Synthesis of conformationally restricted amino acids and their use in the preparation of biologically active peptides and peptidomimetics

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SYNTHESIS OF CONFORMATIONAL RESTRICTED AMINO ACIDS AND THEIR USE IN THE PREPARATION OF BIOLOGICALLY ACTIVE PEPTIDES AND PEPTIDOMIMETICS

Tesi del Dottor

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To raise new questions, new possibilities, to regard old problems from a new angle, requires creative imagination and marks real advance in science.

Albert Einstein

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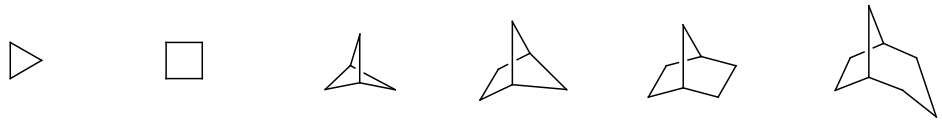
1 Azabicyclo amino acids

1.1 Introduction

The synthesis and the use of conformationally rigid unnatural compounds have attracted the chemists' interest since long time.[1, 2] It is commonly assumed that the restriction of the flexibility by structural modifications, such as the insertion of a cyclic scaffold, is an efficient tool to reduce the possible structural conformations of a molecule. Some examples of these rigid scaffolds are reported here below.

Figure 1



1 **2** **3** **4** **5** **6**

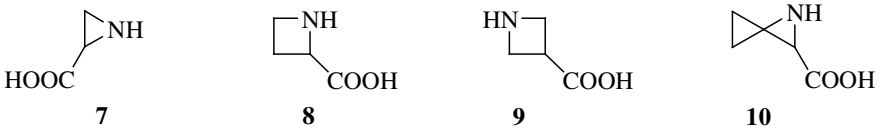
In particular non-natural amino acids containing the rigid backbone moieties indicated in *figure 1* are particularly interesting. With respect to the natural constrained amino acids, *i.e.* proline, rigid amino acids are characterized by "fixed" bond length and torsional angles. Furthermore, cyclic amino acids (CAAs) are versatile molecules as they are used in a wide range of applications in different fields. As single molecules, they often possess biological activity and in many cases they are characterized by higher efficiency, stability and receptor selectivity with respect to the natural ones. Their features are particularly profitable, when these unnatural scaffolds are inserted in peptides, as they can improve the selectivity toward a receptor, the stability to enzymatic hydrolysis and so the bioavailability. Another application field of CAAs is their use as rigid ligands in organometallic catalysis and also as organocatalysts into asymmetric reactions.

In the past decades a huge numbers of constrained amino acids were synthesized. Since the focus of our researches were the preparation of CAAs containing an azabicyclo scaffold, (see paragraph 2) in the following paragraphs are presented selected examples of the constrained amino acids containing an aza-ring and some of their applications.

1.2 3 and 4 member azacyclic amino acids.

This class of compounds is represented by synthetic and naturally occurring products holding a three and four member rings[3] responsible of the conformational rigidity. Some examples of these azacyclo compounds are reported in *figure 2*.

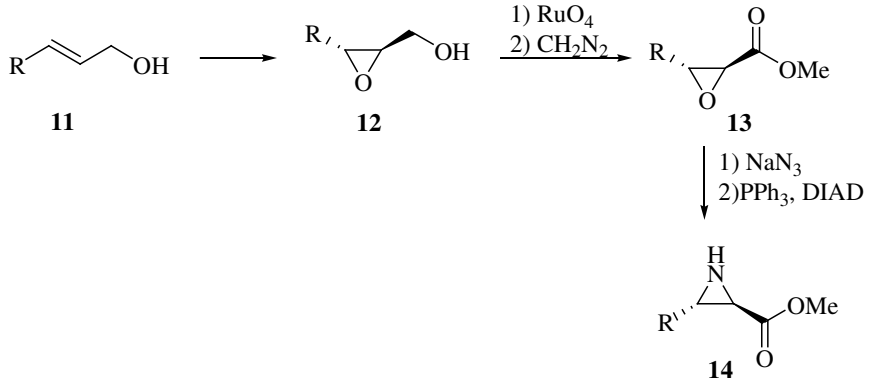
Figure 2



7 **8** **9** **10**

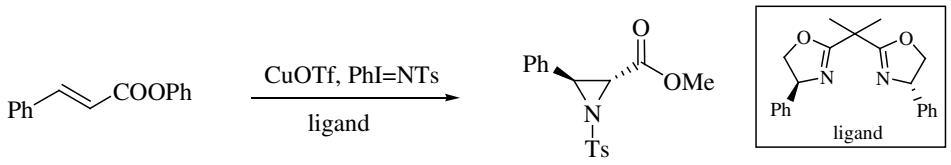
Aziridine constrained amino acids **7** can be considered as fixed glycine derivatives. Chiral aziridinecarboxylic acids **7** are prepared through an asymmetric synthesis. Some examples are reported below. The key stage in these transformations is the Sharpless catalytic asymmetric epoxidation (*Scheme 1*), obtaining the intermediate **12** than converted in few steps in the compound **14**. Alternativelly an asymmetric aziridination of a C=C double bond was performed to give compound **16**. (*Scheme 2*)

Scheme 1



11 **12** **13** **14**

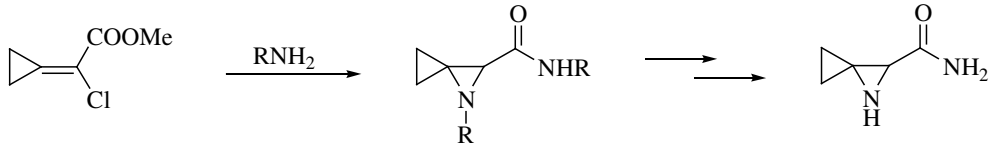
Scheme 2



15 **16** **17**

1-Azaspiro[2.2]pentane-2-carboxylic acid **20** is obtained through the reaction of methyl(chloro)cyclopropylideneacetato with primary amines or ammonia (*Scheme 3*).

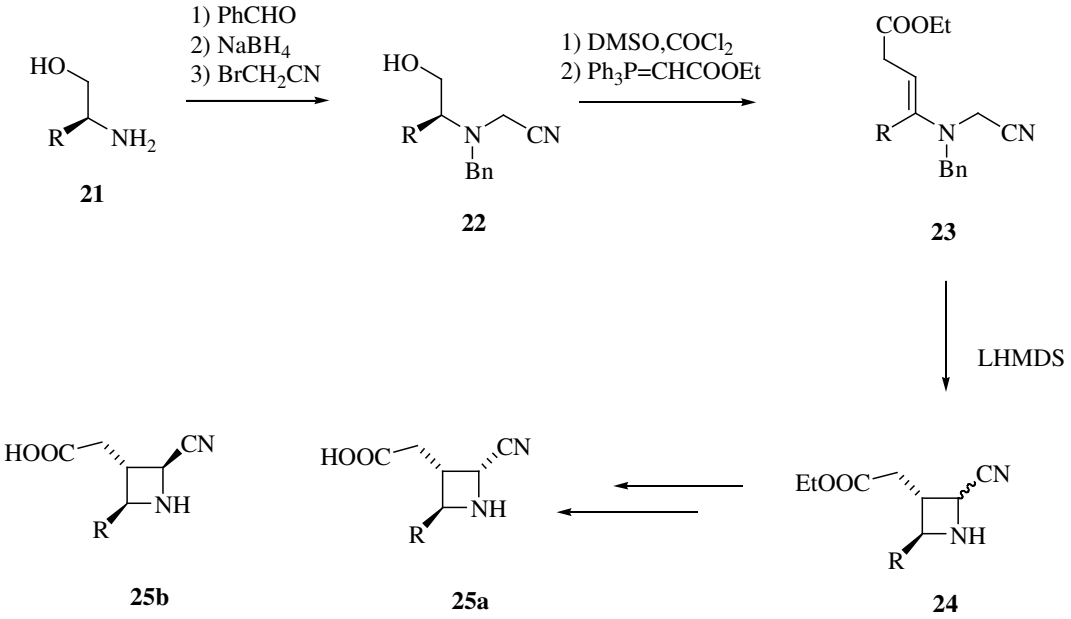
Scheme 3



18 **19** **20**

Four member aza-rings were initially synthesized in racemic form starting from γ-butyrrolactone.[4] Since now, there are only few efficient methods for the preparation of these derivatives in enantiometically pure forms. The first example is reported in the 2003 year. The aza-cyclo compounds **25a,b** were obtained starting from amino alcohol **21** as shown in the scheme 4.[5]

Scheme 4



LHMDS is lithium hexamethylendisilazanide

The development of new methods for the synthesis of these compounds was encouraged for their potential use both as precursors of alkaloids, β-lactam antibiotics and other biological active compounds as well as constrained analogues of natural amino acids which are used in the synthesis of peptidomimetics.[6]

1.3 Bi- and polycyclic conformationally rigid amino acids

Amino acids discussed in the following sections represent bi- or polyclic structures, in which the nitrogen atom is incorporate into the ring. Consequently, these compounds have the φ and χ fixed torsion angles, inducing particular conformations in peptidomimetics.

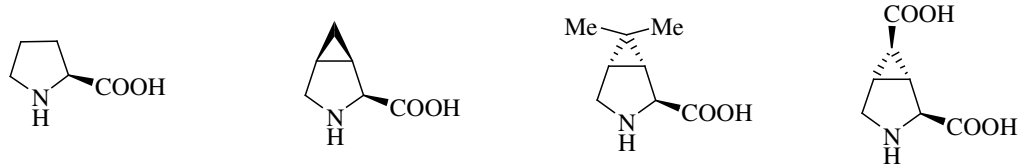
1.3.1 Conformationally rigid bicyclic proline rings

Proline **26** occupies a special place among proteinogenic amino acids due to its unique structure. In this amino acid, the nitrogen atom is incorporated in a five member ring, which gives rise of conformational changes with respect to other natural amino acids. These features are more pronounced for amino acids with a further strained ring.

In order to confer conformational rigidity to the five member-ring, it is necessary to introduce a bridges with a length of 1 or 2 atoms into the molecule. When such a bridge links two adjacent or non adjacent positions in the ring, proline methanologues are formed. Examples of this classes of compounds are reported in *Figures 3 and 4*.

Cis-3,4 propane–L-proline **27** is found in nature. Other derivatives such as amino acids **28-29** were prepared and tested as interesting organocatalysts and also as buildings blocks in peptide sequences.

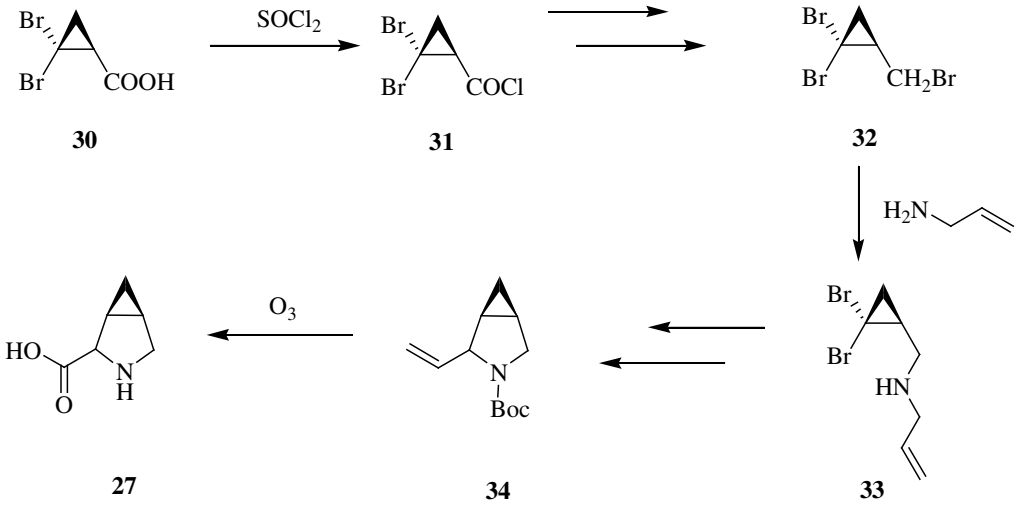
Figure 3



26 **27** **28** **29**

As example of this class, the synthesis of compound **27**[7] is reported below (*scheme 5*). The key reaction step is the cyclization of compound **33** to compound **34** than easily oxidized to compound **27**

Scheme 5

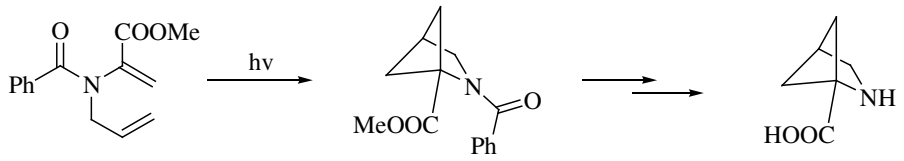


2-Azabicyclo[2.1.1]hexane-1-carboxylic acid **36** was first isolated in 1980 from the seeds of legume *Ateleia Herbert smithii* and more recently from the plant *Bocoa Alterna.* It plays an important role in protecting the seeds from insect predation.

Figure 4

Different synthetic methods are possible to access to this compound taking advantage of a very efficient method such as the photochemical cyclization of compound **37** to **38**, then hydrolyzed to the amino acid **36**.[8] (*Scheme 6*)

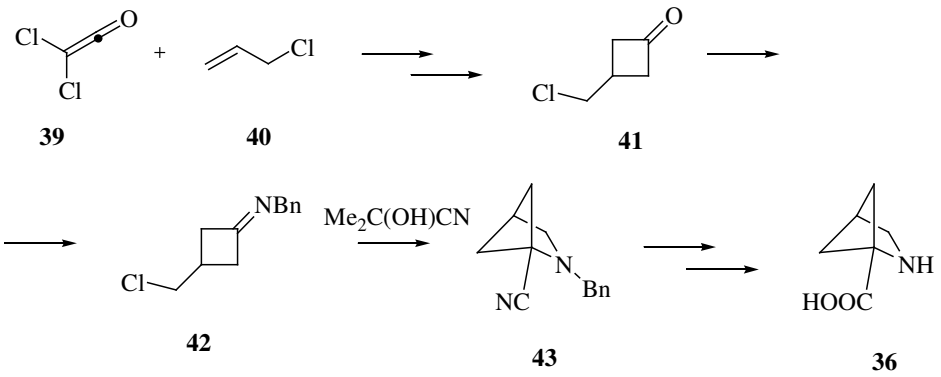
Scheme 6



37 **38** **36**

Alternatively, a new method for the synthesis of the 2,4-methanoproline **36** includes a [2+2]-cycloaddition reaction between dichloroketene **39** and allylchloride **40** to give **41** then transformed into the imine **42.** After reaction with 2-hydroxy-2-methylpropanenitrile, a cyclization occurred giving **43**, the precursor of amino acid **36**.[9]

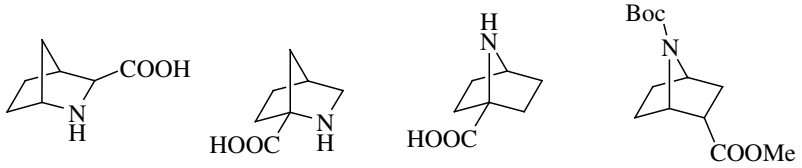
Scheme 7



39 **40** **41** **42** **43** **36**

Azabicyclo[2.2.1]heptane carboxylic derivatives contaning a five member ring are very interesting compounds widely employed in the synthesis of several conformational rigid peptidomimetics (see sections 1.5).[10]

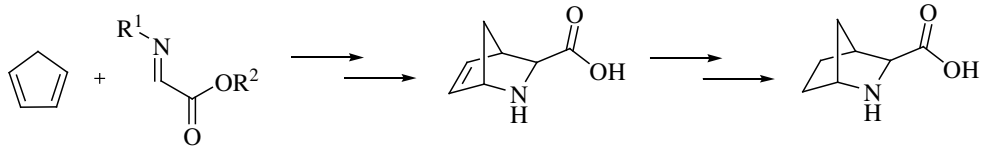
Figure 5



44 **45** **46** **47**

Despite the apparent simplicity of the preparation of 2-azabicyclo[2.2.1]heptane-3-caboxylic acid derivatives, there are only few known representatives of this class. The most utilized method is a Diels-Alder reaction of cyclopentadiene **48** and imine **49**, generated *in situ*. This method allowed to prepare compound **44** in gram scale.[11]

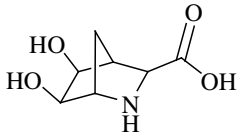
Scheme 8



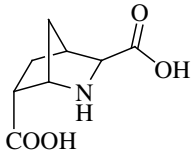
48 **49** **50** **44**

As reported in *figure 6*, it is also possible to generate a series of the above derivative by functionalization of the double bond of the primary cycloadduct **50**.

Figure 6



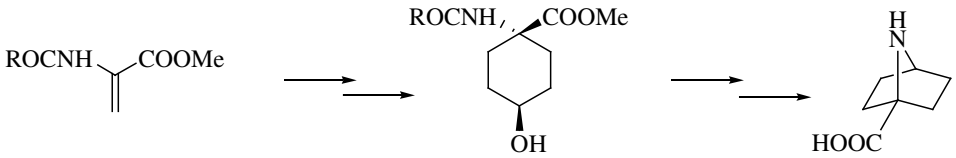
51



52

Concerning the 7-azabicyclo[2.2.1]heptane-3-carboxylic acid **46,** an intramolecular cyclization reaction starting from functionalized cyclohexane derivatives is the useful approach for its preparation.[12] One example is reported below.

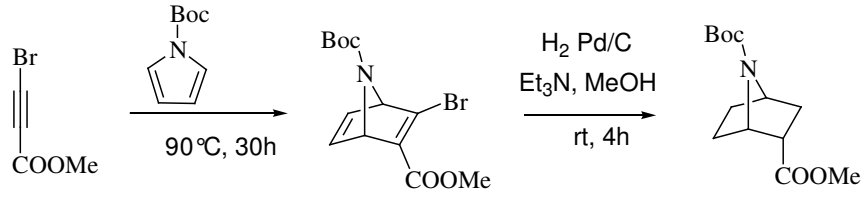
Scheme 9



53 **54** **46**

In alternative, the cycloaddition of alkyne **55**, with N-Boc pyrrole gives the adduct **56** than converted into compound **47**.

Scheme 10

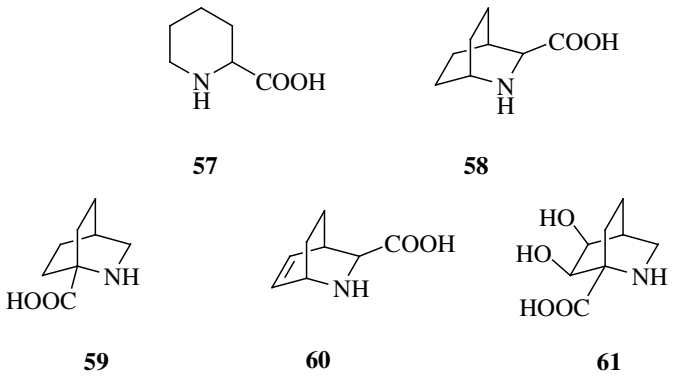


55 **56** **47**

1.3.2 Conformationally rigid bicyclic pipecolic derivatives

Pipecolic acid **57** is a non-natural cyclic α-amino acid that represents a proline homologue. To confer rigidity on the pipecolic acid framework, a bridge linking positions 2 and 5 or 3 and 6 have to be included into the ring. Some examples of 2-aza[2.2.2]octane derivatives are reported here below.

Figure 7

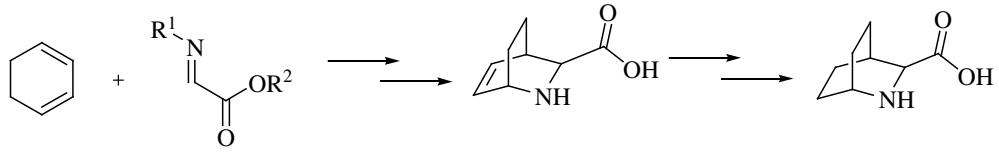


57 **58** **59** **60** **61**

A large number of these derivatives were synthesized and successfully used for the preparation of peptidomimetics and foldamers. (see section 1.5)

The most useful synthetic method to access to these compounds is a Diels-Alder reaction between 1,3-cycloexadiene and an imine. Functionalized derivatives of this class can be obtained by modification of the double bond.

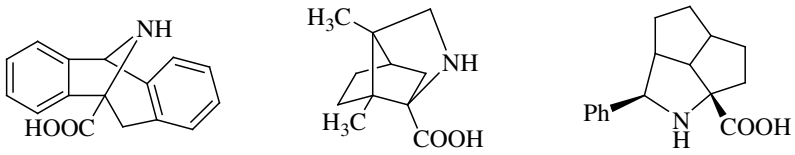
Scheme 11



62 **49** **60** **58**

Aiming to confer rigidity into the proline ring, an other strategy is to insert the proline ring in a more complex policyclic structure (compounds **64, 65**) or to introduce sp^2-hybridised atoms into the ring as compound **63.**

Figure 8



63 **64** **65**

1.4 Rigid Amino acids as drugs

Different conformational constrained unnatural amino acids, were used as single drug molecules. The rigid scaffold oriented the pharmacophore in the space, thus improving the selectivity, the bioavailability and the stability of these compounds in biological systems. Despite the high interest, only few examples of amino acids containing the aza-ring have found an application. Examples are reported in *Table 1*.

Table 1

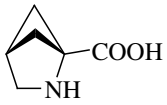
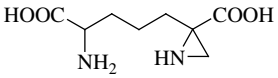
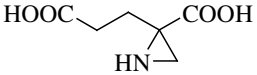
Compound	Name	Therapeutic applications
 **66**	2,4-methanoproline	Agonist for mGluR
 **67**	2-(4-amino-4-carboxybutyl)aziridine-2-carboxylic acid	Irreversible inhibitor of diaminopipemelic acid epimerase[13]
 **68**	2-(2-carboxyethyl)aziridine-2-carboxylic acid	Irreversible inhibitor of glutamate racemase[14]

Table 1. Unnatural amino acids as pharmaceutical and drug components.

1.5 Rigid amino acids in peptidomimetics

The preparation of peptides is the most wide and extended field in which rigid AAs have found applications.

The design and the use of constrained amino acids in the preparation of peptidomimetics with controlled backbone folding is one of the main strategy aiming to preorganize a flexible peptide into a biologically active conformation responsible of the binding to a receptor. Preorganized molecules are expected to play a lower entropic cost upon complexation. As a consequence, an improved selectivity profile and bioavailability can be expected.

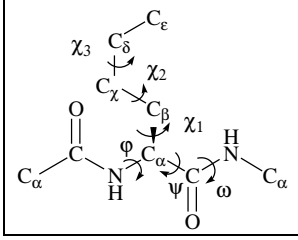
In general rigid amino acids can stabilize a α-, β-, or γ-turn or a β-strand motive that are frequently found in the biologically active conformations.

Since the secondary structure of peptides can be described in terms of the torsion angles φ, ψ and ω, and the conformation of the side chains of amino acids can be described in terms of the torsion angles χ_1, χ_2, χ_3, these parameters play a very important role in the conformation of a peptide fragment. Values of the torsional angles φ, ψ and ω in peptides and proteins containing natural amino acids, were determined as a result of numerous experimental structural data and computational simulations.

By evaluating the values of the torsion angles of a conformationally rigid amino acid, it is possible in general to predict its effect on the secondary structure.

In particular, if the rigid amino acid presents angles close to the native one, a stabilization in the secondary structure is expected, otherwise the destabilization and change in the secondary peptide structure can be predicted with sufficient accuracy.

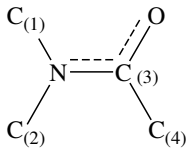
Figure 9



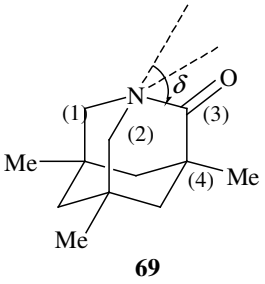
Torsion angle	Notation
C-N-C_α-C	φ
N-C_α-C-N	ψ
N-C_α-C_β-Cγ	ω
C_α-C_β-Cγ-C_δ	χ_1
C_β-Cγ-C_δ-C_ε	χ_2

In the ideal case, the ω_0 angle is 0° for the *cis*-configuration of the peptide bond and 180° for the *trans*-configuration. In the majority natural proteins, the peptide bonds have a *trans*-configuration although bonds with the *cis*-configuration are sometimes encountered. Furthermore, the peptide bonds of cyclic amino acids with a nitrogen atom in the ring, the NH-CO bonds can be non-planar since amino acids form tertiary amides. In fact, these residues, are characterized by noticeable pyramidalization of the nitrogen and carbon atoms of the bond NH-CO and by 'twisting' around this N-C bond. The following parameters have been suggested for quantitative description of the conformation of such a non–planar amide fragment: the *twist* angle τ [τ is the angle between the planes defined by the atoms C(1)NC(2) and OC(3)C(4); parameters ω_0(N) and ω_0(C) which reflect pyramidalization of a bond at the nitrogen and carbon atoms, respectively; the *tilt* angle δ is formed between the N-C(3) bond and the plane defined by the C(1)C(2)N atoms. A strong pyramidalisation of nitrogen atom bonds and 'twisting' of the amide bond can be achieved in a rigid system, such as derivatives of 1-azaadamantan-2-one **69**.

Figure 10



[$\tau = \delta = 0°$, ω_0(N) = 360°]



69

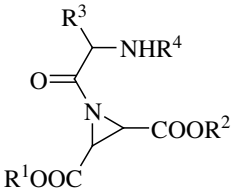
[$\tau = 90°$ $\delta = 57°$, ω_0(N) = 326°]

Here below are reported some examples of peptidomimetics containing the amino acids mentioned in paragraph 1.1 and 1.2 and the above parameters will be discussed for the peptide fragments bearing these rigid moieties.

1.5.1 Conformational rigid cyclic and spirocyclic amino acids with 3 and 4 member rings.

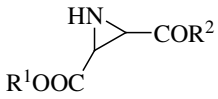
Aziridine amino acids are characterized by strictly fixed φ and χ angles. Since small peptides containing this scaffold present an high toxicity, due to the high reactivity of aziridine ring toward nucleophyles, their use as drugs is very limited.

Figure 11



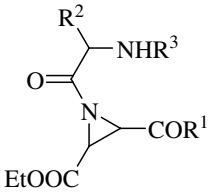
70

R^1 = Et, H; R^2 = Et, Bn;
R^3 is an amino acid residue;
R^4 is a protecting group,
an amino acid or a peptide reidue



71

R^1 = Et, H;
R^2 is an amino
or a peptide
residue.

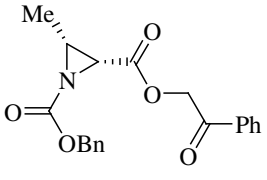


72

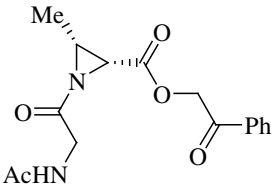
R^1 is an amino acid or
a peptide residue;
R^2 is a side chain of
an amino acid;
R^3 is a protecting group.

Nevertheless, conformational properties of such peptides may be judged from the single-crystal X-ray diffraction studies of compounds **74** and **75** from which is evinced a significant pyramidalization of the nitrogen atom. This is evidently a consequence of the angle strain in the three-member ring. The δ distortion angle in those dipeptides, is about 37-38°, this mean that the nitrogen atom is virtually tetrahedral as in the case sp^3-C hybridisation.

Figure 12



74

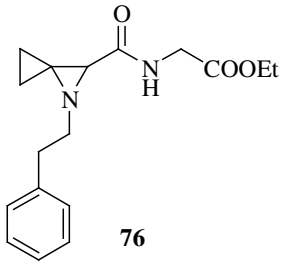


75

Compounds holding a spiro[2.2]pentane skeleton, were characterized by strongly distorted bond lengths and angles. In particular the C-C bond of the spiro atom is 0.07 Å shorter and the angles are strongly distorted (in range of 59 to 134° of distortion angles δ).

Compound **10** is enough stable to permit the preparation of a short peptide sequence. In order to study the secondary structure induced, it was incorporated into the novel simple peptide structure **76**.

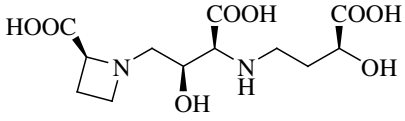
Figure 13



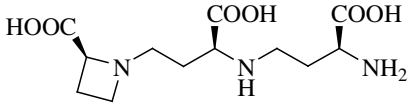
76

Many natural compounds, such as the mugineic acid **77**[15] or the nicotinamide **78**[16], that contain the azetadine-2-carboxylic acids **8** in their structure, have been reported.

Figure 14



77



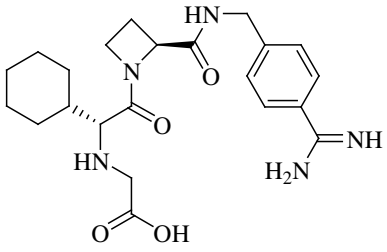
78

The four member ring in compound **8** is conformationally flexible but it acquires conformational rigidity when is inserted in a peptide sequence because the nitrogen atom is involved in the amide bond.[17] Further studies confirm that this ring is virtual planar. For this reason, these amino acids were often incorporated into synthetic peptidomimetic new drugs.

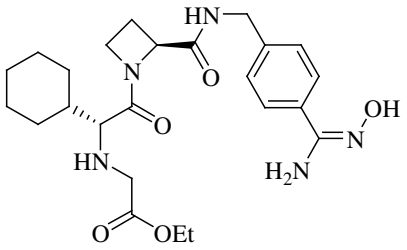
As examples, pharmacologically important molecules such as the thrombin inhibitor Melagatran **79** or Exanta **80**[18], are hire reported which have been prepared from

compound **8** by modification of the known thrombin inhibitor, viz., Boc-D-Phe-Pro-Arg-H **81**.

Figure 15



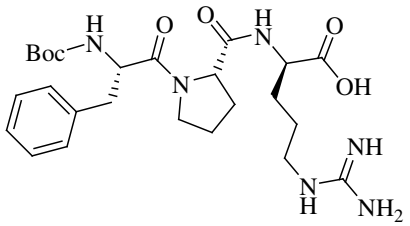
79



80

The replacing of Pro by their conformationally contrained analogous Aze in this tripeptide resulted in a two fold increase in the inhibitory activity.

Figure 16



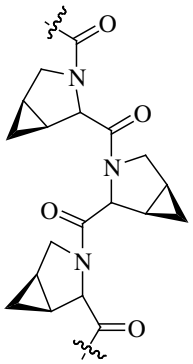
81

1.5.2 Proline derivatives

Rigid proline analogues **27, 28** and **29** may be considered analogues of valine, leucine, and glutamic acids respectively.

Compound **27** is strongly constrained and was successfully employed in the design of mimetics of polyproline II helix. It plays also an important role in the process of intracellular signal trasmission.

Figure 17



82

A detailed conformational computational analysis of di- and tripeptides containing residue **27** or compounds **28** or **29** shown that the torsion angles φ, ψ, χ_1 and χ_2 are –70°, 131°, -57°, and –158°. These values agree with angles typical of the polyproline II helix.

2,4-Methanoprolines (2,4-MePro) **35** and **36** are naturally occurring proline analogues. Their bond lengths are close to those of proline but the angles are in some cases different as reported in *Table 2*.

