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β-AMINO ACIDS AS TOOL FOR THE PREPARATION OF FOLDAMERS AND NANOMATERIALS

Ph.D. Thesis presented by: Andrea BONETTI

R09727

SUPERVISOR: Prof.ssa Maria Luisa GELMI

COORDINATOR: Prof. Ermanno VALOTI

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Possano le tue scelte riflettere le tue speranze,

non le tue paure.

N. Mandela

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Abbreviations

AA. amino acid

Ac. acetyl

ACC. aminocyclopropanecarboxylic acid

ACHC. 2-aminocyclohexanecarboxylic acid

ACPC. aminocyclopentanecarboxylic acid

Aib. Aminoisobutyric acid

Ala. alanine

APC. aminopyrrolidinecarboxylic acid

Bn. benzyl

Boc. *tert*-butyloxycarbonyl

Cbz. benzyloxycarbonyl

CD. circular dichroism

COSY. correlation spectroscopy

DBU. 1,8-Diazabicyclo[5.4.0]undec-7-ene

DCHC. 2,5-diaminocyclohexanecarboxylic acid

DCM. dichloromethane

DIPEA. diisopropylethylamine

DMAP. *N*,*N*-dimethylaminopyridine

DMF. *N*,*N*-dimethylformamide

DMSO. dimethylsulfoxide

ee. enantiomeric eccess

eq. equivalents

ESI.electrosprayionisation

Fmoc.Fluorenylmethyloxycarbonyl

hAla. Homoalanine

HMBC. Heteronuclear Multiple Bond Correlation

HOAc.acetic acid

HSQC. Heteronuclear Single Quantum Coherence

hVal. Homovaline

***i*Bu**.isobutyl

IR.infra-red

Me. methyl

MS. mass spectrometry

Nip. Nipecotic acid

NMM. *N*-methylmorpholine

NMR.nuclear magnetic resonance

NOE.nuclearOverhauser effect

NOESY.nuclearOverhauser effectspectroscopy

Nt. Nanotube

PBS. Phosphate buffer solution

pfb. perfluorobutirrate

Ph. phenyl

Ppm. parts per million

Pro. proline

ROESY.rotating frame NOESY

Rt. room temperature

SPPS.solid phase peptide synthesis

***t*Bu**.*tert*-butyl

TEA. Triethyamine

TEM.transmission electronmicroscopy

TFA.trifluoroacetic acid

THF.tetrahydrofurane

TIC. tetrahydroisoquinoline

TOCSY.total correlationspectroscopy

tpa. tetraphenylacetate

Val. valine

Chapter 1 –General Introduction

1 Beta Aminoacids, Beta Peptides and α,β–Hybrid Peptides: Synthesis, Conformational Analisys and Applications.

β-Amino acids are analogues of α-amino acids (AAs) in which the amino group is linked to the beta-carbon instead of the alpha-carbon (Figure 1).

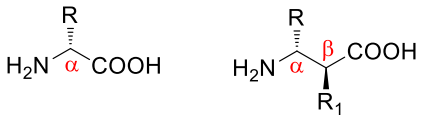


Figure 1. Alpha and Beta amino acids

Differently from proteinogenicα–amino acids, that are costituent of all enzymes which control the metabolism in living matter and are thus an essential prerequisite for life, β–amino acids are present in few natural products, such as peptides, cyclopeptides, glycopeptides, alkaloids or terpenoids. Therefore, these compounds are often characterized by potent biological and physiological activities that are often crucially based on their structures. As a consequence, many natural products with aβ–amino acids (AAs) moiety are potential lead structures for the development of new drugs (Figure 2).

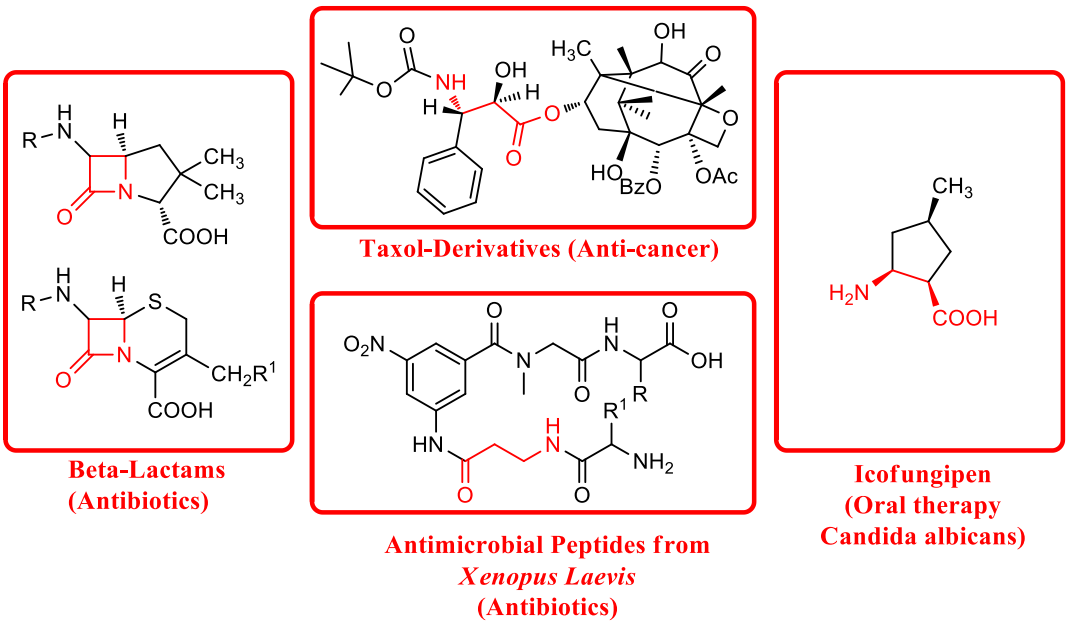


Figure 2. Bio-active compounds containing β-amino acids moiety

Moreover, the incorporation of β–amino acids into peptides instead of α–amino acids increases the stability against degradation by mammalian peptidases. This enhanced stability is caused by a lack of enzymes which induce the cleavage of β–peptidic bond. Therefore β–amino acids are important tools in the development of drugs as single molecules or for the preparation of peptides capable of withstanding hydrolytic degradation for prolonged periods of time. For all these reasons, in recent years,the focus on the synthesis of new β–amino acids is exponentially increased, making this field of great interest and strong expansion (Figure 3).

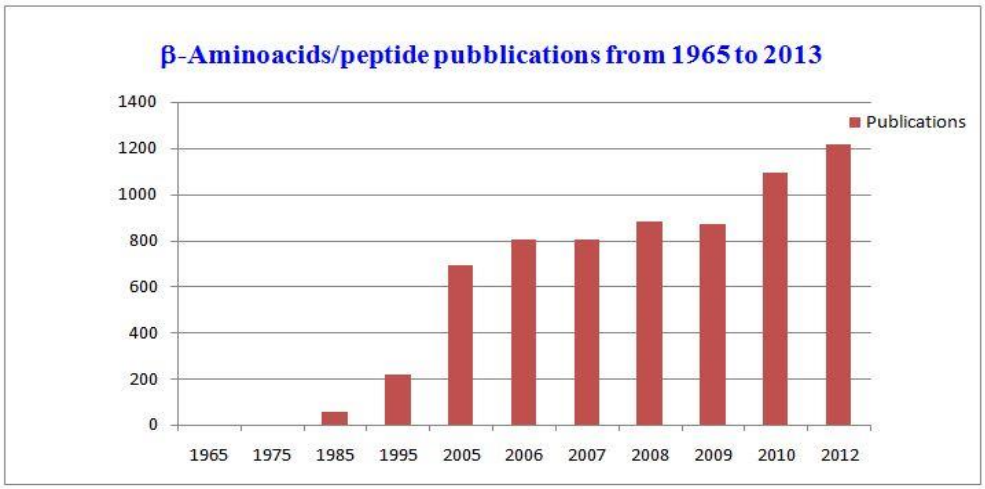


Figure 3

1.1 Acyclic β-Amino Acids Nomenclature and conformational properties

The nomenclature for β-AAs generaly takes in consideration three types of acyclic β–amino acids, depending on whether the sobstitution of side chain takes place, (*i.e.*C_α, C_β, or both). Recently, Seebach and co-workers [1] proposed the terms β^2 and β^3 amino acids, where the numbers indicate the position of the side chain substitution with respect to the carboxylic function, in order to distinguish positional isomer (Figure 4).As regards as β–aminoacidscarringsubstitutionat bothC_α andC_β ($\beta^{2,3}$–AA) the number of possibleregioandstereoisomers becomesgreaterthan β^2 and β^3-AAswhere only enantioisomersare possible. $\beta^{2,3}$-AAs, may exist as apair of diastereisomers generally named*syn*and*anti*depending on the stereoisomeric relationshipof the substituents (Figure 4). Furthermore, position C^2 and C^3can be doubly substituted, giving rise to more complex and constrained β-AAs.

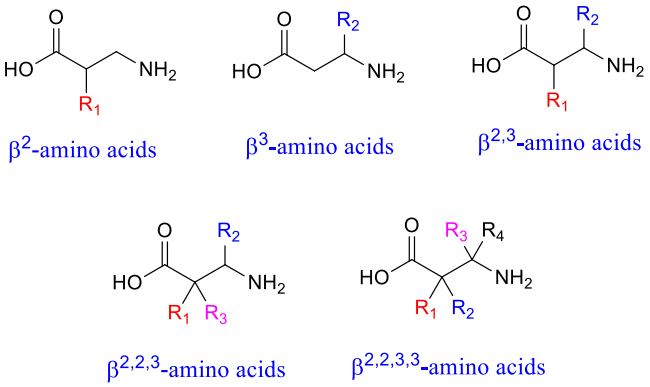


Figure 4

Concerning the use of β-AAs in peptide synthesis, it is of relevance to analyze the amino acid conformation in terms of the main chain torsional angles assigned as φ, θ, and ψ according to the convention of Balaram[2](Figure 5).

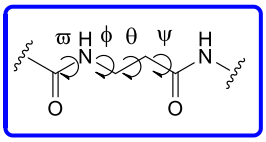


Figure 5

The effects of the substituents on the local conformation of a β-amino acidare summarized in Figure 6. The unsubstitutedβ-amino acid, (*i.e.* β-alanine), is highly flexible, as for Glycine in the α-amino acids. Substituents at position 2 or 3 favor a *gauche* conformation around the C^2-C^3 bond[3].

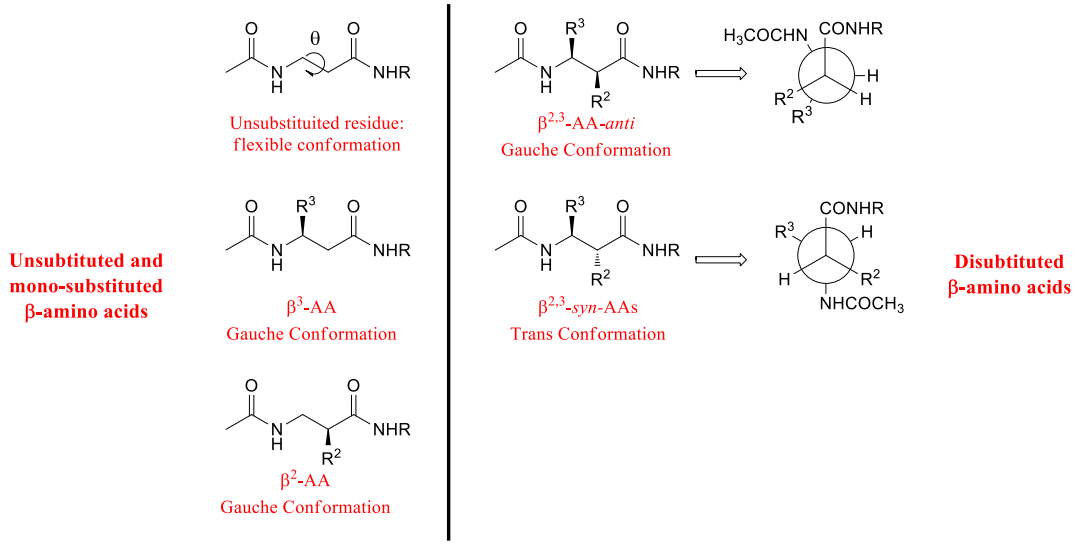
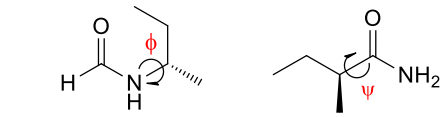


Figure 6. Effects of the substituents on the local conformation of a β-amino acid

$\beta^{2,3}$-amino acids are even more conformationally constrained and a *gauche* or *trans* confortioman is favoured when the substituents are *anti* and *syn* respectively (Figure 6). Computational studies on model fragments of β–amino acids are reported in the literature, and are focused on the comprehension of the effect of substituents on conformation[4]. The torsional effect of methyl substitution on various model has been thoroughly analysed by employing *ab initio*MO quantum chemical calculations. The local effect on φ angleof methyl substitution at C^3 with the S-configuration [(S)-β^3-substitution] was studied in the case of N-(*S*)-*sec*-butylformamide, while the influence of (S)-β^2 substitution on ψ angle was modelled with *sec*-butyramide[5](Figure 7)



N-S-*sec*-butylformamide *sec*-butyramide

Figure 7

The potential energy profile of φ angleindicates that its values are restricted in the region between 60°and 180°. There is also a narrow minimum around -60°, with a relatively high rotational barrier (Figure 8).

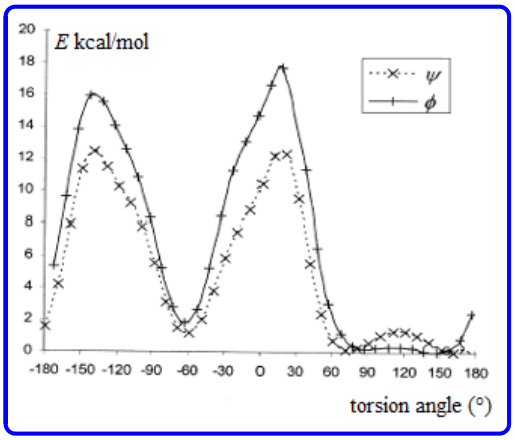


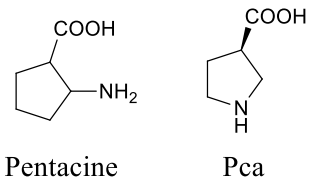
Figure 8: Relative torsional energy profile for ϕ and ψ angles, calculated for (*S*)-β^3–Me and (*S*)-β^2–Me-substituted model system**.** The reduced conformational space for both β^2 and β^3 substitution, explains their significant structuring effect.

The potential energy surface for ψwas found to be rather flat, with two minima, in the range of 60–180°and at around -60°. These results can be transferred to (R)-β^3 and (R)-β^2 substitutions by changing the signs of the dihedral angles. These analyses reveal that a side chain in the β^3 position has a significant structuring effect on the local geometry exerted through the steric interactions along φ angle. On the other hand side chain in the β^2 position has a structuring effect on the global geometry in peptide structures, influencing through the steric interactions along ψ angle. It is interesting to note that all the H-bond-stabilized periodic structures can be found within the range φ

=60–180°or φ= -180-60°that are in fact the preference regions of the (*R*)-β^3 or (*S*)-β^3 substituted β-amino acid.

1.2 Conformational Properties of cyclicβ-Amino Acids

Cyclic β–amino acids may present the amino and carboxylic groups as substituents of a carbocyclic ring or may incorporate the amino group in a heterocyclic ring, as shown in Figure 9 for Pentacine and Pyrrolidine-3-carboxylic acid (Pca).



Pentacine Pca

Figure 9

The use of cyclic β–amino acids in peptide synthesis has been extensively studied in the last decades. These studies are focused mostly on *trans*-2-aminocyclohexanecarboxylic acid (ACHC),[6]*trans*-2,5-diaminocyclohexanecarboxylic acid (DCHC),[7]*trans*-2 aminocyclopentanecarboxylic acid (ACPC),[8]*trans*-3-amino-pyrrolidine-4-carboxylic acid (APC)[9]or cis-pentacine (Figure 10).Clearly, *gauche*-type conformations are even more strongly promoted when C^2 and C^3 substituent are linked at a cyclopropane, cyclopentane or cyclohexane ring, both when amino and carboxylic functions are in *cis* or *trans* relationship. It was evidenced that the ring size determines the precise C^2-C^3 torsional preference, which in turn influences the β-peptide architecture[10].

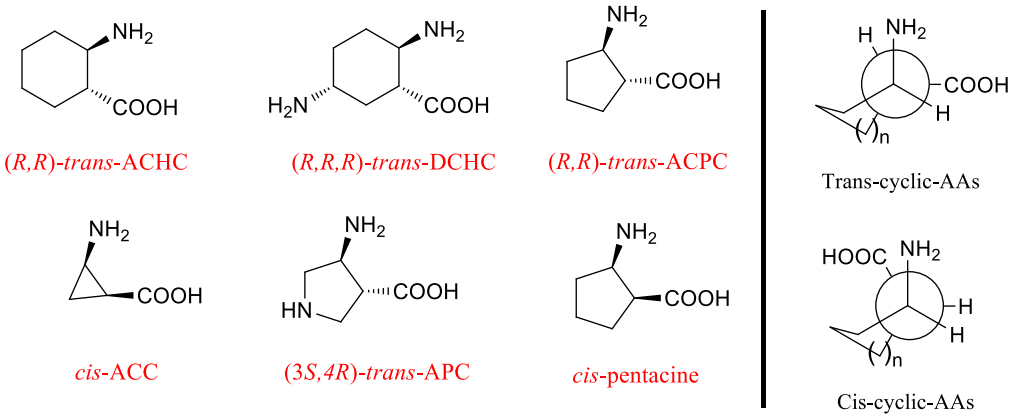
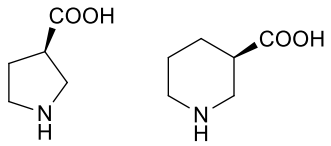


Figure 10

The cyclic amino acids pyrrolidine-3-carboxylic acid (Pca) and nipecotic acid (Nip) containing the nitrogen atom in the ring (Figure 11) are β-substituted equivalents of Proline and pipecolinic acid, respectively, and hence, their conformational properties are similar to those of the corresponding α-amino acids[11]. The θ angle is highly constrained by the aza-ring and contributes to a conformational rigidifying effect of the scaffold.

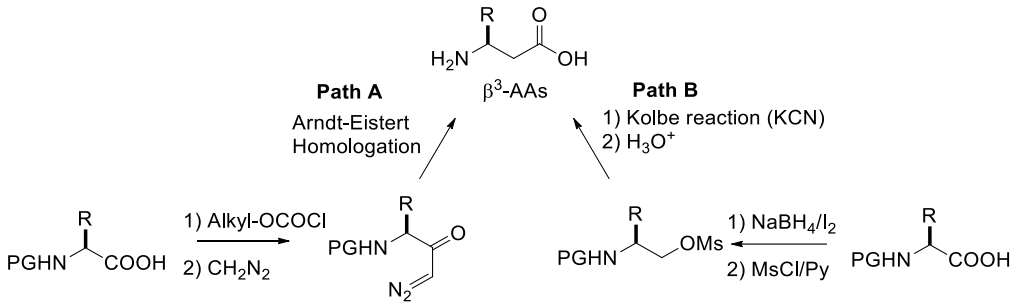


Pca Nip

Figure 11

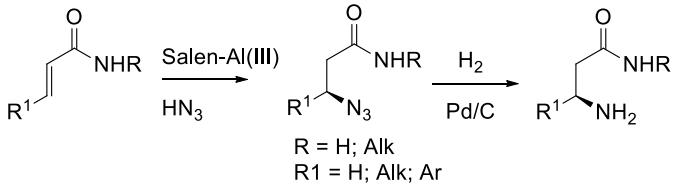
1.3 StandardSynthetic Methodologies for the preparation of β-Amino Acids

A brief overwiev of the main synthetic methods of β-AAs is reported below. β^3-Amino acid were generally obtained by Arndt-Eistert homologation (Scheme 1)[12]starting from an enantiopureα-amino acid. The first step of this method is the transformation of the carboxylic function into diazo-carbonyl moiety. α-Diazoketones are excellent substrates for silver catalyzed Wolff rearrangement (path A) leading to the formation of β^3-AAs in good to excellent yields with retention of the absolute configuration. A valid alternative to the Eistert homologation is the hydrolysis of β^3-amino nitriles, synthetized by the Kolbe nucleophilic substitution (Path B). This method requires the reduction of an enantiopureα-AA to the corresponding amino-alchool, and the transformation of the hydroxyl function in a mesylate. The nucleophilic substitution with cyanide ion affords the β^3-amino nitriles in good yield then hydrolyzed to the β^3-AA. However, the conventional chemical hydrolysis of nitriles suffers from several disadvantages, including the requirement for harsh acidic or basic conditions, high temperatures, the formation of undesirable by-products, racemization, low yields, and environmental problems due to the generation of waste salts.As a result, in recent years, considerable attention has been paid to the enzymatic hydrolysis of nitriles as an alternative route to the chemical synthesis of carboxylic acids.High conversion yields and selective hydrolysis of the CN functionality of compounds containing labile groups such as peptides or aminoacids functionalized on the chain, as well as high chemo-, regio- and stereoselectivity control can be obtained. On the other hand, biocatalysis frequently use a biodegradable catalyst and can be performed under mild reaction conditions (neutral pH, low temperature, and water as solvent).[55]Furthermore, the use of enzymes usually generates less waste and, hence, is both environmentally and economically more attractive than traditional organic syntheses.



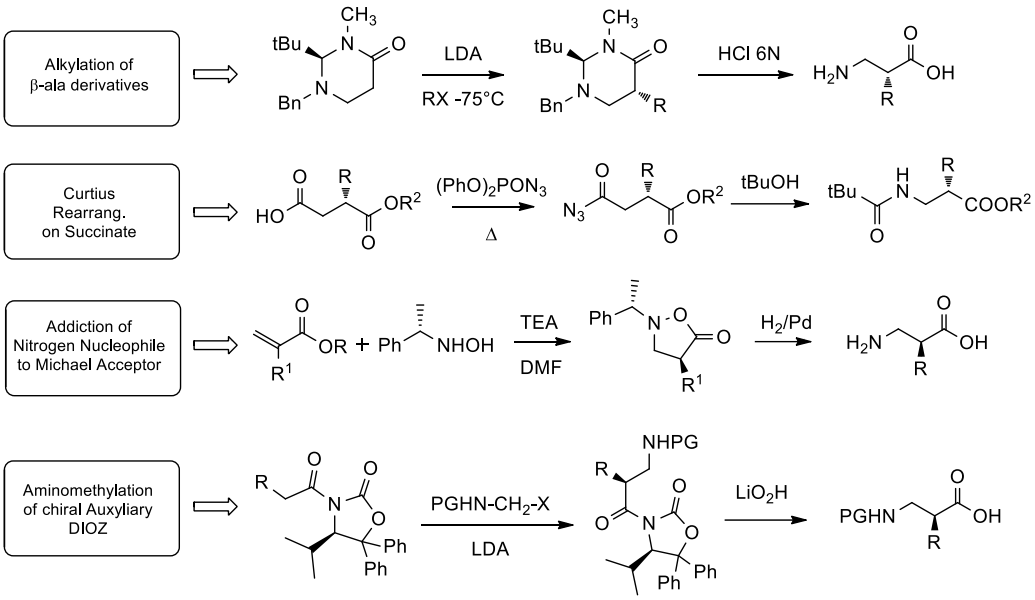
Scheme 1

Alternatively to these methods, the use of azide in conjugate addition of nitrogen nucleophile to aα,β–unsaturated carbonyl compound is also well documented for the synthesis of β^3-AAs. Chiral SalenAl(III) complexes catalyzed conjugate addition of hydrazoic acid (HN_3) to α,β-unsaturated imides was recently described by Jacobsen et al[13]. This procedure provided access to a variety of enantiopureβ^3-alky-l or β^3-aryl-azido-compounds that afford the corrisponding amines through a catalytic hydrogenation. (Scheme 2).



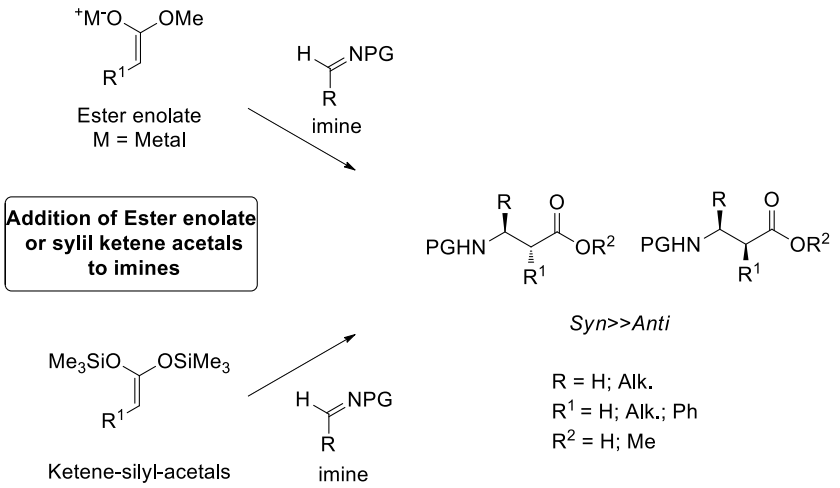
Scheme 2

In the last two decades several methodologies have been suggested for the synthesis of β^2-amino acids (Scheme 3), involving for instance, the diastereoselective alkylation of a chiral derivative of β-alanine,[14]Curtius rearrangement of a chiral succinates,[15] or conjugate addition of nitrogen nucleophiles to Michael acceptors. However, the most useful strategies, based on the pioneering work of Evans, is the aminomethylation of a chiral precursor, which bears the side chain of the amino acid.[16] Noticeably, Seebach*et al.* have successfully used this methodology for the preparation of a wide range of β^2-amino acids using DIOZ as chiral auxiliary[17]. This latter method is of greatest interest because it leads to better yields and enantiomeric excesses.



Scheme 3

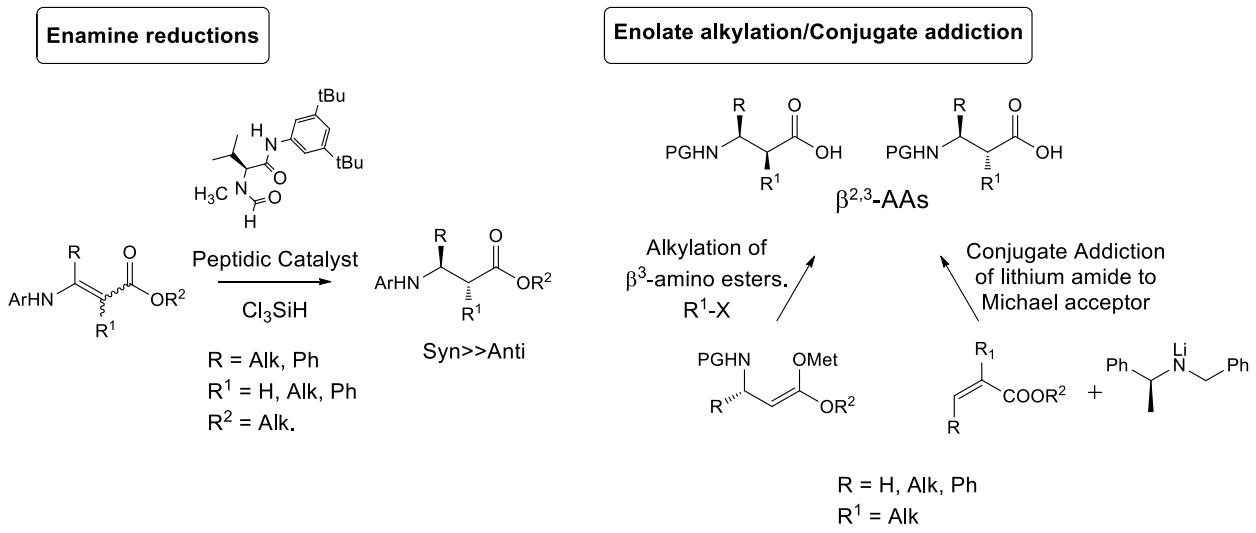
During mywork,I dealtmainly on the β[2,3]-amino acids synthesis. The preparation of these compounds can be performed in many different ways, but the addition of an ester enolate[18] or silyl ketene acetal[19] toan imine has received special attention because ofthe possibility to address the stereoselectivity of the reaction. Bythese Mannich-like reactions, several α,β-dialkyl, orα-phenyl-β-alkyl amino acids have been accessed. On the other hand the preparation of diaryl substituted β-amino acids is scarselyrapresented. With few exceptions[20], a *syn*/*anti* mixtureof β[2,3]-amino esters are formed through these methods, andthe *syn*adduct is the major diastereoisomer (Scheme 4). Many studies carried out using different metals to generate the enolate or changing the reaction solvent showed that these parameters caneffect the stereoselectivity of the reactions. Enantiopure versions of these transformation were developed throught the use of chiral auxiliary, or using Lewis acids in combination with chiral ligands, leading to good enantiomericeccesses.



Scheme 4

Some examples concerning the synthesis of $\beta^{2,3}$-amino esters are reported in literature, using an organocatalytic reduction of enamines[21](Path A). This alternative method works in mild conditions and makes use of an organocatalyst characterized by a peptidic structures, able to induce excellent enantiomericeccesses (Scheme 5).

Other methodologies that can be used for the preparation of $\beta^{2,3}$-AAs are the enantioselective alkylation of β^3-amino esters *via* metal-enolate or the conjugate addiction of a chiral lithium amides to 2,3-disubstituted Michael acceptors. (Path B).



Scheme 5

1.4 β-Oligopeptides and α,β-Hybrid Oligopeptides Conformation overview

Oligomers of non-proteinogenic AAs attracted considerable attention in the last several years because of their capability to form ordered secondary structures[22].In particular, β-peptides or α,β–hybrid peptides have increased our knowledge on proteins folding process, because β-amino acids represent the smallest step away from α-amino acids in "backbone space" and at the same time are more stable than α-AAs to the metabolic degradation. Like α-peptides, β-peptides contain amide bonds capable of forming intramolecular hydrogen bonds, and are therefore able to organize into secondary structures similar to those observed for peptides composed by α-AAs. Early investigations of β-peptides indicated that they were able to adopt stable helical structures,[23] sheet-like or hairpin secondary structures depending on the nature and stereochemistry of the costituentβ-AAs.

The effects of AAs substituents on the conformation of β-peptides have been the subject of extensive experimental studies[24]as well as molecular mechanics/dynamics calculations.Using these methods, it has been persuasively demonstrated that the secondary structure motif of a β-peptide can be efficiently controlled by altering the substituent pattern of β-AAs [25]. In the approach of Wu and Wang[26], the effects exerted by the substituents can be separated into two groups of components. One involves the impact on the local conformational stability at the residue level, referred to as the torsional effect. The other group comprises the medium and long-range effects due to steric and electrostatic interresidue interactions.

1.4.1 β-Oligopeptides Secondary Structures.

1.4.1.1 Helical conformations

To understand the behaviour of β-AAs on the formation of helical structures, polymers constituded by β^2 or β^3-amino acids, which differ from the commonly occurring α-peptides by the insertion of a single methylene group at the AA-level were studied. The insertion of an unsubstituted methylene group would increase the conformational flexibility of the β-amino acid, resulting in a more unfavorable entropy associated with helix formation.[27] On the other hand the energetics of β-hydrogen bonding, which requires the restriction of three dihedral angles, can be energetically more favorable than α-hydrogen bonding, which restricts only two torsions, with the consequent formation of more stable hydrogen bond network[28]. In organic solvents,β-peptides helices are quite stable relative to the α-helical conformation of α-peptides. Helix formation in biopolymers is generally length-dependent, with significant helix formation occurring only after a critical chain length is reached. In organic solvents such as methanol or trifluoroethanol, α−peptides composed of natural amino acids require approximately 8-12 residues to form stable helices, by contrast, different helices are formed in β-peptides composed of monosubstituted amino acids with as few as six residues. Further, highly conformationally constrained β-peptides can form helices with even four residues.[29]

The effects of substituents on the formation of the helical structures have been extensively studied.Polyamide sequences composed of β^2 and/or β^3-substituted β-amino acids can adopt different helical conformations. The nomenclature for these helical structures varied widely in the literature[30]. Here, we adopt a convention that depends on the number of atoms in the hydrogen-bonded ring of the peptide sequence. The main helical secondary structures adopted by oligo-β-peptides are reported in the Table 1 and Figure 12.

HELIX	H-Bond	CD Spectra[a]
14-Helix	NH *i* -- CO *i* + *2*	195(+); 215(-) nm
12-Helix	CO *i* -- NH *i* + *3*	205(+); 190(-) nm
10/12-Helix	CO *i* -- NH *i* + *1* NH*i* -- CO*i* + *3*	205(+) nm

Tab. 1. Helical conformations for oligo-beta peptides; [a] relative max. and min. values change in left-handed (reported) and right-handed helical structures.

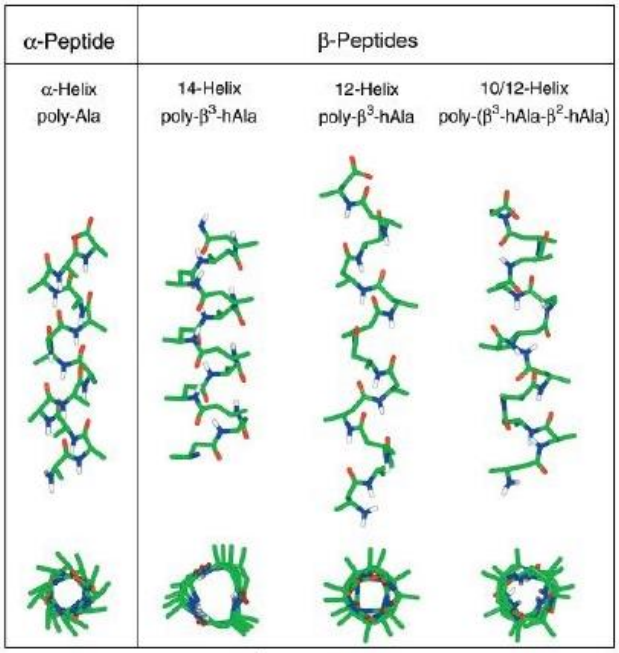


Figure 12. Graphical rapresentation of helical conformations for oligo-beta peptides.

Substitution patterns that favor the formation of a *gauche* conformation at C^2-C^3 bond such in monosubstitutedβ^2 or β^3-AAs, favor helix construct (Figure 12). The inclusion of the C^2- C^3 bond in a ring (as in trans/*cis*-ACHC; -ACC; -ACPC and -APC) is a strong conformational limitation[31]. This condition locks the θ angle to approximately +/- 55° strongly favoring helical architectures (Figure 13. Helix-12 for *trans-(R,R)*-ACPC octapeptide).

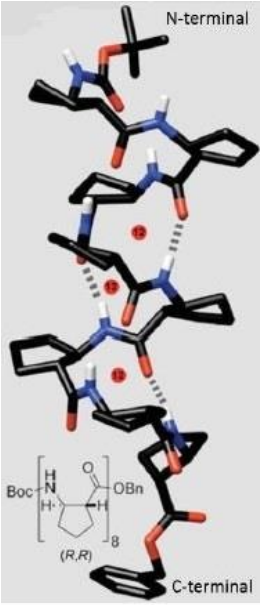


Figure 13

A similar situation is observed for *anti*$\beta^{2,3}$-disubstitution (see Figure 6). By contrast, *syn*-$\beta^{2,3}$-AAs favors a value of θ near 180° and hence strongly destabilizes helix formation. In recent works however[32] it has been shown that*syn*-$\beta^{2,3}$-AAs have a remarkable ability to shift from *anti* to the *gauche* conformation in response to the opportunity to form intramolecular hydrogen bond yielding helical structures.It is also interesting to consider $\beta^{2,2}$ and $\beta^{3,3}$-disubstitutedβ-AAs in light of the well-known tendency of dialkylα–amino acids, such as αα-aminoisobutyric acid (aib), to induce helical conformations in α-peptides. On the other hand, in β-peptides 2,2 or 3,3 disubstitution at the residues level,sterically prevents helix formation. This conformation forces one of the two alkyl groups into an axial position proximal to the helical axis[33] destabilizing this architectures.[34] The use of cyclic AAs with the nitrogen inside the ring such as Nip or Pca (Figure 11) generally breaks helices that require hydrogen bonding between amide NH and carbonyl of the backbone.This phenomenonalso happens for Proline, that is unique among the proteinogenic amino acids in having a disubstituted amino group. Proline linkages are therefore tertiary amides, which cannot serve as hydrogen bond donors. Despite the lack of internal hydrogen bonding, oligomers and polymers of proline adopt discrete secondary structures, such as polyproline I (PPI) and polyproline II (PPII) helices, that appear to be generated by avoidance of steric repulsions.[35] Non-hydrogen-bonded helices can also be formed by unnatural tertiary amide oligomers constructed from Nip and Pca as demonstrated by *Gellman* in the year 1999[36].

1.4.1.2 Sheet-like conformations (extended conformation)

Oligo-β-peptides give also extended structures. In principle two types of sheet secondary structure are available for β-peptides, the first one in which each residue has an *anti*C^2-C^3 torsion angle and the second in which each residue has a *gauche*C^2-C^3torsion angle.[37] The "*anti*" sheet type of β-peptide, generally named fully extended conformation, is characterize by all backbone carbonyls approximately oriented in the same direction, which would endow the resulting sheet with a net dipole (Figure 14). In contrast, β-sheets formed by α-peptides have little or no net dipole because the backbone carbonyls alternate in direction along each strand. Analogously β-peptide sheets formed by residues with *gauche*β^2-β^3 torsion angles would lack a net dipole.

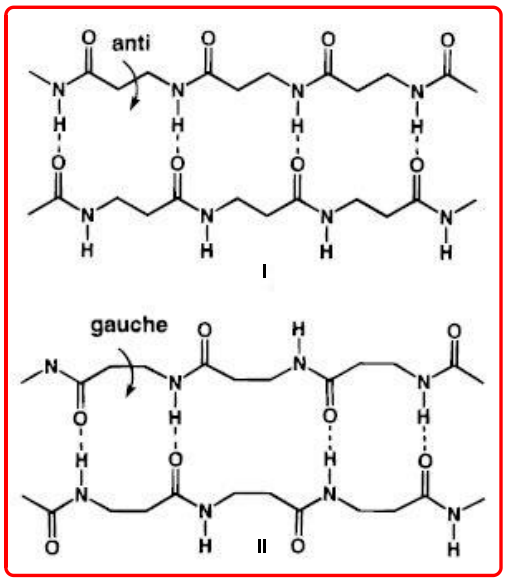


Figure 14. Anti and Gauche types sheet in b-peptide

Many of the findings on β–sheet structures of β–peptides were born by the extraordinary works of Gellman[38] and Seebach[39].In these workswere displayedscaffoldscapable of generatinghairpin-likestructures in solution that are linked to variously subtitutedβ–peptides that can generate parallel or antiparallel sheet (Figure 15).

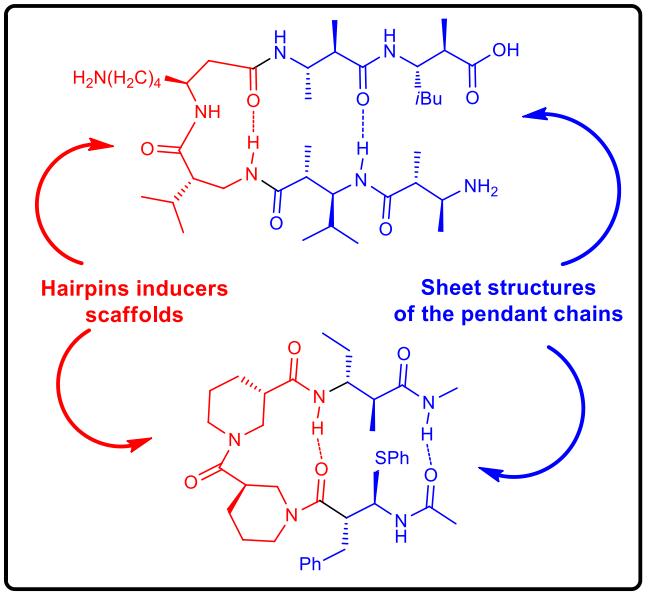


Figure 15

The *syn* configuration of $\beta^{2,3}$-amino acid residue in the pendants chains,favors *anti* C^2-C^3 torsion angles because the alkyl substituents can adopt an energetically favorable *anti*-orientation only

when the N-C^3-C^2-C torsion angle is 180°, generating a full extended sheet. The replacement of the disubstituted$\beta^{2,3}$-amino acid residues with β-alanine led to the formation of a non-polar sheet (Figure 14, type II) in which residues have gauche C^2-C^3 torsion angles, and replacement with β^2 or β^3-residues led to equilibration between the two types of β-peptide sheet. These results suggest that $\beta^{2,3}$ disubstituted residues with the *syn* configuration have the highest sheet-forming propensity. Figure 17 shown the main difference between sheets of α and β-peptides, suggesting that the two types of peptides cannot possibly form 'mixed' sheets.

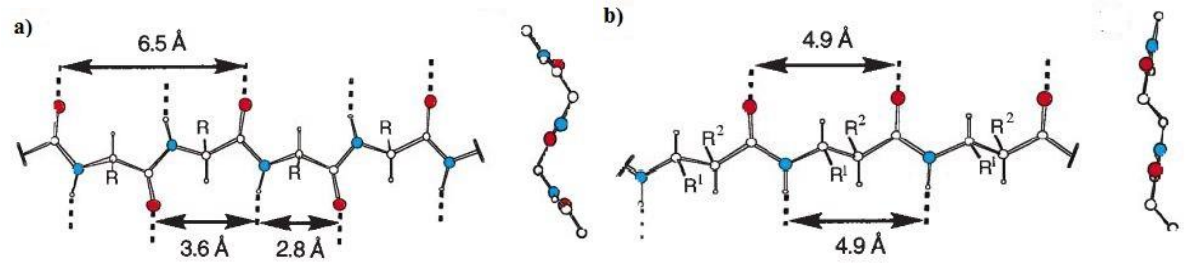


Figure 17. Models of the extended conformation of (a) an α–peptide and (b) a β-peptide which would permit pleated sheet formation.

1.4.1.3 Hairpin conformations

The turn conformation for β-peptides are relatively less studied with respect to other secondary structures, and only few examples are reported in recent leterature. In the year 1998 Seebach*et all*. Report on the formation of a 10-membered ring turn structures in homo-tripeptides composed by 2,2 or 3,3 disubstituted β-amino acids. Double substitution at C^2 or C^3 position sterically prevents helix formation. This conformation forces one of the two alkyl groups into an axial position proximal to the helical axis[40] stabilizing reverse turns motif (Figure 18).[41]

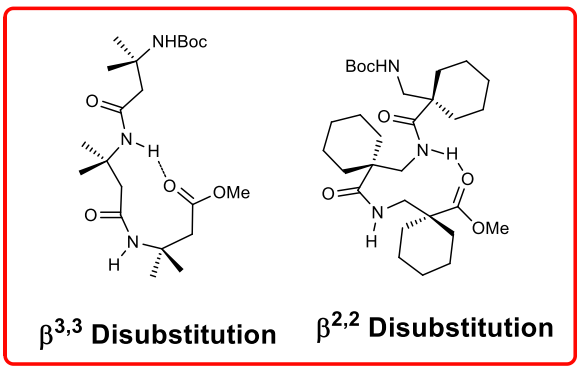


Figure 18. Turn-like structures for homo-tripeptides composed by 3-amino-3-methylbutanoic acidand 1-(aminomethyl)cyclohexanecarboxylic acid.

More recently, Gellman *et all.* first showed the behavior of a heterochiral (*R**,*S**) dipeptide composed of the Proline analogue nipecotic acid (Nip), inserted in the short β-tetrapeptide**A** (Figure 19).[42] This tetrapeptide contains exclusively β-amino acid residues, with the central two residues constituting the β-peptide reverse turnfacilitating antiparallel-sheet formation.The studies carried out on these kinds of amino acids, suggested that a tertiary amide was necessary at the center of the turn-forming segment because this amide adopts an *E* configuration in the reverse turn conformation while secondary amides are largely confined to *Z* configurations.The choice of the stereochemistry of Nipecotic acid is of crucial importance to induce the reverse turn. In facthomochiraldinipecotic acid segments (*i.e.*, both nipecotic acid residues with the same absolute configuration) prevented sheet interactions between the terminal residues of the peptide avoiding the formation of an ordered hairpin structures. Subsequently, Seebach*et al.*[43] found that a dipeptide sequence containing a β^2-AA followed by a β^3-AA stabilized a reverse turn when inserted in a $\beta^{2,3}$-AAs sequence. This motif is different from the reverse turn formed by the heterochiraldinipecotic acid segment for the dimension of hydrogen bonded ring of the hairpin (12-membered ring hydrogen bond in **A***vs* 10-membered ring hydrogen bond in **B**) (Figure 19). The availability of two distinct types of reverse turn among β-peptides highlights the greater conformational diversity in this foldamer family relative to α-peptides in which only a single type of reverse turn (β-turn) is commonly observed.

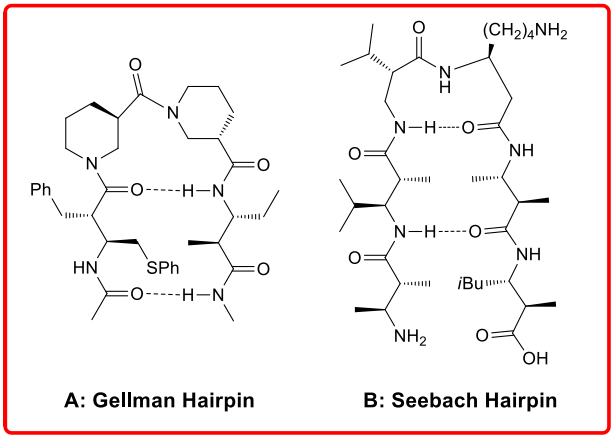


Figure 18. Turn structures for β-peptides

1.4.2 α,β-Hybrid OligopeptidesFoldamers: Secondary Structures.

α/β-Hybrid Peptides significantly expand the "foldamers family" in terms of variations in the backbone pattern with respect to homo-oligomers such as α- or β-peptides. A greater range of possible secondary structures can be obtained depending on the alternation of α and β residues

(αβ; ααβ; αββ etc). A series of examples relating to the formation of different ordered foldamersare reported in the literature. Differently from β–peptides, there isn't a well-defined classification of the possible secondary structures induced by α/β hybrid peptides considering the large number of obtainable folded pattern.

In the field of mixed α/β-peptide foldamers , several new helix types have been discovered. In the year 2001 *Hofmann et al*. carried out calculations upon the theoretical prediction of the basic helix types in α/β-hybrid peptides[44].Experimental studies haveshown thatthe presence of substituents on β-residues, their stereochemical contribution and their nature, dramatically influence also in this case the type of helix that is formed. The most known helical structures reported in the literature are: 9/11-Helix[45], 11-Helix[46], 13-Helix[47], 14/15[48]-Helix and 16/18[49]-Helix and are generally found in hybrids formed by α and β AAs in 1:1 ratio, which are the most studied.Differently from what is observed for α or β peptides, in hybrid peptides a rapid switching of two different helical structures is often observed depending on the nature of the solvent.

Recently, a large number of papers appeared related to α,β-peptides containing constrained cyclic β-amino acids (Figure 19).

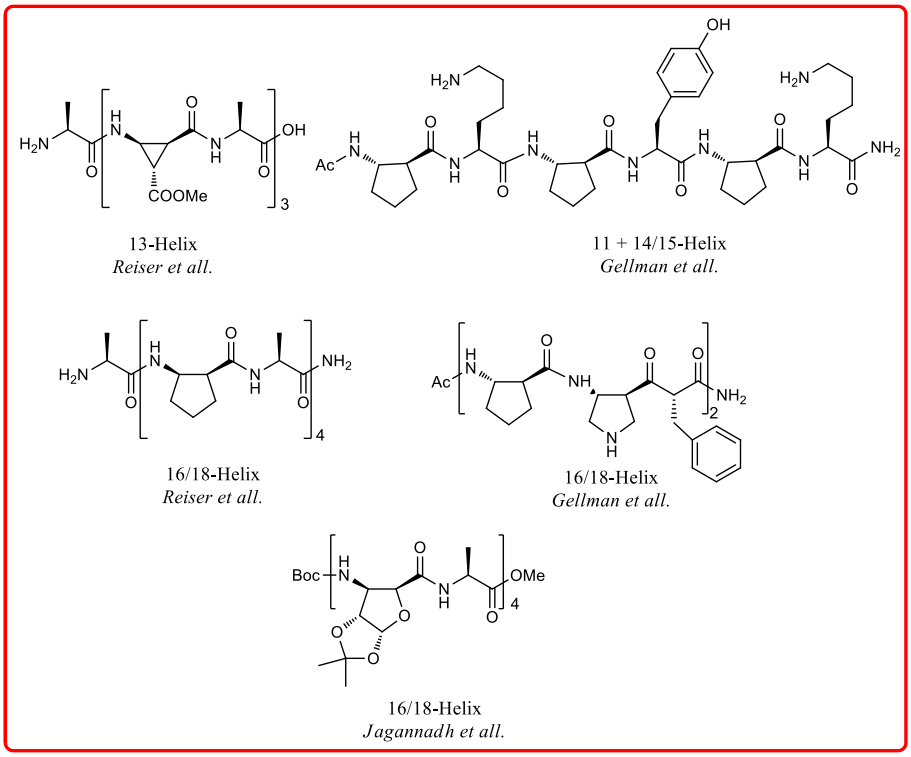


Figure 19. Overview of some α/βfoldamers with a 1:1 backbone alternation containing constrained β-amino acids along with the corresponding helix type.

In the year 2004, two groups independently investigated the solution properties of α/β-peptides with a 1:1 backbone alternation. By combination of rigid *cis*-β-aminocyclopropanecarboxylic acid (β-ACC) residues with (*L*)-alanine, *Reiser et al.* were able to construct relatively short oligomers revealing a 13 helix in methanol solution.[50] At the same time, *Gellman et al.* explored 1:1 α/β-peptides containing *trans*-aminocyclopentanecarboxylic acid residues along with several proteinogenicα-amino acid building blocks. Surprisingly, these short oligomers revealed a 'split personality', which means that there was a coexistence of an 11 helix along with a 14/15 helix, rapidly interconverting in solution[51]. Similar to natural α-peptides with 3_{10}- and α-helical conformations, the length of the foldamers determines which conformation is favored.[52] For relatively long foldamers, the 14/15 helix is favored over the 11 helix, analogous to the α-helix, that is favored over the 3_{10}-helix in larger α-peptide structures.[53]

Very recently, *Reiser et al.* investigated mixed α/β-peptides containing *cis*-ACPC residues instead of β-ACCs used before. These peptides were shown to exhibit a large ring size 16/18 helix in methanol solution.[54]*Gellman et al.* have presented various examples for backbone alternation sequences like α–α–β or α–β–β containing *trans*-ACPC and APC residues, that revealed well defined helical conformations in methanol solution.[55]

Sugar derived β-amino acid units along with alanine residues were introduced in α/β-peptides by *Jagannadh et al.*, exhibiting 11 and 14/15 helical conformations in both chloroform and dimethylsulfoxide (DMSO), similar to those of *Gellman*.[56]

α,βfoldamer were olso prepared starting from acyclic β-amino acids. *Kunwar et al.* presented heterochiral (alternating stereochemistry of amino acids) α/β-peptides with acyclic residues (R-Phe/S-hVal), that revealed a 9/11 helix in chloroform solution.[57] Sugar derived β^3-amino acid units along with alanine residues were introduced in α/β-peptides by the same group in 2005, exhibiting 9/11 helical conformations[58].

In the year 2012 *Balamurugan* and *Muraleedharan*report on the preparation of α,βfoldamer containing *syn*-$\beta^{2,3}$aminoacids and aib. In their case the syn–βaminoacid adopt a torsional preference for the *gauche* conformation when was inserten in the peptides, generating an 11-Helix (Figure 20).

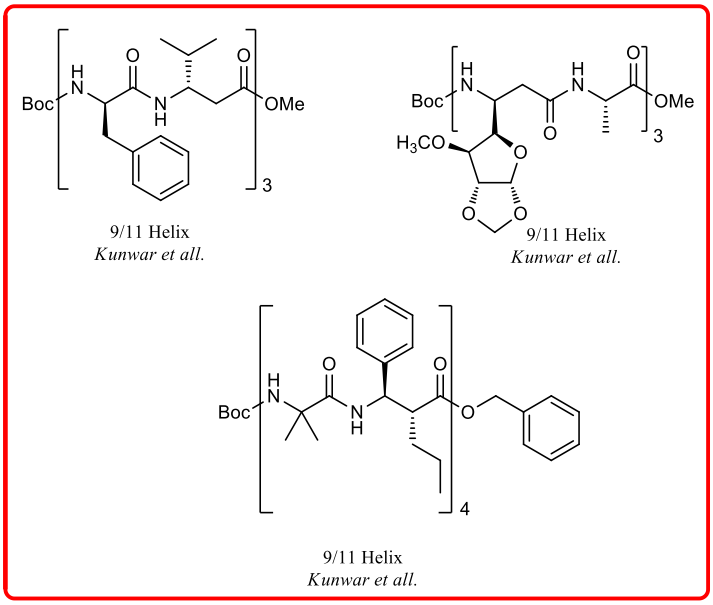
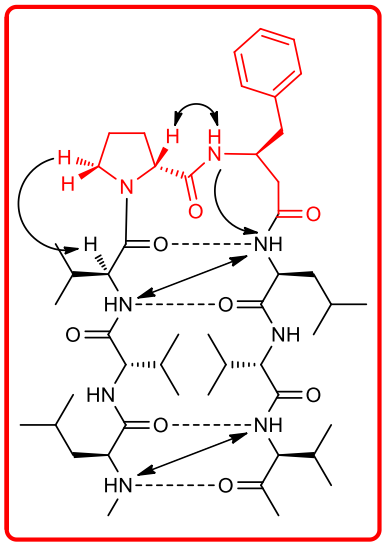


Figure 20

Concerning the formation of other secondary structures for hybrid peptides, only few examples focused on the insertion of a β^3-AA in anα-peptides sequence to induce reverse turn are present in the literature. (*S*)-β^3Phe was linked at the carboxylic function of D-Proline, forming a reverse turn in organic solvent stabilized by a 10-member hygrogen bonded ring as demonstratedby the analysis of model peptides **I**[59].(Figure 21).



I) Boc-Leu-Val-Val-**DPro**-**βPhe**-Leu-Val-Val-OMe

Figure 21. N.O.E.S.Y experiment on model peptide **I**, reported by *Balaram et al*

To the best of our knowledge, no examples of sheet-like structures for α/β (1:1)–foldamers are reported in the literature, probably due to their strong tendency to give helical conformation or simply because they are much less studied than the corrisponding homo-oligomers.

1.5 Applications of β-peptides or α,β-Foldamers

1.5.1 Biological and Pharmaceutical Applications

One of the major concerns of pharmaceutically potent α-peptides (peptide drugs) is their poor proteolytic stability and their rapid degradation in vivo. It is known that β-peptides are inherently stable against proteolysis.[60] *Seebach et al.* were able to show that β-peptides made of acyclic building blocks with incorporated α-amino acids are stable against proteolytic attacks as well.[61] The position of the α-amino acids inside the peptide as well as their nature affect the interactions with the corresponding proteases.

The proteolytic stability of mixed α/β-peptides containing cyclic β-residues was first investigated by *Gellman et al.*[36] The effects of the three proteases trypsin, chymotrypsin and pronase were studied herein on three different α/β-peptides containing trans-β-ACPC and trans-β-APC residues. These results showed that oligomers containing a 1:1 alternation of α- and β-residues are highly resistant to proteolytic degradation. There are also experiments that show that incorporated β-amino acids within the peptide chain can protect the amide bonds of neighboring α-amino acids.[37] All of these results are very promising in terms of the major long term goal of mixed α/β-peptide foldamers, en route to pharmaceutically viable and stable peptides.

There are several examples in literature for the generation of artificial model peptides, where a single β-amino acid had been incorporated within a natural occurring structure. For example, *Reiser et al.* were able to generate active neuropeptide Y (NPY) analogs as selective nanomolar ligands for the Y1 receptor, by removing 24 AAs at the *N*-terminus of natural peptide and by incorporation of conformationally constrained *cis*-β-ACC residues replacing Thr 32 and Gln 34 (Figure 22).[62]

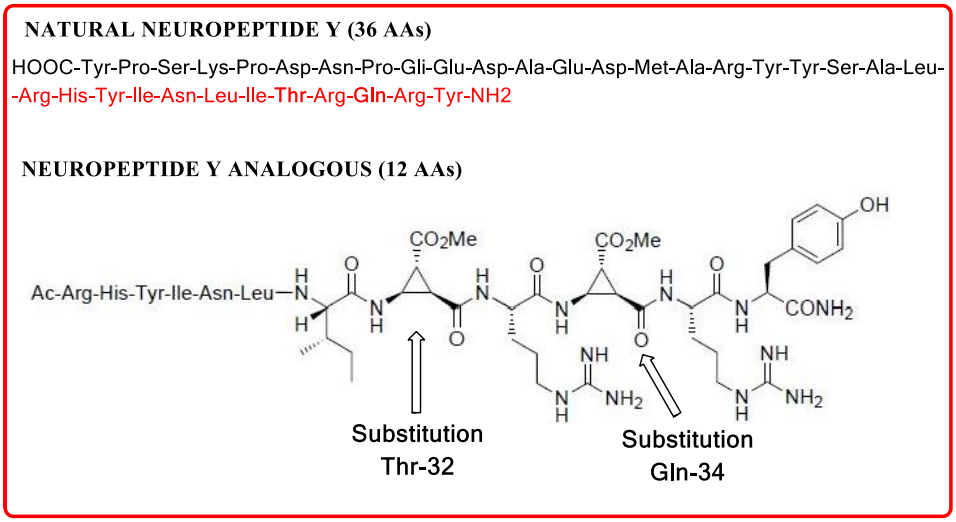


Figure 22

Due to its rigidifying and thus structure stabilizing properties, β-ACC residues were also successfully introduced into several biologically relevant scaffolds. Especially neuropeptides, such as RGD (arginine-glycine-aspartate) peptides as highly active and selective integrin ligands,[63]calcitonine gene related peptide (CGRP) analogs[64] and orexin mimics have been developed.[65]

1.5.1.1 Inhibition of Protein Protein Interaction

Proteins tend to interact with binding partners via folded subdomains, *i.e.* protein secondary structures. Hence, targeting large surfaces of bio macromolecules like proteins or nucleic acids with small molecules found limited success. Recognition of specific secondary structure motifs on a protein surface is the main task for a synthetic oligomer in terms of disrupting protein-protein interactions (PPI), responsible for a giant field of diseases. Therefore, structural motifs are required that are able to mimic typical helical constructs of α-peptides, and several examples of β-peptides helices that accurately reproduces this spatial arrangement are reported in literature.

A major progress towards biologically active foldamers was the identification of a chimeric (α/β+α)-peptide containing *trans*-ACPC that binds the BH_3-recognition site of the anti-apoptotic protein Bcl-XL. Hence, the new peptide foldamer was able to inhibit the interaction between Bcl-xL and its proapoptoticpartner, that can trigger cancer.

The obtained crystal structure of the foldamer-protein complex revealed that a foldamer helix is able to mimic anα-helix. The spatial arrangement of the α-peptide side chains can be reproduced accurately (Figure 23).[66]

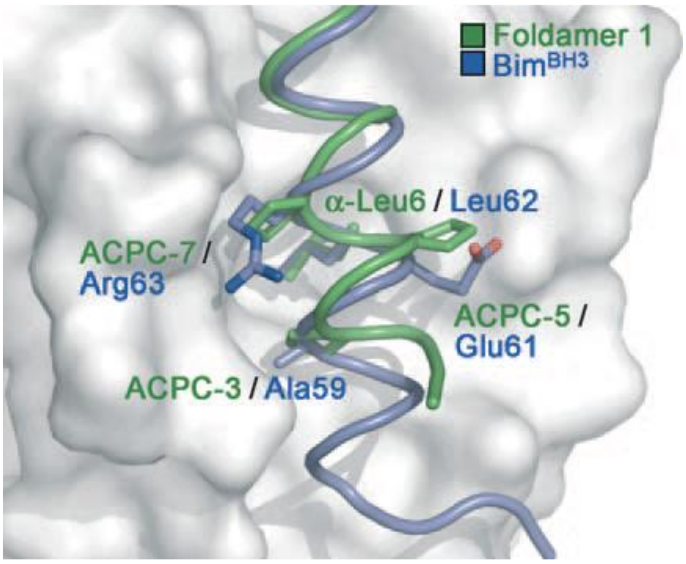


Figure 23. Overlay of the crystal structures of a BH3-recognition site with endogenous α-peptide ligand (blue ribbon) and of an α/β+α chimeric peptide foldamer (green ribbon) bound to the BH3-recognition site of Bcl-xL (in gray).

An even more impressive example for mimicking a biologically active scaffold was illustrated recently by *Gellman et al.*[67] They were able to generate an α/β-oligomer that structurally and functionally mimics a long α-helical segment (~ 10 turns), the CHR region of the HIV membrane protein gp41. Due to conformational changes of gp41, that forms a six-helix bundle during cell penetration, viral entry is facilitated. The synthetic α/β-peptides were shown to exhibit potent activity in the low nanomolar region in cell-cell fusion and HIV anti-infectivity assays by disrupting the undesired helix bundle formation (Figure 24).

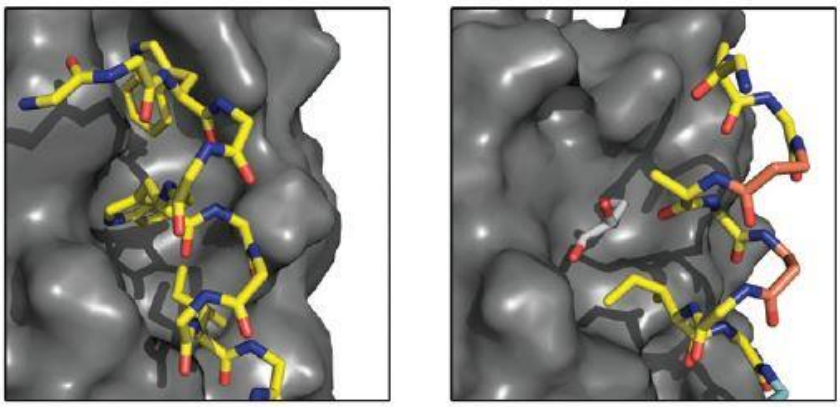


Figure 24. Packing interactions of CHR residues Trp-3, Trp-6, and Ile-10 (shown as sticks) to form 6-helix bundle in gp-41 protein. The interaction betweenhelices of the analousα,β-peptides are sterically prevented

The above mentioned precedents give an insight into the great potential of peptide foldamers with heterogeneous backbones compared to small molecules or foldamers with homogeneous backbones (β-peptides). Mimicry of larger surface structures may lead to more efficient inhibitors of protein-protein interactions involved in many diseases.

β–Amino acids alone andα/β-Peptides with a 1:1 backbone alternation were also shown to exhibit distinct antibacterial activity[68]. Some molecules with β–amino acidic structure as *cis-pentacin,* or*orizocin* are right now in the early stages of preclinical testing as antibiotics.

1.5.2 Nanomaterial Applications

A major challenge in foldamer chemistry is to prove that higher-order structural levels are available for β-peptides such as nanotubes, nanofibers, vescicles and menbrane-like structures, and that their formation can be tailored by the β-amino acid residues.

However, an increasing amount of evidence demonstrates that biomimickingβ-amino acid sequences (β-peptide foldamers or α/βfoldamers) are also fully capable of forming sopramolecular assemblies.

The best known products of self assembling for beta-AA are nanotubes, that represent a new class of synthetically readily accessible peptide-based biomaterials having unique structural and functional properties. Peptide nanotubes are constructed by highly convergentnoncovalent processes by which cyclic or acyclic peptides rapidly self-assemble and organize into ultralargewell ordered three-dimensional structures, upon an appropriate chemical- or medium-induced triggering. The properties of the outer surface and the internal diameter of peptide nanotubes can be adjusted simply by the choice of the amino acid side chain functionalities[69]. This design flexibility, which is unique to this class of tubular structures, has already enabled application of peptide nanotubes to the design of biologically-relevant transmembrane ion channels and pore structures without the need to install metabolic protections, or to the development of new drug delivery systems. (Figure 25)

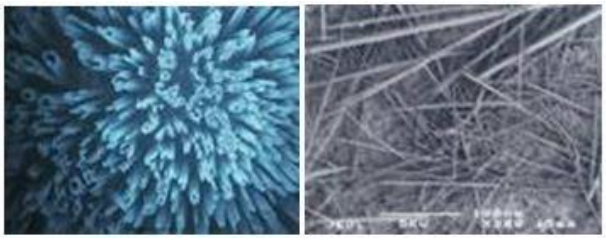


Figure 25. Peptides-based Nanotubes

One drawback of using peptides for biomedical applications is their high susceptibility toward proteases and their short half-lives. The use of unnatural amino acids, such as alpha, alpha disubsituted, beta, and dehydro amino acids, is a common strategies to overcome these issues.

In the year 2008, *Alves* and *Reiches* .[70]reported on the preparation of micro and nanotubes usind L-Diphenylalanine, in particular as vehicle for the delivery of hydrophilic compound. The biological marker Rhodamine B was use as model drug. Using Microscopy, XRD and Raman measurement, they demonstered that the hydrophilic load was conjugates to the self ordered structures. Toxicity assay demonstrated that the damage caused by the nanotubes was relatively low, and kinetic studies demonstrated that bis-phenyalanine nanotubes are able to modulate the release of cargo. Studies on thesenanotubeshaveshown, however,their highdegradationin the presence ofprokaryota and mammal protease.

Other studies reported by *Banerjee* and *Chauhan*[71] explain that nanotubes composed by α/β dipeptides (dehydroalanine-β-alanine or dehydroalanine-β-phenylalanine) are thermally and enzimatically stable in a large range of pH included the physiological. Also in these cases, toxicity assay demonstrated that the damage caused by the nanotubes was relatively low in different cell lines. Thismakes thesesystemsmoreappealingasdrug delivery approach with respect to nanotubes composed exclusively of α–amino acids.

1.6 Aim of my Ph.D thesis

Out of this pool of accessible new secondary structure motifs, the generation of scaffolds able to mimic biologically relevant structures should be straightforward. Although there are already some examples for artificial tertiary and quaternary structures, as well as some biologically active β– or α,β–foldamers. The field of foldamer science is still in its beginning and a lot of work remains to be done. Expanding the scope of the accessible secondary structures is still a major task in order to develop potent biomedical compounds.

