Design, synthesis and structural evaluation of peptidomimetics with a defined secondary structure

Dissertation

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Régis Delatouche

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This work was supervised by Prof. Dr. Oliver Reiser and Prof. Dr. Umberto Piarulli

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Examination committee: Prof.Dr. Sigurd Elz
Prof. Dr. Oliver Reiser
Prof. Dr. Umberto Piarulli
Prof. Dr. Burkhard König

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To my spouse Virginie, for her support and encouragements, my son Erwan and my family.

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Abbreviations

9-BBN	9-borabicyclo[3.3.1]nonane
Ala	alanine
Bn	benzyl
Boc	tert-butyloxycarbonyl
CAN	cerium(IV) diammonium nitrate
Cbz	carboxybenzyloxy
CH_3CN	acetonitrile
d.e.	diastereoisomeric excess
d.r.	diastereoisomeric ratio
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	N,N'-dicyclohexyl carbodiimide
DCM	dichloromethane
DIBAL-H	diisobutyl aluminium hydride
DIC	N,N'-diisopropyl carbodiimide
DKP	diketopiperazine
DMAP	dimethylaminopyridine
DMSO	dimethylsulfoxide
e.e.	enantiomeric excess
EDC	N-ethyl-N'-dimethylaminopropyl carbodiimide
Et_3N	triethylamine
Fmoc	9-fluorenylmethylchloroformate
Gln	glutamine
Gly	glycine
HATU	2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium) hexafluorophosphate
HBTU	O-benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate
HMDS	hexamethyldisilazane
HMPA	hexamethylphosphoramide
HOAt	hydroxyazabenzotriazole
HOBt	hydroxybenzotriazole
LDA	lithium diisopropylamide
mCPBA	3-chloroperoxybenzoic acid
Me	methyl
MEM	methoxyethoxymethyl
Phe	phenylalanine
ppb	part per billion
ppm	part per million
Pro	proline
Ser	serine
tBu	tert-butyl
TEMPO	2,2,6,6-tetramethylpyridine-1-oxyl
TFA	trifluoroacetic acid
THF	tetrahydrofurane
TMU	tetramethylurea
Tyr	tyrosine
Val	valine

Chapter 1: Design, synthesis and structural evaluation of peptidomimetics containing new δ-amino acids

I. Introduction

1. Generalities about peptides

Peptides and proteins are at the base of life and are necessary to living organisms as they fulfil multiple functions. They are involved in cell recognition, cell adhesion, signal transduction, structure in the intracellular and extracellular matrix and are components of hormones and enzymes. These multiple roles can be explained by the immense diversity of peptides and proteins coming from the unlimited combination of naturally occurring amino acids.

Natural peptides and proteins are mainly composed of 20 α-amino acids along with a few other relatively scarce ones. Amino acids are organic molecules which possess an amine and a carboxylic acid. Their nomenclature uses Greek letters according to the number of carbons which separate the carboxylic function to the amine function: α (one carbon), β (2 carbons)… α-amino acids can be substituted on the C_α giving birth to a great diversity in amino acids' chemical and physical properties. The simplest α-amino acid, glycine or 2-amino ethanoic acid, is the only achiral amino acid as it bears no substituent in α-position. All of the other α-amino acids derive from glycine, with substituents comprised of aliphatic chains, aromatic chains or polar aliphatic chains. Natural α-amino acids have been classified in five categories: acidic, neutral, basic, hydrophobic and hydrophilic. When the amino acid is not glycine, the C_α is a chiral center and natural amino acids are all present in the L-configuration in Fischer nomenclature. A few examples extracted from exotic molluscs or in cell walls of some bacterias are in a D-configuration but their occurrence in nature is rare in comparison to the supremacy of L-amino acids (Figure 1).

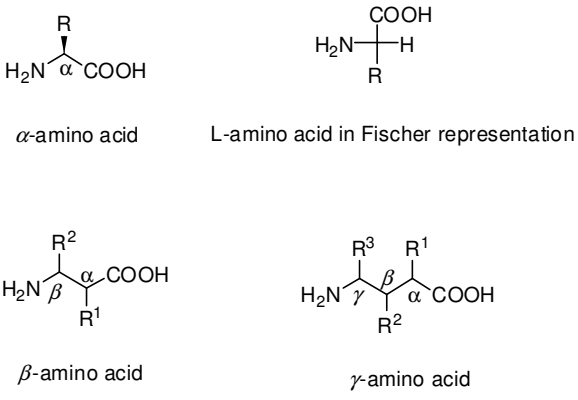


Figure 1: α-, β-, γ-amino acids and representation of an α-amino acid in Fischer representation

Amino acids are the building blocks of peptides and proteins; they are linked together by an amide bond resulting from the condensation of the carboxylic acid of an amino acid with the amino functionality of another amino acid. Peptides and proteins can be considered as polymers of amino acids and those containing less than 10 amino acids are called oligopeptides. Those that contain up to 50 amino acids are called peptides and beyond 50, they are called proteins. Starting from only 20 amino acids, there are therefore 20^n possibilities of peptide sequences for a peptide containing n amino acids. This makes proteins the biggest family of macromolecules existing on Earth and the number of combinations can be considered to be unlimited.

a. Primary structure

To fully describe peptides and proteins, four types of structures are required. The primary structure describes the arrangement of amino acids in the peptide starting from the amino acid having a free or protected amino residue on the left (N terminus), and finishing with the amino acid having a free or protected carboxylic acid (C terminus). The primary structure defines the sequence of amino acids composing a peptide.

b. Secondary structure

Peptides and proteins do not remain linear in liquid phase or in solid state, the interactions they have with their environment cause the chain to fold on itself. This folding can be very organised and several distinct secondary structures have been described. Folding is not only driven by the environment, but it is also favoured and stabilised by intramolecular hydrogen bonding. The amide protons composing the peptide can easily be involved in hydrogen bonding with the carbonyl group of another amide in the chain. This web of hydrogen bonding can present a periodicity which is typical for a subclass of secondary structure. The way the hydrogen bonding is organised determines the secondary structure adopted by the peptide. It is important to note that large proteins do not adopt the same secondary structure along their backbone. They will present some domains with a defined secondary structure and others that will not be organised at all. Three major secondary structures can be defined: helices, β-sheets and turns, all of which having their own nomenclature to describe their hydrogen bonding. A non organised secondary structure is called random coil.

- Helices

Helices present a periodic folding having a curly shape. Most of the time, the helix turns clockwise and is called right-handed helix. In the other case it is a left-handed helix. Within the family of helices, a few subcategories can be described depending on the periodicity of the helix.

α-helices are the most common helices adopted by proteins and peptides, they are characterised by a hydrogen bond between the CO of an *i* residue and the NH of the *i+4* residue forming a 13-membered ring. The mean complete helix loop contains 3.6 residues and is 0.54 nm long, which corresponds to a translation of 0.15 nm per residue. The dihedral angles φ and ψ of the peptidic chain are of -184° and -123°. An α-helix is a very compact structure where the maximum of hydrogen bonds is formed and the lateral amino acids chains point out of the helix (Figure 2).

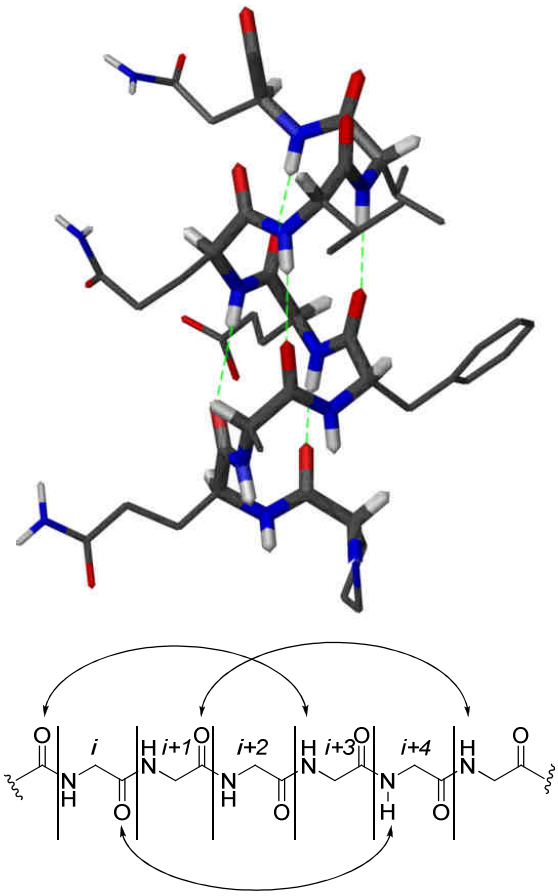


Figure 2: α-helix and hydrogen bond pattern

The 3_{10} helix is a helix presenting hydrogen bonding between the CO of the residue *i* and the NH of the *i+3* residue forming a ten-membered ring. The helix step is 0.60 nm and contains three residues, the dihedral angles φ and ψ are of -49° and -26° respectively and therefore the helix is more narrow and a little longer than an α-helix. This structural type occurs rarely in nature and is usually observed on short distances (2-3 steps).

Some other less common helical structures have been described such as the Π-helix or the left-handed type II helix, which are very specific to a few peptides. Other types of helices have been observed in β-peptides like the 14-helix or the $2.7_{12\text{-}10}$ helix with β^2-β^3-peptides which is a helix presenting alternation of 10- and 12-membered ring with 2.7 residue per turn.[1]

- β-sheets

β-sheets are the result of hydrogen bonding between two fragments that are far from one another. They can differ in their orientation which can be parallel (both fragments are oriented in the same direction) or antiparallel when a fragment is oriented from the N terminus to the C terminus and the other one is oriented from C terminus to the N terminus (Figure 3). In both cases, the sheet is stabilised by interfragment hydrogen bonding.

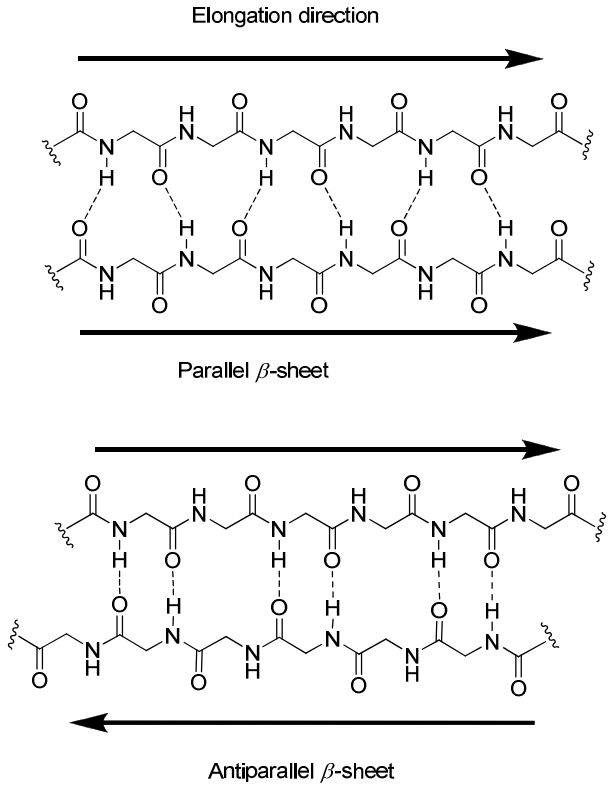


Figure 3: Parallel and antiparallel β-sheet

- Turns

Turns are small secondary structures that form an elbow in the peptide sequence and can induce an antiparallel β-sheet conformation by placing two fragments in front of one another. They are classified depending on the ring size of the hydrogen bond forming the turn.

β-turns are the most common turns and involve hydrogen bonding between the *i* residue and the *i+3* residue within a ten-membered ring. There are three types of β-turns depending on the dihedral angles: I, II and III (the type III corresponds to a single turn of 3_{10} helix). The mirror images of these turns are called I', II' and III'. On the other hand, γ-turns involve hydrogen bonding between the *i* and *i+2* residue by a seven- membered ring. As observed for β-turns, γ-turns are the mirror image of γ-turns and therefore have opposed dihedral angles.

c. Tertiary structure

The tertiary structure is basically the way proteins fold themselves three-dimensionally so it is possible to see how the different secondary structures are organised one to each other. Some domains which are very distant in the primary structure can be neighbours when the protein is folded in its active form. A protein can fold spontaneously just by hydrophobic and hydrophilic effects, the domains containing hydrophobic residues having the tendency to migrate to the centre of the protein with hydrophilic residues appearing at the surface of the protein in aqueous environments. Most of the time, proteins need to be matured in cells by enzymes which induce their active form. Some disulfide

bridges between two cysteines can be created for example to give more stability to the three-dimensional structure.

d. Quaternary structure

The quaternary structure exists only in proteins that are composed of at least two polypeptidic chains or subunits. The quaternary structure describes the way the subunits are organised relative to one another. The quaternary structure is stabilised by non-covalent interactions (e.g. hydrogen bonding and hydrophobic interactions).

2. Conformational studies for secondary structure characterisation

As we have seen, secondary structures are mainly stabilised by hydrogen bonding and their characterisation involves many different analyses.[2]

a. NMR characterisation

NMR spectroscopy is the most useful technique used to characterise secondary structures. Since hydrogen bonding is in great part responsible for the secondary structure, the NMR signals of the amide protons can give significant information on how the peptide is organised.

- Solvent choice

The nature of the solvent can strongly affect the secondary structure adopted by a peptide. For example, a peptide may not organise itself in one solvent but could have a well defined secondary structure in another solvent or even adopt different structures in two different solvents. ^{1}H-NMR spectroscopy can give insights into the hydrogen-bonded state of protons: hydrogen-bonded amide protons, either intramolecularly or with the solvent, appear downfield in comparison to non-hydrogen-bonded amide protons. In non-competitive solvents (e.g. $CDCl_3$), significantly deshielded protons often indicate that they are in a hydrogen-bonded state.[3]

- Dilution studies

NMR spectroscopy can also be used to make dilution analyses as peptides can aggregate together by hydrogen bonding, giving misleading information because the intermolecular hydrogen bonding could be mistaken with intramolecular hydrogen bonding. A peptide is considered to not be aggregated if in a concentration range (typically in the range of 1 to 10 mM) the amide proton chemical shifts remain identical. If a variation is observed at a given concentration in comparison to a lower concentration, this means that some aggregation occurs at this concentration. This aggregation effect is solvent-dependent as a peptide is more prone to aggregation in non competitive solvents like $CDCl_3$. For example, a peptide may easily aggregate in $CDCl_3$ at a given concentration whereas it may not aggregate in methanol at the same concentration which is a very polar and competitive solvent.

- Variation of temperature

The coefficient of temperature dependence ($\Delta\delta/\Delta T$) can also be measured by NMR spectroscopy giving information on the hydrogen-bonded or non-hydrogen-bonded state of the amide protons. These studies must be made at a concentration where no aggregation occurs. According to some authors, amide protons presenting a small temperature dependence coefficient are either locked in hydrogen-bonding or non-hydrogen-bonded ($0<\Delta\delta/\Delta T<3$ ppb/K) whereas the NH protons with a higher coefficient (between -4 and -8 ppb/K) are in an equilibrium between hydrogen-bonded and a non-hydrogen-bonded state.[4-8] By contrast, some authors conclude that an amide proton is intramolecularly hydrogen-bonded if the absolute value of its temperature dependence coefficient is lower than 2.6 ppb/K, without taking into consideration other parameters such as the relative chemical shift compared to the other NH protons and that coefficients higher than 2.6 ppb/K indicate a non-hydrogen-bonded state.[9] It is generally belived that amide protons having a low temperature dependence coefficient ($\Delta\delta/\Delta T<3$ ppb/K) are intramolecularly hydrogen-bonded, NH protons exhibiting a $\Delta\delta/\Delta T$ between 3 and 8 ppb/K are in an equilibrium between hydrogen-bonded and non-hydrogen-bonded state and that coefficents higher than 8 ppb/K reveal a non-hydrogen-bonded state. Again, the solvent nature can affect these measurements as a polar solvent will be more competitive in creating hydrogen bonding with the peptide. It is advised to use $CDCl_3$ for these types of experiments, but if the peptide is very polar, it will aggregate in this solvent even at low concentrations making the experiment invalid.

- Deuterium proton exchange

Another technique that gives information on intramolecular hydrogen bonding is proton-deuterium exchange. CD_3OD can be added to a peptide dissolved in a solvent possessing no exchangeable deuterium and the deuterium on the hydroxyl group of MeOH-d_4 can exchange with amide protons of the peptide. The more rapidly it exchanges, the less hydrogen bonded NH proton is considered to be. Some differences in exchange rates can be observed between protons involved in hydrogen bonding and protons not involved because the protons stabilised by hydrogen bonding will be almost blocked making the exchange time with deuterium longer.

- 2D NMR

2D-NMR is a very useful technique as it allows detecting intramolecular long-range couplings, therefore giving information on the neighbouring protons involved in hydrogen bonding. If some protons are intramolecularly hydrogen bonded, the 2D analysis by NOESY or ROESY will show contacts of the NH proton involved in hydrogen bonding with other protons which are far from it in the molecule. For example, in the case of an α-peptide forming an α-helix, an amide proton, in addition to coupling with the proton in α-position of the same amino acid which can be seen as well by COSY experiment, will couple with the Hα of the neighbour amino acid, and with the Hα of the amino acid *i-4* because this amide proton involved in hydrogen bonding with the CO of the amino acid *i-4* will be close to its Hα (Figure 4). NOESY can detect contact between two protons in a range of 5Å. To

analyse a 2D spectrum, it is useful to have a system where the signals are well dispersed to avoid errors in the attribution.

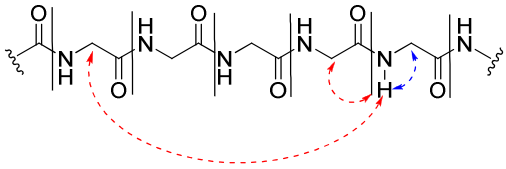


Figure 4: Possible couplings by NOESY or ROESY in an α-helix, blue: COSY signal

b. Circular dichroism

Circular dichroism is a physical measurement that can help in finding the secondary structure of peptides.[10] This analysis is performed in solution in high dilution (typically <1 mM) in the absorption band of amide bonds (180-250 nm). Circular dichroism measures the ellipticity of a peptide in this band with polarised UV light. In this band, the $\pi \rightarrow \pi^*$ absorption of amides can be observed and it will vary according to the hydrogen bonded or non hydrogen bonded state of the amides, therefore it will give information on the presence of a secondary structure. Indeed, since a peptide is a chiral molecule, it will present an optical rotation on polarised light but this optical rotation can vary with the wavelength. The analysis is performed in quartz cells of 1 mm length or less as the solvent absorption can create some parasite noise. Not all solvents can be used in CD spectroscopy as the circular dichroism must be measured in a band where the solvent does not absorb; methanol (limit at 195 nm for a 1 mm-long cell), trifluoroethanol (TFE) or mixtures methanol/water can be used. Chloroform or dichloromethane can not be used in this case as they absorb in the same region as amides.

The ellipticity θ follows the Beer-Lambert law and can be calculated as follows:

$\theta = CD_{measured}/(C \times L \times n)$

With: θ: ellipticity in $deg.cm^2.dmol^{-1}$

C. concentration in $mol.L^{-1}$

L: length of the cell in dm

n: number of NH in the molecule

This technique has the advantage of being fast and one can see almost immediately if the peptide adopts a secondary structure or not. The limitation is that one can not deduce exactly which amides are involved in hydrogen bonding. It is nevertheless very useful since CD curves of α-peptides are typical of a certain secondary structure and so new peptide curves can be compared to reference spectra. There has been also a lot of work in the field of β-peptides and some references are available but when a peptide containing unnatural amino acids is analysed, this comparison can not always be performed with certainty as the curves may differ for identical secondary structures.

c. IR in solution

Solution IR spectroscopy is another simple and fast way to determine if hydrogen bonding is present. This measurement can be done in chloroform or dichloromethane at low concentration (1-2 mM) and the absorption of amides can be observed. The hydrogen bonded amides absorb at wave

numbers <3400 cm^{-1} and non hydrogen bonded amide absorb at >3400 cm^{-1}. This analysis allows direct observation of whether or not the molecule undergoes hydrogen bonding in solution.[7] The disadvantage is that IR in solution can not be performed in solvents like methanol as the OH group of methanol absorbs in the same area as amides and their absorption would be masked by the solvent absorption. The other disadvantage, as for circular dichroism, is that it does not give any information regarding which amide protons are involved in hydrogen bonding.

d. X-ray crystallography

X-ray crystallography is a direct way of observing the structure of the peptide. The primary difficulty of X-ray crystallography is crystallising the peptides, particularly linear ones which have so much mobility that getting a stable crystal is quite challenging. In addition, the peptide may not crystallise in the same structure as it is in solution, but the crystal structure can give good information on how the peptide folds itself, hydrogen bonding taking place and its secondary structure.

e. Computational studies

Computational studies can give the preferred conformation of a peptide utilising minimisation calculations. The result can confirm experimental results or give an idea of what the structure could be. The results can be refined by doing calculations with constraints based on observed long range coupling by 2D-NMR.

3. Peptide coupling: overview

The synthesis of peptides has interested chemists for many years and the actual knowledge in this field is due to decades of research and improvements. An amino acid contains two reactive functionalities in the simplest cases, the additional functionalities present on the lateral chain making the synthesis more difficult and complex. To form an amide bond, the condensation of the carboxylic acid of an amino acid with the amine of another amino acid is required but can be problematic. If one considers a mixture of two free amino acids A an B, controlling the reaction to get only the dipeptide H_2N-A-B-COOH would be impossible as all possible combinations would take place giving dipeptides, tripeptides… To avoid these multiple reactions, chemists have examined the reaction of an N-terminal protected amino acid with another C-terminal protected amino acid. In this way, the only possible reaction would be between the COOH of the first amino acid and the NH_2 of the second amino acid. Furthermore, if one wanted to elongate this peptide, one should be able to deprotect only one function of the new peptide formed. Then, to synthesise a peptide, one must think of using protecting groups that can be removed selectively. These types of protecting groups are called orthogonal.

The condensation of an amine with a carboxylic acid to give an amide is not spontaneous, so is it required to use coupling agent that will favour the amide formation. The coupling agents activate the carboxylic acid moiety, which then react with the amine to form the amide bond. A multitude of coupling reagents and protecting group have been developed to improve peptide synthesis and some of them are specific for difficult couplings like with a secondary amine.

a. Coupling reagents

A coupling reagent will react with the free carboxylic acid functionality creating an activated carbonyl group which will then react with the free amino group of another amino acid. The coupling reagent does not appear in the reaction and a molecule of water is lost in the condensation. The coupling reaction also involves the use of a base which deprotonates the free carboxylic acid (Figure 5).

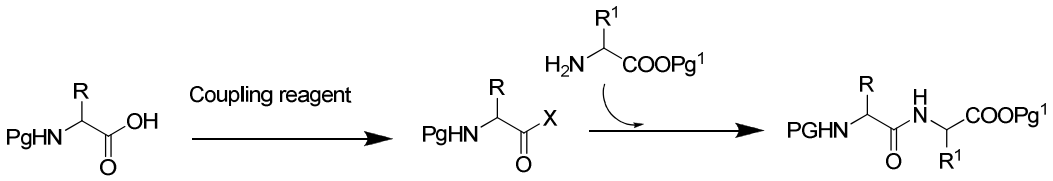


Figure 5: General peptide coupling mechanism

Use of carbodiimides is widespread in peptide synthesis as they are a cheap and efficient way for classical couplings and many diverse carbodiimides can be used depending on the solvent used for the coupling. The most common carbodiimides are EDC (N-ethyl-N'-dimethylaminopropyl carbodiimide), DCC (N,N'-dicyclohexyl carbodiimide) and DIC (N,N'-diisopropyl carbodiimide) and these can be used in liquid-phase and solid-phase peptide synthesis. But these reagents suffer from some drawbacks such as epimerisation of the activated amino acid. Indeed, the activation of the carboxylic acid by a carbodiimide leads to the formation of a very reactive O-acylisourea which then reacts with the amine to form the amide bond and an N,N'-disubstituted urea (Figure 6). The problem of racemisation occurs when the O-acylisourea is formed. The Hα of the activated amino acid becomes very acidic and the base present in the reaction mixture can easily deprotonate it since the anion formed is stabilised by the O-acylisourea. The anion can then be reprotonated non stereoselectively, effecting racemisation of the amino acid. Some additives can be used to diminish or suppress racemisation and accelerate couplings, such as HOSu (hydroxysuccinimide), DMAP (dimethylaminopyridine), HOBt (hydroxybenzotriazole) or HOAt (hydroxyazabenzotriazole). Carbodiimides can also generate symmetrical acid anhydrides by the reaction of the activated amino acid with another N-protected amino acid. The solvent nature also has an effect on racemisation (Figure 7). Nevertheless, carbodiimides are limited in reactivity and couplings with secondary amine are not that efficient so other coupling reagents are required to perform difficult couplings.

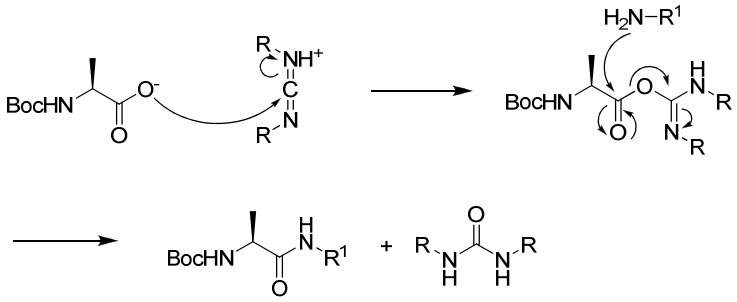


Figure 6: Peptide coupling mediated by carbodiimides

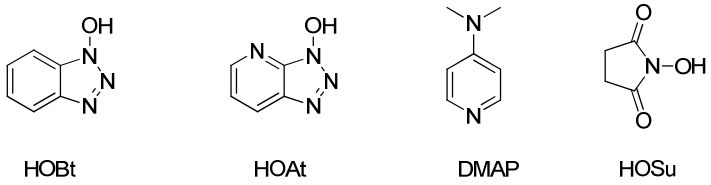


Figure 7: Additives in peptide coupling

Uronium salts are very reactive reagents and can be used to perform difficult couplings. The most common are uronium salts of HOBt and HOAt and, although they are higher priced compared to carbodiimides, are very efficient reagents for difficult couplings. These reagents exist in two forms: uronium and guanidinium: the guanidinim is the predominant form but the uronium is the most reactive species (Figure 8). HBTU (uronium PF_6^- salt of HOBt, widely used in solid phase synthesis) and HATU (uronium PF_6^- salt of HOAt, the most active and expensive) are the most commonly used uronium salts and generally assure high yields in peptide coupling. They are usually used as a last resort (especially HATU) and on small scale as an industrial scale use would not be profitable. Their reactivity comes from their saline nature and good solubility in organic solvents, the presence of the benzotriazole ring being advantageous for the approach of the free amine to the activated carboxylic acid. In the case of HATU, this approach is even more favoured as the nitrogen in position 7 can stabilise the free amine close to the activated carboxylic acid (Figure 9). Once they have reacted, HOBt or HOAt are released in the solution and are easily removed from the organic phase by aqueous washing.

Some other types of coupling reagents are used such as phosphonium salts which are particularly efficient for the coupling of N-methylated amino acids. They are similar to uronium reagents but a phosphonium group replaces the uronium group on the HOAt or HOBt ring.

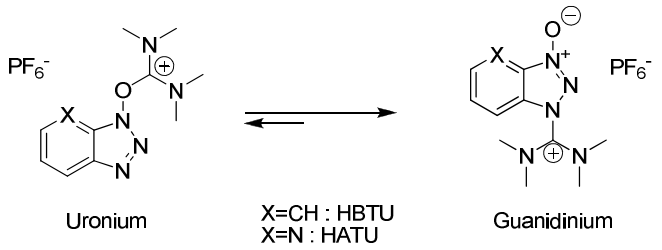


Figure 8: Uronium and Guanidinium forms of HATU and HBTU

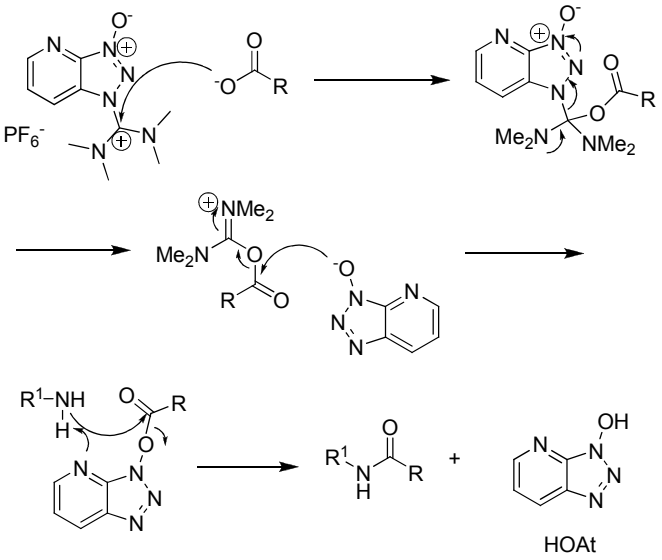


Figure 9: Peptide coupling mediated by HATU

b. Strategies for peptide coupling

For peptide synthesis, two major approaches can be adopted according to the amount of peptide desired and its length.

- Liquid-phase synthesis

This strategy is adopted when the synthesis of a short peptide is desired. It has the advantage of being inexpensive and to be easily applicable on gram-scale. The disadvantage is that it is time consuming because purification has to be performed for every step. It can be realised by the Boc or Fmoc strategy. The synthesis starts from an *N*-Boc or *N*-Fmoc protected amino acid where the C terminus is protected as an ester. Removal of the Boc group is achieved in a mixture TFA/DCM or HCl in ethyl acetate or dioxane affording the corresponding ammonium salt. The Fmoc group is removed by 20-50% piperidine or morpholine in DMF. The *N*-protected amino acid to be added to the chain is then activated and added to the previous ammonium salt. Once the reaction is finished, extraction and column chromatography affords the new peptide which is ready to be subjected to the same reaction sequence until the desired peptide is obtained. Subsequent deprotection of the N-terminal and C terminal may be required if a non protected peptide is desired.

- Solid-phase synthesis

This technique has revolutionised peptide synthesis as it is particularly convenient for long peptide synthesis. The first examples reported by Merrifield[11] on resin supported peptide synthesis using a modified polystyrene as a support for peptide synthesis allowed the fast synthesis of complex and long peptides. The peptide chain being elongated remains attached to the resin through a linker allowing an easy separation as a simple filtration of the unsoluble resin permits removal of the excess reagents which are soluble in the mixture. The resin can be immediately used for the next step, and, when synthesis is achieved, the peptide can be cleaved from the resin support and affords the desired peptide with few impurities. Both Boc and Fmoc strategies can be used but the Fmoc strategy is usually preferred as it is realised under milder conditions. The most significant advantage of solid phase peptide synthesis is that it can be automated and just a single purification is required to obtain pure long peptides. Different types of resins and methods have been developed to improve yields and resin supported synthesis has been applied to many fields other than peptide chemistry.

4. Synthesis of unnatural amino acids

For many years, chemists have been interested in creating new molecules that could mimic natural products in order to create synthetic drugs that would be more efficient than natural products or to avoid tedious extraction from natural sources. In the field of peptide chemistry, scientists were also curious to know how a peptide or a protein would behave if it was partially or totally constituted of synthetic amino acids. Many examples of unnatural α-amino acids exist and the field of β-amino acids has been largely investigated, from simple cases to more and more complex applications giving birth to the term of “Foldamers” (Foldable polymers) by Gellman who defined them as unnatural peptides or polymers that can fold like natural peptides.[12] He also described the approach, the scope and the possible applications of this new way of obtaining peptides and their secondary structure characterisation. The main motivation of creating unnatural amino acids is that they can adopt secondary structures like natural peptides and can be designed to be more effective and selective towards a given target involved in a disease, but, being partially or totally unnatural, they would be less prone to peptide catabolism. Indeed, several drugs are based on a peptide structure but they can be quickly cleaved by proteases before arriving to their target, making them useless even if in in-vitro tests they had shown a high activity. The presence of unnatural amino acids in these drugs would slow down the action of proteases increasing therefore the drug half-life, and consequently, increasing its activity and diminishing the dose required for getting the same efficiency. The introduction of unnatural amino acid would also have the effect of being more selective than natural peptide drugs.

The other interest is more fundamental, creating unnatural peptides and study the structure they can adopt would give a better knowledge of the parameters that control specific folding of these peptides and, as a consequence, this would lead to a better understanding on how natural peptides adopt only one conformation and not another one. The final application would be to prepare unnatural peptides “on demand” designed to adopt a specific secondary structure and to have a specific activity

where every parameter would have been judiciously chosen to obtain the desired peptide with the desired secondary structure.

II. Syntheses of δ-amino acids

1. Synthesis of linear δ-amino acids

a. Non enantioselective synthesis of linear δ-amino acids

The substitution of peptide backbone by a totally or partially non-peptidic sequence is relatively new and δ-amino acids have proved to be good candidates since a δ-amino acid could be seen as an α-dipeptide mimic. The dipeptide isosteric replacement of an amide bond was first theorised in 1976[13] but investigations regarding this had been relatively scarce until it was demonstrated to be a powerful concept in 1980 by Sammes et al.[14] for the preparation of double bond isosteres of peptide bond towards enkephalin analogues. By a simple approach, they showed that the spatial disposition of amide and *trans*-olefin bonds have very similar angles and lengths and that *trans*-olefin isosteres, if inserted in strategic positions into peptide backbones, could exhibit an enhanced resistance towards peptidases and a better cell permeability (Figure 10).

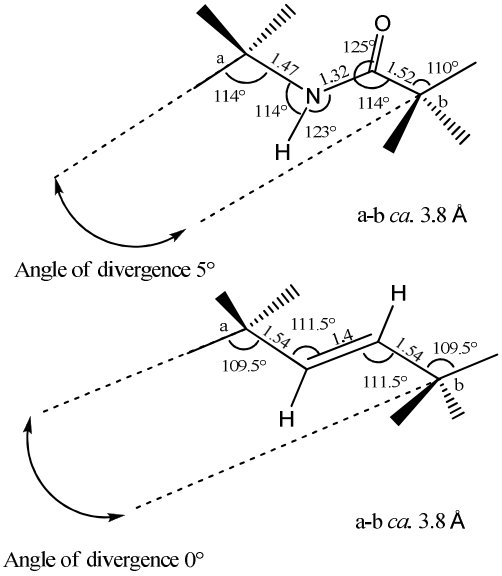
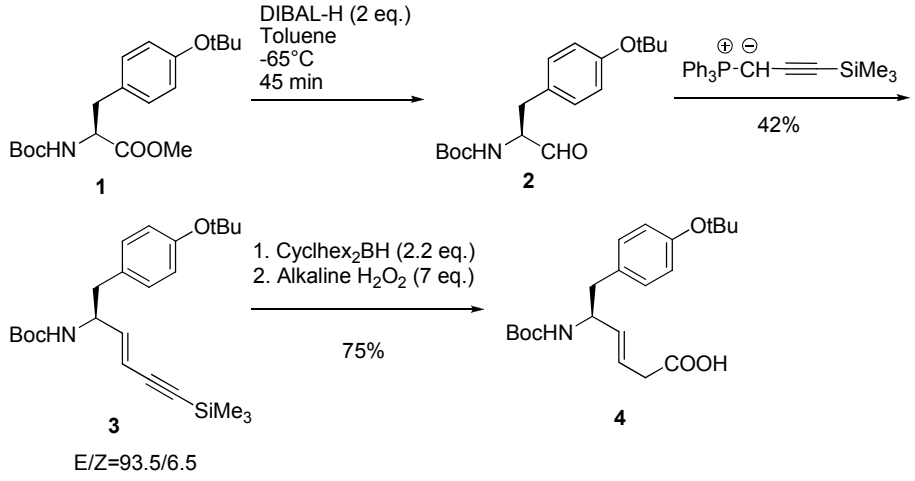


Figure 10: Geometric comparison of amide and trans-olefin backbones

To illustrate their theory, they prepared a trans-olefin dipeptide isostere that would substitute the enkephalin amide bond Tyr1-Gly2 which is known to be easily hydrolysed in vivo by amino peptidases. Starting from L-tyrosine, the O-t-butyl-N-Boc derivative **1** was prepared and submitted to reduction in toluene by DIBAL-H giving the resulting aldehyde **2**. After purification, **2** was condensed with an alkyne ylide. The enyne **3** was obtained primarily in the *trans*-configuration, only 6.5% of the crystalline *cis*-isomer was formed and could be separated. After hydroboration and oxidation with alkaline hydrogen peroxide, the desired *trans*-alkene dipeptide isostere of Tyr-Gly **4** was obtained for further introduction into enkephalin analogues (Scheme 1). This first attempt is considered as a lead in the chemistry of *trans*-alkene dipeptide isosteres and the approach was reused two years later.[15] In

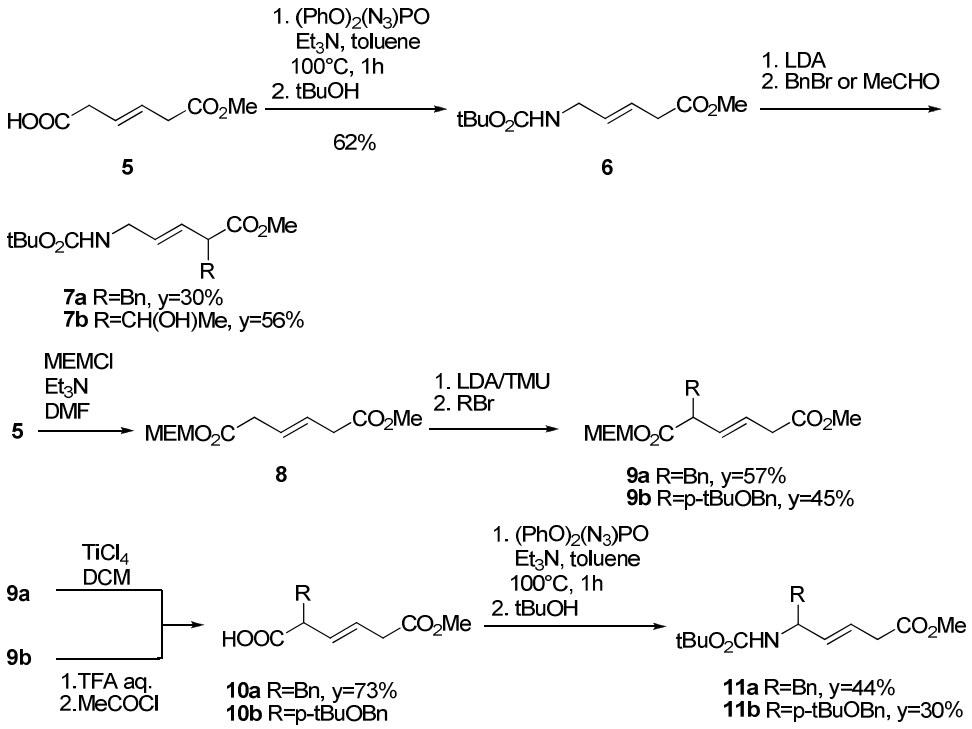
1985, they adapted their method for preparing Pro-Gly, Pro-Leu and Pro-Phe dipeptide isosteres.[16] Although this method suffered from racemisation of the substituting group of the originally optically pure amino acid,[17] it was the first to be successfully utilised to mimic peptide proteases sensitive domains.



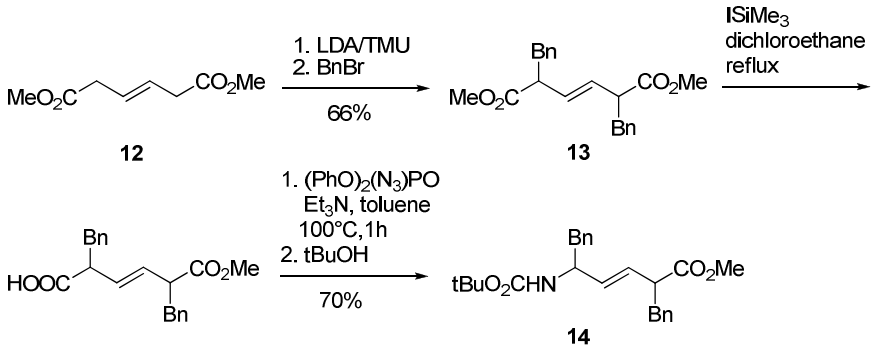
Scheme 1: Synthesis of *trans*-alkene dipeptide isostere by Sammes et al.

After this first example of *trans*-olefin isosteres, many variations have been proposed with various substituents. Later, Cox et al. published another synthesis of that promising genre of δ-amino acids.[18] Starting from commercial *trans*-hex-3-enedioic acid, after methyl esterification and a modified Curtius reaction, they could access the fully protected δ-amino acid **6** that could subsequently be functionalized by enolate formation with LDA and subsequent reaction with alkyl halides or aldehydes in a racemic manner. Products **7a** and **7b** were obtained with moderate yields. Alternatively, the monoester of *trans*-hex-3-enedioic acid **5** can be esterified with MEMCl to give a mixed diester **8** which can be regioselectively alkylated in α-position of the MEM ester. Due to the slow nature of the reaction, the use of additives like HMPA is required, but, for toxicity reasons, HMPA was substituted by tetramethylurea (TMU). Once the diester was alkylated with benzyl bromide and the MEM group removed by $TiCl_4$, the acid was submitted to the same modified Curtius reaction giving the 5-substituted δ-amino acid **11a**. Because the selective deprotection of the MEM group revealed impossible with classic procedure, the p-^{t}BuOBn analogue was reacted with aqueous TFA and then the free OH was acetylated with acetyl chloride. Subsequent modified Curtius reaction gave the final p-AcOBn substituted amino acid **11b** (Scheme 2). The diester **12**, which was formed as a secondary product when preparing **5**, could also be used to prepare 2,5-dibenzylated dipeptide isostere **13** by a double alkylation with LDA in THF/TMU and benzyl bromide. One diastereomer was crystallized and the difficult partial hydrolysis was performed using iodotrimethylsilane in dichloroethane at reflux. The subsequent Curtius reaction led to the protected isostere **14** (Scheme 3). Despite the lack of stereoselectivity of the syntheses, Cox et al. proposed the first route to substituted *trans*-olefin

isosteres that could give new perspectives for the diversity of products and potential medical applications.

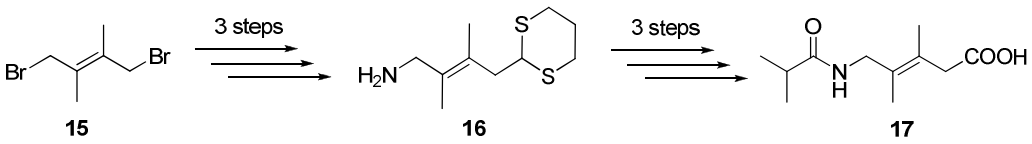


Scheme 2: Route for monosubstituted trans-olefin isosteres by Cox et al.



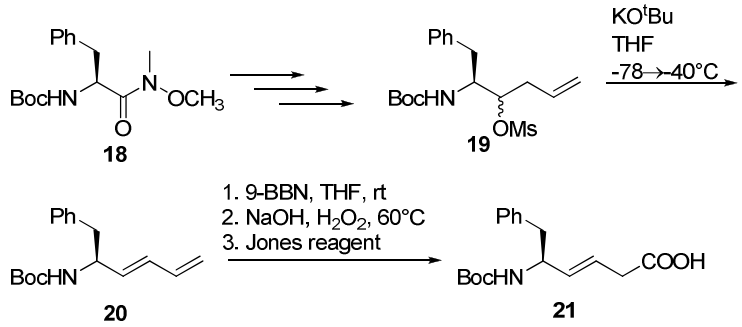
Scheme 3: Route for disubstituted trans-olefin dipeptide isostere

Gellman et al. have also prepared alkene isosteres for studying their secondary structures. Starting from 1,4-dibromo-2,3-dimethylbut-2-ene **15** which was reacted with lithio dithiane, the amino functionality was introduced by substitution of the remaining bromine by an azido group and subsequent Staudinger reaction afforded **16**. After coupling the amine with isobutyryl chloride, the dithiane ring was removed and the carboxylic end was obtained by oxidation leading to the tetrasubsituted dipeptide isostere **17** (Scheme 4).[19,20]



Scheme 4: Gellman's dipeptide isostere synthesis

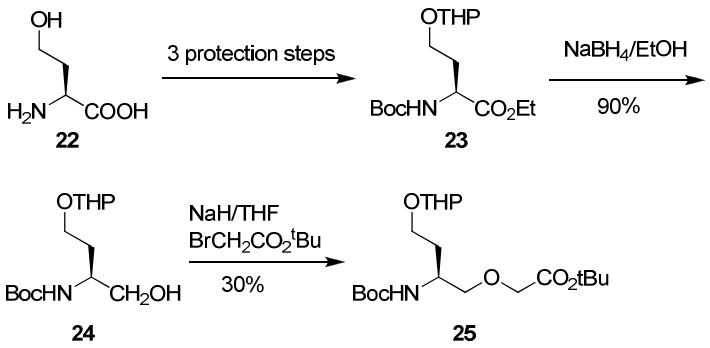
In 1996, Kessler and coworkers developed their own synthesis of Phe-Gly *E*-alkene dipeptide isostere.[21] It is distinctive in that they use a β-elimination to generate the *E*-double bond. Starting from Boc-Phe-OH, they prepared the *N*-methoxy-*N*-methylcarboxamide derivative **18** using a known method bringing into play propylphosphinic anhydride for C-terminus activation. After allylation with allylmagnesium bromide and reduction to the allylic alcohol, the alcohol was mesylated giving **19** as a diastereomeric mixture. Subsequent reaction with potassium tert-butylate afforded the desired diene **20** in good yields. The low temperature proved to be essential since higher temperatures led to the formation of by-products in non negligible amounts. Regioselective hydroboration of the diene with 9-BBN and then further oxidation with Jones reagent allowed for the isolation of the desired *trans*-dipeptide isostere **21** (Scheme 5).