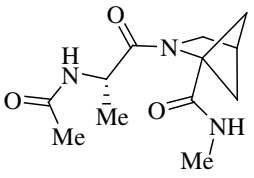
Table 2

Angle	Value (deg)	
	2,4-MePro (compounds **66**)	Proline
N-C(5')-C(4')	97.0	103
C(3')-C(4')-C(5')	103.1	103
C(3')-C(4')-C(6)	100.5	103
C(2')-C(3')-C(4')	82.4	103
C(2')-C(6)-C(4')	81.9	

Small peptides containing a 2,4-MePro residue (compounds **83-86**) are characterized by fixed torsion angles φ and χ. The distortion of these two angles is generated by the

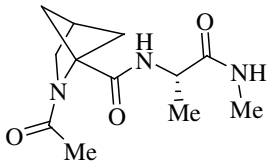
deformation of the framework. Some examples of these compounds have been deeply investigated by NMR spectroscopy and X-ray diffraction.[19]

Figure 18



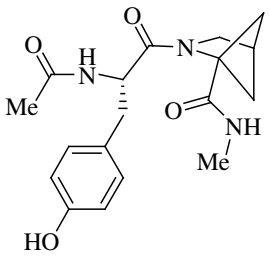
84

Ac-Ala-2,4MePro-NHMe



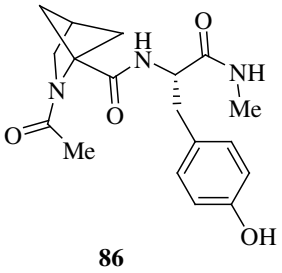
85

Ac-2,4-MePro-Ala-NHMe



83

Ac-Tyr-2,4MePro-NHMe



86

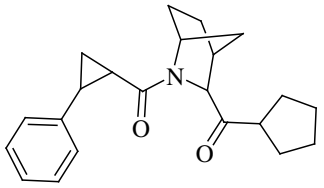
Ac-2,4-MePro-Tyr-NHMe

1.5.3 Azabicyclo[2.2.1]heptane derivatives

Azabicyclo[2.2.1]heptane derivates were widely employed in the synthesis of different conformationally rigid substances. In particular, aza-derivates of norbornane were used in the design of rigid amino acids proline analogues.

The analysis of the bond length and the angles of structure **44** reveled that they are virtually identical to those in proline except for the pyramidalization at the nitrogen atom ($\delta = 14°$ and $\tau = 8°$). However the corresponding peptide bonds are non-planar. The non-planarity of the amide bonds is evidenced by the values of angles (compound **87,** $\omega_0 = 358°$).

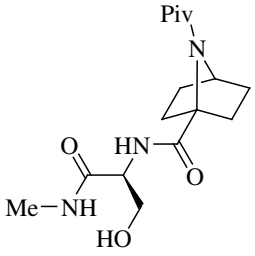
Figure 19



87

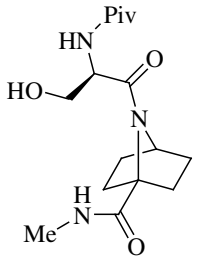
For the sake of structural elucidation, two model oligopeptides containing residue **46** (for this residue, a notation Ahc is adopted) has been synthesized. According to the X-ray diffraction data, the angle φ in the protected dipeptides **88** and **89** are strongly deviated from the theoretical value of 0°.

Figure 20



Piv-Ahc-Ser-NHMe

88



Piv-Ser-Ahc-NHMe

89

1.5.4 Pipecolic and analogue Bicyclo[2.2.2]octane derivatives

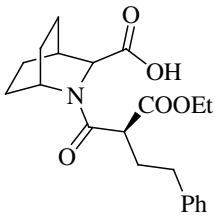
Compound **58** is quite often used as an analogue of proline although it is actually an analogue of pipecolic acid and no substantial difference in the bond angles is observed. X-ray diffraction analysis indicates that the C-C bonds are slightly longer (0.03 Å) than those of pipecolic acid. Instead, no substantial difference in the bond angles were observed.

Amino acids containing a bicyclo[2.2.2]octane framework are widely studied considering the huge number of compounds containing this scaffold. An analysis of

the structural data indicates that amino acids with this framework are characterized by distorted torsion angles especially in the case bulky substituted compounds. Theoretically, the value of the φ angle in derivates of 2-azabicyclo[2.2.2]octane-3-carboxylic acid, assumed the symmetrical framework and the undistorted amide bond, is –60°. According to X-ray diffraction data, this value is – 65° for compound **91**. In Zabicipril **90**, the respective torsion angle is –63°. The pyramidalization parameter (ω_0) of the nitrogen atom in **92** is 359.7°. Distortion of the amide bond is also insignificant (τ = 4° and δ = 9°), i.e., the amide bond in derivatives of 2-aza-bicyclo[2.2.2]octane is virtually planar.

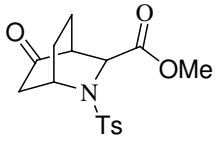
According to X-ray diffraction data, the amide bond is characterized by the *trans*-configuration (ω = -172° and ω = -174° for **92** and **90**, respectively).

Figure 21

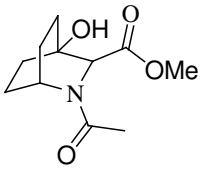


90

Zabicipril



91



92

1.6 Rigid amino acids in organocatalysis

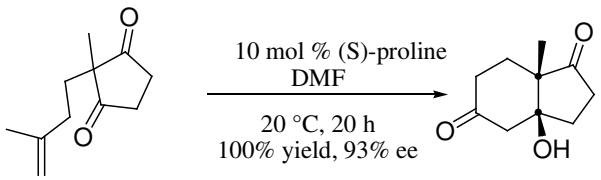
Organocatalysis represents one of the most active research field in the last decade. A range of catalysts of varying structural complexity were developed. An organocatalyst is "an organic compound of relatively low molecular number and a simple structure capable of promoting a given trasformation in substoichiometric quantities".[20] Organic catalysts offer several advantages with respect to the traditional transitional metal mediate reactions. Organocatalysts are generally cheaper because in many cases are naturally derived compounds. They can often easily handle under wet and aerobic conditions, and there are not associated to toxic problems. However in some cases in order to have good performance the catalytic loading is too high.

Examples of organocatalysts were reported first in 1960's in which natural organic compounds such as alkaloids (chinchona) or natural amino acids (proline) were used as the catalyst in asymmetric reactions. Aiming to developed better catalysts, amino acids, and in particular the constrained ones, have found a central role and also interesting applications.

The most readily available catalytic systems within this field are undoubtedly the proteinogenic α-amino acids, with proline derivatives arguably the most commonly used as organocatalysts.

The first pioneering example of an amino acid used as catalyst, was the asymmetric Robinson annulation reported in the early 1970s. A catalytic amount of (S)-proline was enough to mediate the aldolcyclization of compound **93** in 100 % yield and 93 % ee.[21] The polar aprotic DMF was found to give the shorter reaction time and the highest enantioselectivity and yield.

Scheme 12

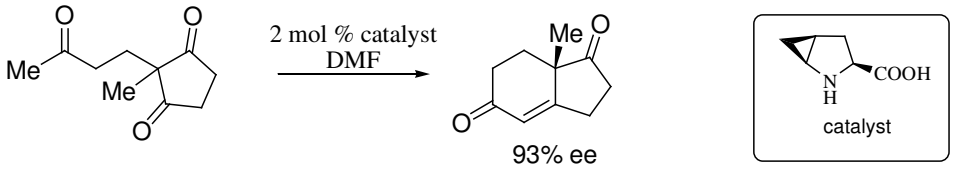


93 **94**

Advantages on the use of proline are that it is cheap, very powerful and easily available in enantiomerically pure form. For these reasons it is the most utilized organocatalyst. Nevertheless some examples were reported in which the catalyst is a fixed proline derivatives that performs similar or better results with respect to proline.

Here below are reported some examples of rigid proline derivatives used as organocatalysts.[22]

Scheme 13

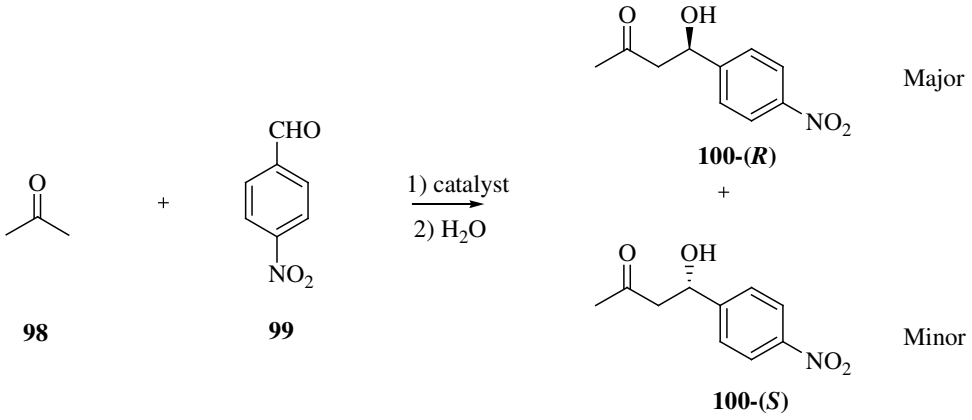


95 **96** **97**

The goal in the use of the rigid proline catalyst is that the loading is much lower compared to the native proline.

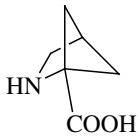
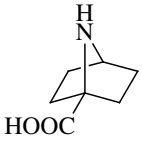
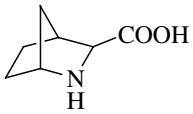
Also aldol reaction was studied using rigid proline derivatives reported in *table 3* which gave a good selectivity also using acyclic ketones.[23]

Scheme 12



98 **99**

Table 3

Catalyst	Yield	ee (%)
 **36**	99 %	87 %
 **46**	100	95 %
 **44**	95%	91 %

Even if, the used of rigid amino acids containing azaring as organocatalyst is a promising application field, since now not many examples are reported in literature.

1.7 Rigid amino acids as metal ligands

Amino acids were used as metal ligands since long time, but this research field is also actual for the "bioorganic applications".[24] In this sense, promising researches pointed out the attention on small peptide containing unnatural rigid amino acids that can conveniently replace a complex enzyme structure and are normally more stable than the native one. Very important, is the active site responsible of the catalytic reaction general makes up a transition metal, coordinated to a serie of amino acids.[25] An evaluation of the intrinsic binding energy ΔE_{int}, between the metal in the metal fragment $[X_nM]^*$, and the ligand L^* (these asterisks designate a simplification that the ligand and the fragment have the some energy in the complex $[X_nML]$), can be obtaining according to the electron transition state (ETS) scheme.[26] The bonding energy between two fragments is described by the three interaction terms shown in *Equation 1*.[27]

Equation 1

$$\Delta E_{int} = \Delta E_{Pauli} + \Delta E_{elsta} + \Delta E_{orb}$$

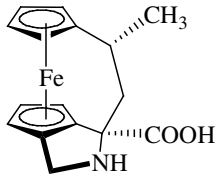
The first term ΔE_{Pauli} quantifies the Pauli repulsion between the electrons on the two fragments. The electrostatic attraction between the two fragments is named ΔE_{elsta} and the ΔE_{orb} represents the orbital interaction term, which quantifies the energy gain upon mixing the orbitals of the two fragments. Only analysing all these energy parameters is possible to evaluate the energy of metal-ligand interaction.

The ΔE_{Pauli} plays an unfavourable role for the ligands with rather extended occupied π orbitals such as C_2H_4, C_2H_2, and CO and is not counterbalanced by a strong electrostatic interaction ΔE_{elsta}. This latter term is also especially large for NHC complexes in fact N atom is a σ donor but does not have an important π retro donation. Furthermore N atom can also change its hardness character, depending on the different functional group in which is involved. As an example, in an oxazoline, N atom revealed a hard character, but in an amine its character is more soft. These characteristics and the different orbital energy ΔE_{orb} make nitrogen atom one of the most interesting useful and versatile "bindings atom" for transition metal.

Natural and unnatural amino acids have been used since long time as ligands for transition metal in order to create interesting chiral organometallic catalysts. However in literature are reported only few examples of azacyclic rigid amino used as transition metal ligands.

In complex **103** the proline ring is condensed in a more complexes structure that became rigid when the iron ion Fe^{2+} was deeply incorporated in the amino structure.[28] Compound **103** could be inserted in a peptidic sequence for the design of mimic enzyme active site.

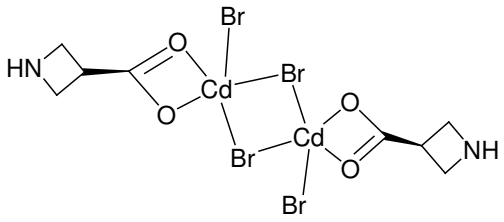
Figure 22



103

In another example the four member azacyclic amino acid **9** was used as a ligand for cadmium ion (Cd^{2+}) forming a very stable complex **104.** [29] The complex is so stable that this system is a very efficient method to chelate the toxic cadmium cation in biological systems.

Figure 23



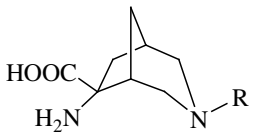
104

2 6-amino-3-azabicyclo[3.2.1]octane-6-carboxylic acids derivatives

2.1 Introduction

As reported in the previous chapter, constrained aza-bicyclo amino acids are noteworthy in particular for their different applications. Since the preparation of constrained amino acids and their possible applications are one of the main synthetic targets of our research group, during my thesis I planned the preparation of a new class of rigid amino acids containing the aza-bicycle-octane scaffold, *i.e.* the 6-amino-3-azabicyclo[3.2.1]octane-6-carboxylic acids named (ABOs) of general formula **105**.

Figure 24

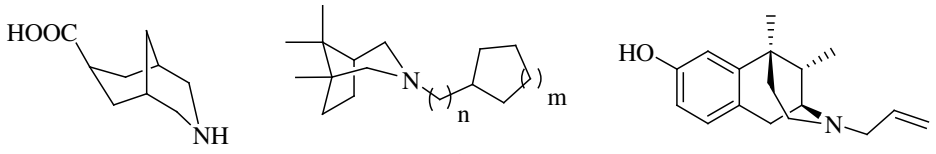


105

The biological interest for aza-bicyclo octane ring is evinced by the recent literature[30] in which a large number of patents point out that this scaffold could be the pharmacophore of a bioactive molecule or a substituent of a different bioactive scaffold.

Depending on the substitution pattern of the ring, different activities were reported. In particular, many of these compounds were used for treating central nervous system disorders. Among these molecules, the carboxylic acid derivatives[31], as well as the amino substituted compounds are of relevance.[32] Some examples of these compounds are reported below.

Figure 25



106 **107** **108**

Due to the importance of this scaffold we developed an efficient synthetic protocol for the preparation of the new 6-amino-3-azabicyclo[3.2.1]octane-6-carboxylic acids, of general structure **105**.

ABO can be considered both an α,γ- and α,δ-diamino acid containing sterical constraints and characterized by α,α-disubstitution. These features make ABO and their derivatives very promising molecules in view of their applications such as:

- bioactive molecules tested on $GABA_A$ receptor
- building blocks in peptidomimetic synthesis
- ligands for transition metal
- organocatalysts both in Diels Alder and Staudinger reactions

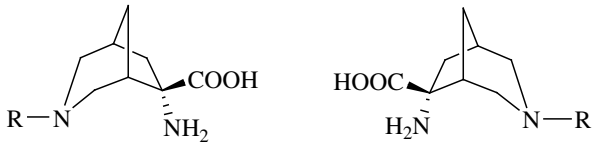
2.2 6-Amino-3-azabicyclo[3.2.1]octane-6-carboxylic acids

Four isomers of the ABO amino acid can be drawn and synthesized *i.e.* a couple of diastereoisomers each present as enantiomers (*Figure 26*) . The availability of all four ABO steroisomers is of importance in view of biological test.

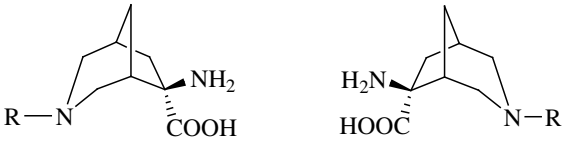
The synthetic protocol for the preparation of ABO amino acids was deeply studied aiming to optimize the synthetic procedure in term of :

- minimization of the synthetic steps
- minimization of cromathographic purification
- orthogonal protection
- preparation of ABO compounds in multi gram scale
- preparation of the four isomers in enantiomerically pure form starting from commercially available compounds

Figure 26



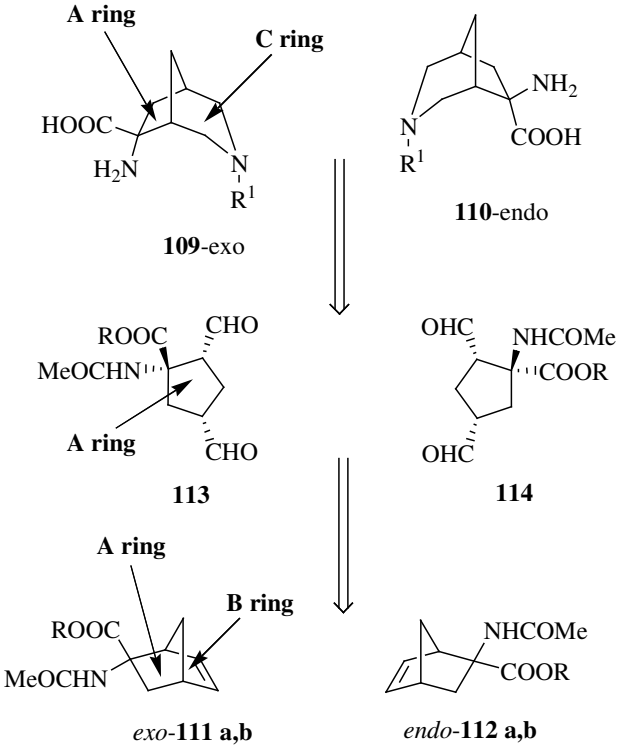
109-*exo* **109'**-exo



110-*endo* **110'**-*endo*

To ensure the preparation of stereomers **109, 109'** and **110**, **110'** in enantiopure form, our retrosynthetic plan features the use of norbornene amino acids **111** and **112** as suitable starting materials.

Scheme 13 Retrosynthetic scheme.



Norbornene amino acids **111** and **112** are readily prepared as endo/exo diastereomers, both in racemic and in enantiopure form, by the Diels-Alder reaction starting from 2-aminoacrylate derivatives and cyclopentadiene **48**. Compounds **111** and **112** are very useful starting materials for the preparation of the aza compounds **109, 110**. In fact, by disconnection of C_5-C_6 bond into *B ring* of norbornenes **111, 112** through an oxidative reaction, the cyclopentyl derivatives **113** and **114** (*A ring* in both compounds **111, 112** and **113, 114**), substituted with the amino acid function and with two formyl groups with the proper regiochemistry and *cis* relationship, were obtained. From these key intermediates, the formation of the C ring of **109** and **110** 3-aza-bicyclo[3.2.1]octane skeleton was assured by a reductive amination reaction.
The objects of these researches were published in:

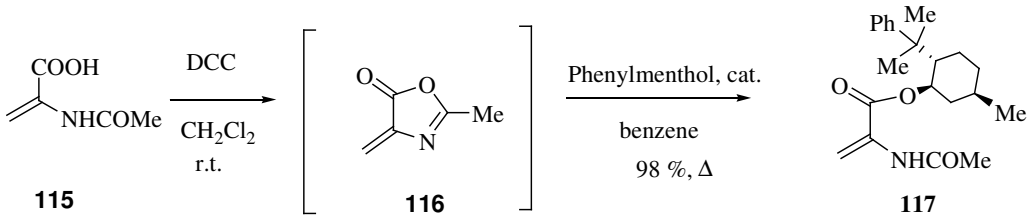
- Francesco Caputo, Cristian Cattaneo, Francesca Clerici, Maria Luisa Gelmi and Sara Pellegrino,*J. Org. Chem.*, **2006,** *71,* 8467-8472
- Maria Luisa Gelmi, Cristian Cattaneo, Sara Pellegrino, Francesca Clerici, Marina Montanari, Claudia Martini, *J. Org. Chem.,* **2007** ,*72* ; 9811-4.

2.2.1 Synthesis of norbornene amino acid derivatives

The preparation of methyl 2-acetamidobicyclo[2.2.1]hept-5-ene-2-carboxylate is reported in literature[33] and shown in *scheme 13*. The key starting reagents for the preparation of the amino acids containing the 3-azabicyclo[3.2.1]octane skeleton were the norbornene derivatives exo-**111a** and endo-**112a** as well as the conresponding enantiopure compounds exo-**111b** and endo-**112b**.
Instead enantiopure norbornene derivatives *exo*-**111b** and *endo*-**112b** were obtained by reaction of aminoacrylates **117** and cyclopentadiene **48** (*Scheme 15*). (-)-8-Phenylmenthyl acrylate **117** is a know compound, which can be obtained in 47 % yield, according to the literature.[34] To improve its yield, a new efficient protocol was adopted, by reacting 2-acetamidoacrylic acid **115** with dicyclohexylcarbodiimide (DCC) in dichloromethane under an inert atmosphere. The corresponding 4-methyleneoxazolone **116** was obtained which is an unstable compound, very sensitive to moisture. For this reason, after filtration of the dicyclohexylurea under a nitrogen atmosphere and solvent elimination, oxazolone **116** was immediately made

to react in refluxing benzene with (-)-8-phenylmenthol in the presence of bis-(dibutylchlorotin)oxide as the catalyst. Chiral acrylate **117** was obtained in an excellent yield (98%) (*Scheme 14*).

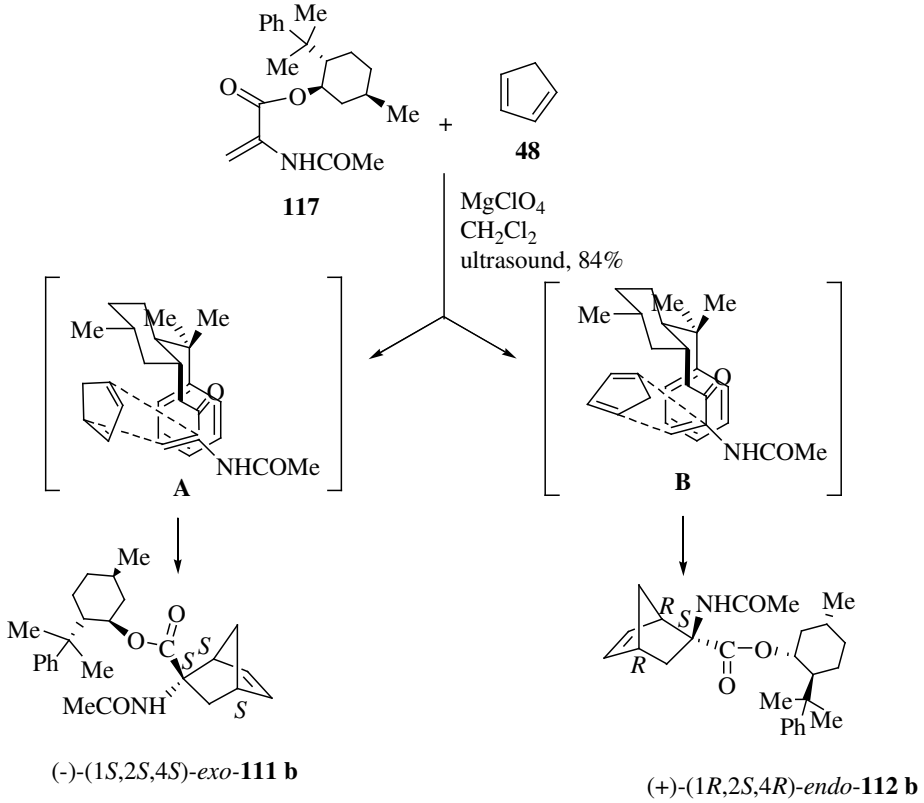
Scheme 14



115 **116** **117**

The asymmetric synthesis of the new cycloadducts *exo*-**111** and *endo*-**112** was carried out starting from enantiopure acrylate **117** performing the Diels–Alder reaction in dichloromethane as the solvent, $Mg(ClO_4)_2$ (0.3 equiv) as the catalyst and with ultrasound (14 h) (*Scheme 15*). A mixture of *exo*-**111b** and *endo*-**112b** (84%) was obtained in 83:17 ratio and with high diastereoselectivity for each *exo* (de 97%) and *endo* (de 96%) couple of diastereomers (HPLC analysis). Pure compounds *exo*-**111b** (de 99%) and *endo*-**112b** (de 99%) were isolated by chromatographic separation and crystallization.

Scheme 15



(-)-(1*S*,2*S*,4*S*)-*exo*-**111 b**

(+)-(1*R*,2*S*,4*R*)-*endo*-**112 b**

The structure of cycloadducts *exo*-**111b** and *endo*-**112b** was determined by NMR experiments (^{1}H, ^{13}C, COSY, NOESY NMRs). ^{1}H NMR spectra revealed differences between the two diastereomers: typical signals at 5.94–5.89 (m, H-6), 6.37–6.33 (m, H-5), 2.84 (m, H-4, H-1), 2.60 (dd, H-3$_{exo}$), 1.15 (dd, H-3$_{endo}$), 1.78 (m, H-7$_s$), 1.38 (m, H-7$_x$), 4.88 (NH), and 1.76 (MeCO) are present for the *exo*-adduct. Signals at 5.78–5.74 (m, H-6), 6.36–6.32 (m, H-5), 2.90 (br s, H-4), 1.76 (dd, H-3$_{exo}$), 2.22 (dd, H-3$_{endo}$), 2.56 (m, H-1), 1.67 (m, H-7$_s$), 1.50 (m, H-7$_x$), 5.42 (NH), and 1.93 (MeCO) characterize the spectrum of the *endo* adduct. As a confirmation of the stereochemistry assigned to the cycloadducts, a significative positive Overhauser effect (NOESY experiments) was observed between the NH and both H-3$_{endo}$ and H-6 in the *exo*-adduct, and between the NH and H-7$_s$ in the *endo*-compound.

The absolute configuration of all the stereocenters in compound *exo*-**111b** was assigned indirectly by the hydrolysis of the (-)-8-phenylmenthyl ester function to the corresponding acid. The known[35] (-)-2-acetylamino-bicyclo[2.2.1]hept-5-ene-2-

carboxylic acid *exo*-**111b** was obtained, characterized by the (1*S*,2*S*,4*S*)-configuration.

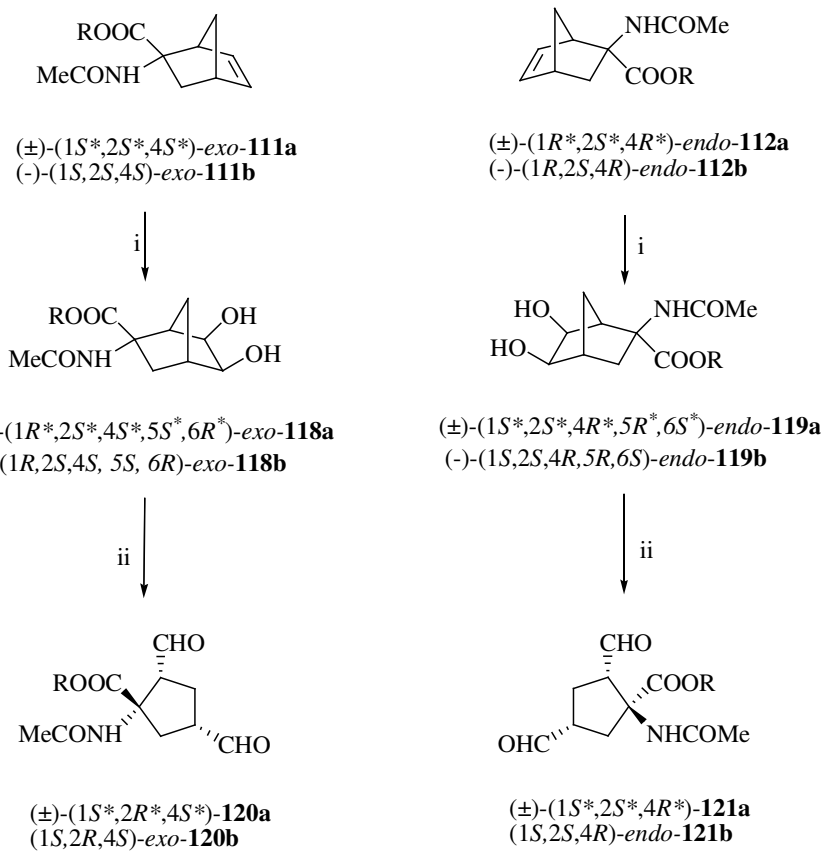
The stereochemical outcome of this reaction showed that the diene attacked the dienophile on the less hindered *Si*-face (intermediates **A** and **B**), which is in agreement with the results observed for the synthesis of the analogous (-)-menthyl[36] or (-)-8-phenylmenthyl derivatives.[33] Accordingly, the stereochemistry assigned to the *endo* adduct (+)-**112b** is 1*R*,2*S*,4*R*.

2.2.2 Synthesis of the 6-amino-3-azabicyclo[3.2.1]octane-6-carboxylic acids and derivatives

The synthesis of ABO derivatives was performed first, starting from racemic compounds *exo*-**111a** and *endo*-**112a** which were transformed into the corresponding dihydroxy derivatives **118a** and **119a** by allowing **111a** and **112a** to react with *N*-methylmorpholine-*N*-oxide (NMO) in acetone/H_2O, in the presence of a catalytic amount of osmium tetroxide (*Scheme 16*). The diol derivatives *exo*-**118a** (86%) and *endo*-**119a** (84%) were obtained in pure form as single diastereomers characterized by the *cis* relationship between both the hydroxy groups and the bridge, as demonstrated by a NOESY experiment on *exo*-**118a**. In fact, spatial proximity between *endo* H-3 (δ 1.34) and H-5 (δ 3.73) and between H-6 (δ 3.97) and the methyl group of the acetyl group (δ 1.94) was observed.

The cyclopentyl derivatives functionalized with two formyl groups with the proper stereochemistry were obtained by cleaving the C_4-C_5 bond of compounds **118a** and **119a** with sodium periodate as the oxidant in dioxane/H_2O (8:1) at room temperature (4 h). Bisaldehydes **120a** and **121a** were quantitatively obtained from *exo*-**118a** and *endo*-**119a**, respectively (*Scheme 16*).

Scheme 16 Synthesis of Bisaldehydes [a]



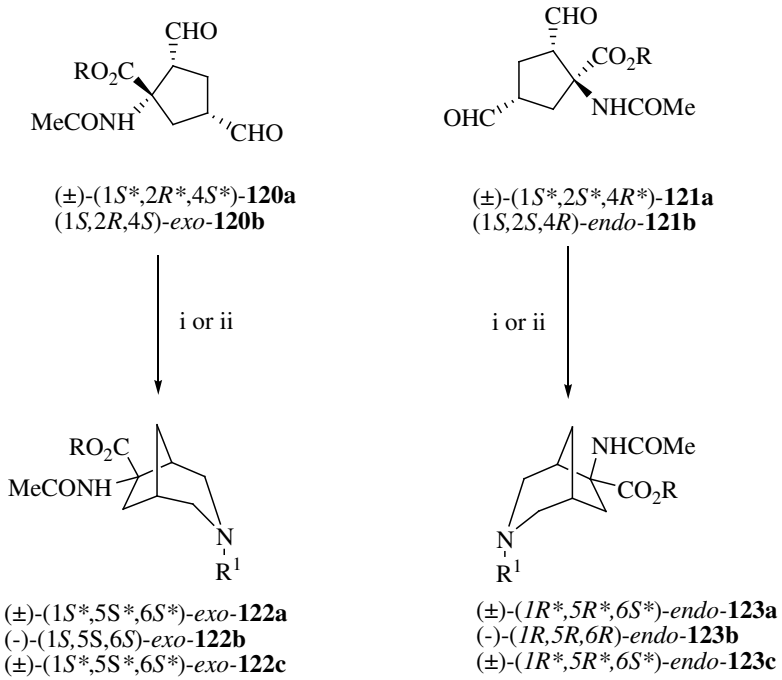
a: R = Me
b: R = (-)-8-fenilmenthyl

[a] Reagents and conditions: (i) OsO_4, NMO, acetone/H_2O, 25 °C; (ii) $NaIO_4$, dioxane/H_2O, 25 °C.

The bisaldehydes are not very stable, and for this reason it was chosen to transform the crude reaction mixtures directly into the corresponding 3-azabicyclo[3.2.1]octane derivatives. A reductive amination was performed using *p*-methoxybenzylamine, as nitrogen donor, and sodium triacetoxyborohydride, as reducing agent (1.3 equiv). The reaction works at room temperature (4 h) in 1,2-dichloroethane and in the presence of a catalytic amount of acetic acid. Methyl 6-acetamido-3-

azabicyclo[3.2.1]octane-6-carboxylate derivative *exo*-**122a** (61%) was obtained from **120a**. Compound *endo*-**123a** was isolated in 53 % starting from **121a**. (*Scheme 17*).