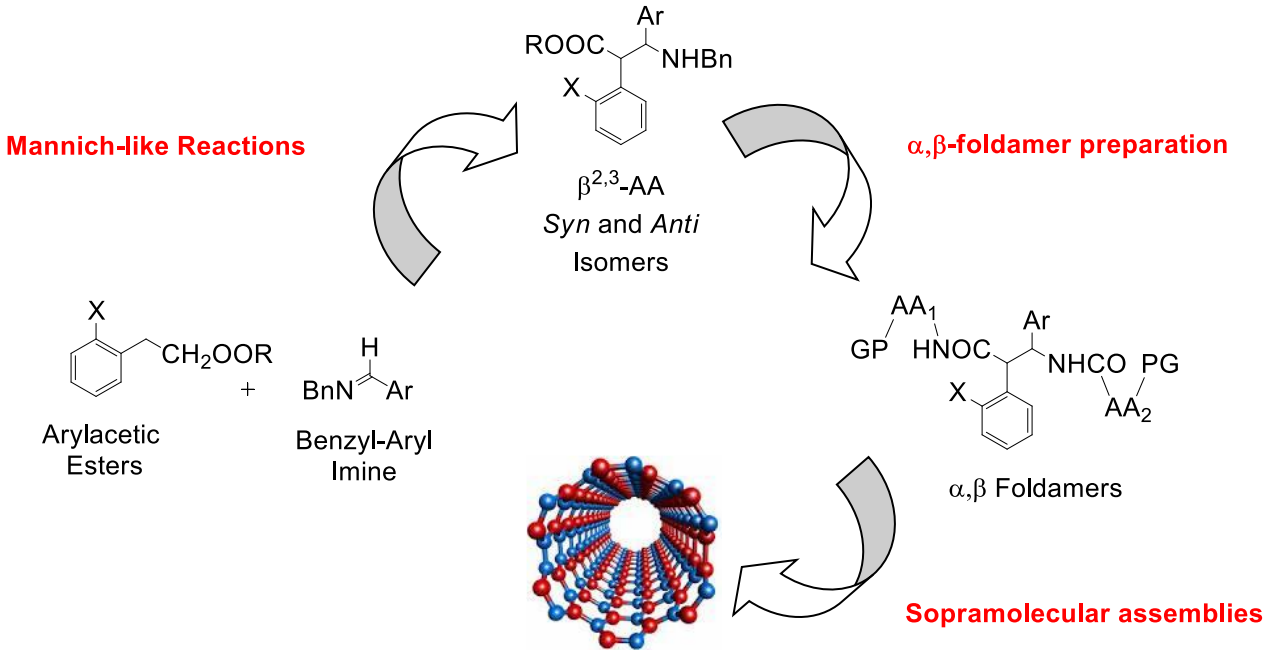
The project developed in this thesis was conceived having as main purpose the preparation and the use in peptides synthesis of two different classes of β-amino acids, that are:

i) $\beta^{2,3}$-Diaryl amino acids

ii) Tetrahydroisoquinoline 4-carboxylic acid.

Concerning $\beta^{2,3}$-Diaryl amino acids my activity was focused on:

- ✓ Development of diastero- and enantioselective synthesis of variously substituted $\beta^{2,3}$-Diaryl amino esters using a $TiCl_4$ catalyzed Mannich-like reation.
- ✓ Preparation of α,β-Hybrid peptides containing $\beta^{2,3}$-Diaryl amino acids and Alanine. Structural characterization of α,β-hybrid using NMR analysis and X-Ray diffraction to understand if their strong aromatic component affect thesecondarystructure of these peptide.
- ✓ Studies on the selfassemblyof thesederivativesin order to obtain sopramolecular architectures (scheme 1).

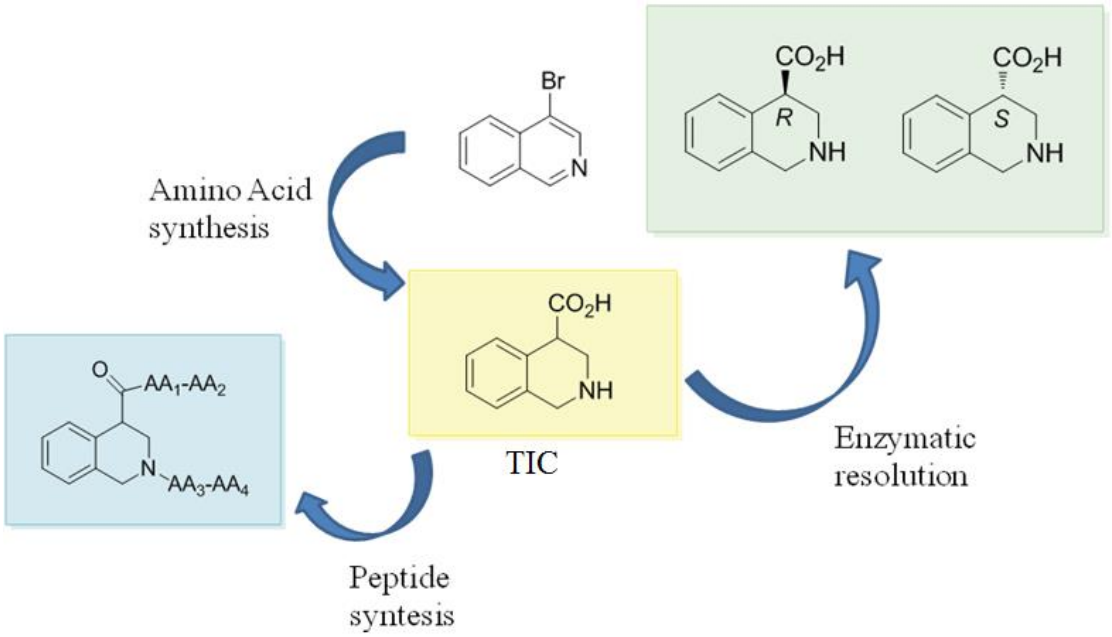


Scheme 1

The secondpurpose of this thesis was focused on Tetrahydroisoquinoline-4-carboxylic acid (TIC), a constrained benzocondensed analogous of Nipecotic acid.

As main:

- ✓ Developmentof asimple and scalablesynthesisoftetrahydroisoquinoline 4-carboxylic acid, solving the problems encountered using the literature synthetic protocol.
- ✓ Bio-enzymatic resolution of TIC.
- ✓ Preparation of model peptides in in order to study TIC conformational behavior. (scheme 2)

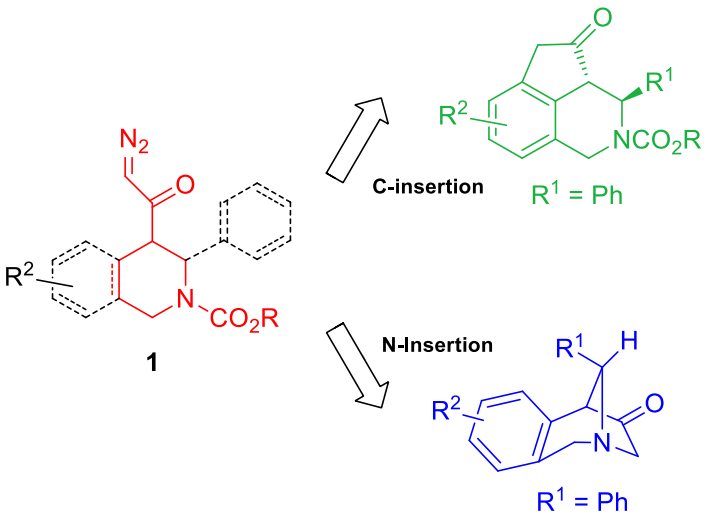


Scheme 2

Finally, during my thesis I exploited the use of α-diazocarbonyl from β-AAs in intramolecular transformation catalyzed by rhodium (II) catalysts (Scheme 3).

The work includes:

- ✓ An efficient preparation of three different classes of α-diazocarbonylpiperidine compounds derived from β-AAs, including the simple Nipecotic acid, its benzocondensate, *i.e.* TIC, and TIC derivatives in which a further aryl moiety is present at position 3.
- ✓ Generation of rhodium carbenes starting from diazocarbonyl derivatives and rhodium-(II) dimer catalysts to give intramolecular carbene insertion.
- ✓ Search ofthebest catalyst and the optimal reaction conditions to drive the chemoselectivity of the insertion reaction.
- ✓ NMR characterization of the polycyclic compounds obtained by intramolecular carbene insertions.


N2
O
R2
N
CO2R
1
C-insertion
O
R1
R2
NCO2R
R1 = Ph
N-Insertion
R1
H
R2
O
N
R1 = Ph

Scheme 3

1.7 Reference

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Chapter 2 – *syn*/*anti* Switching by Specific Heteroatom–Titanium Coordination in the Mannich-Like Synthesis of 2,3-Diaryl-β-amino Acid Derivatives

2. Introduction

The β-amino acid motif[1] is recurrent in biologically active compounds such as β-lactam antibiotics[1a,1b,2] Taxol derivatives and β-peptides;[1,3] (Figure 1) therefore, the preparation of new non-proteinogenc β-amino acids is particularly interesting.

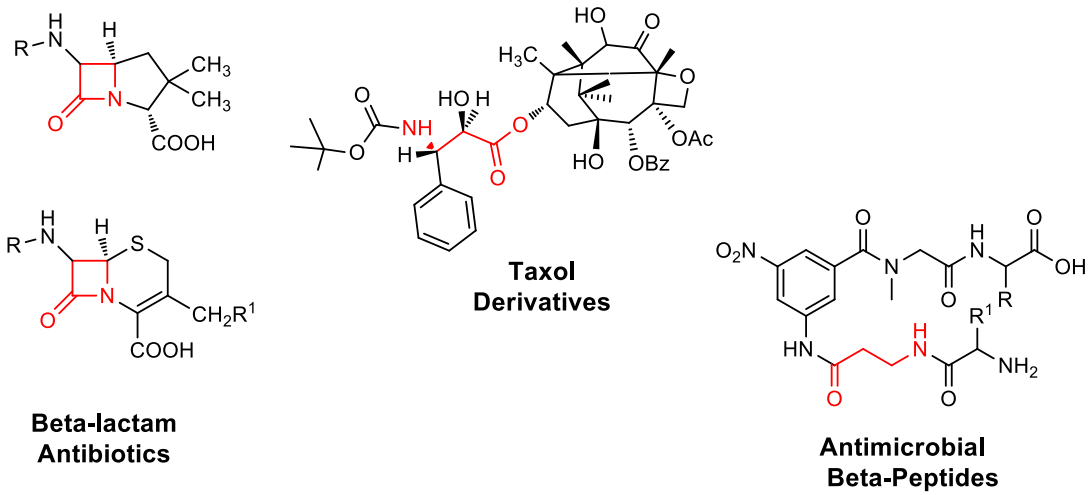
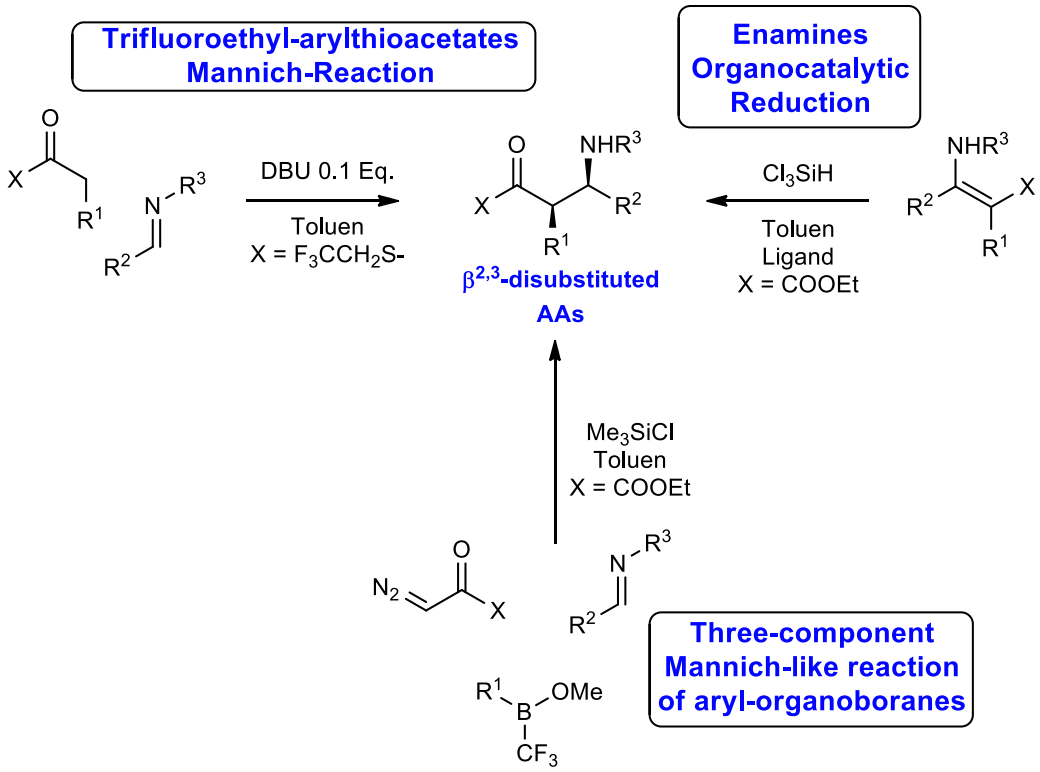


Figure 1. Biologically active Compounds containing β-AAs motif

In particular, β[2,3]-amino acids, which can enhance the helical content and the resistance to peptidases of a peptide,[3f] have been widely studied (see general introduction). The synthesis of these compounds through the addition of an ester enolate[4] or silyl ketene acetal[5] to an imine has received special attention because of the possibility to address the reaction stereoselectivity. By these Mannich-like reactions, several α-alkyl-, α-unsubstituted-, and α-phenyl-β-aryl-β-amino acids have been accessed, [5c,5d,5f,6–9] whereas compounds containing α-aryl substituents different from phenyl have not yet been prepared, owing to the easy radical dimerization of the starting aryl acetic esters.[10] With few exceptions,[6a,6b,7a] a *syn*/*anti* mixture of β[2,3]-amino esters forms through this method, and the *syn* adduct is the major diastereoisomer. Recently, a series of 2,3-diaryl-3-amino thioesters have been prepared by a base-organocatalyzed Mannich reaction of expensive trifluoroethyl arylthioacetates,[11] an organocatalytic reduction of enamines,[12] or a three component reaction of aryl organoboranes, diazoacetate, and imines.[13] (Scheme 1)



Scheme 1. Known methodologies for the preparation of 2,3 Diaryl-AAs

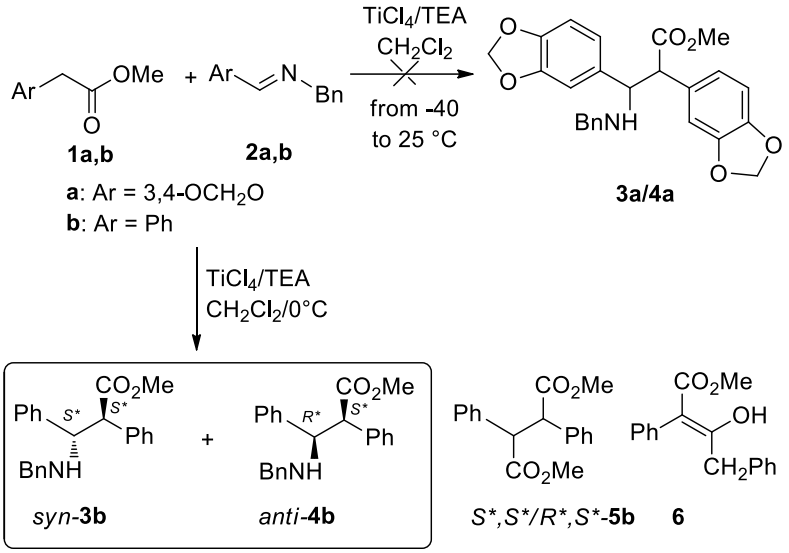
Stimulated by both the literature overview and by our interest in the preparation of nonproteinogenic amino acids,[5a,5b,14] we studied the applicability of the Mannich-like reaction of aryl acetate titanium enolates with N-Benzylimines to the synthesis of $\beta^{2,3}$-amino acids bearing a 2,3-diaryl substitution pattern. The success of this reaction is related to the presence of a coordinating atom at the *ortho* position of the starting aryl acetic ester. Interestingly, by tuning the nature of the heteroatom, the *syn*/*anti* chirality switch can be modulated. On the other hand, the use of the corresponding thioesters gave exclusively the *anti* adducts. To the best of our knowledge, the titanium enolate of an *ortho*-aryl-substituted ester and the coordination concept is innovative in the Mannich-like reaction. Furthermore, the new *ortho*-halogenated $\beta^{2,3}$-diarylamino acids represent a very interesting source for *ortho*-functionalized compounds, which can be generated by both aromatic nucleophilic substitution and coupling.

2.1 Results

Preliminary Experiments

Recently, we were interested in the synthesis of 2,3-diaryl-β-amino esters **3a**/**4a** derived from the condensation of methyl (3,4-methylendioxyphenyl)acetate (**1a**) and piperonal N-benzylimine (**2a**). According to a literature procedure, [9b] we used a Mannich-like $TiCl_4$/triethylamine ($TiCl_4$/TEA)

promoted reaction, but even with different stoichiometries and temperatures, the reaction did not proceed, and only the starting reagents were recovered (Scheme 2).



Scheme 2. Mannich-like reaction of *ortho*-unsubtituted esters **1a,b**

To verify if this behavior was dependent on the use of substrates **1a** and **2a**, we reinvestigated the literature reaction between methyl phenylacetate (**1b**) and benzaldehyde N-benzylimine (**2b**; Scheme 1),[9b] which is reported to give exclusively the *syn*/*anti* adducts **3b**/**4b** (73:27 ratio) in 78% yield. When we reproduced this reaction, in addition to **3b**/**4b**, we found (TLC and ^{1}H-NMR spectroscopic analyses,Figure 2) the starting imine **2b**, the dimethyl 2,3-diphenylsuccinates *S**,*S**-**5b** and *R**,*S**-**5b**, and the Claisen compound **6** [(**3b**/**4b**)/**2b**/**5b**/**6** 1:1.33:0.60:0.24 molar ratio).

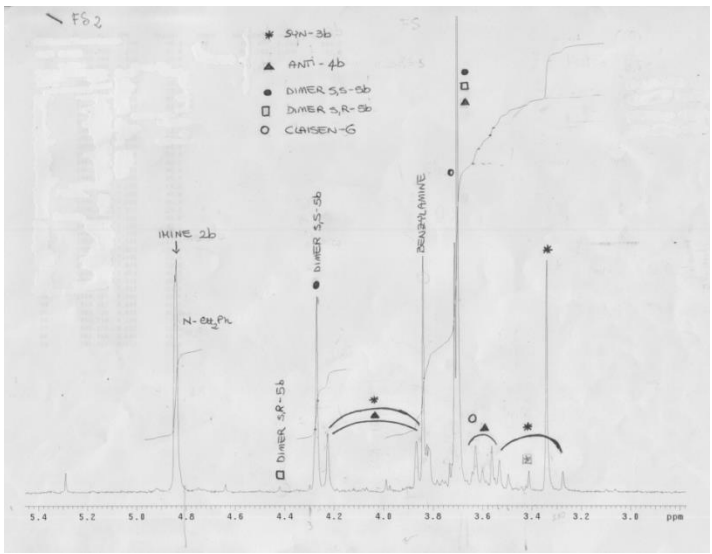


Figure 2. ^{1}H NMR zoom spectrum for the Mannich-like reaction between methyl phenylacetate (**1b**) and imine **2b** in $CDCl_3$ (200 MHz).

After purification, the main *syn* adduct **3b** was isolated in 23% yield only, together with minor amounts of **4b** (10 %), impure of Claisen compound **6** and benzylamine, and dimer **5b** (10 %). Several experiments were performed on the Mannich condensation of **1b** with **2b** at different temperatures or molar ratios without any significant improvement. In addition to this divergent result, we also found that the ^{1}H-NMR spectra of the isolated compounds **3b**/**4b**, inexplicably, do not completely match those reported.[9b] Furthermore, the formation of **5b** and **6** is in agreement with the literature data[10] concerning the radical dimerization or the Claisen condensation of phenylacetic ester **1b** promoted by $TiCl_4$/ TEA[15] which we have also repeated separately with a result similar to that reported (Figure 3. See experimental section: synthesis of dimeric compounds *S**,*S**-**5b** and *R**,*S**-**5b**)

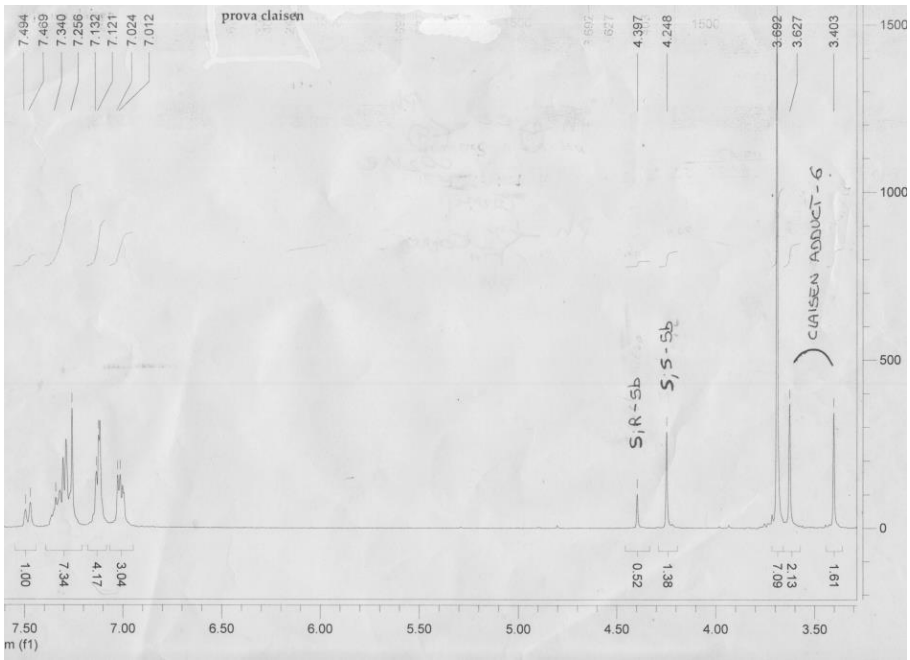
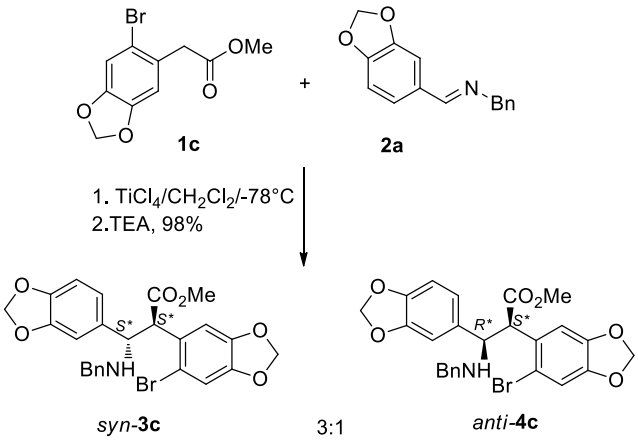


Figure 3. ^{1}H NMR spectrum of crude reaction of **1b** with $TiCl_4$ and TEA in $CDCl_3$ (300 MHz) that affords compound *S***R**-**5b**; *S***S**-**5b**; and **6**.

Mannich-Like Condensation

Adrian et al.[16] reported that methyl (methoxy)acetate titanium enolate reacts with aldimines to afford mainly *anti* β-amino-propionic acid derivatives. From these data, we reasoned that a coordinating group positioned close to the enolate could be crucial for the reaction outcome, probably owing to the formation of a stabilized titanium enolate complex. To confirm our hypothesis, the *ortho*-bromo derivative **1c** and the imine **2a** (Scheme 3) were subjected to the above protocol (Table 1, Entry 1), and a mixture of **3c**/**4c** (3:1 ratio) was isolated in 40% yield.



Scheme 3. Mannich-like reaction of 2-bromophenylacetic ester **1c**.

Several reaction conditions were then tested (Table 1), and the overall results indicate that the same *syn*/*anti* ratio of **3c**/**4c** was always obtained; the lower the reaction temperature, the higher the yields (Table 1, Entries 1–4); lower amounts of $TiCl_4$ and TEA gave lower yields as well as an increased amounts of these reagents gave better yields (Table 1, Entries 5 and 6). The reaction failed when the addition order of the reagents was changed (Table 1, Entry 7) or when N,N-diisopropylethylamine (DIPEA) was used instead of TEA (Table 1, Entry 8). Finally, the best conversion was obtained at a reaction temperature of –78 °C (Table 1, Entry 9).

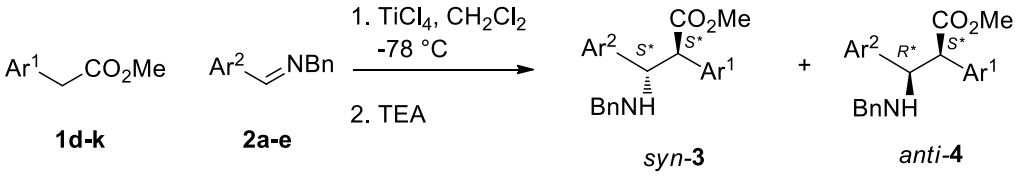
Tab 1. Reaction conditions evaluation of the Mannich-like reaction between **1c** and imine **2a**.

Entry	$TiCl_4$ [mol][a]	T [°C]	TEA [mol]	*t* [min][b]	Yield [%][c]
1	2.1	0	2.1	180	40
2	2.1	-40	2.1	180	60
3	2.1	-50	2.1	180	65
4	2.1	-60	2.1	180	70
5	1.7	-50	1.7	180	50
6	2.5	-50	2.5	180	75
7	2.1[d]	-60	2.1	480	–
8	2.1	-60	2.1[e]	480	–
9	2.1	-78	2.1	75	98[f]

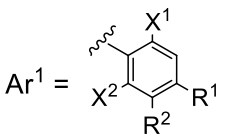
[a] Reaction conditions: ester **1c** (1 mol equiv.), imine **2a** (1 mol equiv.), in DCM (0.1 mol L^{-1}); $TiCl_4$ was dropped in 15 min and the mixture was stirred for 15 min before addition of the base. [b] Reaction time after addition of TEA. [c] Overall yield of isolated compounds *syn*-**3c**/*anti*-**4c** (3:1).[d] The mixture of ester **1c** and $TiCl_4$ in DCM was stirred for 15 min, then TEA (5 min), then **2a** (from -60 to 0 °C), were added.[e] DIPEA was used as a base instead of TEA (from -60 to 0 °C).[f] The diastereoisomers *syn*-**3c** and *anti*-**3c** were separated by flash column chromatography.

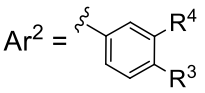
To extend the scope of this condensation to the synthesis of different functionalized 1,2-diaryl-β-amino acid and to evaluate the efficacy of the *ortho* group to coordinate titanium in connection to its relative hardness, a series of *ortho*-substituted arylacetates **1d**–**1k** were reacted with the imines **2a**–**2c** (Scheme 4, Table 2).

As expected, methyl *o*-fluorophenylacetate (**1d**), bearing the hardest and more coordinating *o*-halogen atom, gave high yields of the *syn* adduct **3d** as the largely predominant isomer (Table 2, Entry 1) when reacted with **2c**. A similar behavior was found for the reaction of *o*-chlorophenylacetate (**1e**) with imine **2c** (Table 2, Entry 2), whereas the *o*-bromophenylacetate (**1f**) reacted with imine **2a** (Table 2, Entry 3) to afford a *syn*/*anti* (6.7:1) mixture **3f**/**4f**, which confirms that the bromine atom, less hard than F and Cl, is also less coordinating. The ester **1g**, which contains the more polarizable *o*-iodine atom, reacted with **2b** (Table 2, Entry 4) to afford good yields of a quasi-equimolar syn/anti mixture **3g**/**4g**. Unexpectedly, a complete diastereoselectivity switch toward the anti adduct **4h** was found by using the *o,o*-dichloro ester **1h** and imine **2b** (Table 2, Entry 5). Also surprising was the absence of diastereoselectivity in the reaction of *o*-methoxyphenylacetate (**1i**) with imine **2c** (Table 2, Entry 6), even if the methoxy group is a good titanium-coordinating group.[16] Finally, the Mannich-like reaction did not proceed when esters **1j** and **1k** bearing a non-coordinating electron-withdrawing *ortho* group (*i.e.* -NO_2; -$NHCOCF_3$) were employed.



1d-k **2a-e** *syn*-**3** *anti*-**4**





	X^1	X^2	R^1	R^2
1d	F	H	H	H
1e	Cl	H	H	H
1f	Br	H	MeO	MeO
1g	I	H	H	H
1h	Cl	Cl	H	H
1i	MeO	H	H	H
1j	NO_2	H	H	H
1k	$NHCOCF_3$	H	H	H

	R^3	R^4
2a	OCH_2O	
2b	H	H
2c	Br	H
2d	OMe	H
2e	NO_2	H

Scheme 4. Mannich-like reaction between (*ortho*-substituted) arylacetates **1d**–**1k** and imines **2a**–**2e**.

Tab 2. Mannich-like Reaction between (*ortho*-Substituted) arylacetates **1c-i** and Arylimines **2a-c**.[a]

Entry	Ester[a]	Imine	*t* [min]	Products **3/4**	Yield [%][b]	*syn/anti* [%][c]
1	**1d**	**2c**	15	**d**	91	>97:3
2	**1e**	**2c**	60	**e**	81	92:8
3	**1f**	**2a**	75	**f**	88[d]	87:13
4	**1g**	**2b**	180	**g**	81	55:45
5	**1h**	**2b**	180	**h**	76	<3:97
6	**1i**	**2c**	75	**i**	73	47:53
7	**1j**	**2b**	600	**-**	-	[e]
8	**1k**	**2b**	600	**-**	-	[e]

[a] Reaction conditions: ester **1** (1 mol equiv.) and imine **2** (1 mol equiv.) in DCM (0.1 $molL^{-1}$) at –78 °C, then $TiCl_4$ (2.1 mol equiv.), 15 min; TEA (2.1 mol equiv.). [b] Overall yield of isolated compounds. [c] Determined by ^1H NMR spectroscopic analysis. [d] A *syn*/*anti* mixture (7%) of dimers **5f** was isolated. [e] At 25 °C after the addition of TEA.

The reactions between the imine **2b** and the esters **1g** and **1h** were selected as model reactions, and the screening of both the Lewis acid and the reaction solvent was conducted. The reaction failed in DCM in the presence of $SnCl_4$, $InCl_3$, TiF_4, or $Ti(OiPr)_4$. Moreover, the same behavior was found for $TiCl_4$ in tetrahydrofuran (THF) or acetonitrile (MeCN); therefore, the coordinating solvent probably destabilizes the architecture of the imine/ester/$TiCl_4$ complex.[17]

Further runs were conducted with arylacetic esters **1d**, **1g**, and **1h** and the arylimines **2a** and **2c**–**2e**, characterized by different *para*-substitution patterns for a variety of electronic effects (Scheme 4, Table 3. Figure 4 shown the distribution of products by the *ortho*-group modulation). All these arylimines gave good results, and the yields increased as the electron withdrawing character of the *para* substituent increased, but they had no influence on the diastereoselectivity of the reaction. In contrast, the reaction did not proceed when N-benzylimines of *ortho*-substituted benzaldehydes (2-nitro-, 2-bromo-, 2,4-dimethoxy-, and 2,4-dichlorobenzaldehyde) were used with both **1g** and **1h**. These latter examples indicate that an increased steric crowding near the reactive functions changes the coordination environment of the ester/imine/titanium complex and, thus, prevents the reaction.

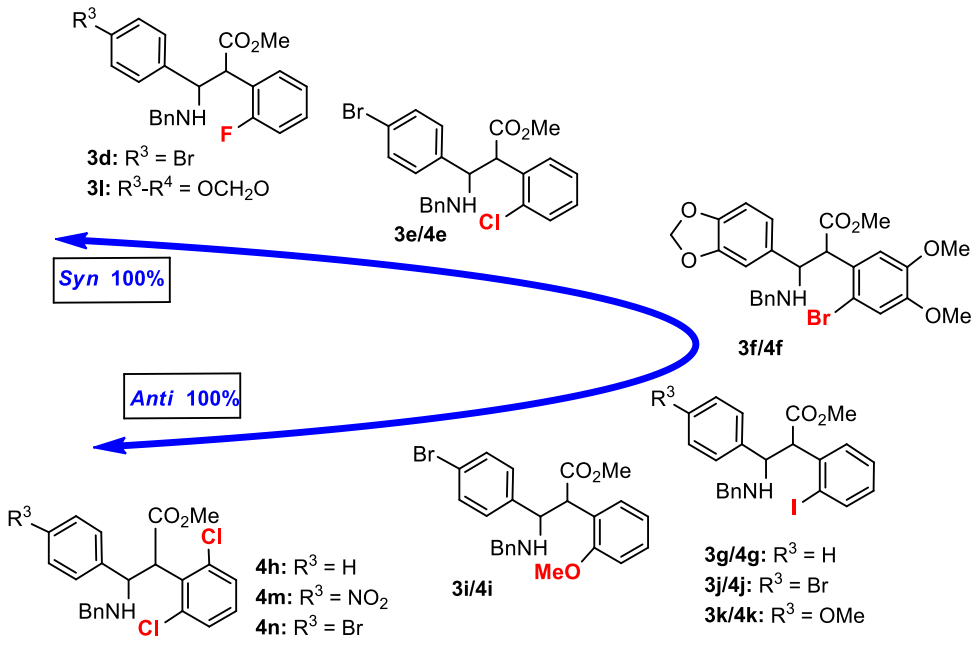


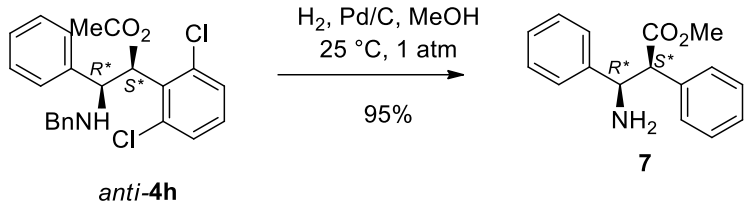
Figure 4. Graphical rapresentation of the Mannich-like reaction diastereoselectivity switch by coordinating *Ortho*-group modulation.

Table 3. Mannich-like reaction of esters **1d**, **1g**, and **1h** with imines **2a** and **2c**–**2e**.[a]

Entry	Ester	Imine	*t* [min]	Products	Yield [%][b]	*syn/anti* [%][c]
1	**1d**	**2a**	80	**3l**	91	>97:3
2	**1g**	**2c**	30	**3j/4j**	97	55:45
3	**1g**	**2d**	75	**3k/4k**	76	59:41
4	**1h**[d]	**2e**	120	**4m**	85	<3:47
8	**1h**	**2c**	150	**4n**	79	<3:47

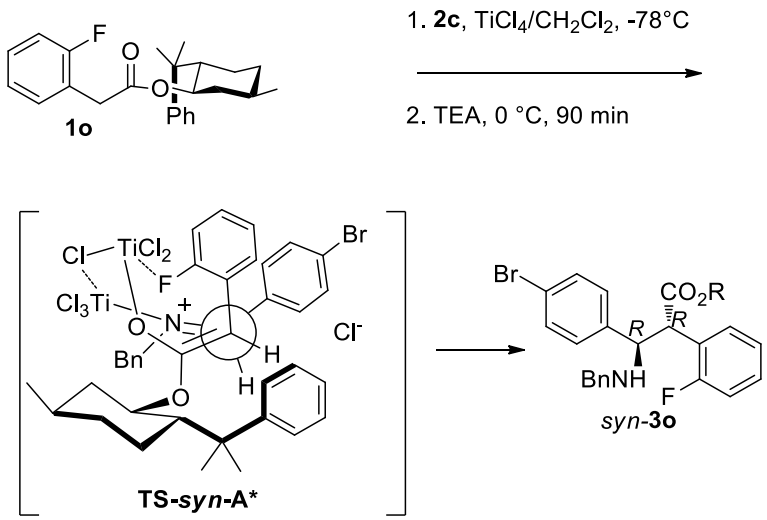
[a] Reaction conditions: ester 1 (1 mol equiv.) and imine 2 (1 mol equiv.) in DCM (0.1 molL–1) at –78 °C, then TiCl4 (2.1 mol equiv.), 15 min; TEA (2.1 mol equiv.). [b] Overall yield of isolated compounds. [c] Determined by 1H NMR spectroscopic analysis. [d] The reaction temperature was increased to –20 °C after the addition of TEA.

To determine the correct configuration of **4h**, a catalytic hydrogenation (H_2, Pd/C, MeOH, 25 °C, 1 atm) was performed to remove both the N-benzyl protection and the aromatic chlorine atoms affording the known 2*S**,3*R**- methyl 3-amino-2,3-diphenylpropionate (7) (Scheme 5).[18]



Scheme 5: Reduction of Mannich Adduct **4h**

The attempts to make enantioselective the condensation of the achiral imine **2c** with the *o*-fluorophenylacetate **1d** by using (–)-sparteine or (–)-quinine as chiral bases was completely unsuccessful, as well as the reaction of benzylidene N-(S)-α-methylbenzylimine with **1d**. Using (–)-sparteine the reaction gave product **3d** in almost quantitative yield. On the other hand using (–)-quinine as base or benzylidene N-(S)-α-methylbenzylimine as chiral auxiliary the reaction was completely blocked. Complete enantioselectivity was reached in the reaction of **2c** with the optically pure (–)-8-phenylmenthyl ester **1o** (Scheme 6), which gave the enantiopure syn adduct (1R,2R)-**3o** as the major diastereoisomer (*syn/anti* 93:7) in 77% yield (see Experimental section for HPLC traces Figure E1, E2 packing diagram Figure E3; and X-Ray analysis Figure 5). On the basis of the proposed reaction mechanism (see below), we can hypothesize that the formation of this enantiomer can be ascribed to the formation of the complex **TS-syn-A*** (Scheme 6) derived from the approach of the imine **2c** toward the less hindered *Si* face of the enolate. This stereochemical result indirectly confirms our hypothesis on the stereochemistry of *syn*-**3** and *anti*-**4** formulated on the basis of ^{1}H NMR analyses (see ^{1}H NMR discussion for 2,3-Diaryl-β-aminoesters 3 and 4).



Scheme 6:Mannich-like reaction of Ester **1o**

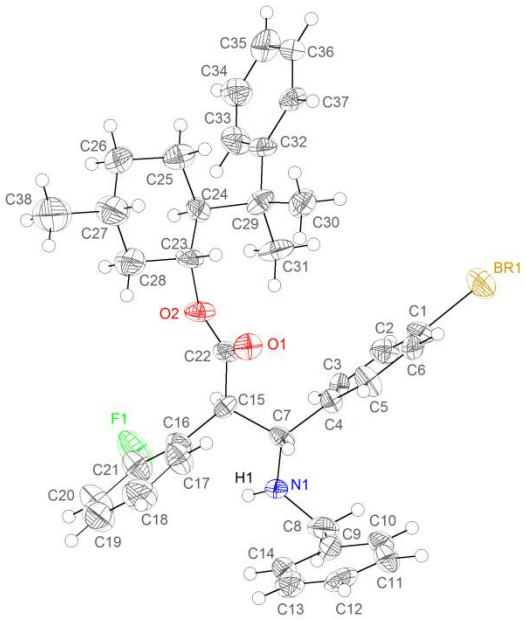
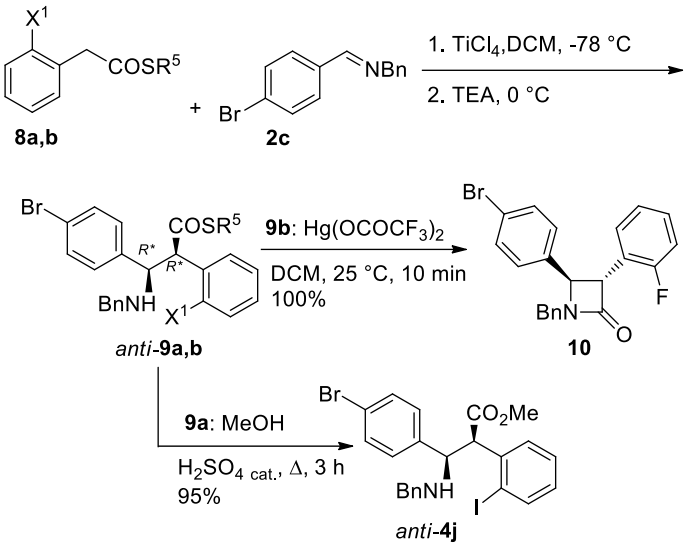


Figure 5:ORTEP plot of **3o** at 180K with atom numbering scheme. Ellipsoids at 50% probability level.[19]

Mannich-like reactions of thioesters with benzylimines in the presence of $TiCl_4$/TEA have also been described in the literature.[20] The corresponding β-amino-thioesters were obtained as *syn/anti* mixtures in variable ratios, depending on the starting reagents and conditions. Notably, examples of 2,3-diaryl-3-amino thioesters prepared under these conditions are unknown. To analyze the reactivity of *ortho*-substituted arylthioacetic esters in the Mannich-like condensation, compounds **8a** and **8b** were prepared and reacted under the usual conditions with imine **2c** (Scheme 7). Surprisingly, the *anti* adducts **9a** and **9b** were isolated in good yields and complete diastereoselectivity in both cases. On the other hand, methyl 2-(2-iodophenyl) ethanedithioate **1p** was completely unreactive in the condensation with **2c**.[21]



	X^1	R^5		*t* [h]	Yield [%]
8a	I	$(CH_2)_2Ph$	*anti*-**9a**	2.5	80
8b	F	CH_2Ph	*anti*-**9b**	6	82

Scheme 7: Mannich-like reaction of thioesters **8** and their transformation.

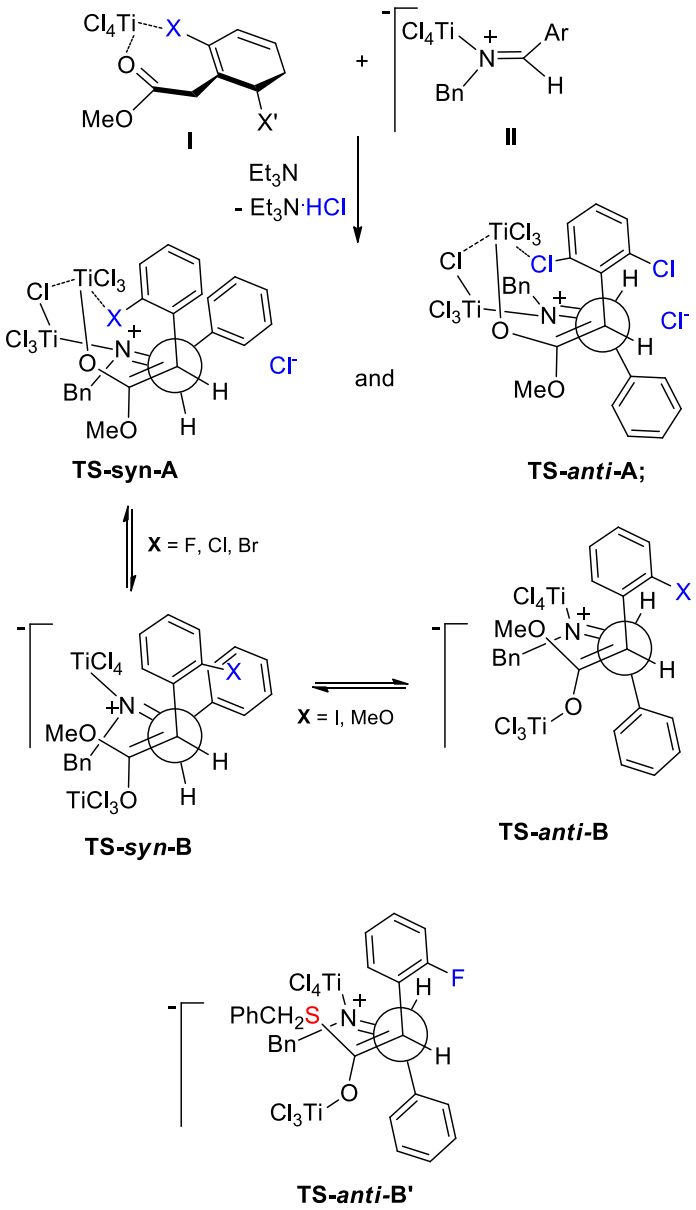
The stereochemistry of thioester **9a** was unambiguously assigned after it was transformed into the corresponding methyl ester anti-**4j** by methanolysis (Scheme 7). The same strategy failed for **9b**, but this compound was quantitatively cyclized to the corresponding β-lactam **10** by treatment with $Hg(OCOCF_3)_2$ (Scheme 6).[22] The ^1H NMR coupling constant between 2-H and 3-H (J_3 = 2.1 Hz) confirmed the trans disposition of the two aryl groups, in agreement with the literature data.[23]

2.2 Discussion

The overall results indicate that the *ortho* substituent of the arylacetate is essential for the outcome of the Mannich-like condensation of these compounds with N-benzylimines. The efficacy of the substituent to promote the reaction depends mainly on its ability to coordinate to the titanium center and seems independent from its electronic effect, which determines only the CH-α acidity. On the contrary, an *ortho* group on the arylimine is detrimental to the reaction; this suggests that the architecture of the transition state is destabilized by the additional steric crowding. To explain the stereochemical results of the reaction, we have postulated a general mechanistic rationale, which takes into account both *ortho*-coordinated and non-coordinated transition states (Scheme 8). As shown by NMR studies (see Experimental Section), in non-coordinating solvents, the iminium species (I) and the hexacoordinate ester– titanium complex (II), in which both the *ortho*-substituent and the carbonyl function are coordinated to the titanium center, are first formed. As further evidence, we found that the reaction did not proceed in a coordinating solvent (THF, MeCN), which can displace the *ortho*-substituent from the titanium coordination sphere.[17] To the best of our

knowledge, the only complexes of titanium with haloorganic ligands reported in the literature, are those with aryl fluorides,[24] acetyl chloride, and acetyl bromide (CH_3COCl >CH_3COBr).[25]

In agreement with the literature data and hard and soft acids and bases (HSAB) theory, we could conclude that the halogen propensity to coordinate to the titanium center follows the hardness of the halogen atom (F>Cl> Br>I). After the addition of TEA,[26] titanium E/Z enolates are formed from the intermediate II. NMR experiments on **1d** (see Experimental Section) confirmed the presence of the E/Z titanium enolate mixture, which by reaction with **I** can afford four possible dimetallic transition states (Scheme 7).[26a,27] With a strongly coordinating *ortho* group (X = F, Cl, Br), most likely both **TS-syn- A** and **TS-anti-B** are initially formed from the E or Z enolate. Complex **TS-syn-A**, which is probably promoted by both titanium/X coordination and π–π stacking,[28] quickly evolves to the kinetically favored *syn* adduct, as also indicated by the short reaction times (e.g., Table 2, Entry 1). On the contrary, the less-reactive non-coordinated **TS-anti-B** probably equilibrates to **TS-syn-A** via the sterically crowded, unstable intermediate **TS-syn-B**. According to previous reports[17] and to NMR experiments (see Experimental Section), if the *ortho* substituent is a less coordinating and/or a more crowding group, the less hindered **TS-anti- B** can form directly from the E enolate or indirectly from **TS-syn-A** via **TS-syn-B**. As a result, a progressive yield increase of the thermodynamically favored *anti* adduct was obtained from *o*-bromo- (**1f**), *o*-iodo- (**1g**), and *o*-methoxyphenylacetic (**1i**) esters. The case of the *o,o*-dichlorophenylacetate (**1h**) is peculiar as it gave exclusively the anti adduct **4h**. We hypothesized that even if one chlorine atom strongly coordinates to the titanium center, the second *ortho* chlorine atom increases the steric crowding and prevents the π–π stacking; thus, the formation of the less hindered **TS-anti-A** is favored. Finally, from *o*-fluoro- (**8a**) and o-iodophenylthioacetate (**8b**), an almost complete *anti* diastereoselectivity was observed, independently from the coordinating ability of the *ortho* substituent. Most likely, **TS-anti-B'** (Scheme 8) is preferred as the steric hindrance between the thioalkyl and N-benzyl groups is minimized in this conformation.



Scheme 8. Transition states for Mannich-like reaction.

2.3 ^{1}H NMR discussion for 2,3-Diaryl-β-aminoesters 3 and 4

All compounds were fully characterized and here are reported the detailed ^{1}H NMR for isomers **3/4c**. *Syn*-adduct 2*S**,3*S**-**3c** is characterized by a AM system at δ 4.46 and 4.04 (*J* = 10.2 Hz) corresponding to H-3 and H-2, respectively, and by an AB system at δ 3.61, 3.32 (*J* = 13.9 Hz) associated to CH_2N. Similar resonances were observed for *anti*-diastereoisomer 2*S**,3*R**-**4c** that is characterized by signals at δ 4.45 and 4.09 (*J* = 10.2 Hz) and atδ 3.62, 3.52 (*J* = 13.4 Hz). The main difference between *syn/anti* isomers are related to OMe group which resonance could be considered diagnostic to establish the *syn/anti* stereochemistry.In fact, a chemical shift at higher

field (δ 3.40) was detected for the *syn* isomer with respect to the *anti* adduct (δ 3.69). Basing on this datum, the stereochemistry of all adducts of the *syn*- and *anti* series was unequivocally assigned.

Furthermore, similar resonances for OMe group for *syn*-**3b** and *anti*-**4b** are also reported in the literature.[5] Nevertheless, comparing the spectra from the literature of derivatives **3b** and **4b** with respect to those recorded on our synthesized compounds, slight differences are detected most of all related to Ph*CH*$_2$. In particular a different multiplicity was observed for the *syn* adduct and differences of chemical shifts were found for the *anti* one.

2.4 Complete set of NMR data for the Mannich-like reaction of 1d and 1i with imine 2c

Even if the structure of titanium enolates has not been unambiguously established in all cases, recent works clarified, by computational and NMR studies, if they are the real intermediates in a specific transformation.[24] To gain insight on the reaction mechanism and stereochemical results of our Mannich reactions, several NMR experiments were performed by monitoring the reaction of imine **2c** with both *ortho*-fluoro ester **1d** and *ortho*-methoxy ester **1i**.

As shown in Table 4 (^{1}H NMR, ^{13}C NMR), and Table 5 (^{1}H NMR ,^{13}C NMR), the behavior of ester **1d** and imine **2c** in the presence of either $TiCl_4$ or $TiCl_4$/TEA was deeply studied (Figures 6-19).

As expected,[24b,c,25] shifts at lower field of both ^{1}H and ^{13}C signals of **1d** were observed in the presence of $TiCl_4$ (Figures 6-9; Tables 4 columns **1d** and **1d**/**TiCl$_4$** (1:1)). In particular Δδ+0.57 (H-2), +6.0 (O*C*H$_3$) and +10.6 (CO) ppm were found indicating a coordination of titanium to the carbonyl group. Even if it is documented by X Ray the coordination of aryl-fluorine to titanium, NMR data are not clear concerning the carbon and fluorine shift resonances.[26] In our case, minor chemical shift changes were found for the aryl carbon nuclei, except for the *ipso*-carbon that is shielded ($\Delta\delta_C$: -3.3 ppm), while a down field signal was detected for fluorine ($\Delta\delta_F$: +1.36 ppm).

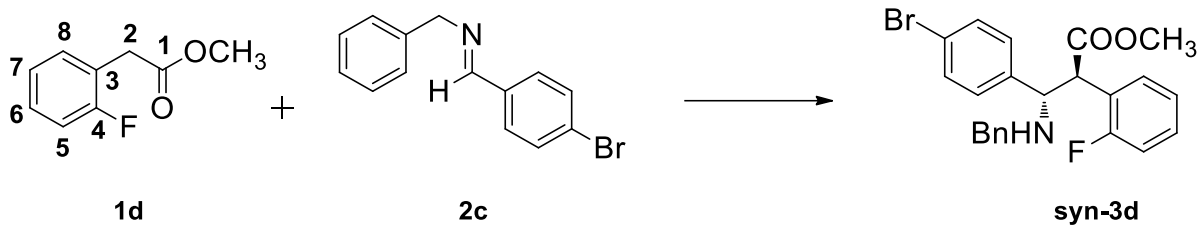
The presence of titanium-enolate after addition of TEA (1 equiv) to **1d**/$TiCl_4$ mixture was supported by the formation of an orange/brown solution containing a solid. As also observed in literature,[24a,c]NMR spectra with broad signals hampering resolution, were thus detected (Figures 10,11). The absence of the *C*H$_2$ signal in the ^{13}C NMR spectrum and the presence of lower field signals indicate the formation of a couple of enolates (HSQC NMR experiment, Figure 12; Tables 4, column **1d**/**TiCl$_4$**/**TEA** (1:1:1)), whose resonances (5.4 ppm and 6.2 ppm) are in agreement with the literature data.[24b,c,25] The formation of two species was confirmed by ^{19}F NMR experiment (data not reported).

To understand the architecture of the **1d**/**2c**/**TiCl$_4$** complex, the mixture imine **2c**/**TiCl$_4$** was first studied (Table 5, column **2c**/**TiCl$_4$** (1:1)), Figures 18, 19). Imine carbon atom is shifted at lower field ($\Delta\delta_C$: +7.4 ppm), whereas no significant chemical shift change was observed for the corresponding proton. Furthermore, a downfield signal was detected for C*H*$_2$ ($\Delta\delta_H$: +0.43 ppm), but the corresponding carbon atom is shielded ($\Delta\delta_C$: -3.8 ppm).

NMR spectra of the **1d**/**2c**/**TiCl$_4$** mixture showed only slight changes with respect to **1d**/**TiCl$_4$** and **2c**/**TiCl$_4$** spectra (Figure 13,14) indicating that both **1d** and **2c** are simultaneously complexed to different titanium units, then after addition of 1 equivalent of triethylamine to the mixture **1d**/**2c**/**TiCl$_4$**, very complicated NMR spectra, with a large number of broad signals, were detected (experiments recorded from 15 min to 6 h without significant changes). Interesting information, moreover, was achieved by addition of two equivalents of TEA, even if broad signals were again obtained (Figures 15,16). Actually, the ^{13}C NMR spectrum showed the absence of the *C*H$_2$ ester signal (a trace of imine *C*H is also present). According to the short reaction time observed for the above reaction, resonances consistent with the formation of Ti-complexed product **3d** were detected (Tables 4 column **1d**/**2c**/**TiCl$_4$/TEA** (1:1:2:2) and Table 5 column **1d**/**2c**/**TiCl$_4$/TEA** (1:1:2:2); HSQC experiment, Figure 17).

Similar NMR studies were then carried out on ester **1i** (Table 6 and Figures 20 and 23). Strong evidences of titanium coordination by both carbonyl and *ortho*-methoxy groups were found, being this last strongly deshielded ($\Delta\delta_H$ +0.62 ppm $\Delta\delta_C$: +15.5 ppm), according to the literature data for other methoxy compounds.[24a] A similar behaviour was found for aryl carbons in the *ortho*- and *para*-position to the methoxy group ($\Delta\delta_{5\text{-}ortho}$: +9 ppm; $\Delta\delta_{7\text{-}para}$: +12.7 ppm). Unfortunately, no information could be deduced by NMR spectra (^{1}H-, ^{13}C-, and HSQC NMR experiments) of **1i**/**TiCl$_4$**/**TEA** mixture (1:1:1 ratio), due to the presence of several signals. Considering the complexity of these spectra and that a different stereochemical result was found for **1d** and **1i**, we hypothesize that a different stability of titanium complex characterizes these esters, as documented by NMR.

All NMR spectra were recorded at 300 MHz in CD_2Cl_2(0.3 M) at -60 °C.



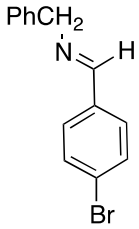
1d **2c** **syn-3d**

Tab 4. Complete ^1H and ^{13}C NMR (CD_2Cl_2, 0.3 M, -60 °C) data referred to ester **1d**

	1d		**1d** /$TiCl_4$ (1:1)		**1d**/$TiCl_4$/TEA (1:1:1) [a]		**1d**/**2c**/$TiCl_4$ (1:1:2)		**1d**/**2c**/$TiCl_4$/TEA (1:1:2:2)[a,b,c]	
	^1H	**^{13}C (J_{CF} Hz)**	**^1H**	**^{13}C (J_{CF} Hz)**	**^1H**	**^{13}C (J_{CF} Hz)**	**^1H**	**^{13}C (J_{CF} Hz)**	**^1H**	**^{13}C (J_{CF} Hz)**
OMe	3.74	53.1	4.08	59.1	4.09	59.7	4.06	59.2	4.01	59.6
CO	-	171.9	-	182.5	-	180.8/184.5[d]	-	182.5	-	181.4
2	3.76	34.9(2.7)	4.33	35.5	6.08/5.37[d]	41.6 /54.9[d]	4.34	35.6	4.33	55.5(overl)
3	_	122.0(16.0)	_	118.7 (15.9)	_	119.9	_	118.7 (15.2)	_	118.6 (15.2)
4	_	161.5 (245.7)	_	161.3 (247.3)	_	160.3 (251.0)		161.3 (247.3)	_	160.5 (236.7)
5	7.14-7.23	115.9(21.2)	7.14-7.24	116.0 (20.6)	7.21 brs	116.1	7.15-7.24	116.1 (20.8)	7.00-7.77	116.0
6		129.9 (8.0)		131.0 (7.2)		131.9		overl		128.8
7	7.29-7.46	124.8(3.6)	7.33-7.57	125.2		125.1	7.33-7.43	125.2		125.1
8		132.4(4.0)		132.6		131.9		132.7		132.9

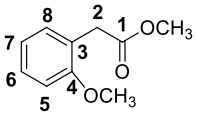
[a] Broad spectrum. [b]Chemical shift of ester framowork in the condensation product. [c] Same ^1H NMR detected at 10 min, 2.40 h, 5.10 h. [d] Resonance of two titanium-enolates.

Tab 5. Complete ^{1}H and ^{13}C NMR (CD_2Cl_2, 0.3 M, -60 °C) data referred to imine **2c**

	2c		**2c**/$TiCl_4$ (1:1)		**1d/2c**/$TiCl_4$(1:1:2)		**1d/2c**/$TiCl_4$/TEA (1:1:2:2)		
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	
CH	8.38	161.2	8.34	168.6	8.38	168.6	5.36[a]	75.8[a]	
CH_2	4.82	65.7	5.25	61.9	5.26	61.9	5.26	62.3	
***m*PhBr**	7.73 (d, *J* 8.5)	132.4(x2)	7.71 (d, *J*8.0)	134.8(x2)	7.69 (d, *J* 7.9)	134.1(x2)	7.77-7.10[b]	136.6-130.1[b]	
***o*PhBr**	7.62 (d, *J* 8.5)	130.2 (x2)	7.50 overlapped	131.6(x2)	7.52 overlapped	131.3(x2)			
***p*PhBr**	-	125.5	-	129.6	-	129.6			
***ipso*PhBr**	-	135.4	-	132.2	-	132.3			
Ph	7.43 (s)	139.7q 127.7 129.1(x2) 128.7(x2)	7.50 (s)	132.6q 130.7 130.2 (x4)	7.52	132.6q 130.8 130.2(x4)			

[a] H-3 in **3f**.[b] Due to the low resolution it is impossible to assign unequivocally aromatic proton and carbon resonances.

Tab 6. ^{1}H and ^{13}C NMR (-60 °C, 0.3 M, 300 MHz) referred to ester **1i**

	1i[a]		**1i** /$TiCl_4$ (1:1) [a]		**1i**/$TiCl_4$/TEA HCl (1:1:1) [b]	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	
CO_2*Me*	3.68	53.2	4.10	58.5	3.94	
1	-	173.5	-	181.6	-	
2	3.62	36.9	4.10	38.1	4.20	
ArOMe	3.80	56.1	4.42	71.6	3.84	
3	-	121.0		122.7	-	
4	-	157.7		159.6	-	
5	6.92	123.0	7.49-7.33	132.0	6.96	
6	7.33	129.5		128.3	7.35	
7	6.94	110.6		123.3	7.11	
8	7.17	131.6		131.5	7.21	

[a] Solvent $CDCl_3$. [b] Solvent CD_2Cl_2

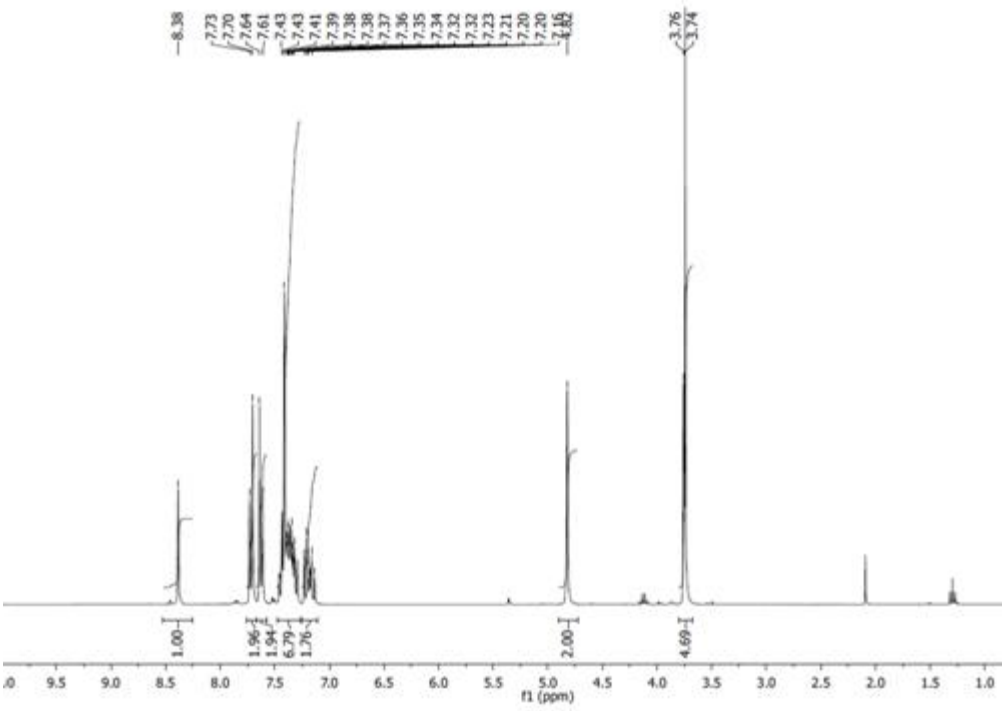


Figure 6. ^{1}H NMR of **1d/2c** mixture (1:1).

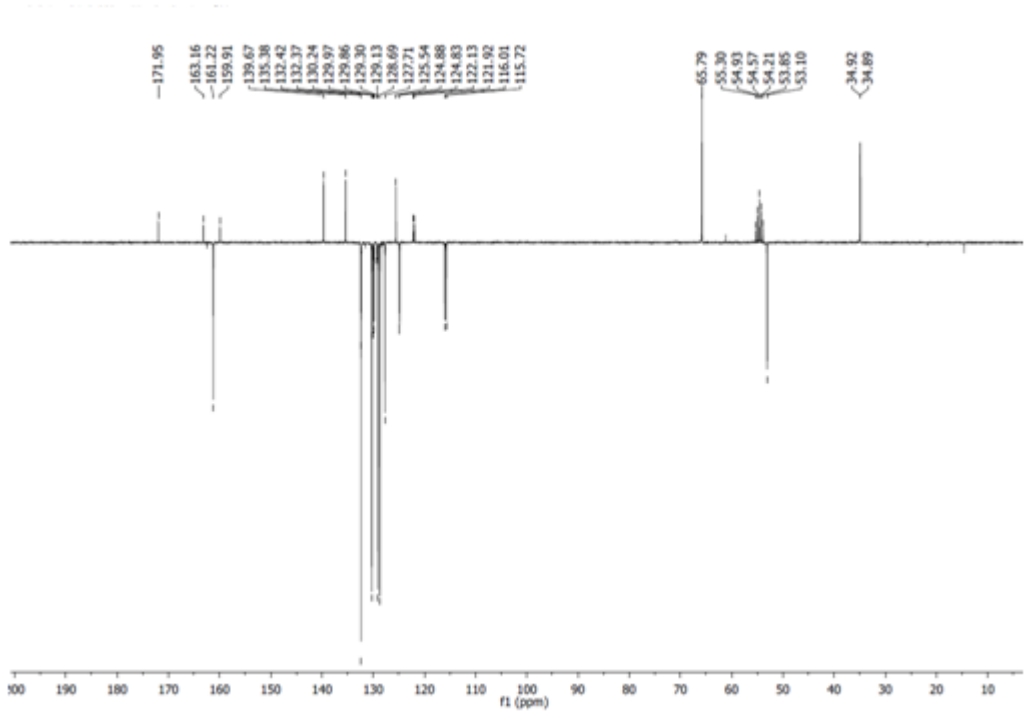


Figure 7. ^{13}C NMR of **1d/2c** mixture (1:1).

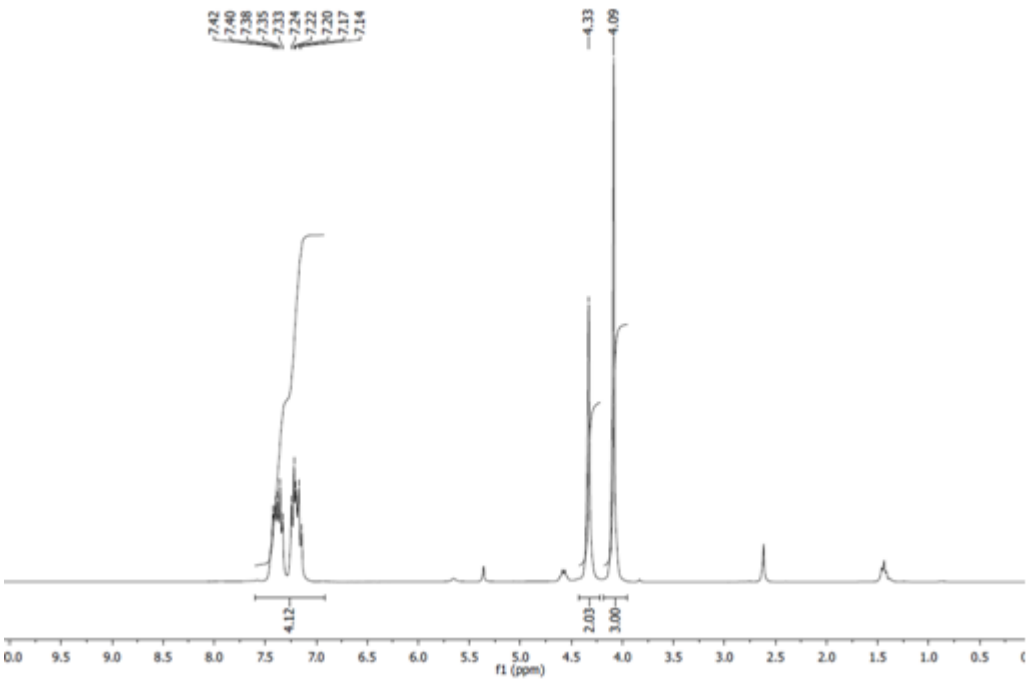


Figure 8. ^{1}H NMR of **1d/TiCl$_4$** mixture (1:1).

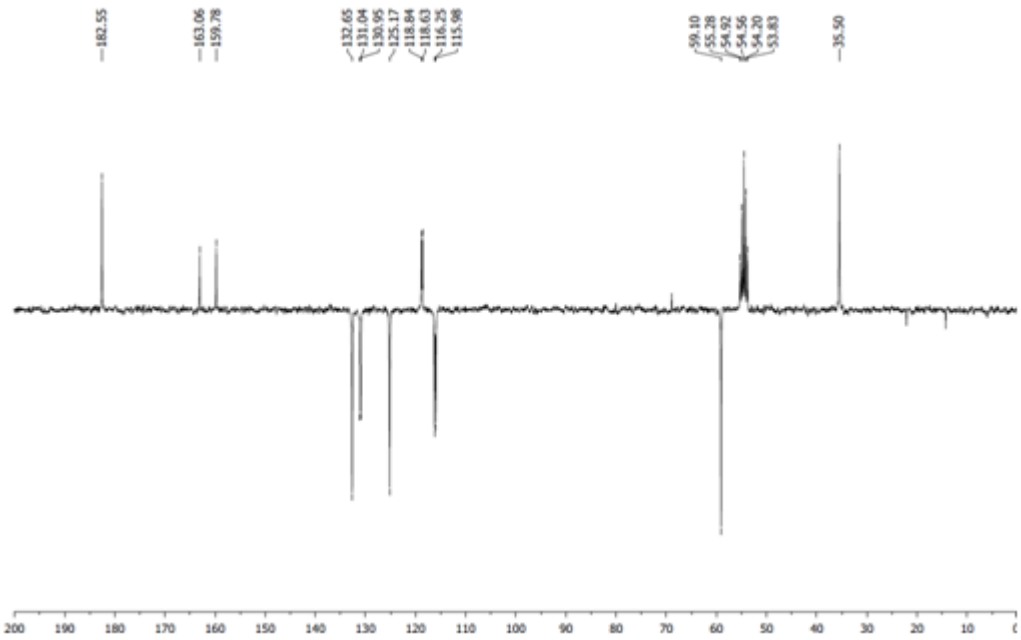


Figure 9. ^{13}C NMR of **1d/TiCl$_4$** mixture (1:1).

