carboxamide functional groups, in order to introduce in complex biologically intermediates the α-amino nitrile moieties, which may serve as a valuable point of diversification. The direct transformation of complex precursors into cyanated compounds, can be used as a starting point for divergent approaches, to explore new region of chemical space around a structure that already possess biological activity.

In summary, in this Ph.D. thesis the following topics are discussed:

- Diversity-Oriented Synthesis of nitrogen-containing polyhydroxylated compounds starting from D-mannose (Chapter 4);
- Application of the mannose-derived library in a cell-based phenotypic screening, follow-up synthesis and identification of novel human breast carcinoma (MDA-MB-231) cell growth modulators (Chapter 5);
- Two-step one-pot synthesis of dihydropyrazinones as Xaa-Ser dipeptide isosteres through morpholine acetal rearrangement (Chapter 6);
- Iridium catalyzed reductive Strecker reaction of tertiary amides and lactams for the generation of α-amino nitriles and application in late stage functionalization of drugs, natural products and proline-containing peptides (Chapter 7).

Part II

DOS and Phenotypic Screening of a Mannose-derived Library

4

Skeletal Diversity from Mannose

4.1 Introduction

As discussed widely in the introduction (2.2), carbohydrates are valuable building blocks in Diversity-Oriented Synthesis for their intrinsic synthetic opportunities.[1] Moreover, the exploitation of sugars for the development of novel small molecules collections is very attractive for drug discovery, considering that several biological communication events (like cellular recognition, attachment and adhesion) are regulated by interactions between glycoproteins and proteins.[2] Despite that, only a limited area in the world of pharmacopeia is covered by sugar-derived compounds. In fact, many important carbohydrate-protein interactions have still to be discovered and, in addition, many difficulties related to their oral availability and plasma stability, need to be solved.

To our knowledge, there is still much room left for developing novel skeletally different scaffolds starting from carbohydrate structure. In particular, as discussed above, even if some contributions on the application of carbohydrates in DOS have appeared in the literature recently, only few of such papers accounted for the exploitation of sugar functional groups to develop skeletal diversity.[3]

For these reasons, we decided to direct our synthetic efforts towards the use of pentoses and hexoses, in order to generate new molecular entities containing polyhydroxylated appendanges, as novel potential glycomimetic interacting

elements. In particular, as part of a major research program devoted to the synthesis of novel potentially bioactive scaffolds, containing both glyco and peptidomimetic moieties, we decided to combine the sugar with an amino acid counterpart, to generate novel polyhydroxylated nitrogen-containing small molecules. In fact, several polyhydroxylated natural products have already been discovered as carriers of relevant biological activities. In particular iminosugars, such as the piperidinic 1-Deoxynojirimycin (DNJ) or the pyrrolidinic hyacinthacine A_2 (Figure 1a), proved to be valuable sugar mimetics, with antibacterial, antitumoral and anti-inflammatory activities.[4] Moreover, several bioactive natural product compounds, as for example the antitumoral Hunanamycin A or the riboflavin precursor 6,7-dimethyl-8-ribityllumazine (Figure 1b), possess long polyhydroxylated chains, revealing the importance of these moieties for their potential in the development of new therapeutics against cancer, infective diseases, diabetes, and metabolic disorders.[5]

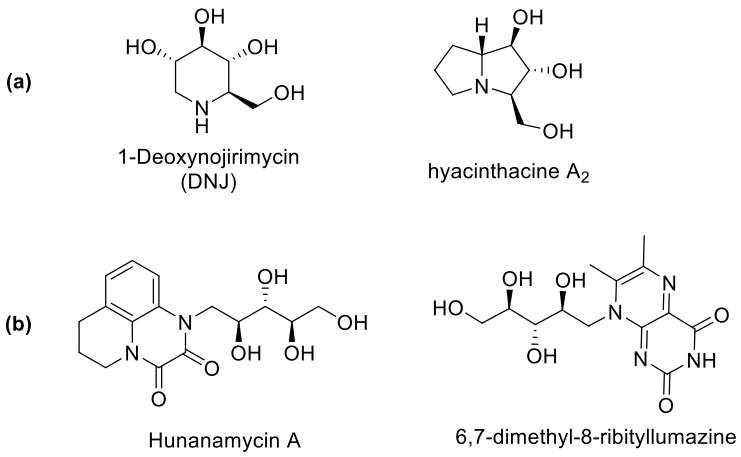


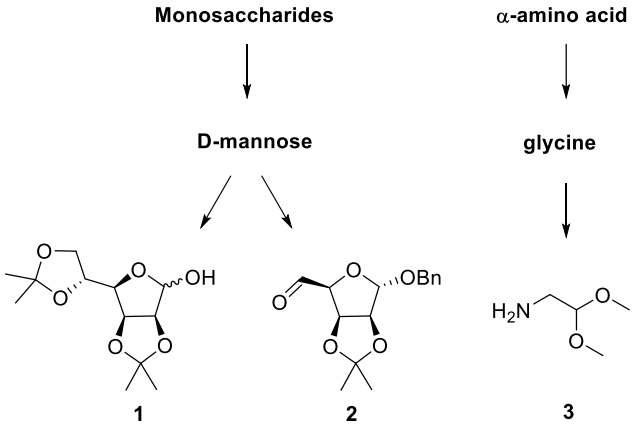
Figure 1: (a) Iminosugars 1-Deoxynojirimycin (DNJ) or the pyrrolidinic hyacinthacine A_2 and **(b)** polyhydroxylated natural products Hunanamycin A and 6,7-dimethyl-8-ribityllumazine.

To this end, we reasoned to develop a DOS strategy following the principles of the coupling/functional group-pairing approaches, starting from D-mannose and glycine, as a first case study of the application of an hexose and an amino acid building block, respectively.

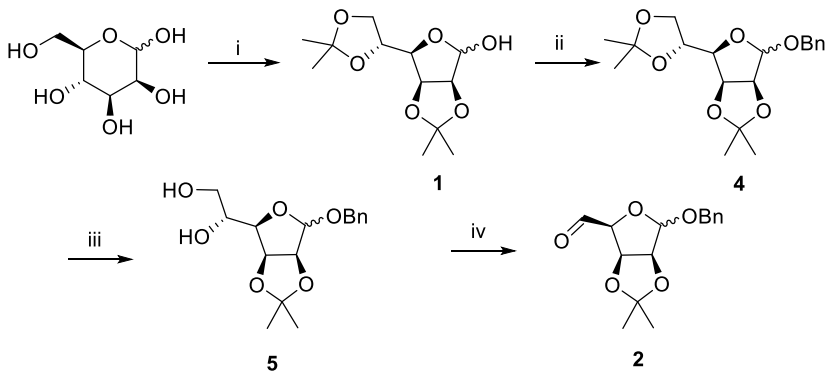
4.2 Results and Discussion[6]

Building:

Two different sugar-derived building blocks have been taken into account. In particular, di-isopropylidene-D-mannofuranose **1** was considered in order to have all the hydroxyl groups protected and benzyl-isopropylidene D-lyxaric aldehyde **2** was selected to explore the different reactivity introduced by the presence of another aldehyde function (Scheme 1). These two compounds can be easily achieved from D-mannose as shown in Scheme 2.



Scheme 1: Selection of building blocks from mannose and from glycine.

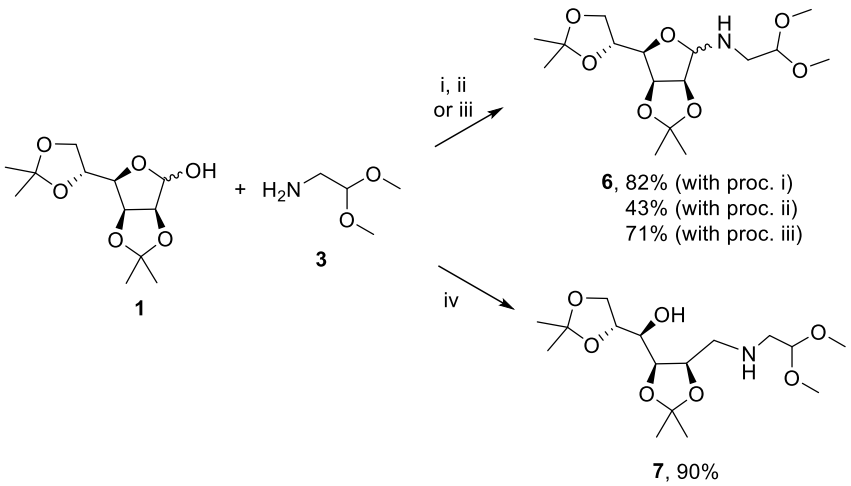


Scheme 2: Synthesis of building blocks **1** and **2**. Reagents and conditions: (i) acetone, I_2, r.t., 2 h; (ii) KOH, BnBr, THF, 18-crown-6, r.t., 16 h; (iii) CH_3COOH, H_2O, r.t., 18 h; (iv) silica gel supported $NaIO_4$, CH_2Cl_2, r.t., 50 min. Overall yield: 82%.[7]

Regarding the amino acid counterpart, glycine-derived aminoacetaldehyde dimethyl acetal **3** was introduced to exploit the reactivity of the sugar hydroxyl group towards the acetal moiety, through the easily tunable and versatile *trans*-acetalization pairing reaction. As the simplest achiral amino acid, glycine was the first choice to prove the potential application of amino acid and sugar derivatives for couple-pair approaches. However, the same strategy can be further applied starting from other chiral amino acids, obtaining the corresponding amino acetals by reduction of the acid functionality and protection of the resulting aldehyde with trimethyl orthoformate.

Coupling:

As a first coupling reaction, the reductive amination between **1** and **3** was studied. However, even under different reaction conditions, i.e. the use of $NaBH(OAc)_3$ or the catalytic hydrogenation with PtO_2, only the hemiaminal intermediate **6** was obtained (Scheme 3).



Scheme 3: Chemical diversity resulting from the coupling stage. Reagents and conditions: (i) $MgSO_4$, MeOH, reflux, 48 h; (ii) H_2SO_4 (1%), MeOH, 50 °C, 6 h, then H_2/PtO_2, MeOH, 50 °C, 20 h; (iii) $NaBH(OAc)_3$, THF, r.t., 3 days; (iv) $MgSO_4$, MeOH, reflux, 48 h, then $LiAlH_4$, dry THF, r.t., 4 h.

The corresponding *δ*-amino alcohol **7** was then achieved upon addition of $LiAlH_4$ as a stronger reducing agent to the hemiaminal **6**. This feature allowed

us to introduce a first point of chemical diversification in the coupling stage, which was further exploited in the pairing step.

Intermediate **6** was obtained as a 3:1 mixture of two unseparable anomers, as shown by the integration of NMR signals corresponding to the CH_2-N moiety, in the region of 3.2 e 2.6 ppm (Figure 2). The stereochemistry of the major α-anomer was established by comparing the values of the coupling constants of CH-1 and CH-2 protons with those of other α-amino-mannofuranosides reported in literature.[8]

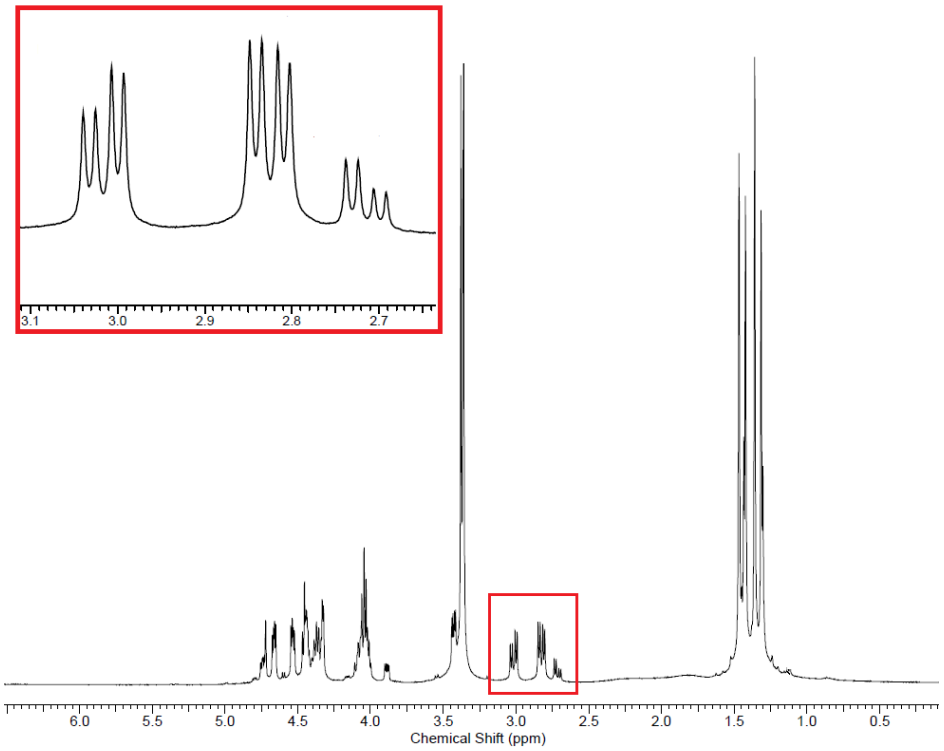
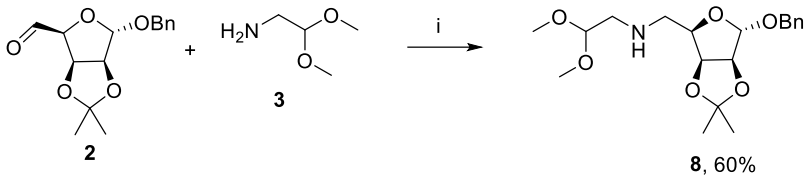


Figure 2: ^{1}H-NMR spectra (400 MHz, $CDCl_3$) and enlargement of the region between 3.2 and 3.6 ppm, corresponding to the proton signals of the CH_2-N moiety of compound **6**.

Finally, reductive amination of benzyl isopropylidene lyxaric aldehyde **2** with aminoacetaldehyde dimethyl acetal using $NaBH_3CN$ as the reducing agent resulted in clean conversion to the corresponding amine **8** in 60% yield (Scheme 4).



Scheme 4: Coupling stage from benzyl isopropylidene lyxaric aldehyde **2**. Reagents and conditions: (i) $NaBH_3CN$, 3Å MS, dry CH_3CN, EtOH, r.t., 48 h.

Study of the functional group pairing step:

With all this pool of compounds in hands, a series of pairing reactions were envisaged in order to achieve skeletal diversity around such mannose- and glycine-derived building blocks. The existence of polyhydroxylated species and the acetal moiety coming from the amino acid derivative **3** opened the way to intramolecular *trans*-acetalizations as the key pairing process. The formation of cyclic acetals is an important issue in the development of novel chemical entities for drug discovery, as this moiety is widespread in the panorama of bioactive natural products. In Figure 3 are reported, as representative examples, the marine toxin pinnatoxin A,[9] the marin didemniserinolipid B,[10] and the pyrrolidine alkaloid broussenetin H.[11]

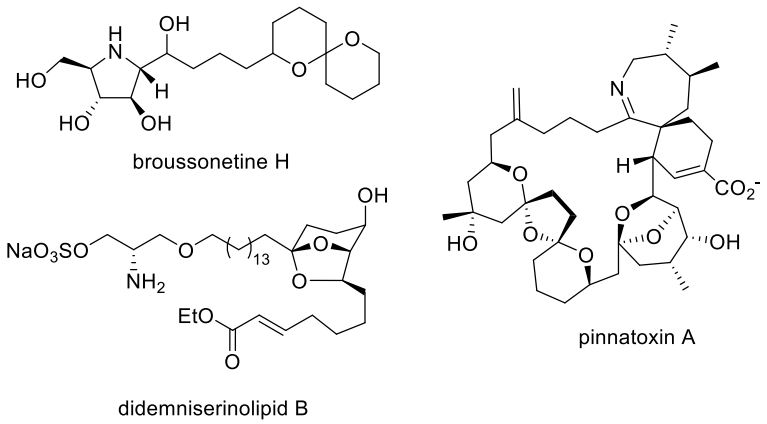
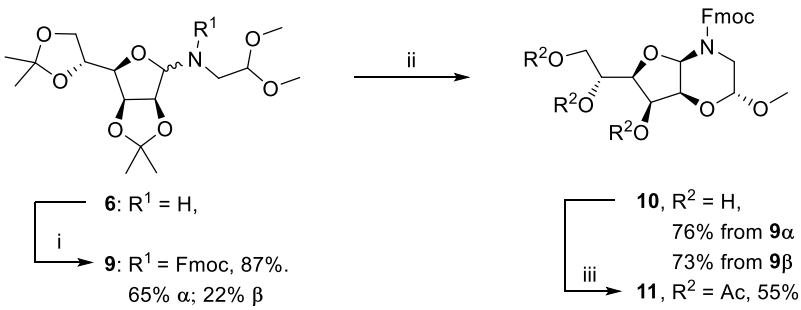


Figure 3: Representative examples of natural products containing acetal moieties: pinnatoxin A, didemniserinolipid B and broussenetin H.

Starting from the hemiaminal coupling intermediate **6**, a first bicyclic scaffold was obtained (Scheme 5). Initially, Fmoc protection of **6** was performed in order to deactivate the basic character of the amino group for the acid-catalized *trans*-acetalization conditions. In this way, the two corresponding

anomeric compounds **9α** and **9β** were obtained with the same 3:1 ratio, but they were easily separated by chromatography.

Upon treatment of the major anomer **9α** under neat TFA condition, the bicyclic *cis*-fused furooxanic scaffold **10** was achieved in 76% yield as a single stereoisomer, as a result of the acetalization of the C-3a hydroxyl group of **9α** to the dimethyl acetal carbon atom.



Scheme 5: Synthesis of scaffold **10** from the hemiaminal coupling intermediate **6**. Reagents and conditions: (i) Fmoc-Cl, H_2O–dioxane, $NaHCO_3$, 0°C to r.t., 24 h; (ii) TFA, neat, r.t., 2 h; (iii) Ac_2O, dry pyridine, r.t., 16 h, 55%.

The same compound, with the same stereochemistry, was achieved also starting from the anomer **9β**, as a consequence of the thermodynamic equilibration of *N*-Fmoc aminals intermediate species under acidic treatment, in agreement with similar *N*-acyl aminals reported in the literature.[12]

The existence of a single anomeric species for **10** consisting of a *cis*-adduct in a 1:1 mixture of rotamers around the C-N bond of the Fmoc group was established by detailed NMR study of the corresponding fully acetylated compound **11** (Figure 4). In particular, the ^{1}H NMR signals at 5.37 and 4.99 ppm attributable to proton H-4a appeared as singlets and showed similar correlation to overlapping carbon signals at 77.3 ppm on the HSQC spectrum, suggesting the existence of a 1:1 ratio of rotamers possessing similar NMR structure. This was ascertained by NOESY1D experiments carried out with a mixing time of 500 ms, considering that NMR signals of same protons from different rotameric species appeared in the same phase when irradiated during chemical-exchange experiments, as reported in the literature.[13] The *cis*-fusion was evinced, as NOESY1D experiments show intense nOe effects between H-4a and H-7a protons, and the *endo* anomer was assessed by nOe effect between H-7a and OCH_3 protons, the latter being found in the axial orientation (Figure 4).

The instability of the *N*-acyl moiety of hemiaminal intermediate allowed the use of only chloroformate as *N*-protecting group. As reported in detail in Chapter 5, benzoyl analogues failed to give the corresponding bicyclic compound, whereas the reaction of the hemiaminal intermediate with sulfonyl chloride was not possible at all. However, preliminary exploration of the appendage diversity of this scaffold, both on the *N*-position and on the polyhydroxylated moiety is discussed in the next chapter.

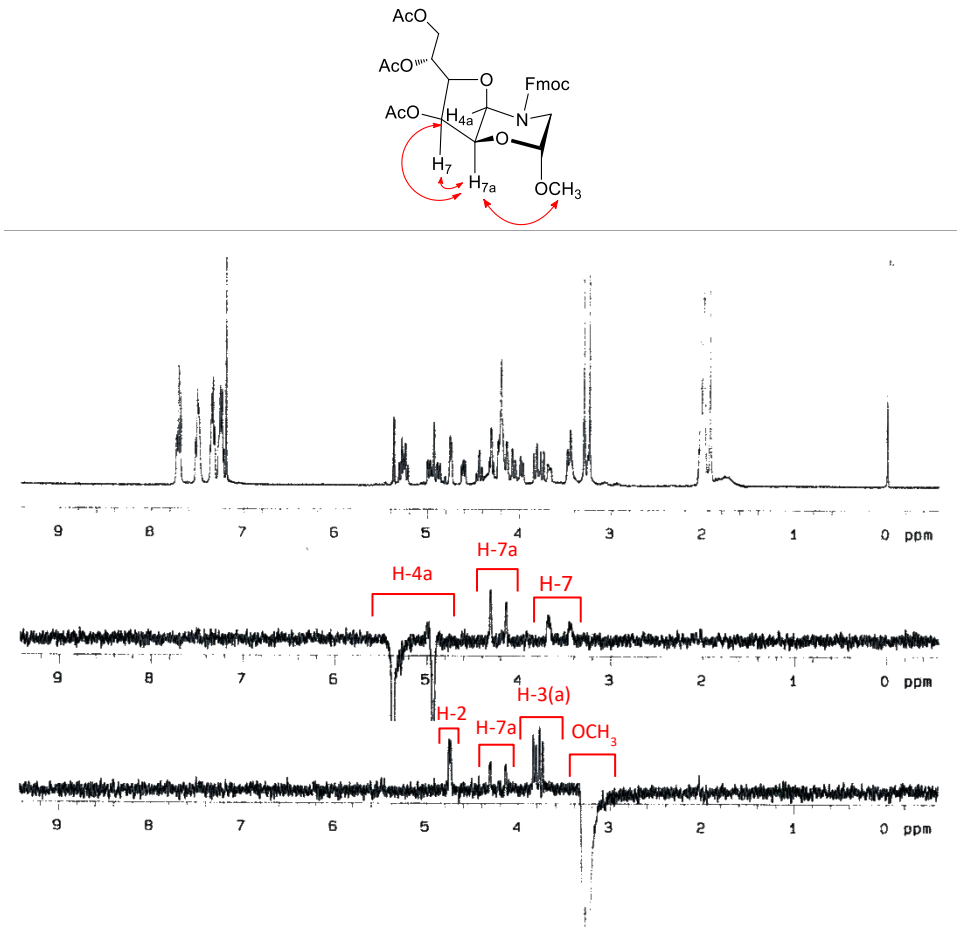
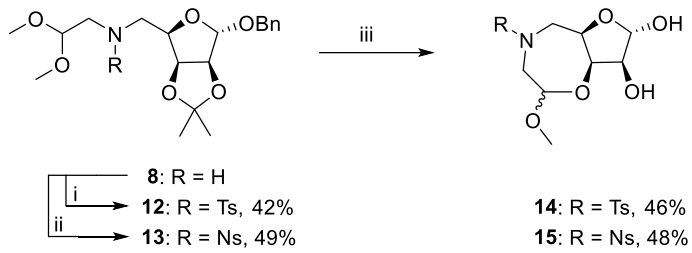


Figure 4: NOE experiments of compound **11** (400 MHz, $CDCl_3$).

Following the principle of the substrate-based approach,[14] the same synthetic strategy was then applied to coupling intermediate **8** derived from D-lyxaric aldehyde. As the amine functionality in this intermediate was not particularly sensitive, we considered standard *N*-sulfonyl protecting group to deactivate the

amine basicity. In particular, the tosylation of **8** produced the corresponding adduct **12** in 42% yield under standard reaction conditions (Scheme 6). Also, the nosyl-derivative **13** was prepared in 49% yield, as it is usually easier to remove than a tosyl group for downstream *N*-functionalization.
In analogy with scaffold **10**, the acetalization of intermediates **12** and **13** gave the corresponding bicyclic scaffolds **14** and **15**, in both cases as a 3:1 mixture of anomers in the acetal moiety.

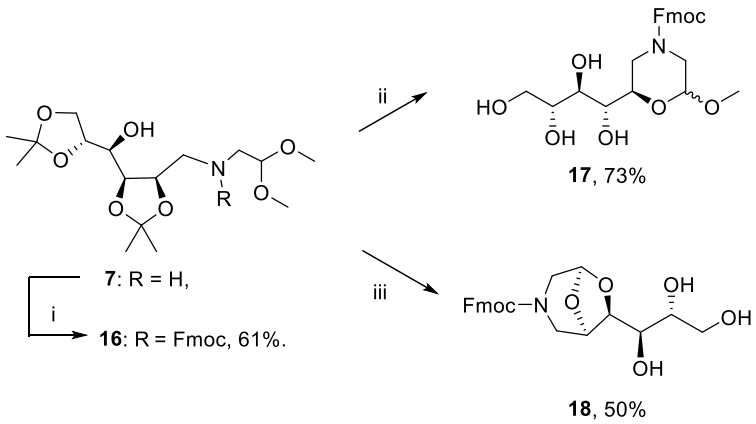


Scheme 6: Synthesis of scaffolds **14** and **15** from the D-lyxaric aldehyde coupling intermediate **8**. Reagents and conditions: (i) TsCl, DIPEA, dry CH_2Cl_2, 0 °C to r.t., 18 h, 42%; (ii) NsCl, DIPEA, dry CH_2Cl_2, 0 °C to r.t., 3 h, 49%; (iii) TFA, neat, r.t., 2 h.

This scaffold, possessing furanose-fused oxazepinic structure, might open the way to the discovery of novel interesting *hit* compounds. In fact, analogue functionalized furanose-fused oxazepines proved to be useful precursors for the synthesis of bioactive nucleosides and glycosidase inhibitors.[15] Even if nosyl deprotection with thiol failed to proceed,[16] the *in situ* deprotection of the anomeric hydroxyl function can be used to introduce further diversity on this position.

On the other hand, the fully reducted coupling intermediate, the δ-amino alcohol **7**, allowed us to exploit more widely the hydroxylic functionalities of the sugar. In particular, Fmoc-protected compound **16** was subjected to same acidic conditions to achieve different pairing reaction between the acetal moieties and the hydroxyls of the parent mannose compound, as a case study of the application of the reagent based approach.[14]
In this event, accurate optimization of the reaction time enabled a divergent synthesis of two different scaffolds (Scheme 7). Specifically, upon treatment of **16** with TFA containing 1% of methanol at room temperature the

corresponding morpholine derivative **17** containing a polyhydroxylated chain at position 5 was obtained in about 20 min. Upon prolonged treatment to 4 h under the same conditions, an intramolecular *trans*-acetalization between the hydroxyl group adjacent to the morpholine ring and the acetal carbon atom of **17** gave the corresponding azabicyclo[3.2.1]octane adduct **18**, together with a small amount of residual morpholine product **17**, easily separable by chromatography (Scheme 7).

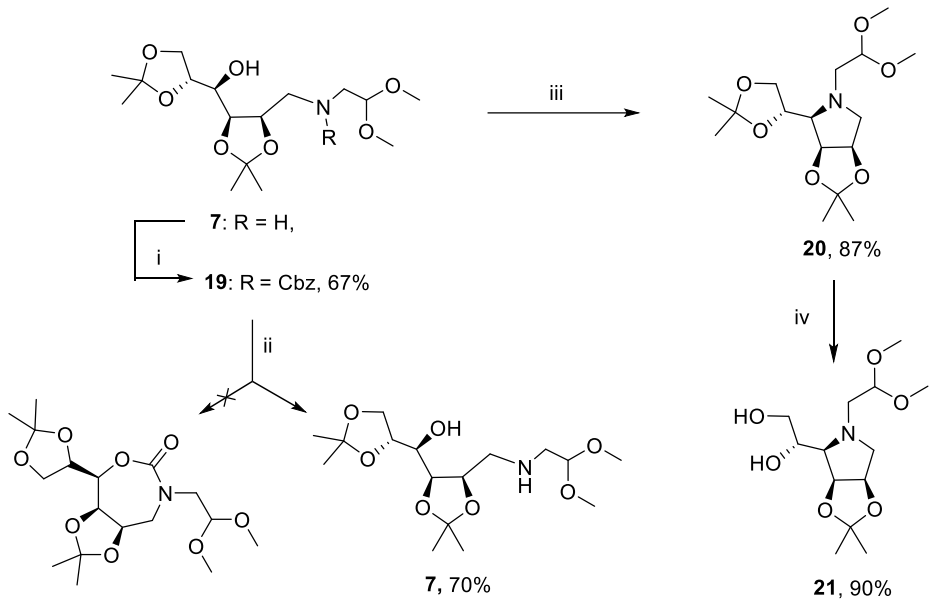


Scheme 7: Chemical diversity resulting from the Fmoc-protected intermediate **16**. Reagents and conditions: (i) Fmoc-Cl, H_2O–dioxane, $NaHCO_3$, 0°C to r.t., 24 h; (ii) TFA–MeOH (99:1), r.t., 20 min; (iii) TFA–MeOH (99:1), r.t., 4 h.

The formation of the bicyclic product was clearly shown by the disappearance of the acetal proton signal of **17** at 3.44 ppm, and the appearance of the diagnostic bridgehead acetal proton signal at 5.45 ppm, in agreement to similar bicyclic compounds reported in the literature.[17] These new compounds possess interesting features. In fact the morpholine ring is a privileged core in medicinal chemistry,[18] being found in several bioactive molecules, such as TACE inhibitors, VLA-4 antagonist and tricyclic benzodiazepines.[19] On the other hand, the azabicyclo[3.2.1]octane structure is an important dipeptide isostere and some compounds possessing this core revealed to be biologically active as aspartyl protease inhibitors and NGF-agonists.[20] Moreover the presence of long polyhydroxylated chains, similar to those possessed by Hunanamycin A and the riboflavin precursor showed in Figure 1, can introduce in these structures new potential interacting elements.

The amine functionality of **7** was then protected with the Cbz group (compound **19**, Scheme 8) in order to explore new functional group pairing approaches. As

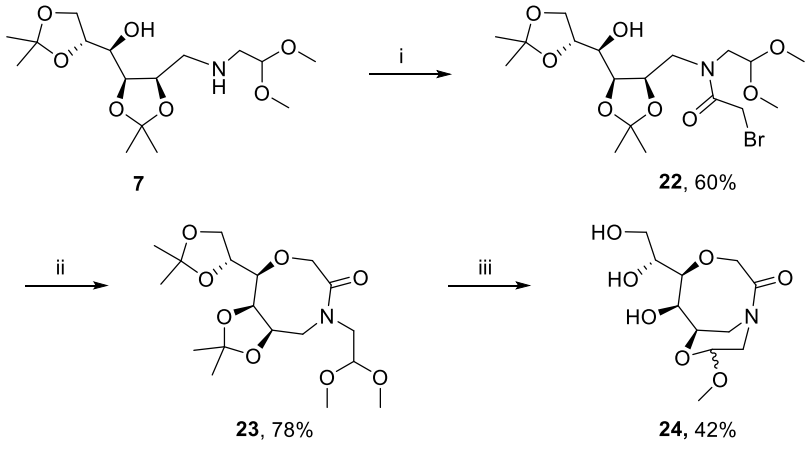
a first attempt, we tried to use the free OH as the nucleophile upon activation with NaH to achieve the corresponding seven-membered cyclic carbamate. Nevertheless, deprotected **7** was obtained, as a consequence of the basic reaction conditions. The same approach was attempted also starting from the ethyl chloroformate derivative using both NaH or DBU as a base, obtaining the same unsuccessful result.



Scheme 8: Synthesis of scaffold **20** and **21** from **19** and further attempted exploitation of Cbz-protected **19**. Reagents and conditions: (i) Cbz-Cl, Et_3N, dry THF, 0 °C to r.t., 24 h; (ii) NaH, dry THF, 0 °C to r.t., 1 h; (iii) PDC, Ac_2O, dry CH_2Cl_2, reflux, 90 min, then H_2, 10% Pd/C, MeOH, r.t., 16 h; (iv) TFA-H_2O (1:1), $CHCl_3$, 0 °C, 1 h.

Hydroxyl group functionalization was then taken into account to exploit the reactivity of the amino group as a nucleophile. No reaction was observed using mostly common reagents, such as Tf_2O, MsCl or TBSCl, thus limiting the use of this group for further elaboration. However, this hydroxyl group was successfully oxidized to the corresponding ketone in high yield using with PDC. Then, an intramolecular reductive amination of the carbonyl moiety with the resulting free amino group, released under reducing conditions, gave the corresponding protected pyrrolidine **20** in 87% yield (Scheme 8). The stereochemistry of the newly formed stereocenter was established by analyzing the values of the coupling constants between the protons of the pyrrolidine ring, according to data reported in literature.[21] To achieve further scaffold

complexity, we tried to perform a second pairing reaction promoted by *trans*-acetalization. However, by reacting compound **20** with a 1:1 TFA-MeOH mixture, compound **21**, with the terminal diol moiety selectively deprotected, was obtained in 90% yield. Upon treatment of **20** under harsher acidic conditions, by refluxing the compound in 6M HCl for 3 hours, unidentified compounds were obtained, possibly resulting from elimination and rearrangement processes. Also, attempts to provide the free aldehyde function from the dimethylacetal moiety of compound **20** using TFA in a biphasic water-chloroform procedure[22] were unsuccessful, as only starting material was recovered. However, the free diol moiety of compound **21** can be potentially oxidized using $NaIO_4$, as reported in Scheme 2 (condition iv), thus achieving a new free aldehyde function as a potential new point of diversification.
Finally, δ-amino alcohol **7** was functionalized with an α-bromoacetyl group, obtaining derivative **22** in 60% yield (Scheme 9). In this case, the intramolecular nucleophilic substitution on the reactive α-bromoacetyl species upon addition of NaH gave the corresponding eight-membered ring lactam **23** in 78% yield.



Scheme 9: Synthesis of oxazocinonic scaffolds **23** and **24** from α-bromoacetylated compound **22**. Reagents and conditions: (i) bromoacetyl bromide, Et_3N, dry CH_2Cl_2, -15°C, 1 h; (ii) NaH, dry THF, 0°C to r.t., 1 h; (iii) TFA, neat, r.t., 2 h.

Even in this substrate the selective deprotection of the aldehyde function failed to proceed. However, a second pairing reaction on **23** through *trans*-acetalization was achieved upon treatment with TFA, giving the resulting deprotected bicyclic scaffold **24** as a 1:1 mixture of anomers.

Chemioinformatic analysis

To computationally assess the structural diversity of this mannose-derived DOS library in the context of the chemical space, two comparative statistical tools were employed: principal component analysis (PCA) and principal moments of inertia (PMI) analysis.

As described in detail in the introduction (paragraph 1.1.1), PCA is used to condense multidimensional chemical properties (for examples molecular weight, logP, ring complexity) into single dimensional numerical values (principal components), to simplify the comparison with different sets of compounds.[23] ChemGPS-NP was chosen for the PCA analysis, as it is an easy web-based public tool useful for the comprehensive chemical space exploration on to a consistent 8-dimensional map.[24] In particular, the first four dimensions of the ChemGPS-NP map capture 77% of data variance. The first dimension PC1 (principal component one), represents size, shape and polarizability (main contribution is size); PC2 is associated with aromatic and conjugation related properties (main influence is aromaticity); PC3 describes lipophilicity, polarity, and hydrogen-bond capacity (major contribution is lipophilicity); PC4 expresses flexibility and rigidity.

In this way, compounds **6**-**24** were analyzed in terms of PCA score prediction for these four physicochemical properties (see Table 2 in the experimental section). These four dimensions of the ChemGPS-NP map were then analyzed in the same way for a reference set of 40 brand-name blockbuster drugs using the protocols employed by Tan (see Table 2 in the Appendix).[25] These numerical data, for these two different compound collections, were then plotted in two different graphs, specifically PC1 versus PC2 graph (Figure 5, right) and PC1 versus PC3 (Figure 5, left), where mannose-derived compounds are shown as black dots and brand-name block buster drugs as blue squares.

The analysis of PC1 vs PC2 resulted in DOS compounds belonging to three different clusters, the first being positioned in the negative direction of both axes, the second in the center of the graph, and the third in the opposite quadrant, due to diverse size (PC1) and aromaticity (PC2) content. Interestingly, the first cluster showed good overlapping with the structures of Fosamax and Topamax, both possessing OH moieties and the latter interestingly showing both acetal and diisopropylidene moieties. The third cluster showed good

overlapping with Serevent, possessing a polyol structure and a hydrophobic chain, suggesting similarity of PC1 and PC2 descriptors with mannose-derived molecules in their *N*-protected form.

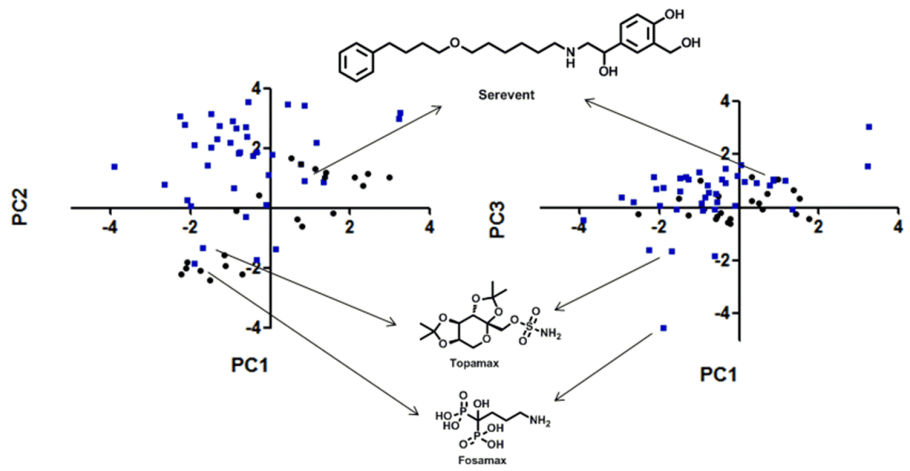


Figure 5. PCA plot resulting from the correlation between PC1 vs PC2 (**left**), and PC1 vs PC3 (**right**), showing the positioning in the chemical space of mannose-derived DOS compounds (**black dots**) with respect to the reference set of brand-name blockbuster drugs (**blue squares**).

In the plot described by PC1 vs PC3 dimensions a grouping into two clusters was observed, mainly due to the effect of the protecting groups, as PC1 is affected mainly by size and PC3 to the calculated logP values, and the number of rings, which together shift the protected compounds in a positive direction along both PC1 and PC3 axes. Although poorer in the number of elements of the library, the mannose-derived collection showed better diversity on the PC3 axis than BB drugs.

Then, principal moment of inertia (PMI) analysis[26] was performed to assess the 3D molecular shape diversity of the polyhydroxylated DOS compounds in comparison with the molecules of the above reference set of blockbuster drugs.[25] PMI analysis was carried out by calculation of the lowest energy conformation of each representative compound using VegaZZ software.[27] Then, the three principal moments of inertia (I_{xx}, I_{yy}, I_{zz}) and the corresponding normalized principal moments of inertia were determined according to Sauer and Schwarz for all polyhydroxylated DOS compounds and the reference

blockbuster drugs.[26a] Specifically, the three calculated principal moments of inertia were sorted by ascending magnitude (the numerical data for mannose-derived compounds are reported in Table 3 in the additional data of the experimental section of this chapter, whereas the numerical values for the blockbuster drugs are reported in Table 3 in the Appendix). Then, all the normalized PMI ratios (I_1/I_3 and I_2/I_3) were plotted on a triangular graph where the vertices (0,1), (0.5,0.5) and (1,1) represent a perfect rod (acetylene), disc (benzene) and sphere (adamantane), respectively.

As we can see from the graph in Figure 6, mannose-derived compounds were found to lie along the center-left side of the triangle, with a preference for the rod-disc side. This positioning showed mannose-derived compounds possessing higher tendency to lie along the rod-disc axis of the triangle and major dispersion in the space, as compared to blockbuster drugs, suggesting higher shape diversity for these compounds.

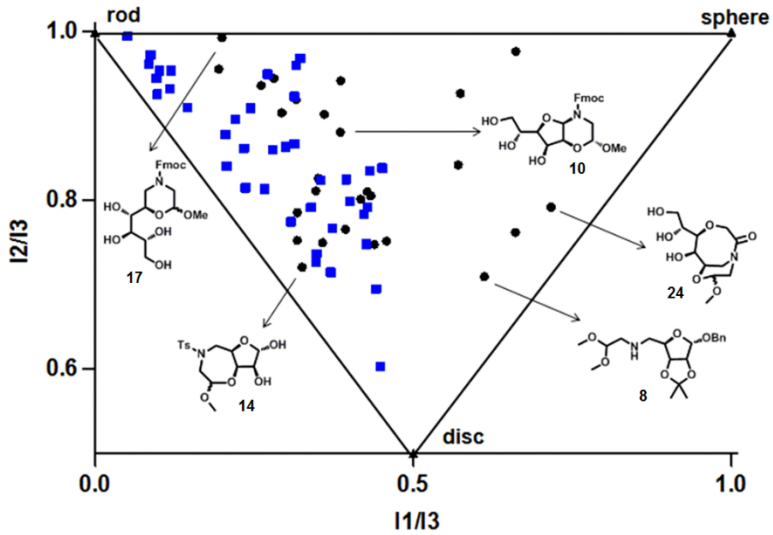


Figure 6. PMI plot showing the skeletal diversity of mannose-derived DOS compounds (**black dots**) with respect to the reference set of brand-name blockbuster drugs (**blue squares**).

Interestingly, compounds **8** and **24** proved to be positioned in the disc-sphere side of the triangle, demonstrating a quite diverse shape as compared to the drugs and the other elements of the library. Also, compounds **10** and **17** are in the rod-sphere region, indicating good coverage within shape diversity as a

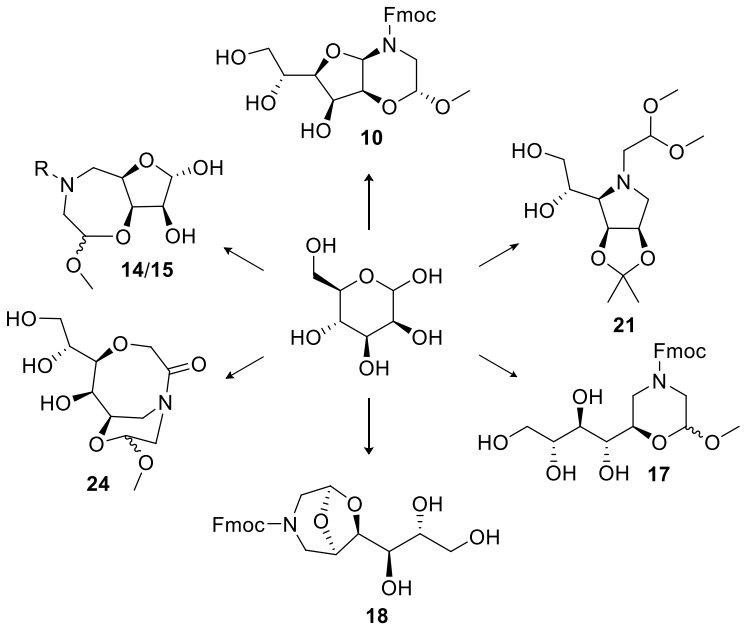
function of the extent of scaffold decoration with hydrophobic groups (i.e. Fmoc) and polyhydroxylated chains.
In conclusion, the PMI analysis suggested that the skeletal diversity coming from the exploitation of mannose and glycine-derived acetal according to the build-couple-pair approach resulted in an array of molecules spanning in the chemical space despite the limited number of representatives, as compared to brand-name blockbuster drugs. This feature is promising in view of expanding the array of reactions on such carbohydrate-derived building blocks and for generating natural product-like chemical libraries carriers of high molecular diversity and complexity.

4.3 Conclusions

In the context of the development of novel small molecules chemotypes for modern phenotypic screening, the Diversity-Oriented Synthesis approach, combined to the exploitation of carbohydrate and amino acid derivatives, proved to be powerful for the achievement of stereochemically dense glyco- and peptidomimetic scaffolds. In particular, mannose was exploited as a case study to develop an array of skeletally diverse molecules in DOS fashion using the build-couple-pair approach. Specifically, the protected sugar derivative was subjected to coupling reaction with aminoacetaldehyde dimethyl acetal as a reference amino acid derivative possessing a protected carbonyl function for subsequent pairing steps. As shown in summary in Scheme 10, six novel polyhydroxylated nitrogen-containg scaffolds (**10**, **14**/**15**, **17**, **18**, **21** and **24**) were thus obtained exploiting synthetic pathways composed of no more than five steps.

The diversity of the pool of scaffolds herein obtained was characterized in terms of shape and chemical properties using PMI and PCA analysis and compared to a reference set of blockbuster drugs, demonstrating mannose as a powerful building block to generate highly diverse compounds when applied to DOS chemistry. Such approach can be beneficial in generating high-quality combinatorial libraries in exploiting the hydroxyl groups for appendage diversity. Also, the combination of polyhydroxylated and hydrophobic moieties may result in novel and unexplored resource for addressing protein-protein

interactions in drug discovery programs. As explained in detail in the next chapter, the ability of these compounds in inducing cell growth inhibition in a human metastatic melanoma cell line (MDA-MB-231) was taken into account as a first biological output, in order to identify novel *hit* chemical entities to further develop in follow-up processes.

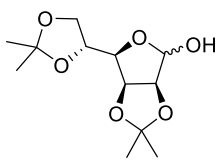


Scheme 10: Summary representation of the six novel scaffolds obtained from D-mannose.

4.4 Experimental Section

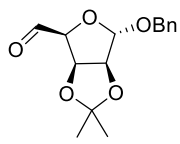
General Analytical grade solvents and commercially available reagents were used without further purification. Reactions requiring inert atmosphere were carried out under nitrogen atmosphere. Flash chromatography was performed using 32−63 μm silica gel (60 Å mesh) with the indicated solvent. Analytical thin layer chromatography (TLC) was performed on 0.25 mm silica gel 60-F plates. Melting points are uncorrected. ^{1}H NMR spectra were acquired on 400 MHz spectrometers. ^{13}C NMR spectra were acquired at 100 and 50 MHz. All chemical shifts are reported in parts per million (δ) referenced to residual nondeuterated solvent. Data are reported as follows: chemical shifts, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet); coupling constant(s) in Hz; integration. ESI mass spectra were carried out on a ion-trap double quadrupole mass spectrometer using electrospray (ES^+) ionization techniques. Elemental analyses were performed on a Perkin Elmer 240 C, H, N analyzer. Optical rotation measurements were performed on a JASCO DIP-370 polarimeter and are given in 10^{-1} deg cm^2 g^{-1}.

Synthesis of (3a*S*,6*R*,6a*S*)-6-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (1)



To a suspension of D-mannose (5.0 g, 27.8 mmol) in acetone (250 mL), iodine (1.47 g, 5.8 mmol) was added and the mixture was stirred for 2 h at room temperature. The reaction mixture was quenched at 0 °C with sodium thiosulfate (saturated solution) and sodium bicarbonate (saturated solution), then extracted with chloroform. The resulting organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **1** as white solid (6.72 g, 25.8 mmol) with a 93% yield. Spectroscopical data are in agreement with the literature values.[28]

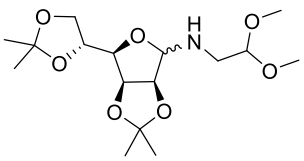
Synthesis of (3a*S*,4*S*,6*S*,6a*S*)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbaldehyde (2)



To a solution of **1** (3 g, 11.5 mmol) in dry THF (25 mL), freshly powdered KOH (1.16 g, 20.7 mmol), 18-crown-6 (121 mg, 0.46 mmol) and BnBr (1.50 mL, 12.7 mmol) were added sequentially. The mixture was stirred at room temperature for

16 h and then diluted with CH_2Cl_2, washed with water and concentrated. Then, the crude mixture was dissolved in glacial AcOH (43 mL) and water (19 mL), left stirring at room temperature for 18 h and then concentrated under reduced pressure. The resulting residue was dissolved in AcOEt, washed sequentially with sodium bicarbonate (saturated solution), water and brine. After the removal of the solvent, the deprotected diol was left vigorously stirring in the presence of silica-gel supported $NaIO_4$ reagent (3.25 g) in CH_2Cl_2 (110 mL) at room temperature for 50 minutes. The reaction mixture was then filtered and washed with $CHCl_3$. The combined filtrates were concentrated under reduced pressure and allowed to stand at 5 °C overnight to give aldehyde **2** (2.64 mg, 9.49 mmol) as a white solid which was pure enough to be employed in the subsequent steps (82% over four steps. Spectroscopical data are in agreement with the literature values.[7]

Synthesis and characterization of (3a*S*,4*R*/*S*,6*R*,6a*S*)-*N*-(2,2-dimethoxyethyl)-6-((*R*)-2,2-dimethyl 1,3-dioxolan-4-yl)-2,2dimethyl-tetrahydrofuro[3,4-*d*][1,3]dioxol-4-amine (6)

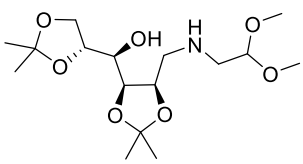


To a solution of di-isopropylidene-D-mannose **1** (200 mg, 0.77 mmol) and 2,2-dimethoxy-ethylamine (125 μL, 1.15 mmol) in MeOH (5 mL), $MgSO_4$ (200 mg, 1.18 mmol) was added and the reaction mixture was left stirring at reflux for 48 h. $MgSO_4$ was then removed by filtration through Celite and the filtrate was concentrated under vacuum to give a yellow crude product. Flash chromatography (EtOAc/Petr. et. = 1:2, buffered with 1% Et_3N; R_f = 0.17) afforded compound **6** (216 mg, 0.62 mmol, 82%) as a yellow oil. NMR spectroscopy revealed that compound **6** was obtained as a 3:1 mixture of α:β anomers.