Scheme 17 Synthesis of 3-Azabicyclo[3.2.1]octane Ring[a]



a: R = Me, R^1 = PMB
b: R = (-)-8-Phenylmethyl, R^1 = PMB
c: R = Me, R^1 = Me

[a] Reagents and conditions: (i) *p*-methoxybenzylamine $NaBH(OAc)_3$, AcOH (cat.), $ClCH_2CH_2Cl$, 25 °C, (ii) $MeNH_2$; $NaBH(OAc)_3$, AcOH (cat.), $ClCH_2CH_2Cl$, 25 °C.

This synthetic protocol suffers from some limitations related to (i) the efficiency of the exo/endo norbornenes **111** and **112** separation, and (ii) the necessity to perform the reaction twice, both starting from the *exo* and the *endo* norbornenes. For this reasons a more efficient synthetic protocol was developed by the preparation of the exo/endo mixture of compounds (±)-**122a** and (±)-**123a** using the same synthetic procedure described for pure diastereoisomers, and in their easy separation by column chromatography in the final step (*Scheme 17*).The mixture of *exo*-**111a** and *endo*-**112a** (7:3) was transformed into a mixture of *exo*/*endo*-3-

azabicyclo[3.2.1]octane amino acid derivatives *exo*-**122a** (41%) and *endo*-**123a** (15%), through the diol derivatives **118a, 119a** and bisaldehydes **120a/121a**.

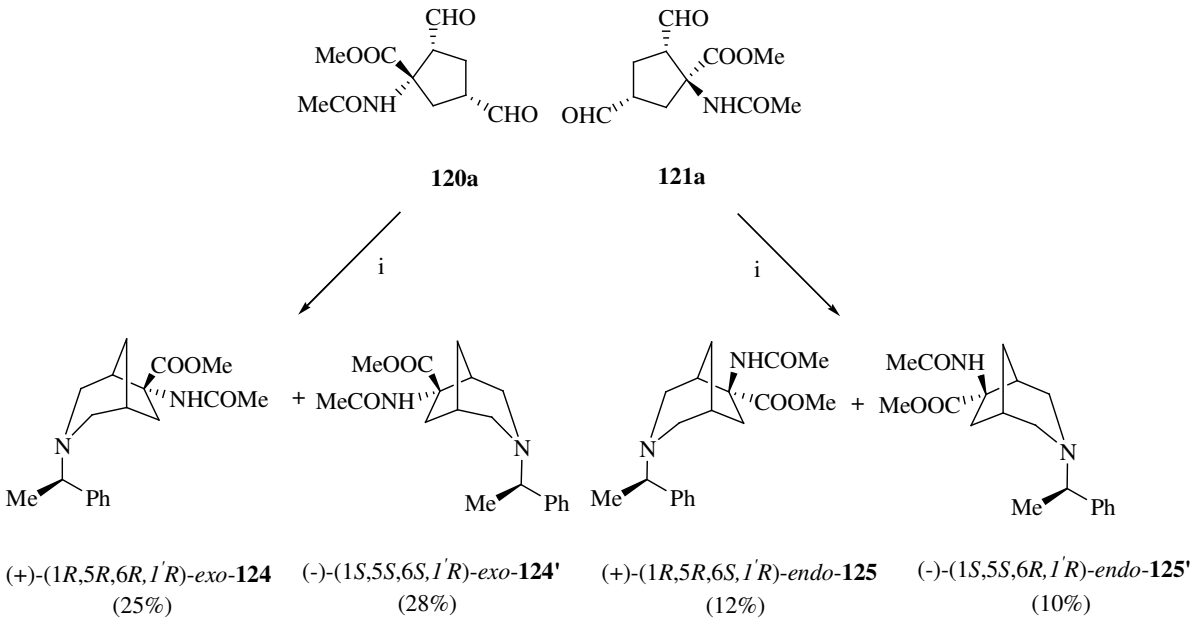
The above method was also applied to the preparation of the 3-*N*-methyl compounds (±)-*exo*-**122c** (24%) and (±)-*endo*-**123c** (11%), which were obtained in poor yields from the mixture of aldehydes **120a** and **121a** and using methylamine as a nitrogen donor in the standard reductive conditions ($NaBH(OAc)_3$, AcOH (cat.), $ClCH_2CH_2Cl$, 25 °C) (*Scheme 17*).

The enantiopure amino acids (-)-*exo*-**122b** (57%) and (-)-*endo*-**123b** (58%) (*Scheme 17*) were prepared following the same synthetic protocol starting from enantiopure compounds (-)-*exo*-**111b** and (+)-*endo*-**112b**, transformed into the diol derivatives (-)-*exo*-**118b** and (+)-*endo*-**119b**, oxidized to bisaldehyde **120b** and **121b**, respectively (*Scheme 16*).

As reported above, enantiopure compounds *exo*-**111b** and *endo*-**112b** were obtained in 83 : 17 *exo*/*endo* ratio through a Diels-Alder reaction and this result obviously disfavored the preparation of the endo series of compounds **123b** when starting from (-)-phenylmenthyl ester *endo*-**112b**. For this reason and also considering the possibility to synthesize all stereomers of compounds **122** and **123**, we chose to start from racemic methyl esters of *exo*-**111a** and *endo*-**112a** which can be prepared in 70:30 ratio and in grams scale.[37]

Following the above reported procedure, the mixture of compounds **111a** and **112a** was transformed into the four enantiopure stereoisomers *exo*-**124b** (25%), *exo*-**124b'** (28%), *endo*-**125b** (12%), and *endo*-**125b'** (10%). The preparation of the above amino acids was performed on the gram scale (7 g), using the cheaper of (+)-(*R*)-1-phenylethylamine as nitrogen donor and resolving reagent when the reductive amination reaction ($NaBH(OAc)_3$, AcOH (cat.), $ClCH_2CH_2Cl$, 25 °C) was performed from aldehydes **120a, 121a** (*Scheme 18*).

Scheme 18 Reductive Amination Procedure[a].



(+)-(1*R*,5*R*,6*R*,1′*R*)-*exo*-**124** (25%) (-)-(1*S*,5*S*,6*S*,1′*R*)-*exo*-**124'** (28%) (+)-(1*R*,5*R*,6*S*,1′*R*)-*endo*-**125** (12%) (-)-(1*S*,5*S*,6*R*,1′*R*)-*endo*-**125'** (10%)

[a] Reagents and conditions: (i) (+)-(*R*)-1-phenylethylamine, $NaBH(OAc)_3$, AcOH (cat.), $ClCH_2CH_2Cl$, 25 °C.

2.2.3 Studies on the deprotection and functionalization of the N-3 atom.

In order to prepare a series of ABO derivatives characterized by a different substitution pattern on *N*-3, which are useful for biological tests, both 3-*NH*- and 3-*N*-alkyl compounds were prepared.

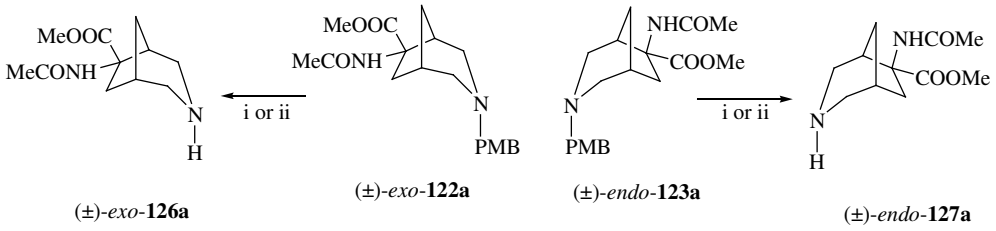
The *p*-methoxybenzyl derivatives *exo*-**122a** and *endo*-**123a** (*Scheme 19*) were first selected as key reagents aiming to find a general procedure to minimize the synthetic steps. This allowed the *NH*-compounds as well as a series of *N*-alkyl compounds to be synthesized efficiently.

Aiming to prepare the NH azabicyclo[3.2.1]octane derivatives, which are also valuable and alternative starting materials for the preparation of 3-*N*-substituted amino acids, the *N*-*p*-methoxybenzyl group (PMB) on compounds *exo*-**122a** was removed by an oxidative process using cerium ammonium nitrate (CAN) in

acetone/H_2O (9:1) at room temperature. This method is reported in the literature, but its straightforward application in the present case caused difficulties because of the solubility of the amino acid derivative in water. The problem was overcome by treating the reaction mixture with solid $NaHCO_3$ followed by its chromatography on silica gel. Compound *exo*-**126a** was isolated in 90% yield. The same synthetic protocol allowed us to transform *endo*-**123a** into amino acid *endo*-**127a** (62%) (*Scheme 19*).

The deprotection of *N*-3 was also performed by hydrogenolysis which made possible an easy purification and better reaction yields of the products (*Scheme 19*). The hydrogenolysis of (±)-*exo*-**122a** and (±)-*endo*-**123a** (Pd/C, MeOH, 25 °C, 1 atm, 2 days) gave the *NH* derivative (±)-*exo*-**126a** (97%) and (±)-*endo*-**127a** (93%), respectively.

Scheme 19 Orthogonal deprotection of the N-*3* amino function [a]



(±)-*exo*-**126a** (±)-*exo*-**122a** (±)-*endo*-**123a** (±)-*endo*-**127a**

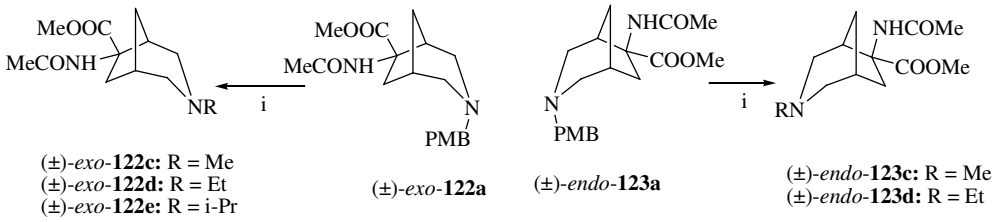
[a] Reagents and conditions: (i) CAN, acetone/H_2O, 25 °C; (ii) Pd/C, H_2, MeOH, 25 °C, 1 atm, 2 days

Since, as reported before, the preparation of *N*-methyl derivatives from aldehydes **120a** and **121a** gave pure results in term of yield, an alternative and more general procedure was planned to prepare, in general, *N*-alkyl compounds. This synthetic target was achieved using a "one pot" deprotecton and alkylation reaction of the nitrogen atom on *N*-3, taking advantage of the use of catalytic hydrogenation in presence of a carbonyl compound, starting from reagents **122a** and **123a** or enantiopure compounds **124/124'** and **125/125'**.

When the hydrogenolysis was performed in presence of a methanolic solution of formaldehyde (37%), the *N*-methyl derivatives (±)-*exo*-**122c** (74%) and (±)-*endo*-

123c (70%) were isolated, respectively, from (±)-*exo*-**122a** and (±)-*endo*-**123a** (Scheme 17). It has to be emphasized that this synthetic approach is a valuable alternative to the reductive amination of *bis*-aldehydes **120a** and **121a** in the presence of $MeNH_2$ which gave the same *N*-methyl derivatives **122c** and **123c** but in very low yield (*Scheme 17*). The reductive alkylation of (±)-*exo*-**122a** was also performed in EtOH/acetaldehyde and in acetone and the expected *N*-ethyl and *N*-isopropyl derivatives (±)-*exo*-**122d** (93%) and (±)-*exo*-**122e** (86%) were isolated, respectively (*Scheme 20*). Compound (±)-*endo*-**123d** (85%) was obtained from (±)-*endo*-**123a**.

Scheme 20 Synthesis of 3-*N*-Alkyl-6-amino-3-azabicyclo[3.2.1]octane-6-carboxylic Acids by a "One-Pot" Deprotection-Alkylation Procedure[a]



(±)-*exo*-**122c:** R = Me
(±)-*exo*-**122d:** R = Et
(±)-*exo*-**122e:** R = i-Pr

(±)-*exo*-**122a**

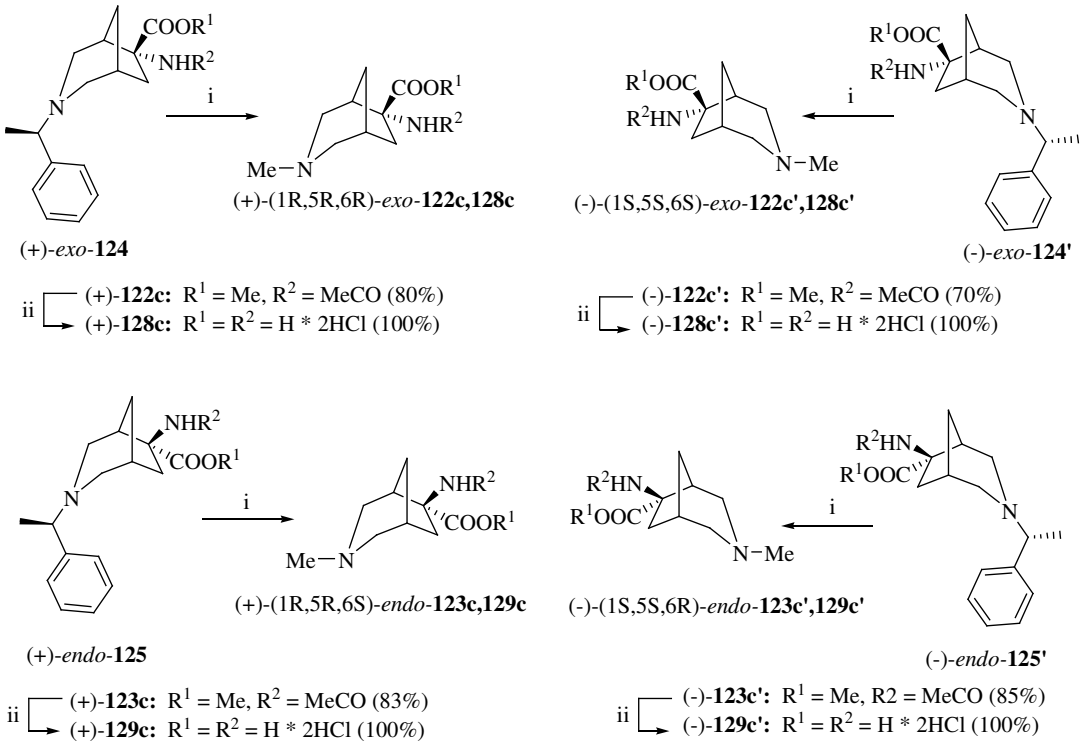
(±)-*endo*-**123a**

(±)-*endo*-**123c:** R = Me
(±)-*endo*-**123d:** R = Et

[a] Reagents and conditions: (i) H_2, Pd/C (**122c**,**123c**: MeOH/HCHO; **122d**,**123d**: EtOH/MeCHO; **122e**: MeCOMe)

The "one-pot" deprotection-methylation reaction of the *N*-3 atom of the four stereoisomers (+)-*exo*-**124**, (-)-*exo*-**124'**, (+)-*endo*-**125**, and (-)-*endo*-**125'** using formaldehyde gave the pure enantiomers (+)-*exo*-**122c** (80%), (-)-*exo*-**122c'** (70%), (+)-*endo*-**123c** (83%), and (-)-*endo*-**123c'** (85%), respectively (*Scheme 21*).

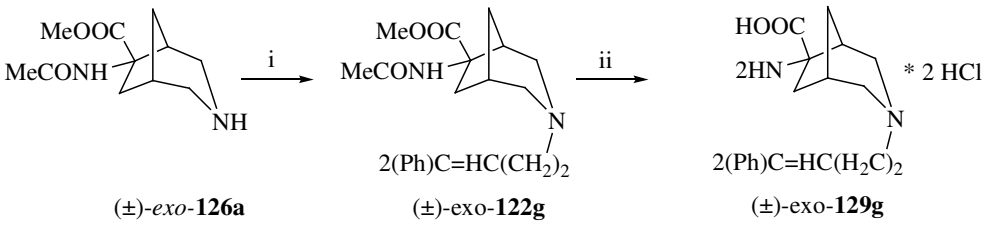
Scheme 21 Enantiopure 6-Amino-3-azabicyclo[3.2.1]octane-6-carboxylic Acids and Their 3-*N*-Methyl Derivatives[a]



[a] Reagents and conditions: (i) R = Me: H_2, Pd/C, MeOH, HCHO; (ii) 6 N HCl,Δ.

Finally, considering that the 4,4-diphenylbut-3-en-1-yl group is a typical residue that increases selectivity (see compound SK&F 89976-A) on GABA receptors and taking into account that the appropriate aldehyde is not available, compound (±)-*exo*-**122g** (30%) was prepared starting from (±)-*exo*-**126a** and 4,4-diphenylbut-3-en-1-yl bromide operating in DMF and in the presence of K_2CO_3 and NaI (*Scheme 22*).

Scheme 22. Synthesis of 3-*N*-(4,4-diphenylbut-3-en-1-yl)-6-amino-3-azabicyclo[3.2.1]octane-6-carboxylic Acids [a]



(±)-*exo*-**126a** (±)-exo-**122g** (±)-exo-**129g**

[a] Reagents and conditions: (i) $(Ph)_2C{=}CH(CH_2)_2Br$, K_2CO_3, NaI, DMF; (ii) 6 N HCl,Δ;

2.2.4 Selective deprotection of amino acid function

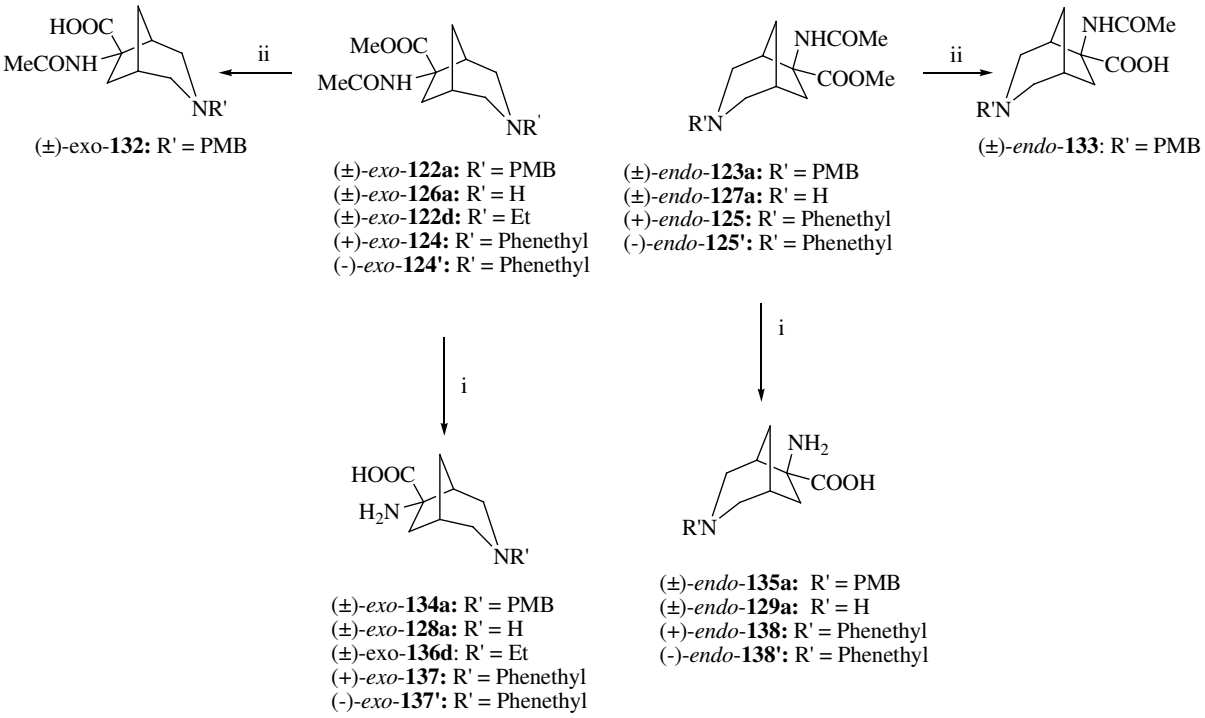
Methyl ester group of (±)-*exo*-**122a** and (±)-*endo*-**123a** was selectively hydrolyzed using basic conditions (EtOH/KOH). The corresponding acids (±)-*exo*-**132** (95%) and (±)-*endo*-**133** (99%) were isolated, respectively (*Scheme 23*).

Both the amino and the carboxy functions were deprotected in acid conditions (6 N HCl at 100 °C, 24 h). Amino acids *exo*-**134a ,134d, 128a** *endo*-**135a, 129a** were obtained in quantitative yield, as bishydrochlorides, from (±)-*exo*-**122a ,122d, 122a** (±)-*endo*-**123a, 127a** respectively (*Scheme 23*). The same protocol was also used efficiently (6 N HCl at 100 °C, 36 h) to deprotect the phenethyl derivatives (+)-*exo*-**124** and (-)-*exo*-**124'**, (+)-*endo*-**125** and (-)-*endo*-**125'**, obtaining compounds (+)-*exo*-**137** and (-)-*exo*-**137'** , (+)-*endo*-**138** and (-)-*endo*-**138'** respectively (*Scheme 23*).

Compounds (+)-*exo*-**122c** and (-)-*exo*-**122c'**, (+)-*endo*-**123c** and (-)-*endo*-**123c'**, were also hydrolysed obtaining compounds (+)-*exo*-**128c** and (-)-*exo*-**128c'** , (+)-*endo*-**129** and (-)-*endo*-**129c'** respectively (*Scheme 21*).

The hydrolysis of ester functions of compounds (±)-*exo*-**122a** and (±)-*endo*-**123a** was successfully achieved with KOH in EtOH (reflux for 50 h). Amino acids (±)-*exo*-**132** (45%) and (±)-*endo*-**133** (60%) were obtained respectively. (*Scheme 23*)

Scheme 23 Orthogonal Deprotection[a]



(±)-exo-**132:** R' = PMB

(±)-*exo*-**122a:** R' = PMB
(±)-*exo*-**126a:** R' = H
(±)-*exo*-**122d:** R' = Et
(+)-*exo*-**124:** R' = Phenethyl
(-)-*exo*-**124':** R' = Phenethyl

(±)-*endo*-**123a:** R' = PMB
(±)-*endo*-**127a:** R' = H
(+)-*endo*-**125:** R' = Phenethyl
(-)-*endo*-**125':** R' = Phenethyl

(±)-*endo*-**133**: R' = PMB

(±)-*exo*-**134a:** R' = PMB
(±)-*exo*-**128a:** R' = H
(±)-exo-**136d**: R' = Et
(+)-*exo*-**137:** R' = Phenethyl
(-)-*exo*-**137':** R' = Phenethyl

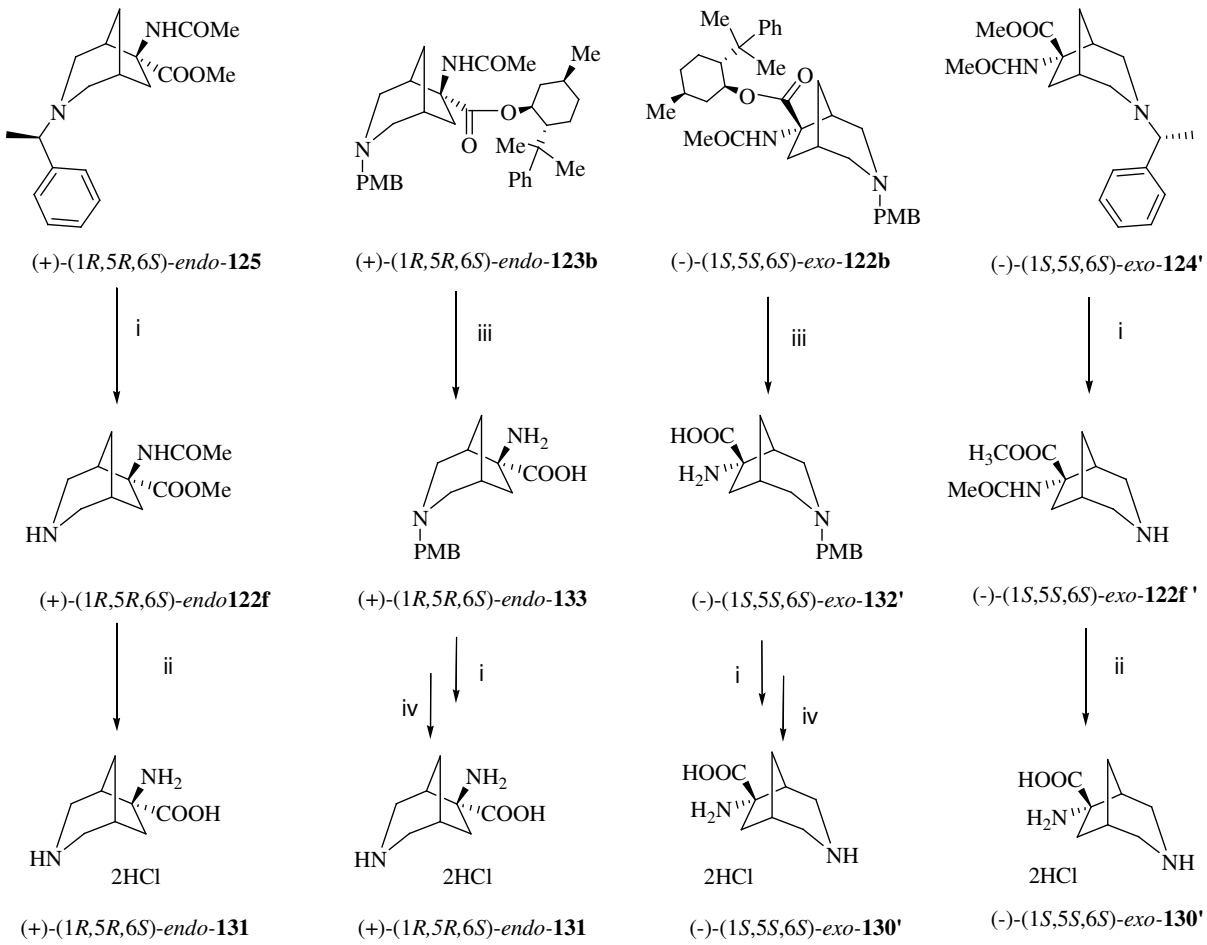
(±)-*endo*-**135a:** R' = PMB
(±)-*endo*-**129a:** R' = H
(+)-*endo*-**138:** R' = Phenethyl
(-)-*endo*-**138':** R' = Phenethyl

[a] Reagents and conditions: (i) 6 N HCl, 100 °C; (ii) KOH, EtOH (95%), 120 °C

2.2.5 *Stereochemical assignment for N-phenethyl derivatives*

Aiming to ensure the correct stereochemistry of each stereoisomer of the phenethyl series, isomers (-)-*exo*-**122f'** and (+)-*endo*-**122f** were hydrolyzed in 6 N HCl (100 °C, 24 h) to give (-)-*exo*-**130'** and (+)-*endo*-**131**, respectively, in quantitative yield as the bishydrochlorides (*Scheme 24*). Compounds (-)-*exo*-**130'** ($[\alpha]_D$ = -9.3) and (+)-*endo*-**131** ($[\alpha]_D$ = +4.9) were correlated to the same compounds (-)-*exo*-**130'** ($[\alpha]_D$ = -9.0) and (+)-*endo*-**131** ($[\alpha]_D$ = +4.0) derived from the catalytic hydrogenolysis (*Scheme 24*) of the known amino acids (-)-*exo*-**132'** and (-)-*endo*-**133** followed by their treatment with anhydrous HCl.

Scheme 24: Stereochemical assignment [a]

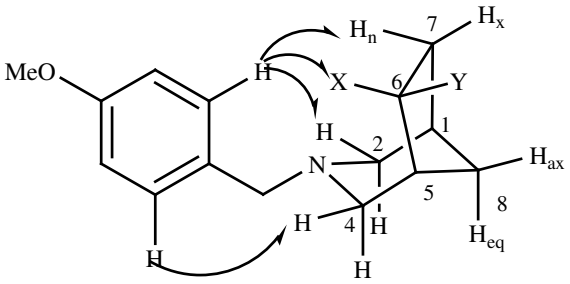


[a]Reagents and conditions: (i) H_2, Pd/C, MeOH; (ii) 6N HCl, Δ; (iii) Na, MeOH at 0 °C, then 100 °C; (iv) 6N HCl, r.t.

2.2.6 Spectroscopic characterization

All compounds were characterized by spectroscopic data (^{1}H NMR, ^{13}C NMR, COSY, NOESY; Hetcor) and, as examples, the spectra of compound **122a** and **123a** are here detailed. It is proposed a predominant chair-envelope conformation for 3-azabicyclo[3.2.1]octane from which it is evinced that the benzyl group is in the equatorial position.[38, 39] Accordingly, we assumed that the more probable conformation of compounds **122a** is that shown in *Figure 28.*

Figure 28 . Conformation and selected NOESY interactions for compounds **122a/123a**



exo-**122a** X = NHCOMe; Y = COOMe

endo-**123a** X = COOMe; Y = NHCOMe

Typical signals for compound *exo*-**122a** are at δ 1.50 (H_{eq}-8) and 2.00 (H_{ax}-8) and at δ 2.20 (H-1) and 2.72 (H-5). Methylene protons resonate at δ 2.35 (H_{β}-2), 2.80 (H_{α}-2), 2.00 (H_{β} –4), 2.53 (H_{α}-4), 2.35 (H_x-7), 1.72 (H_n-7) confirming the formation of constrained ring. An AB system at 3.55, 3.17 δ (J = 12.5 Hz) characterizes the presence of the benzyl protons. Selected signals in the ^{13}C NMR spectrum are δ 65.9 (C-6), 62.2 (CH_2Ar), 61.0 (C-2), 55.6 (MeOAr), 54.9 (C-4), 52.5 (MeO), 42.0 (C-7), 40.0 (C-5), 37.1 (C-8), 34.7 (C-1), 22.5 (MeCO). ^{1}H NMR spectrum of diastereoisomer *endo*-**5a** shows signals δ 3.42, 3.33 (CH_2Ar), 3.01 (H_n-7), 1.90 (H_x-7), 2.84 (H_{α}-4), 2.18 (H_{β} –4), 2.71 (H_{α}-2), 2.02 (H_{β} –4), 2.27 (H-1), 2.19 (H-5), 2.01 (H_{ax}-8), 1.51 (H_{eq}-8). The equatorial position of H-8 was univocally assigned through a COSY experiment. In fact, it is evinced a coupling with H_n-7 at 3.01 (J = 1.8 Hz).

The carbon atoms resonate at δ 8.5 (C-6), 62.0 (CH_2Ar), 59.0 (C-2), 55.9(C-4), 55.6 (MeOAr), 52.5 (MeO), 46.8 (C-5), 39.9 (C-7), 37.1 (C-8), 35.1 (C-1), 23.7 (MeCO). NOESY experiments confirmed the assigned configuration. Significant special proximity on *exo*-**122a** was observed between H_{orto} protons of aryl ring at (δ 7.16) and H_α-2, H_α-4, and most of all, with NH (δ 6.44) and Me of acetamido group (δ 1.59). Overhauser effect was observed in compound *endo*-**122a** between H_{orto} protons of aryl ring at (δ 7.26) and proton of CH_2 groups at lower field (H_α-2: 2.71; H_α-4: 2.84; H_n-7: 3.01 δ) and Me group of carbonylic function (3.69 δ). Furthermore, the NH group shows an Overhauser effect with H_{ax}-8.

