SYNTHESIS AND CONFORMATIONAL ANALYSIS OF PEPTIDOMIMETICS CONTAINING UNNATURAL AMINO ACIDS BASED ON A PIPERAZINONE AND γ-BUTYROLACTONE SCAFFOLDS

Dissertation

Zur Erlangung des Doktorgrades der Naturwissenschaften

Dr. rer. nat.

der Universität Insubria COMO (Italy)

und

der Fakultät für Chemie und Pharmazie der Universität Regensburg

vorgelegt von

Karine Guitot

aus

Paris (Frankreich)

COMO 2008

UNDER THE SUPERVISION OF:

PROFESSOR UMDERTO PIARULLI

PROFI

MARIE CURIE ACTIONS

A Vincent Havage,
A mes parents, à ma sœur,

Acknowledgements

I would like to thank *Professor Umberto Piarulli*, my Supervisor from Italy who gave me the opportunity to participate in this research project. I would like to thank him in particular for his extreme patience and support from the first day of my thesis until the end. I would like also to thank *Professor Oliver Reiser* my Supervisor from Germany who welcomed me in his research team for the second part of this Thesis.
I am grateful to *Professor Chiara Cabrele*, *Professor Sames Sicsic* and *Dr. Sandrine Ongeri*, for their precious advices during these three years.

For the financial support, I am grateful to «Marie Curie Early Stage training Fellowship of the European Community's sixth Framework Programme».

Ringrazio particolarmente tutto il gruppo di ricerca di Como, in particolare Angelo e Enrica che mi hanno sempre fatto degli spettri NMR « artistici ». Patrizia, Sonia, Gianni, Barbara e Damiano per la loro accoglienza e per la simpatica atmosfera in ufficio e in laboratiorio.

Desweiteren möchte ich mich bei *Herr Burgermeister, Herr Kastner, Frau Schramm, Frau Stülhe* für ihre freundliche Unterstutzung bedanken.
Für ihre große Hilfe bin ich auch *Herr Mayer, Herr Kiermeier, Herr Söllner, Herr Vasold Herr Lautenschlage Herr Kreitmeier* sehr dankbar.

Ringraziamenti speciali vanno alle mie due collaboratrice preferite con chi ho passato tallmente tempo dentro e fuori dall laboratorio: Ana Sophia "Portogallo"und meine Mitbewohnerin Lucia. Grazie a tutte due per la vostra amicevole presenza in Italia, in Germania e finallemente in Francia in questi ultimi tempi.

Ringraziamenti anche ai miei colleghi e amici Italiani: Chiara, Chicco, Alice, Marco, Monsieur Marcel, Matteo e tanti altri che ho in contrato durante questo tempo fuori dall mio paese!

Insbesondere möchte ich meine Deutsche Kollegen Sebastian, the little Florian, Dominik, Christopher, Ina, Hans und Flo fur die angenähme Arbeitsatmosphere im labor meinen Dank aussprechen. Vielen dank auch fur Kirsten, Clement Sylvia Carolina und Jack……..und Cristian Cattaneo il mio chouchou!

Je voudrais remercier bien sur mes amis français pour leur fidélité et leur aide incessante malgré l'éloignement. Mes pensées vont en particulier à Coralie « Bridget » qui ne cesse de m'encourager depuis si longtemps déjà.

Enfin je voudrais remercier mon Vincent qui a compris et accepté tous mes choix, malgré la distance, ainsi que ma famille: mes parents et ma sœur qui ont participé à chacune des étapes de cette thèse.

Summary

The design and synthesis of conformationally restrained amino acids has been the focus of extensive research, because these compounds mimic or induce specific secondary structural features of peptides and proteins. Since the discovery of the crucial role of proline in protein structure, cyclic α-amino acids containing a heterocyclic ring have attracted considerable attention both from synthetic and medicinal chemists. The first part of this Ph D project is dedicated to the design and synthesis of a new cyclic α-amino acid; 5-Oxopiperazine carboxylic acid (so called "Pca"). Herein we will report a practical synthesis of (*R*)- and (*S*)-*N*-Boc-5-Oxo-piperazine-2-carboxylic acid, its introduction into peptidomimetic structures by a solution-phase peptide-synthesis strategy, and a conformational analysis of tetrapeptide mimics incoroporating a Pca residue.

Oligomers that adopt predictable conformations (so called "foldamers") are subject of increasing interest from the perspectives of both fundamental research and applications. In this context, the second part of this Ph D work is focused on the structural behaviour of another unnatural cyclic amino acid, previously synthesized in the group of Pr. Reiser. This second scaffold is a delta amino acid constrained in its center by a γ–butyro lactone ring. We have investigated the structural role of this building block in homo-δ-oligomers as well as dipeptide surrogates in α-amino acid sequences. These oligomers were synthesized using both solution and solid phase peptide chemistry and secondary structural features of these new molecules were defined using NMR, IR, CD data as well as molecular modelling.

TABLE OF CONTENTS

ABBREVIATIONS **7**

AMINO ACID CODES **8**

I - INTRODUCTION **9**

UNIVERSITA' DEGLI STUDI DELL'INSUBRIA **12**

II - PROLINE **13**

II-1 PROLINE IN PEPTIDES AND PROTEINS: BIOLOGICAL ACTIVITY 15
II-1.1 Proline in peptides biological activity *15*
II-1.2 Peptides containing proline toward enzymes *16*
II-2 PROLINE IN PROTEINS 17
II-2.1 Proline rich proteins (PRPs) *17*
II-2.1.1 Tandemly repeated sequences: Binding and coating function 17
II-2.1.2 Multi Proline rich regions (PRR) systems: Transcription initiation 18
II-2.1.3 Proline/Hydroxyproline (Hyp) rich proteins : A structural role 19
II-3 PROLINE ROLE IN SECONDARY STRUCTURES 20
II-3.1 Proline in helices *20*
II-3.1.1 Polyproline helices 20
II-3.1.1.1 Polyproline I (PPI) 20
II-3.1.1.2 Polyproline II 21
II-3.1.2 α-helices 22
II-3.2 Proline in turns *22*
II-3.2.1 Proline in Type II β-turns 22
II-3.2.2 Proline in VI β- turns: cis proline turns 23
II-3.2.3 Proline in new foldamers 23

III- CYCLIC α-AMINO ACIDS CONTAINING A NITROGEN ATOM IN THE RING: TOOLS FOR THE DESIGN OF PROLINE MIMICS **25**

III-1 C3 AZIRIDIN-2- CARBOXYLIC ACIDS AND DERIVATIVES 26
III-1.1 Synthetic routes *26*
III-1.2 In the field of peptide and petido mimetics *29*
III-2 C4 AZETIDINE-CARBOXYLIC ACIDS AND DERIVATIVES 31
III-2.1 Synthesis *31*
III. 2. 2 Peptidomimetics *34*
III-3 C5 DECORATED PYRROLIDINE RINGS, A ROUTE TO MODIFIED PROLINE 35
III-3.1 Synthesis *36*
III-3.1.1 2-subsituted prolines 36
III-3.1.2 3-substituted proline 40
III-3.1.3 4-substituted proline 41
III-3.1.4 5-substituted proline 41
III-3.2 Structural features *42*
III.4. C-6, 6-MEMBERED RING CYCLIC α-AMINO ACIDS 44
III-4.1 Synthesis *44*
III-4 1 1 Pipecolic acids and derivatives 44
III-4.1.2 Piperazines carboxylic acids 44
III-4.1.3 Piperazic acid 50
III-4.1 4 Morpholine-3-carboxylic acid 51
III- 4. 2 Structural features *52*

IV – DISCUSSION **53**

IV-1 SYNTHESIS OF THE 5-OXO-PIPERAZIN-2-CARBOXYLIC ACID 53
IV-2 COUPLING STUDIES ON THE 5-OXOPIPERAZINE-2-CARBOXYLIC ACID 67
IV-3 BUILDING OF THE PIPERAZINONE RING WITHOUT THE BENZYL SUBSTITUENT: 74
IV-3.1 removal of the Bn or PMB group: *74*
IV-3.2 Alternative synthesis: *76*
IV-4 CONFORMATIONAL STUDIES ON TETRAPEPTIDES CONTAINING PCA: 84
IV-4.1 Tetrapeptides containing the Bn-Pca: *84*

IV-4.2 Tetrapeptides containing non substituted Pca: *88*

V - EXPERIMENTAL DATA: **90**

V-1 MATERIALS AND METHODS: 90
V-2 SYNTHESIS OF (BN)-PCA DERIVATIVES: 91
V-3 COUPLING STUDIES ON THE (BN)5-OXOPIPERAZINE-2-CARBOXYLIC ACID 112
V-4 SYNTHESIS OF PCA: 125
V-5 TETRAPEPTIDES 139

VI – REFERENCES – CHAPTER II,III,IV,V **144**

UNIVERSITÄT REGENSBURG **150**

VII - δ-AMINO ACIDS AS INDUCER OF SECONDARY STRUCTURES: **151**

VII-1 δ-AMINO ACIDS AND FOLDING PROPERTIES: 151
VII-1.1 α-Helix peptidomimetics containing δ-amino acids *151*
VII.1.2 δ-Amino-acids in turns : *153*
VII-1.2.1 Isosteres containing a double bond in C(β)-C(γ) position: 154
VII-1.2.2 Linear δ-amino acids: 156
VII-1.2.3. Bicyclic dipeptide isosteres: 158
VII-1.2.4. Sugar-amino acids as β-turn mimics: 160

VIII – NEW δ-AMINO ACIDS TOWARDS NEW FOLDAMERS **163**

VIII-1 INTRODUCTION 163
VIII-2. MODEL PEPTIDES CONTAINING δ-AMINO ACID UNITS 164
VIII-2.1. Previous work in the laboratory *164*
VIII-2.2. Conformational investigations *167*
VIII-2.3. Results on alternated α/δ amino acids *174*
VIII-3 NEW PEPTIDES CONTAINING δ-AMINO ACIDS: OBJECTIVES AND DESIGN 183
VIII.4 SYNTHESIS OF PEPTIDES 32-34 185
VIII-5 RESULTS AND DISCUSSION 190
VIII.5.1 Alternated α/(S,S)-δ-unit peptide *190*
VIII.5.2. Alternated α/(R,R)-δ-unit peptides *192*
VIII.6 MONOMER STUDIES 196
VIII.6.1 Previous work on the δ-butyrolactone amino acid *196*
VIII.6.2. New analyses on the monomer *198*
VIII.7 INFLUENCE OF THE (R,R)-δ-LACTONE AMINO ACID ON THE A-HELIX STABILITY 203
VIII.7.1 Aim of the study *203*
VIII.7.2 Design of the modified peptides *205*
VIII.8 SYNTHESIS OF PEPTIDES 45-50 206
VIII.9 RESULTS AND DISCUSSION 207
VIII.10. CONCLUSION 211

XIX - EXPERIMENTAL PART **212**

XIX-1 EXPERIMENTAL PART CHAPTER VIII 212

X - REFERENCES – CHAPTER VII, VIII, XIX **227**

ABBREVIATIONS

Ac	acetyl
CAN	acetonitrile
Bn	benzyl
Boc	tert-Butyloxycarbonyl
CAN	cerium ammonium nitrate
Cbz	benzyloxycarbonyl
CD	circular dicroism
COSY	correlation spectroscopy
DCM	dichloromethane
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethylsulfoxyde
DNA	desoxyribonucleic acid
EDC	N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
ESI	electrospray ionization
Fmoc	fluorenyl-9-methoxycarbonyl
HATU	*O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HBTU	O-(1-benzotriazolyl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate
HOBt	hydroxybenzotriazole
HOAt	1-Hydroxy-7-azabenzotriazole
HPLC	high pressure liquid chromatography
IBX	2-Iodoxybenzoic acid
IR	infra red spectroscopy
LDA	Lithium diisopropylamide
MALDI-Tof	matrix-assisted-laser-desorptionionization time of flight
MBHA	methylbenzhydrylamine
Mp	melting point
NBS	*N*-Bromosuccinimide
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect
Pbf	2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl
PMB	Para-Methoxy-Benzyl
Pca	Piperazinone carboxylique acid
SN_2	Nucleophilic substitution of type 2
RNA	Ribonucleic acid
TEA	Triethylamine
TFA	trifluoroacetic acid
TFE	2,2,2-trifluorethanol
THF	tetrahydrofurane
TIS	triisopropylsilane
TOCSY	total correlation spectroscopy

tR	retention time
UV	ultraviolet
TACE	TNF-a Converting Enzyme
TNF-α	Tumor necrosis factor
TLC	Thin-layer chromatography

Amino Acid Codes

One-Letter Code	Three-Letter Code	Amino Acid
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
Unspecified amino acid	X	Xaa

I - INTRODUCTION

The function of peptides and proteins in living systems is strictly related to their three-dimensional architecture. As it is now well described, the structural organisation of proteins can be divided in three subtypes of structures. Primary structure involves the direct sequence of amino acids. The term “secondary structure” refers to a local conformational preferences of the poly (α-amino acid) backbone. The most common regular elements of secondary structures include α-helices, β-turns and β-strands. The tertiary structure of a protein is the way in which various elements of regular secondary structure, and irregular connecting segments are packed together. Scientists from different fields, have worked together to study and understand these incredible folding properties of proteins, but it is until now, still not possible to predict the exact organisation that will take a simple sequence of amino acids to fold in a extremely complicated tertiary structure, leading to the biological activity.

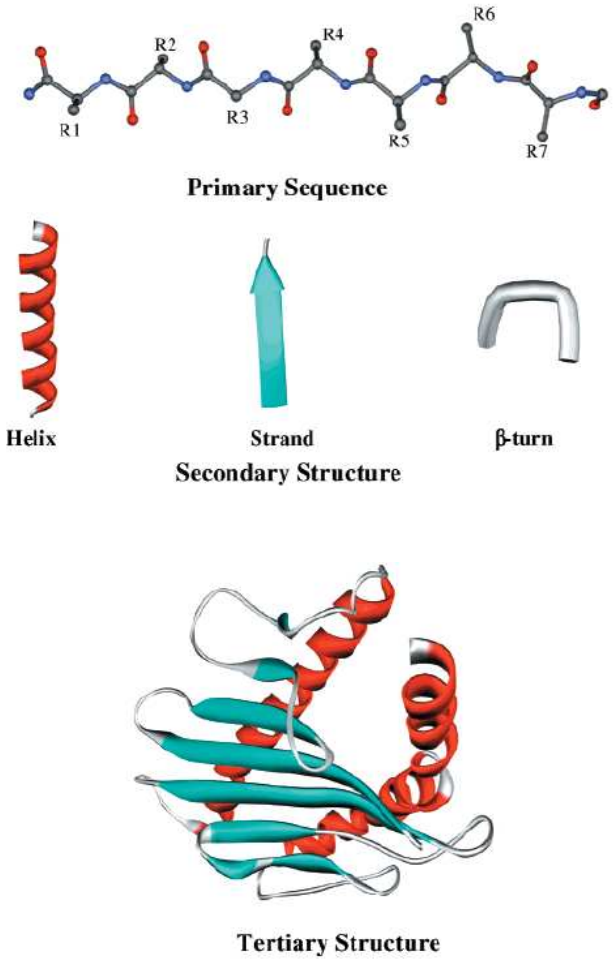
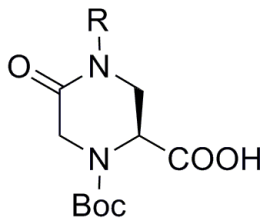


Figure I-1 Various level of structural organisation observed in protein structures
(Reproduced with the agreement of ACS publisher)

It is therefore always more and more important to develop new synthetic tools mimicking the structure and activity of biologically interesting peptide and protein in order to design new effective drugs on the one hand and on the other hand to understand better the architecture of these crucial polymers that are proteins.
In the last decade, many efforts have been made to design oligomers, so-called foldamers, which are able to adopt predictable and well-defined conformations.[1,2]

This PhD project is tightly connected to this context. The whole work performed during this PhD thesis deals with the study of modifications, synthetically introduced into the natural amino acids backbone, and its influence on the conformation of natural peptide sequences.

Two classes of peptidomimetics were studied.
During the first part of my PhD thesis, in Como we focused on the design, synthesis and structural evaluation of a new cyclic α-amino acid; 5-Oxopiperazine carboxylic acid.
Considering the widespread use of cyclic aminoacids in peptidomimetics, we focused our efforts towards the synthesis of new chiral piperazinone-5-carboxylic acids as proline analogs (Figure I-2). The synthesis of 4-*tert*-Butoxycarbonyl-piperazin-2-one-5-carboxylic acid derivatives was obtained in few steps and good yields starting from Boc-L-serine *tert*-butyl ester.



Pca: R= Bn,H

Figure I-2 Structure of 4-*tert*-Butoxycarbonyl-piperazin-2-one-5-carboxylic acid

The synthesis of peptidomimetics containing our piperazin-2-one-5-carboxylic acid was then realized, and their conformation in solution was studied by analytical and computational methods. [3]

In the second part of this PhD we focused our studies on structural properties of a new class of lactone δ-amino acid derivatives as inducer of secondary structures. Two pentalactone-containing derivatives, **1a** and **1b** (Figure I-3) were prepared and their strucutral features studied.

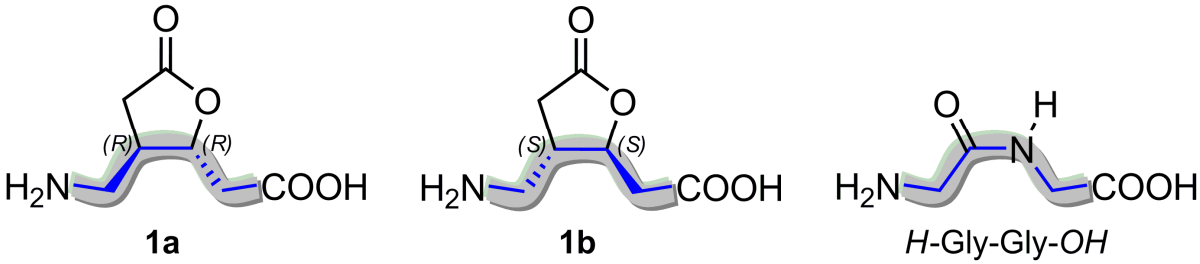


Figure I-3 Structures of pentalactone compounds **1a** and **1b** and of glycyl-glycine.

On the one hand, these cyclic scaffolds can be described as delta-amino acids bearing a lactone ring at gamma- and delta-positions. On the other hand, they mimic a glycyl-glycyl dipeptide unit, in which the amide bond is replaced by a lactone ring.

We have investigated, by CD, NMR, FT-IR and molecular modelling, the properties of these building blocks as dipeptide surrogates in α-amino acid sequences and revealed that they possess a high propensity to induce stable secondary structures.

(1) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173-180.
(2) Venkatraman, J.; Shankaramma, S. C.; Balaram, P. *Chem. Rev.* **2001**, *101*, 3131-3152.
(3) Hayashi, T.; Asai, T.; Ogoshi, H. *Tet. Lett.* **1997**, *38*, 3039-3042.

UNIVERSITA' DEGLI STUDI DELL'INSUBRIA

Sede di Como

Facoltà di Scienze Matematiche Fisiche e Naturali

Corso di Dottorato in Scienze Chimiche

SYNTHESIS AND CONFORMATIONAL ANALYSIS OF PEPTIDOMIMETICS CONTAINING UNNATURAL AMINO ACIDS BASED ON A PIPERAZINONE SCAFFOLD

Under the supervision of Prof. U. PIARULLI

II - PROLINE

Among the twenty amino acids that are coded in proteins, proline occupies a unique position. Indeed, it is the only mammalian amino acid where the side chain is cyclised onto the backbone α-nitrogen atom. The cyclic structure of proline itself leads to three important consequences.

19 α-amino acids

1 cyclic α-amino acid proline

Scheme II-1

First, the amino acid skeleton itself is considerably restricted. Thus the characteristic amino acid ϕ and ψ angles in proline take only particular values (Scheme II-**1**). Since ϕ dihedral angle is part of the ring, it is tightly restricted to a small range around -65°. As for ψ, it is able to populate either the α–helical region (ψ=-40°), or the β-sheet region(ψ= +150°) see figure 1.[1,2]

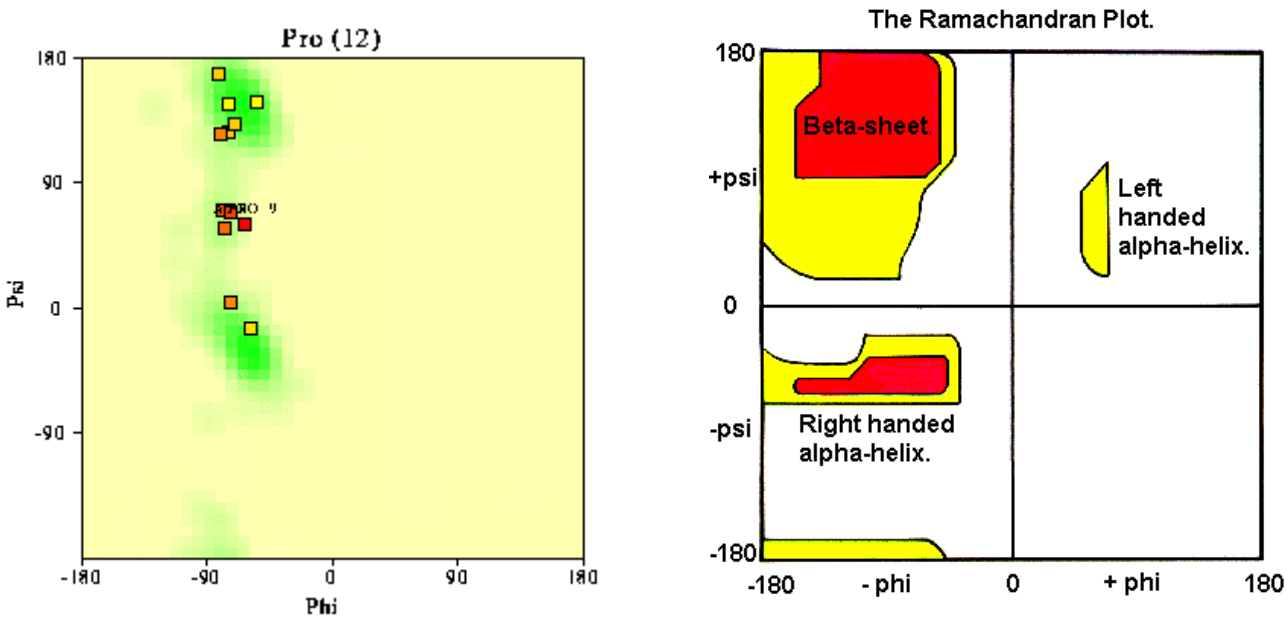


Figure II- 1 : Ramachandran plot for Proline and proteine secondary structures

Second, the bulkiness of CH_2-N region of the ring induce a consequent restriction in the amino acid preceding proline.[3] For instance, in α-helices the presence of a proline residue will sterically affect the conformational behaviour of its C-terminal neighbour preventing it from making a hydrogen bond with a carbonyl in the preceding turn of the helix . This will create a kink in the helix and introduce a deviation of 20° in the helix tube. This disfavouring effect of proline over α-helix conformation,[4] is illustrated in Figure II-2.

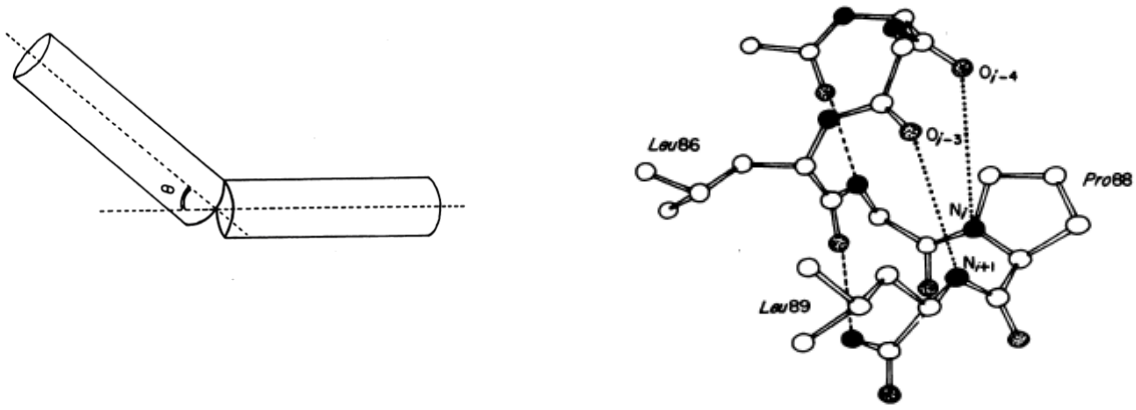
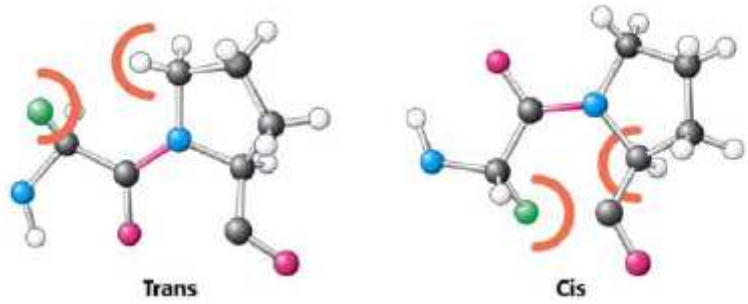


Figure II-2 : Deviation introduced by the presence of a proline residue in a helical structure

Third, in peptides and proteins, the α-nitrogen of proline is involved in three covalent bonds; so that the nitrogen amide can not act as a hydrogen donor.

These three facts point out that proline structure will have a direct impact on peptides and proteins secondary structures.

The other facet of proline is its ability to adopt *cis* or *trans* amide conformation at its N-terminal part (Scheme II-2). Unlike non cyclic natural amino acids that are linked to each other by *trans* peptide bonds. Proline introduces heterogeneity in protein backbones since both *cis* and *trans* conformations of the peptide bond are allowed. The lack of one hydrogen on the amide bond in Xaa-Pro segments significantly lowers the energy difference between the *cis* and *trans* rotamers, while the activation barrier for isomerisation remain significant (Scheme II-2).



Scheme II-2 : Cis ans trans confromations of proline in peptides

It is also to note that the *cis /trans* conversion in Xaa-proline segments in nature is catalysed by the specific peptidyl-prolyl cis trans isomerase[5] wich attest the importance of the geometry around this amino acid. All these caracteristics combined, are giving to proline a crucial role in the recognition, reactivity, and stability of polypeptides and proteins.

II-1 Proline in peptides and proteins: biological activity

II-1.1 Proline in peptides biological activity

Proline is found in many biologically synthetic and natural important peptides.[6] Most of the small peptides showing an immuno-pharmaceutical activity contain proline residues. By interfering with immuno modulation processes, such peptides lead to immuno suppression or immuno stimulation.[7,8] Oligopeptides containing proline are also found to be active in coagulation phenomena: actually the tetrapetide Gly-Pro-Arg-Pro prevent polymerisation of fibrin and therefore its aggregation with platelets to form fibrinogen clots.[9] And bactenecins: peptides located in neutrophiles granules, repeating several times the Arg-Pro-Pro motif have shown antimicrobial activity enhancing permeabilization of bacterian membranes (Table II-1).[10]

Structure	Activity
Thr-Lys-Pro-Arg (tuftsin)	**Enhances the phagocytosis by monocytes and macrophages[8]**
Leu-Pro-Pro-Ser-Arg	**Drives B lymphocytes to immunoglobulin secretion[7]**
Gly-Pro-Arg-Pro	**Prevents polymerization of fibrin and association with platelets[9]**
Arg-Phe-Arg-Pro-Pro-Ile-Arg-Arg-Pro-Pro-Ile-Arg-Pro-Pro	**Bactenin 5 potent antimicrobial and cytotoxic activity[10]**

Table II-1 : Peptides containing proline with an immuno pharmaceutical activities

II-1.2 Peptides containing proline toward enzymes

Proline plays a crucial role in the proteolytic maturation of peptides[11] from their inactive precursors to its biologically active form. It is the case for the peptidic hormone Angiotensin (involved in blood pressure regulation) where the presence of proline direct the cleavage of the peptide precursor Angiotensin I to form the active molecule Angiotensin II. This example highlights the specificity and versatility of proline regarding enzymes. Only a limited number of peptidases are known to be able to hydrolyze proline adjacent bonds (table 2). On the one hand proline may serve as recognition site for prolyl specific peptidase (angiotensin, substance P, neuropeptide Y….), but on the other hand the restricted skeleton of this cyclic amino acid can be used to protect peptides from non specific proteolysis degradation (especially useful for the preservation of peptide or protein extremities) table 2. For instance it has been previously demonstrated that a great number of active peptides, like Growth factor and interleukins[12] possess a N-terminal X-Pro motif protecting the end of peptides from degradation.

Motifs	Examples of biological function
Proline trans configuration	
X-Pro-Y	**Protection against specific N-terminal degradations**
-Pro-X-	**Brain: Hydrolysis of post Pro bond in hormones by prolyloligopeptidase.**

X-Pro-Y-Pro-Z	**By two step action of dipeptidyl peptidase IV, maturation of substance P and Inter leukin 6**
-Pro-Pro-	**Bonds between to Pro possess a high resistance to all proteolytic systems in human**
Cis conformation of proline	
-X-Pro	**Protein folding and intracellular signalling by peptidyl prolyl cis-trans isomerases, translocation of ions in transport channels**

Table II-2 : Effects and function of proline motifs

II-2 Proline in proteins

II-2.1 Proline rich proteins (PRPs)

PRPs [13] are widely found in both procaryote and eucaryote proteins. These sequences containing proline can be located in different part of the proteins and occupy from small domains to the entire part of the protein chain. Depending on their position and their length these proline rich regions (PRRs) lead to different functions.

II-2.1.1 Tandemly repeated sequences: Binding and coating function

Salivary PRPs which constitutes 70% of the Saliva proteins contain PQGPPQQGG sequences of great length repeated in tandem many times. In such proteins, the proline section constitutes almost the entire protein. These proteins's major function is to capture polyphenols (Figure II-3) coming from the diet and decrease their harmful effects by forming precipitates[14]. Mamalian epithelial mucins are made of large proteins containing although quite long, tandemly repeated sequences of prolines. These proteins are forming large networks coating and lubricating the epithelial layer[15].

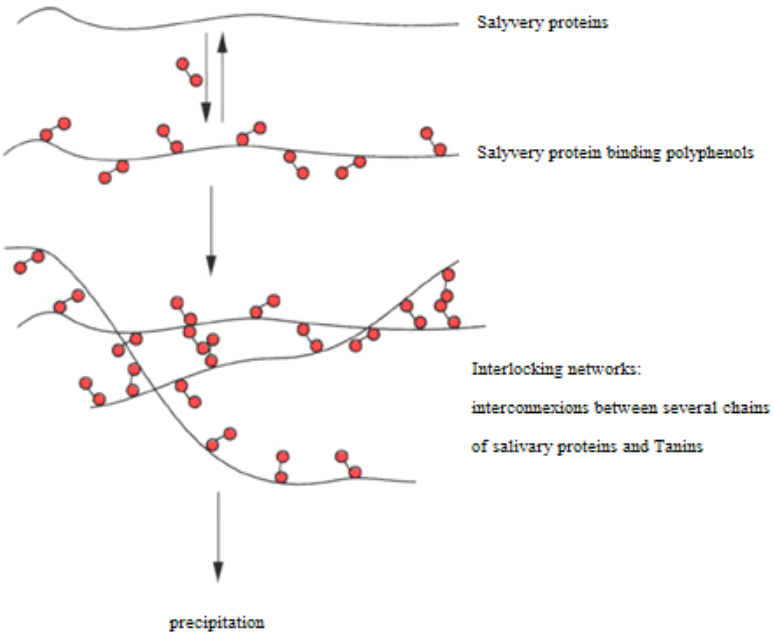


Figure II-3 Capture of polyphenols by proline rich region in salivery proteins.

II-2.1.2 Multi Proline rich regions (PRR) systems: Transcription initiation

As it has been described before proline sequences are involved in binding phenomena interacting with different motifs. But there are also examples showing protein-protein interaction involving the contact between two different PRRs. Thus RNA-Polymerase II, which possess in its C-terminal domain 26 or 27 copies of the Y-S-P-T-Y-S-P-S motif, will interact with DNA by means of another protein TATA box containing also proline rich sequences (Figure II-4). This protein-protein interaction will lead to the initiation of transcription. [16]

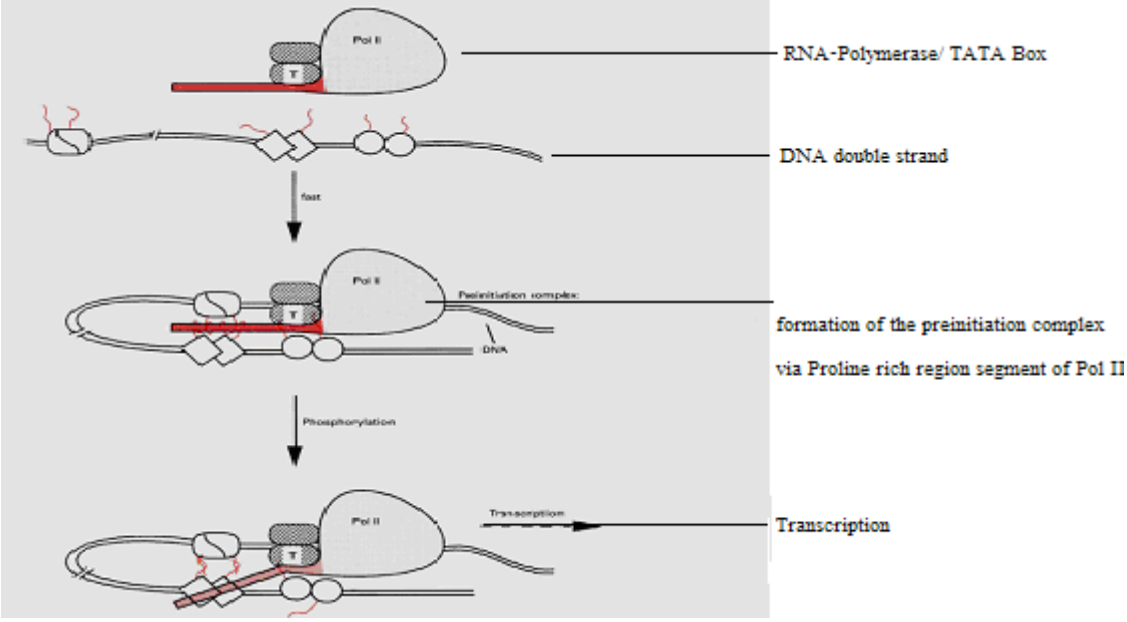


Figure II-4 : Transcription initiation, role of the proline rich segment in RNA polymerase

II-2.1.3 Proline/Hydroxyproline (Hyp) rich proteins : A structural role

Collagen is the major class of insoluble fibrous protein in extracellular matrix and connective tissue such as tendons and skin. The primary structure of collagen is mainly composed of three amino acids occurring as trimer repeats Gly-X-Y. Glycin constitute one third of the protein, the imino acids Proline and Hydroxyproline (Hyp) are then located respectively in X and Y position. Hyp derived from proline residue post-traductionnally modified by proline hydroxilases. The secondary structure of a single strand of collagen is adopting a left handed extended polyproline II helix conformation. In the tertiary structure, three polyproline II chains are interlaced around each other to form a right handed triple helix.[17] This triple helix packing is then stabilized by hydrogen bonds between glycine of each chain (Figure II-5). This specific shape is known to be essential to cell adherence and migration. [18]

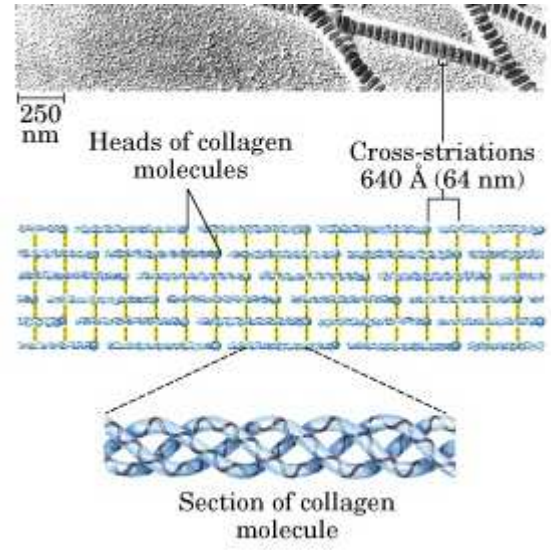


Figure II- 5 : Collagen fibre organisation

II-3 Proline role in secondary structures

In the past years, studies of biological phenomena have shown that the incredible properties of proline are mostly related to its ability to induce specific secondary structures in peptides and proteins.

II-3.1 Proline in helices

II-3.1.1 Polyproline helices

There are two types of polyproline helices: the polyproline I (PPI) and polyproline II (PPII) helices. The originality of such polypeptide is that both helices are made of a succession of proline residues unable to make H-bonds. Therefore, the stabilization of these particular secondary structures results from the restriction induced by proline pyrrolidine ring only.

II-3.1.1.1 Polyproline I (PPI)

This helix adopts a right handed compact helical conformation (Figure II-6) and contain 3,3 residues per turns (10_3 helix). In this particular structure proline residue's dihedral angles

adopt the same repetitive values (-75°, 165°), the dihedral angle of the peptidic bond is always in *cis*.

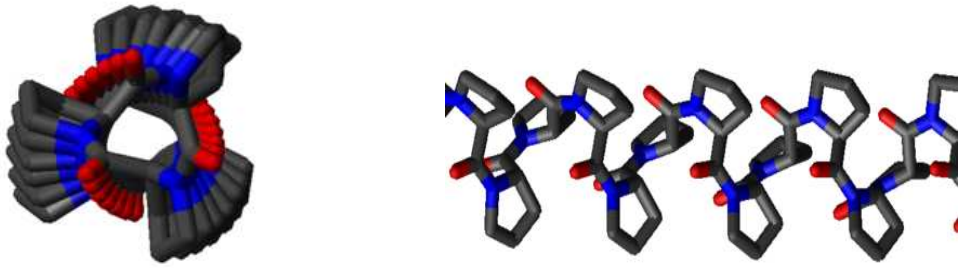


Figure II-6 : Different perspectives of PPI helix

Anyway this succession of cis peptide bonds are quite unfavourable energetically especially in water, so that this kind of helices is poorly populated in nature.

II-3.1.1.2 Polyproline II(PPII)

PPII consist in an extended structure with three proline residues per turns (3_1 helix) in which Φ and Ψ angles adopt -75° and + 145° values. The major difference between both PPI and PPII structure is that PPII adopt a left handed helicoidal structure with the amide bond between two proline residues in *trans*.

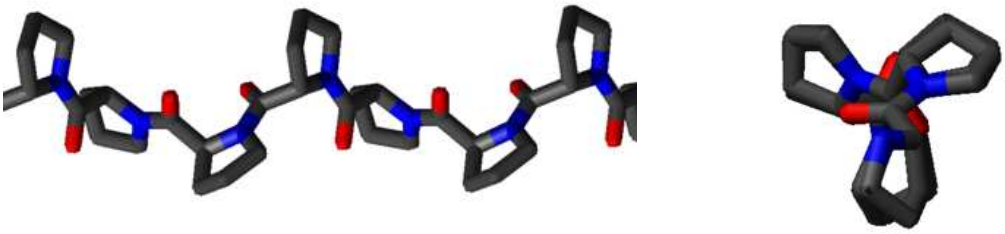


Figure II-7 : Different perspectives of PPII helix

Unlike PPI, PPII helices occur quite often in nature. It has an important role in several biological functions, as signal transduction, cell motility, and immune response[19]. As described in the precedent section, the polyproline II is also component of structural proteins like collagen, and it is believed to be the dominant conformation in proline rich sequences.

II-3.1.2 α-helices

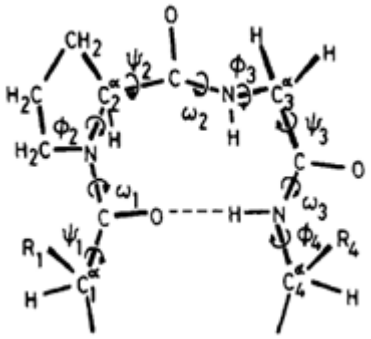
Proline amino acids are mostly known for having a disrupting effect on helices, nevertheless numerous proline residues are found in transmembranar helicoidal segments of proteins acting as receptors subunits or transporters[20]. Indeed, the wide family of protein G coupled receptors possess transmembrane helicoidal units containing numerous proline residues. Mutagenesis studies demonstrated that these residues play a key role in receptor activation, and ligand binding. These experiments led to the hypothesis that the *cis/trans* isomerisation properties of proline could be at the origin of conformational changes in proteins necessary for the regulation of a transport channel. In addition, presence of intrahelical proline residues favours the exposure of the previous amino acid carbonyl groups outside of the helix tube. In this way, the CO of the previous residues have the possibility to bind other ligands such as metal cations (Na^+ or Ca^{2+}). Transmembranes helices containing proline could thus play a role in ionic pumps.

II-3.2 Proline in turns

Turns in peptides and proteins are important structural and biological features. It is widely present in the extremity of proteins, and have proved to participate in protein-protein interactions and recognition phenomena. Because the pyrrolidine ring of the Proline residue forces the Φ angle to be centred on the -60° (+/- 15°), proline presents a ready opportunity to change the direction of the polypeptide chain. It is now widely known that proline serves as turn inducer in natural peptides and proteins.

II-3.2.1 Proline in Type II β-turns

Proline is often present at the i+1 position of β II- turns (Figure II-8). This turn is widely found in peptides and proteins since it is connectings anti parallel β- strands.[21]



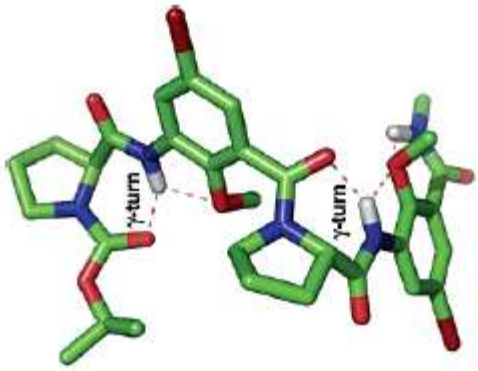
FigureII-8 : β- turn containig proline in the i+1 position

II-3.2.2 Proline in VI β- turns: cis proline turns

Although if this this kind of turn is less common in proteins it seems to play an important role in protein folding, it has been found in the terminal part of a helical segments[22,23]and it appear to be important in protein ligand recognition phenomenon.[24] This motif is constituted of 4 amino acids like the II β-turn above but in this secondary structure the proline occupies the i+2 position. Another particularity of this secondary structure is that the proline preceding amino acid and proline it self are linked trough a *cis* peptide bond.

II-3.2.3 Proline in new foldamers

Recently, proline has been used for the design and synthesis of new foldamers. Baruah & al. in 2007,[25] reported the synthesis of a new hybrid peptide (Figure II-9) that adopt a compact three dimensional structure that display periodic γ-turn.



FigureII-9 (reproduced with the agreement of ACS publisher)

Srinivas *et al.*[26] at the same period described another hybrid foldamer repeating the Aib-Pro-Adb (3-amino 4,6-dimethoxy benzoic acid) sequence that was folding alterning β-turns motifs (Figure II-10).

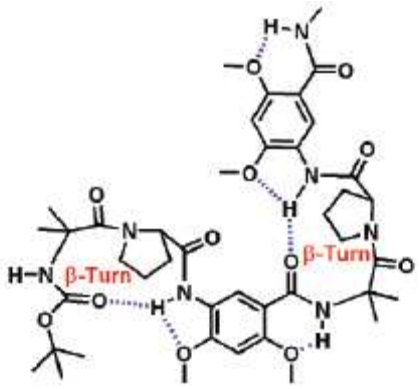


Figure II-10 (reproduced with the agreement of ACS publisher)

These two new peptidomimetics highlights that the importance of this natural amino acid is not limited to natural proteins but also as synthetic tool for scientist to expand the conformational space available for foldamer design.

III- CYCLIC α-AMINO ACIDS CONTAINING A NITROGEN ATOM IN THE RING: TOOLS FOR THE DESIGN OF PROLINE MIMICS

Peptides are bioactive compounds involved in numerous biological pathways in nature. They are thus attractive targets for the design of new drugs. Unfortunatly, they are not suitable for the development of pharmacologically active compounds, since they are highly exposed to proteolytic degradation. The great properties of proline provided the scientists a starting point for the development of new synthetic tools that would mimic and improve on its own properties. Actually the design and synthesis of new conformationally restrained tools, such as Cyclic Amino-Acids (CAA) as well as their incorporation in peptides and proteins, would give the opportunity to acquire a better understanding of protein folding phenomenon as well as protein biological behaviour. In addition, the non proteinogenic character of CAA amino acids would be an asset for the development of new peptide-like drugs. Actually, the presence of a non natural amino acid-like molecule in a peptidic segment could reduce the sensitivity of peptides toward enzymes, and thus overcome the problem of bioavailability of peptide-based drugs. Another advantage of cyclic amino acids containing peptides is the structural control they confer to peptidomimetics, and this could also augment the selectivity for specific receptors.

Thanks to proline, Cyclic Amino Acids have become molecules of increasing interest in both fields of synthetic and medicinal chemistry. Cyclic secondary amino acids have been applied to several biological issues, and their incorporation into bioactive peptides has been reported over the past years.[27]

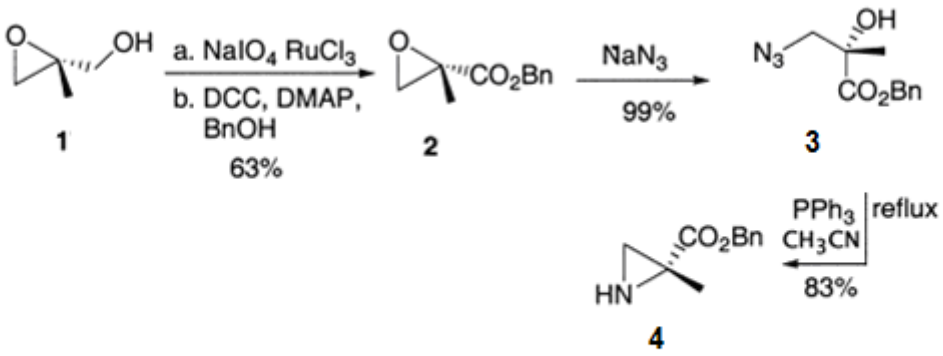
CAA synthesis has been already well commented in a previous review,[28] I will here report some of the major synthetic pathways described in the literature focussing in particular on 6-membered ring cyclic amino acids in which we were interested for the present work.

III-1 C3 Aziridin-2- carboxylic acids and derivatives

Aziridines rings are versatile and powerful building blocks in organic synthesis due to their high reactivity. Synthetic aziridines exhibit multiple biological properties, such as enzyme inhibition[29] and DNA alkylation. Their susceptibility to regio- and stereoselective ring opening,[30] renders these compounds useful precursors for the synthesis of various nitrogen containing compounds, notably chiral amino acids.[31] In particular, the aziridine-2 carboxylic acids could be important components for the preparation of a variety of conformationally constrained peptidomimetics building block.

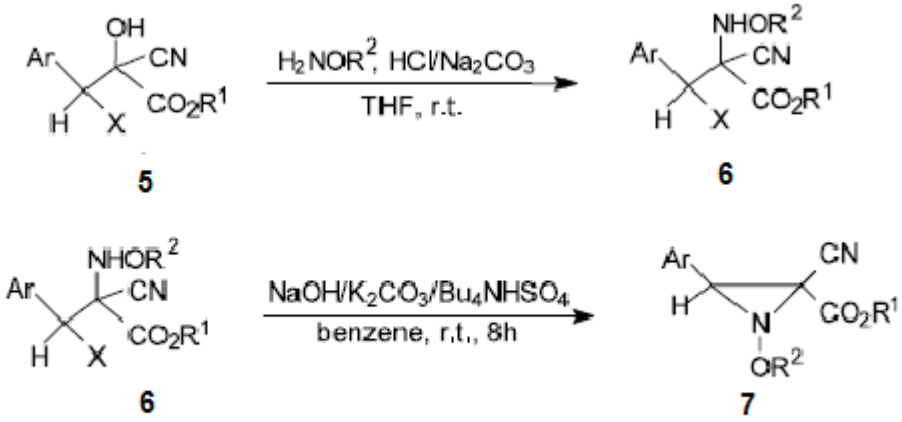
III-1.1 Synthetic routes

In 1996, Goodman and coworkers,[32] proposed a three step procedure providing the mono substituted aziridine carboxylic derivative **4** in good yields and as an optically pure material. Coming from the corresponding allylic alcohol via Sharpless asymmetric epoxidation, R-2-methylglycidol **1**, was oxidized to its carboxylic acid derivative. The acid was then protected by a benzyl group, and subsequent ring opening was performed with NaN_3 to form the azido alcohol **3.** Refluxing the azide derivative with triphenyl phosphine provided the enantiomerically pure aziridine carboxybenzyl **4** (Scheme III-3).



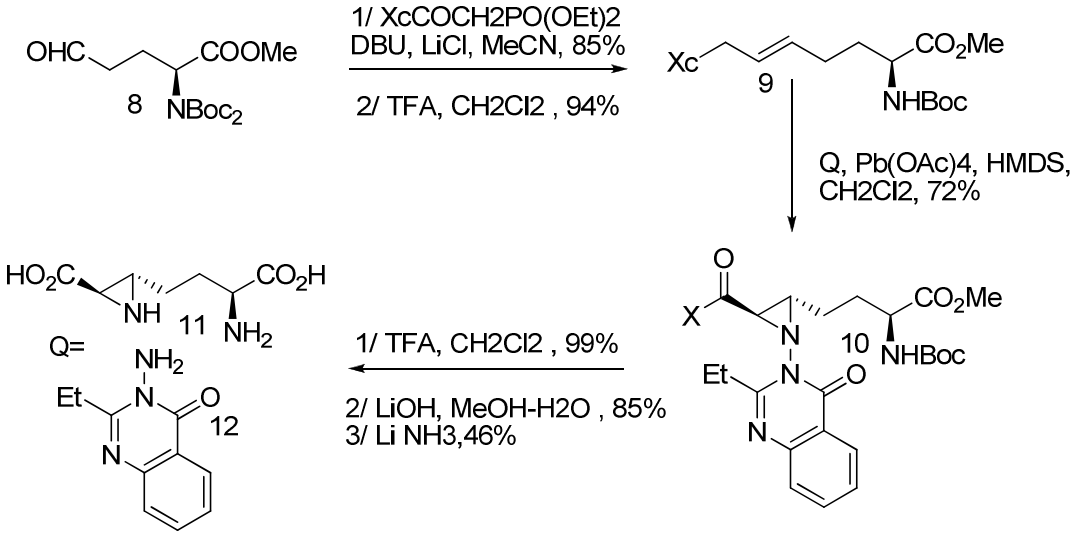
Scheme III-3

Boukris *et al*. in 2003[33] described a two step synthesis of tri-substituted aziridine carboxyester. In a first step the halohydrins **5** prepared from the corresponding epoxy esters, were treated with hydroxylamine derivative to give the cyclisation precursor **6**. In a second step, the N-protected β-halo α-aminoesters were converted into the corresponding aziridine carboxylic ester **7** in good yields (Scheme III-4).



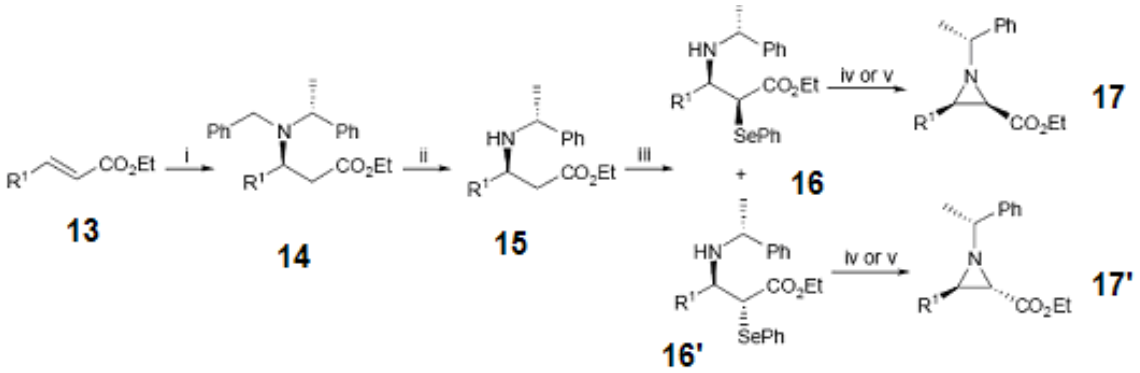
Scheme III-4

Diaper *et al*. in 2005,[34] introduced the Aziridine-2-carboxylic acid motif in analogues of the antibiotic diaminopemilic acid (DAP). Reaction of phosphonate ester $XcCOCH_2PO(OEt)_2$ with N,N-di-Boc glutamate semi aldehyde using a Horner-Wadsworth-Emmons type coupling gives exclusively the trans alkene. Removal of one of the Boc groups generated the aziridination precursor **9**. Subsequent aziridination performed in presence of 3-Amino-2-ethyl-3,4-dihydroquinozolinone, led to the Aziridine derivative as a diastereoisomeric mixture (9:1). The major diastereoisomer was then purified by recristallisation and deprotected to afford the desired molecule with the aziridine-2-carboxylic moiety **11** as an optically pure compound.



Scheme III-5

More recently, Minijew & al. in 2006[35] developed two different diastereoselective procedures based on the cyclisation of selanyl esters. First of all, a chiral center was introduced to induce diastereoselective reactions. Addition of a chiral lithium amide to α,β-unsaturated esters afforded the product **14** as only one diastereoisomer. Then, the debenzylation with CAN selectively provided the corresponding amine **15**. Then deprotonation by LDA followed by addition of benzene selenienyl bromide afforded β-amino α-selanylesters, that was obtained as two diastereoisomers **16** and **16'**. The two diastereoisomers were separated by chromatography, and each pure diastereoisomer was cyclised after activation of selanyl amino esters with Meeerwein salt or NBS providing the trans aziridine compound **17** and **17'**.



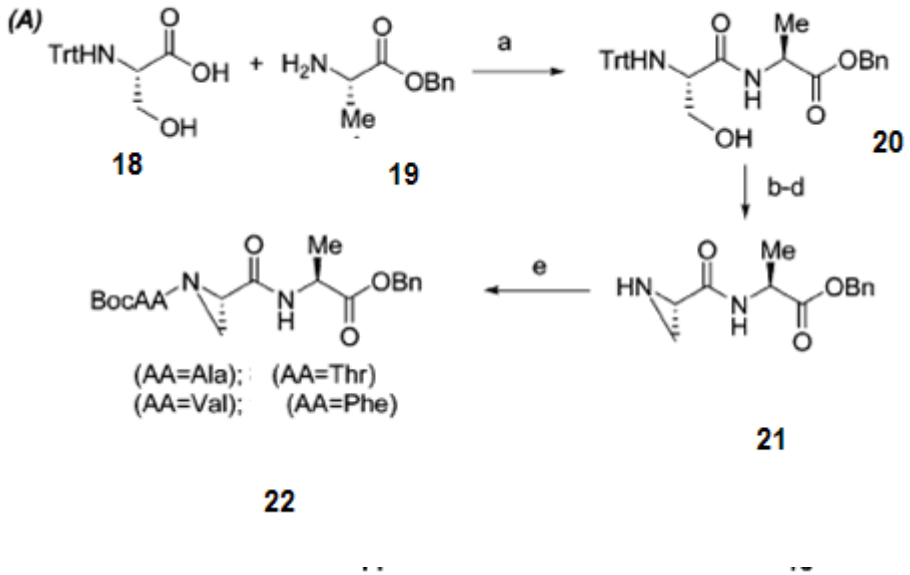
Scheme 5. (i) [(*R*)-BPEA (1.3 equiv), *n*-BuLi (1.3 equiv)], THF, −78 °C, 40 min; (ii) CAN (2.1 equiv), CH_3CN/H_2O, 20 °C, 1 h; (iii) LDA (2.1 equiv), THF, −78 °C then PhSeBr (1.3 equiv), −78 °C, 20 min; (iv) Me_3OBF_4 (2 equiv), CH_2Cl_2, 12 h then NaOH aq 1 N; (v) NBS (1.1 equiv), CH_3CN, 5 min then Na_2CO_3.

Scheme III-6

III-1.2 In the field of peptide and petido mimetics

Aziridine 2-carboxylic acid is a useful tool in peptide chemistry. Its introduction in the backbone or in the lateral chain of peptides, afford reactive electrophilic site for further modifications (conjugation of glucide moieties, introduction of modified lateral chains). Anyway, this great reactivity toward nucleophiles creates difficulties in maintaining the ring integrity during peptide coupling synthesis. Therefore only few methodologies are at the moment available to build this kind of peptidomimetics containing Aziridine carboxylic acid motif.

Danica *et al.* in 2005, reported a strategy to incorporate the azyridine carboxylic acid motif in peptides (Scheme III-7).



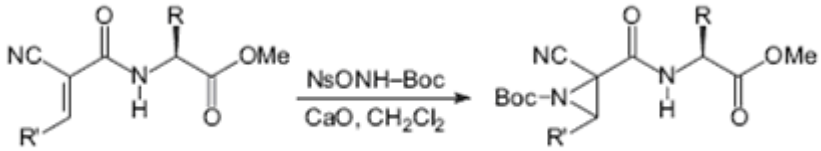
[a] Reagents and conditions: (a) BOP, $^{i}Pr_2NEt$, $CHCl_3$ (92%); (b) MsCl, Et_3N, CH_2Cl_2; (c) Et_3N, THF (91% over two steps); (d) TFA, MeOH, $CHCl_3$ (66% for **6**); (e) BocAAOH, BOP, $^{i}Pr_2NEt$, $CHCl_3$ (88% for **7**, 76% for **8**, 81% for **9**, 89% for **10**); (f) $BnNH_2$, EDC·HCl, HOBt, CH_2Cl_2 (96%); (g)

Scheme III-7

Tripeptides containing a central Aziridine moiety **22**, were prepared by initial coupling of N-Trityl protected serine **18** with alanine benzyl ester. The resulting dipeptide, was reacted with methanesulfonyl chloride and subsequently treated with triethylamine in THF to afford the corresponding N-tritylaziridine-containing dipetide. Further deprotection of the trityl group

followed by acylation of the free azyridine with various Boc protected amino acids led to the generation of tripeptides that possess a central aziridine moiety **22**.

Another synthesis of aziridiidine containing peptides has been reported by Fioravanti et al in 2008[36]. In this case the aziridine ring was introduced by reacting (E)-acrylonitrile with Nosyl-oxycarbamate (Nosyl=Ns=4-$NO_2C_6H_4SO_2$) in the presence of CaO as a base (Scheme III-8). During this reaction, the E configuration of the double bond was conserved. Subsequent deprotection of the aziridine nitrogen, followed by coupling of a third aminoacid (under mild conditions), afforded a library of aziridyl-tripeptide sequences. These kind of procedure allowed the creation of libraries of di- to tetrapeptides containing aziridines, but longer peptides containing aziridine-2-carboxylic acid is still a challenging field.



Scheme III-8

Regarding foldamers synthesis, Takashi *et al*. reported the synthesis of a β-sheet mimic, where the two arms were linked through an aziridine motif (figure III-11). But no conformational studies were reported on this structure.[37-39]

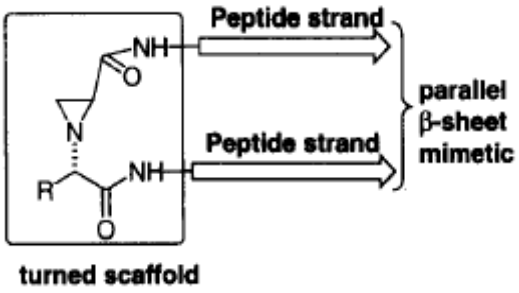


Figure III-11

III-2 C4 Azetidine-carboxylic acids and derivatives

Azetidine-2-carboxylic acid (L-Aze), has first been discovered and isolated from *convallaria majalis* by Fowden in 1955[40]. Later it appeared to be a constituent of several natural products like mugineic acid[41] or nicotiamine [42]. It has also been recently incorporated in the pharmacologically active molecules Melagatran or Extenta that act as Thrombin inhibitors[43].

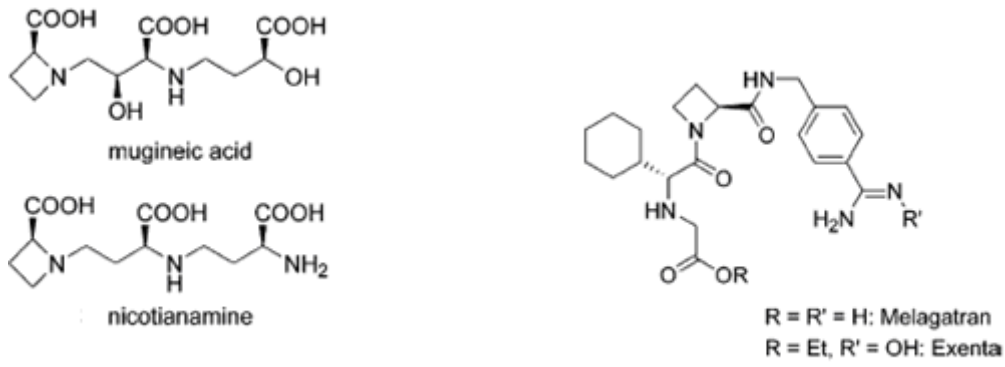


Figure III-12

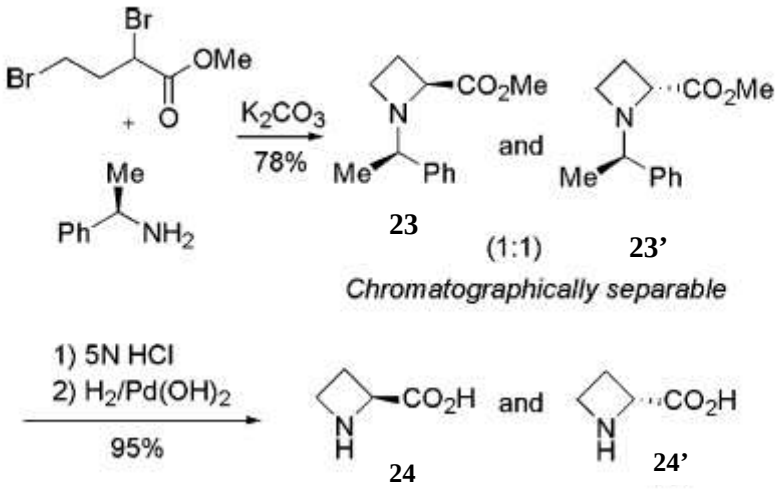
III-2.1 Synthesis

Because of its numerous possibility of functionalisation and its conformational restriction properties, this 4-membered-ring heterocycle has become a convenient building bloc in organic synthesis. Its preparation has been reviewed in 2002 by Kurth *et a.l* [44], and more recently by Cordero & al.,[45] so I will report here only some examples of synthetic strategies providing the "chimera" amino acid enantiomerically pure.

Chromatographic separation of diastereoisomers :

Ma *et al.*[25] developed a synthetic procedure to obtain both enantiomers of the L-Aze **24** and **24',** (Scheme III-9). This route involve Methyl 2, 4-dibromobutanoate, dihalogenated compound that will react with (R)-phenylethylamine an inexpensive source of chirality. The base-promoted cyclisation led to a diastereoisomeric mixture of the *N*-protected azetidine-2-carboxylic acid **23** and **23'.** Diastereoisomers were then separated by chromatography and further deprotection gave both enantiomers of the desired optically pure free amino acid in

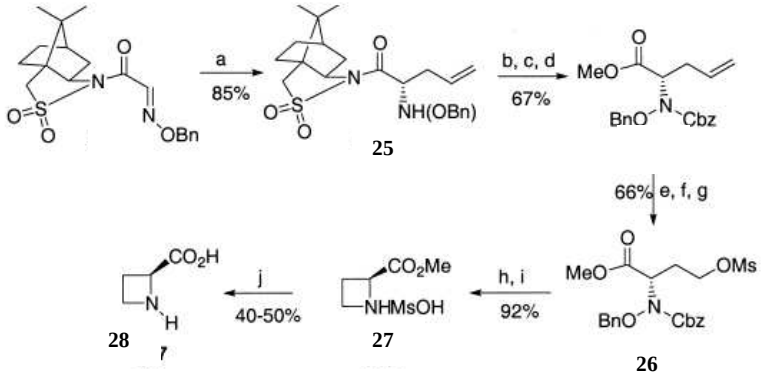
good yields. Similar strategy had been developed previously by Couty & al[46] and Futamura et al[47] .



Scheme III-9

Procedure using chiral auxiliaries:

Hanessian *et al.*[48] (Scheme III-10) described a selective synthesis of L-Aze using the Oppolzer sultam derivative of glyoxylic acid oxime and allyl bromide to form allyl glycine **25**, and thus introducing the stereocenter. The chiral auxiliary was then removed, and the carboxylic and amino groups were orthogonally protected. Ozonolysis of the double bond followed by reduction of the new formed aldehyde function gave the alcohol derivative that was then transformed into the corresponding mesyl group **28**. Finally deprotection of the amino group and cyclisation afforded the desired azetidine carboxylic skeleton **27** which was involved in the final steps of deprotection and DOWEX purification to give pure L-Aze **28**.

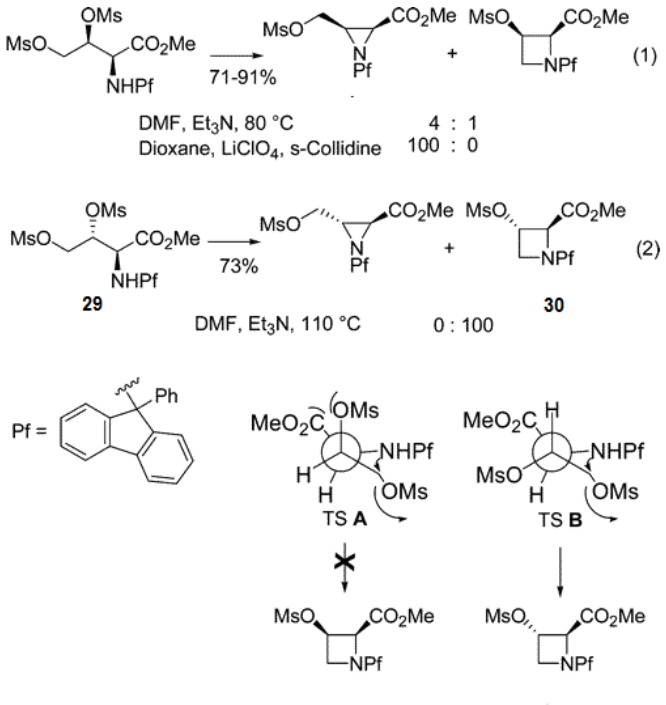


Scheme 48. (a) Allyl bromide, Zn, NH_4Cl; (b) LiOH/THF; (c) CH_2N_2–Et_2O, MeOH; (d) Cbz-Cl, $NaHCO_3$; (e) O_3, MeOH; (f) $NaBH_4$; (g) MsCl, pyridine; (h) H_2, 10% Pd/C; (i) $NaHCO_3$; (j) 3N HCl, Dowex.

Scheme III-10

Sterically directed cyclisation :

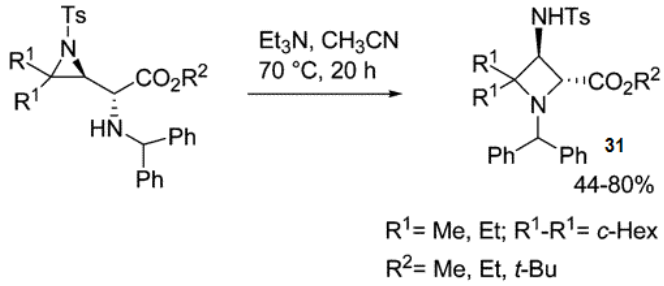
An efficient and unexpected cyclisation leading to the formation of an azetidine-2-carboxylic acid derivative was described by Sardina *et al.*[49] In this route protected 2*S*,3*S*-hydroxyaspartate was converted in two steps in its dimesylate derivative **29**, which was cyclised under basic condition to afford the Trans-substituted azetidine **30** as unique product (Scheme III-9). The epimeric 2*S*,3*R*-derivative, under the same conditions, led to the formation of the aziridine derivative as a major product.



Scheme III-11

Rearrangement of already existing cycles :

More recently, *anti*-aziridino amino esters were thermally rearranged upon treatment with Et_3N to *trans*-*N*-protected alkyl-3-aminoazetidine-2-carboxylic esters **31** (Scheme III-12)[50]



Scheme III-12

III. 2. 2 Peptidomimetics

L-Aze as proline surrogate:

L-Aze and L-proline adopt similar conformation in peptides since they are both constraining the conformational space of the previous residue by steric interaction with the ring. Therefore Aze has been early incorporated in vivo and in vitro into cellular proteins using the cellular protein synthesis machinery.[51,52] Anyway some differences are detected: particularly in dihedral angles and bond distances. Comparative calculations on both rings have shown that, L-Aze in dipeptides is presenting more energetic minima than proline, meaning that it could introduce more flexibility in polypeptides. L-Aze, has earlier been incorporated in collagen-like polytripeptide molecules instead of proline.[53-55] It has been reported that collagen containing L-Aze , present modified biological and physiological properties. It is mainly retained in the cell so that the extrusion to form intercellular matrix is reduced preventing the incorporation into tissues. It seems that this is due this enhanced flexibility induced by L-Aze thus destabilizing the triple helix of collagen.

In a more recent paper the effect of proline substitution by L-Aze was studied in the polypetides $[(AlaGly)ProGluGly]_{16}$. The interesting outcome of this investigations is that proline containing peptides were disordered, whereas L-Aze containing sequences promoted the formation of β-structures.[56] This tendency was confirmed by Ogoshi & al[37] who performed comparative conformational studies on tetrapeptides containing L-Aze. Finally Baeza & al[57,58], demonstrated recently the propensity of L-Aze to induce γ-turns in short peptides (Figure III-13).

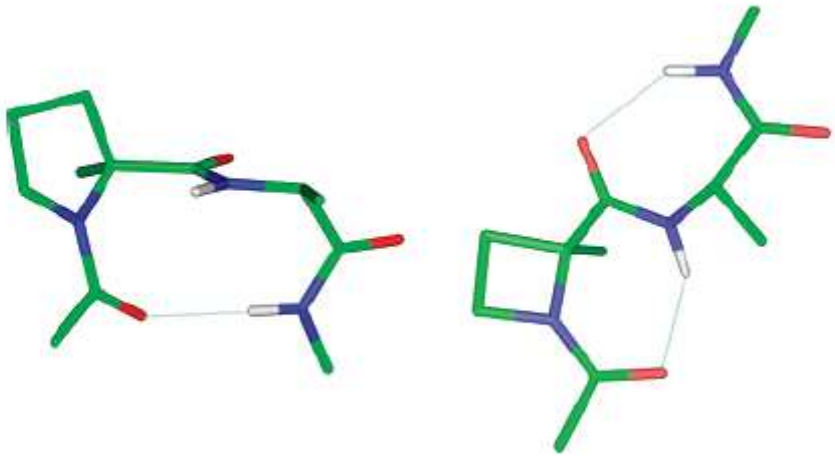


Figure III-13 Conformational preferences of proline and azetidine dipeptides
(reproduced with the agreement of ACS publisher)

III-3 C5 Decorated pyrrolidine rings, a route to modified proline

In term of mimics, what more than a five membered heterocyclic ring could mimic proline ? Actually, nature has already produced a plethora of modified prolines in which the most famous one is hydroxy proline where the presence of the hydroxyl substituent on the pyrrolidine ring is stabilizing the collagen triple helix[59]. Subtituted proline mimic have been used in particular to study stabilization mechanism of hydroxyproline replacing the hydroxyl substituent by different functional groups. Substituted proline, have been found in biologically active molecules, anticancer agents (Figure III-14): are also convenient scaffolds for the development of new pharmacogically active molecules.

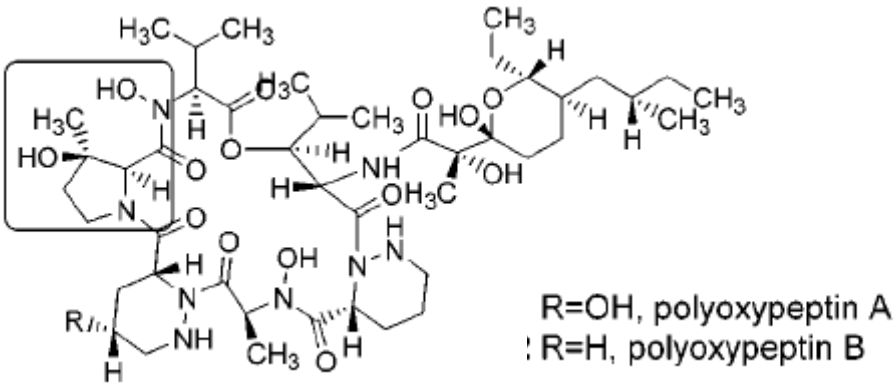


Figure III-14 : 3-substituted proline in anti proliferative agent, polyoxypeptin

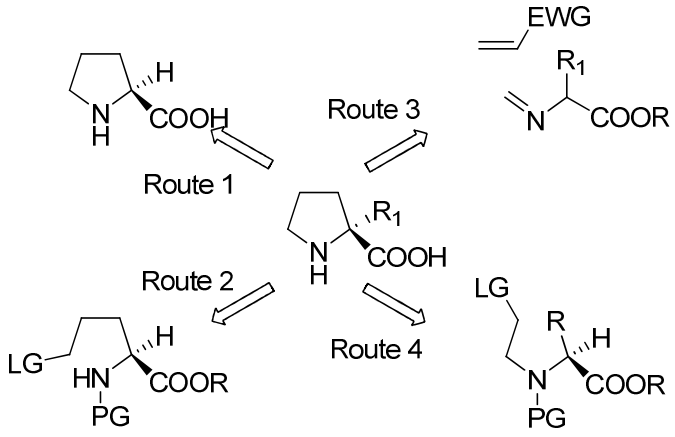
Various approaches are now available to build substituted proline derivatives. And it has been reviewed in 2004 by Karoyan *et al.*[60] and I will repport some synthesis examples as well as structural preferences of this constrained amino acids.

III-3.1 Synthesis

III-3.1.1 2-subsituted prolines

Asymmetric synthetic procedures for the preparation of the so called quaternary prolines have been recently reviewed by Calaza & al, [61] so I will report here only some recent examples.

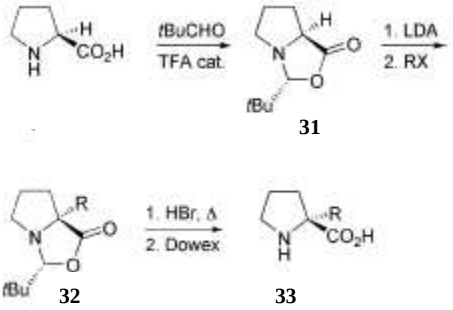
Scheme III-13 reports the different strategies elaborated to perform the synthesis of these highly constrained compounds.



Scheme III-13

α-functionalization of L-proline (Route1,Scheme III-13)

The challenging parameter of this reaction is to maintain the optical purity of the previous proline compound. Therefore Seebach *et al.* in 1983[62] provided a methodology to perform alpha alkylation of proline, inventing the concept of self-reproduction of chirality. In this procedure (Scheme III-14) L- proline was condensed with piruvalaldehyde to afford a single conformer of 2-tert-butyl-1-Aza-3-Oxabicyclo[3.3.0]octane-4-one **31**. Subsequent deprotonation with LDA gave then a non racemic enolate intermediary which was combined with alkyl halides to provide the α-alkylated proline skeleton **32** with complete diastereoselection. The oxazolidinone ring was then opened under hydrolytic conditions providing the desired alkylated proline **33** with good enantioselectivity.



RX	Yield **3** (%)	Ref.
MeI	93[a]	[17]
CH_2=$CHCH_2Br$	87[a]	[17]
BnBr	91[a]	[17]
Me_2N^+=CH_2 Cl^-	56[a]	[17]
MeO_2CCH_2Br	40[a]	[17]
$Me_2N(O)CCH_2Cl$	70[a]	[17]
$BnOCH_2Cl$	62[b,c]	[20]
$MeCH$=$CHCH_2Br$	64[b]	[21]

[a] *dr* > 95:5
[b] Single diastereoisomer
[c] Yield from L-proline

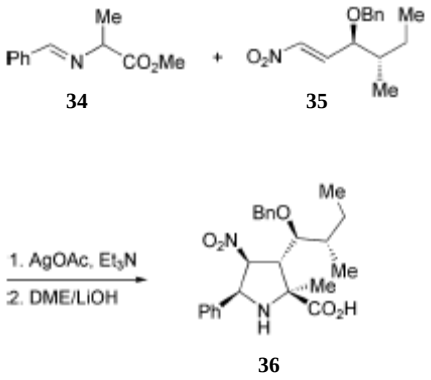
Scheme III-14

Synthesis with the formation of the pyrrolidine ring (routes 2, 3 and 4)

1, 3 dipolar addition cycloaddition: Azomethine ylides (route 3, Scheme III-13).

1,3- dipolar cycloadditions of azomethine ylides and alkenes is a powerful method to afford the stereocontrolled formation of polysubstituted pyrrolidine rings. These kind of reactions involves 2 partners Scheme III-13: An alkene bearing an electron withdrawing group (EWG) although usually called dipolarophile, and an ylide. The stereocontrol of the reaction can be induced either by the presence of stereocenters on one of the partner by a chiral auxiliary, or by employing chiral catalysts. The example chosen here show the [3+2] cycloaddition between a chiral *E*-nitroalkene **34** and an imine **35** (Scheme III-15) that afford polysubstituted

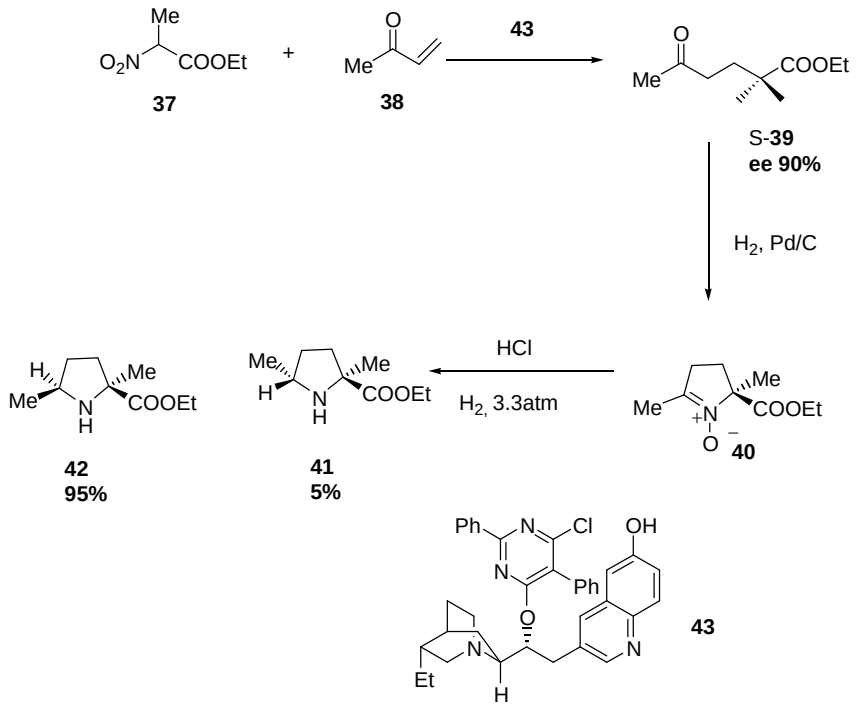
prolines, that belong to a new family of inhibitors of α4β1-integrin-mediated-hepatic melanoma metastasis[63].



Scheme III-15

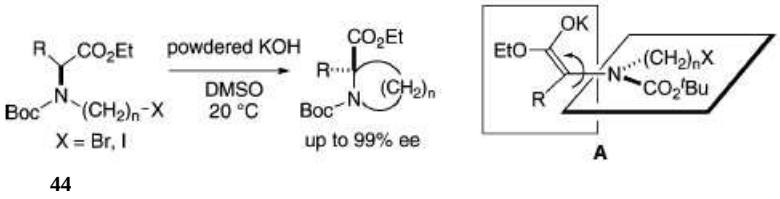
C-N bond formation by intramolecular cyclisation of α-amino acid derivatives (Route 2, Scheme III-13)

Another methodology achieving the formation of α-alkyl prolines, propose the closure of the ring, by intramolecular cyclisation of a geminally disubstituted glycine equivalent with an appropriate leaving group on the side chain. As an example, Snider *et al.*[64] used this strategy to synthesise of a (-)- 2,5-dimethylproline ethyl ester 42, a key intermediate in the synthesis of (+)NP-25302 acting as cell-cell adhesion inhibitor. This procedure starts with an enantioselective Michael addition between the ethyl 2-nitropropionate **37** and a methyl vinyl ketone **38** in the presence of the hydroquinone catalyst **43**. The produced disubstituted glycine derivative **39**, was then cyclised under reductive hydrogenation conditions, using H_2 and Pd/C in ethanol to give the nitro compound **40**. And the desired quaternary proline **42** was obtained "pushing" the conditions to 3,3 atmosphere of H_2 in the presence of HCl, (Scheme III-16).



Scheme III-16

C-C bond formation involving memory of Chirality (Route 4, Scheme III-13)

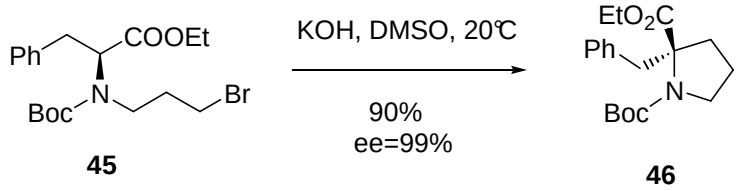


Scheme III-17

Starting from readily available α-amino acids a recent cyclization method via "memory of chirality" has been developed to provide at room temperature without the aid of external chiral sources cyclic amino acid with a tetrasubstituted stereocenter in alpha. [65-67]. In this case the treatment of N-ω-bromoalkyl-N-terbutoxycarbonyl α-aminoacids **44** derivatives with KOH powdered in DMF at 20°C provided 4, 5 and 6 membered ring cyclic amino acids with excellent yields and enantioselectivity.

Key features of this method are the preservation of the chirality of the starting material due to the high reactivity of axial enolate intermediary **A** (Scheme III-17***).*** As an example, The

cyclisation of the N-ω-bromoalkyl-N-terbutoxycarbonyl phenylalanine derivative **45** afforded the corresponding substituted proline **46** in excellent yields and enantiomerically pure (Scheme III-18).



