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DESIGN AND SYNTHESIS OF NOVEL SCAFFOLDS AND BUILDING BLOCKS

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Medicinal chemistry focuses on the aspect related to the structural design, synthesis and identification of therapeutically interesting compounds, *i.e.* pharmaceuticals, as well as the molecular reasons of their mechanism of action, including the understanding of the factors involved in the structure-activity relationships, absorption, distribution, metabolism, elimination and toxicity.

A number of studies have appeared in the recent literature on the identification of molecular frameworks, which correspond to the minimum structural subunit, in several drugs or lead-compounds, able of providing ligand points for more than one type of bioreceptor. Since Evans first introduced the concept of "privileged structure", privileged-based drug discovery has emerged as a fruitful approach in medicinal chemistry. Privileged scaffolds increase hit rates for biological targets of interest, leading to the discovery of other biologically active targets and generating leads with enhanced drug-like properties. Consequently, medicinal chemists value privileged structures as core scaffolds for viable starting points in exploration design and synthesis.

Despite the identification of numerous recurring molecular frameworks in bioactive molecules, there is a restricted availability of privileged structures. Toward this concern, a subject of great fascination and importance, dealing with the need to identify novel chemotypes, have been addressed the studies conducted during this PhD thesis. Research into drug-like and lead-like concepts has explored a range of

ideas looking at structural characteristics and physicochemical properties. The selected structures might represent potential replacements of frequently occurring structural motifs. The development of efficient methodologies for the synthesis of the identified compounds has provided the suitable tools to open up an investigation about the behaviour of such a kind of molecules towards biological systems.

List of Abbreviations

Ac	acetyl
ACE-Cl	1-chloroethyl chloroformate
ADME	absorption, distribution, metabolism, excretion
All	allyl
aq.	aqueous
BB	building block
BEMP	2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine
BINAP	2,2′-bis(diphenylphosphino)-1,1′- binaphthalene
Bn	benzyl
Boc	*t*-butoxycarbonyl
br	broad (NMR)
Cbz	benzyloxycarbonyl
CbzCl	benzyl chloroformate
CNS	central nervous system
COSY	correlation spectroscopy
CV	cardiovascular
d	doublet (NMR)
DABO	2,7-diazabicyclo[3.3.0]octane
DAST	diethylaminosulfur trifluoride
dba	dibenzilideneacetone
DBU	1,8-diazabicycloundec-7-ene
DCE	1,2-dichloroethane
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIBAL-H	diisobutylaluminium hydride
DIPA	diisopropylamine

DIPEA	diisopropylethylenamine
DMAP	4-dimethylaminopyridine
DMF	*N, N*-dimethylformamide
DMSO	dimethyl sulfoxide
DPPF	1,1'-bis(diphenylphosphino)ferrocene
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
Etp	petroleum ether
FDA	Food and Drugs Administration
GPCR	G protein-coupled receptor
HMDS	hexamethyldisilazane
HMPA	hexamethylphosphoramide
HOBT	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HTS	high throughput screening
IP	intellectual property
LDA	lithium diisopropylamide
LO	lead optimization
m	multiplet (NMR)
min	minute(s)
Ms	mesyl
mw	microwave
NCEs	new chemical entities
NIS	*N*-iodosuccinimide
NMP	1-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser enhancement
N-PhGly	*N*-phenylglycine
Ph	phenyl
PK	pharmacokinetic
PTSA	*p*-toluensulfonic acid

Py	pyridine
q	quartet (NMR)
Ra-Ni	raney nichel
rfx	reflux
RT	retention time
s	singlet (NMR)
SAR	structure-activity relationship
sat.d	satured
t	triplet (NMR)
TBTU	*o*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate
TEA	triethylamine
TEMPO	2,2,6,6-tetramethylpiperidine 1-oxyl
TFA	trifluoroacetic acid
TFMSA	trifluoromethanesulfonic acid
THF	tetrahydrofuran
Ti	internal temperature
TMEDA	*N*,*N*,*N*',*N*'-tetramethyl-ethane-1,2-diamine
Ts	tosyl
UPLC	ultra performance liquid chromatography

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1 PRIVILEGED STRUCTURES

The term "privileged structure" has been adopted in several concepts, designing compounds highy represented in the overall bioactive compound population, or scaffolds able to present functional groups in a favourable arrangement. While many classes of privileged compounds such as 1,4-dihydropyridines, biphenyls and benzodiazepines produce leads with enhanced drug-like properties, other structures such as pyrrolinone compounds were classified as "privileged" since they own rigid framework able to direct functional groups in a well defined space.

Several researches dealing with privileged structures highlighted the reason for their privileged status and can help in a more rationale use of these structures in drug discovery. This new tool of understanding bioactive molecular diversity is employed in the "colonization" of the existing therapeutic space for each molecular framework and in association with other classical medicinal chemistry concepts assists the rational design of new drug-candidate prototypes.

1.1 *THE DRUG DISCOVERY PROCESS*

The traditional process of drug discovery has been driven largely by phenotypic observations of the effects of a natural product or a derived synthetic agent on a physiological process or pathological state. This was followed by a process of iterative synthesis and biological testing. Typically, the molecular nature of the biological target was not known, the biological testing was carried out significantly *in vivo*, and chemical synthesis was a hand-crafted 'one molecule at a time process'. It has been a very successful approach yielding antibiotics, antidepressants, antihypertensive and anticancer agents, to name but four principal classes of therapeutic agents.

This traditional scheme is contrasted with the genomic-based approach where genome interrogation was assumed to lead to thousands of new targets, a significant increase over the approximately 500 targets in the current pharmacopoeia.

The advent of genomics and the molecular biology revolution has permitted both the definition of new targets and the characterization of the genetic basis of disease states. The complexity of human cellular organization is not based upon a simple 'one gene = one protein' model, but rather by multiple use of the same gene-splice variants, population alleles and post-translational modification, and by the combinatorial diversification of signalling pathways. From this relatively limited gene repertoire the human probably expresses in spatial and temporally heterogeneous manner some 150,000 proteins. However, a protein target may not be druggable because of its intrinsic properties or expression, but also because it may play a role in multiple signaling pathways other than the one of pathological interest. The deciphering of this signalling network is thus of critical importance to the process of target validation. The systems biology approach is an integrated rather than a reductionist approach used to understand the relationships between function of a biological system and the effects of perturbations such as a disease or the addition of a drug or potential drug molecule. This approach suggests that there may be only a few thousand druggable targets rather than the 150,000 or so once confidently predicted.[1]

Presently, GPCRs are the predominant target family addressed, and more than 600 genes encoding GPCRs have been identified from human genome sequencing efforts.[2] The balance of such targets and their relative novelty are the domain of the companies' early project-portfolio management strategy.[3] Of course, a balance always has to be struck between the requirements of the disease area for efficacious new therapies, business considerations and, most crucially, the chemical tractability or drugability of targets for small-molecule intervention.[4] It is well accepted within the medicinal chemistry community that, independently of the technology applied, certain protein families are more readily modulated by small-molecule intervention than others. In this context, target selection plays a pivotal role in the final outcome

of hit and lead identification activities. A retrospective analysis of past discovery programmes reveals that much higher success rates have been demonstrated for aminergic GPCRs compared with large peptide receptors, for example. This is not surprising, as modulating protein-protein interactions - often involving large surface area- by a small chemical entity is far move demanding than competing against an endogenous small-molecule ligand.

Apart from the intrinsic biochemical and kinetic challenges in identifying an appropriate modulator for a target, the range of meaningful assays and ligand-identification technologies significantly influences the chances of success. *High throughput screening* (screening of a compound collection to identify hits in an *in vitro* assay, usually performed robotically) is presently the most widely applicable technology delivering chemistry entry points for drug discovery programmes. The potential for success is nevertheless demonstrated by a variety of development candidates and marketed drugs that have resulted from hits generated by HTS campaigns. It is evident that in the future the growing number of emerging targets will increase the demands put on compound collections and HTS and that this will call for new hit and lead generation strategies to curb costs and enhance efficiency.[5]

1.2 *MULTI-PROPERTY OPTIMIZATION*

During the past few years, there has been an increasing awareness of the need for developing drug-like properties of a molecule. These are the balance of biophysicochemical requirements for the molecule to reach its site of action in man at the given concentration, for the necessary duration and in an adequate safety window in order to answer the therapeutic principle hypothesis.[6]

In the past, lead-finding activities were mainly directed towards affinity and selectivity rather than molecular properties, metabolic liabilities and so on. It was not uncommon for a confirmed single primary active compound to be considered a ‘lead’ structure, or, in the case of a cluster of activies with SAR, a ‘lead series’. Frequently, attention was not paid to characteristics of the molecules other then

perhaps their chemical stability and synthetic accessibility. A consequence of these insufficient lead criteria was that full project teams were assembled with only a single superficially evaluated 'lead'. A thorough consideration of other important drug features was often postponed later in the optimization phase, when the *in vitro* affinity and selectivity had been fully optimized at the expense of other facets, such as solubility, permeability or metabolic stability.

Unfortunately, as the lead molecules becomes increasingly more potent, selective and tailored for the target, there is generally less tolerance for introducing significant changes to affect biophysical properties without a large intrinsic affinity penality. Such unbalanced, sub-optimal candidates had poor ADME attributes which often preclude them from progressing and being fully evaluated in the clinic due to, for example, dose-limiting solubility, poor absorption, cytochrome P_{450} interactions or metabolic instability. Clearly, poor initial leads with weak entry criteria into lead optimization often can not be refined to generate compounds with an appropriate profile, resulting in high attrition rates at the clinical candidate selection stage. This point has been highlighted in a recent analysis of launched drugs, which indicates that, generally, relatively minor changes in structural and physical molecular properties have been made between the lead and the launched drug candidate.[7] This emphasizes once more that quality of the lead is crucial in most cases to the success of the refinement and development process. If the clinical entry criteria are lax, the attrition is moved further into pilot safety testing or early clinical-phase studies. The optimization process has historically been largely sequential in nature, addressing one issue at time, with the hope that all necessary modifications could be accommodated within the pharmacophore optimized for affinity only. This approach led to a very high and expensive failure rate in the clinic. During the mid-90s, this view changed to embrace a more holistic attitude towards lead optimization and subsequently to hit-to-lead generation and hit identification. The required trade-off for balancing these properties, in conjuction with pure affinity to achieve an equilibrated potential therapeutic drug molecule, resulted in a change of approach from sequential to multi-dimentional optimization.

1.3 *HIT AND LEAD GENERATION STRATEGIES*

The entry point for any chemistry programme within drug discovery research is generally the identification of specifically acting low-molecular-weight modulators with an adequate activity in a suitable target assay. Such initial hits can be generated in a number of ways, depending on the level of information available[8] (Figure 1). It is therefore important to employ alternative hit-identification strategies that are able to tackle a variety of biological macromolecular targets effectively, and to identify proprietary, synthetically tractable and pharmacologically relevant compounds rapidly.

These methods can be subdivided into those that require very detailed ligand and/or target information, and those that do not. The former include techniques such as mutagenesis, NMR and X-ray crystallography, as well as the recognition information that can be derived from endogenous ligands or non-natural small molecule surrogates retrieved from literature and patents. At the other extreme are the technologies that do not require any prior information on target or ligand, and which use serendipity-based search strategies in either a given physical or virtual compound subset. Example of so called “random” or pseudo-biased hit-identification strategies include biophysical and biochemical testing that employ one or more method of detecting a molecular-binding event, usually in high-throughput format[9].

Between these extremes are more integrated approaches, including targeted libraries and chemogenomics[10]. The marriage of HTS with computational chemistry methods[11] allows for more meaningful and directed iterative rapid-feedback searches of subsets and focused libraries. The prerequisite for success of both approaches is the availability of the highest-quality compounds possible for screening, either real or virtual.

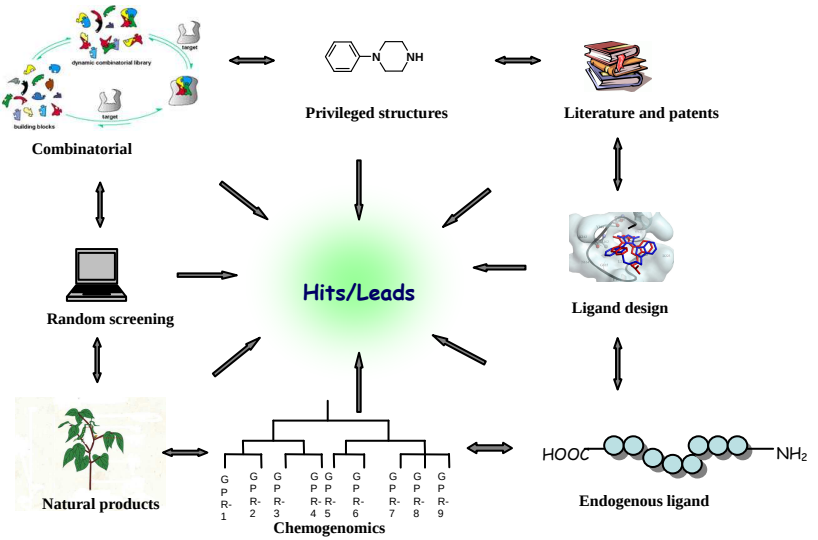

Figure 1- *Hit/lead identification strategies. The most commonly applied hit-identification strategies today range from knowledge-based approaches, which use literature- and patent-derived molecular entities, endogenous ligands or biostructural information, to the purely serendipity-based 'brute force' methods such as combinatorial chemistry and high-throughput screening*

1.4 *FOCUSING FOR LIBRARIES*

The 'combinatorial explosion'- meaning the virtually infinite number of compounds that are synthetically tractable- has fascinated and challenged chemists ever since the inception of the concept. Independent of the library designs, the question of which compounds should be made from the huge pool of possibilities always emerges immediately, once the chemistry is established and the relevant building blocks are identified.[12]

The original concept of 'synthesize and test', without considering the targets being screened, was frequently questioned by the medicinal chemistry community and is

nowadays considered to be of much lower interest due to the unsatisfactory hit rates obtained so far. The days in which compounds were generated just for filling up the companies inventories, without taking any design or filtering criteria into account, have passed. Focus has mooved from huge and diverse 'random' combinatorial libraries towards smaller and focused drug-like subsets. Although the discussion of how much focused or biased a library should be is still an ongoing debate, the low hit rate of large, random combinatorial libraries, as well as the steady increase in demand for screening capacity, has instead set the stage for efforts towards small and focused compound collections.

Guided by the target. Biostructural information derived from mutagenesis data, as well as NMR or X-ray crystallographic analysis, has long been used for drug discovery purposes. Understanding of the mechanism of action of a biological target is an important aid in biasing compound collections. These mechanism-based libraries have been applied successfully to a variety of proteins to generate transition-state mimics using either parallel solution- or solid-phase synthesis techniques[13].

'Cherry picking' from virtual space. A highly sophisticated way to avoid the synthesis of trivial analogues is the application of virtual screening tools in order to search through chemical space for topologically or pharmacophoric similar entities using known actives (seed structures) as references. Biostructural information can also be applied if available[14].

Privileged structures or motifs. This approach is particularly relevant for targets for which very limited or no biostructural informations are available. It is in this case where elements of known biologically active molecules are used as "the core" for generating libraries encompassing these 'privileged structures'. One example is provided by the benzodiazepine nucleus which is found in ligands of both ion channel and G-protein coupled receptors (GPCRs) and in addition to being the basis

for Valium and many other antianxiety and muscle relaxant agents can also be directed against other biologically distinct targets (Figure 2).[15]

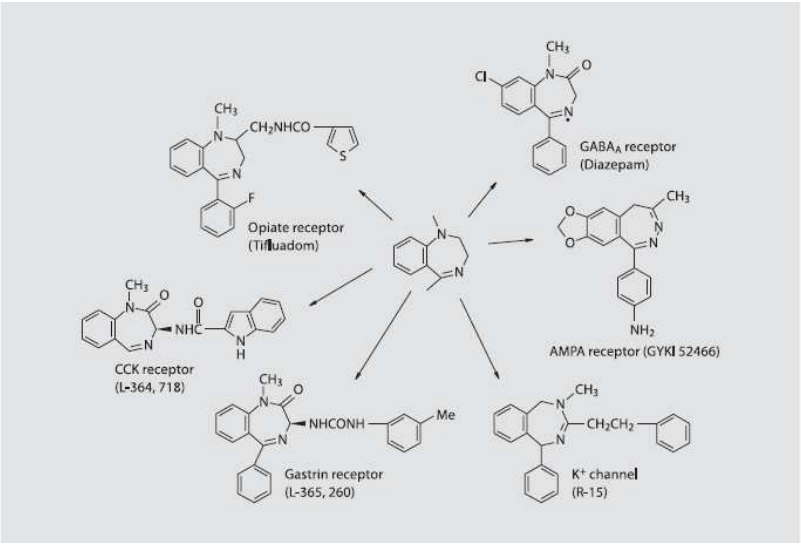

Figure 2- *The benzodiazepine skeleton as a 'privileged' structure generating molecules that interact at diverse and unrelated targets*

The restricted availability of privileged structures clearly limits the scope of this ligand-based approach to some extent. As a result, there is a continued need to identify novel chemotypes.

1.5 *PRIVILEGED STRUCTURES*

In summarizing the successful use of benzodiazepines, Evans and colleagues concluded that "judicious modification of such structures could be a viable alternative in the search for new receptor agonists and antagonists". In 1988 they introduced the term privileged structure in relation to the heterocycle 1,4-benzodiazepine-2-one, defined as "a single molecular framework able to provide ligands for more than one receptor"[16]. This concept of privileged structures was

improved later by Patchett and Nargund,[17] who identified properties in these structures that make their interaction with biomacromolecules easier and occasionally distinct from the ones that involve the respective endogenous ligands.

Prior to the seminal paper of Evans coining the privileged structure term, the notion of these types of stuctures had been emerging for some time. Early various research groups have recognized the presence of recurring structural units in many receptor ligands. For example, Ariëns et al. noted the presence of hydrophobic double-ring systems in many biogenic amine antagonists, which they suggested must interact with accessory hydrophobic binding sites. They also observed multiple actions of some molecules and suggested this was related to conformational flexibility.[18] Subsequently, Andrews and Lloyd described a number of common topological arrangements for biogenic amine antagonists. They concluded that a common pharmacophore existed throughout diverse drug classes, and that specificity resulted from secondary binding groups attached to the basic pharmacophore.[19]

Priviliged structures need to be distinguished from 'frequent hitters' such as those described by Roche,[20] which either bind nonspecifically to a variety of targets or interfere with the read-out of biological assays. Moreover, a structural rather than biological definition of a privileged structure was provided by IUPAC as a 'substructural feature which confers desirable (often drug-like) properties on compounds containing that feature. It often consists of a semi-rigid scaffold, which is able to present multiple hydrophobic residues without undergoing hydrophobic collapse'[21,22].

Privileged structures represent an ideal source of lead compounds. A single library based upon privileged structures might lead to active compounds at a variety of receptors. Several groups have utilized these structures in this manner. For example, combinatorial libraries based upon privileged structures have been synthesized by Nicolau and colleagues, who utilized a benzopyran scaffold,[23,24] Schultz and co-workers, who made use of the purine scaffold,[25,26,27,28] and Hirschmann and Smith, who have worked with glycosides.[29,30,31] Patchett and co-workers utilized privileged structures as "hydrophobic anchors" (harnessing their capabilities to bind to

proteinaceous surfaces) to which they appended peptide functionality to gain specificity.[32,33,34] Hirschmann et al. also believed that the attachment of genetically encoded and uncoded aminoacid side chains to privilegd structures are a promising means to produce diverse libraries of compounds.[24] Whereas not every group intends to use these scaffolds in such a fashion, several groups have focused on privileged structures to improve the efficiency of drug discovery. For example, Hirschmann, Smith and colleagues have actively pursued the design and development of new privileged scaffolds and have made hybrids of existing privileged structures.[35]

There has therefore been significant interest in the identification of new privileged structures, and many groups have utilized computational procedures to aid this endeavor. For example, Nilson et al. explored databases of drugs to identify structural motifs that have broad biological activities and developed synthetic processes to prepare arrays of such compounds.[36] Another example is RECAP, a computational technique that has been developed to identify privileged structures from biologically active molecules for use in library development.[37] Mason et al. also developed a four-point pharmacophore method for the design of focused combinatorial libraries of molecules with privileged structure characteristics.[38]

Natural products can also possess the "privileged structure" characteristics. In general, natural products are considered to contain scaffolds with the potentiality to be privileged structures because in many cases they are synthesized by biological systems to specifically interact with protein targets. Natural products differ from synthetic substances under several aspects: they tend to contain fewer nitrogen, halogen or sulphur but more oxygen atoms; they are likely to contain a larger number of rings and more chiral centers[39,40,41]; thus natural product scaffolds can be used to explore a significant portion of drug-relevant pharmacophoric space.[42]

1.6 *CHARACTERISTICS OF PRIVILEGED STRUCTURES*

The understanding of the molecular determinants that underline the privileged structure-target relationships should be the key to apply the full potential of the privileged structure concept in the design of novel targeted compounds sets. However, the reason for which these compounds are "privileged" is not completely clear.

Hirschmann and colleagues have stressed that "no unifying three-dimentional structural feature for privileged structures has been identified". However, observations in biology and chemistry fields have suggested those molecules do display such characteristics. By the term privileged scaffold it is intendeded a substructure or template (sometimes also referred as "motifs" or fingerprings) that when incorporated in a pharmacophore has a high degree of drug likeness due to the presence of atom or functional group properties that are relevant in ligand binding such as: volume, hybridization, partial atomic charge, electronegativity, polarizability, hydrophobicity, hydrogen-bonding potential; local properties that are often parameterized via the use of global physicochemical properties, such as: molecular weight, logP, molar refractivity, and so on. Viable "privileged scaffolds" often consist of a relatively rigid ring system, which is able to present multiple hydrophobic residues in predictable orientations in space and consequently, without undergoing hydrophobic collapse.[43] They provide molecular rigidity, allowing less entropic energy to be lost upon binding, and also provide better bioavailability. Bicyclic and tricyclic scaffolds are therefore an ideal size for compound array. They have a small enough molecular weight to provide scope for improved specificity and affinity through the attachement of suitable substituents (which will consequently increase molecular weight, yet retain drug-like character) in a wide variety of topologies.

The size of privileged structure relative to the entire molecule is an important factor. Functional groups such as amides and carboxylic acids are too small and too ubiquitous to be classified as privileged structures. Small monocycles such as

benzene, furan or tiophene are also clearly capable of being privileged structures, but when they form part of structures with a molecular weight of around 500 Da, the nature and extent of their contribution to the overall molecule is uncertain. However, larger structures clearly display all of the characteristics of a privileged structure, if on a macromolecular scale. Hence, a privileged structure should constitute a significant portion of the total mass of the molecule, and represent its core element. Bicyclic and tricyclic compounds are capable of fulfilling these requirements. However, it was noted that many privileged structures larger than bicycles were merely combination of hybrids of two or more bicyclic privileged structures. Hence, bicyclic privileged structures may represent the core elements of an entire suite of privileged structures.

Even in absence of a clear understanding of the reason for this status, the use of "privileged structures" provides an empirical alternative to blind screening attempts and might represent a useful tool for the development of new, selective compounds by its application in structure-based drug design.

1.7 *USE OF PRIVILEGED STRUCTURES CONCEPT IN THE RATIONAL DESIGN OF NEW CHEMICAL DRUGS*

Since the 1990's, with the use of the combinatorial chemistry and robotyzed screening (*high throughput synthesis and high throughput screening- HTS*), the design of new chemical libraries for bioassays has become a great challenge for the medicinal chemists, leading to some paradigm changes, especially in the level of research conducted in the industrial laboratories.

It is worth highlighting that, despite the substancial investment made by the pharmaceutical industry sector in these new technologies, the discovery of new chemical entities (NCEs) that effectively represent an opportunity to reach the market as authentic therapeutic innovations, has not fulfilled the expectations of the sector[44]. Infact, in 2005, among the 14 new medicines that came into the market,

approved by the American regulatory agency (*FDA- Food and Drugs Administration*), with the exception of product with diagnosis purpose, only *ca.* 50% of them had the status of authentic NCEs.

In this context, the pharmaceutical industrial sector, involved in the research of new drugs and medicines, redirected the structural design of collections of synthetic derivatives to be bioassayed by HTS, aiming at increasing the probability of obtaining new *hits* candidate to drug prototypes, which presented not only the appropriate pharmacophoric requisites, but also adequate solubility properties. The development of strategies with this purpose has been widely described in the literature and has led to the production of high impact works. Among others, the work of Lipinski and collaborators[45] described the "rule of the five", which is an excellent working hypothesis for predicting good druglike properties in new compounds[*]. Thus, close attention needs to be paid to molecular weights, as well as to the physicochemical properties of lead molecules, such as lipophilicity (logP) and aqueous solubility (which will affect oral bioavailability and the feasibility of generating a parenteral formulation), together with animal pharmacokinetics, which can be extrapolated with caution to predict corresponding behavior in humans. The latter is particularly important in providing some assurance that the candidate drug molecule will exhibit linear pharmacokinetics in humans, with appropriate dose size and elimination characteristics for the intended route and frequency of drug administration.

The employment of the *privileged structures* concept in the planning of new compound sets[46,47] has been associated with the use of computational methods and pharmacophoric models[48,49] or with the fragmentation of bioactive molecules, prototype or drugs, and has allowed the identification of relevant structural patterns that represent authentic biophores, providing useful frameworks to the building of new compound databases[50].

[*] In the discovery setting, the rule of five predicts that poor absorption or permeation of drugs is more likely when a drug molecule possess either (i) more than 5 hydrogen bond donors, (ii) 10 hydrogen bond acceptors, (iii) a molecular weight greater than 500, or (iv) a calculated logP greater than 5.

1.8 *BIOISOSTERISM AS GUIDE LINE FOR THE DESIGN OF PRIVILEGED STRUCTURES*

The principle of bioisosterism has long helped to guide drug discovery. In their attempts to optimize lead structures, medicinal chemists intuitively followed the principles of Darwinist evolution. The biological activity and, in later stages, the target selectivity, toxicology or pharmacokinetic behavior serve as the "fitness function" for the "survival" of certain structural entities. Privileged scaffolds contain structures, features giving better chances of survival.

Bioisosterism[51] is based on the assumption that "similar" molecules tend to exert "similar" biological activities and since long time, medicinal chemists have used this concept to modify the structures of biologically active compounds. While similarity of chemical structures cannot be defined in an objective manner, bioisosteric replacements of atoms or functional groups have paved the way from lead structures to therapeutically useful molecules.

Lead optimization needs similar analogs that cover the chemical space around the leads. In this new context, the principle of isosteric replacement of functional groups serves as a successful optimization strategy. Its systematic application has resulted in a broad variety of therapeutically used drugs, many of them finally having the desired combination of favorable properties.[52]

To date there is a restricted availability of isosteres of natural motifs and their applicability is often limited to a specific lead series SAR. Although novel replacements might be identified, their behaviour towards biological systems has ultimately to be verified.

1.9 *RESEARCH OBJECTIVES*

Hit and lead generation are key processes involved in the creation of successful new medicinal entities, and it is the quality of information content imparted through their

exploration and refinement that largely determined their fate in the later stages of clinical development. It is in the early phases of drug discovery that changes in process, such as the early interception of key ADME parameters, can have the maximum impact on later-stage success and timelines. The present high attrition rates, especially after lead-optimization phases, indicate that drug discovery as a sequential alignment of independent disciplines is ineffective for delivering high-quality medicines of the future, and that issues beyond activity and selectivity must be addressed as early as possible in a flexible, parallel fashion. Iterative medicinal chemistry activities in conjuction with multi-dimensional compound-property optimization generate a much-improved basis for proper and timely decisions about which lead series to pursue further.