^{1}H NMR (400 MHz, $CDCl_3$) 3:1 mixture of anomers: δ 4.71 (m, 0.5H, minor), 4.70 (s, 0.75H, Major), 4.63 (dd, *J* = 6.1, 3.3 Hz, 0.75H, Major), 4.58 (d, *J* = 5.6 Hz, 0.25H, minor), 4.53 (dd, *J* = 6.7, 3.3 Hz, 0.75H, Major), 4.47–4.30 (m, 3H), 4.05–4.01 (m, 2H), 3.87 (dd, *J* = 11.1, 7.8 Hz, 0.25H, minor), 3.38 (dd, *J* = 7.0, 3.4 Hz, 0.75H, Major), 3.36 (s, 3H), 3.34 (s, 3H), 2.99 (dd, *J* = 13.2, 5.7 Hz, 0.75H, Major), 2.80 (dd, *J* = 13.2, 5.7 Hz, 1H), 2.69 (dd, *J* = 12.9, 5.7 Hz, 0.25H, minor), 1.44 (s, 3H), 1.41 (s, 0.75H, minor), 1.40 (s, 2.25H, Major), 1.34 (s, 3H), 1.29 (s, 2.25H, Major), 1.28 (s, 0.75H, minor). ^{13}C NMR (50 MHz, $CDCl_3$) 3:1 mixture of anomers: δ 112.6 and 112.4, 109.2 and 109.0, 104.6 and 103.7, 95.1 and 92.0,

85.7 and 80.2, 79.8 and 79.6, 79.1 and 77.5, 73.4 and 73.3, 66.93 and 66.86, 54.2 and 54.0, 53.8, 48.3 and 47.0, 26.9 and 25.7, 26.0, 25.3 and 24.6, 25.2. MS (ESI) *m/z* (%): 348.05 [(M+H)$^+$, 100]. Anal. Calcd. for $C_{16}H_{29}NO_7$: C, 55.32; H, 8.41; N, 4.03. Found: C, 55.38; H, 8.45; N, 4.00.

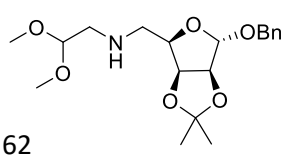
Synthesis and characterization of (*R*)-((4*S*,5*R*)-5-(((2,2-dimethoxyethyl)amino)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)((*R*)-2,2-di methyl-1,3-dioxolan-4-yl)methanol (7)



To a solution of di-isopropylidene-D-mannose **1** (115 mg, 0.43 mmol) and 2,2-dimethoxy-ethylamine (60 μL, 0.52 mmol) in MeOH (5 mL), $MgSO_4$ (100 mg, 0.86 mmol) was added and the reaction mixture was left stirring at reflux for 48 h. $MgSO_4$ was then removed by filtration through celite and the filtrate was concentrated under vacuum to give a yellow crude product. The crude product was dissolved in dry THF (4 mL), then a stirred suspension of $LiAlH_4$ (49 mg, 1.30 mmol) in dry THF (4 mL) was added dropwise at 0 °C. The mixture was left reacting at room temperature for 4 h. $LiAlH_4$ was quenched with MeOH (5 mL) and H_2O (5 mL). The resulting salts were removed by filtration through celite and the filtrate was concentrated under vacuum, to give a crude product, which was purified by flash chromatography (EtOAc/Petr. et. = 3:1; R_f = 0.29) affording compound **7** (135 mg, 0.39 mmol, 90%), as a white powder.

M.P. 83.7–84.8 °C. $[\alpha]_D^{24}$ = +14.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.48 (t, *J* = 5.5 Hz, 1H), 4.44 (d, *J* = 8.0 Hz, 1H), 4.40-4.32 (m, 1H), 4.16-4.12 (m, 2H), 4.07-4.04 (m, 1H), 3.50 (d, *J* = 8.0 Hz, 1H), 3.38 (s, 6H), 3.10 (dd, *J* = 12.9, 4.1 Hz, 1H), 2.84-2.81 (m, 1H), 2.78 (d, *J* = 5.5 Hz, 2H), 1.51 (s, 3H), 1.40 (s, 3H), 1.38 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 109.1, 107.8, 103.2, 76.3, 75.9, 75.3, 70.5, 67.8, 54.3, 54.2, 50.6, 47.9, 26.9, 26.2, 25.3, 24.4. MS (ESI) *m/z* (%): 350.10 [(M+H)$^+$, 100]. Anal. Calcd. for $C_{16}H_{31}NO_7$: C, 55.00; H, 8.94; N, 4.01. Found: C, 55.11; H, 8.99; N, 3.92.

Synthesis and characterization of *N*-(((3a*S*,4*R*,6*S*,6a*S*)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2,2 dimethoxyethan-amine (8)

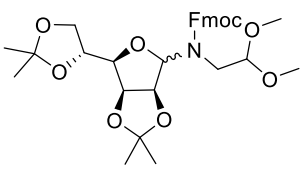


To a solution of D-lixaric aldehyde **2** (500 mg, 1.80 mmol) in dry CH_3CN (3.5 mL), 2,2-dimethoxy-

ethylamine (215 μL, 1.98 mmol) and 3Å molecular sieves were added under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 1 h, then $NaBH_3CN$ (338 mg, 5.4 mmol) and EtOH (2.6 mL) were added. The reaction mixture was left stirring at room temperature for 2 days under a nitrogen atmosphere, and then concentrated under vacuum. The crude compound was purified by flash chromatography (EtOAc/Petr. et. = 2:1; R_f = 0.21) to obtain **8** (386 mg, 1.05 mmol, 60%) as a pure orange oil.

$[\alpha]_D^{21}$ = +33.7 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33-7.30 (m, 5H), 5.09 (s, 1H), 4.73–4.66 (m, 3H), 4.52–4.69 (m, 2H), 4.15 (q, *J* = 6.3 Hz, 1H) 3.40 (s, 6H), 3.40 (s, 2H), 2.98 (d, *J* = 6.3 Hz, 1H), 2.81 (d, *J* = 5.4 Hz, 1H), 1.45 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.4, 128.4 (2C), 128.0 (2C), 127.8, 112.4, 105.2, 103.7, 85.1, 80.1, 79.2, 68.9, 53.9 (2C), 51.3, 48.4, 26.0, 24.8. MS (ESI) *m*/*z* (%): 368.18 [$(M+H)^+$, 100]. Anal. Calcd. for $C_{19}H_{29}NO_6$: C, 62.11; H, 7.96; N, 3.81. Found: C, 62.31; H, 7.99; N, 3.75.

Synthesis and characterization of 9*H*-fluoren-9-yl)methyl-(2,2-dimethoxyethyl)((3a*S*,4*R*,6*R*,6a*S*)-6-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)carbamate (9α) and (9*H*-fluoren-9-yl)-methyl-(2,2-dimethoxyethyl)-((3a*S*,4*S*,6*R*,6a*S*)-6-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)carbamate (9β)



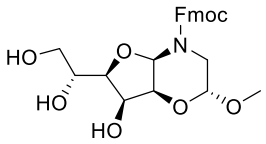
To a solution of **6** (214 mg, 0.61 mmol) in dioxane (3 mL) and water (6 mL) was added $NaHCO_3$ (103 mg, 1.23 mmol). The mixture was cooled to 0 °C, then a solution of Fmoc-Cl (159 mg, 0.61 mmol) in dioxane (3 mL) was added slowly. The mixture was left reacting at room temperature for 24 hours under a nitrogen atmosphere, then it was diluted with EtOAc (20 mL). The organic phase was washed with 1M HCl solution, brine, and dried over anhydrous Na_2SO_4. After solvent evaporation, the crude oil was purified by flash chromatography (EtOAc/Petr. et. = 1:3; R_f **9β** = 0.18, R_f **9α** = 0.32), thus affording compound **9β** (227 mg, 0.40 mmol, 65%) and compound **7a** (78 mg, 0.14 mmol, 22%), both as pure colorless oils.

9α: $[\alpha]_D^{24}$ = +13.6 (*c* 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: δ 7.76 (d, *J* = 7.4 Hz, 2H), 7.61 (br s, 2H), 7.40 - 7.33 (m, 4H), 5.03–4.93 (m, 2H), 4.56 (br, 2H), 4.42–4.22 (m, 4H), 4.12–3.98 (m, 4H), 3.28-3.26 (m, 1H), 3.24 (s, 6H), 1.45–1.24 (m, 12H). ^{13}C NMR (50 MHz, $CDCl_3$) mixture of rotamers: δ 156.0,

143.7 (2C), 141.4 and 141.3 (2C), 127.8 (2C), 127.2 (2C), 124.80 and 124.75 (2C), 120.0 (2C), 112.3, 108.8, 103.6, 96.6, 85.6, 84.3, 81.4, 76.5 and 76.3, 74.0, 66.7 and 66.5, 55.3, 54.9, 50.6, 47.4, 26.8, 26.1, 25.2, 24.4. MS (ESI) *m/z* (%):592.32 [$(M+Na)^+$, 100]. Anal. Calcd. for $C_{31}H_{39}NO_9$: C, 65.36; H, 6.90; N, 2.46. Found: C, 65.49; H, 6.96; N, 2.39.

9β: $[\alpha]_D^{24}$ = +40.3 (*c* 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.75 (d, *J* = 7.4 Hz, 2H), 7.58 (br s, 2H), 7.34–7.29 (m, 4H), 5.40 (br s, 1H), 4.78–4.44 (m, 7H), 4.28 (t, *J* = 5.9 Hz, 1H), 4.12–3.81 (m, 3H), 3.60 (dd, *J* = 14.7, 5.3 Hz, 1H), 3.30 (s, 3H), 3.27 (s, 3H), 1.62–1.29 (m, 12H). ^{13}C NMR (50 MHz, $CDCl_3$) mixture of rotamers: *δ* 160.3 and 159.6, 143.9 (2C), 141.3 (2C), 127.7 and 127.6 (2C), 127.2 and 127.1 (2C), 124.9 (2C), 119.8 (2C), 112.6, 109.2, 105.3 and 102.8, 87.0 and 86.9, 79.3, 78.9, 78.5, 73.0, 67.4, 66.6, 53.1 (2C), 47.2, 45.5 and 45.4, 26.8, 25.5, 25.2, 23.9. MS (ESI) *m/z* (%): 592.32 [$(M+Na)^+$, 100]. Anal. Calcd. for $C_{31}H_{39}NO_9$: C, 65.36; H, 6.90; N, 2.46. Found: C, 65.52; H, 6.97; N, 2.37.

Synthesis and characterization of (2*R*,4a*R*,6*R*,7*S*,7a*S*)-(9*H*-fluoren-9-yl)methyl-6-((*R*)-1,2-dihydroxyethyl)-7-hydroxy-2-methoxy-tetrahydro-2*H*-furo[3,2-*b*][1,4]oxazine-4(3*H*)-carboxylate (10)

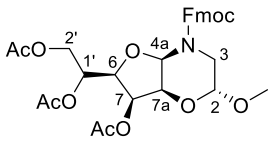


Compound **9β** (78 mg, 0.14 mmol) was dissolved in trifluoroacetic acid (1 mL) and stirred at room temperature for 2 h. After TFA evaporation, the crude powder was dissolved in MeOH and filtered through Amberlite XAD-2 resin. After solvent evaporation, the crude product was purified by flash chromatography (CH_2Cl_2 / MeOH = 30:1, R_f **10** = 0.22), thus affording compound **10** as a colorless oil (45 mg, 0.10 mmol, 73%). With the same procedure, compound **10** (45 mg, 0.10 mmol, 76%) was obtained also from the diastereomer **9α** (76 mg, 0.13 mmol). HPLC analysis revelead that compound **10** was achieved as single anomer.

$[\alpha]_D^{20}$ = -30.2 (*c* 0.8, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.68 (d, *J* = 7.6 Hz, 2H), 7.48 (br s, 2H), 7.33-7.30 (m, 2H), 7.22-7.20 (m, 2H), 5.27 and 4.93 (s, 1H), 4.84 and 4.67 (s, 1H), 4.78–3.98 (m, 5H), 3.98–3.02 (m, 6H), 3.45 and 3.32 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) mixture of rotamers: *δ* 155.6, 143.7 (2C), 141.3 (2C), 128.0 (2C), 127.1 (2C), 124.8 (2C), 120.0 (2C), 95.7 and 95.2, 78.7, 77.9, 73.2, 68.2, 67.7, 66.9 and 66.3, 62.1, 54.9, 46.9, 42.7. MS

(ESI) *m/z* (%): 480.23 [(M+Na)$^+$, 100], 457.87 [(M+H)$^+$, 27]. Anal. Calcd. for $C_{24}H_{27}NO_8$: C, 63.01; H, 5.95; N, 3.06. Found: C, 63.12; H, 5.99; N, 3.00.

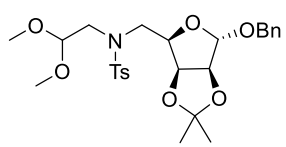
Synthesis and characterization of (*R*)-1-((2*R*,4a*R*,6*R*,7*S*,7a*S*)-4-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-7-acetoxy-2-methoxy-hexahydro-2*H*-furo[3,2-*b*][1,4]oxazin-6-yl)ethane-1,2-diyl diacetate (11)



Compound **10** (54 mg, 0.12 mmol) was dissolved in pyridine (2 mL) and Ac_2O (1 mL) and stirred at room temperature overnight, then the solvent was evaporated under reduced pressure and the crude purified by flash chromatography (Et_2O/Petr. et. = 1:1, R_f = 0.20) affording pure compound **13** (38 mg, 0.07 mmol, 55%) as a colorless oil.

$[\alpha]_D^{20}$ = -32.8 (*c* 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) 1:1 mixture of rotamers: *δ* 7.78-7.76 (m, 2H, 2xCHAr), 7.58-7.56 (m, 2H, 2xCHAr), 7.42-7.40 (m, 2H, 2xCHAr), 7.33-7.31 (m, 2H, 2xCHAr), 5.37 and 4.98 (s, 1H, H-4a), 5.35 and 5.31 (t, *J* = 10.1 Hz, 1H, H-6), 5.05 and 4.94 (dd, *J* = 10.1, 3.2 Hz, 1H, H-1'), 4.81 (d, *J* = 5.9 Hz, 1H, H-2), 4.67 and 4.37 (dd, *J* = 9.7, 6.2 Hz, 1H, H-2'a), 4.51 and 4.27 (dd, *J* = 9.7, 2.6 Hz, 1H, H-2'b), 4.38 and 4.20 (s, 1H, H-7a), 4.28-4.27 (m, 1H, CH-Fmoc), 4.12 and 4.04 (d, *J* = 10.1 Hz, 2H, CH_2-Fmoc), 3.88 and 3.82 (d, *J* = 13.1 Hz, 1H, H-3a), 3.72-3.70 and 3.52-3.50 (m, 1H, H-7), 3.52-3.50 (m, 1H, H-3b), 3.36 and 3.30 (s, 3H, OCH_3), 2.10–2.05 (m, 9H, 3x$COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) 1:1 mixture of rotamers: *δ* 170.7 (CO), 170.1 (CO), 169.6 and 169.5 (CO), 155.2 and 154.7 (CO-Fmoc), 143.5 (2C, C_q-Fmoc), 141.3 (2C, C_q-Fmoc), 127.8 and 127.1 (2C, 2xCHAr), 125.5 and 125.0 (2C, 2xCHAr), 124.8 (2C, 2xCHAr), 120.0 (2C, 2xCHAr), 95.6 and 95.2 (C-2), 77.6 (C-4), 73.3 (C-7), 72.2 (C-6), 68.2 and 67.6 (C-1'), 65.1 and 64.9 (C-7a), 64.0 (CH_2-Fmoc), 62.2 (C-2), 56.8 and 54.9 (OCH_3), 46.9 (CH-Fmoc), 42.7 and 42.0 (C-3), 20.8 (3C, $COCH_3$). MS (ESI) *m/z* (%): 1188.55 [(2M+Na)$^+$, 100], 606.31 [(M+Na)$^+$, 22]. Anal. Calcd. for $C_{30}H_{33}NO_{11}$: C, 61.74; H, 5.70; N, 2.40. Found: C, 62.01; H, 5.87; N, 2.31.

Synthesis and characterization of *N*-(((3a*S*,4*R*,6*S*,6a*S*)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-*N*-(2,2-dimethoxyethyl)-4-methylbenzenesulfonamide (12)

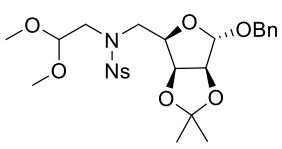


To a solution of **8** (300 mg, 0.81 mmol) and DIPEA (306 μL, 1.62) in dry CH_2Cl_2 (7.5 mL), a solution of TsCl (200 mg, 1.06 mmol) in dry CH_2Cl_2 (7.5 mL) was added

slowly at 0 °C. The mixture was allowed to reach room temperature and was left stirring under a nitrogen atmosphere overnight. Then, water was added slowly and the resulting mixture was washed with a saturated solution of $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by flash chromatography (EtOAc/Petr. et. = 1:4; R_f = 0.47) to give pure **12** (180 mg, 0.34, 42%).

$[\alpha]_D^{20}$ = +40.7 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.8 Hz, 2H), 7.36–7.25 (m, 7H), 4.98 (s, 1H), 4.67 (dd, *J* = 5.9, 3.9 Hz, 1H), 4.58 (d, *J* = 5.9 Hz, 1H), 4.58-4.57 (m, 1H), 4.53 (d, *J* = 11.7 Hz, 1H), 4.34 (d, *J* = 11.7 Hz, 1H), 4.20-4.16 (m, 1H), 3.75 (dd, *J* = 15.6, 3.4 Hz, 1H), 3.54–3.46 (m, 2H), 3.42 (dd, *J* = 11.2, 5.4 Hz, 1H), 3.37 (s, 3H), 3.35 (s, 3H), 2.37 (s, 3H), 1.40 (s, 3H), 1.27 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.2, 137.2 (2C), 129.5 (2C), 128.5 (2C), 127.9 (2C), 127.8 (2C), 127.3, 112.5, 105.0, 103.8, 85.0, 80.1, 79.1, 68.7, 54.6, 54.4, 49.9, 47.9, 26.0, 24.8, 21.5; MS (ESI) *m/z* (%): 1064.50 [$(2M+Na)^+$, 100], 544.33 [$(M+Na)^+$, 82]. Anal. Calcd. for $C_{26}H_{35}NO_8S$: C, 59.87; H, 6.76; N, 2.69. Found: C, 60.12; H, 6.80; N, 2.54

Synthesis and characterization of *N*-(((3a*S*,4*R*,6*S*,6a*S*)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-*N*-(2,2-dimethoxyethyl)-4-nitrobenzenesulfonamide (13)

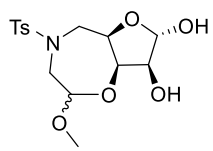


To a solution of **8** (120 mg, 0.33 mmol) and DIPEA (113 μL, 0.66 mmol) in dry CH_2Cl_2 (3 mL), a solution of *p*-NsCl (86 mg, 0.39 mmol) in dry CH_2Cl_2 (3 mL) was added slowly at 0 °C. The mixture was allowed to reach room temperature and was left stirring under a nitrogen atmosphere for 3 hours. Then, water was added slowly and the resulting mixture was washed with a saturated solution of $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by flash chromatography (EtOAc/Petr. et. = 1:3; R_f = 0.51) to give pure **13** (90 mg, 0.16 mmol, 49%).

$[\alpha]_D^{23}$ = +46.9 (*c* 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, *J* = 8.6 Hz, 2H), 7.98 (d, *J* = 8.6 Hz, 2H), 7.27–7.17 (m, 5H), 4.90 (s, 1H), 4.63 (dd, *J* = 5.9, 3.7 Hz, 1H), 4.53 (d, *J* = 5.9 Hz, 1H), 4.49-4.45 (m, 1H), 4.42 (d, *J* = 11.8 Hz, 1H), 4.28 (d, *J* = 11.8 Hz, 1H), 4.17-4.13 (m, 1H), 3.69 (dd, *J* = 15.6, 3.4 Hz, 1H), 3.52 (dd, *J* = 15.6, 8.0 Hz, 1H), 3.46 (dd, *J* = 15.1, 5.1 Hz, 1H), 3.36 (dd, *J* = 15.1, 5.1 Hz, 1H),

3.28 (s, 3H), 3.27 (s, 3H), 1.35 (s, 3H), 1.22 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 149.8, 146.2, 137.0, 128.6 (2C), 128.5 (2C), 127.9 (2C), 127.7, 123.9 (2C), 112.7, 105.3, 103.5, 85.0, 80.0, 78.8, 69.0, 54.8, 54.3, 49.4, 47.7, 26.0, 24.8; MS (ESI) *m/z* (%): 575.26 [$(M+Na)^+$, 100]; MSMS (ESI) *m/z* (%): 575.26 [$(M+Na)^+$, 20], 388.21 (22), 314.17 (100). Anal. Calcd. for $C_{25}H_{32}N_2O_{10}S$: C, 54.34; H, 5.84; N, 5.07. Found: C, 54.61; H, 5.89; N, 5.01.

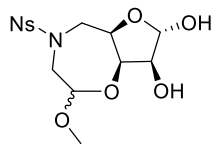
Synthesis and characterization of (5a*R*,7*S*,8*S*,8a*R*)-2-methoxy-4-tosyloctahydrofuro[2,3-f][1,4]oxazepine-7,8-diol (14)



A solution of compound **12** (50 mg, 0.10 mmol) in trifluoroacetic acid (1 mL) was left stirring at room temperature for 2 h. Then, TFA was evaporated under vacuum and the crude product was purified by flash chromatography (EtOAc/hexane = 2:1, R_f = 0.20), affording compound **14** as a pure colorless oil (16 mg, 0.04 mmol, 46%).

^{1}H NMR (400 MHz, $CDCl_3$) 3:1 mixture of anomers: δ 7.64 (d, *J* = 7.8 Hz, 2H), 7.31 (d, *J* = 7.8 Hz, 2H), 5.31 (s, 0.25H, minor), 5.19 (s, 0.75H, Major), 4.57-4.55 (m, 0.5H, minor), 4.46 (dd, *J* = 8.7, 8.6 Hz, 1.5H, Major), 4.24–4.17 (m, 2H), 3.98 (d, *J* = 12.1 Hz, 0.75H, Major), 3.90 (dd, *J* = 13.7, 6.4 Hz, 0.75H, Major), 3.49 (s, 2.25H, Major), 3.45 (s, 0.75H, minor), 3.43–3.34 (m, 0.5H, minor), 2.86 (dd, *J* = 13.7, 11.2 Hz, 0.75H, Major), 2.77 (dd, *J* = 12.7, 10.4 Hz, 0.25H, minor), 2.67–2.61 (m, 1H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.9 (2C), 129.9 (2C), 126.9 (2C), 107.6, 96.5, 80.7, 76.7 and 76.4, 72.3, 56.4 and 54.9, 50.9, 29.7, 21.5. MS (ESI) *m/z* (%): 382.17 [$(M+Na)^+$, 100]. Anal. Calcd. for $C_{15}H_{21}NO_7S$: C, 50.13; H, 5.89; N, 3.90. Found: C, 50.39; H, 5.97; N, 3.79.

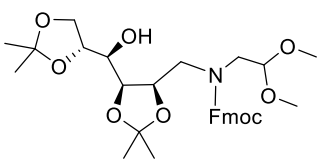
Synthesis and characterization of (5aR,7S,8S,8aR)-2-methoxy-4-((4-nitrophenyl)sulfonyl)octahydrofuro[2,3-f][1,4]oxazepine-7,8-diol (15)



A solution of compound **13** (40 mg, 0.072 mmol) in trifluoroacetic acid (0.5 mL) was left stirring at room temperature for 2 h. Then, TFA was evaporated under vacuum and the crude product was purified by flash chromatography (EtOAc/hexane = 1:1, R_f = 0.21), affording compound **15** as a pure white solid (13 mg, 0.035 mmol, 48%).

^{1}H NMR (200 MHz, $CDCl_3$) 3:1 mixture of anomers: δ 8.34 (d, *J* = 8.4 Hz, 2H), 7.93 (d, *J* = 8.4 Hz, 2H), 5.21 (d, *J* = 3.6 Hz, 0.25H, minor), 5.15 (d, *J* = 3.6 Hz, 0.75H, Major), 4.46 -4.41 (m, 2H), 4.24–4.14 (m, 2H), 3.87 (d, *J* = 13.2 Hz, 0.75H, Major), 3.77 (dd, *J* = 13.6, 6.2 Hz, 0.75H, Major), 3.47 (s, 2.25H, Major), 3.44 (s, 0.75H, minor), 3.37-3.34 (m, 0.5H, minor), 3.01 (dd, *J* = 13.9, 10.6 Hz, 1H), 2.70 (dd, *J* = 12.7, 10.4 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) major anomer: δ 149.7, 145.2, 128.0 (2C), 124.5 (2C), 107.2, 96.1, 80.6, 76.3, 72.3, 56.0, 54.6, 50.0. MS (ESI) *m/z* (%): 413.16 [(M+Na)$^+$, 100]. Anal. Calcd. for $C_{14}H_{18}N_2O_9S$: C, 43.07; H, 4.65; N, 7.18. Found: C, 43.16; H, 4.68; N, 7.12.

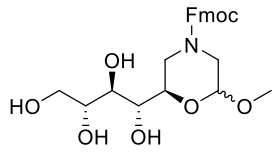
Synthesis and characterization of (9*H*-fluoren-9-yl)methyl-(2,2-dimethoxyethyl)(((4*R*,5*S*)-5-((*R*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(hydroxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)carbamate (16)



To a solution of **7** (100 mg, 0.40 mmol) in dioxane (2 mL) and water (4 mL) was added $NaHCO_3$ (49 mg, 0.58 mmol). The mixture was cooled to 0 °C, then a solution of Fmoc-Cl (159 mg, 0.61 mmol) in dioxane (3 mL) was added slowly. The mixture was left reacting at room temperature for 24 h, then it was diluted with EtOAc (20 mL). The organic phase was successively washed with 1M HCl solution, brine, and dried over anhydrous Na_2SO_4. After solvent evaporation, the crude oil was purified by flash chromatography (EtOAc/Petr. et. = 2:3; R_f = 0.32), giving compound **16** (102 mg, 0.18 mmol, 61%) as a pure colorless oil.

$[\alpha]_D^{24}$ = +11.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.61 (d, *J* = 7.3 Hz, 1H), 7.57 (d, *J* = 7.3 Hz, 1H), 7.42-7.38 (m, 2H), 7.34-7.30 (m, 2H), 4.61–4.53 (m, 2H), 4.46–4.33 (m, 2H), 4.23 (t, *J* = 5.6 Hz, 1H), 4.10-4.07 (m, 3H), 4.01–3.99 (m, 2H), 3.83–3.40 (m, 2H), 3.35 and 3.32 (s, 3H), 3.23 e 3.20 (s, 3H), 2.17 and 2.01 (d, *J* = 9.3 Hz, 2H), 1.57–1.21 (m, 11H), 0.89–0.84 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: δ 156.1, 143.9 (2C), 141.3 (2C), 127.6 (2C), 127.1 (2C), 124.7, 124.5, 119.8 (2C), 109.3, 108.2, 103.5 and 103.0, 76.3, 76.1, 75.2, 70.2, 67.0, 66.5, 60.2, 54.7 and 54.3 (2C), 49.7 and 49.1, 48.2, 26.8, 25.2, 24.6, 14.1. MS (ESI) *m/z* (%): 1164.73 [(2M+Na)$^+$, 8], 594.33 [(M+Na)$^+$, 100]. Anal. Calcd. for $C_{31}H_{41}NO_9$: C, 65.13; H, 7.23; N, 2.45. Found: C, 65.28; H, 7.30; N, 2.40.

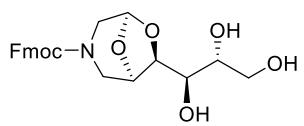
Synthesis and characterization of (6*R*)-(9*H*-fluoren-9-yl)methyl-2-methoxy-6-((1*R*,2*R*,3*R*)-1,2,3,4-tetrahydroxybutyl)-morpholine-4-carboxylate (17)



A solution of compound **16** (76 mg, 0.13 mmol) in trifluoroacetic acid (1 mL) and MeOH (100 μL) was left stirring at room temperature for 20 minutes, until complete disappearance of the starting material (TLC control) was observed. Then, TFA was rapidly quenched by adding a saturated aqueous solution of $NaHCO_3$ (20 mL) until pH = 7. The crude product was extracted in EtOAc and, after solvent evaporation, purification by flash chromatography (EtOAc/MeOH = 10:1, R_f = 0.20), afforded compound **17** as an amorphous solid (44 mg, 0.09 mmol, 73%). HPLC analysis revealed that **17** was obtained as a 10:1 mixture of anomers.

^{1}H NMR (400 MHz, CD_3CN) major anomer, major rotamer: δ 7.81 (d, *J* = 7.4 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.40-7.38 (m, 2H), 7.33-7.31 (m, 2H), 4.82 (s, 1H), 4.80 (br s, 1H, OH), 4.43 (dd, *J* =10.0, 6.5 Hz, 1H), 4.40 (br s, 1H, OH), 4.36 (s, 1H), 4.25 (t, *J* = 6.3 Hz, 1H), 4.10 (br s, 1H, OH), 4.03 (d, *J* = 13.2 Hz, 1H), 3.87 (d, *J* = 13.4 Hz, 1H), 3.81 (br s, 1H, OH), 3.66–3.53 (m, 5H), 3.40 (s, 3H), 3.31-3.30 (m, 2H), 3.21 (s, 1H). ^{13}C NMR (100 MHz, DMSO) major anomer, major rotamer: δ 154.6, 144.2 (2C), 141.1 (2C), 128.2 (2C), 127.6 (2C), 125.6 (2C), 120.7 (2C), 99.1, 95.0, 71.3, 70.0, 67.3, 66.2, 64.2, 55.8, 54.4, 47.0, 46.4. MS (ESI) *m/z* (%): 940.64 (20, $[2M + Na]^+$), 482.31 ($[(M+Na)^+$, 100]; MS (ESI) *m/z* (%): 482.31 ($[(M+Na)^+$, 30], 450.10 (60), 304.26 (32), 272.05 (34), 260.19 (62), 228.14 (100). Anal. Calcd. for $C_{24}H_{29}NO_8$: C, 62.73; H, 6.36; N, 3.05. Found: C, 62.97; H, 6.39; N, 3.01.

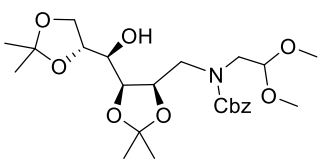
Synthesis and characterization of (1*R*,5*R*,7*S*)-(9*H*-fluoren-9-yl)methyl-7-((1*R*,2*R*)-1,2,3-trihydroxypropyl)-6,8-dioxa-3-aza bicyclo[3.2.1]octane-3-carboxylate (18)



A solution of compound **16** (90 mg, 0.16 mmol) in trifluoroacetic acid (1 mL) and MeOH (100 μL) was left stirring at room temperature for 4 h, until most of the starting material was converted to the bicyclic product, with respect to the monocyclic product (TLC control). Then, TFA was quenched with a saturated $NaHCO_3$ solution (20 mL) until neutral pH. The crude product was extracted in EtOAc and, after solvent evaporation, purification by flash

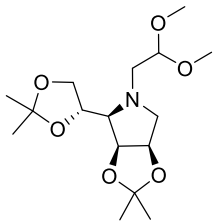
chromatography (EtOAc/MeOH = 100:1, R_f **18** = 0.41), afforded compound **18** as a pure colorless oil (33 mg, 0.07 mmol, 50%).
$[\alpha]_D^{20}$ = +49.5 (*c* 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.75 (d, *J* = 7.3 Hz, 2H), 7.56-7.54 (m, 2H), 7.39-7.28 (m, 4H), 5.50 and 5.46 (s, 1H), 4.46–4.39 (m, 3H), 4.20 (s, 1H), 4.11-4.08 (m, 1H), 3.95 (dd, *J* = 9.0, 4.6 Hz, 1H), 3.80 (s, 1H), 3.74–3.48 (m, 3H), 3.38–3.19 (m, 2H), 3.04 (d, *J* = 7.3 Hz, 1H), 3.02-3.00 (br s, 2H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) mixture of rotamers: *δ* 155.7, 143.3 (2C), 140.9 (2C), 127.5 (2C), 126.7 (2C), 124.6 (2C), 119.7 (2C), 97.1 and 97.0, 80.3 and 80.2, 72.5 (2C), 70.1, 67.4, 63.4 and 63.3, 47.9, 46.8, 43.8. MS (ESI) *m/z* (%): 876.58 [$(2M+Na)^+$, 34], 450.14 [$(M+Na)^+$, 100]. Anal. Calcd. for $C_{23}H_{25}NO_7$: C, 64.63; H, 5.90; N, 3.28. Found: C, 64.97; H, 6.02; N, 3.21.

Synthesis and characterization of benzyl-(2,2-dimethoxyethyl)(((4*R*,5*S*)-5-((*R*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4yl)(hydroxy)-methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)carbamate (19)



To a solution of **7** (500 mg, 1.43 mmol) and Et_3N (600 μL, 4.30 mmol) in anhydrous THF (5 mL), a solution of Cbz-Cl (408 μL, 2.86 mmol) in anhydrous THF (5 mL) was added at 0 °C. The mixture was allowed to reach room temperature and was left stirring under a nitrogen atmosphere for 24 h. Successively, the mixture was diluted with Et_2O, washed with $NaHCO_3$ saturated solution, 1M HCl solution and brine. Then, the organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Crude compound was purified by flash chromatography (EtOAc/Petr. et. = 1:3; R_f = 0.39) to give pure **19** (462 mg, 0.96 mmol, 67%) as a colorless oil.
$[\alpha]_D^{20}$ = +30.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.35-7.32 (m, 5H), 5.16-5.12 (m, 2H), 4.54–4.25 (m, 3H), 4.08–3.87 (m, 3H), 3.76 and 3.72 (s, 1H), 3.65 and 3.62 (s, 1H), 3.49–3.21 (m, 7H), 2.16 and 2.08 (d, *J* = 11.6 Hz, 1H), 1.74 (d, *J* = 11.6 Hz, 1H), 1.45–1.30 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: *δ* 156.2, 136.6, 128.5 (2C), 128.0 (2C), 127.9 and 127.7, 109.4, 108.3, 103.7 and 103.3, 76.3, 76.1, 75.3 and 75.2, 70.3, 67.3, 67.1 and 66.9, 54.7, 54.2, 50.0 and 49.8, 49.2 and 48.7, 26.9, 26.8, 25.2, 24.5. MS (ESI) *m/z* (%): 988.90 [$(2M+Na)^+$, 26], 506.38 [$(M+Na)^+$, 100]. Anal. Calcd. for $C_{24}H_{37}NO_9$: C, 59.61; H, 7.71; N, 2.90. Found: C, 59.73; H, 7.82; N, 2.83.

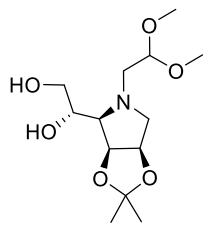
Synthesis and characterization of (3a*S*,4*R*,6a*R*)-5-(2,2-dimethoxyethyl)-4-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-tetrahydro-3a*H*-[1,3]dioxolo[4,5-*c*]pyrrole (20)



Compound **19** (170 mg, 0.35 mmol), Ac_2O (115 μL, 1.23 mmol) and 3Å molecular sieves (10 mg) were dissolved in anhydrous CH_2Cl_2 (10 mL), then pyridinium dichromate (93 mg, 0.25 mmol) was slowly added. The mixture was left stirring under reflux for 90 min, and then filtrated through Silica Gel (EtOAc/Petr. et. = 1:1) affording the intermediate ketone as a colorless oil [^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: δ 7.38-7.35 (m, 5H), 5.31 and 5.17–5.11 (m, 2H), 4.79–3.62 (m, 8H), 3.40–3.27 (m, 7H), 3.17–3.06 (m, 1H), 1.59–1.19 (m, 12H)]. The Cbz-protected ketone (140 mg, 0.29 mmol) was then dissolved in MeOH (20 mL), Pd/C (25 mg, 0.23 mmol) was added and the resulting mixture was left stirring overnight at room temperature under a hydrogen atmosphere. The catalyst was filtered through Celite and the filtrate was concentrated under vacuum, to yield a yellow oil, which was purified by flash chromatography (EtOAc/Petr. et. = 1:2; R_f = 0.65). Compound **20** (100 mg, 0.30 mmol) was thus obtained as a pure yellow oil in 87% yield over these two steps.

$[\alpha]_D^{20}$ = -23.9 (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.60–4.45 (m, 4H), 4.25 (t, *J* = 7.0 Hz, 1H), 3.97 (t, *J* = 7.7 Hz, 1H), 3.39 (s, 3H), 3.37-3.30 (m, 2H), 3.35 (s, 3H), 2.76 (d, *J* = 4.4 Hz, 1H), 2.32–2.22 (m, 2H), 1.44 (s, 3H), 1.42 (s, 3H), 1.31 (s, 3H), 1.26 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 112.3, 110.0, 103.2, 80.2, 77.5, 72.4, 65.3, 59.3, 56.3, 54.6, 52.4, 44.6, 26.0, 25.8, 25.6, 23.8; MS (ESI) *m/z* (%): 354.30 [$(M+Na)^+$, 80], 332.14 [$(M+H)^+$, 100]. Anal. Calcd. for $C_{16}H_{29}NO_6$: C, 57.99; H, 8.82; N, 4.23. Found: C, 58.09; H, 8.95; N, 4.09.

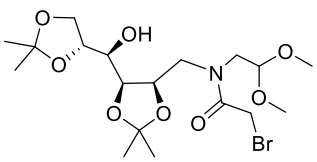
Synthesis and characterization of (*S*)-1-((3a*S*,4*R*,6a*R*)-5-(2,2-dimethoxyethyl)-2,2-dimethyltetrahydro-3a*H*-[1,3]dioxolo[4,5-*c*]- pyrrol-4-yl)ethane-1,2-diol (21)



A solution of compound **20** (105 mg, 0.32 mmol) in trifluoroacetic acid (0.5 mL) and MeOH (0.5 mL) was left stirring at room temperature for 2 h. Then, TFA was quenched with $NaHCO_3$ saturated solution (20 mL) until neutral pH. The crude product was extracted in EtOAc and, after solvent evaporation, purification by flash

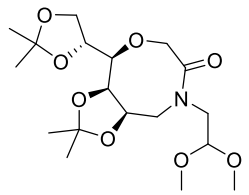
chromatography (CH_2Cl_2/MeOH = 20:1, R_f = 0.35), afforded compound **21** as a pure orange oil (84 mg, 0.29 mmol, 90%).
$[\alpha]_D^{20}$ = -25.9 (*c* 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.69 (dd, *J* = 6.3, 4.3 Hz, 1H), 4.60 (dd, *J* = 6.3, 5.1 Hz, 1H), 4.51 (t, *J* = 5.7 Hz, 1H), 4.01-3.98 (m, 1H), 3.84 (dd, *J* = 11.3, 6.6 Hz, 1H), 3.73 (dd, *J* = 11.3, 4.3 Hz, 1H), 3.46 (s, 2H, OH), 3.39 (s, 3H), 3.37 (s, 3H), 3.35 (d, *J* = 10.9 Hz, 1H), 3.04 (dd, *J* = 13.2, 5.4 Hz, 1H), 2.33–2.25 (m, 3H), 1.50 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 111.5, 103.1, 80.8, 78.0, 69.7, 67.6, 64.9, 59.6, 54.4, 53.8, 53.2, 25.9, 24.6. MS (ESI) *m*/*z* (%): 604.87 [$(2M+Na)^+$, 30], 314.25 [$(M+Na)^+$, 70], 292.12 [$(M+H)^+$, 100]. Anal. Calcd. for $C_{13}H_{25}NO_6$: C, 53.59; H, 8.65; N, 4.81. Found: C, 53.81; H, 8.78; N, 4.65.

Synthesis and characterization of 2-bromo-*N*-(2,2-dimethoxyethyl)-*N*-(((4*R*,5*S*)-5-((*R*)-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-(hydroxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)acetamide (22)



To a solution of bromoacetyl bromide (50 μL, 0.57 mmol) and Et_3N (120 μL, 0.86 mmol) in CH_2Cl_2 (2 mL), a solution of **7** (200 mg, 0.57 mmol) in CH_2Cl_2 (2 mL), was added slowly at -15 °C. The resulting mixture was left stirring at the same temperature for 1 h. Then, the solvent was evaporated under reduced pressure and the crude purified by flash chromatography (EtOAc/Petr. et. = 1:1) affording pure compound **22** (161 mg, 0.34 mmol, 60%) as a yellow oil.
$[\alpha]_D^{22}$ = +12.5 (*c* 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: δ 4.51 (t, *J* = 5.8 Hz, 1H), 4.47-4.42 (m, 1H), 4.37–4.15 (m, 3H), 4.07–3.93 (m, 4H), 3.85-3.82 (m, 1H), 3.69-3.64 (m, 1H), 3.51-3.47 (m, 1H), 3.42 and 3.39 (s, 3H), 3.38 (s, 3H), 3.24-3.18 (m, 1H), 2.20 (br, 1H, OH), 1.47 and 1.45 (s, 3H), 1.39 and 1.37 (s, 3H), 1.32 and 1.31 (s, 3H), 1.11 and 1.09 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: δ 168.2 and 168.1, 109.4, 108.6 and 108.3, 103.3 and 103.1, 76.0, 75.2 and 75.0, 74.9 and 74.3, 70.5 and 70.4, 66.9, 56. 3 and 55.7, 55.3 and 55.2, 52.2, 50.3, 49.5 and 48.9, 27.3 and 27.2, 26.8 and 26.7, 25.3 and 25.2, 24.3 and 24.1. MS (ESI) *m*/*z* (%): 962.75 [$(2M+Na)^+$, 100], 492.17 [$(M+Na)^+$, 60], 469.92 [$(M+H)^+$, 90]). Anal. Calcd. for $C_{18}H_{32}BrNO_8$: C, 45.96; H, 6.86; N, 2.98. Found: C, 46.09; H, 6.94; N, 2.90.

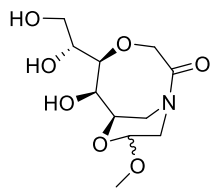
Synthesis and characterization of (3a*R*,4*R*,9a*R*)-8-(2,2-dimethoxyethyl)-4-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-tetrahydro-3a*H*-[1,3]dioxolo[4,5-*f*][1,4]oxazocin-7(4*H*)-one (23)



Sodium hydride (60% in oil, 22 mg, 0.56 mmol) was added to a solution of **22** (130 mg, 0.28 mmol) in anhydrous THF (5 mL) at 0 °C, and the mixture was left stirring for 1 h at room temperature. NaH was quenched with MeOH (2 mL) and filtered through Celite. The filtrate was concentrated under vacuum, to yield a yellow oil which was purified by flash chromatography (EtOAc/Petr. et. = 1:1; R_f = 0.39). Compound **23** (83 mg, 0.21 mmol, 78%) was thus obtained as a pure colorless oil.

$[\alpha]_D^{22}$ = +28.9 (*c* 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.60 (dd, *J* = 5.8, 4.3 Hz, 1H), 4.51 (d, *J* = 16.4 Hz, 1H), 4.49 (dd, *J* = 14.4, 11.4 Hz, 1H), 4.36 (d, *J* = 16.4 Hz, 1H), 4.30 (dd, *J* = 5.8, 3.1 Hz, 1H), 4.28–4.20 (m, 2H), 4.07–3.97 (m, 2H), 3.67 (dd, *J* = 5.9, 1.2 Hz, 1H), 3.53 (dd, *J* = 13.7, 4.3 Hz, 1H), 3.40 (s, 3H), 3.39 (s, 3H), 3.31 (dd, *J* = 13.7, 6.2 Hz, 1H), 3.11 (dd, *J* = 14.4, 2.7 Hz, 1H), 1.42 (s, 3H), 1.38 (s, 3H), 1.34 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 108.9, 108.3, 102.4, 76.7, 75.7, 74.1, 73.5, 73.0, 65.7, 55.1, 54.9, 50.8, 49.4, 27.8, 26.5, 25.5, 25.2; MS (ESI) *m/z* (%): 800.93 [$(2M+Na)^+$, 100], 412.33 [$(M+Na)^+$, 30]. Anal. Calcd. for $C_{18}H_{31}NO_8$: C, 55.51; H, 8.02; N, 3.60. Found: C, 55.64; H, 8.09; N, 3.52.

Synthesis and characterization of (1*R*,6*R*,7*R*,9*R*/*S*)-5-((*R*)-1,2-dihydroxyethyl)-6-hydroxy-9-methoxy-4,8-dioxa-1-azabicyclo[5.3.1]undecan-2-one (24)



A solution of compound **23** (100 mg, 0.27 mmol) in trifluoroacetic acid (0.5 mL) was left stirring at room temperature for 2 h. Then TFA was evaporated under vacuum and the crude residue was purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH (10%) = 4:2:2, R_f = 0.17), affording compound **24** as a colorless oil (36 mg, 0.12 mmol, 42%).

^{1}H NMR (400 MHz, D_2O) 1:1 mixture of anomers: δ 5.42 (s, 0.5H), 5.11-5.08 (m, 0.5H), 4.98 (s, 0.5H), 4.44–4.21 (m, 3H), 4.05-4.02 (m, 0.5H), 3.87–3.75 (m, 2H), 3.72 (s, 1.5H), 3.68–3.55 (m, 2H), 3.51 (s, 1H), 3.45–3.38 (m, 1H), 3.28 (s, 1.5H), 3.23-3.21 (m, 0.5H), 3.10-3.05 (m, 0.5H), 2.98-2.95 (m, 0.5H), 2.82-2.80 (m, 0.5H). ^{13}C NMR (100 MHz, D_2O): δ 174.4 and 172.8, 95.0 and 94.0, 90.1 and

87.2, 78.3 and 78.2, 70.6 and 70.5, 70.2, 70.0, 64.4 and 63.4, 62.5 and 62.3, 57.0 and 55.3, 45.9 and 44.9. MS (ESI) *m/z* (%): 277.19 [$(M+H)^+$, 100]; MSMS (ESI) *m/z* (%): 277.19 [$(M+H)^+$, 8], 246.03 (100), 228.04 (20), 186.02 (22), 170.06 (18), 152.03 (12). Anal. Calcd. for $C_{11}H_{19}NO_7$: C, 47.65; H, 6.91; N, 5.05. Found: C, 47.87; H, 7.01; N, 4.97.

4.4.1 Chemioinformatics:

PCA Analysis. The web-based public tool ChemGPS-NP[24] was used for the PCA analysis of compounds **6**-**24**, to compare their chemical properties with those of a reference set of 40 brand-name blockbuster (BB) drugs as reported by Tan.[25] The first four dimensions of the ChemGPS-NP map capture 77% of data variance. Chemical compounds were positioned onto this map using interpolation in terms of PCA score prediction. SMILES codes for all compounds **6**-**24** (Table 1) and the 40 BB drugs of the reference set (Table 1 in the Appendix) were retrieved using ChemBioDraw Ultra 12.0 and submitted to ChemGPS-NP for achieving the corresponding PC scores.