2.3 Biological evalutation of 6-Amino-3-azabicyclo[3.2.1]octane-6-carboxylic acids and derivates

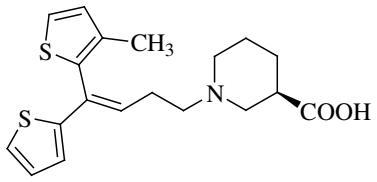
Compounds **105** can be considered constrained analogues of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the vertebrate central nervous system. GABAergic neurotransmission, which is mediated through the activation of three receptor subtypes ($GABA_A$, $GABA_B$ and $GABA_C$),[40] has been implicated in many physiological (feeding, cardiovascular regulation) as well as pathological (epilepsy, alcoholism, schizophrenia, sleep disorders, depression and anxiety) events.[41] $GABA_A$ receptor gates a Cl^--selective channel in response to the binding of the transmitter and contains multiple sites for pharmacologically distinct classes of allosteric modulators. $GABA_A$ receptor–mediated Cl^- conductance is positively modulated at the GABA recognition site, as well as at allosteric sites that bind benzodiazepine, barbiturates, and steroids.[42]

Selective targeting and modulation of GABA transporter subtypes is of biological interest and additionally of fundamental importance for the elucidation of their specialized physiological function and individual structure. Different constrained amino acids have been prepared and tested on GABA receptors and it was found that the stereochemistry and the substituent linked to nitrogen atom are important features to increase selectivity and activity. Between them, nipecotic acid **138** and guvacine **139** are inhibitors of GABA transport with anticonvulsant activity,[43,44] as well as several potent GABA uptake inhibitors including tiagabine **137c**[45] and SK&F 89976-A **137b**[46] which are selective molecules for GAT-1. Recently, derivatives of

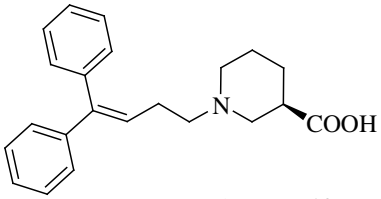
piperidine-3-alkanoic acids **137d**, characterized by highest affinity at GAT-3, were prepared.[47]

Figure 29

GAT-1-selective:

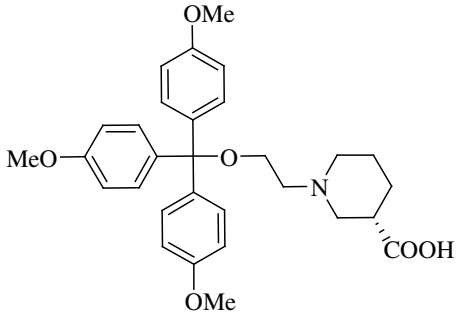


tiagabine [(*R*)-**137c**]

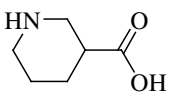


SK&F 89976-A [(*R*)-**137b**]

GAT-3-selective:

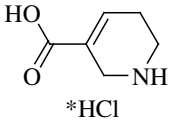


(*S*)-SNAP-5114[(*S*)-**137d**]



Nipecotic acid

138



*HCl

guvacine

139

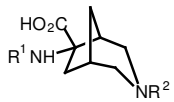
Considering the general biological interest toward new derivatives possessing affinity for GABA receptors, we planned the biological evaluation of GABA mimic amino acids containing the azabicyclooctane skeleton.

2.3.1 Biological results

Amino acids **134a, 128c, 128c', 128a, 129g, 132', 135a, 129c, 129c',129a, 133**, as well as some of the corresponding *N*-acetyl derivatives (see Table 4) were tested for their binding activity on $GABA_A$ receptors in rat cerebral cortical membranes using labelling tests with [^{3}H]muscimol (for $GABA_A$ sites) and [^{3}H]flunitrazepam (for the

benzodiazepine site). The binding affinity of newly-synthesized compounds was determined in rat cerebral cortical membranes by competition experiments. Aliquots of the membrane preparation (~100 μg protein) were incubated in 50 mM Tris-citrate buffer (pH 7.1) at 0 °C for 90 min with [^{3}H]muscimol (5 nM) and the tested compound (10 μM); nonspecific [^{3}H]muscimol binding was determined in the presence of 100 μM GABA. Radioactivity bound to membranes was determined after rapid filtration.

Table 4 Binding affinity of compounds **134a, 128c, 128c', 128a, 129g, 132', 135a, 129c, 129c',129a, 133** on GABA receptors [48]

	R^1/R^2	% Inibition (10 μM)		R^1/R^2	% Inibition (10 μM)
(±)-*exo*-**134a**	R^1 = H, R^2 = PMB	ni	(±)-*endo*-**135a**	R^1 = H, R^2 = PMB	ni
(+)-*exo*-**128c**	R^1 = H, R^2 = Me	14	(+)-*endo*-**129c**	R^1 = H, R^2 = Me	7
(-)-*exo*-**128c'**	R^1 = H, R^2 = Me	ni	(-)-*endo*-**129c'**	R^1 = H, R^2 = Me	ni
(±)-*exo*-**128a**	$R^1 = R^2$ = H	ni	(±)-*endo*-**129a**	$R^1 = R^2$ = H	ni
(±)-*exo*-**129g**	R^1 = H, R^2 = $(Ph)_2C{=}CH(CH_2)_2$	ni	-	-	-
(±)-*exo*-**132**	R^1 = COMe, R^2 = PMB	ni	(±)-*endo*-**133**	R^1 = COMe, R^2 = PMB	ni

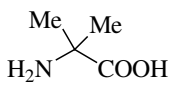
In general these compouds do not display an appreciable affinity for $GABA_A$ receptor. Only *N*-Me derivatives **128c** and **129c** displayed low affinity for $GABA_A$ receptors and in particular, the biological activity is solely due to the (+) enantiomer (*i.e.* (+)-(1*R*,5*R*,6*R*)-*exo*-**128c** and (+)-(1*R*,5*R*,6*S*)-*endo*-**129c**) thus confirming that the stereochemistry of the skeleton is an important feature in the binding with GABA receptors.

3 ABO in peptidomimetics

3.1 Introduction

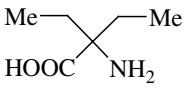
One of the finality of my thesis concern the use of ABO unnatural amino acids in peptide synthesis. In particular we were interested to insert these amino acids in short peptide sequences aiming to evaluate their ability to induce a secondary structure. The features of these compounds, *i.e.* the presence of constraints and heterosubstitutions, make them suitable for this kind of application. ABO compounds are members of the family of $C_i^{\alpha} \leftrightarrow C_i^{\alpha}$ cyclized, C^{α} tetrasubstituted α-amino acids (AC_nC: 1-aminocycloalkane-1-carboxylic acids). The use of $C^{\alpha,\alpha}$-disubstituted glycines in the synthesis of peptides with restricted conformational flexibility has acquired increasing importance in the design of analogues of bioactive compounds. In fact, it is well known that C^{α} tetrasubstituted amino acids belonging to this class are able to reduce drammatically the available conformational space by stabilizing specific secondary structures,[49,50] such as β-[51,52,53] and γ-turns[54,55] and 3_{10}- and α-helixes.[56,57] The main rapresentative of this class of amino acids is Aib **140** (α-aminoisobutyric acid) and its congeners such as compounds **141**, **142-145** (reported in *Figure 30*)**.**[58]

Figure 30

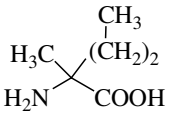


Aib

140

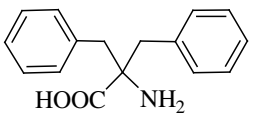


141

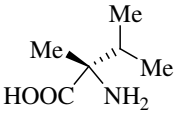


(αMe)Nva

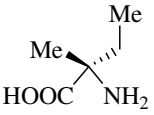
142



143



144



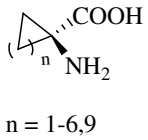
145

Aib has been use since 1964[59] for the preparation of peptides because it was shown that in general it favours α- or 3_{10}-helix, which are the two most prevalent helices found in proteins[60, 61]. The 3_{10}-helix, which accounts for approximately 10% of all protein helical structures, is considered to be a protein folding intermediate to the α-helical conformation.

The backbone torsional angles (φ, ψ) of Aib and several other α,α-dialkylated amino acids are severely restricted, resulting in the pronounced propensity of this residue to favor helical structures, even in short peptides.[62, 63,64]

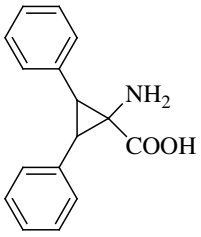
In this context the family of the Ac,c **146** (l-amino-1-cycloalkane-carboxylic acid, *n* = 1-6, 9) alicyclic residues proved to be valuable in the preparation of conformationally constrained peptide backbones.

Figure 31

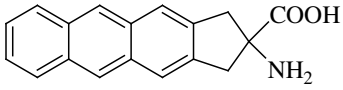


n = 1-6,9

146



147



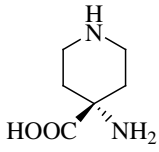
148

More specifically, the three-membered ring Ac3c residue compounds **146** (n = 1) and **147**[65] exhibits a marked preference for the *bridge* region of the conformational space, charaterized by φ = ±90, ψ = 0°. This small-ring residue can also be accommodated in *distorted* type III (III') β-turns and 3_{10}-helices. Interestingly, however, the preferred conformations [*regular* type III-(III') β-turns and 3_{10}-helices],[66] found for Ac_4c-Ac_9c and $Ac_{12}c$ residues, having the cyclic moieties significantly larger than that of Ac_3c, are closely parallel to those of Aib. The conclusion of these studies are that the alicyclic $C^{\alpha,\alpha}$-disubstituted glycines Ac,c with *n* = 1-6, 9 have different effective volume and hydrophobicity, but they exhibit a strictly comparable conformational preference.

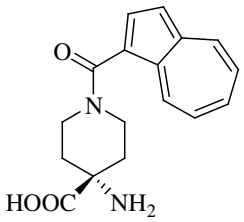
Among $C_i^{\alpha} \leftrightarrow C_i^{\alpha}$ cyclized, C^{α} tetrasubstituted α-amino acids, few example of nitrogen containing ring are reported in the preparation of peptide, *i.e.* the achiral

piperidine-4-amino-4-carboxylic acid Api **149** and derivatives,[67] and its correspnding *N*-oxide (TOAC[68,69]).

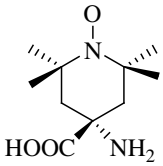
Figure 32



149



150



151

3.2 Synthesis of peptides

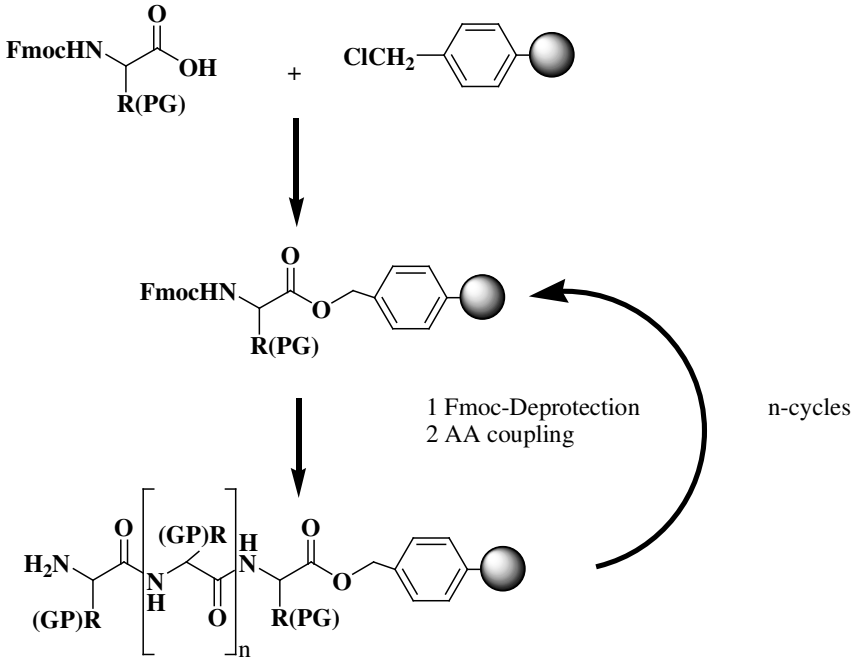
The synthesis of peptides containing multiple α,α-bisubstituted amino acids are inherently difficult due to severe steric hindrance caused by tetrasubstitution at the α-carbon. For this reason, studies on the synthesis of peptides rich in ABO, both in solid phase and in solution, were performed. This part of my research was developed in collaboration with the German group of Dr. Chiara Cabrele of the University of Regensburg (Germany) who introduced me in this field of chemistry. Peptide sequences can be synthesized by two different techniques:

(i) solid phase synthesis (SPPS)
(ii) solution phase synthesis (SPS)

Each of these methodologies has intrinsic advantages and disadvantages and it is commonly thinking that one is the complement to the other one.
Even if the most useful techniques for the chemical peptide synthesis is the solid phase, especially when it is necessary to synthesized long peptide sequences, the use of a very steric hindered amino acid residues generally gives better reaction yields operating in solution.
In the solid phase methodology using a Fmoc strategy, the peptide grows up over a solid polymeric support and therefore, after each coupling step, the by-products are

simply removed by filtration and washing. Furthermore, because of the repetitive nature of this synthesis (deprotection, washing, coupling, washing, deprotection, …), the use of an insoluble support in a single reaction vessel allows the easy automatization of the process.

Scheme 25

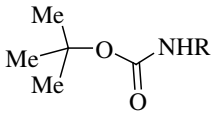


In contrast, the solution phase synthesis is not so versatile. In fact, the peptide grows up in solution and, after each step, the purification of the growing peptide is more complicated. Furthermore all the tedious coupling process cannot be automatized. For these reasons this method found application in the preparation of small peptides or to join pre-synthesized peptide sequences.

In particular, this synthetic methodology presents some advantages since the coupling of "difficult sequences" can be better controlled. This methodology is indicated for the coupling with steric hindered amino acids for which the yield of coupling is very low. Solution phase synthesis is based on the use of the acid-labile *tert*-butyl carbamoyl (Boc) group for the protection of the α-amino group, thereby

allowing the orthogonal protection of side-chain groups through the use of a basic-labile protection.

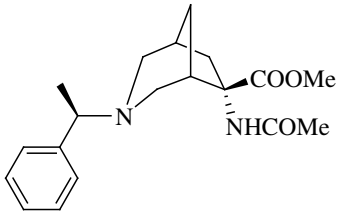
Figure 33



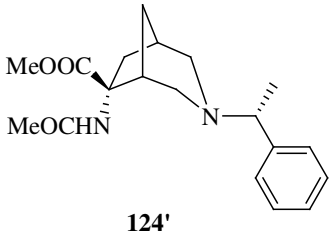
3.2.1 ABO in peptide synthesis

In the preliminary studies we chose to start from the more available enantiopure compounds *exo* **124** and **124'** which were used as α-amino acids.

Figure 34



124



124'

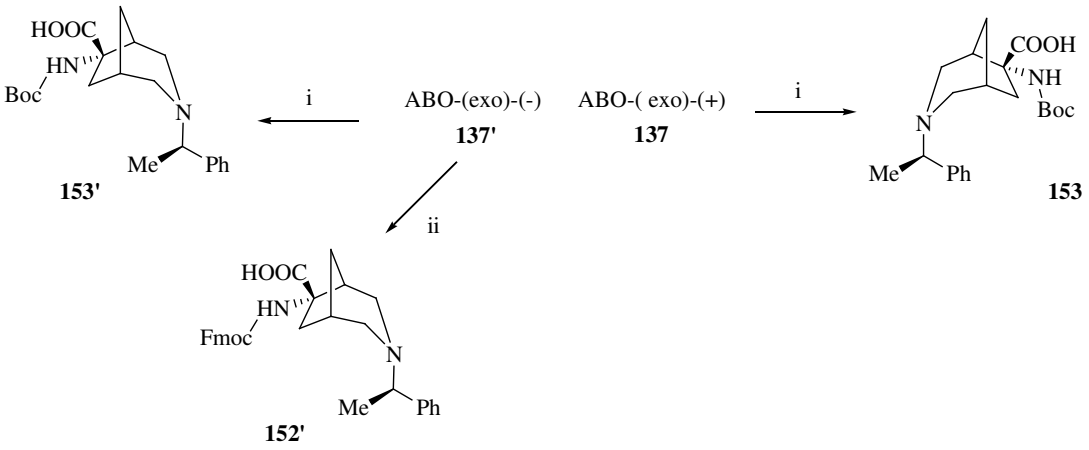
*3.2.2 Use of **124*** or ***124'*** *as α-amino acids.*

It is well known that the N-acetyl and the O-methyl protecting groups were not useful as protecting groups for the peptide synthesis, due to the too hard cleavage condition. For this reason, we replaced these groups with more useful functions.

As reported before in *scheme 23* starting from **124** and **124'**, the hydrolysis of both amino and carboxy groups give compounds **137** and **137'**. In order to investigate the behaviour of the ABO scaffold both in solid phase and in solution phase peptide synthesis, we prepared compounds the Fmoc derivatives **152',** and the Boc protected

compounds **153'** and **153** starting from compounds **137'**. The free amino function was protected using Fmoc-OSu in presence of an aqueous solution of $NaHCO_3$ and 1,4-dioxane. The reaction works overnight at 25 °C, giving the corresponding compounds **152'** (70%). Alternatively, we protected the free amino function of the compounds **137** and **137'** using Boc-anhydride in aqueous NaOH and t-BuOH. The solution was stirred at 25 °C overnight giving the corresponding compounds **153** (60%) and **153'** (58%), respectively.

Scheme 26: Boc and Fmoc Protection[a]

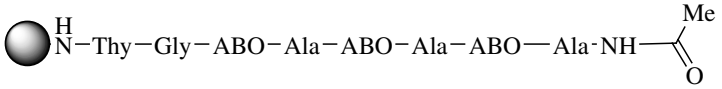


[a] i) Boc anhydride in a Bu^tOH/NaOH aqueous solution, 25 °C, overnight; ii) FmocOSu (N-(9-Fluorenylmethoxycarbonyloxy) succinimide) in dioxane solution/$NaHCO_3$ aqueous solution, 25 °C, overnight

3.2.3 Solid phase peptide synthesis (SPPS)

In order to understand the reactivity of compound (-)-exo-FmocABO **152'** by the solid phase coupling condition, we planned the synthesis of the sequence **154**.

Figure 35

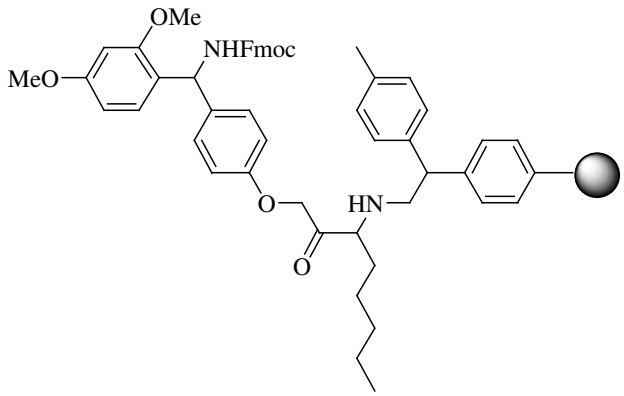


154

In the sequence **154,** thyrosine was inserted making possible to calculate the exact concentration of the peptide in solution by a simple UV/VIS analysis, since is know its ε (estintion molar coefficient). Furthermore glycine, was the spacer between the thyrosine and the peptide, containing the ABO-Ala sequence.

The synthesis of **154** (*Figure 36*) was carried out by manual solution coupling on Rink amide (MBHA resin (loading 0.58 mmol g^{-1}) which is in the Fmoc-protected form. For this reason, to obtain N-free linker, the resin was first treated with piperidine in DMF/NMP (80:20 v/v).

Figure 36 Rink amide MBHA resin



The natural amino acids of this sequence (L-Tyr, L-Ala and Gly), were coupled activating *in situ* the carboxylic function with HBTU (3.9 equiv.), HOBt (4 equiv.) in DMF in the presence of DIPEA (8 equiv.). The (-)-exo-FmocABO was coupled using different conditions, *i.e.* HBTU (2.2 equiv.), HOAt (2.2 equiv.), instead of HOBt, in DMF in the presence of DIPEA (5 equiv.). Every coupling reaction is also followed by an acetylation reaction to block the incomplete coupling reaction. Fmoc cleavage was accomplished by treating the peptidyl-resin with 20% piperidine in DMF.

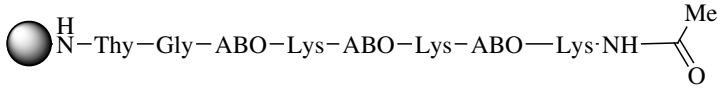
To control the progress of the synthesis, small scale cleavage was performed and the resulting sample was analyzed by MALDI-TOF-MS. The last step of the synthesis was the N-terminal acetylation using acetic anhydride (5 equiv.) and DIPEA (2.5 equiv.). Final cleavage of the peptide from the resin and simultaneous side-chain

deprotection was achieved by treatment with a TFA/water/triisopropylsilane (TIS) mixture (90:5:5) for 2.5 hours.

The purification of the peptide was difficult. First the peptide mixture was treated with cold diethyl ether, centrifugation and further three ether-washing/centrifugation cycles to remove the scavengers. In these steps we have many problems since our final compound **154** is a very apolar compound which was partially soluble in ether. For these reasons we obtained a dirty mixture and in few amount. The mass analysis reveal the presence of the expected product but other picks corresponding to the incomplete ABO coupling were also detected.

To overcome this problem we tried to synthesized the sequence **155** in which we replaced the L-alanine with a L-lysine aiming to increase the polarity end to promote the precipitation process. Unfortunately, we were not able to obtain sequence **155** probably for steric problems.

Figure 37

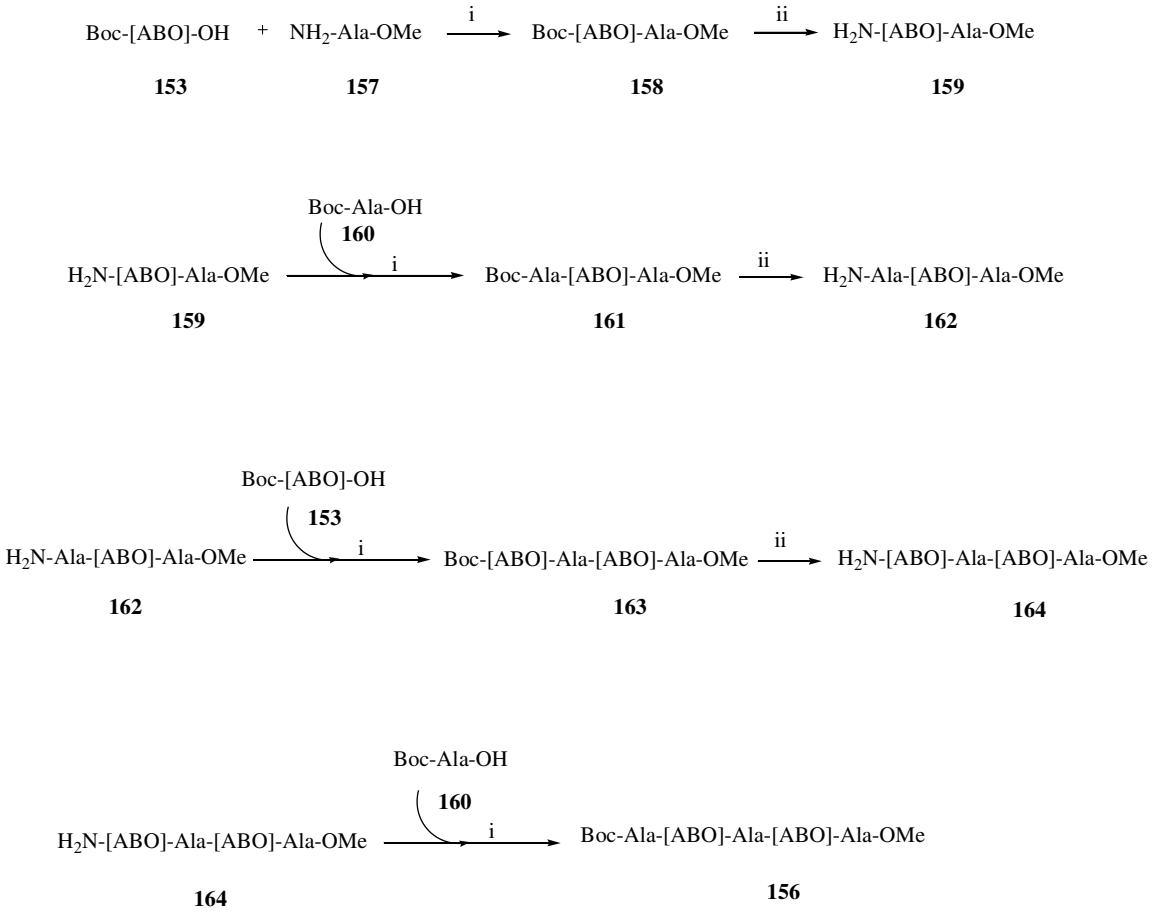


155

3.2.4 Solution phase synthesis (PSS)

To better study the ABO reactivity and the yield of each coupling step, we have developed the synthesis of a short peptide sequence using a Boc solution phase synthesis. We started to synthesized the short penta-aminoacidic sequence **156** containing the repetition of ABO-Ala.

Scheme 27[a]



[a] reaction conditions: i) EDC (10 eq.)/HOBT (10 eq.)/DIPEA(15 eq.) in CH_2Cl_2, 25 °C for 2 days; ii) TFA/CH_2Cl_2 (1 : 2), 25 °C for 24h.

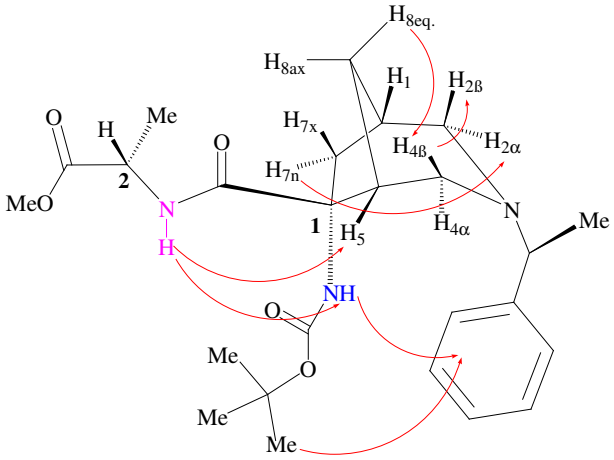
Each coupling step was performed in CH_2Cl_2 using as coupling reagents EDC (10 eq.), HOBT (10 eq.) and DIPEA(15 eq.). The coupling reactions works in 2 days allowing to give pure peptide after washing the crude mixture with water, drying over Na_2SO_4 and column chromatographic purification on SiO_2. The Boc-cleavage was performed using a solution of TFA/DCM (1:2, reaction time 24h). After neutralization the product was extract and analyzed without further purification. Using this synthetic protocol we synthesized step by step compound **156**.

3.3 Spectroscopical characterization of ABO-peptides

Peptides **158**, **161**, **164**, and **156** were studied performing analytic and spectroscopic experiments. The mass (ESI) experiments confirmed the molecular weight of each peptide.

The dipeptide **158** (*Figure 38*) was characterized by spectroscopic data (^{1}H NMR, ^{13}C NMR, COSY, NOESY; Hector). The results are reported in *Table 6* .

Figure 38



158

Table 6 NMR data of the dipeptide **158**

Residue	**atom**	**δ^{1}H**	**Multiplicity *J* (Hz)**	**δ ^{13}C**	**Noesy/Roesy**
1	CO			175.0	
	1	2.18-2.08	overlapped	34.3	
	2α	2.83-2.75	m	56.5	*Me*CH(m), H-7n(vw)
	2β	2.18-2.08	overlapped		
	4α	2.58	d, *J* 10.6	53.3	H-5(w)
	4β	1.91 d	*J* 11.4		H-5(m), H-2β(w)
	5	2.3	brs	41.0	H-4α(w)
	6			66.1	
	7x	2.5		39	
	7n	1.60	d, *J* 13.2	overlapped	H-2α(vw),
	8a	1.69	brs	36.3	
	8e	1.15	d, *J* 7.3		H-4β(m), H-5
	Ph*CH*	3.38-3.36	m	63.7	H-5
	*Me*CH	1.26	d, *J* 6.5	20.1	H-2α(s), H-4α(vw)
	Arom	7.35-7.20	m	127.3, 127.8 128.9, 144.9	*Me*CH(s), *Me*C, H-4β(vw), H-4α(w)

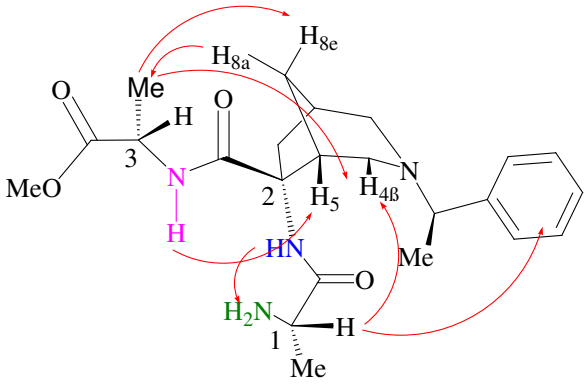
	NH	6.28		brs		Arom, NH_{Ala}(vw), H-7n, H-4α
	Boc	Me	1.40	s	28.8	
		C			78.7	
		CO			155.7	
2	CO				173.7	
	CH	4.25-4.19		m	64.7	Me_{Ala}
	Me	1.21		d, *J* 6.7	17.8	
	NH	7.67		d, *J* 7.5		H-5, CH_{Ala}, Me_{Ala}, NH_{ABO}(vw)
	OMe	3.58		s	52.4	

In particular, NOEs effects were observed between H-4β and H-2β, between H-8e and H-4β and between H-7n and H-2α. These spatial proximity allowed to assigned unequivocally the chemical shift to each proton of the ring. These results are in agreement with the NOEs observed for simple ABO compounds [70] where H-2α, H-4α, H-7x and H-8a resonate at low field with respect to their counterpart. This observation was used for the assignment of the ABO-protons for the other peptides when the certain stereochemistry could not be assigned since the signals are overlapped.

Spatial proximity was also observed between the two NH, between NH_{Ala} and H-5 and between both the methyl and NH of Boc group and aromatic protons.

The NMR data of compound **161** were reported in *Table 7*. The more significant NOEs correlations are resumed in the *Figure 39*.

Figure 39 Tripeptide **161**



161

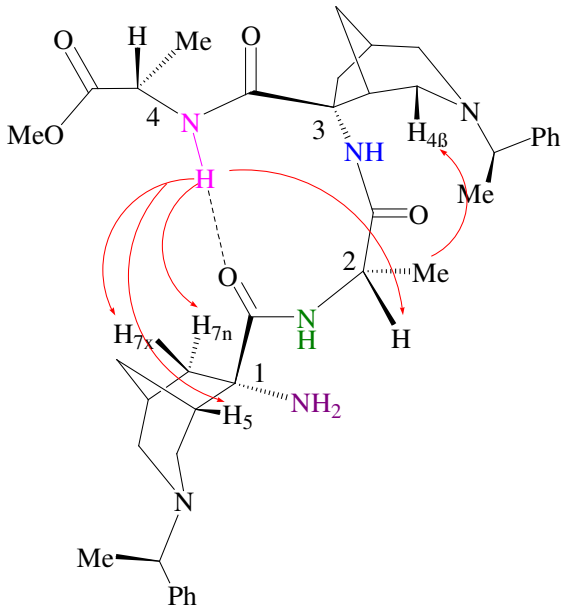
Table 7 the NMR data of the tripeptide **161**

Residue	atom	δ ^{1}H	Molteplicity *J* (Hz)	δ ^{13}C	Noesy
1	CO			173.0[1]	
	CH	3.44	q, *J* 6.9	49.9	Arom(7.35,w), H-4(2.38,s; 2.72,vw), Me(1.33)
	Me	1.33	d, *J* 7.0	20.7	
	NH_2	1.70	overlapped		NH_{ABO}(8.50-7.80,vw)
2	CO			176.3[1]	
	1	2.25	brs	32.9	H-8e(1.15,s)
	2α	2.80	brs	54.2	
	2β	2.33	d, *J* 10.1		
	4α	2.72	d, *J* 13.4	52.4	Arom (7.36,vw)
	4β	2.38	d, *J* 11.5		Arom (7.36,m)
	5	2.90	brs	40.1	NH (7.49), Me_{Ala3}(1.38,s), H-8(2.1,s), H-4(2.72,w),
	6			65.7	
	7x	2.69	d, *J* 13.4	39.4 brs	
	7n	1.90-1.70	m		
	8a	2.20-1.98	m	35.4	Me_{Ala3}(1.38,w), H-1
	8e	1.38	overlapped		Me_{Ala3}(1.38,w)
	Ph*CH*	3.66	q, *J* 6.4	63.4	*Me*CH(1.44)
	*Me*CH	1.44	d, *J* 6.4	15.5	
	Arom	7.42-7.33(*o,m*) 7.33-7.28 (*p*)	m	141.4(C), 127.6, 127.3, 126.7(*p*)	*Me*CH(1.44), H-4(2.38,s, 2.72,vw), CH_{Ala1} (3.44)
	NH	8.50-7.80			CH_{Ala1}(3.44, vw), NH_2(1.70,vw)[2]
3	CO			172.9[1]	
	CH	4.52-4.46	m	47.5	NH_{Ala3}(7.45,s), Me (1.38,s)
	Me	1.38	d, *J* 7.1	17.4	NH_{Ala3}(7.45,s), H-8, H-5(w)
	NH	7.45	d, *J* 6.9		H-5(m), H-7, Me_{Ala3}(1.38,s), CH_{Ala3}(4.50,m)
	OMe	3.72	s	51.5	

[a] spectra in $CDCl_3$ [1]The carbonyl chemical shifts are tentatively assigned; [2]exchange pick.