The research program here described aimed at the identification and synthesis of novel bicyclic compounds, able to represent the core elements of an entire suite of privileged structures. The program consisted in a first step of design of scaffolds which comprised developability characteristics (judged in terms of expected metabolic stability and their physicochemical properties). Also consideration of literature motifs and drugs in development/market aided the selection of structural motifs to investigate.

In *Chapter 2* the synthesis of potential replacements of Ph- and Bn-piperazine/piperidine is reported. These heterocycles and their congeners often occur in active compounds for aminergic, and non-aminergic, CNS targets. Because of their prevalence in the field of pharmacologically active compounds and their drug-like properties, the synthesis of conformationally restricted analogues could be an interesting approach to gain insight into the influence of ring conformation on biological activity.

In *Chapter 3* the synthesis of some conformationally restricted amino acids and bis-amines is described. Target compounds were designed introducing a further constraint in natural cyclic structures and it might be expected that the additional conformational rigidity introduced in the molecule could result in improved pharmacokinetic properties of lead compounds.

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2 REPLACEMENT of Ph- and Bn- PIPERAZINE/ PIPERIDINE

Phenyl- and benzyl- piperazine and piperidine rings are very common structural motifs in drug discovery, with a high number encountered in compounds in the market. Because of their prevalence in the field of pharmacologically active compounds, they are regarded as privileged structural elements for the construction of drug-like molecules. For this reason, the synthesis of conformationally restricted piperazine/ piperidine analogues could be an interesting approach to gain insight into the influence of ring conformation on biological activity. Also modulation of N-atom basicity, as well as hindrance around it, are seen as valuable strategies to modulate activity at biological target and to influence physicochemical properties, selectivity, toxicity and PK characteristics.

Some examples of such structures are reported in Figure 1.

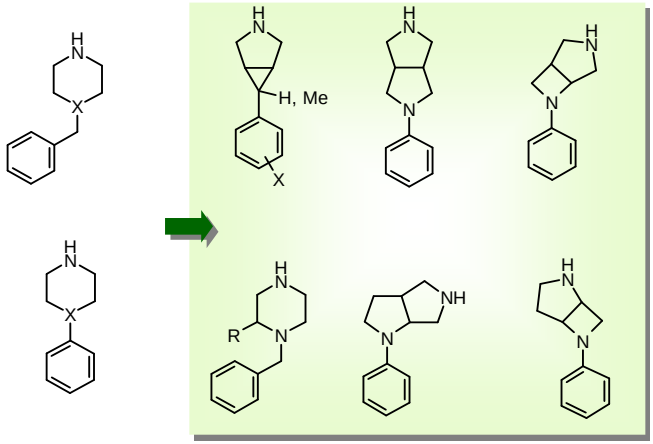


Figure 1 – *Examples of replacements of phenyl- and benzyl- piperazine/piperidine*

In this chapter the synthetic approach to some of these molecules is reported. The goal was to generate with highly efficient syntheses a wide range of building blocks

suitable for being used in the preparation of new chemical entities with biological activities.

2.1 *2- SUBSTITUTED BENZYL PIPERAZINES*

The piperazine template already contains some building features and pharmacological points and provides potent and selective ligands for a range of different biological targets in medicinal chemistry.[1] In particular, it is a common pharmacophore found in a large number of drugs for the treatment of disorders of the central nervous system associated with imbalances in dopaminergic, cholinergic or serotonergic signal transmission. Among these, CNS active agents are antipsychotics such as bifeprunox (**1**), antidepressants such as clozapin (**2**) and anticonvulsivants such as ropizine (**3**) (Figure 2).

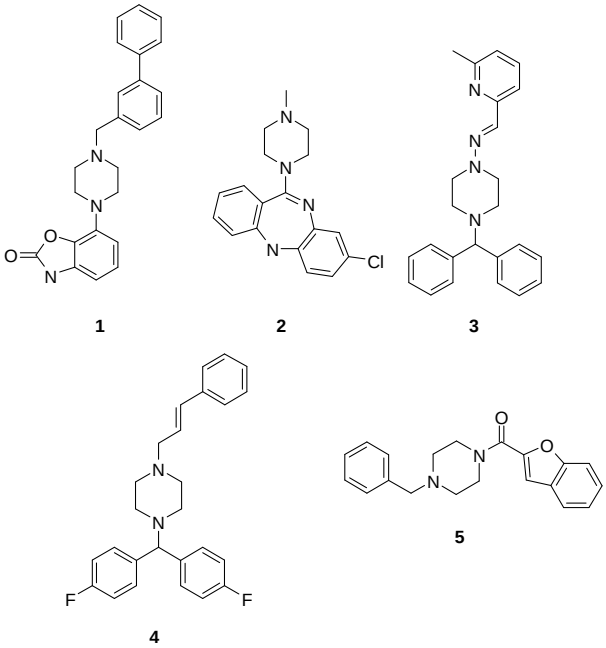
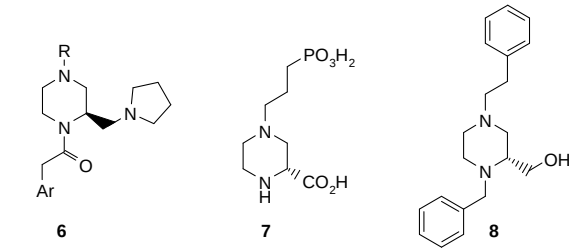


Figure 2 - *Drugs containing the piperazine ring*

The piperazine moiety is also contained in antihypertensive agents such as prazosine, calcium channel blockers such as flunarizine (**4**), H_1-blockers such as oxatomide, and antidepressant agent befuraline (**5**). Because of its prevalence in the field of pharmacologically active compounds, it is regarded as a privileged structural element for the construction of bioactive molecules.[2]

Whereas the synthesis of simple 1,4-disubstituted piperazines has received considerable attention, only a comparatively small number of *C*-substituted derivatives have been prepared and evaluated for their pharmacological properties.[3] It was found that piperazine derivatives bearing substituents in C-2 position can strongly interact with various receptors within the CNS. In Figure 3 some examples of piperazine derivatives with nanomolar receptor affinity are given.[4]



6 **7** **8**

k agonist NMDA antagonist σ ligand

Figure 3 - *C-substituted piperazines*

We were interested in preparing a diverse set of 2-substituted piperazines (**9a** and **10a**) and achieving a suitably flexible synthetic route to allow for the introduction of substituents able to modulate the pKa of the basic nitrogen. pKa_2 values for benzyl piperazine and target compounds were predicted with the ACD/labs software[5] (Figure 4).

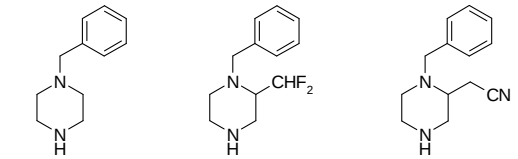


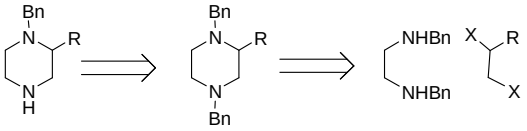
Figure 4 - *2-Substituted piperazines with a side chain able to modulate the basicity of the near N-atom*

2.1.1 *CHEMISTRY*

2-Substituted piperazines are commonly prepared by ring construction and reduction of diketopiperazines[6,7] or 2-ketopiperazines[8], *via* alkylation and reduction of 2-methylpyrazines[9], or by α-lithiation and alkylation of *N*-Boc piperazines[10]. In many of these cases, the piperazine derivatives must then be selectively protected prior to further modification.[11]

We investigated a new synthetic route involving the use of the same protecting group to the two nitrogen atoms: the isolation of the only monoprotected compounds **9a** and **10a** during the deprotection step could demonstrate the steric and/or electronic influence of the side chain on the reactivity of the nitrogen atoms.

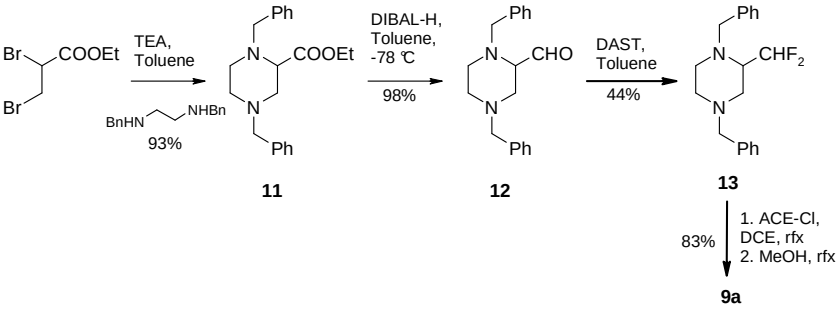
From a retrosynthetic point of view, piperazine ring could be prepared starting from the simple and commercially available *N,N'*-dibenzylethylendiamine (Scheme 1).



Scheme 1 - *Retrosynthetic analysis of **9a** and **10a***

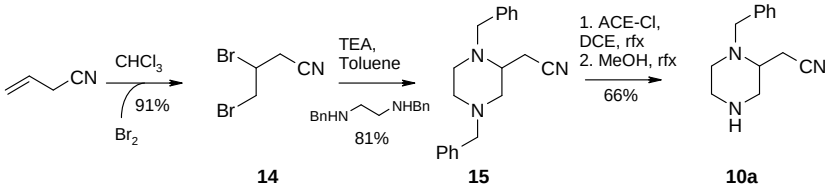
The double alkylation of the *N,N'*-dibenzylethylendiamine with ethyl 2,3-dibromopropenoate afforded in good yield the diaminoester **11**, that was

converted into **13** through controlled ester reduction with DIBAL-H at -78 °C followed by double fluorine-substitution with DAST. Both classical catalytic hydrogenation conditions and *N*-deprotection *via* carbamate (ACE-Cl, DCM then MeOH at reflux) gave the monoprotected derivative **9a** in satisfactory yield, supporting our initial hypothesis. The target compound **9a** was obtained in four steps with 23% overall yield (Scheme 2).



Scheme 2 - *Synthesis of difluoromethylenbenzylpiperazine **9a***

With a similar synthetic route compound **10a** was readily prepared with 46% overall yield (Scheme 3).

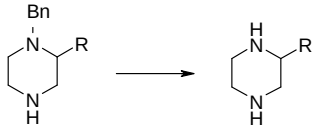


Scheme 3 - *Synthesis of **10a***

The double alkylation of the *N,N'*-dibenzylethylendiamine with 3,4-dibromo butyronitrile (**14**) afforded in good yield the full protected piperazine **15**, suitable to a regioselective deprotection using the methods described above.

In order to exemplify the possibility of introducing an eventual substituent on the N-1, the full deprotection of compounds **9a** and **10a** was tested. Table 1 summarizes

the tested reaction conditions; compounds **9b** and **10b** were obtained with the highest yields by catalytic hydrogenation with ammonium formate in presence of Pd/C.



9a R=CHF_2 **9b** R=CHF_2

10a R=CH_2CN **10b** R=CH_2CN

Table 1. *N-deprotection reaction conditions*

Entry	*Conditions*	*Yield (%)*
9a	H_2, Pd/ C, EtOH	68
9a	H_2, Pd/ C, EtOH, AcOOH	61
9a	1) ACE-Cl, DCE, reflux, 48 h; 2) MeOH, reflux	69
9a	Pd/C, $HCOONH_4$, MeOH, reflux	73
10a	H_2, Pd/ C, MeOH, rt	-
10a	1) ACE-Cl, DCE, 24 h reflux; 2) MeOH, reflux	83
10a	Pd/C, $HCOONH_4$, MeOH, reflux	88

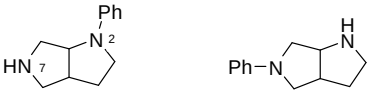
In conclusion, the developed synthetic route has the advantage of not requiring orthogonal protection at the two piperazine nitrogen atoms, therefore several different 2-functionalized piperazines can be readly prepared starting from the simple *N,N'*-dibenzylethylendiamine.

2.2 *ARYL- 2,7-DIAZABICYCLO[3.3.0]OCTANE*

The synthesis of polysubstituted and fused pyrrolidines, pyrrolizidines, indazolidines, pyranoquinolines and pyrrolo β-carboline ring systems has received synthetic chemists' attention over the years since these heterocycles form the

structural subunits of biologically important alkaloids[12] and pharmaceutically important compounds.[13] Specifically, 2,7-diazabicyclo[3.3.0]octane and its derivatives are used as adenosine kinase inhibitors[14] and serve as prime intermediates in the synthesis of 4-quinolone carboxylic acid and uracil-based antibacterials[15]. Recent studies have also demonstrated the success of racemic 2,7-diazabicyclo[3.3.0]octane (DABO) as homopiperazine isoster in the context of 5-HT_{2C} agonism.[16]

The wide range of bioactivities of DABO-based systems supported our plan to devise a synthetic route to its aryl derivatives that would be able to act as replacements of phenyl-piperazine/homopiperazine cores. DABO was selected as privileged structure also because of several attractive features: two potential sites (2-N and 7-N) for attachment to the aryl core (Figure 5), the ability to introduce chirality into the compounds, and the ease of modulation to introduce additional functionality.

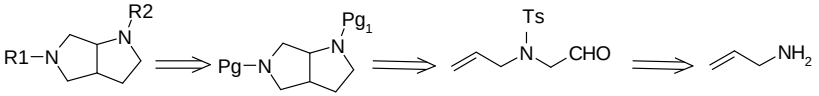


16 **17**

Figure 5 *–2,7-Diazabicyclo[3.3.0]octane aryl derivatives*

2.2.1 *CHEMISTRY*

The retrosynthetic analysis was based on reactions of aldehyde **18** as key intermediate.



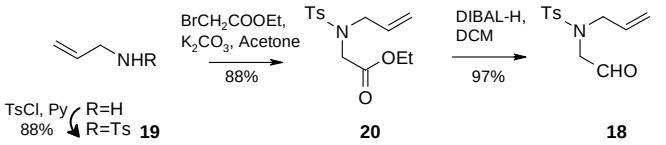
R1=H, R2=Ph
R1=Ph, R2=H

18

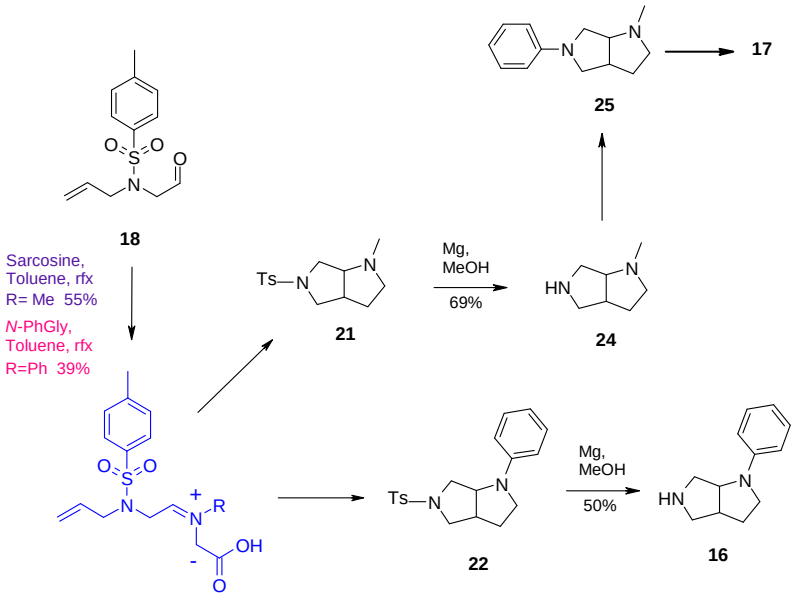
Scheme 4 - *Retrosynthetic analysis*

The crucial step of the strategy is the well documented [3+2] intramolecular cycloaddition of azomethine ylides generated by the decarboxylative condensation of amino acids with alkenyl aldehyde **18** (Scheme 4).[17,18,19,20]

The desired olefinic aldehyde **18** was synthesized in four steps in excellent yield (Scheme 5). Tosylation of allylamine gave sulphonamide **19**, which was treated with ethyl bromoacetate to afford amino ester **20** in 88% yield. Reduction with DIBAL-H at -78 °C afforded aldehyde **18** almost quantitatively.

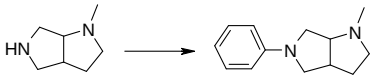


Scheme 5 - *Synthesis of the intermediate amino aldehyde **18***



Scheme 6 – *1,3-Dipolar cycloaddition as key step for the construction of DABO core*

Condensation of **18** with sarcosine and *N*-PhGly in refluxing toluene under Dean-Stark reaction conditions generated the azomethine ylides which cyclised to yield the *cis* adducts **21** and **22** respectively (Scheme 6). Treatment of compounds **21** and **22** with magnesium ribbons in MeOH gave respectively the target compound **16** and the intermediate **24** in good yields. In order to obtain **25**, several different conditions were tested for *N*-arylation of **24**.



24 **25**

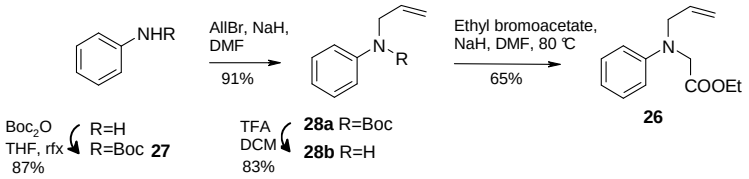
Table 2 - *Aryl amination on the 7-N position of DABO*

Conditions	***Yields (%)***
Cs_2CO_3, PhI, $Pd(OAc)_2$, BINAP, Toluene, 100 °C, 28 h	/
Cs_2CO_3, PhBr, $Pd(OAc)_2$, BINAP, Toluene, 120 °C mw,1 h	/
K_2CO_3, PhBr, DMSO, ultrasound	/
K_2CO_3, PhBr, DMSO, 100 °C	/
KOt-Bu, PhBr, DMF, 150 °C, mw	/
CuI, PhBr, K_2CO_3,, BINOL, toluene, 120 °C, 22 h	/
$PhB(OH)_2$, $Cu(OAc)_2$, TEA, Molecular Sieves, DCM, 16h, rt	7
$PhB(OH)_2$, $Cu(OAc)_2$, TEA, Molecular Sieves, TEMPO, DCM, 16 h, rt	14
$PhB(OH)_2$, $Cu(OAc)_2$, Py, Molecular Sieves, TEMPO, DCM, 16 h, rt	17
$PhB(OH)_2$, $Cu(OAc)_2$, K_2CO_3,Molecular Sieves, TEMPO, DCM, 16 h, rt	8
$PhB(OH)_2$, $Cu(OAc)_2$, DBU, Molecular Sieves, TEMPO, DCM, 20 h, rt	9
$PhB(OH)_2$, $Cu(OAc)_2$, DMAP, Molecular Sieves, TEMPO, DCM, 20 h, rt	/
$PhB(OH)_2$, $Cu(OAc)_2$, DIPEA, Molecular Sieves, TEMPO, DCM, 20 h, rt	Traces
$PhB(OH)_2$, $Cu(OAc)_2$, DIPA, Molecular Sieves, TEMPO, DCM, 20 h, rt	10
NaH, PhF, DMSO, 20 h, rt or 100°C, 24 h	/

As summarized in Table 2, classical Buckwald-Hartwig and Ullmann reaction conditions failed; better results were obtained using phenylboric acid in presence of $Cu(OAc)_2$ and TEMPO to regenerate the catalyst. The yield of reaction was strongly dependent by the nature of the base: pyridine was found the most effective base for

our pourpose, even if phenyl derivative **25** was isolated in yield that did not exceed 17%.

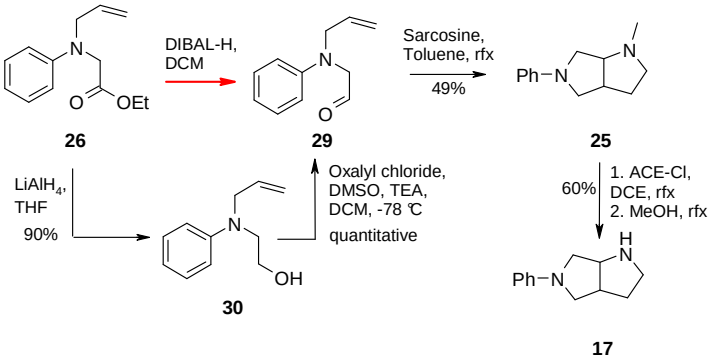
Our need for large quantities of material led us to pursue a longer synthetic route for **25** which had the advantage of *de novo* building of diazabicyclic core already functionalized with the phenyl group. The synthesis was carried out starting from the intermediate ester **26** prepared as outlined in Scheme 7.



Scheme 7 - *Synthesis of intermediate **26***

Controlled reduction with DIBAL-H failed when applied on the ester **26**, therefore aldehyde **29** was obtained *via* reduction to alcohol **30** followed by Swern oxidation. Condensation of **29** with sarcosine followed by *N*-demethylation gave the awaited compound **17** in satisfactory yield (Scheme 8).

The assignment of *cis* stereochemistry to the ring junctions of all cycloadducts was made by analogy with the stereochemistry observed for conventional azomethine ylide cycloaddition in similar systems.[21,22]



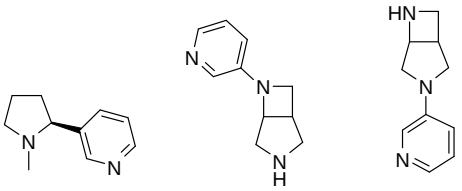
Scheme 8 - *Synthesis of **17***

In conclusion, it was accomplished an efficient synthesis of two novel octahydro aryl-DABO derivatives with high stereoselectivity by tandem ylide generation followed by intramolecular trapping by an *N*-tethered alkenyl group.

2.3 *FUSED AZETIDINES*

While fused 2-azetidinones, commonly known as β-lactams, are among the most useful azaheterocyclic compounds from both synthetic and medicinal chemistry points of view, the potential application of fused azetidines is only partially explored.

Azetidines are an interesting and important class of four membered heterocyclic compounds because of their reactivity[23] and biological activity[24]. In the last years Jianguo et al.[25] have synthesized some nicotinic acetylcholine receptor ligands derived from 3,6-diazabicyclo[3.2.0]heptane and demonstrated their potent neuronal activity. That study showed the ability of the rigidified –NCCN- motif linker, such as 3,6-diazabicyclo[3.2.0]heptanes **32** and **33**, in reducing the conformational complexity of nicotinic ligands (*i.e.* nicotine, **31**) and in enhancing the ligand subtype affinity (Figure 6).

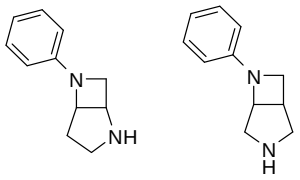


31 **32** **33**

Figure 6 - *Nicotinic acethylcoline receptor ligands*

In the light of those results we hypothesized that diazabicyclo[3.2.0]heptane framework could be an useful core not only for the optimization of novel selective nicotinic acetylcholine receptor ligands, but also for ligands to diverse targets. We synthesised 6-phenyl-2,6-diazabicyclo[3.2.0]heptane (**34**) and 6-phenyl-

3,6-diazabicyclo[3.2.0]heptane (**35**), hypothesizing that the introduction of the conformational constraint into the basic backbone could result in enhancing of the selectivity of the final ligand to the target receptorial site (Figure 7).

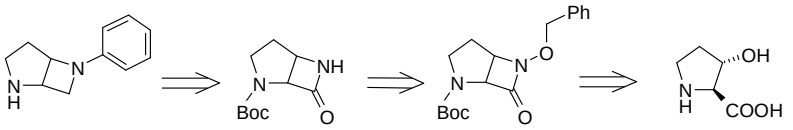


34 **35**

Figure 7 - *Diazabicyclo[3.2.0]heptanes as constrained phenyl piperazine replacements*

2.3.1 *6-PHENYL-2,6-DIAZABICYCLO[3.2.0]HEPTANE*

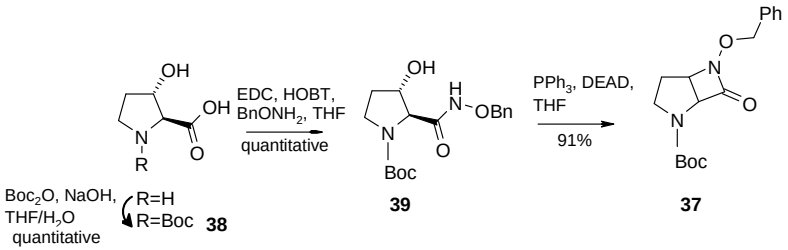
From a rapid retrosynthetic analysis, **34** can be derived from azetidinone **36** prepared starting from commercially available *trans*-3-hydroxy L-proline *via* hydroxamate-mediated cyclization[26] (Scheme 9).



34 **36** **37**

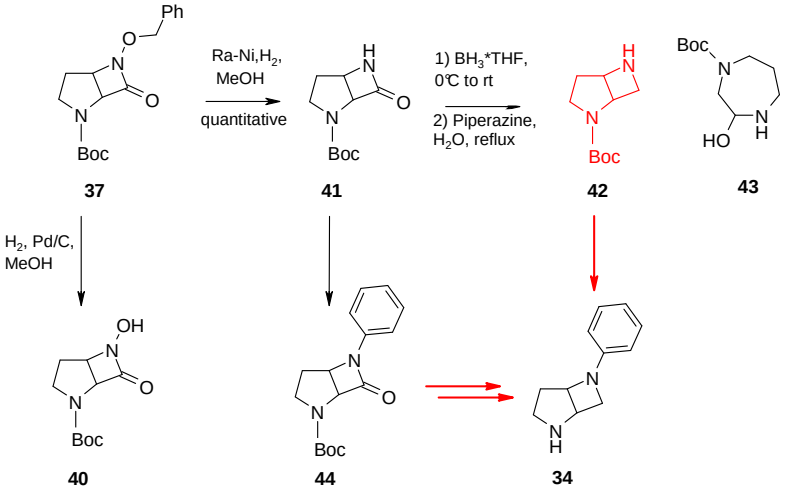
Scheme 9 - *Retrosynthetic analysis*

trans-3-Hydroxy L-proline was protected as *tert*-butylcarbamate and then coupled with *O*-benzylhydroxylamine using the water soluble EDC to afford hydroxamate **39** in good yield. Cyclization under Mitsunobu reaction conditions gave bicyclic β-lactam **37** in satisfactory yield (Scheme 10).



Scheme 10 - *Synthesis of the intermediate lactam **37***

Direct cleavage of the N-O bond of substituted 2-azetidinones could not be easily accomplished using most common N-O bond reduction protocols. The samarium diiodide-mediated reduction reported by Romo[27] was found unsuccessful. However, whilst the catalytic hydrogenolysis of **37** on Pd/C afforded the hydroxylamino adduct **40**, the reaction carried out using Ni-Ra as catalyst gave in good yield the awaited lactam **41** (Scheme 11).



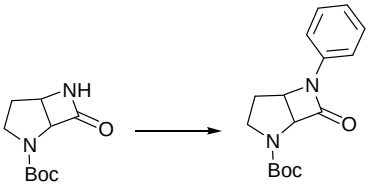
Scheme 11 – *Attempts to obtain **34***

Unfortunately, all attempts to reduce **41** to amine **42** were unsuccessful. Reduction with $LiAlH_4$ in THF or Et_2O didn't work, whereas the treatment with borane-THF complex, which quantitatively lead to the full ring opened product **43**.

Hypothesizing that the difficulties associated to the reduction step were due to the ability of reductive agents to act as bases instead of as nucleophiles on lactam **41**, we decided to revert the strategy, first introducing the aromatic side chain on **41** and then testing the reduction of the lactam to the corresponding amine **34**.

As showed in Table 3, the use of Buchwald-Hartwig's reaction conditions in the presence of $Pd_2(dba)_3$ as catalyst was found to be essential for the *N*-arylation, even if **44** was isolated in very low yield.

All attempts to reduce **44** proved ineffective thereby precluding further transformation to **34**.