Table 1. SMILES code of compounds 6-24

- CC1(O[C@H](CO1)[C@@H](O2)[C@H](O3)[C@H](OC3(C)C)C2NCC(OC)OC)C [**6**]
- O[C@H]([C@@H](CO1)OC1(C)C)[C@H](O2)[C@@H](CNCC(OC)OC)OC2(C)C [**7**]
- CC1(O[C@@H]([C@H]([C@@H](CNCC(OC)OC)O2)O1)[C@H]2OCC3=CC=CC=C3)C [**8**]
- CC1(O[C@@H](CO1)[C@H]2[C@@H](O3)[C@@H](OC3(C)C)[C@@H](N(C(OCC4C5=C(C=CC=C5)C6=C4C=CC=C6)=O)CC(OC)OC)O2)C [**9**]
- O[C@H]1[C@@H]([C@@H](CO)O)O[C@@H]2[C@H]1OC(OC)CN2C(OCC3C4=C(C=CC=C4)C5=C3C=CC=C5)=O [**10**]
- COC(CN1C(OCC2C3=C(C4=C2C=CC=C4)C=CC=C3)=O)O[C@@H]5[C@H]1O[C@H]([C@H](OC(C)=O)COC(C)=O)[C@@H]5OC(C)=O [**11**]
- CC1(O[C@@H]([C@H]([C@@H](CN(S(C2=CC=C(C)C=C2)(=O)=O)CC(OC)OC)O3)O1)[C@H]3OCC4=CC=CC=C4)C [**12**]
- CC1(O[C@@H]2[C@@H](OCC3=CC=CC=C3)O[C@H](CN(S(=O)(C4=CC=C([N+]([O-])=O)C=C4)=O)CC(OC)OC)[C@@H]2O1)C [**13**]
- O[C@@H]([C@H]1OC(OC)CN(S(C2=CC=C(C)C=C2)(=O)=O)C[C@H]1O3)[C@H]3OCC4=CC=CC=C4 [**14**]
- O[C@@H]1[C@H]([C@@H]2[C@@H](CN(S(C3=CC=C([N+]([O-])=O)C=C3)(=O)=O)CC(OC)O2)O1)O [**15**]

- O[C@H]([C@@H]1OC(C)(C)OC1)[C@H](OC(C)(C)O2)[C@H]2CN(C(OCC3C4=C(C=CC=C4)C5=C3C=CC=C5)=O)CC(OC)OC [**16**]
- O[C@H]([C@H](O)[C@H](O)CO)[C@H]1CN(C(OCC2C3=C(C=CC=C3)C4=C2C=CC=C4)=O)CC(OC)O1 [**17**]
- O[C@@H]([C@H]1[C@H](O2)CN(C[C@H]2O1)C(OCC3C4=C(C=CC=C4)C5=C3C=CC=C5)=O)[C@H](O)CO [**18**]
- O[C@H]([C@@H]1OC(C)(C)OC1)[C@H](OC(C)(C)O2)[C@H]2CN(C(OCC3=CC=CC=C3)=O)CC(OC)OC [**19**]
- CC1(C)O[C@@H]([C@@H]2[C@H](O3)[C@@H](CN2CC(OC)OC)OC3(C)C)CO1 [**20**]
- O[C@@H]([C@@H]1[C@H](O2)[C@@H](CN1CC(OC)OC)OC2(C)C)CO [**21**]
- O[C@H]([C@@H](CO1)OC1(C)C)[C@H](O2)[C@@H](CN(C(CBr)=O)CC(OC)OC)OC2(C)C [**22**]
- CC1(C)O[C@@H]([C@@H]2[C@H](OC(C)(C)O3)[C@H]3CN(CC(OC)OC)C(CO2)=O)CO1 [**23**]
- O[C@H](CO)[C@@H]1[C@@H]([C@@H]2OC(OC)CN(C(CO1)=O)C2)O [**24**]

MOLID	PC1	PC2	PC3	PC4
[22]	-0.721276	-2.202219	-0.515466	0.447612
[9]	2.113689	1.025436	1.746083	-0.417978
[11]	2.974673	1.039039	1.348310	0.634842
[15]	-0.277589	0.440170	-1.534655	0.353267
[12]	1.379546	1.185196	1.519654	0.351087
[6]	-1.766170	-2.097457	-0.549029	-0.322918
[24]	-2.075691	-1.795427	-2.525690	-0.245074
[8]	-0.874185	-0.078841	0.317862	0.231161
[17]	1.371461	1.030234	-0.600329	-0.412785
[7]	-1.528225	-2.398319	-0.887160	-0.082554
[19]	0.775478	-0.595027	0.477981	0.171517
[13]	2.450356	1.131515	0.958069	1.050073
[21]	-2.247633	-2.207722	-1.287356	-0.271641
[23]	-1.143815	-1.943431	-0.460395	-0.179870
[16]	2.332051	0.751034	1.428504	-0.239439
[10]	1.127785	1.311264	-0.241421	-0.625869
[20]	-2.109106	-1.995557	-0.240800	-0.419191
[18]	0.754635	1.454661	-0.292545	-0.557017
[14]	0.506196	1.646954	0.567557	-0.084310

Table 2: PCA results for the first four dimensions of **6**-**24** (77% of data variance)

PMI Analysis. Principal moment of inertia analysis was carried out by calculation of the lowest energy conformation of each compound **6**-**24** and each compound from the reference set of 40 brand-name blockbuster drugs,[25] using the built-in AMMP molecular mechanics algorithm with default parameters of the VEGA ZZ molecular modelling software package v.3.0.1.[27] Once the lowest energy conformer was calculated, the three principal moments of inertia (I_{xx}, I_{yy}, I_{zz}) and normalized principal moments of inertia, npr1 (I_{xx}/I_{zz}) and npr2 (I_{yy}/I_{zz}) were determined, and PMI ratios were calculated for **6**-**24** (Table 3 below) and BB drugs (Table 3 in the Appendix).

MOLID	I_x	I_y	I_z	I_x/I_y	I_y/I_z
[14a]	10376,2662	7479,7559	3380,3735	0,325779	0,720852
[14b]	9840,7807	7405,1523	3129,1348	0,317976	0,752496
[24a]	1896,856	1445,6552	1253,7451	0,66096	0,762132
[24b]	1715,9967	1359,3705	1229,6036	0,716554	0,792175
[10]	6980,0609	6572,8015	2698,1673	0,386554	0,941654
[17a]	10334,0337	10265,4869	2060,6368	0,199403	0,993367
[17b]	6937,7315	5217,1686	3178,1488	0,458096	0,751999
[18]	7450,6933	6974,9361	1946,825	0,261294	0,936146
[6a]	4720,5805	3829,0994	1637,9007	0,34697	0,81115
[6b]	5573,2969	4379,1803	1771,542	0,317862	0,785743
[9]	11927,7586	10782,0478	3505,6157	0,293904	0,903946
[7]	5926,031	5663,7907	1150,9566	0,19422	0,955748
[16]	12236,853	9179,1372	4380,614	0,357985	0,750122
[20]	3722,5673	2785,0585	1634,8372	0,439169	0,748155
[21]	2762,8317	2214,5066	1153,9329	0,417663	0,801535
[8]	6733,5953	4120,4904	2924,0884	0,61193	0,709646
[19]	7836,158	6001,4028	3082,3174	0,393345	0,765860
[22]	6496,4887	5230,9832	2815,1678	0,433337	0,805202
[12]	7784,571	7214,4204	4468,7133	0,574047	0,926759
[23]	3526,737	2971,2109	2013,052	0,570797	0,842482
[13]	12023,7331	9932,1051	3483,2614	0,350707	0,826042
[15]	5375,6223	4850,2880	1937,7523	0,36047	0,902275

Table 3: PMI results table of compounds **6**-**24** by calculation of the lowest energy conformation.

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5

From the Phenotypic Screening of Mannose-derived Compounds to the Identification of Novel Breast Carcinoma Cell Growth Modulators

5.1 Introduction

As discussed in Chapter 1, the application of DOS libraries in phenotypic screening and chemical genetics studies proved to be particularly successful for lead discovery in complex disorders, such as cancer and infective pathologies.[1] In particular, phenotypic drug discovery has received increasing interest in oncology, as this therapeutic area presents unique challenges, due to the very large number of molecularly and phenotypically distinct diseases occurring within the same pathology.[2] In fact, despite the high number of validated targets that have been discovered in these decades, only few targeted agents are capable of inducing a significant impact on malignant cells, because, generally, compensatory and feedback effects tend to diminish the final outcome of the drug. For these reasons, phenotypic screening, focusing on functional effects, more than on molecular targets, is a valuable alternative

approach in cancer drug discovery. Although several limitations still need to be solved, some success have been already reported. Among the 48 oncology NMEs approved by the FDA between 1999 and 2013, four of them could be considered as arisingly purely from phenotypic drug discovery approach (PDD), without having prior knowledge of their biochemical activity or their mode of action.[2a] Thalidomide-analogues Lenalidomide and Pomalidomide (Figure 1),[3] for example, were selected as clinical candidates, thanks to their ability in inducing a potent downregulation of tumor necrosis factor (TNF) production, even if their molecular target, ubiquitin ligase Cereblon, was identified only some years later.[4] Vorinostat was serendipitously found to induce erythroid differentiation in cell, and then correlated to an inhibition effect on histone deacetylase (HDAC).[5] In an analogue way, Romidepsin was identified from a screen for cytotoxic activity, and only later, it was shown to be another HDAC inhibitor.[6]

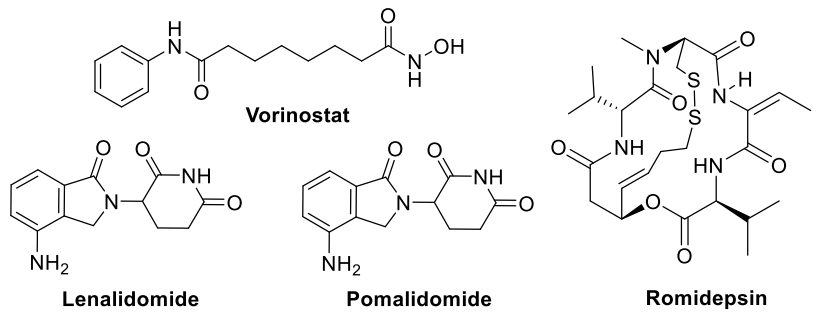


Figure 1: Cancer drugs discovered by completely pure PDD approaches and approved by the FDA between 1999 and 2013.

The numbers of NME in clinical trials or already approved by FDA is even bigger, if we considered the so-called "mechanism-informed phenotypic drug discovery", which includes inhibitors of known molecular targets that are identified or optimized taking advantage of the use of phenotypic *in vitro* models (eribulin, enzalutamide, fulvestrant are just some examples).[7] As in the last decades several improvements have been made into the molecular basis and the classification of the typical cancer hallmarks,[8] different assays has been developed to detect the modulation of different cancer phenotypes (Figure 2).

General classification	Types	Assays
Intracellular signalling and transcription	Gene expression	Pathway-driven reporter gene
		mRNA profiling
		Active promoter reporter gene
	Biochemical events	Protein expression and localization
		Protein degradation
		Post-translational modification
Cell growth and death	Cytotoxicity	ATP assay
	Synthetic lethality	Genetic interaction
		Drug interaction
	Cell cycle	Mitotic defects
		DNA damage
		DNA replication
'Classical' phenotypes	Morphology	Endothelial tubulogenesis
	Motility	Invasion
		Migration
	Differentiation	Myeloid differentiation
		Epithelial-to-mesenchymal transition

Figure 2: Approaches to cell phenotypic screening in the context of cancer threpeutics. Reproduced from ref. 2a with permission from Nature Publishing Group.

In this context, as a first biological output of the mannose-derived DOS library described in Chapter 4 and reported in summary in Figure 3, we reasoned to take into account cancer cell growth as a phenotype, focusing in particular on the ability of these compounds of inducing cell growth inhibition in a human metastatic melanoma cell line (MDA-MB-231).

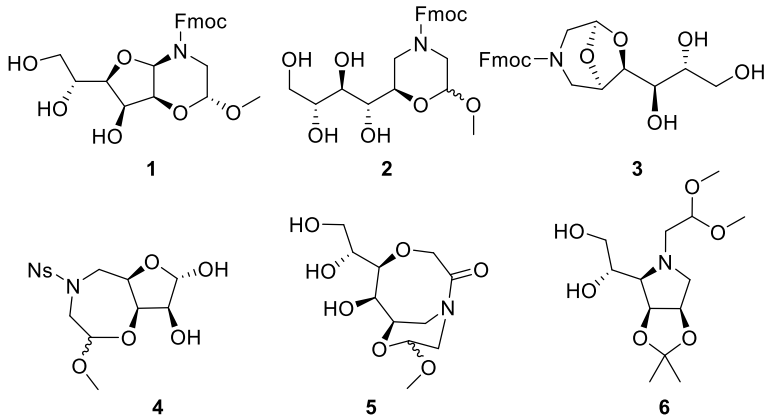


Figure 3: Polyhydroxylated nitrogen-containing scaffolds derived from D-mannose and glycine-derived aminoacetaldehyde.

Considering that these compounds present similarity with polyhydroxylated iminosugars,[9] breast carcinoma cells were selected, as some preliminary evidence about the ability of iminosugars to inhibit breast cancer cell growth has recently appeared in literature. In particular, different types of pyrrolidinic compounds have shown significant cell growth inhibition in breast tumoral cell lines, such as T-470,[10] and MCF-7 line,[11] targeting glycosidase enzymes and molecular pathways related to molecular chaperones and ion channels. In addition, iminosugar-ferrocene conjugates proved to inhibit MDA-MB-231 cells proliferation.[12] This particular cell line (MDA-MB-231) is a simple model system for the study of the triple-negative breast cancer (TNBC).[13] This type of cancer shows a major tendency toward early metastasis, and does not respond to hormonal chemotherapy, as it lacks the three main molecular targets, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER-2/Neu).[14] Therefore, there is a need for the development of new treatments against such type of cancer, which accounts for 15% of all type of breast carcinomas. In this view, the library of skeletally different glycomimetics developed from mannose and glycine-derived amino aldehyde was submitted to a MDA-MB-231 cell-based assay, aiming to identify valuable *hit* compounds for the modulation of breast carcinoma cell cycle mechanism.

5.2 Results and Discussion[15]

A first screening on MDA-MB-231 cells was performed for the six compounds at 10 μM concentration. After the first 24h of treatment, no particular effects were observed on cell growth (data not shown). Interestingly, after 48h incubation, although a slightly induction in cell proliferation was detected for compound **2**, **3** and **6**, a 40% inhibition of cell growth was observed following the treatment with compound **1**, containing the hexahydro-2*H*-furo[3,2-*b*][1,4]oxazine scaffold (Figures 4). This range of inhibition is in line with those observed in other studies conducted on the same cell line.[16]

Also, the examination of cell morphology, which can reveal essential information regarding the healthy status of the cell population, indicated that after 48h incubation MDA-MB-231 cells were not only reduced in number but also significantly changed.

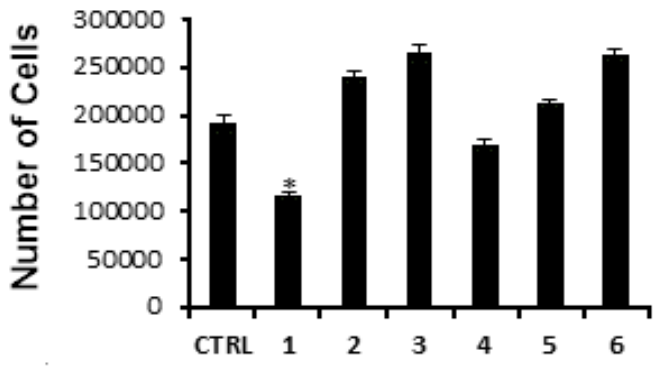


Figure 4: MDA-MB-231 cell number after 48h of incubation with the selected molecules at 10 µM concentration. Values correspond to the mean of three independent experiments. Error bars indicate the corresponding standard deviations values. Student's t test was used to evaluate the data significance,*, $p < 0.05$

In particular, whereas part of the cell population showed a regular appearance, major part exhibited a round shape, though still adhering to the substrate (Figure 5). This observation suggests that the effect of compound **1** might induce a cell cycle slowdown and might not be related to the perturbation of adhesive properties of the cell.

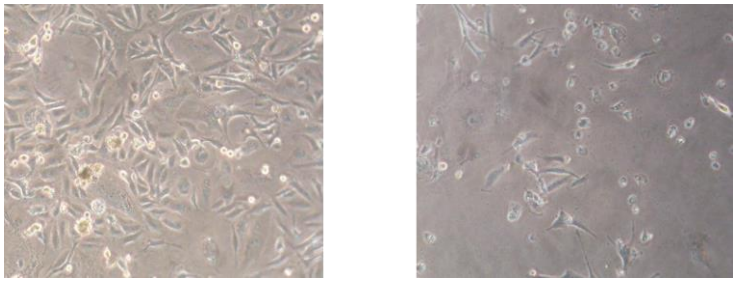


Figure 5: Control cells after 48h (**left**), and cells after 48h of incubation with compound **1** at 10 µM concentration (**right**).

In agreement with this, the biological effect was confirmed to be dose-dependant, thus validating the specificity of compound **1** in inhibiting cell growth (Figure 6, left). Also, significant reduction in cell proliferation was observed at a concentration higher than 3 µM, revealing that compound **1** possess an IC_{50} of 0.6 µM (Figure 6, right).

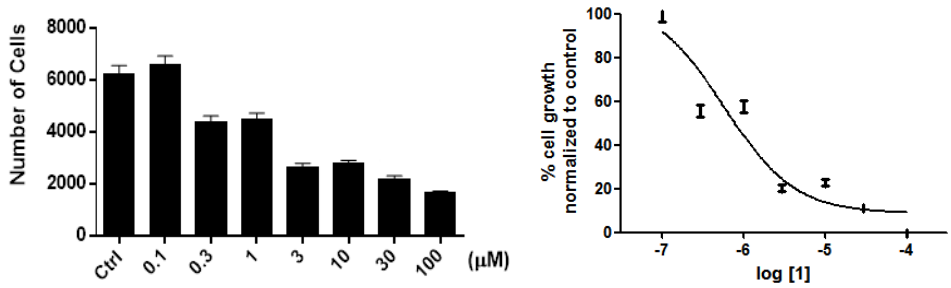
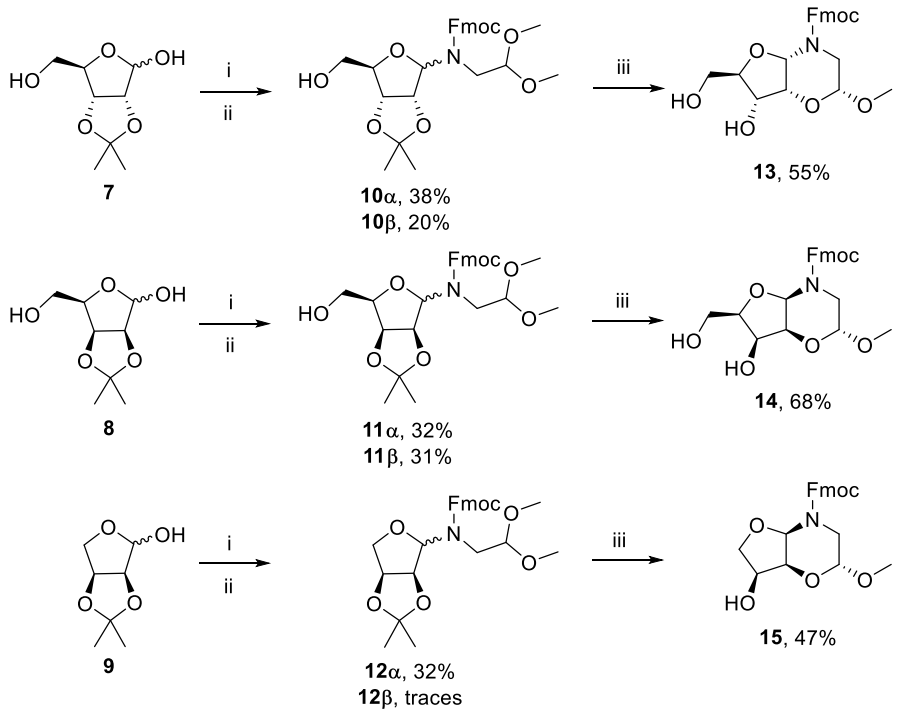


Figure 6: Cell growth inhibition in MDA-MB-231 cells after 48h of incubation with compound **1** at the reported concentration (**left**); dose-response curve of log[**1**] vs % cell growth normalized to control (**right**). Values indicate the mean of three independent experiments. Error bars show the corresponding standard deviation values.

Starting from these results, in order to improve the activity of this compound, we reasoned to develop a focused library around this structure, trying to explore different amine functionalities, and different polyhydroxylated chains.
In details, hexahydro-2*H*-furo[3,2-*b*][1,4]oxazine compounds **13**, **14**, **15** (Scheme 1), with different polyhydroxylated chains, were obtained starting from appropriate sugar derivatives, namely 2,3-*O*-isopropylidene-D-ribofuranose (**7**), 2,3-*O*-isopropylidene-D-lyxofuranose (**8**) and 2,3-*O*-isopropylidene-L-erythrofuranose (**9**). The synthesis of these compounds was obtained following the same synthetic strategy adopted for the achievement of mannose-derived compound **1**, as already explained in Chapter 4.

The reaction of the sugar derivatives **7**-**9** with glycine-derived amino acetaldehyde, by formation of the glycosyl amine and the direct *N*-acylation of the crude hemiaminal coupling intermediates, resulted in the achievement of Fmoc-protected intermediates **10**-**12** with a clean conversion and a successful separation of the two anomers. Then, under acid-catalyzed *trans*-acetalization conditions, the reaction of the *C*-3a hydroxyl group of the intermediate with the dimethyl acetal carbon atom, led to the synthesis of the corresponding bicyclic compounds **13**-**15**. The preferred anomer were found to be the α-anomer, similarly to what observed for mannose-derived intermediate, even though starting from lyxose the ratio between the two anomeric compounds became approximately 1:1. On the other hand, the instability of the erythrose-

derived coupling intermediate was evinced by the low yield of the reaction and in the achievement of only the most stable α-anomer product **12α**.

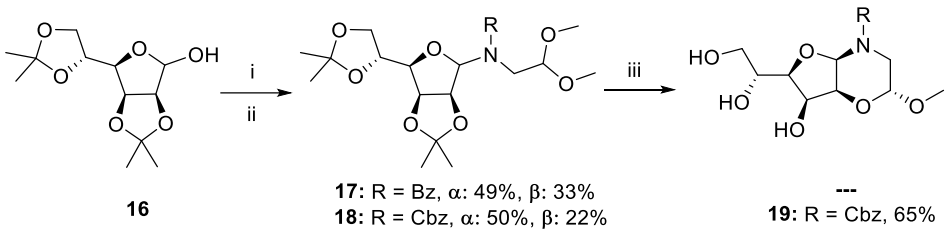


Scheme 1: Synthesis of hexahydro-2*H*-furo[3,2-*b*][1,4]oxazine compounds **13**-**15** from the corresponding sugar derivatives **7**–**9**. Reagents and conditions: (i) $NH_2CH_2CH(OMe)_2$, $MgSO_4$, MeOH, reflux, 48 h; (ii) Fmoc-Cl, H_2O–dioxane, $NaHCO_3$, 0°C to r.t., 24 h; (iii) TFA, neat, r.t., 2 h.

Upon treating both the anomers of the coupling intermediates under neat TFA conditions, the corresponding bicyclic *cis*-fused scaffolds **13-15** were achieved, even if in less yield as compared to the mannose-derived bicycle **1**. In all cases, the same *cis*-fused scaffold was achieved from both the anomers in similar yields, as already discussed in the previous chapter, as a consequence of the thermodynamic equilibration of the Fmoc-protected compounds. Also, in all cases only the *endo* anomer was recovered, which is the most stable considering stereoelectronic effects.

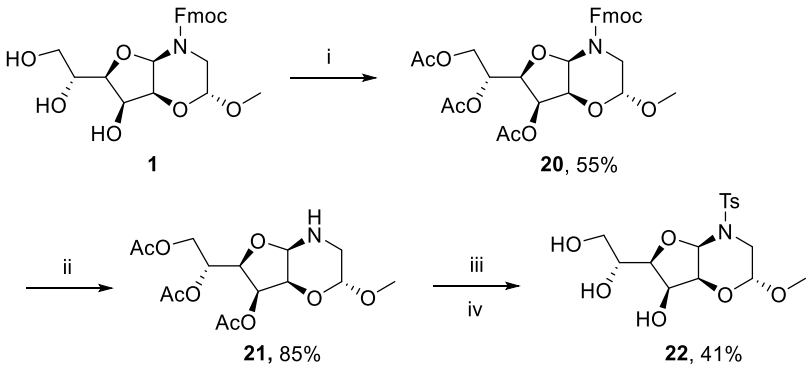
On the other hand, the exploration of the amine functionality resulted to be more difficult, considering the low reactivity of the hemiaminal position. In fact, the derivatization of the unstable hemiaminal coupling intermediate was possible only using benzoyl chloride and chloroformates. However, even if the

use of chloroformate resulted in a clean cyclization to the final product in good yield (compound **19**, Scheme 2), benzoyl analogues (**17α**/**17β**) failed to give the corresponding bicyclic compound, as a consequence of the reduced stability of the *N*-acyl moiety.



Scheme 2: Synthesis of *N*-functionalized hexahydro-2*H*-furo[3,2-*b*][1,4]oxazine compound **19**. Reagents and conditions: (i) $NH_2CH_2CH(OMe)_2$, $MgSO_4$, MeOH, reflux, 48 h; (ii) Fmoc-Cl, H_2O–dioxane, $NaHCO_3$, 0°C to r.t., 24 h; (iii) TFA, neat, r.t., 2 h.

The reaction of the hemiaminal intermediate with sulfonyl chloride was not possible at all. However, in order to explore the possibility of introducing different moieties linked to the amine and studying their biological effect, the tosyl derivative **22** was obtained from the corresponding per-*O*-acetylated *N*-deprotected compound **21**, subsequent to Fmoc deprotection of **20** (Scheme 3).



Scheme 3: *N*-tosylation of compound **1**. Reagents and conditions: (i) Ac_2O, pyr., r.t., 16 h; (ii) 30% Et_2NH, CH_3CN, r.t., 2 h; (iii) TsCl, DIPEA, DCM dry, r.t., 16 h; (iv) Ambersep 900 OH, MeOH, r.t., 2h.

Finally, in order to assess the stability of this class of bicyclic compounds, concerning the hemiaminal moiety under acidic conditions, a study by HPLC analysis was performed. Compound **14** was selected as a representative bicyclic compound and the stability studies were conducted in different solvents,

MeOH and acetonitrile, in the presence of 1M HCl, after 1h (Figure 7) and after 24h (Figure 8). In all cases, neither the formation of by-products nor the reduction of peak intensity was evinced, as compared to benzophenone (internal standard).

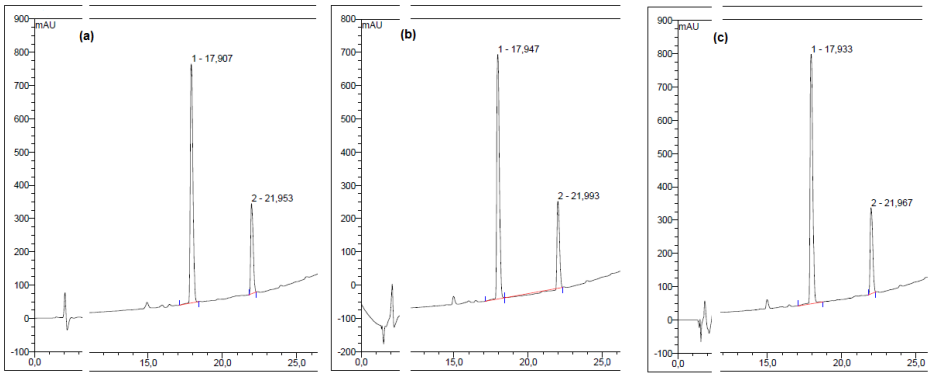


Figure 7: HPLC runs of compound **14** (0.5 mg/mL, final conc.) after 1h incubation in (**a**) CH_3CN; (**b**) 1:1 mixture of CH_3CN and HCl 1M; (**c**) 1:1 mixture of MeOH and HCl 1M. A 0.1 mg/mL quantity (final conc.) of benzophenone (rt = 22 min) was used as an internal standard.

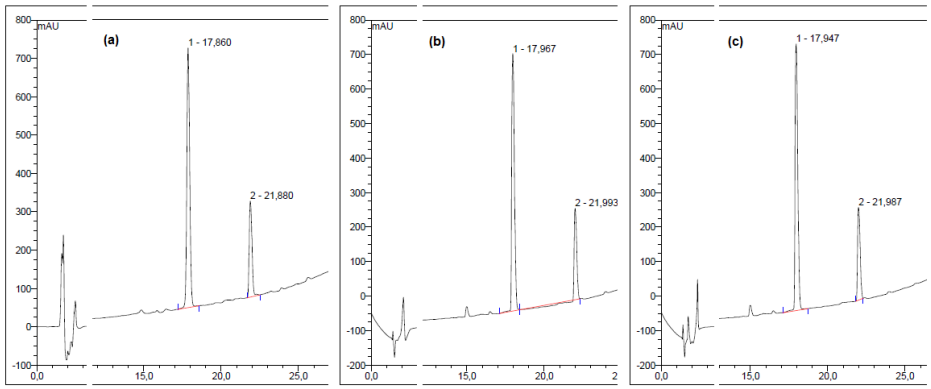


Figure 8: HPLC runs of compound **14** (0.5 mg/mL, final conc.) after 24h incubation in (**a**) CH_3CN; (**b**) 1:1 mixture of CH_3CN and HCl 1M; (**c**) 1:1 mixture of MeOH and HCl 1M. A 0.1 mg/mL quantity (final conc.) of benzophenone (rt = 22 min) was used as an internal standard.

These new compounds, representing some preliminary representative examples of varying both polyhydroxylated chains and *N*-functionalization, were then tested for their inhibition activity on the same MDA-MB-231 cell line, at 10 µM concentration and with 48h of incubation. As reported in Table 1,

however, none of these compounds proved to be as interesting as **1**, revealing a specific structural requirement for an optimal inhibition profile. The importance for the activity of the number of hydroxyl groups and their stereochemistry was evinced by comparing the inhibition activities of Fmoc-derivatives **1** and **13**-**15**, and those of **1** and the corresponding fully acetylated derivative **20**.

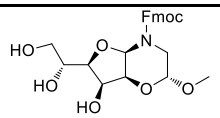
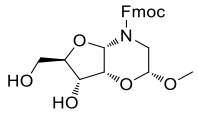
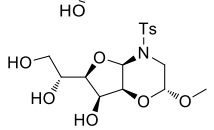
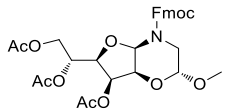
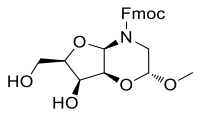
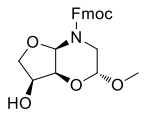
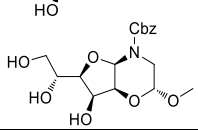
Cmpd	Structure	% inhibition[a]
1		41 ± 3
13		13 ± 1
22		13 ± 3
20		13 ± 2
14		15 ± 11
15		12 ± 6
19		10 ± 4

Table 1: Cell growth inhibition of MDA-MB-231 cell line at 10 μM concentration after 48h of incubation. (a) Cell growth inhibition (% vs control) in MDA-MB-231 cells after 48h of incubation with compound **1** at the reported concentration. Values ± SD indicate the mean of three independent experiments.

Finally, an indication of the importance of the functionalization of the hemiaminal nitrogen atom was shown by comparing the inhibition activities of **1**, **19**, and **22**, all possessing similar scaffold and polyhydroxylated tail, and differing by the Fmoc, Cbz and tosyl group at the nitrogen atom, respectively. All in all, the biological data suggested a specific effect of the bicyclic compounds towards cell growth inhibition of MDA-MB-231 cells, as any small

change in the structure or appendage of the lead compound **1** caused a drop in the biological activity.

Thus, in order to obtain more biological data necessary for the optimization of this lead compound, further biological studies were conducted. Firstly, we investigated the effect of compound **1** in cell viability using the WST-1 tetrazolium salt assay. In analogy to MTT, the stable tetrazolium salt WST-1 is cleaved to a soluble formazan by a mechanism that is largely dependent on the glycolytic production of NAD(P)H in viable cells, thus, the amount of formazan dye produced directly correlates with the number of metabolically active cells. We tested different concentrations of compound **1**, and we found that the number of dead cell was not significantly different as compared to the control (Figure 9), thus indicating that the cell growth inhibition was not related to the cytotoxicity of the molecule.

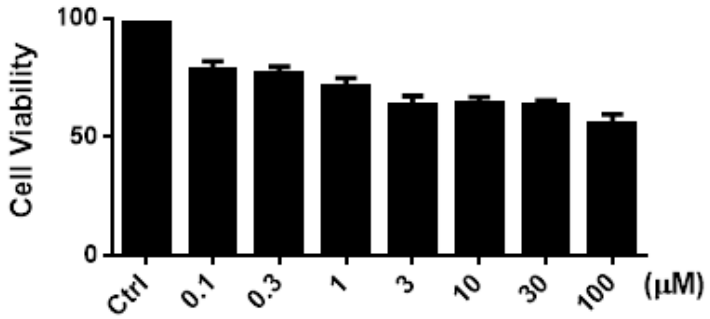


Figure 9: Cell viability assay (WST-1) in MDA-MB-231 cells after 48h of incubation with compound **1** at the reported concentration. Values indicate the mean of three independent experiments and are expressed as percentage compared to control cells, error bars indicate the corresponding standard deviations values.

This result was confirmed also by a cytofluorimetric assay for the apoptosis evaluation using the Annexin V FITC protocol. Annexins are a family of phospholipid-binding proteins that preferentially bind phosphatidylserine (PS), a receptor located in the inner leaflet of the plasma membrane under normal physiologic conditions. Upon initiation of apoptosis, PS is translocated to the extracellular membrane leaflet, to mark the cells as target of phagocytosis, and can be detected by fluorescently labeled Annexin V (FITC-A).[17] In this way, in the plot resulting from cytofluorimetric analysis (Figure 10, top), cells in early-stage apoptosis are found in the right bottom corner (Q4-1: Annexin +/ PI -), as

the plasma membrane binds Annexin V and excludes viability dyes such as propidium iodide (PI). On the other hand, necrotic cells, possessing damaged cell membrane, tend to internalize propidium iodide (PI), resulting in the left top corner (Q1-1: Annexin -/ PI +), whereas cells in the late-stage apoptotis are found in the top right corner as they result positive for both dyes (Q2-1: Annexin +/ PI +).

Using this protocol, the effect on cell apoptosis was evaluated in the entire MDA-MB-231 cell population treated with compound **1** at different concentration (Figure 10, bottom). As mentioned before, no significative effect on cell adhesion was found, however, after the 48h treatment, the very few cells present in the supernatant and the adherent cells were collected together and no differences were found in the diverse cell treatments in term of apoptosis, even at 10 µM concentration.

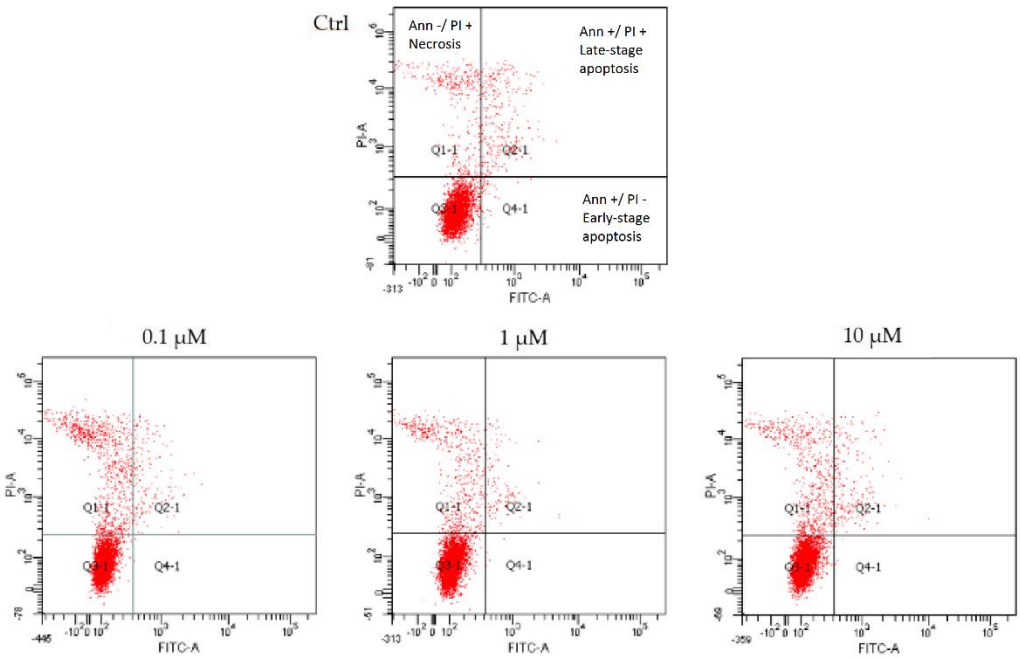


Figure 10: Cytofluorimetric assay for the apoptosis evaluation using the Annexin V FITC protocol in MDA-MB-231. Representative cell plot analysis of apoptosis in MDA-MB-231 of control (**top**) and after 48h of incubation with compound **1** at the reported concentration (**bottom**).

We then investigated the effect of compound **1** on MDA-MB-231 cell cycle, finding that, starting from 10 μM concentration, this compound clearly induced an arrest of MDA-MB-231 cell cycle in the G0/G1 phase (Figure 11). From these preliminary results, it is possible to assume that the growth inhibition induced by compound **1** on MDA-MB-231 cells might be correlated to a cytostatic effect, rather than to a cytotoxic one.

	G0/G1	S	G2/M
Ctrl	38-40	31-33	28-30
0,1 μM	33-35	26-27	36-38
1 μM	40-41	25-27	32-33
10 μM	45-47	25-27	32-35
100 μM	46-48	23-25	25-26

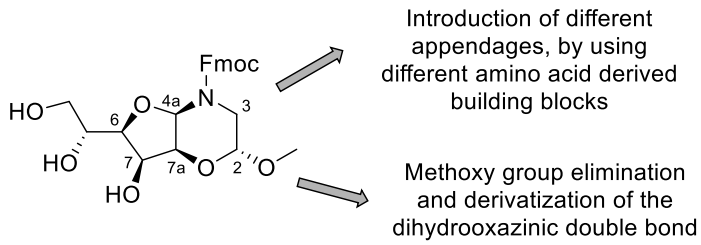
Figure 11: Cell cycle assay in MDA-MB-231 cells after 48h of incubation with compound **1** at the reported concentration. Values indicate the range of cell population percentage in different cell cycle phases of three independent experiments.

Thus, the exploration of the fine mechanisms that regulate the cell cycle will be performed in future to identify the protein target of compound **1**, and future experiments on non-transformed epithelial cells, such as MCF10A,[18] a non-tumorigenic breast epithelial cell line, will be performed to ensure the selectivity and specificity of the compounds.[19]

5.3 Conclusions

In conclusion, in this chapter, a first attempt on the identification of novel potential *lead* antitumor compounds against breast carcinoma phenotype was described. Specifically, the application of a cell-based growth inhibition assay on a library of skeletally different glycomimetics allowed for the selection of a novel chemotype based on the hexahydro-2H-furo[3,2-*b*][1,4]oxazine scaffold as an active inhibitor of MDA-MB-231 cell growth. Subsequent follow-up synthesis of parent compounds and preliminary biological studies validated the selection of **1** as a valuable lead compound for the modulation of breast carcinoma cell cycle mechanism.

Although follow-up compounds showed reduced activity as compared to **1**, interesting structure-activity analysis showed a quite specific structural requirement, revealing that both the three hydroxyl group and the Fmoc group are crucial for inhibition. However, further point of chemical diversification on this scaffold can be included. For example, the use of other amino acid derived amino acetal building blocks can introduce different group in position 3 of the scaffold, whereas the acid-catalyzed elimination of the methoxy group at position 2 can generate a dihydro-oxazinic double bond, which can be used to achieve further complexity (Scheme 4).



Scheme 4: New potential points of diversification of scaffold **1**.

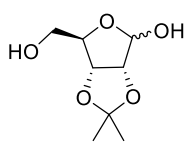
Preliminary biological test revealed that compound **1** clearly induced a significant arrest of MDA-MB-231 cell cycle. From the cell viability assay and the cell cycle analysis, we also assumed that the growth inhibition induced by compound **1** on MDA-MB-231 cells might be correlated to a cytostatic effect and no significative effect on cell adhesion and apoptosis was found, by far. Future experiments on non-transformed epithelial cells will be performed, for this reason, to ensure the selectivity and specificity of compound **1** in inducing this biological effect. The identification of the target responsible for the observed phenotype, among the many different growth factor signals and cyclin-dependent kinases, will shed light in the understanding the mode of action of this compound.[20] For these reasons, further investigations will be attempted in view to characterize the signaling pathways underlying the biological effect,[21] particularly investigating the role of caspases and the modulation of their phosphorylation.[22] Finally, further biological tests will be carried out starting from flow cytometry profiling and the use of reverse chemical genetics approaches in order to collect data for a further optimization of the structure of compound **1**.

5.4 Experimental Section

5.4.1 Synthesis

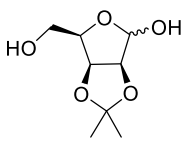
General Analytical grade solvents and commercially available reagents were used without further purification. Flash column chromatography (FCC) purifications were performed manually using glass columns with Merck silica gel (0.040–0.063 mm), or using the Biotage Isolera system and SNAP silica cartridges. TLC analyses were performed on Merck silica gel 60 F254 plates. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), or a Varian Gemini 200 (^{1}H: 200 MHz, ^{13}C: 50 MHz). All chemical shifts are reported in parts per million (δ) referenced to residual nondeuterated solvent. Data are reported as follows: chemical shifts, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constant(s) in Hz; integration). ESI mass spectra were carried out on a ion-trap double quadrupole mass spectrometer using electrospray (ES+) ionization techniques, and a normalized collision energy within the range of 25−32 eV for MSMS experiments. IR spectra were recorded with a FTIR-1600 Perkin-Elmer spectrophotometer. Elemental analyses were performed on a Perkin Elmer 240 C, H, N analyzer. Optical rotation measurements were performed on a JASCO DIP-370 polarimeter and are given in 10^{-1} deg cm^2 g^{-1}. Synthesis of compound **1**, **2**, **3**, **4**, **5**, **6**, **16** and **20** are reported in Chapter 4.

Synthesis of (3a*R*,6*R*,6a*R*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (7)



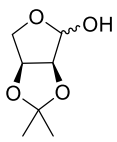
A suspension of D-ribose (2.9 g, 19.3 mmol) in acetone (38 mL) was cooled to 0 °C and treated with 2,2-dimethoxypropane (2.6 mL, 21.2 mol) and *p*-toluenesulfonic acid (37 mg, 0.19 mmol). After being stirred at room temperature for 1 h under nitrogen atmosphere, the clear resulting mixture was neutralized with $NaHCO_3$ and filtered over a pad of celite. The filtrate was concentrated under vacuum and the residue was dissolved in EtOAc, washed with H_2O, and extracted again with EtOAc. The combined organic layer was dried over Na_2SO_4, filtered and concentrated, affording compound **7** (3.2 g, 88%) as a colorless oil, pure enough to be used without further purification. Spectroscopical data are in agreement with the literature values.[23]

Synthesis of (3a*S*,6*R*,6a*S*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (8)



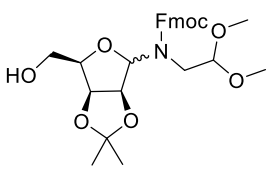
A suspension of D-lixose (2.5 g, 16.6 mmol) in acetone (30 mL) was cooled to 0 °C and treated 2,2-dimethoxypropane (2.6 mL, 21.2 mol) and *p*-toluenesulfonic acid (37 mg, 0.19 mmol). After being stirred at room temperature for 15 h, the clear resulting mixture was neutralized with $NaHCO_3$ and filtered over a pad of celite. The filtrate was concentrated under vacuum and the residue was dissolved in EtOAc, washed with H_2O, and extracted again with EtOAc. The combined organic layer was dried over Na_2SO_4, filtered and concentrated, affording compound **8** (3.3 g, 88%) as a white solid, pure enough to be used without further purification. Spectroscopical data are in agreement with the literature values.[24]

Synthesis of (3a*S*,6a*S*)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (9)



A suspension of L-arabinose (5 g, 33.3 mmol) in DMF (70 mL) was treated with 2,2-dimethoxypropane (12.5 mL, 101.6 mmol) and *p*-toluenesulfonic acid (115 mg, 0.3 mmol) and stirred under nitrogen for 90 minutes. The mixture was then neutralised with a Na_2CO_3 solution, evaporated and washed with hexane. $NaIO_4$ (18.0 g, 8.5 mmol) was added to the aqueous layer and the mixture was stirred for 2 h. Na_2CO_3 was added until basic pH was reached and the slurry mixture was stirred for another 1 h. The mixture was then extracted with EtOAc, dried over Na_2SO_4, filtered and concentrated, affording compound **9** (3.4 g, 65%) as a white solid, pure enough to be used without further purification. Spectroscopical data are in agreement with the literature values.[25]

Synthesis of (9*H*-fluoren-9-yl)methyl (2,2-dimethoxyethyl)((3a*R*,4*S*,6*R*,6a*R*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4yl)carbamate (10α) and (9*H*-fluoren-9-yl)-methyl-(2,2-dimethoxyethyl)((3a*R*,4*R*,6*R*,6a*R*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4yl)carbamate (10β)



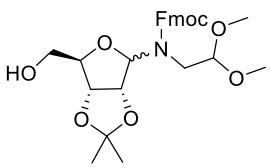
To a solution of 2,3-*O*-isopropylidene-D-ribofuranose **7** (800 mg, 4.20 mmol) and 2,2-dimethoxy-ethylamine (0.55 mL, 5.04 mmol) in MeOH (24 mL), $MgSO_4$ (1.010 g, 8.40 mmol) was added and the reaction mixture

was left stirring at reflux for 48 h. $MgSO_4$ was then removed by filtration through Celite and the filtrate was concentrated under vacuum to give the crude hemiaminal intermediate, unstable under silica gel column chromatography condition. The crude compound was then dissolved in dioxane (5 mL) and in a solution of $NaHCO_3$ (705 mg, 8.40 mmol) in water (10 mL). The mixture was cooled to 0 °C, then a solution of Fmoc-Cl (1.090 g, 8.40 mmol) in dioxane (5 mL) was added slowly and the resulting suspension was left reacting at room temperature for 24 hours under a nitrogen atmosphere, then it was diluted with EtOAc (30 mL). The organic phase was washed with 1M HCl solution, brine, and dried over anhydrous Na_2SO_4. After solvent evaporation, the crude oil was purified by flash chromatography (EtOAc/Petr. et. = 1 : 2; R_f **10β** = 0.31, R_f **10α** = 0.14), thus affording compound **10β** (418 mg, 0.84 mmol, 20%) and compound **10α** (796 mg, 1.59 mmol, 38%), both as colorless oils.

10α: $[\alpha]_D^{20}$ = -50.6 (*c* 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.68 (d, *J* = 7.4 Hz, 2H), 7.64-7.57 (m, 2H), 7.32 (pt, *J* = 7.3 Hz, 2H), 7.29 (pt, *J* = 7.4 Hz, 2H), 5.77 (br s, 0.5H), 5.20 (br s, 0.5H), 4.82-4.41 (m, 5H), 4.27 (pt, *J* = 5.2 Hz, 1H), 4.18 (br s, 0.5H), 3.85 (br s, 0.5H), 3.69-3.54 (m, 3H), 3.43–3.37 (m, 1H), 3.20 (s, 6H), 2.05 (br s, 1H, OH), 1.41–1.38 (m, 3H), 1.24-1.18 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: *δ* 156.0, 143.9 (2C), 141.3 (2C), 127.6 (2C), 127.1 (2C), 124.9 (2C), 119.8 (2C), 112.7, 103.4 e 102.5, 95.0, 86.2, 82.6, 80.0, 67.2 and 67.0, 63.2, 53.4 (2C), 47.2, 45.1 and 45.0, 26.0, 24.1. MS (ESI) *m/z* (%): 521.60 [$(M+Na)^+$, 100]. IR ($CDCl_3$): *ν* = 3608, 2940, 1701, 1452, 1384, 1215 cm^{-1}. Anal. Calcd. for $C_{27}H_{33}NO_8$: C, 64.92; H, 6.66; N, 2.80. Found: C, 65.22; H, 6.71; N, 2.63.

10β: $[\alpha]_D^{19}$ = -12.2 (*c* 1.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.75 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.38 (pt, *J* = 7.0 Hz, 2H), 7.31 (pt, *J* = 7.4 Hz, 2H), 4.79 (br s, 1.5H), 4.60 (d, *J* = 7.4 Hz, 2H), 4.38 (s, 0.5H), 4.21 (pt, *J* = 5.2 Hz, 1H), 4.02 (br s, 2H), 3.81 (d, *J* = 12.7 Hz, 1H), 3.66 (d, *J* = 7.4 Hz 1H), 3.45–3.08 (m, 4H), 3.23 (s, 6H), 1.51 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: *δ* 155.4, 143.6 (2C), 141.4 and 141.3 (2C), 127.8 (2C), 127.2 and 127.1 (2C), 124.8 (2C), 120.0 (2C), 112.5, 103.6, 96.5, 88.0, 85.5, 79.8, 67.8, 63.9, 56.9 and 55.2 (2C), 50.7, 47.2, 27.3, 25.4. MS (ESI) *m/z* (%): 521.88 [$(M+Na)^+$, 100]. IR ($CDCl_3$): *ν* = 3590, 2939, 1706, 1472, 1386, 1261 cm^{-1}. Anal. Calcd. for $C_{27}H_{33}NO_8$: C, 64.92; H, 6.66; N, 2.80. Found: C, 65.26; H, 6.74; N, 2.61.

(9*H*-fluoren-9-yl)methyl (2,2-dimethoxyethyl)((3a*S*,4*S*,6*R*,6a*S*)-6-(hydroxylmethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)carbamate (11α) and (9*H*-fluoren-9-yl)-methyl-(2,2-dimethoxyethyl)((3a*S*,4*R*,6*R*,6a*S*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4yl)carbamate (11β)

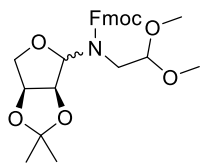


Compounds **11α**/**11β** were synthesized from 2,3-*O*-isopropylidene-D-lyxofuranose **8** (826 mg, 4.34 mmol) as reported for **10α**/**10β**. After solvent evaporation, the crude oil was purified by flash chromatography (EtOAc/Petr. et. = 1 : 1; R_f **11β** = 0.52; R_f **11α** = 0.17), thus affording compound **11β** (776 mg, 1.55 mmol, 31%) and compound **11α** (816 mg, 1.63 mmol, 32%), both as white foam.