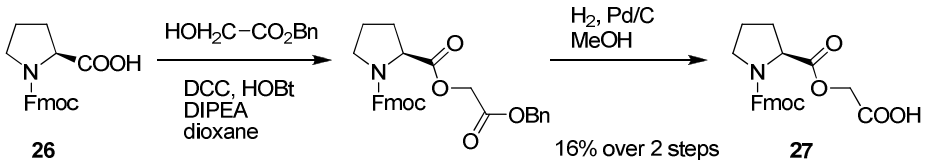
Scheme 5: Kessler's *trans*-alkene dipeptide isostere

Although alkene dipeptide isosteres have received a great deal of attention, some groups focused on creating non alkene dipeptide isosteres. Kuwahara et al.[22] have worked on the synthesis of ether dipeptide isosteres that would have the advantage of being more polar than an alkene dipeptide isotere. To do so, they started from L-homoserine **22** and after adequate protection of the amine, acid and alcohol functionalities, the fully protected homoserine ester **23** was reduced to the corresponding alcohol **24**. Reaction of the alcohol with sodium hydride and bromoacetic acid tert-butyl ester afforded the expected protected δ-amino acid **25** with an ether linkage in β-position (Scheme 6).

Other groups have tried to replace the amide bond by an ester function as this would mimic even better the amide bond polarity. The preparation of this type of derivatives is relatively easy. To replace the fragment Pro-Gly, Raines et al. coupled Fmoc-Pro **26** with benzyl 2-hydroxyacetate[23] and subsequent debenzylation afforded the dipeptide isostere **27** for solid-phase synthesis (Scheme 7).

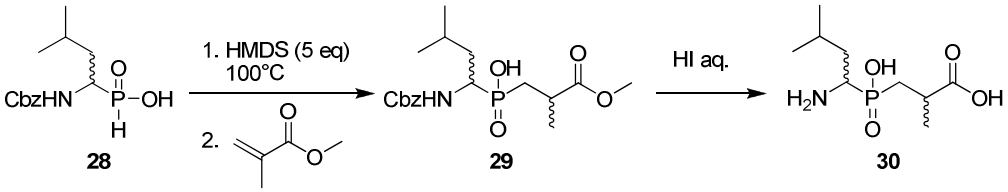


Scheme 6: Ether linkage containing δ-amino acid synthesis



Scheme 7: Synthesis of ester containing dipeptide isosteres

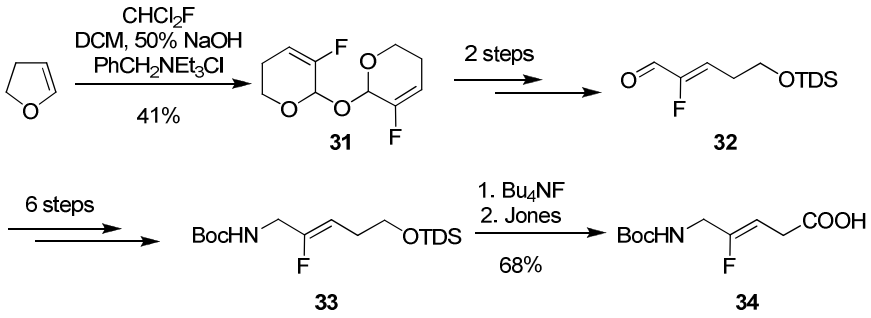
An alternative replacement of the amide bond can be achieved by introducing a phosphinic acid functionality giving birth to phosphino dipeptide isosteres as Kessler et al. reported.[24] The synthesis is straightforward, using the racemic phosphinic acid **28** derived from Cbz protected leucine, it was activated with HMDS and subsequently reacted with α-methyl acrylate in a Michael type reaction giving the fully protected phosphino dipeptide isostere **29**. Deprotection with aqueous HI afforded the deprotected phosphino δ-amino acid **30**. The drawback of the synthesis is that the dipeptide isostere was obtained under its four diastereoisomeric forms (Scheme 8).



Scheme 8: Synthesis of phosphino dipeptide isostere

Fluoroolefin isosteres can also be a good mimic of dipeptide amide bonds regarding geometry and polarity. The first example of this type was reported by Allmendinger et al.[25] who first calculated the geometry of a simple fluoroolefin to compare it with a *trans*-alkene and an amide and came to the conclusion that a fluoroolefin was even closer to the amide backbone than the *trans*-alkene backbone was. Using dihydrofurane as a starting material, it was converted to the cyclic acetal **31**. An acid catalysed hydrolysis accompanied by concomitant double bond isomerisation and protection of the

alcohol by TDS chloride afforded the aldehyde **32**. A sequence of reduction, bromination, azidation, reduction and Boc protection allowed the introduction of the nitrogen moiety to form **33**. A subsequent cleavage of the silyl ether and Jones oxidation gave the fluoroolefin Gly-Gly dipeptide isostere **34** in good yields. Using **32**, they also prepared the Phe-Gly fluoroisostere in *E* and *Z* conformations (Scheme 9).



Scheme 9: Synthesis of fluoroolefin isostere

b. Enantioselective synthesis of linear δ-amino acids

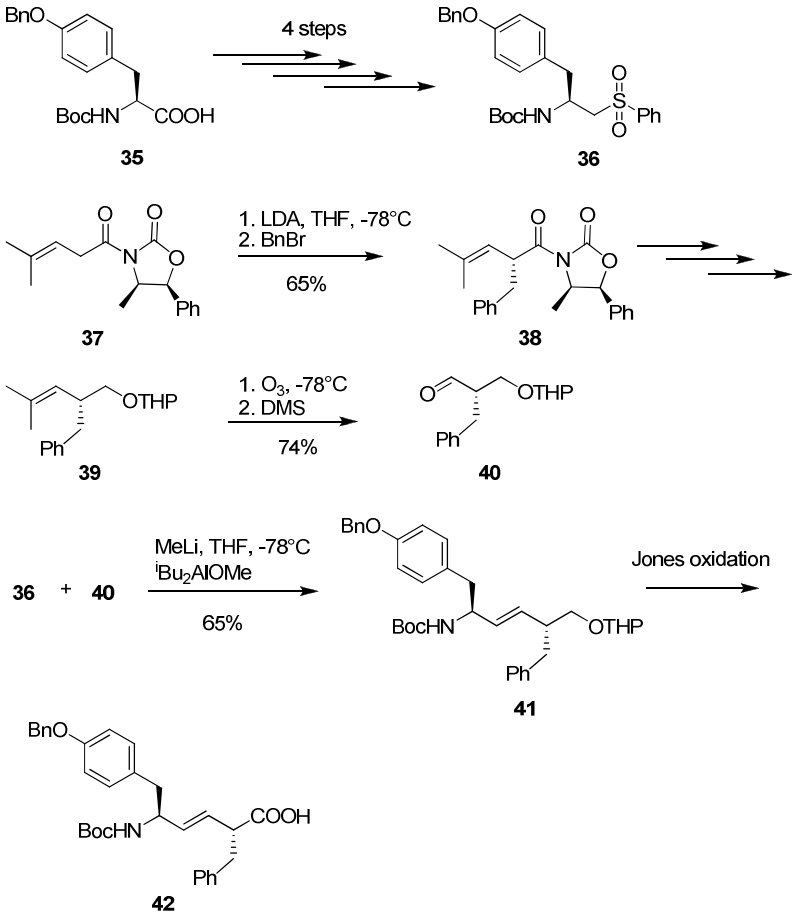
Synthesising linear δ-amino acids with one or more chiral centers created during the synthesis is quite challenging. Most of the time, the chiral centers are introduced using commercially available enantiomerically pure starting materials. Nevertheless, many groups have developed enantioselective ways of syntheses that could allow a higher efficiency for the preparation of the desired biologically active product by the asymmetric introduction of substituents that are difficult to obtain via natural amino acids.

The first stereocontrolled synthesis of *trans*-alkene dipeptide isosteres was reported by Spaltenstein and coworkers who used the Julia olefin synthesis to prepare Tyr-Ala isosteres.[26] The sulfone **36** was prepared from the protected tyrosine derivative **35**. The chiral aldehyde **39** was prepared using the Evans oxazolidinone **37**. The Julia olefin synthesis proceeded well and afforded the dipeptide isostere **41** which led to the corresponding δ-amino acid **42** (Scheme 10).

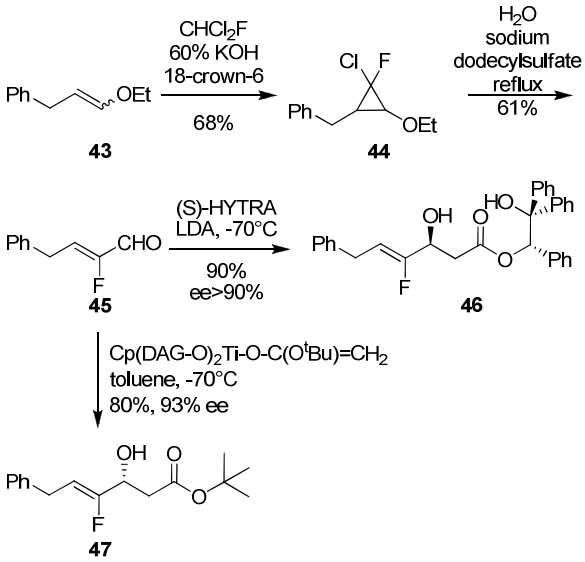
A variety of alkyl substituted alkene dipeptide isosteres have been developed and some additional efforts have been reported for fluoroalkene isosteres since they were predicted to exhibit interesting properties. Allmendinger et al., who were the first to publish the synthesis of fluoroalkene isosteres in a racemic form, reported at the same time an enantioselective method for the preparation of fluoroolefin isosteres.[27] The enol ether **43** was transformed to the chlorofluorocyclopropane **44** which was then converted to the aldehyde **45**. The hydroxyesters **46** and **47** could be obtained by addition of chiral reagents giving the compounds with the hydroxyl groups in either configuration (Scheme 11).

46 and **47** could be submitted to the same reaction sequence involving the cleavage of the chiral auxiliary and conversion to the bis-iminoester **48**. This bis-iminoester was then rearranged by a hetero-Cope mechanism to the corresponding acetamide **49** in refluxing xylene with conservation of

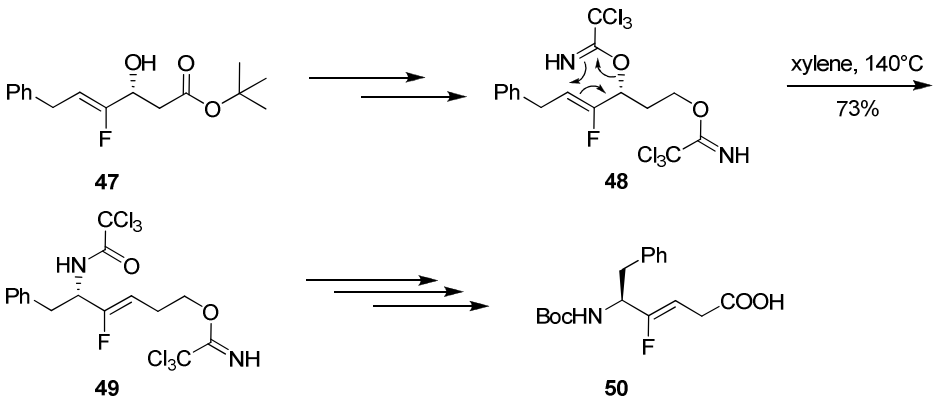
the chirality. The end of the synthesis consisted of adequate deprotection, protection and Jones oxidation affording **50** (Scheme 12).



Scheme 10: First stereocontrolled synthesis of trans-alkene dipeptide isosteres

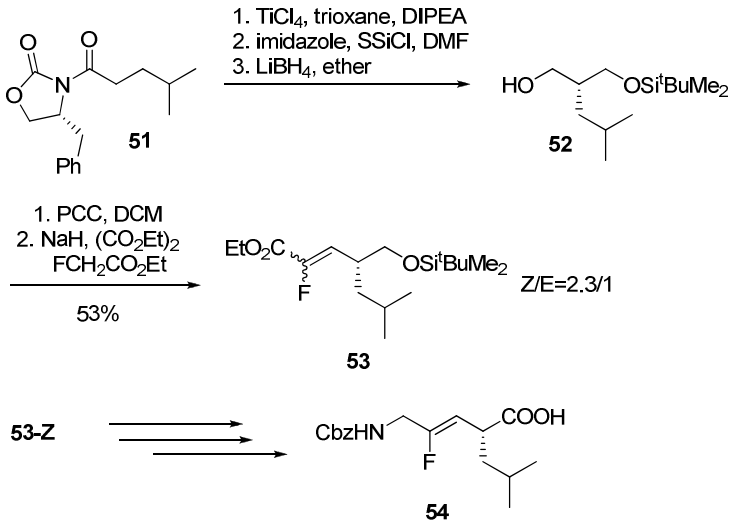


Scheme 11: Synthesis of chiral precursors of fluoroolefin isosteres



Scheme 12: Enantioselective synthesis of fluoroolefin dipeptide isosteres

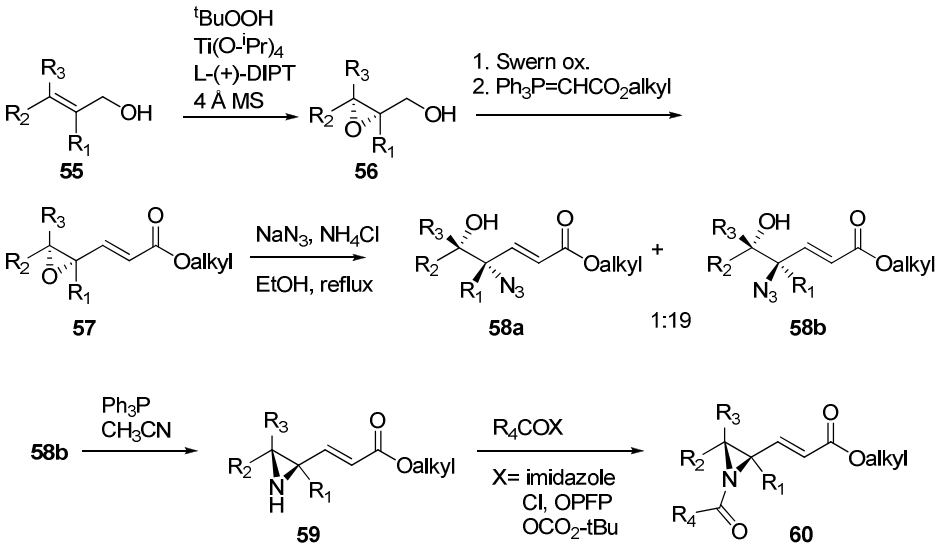
Bartlett and Otake have also reported the synthesis of a fluoroalkene. Starting from an optically pure oxazolidinone **51**,[28] they prepared an enantiopure silyl alcohol **52** which was then oxidised and condensed with diethyl fluorooxaloacetate to give the fluoroacrylate **53** in a 2.3:1 mixture of *Z:E* isomers. After separation, the *Z*-isomer could be aminated, reduced to an allylic amine and protected to afford the Cbz protected amino group. Deprotection of the silyl ether and further oxidation of the alcohol afforded the desired Cbz protected fluoroalkene dipeptide isostere **54** (Scheme 13).



Scheme 13: Synthesis of fluoroalkene dipeptide isostere

Wipf et al. have been examining the preparation of chiral aziridines and have reported an elegant method for the preparation of a variety of polysubstituted (*E*)-alkene isosteres by a cuprate-based approach.[29] Starting from a substituted allylic alcohol **55**, they could synthesize enantiomerically pure epoxy alcohols **56** by Sharpless asymmetric epoxidation in the presence of (+)-diisopropyl tartrate. The epoxy alcohol was then submitted to a Swern oxidation followed by a Wittig chain extension. The epoxide ring was then selectively opened using sodium azide and ammonium chloride affording **58** in a high d.r. (19:1). A Staudinger reaction on the azido alcohol **58** allowed the formation of the aziridine **59** which was then *N*-acylated to give the desired alkenylaziridine **60** (Scheme 14).

Using this method, the authors prepared a variety of polysubtituted alkenylaziridines with overall yields ranging from 24 to 47% from epoxy alcohols. Formation of trans-olefin isostere was then realized by a cuprate-based $SN_{2'}$ reactions which, depending on the substrate and the cuprate used, led to α-alkylation and subsequent ring opening giving the desired dipeptide isostere **61** and other by-products, including the δ-amino acids **62** and **66** (Scheme 15).

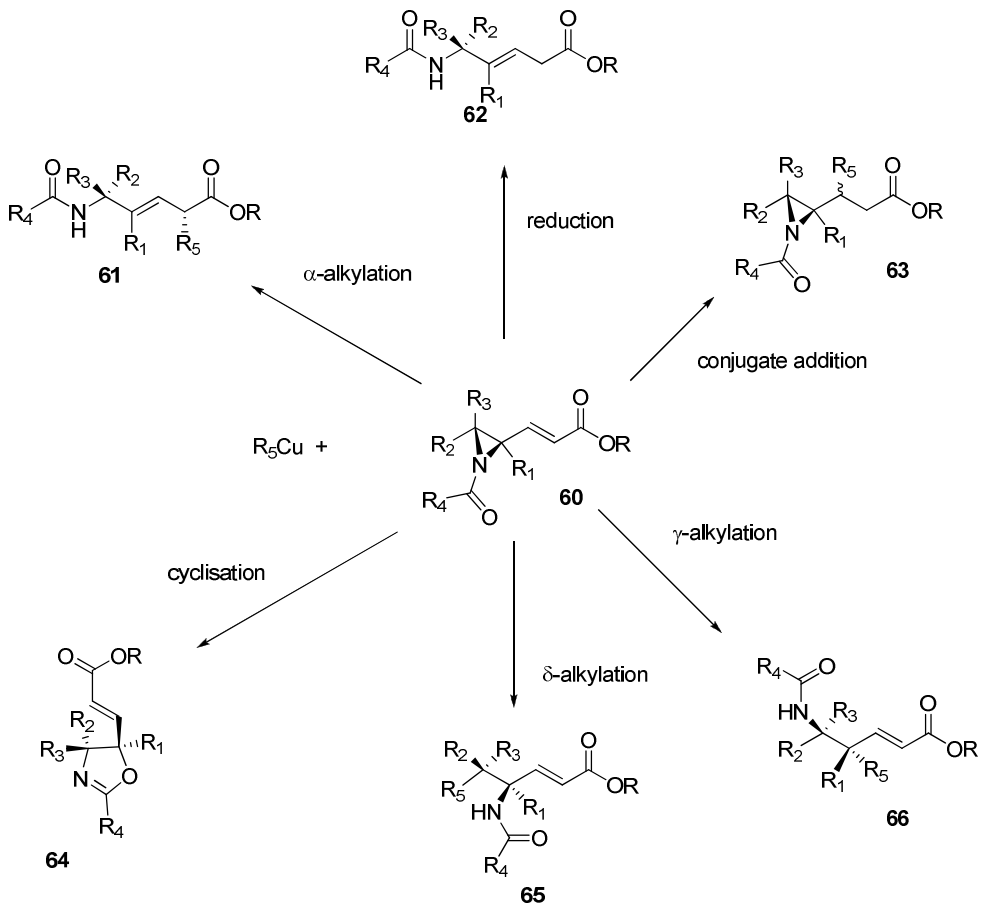


Scheme 14: Wipf's route for polysubstituted alkenyl aziridines

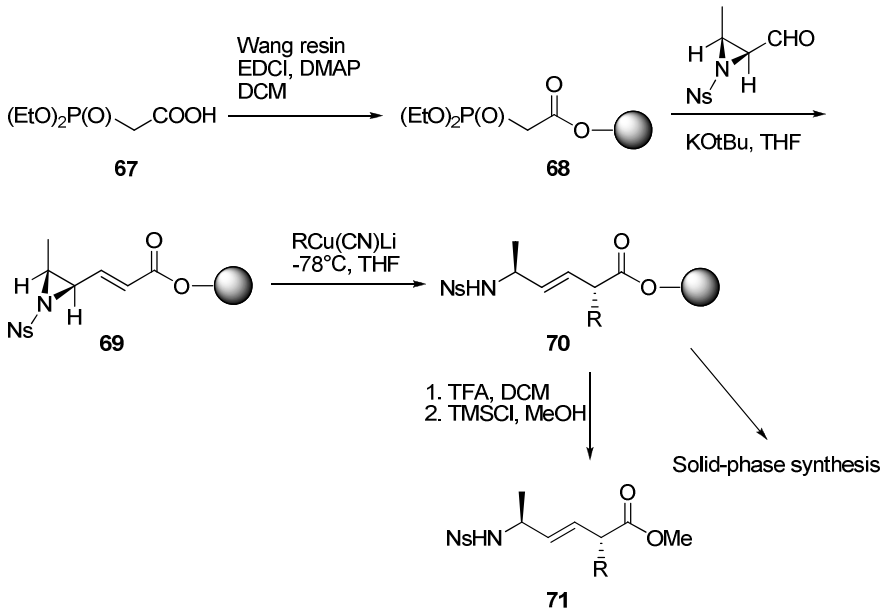
A few years later, the same group applied a similar methodology for a variety *E*-alkene dipeptide isosteres from alkenylaziridines utilising solid phase techniques. The δ-amino acid **70** synthesized could be cleaved from the resin to give **71**, or readily useable for peptide solid phase synthesis (Scheme 16).[30]

The authors also prepared, utilizing their aziridine method,[31] the optically pure (trifluoromethyl)alkene peptide isostere **79** and compared it with the non-fluorinated ones **74** and **83**. To do so, some α, δ- and α, γ, δ-substituted isosteres were synthesised by different methods having in common the use of chiral epoxides and aziridines (Scheme 17).

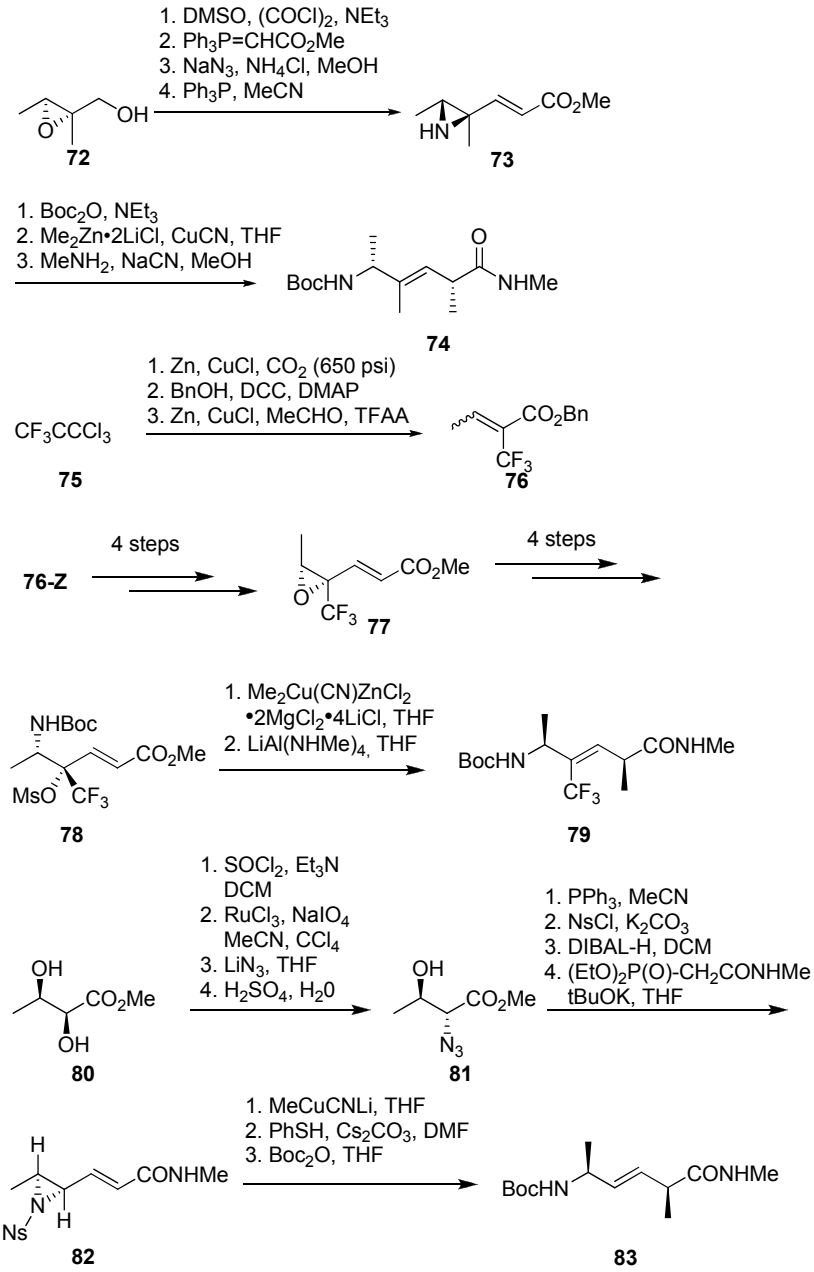
Embrey et al. developed a method for synthesising γ-alkylated (*E*)-olefin dipeptide isosteres using the oxazolidinone **84** as a starting material.[32] An asymmetric aldol reaction allowed for two chiral centers to be created in the presence of dibutylboron triflate and triethylamine in dichloromethane. After replacement of the oxazolidinone by a methyl ester with LiOOH and diazomethane, the alcohol functionality was converted to the acetimidate **87** with trichloroacetonitrile and DBU in DCM. A stereospecific [3,3] rearrangement of the acetimidate **87** in refluxing xylene resulted in the quantitative formation of the *E*-olefin backbone of the product **88**. The δ-amino acid **89** could be obtained by acetamide and ester hydrolysis and Boc-protection. The good overall yield and the synthesis' efficiency make this a good method for getting enantiomerically pure γ-substituted *E*-olefin isosteres (Scheme 18).



Scheme 15: Cuprate based reaction of Wipf leading to substituted (*E*)-alkene isosteres and by-products detected in the reaction

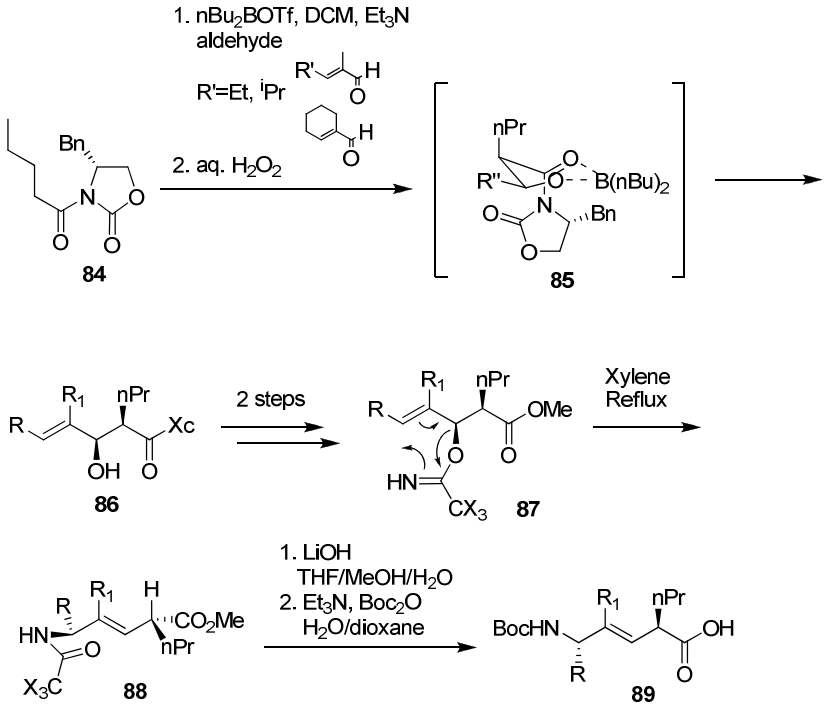


Scheme 16: Solid-phase synthesis of *E*-alkene isosteres derived from alkenylaziridines

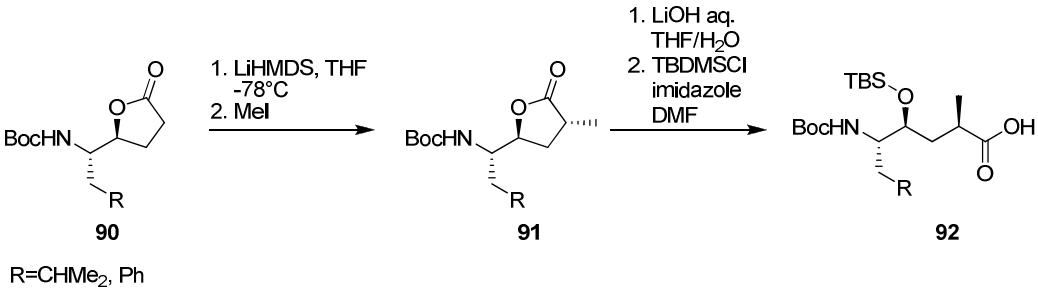


Scheme 17: Stereoselective syntheses of methyl and trifluoromethyl alkene isosteres

Ghosh et al. have prepared a trisubstituted δ-amino acid in few steps.[33] Starting from two differently substituted chiral γ-lactones **90**, a simple stereoselective alkylation by LiHMDS and methyl iodide in THF permitted the introduction of the third substituent with a high diastereoselectivity (d.r.>95%). Subsequent saponification of the lactone **91** and protection of the γ-hydroxy group with *tert*-butyldimethylsilyl chloride afforded the desired hydroxyethylene isostere **92** (Scheme 19).



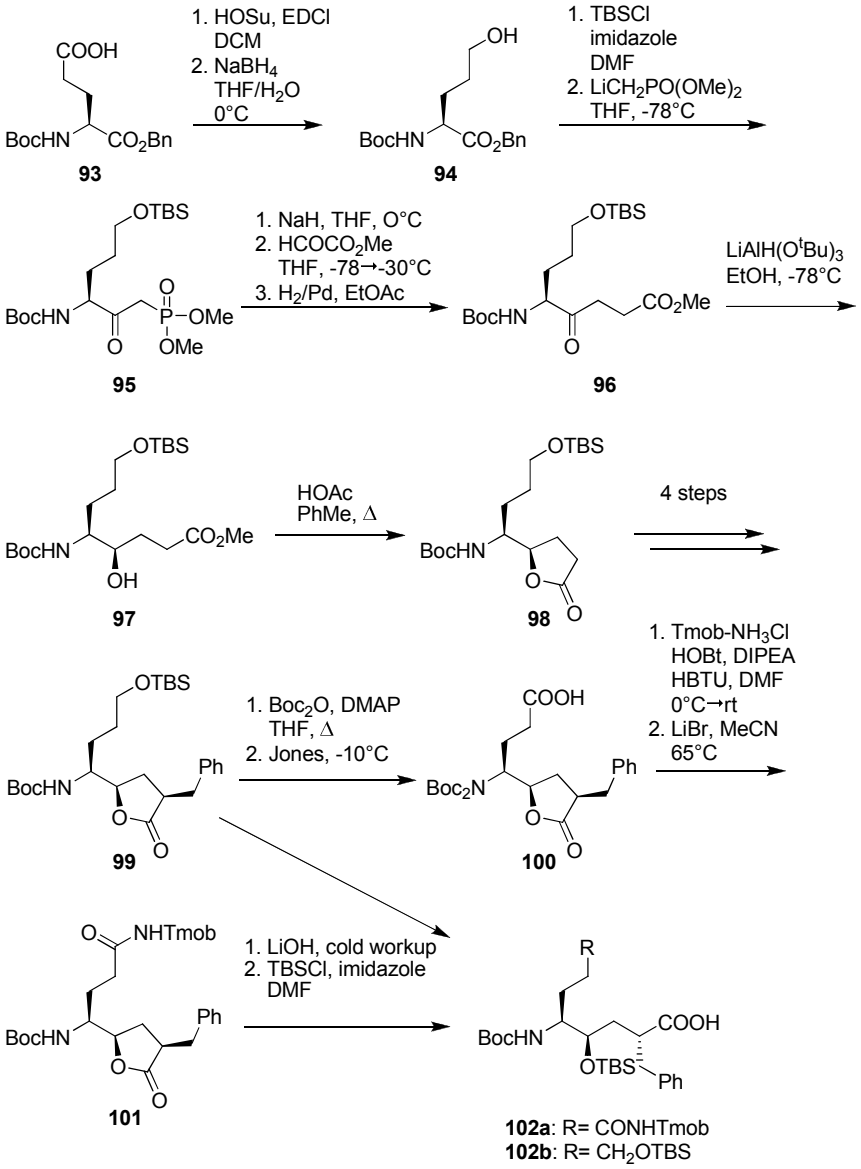
Scheme 18: Synthesis of chiral γ-substituted alkene dipeptide isosteres



Scheme 19: Synthesis of chiral trisubstituted δ-amino acid

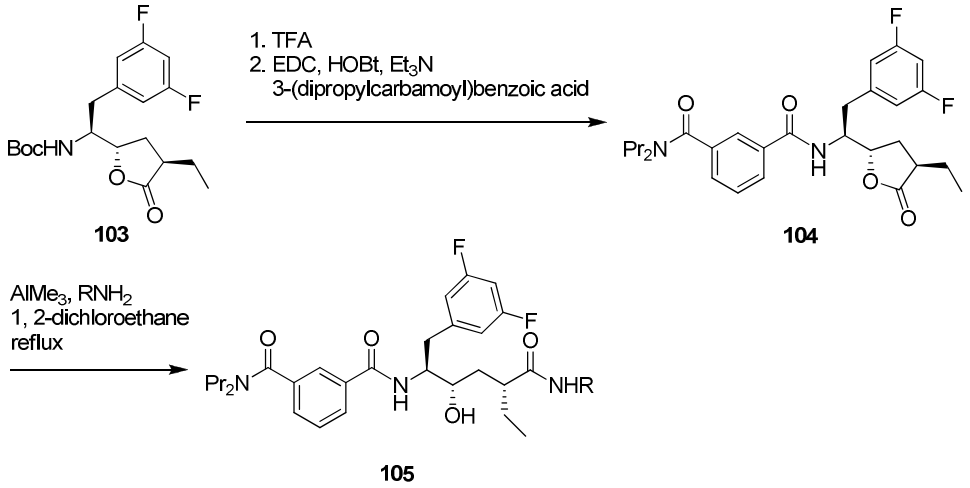
In 2004, Haug and Rich have published a long synthesis of Gln-Phe hydroxyethylene dipeptide isostere.[34] Utilising Boc-Glu-OBn **93** as a starting material, they prepared the succinimide ester derivative, which was then reduced to give the free hydroxylated product **94**. After protection with TBSCl, the reaction with lithio dimethyl methylphosphonate afforded the corresponding keto phosphonate **95** which was then utilised for a Horner-Wadsworth-Emmons reaction with methyl glyoxolate to give a mixture of *cis*- and *trans*-alkenes in a 2/3 ratio. Subsequent hydrogenation led to the saturated ketoester **96** in good yields. A stereoselective reduction of the ketone with $LiAlH(O^tBu)_3$ under chelation control conditions gave almost exclusively the anti-amino alcohol derivative **97** which was submitted to lactonisation with acetic acid in refluxing toluene and the expected separable lactone **98** could be obtained with only traces of its diastereomer. Attempts to alkylate the lactone with LDA and alkyl halides gave the undesired (2*S*)-diastereoisomer and another method was envisaged.

Thanks to an aldol-elimination-hydrogenation sequence, they could obtain the (2*R*)-diastereoisomer **99** and, as a consequence, all the stereocenters they desired were already introduced. Introduction of a second Boc group on the amine was required to avoid cyclisation or epimerisation of the substrate when submitted to a Jones oxidation which allowed the formation of the carboxylic acid **100** in place of the silyl ether. The amide functionality of Gln was then introduced by coupling with 2,4,6-trimethoxy benzylamine. Selective removal of one of both Boc groups could be performed by using LiBr affording **101**. The direct use of LiOH to hydrolyse the lactone was accompanied by Boc-cleavage and epimerisation of the α-carbon atom. Hydrolysis of the lactone **101** finally gave the expected Gln-Phe hydroxyethylene isostere **102a**. It is noteworthy that using the benzylated lactone intermediate **99** in the same conditions afforded a second hydroxyethylene dipeptide isostere **102b** correctly protected for peptide synthesis (Scheme 20).



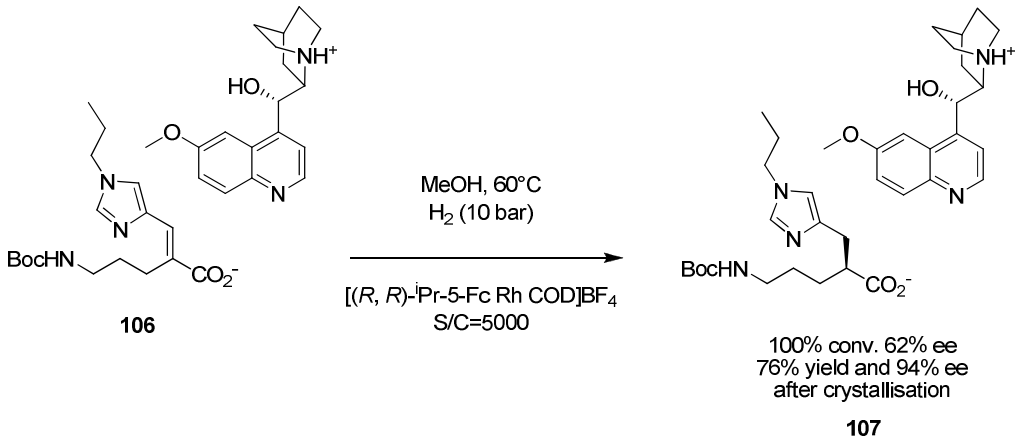
Scheme 20: Haug and Rich's synthesis

Hom et al. also prepared hydroxyethylene dipeptide isosteres containing fluorine.[35] They prepared the lactone precursor **103** starting from Boc-3,5-difluorophenylalanine and alkylated it by classical procedures as in the previous example in order to introduce various substituents. Once coupled to dipropylcarbamoyl benzoic acid, the lactone ring was opened with trimethyl aluminium and a primary amine affording the fluorinated hydroxyethylene dipeptide isostere **105** already coupled on its carboxy and amine ends (Scheme 21).



Scheme 21: Example of fluorinated hydroxyethylene dipeptide isostere

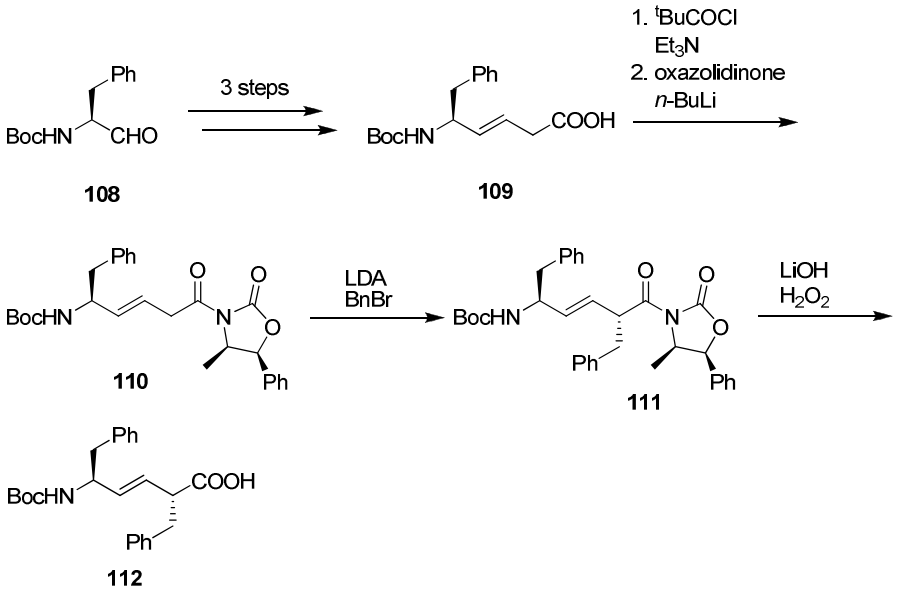
The interest for δ-amino acids is not only academic as even Pfizer has worked on a method for obtaining chiral imidazole substituted δ-amino acids.[36] After testing two routes (crystallisation and enantioselective hydrogenation) to obtain the desired enantiomer of **107**, they combined these two approaches to obtain **107** in high yields and high ee's. The best compromise found was the combination of a rhodium cyclooctadiene complex with a quinidine salt where a total asymmetric induction of with 62% ee was observed using only 0.02 mol% of catalyst (Scheme 22).



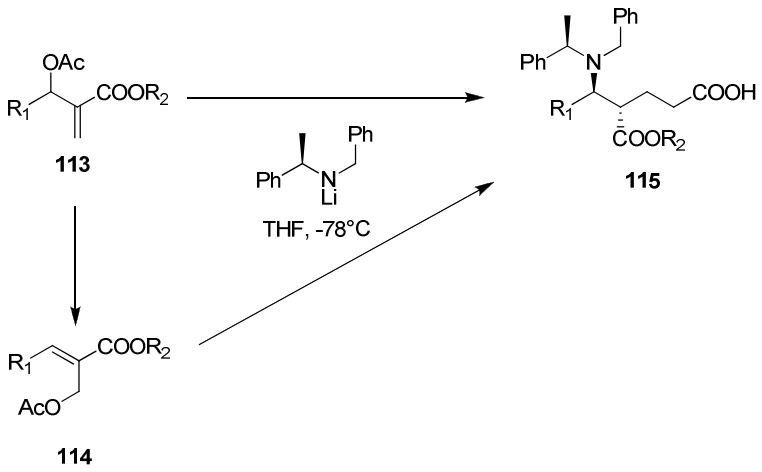
Scheme 22: Pfizer's asymmetric hydrogenation combined to crystallisation

In the synthesis of δ-amino acids, and particularly for dipeptide isosteres, the use of Evans oxazolidinone method has been widely accepted because of its efficiency and ease. Kelly et al. used it in 2005 [37] for preparing the Phe-Phe alkene dipeptide isostere **112**. Starting with the aldehyde **108** obtained from phenylalanine, a Wittig reaction followed by hydroboration afforded the *N*-Boc-Phe-Gly dipeptide isostere **109**. The acid, condensed with a chiral oxazolidinone, was diastereoselectively alkylated in an 11/1 d.r. and then, the oxazolidinone was cleaved to give the Phe-Phe alkene dipeptide isostere **112** (Scheme 23).

Urones et al. have recently developed an original way to access to highly optically active δ-amino acids.[38] Starting from a variety of acetylated Baylis-Hillman adducts **113**, they were reacted with a chiral lithium amide in THF at low temperature and the desired δ-amino acid **115** could be obtained in high diastereomeric excess (from 72 to 95%) (Scheme 24).



Scheme 23: Use of Evans oxazolidinone for chiral induction in alkene dipeptide isostere synthesis



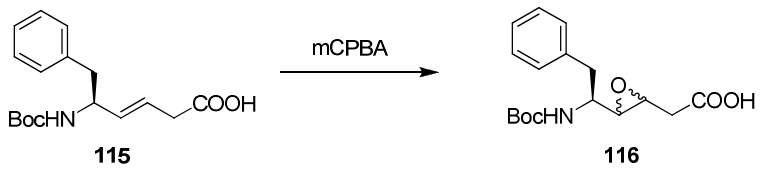
Scheme 24: δ-amino acid obtained by Ireland-Claisen rearrangement

2. Synthesis of cyclic δ-amino acids

The syntheses of linear δ-amino acids is very diversified as the methods and reactions used are very broad. The preparation of cyclic δ-amino acids has been investigated more thoroughly as their inner rigidity is very attractive for obtaining peptides presenting secondary structures. The synthesis of cyclic δ-amino acids also involves different types of chemistry and starting materials allowing the preparation of cyclic products with various ring sizes and conformations.

a. Three membered ring δ-amino acids

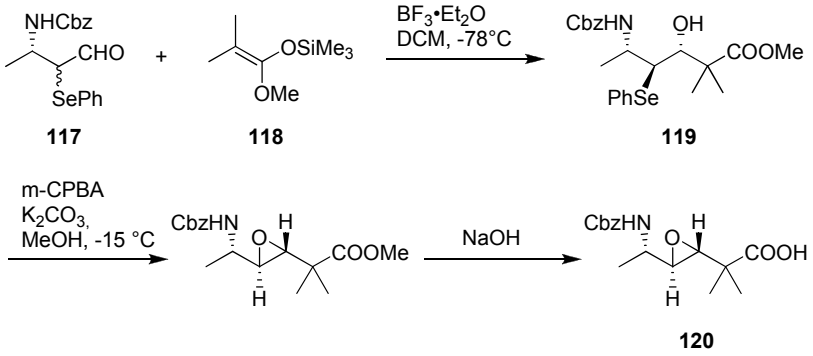
Starting from the linear δ-amino acid **115**, Kaltenbrom et al. prepared the three membered ring dipeptide isostere **116** as a mixture of diastereomers using an epoxidation with mCPBA (Scheme 25).[39]



Scheme 25: Synthesis of an epoxide δ-amino acid

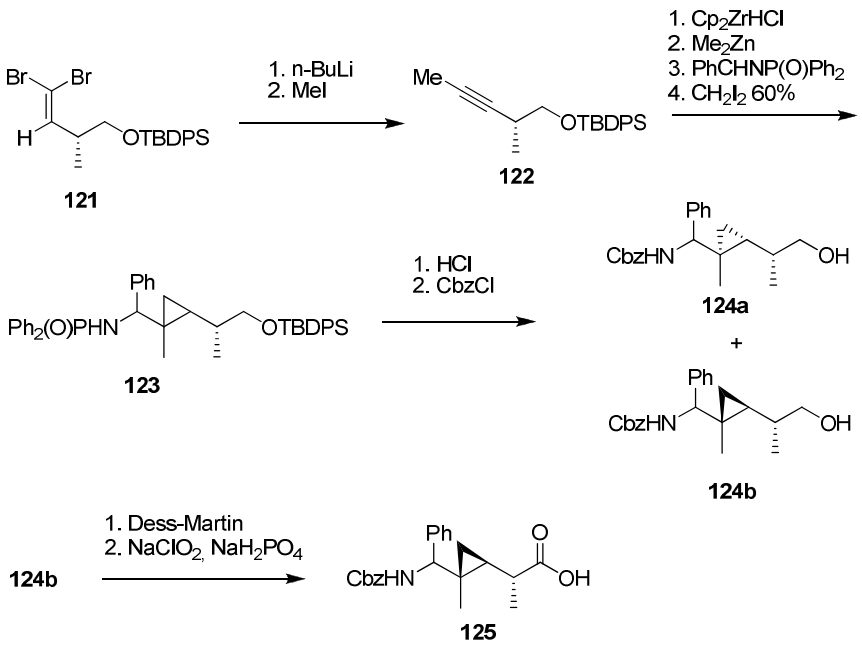
This first example gave rise to similar products having different substituents and diastereomeric ratios.[40-42]

In 1996, Mann published another method which had the advantage of affording the epoxide isostere in an enantioselective manner.[43] The synthesis involved the preparation of the intermediate **119** from the silylketene **118** and the chiral aldehyde **117** by a Mukaiyama aldol type reaction. The intermediate **119** could be cyclised with mCPBA and the ester saponified with sodium hydroxide affording the epoxy δ-amino acid **120** as a single diastereomer (Scheme 26).