45 **46**

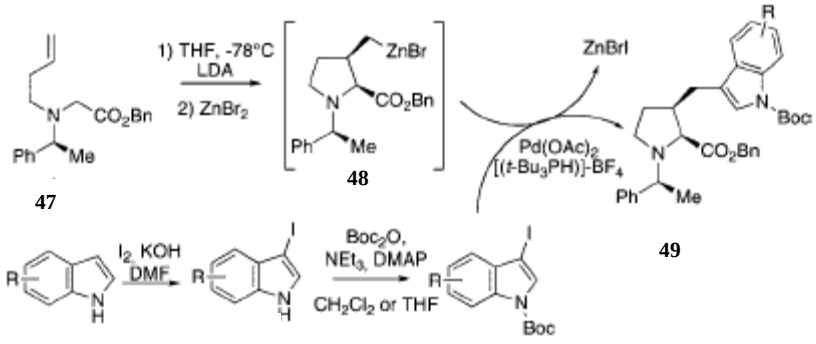
Scheme III-18

III-3.1.2 3-substituted proline

:

Synthesis with the formation of the pyrrolidine ring

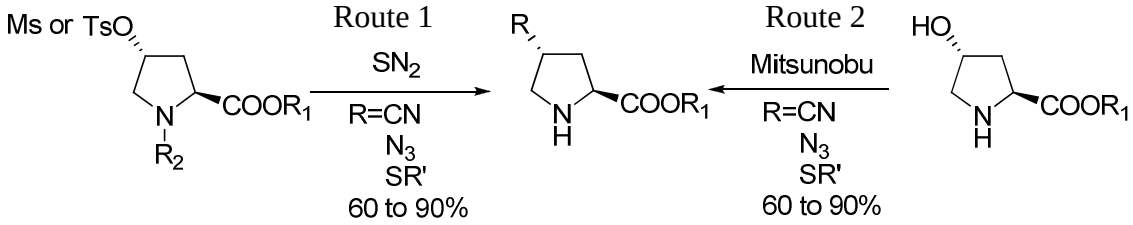
Mothes *et al.* in 2008[68] developed a convenient synthetic procedure to obtain in three steps and good yields 3-substituted proline- homotriptophane. This method is based on the amino-zinc-ene-enolate cyclization(AZEE). In a first step, the zinc intermediate **48** was generated starting from the commercially available olefin **47**. Carbocyclization was performed by the deprotonnation/transmetallation sequence using LDA and $ZnBr_2$. The strereochemistry of the two stereocenters (*cis* in this case) created during the cyclistion, was directed by the chiral auxiliary initially on the molecule(*S*-α-methylbenzylamide). Finally Neigishi cross-coupling of the zinc intermediate with indole rings, was achieved using Pd and Fu's catalyst (Fu's catalyst= [*t*-Bu_3PH)]-BF_4). And the orthogonally protected proline homotriptophane **49** was obtained optically pure (Scheme III-19). Following similar procedures, the same group, used similar methodology, to build 3-substituted prolino dervatives, bearing polar,[69] and alkyl [70] side chains of natural amino acids.



Scheme III-19

III-3.1.3 4-substituted proline

In the case of 4-substituted proline the commercial availability of the starting reagent *trans*-4-hydroxyproline made of it a reagent of choice towards the synthesis of 4- substituted proline. Actually the *cis* 4- substituted proline can be in this case easily obtained by SN_2 diplacing the Tosylate or Mesylate derivative of *trans* -4 hydroxyproline (Scheme III-20, route 1),[71] and Mitsunobu reactions (Scheme III-18 route 2),[72] replacing the alcohol by different functional groups.

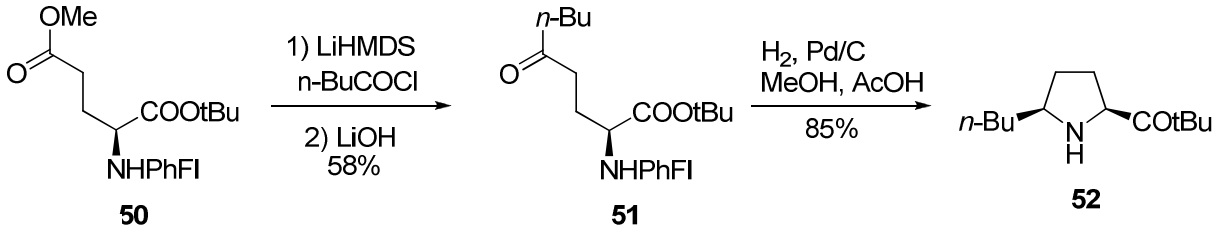


Scheme III-20

III-3.1.4 5-substituted proline

Intramolecular cyclisation

Lubell and coworkers reported in 1993 the synthesis of the 5-substituted *n*-butyl proline starting from the conveniently protected glutamate derivative. In a first step, the enolate formation followed by the acylation with valeryl chloride of the glutamate derivative led to compound **51**. Then the intramolecular imine formation followed by a selective reduction of the imine intermediate led to the 5-substituted proline enantiomerically pure. SchemeIII-21.[73]

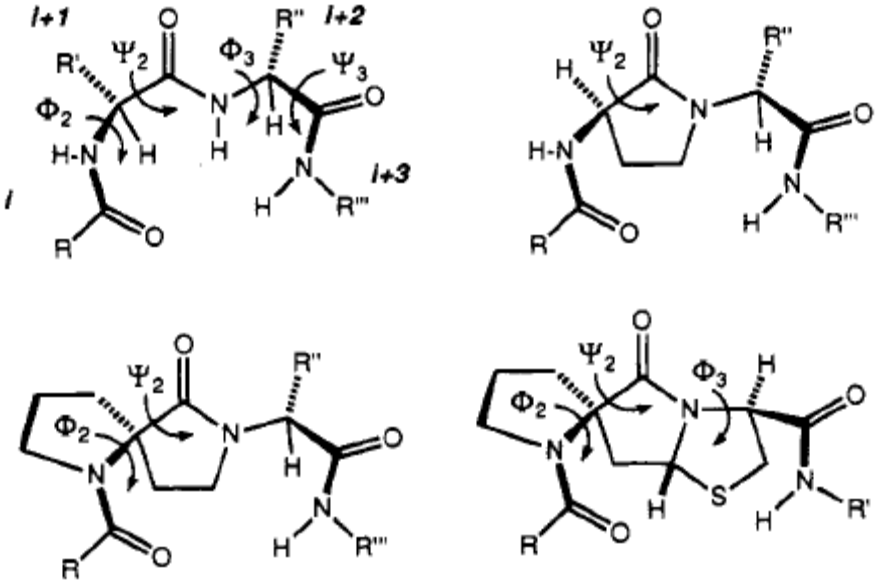


Scheme III-21

III-3.2 Structural features

Substitution of the pyrrolidine ring induces changes in the conformation of the proline cycle, and thus can influence the conformation of peptides. Depending on the substitution position, these amino acids chimeras are attractive tools to modulate secondary structure formation in peptides.

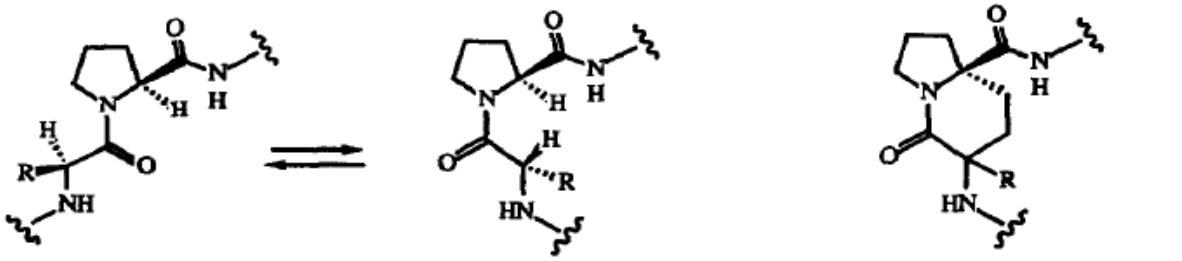
Studies on Ac-L-2-MePro-NHMe model peptide[74] showed that α-methyl proline has a tendency to form γ-turn in non polar solvents as well as destabilization the *cis* preceeding amide bond. In biologically active peptides, β-I turns were stabilized by introduction of α-methylproline derivatives in Tyr-Pro-Tyr-Asp,[75,76] an antigene peptide and in tandemly repeated sequences Asn-Pro-Asn-Ala motifs respectively.[52] Examples of peptidomimetics containing α-substituted proline have been used to mimic β-turns. One of the widely studied motif used as β–II-turn mimic are spirolactam-derivatives. Different bi or tricyclic [77,78]structures containing a spirolactam at the (i+1) position have been designed and synthetized over the past years (Scheme III-22), folding each time as efficient β-II turns mimics.



Scheme III-22 (reproduced with the agreement of ACS publisher)

Benefitting from the decrease of the number of degree of freedom due to the presence the spirolactam introduced in the α-position of proline [78], these kind of bicyclic proline could act as β-II turn nucleator. Actually the spirolactam is rigifying the Ψ-angle of the proline natural amino acid, meaning that in this compound both dihedral angles are constrained. So that, the pyrrolidine ring will restrict the conformational space of its N-terminal neighbour, and the spirolactam ring will reduce the conformational space of its C-terminal neighbour amino acid. In this case conformational analyses based on Variable Temperature (VT) NMR analysis, as well as IR analyses both confirmed the existence of an H-bond between the C-terminal Boc-carbonyl, and the NH hydrogen of the Methyl amide. These observations together with additional computational calculations are in agreement with the presence of a type β-II structure.

Gramberg et al in 1994[79], designed and synthetized a spirolactam bicyclic proline linking this time the α-position of the proline to the α-position of the N-terminal amino acid. Thus the Xaa proline peptide bond was constrained to a cis-conformation forming a β-VI turn mimic.



Substituents in position 3 have shown different structural feature depending on the length and bulkyness of the substituted chain and on the stereochemistry of the trisubstituted carbon. It seems like *cis* 3- substituted prolines stongly destabilize γ-turn conformations because of disfavouring interactions with the carboxamide group, whereas the γ-turn conformation predominates in *trans* 3substituted prolines.[74]

4-Substituted prolines: as it as been reported before, substitution in position 4 is famous for directing the folding of collagen through a PPII helix. Studies of fluorosubstituted proline derivatives have shownn enhancement of the PPII structure stabilisation.

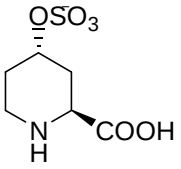
It has been shown that the introduction of substituents in position 5 of proline strongly destabilized the the trans conformation of the preceding peptide bond. So that this kind of modification, tends to stabilize type IV β-turn structures.[80]

III.4. C-6, 6-membered ring cyclic α-amino acids

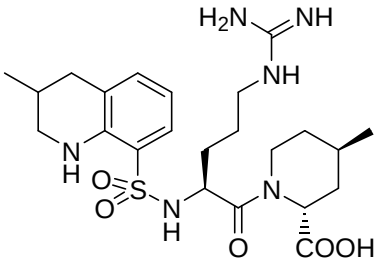
III-4.1 Synthesis

III-4 1 1 Pipecolic acids and derivatives

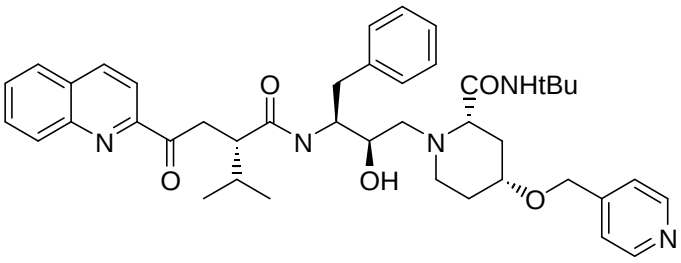
The natural non proteinogenic pipecolic acid (also called homoproline) is an attractive synthetic target because it is a key constituent of many natural and synthetic molecules. For instance, biologically important natural products such as immunosuppressant FK506[81], anticancer agent VX710[82], antitumour antibiotic sandramycin[83], histone deacylase inhibitor apicidin[84] contain a pipecolic acid moiety. Research in the area of 4-substituted pipecolic acids alone has resulted in novel therapeutically relevant agents in areas such as HIV-1 protease inhibition (Palivavir),N methyl-D-aspartic acid receptor antagonism (Selfotel), and thrombin inhibition (Argatroban) See scheme III-23 .[85]



Selfotel



Argatroban

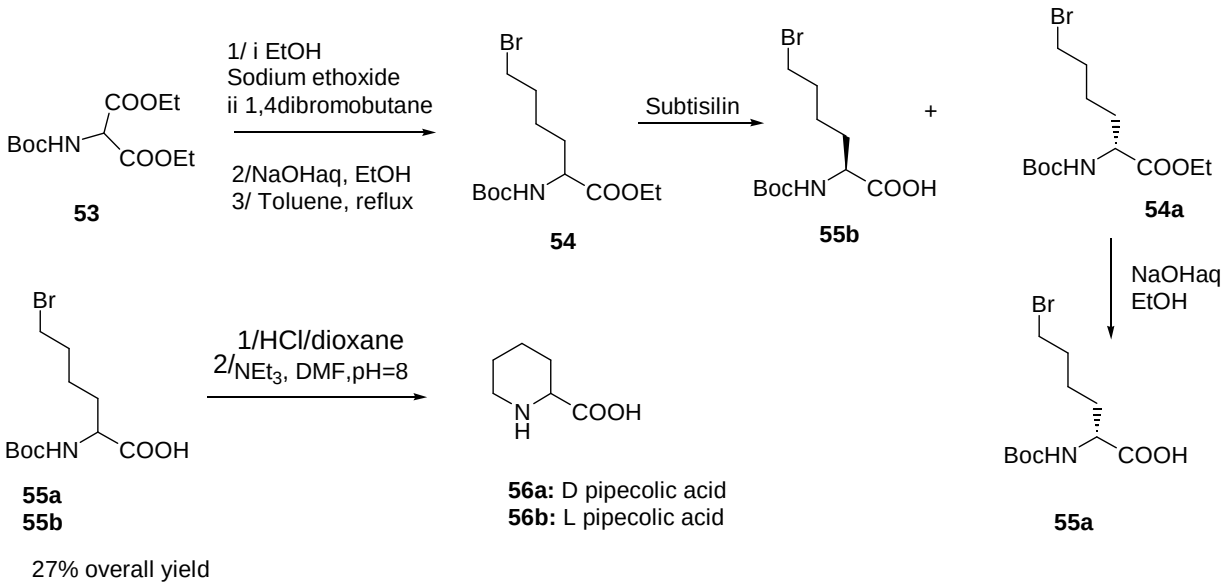


Palinavir

Scheme III-23

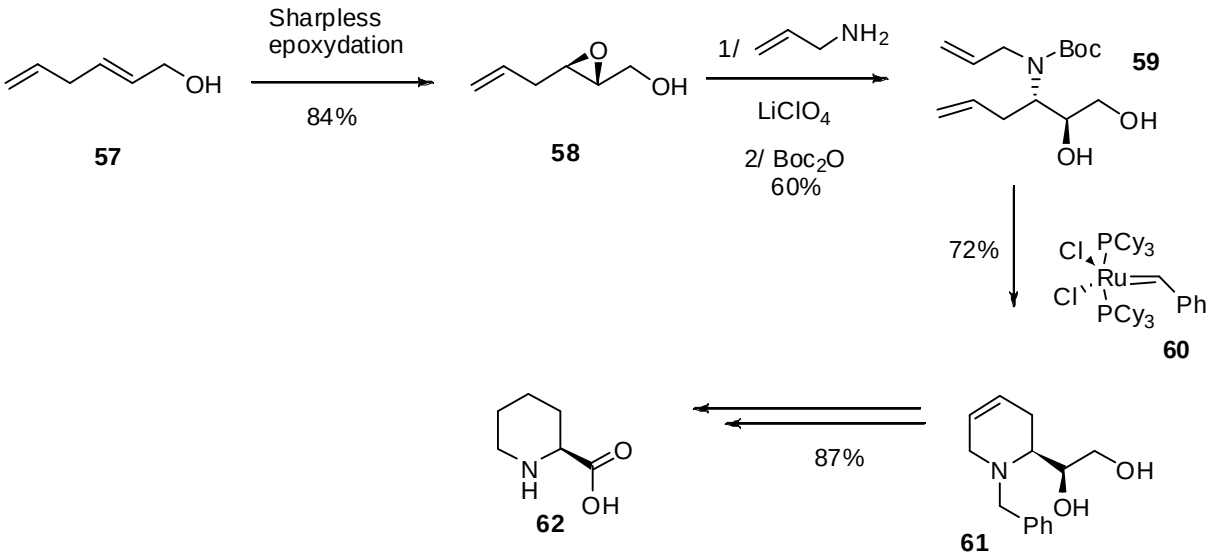
Furthermore it is a interesting tool for building of new petidomimetics.[86,87]
The synthesis of pipecolic acid and derivatives has thus received a huge attention in the last 20 years. Synthetic routes have been already extensively reviewed by Park *et al.* in 2002[28] and by Kadouri-Pouchot *et al*. in 2005,[88] so I will repport here only on more recent synthetic pathways.

In 2005 Watanabe *et al*.[89] proposed a simple and efficient route to access both enantiomers of pipecolic acid. Refluxing dibromoethane with diethyl Boc-aminomalonate **53**, in basic conditions led to the monoacid monoester derivative, decarboxylation at high temperature afforded then the cyclisation precursor **54** as a racemic mixture. Resolution using subtilisin Carlsberg from *bascillus licheniformis* afforded both pure enantiomers **55b** and **54a**. The ethyl ester of Boc-D-amino-6-bromohexanoic acid **54a** was then removed, and deprotection of the Boc group followed by an intramolecular cyclisation in presence of triethyl amine, afforded both enantiomers of pipecolic acid **56a** and **56b** in 27% overall yield (Scheme III-24).



Scheme III-24

Asymetric catalysis has also proven to be a valuable method to produce the piperidine ring. Ginestra *et al.* in 2002[90] reported the synthesis of pipecolic acid involving a cyclisation via ring closing metathesis (RCM). From the known enriched epoxyalcohol **58** synthesised from the allylic alcohol **57** via Sharpless epoxydation, nucleophilic ring opening of the epoxide using allyl amine was followed by the protection of the amino group with Boc_2O. The key intermediate **61** was obtained by ring closure metathesis catalyzed by the Grubb's reagent **60**, of the doubly unsaturated amine 24 with 72% yield. Further hydrogenation and oxidation led to *N*-Boc pipecolic acid **62** in 99% ee after recrystallisation (Scheme III-25).



Scheme III-25

Increasing the ring of the cycle of cyclic amino acids, offers larger possibilities of substitutions, and as we can see on the represented compounds in the scheme III-21, 4-substituted pipecolic acid have shown high pharmacollgical activity. Increasing efforts has been developed by synthetic chemists to provide also routes to these 4-substituted compounds. The same method as described before involving RCM has been recently applied to afford the trans-4 methyl pipecolic acid.[91]

Another widely used component of therapeutics is the *cis*-3-hydroxy pipecolic acid, that is included in the antitumor antibiotic tetrazomine (Figure III-15).

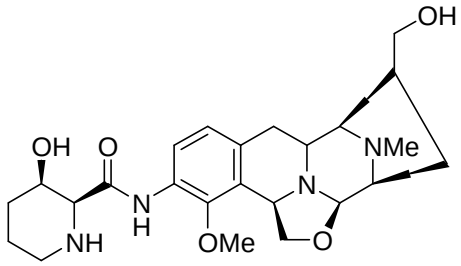
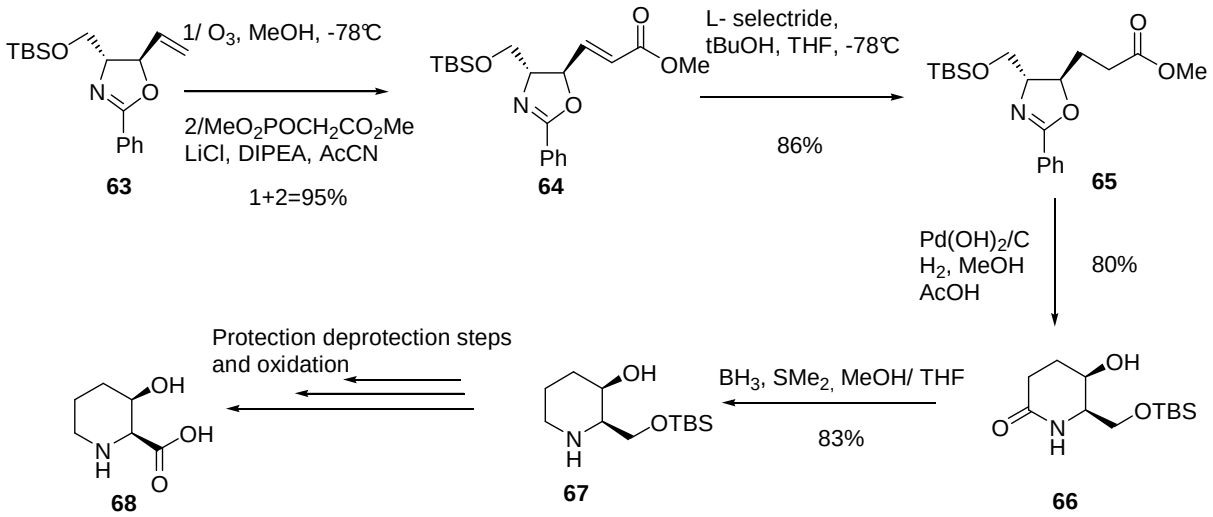


Figure III-15

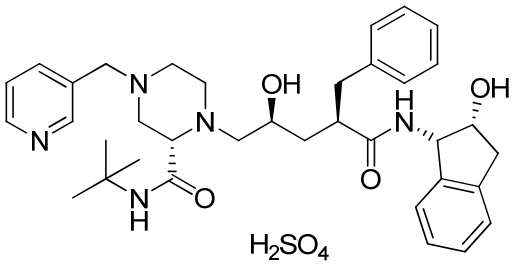
Recently Pham *et al.*[92] reported a new synthesis to access this type of compounds. Starting from the ozonolysis of oxazoline **63**, the subsequent addition of trimethyl phosphonoacetate to the resulting aldehyde yield α-β unsaturated methyl ester **64**. Reduction of **64** with L-selectride and subsequent hydrogenolysis provided, by intramolecular cyclisation, the desired piperidine skeleton **66**. The carbonyl group was then reduced with borane methyl sulphide complex to give **67** in good yields. The dihydroxyl monosilyl compound was then submitted to several steps of protection deprotection steps to afford the free primary alcohol. And finally oxidation with $RuCl_3$ and $NaIO_4$ led to the corresponding cis hydroxyl pipecolic acid **68** (scheme III-26).



Scheme III-26

III-4.1.2 Piperazines carboxylic acids

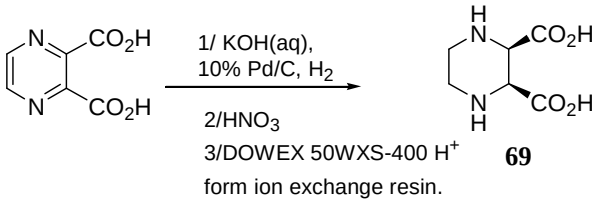
The piperazine ring is a favored structural element in medicinal chemistry, and is often encountered in the structure of enzymes inhibitors, and clinical therapeutics. Piperazine-2-carboxylic acid, has been employed to form biologically active peptidomimetic anti HIV protease inhibitor indinavir (Figure III-16). [93] This useful synthetic building block and related hydroxymethyl derivatives have since been employed in the preparation of a variety of biologically active compounds, antibacterial, antineoplasic , antinociceptive agents.



Indinavir Sulfate

Figure III-16

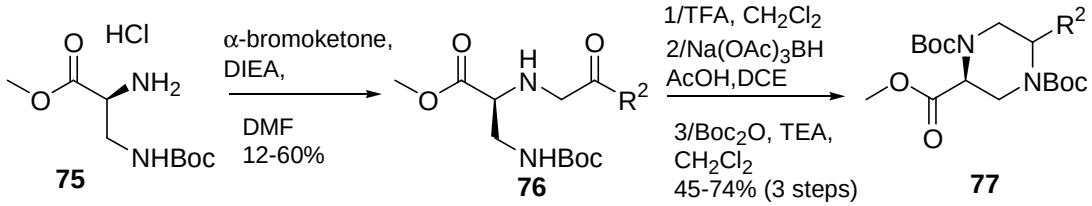
This skeleton is traditionally prepared from pyrazine carboxylic acid by hydrogenation to produce the racemate. The racemate is then resolved by fractional crystallization of diastereomeric salt as camphor sulphonic acid salt.[94] This methodology has been used more recently to provide the piperazine-2,3-dicarboxylic acid **69** that is a precursor of NMDA (N-Methyl D-Aspartate receptor) antagonist (scheme III-27).[95]



Scheme III-27

Enzymatic methods resolution of the corresponding carboxamide precursor using stereoselective amidases led to both S and R pure enantiomers of the piperazine carboxylic acid but a disadvantage of these enzymatic methods is the low amount of product available. [96]

In 2005, Palucki *et al.* reported a synthesis of 5-substituted piperazine, as a precursor of melanocortin receptor agonist. The 5-alkylated piperazine compounds were prepared in a linear fashion illustrated in Scheme III-28. Starting with commercially available methyl N-β-Boc-L-α,β-diaminopropionate hydrochloride **75**, conversion to diamine **76** was achieved via alkylation with various substituted α-bromoketones. Removal of the Boc group followed by reductive amination provided the optically pure piperazine **77**.[97]



Scheme III- 28

III-4.1.3 Piperazic acid

Piperazic carboxylic acid, is also found is several biologically active compounds, the L-enantiomer residue, for example is present within the bicycle ring system of many bioactive synthetic product like cilazapril which is a drug for the treatment of hypertension[98](Figure III-17) and pranalcazan, an antinflamatory.[99]

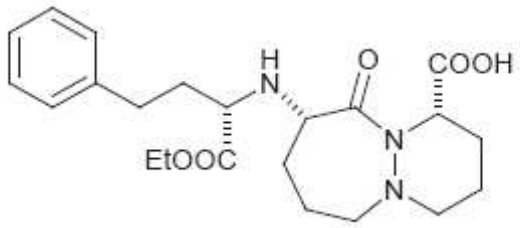
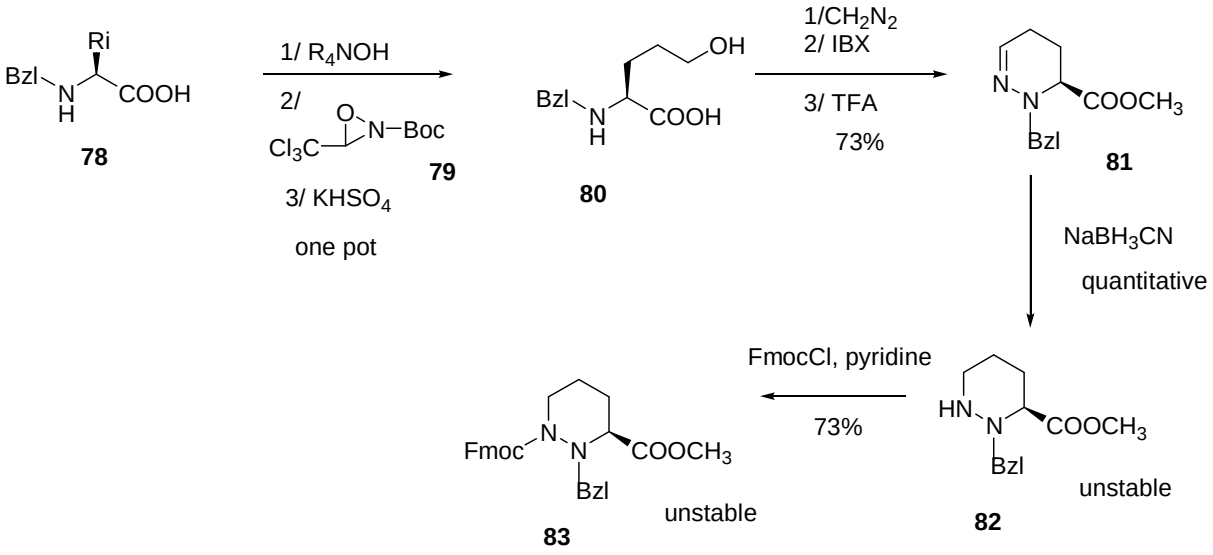


Figure III-17 : Cilazapril

This building block has also shown interesting structural properties in peptides.[100] Its synthesis has been reviewed in1998 by Ciufolini *et al.*[101] and I will report here a recent synthesis from Hanachi *et al.* [102] in 2004 who described an efficient synthesis of piperazic acids of different ring sizes. the hydrazino acid precursor **80** was previously obtained from electrophilic amination of the benzyl protected amino acids **78** with oxaziridine **79**. Then diazomethane esterification followed by mild oxidation and TFA treatment lead to the unsaturated compound **81** in 76% yield. Finally the piperazic acid **82** was obtained by reduction of the hydrazone function with $NaBH_3CN$. The present compound was unstable and was thus Fmoc protected affording finally the desired orthogonally protected piperazic derivative **83** (Scheme III-29).



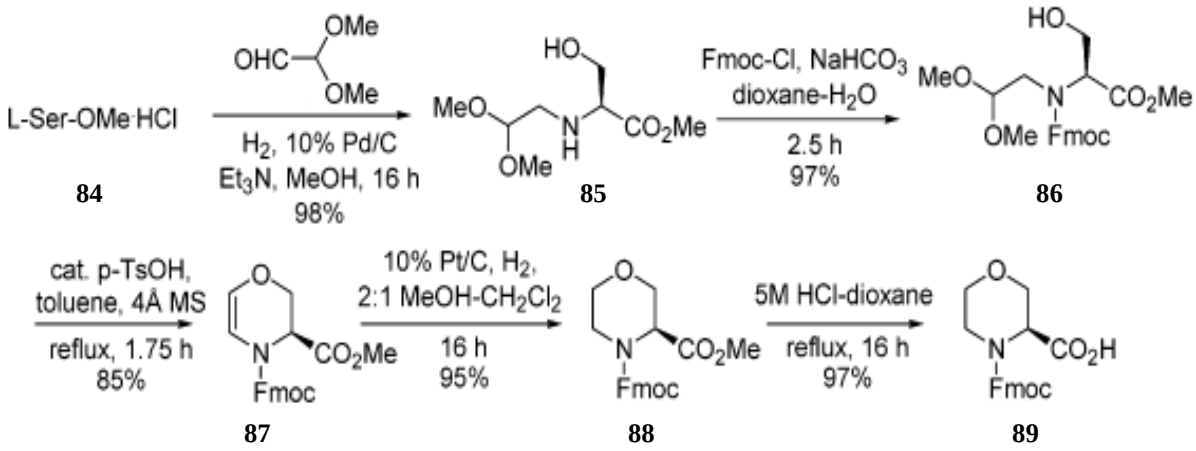
Scheme III-29

III-4.1 4 Morpholine-3-carboxylic acid

This scaffold has been used to build several bioactive molecules, such as TACE[103], MMP and TNF-α inhibitors,[104] and a potent orally active VL-4 antagonist.[105] The first synthesis of morpholine-3-carboxylic acid was reported in 1981 by Anteunis *et al.*[106] providing the racemate. This firs attempt was followed by the synthesis proposed by Brown et al. in 1985, describing the preparation of the amino acid as the *N*-benzyl ethyl ester derivative from serine in 6% overall yield. In the same years, Kogami and Ogawa reported the most convenient synthesis of morpholine-3-carboxylic acid wich employed chiral benzyl *N*-Cbz-2-aziridine carboxylate derived from serine, to give the title amino acid after three steps in 66% yields.[107] And recently an enzymatic synthesis has been proposed.[108]

Guarna et al in 2007, proposed a really convenient synthesis starting from serine methyl ester **84**, and giving in five steps the corresponding morpholine carboxylic acid Fmoc protected **89** in good yields. Reductive amination of dimethoxy acetaldehyde with L-serine methyl ester hydrochloride was carried out under one hydrogen atmosphere in the presence of catalytic amounts of Pd/C and triethyl amine, giving the compound **85**. Subsequent Fmoc protection, afforded the cyclisation precursor **86**. The cyclisation was then conducted in acid conditions *via* acetalization, and in situ elimination of the methoxy group was achieved by adding catalytic amounts of p-toluene sulfonic acid and refluxing. Hydrogenation of the double bond

and final deprotection of the methyl ester give the corresponding morpholine derivative **89** conveniently protected for petide synthesis (Scheme III-30).



Scheme III-30

III- 4. 2 Structural features

As it has been shown during this report, all these six membered ring amino acids have been developed for medicinal chemistry needs, and have shown efficiency especially in structure activity researches, for the discovery of new bioactive compounds.

Their conformational behaviour as inducer of secondary structure has retained less attention. Nonetheless it has been shown that in spite of their higher flexibily because of their larger ring size in respect to proline, they are interesting tools as folding nucleators. Pipecolic acids seems to be less efficient as proline because of its flexibility[109] but Piperazic acid and the D enantiomer of morpholine carboxylic acid[110] have shown propensities to form different turn sructures.[111]

5-Oxo-piperazine-2-carboxylic acid (Pca), has received much less attention, and only few reports deal with its synthesis, structural and biological properties. [112-114]

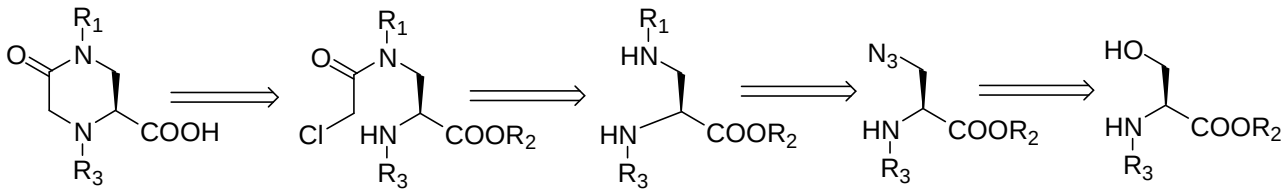
Herein we report a practical synthesis of *N*-Boc-5-Oxo-piperazine-2-carboxylic acid derivatives, their introduction into peptidomimetic structures by a solution-phase peptide-synthesis strategy, and a conformational analysis of tetrapeptide mimics incoroporating a PCA residue.

IV – DISCUSSION

IV-1 Synthesis of the 5-Oxo-piperazin-2-carboxylic acid

In the frame of our studies directed towards the synthesis of peptidomimetics with well defined conformational properties, we became interested in the synthesis of 5-Oxo-piperazinone-2-carboxylic acid, and its introduction into peptide sequences as inducer of peptide's secondary structures.

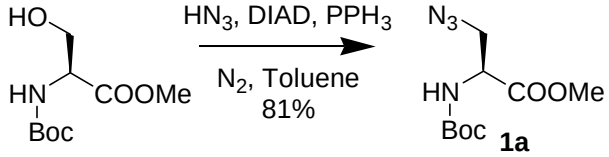
A synthetic plan was envisaged starting from L- or D- serine, requiring the replacement of the hydroxyl by an amino group (possibly via an azide intermediate), acylation with a chloroacetyl derivative and, eventually, cyclization to the piperazinone ring (Scheme IV-1).



Scheme IV-31

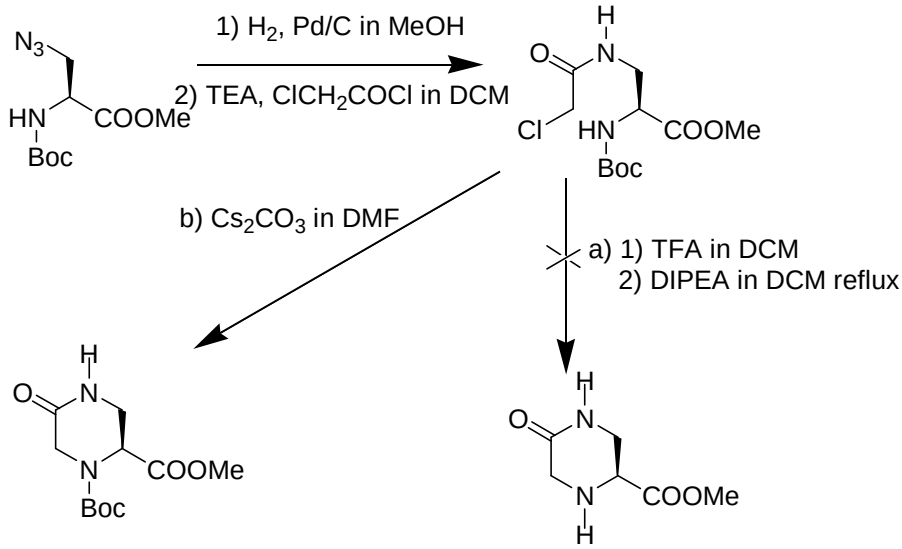
L-Serine was protected both at the nitrogen and carboxylic groups. The α-nitrogen position was protected with a Boc group and the carboxylic acid function was transformed in the methyl ester by reaction with methanol in the presence of acetylchloride (2.7equivalents).[115] The acetylchloride reacts with methanol forming methylacetate and releasing a known amount of gaseous HCl in the methanol, which acts as an acidic catalyst for the esterification reaction. *N*-Boc-serine methyl ester was thus ready for the introduction of the nitrogen functionality. It was reported in the literature that this transformation could be achieved by activation of the OH of serine either as sulfonate ester[116] or via a Mitsunobu type reaction.[117,118] Unfortunately, the formation of sulfonate esters of serine, in our hands, gave only the elimination product, i.e. methyl α-*tert*-butyl-carbamoylacrylate. The Mitsunobu reaction required the use of a titrated solution of hydrazoic acid in a hydrocarbon solvent (originally benzene but toluene was used instead) which was obtained by reaction of sodium azide and concentrated sulfuric acid, followed by extraction of the resulting hydrazoic acid into the organic phase.[119]

The subsequent Mitsunobu reaction was then conducted in toluene and other solvent mixtures in order to determine if any solvent effect would be operating. The best experimental conditions involved running the reaction in toluene at room temperature; in this way excellent yields of the azide derivative **1a** were obtained (Scheme IV-2).



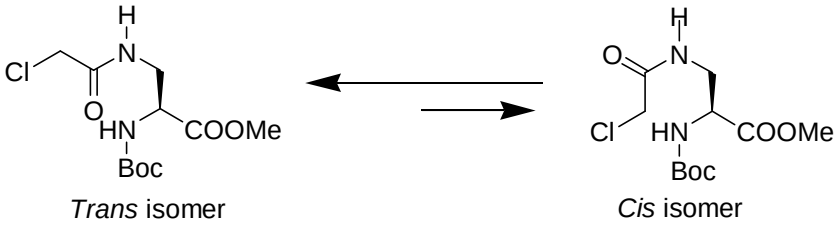
Scheme IV-32

The azide **1a** was then converted in the primary amine *via* a catalytic hydrogenation using Pd/C and the cyclisation precursor was subsequently obtained by coupling the amine with chloracetyl chloride (Scheme IV-3). The subsequent cyclization reaction was then done utilizing two diverse strategies: a) deprotection of the α-nitrogen and further cyclisation under basic conditions, or, b) α-nitrogen deprotonation and further closure of the ring via intramolecular nucleophile substitution of the chlore atom (Scheme IV-3).



Scheme IV-33

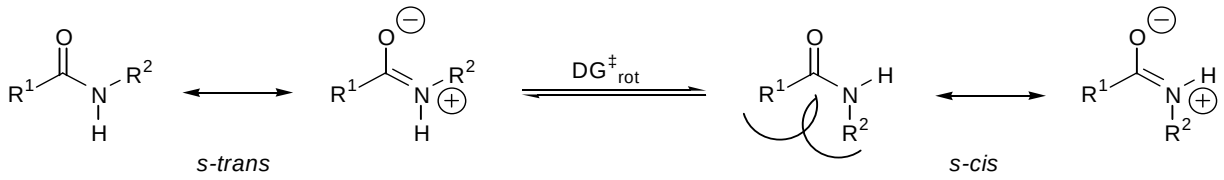
In case a) we could not isolate any cyclised product, and in the second case b) the cyclisation occurred in low yields (about 30%). We related this failure to the presence in the cyclisation precursor of two conformers in a *cis*/*trans* equilibrium (Scheme IV-4).



Scheme IV-34

Indeed the *cis-trans* equilibrium in amide bonds is well known in particular in polypeptides, where it is mainly present under its *trans* configuration. It has been reported that the presence of *cis* isomers in polypeptides containing natural amino acids (proline excluded) is around 0,06%.[4] No *cis* peptide bonds are detected in X-Ray structures of proteins except when the nitrogen is tertiary in proline residues. Some well-established features for the peptide bond are the coplanarity of the group attached to it, its partial double bond character that is reflected in shortened C-N distances and moderate free energy barriers ($\Delta G^{\neq}$=16-22 kcal.mol^{-1}) (Scheme IV-5).[120]

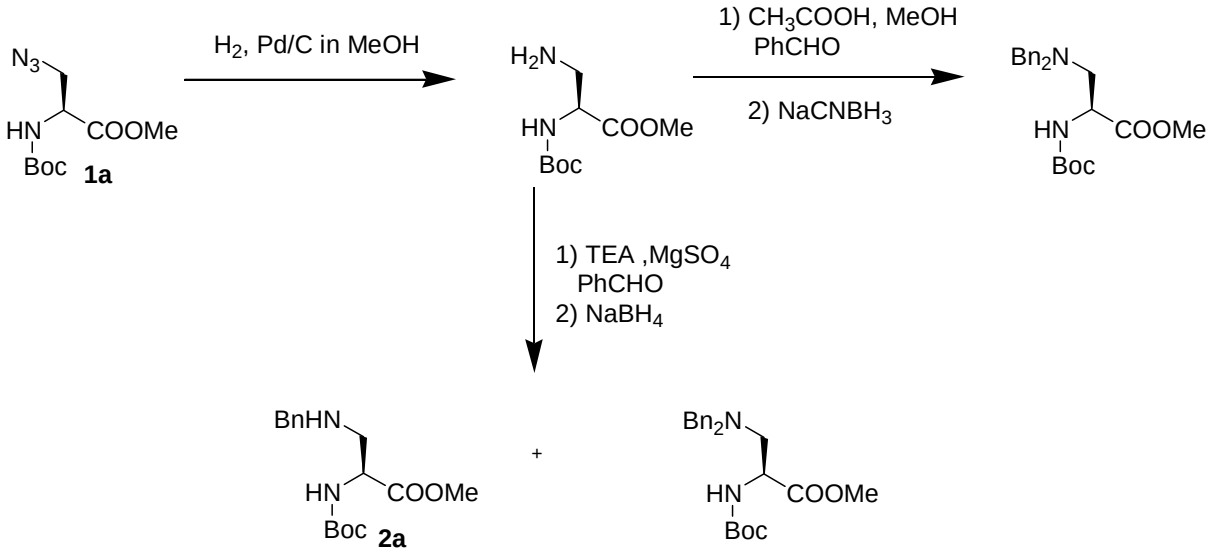
The energetic difference ($\Delta G°$) between both *cis* and *trans* isomers is described to be around 2,5 kcal/mol^{-1} for secondary amides.[121] This barrier is sufficiently strong that no more than a few per cent of the *cis* form is detected in experimental studies of secondary amides. One explanation for the low occurrence of *cis* conformers in nature is the steric hindrance between R^1 and R^2 when they are on the same side of the double bound (Scheme IV-5).



Scheme IV-35

To circumvent the failure due to the *trans* preferred conformation of the amide bond in our acylated derivative, an alternative synthetic way was proposed. To favour the presence of the convenient *cis* conformer and thus the closure of the ring, it was decided to introduce before the acylation step, a new bulky substituent on the amino group. In this way a tertiary amide would be obtained after introduction of the chloracetyl residue and this would favour ring closure by increasing the population of the *cis*-rotamer. We first thought to introduce a benzyl group on the amine position, which would be easily removable at the end of the synthesis. Therefore we proceed to the reduction of the azide group using standard hydrogenation

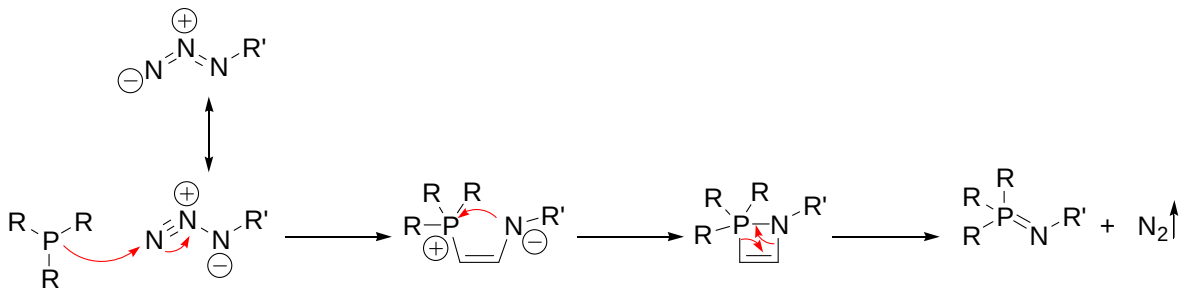
conditions to obtain the corresponding primary amine. We performed the benzyl substitution by reductive alkylation, using benzaldehyde and a reducing agent (scheme IV-6). Unfortunatly, it was not possible to avoid completely the formation of the dibenzylated byproduct, even by the use of stoichiometric amounts of benzaldehyde.



Scheme IV-36

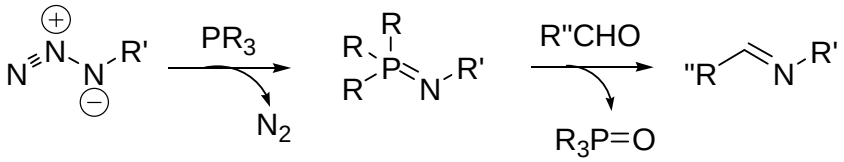
To avoid the dibenzylation of the primary amine we thought about another synthetic strategy starting from the azide, that could give us in "one pot" the monosubstituted compound. This new route is combining two new intermediates: in a first step the azide would be converted directly in an imminophosphorane derivative: "Staudinger intermediate", and the following addition of benzaldehyde should give us the "Aza-Wittig product": the benzyl imine. The imine would be then simply reduced to afford the desired monosubstituted product (SchemeIV- 6).

In a Staudinger reaction[122] the azide derivative is reacted with a phosphine to give the corresponding iminophosphorane intermediate and one diazote molecule is liberated (Scheme IV-7). This derivative reacts usually with the electrophile "H^{+}" to give the primary amine.



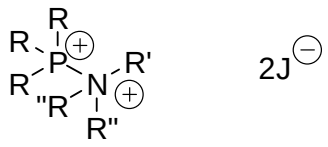
Scheme IV-37

Of course other kinds of electrophiles can be used at this point of the reaction. These electrophiles can be aldehydes or halogenated alkyls (Aza-Witttig reaction),[123] chloroformates (introduction of protective groups),[124] anhydrides to afford rapidly substituted amine derivatives. In our case, the iminophosphorane was directly reacted with benzaldehyde in the same reaction medium to form the imine (Scheme IV-8).



Scheme IV-38

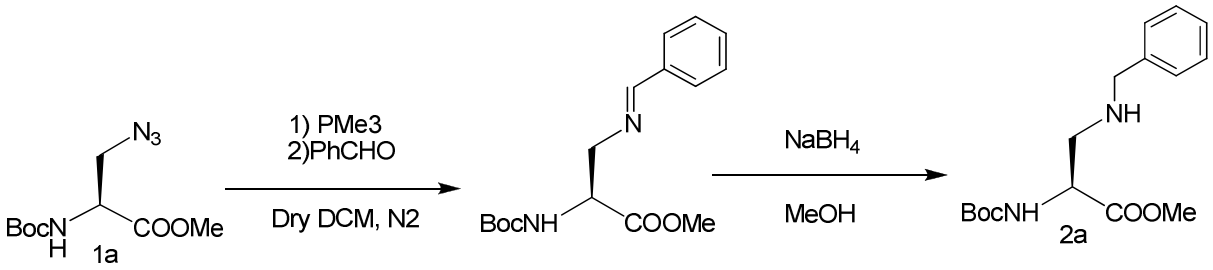
With this methodology the dialkylation of the amine is not permitted since the alkylation of the iminophosphorane would lead to an unfavoured doubly charge salt (Scheme IV-9).[125]



Scheme IV-39

To achieve this step in “one pot” we first screened different conditions. We started our work on this reaction using triphenyl phosphine[126] but the formation of the iminophosphorane was slow, and the removal of the triphenyl phosphine oxide was difficult. So we rapidly switched to the much more reactive trimethyl phosphine, which is also producing a water soluble phosphine oxide. This new reagent gave us the corresponding iminophosphorane in 30 minutes.[127] The following addition of benzaldehyde in quasi stoichiometric amounts was directly poured in the reaction medium, and the imine formation occurred in one hour. Finally, we pitched on the in situ reduction of the imine, using $NaBH_4$ as reducing agent and methanol as cosolvent,[123] with this route we obtained the secondary amine in 55% yield. A better yield was then obtained removing completely the solvent and dissolving the crude mixture in methanol. In this way the mono-benzylated amine was obtained in a three-step-one-pot procedure with an overall yield of 69% Scheme IV-10.

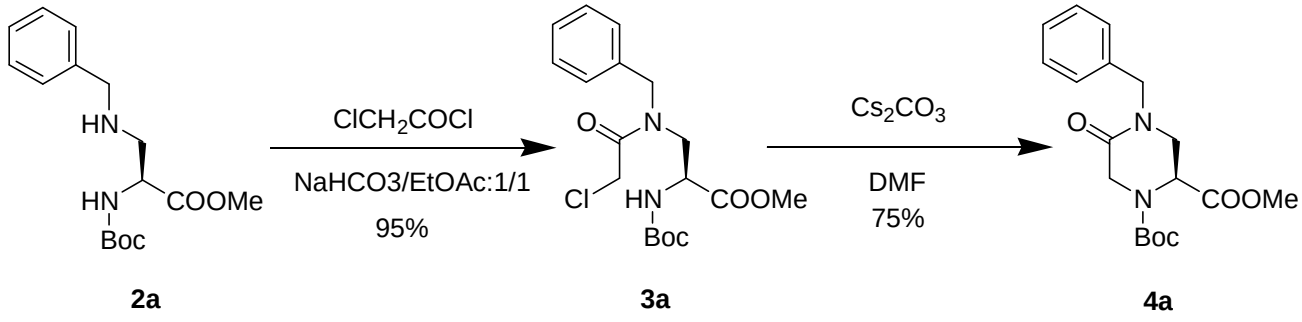
.



Overall yield= 69%

Scheme IV-40

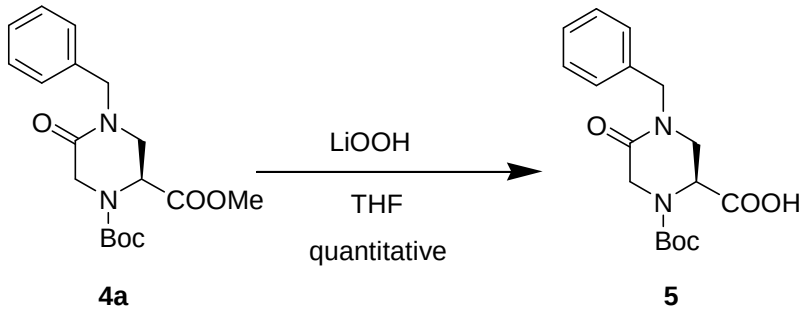
The cyclisation precursor was then formed by addition of chloracetyl chloride in a two phase medium composed of a saturated solution of sodium bicorbonate and ethyl acetate in 95% yields.[128] Thus the following base promoted ring closure using cesium carbonate to enhance the nucleophilicity of the Boc protected nitrogen in DMF at 65°C[129] occurred successfully in a 75% yield (Scheme IV-11).



2a **3a** **4a**

Scheme IV-41

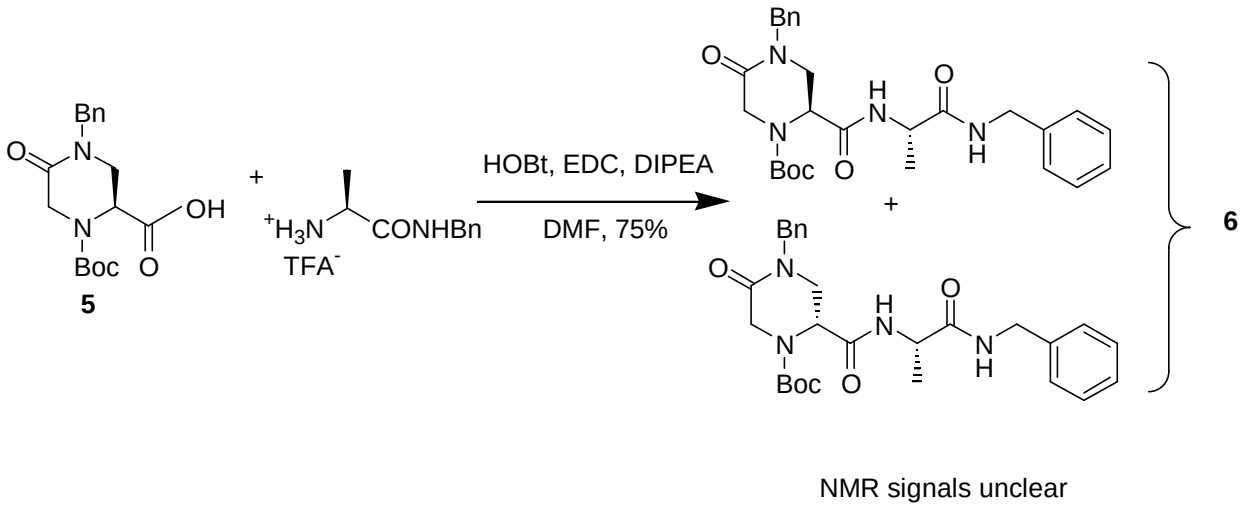
Following the presently described synthesis we were able to obtain our scaffold in good amount. To obtain now the non proteinogenic amino acid in a suitable form to be incorporated in peptides sequences, we had to deprotect the methyl ester to provide the corresponding carboxylic acid. The methyl ester protecting group is widely used in organic synthesis and there are many way to cleave it. One of the most used, involves basic conditions using small amount of aqueous bases NaOH or LiOH. Although rather mild, these conditions are still basic, and could cause epimerization of the stereocenter in the α-position of the ester, prior to hydrolyis. In order to decrease the basicity of this method we prepared and used LiOOH, which is milder as it is less basic and more nucleophilic(Scheme IV-12).[130]



4a **5**

Scheme IV-42

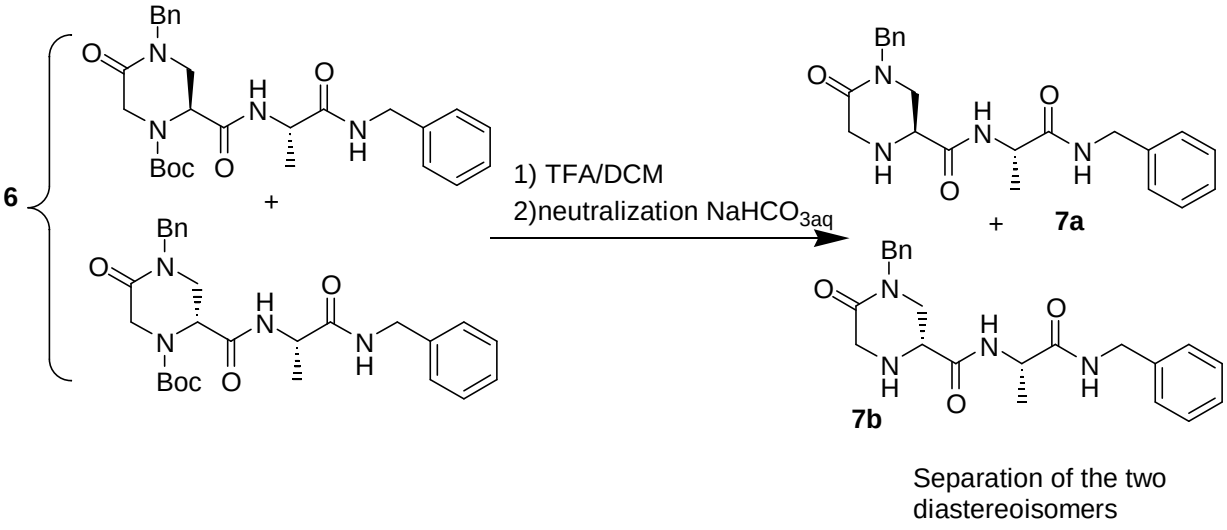
With our scaffold, 5-Oxo-piperazine-2-carboxylic acid, in hand we tried then to check its compatibility with peptide synthesis. Therefore we coupled L-alanine benzyl amide to the 5-Oxo-piperazine-2-carboxylic acid, using HOBt and EDC as coupling reagent, DIPEA as a base and DMF as solvent.[131] The product was obtained in good yields, but, unfortunately, we could detect by NMR the presence of two different signals for the methyl group of the alanine in the dipeptide. To exclude that the splitting of the signal could be due to the presence of rotamers at both Boc and benzyl groups, the NMR analyses on this Boc protected dipeptide were performed at higher temperature, but no significant difference was noticed. (Scheme IV-13).



Scheme IV-43

The evidences for the presence of the two isomers finally came from further analyses performed on the correspondent deprotected dipeptide. The removal of the Boc was performed using a mixture of TFA in DCM, and the free amine was isolated after neutralization of the TFA salt using aqueous $NaHCO_3$ (Scheme IV-14). At this point of the

synthesis we were able to see two close spots on TLC plates and we could isolate both isomers by flash chromatography.



Scheme IV-44

We first related this epimerization problem to the acidity of the α-proton of the scaffold during the base-promoted cyclisation. We therefore performed different cyclisation tests using different bases and solvent conditions (table IV-1). NaH conditions in DMF/THF have been reported for previous ring closure of piperazinone rings.[132] The NaH assisted intramolecular cyclisation occurred rapidly, but the obtained product was a mixture of starting material, cyclised compound and saponified one. Cs_2CO_3 at high temperature in DMF that had been also reported for the cyclisation of piperazinone rings gave us the better results in term of yields but in term of enantiomeric excess K_2CO_3 in Acetonitrile at 80°C[133,134] was more efficient. We chose this K_2CO_3 procedure as it had been reported for the *N*-methylation of α amino acids with no traces of epimerisation.[21] Such differences in the enantiopurity with so similar bases let us notice the influence of the solvent. It has been previously reported that the use of DMF and Acetonitrile in base assisted intramolecular alkylation of nitrogen group, could influence the enantioselectivity.[135]

Base	Solvents	T (°C)	Yields	D. R.*
Cs_2CO_3 (2eq)	DMF	65°C	75 %	1/1
Cs_2CO_3 (1eq)	DMF	RT	44%	1/1
NaH(3eq)	DMF/THF (1/1)	RT	55 %	1/1
NaH(1eq)	DMF/THF (1/1)	RT	33 %	3/1
K_2CO_3(2eq)	Acetonitrile	80°C	70 %	3/1
K_2CO_3(1eq)	Acetonitrile	80°C	38 %	3/1
*t*BuOK(1eq)	THF	RT	64 %	3/1

* The diastereoisomeric ratio D. R. was calculated by ^{1}H-NMR after coupling with L-alanine benzyl amide: i.e. on the product: Boc-Pca-Ala-Bn

Table IV-1

tBuOK was also tried as the base in this cyclisation procedure to test the effect of a bulky base, but, again, the enantiomeric excess remained the same.

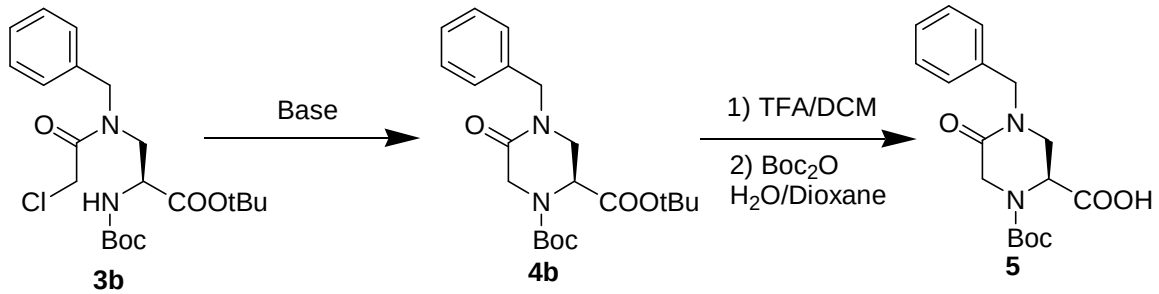
Despite our numerous trials with various basic conditions we were not able to produce the enantiomerically pure scaffold, following this synthetic scheme.

To avoid this problem we decided then, to change protecting group. Consequently, we applied the same synthetic route to the Boc L-Serine *tert*-butyl ester. Since this protecting group is more bulky and electron donating, it could have been suitable to prevent the epimerization phenomena from our previous synthetic pathway. This route produced the cyclisation precursor, starting from the serine protected as Boc and tert-butyl ester, with enhanced yields for each synthetic step (see tables IV-2 and IV-3).

Steps	Methyl ester	*Tert*-butyl ester
Mitsunobu rection Azide synthesis	81%	93%
Aza-Wittig Substitution of the α-amine	68%	83%
Acylation	92%	96%

Table IV-2

The cyclisation reaction was then performed using the same basic condition described above for the methyl ester derivative scheme (table IV-3). The corresponding piperazinone derivative was then deprotected at the same moment in its amino part and in its carboxylic part mediating a reaction with trifluoroacetic acid. The ecxess of TFA was removed and the nitrogen atom was directly reprotected using Boc-anhydride. Thus we could avoid the use of basic conditions for the deprotection(SchemeIV-15).



Scheme IV-45

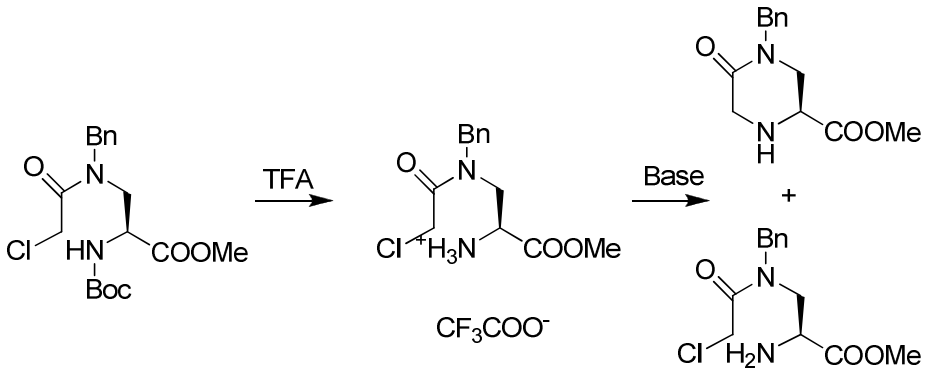
The peptide coupling to the L-Alanine benzyl amide gave us a better diastreoisomeric ratio (table IV-3), but the closure of the ring was anyway not giving us the scaffold with satisfying optical purity.

Bases	Solvent	T (°C)	Yields	D. R.
Cs_2CO_3(2eq)	DMF	65°C	97 %	1/1
K_2CO_3(1eq)	Acetonitrile	80°C	52 %	3/1
tBuOK(1eq)	THF	RT	93 %	3/1
NaOHaq	DCM	RT	N.R.	N.R.

* The diastereoisomeric ratio was calculated by ^{1}H-NMR after coupling with alanine benzyl amide: i.e. on the product: Boc-Pca-Ala-Bn

Table IV-3

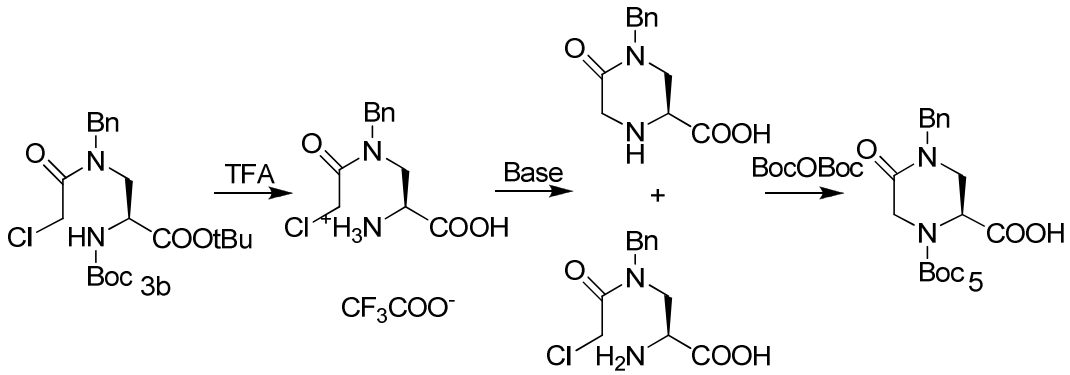
To further decrease the lability of the α-proton in the cyclisation step, we decided to deprotect the Boc-amino group of the cyclization precursor. In this way the cyclization would occur on the free amine which is definitely less acidic at the α-position that the Boc-derivative. A base was then used in the ring closure step to quench the TFA salt and control the formation of HCl.[136,137]



Scheme IV-46

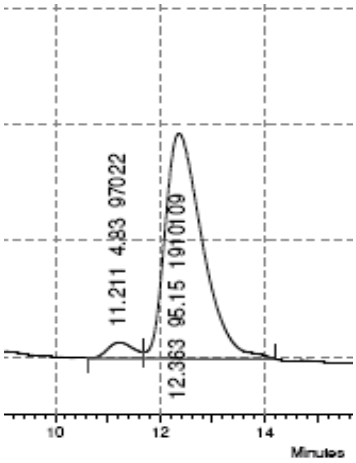
Unfortunately, this method was not suitable in terms of yield, since we recovered substantial amount of the amine uncyclised.

The cyclisation of a completely deprotected scaffold (both at the carboxylic and amino-group) was then envisaged. To this purpose the precursor **3b** was deprotected with TFA and dissolved in a NaOHaq solution (in variable quantities). The cyclisation occurred in this basic aqueous medium. Subsequent addition of Boc-anhydride and dioxane provided the desired cyclic compound in good yields and conveniently protected for peptide coupling reactions.

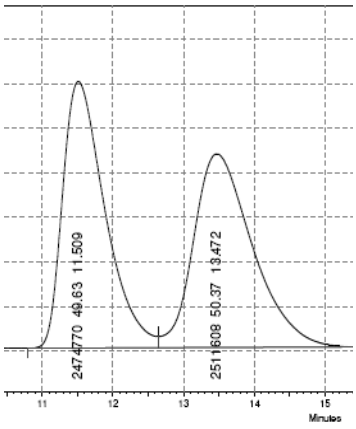


Scheme IV-47

At this point of our investigations we set up chiral HPLC conditions,[138,139] using the column CHIRALCEL OD-H and a mixture of hexane/ isopropanol: 9:1 and 0.1% of TFA[140] (figure IV-1, Scheme IV-18).

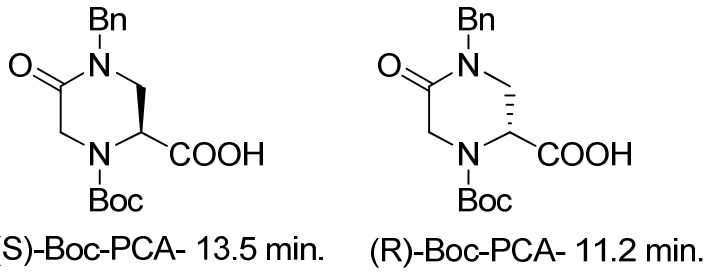


ee of 91% after cyclisation with NaOH 3 eq on the complete deprtotected acyl compound



Racemic mixture
Cyclisation with Cs_2CO_3 on the acyl compound Boc methyl ester

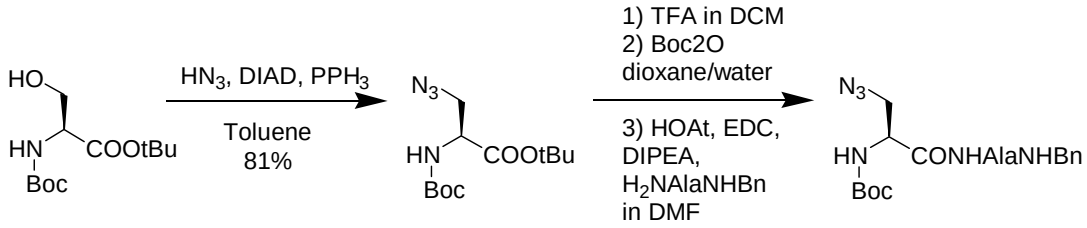
Figure IV-18



Scheme IV-48

The cyclisation reaction has a crucial influence on the enantiomeric excess, and we screened this time again different conditions utilizing from 5 to 3 equivalents of NaOH or even buffered solutions to control precisely the pH of the solution. These different cyclisation conditions gave various results in term of yields and enantiomeric excess. Unfortunately, we noticed that these results were not always reproducible, and in some cases the enantiomeric excess was not in agreement with the strength of the base used. We attributed this erratic behaviour of the cyclization reactions to a different optical purity of the starting material depending on the different batches and this observation prompted us to reconsider carefully each step of the synthesis and check the enantiomeric purity of each intermediate of our synthetic sequence.

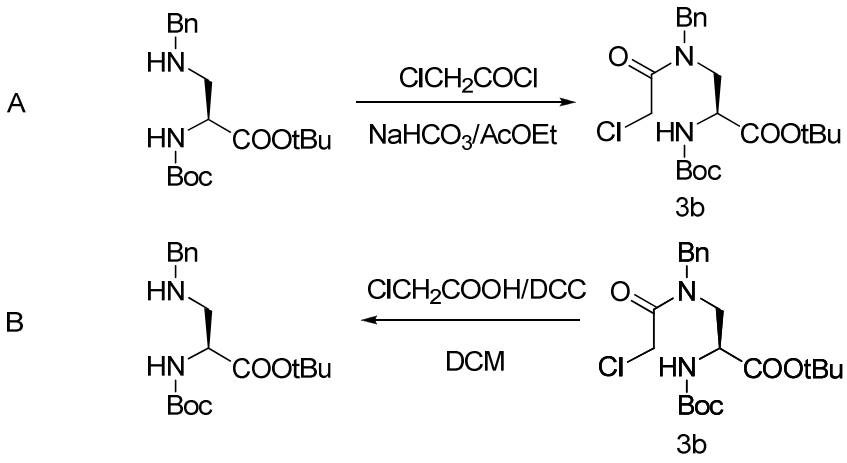
First of all we checked the enantiopurity of the azide obtained from the serine Boc tert-Butyl ester from the Mitsunobu reaction. Therefore we coupled the azide with the L-alanine benzyl amide, using classical conditions Scheme IV-19.



Scheme IV-49

After NMR analysis on the coupling product we could see only one diastereoisomer, thus confirming the enantiopurity of the previous protected azide.

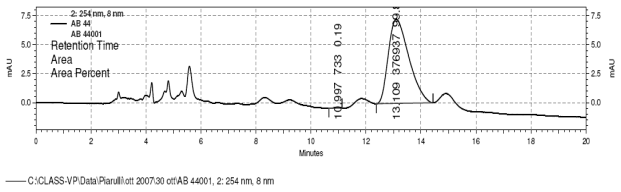
Following the synthetic procedure we then controlled the acylation step that required the use of basic conditions. In this case two different reaction conditions were compared: on the one hand the benzyl amine was submitted to the same conditions developed before, using chloracetylchloride in a biphasic medium (ethyl acetate - saturated solution of sodium bicarbonate) (A). On the other hand the benzylated compound was coupled to chloracetic acid mediating DCC and avoiding indeed the use of a base (B)[141]. Going ahead in the synthetic route, both carboxylic acids were obtained with no significative changes in the enantiomeric excess (Scheme IV-20). These results exclude the acylation step for being responsible of the epimerisation; nevertheless we chose the conditions involving DCC and chloracetic acid to proceed with the synthesis since it was giving us the desired acylated product in good yields without base.



Scheme IV-50

To finish with these controls we evaluated the Aza-Wittig step. Actually, the use of $NaBH_4$ in methanol could lead to the formation of sodium methylate that could be responsible of undesired removal of the α-proton of the imine. To avoid this situation, milder conditions using catalytic hydrogenation were choosen to reduce the imine. This last modification gave us better yields and after all the analysis of the corresponding carboxylic acid by HPLC, gave us the evidence that we had synthetized only one enantiomer(see figure IV-2).

S)-Boc-PCA registrato a 254 nm.



(*S*)-Boc-PCA registrato a 230 nm.

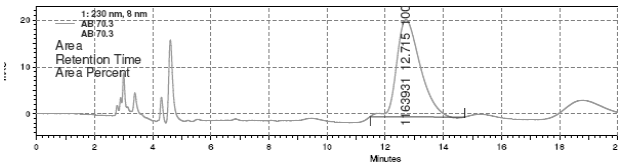
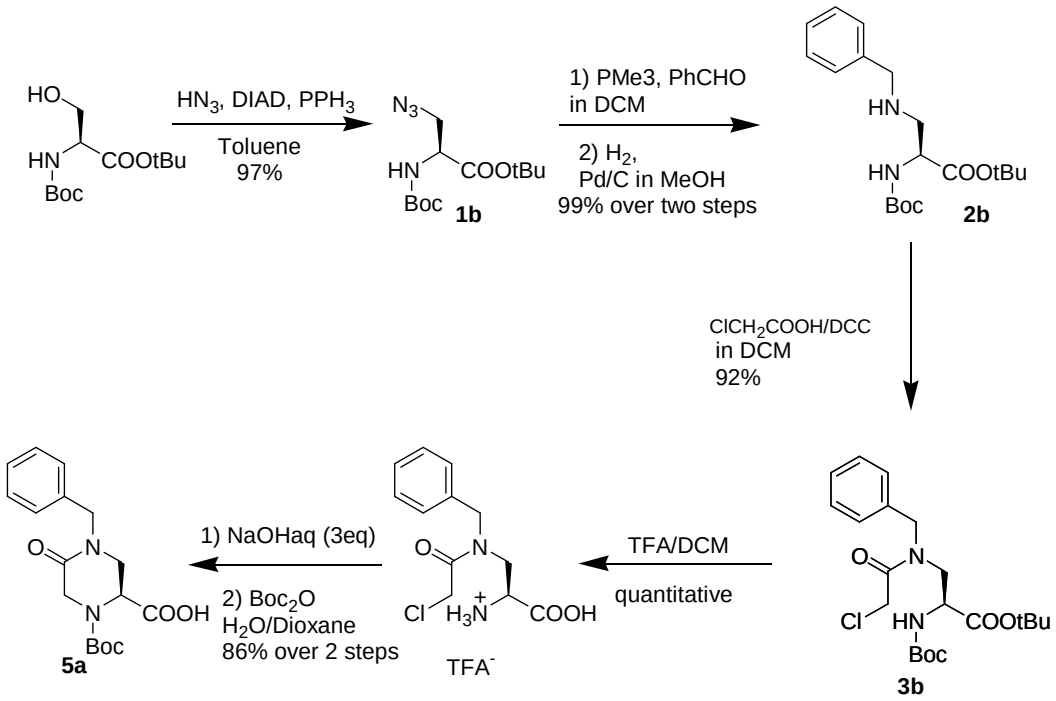


Figure IV-19

In summary, we developed an efficient (yields of each steps > 85%) and simple synthesis to produce the *N*-Boc-5-Oxo-piperazine-2-carboxylic acid. The final synthetic scheme is described below (Scheme IV-21):

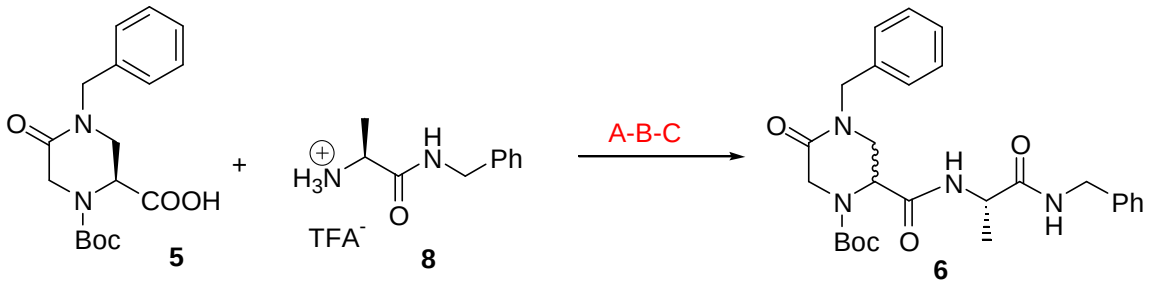


Scheme IV-51

IV-2 Coupling studies on the 5-oxopiperazine-2-carboxylic acid

Having now our 5-oxopiperazine-2-carboxylic acid enantiomerically pure in hands, we wanted to test its compatibility with peptide synthesis, in order to build new peptidomimetics incorporating our non proteinogenic aminoacid in natural amino acid sequences. In order to study the reactivity of our piperazinone toward peptide coupling reactions and determine its stability to epimerization.

We begun our investigations, synthetizing a dipeptide containing our scaffold and a natural amino acid with an alkyl chain. We choosed the alanine for its commodity of detection in NMR spectra. The alanine was first benzylated to protect its carboxylic part and the BOC was removed under acidic conditions using TFA and DCM.

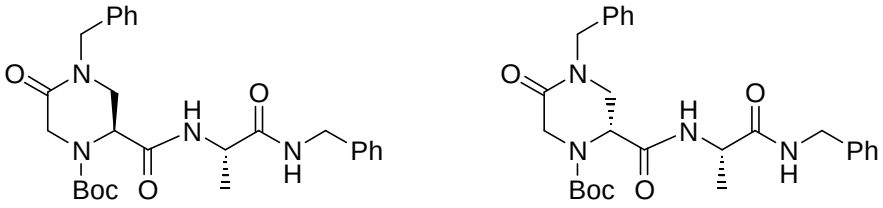


Scheme IV-52

The dipeptide was then formed using 3 different procedures:

- Method A: DIPEA, HOAt, EDC in DMF.
- Method B: DIPEA, HOBt, EDC in DMF.
- Method C: DIPEA, HATU in DMF.

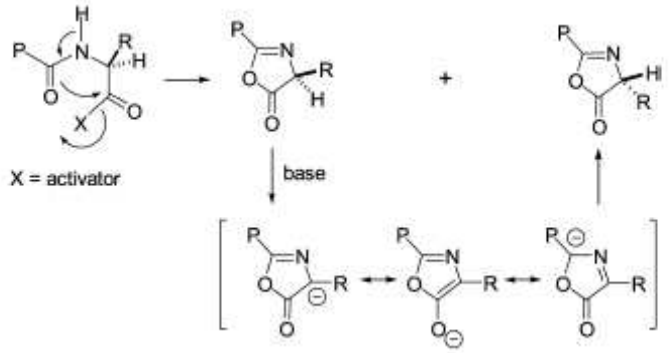
The yield of the coupling step was always satisfying, but in all three cases we obtained a mixture of 2 diastereoisomers (Scheme IV-22). We could detect it by ^{1}H NMR, where we could see some of the proton signals that were doubled



S-Boc-PCA-L-Ala-NHBn *R*-Boc-PCA-L-Ala-NHBn

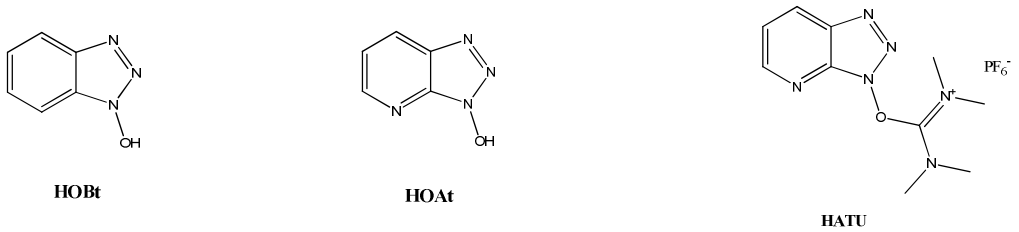
Scheme IV-53

In order to exclude any doubts we decided to analyze directly the deprotected dipeptide to avoid the confusion due to the presence of the two rotamers of the Boc group. The dipeptide was deprotected under acidic conditions using TFA, and the TFA salt was neutralized using $NaHCO_3$ (see scheme IV-14). The further analysis of the ^{1}H NMR spectra confirmed us that we had two sets of signals belonging to two different products. Actually the racemization phenomenon during peptide coupling reactions, is known and described in the literature. [142]In the course of a coupling reaction, racemisation can occur especially at the C-terminal aminoacid residue through the ionisation of the α-hydrogen and the formation of an oxazolone intermediate (Scheme IV-24). This ease of racemisation is emphased in the case of disubstituded amino acids, this problem is attributed to the absence of an acidic amide or urethane proton that would normally ionize first and suppress the α-deprotonation.[143]



Scheme IV-54: Racemisation through oxazolone formation.

We begun thus, coupling studies to elucidate which are, in our case, the causes of the epimerisation of the (Bn)-Pca during peptide synthesis. Different parameters are recognized to influence the amino acid behaviour towards epimerisation in peptide synthesis: Actually, coupling reagents like HOBt have shown a limited efficiency in cases of racemisation issues. Due to the presence of a pyrimidic nitrogen, HOAt and its uronium salt derivative HATU,[131,144,145] have shown to be superior in term of yields and toward the reduction of racemisation (Scheme VI-25)

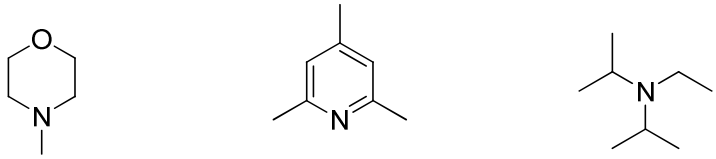


Scheme IV-55 common coupling reagents for peptide synthesis, HOBt, HOAt, HATU

.

But in our case the change of coupling reagent did not lead to better results. We decided thus to screen different coupling conditions, basing our studies on different parameters: basic conditions, coupling reagent, solvent and structure of our molecule. A parameter that is often cited in the literature, as responsible for epimerization phenomenon is the basic conditions used to promote coupling reactions; so in this case we choose different amines with lower pKa than the classical DIPEA (Scheme IV-26). It is to note, that the use of TMP in association with HATU have been widely used to decrease epimerization in both solution and solid state peptide synthesis.[146] To set up the conditions of our study, we based our work on coupling reaction performed by Di Fenza et al.[147] who reported a screening of different coupling reagents and bases conditions to avoid epimerization of serine residues in solid state.

3b



NMM
pKa=7.41

TMP
pKa=7.33

DIPEA
pKa=10.5

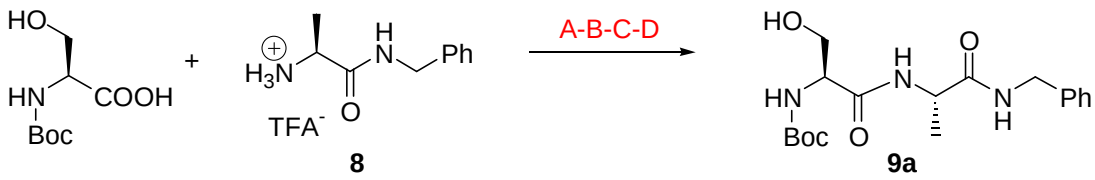
Scheme V-56

These studies were carried out synthesising dipeptides containing the Boc-(S)-Pca **5** and the Alanine benzylamide **8** (Scheme IV-22). As a comparison point, we synthesized at the same time, using the same procedures dipeptides containing the L-serine. In fact, the serine has been described to be sensitive to epimerisation during coupling procedures.[147] As it is shown in (Table IV-4), independently on the coupling conditions the analyses of the dipeptide afforded us the product with a diastereisomeric ratio of 8 to 2 (Table IV-4).