11α: $[\alpha]_D^{21}$ = +50.4 (*c* 1.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers *δ* 7.68 (d, *J* = 7.2 Hz, 2H), 7.59-7.41 (m, 2H), 7.32 (pt, *J* = 7.2 Hz, 2H), 7.26-7.22 (m, 2H), 5.56 (br s, 0.5H), 5.31 (br s, 0.5H), 4.97 (br s, 0.5H), 4.70-4.37 (m, 4.5H), 4.19 (pt, *J* = 5.2 Hz, 1H), 3.90-3.63 (m, 2H), 3.46–3.40 (m, 1H), 3.38 (s, 6H), 3.25-3.19 (m, 2H), 2.15 (br s, 1H, OH), 1.36–1.13 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: *δ* 155.7, 143.9 (2C), 141.3 (2C), 127.8 and 127.7 (2C), 127.2 and 127.1 (2C), 125.0 and 124.6 (2C), 120.0 and 119.8 (2C), 112.7, 103.4 e 102.6, 86.8, 85.0, 79.9 and 79.5, 78.2, 68.1 and 67.5, 60.6, 52.5, 50.7, 47.2, 45.5, 25.5 and 25.1, 23.9 and 23.7. MS (ESI) *m*/*z* (%): 522.28 [$(M+Na)^+$, 100]. IR ($CDCl_3$): *ν* = 3075, 2939, 1706, 1261 cm^{-1}. Anal. Calcd. for $C_{27}H_{33}NO_8$: C, 64.92; H, 6.66; N, 2.80. Found: C, 65.23; H, 6.70; N, 2.64.

11β: $[\alpha]_D^{22}$ = +4.1 (*c* 1.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.76 (d, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.2 Hz, 2H), 7.40 (pt, *J* = 7.3 Hz, 2H), 7.33 (pt, *J* = 7.3 Hz, 2H), 5.06-4.80 (m, 4H), 4.60-4.45 (m, 3H), 4.21 (pt, *J* = 5.2 Hz, 1H), 4.05-4.02 (m, 1H), 3.90–3.78 (m, 3H), 3.26 (s, 6H), 2.06 (br s, 1H, OH), 1.46 (m, 3H), 1.30 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: *δ* 155.8, 143.6 (2C), 141.4 and 141.3 (2C), 127.8 (2C), 127.2 (2C), 124.7 (2C), 120.0 (2C), 112.4, 103.5, 96.4, 85.8, 83.6, 82.4, 66.7, 62.0, 55.2 (2C), 50.4, 47.3, 26.2 and 25.9, 24.3. MS (ESI) *m*/*z* (%): 522.28 [$(M+Na)^+$, 100]. IR (CDCl3): *ν* = 3567, 2901, 1703, 1448, 1354, 1216 cm^{-1}. Anal. Calcd. for $C_{27}H_{33}NO_8$: C, 64.92; H, 6.66; N, 2.80. Found: C, 65.26; H, 6.78; N, 2.60.

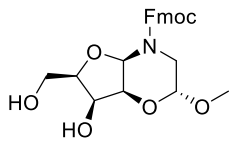
(9*H*-fluoren-9-yl)methyl (2,2-dimethoxyethyl)((3a*S*,4*S*,6a*S*)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)carbamate (12α)



Compound **12α** was obtained from 2,3-*O*-isopropylidene-L-erythrofuranose **9** (920 mg, 5.74 mmol) as a single product with the same procedure reported for **10α**/**10β**. After solvent evaporation, the crude oil was purified by flash chromatography (EtOAc/Petr. et. = 1:3; R_f **12α** = 0.29), thus affording the α-anomer **12α** (834 mg, 1.78 mmol, 32%) as a colourless oil. Anomer β was recovered only in traces (< 10 mg).

$[\alpha]_D^{22}$ = +72.9 (*c* 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.76 (d, *J* = 7.5 Hz, 2H), 7.69-7.58 (m, 2H), 7.40 (pt, *J* = 7.4 Hz, 2H), 7.34-7.29 (m, 2H), 5.32 (br s, 1H), 4.73-4.39 (m, 5H), 4.28 (t, *J* = 5.9 Hz, 1H), 4.10–3.99 (m, 1.5H), 3.81 (br s, 0.5H), 3.56 (br s, 2H), 3.30 (s, 6H), 1.47-1.24 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: *δ* 155.8, 144.0 and 143.9 (2C), 141.3 (2C), 127.7 and 127.6 (2C), 127.2 and 127.1 (2C), 124.9 (2C), 119.8 (2C), 112.4, 103.5, 87.8, 79.3, 78.8, 69.7, 67.2, 53.5 (2C), 47.2, 45.4, 25.8, 24.1. MS (ESI) *m/z* (%): 492.36 [$(M+Na)^+$, 100]. IR (CDCl3): *ν* = 2939, 1703, 1451, 1216 cm^{-1}. Anal. Calcd. for $C_{26}H_{31}NO_7$: C, 66.51; H, 6.65; N, 2.98. Found: C, 66.80; H, 6.71; N, 2.89.

(2*R*,4a*S*,6*R*,7*R*,7a*R*)-(9*H*-fluoren-9-yl)methyl 7-hydroxy-6-(hydroxymethyl)-2-methoxytetrahydro-2*H*-furo[3,2-*b*][1,4]oxazine-4(3*H*)-carboxylate (13)

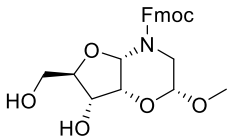


Compound **10α** (134 mg, 0.27 mmol) was dissolved in trifluoroacetic acid (1 mL) and MeOH (100 µL) and stirred at room temperature for 2 h. After TFA evaporation, the crude powder was purified by flash chromatography (EtOAc/Petr. et. = 1 : 1; R_f **13** = 0.40), thus affording compound **13** as a white foam (62 mg, 0.15 mmol, 55%).

$[\alpha]_D^{19}$ = +52.9 (*c* 0.09, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.69 (d, *J* = 7.5 Hz, 2H), 7.50 (pt, *J* = 6.9 Hz, 2H), 7.33 (pt, *J* = 7.5 Hz, 2H), 7.26-7.22 (m, 2H), 5.13 and 4.57 (s, 1H), 4.74 (d, *J* = 3.9 Hz, 1H), 4.77-4.74 and 4.62-4.57 (m, 1H), 4.41-4.36 (m, 1H), 4.20 (t, *J* = 7.4 Hz, 1H), 4.21-4.15 (m, 1H), 4.05-3.96 (m, 2H), 3.86-3.77 (m, 1H), 3.67-3.65 (br s, 1H), 3.59-3.46 (m, 1.5H), 3.38 and 3.32 (s, 3H), 3.41-3.27 (m, 0.5H), 2.31 (br s, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) mixture of rotamers: *δ* 155.4, 143.9 and 143.7 (2C), 141.4 (2C), 127.8 (2C), 127.1 (2C), 125.0 (2C), 120.0 (2C), 95.6 and 95.5, 78.8, 73.0, 69.0, 68.5 and 68.4,

67.7, 66.6, 55.0 and 54.9, 47.1, 42.4 and 42.3. MS (ESI) *m*/*z* (%): 449.49 [(M+Na)$^+$, 100]. IR ($CDCl_3$): *v* = 3608, 3157, 1474, 1382, 1216 cm^{-1}. Anal. Calcd. for $C_{23}H_{25}NO_7$: C, 64.63; H, 5.90; N, 3.28. Found: C, 64.80; H, 5.97; N, 3.21.

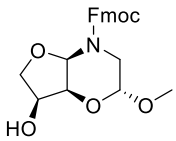
(2*R*,4a*R*,6*R*,7*S*,7a*S*)-(9*H*-fluoren-9-yl)methyl 7-hydroxy-6-(hydroxymethyl)-2-methoxytetrahydro-2*H*-furo[3,2-*b*][1,4]oxazine-4(3*H*)-carboxylate (14)



Compound **11β** (250 mg, 0.50 mmol) was dissolved in trifluoroacetic acid (1 mL) and MeOH (100 µL) and stirred at room temperature for 2 h. After TFA evaporation, the crude powder was purified by flash chromatography (EtOAc/Petr. et. = 1 : 1; R_f **14** = 0.43), thus affording compound **14** as a white foam (142 mg, 0.33 mmol, 66%). With the same procedure, compound **14** (153 mg, 0.36 mmol, 68%) was obtained also from the diastereomer **11α** (264 mg, 0.53 mmol).

$[\alpha]_D^{19}$ = -51.1 (*c* 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.70 (d, *J* = 7.5 Hz, 2H), 7.51 (pt, *J* = 6.6 Hz, 2H), 7.34 (pt, *J* = 7.3 Hz, 2H), 7.25 (pt, *J* = 6.9 Hz, 2H), 5.21 and 4.82 (s, 1H), 4.72 (d, *J* = 8.9 Hz, 1H), 4.59-4.56 (m, 1H), 4.42-4.13 (m, 2H), 3.99-3.76 (m, 3H), 3.54–3.39 (m, 2H), 3.38 and 3.32 (s, 3H), 3.26-3.19 (m, 1H), 3.02 (pt, *J* = 10.1 Hz, 1H), 2.24 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: *δ* 156.0, 143.8 (2C), 141.3 (2C), 127.8 (2C), 127.1, 125.0, 120.0, 95.0, 78.8, 73.9, 68.3, 67.7, 66.6, 66.0, 55.0, 47.1 and 46.7, 42.6 and 41.5. MS (ESI) *m*/*z* (%): 450.24 [(M+Na)$^+$, 100], 876.66 [(2M + Na)$^+$, 60]. IR ($CDCl_3$): *v* = 3610, 3155, 1472, 1382, 1216 cm^{-1}. Anal. Calcd. for $C_{23}H_{25}NO_7$: C, 64.63; H, 5.90; N, 3.28. Found: C, 64.87; H, 5.99; N, 3.22.

(2*R*,4a*R*,7*S*,7a*S*)-(9H-fluoren-9-yl)methyl 7-hydroxy-2-methoxytetrahydro-2H-furo[3,2-b][1,4]oxazine-4(3H)-carboxylate (15)

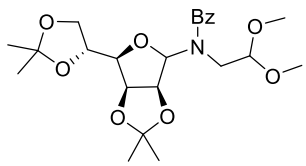


Compound **12α** (203 mg, 0.43 mmol) was dissolved in trifluoroacetic acid (1 mL) and MeOH (100 µL) and stirred at room temperature for 2 h. After TFA evaporation, the crude powder was purified by flash chromatography (EtOAc/Petr. et. = 1:1; R_f **15** = 0.32), thus affording compound **15** as a white foam (80 mg, 0.20 mmol, 47%).

$[\alpha]_D^{19}$ = -56.7 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.68 (d, *J* = 7.5 Hz, 2H), 7.52-7.50 (m, 2H), 7.34-7.21 (m, 4H), 5.70 and 5.60 (s, 1H), 4.76 (d, *J* = 8.9 Hz, 1H), 4.48-4.18 (m, 4H), 4.07-4.01 (m, 2H), 3.68 (pt, *J* =

14.6 Hz, 1H), 3.72 (dd, *J* = 16.6 Hz, 8.6 Hz, 2H), 3.40 and 3.32 (s, 3H), 3.28-3.21 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: *δ* 155.8, 143.8 and 143.6 (2C), 141.3 and 141.2 (2C), 127.7 (2C), 127.1 (2C), 125.2 and 125.0 (2C), 119.9 (2C), 95.3 and 94.9, 81.2 and 80.8, 71.8, 71.6, 68.2, 66.5 and 66.1, 55.3 and 54.5, 47.0, 42.2 and 41.7. MS (ESI) *m/z* (%): 420.35 [$(M+Na)^+$, 100]. IR ($CDCl_3$): *ν* = 3619, 3020, 1714, 1451, 1221 cm^{-1}. Anal. Calcd. for $C_{22}H_{23}NO_6$: C, 66.49; H, 5.83; N, 3.52. Found: C, 67.01; H, 5.90; N, 3.40.

_N_-(2,2-dimethoxyethyl)-_N_-((3a_S_,4_S_,6_R_,6a_S_)-6-((_R_)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[3,4-_d_][1,3]dioxol-4-yl)benzamide (17α) and _N_-(2,2-dimethoxyethyl)-_N_-((3a_S_,_4R_,6_R_,6a_S_)-6-((_R_)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[3,4-_d_][1,3]dioxol-4-yl)benzamide (17β)



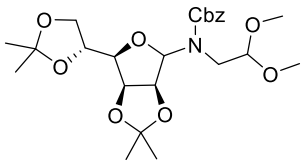
To the crude hemiaminal coupling intermediate, prepared from 2,3:5,6-*O*-di-isopropylidene-D-mannose **16** (60 mg, 0.23 mmol) as reported for **10α/10β**, and Et_3N (70 µL, 0.51 mmol) in dry THF (0.5 mL), a solution of benzoyl chloride (31 µL, 0.27 mmol) in dry THF (0.5 mL) was added dropwise at 0°C. The mixture was allowed to reach room temperature and was left stirring for two days under a nitrogen atmosphere. Successively the mixture was washed with a saturated solution of $NaHCO_3$, a solution of 1N HCl and brine. After solvent evaporation, the crude oil was purified by flash chromatography (EtOAc/Petr. et. = 1 : 1; R_f **17β** = 0.61, R_f **17α** = 0.39), thus affording compound **17β** (35 mg, 0.08 mmol, 33%) and compound **17α** (50 mg, 0.11 mmol, 49%), both as colorless oils.

17α: $[\alpha]_D^{23}$ = +63.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.48-7.38 (m, 5H), 4.71 (br s, 2H), 4.43 (q, *J* = 5.8 Hz, 1H), 4.13-4.09 (m, 3H), 3.66–3.61 (m, 3H), 3.43 (s, 1H), 3.28 (br s, 3H), 1.52 (s, 3H), 1.44 (s, 3H), 1.38 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: *δ* 172.1, 136.0 and 132.8, 131.2 and 129.6, 128.2 and 128.0 (2C), 126.8 and 126.6 (2C), 112.5, 108.9, 103.4 and 102.4, 87.2 and 79.4, 78.8, 78.2, 77.4, 72.7, 66.3, 54.3 and 53.6, 53.5 and 53.4, 47.2 and 41.2, 26.6, 25.4, 24.9, 23.7. MS (ESI) *m/z* (%): 474.20 [$(M+Na)^+$, 100], 924.59 [$(2M+Na)^+$, 16]. IR ($CDCl_3$): *ν* = 3019, 1626, 1521, 1423, 1216, 1046 cm^{-1}. Anal. Calcd. for $C_{23}H_{33}NO_8$: C, 61.18; H, 7.37; N, 3.10. Found: C, 61.43; H, 7.45; N, 3.00.

17β: $[\alpha]_D^{23}$ = +8.0 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.41-7.36 (m, 5H), 5.20 (d, *J* = 7.0 Hz, 2H), 5.01 (br s, 1H), 4.54 (br s, 2H), 4.32

(q, *J* = 5.9 Hz, 1H), 4.06 (dd, *J* = 8.1, 6.3 Hz, 1H), 3.99 (dd, *J* = 8.1, 5.2 Hz, 1H), 3.57 (dd, *J* = 14.6, 5.6, 1H), 3.38 (s, 3H), 3.31 (s, 3H), 3.38-3.22 (m, 1H), 1.43 (s, 6H), 1.36 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) major rotamer: *δ* 168.6, 135.8, 130.1, 128.4 (2C), 127.4 (2C), 108.9, 103.8, 95.0, 85.7, 85.1, 84.9, 81.5, 74.0, 66.5, 56.1 (2C), 48.4, 26.9 (2C), 26.2, 25.2. MS (ESI) *m*/*z* (%): 474.19 [$(M+Na)^+$, 100]. IR ($CDCl_3$): *ν* = 3016, 1625, 1520, 1427, 1220, 1041 cm^{-1}. Anal. Calcd. for $C_{23}H_{33}NO_8$: C, 61.18; H, 7.37; N, 3.10. Found: C, 61.46; H, 7.49; N, 2.98.

Benzyl (2,2-dimethoxyethyl)((3a*S*,4*R*,6*R*,6a*S*)-6-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)carbamate (18α) and Benzyl (2,2-dimethoxyethyl)((3a*S*,4*S*,6*R*,6a*S*)-6-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)carbamate (18β)

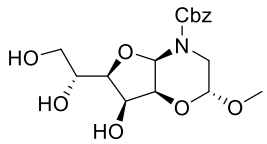


The crude hemiaminal coupling intermediate, prepared from 2,3:5,6-*O*-di-isopropylidene-D-mannose **16** (107 mg, 0.41 mmol) as reported for **10α**/**10β**, and $NaHCO_3$ (70 mg, 0.82 mmol) were dissolved in a mixture of H_2O (4 mL) and EtOAc (2 mL), then Cbz-Cl (68 µL, 0.48 mmol) in EtOAc (2 mL) was added dropwise at 0 °C. The mixture was allowed to reach room temperature and was left stirring overnight under a nitrogen atmosphere. Successively, the mixture was washed with aqueous 1N HCl and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. After solvent evaporation, the crude oil was purified by flash chromatography (EtOAc/Petr. et. = 1 : 3; R_f **18β** = 0.55, R_f **18α** = 0.42), thus affording compound **18β** (43 mg, 0.09 mmol, 22%) and compound **18α** (99 mg, 0.21 mmol, 50%), both as colorless oils.

18α: $[\alpha]_D^{23}$ = +45.9 (*c* 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.35 (s, 5H), 5.11 (br s, 5H), 4.69 (s, 0.5H), 4.42-4.31 (m, 2.5H), 4.07-4.00 (m, 1H), 3.89 (br s, 0.5H), 3.80-3.78 (m, 0.5H), 3.69 (br s, 1H), 3.36-3.33 (m, 7H), 1.49-1.43 (m, 6H), 1.36-1.32 (m, 6H). ^{13}C NMR (50 MHz, $CDCl_3$) mixture of rotamers: *δ* 155.7, 135.9, 129.0 and 128.9, 128.8 and 128.6 (2C), 128.2 and 128.1 (2C), 112.6 and 112.4, 108.9, 103.8 and 103.7, 96.7 and 96.6, 85.8, 84.3, 82.1 and 81.5, 74.0 and 71.2, 67.9 and 66.6, 64.6, 55.1 and 55.0 (2C), 50.6, 26.8, 26.2, 25.3, 24.4. MS (ESI) *m*/*z* (%): 504.08 [$(M+Na)^+$, 100]. IR ($CDCl_3$): *ν* = 3453, 2940, 1709, 1383, 1211 cm^{-1}. Anal. Calcd. for $C_{24}H_{35}NO_9$: C, 59.86; H, 7.33; N, 2.91. Found: C, 60.12; H, 7.41; N, 2.84.

18β: $[\alpha]_D^{23}$ = +8.0 (*c* 0.8, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.31-7.29 (m, 5H), 5.61 and 5.45 (s, 1H), 5.14-5.07 (m, 2H), 4.71-4.64 (m, 2H), 4.39-4.29 (m, 2.5H), 4.06-3.78 (m, 3H), 3.61-3.42 (m, 1.5H), 3.30-3.18 (m, 3H), 3.23 (s, 3H), 1.43-1.21 (m, 12H). ^{13}C NMR (50 MHz, $CDCl_3$) mixture of rotamers: *δ* 155.2, 135.7, 130.5, 128.6 and 128.4 (2C), 128.1 and 127.2 (2C), 113.3, 109.3 and 108.4, 85.5 and 85.4, 79.9, 79.7 and 79.6, 78.4, 72.9, 69.9 and 69.8, 68.2, 66.9 and 66.8, 64.3, 52.7 and 52.6, 52.5, 30.8 and 29.7, 28.4, 26.9, 26.3 and 25.1. MS (ESI) *m/z* (%): 504.08 [$(M+Na)^+$, 100]. IR ($CDCl_3$): *v* = 3456, 2985, 1703, 1261 cm^{-1}. Anal. Calcd. for $C_{24}H_{35}NO_9$: C, 59.86; H, 7.33; N, 2.91. Found: C, 60.16; H, 7.44; N, 2.81.

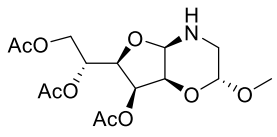
(2*R*,4a*R*,6*R*,7*S*,7a*S*)-benzyl 6-((*R*)-1,2-dihydroxyethyl)-7-hydroxy-2-methoxy tetrahydro-2*H*-furo[3,2-*b*][1,4]oxazine-4(3*H*)-carboxylate (19)



Compound **18α** (40 mg, 0.08 mmol) was dissolved in trifluoroacetic acid (1 mL) and stirred at room temperature for 2 h. After TFA evaporation, the crude powder was purified by flash chromatography (EtOAc, R_f **19** = 0.19), thus affording compound **19** as white foam (19 mg, 0.05 mmol, 65%).

$[\alpha]_D^{23}$ = -51.6 (*c* 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) mixture of rotamers: *δ* 7.28 (s, 5H), 5.29 and 5.16 (s, 1H), 5.15-5.08 (m, 2H), 4.73 and 4.64 (s, 1H), 4.10 (s, 1H), 3.82-3.58 (m, 5H), 3.47-3.44 (m, 1H), 3.32 (s, 3H), 3.33-3.26 (m, 1.5H), 3.10-3.07 (m, 0.5H), 2.43 br s, 3H, OH). ^{13}C NMR (100 MHz, $CDCl_3$) mixture of rotamers: *δ* 155.5, 136.0, 128.6 (2C), 128.3 (2C), 128.0, 96.0 and 95.5, 73.6, 69.9, 68.9 (2C), 67.9, 66.6 and 66.2, 62.7 and 62.6, 54.9, 42.7 and 42.6. MS (ESI) *m/z* (%): 392.05 [$(M+Na)^+$, 100]. IR ($CDCl_3$): *v* = 3628, 3618, 3020, 1793, 1472, 1384, 1216 cm^{-1}. Anal. Calcd. for $C_{17}H_{23}NO_8$: C, 55.28; H, 6.28; N, 3.79. Found: C, 55.98; H, 6.40; N, 3.70.

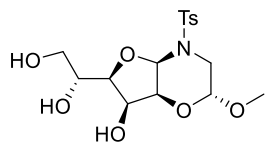
(*R*)-1-((2*R*,4a*R*,6*R*,7*S*,7a*S*)-7-acetoxy-2-methoxyhexahydro-2*H*-furo[3,2-b][1,4] oxazin-6-yl)ethane-1,2-diyl diacetate (21)



Compound **20** (30 mg, 0.05 mmol) was treated with 30% solution in CH_3CN (1 mL). The Fmoc deprotection was monitored by TLC. When complete conversion was obtained, volatiles were removed under reduced pressure and the residue was purified by flash chromatography (AcOEt/Petr. et.

= 1:2, buffered with 1% Et_3N; R_f **21** = 0.14) affording pure compound **21** (15 mg, 0.047 mmol, 85%) as a yellow oil.
$[\alpha]_D^{23}$ = -42.8 (*c* 0.4, $CHCl_3$); ^{1}H-NMR (200 MHz, $CDCl_3$): δ 5.30 (t, *J* = 9.9 Hz, 1H), 4.99 (dd, *J* = 9.9, 3.7 Hz, 1H), 4.72 (d, *J* = 2.2 Hz, 1H), 4.40 (s, 1H), 4.36 (d, *J* = 10.9, 1H), 4.20-4.18 (m, 2H), 3.64-3.61 (m, 2H), 3.40 (dd, *J* = 11.8, 2.9 Hz, 1H), 3.37 (s, 3H), 2.09 (s, 6H), 2.05 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.7, 170.1, 169.6, 96.5, 81.4, 73.1, 72.7, 66.1, 65.4, 63.0, 54.8, 41.9, 20.7 (3C). MS (ESI) *m/z* (%): 384.10 [$(M+Na)^+$, 100]. Anal. Calcd. for $C_{15}H_{23}NO_9$: C, 49.86; H, 6.42; N, 3.88. Found: C, 49.97; H, 6.49; N, 3.79.

(*R*)-1-((2*R*,4a*R*,6*R*,7*S*,7a*S*)-7-hydroxy-2-methoxy-4-tosylhexahydro-2*H*-furo[3,2-*b*][1,4]oxazin-6-yl)ethane-1,2-diol (22)



To a solution of **21** (45 mg, 0.14 mmol) and DIPEA (50 µL, 0.28 mmol) in dry CH_2Cl_2 (2 mL), a solution of TsCl (54 mg, 0.09 mmol) in dry CH_2Cl_2 (2 mL) was added slowly at 0 °C. The mixture was allowed to reach room temperature and was left stirring under a nitrogen atmosphere for 24 hours. Then, water was added slowly and the resulting mixture was washed with a saturated solution of $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude compound was left stirring in MeOH (2 mL) in presence of a catalitic amount of Ambersep 900 OH at room temperature for 1 hour. The resin was then filtered and, after solvent evaporation, the crude mixture was purified by flash chromatography (EtOAc/Petr. et. = 1 : 1; R_f **22** = 0.19) thus affording pure compound **22** (23 mg, 0.06 mmol, 41%) as a white foam.
$[\alpha]_D^{19}$ = -41.7 (*c* 0.6, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.71 (d, *J* = 8.2 Hz, 2H), 7.25 (d, *J* = 8.2 Hz, 2H), 5.15 (s, 1H), 4.75 (s, 1H), 4.21 (d, *J* = 4.5 Hz, 1H), 3.72 (dd, *J* = 11.8, 3.5 Hz, 1H), 3.61 (dd, *J* = 9.7, 3.4 Hz, 1H), 3.56-3.50 (m, 3H), 3.39 (s, 3H), 3.31-3.27 (m, 1H), 3.08 (dd, *J* = 11.8, 2.6 Hz, 1H), 2.41 (s, 3H), 1.99 (br s, 3H, OH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 143.2 (2C), 134.7 (2C), 128.4 (2C), 127.0 (2C), 94.4, 78.1, 75.3, 72.5, 67.7, 66.4, 61.6, 54.1, 42.2, 28.7. MS (ESI) *m/z* (%): 412.23 [$(M+Na)^+$, 100]. IR ($CDCl_3$): *v* = 3618, 3054, 1472, 1354, 1218, 1174 cm^{-1}. Anal. Calcd. for $C_{16}H_{23}NO_8S$: C, 49.35; H, 5.95; N, 3.60. Found: C, 49.91; H, 6.04; N, 3.48.

5.4.2 Biological studies

Cell culture

Human MDA-MB-231 breast cancer cell lines are a model for aggressive, hormone-independent breast cancer. Cells were obtained from the American Tissue Culture Collection (Manassas, VA, USA) and maintained in DMEM (from Life Technologies) containing 10% heat inactivated fetal calf serum (FCS, from Life Technologies) at 37 °C in a humidified atmosphere of 5% CO_2 /95% air. Cells were harvested from subconfluent cultures by incubation with a trypsin–EDTA solution, and propagated every 3 days at a ratio between 1:4 and 1:8.

Cell growth assay

MDA-MB-231 cells were plated at 5×10^4 cells/mL in 24-well plates under standard culture conditions. After 4h adhesion, cells were treated with either vehicle alone or different compounds. After 24h or 48h of treatments, cells were trypsinized, collected, and counted using a hemocytometer.

Cell viability

Cell viability assay was performed using the WST-1 assay, a tetrazolium salt analog to MTT or XTT. Briefly, MDA-MB-231 cells were plated in in triplicate in a 96-well at 10-15x10^3 cells/ml density. After 4h adhesion, cells were treated with different concentration of compound **1**. Following 24h or 48h incubation, cells were exposed to 10 µL of the reagent directly to the cell cultures (200 µL final volume). The plates were incubated for 120 minutes at 37 °C in a humidified 5% CO_2 environment. The WST-1 formazan product was measured at 460 nm (reference wavelength of 630 nm) with an ELx800 Universal Microplate Reader from Bio-Tek Instruments Inc.

Cytofluorimetric assay for the apoptosis evaluation

MDA-MB231 cells were plated at 5×10^5 cells/mL in 6-well plates under standard culture conditions. After 4h adhesion, cells were treated with different concentration of compound **1.** At the end of the treatment, apoptotic cell death was measured by staining trypsinized cells with annexin-V-fluorescein isothiocyanate (FITC), according to manufacturer's instructions (annexin V/FITC kit; Bender MedSystem, Wien, Austria) and read by FACScan flow cytometer (Becton Dickinson). Dot plots of annexin/propidium iodide (PI) were derived

and analyzed for regions of early apoptosis (PI− and annexin+ cells), apoptosis (annexin+ and PI+ cells), and necrosis (annexin− and PI+ cells).

Cytofluorimetric cell cycle analysis

MDA-MB231 cells were plated at 5×10^5 cells/mL in 6-well plates under standard culture conditions. After 4h adhesion, cells were treated with different concentration of compound **1.** After 48h cells were harvested by trypsinization, and then permeabilized with 70% ice-cold ethanol on ice for 30 min. Cells were then washed and incubated in staining buffer with 50 µg/ml propidium iodide (PI), 10 µg/ml RNase A and 0.1% Triton X-100 for 30 min in the dark. Subsequently, the cell cycle was analyzed by flow cytometry (FACSCan; BD Biosciences).

Data analysis

The results are expressed as mean ± standard deviation (SD). Differences in growth rates between groups were analyzed using the two-tailed T-Test, statistical significance at *p*-values <0.05 were presented using respective symbols in the figure legends.

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Part III

Synthesis of *N*-containing Heterocycles and Building Blocks

6

Synthesis of Dihydropyrazinones by Morpholine Acetal Rearrengement

6.1 Introduction

The interest of nitrogen-containing building blocks for the development of novel biologically active scaffolds, has been widely discussed in the introduction (Chapter 2.3). In this context, amino acids and derivatives, thanks to the intrinsic chemical diversity of the side chains and their synthetic versatility, play a key role in the generation of heterocyclic scaffolds with peptidomimetic applications.[1] These structures, developed initially for their property of preventing degradation and improving oral bioavibility of peptide-based drugs, have been receiving new interests as tools for probing protein-protein interactions in chemical biology studies. For this reason, in view of expanding the chemical diversity of the central scaffold, several Diversity-Oriented Synthesis of peptidomimetic compounds have been reported in the last years. In this field, our group has been involved in the exploitation of amino acid derivativs, and particurlarly morpholine-3-carboxylic acids, for the generation of skeletally different nitrogen-containing heterocycles.[2]

Morpholine represent in fact a common motif in medicinal chemistry,[3] being found in several natural products, such as in the alkaloid chelonin A, isolated from *Chelonaplysilla sponge* and possessing antimicrobial activity,[4] and in the spiroalkaloid acortatarin A isolated from the rhizome of *Acorus tatarinowii*.[5]

(Figure 1, top). Also, the morpholine ring is contained in many bioactive molecules and drugs, such as the antidepressant Moclobemide,[6] and the antitumoral Gefitinib (Figure 1, bottom),[7] thus demonstrating the high interest in the biomedical field towards this heterocycle.

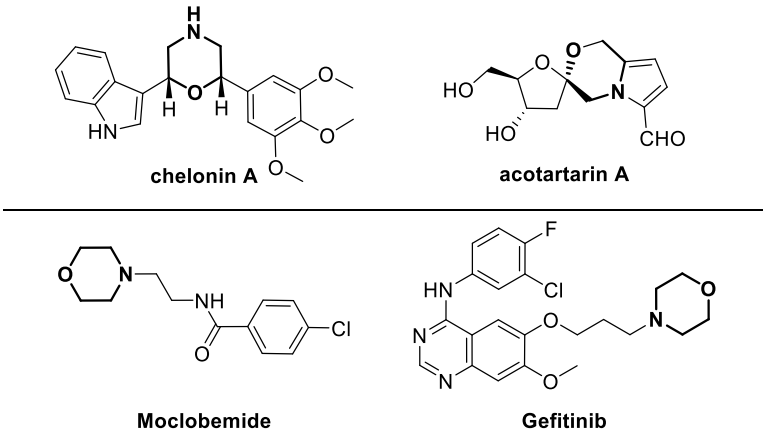
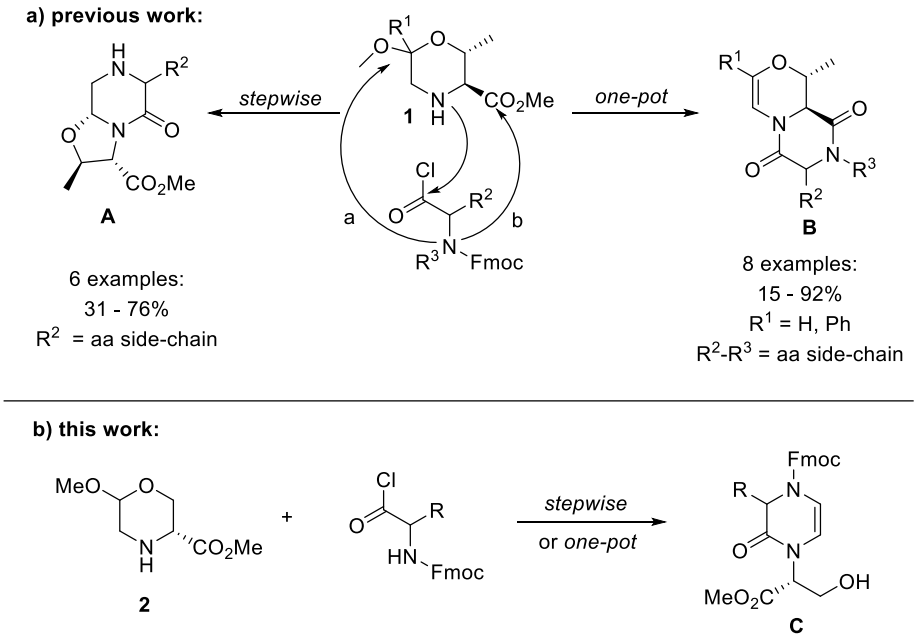


Figure 1. (**Top**) Examples of natural products possessing a morpholine ring. (**Bottom**) Examples of drugs containing a morpholine ring.

Moreover, morpholine acetals possess a characteristic reactivity that can be exploited for the generation of new molecular scaffolds. As shown in scheme 1a, starting from threonine-derived morpholines **1** we reported the achievement of bicyclic 2-oxopiperazines **A** and bicyclic diketopiperazines **B,** by tuning a three-step process, consisting in a coupling of an amino acid, followed by acid-mediated cyclization and Fmoc-deprotection, depending on the reaction mode, from stepwise to sequential one-pot.[8] This process was applied to several amino acids, thus achieving a small library of compounds possessing interesting biochemical features, given that 2,5-diketopiperazine nucleus[9] and 2-oxopiperazine core[10] are considered privileged scaffolds in medicinal chemistry, too. The application of this library to a phenotypic screening on *S. Cerevisiae* wild-type allowed for the discovery of two hit compounds able to induce a significant decrease in yeast cell growth. Also, the use of mutant strains, in a forward chemical genetic approach, resulted in the identification of these compounds as probes for understanding mitochondria metabolism and respiration events.[11]



Scheme 1: (**a**) Skeletal diversity by sequential stepwise or one-pot routes starting from morpholine ester building block **1**, leading to the achievement of bicyclic 2-oxopiperazines **A** and bicyclic diketopiperazines **B**. (**b**) Achievement of dihydropyrazinone scaffold from serine-derived morpholine acetal **2.**

Starting from this result, we envisioned to apply the same process starting from serine-derived morpholine acetal **2**, to explore if serine might serve as a different player in these processes. Interestingly, we found that the major compound obtained, from both the stepwise and the one-pot method, was a novel heterocycle, the uncommon dihydropirazinone scaffold **C**, even if as a mixture with the oxopiperazine compound **B**. The achievement of this heterocycle worths process optimization, considering that this heterocycle can act as a dipeptide isostere and its synthesis is reported in very few papers.

In fact, dipeptide isosteres[12] find special interest in the development of peptidomimetic compounds. A major contribution to this field has been made by the work of Freidinger, who conceived the idea of utilizing dipeptide lactams as conformational constraints in peptides to restrict the peptide bond to the *trans* conformation and to limit the backbone dihedral angles.[13] Results from the research groups of Freidinger and others indicated that dipeptide lactams are useful for potency enhancement, greater receptor selectivity and in increasing the stability towards protease degradation.[14] As a noteworthy

example, Thorsett pioneered the application of dipeptide lactams in the context of angiotensin converting enzyme inhibitors.[15] Following this concept, diverse molecules, such as lactams, piperazinones, pyrrolidinones and imidazolinones have been employed as molecular scaffolds capable of constraining the conformation around a dipeptide unit and of improving the stability of the central peptide bond by including it in the lactam moiety (Figure 2).

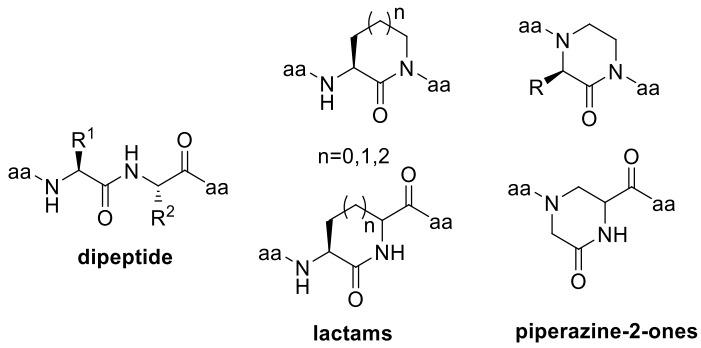


Figure 2: Representative molecular scaffolds used as constrained dipeptide isoster.

Among all these heterocyclic motifs, the piperazine-2-one scaffold has been widely exploited as a peptidomimetic scaffold,[16] taking advantage of many synthetic routes for heterocycle formation[17] and its isosteric capability of acting as a locked *N*-*N* moiety in contiguous amino acids. For example, Tian and collaborators developed a series of potent and selective 1,3,4-trisubstituted-piperazine-2-one-based melanocortin-4 receptor (MC4R) agonists possessing a D-Phe-Arg-2-Nal-$NHCH_3$ tripeptide fragment.[18]

Despite widespread application of piperazine-2-one scaffold in peptidomimetic chemistry, the corresponding unsaturated dihydropyrazinone compounds were reported in very few papers. Neverthless, this heterocyclic structure has been already applied in the design and synthesis of serine protease Factor Xa inhibitor,[19] and it is present in the GG-Tasa-I inhibitor with anticancer activity (Figure 3, left).[20]

Moreover, the specific dihydropyrazinone scaffold **C** obtained from the rearrangement of the morpholine acetal is acting as an isostere of the Xaa-Ser sequence (Figure 3, right). This sequence is particularly relevant in medicinal chemistry, because it is contained in various inhibitors and biologically relevant molecules. For examples, Galanda and coworkers recently reported on the

importance of Ser-Pro dipeptide sequence,[21] which is frequently found in gene regulatory and DNA-binding proteins,[22] and has the ability to induce a type I β-turn in proteins. Also, such dipeptide sequence is recognized as a substrate by several kinases and forms a preferred site for protein phosphorylation.[23]

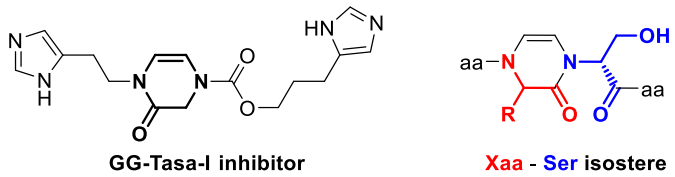
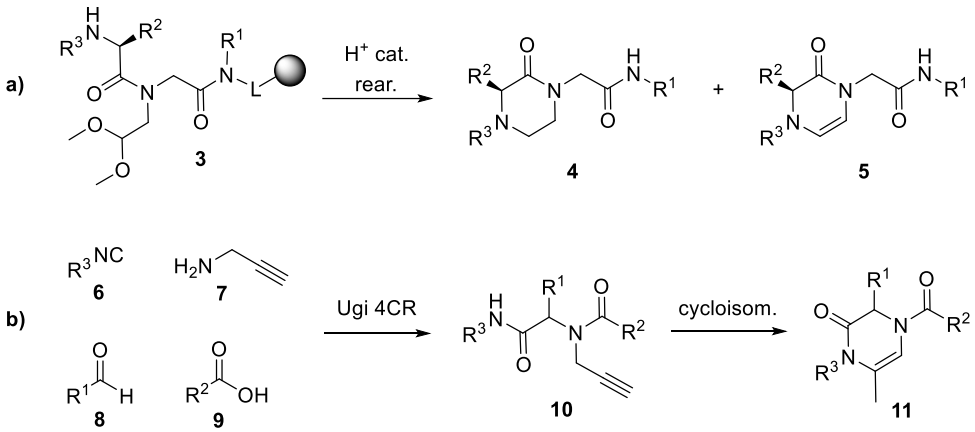


Figure 3: (**left**) Dihydropyrazinonic structure in GG-Tasa-I inhibitor; (**right**) dihydropirazinonic scaffold as Xaa-Ser dipeptide isostere.

Despite these features, the synthesis of these compounds is reported in very few papers, which mainly exploit *N*-acyliminium ion chemistry,[24] or the Ugi multi-component reaction.[25] In scheme 2a is reported, as representative example, the one-step synthesis of trisubstituted piperazinones **4** and dihydropyrazinones **5** from polymer-supported acyclic precursors **3** through the acid-mediated unmasking of the aldehyde and cyclic iminium formation.[26]



Scheme 2: (**a**) Synthesis of trisubstituted piperazinones **4** and dihydropyrazinones **5** coming from *N*-acyliminium ion chemistry reported by Krchňák and coworkers;[26] (**b**) synthesis of 6-methyl-3,4-dihydropyrazinones **11** using a Ugi 4 component reaction reported by Miranda and coworkers.[27]

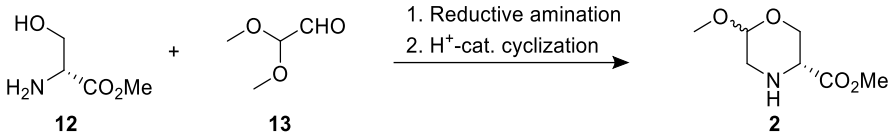
In scheme 2b is reported, on the other hand, the synthesis of 6-methyl-3,4-dihydropyrazinones **11** using a Ugi 4-CR/allenamide cycloisomerization process

starting from different isocyanides **6**, propargylamine **7**, aliphatic aldehydes **8** and carboxylic acids **9**.[27]

For these reasons, the synthesis of this uncommon dihydropyrazinonic heterocycles through the serine-derived morpholine acetal rearrangement has been studied and optimized. Finally, chemioinformatic analyses have been used to assess the chemical diversity and the distribution in the chemical space of these dihydropyrazinonic compounds, as compared to the diketopiperazines and 2-oxopiperazines previously obtained from the threonine-derived morpholine acetal **1**.

6.2 Results and Discussion[28]

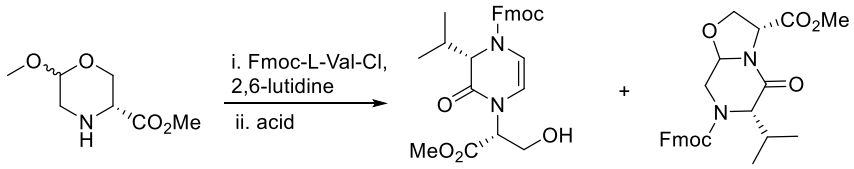
Serine-derived morpholine **2** was easily prepared in multigram scale from D-serine **12** and glyoxal dimethylacetal **13**, following a reported procedure based on reductive amination and *trans*-acetalization (Scheme 3).[29]



Scheme 3: Synthesis of morpholine acetal **2**.

Then, a two-step process consisting of a coupling reaction with a Fmoc-aa-chloride, followed by acid catalyzed *trans*-acetalization reaction, has been applied starting from morpholine acetal **2**. Fmoc-L-Val-Cl was chosen as a model coupling agent and the process was studied, as a one-pot method, by changing the reaction conditions in both coupling and cyclization processes in order to improve the selectivity towards dihydropyrazinone **14** with respect to the bicyclic compound **15** (Table 1).

In details, the coupling of the Fmoc-amino acid residue to the morpholine acetal was achieved by using 2,6-lutidine as a base, as described by Carpino,[30] because previous studies demonstrated that the use of other standard COOH activators was less efficient.



2 14 15

Entry	Conditions[a]	i. Coupling time; temp.	ii. Cyclization time	Yield 14 (%)	Yield 15 (%)
1	pTsOH, 5 eq., toluene	2h, 60 °C	2h	10	27
2	pTsOH, 10 eq., toluene	2h, 60 °C	2h	13	19
3	pTsOH, 10 eq., benzene	2h, 60 °C	2h	5	13
4	Camphorsulfonic acid, 10 eq., toluene	2h, 60 °C	2h	26	50
5	CH_3SO_3H, 10 eq., toluene	2h, 60 °C	2h	39	13
6	CH_3SO_3H, 10 eq., toluene	4h, 25 °C	2h	55	5
7	CH_3SO_3H, 10 eq., toluene	4h, 25 °C	4h	40	10
8	CH_3SO_3H, 10 eq., toluene	4h, 25 °C	15 min[b]	52	3
9	CH_3SO_3H, 10 eq., acetonitrile	4h, 25 °C	15 min[b]	35	8
10	CH_3SO_3H, 10 eq., dichlorobenzene	4h, 25 °C	15 min[b]	34	5

Table 1. Study of the reaction conditions to optimize the conversion to the dihydropyrazinone **14**. (a) The coupling stage was carried out in dichloromethane (2.5 mL/mmol) with 2,6-lutidine (2 eq.). Then the indicated acids and solvents (in order to form a 4:1 solvent/dichloromethane mixture) were added to perform the cyclization/ring rearrangement process; (b) the reaction was conducted under microwave irradiation.

Then, different reaction conditions were screened for the cyclization step, using different acids and different high boiling point solvents, in presence of 4 Å molecular sieves, in order to favour the azeotropical elimination of methanol and the formation of the intermediate 1,4-dihydroxazine ring. The application of *p*-toluenesulfonic acid (pTsOH) proved unsuccessful in the overall yield of **14** and in the selectivity ratio with respect to bicyclic compound **15** (Table 1, entries 1-3), irrespective of solvent replacement in the cyclization process (entry 3) or equivalent amounts of the acid (entry 2). The use of

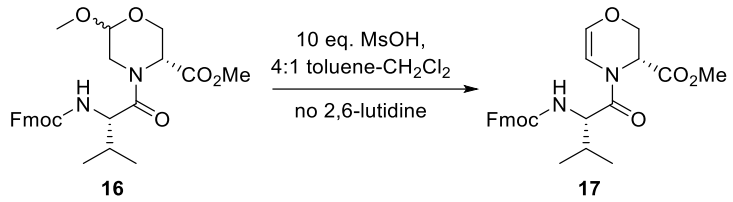
camphorsulfonic acid showed a slight increase in the yield, although the selectivity ratio was found still in favour of **15** (entry 4). The choice of methanesulfonic acid in the cyclization step proved beneficial to reverse the selectivity towards dihydropyrazinone **14** (entry 5), whereas prolonging the cyclization time proved to reduce the overall yield, possibly due to the generation of unidentified degradation products (entry 7). The optimization of the coupling reaction time (from 2 to 4 h) and temperature (lowering from 60 to 25 °C) gave better results in terms of yield and selectivity of **14** (entry 6). Finally, the cyclization process was also tried under microwave irradiation conditions, resulting in similar optimal yield and selectivity as found with conventional heating when carried out in toluene (entry 8), and with lower performance when acetonitrile and dichlorobenzene were used for the ring rearrangement process (entries 9 and 10, respectively).

Overall, the optimal conditions were found as those indicated in Table 1, entry 6, consisting of the coupling reaction in dichloromethane in the presence of 2 equivalents of 2,6-lutidine, followed by solvent evaporation and addition of 10 equivalents of methansulfonic acid in a 4:1 mixture of toluene and dichloromethane.

The stepwise process was also studied to compare the achievement of the desired dihydropyrazinone **14** with respect to the one-pot route. Thus, the coupling adduct **16** (Scheme 4) resulting from **2** and Fmoc-L-Val-Cl was isolated with sufficient purity (checked by NMR) through acid-base work-up, and used for the cyclization/ring rearrangement process under four different reaction conditions.

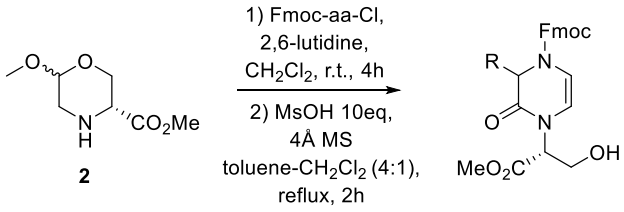
The application of catalytic quantities of methanesulfonic acid (0.1 eq) under refluxing toluene (2h) resulted in the recovery of unaltered starting material. Similar result was achieved when excess of the acid was applied (10 eq) and using only toluene as solvent. When 2,6-lutidine (2 eq) was added in the mixture containing excess CH_3SO_3H, about 10% of the desired product was observed together with the starting material. The application of a 4:1 toluene-dichloromethane mixture as the solvent resulted in similar outcome as for the one-pot process, demonstrating the importance of both solvents in guaranteeing sufficient solubility of the starting material and optimal rearrangement process. The repetition of this experiment in absence of 2,6-lutidine resulted in the dihydroxazine scaffold **17** (Scheme 4), as a consequence of methoxy group elimination from the morpholine acetal moiety of **16**. Thus,

both the solvent mixture and the base were found to be crucial in promoting acid-mediated ring rearrangement processes to give the corresponding dihydropyrazinone scaffold.



Scheme 4: Effect of the absence of 2,6-lutidine resulting to the synthesis of dihydroxazine **17** through stepwise process from **16**.