Scheme 26: Enantioselective synthesis of an oxirane δ-amino acid

Wipf et al. also contributed to the synthesis of three-membered ring δ-amino acids by preparing the cyclopropane amino acid **125**.[44] The methyl alkyne **122** was hydrozirconated with Cp_2ZrHCl, transmetallated to Me_2Zn and added to (diphenylphosphinylimino)phenylarene and the resulting allylic phosphamide was cyclopropanated to give the cyclopropane **123**. N- and O-deprotection followed by Cbz protection of the amine afforded the alcohols **124a** and **124b** as a separable mixture of diastereomers. The OH group of **124b** was oxidised to the carboxylic acid functionality to give the δ-amino acid **125** (Scheme 27).

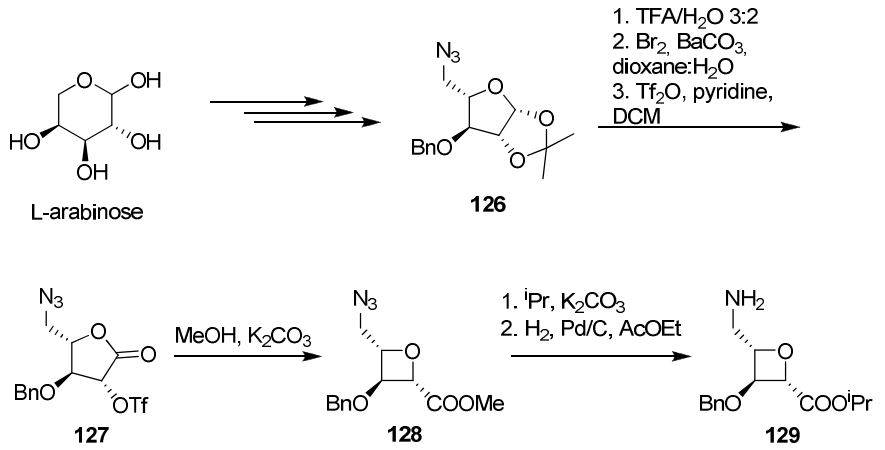


Scheme 27: Wipf's route to cyclopropane δ-amino acids

b. Four-membered ring δ-amino acids

Very few examples of four-membered ring δ-amino acids can be found in the literature. In this field, the Fleet group has been very prolific and has developed syntheses starting from naturally occuring and inexpensive sugars like rhamnose and arabinose. The advantage of using sugars as starting materials is that complex products can be accessed in a few steps. As an example, they recently prepared the 2,4-*cis*-oxetane δ-amino acid from L-arabinose.[45] The key step involved the ring

closure of the lactone **127** in basic conditions to obtain the oxetane azide methyl ester **128** which could be simply trans-esterified and reduced to afford the corresponding amino acid **129** (Scheme 28).



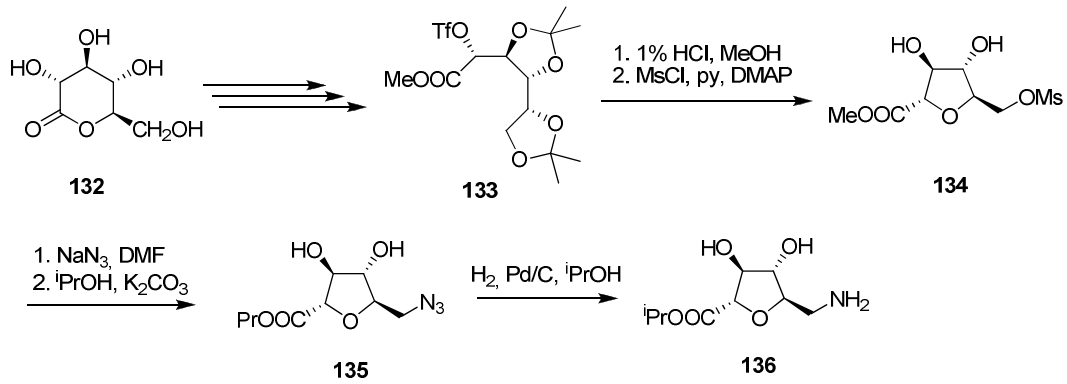
Scheme 28: Fleet's synthesis of oxetane δ-amino acids

Using the same procedure, they could prepare various substituted oxetane amino acids starting with L-rhamnose or D-xylose (Scheme 29).[46,47]

Scheme 29: Oxetane δ-amino acids obtained from L-rhamnose and D-xylose

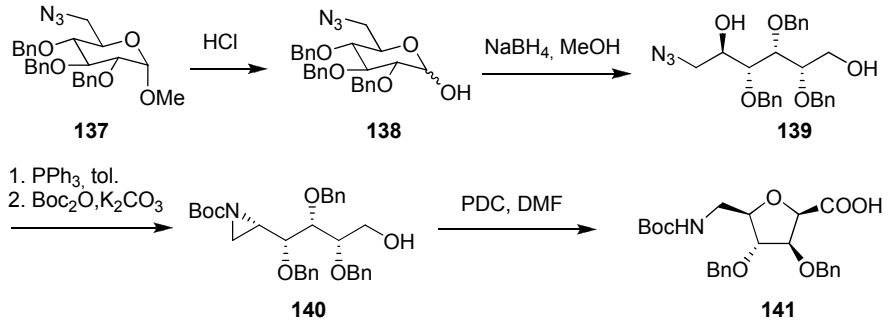
c. Five-membered ring δ-amino acids

In 1999, Smith et al. published the synthesis of the tetrasubstituted tetrahydrofuran δ-amino acid **136**.[48] The treatment of the open chain triflate **133** with HCl in methanol and subsequent protection of the hydroxyl group afforded the substituted furan product **134** which, after substitution of the mesylate with sodium azide and reduction, was converted to the cyclic *trans*-δ-amino acid **136** (Scheme 30).



Scheme 30: Synthesis of tetrasusbtituted tetrahydrofurane δ-amino acid

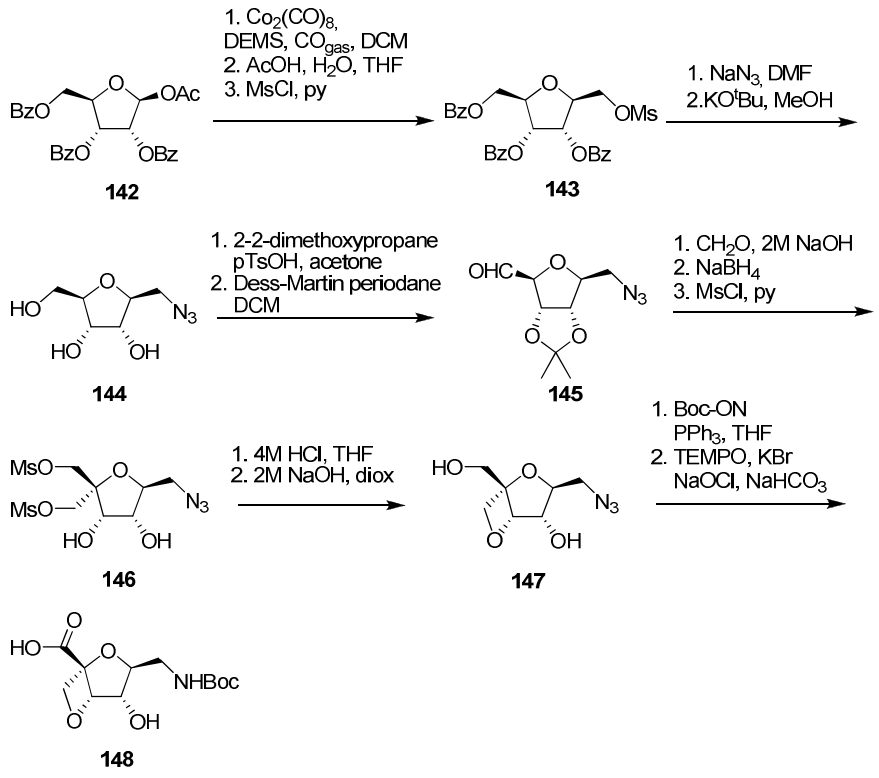
Chakraborty et al. published another synthesis of a furan amino acid using the hexose **137** as a starting material.[49] The key step of the synthesis was the cleavage of the sugar ring and formation of a terminal aziridine which could then be cyclised selectively affording the N-protected furan amino acid **141** (Scheme 31).



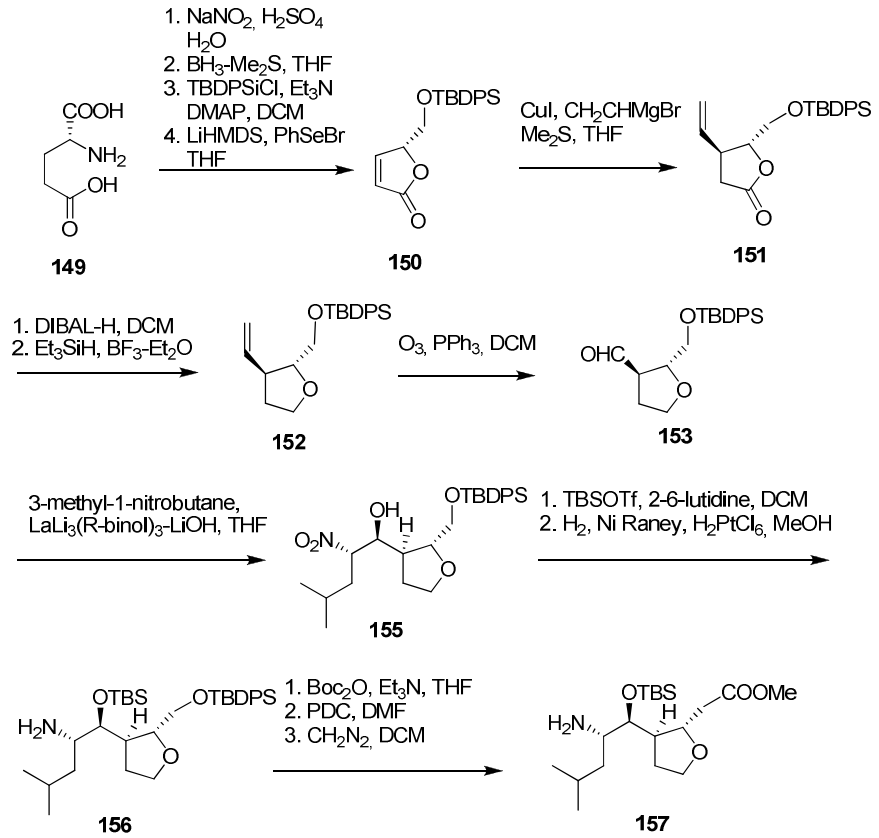
Scheme 31: Synthesis of furanoid δ-amino acid by Chakraborty

Van Well et al. synthesised a locked furan amino acid by the regioselective ring closing of the dimesylated compound **146** in basic conditions after prior deprotection.[50] The final Staudinger reaction and TEMPO oxidation afforded the locked furanoid δ-amino acid **148** (Scheme 32).

In 2004, Hanessian and Brassard[51] published the synthesis of the *trans*-furan δ-amino acid **157** by preparing the dehydrobutyrolactone **150** which was selectively vinylated and reduced to the corresponding furan **152** with DIBAL-H. Ozonolysis and diastereoselective nitroaldol reaction afforded the compound **155** which was protected and reduced before oxidation of the TBDPS protected alcohol and esterification with diazomethane to afford the cyclic hydroxyethylene isostere **157** (Scheme 33).



Scheme 32: Synthesis of locked furan amino acid

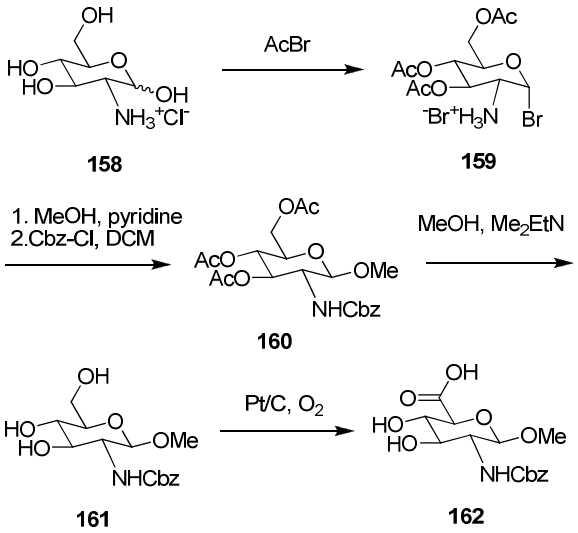


Scheme 33: Hanessian synthesis of a hydroxyethylene dipeptide isostere

d. Six-membered ring δ-amino acids

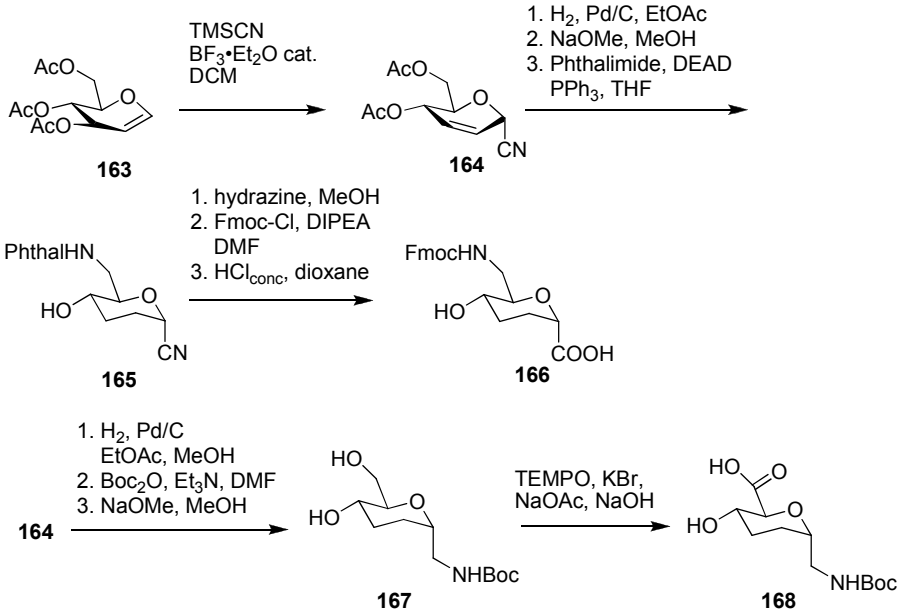
The syntheses of six-membered ring δ-amino acids use in many cases natural sugars as starting materials due to their large diversity which permits the synthesis of highly functionalised amino acids.

Graf von Roedern et al. synthesised a pyranoid δ-amino acid using glucosamine **158** as a starting material,[52] in which the amino functionality, after α-bromination of the anomeric hydroxyl group and concomitant acetylation of the other hydroxyl groups, was transformed to the β-methoxy NHCbz derivative **160**. The deprotection of the hydroxyl groups and oxidation of the C6 alcohol afforded the cyclic δ-amino acid **162** (Scheme 34).



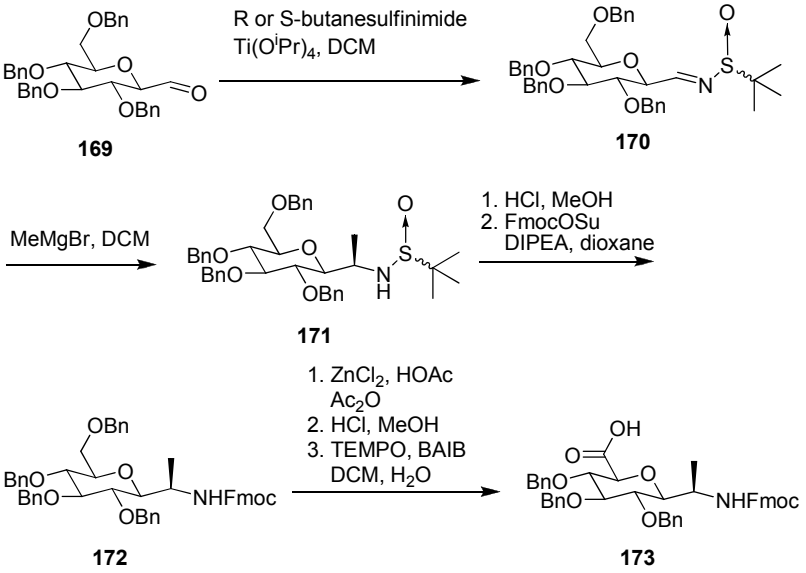
Scheme 34: Synthesis of a pyranoid δ-amino acid

In 1999, Overkleeft et al. published the synthesis of a sugar δ-amino acid starting from 3,4,6-tri-O-acetyl-D-glucal **163**.[53] It was subjected to a Ferrier rearrangement with trimethylsilyl cyanide and a catalytic amount of BF_3•Et_2O that gave a separable mixture of cyanides. The major isomer **164** could be used in two different reaction sequences affording the amino acids **166** and **168** which were opposite δ-amino acids regarding the positions of the carboxylic acid and amine moieties (Scheme 35).



Scheme 35: Syntheses of two opposite δ-amino acids from the same starting material

Risseeuw et al. proposed in 2007 an original synthesis of an alkylated sugar δ-amino acid.[54] The synthesis started with benzyl protected formyl glucopyranose **169** which, after reaction with (*R*)- or (*S*)-butanesulfinimide in the presence of $Ti(O^iPr)_4$ and subsequent alkylation with MeMgBr afforded the adduct **171** with an excellent diastereoisomeric excess. Hydrolysis with HCl in methanol and Fmoc protection of the amine followed by the selective deprotection of the OH on the C6 and TEMPO oxidation yielded the new sugar δ-amino acid **173** which had the unusual feature of having a chiral centre in the δ-position (Scheme 36).



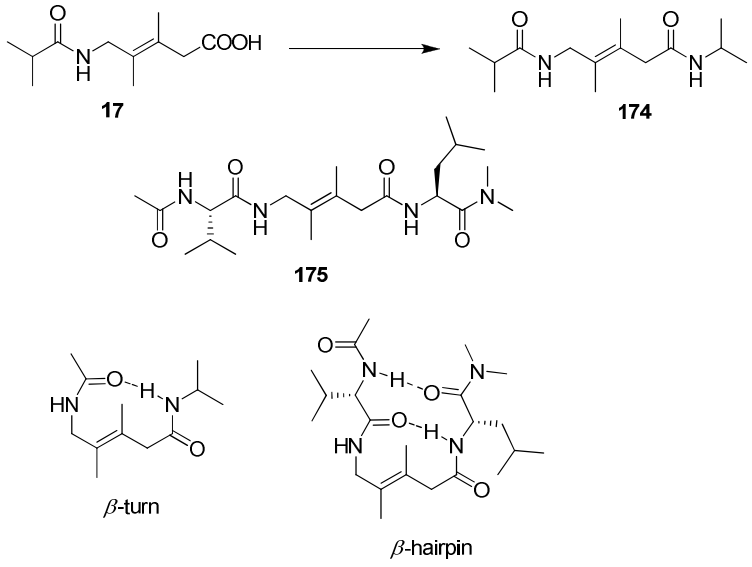
Scheme 36: Synthesis of alkylated sugar δ-amino acid

III. δ-amino acis in foldamers

The major application of δ-amino acids can be found in the synthesis of inhibitors since their backbone corresponds to an α-dipeptide and their introduction into peptidomimetics as ground state analogues in order to inhibit specific targets has been widely investigated. They have found applications as enkephalin analogues[14,15,50,55,56], human β-secretase inhibitors[24,33,35,57,58] as well as in the very potent non peptidic renin inhibitor Aliskiren® [59] developed by Novartis which has received the approval of the U.S. Food and Drug Administration for the treatment of hypertension. Most of the time, the δ-amino acids inserted in inhibitors are placed in strategic domains where they do not participate in the secondary structure of the peptidomimetic as their role is limited to inhibiting the cleavage by a diverse array of enzymes.

Nevertheless, δ-amino acids have a great potential for secondary structure induction for the same reasons as they have been used in inhibitors: their backbone mimics α-dipeptides and they may offer folding properties similar to α-peptides. This potential has been described computationally by Hofmann et al. who calculated the possible secondary structures that could be adopted by δ-amino acids.[60] The calculations revealed that homopeptides of δ-amino acids could adopt a large variety of helical structures presenting 8-, 10-, 14-, 16-, 20- and 22-membered rings closed by hydrogen bonding. In addition, δ-amino acids can induce β-turns, especially the cyclic analogues which confirmed the importance of a blocked conformation of the δ-amino acid for the stabilisation of secondary structures.

Gellman et al., who prepared the alkene dipeptide isostere **17**, investigated its secondary structure induction ability by preparing the protected monomer **174** and the tripeptide **175**. NMR and IR spectroscopic studies in solution showed that both formed a β-hairpin resulting from a ten-membered ring and that for **175**, the flanking α-amino acids were engaged in hydrogen bonding as well, therefore initiating a β-sheet (Scheme 37).[19]

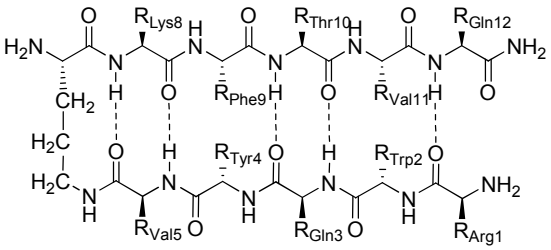


Scheme 37: β-turn and β-hairpin induced by the alkene isostere **17**

Still using alkene dipeptide isosteres, they have observed analogous features with a range of tetrasubsituted alkene isosteres and non-substituted dipeptide isosteres.[20] It was proposed that the hairpin structures are stabilised by the avoidance of allylic strain compared to the non substituted analogues which exhibited less stable structures.

Ornithine is a natural linear α-δ-diamino acid and Nowick and coworkers have prepared hairpin mimicks using it as a δ-amino acid.[61] It was able to induce a β-hairpin in the linear peptide **176** as well as in the cyclic peptide **177** which was closed with a second ornithine building block (Scheme 38).

Still utilising peptides containing δ-ornithine that induced β-hairpin formation, the same authors were able to mimic quarternary structures. These quaternary structures could be adopted by a family of linear and cyclic peptides.[62,63] Peptide **179** was able to form a β-sheet that could dimerise with another β-sheet and the cyclic peptide **180** could as well form dimers of β-sheets that could organise in a quaternary structure by formation of tetramers (Scheme 39).



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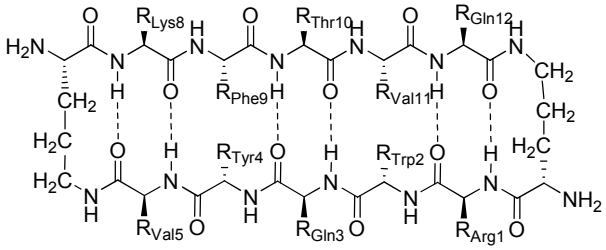
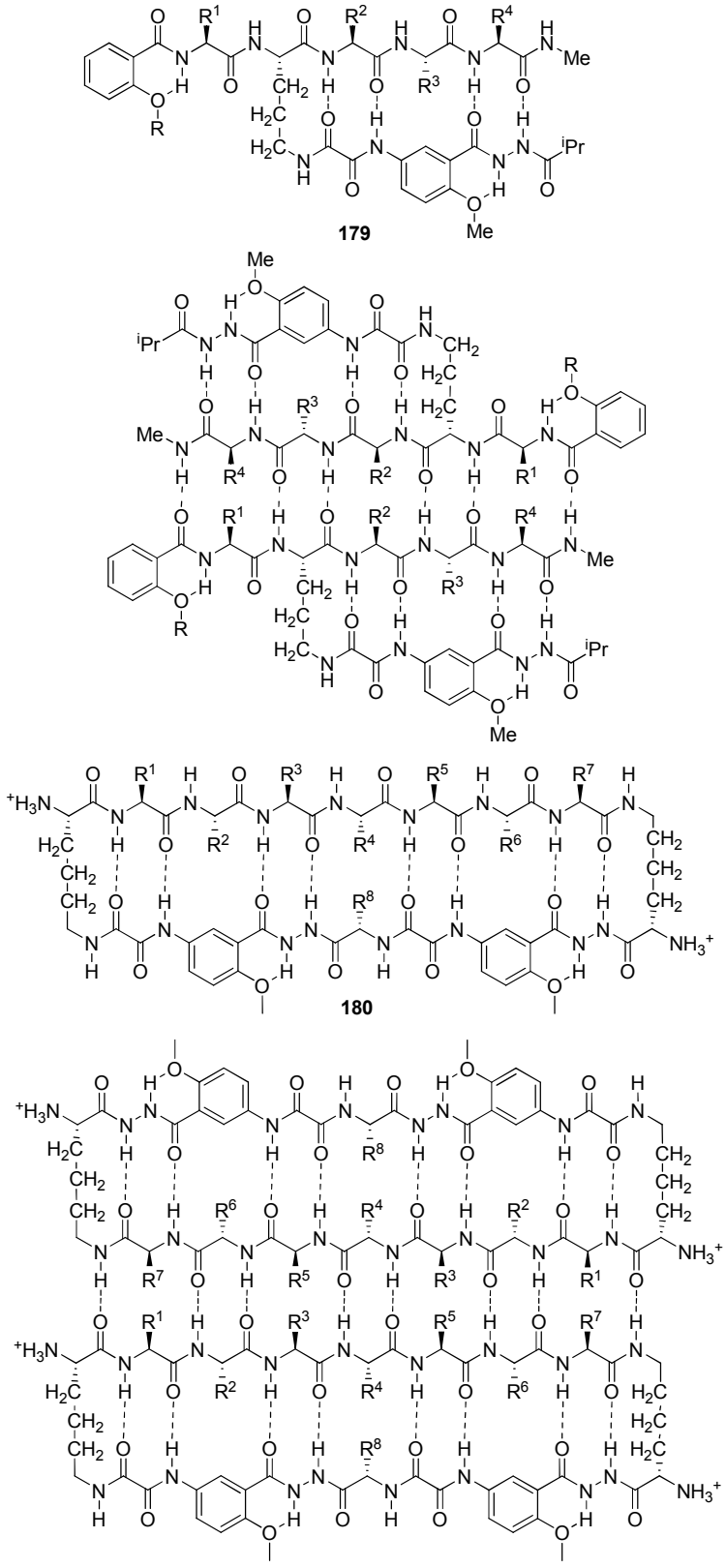


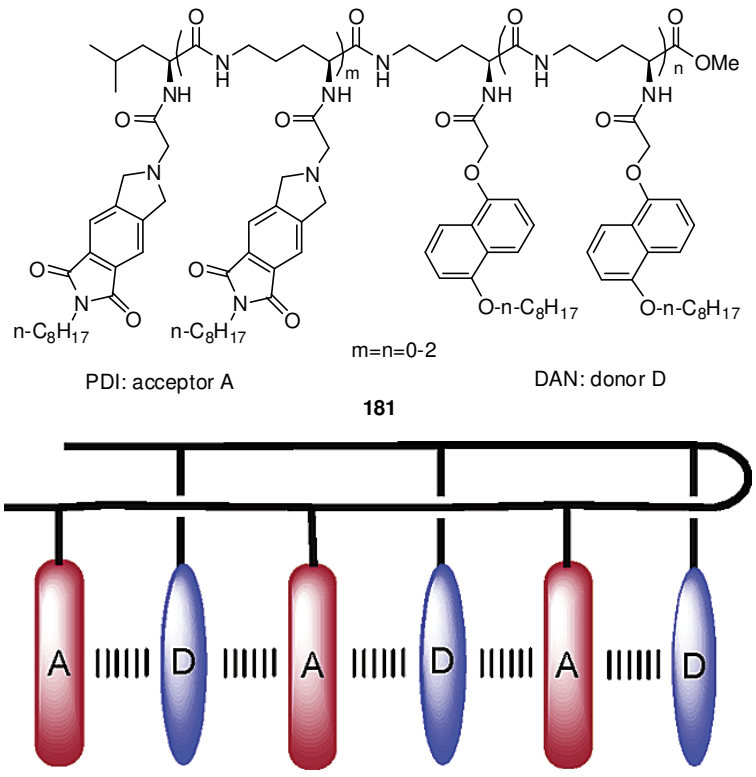
178

Scheme 38: β-hairpin structures based on δ-ornithine

Zhao et al. reported the folding of δ-peptides driven by donor-acceptor interactions.[64] The peptide was composed of L-ornithine which was substituted either by a donor substituent or an acceptor substituent. The peptide could adopt a zipper-featured foldamer-like structure since the secondary structure was stabilised by π-stacking between the electron-rich 1,5-dioxynaphtalene (DAN) and the electron deficient pyromellitic diimide (PDI). The peptide **181** was named a zipper-featured foldamer as the appendages DAN and PDI linked to the α-NH of ornithine were arranged alternatively one between each other as in a zipper (Scheme 40).



Scheme 39: Nowick's peptides forming β-sheet dimers

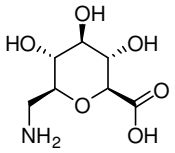


181

Scheme 40: Zipper featured δ-peptide (Reproduction by courtesy o Dr Zhan Ting Li)

Sugar amino acids have also found many applications as their rigid structure, when compared to linear δ-amino acids, is more prone to stabilise secondary structures.

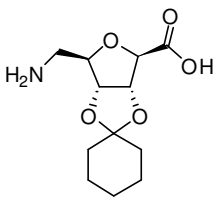
Kessler's group has investigated the potential of sugar amino acids in inducing secondary structures and have showed that the sugar δ-amino acid **182**[65] could induce a flexible β-turn (Scheme 41).[66]



182

Scheme 41: Sugar δ-amino acid inducing a β-turn

Fleet's group has also prepared a variety of sugar δ-amino acids that adopted secondary structures such as **183**, whose tetramer and octamer adopted a succession of β-turns.[67] The octamer of **184** induced a helical secondary structure with a repetition of 16-membered rings[68] and the tetramer and hexamer of the oxetane amino acid **129** exhibited a β-bend ribbon like structure[69] as well as did the tetramer of **185** (Scheme 42).[70]



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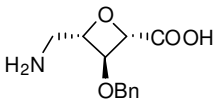

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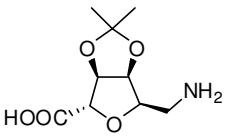

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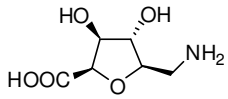
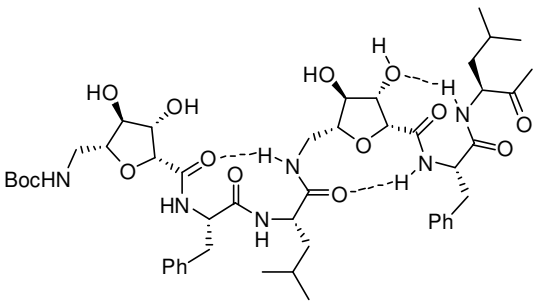


185

Scheme 42: Fleet's sugar δ-amino acids inducing diverse secondary structures

When introduced into mixed peptides, δ-amino acids have also shown a significant potential for inducing secondary structures as demonstrated by Chakraborty via the preparation of the short α-δ-peptide **186**. A succession of β-turns could be observed in addition to a 9-membered ring at the C terminal of the peptide between the terminal leucine and an OH group of the furanoid δ-amino acid.[71] This peptide had the ability of forming organogels in non-polar solvents and displayed interesting nanostructures (Scheme 43).



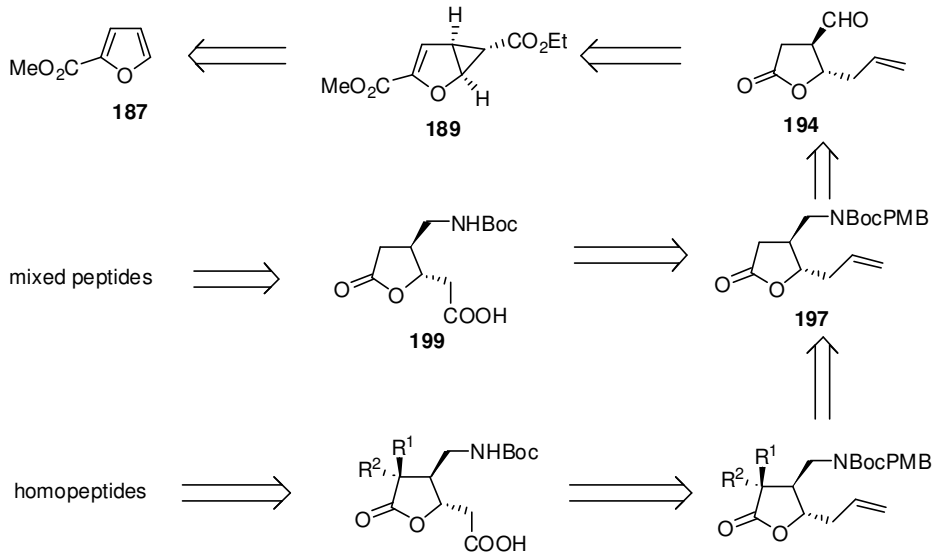
186

Scheme 43: Mixed α-δ-peptide presenting an alternation of β-turns

IV. Aim of this work

Previously in our group, a δ-amino acid based on a γ-butyrolactone ring was prepared and coupled to a tetramer, CD spectroscopy revealed that it could adopt a helical conformation but no other evidence could be collected, especially the 2D-NMR was not very well dispersed. The δ-amino acid can be obtained starting from the asymmetric cyclopropanation developed in our group. The cyclopropanated compound **189** is then submitted to ozonolysis and Sakurai allylation affording a chiral allylated cyclopropane alcohol. This compound can be lactonised very easily to form the

corresponding γ-butyrolactonaldehyde **192** on which is added a paramethoxybenzylamine group by reductive amination. After Boc protection, PMB cleavage and oxidation can be obtained the δ-amino acid **199** ready for peptide coupling. Alternative procedures for the synthesis of the building block have been investigated as well as its α-substitution in order to improve the NMR dispersion of the building block. The introduction of the building blocks into homopolymers and mixed peptides has been investigated for structural characterisation of the resulting peptides. The peptides' structures were characterised by NMR spectroscopy, CD spectroscopy, IR in solution and computational studies (Scheme 44).



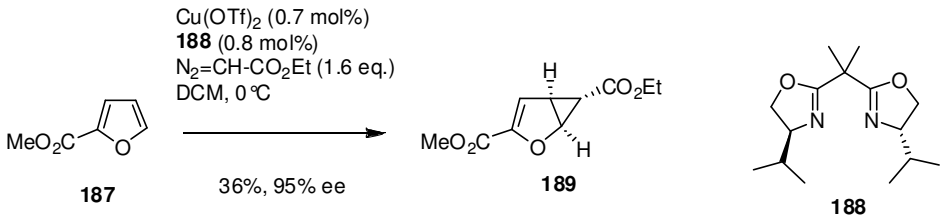
Scheme 44: Retrosynthesis of the building blocks and peptides syntheses

V. Synthesis of γ-butyrolactonaldehyde

1. Asymmetric cyclopropanation of furan methyl ester

Substituted cyclopropanes are an important class of compounds present in many natural products. They are also useful building blocks in organic synthesis as they can give access to diverse chiral compounds. Particularly, cyclopropanes vicinally substituted with donor and acceptor moieties can be very useful since they are subject to an easy ring opening giving rise to reactive intermediates that can be transformed into interesting compounds.

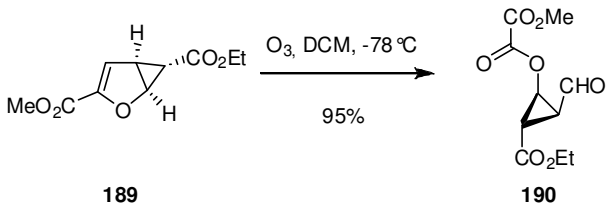
In our group has been developed the enantioselective cyclopropanation of furan methyl ester **187** catalysed by $Cu(OTf)_2$. The cyclopropanation of **187** with ethyl diazoacetate in the presence of a catalytic amount of $Cu(OTf)_2$, phenylhydrazine and the bis-oxazoline **188** occured regio- and enantioselectively.[72] The bis-oxazoline, complexed to the copper carbene, allows the approach of the substrate only by the less hindered double bond. Then, the positioning of the substrate is controlled by the steric hindrance of the isopropyl groups of the bis-oxazoline ligand and the cyclopropanation of the double bond is then directed almost only on one face of the substrate. The cyclopropanated compound **189** could be obtained in 36% yield and 95% ee. A single recrystallisation permitted to get the cyclopropanated furan methyl ester **189** in more than 99% ee. Despite a moderate yield, this reaction can be run on a 50 g scale of **187** and can afford in one reaction 30 g of **189** enantiomerically pure(Scheme 45).



Scheme 45: Cyclopropanation of furan methyl ester

2. Ozonolysis

Ozonolysis of **189** could be realised easily in dichloromethane at -78°C. After ozonolysis, dimethylsulfide was added and the flask was equipped with a drying tube to avoid entrance of water that would react with the peroxide intermediate and oxidise the aldehyde to the carboxylic acid. The reaction afforded **190** in 95% yield after simple extraction (Scheme 46).

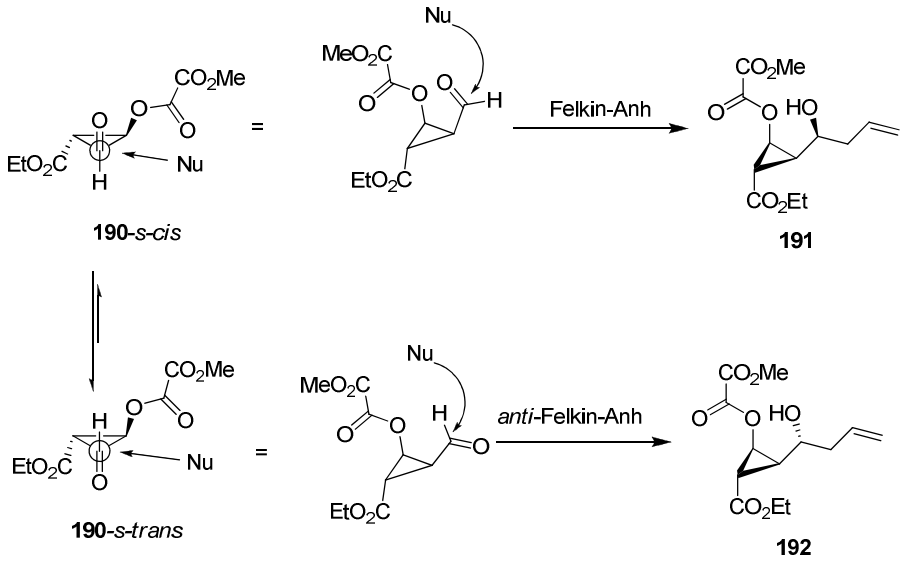


189 **190**

Scheme 46: Ozonolysis reaction

3. Sakurai allylation

The stereoselectivity of addition of nucleophiles to α-chiral carbonyl compound was first postulated by Cram[73] and its mechanism was improved by Felkin and Anh.[74,75] For stereoelectronic reasons, cyclopropyl-substituted carbonyl compounds are more stable in bisected conformations. In our case, two bisected conformations are possible: *s-cis* and *s-trans*. The *s-cis* conformation should be disfavoured because of steric interactions and the anti-Felkin-Anh product **192** should be obtained. But the resulting compound which was obtained was in fact the Felkin-Anh product **191** and some interactions specific to our product would explain the higher stability of the *s-cis* compound. The reaction of **190** with $BF_3 \bullet Et_2O$ and allytrimethylsilane afforded quantitatively the allylated cyclopropane alcohol **191** in a 95/5 ratio (Scheme 47).



190-*s-cis* **191**

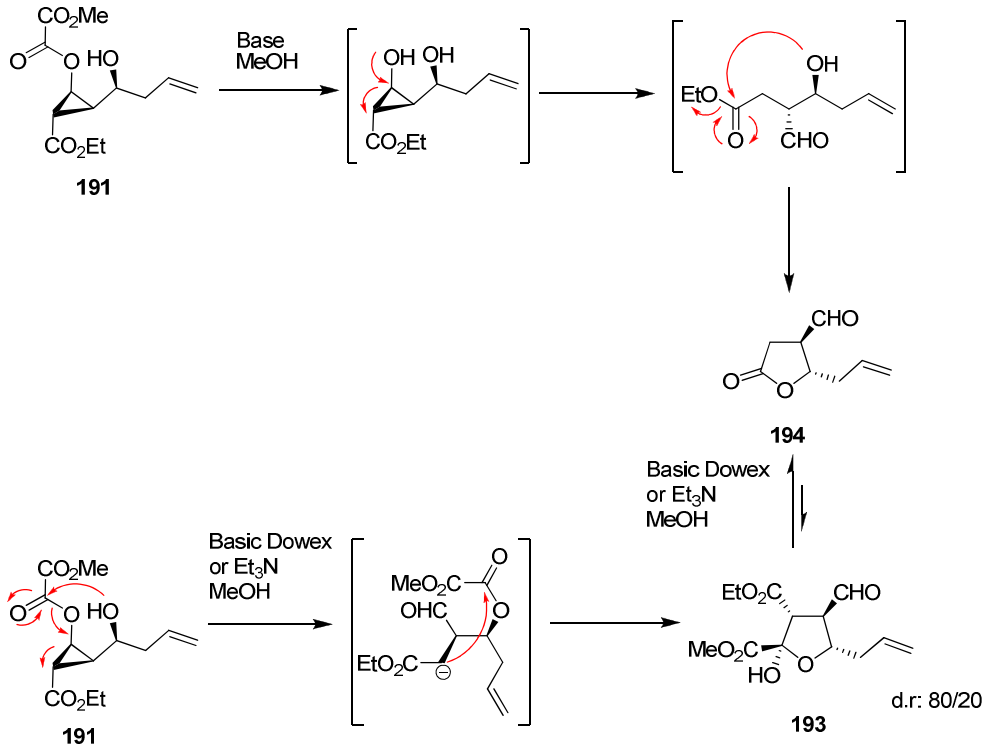
190-*s-trans* **192**

Scheme 47: Mechanism of the nucleophilic attack of an aldehyde

4. Retroaldol lactonisation

The cyclopropane compound **191** presents many interesting features for further synthetic transformations. The free OH group is located in γ-position relatively to the ethyl ester and the cyclopropyl-methyl oxalic diester should saponify easily under basic conditions. The retro-aldol lactonisation could be realised with barium hydroxide in methanol affording the γ-butyrolactonaldehyde **194** in good yields (60%) with the same diastereoisomeric ratio as the starting material. Nevertheless,

lower yields could be observed when the reaction was scaled up to a few grams. Moreover, the presence of barium in the separation mixture was creating emulsion problems and long extraction time. We investigated on using alternative to this procedure by using another base. The use of strongly basic Dowex resin in methanol increased the yield up to 75% on much higher scale (10 g) and with an easier workup as a simple filtration of the insoluble resin afforded the crude mixture whose solvent was evaporated and the residue immediately purified by column chromatography. The reaction time was shortened to two hours. Unfortunately, after having used intensively the resin, yields were decreasing and non negligible amounts of the product **193** were found. The compound **193** could be purified and fully characterised, it resulted from the rearrangement of the starting material to an acetal in a diasteromeric ratio of 80/20. The substitution of the Dowex resin by triethylamine could afford as well **194** in 70% yield on high scale (30 g of starting material) still without extraction. The product **193** could be reused for lactonisation with Dowex resin or Et_3N to form **194** but it was impossible to have a total conversion suggesting that the formation of **193** was a secondary reaction in equilibrium with the lactonisation process (Scheme 48).

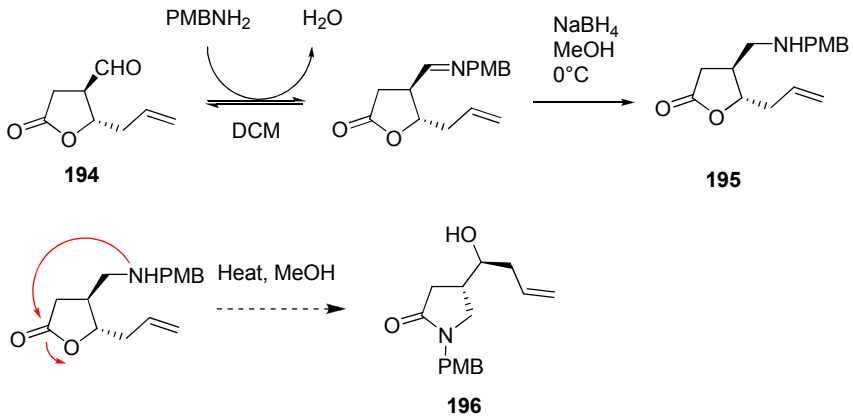


Scheme 48: Lactonisation mechanism and secondary reaction

VI. Synthesis of the δ-amino acid

1. Introduction of the nitrogen moiety by reductive amination

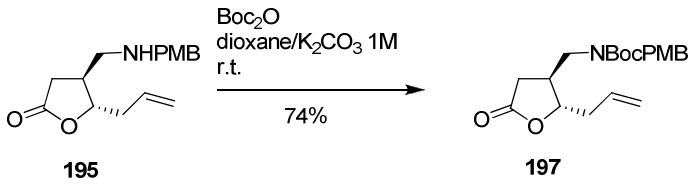
The reductive amination of aldehydes is a common reaction to create secondary amines. It can be used in order to insert a protected nitrogen which avoids difficult purification. There are very few efficient methods of reductive amination of lactonaldehydes.[76] The method involves the formation of the imine resulting from the condensation of the aldehyde with the amine, which is then reduced by $NaBH_3CN$[76], $NaBH_4$[77], $LiAlH_4$… The imination step releases water and there is an equilibrium between the aldehyde and the imine, trapping the water formed can help in having an almost total conversion to the imine, a drying agent can be used like activated molecular sieves 4Å or sodium sulphate. The reductive amination is usually realised in methanol as a proton source is required to complete the reduction. The reductive amination of **194** was first attempted with benzylamine but yields were not satisfying and para-methoxy benzylamine was used instead of benzylamine. The reductive amination in pure methanol did not afford good yields and the imination step was then performed in dichloromethane, methanol and $NaBH_4$ were added only at the reduction step. $NaBH_4$ was found to be more effective than $NaBH_3CN$ and was preferred for the reaction. The reduction step was performed at 0°C as the addition of MeOH and $NaBH_4$ in the reaction mixture is exothermic. Although good yields could be obtained on a 400 mg scale, the reaction showed variable yields sometimes affording much less product than expected. The scaling up of the reaction was giving even more unexpected yields. The isolation of a secondary product **196** could explain the versatility of the reaction. During the reduction step, the resulting secondary aminobutyrolactone **195** can rearrange easily to form the more stable γ-lactame **196** which has a similar NMR to the aminobutyrolactone **195** as well as their Rf are very close by TLC. After investigation, we came to the conclusion that molecular sieves in powder which were used as drying agent could make the yield drop dramatically when the reaction was scaled up by forming emulsion, Na_2SO_4 was then used to dry the reaction solution. Moreover, Na_2SO_4 was not provoking emulsion of the extraction solution. The reduction step time had to be reduced in order to avoid the spontaneous formation of the lactame **196** and that a column chromatography was not the best way to separate the aminobutyrolactone **195** from the lactam. A simple acidic and basic extraction could solve the purification problem and slow addition of methanol dropwise to the reaction mixture containing $NaBH_4$ avoided local heating in the solution forming the lactame. Thanks to these modifications, the reductive amination could be scaled up to a 2 gram scale with 70% yield (Scheme 49).