	Method **A**	Method **B**	Method **C**	Method **D**	Method **E**	Method **F**
PCA	1 eq	1 eq	1 eq	1 eq	1 eq	1 eq
TFA salt AlaNHBn	1.1 eq	1.1 eq	1.1 eq	1.1 eq	1.1 eq	1.1 eq
Coupling reagent	HATU 1.1 eq	HATU 1.1 eq	HBTU 1.1 eq	HBTU 1.1 eq	HATU 1.1 eq	HBTU 1.1 eq
Base	DIPEA 3 eq	TMP 3 eq	DIPEA 3 eq	TMP 3 eq	DIPEA 3 eq	DIPEA 3 eq
Solvent	DMF	DMF	DMF	DMF	THF	THF
Diasteroisomeric ratio D/L	**8/2**	**8/2**	**8/2**	**8/2**	**8/2**	**8/2**

TableIV-4 Coupling conditions for the formation of dipeptide **6**

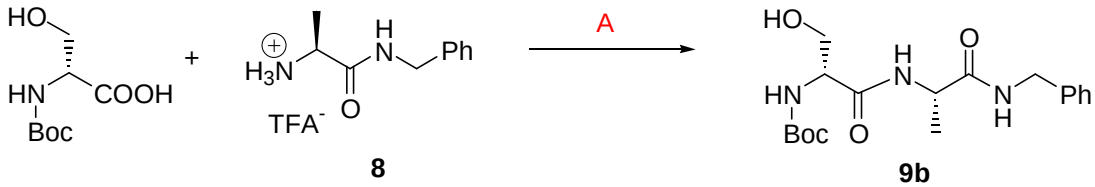
Coupling procedures involving the serine instead of (Bn)-Pca led respectively to the optically pure dipeptide (Table IV-5).



Scheme IV-57

	Method **A**	Method **B**	Method **C**	Method **D**
NH-Boc(L)Ser	1 eq	1 eq	1 eq	1 eq
TFA Salt	1.1 eq	1.1 eq	1.1 eq	1.1 eq
Coupling reagent	HATU 1.1 eq	HATU 1.1 eq	HBTU 1.1 eq	HBTU 1.1 eq
Base	DIPEA 3 eq	TMP 3 eq	DIPEA 3 eq	TMP 3 eq
Diastereoisomeric ratio (L)/(D)	**10/0**	**10/0**	**10/0**	**10/0**

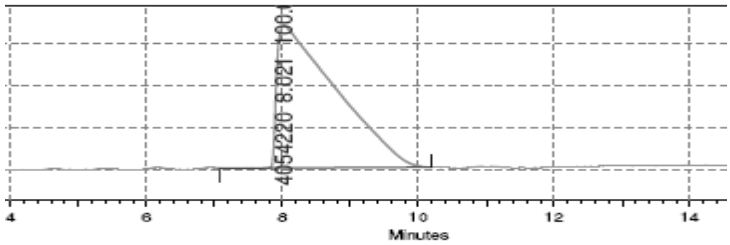
Table IV-5 Coupling conditions for the formation of dipeptide **9a**



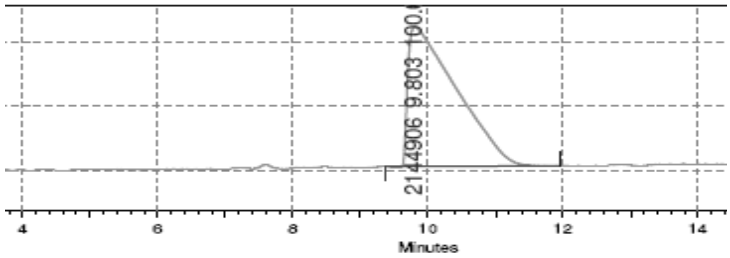
Scheme IV-58

To confirm these last analyses on the serine, and validate our analytical method, we synthesised the other diastereoisomer **9b** (Scheme IV-28) containing the D-Serine this time. The corresponding dipeptide was analysed by HPLC, and afforded us only one peak with a different retention time. Further co-injection of the two diastereoisomers provided us the definitive proof that the serine in these cases was not submitted to epimerization.

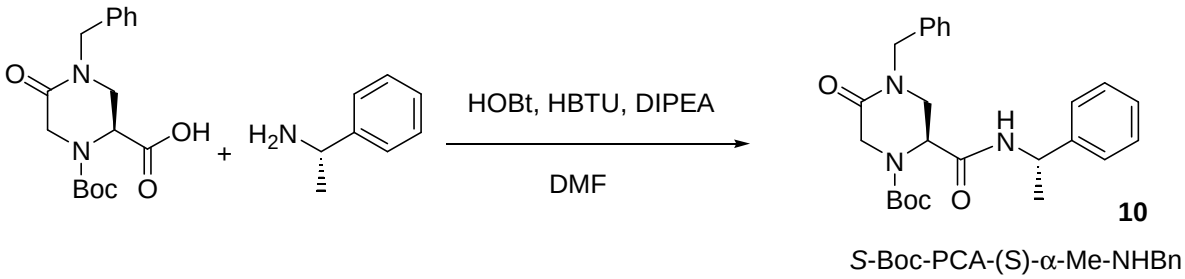
- Dipeptide (L)Ser(L)Ala **9a**, obtained with the method A Tr = 8-8.31 min.



- Dipeptide (D)Ser(L)Ala **9b**, obtained with the method A, Tr = 9.08 min.



Finally to exclude also an epimerization coming from the alanine benzyl amide **8** we changed the coupling partner, switching to a chiral amine: The (S)-α-methyl benzylamine was coupled to the piperazinone scaffold mediating HBTU, HOBt, and DIPEA.



S-Boc-PCA-(S)-α-Me-NHBn

Scheme IV-59

In this case again the dipeptide was analysed by reverse phase HPLC, after deprotection of the Boc , and revealed the formation of two diastereoisomers, The diastereoisomeric excess was calculated from the HPLC trace (registered at 220nm) giving, in a 7 to 3 proportions, both diastereoisomers.

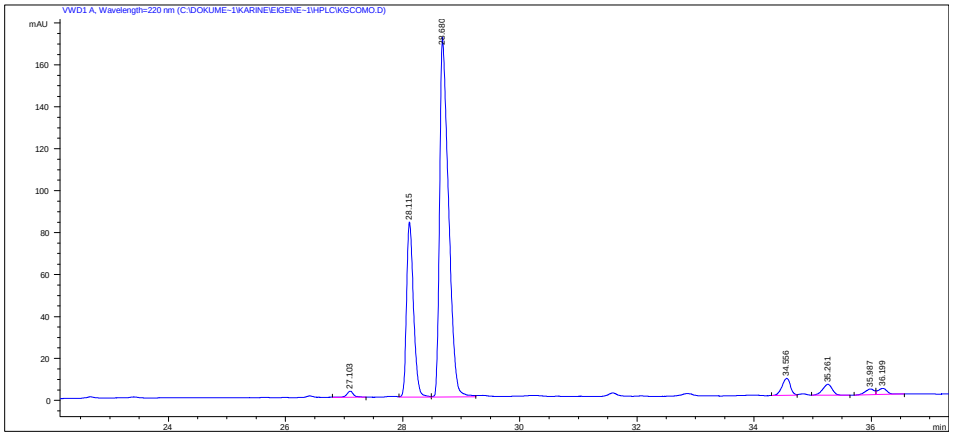


Figure IV-20

(L)PCA (S)-α-methyl benzylamide Tr= 28.68 min.

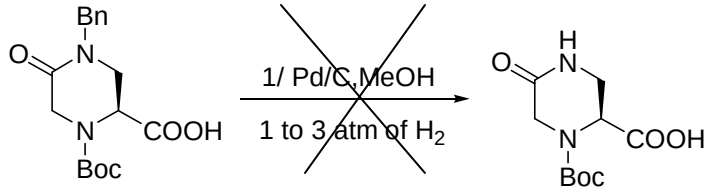
(D)PCA (S)-α-methyl benzylamide Tr=28.11 min.

To conclude, these coupling studies reveal that our compound (Bn)-PCA is really sensitive to racemisation during peptide coupling reactions. Unfortunately, none of our coupling assays afforded us the diastereoisomerically pure compound. And what is really unusual, the diastereoisomeric excess of our coupled derivatives was always the same.

IV-3 Building of the piperazinone ring without the Benzyl substituent:

IV-3.1 removal of the Bn or PMB group:

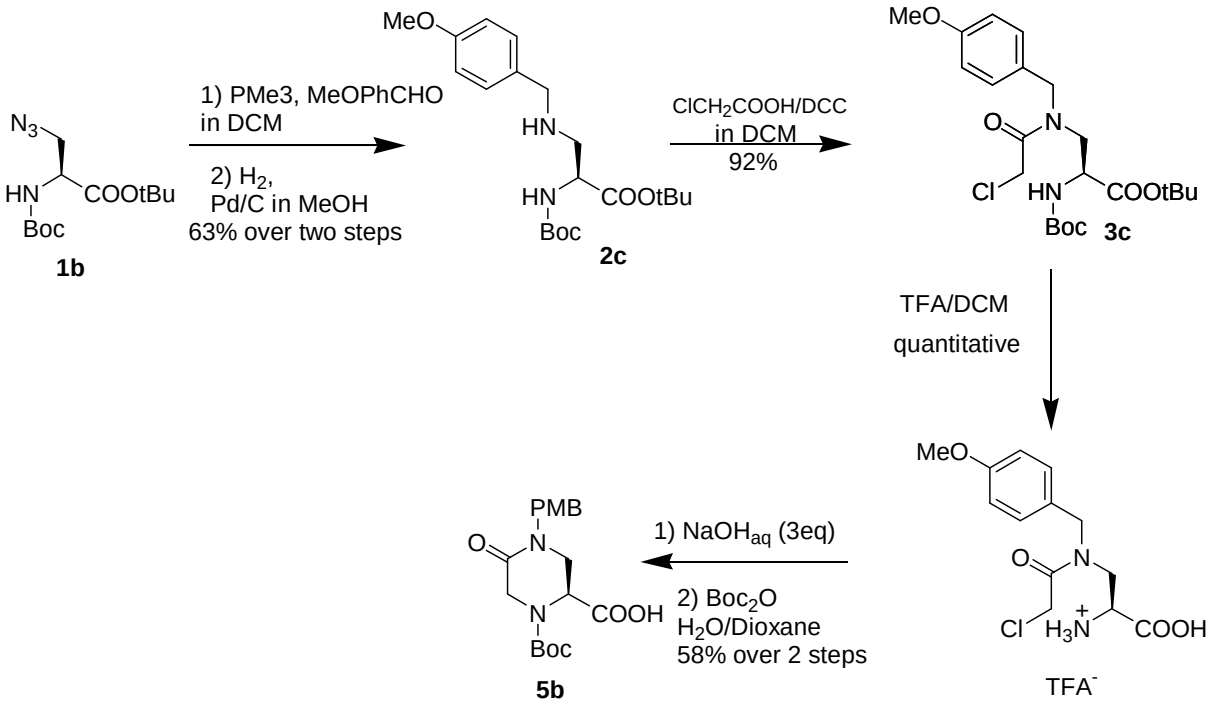
To achieve our initial aim and prepare the piperazinone ring carboxylic acid as proline mimic the final step of the synthesis would have been the removal of the benzyl group. To perform this deprotection, we tried different methodologies based on hydrogenolysis.



5a **12**

Scheme IV-60

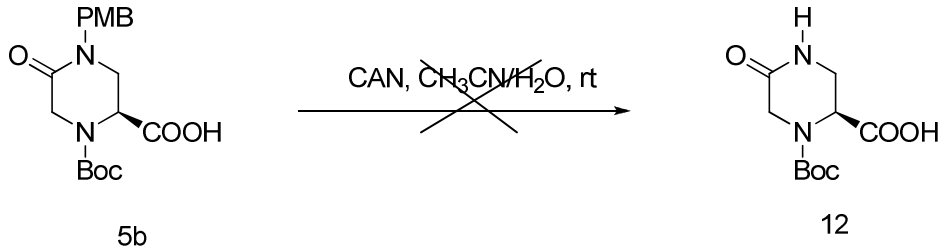
We tested several conditions, including classical hydrogenolysis using from 1 to 3 atmospheres of hydrogen, and Pd/C but all these failed in removing the benzyl substituent. It is known from literature that benzyl group is a suitable protecting group in organic chemistry especially because of its clean deprotection conditions. However whith benzyl amide, classical deprotection condition are sometimes not suitable.[148] Other methods are known to deprotect an amidic benzyl group but it involves drastically basic[149] or acidic conditions.[150] On the one hand, the use of acidic conditions[151] could be problematic in our case because it could contemporaneously deprotect the Boc group which is sensitive to acidic medium, and on the other hand basic conditions would be problematic in our synthesis because of risks towards loss of chirality that we already reported (part IV). The most convenient alternative to solve this issue appeared to be a change of protecting group. In this case we opted for the *para*-methoxy benzylgroup, that can be removed under milder oxidative conditions using CAN (cerium ammonium nitrate).[152,153] Following this idea we applied the same synthesis described in section IV.1 (scheme IV-31) with the PMB in the amide position.



Scheme IV-61

The PMB was introduced in the molecule during the Aza-Wittig reaction using *para*-methoxybenzaldehyde in place of benzaldehyde. All the subsequent reactions were run uneventfully and in comparable yields and the *para*-methoxybenzyl-5-oxo-2-piperazinonecarboxylic acid was thus obtained.

The deprotection of the *para*-methoxybenzyl was then tested in several conditions using ceric ammonium nitrate in acetonitrile in the presence of water, but unfortunately only decomposition products were found, probably arising from deprotectionof both the PMB and Boc groups. Actually these two protecting groups should be orthogonal, but the pH of the solution at the end of the reaction was very acidic (pH < 1) and therefore we suspect that this caused deprotection of the Boc group as well. An *in* situ re-protection of the secondary amine was tried by neutralization and addition Boc_2O goup, but without results

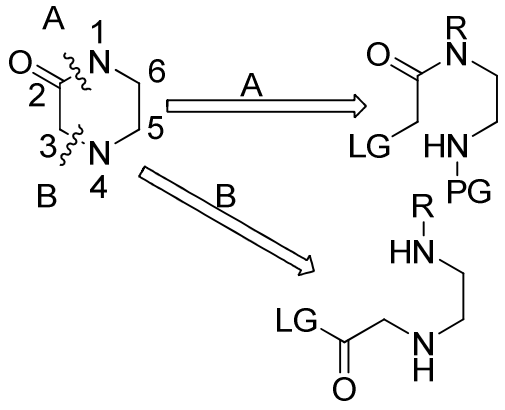


SchemeVI-62

Using this synthetic pathway we were not able to product the unsubstituted scaffold **11**.

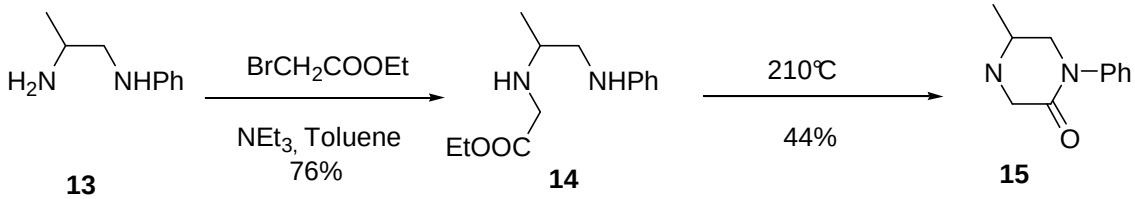
IV-3.2 Alternative synthesis:

As described in our previous chapter III reviewing synthetic strategies for the elaboration of cyclic amino acids, there are several widely used methods to form this kind of heterocyclic compounds. Most of the described reactions involve the intramolecular C-N bond formation (Scheme IV-33).[154]



Scheme IV-63

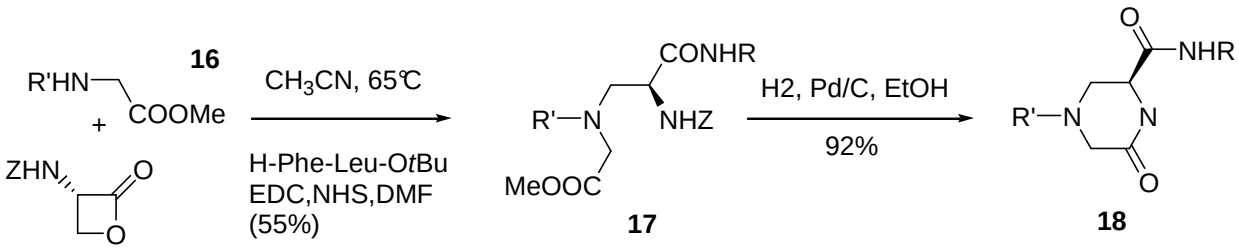
In parallel to our investigations on the deprotection of the amide benzylated compound, we thought about another synthetic strategy, to close the ketopiperazine ring in a different way.
One of the most used methods to access to ketopiperazines rings involves an intramolecular addition of an amine to an acid or ester to form the N1-C2 bond (procedure B scheme VI-2). The construction of the cyclisation precursor can then occur in different way. As an example, Cignarella and coworkers[155] in 1979, reported a convenient procedure starting from the 1,2-diaminoethane. Alkylation of the 1,2-diaminopropane 1,2-propane **13** provided the ester **14** which was heated at 210° to afford the piperazinone ring **15** with loss of ethanol.(scheme IV-34)



13 **14** **15**

Scheme IV-64

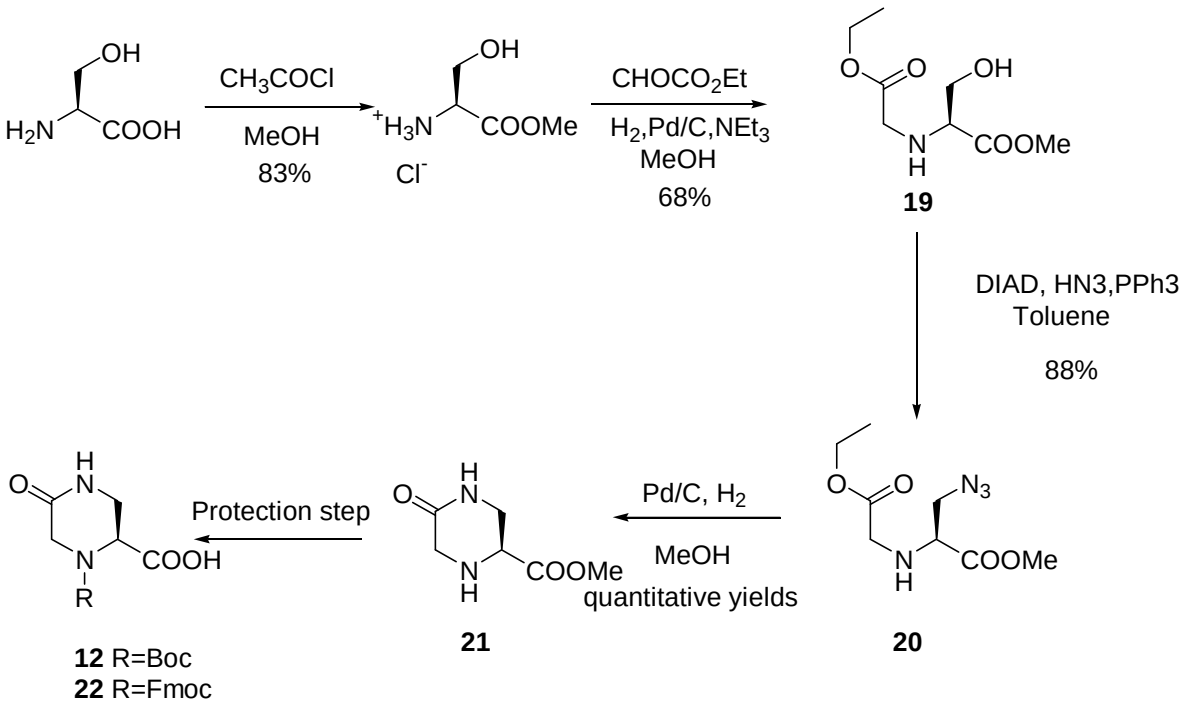
More recently Goodmann's synthesis[156] of the constrained Leu–enkephaline analogue **18**, consist in opening the ring of the Vederas lactone with the amino ester **16** and further coupling of the free amino acid with a dipeptide. Catalytic hydrogenation promoted then the cyclisation that occurred in mild conditions and high yield (scheme IV-35).



Scheme VI-65

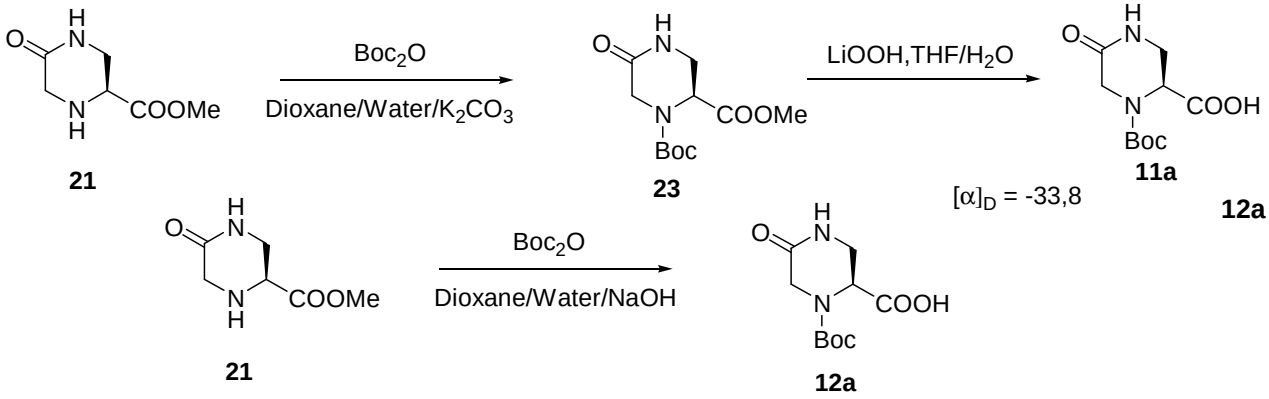
Inspired by these examples we decided to build our scaffold starting from the L-serine which was transformed into the corresponding methylester. Thus the carboxyester segment was added by reductive amination using ethyl glyoxalate giving the corresponding diester derivative **19** in 43% to 68% yield. Actually, the yield of the reaction is rather low, and this was attributed to the possible polymerisation of the ethyl glyoxalate, which could decrease the availability of the reagent. Thus, heating ethyl glyoxalate in toluene at reflux for 30 min depolymeriztion took place and ethyl glyoxalate was obtained as a pure monomer. This was directly added to the reaction medium, and in this way the compound was obtained in a 68% yield. We tried then to study the reaction controlling the formation of the imine, sampling aliquots of the reaction medium and measuring an NMR spectrum, after 30 minutes, we could see the complete disappearance of the aldehyde peak at 9,5 ppm, after 30 minutes, and the formation of a peak at 8.2 ppm corresponding to the formation of the imine. At this point, the reduction of the imine was performed under 1 atmosphere of hydrogen with Pd/C in catalytic amounts but, independently from the reaction time, we were not able to increase the yield. To generate our cyclisation precursor **20** we introduced via a Mitsunobu reaction the second amino group as an azide. Therefore we used the previously described conditions (Scheme IV-2) and here again the azide was obtained in good yields. Finally, the transformation of the azide **20** into its corresponding amine derivative occurred under catalytic hydrogenation conditions, promoting at the same time the cyclisation reaction in excellent yields. Following

this new procedure we manage to build the unsubstituted piperazinone ring **21** in few steps and good yields.



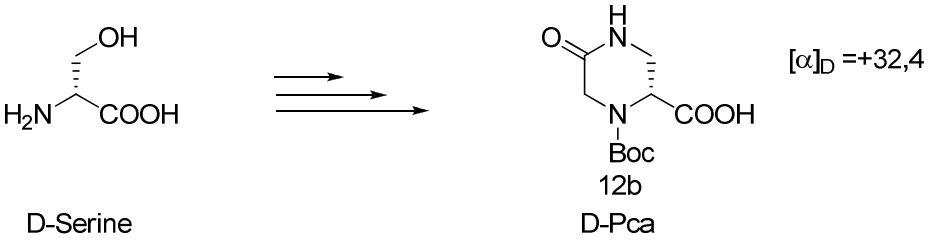
Scheme IV-66

To go ahead with this scaffold in peptide synthesis, we introduced a Boc protecting group in the amino position by two different procedures. In the first case, using Boc_2O in a mixture of water and dioxane with K_2CO_3 as base. This method afforded the corresponding compound protected both at the amino and carboxylic part **23**. In a second procedure, the replacement of K_2CO_3 by the more basic NaOH, afforded at the same time protection of the amino group and hydrolysis of the methyl ester and formation of the free carboxylic group (Scheme IV-37) with no detectable changes in the optical purity.



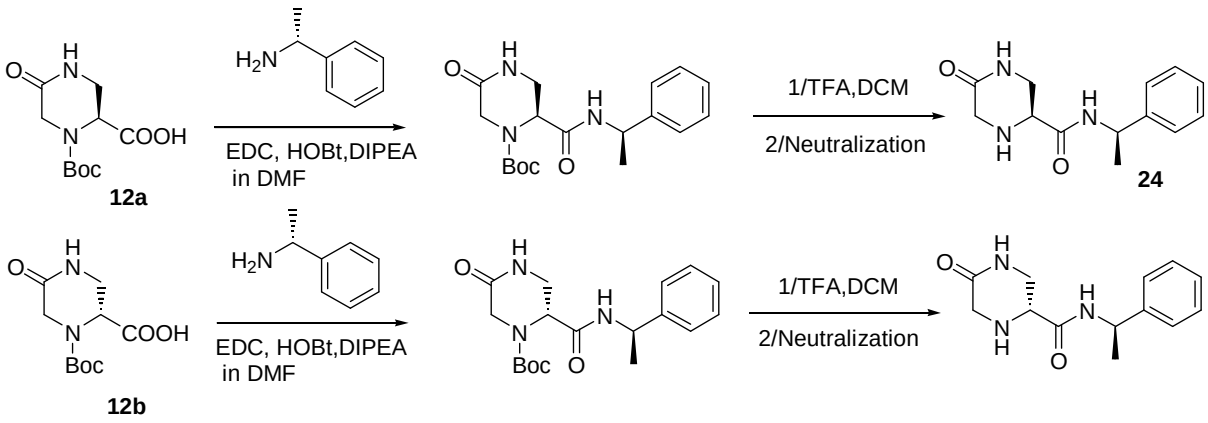
Scheme VI-67

Having this scaffold in hands we first tried to control that no loss of chirality had occurred during the synthetic procedure. Therefore we synthesised the other enantiomer starting from D-serine. Measuring the optical rotatory power of each synthetic intermediate and of the final carboxylic acids we could see that these showed an opposite $[\alpha]^D$ value with respect to corresponding derivatives obtained with L-series (Scheme IV-38).



Scheme VI-68

With both enantiomers of Boc-Pca in hands, we decided to test the compatibility of our scaffold with peptide coupling procedure. Therefore, the carboxylic group of both enantiomers of Boc-Pca was coupled to (*R*)-1-phenylethylamine. The coupling was achieved using HOBt and EDC as coupling reagents and DIPEA as the tertiary amine base. The coupling procedure worked well and the desired substituted compound was obtained in 12 h and acceptable yields. The optical purity was checked by NMR, but no conclusion could be drawn with this analysis since two set of signals were obesrved for each compound, which were attributes to the rotation of the Boc group. We therefore proceeded to removal of the Boc group followed by a neutralization of the amino group (Scheme IV-39). This manipulation afforded the corresponding compounds with the free NH groups in quantitative yields. Their NMR proton spectra showed only one set of signals for both isomers.



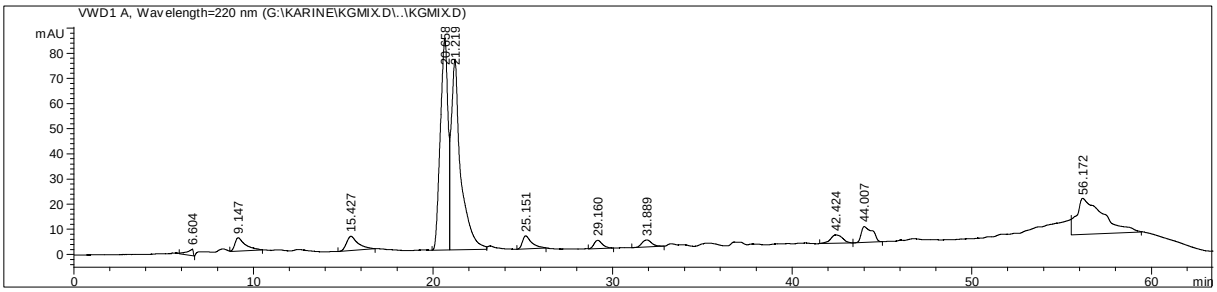
Scheme IV-69

The optical purity of these compounds was also assessed by HPLC anlyses, using the conditions previously developed for the benzylated compound. We used an analytical HPLC containing a C18 column in reverse phase with different solvent gradients. Our better results are presented in Figure 1.

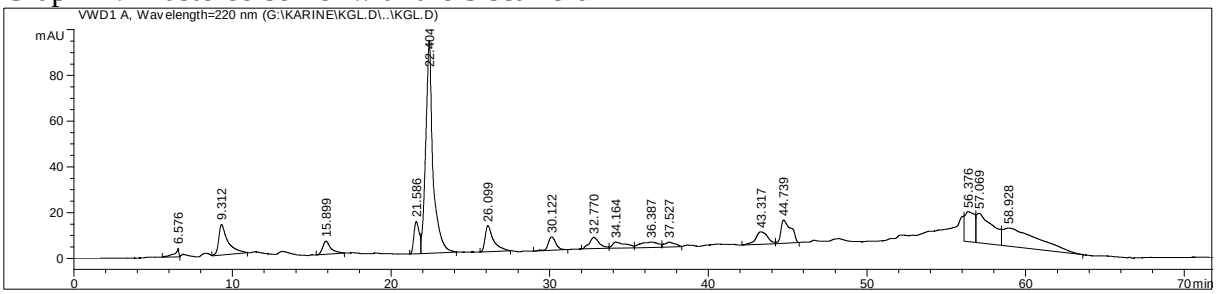
5% of Acetinitrile (0.01%TFA)in 5min

5-40% of Acetonitrile (0.01%TFA)in 55min .

Graph 1: Mixture of both diastereoisomers



Graph 2 : Diastereoisomer with the S scaffold



Graph 3 :Diastereoisomer with the R scaffold

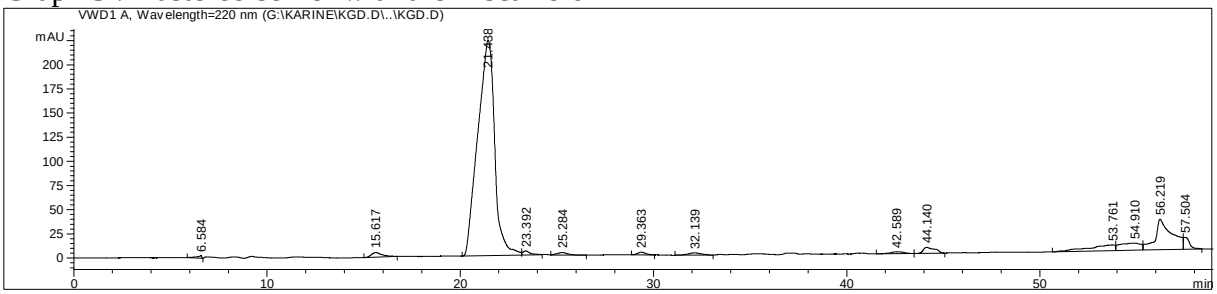


Figure IV-21

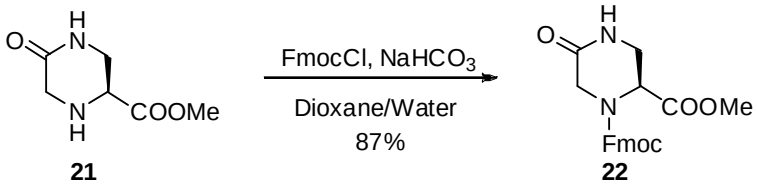
As we can see in the figure **IV-4**, the separation of the peaks was only partial as we could not recover the baseline between the two peaks, anyway it seems to us that both products show different retention time:

(*S*)-Pca-α-Methylbenzylamide=22,4min

(*R*)-Pca-α-Methylbenzylamide=21,4min

From trace 2 we can detect a small peak at 21.5min that could be a small amount of the other diastereoisomer. Anyway such a small amount of the other diastereoisomer would be in agreement with 5% reported epimerisation during coupling procedures.[157]

With these positive results we wanted also to check the compatibility of our piperazinone ring with solid phase peptide synthesis. Therefore, we attempted to protect the amino group as fluorenylmethyl urethane, using Fmoc chloride in dioxane (scheme IV-40),[158] the protection occurred in good yields and the product was crystallized in a mixture of ether and methanol.



21 **22**

Scheme VI-70

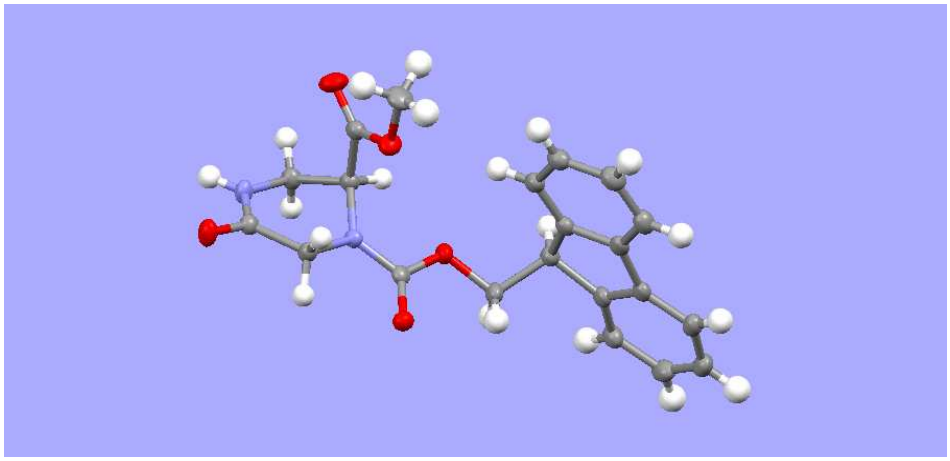
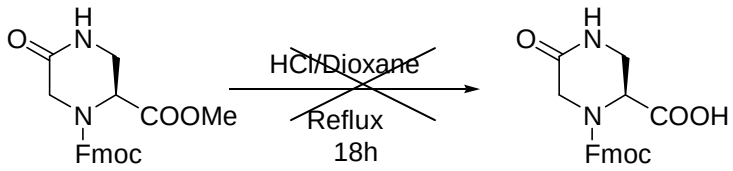


Figure IV-22 Crystal structure of the compound **22.**

The X-ray structure reveals that the piperazinone ring adopts a half chair conformation with the carboxymethyl group in a quasi-axial orientation, whereas the fluorenylmethoxycarbonyl group shows an almost equatorial disposition. The intracyclic amide group has a planar geometry with the N-H bond bisecting the angle of the vicinal CH_2 group.

Unfortunatly assays of deprotection of the methyl ester in acidic conditions failed, leading to the quasi complete recovery of the starting material.



22

Scheme IV-71

IV-4 Conformational studies on tetrapeptides containing Pca:

As anticipated in chapter II, proline is the only cyclic proteinogenic amino acid that plays a significant role in the structural and conformational properties of petides and proteins.[159] Proline is quite often observed at the i+1 position of turn structures, and thus imposes changes in the direction of peptide chains. It has been shown that this structural feature is of particular interest to expose recognition site for the protein protein interaction at the surface of proteins.[160] These kinds of turns in peptides and proteins can be of different types and proline can nucleate some of them. γ and β turns, may or may not be stabilized by intramolecular H-bonds. In γ-turns, the CO of the first residue i may be H-bonded to the NH of the i+2 residue, giving rise to a 7-membered hydrogen bond ring. In β-turns, the CO of the first residue i may be hydrogen-bonded to NH of the fourth residue (i+3), forming thus a ten membered ring (FigureIV-6).

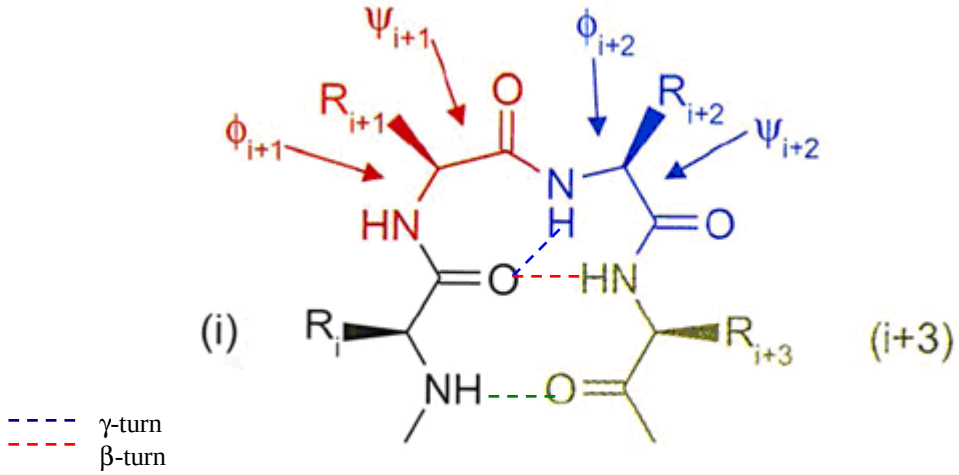


Figure IV-23

The further β- or γ-turn classes are based on the geometry of the peptide backbone. So that these different turns are described by the values of the characteristic backbone torsion angles ϕ and ψ in the residues i+1 and i+2 for β-turns or in residue i+1 in γ-turns (table).

conformation	Φ_{i+1}	Ψ_{i+1}	Φ_{i+2}	Ψ_{i+2}
βI-turn	-60°	-30	-90	0
βI'-turn	60	30	90	0
βII-turn	-60	120	80	0
βII'-turn	60	-120	-80	0
βVI-turn	-60	120	-90	0
βVI'-turn	-120	120	-60	150
γ-turn	70 to 85	-60 to -70		
γ'–turn	-70 to -85	70 to 70		

Table IV-6

β-hairpins are widely occurring β-structures, consisting of two adjacent strands of anti parallel β-sheet and a connecting loop. The shortest common loop involves two residues, in which case the loop and the two adjacent residues form a β-turn. A tetrapeptide can adopt a minimal β-haipin structure, defined by the presence of a 10 and a 14-membered ring N-H….C=O hydrogen bond. Turns that serve in minimal β-hairpins are called reverse turns and are noted βI' or βII'-turns where the prime indicates that the ϕ and ψ torsion angles of the central residues are opposite to those in the common turn. The type VI turn is a unique member of the β-turn family because it is the only turn that incorporate a *Cis*-peptide bond. Natural type VI β-turns always contain a proline residue at the i+2 position (Figure IV-8), since this is the only one that can exist in the *cis* configuration.

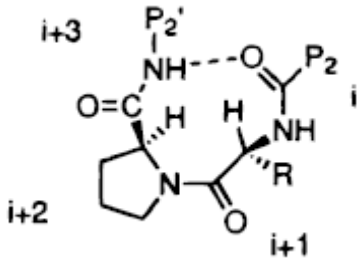


Figure IV-24

It is therefore of great interest to create new templates to mimic those kinds of effective structures. In the literature, numerous compounds have been developed to stabilize β-turns in peptide chains. β- and γ-turns mimetics have been taken into account for peptidomimetics design, as they allow presenting in a stereocontrolled fashion 2 to 4 side chains of amido acids

involved in biological interactions. In particular, because of their similarity with proline cyclic amino acids have been introduced into tetrapeptides and evaluated as turn inducer.[109,110,161]
Because of the low amount of suitable methods to produce this C6 cyclic amino acid, there were no studies available investigating the oxopiperazine ring with a carboxylic group in position 2 as proline mimic and thus as inducer of secondary structures in peptides.
So we will present here our investigations to elucidate the preferred conformations in solution, of tetrapeptides containing our amino acid mimic in i+1 position.

IV-4.1 Tetrapeptides containing the Bn-Pca:

We decided to synthesize new tetrapeptides inserting both Pca derivatives with and without the Benzyl group in the i+1 position of tetrapeptides, in order to evaluate their properties as secondary structure inducer. In the case of the benzyl derivative we had to pay particular attention to the separation of the peptide chain containing the epimerized Pca since this problem was not solved.
First of all we chose the sequence of natural aminoacids based on previous work of Prins *et al*,[162] that had demonstrated that the incorporation of Proline in i+1 position followed by a D-amino acid in the i+2 position could enhance the probability of turn formation. The terminal residues were chosen based on their tendencies in proteins to occupy the first and fourth positions of β-turns embedded in β-hairpin.[21] So we begun our conformational investigation synthesising the tetrapeptide with the following sequence Ac-Val-(Bn)Pca-(D)Ala-LeuNHBu, (Figure IV-9). The synthesis was performed in solution using a Boc strategy, and we separated both diastereoisomers by flash column chromatography after the first coupling between the scaffold and the chain of natural amino acids. The difficulties encountered during the purification together with difficult coupling of the secondary amine of Bn-Pca afforded us the desired product in small amounts. Conformational analyses were performed using variable temperature NMR studies and bi-dimensional ROESY experiments at low concentration to avoid aggregation phenomenon and in a non polar solvent to decrease the interactions of the compound with its environment.
NMR data afforded us some informations about the tetrapeptide conformational behaviour in solution. Two NOE contacts were detected, (Figure IV-8) one between the Valine α-H and H of the (Bn)-Pca cycle, which is described to be a typical signal for a *trans* amino acid amide bond. Another one was detected between α-H of Pca and the H amide of the D-Alanine which

is typical for β-turn conformations. These contacts are characteristic from turn structures but they are not sufficient to conclude for a specific type of turn structure.[163]

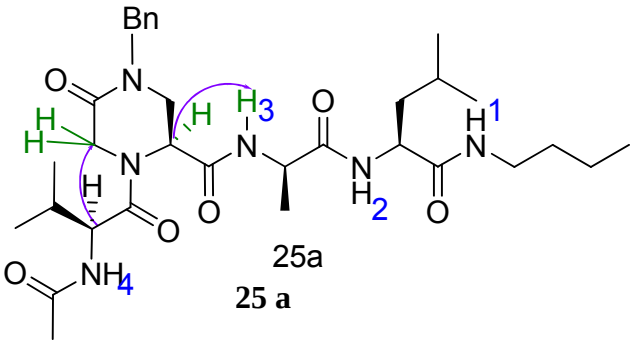


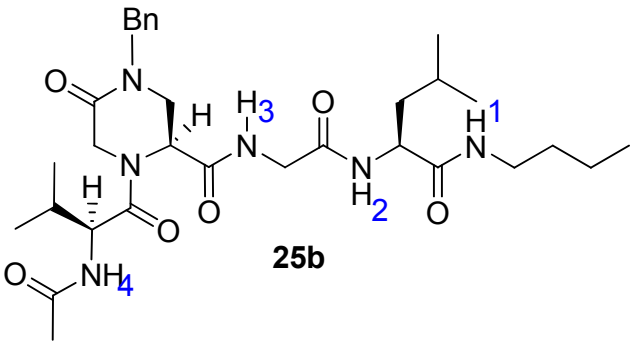
Figure IV-25: tetrapeptide Ac-Val-PCA-(D)Ala-LeuNHBu, violet arrows represent NOEs contacts.

In addition, the informations coming out of the analysis of temperature dependence coefficients and 1H NMR spectrum were contradictory. Temperature coefficient ΔδNH/ΔT of H_2 was inferior (in absolute value) to ΔδNH/ΔT H_3 but on the contrary, H_3 was appearing at higher chemical shifts than H_2 so that we could not have any definitive conclusion about the secondary structure of our tetrapeptide. Based on these NMR data, we can put forward the hypothesis that there was in this peptide exist as an equilibrium between a γ and a β-turn in solution.

N-H	δ (ppm)[a]	ΔδNH/ΔT (ppb/K)[b]
H_1	6.6	-6,00
H_2	6,78	-3.25
H_3	7,47	-4,82
H_4	6,14	-7,18

Table IV-7 [a] Complete proton resonance assignments were made with the aid of COSY experiments; [b] The temperature coefficients were determined between 238 and 298 K (where a linear dependence was observed);.and at a concentration of 2mM (no significant aggregation).

Having a look again in the literature we decided to follow then the Gellman *et al*, paper of 1996 [164] for the design of our new tetrapeptide. Gellman has demonstrated the efficiency of the Proline i+1 and Glycine i+2 for the stabilization of minimal β-hairpin structures. So we decided to incorporate our scaffold in the following sequence Ac-Val-(Bn)Pca-Gly-Leu-NHBu.



25b

Figure IV-26

The tetrapeptide was synthesized as described above and analysed by NMR studies in $CDCl_3$. First of all dilution studies afforded the evidence that there was no significant change in the chemical shift of amide protons in the considered range of concentration (1-10mM), indicating the absence of intermolecular interaction. Then variable temperature studies at low concentration (5mM) were perforemed in a range of temperature between 238 and 298K to study the resonance sensitivity of amide protons towards temperature.

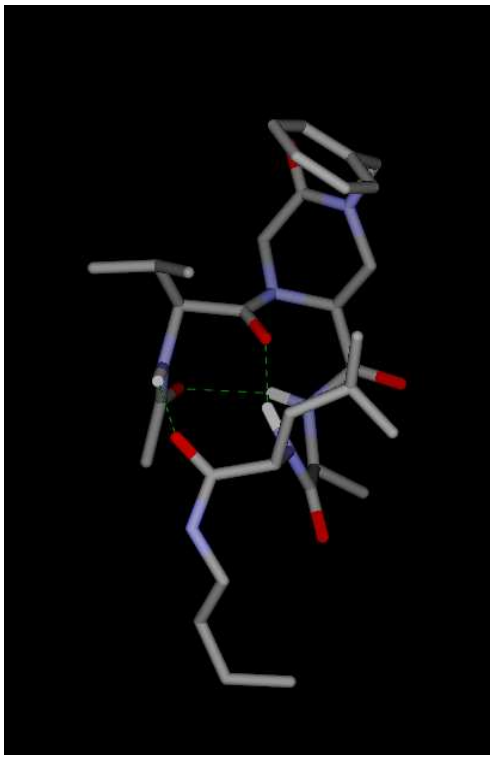
N-H	δ (ppm)[a]	ΔδNH/ΔT (ppb/K)[b]
H_1	6.08	-6,00
H_2	6,78	-2.90
H_3	7,80	-4,60
H_4	6,50	-3.40

Table IV-8 [a] Complete proton resonance assignments were made with the aid of COSY experiments; [b] The temperature coefficients were determined between 238 and 298 K (where a linear dependence was observed);.and at a concentration of 2mM (no significant aggregation).

Here again the informations collected out of the analysis of temperature dependence coefficients and ^1H NMR spectrum were not conclusive. Temperature coefficient $\Delta\delta NH/\Delta T$ of H_2 was lower (in absolute value) to $\Delta\delta NH/\Delta T$ H_3 but at the same time, H_3 was appearing at higher chemical shifts than H_2 so that we do not have any definitive conclusion about the secondary structure of our tetrapeptide.

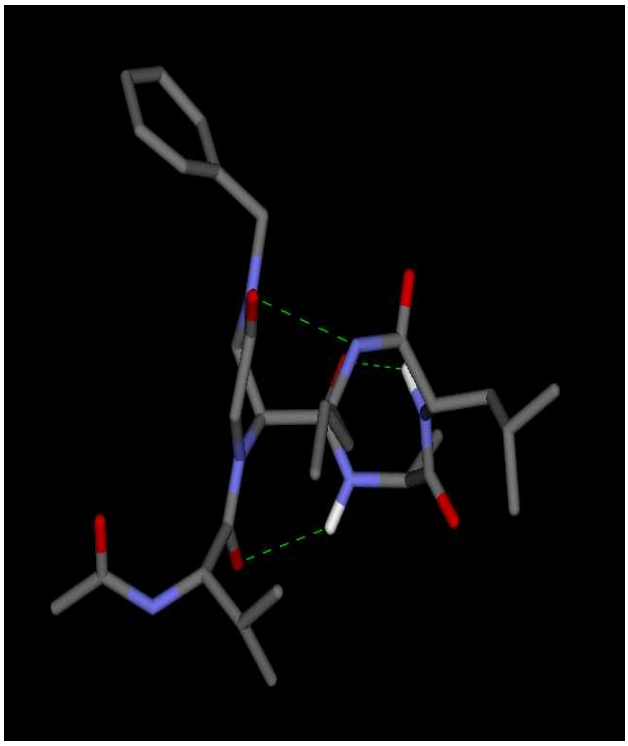
To get further informations on the possible conformations of our tetrapeptide, molecular dynamics in vacuo were performed at the University of Chateney Malabry by Cyril Bauvais. In these complementary studies, molecular dynamics experiments were performed at to different temperatures revealing here again two possible structures for this tetrapeptide. Analyses of the conformer of lower energy at 300K showed a preferred β-turn structure whereas at high temperature, 700K, the conformer of lower energy showed a preferred γ-turn structure.

MD studies at 300 K:



β-turn

MD studies at 700 K:



γ-turn

Figure IV-27: molecular dynamics calculations were performed at two different temperatures in vacuo with the Tripos force field

These conformationnal studies based essentially on NMR experiments afforded us some evidences on the propensity of our scaffold to initiate turn secondary structures, unfortunately racemisation problems of our scaffold during peptide coupling studies led to complicated separation of diastereoisomers that gave us both studied compounds in mg scale and did not allow us to perform complete conformational studies. Nevertheless, analyses of both synthesized oligopeptides seemed to show that Bn-Pca has a role on tetrapeptides folding, but

we are until now not able to conclude for the most populated γ- or β-turn conformation adopted in the case of our benzyl substituted Pca (Figure.IV-11).

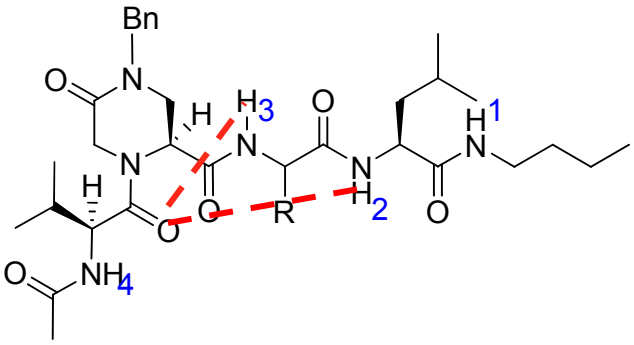


Figure IV-28

IV-4.2 Tetrapeptides containing non substituted Pca:

As we mentioned above, we also prepared both enantiomers of the oxopiperazinone carboxylic acid without the benzyl group onto the intracyclic amide nitrogen and, for still not completely understood reasons, this compound was not suffering from the epimerization problems we encountered with the *N*-benzyl derivative. Both enantiomers of the Pca were introduced in the same tetrapeptide sequence described above. Thus Boc-Val-(*R*)Pca-Gly-LeuCOOMe **26** and Boc-Val-(*S*)Pca-Gly-LeuCOOMe **27** were synthesized and conformational studies are now in course in our laboratory in Como. But first NMR studies showed already structural features about these two compounds.

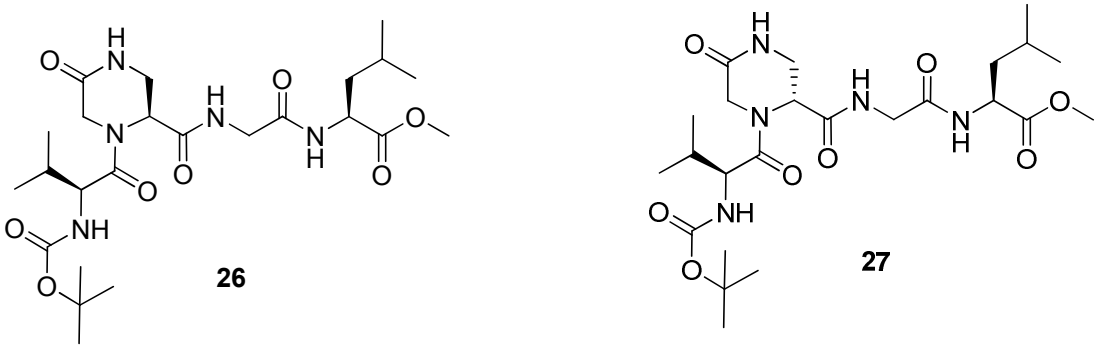


Figure IV-29

The tetrapeptide containing the (L) piperazinone ring showed the presence of two conformers in solution, by NMR studies in $CDCL_{3,}$ whereas a single conformer was detectable in the case of the tetrapeptide containing the (D) scaffold. This preliminary results could be convenient with the presence of a stable turn structure induced by our (D)scaffold, and structural investigation are still in course in our laboratory to determine the structural preference of the ketopiperazine ring.

Conclusions

To conclude with these work performed in two years in Como and followed the third one in Regensburg, we manage to set up the reaction conditions for the synthesis of two oxo-piperazine carboxylic acid derivatives. The first one substituted in its amide position by a benzyl group showed a high tendency to racemisation during peptide formation, in spite of our numerous studies to understand this phenomenon we were not able to set up suitable coupling conditions for its insertion in peptides. Nevertheless, the studies of purified tetrapeptides containing this scaffold was performed showing a certain tendency for turn shape.

The second synthetic route afforded us the unsubstituted Pca in few steps and good yields. In addition, this scaffold did not demonstrate any issues toward racemisation, neither during the synthesis, nor during the coupling procedure. And it seems from last studies currently performed in our laboratory that this scaffold could act as a turn nucleator.

V - EXPERIMENTAL DATA:

V-1 Materials and methods:

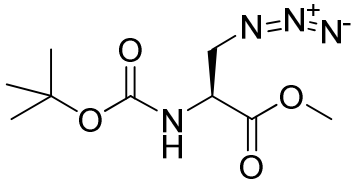
All manipulations requiring anhydrous conditions were carried out inflame-dried glassware, with magnetic stirring and under a nitrogen atmosphere. All commercially available reagents were used as received. Anhydrous solvents were purchased from commercial sources and withdrawn from the container by syringe, under a slight positive pressure of nitrogen. Reactions were monitored by analytical thin layer chromatography using 0.25 mm pre-coated silica gel glass plates (DURASIL-25 UV_{254}) and compounds visualized using UV fluorescence, aqueous potassium permanganate or ninhydrin. Flash column chromatography was performed according to the method of Still and co-workers[165] using Chromagel 60 ACC (40-63 μm) silica gel.

Melting points were obtained in an open capillary apparatus and are uncorrected. Proton NMR spectra were recorded on a spectrometer operating at 400.16 MHz. Proton chemical shifts are reported in ppm (δ) with the solvent reference relative to tetramethylsilane (TMS) employed as the internal standard. The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal, dd = doublet of doublet. Carbon NMR spectra were recorded on a spectrometer operating at 100.63 MHz, with complete proton decoupling. Carbon chemical shifts are reported in ppm (δ) relative to TMS with the respective solvent resonance as the internal standard. Infrared spectra were recorded on a standard FT-IR and peaks are reported in cm^{-1}. Optical rotation values were measured on an automatic polarimeter with a 1 dm cell at the sodium D line and are given in units of 10^{-1} $deg.cm^2.g^{-1}$. Elemental analyses were performed using a Perkin Elmer 2400 Series II CHNS/O Analyzer. High resolution mass spectra (HRMS) were performed on a hybrid quadrupole time of flight mass spectrometer equipped with an ESI ion source. A Reserpine solution 100 pg/ml (about 100 count/s), 0.1% $HCOOH/CH_3CN$ 1:1 was used as reference compound (Lock Mass). FAB mass spectra were recorded using a glycerol matrix. HPLC analytic analyses were performed in direct or reverse phase on a Shimadzu instrument equipped with a UV detector.

V-2 Synthesis of (Bn)-Pca derivatives:

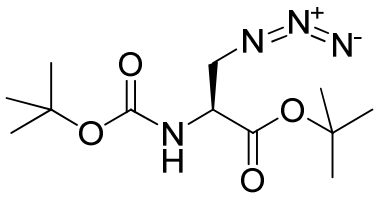
General procedure for the Mitsunobu reaction:

Under nitrogen and at room temperature a stirred solution of protected L-serine in toluene (10mL per mmol), was treated with PPh_3 (1.4 equiv.) and HN_3 (2 equiv.).
After complete dissolution of the reagents, the solution was cooled at 0°C and DIAD (1.4 equiv.) was added. After 2h stirring under 10°C the reaction medium was poured in the column and the product was purified by flash chromatography, giving the desired azide.



1a

3-Azido-2-tert-butoxycarbonylamino-propionic acid methyl ester 1a:
Compound **1a** was prepared from the Boc (L) serine Methyl ester (333mg, 1,52mmol) and purified by flash column chromatography (PE/EA, 8/2) to give the corresponding azide as a transparent oil (285mg, 81%), R_f = 0,45 (PE/EA, 8/2). $[\alpha]^{20}_D$ = 43.32 (c=1, $CHCl_3$). ^{1}H NMR (400MHz, $CDCl_3$): δ=5.36 (br, 1H), 4.49(br, 1H), 3,82 (s, 3H), 3,74 (d, *J*=2.92 Hz, 2H), 1.48 (s, 9H) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=170.6, 155.4, 80.8, 53.9, 53.2, 53.0, 28.6 ppm. IR in $CHCl_3$: 3366, 2109, 1749, 1716. MS (ESI^+): *m/z*= 267,0 [MH^+]



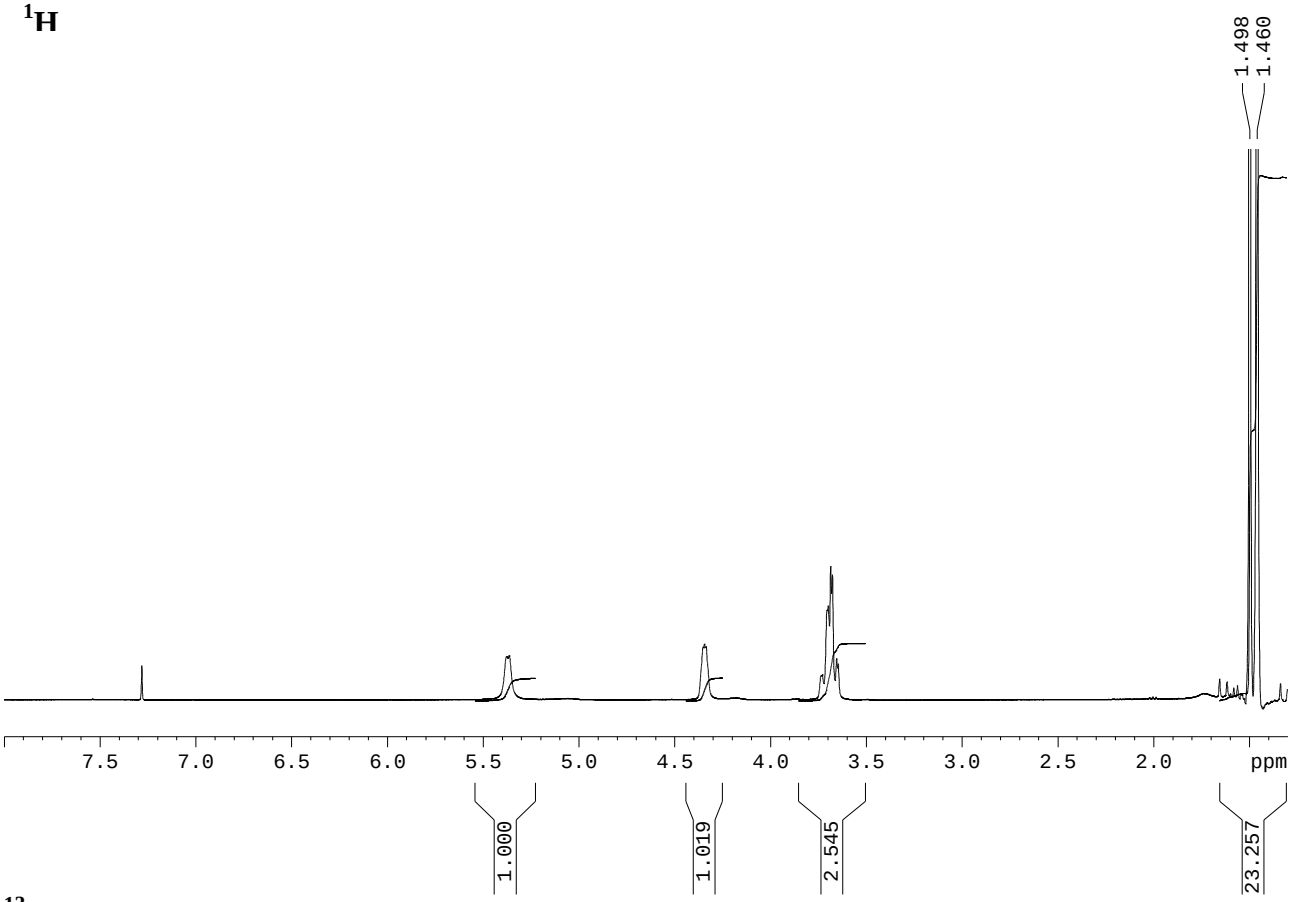
1b

3-Azido-2-tert-butoxycarbonylamino-propionic acid tertbutyl ester (1b):

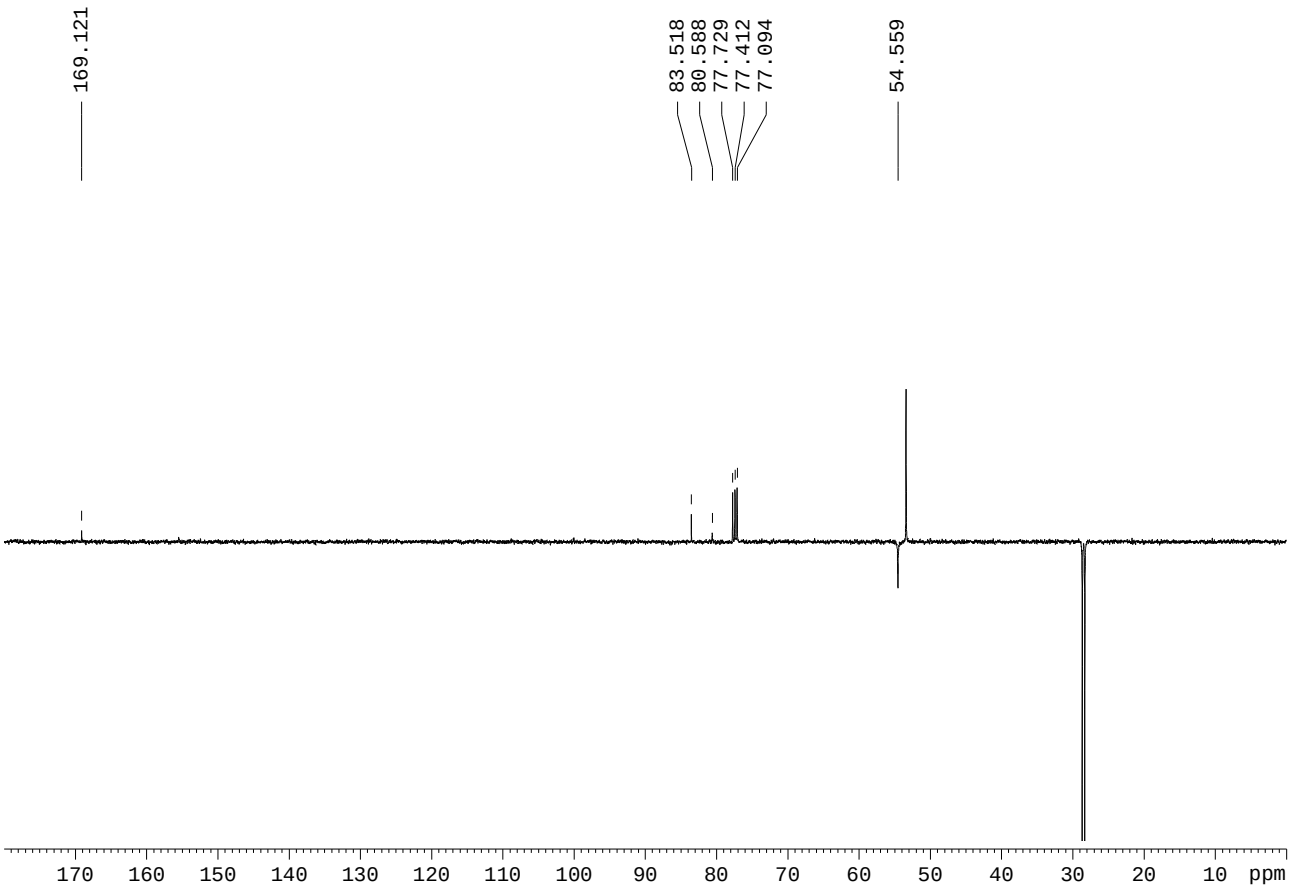
The compound **1b** was prepared from the L serine Boc and tBu (1g, 3,83mmol)and purified by flash column chromatography (PE/EA, 85/15) to give a transparent oil (1.06g, 97%), R_f = 0,41 (PE/EA, 8/2). $[\alpha]^{20}_D$ = 18,01 (c=0,99, $CHCl_3$). ^{1}H NMR (400MHz, $CDCl_3$): δ=5.37 (d, *J*=5.52 Hz, 1H), 4.34(t, *J*=3.19 Hz, 1H), 3,71 (dd, *J*=12.48 Hz, *J*=2.65 Hz 1H)), 3,66 (dd, *J*=12.29 Hz, *J*=3.44 Hz 1H), 1.49 (s, 9H)), 1.46 (s, 9H) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=169.1, 155.4, 83.5, 80.5, 54.5, 53.4, 28.6, 28.3 ppm. IR in $CHCl_3$: 3368, 2098, 1728, 2523.$C_{12}H_{22}N_4O_2$ (286.33): MS (ESI^+): *m/z*= 309,15 [MH^+]

(*S*)-3-azido-2-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester (3b)

^{1}H



^{13}C



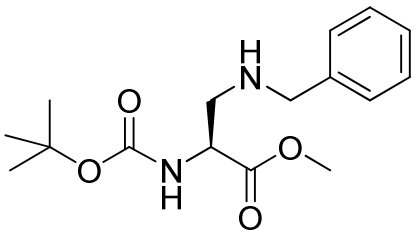
General procedure for the Aza-Wittig reaction:

Under nitrogen and at room temperature a stirred solution of azide **1** in dichloromethane (5mL per mmol), was treated with a solution of PMe_3 1M in THF (2 equiv). After 45 min stirring, freshly distilled benzaldehyde (1.1 equiv) was added and the solution was stirred 1h more. The solvent was then removed using an aqueous pump and a intermediary Dewar.

Reduction of the imine:

Procedure A:
The obtained yellow paste was then dissolved in methanol (5mL per mmol). To this new medium $NaBH_4$ (O,5 equiv) was slowly added over a period of 30min. The reaction was quenched with a saturated solution of $NaHCO_3$ and extracted with DCM 5 times. The organic phase was then evaporated and dried over Na_2SO_4 before purification by flash column chromatography.

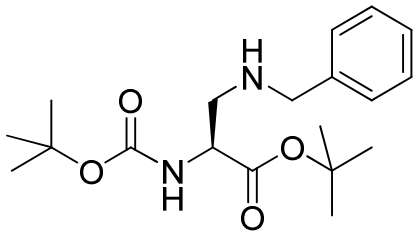
Procedure B:
The obtained yellow paste was then dissolved in methanol (5mL per mmol), Pd/C (10%, 100mg per mmol) was added and the solution was stirred overnight under one atmosphere of H_2. Pd/C was removed by filtration over celite and the pure product was obtained after purification by flash column chromatography.



2a

3-Benzylamino-2-tert-butoxycarbonyl amino-propionic acid methyl ester 2a:

This compound was prepared from the azide **1a** (174mg, 0.71mmol) following procedure A and purified by flash column chromatography (PE/EA, 6/4) to give a transparent oil (151mg, 68%), R_f =0.46 (PE/EA, 6/4). ^{1}H NMR (400MHz, $CDCl_3$): δ=7.34-7,23 (m, 5H), 5.48(br, 1H), 4.41 (br, 1H), 3.78 (m, 1H), 3.74 (s, 3H), 2.98 (dd, *J*=13.36 Hz, 2H), 1.46 (s, 9H), ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=172.7, 155.9, 140.1, 128.8, 128.5, 127.6, 80.3, 53.9, 53.7, 52.7, 50.3, 28.7 ppm. IR in $CHCl_3$: 3348, 2978, 1730, 1714.



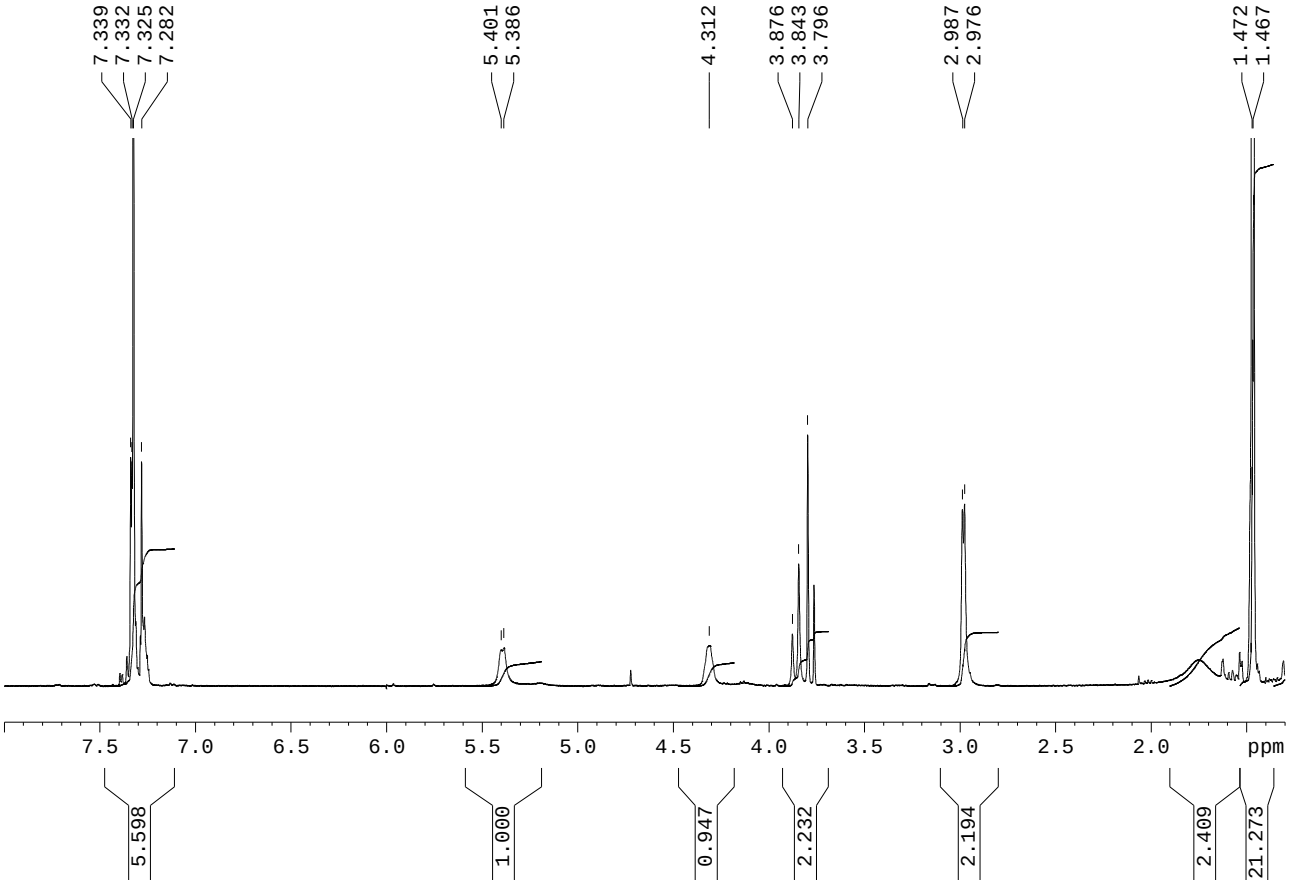
2b

3-Benzylamino-2-tert-butoxycarbonyl amino-propionic acid tert-butyl ester 2b :

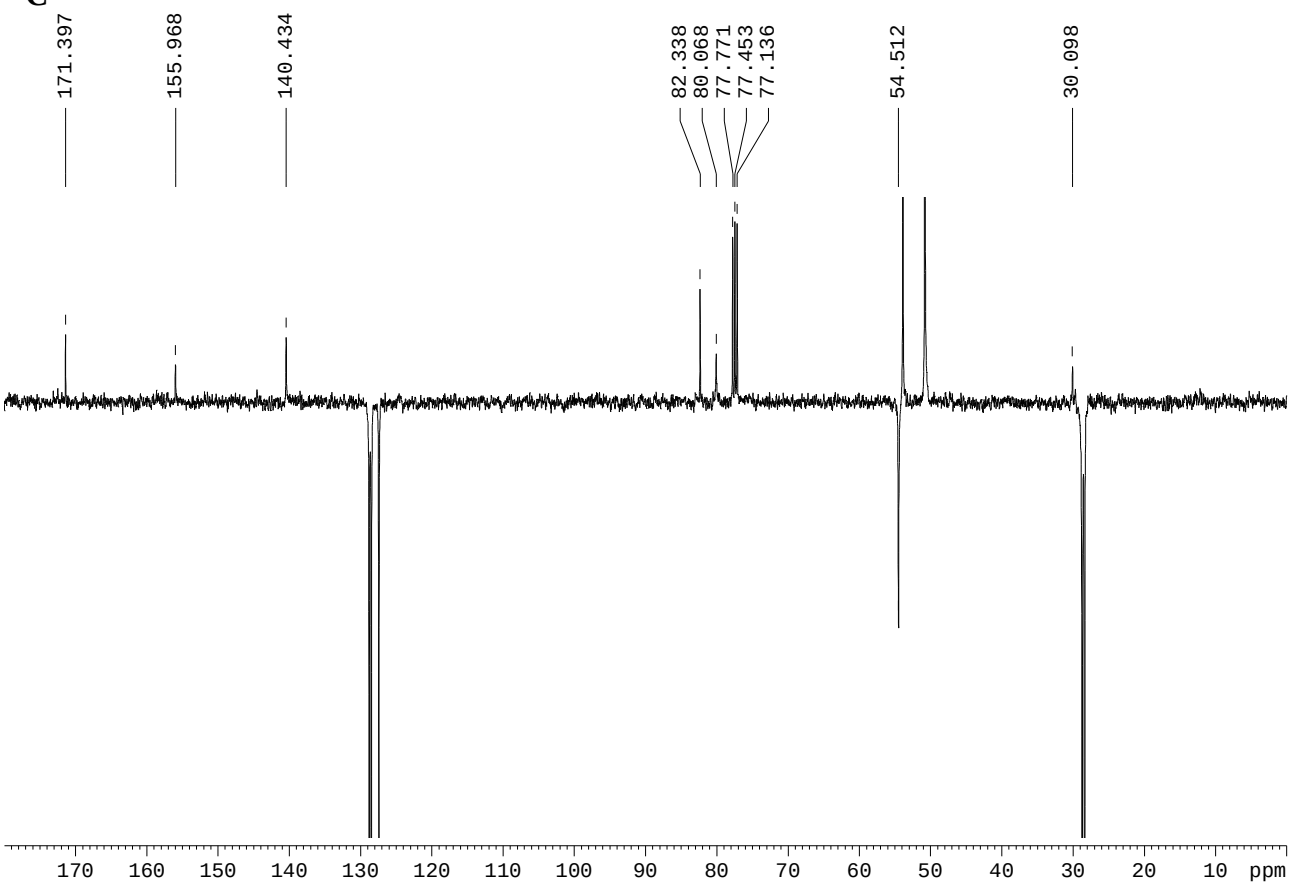
This compound was prepared from the azide **1b** (1.06g, 3.71mmol) following procedure B and purified by flash column chromatography (PE/EA, 85/15) to give **2b** as a transparent oil (1.29g, 99% over two steps), R_f =0.38 (nHex/EA, 7/3). $[\alpha]^{20}_D$ = 8.82 (c=0.46, $CHCl_3$). ^{1}H NMR (400MHz, $CDCl_3$): δ=7.35-7,25 (m, 5H), 5.39(d, *J*=5.60 Hz, 1H), 4,31 (br, 1H), 3,86 (d(AB), *J*=13.30 Hz, 1H), 3,77 (d, *J*=13.22 Hz, 1H), 2.98 (d, J=4.42, 2H)), 1.47 (s, 9H), 1.46 (s, 9H) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=171.3, 155.9, 140.3,128.7, 128.4, 127.4, 82.3, 80.0, 54.5, 53.8, 50.8, 28.7, 28.4 ppm. $C_{19}H_{30}N_2O_4$ (350.46): calcd. C 65.12, H 8.63, N 7.99, O 18.26; found C, 65.29, H 8.68, N 7.67. IR in $CHCl_3$: 3348, 2978, 2932, 1716.

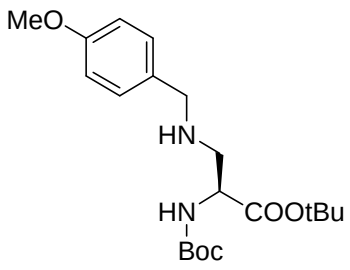
(*S*)-3-benzylamino-2-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester (2b)

^{1}H



^{13}C



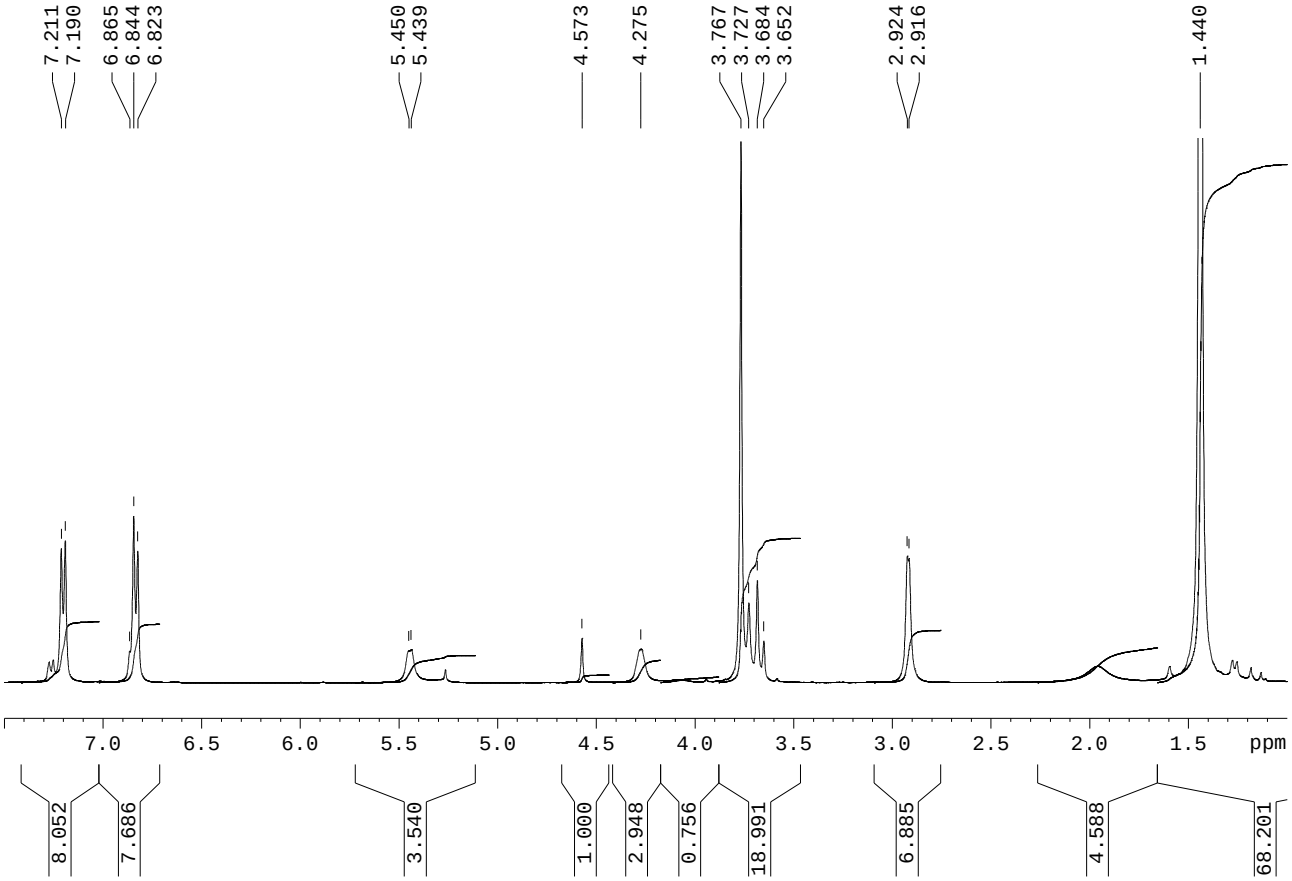


2c

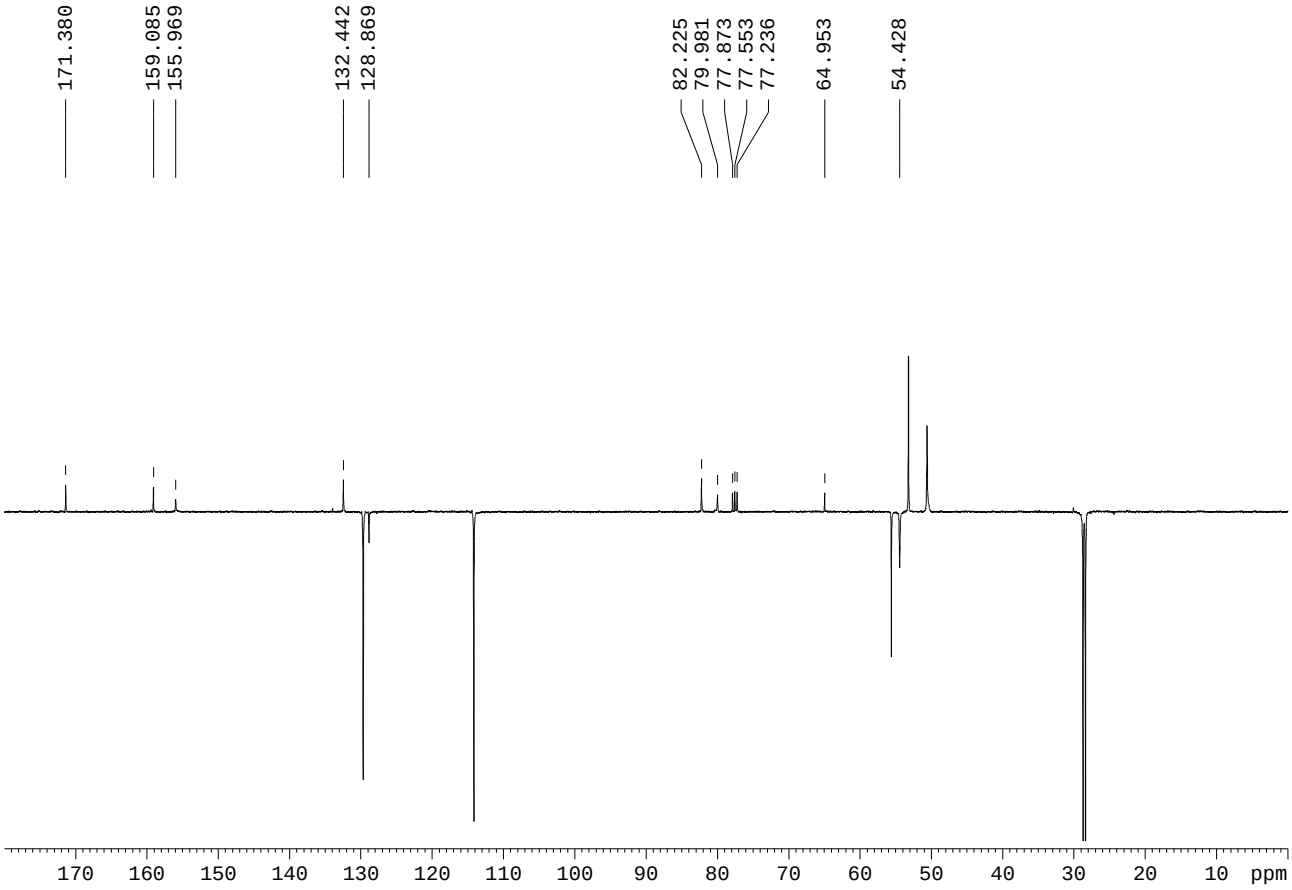
(*S*)p-metoxy-3-benzylamino-2-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester (2c). Azide (595 mg, 2,08 mmol) was dissolved in dichloromethane (9 mL), under nitrogen atmosphere and at room temperature, and treated with PMe_3 (4,16 mL of 1 M solution in THF, 4,16 mmol, 2 equiv). The resulting mixture was stirred at room temperature for 45 min and freshly distilled p-anisaldehyde (3.90mL, 3.71 mmol, 1.1 equiv) was added and the resulting solution was stirred for additional 60 min. The solvent was then removed under reduced pressure, and the residue was dissolved in methanol (18,5 mL). Palladium on charkoal (10% Pd/C, 100 mg) was then added and the resulting solution was filled with H_2 by three vacuum/H_2 cycles. The reaction mixture was then stirred under a hydrogen atmosphere overnight. Pd was removed by filtration over a pad of celite and the filtrate evaporated under reduced pressure. The residue was purified by flash chromatography (Pet. Ether/EtOAc, 6:4) to give **2c** as a transparent oil (500 mg 63% over two steps). Rf =0.46 (Pet. Et./EtOAc, 6/4). R ($CHCl_3$): 2926, 1718, 1460, 1367,1155,1037 .$C_{20}H_{32}N_2O_5$ (350.46): calcd. C 63.14, H 8.48, N 7.36; found C 63.98, H 7.22, N 6.69 . ^{1}H NMR (400MHz, $CDCl_3$): δ=7.2 (d, *J*=8 Hz, 2H), δ=6.83 (d, *J*=8 Hz, 2H) , 5.44 (br, 1H), 4.27 (br, 1H), 3.77 (s, 3H), 3.74 (d, *J*=13.2 Hz 1H), 3.66 (d, *J*=13.2 Hz, 1H), 2.91 (d, *J*=3.6 Hz, 2H), 1.44 (s, 9H), ppm. ^{13}C NMR (400MHz, CDCl3): δ=171.4, 159.0, 156.0, 132.6, 129.7, 128.7, 114.0, 82.1, 80.1, 65.0, 55.7, 54.5, 53.2, 50.7, 28.7, 28.3ppm.