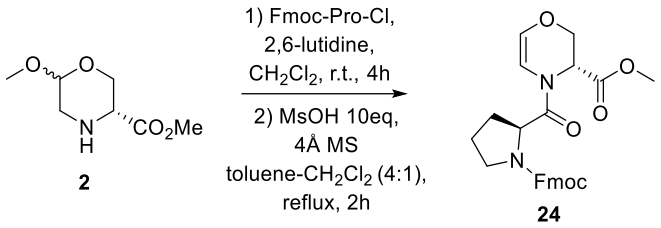
With optimized reaction conditions established, the scope of the synthetic process was studied by varying the amino acids and their stereochemistry in the coupling stage (Table 2). The one-pot process under optimized conditions using different Fmoc-amino acid chlorides as the coupling agents resulted in moderate yields in all cases with similar selectivity ratios observed for valine (Table 1, entry 6, results not shown for Table 2).



Compound	α-amino acid derivative	R	Yield (%)
14	L-Val	$(CH_3)_2CH$	55
18	D-Val	$(CH_3)_2CH$	45
19	L-Phe	$PhCH_2$	32
20	D-Phe	$PhCH_2$	40
21	L-Leu	$(CH_3)_2CH_2CH$	32
22	L-Ala	CH_3	40
23	L-Ile	$CH_3CH_2(CH_3)CH$	29

Table 2. Scope of the one-pot process with α-amino acid chlorides.

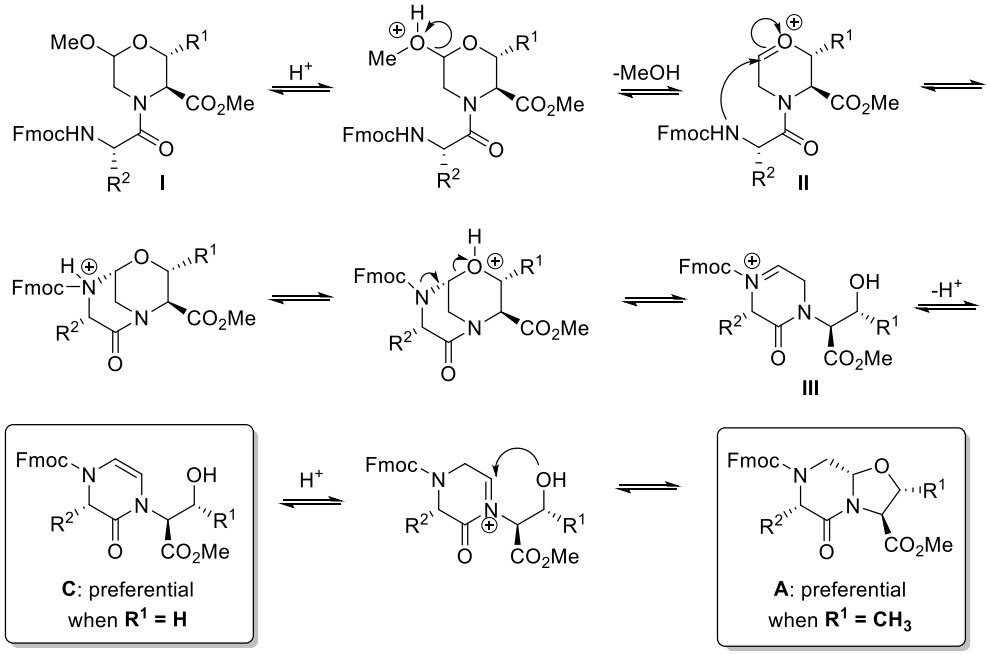
Amino acid containing sensitive functional groups, such as serine or methionine, failed to give the corresponding dihydropiperazinonic compound, as previously observed in the generation of diketopiperazines and 2-oxopiperazines.[8] Interestingly, using the secondary amino acid Fmoc-Pro-Cl, dihydroxazine **24** was obtained, instead of the desired dihydropyrazinone (Scheme 5).



2 24

Scheme 5: Synthesis of dihydrooxazinic compound **24** with Fmoc-Pro-Cl.

This result validated the proposed mechanism for the ring rearrangement, which requires a nucleophilic nitrogen atom of the Fmoc-amino acid component (Scheme 6).



Scheme 6: Hypothesized mechanism for the ring rearrangement and the preferential formation of dihydropyrazinone **C** or oxopiperazine **A.**

Specifically, during the second step of the process, adduct **I**, after protonation, is thought to undergo an intramolecular attack by the protected nitrogen atom to the oxonium moiety **II**, followed by ring-opening of the morpholine ring to the iminium **III**. This species isomerizes by a deprotonation-protonation process to the final dihydropyrazinone **C**. The different reactivity of the serine-derived morpholine acetal as compared to the threonine one could be related to the presence of the methyl group which seems to assist the hydroxyl group in the cyclization to yield the oxopiperazine **A** preferentially.

Structural investigation by X-ray crystallographic analysis of compound **18** deriving from Fmoc-D-Val (Figure 4)[31] confirmed the hypothesized structure coming from ring rearrangement of the aminoacyl-morpholine adduct, and showed interesting features of the dihydropyrazinone coming from D-valine. In particular, isopropyl side-chain was found in pseudoaxial position with respect of the flat scaffold, suggesting a potential exploitation of this scaffold as a tridimensional molecular framework for addressing interactions with protein target in the three dimensions.

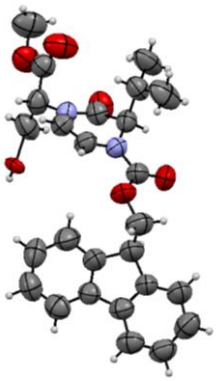
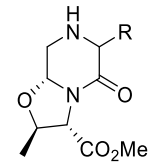


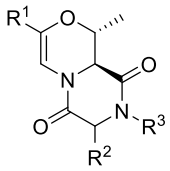
Figure 4: X-ray crystallographic structure of D-Val-derived dihydropyrazinone **3**.

Chemioinformatic analysis

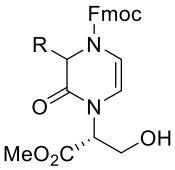
Finally, to assess the chemical and structural diversity of these novel dihydropyrazinonic scaffold **C**, as compared to the 2-oxopiperazines **A** and diketopiperazines **B** previously obtained from morpholine acetal **1** (see Table 3 for a summary of the structures) principal component analysis (PCA)[32] and principal moments of inertia (PMI) analysis[33] were exploited.



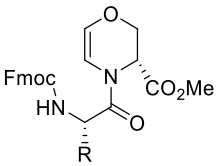
2-oxopiperazines A



diketopiperazines B



dihydropyrazi-nones C



dihydrooxazines D

Cmp.	aa	Cmp.	aa	Cmp.	aa	Cmp.	aa
25	L-Val	**31**	L-Val[a]	**14**	L-Val	**17**	L-Val
26	L-Ala	**32**	L-Pro[a]	**18**	D-Val	**24**	L-Pro
27	L-Leu	**33**	D-Val[a]	**19**	L-Phe		
28	L-Phe	**34**	αMeAla[a]	**20**	D-Phe		
29	L-Phg	**35**	L-OBnSer	**21**	L-Leu		
30	αMeAla	**36**	α-Me-Ala	**22**	L-Ala		
		37	L-Phe	**23**	L-Ile		
		38	L-Pro				

Table 3. Summary table of morpholine-derived compounds. (a) Diketopiperazines derived from phenyl-substituted morpholine, with R^1= Ph.

As already explained in Chapter 4, the structural diversity of this morpholine-derived library, composed of 2-oxopiperazines **25**–**30**, diketopiperazines **31**–**38** and dihydropirazinones **14**–**23** and dihydrooxazines **17** and **24**, were analyzed in terms of PCA score prediction for the first three physicochemical properties. The numerical data (see Table 5 in the experimental section) were then plotted in a graph (Figure 5), reporting PC1 (representing size, shape and polarizability (main contribution is size) versus PC2 (associated with aromatic and conjugation related properties), where morpholine-derived compounds are shown as blue diamonds and brand-name blockbuster drugs (the same 40 compounds already used as reference in Chapter 4)[34] as red squares.

This analysis shows that the three different clusters of the three different heterocycles deriving from serine-derived and threonine-derived morpholine acetals are spanning in different areas of the chemical space, overlapping considerably with those explored by top-selling drugs. In particular, the achievement of the novel dihydropyrazinones **14**–**23** allowed to explore a new area, positioned in the positive direction of both axes which is not particularly

crowded of commercial drugs, thus potentially opening the way to the discovery of novel chemical entities.

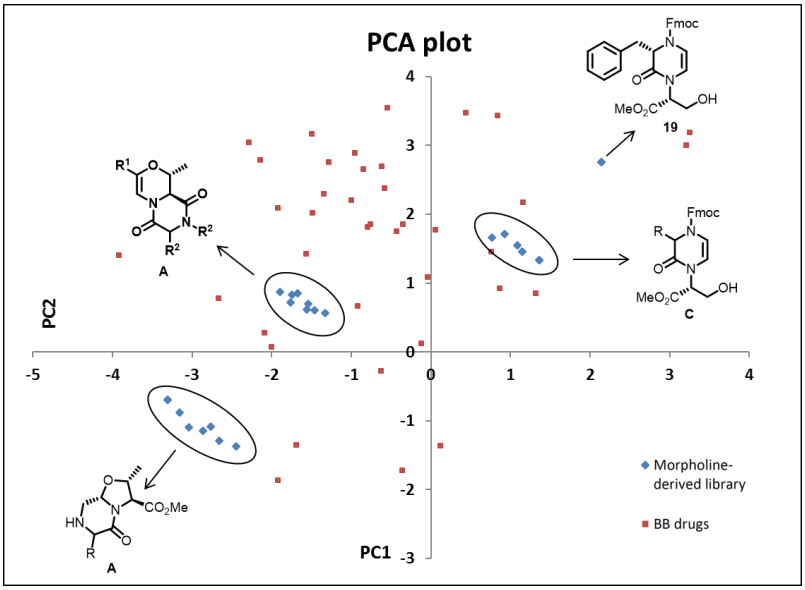


Figure 5. PCA plot resulting from the correlation between PC1 vs PC2, showing the positioning in the chemical space of morpholine-derived compounds (**blue diamonds**) as compared to the reference set of brand-name blockbuster drugs (BB) (**red squares**).

Then, principal moment of inertia (PMI) analysis was performed to assess the skeletal diversity of these nitrogen-containing heterocycles, in comparison with the molecules of the above reference set of BB drugs, as already described in Chapter 4. The three normalized principal moments of inertia (see Table 6 in the experimental section for numerical data for compounds **14**-**38**) were plotted on the characteristic triangular graph (Figure 6), finding that morpholine-derived compounds tend to lie along the center-left side of the triangle, with a preference for the rod-disc side. However, even from this graph, it is possibile to see how these four different skeletons cover large area of the chemical space explored by the 40 BB drugs, thus highlighting how morpholine acetal proved to be a valuable building block in achieving significant skeletal diversity.

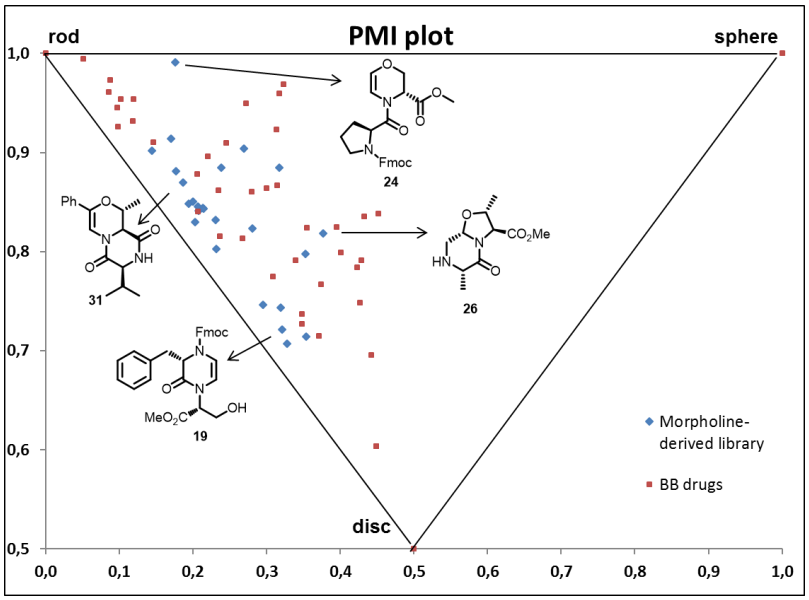
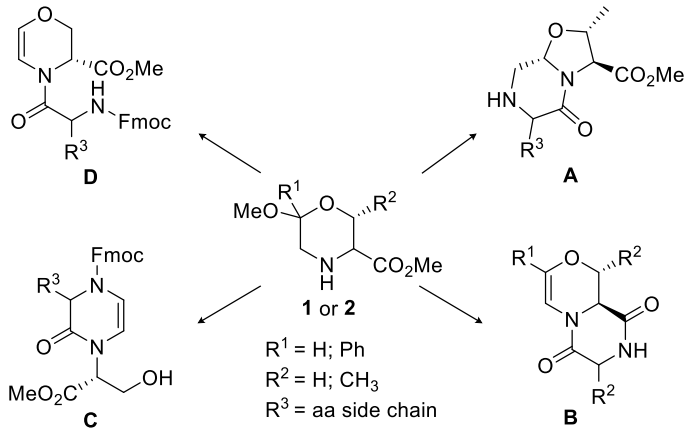


Figure 6. PMI plot showing the skeletal diversity of morpholine-derived compounds (**blue diamonds**) with respect to the reference set of brand-name blockbuster drugs (**red squares**).

6.3 Conclusions

In summary, four different heterocyclic scaffolds were obtained (Scheme 7) through processes involving morpholine acetals and Fmoc-amino acid derivatives. Specifically, in previous works, starting from threonine-derived morpholine acetals **1** the three-step one-pot process led preferentially to the bicyclic diketopiperazine **B**, whereas the stepwise route, by removing 2,6-lutidine and using the same reagents, gave a different bicyclic skeleton containing the 2-oxopiperazine ring **A**. In addition, the two step one-pot process starting from serine-derived morpholine acetal **2** allowed to obtain preferentially the uncommon dihydropyrazinone heterocycle according to a ring rearrangement process. The role of 2,6-lutidine as the base was found essential for promoting the ring rearrangement to give the corresponding dihydropyrazinone scaffold **C**, because when lutidine was missing dihydrooxazines **D** were obtained.



Scheme 7: Skeletal diversity from threonine-derived morpholine acetal **1** and from serine-derived morpholine acetal **2**.

All these heterocycles show interesting biological features. In particular, dihydropyrazinones proved to be interesting Xaa-Ser dipeptide isosteres, with potential application in the generation of peptidomimetic libraries for medicinal chemistry. In addition, X-ray analysis of the D-valine-derived dihydropyrazinone showed that these compounds possess a significant tridimensional framework, potentially useful in addressing interactions with protein target in the three dimensions.

The concept of obtaining skeletal diversity by using same reagents in a different manner may provide new efficient ways to expand the access to chemical diversity. In this context, morpholine acetal and the related reactivity of the *N*-acyl iminium chemistry proved to be extremely interesting for the achievement of novel skeletally different peptidomimetic scaffolds, in a diversity-oriented fashion, using versatile and efficient couple/pair processes. Chemioinformatic analysis proved that, despite the limited number of representatives, morpholine-derived compounds cover large areas of the chemical space, overlapping considerably with those explored by top-selling drugs. These features are promising in view of exploiting mopholine acetal building blocks in achieving new scaffolds to generate peptidomimetic libraries carriers of high molecular diversity and complexity.

6.4 Experimental Section

General

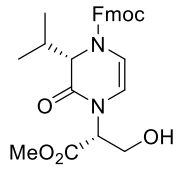
Analytical grade solvents and commercially available reagents were used without further purification. Flash column chromatography (FCC) purifications were performed manually using glass columns with Merck silica gel (0.040–0.063 mm), or using the Biotage Isolera system and SNAP silica cartridges. TLC analyses were performed on Merck silica gel 60 F254 plates. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), or a Varian Gemini 200 (^{1}H: 200 MHz, ^{13}C: 50 MHz). HSQC experiments were carried out to define the multiplicity of ^{13}C signals. All chemical shifts are reported in parts per million (δ) referenced to residual nondeuterated solvent. Data are reported as follows: chemical shifts, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constant(s) in Hz; integration). ESI mass spectra were carried out on a ion-trap double quadrupole mass spectrometer using electrospray (ES+) ionization techniques, and a normalized collision energy within the range of 25−32 eV for MSMS experiments. IR spectra were recorded with a FTIR-1600 Perkin-Elmer spectrophotometer. Elemental analyses were performed on a Perkin Elmer 240 C, H, N analyzer. Optical rotation measurements were performed on a JASCO DIP-370 polarimeter and are given in 10^{-1} deg cm^2 g^{-1}.

General procedure for the one-pot synthesis of dihydropyrazinones

Morpholine acetal **2** (1 eq) and 2,6-lutidine (2 eq) were dissolved in anhydrous CH_2Cl_2 (2.5 mL/mmol), then a solution of Fmoc-aa-Cl (1.1 eq) in CH_2Cl_2 (2.5 mL/mmol) was added dropwise at 0 °C. The reaction mixture was left stirring at room temperature until most of the starting material was converted to the coupling intermediate (TLC control). Then CH_2Cl_2 was removed under *vacuo* and the crude product was dissolved in a 4:1 toluene / CH_2Cl_2 mixture (10 mL/mmol). The reaction mixture was placed in a single-necked round-bottomed flask equipped with a reflux condenser and a dropping funnel containing 4 Å molecular sieves and methanesulfonic acid (10 eq) was added. The mixture was left reacting at reflux for 2 h. Successively, the mixture was brought back at room temperature, diluted with EtOAc, washed with 5% HCl solution, $NaHCO_3$ saturated solution and brine. Then, the organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The

crude was purified by flash chromatography to give the title dihydropirazinone compound.

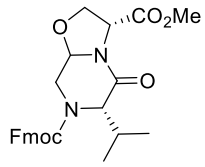
Synthesis and characterization of (*S*)-(9*H*-fluoren-9-yl)methyl 4-((*R*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)-2-isopropyl-3-oxo-3,4-dihydropyrazine-1(2*H*)-carboxylate (14)



Compound **14** was obtained from morpholine acetal **2** (80 mg, 0.46 mmol) and Fmoc-L-Val-Cl (181 mg, 0.51 mmol) following the general procedure. After flash chromatography purification (EtOAc/Petr. et. = 1:3; R_f = 0.29), pure **14** (115 mg, 0.26 mg) was achieved as a white foam in 55% yield.

$[\alpha]_D^{21}$ = +66.9 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) 2:1 mixture of rotamers: *δ* 7.77 (d, *J* = 7.6 Hz, 2H), 7.58–7.56 (m, 2H), 7.41–7.31 (m, 4H), 6.39 (d, *J* = 5.8 Hz, 0.25H), 6.20 (d, *J* = 6.3 Hz, 0.75H), 5.89–5.81 (m, 1H), 4.98 (t, *J* = 6.4 Hz, 0.25H), 4.89 (t, *J* = 6.2 Hz, 0.75H), 4.67–4.57 (m, 2H), 4.51–4.47 (m, 1H), 4.43–4.36 (m, 1H), 4.30–4.21 (m, 1H), 4.01 (d, *J* = 7.1 Hz, 0.75H), 3.96 (d, *J* = 7.1 Hz, 0.25H), 3.77 (s, 2.25H), 3.75 (s, 0.75H), 2.14–2.04 (m, 1H), 1.06 (d, *J* = 6.8 Hz, 0.75H), 1.00 (d, *J* = 7.1 Hz, 2.25H), 0.90 (d, *J* = 7.2 Hz, 2.25H), 0.84 (d, *J* = 6.9 Hz, 0.75H). ^{13}C NMR (100 MHz, $CDCl_3$) 2:1 mixture of rotamers: *δ* 167.8, 164.5 and 163.5, 153.0, 143.3 (2C), 141.4 (2C), 127.9 (2C), 127.2 (2C), 124.6 (2C), 120.1 (2C), 114.0, 112.5 and 109.3, 68.0, 62.8 and 62.4, 62.1, 58.5, 53.0, 47.1, 41.9, 30.1 and 29.6, 19.1 and 18.6. MS (ESI) *m/z* (%): 487.12 [$(M+Na)^+$, 100]. IR ($CHCl_3$): ν = 3188, 2975, 2820, 1825, 1719, 1381, 1221, 1122 cm^{-1}. Anal. Calcd. for $C_{26}H_{28}N_2O_6$: C, 67.23; H, 6.08; N, 6.03. Found: C, 67.36; H, 6.14; N, 5.96.

Synthesis and characterization of (3*R*,6*R*,8a*R*/*S*)-7-((9*H*-fluoren-9-yl)methyl) 3-methyl 6-isopropyl-5-oxotetrahydro-2*H*-oxazolo[3,2-*a*]pyrazine-3,7(3*H*)-dicarboxylate (15)

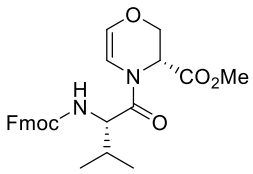


Compound **15** was obtained as a by-product performing the reaction between morpholine acetal **2** and Fmoc-L-Val-Cl. After chromatography purification (EtOAc/Petr. et. = 1:3; R_f = 0.17), it was achieved in variable yields (see Table 1), depending on the reaction conditions.

^{1}H NMR (400 MHz, $CDCl_3$) major rotamer, 1:1 mixture of epimers: *δ* 7.75 (d, *J* = 6.9 Hz, 2H), 7.55-7.53 (m, 2H), 7.39 (pt, *J* = 7.6 Hz, 2H), 7.31 (pt, *J* = 7.5 Hz, 2H), 4.80 (dd, *J* = 10.7, 4.7 Hz, 1H), 4.63–4.43 (m, 3H), 4.40 (d, *J* = 6.8 Hz, 1H), 4.24–

4.13 (m, 3H), 4.03–3.96 (m, 1H), 3.73 (s, 3H), 2.96 (dd, *J* = 11.4, 10.6 Hz, 1H), 2.52 (br s, 0.5H), 2.20 (br s, 0.5H), 1.00 (d, *J* = 6.8 Hz, 1.5H), 0.92 (d, *J* = 6.8 Hz, 1.5H), 0.77 (d, *J* = 6.5 Hz, 1.5H), 0.71 (d, *J* = 6.8 Hz, 1.5H). ^{13}C NMR (100 MHz, $CDCl_3$) major rotamer, 1:1 mixture of epimers: *δ* 169.7, 166.5, 156.0 and 155.6, 143.6 and 143.5 (2C), 141.3 (2C), 127.8 and 127.7 (2C), 127.2 (2C), 124.6 (2C), 120.1 (2C), 84.8 and 84.6, 69.6, 67.4, 60.8, 56.8, 52.7, 47.6 and 47.4, 45.3 and 44.5, 32.2, 20.1 and 19.7, 18.6 and 18.3. MS (ESI) *m/z* (%): 487.00 [(M+Na)$^+$, 100]. IR ($CHCl_3$): ν = 1689, 1411, 1211 cm^{-1}. Anal. Calcd. for $C_{26}H_{28}N_2O_6$: C, 67.23; H, 6.08; N, 6.03. Found: C, 67.36; H, 6.15; N, 5.95.

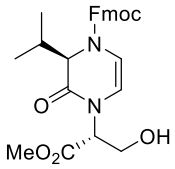
Synthesis and characterization of (*R*)-methyl 4-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanoyl)-3,4-dihydro-2*H*-1,4-oxazine-3-carboxylate (17)



Compound **17** was obtained from morpholine acetal **2** (100 mg, 0.57 mmol) and Fmoc-L-Val-Cl (225 mg, 0.63 mmol) using the general procedure in the absence of 2,6-lutidine. After flash chromatography purification (EtOAc/Petr. et. = 1:3; R_f = 0.17), pure compound **16** (170 mg, 0.37 mmol) was achieved as a white foam in 65% yield.

$[\alpha]_D^{21}$ = +55.6 (*c* 1.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.78 (d, *J* = 7.3 Hz, 2H), 7.61 (d, *J* = 7.3 Hz, 2H), 7.44–7.26 (m, 4H), 6.23 (d, *J* = 5.1 Hz, 1H), 6.00 (d, *J* = 5.1 Hz, 1H), 5.61 (d, *J* = 9.5 Hz, 1H), 5.22 (s, 1H, NH), 4.74 (d, *J* = 12.3 Hz, 1H), 4.67 (dd, *J* = 12.3, 5.5 Hz, 1H), 4.43–4.36 (m, 2H), 4.27–4.23 (m, 1H), 3.95 (dd, *J* = 11.4, 2.9 Hz, 1H), 3.75 (s, 3H), 2.12–2.05 (m, 1H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): *δ* 168.3, 167.3, 156.3, 143.9 (2C), 141.3 (2C), 130.3, 127.6 (2C), 127.1 (2C), 125.1 (2C), 119.9 (2C), 104.9, 67.1, 66.0, 55.6, 52.8, 51.9, 47.3, 31.4, 19.5, 17.2. MS (ESI) *m/z* (%): 487.04 [(M+Na)$^+$, 40], 950.17 [(2M + Na)$^+$, 100]. IR ($CHCl_3$): ν = 2972, 2811, 1718, 1527, 1380, 1220, 1102 cm^{-1}. Elemental analysis calcd (%) for $C_{26}H_{28}N_2O_6$: C, 67.23; H, 6.08; N, 6.03. Found: C, 67.53; H, 6.20; N, 5.93.

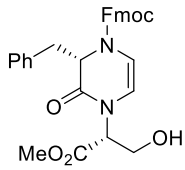
Synthesis and characterization of (*R*)-(9*H*-fluoren-9-yl)methyl 4-((*R*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)-2-isopropyl-3-oxo-3,4-dihydropyrazine-1-(2*H*)-carboxylate (18)



Compound **18** was obtained from morpholine acetal **1** (100 mg, 0.57 mmol) and Fmoc-D-Val-Cl (225 mg, 0.63 mmol) following the general procedure. After flash chromatography purification (EtOAc/Petr. et. = 1:3; R_f = 0.24), pure **18** (119 mg, 0.25 mmol) was achieved as a white foam in 45% yield.

$[\alpha]_D^{21}$ = +104.9 (*c* 1.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) 2:3 mixture of rotamers: *δ* 7.75 (d, *J* = 7.6 Hz, 2H), 7.51–7.48 (m, 2H), 7.34-7.29 (m, 4H), 6.30 (d, *J* = 6.1 Hz, 0.4H), 6.12 (d, *J* = 4.7 Hz, 0.6H), 5.59–5.51 (m, 1H), 4.88 (t, *J* = 6.6 Hz, 0.6H), 4.79 (t, *J* = 5.1 Hz, 0.4H), 4.63–4.44 (m, 2H), 4.41 (dd, *J* = 10.6, 3.9 Hz, 0.6H), 4.19 (t, *J* = 6.6 Hz, 0.6H), 4.16 (t, *J* = 5.6 Hz, 0.4H), 4.05 (d, *J* = 7.8 Hz, 0.4H), 3.92 (d, *J* = 7.1 Hz, 1.2H), 3.87 (dd, *J* = 8.3, 3.1 Hz, 0.8H), 3.68 (s, 1.8H), 3.66 (s, 1.2H), 1.99 (q, *J* = 6.8 Hz, 0.6H), 1.73 (q, *J* = 7.1 Hz, 0.4H), 0.91 (d, *J* = 6.8 Hz, 1.8H), 0.82 (d, *J* = 6.8 Hz, 1.8H), 0.75 (d, *J* = 6.8 Hz, 1.2H), 0.57 (d, *J* = 6.8 Hz, 1.2H). ^{13}C NMR (100 MHz, $CDCl_3$) 2:3 mixture of rotamers: *δ* 167.8 and 167.7, 164.5 and 164.0, 153.4 and 153.3, 143.6 and 143.4 (2C), 141.5 and 141.4 (2C), 127.9 (2C), 127.2 (2C), 125.0 and 124.9 (2C), 120.1 (2C), 112.7 and 112.5, 110.0 and 109.2, 68.0 and 67.7, 62.7, 62.0, 58.8 and 58.5, 53.0, 47.1, 41.8 and 41.7, 30.4 and 30.1, 19.1 and 18.6. MS (ESI) *m/z* (%): 487.04 [$(M+Na)^+$, 100]. IR ($CHCl_3$): ν = 3189, 2973, 2821, 1823, 1719, 1381, 1221, 1104, 1007 cm^{-1}. Anal. Calcd. for $C_{26}H_{28}N_2O_6$: C, 67.23; H, 6.08; N, 6.03. Found: C, 67.53; H, 6.11; N, 5.97.

Synthesis and characterization of (*S*)-(9*H*-fluoren-9-yl)methyl 2-benzyl-4-((*R*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)-3-oxo-3,4-dihydropyrazine-1-(2*H*)-carboxylate (19)

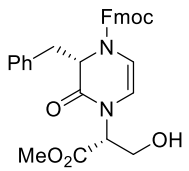


Compound **19** was obtained from morpholine acetal **2** (100 mg, 0.57 mmol) and Fmoc-L-Phe-Cl (254 mg, 0.63 mmol) following the general procedure. After flash chromatography purification (EtOAc/Petr. et. = 1:3; R_f = 0.43), pure **19** (125 mg, 0.24 mmol) was achieved as a white foam in 43% yield.

$[\alpha]_D^{23}$ = +69.2 (*c* 1.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) 2:3 mixture of rotamers: *δ* 7.80–7.78 (m, 2H), 7.74–7.72 (m, 2H), 7.43–7.40 (m, 2H), 7.34–7.30 (m, 2H), 7.26–7.01 (m, 5H), 6.45 (d, *J* = 6.0 Hz, 0.4H), 6.20 (d, *J* = 6.0 Hz, 0.6H), 5.75 (d, *J* = 6.0 Hz, 0.4H), 5.63 (d, *J* = 6.0 Hz, 0.6H), 5.14 (t, *J* = 6.6 Hz, 0.4H), 5.04–4.98 (m,

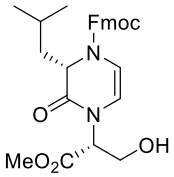
1H), 4.84 (t, *J* = 7.2 Hz, 0.6H), 4.51–4.47 (m, 1H), 4.32–4.25 (m, 1H), 4.21 (t, *J* = 6.6 Hz, 0.6H), 4.19 (t, *J* = 6.6 Hz, 0.4H), 4.06–3.86 (m, 2H), 3.83 (s, 1.2H), 3.80 (s, 1.8H), 3.03 (pt, *J* = 6.7 Hz, 1H), 2.84 (pt, *J* = 7.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) 2:3 mixture of rotamers: *δ* 167.6, 164.5 and 164.0, 153.4, 143.5 and 143.4 (2C), 141.3 (2C), 135.6, 129.6 and 129.3 (2C), 128.4 (2C), 127.9 (2C), 127.1, 126.9 (2C), 124.9 (2C), 120.1 (2C), 111.9 and 111.4, 109.4 and 108.7, 68.1, 59.1, 58.5, 53.0, 47.1 and 46.9, 41.9, 35.9. MS (ESI) *m/z* (%): 535.50 [$(M+Na)^+$, 100]. IR ($CHCl_3$): ν = 3038, 2955, 1813, 1685, 1451, 1428, 1324, 1246, 1201, 1124 cm^{-1}. Anal. Calcd. for $C_{30}H_{28}N_2O_6$: C, 70.30; H, 5.51; N, 5.47. Found: C, 70.45; H, 5.58; N, 5.42.

Synthesis and characterization of (*R*)-(9*H*-fluoren-9-yl)methyl 2-benzyl-4-((*R*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)-3-oxo-3,4-dihydropyrazine-1-(2*H*)-carboxylate (20)



Compound **20** was obtained from morpholine acetal **2** (80 mg, 0.46 mmol) and Fmoc-D-Phe-Cl (207 mg, 0.51 mmol) following the general procedure. After flash chromatography purification (EtOAc/Petr. et. = 1:3; R_f = 0.42), pure **20** (93 mg, 0.18 mmol) was achieved as an incolour oil in 40% yield. $[\alpha]_D^{22}$ = –62.7 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) 1:1 mixture of rotamers: *δ* 7.81–7.76 (m, 2H), 7.56–7.52 (m, 2H), 7.44-7.30 (m, 4H), 7.23–7.02 (m, 5H), 6.38 (d, *J* = 6.0 Hz, 0.5H), 6.11 (d, *J* = 6.0 Hz, 0.5H), 5.68 (d, *J* = 6.1 Hz, 0.5H), 5.54 (d, *J* = 5.6 Hz, 0.5H), 5.13 (t, *J* = 5.6 Hz, 0.5H), 4.95 (t, *J* = 5.2 Hz, 0.5H), 4.90–4.86 (m, 1H), 4.61–4.56 (m, 1H), 4.33–4.26 (m, 1H), 4.15 (t, *J* = 6.6 Hz, 0.5H), 4.12 (t, *J* = 6.6 Hz, 0.5H), 4.03–3.85 (m, 2H), 3.79 (s, 1.5H), 3.75 (s, 1.5H), 3.02–2.99 (m, 1H), 2.87 (dd, *J* = 13.4, 4.8 Hz, 0.5H), 2.74 (dd, *J* = 13.2, 4.6 Hz, 0.5H). ^{13}C NMR (50 MHz, $CDCl_3$): *δ* 169.5, 165.9, 154.7 and 154.2, 143.7 and 143.4 (2C), 141.4 (2C), 136.6 and 136.5, 129.8 and 129.7 (2C), 128.5 and 128.4 (2C), 127.9 (2C), 127.3 and 127.1, 127.0 and 126.9 (2C), 124.9 and 124.7 (2C), 120.2 and 120.0 (2C), 113.7 and 113.0, 109.0 and 108.4, 84.5, 68.4 and 67.5, 57.5 and 56.1, 52.9, 47.2, 43.1, 35.8. MS (ESI) *m/z* (%): 535.50 [$(M+Na)^+$, 100]. IR ($CHCl_3$): ν = 3045, 2803, 1803, 1687, 1404, 1304, 1201, 1003 cm^{-1}. Anal. Calcd. for $C_{30}H_{28}N_2O_6$: C, 70.30; H, 5.51; N, 5.47. Found: C, 70.42; H, 5.62; N, 5.40.

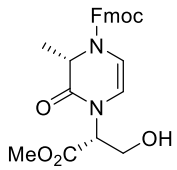
Synthesis and characterization of (*S*)-(9*H*-fluoren-9-yl)methyl 4-((*R*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)-2-isobutyl-3-oxo-3,4-dihydropyrazine-1-(2*H*)-carboxylate (21)



Compound **21** was obtained from morpholine acetal **2** (100 mg, 0.57 mmol) and Fmoc-L-Leu-Cl (233 mg, 0.63 mmol) following the general procedure. After flash chromatography purification (EtOAc/Petr. et. = 1:3; R_f = 0.53), pure compound **21** (87 mg, 0.18 mmol) was achieved as a yellow oil in 32% yield.

$[\alpha]_D^{22}$ = +36.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) 2:3 mixture of rotamers: *δ* 7.76 (d, *J* = 7.3 Hz, 2H), 7.63–7.54 (m, 2H), 7.40–7.30 (m, 4H), 6.21 (d, *J* = 7.1 Hz, 0.6H), 6.11 (d, *J* = 7.1 Hz, 0.4H), 5.70–5.61 (m, 1H), 4.89 (t, *J* = 7.2 Hz, 0.6H), 4.79 (t, *J* = 7.1 Hz, 0.4H), 4.60–4.48 (m, 3H), 4.40 (d, *J* = 8.7 Hz, 1H), 4.27–4.21 (m, 1H), 4.10–3.99 (m, 1H), 3.79 (s, 1.8H), 3.78 (s, 1.2H), 1.65–1.33 (m, 3H), 0.98–0.81 (m, 6H). ^{13}C NMR (50 MHz, $CDCl_3$) 2:3 mixture of rotamers: *δ* 167.2, 164.9, 157.0, 143.3 (2C), 141.3 (2C), 127.9 (2C), 127.4 (2C), 124.9 (2C), 120.1 (2C), 113.1, 108.1 and 108.0, 68.0, 62.3, 55.6, 52.5, 47.2, 41.9, 38.3, 32.4, 29.6, 25.7. MS (ESI) *m/z* (%): 501.50 [$(M+Na)^+$, 100]. IR ($CHCl_3$): ν = 3079, 2984, 2805, 1803, 1745, 1379, 1206, 1011 cm^{-1}. Anal. Calcd. for $C_{27}H_{30}N_2O_6$: C, 67.77; H, 6.32; N, 5.85. Found: C, 67.90; H, 6.38; N, 5.77.

Synthesis and characterization of (*S*)-(9*H*-fluoren-9-yl)methyl 4-((*R*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)-2-methyl-3-oxo-3,4-dihydropyrazine-1-(2*H*)-carboxylate (22)

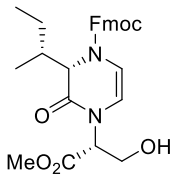


Compound **22** was obtained from morpholine acetal **2** (80 mg, 0.46 mmol) and Fmoc-L-Ala-Cl (168 mg, 0.51 mmol) following the general procedure. After flash chromatography purification (EtOAc/Petr. et. = 1:3; R_f = 0.38), pure **22** (80 mg, 0.18 mmol) was achieved as a yellow oil in 40% yield.

$[\alpha]_D^{21}$ = +14.9 (*c* 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) 1:1 mixture of rotamers: *δ* 7.75 (d, *J* = 7.4 Hz, 2H), 7.59–7.57 (m, 2H), 7.39–7.28 (m, 4H). 6.13 (pt, *J* = 4.4 Hz, 0.5H), 6.00 (pt, *J* = 4.9 Hz, 0.5H), 5.80 (d, *J* = 7.3 Hz, 1H), 5.19 (s, 0.5H), 5.08 (s, 0.5H), 4.79–4.70 (m, 2H), 4.38–4.36 (m, 1H), 4.22–4.20 (m, 1H), 3.95 (dd, *J* = 11.2, 2.9 Hz, 0.5H), 3.89 (dd, *J* = 11.3, 3.0 Hz, 0.5H), 3.77 (s, 1.5H), 3.76 (s, 1.5H), 3.09 (q, *J* = 7.3 Hz, 1H), 1.42 (d, *J* = 6.8 Hz, 1.5H), 1.37 (d, *J* = 6.7 Hz, 1.5H). ^{13}C NMR (50 MHz, $CDCl_3$) 1:1 mixture of rotamers: *δ* 171.1, 167.4, 155.5, 143.5

(2C), 141.5 (2C), 127.7 (2C), 127.0 (2C), 125.0 (2C), 119.4 (2C), 113.6, 107.8, 67.0, 65.8, 53.0, 52.0, 47.2 and 47.0, 29.7 and 29.0, 19.1. MS (ESI) *m/z* (%): 459.32 [(M+Na)$^+$, 100], 895.44 [(2M+Na)$^+$, 40]. IR ($CHCl_3$): ν = 3175, 2980, 2821, 1798, 1719, 1390, 1219, 1102, 1012 cm^{-1}. Anal. Calcd. for $C_{24}H_{24}N_2O_6$: C, 66.04; H, 5.54; N, 6.42. Found: C, 66.27; H, 5.58; N, 6.31.

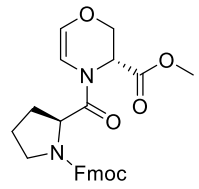
Synthesis and characterization of (*S*)-(9*H*-fluoren-9-yl)methyl 2-((*S*)-sec-butyl)-4-((*R*)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)-3-oxo-3,4-dihydropyrazine-1-(2*H*)-carboxylate (23)



Compound **23** was obtained from morpholine acetal **2** (100 mg, 0.57 mmol) and Fmoc-L-Ile-Cl (233 mg, 0.63 mmol) following the general procedure. After flash chromatography purification (EtOAc/Petr. et. = 1:4; R_f = 0.42), pure **23** (78 mg, 0.16 mmol) was achieved as a white foam in 29% yield.

$[\alpha]_D^{28}$ = +49.4 (*c* 1.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) 1:1 mixture of rotamers: *δ* 7.77 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 6.7 Hz, 2H), 7.42–7.29 (m, 4H), 6.27 (d, *J* = 5.1 Hz, 0.5H), 6.00 (d, *J* = 5.0 Hz, 0.5H), 5.56 (d, *J* = 9.0 Hz, 1H), 5.22 (s, 1H), 4.76–4.67 (m, 2H), 4.42-4.37 (m, 2H), 4.25–4.21 (m, 1H), 3.94 (dd, *J* = 11.3, 2.9 Hz, 1H), 3.74 (s, 3H), 1.91 (br s, 0.5H), 1.46 (br s, 0.5H), 1.33–1.14 (m, 2H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.93–0.89 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) 1:1 mixture of rotamers: *δ* 168.3, 166.2, 156.2, 143.9 and 143.7 (2C), 141.2 (2C), 127.7 (2C), 127.1 (2C), 125.1 (2C), 119.9 (2C), 110.5, 109.5, 67.1, 66.0, 54.9, 52.9, 51.7, 47.1, 39.0, 24.0, 15.9, 11.4. MS (ESI) *m/z* (%): 501.72 [(M+Na)$^+$, 100]. IR ($CHCl_3$): ν = 3189, 2973, 2812, 1822, 1705, 1381, 1221, 1104, 1007 cm^{-1}. Anal. Calcd. for $C_{27}H_{30}N_2O_6$: C, 67.77; H, 6.32; N, 5.85. Found: C, 67.88; H, 6.36; N, 5.79.

Synthesis and characterization of (*R*)-methyl 4-((*S*)-1-(((9*H*-fluoren-9-yl)methoxy)carbonyl)pyrrolidine-2-carbonyl)-3,4-dihydro-2*H*-1,4-oxazine-3-carboxylate (24)



Compound **24** was obtained from morpholine acetal **2** (115 mg, 0.66 mmol) and Fmoc-L-Pro-Cl (257 mg, 0.72 mmol) following the general procedure. After flash chromatography purification (EtOAc/Petr. et. = 1:3; R_f = 0.10), pure **24** (110 mg, 0.24 mmol) was achieved as a yellow foam in 37% yield.

$[\alpha]_D^{23}$ = +49.4 (*c* 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) 2:1 mixture of rotamers:

δ 7.69 (d, *J* = 7.5 Hz, 2H), 7.58-7.48 (m, 2H), 7.34–7.24 (m, 4H), 6.59 (d, *J* = 5.8 Hz, 0.25H), 6.20 (d, *J* = 6.2 Hz, 0.25H), 6.08–6.05 (m, 0.75H), 5.90 (d, *J* = 5.1 Hz, 0.75H), 5.43 (s, 0.25H), 5.19 (d, *J* = 7.6 Hz, 0.75H), 4.76 (td, *J* = 8.5, 3.0 Hz, 0.25H), 4.64 (td, *J* = 8.5, 3.0 Hz, 0.75H), 4.40–4.15 (m, 3H), 3.96 (dd, *J* = 14.2, 6.8 Hz, 0.25H), 3.96 (dt, *J* = 14.2, 6.9 Hz, 0.75H), 3.74 (s, 0.75H), 3.68 (s, 2.25H), 3.70–3.50 (m, 1H), 3.39 (s, 0.50H), 3.38 (s, 1.5H), 2.33–2.25 (m, 0.75H), 2.23–2.17 (m, 0.25H), 2.11–1.86 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) 2:1 mixture of rotamers: *δ* 169.0, 164.6, 156.3, 143.9 (2C), 141.3 (2C), 132.0 and 130.0, 127.7 and 127.6 (2C), 127.0 (2C), 125.1 (2C), 119.9 (2C), 105.0 and 104.8, 67.8 and 67.6, 66.0 and 65.4, 56.9, 55.0, 53.0, 51.7, 47.2 and 47.1, 30.6 and 29.7, 25.0 and 24.3. MS (ESI) *m/z* (%): 485.03 [$(M+Na)^+$, 100]. IR ($CHCl_3$): ν = 3040, 2995, 1688, 1419, 1215 cm^{-1}. Anal. Calcd. for $C_{26}H_{26}N_2O_6$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.72; H, 5.79; N, 5.99.

6.4.1 Chemioinformatics:

PCA analysis. The web-based public tool ChemGPS-NP was used for PCA analysis of compounds **14**-**38**, to compare their chemical properties with those of a reference set of 40 brand-name blockbuster (BB) drugs as reported by Tan.[34b] ChemGPS-NP can be applied for comprehensive chemical space navigation and exploration in terms of global mapping on to a consistent 8-dimension-al map of structural characteristics. The first three dimensions of the ChemGPS-NP map capture 77% of data variance. Chemical compounds were positioned onto this map using interpolation in terms of PCA score prediction. SMILES codes for all compounds **14**-**38** (Table 4) and the 40 BB drugs of the reference set (Table 1 in the Appendix) were retrieved using ChemBioDraw Ultra 12.0 and submitted to ChemGPS-NP for achieving the corresponding PC scores (see Table 5 for the morpholine-derived compounds **14**-**38** and Table 2 in the Appendix for the blockbuster drugs).

Table 4. SMILES code of compounds 14-38

- CC(C)[C@H]1C(N([C@H](CO)C(OC)=O)C=CN1C(OCC2C3=C(C=CC=C3)C4=C2C=CC=C4)=O)=O [**14**]
- O=C(OC)[C@@H]1N(C([C@@H]2CCCN2C(OCC3C4=C(C=CC=C4)C5=C3C=CC=C5)=O)=O)C=COC1 [**17**]
- CC(C)[C@@H]1C(N([C@H](CO)C(OC)=O)C=CN1C(OCC2C3=C(C=CC=C3)C4=C2C=CC=C4)=O)=O [**18**]
- O=C1[C@H](CC2=CC=CC=C2)N(C(OCC3C4=C(C=CC=C4)C5=C3C=CC=C5)=O)C=CN1[C@H](CO)C(OC)=O [**19**]
- O=C1[C@@H](CC2=CC=CC=C2)N(C(OCC3C4=C(C=CC=C4)C5=C3C=CC=C5)=O)C=CN1[C@H](CO)C(OC)=O [**20**]
- O=C1[C@H](CC(C)C)N(C(OCC2C3=C(C=CC=C3)C4=C2C=CC=C4)=O)C=CN1[C@H](CO)C(OC)=O [**21**]
- C[C@H]1C(N([C@H](CO)C(OC)=O)C=CN1C(OCC2C3=C(C=CC=C3)C4=C2C=CC=C4)=O)=O [**22**]
- O=C1[C@H]([C@@H](CC)C)N(C(OCC2C3=C(C=CC=C3)C4=C2C=CC=C4)=O)C=CN1[C@H](CO)C(OC)=O [**23**]
- O=C(OC)[C@@H]1N(C([C@@H](NC(OCC2C3=C(C=CC=C3)C4=C2C=CC=C4)=O)C(C)C)=O)C=COC1 [**24**]
- O=C1[C@H](C(C)C)NC[C@H]2N1[C@H](C(OC)=O)[C@@H](C)O2 [**25**]
- O=C1[C@H](C)NC[C@H]2N1[C@H](C(OC)=O)[C@@H](C)O2 [**26**]
- O=C1[C@H](CC(C)C)NC[C@H]2N1[C@H](C(OC)=O)[C@@H](C)O2 [**27**]
- O=C1[C@H](CC2=CC=CC=C2)NC[C@H]3N1[C@H](C(OC)=O)[C@@H](C)O3 [**28**]
- O=C1[C@H](C2=CC=CC=C2)NC[C@H]3N1[C@H](C(OC)=O)[C@@H](C)O3 [**29**]
- O=C1C(C)(C)NC[C@H]2N1[C@@H](C(OC)=O)[C@@H](C)O2 [**30**]
- O=C([C@H](C(C)C)N1)N2C=C(C3=CC=CC=C3)O[C@H](C)[C@H]2C1=O [**31**]
- O=C([C@H]1N2CCC1)N3C=C(C4=CC=CC=C4)O[C@H](C)[C@H]3C2=O [**32**]
- O=C([C@H](CC(C)C)N1)N2C=C(C3=CC=CC=C3)O[C@H](C)[C@H]2C1=O [**33**]
- O=C(C(C)(C)N1)N2C=C(C3=CC=CC=C3)O[C@H](C)[C@H]2C1=O [**34**]
- O=C([C@H](COCC1=CC=CC=C1)N2)N3C=CO[C@H](C)[C@H]3C2=O [**35**]
- O=C([C@H](CC1=CC=CC=C1)N2)N3C=CO[C@H](C)[C@H]3C2=O [**36**]
- O=C([C@H](CC(C)C)N1)N2C=CO[C@H](C)[C@H]2C1=O [**37**]
- O=C([C@H]1N2CCC1)N3C=COC[C@H]3C2=O [**38**]

MOLID	PC1	PC2	PC3
[37]	-2,765560	-1,084712	-0,9084410
[28]	-1,5557160	0,6124250	-0,1643630
[24]	1,0850890	1,5534410	1,0451550
[38]	-3,3032900	-0,694739	-1,2941830
[17]	0,9245110	1,7131750	1,0818880
[25]	-2,6571110	-1,284442	-0,7541370
[18]	1,1467100	1,4604690	0,8897480
[29]	-1,7670950	0,7229860	-0,3607650
[32]	-1,6739430	0,8578980	-0,0817060
[23]	1,3571790	1,3396640	1,1040300
[20]	2,1432290	2,757265	1,4445330
[26]	-3,0429640	-1,094524	-1,2147560
[22]	0,7639610	1,6667540	0,4422900
[35]	-1,4572190	0,6034880	-0,4913730
[14]	1,1467100	1,4604690	0,8897480
[21]	1,3571720	1,3372350	1,0930040
[31]	-1,5411480	0,7036630	-0,0860070
[30]	-2,8666800	-1,141459	-1,0790910
[36]	-1,8990640	0,8741890	-0,5385440
[34]	-1,7487610	0,8335390	-0,4057590
[19]	2,1432290	2,7572650	1,4445330
[33]	-1,3268820	0,5648610	0,1260710
[27]	-2,4459060	-1,374578	-0,5472530

Table 5: PCA results table for the first four dimensions of compounds **14-38**

PMI analysis. Principal moment of inertia analysis was carried out by calculation of the lowest energy conformation of each compound **14**-**38** and each compound from the reference set of 40 brand-name blockbuster drugs,[34b] using the built-in AMMP molecular mechanics algorithm with default parameters of the VEGA ZZ molecular modelling software package v.3.0.1. Once the lowest energy conformer was calculated, the three principal moments of inertia (I_{xx}, I_{yy}, I_{zz}) and normalized principal moments of inertia, npr1 (I_{xx}/I_{zz}) and

npr2 (I_{yy}/I_{zz}) were determined, and PMI ratios were calculated for **14**-**38** (Table 6 below) and BB drugs (Table 3 in the Appendix).