Scheme 49: Reductive amination mechanism and by-product isolated

2. Boc protection of the secondary amine

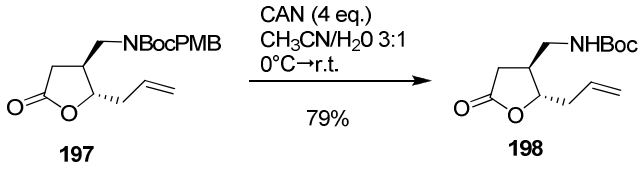
The tert-butoxy carbonyl group is an amine protecting group widely used in liquid phase peptide synthesis as it can be removed easily under acidic conditions. The Boc protection of **195** could be realised efficiently in a mixture of dioxane and a 1M solution of K_2CO_3 with Boc anhydride affording the Boc protected secondary amine **197** in 74% yield. (Scheme 50)



Scheme 50: Boc protection of the secondary amine

3. PMB group removal by cerium ammonium nitrate

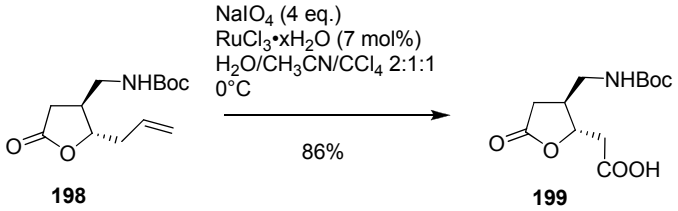
The PMB group can be selectively removed by oxidation with cerium ammonium nitrate in mild conditions by the method of Yoshimura[78,79] who published the optimal conditions for the removal of the PMB group using CAN. The efficiency of the deprotection is concentration dependant and optimal yields can be obtained using 4 equivalents of CAN at a concentration of 0.25 M in a mixture acetonitrile/water 3:1 at 0°C. In the same conditions, the PMB removal by CAN on **197** afforded the carbamate **198** in 79% yield (Sheme 51).



Scheme 51: Oxidative PMB removal by CAN

4. Oxidation of the allylic double bond

The allylic double bond could be transformed to a carboxylic acid by a ruthenium catalysed oxidative cleavage[80] with $RuCl_3 \cdot xH_2O$ (7 mol%) and $NaIO_4$ in a mixture Water/CH_3CN/CCl_4 2:1:1 at 0°C in two hours. The pure protected amino acid **199** was purified by a simple extraction with diethyl ether and washing with water. The reaction afforded **199** in 86% yield (Scheme 52).



Scheme 52: Oxidative cleavage of the allylic double bond

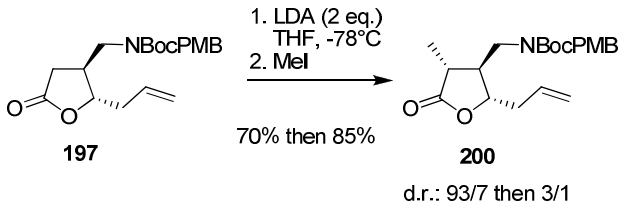
VII. Investigations in the α-substitution of the lactone ring

In order to get a better dispersion of the proton signals of our lactone amino acid, α-substitution was envisaged so that the signals would be splitted and the characterisation of the peptidomimetics facilitated. There are many examples of α-subtitution of lactones and we decided to prepare methyl and fluorine substituted lactones as the added functionality would not overlap the other signals.

1. Monomethylation of the lactone ring

Methylation of lactones is very common in organic synthesis as many examples of diastereoselective methylation of lactones can be found in the literature. The reaction is usually performed by deprotonation of an α-proton with strong bases like lithium diisopropyl amide (LDA) or lithium hexamethylsilylazane (HMDS) forming the lithium enolate which can be then methylated with methyl iodide. The methylation can be highly diastereoselective when realised on a chiral lactone having bulky substituents at low temperatures.[81-83] The methylation step was realised on the compound **197** as the fully protected nitrogen would not be methylated and the bulkiness of the substituents should help for getting a good diastereoselectivity. Our first attempts with LDA in THF at low temperature were successful as we could get the methylated lactone **200** in good yields (70%) and excellent diastereoselectivity (d.r.: 93/7) as our compound before methylation was in a 95/5 d.r. In addition, the NMR dispersion was very interesting for further peptide analyses. But the dry THF used was purified by a solvent purification system and some degradation of the solvent quality could be observed after a few months of service. The THF apparently contained traces of water and we had noticed a gel formation when the fresh LDA was added to the reaction mixture, probably coming from the presence of water. Moreover, two or three equivalents of LDA were required to complete the

reaction and yields were not as satisfying and it was decided to use freshly distilled THF. The first visible effect was that no gel formation occured in the reaction mixture and that only one equivalent of LDA was enough to complete the reaction in better yields (85%). But, when analysed by NMR, the diastereomeric ratio changed drastically from 93/7 to 3/1. The use of cosolvents like DMPU or quenching at low temperature did not improve the diastereoselectivity. When the SPS was fixed, the use of its THF led to the same d.r. of 3:1. Having the compound **200** in a moderate d.r. was not conceivable for further peptidomimetics conformational analyses and the monomethylation was given up (Scheme 53).

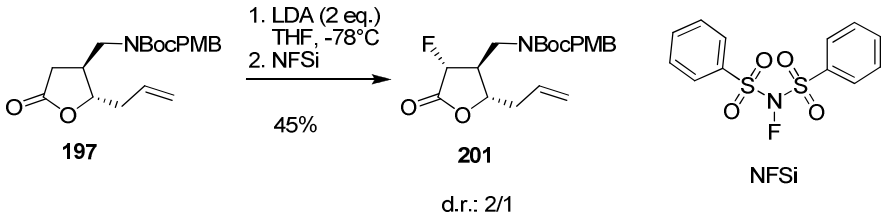


Scheme 53: α-methylation of the lactone ring

Nevertheless, our idea to substitute the lactone in α-position was strengthened as the NMR dispersion of the substituted product was good.

2. Fluorination of the lactone ring

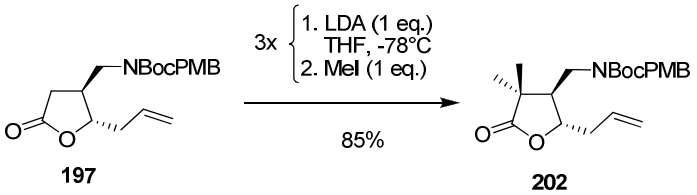
Fluorination of the lactone ring was envisaged as it was an attractive idea as we would benefit from an additional tool for analysing peptides by ^{19}F-NMR. Fluorine being very electronegative compared to a proton or a methyl carbon, the fluorination of lactones necessitates specific fluorinating reagents that can perform electrophilic fluorination like *N*-fluorodibenzene sulfonimide (NFSi) which is a source of electrophilic fluorine by *Umpolung*. A few examples of diastereoselective electrophilic fluorination of lactone[84] can be found and, usually, the reaction is realised with LiHMDS or LDA in THF at low temperatures as in the methylation case. The fluorination of **197** was performed in the same conditions as for the methylation and the monofluorinated compound **201** could be obtained in 45% yield. The ^{1}H-NMR showed a very nice dispersion and ^{19}F-NMR was used to determine the diastereomeric ratio and a set of two doublets of doublets could be observed. The signal was a doublet of doublet as the fluorine was coupling with the α-proton ($^{2}J_{H\alpha-F\alpha}$) and the β-proton ($^{3}J_{H\beta-J\alpha}$). The ratio of both dd was 2/1 meaning that the fluorination was not very diastereoselective and that we could not use it for peptide conformational analysis (Scheme 54).



Scheme 54: Electrophilic fluorination of the lactone ring

3. Dimethylation of the lactone ring

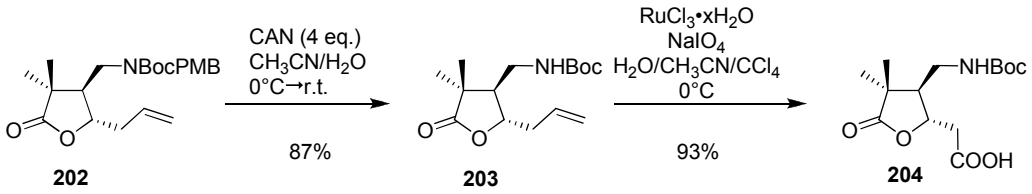
As it seemed difficult to have an excellent diastereoselectivity in monosubstituting the lactone **197**, we simply decided to dimethylate it as we knew that the methylation could give good yields. The lactone **197** was submitted twice to a deprotonation with one equivalent of LDA followed by addition of one equivalent of MeI. A third addition of LDA and addition of 3 equivalents of iodomethane was required to complete the reaction as some monomethylated compound was still present in the reaction mixture and the mono- and dimethylated compound were difficult to separate by flash chromatography. The dimethylated lactone **202** could be obtained in 82% yield and exhibited a very well dispersed NMR (Sheme 55).



Scheme 55: Dimethylation of the lactone

4. Synthesis of the dimethylated δ-amino acid

The dimethylated δ-amino acid was prepared by the same procedures as for the non-methylated lactone. The PMB removal afforded **203** in 87% yield and the ruthenium chloride catalysed oxidation yielded the Boc-protected δ-amino acid **204** in 93% yield. All of these steps were performed with better yields in comparison to the non-methylated lactone. The final δ-amino acid had a very interesting proton NMR and the synthesis of peptide based on the dimethylated and the non-methylated δ-amino acid could be envisaged (Scheme 56).



Scheme 56: Synthesis of the dimethylated δ-amino acid

VIII. Introduction of the δ-amino acid into peptides

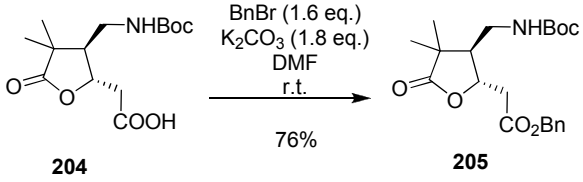
Our building blocks being *N*-Boc protected, the liquid phase strategy was chosen to prepare short peptides. The synthesis of homopeptides as well as mixed peptides was investigated to determine the secondary structure potential of our building block.

1. Synthesis of the homopeptide of dimethylated amino acid

The synthesis of the tetramer **208** based on the δ-amino acid was envisaged. A stepwise synthesis was chosen as the number of steps was the same compared to a fragment condensation of two dimers.

a. Benzyl protection of the δ-amino acid

The protection of the C terminus of **204** was required to initiate peptide elongation. The benzyl ester group is a convenient C protecting group as it can be added easily by reaction of the free carboxylic acid with benzyl bromide and potassium carbonate in DMF. The removal of the benzyl protecting group can be realised with hydrogen at atmospheric pressure with Pd/C in methanol. The benzyl protection of **204** in these conditions afforded the protected δ-amino acid **205** in 76% yield (Scheme 57).

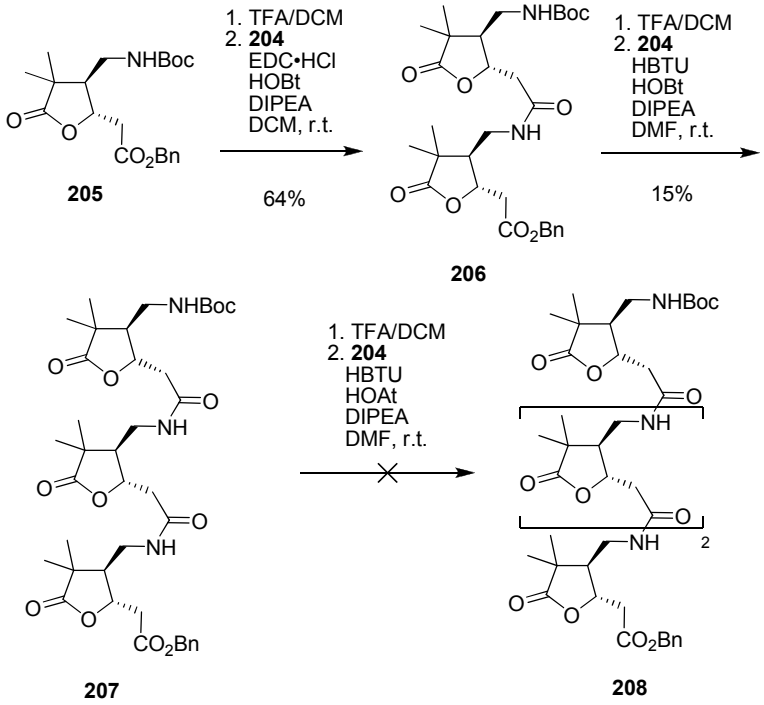


Scheme 57: Benzyl protection of the δ-amino acid

b. Synthesis of the δ-peptides

For the liquid phase synthesis, the benzyl protected fragment was Boc deprotected in TFA/DCM 1:1 affording the corresponding TFA salt after washing and drying. To this TFA salt was added the δ-amino acid **204** beforehand activated by coupling reagent like EDC•HCl or HBTU. The coupling of the TFA salt of **205** with **204** and HOBt and EDC•HCl in DCM to form the dimer **206**

proceeded well with 64% yield. The NMR of **206** was very nice regarding the dispersion and the coupling to prepare the trimer **207** was realised. At this point were encountered coupling difficulties. The coupling with EDC•HCl and HOBt did not work. Substitution of HOBt by HOAt did not afford the trimer as well. Dichloromethane was replaced by DMF and still the trimer could not be obtained. Only the use of HBTU and HOAt in DMF afforded the trimer **207** in low yield (15%). The trimer was particularly insoluble in most solvents and that may be a reason for the difficulties to obtain it. In the same conditions, the coupling of the trimer **207** with another amino acid **204** revealed even more difficult as a few milligrams of a white product were obtained but this was not the pure compound. LC/MS revealed the presence of the tetramer in the white residue but it was impossible to purify it completely (Scheme 58).

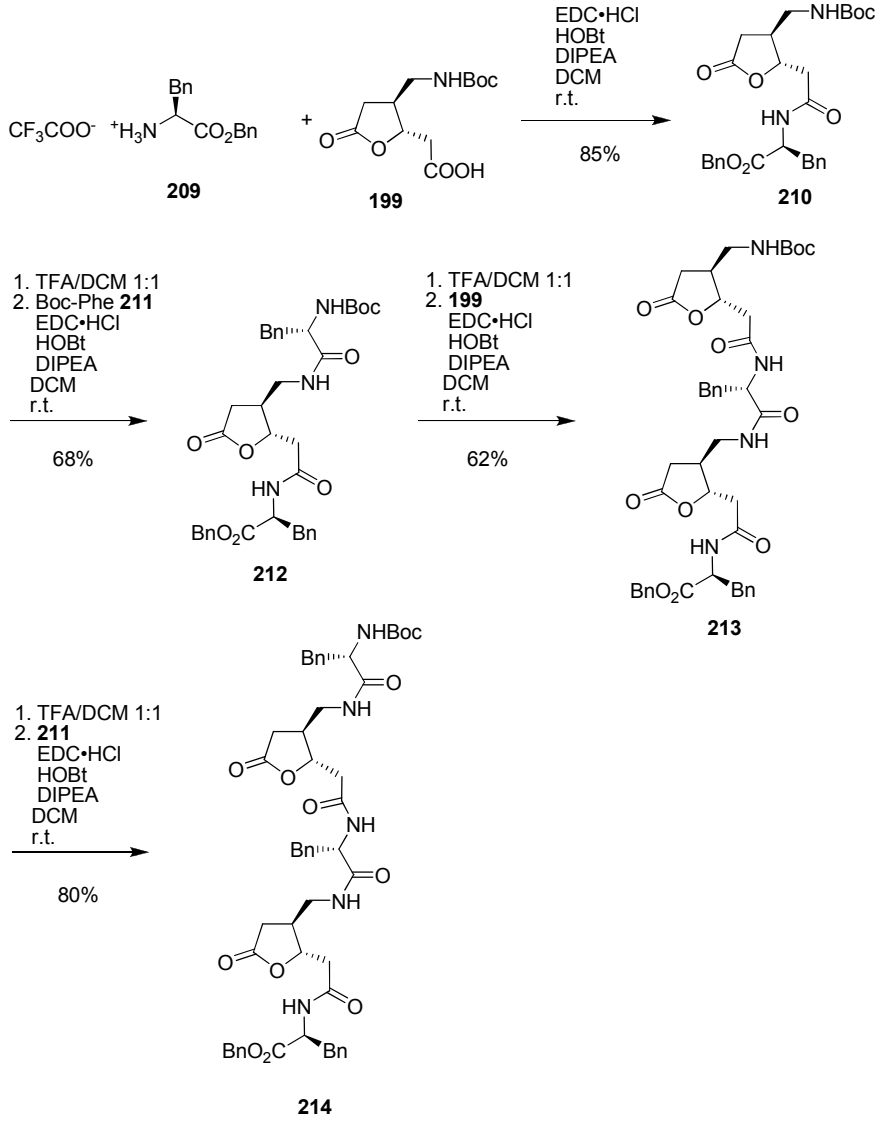


Scheme 58: Synthesis of δ-peptides

2. Synthesis of alternated α-δ-peptides

The conformational analysis of a mixed peptide containing α- and δ-amino acids can be very interesting to observe the behaviour of an unnatural amino acid with α-amino acids as it opens perspectives for its introduction into natural peptides. The α-amino acid chosen for the synthesis was phenylalanine as its signal would not overlap at all with the non-methylated building block. The synthesis of an oligomer of four or five amino acids was envisaged as it would reach a sufficient length to adopt a secondary structure as an helical structure can be observed when 6 and more α-amino acids are coupled together. The synthesis started by coupling the TFA salt of benzyl phenylalanine **209** and **199** with EDC•HCl and HOBt which afforded the dimer **210** in 85% yield. Addition of Boc-Phe

211 to **210** formed the trimer **212** in 68% yield. Addition of a second δ-amino acid afforded the tetramer **213** in 62% yield. The last coupling with Boc-Phe allowed the isolation of the pentamer **214** in 80% yield (Scheme 59).

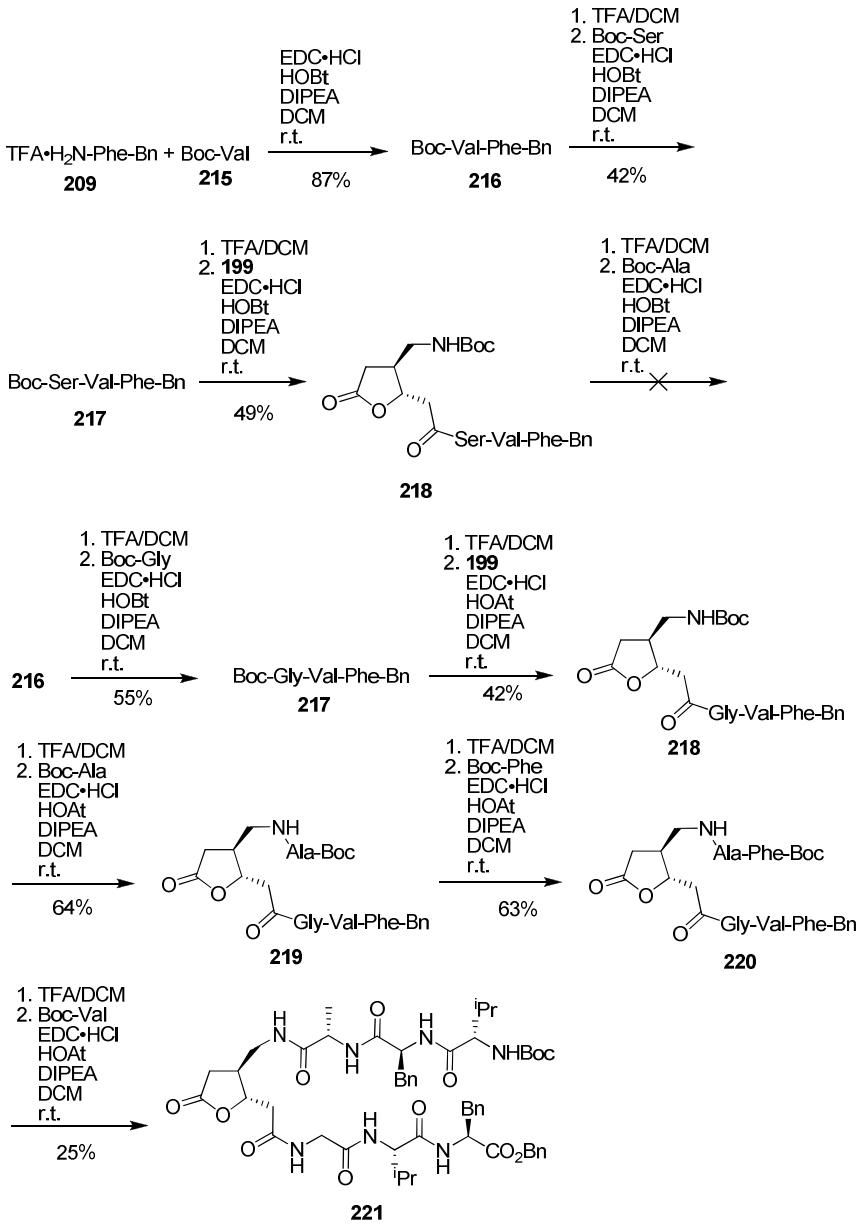


Scheme 59: Synthesis of α-δ-petides

3. Synthesis of a hairpin-like peptide

Our building block presenting two arms relatively close one to each other, it was interesting for us to evaluate its potential as a hairpin inducer. In the computational work of Hofmann et al.[60], it had been demonstrated that cyclic δ-amino acids could induce β-hairpin formation and some hairpin based on δ-amino acids have already been described in the literature[2,19,20,61,85] confirming the Hofmann's calculations. The synthesis of a heptapeptide hairpin mimic with a central δ-amino acid was envisaged using diverse α-amino acids to get the best dispersion possible. For reasons of efficiency, relatively

non polar amino acids were selected. A stepwise synthesis was realised initiating with the TFA salt of benzyl phenylalanine **209** which was coupled to Boc-Val **215** affording the dipeptide **216** in 87% yield. Addition of Boc-serine to this dipeptide gave the tripeptide **217** in moderate yield (42%). The addition of **199** proceeded as well in the formation of the tetrapeptide **218** in moderate yield (49%). When trying to couple Boc-Ala to **218**, the reaction failed to give the expected pentamer. The tetrapeptide was actually very polar because of the presence of serine and we decided to replace it by glycine. The coupling of Boc-Glyc to the dipeptide **216** afforded the expected tripeptide **219** in 55% yield. The addition of **199** to this tripeptide gave the tetrapeptide **220** in 42% yield. Subsequent addition of Boc-Ala was successful and the pentapeptide **221** could be obtained in 64% yield. The hexapeptide **223** was obtained in 63% yield by addition of Boc-Phe. The last coupling step afforded the heptapeptide **224** in low yield (25%) by addition of Boc-Val (Scheme 60).



Scheme 60: Synthesis of hairpin-like peptide

IX. Conformational analyses of the peptides synthesised

1. Conformational analyses of the α-δ-peptide

The secondary structure of the mixed α-δ-peptide was investigated using typical analyses: 2D-NMR, CD spectroscopy, IR in solution, molecular dynamics.

a. CD spectroscopy

CD measurements were realised on the pentamer **214** which showed interesting features suggesting a secondary structure. In methanol at the concentration of 0.1 mM, two positive peaks at 201 nm and 219 nm and a negative peak at 193 nm could be observed suggesting that an organised secondary structure was adopted by the pentapeptide **214** (Figure 11).

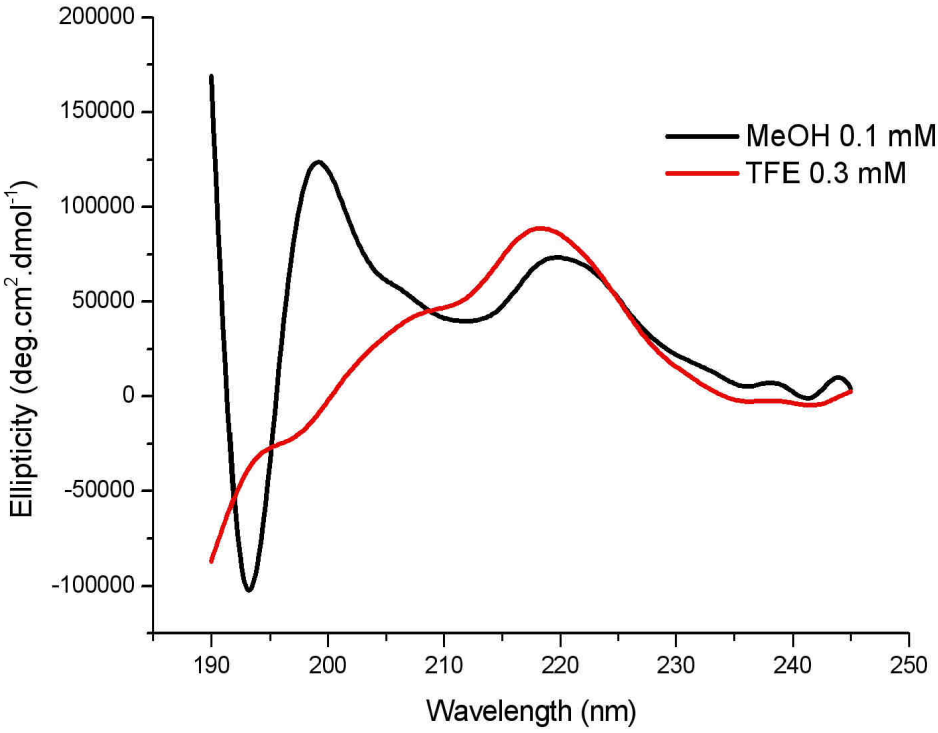


Figure 11: CD spectroscopy of the α-δ-pentamer **214** in MeOH and TFE

On the other hand, the CD spectrum collected in TFE is very different and only the positive peak at 220 nm similar to the one in methanol suggested a changing of conformation. Because of the lack of data on secondary structures adopted by mixed α-δ-peptides, no comparison with known structures could be done.

b. IR in solution

The pentapeptide **214** being soluble in dichloromethane, it was studied by IR in solution in order to determine whether hydrogen bonding was taking place in the molecule. The analysis at 4 mM in dichloromethane showed two bands in the NH amide absorption area, one at 3420 cm^{-1}, revealing the presence of non-hydrogen bonded amide NHs and a band at 3314 cm^{-1}, significant of intramolecular hydrogen bonding. This analysis confirmed the folding of the peptide observed by CD spectroscopy (Figure 12).

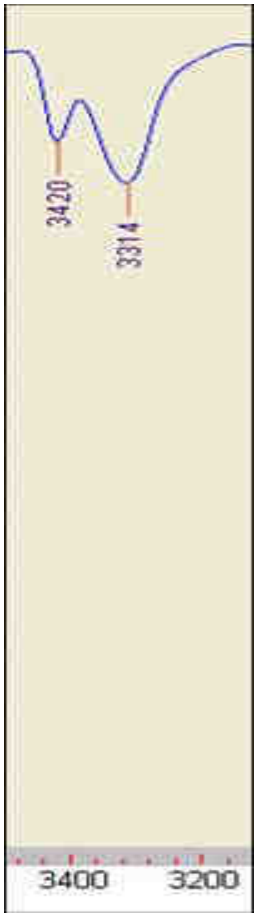


Figure 12: IR in solution of **214** at 4 mM in dichloromethane

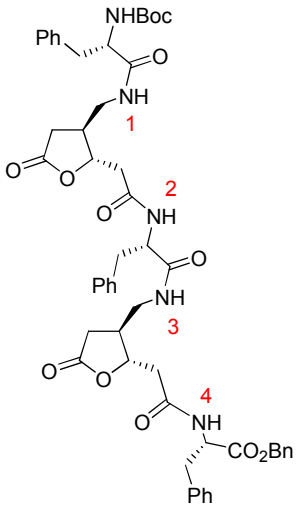
c. NMR studies

The pentapeptide was analysed by NMR using temperature variation, proton deuterium exchange and 2D-NMR

- Temperature variation

The pentapeptide was analysed in $CDCl_3$ and MeOH-d_3 and the temperature variation coefficient ($\Delta\delta/\Delta T$) was calculated for both solvents. Unfortunately, in both solvents, some amide protons were overlapped and in $CDCl_3$, some amide protons were hidden under the aromatic protons on a big range of temperature, making the calculation of their $\Delta\delta/\Delta T$ approximative using only two points. (Scheme 61 and table 1).

In MeOH-d_3, the $\Delta\delta/\Delta T$ calculated was pretty high, the only amide protons which would be in equilibrium between hydrogen-bonded and non hydrogen-bonded state would be the amide protons of both lactones with a $\Delta\delta/\Delta T$ of 7.39 ppb/K, the other amide protons being at more than 8 ppb/K. Methanol being a competitive solvent, these values must be considered with precaution as they do not reflect entirely the bonded or non bonded state of the amide protons. In $CDCl_3$, a totally different behaviour is observed as all the amide protons had a high $\Delta\delta/\Delta T$ suggesting a non-hydrogen bonded state except the carbamate which was almost blocked ($\Delta\delta/\Delta T$=3.37 ppb/K).



Scheme 61: NH numbering of pentapeptide **214**

Methanol-d_3				
NH	1 and 3	2	4	NHBoc
δ (ppm) at 273 K	8.295	8.475	8.585	6.995
Δδ (ppb/K)	-7,39	-8,35	-8	-10,29

$CDCl_3$				
NH	1 or 3	1 or 3 + 2 or 4	2 or 4	NHBoc
δ (ppm) at 278 K	7,61	7,925	7,10<δ<7,00	5,425
Δδ (ppb/K)	-10,8	-12,26	-12	-3,37

Table1: Temperature variation coefficient of the pentapeptide **214**'s NHs in MeOH-d_3 and $CDCl_3$

- Proton exchange with deuterium

The pentapeptide was dissolved in pure methanol-d_4 and the ^{1}H-NMR was realised at different moments after the solubilisation in methanol. The phenylalanine NH protons all disappeared after 15 minutes meanwhile the amide protons of the lactone rings disappeared after 125 minutes as a light

signal was still visible. This result is quite surprising as in very harsh conditions, these amide protons resisted pretty well to the exchange with deuterium despite a $\Delta\delta/\Delta T$ relatively high. This experiment demonstrated that the amide protons belonging to the lactones could be involved in relatively strong hydrogen bonding (Figure 13).

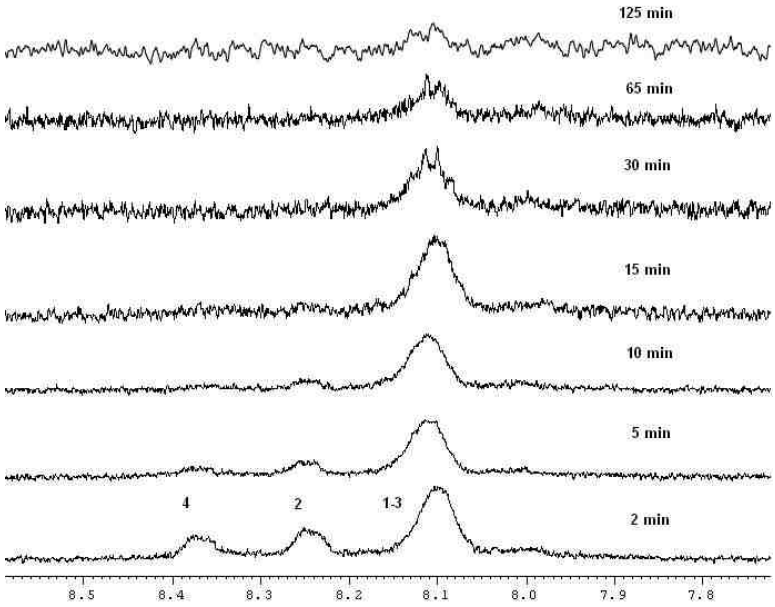


Figure 13: Deuterium proton exchange between **214** and pure methanol-d_4

- 2D-NMR

NOESY and ROESY experiments could be realised in MeOH-d_3 and $CDCl_3$. In $CDCl_3$, some amide protons were hidden by the aromatic protons and the assignment was impossible. MeOH-d_3 provided a better dispersion despite some amide protons were overlapping, making the assignment difficult (Figure 14).

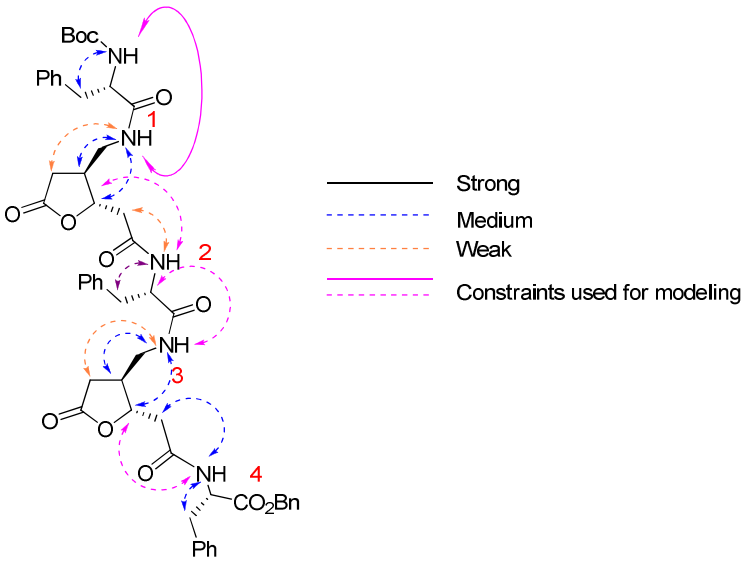


Figure 14: NOE contacts observed in methanol-d_3 at 6 mM (Assignment by K. Guitot)

As the amide 1 and 3 were overlapping, the assignment was difficult but some contacts could be observed. Amides 1 and 3 were found to be close to the CHγ of their own butyrolactone ring. In addition, the amide 1 had a strong contact with the carbamate of Phe1 meaning a folding in this part. Amides 2 and 4 were showing as well contacts with the CHγ of the upper vicinal butyrolactone ring. These different contacts could suggest a large helical structure and were used as constraints in computational studies.

d. Computational studies

Using the NOE constraints above, calculations were realised and the minimum found for the pentapeptide **214** suggested an extended helical conformation (Figure 15).

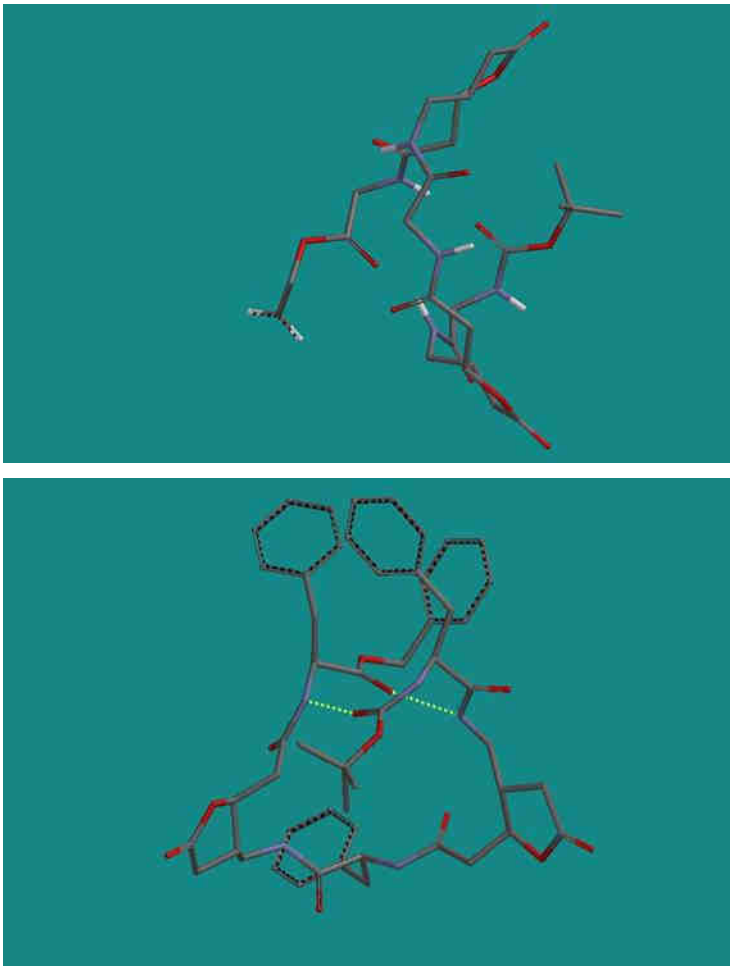


Figure 15: Minimum found by computational studies based on NOE constraints (Calculations realised by K. Guitot and L. Formicola)

The calculations suggested that the CO of the carbamate could create hydrogen bonding with the NH^4 thus forming a 22-membered ring and that the amide proton 1 of the lactone would create hydrogen bonding with the CO of the benzyl ester forming therefore a 20-membered ring. In the computational work of Hofmann,[60] these large 20- and 22-membered ring were described as possible

features in secondary structures adopted by δ-amino acids. In these calculations, the amide proton 1 involved in hydrogen bonding corresponds to the most stable amide proton in the deuterium-proton exchange analysis.

e. X-ray studies

To confirm the diverse data collected on the pentapeptide **214**, its crystal structure would have been of a great help to fully characterise the secondary structure as we would have had some common points between the crystal structure and the secondary structure adopted by **214**. Some nice crystals could be obtained in a solution of pentane and methanol at -20°C in a biphasic system, but, unfortunately, the crystals were very unstable when removed from the mother solution for X-ray analysis. After a few minutes, they were drying and losing their crystallinity making X-ray analysis impossible as only a couple of diffraction points could be collected. Attempts of crystallisation in diverse solvents led to the same result and no X-ray data could be collected. Linear peptide crystals are usually difficult to obtain and to stabilise as they have a lot of movement freedom and slight changes can destroy their structure.

2. Conformational studies of the hairpin-like peptide

The heptapeptide **221** was synthesised in order to investigate on its behaviour when inserted in a peptide containing α-amino acids. The closeness of both arms could favour a hairpin like structure or a helical structure. The two arms of our building block could induce a hairpin structure by putting two chains of amino acids one to each other. Nevertheless, the *trans*-configuration may disfavour this structure but could induce a helical conformation. Its secondary structure was analysed by CD spectroscopy, IR in solution and 2D-NMR.

a. CD spectroscopy

The heptapeptide **221** was first analysed by CD spectroscopy in MeOH at 0.5 mM. The CD spectrum showed three negative peaks at 193 nm, a strong one at 200 nm and a small one at 207 nm with a shoulder at 213 nm. A positive peak could also be observed at 225 nm (Figure 16).

The comparison of the CD spectrum of our compound with a β-hairpin induced by δ-amino acid prepared by Aguilera et al.[85] did not match at all. But the amino acids composing our product and the hairpin of this publication are very different and we could not affirm that **221** did not adopt a hairpin-like structure. The solvent being different, they might as well have a different spectrum. Moreover, CD spectra of secondary structures of δ-peptides are still very scarce and one can not deduce a secondary structure just by comparing with reference curves. Nevertheless, the aspect of the CD spectrum of **221** and the irregular peak shapes could suggest the presence of more than one conformation or a non-defined secondary structure.

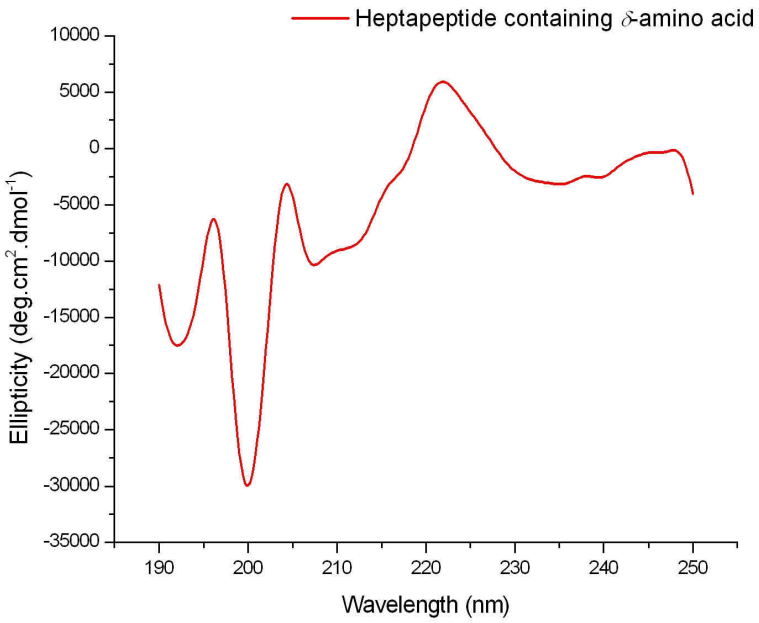


Figure 16: CD spectroscopy of 221 at 0.5 mM in MeOH

b. IR in solution

Despite the heptapeptide **221** was not very soluble in dichloromethane, its analysis by IR in solution could be realised at a concentration of 1 mM. The IR spectrum exhibited two distinct bands at 3420 cm^{-1} and 3326 cm^{-1} meaning that our heptapeptide presented intramolecular hydrogen bonding (Figure 17).

Figure 17: IR in solution of **221** at 1 mM in DCM

c. 2D-NMR

The heptapeptide was analysed by 2D-NMR in methanol-d_3. The low solubility of the compound in chloroform prevented NMR analyses in $CDCl_3$. Many NOE contacts could be observed and their assignment confirmed that **221** adopted a secondary structure (Figure 18). The NHBoc of Val1 showed a contact with amide 1. Amide 2 had a contact with amide 3 and amide 3, like in the pentapeptide **214**, showed a contact with the CHγ of its own butyrolactone ring. More interesting, the amide 4 of Gly 5 showed a long range coupling with the CHα of Phe2, confirming an interstrand hydrogen bonding. This suggested the formation of a 13-membered ring between the NH4 of Gly5 and the CO of Phe2. It is interesting to notice that a 13-membered ring is a loop of α-helix. Finally, the NH 5 of Val6 showed a contact with the NH6 of Phe7. The different coupling observed suggest that an irregular helical conformation is adopted by the heptapeptide **221**.

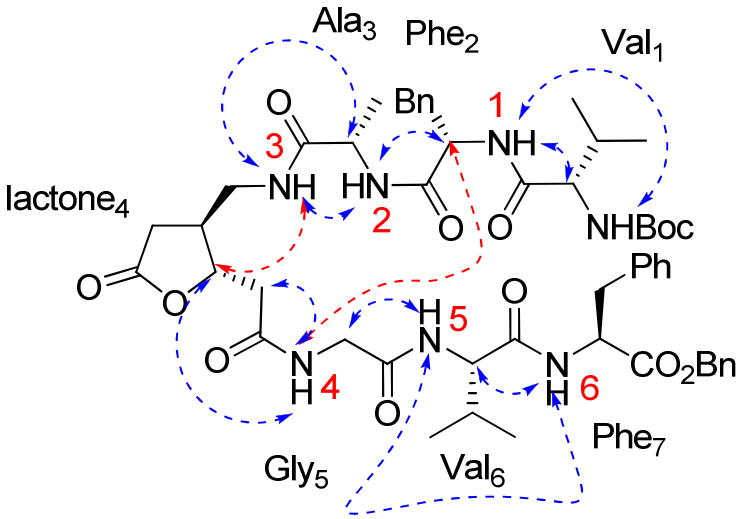


Figure 18: NOE contacts observed for the heptapeptide **221** (assignment by K. Guitot and L. Formicola)

The diverse contacts observed resulting from the irregular helical structure adopted by the heptapeptide can explain the irregular shape of the CD spectrum. The lactone building block seems to disorganise the peptide structure, but does not disrupt it. Moreover, the 13-membered ring observed constitutes a loop of α-helix, this demonstrates the potential of our scaffold as helix-inducer.

X. Conclusion

Based on the synthesis of a new δ-amino acid scaffold in our group, we have improved the synthesis by changing the conditions and reagents leading to improved yields and higher scales.

The synthesis of substituted lactone δ-amino acids gave disappointing results for the monomethylation and monofluorination in terms of diastereoselectivity.

The dimethylation afforded the new δ-amino acid **204** presenting a much more dispersed NMR than the non methylated one, making this scaffold an interesting product for the preparation of homopeptide. Unfortunately, the synthesis of the homopeptide revealed difficult and the envisaged tetramer could not be isolated.

The introduction of the δ-amino acid **199** into alternated α-δ-peptides has shown interesting folding properties although some NOESY data and X-ray crystallography miss to describe the helical secondary structure with certainty.

The introduction of only one δ-amino acid **199** does not induce hairpin structure as supposed at the beginning, but an irregular helical secondary structure featuring, among other things, an interstrand hydrogen bond forming a 13-membered ring. This 13-membered ring being a loop of α-helix, we can deduce that our scaffold can be a helix inducer when introduced into peptides containing α-amino acids.

The investigations on this building block demonstrate its ability to induce secondary structures, even though their characterisation can be difficult.

Chapter 2: Synthesis of rigidically constrained diketopiperazine scaffolds towards foldamers

I. Introduction

Diketopiperazines (DKP) are a class of 6-membered-ring compounds containing 2 amide functionalities in a 1,4-spatial relationship. They are the smallest cyclic peptides, resulting from the cyclisation of two α-amino acids (Figure 19).