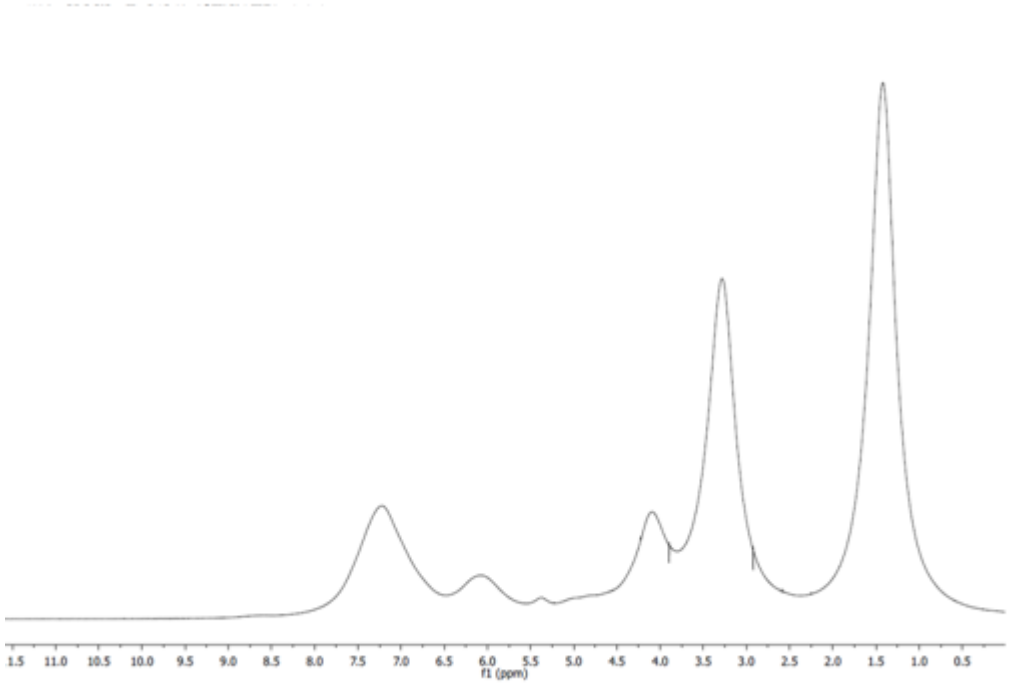


Figure 10. ^{1}H NMR of **1d/TiCl$_4$/TEA** mixture (1:1:1).

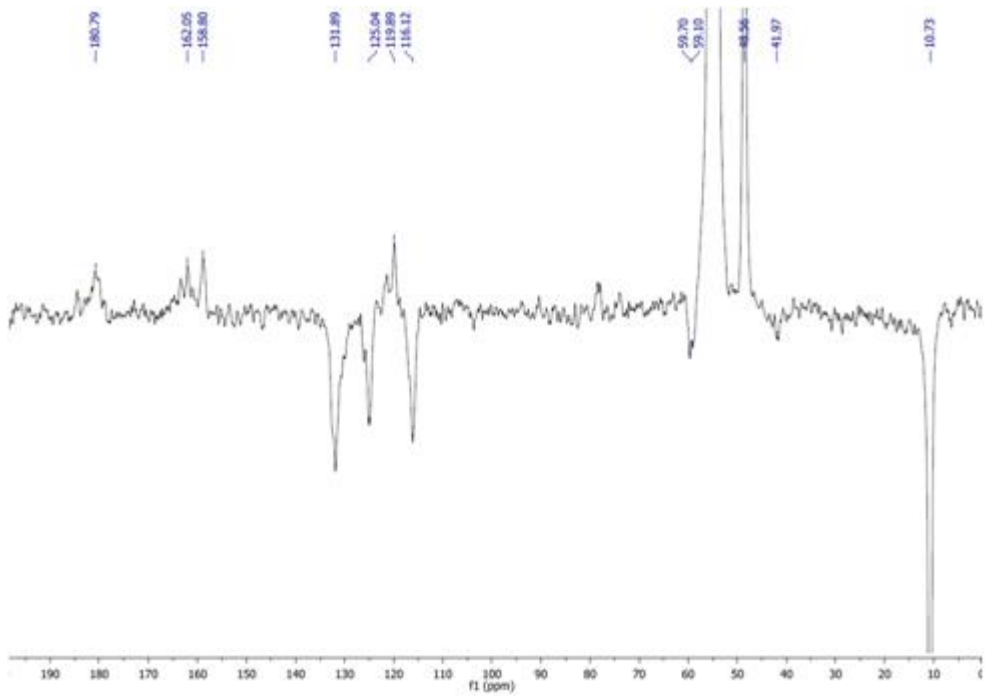


Figure 11. ^{13}C NMR of **1d/TiCl$_4$/TEA** mixture (1:1:1).

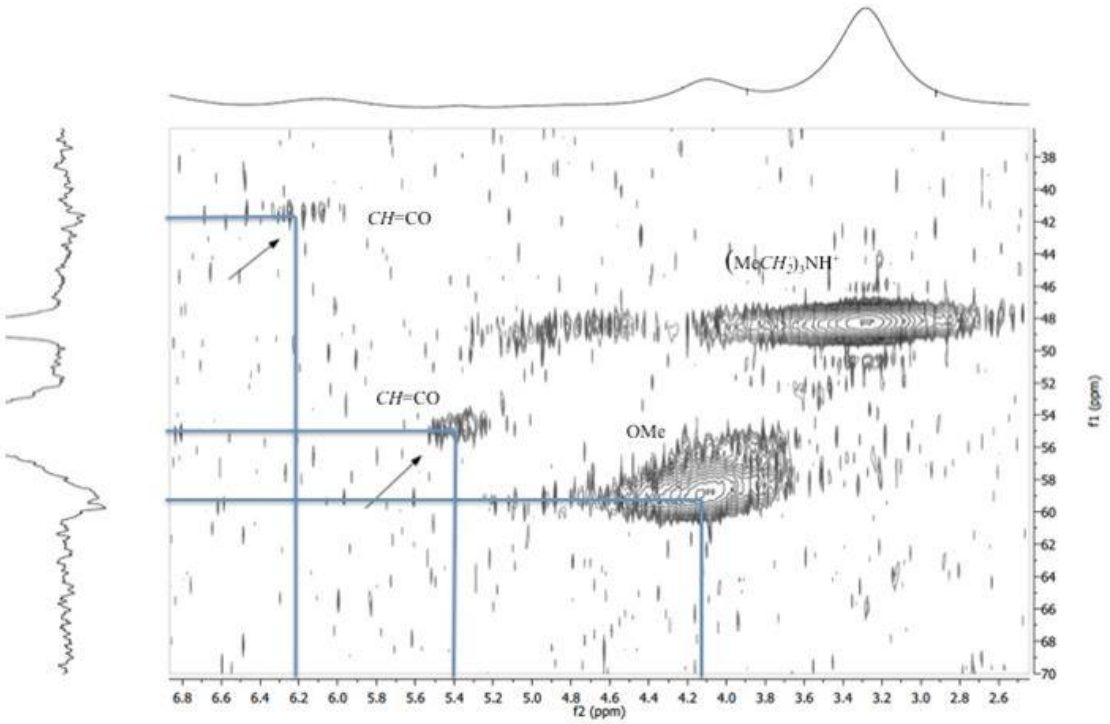


Figure 12. HSQC of **1f/TiCl$_4$/TEA** mixture (1:1:1).

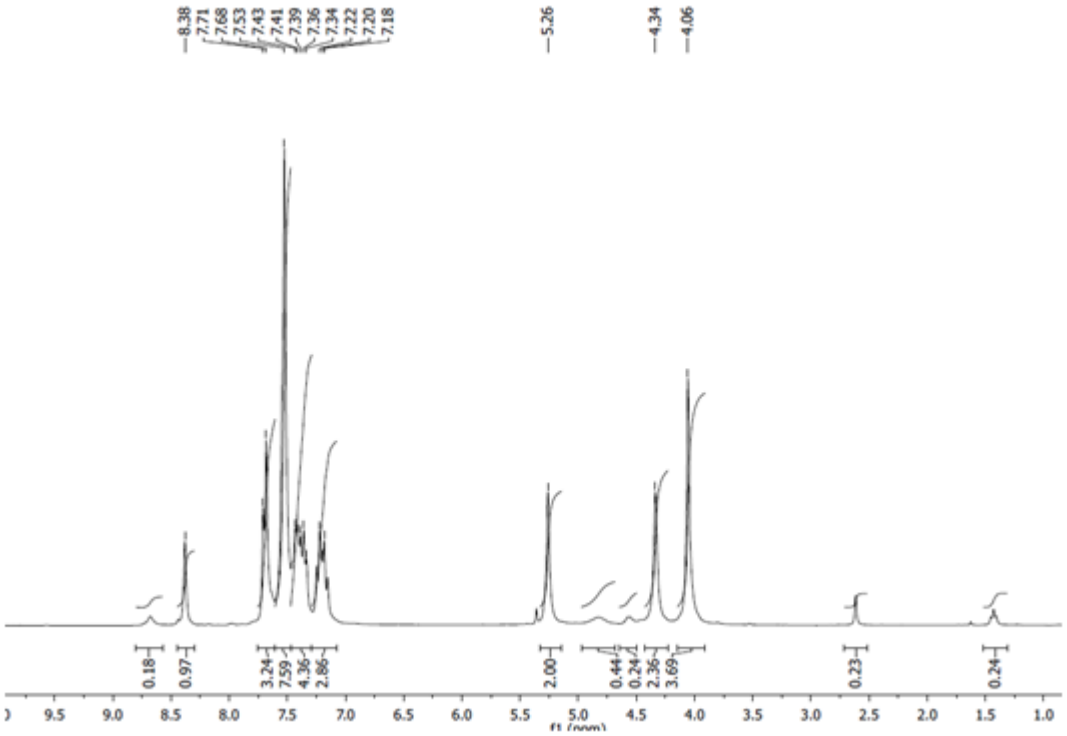


Figure 13. ^{1}H NMR of **1d/2c/TiCl$_4$** mixture (1:1:2).

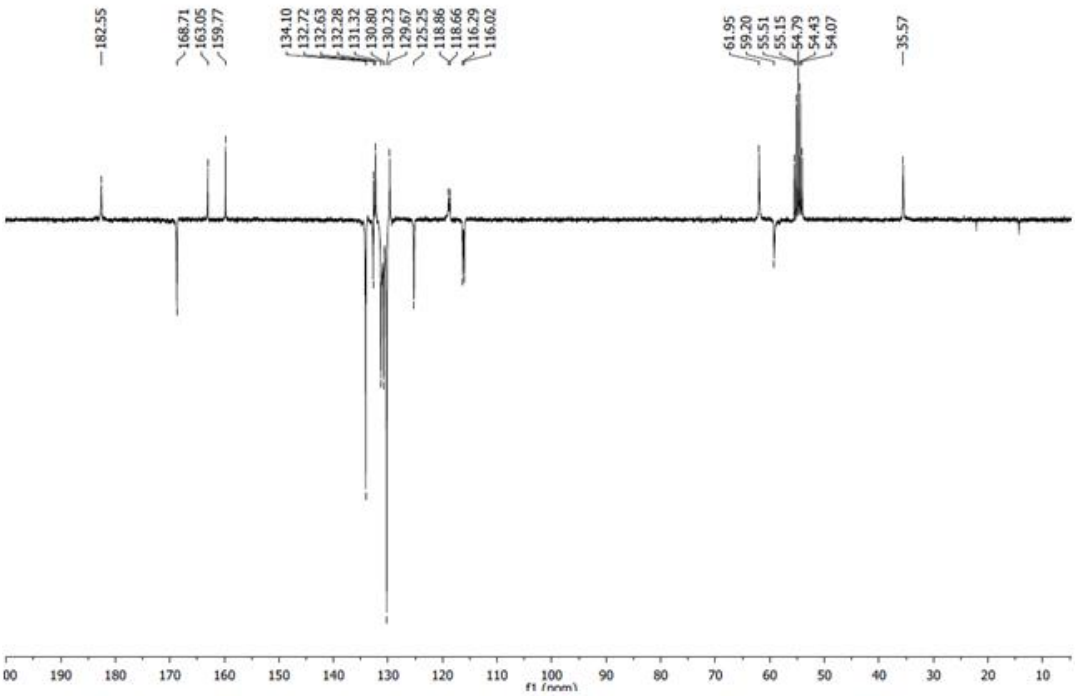


Figure 14. ^{13}C NMR of **1d/2c/TiCl$_4$** mixture (1:1:2).

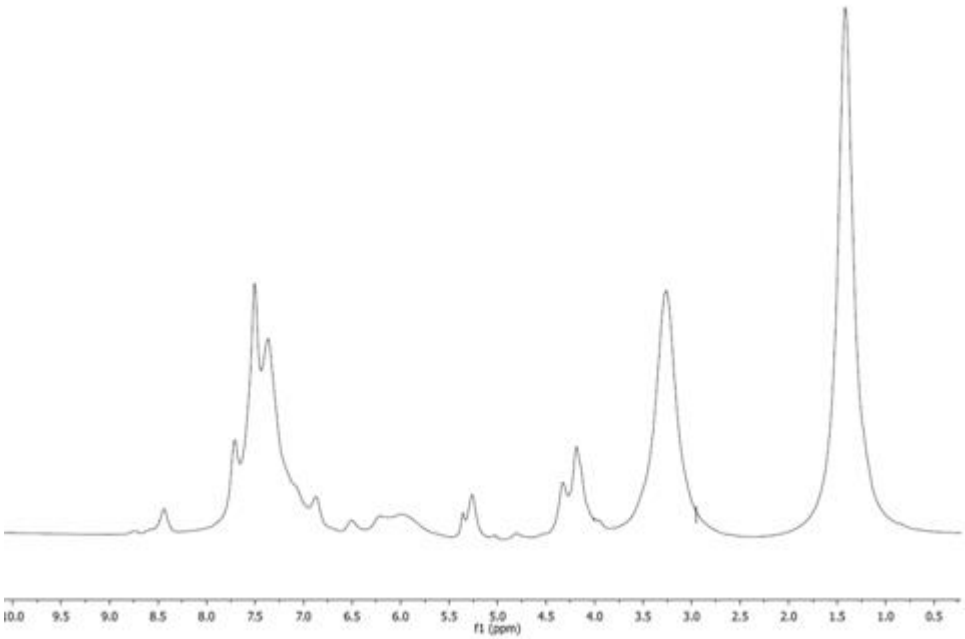


Figure 15. ^{1}H NMR of **1d/2c/TiCl$_4$/TEA** mixture (1:1:2:2).

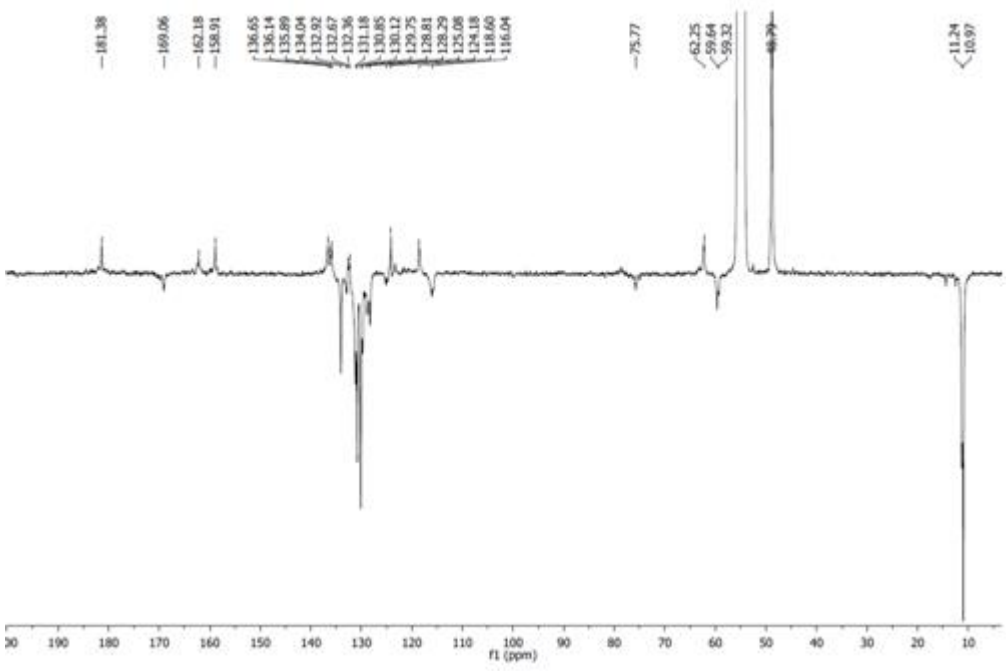


Figure 16. ^{13}C NMR of **1d/2c/TiCl$_4$/TEA** mixture (1:1:2:2).

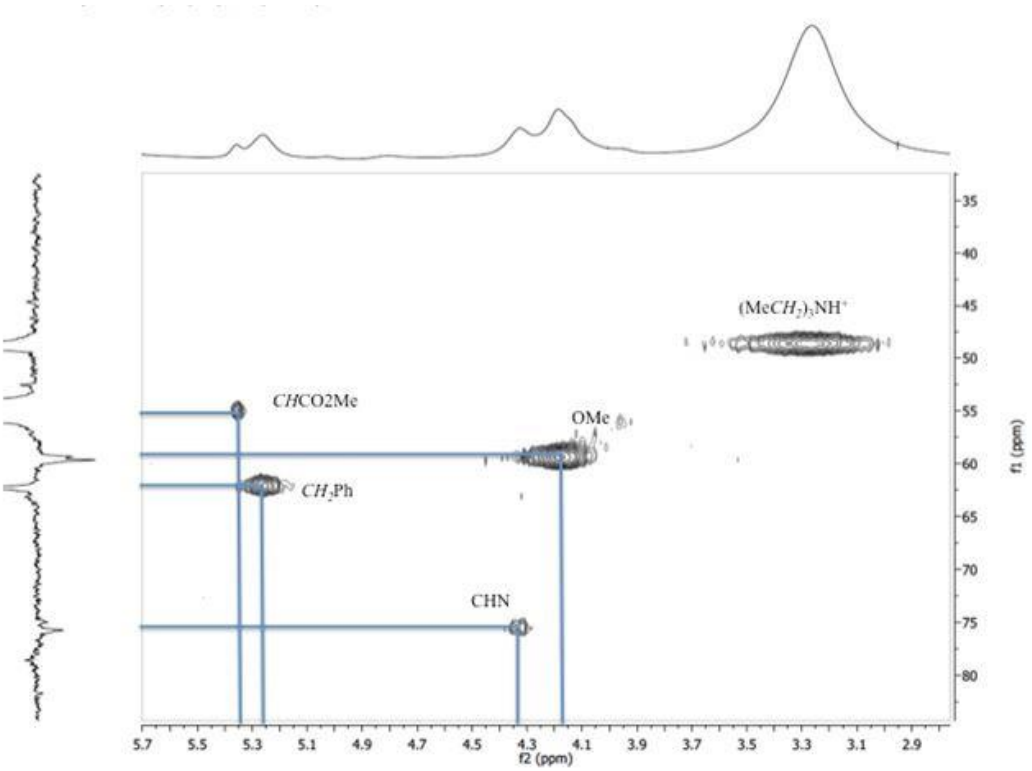


Figure 17. HSQC **1d/2c/TiCl$_4$/TEA** Mixtur mixture (1:1:2:2).

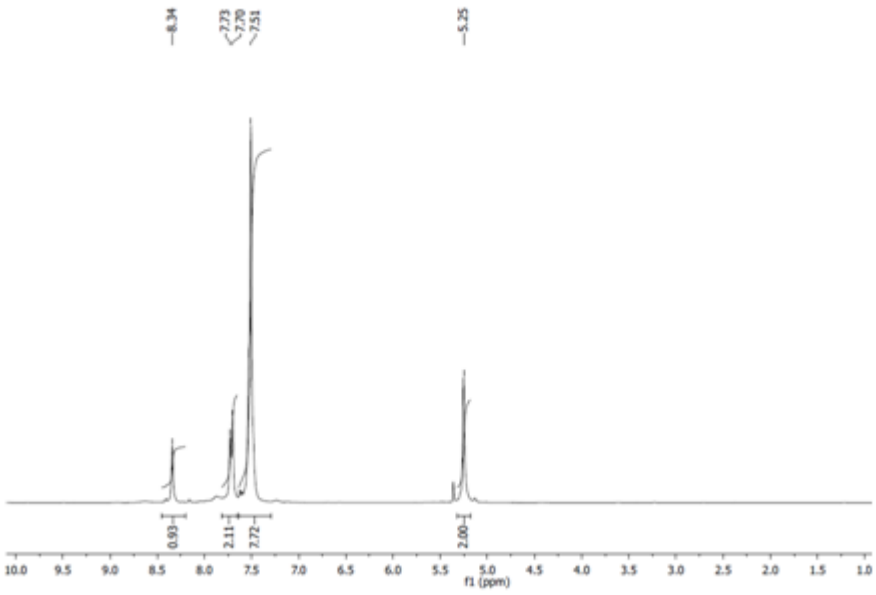


Figure 18. ^{1}H NMR of **2c/TiCl$_4$** (1:1).

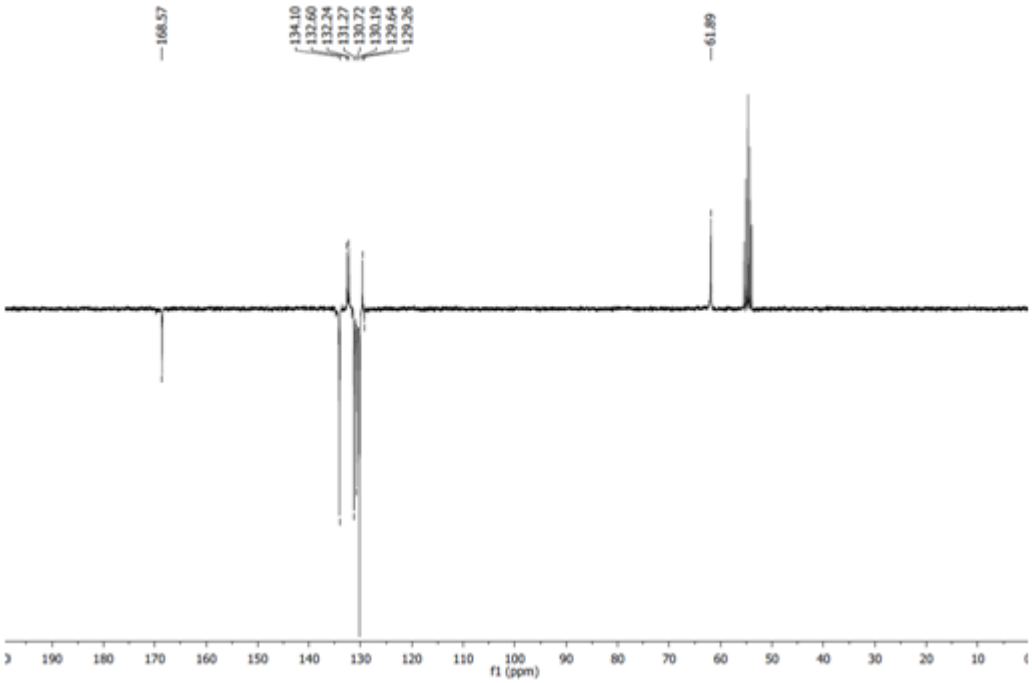


Figure 19. ^{13}C NMR of **2c/TiCl$_4$** (1:1).

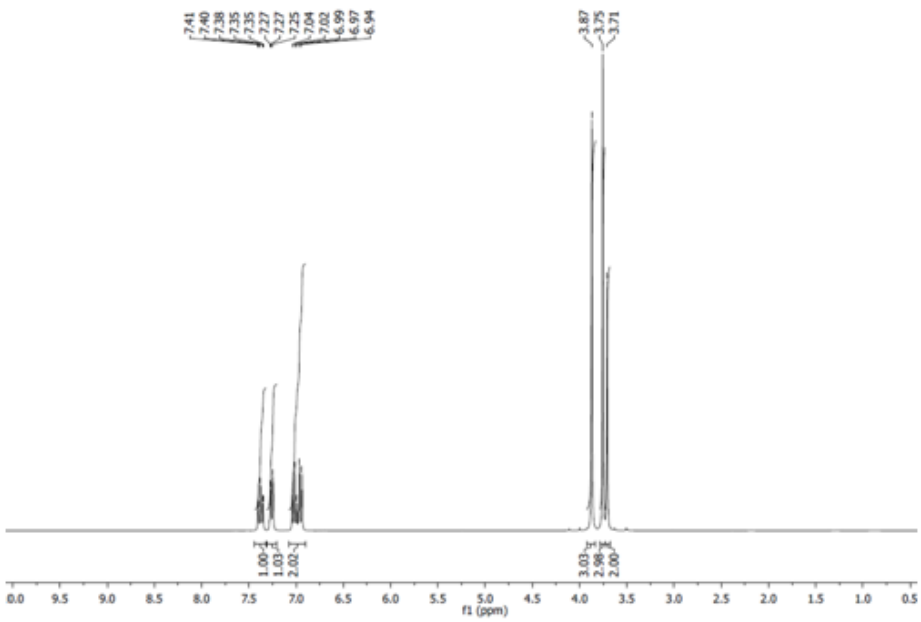


Figure 20.^{1}H NMR of **1i** in $CDCl_3$.

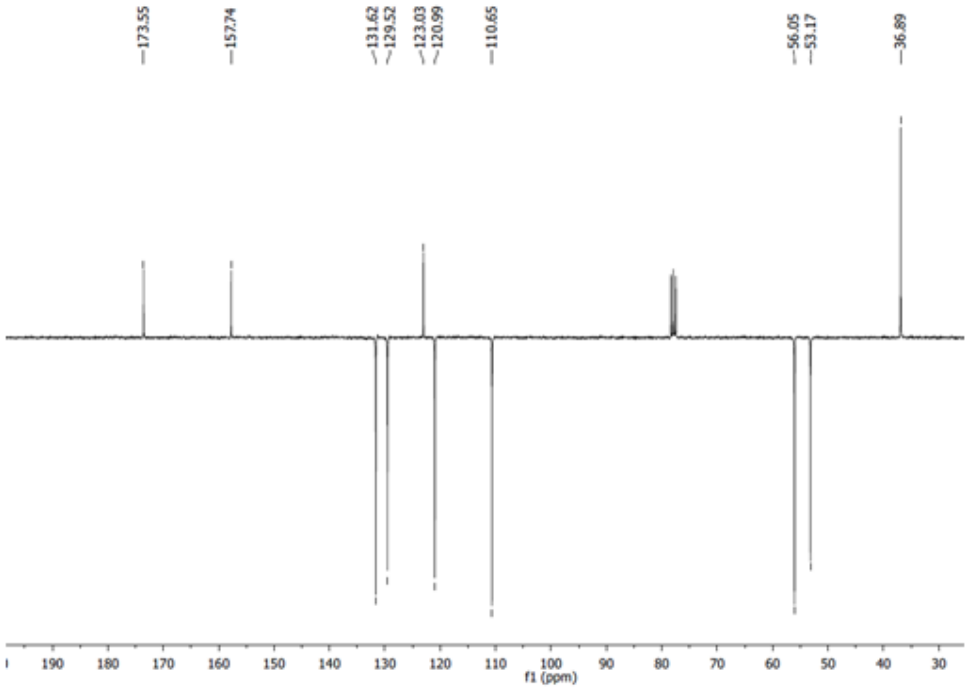


Figure 21. ^{13}C NMR of **1i** in $CDCl_3$.

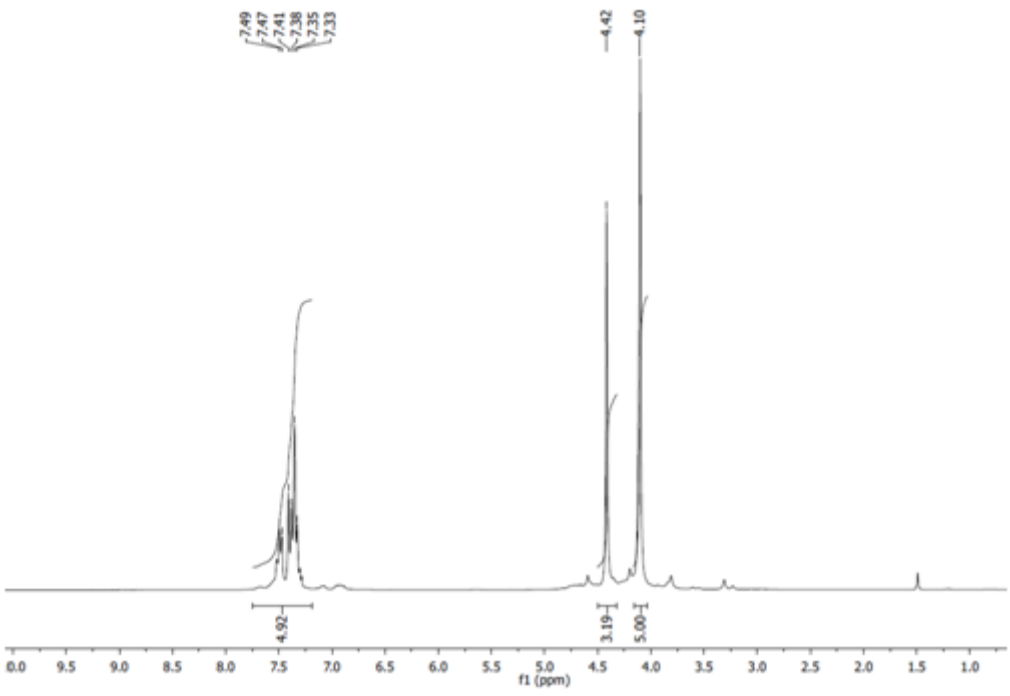


Figure 22. ^{1}H NMR of **1i/TiCl₄** (1:1) in $CDCl_3$.

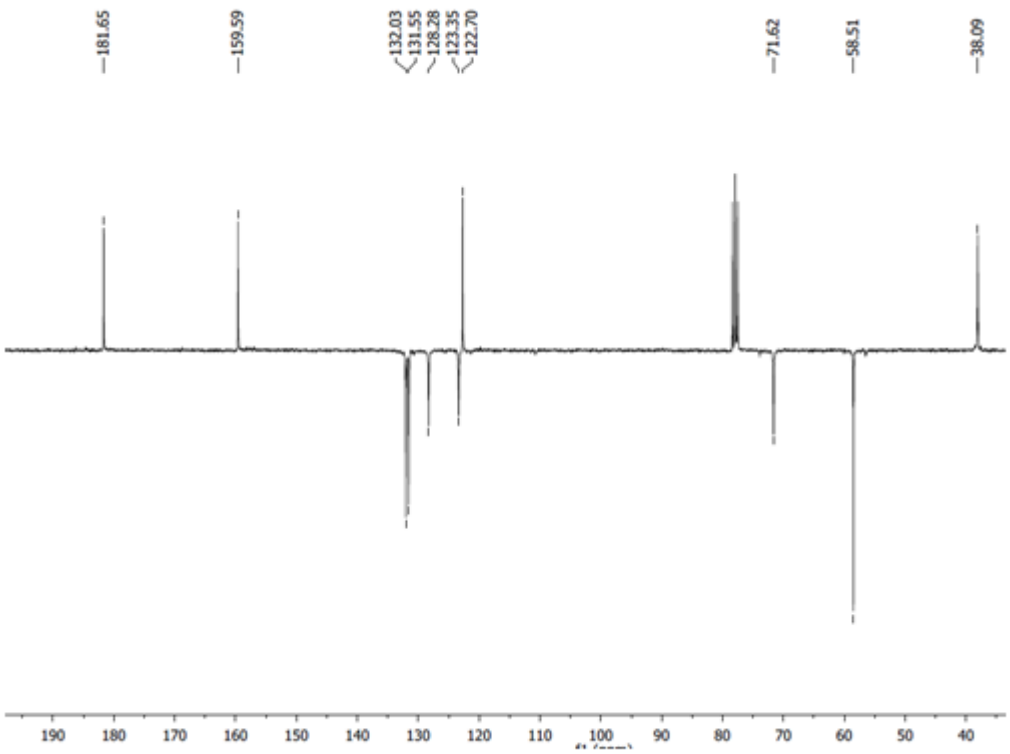


Figure 23. ^{13}C NMR of **1i/TiCl$_4$** (1:1) in $CDCl_3$

2.5. Conclusions

A very efficient synthesis of $\beta^{2,3}$-diarylamino acids was realized by using a Mannich-like reaction. The use of *ortho*-halogen substituted arylacetic esters allowed a chirality switch by tuning the *syn/anti* diastereoselectivity. On the other hand, independently from the ortho heteroatom, the use of the corresponding thioesters gave exclusively the *anti* adducts, which are interesting starting materials for the preparation of trans-β-lactam derivatives. The presence of a halogen atom on the aryl group represents a very interesting tool for further functionalization of the ortho position.

The work disclosed herein has been the subject of a publication on *European Journal of Organic Chemistry*, **2014**, 3203-3209, DOI: 10.1002/ejoc.201400142

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Experimental section

General Procedure for the Bromuration of Arylacetic Acids

Methylenedioxy phenylacetic acid or 3,4-dimethoxy-phenylacetic acid (11.9 mmol) was dissolved in acetic acid (30 mL) under stirring. Bromine (1.3 mL, 24.6 mmol) was added and a solid was formed. After 3 h, H_2O was added (20 mL), the solid was filtered, washed with water and a small amount of CH_2Cl_2. Pure *o*-bromo acid was obtained as a white solid.

2-Bromo-4,5-metylenedioxy-phenylacetic acid: (2.72 g, 90%). Mp 102-103°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ = 7.03 (s, 1H), 6.78 (s, 1H), 5.98 (s, 2H), 3.74 (s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ = 41.1, 103.0, 112.3, 112.9, 115.3 127.2, 147.7, 147.6, 171.2; IR (KBr) ν_{max}= 3456, 1723, cm^{-1}; elemental analysis calcd (%) for $C_9H_7BrO_4$: C, 41.73; H, 2.72; found: C, 41.50; H, 2.85.

2-Bromo-4,5-dimethoxy-phenylacetic acid 952 mg, 89%). Mp 117-118 °C; ^{1}H NMR ($CDCl_3$, 200 MHz):δ =8.35 (broad, 1H), 7.01 (s, 1H), 3.83 (s, 6H), 3.74 (s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz):δ = 41.1, 56.3, 56.4, 114.2, 115.3, 115.7, 125.5, 148.6, 149.2, 176.9; IR (KBr) ν_{max}= 3435, 1741 cm^{-1} ;elemental analysis calcd (%) for $C_{10}H_{11}BrO_4$: 43.66; H, 4.03; found: 43.50; H, 4.12.

***General Procedure for the Esterification of Arylacetic Acids*.**

2-Bromo-4,5-methylenedioxy-phenylacetic acid or 2-bromo-4,5-dimethoxyphenylacetic acid (23.3 mmol) was dissolved in MeOH (40 mL). Few drops of H_2SO_4 were added and the solution was refluxed for 1 h after which MeOH was removed under reduced pressure. The crude mixture was dissolved in CH_2Cl_2 (20 mL) and washed with $NaHCO_3$ (10 mL) and brine (10 mL). The solution was dried with Na_2SO_4, and the solvent removed in vacuo. Pure methyl ester **2c** (6.2 g, 98%) was obtained as a white solid after crystallization (CH_2Cl_2/hexane).

*Methyl 2-bromo-4,5-methylenedioxy-phenylacetate (**1c**):* 6.2 g (98%). mp 81-82 °C;^{1}H NMR ($CDCl_3$, 200 MHz):δ = 7.00 (s, 1H), 6.76 (s, 1H), 5.96 (s, 2H), 3.70 (s, 3H), 3.69 (s, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz):δ = 41.4, 52.4, 102.0, 111.2, 112.9, 115.6, 127.2,147.6, 147.9, 171.3; IR (KBr) ν_{max}= 3435, 1718 cm^{-1};elemental analysis calcd (%) for $C_{10}H_9BrO_4$: C, 43.98; H, 3.32; O, 23.44; found: C, 43.85; H, 3.49; O, 23.31.

*Methyl 2-bromo-4,5-dimethoxy-phenylacetate (**1f**):* 95%. Mp 72-73 °C;^{1}H NMR ($CDCl_3$, 200 MHz):δ = 7.02 (s, 1H) 6.79 (s, 1H), 3.85 (s, 6H), 3.72 (s, 2H). 3.71 (s, 3H); ^{13}C NMR ($CDCl_3$,50 MHz)δ 41.1, 52.3, 56.2, 56.3, 114.0, 115.1, 115.6, 126.1, 148.5, 148.9, 171.4; IR (KBr) ν_{max}= 3441, 1732, cm^{-1}; elemental analysis calcd (%) for $C_{11}H_{13}BrO_4$: C, 45.70; H, 4.53; O, 22.14; found:C, 45.61; H, 4.63; O, 22.08.

(1R,2S,5R)-5-Methyl-2-(2-phenylpropan-2-yl)cyclohexyl-2-(2-fluorophenyl)acetate (1o).

2-Fluoro-phenylacetic acid (154.14 mg, 1 mmol) was dissolved in dry CH_2Cl_2 (10 mL) under nitrogen atmosphere. Thionyl chloride (217 μL, 3 mmol) was added at 0 °C and then the reaction was stirred at 25 °C overnight. CH_2Cl_2 was removed under reduced pressure and the crude, dissolved in dry toluene (2 mL), was dropped at 0°C to a solution of (-)-8-phenylmenthol (278.8 mg, 1.2 mmol) dissolved in dry toluene (10 mL). The reaction was stirred at 25 °C for 5 h. Toluene was removed under reduced pressure and the crude mixture was purified by column chromatography (cyclohexane) affording **1o** (309 mg, 84%) as a colourless oil. $[\alpha]^{25}_D$ -31.6 (*c* 20 mg/mL, $CHCl_3$);^{1}H NMR ($CDCl_3$, 200 MHz) δ = 7.31-6.95 (m, 9H), 4.82 (dt, *J*= 10.6 Hz, 6.2, 1H), 3.04 (AB system, *J* = 19.0, 4.0 Hz, 2H), 1.12-0.80 (m, 17H);MS (ESI+): *m/z*:369.2 $[M+1]^+$.

Phenethyl 2-(2-iodophenyl)ethanethiolate (8a).

Operating under argon atmosphere, a mixture of 2-iodophenylacetic acid (262 mg, 1 mmol), DCC (227 mg, 1.1 equiv) and DMAP (12.2 mg, 0.1 equiv.) in dry CH_2Cl_2 (15 mL) was stirred at 0°C for 2 min. 2-Phenylethanethiol (207 mg, 1.5 mmol) was slowly added in 15 min at 0°C. The reaction was stirred at 25 °C for 3 h and than it was filtered over a short pad of celite. The organic layer was washed with brine (15 mL) and dried over Na_2SO_4. The crude mixture was purified by column chromatography (cyclohexane/AcOEt, 10:3) affording **8a** (340 mg, 89 %) as a colourless oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ = 7.85 (d, *J* 8, 1H), 7.45-7.20 (m, 6H), 6.97 (m, 2H), 3.17 (t, *J*= 5.0 Hz, 2H), 3.26 (t, *J* = 5.0 Hz, 2H); MS (ESI+):*m/z*:382.9$[M+1]^+$.

Benzyl 2-(2-fluorophenyl)ethanethioate (8b).

Operating under nitrogen atmosphere, a mixture of 2-fluorophenylacetic acid (1 g, 6.48 mmol), DCC (1.47 g, 7.14 mmol) and DMAP (79 mg, 0.64 mmol) in dry DCM (50 mL) was stirred at 0°C for 2 min.. Benzylthiol (1.61 g, 7.13 mmol) was slowly added in 15 min at 0°C. The reaction was stirrer at 25 °C for 4 h and than it was filtered under vacuum. The organic layer was washed with 0.1 M HCl (50 mL), $NaHCO_3$ (50 mL), brine (50 mL) and dried over Na_2SO_4. The crude mixture was purified by column chromatography (hexane/AcOEt, 50:1) affording **8b** (1.32 g,79 % yield)as a colourless oil.^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.39-6.97 (m, 9H), 4.13 (s, 2H), 3.90 (s, 2H); MS (ESI+):*m/z*:261.1 $[M+1]^+$.

Methyl 2-(2-iodophenyl)ethanedithioate (1p).

2-Iodio phenylacetic acid (1.00 g, 3.81 mmol) was dissolved in chlorobenzene (20 mL) under stirring. Davy's reagent (2.17 g, 7.63 mmol) was added and the reaction was stirred at 140 °C for 8 h.The solvent was removed in vacuo and pure methyl 2-(2-iodophenyl)ethanedithioate **1p** (728 mg, 65%) was obtained as a colourless oil after chromatographic column (hexane). ^{1}H NMR (CDCl3, 200 MHz): δ = 7.8 (d, *J*= 8.0 Hz, 1H), 7.32 (m, 2H), 6.99 (m, 1H), 4.47 (s, 2H), 2.63 (s, 3H); elemental analysis calcd (%) for C, 35.07; H, 2.94. Found C, 34.93; H, 3.09.

General procedure for the preparation of imines 2a-2e

Aldehyde (1 mmol) and amine (1 mmol) were heated in microwave oven at 100°C (200W) for 15 min in solvent free conditions. The crude mixture was taken up with DCM and water was separated. The organic layer was dried with Na_2SO_4 and the solvent removed under reduced pressure. Crystallization from Et_2O gave pure imine as white needles unless otherwise specified.

Methyl 3-(Benzylamino)-2,3-diphenylpropanoates (3b, 4b)

Operating under N_2 atmosphere,methyl 2-phenylacetate **1b** (315 mg, 2.1 mmol) and *N*-benzylidene-1-phenylmethanamine **2b** (390 mg, 2 mmol) were dissolved in CH_2Cl_2 (16 mL) and the mixture was cooled at 0°C. $TiCl_4$ (4.8 mmol), dissolved in CH_2Cl_2 (4 mL) was added in 15 min and the mixture was stirred for 0.5 h after which triethylamine (280 μL, 2 mmol) was added and stirring was continued for a further 3 h. A saturated solution of K_2CO_3 was dropped and the temperature was raised at 25 °C. The solid was filtered, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude residue was purified by column chromatography on a neutral alumina column using hexane/EtOAc (99/1) as eluent affording **3b** (148 mg,23%), **4b** (123 mg,impure of Claisen compound and benzylamine) and dimer **5b** (65 mg, 11%).

2*S**,3*S****-3b**:Mp113-114 °C (110-112 °C);[51]H NMR (300 MHz, $CDCl_3$) δ = 7.58-6.95 (m, 15H), 4.25, 3.86 (AM system, *J* = 10.2 Hz, 2H), 3.56, 3.31 (AM system, *J* = 13.8 Hz, 2H), 3.34 (s, 3H), 1.63 (brs, 1H, exch.); elemental analysis calcd (%) for C, 79.97; H, 6.71; N, 4.05; found C, 79.83; H, 6.61; N, 4.22.

2*S**,3*R**-**4b**: (impure of Claisen compound and benzylamine). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.40-7.00 (m, 15H), 4.25, 3.85 (AM system, *J*= 10.4 Hz, 2H), 3.67, 3.53 (AM system, *J*= 13.0 Hz, 2H), 3.71 (s, 3H), 2.06 (brs, 1H, exch.);MS (ESI+):*m/z*:346.1 $[M+1]^+$. Significant signals for Claisen compound (enole form): δ= 3.64 (s, 3H), 3.40 (s, 2 H).

2*S**,3*S**-**5b**: ^{1}H NMR (300 MHz, $CDCl_3$):δ = 7.13-7.05 (m, 6H), 7.01-6.96 (m, 4H), 4.25 (s, 2H), 3.69 (s, 6H).[6]

Synthesis of Dimeric Compounds R,S* and S*,S*-**5b** from Ester **1b**:* Compounds **5/5'b** (2.5:1 ratio) were prepared according to the reported procedure starting from **1b**.[6] 1H NMR spectrum of the crude mixture was shown in Figure 3. ^{1}H NMR ($CDCl_3$, 300 MHz), main *S**,*S**-isomer: δ = 7.94-7-01(m, 5H), 4.25 (s, 2H), 3.69 (s, 3H); minor *S**,*R**-isomer δ= 7.94-7-01(m, 5 H), 4.40 (s, 2H), 3.69(s, 3 H).

General Procedure for the Preparation of 2,3-Diarylpropanoates.

In a three necked round bottom flask equipped with a magnetic stirrer, thermometer, a nitrogen inlet and a dropping funnel, ester 1 or thioester 8 (2.56 mmol) and imine 2 (2.56 mmol) were dissolved in dry CH_2Cl_2 (25 mL) and the solution was cooled to -78 °C. A solution of $TiCl_4$ (560 μL, 5.12 mmol) in dry CH_2Cl_2 (5 mL) was added slowly in 15 min. After 5 min, freshly distilled triethylamine (714 μL, 5.12 mmol) was slowly added giving a deep purple solution. Temperature and reaction time were indicated in the single example. A saturated solution of K_2CO_3 was dropped and the temperature was raised at 25 °C. The mixture was stirred for a 10 min after which TiO_2 precipitated that was filtered off over a celite pad. The organic layer was separated, washed with brine and dried over Na_2SO_4. The crude mixture was purified by column chromatography

Methyl 3-(N-Benzylamino)-2-(2-bromo-4,5-methylenedioxyphenyl)-3-(3,4-methylenedioxy-phenyl)propanoates(3c/4c).

T = -78 °C; t = 15 min. Column chromatography (cyclohexane/Et_2O, 10:3): **3c** (45%), mixture of isomers **3c/4c** (26%), **4c** (27%).

2*S**,3*S**-**3c:** white solid; m.p. 129-133 °C (CH_2Cl_2/Et_2O); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.26-6.77 (m, 10H), 6.02 (d, *J* = 1.5 Hz, 1H) 5.97 (s. 3H), 4.46, 4.04 (AX system, *J* = 10.2 Hz, 2H), 3.61, 3.32 (AB system, *J* = 13.9 Hz, 2H), 3.40 (s, 3H), 1.6 (brs, 1H, exch.); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 30.1, 50.9, 52.2, 57.4, 64.2, 101.3, 102.1, 108.3, 108.5, 108.9, 113.1, 117.1, 122.4, 127.2, 128.3, 128.6, 128.9, 135.1, 140.5, 147.5, 148.2, 172.2; IR (KBr) ν_{max} = 3332, 1448 cm^{-1}; MS (ESI+):

m/z:513.9 $[M+1]^+$; elemental analysis calcd (%) for $C_{25}H_{22}BrNO_6$: C, 58.61; H, 4.33; N, 2.73; found: C, 58.48; H, 4.48, N, 2.50.

2*S**,3*R****-4c:** white solid; m.p. 82-84 °C (AcOEt/n-Hexane); ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.34-6.53 (m, 10H), 5.93-5.89 (m. 4H), 4.45, 4.09 (AM system, *J* = 10.2 Hz, 2H), 3.69 (s, 3H), 3.63, 3.52 (AB system, *J* = 13.4 Hz, 2H), 1.70 (brs, 1H, exch.); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 29.9, 51.3, 52.4, 56.5, 65.1, 101.1, 102.0, 107.9, 108.0, 112.7, 115.9, 121.9, 127.1, 128.2, 128.3, 128.5, 129.0, 133.8, 140.4, 146.9, 147.5, 147.8, 173.4; IR (KBr) ν_{max} = 3454, 1734 cm^{-1}; MS (ESI): *m/z*:513.9 $[M+1]^+$; elemental analysis calcd (%) for $C_{25}H_{22}BrNO_6$: C, 58.61; H, 4.33; N, 2.73; found: C, 58.45; H, 4.45; N, 2.53.

Methyl-3-(benzylamino)-2-(2-bromo-4,5-dimethoxyphenyl)-3-(3,4-methylenedioxy-phenyl)-propanoates (3f,4f)

T = -78 °C; t = 15 min. **3c** (66%; obtained directly from crystallization of the reaction mixture with MeOH); Column chromatography (cyclohexane/Et_2O, 10:3): **3f** (11%), **4f** (11%).

2*S**,3*S****-3f:** mp 134-136 °C (MeOH);^{1}H NMR ($CDCl_3$, 200 MHz):δ= 7.26-6.77 (m, 10H), 6.02 (d. *J* = 1.47 1H) 5.97 (s, 3H), 4.46, 4.04 (AX system, *J* = 10.2 Hz, 2H), 3.61, 3.32 (AB system, *J* =13.9 Hz, 2H), 3.40 (s, 3H);^{13}C NMR ($CDCl_3$, 200 MHz):δ= 50.5, 52.2, 56.4, 56.5, 57.1, 63.6, 101.4, 108.3, 108.6, 111.2, 115.7, 116.6, 122.5, 127.3, 128.5, 128.6, 135.0, 140.4, 147.5, 148.2, 149.2, 149.4, 172.5; IR (KBr) ν_{max}= 3354, 1730 cm^{-1}; MS (ESI+):*m/z*:529.1 $[M+1]^+$; elemental analysis calcd (%) for $C_{26}H_{26}BrNO_6$:C, 59.10; H, 4.96; N, 2.65; found: C, 58.81; H, 5.17; N, 2.38.

2*S**,3*R****-4f:** mp 125-126 °C, (AcOEt/n-hexane);^{1}H NMR ($CDCl_3$, 200 MHz):δ= 7.34-7.24 (m, 4H), 6.99 (s, 1H), 6.84 (s, 1H), 6.79 (s,1H), 6.63-6.64 (m, 2H), 5.91 (d, *J* = 3.51 Hz, 2H), 4.44, 4.20 (AX system, *J* = 9.8 Hz, 2H), 3.85 (s, 3H), 3.80 (s, 3H) 3.71 (s, 3H), 3.68, 3.55 (AB system, *J* = 13.2 Hz, 2H);^{13}C NMR ($CDCl_3$,50 MHz):δ= 51.6, 52.5, 56.3, 56.5, 56.9, 65.5, 101.3, 108.1, 108.2, 112.6, 115.5, 121.9, 127.3, 128.3, 128.5 (x2), 128.6 (x2), 134.1, 140.6, 147.1, 147.9, 148.6, 148.9, 173.8; IR (KBr) ν_{max} = 3437, 1733 cm^{-1}; MS (ESI):*m/z*:552 $[M+Na]^+$, 529.1 $[M+1]^+$; elemental analysis calcd (%) for $C_{26}H_{26}BrNO_6$: C, 59.10; H, 4.96; N, 2.65; found: C, 58.79; H, 5.20; N, 2.32.

Methyl 3-(benzylamino)-2-(2-iodophenyl)-3-phenyl-propanoates (3g, 4g).

T = -78 °C; t = 180 min. Column chormatography (hexane/ethyl acetate/TEA, 11:1:0.01): **3g** (45%) and **4g** (36%).

2*S,3*S**-3g:** clearwax; ^{1}H NMR ($CDCl_3$, 300 MHz):δ= 7.90 (dd, *J* = 1.2, 7.8, 1H), 7.52-7.21 (m, 12H), 7.02 (dd, *J* = 2.1 Hz, 7.8, 2H), 4.54, 4.24 (AB system, *J* 9.9 Hz, 2H), 3.62, 3.32 (AB system, *J* = 14.1 Hz, 2H),

3.37 (s, 3H), 1.76 (bs, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 50.2, 51.3, 61.6, 64.0, 103.0, 126.3, 127.3, 127.5 (x2), 127.7 (x2), 127.9 (x4), 128.1, 128.2, 128.9, 138.7, 139.3, 139.6, 140.3, 171.4; IR (neat) ν_{max}= 3373, 1690 cm^{-1}; elemental analysis calcd (%) for $C_{23}H_{22}INO_2$: C, 58.61; H, 4.70; N, 2.97; found C, 58.39; H, 4.91; N, 2.65.

2*S,3*R**-4g:** clearwax; ^{1}H NMR ($CDCl_3$, 300 MHz):δ= 7.64 (dd, *J* = 7.8, 1.2Hz, 1H), 7.50 (dd, *J* = 1.2 Hz, 7.8, 1H), 7.32-7.16 (m, 12H), 6.80 (dt, *J* = 1.5, 7.58, 9.3 Hz, 1H), 4.49, 4.28 (AB system, *J* 9.9 Hz, 2H), 3.70, 3.69 (AB system, *J* 13.5 Hz, 2H), 3.69 (s, 3H), 2.14 (bs, 1H,); ^{13}C NMR ($CDCl_3$, 75 MHz):δ= 50.1, 51.4, 61.6, 77.7, 103.4, 127.1, 127.3-128.9 (x11), 130.0, 138.7, 139.1, 139.7, 141.0, 171.4; IR (neat) ν_{max}= 3406, 1687 cm^{-1}; elemental analysis calcd (%) for $C_{23}H_{22}INO_2$: C, 58.61; H, 4.70; N, 2.97; found C, 58.36; H, 4.95; N, 2.60.

Methyl 2S*,3S*-3-(benzylamino)-2-(2-fluorophenyl)-(4-bromophenyl)-propanoate (3d).

T = -78 °C; t = 40 min. Column chormatography (hexane/ethyl acetate/TEA, 30:1): 91%. Mp 78-79 °C (MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz):δ = 7.49-7.21 (m, 13H), 4.29, 4.22 (AB system, *J* = 9.5 Hz, 2H), 3.59, 3.47 (AB system, *J* = 14.1 Hz, 2H), 3.56 (s, 3H), 1.65 (brs, 1H, exch.); ^{13}C NMR ($CDCl_3$, 50 MHz):δ = 50.6, 50.9, 52.1, 62.9, 115.7 (*J* = 22.9), 121.9, 122.9 (*J* = 14.1), 124.7 (*J* 3.5), 127.2, 128.2 (x2), 128.5 (x2), 129.6, 129.7 (*J*= 8), 130.2 (x2), 131.7 (x2), 140.1, 161.4 (*J*= 247.1), 171.7; IR (KBr) ν_{max}= 3459, 1736 cm^{-1}; MS (ESI) *m/z* 444.4 $[M+2]^+$; Anal. Calcd for $C_{23}H_{21}BrFNO_2$: C, 62.45; H, 4.79; N, 3.17; found C, 62.36; H, 4.91; N, 3.07.

Methyl 3-(2S*,3S*-Benzylamino)-2-(2-chlorophenyl)-3-(4-bromophenyl)-propanoate (3e, 4e).

T = -78 °C; t = 90 min. Column chromatography (hexane/ethyl acetate, from 50:1 to 25:1): **3e** (75%) and **4e** (6%).

2*S**,3*S**-**3e*:*** mp 111 °C (MeOH/Et_2O); ^{1}H NMR ($CDCl_3$, 200 MHz):δ= 7.53-7.23 (m, 11H), 7.03-6.98 (m 2H), 4.62, 4.23 (AB system, *J* = 9.9 Hz, 2H), 3.61, 3.32 (AB system, *J* = 13.7 Hz, 2H), 3.40 (s, 3H), 1.90 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 50 MHz):δ= 50.9, 52.2, 54.2, 63.5, 121.8, 127.2, 127.5, 128.2 (x2), 128.5 (x2), 129.2, 129.4, 130.0, 130.4 (x2), 131.8 (x2), 133.9, 135.6, 139.9, 140.2, 171.9; IR (NaCl) ν_{max}= 3337, 1738 cm^{-1}; MS (ESI):*m/z*:460.2 $[M+1]^+$, 482.1 [M+23]; elemental analysis calcd (%) for $C_{23}H_{21}BrClNO_2$: C, 60.21; H, 4.61; N, 3.05; found C, 60.00; H, 4.69; N, 2.89.

2*S**,3*R**-**4e:** mp 102 °C (EtOAc/*i*Pr_2O);^{1}H NMR ($CDCl_3$, 200 MHz):δ= 7.41-7.01 (m, 13H), 4.53, 4.26 (AB system, *J* = 9.9 Hz, 2H), 3.69 (s, 3H), 3.65, 3.49 (AB system, *J* = 13.2 Hz, 2H), 2.17 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 50 MHz):δ= 51.4, 52.5, 54.0, 64.5, 121.4, 126.9, 127.2, 128.3 (x2), 128.5 (x2), 128.8, 129.8, 129.9(x2), 130.0, 131.4 (x2), 134.1, 134.4, 139.0, 140.1, 173.1; IR (NaCl)

ν_{max} = 3304, 1736 cm^{-1}; MS (ESI):*m/z*:460.1 $[M+1]^+$;elemental analysis calcd (%) for $C_{23}H_{21}BrClNO_2$: C, 60.21; H, 4.61; N, 3.05; found C, 59.97; H, 4.71; N, 2.85.

Methyl 2S*,3R*-3-(benzylamino)-2-(2,6-dichlorophenyl)-3-phenyl-propanoate (4h).

T = -78 °C; t = 3h. Column chormatography (cyclohexane/ethyl acetate, 30:1): 76%. Mp 121 °C (Et_2O/n-hexane); ^1H NMR ($CDCl_3$, 200 MHz):δ= 7.38-7.14 (m, 7H), 7.13-7.08 (m, 5H), 6.92 (t, *J* = 8.0 Hz, 1H), 4.87 (s, 2H), 3.70 (s, 3H), 3.64 (s, 2H), 3.25 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 50 MHz):δ= 52.2, 52.9, 53.9, 62.2, 127.2, 127.8, 128.0 (x2), 128.5 (x2), 128.7, 128.8, 128.9, 134.9, 135.6, 136.6, 139.8, 141.0, 173.2; IR (neat) ν_{max}= 3418, 1740, 1038 cm^{-1}; elemental analysis calcd (%) for $C_{23}H_{21}Cl_2NO_2$: C, 66.67; H, 5.11; N, 3.38; found C, 66.50; H, 5.25; N, 3.22.

Methyl 3-(benzylamino)-2-(2-methoxyphenyl)-(4-bromophenyl)-propanoates (3i, 4i).

T = -78 °C; t = 80 min. Column chromatography (hexane/ethyl acetate, 25:1): **3i** (34%) and **4i** (39%).

2*S**,3*S**-**3i:** mp 71 °C (MeOH);^1H NMR ($CDCl_3$, 300 MHz):δ= 7.48 (dd, *J*= 8.4, 1.8 Hz, 2H), 7.38-7.22 (m, 7H), 7.02-6.90 (m, 4H), 4.48, 4.22 (AB system, *J* = 9.6 Hz, 2H), 3.80 (s, 3H), 3.58, 3.32 (AB system, *J* = 13.8 Hz, 2H), 3.42 (s, 3H), 1.29 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 75 MHz):δ= 50.7, 51.1, 52.1, 56.1, 63.2, 111.4, 121.3, 121.6, 124.4, 127.2, 128.3 (x2), 128.6 (x2), 129.2, 129.3, 130.6 (x2), 131.7 (x2), 140.4, 141.1, 158.1, 172.9; IR (KBr) ν_{max}= 3337, 1732 cm^{-1}; MS (ESI+):*m/z*:478.1 $[M+Na]^+$, 456.1 $[M+1]^+$; elemental analysis calcd (%) for $C_{24}H_{24}BrNO_3$: C, 63.44; H, 5.32; N, 3.08; found: C, 63.12; H, 5.55; N, 2.97.

2*S**,3*R**-**4i:** oil; ^1H NMR ($CDCl_3$, 300 MHz):δ= 7.35-7.22 (m, 8H), 7.15-7.10 (m, 1H), 7.05-6.95 (m, 2H), 6.85-6.79 (m, 1H), 6.63 (d, *J*= 7.6 Hz, 1H), 4.41, 4.22 (AB system, *J* = 9.8 Hz, 2H), 3.69 (s, 3H), 3.63, 3.53 (AB system, *J* = 13.2 Hz, 2H), 3.54 (s, 3H), 2.05 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 75 MHz):δ= 50.8, 51.7, 52.4, 55.7, 64.9, 111.1, 120.9, 121.0, 125.2, 127.2, 128.5 (x2), 128.7 (x2), 128.8, 129.3, 130.2 (x2), 131.1 (x2), 149.9, 140.6, 155.9, 174.2; IR (KBr) ν_{max}= 3342, 1730 cm^{-1}; MS (ESI+):*m/z*:478.1 $[M+Na]^+$, 456.1 $[M+1]^+$; elemental analysis calcd (%) for $C_{24}H_{24}BrNO_3$: C, 63.44; H, 5.32; N, 3.08; found: C, 63.12; H, 5.55; N, 2.97.

Methyl 3-(benzylamino)-2-(2-iodophenyl)-3-(4-bromophenyl)-propanoates (3j, 4j).

T = -78 °C; t = 30 min. Column chormatography (hexane/AcOEt/TEA, 15:1:0.08): **3j** (53%) and **4j** (44%).

2*S**,3*S**-**3j:** mp 69-72 °C (AcOEt/n-hexane); ^{1}H NMR ($CDCl_3$, 200 MHz):δ= 7.93 (dd, *J* 9.9, 0.7 Hz, 2H), 7.54-7.45 (m, 3H), 7.38-7.31 (m 3H), 7.30-7.23 (m, 3H), 7.05-6.98 (m, 3H), 4.50, 4.23 (AB system, *J* = 10.0 Hz, 2H), 3.61, 3.52 (AB system, *J* = 14.0 Hz, 2H), 3.43 (s, 3H), 1.67 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 50 MHz):δ= 50.9, 52.3, 62.3, 64.1, 103.8, 121.9, 127.3, 128.3 (x2), 128.7 (x2), 128.8, 129.2, 129.9, 130.6, 131.9, 139.1, 140.1, 140.3, 172.1; IR (KBr) ν_{max}= 3332, 1732 cm^{-1}; MS (ESI):*m/z*:550.0 $[M]^+$; elemental analysis calcd (%) for $C_{23}H_{21}BrINO_2$: C, 50.21; H, 3.85; N, 2.55; found C, 50.01; H, 3.99; N, 2.49.

2*S**,3*R**-**4j:** mp 86-88 °C (MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz):δ = 7.69 (dd, *J*= 8.0, 1.1 Hz, 1H), 7.49 (dd, *J*= 7.8, 1.5 Hz, 1H), 7.35, 7.12 (AA'XX'system, *J*= 8.4 Hz, 4H), 7.31-7.23 (m, 6H), 6.85 (dd, *J* = 7.5, 1.5 Hz, 1H), 4.47, 4.29 (AB system, *J* = 9.6 Hz, 2H), 3.71 (s, 3H), 3.69, 3.53 (AB system, *J* = 13.2 Hz, 2H), 2.27 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 50 MHz):δ= 51.6, 52.6, 61.5, 65.1, 102.8, 121.6, 127.4, 128.5 (x2), 128.6 (x2), 128.7 (x2), 129.5, 130.3, 131.6, 139.0, 139.3, 140.2, 140.3, 173.3; IR (KBr) = ν_{max} 3433, 1725 cm^{-1}; MS (ESI):*m/z*:550.0 $[M]^+$; elemental analysis calcd (%) for $C_{23}H_{21}BrIO_2$: C, 50.21; H, 3.85; N, 2.55; found: C, 49.98; H, 4.02; N, 2.38.

Methyl 3-(benzylamino)-2-(2-iodophenyl)-3-(4-methoxyphenyl)-propanoates (3k, 4k).

T = -78 °C; t = 1h. Column chormatography (hexane/Et_2O, 7:3): **3k** (45%) and **4k** (31%).

2*S**,3*S**-**3k:** mp 107-109 °C (MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz):δ= 7.92 (dd, *J*= 8.0, 1.1 Hz, 1H), 7.49 (dd, *J*= 7.8, 1.1 Hz, 1H), 7.39, 6.93 (AA'XX', *J*= 8.7Hz, 4H), 7.42-7.30 (m, 1H), 7.30-7.20 (m, 3H), 7.05-6.98 (m, 3H), 4.51, 4.20 (AB system, *J* = 10.0 Hz, 2H), 3.85 (s, 3H), 3.62, 3.32 (AB system, *J* = 14.0 Hz, 2H), 3.40 (s, 3H), 1.61 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 75 MHz):δ= 50.8, 52.2, 55.6, 62.6, 64.1, 103.9, 114.1 (x2), 127.1, 128.4 (x2), 128.6, (x2), 128.9, 129.1, 129.7, 129.8 (x2), 132.9, 139.5, 140.2, 159.5, 172.4; IR (KBr) ν_{max}= 3320, 1729 cm^{-1};elemental analysis calcd (%) for $C_{24}H_{24}INO_3$: C, 57.50; H, 4.83; N, 2.79. Found C, 57.31; H, 5.05; N, 2.48.

2*S**,3*R**-**4k:** mp 81-83 °C (Et_2O/*n*-pentane); ^{1}H NMR ($CDCl_3$, 300 MHz):δ= 7.67 (dd, *J*= 7.9 Hz, 1.0, 1H), 7.49 (d, *J*= 7.6 Hz, 1H), 7.33-7.21 (m, 6H), 7.13, 6.74 (AA'XX', *J*= 8.7, 4H), 6.83 (dt, *J*= 7.8, 1.6 Hz, 1H), 4.47, 4.25 (AB system, *J* = 9.9 Hz, 2H), 3.76 (s, 3H), 3.71 (s, 3H), 3.69, 3.52 (AB system, *J* = 13.3 Hz, 2H), 1.28 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 75 MHz):δ= 51.5, 52.5, 55.5, 61.9, 65.1, 102.8, 113.8 (x2), 127.2, 128,4, 128.5 (x2), 128.6 (x2), 129.2, 129.5 (x2), 129.6, 131.8, 139.5, 140.0, 140.7, 159.1, 173.6; IR (KBr) ν_{max}= 3332, 1730 cm^{-1};elemental analysis calcd (%) for $C_{24}H_{24}INO_3$: C, 57.50; H, 4.83; N, 2.79; found C, 57.31; H, 5.05; N, 2.48.

Methyl 2S*,3S*-3-(benzylamino)-2-(2-fluorophenyl)-3-(3,4-methylenedioxy-phenyl)-2-propanoate (3l)

T = -78 °C; t = 80 min. Column chromatography (hexane/ethyl acetate, 40:1): (91%). Mp 106-108 °C (MeOH/Et_2O); ^{1}H NMR ($CDCl_3$, 300 Mz):δ= 7.55-7.45 (m, 1H), 7.38-6.98 (m, 8H), 6.99 (s, 1H), 6.79, 6.86 (AB system, *J* = 7.9 Hz, 2H), 5.99 (s, 2H), 4.28, 4.18 (AB system, *J* = 9.0 Hz, 2H), 3.62, 3.47 (AB system, *J* = 10.2 Hz, 2H), 3.45 (s, 3H), 1.62 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 75 Mz):δ= 51.0, 51.1, 52.3, 63.3, 101.4, 108.4 (d, *J* 9.4 Hz), 115.9 (d, *J* 22.8 Hz), 122.2, 123.6 (d, *J* 14.4 Hz), 124.8 (d, *J* 3.4 Hz), 127.2, 128.4 (x2), 128.6 (x2), 129.6, 129.7 (d, *J* 13.4 Hz), 135.1, 140.4, 147.4, 148.2, 161.5 (d, *J* 246.7 Hz) 172.1; ^{19}F NMR ($CDCl_3$, Mz 282.4):δ= -117.63; IR (KBr) ν_{max}= 3461, 1738 cm^{-1}; MS (ESI+):*m/z*:408.1 $[M+1]^+$; elemental analysis calcd (%) for $C_{24}H_{22}FNO_4$: C, 70.75; H, 5.44; N, 3.44; found: C, 70.50; H, 5.58; N, 3.31.