(*S*)p-metoxy-3-benzylamino-2-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester (2c).

^{1}H



^{13}C



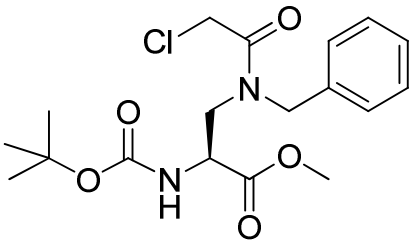
Acylation procedures:

Procedure A:

At room temperature the benzylated derivative was diluted in a 1 to 1 mixture of ethyl acetate (2mL per mmol) and a saturated solution of $NaHCO_3$ (2mL per mmol) and stirred for 24h at room temperature. The two phases were then separated and the aqueous phase was extracted with ethyl acetate 5 times. The combined organic phases were dried over Na_2SO_4, and concentrated in vacuo, to give the desired product without further purification.

Procedure B:

Chloroacetic acid 2eq, and DCC 2eq, were stirred for 1h in DCM (2,5mL per mmol). To these solution the benzyl derivative was added, and these final mixture was reacted overnight.
The solvent was then removed under reduced pressure, and the pure product was obtained after flash column chromatography.

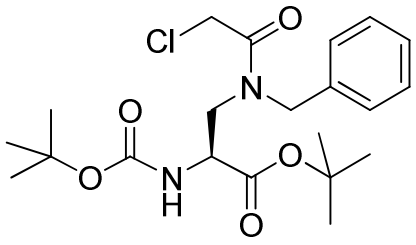


3a

3-[Benzyl-(2-chloro-acetyl)-amino]-2-tert-butoxycarbonylamino-propionic acid methyl ester (3a) :

This compound was prepared from the benzylated derivative (336mg, 1,1mmol) following the procedure A and purified by flash column chromatography (PE/EA, 7/3) to give a transparent oil (388mg, 92%), R_f =0,49(PE/EA, 7/3). NMR analysis gave us evidence of the presence of the product in a two rotamers form A: major rotamer, B minor rotamer. ^{1}H NMR (400MHz, $CDCl_3$): δ=7.40-7.18(m, 5H), 5.48(d, *J*=7.17 Hz, $1H_A$),5.30 (br, $1H_B$), 4.67 (m, $2H_A$), 4.55 (m, $2H_B$), 4.22 (s, $2H_B$), 4.07 (s, $2H_A$), 3.97 (m,1 H_A), 3.78 (s, $3H_B$), 3.76(s, $3H_A$), 3.69(dd, $2H_B$), 3,58 (dd, J=4.72Hz, J=14.20 $1H_A$), 1.59 (s, 9H), 1.45 (s, 9H) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=171.3, 168.7, 135.8, 129.6, 129.1, 128.5, 128.3, 128.1, 126.7, 116.5, 53.3 53.1, 52.6, 52.2, 48.9, 47.9, 41.5, 41.3, 30.028.6 ppm.

IR in CH_2Cl_2: 3342, 3032, 2978, 1749, 1712, 1657



3b

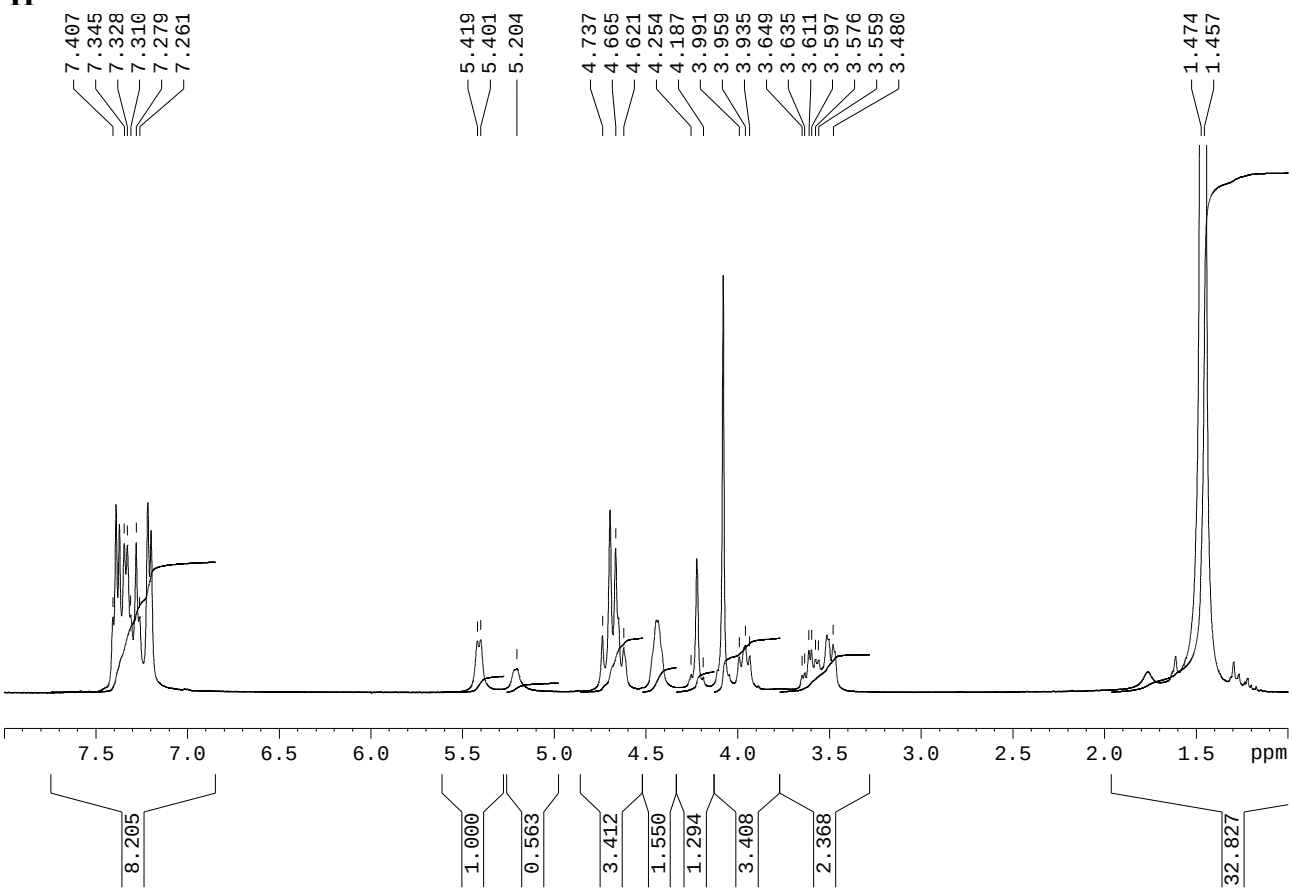
3-[Benzyl-(2-chloro-acetyl)-amino]-2-tert-butoxycarbonylamino-propionic acid tertbutyl ester (3b):

This compound was prepared from benzyl derivative **2a** (1,512g, 4,32mmol) following procedure B and purified by flash column chromatography (PE/EA, 7/3) to give **3a** as a white solid (1,7g, 92%), R_f =0.31(nHex/EA, 8/2). mp= 110-112°C $[\alpha]^{20}_D$ =4.7 (c=0.83, $CHCl_3$). NMR analysis gave us evidence of the presence of the product in a two rotamers form A, B in a 3 to 1 proportion.^{1}H NMR (400MHz, $CDCl_3$): A(67%) and B(33%) δ=7.40-7.19(m, 5H), 5.41(d, *J*=7.68 Hz, $1H_A$),5.20 (bs, $1H_B$), 4.73-4.62 (m, $2H_{A\&B}$), 4.43 (d, *J*=3.7Hz, $2H_{A\&B}$), 4.22 (s, $2H_B$) 4.07 (s, $2H_A$), 3.97 (dd, *J*= 10.1Hz *J*= 13.5, $2H_B$), 3.59 (dd, *J*= 14.3Hz, *J*= 5.7Hz $1H_A$), 3.49 (dd, *J*=14,0Hz, *J*=3.8Hz $1H_A$), 1.47 (s, 9H), 1.45 (s 1.45) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=169.9, 168.4 (C_A), 167.6 (C_B), 156.0, 136.6 (C_B), 135.9 (C_A), 129.5, 129.1, 128.5, 128.4, 128.0, 126.8, 34.0, 83.0, 80.2, 53.3, 51.9, 49.1 (C_B), 48.8 (C_A), 47.8, 41.8(C_A), 41.5 (C_B), 28.7, 28.3 ppm. $C_{12}H_{22}N_4O_2$ (426.94): calcd. C 59.08, H 7.32, N 6.56, O 18.74; found C59,12, H7,24, N6,49.

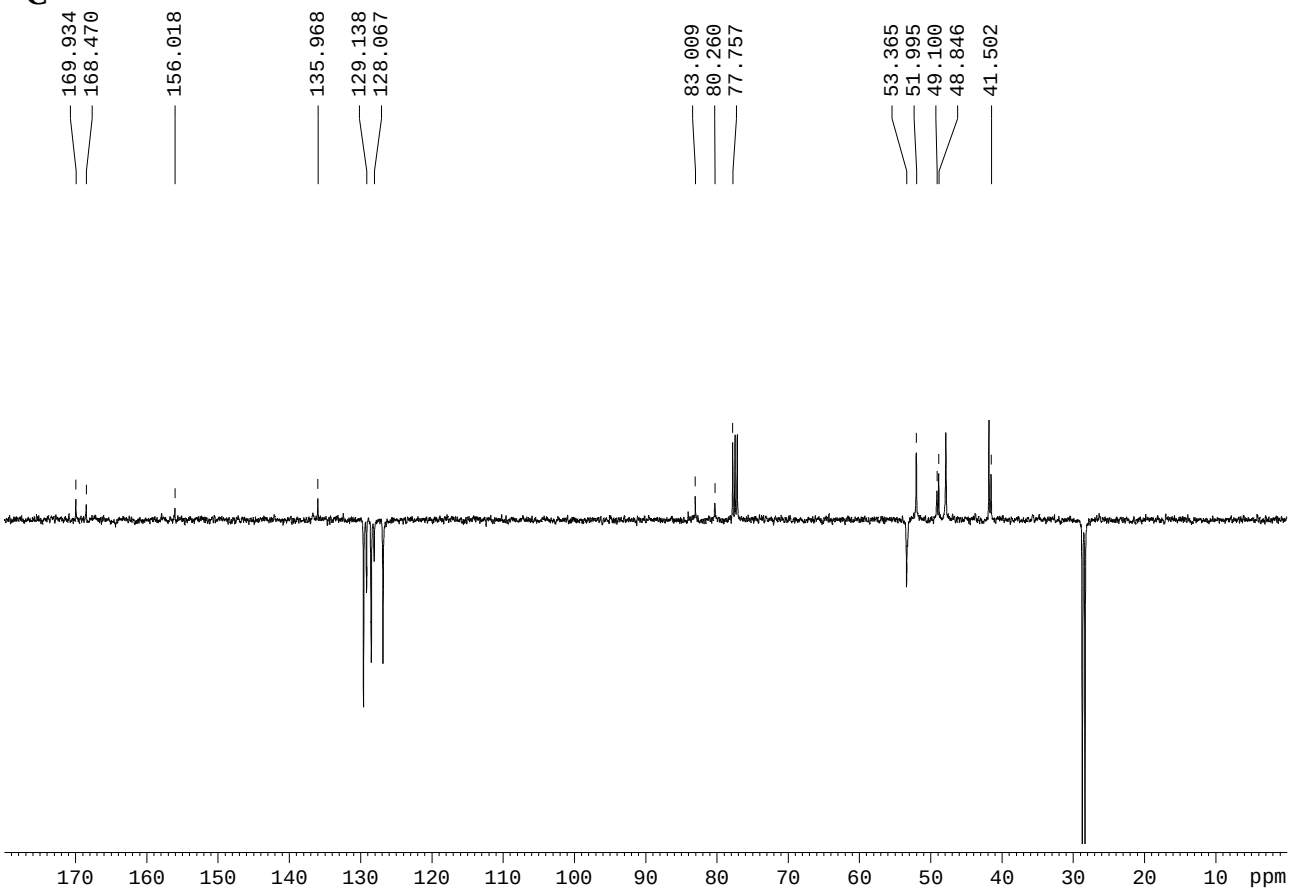
IR ($CHCl_3$):3395, 2924, 2854, 1711, 1655

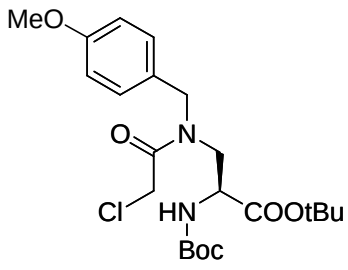
3-[Benzyl-(2-chloro-acetyl)-amino]-2-tert-butoxycarbonylamino-propionic acid tertbutyl ester (3b).

^{1}H



^{13}C





3c

3-[p-metoxy-benzyl-(2-chloro-acetyl)-amino]-2-tert-butoxycarbonylamino-propionic acid tertbutyl ester (3c):

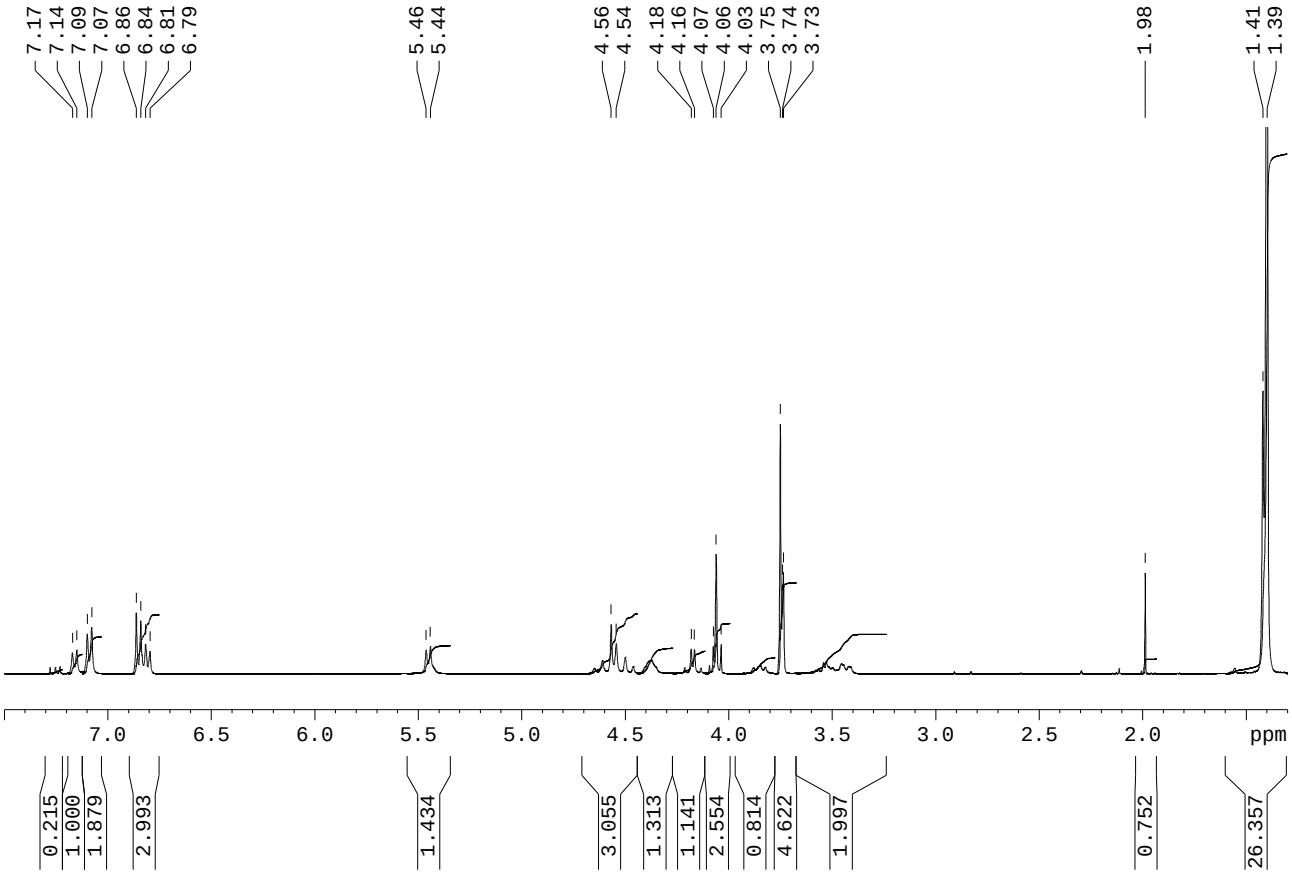
DCC (372mg, 1.409mmol, 1.2equiv) and chloroacetic acid (220mg, 2.34mmol, 2equiv) where dissolved in dichloromethane (5 mL) and stirred for 1h at room temperature. To this solution Boc-(L)-serine amino p-metoxy-benzyl *tert*-butyl ester (447mg, 1.175mmol) previously dissolved in dichloromethane (5mL), was added. The corresponding solution was reacted overnight, the solvent was then removed under reduced pressure and the obtained residue was purified by flash chromatography (petroleum ether/EtOAc, 7:3) to give **3c** as a white solid (480mg, 93%), R_f =0.31(nHex/EA, 8/2). mp= 123-125°C $[\alpha]^{20}_D$ =7.75 (c=0.57, $CHCl_3$).

$C_{12}H_{22}N_4O_2$ (426.94): calcd. C 57.07, H 6.84, N 6.34; found C 57.63, H 7.41, N 5.78. ^{1}H NMR (400MHz, $CDCl_3$):A(67%) and B(33%) δ=7.12 (d, *J*=8 Hz, 2 H), 6.85(d, *J*= 8, 2H), 5.41(d, *J*=7.68 Hz, $1H_A$),5.20 (br, $1H_B$), 4.73-4.62 (m, $2H_{A\&B}$), 4.43 (d, *J*=3.7Hz, $2H_{A\&B}$), 4.22 (s, $2H_B$) 4.07 (s, $2H_A$), 3.97 (dd, *J*= 10.1Hz *J*= 13.5, $2H_B$), 3.80 (d, *J*= 14.2 Hz , 3H), 3.59 (dd, *J*= 14.3Hz, *J*= 5.7Hz $1H_A$), 3.49 (dd, *J*=14,0Hz, *J*=3.8Hz $1H_A$), 1.47 (s, 9H), 1.45 (s 1.45) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ= 169.9, 168.3, 129.9, 128.3, 127.6, 55.5, 53.1, 51.8, 48.1, 47.4, 41.5, 28.6 ppm. $C_{12}H_{22}N_4O_2$ (426.94): calcd. C 59.08, H 7.32, N 6.56, O 18.74; found C59,12, H7,24, N6,49.

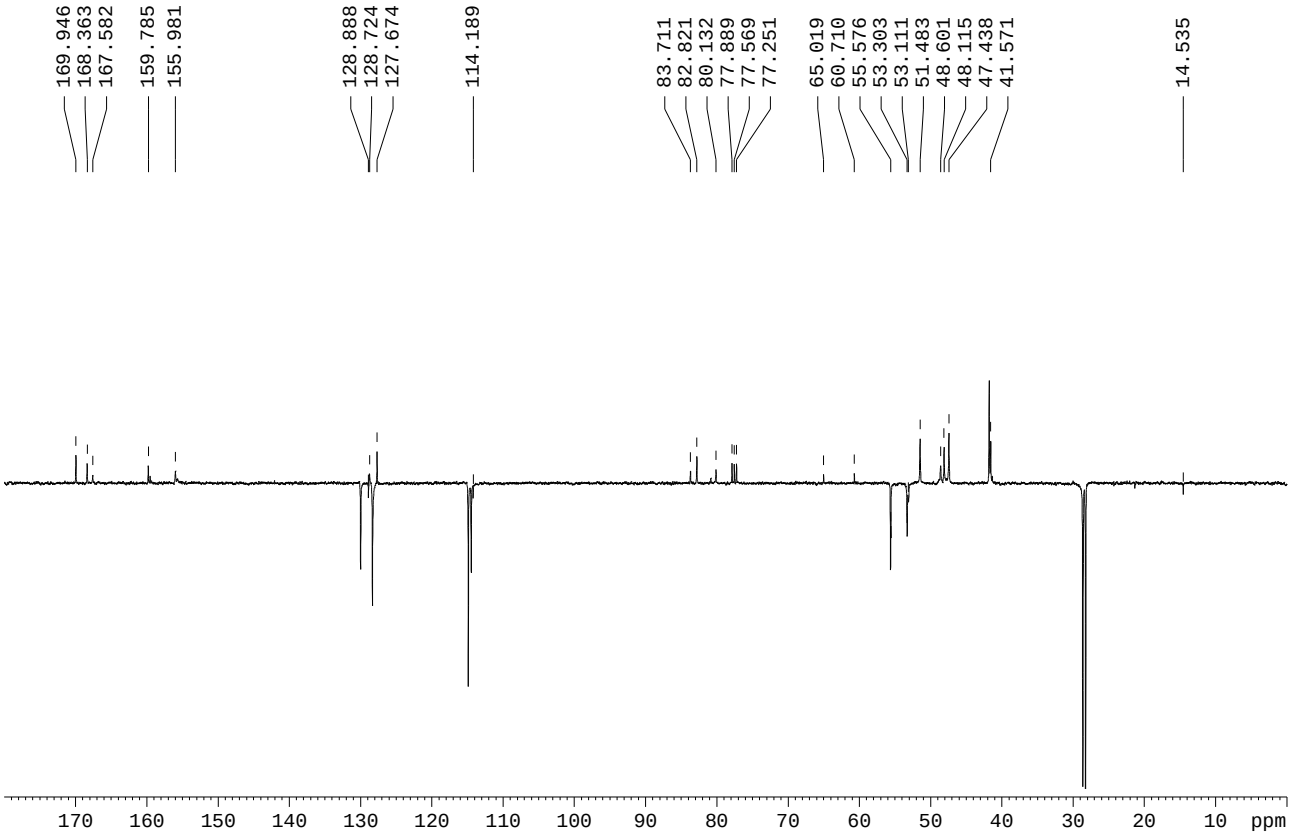
IR ($CHCl_3$) :3350, 2974, 1658, 1460, 1249, 1155, 1033

3-[p-metoxy-benzyl-(2-chloro-acetyl)-amino]-2-tert-butoxycarbonylamino-propionic acid tertbutyl ester (3c)

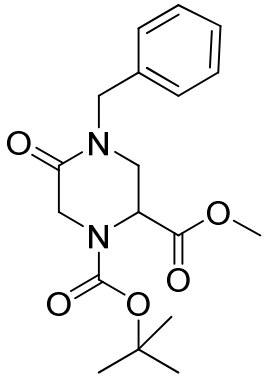
^{1}H



^{13}C



Cyclisation procedures:

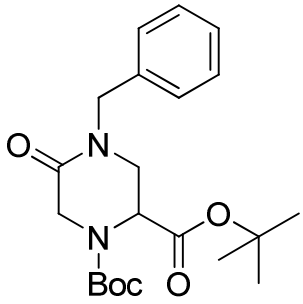


4a

4-Benzyl-5-oxo-piperazine-1,2-dicarboxylic 1-tert-butyl ester 2-methyl ester 4a :

To a stirred solution of the acylated derivative **3a** (380mg, 0.98mmol) in DMF (4,9mL), Cs_2CO_3 (638.60mg, 1.96mmol) was added at 0°C. The solution was stirred over night at room temperature, the solution was then diluted with ethyl acetate 10ml and washed twice with 5mL of a 1M solution of $KHSO_4$ in water and brine. The solvent was then dried over Na2SO4 and removed under reduced pressure to give a **4a** white solid (235mg, 69%),mp= 126°C, R_f = 0,42 (nHex/EA, 7/3). $[\alpha]^{20}_D$ =0 (c=1, $CHCl_3$). ^{1}H NMR (400MHz, DMSO, 60°C): δ=7.36-7.21(m, 5H), 4.72(d, *J*=14.7 Hz, 1H),4.7 (s, 1H), 4.29 (d, *J*=14.7 Hz, 1H), 4.08 (d, *J*=17.38 Hz, 1H), 3.95 (d, *J*=17.38 Hz 1H), 3.74 (dd, J_1=13,24 Hz, J_2=4,63, 1H), 3.54 (dd, J_1=13,19 Hz, J_2=2,67, 1H), 3.51(s, 3H), 1.42 (s, 9H) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=170.1, 165.6, 154.8, 136.2, 129.1, 128.8, 128.3, 82.0, 53.9, 52.9, 52.3, 50.1, 47.0, 46.9, 46.4, 28.6 ppm. $C_{18}H_{24}N_2O_5$ (348.40): calcd. C 62.05%, H 6.94%, N 8.04%; found C61,81%, H6,93%, N7,86%.

IR =2978, 1747, 1703, 1660

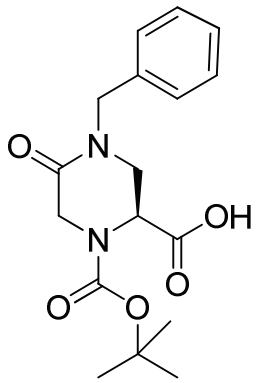


4b

4-Benzyl-5-oxo-piperazine-1,2-dicarboxylic di-tert-butyl ester 4b :

To a solution of the **3b** derivative (90mg, 0.21mmol) in dry THF (3mL), tBuOK in solution in THF (21Oµ L, 0,21mmol) was added at 0°C under nitrogen. The reaction was carried out for 2h at room temperature, and quenched with an aqueous solution 1M $KHSO_4$. The solvent was evaporated under reduced pressure, and the residue was partitioned between dichloromethane 20mL and aqueous solution of $KHSO_4$ 2mL. The organic layer was separated and washed twice with water, and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (PE/EA, 7/3) to give a white solid (80mg, 97%), R_f =0,53 (PE/EA, 6/4).mp:102°C NMR analysis gave us evidence of the presence of the product in a two rotamers form A 66%, B 34%^{1}H NMR (600MHz, $CDCl_3$):A(%) δ=7.30-7.24(m, 5H), 4.78(d, *J*=3Hz, $1H_A$), 4.66 (d, *J*=14.4Hz, $1H_A$), 4.60 (d, *J*=15.0Hz $1H_B$), 4.56 (br, $1H_B$), 4.55 (d, *J*=14.4Hz $1H_B$), 4.47 (d, *J*=14.4Hz, $1H_A$), 4.25 (d, *J*=18.6Hz $1H_A$), 4.22 (d, *J*=19.8Hz, $1H_B$), 4.08 (d, *J*=18.0Hz $1H_A$), .08 (d, *J*=18.6Hz $1H_B$), 3.65 (d, *J*=11.4Hz $1H_A$), 3,59 (d, *J*=12.6Hz $1H_B$), 3,54 (m, $1H_B$, $1H_A$), 3.52 (dd, *J*= 4.5Hz, *J*=12.0 Hz $1H_A$), 1.47 (s, $9H_A$), 1.43 (s, $9H_B$), 1.35 (s, $9H_B$), 1.32 (s, $9H_A$) ppm. ^{13}C NMR (600MHz, $CDCl_3$): δ=168.5, 168.1, 165.6, 165.2, 154.4, 135.9, 135.8, 128.7, 128.5, 128.4, 128.1, 127.8, 82.86, 82.81, 81.2, 81.07, 53.9, 52.3, 50.0, 49.9, 46.8, 46.7, 46.5, 46.0, 28.2, 27.7ppm. EI-MS ($C_{21}H_{30}N_2O_5$) 390.2155 (calcd) 390,2152. (obsd)

IR=2973, 2932, 1733, 1696, 1655cm^{-1}



5

4-Benzyl-5-oxo-piperazine-1,2-dicarboxylic acid 1-tertbuthyl ester (5) :

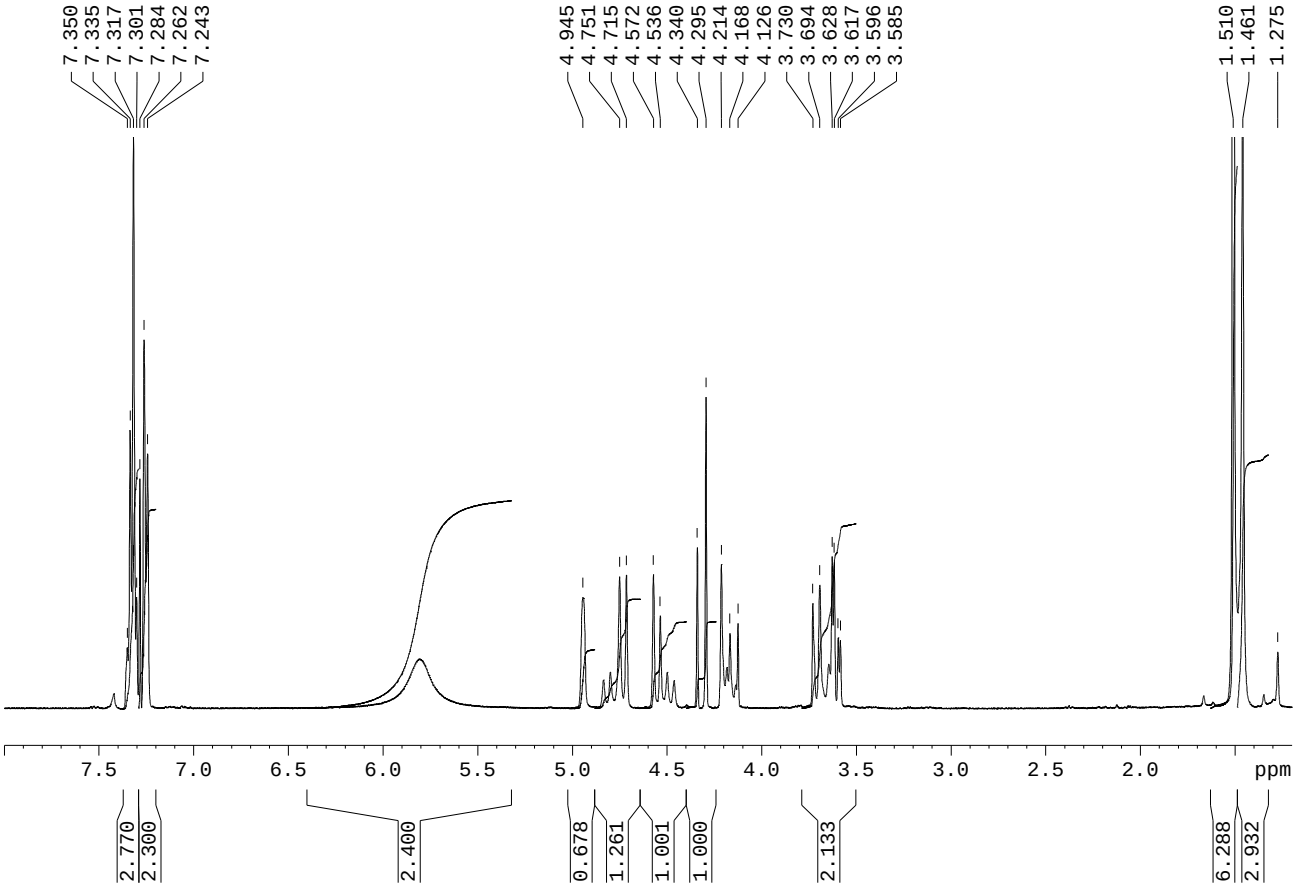
A solution of **3b** (422mg, 0.98mmol) in dichloromethane (3mL) and TFA (3mL) was stirred for 2.5h at RT. The solvent was then evaporated, methanol (5mL) was added and evaporated twice, to remove the excess of TFA, ether (5mL) was added and evaporated to give the unprotected intermediate under its TFA salt's form in quantitative yield. This salt was then dissolved in water (2mL) and an aqueous solution of NaOH 1M (2.94mmol, 2.94mL) and stirred over night at room temperature. Dioxane (4.94mL) was then added to the reaction medium followed by the addition of Boc anhydride (1.97mmol, 432mg) at O°C, this solution was stirred for 24 hours. The reaction was then diluted in water (10mL) and washed twice with ethyl acetate (5ml). The aqueous layer was cooled at 0°C, acidified to pH= 3 with a solution of $KHSO_4$ (1M), and extracted with ethyl acetate 5 times (5mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to afford the desired Boc protected piperazinone (209mg, 64%) as a white solid.

R_f = 0.2 (DCM/MeOH 9/1) $[\alpha]^{20}_D$ = -11.07(c=0.84, $CHCl_3$). m.p.174-176°C.

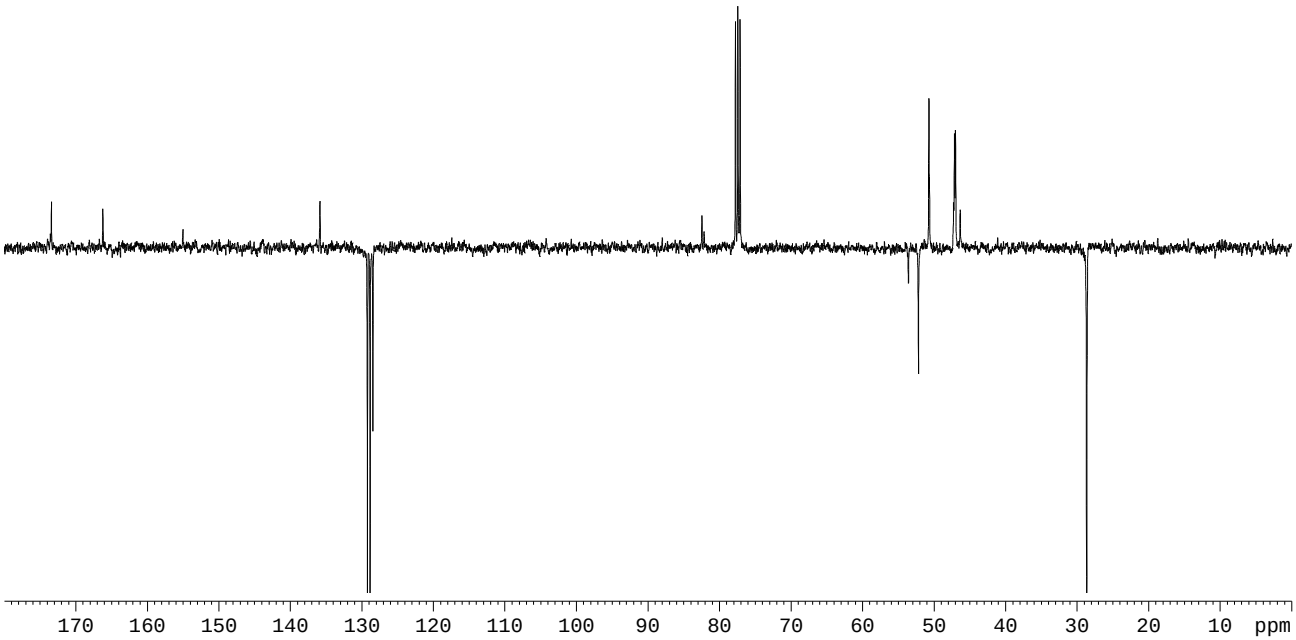
^{1}H NMR (400MHz, $CDCl_3$, 50°C): δ=7.35-7.25(m, 5H), 4.86(br, 1H), 4.72 (d, *J*=14.54 Hz, 1H), 4.73 (d, *J*=14.57 Hz, $1H_B$), 4.55 (d, *J*=14.54 Hz $1H_A$), 4.48 (d, *J*=14,77 Hz, $1H_B$), 4.31 (d, *J*=18.34 Hz, 1H), 4.19(d, *J*=18.24 Hz, 1H), 3.71 (d, *J*=14.50Hz, 1H), 3.60 (dd, J_1=12.86Hz, J_2=4.48Hz 1H),1.50 (s, $9H_A$), 1,46 (s, $9H_B$) ppm. ^{13}C NMR (100MHz, $CDCl_3$): δ=173.3, 166.2, 154.9, 135.7, 129.1, 128.8, 128.4, 82.4, 82.1,.53.5, 52.1, 50.9, 47.1, 46.9, 28.6 ppm. $C_{12}H_{22}N_4O_2$ (334.38): calcd. (+ 0.25%H_2O) C 60.25, H 6.71, N 8.27 found C60.52%, H6.85%, N7.98%. IR (CH_2Cl_2) =3343, 2924, 2854, 1462, 1377

4-Benzyl-5-oxo-piperazine-1,2-dicarboxylic acid 1-tertbuthyl ester (5a) :

^{1}H



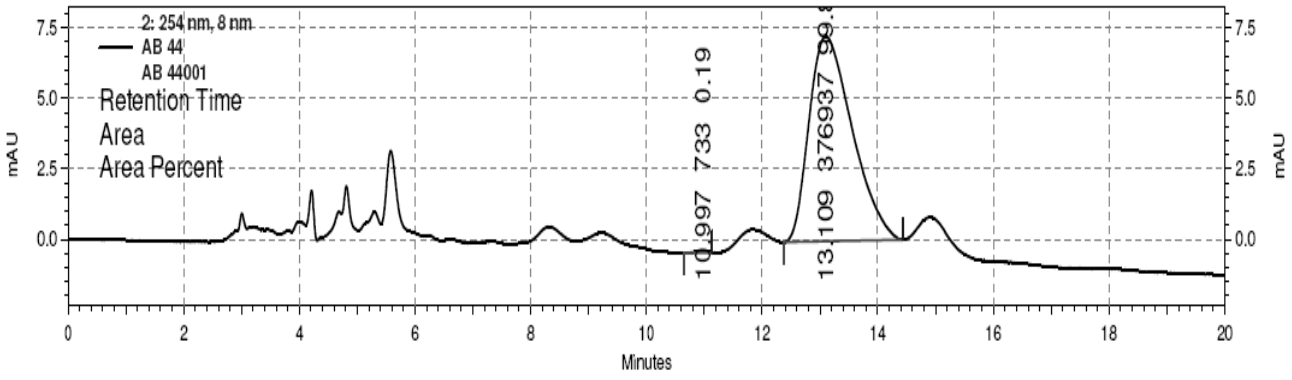
^{13}C



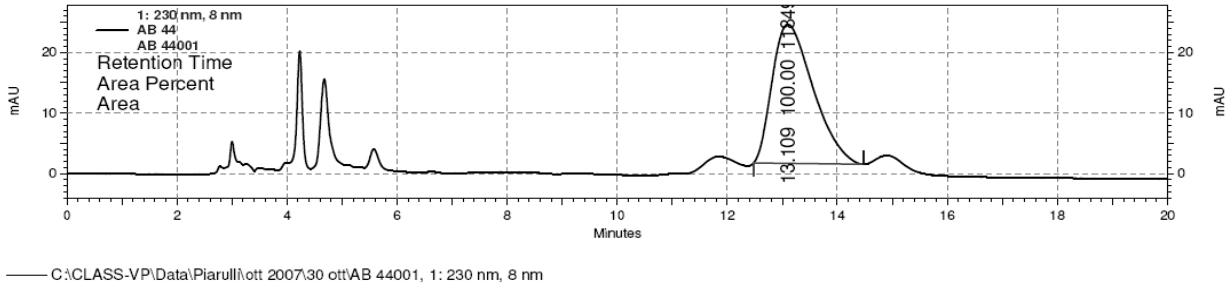
HPLC Analyses:

The optical purity of the compound **5**, was then confirmed by chiral HPLC with Chiracel OD-H column (25cm × 0.46cm) and Isopropanol/ hexane (9/1) in isocratic conditions 1mL.min^{-1}.

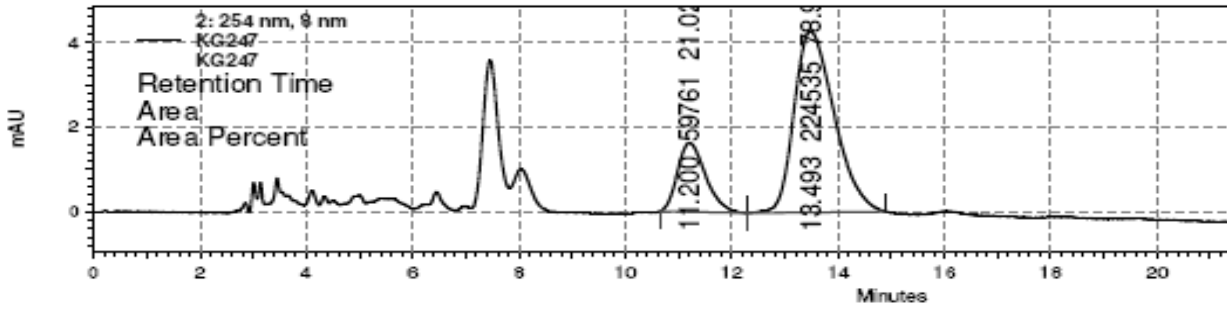
HPLC trace for the product **5** registered at 230 nm.

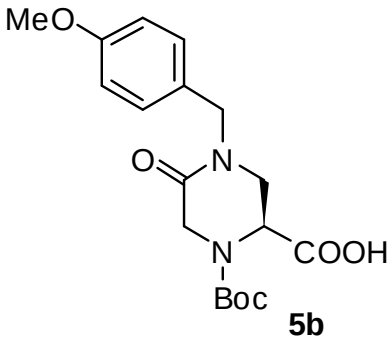


HPLC trace for the product **5** registered at 230 nm.



For comparison, HPLC trace of the same cyclic product obtained with an ee% of 57% registered at 254 nm. In this figure we can note the retention times of both enantiomers.
(*S*)-Boc-PCA- 13.5 min.
(*R*)-Boc-PCA- 11.2 min





5b

4-p-methoxy-benzyl-5-oxo-piperazine-1,2-dicarboxylic acid 1-tertbuthyl ester 5b:

To a solution of the acyl derivative **3c** (400mg, 0.905mmol) in dichloromethane (3mL), TFA (3mL) was added and the resulting mixture was stirred for 2.5h at RT. The solvent was then evaporated, methanol (5mL) was added and evaporated twice, to remove the excess of TFA, ether (5mL) was added and evaporated to give the unprotected intermediate under its TFA salt's form in quantitative yield. This salt was then dissolved in water (10.86mL) and an aqueous solution of NaOH 1M (2.72mmol, 2.72mL) and stirred over night at room temperature. Dioxane (10.8mL) was then added to the medium followed by the addition of Boc anhydride (590mg, 2.72mmol, 3equiv) at O°C, this solution was stirred for 24 hours. The reaction was then diluted in water (10mL) and washed twice with ethyl acetate (5ml). The aqueous layer was cooled at 0°C, acidified to pH= 3 with a 1M solution of $KHSO_4$, and extracted with ethyl acetate 5 times (25mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to afford the desired Boc protected piperazinone **5b** (190mg, 58%) as a white solid.

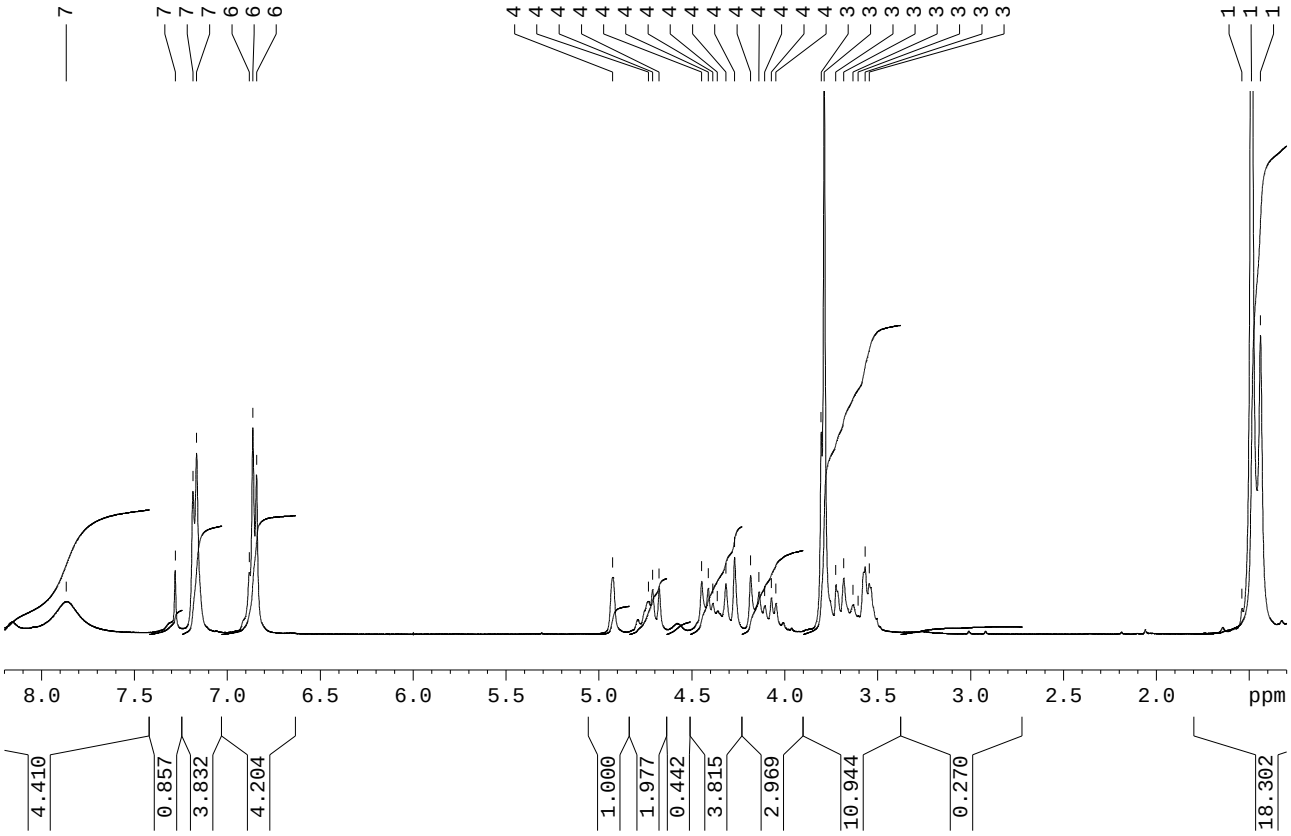
R_f = 0.2(DCM/MeOH 9/1) m.p. 240-242°C.

^{1}H NMR (400MHz, $CDCl_3$, 50°C): δ 7.16(d, *J*= 8 HZ, 2 H), 6.85 (d, *J*= 8.2 Hz, 2H), 5.45 (d, J= 8 Hz, 1H), 4.72 (d, *J*=14.54 Hz, 1H), 4.73 (d, *J*=14.57 Hz, $1H_B$), 4.55 (d, *J*=14.54 Hz $1H_A$), 4.48 (d, *J*=14,77 Hz, $1H_B$), 4.31 (d, *J*=18.34 Hz, 1H), 4.19(d, *J*=18.24 Hz, 1H), 3.80 (d, J=14 Hz, 3H), 3.71 (d, *J*=14.50Hz, 1H), 3.60 (dd, J_1=12.86Hz, J_2=4.48Hz 1H),1.50 (s, $9H_A$), 1,46 (s, $9H_B$) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=173.1, 166.5, 159.5,154.9, 130.3, 114.5, 55.6, 50.1, 46.8, 42.4,28.7 ppm.

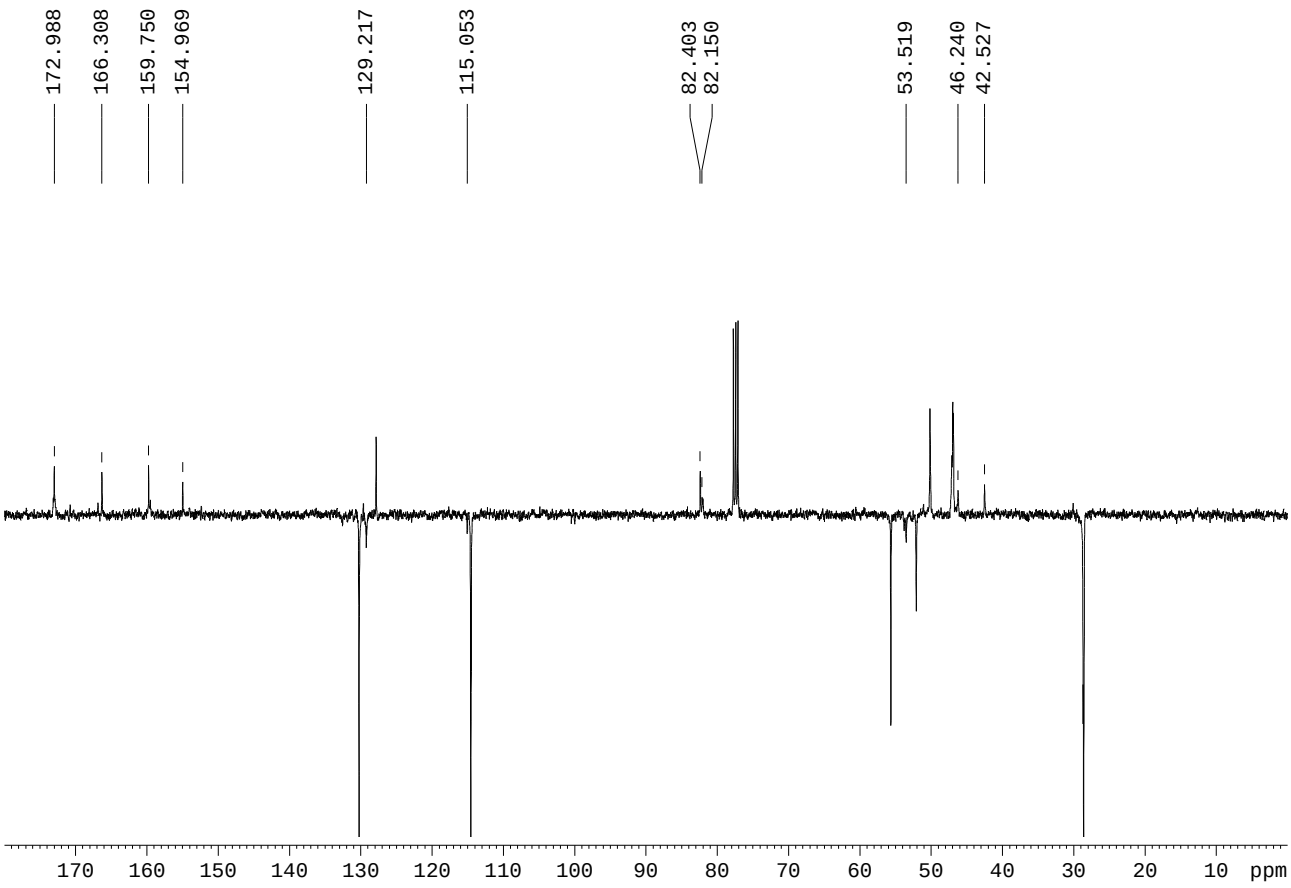
IR in CH_2Cl_2=2922, 1460, 1377,723.

4-p-methoxy-benzyl-5-oxo-piperazine-1,2-dicarboxylic acid 1-tertbuthyl ester 5b

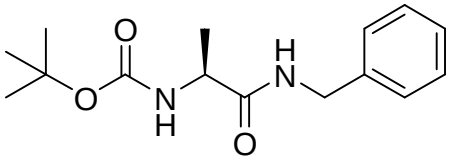
^{1}H



^{13}C



V-3 Coupling studies on the (Bn)5-oxopiperazine-2-carboxylic acid



Boc-Alanine benzylamide:

L-Boc-Ala (255 mg,1.32 mmol) was first dissolved in anhydrous DMF (13.2 ml). Under nitrogen, the benzyl amine (139 µl,1.32 mmol,1eq) was added, followed by the addition of the coupling reagent HOBt (217 mg,1.452 mmol,1.1 eq).

The solution was then cooled at 0°C with an ice bath, and the EDC (253 µl,1.452 mmol,1.1 eq) was added dropwise. The obtained mixture was then reacted for 2h at 0°C and then at room temperature for 22h.

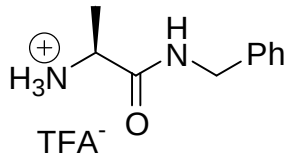
The DMF solution was then diluted with 5 mL of DCM and washed twice with 2mL of $KHSO_4$ (1M), twice with 2mL of $NaHCO_{3sat}$, and once with brine. The solution was then dried over dried Na_2SO_4 and the solvent was removed under reduced pressure to give the Boc protected compound as a white solid (349mg, 95%).

mp= 96-98°C.

^{1}H NMR (400MHz, $CDCl_3$):

δ=7.36-7.26(m,5H), 6.67 (s,1H), 5.10(s,1H), 4.44(d,J=5.3 Hz,2H), 4.21(m,1H), 1.46(s,9H), 1,40(d,J=7.06 Hz,3H) ppm.^{13}C NMR (100MHz, $CDCl_3$):δ=172.98, 138.49, 129.06, 127.85, 43.79, 28.68, 18.7 ppm. $C_{15}H_{22}N_2O_3$ (278.35): calcd. C 64.73%, H 7.97%, N 10.06%; found C 64.16%, H 8.00%, N 9.32%.

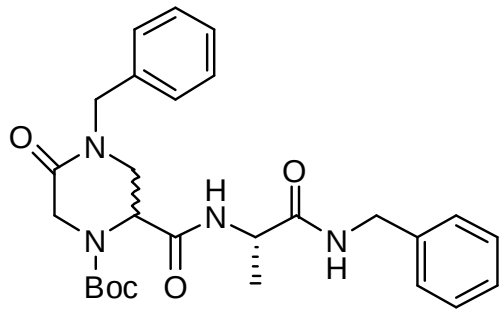
IR (CH_2Cl_2): 2926, 1377, 1298, 723.



8

The Boc-Ala-benzyl amine (40 mg, 0.143mmol) was dissolved in DCM (0.8 ml). To this solution, TFA (0.8mL, 8.6mmol)was added, and the mixture was stirred for 2h at ambient temperature. The obtained solution is then diluted with MeOH, and evaporated 3 times to eliminate the excess of TFA, and finally the TFA salt is precipitated in diethyl ether and dried

under vacuo. The corresponding salt **8** was obtained in quantitative yields and used with out further characterisation, in coupling studies.



6

Boc-PCA-(L)Ala-benzylamide (6):

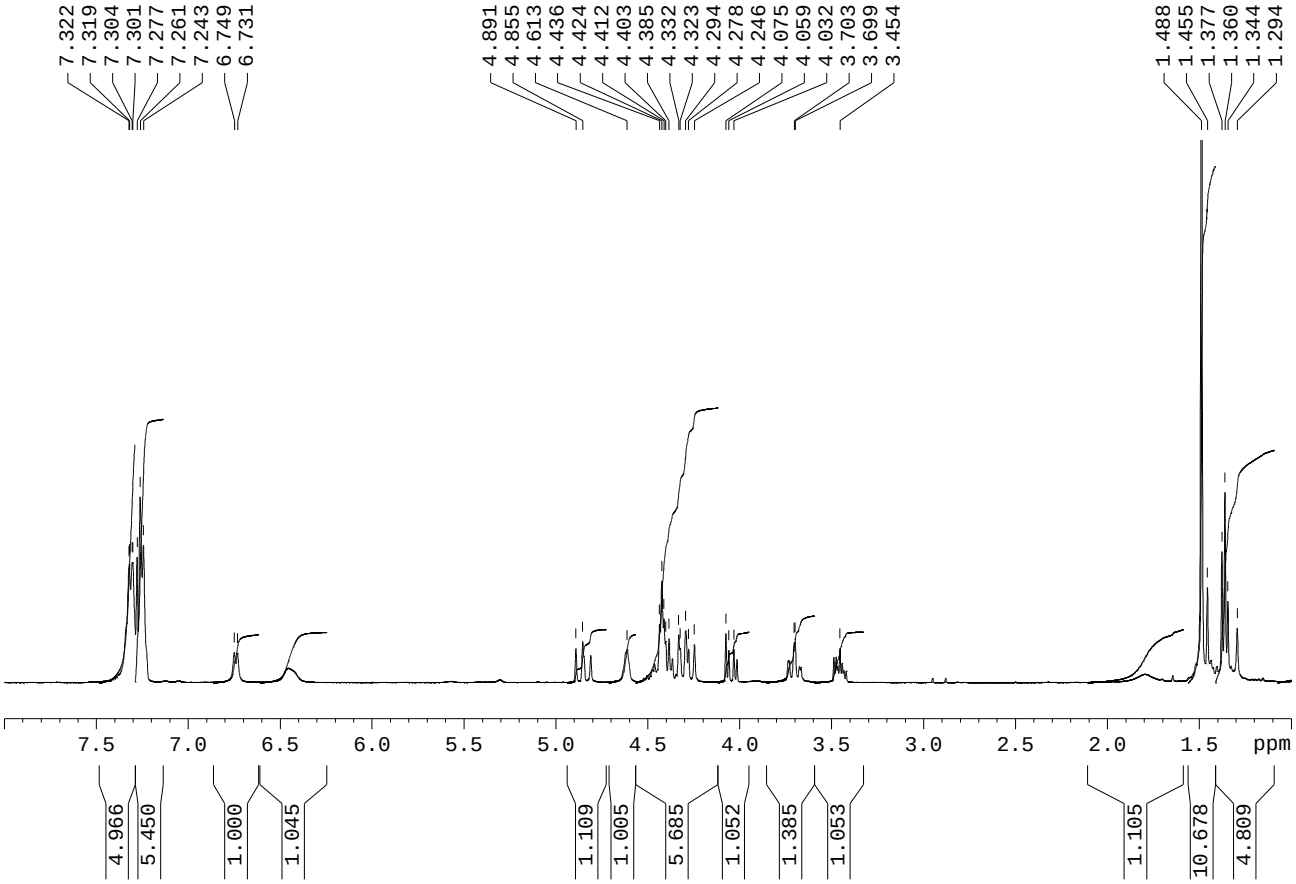
To a solution of **5** (25mg, 0.075mmol) in 1 mL of DMF, HOAt (11mg, 0.082mmol), the alanine benzylamide under it's TFA salt form (24mg, 0.082mmol), and DIPEA (35µL,0.224mmol) were added. The solution was cooled in an ice bath and treated with EDC (15µL,0.082mmol). The mixture was stirred for 1h at 0°C and at room temperature overnight. The DMF solution was then diluted with 5 mL ethyl acetate and washed twice with 2mL of $KHSO_4$ (1M), twice with 2mL of $NaHCO_{3sat}$, and once with brine. The solution was then dried over dried Na_2SO_4 and the solvent was removed under reduced pressure to give the crude product. Further purification by flash column chromatography (DCM/MeOH:95/5) gave us the desired product **(6)** in a 70% yield, as a mixture of two diastereoisomers (26mg, 0.05mmol).

R_f = 0.42(DCM/MeOH 9/1), mp= 68-72°C.

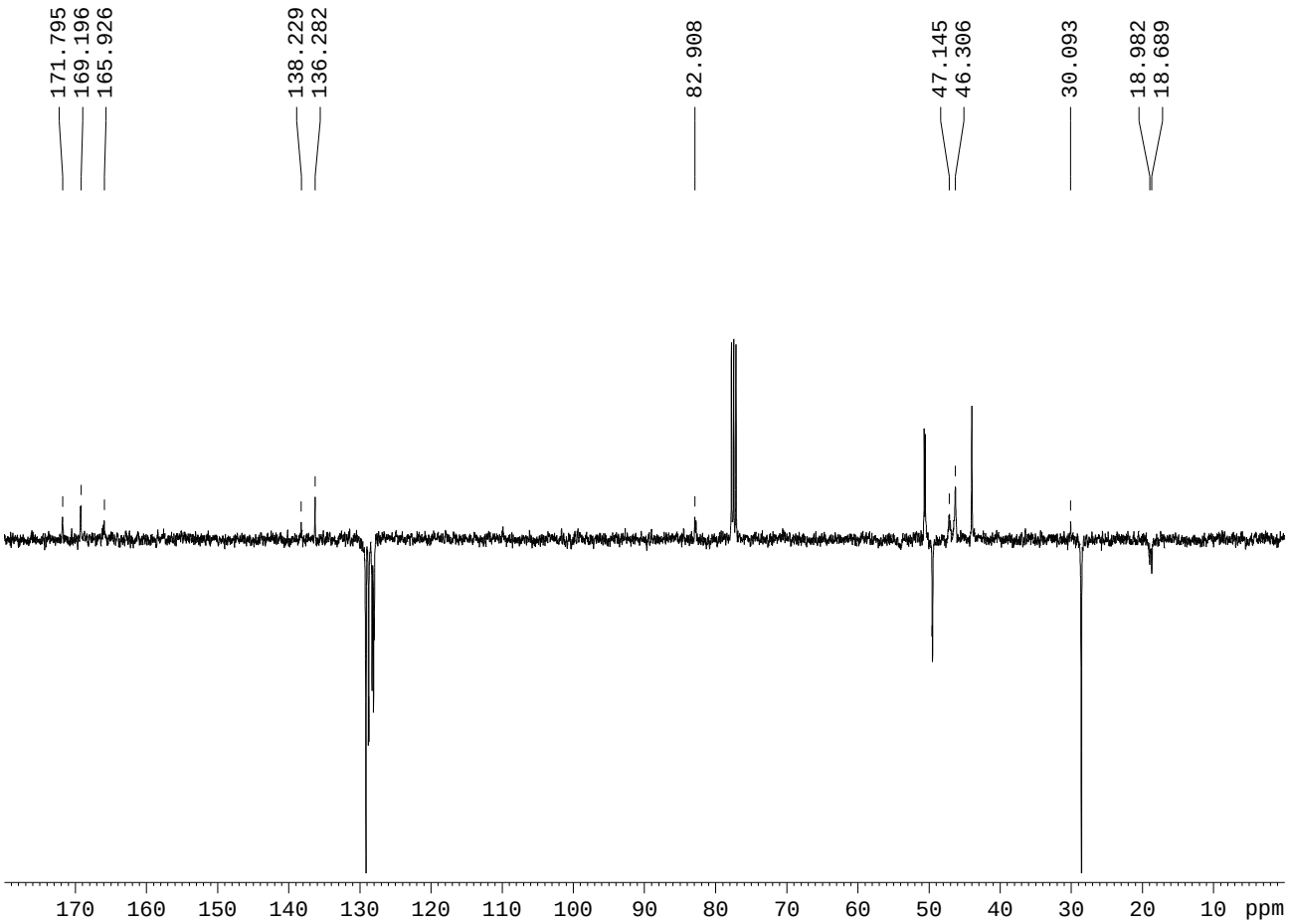
^{1}H NMR (400MHz, $CDCl_3$, 45°C): δ=7.32-7.24(m, 10H), 6.74(m, $1H_{DIA1\&DIA2}$), 6.45 (br, 1H), 4.85 (m, $1H_{DIA1\&DIA2}$), 4.46-4.24 (m, $5H_{DIA1\&2}$), 4.04 (dd, *J*=6,47 Hz, *J*=17,50 Hz 1H), 3.70 (2dd, $1H_{DIA1\&DIA2}$), 3.45(2dd, *J*=18.24 Hz, 1H), 3.71 (d, *J*=14.50Hz, $1H_{DIA1\&DIA2}$), 1.48, 1.45 (2s, $9H_{DIA1\&DIA2}$),1.36 (2d, $3H_A$), 1,46 (s, $3H_{DIA1\&DIA2}$) ppm. ^{13}C NMR (100MHz, $CDCl_3$): δ=171.7, 169.2, 165.9, 138.2, 136.2, 129.1, 128.77, 128.73, 128.2, 128.0, 127.9, 82.9, 56.6, 50.6, 50.5, 49.5, 47.1, 46.2, 43.9, 28.5ppm. $C_{27}H_{34}N_4O_5$ (494.60): calcd. C 65.57%, H 6.93%, N 11.33%; found C 62.18%, H 7.57%, N 8.73%. IR (CH_2Cl_2):2854, 2725, 2361, 1462, 1377, 802, 723, 422.

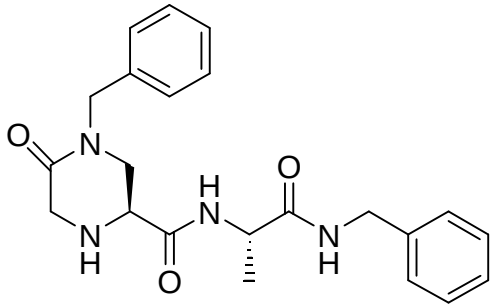
Boc-PCA-(L)Ala-benzylamide (6)

^{1}H



^{13}C





7a

4-Benzyl-5-oxo-piperazine-2-carboxylic acid (1-benzylcarbamoyl-ethyl)-amide (7a):

Deprotection of the dipeptide was carried out in a 50% TFA solution in DCM for 2 hours, following evaporations with methanol and ether were done to remove the excess of acid. Subsequent neutralization using $NaHCO_3$ and extractions with ethyl acetate 5 times gave us the mixture of the two diastereoisomers without Boc.

They were then separated by flash chromatography 97/3 DCM/ MEOH

To afford us both pure desired compounds in mg scale.

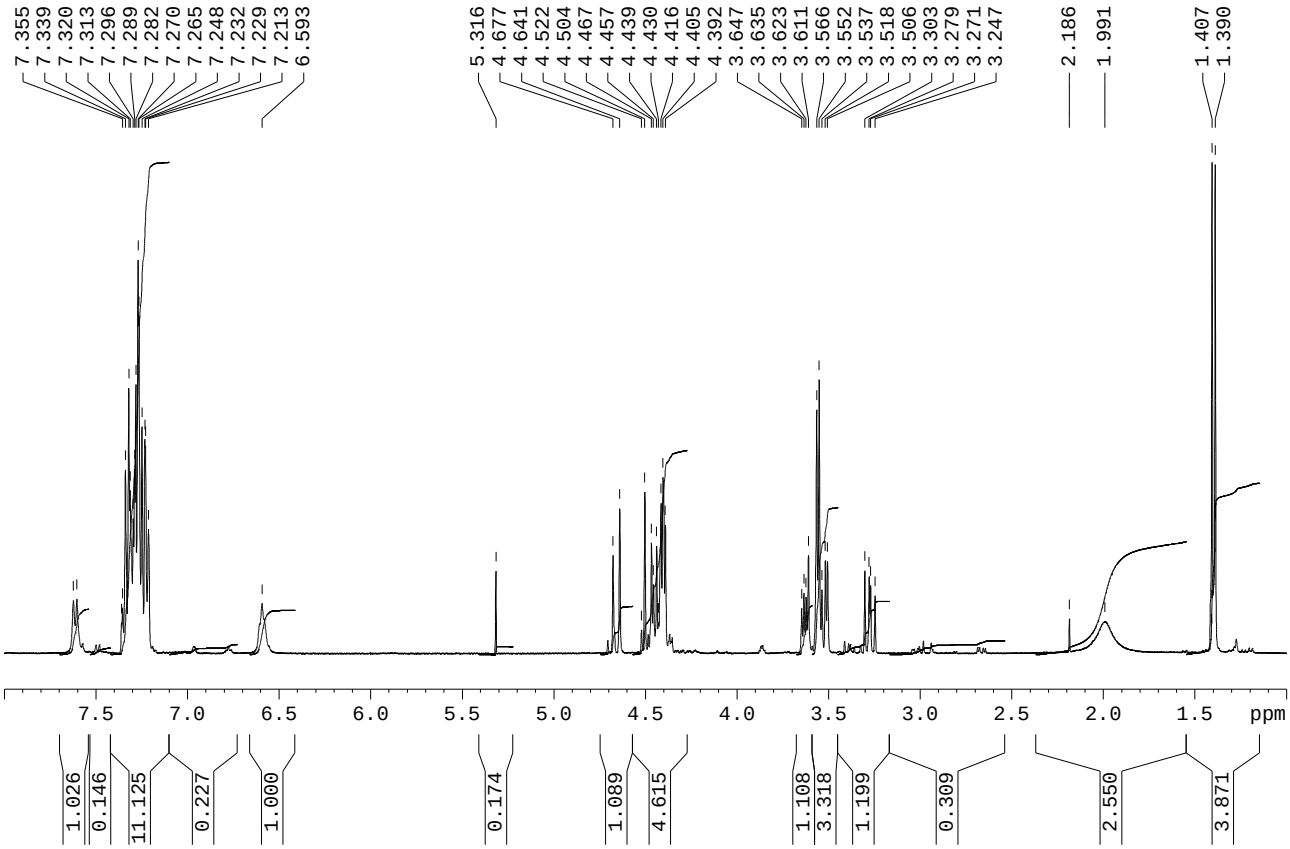
Major diastereoisomer **7a**

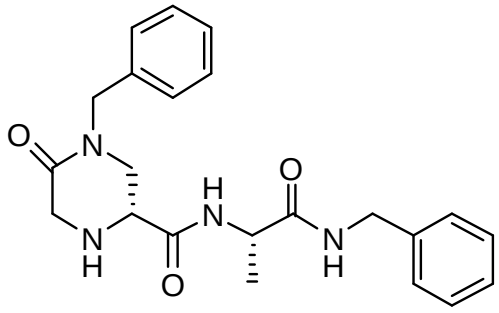
Rf= 0.58 (DCM/MeOH: 96/4)

^{1}H NMR (400MHz, $CDCl_3$): δ=7.61 (d, *J*= 7.61Hz, 1H), 7.33-7.21 (m, 5H), 6.59 (br, 1H), 4.65 (d, *J*= 14.61Hz 1H), 4.48 (d, *J*=14,48Hz, 1H), 4.31 (d, *J*=18.34 Hz, 1H), 4.19(d, *J*=18.24 Hz, 1H), 3.71 (d, *J*=14.50Hz, 1H), 3.60 (dd, J_1=12.86Hz, J_2=4.48Hz 1H),1.50 (s, $9H_A$), 1,46 (s, $9H_B$) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=173.3, 166.2, 154.9, 135.7, 129.1, 128.8, 128.4, 82.4, 82.1,.53.5, 52.1, 50.9, 47.1, 46.9, 28.6 ppm.

H-PCA-(L)Ala-benzylamide (7a)

^{1}H





7b

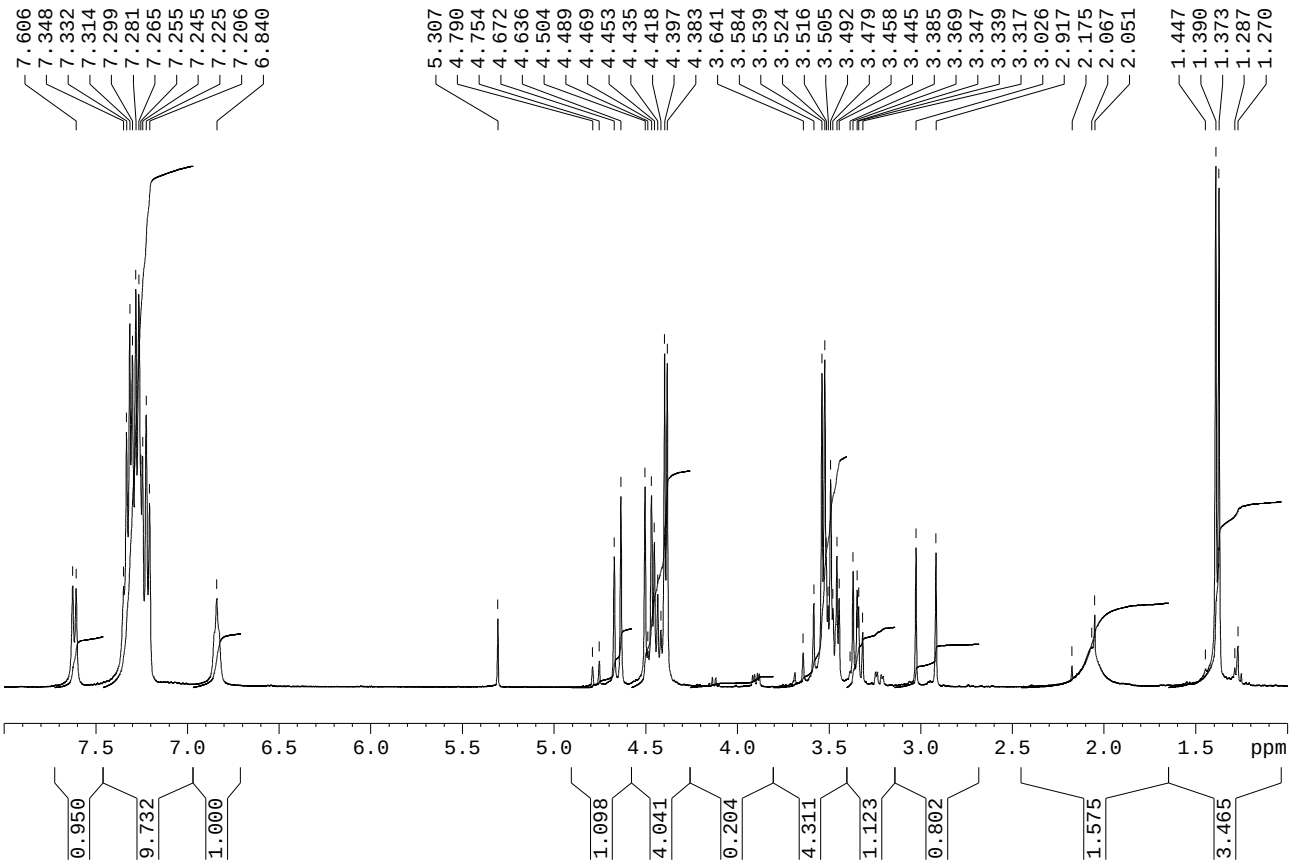
Minor diastereoisomer (7b)

Rf= 0.55 (DCM/MeOH: 96/4)

^{1}H NMR (400MHz, $CDCl_3$): δ=7.61 (d, *J*= 7.64Hz, 1H), 7.34-7.20 (m, 5H), 6.84 (br, 1H), 4.65 (d, *J*= 14.52Hz 1H), 4.48 (d, *J*=14,08Hz, 1H), 4.31 (d, *J*=18.34 Hz, 1H), 4.19(d, *J*=18.24 Hz, 1H), 3.71 (d, *J*=14.50Hz, 1H), 3.60 (dd, J_1=12.86Hz, J_2=4.48Hz 1H),1.50 (s, $9H_A$), 1,46 (s, $9H_B$) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=173.3, 166.2, 154.9, 135.7, 129.1, 128.8, 128.4, 82.4, 82.1,.53.5, 52.1, 50.9, 47.1, 46.9, 28.6 ppm.

H-PCA-(L)Ala-benzylamide dia (7b)

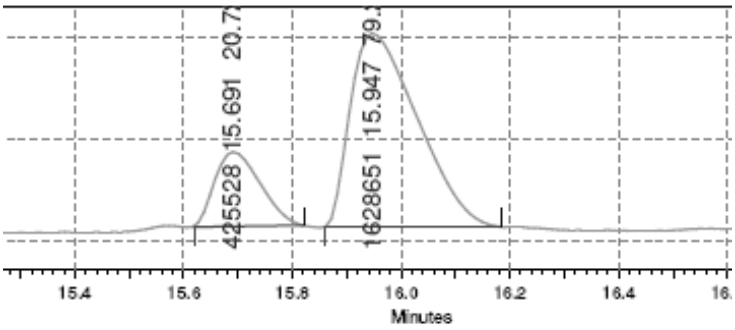
^{1}H



HPLC Analyses:

For the need of coupling studies, the purity of product **7a** and **7b** was evaluated by HPLC analyses, in reverse phase conditions with the column C-18 (5 cm × 0.46 cm, 5µm), with a constant flux: 1mL.min^{-1}

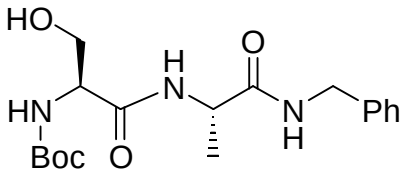
Solutions	Gradient:
A = H_2O + 0.1% TFA **B** = CH_3CN + 0.1% TFA	• **A:** 0-40% in 40 min • **B**: 100% for 10 min.



The spectrum was registered at 230nm

Retention times:
7a: Dipeptide (L)PCA(L)Ala Tr= 15.9 min.

7b: Dipeptide (D)PCA(L)Ala Tr= 15.6min.



9a

NH-Boc(L)Ser(L)Ala benzylamide (9a):

To a solution of Boc-L-Serine (140 mg, 0.683mmol) in anhydrous DMF (7mL), at 0°C, HATU (285mg, 0.75mmol), TFA salt of the Alanine benzylamide(219mg,0.75mmol) and TMP(270mL, 0.75mmol) were added. After 1h stirring at 0°C, the mixture was stirred for 3h at ambient temperature. The solution was then diluted with ethyl acetate and washed subsequently with $KHSO_4$, $NaHCO_3$, and NaCl. The organic phase was then dried over Na_2SO_4, and the organic solvent was evaporated to afford the product **9a** as a white solid (186mg, 77%).

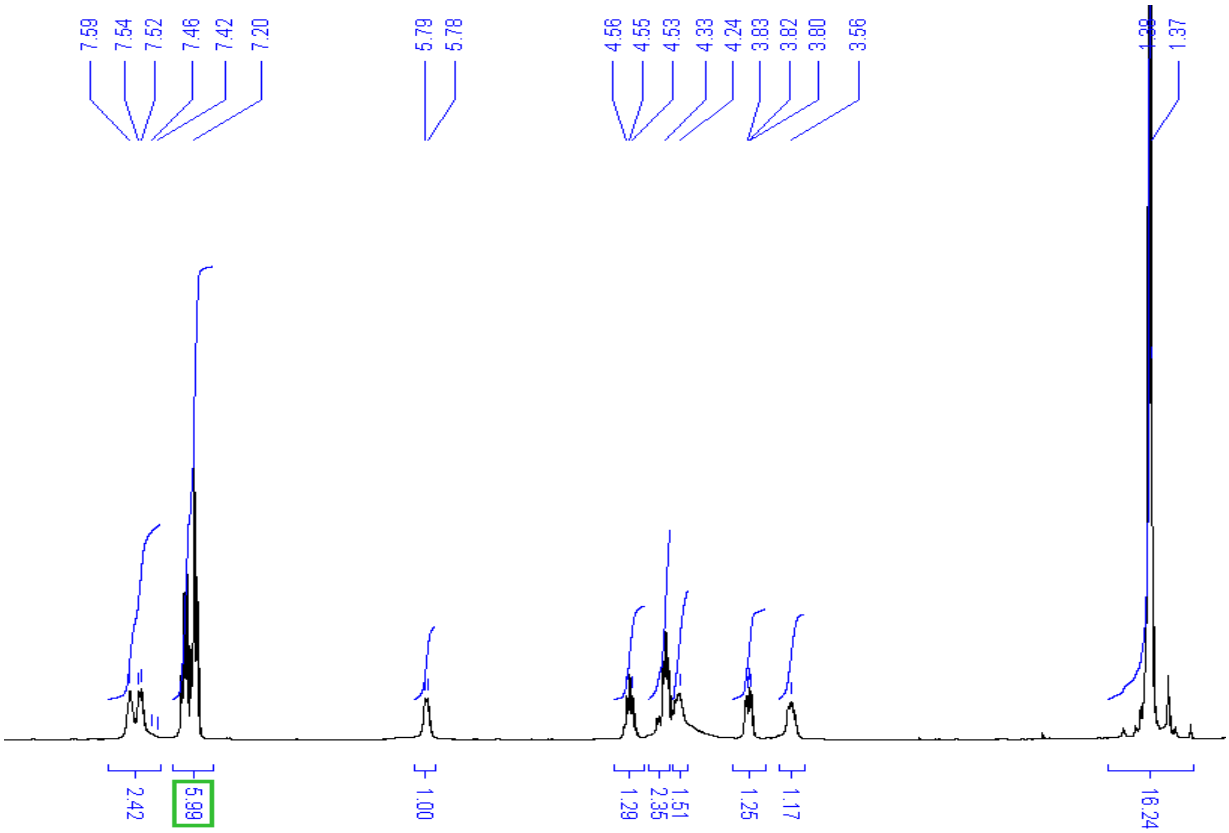
mp= 142-144°C.