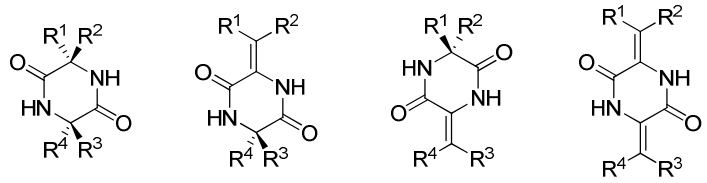


Figure 19: General structure of diketopiperazines

Although known since a hundred years, they have been considered as by-products in peptide synthesis for a long time. They have regained interest from chemists and pharmacologists thanks to their biological properties. Their characteristic backbone, which can also be found in numerous natural products, constitutes a rich source of new biologically active compounds with diverse therapeutic applications.[86] Their chemical properties, such as their resistance to proteolysis, possibility of mimicking pharmacophore peptide groups, their conformational rigidity and the presence of hydrogen bonding donors and hydrogen bonding acceptors are extremely valuable for medicinal chemistry. The variety of substituents on the cycle's carbons as well as on the ring's nitrogens makes them interesting scaffolds for drug design and peptide chemistry. To prepare them, natural α-amino acids can be used affording disubstituted diketopiperazines but the diversity of the substituents can be extended using disubstituted α-amino acids or more complex amino acids. In addition, the relative planarity of the cycle and the presence of two potential stereocenters are an advantage for getting rigid structures with interesting properties.

1. Diketopiperazines in natural products and biologically active compounds

In nature, diketopiperazines are biosynthesised by different organisms, including mammals and are considered as secondary products of terminal peptide cleavage. They can be extracted from natural sources, but can as well be synthesised. The number of biologically active DKP based compounds biosynthesised is very large and they have many different activities depending on their substituents and a small overview can be useful to realise their importance in therapeutics.

The DKP are known for having antitumor, antiviral, antifungal and antibacterial activities but their most known properties are related to the inhibition of plasminogen activator inhibitor-1 (PAI-1)

and alteration of cardiovascular and blood-clotting functions. They also have affinities for calcium channels and opioid, GABAergic, serotonin-ergic-5-HT_{1A} and oxytocin receptors.

PAI-1 is the main physiological inhibitor of the serine proteases, urikinase plasminogen activator (uPA) and tissue plasminogen activator (tPA). A correlation between PAI-1 and cancer has been demonstrated in mice and high levels of circulating PAI-1 led to increased metastases in animals. Diketopiperazines are the most potent PAI-1 inhibitors and the metabolite **222**, isolated from *Streptomyces* sp., was the first low-molecular-weight inhibitor of PAI-1 and was used as a model for designing new inhibitors based on this structure that could be more efficient. Combining bioisosteric substitutions and combinatorial chemistry, a new inhibitor containing a diketopiperazine connected to the side chains by exocyclic double bonds **223** was found to be particularly active (IC_{50}=3.5 µM). Changing the thienyl-thioether group by a phenyl-ether linkage did not cause any loss in the activity. The final addition of an alkyl carboxylic group and a heterocyclic substituent on the other arm led to the product **224** which had an increased inhibition of PAI-1 (IC_{50}=0.2 µM) (Figure 20).

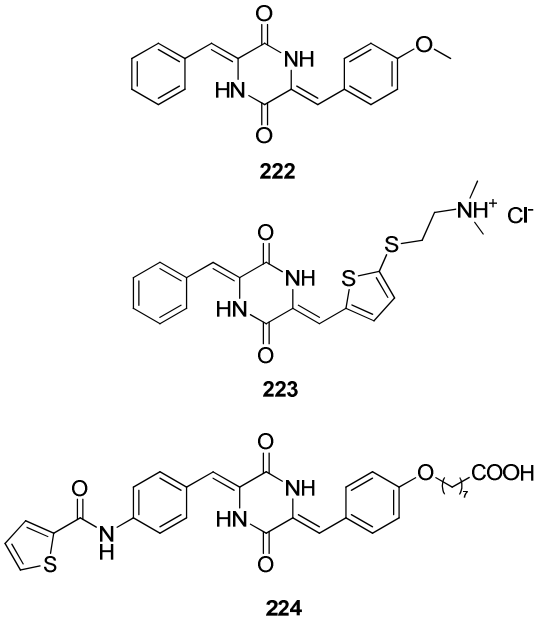
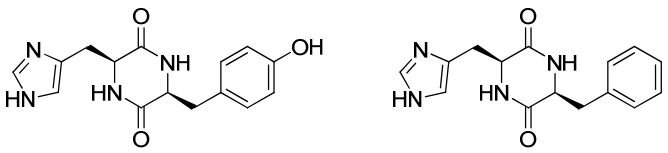


Figure 20: Diketopiperazines inhibitors of PAI-1

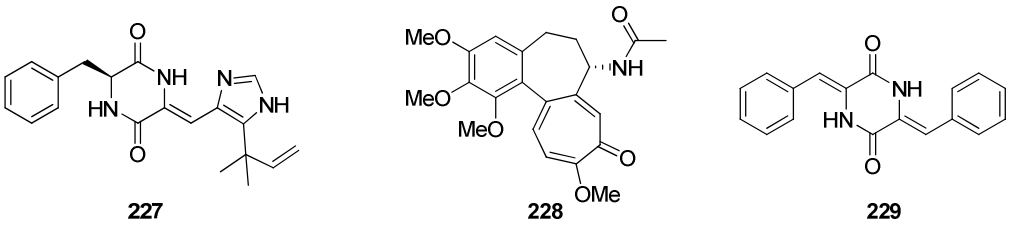
Blood clotting is considered to favour metastatic processes and the *cyclo*[L-His-L-Tyr] diketopiperazine **225** increases the clotting time and prevents platelet adhesion and aggregation induced by adenosine diphosphate. Some other blood-clotting-inhibiting diketopiperazines have been reported. *Cyclo*[L-His-L-Phe] showed an antitumor activity (Figure 21). In addition to antitumor activity, molecules **225** and **226** have displayed a potential activity for the treatment of cardiovascular dysfunctions and could be employed as antiarythmic agents and reduce mortality by ventricular fibrillation in myocardial infarction.



225 226

Figure 21: Histidine-containing diketopiperazines having anti-clotting activity

Phenylahistin **227**, very similar to **226**, isolated from *Aspergillus ustus*, is involved in microtubule depolymerisation in human A459 lung carcinoma and is cytotoxic to several lines of tumor cells. Being structurally very different from colchicine **228**, it interacts at the same site in tubulin provoking microtubule depolymerisation and inhibiting the progression of cellular cycle.[87] The diketopiperazine **229**, an achiral synthetic analogue of **227** also binds to microtubules and has exhibited cytotoxic activity against tumor cell lines from prostate, breast, lung, leukaemia and colorectal tumors with IC_{50} from 4.3 to 18 nM. It had also cytotoxic activity against multidrug cell lines. It was demonstrated that it was even more potent than colchicine and vincristine that it is being tested in preclinical studies (Figure 22).



227 228 229

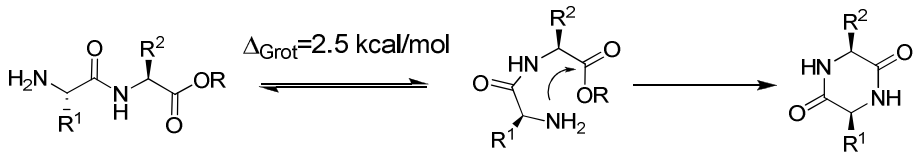
Figure 22: Diketopiperazines **227** and **229** act both at the same tubulin site as colchicine **228**

2. Syntheses of diketopiperazines

Diketopiperazines can be synthesised easily and synthetic methods are mainly based on reactions on dipeptides. These dipeptides can be simply obtained by α-amino acids by conventional methodologies. The interest of using α-amino acids is that the diketopiperazines obtained can be diversely substituted, opening to many applications in parallel synthesis and solid-phase synthesis.[88]

The first diketopiperazine synthesised was a *cyclo*-[Gly-Gly] which was realised by simply heating glycine under a flow of CO_2 or HCl.[89] The first examples of DKPs containing unnatural amino acids were already described in 1888.[90] Chemists realised soon that DKPs could be obtained in an efficient manner when the spontaneous formation of *cyclo*-[Gly-Gly] from glycine ethyl ester was described.[91,92] Diketopiperazines have been used to determine peptide sequences since the controlled hydrolysis of peptides was affording DKPs. Most of them being crystalline, it was then easy to compare them with a library of synthetic DKPs. The most common and direct syntheses of diketopiperazines involve the cyclisation of a dipeptide ester in basic conditions (Scheme 62). This

cyclisation can be accompanied by partial epimerisation of the C terminal amino acid.[93] In order to diminish epimerisation, a concomitant deprotection of the N terminal amine function and cyclisation can be performed using for example a Boc-protected dipeptide ester. In boiling isobutanol and toluene, the Boc group decomposes itself and lets the N-deprotected dipeptide spontaneously attack the ester functionality to form the diketopiperazine scaffold.

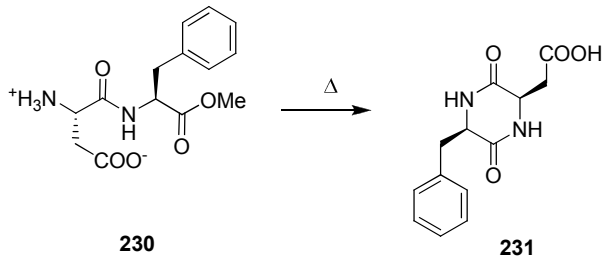


Scheme 62: Mechanism of dipeptide cyclisation giving the diketopiperazine scaffold

Synthesis of diketopiperazines can also be performed by solid-phase synthesis employing linkers based on nitrophenyl,[94] *p*-thiophenyl (with oxidation of the thiol to the sulfone prior to cyclization),[95] and 4-bromomethyl-3-nitrobenzoylaminobenzyl[96] esters. Even standard Merrifield peptide synthesis resin can, under optimized reaction conditions, be used to synthesise DKPs.[97]

The cyclisation of the dipeptide necessitates an isomerisation of the amide bond from the *s-trans*-configuration, more stable, to the *s-cis*-configuration. Studies have shown that in peptides, peptide bond in *s-cis*-configuration is very scarce, less than 2%, and it has been suggested that the presence of bulky substituents on the C_α of the amino acids should prevent even more strongly this isomerisation making the cyclisation more difficult. The energy barrier of rotation of the amide bond between the *s-cis* and *s-trans* configuration has been calculated to be 2.5 kcal/mol.[98] In fact, the steric hindrance of the α-substituents has a negligible effect on the conformation of the amide bond as *cis*-amides are less frequent in Gly-Gly sequences. As a consequence, the energy barrier is easily surmountable at room temperature making the cyclisation feasible. The nature of the leaving alcohol influences the reactivity, methyl esters forming diketopiperazines more easily than longer alkyl chains. The cyclisation efficiency is also very dependant on the nature of α-substituents and the Cα configuration since formation of *trans*-DKPs is easier than for the *cis*-ones.

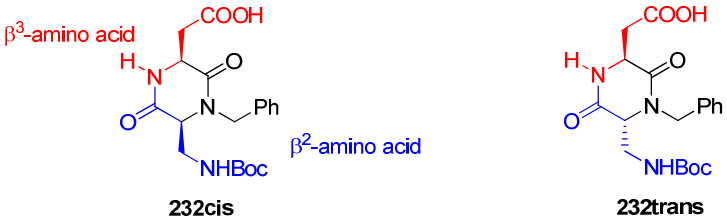
A typical example of spontaneous cyclisation with heating is the case of the artificial sweetener aspartame **230**. This sugar substitute is used by people under diet as it has a high sweetening power, 200 times higher than sucrose. It is a dipeptide of aspartic acid and phenylalanine methyl ester. When one takes a look at the advices of use, it is suggested on the packaging to sweeten cakes after having cooked it in the oven in order to make the cake taste better. The reason is pretty simple, under heating, the aspartame will cyclise, forming the corresponding diketopiperazine which does not have any sweet taste. Aspartame is also subject to pH-dependant epimerisation.[99] After a long time of storage, diet drinks containing aspartame were presenting traces of diketopiperazine **231**. According to some studies, this diketopiperazine presents a little toxicity and the acceptable daily intake has been fixed to 7.5 mg/kg/day but its toxicity is still very controversed. (Scheme 63)



230 **231**

Scheme 63: Cyclisation of aspartame resulting in the formation of a diketopiperazine

In our group, the practical synthesis of a *cis*-DKP formally derived from the cyclisation of aspartic acid and serine has been developed. By a Mitsunobu reaction, the hydroxyl group of the serine was replaced by an azido functionality, giving birth to the DKP **232** which bore a carboxylic acid and an amino functionalities in a cis relationship and could be seen as the cyclisation of aspartic acid with 2,3-diaminopropionic acid. Although being prepared from 2 α-amino acids, this new scaffold can also be considered as a conformationnally constrained dipeptide formed by two β-amino acids, and in articular a β^2-amino acid and a β^3-amino acid (according to Seebach nomenclature).[100] Using L- or D-serine as starting material could give access to the *cis*-DKP **232cis** or the *trans*-DKP **232trans** (Figure 23).[101]



232cis **232trans**

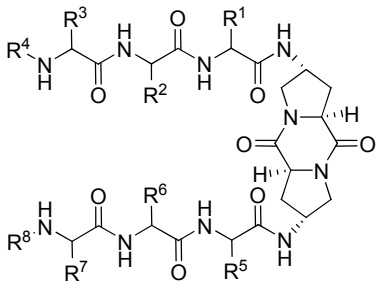
Figure 23: Diketopiperazine scaffold of Piarulli's group

3. Diketopiperazines in peptidomimetics

Diketopiperazine scaffolds are interesting building blocks for peptidomimetics synthesis since the rigidity of its ring can greatly favour secondary structure formation by limiting the conformational freedom of its lateral chains. The *cis*-conformation is therefore particularly interesting as it would force both peptidic arms to stay close one to each other.

The work of Wennemers demonstrates very well the potential applications of this scaffold in peptidomimetics. They have prepared a library of symmetrical diketopiperazine two-armed receptors derived from 4-aminoproline where both tripeptide arms were disposed in a cis-relationship (Figure 24). The receptor **233** was coupled with a dye in order to see whether a binding was occurring or not.[102] These receptors were screened toward a library of 5000 tripeptides and exhibited high binding affinity towards only a few peptide sequences. In addition, every type of receptor had a specifity for a kind of

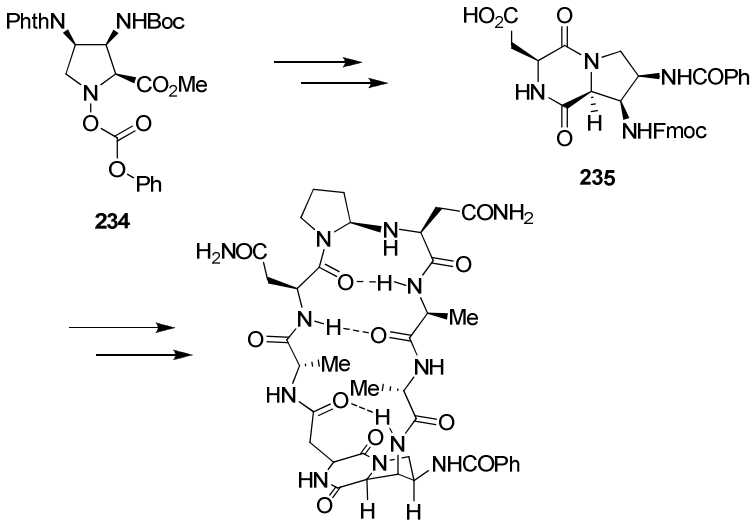
tripeptide. This selective binding property was attributed to the specific turn geometry adopted by the receptors.[103]



233

Figure 24: Diketopiperazine based receptors

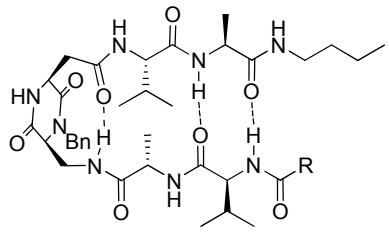
Robinson et al. have prepared the bicyclic diketopiperazine scaffold **235** from L-aspartic acid and (2S,3R,4R)-diaminoproline **234**, a chiral amino acid derived from the transformation of vitamin C. This DKP, when introduced into the cyclic peptide **236**, stabilised a β-hairpin conformation (Scheme 64).[104]



234 **235** **236**

Scheme 64: Diketopiperazine inducing a β-hairpin

Recently in our group, the hexapeptide **237** containing the cis-DKP **232cis** also exhibited a β-hairpin secondary structure.[101] Conformational analysis was carried out using ^{1}H-NMR spectroscopy, IR spectroscopy, CD spectroscopy and molecular modelling and revealed the formation of a β-hairpin mimic involving 10-membered, 18-membered and 24-membered rings by hydrogen bonding between the two arms of the diketopiperazine (Figure 25).



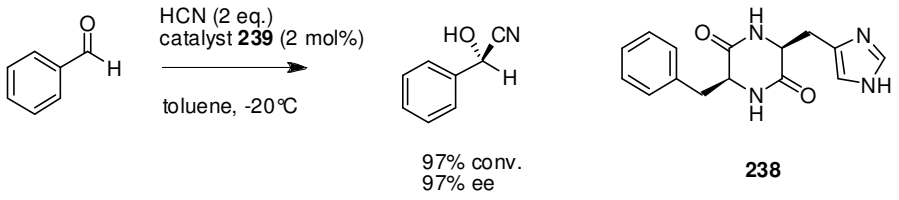
237

Figure 25: Hexapeptide containing the *cis*-DKP **237** exhibting a β-hairpin conformation

4. Diketopiperazines as organocatalysts

Peptides play an important role in living organisms, they have a multitude of roles like hormones, receptors, extracellular matrix components and enzymes. These enzymes are of great importance since they catalyse biochemical reactions with high efficiency and enantioselectivity. For centuries, human have taken benefits of enzymes like in the process of vinification for example and chemists have also been very interested in using them in chemical processes, *i.e* resolution of racemics. Since it has been found that simple amino acids or short peptides could have the same catalytic properties as enzymes, they have attracted a lot of attention[105] as they can catalyse reactions in very mild conditions as efficiently as metal catalysts and have a much lower toxicity which is an advantage for pharmaceutical applications. Furthermore, they are generally much cheaper than the metals and ligands used. The catalytic activity can be explained by the secondary structure they can adopt which recreates enzymes active site. Nevertheless, there is a need for designing organocatalysts in order to get the best efficiency possible. In addition, there are very few polyvalent organocatalysts, they are most of the time effective on a kind of reactions with certain substrates and the need of new organocatalysts to broaden the scope of applications is very stimulating.

Diketopiperazine scaffolds are good candidates for organocatalysis since their rigid structure can favour turn formation which mimics the active sites of enzymes. Their role as efficient organocatalyst is noteworthy in hydrocyanation of aldehydes with the work of Inoue who prepared the *cyclo*[L-Phe-L-His] **238** which exhibited a very high enantioselectivity in the addition of HCN on benzaldehyde with a 97% conversion and 97% ee (Scheme 65).[106]

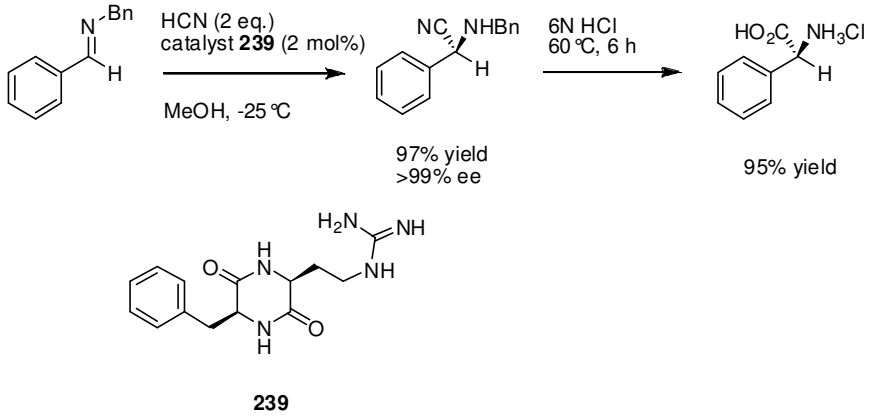


Scheme 65: Hydrocyanation of benzaldehyde catalysed by DKP **238**

This catalysis demonstrated the high potential of *cis*-DKP in organocatalysis and the hydrocyanation by DKP **238** is still the most efficient organocatalysed hydrocyanation.

Another good example of DKPs as organocatalyst has been reported by Lipton where the similar DKP **239** composed of phenylalanine and (*S*)-norarginine showed exceptional results in asymmetric Strecker reaction. In this reaction, HCN could be added to N-benzhydryl aromatic imines in 97% yield and >99% ee.[107] This reaction afforded, in three steps, (*S*)-phenylglycine in 97% ee and 92% overall yield which is an useful unnatural α-amino acid (Scheme 66).

Nevertheless, these results are still controversial as Kunz et al. repeated the reaction with the same diketopiperazine and substrates in the same conditions and found that the conversion was much lower (34%) and that the resulting catalysis product was racemic.[108] Moreover, the reaction performed without **239** afforded the racemic product with a better conversion (42%).



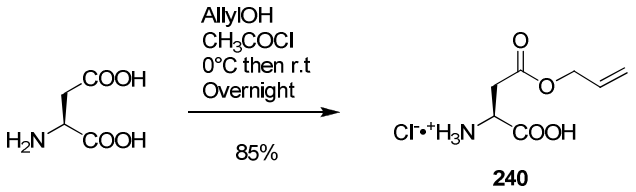
Scheme 66: Strecker reaction catalysed by DKP **239**

II. Synthesis of the diketopiperazine scaffold

The synthesis of the diketopiperazine **232** was realised following the procedures published a few months ago.[101] The synthesis of the **232cis** and **232trans** is exactly the same and yields are similar except for one step.

1. Allylation of aspartic acid

The aspartic acid was specifically β-allylated using allyl alcohol as a solvent and adding acetyl chloride dropwise at 0°C.[109] Maintaining the reaction flask at this temperature during the addition is very important as formation of bis-allylated aspartic acid can be observed. The lab temperature is also very important since in summer time, more bis-allylated compound formation could be observed. The reaction afforded the hydrochloride salt of β-allyl (*S*)-aspartic acid **240** in 85% yield (Scheme 67).

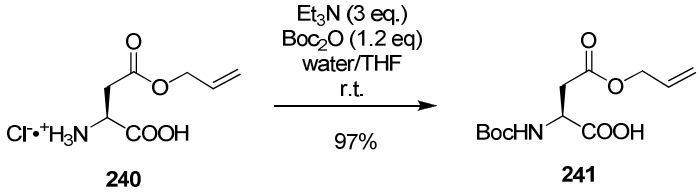


240

Scheme 67: Allylation of aspartic acid

2. Boc protection of *β*-allyl aspartic acid

The *β*-allyl ester of aspartic acid was Boc protected by a conventional procedure using Boc_2O and triethylamine in a mixture of water/dioxane. Alternatively can be used a mixture water/THF making the drying of the product easier. The Boc protected *β*-allyl aspartic acid **241** could be obtained easily in excellent yields (97%) (Scheme 68).

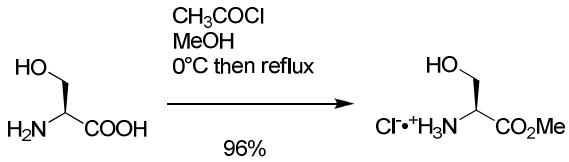


240 **241**

Scheme 68: Boc protection of *β*-allyl aspartic acid hydrochloride

3. Methyl esterification of serine

Serine was esterified in the same manner as aspartic acid except that the solvent used was methanol. The procedure was a little shorter as after addition of acetyl chloride, the reaction mixture was refluxed for 2.5 hours affording the hydrochloride methyl serine salt **242** in 96% yield (Scheme 69). To prepare the *cis*- and *trans*-diketopiperazines, L-serine and D-serine were used respectively.

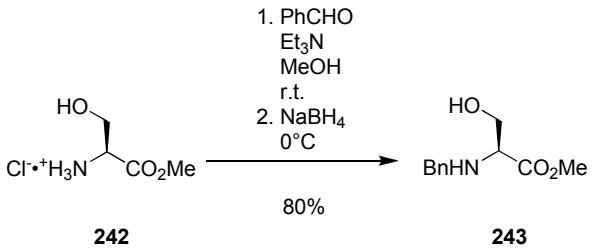


242

Scheme 69: Methyl esterification of serine

4. Reductive amination

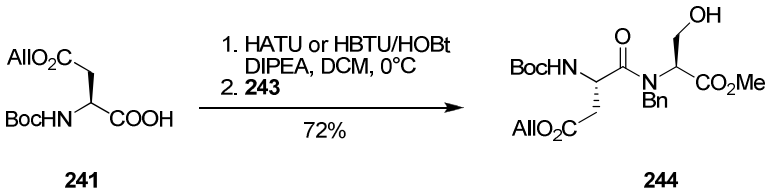
The serine methyl ester hydrochloride salt was benzylated on the N terminal by reductive amination with benzaldehyde using a known procedure.[110] The reaction involved first the formation of the benzylimine in methanol which was then reduced using $NaBH_4$. $NaBH_4$ must be added slowly and portionwise as its addition in methanol is very exothermic and can lead to partial racemisation of serine. The use of freshly distilled benzaldehyde afforded the *N*-benzylserine methyl ester **243** in 80% yield (Scheme 70).



Scheme 70: Reductive amination of the serine methyl ester HCl salt

5. Coupling of *N*-Boc-β-allyl aspartic acid and *N*-benzyl serine methyl ester

The coupling of the N-protected aspartic acid with *N*-benzyl serine methyl ester was investigated. Attempts with EDC and HOBt led to low yields (40%) probably due to the fact that the secondary amine of serine is difficult to couple. The use of HATU in DCM afforded the dipeptide **244** good yields (72%) and little epimerisation of the serine CHα could be observed. The epimerisation comes mainly from the reductive amination step and little from the coupling conditions. More epimerisation was observed with dimethylformamide as solvent. The use of HBTU instead of HATU with HOBt in dichloromethane led to similar yields at much lower costs. The two diastereoisomers could not be separated by flash chromatography (Scheme 71).

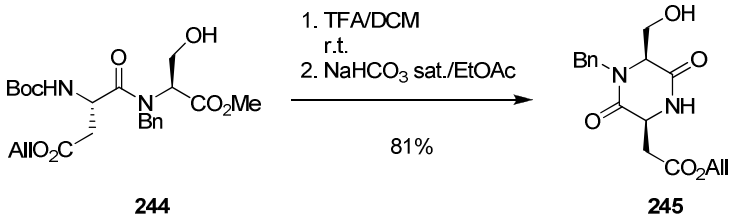


Scheme 71: Coupling between Boc-aspartic acid and benzyl serine

6. Cyclisation of the dipeptide

The dipeptide **244** was cyclised by first removing the Boc group in a mixture of TFA/DCM at room temperature. The TFA salt was then solved in a biphasic mixture of ethyl acetate and saturated $NaHCO_3$.[111] The diketopiperazine **245** was obtained in good yields (81%) and the diastereomers,

which were formed during the previous coupling, could be separated easily affording the pure *cis*- or *trans*-diketopiperazine (Scheme 72).

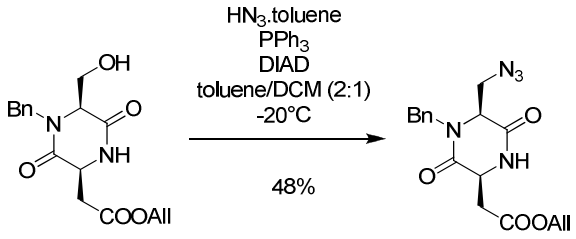


244 **245**

Scheme 72: Dipeptide cyclisation

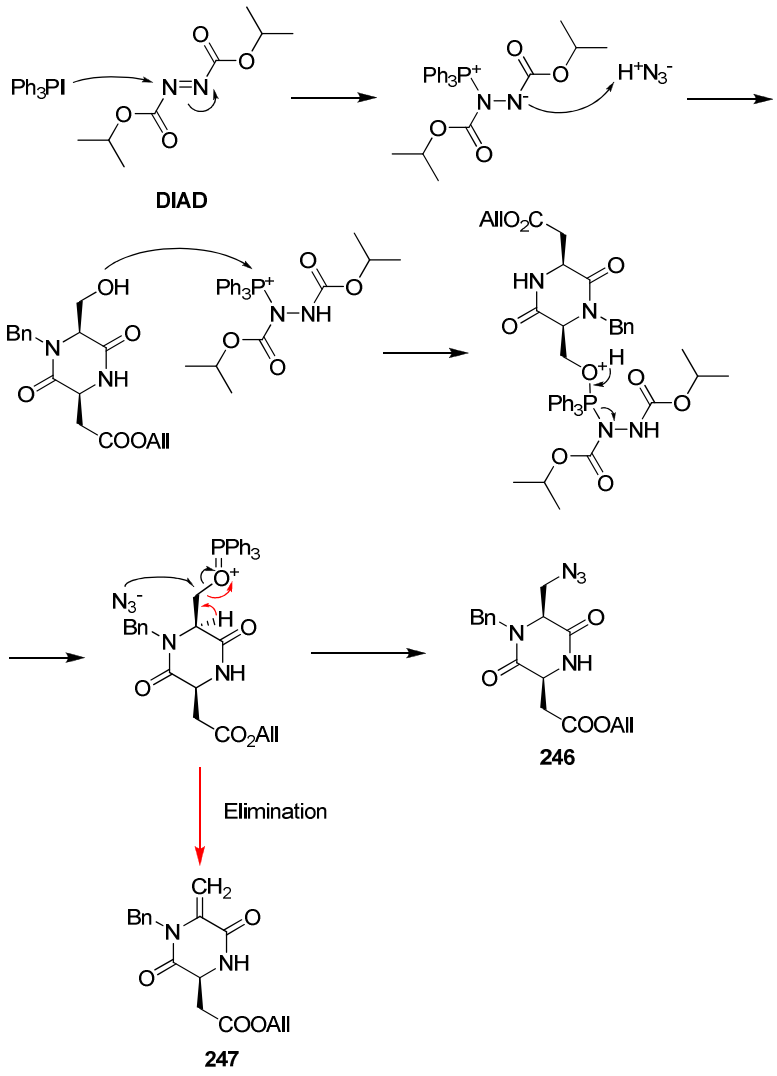
7. Introduction of the nitrogen moiety by Mitsunobu reaction

The nitrogen moiety was introduced by a Mitsunobu reaction with an hydrazoic acid solution in toluene, diisopropylic dicarboxylate (DIAD) and triphenylphosphine following procedures for the synthesis of 2,3-diaminopropionic acid.[112,113] The reaction was performed in a mixture of toluene and dichloromethane at -20°C and gave moderate yields of the azido compound **246** (48%) for the *cis*-DKP **245** (Scheme 73). In fact, a competition between the formation of the azido compound and the elimination product **247** was found to be responsible for this moderate result. The *cis*-conformation favoured elimination in comparison to the *trans*-conformation which gave the *trans*-azido-diketopiperazine in much higher yield (82%). Formation of **247** was also favoured by temperatures higher than -10°C and by higher amounts of dichloromethane in the reaction mixture, which was nevertheless required to dissolve the starting material (Scheme 74).



245 **246**

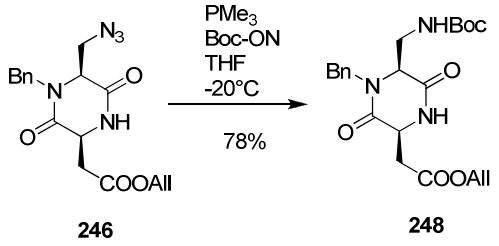
Scheme 73: Mitsunobu reaction



Scheme 74: Mechanism of the Mitsunobu reaction and by-product formation

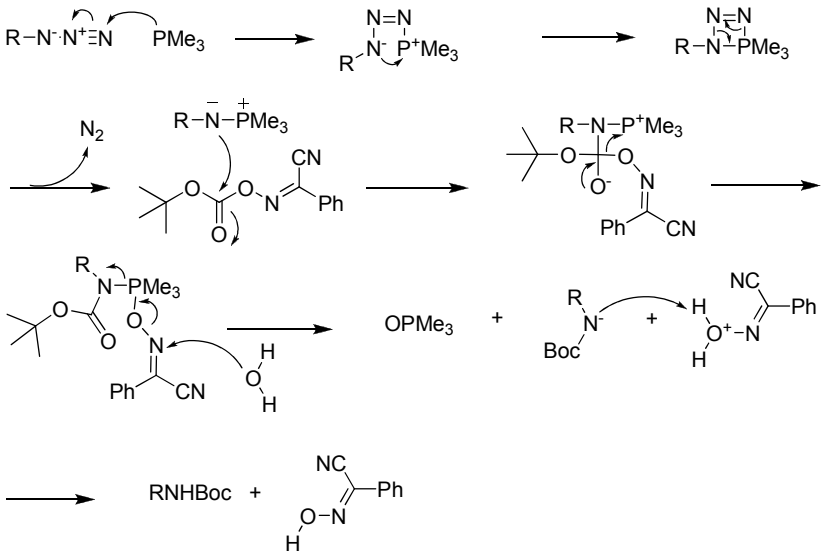
8. Reduction of the azide to the protected amine by tandem Staudinger-Boc protection reaction

The azido functionality was reduced by Staudinger-like reaction with trimethylphosphine and 2-(tert-Butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) in dry THF at -20°C allowing the isolation of the *N*-protected diketopiperazine **248** in 78% yield (Scheme 75).[114] The reaction goes through the formation of a phosphazide which rearranges by liberating N_2 to form the iminophosphorane. The Boc-ON is then attacked by the iminophosphorane. Reaction with water finishes the process liberating the Boc-protected diketopiperazine **248**, trimethylphosphine oxide and *N*-hydroxybenzimidoyl cyanide (Scheme 76).



246 **248**

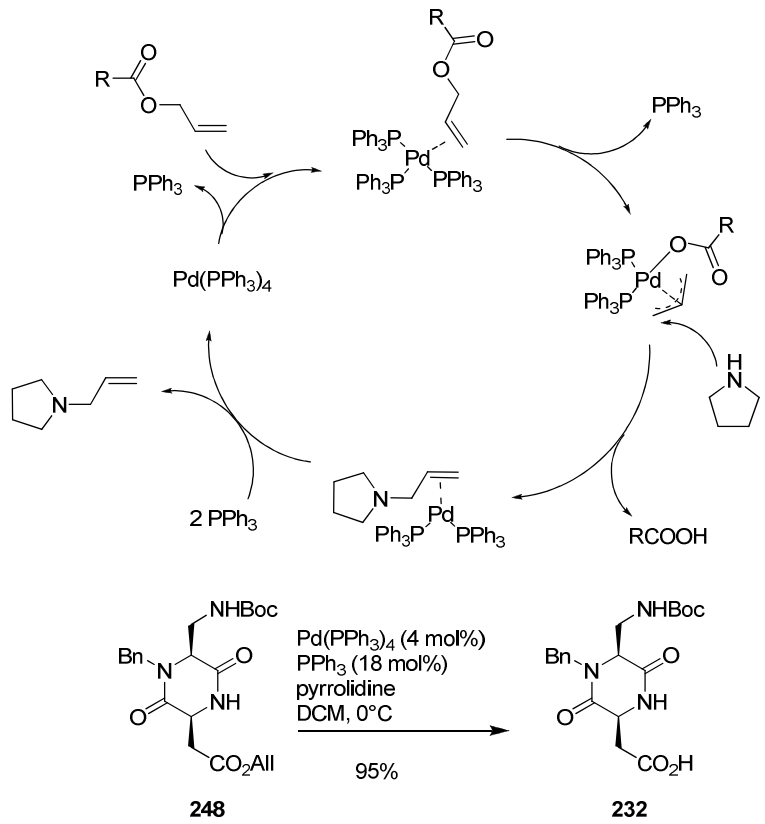
Scheme 75: Reduction and concomitant Boc protection of the azide



Scheme 76: Mechanism of the Staudinger reaction

9. Deallylation catalysed by $Pd(PPh_3)_4$

The diketopiperazine **248** was deallylated by a catalytic amount of palladium tetrakis(triphenylphenylphosphine) (4 mol%) and PPh_3 (18 mol%) in presence of pyrrolidine in dichloromethane at 0°C.[115] The catalysis goes through the formation of a π-allyl complex and oxidative addition of the allyl ester to the palladium catalyst. Protons are furnished by the pyrrolidine finishing therefore the catalytic cycle by the reductive elimination of the carboxylic acid **232** and *N*-allyl-pyrrolidine. The catalysis afforded **232** in 95% yield. (Scheme 77)



Scheme 77: Deallylation of the diketopiperazine and proposed mechanism

The diketopiperazine building block was then ready to be used in liquid phase peptide synthesis. The synthesis of both *cis*- and *trans*-diketopiperazine was performed for this purpose.

III. Introduction of the diketopiperazine building block into peptides and conformational analyses

1. β-bend ribbon

The results obtained with the hexapeptide **237** prompted us to consider taking advantage of the formation of the ten-membered ring by the DKP scaffold into different peptidomimetics. Actually, many secondary structures exhibit ten-membered ring, the most known being the 3_{10} helix. A particular type of 3_{10} helix is the β-bend ribbon. It is characterised by a succession of β-turns forming therefore a linear peptide with a ribbon-like shape (Figure 26). β-bend ribbons are not so common but they do exist in several natural peptides like peptaibol antibiotics zermavicins. Zermavicin IIA is a membrane active peptide antibiotic whose crystal structure, first described by Balaram et al.[116], exhibited a continuous spiral beginning by a 3_{10}-helix at the N-terminus, changing to an α-helix for two turns, and ending in a spiral of three β-bends in a ribbon. Each of the β-bends contained a proline residue at one of the corners. Zermavicin has the particularity to contain α-aminoisobutyric acid (Aib), proline, and

polar amino acids like glutamine. A hexadecapeptide analogue of zermavicin, where Gln, Thr and hydroxyproline (Hyp) were replaced by Ala, Val and Pro respectively, was synthesised and exhibited the same β-bend ribbon structure at the end of the peptide chain. The presence of Aib and Pro was proved decisive for the secondary structure stability.

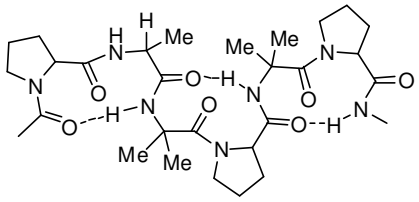
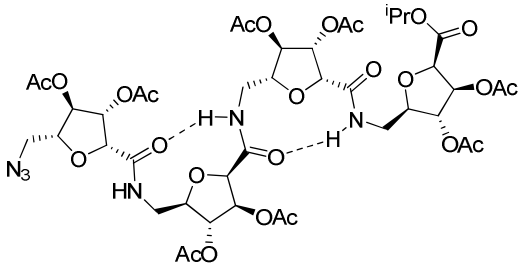


Figure 26: Alternation of ten-membered ring in β-bend ribbon

Inspired by this work, Toniolo et al. have prepared protected polymers repeating the sequence (*L*-Pro-Aib)$_n$ and have also observed a stable β-bend ribbon which was analysed by crystallography and CD spectroscopy (Figure 26).[117]

In general, it is considered that a rigid *cis*-conformation can greatly stabilise a β-bend ribbon and most of the examples of synthetic β-bend ribbon verify this observation. But sometimes, peptides non having a blocked conformation can as well adopt that kind of secondary structure.[118]

Fleet et al. have also observed this structure when preparing the homopolymer **250** of a tetrahydrofuran δ-amino acid exhibiting a cis-conformation.[70] This peptide adopted a stable conformation from 4 building blocks (Figure 27).



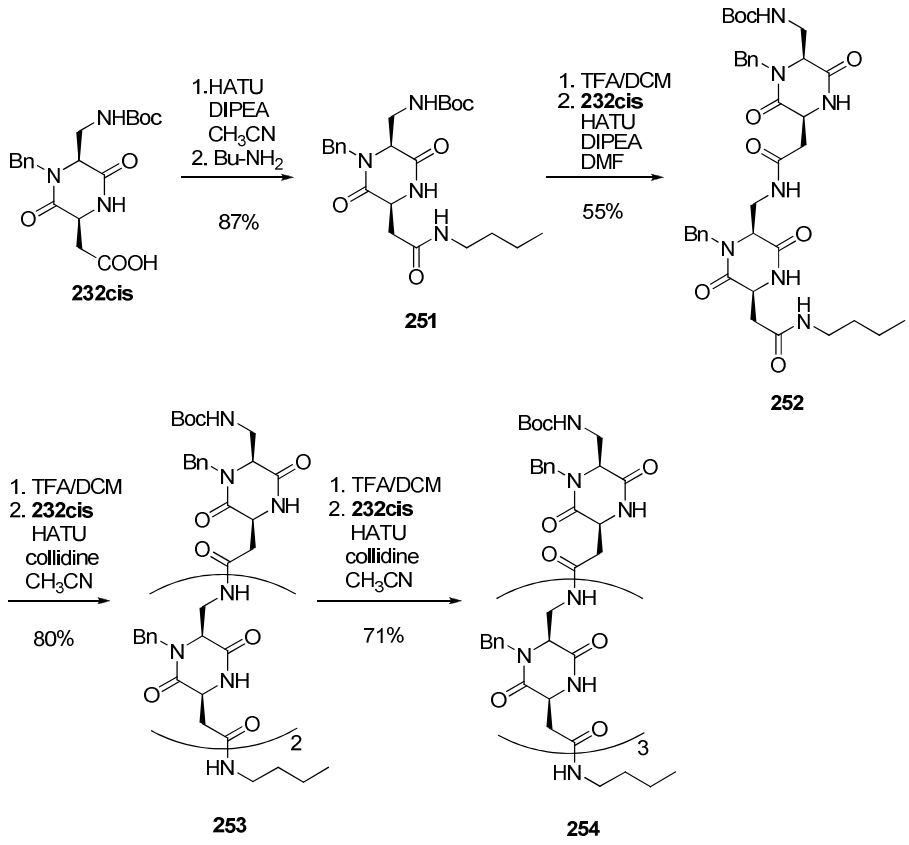
250

Figure 27: Fleet's β-bend ribbon **250** based on tetrahydrofuran amino acid building blocks

With this numerous examples, we were prompted to investigate the potential of our scaffold for β-bend ribbon formation since the 10-membered ring we had observed in the β-hairpin **237** could be repeated in a homopolymer of the diketopiperazine **232cis**. The experience of Fleet et al. suggests that peptide of at least three building blocks had to be prepared to get a stable secondary structure. Thus, we envisaged to prepare homopolymers having at least three or four building blocks. We selected the peptide liquid phase synthesis strategy as it is a convenient procedure for short peptides. Once the polymers were obtained, they would be studied by 2D-NMR, CD spectroscopy and IR to determine whether or not they adopt a β-bend ribbon conformation.

2. Synthesis of homopolymers of *cis*-diketopiperazine

The first reaction of the synthesis was to definitively protect the carboxylic functionality of the starting peptide building block. To do so, we decided to couple **232cis** with butylamine as it would create an additional amide functionality that could participate in the secondary structure. The first attempts to couple the diketopiperazine with butylamine were perfomed using EDC. The yields were very low (20%) and we decided to use HBTU. Unfortunately, with HBTU, the coupling yield was in the same order of magnitude and only the use of HATU in acetonitrile afforded the butylamine-protected diketopiperazine **251** in very good yields (87%). The addition of a second building block to **251** after having transformed it to its TFA salt also gave low yields of the dimer **252** with EDC and HBTU. The best yields were obtained with HATU in DMF but remained moderate (54%). One explanation of this low yield could be given by its little solubity and we found out that it aggregated in $CDCl_3$ for concentrations higher than 2 mM. The formation of the trimer **253**, contrary to the dimer **252**, gave much higher yields (80%) using HATU in acetonitrile. The synthesis of the tetramer **254** led to some reactivity problems as reactions realised in DMF never afforded **254**. It was found that the spontaneous cleavage of DMF released dimethylamine in the solution which was reacting with the diketopiperazine **232cis** preventing therefore the coupling with the trimer TFA salt. As we supposed the coupling to be difficult, the reaction was performed in acetonitrile with HATU, but we had to avoid as well the use of HOAt, even if it should have given best yields, as our HOAt was in DMF solution and the small amount of dimethylamine in the HOAt solution was able to inhibit the reaction. Therefore, the coupling in acetonitrile with HATU and without HOAt finally gave the tetramer **254** in good yields (71%). It was found that the use of collidine instead of DIPEA for the formation of the trimer and tetramer gave higher yields (Scheme 78).