To achieve the better resolution regarding the proton chemical shifts of tetrapeptide **164**, differents solvent were tested ($CDCl_3$, CD_3CN) and CD_3CN was then selected. Using NMR analyses (^{1}H, ^{13}C, TOXY, COSY, HSQC, NOESY and HMQC, see *Table 8*) we first unequivocally assigned all chemical shifts to each atom.

Figure 40 Tetrapeptide **164**



164

Table 8 the NMR data for the Tetrapeptide **164**

Residue	atom	δ ^{1}H	Molteplicity *J* (Hz)	δ ^{13}C	Noesy	HMBC
1 **Broad**	CO			173.2		
	1	1.80-1.70	brs	33.1		
	2α	2.90-2.78	brs	54.9 brs		
	2β	2.25	overlapped		*Me*CH$_{ABO1}$(1.35)	
	4α	2.52	d, *J* 11.7	52.3	PhC*H*$_{ABO1}$(3.59-3.51), *Me*CH or H-8	
	4β	2.17	overlapped			
	5	2.58	brs	40.2	H-8a$_{ABO1}$(1.30), H-8a$_{ABO1}$(1.96)	
	6			65.2 brs		
	7x	1.77		39.9 brs	H-7n$_{ABO1}$(2.41,vw)	
	7n	2.41	d, *J* 12.9		H-7x$_{ABO1}$(1.77,vw)	
	8a	1.30	overlapped	35.3		
	8e	1.96	overlapped			
	Ph*CH*	3.59-3.51	brs	63.9 br	*Me*CH$_{ABO1}$(1.36,s), H-4α$_{ABO1}$ (2.52,w)	
	*Me*CH	1.39-1.34	brs	16.3		
	Arom	7.40-7.28	m	127.9-126.1 (C)142.9		
	NH_2	2.25[1]	overlapped			
2	CO			171.9		CH$_{Ala2}$(4.15, vw), Me$_{Ala2}$(1.33s)
	CH	4.24-4.15	m	48.5	Arom(7.36, 7.31), Me$_{Ala2}$(1.33,s), NH$_{Ala4}$(7.08,vw), NH$_{Ala2}$(7.82,s)	

	Me	1.33	d, *J* 7.0	16.9	CH_{Ala2}(4.24-4.15,s)	CH_{Ala2}(4.24-4.15,w)
	NH	7.82	brs		CH_{Ala2}(4.24-4.15,s)	
3 Sharp	CO			177.1		H-7x_{ABO3}(2.40)
	1	2.22	brs	34.3		
	2α	2.99	dd, *J* 10.4, 3.9	55.5	arom (vw), Ph*CH*$_{ABO3}$(3.48,m), H-7n $_{ABO3}$(1.46,m), Me_{ABO3}(1.40,s)	C-4$_{ABO3}$(52.8,w)/C-8$_{ABO3}$(vw), H-7x_{ABO3}(2.40,s)
	2β	2.29	d, *J* = 10.3			
	4α	2.78-2.73	m	52.8	H-4β$_{ABO3}$(2.06), Ph*CH*$_{ABO3}$(3.48), *Me*CH_{ABO3} (1.40)	Ph*CH*$_{ABO3}$(w), H-2α$_{ABO3}$(2.99,w)
	4β	2.06	d, *J* 11.1		Me_{Ala2}(1.33,m), Ph*CH*$_{ABO3}$(3.48,s), H-4α$_{ABO3}$(2.75,s), Arom (7.36w)	C-8$_{ABO3}$(36.7,vw)
	5	1.97	overlapped	42.8		H-4α$_{ABO3}$(2.75,w)
	6			64.1		H-7x_{ABO3}(2.40), H-4β$_{ABO3}$ (2.06)
	7x	2.40	d, *J* 12.9	43.0		H-2β$_{ABO3}$(2.29,s)
	7n	1.46	dd, *J* 13.0, 2.0		H-2α$_{ABO3}$(2.99,m)	C-8 $_{ABO3}$(36.7), C-2(55.5,vw)
	8a	2.18	overlapped	36.7		H-2β$_{ABO3}$(2.29,w), H-4β$_{ABO3}$ (2.06,w), H-7n$_{ABO3}$(1.46,w)
	8e	1.35	overlapped			
	Ph*CH*	3.48	q, *J* 6.8	64.0	H-2β$_{ABO3}$(2.99,m), H-2α$_{ABO3}$(2.29,m), H-4α$_{ABO3}$ (2.75,m), H-4β$_{ABO3}$(2.06,m), *Me*CH_{ABO3}(1.40)	H-4β$_{ABO3}$(2.06), H-7x_{ABO3} (2.40), Me $_{ABO3}$(1.40)
	*Me*CH	1.40	d, *J* 6.8	17.5	Ph*CH*$_{ABO3}$(3.48), H-2α$_{ABO3}$ (2.99,s), H-4α$_{ABO3}$(2.75)	Ph*CH*$_{ABO3}$ (3.48,vw)
	Arom	7.40-7.28 7.23-7.18 (*p*)	m	127.9-126.1 (C)142.9		
	NH	7.40-7.28				
4	CO			172.5		CH $_{Ala4}$ (4.22), Me_{Ala4} (1.28), OMe(3.62)
	CH	4.31-4.24	m	47.4	Me_{Ala4}(1.28,s), NH $_{Ala4}$(7.08)	CO_{Ala4}(172.5), Me_{Ala4}(1.28)
	Me	1.28	d, *J* 7.2	15.8	CH_{Ala4}(4.28,s)	CO_{Ala4} (4.31)
	NH	7.08	brs		CH_{Ala4}(4.28,s), CH_{Ala2}(4.24-4.15,vw), H-5$_{ABO1}$(2.58,m), H-7$_{ABO1}$(2.40,w), H-7$_{ABO1}$(1.77,vw), Me_{Ala4}(1.28,s)	
	OMe	3.63	s	50.8		CO_{Ala4}(172.5)

[1] positive NOESY signal cross-peak between the shift of exchanging site.

In particular, it is evinced that the signals of two ABO moieties, both in ^{1}H and ^{13}C experiments, possess a different shape, one of them is characterized by sharp signals, the other by broad signals. These data suggest that one of the ABO scaffold is present in different conformations.

Concerning to the broad signals, our hypothesis is that an electronic repulsion between the two basic nitrogen, the nitrogen of the ring and of NH_2 of ABO-1, exists inducing an equilibrium between the chair-envelope and the boat conformations of piperidine ring in the ABO which is reported in the literature having in general a chair conformation with the nitrogen substituent in the equatorial position.[70]

HMQC experiment allowed to assign unequivocally the chemical shift of the carbonyl group of each alanine because a correlation with both CH and Me of each corresponding alanine was observed. The carbonyl group of the ABO-3 resonates at δ 177.1 and posses a long range correlation with H-7_n of ABO-sharp. As a consequence the signal at δ 173.2 was assigned to ABO-1. Concerning NH signals, COSY and TOXY experiments allowed to assigned unequivocally the correlation with the corresponding alanine moiety. Instead, the NH_{ABO-3} is overlapped to the aromatic protons.

The ability of each NH to be involved in an intramolecular hydrogen bond was evaluated both at variable temperature and by calculating their exchange rate with D_2O.

The exchange process respect a second kinetic order (equation 2) but, considering constant the $[D_2O]$ concentration (large excess), the system was simplified to a first apparent reaction order (equation 4 and 5).

2) $\upsilon = k\,[NH]\,[D_2O]$

3) $k_{app} = k\,[D_2O]$

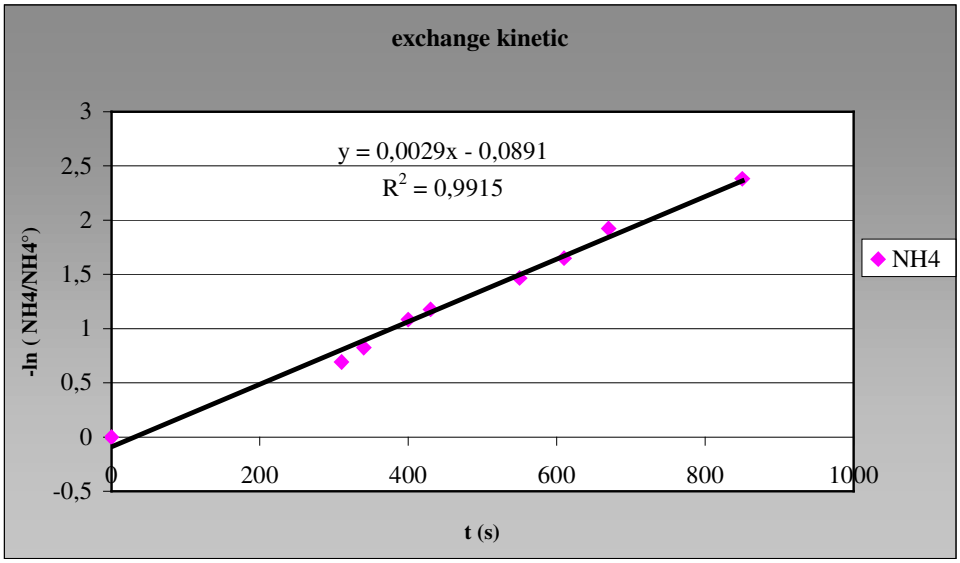
4) $\upsilon = k_{app.}\,[NH]$

5) $-\ln([NH]/[NH^{\circ}] = k_{app.}\,[NH]$

Here below is reported the graphic representation of the equation 5 for the NH-4 considering the decreased value of the NH integral against the time (*Graphic 1*).

Instead, NH-2 was not detected because the proton was totally exchanged after two minutes.

Graphic 1[a] titration data of NH-4 of tetrapeptide **164**



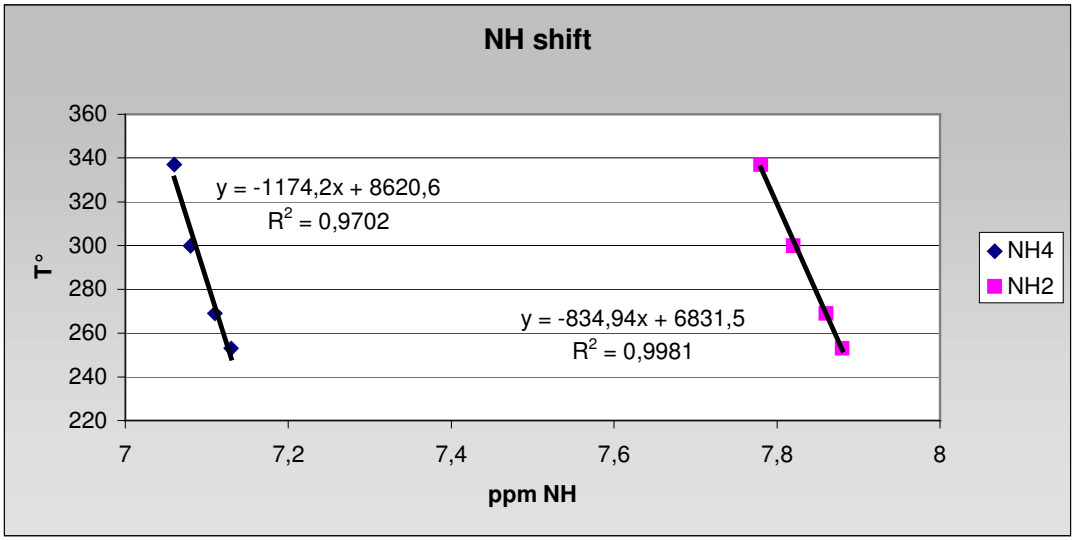
[a][peptide **164**] = 14,8 mM in CD_3CN (700 µL) then D_2O (20 µL), T = 299.94 K

The value of the k_{app} for NH-4 ($3 \cdot 10^{-3}$ $s^{-1}M^{-1}$) at T = 299.94 K revels a very week hydrogen bond interaction, compared to the corresponding value found for the corresponding NH-5 in the pentapeptide **156** [k_{app} for NH-5 ($6 \cdot 10^{-5}$ $s^{-1}M^{-1}$), see below].

The variation of NH chemical shift with respect to the variation of the temperature is a very useful method to evaluate the presence of an hydrogen bond and its stability. ^1H NMR spectra of compound **164** in CD_3CN were recorded at different temperature (from 220 K to 440 K). The δ NH values *versus* the temperature for NH-4 and NH-2 (the signals NH-3 and NH_2 are overlapped) are reported in *Graphic 2*.

By interpolating the experimental values, using the linear equation y = mx + b, the 1/m parameter was calculated from which it is evinced that $\Delta\delta/\Delta T$ = - 0.85 ppb/K for NH-4, and $\Delta\delta/\Delta T$ = - 1.20 ppb/K for NH-2.

Graphic 2[a]



[a] δNH against the temperature (from 253 K to 337 K) of [peptide **164**] = 14,8 mM in CD_3CN (700 µL).

These data, together with those of hydrogen exchange, are indicative of an equilibrium between an intramolecular hydrogen bonded status and a non-hydrogen bonded status for NH-4, and that NH-1 is not involved in hydrogen bond.
Interesting NOE effects were detected which confirm a possible involvement of NH-4 in a hydrogen bond with CO of ABO-1.
Furthermore positive Overhauser effects were detected between CH_{Ala2} and NH_{Ala4} [$d_{\alpha,N}(i, i+2)$, vw], which is in general, diagnostic for the presence of a turn which confirms spatial proximity between ABO-1 and Ala-4 moieties, and between NH_{Ala4} and H-7 and H-5 of ABO-1.

The secondary structure of pentapeptide **156** was deeply studied using NMR technique. To achieve the better resolution regarding the proton chemical shifts, different solvent were tested (DMSO, $CDCl_3$, CD_3CN, CD_3OH) and CD_3CN was then selected. Using NMR analyses (^1H, ^{13}C, TOXY, COSY, HSQC, NOESY and HMQC, *Table 9*) we first unequivocally assigned all chemical shifts to each atom as well as the sequence of the amino acids. Finally, NMR studies at variable temperature as well on the *NH*-exchange rate were performed.

Figure 41: Noesy peptide **156**

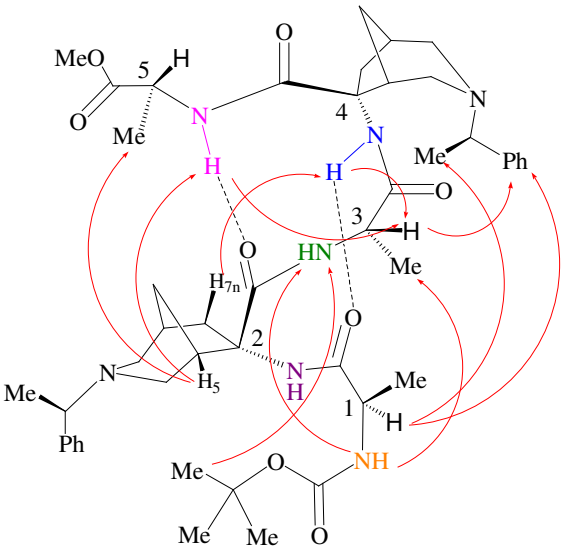


Figure 42: 3D peptide **156**

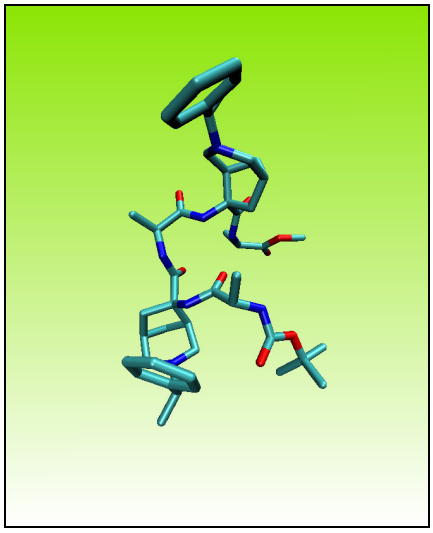


Table 9 the NMR data for the Pentapeptide **156**

Residue	atom		δ ^{1}H	Multiplicity *J* (Hz)	δ ^{13}C	Noesy	HMBC
1	CO				173.6		CH(4.05), Me(1.39)
	CH		4.05	overlapped	51.1	*o*Arom. $_{ABO2}$ (7.42), Me$_{Ala1}$ (1.39), Ph*CH*$_{ABO-4}$ (3.65)	
	Me		1.41-1.37	overlapped	16.4		
	NH		6.03	s		Arom. (7.42), CH$_{Ala1}$(4.05,m), Me(1.39,s), (*Me*)$_3$C(1.50,w), NH $_{Ala3}$(7.49,vw)	
	Boc	Me	1.50	s	26.9	NH$_{Ala3}$(7.49,w), *m*Arom$_{ABO2}$ (7.32), *o*Arom $_{ABO2}$(7.42)	
		C			79.2		Me(1.50)
		CO			158.8		CH(4.05), Me(1.39)
2 broad	CO				175.6 brs		
	1		2.17	overlapped	33.1		
	2α		2.68	overlapped	53.7 brs		H-4(2.58), H-1(2.17)
	2β		2.37	overlapped		*o*Arom $_{ABO2}$(7.42)	
	4α		2.59	dd, *J* 8.5, 3.2	51.7	*o*Arom$_{ABO2}$(7.42,m), Ph*CH*(3.80,m), H-5(2.75,m), H-2 or/and H-4 (2.37,s), Me(1.43,m)	C-8(36.3), C-5(39.7), C-2 (53.5)
	4β		2.37	overlapped			

	5	2.75	brs	39.7	NH_{Ala5}(7.50,vw), H-4β(2.37), H-4α(2.59)	H-4(2.58)
	6			64.9 brs		
	7x	2.18	overlapped	41.7 brs		
	7n	1.86-1.78	brs		H-2α (2.65vw), NH_{Ala3}(7.49w)	
	8a	1.38	overlapped	36.3		
	8e	2.18	overlapped			
	Ph*CH*	3.80	brs	62.9	*o*Arom.$_{ABO2}$(7.42), H-4α(2.59), H-2/H-4(2.35), Me(1.43)	
	*Me*CH	1.43	brs	14.0 brs	Ph*CH* (3.80)	
	Arom	7.44-7.40(*o*) 7.36-7.28 (*m,p*)	m	127.6(*o*)126.6(*o*) (CH), o140.9(C)	7.42 (*o*Arom), PhCH_{ABO2}(3.80,w), H-2 or H-4 or H-5(2.69,w), H-4α_{ABO2}(2.59,w), H-2 or H-4$_{ABO2}$(2.37), H-7n$_{ABO2}$(1.77,vw)	7.42 (*orto*Arom) *Me*CH(1.43)
	NH	7.36	overlapped		H-7n(1.77), H-4α(2.59,w)[1]	
3	CO			171.6		CH(4.06), Me(1.42)
	CH	4.06	overlapped	50.3	Arom. (7.29), NH_{Ala5}(7.50 vw), $MeAla_3$(1.42)	
	Me	1.42	overlapped	15.4		
	NH	7.49 scambio veloce	brs		Me_{Ala3}(1.42), CH_{Ala1}(4.06,s), H-7n$_{ABO2}$(1.86), H-5$_{ABO2}$(2.75,vw), NH_{Ala1}(6.03,vw)	
4 sharp	CO			174.9		NH_{Ala5}(7.50), CH_{Ala5}(4.22), CH_{Ala3}(4.06)
	1	2.09	brs	33.1	H-8e(1.15,m)	H-2(2.18), H-5(2.65), H-8(2.28, 1.15); C-6(65.4)
	2α	2.69	overlapped	55.9	NH_{ABO4}(7.55)	H-8(1.15), H-5(2.65), Ph*CH*(3.65)
	2β	2.18	overlapped			
	4α	2.68	overlapped	49.7		
	4β	2.06	*J* 11.0		H-8e(1.15), Ph*CH*(3.65) *o*Arom$_{ABO4}$(7.34)	
	5	2.65	overlapped	40.2		
	6			65.4		H-8(1.15), H-7(2.16), H-4(2.06)
	7x	2.18	overlapped	40.5		H-8(1.15)
	7n	2.18	overlapped			
	8a	2.18	overlapped	36.6		
	8e	1.15	d, *J* 10.9		H-4ß(2.06), H-1(2.09), H-5(2.65)	C-1/33.1), C-7(40.5), C-4(49.7), C-2(55.9), C-6(65.4)

	Ph*CH*	3.65	q, *J* 7.7	62.9	*o*Arom$_{ABO4}$(7.34), H-4β (2.06), H$_{Ala1}$(4.05w), H-2β (2.69s), *Me*CH (1.40)	Arom(7.3), Me(1.40), C-4(49.7), C-2(55.9), *Me*CH(16.4)
	*Me*CH	1.41-1.39	overlapped	16.4		
	Arom	7.44-7.40(*m*) 7.36-7.28 (*o*), 7.27-7.22(*p*)	m	126.0(*p*)128.0-126.0 (CH), 140.8(C)	*m*Arom$_{ABO4}$7.42: CH$_{Ala3}$(4.06 w) *o*Arom$_{ABO4}$7.34: Ph*CH*$_{ABO4}$(3.65), H-4β $_{ABO4}$2.06),	140.8: *orto*Arom(7.34) Me(1.39), Ph*CH*(3.65)
	NH	7.55	brs		CH$_{Ala3}$(4.06s), H-4α(2.68),	CO$_{Ala3}$(171.6)
5	CO			172.6		CH(4.22), Me(1.33), OMe(3.62)
	CH	4.25-4.19	m	47.6	NH(7.50), Me(1.33)	
	Me	1.33	d, *J* 7.3	15.8	NH(7.50), CH(4.22), H-5$_{ABO2}$ (2.75vw)	
	NH	7.50	brs		CH$_{Ala5}$(4.22,s), Me$_{Ala5}$(1.33,s), CH$_{Ala3}$(4.06,w)	
	OMe	3.62	s	50.6		

As for pentapeptide **164**, it is evinced that the signals of two ABO moieties, both in ^{1}H and ^{13}C experiments, possess a different shape, one of them is characterized by sharp signals, assigned to ABO-4 the other by broad signals assigned to ABO-2.

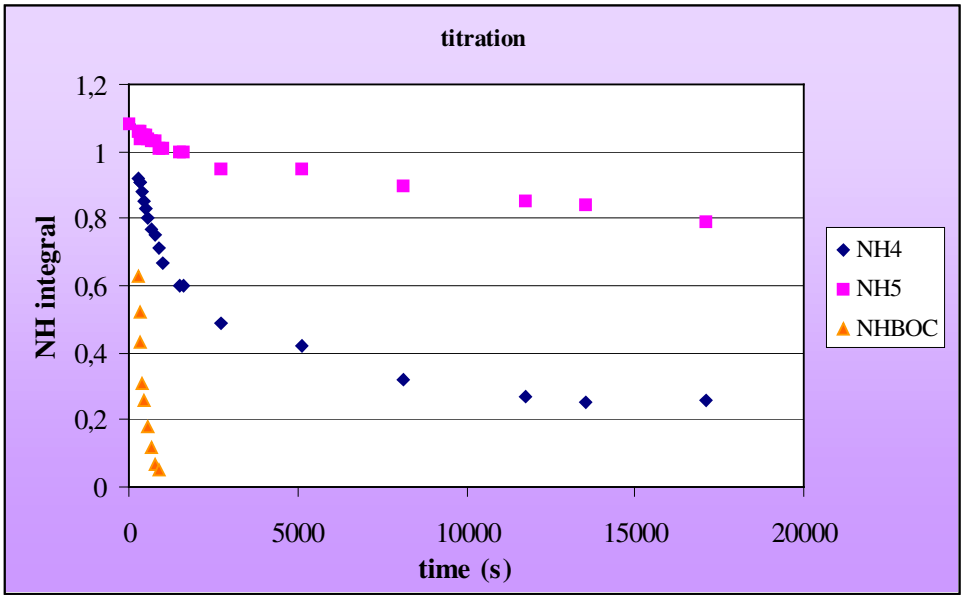
HMQC experiment allowed to assigned unequivocally the chemical shift of the carbonyl group of each alanine because of its correlation with both CH and Me of the some alanine moiety. The carbonyl group of the ABO-4 resonates at δ 174.9 and possess a long range correlation with NH$_{Ala5}$(δ 7.50), CH$_{Ala5}$(δ 4.22), as well as with CH$_{Ala3}$(δ 4.06). Concerning NH signals, COSY and TOXY experiments allowed to assigned unequivocally the correlation with the corresponding alanine. Instead, a correlation was observed between NH$_{ABO4}$ at δ 7.55 and CO$_{Ala3}$ (δ 171.6) which is correlated both to CH (δ 4.06) and Me (δ 1.42) of its alanine moiety. As a consequence, the chemical shifts at δ 175.6 (brs) was assigned to the carbonyl carbon of ABO-2 and at δ 7.36 (overlapped) at NH$_{ABO-2}$.

The NOESY experiments (only certain significative of non-overlapped signals are reported), gave very interesting informations concerning the δ values of ABO protons and their spatial relationship. Concerning protons of ABO-4, positive

Ovehauser effects were observed between H-4β (δ 2.06) and H-8e (δ 1.15), and between H-4α (δ 2.68) and NH (δ 7.55), thus assuring the NH assignment for ABO-4. Instead, no informations are given regarding H-2 since they are overlapped. The δ values are tentatively assigned to these protons considering the data available from the simple ABO- 3 and the other peptides.[33] Concerning ABO-2, NOE effects were found between H-7n (δ 1.86-1.78) and H-2α (δ 2.68; this signal is overlapped but it is the sole one belonging to ABO-2) and between NH (δ 7.36) and both H-7n and H-4α (δ 2.59) indicating their *cis* relationship. Since the two ABO moieties do not have spatial proximity (see model), the *orto* protons of phenyl ring in ABO2 (δ 7.42) confirms the above assigned stereochemistry. In fact, NOE effects were observed between *orto* protons and Ph*CH*$_{ABO2,}$ H-4α$_{ABO2,}$ H-2 or H-4$_{ABO2}$ (overlapped) and H-7n $_{ABO2}$ (1.77 vw). Concerning the aromatic region at δ 7.36-7.28, of relevance for ABO-4 are the spatial proximity between the signal centered at δ 7.34 (*o*Arom$_{ABO4}$) and Ph*CH*$_{ABO4}$ (δ 3.65), and H-4β$_{ABO4}$.

Concerning the titration, this experiment was performed on pentapeptide **156** (16,8 mM in CD_3CN, 700 μL) in presence of D_2O (20 μL). A series of NMR spectra were recorded at 299.94 K at different times (from 0 to 5h). No information were given for NH_{ABO2} because the signal is overlapped with the aromatic signals but, as evidenced in a NOESY experiment of **156** in CD_3CN/D_2O, it easily exchange with D_2O. Furthermore, the kinetic exchanges of NH-3 its so fast that the signal was lost in few minute. Concerning NHBoc, NH-4 and NH-5, integral values were reported in the graphic below (*Graphic 3*).

Graphic 3. Plot of the variation of NH integrals in the ^{1}H NMR spectra of **156** vs. time.[a]

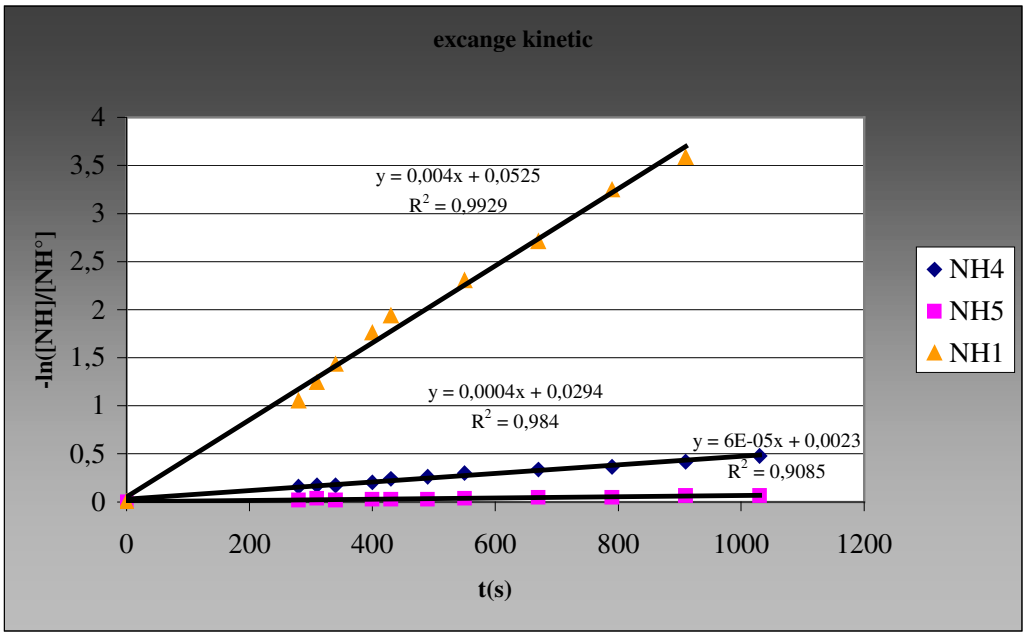


[a][peptide 156] = 16,8 mM in CD_3CN (700 µL) then D_2O (20 µL), T = 299.94 K

From these data it is clear that the NH-5 is involved in a very slow exchange process. In fact, after a 8h, the NH integration area was reduced of about 20%. NH-4 slowly exchange too, but the integration area was reduced of 80% after 8h. Concerning NH-1 of carbamate, even if a comparable kinetic with the other signals is not possible because this proton is different with respect to the others NH, it was found that its deuteration is very fast.

Here below is reported the graphic representation of the pentapeptide **156** titration considering the decreased value of the NH integrals against the time (*graphic 4*).