1H NMR (400MHz, CDCl3):δ=7.59 (d, *J*=7.2 Hz,1H), 7.53(d, *J*=7 Hz,1H),7.33-7.23 (m,5H) ,5.78(d, *J*=5.77 Hz, 1H), 4.56 (t, 1H), 4.41(d, *J*=5.47Hz,2 H),4.19(m,1H),3.93(dd,*J*=3.8Hz, *J*=11.07 Hz,1H), 3.60(dd,*J*=7.16 Hz,*J*=14 Hz,1H),1.38(s,12H) ppm.

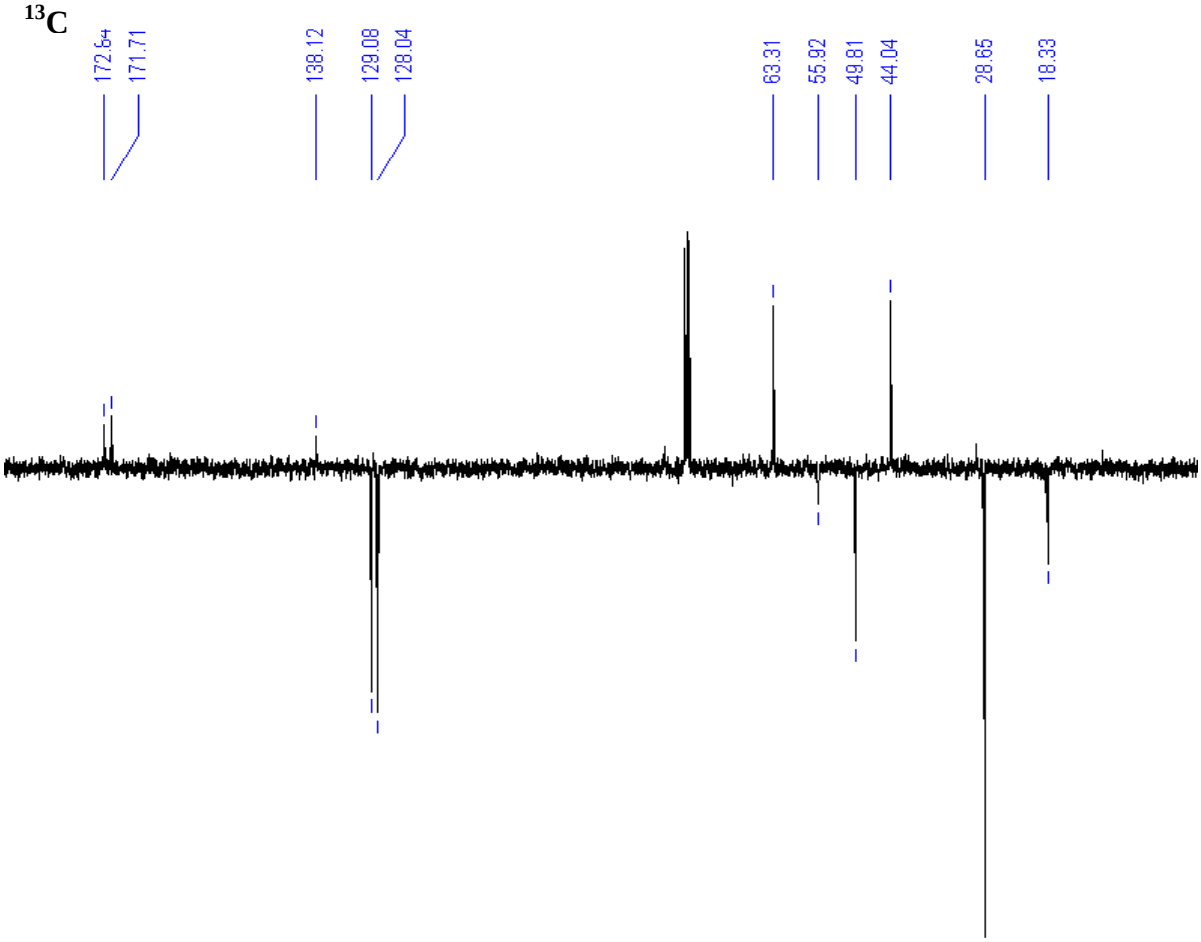
13CNMR(100MHz,CDCl3):δ=172.83,171.71,138.11,128.07,127.9,63.31,55.91,49.81,44.03,28.65,18.32ppm. C18H27N3O5 (365.43): calcd. C 59.16%, H 7.45%, N 11.50%; found C 58.25%, H 7.38%, N 10.55%. IR (CHCl3): 2924, 2854, 1462, 1377, 1091, 723.

NH-Boc(L)Ser(L)Ala benzylamide (9a):

^{1}H



^{13}C



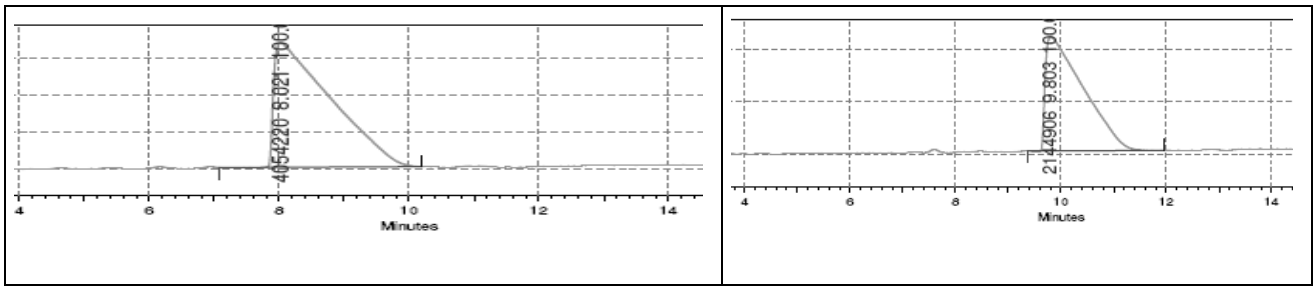
Starting from the D-Serine (110mg, 0.54mmol) the same dipeptide **9b** was synthesized following the same procedure and afforded the corresponding dipeptide in a 90% yield (170mg)

HPLC Analyses:

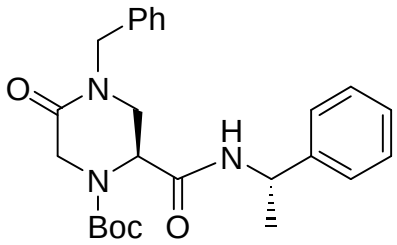
For the need of coupling studies, the purity of product **9** was evaluated by HPLC analyses on its corresponding unprotected derivative under its TFA salt form. The analyses were carried out in reverse phase conditions with the column C-18 (5 cm × 0.46 cm, 5µm), with a constant flux: 1mL.min^{-1}

Solutions	Gradient:
A = H_2O + 0.1% TFA **B** = CH_3CN + 0.1% TFA	• CH_3CN da 0-10% in 15 min • CH_3CN da 10-15% in 5 min • H_2O 100% per 5 min.

The corresponding TFA salts diluted in water were injected for HPLC characterisation. And HPLC traces were registered at 230nm.



(L)Ser(L)Ala Tr = 8-8.31 min. (D)Ser(L)Ala, Tr = 9.08 min.



10

N-Boc(L)PCA-(S)-α-methyl benzylamide (10):

To a solution of N-Boc-(S)-Pca **5** (30 mg, 0.083mmol) in anhydrous DMF (1mL), at 0°C, HBTU (40mg, 0.11mmol), HOBt (23mg, 0.11mmol), freshly distilled -(S)-α-metil benzylamide (12µL, 0.1mmol) and DIPEA (46µL, 0.267mmol) were added. After 1h stirring at 0°C, the mixture was left stirring for 5h at ambient temperature. The solution was then diluted with ethyl acetate and washed subsequently with $KHSO_4$, $NaHCO_3$, and NaCl. The organic phase was then dried over Na_2SO_4, and the organic solvent was evaporated to afford the product **10** as a white solid(22.7mg, 58%),

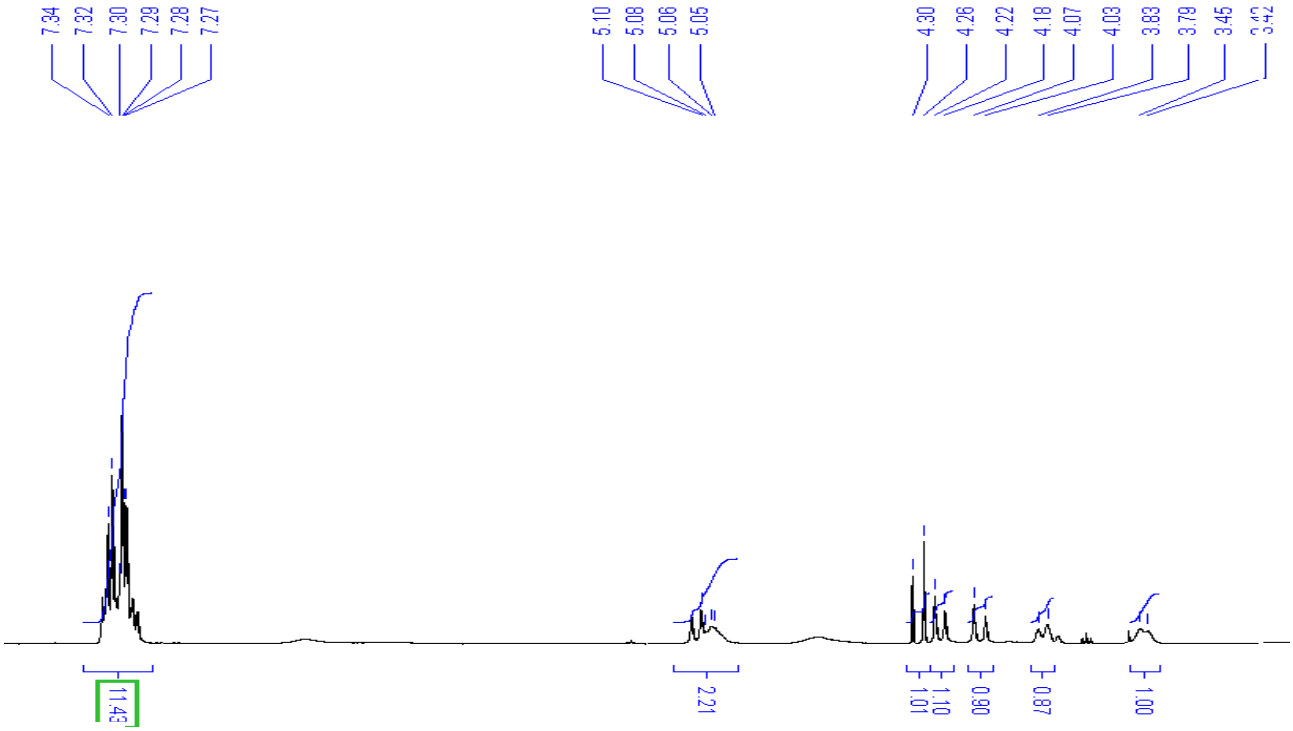
Rf=0.6 (DCM/MeOH=95/5)

^{1}H NMR (400MHz, $CDCl_3$): δ=7.36-7.22(m,10H), 6.64(m,1H), 5.12(d,J=14.6 Hz,1H), 5.06(m,1H), 4.66(m,1H), 4.28(d, J=14.6Hz,1H), 4.19(d,J=15.5 Hz,1H), 4.05(d,J=17.5Hz,1H), 3.8(dd,J=13.7Hz,1H), 3.42(dd,J=12.1Hz,1H), 1.46(d,12H)ppm.

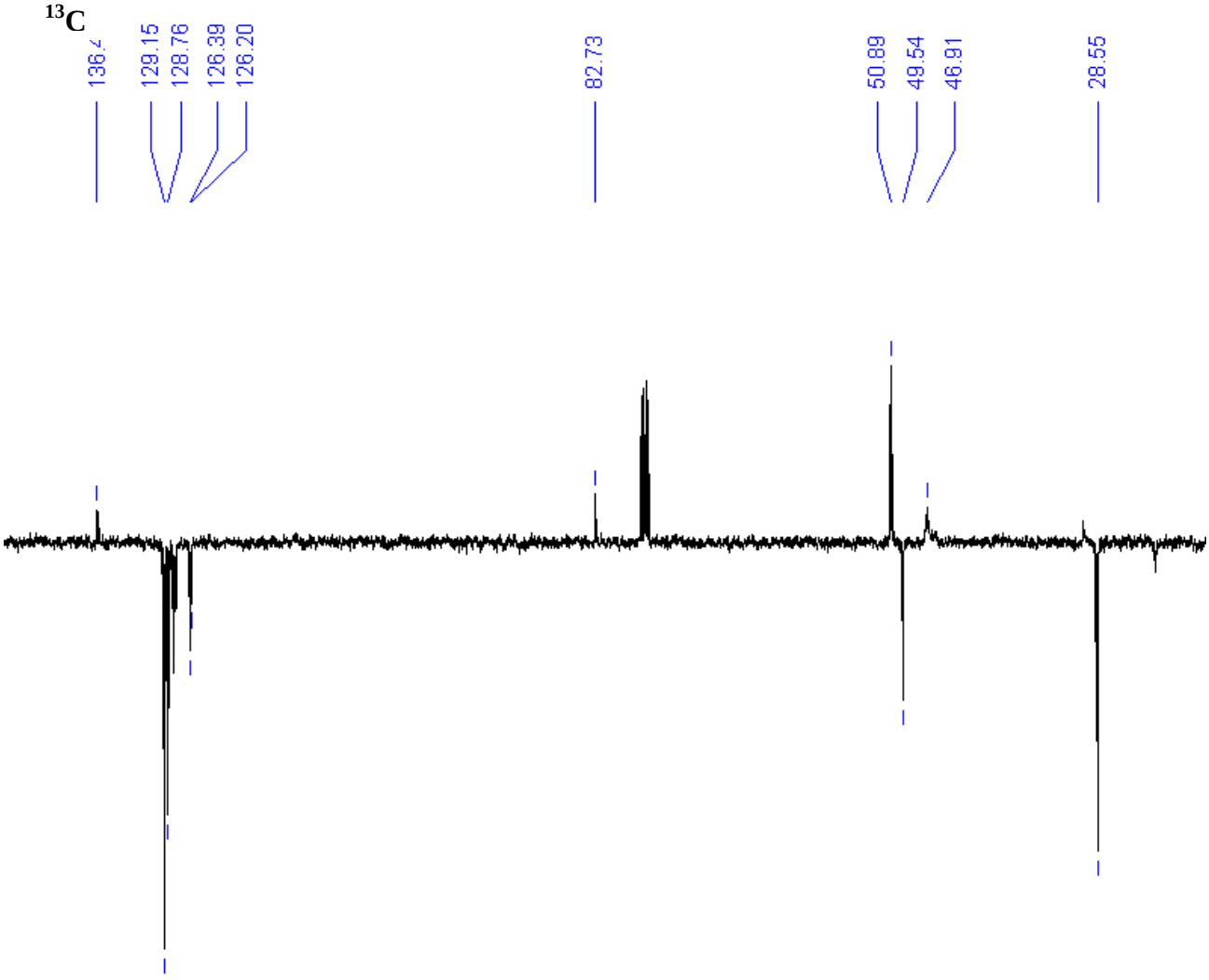
^{13}C NMR (100MHz, $CDCl_3$): δ=136.4,129.15,128.76,128.22,127.93,126.38,126.2, 82.73,50.88,49.53,46.91,28.55, 22.30ppm.

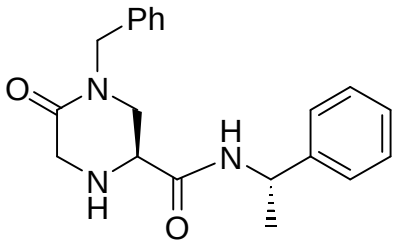
N-Boc(L)PCA-(S)-α-methyl benzylamide (10):

^{1}H



^{13}C





11

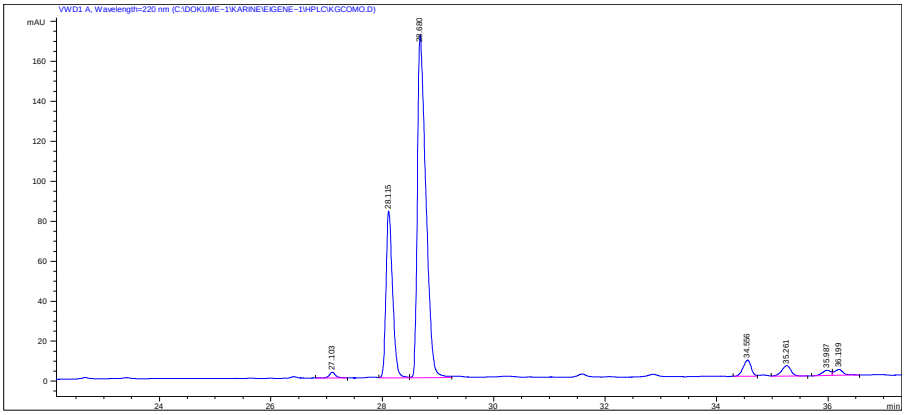
Deprotection of the dipeptide **10** was carried out in a 50% TFA solution in DCM for 2 hours, following evaporations with methanol and ether were done to remove the excess of acid. Subsequent neutralization using $NaHCO_3$ and extractions with ethyl acetate 5 times gave us the mixture of the two diastereoisomers without Boc

HPLC Analyses:

For the need of coupling studies, the purity of product **10** was evaluated by HPLC, on its corresponding unprotected derivative **11**, in reverse phase conditions with the column C-18 (5 cm × 0.46 cm, 5µm), with a constant flux: $1mL.min^{-1}$

Solutions	Gradient:
A = H_2O + 0.1% TFA **B** = CH_3CN + 0.1% TFA	• CH_3CN da 0-10% in 15 min • CH_3CN da 10-15% in 5 min • H_2O 100% per 5 min.

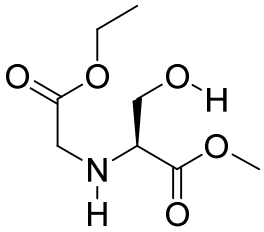
The product **11**, diluted in H_2O was then injected.
And the analyses of the corresponding HPLC trace showed us a 3 to 7 diastereoisomeric ratio.



(L)PCA (S)-α-metil benzylamide Tr= 28.68 min.

(D)PCA (S)-α-metil benzylamide Tr=28.11 min.

V-4 Synthesis of Pca:



19

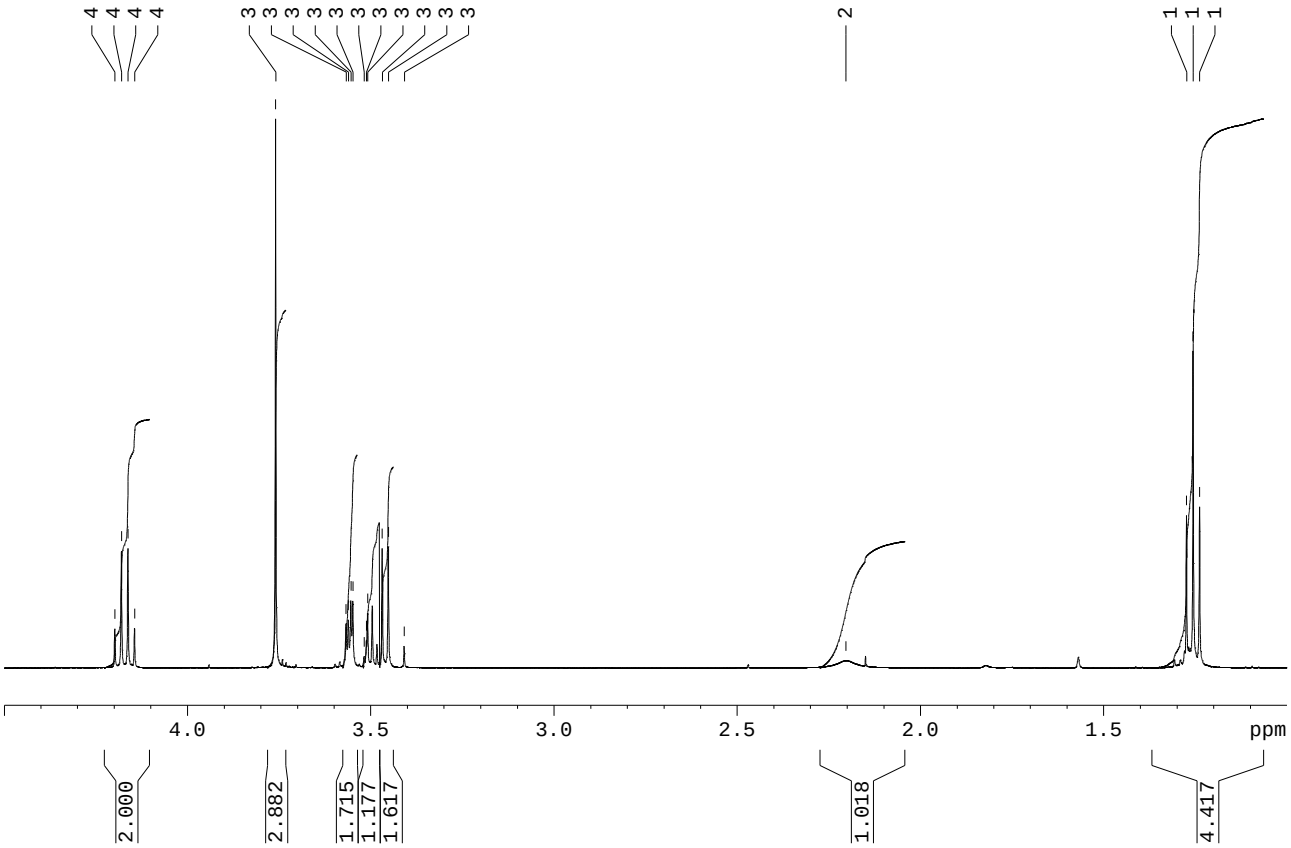
2-(Ethoxycarbonylmethyl-amino)-3-hydroxypropionic acid methyl ester (19):

L-serine methyl ester hydrochloride (1g, 6.47mmol) was dissolved in methanol, then triethylamine (902µL, 6.47mmol), a 50% solution of ethyl glyoxalate in toluene, and 10% Pd/C (90mg), were successively added, and the resulting mixture was stirred overnight under a hydrogen atmosphere. Next the suspension was filtered over a pad of Celite, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH:98/2) to yield **19** as a color less oil (902mg, 68%) Rf= 0.25(CH_2Cl_2/MeOH:95 /5) $[\alpha]^{20}_D$ =-27.8 (c=1, $CHCl_3$).

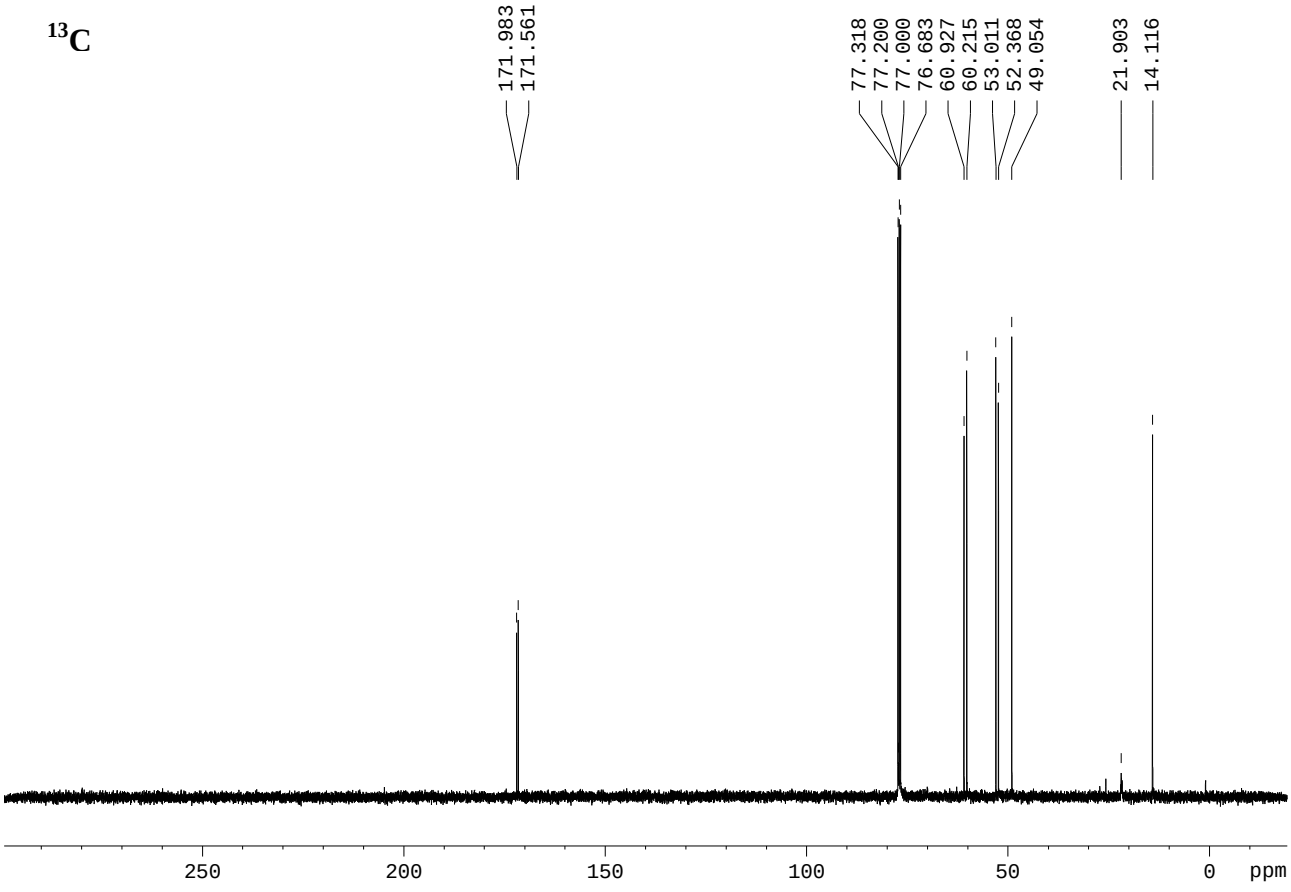
^{1}H NMR (400MHz, $CDCl_3$): δ=4.20-4.15 (q, *J*=8.74 Hz, 2H), 3.79 (dd, J_1=11.16 Hz, J_2=4.56Hz 1H), 3.74 (s, 3H), 3.69 (dd, *J*=5.86Hz, *J* =11.15 Hz 1H), 3.52 (d, *J*=17.4 Hz, 1H), 3.40 (d, *J*=17.5 Hz, 1H), 2.76(br, 1H), 1.26 (t, *J*=7.14Hz, 3H), ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=173.1, 172.4, 62.8, 62.6, 61.2, 52.4, 49.2, 14.4 ppm. $C_8H_{15}NO_5$ (205.21): calcd. C 46.82%, H 7.37%, N 6.83%; found C46,74%, H7,32%, N6,46%.

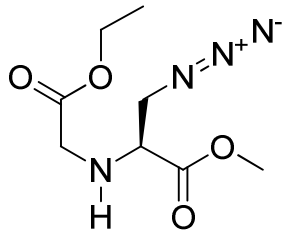
2-(Ethoxycarbonylmethyl-amino)-3-hydroxypropionic acid methyl ester (19):

^{1}H



^{13}C





20

3-Azido-2-(ethoxycarbonylmethyl-amino)-propionic acid methyl ester 20:

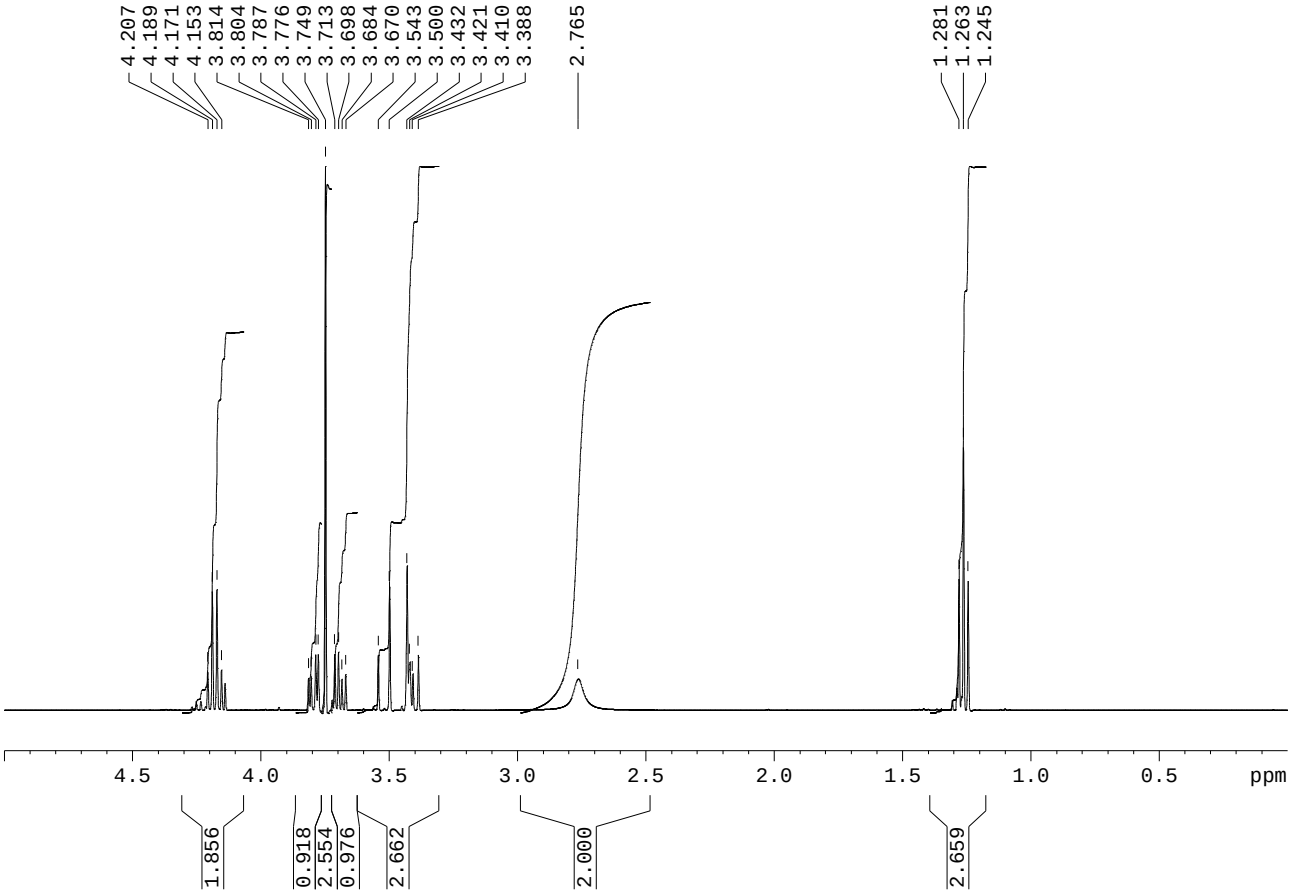
Under nitrogen, to a solution of **19** (807mg, 3.93mmol) in dry toluene 30mL, triphenylphosphine (1.44g, 5.50mmol) was added. After complete dissolution of the phosphine, HN_3(0.5M) (7,86mmol, 15.70mL) was added, followed by DIAD (5.50, 1.10mL). After 2 hours of reaction the mixture was directly poured into a column and purified by flash column chromatography (CH_2Cl_2/MeOH, 99/1) to give the azide as a transparent oil (796mg, 88%).

Rf=0.65(CH_2Cl_2/MeOH:95/5) $[\alpha]^{20}_D$ =-37.6 (c=0.5, $CHCl_3$). ^{1}H NMR (400MHz, $CDCl_3$): δ=4.17(q, *J*=7.1 Hz, 2H), 3.75(s, 3H),3.53 (dd, J_1=12,16 Hz, J_2=5,25 Hz, 1H), 3.49 (dd, J_1=12,16 Hz, J_2=5,25 Hz, 1H), 4.08 (d, *J*=17.38 Hz, 1H), 3.95 (d, *J*=17.38 Hz 1H), 3.74 (dd, J_1=12,16 Hz, J_2=4,95Hz, 1H), 3.49 (d, *J*=17,30 Hz, 1H), 3.43(d, *J*=17,30 Hz, 1H), 1.25 (t, *J*=7,15Hz, 3H) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=171.9, 171.5, 60.9, 60.2, 53.0, 52.3, 49.0, 14.11 ppm. HRMS (ESI) m/z calcd for $[C_8H_{14}N_4NaO_4]^+$ 253.O9073 $[M+Na]^+$ found 253.09097

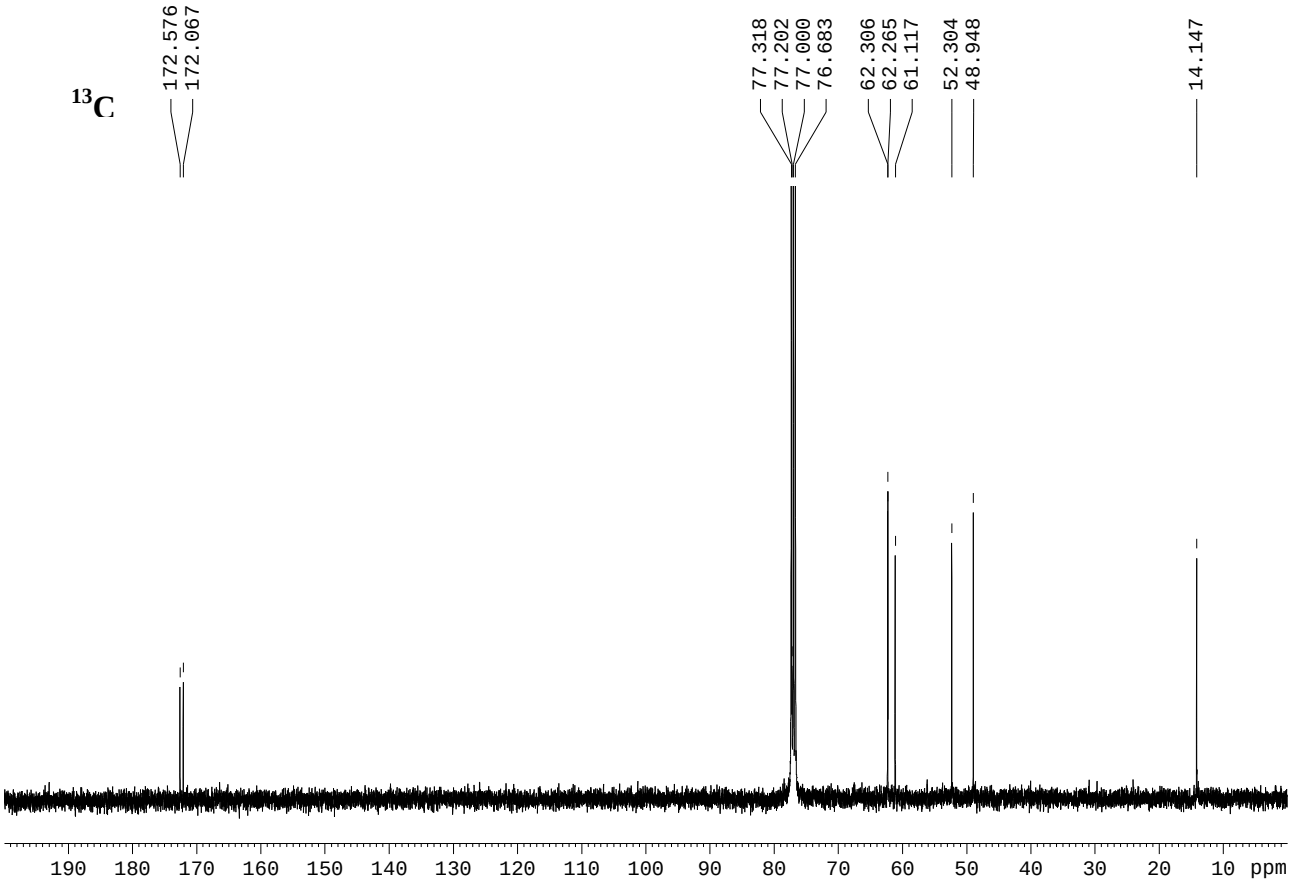
IR (CH_2Cl_2) =3352, 3063, 2108, 1744, 1445

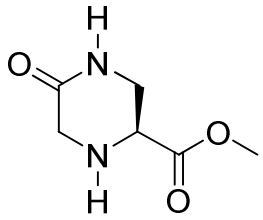
3-Azido-2-(ethoxycarbonylmethyl-amino)-propionic acid methyl ester 20:

^{1}H



^{13}C





21

5-Oxo-piperazine-2-carboxylic acid methyl ester 21:

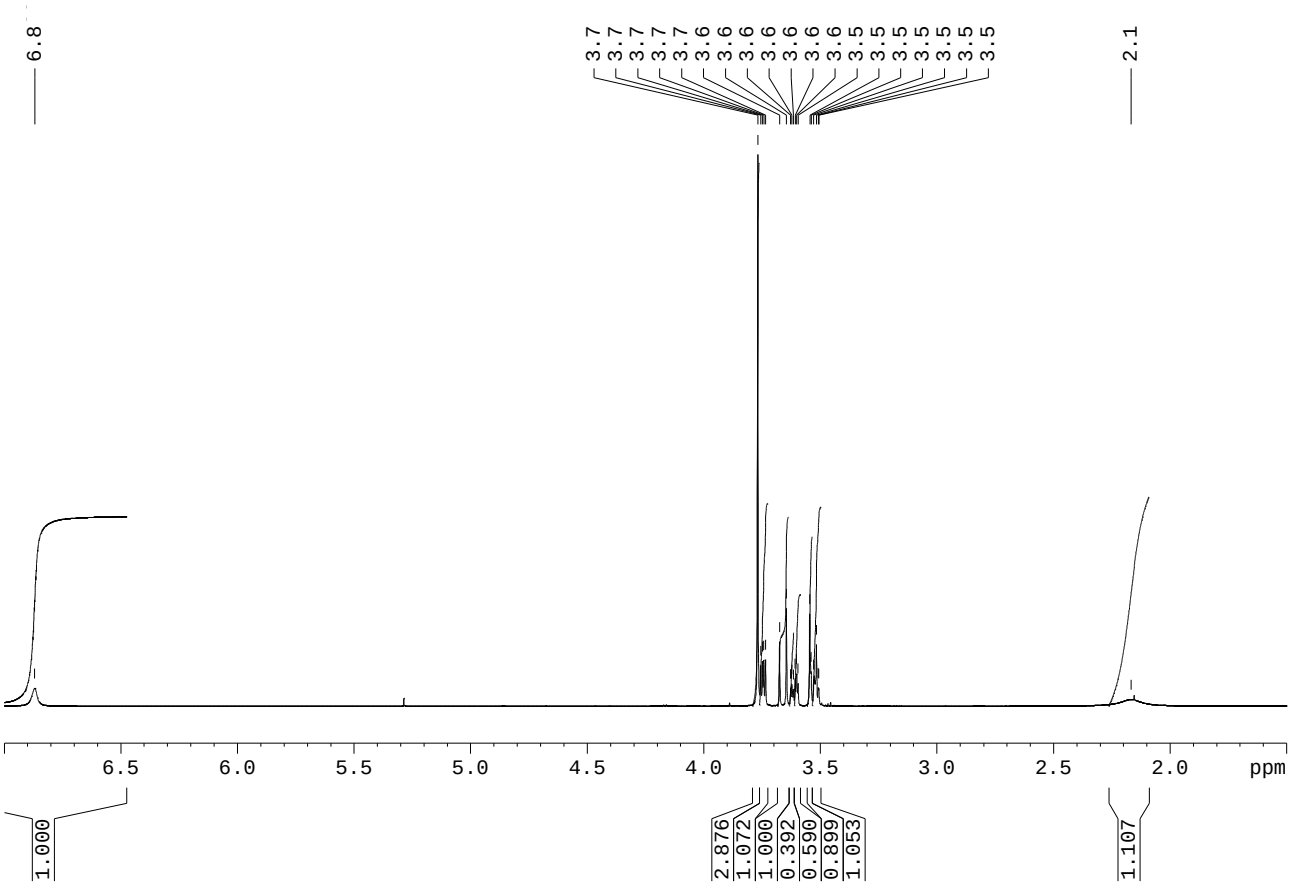
To a solution of **20** (260mg, 1.12mmol) in MeOH (5mL) Pd/C (0.112 mmol 0.1eq) was added. The reaction was stirred overnight at RT under 1atm of H_2. The solution medium was then filtrated through a pad of celite to remove the Pd/C, the solvent was evaporated under reduced pressure to obtain **21** (177 mg, 1.11 mmol, 99%) as a white paste.

Rf= 0,3 (DCM/MeOH:92/8), $[\alpha]^{20}_D$ =-46.3 (c=1, $CHCl_3$). ^{1}H NMR (400MHz, $CDCl_3$): δ= 3.76(s, 3H),3.74 (dd, J_1=8,06 Hz, J_2=4,48 Hz, 1H), 3.66 (d, J=17.1 Hz, 1H), 3.61 (ddd, J_1=11,84 Hz, J_2=4,48 Hz, J_3=3.13 Hz, 1H), 3.53 (d, J=17.44 Hz 1H), 3.52 (ddd, J_1=11,85 Hz, J_2=8,06, J_3=1.84 Hz 1H) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=171.4, 170.1, 54.2, 52.9, 48.2, 44.1ppm. HRMS (EI-MS) m/z calcd for [$C_6H_{10}N_2O_3$] 158.06910 $[M]^+$ found 158.06920

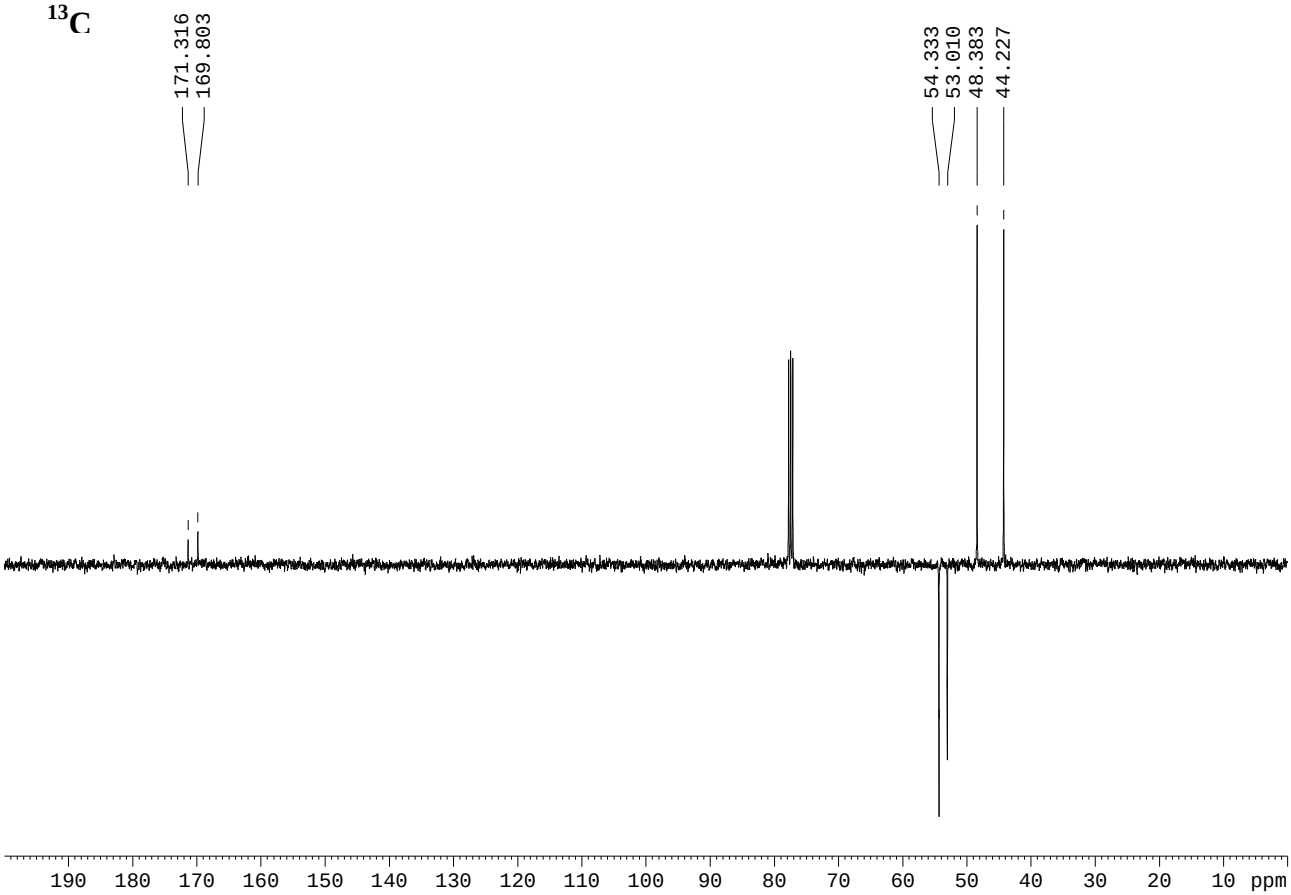
IR (CH_2Cl_2) =3406, 3354, 3211, 1744, 1678

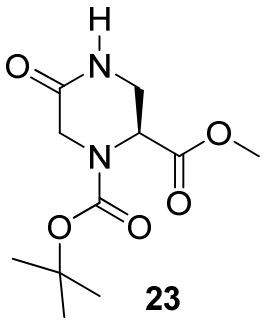
5-Oxo-piperazine-2-carboxylic acid methyl ester 21:

^{1}H



^{13}C





23

5-Oxo-piperazine-1,2-dicarboxylic acid 1-tert-butyl ester 2-methyl ester :

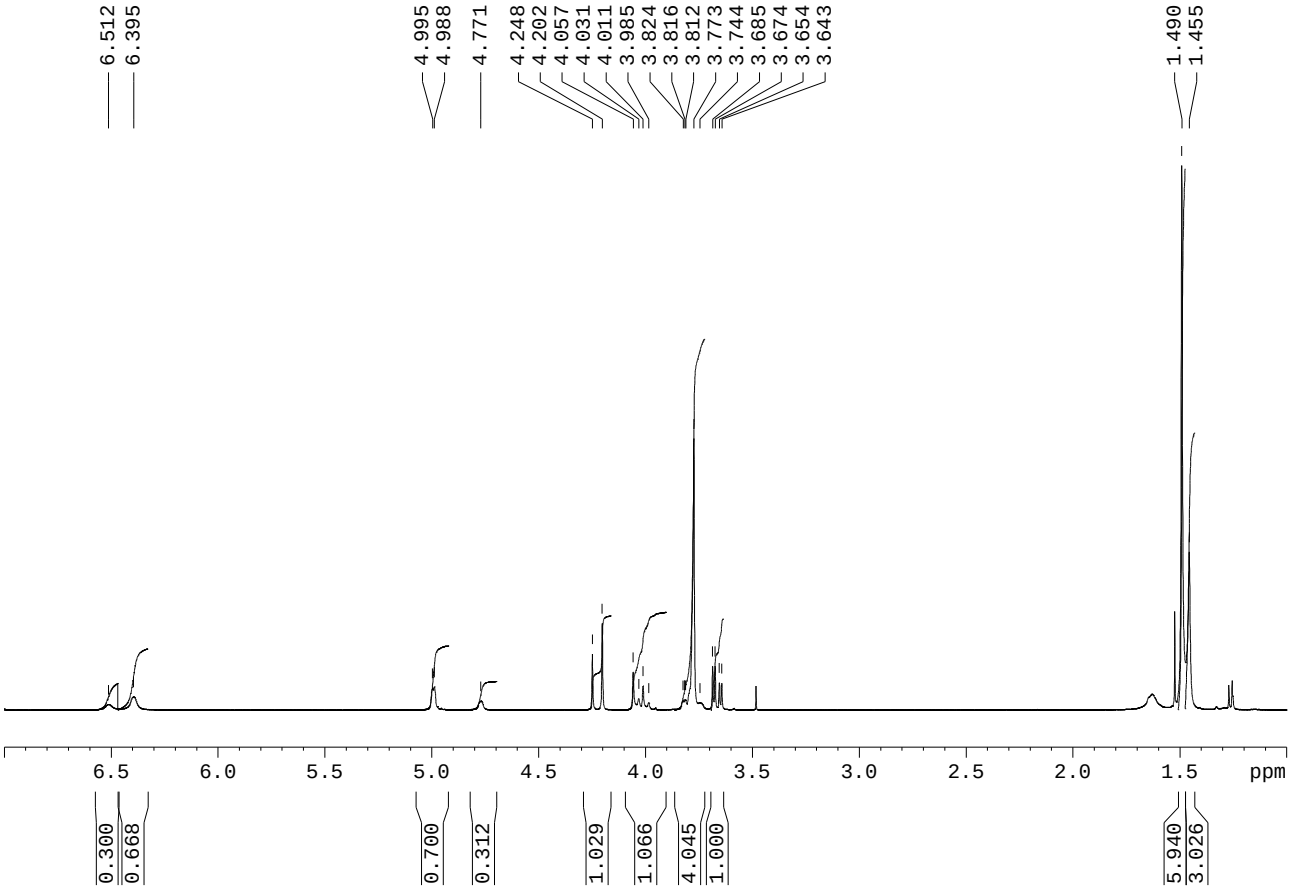
To a solution of **21** (100mg, 0.63mmol)in Dioxane /1M solution of K_2CO_3 in water (3mL/3mL) mixture, at 0°C Boc anhydride was added. After 15min the ice bath was removed and the solution was stirred over night. Water 5mL was added and the solution was extracted with ethyl acetate. Solvents were removed under reduced pressure and the crude product was purified by flash column chromatography to afford the desired Boc protected compound **23** (65mg, 40%)

Rf=0.32(CH_2Cl_2/MeOH:97/3) $[\alpha]^{20}_D$ =-20.8 (c=1, $CHCl_3$).mp= 85°C. ^{1}H NMR (400MHz, $CDCl_3$): δ= 6.65(bs, $1H_B$), 6.50(bs, $1H_A$), 4.99 (dd, J_1=4,63Hz, J_2=1,85Hz, $1H_A$),4.77 (br, $1H_B$), 4.23 (d, J=18.49Hz, $1H_A$), 4.22 (d, J=18,62Hz, $1H_B$), 4.03 (d, J=18.49Hz $1H_A$), 4.00 (d, J_1=18,62Hz, $1H_B$) 3.80 (ddd, J_1=12.55Hz, J_2=4.97Hz, J_3=1.85Hz, $1H_A$), 3.78 (s, $3H_A$), 3.77(s, $3H_B$), 3.66(dd, J_1=12.55Hz, J_2=4.63Hz, $1H_B$)ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=169.9, 167.5, 81.6, 52.9, 51.4, 46.3, 42.3, 28.22ppm. $C_{11}H_{18}N_2O_5$+0.4H_2O(258.27): calcd. C 49.76%, H 7.15%, N 10.55%; found C49,93%, H7,42%, N10,06%.

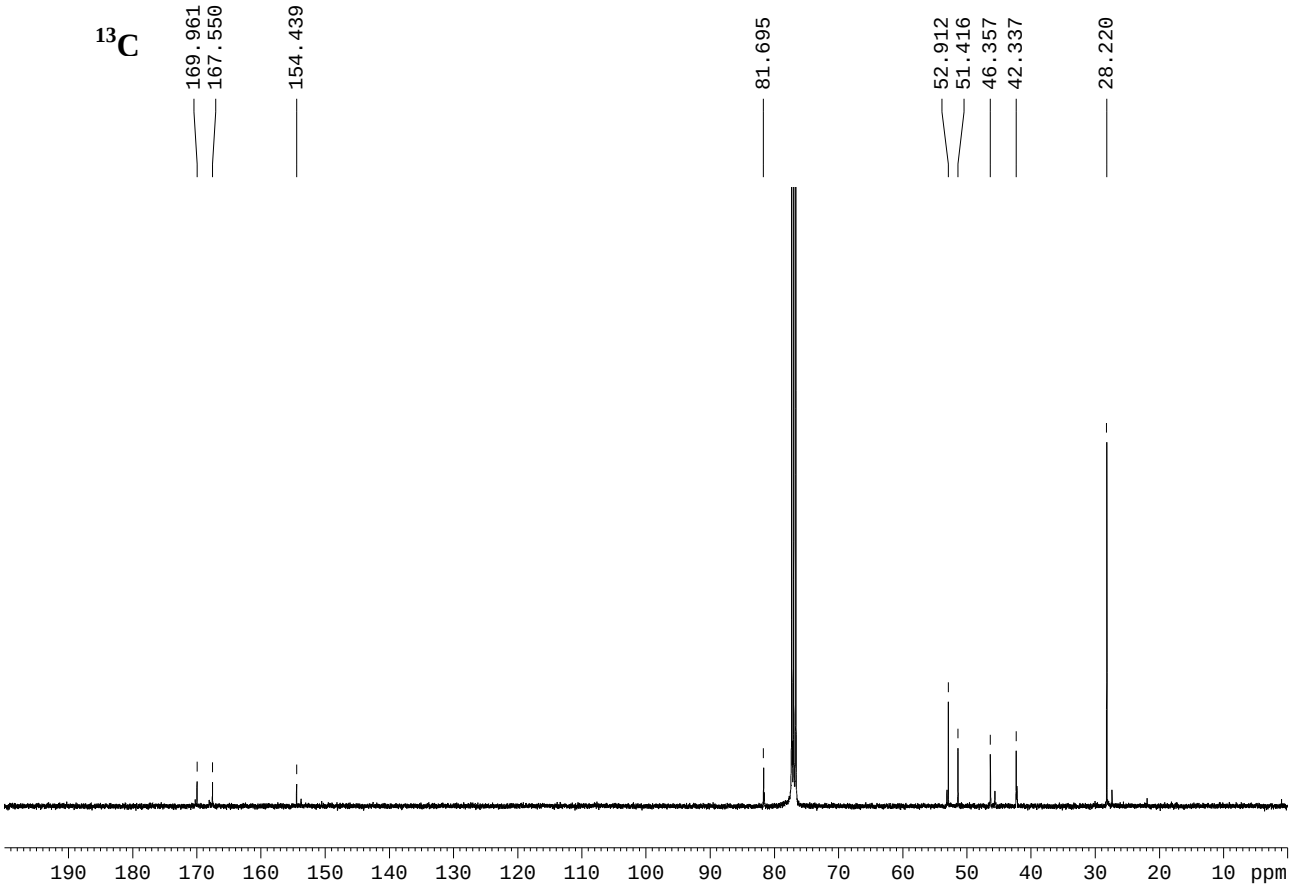
IR (CH_2Cl_2) =3410, 2980,1749, 1693.

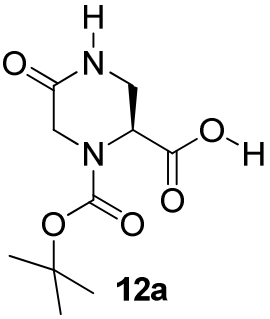
5-Oxo-piperazine-1,2-dicarboxylic acid 1-tert-butyl ester 2-methyl ester :

^{1}H



^{13}C





12a

5-Oxopiperazine-1,2-dicarboxylic acid 1-tert-butyl ester (12a)

Procedure A from the Boc Methyl ester **23.**

A solution of **23** (65mg, 0.25mmol),in 3mL of THF was treated with a solution of LiOOH (3mL, 2.7M of LiOH in 30% H_2O_2) at 0°C. The reaction was stirred for 2h (25°C).
The reaction mixture was acidified until pH=1 with saturated solution of $KHSO_4$ and the aqueous phase was extracted with EtOAc (5X10mL). The Organic phase was then dried over Na_2SO_4 and Dried under vacuo to afford the corresponding acid as a white powder (53mg, 87%)

Procedure B starting from the compound **21** one pot Boc and Methylester deprotection:

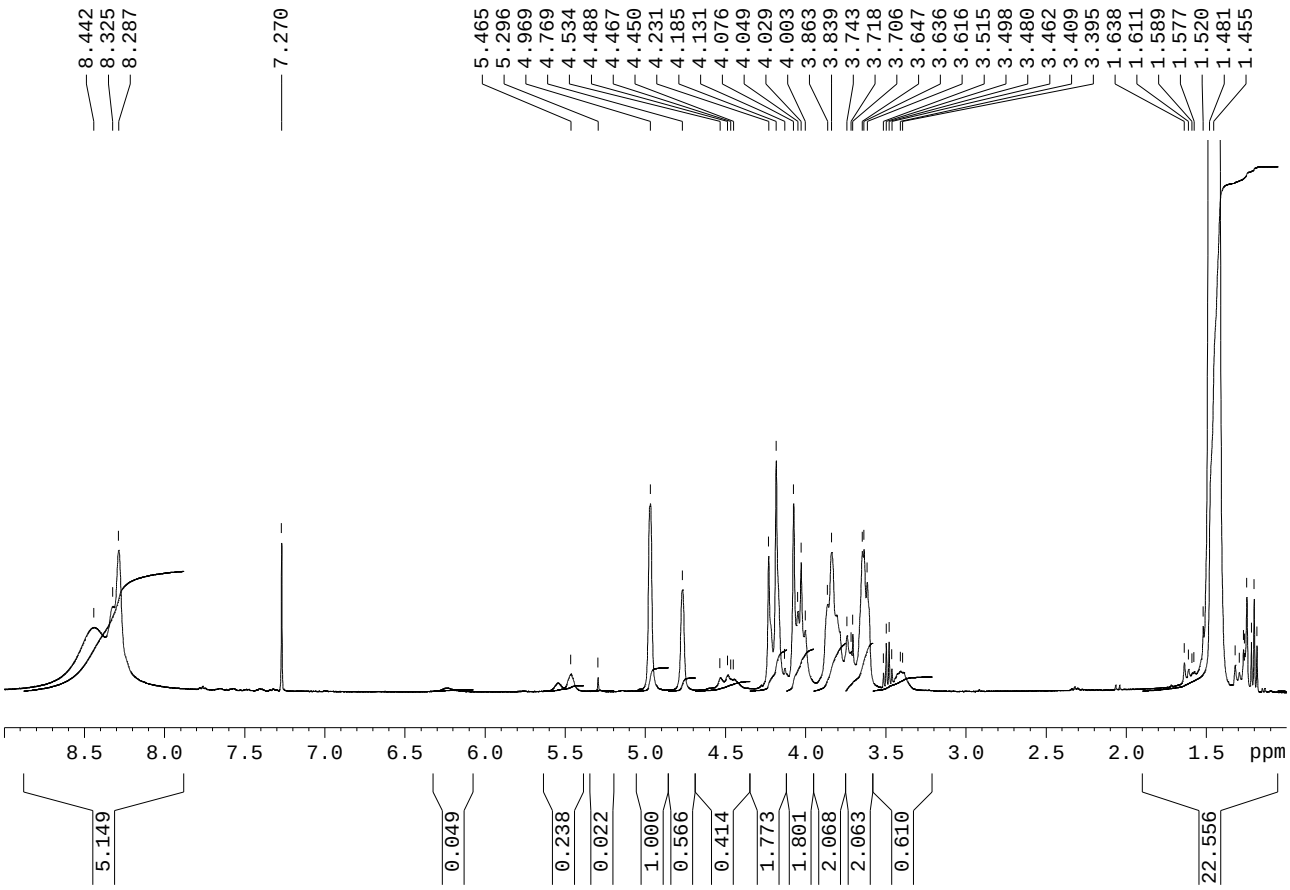
To a solution of **21** (120mg, 0.76mmol) in Dioxane 2mL/ NaOH(1M),1mL/water 1mL,at O°C, Boc_2O (331mg, 1.52mmoles) was added. After 15minutes, the ice bath was removed and the reaction was stirred for 10 h. The reaction was quenched by addition of water 5ml, and the aqueous phase was washed 2 times with ethyl acetate 3mL. At O°C, then the aqueous solution was acidified until pH=3 mediating addition of a 1M solution of KHSO4. The acid phase was finally extracted 5 times with ethyl acetate 5mL, and dried over Na_2SO_4. Subsequent evaporation under reduced pressure of the solvent, afforded the pure product **11** (135mg, 88%)as a thin white powder.

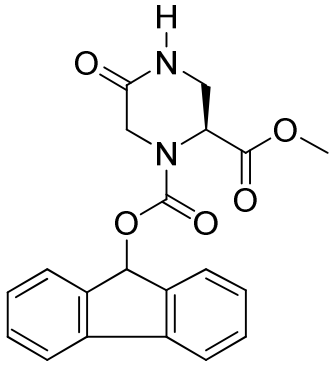
Rf=0.15(CH_2Cl_2/MeOH:97/3) $[\alpha]^{20}_D$ =-33,8 (c=0,5, CH_3OH).mp= 103,5°C. ^1H NMR (400MHz, $CDCl_3$): δ= 8.61 (d, *J*=3.21Hz, $1H_B$), 8.55 (d, *J*=4.33Hz, $1H_A$), 4.99(d, *J*=4,38Hz, $1H_A$), 4.79 (d, *J*=2,98, $1H_B$), 4.23 (d, *J*=18.88Hz, $1H_{A\&B}$), 4.07 (d, *J*=18,83Hz, $1H_A$), 4.07 (d, *J*=19.14Hz, $1H_B$), 3.88 (dd, J_1=12,60Hz, J_2=3.98Hz $1H_A$) 3.84 (dd, J_1=17.32Hz, J_2=4.10Hz, $1H_B$), 3.65 (dd, J_1=17.37Hz, J_2=4.95Hz, $1H_{A\&B}$), 1.50(s, $9H_A$), 1.48 (s, $9H_B$)ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=174.8C_A, 174.6C_B,171.1C_A, 170.6C_B, 154.8C_A, 154.1C_B, 82.3$C_{A\&B}$, 73.7C_A, 73.3C_B, 52.9C_A, 51.4C_B, 46.2C_A, 46.2C_A, 45.6C_B, 28.6$C_{A\&B}$ ppm. HRMS (EI-MS) m/z calcd for [$C_{10}H_{16}N_2O_5$] 244.1059 [M] found 244.10602

IR (CH_2Cl_2) =3408, 3230, 1707, 1647.

5-Oxopiperazine-1,2-dicarboxylic acid 1-tert-butyl ester (12a)

^1H





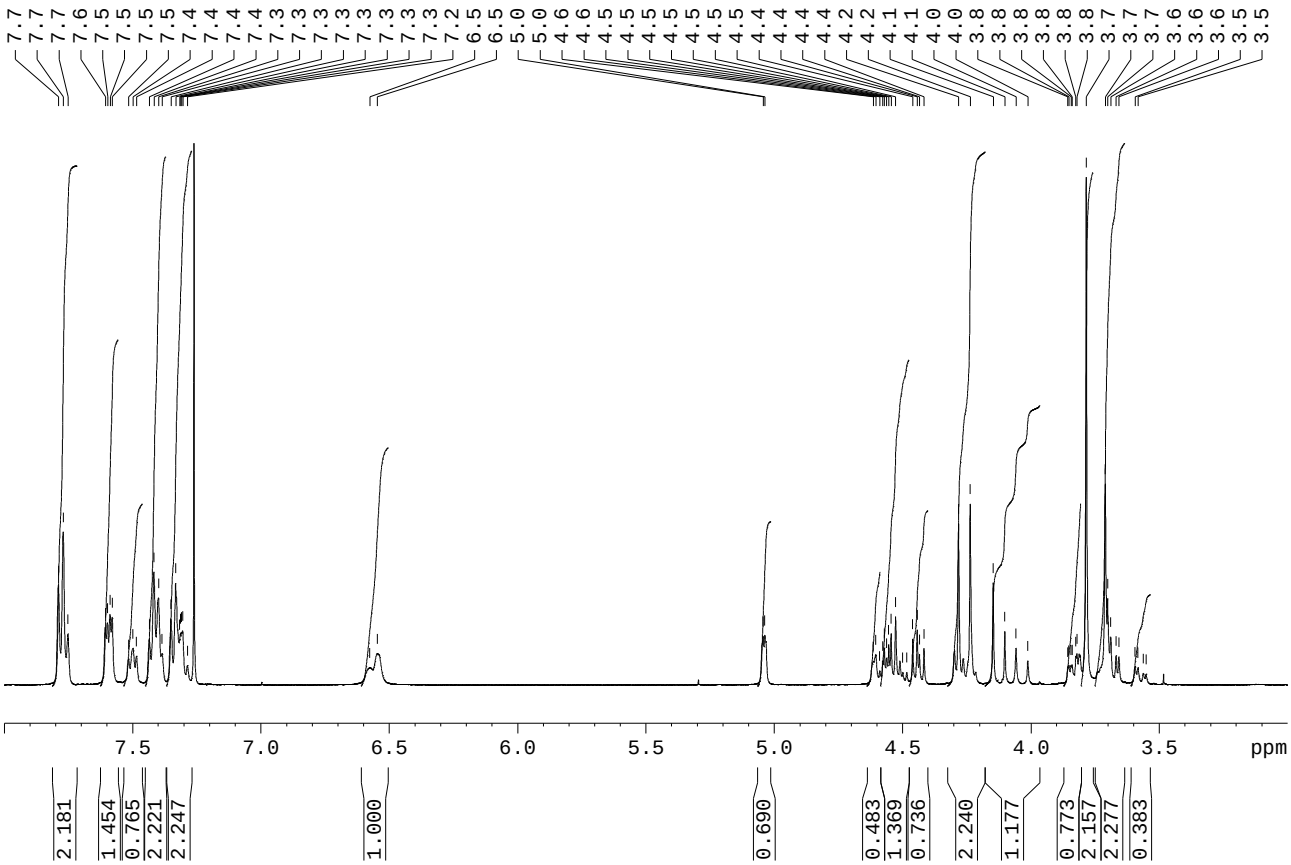
22

5-Oxopiperazine-1,2-dicarboxylic acid 1-(fluoren-9-yl) ester 2-methyl ester 22.

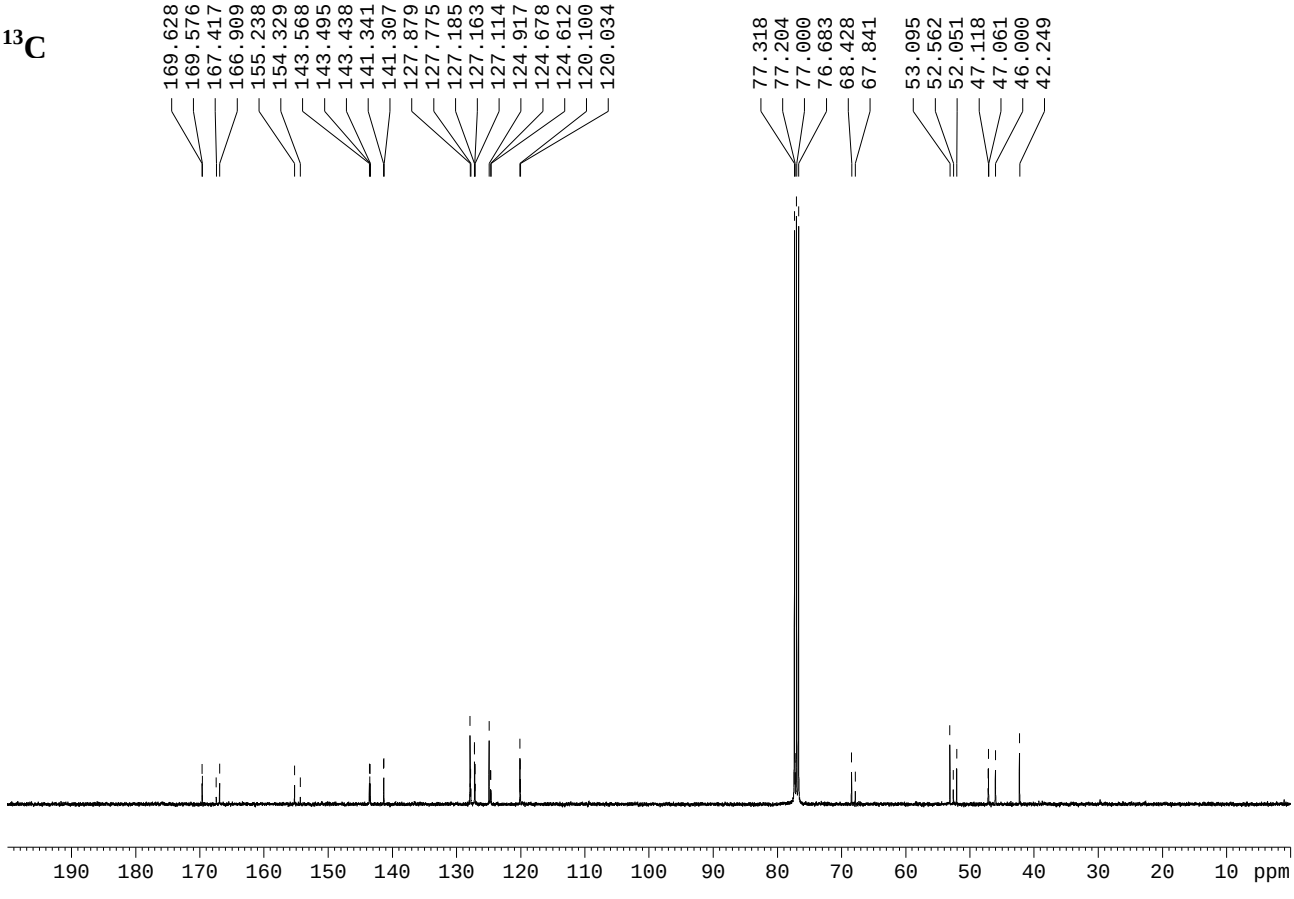
To a solution of **21** (128mg, 0.81mmol), in 2:1 water-dioxane (3mL) $NaHCO_3$ (136mg, 1.61mmol)was added. The mixture was cooled to 0°C with a ice bath, then a ,solution of Fmoc-Cl (209mg, 0.81mmol) in 1.5mL of dioxane was added dropwise over 15 min. The ice bath was removed and the reaction mixture was left stirring for 2.5h. Successively, the mixture was partitioned between EtOAc 14mL and water 7mL, and the organic phase was washed with with 1M HCl and brine, then dried over Na_2SO_4. The organic solvents were removed and the crude product was purified by flash column chromatography (DCM/MeOH: 95/5) thus giving the pure fmoc protected compound **22** (258mg, 87%).
Rf=0.45(CH_2Cl_2/MeOH:96/4) $[\alpha]^{20}_D$ =-11.9 (c=1, $CHCl_3$).mp= 144°C. ^{1}H NMR (400MHz, $CDCl_3$): δ= 6.57(bs,1H_B), 6,54(bs, 1H_A), 5,04 (t, *J*=2Hz, 1H_A), 4.61(bs, 1H_B),4.60-4.49(m, 2$H_{A\&B}$), 4.44 (dd, J_1=7.2Hz,J_2=3.6Hz, 1H_A), 4.28-4.15 (m, 1H_A+1$H_{A\&B}$), 4.12 (d, *J*=18Hz 1H_A), 4.03 (d, *J*=18.4Hz, 1H_B) 3.84 (dd, J_1=5.2Hz, J_2=1.6Hz, 1H_B), 3.81 (dd, J_1=3.6Hz, J_2=1.6Hz 1H_B), 3.78(s, 3H_A), 3.71(s, 3H_B), 3.68(dd, J_1=13.2Hz, J_2=4.8Hz, 1H_A), 3.57(dd, J_1=12.8Hz, J_2=4.4Hz, 1H_A) ppm. ^{13}C NMR (400MHz, $CDCl_3$): δ=169.6(A), 169.5(B), 167.4(B), 166.9(A), 155.2(A), 154.3(B), 143.5(A&B), 143.4(A&B), 141.3(A&B), 141.3(A&B), 127.8(A), 127.7(B), 127.1(A), 127.1(B), 127.1(A&B), 124.9(A), 124.6(B), 120.1(A), 120.0(B), 68.4(A), 67.8(B), 53.1(A&B), 52.5(B), 52.0(A), 47.1(A), 47.0(B), 46.0(A&B), 42.2(A&B) ppm. $C_{21}H_{20}N_2O_5$+1.5H_2O (380.40): calcd. C 61.90%, H 5.70%, N 6.88%; found C61,95%, H5,31%, N6,69%.

5-Oxopiperazine-1,2-dicarboxylic acid 1-(fluoren-9-yl) ester 2-methyl ester (22).

^{1}H



^{13}C



X-Ray crystallographic data : C_{21} H_{20} N_2 O_5 ; MW=380.39g.mol^{-1}; T= 123 K; λ(Mo Kα)=1.54184 A; Orthorhombic, space group P 21 21 21, a = 6.5176(2) A, b = 11.6690(5) A, c=23.9190(9) A, α = 90°,.β= 90°, γ = 90°.,V=;1819.13(12) A^3, ρ_{calc}=1.389 mg.m^{-3}, Z=4; μ(Mo, Kα)= 0.828mm^{-1}, R1 = 0.0283, wR2 = 0.0713, for 2679 unique data collected in the 3.70-66.5° θ range.

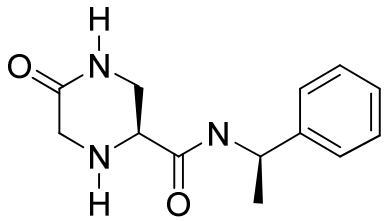
Selected bond distances and bond angles:

Bond Distances

O(1)-C(14);1.453(2)
O(1)-C(15);1.345(2)
O(2)-C(15);1.2206(18)
O(3)-C(17);1.227(2)
O(4)-C(20);1.335(2)
O(4)-C(21);1.449(2)
O(5)-C(20);1.199(2)
N(1)-C(15);1.353(2)
N(1)-C(16);1.459(2)
N(1)-C(19);1.451(2)
N(2)-C(17);1.338(2)
N(2)-C(18);1.457(2)
N(2)-H(2A);0.8800
C(1)-C(13);1.511(3)
C(1)-C(6);1.401(3)
C(16)-C(17);1.508(3)
C(18)-C(19);1.515(3)
C(19)-C(20);1.531(2)
C(16)-H(16A);0.9900
C(16)-H(16B);0.9900
C(18)-H(18A);0.9900
C(18)-H(18B);0.9900
C(19)-H(19);1.0000
C(21)-H(21A);0.9800
C(21)-H(21B);0.9800
C(21)-H(21C);0.9800

Bond angles

C(14)-O(1)-C(15);115.07(12)
C(20)-O(4)-C(21);115.75(14)
C(15)-N(1)-C(16);117.02(13)
C(15)-N(1)-C(19);125.58(13)
C(16)-N(1)-C(19);116.18(13)
C(17)-N(2)-C(18);124.93(15)
C(17)-N(2)-H(2A);118.00
C(18)-N(2)-H(2A);118.00
O(2)-C(15)-N(1);123.20(15)
O(1)-C(15)-N(1);112.43(13)
O(1)-C(15)-O(2);124.37(14)
N(1)-C(16)-C(17);114.02(15)
N(2)-C(17)-C(16);119.06(15)
O(3)-C(17)-N(2);121.64(17)
O(3)-C(17)-C(16);119.30(17)
N(2)-C(18)-C(19);109.62(15)
N(1)-C(19)-C(20);112.09(15)
C(18)-C(19)-C(20);110.91(14)
N(1)-C(19)-C(18);108.29(13)
O(5)-C(20)-C(19);124.22(17)
O(4)-C(20)-O(5);124.18(16)
O(4)-C(20)-C(19);111.59(15)
N(1)-C(16)-H(16A);109.00
N(1)-C(16)-H(16B);109.00
C(17)-C(16)-H(16A);109.00
C(17)-C(16)-H(16B);109.00
H(16A)-C(16)-H(16B);108.00
N(2)-C(18)-H(18A);110.00
N(2)-C(18)-H(18B);110.00
C(19)-C(18)-H(18A);110.00
C(19)-C(18)-H(18B);110.00
H(18A)-C(18)-H(18B);108.00
N(1)-C(19)-H(19);108.00
C(18)-C(19)-H(19);109.00
C(20)-C(19)-H(19);109.00
O(4)-C(21)-H(21A);109.00
O(4)-C(21)-H(21B);109.00
O(4)-C(21)-H(21C);109.00
H(21A)-C(21)-H(21B);109.00
H(21A)-C(21)-H(21C);109.00
H(21B)-C(21)-H(21C);109.00

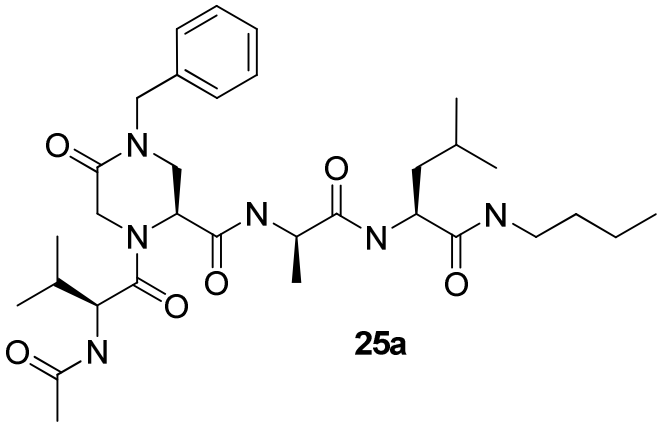


24

5-Oxo-piperazine-2-carboxylic acid (1-phenyl-1ethyl)-amide 24 :

To a solution of **12a** (30mg, 0.123mmol)in DMF(2mL), HOBt (21mg,0.135mmol), (R)α-Methylbenzylamine (24µL,0.184mmol) and DIPEA(4.2µL, 0.246mmol) were added. The solution was cooled to 0°C with an ice bath and EDC.HCl (26mg, 0.135mmol) was added. The reaction mixture was stirred overnight letting the reaction medium rise at room temperature. 5mL of DCM were added to the medium, and the organic phase was washed sequentially with an acid solution of $KHSO_4$ (2times, 3mL), with a saturated solution of $NaHCO_3$ (2 times 3mL) and finally with brine (2 times 3mL). the Organic layer was then dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was analysed by NMR, and directly diluted in a solution of DCM/TFA (1mL/1mL). The solution was stirred for 2 hours at room temperature. The excess of TFA was then removed under reduced pressure using several additions of methanol. The TFA salt was then diluted in water (2mL) and the solution was cooled at O°C with an ice bath. At this temperature, the solution was neutralized mediating a saturated solution of $NaHCO_3$ until pH=7. And the neutral aqueous phase was extracted 5 times with 4mL of ethyl acetate. The combined organic phases were then dried over Na_2SO_4 and the solvent was removed under vacuo to give the desired unprotected product **24** in acceptable yield (22mg, 72%).

V-5 Tetrapeptides

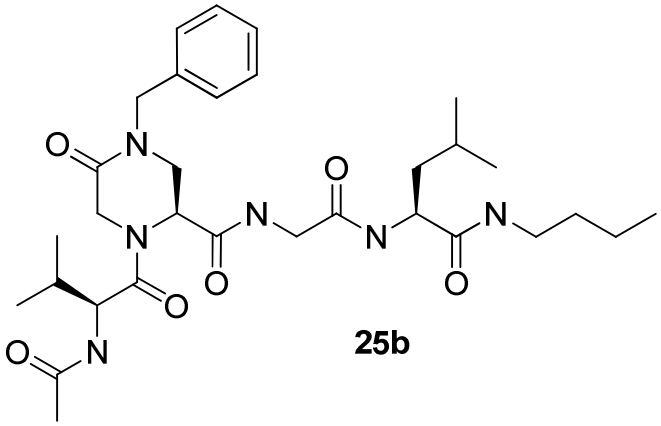


25a

AcVal-L-(Bn)Pca-D-Ala-LeuNBu (25 a)

To a solution of Ac-L-Val (26 mg, 0.165 mmol) in dry DMF (200 μL) and collidine (22 μL, 0.165mmol), under a nitrogen atmosphere and at 0°C, HATU (63 mg, 0.14 mmol) was added. After 1h a solution of H-Pca-Gly-L-Leu-NHBu (50 mg, 0.11 mmol) in dry DMF (300 μL) and DIPEA (28μL, 0.165 mmol) was slowly added and the reaction was stirred at 0°C for 10 minutes and at RT for a week-end. The mixture was diluted with EtOAc (15 mL) and the organic phase was washed in order with: 1 M $KHSO_4$ (2×10 mL), aqueous $NaHCO_3$ (2×10 mL) and brine (2×10 mL), dried over Na_2SO_4 and volatiles were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (DCM/MeOH 94:6) several times to afford the desired product as a white solid in mg scale.

^{1}H NMR (400MHz, $CDCl_3$): δ=7.53 (d, *J*=8Hz, 1H), 7 .48-7.24(m, 5H), 6.89 (d, *J*=8.28Hz, 1H), 6.70 (br s, 1H), 6.59 (br s, 1H), 5.10(m, 2H), 4.66(d, *J*=16.78, 1H), 4.68-4.35(m,3H), 4.20-4.16 (m,2H), 3.94 (dd, *J* =13.11 Hz, *J*=3.04Hz, 1H), 3.40 (dd, *J* =13.01 Hz, *J*=4.41Hz, 1H), 3.20 (m, 2H), 2.10-2.02(m,4H), 1.80-1.77(m, 1H), 1.61-1.57(m, 2H), 1.54-1.43(m, 3H), 1.40-1.27(m,4H), 1.08(d, *J*=6.64Hz, 3H), 1.00 (d, *J*=6.73, 3H), 0.9-0.8(m, 9H)ppm. ^{13}C NMR (100MHz, $CDCl_3$): δ=172.8, 172.4, 172.2, 168.2, 164.3, 135.9, 129.2, 128.6, 128.3, 56.8, 53.3, 52.4, 50.9, 50.4, 48.3, 46.4, 40.9, 39.6, 31.9, 30.3, 25.3, 23.3,22.9, 22.2, 20.4, 19.9, 19.4, 18.0, 14.16 ppm.

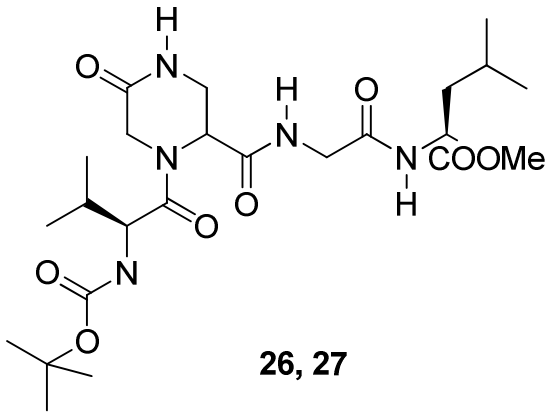


25b

AcVal-L-(Bn)Pca-Gly-LeuNBu (25 b):

To a solution of Ac-L-Val (23 mg, 0.14 mmol) in dry DMF (200 μL) and collidine (18.5 μL, 0.14mmol), under a nitrogen atmosphere and at 0°C, HATU (53 mg, 0.14 mmol) was added. After 1h a solution of Pca-Gly-L-Leu-OMe TFA salt (43 mg, 0.093 mmol) in dry DMF (300 μL) and DIPEA (24 μL, 0.14 mmol) was slowly added and the reaction was stirred at 0°C for 10 minutes and at RT for a week-end. The mixture was diluted with EtOAc (15 mL) and the organic phase was washed in order with: 1 M $KHSO_4$ (2×10 mL), aqueous $NaHCO_3$ (2×10 mL) and brine (2×10 mL), dried over Na_2SO_4 and volatiles were removed under reduced pressure. The residue was purified by flash chromatography on silica gel several times (DCM/MeOH 94:6) to afford the desired product as a white solid in mg scale.