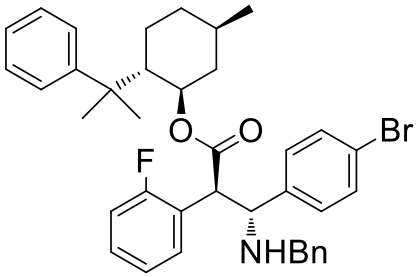
Methyl 2S*,3R*-3-(benzylamino)-2-(2,6-dichlorophenyl)-3-(4-nitro-phenyl)-propanoate (4m).

T = -20 °C; t = 24h. Column chromatography (hexane/ethyl acetate/TEA, 10:1:0.01): 85%. Mp 114-116 °C (AcOEt/*n*-hexane); ^{1}H NMR ($CDCl_3$, 200 MHz):δ= 7.96-7.94 (m, 2H), 7.51-7.49 (m, 2H), 7.34-6.92 (m, 8H), 4.92, 4.79 (AB system, *J* = 6.3 Hz, 2H), 3.69 (s, 3H), 3.58, 3.54 (AB system, *J* = 8.6 Hz, 2H), 3.05 (bs, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 51.9, 52.7, 52.8, 61.2, 122.9, 127.1-129.1 (x14), 133.5, 139.7, 145.4, 172.2; IR (nujol) ν_{max}= 3434, 1740 cm^{-1}; MS (ESI+):*m/z*:459.2 $[M+1]^+$; elemental analysis calcd (%) for $C_{23}H_{20}Cl_2N_2O_4$: C, 60.14; H, 4.39; N, 6.10; found C, 59.89; H, 4.53; N, 4.10.

Methyl 2S*,3R*-3-(benzylamino)-2-(2,6-dichlorophenyl)-3-(4-bromo-phenyl)-propanoate (4n).

T = -20 °C; t = 24h. Column chromatography (hexane/ethyl acetate/TEA, 9:1:0.01): 79%. Yellow wax; ^{1}H NMR ($CDCl_3$, 200 MHz):δ= 7.37-6.90 (m, 12H), 4.81 (s, 2H), 3.57 (s, 2H), 3.67 (s, 3H), 1.90 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 50 MHz):δ= 51.8, 52.6, 53.1, 61.2, 121.3, 126.9-130.8 (x14), 134.1, 138.5, 140.1, 171.6; MS (ESI):*m/z*:494.0 [M+1], 515.9 [M+23]; IR (neat) ν_{max}= 3421, 1649, 1073 cm^{-1}; elemental analysis calcd (%) for $C_{23}H_{20}BrCl_2NO_2$: C, 56.01; H, 4.09; N, 2.84; found C, 55.79; H, 4.24; N, 2.61.

(-)-Phenylmentyl 3-(benzylamino)-3-(4-bromophenyl)-2-(2-fluorophenyl)-propanoates (3o).

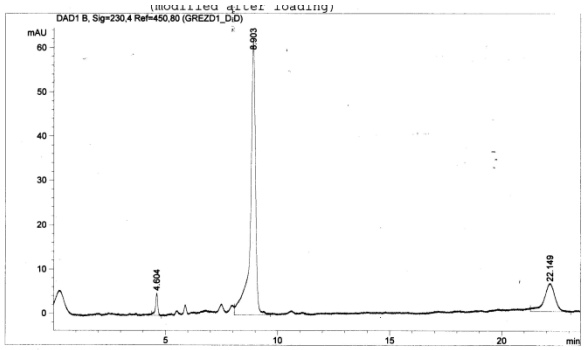


T = 0 °C; t = 90 min. Column chormatography (hexane/ethyl acetate, 40:1 to 30:1). HPLC analysis: ASCENTIS Si column (3 μm, 150 x 4.6 mm; flow rate: 1 mL/min.); mobile phases: *n*hexane/*i*PrOH: 99:1.

2*R*,3*R*-**3o:** 77%.$[\alpha]^{25}_{D}$ +56.7 (*c* 10 mg/mL, $CHCl_3$);mp 109 °C (MeOH/H_2O); ^{1}H NMR ($CDCl_3$, 300 Mz):δ= 7.48, 7.31 (AA'XX', *J*= 8.4 Hz, 4H), 7.33-7.00 (m, 14H), 4.65-4.55 (m, 1H), 4.26, 3.76 (AM system, *J*= 9.0 Hz, 2H), 3.58, 3.36 (AB system, *J* = 13.7 Hz, 2H), 1.85-1.70 (m, 1H), 1.65-1.55 (m, 1H), 1.53-1.42 (m, 1H), 1.40-1.25 (m, 2H + 1H exch.), 1.03 (s, 3H), 0.97 (s, 3H), 0.95-0.85 (m, 1H), 0.75 (d, *J*= 6.4 Hz, 3H), 0.72-0.63 (m, 1H), 0.65-0.55 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz):δ= 22.1, 25.6, 27.3, 27.7, 31.6, 34.8, 40.1, 40.8, 50.3, 51.2, 51.5, 62.4, 75.8, 115.9 (d, *J* 22.8 H), 121.8, 123.2 (d, *J* 14.9 Hz), 124.6 (d, *J* 3.6 Hz), 125.5, 125.9 (x2), 127.2, 128.3 (x4), 128.6 (x2), 129.6 (d, *J* 8.2 Hz), 130.0 (d,*J* 3.3 Hz), 130.9 (x2), 131.7 (x2), 140.4, 140.7, 151.4, 161.5 (d, *J* 247.2 Hz), 171.0; IR (KBr) ν_{max} = 3461, 1738 cm^{-1}; MS (ESI+):*m/z*:644.2$[M+1]^+$;elemental analysis calcd (%) for $C_{38}H_{41}BrFNO_3$: C, 71.02; H, 6.43; N, 2.18; found: C, 70.89; H, 6.59; N, 2.00.

Second crop: 14 mg (from 0.45 mmol of ester **1o**: mixture of a second diastereoisomer and of the dimer **5o** of starting ester (70:30; unseparable compounds);^{1}H NMR ($CDCl_3$, 300 Mz):δ= 7.45, (d, *J*= 8.3, 2H), 7.33-7.00 (m, 16H), 4.88-4.78 (m, 1H), 4.17, 3.71 (AM system, *J* = 9.6 Hz, 2H), 3.55, 3.32 (AB system, *J* = 13.6 Hz, 2H), 2.05-1.90 (m, 3H), 1.70-1.60 (m, 2H), 1.50-1.43 (m, 1H), 1.40-1.30 (m, 1H + 1H exch.), 1.18 (s, 3H), 1.16 (s, 3H), 1.15-1.05 (m, 1H), 0.88 (d, *J*= 6.4 Hz, 3H), 0.72-0.63 (m, 1H), 0.65-0.55 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz):δ= 22.2, 26.0, 27.1, 27.7, 31.6, 34.9, 40.1, 41.4, 50.5, 51.0, 51.3, 62.4, 77.0, 115.8 (d, *J* 22.0 Hz), 121.8, 122.2 (d, *J* 14.9 Hz), 124.5 (d, *J* 3.4 Hz), 125.3-125.9 (x3), 127.2, 128.3-128.6 (x6), 129.5 (d, *J* 8.2 Hz), 130.0 (d,*J* 3.3 Hz), 130.9 (x2), 131.7 (x2), 140.2, 140.7, 151.4, 161.1 (d, *J* 247.5 Hz), 170.8. Dimer of **1o**,significative signals:δ= 4.55-4.45 (m, 1H), 4.4 (s, 1H).

HPLC spectra for the reaction of 1o and 2c



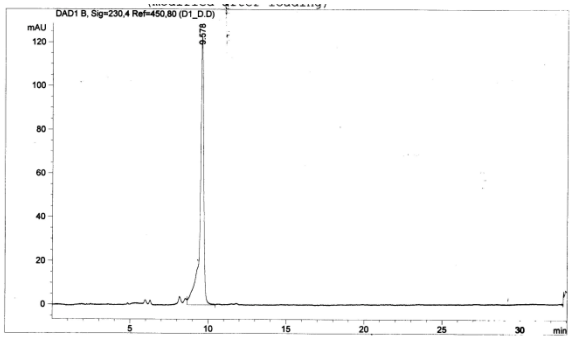
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 B, Sig=230,4 Ref=450,80.

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.604	BP	0.1123	36.22718	5.01133	2.9153
2	8.903	VB	0.2367	993.64343	61.43357	79.9612
3	22.149	BP	0.5039	212.78564	6.20828	17.1235
Totals :				1242.65626	72.65318	

Figure E1. HPLC of the crude reaction mixture between **1o** with **2c**.



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 B, Sig=230,4 Ref=450,80

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.578	VV	0.2405	2015.51025	122.17067	100.0000
Totals :				2015.51025	122.17067	

Figure E2. HPLC of pure *syn*-**3o**.

Single crystal X-ray crystallography of 3o

Colourless, anhydrous, $C_{38}H_{41}BrFNO_2$, hexagonal, crystal size 0.20 × 0.10 × 0.08 mm, $a = b = 22.127(3)$ Å, $c = 6.0021(12)$Å, $V = 2545.0(7)$ Å^3, $Z = 3$, reflections collected 50647, reflections unique 6577, final R indices for 2059 reflections with $I>2\sigma(I)$ R1 = 0.0601, $wR^2 = 0.1049$, completeness to 2θ (25.96) 99.0%. The asymmetric unit includes one moleculeand it crystallizes in the chiral space group $P3_1$. Along the *c* axis a C-H···F intermolecular hydrogen bond (HB) occurs, connecting the parent molecule with the molecule in x, y, z-1. The H28A···F1 distance amounts to 2.517(7) Åand the angle C28-H28A···F1 is as large as 154.2(7)°. Two C-H···O and one N-H···O intermolecular HBs are also present. The data collection was performed ona Bruker Smart Apex CCD diffractometer with graphite-monochromatedMo Ka radiation λ=0.71073 Å at T = 180 K. The structure was solvedby direct methods using the program SHELXS-97.[lxxii] Refinementmethod was full-matrix least-squares on F^2, and goodness-of-fit onF^2 is 0.871. Crystallographic data (excluding structure factors) for the **3o** structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC949330.

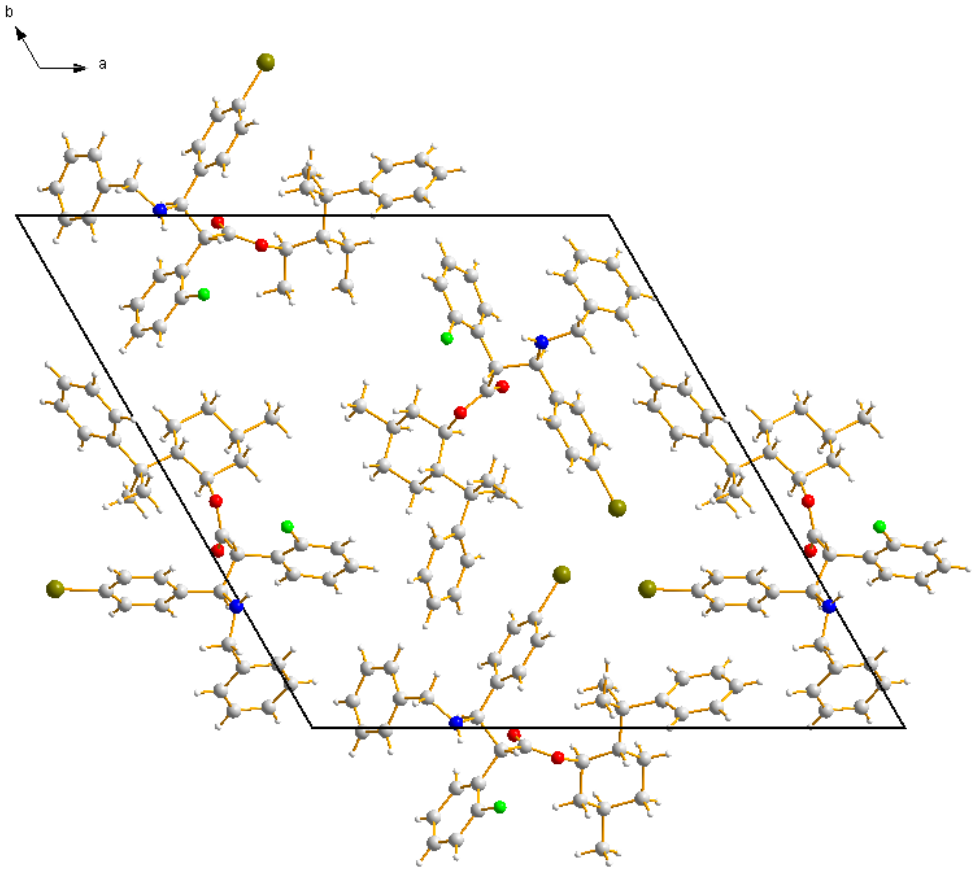


Figure E5. Packing diagram of compound **3o** viewed down the *c* axis.

Mixture of dimeric compounds 5d of ester 1d: 2.5:1 mixture.

IR (KBr) ν_{max} 1734 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (s, 3 H), 3.77 (s, 3 H), 3.71 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 51.4, 52.9, 56.3, 56.42, 111.4, 115.5, 115.7, 127.0, 148.7, 149.1. Minor dimer:δ 7.06 (s, 2 H), 7.01 (s, 2 H), 4.95 (s, 2H), 3.86 (s, 6 H), 3.54 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 52.5, 52.6, 56.46, 56.5, 112.4, 115.8, 116.4, 127.4, 148.9, 149.5; MS (ESI+) *m/z* 599.3 $[M+23]^+$.

2-Phenylethyl 2S*,3R*-3-(benzylamino)-3-(4-bromophenyl)-2-(2-iodophenyl)-thiopropionate (9a).

T = 0 °C; t = 2.40h. Column chromatography (hexane/AcOEt, 30:1): 80%. Mp 117 °C (AcOEt/n-hexane); ^{1}H NMR ($CDCl_3$, 300 MHz):δ= 7.68, (d, *J*= 7.9 Hz, 1H), 7.47 (dd, *J*= 7.8 Hz, 2.1, 1H), 7.30, 7.08 (AA'XX' system, *J*= 8.3 Hz, 4H), 7.28-7.05 (m, 6H), 6.85 (dt, *J*= 7.9, 2.0 Hz, 1H), 4.65, 4.38 (AB system, *J* = 9.8 Hz, 2H), 3.64, 3.50 (AB system, *J* = 13.3 Hz, 2H), 3.25-3.05 (m, 2H), 2.86 (t, *J*= 7.4 Hz, 2H), 1.98 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 75 MHz): δ= 31.2, 36.0, 51.6, 65.4, 69.5, 103.3, 121.6, 126.9, 127.4, 128.4 (x2), 128.6, 128.7 (x2), 128.8 (x2), 129.0 (x2), 129.4, 129.5, 130.3 (x2), 131.5 (x2), 138.5, 138.9, 140.1, 140.2, 199.1; IR (KBr) ν_{max}= 3436, 1674, 1638 cm^{-1}; MS (ESI):*m/z*:658.1 $[M+1]^+$; elemental analysis calcd (%) for $C_{30}H_{27}BrINOS$: C, 54.89; H, 4.15; N, 2.13; found C, 54.70; H, 4.23; N, 2.01.

Benzyl 2S*,3R*-3-(benzylamino)-2-(4-bromophenyl)-3-(2-fluorophenyl)-thiopropanoate (9b).

T = 0 °C; t = 6 h. Column chromatography (*n*hexane): 83% (yield was given on reacted ester. 25% of recovered starting thioester **8b** was isolated).Mp 129 °C (MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz):δ= 7.38-6.75 (m, 13 H), 6.78 (dt, *J*= 8.1, 1.1 Hz, 1H), 4.65, 4.41 (AB system, *J* = 11.4 Hz, 2H), 4.26, 4.07 (AB system, *J* = 17.7 Hz, 2H), 3.58, 3.48 (AB system, *J* = 13.2 Hz, 2H), 1.43 (s, 1H, exch.); ^{13}C NMR ($CDCl_3$, 50 MHz):δ = 34.0, 36.6, 50.5, 55.8, 115.6 (d, *J* = 22.5 Hz), 121.2, 123.1 (d, *J* = 14.1), 124.4 (d, *J* = 3.4 Hz), 127.4 (d, *J* = 8.4 Hz), 128.6 (x2),128.8 (x2), 129.0 (x2), 129.3 (x2), 129.5, 129.7, 130.2 (x2), 131.5 (x2), 132.0 (d, *J* = 2.7 Hz), 137.0, 137.3, 138.6, 160.4 (d, *J*= 240Hz),196.8; IR (KBr) ν_{max}= 3370, 1682 cm^{-1}; elemental analysis calcd (%) for $C_{29}H_{25}BrFNOS$: C, 65.17; H, 4.71; N, 2.62; found C, 65.06; H, 4.83; N, 2.53

References for Experimental section.

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Chapter 3 – 2,3-Diaryl-β-amino acid for the preparation of α,β Foldamers and Nanomaterials

3. Introduction

The preparation of synthetic polymers containing β-aminoacids (β-AAs) is of great interest as documented by recent literature.[1] Different applications such as gelating agent, or nanostructured materials inducers, have been recently reported for these kinds of foldamers[2].Construction of well-defined nanostructures from the self-assembly of small organic molecules has gained increasing attention, with the development of a broad range of application, starting from catalysis to elettrochemistry, biology and nanomedicine.

As reported in Chapter 1, few example of α,β-peptides are reported in the literature, the majority of them containing cyclic amino acids. A couple of paper reported on the preparation and conformational study using α,β^3-sequences and only a single example report on α,$\beta^{2,3}$-sequences containing the helicogenic Aib, a α,α-substituted amino acids. As reported above, the conformation of the $\beta^{2,3}$- oligomeric peptide is connected with the stereochemistry of the amino acid. The *syn*-$\beta^{2,3}$-amino acid, preferring a trans conformation, favors extended conformations and the *anti*-amino acid, preferring a gauche conformation, favors helix contents. In the above examples 9/11-helix is reported usingβ^3-amino acids and an 11- helix is reported using a $\beta^{2,3}$-amino acid characterized by *S,S*- configuration.

As showed in chapter 2 we have recently reported on the diastreoselective synthesis of a series of *syn*-*S**,*S**-$\beta^{2,3}$-diarylamino acids that represent a new class of amino acids to be used in peptide synthesis.Considering the paucity in literature of α,$\beta^{2,3}$-peptide sequences, we prepareddifferent α,$\beta^{2,3}$-peptides models and we studiedthe ability of our*syn*-$\beta^{2,3}$-diarylamino acid to induce secondary structures. Amino acid **1** was selected as model both for the presence aryl groups, which could induce the conformation stabilization throughπ-stacking, and the presence of fluorine making possible the formation of further H-bonds.

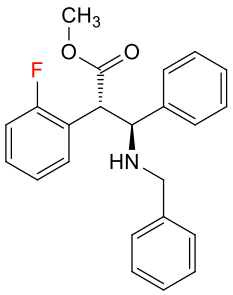


Figure 1. (2*S*,3*S*)-methyl-3-(benzylamino)-2-(2-fluorophenyl)-3-phenyl-propanoate (**1**)

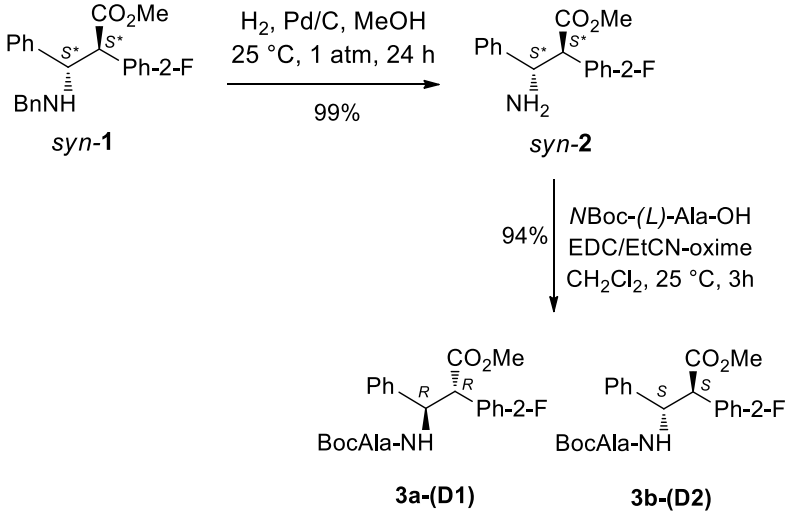
Since it is not possible *a priori* to predict with is the correct stereochemistry to be combined with a *L*-α-amino acid affording a peptide characterized by a stable conformation, here we report on the preparation of α,$\beta^{2,3}$-peptide sequences containing *L*-Ala alternated with both *R,R*-$\beta^{2,3}$- and *S,S*-$\beta^{2,3}$-diarylamino acids. Di- tetra and hexa-α,$\beta^{2,3}$-peptides were prepared for both series and their conformation was studied by using NMR, CD and UV analyses.

Interestingly,dipeptide containing *L*-Ala and the new *S,S*-$\beta^{2,3}$-diarylamino acid **1** with a fluorine substituent on the β^2-phenyl group self-assembled allowing the preparation of nanotubes.

3.1 Result and Discussion

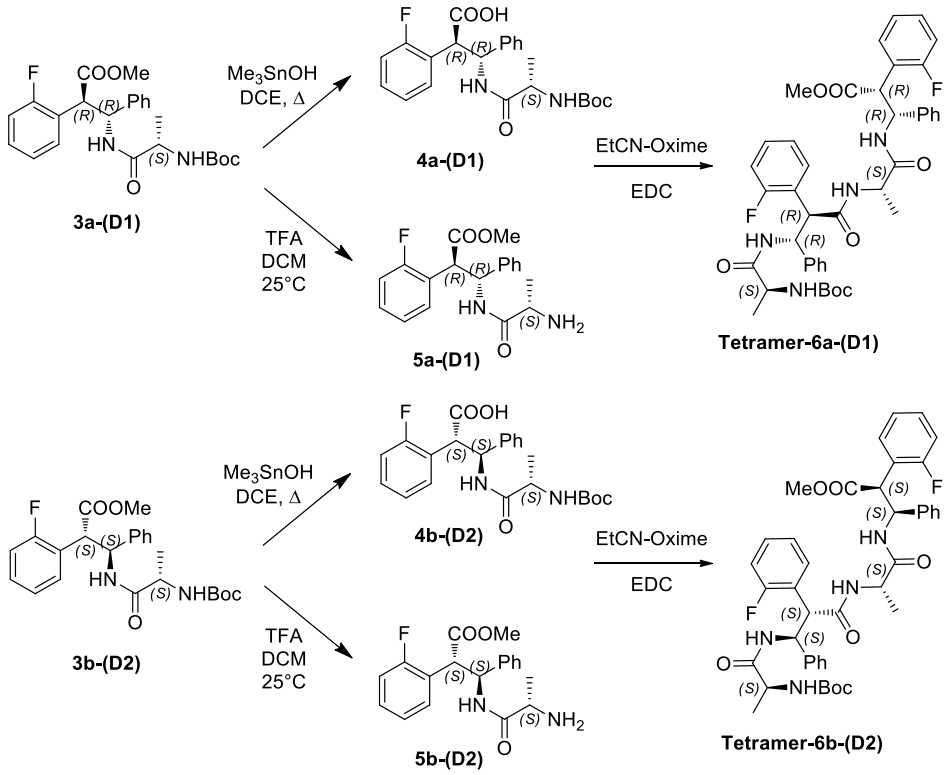
3.1.1 Chemistry

The two dipeptides **3a-D1** and **3b-D2** were efficiently synthesized in gram scale from racemic *syn*-**1** (Scheme 1). Amino acid **1** was first deprotected at nitrogen atom by catalytic reduction (H_2, Pd/C, MeOH, 25 °C, 1 atm, 24 h) affording *S*,S**-$\beta^{2,3}$-diarylamino acids **2** in quantitative yield. The condensation reaction of **2**with *N*Boc-(*L*)-AlaOHwas studied following different reaction conditions. HOBT(1.1 equiv.)/EDC(1.1 equiv.) in CH_2Cl_2 (25 °C, 8h) gave diastereoisomer **3a-(D1)** and **3b-(D2)** in 66% overall yield. The yield increased to 80% using HOAT/(1.1 equiv.)/EDC(1.1 equiv.) (CH_2Cl_2, 25 °C, 8h).The best result (94%) was achieved using EDC(1.1 equiv.)/EtCN-oxime/(1.1 equiv.) (CH_2Cl_2, 25 °C, 3h).The couple of diastereoisomers **3a-(D1)** and **3b-(D2)** were easily separated by column chromatography on silica gel (Scheme 1).



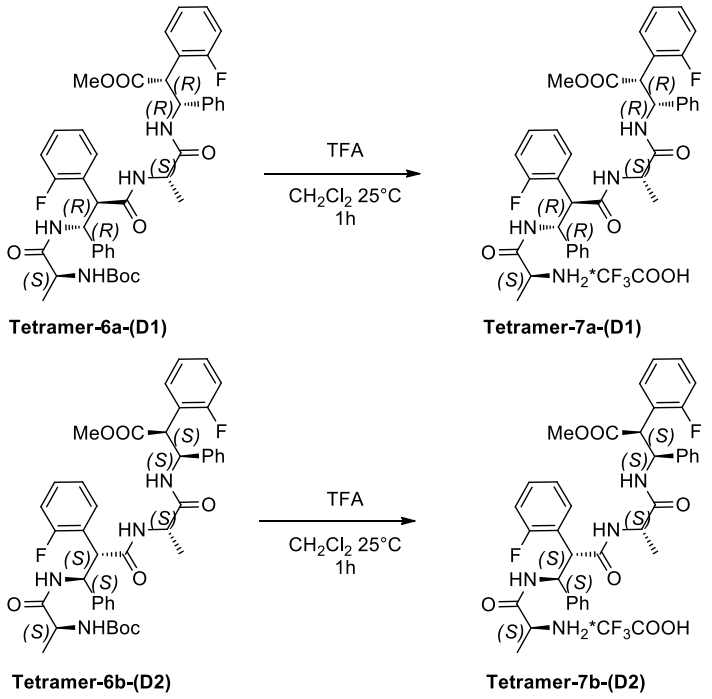
Scheme 1. Preparation of Dipeptides **3a-(D1)** and **3b-(D2)**

The deprotection of carboxyl group on dipeptides **3a-(D1)** and **3b-(D2)**with LiOH or KOH gave some problems since a partial racemization was observed for the alanine stereocentre even when operating at low temperatures. On the other hand, the use of trimethyl-tin-hydroxyde (8 equiv.; DCE, 80°C, 24h) gave compounds **4a-(D1)** (76%) and **4b-(D2)** (69%) when starting from **3a-(D1)** and **3b-(D2)**, respectively (Scheme 2).The deprotection of nitrogen atom on dipeptides **3a-(D1)** and **3b-(D2)** was easily performed using TFA in CH_2Cl_2 at room temperature for 1h, affording **5a-(D1)** and **5b-(D2)** in quantitative yields. By coupling dipeptide **4a-(D1)** with **5a-(D1)** in CH_2Cl_2/DMF (10:1) as solvent and using EDC(1.1 equiv.)/EtCN-oxime(1.1 equiv.) as condensing agents (25°C, 24h), tetrapeptide **6a-(D1)** was isolated in 74% yield after purification on silica gel. Tetrapeptide **6b-(D2)** (76%) was obtained in an analogue way starting from **4b-(D2)** and **5b-(D2)** (Scheme 2).



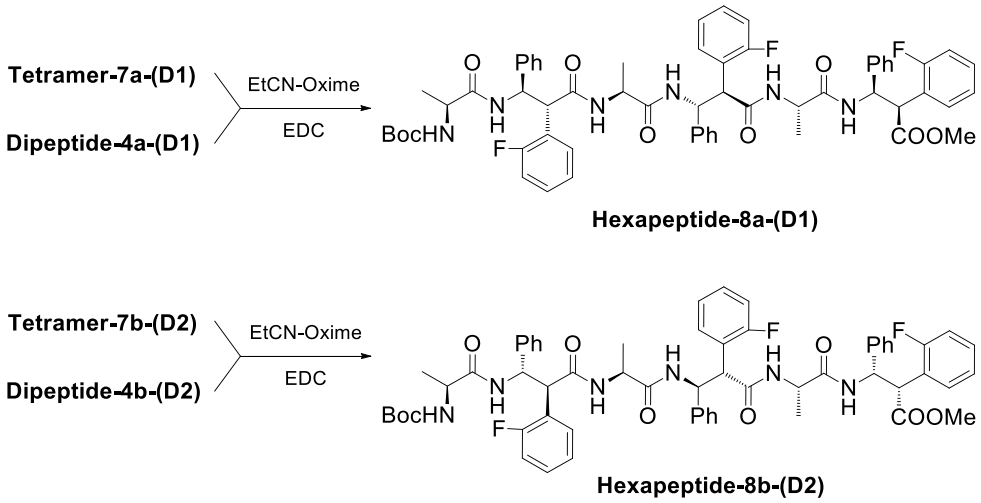
Scheme 2. Synthesis of Tetramers **6a-(D1)** and **6b-(D2)**

Operating with an analogous protocol as described above for nitrogen deprotection, peptides **6** were transformed into**7a-(D1)** and **7b-(D2)** (quantitative yields),obtained as trifluoroacetic salts.(Scheme 3)



Scheme 3. Preparation on *N*-deprotected peptides **7a-(D1)** and **7b-(D2)**

By coupling **7a-(D1)** with **4a-(D1)** and **7b-(D2)** with **4b-(D2)**, adopting the above protocol, the expected hexapeptides **8a-(D1)** (55 %) and **8b-(D2)** (62 %) were obtained and purified by column chromatography (Scheme 4).



Scheme 4. Synthesis of hexapeptides**8a-(D1)** and **8b-(D2)**

Any attempt to merge two tetrameric residues, in order to obtain octamers of both series **(D1)** and **(D2),** gave poor results in terms of yield and the products are difficult to purified with conventional methods probably due to the strong lipophilicity.

3.2 Dipeptides 3a-(D1) and 3b-(D2) characterization.

3.2.1 Solid state (X-ray analysis).

Dipeptide **3b-D2** crystals suitable for X-ray analysis, were obtained from a slow evaporation of a solution acetonitrile/water (1:1). Despite numerous attempts, we were unable to grow any good single crystal from dipeptide **3a-D1** solutions.

3a-D2 crystallizes in the space group *R3* and the structure was obtained without disorder and with a single molecule in the asymmetric unit. The unit cell contains 9 molecules, showing chains around the helicoidal 3_1 axes. The conformation of the dipeptide and the arbitrary numbering scheme are shown in Figure2. First, the crystallographic analysis provided the establishment of the stereochemistry of atoms C7(*S*) and C8(*S*) of the beta residue. From a geometrical point of view, the backbone adopts a curled conformation, with the Boc protecting group bended with respect to the plane of the beta-peptide link, as shown by the torsion angles N2-C17-C18-N1 of -33(1)° and C17-C18-N1-C20 of -69(1)°. The alanine side chain protrudes on the same side of the fluorine substituted ring, but it is opposite oriented with respect to the C1-C6 phenyl moiety. The conformation of the molecule shows that the two aromatic rings are nearly parallel, forming a dihedral angle of 11(1)°. The two peptidelinks are in *trans* configuration and show a slight deviation from planarity, as indicated by the torsion angles C7-N2-C17-C18 of 176(1)° and C18-N1-C20-O1 of 172(1)°.

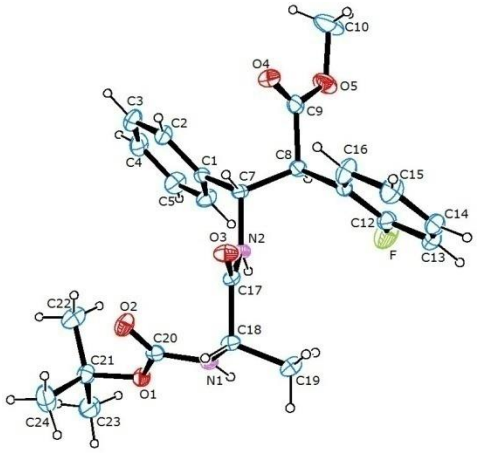


Figure2.ORTEP view of the compound **3b-D2** showing atom-labeling scheme. Displacement are at the 40% probability level.

In the solid state, the strategic position of the diaryl moiety did not lead to stacking interactions able to influence the peptide conformation. The fluorine placed in order to direct the conformation of the beta-peptide bond did not gave an intramolecular hydrogen bond, but only contributed to enforce the molecular packing through the contact O5-H5...F^{I} (symmetry code = ^{I} at -y+2/3+1, x-y+1/3,

z+1/3), distance 3.003(3)Å, angle 153(1)°.

The strongest hydrogen bond in this crystal structure is intermolecular, between the peptidic carbonyl group and the amidic hydrogen of a neighbor molecule: N1-H...O3^{II} (^{II} at x,+y,+z-1), 2.200(9) Å, angle 147(1)°, leading to chains of hydrogen-bonded molecules along the *c*axis (Figure3).

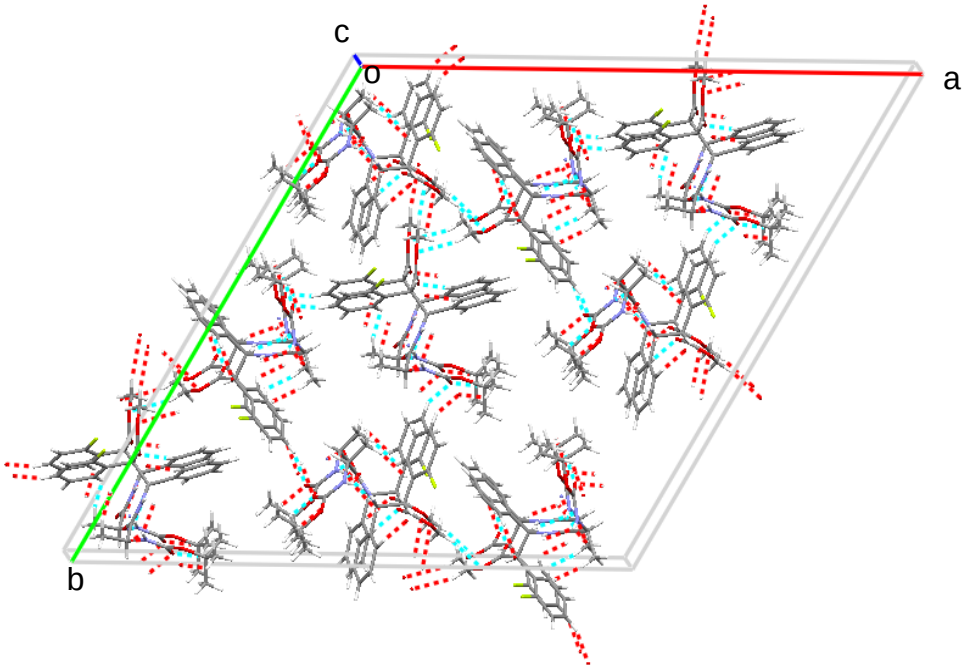


Figure3. Crystal packing viewed about along the *c* axis. Intermolecular interactions are represented as dashed lines.

These molecular chains are consolidated by C6-H...O4^{II}, 2.394(9)Å, angle 173(1)°, and N2-H...O4^{II}, 2.742(9) Å, angle 149(1)° interactions. In the crystal packing, weak contacts are also present between C13-H...O2^{III}, C14-H...O2^{III} and C14-H...O3^{III} (^{III} at -x+y+1,-x+2,+z), as depicted in Figure 4.

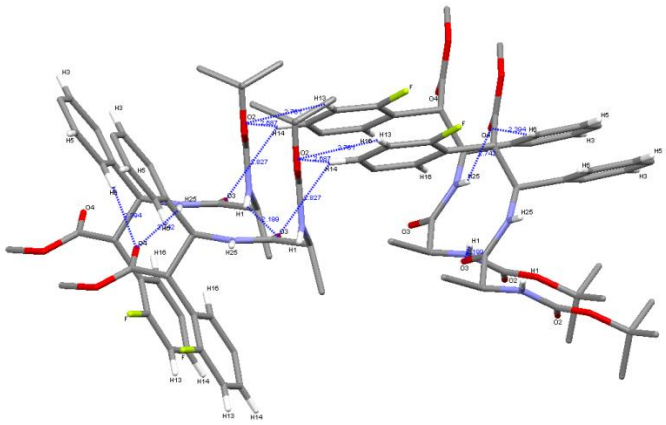


Figure 4. Intermolecular hydrogen bonds interactions of **3b-D2** (only the hydrogens involved are shown in sake of clarity).

3.2.2 In solution characterization

Pipeptides**3a-(D1)** and **3b-(D2)** were fully characterized in solution (UV, CD, NMR).

3.2.2.1 UV and CD analysis

Two peaks are present at 218 and 260 nm in the far UV spectrum both for**3a-(D1)** and **3b-(D2)**, whichare typical of phenyl absorption (Figure E1 experimental section).

The CD signature of **3a-(D1)**in H_2O/TFE (1:1) solution showed a negative band below 190 nm (π–π* transition) and two positive bands at around 197 nm (π–π∗ transition) and 215 nm (n-π* transition), suggesting a probable turn like structure in solution.[3]**3b-(D2)** spectrum was characterized by the same Cotton effect but with opposite sign and slightly lesser intensity (Figure 6). Considering that in both dipeptides alanine possesses the *S*-stereochemistry, this result indicates that in solution the adopted conformation is driven by the stereochemistry of the unnatural residue (*R,R*for **3a-D1** and *S,S* for **3b-D2**). The spectra where recorded at different concentration (from 1mM to 50 μM) without significant changes, indicating that at these concentrations no aggregation occurred.

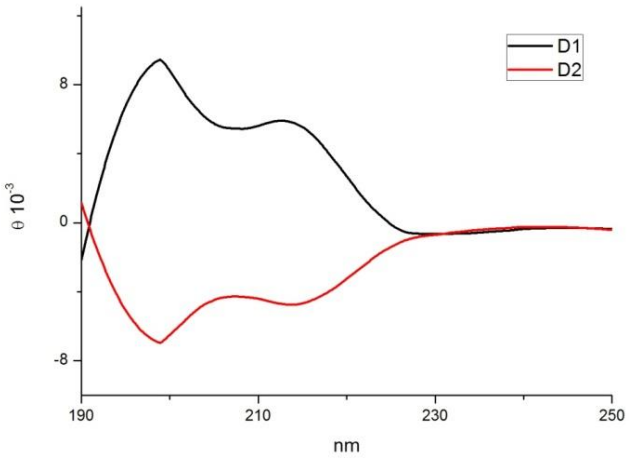


Figure 6. CD Spectra for Dipeptides **3a-(D1)** and **3b-(D2)**

3.2.2.2 NMR characterization.

Dipeptides **3a-D1** and **3b-D2** were fully characterized by NMR ($CDCl_3$, 500 MHz). Only slight differences on the chemical shifts as well as on the N.o.e.s were observed (Figure 7). It is well known that the substituent effect is of relevance on the local conformation of a β-aminoacid. Concerning $\beta^{2,3}$amino acids, it is reported that the *syn* stereoisomer strongly favours the *trans* conformation.[4] As expected, and according to $^3J_{C\beta H\text{-}C\alpha H}$ values (10 Hz), the β-amino acid in both dipeptides is characterized by a *trans* conformation. This datum is consistent with the conformation of the β-amino acid shown in the crystal of **3b-D2** (see figure 2). Noes between CH_{Ala1} and NH-2 (vs) as well as between NH-1/NH-2 (vw) are observed in **3b-D2**as well as for **3a-D1**.

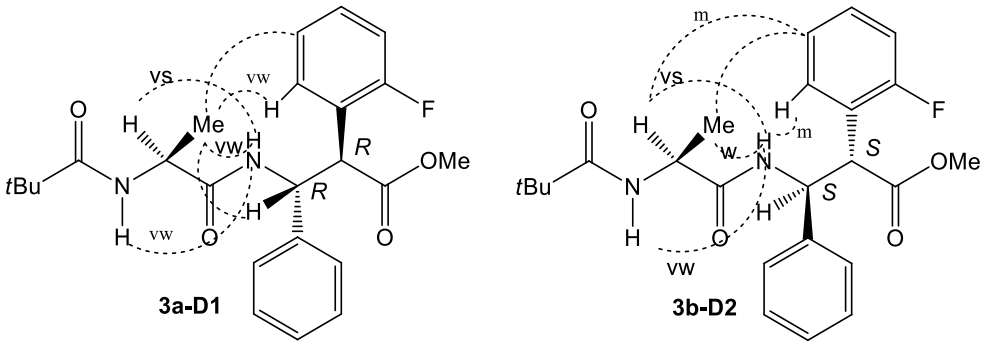


Figure 7. Significant Noesy (300 MH, 10mM in CD_3CN,300 ms) for dipeptides **3a-D1**and **3b-D2.**

The experiment at variable temperature showed Δδ/ΔT values larger of 5 ppb K^{-1} for both NH of **3b-D2** dipeptide. The same experiments were performed on **3a-D1**.Lower Δδ/ΔT values were detected with respect **3b-D2** dipeptide (Ala-1: -4 ppb K^{-1}; beta-2: -3.8 ppb K^{-1}, Figure 8).

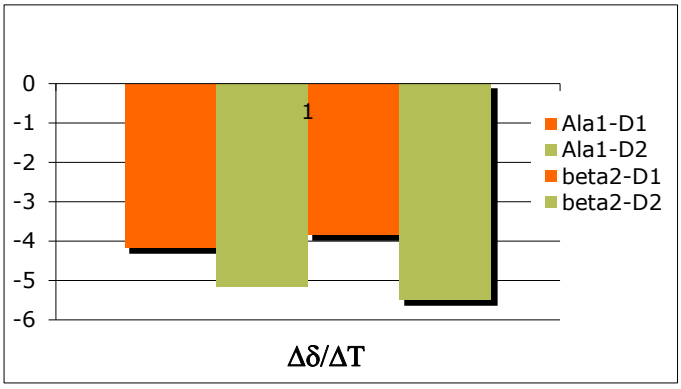


Figure 8. Δδ/ΔT values for dipeptides **3a-D1** and **3b-D2** ($CDCl_3$)

The solvent titration of the NH proton chemical shifts of **3b-D2** and **3a-D1** was also performed indicating similar results to those found at variable temperature. The polar solvent dimethylsulfoxide (DMSO) added to the $CDCl_3$ solution induces a strong downfield shift in the resonances of both NHs (Δδ >1) for **3b-D2**, and Δδ NH larger then 0.7 (Ala-1: 0.74; beta-2: 1.1) for **3a-D1** indicating their solvent exposure (Figure 9).

These data suggest that hydrogen bond are absent for **3b-D2** and **3a-D1** in solution.

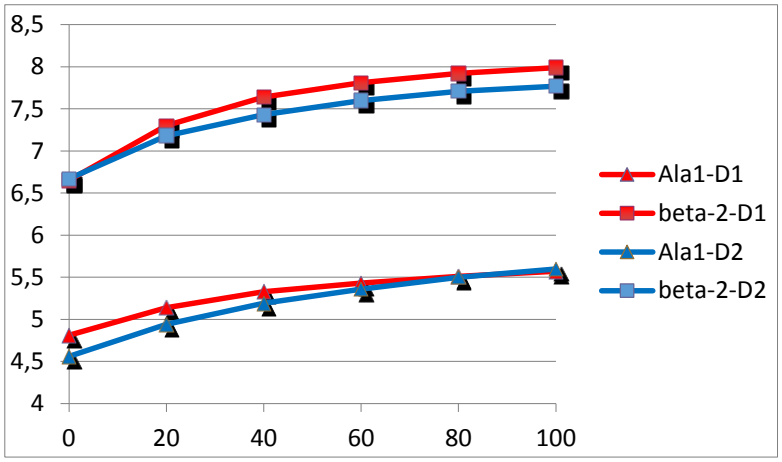


Figure 9. DMSO Titrations of peptides **3a-D1** and **3b-D2** in $CDCl_3$

Taken together, CD and NMR data suggest that in solution both **3a-D1** and **3b-D2** do not possess a preferred conformation, with different contributions of turn and extended structures.

NMR analysis of **3b-D2**does not match with its X-Ray information indicating a different behaviour of this dipeptide in solution and in solid state. In fact, the alanine moiety possesses a different orientation in the crystal. An intermolecular H-bond between NH of alanine of a dipeptide sequence with the carbonyl group of β-AA of a second sequence occurred in the solid state. On the other hand, NH involvement in hydrogen bond was not confirmed in NMR solution studies.

3.3 Self organization studies on Dipeptides 3a-D1 and 3b-D2

In order to obtain sopramolecular structures, self assembly studies on dipeptides **3a-D1** and **3b-D2**were conducted in collaboration with Professor *Reiches* at the *Hebrew University of Jerusalem.*

According with the standard procedure[5], dipeptides **3a-D1** and **3b-D2** were dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (100 mg/ml) and diluted with water up to a concentration of 2 mg/ml. The solutions were stand for 24 hours, and subsequently filtered.The precipitates deriving from **3a-D1** and **3b-D2**solutions,were analyzed using different microscopy techniques. Evidence in the formation of nanotubes are present exclusively for dipeptide **3b-D2** (Figures 10,11, and 12)**.**All analyzes shown the formation of nanotubes with an average external diameter of 140nm.

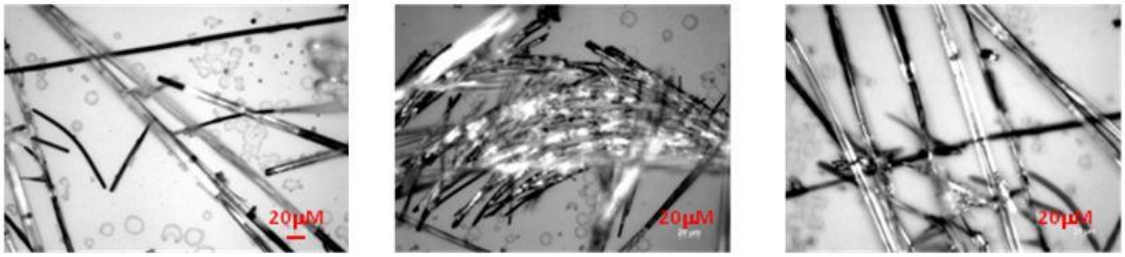


Figure 10. Optical microscopic images with 50X optical zoom for **3b-D2** nanotubes.

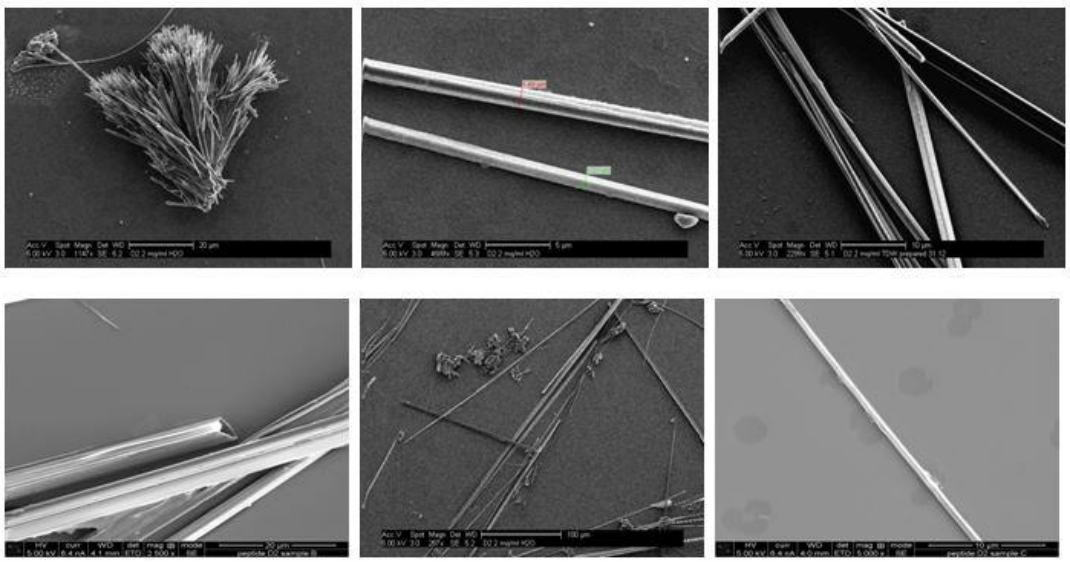


Figure 11. SEM/TEM microscopic images of **3b-D2** nanotubes.

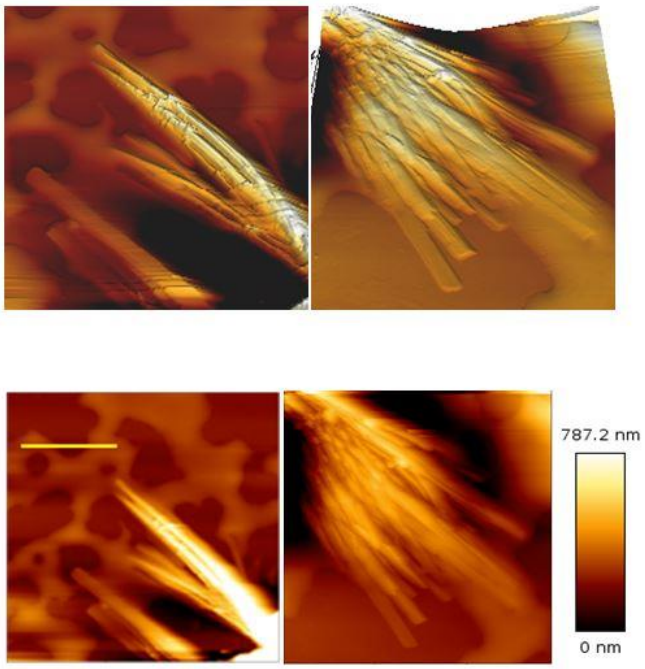


Figure 12. AFM microscopy images of **3b-D2**nanotubes.This technique allowed to determine the height of **3b-D2**tubes.

3.3.1 Proteolytic and Thermal stability of 3b-D2 Nanotubes

Susceptibility to proteolysis has been a major drawback of peptides, especially for *in vivo* delivery applications. The introduction of unnatural amino acids in peptide sequences provides enhanced stability to proteolytic degradation. Considering that our dipetide **3b-D2** contains a non natural β-amino acids (*i.e*methyl 3-amino-2-(2-fluorophenyl)-3-phenylpropanoate), we investigated its stability to highly non-specific protheolytic enzymes Pronase (from *Streptomyces griseus*) and Trypsin (*vertebrates protease*). Different solution of dipeptide **3b-D2,** dissolved in a PBS/DMSO mixture (DMSO 20% *v/v*, PBS), were prepared at different pH (pH = 5, 6, 7, 8) that were incubated with Pronase or Trypsin for 48h at 25 °C and 37°C, respectively. The reaction mixtures were then analyzed to reversed phase HPLC. No significant change in the peak position or the peak area was observed both in absence and in presence of enzymes, indicating that **3b-D2** nanotubesare stable to proteolytic degradation induced by proteases. Furthermore, thetested conditions did not induce the spontaneous hydrolysis of peptide in a large pH range. (Figure E2, E3 experimental part)

The thermal stability of **3b-D2** nanotubes in solution was tested in DMSO-d_6 and in a large range of temperature (30-120°C; Figure E4 experimental section) NMR experiments. The sample wasalso maintained at the 120 °C for 4 hours. No significant structural changes were observed up to 120°C, demonstrating the thermal stability of our nanotubes in solution (Figure E4).

The molecular interaction involved in the self assembly of **3b-D2** were investigated also by fluorescence spectroscopy. For diluted solutions of preformed dipeptide-nanotubes in ethanol the

analysis support the existence of aromatic π–π stacking interaction with typical emission at 290 nm after axcitation at 260 nm (Figure E5).

The efficient synthesis of dipeptides **3b-D2** together with the presence of an unnatural moiety, that gives protheolytic and thermal stability to its nanotubes assemblies make these sopramolecular architectures exploitable in future biomedical applications as drug delivery system.

3.4 NMR Characterization of tetrapeptides and hexapeptides of D1 and D2 series.

All synthesized peptides are fully analysed in $CDCl_3$ by NMR (^1H, ^{13}C, ecc). As general consideration we found that in many cases broads signal were detected both for CH and NH thus avoiding to have clear information on the Noesy experiment. To ascertain an effective Noe, the row is reported confirming our hypothesis. Furthermore, the presence of many aryl groups in some case avoids having clear information in experiments like NH mobility using experiments at variable temperature or DMSO-titration. As a result, only qualitative information are given.

As reported in the introduction (Chapter 1), the stereochemistry of the $\beta^{2,3}$-aminoacids defines the preferred conformation of the amino acid driving the configuration of the peptide. *J* value analysis of each $\beta^{2,3}$-aminoacid help to understand and predict the secondary structure of the peptide. The antiperiplanar arrangement of the corresponding NH/C_β-H and C_α-H/C_β-H are indicative of an extended conformation. On the other hand, a relatively small $J_{C\alpha H/C\beta H}$ is indicative of a *gauche* conformational that induce helical constructs. Considering *J* values reported in the table 1, we can find that an extended conformation was detected for β-amino acid at *C*-termini in all peptides. Instead a different behaviour was found for the internal amino acids depending on the length of the peptide. Focusing on β-amino acid at position two of tetrapeptides, a gauche conformation was detected in both **D1** and **D2** series giving information on the formation of a possible helix construct. A different behaviour was found for the same amino acid for **D1** and **D2** hexapeptides. As shown in the Table 1, a larger $J_{C\alpha\text{-}H/C\beta\text{-}H}$ values was detected for **D1** with respect to **D2** serie indicating an extended conformation for the firth one and a gauche conformation for the second. Finally, β-amino acid at position four in hexapeptide possess an extended conformation in both series.

These data suggests that turn or helixes could be expected for tetra-peptides of each series and a fully extended or partially extended conformation for hexapeptides. Of relevance, the *R,R*-stereosomer seems to be more prone to stabilize the extended conformation with respect the *S,S*-one. Interestingly, analysing Alanine moieties, we found that CH signal is broad, corresponding to a small *J* value, when the alanine is located in a turn region. Instead, defined doublets are present for those residues present in an extended region.

Table 1. *J* values (Hz) for di- tera- and hexapeptides of **D1** and **D2** series.

		D1 serie			**D2 serie**		
	Amino acid	*J*(NH,C_αH)	*J*(NH,C_β-H)	*J*(C_α-H/C_β-H)	*J*(NH, C_α-H)	*J*(NH, C_β-H)	*J*(C_α-H/C_β-H)
Dipept.	Ala-1	6.7	-	-	brs	-	-
	Beta-2	-	10.8	10.9	-	9.9	10.5
Tetra pept.	Ala-1	brs	-	-	brs	-	-
	Beta-2	-	8.2	4.5	-	9.0	4.1
	Ala-3	6.9	-	-	7.2	-	-
	Beta-4	-	9.5	10.7	-	9.4	10.5
Hexapept.	Ala-1	7.0	-		brs	-	
	Beta-2	-	7.5	9.4	-	5.1[a]	6.3
	Ala-3	6.9	-	-	brs	-	-
	Beta-4	-	8.0	10.7	-	6.7	9.8
	Ala-5	6.5	-	-	[b]	-	-
	Beta-6	-	10.6	11.2	-	8.6	9.6

[a]Extimatevalue; [b]Overlapped signals

3.4.1 Tetrapeptides 6a-(D1) and 6b-(D2)

3.4.1.1 NMR Characterization of tetrapeptide6b-(D2)

A complete set of strong CH/NH (i,i+1) NOEs are present in peptide ***6b-(D2)***(Figure 13a).Very weak spatial proximity was found between $CH_{\beta 2}$-3/NH_{Ala3},CH_{Ala1}/NH_{Ala3}, $CH_{\beta 2}$-2/$NH_{\beta 4}$[6].These non-sequential NOEs are the early signs of the presence of a 11-helical structure that typically characterized α,β-peptide sequences.[7] The two last amino acids, placed in the i/(i+2) position, are faced each other on the same side of the helix in agreement with the proposed structure (Figure 13).

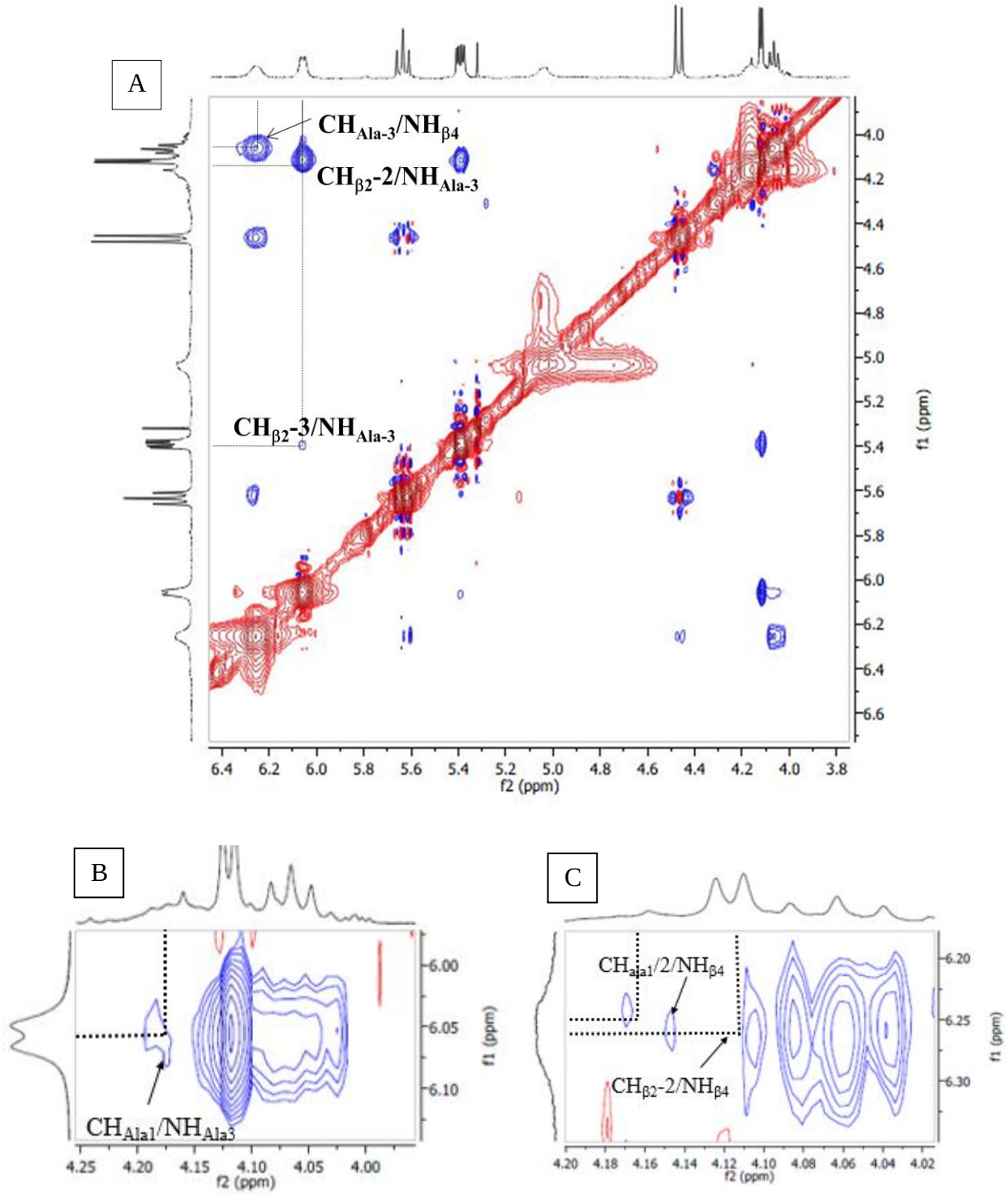


Figure 13. Significant NOEs for 11-Helix structure of **6b-D2** ($CDCl_3$, 10 mM, 450ms)**.** A) complete set of strong CH/NH (i,i+1). B) Zoom of CH_{Ala1}/NH_{Ala3} Noe; C) Zoom of $CH_{\beta2}$-2/$NH_{\beta4}$ and $CH_{Ala1}/NH_{\beta4}$ NOEs.

A further Noe was detected between $NH_{Ala1}/NH_{\beta 2}$ (Figure 14) and $CH_{Ala1}/NH_{\beta 4}$ (Figure 13c), that is in agreement with a 9-helix.

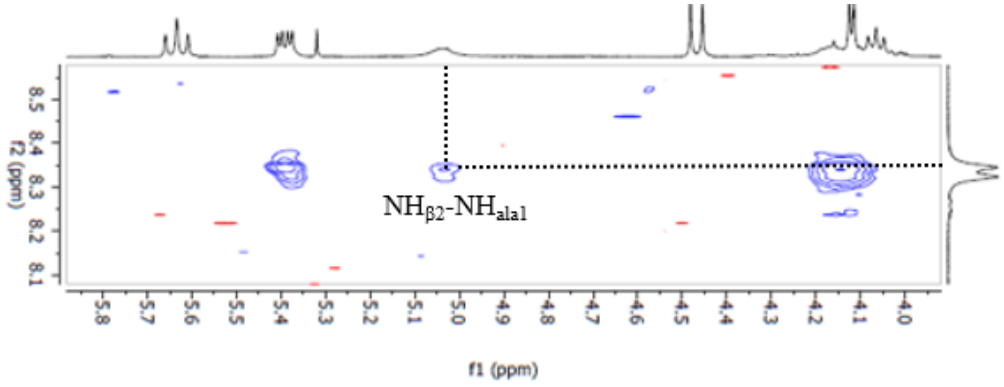


Figure 14. Zoom of $NH_{Ala1}/NH_{\beta 2}$ Noe ($CDCl_3$, 10 mM, 450ms)

These data suggest the formation of 9/11-type helix according to the convention of Hoffmanet all[8]. A mixed helix type was proposed, with alternating change of the hydrogen-bond between NH of Ala-1 and CO of β-2 amino acid (1→2) and between NH of β-4 amino acid and CO of Ala-1(1←4) (Figure 15).

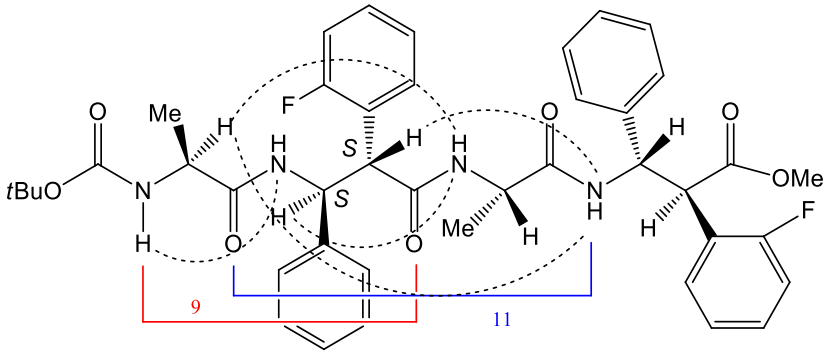
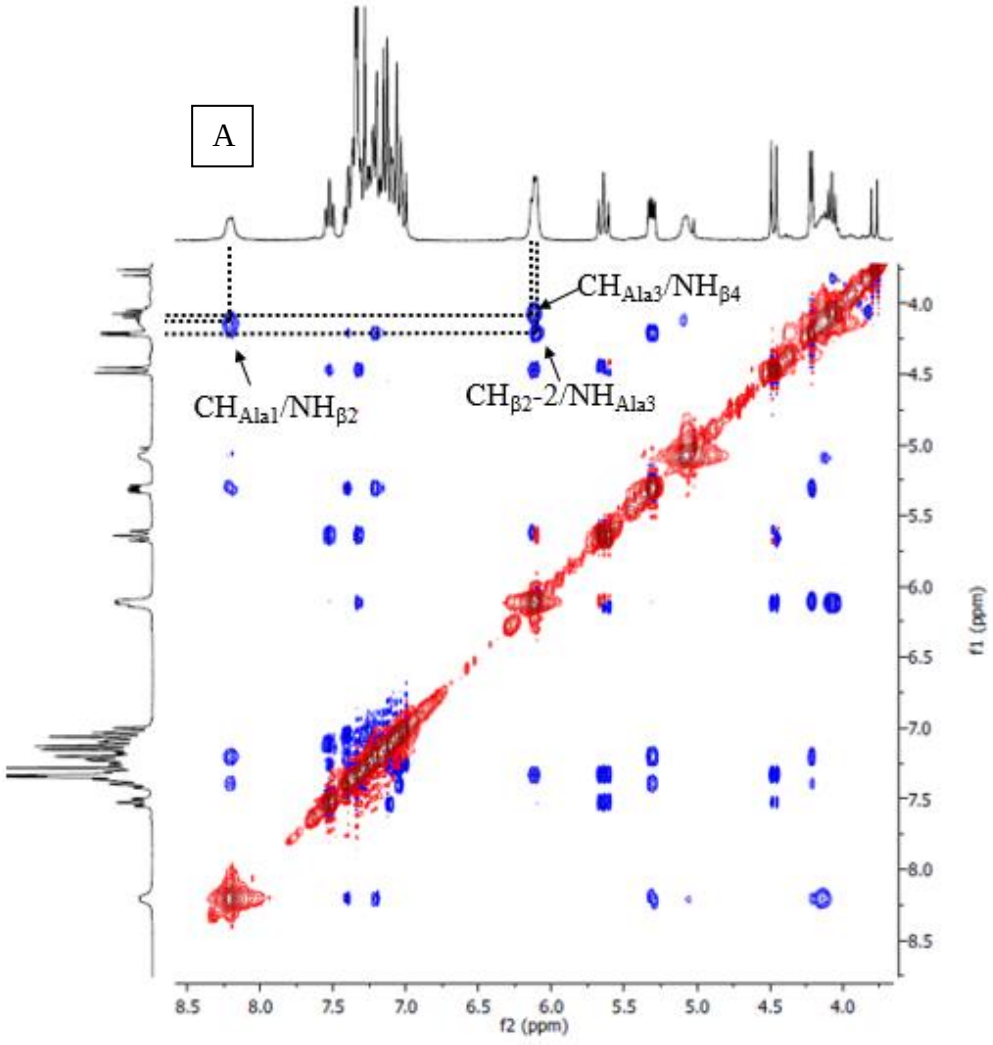


Figure 15. Dotted lines indicated non-sequential NOEs in the Noesy spectrum. Solid lines indicate C=O···HN hydrogen bonds.

Our proposal is consistent with studies on temperature dependences of the NH proton chemical shifts (273-333 K), which provide information on inaccessible or intramolecular H-bonds. Values of -1.1 ppb K^{-1} for β-4, -3.1 ppb K^{-1} for Ala-1, -3.5 ppb K^{-1} for Ala-3, and -4.5 ppb K^{-1} for β-2 were detected (Figure 16), indicating that NH of β-4 falls within the typical range for intramolecular H-bonded. Instead, moderately solvent-shielded/intramolecular hydrogen bonded amides are those of Ala-1>Ala-3>>β-2.Thase data, comfirm the proposed H-bond network (Figure 20).

3.4.1.2NMR Characterization of tetrapeptide 6a-(D1)

The behavior of isomer **6a-(D1)** is similar to that of **6b-(D2).** In this case too in fact, a complete set of strong CH/NH(i,i+1) Noes are present (Figure 16a). Weak spatial proximity were found between $CH_{\beta 2}$-3/NH_{Ala3}, $CH_{\beta 2}$-2/$NH_{\beta 4}$. Even if in many cases broad signal are present, with a low responce in terms of resolution, we found that the detected noes inequivocally confirmed the presence of a 11-helix structure for **6a-(D1)**.[9](Figure 16).