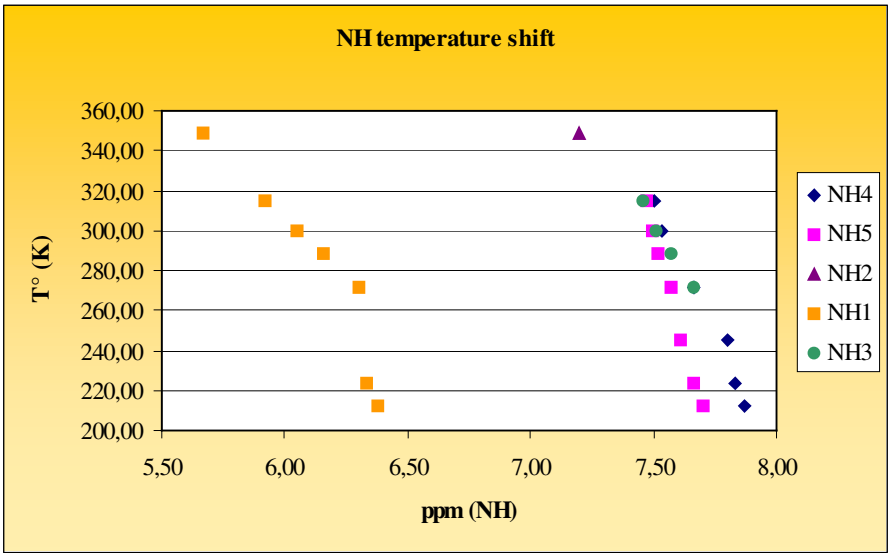
Graphic 4: Plot of the variation of –ln(NH/NH°) of compound **156** vs. time.[a]



[a][peptide **156**] = 16,8 mM in CD_3CN (700 µL) then D_2O (20 µL), T = 299.94 K

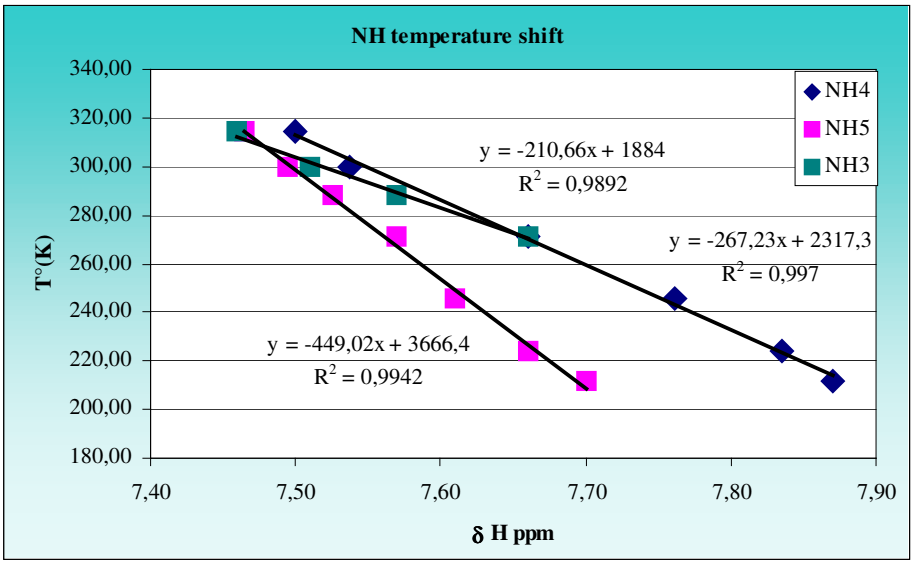
Using *Equation 5* it is possible to calculate the k_{app} for NH-4 ($4 \cdot 10^{-4}$ $s^{-1}M^{-1}$) NH-5 ($6 \cdot 10^{-5}$ $s^{-1}M^{-1}$) and NH-1 ($4 \cdot 10^{-3}$ $s^{-1}M^{-1}$). This means that NH-1 exchanges 10 time faster than NH-4, and NH-4 exchanges 10 time quicker that NH-5 at T = 299.94 K. (*Graphic 4*).The variation of NH chemical shift with respect to the variation of the temperature is also performed. ^1H NMR spectra of compound **156** in CD_3CN were recorded at different temperature (from 212.04 K to 348.33 K) and the results of these experiments confirm the data deduced from titration experiments.The δ NH values *versus* the temperature for all NH, except for NH-2 (overlapped with aromatic hydrogens), are reported in *Graphic 5*. The plot in *Graphic 6* represents the expanded area of NH chemical shifts (from 7.40 to 7.90 ppm) *versus* temperature (from 212.51 K to 314.57 K).

Graphic 5: NH chemical shift against temperature[a]



[a] δNH against the temperature (from 212.04 K to 348.33 K) of [peptide **156**] = 16,8 mM in CD_3CN (700 µL)

Graphic 6: expansion of the *graphic 5*[a]

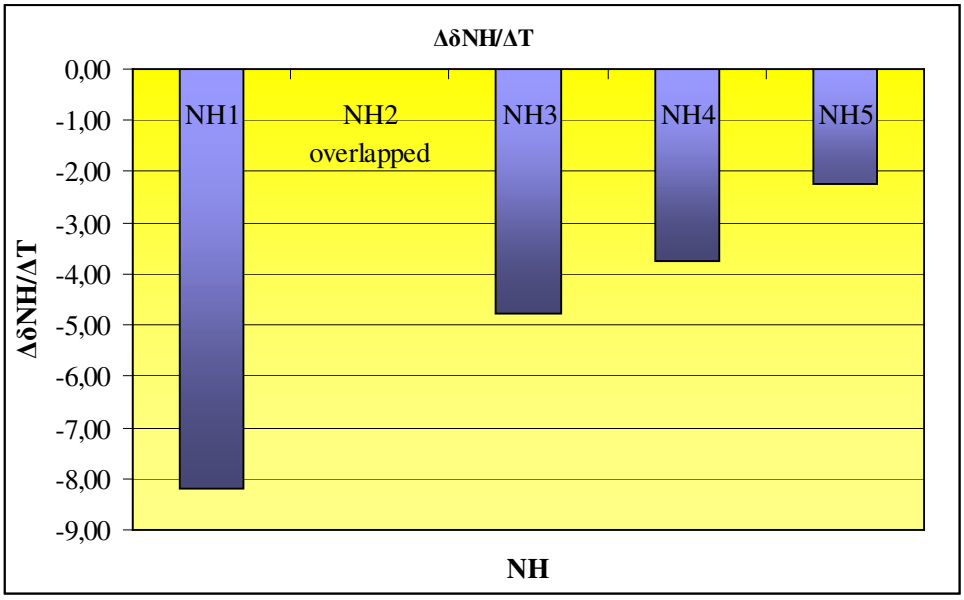


[a] δNH against the temperature(from 212.51 K to 314.57 K) of [peptide **156**] = 16,8 mM in CD_3CN (700 µL).

As you can see the linearity was not maintained in the large range of temperature analyzed (from 212.04 K to 348.33 K). Only in the 271.51 K to 314.57 K range (expansion in *graphic 6*) a linear relationship between the NH shift and the temperature was observed. When the temperature is higher, probably, a "denaturation" process is involved and the peptidic secondary structure change. Instead, at lower temperature we observed the formation of other hydrogen bonds that again could change the secondary structure.

The above data were reported here below in the histogram of ΔδNH/ΔT in which is evident the low δ variation for NH-4 and NH-5. (*Graphic 7*)

Graphic 7 variations of the NH proton chemical shifts for peptide **156** toward the temperature.[a]



[a] ΔδNH/ΔT considering the linearity ΔT (271.51 K to 314.57 K) range values.

In *Table 5* are resumed all the data for the pentapeptide **156.**

Table 5 reports on the resumes data

N-H	δ (ppm)[a]	ΔδNH/ΔT (ppb/K)[b]	k_{app}(1/s) exchange reaction with D_2O[c]
H1	6.03	-8.27	$4*10^{-3}$
H2	7.42	-[d]	-[d]
H3	7.47	-4.76	-[e]
H4	7.55	-3.74	$4*10^{-4}$
H5	7.52	-2.23	$6*10^{-5}$

[a] δ value determined to T° = 299,93(K);

[b] (ΔδNH/ΔT) coefficients were determined between 271 and 314(K) (were a linear dependence of all signals was observed) and [peptide **156**]= 16,8 mM in CD_3CN;

[c] N-H/N-D exchange rate [peptide **156**]= 16,8 mM in CD_3CN (700 μL) D_2O (20 μL)T° = 299,93(K);

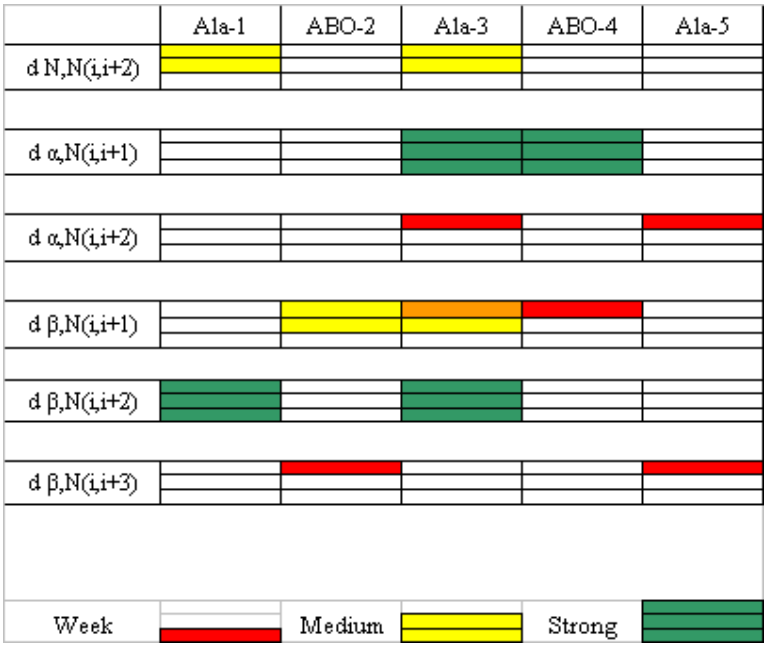
[d] Not determined because overlapped with other resonances;

[e] Not determined because the excange is too fast;

From these data appears that the amide protons NH-5 and NH-4 are in an intermolecular hydrogen-bonded status. As reported in *Table 5*, the temperature dependence of the NH-5 chemical shifts falls within the typical values for intramolecularly hydrogen bonded protons. A slightly higher is observed for NH-4 instead, amide protons NH-1 and NH-3 exhibit a larger temperature dependence as well as a quick deuterium exchange with respect to the other amide protons. Furthermore, the good linearity of the chemical shift vs. temperature (271.51 K to 314.57) of NH-4 and NH-5 indicates the absence of conformational changes over the temperature range swept.[71] Our hypothesis is that NH of ABO-4 is involved in a medium hydrogen bond with the carbonyl group of Ala-1 and that a strong hydrogen bond was effective between NH Ala-5 and the carbonyl group of ABO-2. Since CO_i-NH_{i+3} hydrogen bond are typical of a 3_{10}-helix, this secondary structure was assigned to pentapeptide **156**.[72] This hypothesis was supported by NOESY experiments

which were performed on a sample of **156** dissolved both in CD_3CN and in CD_3CN/D_2O (in this case we ensured the NOE effects for NH_{Ala5} since the other NH disappeared). Interesting informations are given regarding to the secondary structure. Of relevance are the NOESY effects between the NH_{Ala3} (δ 7.49) with NHBoc group (both with Me and NH [$d_{N,N}(i, i+2)$, w]. Positive Overhauser effects were observed between the H-7n of ABO-2 with NH_{Ala3} [dβ,N(i,i+1), w] and between H-5 with both NH_{Ala5} [dβ,N(i,i+3), vw] and Me_{Ala5} (vw). Other NOE contacts of relevance are those of NH_{ABO4} and CH_{Ala3} [dα,N(i,i+1, s], and between this last and NH_{Ala5} [$d_{\alpha,N}(i, i+2$, vw]. The proposed secondary structure is also consisted with the spatial proximity observed between CH_{Ala3} (δ 4.06) and the signals δ at 7.42 (w) and at δ 7.34 assigned to H-*meta*$_{ABO4}$ and H-*orto*$_{ABO4}$, respectively.

Figure 43 Summary of the NOEs-derived backbone obtained for peptide **156** in CD_3CN[a]



[a] Peaks are grouped into three classes based upon their integrated volume

As reported above, differently to the signals of ABO-2, ABO-4 shows broad signals suggesting that more than one conformation is possible for the nitrogen ring in this scaffold. This result is comfirmed by the peptide molecular model in which it is evinced that when the benzyl group of ABO-4 is located in the equatorial position it is more crowded with respect to its location in the axial position.

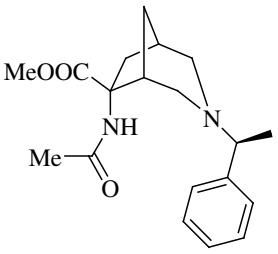
4 Metallorganic ligands

4.1 ABO as a metal ligand

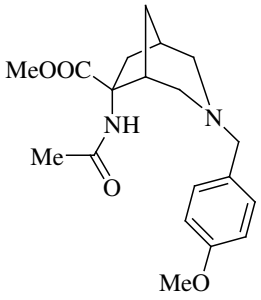
As reported in the introduction, a promising research field is the bio-organometallic chemistry. In fact small peptidomimetics containing a metal center can be considered synthetic enzyme analogues.[73] In this view we have tested compounds ABO (exo)-(-) **124'** and ABO (exo)-(±) **122a** as ligands into transition metal complexes (figure 44). Furthermore, we studied also a new organometallic ligand characterized by norbornene structure.[74] (See figure 45)

This part of my researches was developed under the supervision of Professor Oliver Reiser of the University of Regensburg (Germany) who introduced me into the "World" of metallo-organic chemistry.

Figure 44



124' ABO (exo)-(-)



122a ABO (exo)-(±)

ABOs attract our interest because they are polifunctional compounds, with different possible points of interaction with a metal, *i.e.* the amino, amido and phenyl groups which can be conveniently used to stabilize the metal-ligand interactions. Nitrogen compounds are highly attractive as ligands for transition-metal complexes, and their application in catalysis become one of the major research topic.[75] Amines in fact do not suffer from the intrinsic drawbacks of phosphanes, such as their oxygen

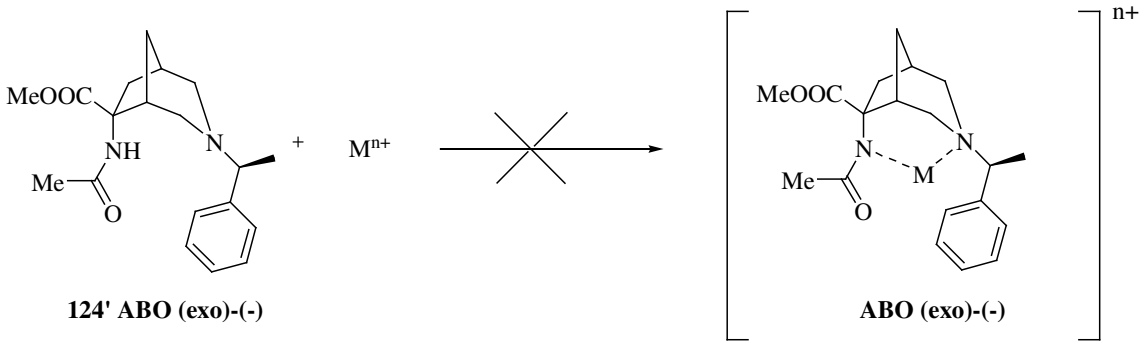
sensitivity. Moreover the biological systems are rich of chiral nitrogen compounds. However late-transition–metal amine complexes are often not very stable and the amine is rather easily displaced by a σ donor. Complexes with sp^2 valence electron configured nitrogen donors seem to have a higher stability.[76]

Preliminary considerations about ABOs take into account the hard/soft character of the binding site. Particularly, the N atoms present in these molecules possess different features and an hard-borderline character; furthermore, the phenyl group can operate in the complex formation too.[77] As reported in the sperimental section by the spectroscopic data (^{1}H NMR, ^{13}C NMR, COSY, NOESY; Hector) a predominant chair-envelope conformation for 3-azabicyclo[3.2.1]octane is proposed.

Geometrical consideration revealed that the N amine and the N amidic lone pairs pointed in a convenient direction and computational studies analyzed the distance between this two groups.

All these informations have been used to focalize our studies into a small number of transition metals. Our first choice was directed toward borderline metals characterized by square planar geometry such as Cu^{2+}, Zn^{2+}, Ni^{2+} Pd^{2+}, Ru^{+1} and Co^{2+}. Different reaction conditions *i.e.* temperature (25°C , 40°C, 70°C) and solvents (CH_2Cl_2, and MeOH or EtOH) were evaluated (see *table 14*).[78] In order to archived the formation of the complex both starting from **124'** and **122a**

Scheme 28



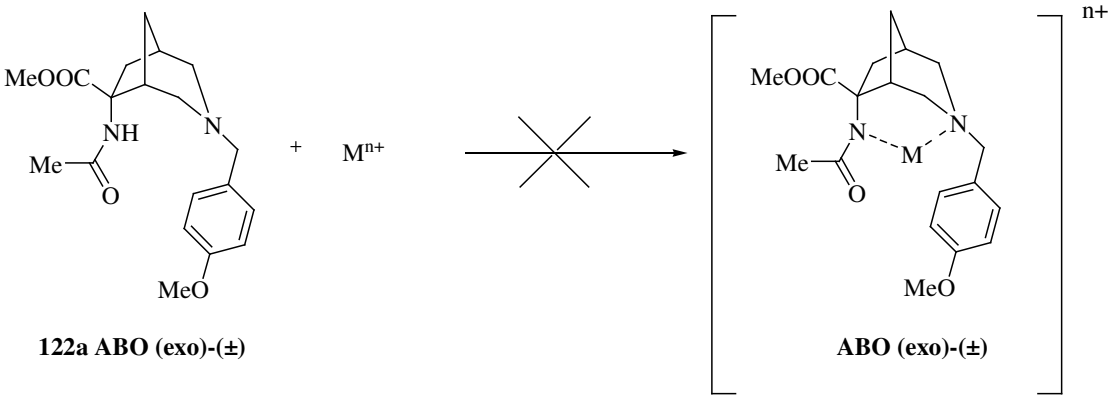


Table 14 reagents and conditions

ligand	metal salt	Ratio Ligand/metal	solvent	T°C	time (s)
122a ABO (±)	$NiCl_2*6H_2O$	2:1	EtOH	60 and 25	24
122a ABO (±)	$Cu(ClO_4)_2*6H_2O$	2:1	EtOH	60 and 25	24
122a ABO (±)	$CoCl_2*6H_2O$	2:1	EtOH	60 and 25	24
122a ABO (±)	$ZnCl_2$	2:1	EtOH	60 and 25	24
122a ABO (±)	$MnCl_2$	2:1	EtOH	60 and 25	24
122a ABO (±)	$FeCl_2*4H_2O$	2:1	EtOH	60 and 25	24
124' ABO exo (-)	$Cu(ClO_4)_2*6H_2O$	2:1	EtOH	60 and 25	24
124' ABO exo (-)	$Pd(OAc)_2$	2:1	EtOH	60 and 25	24
124' ABO exo (-)	$NiCl_2$	2:1	EtOH	60 and 25	24
124' ABO exo (-)	$CuCl_2*2H_2O$	2:1	CH_2Cl_2	25	24
124' ABO exo (-)	$[Ru(p\text{-}Cymene)Cl]_2$	2:1	CH_2Cl_2	25	24
124' ABO exo (-)	$[Ru(p\text{-}Cymene)Cl]_2$	2:1	CH_2Cl_2	Reflux	24

The solvent was removed and the row reaction mixture was analyzed by different techniques, *i.e.* NMR, IR, and MASS (ESI) spectroscopy.

Our explorative researches did not give the expected results, ABO scaffold was not useful as a ligand for a stable metal complex.

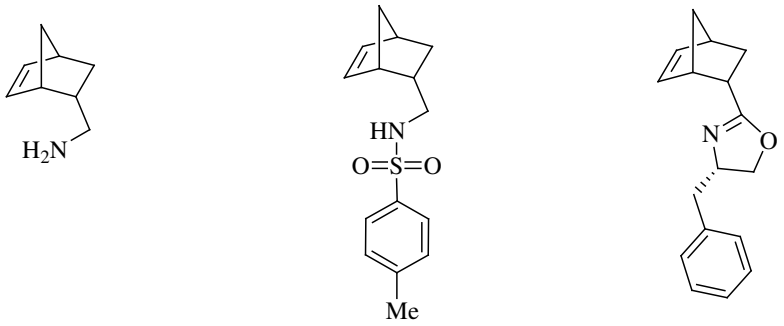
Our failure could be caused by the rigidity of the ligand structure that increase the transition state energy in the formation of the metal complex, or also by a steric hindrance of the ligand can interfered in the chelation process.

Another possibility could be the not optimal overlapping of the metal and ligand orbitals and, consequently, a week interaction which makes instable the M-L complex, more sensitive to the solvent and temperature effects.

4.2 norborne oxazoline ligands

In the second part of my experience I changed the ligand and I studied a new class of bidentate ligand containing both Hard and Soft binding sites, these molecules are characterized by a double bond (Soft) and a nitrogen (Hard) binding sites. The interest toward this class of compounds is related to the scarce literature data regarding complexes with molecules having both Hard/Soft binding sites. Furthermore they are considered very instable complexes.

Figure 45



165 **166** **167**

First we have synthesized a series of compounds **165, 166** and **167** as shown in *figure 45*.

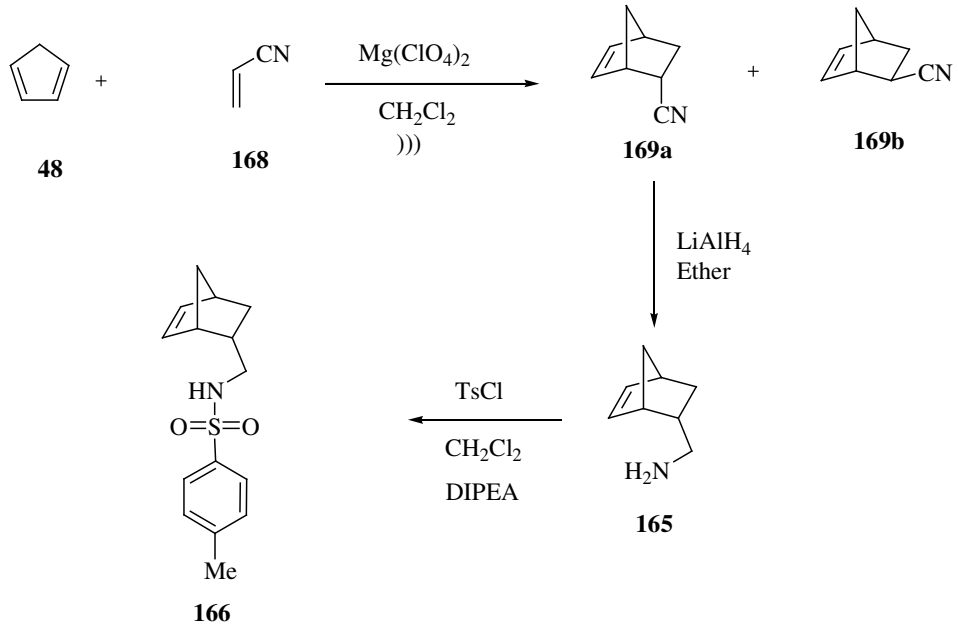
These three compounds present a different nitrogen atom characterized by different hardness. In compound **165,** the nitrogen can be considered an hard sp^3 weak σ-donor, in compound **166** the nitrogen atom has a more soft character because its lone pare delocalizes in the sufamidic function, and in the oxazoline derivative **167** the nitrogen atom was characterized by a hard sp^2 good σ-donor. Furthermore, the phenyl group in compounds **166** and **167** can interact with the metal improving the stabilization of the metal complex.

I developed two different synthetic strategies to access in few steps to these compounds.

The first synthetic strategy is reported in the *scheme 29* and consists in the Diels-Alder reaction between acrylonitrile **168** and cyclopentadiene **48** which operating in

CH_2Cl_2 using $Mg(ClO_4)_2$ as the catalyst and ultrasound. A mixture of cycloadducts **169b**-*exo* and **169a**-*end* were obtained which can be easy separated by column chromatography. Only the major *endo* racemic compound was useful for our further studies. The reduction of the nitrile function using $LiAlH_4$ in diethyl ether to the corresponding amine gave compound **165**. Compound **165** was then transformed into the corresponding tosylamide **166** by reaction with TsCl.

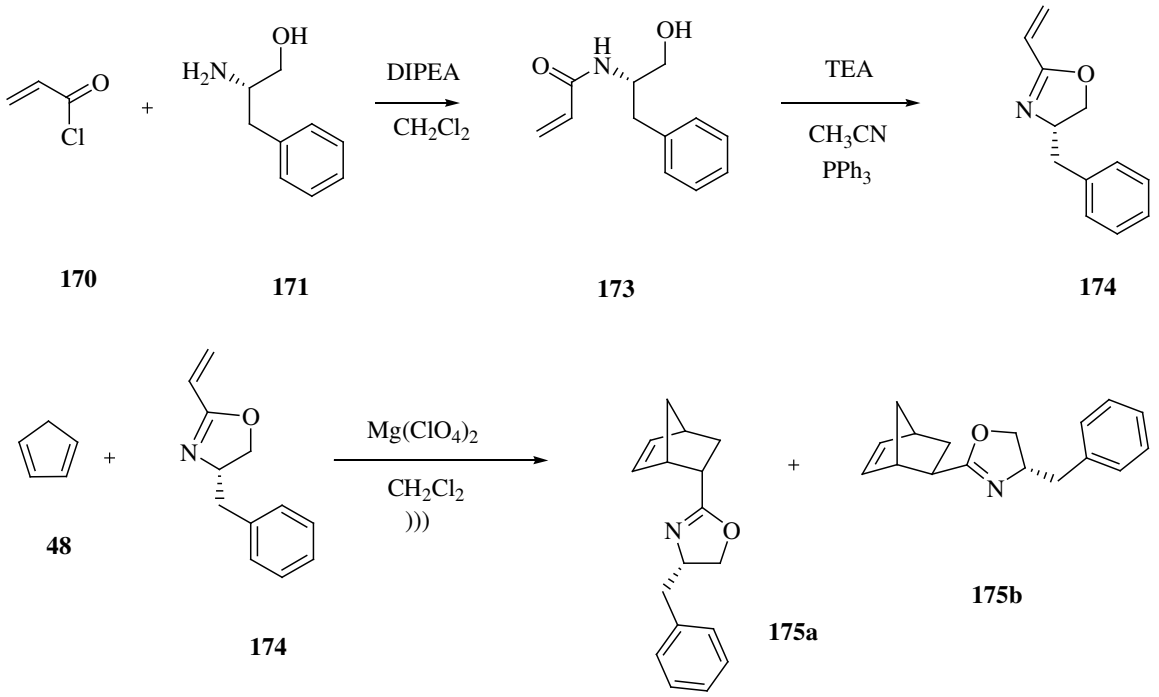
Scheme 29



48 **168** **169a** **169b** **165** **166**

In order to obtain compound **167**, we have developed a different synthetic strategy (Scheme 30). First we prepared the oxazoline compound **174** and then by a Diels-Alder reaction with cyclopentadiene it is possible to obtain a mixture of compounds **175a** and **175b** easily separed by column cromatography. Also in this case, the *endo* compound is of our interest.

Scheme 30



170 **171** **173** **174**

48 **174** **175a** **175b**

Studies on the formation of the complexes was performed using different kind of solvents and metals as reported in the table below.

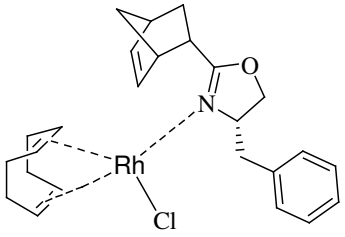
Table 15

ligand	metal salt	Ratio Ligand/metal	solvent	T°C	time (s)
167	$Cu(AcO)_2$	2:1	EtOH	60 and 25	24
167	$[RhCl(COD)]_2$	2:1	EtOH	60 and 25	24
165	$[Ru(p\text{-}Cymene)Cl]_2$	2:1	THF	RT	16
166	$[Ru(p\text{-}Cymene)Cl]_2$	2:1	THF	RT	16
165	$RuCl_3$	2:1	CH_2Cl_2	25	24
165	$[Ru(p\text{-}Cymene)Cl]_2$	2:1	MeOH	25	24
166	$[Ru(p\text{-}Cymene)Cl]_2$	2:1	CH_2Cl_2	Refluxed	24

All reactions mixture (see *table 15*) were analyzed by MASS techniques which is diagnostic for the formation of the complex. Only using NOXA ligand **175a** (Norbornene OXAzoline compounds) and Rh^{+1} as a metal was observed the formation of a complex. Furthermore, ^{1}H NMR analysis confirmed the ligand chelation, since a significant shift of the oxazoline signals (δ ^{1}H and δ ^{13}C) was observed. Instead, the interaction between the metal and the double bond was not

observed (no significant shift of the double bond signals). Our hypothesis is that complex **176** reported below could be formed.

Figure 46



176

Further studies are in progress modifying the ligand structure **175a** in order to obtain an optimal distance and a favorable geometry between the two function (nitrogen and the double bond), aiming to synthesized a bidentate ligand. Also further transition metal, with a different hardness and geometry, have to be evaluated.

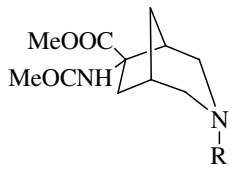
5 ABO in Organocatalysis

5.1 Introduction

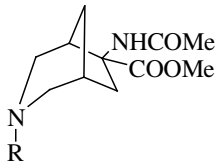
The use of ABO as an organocatalyst was performed in collaboration with Prof.ssa Clerici and Prof.ssa La Rosa of the University of Milano, and was a part of a large ongoing program in which the study of Diels-Alder and Staudinger reactions were one of the main research field. Our compounds together with other amine derivatives were tested in the above reactions.

The presence of an amine in the azabicyclo[3.2.1]octane ring makes ABO an interesting tool in organo catalyst-mediate asymmetric reactions. In the literature there are a huge numbers of reaction catalyzed by a small rigid molecule, in which, the secondary amine function is the active site responsible of the substrate activation. Furthermore, these catalysts tagged the reagents in a particular spatial position inducing a particular stereochemistry on the product. Since ABO derivatives are rigid molecules available in enantiopure form too, they are interesting and potential organocatalyst.

Figure N



exo-**128**



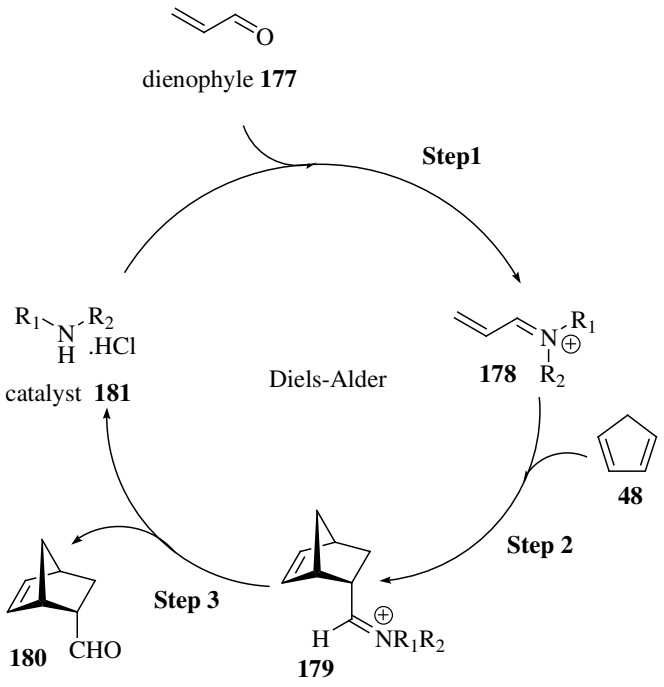
endo-**125/125'**
endo-**123a**

128: R = H,
125/125': (+)-Phenethyl
123a: PMB

5.2 Studies on Diels-Alder reaction

The catalytic cycle of amino organo-catalyzed Diels-Alder reactions is reported below. The condensation of the amine **181** with an aldehyde **177** leads to the formation of an iminium ion (step 1) that is sufficiently activated to engage a diene reaction partner[79] with cyclopentadiene (step 2). The hydrolysis of compound **179** allows to obtain the product and to regenerate the free catalyst for the next catalytic cycle (step 3) .

Scheme 31 Diels-Alder catalytic circle

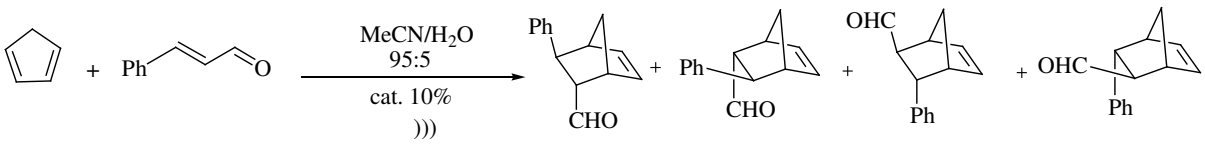


It is well known, that in general, secondary amines are requested to improve significantly the yield. In a preliminary study we planned to test the racemic mixture of both *exo*-ABO and *endo*-ABO.

The ability of our amines to catalyze the Diels-Alder reaction was tested in the reaction of cypentadiene **48** and an α,β-insatured-aldehyde (E-cynnamaldehyde **182**) as a dienophile. Which do not work without the catalyst [80].

The reaction was performed in a screw-cap-tube in which the adehyde **182** (1 eq) was dissolved in MeCN/H_2O (95:5) with ABO catalyst (**128**). The cyclopentadiene **48** (3 eq. in MeCN) was added and the reaction was sonicated for 42 h. The cycloddition products were isolated by chromatography and analyzed by HPLC to evaluate the enantiomerically excess.