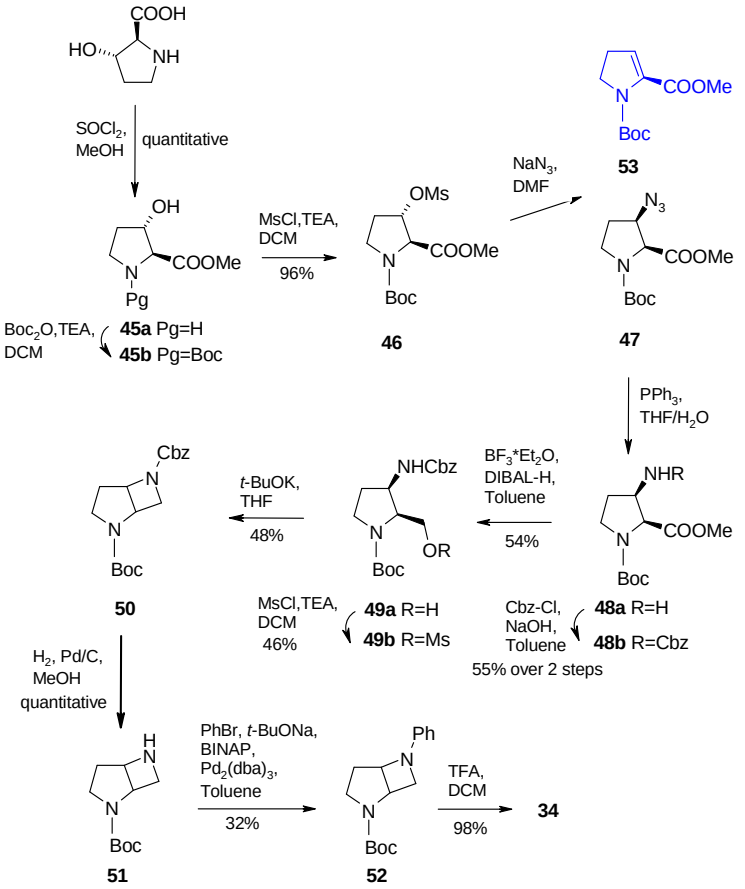
41 **44**

Table 3 -*Arylation of the β-lactam*

Conditions	*Results*
$Cu(OAc)_2$, $PhB(OH)_2$, TEMPO, Py, Molecular sieves, DCM, rt	No reaction
PhBr, DPPF, $Pd(OAc)_2$, *t*-BuONa, toluene, 120 °C, 48 h	Degradation
Cs_2CO_3, PhBr, silica gel	No reaction
K_2CO_3, PhBr, toluene, reflux, 80 h	No reaction
PhBr, *rac*-BINAP, $Pd_2(dba)_3$, *t*-BuONa, toluene, 100 °C, 10 h	13%

In the light of these problems, we sought an alternative route to the required bicyclic amine. *trans*-3-Hydroxy L-proline was protected as *N*-Boc amino ester **45b** and then activated to mesylate **46**. The treatment with NaN_3 afforded a mixture of azide **47**

and unsatured ester **53** in 2:1 ratio. The undesired elimination reaction is particularly favourited due to the high conjugation degree shown by product **53** (Scheme 12).



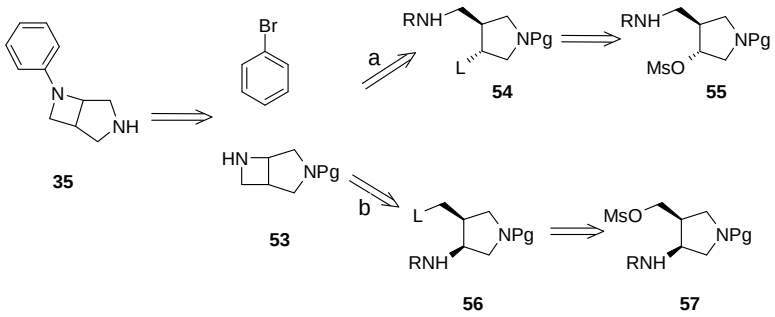
Scheme 12 - *Total synthesis of 6-phenyl-2,6-diazabicyclo[3.2.0]heptane*

After Staudinger reduction of **47**, amino ester **48a** was protected as *N*-Cbz derivative and reduced to alcohol. Whilst classical reduction using $LiAlH_4$ in THF failed, reduction with DIBAL-H in presence of BH_3*Et_2O gave amino alcohol **49a** in good

yield. The alcohol was converted in mesylate **49b**, suitable to undergo an intramolecular cyclisation to afford the bicyclic bis-amine **50**. After *N*-Cbz deprotection, **51** was condensed with bromobenzene *via* the palladium-mediated procedure developed by Buchwald and Hartwig.[28] Amination of bromobenzene proceeded smoothly and provided the corresponding coupling product with moderate chemical yield (32%). The protecting group of the coupling product **52** was finally removed under acidic conditions (TFA) to afford the target compound **34** in almost quantitative yield.

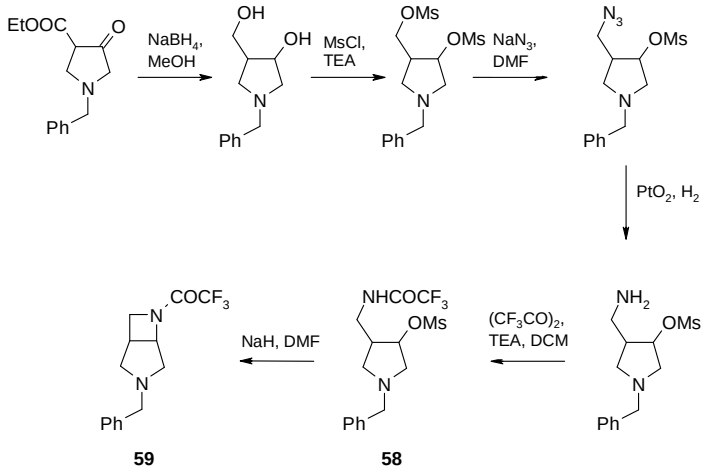
2.3.2 *6-PHENYL-3,6-DIAZABICYCLO[3.2.0]HEPTANE*

From a retrosynthetic point of view, **35** is straightforwardly disconnected at the C-N bond between the phenyl group and fused azetidine. Upon this disconnection, 3,6-diazabicyclo[3.2.0]heptane **53** becomes the key precursor (Scheme 13).



Scheme 13 – *Possible approaches to **35***

Of the many possible synthetic routes toward **53**,[29] two different approaches were initially chosen for further investigastions: (a) a *trans*-pyrrolidine approach, constructing the azetidine ring from *trans*-3,4-disubstituted pyrrolidine **55** originally reported by Jacquet et al.;[30] (b) a *cis*-pyrrolidine approach, assembling azetidine ring from the *cis*-3,4-disubstituted pyrrolidine **57**.



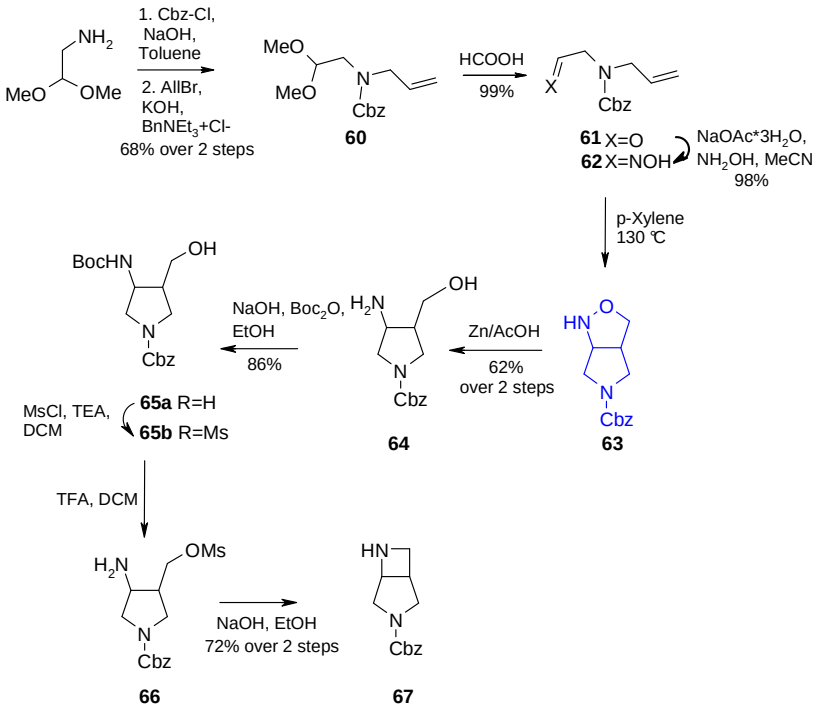
Scheme 14 - *Jacquet synthesis of 3,6-diazabicyclo[3.2.0]heptane core*

Jacquet et al. reported a synthesis of 3,6-diazabicyclo[3.2.0]heptane core **59** *via* intramolecular cyclization of 3,4-disubstituted *N*-benzyl pyrrolidine **58** (Scheme 14). Their synthesis involved a multistep sequence toward the preparation of **58** and required a key ring construction step via C-N bond formation through substitution of the mesylate with the primary amino group. The limiting step of that synthesis was the inefficient intramolecular displacement of the secondary mesylate **58** with the amide ion nucleophile.

To overcome that problem, we considered translocation of the reacting groups, so that ring closure involved more facile displacement of the primary mesylate by the secondary amine. This, in turn, required access to the *cis*-substituted pyrrolidine **56**.

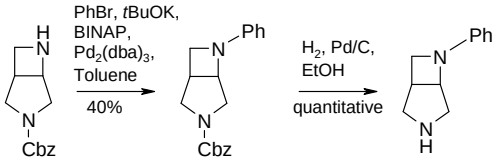
Scheme 15 outlines the synthesis of the intermediate 3,6-diazabicyclo[3.2.0]heptane **67**. Oxime **62** was synthesized through a convenient process[31] including the *N*-Cbz protection of 2,2-dimethoxyethylamine and the subsequent *N*-allylation to offer dimethyl acetal **60**, hydrolysis of **60** to produce aldehyde **61**, and oxime formation with hydroxylamine. Intramolecular 1,3-dipolar cycloaddition of oxime **62** afforded the *cis*-racemic intermediate isoxazolidine **63**, which was then converted to

cis-3-amino-4-(hydroxymethyl)pyrrolidine (**64**) by reductive cleavage of the N-O bond using Zn/HOAc. To minimize the facile air oxidation of **63** to isoxazoline[32] impurities as often detected by LC/MS, cyclization of **62** and reduction of **63** were carried out in a single pot providing **64** in 62% overall yield. The optimized six step process from 2,2-dimethoxyethylamine to **64** required neither distillation nor chromatographic purification. After *N*-protection, the reaction of **65a** with mesyl chloride gave **65b**, which was smoothly transformed to **67** by removal of the *N*-Boc protecting group under acidic conditions, followed by basification to liberate the nucleophilic amine.



Scheme 15 – *Synthesis of the 3,6-diazabicyclo[3.2.0]heptanyl core*

Monoprotected 3,6-diazabicyclo[3.2.0]heptanes **67** was condensed with bromobenzene *via* the palladium-mediated procedure with a satisfactory yield (40%). The protecting group of the coupling product was then removed under catalytic hydrogenation conditions (Pd/C, H_2) to afford the target compound **35** in excellent yield (Scheme 16).



67 **68** **35**

Scheme 16 – *Derivatization to **35***

2.4 *AZABICYCLO[3.1.0]HEXANE HETEROCYCLES*

3-Azabicyclo[3.1.0]hexane is a basic structure of biologically active natural products such as CC-1065,[33] duocarmycin,[16] and indolizomycin,[34] and also a framework of a pharmacologically important class of compounds such as 3,4-methanoprolines,[35] poly-L-proline type II peptide mimetics,[36] and conformationally rigid analogues of [1,4'-bipiperidine]-4'-carboxamides (Figure 8).[37] Moreover, recently Renslo has reported the synthesis of potential antibacterial bicyclo[3.1.0]hexane derivatives, which viewed as conformationally constrained isosters of morpholine-, thiomorpholine- and piperazine-substituted phenyloxyoxazolidinones.[38]

Due to the good lipophilicity properties and the possibility of several kinds of decoration of the molecular framework, 3-azabicyclo[3.1.0]hexane system represents an interesting framework for our synthetic activities.

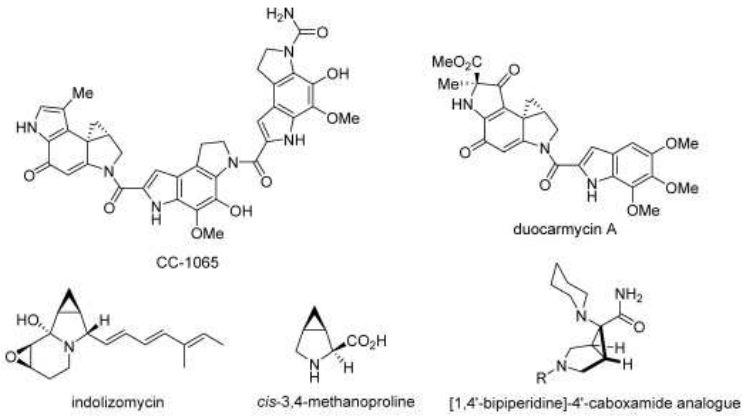


Figure 8- *Representative pharmacologically important compounds having a 3-azabicyclo[3.1.0]hexane framework*

We engaged in a program aimed at the preparation of several 3-azabicyclo[3.1.0]hexanes substituted with an aromatic ring (Figure 9).

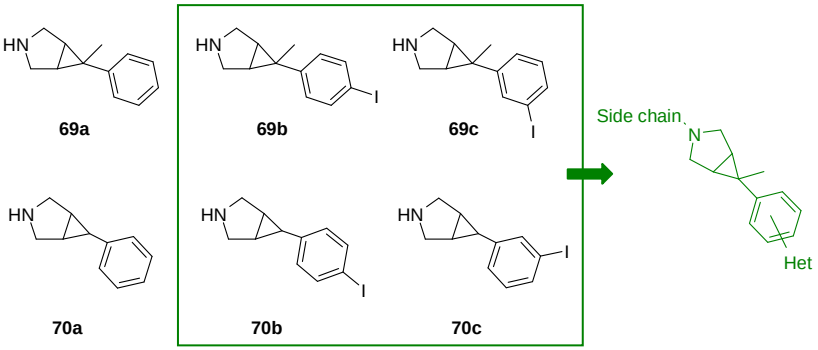
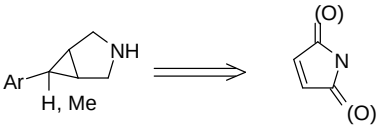


Figure 9 - *Azabicyclo[3.1.0]hexane heterocycles*

Halo-compounds are interesting derivatives in the perspective of decorating the basic framework with heterocyclic rings *via* C-C and C-N coupling.

2.4.1 *CHEMISTRY*

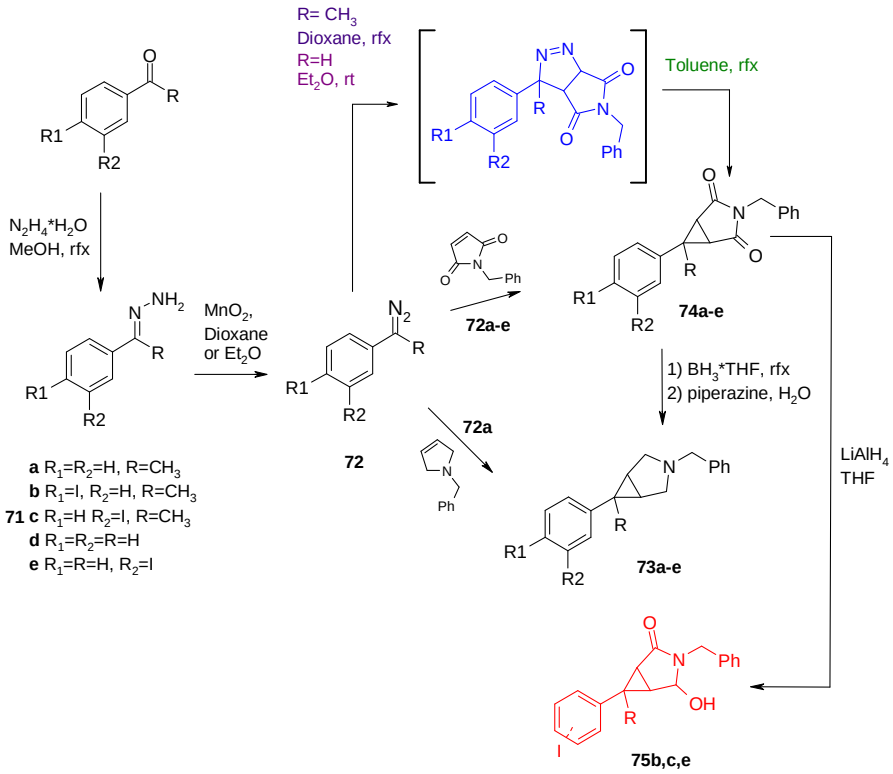
A survey of literature data shows that the azabicyclo[3.1.0]hexane[39] ring system has been prepared through intermolecular cyclopropanation of 3-pyrroline or maleimide substrates.[40] The most direct approach involves rhodium(II) acetate-mediated cyclopropanation of an *N*-protected pyrroline with diazoesters. Unfortunately, these reactions often produce mixtures of *exo* and *endo* diastereomers and, furthermore, could not provide ready access to the desired *aryl*-substituted cyclopropanes.
Since the preparation of the more highly oxidized 3-azabicyclo[3.1.0]-hexane-2,4-dione systems *via* reaction of a protected maleimide with a diazoester followed by pyrolysis of the resulting pyrazoline had been well reported,[41] we decided to follow this approach to achieve the *exo*-stereoselective synthesis of 6-aryl azabicyclo[3.1.0]hexane framework (Scheme 17).



Scheme 17 - *Retrosynthetic analysis of 3-azabicyclo[3.1.0]-hexane core*

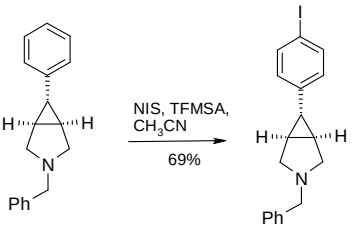
Hydrazones **71a-e** were prepared in excellent yields refluxing corresponding commercially available aldehydes/ ketones with hydrazine monohydrate. Oxidation to diazo derivatives **72** was carried out using a strong excess of MnO_2. In a preliminary effort, **72a** was cycloadditioned to *N*-benzyl pyrroline; after pyrolysis of the resulting pyrazoline, the bicyclic amine **73a** was obtained in very modest yield (5%). However, using a more electrophilic system, such as the *N*-benzylmaleimide, cycloaddition followed by thermolysis provided pure intermediates **74** in moderate to good yields after a simple work up procedure involving slurrying the reaction mixture in diethyl ether. Compounds **72d,e** needed softer reaction conditions than derivatives **72a-c**: the cycloaddition already worked at room temperature and the heating needed exclusively for the thermolysis step. Adjustment of the succinimide

functionality to the desired pyrrolidine was carried out by exhaustive reduction using borane-THF complex. The use of $LiAlH_4$ guaranteed the overreduction only of the non-iodinate compounds **74a,d**. In those conditions partial reduction to derivatives **75** was observed for iodinate compounds (Scheme 18).



Scheme 18 - *Synthesis of 3-azabicyclo[3.1.0]-hexane framework*

Compound **73f** was readly obtained through *p*-ioduration of **73d** with NIS and trifluoromethansulfonic acid (Scheme 19).



Scheme 19 - *Derivatization through p-ioduration*

Isolation of only one cyclopropane isomer for all isolated compounds led to tentative assignment of the phenyl ring to the less hindered *exo* face. For compounds **74d,e** and **73f** the assignment was supported by a 3.2 Hz coupling constant between the H-6 and H-1/H-5. For compounds **73a-c** the *exo* stereochemistry is confirmed by the clear NOE effect between the hydrogen atoms of methyl group and H-2endo/ H-4endo, readily identified in ^{1}H NMR spectra of intermediates **74** (Figure 9).

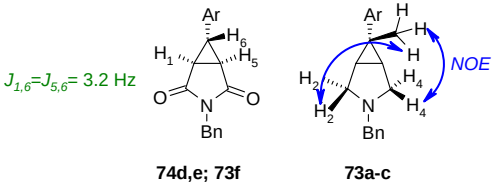
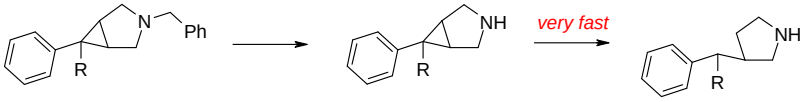


Figure 9 - *Stereochemistry assignment*

As pyrrolidine nitrogen required eventual deprotection, it was tested the *N*-debenzylation reaction on **73a** and **73d** samples. The *N*-deprotection step was not so easy as expected, because both **69a** and **70a** underwent a fast ring opening to give monocycles **76** (Scheme 20).



73a R=CH_3
73d R=H

69a R=CH_3
70a R=H

76a R=CH_3
76d R=H

Scheme 20 - ***N*-Debenzylation**

Several attempts were made in order to isolate the deprotected amines **69a** and **70a**. Optimization efforts included reaction conditions and purification methods screening. Table 3 summarizes the results obtained for **73a**: in all cases of successful deprotection ^{1}H NMR analysis showed a mixture of **69a** and **76a**. Moreover, it was observed that the use of silica gel as stationary phase for chromatographic purification promoted the further degradation of **69a** probably due to its acidity. We found that the use of neutral florisil as stationary phase allowed the purification of **69a** and arrested the ring opening process.

These optimized working conditions were then tested on compound **73d**: the treatment with $NH_4^+HCOO^-$ in presence of Pd/C followed by purification over florisil allowed to isolate amine **70a** in good yield (69%).

Table 3 - *N-Debenzylation of **73a**: tested conditions*

Reaction conditions	*Purification*	*Yields of 69a (%)*
H_2, Ni/Ra, EtOH	-	-
$AlCl_3$, benzene, reflux	-	-
H_2, Pd/C, MeOH, 65 °C	Florisil	30%
1) ACE-Cl, DCE, reflux, 48h; 2) MeOH, reflux	Silica gel	Degradation of TM
1) ACE-Cl, DCE, reflux, 48h; 2) MeOH, reflux	Florisil	20%
$NH_4^+HCOO^-$ (5 eq), Pd/C, MeOH, room temperature	-	-
$NH_4^+HCOO^-$ (3 eq), Pd/C, MeOH, reflux	Silica gel	Degradation of TM
$NH_4^+HCOO^-$ (3 eq), Pd/C, MeOH, reflux	Florisil	51%

2.5 *EXPERIMENTAL SECTION*

All moisture-sensitive reactions were performed under argon or nitrogen atmosphere using oven-dried glassware. All chemicals and solvents were purchased from commercial sources and used without purification, unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded either on Varian instruments at 200, 400, 500 or 600 MHz or on Bruker instruments at 400 MHz and 50, 100, 125 or 150 MHz or 125 Mz, respectively. Solvent was $CDCl_3$ unless otherwise specified. Mono- (^{1}H and ^{1}H with homonuclear decoupling) and two-dimensional techniques (^{1}H–^{1}H COSY, ^{1}H–^{1}H ROESY, ^{1}H-^{13}C HSQC) were used for stereochemistry investigation. Chemical shifts are reported in ppm (δ) using the residual solvent line as internal standard. The NMR spectra were recorded at a temperature ranging from 25 to 90 ºC. When more than one conformer was detected the chemical shifts for the most abundant one is usually reported. Mass spectra (MS) were taken on a Micromass ZMD 2000 Mass Spectrometer, operating in ES (+) ionization mode. Total ion current (TIC) and DAD UV chromatographic traces together with MS and UV spectra associated with the peaks were taken on a UPLC/MS Acquity^{TM} system equipped with 2996 PDA detector and coupled to a Waters Micromass ZQ^{TM} mass spectrometer operating in positive or negative electrospray ionisation mode. [LC/MS - ES (+/-): analyses performed using an Acquity^{TM} UPLC BEH C18 column (50 x 2.1 mm, 1.7 µm particle size), column temperature 40 ºC, mobile phase: A -water + 0.1% HCOOH / B - CH_3CN + 0.06% HCOOH, Flow rate: 1.0 mL/min, Run time= 1.5 min, Gradient: t=0 min 3% B, t=0.05 min 6% B, t= 0.57 min 70% B, t=1.06 min 99% B, t=1.449 min 99% B, t=1.45 min 3% B, stop time 1.5 min. Positive ES 100-1000, Negative ES 100-800, UV detection DAD 210-350nm. The usage of this methodology is indicated by "UPLC/MS" in the analytic characterization of the described compounds. Unless otherwise specified, preparative HPLC-MS conditions, indicated by "HPLC/MS" – basic method: Column: Gemini C18 AXIA, 50 x 21 mm, 5 µm; mobile phase: A: 10 mM aq. $NH_4HCO_3^+$ ammonia (pH 10); B:

CH_3CN; Gradient: specified each time, Flow rate: 17 ml/min; UV range: 210-350 nm; Ionization: ES+/-; Mass range: 100-900 amu. The fractions so obtained are typically evaporated to give the compound as free base. For reactions involving microwave irradiation, a Personal Chemistry Emrys™ Optimizer was used. For some hydrogenations, a H-Cube Tutor™ reactor was used. Chromatography was carried out on silica gel 70-230 mesh (supplied by Merck AG Darmstadt, Germany) or florisil 60-100 mesh (supplied by Fluka AG Buchs, Switzerland). Flash chromatography was carried out on silica gel 230-400 mesh (supplied by Merck AG Darmstadt, Germany). In a number of preparations, purification was performed using Vac Master systems. SPE-SCX cartridges are ion exchange solid phase extraction columns supplied by Varian. The eluent used with SPE-SCX cartridges is methanol followed by 2N ammonia solution in methanol. SPE-Si cartridges are silica solid phase extraction columns supplied by Varian.

Compound 11. A solution of DIPEA (4.73 mL, 27.2 mmol) and *N,N'*-dibenzylethylendiamine (2 mL, 8.5 mmol) dissolved in 13.5 mL of dry toluene was added portionwise to a solution of ethyl 2,3-dibromopropionate (2.48 mL, 17.0 mmol) in 20 mL of dry toluene warmed to 50 °C. The mixture was heated to reflux for 21 h, then cooled to room temperature and filtered from the solid. Organic layer was evaporated under vacuum and the crude residue purified by chromatography over silica gel (grad. Etp to Etp/Et_2O= 85:15) to afford the pure **11** (2.68 g, 93%). ^1H NMR (400 MHz) δ 7.22-7.34 (m, 10H), 4.08-4.20 (m, 2H), 3.92 (d, *J*= 13.6 Hz, 1H), 3.48-3.62 (m, 2H), 3.38 (d, *J*= 13.2 Hz, 1H), 3.24-3.35 (m, 1H), 3.02-3.12, (m, 1H), 2.59-2.80, (m, 2H), 2.38-2.57 (m, 3H), 1.24 (t, *J*= 7.2 Hz, 3H).

Compound 12. 25% wt DIBAL-H solution in toluene (1.58 mL, 2.36 mmol) was added dropwise at – 78 °C to a solution of **11** (400 mg, 1.18 mmol) in 10 mL of dry toluene. The mixture was stirred at that temperature for 15 min, then quenched with sat.d NH_4Cl aq. solution. EtOAc was added; the organic layer was washed twice

with sat.d aq. NH_4Cl solution, dried (Na_2SO_4) and filtered. Solvent removal under reduced pressure afforded **12** (340 mg, 98%), which was used for next step without purification.