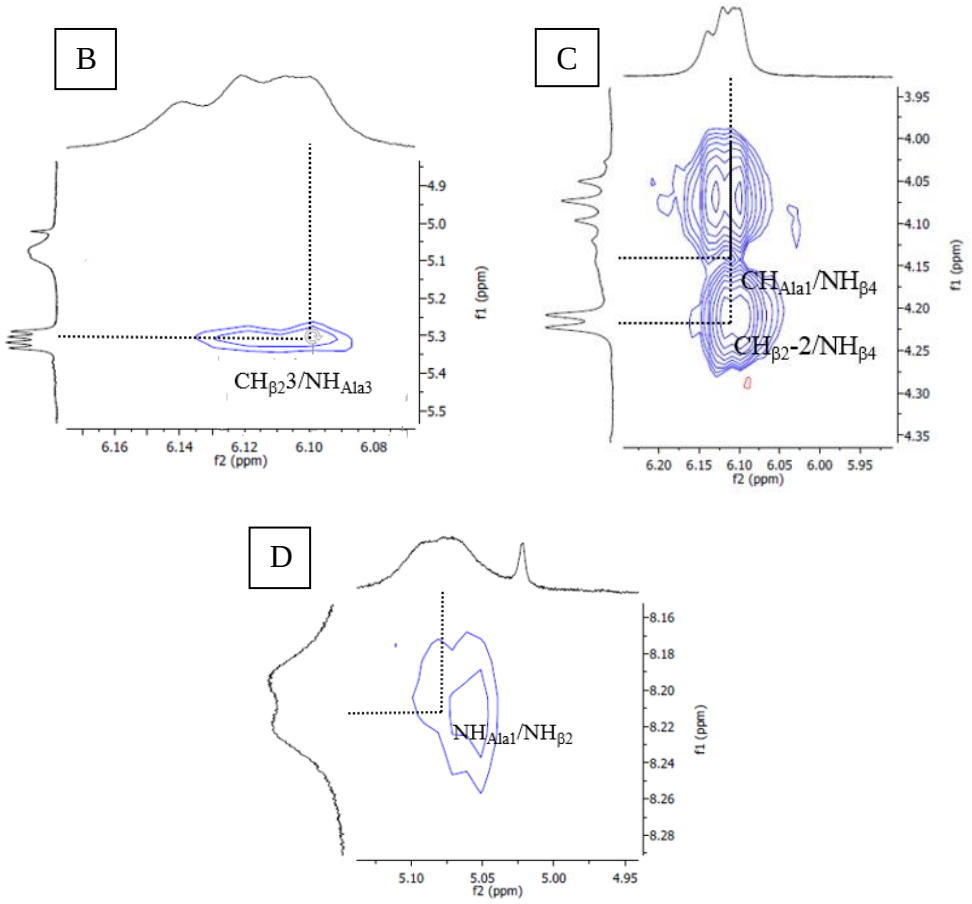


Figure 17.Significant Noes for 11-Helix structure of **6a-D1**($CDCl_3$, 10 mM, 450ms)**.**A) complete set of strong CH/NH (i,i+1) NOEs. B) Zoom of $CH_{\beta 2}$-3/NH_{Ala3}Noe; C) zoom of $CH_{\beta 2}$-2/$NH_{\beta 4}$ and CH_{Ala1}/$NH_{\beta 4}$ Noes. D)zoom of NH_{Ala1}/$NH_{\beta 2}$ Noe

A further Noe was detected also for this isomer, between NH_{Ala1}/$NH_{\beta 2}$ and CH_{Ala1}/$NH_{\beta 4}$ (Figure 17d, very weak signal), that is in agreement with a 9-helix.

The formation of 9/11-type helix was also supported by spatial proximity of Boc group with fluorinated ring of β^2 and β^4 residues (Figure 18).

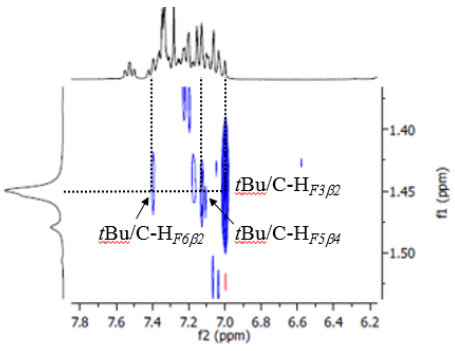


Figure 18. Spatial proximity of Boc group with fluorinated ring of β^2 and β^4 residues. Noesy ($CDCl_3$, 10 mM, 450ms).

A mixed helix type was proposed, with alternating change of the hydrogen-bond between NH of Ala-1 and CO of β-2 amino acid (1→2) and between NH of β-4 amino acid and CO of Ala-1(1←4) (Figure 19).

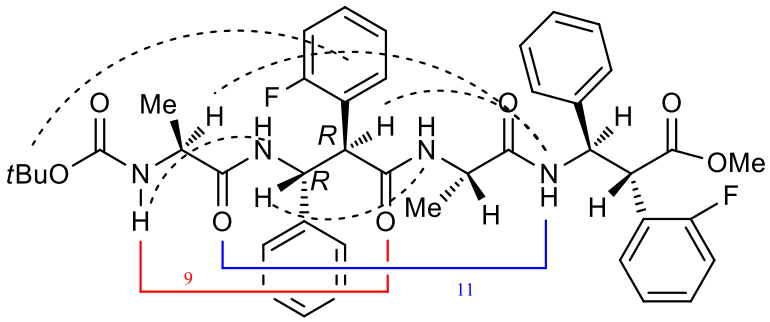


Figure 19. Proposed helix structure for isomer **6a-D1.** Dotted lines indicated non-sequential NOEs in the Noesy spectrum. Solid lines indicate C=O···HN hydrogen bonds.

The temperature dependence of the NH proton chemical shifts (273-333 K) for **6a-D1** terapeptide ranges in 3.1-3.5 ppb K^{-1} for Ala-1, Ala-3, and β–4 that represent the typical range for medium/weak intramolecular H-Bond. Instead, also in this case, NH of beta-2 is not involved in the intramolecularly H-bond. Comparing **6a-D1** with respect to **6b-D2** we found a similar behaviour except for NH-4 that is involved in a strong H-Bond in the case of **6b-D2** and in a medium H-Bond for **6a-D1.**This datum suggest that the isomer **6b-D2** matched with L-AAs gave a more stable 9/11 helix with respect to **6a-D1.**

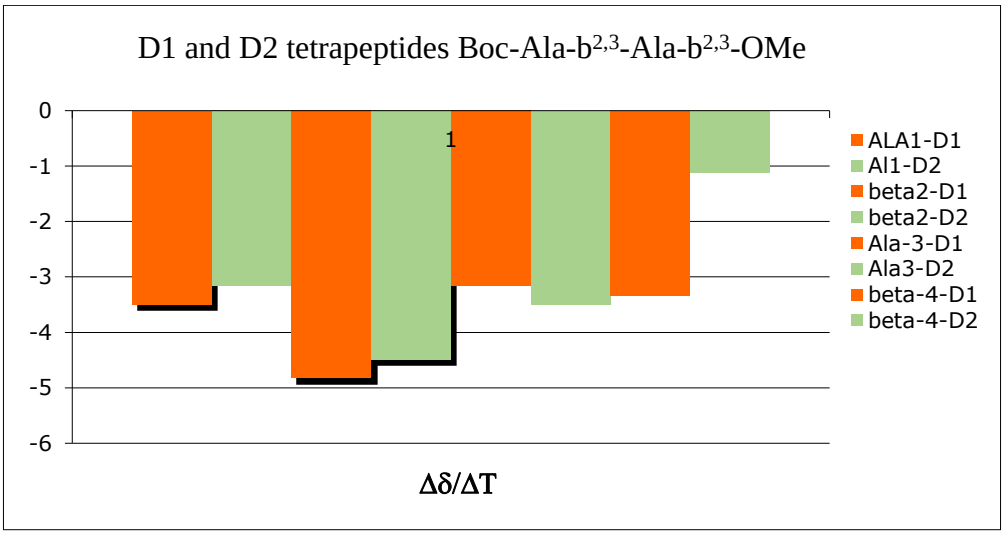


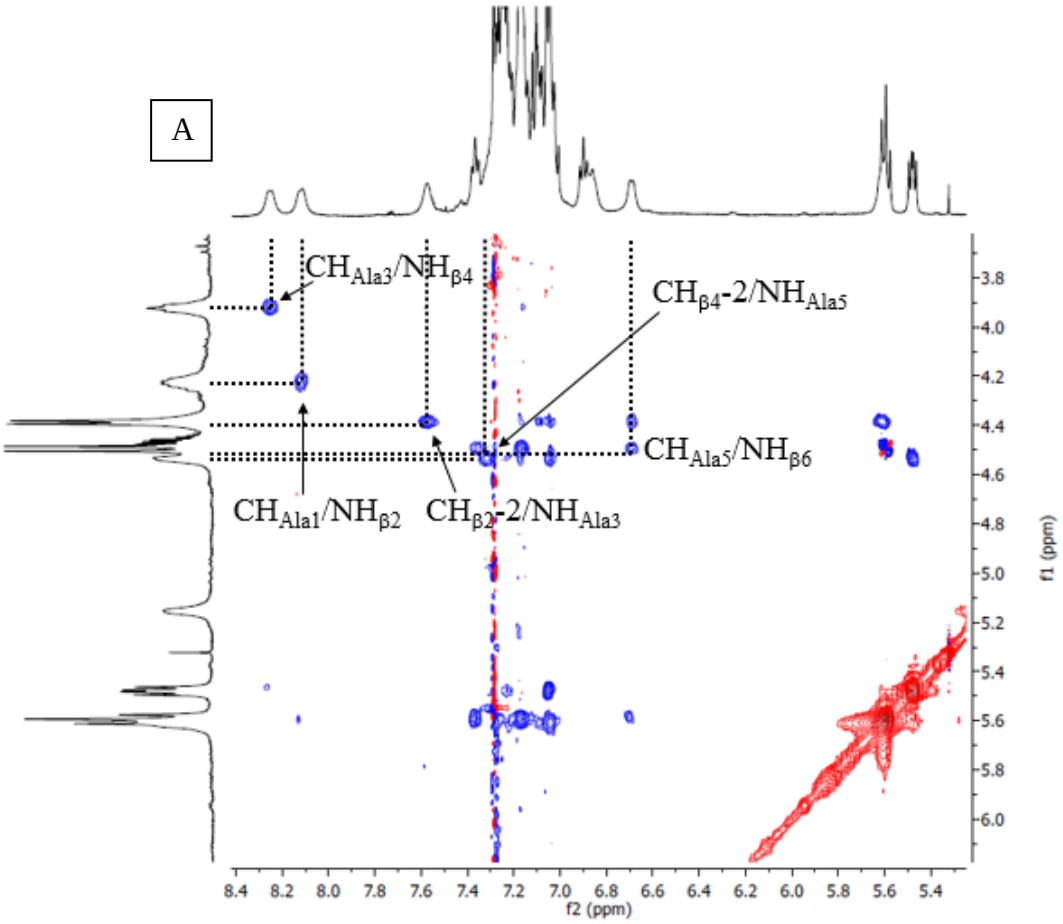
Figure 20. Temperature dependence of the NH proton chemical shifts (273-333 K) for **6b-D2** and **6a-D1** terapeptides.

It is well known that the solvent plays an important role in solution-phase structure refinement shifting from low polar solvents such as $CHCl_3$ or CH_2Cl_2 to polar solvent[10], whereas non polar solvents are destructive if hydrophobic forces cannot facilitate folding at the tertiary/quaternary structure level. Since the helicogenic structure of our peptide is in contrast with the *trans* stereochemistry of the β-peptide, we tested the behaviour of tetrapeptide **6a-D1** in the more polar solvent (THF). NMR experiments at variable temperature were performed showing a significant variation of chemical shift for all NH ($\Delta\delta$ NH larger then-5 ppb K^{-1}) indicating the competition of the solvent that could be involved in H bonds destroying the helical structure.

3.4.2 Characterization of hexapeptides 8a-(D1) and 8b-(D2)

3.4.2.1 NMR Characterization of hexapeptide 8b-(D2)

Noesy experiment on hexapeptide **8b-D2** show that only strong CH/NH (i,i+1) Noes are present. As exception, spatial proximity was observed between methyl group of Boc-moiety and $H_{\beta 2}$-2 as well as NH/NH (i,i+1) between Ala-1 and β_2-amino acid (Figure 21).



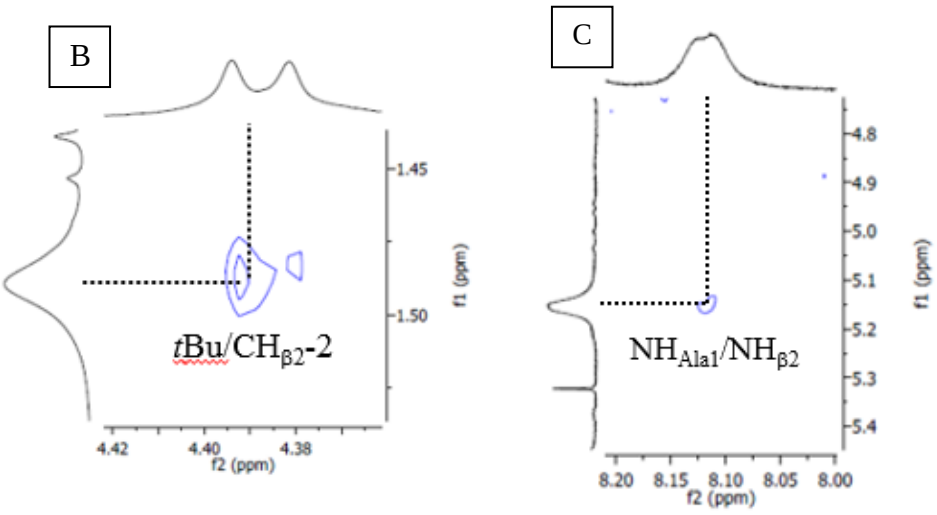


Figure 21. Significant Noes for **8b-D2** ($CDCl_3$, 10 mM, 450/600 ms)**.** A) complete set of strong CH/NH (i,i+1) Noes. B) Zoom of Boc-moiety and $H_{\beta 2}$-2Noe. C) Zoom of NH/NH Noe between Ala-1 and β_2-amino acids

The experiment at variable temperature (273-318 K; Figure 22) shows that only NH of Ala-1 posses a $\Delta\delta/\Delta T$ value in the range of a weak H-bond (-3.3 ppb K^{-1}). Larger values were detected for other NHs indicating the absence of H-Bond. Due to is overlapping, we dont't have information for NH-5 (Figure 22).

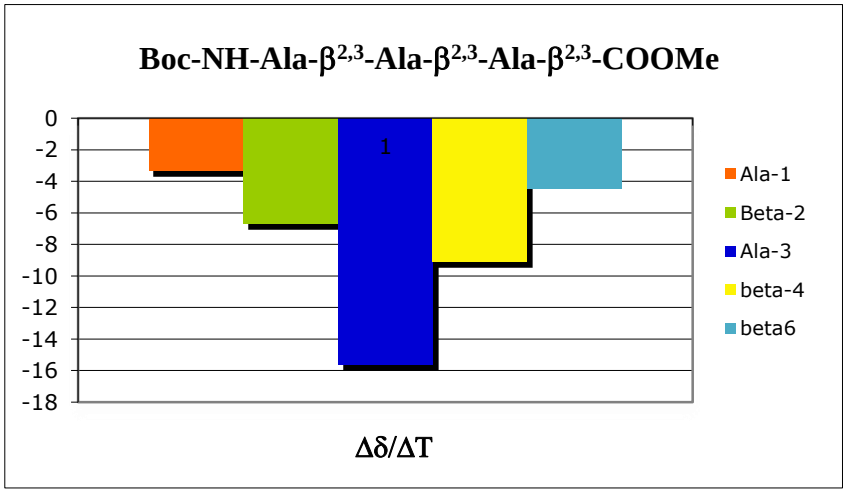


Figure 22.Temperature dependence of the NH proton chemical shifts (273-333 K) for **8b-D2.**

Our hypothesis is that a hydrogen bond occurs between Ala-1 and CO of β-2 amino acid (1→2). Interestingly, we found small $J_{NH,C\beta\text{-}H}$ and $J_{C\alpha\text{-}H/C\beta\text{-}H}$ values (5-6 Hz) for β-2 amino acid,indicating its gauche conformation compatible with a turn-like structure. On the other hand, typical large values were detected for both β-4 and β-6 indicating their *anti* conformation. Our hypothesis is that a turn was formed at *N*-termini in which Ala-1/β-2 are involved, with the formation of a 9-member ring, being the sequence of the other four amino acids extended (Figure 23).

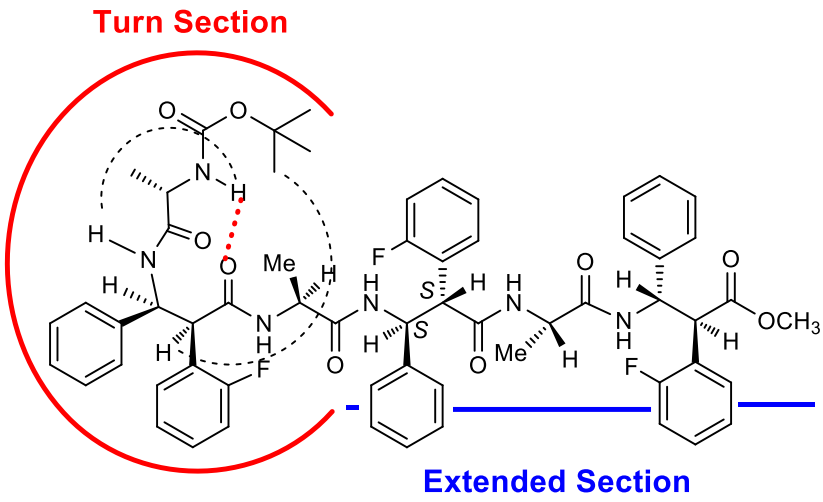


Figure 23. Proposed turn structure for isomer **8b-D2.** Black-dotted lines indicated non-sequential NOEs in the Noesy spectrum. Red-dotted lines indicate C=O···HN hydrogen bonds.

Interestingly, during the NH solvent titration of hexamer **8b-D2**, the polar solvent dimethylsulfoxide (DMSO) added to the $CDCl_3$ solution induces a switch of the conformation. In fact large $J_{NH,C\beta\text{-}H}$ and $J_{C\alpha\text{-}H/C\beta\text{-}H}$ values were found for all three β-residues indicating the formation of a fully extended conformation. Considering these data we can conclude that the presence of a turn structure at *N*-terminus was solvent dependent.

The polar solvent dimethylsulfoxide (DMSO) induces a strong downfield shift in the resonances of NH-6 ($\Delta\delta > 1$), and $\Delta\delta$ NH larger then 0.6 for NH-1 indicating their solvent exposure. Instead the analysis showed that NH-2 and NH-4 are strongly involved in a H-bond ($\Delta\delta$ NH < 0.08) indicating that these protons are solvent shielded (Figure 24). Only qualitative information can be given for Ala-3 and Ala-5 due to their overlapped signals. However, a qualitative analysis based on the difference of detectable chemical shift values (before 120μL of DMSO) gave information on their involved in hydrogen bonds of medium intensity.

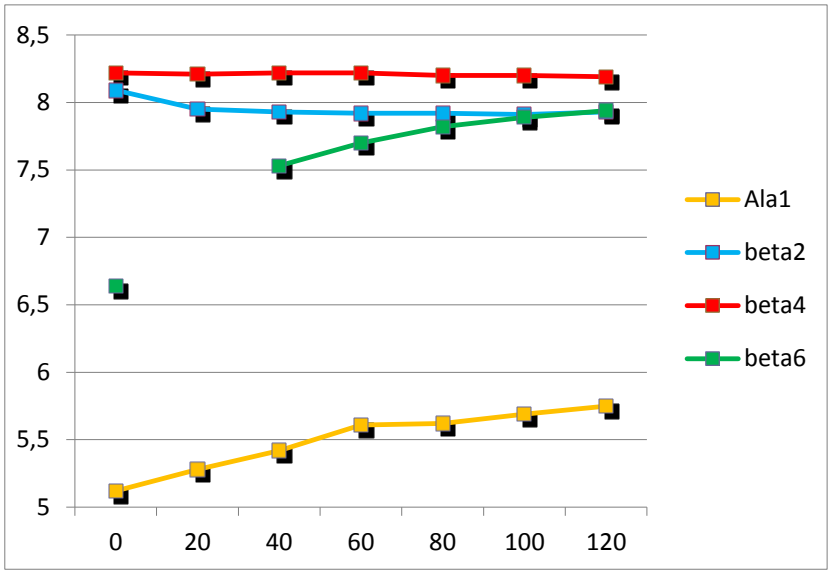


Figure 24. DMSO Titrations of peptide**8b-D2** in $CDCl_3$

These results suggest the formation of a parallel or antiparallel sheet aggregation for peptide **8b-D2** in polar solvent. Noesy analysis in DMSO are in progress in order to understand the sheet orientation.

The hypothesis of structural switching for peptide **8b-D2** in function of the solvent is further supported by preliminary IR analyses. As shown in Figure 25, IR spectrum of **8b-D2** was first gained after MeOH evaporation (blue trace) showing exclusively an absorption peak corresponding to a helix construct. After addition of H_2O or D_2O (green and black traces) the typical absorption bands of a sheet-structure appears. The switching process is also reversible as demonstrated by the spectrum registered after water exposure and re-filming process from MeOH evaporation (red trace).

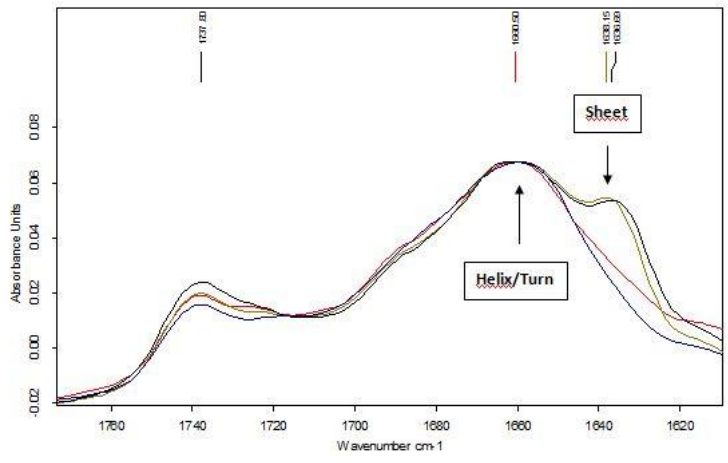


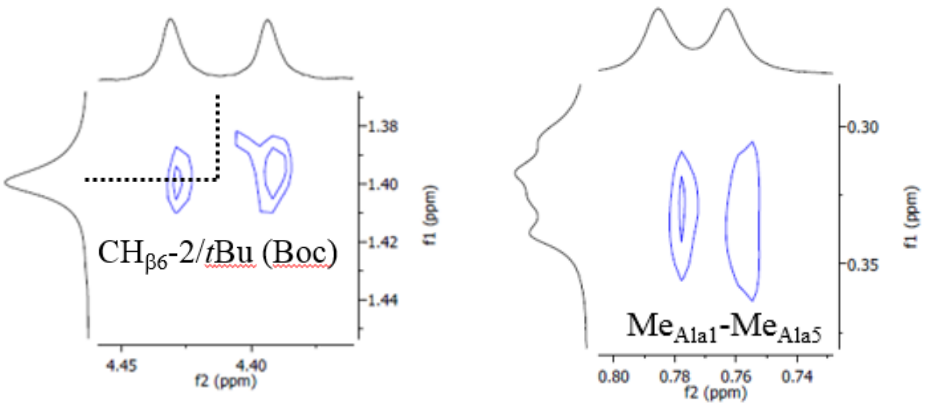
Figure 25. IR analysis of hexamer **8b-D2** shown the structural switching in polar solvents.

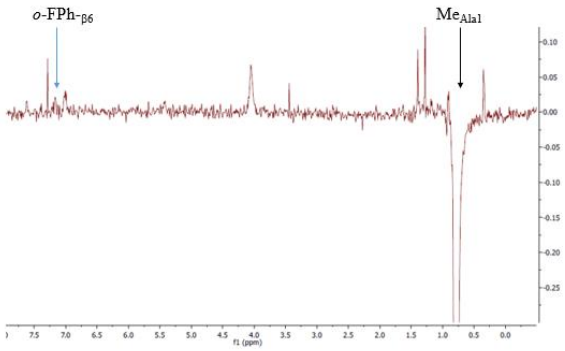
This behaviour appears different from the tetrapeptide for which a 9/11-helix was proposed. Our hypothesis is that the helix behaviour is induced mostly by the α-amino acid in a short sequence but elongating the sequence the trans β-amino acid force the peptide in an extended conformation.

3.4.2.2 NMR Characterization of hexapeptide 8a-(D1)

Comparing as reported considering the hexapeptides **8b-D2,** a main difference is evident being **8a-D1** fully extended already in $CDCl_3$ as shown by large NH/C_β-H and C_α-H/C_β-H *J* values (Table 1) indicating the antiperipalanar orientation between these hydrogens. This is also confirmed by *J* values (6.5-9 Hz) for alanines that are present as doublet. On the other hand Alanine involved in a turn show broad signals.

Interestingly, spatial proximities were detected in Noesy/Roesy experiments: *i.e.* $CH_{\beta 6}$-2/Boc group, *o*-FPh of β-6amino acid/Me_{Ala1},*o*-FPh of β-6amino acid/Boc and Me_{Ala1}/Me_{Ala5}(s) (Figure 26).





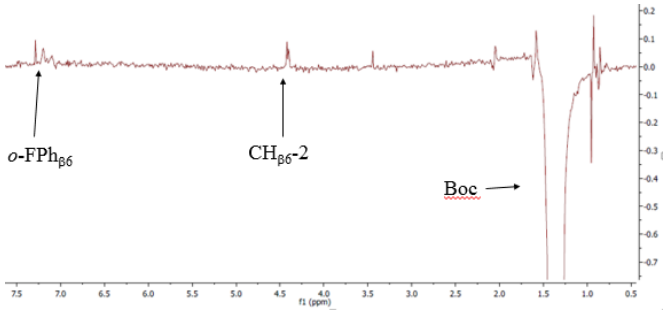


Figure 26. Significant Noes ($CDCl_3$, 10mM, 450ms)to explain thespatial proximitybetween interstrand*C*-terminus and *N*-terminus. A) Zoom of $CH_{\beta 6}$-2/BocNoe B) Zoom of Me_{Ala1}/Me_{Ala5}Noe C) Row for *o*-FPh of β-6 amino acid/Me_{Ala1}Noe D) Row for *o*-FPh of β-6 amino acid/Boc Noe

Due to the low resolution of the proton of beta amino acid in position 2 and 4, only weak noesy were detected, even if these Noes support the formation of an antiparallel pleated sheet arrangementin which the *C*-termini of one strand is faced on the *N*-termini of a second strand. As a result, three H-bonds are predictable as shown in figure 27.

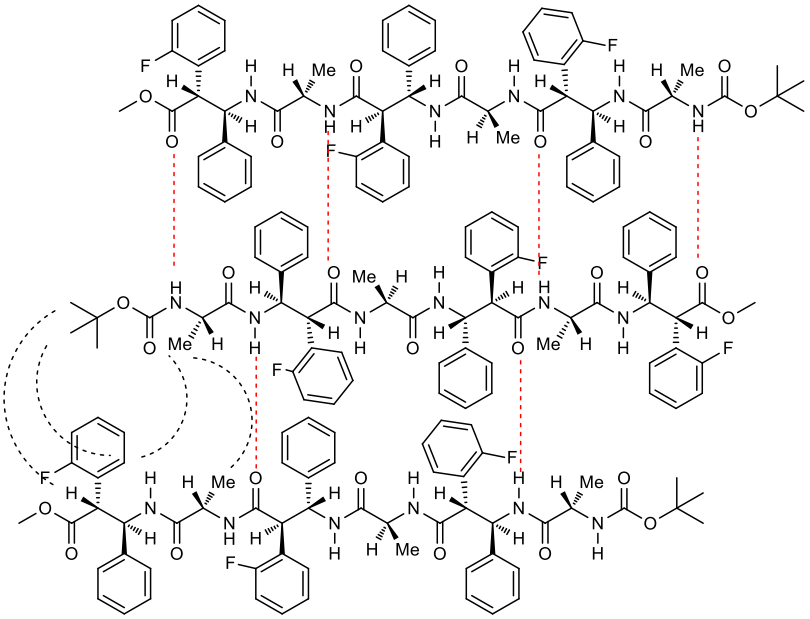


Figure 27. Black-Dotted lines indicated non-sequential NOEs in theNoesy spectrum. Red-Dotted lines indicate C=O···HN hydrogen bonds

Even if some NH protons are overlapped with aromatic protons, both solvent titration studies and temperature dependence of the NH proton chemical shifts were performed. Some interesting information were achieved even if in some cases only qualitative evidences were detected.

Due to the partial overlapping of NH protons with aromatic protons, only general and qualitative information can be done both in the NH temperature dependence and DMSO titration.

In the first experiment, NH proton chemical shifts (273-333 K) ranges in 3.1-3.5 ppb $K^{-}$for β-2 and Ala-5. Instead, NH of and β-4 is not involved in the intramolecular H-bond. Data for Ala-1, Ala-3 and β-6 can not be evaluated due to the overlapped signals.

Solvent titration studies were matched with Toxy experiments [**8a-D1**(10 mM);0%, 9% and 20% *v/v* of DMSO] to ensure the correct correlation between NH proton with the corresponding amino acid and to detect the correct chemical shift. Figure 28 shows the results related the NH mobility. Clearly they are only qualitative results but essential to assign the total Δδ to NHs. The polar solvent dimethylsulfoxide (DMSO) added to the $CDCl_3$ solution induces a strong downfield shift in the resonances of NH-6 (Δδ >1), and Δδ NH larger then 0.5 for Ala-3 (0.54) and beta-4 (0.89) indicating their solvent exposure. Instead the analysis showed that NH-1 and NH-5 are strongly involved in a H-bond (Δδ NH < 0.05) indicating that these protons are solvent shielded. NH-2 is involved in a medium-weak H Bond (Δδ = 0.35).The obtained results are in agreement with the proposed H-Bond Network shown in Figure 27

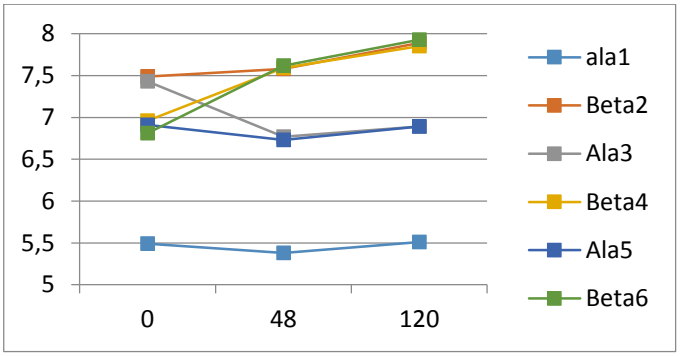


Figure 28. DMSO Titrations of peptide**8a-D1** in $CDCl_3$. NH chemical shift were evaluated by TOCSY analysis

3.5.Conclusion

α,$\beta^{2,3}$-Sequences synthetized during my thesis, represent, to the best of our knowledge, the first examples of combination of chiral α-amino acids and $\beta^{2,3}$-amino acids.

Interesting information were achieved by *J* value analysis indicating that the *trans/gauche* conformation of the β-amino acid is driven by the length of the peptide.

In short di- and tetrapeptides the secondary structure is mostly driven by the helicogenicity of Ala thus forming preferably helix construct.

Examples reported in literature focused on α,β-peptide sequences showed the formation of 11/9-

type helixes. On the other hand, 9/11-helix are reported when inverting the amino acid sequence, *i.e.* β,α-peptides.[11,12]In all reported examples, β^3-amino acids were used. Examples of peptides containing a trans cyclic β-amino acids, characterized by gauche conformation, reflect the same H-bond network[13]. As reported in the introduction, *Balamurugan at al*.[14] recently reported on the use of α,$\beta^{2,3}$-sequence where Aib is present resulting in the formation of a 11 helix architecture. Our results on tetrapeptides appear very original with respect to those reported in literature. Neither a 11/9-type helixes, characterizing β^3,α-peptides, nor 11 helixes, characterizing Aib,$\beta^{2,3}$-sequences, where detected.In our case, the (L-Ala,$\beta^{2,3}$)$_2$sequence gave a 9/11-helixes, independently on the stereochemistry of the β-amino acid.

Elongating the peptide sequence an extended conformation is preferred indicating that the presence of more then two β-amino acids drive the conformation of the peptide. Furthermore, the *R,R*-β-amino acid is more prone to induce an extended conformation with respect to the *S,S*-one when combined with a *L*-amino acid.

Comparing our result with the Aib,$\beta^{2,3}$-sequences, for which a 11-helix is proposed independently from their length, we can deduce that their behaviour is strictly dependent on the strong helicogenic effect of Aib.

3.6 References

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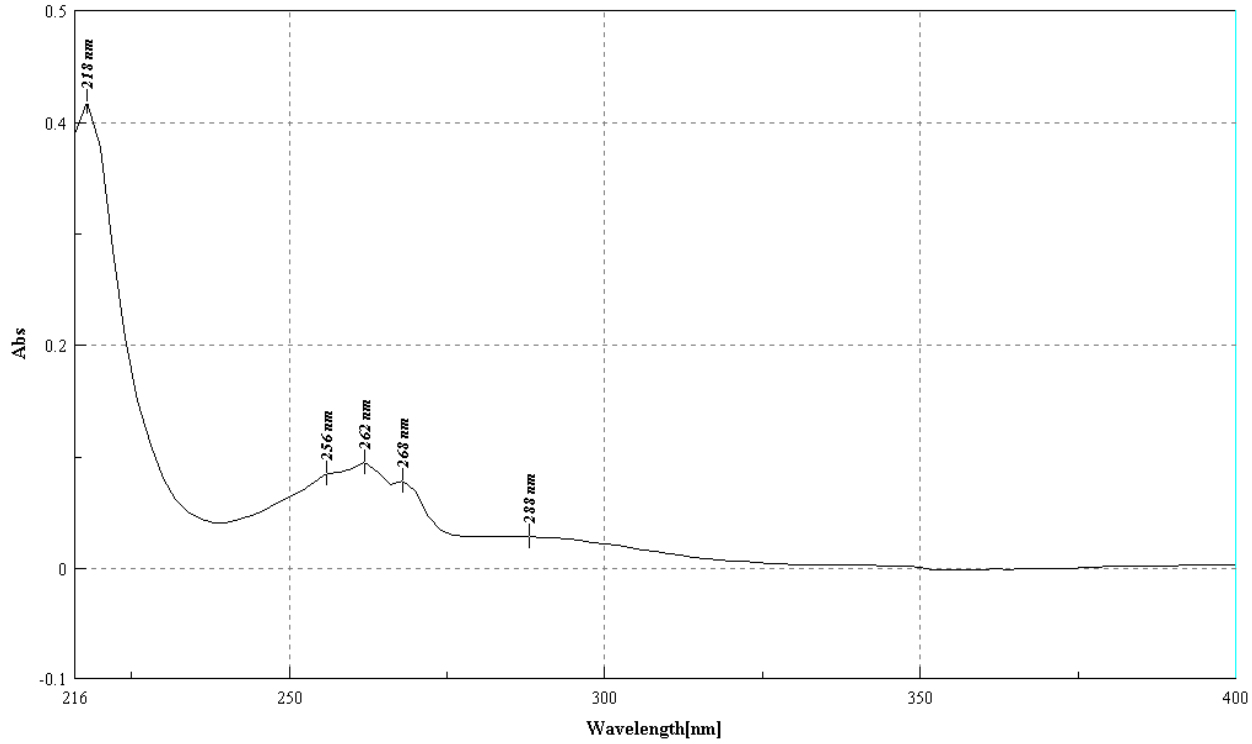
Esperimental section



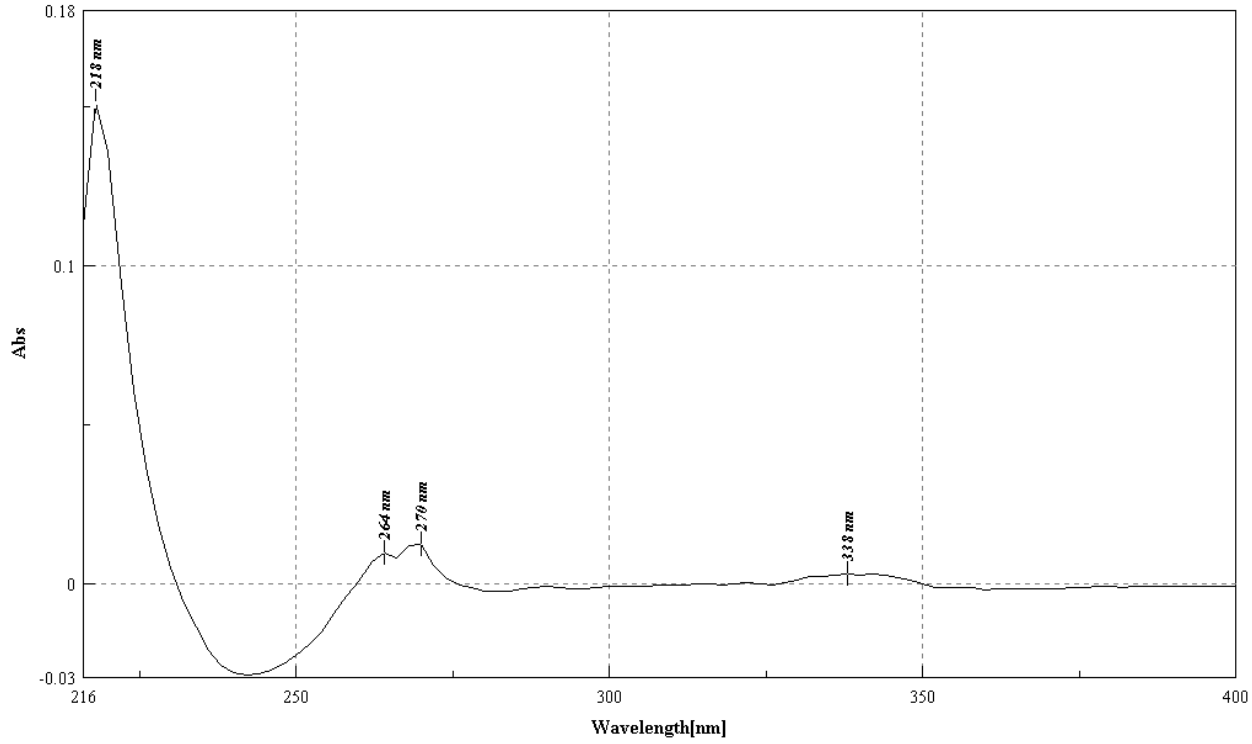


Figure E1. UV spectra for dipeptides **3a-D1** and **3b-D2**(H_2O/TFE 1:1, 50μM)

Complete NMR Characterization for dipeptide **3a-D1**($CDCl_3$, 10 mM, 293 K, 500MHz)

AA	atom		^{1}H δ	Molteplicity *J* (Hz)	^{13}Cδ	Noesy
Ala-1	CO				171.3[a]	
	CH		3.80	m	50.1	Me_{Ala1}(s), NH-2(vs), NH-1(vw)
	Me		0.81	d, *J* 6.7	17.8	Arom(7.54, 7.43vw), NH-2(vw), H-3_{beta2}(vw), NH-1(m), CH_{Ala1}(s)
	NH		5.38	brs		Me_{Ala1}(vs), H_{Ala}(m), NH-2(w)
	Boc	Me	1.35		27.9	
		C			79.1	
		CO			155.5	
2 ***beta***	CO				172.2[a]	
	2		4.50	d,*J*10.9	49.6	H-3(m),Arom(7.54, 7.43m), NH-2(s)
	3		5.69	dd, *J* 10.9, 10.8	54.1	H-2(m), Arom(7.54, 7.43, 7.11s), Me_{Ala1}(vw)
	NH		7.07	d, *J*10.8		Me_{Ala1}(m), CH_{Ala1}(vs), CH-2 (s), NH_{Ala1}(w)
	Arom		7.54 *F-6* 7.44-7.31 7.20 *F-5* 7.13 *F-3*	ddd*J* 7.6, 1.5 m ddd*J*7.6, 1.3 ddd*J*8.3, 1.3?	130.1 (*J* 12.0) 127.8, 128.2, 128.9, 130.2 124.9 (*J* 3.6) 115.7 (*J* 22.8) 117.7(q) 140.9 (q) 161.2 (C_{F-2}, *J* 245.2)	Me_{Ala1}(vw), H-2(m), H-3(s)
	OMe		3.46	52.2		Arom (7.54,7.42)

[a]Tentativelyassigned

Complete NMR Characterization for dipeptide **3b-D2**($CDCl_3$, 10 mM, 293 K, 500MHz)

AA	atom		^{1}H δ	Molteplicity *J* (Hz)	^{13}Cδ	Noesy
Ala-1	CO				171.4	
	CH		3.76	M	51.1	Me_{Ala1}(s), NH-2(vs), NH-1(vw), Arom(7.4m)
	Me		0.92	d, *J* 6.1	17.6	Arom(7.3, 7.13vw), NH-2(w), NH-1(m), CH_{Ala1}(s)
	NH		5.38	Brs		Me_{Ala1}(w), H_{Ala}(vw), NH-2 (w)
	Boc	Me	1.37		27.9	Arom
		C			79.4	
		CO			155.5	
2 ***beta***	CO				172.5	
	2		4.52	d, *J* 10.5	49.6	H-3(m),Arom(7.56m, 7.4s), NH-2(s)
	3		5.68	dd, *J* 10.5, 9.9	53.9	H-2(m), Arom(7.54, 7.43s), NH-2(w)
	NH		7.07	d, *J*9.9		Me_{Ala1}(vw), CH_{Ala1}(vs), CH-3 (m),CH-2 (s),NH-1(vw), Arom(7.35)
	Arom		7.54 $_{F-6}$ 7.37$_{F-4}$ 7.58-7.30 7.19$_{F-5}$ 7.13$_{F-3}$	ddd*J*7.6, 1.4 overl mult ddd*J*7.6, 1.2 ddd*J*9.6, 8.3, 1.3	130.3 (*J* 3.3) 130.1 (*J* 6.4) 128.8, 128.2, 127.7 124.8 (*J* 3.5) 115.7 (*J* 22.8) 117.7($_{F-6}$) 140.9 (q) 161.2 (C_{F-2}, *J* 245.1)	H-2(m), H-3(s), NH-2(m), H_{F-5}(m)
	OMe		3.46		52.1	

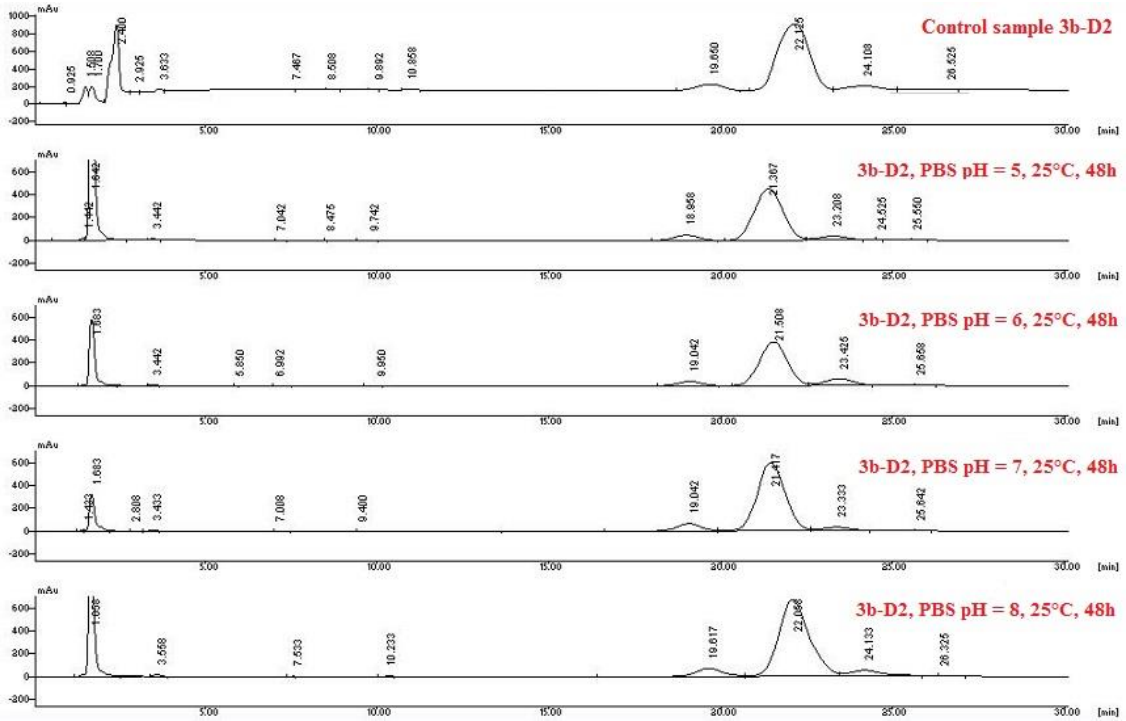


Figure E2**. 3b-(D2)** solution incubated for 48h in PBS pH 5/6/7/8 + DMSO 20% *v/v*.

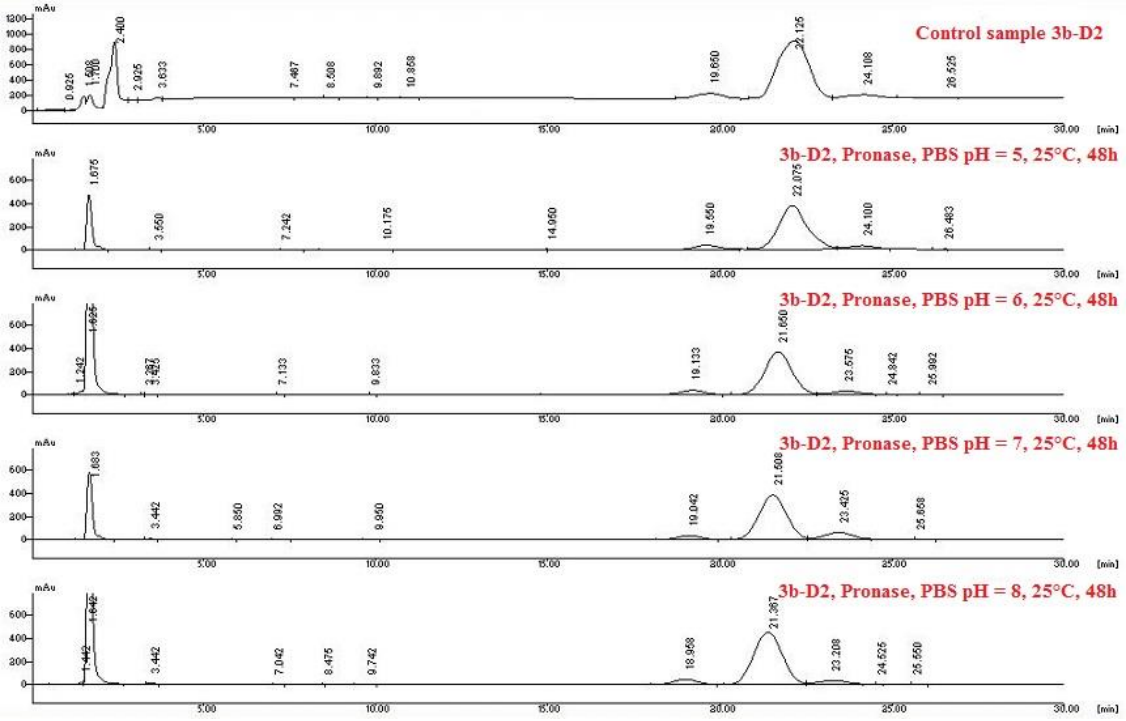


Figure E3**. 3b-(D2)**Nanotubes solution incubated for 48h with Pronase from *Streptomyces griseus* (PBS pH 5/6/7/8 + DMSO 20% *v/v*).

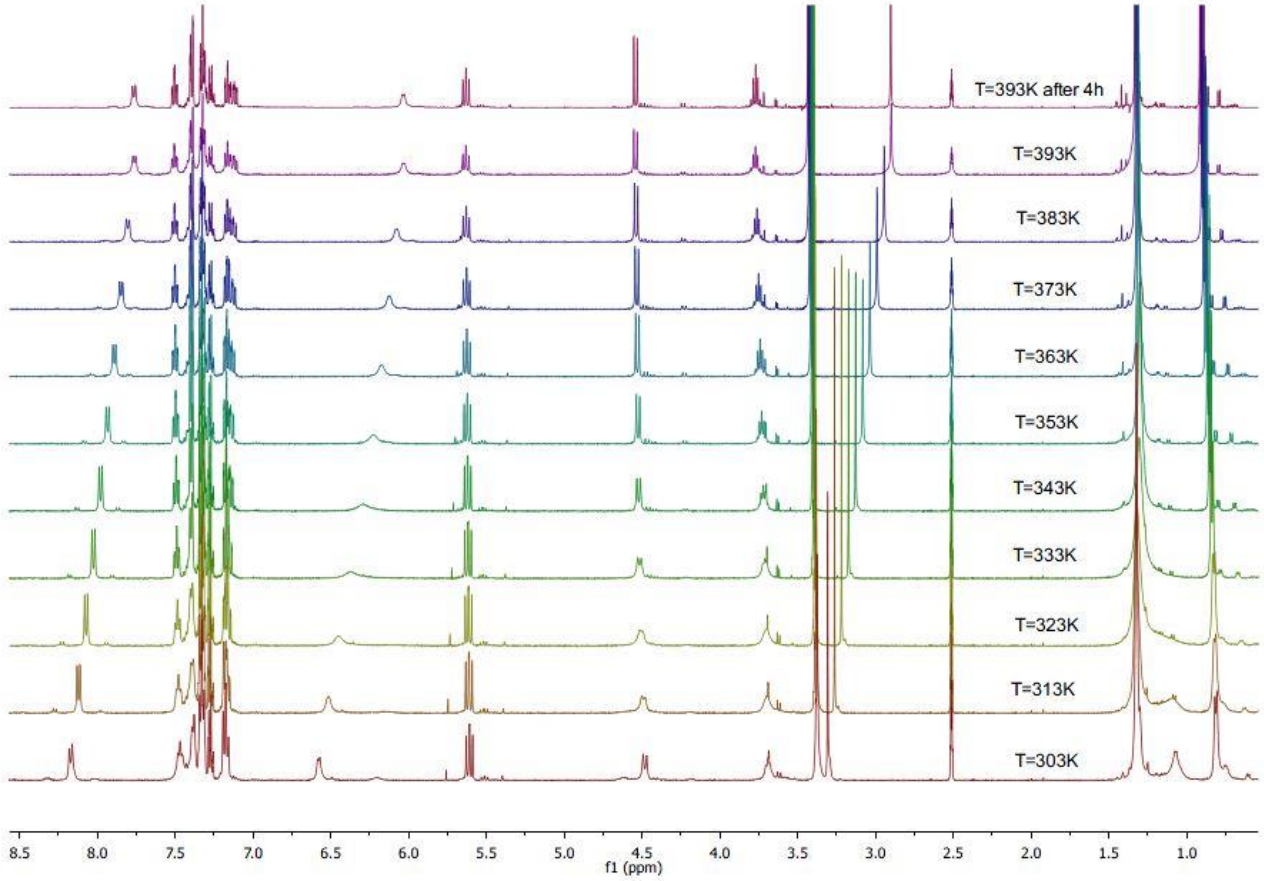


Figure E4. Thermal stability for D2 Nanotubes.

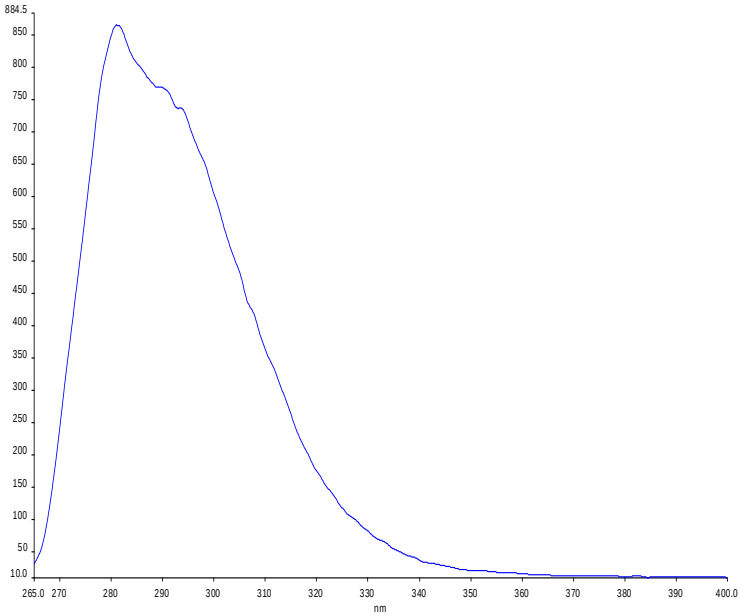


Figure E5. Fluorescence emission for 3b-D2 Nanotubes

Complete NMR Characterization for Tetrapeptide**6a-D1** ($CDCl_3$, 10 mM,293 K, 500MHz)

AA	atom		^{1}H	Molteplicity *J* (Hz)	^{13}C	NoesyCH[a]
Ala-1	CO				172.3	
	CH		4.14	brs	50.5brs	$NH_{\beta 2}$(s), Me_{Ala1}(vs), NH_{Ala1}(vw)
	Me		1.18	*J* 7.0	18.8	NH_{Ala1}(m), CH_{Ala1}(s), $NH_{\beta 2}$(vw), Ar(7.45)
	NH		5.07	brs		Me_{Ala1}(m), CH_{Ala1}(vw), $NH_{\beta 2}$(w)
	Boc	Me	1.45		28.7	Ar (7.39w, 7.0w),NH_{Ala1}(vw)
		C			80.1	
		CO			155.6	
Beta-2	CO				171.0	
	2		4.22	d, *J* 4.5	49.0 (J_{CF} 2.2)	$NH_{\beta 2}$(vw), $H_{\beta 2}$-3(vs),Ar(7.39w, 7.21s, 7.15s), NH_{Ala3}(vs),F(-118.16, vs)
	3		5.31	dd, *J* 8.9, 4.5	55.7	$H_{\beta 2}$-2(s),Ar(7.39m, 7.21vs, 7.17m), $NH_{\beta 2}$(m), F (-118.16 vs)
	NH		8.20	d, *J*8.9		CH_{Ala1}(s), $H_{\beta 2}$-3(m), $H_{\beta 2}$-2(w), Me_{Ala1}(w), Ar(7.19s, 7.39w), NH_{Ala1}(w),Boc(w)
	Arom		$7.39_{F\text{-}6}$ $7.23_{F\text{-}4}$ $7.06_{F\text{-}5}$ $7.03_{F\text{-}3}$ - 7.30-7.25		$129.5_{F\text{-}6}$ (brs) $129.7_{F\text{-}4}$ (*J* 8.5) $124.5_{F\text{-}5}$ (*J* 3.5) $116.0_{F\text{-}3}$ (*J* 22.2) 157.2_{F2} (*J* 246.1) 122.7_{F1} (*J* 14.1) 127.8[c] 128.8[c] 126.8	F_6: $NH_{\beta 2}$(s), Ar(7.06vs),$H_{\beta 2}$-3(s), $H_{\beta 2}$-2(m), Me_{Ala1}(m), Me_{Ala3}(m),Boc(w) F_3: F (-118.43vs)

				140.4 (q)	
Ala-3	CO			170.5	
	CH	4.07	m7.0	52.6	Me_{Ala3}(vs), $NH\beta_4$(s)
	Me	0.83	d, *J* 6.9	14.5	H_{Ala3}(vs),NH_{Ala3}(vs), Ar(7.39m, 7.06vw)
	NH	6.11	Overl.		$H\beta_2$-2(s), Me_{Ala3}(vs),F(-118.45w)
Beta-4	CO			171.1	
	2	4.47	d,*J*10.7	48.9(J_{CF} 1.9)	$H\beta_4$-3(vs), $NH\beta_4$ (s), NH_{Ala3}(s), Ar(7.33 vs; 7.52w), F (s, -118.43vs)
	3	5.64	dd, *J*10.7, 9.5	55.2	$NH\beta_4$(w), $H\beta_4$-2(m), Ar(7.52s, 7.34vs, 7.31w), F (-118.45 vs)
	NH	6.13	Overl.		H_{Ala3}(vs), $H\beta_4$-2(m), $H\beta_4$-3(m), Ar(7.32 m)
	Arom	7.52_{F-6} 7.25_{F-4} 7.13_{F-5} 7.06_{F-3} - - 7.30- 7.25		129.9_{F-6} (*J* 2.0) 130.1_{F-4} (*J* 7.4) 125.0_{F-5} (*J* 3.5) 115.7_{F-3} (J 22.9) 161.1_{F2} (*J* 245.0) 124.3_{F1} (*J*13.9) 129.2[b] 128.6[b] 127.6 139.7(q)	F_6: Ar(7.13s), $H\beta_4$-3(s), $H\beta_4$-2(vw), OMe(w) F_5: Ar(7.50s) F_3: OMe(w), F(-118.45)
	OMe	3.52	s	52.6	Ar (7.53s, 7.06w), $H\beta_4$-3(s), $H\beta_4$-2(vs)

[a]500 MHz /450ms at 300 K. [b]Tentatively assigned.

Complete NMR Characterization for Tetrapeptide**6b-D2** ($CDCl_3$, 10 mM, 293 K, 400MHz)

AA	atom		^{1}H	Molteplicity *J* (Hz)	^{13}C	NoesyCH[a]
Ala-1	CO				172.4	
	CH		4.16	brs	49.2	$NH\beta_2$(s),NH_{Ala3}(w), Me_{Ala1}(vs)$NH\beta_4$(vw),
	Me		1.28		19.2	$NH\beta$□(m), $H\beta_2$-3(s), CH_{Ala1}(vs),
	NH		5.02	brs		$NH\beta_2$(w)
	Boc	Me	1.47		28.7	
		C			80	
		CO			155.6	
***Beta*-2**	CO				170.8	
	2		4.11	d, *J* 4.1	49.2	$H\beta_2$-3(vs),$NH\beta_4$(vw),Ar(7.37vw,7.28s), NH_{Ala3}(vs),F(-118.13, s)
	3		5.38	dd, *J* 9.0, 4.1	55.3	$H\beta_2$-2(vs), Arom(7.38vw, 7.28vs), $NH\beta_2$(m), NH_{Ala3}(vw)
	NH		8.32	d, *J*9.0		Ar(7.37s,7.28s),$H\beta_2$-3(s), $H\beta_2$2(m),CH_{Ala1}(vs),CH_{Ala3}(w),NH_{Ala1}(vw), Me_{Ala1}(s), Boc(vw)
	Arom		7.37_{F-6} 7.22_{F-4} 6.98_{F-5} 7.00_{F-3} - - 7.30-7.25		129.3 $_{F-6}$ (*J*3.1) 129.7$_{F-4}$(*J*8.4) 124.6 $_{F-5}$(*J* 3.0) 116.0$_{F-3}$ (*J*22.9) 160.6$_{F2}$ (*J* 245.0) 122.7 $_{F1}$ (*J* 14.4)	F_6: $NH\beta_2$(m), Ar(6.99vs),NH_{Ala3}(vw), $H\beta_2$-3(w), $H\beta_2$-2(m) F_5: Ar(7.38s) F_3: F(-118.42m)

				127.5[b] 128.5[b] 126.2 140.5 (q)	
Ala-3	CO			170.5	
	CH	4.06	m	48.9	Me_{Ala3}(vs), $NH\beta_4$(m), NH_{Ala3}(vw), Ar(7.28m), $NH\beta_2$(w),
	Me	0.70	d, *J* 6.9	17.9	H_{Ala3}(vs),NH_{Ala3}(s), $NH\beta_4$(m), Ar(7.28m)
	NH	6.05	d, *J*7.2		$H\beta_2$-2(vs), H_{Ala3}(w), Me_{Ala3}(vs), $Ar\beta_2$(7.28vw), $Ar\beta_2$(7.37w),$H\beta_3$-2(vw), H_{Ala1}(w),
Beta- 4	CO			171.1	
	2	4.46	d, *J*10.5	49.0 (d, J_{CF} 5.3)	$H\beta_4$-3(vs), Ar(7.29 vs; 7.50w), $NH\beta_4$(s), F (-118.43vs)
	3	5.63	dd, *J*10.5, 9.4	55.1	$NH\beta_4$-(w), $H\beta_4$-2(vs), Ar(7.50s, 7.28vs)
	NH	6.24	d, *J* 9.4		Me_{Ala3}(m), H_{Ala3}(s), $H\beta_4$-2(s), $H\beta_4$-3(m),$H\beta_2$-2(vw), H_{Ala1}(vw), Ar(7.28 s)
	Arom	7.51_{F-6} 7.27_{F-4} 7.13_{F-5} 7.07_{F-3} - - 7.30-7.25		130.0_{F-4} (*J*2.8) 129.9_{F-4} 125.0_{F-5} 115.6_{F-3} (*J*23.2) 161.1_{F2}(*J* 245.6) 123.8_{F1} 128.1[b] 128.7[b] 127.2	F_6: Ar(7.13s), $H\beta_4$-3(s), $H\beta_4$-2(m), OMe(w) F_5: Ar(7.50s) F_3: OMe(w)

				139.7(q)	

Complete NMR Characterization for Hexaapeptide**8b-D2** ($CDCl_3$, 10 mM,293 K, 500MHz)

AA	atom		^1H	Molteplicity *J* (Hz)	^{13}C	NoesyCH[a]and F chemical shift
Ala-1	CO				172.4	
	CH		4.22	brs	51.1	$NH_{\beta 2}$(s),NH_{Ala1}(vw), Me_{Ala1}(vs)
	Me		1.35	brs	17.6	NH_{Ala1}(s), CH_{Ala1}(vs)
	NH		5.14	brs		Me_{Ala1}(m), CH_{Ala1}(vw), $NH_{\beta 2}$(w)
	Boc	Me	1.50		28.3	$H_{\beta 2}$-2 (w)
		C			80.3	
		CO			155.6	
Beta-2	CO				170.41	
	2		4.39	d, *J* 6.3	47.3(d)	$H_{\beta 2}$-3(vs),NH_{Ala3}(vs), Me Boc(w) F (-118.0)
	3		5.62	Overl.	55.1	H_{β}-2(vs), $NH_{\beta 2}$(m),Arom(7.06vs)
	NH		8.12	d, *J* 5.1		$H_{\beta 2}$-3(s),$H_{\beta 2}$-2(vw), CH_{Ala1}(s),Ar(7.05s),NH_{Ala3}(w), NH_{Ala1}(vw)
	Arom		7.37_{F-6} 7.22_{F-4} 6.98_{F-5} 7.00_{F-3} - - 7.30- 7.25		129.3_{F-6} (*J*3.1) 129.7_{F-4}(*J*8.4) 124.6_{F-5} (*J* 3.4?) 116.0_{F-3} (*J* 22.8) 160.7_{F2}(*J* 245.0) 122.7_{F1} (*J* 14.4) 127.5^b 128.5^b 126.2	F_6: $NH_{\beta 2}$(m), Ar(6.99vs),NH_{Ala3}(vw), $H_{\beta 2}$-3(w), $H_{\beta 2}$-2(m) F_5: Ar(7.38s) F_3: F(-118.42m)

				140.5 (q)	
Ala-3	CO			173.8	
	CH	3.94	br	50.7	Me_{Ala3}(s), NH_{Ala3}(w), $NH\beta_4$(s), Arom(7.17w)
	Me	1.22	br	16.8	H_{Ala3}(vs),Ar(7.16vw), NH_{Ala3}(s)
	NH	7.57	br		Me_{Ala3}(vs), H_{Ala3}(vw), Hβ□-2(vs), NHβ□-(w)
Beta- 4	CO			169.7	
	2	4.54	br	47.1(d, J_{CF} 5.3)	$H\beta_4$-3(vs), NH_{Ala5}(s),Ar(7.06s; 7.17w) F (-118.8w)
	3	5.48	dd, *J*9.8, 6.7	56.1	$NH\beta_4$(m), $H\beta_4$-2(vs), Ar(7.04vs, 7.24s) F (-117.8)
	NH	8.24	*J* 6.7		H_{Ala3}(s), $H\beta_4$-3(w),Ar(7.07vs),NH_{Ala5}(m)
	Arom	7.51_{F-6} 7.27_{F-4} 7.13_{F-5} 7.11_{F-3} - - 7.30- 7.25		130.0_{F-4} (*J*2.8) 129.9_{F-4} (*J* 8.3) 125.0_{F-5}(*J*3.0??) 115.6_{F-3} (*J*23.0) 160.6_{F2}(*J* 245.6) 123.8_{F1} (*J*14.4)? 128.1[b] 128.7[b] 127.2 139.7(q)	F_6: Ar(7.13s), $H\beta_4$-3(s), $H\beta_4$-2(m), OMe(w) F_5: Ar(7.50s) F_3: OMe(w)
Ala-5	CO			172.6	
	CH	4.39	overl	49.3	Me_{Ala5}(vs)[b], $NH\beta_6$(s),
	Me	1.01	d, *J* 7.2	16.5	H_{Ala5}(vs), NH_{Ala5}(vs), $NH\beta_6$(vs), Ar(7.28w, 7.17n, 7.10s,7.05 vw)

	NH	7.32	overl		Me_{Ala5}(vs), $H\beta_2$-2(s)
Beta- 6	CO			171.1	
	2	4.49	d, *J* 9.6	48.9(d, J_{CF} 5.3)	$H\beta_6$-3(s), $NH\beta_6$(vs), Ar(7.37 vs; 7.18s, 7.04w), OMe(w); F (-117.8)
	3	5.59	t, *J* 9.3	55.1	$NH\beta_6$(m), $H\beta_6$-2(m), Ar(7.28w, 7.18vs, 7.38s), $NH\beta_2$(vw), F (-117.8)
	NH	6.70	d, *J* 8.6		Me_{Ala5}(s), H_{Ala5}(m),[c] $H\beta_6$-2(s), $H\beta_6$-3(s), Ar(7.18 m)
	Arom	$7.51_{F\text{-}6}$ $7.27_{F\text{-}4}$ $7.13_{F\text{-}5}$ $7.03_{F\text{-}3}$ - - 7.30- 7.25		$130.0_{F\text{-}4}$ (*J* 2.8) $129.9_{F\text{-}4}$ (*J* 8.3) $125.0_{F\text{-}5}$(*J* 3.0??) $115.6_{F\text{-}3}$ (*J* 23.0) 160.6_{F2}(*J* 245.6) 123.8_{F1} (*J* 14.4) 128.1[b] 128.7[b] 127.2 139.7(q)	F_6: Ar(7.13s), $H\beta_4$-3(s), $H\beta_4$-2(m), OMe(w) F_5: Ar(7.50s) F_3: OMe(w)
	OMe	3.56	s	52.3	

[a]NOESYat293K.[b]Tentativelyassigned.