Scheme 78: Homopolymers syntheses

3. Conformational analyses of the homopolymers

The conformational analyses of the homopolymers of cis-diketopiperazine were studied by means of 2D-NMR and CD spectroscopy. The dimer **252** was first analysed, it was found that it aggregated in $CDCl_3$ for concentrations higher than 2 mM. Moreover, its 2D-NMR analysis did not reveal any interesting NOE contact, therefore, its conformational study will not be exposed in the following part.

a. Dilution studies of the trimer

The trimer's aggregation was studied in $CDCl_3$ for concentration going from 1 to 5 mM. No aggregation could be observed in this range of concentrations and the trimer **253** was then studied at the concentration of 5 mM.

b. Temperature variation coefficient of the trimer

The measurement of the temperature variation coefficient of the trimer's amide protons in $CDCl_3$ revealed that many amide protons were involved relatively strongly in hydrogen bonding. The first evidence of hydrogen bonding is the chemical shifts of all NH protons, including the carbamate proton which had a chemical shift of 6.16 ppm at room temperature. All the amide protons were particularly deshielded showing chemical shifts higher than 8 ppm except the amide A4 with a

chemical shift of 7.77 ppm. The most interesting $\Delta\delta/\Delta T$ values concerned the extracyclic amide proton A4 and the carbamate proton which had values (4.4 and 5.3 ppb/K) much lower than the $\Delta\delta/\Delta T$ of A2 (7 ppb/K) meaning that the NHBoc and the NH4 should be involved in an equilibrium between an hydrogen-bonded state and a non-hydrogen bonded state meanwhile NH2 is less involved in hydrogen bonding. Low values were found as well for the intracyclic amide protons A1 and A5 (4.9 and 3.4 ppb/K) and the lowest $\Delta\delta/\Delta T$ found was for the intracyclic amide A3 with a value of 2.3 ppb/K meaning that it is strongly involved in hydrogen bonding. On the other hand, NH6 had the highest $\Delta\delta/\Delta t$ (8.8 ppb/K) suggesting a totally non-hydrogen bonded state (Table 2 and figure 28).[2]

	Δδ/ΔT (ppb/K)	**δ (ppm)**
A1	4.9	8.65
A2	7	8.07
A3	2.3	8.63
A4	4.4	7.77
A5	3.4	8.43
A6	8.8	8.43
NHBoc	5.3	6.16

Table 2: Temperature variation of the NH of the trimer **253** in $CDCl_3$ at 5 mM

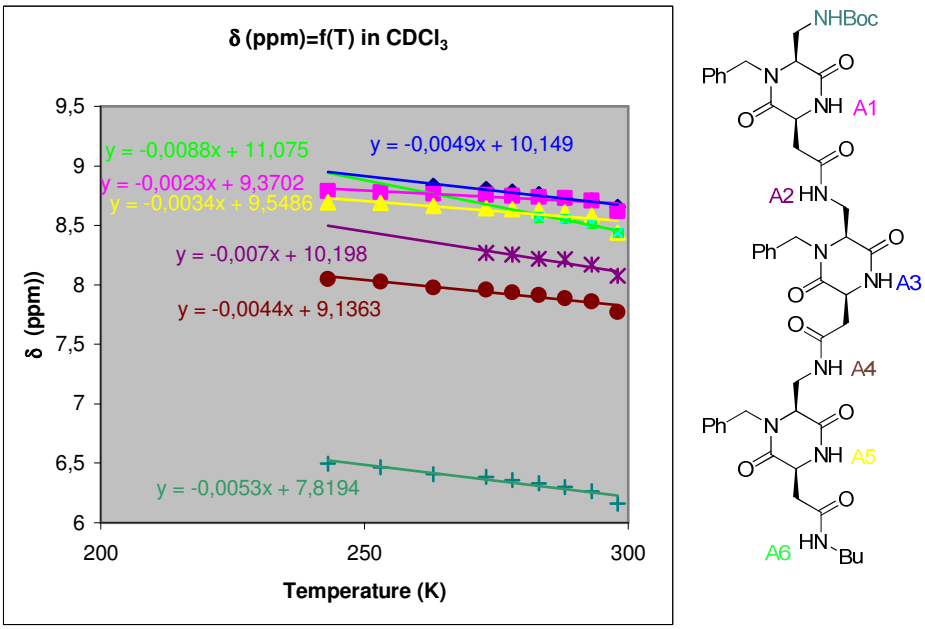


Figure 28: Graph of temperature variation and amide numbering in **253**

c. 2D-NMR analysis of the trimer

The trimer was analysed in $CDCl_3$, DMSO-d_6 and methanol-d_3. In $CDCl_3$, the dispersion of amide protons was not exploitable, some of them being hidden by the aromatic protons. Moreover, some of them were overlapping making the assignment impossible. In DMSO-d_6, the dispersion was more satisfying and some contacts could be seen but the dispersion of the protons "e" made again the analysis difficult. In methanol-d_3, on the other hand, the dispersion of the amides and protons "e" allowed assignment with certainty. The carbamate proton showed a NOE contact with the proton "e1", suggesting that a 10-membered ring was formed on the first building block (Figure 29). For the amide proton A2, no NOE contact could be observed with "e2" or with another proton except with "e1" which is spatially close to it. Surprisingly, the amide proton A3 had a NOE contact with the proton "d3" (Figure 30). The reason of this unusual coupling is unclear but was undoubtedly observed. These couplings show clearly that the central building block did not form a 10-membered ring such as the upper building block. Finally, the amide A4 had a contact with the proton "e3", revealing the presence of a 10-membered ring in the lower building block. These results taught us that the trimer adopted therefore a partial β-bend ribbon, with only the central building block not forming a ten-membered ring (Figure 31).

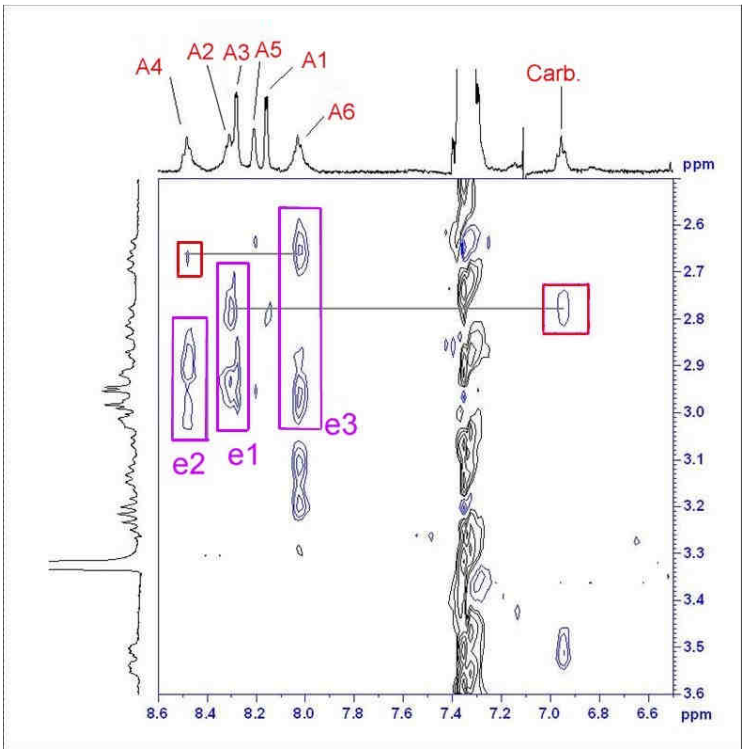


Figure 29: NOESY expansion showing the contacts of NHBoc and A4 with "e1" and "e3" respectively

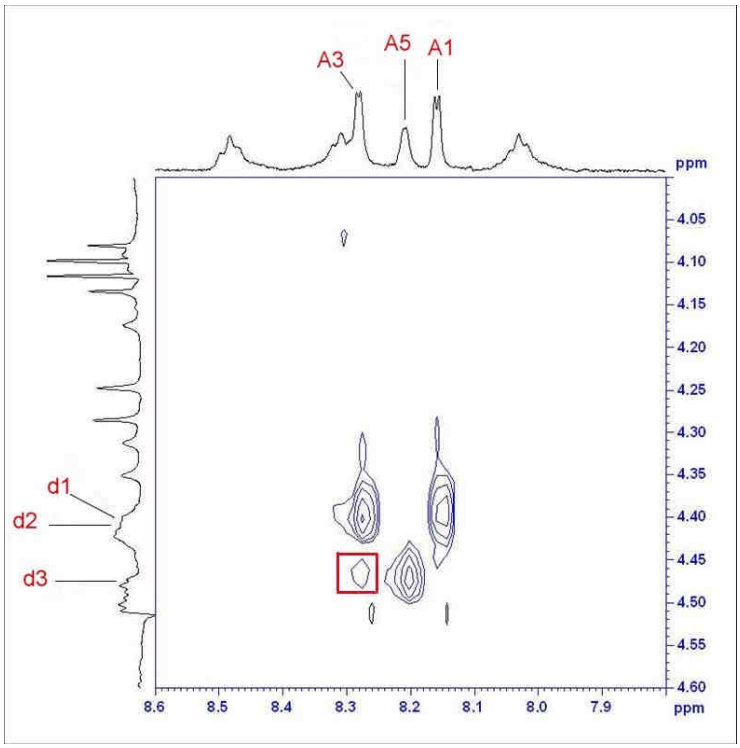


Figure 30: NOESY expansion showing the coupling of A3 with “d3”

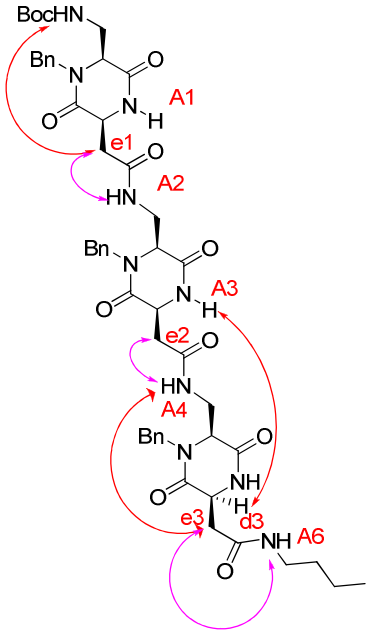


Figure 31: NOE contacts observed for **253** in MeOH-d_3 at 5 mM

d. 2D-NMR conformational analysis of the tetramer 254

The tetramer **254** was analysed by NMR in different solvents but its low solubility in $CDCl_3$ did not allow temperature variation studies. The NMR measurement in methanol-d_3 showed that the intracyclic amide protons were not visible and assignment could not be done. In DMSO-d_6, the

dispersion of the amide protons was very nice but the dispersion of the protons “e” was not good enough to assign all the amides with certainty. Only one NOE contact could be assigned with certainty, it showed that the carbamate proton had a NOE contact with the proton “e1” and so that it was involved in a hydrogen bond with the CO of the same building block, forming a ten-membered ring typical of β-bend ribbons. For the other extracyclic amides, unfortunately, no assignment could be deduced. A 2D-NMR was attempted in a mixture of $CDCl_3$ and DMSO-d_6 affording as well a good dispersion for the amide protons, but still, no good dispersion was obtained for the protons “e”, making assignment impossible. Again, only the contact between the carbamate proton and the proton “e1” could be observed revealing the presence of at least one ten-membered ring. In DMSO-d_6 and methanol-d_3, more than one signal was observed for the extracyclic amides, suggesting an equilibrium between at least two conformations, one being in great majority. For the intracyclic amides, no particular contact could be observed (Figure 32). Since no conclusive proof of a secondary structure was obtained by NOE studies, we moved forward and decided get some insights from different spectroscopic evidences.

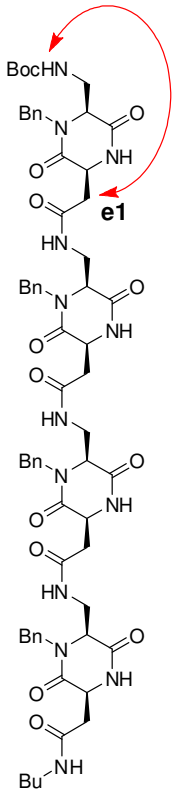


Figure 32: NOE contact observed for **254** showing the formation of a ten-membered ring by hydrogen bonding

e. Proton exchange analysis on 254

A proton exchange analysis was realised by adding 0.19mL of methanol-d_4 to 0.75 ml of a 5 mM solution of **254** in DMSO-d_6. The ^{1}H-NMR was measured at different moments and different exchange times were observed for the amide and carbamate protons. It was found that all the

intracyclic amides exchanged almost instantly (7 minutes after the addition). The extracyclic amides including the NH belonging to the butylamine fragment disappeared 48 minutes after the addition. The NHBoc was the last proton to disappear after 169 minutes. This experiment could allow us to deduce that the intracyclic amides are not involved at all in hydrogen bonding meanwhile the extracyclic amides showed a much longer resistance to proton exchange, meaning that they must be involved in hydrogen bonding (Table 3).

Time stability of the NH protons of the tetramer 254	
NHBoc	169 min.
Intracyclic NH	7 min.
Extracyclic NH	48 min.
NHBu	48 min.

Table 3: Proton exchange time of the NH protons of the tetramer **254**

f. CD spectroscopy of the trimer and tetramer

The trimer **253** and the tetramer **254** were analysed by CD spectroscopy in methanol and TFE (Figure 33 and 34). The CD spectrum realised in MeOH at 0.2 mM for both homopolymers showed that the trimer and tetramer did not adopt the same structure, the secondary structure of the tetramer being much more defined. The tetramer CD spectrum showed an intense negative peak at 198 nm and a second negative peak at 220 nm. On the other hand, the trimer exhibited 3 negative peaks, one at 197 nm, less intense than the tetramer's peak at 198 nm and two intense peaks at 208 nm and 221 nm, revealing therefore a very different secondary structure. Having characterised the trimer by NMR, we could affirm that the tetramer's secondary structure had just a few common points with the trimer's secondary structure. A comparison of the curve shape of the hairpin **237** with the tetramer's curve showed that their CD spectra were very similar.[101] The intense peak at 198 nm was attributed to the ten-membered ring formed between both arms of the diketopiperazine, suggesting that the tetramer **254** features as well ten-membered rings. The intensity of the peak at 203 nm of the hairpin **237** was around 6500 deg.cm^2.dmol^{-1} at the concentration of 0.2 mM and the intensity of the same peak for the tetramer **254** was of 120000 deg.cm^2.dmol^{-1} at the same concentration, this means that there were many more ten-membered rings formed (Figure 35). In the literature, β-bend ribbon CD spectra are available and they all exhibit similar CD spectra with an intense negative band around 200 nm and another one, less intense, around 225 nm (Figure 36 and 37).[117,119] In addition the CD spectrum of the tetramer in TFE exhibited almost the same curve except an additional shoulder around 205 nm revealing that this secondary structure could be adopted in methanol as well as in TFE. The trimer exhibited a very different CD curve in TFE compared to its curve in methanol. It had 3 intense negative peaks at 197, 204 and 221 nm showing that the secondary structure adopted by the trimer is different in methanol and TFE.

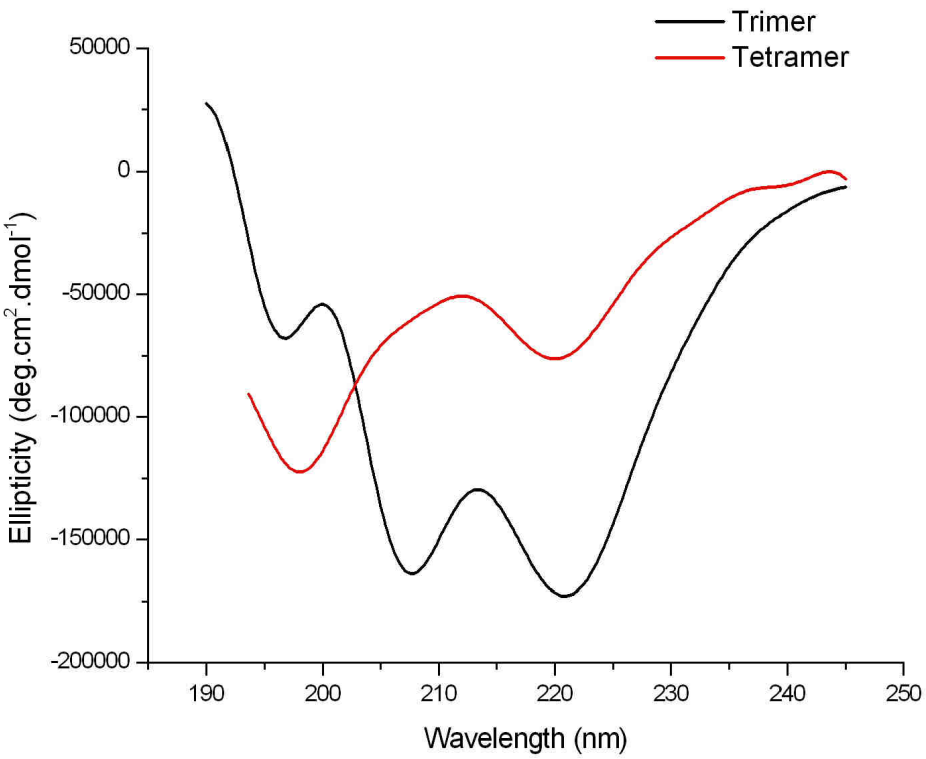


Figure 33: CD spectroscopy of the trimer and tetramer in MeOH at 0.2 mM

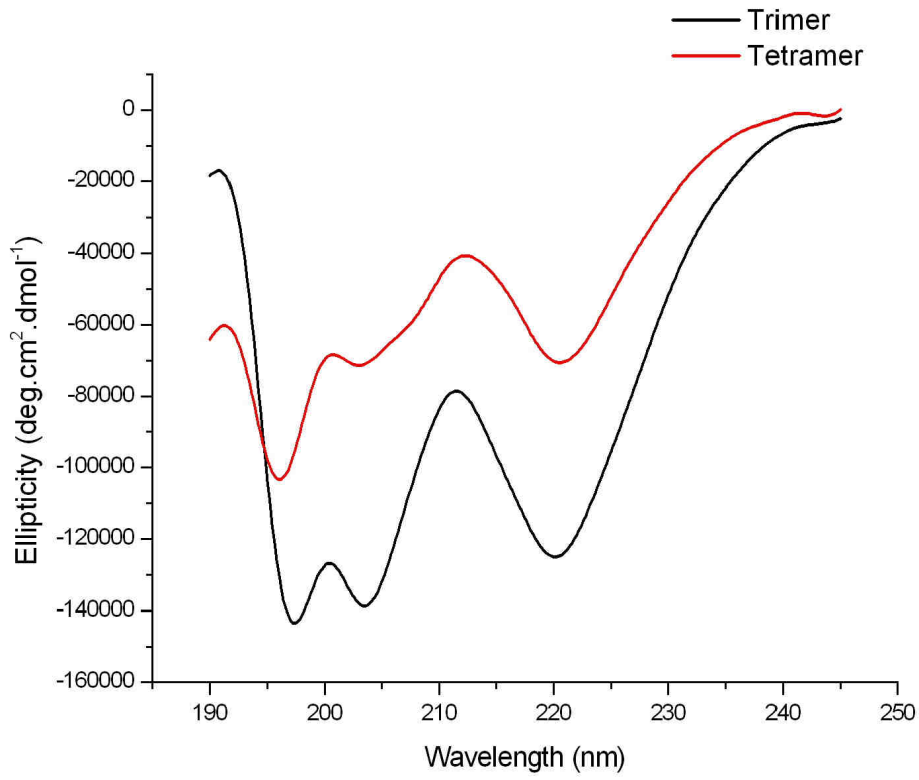


Figure 34: CD spectroscopy of the trimer and tetramer in TFE at 0.2 mM

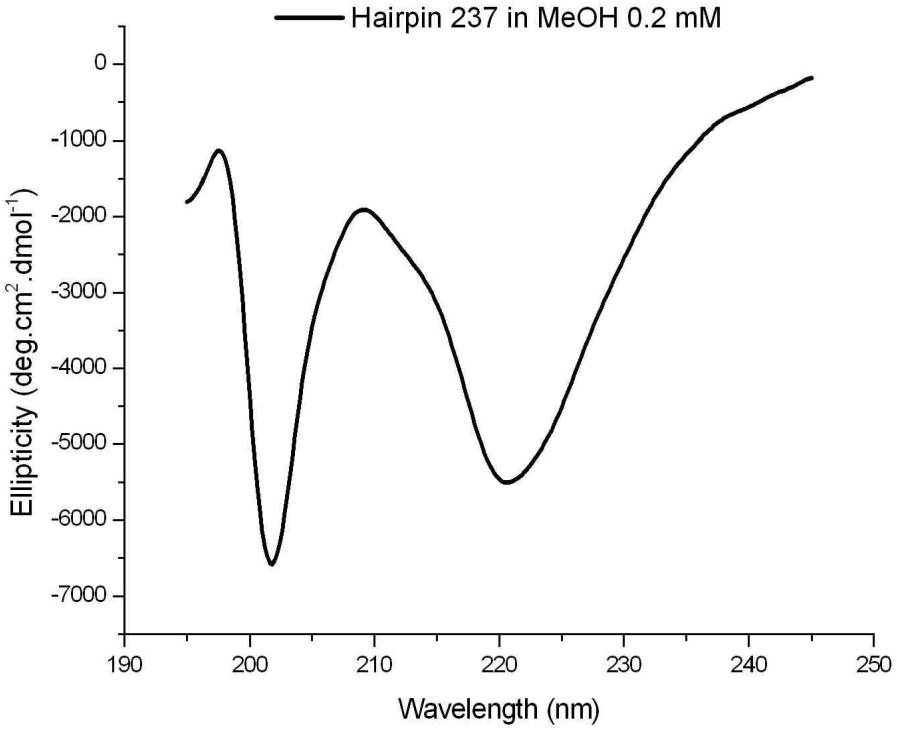


Figure 35: CD spectroscopy of the hairpin **237** containing a diketopiperazine

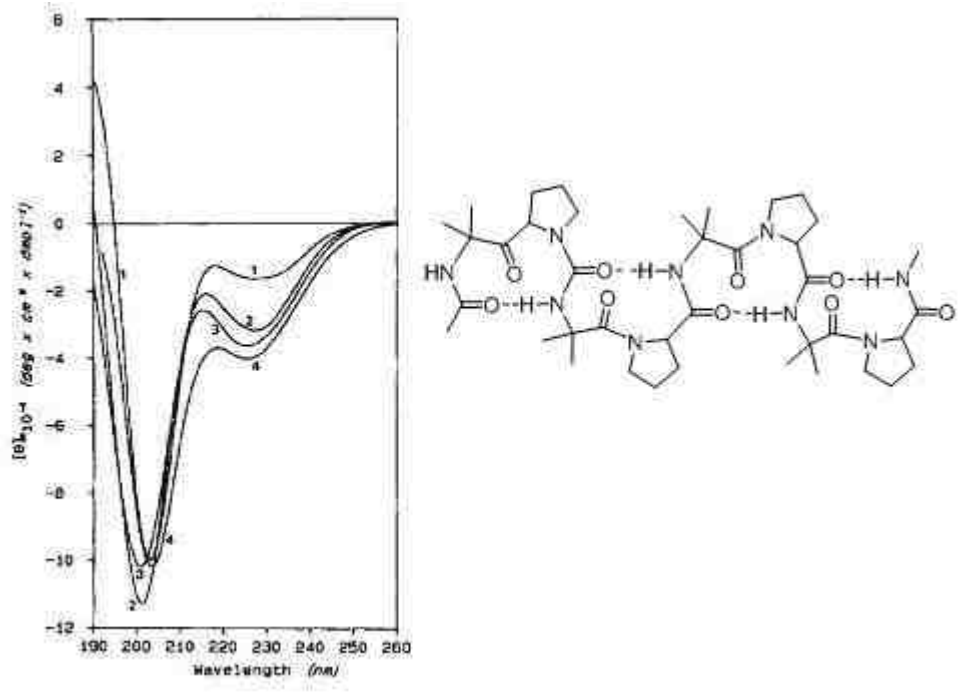


Figure 36: β-bend ribbon by Toniolo[117] exhibiting the same CD spectrum as **254**

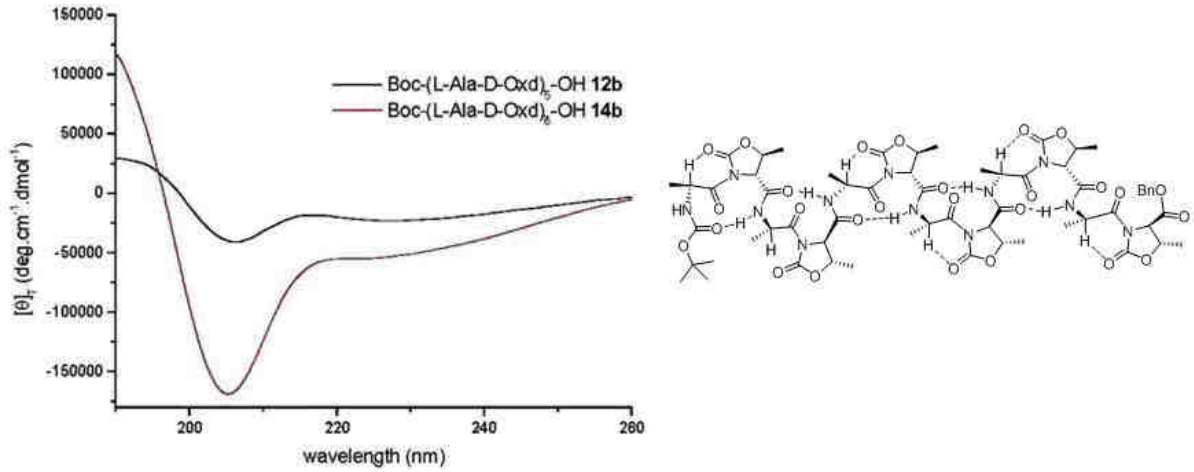


Figure 37: β-bend ribbon CD spectrum by Tomasini[119] et al.

g. Structures of the homopolymers

The secondary structure of the trimer **253** was characterised by 2D-NMR and it was found to adopt a partial β-bend ribbon structure, the central building block not forming the expected ten-membered ring. The secondary structure of the tetramer **254**, on the other hand could not be fully characterised by 2D-NMR because of NMR dispersion problems. Nevertheless, the presence of a ten-membered ring on the N-terminus building block showed that at least one of the building block tended to form a β-bend ribbon. The CD spectroscopy analysis showed that the tetramer had a much more defined secondary structure than the trimer and that it exhibited CD curve typical of a β-bend ribbon structure.

4. Synthesis of cyclic peptides based on the *trans*-diketopiperazine building block

The ability of secondary structure induction of peptides based the *trans*-diketopiperazine **232trans** has been studied in our group.[120] Some linear peptides having one *trans*-diketopiperazine coupled to α-amino acid tripeptides have been synthesised. Even if some NOE contacts had been observed, no particular secondary structure could be deduced, probably because of the *trans*-conformation of both arms which hardly favours secondary structure formation (Figure 38).

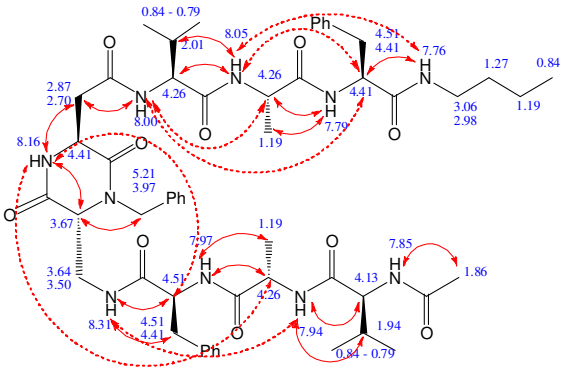
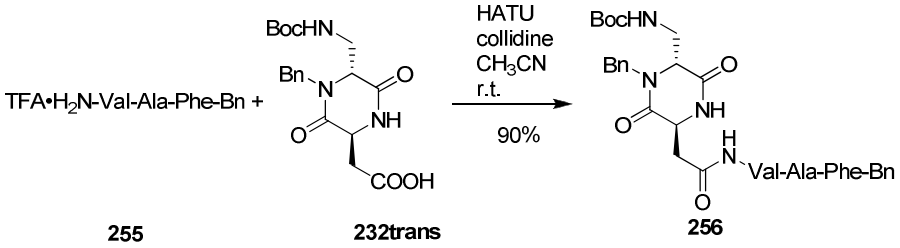


Figure 38: Linear peptide based on the *trans*-DKP scaffold

Nevertheless, still in our group, the successful synthesis of RGD cyclic peptides based on the *trans*-diketopiperazine scaffold with interesting biological activity against integrins motivated us to prepare a simpler cyclic peptide and to study its secondary structure as the *trans*-conformation should favour intramolecular hydrogen bonding and the conformational analysis of a simpler cyclic peptide should give precious informations on its preferred conformation which should not be much different from the cyclic RGD. We chose as well to prepare a cyclic peptide with the *trans*-diketopiperazine and three α-amino acids to have a similar system. The tripeptide Boc-Val-Ala-Phe-Bn being available, it was decided to prepare the cyclic peptide containing this sequence.

a. Coupling of the diketopiperazine with the tripeptide

The trans-diketopiperazine **232trans** was coupled with the TFA salt of the tripeptide **255** using HATU and collidine in acetonitrile affording the desired pentapeptide **256** in excellent yields (90%). (Scheme 79)

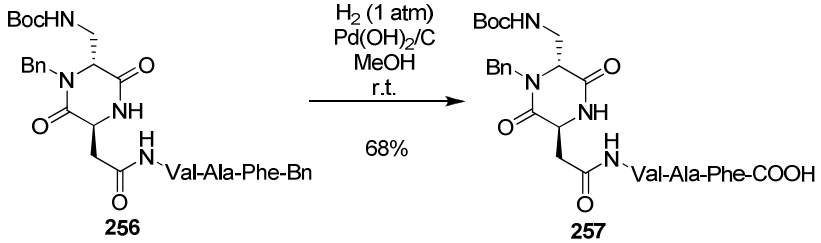


255 **232trans** **256**

Scheme 79: Coupling of the *trans*-diketopiperazine scaffold with the tripeptide

b. Debenzylation of the pentapeptide

The pentapeptide **256** was debenzylated by hydrogenation mediated by $Pd(OH)_2/C$ (10 mass%) in methanol affording the C-deprotected pentapeptide **257** in 68% yield. (Scheme 80)



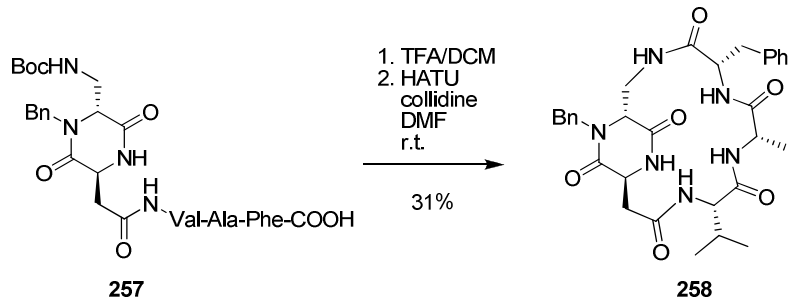
256 **257**

Scheme 80: Debenzylation of the pentapeptide

c. Cyclisation of the peptide

The pentapeptide **257** was first Boc deprotected with TFA/DCM in a quantitative yield. The cyclisation was realised in DMF with HATU and collidine. In order to favour the intramolecular cyclisation, the reaction must be performed at low concentration to avoid intermolecular coupling. The concentration chosen was 2 mM based on the experience of our group on similar reactions. DMF was

prealably degassed by a sequence of vacuum and nitrogen bubbling so that the dimethylamine which could be present in the solution would be removed and, as a consequence, would not parasite the reaction. Seen the unfavourable *trans*-conformation, a long reaction time was required to have the maximum completion of the reaction. The reaction was run for three-four days and the cyclised compound **258** could be obtained in 31% yield. This moderate yield can be explained by the high cycle tension in the peptide making the coupling difficult. (Scheme 81)



257 **258**

Scheme 81: Cyclisation of the pentapeptide

5. Conformational studies of the cyclic peptide

The cyclic pentapeptide **258** was analysed by 2D-NMR, due to its low solubility in $CDCl_3$, methanol-d_3 was used for conformational studies at a concentration of 5 mM. The dispersion of amide protons was satisfying and a NOESY experiment allowed us to define its secondary structure. The proton NMR revealed the presence of a major conformation and of at least a second conformation as some amide protons presented two peaks corresponding to the same residue, particularly the valine residue which had a major peak at 8.52 ppm and a second one at 8.79 ppm. The ratio for both peaks was of 77/23. Plenty of NOE contacts could be seen and revealed a close neighbouring between the α-amino acid residues. The amide A1 of the diketopiperazine, in addition to the protons “a”, had a NOE contact with the amide A2. The intracyclic amide A2 had no long range coupling with protons which were not neighbours except the A1 amide. The major amide peak of A3 (Val H^N) showed a contact with the amide proton A4 (Ala H^N). The amide A4 (Ala H^N) showed NOE contacts with the amide protons A3 major (Val H^N) and A5 (Phe H^N) as well as with all the protons of the isopropyl group. The amide proton A5 (Phe H^N) showed a NOE contact with A4 (Ala H^N) and the methyl protons “k” of the Ala (Figure 39 and 40). All of these NOE couplings show that the cyclic peptide **258** adopts at least two conformations, the major one presenting a close neighbouring of the valine, alanine and phenylalanine residues, the minor being difficult to analyse because of the little intensity of its amide peaks. The structure adopted is very compact due to the *trans*-configuration of the diketopiperazine arms forcing the peptide to adopt a constrained conformation (Figure 41).

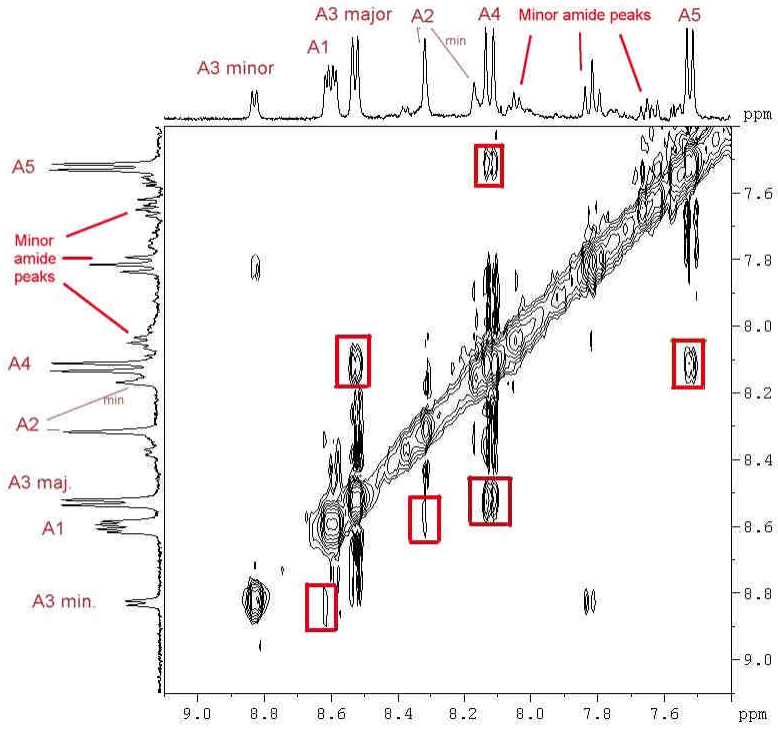


Figure 39: NOESY expansion in the region of the amide protons

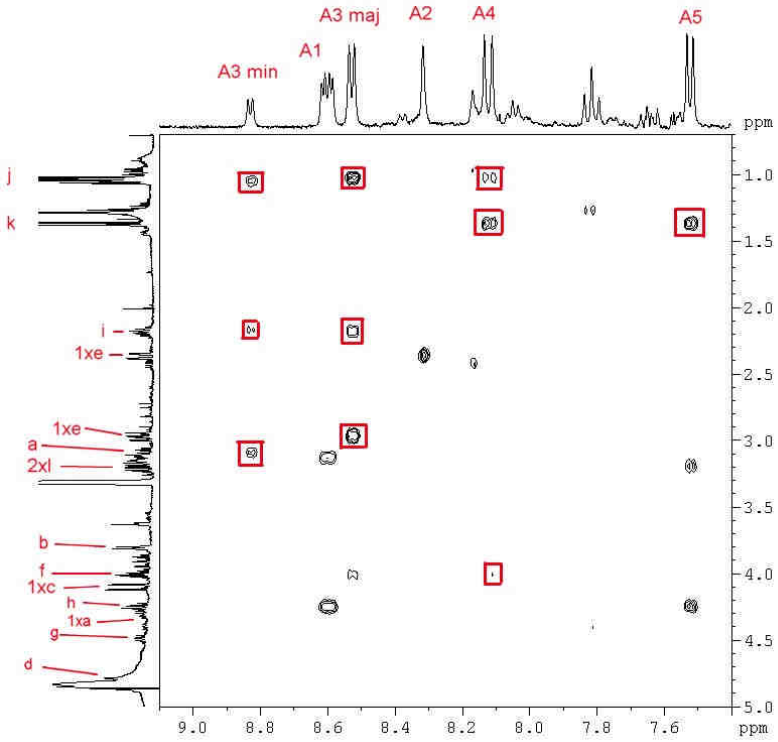


Figure 40: NOESY expansion showing the principal couplings between amides and CH_3, CH_2 and CH protons

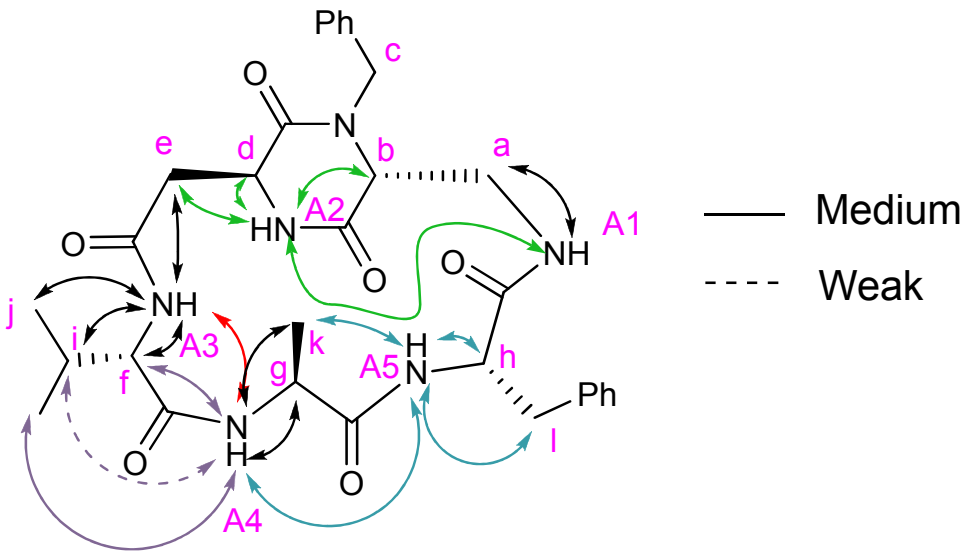


Figure 41: NOE contacts observed in the cyclic peptide

Calculations realised in the group of Laura Belvisi in the University of Milano on the cyclic peptide *cyclo*[DKP-Ala-Gly-Ala] revealed that the minimised cyclic peptide conformation should form a γ-turn between the CO of the DKP and the Gly H^N. Distance measurements on the minimised structure revealed that the Val H^N and Ala H^N was 3.772 Å, the distance between Ala H^N and Phe H^N was 3.814 Å and the distance between A1 and A2 would be 4.071 Å. All these distances are in accordance with the NOE contacts observed and would confirm the formation of a γ-turn between the CO of the DKP linked to Val H^N and the Ala H^N (Figure 42 and 43).

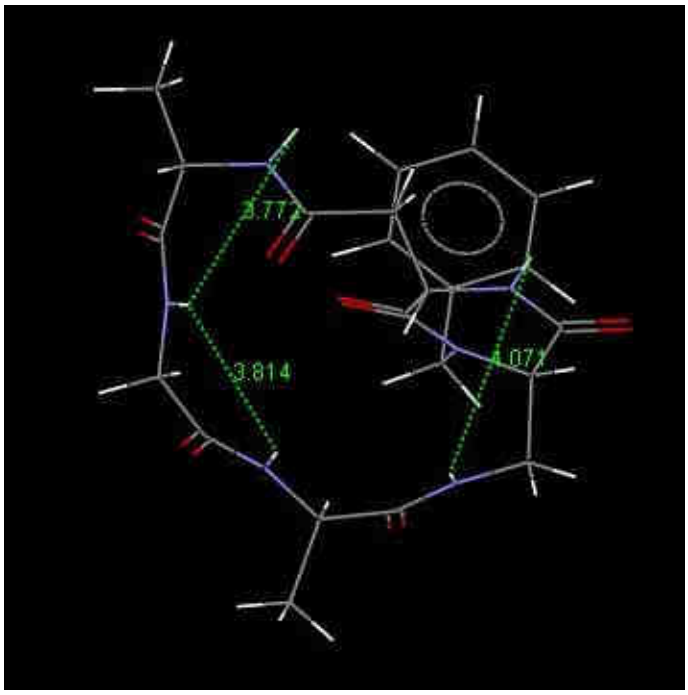


Figure 42: Minimised structure of the *cyclo*[DKP-Ala-Gly-Ala]

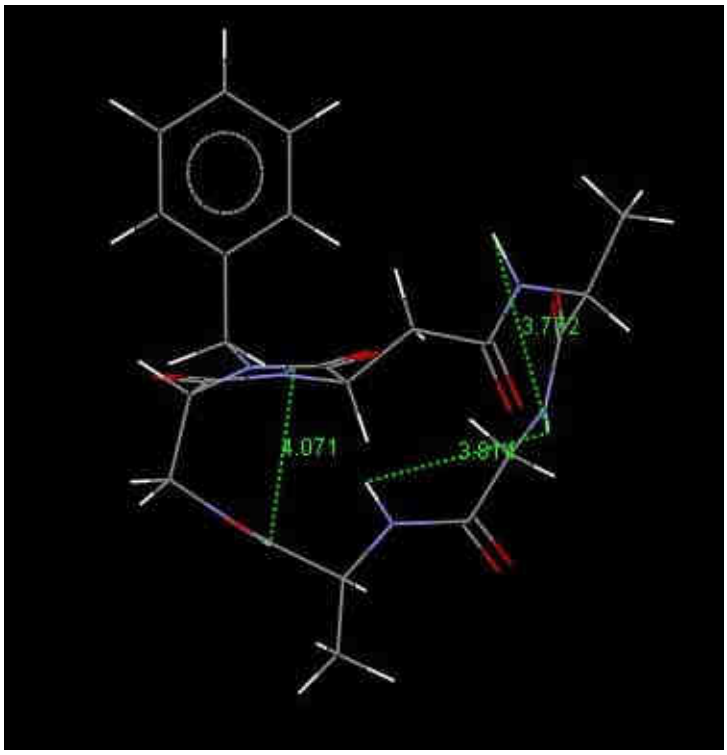


Figure 43: Second view of the minimised structure of the cyclic peptide

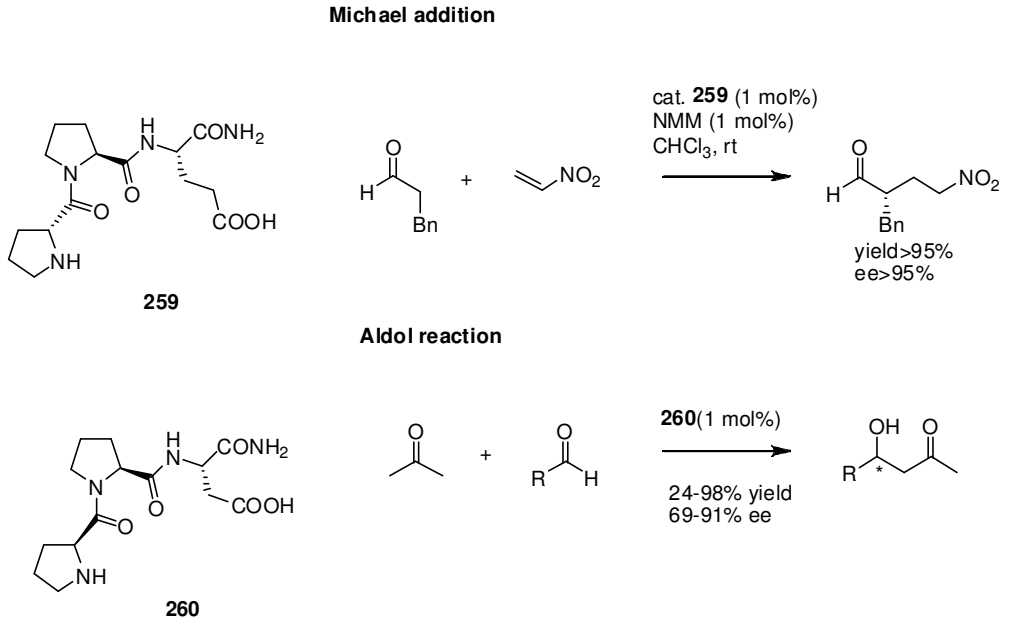
Many rigid cyclic peptides have been developed in the field of RGD sequences and conformational studies have been essential for the better understanding of their activity.[121] One publication of Sewald et al. attracted our attention in particular since it was dealing with the conformational preferences of 2 RGD cyclo-pentapeptides, namely *cyclo*[D-β-Phe-Val-Arg-Gly-Asp] and *cyclo*[D-Phe-β-Leu-Arg-Gly-Asp] containing a scaffold comprising one β-amino acid (either L or D) and one α-amino acid (either D or L), as higher homologs of the well known Kessler's *cyclo*-[RGDfV].[122] The authors showed, by NMR studies and molecular mechanics calculations, that these cyclic peptides were able to adopt a modified γ-turn conformation called $\Psi\gamma$-turn. In particular, *cyclo*[D-β-Phe-Val-Arg-Gly-Asp] presented two turns: a β-turn was adopted by the RGD sequence as well as a $\Psi\gamma$-turn conformation with the β-amino acid in central position. This conformation was essential, according to the authors, to establish a good interaction of the pharmacophoric groups with the integrin receptors for which these derivatives showed binding values in the nanomolar range. The main differences between this peptide and **258**, reside in the fact that Sewald's cyclic RGD contains only one β-amino acid thus forming a 16-membered cyclic peptide while our peptide **258** contains 2 β-amino acids with a 17-membered cyclic peptide. As a consequence, we do not observe exactly the same secondary structure. Moreover, the presence of the *trans*-DKP added more constraints in the peptide favouring only the formation of a γ-turn between the CO of the DKP and the Ala H^N. The data collected by 2D-NMR experiments and computational studies on the *cyclo*[DKP-Ala-Gly-Ala] are in accordance with the presence of a γ-turn in the cyclic peptide **258**. By analogy, we can imagine that a

cyclic RGD peptide containing the *trans*-DKP would as well induce a γ-turn between the CO of the DKP and Gly H^N.

IV. Syntheses of potential organocatalysts based on a diketopiperazine scaffold

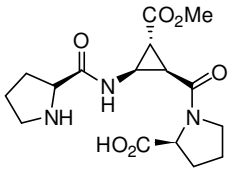
The potential of diketopiperazines as organocatalysts has been described,[106,107] even if some results remain controversial.[108] In organocatalysis, a lot of efforts are now dedicated to asymmetric organocatalysis by simple peptides, especially tripeptides. One of the most notable example is the work of Wennemers et al. who have developed a simple tripeptide H-*D*-Pro-Pro-Glu-NH_2 **259** which exhibited a very high enantioselectivity in Michael addition[123] and the tripeptide H-Pro-Pro-Asp-NH_2 **260** which was active in asymmetric aldol reaction (Scheme 82).[124] The interest of these organocatalysts is their ability to catalyse reactions with very low catalyst loading (1 mol%).

In general, organocatalysts have a specificic activity on a specific substrate and it is relatively hard to find polyvalent catalysts. Catalyst **259**, very active in Michael addition was also active in aldol reaction, but the enantioselectivities observed were lower than with **260**.