MOLID	I_x	I_y	I_z	I_x/I_y	I_y/I_z
31	4018,7043	3409,6643	778,4491	0,8484	0,1937
32	3794,9786	3148,4629	767,5048	0,8296	0,2022
33	4545,0872	3835,5450	969,5869	0,8439	0,2133
34	3278,8946	2727,6564	754,9941	0,8319	0,2303
35	4944,0078	4457,7067	709,9298	0,9016	0,1436
36	3071,3630	2583,7069	636,3124	0,8412	0,2072
37	2040,9499	1679,9960	571,5791	0,8231	0,2801
38	1325,4968	947,0934	468,4714	0,7145	0,3534
25	2110,6662	1868,0213	669,9698	0,8850	0,3174
26	1572,0547	1286,8744	592,1863	0,8186	0,3767
27	2807,4659	2483,8466	667,7233	0,8847	0,2378
28	4184,4395	3824,0299	711,0117	0,9139	0,1699
29	3112,1952	2814,2532	838,1639	0,9043	0,2693
30	1712,0665	1365,5284	603,2403	0,7976	0,3523
14	10434,2491	8799,1129	2230,6997	0,8433	0,2138
18	11183,2011	9511,2469	2228,4934	0,8505	0,1993
19	11704,2436	8704,4363	3735,5247	0,7437	0,3192
20	13572,2591	10126,6573	4004,2404	0,7461	0,2950
21	12069,7151	9686,7558	2794,2078	0,8026	0,2315
22	9709,0293	8558,5112	1718,9028	0,8815	0,1770
23	11921,6140	10075,9013	2456,6999	0,8452	0,2061
17	10917,5141	10816,8849	1914,3071	0,9908	0,1753
24	10172,7598	8844,3421	1891,1211	0,8694	0,1859

Table 6: PMI results table of compounds **14**-**38** by calculation of the lowest energy conformation

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measured using the ω scan mode, were corrected for Lorentz and polarization effects (Walker, N.; Stuart, D. *Acta Cystallogr*. *Sect .A* **1983**, *39*, 158). The substantial redundancy in data allows empirical absorption corrections to be applied using multiple measurements of symmetry-equivalent reflections. Structure was solved by direct methods of SIR2014 and refined using the full-matrix least squares on F^2 provided by SHELXL2013 in WinGX platform (Sheldrick. *Acta Cryst.* **2008**, *A64*, 112). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned in calculated positions, all of them were refined as isotropic.

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7

Synthesis of α-Amino Nitriles from Tertiary Amides and Lactams

7.1 Introduction

The interest of α-amino nitriles as a good starting point for Diversity-Oriented Synthesis has been already pointed out in Section 2.3, where it has been underlined the possibility to use the nitrile group to generate a wide range of functional groups including amines, amides, carbonyl compounds, carboxylic acid derivatives and various heterocycles.[1] In particular, α-amino nitriles, with their several different modes of reactivity, as masked iminium ions, α-amino carbanions and acyl anions equivalent, can serve in a variety of different synthetic applications, especially for the achievement of novel methods of cyclization.[2]

In addition, the interest in these building blocks for library development is not solely connected to their synthetic versatility, but also to the intrinsic biological properties of the nitrile group, which can be involved into hydrogen-bonding or π-π interactions, due to its high polarity and the characteristic linear geometry.[3]

In fact, α-amino nitriles are a recurrent scaffold in many biologically active molecules, natural compounds and also drugs. Just to give some examples, *Aspidofractinine* and *Streptomyces* metabolites, such as saframycin A and cyanocycline A, and lahadinines A and B, extracted from *Kopsia pauciflora*, contain an α-amino nitrile moiety in their structure (Figure 1, top).[4] In addition,

Vildagliptin is an amido nitrile-containing antidiabetic drug, developed as a dipeptidyl peptidase (DPP IV) reversible inhibitor,[5] and Odonacatib has been investigated by Merck for the treatment of osteoporosis (Figure 1, bottom).[6]

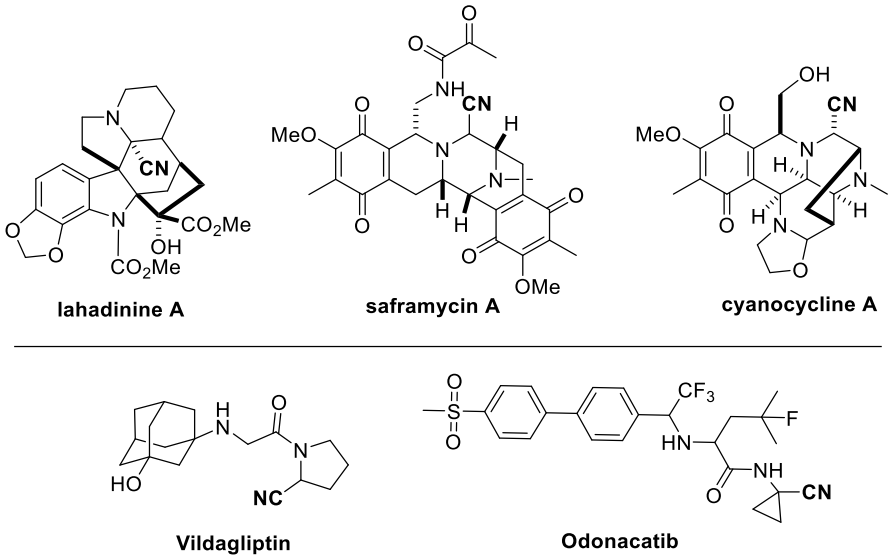
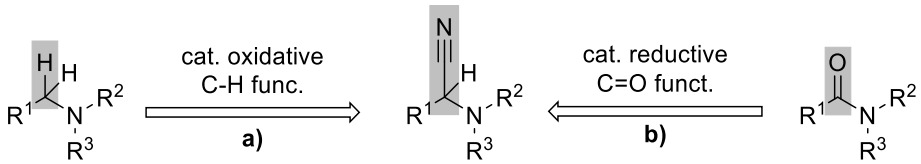


Figure 1. (**Top**) Examples of natural products possessing an α-amino nitrile moiety. (**Bottom**) Examples of drugs containing an α-amido nitrile moiety.

Since Strecker firstly described in 1850 the synthesis of α-aminonitriles in his famous three-component reaction with hydrogen cyanide,[7] many efforts have been devoted to the development of efficient ways for the preparation of these compounds. For this reason, during a secondment activity of this PhD in the group of Prof. Darren J. Dixon at the University of Oxford, with the aim of developing new methodologies for the generation of valuable multifuctional building blocks for Diversity-Oriented Synthesis, α-amino nitriles were taken into account. In particular, considering the broad presence of amides and lactams in biologically active compounds, we envisioned to develop a methodology for the selective reductive cyanation of carboxamide functional groups, in order to introduce in complex biologically intermediates the α-amino nitrile moieties, which may serve as a valuable point of diversification, in a diversity-oriented fashion. Moreover, amides and lactams are widespread also within the suppliers' catalogues and the compound libraries of pharmaceutical and agrochemical companies, thus a mild reductive method that could efficiently and chemoselectively target such functional groups would likely find

numerous applications even in library generation, late stage functionalization and total synthesis.

In recent years, direct α-C-H functionalization reactions of amines has attracted significant attention,[8] and great strides have been made in both sp^2 and sp^3 photochemical, electrochemical and transition-metal-catalyzed oxidative C-H cyanation procedures (Scheme 1a).[9] However, significant challenges in relation to improvements to catalytic turnover, site selectivity and in particular substrate scope and functional group tolerance remain to be addressed for this approach to be generally applicable. For these reasons, the development of a reductive Strecker-type (reductive cyanation) reaction of carboxamides is a direct and synthetically powerful solution for the synthesis of α-amino nitriles (Scheme 1b).



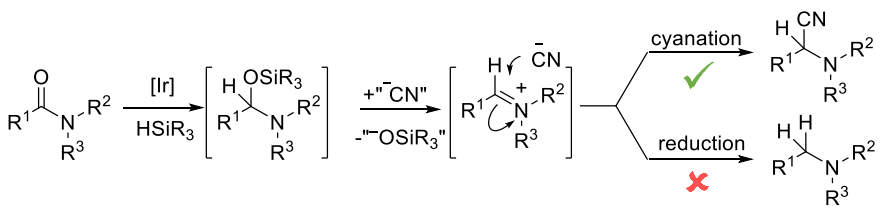
Scheme 1. (**a**) catalytic oxidative C-H functionalization of amine and (**b**) catalytic reductive cyanation of amide for the synthesis of α-amino nitriles.

To this end, in recent years some examples of reductive cyanation reactions at the amide and lactam carbonyl carbon functionality have been described. In particular Suh and coworkers exploited the preactivation of *N*-acyl protected amides by partial reduction with DIBAL and trapping with TMSOTf, which then can undergo Lewis acid catalyzed cyanation. Huang improved this methodology using an *in situ* activation of the amide with Tf_2O, but the reaction is restricted to secondary amides.[10] Chida managed to avoid the preactivation step using *N*-alkoxyamides and DIBAL as reducing agent.[11] However the yields proved to be lower with *N*-alkylamides. Due to its oxophilicity, Schwartz´s reagent has also been used to activate both secondary and *N*-methoxy amides.[12] Catalytic versions has been described with IrI_3[13] and $IrCl(CO)(PPh_3)$[14] for *N*-sulfonyl and *N*-methoxy amides respectively, however these methodologies are restricted to *N*-functionalized amides. In summary, in all these approaches, to overcome the low inherent electrophilicity of the amide/lactam carbonyl group, superstoichiometric amounts of powerful metal hydride reducing agents or strong electrophiles for pre-activation are necessary. The use of these reactivity

auxiliaries bring with them issues of chemoselectivity and functional group intolerance and therefore we envisioned to develop a mild, catalytic, chemoselective and direct reductive cyanation reaction of carboxylic amides and lactams.

7.2 Results and discussion[15]

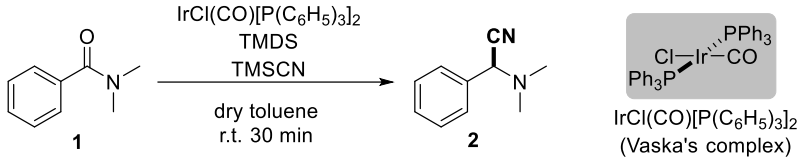
Dixon research group recently reported that Vaska's complex $[IrCl(CO)(PPh_3)_2]$ in the presence of tetramethyldisiloxane (TMDS) is able to catalyze the intramolecular reductive nitro-Mannich reaction of lactam substrates possessing *N*-linked nitro-alkyl groups.[16] That work demonstrated that reactive iminium species could be readily generated, then efficiently trapped by the pendant nitronate nucleophile to afford bicyclic tertiary amines. Furthermore, the remarkable chemoselectivity of the Vaska's catalyst for lactam carbonyl groups over ester functionalities and C=C double bonds has been also demonstrated,[17] even if analogous reactions of acyclic carboxylic acid amides had not been explored yet. Following the formation of an intermediate hemiaminal by the action of Vaska's complex and a silane reductant, we reasoned to find the right conditions for an efficient intermolecular cyanation reaction that out-competed any over-reduction of the iminium species (Scheme 2).[18]



Scheme 2. Catalytic reductive cyanation strategy for the synthesis of α-amino nitriles.

N,*N*-dimethylbenzamide was chosen as a model substrate to carry out feasibility studies and TMSCN as a convenient source of cyanide. Pleasingly, after addition of 1.1 equivalents of TMDS to a solution of **1** and 1 mol% Vaska's complex, followed by addition of 1.1 equivalents of TMSCN, the desired α-amino nitrile product **2** was isolated after 30 minutes in 43% yield (Table 1, entry 1). Increasing the amounts of TMDS and TMSCN to 2 equivalents each resulted in an increase in the yield to 92% (Table 1, entry 5). Lowering the

amount of catalyst (entry 7-8) or increasing the concentration (entry 9) proved to be detrimental for the yield. Moreover, the reaction with model amide **1** was scaled-up to 1 g with no yield erosion (92%, Table 1, entry 10) and also scaled-down to 4 mg with acceptable yield (58%, Table 1, entry 11), which makes the transformation very attractive, not only for the preparation of α-aminonitrile compounds in large scale, but also for the functionalization of small amounts of complex materials.



Entry[a]	%mol cat	TMDS (eq)	TMSCN (eq)	Yield (%)
1	1	1.1	1.1	43
2	1	1.5	1.1	57
3	1	2	1.1	75
4	1	2	1.5	79
5	1	2	2	92
6[b]	1	2	2	86
7	0.5	2	2	58
8[c]	0.5	2	2	23
9[d]	1	2	2	65
10[e]	1	2	2	92
11[f]	1	2	2	58

Table 1. Reaction optimization: (a) reactions were run at 0.05M concentration; (b) overnight; (c) the reaction was stirred for 30 min before TMSCN addition; (d) 0.1 M concentration; (e) 1 g scale; (f) 4 mg scale.

Every step of the reaction can be monitored by observable physical changes in the reaction media. Consumption of the starting material is characterized by the production of H_2 bubbles and the disappearing of characteristic Vaska's yellow color (Figure 2b and c). The formation of the final product, which takes

place in less than 5 minutes, is also recognizable by the pink color appearance (Figure 2d).

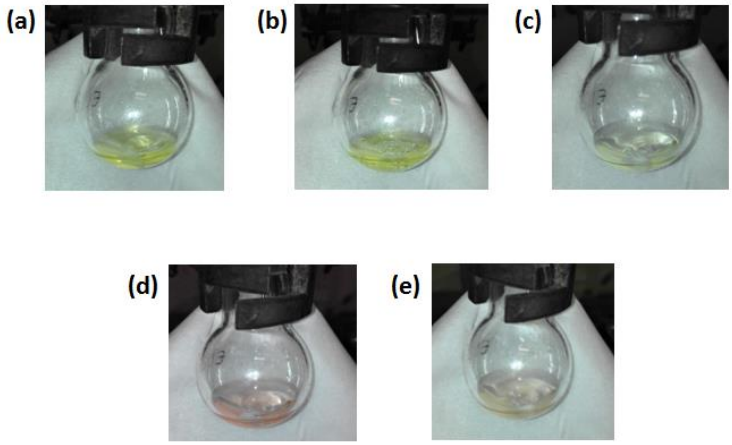


Figure 2: (**a**) Reaction mixture with amide **1** and Vaska's catalyst; (**b**) TMDS addition: bubbles formation; (**c**) 5 min after TMDS addition: yellow colour disappearance; (**d**) TMSCN addition: pink colour (only visible with aromatic amides); (**e**) 5 min after TMSCN addition: disappearance of pink colour which indicates reaction completion.

With optimized reaction conditions being established, the scope of the reductive Strecker reaction with respect to the carboxylic acid moiety was studied (Figure 3, blue highlights). Pleasingly, the introduction of an additional aromatic ring in the naphthyl amide derivative increased the yield up to 98% (**3**). Both electron-rich and electron-deficient substituents were well-tolerated in the aromatic counterpart (**4**-**6**), although the yield was slightly lower for the electron-deficient *N*,*N*-dimethyl-4-nitrobenzamide, recovering some starting material. The reaction proved to be applicable to furanyl heterocycle (**7**), as well as to conjugated alkenes such as a cinnamic acid-derived amide (**8**) and also with aliphatic carboxylic acid moieties (**9**-**12**). Importantly, bulky substituents adjacent to the carbonyl group, such as *tert*-butyl (**9**) and adamantyl (**10**), did not dramatically decrease the yield, and secondary and primary alkyl amides also worked well, showing that enolizable substrates did not derail the reductive cyanation reaction through possible irreversible formation of enamine intermediates.[19] Different amines were also explored and the majority were well tolerated (Figure 3, green highlights); diethyl amine (**13**),

pyrrolidine (**14**) and Boc-piperazine (**18**) afforded the products with yields between 81-88%.

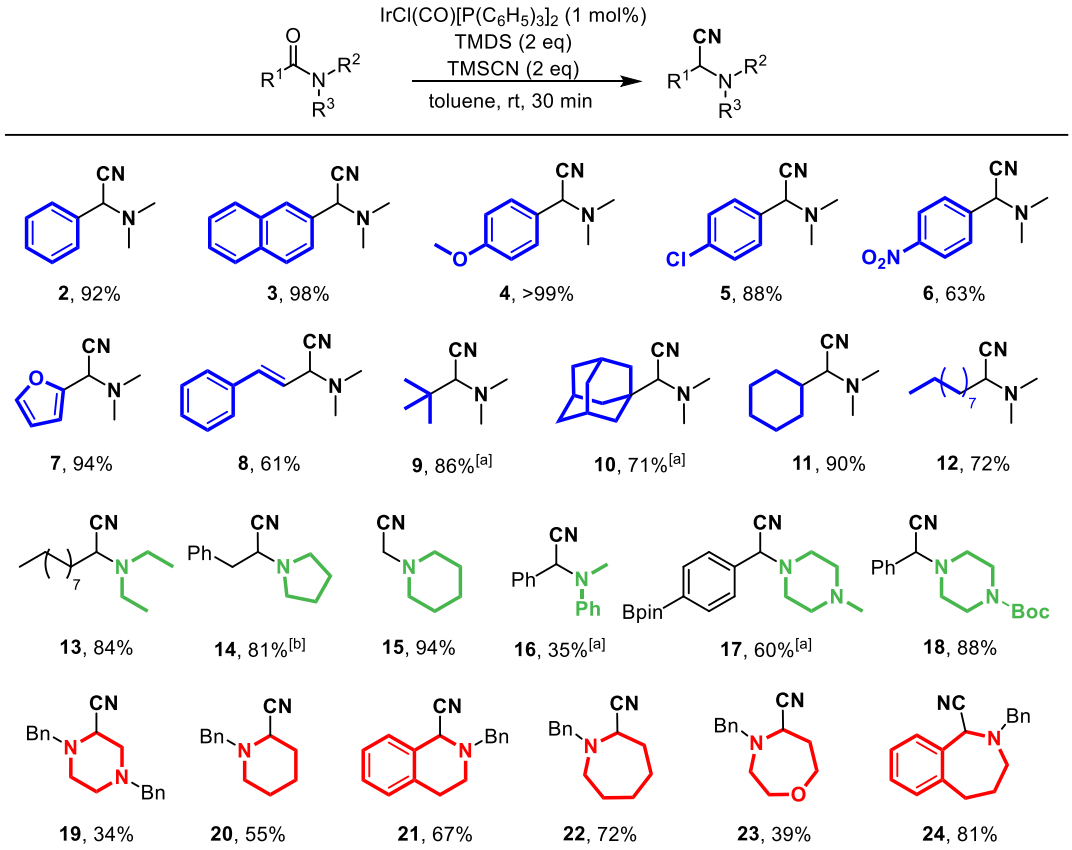


Figure 3. Scope of the reaction: (a) 2 mol% $IrCl(CO)[P(C_6H_5)_3]_2$ and 4 eq of TMDS were used; (b) 4 eq TMSCN were used.

Single crystal X-ray analysis studies of compound **18** unambiguously established its structure as shown in Figure 4.[20] On the other hand, a substrate possessing an aromatic amine resulted in a considerably reduced yield (**16**, 35%), probably due to a higher steric hindrance effect in the formation of the iminium intermediate, or because of the reduced Lewis basicity of the carboxylic amide group.[21] A formic acid-derived amide was a good substrate, affording product **15** in near quantitative yield. Furthermore, lactams (Figure 3, red highlights) were generally well-tolerated with yields ranging from modest to high (**19**-**24**), although usually the reactions proved to be more challenging due to enamine

and self-condensation products formations. Notably, chloro substituents, nitro groups, alkenes, esters, carbamates and boronate esters remained essentially untouched under the reaction conditions, clearly demonstrating the wide functional group tolerance of this transformation.

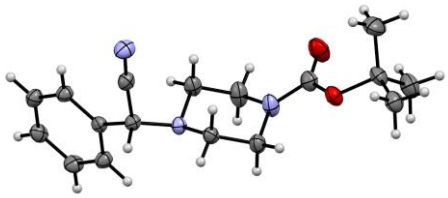


Figure 4: X-ray structure of compound **18**.

For sake of clarity, in figure 5 are reported some compounds that failed to undergo reductive cyanation. In particular, urea and carbamate did not react at all (**25**, **26**), whereas system where the resulting enamine was too stable, as in 1-methylindolin-2-one **28**, the addition of cyanide failed to proceed. Also, morpholinone **29** gave the dimeric adduct as the only product, due to enamine/iminium condensation processes, whereas the reaction performed on more complex system, such as McMillan's imidazolidinone **30**, diketopiperazine **31** and quinolinone **32**, resulted in a complex mixture of degradation products.

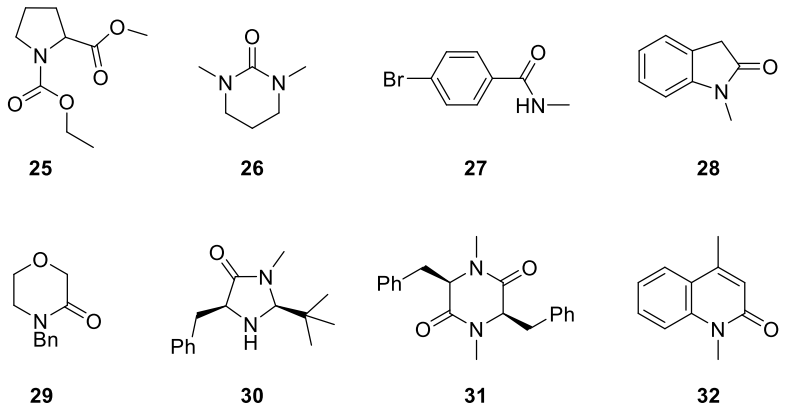


Figure 5: Substrates that failed to undergo reductive cyanation reaction.

Interestingly, less basic and less reactive secondary amides, as compound **27**, did not undergo any reaction at all, although their presence in the reaction media did not impede the cyanation of compound **1** (Figure 6).

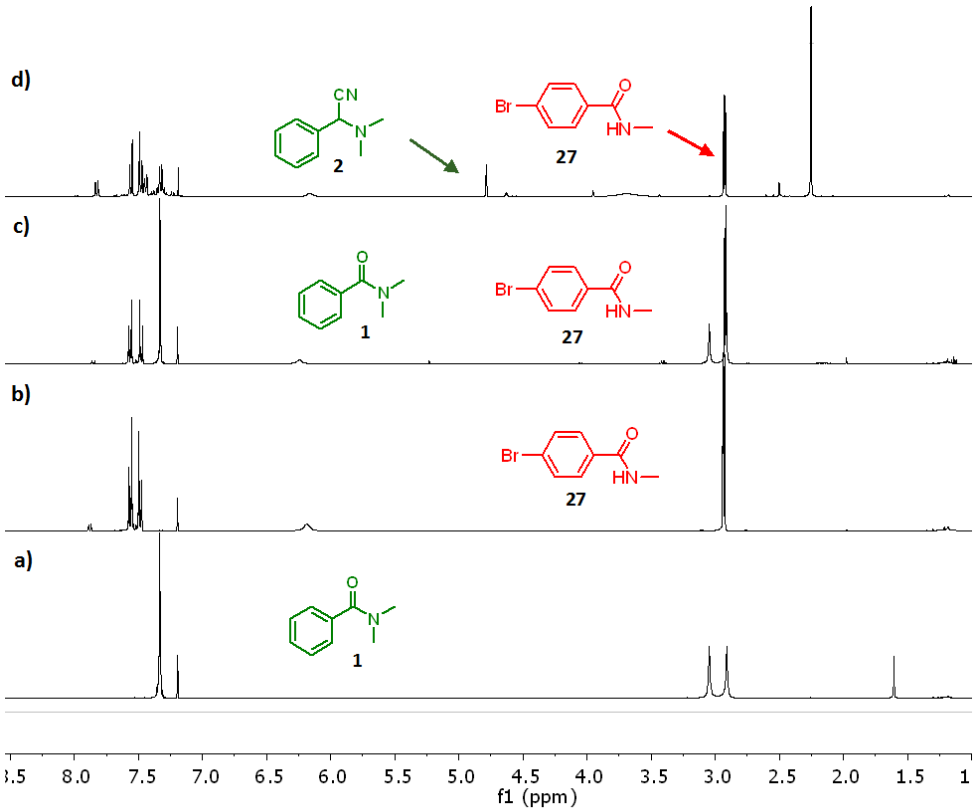


Figure 6: (**a**) ^{1}H-NMR of tertiary amide **1** in $CDCl_3$; (**b**) ^{1}H-NMR of secondary amide **27** in $CDCl_3$; (**c**) ^{1}H-NMR of a 1:1 mixture of tertiary and secondary amides **1** and **27** used as starting material for the [Ir]-catalyzed cyanation; (**d**) ^{1}H-NMR of the crude mixture: tertiary amide **1** is fully converted into the cyanated product **2**, with complete selectivity towards the secondary amide **27** which is completely recovered after the reaction.

Starting from this result, we envisioned that this chemoselective cyanation of tertiary amides could potentially functionalize the carbonyl carbon linked to the *N*-atom of a proline residue within a peptide in a selective way, as proline is the only proteinogenic amino acid which forms tertiary amides.[22] Firstly, we tested our reaction on a simple benzoylated proline methyl ester, and very pleasingly the cyanated product **33** was obtained as a 5:1 mixture of diastereomers in 82% yield (Figure 7). The introduction of a bulkier benzhydryl group in place of the

methyl ester resulted in the formation of only one diastereomeric α-amino nitrile product, albeit with lower yield (**34**, 35%). Importantly, the presence of a 'free' NH in the dipeptide Boc-Val-Pro-OMe did not prevent the reaction from proceeding; the nitrile product **35** was formed in 38% yield as a mixture of diastereomers, likely as a result of iminium-enamine interconversion with concomitant epimerization of the former valine residue α-carbon.

Boc-Gly-Pro-OMe dipeptide yielded again a 5:1 mixture of diastereomers **36** with good yield. Interestingly, the tripeptide Boc-Gly-Pro-Phe-OMe generated the cyanation product **37** exclusively in the carbonyl carbon linked to the proline *N*-atom in 54% yield, in a completely chemo- and diastereoselective fashion. This is, to our knowledge, the first time that a peptide has been selectively functionalized with a CN group in the carbonyl group attached to a proline *N*-atom. This significant result points to the possibility of allowing selective functionalization of tertiary amides within peptides and proteins, which could have important applications in both drug design and labelling, as CN stretching vibration has emerged as a valuable IR probe of the conformation and local environment of protein and enzymes.[23]

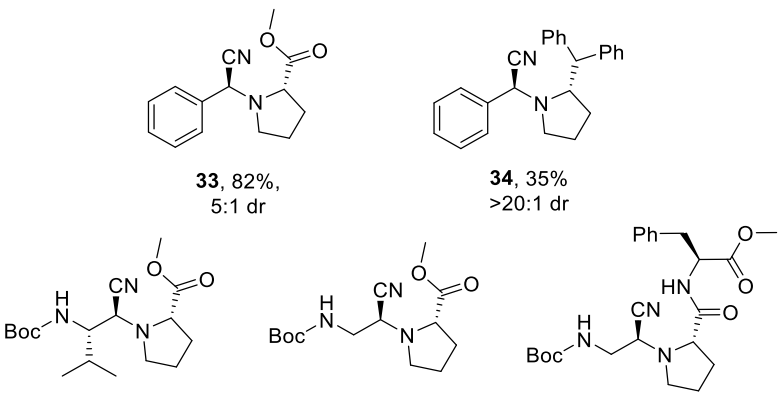
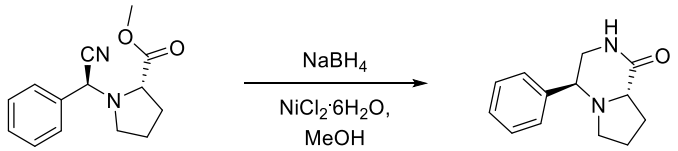


Figure 7: Cyanation of proline, di- and tripeptides.

The stereochemistry of the major diastereomer of cyanated proline **33** was evinced through reduction of the nitrile functionality, which spontaneously cyclize into the constrained product **38**, suitable for NOE studies (Scheme 3 and Figure 8).



Scheme 3: Reduction of the major diastereomer of compound **33** into the cyclized product **38**.

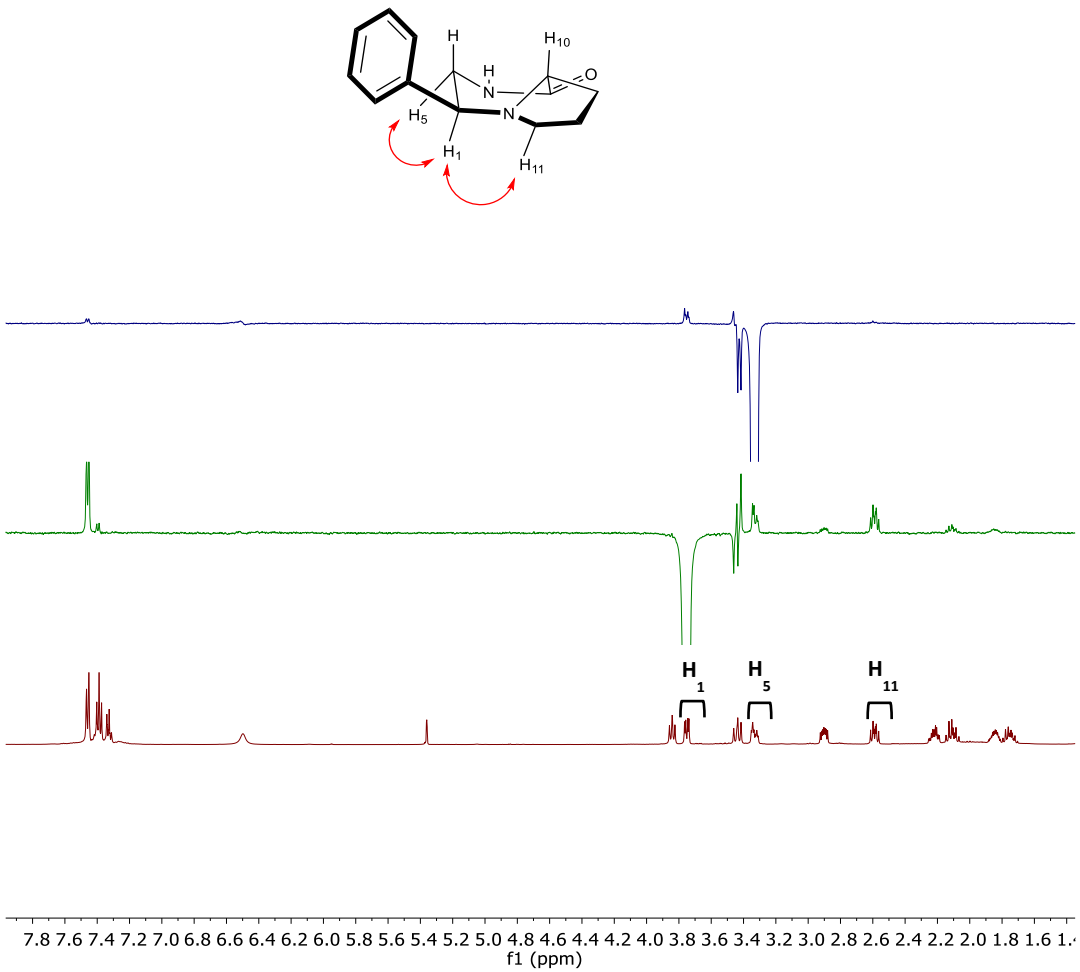
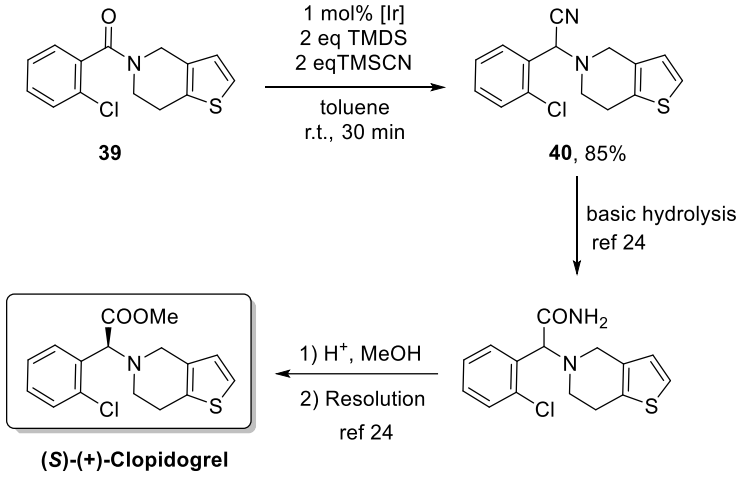


Figure 8: NOE experiment to determine the absolute configuration of compound **38** and hence of the major diastereomer of compound **33**.

Moreover, the synthetic utility of this new methodology was proven in the reductive cyanation of amide **39** (Scheme 4). The cyanated product **40**, a known precursor of the antiplatelet agent Clopidogrel (Plavix), was obtained in 85% yield, thus offering an alternative route for the first step of the synthesis of this drug.[24]



Scheme 4: Application of the Iridium-catalyzed reductive cyanation of amide **39** in the synthesis of Clopidogrel.

Finally, the late stage cyanation of a selection of alkaloids and drugs possessing tertiary amide or lactam residues was approached (Figure 9). Good yields were obtained in all cases and the reductive cyanation reaction proved to be diastereoselective for chiral alkaloids brucine and matrine, as proved by NOE studies conducted on the corresponding cyanated compounds **41** and **42** (see Figure 11 and 12, respectively, in the Experimental Section).

Drug molecules containing various heterocycles were well-tolerated (**46**, **48**). Also, alkene (**41**, **45**) and ester (**43**-**46**) functionalities proved to be inert under the reaction conditions. The presence of a free hydroxyl group, as in Tropicamide, is compatible with the reaction, but results in the formation of the *O*-silylated product, thus requiring higher amount of TMDS. However, even when the OH group is protected with an acetyl group, the presence of the pyridine ring seems to poison the catalyst, giving the cyanated product **43** in lower conversion.

Interestingly, the reaction was successful even working at μmol scale, and Fentanyl and Zolpidem were cyanated in 91 and 80% yield (**47**-**48**), respectively, thus validating the versatility of this methodology in late stage functionalization.[25] In fact, the reaction is simple to perform and highly chemoselective, therefore the corresponding cyanated product can be recovered easily from the reaction mixture after basic neutralization of TMSCN and extraction with organic solvent.

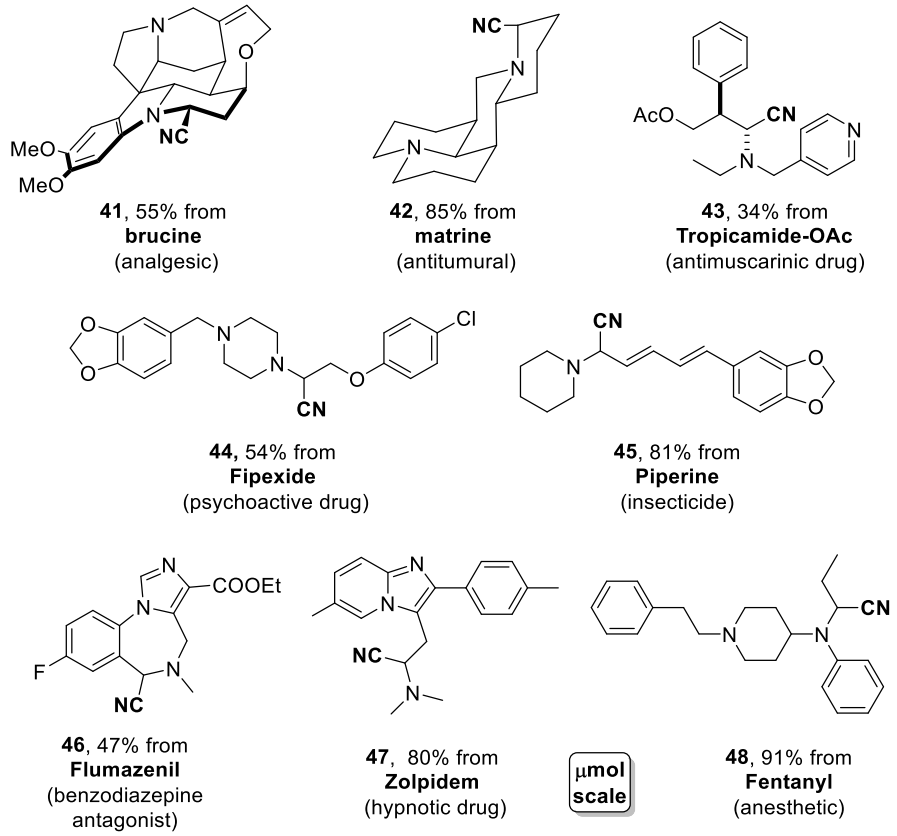


Figure 9: Late stage cyanation of natural products and drugs.

Although we have demonstrated that the reductive cyanation occur with broad scope and can be conducted on complex molecules, there are also some limitations. As reported previously, the reductive cyanation reaction is not working on carboxylic amide group with reduced Lewis basicity, as those contained in Dichlormid or Loperamide (Figure 10). Praziquantel, as other substrates with double amide functionalities, gave a complex mixture of

degradation and self condensation products, whereas the tetrazole moiety of Valsartan poisoned the catalyst irreversibly and did not allow to perform the reaction.

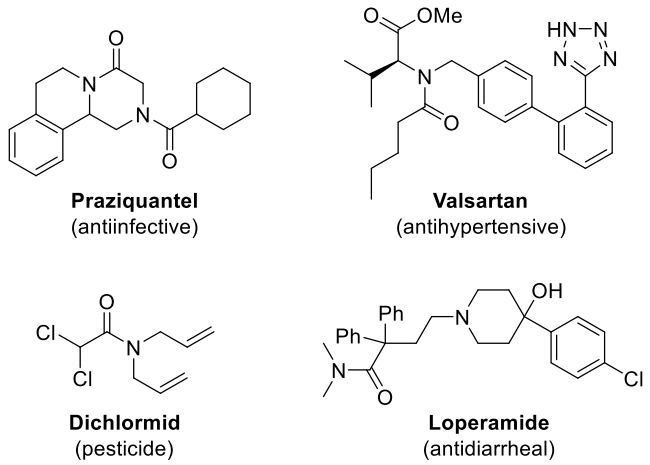
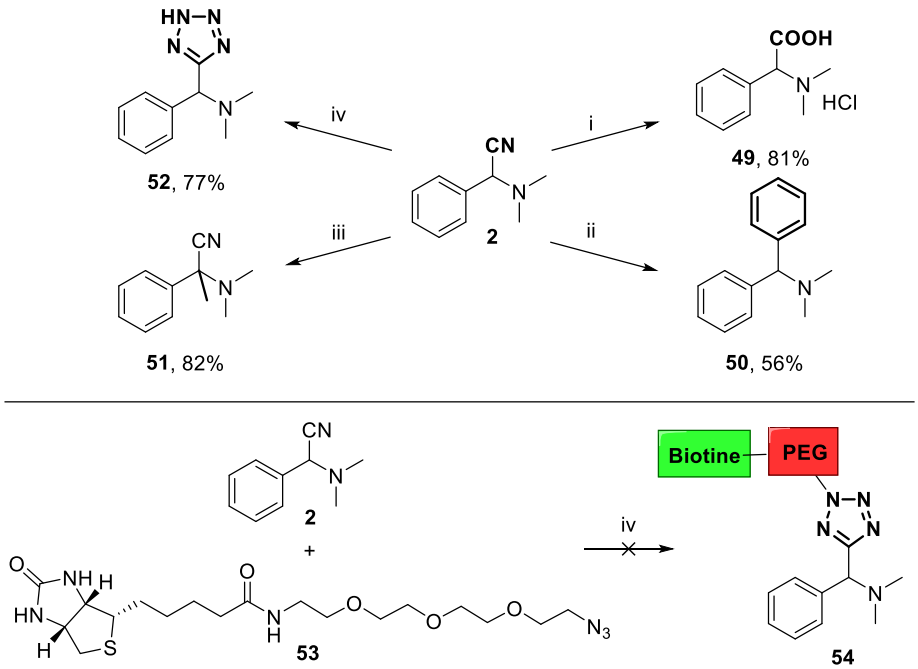


Figure 10: Substrates that did not react in the reductive cyanation.

As discussed in the introduction, the nitrile group is a valuable and versatile precursor to a wide range of functional groups and α-amino nitriles, possessing several modes of reactivity, can serve as key precursor for many synthetic applications (see Scheme 14 in Chapter 2.3). To prove that and to show the versatility of the late stage cyanation for further derivatization, we tried to apply different reaction conditions to a simple model substrate, as compound **2** (Scheme 5, top). Firstly, in an historical point of view,[7] the nitrile group of compound **2** was hydrolyzed to the carboxylic acid, thus achieving the corresponding dimethylated amino acid analogue **49** in nearly quantitative yield. In addition, the characteristic behavior of the α-amino nitriles of acting as masked iminium ion was proven by the successful substitution of the cyanide by a phenyl ring with PhMgBr, in analogy with Bruylant reaction conditions (**50**).[26] Furthermore, as already mentioned, α-amino nitriles bearing an α-hydrogen can be deprotonated under basic conditions generating α-amino carbanions, with formally reversed polarity and *umpolung* reactivity at α-carbon, capable of nucleophilic attack on a number of different electrophilic reagents.[27] This third mode of reactivity was exploited, in a representative example of deprotonation/methylation of compound **2** using KHMDS and MeI (**51**). Finally, the nitrile group was transformed into a tetrazole (**52**), using large

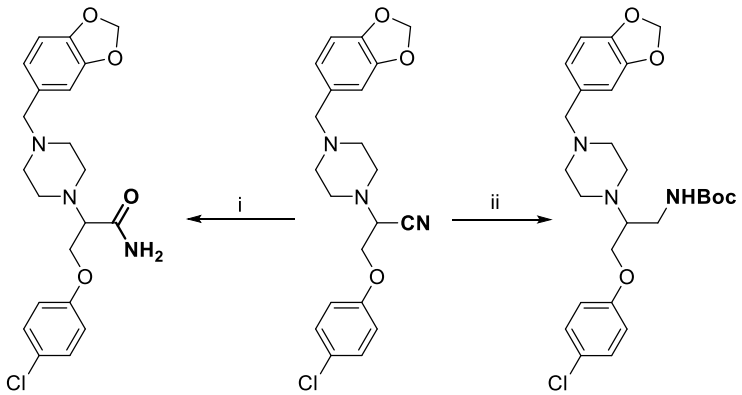
eccess of $TMSN_3$ at 70 °C in the presence of dibutyl oxide. No reaction was observed using sodium azide, neither exploiting the addition of different auxiliaries, as tris(pentafluorophenyl)borane or zinc chloride. Starting from this result, we envisioned if the cyano group could be used in bioconjugation chemistry, considering that several azide-linked probes, labelling reagents and other biologically relevant small molecules, are now commercially available, thanks to the wide use in chemical biology of the copper-catalyzed 1,3 dipolar cycloaddition (CuAAC) click chemistry and the copper-free click chemistry with cyclooctyne derivatives. For this reason, we tried to apply the same reaction conditions using azide-PEG_3-biotin conjugate **53**, in order to promote the formation of bioconjugated adduct **54** (Scheme 5, bottom). However, the harsh reaction conditions required for the tetrazole formation, resulted in breaking the labile azide linker and no reaction was observed with this substituted azide.



Scheme 5: Derivatization of compound **2**. Reagents and conditions: (i) HCl conc, reflux, 24 h; (ii) PhMgBr, THF, r.t., 3 h; (iii) KHMDS, MeI, THF, r.t., 30 min; (iv) $TMSN_3$, Bu_2SnO, toluene, 70 °C, 4 days.

Finally, to prove that these kind of functionalization were also possible on complex cyanated compounds, such as drug derivatives, the cyanation of Fipexide was scaled up with no yield erosion. Then, different ways of

functionalization were attempted. However, this substrate proved to be more challenging, as the application of not mild conditions results in many cases in the elimination of *p*-chloro-phenol, which proved to act as a good leaving group. In fact, the deprotection/alkylation approach or the addition of Grignard reagents were not compatible with this substrate, because of the strong basic conditions, and the tetrazole formation was not possible, due to the high temperature required. No reduction or acidic hydrolysis was also observed using DIBAL and HCl. However, cyanated Fipexide **44** was converted to amide **55** through partial hydrolysis with H_20_2 under mild conditions (Scheme 6). In addition, the reduction of α-amino nitrile moiety to the corresponding diamines was obtained with $NaBH_4$ and $NiCl_2$ with 38% yield. Pleasingly, the *in situ* protection with Boc_2O of the resulting amine functionality allowed to increase the yield of the final *N*-Boc-protected compound **56** to 57% (Scheme 6), thus validating the introduction of the cyano moiety, even in complex molecules, as an interesting point of diversification to be exploited for functional group interconversion and further chemical manipulations.



Scheme 6: Derivatization of cyanated Fipexide **44**. Reagents and conditions: (i) K_2CO_3, H_2O_2, DMSO, r.t., 24 h; (ii) $NiCl_2{\cdot}6H_2O$, $NaBH_4$, Boc_2O, MeOH, 0 °C, 1 h.

7.3 Conclusions

In conclusion, a new iridium catalyzed reductive Strecker reaction for the introduction of a nitrile residue into amide and lactam containing substrates has been developed. The reaction is simple to perform, chemoselective,

functional group tolerant, requires low catalyst loading, and has proven to be successful for the generation of a wide variety of novel α-amino nitriles, starting from their corresponding tertiary amides and lactams. As discussed in details in Chapter 2.3, these compounds proved to be versatile intermediates for a wide range of synthetic applications, and can be used as polyfunctional *N*-containing building blocks for the development of novel skeletally different scaffold in a diversity-oriented fashion. In particular, considering the importance of *N*-heterocycles in drugs and biologically active compounds and the relevance of the nitrile moiety in medicinal chemistry, these building blocks can be particularly useful for library generation for drug discovery issues.

Moreover, taking advantage of the high chemoselectivity of this methodology for tertiary amides, the selective cyanation of the carbonyl carbon linked to the *N*-atom of a proline residue in di- and tripeptides has been achieved with good yields and selectivities, thus opening the way to important applications in both drug design and labelling.

Finally, the late stage cyanation of a selection of alkaloids and commercially available drugs possessing tertiary amide or lactam residues was approached, even working at μmol scale. The potentialilty in introducing nitrile compound in complex molecules is extremely interesting for Diversity-Oriented Synthesis. In fact, the direct transformation of complex precursors into cyanated compounds, can be used as a starting point for divergent approaches, to explore new region of chemical space around a structure that already possess biological activity.

7.4 Experimental section

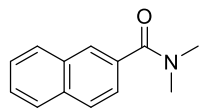
General Analytical grade solvents and commercially available reagents were used without further purification. Toluene was distilled twice over calcium hydride. Reactions requiring inert atmosphere were carried out under argon atmosphere. Chromatographic purification was performed on VWR 60 silica gel 40-63 μm using HPLC grade solvents that were used as supplied. All reactions were followed by thin-layer chromatography (TLC) when practical, using Merck Kieselgel 60 F_{254} fluorescent treated silica. Visualisation was accomplished under UV light (λ_{max}= 254 nm) and by staining with *p*-anisaldehyde staining dip. Melting points were obtained on a Leica Galen III Hot-stage microscope apparatus equipped with a digital thermometer and are reported as uncorrected. NMR spectra were recorded on Bruker spectrometers operating at 400 or 500 MHz (^{1}H resonance). Proton chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (TMS) with the solvent resonance as internal standard. ^{13}C-NMR spectra were recorded with complete proton decoupling. High resolution mass spectra (HRMS) were recorded on a Bruker Daltonics MicroTOF mass spectrometer equipped with an ESI source or on a Micromass GCT equipped with an EI source unless otherwise specified. Infrared absorption spectra (IR) were recorded on a Bruker Tensor 27 FT-IR spectrometer from a thin film on a diamond ATR module. Optical rotations were recorded using a Schmidt Haensch UniPol polarimeter. $[\alpha]_D^T$ values, reported in 10^{-1} deg cm^2 g^{-1}, are calculated on the average value of ten consecutive readings.

7.4.1. Synthesis and characterization data for starting materials

General procedure for the synthesis of amides

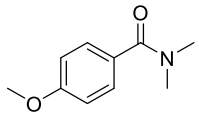
To a solution of the amine (1.1 eq) and Et_3N (2.25 eq) in dichloromethane (2 mL/mmol), acyl chloride (1 eq) was added in one portion at 0 °C. The reaction mixture was stirred overnight at room temperature until TLC showed disappereance of starting material. The solution, diluted with dichloromethane, was washed with 1N HCl, dried over Na_2SO_4 and concentrated under reduced pressure.