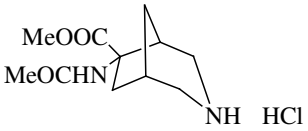
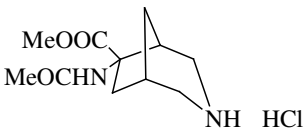
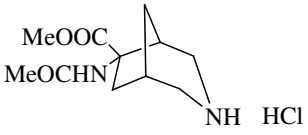
Scheme 32



48 **182** *endo*-**183** *endo*-**183'** *eso*-**184** *eso*-**184'**

In principle four stereoisomers could be formed: a couple of *endo* enantiomers **183** and **183'**, and a couple of *exo* **184** and **184'.** But this reaction do not work without catalyst.

Table 16 results and conditions of Diels-Alder reaction

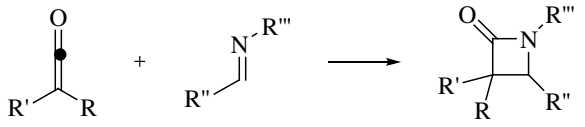
Dienophile eq.	Diene Eq.	Catalyst	Solvent	Time	Temperature Condition	exo:endo ratio	yield
1	3	 **10% mol 128**	CH_3CN/H_2O 95/5	42h	Ultrasound	1:1	18 %
1	3	 **15% mol 128**	CH_3CN/H_2O 95/5	72h	ultrasound	1:1	28 %
1	3	 **10% mol 128**	CH_3CN/H_2O 95/5	42h	ultrasound	1:1	28 %

The results shown in *Table 16* evinced that the reaction yields are modest improved compared to the simple acid catalysis reaction (10 %)[81] but are lower compared to other organo-catalyst such as McMillan catalyst (0-94%)[82] or proline derivatives (10-86%)[81]. Concerning the *exo*:*endo* ratio, we don't have significant results which is an analogues ratio found by Mc Millan[82].

5.3 Staudinger reaction.

Concerning the Staudinger reaction, ketene **185** was made to react with the imine **186** to generate a β-lactam derivatives **187**.

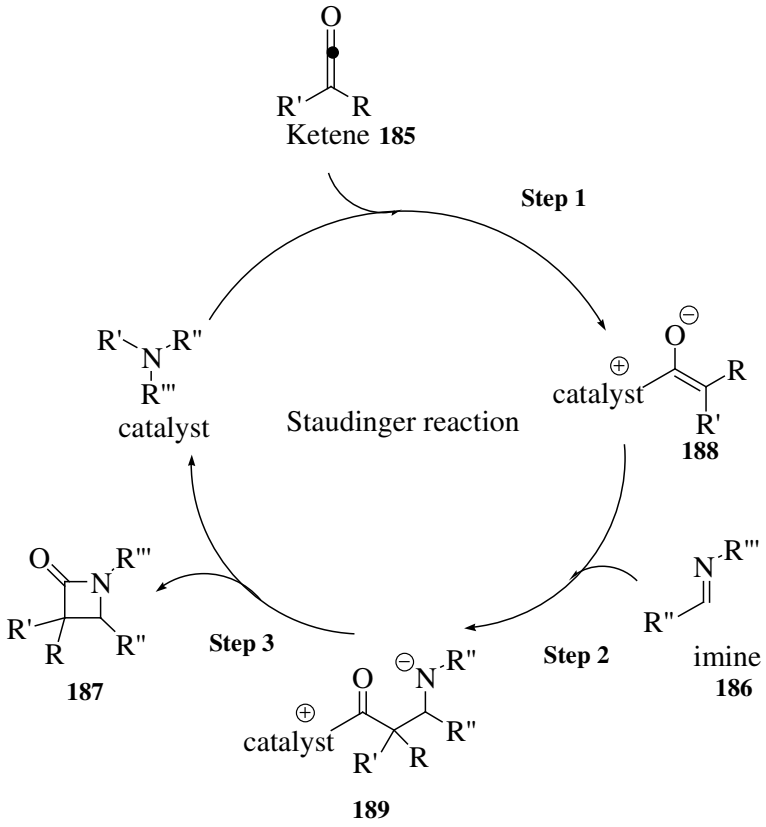
Scheme 33



185 **186** **187**

The catalytic cycle for the above Staudinger like reaction is reported below. First, ketene N reacts with a catalytic tertiary amine (step 1) forming the active intermediate N which gives a nucleophilic attack on the electron poor imine N (step 2). Finally, compound N was formed by cyclization (step 3). Recent result demonstrate[83] that a chiral nucleophilic base could dramatically enhance the rate and the yields in the formation of β-lactam derivatives obtained a good diastereoselectivity and enantioselectivity for analogue reaction.

Scheme 34



In our case we studied the reaction of ketene **193** with imine **194** racemic ABO **123a** and enantiopure compound (+)-**125** were tested as catalysts. To generate the ketene **193** tioazolidinic acid **190** (2 eq.) reacted in the presence of the Mukayama activator **191** (2.31 eq.) and a non nucleophylic base protonsponge **192** (2 eq.). This reaction was perfomed in toluene at 70°C. In the second step the electron poor imine **194** (1 eq.) and the tertiary catalytic base (ABO **123a** or (+)-**125:** 0.1 eq.) and a further amount of a protonsponge base **192** (4 eq.) were added and mixture of diastereoisomeric compounds **195** β_1, **195** β_2 was formed.

Scheme 35

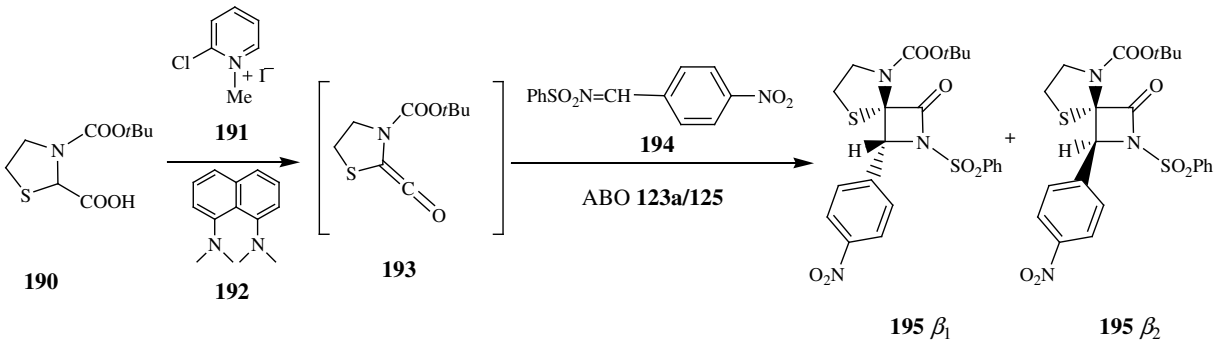


Table 17

	Base catalyst	T, °C	Ratio Acid:imine	yield	de $\beta_1 : \beta_2$	e e β_1
1	(±)-ABO 123a	70	1 : 1	1.5	//	//
2	(±)-ABO 123a	70	2 : 1	12.3	75 : 25	//
3	(+)-ABO 125	r.t	2 : 1	4.8	86 : 14	0
4	(+)-ABO 125	70	2 : 1	14.1	71 : 29	0

The results evinced that the reaction does not work very well. In particular yields are very low probably because the steric hindrance of the reactants make difficult the cyclization (catalytic cycle, step 3).

6. Experimental Section

Compounds *exo*-**111a**, *endo*-**112a**, *exo*-**111b** and *endo*-**112b** were prepared according to a know procedure.

General Procedure for the Synthesis of Diols 118, 119. Compound **111, 112** [*exo*-**111a** or *endo*-**112a** or a mixture of *exo*-**111a**/*endo*-**112a** (7 : 3) (209 mg, 1 mmol); *exo*-**111b** or *endo*-**112b** (409.6 mg, 1 mmol)] was suspended in a mixture of acetone/H_2O (6 mL, 10 : 1). After addition of *N*-methylmorpholine-*N*-oxide (176 mg, 1.5 mmol) and OsO_4 (1.35 mg, 0.005 mmol) the solution turned brown. The reaction mixture was stirred at room temperature for 8 h after which the solvent was evaporated. The crude reaction mixture was filtered on silica gel (CH_2Cl_2/MeOH = 5 : 1). Pure compound **118, 119** (*exo*-**118a**: 209 mg, 86 %; *endo*-**119a**: 204 mg, 84 %; *exo*-**118a**/*endo*-**119a**: 202 mg, 83 %) was obtained after crystallization. The crude reaction mixture containing **2b** was taken up with a saturated solution of $Na_2S_2O_4$ (5 mL) and extracted with CH_2Cl_2 (3 x 5 mL). After drying of the organic layer with Na_2SO_4 and column chromatography on silica gel (CH_2Cl_2/MeOH = 20 : 1) pure compound **118b, 119b** (*exo*-**118b**: 404 mg, 90%; *endo*-**119b**: 430 mg, 97%) was isolated.

Methyl (1*R, 2*S**, 4*S**, 5*S**, 6*R**)-2-Acetylamino-5,6-dihydroxybicyclo[2.2.1]heptane-2-carboxylate *exo*-118a.** Mp 161 °C (MeOH/*i*Pr_2O); IR ν_{max} 3500-3100, 1735, 1651 cm^{-1}; ^1H NMR (CD_3OD) δ 3.97 (d, J = 6.2, 1 H), 3.73 (d, J = 5.0, 1 H), 3.67 (s, 3 H), 2.71 (bs, 1 H), 2.22 (dd, J = 13.8, 5.0, 1 H), 2.09 (bs, 1 H), 1.94 (s, 3 H), 1.94-1.91 (m, 1 H), 1.64 (d, J = 10.9, 1 H), 1.34 (dd, J = 13.8, 2.9, 1 H); ^{13}C NMR (CD_3OD) δ 174.6, 172.9, 73.9, 69.3, 62.6, 51.8, 50.4, 43.3, 38.3, 31.3, 21.0; *m/z* 244.2 [M^+]; Anal. Calcd for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.04; N, 5.76. Found: C, 54.28; H, 7.10; N, 5.68.

Methyl (1*S, 2*S**, 4*R**, 5*R**, 6*S**)-2-Acetylamino-5,6-dihydroxybicyclo[2.2.1]heptane-2-carboxylate *endo*-119a.** Mp 186 °C (MeOH/*i*Pr_2O); IR ν_{max} 3346, 1731, 1694 cm^{-1}; ^1H NMR (CD_3OD) δ 3.74 (dd, J =

6.2, 1.4, 1 H), 3.66 (s, 3 H), 3.57 (dd, *J* = 5.8, 1.4, 1 H), 2.38 (dd, *J* = 13.9, 2.5, 1 H), 2.23 (bs, 1 H), 2.16-2.14 (m, 1 H), 1.97 (dt, *J* = 10.6, 1.1, 1 H), 1.88 (s, 3 H), 1.65 (dt, *J* = 10.6, 1.5, 1 H), 1.54 (ddd, *J* = 14.0, 5.1, 0.7, 1 H); ^{13}C NMR (CD_3OD) δ 173.1, 171.5, 72.8, 68.9, 63.8, 52.3, 51.6, 43.9, 38.8, 31.8, 20.8; *m/z* 244.2 [M^+]; Anal. Calcd for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.04; N, 5.76. Found: C, 54.25; H, 7.12; N, 5.67.

(-)-8-Phenylmenthyl (1*R*, 2*S*, 4*S*, 5*S*, 6*R*)-2-Acetylamino-5,6-dihydroxybicyclo[2.2.1]heptane-2-carboxylate *exo*-118b. Mp 235 °C (CH_2Cl_2/*i*-Pr_2O); $[\alpha]_D^{25}$ = -9.1° (c 0.5, MeOH); IR ν_{max} 3360-3300, 1715, 1647 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.34-7.11 (m, 5 H), 4.83-4.71 (m, 1 H), 3.97 (d, *J* = 6.2, 1 H), 3.66 (d, *J* = 5.5, 1 H), 2.61 (bs, 1 H), 2.26 (dd, *J* = 13.9, 5.1, 1 H), 2.05-1.77 (m, 3 H), 1.90 (s, 3 H), 1.55-0.72 (m, 9 H), 1.35 (s, 3 H), 1.24 (s, 3 H), 0.86 (d, *J* = 6.6, 3 H); ^{13}C NMR (CD_3OD) δ 172.9, 171.6, 153.3, 128.5, 125.9, 124.9, 77.4, 73.8, 69.3, 62.6, 50.8, 50.2, 43.7, 40.9, 39.7, 38.1, 35.0, 31.5, 29.9, 29.6, 26.8, 24.1, 23.5, 21.9; *m/z* 466.4 [+Na]; Anal. Calcd for $C_{26}H_{37}NO_5$: C, 70.40; H, 8.41; N, 3.16. Found: C, 70.33; H, 8.45; N, 3.10.

(-)-8-Phenylmenthyl (1*S*, 2*S*, 4*R*, 5*R*, 6*S*)-2-Acetylamino-5,6-dihydroxybicyclo[2.2.1]heptane-2-carboxylate *endo*-119b. 249 °C; $[\alpha]_D^{25}$ = +13 (c 0.5, MeOH); IR ν_{max} 3375, 1704, 1649 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.40-7.16 (m, 5 H), 5.00 (s, 1 H, exch.), 4.69-4.59 (m, 1 H), 3.90 (d, *J* = 5.9, 1 H), 3.82 (d, *J* = 6.3, 1 H), 2.22-2.00 (m, 3 H), 2.00-1.80 (m, 4 H), 1.81 (s, 3 H), 1.79-1.42 (m, 4 H), 1.34 (s, 3 H), 1.30-0.80 (m, 2 H), 1.17 (s, 3 H), 0.87 (d, *J* = 6.7, 3 H); ^{13}C NMR ($CDCl_3$) δ 171.8, 169.9, 152.9, 128.4, 125.9, 125.1, 77.7, 73.2, 69.4, 63.7, 52.3, 49.8, 44.4, 41.0, 39.8, 39.2, 35.0, 32.5, 31.6, 28.9, 27.0, 25.1, 23.3, 22.0; *m/z* 466.4 [+Na]; Anal. Calcd for $C_{26}H_{37}NO_5$: C, 70.40; H, 8.41; N, 3.16. Found: C, 70.47; H, 8.39; N, 3.13.

General Procedure for the Preparation of *bis*-Aldehydes 120, 121. Compound **118, 119** (**118a**: 243 mg, 1 mmol; **119b**: 443 mg, 1 mmol) was dissolved in a mixture of dioxane/H_2O (5 mL, 8 : 1). $NaIO_4$ was added (1.7 g, 7.92 mmol) and the mixture was stirred at room temperature for 2 h. The solvent was evaporated and the reaction mixture was taken up with $CHCl_3$ (10 mL) and the salts were filtered and washed with $CHCl_3$. The organic solution was collected and, after solvent elimination, the crude aldehydes **120** or **121** [*exo/endo*-**118a/119a**: **120a, 121a** (240 mg); *exo*-**118a**: **120a**

(232 mg); *endo*-**119a**: **121a** (234 mg); *exo*-**118b**: **120b** (395 mg), *endo*-**119b**: **121b** (398 mg)] were isolated and used without further purification.

Methyl (1*S, 2*R**, 4*S**)-1-Acetylamino-2,4-diformyl-cyclopentanecarboxylate 120a:** crude compound. IR ν_{max} 3346, 1731, 1694 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.76 (s, 1 H), 9.71 (s, 1 H), 6.50 (s, H, exch.), 3.77 (s, 3 H), 3.25-3.05 (m, 1 H), 2.50 (dd, *J* = 13.9, 3.7, 1 H), 2.40-1.90 (m, 4 H), 1.91 (s, 3 H); ^{13}C NMR δ 202.7, 172.0, 170.8, 66.6, 57.2, 53.3, 47.4, 37.0, 24.7, 22.9; *m/z* 242.1 [M^+].

Methyl (1*S, 2*S**, 4*R**)-1-Acetylamino-2,4-diformyl-cyclopentanecarboxylate 121a:** crude compound. IR ν_{max} 3391, 1728, 1658 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.68 (s, 1 H), 9.67 (s, 1 H), 6.70 (s, H, exch.), 3.69 (s, 3 H), 3.20-2.90 (m, 1 H), 2.60-1.90 (m, 4 H), 2.01 (s, 3 H); ^{13}C NMR δ 201.7, 200.0, 173.0, 170.5, 65.7, 58.4, 53.2, 48.9, 36.8, 27.2, 23.9; *m/z* 242.1 [M^+].

(-)-8-Phenylmenthyl (1*S*, 2*R*, 4*S*)-1-Acetylamino-2,4-diformyl-cyclopentanecarboxylate 120b: crude compound. IR ν_{max} 3428, 1759, 1633 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.73 (s, 1 H), 9.48 (d, *J* = 0.81, H), 7.39-7.11 (m, 5 H), 6.35 (s, 1 H, exch.), 4.93-4.88 (m, 1 H), 3.02-2.92 (m, 1 H), 2.40-2.31 (m, 1 H), 2.25 (dd, *J* = 14.0, 2.3, 1 H), 2.20-2.13 (m, 1 H), 2.12-1.75 (m, 5 H), 1.84 (s, 3 H), 1.68 (dd, *J* = 14.0, 10.1, 1 H), 1.60-1.50 (m, 1 H), 1.32 (s, 3 H), 1.28-1.18 (m, 1 H), 1.14 (s, 3 H), 1.10-0.97 (m, 2 H), 0.93 (d, *J* = 6.6, 3 H); ^{13}C NMR ($CDCl_3$) δ202.2, 197.3, 169.6, 168.8, 152.1, 127.4, 124.9, 124.3, 76.6, 65.5, 55.9, 48.5, 45.3, 40.1, 38.7, 35.0, 34.0, 30.7, 30.3, 25.8, 23.3, 21.7, 21.1, 20.8; *m/z* 442.5 [M^+].

(-)-8-Phenylmenthyl (1*S*, 2*S*, 4*R*)-1-Acetylamino-2,4-diformyl-cyclopentanecarboxylate 121b: crude compound. IR ν_{max} 3390, 1727, 1651 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.69 (d, *J* = 1.5, 1 H), 9.61 (d, *J* = 1.3, 1 H), 7.32-7.15 (m, 5 H), 6.05 (s, 1 H, exch.), 5.04-4.95 (m, 1 H), 3.27-3.12 (m, 1 H), 2.43 (dd, *J* = 14.0, 10.0, 1 H), 2.33 (dd, *J* = 9.3, 9.2, 1 H), 2.12-1.95 (m, 2 H), 1.97 (s, 3 H), 1.90-1.80 (m, 2 H), 1.72-1.55 (m, 3 H), 1.50-1.40 (m, 1 H), 1.36 (s, 3 H), 1.22 (s, 3 H), 1.18-0.80 (m, 3 H), 0.90 (d, *J* = 6.5, 3 H); ^{13}C NMR ($CDCl_3$) δ 202.0, 200.1, 171.5, 170.3, 151.7, 128.7, 125.9, 78.8, 67.1, 57.9, 50.3, 48.9, 41.8, 40.3, 35.6, 34.6, 31.8, 27.7, 27.3, 27.0, 26.5, 24.5, 22.1; *m/z* 442.5 [M^+].

General Procedure for the Reductive Amination: Synthesis of 3-Aza-bicyclo[3.2.1]octane Derivatives 122/123 a,b. Aldehyde [**120a/121a** (242 mg, 1

mmol); **120b/121b** (442 mg, 1 mmol)] was dissolved in anhydrous dichloroethane (4 mL) under nitrogen. Operating under stirring at room temperature, *p*-methoxybenzylamine (137.2 mg, 1 mmol), $NaBH(OAc)_3$ (487.0 mg, 2.3 mmol) and a catalytic amount of AcOH were added. After 4 h, the solvent was evaporated and the crude reaction mixture was taken up with CH_2Cl_2 (5 mL). The organic layer was washed with H_2O (5 mL) and dried over $MgSO_4$. The crude reaction was chromatographed on silica gel (**122a**: CH_2Cl_2/MeOH = 20 : 1; **123b**: CH_2Cl_2/MeOH = 50 : 1) giving pure 3-aza-bicyclo[3.2.1]octane derivatives **122/123** after crystallization [**120a**: *exo*-**122a** (211 mg, 61%); **121a**: *endo*-**123a** (183 mg, 53%); **120a/121a**: *exo*-**122a** (141 mg, 41%), *endo*-**123a** (52 mg, 15%); **120b**: *exo*-**122b** (311 mg, 57%); **121b**: *endo*-**123b** (317 mg, 58%)] .

Methyl (1*S, 5*S**, 6*S**)-6-(Acetylamino)-3-(4-methoxybenzyl)-3-azabicyclo[3.2.1]octane-6-carboxylate *exo*-122a.** Mp 143-145 °C (benzene); IR ν_{max} 3344, 1737, 1637 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.16, 6.84 (AA'XX' system, J = 8.4, 4 H), 6.44 (s, 1H, exch.), 3.78 (s, 3 H), 3.67 (s, 3 H), 3.55, 3.17 (AB system, J = 12.5, 2 H), 2.83-2.75 (m, 1 H), 2.72 (brs, 1 H), 2.56-2.50 (m, 1 H), 2.40-2.30 (m, 2 H), 2.20 (bs, 1 H), 2.03-1.98 (m, 2 H), 1.72 (dd, J = 13.5, 2.5, 1 H), 1.57 (s, 3 H), 1.50 (dd, J = 11.1, 2.5, 1 H); ^{13}C NMR ($CDCl_3$) δ 174.4, 171.0, 159.2, 130.6, 130.6, 114.2, 65.9, 62.2, 61.0, 55.6, 54.9, 52.5, 43.0, 40.0, 37.1, 34.7, 22.5; *m/z* 347.3 [M^+]; Anal. Calcd for $C_{19}H_{26}N_2O_4$: C, 65.87; H, 7.56; N, 8.09; Found: C, 65.85; H, 7.55; N, 8.06.

Methyl (1*R, 5*R**, 6*S**)-6-(Acetylamino)-3-(4-methoxybenzyl)-3-azabicyclo[3.2.1]octane-6-carboxylate *endo*-123a.** Mp 155-157 °C (benzene); IR ν_{max} 3265, 1747, 1640 cm^{-1}; ^{1} H NMR ($CDCl_3$) δ 7.23, 6.84 (AA'XX' system, J = 8.5, 4 H), 5.72 (s, H, exch.), 3.80 (s, 3 H), 3.69 (s, 3 H), 3.42, 3.33 (AB system, J = 13.0, 2 H), 3.01 (dd, J = 13.9, 1.8, 1 H), 2.84 (d, J = 10.2, 1 H), 2.71 (d, J = 8.4, 1 H), 2.30 (bs, 1 H), 2.30-1.85 (m, 5 H), 1.96 (s, 3 H), 1.51 (dd, J = 11.3, 2.2, 1 H); ^{13}C NMR ($CDCl_3$) δ 172.5, 169.7, 159.0, 131.1, 130.4, 113.9, 68.5, 62.0, 59.0, 55.9, 55.6, 52.5, 46.8, 39.9, 37.1, 35.1, 23.7; *m/z* 347.3 [M^+]; Anal. Calcd for $C_{19}H_{26}N_2O_4$: C, 65.87; H, 7.56; N, 8.09. Found: C, 65.91; H, 7.54; N, 8.08.

(-)-Phenylmenthyl (1*S*, 5*S*, 6*S*)-6-(Acetylamino)-3-(4-methoxybenzyl)-3-azabicyclo[3.2.1]octane-6-carboxylate *exo*-122b. Mp 186 °C (*i*-Pr_2O); $[\alpha]_D^{25}$ = -

46.5° (c 5.24, $CHCl_3$); IR ν_{max} 3400, 1730, 1657 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ7.28-7.08 (m, 6 H), 7.08-6.98 (m, 1 H), 6.86 (d, *J* = 8.4, 2 H), 5.94 (s, 1 H, exch.), 4.81-4.65 (m, 1 H), 3.80 (s, 3 H), 3.54, 3.16 (AB system, *J* = 12.5, 2 H), 2.90-2.70 (m, 1 H), 2.65-2.00 (m, 4 H), 2.00-1.80 (m, 2 H), 1.60-0.70 (m, 11 H), 1.58 (s, 3 H), 1.30 (s, 3 H), 1.19 (s, 3 H), 0.83 (d, *J* = 6.2, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.3, 170.6, 159.2, 151.7, 131.0, 130.5, 128.1, 125.9, 125.2, 114.1, 76.7, 66.2, 62.2, 60.9, 55.6, 54.8, 50.4, 42.1, 41.1, 40.3, 36.7, 34.9, 34.7, 31.5, 28.4, 27.6, 25.0, 23.1, 22.7, 22.0; *m/z* 547.3 [M^+]; Anal. Calcd for $C_{34}H_{46}N_2O_4$: C, 74.69; H, 8.48; N, 5.12. Found: C, 74.53; H, 8.50; N, 5.09.

(-)-Phenylmenthyl (1*R*, 5*R*, 6*S*)-6-(Acetylamino)-3-(4-methoxybenzyl)-3-azabicyclo[3.2.1]octane-6-carboxylate *endo*-123b. Mp 86 °C (CH_2Cl_2/*i*Pr_2O); $[\alpha]_D^{25}$ = -3.5° (c 6.4, $CHCl_3$); IR ν_{max} 3400, 1730, 1657 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ7.42-7.05 (m, 6 H), 7.05-6.95 (m, 1 H), 6.88 (d, *J* = 8.8, 2 H), 5.42 (s, 1 H, exch.), 4.78-4.66 (m, 1 H), 3.82 (s, 3 H), 3.55, 3.22 (AB system, *J* = 13.0, 2 H), 2.90-2.80 (m, 1 H), 2.58-162 (m, 10 H), 1.57 (s, 3H), 1.58-0.70 (m, 7 H), 1.29 (s, 3 H), 1.15 (s, 3 H), 0.84 (d, *J* = 6.2, 3 H); ^{13}C NMR ($CDCl_3$) δ 171.0, 169.4, 158.8, 152.3, 131.0, 130.2, 128.1, 125.8, 125.2, 113.7, 77.3, 68.5 61.5, 58.1, 55.7, 55.4, 50.2, 41.1, 40.3, 39.1, 37.1, 35.0, 34.6, 31.6, 28.3, 27.6, 25.4, 23.5, 23.1, 22.1; *m/z* 547.3 [M^+]; Anal. Calcd for $C_{34}H_{46}N_2O_4$: C, 74.69; H, 8.48; N, 5.12. Found: C, 74.50; H, 8.51; N, 5.07.

General Procedure for the Oxidative Deprotection of 122a/123a. To a solution of compound **122a/123a** (346 mg, 1 mmol), in acetone/H_2O (23 mL, 9 : 1) at 0 °C, $(NH_4)_2Ce(NO_3)_6$ (2.19 g, 4 mmol) was added in several portion in 1 h. The mixture was stirred at room temperature for 3 h and quenched with a saturated solution of $NaHCO_3$ (pH 10). A solid was formed. Acetone was evaporated and the aqueous layer was filtered trough a celite pad. After elution with warm EtOH, the organic solution was concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH = 5 : 1 : 0.025). Compound **126/127** (*exo*-**126a**: 204 mg, 90%; *endo*-**127a**: 141 mg, 62%) was isolated after recrystallization.

Methyl (1*S, 5*S**, 6*S**)-6-(Acetylamino)-3-azabicyclo[3.2.1]octane-6-carboxylate *exo*-126a.** Mp 110 °C dec. (EtOH); IR ν_{max} 3400, 1727, 1632 cm^{-1}; ^{1}H NMR (D_2O) δ

3.58 (s, 3 H), 3.10 (brs, 4 H), 2.86 (brs, 1 H), 2.55 (dd, *J* = 15.4, 7.3, 1 H), 2.39 (brs, 1 H), 2.11-1.98 (m, 1 H), 1.92 (s, 3 H), 1.83 (dd, J = 15.4, 3.0, 1 H), 1.71 (dd, *J* = 12.5, 2.6, 1 H); ^{13}C NMR (D_2O) δ 176.5, 175.5, 65.0, 53.5, 49.2, 45.9, 39.2, 38.8, 34.2, 32.0, 21.9; *m/z* 227.2 [M^+]; Anal. Calcd for $C_{11}H_{18}N_2O_3$: C, 58.39; H, 8.02; N, 12.38. Found: C, 58.21; H, 8.18; N, 12.22.

Methyl (1*R, 5*R**, 6*S**)-6-(Acetylamino)-3-azabicyclo[3.2.1]octane-6-carboxylate *endo*-127a.** Mp 99 °C dec. (EtOH); IR ν_{max} 3420, 1720, 1645 cm^{-1}; ^{1}H NMR (D_2O) δ 3.66 (s, 3 H), 3.21 (d, *J* = 12.5, 1H), 3.18-3.00 (m, 3 H), 2.56 (d, *J* = 15.7, 1 H), 2.47 (brs, 2 H), 2.21-2.00 (m, 2 H), 1.93 (s, 3 H), 1.75 (d, *J* = 12.5, 1 H); ^{13}C NMR (D_2O) δ 175.1, 173.9, 66.9, 53.9, 49.6, 45.8, 42.3, 39.8, 34.4, 32.5, 21.7; *m/z* 227.1 [M^+]; Anal. Calcd for $C_{11}H_{18}N_2O_3$: C, 58.39; H, 8.02; N, 12.38. Found: C, 58.30; H, 8.20; N, 12.14.

6-Acetylamino-3-(4-methoxybenzyl)-3-azabicyclo[3.2.1]octane-6-carboxylic Acid (±)-132/133. Operating in a sealed tube, compound **122a/123a** (346 mg, 1 mmol) was dissolved in EtOH (95%, 5 mL) and KOH (112.2 mg, 2 mmol) was added. The solution was heated at 120 °C under stirring for 2h. The reaction mixture was treated with HCl (6 N, pH 7) and the solvent was removed. The crude material was filtered through a pad of silica gel with CH_2Cl_2/CH_3OH (5 : 2). Crystallization from absolute EtOH afforded the pure carboxylic acid derivative **132/133** [(±)-*exo*-**132**: (315 mg, 95%); (±)-*endo*-**133**: (330 mg, 99%)].

(1*S, 5*S**, 6*S**)-(±)-*exo*-132**: mp 200 °C (EtOH); IR ν_{max} 3412, 1740, 1661 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.61, 7.00 (AA'XX' system, *J* = 8.4, 4 H), 4.42, 4.01 (AB system, *J* = 11.0, 2 H), 3.83 (s, 3H), 3.56, 3.17 (AB system, *J* = 11.0, 2 H), 3.10-1.90 (brs, 2 H), 2.81 (d, *J* = 12.2, 1 H), 2.63 (d, *J* = 14.2, 1 H), 2.50 (brs, 1 H), 2.41 (dd, *J* = 14.7, 7.0, 1 H), 2.38-2.29 (m, 1 H), 1.92 (s, 3 H), 1.78 (d, *J* = 11.2, 1 H); ^{13}C NMR (CD_3OD) δ 176.9, 172.7, 160.2, 131.5, 120.9, 113.4, 65.0, 59.7, 57.6, 53.8, 53.0, 40.0, 36.4, 33.9, 32.8, 20.9; *m/z* 333.2 [M^+]; Anal. Calcd for $C_{18}H_{24}N_2O_4$: C, 65.04; H, 7.28; N, 8.43. Found: C, 65.12; H, 7.38; N, 8.50.

(1*R, 5*R**, 6*S**)-(±)-*endo*-133**: mp 202 °C dec. (EtOH); IR ν_{max} 3435, 1613, 1516 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.42, 6.98 (AA'XX' system, *J* = 8.4, 4 H), 4.16, (brs, 2 H), 3.80 (s, 3 H), 3.40-3.20 (m, 3 H), 3.11 (d, *J* = 12.1, 1 H), 2.66 (d, *J* = 15.0, 1 H), 2.53 (brs, 2 H), 2.30-2.02 (m, 2 H), 1.92 (s, 3 H), 1.88 (d, *J* = 11.5, 1 H); ^{13}C NMR

(CD_3OD) δ 177.2, 170.9, 160.2, 131.1, 120.7, 113.6, 67.6, 58.7, 56.2, 54.4, 53.8, 42.3, 41.2, 33.8, 33.6, 20.8; *m/z* 333.3 [M^+]; Anal. Calcd for $C_{18}H_{24}N_2O_4$: C, 65.04; H, 7.28; N, 8.43. Found: : C, 65.00; H, 7.30; N, 8.39.