Complete NMR Characterization for Hexaapeptide**8a-D1** ($CDCl_3$, 10 mM,293 K, 500MHz)

AA	atom		^{1}H	Molteplicity *J* (Hz)	^{13}C	Noesy CH
Ala-1	CO				172.1	
	CH		4.05		49.7	Me_{Ala1}(vs)
	Me		0.77	*J* 7.0	19.6	NH_{Ala1}(w), CH_{Ala1}(s), Ar(7.1m, 7.16m, 7.61m), Me_{Ala5}(s)
	NH		5.49	*J* 6.8		Me_{Ala1}(vw)
	Boc	Me	1.40		28.8	$H\beta_6$-2(s),Arom (7.17)
		C			79.8	
		CO			155.8	
***Beta*-2**	CO				171.0	
	2		4.55	*J* 9.4	50.7(J_{CF} 2.2)	
	3		5.97	t*J*11.1	53.9	NH_{Ala3}(vs),Arom(7.61vs)
	NH		7.49	overl.		
	Arom		$7.61_{F\text{-}6}$ 7.16, 6.97 Ph: 7.43, 7.19, 7.15, 7.00		$129.5_{F\text{-}6}$ (brs) $129.7_{F\text{-}4}$ (*J* 8.5) $124.5_{F\text{-}5}$ (*J* 3.5) $116.0_{F\text{-}3}$ (*J* 22.2) 157.2_{F2} (*J* 246.1) 122.7_{F1} (*J* 14.1) 127.8^b 128.8^b 126.8 140.4 (q)	F_6: $NH\beta_2$(s), Ar(7.00vs),$H\beta_2$-3(s), $H\beta_2$-3(vs),Me_{Ala3}(w)
Ala-3	CO				171.4	
	CH		4.19		48.9	Me_{Ala3}(m),

	Me	0.32	*J* 6.9	18.8	H_{Ala3}(m), 6.96(vw), 7.17(vs),NH_{Ala3}(w), 7.49(m)
	NH	7.43	overl.		
Beta- 4	CO			171.1	
	2	4.27	d,*J*10.7	51.3	
	3	5.92	t*J*11.4	53.6	$H\beta_4$-2(w), $H\beta_2$-3(w),7.48(vs), 7.16(vs)
	NH	6.88	overl.		H_{Ala3}(vs), $H\beta_4$-2(m), $H\beta_4$-3(m), Ar(7.32 m)
	Arom	$7.48_{F\text{-}6}$ $7.25_{F\text{-}4}$ $7.13_{F\text{-}5}$ $7.06_{F\text{-}3}$ 7.00, 6.94 - 7.16- 7.25m		$129.9_{F\text{-}6}$ (*J* 2.0) $130.1_{F\text{-}4}$ (*J* 7.4) $125.0_{F\text{-}5}$ (*J* 3.5) $115.7_{F\text{-}3}$ (J 22.9) 161.1_{F2} (*J* 245.0) 124.3_{F1} (*J*13.9) 129.2[b] 128.6[b] 127.6 139.7(q)	F_6: Ar(7.13s), $H\beta_4$-3(s), $H\beta_4$-2(vw), OMe(w) F_5: Ar(7.50s) F_3: OMe(w), F(-118.45)
Ala-5	CO			171.0	
	CH	4.09		48.7	Me_{Ala5}(vs)
	Me	0.35	*J* 6.5	18.8	H_{Ala5}(vs), 6.92(vw), 7.02(s), 7.15 (vs), 7.50(w). 6.93(w), Me_{Ala1}(s)
	NH	6.91	overl.		
Beta- **6**	CO			171.0	
	2	4.41	*J*11.2	48.9	Boc(s), 7.19(vs), 7.50(s), $H\beta_6$-3(s),6.77(vwNH). OMe(m)
	3	5.69	t*J*10.6	54.1	$H\beta_6$-2(s),7.22(s), (7.50w, 7.19 vs)
	NH	6.76	overl.		

	Arom	Ph_{6F} 7.50, 7.17, 7.01 Ph: 7.22		Ph_F: 122.9 (*J*q), 129.6, 161.1 Ph:126,9, 139.6q	$H\beta_6$-3(vs),$H\beta_6$2(m),H_{Ala5}(w),
	OMe	3.44		52.4	

[a]NOESYat293K.[c]Tentativelyassigned.

Chapter 4 –Tetrahydroisoquinoline-4-carboxylic acid/β-Alanine, a β-Peptide Reverse Turn that Promotes Hairpin formation

4.Introduction

It is of general knowledge that different peptide shapes, mostly α- and 3_{10}-helixes, β-sheets, loops and turns typically characterize proteins structure.[1] Loops and turns, acting as linking motifs between different secondary structures such as α-helix and/or β-strands, represent a favourable tool to reverse the peptide backbone direction. Even if these structures are called random coils, they exhibit a significant amount of regular structural patterns[2]involving polypeptide segments of varying length. Short loops involving three, four, and five residues are well studied as β-turn[3]and α-turn,[4] respectively. A loop involving six residues, also called a α-turn, is assuming significant interest because it is found to be important as a helix-terminating motif in proteins.[5] Considering the enormous variety of sequences that can form such motifs, researches working on their identification are of relevance.[6]Focusing on the β-turn, that is generally constituted by four amino acids giving hydrogen bond between amino acid *i* and *i* + 3, it is known that it acts as folding nucleator by pre-organizing the pendant polypeptide chains, thereby lowering the activation barrier for β-sheet formation.[7]Small-molecule capable of mimicking peptide turn structures continue to be valuable in defining the residues and secondary structure responsible for the binding recognition and affinity, useful in validating new drug targets, and central to the subsequent development of new therapeutics[8;9](Figure 1).

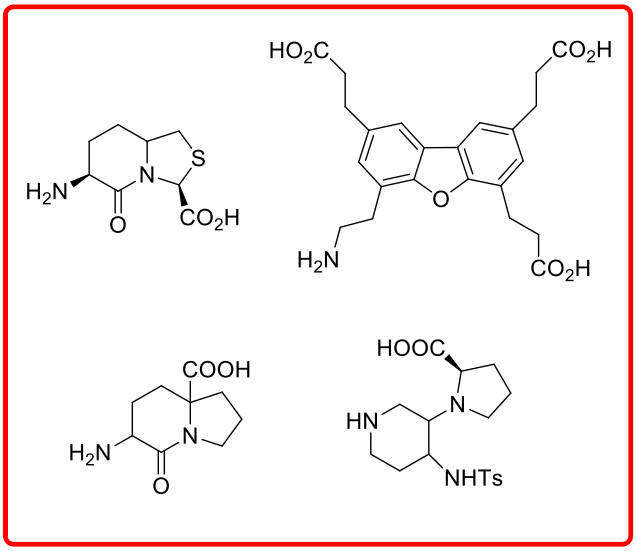
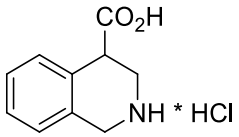


Figure 1

One of the most promising application for unnatural peptides containing turn-mimics is the modulation of protein-protein interactions (PPIs). Nevertheless, considering the flexibility of proteins and their adaptability in the formation of the protein-protein complex, peptidomimetics are supposed to be rigid only if the obtained molecular shape is exactly complementary to the binding partner. On the contrary, the use of semirigid-mimetics of secondary protein structures is a much more convenient approach[10].

The preparation of non natural amino acids,[11] and of scaffolds to be used as turn-mimicsas well as their use to prepare peptide model sequences is one of our main research field. In this work we focused on tetrahydroisoquinoline-4-carboxylic acid (**1**), named TIC (Figure 2), a constrained cyclic β-amino acid. To or knowledge, study on its ability to induce well defined structure when inserted in a peptide sequence are not reported. Furthermore, TIC is a very expensive commercially available compound which preparation was reported in the literature.Owing to the difficulty to repeat the known procedure,[12]here we focused on an easily new synthetic protocol to obtain its ester prepared in gram scale as well as on its enzymatic resolution.

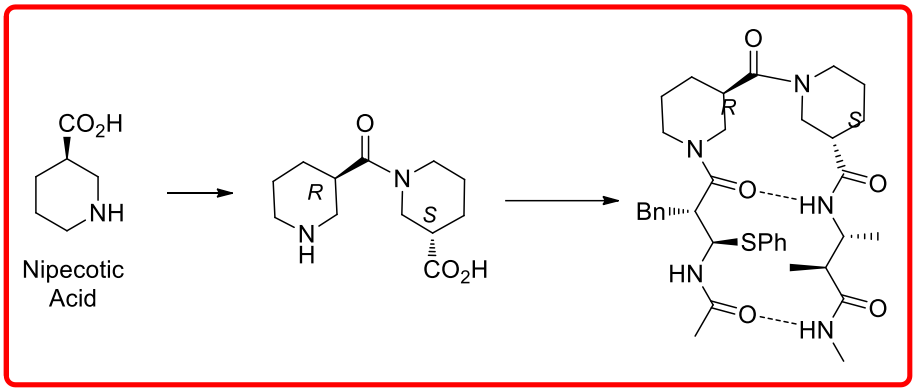


1-TIC

Figure 2

Even if it is reported in the literature that segments composed of acyclic and cycloalkane β-amino acids did not display revers turn propensity[13], our challenge has been to use compound **1** (TIC) in combination with β-alanine to generate this motif. This synthetic result is of relevance in view of the increased stability of β-amino acids toward proteases.

Gellman *at all.*[14] reported on the use of its parent amino acid, *i.e.* the pipecotic acid (Nip), as turn inducer. It is reported that when a single Nip unit is matched with an acyclic β-amino acid the formation of the turn was not observed. Instead, *R*-Nip-*S*-Nip dipeptide segment or the *S, R* counterpart, gives β-peptide reverse turns that promote the hairpin formation (Scheme 1). Instead, fragments containing the same stereochemistries are ineffective. This behaviour is also supported by other authors.[15]



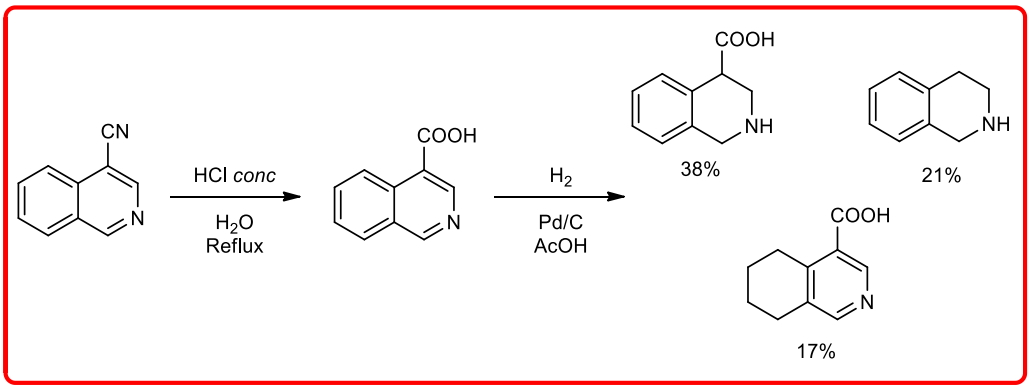
Scheme 1

On the other hand, the use of TIC for the preparation of peptide is reported on a data bank but without references. As a result, we explored the possibility to use this amino acid as turn inducer when inserted in short peptide sequences, *i.e.* the tetrapeptides Fmoc-NH-(*L*)-Ala-TIC-β-Ala-(*L*)-Val-OBn or Ac-NH-(*L*)-Ala-TIC-β-Ala-(*L*)-Val-NH_2. In order to probe the correlation between the configuration of the loop and strand residues, both TIC-stereoisomers were tested. As second amino acid to be inserted in the turn, β-Alanine was chosen since, like glycine, has a strong conformational flexibility and mostly does not possess stereocentre avoiding an increased number of isomers.

4.1 Results and discussion

4.1.1 Synthesis of amino acid 1 and its N-Boc derivative 5.

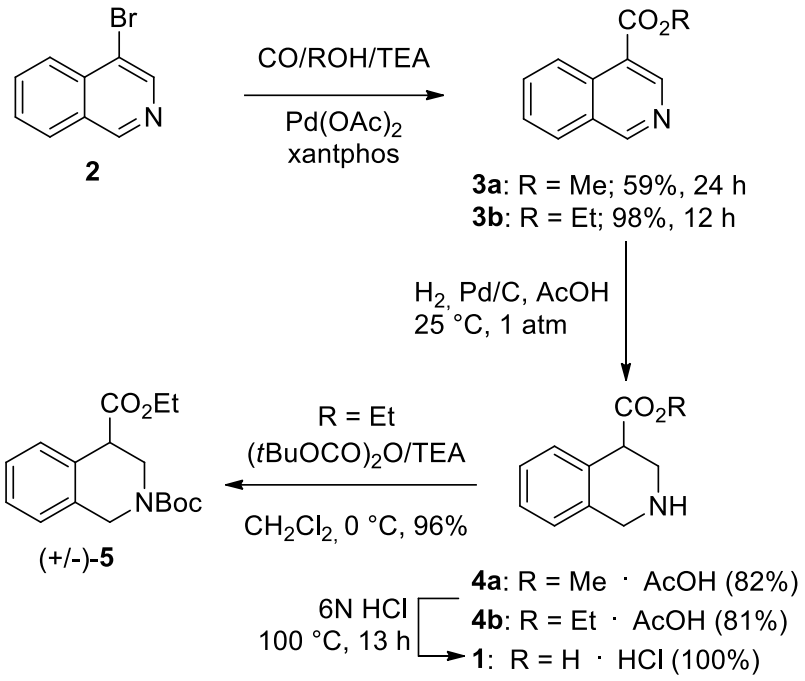
The preparation of TIC **1** is reported in the literature from 4-cyanoisoquinoline after acid hydrolysis followed by catalytic reduction.Nevertheless, we found that a decarboxylation occurs in the reductive step affording tetrahydroisoquinoline as by-product isolated in 21% yield together with the product reduced at the carbocycle (17%) (Scheme 2).



Scheme 2.

Owing to this difficulty we planned a different synthetic procedure using a protocol described for analogue compounds[16]. Compound **2** was transformed into methyl ester **3a** (59%) using a carbonylation reaction with CO operating in MeOH/TEA and in the presence of $Pd(OAc)_2$ and Xantphos as ligand (Scheme 3). Using EtOH instead of MeOH, ethyl ester **3b** was obtained in better yield (98%). The reduction of pyridine ring with H_2 in the presence of Pt_2O in AcOH gave the expected compounds **4a** (82%) and **4b** (81%) from esters **3a** and **3b**, respectively.

The preparation of the Boc-derivative **5 (**60%) was achieved staring from **4b** operating in dioxane/H_2O solution at 80 °C and in the presence of $NaHCO_3$ (2.2 eq.). The yield of **5** was dramatically increased (96%) using TEA (2.2 eq.) as the base and operating in CH_2Cl_2 at 25 °C for 26 h. To obtain the free amino acid **1** (HCl salt, quantitative yield), the hydrolysis of esters **4** was performed with 6N HCl (100 °C, 13h). It is to be underlined that, starting from the cheap commercially available 4-bromoisochinoline **2**, amino acid **1** was prepared in gram scale in three steps and 80% overall yield.



Scheme 3. Synthesis of tetrahydroisoquinoline-4-carboxylic acid derivatives

4.2 Studies on enzymatic resolution of N-Boc derivative 5.

Biocatalysis, year after year, is gaining a prominent role as a very efficient tool for the synthesis of target molecules not only in the lab,[17] but also at industrial scale, especially because it fits perfectly in the Green Chemistry approach,[18] which is gradually becoming a dominant paradigm for the chemical and

pharmaceutical industry. Moreover, thanks to the extraordinary and continuous progress of protein engineering, enzymes are becoming more and more efficient in terms of stability, specificity and selectivity, and also their availability as "off-the shelf" reagents is rapidly increasing.[19]

Among the six class of enzymes, hydrolases are by far the most employed and studied biocatalysts,[20] in terms of known sources, protein characterization, enzyme production, protein engineering, reaction optimization and industrial application. Overall, as noted by Faber, about two thirds of the total research in the biocatalysis field has been carried out using hydrolytic enzymes.[21]

In this context, we decided to devise and attempt a biocatalytic route for the resolution of TIC. The latter falls into the class of β^2-aminoacids, which is quite peculiar because, in contrast with their β^3 homolog, doesn't share a general approach for its enantioselective synthesis. In a comprehensive review covering the role of biocatalysis in providing enantiopure β-aminoacids, the authors reported that, to their knowledge, only two enzymatic resolutions of β^2-aminoacids had been described at the time, both of them providing poor results in terms of enantioselectivity and efficiency.[22] More recently, *Mohan* and coworkers have proposed oxazinones as suitable precursors for the enzymatic dynamic kinetic resolution of β^2-aminoacids:[23] unfortunately, this approach cannot be employed for our target compound because it would be necessary that the amino group was a primary one, which is not the case. The most similar analogues on which a kinetic resolution have been carried out, although with scarce success, have, to our knowledge, only been reported in a paper by *Kanerva et al*.[24]

Despite these not so encouraging premises, we established it would have been worthy to attempt the enzymatic resolution, first of all because of its simplicity, but also for the interesting potential that would disclose in case of success. In particular, we devoted our attention to the NBoc-protected ethyl ester **5**: this precursor proved to be stable to spontaneous hydrolysis in the chosen conditions (buffer at pH 8, 37°C), and at the end of the resolution would be easily employed in the coupling with other aminoacids. Moreover, the hydrogen in the α position respect to the carboxylic moiety is sufficiently acidic to permit the continuous racemization of the substrate,[25] and would put a base for try to work under dynamic kinetic resolution conditions.[26]

After an intensive screening, that involved about 40 commercial hydrolases belonging to various subclasses (esterases, lipases, proteases, peptidases), Pronase (a mixture of proteases extracted from the extracellular fluid of *Streptomyces griseus*) proved to be the most convenient catalyst in terms of activity and specificity, being able to mediate the stereoselective hydrolysis of the precursor with a not outstanding, yet significative, enantioselectivity (E=17). The hydrolysis was carried out in a water/DMSO mixture kept at pH 8 by means of an automatic titrator: when the conversion reached about 65-70%, we were able to isolate the starting ester with a very high enantiopurity (e.e.> 99%), together with the enantioenriched acid (e.e. 48%).

Thus, the enzymatic kinetic resolution was able to provide an enantiopure material which could be further employed in the synthesis of the target oligopeptide.

Enzyme screening

Enzyme screening have been carried out in microscale (500 µL) in an aqueous buffer at pH 8.0 containing 10% of DMSO in order to help to solubilize the substrate, which otherwise would stick to the vial walls. The reaction vials were inserted in a thermostated shaker and kept at 40°C together with a "blank" (without any enzyme) in order to monitor the progress of the possible spontaneous hydrolysis.

The reactions were analysed by TLC at determined intervals: in case of the presence of any hydrolytic activity in the vial, an aliquot was withdrawn and, after a suitable workup, submitted to chiral HPLC analysis. After having evaluated both the substrate and the product enantiomeric excesses, the parameter E (enantiomericratio)[27] was calculated together with the estimated conversion, the former representing the enzyme selectivity and the latter roughly indicating the activity.

In total, 48 hydrolitic enzymes, listed in Table 1, including lipases, proteases, esterases and a couple of enzymatic extracts have been tested. As a general trend, lipases are not active towards our substrate, while most proteases are active although not very selective.

After the preliminary screening , six different enzymes have been considered, which are evaluated in terms of price, activity, selectivity and commercial availability (Figure 3, red arrows). Pronase and yeast enzyme extract type III are the two preparation with greater selectivity, the first against the starting ester and the second against the product. Despite yeast enzyme extract type III has higher selectivity and activities with respect to Pronase, the extract is unfortunately no longer commercially available, for this reasons we have started our study on the resolution of ester **5** using Pronase

Enzyme class	ENZYME	Supplier	Active	e.e.(S) %	e.e.(P) %	Conv. %	E *	time (hours)
Esterases	ESTERASE BS2	CLEA Technologies	Yes	39	3	33	1,4	24
	ESTERASE BS3	CLEA Technologies	Yes	94	25	79	-4,9	24
	ESTERASE, from Hog Liver (code 46064)	Sigma-Aldrich	Yes	16	13	55	1,5	72
	ESTERASE, from Porcine Liver (code 3019)	Sigma-Aldrich	Yes	9	1	90	-1,1	50
	NAPROXENE ESTERASE	Chemi	No					
Lipases	AMANO LIPASE AK, from Ps. Fluorescens	Sigma-Aldrich	No					
	AMANO LIPASE G, from Pe. Camemberti	Sigma-Aldrich	No					
	CAL-A, adsorbed on celite	Novozymes	Yes	4	63	6	-4,6	24
	LIPASE AP6	Amano	No					
	LIPASE AY	Amano	No					
	LIPASE F-AP15	Amano	No					
	LIPASE, from Aspergillus Niger	Sigma-Aldrich	No					
	LIPASE, from Candida Lypolytica	Sigma-Aldrich	No					
	LIPASE, from Candida Rugosa	Sigma-Aldrich	No					
	LIPASE, from Rhizopus Niveus	Sigma-Aldrich	No					
	LIPASE, type I from Wheat Germ	Sigma-Aldrich	No					
	LIPASE, type VII from Candida Cylindracea	Sigma-Aldrich	No					
	LIPASE, type XI from Rhizopus Arhizus	Sigma-Aldrich	No					
	LIPASE PGE	Amano	No					
	LIPASE PS	Amano	No					
	LIPASE, from Mucor Javanicus	Sigma-Aldrich	No					
	LYPOZIME CALB-L	Novozymes	No					
	NOVOZYM 435	Novozymes	No					
Peptide amidase	PEPTIDE AMIDASE, from Citrus Sinensis	Juelich Fine Chemicals	No					
Proteases	ALCALASE 2,5 L	Novozymes	Yes	55	47	54	4,7	30
	ALCALASE 3,0 T	Novozymes	Yes	37	36	45	3,8	90
	ALKALINE PROTEASE	Enzeco	Yes	24	59	29	4	4
	CHIMOTRYPSIN	Novozymes	Yes	1	53	2	3,3	72
	ESPERASE 4,0T	Novozymes	Yes	6	81	7	10	24
	IMMOZYME ALC-T2-150	Chirazymes	Yes	14	67	17	5,8	72
	NEUTRASE 1,5 M	Novozymes	No					
	PROLEATHER FG-F	Novozymes	Yes	2	64	3	4,6	6
	PROLYVE 1000	Novozymes	Yes	14	49	22	3,3	24
	PROLYVE BS conc	Novozymes	No					
	PRONASE, from Streptomyces Griseus	Novozymes	Yes	89	72	55	17	50
	PROPERASI 1600L	Novozymes	Yes	6	77	7	8,2	24
	PROTEASE N	Amano	No					
	PROTEASE NL	Amano	No					
	PROTEASE S	Amano	No					
	PROTEASE type VII, from B. Licheniformis	Sigma-Aldrich	Yes	64	51	56	5,8	50
	PROTEINASE K, from Tr. Album	Sigma-Aldrich	Yes	30	74	29	8,9	24
	PURAFECT 4000L	Genencor	Yes	5	70	7	6	24
	PYRASE 250 MP	Novozymes	Yes	9	72	11	6,7	24
	SAVINASE (CLEA)	CLEA Technologies	Yes	18	85	17	14	24
	TRYPSIN 6.0	Novozymes	No					
	TRYPSIN, from Hog Pancreas	Sigma-Aldrich	Yes	2	65	3	4,8	24
Raw preparations	YEAST ENZYME CONCENTRATE, type II	Sigma-Aldrich	Yes	2	6	25	1,1	50
	YEAST ENZYME CONCENTRATE, type III	Sigma-Aldrich	Yes	31	96	24	-66	50

Table 1. Screening to assess the activity of different enzymes on the substrate 5 under the same reaction conditions

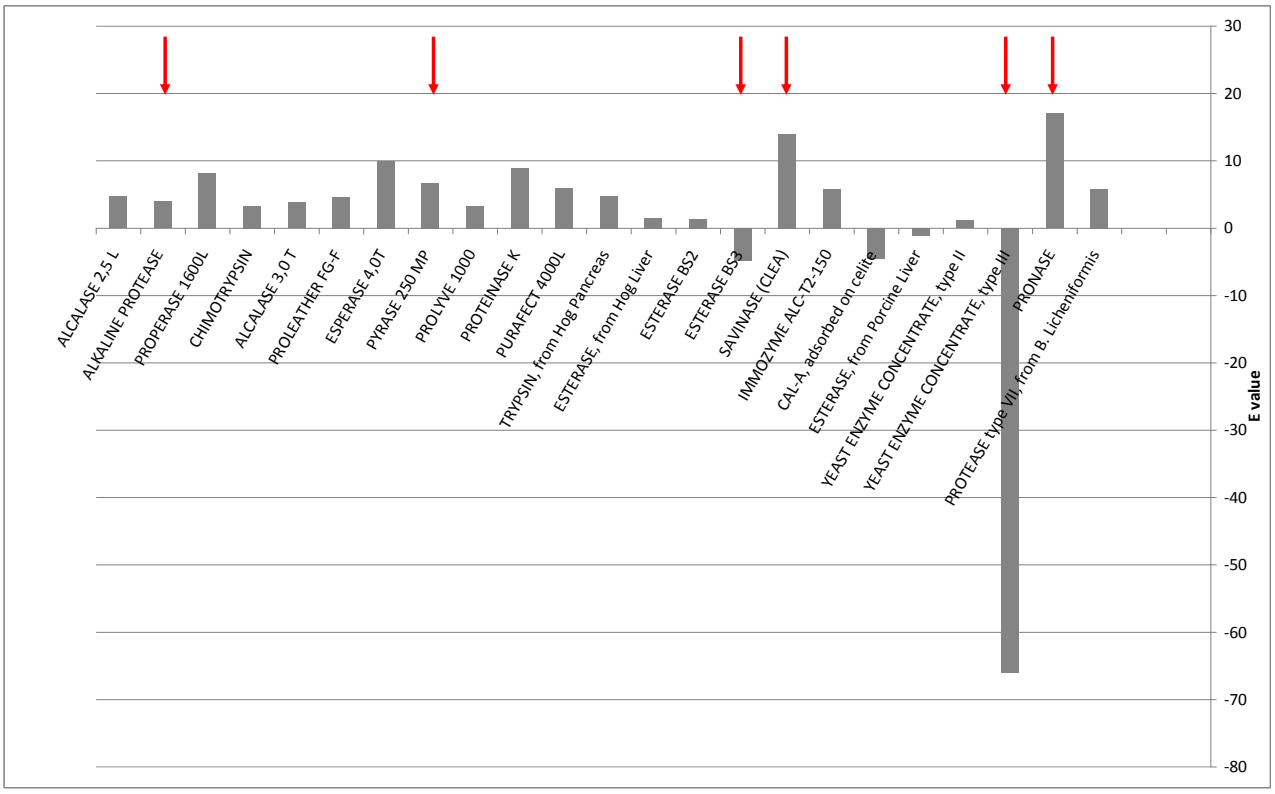


Figure 3. Screening of enzymatic activities on the substrate **5**. The arrows indicate enzymes with good selectivity for kinetic resolution.

4.2.1 Pronase

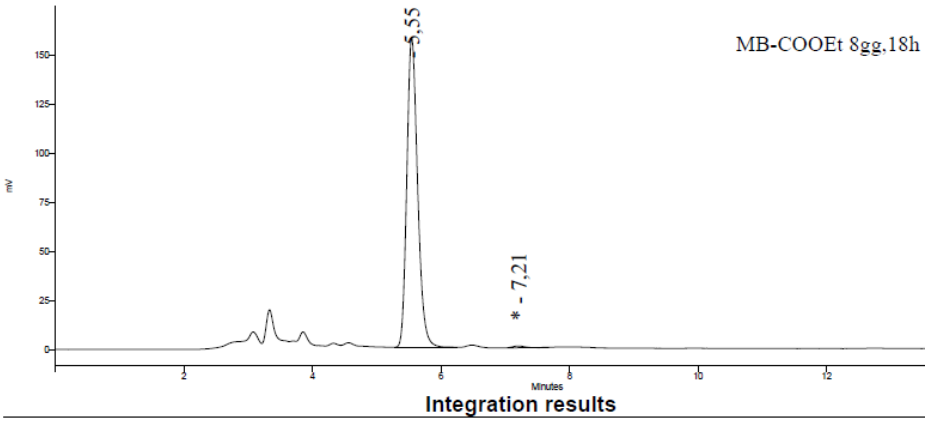
Pronase is the commercial name for a mixture of proteases extracted from the extracellular fluid of *Streptomyces griseus*. Despite the fact that this preparation is used for the degradation of peptides because it acts non-specifically on all the aminoacylic residues, Pronase proved to be one somehow selective against our substrate. Even more, among the commercially available preparations, it possessed the most desirable characteristics.

From this starting point, we began to explore new reaction conditions in terms of cosolvent and pH. Water-miscible cosolvents with a negative LogP usually constitute a good possibility for increasing the substrate concentration in the mixture without resulting too detrimental against the enzyme activity. DMSO is often a good choice, but also ethanol and DME (1,2-dimethoxyethane) can be very useful in this context. Moreover, the reaction pH can show a significant effect on the reaction outcome, especially affecting the enzyme activity. In Table 2 we present the results of the cosolvent and pH screening.

Table 2. Screening to assess the activity of Pronase*from Streptomyces Griseus*on the substrate **5** under different reaction conditions.

e.e. (S) %	e.e. (P) %	Conv. %	E *	cosolvent	pH
49	75	40	11	None	8.0
52	74	41	11	DMSO 5%	8.0
78	72	52	14	DMSO 10%	8.0
68	76	47	14	DMSO 20%	8.0
49	76	39	11	DMSO 5%	8.0
30	69	30	7.3	EtOH 5%	8.0
46	75	38	10	DME 5%	8.0
76	63	55	9.8	DMSO 10%	7.0
44	77	36	11	DMSO 10%	9.0
51	51	50	5	DMSO 10%	10.0

Unfortunately, it was not possible to get any improvement with respect to the initial conditions, where Pronase shows an enantiomeric ratio in the range 14-17. This behaviour, though being not optimal, permits to obtain an enantiopure residual ester at around 65% of conversion. Indeed, carrying out the reaction on preparative scale (1.5 g of **5**) employing a pH-state to monitor the reaction progress, we were able to make use of Pronase for the obtainment of an optically pure ester **(-)-5** (*ee*> 99%, Figure 4) which was in turn hydrolysed to yield the enantiopure acid **(-)-7** (Scheme 4).



Integration results

#	Peak name	Rt.	Area	% Area
1		5,55	1844,21	**99,54**
2		7,21	8,55	**0,46**
SUM			**1852,75**	**100,00**

Figure 4. Elution profile of the substrate **5** after the kinetic resolution with *Pronase*.

Non-selective hydrolysis in mild conditions

During the chemical hydrolysis of the enantiopure ester **(-)-5** in basic conditions (LiOH in MeOH/H_2O, 0 °C), a significative decrease in the optical purity of the obtained acid was observed. This was attributed to the basic enolization of the ester, promoted by the base, followed by a partial racemization. In order to solve this problem, we exploited the scarce selectivity shown by some enzymes during the screening, carrying out a non selective hydrolysis of the enantiopure ester in mild conditions (pH 8, 40°C), where the optical purity is conserved. In particular, employing the Enzeco *Alkaline Protease*, which possesses good activity ad very low selectivity, it was possible to convert the enantiopure *N*-Boc-aminoester (-)-**5** into the corresponding acid **(-)-7** preserving the enantiomeric excess (Scheme 4).

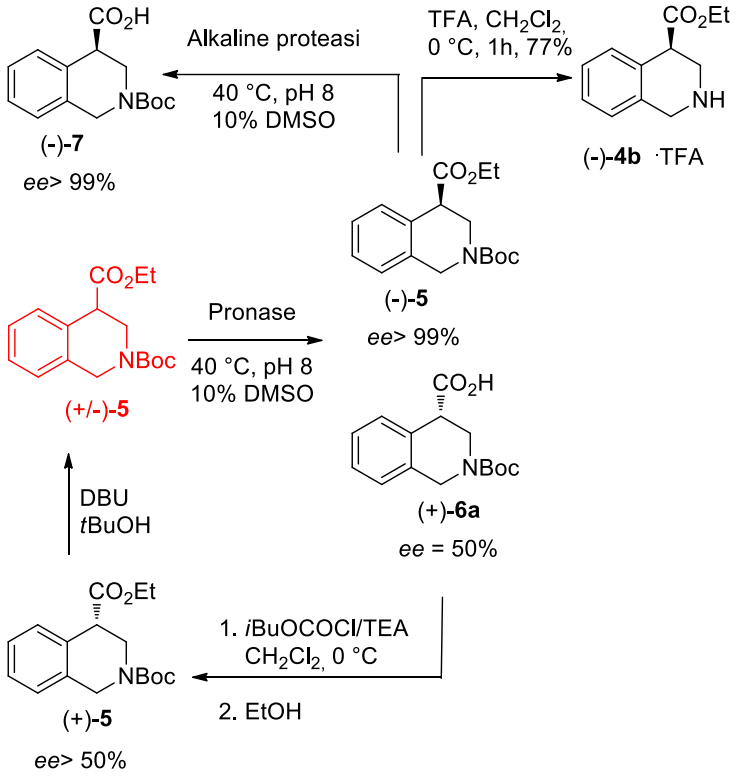
Recycle of the enantioenriched acid

At the end of the enzymatic resolution we could isolate the enantiopure *N*-Boc-aminoester **(-)-5** and an enantioenriched mixture of *N*-Boc-aminoacid **(+)-6** whose optical purity is however too scarce for its employment in a coupling. Thus, we decided to carry out an esterification of **(+)-6** (isobutyl chloroformate/TEA followed by ethanol) which yields an enantioenriched ethyl ester; the latter was racemized again employing DBU in *t*-BuOH (one night at 25 °C) in order to provide some further starting material for a successive resolution (Scheme 4).

General scheme of enzymatic resolution

A preparative protocol yielding one of the two enantiomers with high optical purity was developed and applied to the resolution of ester **5**. Employing two enzymatic preparation (Pronase and Enzeco Alkaline Protease), the former selective and the second unselective, we managed to obtain an enantiopure acid **(-)-7** which was employed in the peptide synthesis. The by-product, the enantioenriched acid of opposite

configuration **(+)-6**, was recycled back to be employed as starting material for the successive batch. The whole process is depicted in Scheme 4.



Scheme 4

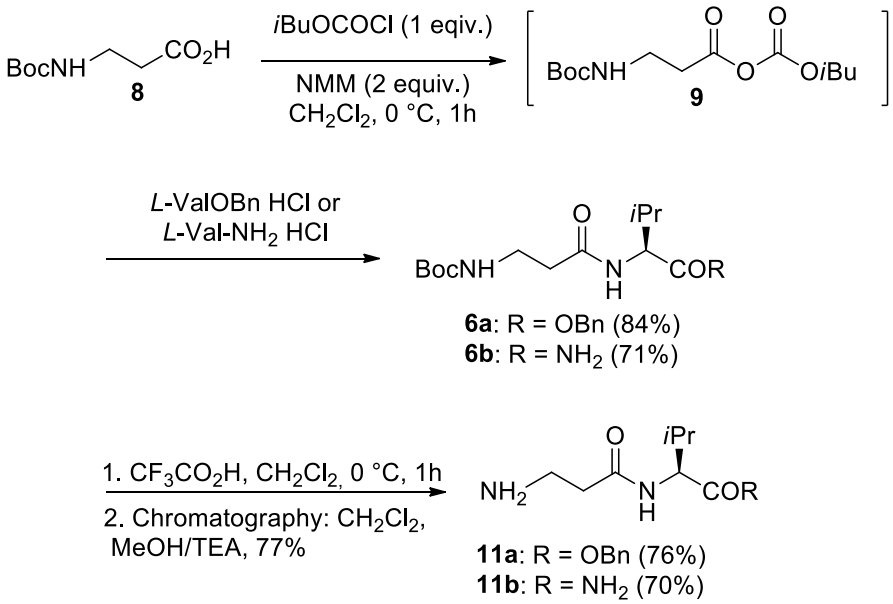
It must be emphasized that the absolute stereochemistry of the two enantiomers was not determined and will constitute the object for a future characterization.

4.3 Preparation of model tetrapeptides. Fmoc-NH-(L)Ala-TIC-βAla-(L)Val-OBn and Ac-NH-(L)Ala-TIC-βAla-(L)Val-NH₂.

To evaluate the ability of TIC to induce a turn and to understand the effect of its chirality, two model tetrapeptides, *i.e. FmocNH-(L)Ala-TIC-β-Ala-(L)Val-OBn*, and *Ac-NH-(L)Ala-TIC-βAla-(L)Val-NH₂*. containing both the *R*- and *S*- stereoisomer, were prepared.

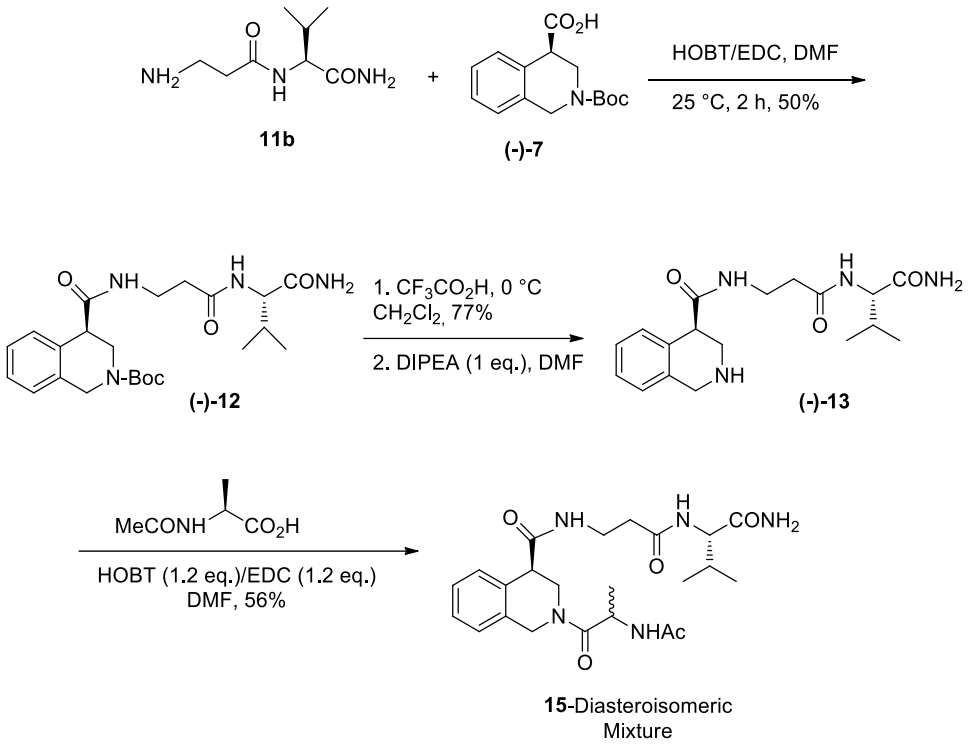
The synthesis of dipeptides Boc-NH-β-Ala-(*L*)-Val-OBn **6a** and Boc-NH-β-Ala-(*L*)-Val-NH_2 **6b** (Scheme 5) is not reported in the literature. Their preparation from Boc-β-alanine (**8**) and the benzyl ester of *L*-valine hydrochloride or Valinamide using a standard protocol [HOBT (1.2 equiv.)/EDC (1.2 equiv.), DIPEA (3 equiv.), CH_2Cl_2, 0.1 M] gave poor yields since a 2,6-dioxotetrahydropyrimidine by-product was formed. An increase of the yields for compounds **6a** and **6b** was achieved by transforming first **8** into its corresponding

anhydride **9** [*i*BuOCOCl (1 equiv),*N*-methylmorpholine (NMM, 2 equiv.), CH_2Cl_2, 0 °C, 1h)] that was not isolated. Its condensation with *L*-valineOBn·HCl gave dipeptide **6a** in 84% yield. On the other hand, the condensation with Valinamide affords compound **6b** in 71% yield. After nitrogen deprotection [CF_3CO_2H, CH_2Cl_2, 0 °C, 1h], dipeptide **11a** and **11b** was obtained respectively in 76% and 70% yields after column chromatography. (Scheme 5)



Scheme 5. Synthesis of dipeptide β-Ala-(*L*)-Val-COR

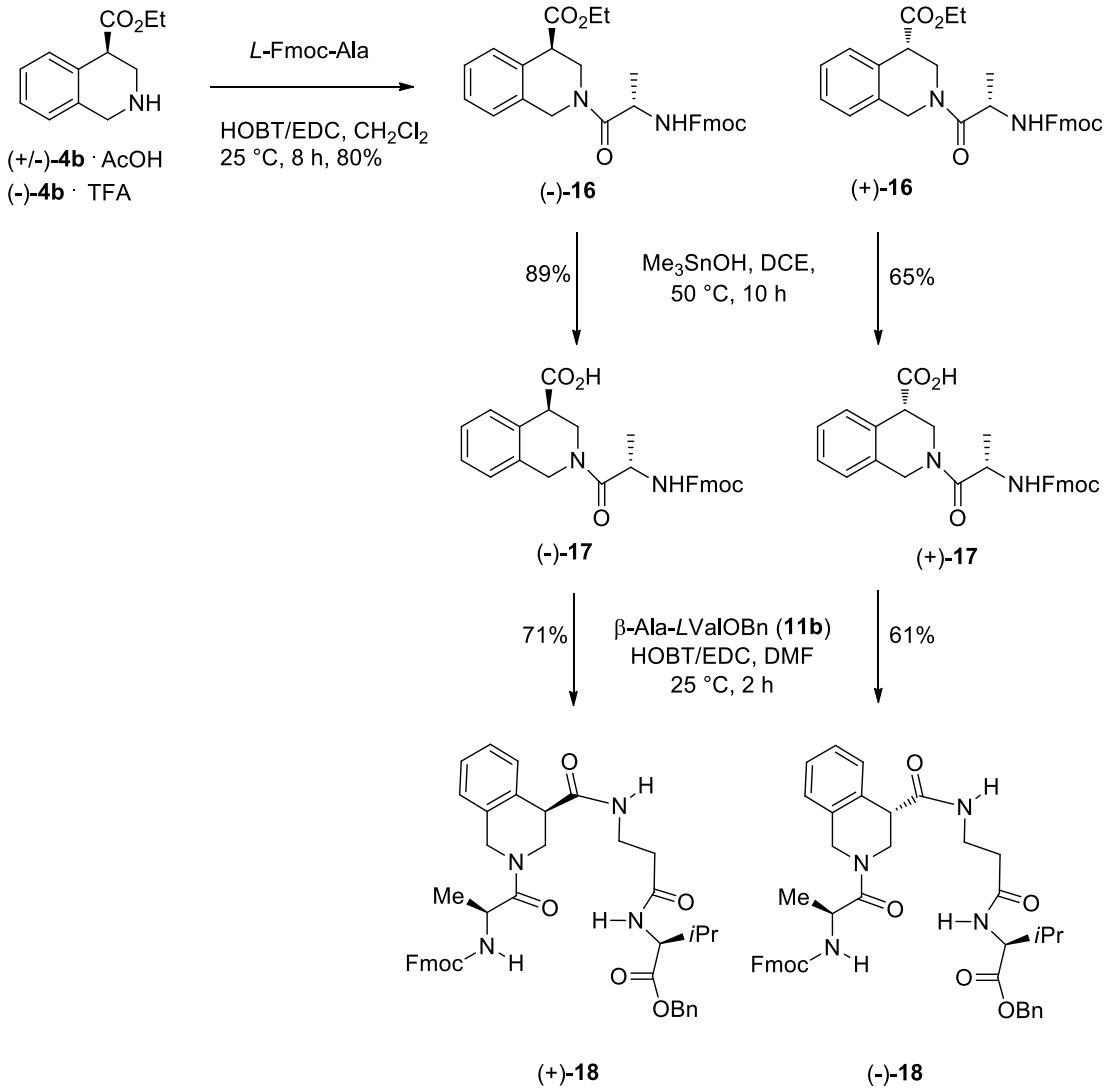
The reaction of **11b** with enantiopure acid (-)-**7** (Scheme 6) was performed in the presence of DCC in CH_2Cl_2 (25 °C, 3h). Unfortunately the reaction did not work. Instead, tripeptide Boc-*N*-(-)-TIC-β-Ala-(*L*)-Val-NH_2 (-)-**12** was obtained in satisfactory yield (50%) using HOBT/EDC as condensing agents (DMF, 25 °C, 2 h). Its deprotection at nitrogen atom with CF_3CO_2H (0 °C, CH_2Cl_2, °C, h) afforded (-)-**13** (77%) that was directly used. It was first treated with DIPEA (1 equiv.) in DMF and then made to react with *N*Ac-(*L*)-Alanine using the above condensing conditions (12h). Tetrapeptide AcNH-Ala-(-)-TIC-βAla-(*L*)Val-NH_2 (**15**) was isolated in 56% yield, but unfortunately, these reaction conditions result in the racemization of the Alanine residue, with the formation of unseparable isomers.



Scheme 6. Synthesis of tetrapeptide AcNH-Ala-TIC-βAla-Val-NH_2 (**15**)

In a second approach, ethyl ester of TIC (-)-**4b**·TFA was treated with Fmoc-*L*-Ala (HOBT/EDC/CH_2Cl_2, 25 °C, 8h) giving (-)-**16** in 80% yields (Scheme 7). The reaction condition for the hydrolysis of the ester must be carefully chosen because of the easily racemization of TIC stereocenter. Several conditions were tested (LiOH/MeOH; LiOH/THF/H_2O; NaOH/MeOH/DCM; KOH/H_2O), but acid (-)-**17** (89%) was successfully performed without racemisation only using Me_3SnOH (DCE, 50 °C, 10 h). Its reaction with NH_2–β-Ala-(*L)*-Val-OBn (**11a**)[HOBT/EDC, DMF, 25 °C, 2 h] gave tetrapeptide (+)-**18** (71%).

To understand the stereochemical effect of TIC when inserted into peptides, we repeated the above synthetic scheme starting from the racemic ester (+/-)-**4b**·AcOH aiming to obtain both isomers of **18**. A mixture of (+)-**16** and (-)-**16** were obtained from (+/-)-**4b** which were partially separated by column chromatography. Their isolation allowed assigning unequivocally the stereochemistry to each isomer by comparing their α_D values with the isomer (-)-**16**. Pure isomer (+)-**16** was then transformed into (-)-**18** (61%), *via* (+)-**17** (65%) (Scheme 7). The whole protocol could be performed from the racemic compound **4b** without separation of intermediates whereas the compounds **16** and **17** have a marked instability. Tetrapeptides (-)-**18** and (+)-**18** were easily separated and isolated in 64 and 67 % overall yield, respectively.



Scheme 7: Synthesis of Tetrapeptides 18

4.4 NMR Characterization

NMR characterization of peptides (+)-**18**

Both isomeric peptides (-)-**18** and (+)-**18** were fully characterized by NMR (^{1}H, ^{13}C, HMQC, HMBC, TOXY and ROESY experiments in $CDCl_3$, 20Mm, 500 MHz). As reported in the experimental section all resonances were assigned. ^{1}H NMR spectrum of peptides (+)-**18** showed the presence of two rotamers equally populate. It was found that the NHs of valine in each rotamer resonate at low field (δ 7.90 and 7.16, respectively) and that of β-alanine (δ 6.62 and 6.10) and alanine (δ 5.70 and 5.90) at higher fields. According to the literature data for β-peptides,these values suggest that NH of valine is involved in a H-bond. On the other hand, the chemical shifts of β-Ala suggest a completely non H-bonded amide protons. Being the alanine moiety functionalized with Fmoc, we can not define *a priori* the behaviour of its NH. Interestingly, the NH resonances of rotamers of each amino acid fall in the same region.

Noesy/Roesy experiments at different mixing times (300, 450, 600 ms) showed interesting information on the secondary structure of the peptide. Considering rotamer (+)-**18-A** significant Noes supporting the formation of a turn are those of H_{TIC}-3 with both H-3 of β-Ala and NH of valine. Furthermore, Me of Alanine shows proximity to H-1 of TIC (Figure 4).

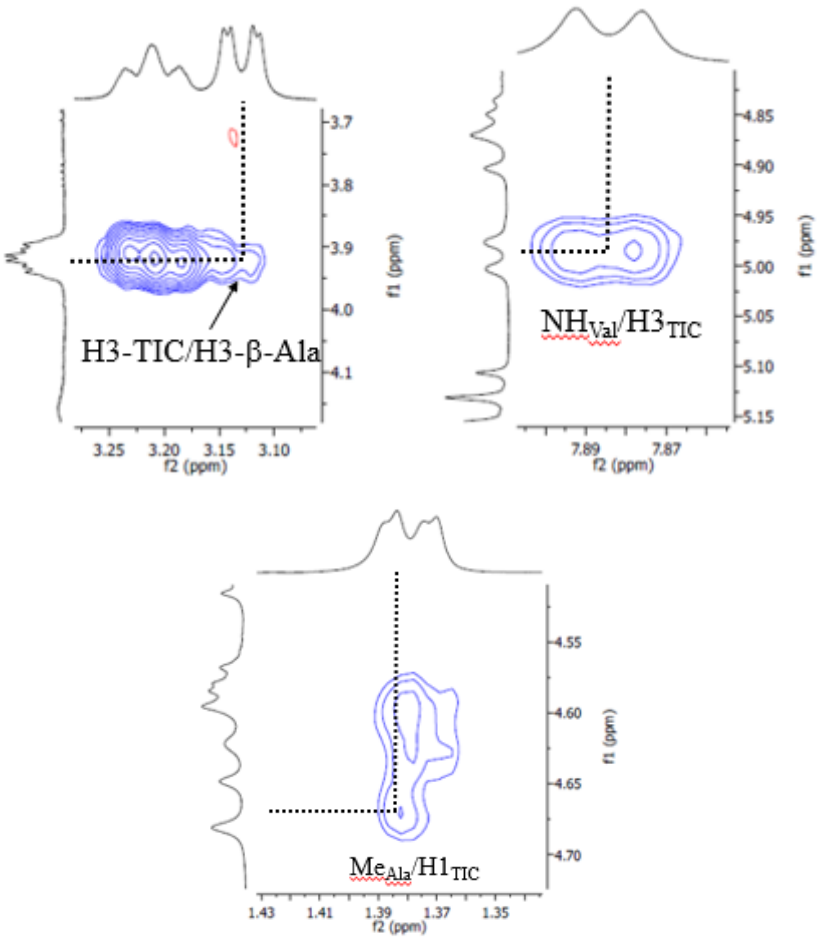


Figure 4. Significant Noes ($CDCl_3$, 600ms, 20mM) to define turn structures in rotamer **(+)-18-A**. A)$H3_{TIC}/H3_{\beta Ala}$Noe Zoom B) $NH_{Val}/H3_{TIC}$ Noe Zoom C) $Me_{Ala}/H1_{TIC}$Noe Zoom.

On the other hand rotamer (+)-**18-B** did not present interstrand Noes. Both H-α and Me of alanine show spatial proximity with H-3 of TIC indicating the formation of rotamers at tertiary amide accordingly with the literature data (Figure 5).[28]

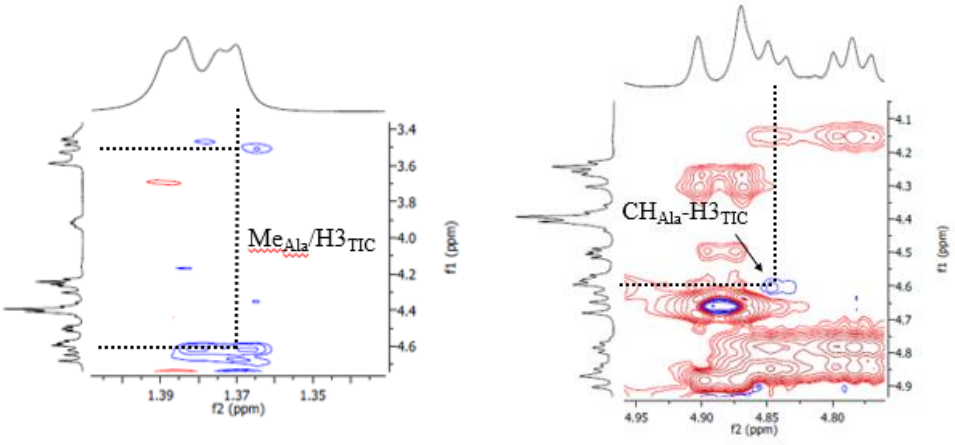


Figure 5. $Me_{Ala}/H3_{TIC}$ and $CH_{Ala}/H3_{TIC}$ Noes zoom in rotamer **(+)-18-B** ($CDCl_3$, 600ms, 20mM).

Since a different molteplicity was observed for NH signal of β-Ala (t and dd, respectively),our hypothesis is that the backbone of β-Ala is present in a *gauche* conformation in the first rotamer due to the formation of the 12-membered turn (Figure 6). On the other hand, considering *anti* conformation present in the second regioisomer, our hypothesis is that a bigger turn (15-membered ring) was formed between the NH of valine and CO of the Fmoc group (Figure 6).

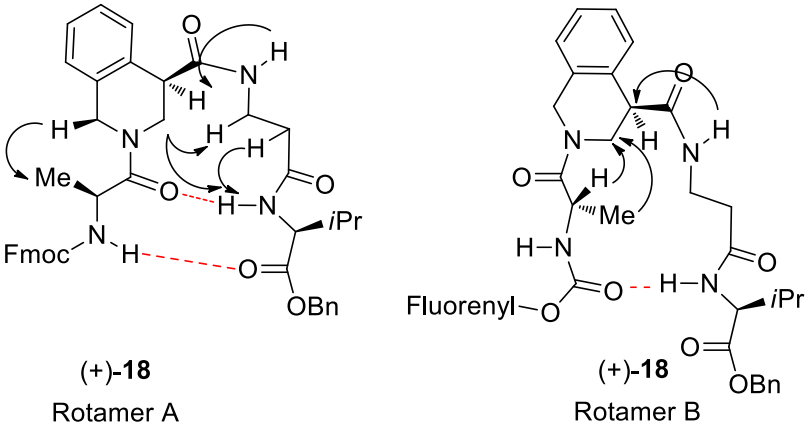


Figure 6. Secondary structures proposed for peptide (+)-18. Black arrows indicate the Noes in the NOESY spectrum. Red dotted line indicate H-Bonds

The solvent titration of the NH proton chemical shifts was also performed (Figure 7). The polar solvent DMSO added to the $CDCl_3$ solution induces similar chemical shift changes in both rotamers. A pronounced down field shift with increasing concentrations of DMSO was observed for β-Ala (Δδ–NH : 0.66 and 1.02) indicating its solvent exposure. The chemical shifts of NH-Val is insensitive (0.14 and 0.19, respectively), confirming its shielding from the solvent and its implication in intramolecular hydrogen bonding for both rotamers.The intermediate value for NH-Ala (Δδ 0.47 and 0.69) suggests that this NH is partially solvated, being at the *N*-termini, but it contributes to the H-network. Comparing the stability of the H-network of the

two rotamers, it is evident that the rotamer containing the *gouche*-β-Ala is less sensitive to DMSO indicating a stronger H-bond network.

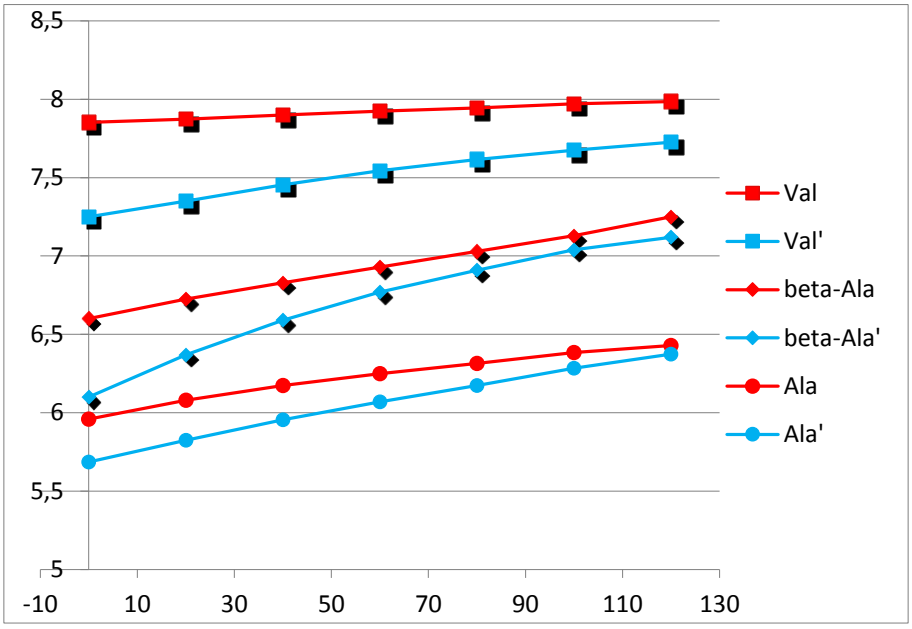


Figure 7. Plots of the NH proton chemical shifts in the ^{1}H NMR spectra of (+)-**18** as a function of the increasing amount of DMSO added to the $CDCl_3$ solution (20% v/v) (peptide concentration: 20 mM).

NMR characterization of peptides (-)-**18**

^{1}H NMR spectrum of peptides (-)-**18** showed the presence of two rotamers equally populate. Different from isomer **(+)-18** there is a large number of overlapped signals, for this reason a complete characterization of both rotamers is very difficult. Noesy/Roesy experiments were performed at different mixing times (300, 450, 600 ms) showed only qualitative information. Rotamer (-)-**18-A** did not present interstrand Noes and only a complete set of medium-weak i(i+1) Noes are present. Considering rotamer (-)-**18-B** significant Noe is beetween H_{TIC}-3 NH and of valine. (Figure 8).

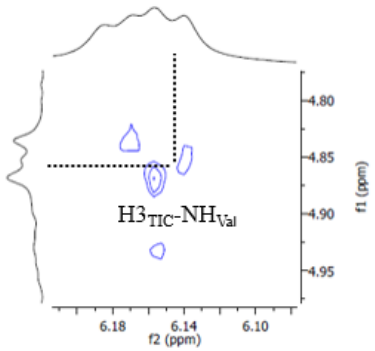


Figure 8. Zoom of $H3_{TIC}$-NH_{Val} Noe. ($CDCl_3$, 20mM, 600ms)

Even if some NH protons are overlapped with aromatic protons, solvent titration studies of NH proton chemical shifts were performed. Some information were achieved even if in some cases only qualitative evidences were detected.

The polar solvent DMSO added to the $CDCl_3$ solution induces similar chemical shift changes in both rotamers. A pronounced down field shift with increasing concentrations of DMSO was observed for β-Ala (Δδ–NH : 0.87 and 0.83) and Alanine (Δδ–NH : 0.74 and 1.7) indicating their solvent exposure. The chemical shifts of NH-Val is quite sensitive (0.54 (A) and 0.39 (B)), confirming its partial shielding from the solvent and its implication in weak intramolecular hydrogen bonding especially for rotamer (-)-**18-B** (Figure 9).

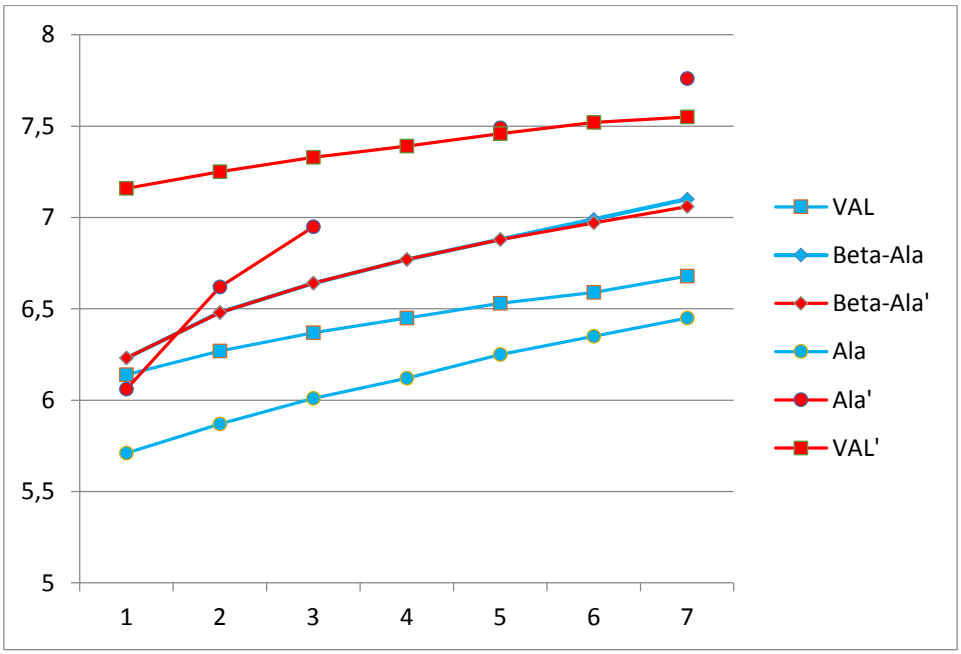


Figure 9. Plots of the NH proton chemical shifts in the ^{1}H NMR spectra of (-)-**18** as a function of the increasing amount of DMSO added to the $CDCl_3$ solution (20% v/v) (peptide concentration: 20 mM).

Despite further analysis are needed to univocally determine the structure of the peptide **(-)-18**, the collected data allow us to hypothesize an unfolded structures for rotamer **(-)-18-A** and a turn-like structures for rotamer **(-)-18-B** (Figure 10)

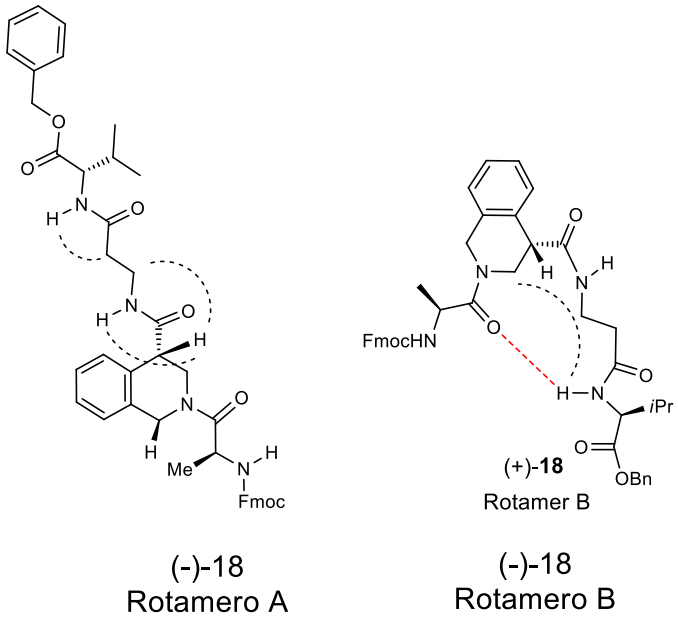


Figura 10. Proposed structures for rotamers (-)-18

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Experimental Section

Methyl 1,2,3,4-tetrahydroisoquinoline-4-carboxylate acetate (4a)

In a round bottomed flask Pt_2O (141 mg, 20 mol%) was suspended in acetic acid (62 mL). The solution was stirred under H_2 atmosphere for 15 min. and then methyl isoquinoline-4-carboxylate **3a** (1.25 g, 6.2 mmol) was added. The mixture was stirred under H_2 atmosphere for 24 hours at 25 °C for 24 h. After filtration over a celite pad, the solvent was removed under reduce pressure. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH, from 80:1 to 20:1) giving pure compound **4a**

4a:yellow oil (1.06 mg, 82%).^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.1 (s, 2H, exch), 7.29 - 7.05 (m, 4H), 4.15 (q, 2H, *J* = 6.9), 3.78, 3.63, 3.34 (ABX system, 3H, J_1 = 13.7 Hz, J_2= 4.9 Hz, J_3 = 2.9 Hz), 3.71 (s, 3H), 1.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) : δ = 21.6, 41.9, 44.3, 45.8, 52.3, 126.6, 126.9, 127.7, 129.6, 130.2, 133.7, 173.6, 175.8 (ppm); MS (GM): *m/z*: 191.0; elemental analysis calcd (%) for $C_{13}H_{17}NO_4$: C,62.14; H, 6.82; N, 5.57; found C, 62.32; H, 7.00; N, 5.75.

Ethyl 1,2,3,4-tetrahydroisoquinoline-4-carboxylate acetate (4b)

4b: pale yellow oil (78%). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.64 (s, 2H, exch), 7.33-7.20 (m, 3H), 7.18-7.04 (m, 1H), 4.15 (q, 2H, *J* = 7.0 Hz), 4.07 (s, 2H), 3.73, 3.54, 3.15 (ABX system, 3H, J_1=13.6 Hz, J_2= 4.0 Hz, J_3= 2.9 Hz), 1.97 (s, 3H), 1.24 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz) : δ = 14.3, 22.2, 42.1, 44.6, 46.2, 61.6, 126.8, 127.0, 127.9, 129.8, 130.6, 134.2, 173.6, 176.1; IR (NaCl) ν_{max} = 3584, 2981, 1728 cm^{-1}; MS (ESI): *m/z*: 206.2 $[M]^+$; elemental analysis calcd (%) for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28; found C, 63.68; H, 7.40; N, 4.67.

4-carboxy-1,2,3,4-tetrahydroisoquinolin-2-ium chloride (1)

Operating in a sealed tube, compound **4b**(1.10 mg, 4.0 mmol) was suspended in HCl (6 N, 25 mL) and the mixture was heated at 120°C for 9 hours. The solvent was removed under reduced pressure and the residue was crystallized affording pure **1**(700 mg, 3.28 mmol, 82.5%) .

1: mp 237.4°C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 10 (brs, 3H), 7.21-7.45 (m, 4H), 4.23 (s, 2H), 4.10 (t, 1H, *J* = 5.5 Hz), 3.14-3.61 (m, 2H), 2.49; ^{13}C NMR (DMSO-d_6, 50 MHz) : δ = 41.1, 42.7, 44.2, 127.7, 128.2, 128.3, 129.6, 129.8, 130.3, 173.2; IR (KBr) ν_{max} = 3030, 1725, 1609 cm^{-1}; MS (ESI): *m/z*: 178.1 $[M]^+$; elemental analysis calcd (%) for $C_{10}H_{12}ClNO_2$: C, 56.21; H, 5.66; Cl, 16.59; N, 6.56; O, 14.98 found C, 56.43; H, 5.75; Cl, 16.70; N, 5.92; O, 15.20.

2-*tert*-butyl-4-ethyl 3,4-dihydroisoquinoline-2,4(1H)-dicarboxylate (5)

In a three necked round bottom flask equipped with a magnetic stirrer, nitrogen inlet, and thermometer, compound **4b** (260 mg, 1.0 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and the solution was cooled to 0°C. Triethylamine (0.9 mL, 2.1 mmol) was added. After 10 min., di-*tert*-butyl dicarbonate (187 mg, 1.2 mmol) was slowly added and the stirring was continued for further 60 min. at 25 °C. The reaction mixture was washed with $KHSO_4$ (pH=3, 0.5 N, 5 mL) and then dried over Na_2SO_4.The solvent was removed under reduced pressure and product **5** was obtained as a colorless oil (330 mg, 92%).

5[1]: α_D ($CHCl_3$) = -56.1 ;^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.27-12 (m, 4H), 4.73, 4.48 (AB system, 2H, *J* = 17.0 Hz), 4.24, 3.59 (ABX system, 2H, J_1= 13.2 Hz, J_2= 5.1 Hz, J_3= 4.4 Hz), 4.17 (q, 2H, *J* = 7.1 Hz), 3.79 (m, 1H), 1.48 (s, 9H), 1.27 (t, 3H, *J* = 3. 65); ^{13}C NMR ($CDCl_3$, 200 MHz): δ = 14.4, 28.6 (x3), 44.1, 45.1, 46.0, 61.3, 80.3, 126.7 (x2), 127.7, 129.1, 131.9, 133.9, 154.9, 172.2 ; IR (NaCl) υ_{max} = 2979, 1736, 1699 cm^{-1}; MS (ESI): *m/z*: 328.2 $[M + Na]^+$; elemental analysis calcd (%) for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59; O, 20.96; found C, 66.06; H, 7.80; N, 4.10; O, 21.04.

2-(tert-butoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (7)

Racemic Compound **5**(500 mg, 1.6 mmol) was dissolved in a mixture of H_2O/ MeOH (1:3, 20 mL), the solution was cooled to 0°C and LiOH *H_2O (275 mg, 6.5 mmol) was added. The reaction was stirred overnight at room temperature. AcOEt (30 mL) was added. The aqueous layer was separated, acidified with $KHSO_4$ (20 mL, ss) and extracted with AcOEt (30 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the product **7**was obtained as a colorless oil (440 mg, quantitative).