1H NMR (400MHz, CDCl3):δ=7.49 (d, J=7.7 Hz,1H), 7.34(d, J=7.08 Hz, 2H), 7.26 (d, J=6.7 Hz, 2H), 6.8 (d, J=8 Hz, 1H), 6.61 (m, 1H), 6.29 (m, 1H), 5.1 (dd, J=10 Hz, J=4.47 Hz, 2H), 4.65(d, J=16 Hz,1H), 4.38(m,2H), 4.34 (s,1H), 4.18 (m,1H), 4.16(s,1H),3.96(dd,J=12Hz,1H),3.83(s,1H), 3.66 (s,1H), 3.40 (dd, J=4 Hz, J=12.7 Hz, 1H), 3.22 (m, 2H), 2.06 (s, 3H), 1.17 (m, 2H), 1.6 (m, 2H), 1.47(m, 2H), 1.34 (m,2H), 1.27(s, 1H), 1.1 (d, J=6.6 Hz, 3H), 1.01(d, J= 6.6 Hz, 3H), 0.96 (d, J=6 Hz, 3H), 0.92(d, J=7 Hz, 6H) ppm.13C NMR (400MHz, CDCl3): δ=172.72, 172.22, 169.53, 169.28, 164.64, 129.25, 128.80, 128.53, 56.87, 54.79, 52.62, 50.89, 48.53, 46.13, 44.13, 40.68, 39.69, 31.76, 30.36, 30.13, 25.28, 23.56, 22.86, 21.85, 20.39, 19.37, 14.18 ppm.



26, 27

Boc-Val-Pca-Gly-Leu-COOMe 26, 27.

To a solution of Boc-L-Val (15 mg, 0.07 mmol, 1.5 eq) in dry DMF (200 μL) and DIPEA (16 μL, 0.094 mmol, 2.0 eq), under a nitrogen atmosphere and at 0°C, HATU (27 mg, 0.07 mmol, 1.5 eq) was added. After 1h a solution of PCA-Gly-L-Leu-OMe TFA salt (20 mg, 0.047 mmol) in dry DMF (300 μL) and DIPEA (24 μL, 0.14 mmol, 3.0 eq) was slowly added and the reaction was stirred at 0°C for 10 minutes and at RT for a week-end. The mixture was diluted with EtOAc (15 mL) and the organic phase was washed in order with: 1 M $KHSO_4$ (2×10 mL), aqueous $NaHCO_3$ (2×10 mL) and brine (2×10 mL), dried over Na_2SO_4 and volatiles were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (DCM/MeOH 94:6) to afford the desired product as a white solid (15 mg, 68% (**L-Pca**); 14 mg, 64% (**D-Pca**)

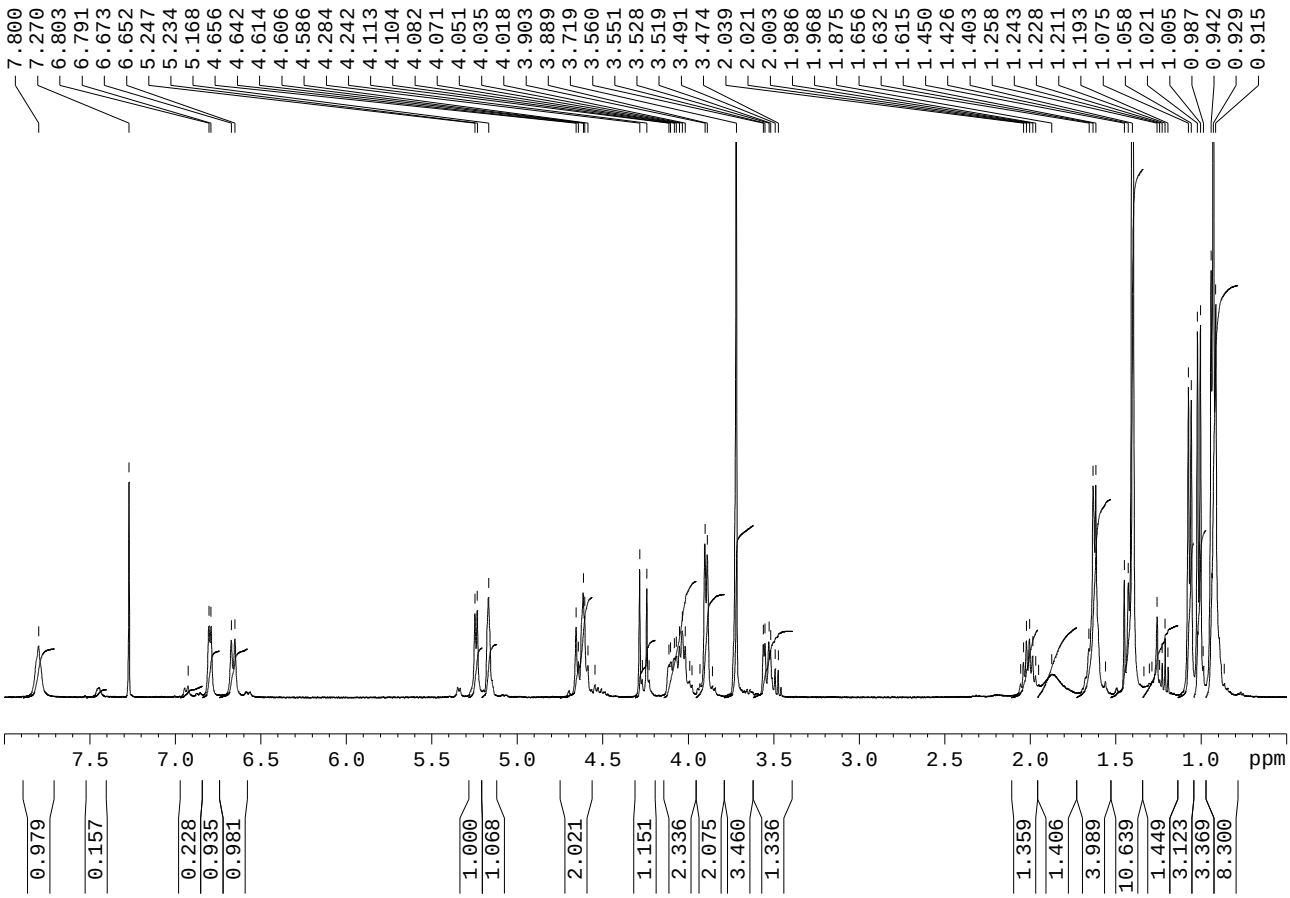
.

Boc-Val-(D)-Pca-Gly-Leu-COOMe 26

^{1}H NMR (400MHz, $CDCl_3$): δ=7.80 (br s, 1H), 6.79(d, *J*=4.8Hz, 1H), 6.66 (d, *J*=8.4Hz, 1H), 5.24 (d, *J*=5.2Hz 1H), 5.16 (br s, 1H), 4.65-4.64 (m, 2H), 4.25(dd, *J*=16.8Hz, *J*=6.0Hz, 1H), 4.11-3.90(m, 2H), 3.89 (d, *J*=5.6Hz, 2H), 3.71 (s, 3H), 3.53 (dd, *J* =12.80 Hz, *J*=3.6Hz, 1H), 2.02 (m, 1H), 1.64 (m,2H), 1.40 (s, 9H), 1.06 (d, *J*=6.8Hz, 3H), 1.01 (d, *J*=6.8Hz, 3H) 0.9 (m, 6H)ppm

Boc-Val-(D)-Pca-Gly-Leu-COOMe 26

^{1}H



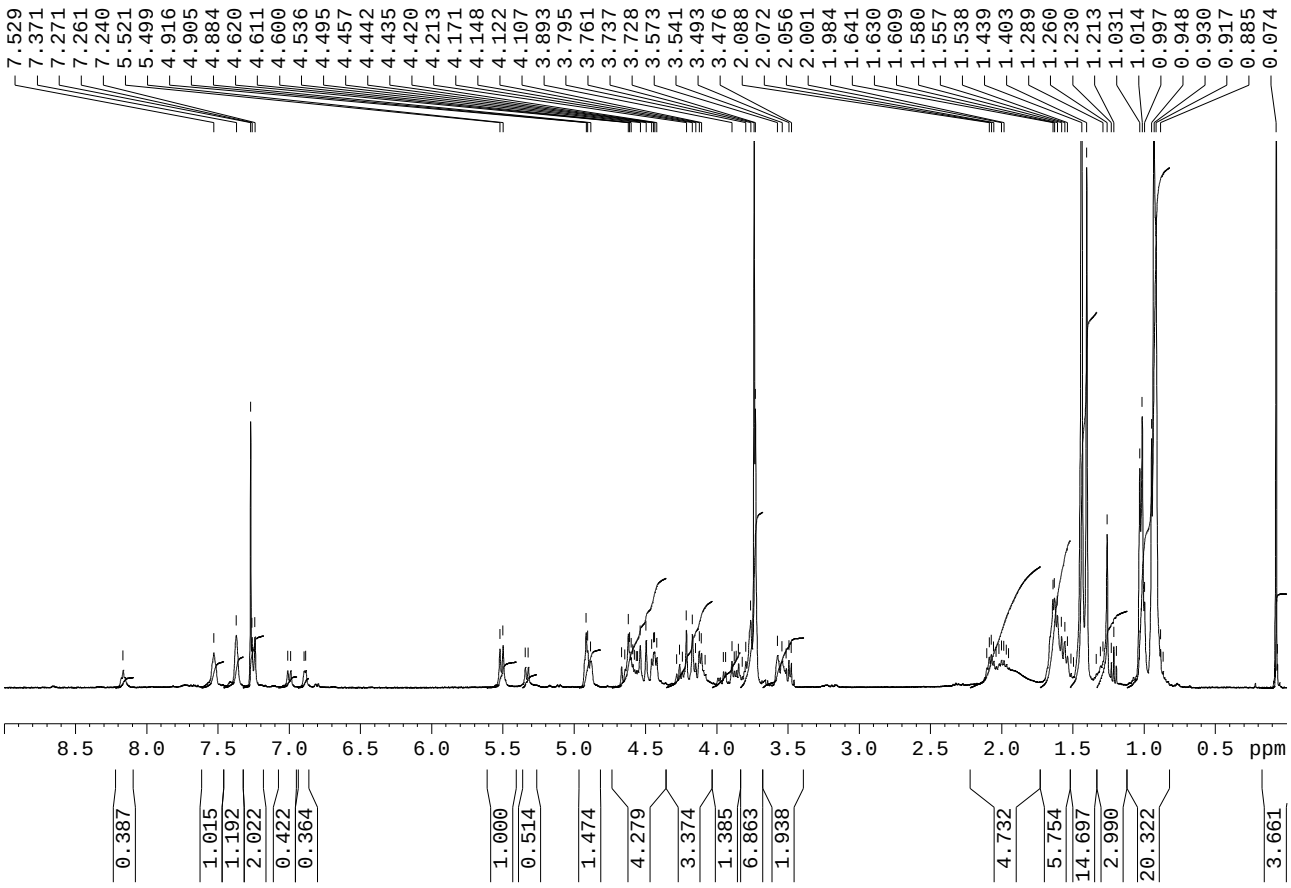
.

Boc-Val-(L)-Pca-Gly-Leu-COOMe 27

Two conformers in proportions 2 (conformer a) to 1(conformer b) ^{1}H NMR (400MHz, $CDCl_3$): δ=8.16 (br s, 1Hb), 7.53(br s, 1Ha), 7.37(br s, 1Ha), 7.25 (d, *J*=8.4Hz 1Ha), 7.00 (d , , *J*=8Hz,1Hb), 6.89 (d, *J*=4.8Hz 1Hb), 5.51(d, *J*=8.0Hz, 1Ha), 5.33 (d, 1Hb), 4.91-4.88 (m, 1Ha and 1Hb), 4.66-4.42 (m, 4Ha and b), 4.28-4.08 (m, 3Ha and b), 3.99-3.79 (m, 1Ha and b), 3.77-3.72 (m, 5Ha and b) 1.64-1.49 (m, 3Ha and b), 1.43(s, 9Ha) 1.40(s, 9Hb), 1.31-0.86 (m,12 H)ppm.

Boc-Val-L-Pca-Gly-Leu-COOMe 27

^{1}H



VI – REFERENCES – CHAPTER II,III,IV,V

(1) Balasubramanian, R.; Lakshminarayanan, A. V.; Sabesan, M. N.; Tegoni, G.; Venkatesan, K.; Ramachandran, G. N. *Int. J. Protein Res.* **1971**, *3*, 25-33.

(2) Morris, A. L.; MacArthur, M. W.; Hutchinson, E. G.; Thornton, J. M. *Proteins* **1992**, *12*, 345-364.

(3) Hurley, J. H.; Mason, D. A.; Matthews, B. W. *Biopolymers* **1992**, *32*, 1443-1446.

(4) MacArthur, M. W.; Thornton, J. M. *J. Mol. Biol.* **1991**, *218*, 397-412.

(5) Galat, A. *Eur. J. Biochem.* **1993**, *216*, 689-707.

(6) Vanhoof, G.; Goossens, F.; De Meester, I.; Hendriks, D.; Scharpe, S. *Faseb. J.* **1995**, *9*, 736-744.

(7) Hobbs, M. V.; Morgan, E. L.; Houghten, R. A.; Thoman, M. L.; Weigle, W. O. *J. Immunol.* **1987**, *138*, 2581-2586.

(8) Fridkin, M.; Najjar, V. A. *Crit. Rev. Biochem. Mol. Biol.* **1989**, *24*, 1-40.

(9) Kawasaki, K.; Hirase, K.; Miyano, M.; Tsuji, T.; Iwamoto, M. *Chem. Pharm. Bull.* **1992**, *40*, 3253-3260.

(10) Frank, R. W.; Gennaro, R.; Schneider, K.; Przybylski, M.; Romeo, D. *J. Biol. Chem.* **1990**, *265*, 18871-18874.

(11) Mentlein, R. *FEBS Lett.* **1988**, *234*, 251-256.

(12) Ju, G.; Collins, L.; Kaffka, K. L.; Tsien, W. H.; Chizzonite, R.; Crowl, R.; Bhatt, R.; Kilian, P. L. *J. Biol. Chem.* **1987**, *262*, 5723-5731.

(13) Williamson, M. P. *Biochem. J.* **1994**, *297 (Pt 2)*, 249-260.

(14) Hagerman, A. E.; Butler, L. G. *J. Biol. Chem.* **1981**, *256*, 4494-4497.

(15) Gendler, S. J.; Lancaster, C. A.; Taylor-Papadimitriou, J.; Duhig, T.; Peat, N.; Burchell, J.; Pemberton, L.; Lalani, E. N.; Wilson, D. *J. Biol. Chem.* **1990**, *265*, 15286-15293.

(16) Koleske, A. J.; Buratowski, S.; Nonet, M.; Young, R. A. *Cell* **1992**, *69*, 883-894.

(17) Ramachandran, G. N.; Kartha, G. *Nature* **1955**, *176*, 593-595.

(18) Vandenberg, P.; Kern, A.; Ries, A.; Luckenbill-Edds, L.; Mann, K.; Kuhn, K. *J. Cell. Biol.* **1991**, *113*, 1475-1483.

(19) Chellgren, B. W.; Creamer, T. P. *Biochemistry* **2004**, *43*, 5864-5869.

(20) Williams, D. A., Deber, C. M. *Biochemistry* **1991**, *30*, 8919-8923.

(21) Wilmot, C. M.; Thornton, J. M. *J. Mol. Biol.* **1988**, *203*, 221-232.

(22) Chou, P. Y.; Fasman, G. D. *J. Mol. Biol.* **1977**, *115*, 135-175.

(23) Frömmel, C.; Preissner, R. *FEBS Lett.* **1990**, *277*, 159-163

(24) Fischer, G. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1415-1436

(25) Baruah, P. K.; Sreedevi, N. K.; Gonnade, R.; Ravindranathan, S.; Damodaran, K.; Hofmann, H. J.; Sanjayan, G. J. *J. Org. Chem.* **2007**, *72*, 636-639.

(26) Srinivas, D.; Gonnade, R.; Ravindranathan, S.; Sanjayan, G. J. *J. Org. Chem.* **2007**, *72*, 7022-7025.

(27) Che, Y. M., G. R. *J. Med. Chem.* **2006**, *49*, 111-124.

(28) Park, K.-H., Kurth, M. J. *Tetrahedron* **2002**, *58*, 8629-8659.

(29) Toda, A.; Aoyama, H.; Mimura, N.; Ohno, H.; Fujii, N.; Ibuka, T. *J Org Chem* **1998**, *63*, 7053-7061.

(30) Mc Coull, W. Davies, F. A. *Synthesis* **2000**, *10*. 1347-1345

(31) Lee, K. D. Suh., J.M.; Park, J.H.; Ha, H.J.; Choi, H.G.; Park, C.S.;. Chang, J.W.; Lee, W.K.; Dong, Y.; Yun, H. *Tetrahedron* **2001**, *57*, 8267-8276.

(32) Goodman, M.; Shao, H. *Pure and Applied Chemistry* **1996**, *68*, 1303-1308.

(33) Boukhris, S.; Souizi, A. *Tet Lett.* **2003**, *44*, 3259-3261.

(34) Diaper, C. M.; Sutherland, A.; Pillai, B.; James, M. N.; Semchuk, P.; Blanchard, J. S.; Vederas, J. C. *Org. Biomol. Chem.* **2005**, *3*, 4402-11.

(35) Miniejew, C.; Outurquin, F.; Pannecoucke, X. *Tetrahedron* **2006**, *62*, 2657-2670.

(36) Fioravanti, S.; Massari, D.; Morreale, A.; Pellacani, L.; Tardella, P. A. *Tetrahedron* **2008**, *64*, 3204-3211.

(37) Hayashi, T. Asai, T.; Ogoshi, H. *Tet. Lett.* **1997**, *38*, 3039-3042.

(38) Henery-Logan, K. R.; Limburg, A. M. *Tet. Lett.* **1966**, *38*, 4615-4620.

(39) Korn, A.; Rudolphbohner, S.; Moroder, L. *Tetrahedron* **1994**, *50*, 1717-1730.

(40) Fowden, L. *Nature* **1955**, *176*, 347-348.

(41) Takemoto, T.; Nomoto, K.; Fushiya, S.; Ouchi, R.; Kusano, G.; Hikino, H.; Takagi, S. I.; Matsuura, Y.; Kakudo, M. *Proceedings of the Japan Academy Series B-Physical and Biological Sciences* **1978**, *54*, 469-473.

(42) Kinoshita, E.; Yamakoshi, J.; Kikuchi, M. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 1107-1110.

(43) Ericksson, B. I. Carlsson., S.; Halvarsson, M.; Risberg, B.; Mattsson, C. *Thromb. Haemostasis* **1997**, *78*. 1404-1407.

(44) Park, K. H.; Kurth, M. J. *Tetrahedron* **2002**, *58*, 8629-8659.

(45) Brandi, A.; Cicchi, S.; Cordero, F. M. *Chem. Rev.* **2008**, *108*, 3988-4035.

(46) Couty, F.; Evano, G.; Vargas-Sanchez, M.; Bouzas, G. *J. Org. Chem.* **2005**, *70*, 9028-9031.

(47) Futamura, Y.; Kurokawa, M.; Obata, R.; Nishiyama, S.; Sugai, T. *Biosci. Biotechnol., Biochem.* **2005**, *69*, 1892-1897.

(48) Hanessian, S.; Bernstein, N.; Yang, R. Y.; Maguire, R. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1437-1442.

(49) Fernandez-Megia, E.; Montaos, M. A.; Sardina, F. J. *J. Org. Chem.* **2000**, *65*, 6780-6783.

(50) Kiss, L.; Mangelinckx, S.; Fulop, F.; De Kimpe, N. *Org. Lett.* **2007**, *9*, 4399-4402.

(51) Takeuchi, T.; Prockop, D. J. *Biochim. Biophys. Acta.* **1969**, *175*, 142-55.

(52) Takeuchi, T.; Rosenbloom, J.; Prockop, D. J. *Biochim. Biophys. Acta* **1969**, *175*, 156-64.

(53) Zagari, A.; Nemethy, G.; Scheraga, H. A. *Biopolymers* **1990**, *30*, 951-9.

(54) Zagari, A.; Palmer, K. A.; Gibson, K. D.; Nemethy, G.; Scheraga, H. A. *Biopolymers* **1994**, *34*, 51-60.

(55) Zagari, A.; Nemethy, G.; Scheraga, H. A. *Biopolymers* **1990**, *30*, 967-74.

(56) Timothy J. Deming, M. J. F., Thomas L. Mason, and David A. Tirrell *Macromolecules* **1996**, *29* 1442 -1444.

(57) Baeza, J. L.; Gerona-Navarro, G.; de Vega, M. J. P.; Garcia-Lopez, M. T.; Gonzalez-Muniz, R.; Martin-Martinez, M. *Tet. Lett.* **2007**, *48*, 3689-3693.

(58) Baeza, J. L.; Gerona-Navarro, G.; Perez de Vega, M. J.; Garcia-Lopez, M. T.; Gonzalez-Muniz, R.; Martin-Martinez, M. *J Org Chem* **2008**, *73*, 1704-15.

(59) Taylor, C. M. H., R., Edwards, P. J. B. *J. Org. Chem;* **2005**, *70*, 1306-1315.

(60) Karoyan, P.; Sagan, S.; Lequin, O.; Quancard, J.; Lavielle, S.; Chassaing, G. *Targets Heterocyclic. syst.* **2004**, *8*, 216-273.

(61) Calaza, M. I.; Cativiela, C. *Eur. J. Org. Chem.* **2008**, 3427-3448.

(62) Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* **1983**, *105*, 5390-5398.

(63) Zubia, A.; Mendoza, L.; Vivanco, S.; Aldaba, E.; Carrascal, T.; Lecea, B.; Arrieta, A.; Zimmerman, T.; Vidal-Vanaclocha, F.; Cossio, F. P. *Angew. Chem. Int. Ed. Eng.l* **2005**, *44*, 2903-2907.

(64) Duvall, J. R.; Wu, F.; Snider, B. B. *J. Org. Chem.* **2006**, *71*, 8579-90.

(65) Kawabata, T.; Kawakami, S.; Majumdar, S. *J. Am. Chem. Soc.* **2003**, *125*, 13012-3.

(66) Kawataba, T. M., K.; Kawakami, S.; Tsubaki, K. *J. Am. Chem. Soc.* **2008**, *130*, 4153-4157.

(67) Kawabata, T.; Matsuda, S.; Kawakami, S.; Monguchi, D.; Moriyama, K. *J. Am. Chem. Soc.* **2006**, *128*, 15394-15395.

(68) Mothes, C.; Lavielle, S.; Karoyan, P. *J. Org. Chem.* **2008**, *73*, 6706-6710.

(69) Quancard, J.; Labonne, A.; Jacquot, Y.; Chassaing, G.; Lavielle, S.; Karoyan, P. *J. Org. Chem.* **2004**, *69*, 7940-7948.

(70) Karoyan, P.; Quancard, J.; Vaissermann, J.; Chassaing, G. *J. Org. Chem.* **2003**, *68*, 2256-2265.

(71) Tamaki, M.; Han, G. X.; Hruby, V. J. *J. Org. Chem.* **2001**, *66*, 3593-3596.

(72) Gomez-Vidal, J. A.; Silverman, R. B. *Org. Lett.* **2001**, *3*, 2481-2484.

(73) Houda, H. I.; Lubell, W. D. *J. Org. Chem. Soc.* **1993**, *58*, 6438-6441.

(74) Delaney, N. G.; Madison, V. *Int. J. Pep. Prot. Res.* **1982**, *19*, 543-548.

(75) Hinds, M. G.; Welsh, J. H.; Brennand, D. M.; Fisher, J.; Glennie, M. J.; Richards, N. G. J.; Turner, D. L.; Robinson, J. A. *J. Med. Chem.* **1991**, *34*, 1777-1789.

(76) Bisang, C.; Weber, C.; Inglis, J.; Schiffer, C. A.; Vangunsteren, W. F.; Jelesarov, I.; Bosshard, H. R.; Robinson, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 7904-7915.

(77) Genin, M. J.; Johnson, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 8778-8783.

(78) Bittermann, H.; Gmeiner, P. *J Org Chem* **2006**, *71*, 97-102.

(79) Gramberg, D.; Robinson, J. A. *Tet. Lett.* **1994**, *35*, 861-864.

(80) Halab, L.; Lubell, W. D. *J. Org. Chem.* **1999**, *64*, 3312-3321.

(81) Takana, H. K., A.; Marusawa, H.; Hatanaka, H.; Kino,T.; Goto, T.; Hashimoto, M. *J. Am. Chem. Soc.* **1987**, *109*, 5031-5033.

(82) Germann, U. A. S., D.; Mason, V. S.; Zelle, R. E.; Duffy, J. P.; Gallulo, V.; Armistead, D. M.; Saunders, J. O.; Boger, J.; Harding, M. W. *Anticancer Drugs* **1997**, *8*, 125-140.

(83) Boger, D. L. C., J. H.; Saionz, K. W. *J. Am. Chem. Soc.* **1996**, *118*, 1629-1644.

(84) Singh, S. B. Z., D. L.; Liesch, J. M.; Mosley, R. T.; Dombrowsky, A. W.; Bills G. F.; Darkin-Rattray, S. J.; Schmatz, D. M.; Goetz, D. A. *J. Org. Chem.* **2002**, *67*, 815-825.

(85) Keenan, P. T. Y., D.; Holt, D. A. *Tetrahedron: Asymmetry* **1999**, *10*, 4331-4341.

(86) Maison, W. K., R.; Kemp, D. S. *Tet. Lett.* **2001**, *42*, 4975-4977.

(87) Ravindranadh, V. J. S. L. J., R. L. *J. Org. Chem.* **2005**, *70*, 5954-5963.

(88) Kadouri-Puchot, C.; Comesse, S. *Amino Acids* **2005**, *29*, 101-30.

(89) Watanabe, L. A.; Haranaka, S.; Jose, B.; Yoshida, M.; Kato, T.; Moriguchi, M.; Soda, K.; Nishino, N. *Tetrahedron: Asymmetry* **2005**, *16*, 903-908.

(90) Ginesta, X.; Pericas, M. A.; Riera, A. *Tet. Lett.* **2002**, *43*, 779-782.

(91) Alegret, C.; Santacana, F.; Riera, A. *J. Org. Chem.* **2007**, *72*, 7688-92.

(92) Pham, V. T.; Joo, J. E.; Tian, Y. S.; Chung, Y. S.; Lee, K. Y.; Oh, C. Y.; Ham, W. H. *Tetrahedron: Asymmetry* **2008**, *19*, 318-321.

(93) Dorsey, B. D.; Levin, R. B.; McDaniel, S. L.; Vacca, J. P.; Guare, J. P.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Quintero, J. C.; et al. *J. Med. Chem.* **1994**, *37*, 3443-3451.

(94) Stingl, K.; Kottenhahn, M.; Drauz, K. *Tetrahedron: Asymmetry.* **1997**, *8*, 979-982.

(95) Morley, R. M.; Tse, H. W.; Feng, B.; Miller, J. C.; Monaghan, D. T.; Jane, D. E. *J. Med. Chem.* **2005**, *48*, 2627-2637.

(96) Eichhorn, E.; Roduit, J. P.; Shaw, N.; Heinzmann, K.; Kiener, A. *Tetrahedron: Asymmetry* **1997**, *8*, 2533-2536.

(97) Palucki, B. L.; Park, M. K.; Nargund, R. P.; Tang, R.; MacNeil, T.; Weinberg, D. H.; Vongs, A.; Rosenblum, C. I.; Doss, G. A.; Miller, R. R.; Stearns, R. A.; Peng, Q.; Tamvakopoulos, C.; Van der Ploeg, L. H.; Patchett, A. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1993-1996.

(98) Attwood, M. R.; Hassall, C. H.; Krohn, A.; Lawton, G.; Redshaw, S. *J. Chem. Soc. Perkin Trans. 1* **1986**, 1011-1019.

(99) Siegmund, B.; Zeitz, M. *Idrugs* **2003**, *6*, 154-158.

(100) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173-180.

(101) Ciufolini, M. A.; Xi, N. *Chem. Soc. Rev.* **1998**, *27*, 437-445.

(102) Hannachi, J. C.; Vidal, J.; Mulatier, J. C.; Collet, A. *J. Org. Chem.* **2004**, *69*, 2367-2373.

(103) Levin, J. I.; Chen, J. M.; Laakso, L. M.; Du, M.; Du, X.; Venkatesan, A. M.; Sandanayaka, V.; Zask, A.; Xu, J.; Xu, W.; Zhang, Y.; Skotnicki, J. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4345-4349.

(104) Cheng, M. Y.; De, B.; Almstead, N. G.; Pikul, S.; Dowty, M. E.; Dietsch, C. R.; Dunaway, C. M.; Gu, F.; Hsieh, L. C.; Janusz, M. J.; Taiwo, Y. O.; Natchus, M. G.; Hudlicky, T.; Mandel, M. *J. Med. Chem.* **1999**, *42*, 5426-5436.

(105) Chiba, J.; Machinaga, N.; Takashi, T.; Ejima, A.; Takayama, G.; Yokoyama, M.; Nakayama, A.; Baldwin, J. J.; McDonald, E.; Moriarty, K. J.; Sarko, C. R.; Saionz, K. W.; Swanson, R.; Hussain, Z.; Wong, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1259-1259.

(106) Asher, V.; Becu, C.; Anteunis, M. J. O.; Callens, R. *Tet. Lett.* **1981**, *22*, 141-144.

(107) Kogami, Y.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2963-2965.

(108) Yasuda, M.; Ueda, M.; Muramatsu, H.; Mihara, H.; Esaki, N. *Tetrahedron: Asymmetry* **2006**, *17*, 1775-1779.

(109) Hayashi, T.; Asai, T.; Ogoshi, H. *Tet. Lett.* **1997**, *38*, 3039-3042.

(110) Trabocchi, A.; Sladojevich, F.; Guarna, A. *Chirality*, **2008**, 1-11.

(111) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893-4011.

(112) Hardegger, E.; Lietchi, P.; Jackman, L. M.; Boller, A.; Plattner, Pl. A. *Helv. Chim. Acta* **1963**, *46*, 60-74.

(113) Koegel, R. J.; Birnbaum, S. M.; Baker, C. G.; Sober, H. A.; Greenstein, J. P. *J. Biol. Chem.* **1953**, *201*, 547-551.

(114) D. Bogdanovaky, M. B. *Bull. Soc. Chim. Fr.* **1965**, 832-834.

(115) Huang, Y.; Dalton, D. R.; Carroll, P. J. *J. Org. Chem.* **1997**, *62*, 372-376.

(116) McLaughlin, M.; Mohareb, R. M.; Rapoport, H. *J. Org. Chem.* **2003**, *68*, 50-4.

(117) Boger, D. L.; Lee, J. K. *J. Org. Chem.* **2000**, *65*, 5996-6000.

(118) Pickersgill, I. F.; Rapoport, H. *J. Org. Chem.* **2000**, *65*, 4048-57.

(119) Equi, A. M.; Brown, A. M.; Cooper, A.; Ner, S. K.; Watson, A. B.; Robins, D. J. *Tetrahedron* **1991**, *47*, 507-518.

(120) Dugave, C.; Demange, L. *Chem Rev* **2003**, *103*, 2475-532.

(121) Gao, J; Jorgensen, W. L. *J. Am. Chem. Soc.* **1988**, *110*, 4212 - 4216.

(122) Gololobov, Y. G.; Kasukhin. L. F. *Tetrahedron* **1992**, *48*, 1353-1406.

(123) Kato, H.; Ohmori, K.; Suzuki, K. *Synlett* **2001**, 1003-1005.

(124) Ariza, X.; Urpf, F.; Vilarrasa, J. *Tet. Lett.* **1999**, *40*, 7515-7517.

(125) Briggs, E. M.; Brown, G. W.; Jiricny, J.; Meidine, M. F. *Synthesis* **1980**, 295-296.

(126) Sa, M. M. *J. Braz. Chem. Soc.* **2003**, *14*, 1005-1010.

(127) Ariza, X.; Urpi, F.; Vilarrasa, J. *Tet. Lett.* **1999**, *40*, 7515-7517.

(128) Williams, T. M.; Bergman, J. M.; Brashear, K.; Breslin, M. J.; Dinsmore, C. J.; Hutchinson, J. H.; MacTough, S. C.; Stump, C. A.; Wei, D. D.; Zartman, C. B.; Bogusky, M. J.; Culberson, J. C.; Buser-Doepner, C.; Davide, J.; Greenberg, I. B.; Hamilton, K. A.; Koblan, K. S.; Kohl, N. E.; Liu, D. M.; Lobell, R. B.; Mosser, S. D.; O'Neill, T. J.; Rands, E.; Schaber, M. D.; Wilson, F.; Senderak, E.; Motzel, S. L.; Gibbs, J. B.; Graham, S. L.; Heimbrook, D. C.; Hartman, G. D.; Oliff, A. I.; Huff, J. R. *J. Med. Chem.* **1999**, *42*, 3779-3784.

(129) Dinsmore, C. J.; Bergman, J. M.; Bogusky, M. J.; Culberson, J. C.; Hamilton, K. A.; Graham, S. L. *Org. Lett.* **2001**, *3*, 865-8.

(130) Boger, D. L.; Yohannes, D.; Zhou, J. C.; Patane, M. A. *J. Am. Chem. Soc.* **1993**, *115*, 3420-3430.

(131) Louis A. Carpino, A. E.-F., and Fernando Albericio *J. Org. Chem.* **1995**, *60*, 3561 - 3564.

(132) Pohlmann, A. S., V.; Guillaume, D.; Quirion, J.; C.; Husson H. P. *J. Org. Chem.* **1997**, *62*, 1016 - 1022.

(133) Mohamed, N.; Bhatta, U.; Just, G. *Tet. Lett.* **1998**, *39*, 8213-8216.

(134) Attolini, M.; Boxus, T.; Biltresse, S.; Marchand-Brynaert, J. *Tet. Lett.* **2002**, *43* 1187-1188.

(135) Bonache, M. A.; Gerona-Navaro, G.; García-Aparicio, C.; Alias, Martín-Martínez, M.; Garcia-Lopez,. M. T.; López, P.; Cativiela, C.; González-Muñiz, R. *Synlett* **2003,** *14*, 2161-2169.

(136) Lewis, R. T.; Macleod, A. M.; Merchant, K. J.; Kelleher, F.; Sanderson, I.; Herbert, R. H.; Cascieri, M. A.; Sadowski, S.; Ball, R. G.; Hoogsteen, K. *J. Med. Chem.* **1995**, *38*, 923 - 933.

(137) Chambers, M. S.; Street, L. J.; Goodacre, S.; Hobbs, S. C.; Hunt, P.; Jelley, R. A.; Matassa, V. G.; Reeve, J. A.; Sternfeld, F.; Beer, M. S.; Stanton, J. A.; Rathbone, D.; Watt, A. P.; MacLeod A. M.; .*J. Med. Chem.* **1999**, *42*, 691 - 705.

(138) Desikan Rajagopal, K. R., Swaminathan, S. *Tetrahedron: Asymmetry* **1996**, *7*, 2189-2190.

(139) List, B.; Lerner, R. A.; Barbas, C. F.; *J. Am. Chem. Soc.* **2000**, *122*, 2395 - 2396.

(140) Koegel, R. J.;Birnaum, S. M.; Baker, G. C.; Sober, H. A.; Greenstein J. P. .*J. Biol. Chem.* **1953**, *201*, 547-551.

(141) Schanen, V. Cherrier, M. P.; deMelo, S. J. de; Quirion, J. C.; Husson, H. P. *Synthesis* **1996**, 833-837.

(142) Han, S. K., Y. *Tetrahedron* **2004**, *60*, 2447-2467.

(143) MacDermott, J. R.; Benoiton, N. L. *Can. J. Chem.* **1973**, *51*, 2555-2561.

(144) Carpino, L. A. *J. Org. Chem.* **1993**, *115*, 4397-4398.

(145) Humphrey, J. M.; Chamberlain, A. R. *Chem. Rev.* **1997**, *97*, 2243-2266.

(146) Corradini, R.; Sforza, S.; Dossena, A. Palla, G. Rocchi, R. Filira, F. Nastri, F. Marchelli, R. *J. Chem. Soc., Perkin Trans. 1.* **2001**, 2690 - 2696.

(147) Di Fenza, A.; Tancredi, M.; Galoppini, C.; Rovero, P. *Tet. Lett.* **1998**, *39*, 8529-8532.
(148) Chern, C. Y.; Huang, Y. P.; Kan, W. M. *Tet. Lett.* **2003**, *44*, 1039-1041.
(149) Williams, R. M.; Kwast, E. *Tet. Lett.* **1989**, *30*, 451-454.
(150) Baker, S. R.; Parsons, A. F.; Wilson, M. *Tet. Lett.* **1998**, *39*, 331-332.
(151) Baggiolini, E. G.; Lee, H. L.; Pizzolato, G.; Uskokovic, M. R. *J. Am. Chem. Soc.* **1982**, *104*, 6460-6462.
(152) Jarowicki, K.; Kocienski, P. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2109-2135.
(153) Kronenthal, D. R.; Han, C. Y.; Taylor, M. K. *J.Org. Chem.* **1982**, *47*, 2765-2768.
(154) Dinsmore, C. J.; Beshore, D. C. *Org. Prep. Proced. Int.* **2002**, *34*, 367-404.
(155) Cignarella, G.; Pirisino, G. *Farmaco Ed. Sci.* **1979**, *34*, 824-828.
(156) Shreder, K.; Zhang, L.; Goodman, M. *Tet. Lett.* **1998**, *39*, 221-224.
(157) Carpino, L. A.; Elfaham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 3561-3564.
(158) Sladojevich, F.; Trabocchi, A.; Guarna, A. *J. Org. Chem.* **2007**, *72*, 4254-4257.
(159) Schmid, F. X.; Mayr, L. M.; Mucke, M.; Schonbrunner, E. R. *Adv. Protein Chem.* **1993**, *44*, 25-66.
(160) Vanhoof, G.; Goossens, F.; De Meester, I.; Hendriks, D.; Scharpe, S. *Faseb J* **1995**, *9*, 736-44.
(161) Lubell, W. D.; Halab, L. *J. Am. Chem. Soc.* **1999**, *124*, 2474-2484.
(162) Imperiali, B.; Fisher, L. S.; Prins, T. J. *J. Am. Chem. Soc.* **1992**, *114*, 3182-3188.
(163) Hayashi, T.; Asai, T.; Ogoshi, H. *Tet. Lett.* **1997**, *38*, 3039-3042.
(164) Haque, S. T.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 6975-6985.
(165) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

UNIVERSITÄT REGENSBURG

Structural properties of γ-butyrolactone δ-amino acids in peptide sequences

Under the supervision of Prof. O. REISER
In collaboration with Lucia Formicola, Ph D Student

VII - δ-AMINO ACIDS AS INDUCER OF SECONDARY STRUCTURES:

VII-1 δ-amino acids and folding properties:

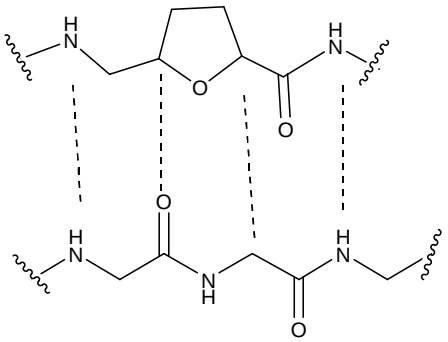
VII-1.1 α-Helix peptidomimetics containing δ-amino acids

As mentioned before, in the past years, the interest in a rational design of amino acid and peptide mimetics has extensively grown due to the pharmacological limitation of bioactive peptides. Numerous example of unnatural oligomeric sequences have been found that fold into well defined conformations in solution.[1,2] Although the oligomers of β- and γ-amino acids are more extensively studied, experimental hints were also obtained for the formation of ordered structures in oligomers of δ-amino acids. However detailed structure information is still missing.

Members of the δ-peptide family are isosteric replacements of dipeptide units. As such, this is the first member of the peptidomimetics lineage in which a single unit represents two or more α-peptide repeats. Thus, δ-amino acid monomers are able to adopt the secondary structures of α-peptide sequences, particularly helices and β-turns.

In 2004, Hofmann and co-workers described on the basis of theoretical studies all possible helix types in oligomers of δ-amino acids (δ-peptides) and their stabilities.[3]

Thus far, the chemical literature has mostly involved carbopeptoid backbones. In fact, carbohydrate research produced interesting examples of new δ-amino acids as building blocks for peptidomimetic design. Sugar amino acids can adopt robust secondary turn or helical structures and they can be used as substitutes for single amino acids or dipeptide isosters **1** (Figure VII-1).

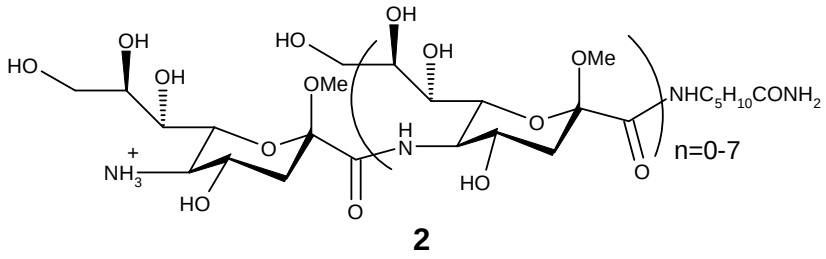


1

Figure VII-1 : Comparison between the α-peptide subunit and the furanose-based subunit as a dipeptide isoster

Carbopeptoids,[4] homooligomers of sugar amino acids, have been prepared from both furanose[5] and pyranose[6] residues.

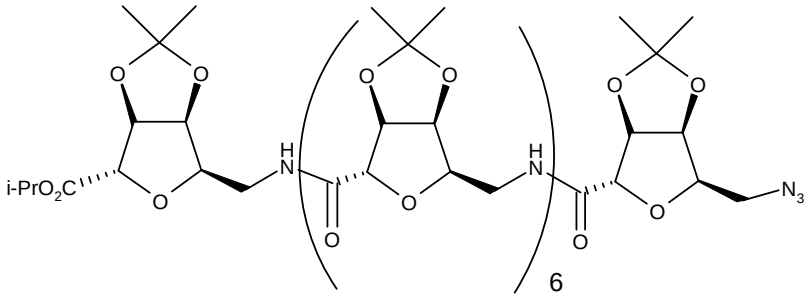
Motivated by the fact that *O*-glycoside oligomers of sialic acid were helical in solution,[7] Gervay and co-workers reported in 1998 that (1→5) amide-linked sialooligomers **2** longer than the trimer formed ordered secondary structures in water[8] (Figure VII-2). The conformational features apparently varied with chain length. After circular dichroism (CD) studies[9] and in combination with molecular modelling, the hypothesis was that for the shorter oligomers, 16-membered hydrogen-bonded rings stabilized a helix involving three residues per turn while for the octamer 22-membered hydrogen-bonding stabilized a helix involving four residues per turn. More recently, Gervay synthesized also new neuraminic acid analogues.[10]



2

FigureVII-2. Amide-linked β-*O*-methoxy neuraminic acid oligomer.

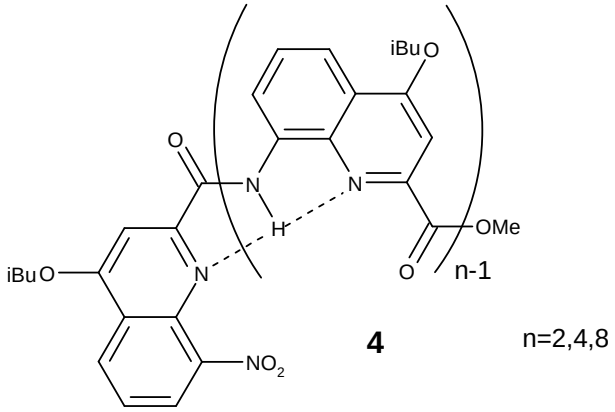
In 2005, on the basis of their conformational investigations, Fleet and co-workers described an octameric chain of carbohydrate amino acids **3** that adopt in solution a left-handed helical secondary structure stabilized by 16-membered (i, i – 3) interresidue hydrogen bonds[11] (Figure VII-3).



3

Figure VII-3. Octameric chain of *C*-glycosyl α-D-furanose configured tetrahydrofuran amino acids 8.

Huc and co-workers reported on the stable helical conformation adopted by oligomers of a new quinoline-derived δ-amino acid **4** (Figure VII-4).[12,13] This class of aromatics foldamers present the advantage that the quinoline amino acid monomers are easily accessible, functionalizable, and assembled into oligoamides. These oligomers adopt unusually stable helical conformations even in polar solvents at high temperature.



4 n=2,4,8

FigureVII-4. Structure of quinoline-derived oligoamide foldamers.

VII.1.2 δ-Amino-acids in turns :

As they did for helical structures, Hofmann & al.[3] envisaged also the β-turns motif as folding possibility. The typical β-turn in α-peptides consists of four consecutive α-aminoacids. The most frequent one is characterized by a hydrogen bond between the peptidic CO of the first and the peptidic NH bond of the fourth amino acid, thus forming a 10-membered ring pseudo cycle. Since the first and the fourth amino acids are involved in periodic structures, the induction of the

β-turn is controlled predominantly by the second and the third amino acid. So that, the replacement of this dipeptide unit by a δ-amino acid could be suitable for the induction of β-folded structures. In this case the C(β)-C(γ) of the δ-amino acid would replace the peptide linkage of the previous dipeptide. Hoffman's calculations, showed in a first time that δ-amino acids introduced in α-amino acid sequences were possible inducers of β-I/βI' and βII/βII'turns (see figure VII-5).

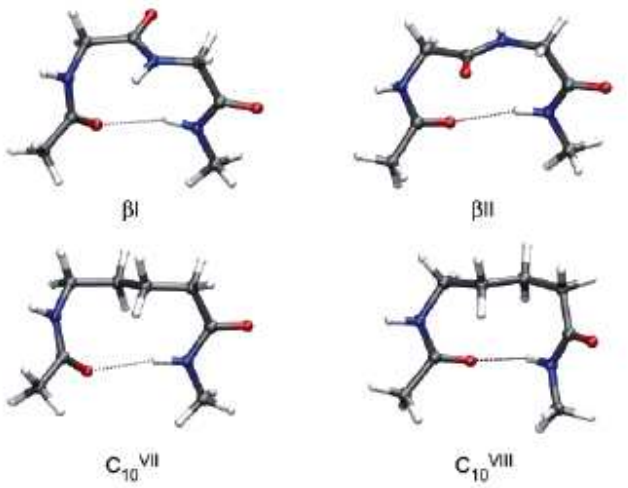


Figure VII-5. Comparison of the βI-and β-II-turns of α-peptides with the two turn conformers C_{10}^{VII} and C_{10}^{VIII} of a blocked δ-amino acid constituent.

They described in a second time, that the incorporation of these non proteinogenic amino acids could generate new turn types that are not permitted in natural amino acids because of the rigidity of the peptide bond. Finally, they confirmed that replacing the C(β)–C(γ) flexible single bond of δ-amino acids by a double bond would be suitable for the stabilisation of β-I/II turn mimics.

This last structural alternative remark, has already been investigated, and experimental data have been reported in the past years.

VII-1.2.1 Isosteres containing a double bond in C(β)-C(γ) position:

The precocious work of Hann et al. in 1980,[14] investigated the replacement of a peptide bond by a trans double bond to form Leu-enkephalin analogues and increase their stability toward proteases. They reported that from a structural point of view both double bonds and amide bonds were

showing similar bond distances, and angles, so that they could direct subsequent attached peptidic strands in a similar way (Figure VII-6).

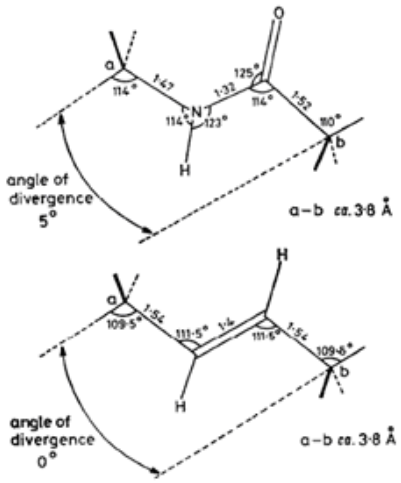


Figure VII-6. Spatial disposition of amide and *trans*–olefin bonds (distances in angstroms)

Based on the previous work of Hann, Wipf and coworkers, reported in 1998,[15] the evaluation of δ-amino acids containing in the C(β)-C(γ) place a (E)-double bond. It was established by X-ray studies that methyl and trifluoromethyl substituted alkenes provided highly preorganized peptide mimetics. The D-L methyl-(E)-alkene isostere **5** sequence prefers an intramolecularly H-bonded type-II' β-turn in the solid state, whereas the trifluoromethyl analogue **6** representing a D-L sequence showed a preference for a type II β-turn.

5 **6**

FigureVII-7. Chimical structure of the D-L methyl-(E)-alkene isostere and its trifluoromethyl analogue.

Gardner *et al*. in 1995,[16] and then in 1999, [17] reported a *trans*-5-amino-3,4-dimethylpent-3-enoate residue (ADPA) glycyl-glycine mimic promoting β-hairpin formation in solution. It came out of this study, that ADPA tetrasubstituted (E)-alkenes favours adoption of folded conformation in the corresponding tetrapeptide mimic **7**, whereas disubtituted alkene (APA), more flexible was not

efficient. In this case, it seems that allylic strains present in ADPA is introducing rigidity in the tetramer and thus blocking the conformation (Figure VII-8).

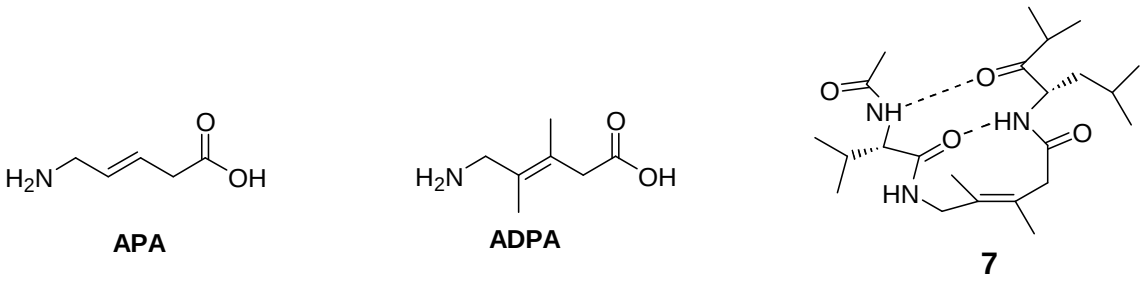


Figure VII-8. APA and ADPA dipeptide isosteres and β-hairpin mimic tripeptide containing ADPA.

VII-1.2.2 Linear δ̃-amino acids:

Balaram and coworkers, in 2006,[18]examined crystal structure of tripeptides comporting δ-aminovaleric acid (δ-Ava) in the i+1 position. In this study, the sequence Piv-Pro-δ-Ava-OMe does not fold into a β-turn like structure, in comparison with the model Piv-Pro-Gly-OMe that provoke in solid state a quasi perfect β-II turn (Figure VII-9).

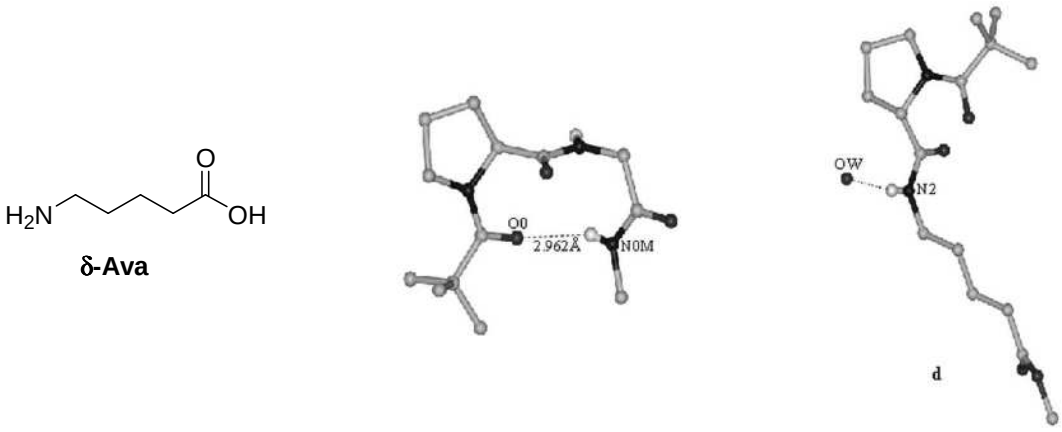


Figure VII-9. δ-aminovaleric acid, Piv-Pro-Gly-OMe turn Piv-Pro-δ-Ava-OMe extended structure.

In 2007,[19]the same group focussed on longer peptides, bearing one residue δ-Ava. The octapeptide Boc-Leu-Val-Val-^{D}Pro-δ-Ava-Leu-Val-Val-OMe was first analysed by NMR that did not achieve complete resolution of the structure. However, these previous conformational analyses were consistent with further crystal structure data, gaving the evidence that this peptide

was folding through a β-hairpin structure. As it is shown in FigureVII-10, it folds in an anti parallel hairpin form that contains a central ^{D}Pro-δ-Ava (α-δ hybrid turn) stabilized by a C_{13} hydrogen bond between the Val_{l3} CO and Leu_7 NH groups. In the crystal structure, the turn structure was visible showing the arrangement of the δ-Ava chain, in an extended loop that spread the continuing strand close to the pyrrolidine ring. Although if the strands part of the molecule have shown to be similar in both solid and liquid state, but it was not possible to get any informations concerning the the turn region in solution because of a lack of NMR data and in particular NOE contacts.

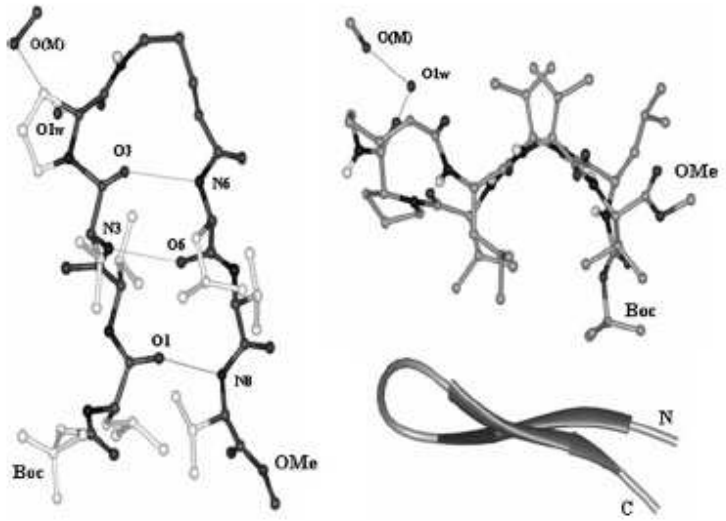


Figure VII-10. Molecular conformation of the peptide Boc-Leu-Val-Val-^{D}Pro-δ-Ava-Leu-Val-Val-OMe. Intramolecular hydrogen bonds are shown as dotted lines. Right: Side view of the molecule showing the pleated sheet formation (top) and ribbon representation.

Nowick & al. described in 2003,[20] a new stable turn structure involving δ-aminoacid: Ornithine ($^{\delta}$Orn) **8** for the stabilization of further β-hairpin structures **10**. To establish this feature two models were chosen for comparison. The first one came from Gellman & al.[21] that had previously described a suitable ^{D}Pro-Gly motif **9** as turn inducer efficient for further hairpin stabilisation. The second β-hairpin model was formed by two complementary α-amino acid sequences locked at the extremity by two δ ornithine segment **11** (see FigureVII-11).

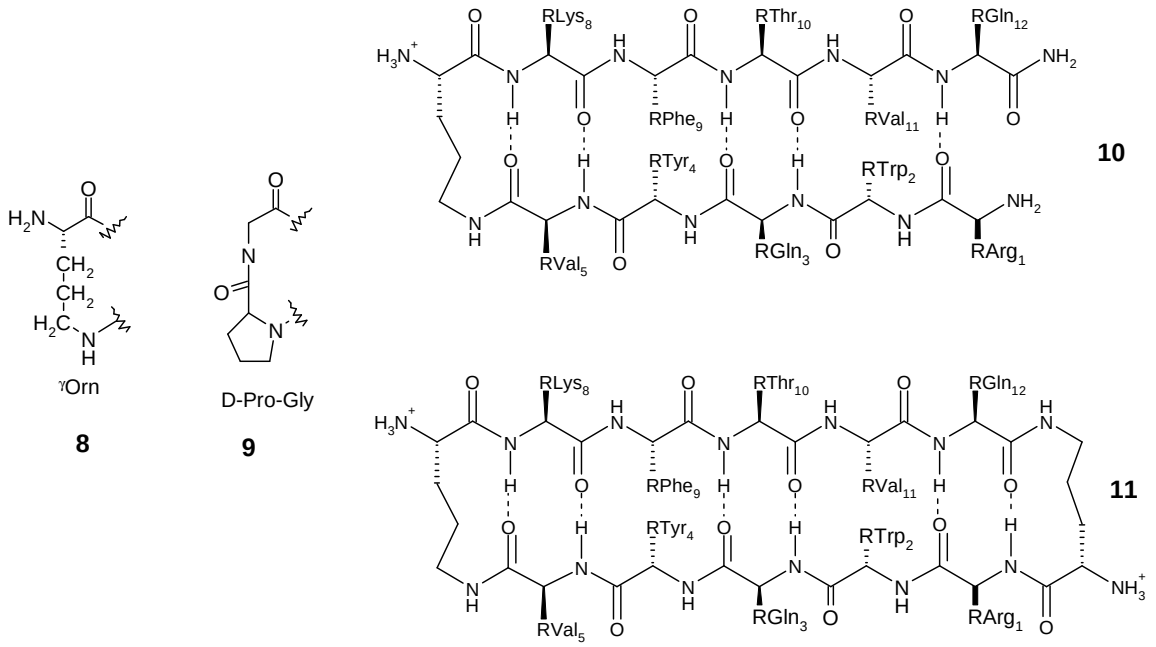


Figure VII-11. (Right) Acyclic and cyclic β-hairpin analogues containing one or two Ornitine dervatives. (Left) D-Pro-Gly turn mimic and dOrn turn mimic.

This study allowed the assignation of the unlinked β-hairpin in which the $^{\delta}$Ornitine that was evaluated to be as good as ^{D}Pro-Gly of Gellman (Figure VII-11), as reverse turn inducer and the hairpin was as folded as the locked one. The properties of such compound were then used for the design and synthesis of extended molecules as mimic of protein quaternary structure.[22]

VII-1.2.3. Bicyclic dipeptide isosteres:

In the last years, Guarna & al.[23] focused on the development of a new class of bicyclic molecular scaffolds. BTAa (Bicycles from Tartaric Acid and amino acids) having a 3-aza-6,8-dioxabicyclo [3.2.1] octane as core structure, and amino acid side chain functionalities. These molecules reveal to be a good template for reverse turn inducer. Their properties were first demonstrated in cyclic peptides. In 2001,[24] Guarna et al synthetized a cyclic peptide mimic of the loop extremity of the BBI protein **12** (FigureVII-12) containing a BTAa motif.

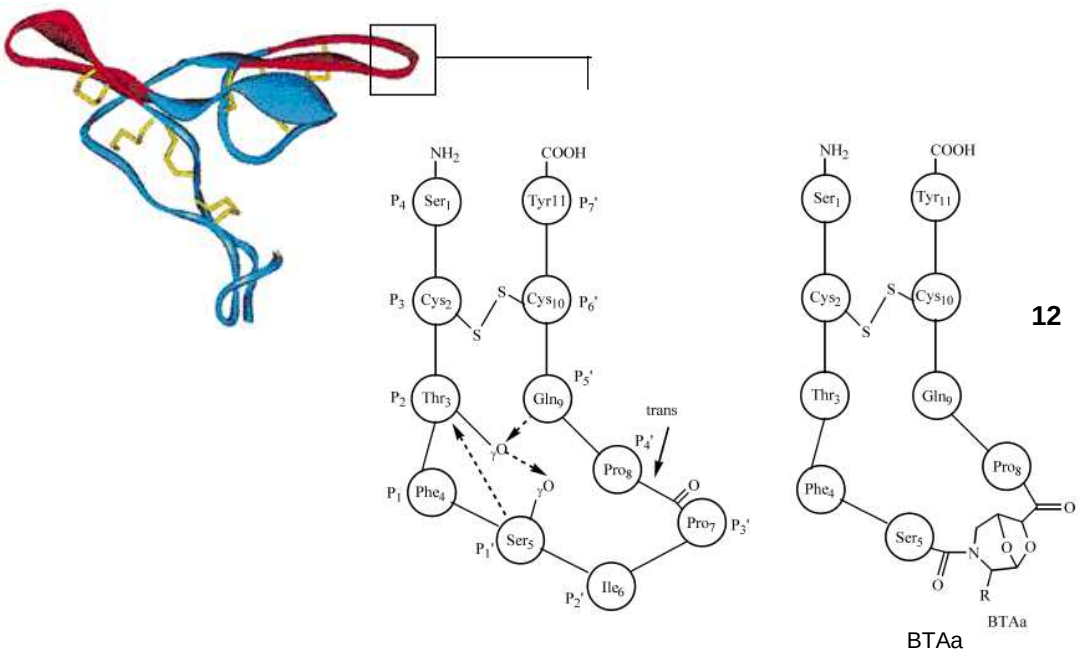


Figure VII-12. Representation of the BBI sequence and in particular, the part of the loop involved in inhibition of proteases in red showing a β-turn structure

The modified BBI peptide containing the 7-endo-BTAa motif (Figure VII-12) led to only one NMR visible conformer. NMR analysis showed also high vicinal coupling constant $J_{NH\text{-}\alpha H}$ and strong sequential NOE correlations which is convenient with extended conformations. Molecular modelling calculations and superimposition of both natural and mimic structures showed the hight similarity between both peptides. These observation lead to conclusion that 7-endo BTAa could be suitable as turn inducer (Figure VII-13).

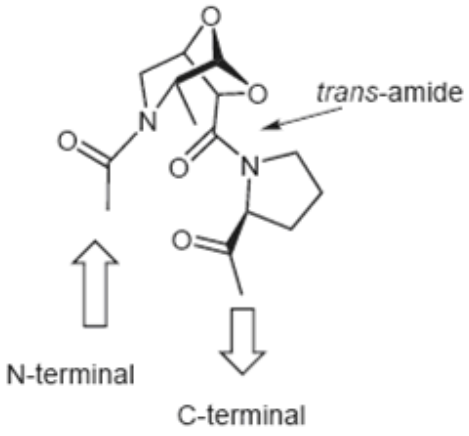


Figure VII-13. Turn inducing property of 7-endo-BTAa

In linear peptides, scaffolds deriving from the combination of leucinol and meso-tartaric acid have been used to test the reverse turn propensity of linear hexapeptidic sequences. Petides containing a BTAa scaffold in the central part of the peptide (see figure VII-14) 7-endo-BTL (Bicycle from Tartaric acid and Leucine), having a leucine side chain at the 4-exo position, showed a mixture of rotamers. One of the rotamer showed a better organized structure, this was supported by sequential NOE contacts between NH and α-H protons indicating a β-strand organisation of the N-terminal main chain. This was reinforced by a 14- membered ring hydrogen bonded structure typical of β-hairpin like structure. The lack of the 10 membered ring H-bond in this particular case indicate that the driving force for nucleation of β-structure is here related to the scaffold structure.

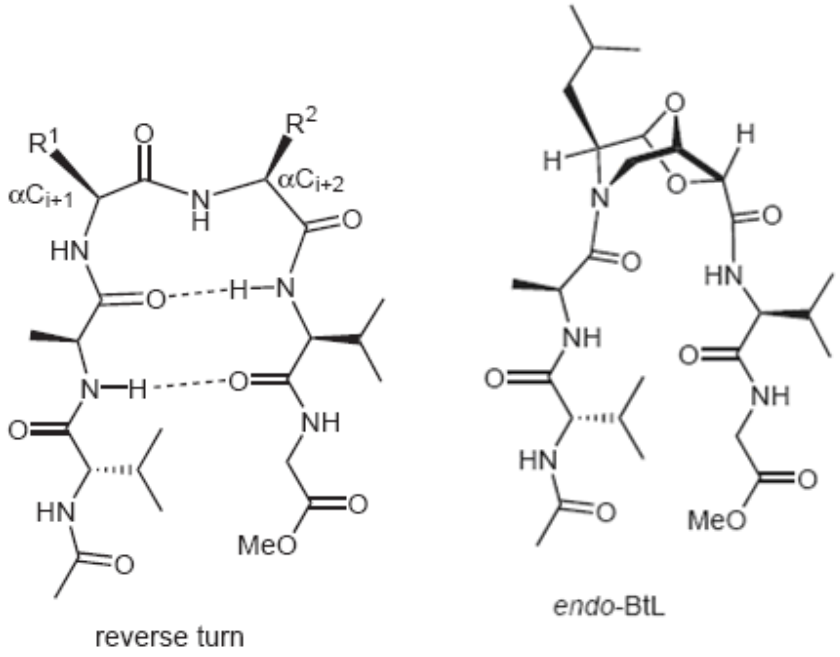


Figure VII-14. Comparison between natural reverse turn and its mimic containing endo-BTL, substituting both positions i+1 and i+2.

VII-1.2.4. Sugar-amino acids as β-turn mimics:

Von Roedern & al.[25] reported in 1994, and in 1996,[26] the design and synthesis of new dipeptide isosteres based on the sugar monomer H-Gum-OH a glucose derivative **13** (Figure VII-15). The cyclic structure of H-Gum-OH fixes the ω_I angle at 180°, which corresponds to a *trans* amide

bond. The φ+1 angle is also fixed in a 180° angles; this is often found in β-sheet structures. According to its structure the H-Gum-OH was used to replace two neighbour natural amino acids in cyclic hexapeptides **14**, derivatives of cyclic somatostatin analogues. Conformational analysis by NMR in solution, and molecular dynamics, lead to the described structure Figure VII-15. The hexapeptide show a β-II'turn type conformation were the sugar residue occupies the i+1 and i+2 positions. In this case the peptidic ring is distinctly bent because of the steric repulsion of the ring oxygen and the carbonyl of the neighbour amino acid. In contrast in "normal" hexapeptides the two β-turns are almost coplanar.

Figure VII-15. (Right) : corespondance between H-Gum-OH skeleton and angles with classical peptide structure. (Left) Turn like structure in cyclic hexapeptide containing H-Gum-OH in i+1 and i+2 position (image from MD simulations)

The same work was applied to linear peptides, but in this case peptides showed a non folded structure.

In 2000, Chakraborky and coworkers[27] reported the design and synthesis of new peptidic turn mimic using new furanoid sugar amino acids **15** and **16**. Furanoids sugars were incorporated in Leu-enkephalin analogues **18** at the place of two neighbouring glycine residues (Figure VII-16). Conformational analysis using NMR, molecular dynamics and circular dichroism showed that two of these analogues (containing the sugar Gaa and Iaa) displayed an unusual β-turn structure involving a C_9 membered ring hydrogen bond between NH-Leu and C_3 OH of the sugar.

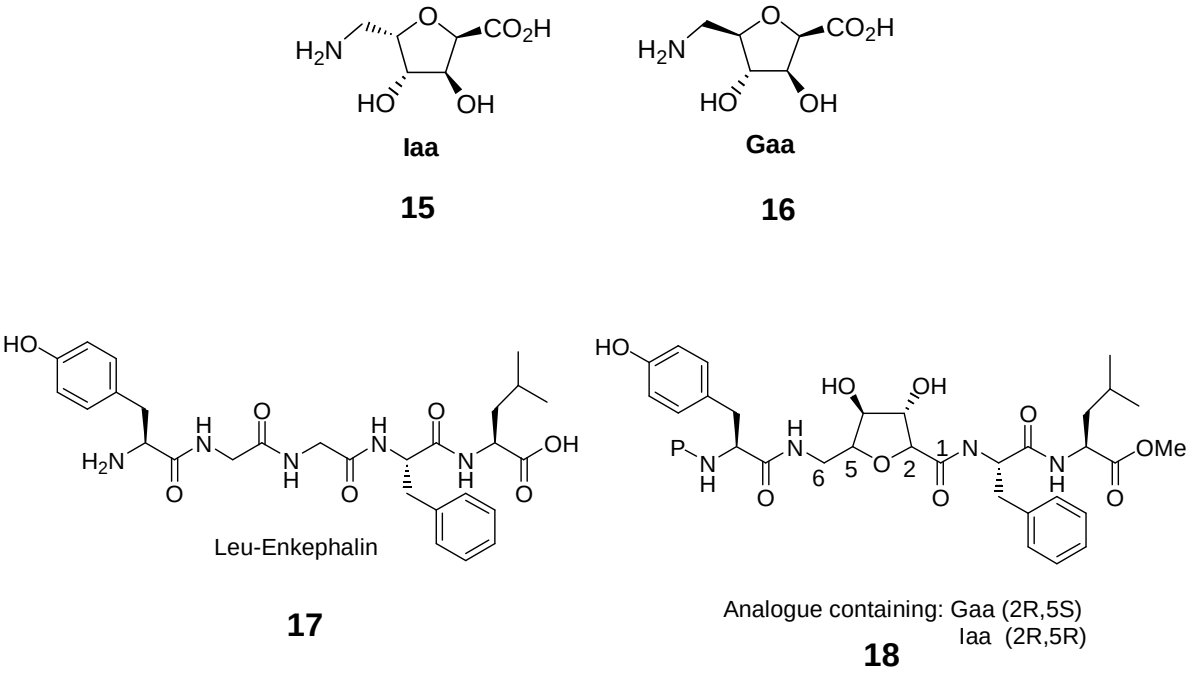


Figure VII-16. Furanoid sugar amino acids and their analogues. Gaa=6-amino-2,5anhydro-6-deoxy-D-gluconic acid, Iaa=6-amino-2,5anhydro-6-deoxy-D-Idonic acid.

It came out of these studies that the driving force for the turn nucleator depends not only from the restriction caused by the ring but also from the substitution of the ring, since unsubstituted furane were not able to fold.

In the course of this year, Fleet and co-worker described oxetane amino acids and oligomers **19** obtained from it[28] (Figure VII-17). These δ-2,4-*cis*-oxetane amino acid oligomers adopt well-defined secondary structure in solution in which the major conformation is dictated by internal 10-membered H-bonded rings.[29] This β-turn motif is repeated along both the tetramer and hexamer.

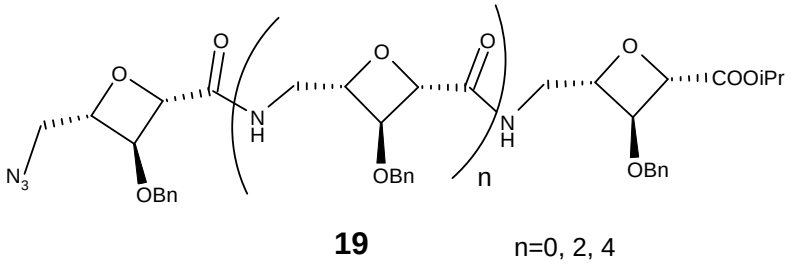


Figure VII-17. Structures of homo-oligomers of δ-2,4-*cis*-oxetane amino acid**.**

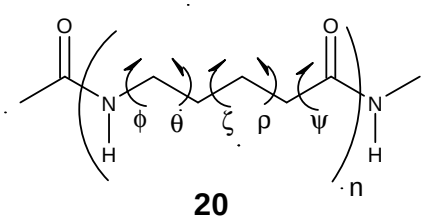
VIII - NEW δ-AMINO ACIDS TOWARDS NEW FOLDAMERS

VIII-1 INTRODUCTION

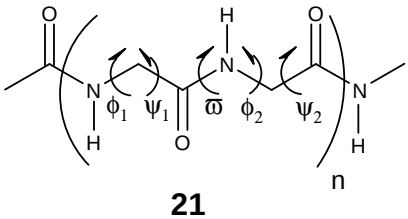
The folding of polypeptide chains into secondary and eventually into a bewildering array of tertiary structures results in protein molecules that are responsible for most of the biological interactions and functions found in nature. Therefore, peptides and proteins are attractive targets for drug design. Poor bioavailability and metabolic stability of peptidic drugs, however, have resulted in significant limitations. A logical next step is to mimic nature and create nonbiologically derived molecules that either fold into well-defined secondary structures, or assemble into larger architectures. The pioneering studies of Gellman and Seebach on β-peptides, a class of unnatural peptidomimetic folding oligomers, have demonstrated the feasibility of folding unnatural oligomers into well-defined conformations. Extending the concept of β-peptides has led to other peptidomimetic foldamers such γ-peptides[30,31] and δ-peptides.[8]

In the presented work we are interested in δ-amino acids towards new foldamers.

As mentioned in the chapter VII, δ-amino acid residues **20** are isosteres of dipeptide elements in α-peptides **21** (Figure VIII-1).



20



21

Figure VIII-1. **Comparison of δ-amino acid residues** 20 **with α-dipeptide elements** 21**. φ, ψ, θ, ζ, ρ, ω are the backbone torsion angles.**

Therefore, δ-amino acids are potential surrogates for α-dipetides and can be useful to improve the resistance of natural peptide chains toward degradation by proteases.

There are some examples of the incorporation of a single δ-amino acid into longer natural amino acid sequences,[32-34] but most of the cases deal with sequences based on repeating δ-amino acid units, particularly on sugar amino acids derived from furanose[35] and pyranose.[6] In fact, homo-oligomers of sugar amino acids have been extensively studied and provide many examples of foldamers.[6,11,29,36]

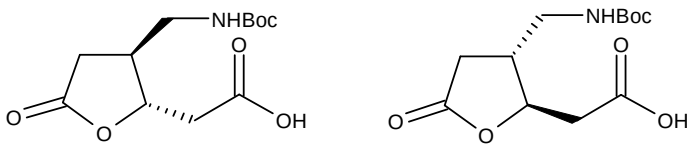
Oligomers of different types of amino acids are also important in conformational design and can be potentially useful to obtain new foldamers. Our approach presented in this chapter is based on the synthesis of peptides containing α- and δ-amino acids with different chain length and α-amino acid composition.

VIII-2. Model peptides containing δ-amino acid units

VIII-2.1. Previous work in the laboratory

The application of unnatural building blocks to induce a predictable conformation requires the investigation of their conformational preferences.

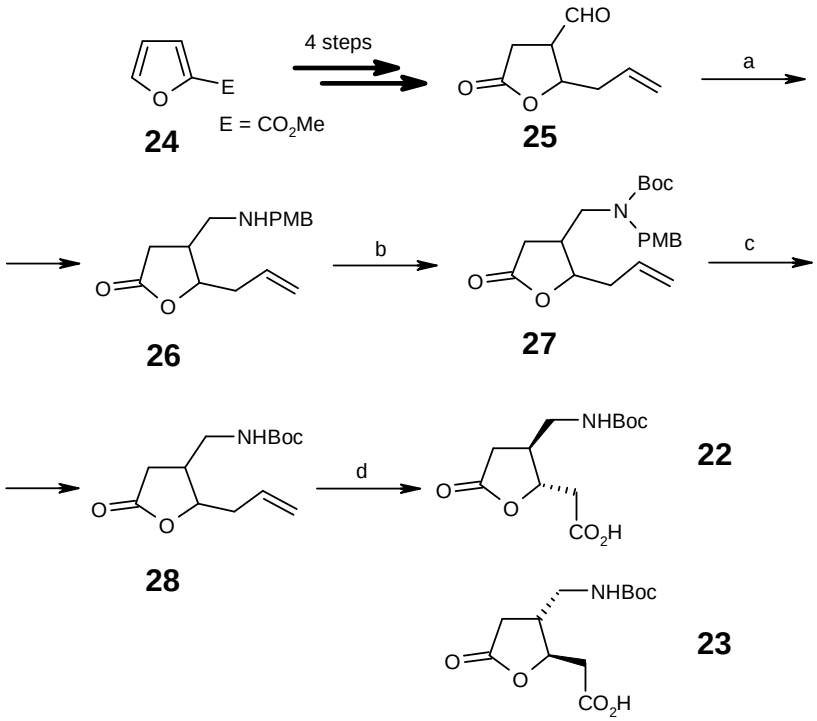
In our group we investigated the conformational preferences of peptide mimics containing the two enantiomers of a novel δ-sugar amino acid **22** and **23** (FigureVIII-2). The synthesis of both enantiomers was previously developed by M. Haque in our group.[37]



22 (*S*,*S*) **23** (*R*,*R*)

Figure VIII-2. (*S*,*S*)-22 and (*R*,*R*)-23 enantiomers of a novel δ-sugar amino acid.

The key intermediate **25** was obtained starting from inexpensive furan-2-carboxylic methyl ester **24** in four steps by using a procedure that has been previously developed in our group.[38] Treatment of γ-butyrolactonaldehyde **25** with 4-methoxybenzylamine to form the corresponding imine, followed by reduction with $NaBH_4$ provided the amine **26**[39] in 76% yield. *N*-Boc protection afforded the protected amine **27**,[40] in which the PMB group was removed with CAN to give the carbamate **28**[41] in 79% yield. Finally, ruthenium catalyzed oxidative cleavage of the allylic double bond with $NaIO_4$ gave the δ-amino acid enantiomers **22** and **23**[42] in 86% yield (Scheme VIII-8).



Scheme VIII-8. Synthesis of compounds 22 and 23. Reagents and conditions:

a) (i) Na_2SO_4, 4-methoxybenzylamine (1.1 equiv), CH_2Cl_2, 3 h; (ii) $NaBH_4$ (2equiv), MeOH, H_2O, 0 ℃, 30 min, 76%; b) $(Boc)_2O$, dioxane-1M K_2CO_3 (1:1.2), r.t., overnight, 74%; c) CAN (4 equiv), CH_3CN-H_2O (1:3), 0 ℃, 1 h and r.t., 2 h, 79%; d) $RuCl_3 \cdot 3H_20$ (6 mol%), $NaIO_4$ (4.0 equiv), CCl_4-CH_3CN-H_2O (1 : 1 : 2), 0 ℃, 1 h and r.t., 2 h, 86%.

Delatouche and Bordessa synthesized two α-δ pentapeptides **29** and **30** (FigureVIII-3) using the scaffolds **22** and **23**, respectively, which were alternated with the natural amino acid phenylalanine. In addition, Bordessa synthesized heptapeptide **31** using the R,R enantiomer and phenylalanine (FigureVIII-3).

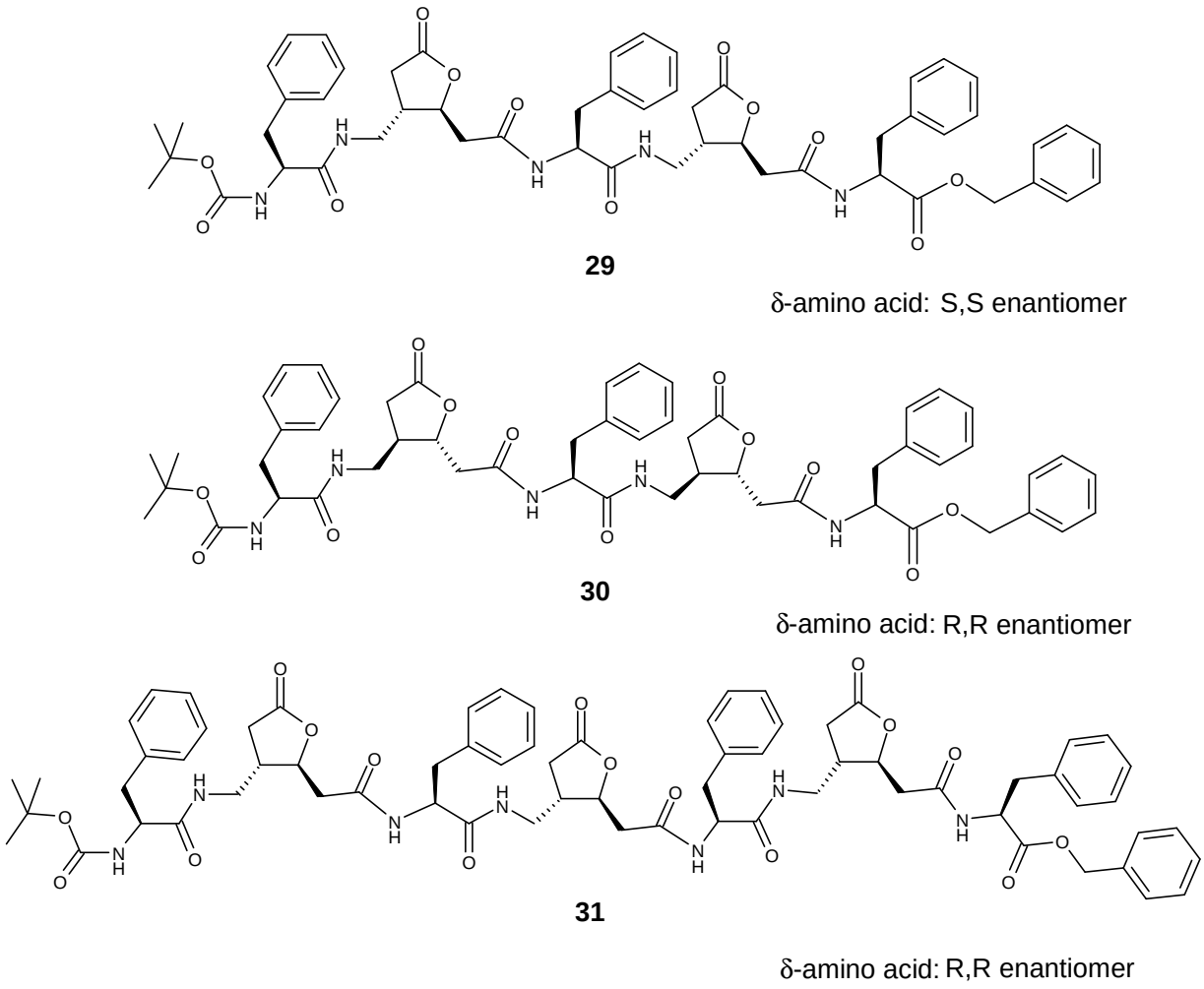


Figure VIII-3. Pentapeptides containing α-δ amino acids 29 and 30 and heptapeptide 31.

The aim of our work has been to investigate the conformational analyses of the two pentapeptides **29** and **30**, and of the heptapeptide **31**, in order to determine their secondary structure preferences.

VIII-2.2. Conformational investigations

The conformation of peptides depends principally on their amino acidic composition and on the sequence of these amino acids. As the physical and biological properties of peptides are determined by their structure, the knowledge of the latter is essential to understand these properties. There are many techniques to investigate the three dimensional structure of peptides. FT-IR and CD spectroscopy are important tools to indicate the presence or absence of secondary structure elements, while NMR spectroscopy and X-ray crystallography allow a detailed description of the localization and spatial arrangement of such elements within the amino acid sequence. However, for the X-ray crystallography single crystals are needed, and there always exists the possibility of a difference in the conformation between the solid state and the one seen in solution. In the present work we employed NMR, CD, FT-IR spectroscopy, and molecular modelling to characterize the peptides synthesized.