Compound 13. DAST (365 µl, 2.76 mmol) was added dropwise to a solution of aldehyde **12** (340 mg, 1.15 mmol) in 17 mL dry toluene cooled to 0 °C. The mixture was gently warmed to reflux; after 3 h the reaction was quenched with sat.d aq. $NaHCO_3$ solution, diluted with EtOAc and washed twice with brine. The organic layer was dried (Na_2SO_4), filtered and concentrated under vacuum. Chromatography of the crude residue over silica gel (grad. Etp to Etp/Et_2O=9:1) gave the pure difluoro derivative **13** (160 mg, 44%). ^{1}H NMR (400 MHz) δ 7.21-7.36 (m, 10H), 6.32 (dt, *J*= 6.0 Hz, *J*= 56.4 Hz, 1H), 3.91 (dd, *J*= 2.8 Hz, *J*= 13.6 Hz, 1H), 3.78 (d, *J*= 14.0 Hz, 1H), 3.52 (d, *J*= 13.2 Hz, 1H), 3.48 (d, *J*= 13.2 Hz, 1H), 2.80-2.98 (m, 2H), 2.67-2.78 (m, 1H), 2.44-2.60 (m, 3H), 2.34-2.40 (m, 1H).

Compound 14. A solution of Br_2 (3.1 mL, 59.6 mmol) in 10 mL of $CHCl_3$ was added dropwise to a solution of allylcyanide (4.8 mL, 59.6 mmol) in 50 mL of the same solvent. After 1 h the organic layer was washed twice with 5N aq. $Na_2S_2O_3$ solution, dried (Na_2SO_4), filtered and evaporated under reduced pressure to afford 14 (12.3 g, 91%) as a yellow pale oil. ^{1}H NMR (400 MHz) δ 4.27 (m, 1H), 3.90 (dd, *J*= 4.4 Hz, *J*= 10.8 Hz, 1H), 3.71 (t, *J*= 10.8 Hz, 1H), 3.22 (d, *J*= 5.2 Hz, 2H).

Compound 15. A solution of TEA (15.0 mL, 108.2 mmol) and *N,N'*-dibenzylethylendiamine (12.7 mL, 54.1 mmol) in 30 mL of dry toluene was added portionwise to a solution of dibromide 14 (12.3 g, 54.1 mmol) in 120 mL of the same solvent warmed to 40 °C. The mixture was heated to reflux for 1 h, then cooled to room temperature and filtered from the solid. The organic layer was evaporated under vacuum and crude residue was treated with EtOH. Piperazine 15 was isolated as a white solid (13.4 g, 81%). ^{1}H NMR (400 MHz) δ 7.22-7.38 (m,

10H), 3.78 (d, *J* = 13.2 Hz, 1H), 3.52 (d, *J*= 13.2 Hz, 1H), 3.42-3.50 (m, 2H), 2.96-3.04 (m, 1H), 2.89 (dd, *J*= 8.0 Hz, *J*= 16.4 Hz, 1H), 2.36-2.66 (m, 7H).

Compounds 9a and 10a. ACE-Cl (6.25 mL, 57.4 mmol) was added dropwise to a solution of **13** (13.9 g, 44.1 mmol) in dry DCE (50 mL) cooled to 0 °C. The mixture was gently heated to reflux. After 2 h volatiles were evaporated in vacuo; the crude residue was dissolved in 20 mL of MeOH and the mixture heated to reflux for 1h. After cooling to rt, the mixture was filtered from the precipitate and volatiles evaporated under vacuum to afford a brown oil. Crystallization from $CHCl_3$/MeOH afforded the hydrochloride salt of **9a** as white solid (9.59 g, 83%). ^{1}H NMR (400 MHz) δ 10.03 (bs, 1H), 9.86 (bs, 1H), 7.26-7.41 (m, 5H), 6.41 (t, *J*= 54.4 Hz, 1H), 4.10 (d, *J*= 13.2 Hz, 1H), 3.69 (d, *J*= 13.2 Hz, 1H), 3.12-3.56 (m, 5H), 3.07 (bd, *J*= 1.68 Hz, 1H), 2.82 (bs, 1H).

Under analogous reaction conditions, hydrochloride salt of **10a** was obtained from **15** as white solid (66% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (bs, 2H), 7.32-7.44 (m, 4H), 7.22-7.30 (m, 1H), 4.06 (d, *J*= 13.6 Hz, 1H), 3.20-3.40 (m, 3H), 3.05-3.18 (m, 1H), 2.96-3.04 (m, 2H), 2.87 (bs, 2H), 2.66-2.78 (m, 1H), 2.38-2.46 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 137.4, 128.8, 128.2, 127.1, 117.9, 56.1, 52.4, 45.9, 45.4, 42.1, 18.1.

Compounds 9b and 10b. Method A: Ammonium formate (0.0752 g, 1.19 mmol) was added to a mixture of **9a** (0.104 g, 0.398 mmol) and Pd/C (0.100 g) in 2 mL of MeOH. The reaction was heated to reflux for 2 h. After cooling to room temperature, the mixture was filtered on a pad of celite and organic layer evaporated under vacuum to afford **9b** (0.038 g, 70%) as yellow pale oil. Method B: ACE-Cl (143 μl, 1.32 mmol) was added dropwise to a solution of **9a** (0.110 g, 0.438 mmol) in 7 mL dry DCE cooled to 0 °C. The mixture was gently heated to reflux. After 15 h volatiles were evaporated in vacuo and the crude residue was dissolved in 10 mL MeOH. The mixture was heated to reflux for 1 h, then cooled to room temperature and filtered from the precipitate. Dihydrochloride salt of **9b** (0.043 g, 73%) was

obtained as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.31 (bs, 3H), 6.49 (dt, *J*= 2.8 Hz, *J*= 53.8 Hz, 1H), 4.17 (m, 1H), 3.62 (d, *J*= 13.2 Hz, 1H), 3.35-3.57 (m, 3H), 3.21-3.33 (m, 1H), 3.16 (t, *J*= 12.8 Hz, 1H).
Under analogous reaction conditions (method B), dihydrochloride salt of **10b** (90% yield) was obtained from **10a** as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.20 (bs, 4H), 3.84-4.00 (m, 1H), 3.15-3.55 (m, 8H).^{13}C NMR (100 MHz, DMSO-d_6) δ 118.0, 48.3, 46.2, 42.5, 41.5, 20.9.

Compound 19. TsCl (19.2 g, 100.9 mmol) was added portionwise to a solution of AllNH_2 (6.57 mL, 87.7 mmol) in 30 mL of Py cooled to 0 °C. The mixture was stirred at room temperature for 3 h, then diluted with EtOAc and washed twice with 2N aq. HCl solution. Organic layer was dried (Na_2SO_4), filtered and concentrated under vacuum. Purification by chromatography over silica gel (DCM) afforded the pure amine **19** (16.2 g, 88%) as yellow solid. ^{1}H NMR (200 MHz) δ ppm 7.76 (d, *J*= 8.0 Hz, 2H), 7.32 (d, *J*= 8.0 Hz, 2H), 5.71-5.88 (m, 1H), 5.06-5.22 (m, 2H), 4.22 (bt, 1H), 3.54-3.63 (m, 2H), 2.44 (s, 3H).

Compound 20. K_2CO_3 (15.9 g, 115.4 mmol) was added portionwise at 0 °C to a solution of amine **19** (16.2 g, 77.0 mmol) in 250 mL of acetone. After 10 min ethyl bromoacetate (11.1 mL, 100.0 mmol) was added and the mixture was gently warmed to room temperature. After 15 h, solvent was evaporated under vacuum and the residue was dissolved in DCM. Organic layer was washed twice with water, then dried (Na_2SO_4), filtered and concentrated under vacuum. Purification by chromatography over silica gel (DCM) afforded the pure **20** (20.3 g, 88%) as yellow pale oil. ^{1}H NMR (200 MHz) δ 7.74 (d, *J*= 8.2 Hz, 2H), 7.30 (d, *J*= 8.2 Hz, 2H), 5.81-5.59 (m, 1H), 5.22 (bs, 1H), 5.11-5.18 (m, 1H), 4.09 (q, *J*= 7.2 Hz, 2H), 4.02 (s, 2H), 3.90 (d, *J*= 6.4 Hz, 2H), 2.43 (s, 3H), 1.20 (t, *J*= 7.2 Hz, 3H).

Compound 18. 1M DIBAL-H solution in hexane (24.0 mL, 24.0 mmol) was added dropwise at –78 °C to a solution of amine **20** (5.95 g, 20.0 mmol) in 40 mL of dry

DCM. The mixture was stirred 30 min at -78 °C, then quenched with sat.d aq. NH_4Cl solution. DCM was added, organic layer was washed twice with sat.d aq. NH_4Cl solution, dried (Na_2SO_4), filtered and concentrated under vacuum to afford the aldehyde **18** which did not need further purification (4.91g, 97%). ^{1}H NMR (200 MHz) δ 9.60 (t, *J*= 1.4 Hz, 1H), 7.71 (d, *J*= 8.2 Hz, 2H), 7.34 (d, *J*= 8.2 Hz, 2H), 5.55-5.82 (m, 1H), 5.11-5.25 (m, 2H), 3.77-3.85 (m, 4H), 2.45 (s, 3H).

Compounds 21 and 22. Sarcosine (1.05 g, 11.8 mmol) was added to a solution of aldehyde **18** (2.0 g, 7.9 mmol) in 120 mL of toluene. The mixture was heated to reflux under Dean-Stark reaction conditions. After 2.5 h solvent was removed under reduced pressure. Purification of the crude residue by chromatography over silica gel (grad. Etp to Etp/Acetone=6:4) afforded the pure **21** (1.23 g, 55%). ^{1}H NMR (200 MHz) δ 7.70 (d, *J*= 8.2 Hz, 2H), 7.32 (d, *J*= 8.0 Hz, 2H), 3.27 (dd, *J*= 1.2 Hz, *J*= 9.8 Hz, 1H), 3.07 (d, *J*= 6.2 Hz, 2H), 2.58-3.04 (m, 4H), 2.44 (s, 3H), 2.16-2.34 (m, 4H), 1.90-2.08 (m, 1H), 1.52-1.73 (m, 1H).
Under analogous reaction conditions, **22** was obtained (39% yield) by condensation of **18** with *N*-PhGly. ^{1}H NMR (400 MHz) δ 7.65 (d, *J*= 8.4 Hz, 2H), 7.29 (d, *J*= 8.4 Hz, 2H), 7.18-7.22 (m, 2H), 6.72 (t, *J*= 7.2 Hz, 1H), 6.43 (m, 2H), 4.03-4.09 (m, 1H), 3.36-3.42 (m, 1H), 3.32 (dd, *J*= 2.8 Hz, *J*= 10.4 Hz, 1H), 3.25 (dd, *J*= 6.4 Hz, *J*= 10.0 Hz, 1H), 3.16-3.21 (m, 3H), 2.88-2.96 (m, 1H), 2.43 (s, 3H), 2.06-2.18 (m, 1H), 1.86-1.95 (m, 1H). MS (ES+) m/z: found 343.1 [MH^+], $C_{19}H_{22}N_2O_2S$ requires 342.4.

Compounds 16 and 24. In a flame dried 500 mL two-necked round bottomed flask were taken activated Mg turnings (2.86 g, 119 mmol). Tosylamine **22** (1.63 g, 4.77 mmol) dissolved in 200 mL MeOH was transferred into the reaction flask and the mixture was heated to reflux for 3 h. Solvent was evaporated under reduced pressure and 300 mL of H_2O were added. The mixture was stirred 15 min at room temperature, then backextracted three times with $CHCl_3$. Combined organics were dried (Na_2SO_4), filtered and concentrated under vacuum to afford amine **16** as

yellow pale oil (0.448 g, 50% yield) . ^{1}H NMR (400 MHz) δ 7.20-7.25 (m, 2H), 6.71 (t, *J*= 8.0 Hz, 1H), 6.59 (d, *J*= 8.4 Hz, 2H), 3.95-4.01 (m, 1H), 3.48-3.55 (m, 1H), 3.13-3.22 (m, 1H), 3.06 (d, *J*= 11.6 Hz, 1H), 2.82-2.95 (m, 4H), 1.96-2.14 (m, 2H), 1.68-1.82 (m, 1H).
Under analogous reaction conditions, amine **24** was obtained (69% yield) from **21**. ^{1}H NMR (200 MHz) δ 2.86-2.98 (m, 2H), 2.83-2.54 (m, 3H), 2.43 (dd, *J*= 4.0 Hz, *J*= 12.2 Hz, 1H), 2.34 (s, 3H), 1.82-2.24 (m, 4H). MS (ES+) m/z: found 127.0 [MH^+], $C_7H_{14}N_2$ requires 126.2.

Compound 25. To a 10 mL round bottomed flask were added in sequence 30 mg of molecular sieves (4 Å, powdered), $PhB(OH)_2$ (49.7 mg, 0.38 mmol), 1 mL of dry DCM, Py (32.2 μL, 0.38 mmol), amine **24** (25.0 mg, 0.2 mmol) dissolved in 2 mL of dry DCM, $Cu(OAc)_2$ (3.6 mg, 0.02 mmol) and TEMPO (34.0 mg, 0.218 mmol). The reaction was allowed to stir under air at room temperature for 24 h. DCM was added and the organic was washed with water and sat.d aq. NH_4Cl solution. The organic layer was dried (Na_2SO_4), filtered and concentrated under vacuum. Purification by chromatography over silica gel of the crude residue (grad. DCM to DCM/Acetone= 1:9) afforded pure **25** (7.0 mg, 17%). ^{1}H NMR (400 MHz) δ 7.17-7.23 (m, 2H), 6.70 (dt, *J*= 0.8 Hz, *J*= 7.2 Hz, 1H), 6.63 (dd, *J*= 0.8 Hz, *J*= 8.8 Hz, 2H), 3.47 (d, *J*= 10.0 Hz, 1H), 3.24-3.35 (m, 2H), 3.08-3.15 (m, 2H), 2.85-2.98 (m, 2H), 2.30-2.45 (m, 4H), 2.09-2.20 (m, 1H), 1.69-1.81 (m, 1H). MS (ES+) m/z: found 203.1 [MH^+], $C_{13}H_{18}N_2$ requires 202.3.

Compound 27. A solution of aniline (5.0 g, 53.7 mmol) in 100 mL of THF containing Boc_2O (14.1 g, 64.5 mmol) was heated to reflux for 4 h. The solvent was removed in vacuo. The crude residue was treated with 7/3 Hexane/Et_2O mixture; the precipitate was filtered off, rinsed with Hexane and collected. Amine **27** was isolated as yellow pale oil (9.06 g, 87%). ^{1}H NMR (200 MHz) δ 7.23-7.39 (m, 4H), 7.03 (m, 1H), 6.46 (bs, 1H), 1.52 (s, 9H).

Compound 28a. NaH (60% oil dispersion 2.06 g, 51.6 mmol) was added portionwise at 0 °C to a solution of **27** (9.06 g, 46.9 mmol) in 100 mL of fresh distilled DMF. After 10 min AllBr (7.38 g, 61.0 mmol) was added. The suspension was stirred 20 min at 0 °C, then warmed to room temperature. After 1.5 h the reaction was quenched with MeOH and diluted with EtOAc; the organic layer was washed twice with 1N aq. HCl solution, dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the pure **28a** (9.97 g, 91%) which did not need any purification. ^{1}H NMR (200 MHz) δ 7.11-7.38 (m, 5H), 5.89 (m, 1H), 5.16 (dd, *J*= 1.4 Hz, *J*= 7.6 Hz, 1H), 5.06-5.09 (m, 1H), 4.21 (dt, *J*= 1.8 Hz, *J*= 5.6 Hz, 2H), 1.43 (s, 9H).

Compound 28b. TFA (120 mL) was added portionwise at 0 °C to a solution of **28a** (9.97g, 42.8 mmol) in 30 mL of DCM. The mixture was stirred at that temperature for 20 min, then heated to reflux for 3 h. Sat.d aq. K_2CO_3 solution was added until neutrality and the aqueous layer was extracted twice with $CHCl_3$. The combined organics were dried (Na_2SO_4), filtered and evaporated under vacuum to afford **28b** (4.72 g, 83%) which did not need any purification. ^{1}H NMR (200 MHz) δ 7.16-7.28 (m, 2H), 6.76 (t, *J*= 7.4 Hz, 1H), 6.43-6.68 (m, 2H), 5.86-6.11 (m, 1H), 5.33 (dd, *J*= 1.4 Hz, *J*= 17.2 Hz, 1H), 5.21 (dd, *J*= 1.6 Hz, *J*= 10.4 Hz, 1H), 3.68-3.87 (m, 3H).

Compound 26. NaH (60% oil dispersion 1.70 g, 42.6 mmol) was added portionwise to a solution of the amine **28b** (4.72 g, 35.5 mmol) in 100 mL of fresh distilled DMF under argon atmosphere. After 10 min, ethyl bromoacetate (5.11 mL, 46.1 mmol) was added. The mixture was stirred 30 min at room temperature, then warmed to 80 °C. After 2 h the reaction was not complete and no improvements were observed after prolonged heating. Sat.d aq. NH_4Cl solution was added and the aqueous layer was extracted twice with EtOAc. The combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification of the crude residue by chromatography over silica gel (grad. Etp to Etp/Et_2O=97:3) gave the pure amine **26** (5.10 g, 65%). ^{1}H NMR (200 MHz) δ 7.18-7.31 (m, 2H), 6.75 (t, *J*= 7.2 Hz, 1H),

6.64-6.71 (m, 2H), 5.82-6.01 (m, 1H), 5.16-5.32 (m, 2H), 4.21 (q, *J*= 7.2 Hz, 2H), 4.03-4.07 (m, 4H), 1.28 (t, *J*= 7.2 Hz, 3H).

Compound 30. $LiAlH_4$ (1.04g, 27.4 mmol) was added at 0 °C to a stirring solution of **26** (3.0 g, 13.7 mmol) in 45 mL of dry THF. The mixture was stirred 30 min at that temperature, then warmed to room temperature. After 1 h, 10% aq. NaOH solution was added to quench the reaction and the aqueous layer was extracted twice with EtOAc. The combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the alcohol **30** (2.18 g, 90%) which did not need any purification (90%). ^{1}H NMR (200 MHz) δ 7.20 (t, *J*=7.2 Hz, 2H), 6.67-6.81 (m, 3H), 5.72-5.97 (m, 1H), 5.19 (bs, 1H), 5.08-5.17 (m, 1H), 3.93-3.99 (m, 2H), 3.80 (t, *J*= 5.6 Hz, 2H), 3.50 (t, *J*= 5.8 Hz, 2H).

Compound 29. DMSO (2.93 mL, 41.0 mmol) was slowly added at – 78 °C to a solution of oxalyl chloride (1.60 mL, 18.8 mmol) in 15 mL of dry DCM. The mixture was stirred at the same temperature under argon for 30 min. Alcohol **30** (1.45 g, 8.20 mmol) dissolved in 5 mL of dry DCM was slowly added and the resulting mixture was stirred at -78 °C for 1 h. After addition of TEA (4.70 mL), the mixture was stirred for 30 min further. The reaction was allowed to stir at room temperature for 2 h, then partitioned between $CHCl_3$ and H_2O. The organic layer was washed with sat.d aq. NH_4Cl solution, dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the aldehyde **29** (quantitative) which did not need any further purification.

Compound 25. Sarcosine (1.40 g, 15.7 mmol) was added to a solution of aldehyde **29** (1.41 g, 8.0 mmol) in 120 mL of toluene. The mixture was heated to reflux under Dean-Stark reaction conditions for 4 h. Sovent was evaporated under reduced pressure and the crude residue was purified by chromatography over silica gel (grad. DCM/Acetone=95:5 to Acetone) to afford the pure **31** (801.1 mg, 49%) as a red-brown oil. ^{1}H NMR (400 MHz) δ 7.20 (t, *J*= 7.2 Hz, 2H), 6.70 (t, *J*= 7.2 Hz, 1H),

6.63 (dd, *J*= 0.8 Hz, *J*= 8.8 Hz, 2H), 3.47 (d, *J*= 10.0 Hz, 1H), 3.24-3.34 (m, 2H), 3.07-3.15 (m, 2H), 2.84-2.98 (m, 2H), 2.38 (s, 3H), 2.29-2.41 (m, 1H), 1.50-1.82 (m, 2H).

Compound 17. ACE-Cl (4.32 mL, 39.6 mmol) was added slowly to a solution of **31** (801.0 mg, 3.96 mmol) in 20 mL of dry DCE cooled to 0 °C. The mixture was stirred at room temperature under argon atmosphere for 20 min, then heated to reflux. After 4 h the mixture was cooled to room temperature and DIPEA (6.89 mL, 39.6 mmol) was added. The resulting mixture was heated to reflux for further 3 h. Volatiles were evaporated under reduced pressure; the crude residue was dissolved in 20 mL of MeOH and heated to reflux. Volatiles were evaporated under reduced pressure to afford hydrochloride salt of **17** (446 mg, 60% yield).

Compound 38. 10% aq. NaOH solution (10.3 mL) was added to a suspension of *trans*-3-hydroxy proline (3.0 g, 22.9 mmol) in 45 mL of a mixture THF/H_2O= 2:1. Boc_2O (5.98 g, 27.5 mmol) was added and the mixture was allowed to stir at room temperature for 18 h. Volatiles were evaporated under vacuum; aqueous layer was acidificated with 2N aq. HCl solution until pH 2-3 and extracted three times with $CHCl_3$. Combined organics were dried (Na_2SO_4), filtered and concentrated under vacuum to afford acid **38** (2.81 g, 53%) which was used in the next step without any purification. ^{1}H NMR (200 MHz, CD_3OD) δ 4.34-4.43 (m, 1H), 4.12 (d, *J*= 14.2 Hz, 1H), 3.46-3.59 (m, 2H), 1.77-2.15 (m, 2H), 1.46, 1.42 (2s, 9H).

Compound 39. HOBT (1.15 g, 8.56 mmol) and EDC (1.63 g, 8.56 mmol) were added to a solution of **38** (1.51 g, 6.60 mmol) in 15 mL of dry THF. After 10 min $BnONH_2$ (812 mg, 6.60 mmol) was added and the mixture was allowed to stir at room temperature for 18 h under argon atmosphere. EtOAc was added, organic layer was washed twice with 0.2N aq. HCl solution, then dried (Na_2SO_4), filtered and concentrated under vacuum. The crude residue was purified by chromatography over silica gel ($CHCl_3$) to afford the pure **39** (2.21 g, quantitative) as colorless wax.^{1}H

NMR (200 MHz) δ 7.28-7.49 (m, 5H), 4.91 (d, 1H), 4.87 (d, 1H), 4.64-4.78m (m, 1H), 4.01-4.12 (m, 1H), 3.28-3.46 (m, 2H), 1.67-2.32 (m, 4H), 1.42 (s, 9H). MS (ES+) m/z: found 337.5 [MH^+], $C_{17}H_{24}N_2O_5$ requires 336.4.

Compound 37. DEAD (436 mg, 2.51 mmol) was added at 0 °C to a solution of **39** (767.4 mg, 2.28 mmol) and PPh_3 (658 mg, 2.51 mmol) in 10 mL of dry THF. The mixture was stirred 1h at 0 °C, then allowed to warm to room temperature. After 16 h volatiles were evaporated under vacuum. The crude residue was purified by chromatography over silica gel (grad. Etp/EtOAc=9:1 to Etp/EtOAc=1:1) to afford the pure **37** (659.1 mg, 91%) as white solid.^{1}H NMR (200 MHz, DMSO-d_6) δ 7.35-7.47 (m, 5H), 4.99 (d, *J*=11.4 Hz, 1H), 4.92 (d, *J*=11.4 Hz, 1H), 4.71-4.92 (m, 1H), 4.23 (t, *J*= 4.4 Hz, 1H), 3.66-3.84 (m, 1H), 2.89-3.11 (m, 1H), 1.82 (dd, *J*= 6.4 Hz, *J*= 14.4 Hz, 1H), 1.52-1.73 (m, 1H), 1.39 (s, 9H).

Compound 40. 10% wt Pd/C was added to a solution of **37** (83 mg, 0.261 mmol) in 4 mL of EtOH. The atmosphere of reaction flask was satured of H_2 and the mixture stirred for 5 h at room temperature. The catalyst was removed by filtration through a pad of celite. Solvent was evaporated *in vacuo* to afford compound **40** (55 mg, 92%) as yellow pale oil. ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.68-4.97 (m, 1H), 4.39 (t, *J*= 4.4 Hz, 1H), 3.75-3.87 (m, 1H), 3.20-3.61 (m, 1H), 2.98-3.19 (m, 1H), 2.02 (dd, *J*= 6.2 Hz, *J*= 13.8 Hz, 1H), 1.57-1.79 (m, 1H), 1.40 (s, 9H). MS (ES+) m/z: found 173.3 [MH^+-56(Boc)], $C_{10}H_{16}N_2O_4$ requires 228.2.

Compound 41. **37** (102 mg, 0.320 mmol) was added to a suspension of Ra-Ni in 5 mL of MeOH. The atmosphere of reaction flask was satured of H_2 and the mixture stirred for 4 h at room temperature. The catalyst was removed by filtration through a pad of celite. Solvent was evaporated *in vacuo* to afford compound **41** (52.8 mg, 78%) as yellow pale oil. ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.12 (bs, 1H), 4.81-5.04 (m, 1H), 4.15 (t, *J*= 4.4 Hz, 1H), 3.74-3.92 (m, 1H), 3.01-3.22 (m, 1H), 1.78 (dd, *J*= 6.4 Hz, *J*= 13.8 Hz, 1H), 1.58-1.78 (m, 1H), 1.39 (s, 1H).

Compound 44. A mixture of amide **41** (150 mg, 0.707 mmol), PhBr (0.112 mL, 1.06 mmol) and *t*BuONa (102 mg, 1.06 mmol) in 5 mL of dry toluene was degasses and purged with argon three times before the addition of BINAP (26.4 mg, 0.042 mmol) and $Pd_2(dba)_3$ (13 mg, 0.014 mmol). The mixture was heated to 100 °C and stirred at that temperature for 10 h. After cooling to room temperature, EtOAc was added and organic layer was washed with H_2O, dried (Na_2SO_4), filtered and concentrated under vacuum. The crude residue was purified by chromatography over silica gel ($CHCl_3$) to afford phenyl derivative **44** (27 mg, 13%) as yellow pale oil. ^{1}H NMR (200 MHz) δ 7.18-7.57 (m, 5H), 5.09-5.48 (m, 1H), 4.68 (t, *J*= 4.6 Hz, 1H), 3.97-4.13 (m, 1H), 3.19-3.38 (m, 1H), 2.25 (dd, *J*= 5.8 Hz, *J*= 13.6 Hz, 1H), 1.72-1.95 (m, 1H), 1.49 (s, 9H). MS (ES+) m/z: found 233.4 [MH^+-56(Boc)], $C_{16}H_{20}N_2O_3$ requires 288.3.

Compound 45a. $SOCl_2$ (916 μL) was gradually added at 0 °C to a solution of *trans*-3-hydroxy proline (1.50 g, 11.4 mmol) in 10 mL of MeOH. The mixture was heated to reflux for 2.5 h, then stirred at room temperature for 16 h. Volatiles were evaporated under vacuum to afford hydrochloride salt of **45a** (2.16 g, quantitative) as white solid. ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.41-4.52 (m, 1H), 4.12 (bs, 1H), 3.74 (s, 3H), 3.29 (bt, *J*= 7.8 Hz, 2H), 1.78-2.04 (m, 2H). MS (ES+) m/z: found 145.9 [MH^+], $C_6H_{11}NO_3$ requires 145.1.

Compound 45b. Boc_2O (3.11 g, 14.26 mmol) was added portionwise to a solution of **45a** (2.16 g, 11.9 mmol) and TEA (3.32 mL, 23.8 mmol) in 40 mL of DCM. The mixture was stirred at room temperature for 4 h, then DCM was added and the organic phase was washed twice with sat.d aq. NH_4Cl solution. The organic layer was dried (Na_2SO_4), filtered and concentrated under vacuum to afford **45b** (2.40 g, 82%) as white solid. ^{1}H NMR (200 MHz) δ 4.37-4.48 (m, 1H), 4.17, 4.29 (2bs, 1H), 3.74 (s, 3H), 3.47-3.68 (m, 2H), 2.00-2.33 (m, 2H), 1.82-1.99 (m, 1H), 1.40, 1.46 (2s, 9H).