7[2]: α_D ($CHCl_3$) = -15.4 ;^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.29-7.12 (m, 4H), 4.79, 4.45 (AB system, 2H, *J* = 16.9), 4.34, 3.59 (ABX system, 3H, J_1= 13.2 Hz, J_2= 4.4 Hz, J_3= 3.6 Hz,), 3.82 (t, 1H, *J* = 3.6 Hz), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 200 MHz): δ = 28.6 (x3), 43.6, 44.7, 45.8, 80.7, 126.9 (x2), 127.9, 129.3, 131.2, 133.8, 155.1, 177.6; IR (NaCl) ν_{max} = 2977, 1694, 1428 cm^{-1}; MS (ESI): *m/z*: 300.1 $[M + Na+]^+$; elemental

[1]Data reported for compound (-)-**5** resolved with Pronase from *Streptomyces Griseus*

[2]The enantiopure compound (-)-**7** was synthesized enzymatically with *Alkaline Protease*

analysis calcd (%) for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05; O, 23.08; found C, 65.20; H, 7.02; N, 4.58; O, 23.21.

(S)-benzyl 2-(3-((tert-butoxycarbonyl)amino)propanamido)-3-methylbutanoate (6a)

(S)-Tert-butyl (3-((1-amino-3-methyl-1-oxobutan-2-yl)amino)-3-oxopropyl)carbamate (6b)

Boc-NH-β-Alanine(500 mg, 2.64 mmol) was dissolved in DCM (20 mL), the solution was cooled to 0°C and then NMM (1 Equiv.)and iBuOCOCl (1 Equiv.) were added. The reaction was stirred at 0°C for 1h. A saturated solution of $NaHCO_3$ (30 mL) was added. The aqueous layer was separated, and extracted twice with DCM (20 mL) .The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the product **9**was obtained as a colorless oil.
Compound ***9*** (400 mg, 1.59 mmol) was dissolved in DCM (15 ml) at 0°C and Valine derivativse (Benzyl ester hydrochloride or carboxamide hydrochloride 1 Equiv.) was added. After 5 min. NMM (1 Equiv.) was added dropwise. The reaction was stirred at room temperature for 12h. A saturated solution of NH_4Cl (30 mL) was then added.The aqueous layer was separated, and extracted twice with DCM (20 mL) . The Organic layer was washed twice with NaCl ss. (30 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product **6a** (84%)or **6b** (71%)were obtained as a colorless oil and used without further purification.

(S)-benzyl 2-(3-aminopropanamido)-3-methylbutanoate (11a)
(S)-2-(3-aminopropanamido)-3-methylbutanamide (11b)

Compound **6a** or **6b**(500 mg) was dissolved in DCM (20 mL), the solution was cooled to 0°C and then TFA (5 ml)was added. The reaction was stirred at 0°C for 1h.The solvent was removed under reduced pressure and the crude mixture purified by column cromatoghraphy.(**11a:** DCM:MeOH 30:1 + 0.3% TEA, yield 76%. **11b:** DCM:MeOH 12:1 + 0.3% TEA yield 70%).

11a:α_D ($CHCl_3$) = +11.3 ;^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.8 (d, *J* = 8.4 Hz, 1H), 7.10-7.40 (m, 5H), 5.18 (AB system, 2H, J_1= 12.4 Hz, J_2= 19 Hz,), 4.58 (dd,*J* = 5.0 Hz,*J* = 9.0 Hz, 1H), 3.76-3.39 (m, 3H), 2.98 (t, 2H, *J* = 6.10 Hz), 2.21 (m, 1H), 1.95 (s, 2H, exch), 0.98-0.76 (m, 6H); IR (NaCl) υ_{max} = 2755, 1731, 1678 cm^{-1}; MS (ESI): *m/z*: 278.1 $[M + H]^+$

11b:α_D ($CHCl_3$) = +37.2 ;^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.83 (d, *J* = 8.1 Hz, 1H), 6.63 (s, 2H), 4.54 (dd,*J* = 4.9 Hz,*J* = 8.6 Hz, 1H), 3.75-3.61 (m, 3H), 2.94 (t, 2H, *J* = 5.2 Hz), 2.16 (m, 1H), 1.89 (s, 2H, exch), 0.93-0.74 (m, 6H); IR (NaCl) υ_{max} = 2731, 1740, 1650 cm^{-1}; MS (ESI): *m/z*: 187.1 $[M]^+$

*HPLC Analysis for Biocatalysis resolution of ester **5***

Micro workup

During the resolution step, 20 µL of the reaction mixture were poured in a 1,5 mL Eppendorf tube together with 250 µL of diethylether and 250 µL of TRIS buffer (100 Mm, pH 8). The tube was capped and shaken: after phase separation, most of the organic layer was carefully transferred using a Pasteur pipette in a new Eppendorf tube together with some anhydrous sodium sulfate. A second extraction of the aqueous phase was carried out, and the ethereal phase has been added to the previous one. After shaking and decanting, the surnatant was filtered and injected in HPLC for the chiral analysis of the ester **5**. The aqueous phase was brought to pH 2 with HCl 1M and extracted twice with 250 µL of diethylether. The organic phases were brought together in a new Eppendorf tube, treated with sodium sulfate, filtered and put in an ice bath. Then, under an efficient hood, a few drops of an ethereal solution of diazomethane were added till a persistent yellowish color was developed. At this point, a gentle nitrogen stream was directed into the Eppendorf tube for evaporating the excess diazomethane and then the solvent. The residue was redissolved in hexane and injected in HPLC for the chiral analysis of the acid **7**.

Analysis conditions

The chiral analysis was performed in isocratic conditions (hexane:2-propanol 9:1, flow 1 mL/min) employing a Chiralcel OD column (250 x 4.6 mm) kept at room temperature and following the absorbance at 210 nm.

*Kinetic enzymatic resolution of **(+/-)-5***

A 250 mL three necked round-bottomed flask was immersed in a thermostatic oil bath, equipped with a magnetic stirrer and connected to an automatic titration device through the necks (burette, electrode, temperature probe). The bath temperature was set to to 40°C and the titrator bottle was filled with NaOH 0,5 M.

In the flask, 75 mg of TRIS (0,6 mmol) were dissolved in water (120 mL), together with 1.00 g of Pronase, and the pH was adjusted to 8 with NaOH 1M. Compound **5** (1.50 g; 5.2 mmol) was separately dissolved in DMSO (12mL) and added dropwiseto the solution under constant stirring.

The titrator was regulated for keeping pH 8, switched on, and the data collection was started. At fixed intervals, aliquots were taken from the solution and analyzed by HPLC: when e.e. (substrate **5**) was found to be about 99% (usually around 65-70% of conversion, after 6-8 days), the titration was switched off and the stirring was interrupted. The reaction mixture was poured in a separatoryfunnel and extracted twice with diethylether. The organic layers were brought together, dried on anhydrous sodium sulfate and the solvent was removed, leaving an oily residue (compound **(-)-5**, 432 mg, e.e. 99%). The aqueous phase was acidified to pH 3 with HCl 6M and extracted twice with diethylether. The organic layers were brought together, dried

on anhydrous sodium sulfate and the solvent was removed, leaving an oily residue (compound (+)-6, 432 mg, e.e. 48%).

Complete NMR Characterization for tetrapeptide (+)-18 rotamer A ($CDCl_3$, 20 mM,293 K, 500MHz)					
AA	**atom**	**^{1}H δ**	**Molteplicity** *J* **(Hz)**	**^{13}C**	**Noesy**
Ala	CO			172.4	
	CH	4.78			Me_{Ala}(s), NH_{Ala}(w)
	Me	1.385	d, *J*6.8	19.1	H-1_{TIC}(4.89,s), CH_{Ala}(s)
	NH	5.70	d, *J*8.0		CH_{Ala}(m), Me_{Ala}(w)
	Fmoc	4.24 (CH) 4.44-,4.36 (CH_2) Arom: 7.78, 7.41, 7.32, 7.61		47.2, 66.9, 155.6 Arom: 120.2, 125.3, 127.3, 144.4, 143.8	Arom (7.61
TIC	CO			171.0	
	4	3.59	m	45.7	Arom: 7.13(m),$NH_{\beta Ala}$(m), H-3_{TIC}(4.99,m; 3.13,m)
	3	4.99, 3.13	dd, *J* 13.3, 3.2	43.5	
	1	4.89, 4.67	d, *J* 16.3	46.2	Me_{Ala}(s), 7.21 H-3_{TIC}(3.13,vw), 7.20
	Arom	7.13		129.8 128.5 127.2 127.1 *C:* 133.6,131.3	H-4_{TIC}(3.81)
β-Ala	CO			173.5	
	2	2.44 2.26	m	35.2	7.18(w), NH_{Val}(m) H-$3_{\beta Ala}$(3.92,vw)
	3	3.92	m	36.5	H-$2_{\beta Ala}$(2.26,vw)

		3.21			H-$2_{\beta Ala}$(2.26,vw), H-4_{TIC}(w)
	NH	6.62	dd, *J* 12.2, 4.7		H-4_{TIC}(s), H-$3_{\beta Ala}$(vw)
Val	CO			173.5	
	CH	4.58	Overl.	59.6	Me_{Val}(1.02,s), CH_{val}(s)
	CH	2.38-2.25	m	30.2	
	Me	1.02 0.98	d, *J*6.8 d, *J*6.8	19.3 18.2	NH_{Val}(m), CH_{val}(s) 7.17
	OCH_2Ph	5.31, 5.17 7.79,7.41, 7.33	d, *J* 12.3	66.9 Arom: 135.4, 128.3,	
	NH	7.90	d, *J*8.2		H-3_{TIC}(w), CH_{Val}(m), Me_{Val}(s), Me_2CH_{Val}(s), H-$2_{\beta Ala}$(vs)

(+)-18 rotamer B ($CDCl_3$, 20 mM,293 K, 500MHz)					
AA	**atom**	^{1}H δ	Molteplicity *J* (Hz)	^{13}C δ	Noesy
Ala	CO			171.8	
	CH	4.85			H-3_{TIC}(4.61,s), Me_{Ala}(m)
	Me	1.38	d, *J*6.8		H-3_{TIC}(4.61,w), CH_{Ala}(vs), NH_{Ala}(s)
	NH	5.96	d, *J*7.1		CH_{Ala}(m), Me_{Ala}(m),
	Fmoc	4.24 (CH) 4.44-,4.36 (CH_2) Arom: 7.78, 7.41, 7.32, 7.61		47.2, 66.9, 155.6 Arom: 120.2, 125.3, 127.3, 144.4, 143.8	Arom: 7.65(s)
TIC	CO			170.9	
	4	3.53	m	46.1	Arom: 7.15(vs),$NH_{βAla}$(m), H-3_{TIC}(4.61,s)
	3	4.61, 3.47	dd, *J* 13.9, 3.6	45.0	CH_{Ala}(vw) H-1_{TIC}(3.47,w)
	1	5.32, 4.28	d, *J* 12.0	44.6	H-3_{TIC}(4.61,s) H-3_{TIC}(3.47,w) Arom: 7.21(w),
	Arom	7.21, 7.15		129.8? 128.5? 127.4? 127.2 *C:* 133.4,131.3	H-4_{TIC} 3.81
β-Ala	CO			172.2	
	2	2.47, 2.32	m	35.2	

	3	3.56, 3.43	m	36.5	
	NH	6.10	dd		H-4_{TIC}(s), H-$3_{\beta Ala}$(vw)
Val	CO			172.5	
	CH	4.51	dd, *J* 8.4, 5.5	59.6	Me_{Val}(s), CH_{Val}(m), 7.10(w)
	CH	2.21-2.13	m	30.2	
	Me	0.88, 0.85	d, *J*6.9 d, *J*6.9	19.1 17.9	
	OCH_2Ph	5.19, 5.12 7.62, 7.42, 7.33	d, *J* 12.2	67.2 Arom: 135.6, 128.2	
	NH	7.16	Overl.		

Complete NMR Characterization for tetrapeptide (-)-18 rotamer A ($CDCl_3$, 20 mM,293 K, 500MHz)					
AA	**atom**	^{1}H δ	Molteplicity *J* (Hz)	^{13}C	Noesy
Ala	CO			172.7	
	CH	4.86		47.1	Me_{Ala}(s), NH_{Ala}(w)
	Me	1.45	d	19.0	H-1_{TIC} (4.89,s), CH_{Ala}(s)
	NH	5.75	d, *J* 8.0		CH_{Ala}(m), Me_{Ala}(w)
	Fmoc	4.38 (CH) 4.22 (CH_2)			
TIC	CO			171.0	
	4	3.73	m	46.2	Arom: 7.13(m), $NH_{\beta Ala}$(m), H-3_{TIC} (4.99,m; 3.13,m)
	3	4.88, 3.39	dd	42.8	
	1	4.53; 3.63	dd	44.9	Me_{Ala}(s), H-3_{TIC} (3.13,vw)
	Arom				
β-Ala	CO			173.5	
	2	2.45 2.41	m	35.2	NH_{Val}(m) H-$3_{\beta Ala}$(3.92,vw)
	3	3.51 3.47	m	35.8	H-$2_{\beta Ala}$(2.26,vw)
	NH	6.28	dd,		H-4_{TIC}(s), H-$3_{\beta Ala}$(vw)
Val	CO			171.7	
	CH	4.56		57.1	
	CH	2.18	m	31.2	
	Me	0.87 0.92	d, d,	17.8 14.0	

	OCH_2Ph	5.19, 5.15	d, *J* 12.3	66.9	
	NH	6.14	d, *J* 8.3		$H\text{-}2_{\beta Ala}$

(-)-18 rotamer B ($CDCl_3$, 20 mM,293 K, 500MHz)					
AA	**atom**	^{1}H δ	Molteplicity *J* (Hz)	^{13}C	Noesy
Ala	CO			171.8	
	CH	4.92		47.3	Me_{Ala}(s), NH_{Ala}(w)
	Me	1.41	d		CH_{Ala}(s)
	NH	6.16	d		CH_{Ala}(m), Me_{Ala}(w)
	Fmoc	4.35 (CH) 4.22 (CH_2)		47.1, 66.9, 155.6 Arom: 120.2, 125.3, 127.3, 144.4, 143.8	
TIC	CO			171.0	
	4	3.59	m	45.7	$NH_{\beta Ala}$(m), H-3_{TIC} (4.99,m; 3.13,m)
	3	4.98, 3.13	dd	43.5	
	1	4.85, 4.62	d	45.4	H-3_{TIC} (3.63m)
	Arom			129.8 128.5 127.2 127.1 *C:* 133.6, 131.3	
β-Ala	CO			173.5	
	2	2.41 2.35	m	35.4	NH_{Val}(m) H-$3_{\beta Ala}$(3.92,vw)
	3	3.67 3.35s	m	35.5	H-$2_{\beta Ala}$(2.26,vw)
	NH	6.29	dd		H-4_{TIC}(s), H-$3_{\beta Ala}$(vw)
	CO			171.8	

Val	CH	4.52	Overl.	57.3	Me_{Val}(0.92,s), CH_{val}(s)2.17, 5.03(vw)
	CH	2.17	m	31.9	
	Me	0.85 0.97	d d	17.7 17.8	NH_{Val}(m), CH_{val}(s)
	OCH_2Ph	5.21, 5.17	d	67.1	
	NH	6.86	d, *J* 7.0		H-4_{TIC}(w), CH_{Val}(m), Me_{Val}(s), Me_2CH_{Val}(s),

Chapter 5 – Rhodium catalyzed transformation of β-(α-diazo carbonyl)-piperidine derivatives

5.Introduction

α-Diazocarbonyl-derivatives are characterized by quite an exceptional flexibility in synthesis, [1] reacting catalytically with numerous transition metals and their salts forming reactive intermediates. Rhodium-(II) carboxylate catalysts induce their transformation into carbenes whose reactivity ranges from cyclopropanation to X-H (X = C, O, N, S) insertion and cyclization/cycloaddition domino cascade reaction.[1b,2] Furthermore, the introduction of these catalysts opened the way to several synthetic studies related to the Büchner reactions.[1b,2f,g,3] The use of diazocarbonyl compounds in the intramolecular reactions is of relevance especially when starting from polyfunctionalized compounds. As a result, the selectivity for C-H insertion in competition with other carbene-based transformations has been a cause of considerable interest.[1b,2a,c,4,5] In this way it is possible to achieve numerous carbocyclic and heterocyclic compounds, otherwise difficult to obtain.[1b,2a,c-g,4] The success of these approaches is related to the high level of regio- and stereocontrol that in general is guaranteed by the selection of the different available Rh(II)-catalysts.[2a,c,d,5b]

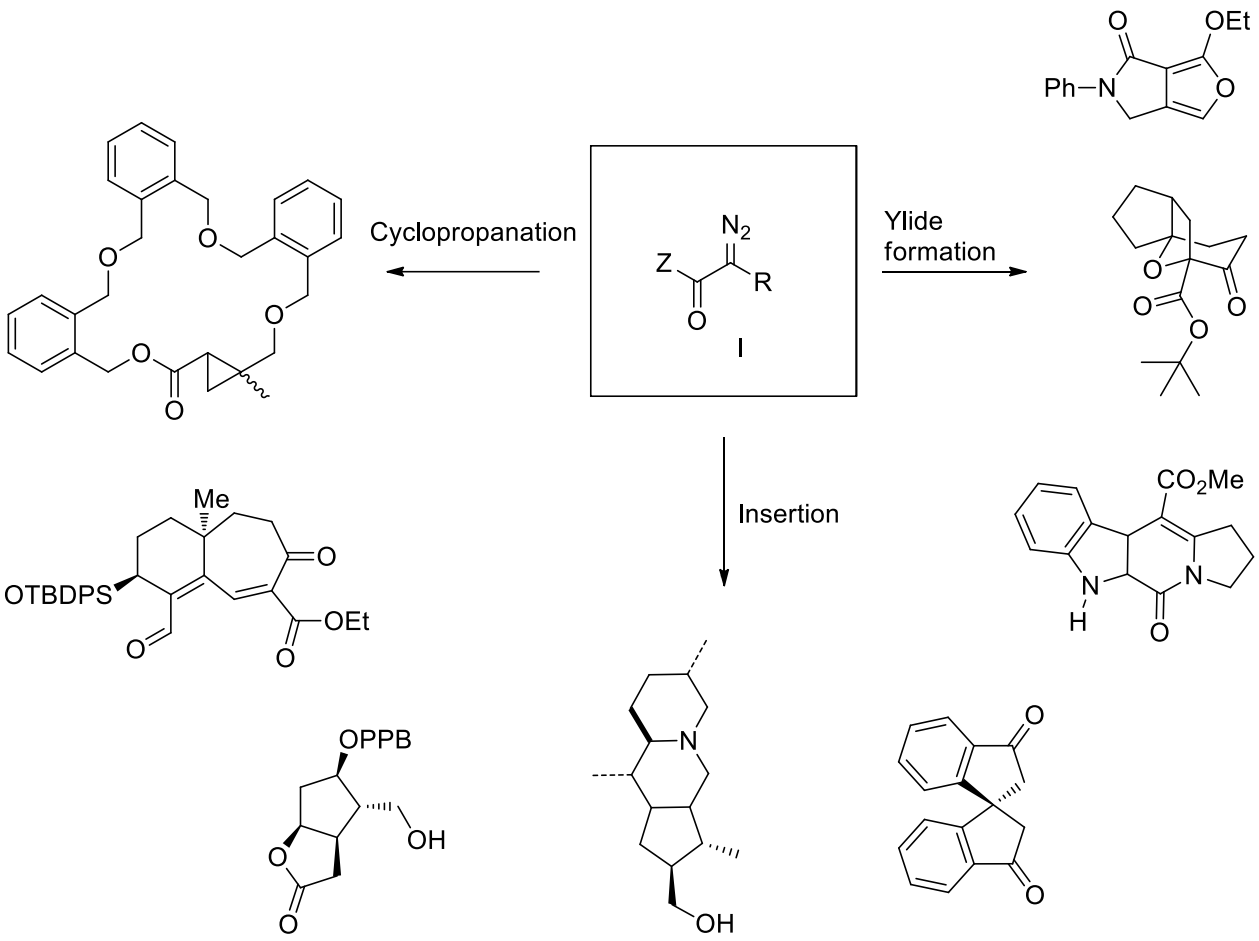
Before to detail my researches, an overview of the potentiality of rhodium (II) catalysts in organic transformations is reported.

5.1 Rhodium catalysts: an overview on their synthetic applications

The use of carbenes as synthetic intermediates has attracted considerable interest since a half centuary most of all because of their potential in forming a carbon-carbon bond. Their generation can occur from diazoalkanes with loss of nitrogen which can be brought about photochemically and thermally or, as was initially reported, by the use of a transition metal, most of all copper catalysts.

In particular, α-diazocarbonyl compounds **I**, react stoichiometrically with many Bronsted acids and electrophiles and catalytically with numerous transition metals and their salts forming reactive intermediates such as free carbenes, carbenoids (complexed carbenes), carbonyl ylides, and diazonium cations.

The most serviceable of diazocarbonyl reactions are cyclopropanation, Wolff rearrangement, insertion into unactivated C-H bonds, aromatic cycloaddition, α,α–substitution, dipolar addition, acid-catalyzed cyclization of unsaturated substrates, dimerization, electrophilic aromatic substitution, oxidation and ylide formation followed by sigmatropic rearrangement. Some applications are reported in the scheme 1.



Scheme 1

Undoubtedly, the high level of interest in diazocarbonyl compounds as synthetic intermediates is due to intramolecular reactions.

The most significant contribution to the usefulness of diazocarbonyls in synthesis was the discovery by Teyssié group of rhodium carboxylate catalysts, as alternative to Cu catalysts. The Belgian group first introduced rhodium(II) acetate and later was able to modulate catalytic activity using the trifluoroacetate, pivalate, and octanoate.

The dirhodium(II) carboxylates and the rhodium (II) carboxamides are especially important for their different electrophilic profiles. Electron-withdrawing ligands such as the carboxylates augment the catalyst's electrophilic character, increasing the reactivity towards diazo decomposition. Catalysts with electron-donating bridged ligands are often recognized as more selective, but on the other hand higher reactivity is required from the diazo compound in order to form the reactive metal-carbene.

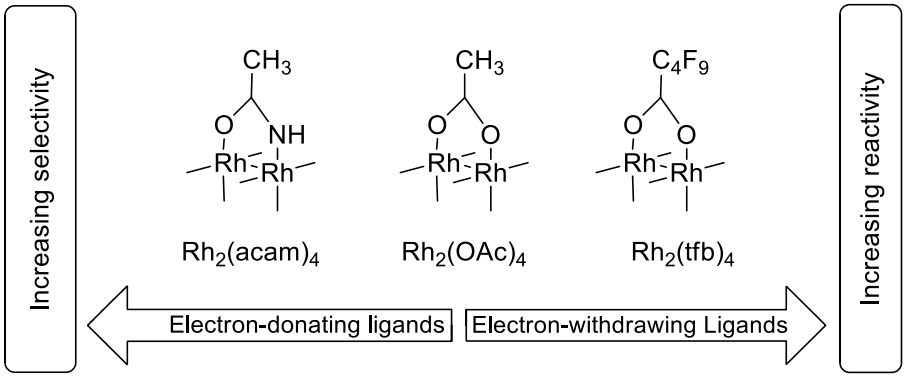


Figure 1

Nowadays, chiral Rh(II) catalysts are available and are used for asymmetric synthesis. Some examples are reported in Figure 2.

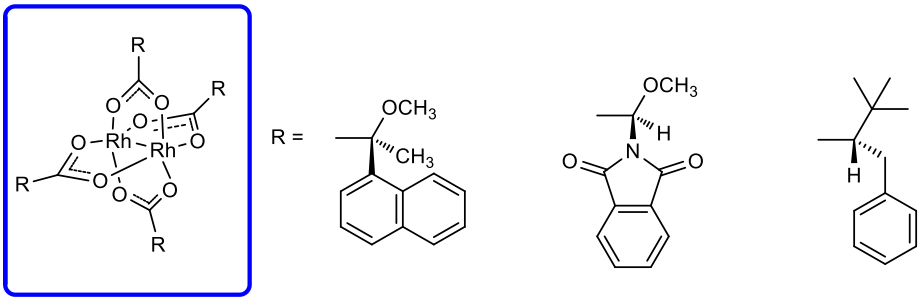
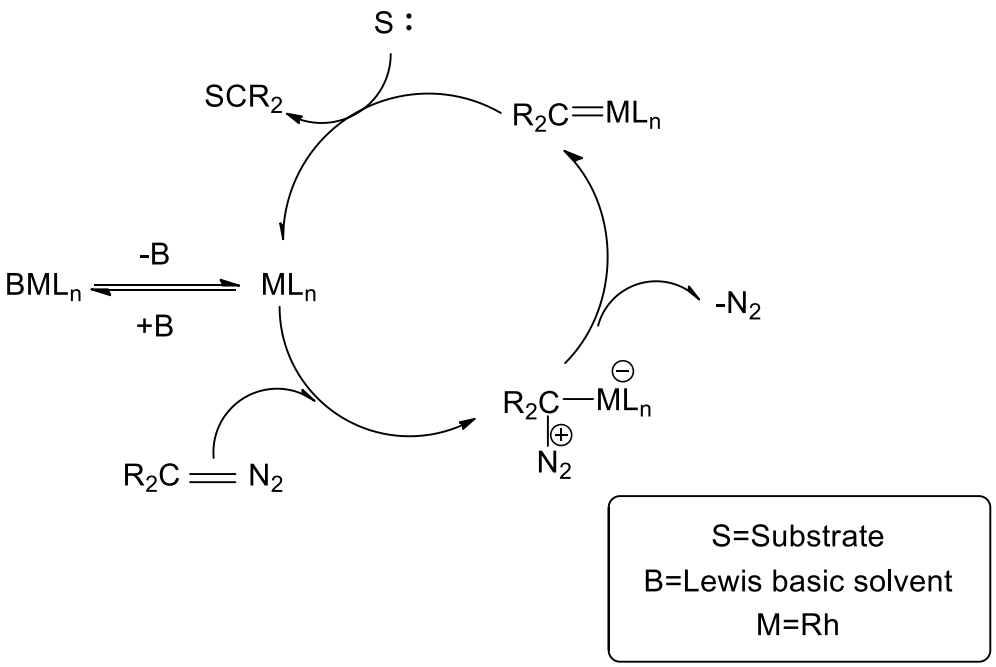


Figure 2

The accepted mechanism for the dirhodium(II)-catalysed transformations of diazo compounds consists in a catalytic cycle (Scheme 2) in which, after solvent decomplexation from the metal atom, a nucleophilic attack of the diazo compound on the electrophilic catalyst occurs, affording the intermediate ylide **II**, which upon nitrogen extrusion generates a metal-stabilized carbene **III**. Product formation takes place when the electrophilic carbene is transferred to an electronrich substrate, thus recovering the catalyst.



Scheme 2

Here below resumed the possible transformations of diazocarbonyl compounds that have been of interest in the course of my work are summarized.

5.1.1 H-CR¹R²R³ insertion

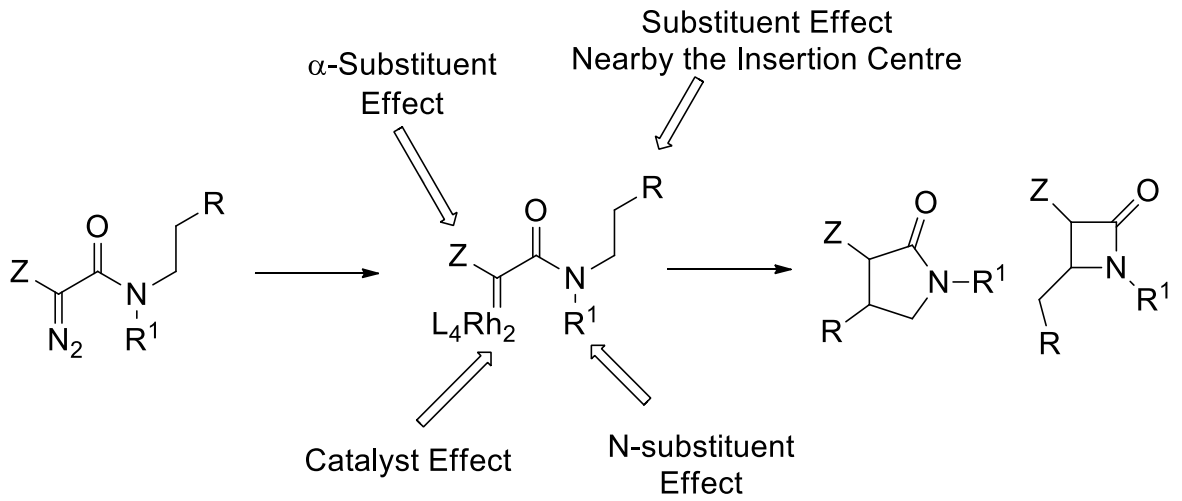
The past 30 years have witnessed a significant increase in the utilization of diazocarbonyl compounds as precursors in carbon-carbon bond-forming reactions (*Ber. Dtsch. Chem. Ges.* **1942**, *75*, 1610).

Intramolecular carbene insertion into C-H bonds has assumed strategic importance in organic synthesis. Insertion reactions of carbenes into C-H bonds have attracted much attention since the first discovery by Meerwein, Rathjen, and Werner.

The insertion process can proceed intermolecularly or intramolecularly. From the synthetic point of view, intermolecular C-H insertion is not generally useful because of low selectivity and competition. Intramolecular insertion of keto carbenes into unactivated C-H bonds sometimes allows transformations which would otherwise be difficult to achieve. In recent years, the dirhodium(II)-catalysed intramolecular C-H insertion has emerged as a general strategy for the construction of numerous cyclic and heterocyclic compounds. The most widely recognized are the stereoselective outcome of the insertion process and the overwhelming preference for five membered-ring formation, even if also four membered-ring formation, *i.e.* β- and γ-lactams, is

reported. (Scheme 3)

The regioselectivity which leads to the control of ring size in a particular molecule depends upon the type of diazo function, the degree of substitution of the carbon where insertion takes place, and steric and electronic factors. In general, the reactivity order is tertiary > secondary >> primary C-H bond. The success of this approach is related to the level of regio- and stereocontrol and, in some cases, to the high enantioselectivity of the C-H insertion process.



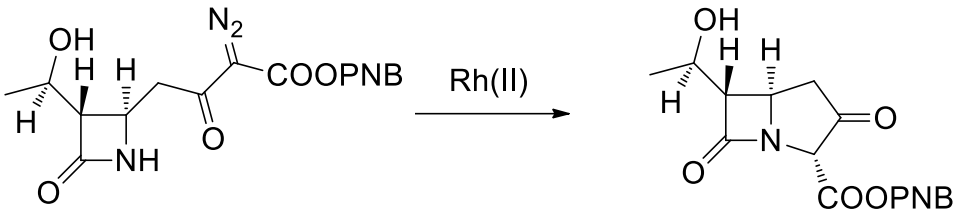
Scheme 3

5.1.2 H-NR[1]R[2] insertion

The insertion of α-keto carbenes or carbenoids into N-H bonds attracted little attention as a synthetic route to α–amino ketones or esters until 1978 when the first example of its use in bicyclic β-lactam synthesis was published from Merck laboratories (*Tetrahedron Lett.* **1978**, 4233).

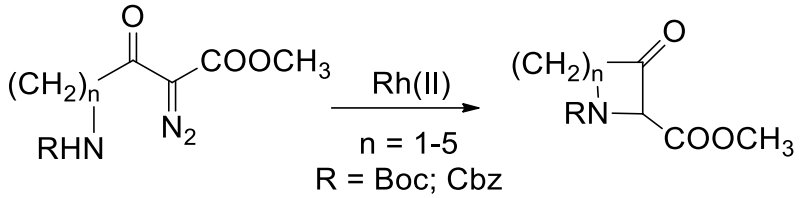
Since then, there have been numerous demonstrations of the power of this reaction, especially the intramolecular version leading to nitrogen heterocycles.

By far the most successful N-H insertions have been in intramolecular reactions leading to small-ring bicyclic heterocycles. The conversion of a penicillin analogue into the carbapenem nucleus via rhodium-catalyzed insertion of a keto carbenoid into the N-H bond of the beta-lactam was the key step in the Merck synthesis of the antibiotic thienamycin (*J. Am. Chem. Soc.* **1980,** *102*, 6161). (Scheme 4)



Scheme 4

In general intramolecular carbenoid N-H insertion has been demonstrated to be a mild, efficient, and regiospecific method for the construction of various-sized aza rings starting from *t*-butoxycarbonyl or benzyloxycarbonyl protected amines. (Scheme 5)



Scheme 5

5.1.3 Ylide Formation and Subsequent Reactions

Carbenoids derived from α-diazocarbonyl compounds exhibit highly electrophilic properties. They can readily react with an available heteroatom to effect ylide formation.

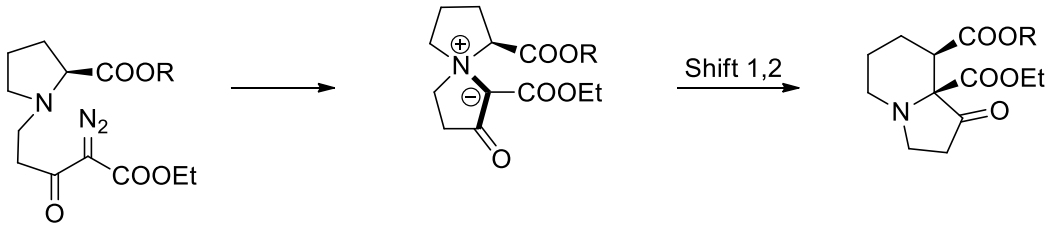
Chemical transformations of ylides have shown great versatility in the synthesis of natural products as well as other complex molecules.

Recently, ylides derived from α-diazocarbonyl compounds have received considerable attention and numerous examples concerning the application of ylide generation via catalytic methods are reported in the literature.

There have been many reports of ylide formation by catalytic methods, followed by Stevens [1,2]-shift leading to the formation of new carbon-carbon bonds. Although concerted [1,2]-shifts are forbidden processes according to the "Woodward-Hoffmann rules", the many reports of apparent [1,2]-shift of ylides derived from reaction of carbenoids and heteroatoms suggest the rearrangements occur via a homolysis-recombination mechanism. Ylides derived from catalytic reactions of diazo carbonyl compounds which undergo [1,2]-shift processes are becoming

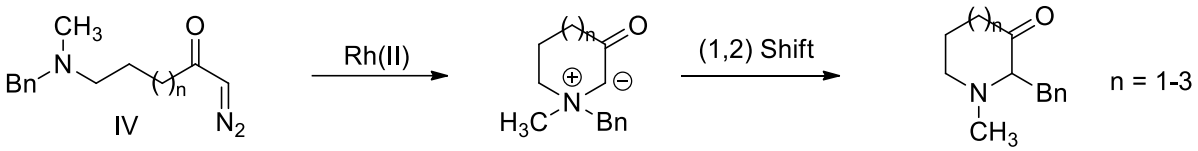
increasingly useful in synthetic chemistry. Some example of intramolecular transformations are reported below.

Enantiomerically pure spiro[5,5]-ammonium ylides were obtained by Rh(II)-catalyzed decomposition of α-diazo-β-carbonylesters (*Tetrahedron* **2006**, *62*, 1459–1466). (Schema 6)



Scheme 6

Ylide-derived [1,2]-shift (or in some cases [1,4]-shift) products were obtained upon treatment of amino diazo ketone substrates **IV** with soluble Cu(II) catalysts. Worst results were found using rhodium catalyst (*Tetrahedron* **2007**, *63*, 12232–12238). (Scheme 7)



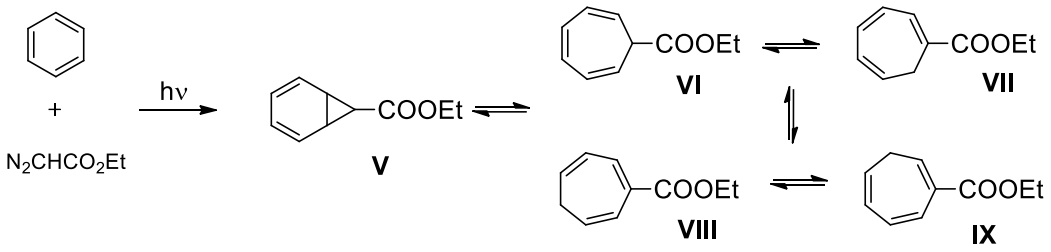
Scheme 7

5.1.4. Reactions with Aromatics: Benzene and Its Derivatives

After the synthesis of ethyl diazoacetate (EDA) in 1893, Buchner commenced an investigation of its reactions with alkenes, alkynes, and aromatics that was to continue for 30 or more years. Initially, Buchner believed that thermal or photochemical decomposition of EDA in benzene furnished a single product to which he assigned the norcaradiene structure **V**, although he was later to discover that hydrolysis of the product yielded a mixture of four isomeric carboxylic acids. We now know that the Buchner reaction in its original form produces four cycloheptatrienyl esters (Scheme 8).

The mechanism proposed for the formation of the above compounds is that carbethoxycarbene adds to benzene forming an unstable norcaradiene intermediate **V** which is in mobile equilibrium with the more stable cycloheptatriene tautomer **VI**; the remaining products, **VII-IX,** are isomers of **VI**

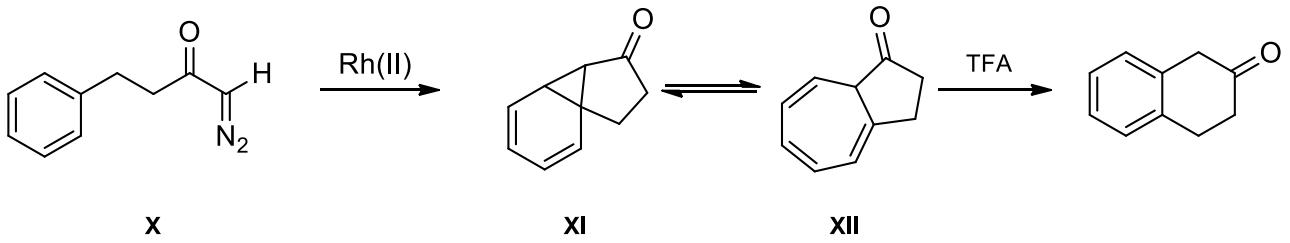
formed by thermally or photochemically induced sigmatropic rearrangement.



Schema 8

Only a few examples of intramolecular Buchner reactions were known prior to the introduction of transition metal catalysts. All employed a copper salt, and although yields of ring-expanded bicyclic products were low, the process was recognized as a potentially useful route to a variety of novel structures. Cyclization of l-diazo-4-phenylbutan-2-one (**X**) with a rhodium catalyst in hot decalin furnished a mixture of products from which cycloheptatriene (**XII**) tautomers were isolated in 13% yield *via* norcaradiene derivative (**XI**) (*Bull. Soc. Chim. Fr.* **1970**, 907) (Scheme 9).

Interestingly, cycloheptatriene tautomers can be transformed into tetralone by addition of trifluoroacetic acid.



Scheme 9

Introduction of rhodium catalyst opened the way to several synthetic studies related to the above reaction starting from substituted benzene derivatives aiming to determine the substituent effect of on the aromatic ring in term of efficiency and regiochemistry in the cyclization. The activating methoxy group was among the substituents selected for investigation. Interesting dynamic equilibria are observed in the cyclization products *via* rearrangements of the methoxy substituted norcaradiene derivatives.

It is well established that variation of the ligand on rhodium carboxylate and carboxamide catalysts can have a significant effect on the reactivity of carbenoids formed from the catalysts; chemo-, regio-, and stereoselectivity can be altered by variation of the ligand. The aromatic addition reaction would be expected to be catalyzed more efficiently by catalysts such as $Rh_2(tfa)_4$ and $Rh_2(pfb)_4$ which form highly electrophilic carbenoids and less efficiently by $Rh_2(cap)_4$ compared to $Rh_2(OAc)_4$. It was found that in practice rhodium(II)-catalyzed cyclization of diazoketones to aromatic rings bearing methoxy substituents proceed efficiently since the activating effect of the methoxy substituent overcomes to a large extent the electronic effect of the ligands on the catalyst and results in relatively minor catalyst sensitivity.

5.1.5. Chemoselectivity and regioselectivity

The use of diazocarbonyl compouds appears very interesting when starting from polifunctionalized compounds in which the carbene could react intramolecularly at different functional groups.

The selectivity for C-H insertion in competition with other carbene-based transformations has been a cause of considerable interest (*Tetrahedron* **1996**, *52*, 2489). Ligands on the metal have been shown to have significant influences, often leading to a complete change in chemoselectivity. For example, aromatic substitution ("aromatic C-H insertion") is favored over cyclopropanation, which, in turn, is favored over C-H insertion for catalysts that include dirhodium (II) acetate, which have electron-withdrawing carboxylate ligands. Increasing the electron-withdrawing ability of the ligand, such as replacing acetate by trifluoroacetate on dirhodium(II), increases the electrophilic character of the intermediate metal carbene and leads to an enhancement in the selectivity of the carbene for the more nucleophilic substrates. Decreasing the electron-withdrawing ability of the ligand, such as replacing acetate by acetamide on dirhodium(II), decreases the electrophilic character of the intermediate metal carbene and leads to an enhancement in the selectivity of the carbene for the less nucleophilic substrates.

5.2 Aim of the work

Here we report on a study on different piperidine systems of general formula **1** easily sinthetized starting from β-amino cids (Figure 3), with an increased complexity and substitution pattern, using the very interesting chemistry that combines the use of α-diazocarbonyl compounds and Rh(II)-catalysts.

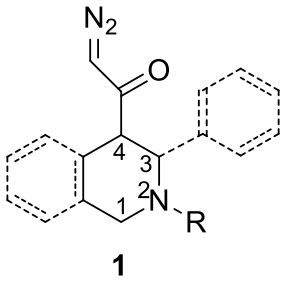


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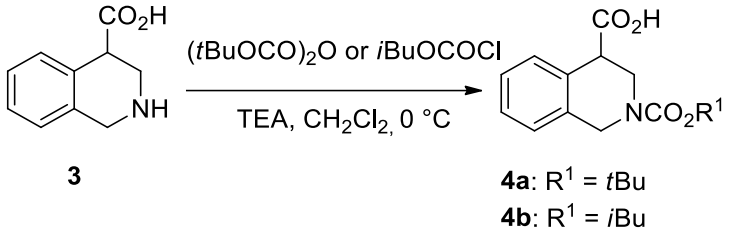
Figure 3. General formula of diazocarbonyl piperidine systems.

Three different classes of α-diazocarbonyl piperidine compounds were prepared including the simple piperidine derivative, its benzocondensed, *i.e.* 1,2,3,4- tetrahydroisoquinoline derivatives, and tetrahydroisoquinoline derivatives in which a further aryl moiety is present at position 3. Both CH(sp3)-insertion and Ar-H reaction could be expected when the aromatic moiety is present. Nitrogen atom on the ring could be a further center of carbene insertion. Our challenge has been to compare the different behaviour of the starting reagents and to find the best catalyst and the optimal reaction conditions to drive the chemoselectivity of the reaction. Using Rh(II)-chemistry and through unprecedented mechanisms, we achieved different compounds. 1,3,4,5- Tetrahydro-2,5-methanobenzo[*c*]azepine ring, that is included in the crinine alkaloids[6] belonging to the class of Amaryllidaceae,[7] was obtained from 1,2,3,4-tetahydroquinoline derivatives. The nitrogen atom of azepino ring is able to generate very stable complexes with Rh(II), opening the way to Rh(II) complexes whose interest has been growing over the last few years.[2a,c,f,8] 1,2,3,3a Tetrahydrocyclopenta[*de*]isoquinolin- 4(5*H*)-one scaffold can also be obtained. To our knowledge, a single example for the preparation of this heterocycle is reported in literature.[9] Finally, starting from the simple piperidine core, an unusual dimerization process occurred giving the hexahydrotetrazine ring. Notably, apart from a few examples related to *CH* insertion [10] and the use of α-diazocarbonyl piperidones as key reagents to generate carbonyl ylides for cycloaddition reaction,[2d,11] systematic studies combining Rh(II)-catalysis and different α-diazocarbonyl piperidine cores are not reported in literature.

5.3 Results and Discussion

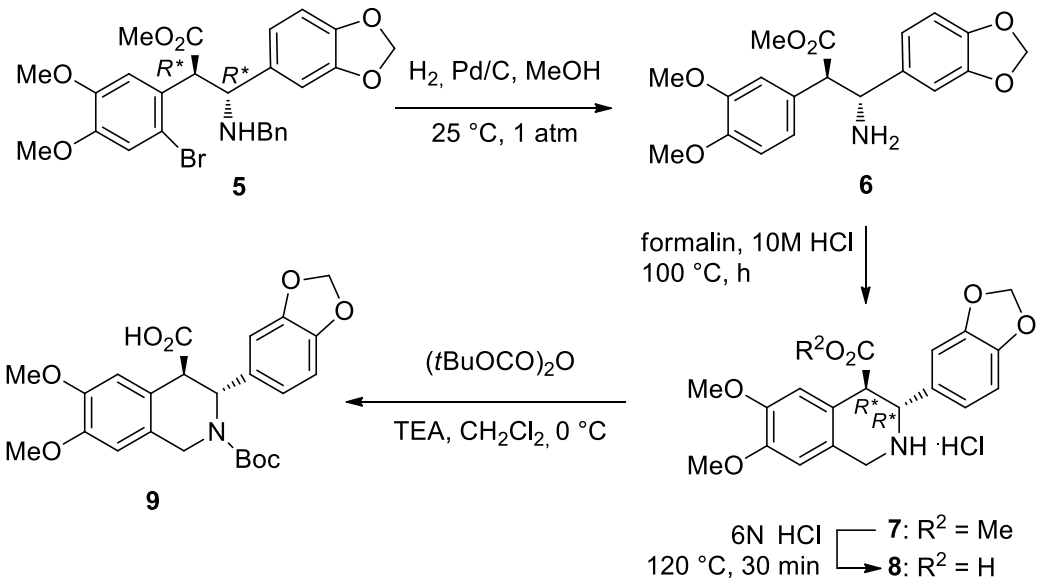
5.3.1 Starting materials preparation.

N-protected piperidine carboxylic acids are the key starting materials to obtain diazoderivatives. *N*-Boc-piperidine-3-carboxylic acid (**2**) and tetrahydroisoquinoline-4-carboxylic acid (**3**) are commercially available compounds. The protection of nitrogen atom of compound **3** with Boc-group was performed using (*t*BuOCO)$_2$O in the presence of TEA in CH_2Cl_2 at 0 °C then at 25 °C (1 h) affording **4a** (92%). Similarly, derivative **4b** (98%) was obtained from **3** and *i*BuOCOCl (TEA/CH2Cl2, 0 °C, 4 h). (Scheme 10)



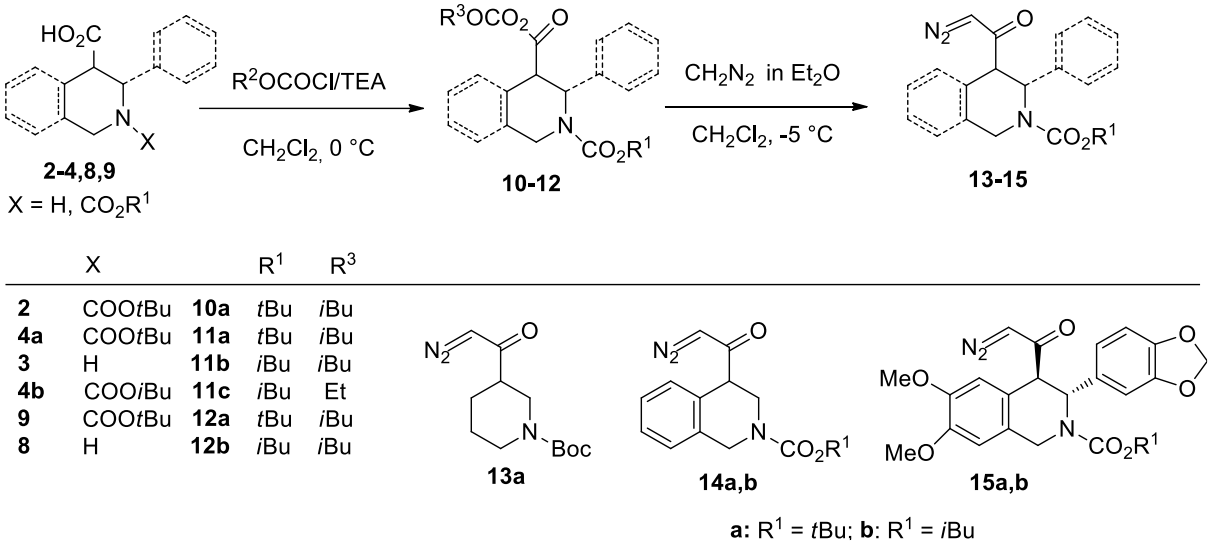
Scheme 10. Synthesis of N-protected derivatives **4a** and **4b**

Tetrahydroisoquinoline-4-carboxylic acid **8**, substituted at C-3 with an aryl moiety, was prepared from β-amino ester **5**[12] using a Pictet-Spengler reaction. (Scheme 11) The reaction failed starting directly from **5** even if several reaction conditions were tested.[13] It is operative from β-amino ester **6**, obtained in 90% yield from **5** after nitrogen deprotection and bromine elimination (H_2, Pd/C in MeOH, 1 atm., 25 °C, 1 h). The treatment of **6** with formalin and 12M HCl (HCOH/HCl, 10:1, 80 °C, 10 min.) gave the tetrahydroisoquinoline derivative 3*R**,4*R**-**7** (83%). The hydrolysis of the ester function was achieved in 6N HCl (120 °C, 30 min) affording amino acid **8** (93%) then transformed into *N*-Boc derivative **9** (84%) according to the standard protocol.



Scheme 11. Synthesis of *N*-Boc-tetrahydroisoquinoline-4-carboxylic acid derivatives **8** and **9**.

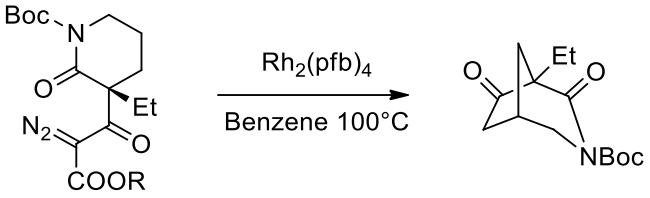
The procedure of Schoth-Eskesen *et al.* was adopted to obtain diazocarbonyl compounds.[14] It consists of the transformation of a carboxylic acid into a mixed anhydride then treated with diazomethane. In general, due to their instability, the anhydrides were prepared and immediately used for the preparation of diazoketone derivatives **13-15** (Scheme 12). For the preparation of diazo-derivative **13a**, *N*-Boc-piperidine-3-carboxylic acid (**2**) was made to react with ClCOO*i*Bu (1.1 equiv.) in the presence of *N*-methylmorpholine (NMM) (THF, from 0 to 25 °C, 2h) giving intermediate **10a**. After cooling at 0°C, the reaction mixture was treated with an Et_2O solution of CH_2N_2 (from -5° to 25 °C, 4 h) affording **13a** in 93% yields. Similarly, **14a** (60%, overall yield) was prepared from **4a**, *via* intermediate **11a** using THF as the solvent. The anhydride **12a**, obtained from **9** (Et_2O, TEA, 0 °C, 30 min.), gave pure diazoketone **15a** (92%) after treatment with CH_2N_2 in Et_2O. It is possible to generate the anhydride and to protect directly the amino group using an excess of ClCOO*i*Bu (2.2 equiv. TEA, 0 °C, 1 h). Starting from amino acids **3** (THF) and **8** (CH_2Cl_2) anhydrides **11b** (81%) and **12b** (91%) were prepared. This latter was made to react with CH_2N_2 (Et2O, -5 °C, 2 h) giving diazoketone **15b** (93%). Instead, **11b** is a very stable compound reacting slowly (3 days) with an excess of CH_2N_2 affording **14b** in unsatisfactory yields (15%). To improve the yields, the less hindered anhydride **11c** was prepared from **4b** and ethyl chlorocarbonate (THF/NMM, 0 °C, 1.30 h) that, after reaction with CH_2N_2 (Et_2O, 0 °C), gave **14b** in a shorter reaction time (12 h) and in 65% yield. It is worth underlining that the adopted protocol to obtain diazoketones **15** is of relevance with respect to the standard synthesis of diazoketones from acyl chlorides. In fact, it is reported that an analogous of the acid **9**, and as we verified on the reagent **9**, gave an oxidation of the tetrahydroquinoline ring with $SOCl_2$.[15] All isolated diazo-derivatives are stable materials that could be manipulated in light, purified by column chromatography and stored at room temperature for a long time without decomposition.



Scheme 12. Synthesis of diazocarbonyl-compouds **13-15**.

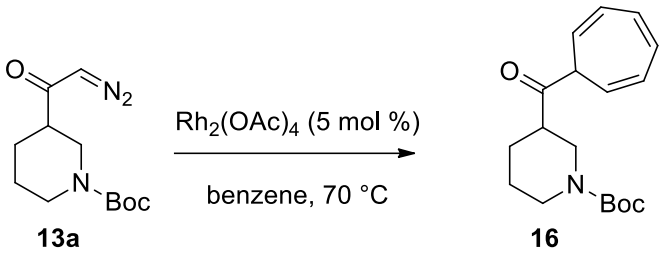
Rh(II)-Catalyzed transformations of diazocarbonyl compouds 13-15.

Having in our hands the diazocarbonylcompouds **13-15**, we started to study their reactivity using Rh(II) catalysts. As a general procedure, Rh(II) catalyst was dissolved in the solvent of choice and heated at reflux for 10 min. The temperature was adjusted at the indicated value and a 0.1 M solution of diazoketone was dropped in 2 h and the reaction stirred for the time indicated (see Tables 1-3). The reactivity of compound **13a** was first tested. Mejia-Oneto *and al.*[10b] reported on the reactivity of a dicarbonyldiazo compound, an analogous of **13a**, using $Rh_2(epf)_4$*2 CH_2Cl_2 catalyst giving a tropone derivative through CH-carbene insertion (scheme 13).



Scheme 13.

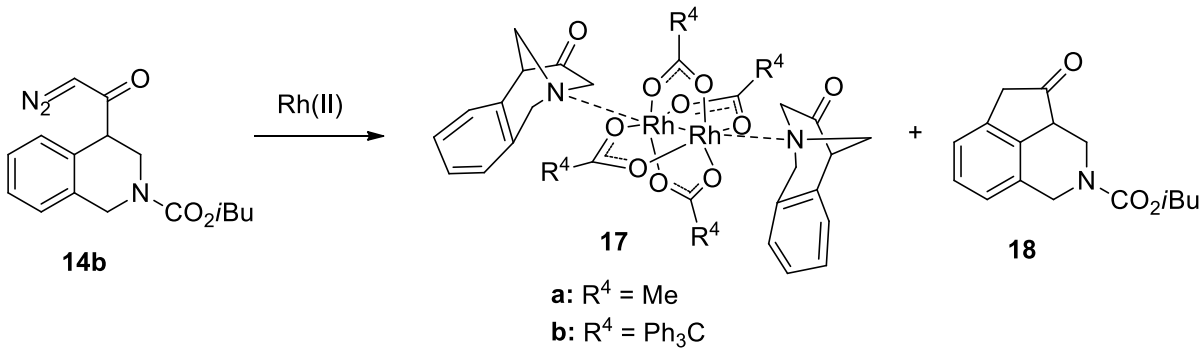
We performed the reaction on **13a** operating in the same reaction conditions. We did not obtain the analogue tropone derivative but only a mixture of compounds. Similar results were found using $Rh_2(OAc)_4$*$2H_2O$ (5 mol %) at different temperatures (from 0 to 110°C) and solvents (CH_2Cl_2, $CHCl_3$, CCl_4 and trifluorotoluene). When benzene was used at 70 °C, the new derivative **16**, a Büchner compound deriving from the carbene insertion into the benzene solvent, was isolated in only 33% yield (Scheme 14).



Scheme 14. Synthesis of ketone 16.

Rh(II) catalyzed transformation of 4-α-diazocarbonyl tetrahydroisoquinoline derivatives **14** could give C-insertion both to aromatic and sp^3 carbons. To understand the behavior of **14** toward Rh(II) catalysis many experiments were done since a complex situation was observed with unexpected results. The reaction of **14a** with $Rh_2(OAc)_4$ .2 H_2O (1 mol %, 0 °C) gave degradation compounds.
The use of 15 mol % of the catalyst required a long reaction time (0 °C, 10 h). TLC and HPLC analyses showed again the formation of a complex mixture but a solid was separated taking up the crude mixture with MeCN. Its ^1H NMR analysis showed the absence of N-Boc-group. The new product is characterized by the presence of a new core derived from the starting reagent, and of the acetate moiety derived from the catalyst, which are in a 2:1 ratio. Our hypothesis was that the new complex **17a** was formed (table 1), as also confirmed by mass spectrometry analysis (MS: m/z 789.1 [M+H]+).
Generally, the formation of N-C bond occurs via NH-carbene insertion.[16,1] The stability of the Boc group toward the catalyst was verified in an independent experiment. Treating the ethyl ester of **4a** with $Rh_2(OAc)_4$*2 H_2O (5 mol %; toluene, 120 °C, 12 h), only the starting material was recovered. From this datum we can conclude that the loss of N-Boc from 14a occurs with an unusual mechanism.
To definitely exclude that the formation of the amine scaffold was dependent from the Boc group, an acid labile *N*-protecting group, we turned to **14b**, which contains the more stable *iso*butylcarbamate and requires fewer synthetic steps for its preparation. To modulate the chemoselectivity, we tested different temperatures, solvents and catalysts (15 mol %, Table 1). The overall results indicate that complex **17a** was obtained in all cases, confirming that its formation is independent from the *N*-protecting group.

Tab 1. Rh(II) catalyzed transformation of 4-α-diazocarbonyl-tetrahydroisoquinoline 14b[a]



Entry	Catalyst	Solvent	T (°C) [b]	t (h)	**17** (%)[c,d]	**18** (%)[c,e]
1	$Rh_2(OAc)_4$* 2 H_2O	CH_2Cl_2	25	3	a: 63	5
2	$Rh_2(OAc)_4$* 2 H_2O	CH_2Cl_2	40	1.30	a: 73	7
3	$Rh_2(OAc)_4$* 2 H_2O	CCl_4	60	3	a: 83	6
4	$Rh_2(tpa)_4$*2 CH_2Cl_2	$CHCl_3$	0	8	b: 65	24

[a] Catalyst (15 mol %) in the indicated solvent at 40 °C (10 min.), then a 0.1 M solution of diazoketone was dropped in 2 h. [b] Reaction temperature after activation of the catalyst. [c] Isolated compound. [d] Yield referred to the catalyst. [e] Yield referred to diazoketone **14b**.

By column chromatography of the mother liquor, it was possible to isolate in low yield the C-insertion compound **18**, i.e. 1,2,3,3a-tetrahydrocyclopenta[de]isoquinoline-4(5H)-one (ESI MS: m/z 274.0 $[M+H]^+$ and NMR analyses). As shown in Table 1, the higher the temperature, the higher the yields for **17a** (entries 1-3; $Rh_2(OAc)_4$ *2 H_2O) with a decrease of the reaction time, except when CCl_4 was used as the solvent. The more hindered $Rh_2(tpa)_4$*2 CH_2Cl_2 catalyst gave a better performance with respect to $Rh_2(OAc)_4$ .2 H_2O working at a lower temperature (entry 4). Complex **17b** was isolated in good yield at 0 °C with a significant increased amount of derivative **18** that is very unstable in solution and degraded. Even if these preliminary data gave only qualitative information, some general considerations can be drawn. First, the free amine scaffold was never isolated, indicating the stability of the complexes **17a** and **17b**. Second, the presence of several by products is correlated to the degradation of the unstable **18**,[17] and, most of all, of the starting diazoketone **14b** whose transformation is prevented by the absence of Rh(II)-catalyst sequestered by azepine scaffold.

Further runs were then conducted increasing the amount of the Rh(II) reagent and expecting a better yield of the complex (Table 2). The reaction of **14b** (1 equiv.) was performed in CH_2Cl_2 at 0 °C by

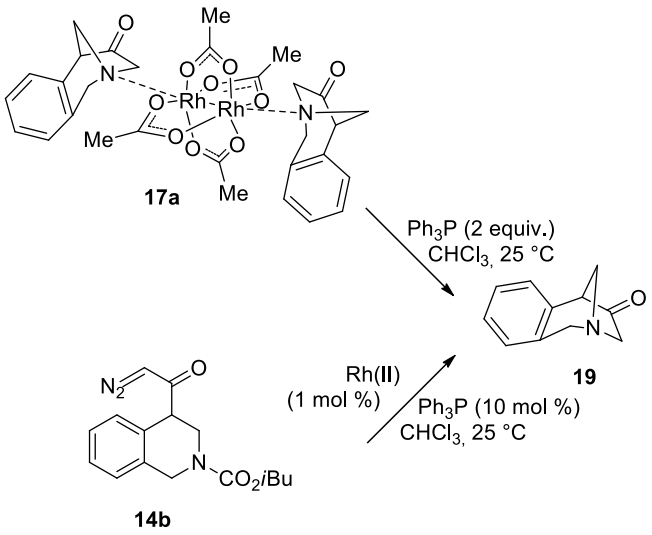
slowly adding a 0.01 M solution of $Rh_2(OAc)_4$*2 H_2O (0.5 equiv.) in CH_2Cl_2. The reaction was monitored by the solution colour change that turned from green to red after addition of the catalyst indicating the formation of the carbene. The reagent disappeared after addition of 0.4 equiv. (0.1 equiv. x 4) of the Rh-catalyst (80 min.). The expected complex **17a** (88%) and the *C*-insertion product **18** (22%) were obtained (Table 2, entry 1).[18] The same protocol at 25 °C (entry 2) afforded **17a** in excellent yield. A similar result was achieved using $Rh_2(tpa)_4$ .2 CH_2Cl_2 that gave **17b** in 98% yield (entry 3). The ability of the complex **17a** to catalyze the transformation of **14b** was tested in CH_2Cl_2 at 0°C (3h), 25°C (3h) and 40°C (2h). Compound **14b** was recovered without any transformation. These data confirm that the formation of the complexes **17** prevents the transformation of diazoketone into the insertion products.

Tab 2. Reaction conditions optimization for the syntheses of complexes **17** and free azepino derivative **19**

Entry	Catalyst	T (°C)	t (h)	**17** (%)[b,c]	**18** (%)[b,d]	**19** (%)[b,d]
1[a]	$Rh_2(OAc)_4$*2H_2O[e]	0	1.20	a:83	22	-
2[a]	$Rh_2(OAc)_4$*2H_2O [f]	25	2.30	a:93	-	-
3[a]	$Rh_2(tpa)_4$.2*CH_2Cl_2	25	1.40	b: 98	-	-
4[g]	$Rh_2(OAc)_4$*2H_2O	25	4.00	-	-	70
5[g]	$Rh_2(tpa)_4$.2*CH_2Cl_2	25	7.30	-	-	96

[a] A 0.01 M solution of the catalyst (0.5 equiv.) was prepared in CH_2Cl_2 and dropped to a 0.1 M solution of diazoketone until the complete transformation of 14b. [b] Isolated compound. [c] Yield referred to the catalyst. [d] Yield referred to diazoketone **14b**. [e] Recovered catalyst: 0.1 equiv. [f] Recovered catalyst: 0.03 equiv. [g] Rh(II)-catalyst (1 mol %)/Ph_3P (10 mol %), $CHCl_3$.

Since phosphorus has a better sigma-affinity toward Rh(II) than nitrogen,[19] the reactivity of **17a** toward Ph_3P (2 equiv.) was verified in $CHCl_3$. As expected, the free amine **19** was formed (25 °C, 5 min.) and isolated in 82% yield (Scheme 15).



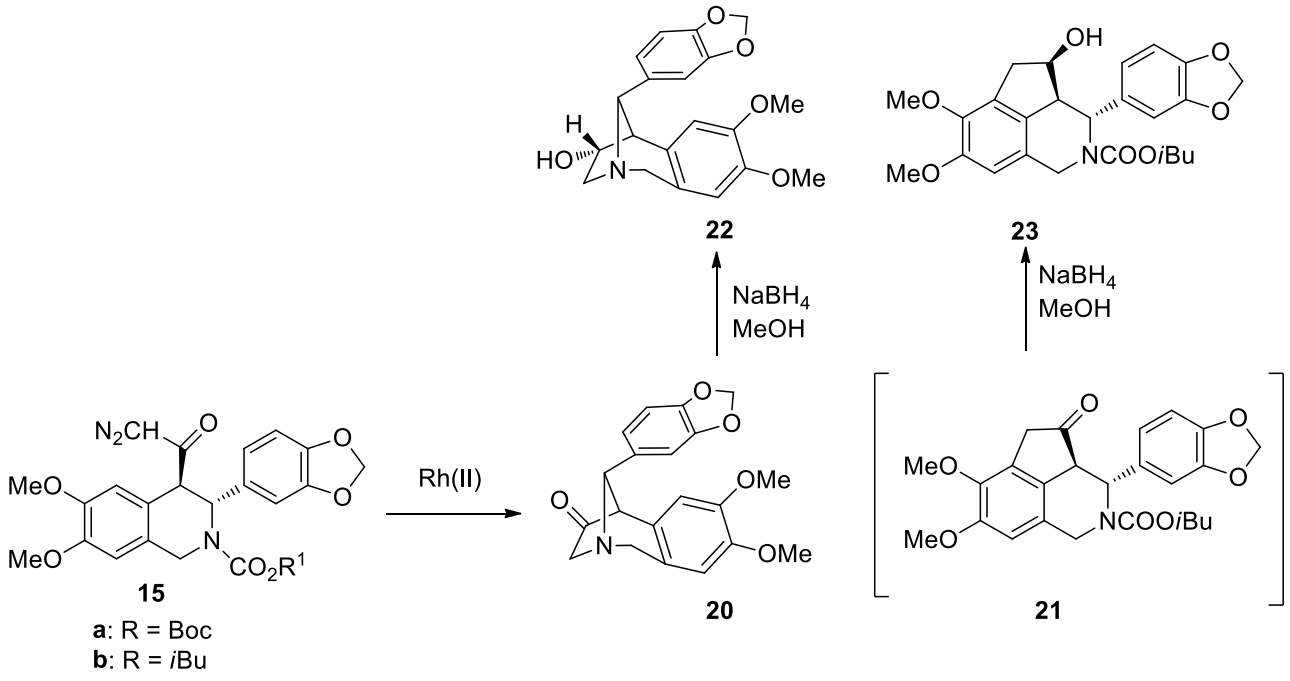
Scheme 15. Synthesis of the amino scaffold **19**.

This result made us test the reactivity of **14b** using a catalytic amount of $Rh_2(OAc)_4$ *2 H_2O in the presence of Ph_3P (Table 2, entry 4). The reaction gave the amino derivative [19] in very good yield (70%). The same protocol was used with $Rh_2(tpa)_4$ .2 CH_2Cl_2 catalyst (Table 2, entry 5) affording the amine **19** in excellent yield (96 %) (Scheme 15). In both cases the ketocompound **18** was not detected, thus indicating that under this reaction conditions the kinetic in the formation of the amino scaffold **19** is faster than the C-insertion to aromatic ring.