Synthesis of *N*,*N*-dimethyl-2-naphthamide



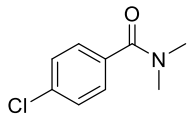
According to the general procedure, using 2-naphthoyl chloride (452 µL, 3 mmol) and dimethylamine hydrochloride (270 mg, 3.3 mmol), *N,N*-dimethyl-2-naphthamide (419 mg, 2.10 mmol) was obtained in 70% yield as a white crystalline solid, after flash column chromatography on silica gel (EtOAc / PE 1:1). Spectroscopical data are in agreement with the literature values.[28]

Synthesis of *N*,*N*-dimethyl-4-methoxy-benzamide



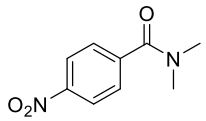
According to the general procedure, using 4-anisoyl chloride (270 µL, 2 mmol) and dimethylamine hydrochloride (180 mg, 2.2 mmol), *N,N*-dimethyl-4-methoxy-benzamide (340 mg, 1.89 mmol) was obtained in 95% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 1:1). Spectroscopical data are in agreement with the literature values.[29]

Synthesis of *N*,*N*-dimethyl-4-chloro-benzamide



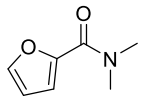
According to the general procedure, using 4-chlorobenzoyl chloride (256 µL, 2 mmol) and dimethylamine hydrochloride (180 mg, 2.2 mmol), *N,N*-dimethyl-4-chloro-benzamide (325 mg, 1.77 mmol) was obtained in 89% yield as a white crystalline solid, after flash column chromatography on silica gel (EtOAc / PE 1:1). Spectroscopical data are in agreement with the literature values.[30]

Synthesis of *N*,*N*-dimethyl-4-nitrobenzamide



According to the general procedure, using 4-nitrobenzoyl chloride (371 mg, 2 mmol) and dimethylamine hydrochloride (180 mg, 2.2 mmol), *N,N*-dimethyl-4-nitrobenzamide (364 mg, 1.88 mmol) was obtained in 94% yield as a white crystalline solid, after recrystallization from dichloromethane/hexane. Spectroscopical data are in agreement with the literature values.[30]

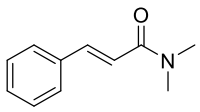
Synthesis of *N*,*N*-dimethylfuran-2-carboxamide



According to the general procedure, using 2-furoyl chloride (295 µL, 3 mmol) and dimethylamine hydrochloride (270 mg, 3.3 mmol), *N*,*N*- dimethylfuran-2-carboxamide (356 mg, 2.56 mmol)

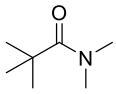
was obtained in 85% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 1:1). Spectroscopical data are in agreement with the literature values.[30]

Synthesis of *N,N*-dimethylcinnamamide



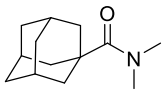
According to the general procedure, using cinnamoyl chloride (333 mg, 2 mmol) and dimethylamine hydrochloride (180 mg, 2.2 mmol), *N,N*-dimethylcinnamide (292 mg, 1.66 mmol) was obtained in 84% yield as a white crystalline solid, after recrystallization from dichloromethane. Spectroscopical data are in agreement with the literature values.[30]

Synthesis of *N,N*-dimethylpivalamide



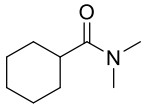
According to the general procedure, using pivaloyl chloride (370 µL, 3 mmol) and dimethylamine hydrochloride (270 mg, 3.3 mmol), *N,N*-dimethylpivalamide (296 mg, 2.29 mmol) was obtained in 76% yield as a yellow oil, after flash column chromatography on silica gel (EtOAc / PE 1:1). Spectroscopical data are in agreement with the literature values.[31]

Synthesis of *N,N*-dimethyl-1-adamantanecarboxamide



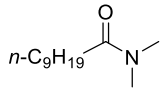
According to the general procedure, using 1-adamantyl chloride (380 mg, 2 mmol) and dimethylamine hydrochloride (180 mg, 2.2 mmol), *N,N*-dimethyl-1-adamantanecarboxamide (365 mg, 1.76 mmol) was obtained in 88% yield as a white crystalline solid, after flash column chromatography on silica gel (EtOAc / PE 1:1). Spectroscopical data are in agreement with the literature values.[32]

Synthesis of *N,N*-dimethylcyclohexanecarboxamide



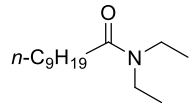
According to the general procedure, using cyclohexyl carbonyl chloride (267 µL, 2 mmol) and dimethylamine hydrochloride (180 mg, 2.2 mmol), *N,N*-dimethylcyclohexylcarboxamide (263 mg, 1.69 mmol) was obtained in 85% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE 2:1). Spectroscopical data are in agreement with the literature values.[33]

Synthesis of *N*,*N*-dimethyldecanamide



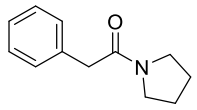
According to the general procedure, using decanoyl chloride (622 µL, 3 mmol) and dimethylamine hydrochloride (270 mg, 3.3 mmol), *N,N*-dimethyldecanamide (455 mg, 2.28 mmol) was obtained in 76% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 1:1). Spectroscopical data are in agreement with the literature values.[34]

Synthesis of *N*,*N*-diethyldecanamide



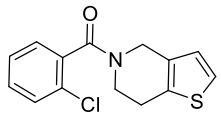
According to the general procedure, using decanoyl chloride (622 µL, 3 mmol) and diethylamine hydrochloride (361 mg, 3.3 mmol), *N,N*-diethyldecanamide (617 mg, 2.69 mmol) was obtained in 89% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE 1:2). Spectroscopical data are in agreement with the literature values.[35]

Synthesis of 2-phenyl-1-(pyrrolidin-1-yl)ethan-1-one



According to the general procedure, using 2-phenylacetyl chloride (0.66 mL, 5 mmol) and pyrrolidine (0.5 mL, 6 mmol), 2-phenyl-1-(pyrrolidin-1-yl)ethan-1-one (860 mg, 4.5 mmol) was obtained in 91% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 2:1). Spectroscopical data are in agreement with the literature values.[36]

Synthesis of 5-chlorobenzoyl-4,5,6,7-tetrahydro-thieno[3,2-*c*]pyridine (39)



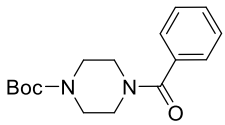
According to the general procedure, using 2-chlorobenzoyl chloride (1.10 g, 6 mmol) and 4,5,6,7-tetrahydro-thieno[3,2-*c*]pyridine (1.6 g, 6.6 mmol), 5-chlorobenzoyl-4,5,6,7-tetrahydro-thieno[3,2-*c*]pyridine (1.46 g, 5.3 mmol) was obtained in 88% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 1:2). Spectroscopical data are in agreement with the literature values.[37]

General procedure for the synthesis of *N*-benzoylated substrates

To a solution of amine (1.1 eq) and triethylamine (1.2 eq) in dichloromethane (4 mL/mmol), under inert atmosphere and at 0 °C, a solution of benzoylchloride

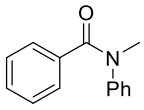
(1.1 eq) in dichloromethane (1 mL/mmol) was added. The solution was stirred overnight at room temperature and then quenched with water. The reaction mixture was extracted with dichloromethane, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure.

Synthesis of *N*-Benzoyl-4-*N*-Boc-piperazine



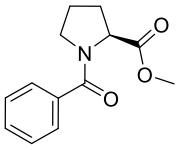
According to the general procedure, using *N*-Boc-piperazine (186 mg, 1 mmol), *N*-Benzoyl-4-*N*-Boc-piperazine (273 mg, 0.94 mmol) was obtained in 94% yield as a white crystalline solid, after recrystallization from EtOAc / PE. Spectroscopical data are in agreement with the literature values.[38]

Synthesis of *N*-Methyl-*N*-phenylbenzamide



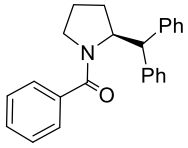
According to the general procedure, using *N*-methylaniline (108 µL, 1 mmol) *N*-methyl-*N*-phenylbenzamide (157 mg, 0.74 mmol) was obtained in 74% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE 1:2). Spectroscopical data are in agreement with the literature values.[39]

Synthesis of *N*-benzoyl-*L*-proline methyl ester



According to the general procedure, using *L*-proline methyl ester hydrocloride (1.4 g, 8.45 mmol), *N*-benzoyl-*L*-proline methyl ester (1.61 g, 6.93 mmol) was obtained in 82% yield as a colourless crystalline solid, after flash column chromatography on silica gel (AcOEt / PE 1:1). Spectroscopical data are in agreement with the literature values.[40]

Synthesis and characterization of (*S*)-(2-benzhydrylpyrrolidin-1-yl)(phenyl)methanone

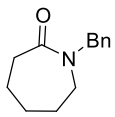


According to the general procedure, using (*S*)-diphenylmethylpyrrolidine (166 mg, 0.7 mmol), (*S*)-(2-benzhydrylpyrrolidin-1-yl)(phenyl)methanone (191 mg, 0.56 mmol) was obtained in 80% yield as a yellow foam, after flash column chromatography on silica gel (Et_2O / PE 1:2).

$[\alpha]_D^{25}$ = - 93.6 (*c* 0.94, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.37 – 6.98 (m, 15H), 5.24 (dq, *J* = 8.9, 5.2, 3.7 Hz, 1H), 4.61 (d, *J* = 6.9 Hz, 1H), 3.20 (ddd, *J* = 12.1, 7.9,

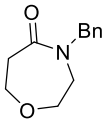
4.7 Hz, 1H), 2.99 (ddd, *J* = 10.9, 7.8, 7.8 Hz, 1H), 2.07 (dq, *J* = 14.6, 7.6 Hz, 1H), 1.86 (dq, *J* = 12.8, 6.8 Hz, 1H), 1.64-1.57 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.0, 142.0, 141.6, 137.2, 129.9, 129.8 (2C), 128.8 (2C), 128.5 (2C), 128.3 (2C), 128.1 (2C), 127.4 (2C), 126.7, 126.4, 59.4, 52.7, 49.8, 27.6, 24.5; IR (film) *v* = 3059, 3028, 2971, 2878, 1626, 1409 cm^{-1}; HRMS (ESI): calcd. for $C_{24}H_{24}ON^+$ $[M+H]^+$ 342.1852, found *m/z* 342.1848.

Synthesis of 1-benzylazepan-2-one



To a solution of sodium hydride (637 mg, 26.6 mmol) in THF (80 mL), Ɛ-caprolactam (2.00 g, 17.7 mmol) in THF (10 mL) and benzylbromide (4.2 mL, 35.4 mmol) were added sequentially at 0 °C. Then, the mixture was let stirring at room temperature. After four hours, water (15 mL) was added under ice conditions and the product was extracted with EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. After flash column chromatography on silica gel (EtOAc / PE 1:1), the product was obtained as a white solid in 75% yield. Spectroscopical data are in agreement with the literature values.[41]

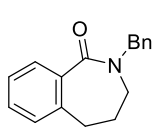
Synthesis and characterization of 4-benzyl-1,4-oxazepan-5-one



To a solution of sodium hydride (208 mg, 5.2 mmol) in THF (10 mL), 1,4-oxazepan-5-one (400 mg, 3.47 mmol) in THF (5 mL) and benzylbromide (0.82 mL, 6.94 mmol) were added sequentially at 0 °C. After stirring at room termperature for 3 hours, water (5 mL) was added at 0 °C and the product was extracted with EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by flash column chromatography on silica gel (EtOAc / PE 1:1), and obtained as a colourless oil in 84% yield**.**

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.14 (m, 5H), 4.53 (s, 2H), 3.76 – 3.68 (m, 2H), 3.53 – 3.46 (m, 2H), 3.36 – 3.29 (m, 2H), 2.79 – 2.72 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.4, 137.2, 128.7 (2C), 128.2 (2C), 127.6, 70.2, 65.3, 51.4, 50.8, 41.1; IR (film) *v* = 2958, 2918, 2853, 1642, 1477, 1427, 1359, 1292 cm^{-1}; HRMS (ESI): calcd. for $C_{12}H_{16}O_2N^+$ $[M+H]^+$ 206.1176, found *m/z* 206.1177.

Synthesis of 2-benzyl-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepin-1-one



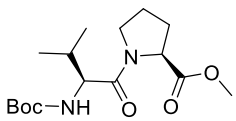
α-Tetralone (1.00 g, 6.84 mmol) was dissolved in concentrated hydrochloric acid (12 mL) in an ice bath and sodium azide (889

mg, 13.7 mmol) was added slowly. After complete addition, the mixture was stirred until it reached room temperature, then it was heated at 50 °C for 16 hours. The solution was poured onto ice-cold water and made alkaline to pH 10 with a 1M K_2CO_3 aqueous solution. After flash column chromatography on silica gel (EtOAc/PE 9:1), 2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepin-1-one (532 mg) was isolated as a pale yellow solid in 48% yield. This compound (343 mg, 2.13 mmol) was dissolved in THF (1 mL) and added to a solution of sodium hydride (76.8 mg, 3.20 mmol) in THF (8 mL). Benzylbromide (0.51 mL, 4.26 mmol) was added sequentially at 0 °C and the mixture was stirred at room temperature for 4 h. Then water (1.5 mL) was added under ice conditions and the product was extracted with EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. After flash column chromatography on silica gel (EtOAc / PE 2:3), the product was obtained (390 mg, 73%) in the form of a white solid. Spectroscopical data are in agreement with the literature values.[42]

General procedure for solution phase peptide coupling

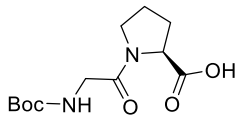
To a solution of Boc-aa-OH (1 eq) in dichloromethane, 1-Hydroxybenzotriazole (HOBt) (1.1 eq) and dicyclohexylcarbodiimide (DCC) (1.2 eq) were added sequentially at 0 °C. After 1 hour, the second aa-OMe hydrocloride, dissolved in DMF (less as possible), was added dropwise, followed by the addition of Et_3N. The reaction mixture was left stirring at room temperature for 24 hours. The precipitated dicyclohexylurea was filtered off and washed with dichloromethane. The filtrate was evaporated under reduced pressure and the crude compound was acidified at 0 °C with 1N HCl. After filtration of the resulting Et_3N salts, the product was extracted with EtOAc, washed with a saturated solution of $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated under reduced pressure.

Synthesis of Boc-*L*-Val-*L*-Pro-OMe



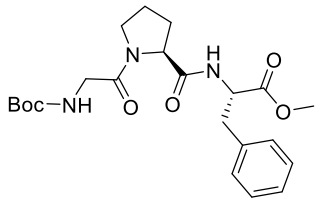
According to the general procedure, using Boc-*L*-valine (1.43 g, 6.6 mmol) and *L*-proline methyl ester hydrochloride (1.2 g, 7.26 mmol), Boc-*L*-Val-*L*-Pro-OMe was obtained in 92% yield as a white foam, after flash column chromatography on silica gel (DCM / MeOH 40:1). Spectroscopical data are in agreement with the literature values.[43]

Synthesis of Boc-*L*-Gly-*L*-Pro-OMe



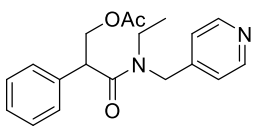
According to the general procedure, using Boc-*L*-glycine (1.15 g, 6.6 mmol) and *L*-proline methyl ester hydrochloride (1.2 g, 7.26 mmol), Boc-*L*-Gly-*L*-Pro-OMe was obtained in 86% yield as a colourless crystalline solid, after flash column chromatography on silica gel (DCM / MeOH 20:1). Spectroscopical data are in agreement with the literature values.[44]

Synthesis of Boc-*L*-Gly-*L*-Pro-*L*-Phe-OMe



Boc-*L*-Gly-*L*-Pro-OMe (1g, 3.5 mmol) was dissolved in methanol (2 mL) and 1N NaOH (4.2 mL) and stirred for 15 hours. Then, methanol was evaporated under reduced pressure and water was added under ice cold conditions. The solution was acidified with 1N HCl, extracted with EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting Boc-*L*-Gly-*L*-Pro-OH (940 mg, 3.45 mmol) was coupled using the general procedure, to *L*-phenylalanine methyl ester hydrochloride (819 mg, 3.80 mmol). Boc-*L*-Gly-*L*-Pro-*L*-Phe-OMe was obtained in this way in 76% yield as a colourless crystalline solid, after flash column chromatography on silica gel (EtOAc / PE 1:1). Spectroscopical data are in agreement with the literature values.[45]

Synthesis and characterization of 3-(ethyl(pyridin-4-ylmethyl)amino)-3-oxo-2-phenylpropyl acetate (Tropicamide-OAc)



Tropicamide (250 mg, 0.87 mmol) was dissolved in 1 mL of pyridine. Acetic anhydride (0.5 mL) was then added dropwise and the mixture was left stirring at room temperature overnight. Volatiles were then removed under reduced pressure using toluene, giving the acylated tropicamide in 92% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 1:1).

^{1}H-NMR (400 MHz, $CDCl_3$) 1:1 mixture of rotamers δ 8.47 (d, *J* = 6.0 Hz, 1H), 8.41 (d, *J* = 6.0 Hz, 1H), 7.35 – 7.12 (m, 5H), 6.96 (d, *J* = 6.0 Hz, 1H), 6.93 (d, *J* = 6.0 Hz, 1H), 4.70 – 4.22 (m, 4H), 4.14 and 3.82 (dd, *J* = 8.9, 5.3 Hz, 1H), 3.68 – 3.57 (m, 0.5H), 3.33 (dtd, *J* = 14.3, 7.2, 1.0 Hz, 1H), 3.20 – 3.00 (m, 0.5H), 1.98

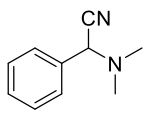
and 1.94 (s, 3H), 1.03 and 0.97 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) 1:1 mixture of rotamers δ 170.9, 170.8, 150.2 and 149.9 (2C), 146.9, 135.4, 129.2 and 129.1 (2C), 128.2, 128.1 and 128.0 (2C), 122.3 and 121.2 (2C), 66.6 and 66.5, 49.2 and 47.8, 48.3 and 47.8, 42.3 and 41.6, 20.9, 14.0 and 12.5; IR (film) *v* = 3028, 2933, 1738, 1643, 1449, 1422, 1371, 1235, 1037 cm^{-1}; HRMS (ESI): calcd. for $C_{19}H_{23}N_2O_3^+$ $[M+H]^+$ 327.1703, found *m/z* 327.1700.

7.4.2. Synthesis and characterization data of α-aminonitrile compounds

General procedure:

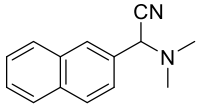
To a solution of the amide/lactam (0.3 mmol) in dry toluene (6 mL), Vaska's catalyst (1 mol% **(a)** or 2 mol% **(b)**) was added. The resulting suspension was stirred for 5 minutes giving a yellow solution. TMDS (2 eq **(a)** or 4 eq **(b)**) was added in one portion and the reaction mixture stirred for 5 minutes until H_2 gas evolution had ceased and the solution turned colourless, then TMSCN (2 eq) was added in one portion and stirred for 30 minutes or overnight. The solution was then washed with 1N NaOH, extracted with EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. Pure α-aminonitrile samples were obtained after flash column chromatography on silica gel.

Synthesis of 2-(dimethylamino)-2-phenylacetonitrile (2)



According to the general procedure **a**, using *N*,*N*-dimethylbenzamide (44.8 mg, 0.3 mmol), compound **2** was obtained in 92% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 1:4). Spectroscopical data are in agreement with the literature values.[46]

Synthesis and characterization of 2-(dimethylamino)-2-(naphthalen-2 yl)acetonitrile (3)

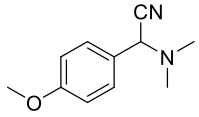


According to the general procedure **a**, using *N*,*N*-dimethyl-2-naphthamide (60 mg, 0.3 mmol), compound **3** was obtained in 98% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE 1:4).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.4 Hz, 1H), 7.81 (m, 2H), 7.71 (d, *J* = 7.1 Hz, 1H), 7.51-7.38 (m, 3H), 5.39 (s, 1H), 2.30 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 134.0, 130.9, 130.1, 129.1, 128.8, 126.9, 126.6, 126.3, 124.8, 123.6, 113.5,

61.6, 41.6 (2C); IR (film) *v* = 3050, 2953, 2785, 2225, 1511, 1458 cm^{-1}; HRMS (ESI): calcd. for $C_{14}H_{15}N_2^+$ $[M+H]^+$ 211.1230, found *m/z* 211.1230.

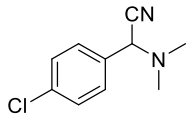
Synthesis and characterization of 2-(dimethylamino)-2-(4-methoxyphenyl)acetonitrile (4)



According to the general procedure **a**, using *N*,*N*-dimethyl-4-methoxy-benzamide (54 mg, 0.3 mmol), compound **4** was obtained in quantitative yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 1:5).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.4 Hz, 2H), 6.78 (d, *J* = 8.7 Hz, 2H), 4.65 (s, 1H), 3.68 (s, 3H), 2.16 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 159.9, 129.1 (2C), 125.7, 115.3, 114.1 (2C), 62.5, 55.4, 41.6 (2C); IR (film) *v* = 2953, 2836, 2786, 2254, 1612, 1512, 1459, 1252 cm^{-1}; HRMS (ESI): calcd. for $C_{11}H_{15}ON_2^+$ $[M+H]^+$ 191.1179, found *m/z* 191.1180.

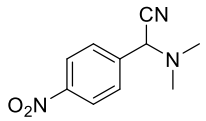
Synthesis and characterization of 2-(dimethylamino)-2-(4-chlorophenyl)acetonitrile (5)



According to the general procedure **a**, using *N*,*N*-dimethyl-4-chloro-benzamide (52 mg, 0.3 mmol), compound **5** was obtained in 88% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 1:5).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.5 Hz, 2H), 7.31 (d, *J* = 8.6 Hz, 2H), 4.74 (s, 1H), 2.24 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 133.8, 131.2, 128.1 (2C), 127.9 (2C), 113.6, 61.5, 40.7 (2C); IR (film) *v* = 2923, 2861, 2788, 2358, 1727, 1488, 1460 cm^{-1}; HRMS (ESI): calcd. for $C_{10}H_{12}ClN_2^+$ $[M+H]^+$ 195.0684, found *m/z* 195.0685.

Synthesis and characterization of 2-(dimethylamino)-2-(4-nitrophenyl)acetonitrile (6)

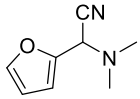


According to the general procedure **a**, using *N*,*N*-dimethyl-4-nitro-benzamide (58 mg, 0.3 mmol), compound **6** was obtained in 63% yield as a colourless oil, after silica gel (washed with 1% Et_3N) column chromatography (Et_2O / PE 1:4).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 9.0 Hz, 2H), 4.86 (s, 1H), 2.27 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 148.3, 140.7, 128.7 (2C), 124.0

(2C), 114.1, 62.6, 41.9 (2C); IR (film) *v* = 2923, 1952, 1523, 1346 cm^{-1}; HRMS (ESI): calcd. for $C_{10}H_{12}O_2N_3^+$ $[M+H]^+$ 206.0924, found *m/z* 206.0926.

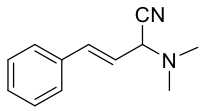
Synthesis and characterization of 2-(dimethylamino)-2-(furan-2-yl)acetonitrile (7)



According to the general procedure **a**, using *N*,*N*-dimethylfuran-2-carboxamide (42 mg, 0.3 mmol), compound **7** was obtained in 94% yield as a pale yellow oil, after flash column chromatography on silica gel (Et_2O / PE 1:3).

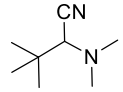
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 1.4 Hz, 1H), 6.48 (d, *J* = 3.3 Hz, 1H), 6.33 (dd, *J* = 3.3, 1.9 Hz, 1H), 4.80 (s, 1H), 2.29 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 146.5, 143.9, 113.5, 110.5, 110.4, 56.8, 41.6 (2C); IR (film) *v* = 2956, 2872, 2834, 2789, 2232, 1458 cm^{-1}; HRMS (ESI): calcd. for $C_8H_{11}N_2O^+$ $[M+H]^+$ 151.0866, found *m/z* 151.0867.

Synthesis of (*E*)-2-(dimethylamino)-4-phenylbut-3-enenitrile (8)



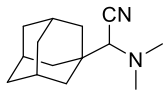
According to the general procedure **a**, using *N*,*N*-dimethylcinnamide (53 mg, 0.3 mmol), compound **8** was obtained in 61% yield as a colourless oil, after flash column chromatography on silica gel (washed with 1% Et_3N) (Et_2O / PE 1:4). Spectroscopical data are in agreement with the literature values.[47]

Synthesis of 2-(dimethylamino)-3,3-dimethylbutanenitrile (9)



According to the general procedure **b**, using *N*,*N*-dimethylpivalamide (39 mg, 0.3 mmol), compound **9** was obtained in 86% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE 1:4). Spectroscopical data are in agreement with the literature values.[48]

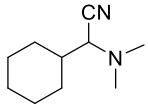
Synthesis and characterization of 2-(adamantan-1-yl)-2-(dimethylamino)acetonitrile (10)



According to the general procedure **b**, using *N*,*N*-dimethyl-1-adamantanecarboxamide (62 mg, 0.3 mmol), compound **10** was obtained in 71% yield as a colourless crystalline solid, after flash column chromatography on silica gel (Et_2O / PE 1:9).

M.P. = 49-50 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.94 (s, 1H), 2.30 (s, 6H), 1.96 (br s, 3H), 1.66-1.53 (m, 12H); ^{13}C-NMR (100 MHz, $CDCl_3$) 115.3, 70.0, 45.6 (2C), 39.6 (3C), 37.4, 36.6 (3C), 28.3 (3C); IR (film) ν = 2905, 2850, 2789, 2223, 1453, 1030 cm^{-1}; HRMS (ESI): calcd. for $C_{14}H_{23}N_2^+$ $[M+H]^+$ 219.1856, found *m/z* 219.1857.

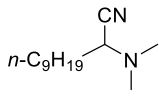
Synthesis and characterization of 2-cyclohexyl-2-(dimethylamino)acetonitrile (11)



According to the general procedure **a**, using *N*,*N*-dimethyl-cyclohexanecarboxamide (47 mg, 0.3 mmol), compound **11** was obtained in 90% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE 1:9).

^{1}H-NMR (400 MHz, CD_3CN) δ 3.20 (d, *J* = 10.9 Hz, 1H), 2.14 (s, 6H), 1.86 - 1.81 (m, 3H), 1.71 – 1.54 (m, 4H), 1.22 – 1.08 (m, 4H), 0.99–0.80 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 116.3, 65.1, 41.9 (2C), 38.2, 30.8, 29.7, 26.3, 25.6, 25.4; IR (film) ν = 2921, 2857, 1733, 1611, 1452 cm^{-1}; HRMS (ESI): calcd. for $C_{10}H_{19}N_2^+$ $[M+H]^+$ 167.1543, found *m/z* 167.1543.

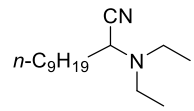
Synthesis and characterization of 2-(dimethylamino)undecanenitrile (12)



According to the general procedure **a**, using *N*,*N*-dimethyldecanamide (60 mg, 0.3 mmol), compound **12** was obtained in 72% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE 1:5).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.33 (dd, *J* = 8.6, 7.1 Hz, 1H), 2.17 (s, 6H), 1.67 – 1.48 (m, 2H), 1.33 – 1.27 (m, 2H), 1.15 – 1.12 (m, 12H), 0.74 (t, *J* = 8.7 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) 116.9, 58.8, 41.8 (2C), 31.9, 31.7, 29.5, 29.4, 29.3, 29.0, 26.0, 22.7, 14.1; IR (film) *v* = 2924, 2855, 2787, 2223, 1459, 1043 cm^{-1}; HRMS (ESI): calcd. for $C_{13}H_{27}N_2^+$ $[M+H]^+$ 211.2169, found *m/z* 211.2168.

Synthesis and characterization of 2-(diethylamino)undecanenitrile (13)

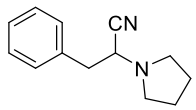


According to the general procedure **a**, using *N*,*N*-diethyldecanamide (68 mg, 0.3 mmol), compound **13** was obtained in 84% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE 1:9).

^{1}H-NMR (400 MHz, $CDCl_3$) 3.53 (t, *J* = 7.8 Hz, 1H), 2.63 (dt, *J* = 14.4, 7.2 Hz, 2H), 2.34 (dq, *J* = 13.6, 6.9 Hz, 2H), 1.75 – 1.58 (m, 2H), 1.42 – 1.32 (m, 2H), 1.25 – 1.22 (m, 12H), 1.01 (t, *J* = 7.1 Hz, 6H), 0.81 (t, *J* = 6.6 Hz, 3H); ^{13}C-NMR (100 MHz,

$CDCl_3$) 118.6, 54.1, 45.2 (2C), 31.9, 31.8, 29.5, 29.4, 29.3, 29.0, 26.1, 22.7, 14.1 (2C), 13.3; IR (film) ν = 2979, 2971, 2926, 2855, 2253, 1467, 1384 cm^{-1}; HRMS (ESI): calcd. for $C_{15}H_{31}N_2^+$ $[M+H]^+$ 239.2482, found *m/z* 239.2482.

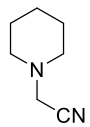
Synthesis and characterization of 3-phenyl-2-(pyrrolidin-1-yl)propanenitrile (14)



According to the general procedure **a** and 4 eq of TMSCN, using 2-phenyl-1-(pyrrolidin-1-yl)ethan-1-one (57 mg, 0.3 mmol), compound **14** was obtained in 81% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE 1:4).

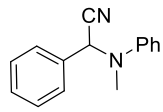
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39 – 6.90 (m, 5H), 3.88 (dd, *J* = 9.9, 5.9 Hz, 1H), 3.05 – 2.89 (m, 2H), 2.77 – 2.56 (m, 4H), 1.88 – 1.71 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 136.1, 129.1 (2C), 128.7 (2C), 127.3, 117.1, 57.8, 50.1 (2C), 39.3, 23.5 (2C); IR (film) *v* = 2965, 2814, 2221, 1493, 1454, 1132 cm^{-1}; HRMS (ESI): calcd. for $C_{13}H_{17}N_2^+$ $[M+H]^+$ 201.1386, found *m/z* 201.1387.

Synthesis of 2-(piperidin-1-yl)acetonitrile (15)



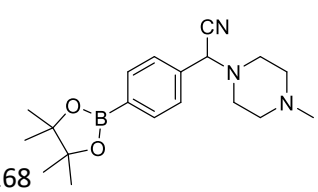
According to the general procedure **a**, using *N*-formylpiperidine (34 mg, 0.3 mmol), compound **15** was obtained in 94% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 1:1). Spectroscopical data are in agreement with the literature values.[49]

Synthesis of 2-(methyl(phenyl)amino)-2-phenylacetonitrile (16)



According to the general procedure **b**, using *N*-methyl-*N*-phenylbenzamide (64 mg, 0.3 mmol), compound **16** was obtained in 35% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE 1:9). Spectroscopical data are in agreement with the literature values.[50]

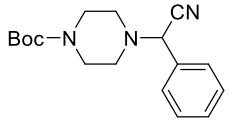
Synthesis and characterization of 2-(4-methylpiperazin-1-yl)-2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetonitrile (17)



According to the general procedure **a**, using (4-methylpiperazin-1-yl)(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanone

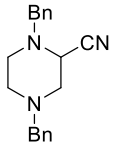
(104.3 mg, 0.32 mmol), compound **17** was obtained in 76% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 7.8 Hz, 2H), 4.78 (s, 1H), 2.54 (br s, 4H), 2.40 (br s, 4H), 2.22 (s, 3H), 1.28 (s, 12H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 135.9, 135.1 (2C), 127.2 (3C), 115.2, 84.0 (2C), 62.1, 54.7 (4C), 45.9, 24.9 (4C); IR (film) *v* = 2977, 2797, 2698, 2359, 1286, 1090 cm^{-1}; HRMS (ESI): calcd. for $C_{19}H_{29}O_2N_3B^+$ $[M+H]^+$ 342.2347, found *m/z* 342.2349.

Synthesis and characterization of *tert*-butyl 4-(cyano(phenyl)methyl)piperazine-1-carboxylate (18)



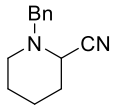
According to the general procedure **a**, using *N*-benzoyl-4-*N*.Boc-piperazine (87 mg, 0.3 mmol), compound **18** was obtained in 88% yield as a colourless crystalline solid, after flash column chromatography on silica gel (Et_2O / PE 1:4).
M.P. = 82-84 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 6.3 Hz, 2H), 7.34 – 7.27 (m, 3H), 4.77 (s, 1H), 3.45 – 3.23 (m, 4H), 2.43 (t, *J* = 4.9 Hz, 4H), 1.36 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 154.5, 132.6, 129.1 (2C), 128.9 (2C), 127.9, 115.1, 80.0, 62.2, 49.5 (2C), 43.8, 43.1, 28.4 (3C); IR (film) *v* = 3029, 2969, 2226, 1596, 1492, 1452 cm^{-1}; HRMS (ESI): calcd. for $C_{17}H_{24}O_2N_3^+$ $[M+H]^+$ 302.1863, found *m/z* 302.1864.

Synthesis of 1,4-dibenzylpiperazine-2-carbonitrile (19)



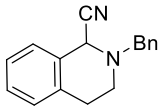
According to the general procedure **a**, using 1,4-dibenzylpiperazin-2-one (84 mg, 0.3 mmol), compound **19** was obtained in 34% yield after flash column chromatography on silica gel. Spectroscopical data are in agreement with the literature values.[51]

Synthesis of 1-benzylpiperidine-2-carbonitrile (20)



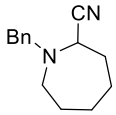
According to the general procedure **a**, using *N*-benzylpiperidone (57 mg, 0.3 mmol), compound **20** was obtained in 55% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 1:9). Spectroscopical data are in agreement with the literature values.[52]

Synthesis of 2-benzyl-1,2,3,4-tetrahydroisoquinoline-1-carbonitrile (21)



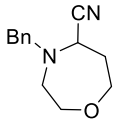
According to the general procedure **a**, using *N*-Benzyl-1,2,3,4-tetrahydroisoquinoline-2-one (71 mg, 0.3 mmol), compound **21** was obtained in 67% yield as a white solid, after flash column chromatography on silica gel (EtOAc / PE 1:9). Spectroscopical data are in agreement with the literature values.[53]

Synthesis of 1-benzylazepane-2-carbonitrile (22)



According to the general procedure **a**, using 1-benzylazepan-2-one (61 mg, 0.3 mmol), compound **22** was obtained in 72% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE 1:9). Spectroscopical data are in agreement with the literature values.[53]

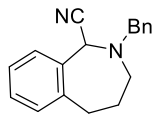
Synthesis and characterization of 4-benzyl-1,4-oxazepane-5-carbonitrile (23)



According to the general procedure **a**, using 4-benzyl-1,4-oxazepan-5-one (62 mg, 0.3 mmol), compound **23** was obtained in 39% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE = 2:5).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.16 (m, 5H), 3.90 – 3.58 (m, 7H), 2.90 (dd, *J* = 14.0, 10.3 Hz, 1H), 2.72 (dd, *J* = 14.2, 3.2 Hz, 1H), 2.24 (ddt, *J* = 14.6, 9.3, 4.7 Hz, 1H), 2.00 (dq, *J* = 15.3, 6.0, 5.0 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 137.3, 128.9 (2C), 128.7 (2C), 127.8, 117.1, 68.9, 65.2, 60.8, 53.8, 52.7, 33.7; IR (film) ν = 3030, 2947, 2863, 2225, 1453 cm^{-1}; HRMS (ESI): calcd. for $C_{13}H_{17}ON_2^+$ $[M+H]^+$ 217.1335, found *m/z* 217.1336.

Synthesis of 2-benzyl-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine-1-carbonitrile (24)

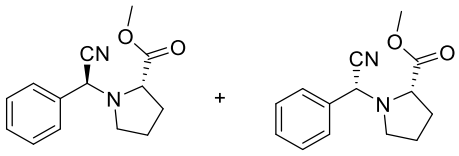


According to the general procedure **a**, using 2-benzyl-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepin-1-one (75 mg, 0.3 mmol), compound **24** was obtained in 81% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE = 1:6).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.22 (m, 5H), 7.22 – 6.82 (m, 4H), 4.76 (br s, 1H), 3.67 – 3.50 (m, 2H), 3.23 – 3.03 (m, 3H), 2.80 (dd, *J* = 15.1, 7.9, 1H), 1.91 – 1.79 (m, 1H), 1.76 – 1.61 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 142.6, 137.6, 134.1, 130.2, 129.5, 129.2, 129.0 (2C), 128.7 (2C), 127.8, 126.7, 116.3, 59.3,

55.2, 34.5, 26.6; IR (film) ν = 2933, 2847, 2825, 2361, 1493, 145 cm^{-1}; HRMS (ESI): calcd. for $C_{18}H_{19}N_2^+$ $[M+H]^+$ 263.1543, found *m/z* 263.1542.

Synthesis and characterization of methyl ((*S*/*R*)-cyano(phenyl)methyl)-*L*-prolinate (33)

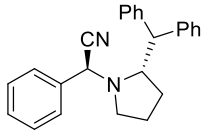


According to the general procedure **a**, using *N*-benzoyl-*L*-proline methyl ester (500 mg, 2.14 mmol), compound **33** was obtained in 82% yield as 5:1 mixture of diastereomers, both as colourless oils, after flash column chromatography on silica gel (Et_2O / PE = 1:4).

Major diastereomer - Methyl ((*S*)-cyano(phenyl)methyl)-*L*-prolinate: $[\alpha]_D^{25}$ = -107.5 (*c* 1.61, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.60 (ddd, *J* = 7.9, 1.9, 0.9 Hz, 2H), 7.47 – 7.31 (m, 3H), 5.38 (s, 1H), 3.81 (s, 3H), 3.62 (dd, *J* = 9.1, 6.8 Hz, 1H), 2.75 (ddd, *J* = 9.0, 6.8, 3.7 Hz, 1H), 2.58 (td, *J* = 8.9, 7.7 Hz, 1H), 2.34 – 2.20 (m, 1H), 2.15 – 2.07 (m, 1H), 1.93 – 1.74 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.3, 133.8, 128.8 (2C), 127.6 (3C), 116.1, 63.0, 58.1, 52.2, 48.2, 28.9, 22.8; IR (film) *v* = 2981, 2928, 2231, 1744, 1381 cm^{-1}; HRMS (ESI): calcd. for $C_{14}H_{17}O_2N_2^+$ $[M+H]^+$ 245.1285, found *m/z* 245.1286.

Minor diastereomer - Methyl ((*R*)-cyano(phenyl)methyl)-*L*-prolinate: $[\alpha]_D^{25}$ = -34.4 (*c* 1.30, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.42 (m, 2H), 7.41 – 7.28 (m, 3H), 5.15 (s, 1H), 3.46 (dd, *J* = 9.4, 4.1 Hz, 1H), 3.38 – 3.27 (m, 1H), 3.29 (s, 3H), 2.93 (td, *J* = 8.9, 6.7 Hz, 1H), 2.22 – 1.82 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.0, 132.9, 129.5, 128.7 (2C), 128.4 (2C), 116.6, 61.0, 58.7, 53.2, 51.6, 30.5, 23.9; IR (film) ν = 2952, 2233, 1736, 1450, 1361, 1277 cm^{-1}; HRMS (ESI): calcd. for $C_{14}H_{17}O_2N_2^+$ $[M+H]^+$ 245.1285, found *m/z* 245.1284.

Synthesis and characterization of (*S*)-2-((*S*)-2-benzhydrylpyrrolidin-1-yl)-2-phenylacetonitrile (34)

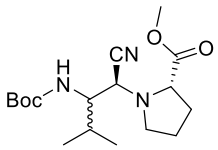


According to the general procedure **a**, using (*S*)-(2-benzhydrylpyrrolidin-1-yl)(phenyl)methanone (102 mg, 0.3 mmol), compound **34** was obtained in 35% yield as a colourless oil, after flash column chromatography on silica gel (washed with 1% Et_3N) (Et_2O / PE 1:5).

$[\alpha]_D^{25}$ = + 17.7 (*c* 0.26, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.42 – 7.36 (m, 2H), 7.33 – 7.16 (m, 10H), 7.17 – 7.05 (m, 3H), 4.22 (s, 1H), 3.89 – 3.78 (m, 1H), 3.85

(s, 1H), 2.61 – 2.44 (m, 2H), 2.06 – 1.93 (m, 1H), 1.71 – 1.55 (m, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 143.4, 143.3, 134.5, 128.8 (4C), 128.6 (2C), 128.4 (3C), 128.3 (2C), 127.6 (2C), 126.8, 126.4, 117.5, 65.8, 60.1, 58.8, 50.2, 31.1, 23.4; IR (film) *v* = 2980, 2888, 2219, 1382, 1252, 1153, 1073 cm^{-1}; HRMS (ESI): calcd. for $C_{25}H_{25}N_2^+$ $[M+H]^+$ 353.2012, found *m/z* 353.2014.

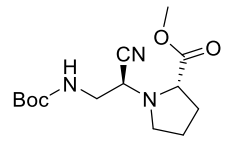
Synthesis and characterization of methyl ((1*S*)-2-((tert-butoxycarbonyl)amino)-1-cyano-3-methylbutyl)-L-prolinate (35)



According to the general procedure **a**, using Boc-*L*-Val-*L*-Pro-OMe (98 mg, 0.3 mmol), compound **35** was obtained as a 5:5:1:1 mixture of diastereomers. The two major diastereomers, were isolated as an unseparable mixture in 38% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE = 1:4).

^{1}H-NMR (500 MHz, C_6D_6) δ 4.76 (br d, *J* = 9.7 Hz, 1H, NH A), 4.20 (br d, *J* = 10.6 Hz, 1H, NH B), 4.02 – 3.98 (m, 1H, A), 3.82 – 3.71 (m, 2H, A + B), 3.67 – 3.57 (m, 2H, A), 3.64 (dd, *J* = 7.6, 3.0 Hz, 1H, A), 3.60 (d, *J* = 7.3 Hz, 1H, B), 3.44 (s, 3H, A), 3.35 – 3.28 (m, 1H, B), 3.31 (s, 3H, B), 2.92 – 2.84 (m, 1H, A), 2.77 (ddd, *J* = 8.8, 7.4, 3.7 Hz, 1H, B), 2.65 (m, 1H, A), 2.56 (m, 1H, B), 1.98 (m, 2H, B), 1.80 – 1.63 (m, 5H, A + B), 1.49 (s, 9H, A), 1.44 (s, 9H, B), 1.43 - 1.40 (m, 1H, A), 0.86 (d, *J* = 6.9 Hz, 3H, A), 0.77 (d, *J* = 6.8 Hz, 3H, A), 0.73 (d, *J* = 6.9 Hz, 3H, B), 0.58 (d, *J* = 6.8 Hz, 3H, B); ^{13}C-NMR (125 MHz, C_6D_6) δ 173.3 (A), 172.1 (B), 155.5 (2C, A + B), 116.3 (A), 115.9 (B), 79.2 (A), 78.8 (B), 63.7 (A), 62.8 (B), 58.2 (2C, A + B), 57.0 (2C, A + B), 52.2 (A), 51.0 (B), 50.8 (A), 47.9 (B), 29.4 (A), 29.3 (B), 28.1 (6C, A + B), 28.0 (2C, A + B), 23.6 (A), 23.1 (B), 19.7 (A), 19.5 (B), 16.8 (2C, A + B); IR (film) *v* = 3657, 3368, 2980, 1717, 1520, 1367, 1169 cm^{-1}; HRMS (ESI): calcd. for $C_{17}H_{30}O_4N_3^+$ $[M+H]^+$ 340.2231, found *m/z* 340.2228.

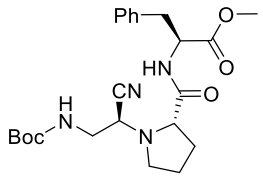
Synthesis and characterization of methyl ((*S*)-2-((*tert*-butoxycarbonyl)amino)-1-cyanoethyl)-*L*-prolinate (36)



According to the general procedure, using Boc-*L*-Gly-*L*-Pro-OMe (86 mg, 0.3 mmol), compound **36** was obtained in 77% yield as a colourless oil as 5:1 mixture of separable diastereomers, after flash column chromatography on silica gel (EtOAc / PE = 1:3).

Major diastereomer: $[\alpha]_D^{25}$ = - 51.8 (*c* 1.09, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.48 (br d, *J* = 7.5 Hz, 1H, NH), 3.90 (dd, *J* = 10.0, 5.2 Hz, 1H), 3.68 (s, 3H), 3.60 (ddd, *J* = 14.5, 8.6, 5.2 Hz, 1H), 3.51 (dd, *J* = 9.9, 3.5 Hz, 1H), 3.13 (ddd, *J* = 9.0, 5.7, 4.1 Hz, 1H), 2.89 (ddd, *J* = 14.2, 10.0, 3.9 Hz, 1H), 2.74 (q, *J* = 8.4 Hz, 1H), 2.14 (dq, *J* = 13.0, 9.4 Hz, 1H), 2.00 (ddt, *J* = 13.3, 8.5, 4.6 Hz, 1H), 1.88 – 1.77 (m, 2H), 1.38 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.2, 155.7, 116.5, 80.0, 60.6, 55.6, 54.4, 52.4, 42.0, 30.6, 28.3, 24.1; IR (film) *v* = 3658, 3401, 2980, 2254, 1710, 1506, 1367, 1166 cm^{-1}; HRMS (ESI): calcd. for $C_{14}H_{23}O_4N_3Na^+$ $[M+Na]^+$ 320.1581, found *m/z* 320.1577.

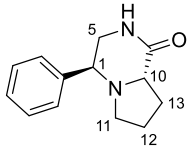
Synthesis and characterization of methyl ((*S*)-2-((*tert*-butoxycarbonyl)amino)-1-cyanoethyl)-*L*-prolyl-*L*-phenylalaninate (37)



According to the general procedure, using Boc-*L*-Gly-*L*-Pro-*L*-Phe-OMe (130 mg, 0.3 mmol), compound **37** was obtained in 54% yield as a single diastereomer as a white solid, after crystallization from EtOAc/PE.

$[\alpha]_D^{25}$ = - 61.8 (*c* 0.50, DMSO); M.P. = 156-158 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.6 Hz, 1H), 7.35 – 7.15 (m, 3H), 7.10 – 7.02 (m, 2H), 6.18 (br s, 1H, NH), 4.76 (td, *J* = 8.8, 5.4 Hz, 1H), 4.10 (dd, *J* = 9.8, 4.8 Hz, 1H), 3.73 (s, 3H), 3.45 – 3.31 (m, 3H), 3.19 (dd, *J* = 14.0, 5.4 Hz, 1H), 3.05 – 2.85 (m, 2H), 2.67 (ddd, *J* = 11.5, 8.9, 5.5 Hz, 1H), 2.09 – 1.93 (m, 1H), 1.74 – 1.33 (m, 2H), 1.39 (s, 9H, 3xCH_3-Boc), 1.14 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.6, 173.5, 156.1, 135.7, 128.9 (2C), 128.8 (2C), 127.4, 116.7, 80.0, 62.2, 55.3, 54.9, 52.9, 52.5, 42.5, 37.7, 31.3, 28.4, 24.0; IR (film) *v* = 3657, 3369, 2978, 2891, 1740, 1689, 1656, 1522, 1452, 1383, 1266, 1165 cm^{-1}; HRMS (ESI): calcd. for $C_{23}H_{33}O_5N_4^+$ $[M+H]^+$ 445.2446, found *m/z* 445.2447.

Synthesis and characterization of (1*S*,10*aS*)-4-phenylhexahydropyrrolo[1,2-a]pyrazin-1(2H)-one (38)

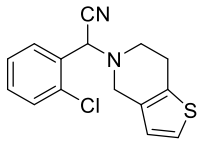


Methyl ((*S*)-cyano(phenyl)methyl)-*L*-prolinate (major diastereomer-**33**) (50.6 mg, 0.21 mmol) and $NiCl_2.6H_2O$ (122 mg, 0.51 mmol) were suspended in MeOH (4 mL) under Ar atmosphere and the solution was cooled to 0 °C. $NaBH_4$ (131 mg, 3.5 mmol) was added portionwise, and the mixture was stirred at 0 °C for one hour. Then, MeOH was evaporated under reduced pressure, ethyl acetate

and $NaHCO_3$ saturated aqueous solution were added and the mixture filtered through a pad of celite. Then the aqueous phase was extracted with EtOAc, dried over Na_2SO_4 and evaporated under reduced pressure. After flash column chromatography on silica gel (MeOH / DCM = 0:1 to 1:4), compound **38** was obtained as a brown solid (8.5 mg, 19% yield).