Compound 46. MsCl (1.52 mL, 19.6 mmol) was gradually added at 0 °C to a solution of **45b** (2.40 g, 9.8 mmol) and TEA (2.0 mL, 19.6 mmol) in 25 mL of dry DCM. The mixture was stirred 20 min at 0 °C, then warmed to room temperature for 3.5 h. DCM was added and organic layer was washed twice with sat.d aq. NH_4Cl solution, dried (Na_2SO_4), filtered and concentrated under vacuum. The crude residue was purified by chromatography over silica gel (grad. DCM to DCM/ Acetone=9:1) to afford **46** (3.03 g, 96%) as white solid. ^{1}H NMR (200 MHz) δ 5.18-5.22 (m, 1H), 4.46, 4.58 (2s, 1H), 3.44-3.80 (m, 5H), 3.09 (s, 3H), 2.18-2.36 (m, 2H), 1.42, 1.47 (2s, 9H).

Compound 48a. NaN_3 (241 mg, 3.71 mmol) was added to a solution of **46** (1.0 g, 3.10 mmol) in 12 mL of dry DMF. The mixture was heated to 100 °C for 7 h. After cooling to room temperature, EtOAc was added and the organic layer was washed twice with sat.d aq. NH_4Cl solution, then dried (Na_2SO_4), filtered and concentrated under vacuum to afford a crude mixture of **47** and **53** (728 mg) in 2:1 ratio as detected by ^{1}H NMR analysis.
PPh_3 (911 mg, 3.48 mmol) was added to a stirred emulsion of that material (728 mg, 2.68 mmol) in 13.2 mL of a THF/H_2O=10:1 mixture. The resulting mixture was heated to reflux for 4 h. Volatiles were evaporated under vacuum. The crude residue was purified by chromatography over silica gel (grad. $CHCl_3$ to $CHCl_3$/MeOH=8:2) to afford the pure **48a** (417 mg, 55% over 2 steps). ^{1}H NMR (200 MHz) δ 4.28 (dd, *J*= 7.8 Hz, *J*= 13.8 Hz, 1H), 3.51-3.86 (m, 5H), 3.23-3.45 (m, 1H), 2.00-2.20 (m, 1H), 1.68-1.98 (m, 1H), 1.39, 1.43 (2s, 9H).

Compound 48b. CbzCl (312 μL, 2.22 mmol) was added dropwise to a mixture of **48a** (417 mg, 1.71 mmol) in 6 mL of toluene and 1 mL 10% aq. NaOH solution. The mixture was stirred at room temperature for 18 h, then EtOAc and H_2O were added. The phases were separated and the organic was washed with H_2O. The organic layer was dried (Na_2SO_4), filtered and concentrated under vacuum. The crude residue was purified by chromatography over silica gel (grad. $CHCl_3$ to $CHCl_3$/MeOH=95:5) to

afford the pure derivative **48b** (607 mg, 94%). ^{1}H NMR (200 MHz) δ 7.25-7.40 (m, 5H), 5.15 (d, *J*= 13.6 Hz, 1H), 5.06 (d, *J*= 13.6 Hz, 1H), 4.80-4.99 (bs, 1H), 4.41-4.59 (m, 2H), 3.47-3.78 (m, 4H), 3.24-46 (m, 1H), 2.08-2.29 (m, 1H), 1.82-2.07 (m, 1H), 1.39, 1.44 (2s, 9H).

Compound 49a. 1M DIBAL-H solution in hexane (4.84 mL, 4.84 mmol) was added dropwise at – 78 °C to a stirred solution of ester **48b** (600 mg, 1.60 mmol) and BF_3*Et_2O (67.2 μL, 0.56 mmol) in 5 mL of dry toluene. The resulting solution was stirred 30 min at – 78 °C, then allowed to warm to room temperature and stirred for additional 3 h. The reaction was quenched with sat.d aq. NH_4Cl solution. The phases were separated and the aqueous extracted twice with $CHCl_3$. The combined organics were dried (Na_2SO_4), filtered and concentrated under vacuum to afford the alcohol **49a** (301 mg, 54%). ^{1}H NMR (200 MHz) δ 7.27-7.41 (m, 5H), 5.11 (bs, 2H), 4.70 (s, 1H), 4.23-4.56 (m, 1H), 3.82-4.03 (m, 2H), 3.58-3.76 (m, 1H), 3.22-3.53 (m, 2H), 2.04-2.29 (m, 1H), 1.79-2.02 (m, 1H), 1.45 (s, 9H).

Compound 49b. MsCl (130 μL, 1.7 mmol) was added dropwise to a solution of alcohol **49a** (300 mg, 0.85 mmol) and TEA (238 μL, 1.70 mmol) in 3 mL of dry DCM. The mixture was stirred at room temperature for 18 h. DCM was added; the organic layer was washed twice with sat.d aq. NH_4Cl solution, then dried (Na_2SO_4), filtered and concentrated under vacuum. The crude residue was purified by chromatography over silica gel (grad. $CHCl_3$ to $CHCl_3$/Acetone=9:1) to afford the pure **49b** (170 mg, 46%). ^{1}H NMR (200 MHz) δ 7.27-7.40 (m, 5H), 5.15 (d, *J*= 13.6 Hz, 1H), 5.08 (d, *J*= 13.6 Hz, 1H), 4.05-78 (m, 4H), 3.18-58 (m, 2H), 2.95 (s, 3H), 1.76-2.32 (m, 2H), 1.46 (s, 9H).

Compound 50. Compound **49b** (100 mg, 0.23 mmol) was dissolved in 2 mL of dry THF and *t*BuOK (38.6 mg, 0.345 mmol) was added. The mixture was stirred during 48 h at room temperature. EtOAc was added and the organic phase was washed

twice with H_2O. The organic layer was dried (Na_2SO_4), filtered and concentrated under vacuum. The crude material was purified by chromatography over silica gel (grad. Etp to Etp/ AcOEt=7:3) to afford the pure **50** (37 mg, 48%). ^{1}H NMR (200 MHz) δ 7.27-7.42 (m, 5H), 5.09 (s, 2H), 4.89 (t, *J*= 5.4 Hz, 1H), 4.24-4.58 (m, 1H), 4.02-4.21 (m, 1H), 3.73-3.97 (m, 1H), 3.40-3.59 (m, 2H), 2.08-2.39 (m, 1H), 1.67-1.82 (m, 1H), 1.44 (s, 9H). MS (ES+) m/z: found 277.4 [MH^+-56(Boc)], $C_{18}H_{24}N_2O_4$ requires 332.4.

Compound 51. 10% Pd/C was added to a solution of **50** (37 mg, 0.11 mmol) in 2.3 mL of MeOH. The mixture was stirred under H_2 atmosphere for 5 h and then filtered from the catalyst through a pad of celite. The volatiles were evaporated under vacuum to afford amine **51** (26 mg, quantitative). ^{1}H NMR (200 MHz) δ 5.14 (t, *J*= 6.2 Hz, 1H), 4.53-4.78 (m, 2H), 4.38 (dd, *J*= 6.2 Hz, *J*= 11.6 Hz, 1H), 3.80-4.05 (m, 2H), 3.67-3.79 (m, 1H), 2.51 (bdd, *J*= 5.6 Hz, *J*= 15.0 Hz, 1H), 1.90-2.19 (m, 1H), 1.43 (s, 9H).

Compound 60. Step 1: CbzCl (3.15 mL, 27.1 mmol) was added gradually to a mixture of aminoacetaldehyde dimethyl acetal (3.0 g, 28.9 mmol) in 15 mL of toluene and aq. NaOH (1.5 g, 36.2 mmol, in 7.5 mL of water) at 10-20 °C. After the addition was completed, the mixture was stirred at room temperature for 4 h. The organic layer was separated and washed with brine. It was then concentrated to afford benzyl 2,2-dimethoxyethyl-carboxylate as an oil (3.0 g, 46% yield). ^{1}H NMR (300 MHz) δ 7.30 (m, 5H), 5.11 (s, 2H), 4.37 (t, *J* = 6.0 Hz, 1H), 3.39 (s, 6H), 3.33 (t, *J* = 6.0 Hz, 2H).

Step 2: Under N_2, powdered KOH (3.10 g, 55.2 mmol) and triethyl-benzylammonium chloride (470 mg, 2.06 mmol) were added to a solution of benzyl 2,2-dimethoxyethylcarbamate (3.0 g, 12.5 mmol) in 50 mL of dry toluene. A solution of AllBr (1.43 mL, 16.6 mmol) in 5 mL of toluene was then added dropwise over 1 h at 20-30 °C. The mixture was stirred at room temperature for 14 h. The reaction was slowly quenched with water at 20-30 °C over 20 min. The organic

layer was separated and the aqueous phase was extracted with toluene. The combined organic extracts were washed with brine and concentrated to give **60** (2.39 g, 68%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.23 (m, 5H), 5.75 (m, 1H), 5.15 (m, 4H), 4.45 (m, 1H), 3.97 (d, *J* = 5.40 Hz, 2H), 3.37 (m, 5H), 3.32 (s, 3H).

Compound 61. A solution of **60** (2.39 g, 8.57 mmol) in formic acid (2.7 mL) was stirred under argon at room temperature for 15 h. Most of the formic acid was then removed under reduced pressure at 40-50 °C. The residue was extracted with EtOAc. The combined extracts were washed with brine until the aqueous phase had a pH of 6-7. The organic solution was then concentrated to provide the pure **61** (2.01 g, 99%). ^{1}H NMR (300 MHz) δ 9.50 (d, *J* = 6.4 Hz, 1H), 7.45 (m, 5H), 5.75 (m, 1H), 5.10 (m, 4H), 4.10 (m, 1H), 3.97 (m, 2H), 3.20 (m, 1H).

Compound 62. A solution of $NaOAc{\cdot}3H_2O$ (1.18 g, 8.70 mmol, in 6 mL of water) was added to a flask containing the solution of **61** (2.01 g, 8.61 mmol) and NH_2OH*HCl (754 mg, 10.85 mmol) in MeCN (10 mL) and then stirred at room temperature for 20 h. The volatiles were then removed under reduced pressure, and the residue was extracted with EtOAc. The combined extracts were washed with brine until the aqueous phase had a pH=7. The organic solution was concentrated to provide **60** (2.10 g 98%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.27-7.36 (m, 6H), 5.62 (m, 1H), 5.30 (m, 4H), 4.17 (d, *J* = 4.41 Hz, 1H), 3.98 (d, *J* = 5.43 Hz, 1H), 3.94 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.1, 155.9, 149.8, 149.4, 147.0, 136.2, 132.8, 132.6, 128.4, 128.0, 127.8, 118.1, 117.8, 117.5, 117.3, 67.5, 50.8, 50.4, 49.5, 49.0, 45.4, 45.1, 42.4, 42.0.

Compound 64. A solution of **62** (950.4 mg, 3.83 mmol) in 10 mL of *p*-xylene was stirred under Ar at 130 °C for 10 h. The brown solution was cooled to room temperature, and 10 mL of AcOH were then added. Zinc powder (500 mg, 7.65 mmol) was added gradually at 0 °C. After the addition was completed, the reaction mixture was stirred at room temperature for 3 h. The inorganic solid was removed

by filtration. The organic solution was then stirred with water for 10 min and separated. The aqueous layer was extracted with EtOAc. The combined extracts were concentrated under reduced pressure. The residue was basified to pH 9-10 by cautious addition of sat.d aq. Na_2CO_3 solution. The precipitated white solid was removed by filtration. The aqueous solution was extracted with $CHCl_3$. The combined extracts were washed with sat.d aq. Na_2CO_3 solution and dried (Na_2SO_4). The mixture was then filtered through a short column of diatomaceous earth and concentrated to provide **64** (590 mg, 62%) as an oil.^{1}H NMR (300 MHz, CD_3OD) δ 7.35 (m, 5H), 5.10 (s, 2H), 3.52-3.80 (m, 5H), 3.32 (m, 2H), 2.40 (m, 1H). MS (ES+) m/z: found 251.5 [MH^+], $C_{13}H_{18}N_2O_3$ requires 250.3.

Compound 65a. A solution of **64** (400 mg, 1.6 mmol) in 5 mL of EtOH was basified to pH~10 with 10% aq. NaOH solution. Boc_2O (523 mg, 2.4 mmol) was cautiously added at 10-20 °C. After the addition was completed, it was allowed to stir at 65 °C for 4 h. The volatiles were removed under reduced pressure, and the residue was extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated under vacuum to give **65a** (482.4 mg, 86%) as a solid.^{1}H NMR (400 MHz, CD_3OD) δ 7.35 (m, 5H), 5.10 (s, 2H), 4.20 (m, 1H), 3.50-3.75 (m, 4H), 3.40 (m, 1H), 3.25 (m, 1H), 2.50 (m, 1H), 1.46 (s, 9H).

Compound 65b. A solution of MsCl (0.373 mL, 4.82 mmol) was added to the solution of **65a** (482.4 mg, 1.93 mmol) and Et_3N (0.537 mL, 3.86 mmol) in 3 mL dry DCM at 0 °C. The reaction was allowed to warm to room temperature, stirred for 1 h and then quenched with water. The organic layer was separated, and the aqueous layer was extracted twice with DCM. The combined organics were washed with brine, dried (Na_2SO_4), filtered and concentrated to give **65b** (326.7 mg, 16% starting from **62**) as an oil.^{1}H NMR (300 MHz) δ 7.30 (m, 5H), 5.16 (s, 2H), 4.75 (m, 1H), 4.10 (m, 2H), 3.70 (m, 2H), 3.40 (m, 2H), 3.08 (s, 3H), 2.80 (m, 1H), 1.46 (s, 9H). MS (ES+) m/z: found 429.6 [MH^+], $C_{19}H_{28}N_2O_7S$ requires 428.5.

Compound 67. A solution of **65b** (151 mg, 0.352 mmol) in 1.5 mL of DCM was stirred with TFA (0.2 mL) at room temperature for 1 h. It was then concentrated under reduced pressure. The residue was dissolved in EtOH (2.5 mL), basified to pH~12 with 10% aq. NaOH solution, and stirred at 60°C for 3 h. It was cooled to room temperature and concentrated under reduced pressure to remove most of the volatiles. The residue was extracted with $CHCl_3$. The combined extracts were washed with brine and then passed through a short column of diatomaceous earth. The filtrate was concentrated to give **67** (75.4 mg, 72%). ^{1}H NMR (200 MHz, CD_3OD) δ 7.26-7.45 (m, 5H), 5.16 (s, 2H), 4.45 (t, *J*= 5.2 Hz, 1H), 3.82 (t, *J*= 2.6 Hz, 2H), 3.77 (d, *J*= 3.4 Hz, 1H), 3.16-3.42 (m, 4H).

Buchwald-Hartwig Coupling Reaction. The mixture of amine (0.325 mmol), bromobenzene (0.051 mL, 0.487 mmol), and ^{t}BuONa (46.8 mg, 0.487 mmol) in dry toluene (3 mL) was degassed and purged with argon three times before the addition of *rac*-BINAP (12.0 mg, 0.019 mmol) and $Pd_2(dba)_3$ (5.95 mg, 0.0065 mmol). It was then heated to 100 °C and stirred at this temperature for 10 h. After it was cooled down to room temperature, it was diluted with EtOAc and washed with brine. Products were isolated after chromatographic purification over silica gel.

Data for compound 52. 32% yield, ^{1}H NMR (200 MHz) δ 7.20 (dd, *J*= 1.2 Hz, *J*= 8.4 Hz, 2H), 6.75 (t, *J*= 7.6 Hz, 1H), 6.46 (dd, *J*= 1.4 Hz, *J*= 8.8 Hz, 2H), 4.65 (t, *J*= 5.4 Hz, 1H), 4.33-4.59 (m, 1H), 3.77-4.02 (m, 2H), 3.55-3.76 (m, 2H), 2.15 (dd, *J*= 6.4 Hz, *J*= 13.0 Hz, 1H), 1.73-1.97 (m, 1H), 1.48 (s, 9H).

Data for compound 68. 40% yield, ^{1}H NMR (200 MHz) δ 7.15-42 (m, 7H), 6.73 (t, *J* = 7.2 Hz, 1H), 6.40 (d, *J* = 8.4 Hz, 2H), 5.07-5.20 (m, 2H), 4.58 (dd, *J* = 3.6 Hz, *J* = 6.6 Hz, 1H), 3.94-4.11 (m, 2H), 3.91 (t, *J*= 7.4 Hz, 1H), 3.63-3.72 (m, 1H), 3.36-3.51 (m, 1H), 3.11-3.33 (m, 2H). MS (ES+) m/z: found 309.5 [MH^+], $C_{19}H_{20}N_2O_2$ requires 308.3.

Compound 34. TFA (250 μL) was added to a solution of **52** (7.1 mg, 0.026 mmol) in 2 mL of DCM. The mixture was stirred at room temperature for 2 h. Volatiles

were evaporated under vacuum to afford **34** as its trifluoroacetic salt (7.5 mg, 98%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.44 (bs, 1H), 9.09 (bs, 1H), 7.17 (t, *J*= 7.4 Hz, 2H), 6.69 (t, *J*= 7.4 Hz, 1H), 6.48 (dd, *J*= 1.0 Hz, *J*= 7.6 Hz, 2H), 4.70 (t, *J*= 4.6 Hz, 1H), 4.11-4.60 (m, 1H), 3.33-4.05 (m, 4H), 2.20 (dd, *J*= 5.6 Hz, *J*= 14.0 Hz, 1H), 1.68-1.91 (m, 1H).

Compound 35. A solution of **68** (30 mg) in 2 mL MeOH was stirred with 10% wt Pd/C under H_2 for 4 h. The catalyst was cautiously removed by filtration through a short column of diatomaceous earth. The filtrate was concentrated to give **35** (18.8 mg, quantitative). ^{1}H NMR (200 MHz) δ 7.19 (t, *J*= 8.2 Hz, 2H), 6.71 (t, *J*= 7.2 Hz, 1H), 6.40 (d, *J*= 7.8 Hz, 2H), 4.42-4.83 (m, 2H), 3.85 (t, *J*= 7.6 Hz, 1H), 3.57 (dd, *J*= 2.8 Hz, *J*= 7.6 Hz, 1H), 3.30 (dd, *J* = 7.8 Hz, *J* = 11.8 Hz, 2H), 2.99-3.17 (m, 1H), 2.77-2.94 (m, 1H), 2.61 (bd, 1H).

Compounds 71a-e. Hydrazine monohydrate (18.3 mL, 37.7 mmol) was added to a solution of acetophenone (4.52 g, 37.7 mmol) in 20 mL MeOH. The solution was heated to reflux. After 4 h solvent was evaporated under reduced pressure to afford **71a** (4.80 g, 95%) which did not need any purification. ^{1}H NMR (400 MHz) δ 7.63-7.66 (m, 2H), 7.29-7.38 (m, 3H), 5.36 (bs, 2H), 2.13 (s, 3H).

Under analogous reaction conditions, **71b** was obtained (97% yield) from *p*-iodoacetophenone: ^{1}H NMR (400 MHz) δ 7.66 (dd, *J*=1.6 Hz, *J*= 6.8 Hz, 2H), 7.37 (dd, *J*= 1.6 Hz, *J*= 6.8 Hz, 2H), 5.39 (bs, 2H), 2.08 (s, 3H).

Under analogous reaction conditions, **71c** was obtained (95% yield) from *m*-iodoacetophenone: ^{1}H NMR (400 MHz) δ 7.58-7.74 (m, 2H), 7.24-7.28 (m, 1H), 7.07-7.13 (m, 1H), 5.31 (bs, 2H), 2.15 (s, 3H).

Under analogous reaction conditions, **71d** was obtained (96% yield) from benzaldehyde: ^{1}H NMR (400 MHz) δ 7.75 (s, 1H), 7.52-7.56 (m, 2H), 7.25-7.37 (m, 3H), 5.50 (bs, 2H).

Under analogous reaction conditions, **71e** was obtained (96% yield) from *m*-iodobenzaldehyde: ^{1}H NMR (400 MHz) δ 7.71-7.75 (m, 1H), 7.61-7.67 (m, 2H), 7.17-7.21 (m, 1H), 7.08-7.14 (m, 1H), 4.57 (s, 2H).

Compounds 74a-c. MnO_2 (14.8 g, 170.0 mmol) was added portionwise at 0 °C to a solution of **71a** (3.80 g, 28.3 mmol) in 70 mL of dioxane. The mixture was stirred 1h at room temperature, then filtered on a pad of celite. To the resulting solution was added *N*-benzylmaleimide (2.64 g, 14.2 mmol) dissolved in 10 mL of dioxane over 30 min. The mixture was stirred at room temperature for 2 h, then refluxed for 18 h. Volatiles were evaporated under vacuum. The crude was treated with MeOH; the precipitate was filtered, rinsed with MeOH and collected. **74a** was obtained as a white solid (1.42 g, 34%). ^{1}H NMR (400 MHz) δ 7.32-7.44 (m, 4H), 7.25-7.31 (m, 6H), 4.61 (s, 2H), 2.77 (s, 2H), 1.27 (s, 3H).
Under analogous reaction conditions, **74b** (37% yield) was obtained from **71b**: ^{1}H NMR (400 MHz) δ 7.64 (d, *J*= 8.4 Hz, 2H), 7.39-7.40 (m, 2H), 7.25-7.30 (m, 3H), 7.02 (d, *J*= 8.4 Hz, 2H), 4.58 (s, 2H), 2.71 (s, 2H), 1.22 (s, 3H).
Under analogous reaction conditions, **74c** (26% yield) was obtained from **71c**: ^{1}H NMR (400 MHz) δ 7.57-7.72 (m, 2H), 7.39-7.48 (m, 2H), 7.24-7.38 (m, 4H), 7.05 (t, *J*= 7.8 Hz, 1H), 4.60 (s, 2H), 2.73 (s, 2H), 1.24 (s, 3H).

Compounds 74d,e. MnO_2 (19.71 g, 226.4 mmol) was added portionwise at 0 °C to a solution of **71d** (4.44 g, 37.0 mmol) in 170 mL of DCM. The mixture was stirred 1h at 0 °C and further 30 minutes at room temperature, then filtered on a pad of celite. Solvent was evaporated under vacuum and residue dissolved in Et_2O (100 mL). *N*-Benzylmaleimide (9.35 g, 50.0 mmol) dissolved in 100 mL Et_2O was added and the mixture was stirred at room temperature for 2 h. Precipitate was filtered off, rinsed with Et_2O and collected. The solid was dissolved in 50 mL of benzene and the reaction mixture heated to reflux for 15 h. Solvent was evaporated under reduced pressure; precipitation of residue from a mixture benzene/hexane=1:1 afforded **74d**

as a white solid (3.58 g, 35%). ^{1}H NMR (400 MHz) δ 7.25-7.36 (m, 8H), 7.07 (dd, *J*= 2.0 Hz, *J*= 7.6 Hz, 2H), 4.57 (s, 2H), 2.76 (d, *J*= 3.2 Hz, 2H), 2.67 (m, 1H).
Under analogous conditions, **74e** (26% yield) was obtained from **71e**: ^{1}H NMR (400 MHz) δ 7.58-7.64 (m, 1H), 7.44 (bs, 1H), 7.28-7.34 (m, 5H), 7.03-7.05 (m, 2H), 4.56 (s, 2H), 2.74 (d, *J*= 3.2 Hz, 2H), 2.58 (t, 1H).

Compounds 73a-e. 1M Borane solution in THF (45.3 mL, 45.3 mmol) was added dropwise to a solution of **74a** (2.18 g, 7.50 mmol) in 70 mL of dry THF cooled to 0 °C. The mixture was stirred 2 h at room temperature and then heated to reflux for further 2 h. After cooling to 0 °C, piperazine (3.89 g, 45.3 mmol) dissolved in 24 of H_2O was added and the solution heated to reflux for 18 h. The reaction mixture was cooled to room temperature, diluted with H_2O and extracted twice with EtOAc. Combined organics were washed with brine, dried (Na_2SO_4), filtered and concentrated under vacuum. Chromatography of the crude material over silica gel ($CHCl_3$) afforded the pure **73a** (1.56 g, 79%). ^{1}H NMR (400 MHz) δ 7.23-7.34 (m, 9H), 7.14-7.17 (t, 1H), 3.70 (bs, 2H), 3.04 (bd, 2H), 2.88 (bs, 2H), 1.79 (bs, 2H), 1.60 (s, 3H).
Under analogous reaction conditions, **73b** (75% yield) was obtained from **74b**: ^{1}H NMR (400 MHz) δ 7.57 (dd, *J*= 2.0 Hz, *J*= 6.4 Hz, 2H), 7.28-7.33 (m, 4H), 7.21-7.27 (m, 1H), 6.98 (dd, *J*= 2.0 Hz, *J*= 6.4 Hz, 2H), 3.69 (bs, 2H), 3.04 (bd, 2H), 2.84 (bs, 2H), 1.73 (bs, 2H), 1.58 (s, 3H).
Under analogous reaction conditions, **73c** (82% yield) was obtained from **74c**: ^{1}H NMR (200 MHz) δ 7.57-7.60 (m, 1H), 7.44-7.47 (m, 1H), 7.17-7.33 (m, 6H), 6.98 (t, *J*= 7.8 Hz, 1H), 3.66 (s, 2H), 3.04 (d, *J*= 9.4 Hz, 2H), 2.76-2.84 (m, 2H), 1.71-1.74 (m, 2H), 1.59 (s, 3H).
Under analogous reaction conditions, **73d** (89% yield) was obtained from **74d**: ^{1}H NMR (400 MHz) δ 7.33-7.35 (m, 4H), 7.23-7.26 (m, 3H), 7.13-7.19 (m, 1H), 7.05 (d, *J*= 8.0 Hz, 2H), 3.68 (s, 2H), 3.15 (bd, *J*= 8.8 Hz, 2H), 2.52 (bd, *J*= 8.4 Hz, 2H), 2.38 (bs, 1H), 1.68 (s, 2H).

Under analogous reaction conditions, **73e** (78% yield) was obtained from **74e**: ^{1}H NMR (200 MHz) δ 7.19-7.53 (m, 7H), 6.85-7.04 (m, 2H), 3.64 (s, 2H), 3.11 (d, *J*= 9.0 Hz, 2H), 2.47 (d, *J*= 8.6 Hz, 2H), 2.30 (t, 1H), 1.64 (bs, 2H).

Compound 73f. TFMSA (1.01 mL, 11.61 mmol) and NIS (1.22 g, 5.41 mmol) were added to a solution of the amine **73d** (0.963 g, 3.87 mmol) in 11 mL of acetonitrile cooled to 0 °C. The mixture was gradually warmed to room temperature. After 22 h, the reaction mixture was quenched with sat.d aq. $NaHCO_3$ solution and extracted twice with EtOAc. Combined organics were dried (Na_2SO_4), filtered and concentrated under vacuum. Flash chromatography of the crude residue over silica gel (DCM) gave the pure **73f** (1.01 g, 69%). ^{1}H NMR (200 MHz) δ 7.53 (d, *J*= 8.4 Hz, 2H), 7.20-7.32 (m, 5H), 6.78 (d, *J*= 8.4 Hz, 2H), 3.64 (s, 2H), 3.11 (d, *J*= 8.6 Hz, 2H), 2.47 (bd, *J*= 8.4 Hz, 2H), 2.30 (t, *J*= 3.2 Hz, 1H), 1.62 (bs, 2H).