Scheme 82: Wennemers' tripeptide organocatalysts

Tripeptide organocatalysts containing proline have proved to be very efficient in organocatalysis as in the work of Reiser et al. where the tripeptide **261** composed of two prolines and a β-amino acid has been described as active in asymmetric aldol reaction. The catalyst loading was relatively high (20 mol%) as it is often the case in organocatalysis but it was effective in intermolecular aldol reaction between acetone and nitrobenzaldehyde as well as on cyclic ketones with aromatic aldehydes and intramolecular aldol reaction with very good yields and ee (Figure 44).[125]



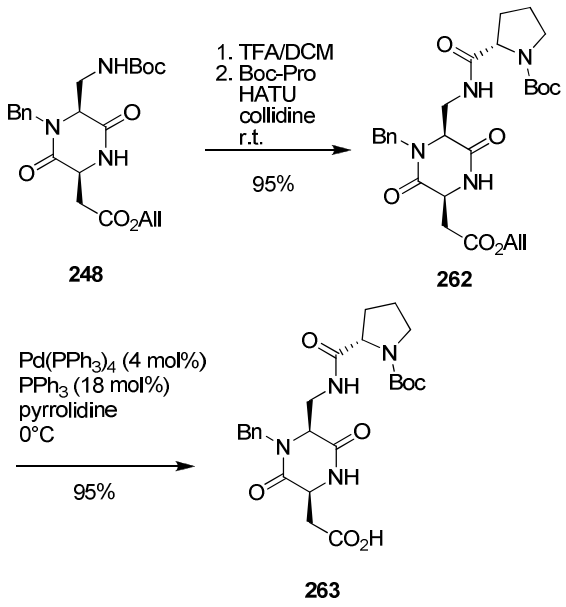
261

Figure 44: Reiser's tripeptide organocatalyst for aldol reaction

Tripeptide catalysts have found many applications in organocatalysis and it is normally accepted that their prefered turn conformation is responsible for their efficiency. It has also been described that these peptides must have a free carboxylic acid and a free amine. Since our diketopiperazine scaffold is a rigid and constrained building block and a dipeptide; it would be interesting to prepare a tripeptide having a proline residue and test it into catalysed reaction as a turn structure could be favoured by the *cis*-conformation. Moreover, we would easily get the free carboxylic acid and the free amine as our building block is Boc protected, giving an easy access to this deprotected tripeptide. It was envisaged to prepare two organocatalysts based on the cis-diketopiperazine coupled by an amide or an ester bond with proline as the changing of the amide bond to the ester bond may change the reactivity or the enantioselectity of the organocatalyst.

1. Amide-bonded organocatalyst synthesis

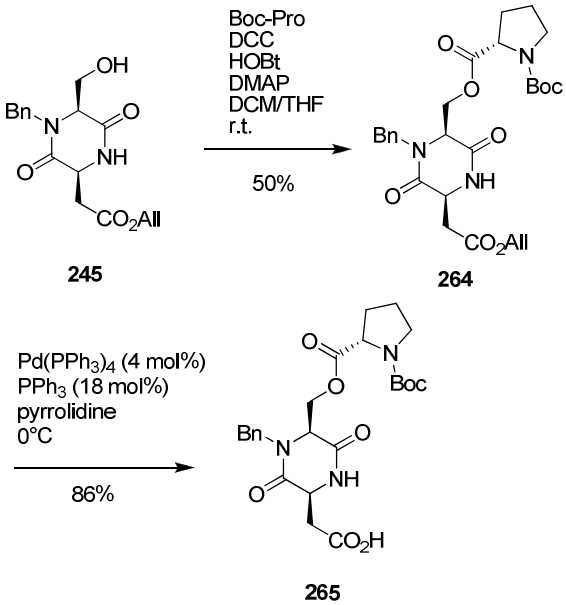
To prepare the organocatalyst having the *cis*-diketopiperazine linked to the proline by an amide bond, the *cis*-diketopiperazine **248** was used as a starting material. Its TFA salt was coupled with proline with HATU and collidine in acetonitrile. The tripeptide **262** was obtained in excellent yields (95%). The deallylation of the tripeptide was realised in the same conditions as **232** with $Pd(PPh_3)_4$, PPh_3 and pyrrolidine affording the free C-terminus tripeptide **263** in 95% yield (Scheme 83).



Scheme 83: Synthesis of amide-bonded organocatalyst

2. Ester-bonded organocatalyst synthesis

To prepare the ester-linked organocatalyst, a different starting material and coupling reagent had to be used. The reaction started from the hydroxydiketopiperazine **245** to which was coupled proline using DCC as a coupling reagent. The proline, DCC, HOBt and DMAP did not dissolve themselves very well in the mixture DCM/THF used and the ester-bonded tripeptide **264** could be obtained in moderate yields (50%). Then, the deallylation catalysed by $Pd(PPh_3)_4$ afforded the C-terminus free tripeptide **265** in very good yields (86%) (Scheme 84).



Scheme 84: Synthesis of the diketopiperazine-ester-bonded organocatalyst

Work is now in progress to investigate their activity in organocatalysed reactions such as enantioselective aldol reaction and Michael addition.

Experimental part

I. Instruments and general techniques

^{1}H-NMR spectra were recorded on Bruker AC 250 (250 MHz), Bruker Avance 300 (300 MHz), Bruker Avance 400 (400 MHz) and Bruker Avance 600 (600 MHz). The chemical shifts are reporter in δ (ppm) relative to chloroform ($CDCl_3$, 7.26 ppm), dimethylsulfoxide (DMSO-d_6, 2.49 ppm), methanol-d_3 (CD_3OH, 3.34 ppm) with presaturation to eliminate attenuate the OH peak and tetramethylsilane (TMS, 0.00 ppm) as an internal standard. The spectra were analysed by first order, the coupling constant (*J*) are reported in Hertz (Hz). Characterisation of signals: s= singlet, bs= broad singlet, d= doublet, t= triplet, q= quartet, m= multiplet, bm= broad multiplet, dd= double doublet, dt= double triplet, ddd= double double doublet. Integration is determined as the relative number of atoms. Diastereoisomeric ratios were determined by comparing the integrals of corresponding protons in the ^{1}H-NMR spectra.

^{13}C-NMR spectra were recorded on Bruker AC 250 (62.9 MHz), Bruker Avance 300 (75.5 MHz), Bruker Avance 400 (100.6 MHz) and Bruker Avance 600 (150.9 MHz). The chemical shifts are reported in δ (ppm) relative to chloroform ($CDCl_3$, 77 ppm), dimethylsulfoxide (DMSO-d_6, 39.52 ppm), methanol-d_3 (CD_3OH, 49 ppm) and tetramethylsilane (TMS, 0.00 ppm) as an internal standard.

2D-NMR spectra (COSY, NOESY, ROESY, HETCORR) were recorded on Bruker Avance 400 (400 MHz) and Bruker Avance 600 (600 MHz).

IR spectra were recorded with a Bio-Rad Excalibur series FT-IR

MS spectra were recorded in the mass spectroscopy departments of Regensburg and Milan

Optical rotations were measured on a Perkin-Elmer-polarimeter 241 with sodium lamp at 589 nm in the specified solvent.

CD spectra were measured on a JASCO model J-710/720 at the Institute of Bioanalytic and Sensoric of the University of Regensburg at 21°C between 250 and 190 nm in the specified solvent, with 10 scans. The length of the rectangular cuvette was 0.1 mm, the resolution was 0.2 nm, the band width 1.0 nm, the sensitivity 10-20 mdeg, the response 2.0 s, the speed 10 nm/min. The background was substracted for each spectrum. The absorption value is measured as molar ellipticity per residue ($deg.cm^2.dmol^{-1}$). The spectra were smoothed by adjacent averaging algorithm or FFT filter with the Origin 6.0 program.

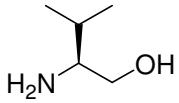
Thin layer chromatography (TLC) was performed on alumina plates coated with silica gel (Merck silica gel 60 F 254, layer thickness 0.2 mm) or glass plates coated with flash chromatography silica gel (Merck silica gel 60 F 254, layer thickness 0.25 mm). Visualisation was accomplished by UV light

(wavelength λ= 254 nm), permanganate solution, ninhydrin/acetic acid solution, vanillin/H_2SO_4 solution and paramethoxybenzaldehyde solution.

In Regensburg, solvents were purified according to standard laboratory method. THF was distilled over sodium/benzophenone before use. After distillation, dry THF was stored in a Schlenk flask under nitrogen over molecular sieves 4 Å. BF_3•Et_2O was distilled under nitrogen and stored in a Schlenk flask under nitrogen in the refrigerator. Diethyl ether and dichloromethane were purified by a solvent purification system apparatus. In Como, all dry solvents were obtained from sealed bottles under nitrogen purchased from Aldrich. DMF was degassed by two sequences of nitrogen bubbling for 15 minutes and vacuum. All reactions with oxygen or moisture sensitive reactants were performed under nitrogen atmosphere.

II. Synthesis of compounds

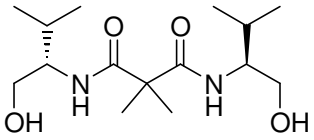
1. Synthesis of δ-amino acids



(L)-amino-3-methylbutan-1-ol

To a solution of $NaBH_4$ (8.1g, 214 mmol, 2.5 eq.) in dry THF (135 mL) was added L-valine (10.0 g, 85.3 mmol, 1.0 eq.) under nitrogen atmosphere. The reaction mixture was cooled to 0℃ in an ice bath and a solution of iodine (21.6 g, 85.3 mmol, 1.0 eq.) in dry THF (50 mL) was slowly added over 1 h, resulting in production of hydrogen. After gas ceased, the reaction mixture was refluxed for 20 h and then cooled to room temperature. Methanol was added cautiously until the stirred solution became clear. The solution was stirred for 30 minutes and concentrated in vacuo to give a white paste, which was dissolved in 20% aqueous KOH (50 mL).The solution was further stirred for four hours and extracted with DCM (3 x 140 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to afford L-valinol (8.15 g, 92%) as a colourless oil.

^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.64 (dd, *J*= 10.6, 8.7Hz, 1H),3.31 (dd, *J*= 10.6, 8.7 Hz, 1H), 2.57 (ddd, *J*= 8.6, 6.4, 3.9 Hz, 1H), 2.20 (bs, 2H), 1.5-1.7 (m, 1H), 0.93 (d, *J*= 6.8 Hz, 3H), 0.91 (d, *J*= 6.8 Hz, 3H).



(-)-(S, S)-N,N'-bis-(1-hydroxymethyl-2-methyl-propyl)-2,2-dimethyl-malonamide

To a cold solution (0°C) of L-valinol (15.4 g, 150.0 mmol, 2.0 eq.) in dry DCM (150 mL) were slowly added triethylamine (52.3 mL, 375 mmol, 5 eq.) and a solution of 2,2-dimethylmalonyl dichloride (10 mL, 75 mmol, 1 eq.) in dry DCM (70 mL). Then, the ice bath was removed and the reaction mixture was stirred for 45 minutes to room temperature, resulting in a colorless precipitate which was dissolved again by addition of dry DCM (350 mL). After addition of 1M HCl (100 mL), the aqueous layer was separated and and extracted with DCM (3 x 50 mL). The combined organic layers were washed with saturated $NaHCO_3$ (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. Crystallisation of the crude product from ethyl acetate (100 mL) and subsequent recrystallisation of the residue of the mother liquor afforded the dimethylmalonamide (18.76 g, 83%) as colourless crystals.

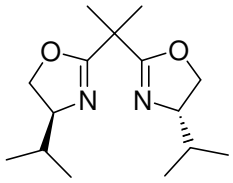
R_f = 0.26 (SiO_2, EA/MeOH 95:5); m.p. = 98-99°C; $[\alpha]_D^{20}$ = -6.3 (c=0.50, DCM).

^{1}H NMR (250 MHz, $CDCl_3$): δ = 6.41 (d, *J*= 8.8 Hz, 2H), 3.84-3.72 (m, 4H), 3.56-3.48 (m, 2H), 3.21 (bs, 2H), 1.80 (hept., *J*= 6.8 Hz, 2H), 1.49 (s, 6H), 0.95 (d, *J*= 6.74, 6H), 0.92 (d, *J*= 6.74 Hz, 6H)

^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 174.6, 64.0, 57.2, 50.1, 29.1, 23.6, 19.7, 18.8

IR (KBr): $\bar{\upsilon}$ = 3326, 2963, 2877, 1642, 1543, 1391, 1368, 1287, 1186, 1071, 1024, 899, 651 cm^{-1}

MS (DCI, NH_3): m/z (%) = 304.5 (16), 303.5 (100) [$M+H^+$].



188

(-)-(S,S)-isopropylbisoxazoline (188):

To a mixture of (-)-(S, S)-N,N'-bis-(1-hydroxymethyl-2-methylpropyl)-2,2-dimethylmalonamide (18.76 g, 620.0 mmol, 1.0 equiv.) and 4-dimethylamino pyridine (0.75 g, 6.2 mmol, 0. 1 equiv.) in dry CH_2Cl_2 (400 mL) was slowly added triethylamine (37.6 mL, 270.0 mmol, 4.4 equiv.) over 15 min. Subsequently a solution of tosyl chloride (23.65 g, 124.0 mmol, 2.0 equiv.) in dry CH_2Cl_2 (50 mL) was added dropwise via an addition funnel. The reaction mixture was stirred for additional 48 h at room temperature where the color changed to yellow and a cloudy precipitate occurred. The precipitate was dissolved in CH_2Cl_2 (150 mL). The reaction mixture was then washed with saturated NH_4Cl (250 mL) followed by water (150 mL) and saturated $NaHC0_3$ (200 mL). The combined aqueous layers were extracted with CH_2Cl_2 (3x200 mL) and the combined organic layers were dried over Na_2SO_4. After filtration and concentration in vacuo the residue was purified by hot n-pentane extraction to afford **188** (7.466 g, 44%) as a colorless oil.

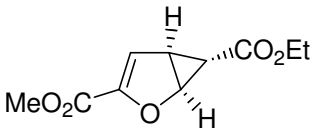
R_f = 0.26 (SiO_2, CH_2Cl_2/MeOH 19: 1); $[\alpha]_D^{20}$ = -108.1 (c = 1.01, CH_2Cl_2)

^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.27-4.09 (m, 2 H), 4.04-3.92 (m, 4 H), 1.91-1.72 (m, 2 H), 1.52 (s, 6 H), 0.92 (d, *J*= 6.84 Hz, 6 H), 0.85 (d, *J*= 6.79 Hz, 6 H);

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 168.8, 71.5, 69.9, 38.6, 32.2, 24.4, 18.5, 17.3

IR (Film): $\tilde{\nu}$ = 3411, 3225, 2960, 1660, 1468, 1385, 1352, 1301, 1247, 1146, 1109, 980, 925, 795, 737 cm^{-1}

MS (DCI, NH_3): m/z (%) = 391.6 (7), 313.5 (7), 268.4 (17), 267.4 (100) [M + NH_4^+]



189

(1S,5S,6S)-(-)-2-Oxabicyclo[3.1.0]hex-3-ene-3,6-dicarboxylic 6-ethylester-3-methyl ester (189)

In a three-neck flask equipped with a slow addition funnel under nitrogen at 0°C were added successively 53.2 g (421 mmol, 1 eq.) of furan-methylester, 0.88 g (3.32 mmol, 0.008 eq.) of ligand **188** and 1.08 g (2.98 mmol, 0.007 eq.) of $Cu(OTf)_2$. The inner walls of the flask were rinsed with a few millilitres of dry DCM to allow all of the copper to dilute in the solution. After 10 minutes, three drops of phenylhydrazine were added in the solution which turned from a deep blue to a dark red colour testifying of the reduction of the metal complex. The slow addition funnel was then filled with 500 mL of a solution of diazoacetate in DCM (153.97g.L^{-1}, 76.98 g, 675 mmol, 1.6 eq.). After half an hour, the solution was added dropwise at the frequency of one drop every 6 seconds and the reaction was let at 0°C for four days. Once the solution was totally added, the reaction mixture was filtered on a 10 cm pad of basic alumina and washed with 500 mL of DCM. The solution was evaporated under vacuum and column chromatographed on silica gel with a solution hexanes/Ethyl acetate 5:1 as eluent. The fractions were collected and the solvent evaporated under vacuum. The product was obtained as a slightly yellow oil and was crystallised in pentane/DCM affording 29.6 g (139.5 mmol, 33%) of enantiomerically pure white crystals (ee>99%).

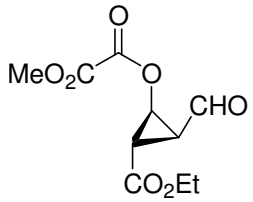
Rf (PE/EE 5:1)= 0.14; m.p. 42 °C; $[\alpha]_D^{20}$ = -272 (c=1.0, CH_2Cl_2)

^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.16 (dd, *J*=2.7, 1.1 Hz, 1H), 1.23 (t, *J*=7.1 Hz, 3H), 2.87 (ddd, *J*=5.3, 2.9, 2.7 Hz, 1H), 3.78 (s, 3H), 4.12 (q, *J*=7.1 Hz, 2H), 4.97 (dd, *J*=5.3, 1.1 Hz, 1H), 6.39 (d, *J*=2.9 Hz, 1H)

^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 14.2, 21.5, 31.9, 52.1, 61.0, 67.5, 116.0, 149.3, 159.5, 171.7

IR (KBr): $\bar{\upsilon}$ = 3118, 2956, 1720, 1617, 1428, 1380, 1297, 1166, 1124, 1041, 954, 831, 725 cm^{-1}

MS (70 eV, EI): m/z (%): 212.1 [M^+] (9.8), 153.0 [M^+-CO_2Me] (11.5), 139.0 [M^+-CO_2Et] (100), 124.9 (24.4), 98.9 (28.6), 96.9 (31.7), 78.9 (11.3), 59.0 (13.5), 52.1 (11.5); elemental analysis calcd (%) for $C_{10}H_{12}O_5$ (212.2): C 56.60, H 5.70; found C 56.51, H 5.73.



190

(1S,2S,3S)-(-)-Oxalic acid 2-ethoxycarbonyl 3-formyl-cyclopropyl ester methyl ester (190):

A solution of **189** (2.50 g, 11.78 mmol) in dry CH_2Cl_2 (125 mL) was cooled to -78°C and treated with ozone until the mixture turned blue. Excess ozone was expelled by passing oxygen through the solution, followed by addition of dimethyl sulfide (4.3 mL, 58.91 mmol, 5.0 equiv). The reaction mixture was allowed to warm to room temperature and stirring was continued for 24 h. Saturated $NaHCO_3$ (10 mL) was added and layers were separated. The organic layer was washed with water (2x10 mL), dried, filtered and evaporated. The residue was recrystallized from Et_2O at -27°C to yield **190** as a colourless solid (2.70 g, 94%).

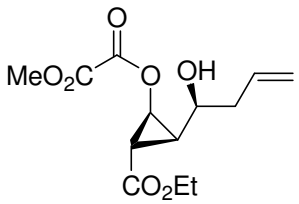
M.p. 52°C; $[\alpha]_D^{20}$=-37.7 (c=1.0, CH_2Cl_2)

^{1}H NMR (250 MHz, $CDCl_3$): δ=1.28 (t, *J*=7.1 Hz, 3H), 2.79 (ddd, J_7.3, 6.0, 4.0 Hz, 1H), 2.90 (dd, *J*=6.0, 3.6 Hz, 1H), 3.91 (s, 3H), 4.19 (q, *J*=7.1 Hz, 2H), 4.83 (dd, *J*=7.3, 3.6 Hz, 1H), 9.45 (d, *J*=4.0 Hz, 1H)

^{13}C NMR (62.9 MHz, $CDCl_3$): δ=14.1, 26.4, 34.9, 54.0, 58.9, 62.0, 156.6, 156.9, 168.1, 192.7

IR (KBr): $\bar{\upsilon}$= 3066, 3015, 2963, 2892, 1785, 1751, 1735, 1706, 1445, 1345, 1313, 1210, 1167, 1086, 1011, 963, 867, 790, 715, 613, 495 cm^{-1}

MS (DCI, NH_3): m/z (%): 262.0 [M^++NH_4] (100), 176.0 (20), 160.0 (55), 120.9 (15); elemental analysis calcd (%) for $C_{10}H_{12}O_7$ (244.2): C 49.19, H 4.95; found C 49.22, H 4.99.

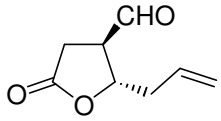


191

(1S, 1'S/R,2S, 3S)-Oxalicacid-hydroxy-but-3'-enyl-3-ethoxycarbonyl-cyclopropylester methylester (191):

A solution of **190** (5.00 g, 20.5 mmol) in dry CH_2Cl_2 (200 mL) was treated with BF_3•Et_2O (3.0 mL, 20.5 mmol) at -78°C. After 10 minutes allyltrimethylsilane (5.0 mL, 30.75 mmol, 1.5 equiv) was added and stirring was continued for 24 h. The reaction was quenched with saturated $NaHCO_3$ (6.0 mL) and the mixture was allowed to warm to 0°C. After separation of the organic layer and drying with $MgSO_4$, the solvent was evaporated under vacuum to yield the corresponding alcohol **191** as a colourless oil (5.82 g, 100% crude yield, dr 95:5).

^{1}H NMR (250 MHz, $CDCl_3$): δ=1.25 (t, *J*=7.0 Hz, 3H), 1.81 ± 1.92 (m, 1H), 2.15 (dd, *J*=6.2, 2.7 Hz, 1H), 2.31 ± 2.51 (m, 4H), 3.70 (ddd, *J*=7.3, 7.3, 5.4 Hz, 1H), 3.88 (s, 3H), 4.13 (q, *J*=7.0 Hz, 2H), 4.72 (dd, *J*=7.5, 2.8 Hz, 1H), 5.14 ± 5.22 (m, 2H), 5.76 ± 5. 93 (m, 1H), characteristic signals of the diastereomer: δ=4.14 (q, *J*=7.0 Hz, 2H), 4.67 (dd, *J*=6.9, 3.0 Hz, 1H).



194

(2S/R,3S)-2-Allyl-5-oxotetrahydrofuran-3-carbaldehyde (194):

A solution of **191** (6.15 g, 21.48 mmol, 1 eq.) in 100 mL dry MeOH was put in an ice bath. 5.96 mL of triethylamine (4.35 g, 42.99 mmol, 2 eq.) were then slowly added and the reaction was let at 0°C for two hours. The ice bath was then removed and the reaction was warmed to room temperature until no more evolution could be seen on TLC. The reaction mixture was evaporated under vacuum and the residue was chromatographed on silica gel (hexanes/ethyl acetate 1:1) and afforded 2.21g of **194** (yield=67%) as a slightly yellow coloured oil with a diastereomeric ratio of 95/5. After the reaction, the stable intermediate **193** could be isolated and fully characterised in a diastereomeric ratio of 80/20.

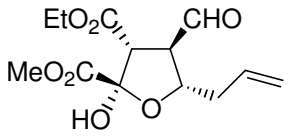
Rf (hexanes/EA 1:1) = 0.17; $[\alpha]_D^{20}$ =-31.7 (c=1.35 in CH_2Cl_2)

^{1}H NMR (250 MHz, $CDCl_3$): δ=2.35 ± 2.59 (m, 2H), 2.71 (dd, J_1=18.2, J_2=9.9 Hz, 1H), 2.89 (dd, J_1=18.2, J_2=7.5 Hz, 1H), 3.19 (dddd, *J*=10.0, 7.3, 6.0, 1.2 Hz, 1H), 4.74 (dd, *J*=11.9, 6.2 Hz, 1H), 5.10 ± 5.27 (m, 2H), 5.75 (dddd, *J*=17.3, 10.0, 7.0, 3.5 Hz, 1H), 9.69 (d, *J*=1.2 Hz, 1H), characteristic signals of the diastereomer (2R): δ = 3.00 (dd, *J*=17.7, 5.8 Hz, 1H), 9.82 (d, *J*=1.7 Hz, 1H)

^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 197.3, 174.0, 130.9, 120.5, 78.0, 51.3, 39.2, 28.9, characteristic signals for the minor compound: δ = 198.0, 131.3, 120.0, 49.6, 39.4, 28.7

IR (film): $\bar{\upsilon}$ = 3080, 2980, 2939, 2841, 1774, 1727, 1642, 1419, 1359, 1193, 1111, 1000, 924 cm^{-1}

MS (EI, 70 eV): m/z (%): 154.2 (5) [M^+], 113.1 (100) [M^+-C_3H_5], 85.1 (95), 57.1 (95); elemental analysis calcd (%) for $C_8H_{10}O_3$ (154.2): C 62.33, H 6.54; found: C 62.36, H 6.83.



193

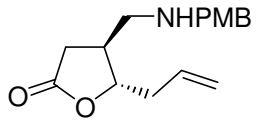
(2R/S, 3S, 4S, 5S)-5-Allyl-4-formyl-2-hydroxy-tetrahydro-furan-2,3-dicarboxylic acid 3-ethyl ester 2-methyl ester (193)

Rf (hexanes/EA 1:1) = 0.67

^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 9.79 (d, *J* = 1.4 Hz, 0.8H, major), 9.75 (d, *J* = 1.9 Hz, 0.2H, minor), 5.83-5.68 (m, 1H), 5.22-5.13 (m, 2H), 4.50 (s, 0.2H, minor), 4.46 (s, 0.8H, major), 4.37-4.07 (m, 3H), 3.95 (dd, *J* = 11.5H, *J* = 1.2 Hz, 1H), 3.87 (s, 2.4H, major), 3.79 (s, 0.6H, minor), 3.65-3.57 (ddd, *J* = 1.4 Hz, *J* = 9.0 Hz, *J* = 11.5 Hz, 1H), 2.77-2.44 (m, 2H), 1.22-1.17 (t, *J* = 7.13 Hz, 3H)

^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 198.3, 169.5, 167.3, 132.7, 119.0, 100.3, 80.6, 61.6, 55.9, 53.9, 53.6, 40.8, 13.9

MS (LR): m/z (%): 304.2 (100) [$M+NH_4^+$], 286.3 (7.35) [$M+NH_4^+-H_2O$]



195

(**4S,5S)-5-Allyl-4-[(4-methoxy-benzylamino)-methyl]-dihydro-furan-2-one (195):**

To a solution of **194** (880 mg, 5.24 mmol, 1.0 eq.) in dry DCM (50 ml) under nitrogen were added sequentially sodium sulfate (1.49 gr, 10.48 mmol, 2.0 eq.) and 4-methoxybenzylamine (750 µL, 5.77 mmol, 1.1 eq.). The reaction mixture was stirred at room temperature for 3 h. To the resulting slightly yellow solution were added $NaBH_4$ (397 mg, 10.48 mmol, 2.0 eq.) and the flask was placed in an ice bath. Dry MeOH (15 ml) was then added dropwise to the reaction mixture which was stirred for further 30 min at 0°C and thereafter filtrated on a celite pad. The solution was cooled to 0°C in an ice bath and a 1M solution of HCl was added until the pH solution was 1. The organic phase was separated and extracted with 1M solution of HCl (2 x 50 ml). The combined aqueous layers were washed with DCM (2 x 50 ml) and cooled at 0°C in an ice bath. Solid sodium hydrogen carbonate was added until the pH of the solution was 8. The aqueous phase was then extracted with diethyl ether (3 x 80 mL), the combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo to give **195** as a slightly yellow oil (1.101 g, 4.00 mmol, 76%) which was used for the next step without further purification. The lactame **196** could be identified as a by-product of the reaction and fully characterised.

R_f = 0.40 (SiO_2, ethylacetate); $[\alpha]_D^{20}$ = -19.42 (c=1.04, DCM)

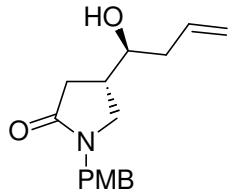
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.23 (dd, *J* = 11.5, 8.5 Hz, 2H), 6.89-6.81 (m, 2H), 5.79 (m, 1H), 5.20-5.13 (m, 2H), 4.34 (dd, *J* = 11.7, 5.4 Hz, 1H), 3.80 (s, 3H), 3.71 (s, 2H), 2.73-2.61 (m, 2H), 2.53-2.20 (m, 5H)

^{13}C-NMR (75.5 MHz, $CDCl_3$): δ = 176.4, 158.8, 132.4, 132.0, 129.2, 119.0, 113.9, 83.1, 55.3, 53.3, 51.4, 39.9, 39.1, 33.2

IR (KBr): $\tilde{\nu}$ = 3329, 3070, 2924, 2831, 2357, 2057, 1772, 1679, 1611, 1508, 1454, 1288, 1176, 1029, 985, 819

MS (CI, NH_3): m/z (%) = 276.3 (100) [M+ H^+]

HRMS (EI, 70 eV): Calculated for [$C_{16}H_{21}NO_3$]: 275.1521, found 275.1515 [M^+]



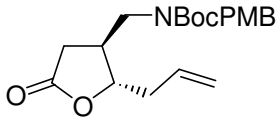
196

4-(1'-Hydroxy-but-3'-enyl)-1-(para-methoxy-benzyl)-pyrrolidin-2-one (196)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.18-7.15 (d, *J* = 8.7 Hz, 2H), 6.87-6.84 (d, *J* = 8.7 Hz, 2H), 5.81-5.68 (dddd, *J* = 6.2 Hz, *J* = 8.2 Hz, *J* = 10.4 Hz, *J* = 16.6 Hz, 1H), 5.19-510 (m, 2H), 4.42-4.32 (d, *J* = 4.2 Hz, 2H), 3.81 (s, 3H), 3.60-3.53 (m, 1H), 3.29-3.23 (dd, *J* = 8.4Hz, *J* = 9.7Hz, 1H), 3.12-3.06 (dd, *J* = 6.6Hz, *J* = 9.8Hz, 1H), 2.54-1.98 (m, 6H)

^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.9, 159.1, 133.9, 129.5, 128.5, 119.1, 114.1, 71.5, 55.3, 48.6, 45.9, 39.7, 36.5, 32.9

MS (LR): m/z (%): 293.2 (9.6) [M+NH_4^+], 276.2 (100) [M+H^+]



197

(2S,3S)-(2-Allyl-5-oxo-tetrahydro-furan-3-ylmethyl)-(4-methoxy-benzyl)-carbamic acid tert-butyl ester (197)

To a solution of **195** (1.28 g, 4.65 mmol, 1.0 eq.) in dioxane / 1M aqueous solution of K_2CO_3 (12 mL / 16 mL) was added di-*tert*-butyldicarbonate (1.52 g, 6.99 mmol, 1.5 eq.). The reaction mixture was stirred at RT overnight and extracted with EtOAc (3 x 50 ml). The combined organic layers were washed with 10% aqueous solution of citric acid (2 x 30 ml) and brine (2 x 30 ml), dried over Na_2SO_4, filtrated and evaporated in vacuo to give a slightly yellow oil which was purified by column chromatography (PE:EtOAc 3:1) to afford **197** (1.294 g, 3.44 mmol, 74%)as a colourless oil.

R_f = 0.35 (SiO_2, PE:EtOAc 3:1) ; $[\alpha]_D^{20}$ = -10.37 (c = 0.96, DCM)

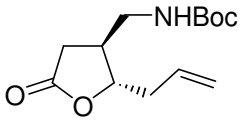
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.20-7.11 (m, 2H), 6.92-6.84 (m, 2H), 5.85-5.67 (m, 1H), 5.23-5.10 (m, 2H), 4.40 (s, 2H), 4.24 (bs, 1H), 3.80 (s, 3H), 3.26 (bs, 2H), 2.59-2.22 (m, 5H), 1.50 (s, 9H)

^{13}C-NMR (75.5MHz, $CDCl_3$): δ = 175.7, 159.1, 155.8, 132.1, 129.6, 128.8, 119.1, 114.1, 82.5, 80.7, 55.3, 48.4, 39.9, 38.6, 33.0, 28.4

IR (film): $\bar{\upsilon}$ = 3076, 2976, 2931, 2837, 2372, 1778, 1690 cm^{-1}

MS (EI, 70 eV): m/z (%) = 375.3 (100) [M^+]

HRMS (EI, 70 eV): Calculated for [$C_{21}H_{29}NO_5$]: 375.2046, found 375.2046 [M^+]



198

(2S,3S)-(-)-(2-Allyl-5-oxo-tetrahydro-furan-3-ylmethyl)-carbamic acid tert-butyl ester (198)

To a cold (0°C) solution of **197** (633 mg, 1.69 mmol, 1.0 eq.) in water / acetonitrile (7mL / 21mL), cerium ammonium nitrate (3.70 g, 6.75 mmol, 4.0 eq, c = 0.25 M) was added. The reaction was stirred at 0°C for 1 h and then at RT for additional 2 h until total consumption of the starting material. Water (30 ml) was added and the aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with a saturated solution of $NaHCO_3$ (30 mL), dried with $MgSO_4$, filtrated and evaporated in vacuo to give an oil which was purified by column chromatography (PE:EtOAc 3:1) to afford **198** (340 mg, 1.33 mmol, 79%) as a colourless solid.

R_f = 0.29 (SiO_2, PE:EtOAc 3:1) ; $[\alpha]_D^{20}$ = -21.58 (c = 1.01, DCM)

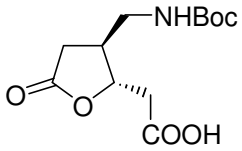
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 5.81 (ddd, *J*= 17.8, 16.5, 7.1 Hz, 1H), 5.25-5.15 (m, 2H), 4.72 (bs, 1H), 4.31 (dd *J* =11.8, 5.8 Hz, 1H), 3.33-3.17 (m, 2H), 2.68 (dd *J* =17.2, 8.1 Hz, 1H), 2.59-2.42 (m, 3H), 2.34 (dd *J*=17.3, 7.1 Hz, 1H), 1.41 (s, 9H)

^{13}C-NMR (75.5MHz, $CDCl_3$): δ = 175.7, 156.0, 131.0, 119.3, 82.3, 80.0, 40.2, 38.7, 38.5, 28.3

IR (KBr): $\bar{\upsilon}$ = 3474, 2976, 2931, 2837, 2372, 1778, 1690, 1168, 910, 855

MS (CI, NH_3): m/z (%) = 273.2 (71.32) [$M+NH_4^+$], 256.1 (1.07) [MH^+]

Elementar analysis calcd (%) for [$C_{13}H_{21}NO_4$]: C 61.16, H 8.29, N 5.49; found C 61.04, H 7.86, N 5.35



199

(-)-(2S,3S)-[3-(tert-Butoxycarbonylamino-methyl)-5-oxo-tetrahydro-furan-2-yl]-acetic acid (-)-GBA (200)

To a cold (0°C) solution of **198** (340 mg, 1.33 mmol, 1.0 eq.) in water / acetonitrile / carbon tetrachloride (6 mL / 3 mL / 3 mL) were added sodium periodate (1.14 g, 5.32 mmol, 4 eq.) and $RuCl_3 \bullet xH_2O$ (25 mg, 0.09 mmol, 0.07 eq.). The solution was stirred for 1 h at 0°C and for 2 h at RT. Water (10 ml) was added and the solution was then extracted with diethyl ether (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtrated through a celite pad and evaporated in vacuo to obtain **199** (317 mg, 1.15 mmol, 86%) as a colourless solid.

R_f = 0.15 (SiO_2, EtOAc); $[\alpha]_D^{20}$ = -7.41 (c = 1.01, DCM)

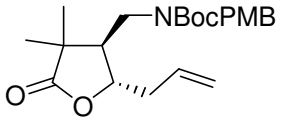
^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.75-12.20 (bs, 1H), 7.07 (t *J* = 5.9 Hz, 1H), 4.55 (m, 1H), 3.15-2.96 (m, 2H), 2.74-2.25 (m, 5H)

^{13}C NMR (75.5MHz, DMSO-d_6): δ = 175.8, 171.3, 155.8, 78.9, 77.8, 41.3, 39.8, 39.0, 31.6, 28.0

IR (KBr): $\bar{\upsilon}$ = 3327, 3101, 2989, 2938, 2569, 1783, 1658, 1256

MS [ESI, DCM/MeOH + 10 mmol/L $NH_4Ac)NH_3$]: m/z (%)= 290.8 (100) [$M+NH_4^+$], 273.3 (4.7) [MH^+]

Elementar analysis calcd (%) for [$C_{12}H_{19}NO_6$]: C 52.74, H 7.01, N 5.13; found C 52.40, H 6.73, N 5.03



202

(2S, 3S)-(2-Allyl-4,4-dimethyl-5-oxo-tetrahydro-furan-3-ylmethyl)-(4-methoxy-benzyl)-carbamic acid tert-butyl ester (202)

To a solution of 1.138 g of **197** (3.03mmol, 1 eq.) in 30 mL of dry THF at -78°C were added 10 mL of a solution of fresh LDA in THF which had been prepared with 1.89 mL of BuLi (0.194 g, 3.03 mmol, 1 eq.) and 0.47 mL of diisopropylamine (0.337 g, 3.33 mmol, 1.1 eq.) in 8 mL of dry THF at 0°C. The mixture was let at -78°C for half an hour and then, 0.19 mL of iodomethane (0.430 g, 3.03 eq.) were added to the mixture. The reaction mixture was let reacting half an hour and addition of LDA and iodomethane were repeated twice with the same time between each addition. When the reaction was complete, the reaction mixture was allowed to warm to room temperature and the solution was diluted in 100 mL of diethyl ether. The organic phase was washed twice with demineralised water and brine. The combined aqueous phases were then extracted with ether and the combined organic phases were dried with $MgSO_4$, filtered and the solvent was evaporated under vacuum. The residue was chromatographed over silica gel using hexanes/ethyl acetate 4:1 as eluent and by increasing slightly the polarity during the column yielding to a colourless oil of **202** (0.999 g, 2.48 mmol, yield=82%).

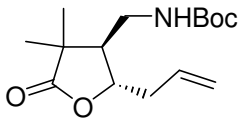
Rf (hexanes/EA 3:1)= 0.33, $[\alpha]_D^{20}$ = -33.2 (c=1.00 in $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.24-7.02 (d, *J*= 8.1Hz, 2H), 6.95-6.76 (m, 2H), 5.92-5.65 (m, 1H), 5.23-4.92 (m, 2H), 4.53-4.39 (d, *J*= 15.4 Hz, 1H), 4.38-4.13 (bs, 2H), 3.77 (s, 3H), 3.14-2.98 (dd, *J*= 14.4 Hz, *J*= 4.3 Hz, 1H), 2.59-2.44 (ddd, *J*= 14.9 Hz, *J*= 6.1 Hz, *J*= 2.9 Hz, 1H), 2.24-2.05 (m, 2H), 1.48 (s, 9H), 1.15 (s, 3H), 1.06 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.9, 159.2,155.6, 132.9, 129.2, 118.7, 114.1, 80.7, 60.4, 55.3, 47.7, 42.4, 28.5, 23.5, 21.1, 18.9, 14.2

IR (film): $\bar{\upsilon}$ = 3200, 2972, 2932, 1771, 1685, 1611, 1512, 1458, 1412, 1395, 1246, 1164, 1032, 984, 918, 877, 814, 756, 668 cm^{-1}

MS (CI MS): m/z (%): 421.2 [MNH_4^+] (100), 404.1 [MH^+] (12.35), 365.1 [MNH_4^+-C_4H_8] (35.51), 348.0 [MH^+-C_4H_8] (12.69)



203

(2S, 3S)-(2-Allyl-4,4-dimethyl-5-oxo-tetrahydro-furan-3-ylmethyl)-carbamic acid tert-butyl ester (203)

To a cold (0°C) solution of **202** (540 mg, 1.34 mmol, 1.0 eq.) in water / acetonitrile (5.3 mL / 15.9 mL), cerium ammonium nitrate (2.938 g, 5.35 mmol, 4.0 eq, c = 0.25 M) was added. The reaction was stirred at 0°C for 1 h and then at RT for additional 2 h until total consumption of the starting material. Water (30 ml) was added and the aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with a saturated solution of $NaHCO_3$ (30 mL), dried with $MgSO_4$, filtrated and evaporated in vacuo to give an oil which was purified by column chromatography (PE:EtOAc 3:1) to afford **203** (331 mg, 1.17 mmol, 87%) as a colourless oil.

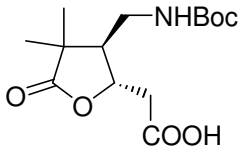
Rf (hexanes/EA 3:1) = 0.19; $[\alpha]_D^{20}$ = -29.6 (c=1.00 in $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.91-5.77 (td, *J* = 7.0 Hz, *J* = 16.9 Hz, 1H), 5.22-5.10 (m, 2H), 4.64-4.50 (bs, 1H), 4.23-4.16 (m, 1H), 3.34-3.19 (m, 2H), 2.67-2.58 (m, 1H), 2.39-2.30 (m, 1H), 2.17-2.08 (m, 1H), 1.44 (s, 9H), 1.27 (s, 3H), 1.15 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): d = 180.90, 132.63, 118.89, 79.94, 60.39, 50.27, 42.52, 38.18, 28.38, 24.45, 19.18, 14.20

IR (film): $\bar{\upsilon}$ = 3350, 3200, 2974, 2932, 1761, 1693, 1517, 1454, 1390, 1366, 1249, 1163, 1043, 983, 865, 781, 613

MS (LR): m/z (%): 301.2 (100) [$M+NH_4^+$], 284.1 (2.8) [MH^+], 245.1 (21.3) [$M+NH_4^+-C_4H_8$]



204

(2S, 3S)-[3-(tert-Butoxycarbonylamino-methyl)-4,4-dimethyl-5-oxo-tetrahydro-furan-2-yl]-acetic acid MGBA (204)

To a cold (0°C) solution of **203** (303 mg, 1.07 mmol, 1.0 eq.) in water / acetonitrile / carbon tetrachloride (5.4 mL / 2.7 mL / 2.7 mL) were added sodium periodate (0.915 g, 4.28 mmol, 4 eq.) and $RuCl_3 \bullet xH_2O$ (22 mg, 0.09 mmol, 0.07 eq.). The solution was stirred for 1 h at 0°C and for 2 h at RT. Water (10 ml) was added and the solution was then extracted with diethyl ether (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtrated through a celite pad and evaporated in vacuo to obtain **204** (300 mg, 0.995 mmol, 93%) as a colourless solid.

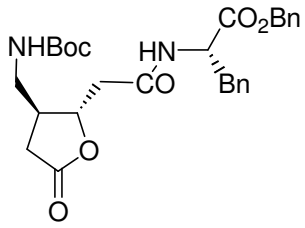
Rf (EA) = 0.13, $[\alpha]_D^{20}$ = -19.4 (c=1.00 in $CHCl_3$)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.44 (s,1H), 7.08-7.04 (t, *J* = 5.72 Hz, 1H), 4.49-4.42 (td, *J* = 9.8 Hz, *J* = 2.0 Hz, 1H), 3.33 (bs, 1H), 3.11-3.04 (m, 2H), 2.83-2.77 (dd, *J* = 16.9 Hz, *J* = 2.4 Hz, 1H), 2.21-2.13 (dd, *J* = 16.8 Hz, *J* = 7.0 Hz, 1H), 1.38 (s, 9H), 1.13 (s, 3H), 1.07 (s, 3H)

^{13}C NMR (75 MHz, DMSO-d_6): δ = 180.49, 171.28, 167.93, 155.43, 76.43, 49.14, 41.65, 37.14, 28.10, 23.58, 18.43, 13.89,

MS (LR): m/z (%): 319.1 (100) [M+NH_4^+], 301.1 (18.3) ([M+NH_4^+-$H_2$0], 263.1 (42.7) [M+NH_4^+-C_4H_8], 245.1 (11.9) [M+NH_4^+-$H_2$0-C_4H_8]

2. Synthesis of peptides containing δ-amino acids



210

N-tert-butoxycarbonyl-(-)-GBA-L-Phe-benzyl ester (210)

A solution of Boc-(L)-Phe-COOBn (297 mg, 1.78 mmol) in DCM/TFA 50:50 (5 ml) was stirred for 1 h at RT. The solvent was evaporated in vacuo and the resulting TFA salt was precipitated by addition of diethyl ether. The solvent was removed and the resulting colourless salt (247 mg, 0.67 mmol, 1.44 eq.) was dissolved in DCM (7 ml) and DIPEA (0.40 mL, 2.3 mmol, 5.0 eq.) was added. Meanwhile, a solution of **199** (127 mg, 0.46 mmol, 1.0 eq.) and EDC•HCl (134 mg, 0.70 mmol, 1.5 eq.) in DCM (5 mL) was stirred for half an hour at RT and added to the phenylalanine solution. After further 5 min, HOBt (107 mg, 0.70 mmol, 1.5 eq.) was added and the solution was stirred for 20 h at RT. The solution was diluted with DCM (50 mL) and washed with an aqueous solution of $KHSO_4$ 1M (2 x 30 mL) and a saturated aqueous solution of $NaHCO_3$ (2 x 30 mL), dried over Na_2SO_4, filtrated and evaporated in vacuo to give a slightly yellow solid which was purified by column chromatography (hexanes/EtOAc 1:1) to give **210** as a colourless solid (201 mg, 0.39 mmol, 85%).