NMR investigation

The most common and useful tool to investigate the secondary and tertiary structure of peptides and proteins in solution is the Nuclear Magnetic Resonance (NMR) spectroscopy.[43]NMR spectroscopy allows an investigation of the structure of biopolymers on an atomic level in solution. Moreover, NMR investigations can highlight the presence of conformational equilibria and the dynamics of the folding. Important structural information can be obtained from both mono- and bidimensional NMR spectra.

Information from 1D-proton NMR spectra

Even though the NMR-spectra of biopolymers are derived from the nuclear spins of the monomers thea are build up from, there is no straightforward correlation between the NMR spectra of the low molecular weight components and the three-dimensional strucutre of the whole polymer.[43] A dispersion of nucleus's chemical shift values is the first indication of the presence of spatial folding in a peptide chain. When a peptide has a defined conformation in solution, a proton in the sequence will have a different microenvironment compared to those in a random coil structure. It will also be different from the same proton contained in an identical residue type

at a different point in the sequence. This fact leads to different specific chemical shift values compared to those for an unfolded structure and to a good dispersion of the signals.

The presence of a secondary structure in solution can lead to a slower exchange rate for labile protons, which can be measured by time dependent NMR-spectroscopy, while in small molecules proton exchange is too fast to be observed. Moreover the slower diffusional motions of the macromolecul in solution can substantially affect the spin relaxation and the Nuclear Overhauser Enhancement (NOE).[43] The presence of hydrogen bonds can be detected from onedimensional NMR spectra. These techniques are based on the assumption that the NHs involved in a H-bond are less sensitive to perturbations such as changing of temperature, concentration or solvent, than the NHs that are exposed to the solvent. The determination of the temperature coefficients ($\Delta\delta/\Delta T$) for the amide protons is based on the observation that, upon warming, NHs involved in hydrogen bonds display shifts at a smaller extent than the NHs that are exposed to the solvent. Generally, values of $-\Delta\delta/\Delta T > 3$ ppb/K indicate amide protons in an equilibrium between a hydrogen-bonded and a non-hydrogen-bonded state, while values of $-\Delta\delta/\Delta T < 2.6$ ppb/K are considered an indication of hydrogen-bonded NH protons or of NH protons locked in a hydrogen-bonded conformation.[44]

The secondary structures of peptides are characterized by distinct torsion angles along the backbone. Since the size of the spin-spin coupling constant $^3J_{HN\text{-}H\alpha}$ depends on the torsion angle NH-CHα, on the basis of Karplus relationship, the measurement of $^3J_{HN\text{-}H\alpha}$ is useful for secondary structure determination, as it can be directly related to the backbone dihedral angle φ.[43,45] Generally, $^3J_{HN\text{-}H\alpha}$ values < 6 Hz indicate turn or helical conformations, whereas values higher than 6 Hz are referred to unordered structure or an extended conformation (Figure VIII-3).

Secondary structure	φ	$^3J_{HN\alpha}$ (Hz)
α-helix	- 57°	3.9
3_{10}-helix	- 60°	4.2
antiparallel β-sheet	- 139°	8.9
parallel β-sheet	- 119°	9.7

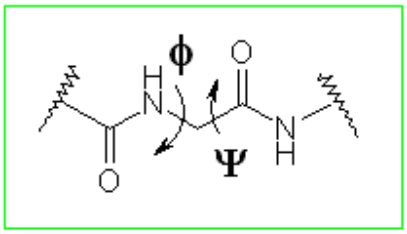


Figure VIII-3: Table of the $^3J_{HN\text{-}H\alpha}$ values in regular secondary structures[43] (left side). Torsion angles φ and ψ present along the backbone of a α-peptide (right side).

Information from 2D-proton NMR spectra

The most useful data relating to a peptide conformation is gained from 2D-NMR investigation. Of particular interest are the correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY) and NOE spectroscopy/rotational frame NOE (NOESY/ROESY) experiments.

The cross peaks observed in the COSY spectrum correspond to proton-proton correlations due to scalar (through-bond) couplings, though the respective protons may only be separated by a maximum of three covalent bonds. The TOCSY spectrum contains all correlations between protons of one spin system and in the case of peptides each amino acid has a particular spin-system pattern that allows the identification of every residue type in the sequence. With the help of these two experiments, it is possible to assign completely all the chemical shifts of the protons in a peptide. Further, NOESY experiment displays cross peaks due to dipolar coupling resulting from through space interactions. NOESY cross peaks depend on the distance between two protons and occur between two nuclei that are close in the space up to 5 Å.A NOE cross peak indicates that two non-covalently bonded nuclei are close enough to interact or to exchange. NOE cross peaks are usually defined as *sequential* distances when they are between backbone protons, or between a backbone proton and a β proton of residues that are nearest neighbours in the sequence. *Medium-range* distances are all non-sequential signals between residues within a segment of five consecutive residues. *Long-range* distances are between the backbone protons of the residues that are at least six positions away in the sequence.[43] Depending on the torsion angles that characterize the secondary structure of a peptide, sequential and medium-range proton-proton distances will have different values.For instance the sequential NH-NH and CHα-NH contacts for a regular α-helix are about 2.8 and 3.5 Å, respectively. The CHβ-NH contacts, since they depend also on the torsion angle on the side chain χi, vary between 2.5 and 4.1 Å for a α-helix.[43] Medium-range contacts in helices are usually i,i+2, i,i+3, and i,i+4. In particular i,i+4 contacts are especially useful since they are present in a regular α-helix and not in a 3_{10}-helix. Tight turns have close *i*,*i*+2 values. In the case of β-structure short *medium-range* distances are not observed, since the polypeptide segments are almost fully extended.[43] Table VIII-1 lists short *sequential* and *medium-range* distances that are found in known α-peptide conformations.

Distance	α-helix	3_{10}-helix	β-sheet
$d_{\alpha N}$	3.5	3.4	2.2
$d_{\alpha N}(i,i+2)$	4.4	3.8	
$d_{\alpha N}(i,i+3)$	3.4	3.3	
$d_{\alpha N}(i,i+4)$	4.2		
d_{NN}	2.8	2.6	4.2-4.3
$d_{NN}(i,i+2)$	4.2	4.1	
$d_{\beta N}$	2.5-4.1	2.9-4.4	3.2-4.7
$d_{\alpha\beta}(i,i+3)$	2.5-4.4	3.1-5.1	

Table VIII-1. Short sequential and medium-range 1H-1H distances in some common secondary structures in α-peptides.[43]

Circular dichroism

Circular Dichroism (CD) spectroscopy is an important technique to investigate the secondary structure of proteins and peptides. It is based on the differential absorption of left and right circularly polarized light. To deliver a CD signal, a compound has to possess a chromophore, which is either inherent chiral or surrounded by a chiral environment and absorbs in the observed wavelength range. These prerequisites are met by the amide bond in peptides and proteins, rendering the CD spectroscopy a suitable methodology for the elucidation of their structural preferences. The observed bands in the CD spectra of peptides and proteins stem from the $n \rightarrow \pi^*$ and the $\pi \rightarrow \pi^*$ transitions of the amide bonds. Every secondary structure gives rise to a specific CD spectrum that represents a survey upon the averaged overall structure of a peptide or protein, while it is impossible to assign structural preferences to an individual residue (Figure VIII.4). However, algorithms based on protein and peptide reference data sets enable the calculation of the secondary structure elements composition of a peptide/protein from its CD spectrum.

In addition to the far-UV region over the range 190-250 nm, also the near-UV region from 250 to 320 nm can deliver useful information, as it shows the contribution of aromatic residues like tyrosine or tryptophan.[46,47]

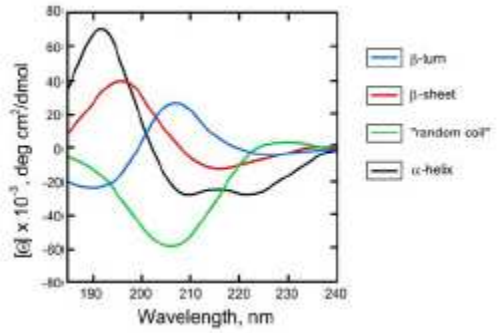


Figure VIII.4: CD spectra characteristic for common secondary structure elements (adapted from Greenfield).[48]

The molar ellipticity $[\Theta]_\lambda$ at the wavelength λ is defined as follows (Equation 1):

$$[\Theta]_\lambda = \frac{100 \cdot \Psi}{l \cdot c} \left[\frac{Deg \cdot cm^2}{dmol}\right] \qquad \textbf{Equation 1}$$

where Ψ is the deviance of left- and right-circularly polarized light in degree, *l* the cell length in cm and c the peptide concentration in M. To compare peptides with different chain lengths, the molar ellipticity per residue is used, which is obtained by dividing $[\Theta]_\lambda$ by the number of amino acids. The CD bands characteristic for helical peptides at 190, 208 and 222 nm stem from the $\pi \rightarrow \pi^*$ transition (190 nm and 208 nm are the components perpendicular and parallel to the helix axis, respectively) and $n \rightarrow \pi^*$ transition (222 nm), with the latter being sensitive to hydrogen bond formation.[49] The ratio R between the two minima at 222 and 208 nm can be used as an indicator for the presence of interacting helices, as the band at 208 nm, corresponding to the parallel component of the $\pi \rightarrow \pi^*$ transition, changes its energy upon an interhelical interaction. Ratios larger than 1 are commonly regarded as the result of interacting helices, as found in coiled coil structures, while ratios lower than 1 indicate the presence of non-interacting helices.[50]

Conformational analysis

In the presented work by using the Sybyl program (version 7.0) we carry out computer simulations in the hope of understanding the properties of assemblies of molecules in terms of their structure and the microscopic interactions between them. This serves as a complement to conventional experiments, enabling us to learn something new. Molecular structures generated *in silico* have to be geometry optimized to find the individual energy minimum state by applying a molecular mechanics method. This is a method employed to calculate molecular geometries and energies. In the framework of the molecular mechanics method the atoms in molecules are treated as rubber balls of different sizes (atom types) joined together by springs of varying length (bonds). For calculating the potential energy of the atomic ensemble Hooke's law is used. In the course of a calculation the total energy is minimized with respect to atomic coordinates where:

$$E_{tot} = E_{str} + E_{bend} + E_{tors} + E_{vdw} + E_{elec}$$

where E_{tot} is the total energy of the molecule, E_{str} is the bond-stretching energy term, E_{bend} is the angle-bending energy term, E_{tors} is the torsional energy term, E_{vdw} is the van der Waals energy term, and E_{elec} is the electrostatic energy term. Molecular mechanics enables the calculation of the total steric energy of a molecule. The set of parameters consisting of equilibrium bond lengths, bond angles, partial charge values, force constants and van der Waals parameters is known as the *force field*. In our work was used the MMFF94 Force Field[51] that was developed as a combined "organic/protein" force field, one which was equally applicable to small molecules as well as proteins and other systems of biological importance.

The technique known as energy minimization is used by molecular mechanics only to find the local energy minimum but not implicitly the global energy minimum. Lower energy states are more stable and are commonly investigated because of their role in chemical and biological processes.

Molecules can be modelled either in vacuum or in the presence of a solvent. Simulations of systems in vacuum are referred to as *gas-phase* simulations, while those that include the presence of solvent molecules are referred to as *explicit solvent* simulations. In another type of simulation, the effect of solvent is estimated using an empirical mathematical expression; these are known as *implicit solvation* simulations. In our work the molecular modelling was performed in vacuum.

As mentioned before, the use of theoretic methods to obtain models that allow predicting structures, properties and molecular interactions is known as “Molecular Modelling”.
Molecular Modelling provides information that is not available directly from conventional experiments, thus it has a complementary role in the field of the experimental chemistry.
There are several simulation technique methods: quantum chemistry, molecular mechanics, molecular dynamics (MD).
It is known that the structures with lower energy states are more stable and take a part in chemical and biological processes. Molecular dynamics simulation offers an effective tool to detect the energetically most favored 3D structure of a system.

In our work simulations incorporating NOE derived distance constraints as well as “free” dynamics were investigated in vacuum (*gas-phase* simulations). MD calculations were performed using Sybyl program (version 7.0) with MMFF94 force field. The temperature was increased progressively from 300 to 700 K and the time period of each simulation was 222 ps, where a molecule was extracted every 500 steps. Among the resulting structures those energetically favored were then minimized and analyzed.

FT-IR spectroscopy

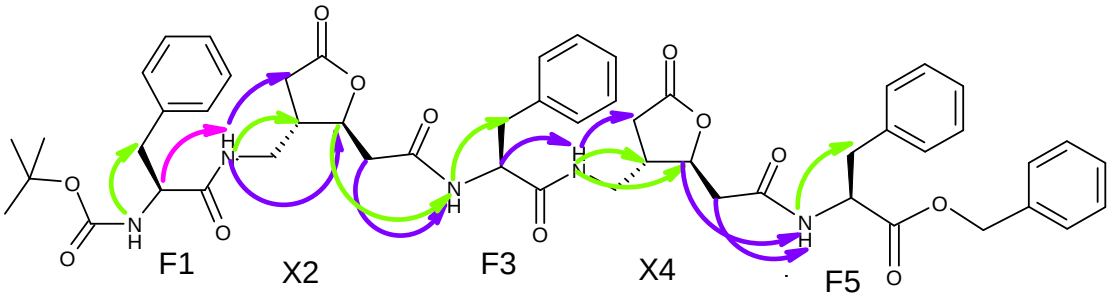
Infrared light is energetic enough to excite molecular vibrations to higher energy levels. The main characteristic bands of the peptide group are called amide A, amide I and amide II. Evidence exists that the position of these bands is related to the conformation of the peptide.[52] The amide A band is mainly due to the N–H stretching vibration. It is very sensitive to the strength of a hydrogen bond, thus providing an opportunity to gain information about any intra- or intermolecular hydrogen bond. Usually, in a non polar solvent, non hydrogen-bonded NHs adsorb above 3400 cm^{-1}, while NHs that are involved in hydrogen-bonds absorb below 3400 cm^{-1}.[53] The amide I and II bands are related to the backbone conformation. The amide I is associated with the C=O stretching vibration, while the amide II results from the N–H bending and from C–N stretching vibrations.

	α-helix	*3_{10}-helix*	*β-sheet*
Amide I	1652	1666-1662	1648-1645
Amide II	1548	1533-1531	1533-1536

Table VIII.2. Frequencies (cm^{-1}) of the amide I and amide II bands found for different α-peptide conformations.

VIII-2.3. Results on alternated α/δ amino acids

The pentamer containing alternated (S,S)-δ-amino acid and phenylalanine was analyzed by NMR in both $CDCl_3$ and methanol-d_3 solvents. Comparing both analyses at different temperatures, the spectrum in $CDCl_3$ seemed to be less comprehensible than the spectrum in methanol-d_3, showing a lower dispersion of the NH signals, and higher temperature coefficients ($-\Delta\delta/\Delta T > 10$ppb/K). We therefore present here only the analyses performed in methanol-d_3. This choice was also supported by the CD experiment, where the CD curve appeared to be much more defined in methanol than in TFE. Thus, the synthesized α/δ-peptide **29** (Figure VIII.5) was dissolved in methanol-d_3 and studied by NMR spectroscopy. 1D experiments were first acquired at five different temperatures (273, 278, 283, 293 and 303 K), and the one that gave the better resolved spectrum (283 K) was chosen to record the 2D experiments. The complete ^{1}H-NMR resonance and sequence assignments were done by using the COSY, TOCSY and ROESY spectra. Sequential and interresidue NOE cross-peak intensities were classified as strong (1.8-2.7 Å), medium (1.8-3.3 Å) and weak (1.8-5 Å) based on the number of contours in the contour plot of the ROESY spectrum. The NOE contacts observed for this peptide are shown in Figure VIII.5.

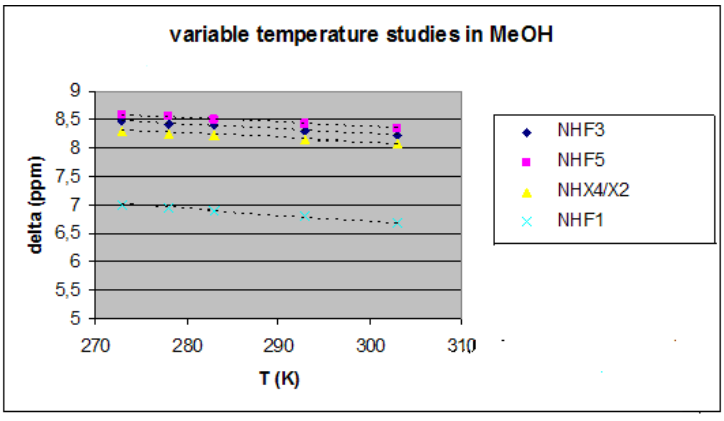


29 δ-amino acid: S,S enantiomer

Figure VIII.5 Summary of the interresidue NOE contacts observed for the peptide 113 in methanol-d_3 at 283 K (pink arrows: strong NOEs; green arrows: medium NOEs; blue arrows: weak NOEs).

The values of the $^3J_{HN\text{-}H\alpha}$ coupling constants were estimated in the 1D ^{1}H-NMR spectrum and these vicinal coupling constants showed always values bigger than 6 Hz (Figure VIII.6).

Amide protons showed high temperature coefficients (Figure VIII.6), suggesting a non-hydrogen-bonded state. However, studies of deuterium exchange in methanol-d_4 showed a slow disappearance of one or both amide protons of the lactone residues. The presence of the hydrogen bond possibility in this molecule was confirmed by IR experiments in CH_2Cl_2, which showed two bands in the amide region: a broad and intense band at 3311 cm^{-1} in the H-bonded region, and a weaker one at 3420 cm^{-1}.



	-Δδ (ppb)/ ΔT(K)	$^3J_{HN\text{-}H\alpha}$ (Hz)
NHF_1	10.3	7.2
NHX_2	7.1	
NHF_3	8.3	6.9
NHX_4	7.1	
NHF_5	8.1	8.2

Figure VIII.6: Variable temperature studies in methanol-d_3 of peptide **29**.

Molecular modelling was performed using molecular dynamics experiments in vacuum. Introduction of four of the NOE contacts found in the ROESY spectrum as constraints in this experiment gave the structure shown in Figure VIII.7 as the conformational energetic minimum, which is characterized by a large loop locked at the extremities by two hydrogen bonds.

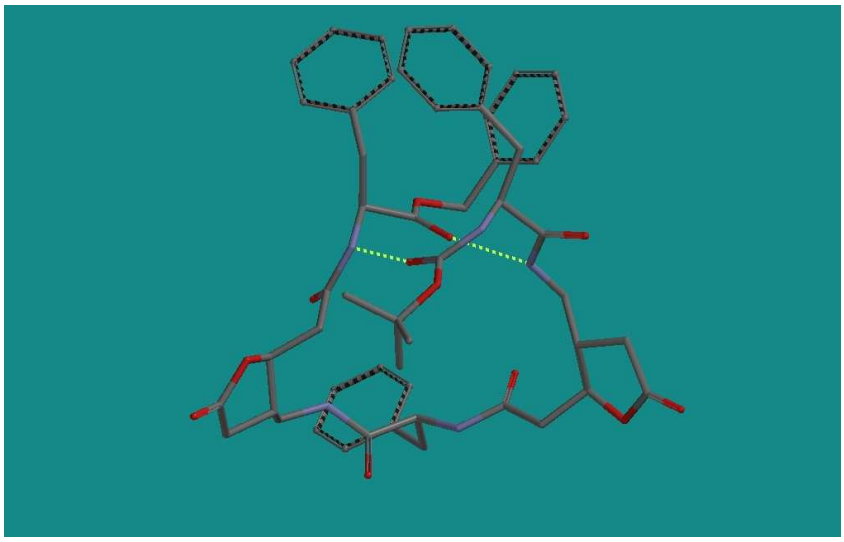


Figure VIII.7: Structure of low-energy conformer calculated for peptide **29**. Hydrogen bonds are indicated with dashed lines and all hydrogen atoms have been omitted for clarity.

The CD spectrum in methanol at 0.1 mM showed two maxima at 200 and 219 nm that were not comparable with any data in the literature (Figure VIII.8).

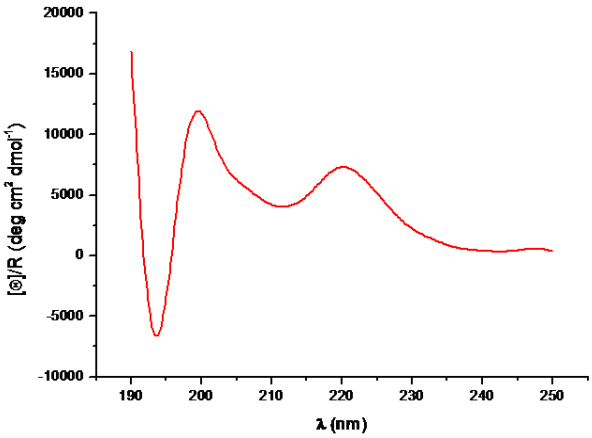


Figure VIII.8: CD spectrum of the pentapeptide **29** in methanol.

The pentamer containing alternated (R,R)-δ-amino acid and phenylalanine **30** (Figure VIII.9) was analyzed by NMR in both $CDCl_3$ and methanol-d_3 solvents. Due to low solubility and low dispersion of the signals in methanol, we used only the analyses in $CDCl_3$. The synthesized α/δ-peptide **30** was thus dissolved in $CDCl_3$ and studied by NMR spectroscopy. 1D experiments were first acquired at five different temperatures (278, 283, 288, 293, 298 and 303 K), and the one that gave the better resolved spectrum (288 K) was chosen to record the 2D experiments. The detected NOE contacts are shown in Figure VIII.9.

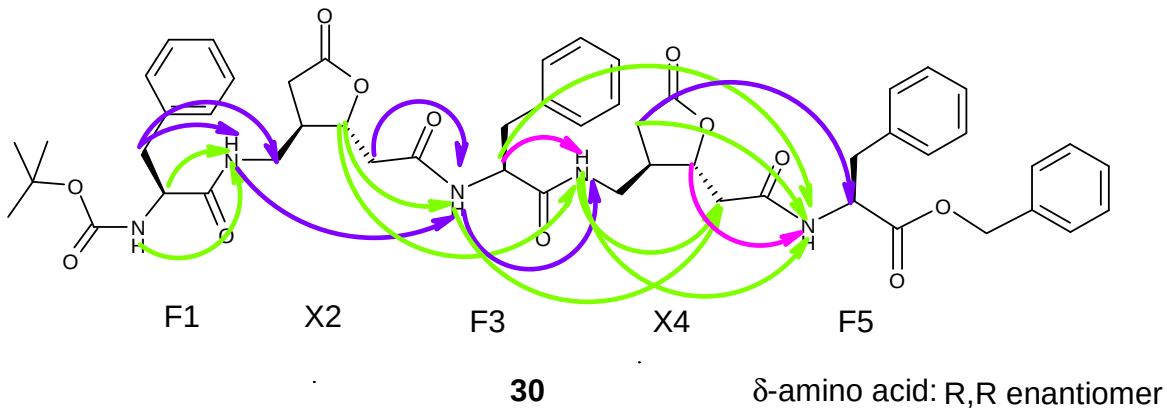
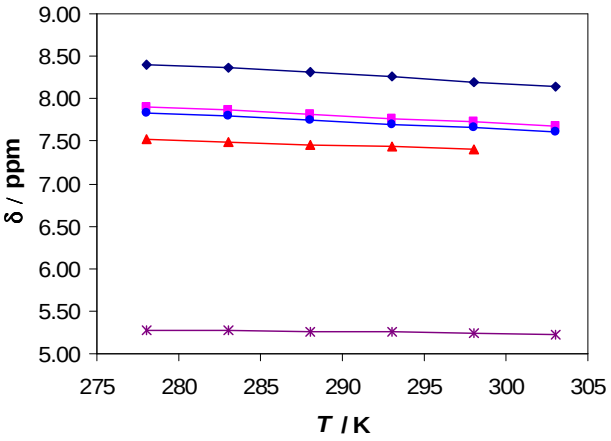


Figure VIII.9: Summary of the interresidue NOE contacts observed for the peptide **30** in methanol-d_3 at 288 K (pink arrows: strong NOEs; green arrows: medium NOEs; blue arrows: weak NOEs).

In comparison with the pentamer containing the (S,S)-δ-amino acid, this oligomer had a better dispersion of the signals in the ^{1}H spectrum with no overlapping of the NH amides, and the ROESY spectrum allowed us to find numerous NOE contacts that are reported in Figure VIII.9. In counterpart, we could see the presence of more than one conformer. We focused our study on the major conformer. NMR analyses showed amide protons at high ppm values (between 7.4 and 8.4 ppm), which is characteristic of protons involved in hydrogen bonding. However, $-\Delta\delta/\Delta T$ were quite high, ranging between 6 and 10.4 ppb/K, and also the ^3J coupling constants were higher than 6Hz (Figure VIII.10).



	$-\Delta\delta$(ppb)/ ΔT(K)	$^3J_{HN\text{-}H\alpha}$ (Hz)
NHF_1	2.0	7.2
NHX_2	8.8	
NHF_3	10.4	6.9
NHX_4	6.0	
NHF_5	8.8	8.2

Figure VIII.10: Variable temperature studies in methanol-d_3 of peptide 30.

The CD spectrum was recorded in TFE (0.3 mM) and gave two maxima at 203 and 218 nm (Figure VIII.11).

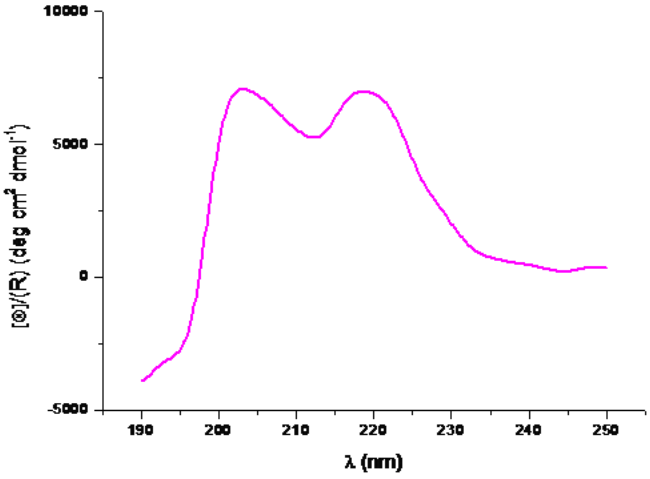


Figure VIII.11: CD spectrum of the pentapeptide **30** in methanol.

Finally, molecular dynamics experiments, taking into account eleven NOE contacts, afforded the structure shown in Figure VIII.12 corresponding to the energetic minimum.

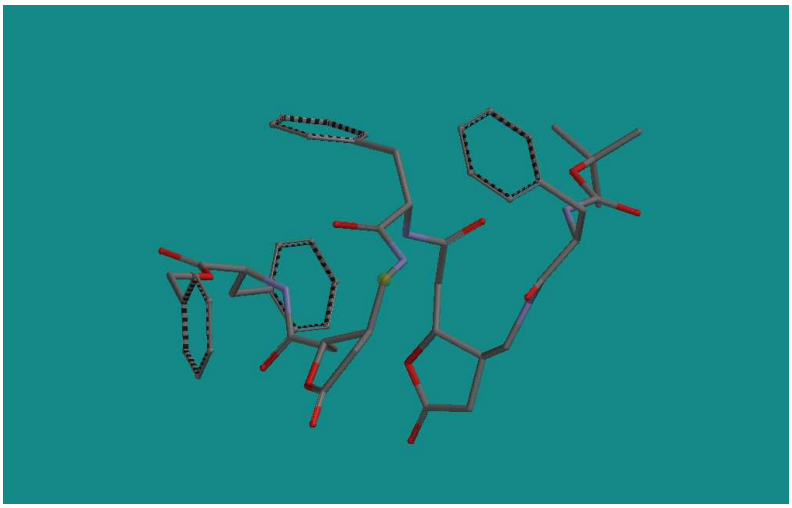


Figure VIII.12: Structure of the low-energy conformer calculated for peptide **30**. For clarity hydrogen bonds are omitted.

The heptamer containing alternated (R,R)-δ-amino acid and phenylalanine was analyzed by 2D NMR only in $CDCl_3$, since it was insoluble in methanol. In this solvent it showed a high dispersion of the signals, as well as numerous NOE contacts, among them long range ROE contacts were also detected. We can remark that the major part of the contacts were found in the N-terminal part of the molecule (Figure VIII.13): in particular, we detected contacts between two non neighbouring lactones, which gave indication about an eventual organisation of this part of the molecule. However, we could not see any repetitive contacts that would have been an evidence for the presence of a periodic structure.

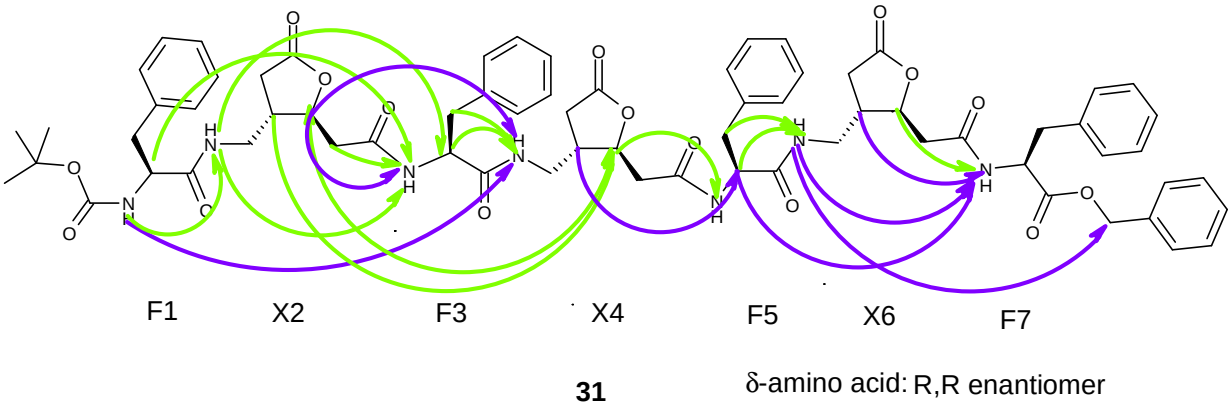
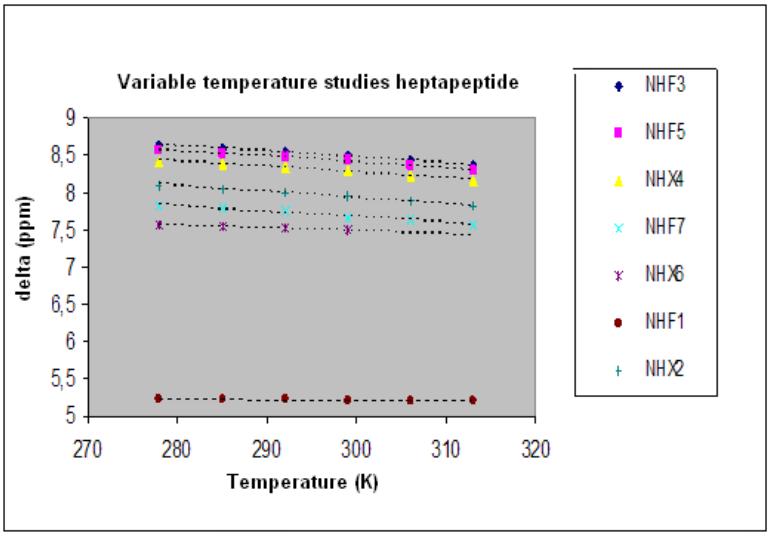


Figure VIII.13: Summary of the interresidue NOE contacts observed for the peptide 31 in $CDCl_3$ at 292 K (green arrows: medium NOEs; blue arrows: weak NOEs).

Here again Δδ/ΔT coefficients adopt quite high absolute values between 7 and 8 ppb/K, excepted for the third lactone and the Boc terminal phenylalanine, and ^{3}J coupling constants were also quite high, excepted for F5 that showed a ^{3}J of 4.8 Hz (Figure VIII.14).



	$-\Delta\delta$(ppm)/ΔT(K)	$^{3}J_{HN\text{-}H\alpha}$ (Hz)
NHF1	0.7	9.6
NHX2	7.8	
NHF3	7.7	8.4
NHX4	7.3	
NHF5	7.8	4.8
NHX6	3.9	
NHF7	7.2	7.2

Figure VIII.14: Variable temperature studies in $CDCl_3$ of peptide **31.**

IR in solution was performed in CH_2Cl_2 at low concentration showing in this case again two bands, a weak one at 3420 cm^{-1} representing the non hydrogen-bonded amide, and an intense band at 3300 cm^{-1}, reflecting the presence of amide protons involved in hydrogen bonds. To gain further information about the amide protons involved in hydrogen bonding, we tried for the penta- and heptapeptides containing the (R,R)-δ-amino acid to perform solvent competition experiments, by progressive addition of DMSO in the NMR tube and controlling the changing in the chemical shifts. Unfortunately, this experiment failed due to the fact that by adding DMSO we were not able anymore to assign the amide protons. Deuterium exchange experiments revealed to be unrealisable, since our compounds were precipitating upon addition of minimum amounts of methanol-d_4. Molecular dynamics was performed including seventeen constraints taken from NOE contacts. Two pictures of the energetic minimum are represented below showing in the first segment, containing Boc-Phe-Lac-Phe-Lac-Phe, a folded part that describes a first loop of helix, while the folding in the C-terminal part seems to be disrupted from the last lactone. One reason for the loss of the folding properties in C-terminal part of the peptide could

be the high spatial freedom of the benzyl ester group that directs the C-head of the peptide in another direction. This is particularly evident in the second view of the structure, in which we can see the compact helical structure imposed by the two first lactones X2 and X4, followed by a reversal of the structure in the C-terminal part (Figure VIII.15).

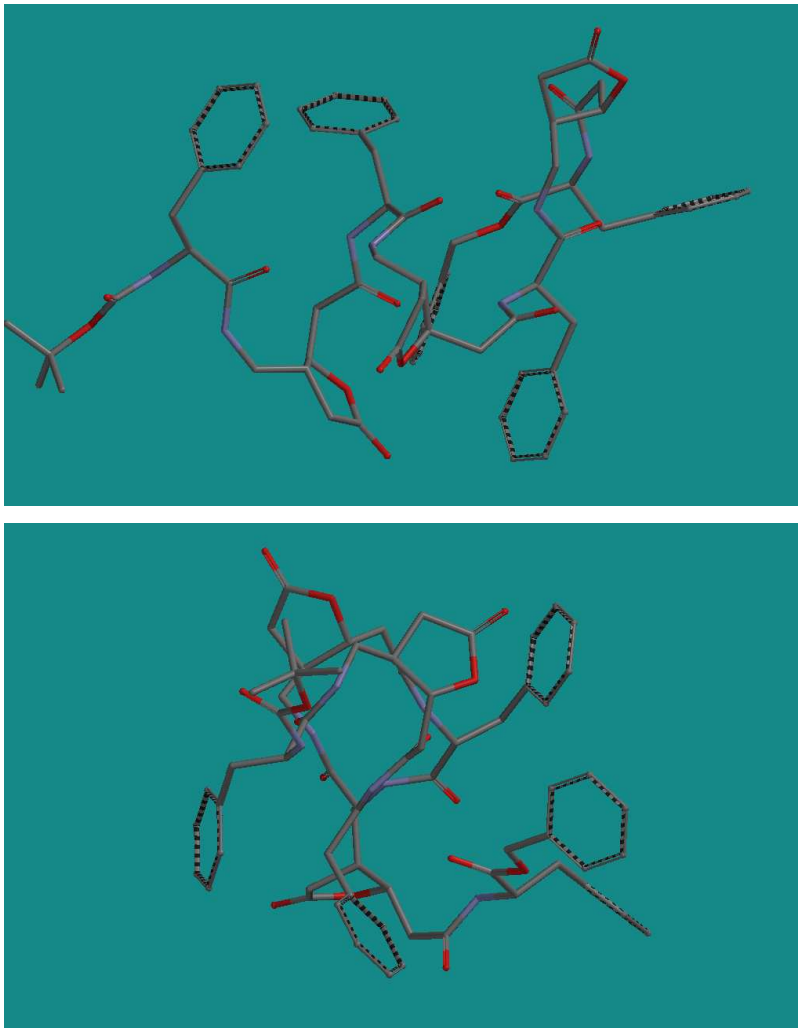
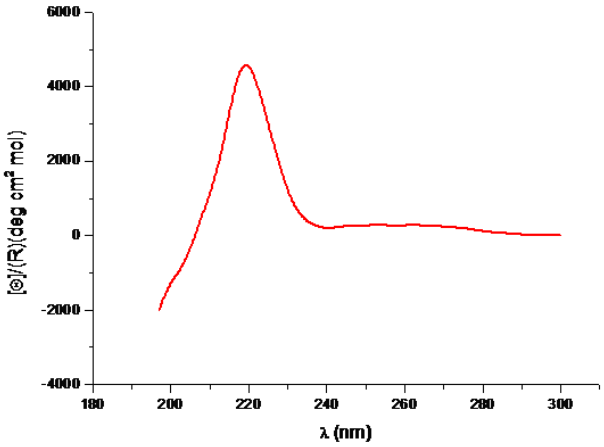


Figure VIII.15: Two views of the structure of low-energy conformer calculated for peptide **31**. For clarity hydrogen bonds are omitted.

In contrast to the CD spectra of the pentamers, the CD spectrum of the heptamer in TFE at 0.5 mM showed only the maximum at 218 nm, whereas the second maximum near 200 nm found for the pentamers disappeared (Figure VIII.16). This might be indicative of the presence of an ordered structure stabilized by the longer peptide chain.

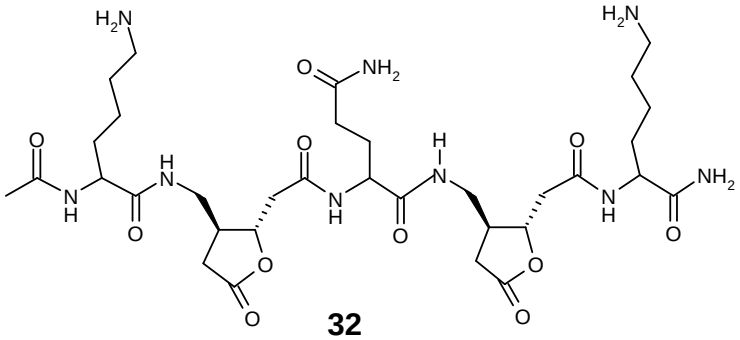


FigureVIII.16: CD spectrum of the heptapeptide **31** in TFE.

The results presented above on the conformational analysis of three peptidomimetics containing aromatic α-amino acids alternated with polar δ-amino acids suggest that these δ-amino acids have the potential to induce structural motifs, which, however, differ from those typically adopted by α-peptides. Interestingly, peptides containing the (S,S)-δ-amino acid showed major solubility in methanol, also showing better folding properties in polar solvents. In contrast, peptides containing the (R,R)-δ-amino acid showed better folding properties in non polar solvents. According to the numerous NOE contacts found in the analysis of pentamer and heptamer containing the (R,R)-δ-amino acid, this enantiomer is likely to have better folding properties than the S,S enantiomer. Finally, increase in the length of the peptide sequence led to a stabilization of the secondary structure.

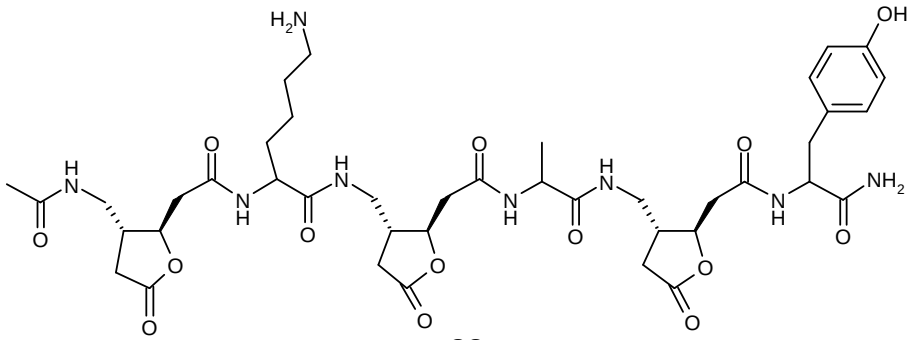
VIII-3 New peptides containing δ-amino acids: Objectives and design

After the results obtained with peptides **29**, **30**, **31** (Figure VIII.3), we decided to synthesize further α/δ-peptides containing a variety of -amino acids ranging from apolar to charged residues. This should avoid the overlapping of the signals in NMR spectra and lead to peptides with different properties. In the case of **32** (Figure VIII.16), we modified the sequence by increasing the number of positively charged residues. Thus, two lysines and one glutamine were alternated with δ-units. To investigate the effect of our scaffold on the secondary structure of longer peptides, we carried out the synthesis of peptides **33** and **34** (Figure VIII.16), which contain both apolar and polar α-amino acids. The synthesis and the results of the 2D-NMR, CD, FT-IR measurements and of the molecular modelling will be shown and discussed in the following paragraphs.



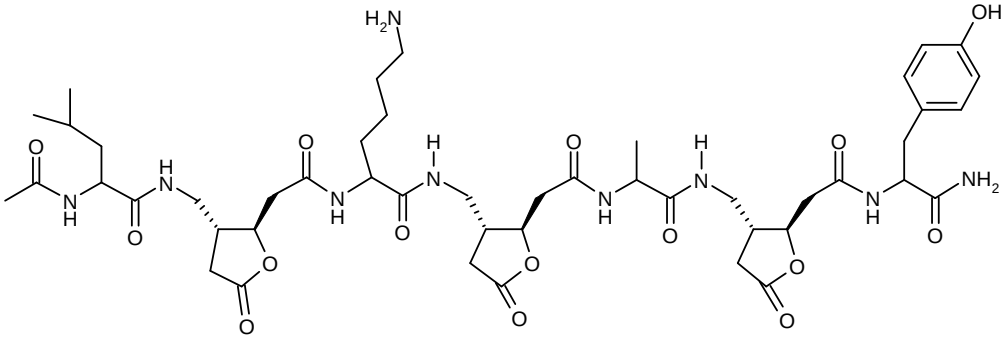
32

δ-amino acid: S,S enantiomer



33

δ-amino acid: R,R enantiomer



34

δ-amino acid: R,R enantiomer

Figure VIII.16. Lactone containing peptides 32, 33 and 34 that have been synthesized and investigated in this work.

VIII.4 Synthesis of peptides 32-34

The model peptides containing the enantiomers (S,S) or (R,R) of our lactone-scaffold that were synthesized and investigated in the course of this study are shown in Figure VIII.16.

The synthesis of the building block, being suitably functionalized to be introduced in peptides, has been carried out as developed in our group from γ-butyrolactonaldehyde **25** in enantiomerically pure form (S,S)[37]-**35** and (R,R)-**36** (Figure VIII.17).

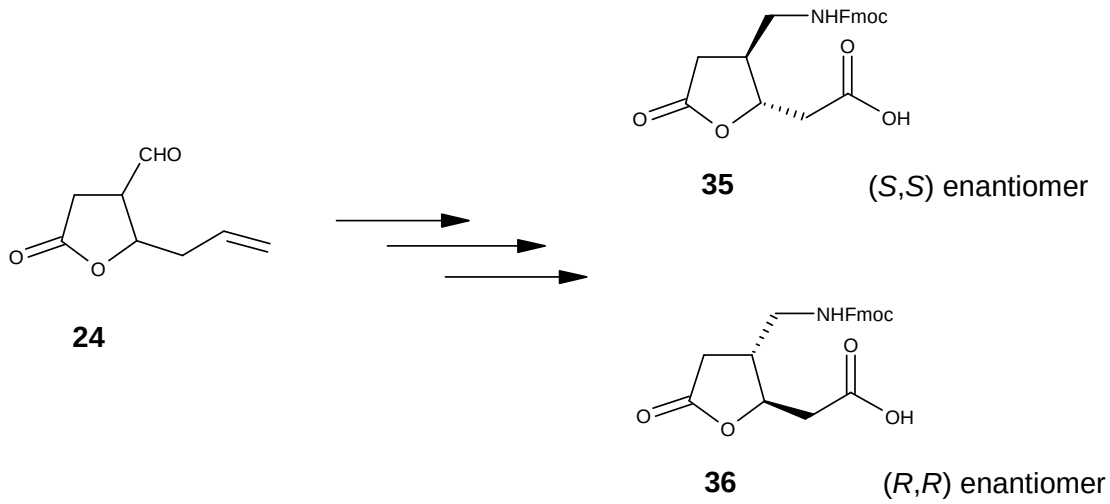
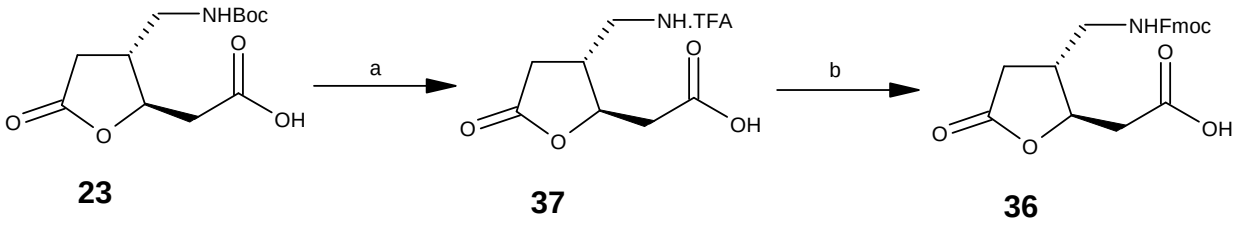


Figure VIII.17. Pentalactone building blocks 35 and 36 for peptide synthesis.

Synthesis of Fmoc protected δ-amino acid (R,R) 36.

The synthesis of the Fmoc protected δ-amino acid (R,R)**36** is described in Scheme VIII.9 **36** was synthesized starting from (R,R) lactone **23** (Figure VIII.2), by cleavage of the Boc protecting group with TFA in dry CH_2Cl_2. The resulted product **37** was treated with Fmoc-OSu in dioxane-1 M K_2CO_3 to afford the desired compound in moderate yield (60%).



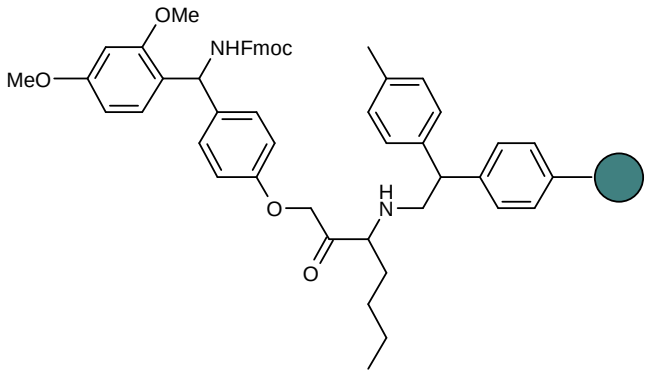
23 **37** **36**

Scheme VIII.9. Synthesis of the Fmoc protected δ-amino acid 36. Reagents:
a) TFA, dry CH_2Cl_2, 100%; b) FmocOSu, dioxane-1M K_2CO_3 (1 : 2), 60%.

Synthesis of alternated α/δ-unit peptides

The peptides **32-34** were synthesized, as shown in Schemes VIII.10 and 11, using a solid phase protocol. The solid phase synthesis can be commonly performed by using two alternative protecting groups, tert-butyloxycarbonyl (Boc)[54] and fluorenyl-9-methoxycarbonyl (Fmoc),[55,56] to mask the α-amino group of the amino acid only temporarily to allow further chain elongation. Generally, the Fmoc strategy is more suitable for solid phase synthesis, as the Fmoc group is a base-labile protecting group which can be removed using secondary amines like piperidine and the cleavage of the peptide from the resin occurs under mild acidic conditions. Another very important advantage of the Fmoc group is its stability upon different conditions used to remove side-chain protecting groups, including strong acidic or reductive conditions.

The products presented in this work were synthesized by manual coupling using Fmoc chemistry on Rink amide MBHA resin **38** (loading 0.6 mmol g^{-1}) (Figure VIII.18). The resin was in the Fmoc-protected form and, for this reason, to obtain N-free linker, the resin was treated with piperidine in DMF/NMP (80:20 v/v).

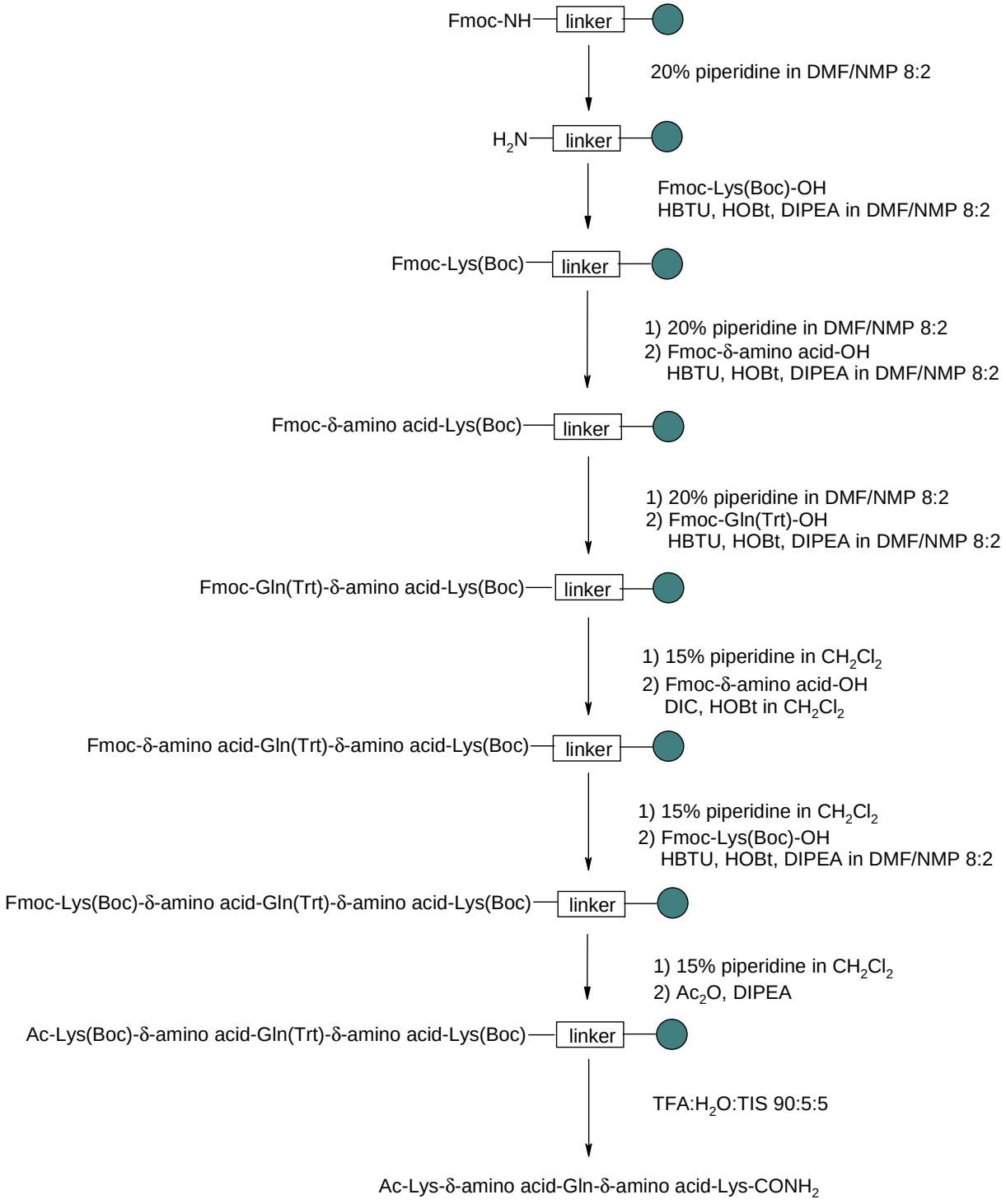


4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucyl-MBHA resin

38

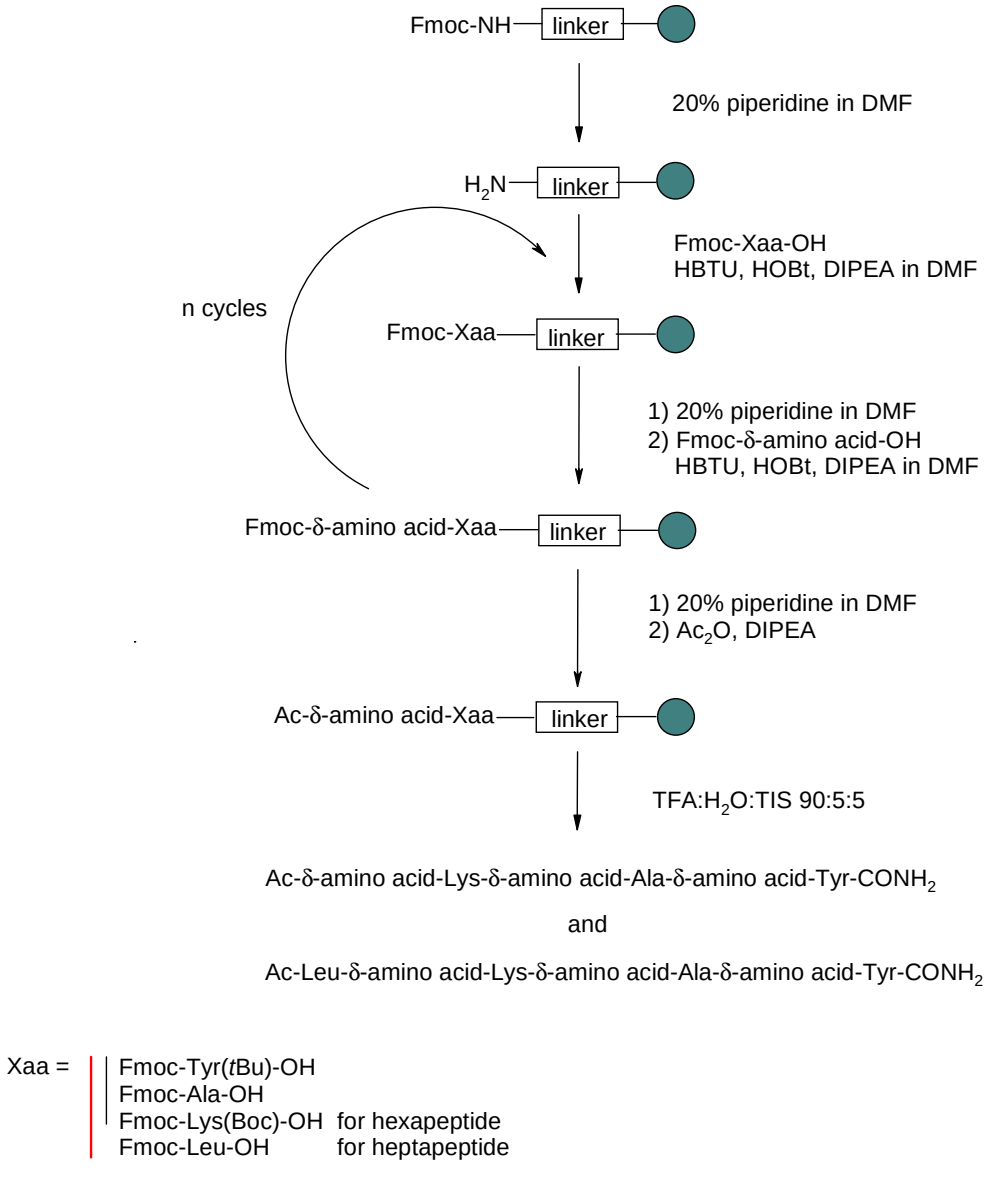
Figure VIII.18. Rink amide MBHA resin 38.

In the case of peptide **32** the synthesis is outlined in Scheme VIII.10, and was performed with (S,S)-δ-amino acid. The first amino acid (4 equiv.), lysine, was coupled activating *in situ* the carboxylic function with HBTU (3.9 equiv.), HOBt (4 equiv.) in DMF/NMP (80:20 v/v), in the presence of DIPEA (8 equiv.). Fmoc cleavage was accomplished by treating the peptidyl-resin with 20% piperidine in DMF/NMP (80:20 v/v). For the coupling of the second amino acid, Fmoc-δ-amino acid, 2.5 equiv. excesswere used, together with HBTU (2.4 equiv.), HOBt (2.5 equiv.) in DMF/NMP (80:20 v/v), and DIPEA (5 equiv.). The following steps, the Fmoc deprotection and the coupling of glutamine, were done with the same conditions used for lysine. To monitor the progress of the synthesis, small scale cleavage was performed and the resulting sample was analyzed by MALDI-ToF-MS. To avoid a potential lactone ring opening, we decided to use milder conditions for the Fmoc deprotection (only 15% of piperidine in CH_2Cl_2 for 3 min. instead of 20% for 5 min. in DMF/NMP) and to change the coupling conditions for the second Fmoc-δ-amino acid: DIC (2.5 equiv.) and HOBt (2.5 equiv.) in CH_2Cl_2 without base. After the coupling of the second lysine and the Fmoc deprotection, the last step was the N-terminal acetylation using acetic anhydride (5 equiv.) and DIPEA (2.5 equiv.). Final cleavage of the peptide from the resin and simultaneous side-chain deprotection was achieved by treatment with a TFA/water/TIS mixture (90:5:5) for 2.5 hours. The peptide was then precipitated from ice-cold diethyl ether, centrifuged and subjected to three ether-washing/centrifugation cycles to remove the scavengers. The characterization was performed by HPLC and MALDI-ToF-MS.



Scheme VIII.10. Solid phase synthesis of 32 using the Fmoc chemistry.

In the case of peptides **33** and **34** containing (R,R)-δ-amino acid, the solid phase protocol used was the same as the one just described for **32**, but this time only DMF was used as the reaction solvent. A different number of equivalents of the amino acids (Xaa 5 equiv.), coupling reagents (HOBt 5 equiv./HBTU 4.8 equiv./DIPEA 10 equiv.) and acetic anhydride/DIPEA (8 equiv./ 7equiv.) were also used (Scheme VIII.11).



Scheme VIII.11. Solid phase synthesis of 33 and 34 using the Fmoc chemistry.

VIII-5 Results and discussion

VIII.5.1 Alternated α/(S,S)-δ-unit peptide

The synthesized α/δ-pentapetide **32** was dissolved in methanol-d_3 and studied by NMR spectroscopy. 1D experiments were first acquired at five different temperatures (283, 288, 293, 298 and 303 K), and the one that gave the better resolved spectrum (303 K) was chosen to record the 2D experiments. The complete ^{1}H-NMR resonance and sequence assignments were done by using the COSY, TOCSY and ROESY spectra. Unfortunately only, medium and weak contacts were found and only between one residue and its neighbour, or intralactone in one case (Figure VIII.19).

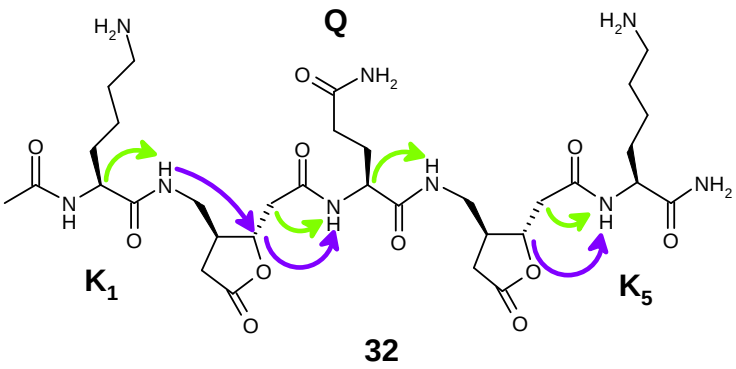
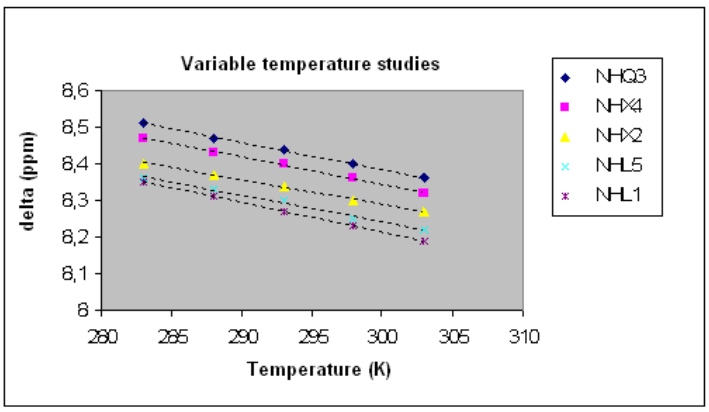


Figure VIII.19. Summary of the interresidue ROE constraints observed for the peptide 32 in methanol-d_3 at 303 K (green arrows: medium ROEs; blue arrows: weak ROEs).

The values of the $^3J_{HN\text{-}H\alpha}$ coupling constants were estimated by using the amide region in the 1D-NMR spectrum and the vicinal coupling constant value is always bigger than 6 Hz. No estimation was possible in the case of the two lactones. These values are typical of an extended or unordered structure (Figure VIII.20).

The temperature coefficients ($\Delta\delta/\Delta T$) for the pentapeptide **32** were also measured, which were in the range of -6.6/-8.0 ppb/K (Figure VIII.20).



	$-\Delta\delta(ppm)/\Delta T(K)$	$^3J_{HN-H\alpha}$ (Hz)
NHQ_3	7.4	7.0
NHX_4	7.4	
NHX_2	7.2	
NHK_5	6.6	7.6
NHK_1	8.0	7.2

Figure VIII.20 Temperature coefficients for the NHs and vicinal coupling constants for HN-Hα in methanol-d_3 for 32.

We used molecular modelling to investigate the possible structures that the peptide could adopt. After the construction of the molecule *in silico* the observed NMR contacts were introduced as constraints before carrying out an energy minimization and Molecular Dynamics. In our analysis we excluded the molecules in which there were violations of the NMR-derived constraints and we considered only the structures energetically favored. The lowest energy conformer of this set of molecules is shown in Figure VIII.21 Clearly the peptide forms a large loop, in which the two lactones are located opposite to each other, with the central residue glutamine acting as a pivot.

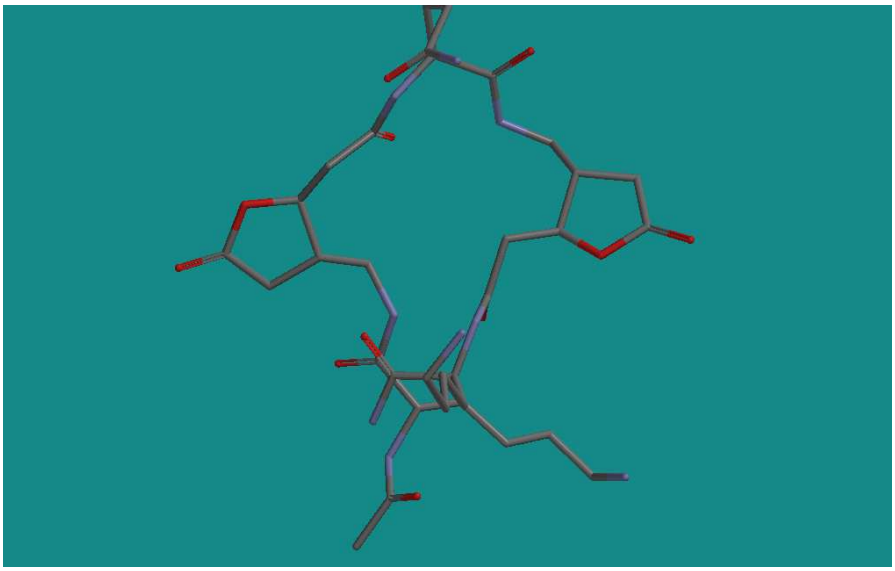
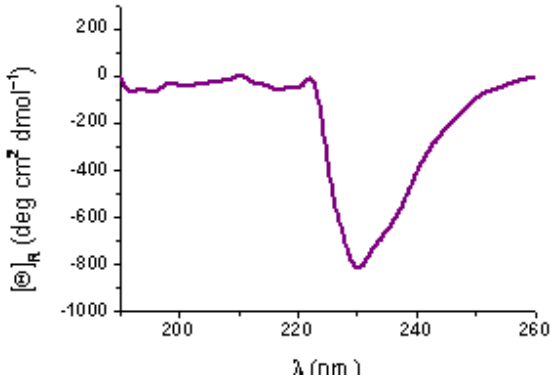


Figure VIII.21 Structure of low-energy conformer calculated for compound 32. Hydrogen atoms are omitted for clarity.

The CD investigation was performed in methanol (5 mM) and the peptide **32** displayed only one negative band at 230 nm (Figure VIII.22). Unfortunately, no similar spectra have been reported either in the literature or in our previous work.

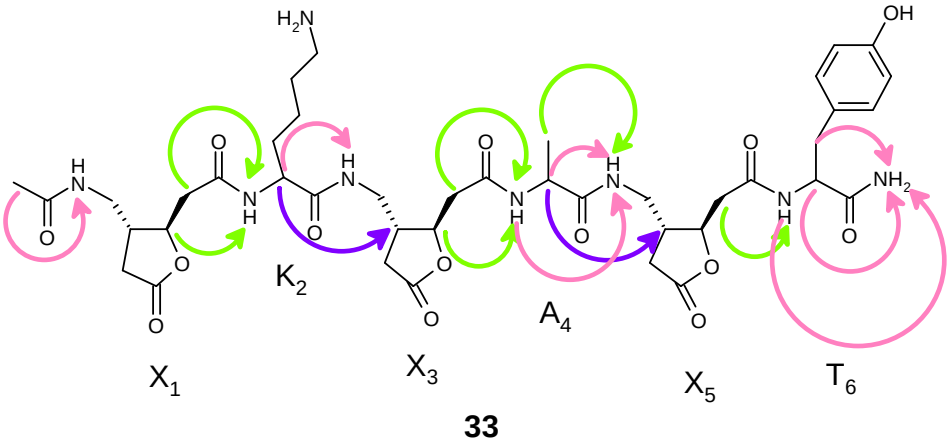


.

Figure VIII.22 CD spectra of compound 116 (5 mM in methanol). The data are normalized for peptide concentration and number of residues.

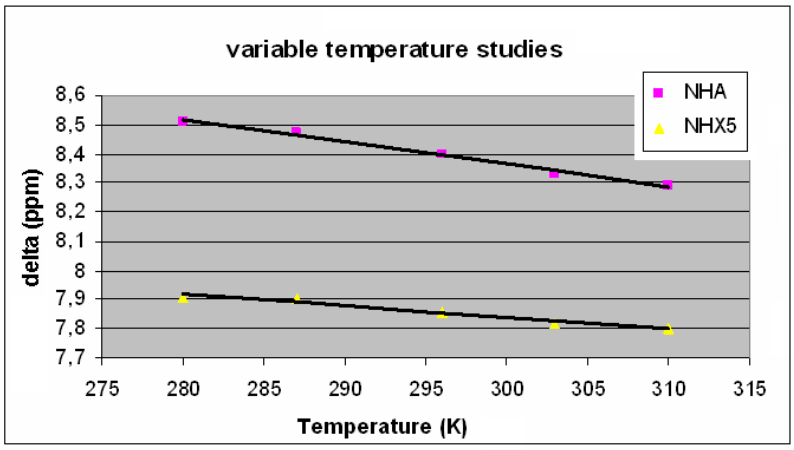
VIII.5.2. Alternated α/(R,R)-δ-unit peptides

Two further peptides containing the (R,R)-δ-unit alternated with α-amino acids like lysine, alanine, tyrosine and leucine were prepared by solid phase methodology.The ^{1}H NMR spectra of hexapeptide **33** were recorded at five different temperatures (280, 287, 296, 303 and 310 K), and the one at which the dispersion of the signals was reasonable (280 K) was chosen to record the 2D experiments. Nevertheless, NMR characterization was complicated from the significant signal overlap in the spectrum. The ROESY spectrum in connection with COSY and TOCSY spectra revealed several contacts, including those between neighbour amino acids (Figure VIII.23). The central part of the peptide seemed to be more structured and the temperature coefficient of lactone X_5 was relatively small (Figure VIII.23).



33

Figure VIII.23. Summary of the interresidue NOE constraints observed for the peptide 33 in methanol-d_3 at 280 K (pink arrows: strong NOEs; green arrows: medium NOEs; blue arrows: weak NOEs).



	$-\Delta\delta$(ppm)/ ΔT(K)	$^3J_{HN\text{-}H\alpha}$ (Hz)
NHA_4	7.6	6.4
NHX_5	4.0	
NHK_2		7.9

Figure VIII.24 Temperature coefficients for the NHs and vicinal coupling constants for HN-Hα in methanol-d_3 for **33.**

Using several NOE contacts as constraints, MD simulations were carried out, but we could not see a periodic structure (Figure VIII.25).

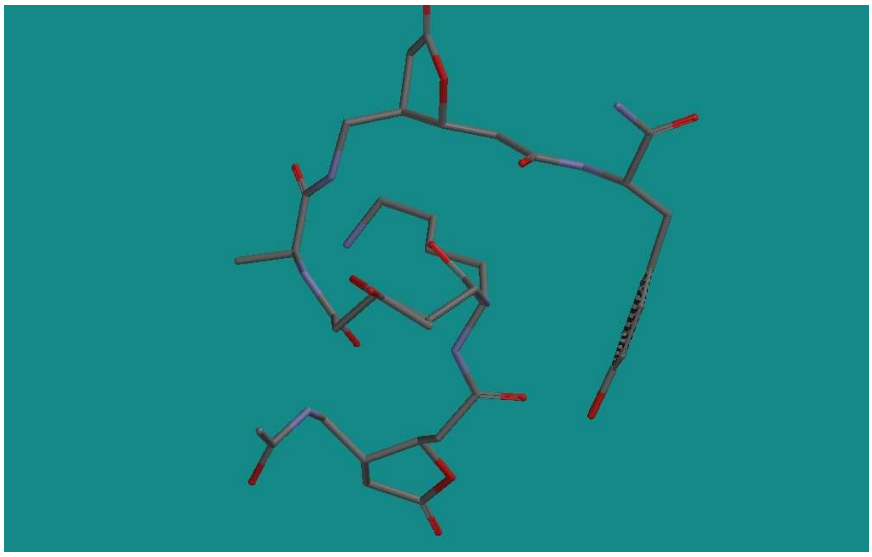


Figure VIII.25 Structure of low-energy conformer calculated for compound **33**. Hydrogen atoms are omitted for clarity

We recorded the circular dichroism spectrum in methanol at concentration of 2 mM (Figure VIII.26), which exhibited a strong positive band at 238 nm.

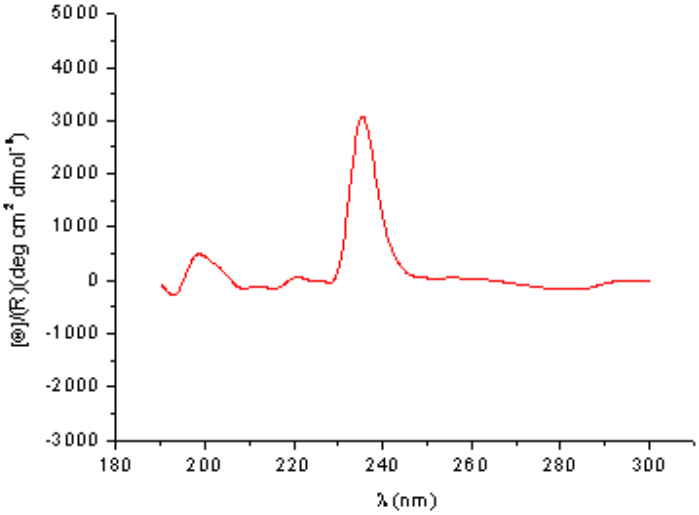
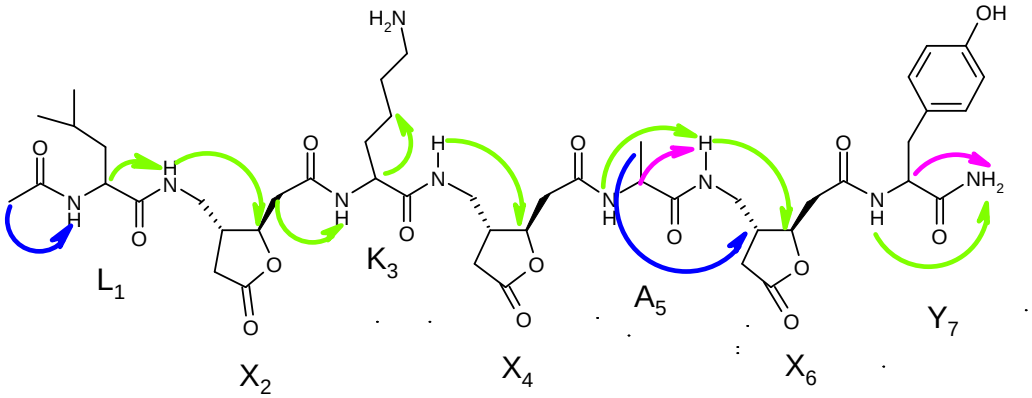


Figure VIII.26 CD spectra of compound **33** (2 mM in methanol). The data are normalized for peptide concentration and number of residues.

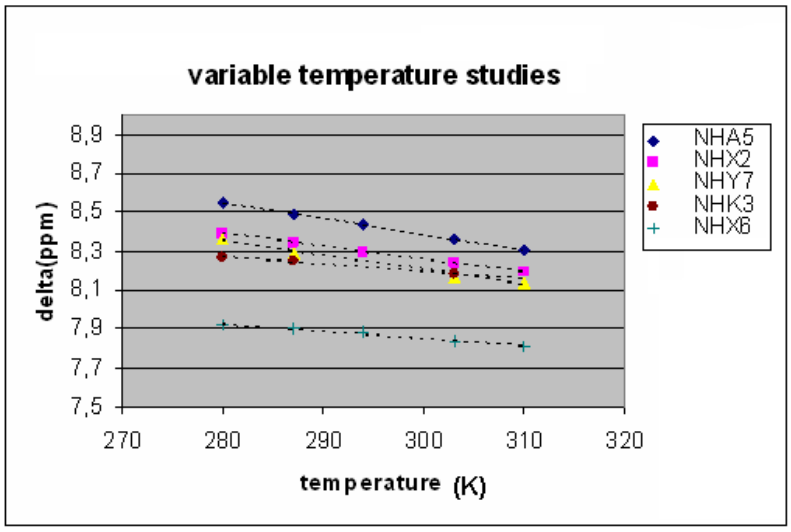
The heptapeptide **34** was obtained from **33** after elongation with an additional α-amino acid, leucine. The 2D NMR analyses at 303 K revealed only few and non-relevant NOE contacts (Figure VIII.27).



34 δ-amino acid: R,R enantiomer

Figure VIII.27 Summary of the interresidue NOE constraints observed for the peptide **34** in methanol-d_3 at 303 K (pink arrows: strong NOEs; green arrows: medium NOEs; blue arrows: weak NOEs).

The temperature coefficients and coupling constants of **34** are reported in Figure VIII.28 It can be noted that the amide protons of the alanine-δ-lactone motif in **33** and **34** show comparable temperature coefficients.



	-Δδ(ppm)/ ΔT(K)	$^3J_{HN-H\alpha}$ (Hz)
NHL_1		
NHX_2	6.6	
NHK_3	4.0	
NHX_4		
NHA_5	8.0	6.6
NHX_6	3.7	
NHY_7	7.4	8.4

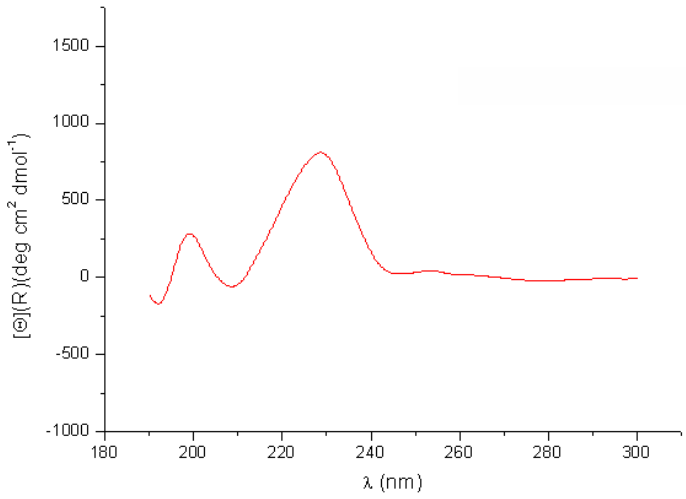
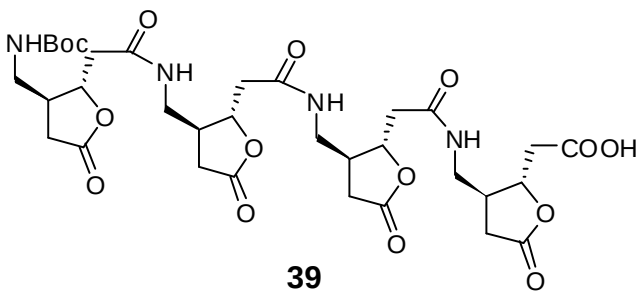


Figure VIII.28 CD spectra of compound 34 (2 mM in methanol). The data are normalized for peptide concentration and number of residues.

VIII.6 Monomer studies

VIII.6.1 Previous work on the δ-butyrolactone amino acid

In a previous work carried out by M. Haque[37] in our laboratory in Regensburg, the tetramer **39** consisting of four (S,S)-δ-amino acid units was synthesized (Figure VIII.29). The NMR investigation was not possible due to signal overlapping especially in the NH region.



39

Figure VIII.29. Tetramer **39**.

Therefore CD analyses in different solvents were performed (Figure VIII.30). The CD spectra were recorded in the far UV region, at the concentration of 5 mM in TFE, methanol and methanol/water 60:40. In TFE the CD spectrum was characterised by a positive band at 191 nm, and by a broad negative band centred at 214 nm, with a crossover at 204nm. Changing TFE with methanol doubled the intensity of the negative band, while the shape and the bands position remained the same. The intensity of the negative band further increased in the mixture methanol/water, becoming more than two times higher than in methanol and more than four times higher than in TFE. Again, the shape of the band remained more or less constant, but a better-defined minimum was present at 214 nm. The fact that the CD spectra were solvent dependent indicates that the conformational properties of the peptide are influenced by the environment. An increase in the intensity of CD bands suggests an improved structural stability. For the δ-oligopeptide a stabilisation effect was observed in methanol rather than in TFE, and in the presence also of water rather than in 100% methanol. This behaviour is quite unusual, as the conformation of peptide mimics is normally destabilized by addition of water that is a strong donor and acceptor of H-bonds. In contrast, TFE is a well-known secondary structure stabilizer, being a strong H-bond donor but a weak H-bond acceptor.

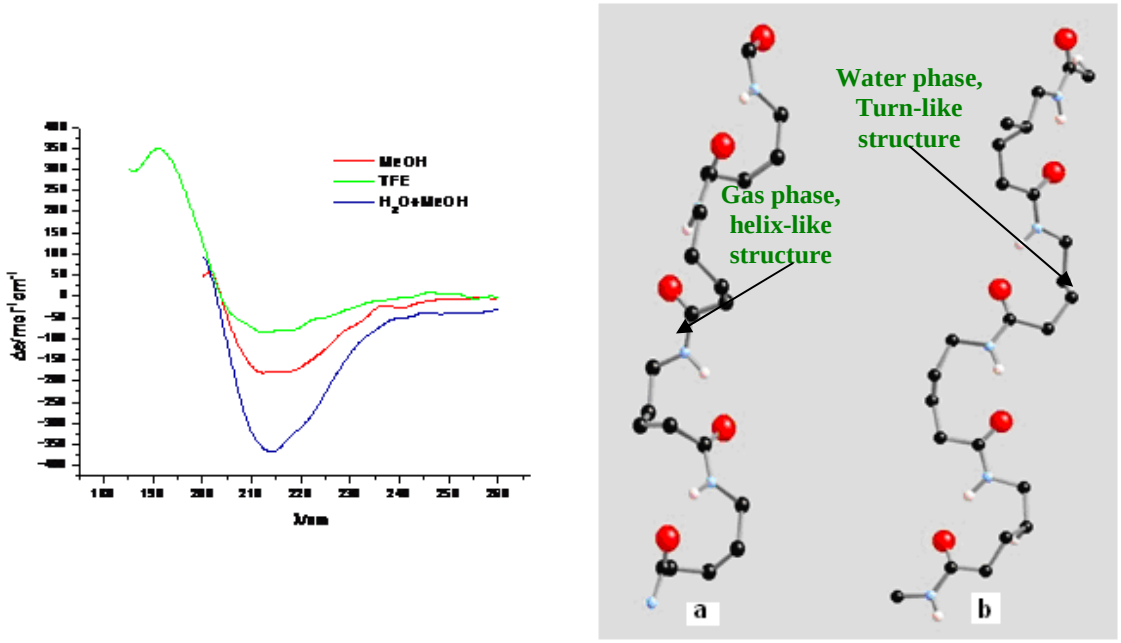


Figure VIII.30 Left: CD spectra in TFE (green), methanol (red) and water/methanol mixture (blue). Right: Hofmann's calculations: a) in gas-phase, helix-like structure; b) in water, turn-like structure.

To reinforce and illustrate these results, molecular modelling experiments were performed at the University of Leipzig by the Hofmann's group and the results showed two families of structures. The first family in gas-phase had a helical tendency (Figure VIII.30 a), whereas the second one in water showed a succession of turn-like structures (Figure VIII.30 b)**.** In both cases, the secondary structures seemed to be stabilized by H-bonds formed between the carbonyl and the NH of the same lactone residue (8-membered hydrogen-bonded ring). Another feature distinguishing both Hofmann's structures is the conformation of the lactone. In the case of the calculations in gas-phase both substituents on the cycle are occupying an axial position, whereas the calculations in water show the two "arms" of the lactone in an equatorial position. It was not possible to confirm by NMR and X-ray any of the information obtained from the computational and CD data.

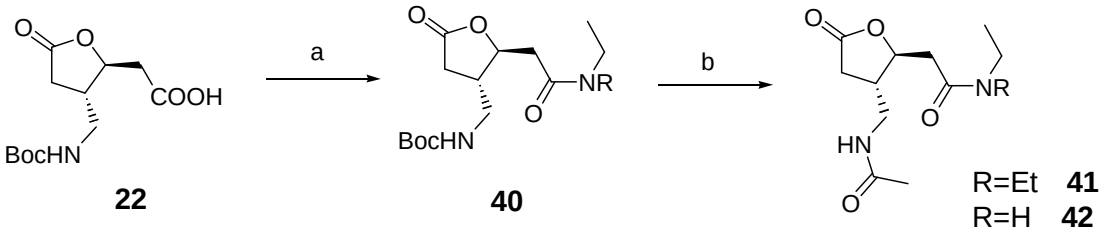
VIII.6.2. New analyses on the monomer

In order to gain further information about such structures in solutions, we decided to perform conformational studies on the monomer. First we wanted to control its ability to form an internal H-bond (8-membered H-bonded ring) that would confirm the possible stabilization of the structures described by Hofmann's calculations and, secondly, we wanted to study the lactone ring conformation as it could play a crucial role in the structural behaviour of further lactone-containing peptidomimetics.