Compounds 69a and 70a. To a suspension of **73a** (248 mg, 1.0 mmol) in 50 mL of MeOH were added first ammonium formate (189 mg, 3.0 mmol) and then 10% wt Pd/C (0.3 mmol). The reaction mixture was heated to reflux for 1 h. The suspension was filtered from the catalyst through a pad of celite and solvent evaporated under vacuum. Chromatography of the crude residue over florisil (grad. DCM to DCM/MeOH=8:2) afforded the pure **69a** (88 mg, 51%). ^{1}H NMR (400 MHz) δ 7.24-7.38 (m, 4H), 7.15-7.22 (m, 1H), 3.28-3.42 (m, 2H), 3.16 (d, *J*= 8.0 Hz, 2H), 2.24 (bs, 1H), 1.82-1.95 (m, 2H), 1.37 (s, 3 H).
Under analogous reaction conditions, **70a** (69% yield) was obtained from **73d**: ^{1}H NMR (400 MHz) δ 7.24-7.31 (m, 2H), 7.12-7.19 (m, 1H), 7.03-7.11 (m, 2H), 3.18 (d, 2H), 3.00-3.09 (m, 2H), 1.82 (bs, 1H), 1.72-1.76 (m, 2H), 1.69-1.72 (m, 1H).

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3 CONSTRAINED AMINO ACIDS AND BIS-AMINES

A common strategy in the practice of medicinal chemistry is the introduction of conformational constraint in the design of new inhibitor structures.[1] The goal is tighter and/or more selective binding of the inhibitor to its target. Reducing the entropic costs of binding can produce the desired effect, but only if enthalpically important contacts are not lost as a result of the structural constraint. The most common tactic toward these ends involves the construction of cyclic isosters of acyclic structures.[1a,b] The further constraint of cyclic structures can be achieved through the introduction of unsaturation, through fusion to a second ring, or by conversion of a (mono)cyclic system into a bicyclic one.[1c,d,2] As examples of the latter approach, cyclopropyl analogues of non cyclopropyl-group containing compounds have been used to modify or even improve the selectivity of biologically active compounds and improve the pharmacokinetic and pharmacodynamic properties of lead compounds.[3,4]

The cyclopropane moiety is a common motif in nature. The importance of cyclopropyl-group containing compounds for industrial applications is also apparent, reflected for example by the tremendously successful of pharmaceutical blockbusters like the antiinfectives ciprofloxacin and moxifloxacin.[5] Several natural and non-natural products with interesting biological activities comprising a cyclopropane moiety are amino acids and bis-amines. Among these, β- and γ-amino acids have gained increasing attention over the past years, not only as conformationally restricted analogues of natural amino acids in peptide/protein synthesis[6] but also in terms of developing new synthetic methodology[7]. Constrained bis-amines are also interesting as rigid scaffolds with two nitrogen atoms held at well defined distance and could be ranked on the base of their ability to act as mimetics of common structural motives, such as azepine, diazepine and piperazine rings.

In view of the biological relevance of these two classes of structures, we prepared constrained bicyclic heterocycles. In particular, both constrained amino acids and bis-amines could give access to a whole series of azabicyclo heteroaromatic derivatives.

3.1 *CONSTRAINED AMINO ACIDS*

The discovery of substances which mimic the action of the mammalian neurotransmitter γ-aminobutyric acid (GABA) at its central receptors may be important not only for understanding the characteristics of the receptors but also for obtaining potentially-useful therapeutic agents. Nipecotic acid and guvacine scaffolds are emerging as novel privileged structures due to their cyclic nature, high degree of functionality, and diverse range of biological activity. These cyclic amino acids, which may be considered as conformationally restricted GABA analogues, display *in vitro* potent activity as inhibitor of [^{3}H]-GABA uptake.[8] On the other hand, it has been reported that the isomeric compounds, isoguvacine and isonipecotic acid, are specific GABA receptors agonists.[9]

The use of conformationally constrained amino acids to probe the binding mode of bioactive molecules to their receptors is an approach to the design of highly selective and active compounds. Reducing the conformational freedom of a ligand may alter the binding affinity of the ligand at a given receptor, the selectivity of the ligand between different receptors, and the stability of the ligand with respect to enzymatic degradation. Examination of the effect of restricting the conformational freedom of a given ligand on these properties may lead to increased insight into the bioactive conformation of the ligand and hence ultimately to the generation of more potent and selective molecules.

Thus, we became interested in 3-azabicyclo[4.1.0] heptane-1-carboxylic acid (**1**) and 3-aza-bicyclo[4.1.0] heptane-6-carboxylic acid (**2**) (Figure 1) as templates for the design of new drugs. Imparting additional conformational rigidity to the piperidine

ring of nipecotic/isonipecotic acids incorporating a cyclopropyl group at C-1/ C-6 positions could result in improved pharmacokinetic properties of lead compounds.

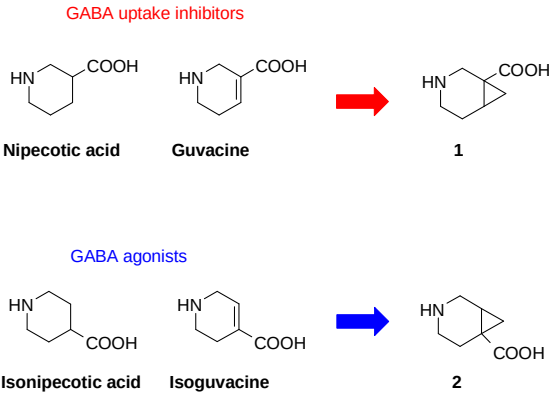
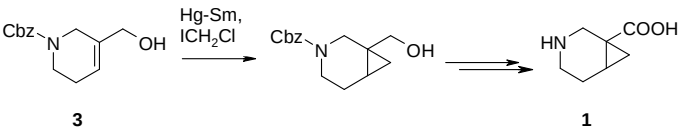


Figure 1 - *Conformationally constrained aminoacids*

3.1.1 *NIPECOTIC ACID MIMETIC*

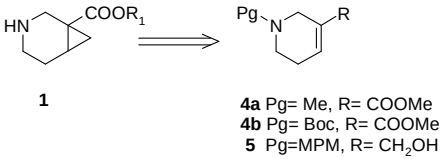
A survey of literature shows that only one approach to the synthesis of **1** has been reported. The key synthetic step was the cyclopropanation of the hydroxymethyl tetrahydropyridine **3** using samarium-mercuric amalgam and iodochloromethane (Scheme 1).[10]



Scheme 1 - *The reported synthesis of nipecotic acid analogues*

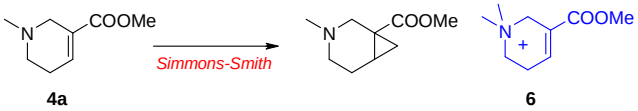
In the light of the problems associated with the use of mercuric amalgams, we sought an alternative route to the required β-amino acid. Following the reported

retrosynthetic approach, **1** can be straightforwardly disconnected at the fused junctions. Upon this disconnection, arecoline (**4a**) or its simple derivatives (**4b**, **5**) become the key precursors (Scheme 2).



Scheme 2 - *Retrosynthetic analysis*

One of the most widely used methods to introduce a cyclopropane ring from an olefin is the Simmons-Smith reaction.[11,12] Despite the coordinative potential of a neighboring amino group, allylic amines or olefins containing an amino group not bearing a chelating group have not been directly applied to the Simmons-Smith reaction to the propensity of these compounds to undergo *N*-ylide formation.[13] It was, therefore, not surprising that cyclopropanation of arecoline (**4a**) was unsuccessfull using those reaction conditions, due to the preferentially formation of ammonium salt **6**.

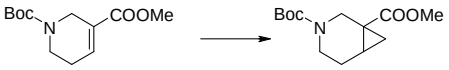


Scheme 3

In an effort to alleviate the formation of complexed ammonium ylide, the methyl amino protecting group of arecoline was converted into the non nucleophilic *tert*-butylcarbamate group. Unfortunately, all attempts to cyclopropanate **4b** were unsuccessful.

As summarized in Table 1, cyclopropanation using diazomethane or a dihalomethane as carbene sources failed; the reaction under Corey conditions[14]

afforded only traces of **7**, as detected by UPLC monitoring, but in all case we were not able to isolate the product.



4b **7**

Table 1 - *Cyclopropanation of **4b***

Conditions	***Results***
CH_2N_2, $Pd(OAc)_2$, Et_2O	SM
CH_2N_2, $Pd(PPh_3)_4$, CH_2Cl_2	SM
$ZnEt_2$, CH_2I_2, DCE	SM
Zn/ Cu, CH_2I_2, DCE	SM
$ZnEt_2$, $ClCH_2I$, DCM	SM
$TMSCHN_2$, toluene, reflux or hν	Different product
tert-BuOLi, CH_2I_2, NMP	SM
$(CH_3)_3SO^+I^-$, NaH, DMSO	Only traces of **7**on UPLC
$(CH_3)_3SO^+I^-$, NaH, THF	Only traces of **7**on UPLC
$(CH_3)_3SO^+I^-$, BEMP, MeCN	Only traces of **7**on UPLC
Dimethylaminophenylsulfoxonium methylide, NaH, DMSO	Only traces of **7**on UPLC
Dimethylaminophenylsulfoxonium methylide, NaH, THF	Only traces of **7**on UPLC
Dimethylaminophenylsulfoxonium methylide, BEMP, MeCN	Only traces of **7**on UPLC
Dimethylaminophenylsulfoxonium methylide, DBU, DCM	Only traces of **7**on UPLC

Treating **4b** with $TMSCHN_2$ in refluxing toluene we did not isolate the awaited pyrazoline **8**, but the regioisomeric hydrazone **9a**, which spontaneously underwent desilylation to give 2-pyrazoline **9b** (Scheme 4). In the light of the literature data,[15] the tautomerization **8→9a** competed with the N_2-extrusion, however we expected that the share of the latter as a unimolecular reaction growed with increasing the temperature. Unfortunately, the N_2-extrusion was not observed as neither under drastic thermal nor photolytic conditions (Table 2).

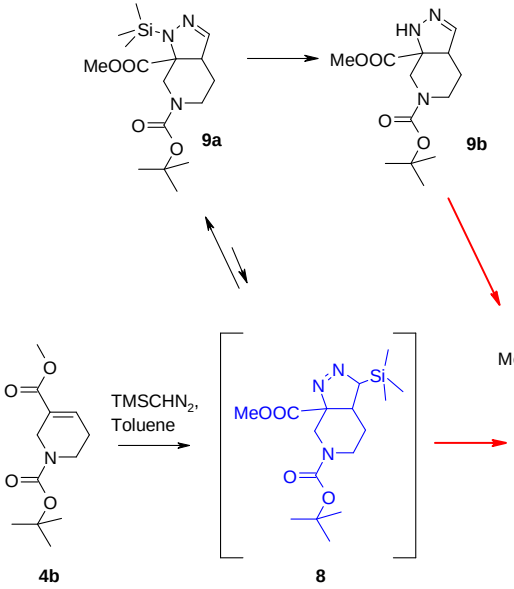
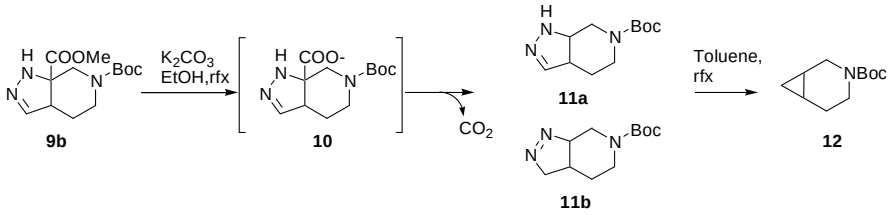


Table 2. *N_2-Extrusion*

Conditions	*Result*
$CuCl_2$ (cat), n-BuOH, reflux	SM
Toluene or DMF, reflux	SM
DMF, mw 200 ℃, 30 min	SM
DMSO, mw, 230 ℃	SM
neat, mw 250 ℃	-
CH_3CN or Toluene, hν	SM
neat, 150 ℃ under vacuum	-

Scheme 4 - *Cyclopropanation with $TMSCHN_2$*

Treating **9b** with a base, such as K_2CO_3 in refluxing EtOH we observed hydrolysis of the ester group, decarboxylation and finally N_2-extrusion to give **12** in very low yield (Scheme 5).



Scheme 5 - *N_2-extrusion in basic media*

A survey of literature revealed few examples that could provide the cyclopropanation of an allylic amine by masking the amine as carbamate in the

absence of a chelating group (OH or OR) under Simmons-Smith conditions.[16] To overcome the absence of a zinc chelating group, the methyl ester **4b** was reduced to the corresponding alcohol **5** by simply treatment with $LiAlH_4$. Less than satisfactory results were obtained using Denmark's activated $IZnCH_2Cl$ reagent[17]; several different cyclopropanation conditions were also tested, but all our attempts were again unsuccessful (Table 3).

5

Table 3 -*Cyclopropanation of* ***5***

Conditions	***Results***
CH_2N_2, $Pd(PPh_3)_4$, Et_2O	SM
CH_2N_2, $ZnCl_2$, DCE	SM
$ZnEt_2$ (2eq), $ClCH_2I$ (4eq), DCE	SM
Zn/Cu, $ClCH_2I$, THF	SM
$AlMe_3$, CH_2I_2, DCM, 0 °C	Only traces of TM on UPLC
$TMSCHN_2$, Toluene, reflux	Only traces of TM on UPLC
$TMSCHN_2$, Toluene, $PdCl_2$, 0 °C	Only traces of TM on UPLC
$TMSCHN_2$, Toluene, 0 °C	Only traces of TM on UPLC
$TMSCHN_2$, CH_2Cl_2, 0 °C	Only traces of TM on UPLC
$TMSCHN_2$, Toluene, hν	Only traces of TM on UPLC

The methyl ester group and the hydroxy methylene group may be interfering with the cyclopropanation either directly through unfavorable steric interactions or indirectly by altering the conformation of piperidine ring. Therefore, to test the substituent effect on direct cyclopropanation of heterocycle, the methyl ester **4b** was converted into nitrile **13** as outlined in Scheme 6. Conversion of methyl ester into the corresponding amide **14**, followed by dehydration with trichloroacetyl chloride/ triethylamine afforded **15** in good yield.[18] As showed in Table 4, the use of Corey's conditions was found to be essential for the cyclopropanation success, even if the

product was isolated in very low yield (3%). Interestingly, implementation of Corey's reaction conditions using dimethylaminophenylsulfoxonium methylide[19] led to a slight improvement in yield (ca. 11%) that, however, was mitigated by the formation of a complicated mixture of byproducts. Hydrolysis of cyano group was accomplished by *N*-Boc deprotection to afford **1** with very low overall yield (3%) (Scheme 6).

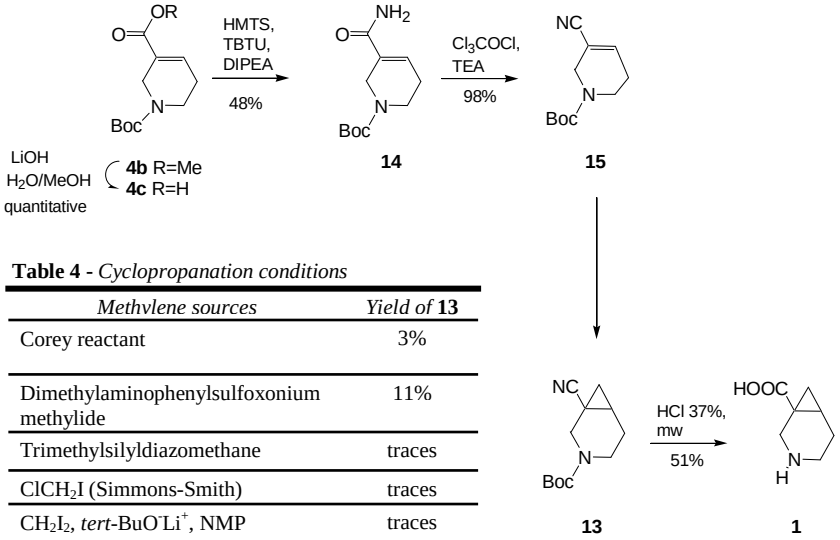
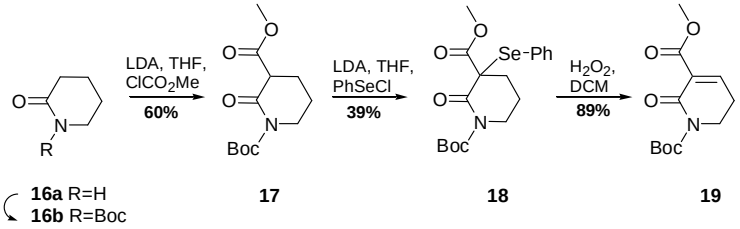


Table 4 - *Cyclopropanation conditions*

Methylene sources	*Yield of* **13**
Corey reactant	3%
Dimethylaminophenylsulfoxonium methylide	11%
Trimethylsilyldiazomethane	traces
$ClCH_2I$ (Simmons-Smith)	traces
CH_2I_2, *tert*-BuO^-Li^+, NMP	traces

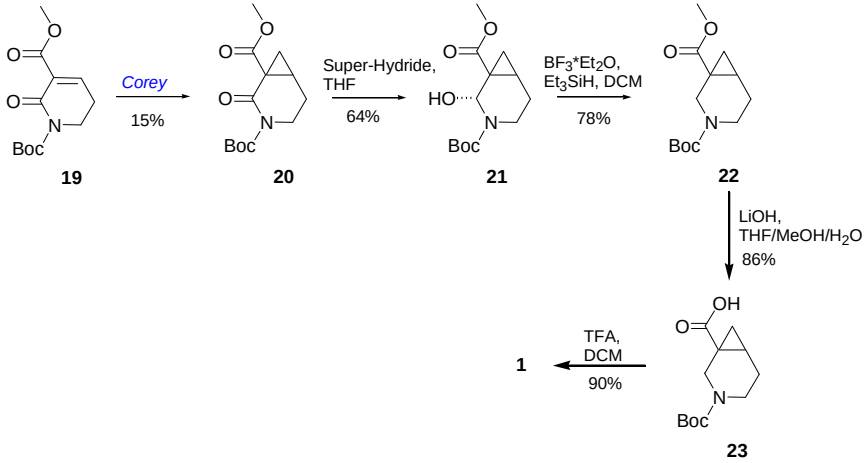
Scheme 6 - *The effect of a linear substituent on the cyclopropanation of piperidine ring*

In a second approach to change the conformation of the piperidine ring to a more favorable one, the arecoline core was exchanged for a piperidone-like structure. The preparation of β–amido ester **19** from δ-valerolactam (**16a**) is well precedented[20] and is outlined in Scheme 7. Initial protection of δ-valerolactam as *tert*-butylcarbamate was carried out straightforwardly, followed by *C*-carboxymethylation to give **17**. Subsequent introduction of the C-3/C-4 unsaturation was carried out *via* selenation and intermediate *syn*-elimination.



Scheme 7 – *Synthesis of the unsatured piperidone **19***

With **19** in hand, we began our investigation of its cyclopropanation. Also in this case Corey's reaction conditions ($(CH_3)_3SO^+I^-$, NaH, DMSO) were found to be essential for the modest observed conversion (5%) to occur. Again, the use of dimethylaminophenylsulfoxonium methylide led to a slight improvement in yield (15%), but no further improvements were registered modifying the base, the solvent or the reaction temperature.



Scheme 8 – *From **19** to **1**: a successful but inefficient methodology*

In order to validate the ability to access the desired β-amino acid **1** with this synthetic plan, we explored chemoselective reduction of the lactam **20** (Scheme 8).

Attempts to selectively reduce the lactam in presence of the ester with $LiBH_4$ or DIBAL gave no reaction. In an improved protocol[21], **20** was reduced with lithium triethylborohydride to a diastereomeric mixture of the *N*-Boc aminals **21**. Further reduction with triethylsilane and $BF_3 \cdot Et_2O$ followed by acidic work-up gave **22** in 50% overall yield from **20**. Subsequent ester hydrolysis followed by *N*-deprotection gave **1** in excellent yield.

The observed chemoselectivity in the reduction step to aminals **21** is explained on the basis of a six-membered transition state (Figure 2). Model was obtained with the Chem3D Ultra software.[22]

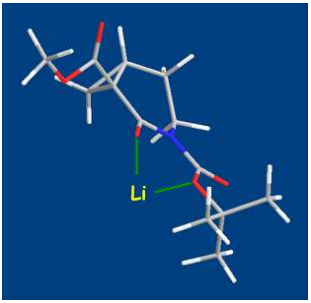
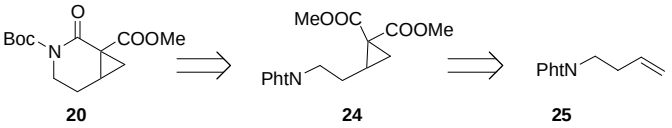


Figure 2 - *Six-membered transition state.*

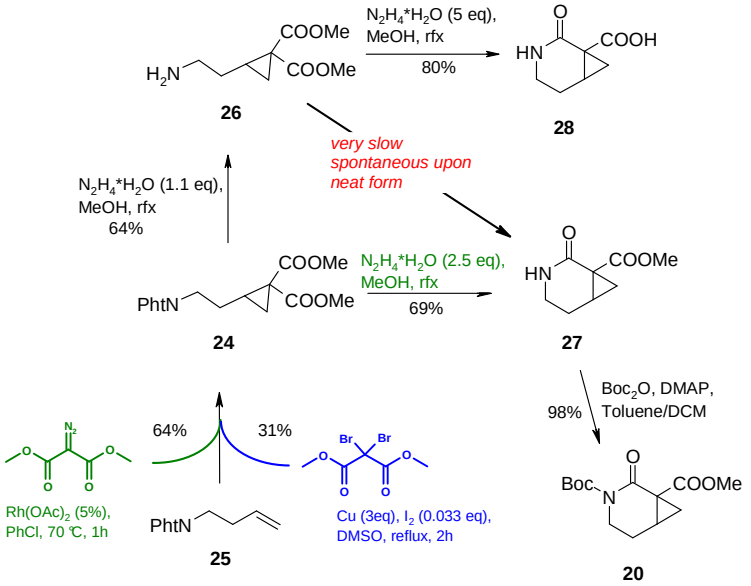
The overall yield of this synthetic pathway did not exceed 2% due to the low efficiency of the cyclopropanation step. Whereas all the other synthetic steps of the achieved plan worked well, we sought an alternative route to the required bicyclic lactam **20** and then converted it into amino acid **1** as described above.

We planned a *de novo* synthesis of **20** *via* reaction of a protected amino olefin with an activate dimethylmalonate followed by intramolecular cyclization (Scheme 9). As the amino group required eventual protection, 4-phtalimido 1-butene (**25**)[23] was employed as starting material for our work.



Scheme 9 - *Retrosynthetic analysis*

The cyclopropyl derivative **24** could be obtained by insertion of a carbene on olefin **25**. We chose testing the ability of dimethyl dibromomalonate[24] and dimethyl diazomalonate[25] as carbene sources.

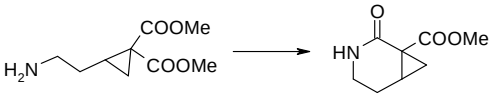


Scheme 10 – *De novo synthesis of intermediate lactam **20***

The copper-promoted insertion of dimethyl dibromomalonate at reported temperature led only to the formation of a small amount of **24** (31% yield). Unsatisfactory yields (13-29%) were obtained also at higher temperatures (150-175 °C) under mw irradiation. Moreover it was found that the yield of **24** dropped substancially on scale-up evidently as a consequence of the heterogeneous reaction

conditions. On the other hand, the use of $[Rh_2(OAc)_4]$ coupled with slow introduction of the dimethyl diazomalonate into the reaction mixture afforded a higher yield of **24** regardless of scale. The reaction required lower temperature (70 °C) and afforded cyclopropyl diester **24** in 64% yield[26] (Scheme 10).

For the construction of azabicycloheptanyl skeleton we were encouraged by a Danishefsky communication[27] of the spontaneous intramolecular cyclization of amino diester **24** after *N*-deprotection. Classical dephtaloylation conditions (1.1 equiv of hydrazine monohydrate in methanol at reflux) gave only deprotected amine **26** in 64% yield. Upon standing in neat form, partial cyclization (15-30%) to **27** was observed by ^1H NMR analysis.



26 **27**

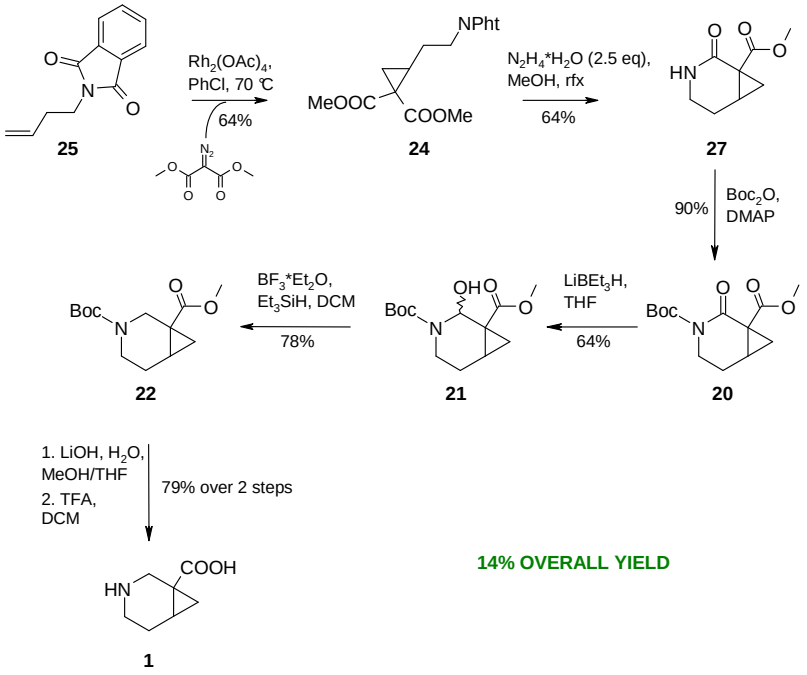
Table 5 - *Cyclization*

Conditions	***Conversion to* 27 *(%)***
SiO_2, MeOH, rt	-
SCX, $CHCl_3$, rt	-
HCl/ MeOH, rt	-
AcOH/ MeOH, rt	-
MeOH, reflux	15
MeOH, 100 °C mw	-
n-BuLi, THF, -78° C	20
n-BuLi, THF, 0 °C	Only degradation
N_2H_4*H_2O (5 eq.), MeOH, reflux	Full conversion to **28**

Initial attempts to effect the total conversion of dephtaloylamine **26** into lactam **27** afforded very low yields of the awaited compound, instead affording multiple products attributed to decomposition of starting material. Optimization efforts included the activation of **26** *via* the addition of additivies or treatment with a base in combination with temperature and solvent screening (Table 5). The most significant

improvements in the efficiency of the reaction were realized employing an excess of hydrazine monohydrate (5 equiv) and running the reaction in refluxing MeOH; in those conditions, undesirable hydrolysis of ester group together with the efficient intramolecular cyclization was observed and carboxylic acid **28** was obtained in good yield (80%).

With these results in hand, we next addressed direct conversion of **24** into lactam **27**. Vigorously hydrazinolysis conditions (2.5 equiv of hydrazine in methanol at reflux for 18 h) provided β-amido ester **27** in 64% yield after crystallization. Finally, after *N*-protection as *tert*-butylcarbamate necessary for the subsequent chemoselective reduction to **22**, ester hydrolysis and *N*-deprotection gave bicyclic β-amino acid **1** in good yield (Scheme 8).

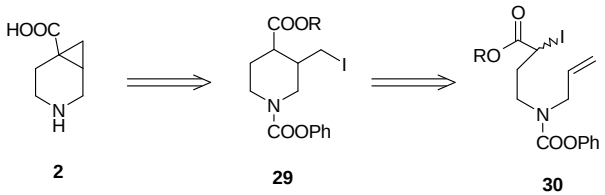


Scheme 11 - *Total synthesis of the 3-azabicyclo[4.1.0] heptane- 1- carboxylic acid (**1**)*

In summary, we have achieved a practical approach to the synthesis of 3-azabicyclo[4.1.0] heptane-1-carboxylic acid (**1**) starting from 4-phtalimido 1-butene (**25**) *via* cyclopropanation of an appropriate olefine, intramolecular cyclization and chemoselective reduction. With the optimized synthetic route, nipecotic acid mimetic **1** was prepared in 7 steps with 14% overall yield (Scheme 11).