General Procedure for the Hydrolysis of Amino Acid Function. *i*) Operating in a sealed tube, compounds (±)-*exo*-**122a,123a** or (±)-*endo*-**122a,123a** (1 mmol) were suspended in HCl (1 mL, 6 M) and the mixture was heated at 120 °C for 24 h. The solvent was removed and the crude amino acids (±)-*exo*-**134a** (mixture of conformers 7 : 1), *exo*-**135a** and (±)-*endo*-**134a,135a** were isolated as *bis*-chlorhydrate in quantitative yield. *ii*) Operating in a sealed tube, MeOH (5 mL) was cooled at 0 °C and Na (161 mg, 7 mmol) was added. Compound **122b** (546.7 mg, 1 mmol) was added and the mixture was heated at 100 °C under stirring for 50 h (TLC: CH_2Cl_2/MeOH, 50 : 1). The solvent was removed and the crude reaction mixture was taken up with distilled H_2O (5 mL) and extracted with AcOEt (3 x 10 mL). The aqueous solution was treated with HCl (6N, pH 1) and was purified by a Dowex 50W x 4-50 ion exchange resin which was first activated with NH_4OH (2N) then with AcOH (2 N, pH 4) followed by washing with H_2O (pH 7). The reaction mixture was deposited on the resin which was rinsed with water. The amino acid was eluted with aqueous NH_4OH (2 N). Ninhydrin positive fractions (T.L.C. CH_2Cl_2/MeOH/NH_4OH(15%) = 5 :3 : 0.9) were pooled and evaporated to give (-)-*exo*-**134b'** (130 mg, 45 %) from (-)-*exo*-**122b'** and (-)-*endo*-**135b'** (172 mg, 60 %) from (-)-*endo*-**123b'**.

6-Amino-3-(4-methoxybenzyl)-3-azabicyclo[3.2.1]octane-6-carboxylic Acids:

(1*S, 5*S**, 6*S**)** (±)-***exo*-124a · 2 HCl**: mixture of isomers (8 : 1). Mp 176 °C (acetone/H_2O); IR ν_{max} 3470-3370, 1742, 1614 cm^{-1}; ^1H NMR (D_2O) δ major isomer: 7.32, 6.92 (AA'BB' system, *J* = 8.8, 4 H), 4.29, 4.13 (AB system, *J* = 12.8, 2 H), 3.70 (s, 3 H), 3.52, 3.37 (AB system, *J* = 14.3, 2 H), 3.15 (brs, 2 H), 2.70-2.55 (m, 2 H), 2.52-2.48 (m, 1 H), 2.30-2.10 (m, 1 H), 1.62 (d, *J* = 4.0 1 H), 1.56 (d, *J* = 8.4, 1 H); minor isomer (significative signals): 7.18, 6.80 (AA'BB' system, *J* = 8.8, 4 H); ^{13}C NMR (D_2O) δ 173.1, 160.5, 133.3 (133.5), 120.3, 114.5 (115.9), 64.6, 61.9, 57.5, 55.5, 52.5, 41.5, 33.8, 33.0, 32.5; *m/z* 291.8 [M^+]; Anal. Calcd for $C_{16}H_{24}Cl_2N_2O_3$: C, 52.90; H, 6.66; N, 7.71. Found: C, 52.50; H, 7.00; N, 7.28.

(1*S*, 5*S*, 6*S*) (-)-***exo*-134b'**: $[\alpha]_D^{25}$ = -36.3° (c 3.7, MeOH).

(1*R, 5*R**, 6*S**)** (±)-***endo*-135a · 2 HCl**: mixture of isomerss (3 : 1). Mp 220-221 °C (EtOH); IR ν_{max} 3405-2950, 1725, 1614 cm^{-1}; ^1H NMR (D_2O) δmajor isomer: 7.35, 7.01 (AA'BB' system, *J* = 8.8, 4 H), 4.18, 4.12 (AB system, *J* = 13.1, 2 H), 3.79 (s, 3 H), 3.44, 3.16 (AB system, *J* = 11.2, 2 H), 3.28-3.13 (m, 2 H), 2.71 (brs, 1 H), 2.66 (brs, 1 H), 2.46 (dd, *J* = 15.7, 2.1, 1 H), 2.17 (dd system, *J* = 15.7, 7.2, 1 H), 2.09-2.07 (m, 1 H), 1.94 (d, *J* = 13.5, 1 H); minor isomer (significative signals): 7.28, 6.90 (AA'BB' system, *J* = 8.8, 4 H); ^{13}C NMR (D_2O) δ 174.6, 160.3, 132.4 (132.2), 121.3 (120.6), 114.9 (116.2), 67.1, 59.96 (60.03), 56.99 (56.93), 55.5, 54.5, 42.8, 38.9, 34.2, 33.9; *m/z* 291.8 [M^+]; Anal. Calcd for $C_{16}H_{24}Cl_2N_2O_3$: C, 52.90; H, 6.66; N, 7.71. Found: C, 52.53; H, 7.69; N, 7.29.

(1*R*, 5*R*, 6*S*) (-)-***endo*-135b'** $[\alpha]_D^{25}$ = -14.0° (c 4.25, MeOH).

(1*S, 5*S**, 6*S**) 6-Amino-3-azabicyclo[3.2.1]octane-6-carboxylic Acid 2 HCl** (±)-***exo*-130:** IR ν_{max} 3600-2950, 1695, 1626 cm^{-1}; ^1H NMR (2 · HCl salt D_2O) δ 3.51 (dd, *J* 14.6, 3.1, 1 H), 3.45 (dt, *J* 14.6, 2.2, 1 H), 3.37-3.24 (m, 2 H), 2.89 (dd, *J* 15.9, 8.0, 1 H), 2.78 (brs, 1 H), 2.62 (brs, 1 H), 2.45-2.28 (m, 1 H), 1.85 (dd, *J* 15.9, 1.9, 1 H), 1.79 (dd, *J* 13.0, 2.1, 1 H); ^{13}C NMR δ 172.6, 63.1, 48.9, 42.9, 39.6, 33.6, 32.2, 31.0; *m/z* 172.2 [$M]^+$; Anal. Calcd for $C_8H_{16}Cl_2N_2O_2$: C, 39.52; H, 6.63; N, 11.52. Found: C, 39.05; H, 6.96; N, 11.30.

(1*R, 5*R**, 6*S**) 6-Amino-3-azabicyclo[3.2.1]octane-6-carboxylic Acid 2 HCl** (±)-***endo*-131.** IR ν_{max} 3600-2950, 1697, 1627 cm^{-1}; ^1H NMR (D_2O) δ 3.35 (dt, *J* 12.7, 2.4, 1 H), 3.27 (brs, 2 H), 3.23 (d, *J* 12.7, 1 H), 2.71 (brs, 1 H), 2.68 (brs, 1 H), 2.57 (dd, *J* 15.9, 2.1, 1 H), 2.24 (dd, *J* 15.9, 7.4, 1 H), 2.20-2.12 (m, 1 H), 1.98 (dd, *J* 13.2, 2.1, 1 H); ^{13}C NMR (D_2O) δ 173.2, 66.3, 48.4, 45.7, 41.4, 37.7, 33.2, 32.6; *m/z* 172.2 [$M]^+$; Anal. Calcd for $C_8H_{16}Cl_2N_2O_2$: C, 39.52; H, 6.63; N, 11.52. Found: C, 39.01; H, 6.98; N, 11.28.

General Procedure for the Reductive Amination from Aldehydes 120a, 121a: Synthesis of 3-aza-bicyclo[3.2.1]octane *exo*-124, 124', 122c and *endo*-125, 125', 123c. A mixture of aldehydes **120a**, **121a** (241 mg, 1 mmol) was dissolved in anhydrous dichloroethane (4 mL) under N_2 and stirring at 25 °C. (*R*)-1-Phenylethylamine (50 μL, 1.1 mmol) or $MeNH_2$ (32.6 mg, 1.05 mmol), $NaBH(OAc)_3$ (530 mg, 2.5 mmol) and AcOH (catalytic amount) were added. After 36 h, the solvent was eliminated, the mixture was taken up with CH_2Cl_2 (5 mL),

washed with H_2O (5 mL) and dryed over $MgSO_4$. In the case of phenylethylamine (1.45 mL, 31.9 mmol) the reaction has been scaled up starting from aldehydes **120a, 121a** (7 g, 29 mmol) to give a mixture of diastereoisomers (7.6 g, 80%) which were chromatographed on silica gel (CH_2Cl_2/MeOH, 20 : 1), affording the mixture *exo*-**124/124'** (5.3 g, 56%) and *endo*-**125/125'** (2.3 g, 24%). Using a Biotage chromatographic system (column: FLASH 65i, KP-SIL, 65x200 mm; *exo*-**124/124'**: cyclohexane/AcOEt, 3:2; *endo*-**125/125'**: CH_2Cl_2/MeOH, 10:1) pure (+)-*exo*-**124** (2.3 g, 25%) was separated from (-)-*exo*-**124'** (2.65 g, 28%) and (+)-*endo*-**125** (1.15 g, 12%) from (-)-*endo*-**125'** (0.95 g, 10%). Pure (±)-*exo*-**122c** (57 mg, 24%) and (±)-*endo*-**123c** (21.6 mg, 11%) were isolated after column chromatography (CH_2Cl_2/MeOH, 50 : 1) and crystallization.

General Procedure for the Deprotection of *N*-3. Compound (±)-*exo*-**122a** or (±)-*endo*-**123a** (346 mg, 1 mmol) or (-)-*exo*-**124'** or (+)-*endo*-**125** (330 mg, 1 mmol) or (-)-*exo*-**132'** or (-)-*endo*-**133** (290 mg, 1 mmol) was dissolved in MeOH (10 mL). Pd/C (10 %, 100 mg, 0.1 mmol) was added and the mixture was hydrogenated (25 °C, 1 atm; *exo*-**122a** and *endo*-**123a**: 48 h; *exo*-**124'** and *endo*-**125**: 36 h; *exo*-**132'** and *endo*-**133**: 24 h). The catalyst was filtrated over a celite pad, the solvent was removed and the mixture was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 1 : 1). Pure compound [from (±)-*exo*-**122a**: (±)-*exo*-**126a** (165 mg, 73%); from (±)-*endo*-**123a**: (±)-*endo*-**127a** (158 mg, 70%); from (-)-*exo*-**124'**: (-)-*exo*-**122f'** (192 mg, 85%); from (+)-*endo*-**125**: (+)-*endo*-**122f** (165 mg, 73 %), from (-)-*exo*-**132'**: (-)-*exo*-**130** (142 mg, 84%); from (-)-*endo*-**133**: (+)-*endo*-**131** (160 mg, 95%)] was isolated after crystallization. Spectroscopic data of known compounds are in agreement with literature.[2]

6-Amino-3-aza-bicyclo[3.2.1]octane-6-carboxylic Acids *exo*-130 and *endo*-131 · 2 HCl. The hydrochloride salt was obtained after treating compounds (-)-*exo*-**130** and (+)-*endo*-**131** with a solution of anhydrous HCl in EtOH.

(-)-(1*S*, 5*S*, 6*S*)-*exo*-130 · 2 HCl: $[\alpha]_D^{25}$ - 9.0 (*c* 0.5, MeOH).

(+)-(1*R*, 5*R*, 6*S*)-*endo*-131 · 2 HCl: $[\alpha]_D^{25}$ + 4.0 (*c* 0.5, MeOH).

General Procedure for the "One Pot" Deprotection-Alkylation Reaction of *N*-3. Compound (±)-*exo*-**122a** or (±)-*endo*-**123a** (150 mg, 0.432 mmol) or (+)-*exo*-**124** or (-)-*exo*-**124'** or (+)-*endo*-**125** or (-)-*endo*-**125'** (100 mg, 0.303 mmol) was

dissolved in the proper solvent (see below: 10 mL). A catalytic amount of AcOH and the carbonyl compound were added [for **c**: MeOH and formaldehyde (37 % MeOH solution, 200 μL, 1.2 mmol); for **d**: EtOH and acetaldehyde (25 μL, 0.476 mmol); for **e**: acetone and water 1:3]. The mixture was hydrogenated with Pd/C (10%, 47 mg, 0.044 mmol) at 25 °C and 1 atm for 24 h. The catalyst was filter over celite, the solvent was eliminated affording the expected compounds of **c** series [from (±)-*exo*-**122a**: (±)-*exo*-**122c** (90 mg, 88%); from (±)-*endo*-**123a**: (±)-*endo*-**123c** (88mg, 85%); from (+)-*exo*-**124**: (+)-*exo*-**122c** (50 mg, 70%); from (-)-*exo*-**124'**: (-)-*exo*-**122c'** (58 mg, 80%); from (+)-*endo*-**123b**: (+)-*endo*-**123c** (60 mg, 83%); from (-)-*endo*-**123b'**: (-)-*endo*-**123c'** (62 mg, 85%)] or (±)-*exo*-**122d** (from (±)-*exo*-**122a**: 100 mg, 90%) or (±)-*endo*-**123d** (from (±)-*endo*-**123a**: 75 mg, 68 %) or (±)-*exo*-**122e** (from (±)-*exo*-**122a**: 81 mg, 70%) after crystallization.

Synthesis of Methyl (±)-(1*S, 5*S**, 6*S**)-6-Acetamido-3-(4,4-diphenylbut-3-enyl)-3-aza-bicyclo[3.2.1]octane-6-carboxylate *exo*-122g.** To a solution of (±)-*exo*-**126d** (297 mg, 1.3 mmol) in DMF (20 mL), 4-bromo-1,1-diphenylbut-1-ene (565 mg, 1.96 mmol), K_2CO_3 (370 mg, 2.68 mmol) and NaI (78 mg, 0.52 mmol) were added. The reaction was stirred at reflux for 48 h (TLC: CH_2Cl_2/*n*-hexane, 4:1). The reaction mixture was poured into water (10 mL) and extracted with Et_2O (3 x 10 mL). The organic layer was washed with a saturated solution of NaCl (3 x 10 mL), dried over Na_2SO_4. After flash column chromatography on silica gel (CH_2Cl_2/MeOH, 40:1) pure compound (±)-*exo*-**122g** was obtained (170 mg, 30%).

General Procedure for Hydrolysis of Amino Acid Function. Operating in a sealed tube, compound (+)-*exo*-**122c** or (-)-*exo*-**122c'** or (+)-*endo*-**123c** or (-)-*endo*-**123c**' or (+)-*exo*-**133** or (-)-*exo*-**122f'** or (+)-*endo*-**122f** or (-)-*endo*-**132**' or (±)-*exo*-**122d** or (±)-*exo*-**122g** (1 mmol) was suspended in 6N HCl (1 mL) and the mixture was heated at 120 °C for 14 h. The solvent was removed and the corresponding acids *exo*-**128,** *exo*-**128'**, *endo*-**129** and *endo*-**129'** , (+)-*exo*-**137,** (-)-*exo*-**137,** (+)-*endo*-**138**, (+)-*endo*-**138'** were isolated in quantitative yield. Spectroscopic data of known compound are in agreement with literature.[2]

(-)-(1*S*, 5*S*, 6*S*)-6-Amino-3-aza-bicyclo[3.2.1]octane-6-carboxylic Acid *exo*-130 · 2 HCl: $[\alpha]_D^{25}$ - 9.3 (*c* 0.5, MeOH).

(+)-(1*R*, 5*R*, 6*S*)-6-Amino-3-aza-bicyclo[3.2.1]octane-6-carboxylic Acid *endo*-131 · 2 HCl $[\alpha]_D^{25}$ + 4.9 (*c* 0.5, MeOH).

General Procedure for the Fmoc protection. Compound (-)-.*exo*-**137**'(226 mg, 0.65 mmol); was suspended in 1,4-Dioxane (10 mL). After addition of aqueous solution $NaHCO_3$ (6mL) the pH turned basic. FmocOSu (240 mg, 0.914 mmol) was dissolved in 5 mL of Dioxane and dropped into the stirring solution. The reaction mixture was stirred (overnight, 25 °C). The solvent was evaporated. The crude reaction mixture was solubilized in HCl 2N (10 mL) to pH acid and the product was precipitated and filtered off. Product (-)-.*exo*-**140**' (332 mg, 0.67 mmol)

(1*S*, 5*S*, 6*S*, 1'*R*) 6-Amino((9H-fluoren-9-yl)methylcarbamoil)-3-azabicyclo[3.2.1]octane-6-carboxylic Acid (-)-***exo*-140'.** $[\alpha]_D^{25}$ = - 18.9° (*c* 0.3, MeOH); IR ν_{max} cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.56 (d, *J* 7.0, 3 H) 1.67 (brs, 2H) 1.8-2.0 (brs 1 H), 2.32-2.54 (m, 2 H), 2.80 (s, 2 H), 3.09 (brs, 2 H), 3.87 (d, *J* 6.2, 1 H), 4. 15 (d, *J* 3.3, 1 H), 4.21 (d, *J* 5.9, 1 H), 4.33 (d, *J* 7.0, 1 H), 4.47 (brs, 1 H), 4.65 (d, *J* 7.0, 2 H), 7.29-7.38 (m, 7 H), 7.6-7.83 (m, 6 H); ^{13}C NMR (CD_3OD) δ 175.2, 157.8, 144.2, 144.0, 141.5, 134.6, 130.0, 129.4, 127.9, 127.4, 125.5, 125.2, 120.0, 67.2, 67.1, 56.8, 52.0, 41.2, 34.7, 33.8, 30.0, 25.4, 14.5 *m/z* 497.2 $[M]^+$; Anal. Calcd for $C_{31}H_{32}N_2O_4$: C, 74.98; H, 6.50; N, 5.64. Found: C, 75.01; H, 6.51; N, 5.63.

General Procedure for the Boc protection of compounds 137 and 137'. Compounds (+)-.*exo*-**137** (398,8 mg, 1.08 mmol) or (-)-.*exo*-**137**'(400 mg, 1.15 mmol); was suspended in Bu^tOH (10 mL). After addition of aqueous solution NaOH 2N (6mL) the pH turned basic. Boc anhydride (1,3 g, 6.3 mmol) was dissolved in 15 mL of H_2O and dropped into the stirring solution. The reaction mixture was stirred (3 days, 25 °C). the solution was concentred and the Boc anhydride was extracted with diethylether. HCl 2N was added in the solution to pH = 4 and cooling the water solution and the product precipitate and was filtered of giving respectively (+)-.*exo*-**N** (mg, mmol yield 40%)or (-)-.*exo*-**N**(mg, mmol yield 35%).

(1*S*, 5*S*, 6*S*, 1'*R*) 6-Amino((*ter*-Buthyl)carbamoil)-3-azabicyclo[3.2.1]octane-6-carboxylic Acid (-)-***exo*-153'.** $[\alpha]_D^{25}$ = -57.97° (c 5.25, MeOH); IR ν_{max} 3600-2950, 1691, 1600, 1500, 1169 cm^{-1}; ^1H NMR (CD_3OD) δ 1.3-1.4 (m, 6 H), 1.44 (s, 9H) 1.53- 1.61 (d,d, *J* 13.6, 2.2, 1 H), 1.93 (brs, 2H), 2.05-2.11(brs, 2H), 2.24 (d,d, *J* 13.5, 6.6, 1 H) 2.49 (d, *J* 6.2, 1 H), 2.73 (s, 1H), 2.99 (d, *J* 7.0, 1 H), 3.20-3.29 (d,d, *J* 1 H), 7.30-7.25 (m, 5 H); ^{13}C NMR (CD_3OD) δ 181.3, 156.9, 145.5, 128.2, 127.2, 126.7, 78.5, 67.6, 65.3, 58.0, 53.3, 43.6, 40.5, 37.3, 34.4, 27.7, 20.4; *m/z* 375.2 $[M]^+$, 397.2 $[M+Na^+]$; Anal. Calcd for $C_{21}H_{30}N_2O_4$: C, 67.35; H, 8.07; N, 7.48. Found: C, 67.38; H, 8.08; N, 7.49.

(1*R*, 5*R*, 6*R*, 1'*R*) 6-Amino((*ter*-Buthyl)carbamoil)-3-azabicyclo[3.2.1]octane-6-carboxylic Acid (+)-***exo*-153.** $[\alpha]_D^{25}$ = +19.5° (c 5.36, MeOH);IR ν_{max} 3600-2950, 1691, 1600, 1500, 1169 cm^{-1}; ^1H NMR (CD_3OD) δ 1.31-1.439 (m, 6 H), 1.39 (s, 9H) 1.61- 1.69 (d,d, *J* 13.6, 2.6, 1 H), 1.95 (d, *J* 9.3, 1H), 2.1 (brs, 2H), 2.18 (d, *J* 9.9, 1H), 2.41 (d,d, *J* 14.0, 6.6, 1H), 2.50 (brs, 1H), 2.57 (d, *J* 11.0, 1H), 2.88 (d, *J* 9.5, 1H), 3.32 (q, *J* 6.6,14.1, 1H) 7.25-7.35 (m, 5H);^{13}C NMR (CD_3OD) δ 181.4, 156.6, 144.1, 128.2, 127.5, 126.6, 67.4, 65.2, 56.8, 53.7, 41.1, 37.1, 34.4, 27.7, 18.6; *m/z* 375.2 $[M]^+$, 397.2 $[M+Na^+]$; Anal. Calcd for $C_{21}H_{30}N_2O_4$: C, 67.35; H, 8.07; N, 7.48. Found: C, 67.36; H, 8.06; N, 7.48.

General Procedure for the synthesis of peptide 154, 155 via solid-phase peptide synthesis.

Peptide **154, 155** were synthesized via solid-phase synthesis using manual coupling steps on a peptide rink amide linker MBHA (0.58 mmol/g) solid support. The resin was swelled for 30 minute in DMF, after which the Fmoc protecting group was cleavage with a DMF/Piperidine solution (1 mL 40% of piperidine, 3 minute). We repeated the cleavage DMF/Piperidine solution (1 mL 20% of piperidine, 10 minute) and the resin was washed with DMF to remove all the Fmoc residue.

Each oligomer was synthesized manually. Double coupling of the N-α-Fmoc natural amino acids was performed using a mixture of amino acid/HOBt/HBTU/DIPEA (4 eq. each) in DMF for all four coupling. A single coupling of the Fmoc-ABO-OH (6 eq.) using HBTU (8 eq.)/HOAt (8 eq.)/Collidine (10 eq.) in DMF for 4h was preferred. After each coupling, the not reacted amino acid coupled chain was stopped

using a acetylation reaction with acetic anhydride (17 μL in 0.5 mL of DMF) DIPEA for 30 minute. Fmoc cleavage was developed using DMF/Piperidine solution (1 mL 40% of piperidine, 3 minute) than we repeated the cleavage DMF/Piperidine solution (1 mL 20% of piperidine, 10 minute) and the resin was washed with DMF to remove all the Fmoc residues.

General Procedure for the synthesis of peptide 156 via solution-phase peptide synthesis.

Coupling reaction

(+)-exo-ABO **153',159,162,164** (1 eq.) was dissolved in CH2Cl2(10 mL) of. DIPEA (eq.), HOBT (10 eq.), EDC (10 eq.) and L-Ala-OMe (5 eq.) were added to the solution mixture and the reaction was stirred at 25°C for two days. The organic phase was washed with water and dried over Na_2SO_4. The solvent was removed and the crude product was purifided over SiO_2 (CH_2Cl_2 : Et_2O). Giving pure compound N (Yield 40%).

Boc deprotection

Compound **158, 161, 163** was solubilized in a solution of TFA/CH_2Cl_2 and stirred overnight at 25°C. The solution was treated with a acqueous solution of Na_2CO_3 and the product was estracted with CH_2CL_2, dried and the solvent was removed under reduced pressure. The compounds were analyzed without further purifications.

ΔδNH/ΔT

We have prepared a sample dissolved compound **156** and **164** in CD_3CN and a series of ^1H spectra to different temperature have been registered. The analytical data of the δNH have been reported below (only the signals without overlapping were reported)in the table 10.

Table 10 tetrapeptide **164**

δNH4	7.13	7.11	7.08	7.06
T°K	253	268.9	299.91	337
δ NH2	7.88	7.86	7.82	7.78
T°K	253	268.9	299.91	337

[a] [peptide **164**] = 14,8 mM in CD_3CN, T° = 253 K to 337 K

Table 11 pentapeptide **156**[a]

δNH5	7.87	7.84	7.80	7.66		7.54	7.50	
T°K	212.04	223.75	245.61	271.51	288.31	299.94	314.57	348.33
δ NH4				7.66	7.57	7.51	7.46	
T°K	212.04	223.75	245.61	271.51	288.31	299.94	314.57	348.33
δ NH3	7.70	7.66	7.61	7.57	7.52	7.50	7.47	
T°K	212.04	223.75	245.61	271.51	288.31	299.94	314.57	348.33
δNH2								7.20
T°K	212.04	223.75	245.61	271.51	288.31	299.94	314.57	348.33
δ NH1	6.38	6.33		6.30	6.16	6.05	5.92	5.67
T°K	212.04	223.75	245.61	271.51	288.31	299.94	314.57	348.33

[a] [peptide **156**] = 16,8 mM in CD_3CN, T° = 212,04 K to 348.33 K

Table 12 Kinetic Exchange data of the tetrapeptide **164**

[peptide **164**] = 14,8 mM in CD_3CN (700 µL) then D_2O (20 µL), T = 299.94 K

			NH4 = 7,08	NH2 = 7,82
N° prova	N° exp.	t (s)		
0	60	0	1,3	1,3
1	61	280		exchanged
2	62	310	0,65	
3	63	340	0,57	
4	64	370		
5	65	400	0,44	
6	66	430	0,4	
7	67	490		
8	68	550	0,3	
9	69	610	0,25	
10	70	670	0,19	
11	71	730		
12	72	790		
13	73	850	0,12	
14	74	910		
15	75	970	exchanged	
16	76	1030		
17	77	1330		
18	78	1630		

Table 13 kinetic exchange data for the pentapeptide **156** with D_2O:

[peptide **156**] = 16,8 mM in CD_3CN (700 µL) then D_2O (20 µL), T = 299.94 K

N°	N°exp	time	NH1	NH2	NH3	NH4	NH5
		s					
				7.55	7.52	7.47	7.42	6.03
					excanged	overlapped		
0		0	1,08	1,08				1,8
1	31	280	0,92	1,06				0,63
2	32	310	0,91	1,04				0,52
3	33	340	0,91	1,06				0,43
4	34	370						
5	35	400	0,88	1,05				0,31
6	36	430	0,85	1,05				0,26
7	37	490	0,83	1,05				
8	38	550	0,8	1,04				0,18
9	39	610						
10	40	670	0,77	1,03				0,12
11	41	730						
12	42	790	0,75	1,03				0,07
13	43	850						
14	44	910	0,71	1,01				0,05
15	45	970						
16	46	1030	0,67	1,01			exchanged	
17	47	1330						
18	48	1630	0,6	1				
19	49	1930						
20	50	1530	0,6	1				
21	51	2130						
22	52	2730	0,49	0,95				
23	53	3930						
24	54	5130	0,42	0,95				
25	55	6330						

26	56	8130	0,32	0,9
27	57	9930		
28	58	11730	0,27	0,85
29	59	13530	0,25	0,84
30	60	17130	0,26	0,79
31	61	20730	exchanged	exchanged
32	62	27930		
33	63	35130		
34	64	49530		

Metallorganic chemistry

(S)-4-benzyl-4,5-dihydro-2-vinyloxazole 174: $[\alpha]_D^{25}$ = -93.7° (c 10.2, $CHCl_3$) IR ν_{max} 3600-3000, 1666, 1645, 1454 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.67 (AB system, *J* 13.8, 8.4, 1 H), 3.15 (AB system, *J* 13.8, 5.5, 1 H), 4.04 (t, *J* 8.06, 1 H), 4.24 (t, *J* 8.43, 1 H), 4.46 (m, *J* 8.4, 5.5, 1 H), 5.68(dd, *J* 10.6, 1.8, 1 H), 6.04 (dd, *J* 17.6, 1.8, 1 H), 6.31 (dd, *J* 17.6, 10.6, 1 H), 7.19-7.35 (m, 5 H); ^{13}C NMR δ 163.4, 129.4, 128.8, 126.7, 126.2, 125.2, 71.7, 67.9, 41.9, 27.14; *m/z* 188.2 $[M + H]^+$; Anal. Calcd for $C_{12}H_{13}NO$: C, 76.98; H, 7.00; N, 7.48. Found: C, 76.96; H, 6.98; N, 7.49.

(*endo*)-(S)-4-benzyl-2-(bicyclo[2.2.1]hept-5-en-2-yl)-4,5-dihydrooxazole 175a: mixture of two diastereoisomers IR ν_{max} 3600-3200, 2971, 2930, 1660, 1453 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80-0.85 (m, 1 H), 1.15-1.6 (m, 9 H), 1.85-2.10 (m, 2 H), 2.4-2.6 (m, 2 H), 2.8-3.2 (m, 10 H), 3.8-4.0 (m, 2 H), 4.0-4.2 (m, 2H), 4.2-4.4 (m, 2 H), 5.8-6.0 (m, 2 H), 6.1-6.3 (m, 2 H), 7.1-7.4 (m, 10 H); ^{13}C NMR δ 170.2, 138.2, 137.63, 137.61, 132.68, 132.51, 129.6, 129.5, 129.57, 128.6, 126.5, 71.5, 71.45, 67.27, 67.15, 49.8, 49.76, 46.03, 45.97, 42.77, 42.74, 41.9, 41.8, 37.5, 37.4, 30.4, 30.3, 30.2, 29.9, 27.12 *m/z* 254.1 $[M + H]^+$; Anal. Calcd for $C_{17}H_{19}NO$: C, 80.60; H, 7.56; N, 5.53. Found: C, 80.58; H, 7.58; N, 5.55.

(*endo*)-(bicyclo[2.2.1]hept-5-en-2-yl)methanamine 165: compound known in licterature and prepared follow the procedure in the L.I. Kasyan, I.N. Tarabara, O.A. Kasyan, O, Sergiy *Tetrahedron,* **2007,** *63(8),* 1790-1797

(*endo*)-(bicyclo[2.2.1]hept-5-en-2-yl)-N-tosylmethanamine 166: mixture of two diastereoisomers; ^{1}H NMR ($CDCl_3$) δ 0.5 (m, 1 H), 1.1(d, 1 H), 1.4 (d, 1 H), 1.8 (m, 1H), 2.2 (m, 1H), 2.45 (s, 3 H), 2.5-2.6 (m, 1H), 2.7-2.75 (m, 2 H), 2.8 (s, 1 H), 2.9 (s, 1 H), 5.2 (bs, 1H), 5.75 (m, 1 H), 6.1 (m, 1H), 7.3(m, 2 H), 7.7 (m, 2 H).

Organocatalysis

General Procedure for the Diels-Alder reaction.

Catalyst **128** (9 mg, 0.05 mmol) and cynnamaldehyde **182** (88 mg, 0.5 mmol) were solubilized in CH_3CN/H_2O solution (1.5 mL, 95:5), distilled cyclopentadiene (99 mg 1.5 mmolo) was added into the solution. The reaction was stirred for 72 h or 42 h at 25°C. The solvent was removed and the product were purified by column chromatography. The pure product mixture was analyze by chiral HPLC.

General Procedure for Staudinger reaction

Tioazolidinic acid **190** (50 mg, 0.214 mmol) was dissolved in toluene (5 mL), Mukayama activator **191** (63.4 mg, 0.247 mmol) and the protonsponge base **192** (45.79 mg, 0.214 mmol) were added in the reaction mixture and warmed to 70°C for 1h allow to obtain the relative chetene **193**. Imid **194** (31.1 mg, 0.107 mmol) protonsponge base **192** (91.58 mg, 0.428 mmol) and catalytic base ABO **123a** and **125** (7,09 mg 0.0214 mmol) were dissolved in toluene (5mL) and dropped in the stirring reaction to 70° C or 25° C. The reaction was stirred overnight. The product mixture was then washed with HCl 2 N solution to remove the excess of base. The organic phase was dried over Na_2SO_4 and the product purified by column cromatography.

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