Design and synthesis

This kind of conformational studies based on monomeric structures have been previously reported by Gellman and co-workers[57] in their attempt to evaluate folding properties of β- and γ-aminoacids. To study the particular H-bond preferred conformation of our lactone we needed two types of compounds. In the first one, only one hydrogen bond would be possible, and in the second one the monomer could adopt two hydrogen bond patterns. Thus, we synthesised compounds **41** and **42** as described in Scheme VIII.12 In the first step the Boc-protected lactone derivative **22** was coupled to a mono or dialkylated amine. The resulting compound **40** was Boc

deprotected by treating it with TFA in dry CH_2Cl_2, and finally the amino group was acetylated to afford our model compounds **41** and **42**.



Scheme VIII.12 Synthesis of compounds 41 and 42. Reagents:
a) NHEtR, EDC, HOBt, DMF, 42% when R=H and 65% when R=Et; b) (i) TFA, dry CH_2Cl_2 ; (ii) Ac_2O, THF, 65% when R=H and 60% when R=Et.

Conformational analyses

We studied both compounds first by NMR and, secondly, by IR in CH_2Cl_2 at low concentration (3 mM) and room temperature (Figure VIII.31).

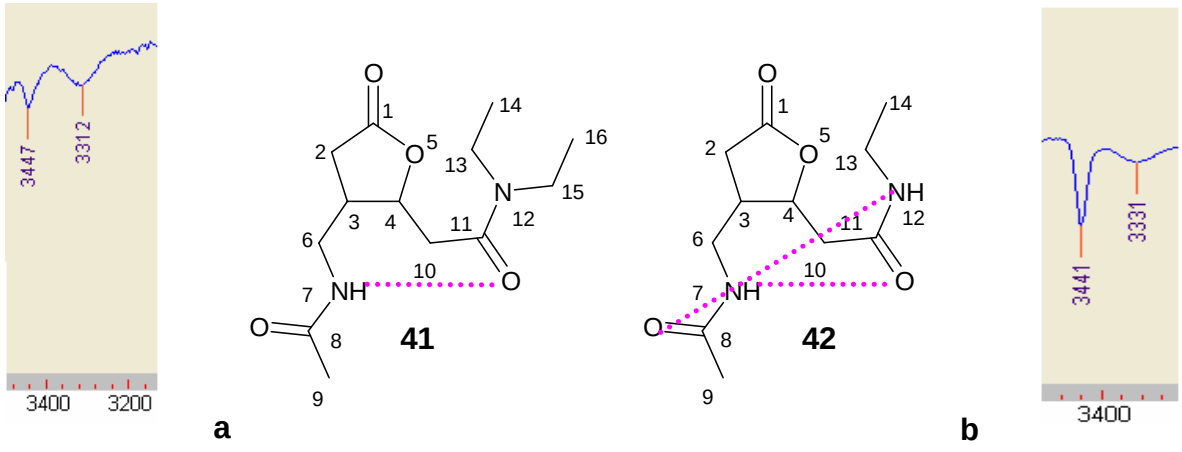


Figure VIII.31 a) IR studies of **41**; b) IR studies of **42**. The possible hydrogen bonds are shown in magenta.

In the IR spectra it is possible to observe two N–H stretch bands for amide protons: the highest one about 3400 cm^{-1} indicates an amide in a non-hydrogen-bonded state, whereas the lowest one appearing near 3300 cm^{-1} corresponds to an amide in hydrogen-bonded state.

The disubstituted amide in **41** presented two IR bands at 3347 and 3412 cm^{-1} with similar intensity (Figure VIII.31 a), whereas the monosubstituted compound **42** showed an intense band in the non-hydrogen-bonded NH region at 3441 cm^{-1} and a weaker band in the region of H-bonded amides at 3331 cm^{-1} (Figure VIII.31 b). These data suggest that the intraresidue H-bond is favoured in the monomer **41** comporting only one H-bond possibility, whereas in the monosubstituted derivative **42** with more possible combinations, the non-H-bonded state is preferred. These analyses confirm that an intraresidue H-bond is possible in our structure, supporting indeed the H-bond pattern described in Hofmann's structures.

In NMR investigations two parameters were considered. On the one hand, variable temperature studies gave the temperature coefficients that in some cases can be correlated with the H-bonding state of amide protons. On the other hand, NOESY experiments gave insight about the distance between two protons in the space.

In variable temperature studies the NH(7) of the disubstituted lactone **41** showed a high temperature coefficient (-9.2 ppb/K), typical value for non-hydrogen-bonded state, and NOESY spectra performed at room temperature did not show any intra-strand NOE contacts. In contrast, the monosubstituted lactone **42** appeared to adopt two distinct behaviours depending on the temperature in which it was studied, at room temperature and at 225 K. The temperature coefficient for the NH(7) (-8.9 ppb/K) was similar to the one in the disubstituted lactone, whereas the NH(12) showed a lower value of $\Delta\delta/\Delta T$ = -6 ppb/K. For this compound NOESY experiments were performed at room temperature and at 225 K, and an intra-strand NOE cross peak was found at low temperature between CH_3(9) and NH(12). These two parameters, NOE contacts and temperature coefficient, found for NH(12) suggest a preferred H-bonding pattern involving NH(12) and CO(8) of **42** at low temperature.

Investigations on the ring conformation

The γ-butyrolactone system possesses great importance in the chemistry of natural products.[58,59] Therefore, considerable interest is given at the synthesis and structural characterization of substituted γ-butyrolactones.

One advantage of using γ-butyrolactone as synthetic intermediate is the low conformational flexibility: indeed, one needs to consider only the interconversion between two envelope forms A

and B, in which $\alpha = \beta = H$[60] (Figure VIII.32). In this case the interconversion barrier is quite low and the lactone adopts both shapes in solution. However, when substituents are introduced on such a small ring, both the ring structure and the equilibrium between the A and B forms are greatly affected, and the analysis in NMR spectra of ring protons turns into a complicated task.

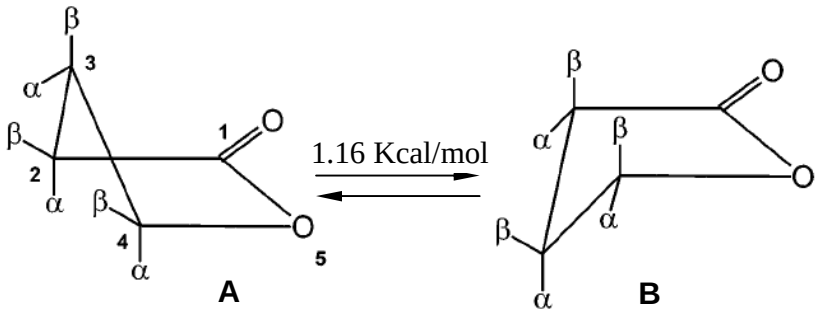
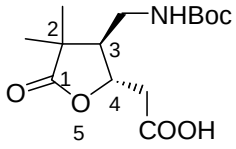


Figure VIII.32 Interconversion of γ-lactone A in B

To evaluate the substituted γ-lactone conformer distribution in solution several methods have been developed based on experimental vicinal and geminal coupling constants combined with conformational studies by molecular mechanics.[60-62] In our case, experimental data gave a really large value of $J_{3\text{-}4}$ for both disubstituted **41** and monosubstituted **42** monomers, which was 12.3 and 13.1 Hz, respectively. Such large coupling constants are in agreement with *trans* protons 3 and 4 occupying both a diaxial position on the lactone ring. We then compared these values with the one of the coupling constant from the dimethylated lactone **43** (Figure VIII.33), which was previously synthesized by Delatouche in our group. In this case the coupling constant value between H_3 and H_4 was 9.8 Hz. It has been earlier demonstrated on 2,4 disubstituted lactones that 1-3 diaxial strains are quite disfavouring in term of energy in γ-butyrolactones.[60] Thus, in our dimethylated compound **43** the conformer A that shows a 1-3 diaxial strain between the methyl group in position 2 and the lactone arm in position 4 should be unstable, while the predominant conformation in solution should be the conformation B, in which H_4 and one methyl group in position 2 are diaxial (Figure VIII.33).



43

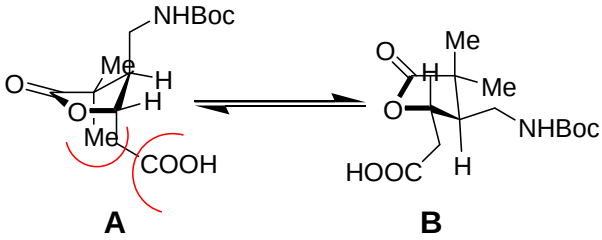
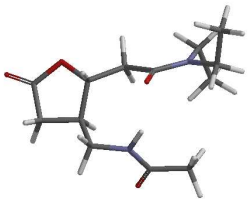


Figure VIII.33 Interconversion between the two envelope conformations A and B of **43**.

The results suggested by the coupling constants were then supported by molecular mechanics calculations using MMFF force field in the program Spartan,[63] which gave the conformation with the two arms in an equatorial position as energetic minimum for both lactones **41** and **42** (Figure VIII.34).

E=-57,62Kcal/mol

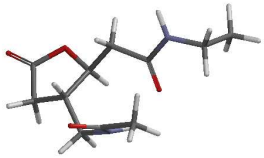
41



H_3CCH_{2A}	157.77°
H_3CCH_{2B}	32.96°
H_3CCH_4	-162.71

E=-67,84Kcal/mol

42



H_3CCH_{2A}	-158.05°
H_3CCH_{2B}	-33.21°
H_3CCH_4	163.23°

Figure VIII.34 Energy minimum of **41** and **43** based on a molecular mechanics study with the force field MMFF of the Spartan program.

Finally, analysis of the crystal structure data[37] obtained by Haque gave further evidence of the equatorial distribution of the two lactone arms (Figure VIII.35).

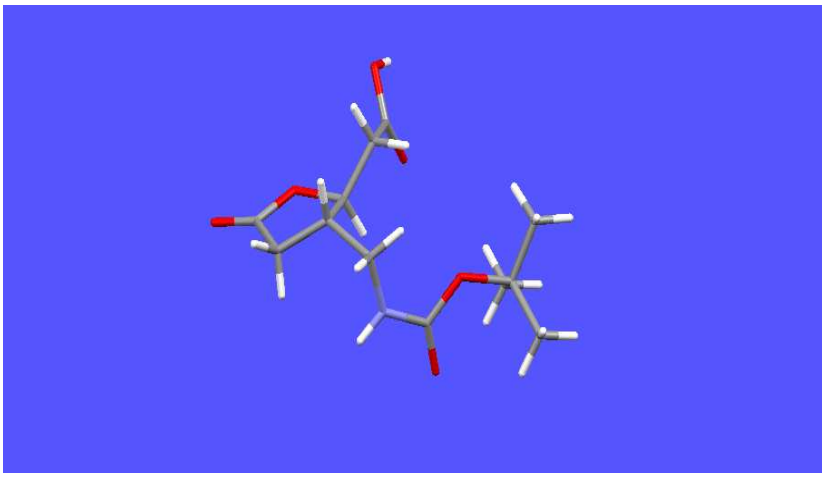
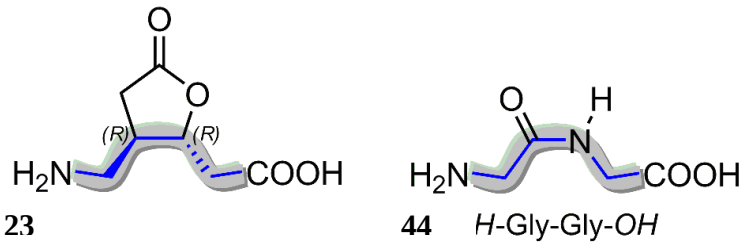


Figure VIII.35 X-ray structure of compound **22**.

VIII.7 Influence of the (R,R)-δ-lactone amino acid on the α-helix stability

VIII.7.1 Aim of the study

As it as been described before, on the one hand, this cyclic scaffold **23** can be described as δ-amino acid bearing a lactone ring at β- and γ-positions (Figure VIII.36). On the other hand, it mimics a glycyl-glycyl dipeptide unit **44**, in which the amide bond is replaced by a lactone ring. We have investigated the folding properties of these building blocks in homo-δ-oligomers[37] as well as in hetero-α,δ-oligomers. We have previously shown that the δ-lactone amino acid unit possesses a tendency to build loop- or turn-like elements.



23 **44** *H*-Gly-Gly-*OH*

Figure VIII.36 Structure of pentalactone-compound 23 and of glycyl-glycine **44**.

The aim of the experiment described in the following paragraphs was to evaluate the influence of this δ-amino acid on the helical structure of α-amino acid sequences. To carry out this study we used as peptide reference the peptide **45**, that was designed by Serrano's group[64] in 1995 and adopts an α-helical structure (Figure VIII.37). This peptide is composed of eighteen amino acids and shows in the *N*-terminal part two crucial motifs for the helix initiation: the hydrophobic staple and the capping box. The hydrophobic staple consists of residues Phe-2 and Leu-7. A second helix inducer effect comes from the presence of the capping box consisting of Ser-3 and Glu-6.[65] NMR analysis of this peptide in aqueous solutions have shown that the helical part of the peptide starts at Lys-4 and stops around Arg-15.

45

Figure VIII.37 Peptide reference **45** used in our studies.

This peptide **45** was analysed using CD spectroscopy and led to a CD spectrum with a maximum at 195 nm and two minima at 208 and 222 nm (Figure VIII.38 b). Replacement of Phe-2 by Ala (peptide **46**, Figure VIII.38 a) led to a destabilization of the helix, as indicated by the reduced intensity of the CD spectrum of **46** (Figure VIII.38 b).

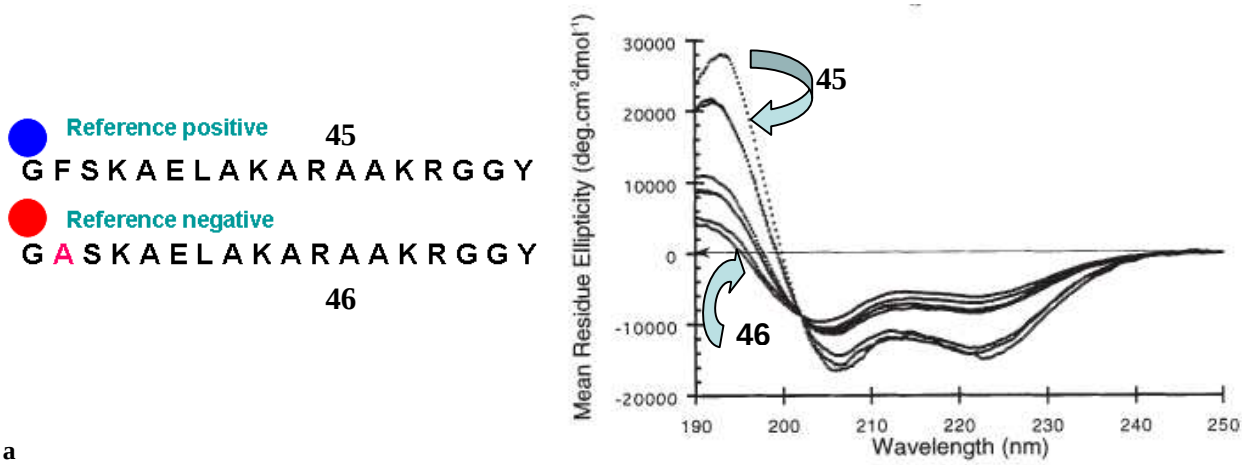


Figure VIII.38 a) Peptide references used in our studies; b) CD spectra of 45 and 46 and other analogs, as reported by Serrano and co-workers.[64](Reproduction by courtesy of Dr. Serrano).

Thus, we chosed these two peptides as references to carry out our studies. The reference called "positive" **45** included Phe-2, whereas the reference called "negative" included Ala-2 (Figure VIII.38 a).

VIII.7.2 Design of the modified peptides

In order to check the compatibility of our amino acid with the helical structure of an α-peptide, we decided to introduce the lactone unit in the C-terminal part of the helix of the reference peptide **46** (from Lys-6 to Arg-14), by substituting either the two residues Arg-11 and Ala-12 (peptide **47**) or Ala-10 and Arg-11 (peptide **48**, Figure VIII.39).

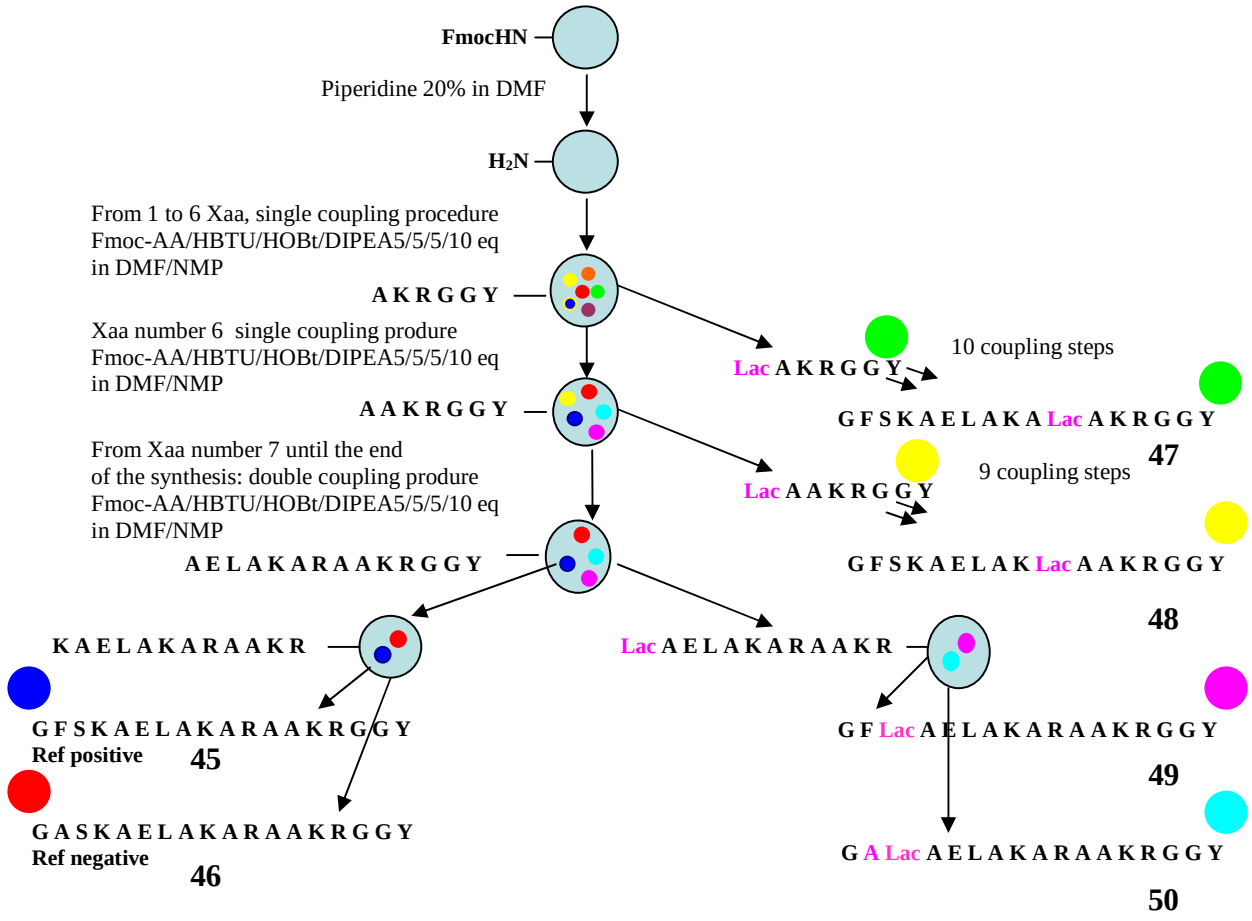
Figure VIII.39 Peptide mimics **47** and **48** containing the lactone unit in the helical part.

Moreover, we introduced one lactone unit in the N-terminal part of the peptide: indeed, we substituted Ser3 and Lys-4, thus introducing the lactone between the two residues that should build the hydrophobic staple (peptide **49**, Figure VIII.40). As a final test, we designed an analog of the latter peptide **49** that contains the substitution Phe-2/Ala, thus replacing one of the two partners of the hydrophobic staple (peptide **50**, Figure VIII.40). The goal of this last test was to see if our lactone would be able to restore the hydrophobic staple properties and to induce the helix.

Figure VIII.40 Peptide mimics **49** and **50** containing the lactone unit in the N-terminal part of the helix.

VIII.8 Synthesis of peptides 45-50

The synthesis of peptides **45-50** was performed on solid phase using Fmoc-chemistry on Rink amide MBHA resin (loading 0.6 mmol g^{-1}) (Scheme VIII.13. The seven first amino acids were introduced using a single coupling procedure (75 min), whereas the following amino acids were introduced by a double coupling procedure (2 x 45 min). We used Fmoc-amino acid/HOBt/HBTU/DIPEA (5:5:5:10 equiv.) in DMF/NMP (80:20, v/v), followed by Fmoc removal with 25% piperidine in DMF/NMP (80:20, v/v) (2 x 15 min). To monitor the progress of the synthesis, test cleavages using small amounts of resin were conducted and the resulting samples were analyzed by MALDI-ToF-MS and analytical HPLC. The last step was the N-terminal acetylation using acetic anhydride (8 equiv.) and DIPEA (7 equiv.). Final cleavage of the peptide from the resin and simultaneous side-chain deprotection was achieved by treatment with a TFA/water/TIS mixture (90:5:5) for 2.5 hours. The peptide was then precipitated from ice-cold diethyl ether, centrifuged and subjected to three ether-washing/centrifugation cycles to remove the scavengers.



Scheme VIII.13 Synthetic procedure for the six peptides **45-50.**

Each peptide was then purified by preparative HPLC. Each pure peptide was obtained in mg scale and submitted to circular dichroism analyses. The circular dichroism was recorded at 5 °C in phosphate buffer at pH 7.0 as it was described by Serrano and co-workers.[64]

VIII.9 Results and discussion

The CD spectra of the synthesized peptides are shown in Figure VIII.41. It can be noted, that introduction of the lactone led to the loss of the α-helical structure in any case. Indeed, the minimum at 222 nm characteristic for α-helices was replaced by a weak shoulder, while the minimum near 208 nm decreased intensity and was slightly blue-shifted to 205 nm. Such CD curves are similar to the one reported in the literature for the 3_{10} helix.[66]

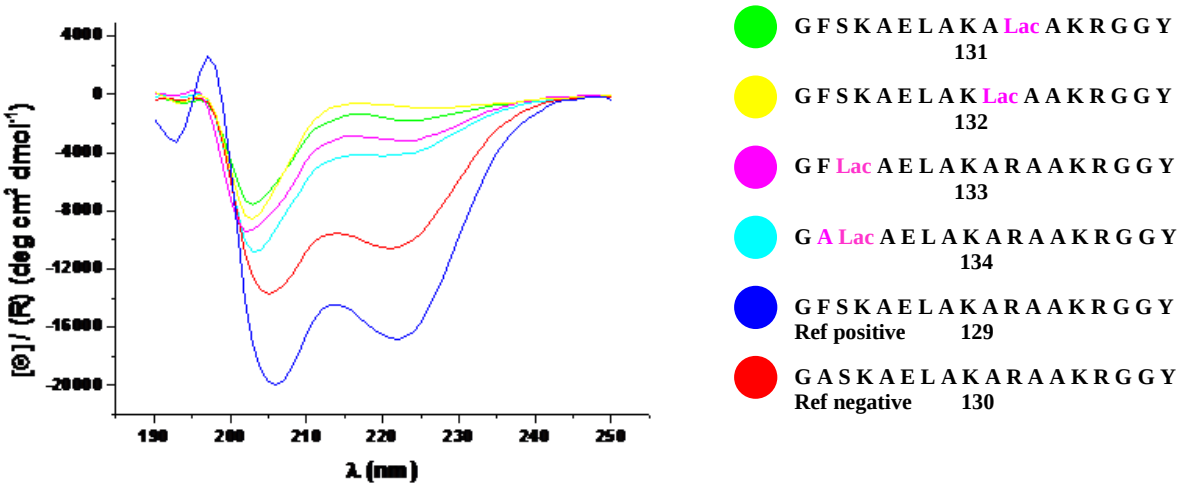


Figure VIII.41 CD spectra recorded at 5 °C in phosphate buffer pH=7 and at 0.5 mM. The concentration of the peptides was controlled previously by UV measurement.

As described before, the α-helix is the most common regular secondary structure of peptides and proteins. However, 3_{10} helices are also observed in nature. The latter are more tightly bond and more elongated than the α-helix. The set of ϕ and ψ torsion angles of the α- and 3_{10}-helices does not differ much, falling within the same region of the Ramachandran map (Table VIII.3).

Parameter	α-Helix	3_{10}-Helix
Φ (°)	-63	-57
Ψ (°)	-42	-30

Table VIII.3 Average torsion angles for α- and 3_{10}-helices based on α-amino acids.

However, their intramolecular C=O··· H-N H-bonding schemes are remarkably distinct, being of the 1←4 type (sub-type III or helical β-turn[67]) in the 3_{10}-helix, and of the 1←5 type (helical α-bend[68]) in the α-helix[69] (Figure VIII.42).

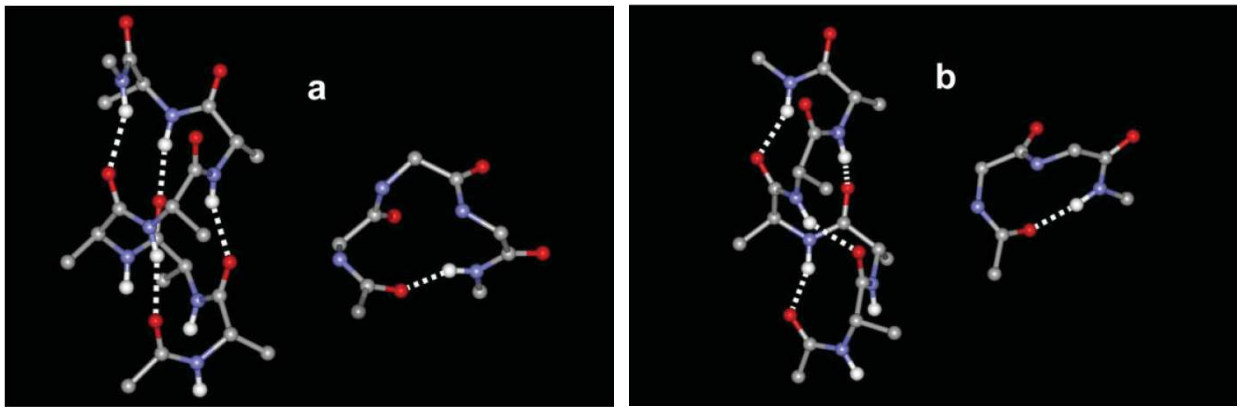


Figure VIII.42 a) The α-helix and its building block, the helical α-turn (or C_{13}-conformation); b) The 3_{10}-helix and its building block, the helical (type III) β-turn (or C_{10}-conformation). From Toniolo *et al*.[69](Reproduction by courtesy of Prof. Toniolo).

Theoretical calculations have predicted a very low conformational energy barrier separating these two secondary structures.[70] It has been shown that factors influencing the folding through one or the other conformation are: the solvent properties, the length of the peptide, the temperature, and, of course, changes in the amino acid composition.

Recently, Toniolo and co-workers[71] reported an example of homooligomer made of α,α-amino acids able to fold either in α-helix or in 3_{10}-helix depending on the solvent. As an example,[71] the Ac-[L-(αMe)Val]$_7$-NH^{i}Pr showed two different folding properties in MeOH, in which it adopts a 3_{10}-helix structure, and in the acidic alcohol HFIP, where it adopts an α-helical structure (Figure VIII.43).

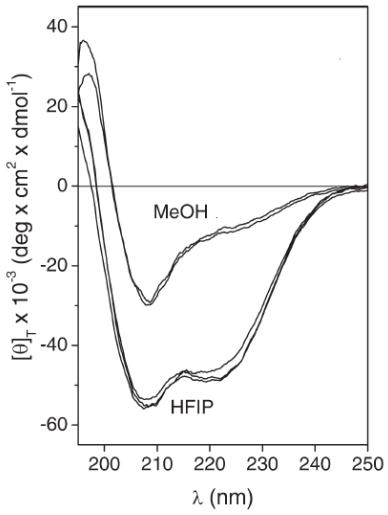


Figure VIII.43 CD spectra of Ac-[L-(αMe)Val]$_7$-NHiPr in methanol and HFIP solutions. Reported from Toniolo and co-workers.[71](Reproduction by courtesy of Prof. Toniolo).

According to the analysis of Toniolo's group that demonstrated the different peptide folding behaviour depending on the solvent, we performed further CD analyses of peptides **45-50** in TFE that is know to be a secondary structure stabilizer, as it is a strong H-bond donor and a weak H-bond acceptor, and, consequently, it interacts with carbonyl groups of peptides which are able to build bifurcated H-bonds without the necessity of breaking the intramolecular H-bond.

In our CD analyses in TFE, we can see differences in the structural behaviour of our modified peptides. While **47**, **49** and **50** showed α-helix-like CD profiles, **48** seemed to maintain the structure it already adopted in water (Figure VIII.42), showing the typical CD profile for a 3_{10}-helix (Figure VIII.44).

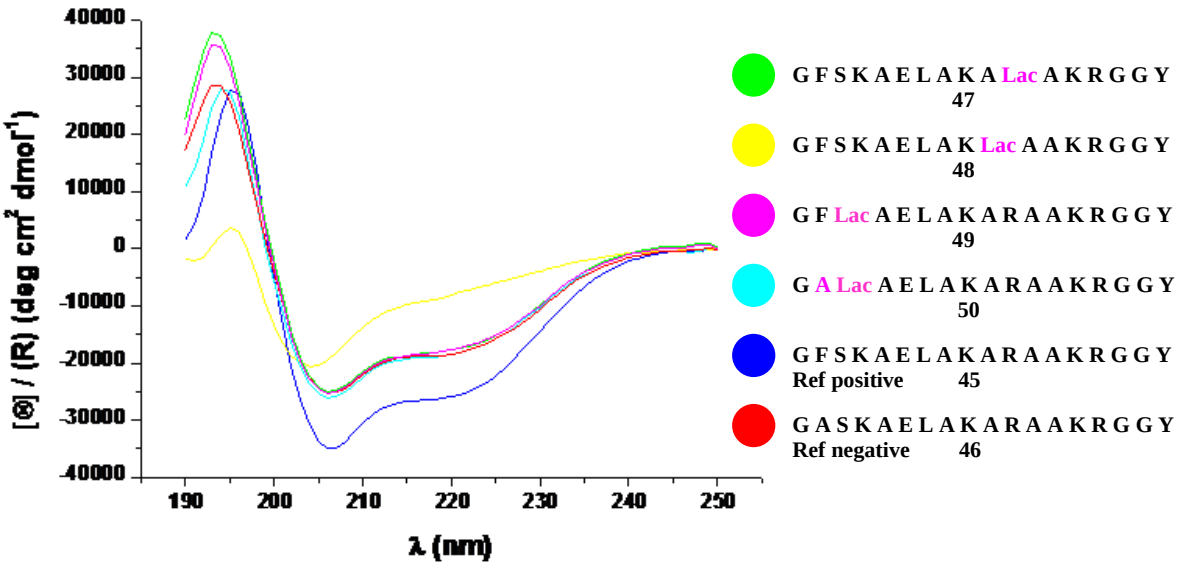


Figure VIII.44 CD spectra recorded at 5 °C in TFE and at 0.3 mM. The concentration of the peptides was controlled previously by UV measurement.

We can conclude from these last experiments, that the introduction of the lactone in already existing α-helical peptides has an influence on their secondary structure preferences. In water, a non-stabilizing solvent, the presence of one lactone unit in the proximity of the N-end or C-end of a α-amino acid sequence with strong α-helix propensity seems to favour the building of the 3_{10}-helix at the expense of the α-helix. In contrast, in the structure-stabilizing solvent TFE, the presence of the lactone unit does not prevent the formation of the α-helix, as indicated by the fact

that the CD spectra of the lactone-containing peptides **47**, **49** and **50** are superimposable with the CD spectrum of the "negative" reference peptide **46**. The striking behaviour of peptide **48**, whose structure preference seems to be less solvent-dependent, might be due to the fact that the shift of the lactone position toward the central part of an α-amino acid sequence prevented the formation of a single α-helix segment, while favouring the formation of short 3_{10}-helical structures of α-amino acids.

VIII.10. Conclusion

The synthetic studies performed on oligomers containing the butyrolactone δ-amino acids have shown that these interesting new scaffolds are compatible with the solid-phase methodology, which allows for a comfortable and efficient preparation of a large number of compounds. Moreover, the structural analyses of the synthesized peptide mimics have given insights in the conformational properties of peptide backbones based on alternated α/δ-units. In fact, the presence of the δ-residue seems to favour the formation of loops or isolated turn-like elements, whereas no periodic structures were observed. However, conformational analyses of δ-homooligomers support the presence of ordered structures, like turn-like repeats stabilized by intra-residue CO-NH H-bonds. Moreover, introduction of a δ-amino acid unit in proximity of the N- or C-end of a α-helical peptide chain seems to destabilize the α-helix in water, but not to prevent its formation in a structure-stabilizing solvent. In contrast, the δ-amino acid unit can act as a helix breaker when introduced into a α-helical segment, thus favouring the 3_{10}-helix over the α-helix.

XIX - EXPERIMENTAL PART

XIX-1 EXPERIMENTAL PART CHAPTER VIII

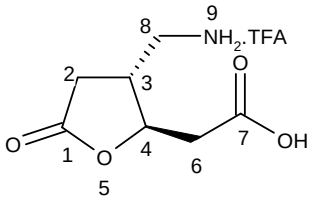
General chemical techniques:

The N^{α}-Fmoc protected amino acids and HBTU were purchased from MultiSynTech (Witten, Germany). Peptide-synthesis grade DMF, NMP, piperidine and diethylether, ACN and TFA for spectroscopy were purchased from Biosolve (Valkenswaard, The Netherlands).
HOBt, DIPEA, TFA, DCM, DMSO, and α-cyano-4-hydroxy-cinnamic acid for mass spectrometry were bought from Fluka (Taufkirchen, Germany). Methanol was obtained from J.T. Baker (Deventer, The Netherlands), TIS from Aldrich (Steinheim, Germany) and TFE from Acros (Geel, Belgium). Acetic anhydride, acetic acid, NaH_2PO_4 and Na_2HPO_4 were purchased from Merck (Darmstadt, Germany). THF was distilled on sodium/benzophenone before use. Pure products were obtained after liquid chromatography using Merck silica gel 60 (40-63 μm mesh). TLC analyses were performed with 0.25 mm 60 F_{254} silica plates (Merck). The plates were visualized with UV light (254 nm) or with a solution of vanillin in ethanol or with a solution of ninhydrin in ethanol. The mass spectra were recorded on Finnigan MAT 95, Varian MAT 311A, Finnigan TSQ 7000 and Future GSG spectrometer (Bruchsal, Germany) for MALDI-ToF-MS analysis. IR spectra were recorded on a Varian 800 FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer-Polarimeter 241 with sodium lamp at 589 nm in the specified solvent. NMR spectra were recorded on a Bruker AVANCE 300 (300.13 MHz) or a Bruker AVANCE 400 (400.13 MHz) or a Bruker AVANCE 600 (600.13 MHz). The 2D-NMR standard experiments DQF-COSY, 80 ms-TOCSY and 500 ms-ROESY the water resonance was suppressed by using the presaturation method. The concentration of the peptide samples prepared for CD measurements were determined UV spectrophotometrically on a Cary 100 Conc from Varian (Darmstadt, Germany) using 1 cm quartz cuvettes obtained from Hellma (Müllheim, Germany). CD spectra were recorded at room temperature or at 5 °C on a JASCO J710 spectropolarimeter between 300 and 200-180 nm in the specified solvent. The path length of the quartz cuvette was 1.0 mm. For each CD spectrum ten scans were accumulated using a step resolution of 1 nm, a bandwidth of 1 nm, a response time of 2 s, a scan speed of 20 nm min^{-1} and

a sensitivity of 10 mdeg, 20 mdeg, depending on the peptide concentration. The background was subtracted to each spectrum. The absorption value is measured as Molar Ellipticity per Residue (deg cm^2 $dmol^{-1}$). The noise reduction was obtained by a Fourier transform filter using the program Origin 6.0 (OriginLab Corporation, Northampton, USA). Analytical and preparative reverse Phase HPLC was performed on Agilent equipment (Böblingen, Germany) by using the columns: Luna C18(2), 3 μm, 4.60 x 150 mm and the Luna C18(2), 90 μm, 21.2 x 250 mm (Phenomenex, Aschaffenburg, Germany). The flow rate was 1 mL/min for the analytical HPLC runs and 21 mL/min for preparative applications. The binary solvent system (A/B) was: (A) 0.012% TFA in water (B) 0.01% of TFA in ACN. The absorbance was detected at 220 nm.

SYNTHESIS OF MOLECULES 37 AND 36

(3-Aminomethyl-5-oxo-tetrahydro-furan-2-yl)acetic acid, trifluoroacetic acid salt 37



$C_7H_{11}NO_4.CF_3COOH$

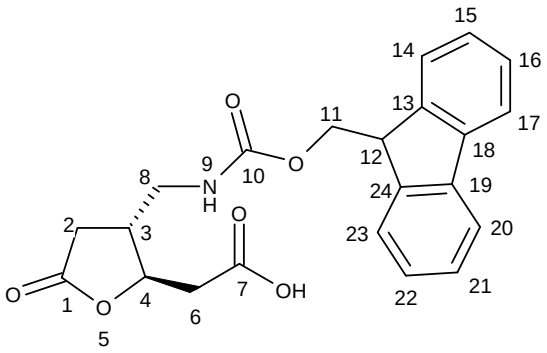
M =287.09 g/mol

Compound **23** (100 mg, 0.37 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and trifluoroacetic acid (1.7 mL, 22.0 mmol, 60.0 eq) was added dropwise at room temperature. The reaction mixture was stirred for 2 h. Solvent was evaporated under reduced pressure to yield a brown solid oil **37** (106 mg). This crude product was used without further purification in the course of the synthesis.

^{1}H NMR (300 MHz, CD_3OD) δ ppm 4.69 (m, 1H, H_4), 3.20 (m, 2H, H_8), 2.96-2.71 (m, 4H, H_3, H_6, H_{2a}), 2.51 (dd, J = 17.0 and 6.9 Hz, 1H, H_{2b}).

^{13}C NMR (75 MHz, CD_3OD) δ ppm 177.1 (C_7), 173.4 (C_1), 80.0 (C_4), 42.7 (C_8), 39.9 (C_6), 39.3 (C_2), 33.6 (C_3).

{3-[(9*H*-Fluoren-9-ylmethoxycarbonylamino)-methyl]-5-oxo-tetrahydro-furan-2-yl}acetic acid **36**



$C_{22}H_{21}NO_6$

M =395.14 g/mol

To a cold (0°C) solution of **37** (106 mg, 0.37 mmol) in dioxane /1M aqueous solution of K_2CO_3 (2 ml /4 ml) was added portionwise FmocOSu (187.2 mg, 0.55 mmol, 1.5 eq.). The reaction was allowed to come at room temperature and stirred overnight. The mixture was poured in water (7 mL) and extracted with EtOAc (3 x 9 mL).

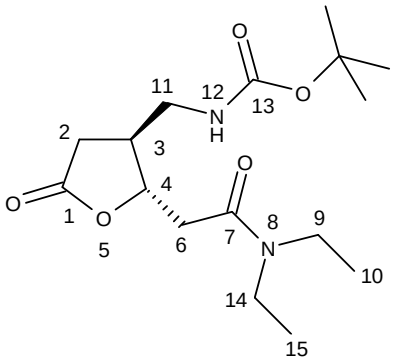
The combined organic layers were dried over Na_2SO_4, filtered and evaporated in vacuo to give a sticky solid wich was purified by column chromatography (PE:EtOAc 2:1) to obtain **36** (86.9 mg, 60%) as a sticky, colorless solid.

^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.80 (d, *J* = 7.4 Hz, 2H, H_{17}, H_{20}), 7.63 (d, *J* = 7.2 Hz, 2H, $H_{14,}$ H_{23}), 7.40-7.27 (m, 4H, $H_{15,}$ $H_{16,}$ H_{21}, H_{22}), 4.64 (m, 1H, H_4), 4.39 (d, *J* = 6.6 Hz, 2H, H_{11}), 4.20 (m, 1H, H_{12}), 3.23 (m, 2H, H_8), 2.73-2.47 (m, 4H, $H_{3,}$ $H_{6,}$ H_{2a}), 2.35 (dd, *J* = 17.2 and 7.0 Hz, 1H, $H_{2b)}$.

^{13}C NMR (75 MHz, CD_3OD) δ ppm 128.8, 128.2, 126.2, 121.0 ($C_{14,}$ C_{15}, $C_{16,}$ C_{17},C_{20}, C_{21}, C_{22}, C_{23}), 81.0 (C_4), 68.2 (C_{11}), 48.6 (C_{12}),43.1 (C_8), 41.9 (C_3), 39.9 (C_2), 33.2 (C_6).

SYNTHESIS OF MOLECULES 41 AND 42

(5-Oxo-tetrahydro-furan-3-ylmethyl)-carbamic acid *tert*-butyl ester; compound with *N*,*N*-diethyl-propionamide 40



$C_{16}H_{28}N_2O_5$

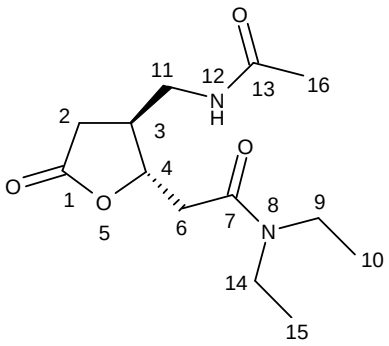
M =328.20 g/mol

To a solution of **22** (142 mg, 0.52 mmol) and diethylamine (41.8 mg, 0.57 mmol, 1.1 eq) in DMF (8 mL) was added DIPEA (180 µL, 1.04 mmol, 2.0 eq), HOBt (88 mg, 0.57 mmol, 1.1 eq) and HBTU (218 mg, 0.57 mmol, 1.1 eq). The mixture was stirred at room temperature under argon atmosphere for 25 h. After removal of the solvent under reduced pressure, the residue was dissolved in EtOAc (20 mL). The organic solution was successively washed with 10% aqueous citric acid (20 mL), water (20 mL), 10% aqueous K_2CO_3 (20 mL), brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. A purification by chromatography on silica gel, eluting with CH_2Cl_2/MeOH (9/1), afforded 110 mg (65%) of **40 a** as a sticky light yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 5.40 (br s, 1 H, H_{12}), 4.78 (m, 1H, H_4), 3.43-3.15 (m, 6H, H_{11}, $H_{14,}$ H_9), 2.86 (dd, *J* = 16.3 and 4.3 Hz, 1H, H_{6a}), 2.73-2.58 (m, 2H, H_{6b}, H_{2a}), 2.52 (m, 1H, H_3), 2.38 (dd, *J* = 18.1 and 5.9 Hz, 1H, H_{2b}), 1.43 (s, 9H, C$\underline{H}_3$Boc), 1.22-1.0 (m, 6H, H_{10}, H_{15}).
^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 175.5 (C_7), 168.1 (C_1), 156.3 (C_{13}), 80.0 ($\underline{C}$Boc), 79.6 (C_4), 42.1, 41.6 (C_9, C_{14}), 40.3 (C_3), 37.9 (C_{11}), 32.4 (C_6), 28.3 ($\underline{C}H_3$Boc), 14.2, 13.0 (C_{10}, C_{15}).
Rf: 0.4 (CH_2Cl_2/MeOH: 90/10)

HRMS (EI) *m/e*: $[M]^+$ calcd 328.1998, found 328.1996

2-[3-(Acetylamino-methyl)-5-oxo-tetrahydro-furan-2-yl]-*N*,*N*-diethyl-acetamide 41.



$C_{13}H_{22}N_2O_4$

M =270.16 g/mol

Compound **40 a** (54 mg, 0.18 mmol) was dissolved in dry CH_2Cl_2 (6 mL) and trifluoroacetic acid (3.0 mL, 10.8 mmol, 60.0 eq) was added dropwise at room temperature. The reaction mixture was stirred for 2 h. Solvent was evaporated under reduced pressure to afford the corresponding TFA salt.

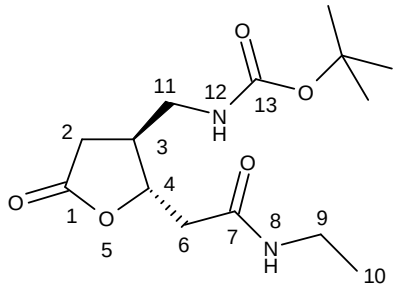
A solution of this salt in dry THF (5 mL) was heated at 70 °C and acetic anhydride (32 µL, 0.22 mmol, 1.2 eq) was added dropwise to this solution. The reaction mixture was stirred for 2 hours at 70 °C. The solvent of reaction was evaporated under reduced pressure. A purification by chromatography on silica gel, eluting with CH_2Cl_2/MeOH (9/1), afforded 29 mg (60%) of **42** as a colorless solid oil.

^{1}H NMR (600 MHz, CD_2Cl_2) δ ppm 7.1 (br s, 1H, H_{12}), 4.77 (m, 1 H, H_4), 3.41-3.21 (m, 6H, H_9, H_{14}, H_{11}), 2.88 (dd, *J* = 16.3 and 4.3 Hz, 1H, H_{6a}), 2.72 (dd, *J* = 18.1 and 9.3 Hz, 1H, H_{2a}), 2.66 (dd, *J* = 16.3 and 8.8 Hz, 1H, H_{6b}), 2.49 (m, 1H, H_3), 2.30 (dd, *J* = 18.1 and 5.9 Hz, 1H, H_{2b}), 1.94 (s, 3H, C_{16}), 1.19 (t, *J* = 7.3 Hz, 3H, H_{10}), 1.11 (t, *J* = 7.2 Hz, 3H, H_{15}).

^{13}C NMR (150 MHz, CD_2Cl_2) δ ppm 175.6 (C_7), 170.7 (C_1), 168.8 (C_{13}), 80.3 (C_4), 42.4, 42.3 (C_9, C_{14}), 40.7 (C_{11}), 40.6 (C_3), 38.3 (C_6), 32.4 (C_2), 23.1 (C_{16}), 14.2, 13.1 (C_{10}, C_{15}).

IR in CH_2Cl_2 (cm^{-1}): 3447 (NH), 3312 (NH), 1781 (C=O), 1671 (C=O), 1629 (C=O).

(2S, 3S)-(2-Ethylcarbamoylmethyl-5-oxo-tetrahydro-furan-3-ylmethyl)-carbamic acid *tert*-butyl ester 40 b.



$C_{14}H_{24}N_2O_5$

M = 300.17 g/mol

To a solution of **22** (71 mg, 0.26 mmol) and ethylamine hydrochloride (23.6 mg, 0.29 mmol, 1.1 eq) in DMF (4 mL) was added DIPEA (90 µL, 0.52 mmol, 2.0 eq), HOBt (44 mg, 0.29 mmol, 1.1 eq) and HBTU (109 mg, 0.29 mmol, 1.1 eq). The mixture was stirred at room temperature under argon atmosphere for 23 h. After removal of the solvent under reduced pressure, the residue was dissolved in EtOAc (10 mL). The organic solution was successively washed with 10% aqueous citric acid (10 mL), water (10 mL), 10% aqueous K_2CO_3 (10 mL), brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. A purification by chromatography on silica gel, eluting with CH_2Cl_2/MeOH (9/1), afforded 33 mg (42%) of **40 b** as a sticky, light yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 6.10 (br s, 1 H, H_8), 5.22 (m, 1 H, H_{12}), 4.64 (dd, *J* = 12.6 and 6.3 Hz, 1H, H_4), 3.31-3.22 (m, 4H, H_9, H_{11}), 2.65 (dd, *J* = 16.6 and 8.3 Hz, 1H, H_{2a}), 2.57 (d, *J* = 6.0 Hz, 2H, H_6), 2.52 (m, 1H, H_3), 2.39 (dd, *J* = 16.6 and 7.7 Hz, 1H, H_{2b}), 1.42 (s, 9H, C$\underline{H}_3$Boc), 1.12 (t, *J* = 7.2 Hz, 3H, H_{10}).

^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 175.8 (C_7), 168.8 (C_1), 156.4 (C_{13}), 80.0 ($\underline{C}$Boc), 79.8 (C_4), 41.6 (C_3), 41.2 (C_6, C_{11}), 34.5 (C_9), 32.4 (C_2), 28.3 ($\underline{C}H_3$Boc), 14.63 (C_{10}).

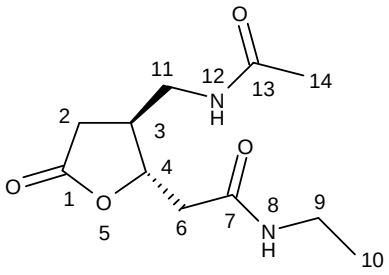
Rf: 0.3 (CH_2Cl_2/MeOH: 90/10)

IR in CH_2Cl_2 (cm^{-1}): 3443 (NH), 1782 (C=O), 1714 (C=O), 1673 (C=O).

HRMS (EI) *m/e*: $[M]^+$ calcd 300.1685, found 300.1685

$[\alpha]^{25}_D$: -19.3 (c 1.0, $CHCl_3$)

2-[3-(Acetylamino-methyl)-5-oxo-tetrahydro-furan-2-yl]-N-ethyl-acetamide 42.



$C_{11}H_{18}N_2O_4$

M = 242.13 g/mol

Compound **40 b** (27 mg, 0.09 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and trifluoroacetic acid (1.5 mL, 5.4 mmol, 60.0 eq) was added dropwise at room temperature. The reaction mixture was stirred for 2 h. Solvent was evaporated under reduced pressure to afford the corresponding TFA salt.

A solution of this salt in dry THF (3 mL) was heated at 70 °C and acetic anhydride (16 µL, 0.11 mmol, 1.2 eq) was added dropwise to this solution. The reaction mixture was stirred for 2 hours at 70 °C. The solvent of reaction was evaporated under reduced pressure. A purification by chromatography on silica gel, eluting with CH_2Cl_2/MeOH (9/1), afforded 14 mg (65%) of **42** as a colorless solid oil.

^{1}H NMR (600 MHz, CD_2Cl_2) δ ppm 6.67 (br s, 1 H, H_{12}), 6.07 (br s, 1 H, H_8), 4.63 (m, 1H, H_4), 3.33 (m, 2H, H_{11}), 3.25 (m, 2H, H_9), 2.66 (dd, *J* = 17.5 and 8.9 Hz, 1H, H_{2a}), 2.58 (d, *J* = 6.2 Hz 2H, H_6), 2.53 (m, 1H, H_3), 2.34 (dd, *J* = 17.5 and 7.5 Hz, 1H, H_{2b}), 1.95 (s, 3H, H_{14}), 1.12 (t, *J* = 7.3 Hz, 3H, H_{10}).

^{13}C NMR (150 MHz, CD_2Cl_2) δ ppm 175.7 (C_1), 171.0 (C_{13}), 169.2(C_7), 80.3 (C_4), 41.3 (C_6), 41.2 (C_{11}), 41.0 (C_3), 34.8 (C_9), 32.7 (C_2), 23.2 (C_{14}), 14.8 (C_{10}).

Rf: 0.3 (CH_2Cl_2/MeOH: 90/10)

IR in CH_2Cl_2 (cm^{-1}): 3441 (NH), 3331 (NH), 1781 (C=O), 1673 (C=O), 1629 (C=O).

HRMS (EI) *m/e*: $[M]^+$ calcd 242.1267, found 242.1269

$[\alpha]^{25}_D$: -17.3 (c 1.0, $CHCl_3$)

METHODS FOR PEPTIDE CHARACTERIZATION

Analytical HPLC

For the analytical HPLC measurements a small amount of the peptide was dissolved in methanol or in millipore water containing 0.1% TFA The binary elution system consisted of 0.012% (v/v) TFA in water (eluent A) and 0.01% (v/v) TFA in ACN (eluent B). The following gradients were applied: 15% B for 3 min, 15-70% B over 40 min, 70-95% B over 50 min and 95% B over 55% (peptides **29** and **30**); 5% B for 5 min, 5-55% B over 45 min, 55-65% B over 50 min and 65-95% B over 60 min (peptides **33**, **34**, **45-50**).

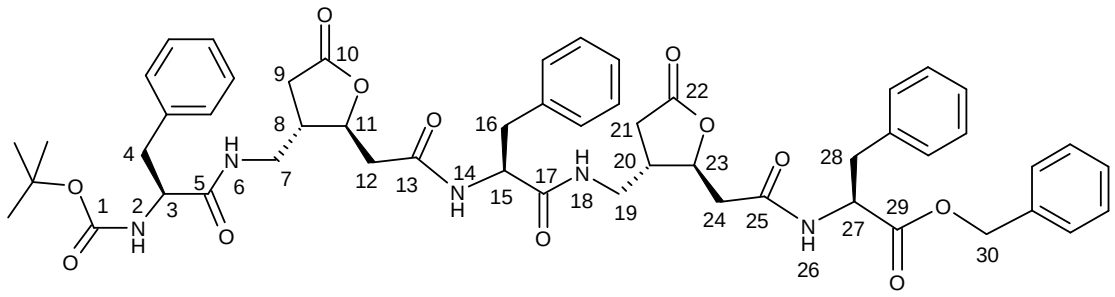
MALDI-ToF-MS

For the mass spectrometry MALDI-ToF measurements a small sample of the peptide was dissolved in methanol or in millipore water containing 0.1% TFA and mixed with a solution of α-cyano-4-hydroxycinnamic acid in MeOH/ACN (1:1, v/v).

UV spectroscopy

The concentration of the peptide samples prepared for CD measurements were determined UV spectrophotometrically. By measuring the tyrosine absorption at 280 nm the concentration was calculated according to the Lambert-Beer law using an extinction coefficient of 1480 M-1 cm-1 per tyrosine residue. The ratio between the peptide concentration values obtained by UV and by weight gave the peptide content. In case of peptides lacking tyrosine or tryptophan residues the peptide contents of similar peptides containing these residues were applied to calculate the concentration.

Boc-F-(S,S)-Lac-F-(S,S)-Lac-F-Bn 29.



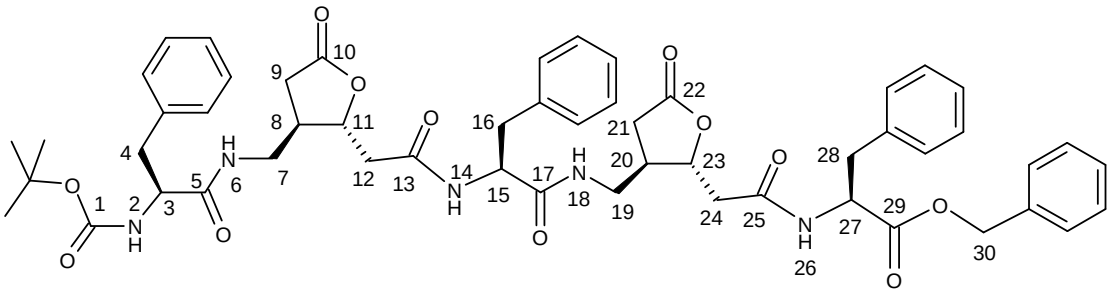
^{1}H NMR (600 MHz, CD_3OH, 283 K) δ ppm 8.51 (d, *J* = 8.4 Hz, 1H, H_{26}), 8.39 (d, *J* = 7.2 Hz, 1H, H_{14}), 8.23 (m, 2H, $H_{18,}$ H_6), 7.31-7.11 (m, 20H, H_{ar}), 6.89 (d, *J* = 7.8 Hz, 1H, H_2), 5.46 (s, 2H, H_{30}), 4.70 (m, 1H, H_{26}), 4.48 (m, 1H, H_{14}), 4.37 (m, 2H, H_6, H_{18}), 4.18 (m, 1H, H_2), 3.10-3.30 (m, 6H, H_9, H_{7a}, H_{19a}, H_{21}), 3.10-2.97 (m, 6H, H_{4a} H_{16a}, H_{28}, H_{7b}, H_{19b}), 2.95-2.79 (m, 2H, $H_{4b,}$ H_{16b}), 2.65-2.59 (m, 2H, H_{12a}, H_{24a}), 2.47-2.34 (m, 2H, H_{12b}, H_{24b}), 2.31-2.17 (m, 2H, H_8, H_{20}), 1.43 (s, 9H, C$\underline{H_3}$Boc).

IR in CH_2Cl_2 (cm^{-1}): 3420 (NH), 3314 (NH), 1783(C=O), 1731(C=O), 1672(C=O).

MALDI-ToF-MS: 983 $[M+Na]^+$, 999 $[M+K]^+$

$t_{R:}$ 36.0 min

Boc-F-(R,R)-Lac-F-(R,R)-Lac-F-Bn 30.



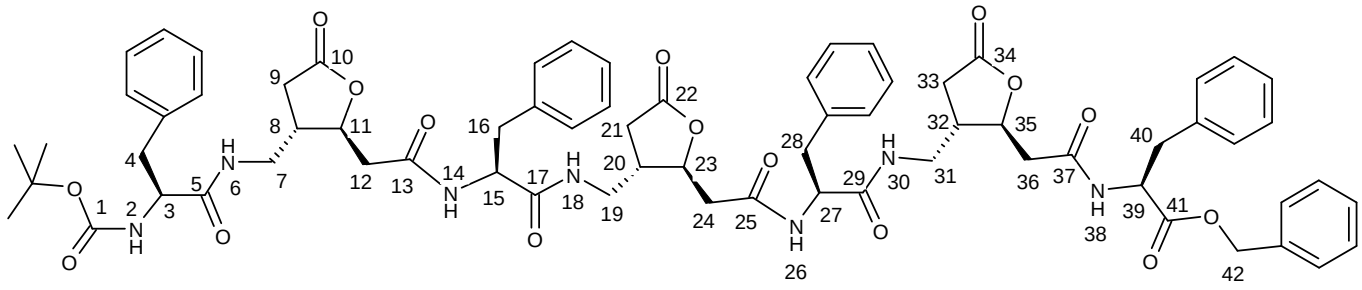
^{1}H NMR (600 MHz, $CDCl_3$, 288K) δ ppm 8.56 (d, *J* = 7.8 Hz, 1H, H_{26}), 8.42 (d, *J* = 7.6 Hz, 1H, H_{14}), 8.22 (m, 1H, H_6), 8.04 (m, 1H, H_{18}), 8.38-7.10 (m, 20H, H_{ar}), 6.90 (d, *J* = 7.8 Hz, 1H, H_2), 5.04 (s, 2H, H_{30}), 4.70 (m, 1H, H_{27}), 4.54 (m, 1H, H_{15}), 4.52-4.42 (m, 2H, H_{11}, H_{23}), 4.26-4.17 (m, 2H, H_3, H_{11}), 3.22-3.16 (m, 3H, H_7, H_{19a}), 3.16-3.10 (m, 3H, H_{19b}, H_{28a}, H_{16a}), 3.0-2.90 (m, 2H, H_{4a}, H_{28b}), 2.93-2.81 (m, 2H, H_{4b}, H_{16b}), 2.56-2.44 (m, 6H, H_{12}, $H_{24,}$ H_{9a}, H_{21a}), 2.35-2.25 (m, 2H, H_{20}, H_8), 2.23-2.14 (m, 2H, H_{9b}, H_{21b}), 1.36 (s, 9H, C$\underline{H}_3$Boc).

IR in CH_2Cl_2 (cm^{-1}): 3421 (NH), 3314 (NH), 1783(C=O), 1733(C=O), 1669(C=O).

HRMS (EI) *m/e*: $[M+H]^+$ calcd 960.4395, found 960.4417

t_R: 37.0 min

Boc-F-(R,R)-Lac-F-(R,R)-Lac-F-(R,R)-Lac-F-Bn 31.

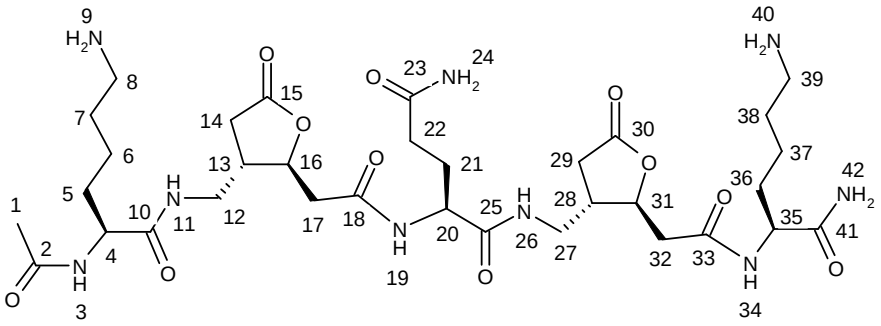


^{1}H NMR (600 MHz, $CDCl_3$, 292K) δ ppm 8.54 (d, *J* = 5.8 Hz, 1H, H_{14}), 8.49 (br s, 1H, H_{26}), 8.34 (d, *J* = 9.5 Hz, 1H, H_{18}), 8.0 (d, *J* = 9.5 Hz, 1H, H_6), 7.74 (d, *J* = 6.8 Hz, 1H, H_{38}), 7.52 (d, *J* = 9.1 Hz, 1H, H_{30}), 7.46-7.0 (m, 25H, H_{ar}), 5.22 (d, *J* = 8.5 Hz, 1H, H_2), 5.17 (d, J_{AB} = 12.1 Hz, 1H, H_{42a}), 5.12 (d, J_{AB} = 12.3 Hz, 1H, H_{42b}), 4.81 (m, 1H, H_{39}), 4.73-4.65 (m, 2H, H_{23}, H_{15}), 4.63 (m, 1H, H_{35}), 4.57 (m, 1H, H_{11}), 4.47 (m, 1H, H_3), 4.22 (m, 1H, H_{27}), 3.90-3.80 (m, 2H, H_{19a}, H_{7a}), 3.72 (m, 1H, H_{31a}), 3.17-2.80 (m, 15H, H_4, H_{16}, H_{24}, H_{36}, H_{28}, H_{40}, H_{31b}, H_{19b}, H_{7b}), 2.80-2.60 (m, 3H, H_{9a}, H_{21a}, H_{33a}), 2.50 (m, 1H, H_8), 2.43-2.10 (m, 5H, H_{33b}, H_{21b} H_{9b}, H_{20}, H_{32}), 1.40 (s, 9H, C$\underline{H}_3$Boc).

IR in CH_2Cl_2 (cm^{-1}): 3420 (NH), 3302 (NH), 1783 (C=O), 1732 (C=O), 1652 (C=O).

MALDI-ToF-MS: 1284 $[M+Na]^+$, 1300 $[M+K]^+$

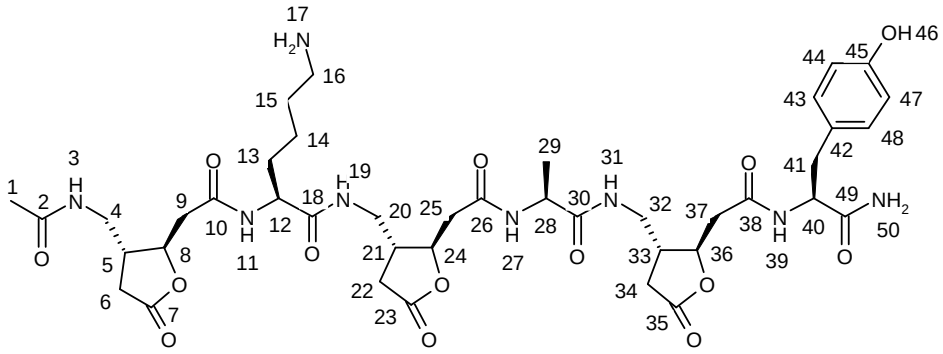
Ac-K-(S,S)-Lac-Q-(S,S)-Lac-K-CONH$_2$ 32.



^{1}H NMR (600 MHz, CD$_3$OH, 303 K) δ ppm 8.36 (d, *J* = 6.3 Hz, 1H, H_{19}), 8.32 (m, 1H, H_{26}), 8.27 (m, NH, 1H, H_{11}), 8.22 (d, *J* = 7.4 Hz, 1H, H_{34}), 8.19 (d, *J* = 6.3 Hz, 1H, H_3), 7.74 (br s, 4H, $H_{9,}$ H_{40}), 7.65-7.45 (m, 2H, H_{24} or H_{42}), 7. 10-7.0 (m, 1H, H_{24} or H_{42}), 6.82 (br s, 1H, H_{24} or H_{42}), 4.71-4.63 (m, 2H, H_{16}, H_{31}), 4.39 (m, 1H, H_{35}), 4.26-4.22 (m, 2H, $H_{20,}$ H_4), 3.38-3.32 (m, 2H, $H_{12,}$ H_{27}), 2.99-2.90 (m, 4H, H_8, H_{39}), 2.79-2.67 (m, 4H, H_{14a}, H_{17a}, H_{29a}, H_{32a}), 2.64-2.50 (m, 4H, H_{13}, H_{17b}, $H_{28,}$ H_{32b}), 2.47-2.39 (m, 2H, H_{14b}, H_{29b}), 2.34 (m, 2H, H_{22}), 2.11 (m, 1H, H_{21a}), 2.0; 1.96 (s, 3H, $H_{1,}$ 2 conf.), 1.95-1.87 (m, 2H, H_{21b}, H_{36a}), 1.82 (m, 1H, H_{5a}), 1.73-1.61 (m, 6H, H_7, H_{38}, H_{5b}, H_{36b}), 1.57-1.36 (m, 4H, $H_{37,}$ H_6).

MALDI-ToF-MS: 753 $[M]^+$, 776 $[M+Na]^+$, 792 $[M+K]^+$

Ac-(R,R)-Lac-K-(R,R)-Lac-A-(R,R)-Lac-Y-CONH$_2$ 33.

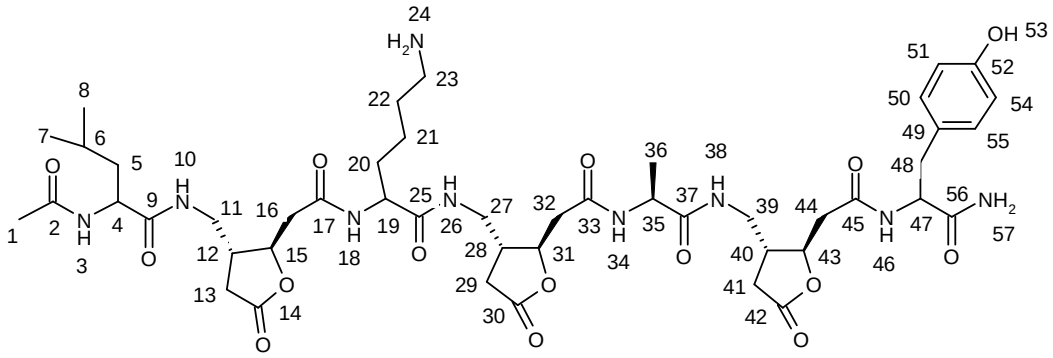


^{1}H NMR (600 MHz, CD$_3$OH, 280 K) δ ppm 9.20 (br s, 1H, H_{46}), 8.51 (d, *J* = 6.4 Hz, 1H, H_{27}), 8.34-8.30 (m, 3H, H_3, $H_{19,}$ H_{39}), 8.25 (d, *J* = 7.9 Hz, 1H, H_{11}), 7.90 (m, 1H, H_{31}), 7.71 (br s, 2H, H_{17}), 7.65 (br s, 1H, H_{50a}), 7.22 (br s, 1H, H_{50b}), 7.10 (d, *J* = 8.6 Hz, 2H, H_{45}, H_{47}), 6.70 (d, *J* = 8.4 Hz, 2H, H_{44}, H_{48}), 4.70 (m, 1H, H_{36}), 4.66-4.58 (m, 2H, $H_{8,}$ H_{24}), 4.51 (m, 1H, H_{40}), 4.29 (m, 1H, H_{12}), 4.22 (m, 1H, H_{28}), 3.45 (m, 2H, H_{20a}), 3.39-3.27 (m, 2H, H_{4a}, H_{32a}), 3.26-3.17 (m, 3H, H_{32b}, H_{20b}, H_{4b}), 3.04 (dd, *J* = 5.3 and *J* = 14.0 Hz, 1H, H_{41a}), 2.90 (br s, 2H, H_{16}), 2.78 (dd, *J* = 9.3 and *J* = 13.9 Hz, 1H, H_{41b}), 2.75-2.65 (m, 4H, $H_{6a,}$ $H_{9a,}$ $H_{22a,}$ H_{34a}), 2.64-2.51 (m, 7H, H_5, H_{9b}, H_{21},H_{25}, H_{37}), 2.47-2.37 (m, 3H, $H_{6b,}$ H_{22b}, H_{33}), 1.94 (s, 3H, H_1), 1.78 (m, 1H, H_{13a}), 1.70-1.61 (m, 2H, $H_{15a,}$ H_{13b}), 1,58 (m, 1H, H_{15b}), 1.41 (m, 2H, H_{14}), 1.33 (d, 3H, *J* = 7.3 Hz, H_{29}).

HRMS (EI) *m/e*: $[M+H]^+$ calcd 887.4151, found 887.4143

$t_{R:}$ 11.5 min

Ac-L-(R,R)-Lac-K-(R,R)-Lac-A-(R,R)-Lac-Y-CONH$_2$ 34.



^{1}H NMR (600 MHz, CD$_3$OH, 303 K) δ ppm 9.10 (br s, 1H, H_{53}), 8.37 (d, *J* = 6.5 Hz, 1H, H_{34}), 8.25 (m, 1H, H_{10}), 8.19-8.12 (m, 4H, H_3, H_{18}, H_{26}, H_{46}), 7.84 (m, 1H, H_{38}), 7.70 (br s, 2H, H_{24}), 7.52 (br s, 1H, H_{57a}), 7.10-7.05 (m, 3H, H_{57b}, $H_{54,}$ H_{51}), 6.7 (d, *J* = 8.6 Hz, 2H, H_{55}, H_{50}), 4.80-4.59 (m, 3H, H_{15}, H_{31}, H_{43}), 4.54 (m, 1H, H_{47}), 4.32 (m, 1H, H_{19}), 4.30-4.22 (m, 2H, H_{35}, H_4), 3.48- 3.37 (m, 3H, H_{11a}, H_{27a}, H_{39a}), 3.36-3.22 (m, 3H, H_{11b}, H_{27b}, H_{39b}), 3.05 (dd, *J* = 5.5 and *J* = 14.0 Hz, 1H, H_{48a}), 2.93 (m, 2H, H_{23}), 2.80 (dd, *J* = 9.1 and *J* = 13.9 Hz, 1H, H_{48b}), 2.76-2.63 (m, 6H, $H_{13a,}$ $H_{16a,}$ $H_{29a,}$ H_{32a}, H_{41a}, H_{44}), 2.63-2.54 (m, 6H, H_{12}, H_{16b}, H_{28},H_{32b}, H_{40}, H_{44b}), 2.49-2.35 (m, 3H, $H_{13b,}$ H_{29b}, H_{41b}), 1.98 (s, 3H, H_1), 1.82 (m, 1H, H_{20a}), 1.72-1.58 (m, H, $H_{20b,}$ $H_{22,}$ H_6), 1,58 (m, 2H, H_5), 1.43 (m, 2H, H_{21}), 1.34 (d, *J* = 7.3 Hz, 3H, H_{36}), 0.96 (d, *J* = 6.7 Hz, 3H, H_7 or H_8), 0.92 (d, *J* = 6.7 Hz, 3H, H_7 or H_8).

MALDI-ToF-MS: 1000 $[M]^+$, 1023 $[M+Na]^+$, 1039 $[M+K]^+$

$t_{R:}$ 16.3 min

Ac-G-F-S-K-A-E-L-A-K-A-R-A-A-K-R-G-G-Y-CONH$_2$ 45.

MALDI-ToF-MS: 1923 $[M+H]^+$

t_R: 20.18 min

Ac-G-A-S-K-A-E-L-A-K-A-R-A-A-K-R-G-G-Y-CONH$_2$ 46.

MALDI-ToF-MS: 1846 $[M+H]^+$

t_R: 17.51 min

Ac-G-F-S-K-A-E-L-A-K-A-Lac-A-K-R-G-G-Y-CONH$_2$ 47.

MALDI-ToF-MS: 1849 $[M]^+$

t_R: 21.0 min

Ac-G-F-S-K-A-E-L-A-K-Lac-A-A-K-R-G-G-Y-CONH$_2$ 48.

MALDI-ToF-MS: 1849 $[M+H]^+$

t_R: 20.51 min

Ac-G-F-Lac-A-E-L-A-K-A-R-A-A-K-R-G-G-Y-CONH$_2$ 49.

MALDI-ToF-MS: 1785 $[M]^+$

t_R: 18.82 min

Ac-G-A-Lac-A-E-L-A-K-A-R-A-A-K-R-G-G-Y-CONH$_2$ 50.

MALDI-ToF-MS: 1862 $[M+H]^+$

t_R: 21.89 min

X - REFERENCES – CHAPTER VII, VIII, XIX

(1) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893-4011.

(2) Sanford, A. R.; Gong, B. *Curr. Org. Chem.* **2003**, *7*, 1649-1659.

(3) Baldauf, C.; Guenther, R.; Hofmann, H.-J. *J. Org. Chem.* **2004**, *69*, 6214-6220.

(4) Nicolaou, K. C.; Florke, H.; Egan, M. G.; Barth, T.; Estevez, V. A. *Tet. Lett.* **1995**, *36*, 1775-1778.

(5) Smith, M. D.; Long, D. D.; Claridge, T. D. W.; Fleet, G. W. J.; Marquess, D. G.; Marquess, D. G. *Chem. Commun.* **1998**, 2039-2040.

(6) Gervay, J.; Flaherty, T. M.; Nguyen, C. *Tet. Lett.* **1997**, *38*, 1493-1496.

(7) Michon, F.; Brisson, J. R.; Jennings, H. J. *Biochemistry* **1987**, *26*, 8399-8405.

(8) Szabo, L.; Smith, B. L.; McReynolds, K. D.; Parrill, A. L.; Morris, E. R.; Gervay, J. *J. Org. Chem.* **1998**, *63*, 1074-1078.

(9) McReynolds, K. D.; Gervay-Hague, J. *Tetrahedron: Asymmetry* **2000**, *11*, 337-362.

(10) Gregar, T. Q.; Gervay-Hague, J. *J. Org. Chem.* **2004**, *69*, 1001-1009.

(11) Claridge, T. D. W.; Long, D. D.; Baker, C. M.; Odell, B.; Grant, G. H.; Edwards, A. A.; Tranter, G. E.; Fleet, G. W. J.; Smith, M. D. *J. Org. Chem.* **2005**, *70*, 2082-2090, and references therein.

(12) Jiang, H.; Leger, J.-M.; Dolain, C.; Guionneau, P.; Huc, I. *Tetrahedron* **2003**, *59*, 8365-8374.

(13) Jiang, H.; Leger, J.-M.; Huc, I. *J. Am. Chem. Soc.* **2003**, *125*, 3448-3449.

(14) Hann, M. M.; Sammes, P. G.; D., K. P.; Taylor, J. B. *J. Chem. Soc., Chem. Commun.* **1980**, 234-235.

(15) Wipf, P.; Henninger, T. C.; Geib, S. J.; *J. Org. Chem.* **1998**, *63*, 6088-6089.

(16) Gardner, R. R.; Liang, G. B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280-3281.

(17) Gardner, R. R.; Liang, G. B.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 1806 - 1816.

(18) Sengupta, A.; Aravinda, S.; Shamala, N.; Raja, K. M.; Balaram, P. *Org. Biomol. Chem.* **2006**, *4*, 4214-22.

(19) Rai, R.; Vasudev, P. G.; Ananda, K.; Raghothama, S.; Shamala, N.; Karle, I. L.; Balaram, P. *Chemistry* **2007**, *13*, 5917-26.

(20) Nowick, J. S.; Brower, J. O. *J. Am. Chem. Soc.* **2003**, *125*, 876-7.

(21) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4105-4106.

(22) Khakshoor, O.; Demelher, B.; Nowick, J. S. *J. Am. Chem. Soc* **2007**, *129*, 5558-5569.

(23) Trabocchi, A.; Menchi, G.; Guarna, F.; Machetti, F.; Scarpi, D.; Guarna, A. *Synlett* **2006**, 331-353.

(24) Scarpi, D.; Occhiato, E. G.; Trabocchi, A.; Leatherbarrow, R. J.; Brauer, A. B.; Nievo, M.; Guarna, A. *Bioorg. Med. Chem.* **2001**, *9*, 1625-1632.

(25) Von Roeder, E. G.; Kessler, H. *Angew. Int. ed. engl.* **1994**, *33*, 687-689.

(26) Von Roeder, E. G.; Lohof, E.; Hessler, G.; Hofmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156-10167.

(27) Chakraborty, T. K.; Ghosh, S.; Jayaprakash, S.; Sarma, J. A.; Ravikanth, V.; Diwan, P. V.; Nagaraj, R.; Kunwar, A. C. *J. Org. Chem.* **2000**, *65*, 6441-6457.

(28) Lopez-Ortega, B.; Jenkinson, S. F.; Claridge, T. D. W.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2008**, *19*, 976-983.

(29) Claridge, T. D. W.; Lopez-Ortega, B.; Jenkinson, S. F.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2008**, *19*, 984-988.

(30) Hanessian, S.; Luo, X.; Schaum, R.; Michnick, S. *J. Am. Chem. Soc.* **1998**, *120*, 8569-8570.
(31) Seebach, D.; Brenner, M.; Rueping, M.; Jaun, B. *Chem. Eur. J.* **2002**, *8*, 573-584.
(32) Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc. Chem. Commun.* **1980**, *24*, 234-235.
(33) Rai, R.; Vasudev, P. G.; Ananda, K. R., S.; Shamala, N.; Karle, I. L.; Balaram, P. *Chemistry* **2007**, *13*, 5917-5926.
(34) Nowick, J. S.; Brower, J. O. *J. Am. Chem. Soc.* **2003**, *125*, 876-877.
(35) Smith, M. D.; Long, D. D.; Marquess, D. G.; Claridge, T. D. W.; Fleet, G. W. J. *Chem. Commun.* **1998**, 2039-2040.
(36) Chakraborty, T. K.; Jayaprakash, S.; Srinivasu, P.; Chary, M. G.; Diwan, P. V.; Nagaraj, R.; Sankar, A. R.; Kunwar, A. C. *Tet. Lett.* **2000**, *41*, 8167-8171.
(37) Haque, M. M. Ph D Thesis, Regensburg **2005**.
(38) Nosse, B.; Chhor, R. B.; Jeong, W. B.; Boehm, C.; Reiser, O.; *Org. Lett.* **2003**, *5*, 941-944, and references therein.
(39) Meindl, W. R.; von Angerer, E.; Schonenberger, H.; Ruckdeschel, G. *J. Med. Chem.* **1984**, *27*, 1111-1118.
(40) Burk, M. J.; Allen, J. G. *J. Org. Chem.* **1997**, *62*, 7054-7057.
(41) Williams, R. M.; Armstrong, R. W.; Maruyama, L. K.; Dung, J. S.; Anderson. O. P.; *J. Am. Chem. Soc.* **1985**, *107* 3246–3253.
(42) Chakraborty, T. K.; Ghosh, A. *Synlett* **2002**, 2039-2040.
(43) Wüthrich, K. *NMR of proteins and nucleic acids, Wiley, New York.*, ***1986.***
(44) Belvisi, L.; Gennari, C.; Mielgo, A.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **1999**, 389-400.
(45) Pardi, A.; Billeter, M.; Wuthrich, K. *J. Mol. Biol.* **1984**, *180*, 741-751.
(46) Chakrabartty, A.; Kortemme, T.; Padmanabhan, S.; Baldwin, R. L. *Biochemistry* **1993**, *32*, 5560-5565.
(47) Lisowski, M.; Olczak, J.; Zabrocki, J. *J. Pept. Sci.* **2006**, *12*, 297-302.
(48) Greenfield, N. J. *Anal. Biochem.* **1996**, *235*, 1-10.
(49) Sewald, N. J.; H.-D., *Peptides: Chemistry and Biology, Wiley, New York* **2002**.
(50) Lau, S. Y.; Taneja, A. K.; Hodges, R. S. *J. Biol. Chem.* **1984**, *259*, 13253-13261.
(51) Halgren, T. A. *J. Am. Chem. Soc.* **1990**, *112*, 4710-4723.
(52) http://www.imb-jena.de/ImgLibDoc/ftir/IMAGE_FTIR.html.
(53) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 4228-4245.
(54) McKay, F. C.; Albertson, N. F. *J. Am. Chem. Soc.* **1957**, *79*, 4686-4690.
(55) Carpino, L. A. *Acc. Chem. Res.* **1987**, *20*, 401-407.
(56) Carpino, L. A.; Han, G. Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748-5749.
(57) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 1054-1052.
(58) Franot, C.; Roberts, D. W.; Smith, R. G.; Basketter, D. A.; Benezra, C.; Lepoittevin, J. P. *Chem. Res. Toxicol.* **1994**, *7*, 297-306.
(59) Tomioka, K.; Cho, Y. S.; Sato, F.; Koga, K. *J. Org. Chem.* **1988**, *53*, 4094-4098.
(60) Jaime, C.; Ortuno, R. M.; Font, J. *J. Org. Chem.* **1986**, *51*, 3946-3951.
(61) Dinares, I.; Entrena, A.; Jaime, C.; Segura, C.; Pont, J. *Electron. J. Theor. Chem.* **1997**, *2*, 160-167.
(62) Haasnoot, C. A. G.; Deleeuw, F.; Altona, C. *Tetrahedron* **1980**, *36*, 2783-2792.
(63) Wavefunction Inc., 18401 Von Karmann Ave., Suite 370, Irvine, CA 92612; J. J. P. Stewart. *J. Comput. Chem.* **1989,** 10, 209.
(64) Munoz, V.; Blanco, F. J.; Serrano, L. *Nat. Struct. Biol.* **1995**, *2*, 380-385.
(65) Harper, J.; Rose, G. D. *Biochemistry* **1993**, *32*, 7605-7609.
(66) Toniolo, C.; Polese, A.; Formaggio, F.; Crisma, M.; Kamphuis, I. *J. Am. Chem. Soc.* **1996**, *118*, 2744-2745.

(67) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein. Chem.* **1985**, *37*, 1-109.
(68) Pavone, V.; Gaeta, G.; Lombardi, A.; Nastri, F.; Maglio, O.; Isernia, C.; Saviano, M. *Biopolymers* **1996**, *38*, 705-721.
(69) Crisma, M.; Formaggio, F.; Moretto, A.; Toniolo, C. *Biopolymers* **2006**, *84*, 3-12.
(70) Millhauser, G. L. *Biochemistry* **1995**, *34*, 3873-3877.
(71) Moretto, A.; Formaggio, F.; Kaptein, B.; Broxterman, B. Q.; Wu, L.; Keiderling, T. A.; Toniolo, C. *Biopolymers* **2007**, *90*, 567-574.