3.1.2 *ISONIPECOTIC ACID MIMIC*

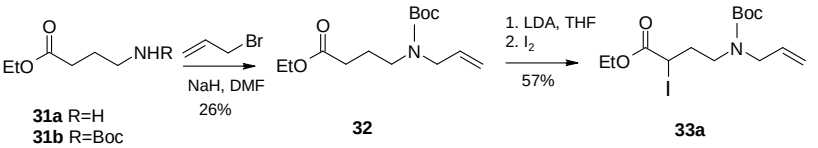
In the light of the studies realized on the scaffold **1**, the best way to synthesize the γ-amino acid **2** is the *de novo* synthesis of its molecular framework. Mori and coworkers reported the synthesis of a full protected derivative of **2** through intramolecular cyclization of the iodomethylen piperidine **29**, deriving *via* ene-halogenation from the unsatured iodocarbonyl derivative **30** (Scheme 12).[28]



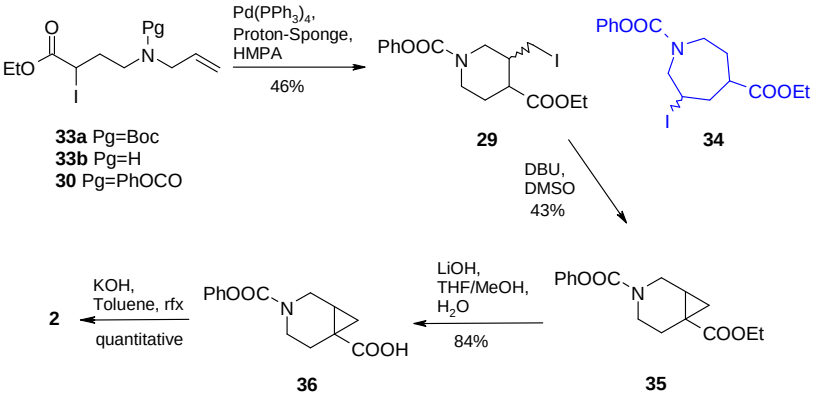
2 **29** **30**

Scheme 12 - *Retrosynthetic analysis*

We optimized that synthetic way in order to increase its overall yield. The starting α-haloester having internal double bond **33a** was prepared from commercially available ethyl γ-aminobutyrate (**31a**) as outlined in Scheme 13. After *N*-protection as *tert*-butylcarbamate, classical alkylation with allylbromide afforded **32** in moderate yield.



Scheme 13 - *Synthesis of the intermediate α-haloester **33***



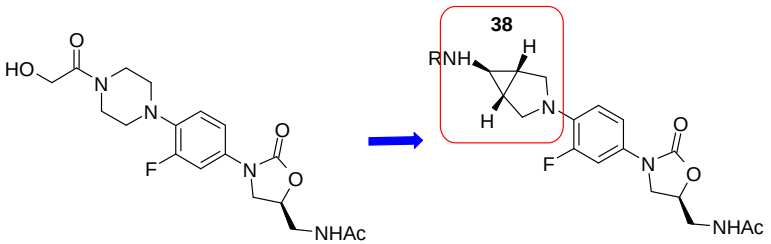
Scheme 14 - *Synthesis of 3-aza-bicyclo[4.1.0] heptane-6-carboxylic acid (**2**)*

It was not so easy to get the α-haloesters having amino group. Halogenation *via* ketene silyl acetal followed by reaction with bromine and subsequent treatment with potassium iodide gave **33a** in 13% yield.[29] Better results were obtained reacting **32** with LDA at – 78 °C followed by treatment with I_2 (57% yield). After changing the *N*-protecting group into phenyloxycarbamate (the Boc-protecting group allowed to prepare **33a** in satisfactory yield, whilst phenyloxycarbonyl group was more suitable for the next steps and guaranteed higher yields) α-iodoester **30** was warmed with 10% $Pd(PPh_3)_4$ at 65 °C in HMPA in the presence of proton-sponge (1,8-dimethylaminonaphtalene) as scavenger of hydrogen iodide to afford with 46% yield iododerivative **29** as unseparable diastereoisomeric mixture. The moderate yield of **29** was due to the formation of 7-membered ring **34**, rationalized on the basis of the radical mechanism of the reaction.[30] **29** was treated with DBU in DMSO

to give the fused three membered ring product **35** in moderate yield. Finally, hydrolysis of ester group followed by phenyloxycarbonyl group cleavage afforded the target compound **2** in good yield (Scheme 14).

3.2 *CONSTRAINED BIS-AMINES*

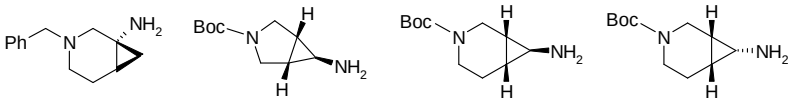
Constrained bis-amines are interesting as rigid scaffolds with two nitrogen atoms held at a well defined distance. Among these, 3-azabicyclo[3.1.0]hexylamine **38** has considered a privileged structure: besides being a key constituent of the highly active antibiotic trovafloxacin[31], this rigid system mimics the piperazine ring of eperezolid as reported by Renslo and coworkers[32] (Figure 3).



eperezolid

Figure 3 - *3-azabicyclo[3.1.0]hexylamine **38** mimics the piperazine ring of eperezolid*

In order to investigate the biological properties of these rigid systems, we prepared a variety of bicyclic bis-amines incorporating the amino-3-azabicyclo[3.1.0]hexane and the homologous 3-azabicyclo[4.1.0]heptane skeletons (Figure 4).



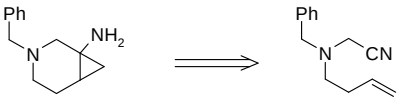
37 **38** **39** **40**

Figure 4 – *Conformationally restricted bis-amines*

Because of these scaffolds have to be used in focused drug-like subsets of compounds containing at least two different substituents on the two nitrogen atoms, these nitrogen atoms would have to be chemically addressable individually and selectively.

3.2.1 *3-(PHENYLMETHYL)-3-AZABICYCLO[4.1.0]HEPTAN-1-AMINE*

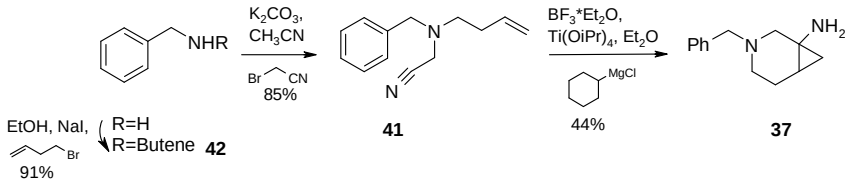
For the preparation of amine **37**, we sought the synthetic route developed by Laroche and coworkers.[33] The *key* step was the Ti(II)-mediated intramolecular coupling of alkene and nitriles moieties of the tertiary amine **41** (Scheme 15).



37 **41**

Scheme 15 - *Retrosynthetic analysis*

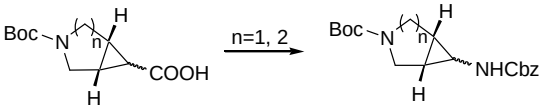
Starting nitrile **41** was readly prepared by alkylation of benzylamine first with 4-bromo-1-butene and then with bromoacetonitrile. Intramolecular reductive cyclopropanation of **41** upon treatment with titanium isopropoxide and cyclohexylmagnesium chloride with subsequent addition of a Lewis acid, such as BF_3*Et_2O, provided the monoprotected bis-amine **37** with 35% overall yield (Scheme 16).



Scheme 16 – *Efficient approach to **37***

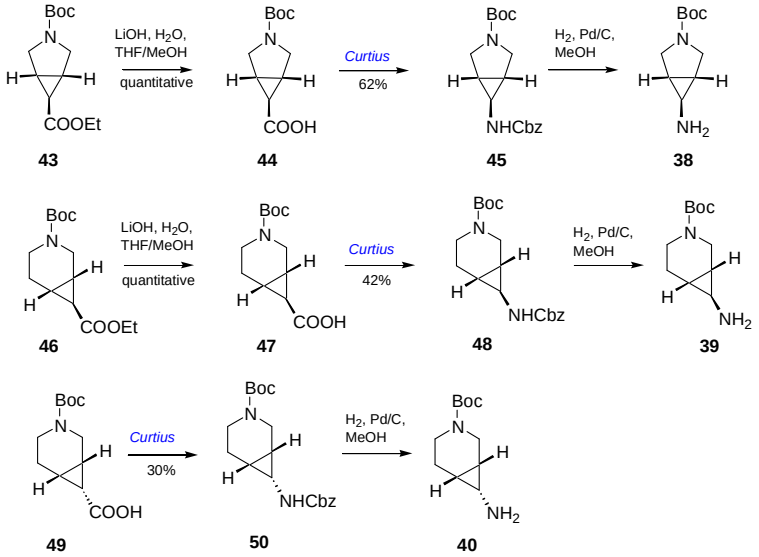
3.2.2 *APICAL BIS-AMINES*

The apical amino group on the bicyclic systems **38**, **39** and **40** was envisioned to arise *via* Curtius rearrangement of the corresponding carboxylic acid **44**[34], **47** and **49**.



Scheme 18 - *Retrosynthetic analysis*

The Curtius rearrangement using diphenylphosphoryl azide in refluxing toluene followed by treatment with benzyl alcohol introduced the requisite amino group at apical position, protected as its Cbz-derivative (Scheme 19).



Scheme 19 – *The simple synthetic plans to **38**, **39** and **40***

Compounds **45**, **48** and **50** serve as useful synthetic fragments, given the differential protection of the two amino groups. For our pourpose, hydrogenation of the Cbz group on the primary amine was carried out, delivering monoprotected **38**, **39** and **40**. ^{1}H NMR determinations carried out on these substrates established that the Curtius rearrangement had proceeded with retention of configuration, as expected.

3.3 EXPERIMENTAL SECTION

Compound 4b. K_2CO_3 (18.3 g, 133 mmol) was added to arecoline hydrobromide (25 g, 106 mmol) in 60 mL of H_2O. After 30 min, the mixture was extracted with Et_2O, the organic layers were dried (Na_2SO_4) and evaporated, and the resulting oil was dissolved in toluene (120 mL). ACE-Cl (14 mL, 128 mmol) was added slowly and the mixture was heated to reflux. After 16 h, HCl (100 mL, 0.1 N) was added and the mixture was extracted with Et_2O. The organic layers were dried (Na_2SO_4), filtered and evaporated under vacuum. The resulting carbamate was dissolved in MeOH (100 mL) and heated to reflux. After 2 h, the solvent was evaporated and the resulting amine was dissolved in 150 mL DCM and cooled to 0 °C. TEA (16.5 mL, 118 mmol) and Boc_2O (31.7 g, 145 mmol) were added. After 24 h, HCl (100 mL, 1 M) was added and the mixture was extracted with DCM. The organic layers were washed with sat.d aq. $NaHCO_3$ solution, dried (Na_2SO_4), filtered, evaporated and purified by flash chromatography, eluting with Etp/EtOAc=9:1, to give the carbamate **4b** (20 g, 78%) as an oil that crystallized at low temperature: mp 29-31 °C. ^{1}H NMR spectrum was superimposible to that reported in literature.[35]

Compound 9a. 2M solution of $TMSCHN_2$ in Et_2O, (622 µl, 1.24 mmol) was added to a solution of **4b** (100 mg, 0.414 mmol) in 4 mL of dry toluene. After stirring at room temperature for 1 h, the mixture was refluxed till the reaction was complete (UPLC monitoring) and the solvent removed under vacuum. The remaining crude material was purified by SPE-Si column (5 g, grad. Hexane to Hexane/EtOAc=9:1) to afford silyl hydrazone **9a** (40 mg, 27%) as yellow pale oil. ^{1}H NMR (400 MHz)

δ 6.34 (d, 1H), 3.91 (d, 1H), 3.76 (s, 3H), 3.69 (dd, 1H), 3.52-3.59 (m, 1H), 3.34-3.51 (m, 1H), 3.06-3.23 (m, 1H), 1.94-2.08 (m, 1H), 1.65-1.82 (m, 1H), 1.44, 1.47 (2s, 9H), 0.22 (s, 9H). UPLC/MS (ES+), m/z: found 356 [MH^+], $C_{16}H_{29}N_3O_4Si$ requires 355. Rt: 0.80 min.

Compound 9b. A solution of hydrazone **9a** (40 mg, 0.113 mmol) in 4 mL of MeOH was stirred at room temperature for 16 h. Solvent was evaporated under vacuum to afford pyrazoline **9b** (27 mg, 86%) which did not need any purification. ^{1}H NMR (400 MHz) δ 6.69 (bd, 1H), 6.01-6.14 (m, 1H), 3.79 (s, 3H), 3.30-3.79 (m, 4H), 1.94-2.06 (m, 1H), 1.63-1.76 (m, 1H), 1.46 (s, 9H). UPLC/MS (ES+), m/z: found 284 [MH^+], $C_{13}H_{21}N_3O_4$ requires 283. Rt: 0.59 min.

Compounds 11a and 11b. K_2CO_3 (68.3 mg, 0.494 mmol) was added to a solution of pyrazoline **9b** (70 mg, 0.247 mmol) in 3 mL of EtOH. The mixture was refluxed under nitrogen atmosphere for 15 h. Volatiles were evaporated under vacuum to afford a mixture of compounds **11a** and **11b** (44.6 mg, 40%), identified by UPLC analysis. UPLC/MS (ES+), m/z: found 226 [MH^+], $C_{11}H_{19}N_3O_2$ requires 225. Rt: 0.45 min and 0.53 min.

Compound 12. A solution of compounds **11** (44.6 mg, 0.198 mmol) in 5 mL of toluene was refluxed for 24 h. Volatiles were evaporated under vacuum to afford the crude product **12** (5 mg, 13%) which no need any purification. UPLC/MS (ES+), m/z: found 198 [MH^+], $C_{11}H_{19}N_3O_2$ requires 197. Rt: 0.62 min.

Compound 4c. To a solution of monohydrate LiOH (1.19 g, 28.2 mmol) in 10 mL of H_2O was added ester **4b** (3.42 g, 14.2 mmol) diluted in 11 mL of a mixture MeOH/THF=10:1 and the mixture was stirred at room temperature. After 4h the reaction was complete. The reaction mixture was acidificated with 1N aq. HCl solution, diluted with H_2O and backextracted three times with DCM. The combined

organics were dried (Na_2SO_4), filtered and concentrated under vacuum to afford the acid **4c** (3.20 g, quantitative) which did not need any purification. ^{1}H NMR (400 MHz) δ 7.40 (bs, 1H), 4.18 (bs, 4H), 2.34 (bs, 2H), 1.58 (s, 9H). UPLC/MS (ES+), m/z: found 228 [MH^+], $C_{11}H_{17}NO_4$ requires 227. Rt: 0.62 min.

Compound 14. Carboxylic acid **4c** (550 mg, 2.42 mmol), DIPEA (1.86 ml, 10.65 mmol) and TBTU (1.71 g, 5.32 mmol) were dissolved in 12 mL of DMF and the solution was stirred at room temperature for 1 h. HMDS (859 mg, 5.32 mmol) was added and stirring was continued for further 18 h. The mixture was diluted with EtOAc and washed with H_2O and with sat.d aq. NH_4Cl solution. The aqueous layers were backextracted three times with EtOAc. The combined organic layers were dried (Na_2SO_4), filtered and concentrated under vacuum. Purification of the crude residue by chromatography over SPE-Si column (25 g, grad. Hexane/Acetone=95:5 to Hexane/ Acetone=1:1) afforded the pure amide **14** (260 mg, 48%). ^{1}H NMR (400 MHz) δ 6.70 (bs, 1H), 5.48 (bs, 2H), 4.17 (dd, 2H), 3.51 (t, 2H), 2.27-2.36 (m, 2H), 1.50 (s, 9H). UPLC/MS (ES+), m/z: found 227 [MH^+], $C_{11}H_{18}N_2O_3$ requires 226. Rt: 0.54 min.

Compound 15. To a stirred mixture of **14** (1.10 g, 4.87 mmol), TEA (1.35 mL, 9.74 mmol) in 8 mL of dry DCM was added dropwise trichloroacetyl chloride (0.974 g, 5.36 mmol) dissolved in 3 mL of dry DCM, between 0-5 °C. After the addition was finished, the mixture was treated with ice-cooled water, 5% aq. NaOH solution, 5% HCl solution and finally with H_2O. The organic solution was dried (Na_2SO_4), filtered and concentrated under vacuum to afford nitrile **15** (1.0 g, 98%) as yellow pale oil. ^{1}H NMR (400 MHz) δ 6.77 (bs, 1H), 4.06 (bs, 2H), 3.53 (t, 2H), 2.33 (bs, 2H), 1.49 (s, 9H). UPLC/MS (ES+), m/z: found 209 [MH^+], $C_{11}H_{16}N_2O_2$ requires 208. Rt: 0.70 min.

Compound 13. NaH (17.3 mg, 0.720 mmol, 60% oil dispersion) was added portionwise to a solution of dimethylaminophenylsulfoxonium methylide (130 mg, 0.480 mmol) in 2 mL of DMSO. After 20 min a solution of **15** (100 mg, 0.480 mmol) in 2 mL of DMSO was added and the mixture was stirred at room temperature for 18 h. Brine was added and the reaction extracted four times with a mixture Hexane/EtOAc=1:1. Combined organics were dried (Na_2SO_4), filtered and concentrated under vacuum. Purification of the crude residue by chromatography over SPE-Si column (2 g, grad. Hexane to Hexane/Acetone=95:5) gave the pure **13** (12.7 mg, 11%).^{1}H NMR (400 MHz) δ 3.97-4.30 (m, 1H), 3.45-3.70 (m, 2H), 2.88-3.03 (m, 1H), 1.99-2.13 (m, 1H), 1.67-1.84 (m, 2H), 1.57 (s, 9H), 1.47 (dd, 1H), 0.83 (t, 1H). UPLC/MS (ES+), m/z: found 223 [MH^+], $C_{12}H_{18}N_2O_2$ requires 222. Rt: 0.70 min.

Hydrolysis of 13. In a 5 mL microwave vial was added **13** dissolved in 1 mL of 37% aq. HCl. The mixture was heated to 150 °C for 10 minutes by mw irradiation. Volatiles were removed under vacuum to afford quantitatively the crude **1** as hydrochloride salt. ^{1}H NMR (400 MHz, D_2O) δ 4.27 (d, 1H), 3.11-3.19 (m, 1H), 3.04 (d, 1H), 2.66-2.77 (m, 1H), 2.22 (ddd, 1H), 1.91-2.03 (m, 2H), 1.66 (dd, 1H), 1.07 (dd, 1H).

Compound 16b. To a solution of δ-valerolactam (1.02 g, 10.3 mmol) in 20 mL of acetonitrile was added DMAP (122 mg, 1.0 mmol) followed by a solution of Boc_2O (2.50 g, 11.5 mmol) in 10 mL of the same solvent. The colorless reaction mixture was stirred at room temperature for 2 h. The resulting orange reaction mixture was concentrated *in vacuo* and the crude was taken up in Et_2O. The mixture was washed with 1N aq. HCl solution. The organic phase was dried (Na_2SO_4), filtered and concentrated under vacuum. Purification of the crude residue by chromatography over silica gel (Hexane/EtOAc= 6:4) afforded the pure **16b** (1.71 g, 83%) as a white crystalline powder. mp: 31-33 °C. ^{1}H NMR (400 MHz) δ 3.50-3.57 (m, 2H), 2.34-

2.44 (m, 2H), 1.66-1.76 (m, 4H), 1.40 (s, 9H). ^{13}C NMR (75 MHz) δ 171.0, 152.4, 82.4, 46.0, 34.6, 27.7, 22.5, 20.2. UPLC/MS (ES+), m/z: found 200 [MH^+], $C_{10}H_{17}NO_3$ requires 199. Rt: 0.61 min.

Compound 17. 2M LDA solution in heptane/THF (5.47 ml, 10.94 mmol) was slowly added at -78 ºC to a solution of **16b** (1.677 g, 8.42 mmol) in 10 mL of dry THF. The resulting solution was stirred at -78 °C for 30 min, then methyl chloroformate (0.978 ml, 12.62 mmol) was added and the reaction mixture was allowed to warm to room temperature over 3 h. Sat.d aq. NH_4Cl solution was added, followed by EtOAc and H_2O. The layers were separated and the aqueous phase was extracted twice with EtOAc. The combined organics were dried (Na_2SO_4), filtered and concentrated under vacuum. Purification of the crude residue by chromatography over SPE-Si column (25 g, Cyclohexane/Acetone= 95:5) afforded the pure **17** (1.310 g, 61%) as a pale yellow oil. ^{1}H NMR (400 MHz) δ 3.79 (s, 3H), 3.71 (t, 2H), 3.54 (dd, 1H), 2.05-2.26 (m, 2H), 1.92-2.04 (m, 1H), 1.77-1.89 (m, 1H), 1.54 (s, 9H).

Compound 18. 2M LDA solution in heptane/THF (3.31 ml, 6.62 mmol) was added dropwise at -78 ºC to a solution of **17** (1.31 g, 5.09 mmol) in 10 mL of dry THF. After 30 min a solution of PhSeCl (1.268 g, 6.62 mmol) in 3 mL of dry THF was added and stirring at -78 ºC was continued for further 1 h. The mixture was then allowed to warm to 0 ºC over 2 h. Sat.d aq. NH_4Cl solution and EtOAc were added and the layers were separated. The organic layer was further washed with brine, then dried (Na_2SO_4), filtered and concentrated under vacuum. Purification of the crude residue by chromatography over SPE-Si column (25 g, grad. Cyclohexane to Cyclohexane/AcOEt=9:1) gave the pure **18** (810 mg , 39%). ^{1}H NMR δ 7.65 (d, 2H), 7.41 (t, 1H), 7.27-7.32 (m, 2H), 3.76 (s, 3H), 3.54-3.66 (m, 1H), 3.41-3.52 (m, 1H), 2.28 (dt, 1H), 1.95-2.06 (m, 1H), 1.79-1.90 (m, 1H), 1.64-1.77 (m, 1H), 1.54 (s, 9H). UPLC/MS (ES+), m/z: found 357 [MH^+-56(Boc)], $C_{18}H_{23}NO_5Se$ requires 412. Rt: 0.82 min.

Compound 19. To a solution of **18** (310 mg, 0.75 mmol) in 4 mL of DCM at 0 °C was added dropwise a 30% aqueous H_2O_2 solution (5.76 mL, 57.0 mmol). The mixture was stirred at 0 °C for 4 h before being quenched with aq. 1N HCl solution. The aqueous layer was extracted three times with DCM and the combined organics were washed with a sat.d aq. $NaHCO_3$ solution, dried (Na_2SO_4), filtered and concentrated under vacuum. Compound **19** (160 mg, 84%) was obtained as yellow pale oil. ^{1}H NMR (400 MHz) δ 7.55 (t, *J*= 3.8 Hz, 1H), 3.88 (t, *J* = 6.2 Hz, 2 H), 3.85 (s, 3H), 2.54 (td, *J*= 6.3 Hz, *J*= 4.4 Hz, 2 H), 1.54 (s, 9 H).

Compound 20. NaH (28.4 mg, 1.185 mmol, 60% oil dispersion) was added portionwise to a solution of dimethylaminophenylsulfoxonium methylide (193 mg, 0.711 mmol) in 3 mL of DMSO. After 20 min a solution of **19** (121 mg, 0.474 mmol) in 3 mL of DMSO was added and the stirring was continued for further 3 h. Brine was added and the reaction extracted three times with EtOAc. The combined organics were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification of the crude residue by chromatography over SPE-Si column (2 g, grad. Hexane to Hexane/EtOAc=95:5) afforded the pure **20** (19.1 mg, 15%) as yellow pale oil. ^{1}H NMR spectrum of isolated compound was superimposible to that described below.

Compound 24. To a solution of **25** (5.28 g, 26.2 mmol) in 50 mL of chlorobenzene was added $Rh_2(OAc)_4$ (0.580 g, 1.31 mmol). The suspension was warmed to an internal temperature of +60 ºC and dimethyl diazomalonate (6.64 g, 42.0 mmol) was added dropwise keeping the Ti below +70 ºC. After 1 h, dimethyl diazomalonate (6.64 g, 42.0 mmol) was added dropwise keeping the Ti below +70 ºC and the reaction mixture was stirred for further 1 h. The suspension was cooled to room temperature, diluted with DCM and filtered from catalyst. Solvent was partially removed under vacuum; crude material was purified by flash chromatography (Cyclohexane/AcOEt 6:4) to afford cyclopropane derivative **24** (5.55 g, 64%) as off-white solid: mp =124-126 °C. ^{1}H NMR (400 MHz) δ 7.82-7.89 (m, 2H), 7.69-7.76 (m, 2H), 3.76-3.87 (m, 5H), 3.73 (s, 3H), 1.90-2.01 (m, 1H),

1.63-1.86 (m, 2H), 1.37-1.48 (m, 2H). ^{13}C NMR (100 MHz) δ 170.4, 168.3, 168.2, 134.0, 132.1, 123.2, 52.7, 52.6, 37.0, 33.7, 27.8, 25.9, 20.5. UPLC/MS (ES+), m/z: found 332 [MH^+], $C_{17}H_{17}NO_6$ requires 331. Rt: 0.68 min.

Compound 26. Hydrazine monohydrate (0.112 mL, 3.59 mmol) was added to a solution of diester **24** (1.08 g, 3.27 mmol) in 40 mL of MeOH and the mixture was heated to reflux. After 18 h the mixture was cooled to room temperature and the solvent was partially evaporated under vacuum. The residue was dissolved in DCM; precipitate was filtered off and washed with DCM. The organic layers were combined and the solvent evaporated under vacuum to afford **26** (420 mg, 64%) as a yellow pale oil. UPLC/MS (ES+), m/z: found 202 [MH^+], $C_7H_9NO_3$ requires 201. Rt: 0.34 min.

Compound 28. Hydrazine monohydrate (0.51 mL, 16.3 mmol) was added to a solution of **26** (1.08 g, 3.27 mmol) in 50 mL of MeOH and the mixture was heated to reflux. After 18 h the mixture was cooled to room temperature and the solvent was partially evaporated under vacuum. The residue was dissolved in DCM; precipitate was filtered off and washed with DCM. The organic layers were combined and the solvent evaporated under vacuum to afford the crude aminoacid **28** (405 mg, 80%) as tallow pale oil. ^{1}H NMR (400 MHz) δ 12.31 (s, 1H), 5.76-5.97 (m, 1H), 3.12-3.31 (m, 2H), 2.67 (t, 1H), 2.02-2.25 (m, 2H), 1.76 (dd, 1H), 1.68 (dd, 1H).

Compound 27. Hydrazine monohydrate (0.257 ml, 8.19 mmol) was added to a solution of diester **24** (1.085 g, 3.27 mmol) in 40 mL of MeOH and the mixture was heated to reflux. After 18 h the mixture was cooled to room temperature and the solvent was partially evaporated under vacuum. The residue was dissolved in DCM; precipitate was filtered off and washed with DCM. The organic layers were combined and the solvent evaporated under vacuum. Recrystallization of the crude

residue from Et_2O/MeOH= 95:5 afforded **27** (353 mg, 69%) as off-white solid: mp= 133-135 °C. ^{1}H NMR (400 MHz) δ 6.58 (bs, 1H), 3.77 (s, 3H), 3.21-3.32 (m, 1H), 3.04-3.15 (m, 1H), 2.09-2.21 (m, 1H), 1.91-2.00 (m, 2H), 1.88 (dd, *J*= 4.0 Hz, *J*= 8.0 Hz, 1H), 1.46 (t, *J*= 4.0 Hz, 1H). ^{13}C NMR (100 MHz) δ 170.5, 168.6, 52.7, 38.0, 28.0, 24.7, 20.3, 16.4. UPLC/MS (ES+), m/z: found 170 [MH^+], $C_8H_{11}NO_3$ requires 169. Rt: 0.37 min.