The generality of the reaction was verified adding a further aryl substituent at C-3 of tetrahydroisoquinoline ring increasing the bulkiness at nitrogen atom as well as the molecular complexity. Differently from the parent compounds **14**, diazoketone **15a** was transformed in the presence of only 1 mol % of activated $Rh_2(OAc)_4$ *2H_2O (CH_2Cl_2, 40 °C) and in a shorter time (15 min.). T.L.C. analysis showed the formation of three main compounds. Unfortunately, during the column chromatography, only the methanobenzo[*c*]azepinone derivative **20** (Scheme 16), derived from the carbene nitrogen insertion, was isolated in good yields (50%; MS: *m/z* 354.4 [M+1]+; NMR analyses), being the other compounds degraded. The reaction was also performed in basic conditions using K_2CO_3 (1 equiv.) to evaluate whether the pH could affect the behaviour of the reaction. In this case too, only **20** was isolated although in lower yield (40%). We moved then into derivative **15b** that gave compound **20** in 48% yield. The poor overall reaction yields are due to the instability of ketone **21** (Scheme 16), as reported for the analogous **18**, giving degradation compounds. We managed to overcome the problem treating the crude mixture of the Rh-catalyzed transformation with $NaBH_4$ (4 equiv.) in EtOH (25 °C, 12 h). Starting from **15b**, compound **22** (51%, *d.e.*72%) and the stable alcohol **23** (11%, *d.e.* 99%) were obtained with good to excellent

diastereoselection, respectively, after column chromatography. Only the main isomers are shown in scheme 16 characterized by a stereochemistry derived from the hydride attack to the less hindered face. The above protocol was used for further studies aimed to modulate the chemoselectivity and the temperature, solvent and catalyst were evaluated (Table 3). The experiments performed from -23 to 60 °C (entries 1-6) showed that the temperature has a strong impact on the chemoselectivity: the lower the reaction temperature, the higher the yields of the *C*-insertion deriving compound **23**. On the other hand, the *N*-insertion deriving compound **22** was absent at -23 °C (entries 1,2) and was obtained in higher yield at 60 °C (entry 6). The solvent did not affect the chemoselectivity (entries 1,2) but influenced the reaction time (entry 6).

The more hindered catalyst $Rh_2(tpa)_4$ *2 CH_2Cl_2 was tested at -23, 0, 25 and 40 °C (entries 7-10). The reaction was not operative at -23 °C (entry 7) but gave exclusively the *C*-insertion at aromatic ring deriving compound **23** at 0 °C (entry 8). Increasing the temperature an increase of the yield of **23** was observed. The nitrogen insertion compound **22** was formed at temperature higher then 25 °C (entries 9,10), even if in low yield.



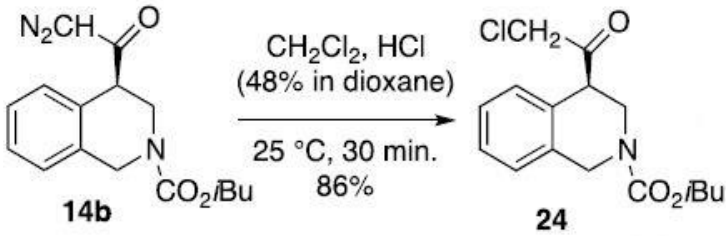
Scheme 16. Rh(II) catalyzed transformation of diazoketones **15** and $NaBH_4$ reduction of insertion compounds.

Tab 3. Rh(II) catalysed transformation of **15b**[a]

Entry	Catalyst	T (°C)[b]	t (h)[c]	solvent	**22** (%)[d,e]	**23** (%)[d,e]
1	$Rh_2(OAc)_4*2H_2O$	-23	7	CH_2Cl_2	-	44
2	$Rh_2(OAc)_4*2H_2O$	-23	7	$CHCl_3$	-	45
3	$Rh_2(OAc)_4*2H_2O$	0	4.30	CH_2Cl_2	41	15
4	$Rh_2(OAc)_4*2H_2O$	25	3	CH_2Cl_2	45	12
5	$Rh_2(OAc)_4*2H_2O$	40	0.15	CH_2Cl_2	51	11
6	$Rh_2(OAc)_4*2H_2O$	60	6	CCl_4	63	11
7	$Rh_2(tpa)_4.2*CH_2Cl_2$	-23	15	$CHCl_3$	-	-
8	$Rh_2(tpa)_4.2*CH_2Cl_2$	0	15	$CHCl_3$	-	47
9	$Rh_2(tpa)_4.2*CH_2Cl_2$	25	8.30	$CHCl_3$	14	51
10	$Rh_2(tpa)_4.2*CH_2Cl_2$	40	4.30	$CHCl_3$	21	59

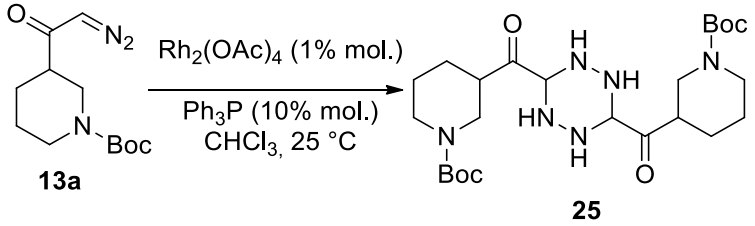
[a] 1 mol % catalyst (0.1 M in the solvent indicated, 40 °C, 10 min); then addition of **15b**; then $NaBH_4$ (4 equiv.) in EtOH (25 °C, 12 h). [b] Reaction temperature after activation of the catalyst. [c] Reaction time of Rh(II) catalyzed transformation. [d] Isolated compound. [e] A fluorescent compound was also formed in variable amount in all tested reactions. Its degraded in solution prevented its characterization.

Hendrickson *et al.*[20] reported on the preparation of methanobenzo[c]azepinone ring starting from a 3,4-dihydroisoquinolin-1(2H)-one derivative, functionalized at C-4 with a diazoketone moiety, and in the presence of anhydrous HCl. We performed the same reaction on **14b** [CH_2Cl_2, HCl (10 equiv., 48% dioxane solution), 25 °C, 30 min.]. In our case, α- chloro ketone 24 was isolated (86% yield) as a single compound, confirming that the Rh(II)-catalyst is essential in the formation of the N-C bond (Scheme 17).



Scheme 17. Reaction of diazoketone **14b** with anhydrous HCl.

Finally, considering the Rh(II) catalyzed transformation of tetrahydroquinoline derivatives **14** and **15**, we came back to the simple piperidine derivative 13a reasoning that the bad result reported above could be due to the coordination of the amine scaffold to the catalyst. The standard protocol was adopted and the amount of rhodium catalyst was gradually increased until 0.5 equiv. Only degradation compounds were detected confirming a different behaviour of **13a** with respect to the tetrahydroquinoline derivatives **14** and **15**. Instead, the new compound 25 (94%) was isolated performing the reaction of **13a** in the presence of Ph_3P (10 mol %) and $Rh_2(OAc)_4$ *2 H_2O (1 mol %, CH_2Cl_2, 25 °C, 2.5 h). Analytical and spectroscopic analyses confirm the formation of the hexahydrotetrazine derivative **13a** formed by the dimerization of the starting diazoketone followed by a reduction (Scheme 18). The starting material **13** was not transformed after treating with Ph_3P ($CHCl_3$, 25 °C, 8 h) in the absence of the rhodium catalyst. Instead, when $Rh_2(OAc)_4$ is present, a red solid was isolated from the reaction whose analytical data (NMR spectra and HRMS analysis) suggest a formulation as a dimeric or oligomeric rhodium hydride phosphine complex, with bridging chloride ligands and no trace of the original acetate moieties (see Experimental section for characterization).

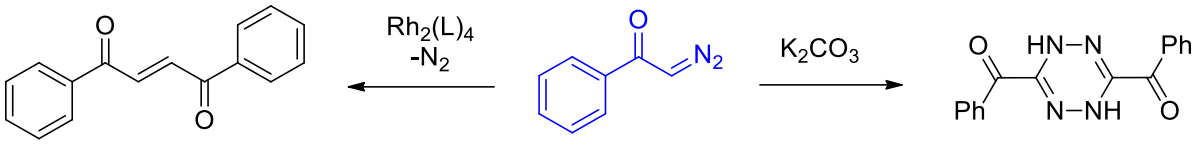


Scheme 18. Rh(II)-catalyzed dimerization/reduction of diazoketone **13a**.

5.3.3 Discussion

Based on the above results, some general considerations can be made on the Rh(II)-catalyzed intramolecular insertion of carbene derived from piperidine derivatives functionalized with

diazoketone moiety. In contrast to the literature data on a similar substrate affording a tropone derivative,[10b] the simple compound **13** did not give the CH-insertion, indicating that the reported result is strictly dependent on the substitution pattern of the molecule. On the other hand, hexahydro-tetrazine derivative **25** was formed but only in the presence of a catalytic amount of Ph_3P. The dimerization of Rh(II)-carbene is known to afford diketo-olefin derivatives with a loss of nitrogen.[16f] Furthermore, the base-promoted dimerization of α-diazo ketones is reported to give dihydrotetrazine ring or acyltetrazoles, depending on the base and the solvent (scheme 19).[21]



Scheme 19. Dimerization of α-diazo ketones

However, the subsequent reduction to yield the saturated ring, to the best of our knowledge, is an unprecedented reaction. The origin of the four hydrogen atoms and the role of the phosphine are at the moment unclear, but depend on the combination of the Rh(II)-catalyst and PPh_3 in $CHCl_3$. In fact, the reaction does not work in the absence of Rh(II)-catalyst. Our hypothesis is that an odd-electron complex [RC(H)NN(Rh2(OAc)4)(L)] is formed in the first reaction step. However, this species could be unstable with regard to the spontaneous dimerization[22] to tetrazine nucleus affording a mixture of degraded compounds. It is known that the formation of phosphine base adducts of dirhodium-carboxylates is controlled by a rate-determining step to form a mono adduct, i.e. $Rh_2(O_2CCH_3)_4(L)PR_3$.[23] The formation of such a monoadduct could alter the reactivity of the diazoketone complex, thus favouring the dimerization reaction. In the presence of chlorform, it is reasonable to assume that dimeric rhodium species are formed, with bridging chloride ligands.[24] Getting onto tetrahydroisoquinoline derivatives **14** and **15**, it was found that, in this case too, products of CH (sp3) insertion were never detected. A different behaviour was observed with respect to compound **13**, indicating that the conformation of the piperidine ring is probably of fundamental importance for the reaction outcome. Compounds of carbon aromatic insertion and the unexpected compounds of nitrogen insertion were obtained. Their distribution is strictly dependent on the steric hindrance of both the starting material and the catalyst. Focusing on nitrogen-insertion products, it is known that the carbene NH-insertion[2,16] is a powerful tool to obtain several heterocyclic derivatives. On the other hand, tertiary amines give carbenoid/ammonium ylide/Stevens[1,2]-shift with alkyl group or ring expansion tandem sequence.[2d,11,25] To our

knowledge, the formation of an amine scaffold via Rh(II)-carbene insertion to nitrogen of a tertiary carbamate with concomitant loss of the carbonate is uncommon.

The formation of N-insertion compounds is independent from the nitrogen protecting carbonate group. The less hindered derivatives **14** are more favourable to giving the *N*-insertion independently from the catalyst. The bulkiness of the reagent and catalyst is of relevance on the formation of coordinated (i.e. compounds **17**) and not coordinated amine derivatives (i.e. compounds **19** and **20**). In fact, the coordination is prevented by the presence of an aryl group at C-3 on the starting reagent.

The temperature has a strong impact on the chemoselectivity. Even if it is difficult to evaluate quantitatively this parameter starting from compound **14b**, we can observe that the higher the temperature, the higher is the *N*-insertion compound (Table 1).

This effect is more evident for the reagent **15b** for which a strong difference in the chemoselectivity was found (Figure 4).

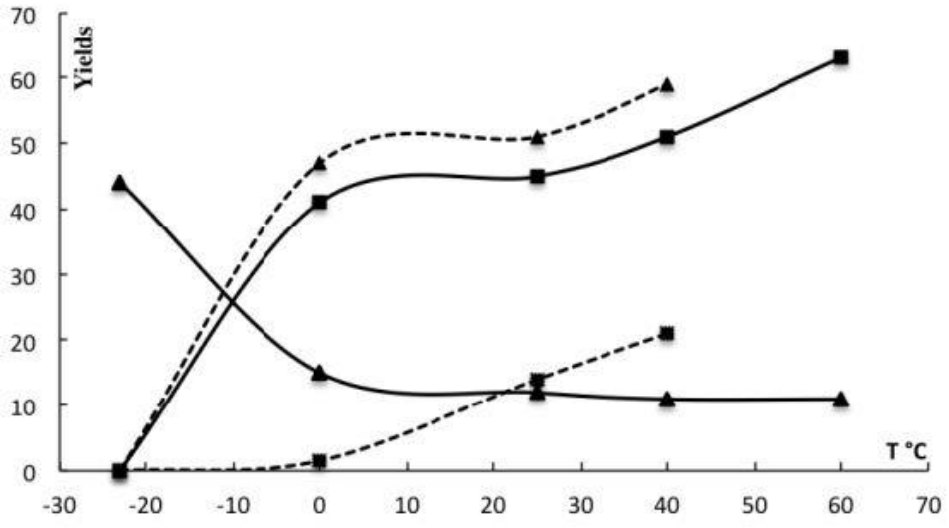
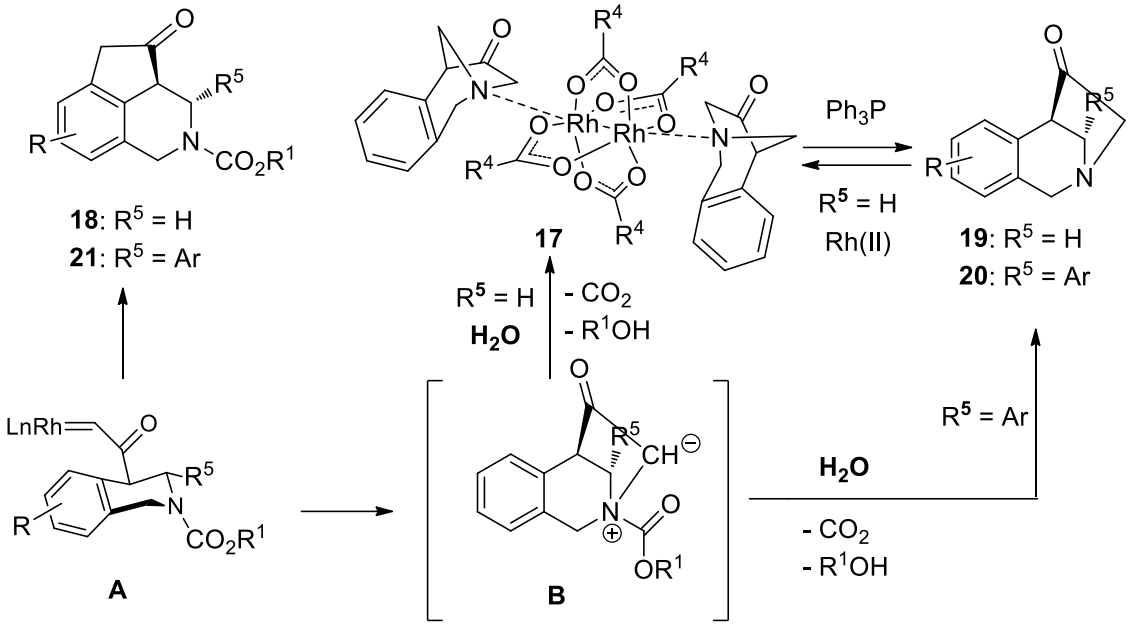


Figure 4. Chemoselectivity toward temperature for diazoketone **15b**. Straight line: $Rh_2(OAc)_4$ *$2H_2O$; Dotted line: $Rh_2(tpa)_4$*$2CH_2Cl_2$; Square: *N*-insertion compound **22**; Triangle: *C*-insertion compound **23**.

Using $Rh_2(OAc)_4 \cdot 2H_2O$ catalyst, the distribution of the *N*-insertion compound **20** with respect to the *C*-insertion compound **21** is directly dependent on this parameter. $Rh_2(tpa)_4 \cdot 2CH_2Cl_2$ catalysts seems to be less efficient, requesting higher temperature and longer reaction times, but with a better selectivity toward the *C*-insertion reaction. This result is probably driven by the steric hindrance of both the catalyst and reagent.

Scheme 20 depicts the proposed mechanism for the formation of *C*- and *N*-insertion products.



Scheme 20. Mechanism for the Rh(II) catalyzed transformation of diazoketones **14** and **15**.

We suggest the formation of Rh-carbene **A** as the common intermediate to achieve both products. An aromatic substitution promoted by the electrophilic attack of the metal-carbene on the piperidine fused aromatic ring, followed by a 1,2-hydride shift, would yield to **18** and **21**, respectively. On the other hand, the nitrogen-ylide intermediate **B** could be formed from **A** that in turn was deprotected at nitrogen by reaction with water forming the *N*-insertion products **19** and **20**. Since this result is independent from the coordination solvent of the catalyst, it must be deduced that the solvent moisture favours this transformation. In the case of 3-unsubstituted compounds (R5 = H), the nitrogen atom coordinates the starting catalyst depleting the original coordination solvent giving complexes **17**. We tend to exclude the intermediacy of free carbene in the formation of the azepino core, since we did not observe any product in the absence of the Rh(II)-catalyst, or by thermal activation. On the other hand, a mechanism based on metallo-carbene decoordination, promoted by the axial coordination of the azepino moiety to the Rh atom,[2a] can not be ruled out at the present stage. Against this proposal, however, is the formation of product **20** in comparable yield. In this latter case we never observed any coordination of the azepino nucleus to the metal atom, probably for steric hindrance.

Characterization of complex 17b.

The formation of complex **17b** was confirmed by mass spectrometry (HRMS: *m/z* 1701.42472 [M+1]+). Typical Rh-O streaking bonds in the IR spectrum[26] -1360 cm^{-1}. A detailed NMR

characterization (CD_2Cl_2, 500 MHz: ^1H, ^{13}C, COSY, HSQC, NOESY) of compound **17b** is reported in the Supporting Information. Of relevance the presence of two carbonyl groups at ^{13}C NMR at δ 192.4 and 208.6, associated to acetate moiety and ketone function. The Noesy experiment of **17b (**Figure 5) showed spatial proximity between the *orto*- and *meta*-protons of the phenyl ring of the catalyst (δ the protons of the aza ring). Attempts to prepare suitable crystals for X-Ray analysis failed since only amorphous materials were generated (Figure 6).

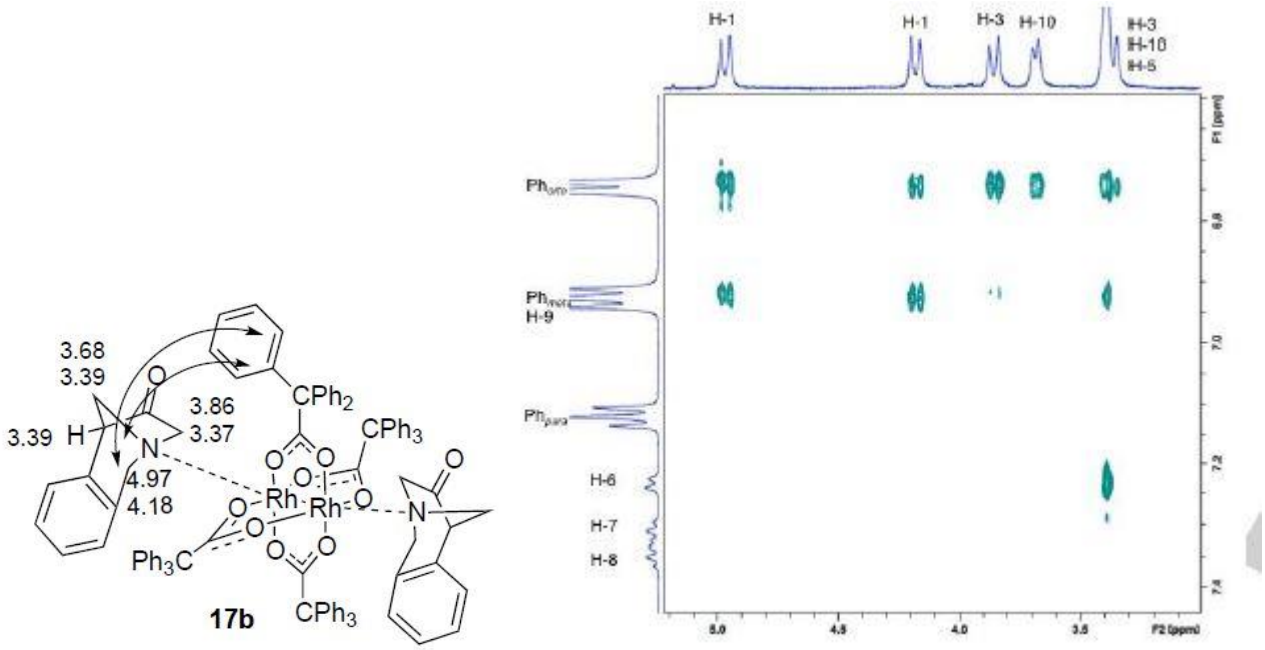


Figure 5. Noesy zoom (CD_2Cl_2, 800 ms, 500 MHz) for compound **17b**

The work disclosed herein has been the subject of a publication on *Chemistry an Europea Journal*, **2014**, *20*, 1 – 13, DOI: 10.1002/chem.201405197

Figure 6.

5.3 Reference

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[17] The behavior of **18** was confirmed by HPLC analysis of a pure simple that gave a complex chromatogram. Instead, complexes **17a** and **17b** are very stable and can be chromatographed on silica gel.

[18] The amount of the recovered $Rh_2(OAc)_4*2H_2O$ (0.2 mmol), not added to the solution, is in agreement with the formation of the amount of **17a**.

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Experimental section

General Experimental details.

ESI MS were recorded on a LCQ Advantage Thermo Finningan, HRMS on LTQ Orbitrap XL (Thermo; **17b**) and FTMS ApexII (Rh-hydride) spectrometers, respectively. NMR spectroscopic experiments were carried out on a Varian MERCURY 200 MHz (200 and 50 MHz for ^{1}H and ^{13}C, respectively) or Bruker *Avance* 300 MHz spectrometers (300 and 75 MHz for ^{1}H and ^{13}C, respectively). Chemical shifts δ are given in ppm and the coupling constants *J* are reported in Hertz (Hz). Ethyl 1,2,3,4- tetrahydroisoquinoline-4-carboxylate, *N*-Boc-piperidine-3-carboxylic acid (**2**) and 1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (**3**) are commercially available compounds. β-Amino ester **5** was prepared according to a know procedure.[12]

Ethyl 4-t-buthoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-4- carboxylate.

Operating in a three necked round bottom flask equipped with a magnetic stirrer, nitrogen inlet, and thermometer, ethyl 1,2,3,4- tetrahydroisoquinoline-4-carboxylate (260 mg, 1.0 mmol) was dissolved in dry DCM (5 mL) and the solution was cooled to 0°C. Triethylamine (0.9 mL, 2.1 mmol) was added. After 10 minutes di-*t*butyl dicarbonate (187 mg, 1.2 mmol) was slowly added. The reaction was stirred for further 60 min at 25 °C. The reaction mixture was washed with $KHSO_4$ (0.5 N, 5 mL, pH = 3) and the organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and product was obtained as a colorless oil (330 mg, 92%).

4a- ^{1}H NMR ($CDCl_3$, 200 MHz): δ= 7.27-7.12 (m, 4H), 4.73, 4.48 (AB system, *J* = 17.0 Hz, 2H), 4.24, 3.81, 3.59 (AMX system, *J* 13.2, 5.1, 4.4 Hz, 3H), 4.17 (q, *J* = 7.1 Hz, 2H), 1.48 (s, 9H), 1.27 (t, *J* = 3.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 200 MHz): δ= 14.4, 28.6 (x3), 44.1, 45.1, 46.0, 61.3, 80.3, 126.7 (x2), 127.7, 129.1, 131.9, 133.9, 154.9, 172.2; IR (NaCl) ν 1736, 1699 cm^{-1}; MS (ESI): *m/z*: 328.2 [M + Na]+; elemental analysis calcd (%) for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59; found C, 66.52; H, 7.80; N, 4.27.

General Procedure for the Synthesis of Carbamates 4.

Operating in a three necked round bottom flask equipped with a magnetic stirrer, nitrogen inlet, and thermometer, compound **3** (2.80 g, 15.8 mmol) was dissolved in dry DCM (22 mL), and the solution was cooled to 0°C. Triethylamine (4.4 mL, 31.6 mmol) was added. After 10 min., di-tert-butyl dicarbonate (2.45 g, 15.8 mmol) or iBuOCOCl (2.07 mL, 15.8 mmol) was slowly dropped. The reaction was stirred at 25 °C (**4a**: 1h; **4b**: 4h) after which it was washed with a saturated

solution of NH_4Cl (15 mL) and of NaCl (10 mL). After drying over Na_2SO_4, the solvent was removed under reduced pressure and product **4a** (92%) or **4b** (98%) was obtained as yellow oil.

4a: crude compound (mixture of conformers); ^{1}H NMR ($CDCl_3$, 200 MHz): δ= 9.82 (s, 1H, exch.), 7.30-7.12 (m, 4H), 4.79, 4.45 (AB system, *J* = 16.8 Hz, 2H), 4.34, 3.82, 3.55 (ABX system, *J* = 13.2, 4.4, 4.0 Hz, 3H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ= 28.6 (x3), 43.6, 44.7, 45.7, 80.7, 126.8 (x2), 127.9, 129.4, 131.2, 133.8, 155.1, 177.6; IR (KBr) ν 3200, 1738, 1701 cm^{-1}; MS (ESI): m/z 300.1 $[M + Na]^+$; elemental analysis calcd (%) for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05; found C, 64.80; H, 7.10; N, 4.87.

4b: crude compound (mixture of conformers); ^{1}H NMR ($CDCl_3$, 200 MHz): δ= 11.05 (s, 1H, exch), 7.32-7.12 (m, 4H), 4.78, 4.52 (AB system, *J* = 17.0 Hz, 2H), 4.35, 3.85, 3.61 (ABX, *J* = 13.6, 4.4, 4.3 Hz, 3H), 3.91 (d, *J* = 6.9, 2H), 2.03-1.87 (m, 1H), 0.92 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 19.3 (x2), 28.2, 43.5, 44.6, 45.8, 72.2, 126.8, 127.0, 127.9, 129.4, 131.1, 133.4, 156.2, 176.9; IR (NaCl) ν 2963, 1730, 1704 cm^{-1}; MS (ESI): m/z 276.3 $[M-H]^-$; elemental analysis calcd (%) for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05; found C, 65.70; H, 7.15; N, 4.78.

Methyl 2R*,3R*-3-amino-2-(3,4-dimethoxyphenyl)-3-(3,4-methylenedioxyphenyl)-propionate (6).

Pd on charcoal (10%, 64 mg, 0.06 mmol) was added to a solution of **5** (338 mg, 0.64 mmol) in MeOH (5 mL). The reaction mixture was hydrogenated under H_2 atmosphere under stirring at 25 °C for 1h. The mixture was filtrated over a celite pad and the solution was concentrated. After Et_2O addition, pure compound **6** (206 mg, 90%) was isolated as a white solid.
6-M.p. 167 °C dec. (MeOH/Et2O); ^{1}H NMR (CD_3OD, 200 MHz): δ= 7.13-7.00 (m, 5 H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.01 (s, 2H), 4.88, 4.18 (AX system, *J* = 11.0 Hz, 2H), 4.82 (brs, 2H, exch.), 3.89 (s, 3H), 3.85 (s, 3H), 3.49 (s, 3H); ^{13}C NMR (CD_3OD, 50 MHz): δ= 51.6, 55.5, 55.6 (x2), 57.2, 101.9, 107.9, 108.5, 112.4, 112.7, 121.6, 122.0, 125.5, 128.7, 148.6, 149.1, 150.2, 150.4, 170.9; IR (KBr) ν 3458, 1734 cm^{-1}; MS (ESI): m/z 381.9 $[M+Na]^+$; elemental analysis calcd for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. Found C, 63.25, H, 6.10, N, 3.75.

Methyl 3R*,4R*-3-(3,4-methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-4-carboxylate HCl (7).

Operating in a sealed tube, compound **6** (808 mg, 2.25 mmol) was suspended in a 10:1 mixture of formalin and HCl (37%, 2 mL). The mixture was heated at 80 °C for 10 min. A solid was formed which was then filtered. Compound **7** was recovered as a white solid (765 mg, 83%).
7-M.p. 221-223 °C; ^{1}H NMR (DMSO-d_6): δ= 10.79 (brs, 1H, exch.), 9.90 (brs, 1H, exch.), 7.35 (d, *J* = 1.2 Hz, 1H), 7.08 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.00 (d, *J* = 8.1 Hz, 1H), 6.98 (s, 1H) 6.61 (s, 1H), 6.09 (d, *J* = 3.2 Hz, 2H), 4.75 (dd, *J* = 11.4, 6.0 Hz, 1H), 4.49 (d, *J* = 11.4 Hz, 1H), 4.51, 4.25 (AM system, *J* = 15.4 Hz, 2H), 3.78 (s, 3H), 7.73 (s, 3H), 3.62 (s, 3H); ^{13}C NMR (DMSO-d6): δ= 44.4, 48.9, 52.3, 55.3, 55.4, 57.7, 101.3, 108.0, 108.3, 109.0, 109.8, 120.5, 121.5, 122.4, 128.1, 147.3, 147.8, 148.2, 148.4, 170.3; MS (ESI): m/z 372.0 $[M+H]^+$; elemental analysis calcd for $C_{20}H_{22}ClNO_6$: C, 58.90; H, 5.44; N, 3.43; found C, 58.55; H, 5.70; N, 3.12.

3R*,4R*-3-(3,4-Methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-4-carboxylic . acid HCl (8).

Operating in a sealed tube, compound 7 (900 mg, 2.20 mmol) was suspended in 6N HCl (75 mL) and the mixture was heated at 120 °C for 30 min. The solvent was removed under reduced pressure. After crystallization of the residue from MeOH/Et_2O (1:10), pure product **8** was isolated as a pail yellow solid (806 mg, 93%).
8-M.p. 163°C dec. (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6, 300 MHz): δ= 11.50- 9.50 (brs, 3H, exch.), 7.31 (d, *J* = 1.6 Hz, 1H), 7.09 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.99 (d, *J* = 8.1 Hz, 1H), 6.96 (s, 1H), 6.77 (s, 1H), 6.08 (s, 2H), 4.69, 4.34 (AM system, *J* = 11.0 Hz, 2H), 4.49, 4.23 (AB system, *J* = 16.0 Hz, 2H), 3.77 (s, 3H), 3.71 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ= 45.6, 50.0, 56.4, 56.5, 59.0, 102.3, 109.3, 109.4, 110.1, 110.9, 121.5, 123.1, 123.6, 129.5, 148.3, 148.8, 149.2, 149.3, 172.5. IR (KBr) ν 3430, 1722 cm^{-1}; MS (ESI): m/z 358 $[M]^+$; elemental analysis calcd. for $C_{19}H_{20}ClNO_6$: C, 57.95; H, 5.12; N, 3.56; found C, 57.51; H, 5.41; N, 3.11.

3R*,4R*-3-(3,4-Methylenedioxyphenyl)-2-(tert-butoxycarbonyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (9).

Operating in a three-necked round bottom flask equipped with a magnetic stirrer, nitrogen inlet, thermometer and a dropping funnel, compound **8** (500 mg, 1.40 mmol) was suspended in dry

CH_2Cl_2 (30 mL). The suspension was cooled to 0 °C after which a solution of triethylamine (215 μL 1.54 mmol) in dry CH_2Cl_2 (2 mL) was added. After 10 min a solution of $(tBuOCO)_2O$ (519 mg, 2.40 mmol) in dry CH_2Cl_2 (3 mL) was slowly dropped. The reaction was stirred for further 8 h at 25 °C. The reaction mixture was washed with brine (30 mL) and a saturated solution of $NaHCO_3$ (30 mL). After drying over Na_2SO_4 and solvent evaporation, the crude mixture was crystallized affording **9** (588 mg, 84%).

9-M.p. 93- 95 °C dec. (toluene/n-hexane); ^{1}H NMR (DMSO-d_6, 200 MHz): δ= 6.73- 6.40 (m, 5H), 5.88 (s, 2H), 5.68 (brs, 1H), 4.51, 4.16 (AB system, *J* = 16.5 Hz, 2H), 3.97 (brs, 1H), 3.69 (s, 3H), 3.64 (s, 3H), 1.36 (s, 9H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ: 28.7 (x3), 46.3, 50.5, 55.1, 56.25, 56.29, 79.8, 101.5, 107.2, 108.5, 110.3(br), 114.0, 119.9, 124.3, 125.8, 136.2, 146.5, 147.8, 148.1, 148.6, 155.1, 174.1; IR (KBr) ν 3450, 1720, 1688 cm^{-1}; MS (ESI): m/z 480.0 $[M+Na]^+$; elemental analysis calcd. For $C_{24}H_{27}NO_8$: C, 63.01; H, 5.95; N, 3.06; found C, 62.65; H, 6.29; N, 2.79.

t-Butyl 3-(2-diazoacetyl)piperidine-1-carboxylate (13a).

Operating in a three necked round bottom flask equipped with a magnetic stirrer, nitrogen inlet, and thermometer, compound **2** (500 mg, 2.2 mmol) was dissolved in dry THF (10 mL). The solution was cooled to 0°C and first iBuOCOCl (315 μL, 2.4 mmol) and then *N*-methylmorpholine (240 μL, 2.2mmol) were slowly added. The reaction was stirred for further 2 h at 25 °C. The crude mixture, containing intermediate **10a** was cooled at - 5 °C. A solution of diazomethane in Et_2O was added until the yellow colour was maintained and the stirring was continued for further 4 h at 25 °C. Et_2O was removed under vacuum and the crude mixture was purified by column chromatography (n-hexane/AcOEt; from 12:1 to 6:1) affording **13a** as a colourless oil (512.6 mg, 93%).

^{1}H NMR ($CDCl_3$, 200 MHz): δ= 5.29 (s, 1H), 4.06 (d, *J* = 13.6 Hz, 1H), 4.01-3.90 (m, 1H), 2.93 (dd, *J* = 12.2, 10.2 Hz, 1H), 2.85-2.72 (m, 1H), 2.49-2.22 (m, 1H), 1.92- 1.88 (m, 1H), 1.76-1.48 (m, 3H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ= 27.7, 28.7 (x3), 29.9, 44.2, 46.1, 47.0, 54.4, 80.0, 154.9, 195.3; IR (NaCl) ν 2104, 1694 cm^{-1}; MS (ESI): m/z 254.3 $[M]^+$; elemental analysis calcd (%) for $C_{12}H_{19}N_3O_3$: C, 56.90; H, 7.56; N, 16.59; found C, 56.68; H, 7.72; N, 16.33.

It is possible to isolate a sample of the anhydride intermediate **10a** after washing the reaction mixture with brine (30 mL) and a saturated solution of $NaHCO_3$ (30 mL). After drying over Na_2SO_4 and solvent evaporation, crude compound **10a** was isolated and analysed by ^{1}H NMR ($CDCl_3$, 200 MHz): δ= 4.35-4.00 (m, 1H), 4.02 (d, *J* = 7.0 Hz, 2H), 3.90-3.73 (m, 1H), 3.25-2.98 (m, 1H), 3.00-2.78 (m, 1H), 2.66-2.48 (m, 1H), 2.15-1.90 (m, 2H), 1.80-1.60 (m, 2H), 1.60-1.40 (m, 1H), 1.43 (s, 9H), 0.94 (d, *J* = 6.6 Hz, 6H).

t-Butyl 4-(2-diazoacetyl)-1,2,3,4-tetrahydroisoquinoline-2- carboxylate (14a).

Operating in a three-necked round bottom flask equipped with a magnetic stirrer, nitrogen inlet, thermometer, compound **4a** (440 mg, 1.6 mmol) was dissolved in dry THF (7.3 mL). The solution was cooled to 0°C and iBuOCOCl (281 mg, 1.6 mmol) was slowly dropped, then *N*-methylmorpholine (174 μL, 1.7 mmol) was added. The reaction was stirred for further 1.5 h at 25 °C. AcOEt (15 mL) and H_2O (7 mL) were added to the reaction mixture. The organic layer was separated, washed with saturated $NaHCO_3$ solution (3.5 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure. The crude mixture, containing **11a**, was dissolved in Et_2O (15 mL) and cooled at 0 °C. A solution of diazomethane in Et_2O was added until the yellow colour was maintained. The reaction was stirred overnight at 25 °C. Et_2O was removed under reduced pressure and the crude mixture was purified by column chromatography (n-hexane/AcOEt from 7:1 to 4:1) affording **14a** as a white solid (163 mg, 60 %).
M.p. 81-82°C (CCl_4); ^{1}H NMR (CD_3CN, 200 MHz): δ= 7.35-7.15 (m, 4H), 5.44 (s, 1H), 4.62, 4.33 (AB system, *J* = 16.9 Hz, 2H), 4.33, 3.39 (ABX system, *J* = 13.6, 4.4, 2.6 Hz, 2H), 3.78 (s, 1H), 1.45 (s, 9H); ^{13}C NMR (CD_3CN, 50 MHz): δ= 27.7 (x3), 44.2 (br), 45.3, 49.9, 54.9, 79.7, 126.8 (x2), 127.7, 129.8, 132.8, 134.6, 154.7, 194.5; IR (KBr) ν_{max} 2014, 1682, 1629 cm^{-1}; MS (ESI): m/z 324.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.36; N, 13.94; found C, 63.51; H, 6.49; N, 13.73.

3-Methylbutanoic anhydride of 2-(iso-butoxycarbonyl)-1,2,3,4- tetrahydroisoquinoline-4-carboxylic acid (11b)

Operating in a three necked round bottom flask equipped with a magnetic stirrer, nitrogen inlet, and thermometer, compound **3** (170 mg, 0.96 mmol) was dissolved in dry THF (3.5 mL). The solution was cooled to 0°C and first iBuOCOCl (1.9 mL, 1.9 mmol) and then TEA (1.47 mL, 1.9 mmol) were then added. The reaction was stirred for 60 min. at 25 °C. AcOEt (7 mL) and H_2O (3.5mL) were added to the reaction mixture. The organic layer was separated, washed with a saturated $NaHCO_3$ solution (3.5 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure affording product **11b** as a pail yellow oil (280 mg, 81%).
^{1}H NMR ($CDCl_3$, 200 MHz): δ= 7.28-7.13 (m, 4H), 4.76, 4.52 (AB system, *J* = 16.9 Hz, 2H), 4.34 (dd, *J* = 13.3, 4.7 Hz, 1H), 3.92-3.86 (m, 5H), 3.59 (dd, *J* = 13.3, 4.4 Hz, 1H), 2.00-1.79 (m, 2H), 0.95 (d, *J* = 6.6 Hz, 6H), 0.86 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ= 19.2 (x2), 19.3 (x2), 27.9, 28.2, 43.6, 45.1, 45.8, 71.4, 71.9, 126.7, 126.8, 127.7, 129.2, 131.8, 133.5, 155.9

(x2), 172.1; IR (NaCl) ν_{max} 1732, 1705 cm^{-1}; MS (ESI): calcd for 377.43; found 416.4+ $[M+K]^+$.

i-Butyl 4-(2-diazoacetyl)-1,2,3,4-tetrahydroisoquinoline-2- carboxylate (14b).

Operating in a three necked round bottom flask equipped with a magnetic stirrer, nitrogen inlet, thermometer, compound **4b** (260 mg, 0.9 mmol) was dissolved in dry THF (5.0 mL). The solution was cooled to 0 °C and EtOCOCl (98 μL, 1.0 mmol) was slowly dropped. *N*-methylmorpholine (103 μL, 1.0 mmol) was then added. The reaction was stirred for further 1.5 h at 0°C. AcOEt (10 mL) and H_2O (5 mL) were added to the reaction mixture. The organic layer was separated, washed with a saturated $NaHCO_3$ solution (5 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude mixture containing intermediate **11c** was dissolved in Et_2O (15 mL). The resulting solution was cooled to 0 °C and a solution of diazomethane in Et_2O was added until the characteristic yellow coloration was maintained. The reaction was stirred for 12 h at 25 °C. Et_2O was removed under reduced pressure and the crude mixture was purified by column chromatography (n-hexane/EtOAc, from 7:1 to 3:1) affording pure **14b** as a colourless oil (126 mg, 65%).

^1H NMR (CD_3CN, 200 MHz): δ= 7.36-7.18 (m, 4H), 5.44 (brs, 1H), 4.83 (d, *J* = 16.9 Hz, 1H), 4.60-4.40 (brs, 1H), 4.38, 3.80, 3.44 (AMX system, *J* = 13.6, 4.1 Hz, 3H), 3.88 (d, *J* = 6.6 Hz, 2H), 1.99-1.86 (m, 1H), 0.96 (d, *J* = 7.0 Hz, 6H); ^{13}C NMR (CD_3CN, 50 MHz): δ= 18.5 (x2), 28.2, 43.9, 45.5, 49.7, 55.1, 71.5, 126.86, 126.89, 127.7, 129.7, 132.7, 134.3, 155.6, 194.3; IR (KBr) ν_{max} 3436, 2104, 1683, 1628 cm^{-1}; MS (ESI): m/z 324.2 $[M+Na]^+$; elemental analysis calcd (%) forc $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.36; N, 13.94; found C, 63.41; H, 6.54; N, 13.57.

t-Butyl 3R*,4R*-4-(2-diazoacetyl)-3-(3,4-methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (15a)

Operating in a three-necked round bottom flask equipped with a magnetic stirrer, nitrogen inlet, thermometer and a dropping funnel, compound **9** (700 mg, 1.53 mmol) was suspended in dry Et_2O (30 mL). After cooling at -10 °C, a solution of TEA (235 μL 1.68 mmol) in dry Et_2O (2 mL) was added. After 10 min a solution of iBuOCOCl (221 μL, 1.68 mmol) in dry Et_2O (3 mL) was slowly dropped. The reaction was stirred for 30 min at 25 °C and then was washed with brine (30 mL), with a saturated solution of $NaHCO_3$ (30 mL) and the organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure. The crude mixture containing compound **12a** (850 mg, 1.52 mmol) was dissolved in dry Et_2O (30 mL). The solution was cooled at -5 °C and a solution

of diazomethane in Et_2O was added until the yellow colour was maintained. The reaction was stirred for further 2 h. The reaction mixture was washed with H_2O (30 mL), a saturated solution of $NaHCO_3$ (30 mL) and the organic layer was dried over Na_2SO_4 The crude mixture was purified by column chromatography (n-hexane/AcOEt, 7:1) affording pure **15a** (658 mg, 90%).

15a- M.p .73-75 °C dec. (Et_2O/n-hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ= 6.66-6.50 (m, 5H), 5.90 (s, 3H), 4.92 (brs, 1H), 4.84, 4.05 (AB system, *J* = 16.0 Hz, 2H), 3.88 (s, 4H), 3.87 (s, 3H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$): δ= 29.3 (x3), 42.3, 54.9, 55.2 (br), 55.9 (br), 56.3, 56.4, 81.0, 101.4, 107.7, 108.4, 109.5, 112.4, 120.4, 122.4, 126.2, 134.2, 147.1, 148.0, 148.8, 149.4, 155.2, 195.0; IR (KBr) ν_{max} 2102, 1720, 1684 cm^{-1}; MS (ESI): m/z 504.0 $[M+Na]^+$; elemental analysis calcd. For $C_{25}H_{27}N_3O_7$ C, 62.36; H, 5.65; N, 8.73; found C, 62.20; H, 5.81; N, 8.59.

3-Methylbutanoic anhydride of 3R*,4R*-3-(3,4- methylenedioxyphenyl)-2-(ibutoxycarbonyl)-6,7-dimethoxy-1,2,3,4- tetrahydroisoquinoline-4-carboxylic acid (12b).

Operating in a threenecked round bottom flask equipped with a magnetic stirrer, nitrogen inlet, thermometer and a dropping funnel, compound **8** (500 mg, 1.27 mmol) was suspended in dry CH_2Cl_2 (30 mL). The suspension was cooled to - 10 °C and a solution of TEA (390 µL 2.79 mmol) in dry DCM (2 mL) was added. After 10 min a solution of iBuOCOCl (367 µl, 2.79 mmol) in dry CH_2Cl_2 (3 mL) was slowly added. The reaction was stirred for further 60 min. The reaction mixture was washed with brine (30 mL), a saturated solution of $NaHCO_3$ (30 mL) and dried over Na_2SO_4. The crude mixture containing compound **12b** was used without further purification for the following reaction.

^1H NMR ($CDCl_3$, 200 MHz): δ= 6.69-6.54 (m, 5H), 6.03-5.89 (m, 1H), 5.89 (s, 2H), 4.80, 4.21 (AM system, *J* = 17.3 Hz, 1H), 4.09 (d, *J* = 1.5 Hz, 1H), 4.02 (d, *J* = 6.9 Hz, 2H), 3.93 (dd, *J* = 6.6, 1.5 Hz, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 2.06-1.83 (m, 2H), 0.94 (d, *J* = 6.6 Hz, 6H). 0.93 (d, *J* = 6.2 Hz, 6H).

i-Butyl-3R*,4R*-4-(2-diazoacetyl)-3-(3,4-methylenedioxyphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (15b).

Operating in a three necked round bottom flask equipped with a magnetic stirrer, nitrogen inlet, thermometer and a dropping funnel, compound **12b** (600 mg, 1.08 mmol) was dissolved in dry Et_2O (30 mL), the solution was cooled at -5 °C and a solution of diazomethane in Et_2O was added until the characteristic yellow colour was maintained. The reaction was stirred for further 2 h. The

reaction mixture was washed with water (30 mL), a saturated solution of saturated $NaHCO_3$ (30 mL). After drying over Na_2SO_4, the crude mixture was purified by column chromatography (n-hexane/AcOEt, 7:1) affording pure **15b** (481 mg, 93%).

15b-M.p. 76-78 °C dec. (Et_2O/n-hexane); ^1H NMR (CDCl3, 500 MHz) mixture of rotamers: δ= 6.57-6.71 (m, 5H), 5.91 (s, 3H), 4.87 (brs, 1H), 4.88, 4.15 (AB system, *J* = 16.7 Hz, 2H), 3.85-3.99 (m, 9H), 1.97 (br, 1H), 0.94 (s, 6H); ^{13}C NMR ($CDCl_3$, 500 MHz) mixture of rotamers: δ= 18.4 (x2), 27.3, 42.0, 53.7 (br), 53.9(br), 54.3(br), 55.2, 55.3, 71.4 (71.5), 100.3, 106.5 (106.6), 107.4 (107.5), 108.4, 111.3 (11.2), 119.3, 133.0 (129.5), 134.7, 164.0 (146.1), 146.1, 146.99 (147.0), 147.8, 148.42 (148.41), 155.1, 193.5; IR (KBr) ν_{max} 2107, 1717, 1679 cm^{-1}; MS (ESI): m/z 480.2 $[M-H]^-$; elemental analysis calcd. for $C_{25}H_{27}N_3O_7$ C, 62.36; H, 5.65; N, 8.73. Found C, 62.14; H, 5.83; N, 8.50.

t-Butyl 3-(cyclohepta-2,4,6-trienecarbonyl)piperidine-1-carboxylate (16).

Operating in a sealed tube under argon atmosphere, $Rh_2(OAc)_4$ (25.7 mg, 0.058 mmol) was dissolved in benzene (0.1 M) and the solution was refluxed at 70 °C for 10 min.. A solution of diazo-compound **13a** (100 mg, 0.39 mmol, 0.1 M in benzene) was slowly dropped in 2h after which the reaction was stirred until under reflux for 6h. The crude mixture was purified by column chromatography (n-hexane/AcOEt, from 20:1 to 1:1) affording **16** (39 mg, 33%) as an oil (trace amount of isomers are present on NMR spectra).

^1H NMR (CD_2Cl_2, 300 MHz): δ= (main isomer): 6.65-6.55 (m, 2H), 6.40-6.28 (m, 2H), 4.94 (brs, 2H), 4.11 (brs, 1H), 3.95 (d, *J* = 13.4 Hz, 1H), 2.98-2.73 (m, 4H), 2.00-1.95 (m, 1H), 1.75-1.38 (m, 3H), 1.46 (s, 9H); ^{13}C NMR (CD_2Cl_2, 75 MHz): δ= 24.8, 27.4, 28.5 (x3), 44.5, 45.9, 46.0, 47.9, 79.7, 103.1 (x2), 126.6, 126.7, 129.7, 129.8, 154.8, 210.1; IR (NaCl) ν_{max} 1693 cm^{-1}; MS (ESI): m/z 326.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{18}H_{25}NO_3$: C, 71.26; H, 8.31; N, 4.62; found C, 70.88; H, 8.53; N, 4.31.

General Procedure for Rhodium (II)-Catalyzed Transformation of Diazocarbonyl Compounds 14.

Operating in a sealed tube under argon atmosphere, a solution of Rh-(II) catalyst (1 mol % or 15 mol %; 0.1 M in the solvent of choice; see Tables 1 or Table 2) was heated at reflux for 10 min. The temperature was adjusted at the indicated value (see Tables 1 or 2). Compound **14a** or **14b** (0.1 M solution) was dropped in 2h. The reaction was stirred for the indicated time. The crude mixture was

purified by column chromatography (n-hexane/EtOAc, from 10:1 to 1:1) affording complex **17** and **18**. The yield of the above compounds for each experiment is reported in Table 1 and Table 2.

17a: m.p. 197-198 dec. (CH_3CN); ^1H NMR ($CDCl_3$, 500 MHz): δ= 7.41- 7.29 (m, 8H), 5.45, 4.72 (AB system, *J* = 17.8 Hz, 4H), 4.45, 3.92 (d, *J* = 18.7 Hz, 4H), 4.30, 3.97, 3.74 (AMX system, *J* = 12.0, 2.5, 0.5 Hz, 6H), 1.88 (s, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ= 23.7 (x4), 50.6 (x2), 55.8 (x2), 60.6 (x2), 63.1 (x2), 127.1 (x2), 127.5 (x2), 127.8 (x2), 128.4 (x2), 132.3 (x2), 133.0 (x2), 190.5 (x4), 210.3 (x2); IR (KBr) ν_{max} 3436, 1755, 1593, 1431 cm^{-1}; MS (ESI): m/z 789.1 $[M+H]^+$, 638.1 [-$C_{11}H_{11}NO$ (**19**) +Na], 496.7 [-$C_{11}H_{11}NO$ (**19**), -2AcO], 365.5 [AcORh-RhOAc,+K], 206 [Rh-Rh], 174.2 [$C_{11}H_{11}NO$ (**19**) +H]; elemental analysis calcd (%) for $C_{30}H_{34}N_2O_{10}Rh_2$: C, 45.70; H, 4.35; N, 3.55; found C, 45.43; H, 4.79; N, 3.30.

17b: m.p. 207-208 dec. (CH_3CN); 1H NMR (CD_2Cl_2, 500 MHz): δ= 7.38- 7.28 (m, 4H); 7.24 (d, *J* = 7.2 Hz, 2H), 7.12 (t, *J* = 7.3 Hz, 12H), 6.93 (t, *J* = 7.8 Hz, 26H), 6.75 (d, *J* = 7.6 Hz, 24H), 4.97, 4.18 (AB system, *J* = 17.6 Hz, 4H), 3.85, 3.37 (AB system, *J* = 18.5 Hz, 4H), 3.68 (d, *J* = 9.9 Hz, 2H), 3.45-3.32 (m, 4H); ^{13}C NMR (CD_2Cl_2, 125 MHz): δ= 50.3(x2), 56.3(x2), 61.2(x2), 63.5(x2), 69.1(x4), 126.5 (x12), 126.9(x2), 127.0(x2), 127.1(x24), 127.4(x2), 128.0(x2), 130.5(x24), 131.1(x2), 132.1(x2), 143.5(x12), 192.4(x4), 208.6(x2); IR (KBr) ν_{max} 3436, 1754, 1706, 1591, 1430 cm^{-1}; MS (HRMS): m/z calcd for $C_{102}H_{82}N_2O_{10}Rh_2$ 1700.41; found 1701.42472 $[M+H]^+$; 1550.32149 [-$C_{11}H_{11}NO$ (**19**)].

18: oil, unstable compound. ^1H NMR ($CDCl_3$, 200 MHz): δ= 7.40-7.10 (m, 3H), 4.99, 4.30 (AX system, *J* = 16.9, 2H), 4.80-4.60 (m, 1H), 3.93 (d, *J* = 6.7 Hz, 2H), 3.78, 3.35 (AB system, *J* = 20.9 Hz, 2H), 3.62-3.58 (m, 1H), 2.98-2.70 (m, 1H), 2.04-1.90 (m, 1H), 0.97 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ= 19.5 (x2), 28.4, 41.8, 44.3, 48.5, 55.7, 72.4, 123.1, 124.5, 128.5, 130.0, 145.9, 147.5, 151.1, 198.1; IR (KBr) ν_{max} 1760.9, 1691.3 cm^{-1}; MS (ESI): m/z calcd for $C_{16}H_{19}NO_3$ 273.33; found 274.0 $[M+H]^+$.

General Procedure for the Stoichiometric Reactions of Rhodium (II)- catalyst with Diazo-carbonyl Compound 14b.

Operating in a sealed tube under argon atmosphere, compound 14b (50 mg; 0.17 mmol) was dissolved in CH_2Cl_2 (1.7 mL) and refluxed for 10 min. The temperature was adjusted at 25 °C. A solution of $Rh_2(OAc)_4$ * $2H_2O$ or $Rh_2(tpa)_4$ *$2CH_2Cl_2$ in CH_2Cl_2 (0.5 equiv., 0.01 M solution) was

added (0.1 equiv. every 20 min. for the first four additions; then 0.01 equiv. every 5 min. until the disappearance of the starting material). The solvent was evaporated to reduced pressure and the crude mixture was purified by column chromatography (n-hexane/AcOEt, from 10:1 to 1:1) affording compound **17a** (70%) or **17b** (96%) as a violet solid.

3,5-Dihydro-2,5-methanobenzo[c]azepin-4(1H)-one (19).

Method A. Operating in a two-necked round bottom flask equipped with a magnetic stirrer, a nitrogen inlet and a dropping funnel under N_2 atmosphere, compound **17a** (50 mg, 0.58 mmol) was dissolved in $CHCl_3$ (5 mL). A solution of PPh_3 (75 mg, 1.16 mmol) in $CHCl_3$ (5 mL) was dropped and the reaction mixture was stirred for 5 min.. The organic layer was extracted with a solution of HCl (0.1 N, 10 mL). The aqueous layer was basified with $NaHCO_3$ (pH = 8) and extracted with CH_2Cl_2 (10 mL x 2). The organic layers were collected and dried over Na_2SO_4. After solvent removing under reduced pressure, compound **19** was obtained as a pail yellow oil (8.3 mg, 82%).

Method B. Operating in a sealed tube under argon atmosphere at 25 °C, compound **14b** (150 mg, 0.5 mmol) was dissolved in dry $CHCl_3$ (0.1 M). A solution of PPh_3 (64 mg, 10 mol %) in dry $CHCl_3$ (0.1 M) was dropped and, after 10 min., Rh-(II) catalyst (1% mol.; Table 2, entries 4 and 5) was added to the solution and the reaction was stirred for time indicated in Table 2 (entries 4,5). The solvent was evaporated under reduced pressure and the crude mixture was purified by column chromatography (n-hexane/EtOAc, 4:1). Pure compound **19** ($Rh_2(OAc)_4$. 2 H_2O: 70%; $Rh_2(tpa)_4$. 2 CH_2Cl_2: 96%) was isolated as an oil.

^{1}H NMR ($CDCl_3$, 200 MHz): δ= 7.40-7.00 (m, 4H), 4.57, 3.86 (AB system, *J* = 17.6 Hz, 2H), 3.58-3.30 (m, 3H) 3.23 (dd, *J* = 5.1, 3.3 Hz, 1H), 3.16 (dd, *J* = 11.2, 3.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ= 56.4, 60.9, 65.6, 69.0, 107.9, 109.7, 111.2, 111.4, 123.4, 125.0, 191.7. IR (KBr) ν_{max} 1760, 1592 cm^{-1}; MS (ESI): m/z 174.2 $[M+H]^+$; elemental analysis calcd (%) for $C_{11}H_{11}NO$: C, 76.28; H, 6.40; N, 8.09; found C, 75.81; H, 6.73; N, 7.75.

Di-t-butyl 3,3'-(1,2,4,5-tetrazinane-3,6-dicarbonyl)bis(piperidine-1- carboxylate) (25).

Operating in a sealed tube under argon atmosphere at 25 °C, compound **13a** (500 mg, 1.96 mmol) was dissolved in dry $CHCl_3$ (0.1 M). A solution of PPh_3 (10 mol %) in dry $CHCl_3$ (0.1 M) was dropped and, after 10 min., Rh-(II) catalyst (1 mol %) was added to the solution. The reaction was stirred for 8 h. A red solid was formed. The solvent was evaporated under reduced pressure and the

crude mixture was purified by column chromatography (n-hexane/EtOAc, 4:1). Pure compound **25** (472 mg, 94%) was isolated as a solid after crystallization.

25-M.p. 107-109 °C dec. (Et_2O); ^{1}H NMR (DMSO-d_6, 500 MHz): δ= 8.50 (s, 4H, exch.), 6.99 (s, 2H), 3.98-3.78 (m, 4H), 3.31-3.27 (m, 2H), 2.98-2.70 (m, 4H), 1.81-1.76 (m, 2H), 1.69-1.62 (m, 2H), 1.51-1.43 (m, 2H), 1.39 (s, 18H), 1.40-1.30 (m, 2H); ^{13}C NMR (CD_3CN, 75 MHz): δ= 199.9 (x2); 154.4 (x2); 135.5 (x2); 78.8 (x2); 46.0 (x2); 44.1 (m, x2); 41.9 (x2); 27.1 (x2); 27.6 (x6); 24.2 (x2); IR (KBr) ν_{max} 2790; 1749 cm^{-1}; MS (ESI): m/z 533.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{24}H_{42}N_6O_6$: C, 56.45; H, 8.29; N, 16.46; found C, 56.61; H, 8.29; N, 15.99.

General Procedure for Rhodium (II)-Catalyzed Transformation of Diazocarbonyl Compounds 15b.

Operating in a sealed tube under argon atmosphere, a solution of Rh-(II) catalyst (1 mol. %; 0.1 M in the solvent of choice; see Table 3) was heated at reflux for 10 min. The temperature was adjusted at the indicated value (see Table 3). Compound **15b** (0.1 M solution) was dropped in 2h and the reaction was stirred for the indicated time. The solvent was evaporated under reduced pressure and the crude mixture was dissolved in dry EtOH (0.1 M) under N_2 atmosphere. The solution was cooled at 0 °C and $NaBH_4$ (4 equiv.) was added. The reaction was stirred at 25 °C for further 12 h. The mixture was filtered over a short silica gel pad and finally washed with AcOEt (100 mL). After solvent evaporation, the crude mixture was purified by column chromatography (n-hexane/AcOEt, 7:1) affording compounds **20** and **21** that were isolated as pure compounds after crystallization. The yields of the isolated compounds were reported in Table 3. Is it possible to isolate pure ketone **20** (36 %; CH_2Cl_2, 0 °C, 4.30 h) by column chromatography avoiding the reductive step. Any attempt to isolate compound **21** failed because of its instability in solution.

(2R,5R*,10R*)-10-(3,4-methylenedioxyphenyl)-7,8-dimethoxy-3,5-dihydro-2,5 methanobenzo[c]azepin-4-(1H)-one (20)*

m.p. 101- 104 °C dec. (Et_2O/n-hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ= 6.84 (s, 1H), 6.78-6.68 (m, 2H), 6.66 (d, *J* = 8.1 Hz, 1H), 6.35 (s, 1H), 5.91, 5.90 (AB system, *J* = 1.4 Hz, 2H), 4.53, 3.61 (AX system, *J* = 2.6 Hz, 2H), 4.11, 3.58 (AX system, *J* = 17.4 Hz, 2H), 3.92 (s, 3H), 3.79, 3.49 (AM system, *J* = 18.3 Hz, 2H), 3.77 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ= 51.7, 52.5, 56.1, 56.4, 63.7, 63.9, 101.4, 108.4, 109.1, 109.4, 110.5, 122.1, 122.3, 124.0, 130.0, 147.1, 147.9, 149.4, 149.5,

209.3; IR (KBr) ν_{max} 1748 cm^{-1}; MS (ESI): m/z 354.1 $[M+H]^+$; elemental analysis calcd (%) for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96; found C, 67.73; H, 5.64; N, 3.78.

(2R*,4R*,5R*,10R*)-10-(3,4-methylenedioxyphenyl)-7,8-dimethoxy- 1,3,4,5-tetrahydro-2,5-methanobenzo[c]azepin-4-ol (22).

Mixture of diastereoisomers: d.e.72%. Main isomer: m.p. 121-123 °C dec. (Et_2O/n-pentane); ^1H NMR ($CDCl_3$, 200 MHz): δ= 6.87 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.79 (d, *J* = 1.7 Hz, 1H), 6.76 (s, 1H), 6.66 (d, *J* = 8.2 Hz, 2H), 6.57 (s, 1H), 5.93 (s, 2H), 4.75-4.68 (m, 1H), 4.33 (d, *J* = 2.3 Hz, 1H), 4.04, 3.89 (AX system, *J* = 18.5 Hz, 2H), 4.02 (dd, *J* = 13.6, 4.3 Hz, 1H), 3.96 (s, 3H), 3.90 (s, 3H), 3.40 (dd, *J* = 2.3, 5.4 Hz, 1H), 2.88 (dd, *J* = 13.6, 5.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ= 48.6 (47.9), 56.4, 56.5, 61.0, 69.0, 70.2, 73.2, 101.5, 108.0, 109.6 (109.8), 111.4 (111.2), 112.6, 113.4, 123.4, 123.5, 125.0 (124.8), 125.9, 128.1; IR (KBr) ν_{max} 3436, 1620, 1612 cm^{-1}; MS (ESI): m/z 356.3 $[M]^+$; elemental analysis calcd (%) for $C_{20}H_{21}NO_5$: C, 67.59; H, 5.96; N, 3.94; found C, 67.40; H, 6.13; N, 3.81. Significant signals for the minor isomer: ^1H NMR ($CDCl_3$, 200 MHz) δ: 6.61 (s, 1H), 5.88 (s, 2H), 2.78 (dd, *J* = 12.4 Hz 1H).

(3R*,3aR*,4R*)-i-Butyl-3-(3,4-methylenedioxyphenyl)-4-hydroxy-6,7-dimethoxy-3,3a,4,5-tetrahydrocyclopenta[de]isoquinoline-2(1H)-carboxylate (23)

m.p. 83-85 °C dec (Et_2O/n-hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ= 6.98 (d, *J* = 1.4 Hz, 1H), 6.91 (dd, *J* = 8.0, 2.1 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.72 (s, 1H), 5.98 (s, 2H) 5.17, 4.12 (AX system, *J* = 15.0 Hz, 2H), 4.83 (d, *J* = 7.7 Hz, 1H), 4.67 (q, *J* = 7.7 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.82 (dd, *J* = 10.4, 7.5 Hz, 1H), 3.73 (dd, *J* = 10.4, 6.8 Hz, 1H), 3.50 (dd, *J* = 15.6, 7.6 Hz, 1H), 3.12 (dd, *J* = 7.6, 7.7 Hz, 1H), 2.96 (dd, *J* =15.6, 8.3 Hz, 1H), 1.87 (brs, 1H, exch), 1.83-1.76 (m,1H), 0.88 (d, *J* = 5.5 Hz, 6H); ^{13}C ($CDCl_3$, 75 MHz): δ= 18.2, 18.3, 27.2, 38.0, 43.5, 53.1, 55.8, 59.6, 61.7, 71.3, 81.6, 100.4, 105.8, 107.71, 107.74, 118.2, 127.8, 128.1, 131.0, 137.9, 144.1, 145.9, 147.5, 151.1, 155.6; IR (KBr) ν_{max} 3436, 1700, 1671 cm^{-1}; MS (ESI): m/z 478 $[M+Na]^+$; elemental analysis calcd (%) for $C_{25}H_{29}NO_7$: C, 65.92; H, 6.42; N, 3.08; found C, 65.79; H, 6.53; N, 2.91.

i-Butyl 4-(2-chloroacetyl)-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (24).

Operating at 25 °C under stirring, compound **14b** (50 mg, 0.166 mmol.) was dissolved in CH_2Cl_2 (5 mL) and a dioxane solution of anhydrous HCl (2 mL, 10 equiv., 48%) was added. After 30 min., the

solvent was evaporated and the mixture was purified by column chromatography (cycohexane/AcOEt, 6:1). Pure compound **24** (46.7 mg, 91%) was isolated as an oil.

^{1}H NMR (CD_3CN, 300 MHz) δ: 7.33-7.16 (m, 4H), 4.86 (d, *J* = 19.2 Hz, 1H), 4.57, 4.47 (AB system, *J* = 16.5, 2H), 4.55 (brs, 1H, overl.), 4.38 (brs, 1H), 4.17 (brs, 1H), 3.88-3.85 (m, 2H), 3.45 (brs, 1H), 1.99-1.86 (m, 1H), 0.95 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (CD_3CN, 50 MHz): δ= 18.7 (x2), 28.3, 43.3, 45.7, 48.9, 49.5, 71.8, 117.6, 117.8, 126.9, 127.3, 128.0, 129.9, 131.9, 201.1; IR (KBr) ν_{max} 1733, 1704 cm^{-1}; elemental analysis calcd (%) for $C_{16}H_{20}ClNO_3$: C, 62.03; H, 6.51; N, 4.52; found 61.78; H, 6.77; N, 4.30.

Pubblications and Conferences

1) *Syn/anti* Switching by Specific Heteroatom–Titanium Coordination in the Mannich-Like Synthesis of 2,3-Diaryl-β-amino Acid Derivatives. Andrea Bonetti; F. Clerici; F. Foschi; D. Nava; S. Pellegrino; M. Penso; R. Soave; M. L. Gelmi; Eur. J. Organic Chem., 2014,15, 3203–3209,(*full Paper*)

2) Unusual chemoselective Rh-(II) catalyzed transformation of alpha-diazocarbonyl piperidine cores. Andrea Bonetti; *E. Beccalli*; *A. Caselli; F.Clerici; S. Pellegrino*; M.L. Gelmi; *Chemistry an European Journal*, Article first published online: 24 NOV **2014** DOI: 10.1002/chem.201405197 (*full Paper*)

3) Beccalli . E; Bonetti. A; Mazza. A; (planned pubblication 2015) *Intramolecular C-N and C-O bond formation.* In S. Ma; S. Gao; (1^{fts} Thieme Edition) *Metal Catalyzed Cyclization Reactions* (Chapter-9). New York. (*Book Chapter*)

4) XXIV Congresso della Divisione di Chimica Organica della SCI Pavia 10-14 Settembre 2012 ***"Alpha- diazocarbonyl-piperidine derivatives: chemoselective Rhodium catalyzed transformations."*** Andrea Bonetti (speaker), Maria Luisa Gelmi, Sara Pellegrino.

5) XXXV Congresso della Divisione di Chimica Organica della SCI Sassari 9-13 Settembre 2013 ***"β^{2,3}-Diaryl aminoacid via Mannich-like Reaction of Arylacetic ester/thioester: Chirality switching Induced by an Aryl-substituted Ortho-Heteroatom"*** Andrea Bonetti (speaker), Francesca Clerici, Francesca Foschi, Sara Pellegrino Michele Penso, Maria Luisa Gelmi.

6) XI Congresso del Gruppo Interdivisionale di Chimica Organometallica Milano 24-27 Giugno 2014 ***"Unusual Chemoselective Rh(II)-Catalyzed Transformations of α-Diazocarbonyl-Piperidine Cores"*** Andrea Bonetti (speaker), Alessandro Caselli,Francesca Clerici, Sara Pellegrino, Raffaella Soave, Maria Luisa Gelmi.

7) S. Pellegrino, A. Bonetti, F. Meneghetti, M. L. Gelmi, M. Reiches "***self-assembly of dipeptide nanotubes constituted by (S,S) beta-diaryl aminoacid and (L)-alanine, as putative drug delivery system****" 7th Annual Thematic Workshop of CRS Italy Chapter. Florence 6-8 November 2014. (Poster)*