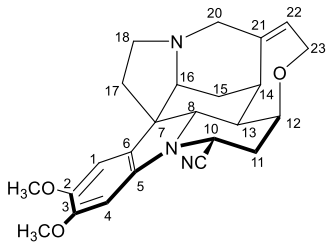
$[\alpha]_D^{25}$ = + 19.6 (*c* 0.58, $CHCl_3$); ^{1}H-NMR (500 MHz, CD_2Cl_2) δ = 7.42 (d, *J* = 7.1 Hz, 2H, CHAr), 7.35 (t, *J* = 7.4 Hz, 2H, 2xCHAr), 7.29 (t, *J* = 7.3 Hz, 1H, CHAr), 6.42 (br s, 1H, NH), 3.80 (t, *J* = 8.5 Hz, 1H, H-10), 3.71 (dd, *J* = 9.9, 3.7 Hz, 1H, H-1), 3.39 (dd, *J* = 12.4, 10.1 Hz, 1H, H-5_{ax}), 3.29 (dt, *J* = 12.6, 4.2 Hz, 1H, H-5_{eq}), 2.86 (ddd, *J* = 10.4, 7.5, 4.7 Hz, 1H, H-11_{ax}), 2.55 (dt, *J* = 10.3, 7.5 Hz, 1H, H-11_{eq}), 2.18 (dtd, *J* = 11.7, 8.1, 3.5 Hz, 1H, H-13a), 2.07 (dq, *J* = 12.9, 8.9 Hz, 1H, H-13b), 1.86 – 1.76 (m, 1H, H-12a), 1.75 – 1.65 (m, 1H, H-12b); ^{13}C-NMR (126 MHz, CD_2Cl_2) δ = 174.0 (CONH), 141.4 (CAr), 129.0 (CHAr), 128.2 (2C, 2xCHAr), 128.1 (2C, 2xCHAr), 63.0 (C-10), 62.0 (C-1), 54.0 (C-5), 48.5 (C-11), 28.9 (C-13), 23.8 (C-12); IR (film) *v* = 3228, 3061, 2968, 2875, 1672, 1490, 1451, 1333, 1267 cm^{-1}; HRMS (ESI): calcd. for $C_{13}H_{17}ON_2^+$ $[M+H]^+$ 217.1335, found *m/z* 217.1334.

Synthesis of 2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)acetonitrile (40)



According to the general procedure **a**, using 5-chlorobenzoyl-4,5,6,7-tetrahydro-thieno[3,2-*c*]pyridine **39** (83 mg, 0.3 mmol), compound **40** was obtained in 85% yield as a colourless oil, after flash column chromatography on silica gel (Et_2O / PE = 1:5). Spectroscopical data are in agreement with the literature values.[54]

Synthesis and characterization of Brucine-CN (41)



According to the general procedure, using commercial Brucine (117.1 mg, 0.30 mmol), compound **41** was obtained in 55% yield as a white solid, after trituration with EtOAc / PE = 1:1. Absolute configuration of new stereocenter was assigned by NOE studies (see Figure 11).

$[\alpha]_D^{25}$ = - 116.5 (*c* 1.05, DMSO); M.P. = 226-228 °C; ^{1}H-NMR (500 MHz, C_6D_6) δ = 7.24 (s, 1H, H-4), 6.64 (s, 1H, H-1), 5.33 (t, *J* = 6.7 Hz, 1H, H-22), 4.43 (q, *J* = 6.7 Hz, 1H, H-24), 3.88 (dd, *J* =13.5, 7.2 Hz, 1H, H-

23a), 3.70 (t, *J* = 2.7 Hz, 1H, H-16), 3.61 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 3.56 – 3.48 (m, 3H, H-8, H-20, H-23), 3.35 – 3.31 (m, 1H, H-12), 3.19 (dd, *J* = 9.8, 8.1 Hz, 1H, H-18a), 2.53 (ddd, *J* =11.8, 10.1, 6.7 Hz, 1H, H-18b), 2.40 (d, *J* = 14.9 Hz, 1H, H-20), 2.05 – 2.12 (m, 2H, H-14, H-17a), 1.83 (dt, *J* = 13.8, 3.8 Hz, 1H, H-15a), 1.71 – 1.67 (m, 2H, H-11a + H-11b), 1.42 (td, *J* = 12.1, 8.0 Hz, 1H, H-17b), 1.13 (d, *J* = 13.8 Hz, 1H, H-15b), 1.02 (d, *J* = 10.8 Hz, 1H, H-13); ^{13}C-NMR (126 MHz, C_6D_6) δ = 151.2 (C-3), 145.0 (C-21), 144.3 (C-5), 144.1 (C-2), 124.0 (C-6), 123.5 (C-22), 119.0 (CN), 111.3 (C-1), 97.2 (C-4), 79.1 (C-12), 65.8 (C-23), 64.4 (C-8), 59.5 (C-16), 57.9 (OCH_3), 56.1 (OCH_3), 54.1 (C-20), 52.1 (C-7), 52.0 (C-18), 42.9 (C-24), 39.7 (C-13), 36.4 (C-17), 35.2 (C-11), 34.9 (C-14), 27.1 (C-15); IR (film) ν = 2948, 2850, 2246, 1660, 1614, 1493, 1453, 1336, 1219 cm^{-1}, HRMS (ESI): calcd. for $C_{24}H_{28}O_3N_3^+$ $[M+H]^+$ 406.2125, found *m/z* 406.2122.

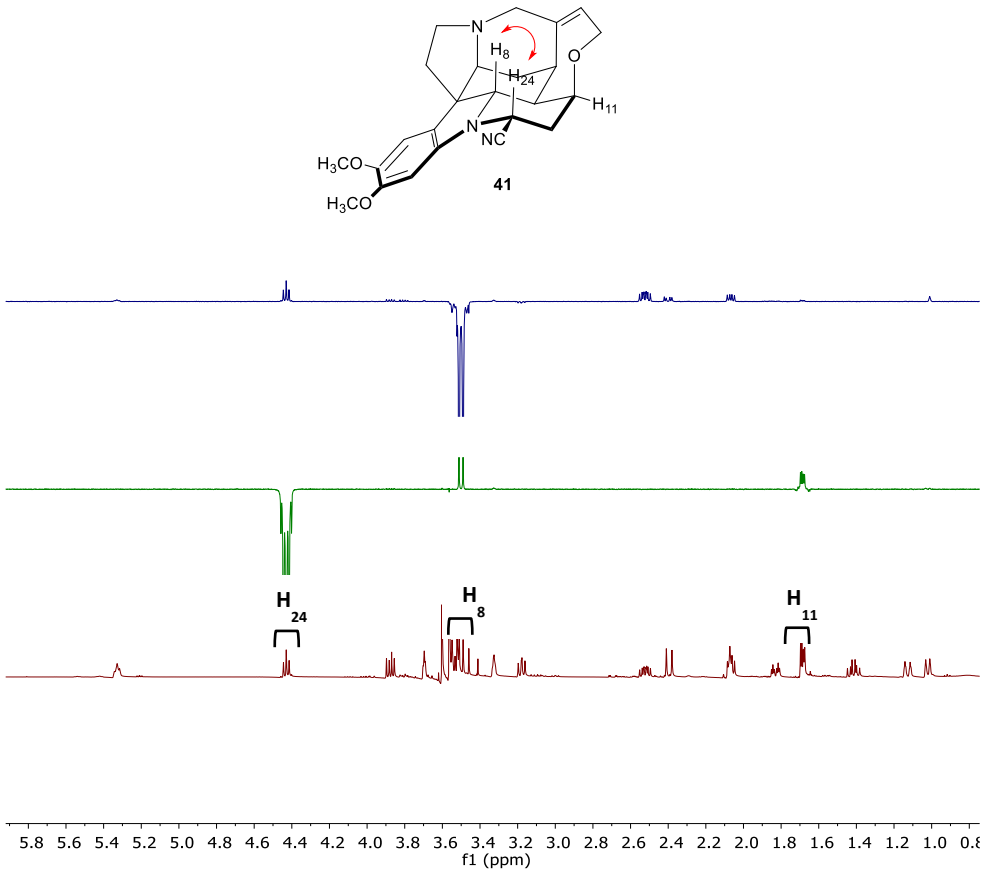
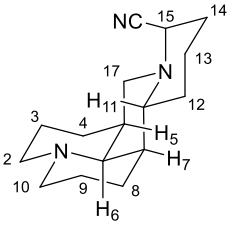


Figure 11: NOE experiments of compound **41** obtained from brucine.

Synthesis and characterization of (5*S*,6*S*,7*R*,11*R*,15R)-dodecahydro-1*H*,5*H*,8*H*-dipyrido[2,1-*f*:3',2',1'-*ij*][1,6]naphthyridine-10-carbonitrile– Matrine-CN (42)



According to the general procedure, using Matrine (45.7 mg, 0.18 mmol), compound **33** was obtained in 85% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE = 1:1). The absolute configuration was assigned by NOE (see Figure 12). $[\alpha]_D^{25}$ = - 16.3 (*c* 1.00, MeOH); ^{1}H-NMR (400 MHz, C_6D_6) δ 3.24 – 3.14 (m, 2H, H-15 + H-17$_{ax}$), 2.80 (td, *J* = 10.5, 2.7 Hz, 1H, H-11), 2.62 – 2.41 (m, 2H, H-10$_{ax}$ + H-2$_{ax}$), 1.79 (dd, *J* = 10.3, 4.0 Hz, 1H, H-17$_{eq}$), 1.72 – 0.93 (m, 18H, H-6 + H-2$_{eq}$ + H-10$_{eq}$ + H-12$_{eq}$ + H-13a + H-13b + H-8a + H-8b + H-9a + H-9b + H-14 + H-14b + H-3a + H-3b + H-4a + H-4b + H-5 + H-7), 0.64 (tdd, *J* = 14.1, 11.2, 4.1 Hz, 1H, H-12$_{ax}$); ^{13}C-NMR (100 MHz, C_6D_6) δ 116.8 (CN), 63.5 (C-6), 57.4 (C-2), 57.2 (C-10), 55.1 (C-15), 53.7 (C-17), 52.8 (C-11), 42.5 (C-7), 35.6 (C-5), 29.3 (C-12), 28.6 (C-13), 28.3 (C-14), 26.2 (C-4), 21.5 (C-3), 21.1 (C-9), 20.7 (C-8); IR (film) *v* = 2933, 2763, 1447, 1356, 1294, 1127 cm^{-1}; HRMS (ESI): calcd. for $C_{16}H_{26}N_3^+$ $[M+H]^+$ 260.2121, found *m/z* 260.2121.

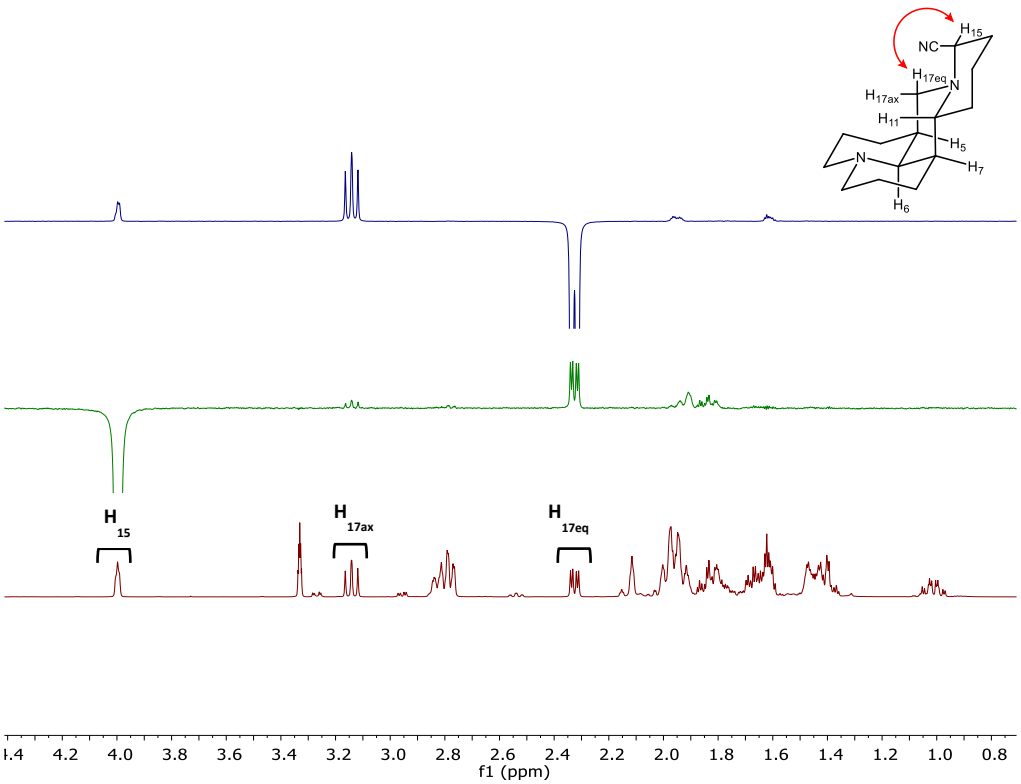
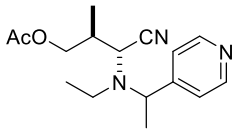


Figure 12: NOE experiments of compound **42** obtained from matrine.

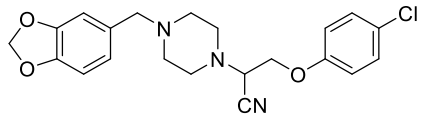
Synthesis and characterization of 3-cyano-3-(ethyl(pyridin-4-ylmethyl)amino)-2-phenylpropyl acetate - Tropicamide-OAc-CN (43)



According to the general procedure, using acetylated Tropicamide (98 mg, 0.3 mmol), compound **43** was obtained in 34% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE = 1:1). The relative stereochemistry was assigned by comparison with a related compound.[55]

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.33 – 8.27 (m, 2H), 7.34 – 7.15 (m, 3H), 7.03 – 6.95 (m, 2H), 6.66 – 6.60 (m, 2H), 4.39 (dd, *J* = 11.5, 5.8 Hz, 1H), 4.30 (dd, *J* = 11.5, 5.4 Hz, 1H), 3.91 (d, *J* = 10.7 Hz, 1H), 3.72 (d, *J* = 14.9 Hz, 1H), 3.34 (dt, *J* = 10.9, 5.6 Hz, 1H), 3.28 (d, *J* = 15.0 Hz, 1H), 2.55 – 2.35 (m, 2H), 1.97 (s, 3H), 0.90 (t, *J* = 7.1 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.6, 149.7 (2C), 147.0, 136.7, 128.6 (2C), 128.4, 127.9 (2C), 123.3 (2C), 116.6, 65.4, 55.4, 54.1, 46.0, 45.7, 20.8, 12.6; IR (film) ν = 3658, 2981, 2888, 1737, 1382, 1250, 1152, 1072 cm^{-1}; HRMS (ESI): calcd. for $C_{20}H_{24}N_3O_2^+$ $[M+H]^+$ 338.1863, found *m/z* 338.1861.

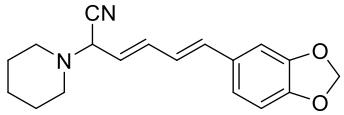
Synthesis and characterization of 2-(4-(benzo[*d*][1,3]dioxol-5-ylmethyl)piperazin-1-yl)-3-(4-chlorophenoxy)propanenitrile –Fipexide-CN (44)



According to the general procedure, using the commercial drug Fipexide (119.7 mg, 0.31 mmol), compound **44** was obtained in 54% yield as a colourless solid, after flash column chromatography on silica gel (Et_2O / PE = 1:1 to 2:1).

M.P. = 130-132 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 9.0 Hz, 2H), 6.94 – 6.81 (m, 3H), 6.77 (d, *J* = 0.9 Hz, 2H), 5.97 (s, 2H), 4.28 – 4.14 (m, 2H), 3.97 (t, *J* = 6.5 Hz, 1H), 3.44 (d, *J* = 1.2 Hz, 2H), 2.79 (dt, *J* = 10.1, 4.7 Hz, 2H), 2.66 (dt, *J* = 10.3, 4.7 Hz, 2H), 2.52 (br s, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.4, 147.7, 146.7, 131.8, 129.5 (2C), 126.9, 122.1, 116.2 (2C), 115.1, 109.4, 107.9, 100.9, 67.1, 62.4, 57.4, 52.4 (2C), 50.7 (2C); IR (film) ν = 2942, 2887, 2821, 2775, 2297, 1592, 1493, 1445, 1244 cm^{-1}; HRMS (ESI): calcd. for $C_{21}H_{23}ClO_3N_3^+$ $[M+H]^+$ 400.1422, found *m/z* 400.1417.

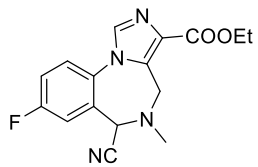
Synthesis and characterization of (3*E*,5*E*)-6-(benzo[*d*][1,3]dioxol-5-yl)-2-(piperidin-1-yl)hexa-3,5-dienenitrile – Piperine-CN (45)



According to the general procedure, using the commercial drug Piperine (83.2 mg, 0.3 mmol), compound **45** was obtained in 81% yield as a yellow solid, after flash column chromatography on silica gel (EtOAc / PE = 1:1) and trituration in petrol ether.

M.P. = 88-90 °C; ^{1}H-NMR (400 MHz, CD_2Cl_2) δ 7.04 (d, *J* = 1.7 Hz, 1H), 6.94 (dd, *J* = 8.0, 1.7 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.82 – 6.60 (m, 3H), 6.04 (s, 2H), 5.81 – 5.69 (m, 1H), 4.41 (dd, *J* = 4.7, 1.4 Hz, 1H), 2.68 (ddd, *J* = 11.0, 6.7, 3.9 Hz, 2H), 2.55 – 2.43 (m, 2H), 1.68 (m, 4H), 1.59 – 1.51 (m, 2H); ^{13}C-NMR (100 MHz, CD_2Cl_2) δ 148.2, 147.7, 134.5, 134.2, 131.2, 125.2 (2C), 121.7, 115.1, 108.3, 105.3, 101.4, 60.3, 51.0 (2C), 25.9 (2C), 24.0; IR (film) *v* = 2937, 2810, 2225, 1605, 1495, 1445, 1250 cm^{-1}; HRMS (ESI): calcd. for $C_{18}H_{21}O_2N_2^+$ $[M+H]^+$ 297.1598, found *m/z* 297.1595.

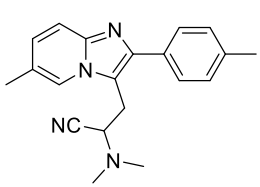
Synthesis and characterization of ethyl 6-cyano-8-fluoro-5-methyl-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate –Flumazenil-CN (46)



According to the general procedure, using Flumazenil (91 mg, 0.3 mmol), compound **46** was obtained in 47% yield as a white solid, after trituration in petrol ether and column chromatography (EtOAc / PE = 1:2).

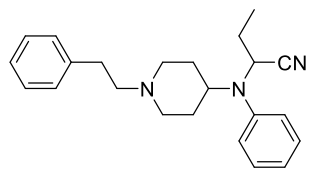
M.P. = 184-186 °C; ^{1}H-NMR (500 MHz, CD_2Cl_2) δ 7.88 (s, 1H), 7.59 – 7.48 (m, 2H), 7.40 (ddd, *J* = 8.7, 7.8, 2.8 Hz, 1H), 4.48 (s, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 4.09 (d, *J* = 14.8 Hz, 1H), 4.03 (d, *J* = 14.8 Hz, 1H), 2.68 (s, 3H), 1.44 (t, *J* = 7.1 Hz, 3H); ^{13}C-NMR (125 MHz, CD_2Cl_2) δ 163.1 and 162.8, 161.1, 135.1, 132.4, 131.5, 131.1, 128.2 and 128.1, 125.1 and 125.0, 118.4 and 118.2, 117.7 and 117.5, 115.7, 60.7, 57.0, 45.8, 42.4, 14.2; IR (film) ν = 3077, 2983, 2802, 2247, 1721, 1573, 1504, 1243, 1158 cm^{-1}; HRMS (ESI): calcd. for $C_{16}H_{16}FN_4O_2^+$ $[M+H]^+$ 315.1252, found *m/z* 315.1240.

Synthesis and characterization of 2-(dimethylamino)-3-(6-methyl-2-(p-tolyl)imidazo[1,2-a]pyridin-3-yl)propanenitrile – Zolpidem-CN (47)



Commercial Zolpidem (4.6 mg, 0.015 mmol) was dissolved in toluene/DCM-d_2 (140 µL / 50 µL) and the mixture purged with vacuum/Ar (x3). Under Ar atmosphere and under continuous stirring, 112 µL of a Stock solution of Vaska's complex in toluene (0.026 M) was added. After 5 min, 112 µL of a Stock solution of TMDS in toluene (0.54 M) was added. 10 min later, 112 µL of a Stock solution of TMSCN (0.54 M) were incorporated and the solution was left stirring at room temperature overnight. Then the solvent was evaporated using a N_2 flow and the crude purified by silica gel column yielding 3.8 mg of compound **47** as a yellow solid (80% yield).
M.P. = 150-152 °C; ^{1}H-NMR (500 MHz, CD_2Cl_2) δ = 7.75 (d, *J* = 1.1 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.42 (d, *J* = 9.1 Hz, 1H), 7.21 (d, *J* = 7.9 Hz, 2H), 7.00 (dd, *J* = 9.2 Hz, 1.6, 1H), 3.76 (dd, *J* = 8.7, 6.2 Hz, 1H), 3.52 (dd, *J* = 15.5, 8.7 Hz, 1H), 3.36 (dd, *J* = 15.5, 6.2 Hz, 1H), 2.32 (s, 3H), 2.29 (d, *J* = 0.9 Hz, 3H), 2.25 (s, 6H); ^{13}C-NMR (126 MHz, CD_2Cl_2) δ = 144.7, 144.6, 138.2, 132.4, 129.9 (2C), 128.5 (2C), 127.9, 122.8, 121.4, 117.5, 116.5, 114.8, 57.7, 42.3 (2C), 27.6, 21.5, 18.7; IR (film) ν = 2951, 2789, 2223, 1647, 1613, 1503, 1455, 1345, 1266 cm^{-1}; HRMS (ESI): calcd. for $C_{20}H_{23}N_4^+$ $[M+H]^+$ 319.1917, found m/z 319.1914.

Synthesis and characterization of 2-((1-phenethylpiperidin-4-yl)(phenyl)amino)butanenitrile – Fentanyl-CN (48)

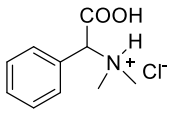


Commercial Fentanyl (6.5 mg, 0.019 mmol) was dissolved in toluene (184 µL) and the mixture purged with vacuum/Ar (x3). Under Ar atmosphere and under continuous stirring, 144 µL of a Stock solution of Vaska's complex in toluene (0.026 M) was added. After 5 min, 144 µL of a Stock solution of TMDS in toluene (0.54 M) was added. 10 min later, 144 µL of a Stock solution of TMSCN (0.54 M) were incorporated and the solution was left stirring at room temperature overnight. Then the solvent was evaporated using a N_2 flow and the crude purified by flash column chromatography on silica gel yielding 6.1 mg of compound **48** as a colourless oil (91% yield).
^{1}H-NMR (500 MHz, CD_2Cl_2) δ = 7.20 – 7.02 (m, 9H), 6.98 (t, *J* = 7.3 Hz, 1H), 3.96 (t, *J* = 7.8 Hz, 1H), 3.13 (tt, *J* = 11.2, 3.9 Hz, 1H), 2.93 – 2.85 (m, 1H), 2.84 – 2.77

(m, 1H), 2.61 (dd, *J* = 9.1, 6.7 Hz, 2H), 2.42 (dd, *J* = 9.1, 6.7 Hz, 2H), 2.00 – 1.81 (m, 3H), 1.64 – 1.35 (m, 5H), 0.86 (t, *J* = 7.4 Hz, 3H); ^{13}C-NMR (125 MHz, CD_2Cl_2) δ = 146.5, 141.4, 129.4 (2C), 129.2 (2C), 128.8 (2C), 126.4, 125.8 (2C), 124.8, 120.8, 60.7, 60.0, 53.3, 53.3, 52.4, 34.2, 32.3, 31.1, 26.3, 11.0; IR (film) ν = 2942, 2807, 2226, 1598, 1496, 1455, 1233 cm^{-1}, HRMS (ESI): calcd. for $C_{23}H_{30}N_3^+$ $[M+H]^+$ 348.2434, found m/z 348.2426.

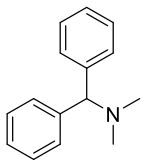
7.4.3. Synthesis and characterization of derivatized compounds

Synthesis and characterization of 1-carboxy-*N*,*N*-dimethyl-1-phenylmethanaminium chloride (49)



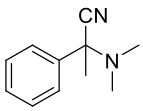
Following a procedure described in literature,[56] compound **2** (58.1 mg, 0.36 mmol) was suspended in 1 mL of concentrated HCl and refluxed for 24 h. Then the solution was washed with CH_2Cl_2 and the water was evaporated under reduced pressure to yield 63.2 mg of the title compound **49** as a white solid (81% yield). Spectroscopical data are in agreement with the literature values.[57]

Synthesis and characterization of *N,N*-dimethyl-1,1-diphenylmethanamine (50)



To a 1M solution of PhMgBr in THF (1 mL) a solution of compound 2 (56.4 mg, 0.35 mmol) in 0.5 mL of THF was added. The mixture was refluxed for 3.5 h, then quenched with 1M aqueous HCl. Then, THF was removed under reduced pressure, NaOH was added to neutralize the solution followed by extraction with DCM. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. 41.9 mg (56% yield) of compound **50** were obtained as a solid flash column chromatography on silica gel (EtOAc / PE = 1:7). Spectroscopical data are in agreement with the literature values.[58]

Synthesis and characterization of 2-(dimethylamino)-2-phenylpropanenitrile (51)

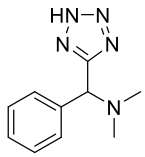


To a solution of compound **2** (50.8 mg, 0.32 mmol) in dry THF (1 mL) under Ar atmosphere was added a 0.5 M solution of KHMDS in toluene (0.64 mL, 0.32 mmol) dropwise at 0 ˚C and the solution was stirred for 20 min. MeI (25 mL, 0.40 mmol) was then added dropwise and,

after 10 min, 1 mL of saturated NH_4Cl aqueous solution was added to quench the reaction. The reaction mixture was extracted with Et_2O (x3), dried over $MgSO_4$ and evaporated under reduced pressure. 45.5 mg (82% yield) of compound 51 were obtained as a yellow oil after purification by flash column chromatography on silica gel (EtOAc / PE = 1:9).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.59 (dd, *J* = 8.3, 1.4 Hz, 2H), 7.45 – 7.28 (m, 3H), 2.29 (s, 6H), 1.72 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 141.2, 128.9 (2C), 128.5, 125.7 (2C), 117.8, 67.5, 41.0 (2C), 29.6; IR (film) *ν* = 2994, 2870, 2831, 2789, 2181 cm^{-1}; HRMS (ESI): calcd. for $C_{11}H_{15}N_2H^+$ $[M+H]^+$ 175.1230, found *m/z* 175.1229.

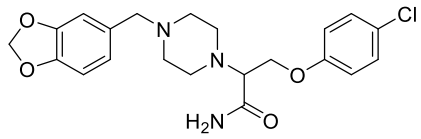
Synthesis and characterization of *N*,*N*-dimethyl-1-phenyl-1-(2*H*-tetrazol-5-yl)methanamine (52)



Compound **2** (50 mg, 0.31 mmol) was dissolved in toluene (3.1 mL), trimethylsilylazide (0.41 mL, 3.1 mmol) and tributyltin oxide (50 mg, 0.2 mmol) were added and the mixture stirred at 70 °C for 4 days. The mixture was diluted with EtOAc and the solid which appeared was filtered, yielding 17.8 mg of compound **52** as a white solid. The mother liquors were concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel (DCM-MeOH), to give 31.2 mg of compound **52** (77% combined yield).

M.P. = 196-198 °C; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.71 – 7.63 (m, 2H), 7.50 – 7.38 (m, 3H), 5.79 (s, 1H), 2.79 (s, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ = 158.7, 134.1, 131.2, 130.8 (2C), 130.4 (2C), 68.2, 42.6 (2C); IR (film) ν = 3387, 3033, 2968, 2631, 2478, 1649, 1486, 1410, 1158 cm^{-1}; HRMS (ESI): calcd. for $C_{10}H_{14}O_4N_5H^+$ $[M+H]^+$ 204.1244, found *m/z* 204.1241.

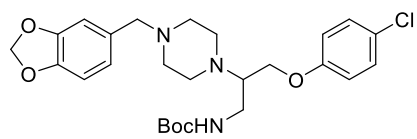
Synthesis and characterization of 2-(4-(benzo[*d*][1,3]dioxol-5-ylmethyl)piperazin-1-yl)-3-(4-chlorophenoxy)propanamide (55)



K_2CO_3 (2 mg, 0.015 mol) and 10% aqueous solution of H_2O_2 (0.02 mL) were sequentially added to a solution of compound **44** (20 mg, 0.015 mmol) in DMSO at 0 °C. The resulting mixture was left stirring at room temperature overnight. Then, H_2O was added (1 mL), the product was extracted with EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. Compound **55**

was obtained in 30% yield as a colourless oil, after flash column chromatography on silica gel (EtOAc / PE = 2:1).
^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.26 (br s, *J* = 5.1 Hz, 1H), 7.19 – 7.12 (m, 2H), 6.81 – 6.74 (m, 3H), 6.71 – 6.62 (m, 2H), 5.87 (s, 2H), 5.41 (br d, *J* = 5.3 Hz, 1H), 4.41 (dd, *J* = 10.4, 3.3 Hz, 1H), 4.20 (dd, *J* = 10.4, 7.4 Hz, 1H), 3.45 – 3.36 (m, 1H), 3.36 (s, 2H), 2.79 (t, *J* = 7.8 Hz, 2H), 2.67 – 2.60 (m, 2H), 2.40 (br s, 2H), 1.26 – 1.11 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.3, 156.8, 147.7, 146.7, 131.7, 129.4 (2C), 126.0, 122.2 (2C), 115.9, 109.5, 107.9, 100.9, 67.2, 65.2, 62.7, 53.5 (2C), 29.7 (2C); IR (film) ν = 3427, 3289, 2920, 1686, 1494, 1244 cm^{-1}; HRMS (ESI): calcd. for $C_{21}H_{25}O_4N_3Cl^+$ $[M+H]^+$ 418.1528, found *m/z* 418.1522.

Synthesis and characterization of *tert*-butyl (2-(4-(benzo[*d*][1,3]dioxol-5-ylmethyl)piperazin-1-yl)-3-(4-chlorophenoxy)propyl)carbamate (56)



Boc_2O (46.9 mg, 0.21 mmol), compound **44** and $NiCl_2.6H_2O$ (30 mg, 0.13 mmol) were suspended in MeOH (1 mL) under Ar atmosphere and the solution was cooled to 0°C. $NaBH_4$ (33.1 mg, 0.87 mmol) was added portionwise, and the mixture was allowed to warm to room temperature until TLC showed complete starting material consumption. Then, MeOH was evaporated under reduced pressure, EtOAc and $NaHCO_3$ saturated aqueous solution were added and the mixture filtered through a pad of celite. Then the aqueous phase was extracted with EtOAc, dried over Na_2SO_4 and evaporated under reduced pressure. After flash column chromatography on silica gel (EtOAc / PE = 1:2), compound **56** was obtained as a colourless oil (14.4 mg, 57% yield).
^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.22 – 7.11 (m, 2H), 6.78 – 6.62 (m, 5H), 5.87 (s, 2H), 4.98 (br s, 1H), 4.00 (dd, *J* = 9.6, 5.2 Hz, 1H), 3.82 (dd, *J* = 9.6, 5.4 Hz, 1H), 3.36 – 3.34 (m, 3H), 3.12 – 3.08 (m, 1H), 2.92 – 2.89 (m, 1H), 2.75 (br s, 2H), 2.53 – 2.38 (m, 4H), 1.39 (s, 9H), 1.36 (br s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) 1:1 mixture of rotamers δ 156.0 and 154.9, 146.6, 145.6, 128.4 (2C), 128.3 (2C), 124.8, 121.3, 114.7, 113.4, 108.5, 106.8, 99.9, 78.3, 64.7, 61.7, 61.1, 52.5 (2C), 37.9, 28.7 (2C), 27.4 (3C); IR (film) *v* = 3417, 2930, 2816, 1708, 1493, 1243, 1167 cm^{-1}; HRMS (ESI): calcd. for $C_{26}H_{35}O_5N_3Cl^+$ $[M+H]^+$ 504.2260, found *m/z* 504.2253

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Part IV

Conclusions

8

Conclusions and Future Perspectives

In conclusion, in this thesis work we presented how the application of Diversity-Oriented Synthesis principles on carbohydrates and *N*-containing building blocks led to the achievement of novel different scaffolds. In the first part of this thesis, the synthesis of six polyhydroxylated nitrogen-containing compounds has been reported and their structural diversity has been assessed by using PMI and PCA analysis. The application of a phenotypic whole cell-based assay, combined with follow up synthesis and further biological studies, allowed for the selection of the hexahydro-2*H*-furo[3,2-*b*][1,4]oxazine structure as an active modulator of MDA-MB-231 cell growth, through cytostatic effect. Even if further investigations are necessary in view to characterize the signaling pathways behind the biological effect, and to collect data for a further optimization of the structure, these preliminary results show the relevance of the application of carbohydrates in DOS strategies for the generation of novel biologically active scaffolds.

In the second part of the thesis, the generation and the application of *N*-containing building blocks has been envisioned with aim of synthetizing new heterocyclic structures with potential peptidomimetic features. In particular, morpholine acetal building blocks have been applied in the synthesis of the uncommon dihydropyrazinone skeleton, which proved to be an interesting Xaa-Ser dipeptide isostere. Chemioinformatic analysis proved that these compounds possess high structural diversity as compared to the 2-oxopiperazines and diketopiperazines previously obtained from threonine-derived morpholine acetals, thus confirming the relevance of morpholine acetal and the related reactivity of the *N*-acyl iminium chemistry, in the achievement

of skeletally different scaffolds. For these reasons, further experimental work is already in development in our laboratories, for the synthesis of novel α-alchenyl-functionalized morpholine acetal building blocks and their application in Diversity-Oriented Synthesis.

Finally, a new iridium-catalyzed methodology for the transformation of tertiary amides and lactams into α-amino nitriles has been reported. These bifunctional compounds possess several modes of reactivity and can serve as key precursors for a wide range of synthetic applications. In particular, taking advantage of the high chemoselectivity, this method can be applied for the late stage functionalization of drugs, natural products and proline-containing peptides. Introducing α-amino nitrile moieties in complex biologically active molecules is extremely interesting for Diversity-Oriented Synthesis, as it can be used as a starting point for divergent approaches, thus creating novel different analogues that still retain the interesting biological features of the parent compound.

Appendix

Abbreviations

° C	Celsius degrees
Å	Angstrom
Ac	Acetyl
Ala	Alanine
Ar	Aromatic group, not phenyl
BB	Block buster
BCP	Build/Couple/Pair
Bn	Benzyl
Boc	t-Butyloxycarbonyl
bs	Broad singolet
d	Doublet
dd	Doublet of doublets
Cbz	Carboxybenzyl
DOS	Diversity-Oriented Synthesis
DIBAL	Diisobutylaluminium hydride
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMEM	Dulbecco's modified eagle medium
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
eq	Equivalents
EDTA	Ethylenediaminetetraacetic acid
ESI	Electrospray Ionisation
Et	Ethyl
Et_2NH	Diethylamine
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
FCC	Flash column chromatography
FCS	Fetal calf serum
FDA	Food and Drug Administration
Fmoc	9H-Fluoren-9-yl-methoxycarbonyl
FITC-A	Fluorochrome-labeled Annexin V

h	Hours
HPLC	High performance liquid chromatography
HTS	High-throughput screening
iBu	iso-Butyl
iPr	iso-Propyl
Im	Imidazolyl
Ile	Isoleucine
KHMDS	Potassium bis(trimethylsilyl)amide
Leu	Leucine
m	Multiplet
m/z	Mass-to-charge ratio
MCR	Multicomponent reaction
Me	Methyl
MeOH	Methanol
MS	Molecular sieves / Mass Spectrometry
Ms	Methanesulfonyl
MsOH	Methanesulfonic acid
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium
NGF	Nerve Growth Factor
NMR	Nuclear Magnetic resonance
NOE	Nuclear Overhauser Effect
o.n.	Over night
PCA	Principal Component Analysis
PDC	Pyridinium dichromate
PDD	Phenotypic Drug Discovery
Ph	Phenyl
Phe	Phenylalanine
Phg	Phenylglycine
PI	Propidium iodide
PMI	Principal Moment of Inertia
Pro	Proline
PS	Phosphatidylserine
q	Quartet
R	Unspecified alkyl group
RCBC	Relay Catalytic Branching Cascade
Ref	Reference
r.t.	Room temperature
Rf	Retention factor

SD	Standard Deviation
Ser	Serine
SMM	Small Molecules Microarray
T	Temperature
tBu	tert-Butyl
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Thr	Threonine
TDD	Target-based Drug Discovery
TLC	Thin Layer Chromatography
TMDS	Tetramethyldisiloxane
TMSCN	Trimethylsilyl cyanide
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
$TMSN_3$	Trimethylsyl azide
TNBC	Triple-Negative Breast Cancer
pTsOH/pTSA	p-Toluenesulfonic Acid
Val	Valine
VLA	Very Late Antigen
WST	Water Soluble Tetrazolium

Additional experimental data

Table 1. SMILES codes of 40 selected BB drugs[1]

- CC(C)C1=C(C(=C(N1CC[C@H](C[C@H](CC(=O)O)O)O)C2=CC=C(C=C2)F)C3=CC=CC=C3)C(=O)NC4=CC=CC=C4 [**D1**] **Lipitor**
- CC1=CN=C(C(=C1OC)C)CS(=O)C2=NC3=C(N2)C=C(C=C3)OC [**D2**] **Nexium**
- CC1=C(C=CN=C1CS(=O)C2=NC3=CC=CC=C3N2)OCC(F)(F)F [**D3**] **Prevacid**
- CCC(=O)O[C@@]1([C@@H](C[C@@H]2[C@@]1(C[C@@H]([C@]3([C@H]2C[C@@H](C4=CC(=O)C=C[C@@]43C)F)F)O)C)C)C(=O)SCF [**D4**] **Flonase**
- C1=CC=C(C=C1)CCCCOCCCCCCNCC(C2=CC(=C(C=C2)O)CO)O [**D5**] **Serevent**
- CC(C)(C1=CC=CC=C1CC[C@H](C2=CC=CC(=C2)\C=C\C3=NC4=C(C=CC(=C4)Cl)C=C3)SCC5(CC5)CC(=O)O)O [**D6**] **Singulair**
- CN(C)CC(C1=CC=C(C=C1)OC)C2(CCCCC2)O [**D7**] **Effexor**
- COC(=O)[C@H](C1=CC=CC=C1Cl)N2CCC3=C(C2)C=CS3 [**D8**] **Plavix**
- CCC(C)(C)C(=O)O[C@H]1C[C@H](C=C2[C@H]1[C@H]([C@H](C=C2)C)CC[C@@H]3C[C@H](CC(=O)O3)O)C [**D9**] **Zocor**
- CCOC(=O)C1=C(NC(=C(C1C2=CC=CC=C2Cl)C(=O)OC)C)COCCN [**D10**] **Norvasc**
- CN(C)CCC[C@@]1(C2=C(CO1)C=C(C=C2)C#N)C3=CC=C(C=C3)F [**D11**] **Lexapro**
- C1CN(CCN1CCOCCO)C2=NC3=CC=CC=C3SC4=CC=CC=C42 [**D12**] **Seroquel**
- COC1=C(C(=NC=C1)CS(=O)C2=NC3=C(N2)C=C(C=C3)OC(F)F)OC [**D13**] **Protonix**
- CC1=CC=C(C=C1)C2=C(N3C=C(C=CC3=N2)C)CC(=O)N(C)C [**D14**] **Ambien**
- CCC1=CN=C(C=C1)CCOC2=CC=C(C=C2)CC3C(=O)NC(=O)S3 [**D15**] **Actos**
- CN[C@H]1CC[C@H](C2=CC=CC=C12)C3=CC(=C(C=C3)Cl)Cl [**D16**] **Zoloft**
- CC(C(=O)C1=CC(=CC=C1)Cl)NC(C)(C)C [**D17**] **Wellbutrin**
- CN(CCOC1=CC=C(C=C1)C[C@H]2C(=O)NC(=O)S2)C3=CC=CC=N3 [**D18**] **Avandia**
- CC1=C(C(=O)N2CCCCC2=N1)CCN3CCC(CC3)C4=NOC5=C4C=CC(=C5)F [**D19**] **Risperdal**
- CC1=CC2=C(NC3=CC=CC=C3N=C2S1)N4CCN(CC4)C [**D20**] **Zyprexa**

- CC1(O[C@@H]2CO[C@@]3([C@H]([C@@H]2O1)OC(O3)(C)C)COS(=O)(=O)N)C [**D21**] **Topamax**
- CC(C)NCC(COC1=CC=C(C=C1)CCOC)O [**D22**] **Toprol**
- C1=CC(=CC=C1[C@@H]2[C@H](C(=O)N2C3=CC=C(C=C3)F)CC[C@@H](C4=CC=C(C=C4)F)O)O [**D23**] **Zetia**
- C(CC(O)(P(=O)(O)O)P(=O)(O)O)CN [**D24**] **Fosamax**
- C1CC(=O)NC2=C1C=CC(=C2)OCCCCN3CCN(CC3)C4=C(C(=CC=C4)Cl)Cl [**D25**] **Abilify**
- C[C@H]1COC2=C3N1C=C(C(=O)C3=CC(=C2N4CCN(CC4)C)F)C(=O)O [**D26**] **Levaquin**
- C1=CC(=C(C(=C1)Cl)Cl)C2=C(N=C(N=N2)N)N [**D27**] **Lamictal**
- CC1=CC=C(C=C1)C2=CC(=NN2C3=CC=C(C=C3)S(=O)(=O)N)C(F)(F)F [**D28**] **Celebrex**
- CCOC(=O)[C@H](CCC1=CC=CC=C1)N[C@H]2CCC3=CC=CC=C3N(C2=O)CC(=O)O [**D29**] **Benazepril**
- C1CN(CCN1CCOCC(=O)O)C(C2=CC=CC=C2)C3=CC=C(C=C3)Cl [**D30**] **Zyrtec**
- COC1=CC=CC=C1OCCNCC(COC2=CC=CC3=C2C4=CC=CC=C4N3)O [**D31**] **Coreg**
- CC(C)[C@@H](C(=O)OCCOCN1C=NC2=C1NC(=NC2=O)N)N [**D32**] **Valtrex**
- CC(CC1=CC=CC=C1)N [**D33**] **Adderall**
- CC1=C(C=CN=C1CS(=O)C2=NC3=CC=CC=C3N2)OCCCOC [**D34**] **Aciphex**
- CNCC[C@@H](C1=CC=CS1)OC2=CC=CC3=CC=CC=C32 [**D35**] **Cymbalta**
- CC(C)C1=NC(=NC(=C1\C=C\[C@@H](C[C@H](CC(=O)O)O)O)C2=CC=C(C=C2)F)N(C)S(=O)(=O)C [**D36**] **Crestor**
- CCCCC(=O)N(CC1=CC=C(C=C1)C2=CC=CC=C2C3=NNN=N3)[C@@H](C(C)C)C(=O)O [**D37**] **Diovan**
- CC(C)OC(=O)C(C)(C)OC1=CC=C(C=C1)C(=O)C2=CC=C(C=C2)Cl [**D38**] **Tricor**
- COC(=O)C(C1CCCCN1)C2=CC=CC=C2 [**D39**] **Concerta**
- CNS(=O)(=O)CC1=CC2=C(C=C1)NC=C2CCN(C)C [**D40**] **Imitrex**

MOLID	PC1	PC2	PC3	PC4
Wellbutrin	-2.947867	0.949240	0.379244	0.395389
Actos	-0.795424	1.814022	0.847535	0.629298
Fosamax	-1.923532	-1.865736	-4.500723	0.641577
Zyprexa	-1.490190	2.025484	0.624590	-0.329180
Lexapro	-1.349387	2.302719	1.157700	0.288288
Cymbalta	-1.496572	3.165058	1.103102	0.199865
Toprol	-1.999916	0.072818	0.080971	0.520913
Zoloft	-2.148413	2.789387	1.148670	-0.093031
Lamictal	-2.284605	3.047376	-1.588002	1.072271
Lipitor	3.207815	3.005286	1.540507	0.424845
Zyrtec	-0.430144	1.758198	1.082486	0.508056
Celebrex	-0.545456	3.551996	0.212534	1.150342
Zocor	-0.358407	-1.714874	1.453941	-0.269053
Prevacid	-0.954760	2.894003	0.140200	1.567204
Zetia	0.441375	3.478700	0.921978	0.463414
Plavix	-1.922749	2.092060	0.766399	0.405654
Imitrex	-1.566291	1.423110	-0.058478	0.629250
Serevent	0.864760	0.926540	1.008021	0.609156
Valtrex	-0.626126	-0.274993	-1.811246	1.533520
Levaquin	-0.921686	0.665634	-0.128226	-0.001559
Risperdal	-0.041411	1.087003	1.174004	0.286604
Aciphex	-0.580862	2.377047	0.522973	0.854566
Singulair	3.249701	3.187256	3.057315	0.285042
Flonase	0.121278	-1.355958	0.985766	0.354296
Effexor	-2.094719	0.277190	0.695032	-0.383218
Avandia	-0.756467	1.861984	0.547909	0.882779
Concerta	-2.664336	0.779642	0.210238	-0.052132
Topamax	-1.695090	-1.344998	-1.659410	-0.148023
Coreg	0.839170	3.431658	1.053189	0.273647
Adderall	-3.917897	1.402990	-0.449306	-0.334897
Crestor	1.320920	0.849008	-0.081762	1.109810
Nexium	-0.852321	2.656151	0.389447	0.481122

Seroquel	-0.348335	1.861663	0.920296	0.037717
Diovan	1.155581	2.170853	1.012792	1.068148
Ambien	-1.286665	2.754190	1.044149	0.076844
Abilify	0.054914	1.771577	1.616710	0.722017
Benazepril	0.758169	1.455439	0.830365	0.846831
Tricor	-1.000666	2.202432	1.344783	0.770577
Norvasc	-0.117803	0.126655	0.081803	1.212919
Protonix	-0.615848	2.694799	-0.059414	1.481745

Table 2: PCA results table for the first four dimensions of the 40 selected BB drugs (77% of data variance, as reported)[2]

BB drugs	**Ix**	**Iy**	**Iz**	**I_1/I_3**	**I_2/I_3**
Abilify	19468,6316	18027,9885	1907,6690	0,097987	0,926002
Aciphex	8604,1896	7833,5804	1253,2256	0,145653	0,910438
Actos	3700,3624	2929,2283	1255,8522	0,339386	0,791606
Addreall	750,3472	672,3761	165,0999	0,220031	0,896087
Ambien	3700,3624	2929,2283	1255,8522	0,428731	0,791606
Avandia	10080,1629	9689,6952	855,6519	0,084885	0,961264
Benazepril	7116,9909	5327,0804	3035,2427	0,426478	0,748502
Celebrex	6504,5945	4728,0579	2261,1954	0,34763	0,72688
Concerta	2054,2651	1428,571	907,4623	0,441745	0,695417
Coreg	7617,6095	5901,818	2345,3039	0,307879	0,77476
Crestor	11878,6363	9684,2605	2808,7465	0,236454	0,815267
Cymbalta	3295,0585	2633,8655	1319,0532	0,400313	0,799338
Diovan	7735,9358	7144,0329	2422,3008	0,313123	0,923487
Effexor	2955,9373	2469,4745	1066,8094	0,431999	0,835429
Flonase	8573,4311	8143,2522	2326,1138	0,271317	0,949824
Fosamax	1564,4737	1226,5064	661,7331	0,422975	0,783974
Imitrex	3763,789	2689,8955	1394,413	0,370481	0,714678
Lamictal	2271,6461	1954,8567	546,986	0,279809	0,860546
Levaquin	5458,2831	4589,3588	1129,1647	0,206872	0,840806
Lexapro	4756,8421	3648,4641	1777,3417	0,373639	0,766993
Lipitor	16079,1994	13083,11	4288,9064	0,266736	0,813667
Nexium	7032,6028	6708,9966	715,4585	0,101735	0,953985

Norvasc	5122,3842	5122,3842	2311,8703	0,451327	0,838675
Plavix	3775,7536	3111,5022	1337,2041	0,354156	0,824074
Prevacid	8198,8612	7641,7607	970,0498	0,118315	0,932051
Protonix	5182,7972	4276,3957	2044,7004	0,394517	0,825113
Risperdal	3769,5912	2274,2153	1689,8372	0,448281	0,603306
Serevent	22065,1553	21469,9341	1924,1721	0,087204	0,973024
Seroquel	7922,1841	6956,4141	1623,9171	0,204984	0,878093
Singulair	18800,0658	17096,7837	4597,0118	0,244521	0,9094
Topamax	3819,7256	3299,4195	1145,2816	0,299833	0,863784
Toprol	6336,0522	6302,3245	321,2545	0,050703	0,994677
Tricor	9201,1394	8696,1598	892,3361	0,096981	0,945118
Valtrex	7133,8108	6805,8912	850,4822	0,119218	0,954033
Wellbutrin	2298,211	1693,4448	800,0743	0,348129	0,736853
Zetia	8560,4071	7421,4181	2684,933	0,313645	0,866947
Zocor	6528,1806	6266,8953	2069,5387	0,317016	0,959976
Zoloft	3213,3139	3112,888	1037,6188	0,322912	0,968747
Zyprexa	3769,5912	2274,2153	1689,8372	0,448281	0,603306
Zyrtec	8297,2452	7152,2397	1942,6328	0,23413	0,862002

Table 3: PMI results of the 40 selected BB drugs

References

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