Compound 20. To a solution of **27** (400 mg, 2.364 mmol) in 15 ml of a 2:1 mixture toluene/ DCM was added DMAP (433 mg, 3.55 mmol) followed by Boc_2O (0.659 mL, 2.84 mmol). The resulting mixture was heated to reflux for 2 h, diluted with DCM and washed with brine. The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by flash chromatography (DCM) of the crude residue afforded **20** (0.57 g, 90%) as a yellow pale oil. ^{1}H NMR (400 MHz) δ 3.76-3.85 (m, 4H), 3.45-3.54 (m, 1H), 2.15-2.32 (m, 1H), 1.98-2.08 (m, 1H), 1.86-1.97 (m, 2H), 1.52-1.57 (m, 9H), 1.49 (t, *J*= 4.0 Hz, 1H). ^{13}C NMR (100 MHz) δ 170.2, 167.1, 152.8, 83.5, 52.8, 42.7, 31.2, 28.0, 25.5, 22.6, 19.0. UPLC/MS (ES+), m/z: found 270 [MH^+], $C_{13}H_{19}NO_5$ requires 269. Rt: 0.63 min.

Compound 22. 1.0 M Superhydride solution (2.495 ml, 2.495 mmol) was added over 30 min to a solution of lactam **20** (0.56 g, 2.08 mmol) in 5 mL of THF cooled to -10 ºC. After 2 h at 0 ºC, Superhydride (0.500 mL) was added and the solution was stirred for further 2 h. The reaction mixture was quenched with H_2O and extracted three times with EtOAc. The organic layer was dried (Na_2SO_4), filtered and concentrated under vacuum. Purification by flash chromatography (Cyclohexane/EtOAc=6:4) afforded **21** (0.36 g, 64%) as unseparable mixture of diastereoisomeric aminals.

A solution of aminals **21** (250 mg, 0.92 mmol) and triethylsilane (0.147 mL, 0.921 mmol) in 8 mL of DCM was cooled to -78 ºC and BF_3*OEt_2 (0.117 mL, 0.921 mmol) was then added dropwise under nitrogen atmosphere. After 30 min, more

triethylsilane (0.147 mL, 0.921 mmol) and BF_3*Et_2O (0.117 mL, 0.921 mmol) were added and the resulting mixture was stirred for further 1.5 h at - 78 ºC. The reaction mixture was warmed to room temperature, diluted with DCM and washed with H_2O. The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by flash chromatography (Cyclohexane/EtOAc=95:5) of the crude residue afforded **22** (0.19 g, 36% for two steps) as yellow pale oil. ^{1}H NMR (400 MHz) δ 3.82-4.12 (m, 2H), 3.66-3.74 (m, 3H), 3.51 (bs, 1H), 2.87-3.03 (m, 1H), 1.94 - 2.08 (m, 1H), 1.66-1.86 (m, 2H), 1.44-1.53 (m, 9H), 1.41 (dd, *J*= 4.0 Hz, *J*= 12.0 Hz, 1H), 0.75 (dd, *J*= 4.0 Hz, *J*= 8.0 Hz, 1H). ESI *m/z* (relative intensity) 278 ([M+ Na]$^+$, 63), 200 (100), 182 (86).

Compound 23. To a solution of LiOH (33.8 mg, 1.410 mmol) in 2 mL of H_2O was added ester **22** (180 mg, 0.705 mmol) diluted in 3 mL of a mixture MeOH/THF=10:1 and the mixture was stirred at room temperature. After 3 h the reaction was complete. The reaction mixture was acidificated with 1N aq. HCl solution, diluted with H_2O and backextracted with DCM (3 x 30ml). The organic layer was dried (Na_2SO_4), filtered and concentrated under vacuum to afford the awaited compound **23** (146 mg, 86%). ^{1}H NMR (400 MHz) δ 3.94 (bs, 2 H), 3.72-3.81 (m, 1H), 3.47 (bs, 1H), 2.94-3.04 (m, 1H), 1.96-2.10 (m, 1H), 1.72-1.85 (m, 2H), 1.42-1.54 (m, 10H), 0.83 (dd, 1H). ^{13}C NMR (100 MHz) δ 179.8, 156.0, 79.9, 44.7, 38.5, 28.0, 26.7, 22.0, 20.9, 19.8. ESI *m/z* (relative intensity) 264 ([M+ Na]$^+$, 61), 186 (100).

Compound 1. Compound **23** (100 mg, 0.415 mmol) was dissolved in 4 mL of a mixture 3:1 DCM/TFA. The solution was stirred at 0 ºC; after 45 min UPLC analysis showed that the reaction was complete. Solvents were evaporated under vacuum to afford compound **1** (95.2 mg, 90%) as trifluoroacetic salt, which did not need any purification. ^{1}H NMR (400 MHz, D_2O) δ 4.27 (d, 1H), 3.11-3.19 (m, 1H), 3.04 (d, 1H), 2.66-2.77 (m, 1H), 2.22 (ddd, 1H), 1.91-2.03 (m, 2H), 1.66 (dd, 1H),

1.07 (dd, 1H). ^{13}C NMR (100 MHz) δ 176.6, 43.1, 38.1, 30.3, 21.2, 19.9, 18.6. UPLC/MS (ES+), m/z: found 142 [MH^+], $C_7H_{11}NO_2$ requires 141. Rt: 0.21 min.

Compound 31b. To a solution of ethyl 4-aminobutanoate (**31a**) (3.76 g, 22.43 mmol) and TEA (3.12 mL, 22.43 mmol) in 100 mL of acetonitrile was added Boc_2O (5.38 g, 24.67 mmol) in one portion. The mixture was stirred 1.5 h at room temperature. Solvent was evaporated under vacuum and the residue was purified by SPE-Si column (50 g, grad. Cyclohexane to Cyclohexane/EtOAc=7:3) to afford the protected amine **31b** (5.10 g, quantitative). ^{1}H NMR (400 MHz) δ 4.63 (bs, 1H), 4.15 (q, 2H), 3.03-3.31 (m, 2H), 2.36 (t, 2H), 1.73-1.99 (m, 2H), 1.45 (s, 9H), 1.27 (t, 3H). UPLC/MS (ES+), m/z: found 232 [MH^+], $C_{11}H_{21}NO_4$ requires 231. Rt: 0.68 min.

Compound 32. Amine **31b** (5.10 g, 22.07 mmol) was dissolved in 80 mL of dry DMF and the resulting solution was cooled to 0 °C. NaH (2.91 g, 121 mmol) was added; after 30 min AllBr (6.29 mL, 72.6 mmol) was added and the mixture was stirred under nitrogen atmosphere at room temperature for 1 h. Sat.d aq. NH_4Cl solution was added and the mixture was extracted twice with EtOAc. Combined organic layers were dried (Na_2SO_4), filtered and concentrated under vacuum. Crude material was purified by SPE-Si column (50 g, grad. Cyclohexane to Cyclohexane/EtOAc=9:1) to afford **32** (1.734 g, 26%). ^{1}H NMR (400 MHz) δ 5.60-5.96 (m, 1H), 4.98-5.24 (m, 2H), 4.13 (q, 2H), 3.81 (bs, 2H), 3.22 (bs, 2H), 2.29 (t, 2H), 1.77-1.93 (m, 2H), 1.46 (s, 9H), 1.26 (t, 3H). UPLC/MS (ES+), m/z: found 172 [MH^+-100(Boc)], $C_{14}H_{25}NO_4$ requires 271. Rt: 0.81 min.

Compound 33a. 2M LDA solution in heptane/THF (5.33 ml, 9.59 mmol) was added *via* a syringe to a solution of **32** (1.73 g, 6.39 mmol) in 50 mL of dry THF cooled to – 78 °C. After being stirred for 30 min at – 78 °C, the reaction mixture was treated with finely crushed iodine (2.11 g, 8.31 mmol) crystals and then stirred at – 78 °C

for 1 h. Brine was added to the solution; aqueous layer was extracted with EtOAc. Combined organic layers were dried (Na_2SO_4), filtered and concentrated under vacuum. Purification by SPE-Si column (25 g, grad. Cyclohexane to Cyclohexane/ EtOAc=95:5) of the crude residue afforded the pure **33a** (1.44 g, 57%). ^{1}H NMR (400 MHz) δ 5.64-5.92 (m, 1H), 5.02-5.22 (m, 2H), 4.32 (t, 1H), 4.22 (q, 2H), 3.80 (bs, 2H), 3.25-3.44 (m, 1H), 3.11-3.26 (m, 1H), 2.01-2.40 (m, 2H), 1.47 (s, 9H), 1.30 (t, 3H). UPLC/MS (ES+), m/z: found 298 [MH^+-100(Boc)], $C_{14}H_{24}INO_4$ requires 397. Rt: 0.92 min.

Compound 30. To a solution of **33a** (1.04 g, 2.61 mmol) in 6 mL of DCM cooled at 0 °C was added TFA (2 mL, 26.0 mmol). The reaction mixture was stirred at 0 °C for 1 h, then volatiles were evaporated under vacuum. Crude residue was dissolved in 3 mL of acetone and potassium carbonate (0.722 g, 5.22 mmol) and phenyl chloroformate (0.426 mL, 3.39 mmol) were added. The reaction mixture was stirred at room temperature for 2 h. Solvent was evaporated under vacuum; the residue was dissolved in H_2O. The aqueous layer was backextracted twice with DCM. Combined organic layers were dried (Na_2SO_4), filtered and concentrated under vacuum. Purification of the crude residue by SPE-Si column (20 g, grad. Cyclohexane to Cyclohexane/EtOAc=85:15) afforded the pure **30** (544 mg, 50%). ^{1}H NMR (400 MHz) δ 7.38 (t, 2H), 7.22 (t, 1H), 7.05-7.18 (m, 2H), 5.74-6.10 (m, 1H), 5.18-5.36 (m, 2H), 4.32-4.59 (m, 1H), 4.08-4.33 (m, 2H), 3.89-4.10 (m, 2H), 3.19-3.72 (m, 2H), 2.40-2.59 (m, 1H), 2.15-2.38 (m, 1H), 1.28 (t, 3H). UPLC/MS (ES+), m/z: found 418 [MH^+], $C_{16}H_{20}INO_4$ requires 417. Rt: 0.87 min.

Compound 29. To a solution of palladium tetrakis (0.085 g, 0.074 mmol) in 10 mL of HMPA was added **30** (1.06 g, 2.54 mmol) and Proton-Sponge (0.987 g, 4.60 mmol) at room temperature under nitrogen atmosphere. The solution was heated to 80 °C for 2 h. EtOAc was added; the organic layer was washed with 1N aq. HCl solution and brine, dried (Na_2SO_4), filtered and concentrated under vacuum. The crude residue was purified by SPE-Si column (20 g, grad. Cyclohexane to

Cyclohexane/EtOAc=85:15) to give **29** (483.3 mg, 46%) as diastereoisomeric mixture. UPLC/MS (ES+), m/z: found 418 [MH^+], $C_{16}H_{20}INO_4$ requires 417. Rt: 0.75 min.

Compound 35. To a solution of **29** (227 mg, 0.544 mmol) in 4 mL dry DMSO was added DBU (0.172 mL, 1.143 mmol). Reaction mixture was stirred at 90 °C over night. EtOAc was added to the solution: the organic layer was washed with 1N aq. HCl solution and brine, dried (Na_2SO_4), filtered and concentrated under vacuum. Purification of the crude residue by SPE-Si column (5 g, grad. Cyclohexane to Cyclohexane/ EtOAc=9:1) gave the pure **35** (67.3 mg, 43%). ^{1}H NMR (400 MHz) δ 7.37 (t, 2H), 7.21 (t, 1H), 7.11 (dd, 2H), 4.16 (q, *J*= 7.4 Hz, 2H), 3.86-4.13 (m, 1H), 3.52-3.88 (m, 2H), 3.08-3.40 (m, 1H), 2.57-2.79 (m, 1H), 1.85-1.98 (m, 1H), 1.82 (bs, 1H), 1.47-1.58 (m, 1H), 1.28 (t, *J*= 7.4 Hz, 3H), 0.89 (bs, 1H).

Compound 36. To a solution of LiOH (15 mg, 0.626 mmol) in 1 mL of H_2O was added ester **35** (46 mg, 0.171 mmol) dissolved in 1.1 mL of a mixture MeOH/THF=10:1. The mixture was stirred at room temperature for 2 h, then volatiles were evaporated under vacuum. Residue was dissolved in DCM and organic layer was washed with 1N aq. HCl solution, then dried (Na_2SO_4), filtered and evaporated under vacuum to afford the crude acid **36** (34.7 mg, 84%), that did not need any purification. ^{1}H NMR (400 MHz) δ 7.37 (t, 2H), 7.21 (t, 1H), 7.11 (dd, 2H), 3.89 (dd, 1H), 3.35-3.63 (m, 2H), 2.89-3.06 (m, 1H), 2.53 (dt, 1H), 1.71-1.87 (m, 2H), 1.54 (dd, 1H), 0.82 (dd, 1H).

Compound 2. LiOH (45 mg, 1.8 mmol) was added to a solution of acid 36 (15 mg, 0.051 mmol) in 3 mL of toluene. The reaction mixture was refluxed for 18 h. After cooling to room temperature, the suspension was filtered from salts and volatiles evaporated under vacuum to afford the crude amino acid **2** (6.9 mg, quantitative),

which did not need any purification. UPLC/MS (ES+), m/z: found 142 [MH^+], $C_7H_{11}NO_2$ requires 141. Rt: 0.17 min.

Compound 42. A solution of benzylamine (7.56 mL, 69.2 mmol), 4-bromo-1-butene (1.50 mL, 14.81 mmol) and EtOH (10 mL) was deoxygenated with nitrogen and treated with NaI (10 mg, 0.067 mmol) at room temperature. The resulting solution was heated at 92 ºC for 3 h, then cooled to room temperature and partitioned between DCM and 1N aq. NaOH solution. The layers were separated and the aqueous phase was extracted with DCM. The combined organic layers were dried (Na_2SO_4), filtered and concentrated under vacuum. The crude residue was purified by SPE-Si column (25 g, Cyclohexane/EtOAc=1:1) to afford the pure **42** (2.39 g, quantitative). ^{1}H NMR (400 MHz) δ 7.31-7.40 (m, 4H), 7.22-7.31 (m, 1H), 5.72-5.89 (m, 1H), 4.98-5.18 (m, 2H), 3.82 (s, 2H), 2.73 (t, 2H), 2.25-2.36 (m, 2H).

Compound 41. To a solution of amine **42** (1.49 g, 9.24 mmol) in 80 mL of acetonitrile were added in sequence potassium carbonate (1.92 g, 13.86 mmol) and bromoacetonitrile (1.93 mL, 27.7 mmol). The reaction mixture was refluxed for 4 h. The mixture was concentrated under vacuum. The residue was dissolved in DCM and organic layer was washed with H_2O, then dried (Na_2SO_4), filtered and concentrated under vacuum. Purification of the crude residue by SPE-Si column (25 g, grad. Cyclohexane/EtOAc=7:3 to Cyclohexane/EtOAc=3:7) gave the pure **41** (1.759 g, 95%). ^{1}H NMR (400 MHz) δ 7.28-7.41 (m, 5H), 5.77-5.92 (m, 1H), 5.04-5.18 (m, 2H), 3.70 (s, 2H), 3.32-3.57 (m, 2H), 2.66-2.80 (m, 2H), 2.28-2.43 (m, 2H). UPLC/MS (ES+), m/z: found 201 [MH^+], $C_{13}H_{16}N_2$ requires 200. Rt: 0.80 min.

Compound 37. To a solution of amine **41** (500 mg, 2.496 mmol) in 20 mL of Et_2O were added successively Ti(O*i*-Pr)$_4$ (0.805 mL, 2.75 mmol) and cyclohexylmagnesium chloride (2.75 mL, 5.49 mmol). After stirring for 45 min, BF_3*Et_2O (0.63 mL, 4.99 mmol) was added at once. Stirring was continued over a

period of 45 min. 1N aq. NaOH solution was added and the mixture was extracted with Et_2O. The combined ether layers were dried (Na_2SO_4), filtered and concentrated under vacuum. Purification of the crude residue by SPE-Si column (10 g, grad. Cyclohexane/EtOAc=9:1 to EtOAc, then Acetone) afforded the pure **37** (156.3 mg, 31%). ^{1}H NMR (400 MHz) δ ppm 7.21-7.38 (m, 5H), 3.43-3.48 (m, 2H), 3.01 (d, *J*= 10.4 Hz, 1H), 2.37-2.51 (m, 1H), 2.22 (d, *J*= 10.4 Hz, 1H), 1.95 (dt, *J*= 17.5 Hz, *J*= 5.2 Hz, 2H), 1.53-1.76 (m, 3H), 0.91-1.06 (m, 1H), 0.62-0.74 (m, 2H). UPLC/MS (ES+), m/z: found 203 [MH^+], $C_{13}H_{18}N_2$ requires 202. Rt: 0.23 min.

Ester hydrolysis. To a solution of LiOH (1.41 mmol) in 2 mL H_2O was added ester (0.705 mmol) diluted in a mixture MeOH/THF=10:1 and the mixture was stirred at room temperature. After 3 h the reaction was complete. The reaction mixture was acidificated with 1N aq. HCl solution, diluted with H_2O and backextracted with DCM. The organic layer was dried (Na_2SO_4), filtered and concentrated under vacuum to afford awaited carboxylic acid which was used for successive step without further purification.

Data for compound 44. quantitative, ^{1}H NMR (400 MHz) δ 3.72 (bd, 1H), 3.64 (bd, 1H), 3.38-3.59 (m, 2H), 2.12-2.17 (m, 2H), 1.52 (t, 1H), 1.44 (s, 9H). UPLC/MS (ES+), m/z: found 228 [MH^+], $C_{11}H_{17}NO_4$ requires 227. Rt: 0.58 min.

Data for compound 47. quantitative, ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.72 (d, 1H), 3.45-3.54 (m, 1H), 3.21-3.32 (m, 1H), 2.88-2.99 (m, 1H), 1.81-1.96 (m, 1H), 1.61-1.71 (m, 1H), 1.51-1.59 (m, 2H), 1.39 (s, 9H), 1.27 (t, 1H). UPLC/MS (ES+), m/z: found 242 [MH^+], $C_{12}H_{19}NO_4$ requires 241. Rt: 0.61 min.

General procedure for Curtius rearrangement. Carboxylic acid (2.0 mmol), TEA (6.03 mmol) and diphenyl azidophosphate (3.01 mmol) were dissolved in 15 mL of toluene and the resulting solution was heated to reflux for 2 h. After cooling to room temperature, benzyl alcohol (10.0 mmol) was added. The solution was heated to reflux for 2 h, then diluted with 50 mL of EtOAc. Organic layer was washed with

1N aq. HCl solution and brine, dried (Na_2SO_4), filtered and concentrated under vacuum. Purification of the crude residue by SPE-Si column (20 g, grad. Cyclohexane to Cyclohexane/EtOAc=8:2) gave the pure *N*-Cbz protected amine.

Data for compound 45. 62% yield, ^{1}H NMR (400 MHz) δ 7.28-7.43 (m, 5H), 5.12 (bs, 2H), 3.63-3.75 (m, 2H), 3.34-3.45 (m, 2H), 2.34-2.41 (m, 1H), 1.66-1.79 (m, 2H), 1.44 (s, 9H). UPLC/MS (ES+), m/z: found 277 [MH^+-56 (Boc)] $C_{18}H_{24}N_2O_4$ requires 332. Rt: 0.75 min.

Data for compound 48. 42% yield, ^{1}H NMR (400 MHz) δ 7.28-7.41 (m, 5H), 5.12 (bs, 2H), 3.88 (d, 1H), 3.46-3.64 (m, 1H), 3.22-3.45 (m, 1H), 2.85-3.06 (m, 1H), 2.25-2.41 (m, 1H), 1.90-2.07 (m, 1H), 1.62-1.90 (m, 1H), 1.46 (s, 9H), 1.33-1.52 (m, 1H), 1.11-1.32 (m, 1H). UPLC/MS (ES+), m/z: found 347 [MH^+], $C_{19}H_{26}N_2O_4$ requires 346. Rt: 0.78 min.

Data for compound 50. 30% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26-7.42 (m, 5H), 4.92-5.19 (m, 2H), 3.59 (dd, 1H), 3.15-3.42 (m, 2H), 2.68-2.84 (m, 1H), 2.36-2.49 (m, 1H), 1.74-1.90 (m, 1H), 1.40-1.59 (m, 1H), 1.37 (s, 9H), 1.05-1.30 (m, 2H). UPLC/MS (ES+), m/z: found 347 [MH^+], $C_{19}H_{26}N_2O_4$ requires 346. Rt: 0.79 min.

General procedure of hydrogenation. Reactions were performed in the H-Cube™. A CatCart™ was washed with only MeOH, and a 2 mL solution of amine was flowed through the catalyst at 1 mL/min at room temperature and 1 bar until TLC monitoring showed the reaction was complete. The resulting solution was concentrated under vacuum to give the desired *N*-Boc protected bis-amine.

Data for compound 38. quantitative, ^{1}H NMR (400 MHz, C_6D_6) δ 1.10-1.25 (m, 2H), 1.46 (s, 9H), 1.68 (t, *J*= 2.0 Hz, 1H), 1.71 (bs, 2 H), 3.10 (dd, *J*= 3.7 Hz, *J*= 10.4 Hz, 1H), 3.25 (dd, *J*=3.9 Hz, *J*= 10.9 Hz, 1H), 3.38 (d, *J*= 10.5 Hz, 1H), 3.65 ppm (d, *J*=10.9 Hz, 1H). ^{13}C NMR (100 MHz) δ 25.4, 26.2, 28.5, 35.5, 48.0, 78.7, 154.2.

Data for compound 39. quantitative, ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.64 (d, 1H), 3.39-3.51 (m, 1H), 3.11-3.29 (m, 1H), 2.74-2.93 (m, 1H), 1.95 (bs, 1H), 1.28-1.42

(m, 1H), 1.46-1.62 (m, 1H), 1.39 (s, 9H), 0.92-1.11 (m, 2H). UPLC/MS (ES+), m/z: found 157 [MH^+-56(Boc)], $C_{11}H_{20}N_2O_2$ requires 212. Rt: 0.52 min.

Data for compound 40. quantitative, ^{1}H NMR (400 MHz) δ 3.32-3.81 (m, 4H), 2.71-2.87 (m, 1H), 2.34 (t, 1H), 1.84-1.97 (m, 1H), 1.55-1.68 (m, 1H), 1.44 (s, 9H), 0.99-1.11 (m, 1H), 0.81-0.96 (m, 1H). UPLC/MS (ES+), m/z: found 157 [MH^+-56(Boc)], $C_{11}H_{20}N_2O_2$ requires 212. Rt: 0.44 min.

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4 CONCLUSIONS AND FUTURE PERSPECTIVES

Medicinal chemistry focuses on the aspect related to the structural design, synthesis and identification of therapeutically interesting compounds, *i.e.* pharmaceuticals, as well as the molecular reasons of their mechanism of action, including the understanding of the factors involved in the structure-activity relationships, absorption, distribution, metabolism, elimination and toxicity.

The molecular recognition of a drug in the biophase is resultant from intermolecular interactions with biomacromolecules, involving electrostatic forces, dispersion and hydrophobic interactions, hydrogen and covalent bonds. The spatial arrangement of structural subunits of micromolecule, responsible for these interactions with the complementary sites of the target bioreceptor, includes the pharmacophoric requisites for its recognition, and defines qualitatively the affinity and selectivity degree of drug-bioreceptor complex.

In the recent literature, it is noticed great interest in the identification of molecular frameworks, which correspond to the minimum structural subunit, usual in several drugs or lead-compounds, able of providing ligand points for more than one type of bioreceptor. Since Evans first introduced the concept of privileged structure, privileged-based drug discovery has emerged as a fruitful approach in medicinal chemistry. Privileged scaffolds increase hit rates for biological targets of interest, leading to the discovery of other biologically active targets and generating leads with enhanced drug-like properties. Consequently, medicinal chemists value privileged structures as core scaffolds for viable starting points in exploration design and synthesis.

Despite the identification of numerous recurring molecular frameworks in bioactive molecules, there is a restricted availability of privileged structures. Toward this concern, a subject of great fashination and importance, dealing with the need to identify novel chemotypes, have been addressed the studies conducted during this PhD thesis. The selected structures might represent potential replacements of

frequently occurring structural motifs. The development of efficient methodologies for the synthesis of the identified compounds has provided the suitable tools to open up an investigation about the behaviour of such a kind of molecules towards biological systems.

Inspired by the literature reports describing numerous bioactive piperidine and piperazine Ph- and Bn-derivatives, as well as by the fundamental knowledge according to which several compounds of these series are included in drugs with central and peripheral neurotropic effects, a general route for the synthesis of alternative bis-amines (Figure 1) has been finely tuned (Chapter 2).

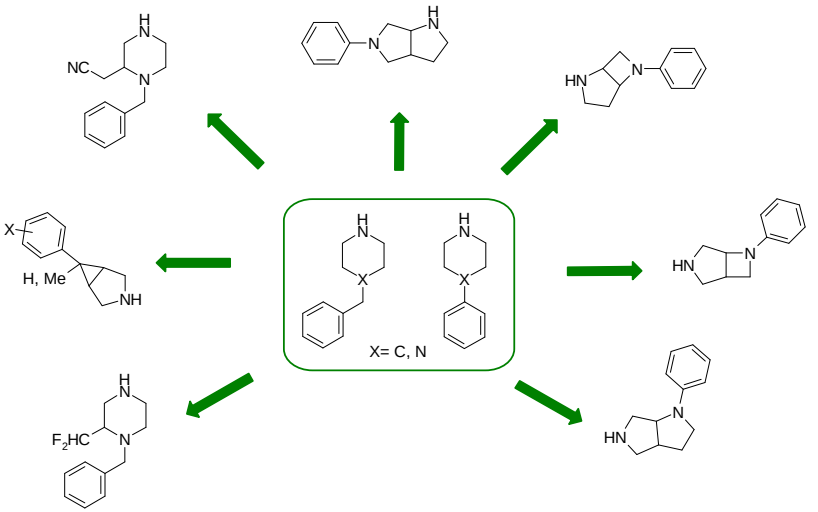

Figure 1

In the context of introducing conformational constraint one strategy may be the construction of cyclic systems, and, in particular, cyclopropyl analogues of non cyclopropyl containing structures. Following this approach, a program aimed to synthesize constrained bicyclic[x.1.0] heterocycles (Figure 2) has been started (Chapter 3).

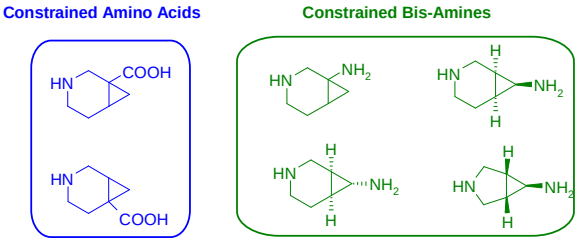

Figure 2

The chemistry presented here has offered high-yielding and scalable routes for the preparation of a wide range of suitably protected building blocks. Because of the *de novo* approach of such synthetic methodologies, the strategies have demonstrated to be highly convergent as well, minimizing the number of synthetic steps. Given the possibility to have easy access to such molecules in large amount, further endeavours could be addressed to their employ as scaffolds for the synthesis of more complex structures for enhancing our understanding of their role in biological environments.