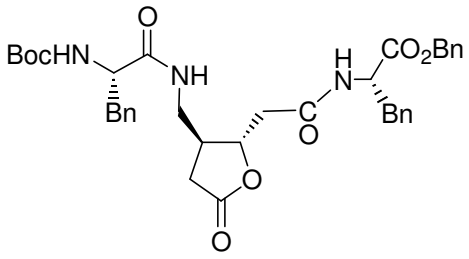
Rf (SiO_2, hexanes/EA 1:1) = 0.19; $[\alpha]_D^{20}$ = + 8.0 (c=1.00 in $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 7.34-7.21 (m, 8H), 7.07-7.01 (m, 2H), 6.40-6.32 (d, J = 7.8 Hz, 1H), 5.19-5.06 (m, 3H), 4.93-4.4.86 (td, J = 7.7 Hz, J = 5.9 Hz, 2H), 4.62-4.56 (q, J = 6.2 Hz, 1H), 3.28-3.03 (m, 4H), 2.65-2.55 (m, 2H), 2.51-2.26 (m, 2H), 1.42 (s, 9H)

^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 175.1, 171.2, 168.4, 135.4, 134.9, 129.4, 128.71, 128.68, 128.66, 127.2, 79.3, 67.5, 53.3, 41.1, 41.0, 37.6, 32.4, 28.4

IR (NaCl): $\bar{\upsilon}$ = 3335, 2199, 1779, 1742, 1667, 1523, 1450, 1363, 1251, 1170, 1009, 752, 699 cm^{-1}

MS (LR): m/z (%): 528.3 (100) [$M+NH_4^+$], 511.3 [MH^+]



212

N-tert-butoxycarbonyl-L-Phe-(-)-GBA-L-Phe-benzyl ester (212)

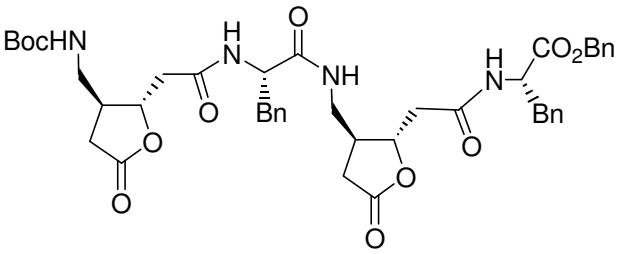
A solution of **210** (220 mg, 0.43 mmol) in DCM/TFA 50:50 (5 ml) was stirred for 1 h at RT. The solvent was evaporated in vacuo and the resulting TFA salt was precipitated by addition of diethyl ether. The solvent was removed and the resulting colourless salt (220 mg, 0.42 mmol, 1 eq.) was dissolved in DCM (7 ml) and DIPEA (0.36 mL, 2.1 mmol, 5.0 eq.) was added. Meanwhile, a solution of Boc-Phe (167 mg, 0.63 mmol, 1.5 eq.) and EDC•HCl (121 mg, 0.70 mmol, 1.5 eq.) in DCM (5 mL) was stirred for half an hour at RT and added to the previous solution. After further 5 min, HOAt (86 mg, 0.63 mmol, 1.5 eq.) was added and the solution was stirred for 20 h at RT. The solution was diluted with DCM (50 mL) and washed with an aqueous solution of $KHSO_4$ 1M (2 x 30 mL) and a saturated aqueous solution of $NaHCO_3$ (2 x 30 mL), dried over Na_2SO_4, filtrated and evaporated in vacuo to give a slightly yellow solid which was purified by column chromatography (hexanes/EtOAc 1:1) to give **212** as a colourless solid (189 mg, 0.29 mmol, 68%).

Rf (hexanes/EA 1:1)= 0.13, $[\alpha]_D^{20}$ = +8.1 (C=1.00 in $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40-6.95 (m, 15H), 6.85-6.80 (d, 1H), 5.25-5.07 (m, 3H), 4.85-4.75 (dd, *J* = 13.2 Hz, *J* = 7.0 Hz, 1H), 4.40-4.25 (m, 2H), 3.45-3.30 (m, 2H), 3.16-2.92 (m, 5H), 2.65-2.30 (m, 4H), 2.24-2.12 (dd, J = 16.7 Hz, J = 9.3 Hz, 1H), 1.40 (s, 9H)

IR (NaCl): $\bar{\upsilon}$ = 3306, 2199, 1660, 1531, 1450, 1170, 1018, 750, 698 cm^{-1}

MS (LR): m/z (%): 658.5 [MH^+]



213

N-tert-butoxycarbonyl-(-)-GBA-L-Phe-(-)-GBA-L-Phe-benzyl ester (213)

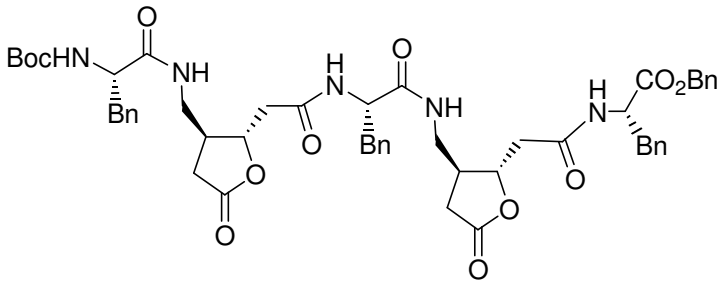
A solution of **212** (208 mg, 0.32 mmol) in DCM/TFA 50:50 (5 ml) was stirred for 1 h at RT. The solvent was evaporated in vacuo and the resulting TFA salt was precipitated by addition of diethyl ether. The solvent was removed and the resulting colourless salt (224 mg, 0.32 mmol, 1 eq.) was dissolved in DCM (7 ml) and DIPEA (0.27 mL, 1.58 mmol, 5.0 eq.) was added. Meanwhile, a solution of **199** (125 mg, 0.46 mmol, 1.44 eq.) and EDC•HCl (91 mg, 0.47 mmol, 1.5 eq.) in DCM (5 mL) was stirred for half an hour at RT and added to the previous solution. After further 5 min, HOAt (65 mg, 0.47 mmol, 1.5 eq.) was added and the solution was stirred for 20 h at RT. The solution was diluted with DCM (50 mL) and washed with an aqueous solution of $KHSO_4$ 1M (2 x 30 mL) and a saturated aqueous solution of $NaHCO_3$ (2 x 30 mL), dried over Na_2SO_4, filtrated and evaporated in vacuo to give a slightly yellow solid which was purified by column chromatography (EtOAc) to give **213** as a colourless solid (161 mg, 0.20 mmol, 62%).

Rf (EA) = 0.10, $[\alpha]_D^{20}$ = -9.3 (c=1.00 in $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 7.40-7.01 (m, 16H), 6.85-6.75 (m, 2H), 5.21-5.05 (m, 3H), 4.89-4.79 (dd, *J* = 13.7 Hz, *J* = 6.6 Hz, 1H), 4.66-4.50 (m, 2H), 4.41-4.36 (dd, *J* = 12.0 Hz, *J* = 5.7 Hz, 1H), 3.30-2.90 (m, 8H), 2.70-2.10 (m, 10H), 1.42 (s, 9H)

IR (NaCl): $\bar{\upsilon}$ = 3418, 2199, 2092, 1644 cm^{-1}

MS (LR): m/z (%) 830.5 (100) [$M+NH_4^+$], 813.4 (43) [$M+H^+$]



214

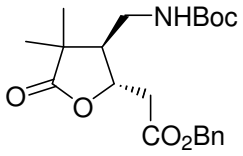
N-tert-butoxycarbonyl- L-Phe-(-)-GBA- L-Phe-(-)-GBA-L-Phe benzyl ester (214)

A solution of **213** in DCM/TFA 50:50 (4 ml) was stirred for 1 h at RT. The solvent was evaporated in vacuo and the resulting TFA salt was precipitated by addition of diethyl ether. The solvent was removed and the resulting colourless salt (115 mg, 0.14 mmol, 1 eq.) was dissolved in DCM (3 ml) and DIPEA (0.12 mL, 0.70 mmol, 5.0 eq.) was added. Meanwhile, a solution of Boc-L-Phe (55 mg, 0.21 mmol, 1.5 eq.) and EDC•HCl (40 mg, 0.21 mmol, 1.5 eq.) in DCM (4 mL) was stirred for half an hour at RT and added to the previous solution. After further 5 min, HOAt (28 mg, 0.21 mmol, 1.5 eq.) was added and the solution was stirred for 20 h at RT. The solution was diluted with DCM (50 mL) and washed with an aqueous solution of $KHSO_4$ 1M (2 x 30 mL) and a saturated aqueous solution of $NaHCO_3$ (2 x 30 mL), dried over Na_2SO_4, filtrated and evaporated in vacuo to give a slightly yellow solid which was purified by column chromatography (EtOAc/MeOH 9:1) to give **214** as a colourless solid (107 mg, 0.11 mmol, 80%).

Rf (EA/MeOH 9:1) = 0.67, $[\alpha]_D^{20}$ = 15 (c=0.1 in $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.71-7.61 (m, 1H), 7.40-7.04 (m, 20H), 5.43-5.36 (d, 1H), 5.21-5.08 (m, 3H), 4.84-4.77 (dd, *J* = 6.7 Hz, *J* = 13.2 Hz, 1H), 4.59-4.35 (m, 4H), 3.59-3.38 (m, 2H), 3.18-2.87 (m, 9H), 2.67-2.35 (m, 8H), 2.26-2.11 (m, 2H), 1.94-1.81 (bs, 1H), 1.39 (s, 9H)

MS (LR): m/z (%) 960.5 [MH+]



205

[3-(tert-Butoxycarbonylamino-methyl)-4,4-dimethyl-5-oxo-tetrahydro-furan-2-yl]-acetic acid benzyl ester (205)

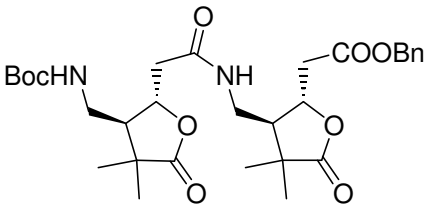
In 5 mL of DMF were added 100 mg of **204** (0.33 mmol, 1 eq.), 0.06 mL of benzyl bromide (0.53 mmol, 1.6 eq.) and 82 mg of K_2CO_3 (0.59 mmol, 1.8 eq.). The reaction was let at room temperature for 36 h. The reaction mixture was diluted in diethyl ether and washed with water twice. The aqueous phases were then extracted with diethyl ether and the organic layers were dried with $MgSO_4$ and filtered. The solvent was evaporated under vacuum and the crude residue purified by column chromatography (PE/EA 3:1) giving 96 mg of **205** (0.25 mmol, 76%).

Rf (PE/EA 3:1) = 0.18; $[\alpha]_D^{20}$ = -23.6 (c=1.00 in $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$) : δ = 7.38-7.32 (m, 5H), 5.16 (s, 2H), 4.69 (bs, 1H), 4.63-4.56 (ddd, *J* = 3.9 Hz, *J* = 7.7 Hz, *J* = 10.0 Hz, 1H), 3.32-3.17 (dd, *J* = 5.9 Hz, *J* = 11.7 Hz, 2H), 2.91-2.84 (dd, *J* = 3.9 Hz, *J* = 16.6 Hz, 1H), 2.76-2.68 (dd, *J* = 7.6 Hz, *J* = 16.6 Hz, 1H), 2.24-2.16 (td, *J* = 6.8 Hz, *J* = 9.9 Hz, 1H), 1.42 (s, 9H), 1.26 (s, 3H), 1.16 (s, 3H)

^{13}C NMR (75 MHz, $CDCl_3$) : δ = 180.3, 169.8, 155.8, 135.3, 128.6, 128.4, 128.3, 66.9, 50.6, 47.9, 42.4, 39.2, 38.3, 35.7, 28.3, 24.2, 19.0

MS (LR) : m/z (%) 409.0 (100) [$M+NH_4^+$], 353.0 (32) [$M+NH_4^+-C_4H_8$]



206

N-tert-butoxycarbonyl-MGBA-MGBA-benzyl ester (206)

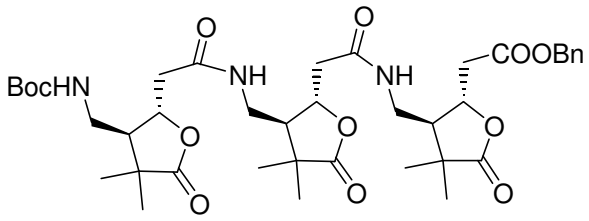
In 5 mL DCM were added 112 mg of MGBA **204** (0.37 mmol, 1.5 eq.) and 37 mg EDC•HCl (0.37 mmol, 1.5 eq.). The amino acid was activated for 30 minutes and added to a solution of 2 mL containing 100 mg of the TFA salt of **205** (0.25 mmol, 1 eq.) and 0.21 mL of DIPEA (1.24 mmol, 5 eq.). Subsequently, 51 mg of HOAt (0.37 mmol, 1.5 eq.) were added and the reaction was stirred for 24h at room temperature. The reaction mixture was dissolved in 50 mL DCM and washed with a solution of $KHSO_4$ 1M and a saturated solution of $NaHCO_3$. The organic phase was then dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was chromatographed on silica gel with PE/EA (1:1) and by increasing gradually the eluent mixture polarity. After column, the fraction were gathered, evaporated and dried under high vacuum affording 92 mg of **206** (0.16 mmol, 64%) as a white powder.

Rf (EA) = 0.78; $[\alpha]_D^{20}$ = -26.6 (c=1.00 in $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$) : δ = 7.38-7.35 (m, 5H), 6.30-6.25 (bs, 1H), 5.17 (s, 1H), 4.85-4.95 (bs, 1H), 4.60-4.46 (m,2H), 3.51-3.20 (m, 4H), 2.96-2.89 (dd, *J* = 5.0 Hz, *J* = 16.6 Hz, 1H), 2.85-2.77 (dd, *J* = 6.3 Hz, *J* = 16.6 Hz, 1H), 2.71-2.63 (dd, *J* = 3.3 Hz, *J* = 15.0 Hz, 1H), 2.47-2.37 (dd, *J* = 7.7 Hz, *J* = 14.9 Hz, 1H), 2.24-2.15 (m, 2H), 1.44 (s, 9H), 1.28-1.26 (m, 6H), 1.17-1.14 (d, 6H).

IR (film) : $\bar{\upsilon}$ = 3375, 2975, 2200, 1770, 1531, 1456, 1393, 1366, 1252, 1159, 1133, 1012, 754 cm^{-1}

MS (LR) : m/z (%) 592.4 (100) [M+NH_4^+], 575.4 (99) [M+H^+], 519.3 (37) [M+H^+-C_4H_8]



207

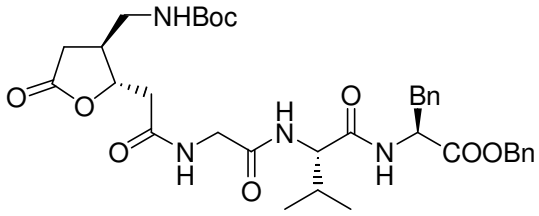
N-tert-butoxycarbonyl-MGBA-MGBA-MGBA-benzyl ester (207)

In 3 mL DMF were added 82 mg of MGBA **204** (0.27 mmol, 1.5 eq.) and 103 mg of HBTU (0.27 mmol, 1.5 eq.). The amino acid was activated for 30 minutes and added to a solution of 1 mL of DMF containing 107 mg of the TFA salt of **206** (0.18 mmol, 1 eq.) and 0.16 mL of DIPEA (0.27 mmol, 5 eq.). Subsequently, 37 mg of HOAt (0.27 mmol, 1.5 eq.) were added and the reaction was stirred for 24h at room temperature. The reaction mixture was dissolved in 50 mL DCM and washed with a solution of $KHSO_4$ 1M, the aqueous phase was then extracted with ethyl acetate and the organic phase were gathered and washed with a saturated solution of $NaHCO_3$. The organic phase was then dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was chromatographed on silica gel with EA/MeOH (9:1) and the fractions were evaporated and dried under high vacuum affording 20 mg of **207** (0.03 mmol, 15%) as a white powder.

Rf (EA/MeOH 9:1) = 0.29

^{1}H NMR (300 MHz, DMSO-d_6) : $\delta =$ 8.13 (bs, 2H), 7.44-7.33 (m, 5H), 7.04 (bt, 1H), 5.14 (s, 2H), 4.57-4.41 (m, 3H), 3.50-3.40 (m, 2H), 3.24-3.15 (m, 2H), 3.09-2.97 (m, 2H), 2.73-2.57 (m, 4H), 2.38-2.30 (m, 2H), 2.20-2.14 (m, 3H), 1.37 (s, 9H), 1.14-1.03 (m, 18H)

MS (LR) : m/z (%) 758.5 (10) [$M+H^+$], 658.5 (100) [MH^+-Boc]



218

N-tert-butyloxycarbonyl-(-)-GBA-Gly-Val-Phe benzyl ester (218)

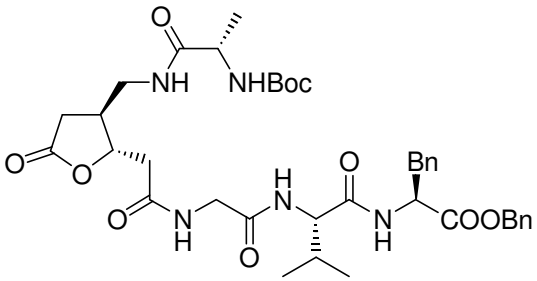
In a 5 ml of a mixture of DCM/TFA (1:1) was added 320 mg (0.63 mmol) of the peptide Boc-Gly-Val-Phe-Bn for half an hour. When the Boc cleavage was achieved, the solvent was evaporated under vacuum and the peptide salt was precipitated and washed in Et_2O. The salt was filtered and dried in vacuo to give 325 mg (0.62 mmol, 1.5 eq.) of the peptide salt as a white powder. In 5 mL DCM were dissolved 113 mg of **(-)-GBA** (0.41 mmol, 1 eq.) and 119 mg of EDC•HCl (0.62 mmol, 1.5 eq.) for half an hour, the solution was added to the peptide salt which had previously been dissolved in 3 mL DCM and neutralized with 0.36 mL of DIPEA (2.07 mmol, 5 eq.). Then, 84 mg of HOAt (0.62 mmol, 1.5 eq.) were added and the reaction was run for 48 h at room temperature. The reaction mixture was diluted in EA (20 mL) and washed subsequently with a solution of $KHSO_4$ 1M and a saturated solution of $NaHCO_3$. The organic phase was dried with $MgSO_4$, filtered and concentrated in a rotary evaporator. The residue was then chromatographed on silica gel with EA and afforded 116 mg of **218** (0.17 mmol, 42%) as a white solid.

Rf (EA) = 0.36; $[\alpha]_D^{20}$ = -10.4 (c=1.00 in $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$) : δ = 7.37-7.01 (m, 8H), 7.06-6.99 (m, 2H), 6.75-6.50 (m, 3H), 5.22-5.07 (m, 3H), 4.95-4.88 (dd, *J* = 6.2 Hz, *J* = 14.1Hz, 1H), 4.71-4.62 (dd, *J* = 6.0 Hz, 1H), 4.35-4.27 (dd, *J* = 6.6 Hz, *J* = 8.6 Hz, 1H), 3.97-3.90 (m, 2H), 3.30-3.15 (m, 2H), 3.13-3.05 (m, 2H), 2.72-2.47 (m, 4H), 2.44-2.33 (dd, *J* = 7.8 Hz, *J* = 16.8 Hz, 1H), 2.10-199 (m, 1H), 1.43 (s, 9H), 0.90-0.81 (dd, *J* = 6.8 Hz, *J* = 11.6 Hz, 6H).

IR (solid) : $\bar{\upsilon}$ = 3308, 3068, 2968, 2200, 1776, 1644, 1531, 1450, 1170, 1002, 754, 699 cm^{-1}

MS (LR) : m/z (%) 684.5 (100) [$M+NH_4^+$], 667.5 (47) [$M+H^+$], 611.4 (9) [$M+H^+-C_4H_8$]



219

N-tert-butyloxycarbonyl-Ala-(-)-GBA-Gly-Val-Phe benzyl ester (219)

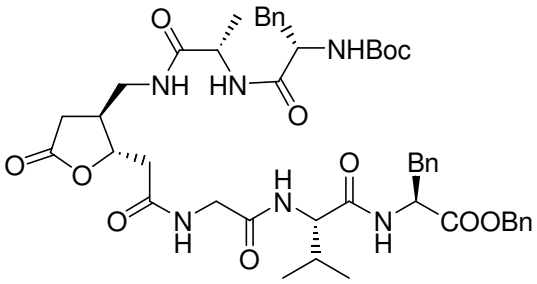
In a 5 ml of a mixture of DCM/TFA (1:1) was added 116 mg (0.17 mmol) of **218** for half an hour. When the Boc cleavage was achieved, the solvent was evaporated under vacuum and the peptide salt was precipitated and washed in Et_2O. The salt was filtered and dried in vacuo to give 122 mg (0.17 mmol, 1 eq.) of the peptide salt as a white powder. In 5 mL DCM were dissolved 48 mg of Boc-alanine (0.26 mmol, 1.5 eq.) and 49 mg of EDC•HCl (0.62 mmol, 1.5 eq.) for half an hour, the solution was added to the peptide salt which had previously been dissolevd in 3 mL DCM and neutralized with 0.15 mL of DIPEA (0.85 mmol, 5 eq.). Then, 35 mg of HOAt (0.26 mmol, 1.5 eq.) were added and the reaction was run for 48 h at room temperature. The reaction mixture was diluted in EA (20 mL) and washed subsequently with a solution of $KHSO_4$ 1M and a saturated solution of $NaHCO_3$. The organic phase was dried with $MgSO_4$, filtered and concentrated in a rotary evaporator. The residue was then chromatographed on silica gel with EA/MeOH (9:1) and afforded 80 mg of **219** (0.11 mmol, 64%) as a white solid.

Rf (EA/MeOH 9:1) = 0.41; $[\alpha]_D^{20}$ = -11.2 (c=1.00 in $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$) : δ = 7.65-7.51 (bs, 1H), 7.36-7.34 (m, 3H), 7.29-7.17 (m, 6H), 7.07-7.00 (m, 2H), 6.87-6.79 (bd, 1H), 6.78-6.70 (bd, 1H), 5.29-5.20 (bd, 1H), 5.16-5.05 (dd, *J* = 12.1 Hz, 2H), 4.90-4.84 (dd, *J* = 6.3 Hz, 1H), 4.65-4.68 (dd, *J* = 6.2 Hz, *J* = 12.3 Hz, 1H), 4.31-4.26 (dd, *J* = 6.7 Hz, *J* = 8.3 Hz, 1H), 4.22-4.16 (m, 1H), 4.05-3.95 (dd, *J* = 5.7 Hz, *J* = 16.5 Hz, 1H), 3.80-3.73 (dd, *J* = 4.6 Hz, *J* = 16.5 Hz, 1H), 3.51-3.41 (m, 1H), 3.20-3.03 (m, 3H), 2.80-2.55 (m, 4H), 2.35-2.24 (dd, *J* = 7.0 Hz, *J* = 16.3 Hz, 1H), 2.10-1.98 (m, 1H), 1.42 (s, 9H), 1.32-1.28 (d, *J* = 7.0Hz, 3H), 0.94-0.85 (dd, *J* = 7.0 Hz, *J* = 8.2 Hz, 6H).

IR (solid) : $\bar{\upsilon}$ = 3413, 3300, 3200, 2974, 1743, 1645, 1532, 1454, 1366, 1245, 1175, 1096, 1070, 984, 866, 739, 554, 489 cm^{-1}

MS (LR) : m/z (%) 755.5 (100) [$M+NH_4^+$], 738.5 (36) [$M+H^+$]



220

N-tert-butyloxycarbonyl-Phe-Ala-(-)-GBA-Gly-Val-Phe benzyl ester (220)

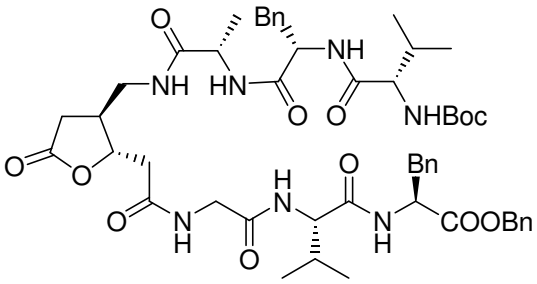
In 5 mL DCM were dissolved 85 mg of Boc-phenylalanine (0.32 mmol, 1.5 eq.) and 62 mg of EDC•HCl (0.32 mmol, 1.5 eq.) for half an hour, the solution was added to the peptide salt of **219** (161 mg, 0.21 mmol, 1.0 eq.) which had previously been dissolved in 3 mL DCM and neutralized with 0.18 mL of DIPEA (1.07 mmol, 5 eq.). Then, 44 mg of HOAt (0.32 mmol, 1.5 eq.) were added and the reaction was run for 48 h at room temperature. The reaction mixture was diluted in EA (20 mL) and washed subsequently with a solution of $KHSO_4$ 1M and a saturated solution of $NaHCO_3$. The organic phase was dried with $MgSO_4$, filtered and concentrated in a rotary evaporator. The residue was then chromatographed on silica gel with EA/MeOH (9:1) and afforded 117 mg of **220** (0.13 mmol, 63%) as a white solid.

Rf (EA/MeOH 9:1) = 0.61; $[\alpha]_D^{20}$ = -23.1 (c=1.00 in MeOH)

^{1}H NMR (300 MHz, $CDCl_3$) : δ = 7.39-6.91 (m, 18H), 6.79-6.60 (m, 2H), 5.40-5.25 (bs, 1H), 5.16-5.05 (m, 2H), 4.93-4.84 (dd, *J* = 7.0 Hz, *J* = 12.7 Hz, 1H), 4.63-4.22 (m, 4H), 3.99-3.85 (m, 2H), 3.60-3.31 (m, 2H), 3.19-2.95 (m, 5H), 2.77-2.48 (m, 2H), 2.32-2.17 (m, 1H), 2.13-1.93 (m, 3H), 1.39 (s, 9H), 1.28-1.26 (d, *J* = 7.2 Hz, 3H), 0.93-0.83 (m, 6H).

IR (solid): $\bar{\upsilon}$ = 3286, 3200, 1777, 1737, 1643, 1525, 1454, 1168, 1009, 748, 697, 566, 495 cm^{-1}

MS (LR): m/z (%) 885.4 (16) [$M+H^+$], 785.5 (100) [$M-C_5H_8O_2+H^+$]



221

N-tert-butyloxycarbonyl-Val-Phe-Ala-(-)-GBA-Gly-Val-Phe benzyl ester (221)

In 5 mL DCM were dissolved 41 mg of Boc-alanine (0.19 mmol, 1.5 eq.) and 36 mg of EDC•HCl (0.19 mmol, 1.5 eq.) for half an hour, the solution was added to the peptide salt of **220** (114 mg, 0.13 mmol, 1.0 eq.) which had previously been dissolved in 3 mL DCM and neutralized with 0.11 mL of DIPEA (0.63 mmol, 5 eq.). Then, 26 mg of HOAt (0.19 mmol, 1.5 eq.) were added and the reaction was run for 48 h at room temperature. The reaction mixture was diluted in EA (20 mL) and washed subsequently with a solution of $KHSO_4$ 1M and a saturated solution of $NaHCO_3$. The organic phase was dried with $MgSO_4$, filtered and concentrated in a rotary evaporator. The residue was then chromatographed on silica gel with EA/MeOH (9:1) and afforded 32 mg of **221** (0.03 mmol, 25%) as a white solid.

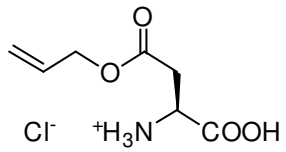
Rf (EA/MeOH 9:1) = 0.27

^{1}H NMR (300 MHz, DMSO-d_6) : δ = 8.55-8.53 (d, 1H), 6.22-8.10 (m, 2H), 8.00-7.77 (m, 3H), 7.36-7.14 (m, 15H), 6.68-6.65 (d, 1H), 5.09-4.99 (q, 2H), 4.65-4.48 (m, 3H), 4.27-4.15 (m, 2H), 3.83-3.67 (m, 3H), 3.07-2.95 (m, 3H), 2.80-2.72 (m, 1H), 2.65-2.26 (m, 5H), 195-1.76 (m, 2H), 1.37 (s, 9H), 1.24-1.03 (m, 6H), 0.88-0.56 (m, 12H)

IR (solid) : $\bar{\upsilon}$ = 3280, 3200, 2962, 2927, 1778, 1740, 1638, 1526, 1455, 1390, 1244, 1213, 1167, 1088, 1011, 928, 874, 800, 740, 696, 567

MS (LR): m/z (%) 984.3 (50) [M+H$^+$], 884.3 (100) [M+H$^+$-Boc]

3. Synthesis of the diketopiperazine scaffold



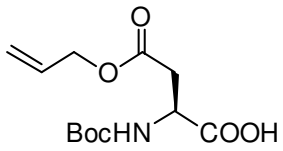
240

(S)-β-allyl aspartic acid hydrochloride (240)

In a round bottom flask cooled at 0°C were diluted 4.160 g (31.2 mmol, 1eq.) of L-aspartic acid in 45 mL of allylic alcohol. Acetyl chloride (8.74 mL, 122.95 mmol, 3.9 eq.) were then slowly added dropwise with a dropping funnel into the reaction mixture. Once the addition was finished, the reaction flask was removed from the ice bath and let react at room temperature for 18 h. The reaction mixture was diluted in Et_2O making the product precipitate entirely. The salt was filtered through a glass filter and washed twice with more Et_2O. The salt was recovered and dried under vacuum affording 5.542 g (26.44 mmol, 85%) of the monoallylated aspartic acid hydrochloride **240** as a white salt.

$[\alpha]_D^{20}$ = +22.7° (MeOH, *c*=1.00)

^{1}H NMR (D_2O, 400 MHz): δ= 6.05-5.85 (m, 1H), 5.36-5.25 (m, 2H), 4.63 (d, 2H, *J*=5.74 Hz), 4.48 (m, 1H), 3.26-3.3.20 (dd, 1H, J_1=5.5 Hz, J_2=18 Hz), 3.14-3.08 (dd, 1H, J_1=4.8 Hz, J_2=18.4 Hz)



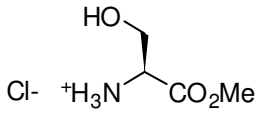
241

N-Boc-(S)-β-allyl aspartic acid (241)

In a round bottom flask were solved 5.54 g (26.4 mmol, 1 eq.) of allyl aspartic acid hydrochloride **240** in 150 mL of THF/water 1:1 solution. The reaction flask was put in an ice bath and 10.99 mL (79.28 mmol, 3 eq.) of triethylamine were added. Then, 6.860 g (31.7 mmol, 1.2 eq.) of Boc_2O were added and the reaction was let at room temperature for 24 h. The reaction mixture was diluted in 200 mL of EtOAc and washed with $KHSO_4$ 1M solution and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was then dried under high vacuum for a few hours affording 7.046 g (25.8 mmol, 97%) of the pure expected product **241** as a pale viscous yellow oil.

$[\alpha]_D^{20}$ = +33.3° ($CHCl_3$, *c*=1.00)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ= 5.80-5.93 (m, 1H), 5.59 (d, 1H, *J*=8.8), 5.19-5.32 (m, 2H), 4.52- 4.60 (m, 3H), 3.01 (dd, 1H, J_1=4.4 Hz, J_2=17.0 Hz), 2.87 (dd, 1H, J_1=4.9Hz, J_2=17.3 Hz), 1.41 (s, 9H)



242

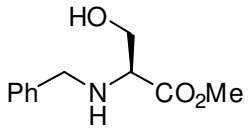
Serine methyl ester hydrochloride (242)

In a round bottom flask at 0°C were dissolved 6 g (57.09 mmol, 1 eq.) of serine in 45 mL MeOH. Acetyl chloride (16.2 mL, 228 mmol, 4 eq.) was added dropwise in the reaction mixture. Once the addition was done, the reaction flask was equipped with a condenser and refluxed for 2.5 h. Reflux was then stopped and the flask was cooled to room temperature. 200 mL of Et_2O were added provoking the precipitation of the resulting salt which was filtered on a glass funnel and dried under vacuum affording 8.57 g (55.08 mmol, 96%) of serine methyl ester hydrochloride **242** as a white powder.

R: $[\alpha]_D^{20}$ = -3.98° (MeOH, *c*=1.00)

S: $[\alpha]_D^{20}$ = +3.98° (MeOH, *c*=1.00)

^{1}H NMR (D_2O, 400 MHz): δ= 4.20-4.22 (t, 1H, *J*=7.6 Hz), 4.01-4.06 (dd, 1H J_1=4.3 Hz, J_2=12.5 Hz), 3.91-3.95 (dd, 1H, J_1=3.4 Hz, J_2=12.5 Hz), 3.69 (s, 3H)



243

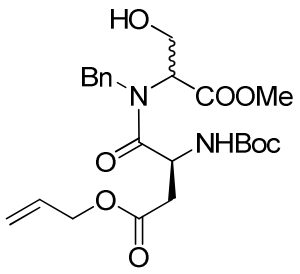
***N*-benzyl-serine methyl ester (243)**

In 18 mL MeOH were added successively 2.54 g (16.3 mmol, 1eq.) of serine methyl ester hydrochloride, 3.2 mL (22.8 mmol, 1.4 eq.) of Et_3N and 1.65 mL (16.3 mmol, 1 eq.) of freshly distilled benzaldehyde. The reaction was stirred for 4 hours. The reaction flask was put in an ice bath and 1.23 g (32.6 mmol, 2eq.) of $NaBH_4$ were added portionwise during 30 min. The reaction mixture was again let react for half an hour, then, reaction was quenched by adding at 0°C HCl 4 M until no more gas formation was observed. The mixture was washed 3 times with Et_2O. The organic phases were combined and extracted with HCl 4 M twice. The aqueous phases were then combined and neutralised by careful addition of saturated $NaHCO_3$ solution until a pH=8 was reached. The aqueous phase was then extracted 4 times with Et_2O. The organic phase was separated and dried over Na_2SO_4, filtered, concentrated under reduced pressure and dried under vacuum affording 2.73 g (13.04 mmol, 80%) of benzyl serine methyl ester **243** as a white oil.

R: $[\alpha]_D^{20}$ = +39.4 ($CHCl_3$, *c*=1.00)

S: $[\alpha]_D^{20}$ = -39.4 ($CHCl_3$, *c*=1.00)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ= 7.26-7.37 (m, 5H), 3.79-3.83 (m, 1H), 3.75 (s, 3H), 3.78 (s, 1H), 3.64-3.68 (dd, 1H, J_1=6.2 Hz, J_2=10.8 Hz), 3.44-3.47 (m, 1H), 2.69 (s, 2H),



244

(S)-*N*-Benzyl-3-*tert*-butoxycarbonylamino-*N*-[(S)-2-hydroxy-1-methoxycarbonyl-ethyl]-succinamic acid allyl ester (244)

To a solution of *β*-allyl (2*S*)-*N*-(*tert*-butoxycarbonyl)aspartate ester **241** (328 mg, 1.2 mmol, 1 equiv) in CH_2Cl_2 (8 mL), under nitrogen atmosphere and at 0 ºC, was added HATU (524 mg, 7.9 mmol, 1.1 equiv) and DIPEA (417 µL, 2.4 mmol, 2 equiv). After 30 min, a solution of (*S*) or (R) -*N*-benzylserine methyl ester **243** (250 mg, 1.2 mmol) in CH_2Cl_2 (1.6 mL) was added and the reaction was stirred at 0 ºC for 1 h and at r.t. for 24 h. The mixture was then diluted with AcOEt (100 mL) and the organic phase was washed in order with: 1 M $KHSO_4$ (2×20 mL), aqueous $NaHCO_3$ (2×20 mL) and brine (2×20 mL), dried over Na_2SO_4 and volatiles were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (Petroleum ether/AcOEt, 75:25) to afford the desired product **244** as a yellow oil (401 mg, 72%).

Rf (Hex/EA 6:4)= 0.31; S,S: $[\alpha]_D^{20}$ = -2.65 ($CHCl_3$, *c*=1.00); S,R: $[\alpha]_D^{20}$ = +11° ($CHCl_3$, *c*=1.00)

Cis:

^{1}H NMR ($CDCl_3$, 400 MHz): *δ*= 7.24-7.34 (m, 5H), 5.83-5.93 (m, 1H), 5.48 (d, 1H, *J*=8.2 Hz), 5.30 (d, 1H, *J*=17.2 Hz), 5.23 (d, 1H, *J*=10.4 Hz), 4.51-4.61 (m, 3H), 4.32-4.48 (m, 2H), 3.88 (d, 1H, *J*=13.1 Hz), 3.75 (s, 3H), 3.73 (d, 1H, *J*=13.1 Hz), 3.55 (t, 1H, *J*=4.7 Hz), 2.99 (dd, 1H, J_1=17.0 Hz, J_2=4.3 Hz), 2.85 (dd, 1H, J_1=17.0 Hz, J_2=4.7 Hz), 2.21 (br s, 1H), 1.45 (s, 9H).

Two set of signals were observed in the ^{13}C spectrum due to the presence of two rotational isomers A:B (20:1 ratio):

^{13}C NMR ($CDCl_3$, 100 MHz) *δ*= 172.8 (A), 172.1 (B), 171.0 (A), 170.3 (B), 155.7 (A), 155.0 (B), 139.6 (A), 138.9 (B), 132.1 (A), 131.4 (B), 128.9 (A), 128.7 (A), 128.1 (B), 127.9 (B), 127.6 (A), 126.9 (B), 119.1 (A), 118.4 (B), 80.6 (A), 79.9 (B), 66.2 (A), 66.1 (A), 65.5 (B), 65.4 (B), 59.5 (A), 58.8 (B), 52.7 (A), 52.2 (A), 51.9 (B), 51.4 (B), 50.3 (A), 49.6 (B), 37.1 (A), 36.4 (B), 28.7 (A), 27.9 (B);

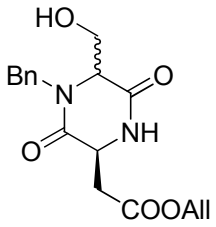
IR (film): $\bar{\upsilon}$= 3438, 3338, 3026, 2983, 2953, 2857, 1739, 1500, 1453, 1378, 1341, 1279, 1247, 1176 cm^{-1}.

HRMS (ESI) m/z calcd for $[C_{23}H_{33}N_2O_8]^+$: 465.22314 $[M+H]^+$; found: 465.22326; Elemental analysis calcd% for $C_{23}H_{32}N_2O_8$: C 59.47, H 6.94, N 6.03; found C 59.07, H 7.01, N 5.91.

Trans:

^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*= 7.26-7.34 (m, 5H), 5.89 (ddd, 1H, J_3=5.8Hz, J_2=11.0Hz, J_1=16.3Hz), 5.48 (d, 1H, *J*=8.3Hz), 5.28 (dd, 2H, J_2=13.8Hz, J_1=26.7Hz), 4.46 (dd, 1H, J_2=4.6Hz, J_1=10.9Hz), 4.34 (dd, 1H, J_2=4.8Hz, J_1=10.9Hz), 3.88 (d, 1H, *J*=13.1Hz), 3.73 (d, 1H, *J*=17.7Hz), 3.54 (t, 1H, *J*=4.7Hz), 3.00 (dd, 1H, J_2=4.3Hz, J_1=17.1Hz), 2.85 (dd, 1H, J_2=4.7Hz, J_1=17.1Hz), 1.45 (s, 9H)

^{13}C-NMR ($CDCl_3$, 100 MHz): *δ*= 172.8, 171.0, 170.9, 155.6, 139.6, 132.0, 129.1, 128.8, 128.6, 128.4, 127.0, 119.0, 80.5, 66.3, 59.4, 52.6, 52.2, 50.3, 37.1, 28.6



245

[(2S,5S)-4-Benzyl-5-hydroxymethyl-3,6-dioxo-piperazin-2-yl]-acetic acid allyl ester (245)

A solution of the dipeptide **244** (1.94 g, 4.2 mmol) in TFA/DCM 1:1 (32 mL) in a tarred flask was stirred for 30 minutes. The solvent was evaporated and dried under vacuum. A few milliliters of diethyl ether were added to wash and precipitate the TFA salt of the dipeptide, the solvent was removed with a filtrating Pasteur pipette and the salt was dried under vacuum affording the TFA salt as a white solid. This salt was dissolved in a mixture of saturated aqueous $NaHCO_3$/EtOAc (0.1 M, 1:1 v/v) and stirred at room temperature for 48 h. Subsequently, the layers were separated and the aqueous layer was extracted with EtOAc (4×). The combined organic layers were washed with brine, dried over Na_2SO_4 and volatiles were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (EA/PE, 8:2) to afford the desired product **245** as a white solid (1.13 g, 81%).

Cis:

Rf (SiO_2, EA) = 0.44; Mp 118-120 ºC; $[\alpha]_D^{20}$ = -72.1 (*c*= 1.00, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 400 MHz): *δ*= 7.25-7.37 (m, 5H), 7.05 (br s, 1H), 5.84-5.94 (m, 1H), 5.25-5.34 (m, 3H), 4.55-4.66 (m, 2H), 4.49-4.51 (m, 1H), 4.07 (d, 1H, *J*= 15.0 Hz), 3.98 (d, 1H, *J*= 11.1 Hz), 3.85-3.89 (m, 2H), 3.21 (dd, 1H, J_1= 17.5 Hz, J_2= 2.8 Hz), 3.16 (br s, 1H); 3.13 (dd, 1H, J_1= 17.5 Hz, J_2= 10.4 Hz);

^{13}C NMR ($CDCl_3$, 100 MHz) *δ*= 171.9, 167.0, 166.3, 135.6, 131.9, 129.5, 128.6, 119.5, 66.4, 61.3, 60.5, 52.8, 47.6, 40.7;

IR (film): $\bar{\upsilon}$= 3388, 3275, 3031, 3017, 2945, 1728, 1680, 1452, 1379, 1336, 1276, 1183, 1124 cm^{-1}. **MS** (FAB$^+$) *m/z* (%) 333 (80) [M+H]$^+$, 275 (11), 154 (57), 136 (48), 91 (100); Elemental analysis calcd% for $C_{17}H_{20}N_2O_5$: C 61.44, H 6.07, N 8.43; found C 61.23, H 5.97, N 8.24.

Trans:

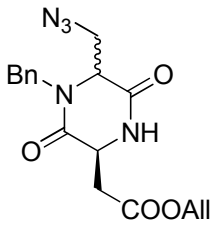
Rf (SiO_2, EA) =0.19; $[\alpha]_D^{20}$ == -35.3° ($CHCl_3$, *c*=1.00)

^{1}H-NMR ($CDCl_3$, 400 MHz): *δ*= 7.26-7.35 (m, 5H), 5.91 (qd, 1H, J_1= 5.8 Hz, J_2= 10.7 Hz), 5.26-5.36 (m, 2H), 4.68 (dd, 1H, J_1= 3.4 Hz, J_2= 8.8 Hz), 4.63 (d, 1H, *J*= 5.8 Hz), 4.05 (dd, 1H, J_1= 1.4Hz, J_2= 11.6 Hz), 3.90 (dd, 1H, J_1= 3.2 Hz, J_2= 11.7 Hz), 3.85 (s, 1H), 3.30 (dd, 1H, J_1= 3.5 Hz, J_2= 17.5 Hz), 2.83 (dd, 1H, J_1= 8.9 Hz, J_2= 17.5 Hz)

^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*= 171.2, 168.8, 167.0, 135.8, 131.9, 129.3, 128.4, 119.2, 66.2, 62.0, 61.7, 51.4, 47.6, 37.4

Preparation of hydrazoic acid

In a three-necked flask were dissolved 3 g of NaN_3 in 3 mL H_2O. Once totally diluted, 20 mL of toluene were added and the reaction flask was put in an ice bath under vigorous stirring. When the solution was at 0°C, 1.2 mL of concentrated H_2SO_4 were added extremely slowly in order not to exceed 10°C for the solution temperature. The reaction was let one hour at 0°C and was then filtered on cotton wool. The salt residue was washed twice with more toluene. The toluene solution was titrated by diluting 1 mL of the toluene solution in distilled water in a total volume of 50 mL. Titration was realised by addition of 0.1 M NaOH controlled with a pH meter allowing to calculate the HN_3 concentration in the mother solution when the equivalence volume was reached.



246

[(2S,5S)-5-Azidomethyl-4-benzyl-3,6-dioxo-piperazin-2-yl]-acetic acid allyl (246)

To a solution of HO-cis-DKP-COOAllyl **245** (559 mg, 1.7 mmol) in CH_2Cl_2/toluene (6.6 mL/12.2 mL), under nitrogen atmosphere and at -20 ºC, was added PPh_3 (535 mg, 2.0 mmol, 1.2 equiv) and the mixture was stirred until a solution was obtained. Hydrazoic acid (0.45 M in toluene, 7.5 mL, 3.4 mmol, 2 equiv) was added followed by dropwise addition of DIAD (0.42 mL, 2.0 mmol, 1.2 equiv) and the reaction was stirred at -20ºC during 3.5 h. The reaction mixture was put on silica gel without previous evaporation and a quick chromatography (petroleum ether/AcOEt, 6:4) was performed to remove the hydrazine and the resulting crude residue was then purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 99:1) to afford the desired product **246** as a colourless oil (291 mg, 48% for the cis compound, 82% for the trans compound). The *cis*-azido DKP can also be separated in one long column in modified flash chromatography condition using 45 cm of silica and Hex/EA 3:2 as eluent.

Cis:

Rf (DCM/MeOH 97:3)= 0.36, elimination Rf=0.31; $[\alpha]_D^{20}$ = -72.7 ($CHCl_3$,*c*=1.90)

^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 7.26-7.39 (m, 5H), 6.91 (br s, 1H), 5.89-5.99 (m, 1H), 5.36 (d, 1H, *J*=17.2 Hz), 5.30 (d, 1H, *J*=10.4 Hz), 5.18 (d, 1H, *J*=15.0 Hz), 4.62-4.71 (m, 2H), 4.51-4.54 (m, 1H), 4.20 (d, 1H, *J*=15.0 Hz), 3.95 (br s, 1H), 3.89 (dd, 1H, J_1=12.7 Hz, J_2=1.7 Hz), 3.68 (dd, 1H, J_1=12.7 Hz, J_2=3.4 Hz), 3.31 (dd, 1H, J_1=17.7 Hz, J_2=2.2 Hz), 3.08 (dd, 1H, J_1=17.7 Hz, J_2=11.2 Hz)

^{13}C NMR ($CDCl_3$, 100 MHz): *δ*= 171.6, 165.7, 165.1, 135.3, 131.8, 129.6, 128.8, 128.6, 119.6, 66.5, 58.8, 52.6, 51.1, 48.0, 40.7

IR (film): $\bar{\upsilon}$= 2984, 2929, 2853, 2119, 1734, 1686, 1667, 1451, 1336, 1274, 1181 cm^{-1}

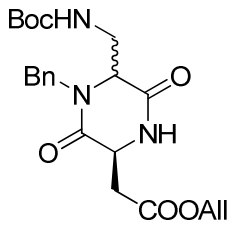
MS (FAB$^+$) *m/z* (%) 358 (12) [M+H]$^+$, 330 (2), 149 (16), 109 (27), 91 (100); Elemental analysis calcd% for $C_{17}H_{20}N_2O_5$: C 57.14, H 5.36, N 19.60; found C 57.39, H 5.28, N 19.25

Trans:

Rf (Hex/EA 3:2)= 0.13; $[\alpha]_D^{20}$ = = -55.9 ($CHCl_3$, *c*=1.00)

^{1}H-NMR ($CDCl_3$, 400 MHz): *δ*= 7.26-7.38 (m, 5H), 5.92 (ddd, 1H, J_3= 5.8 Hz, J_2= 10.6 Hz, J_1= 22.9 Hz), 5.32 (dd, 1H, J_2= 13.8 Hz, J_1= 26.9 Hz), 5.16 (d, 1H, *J*= 15.1 Hz), 4.63-4.66 (m, 2H), 4.26 (d, 1H, *J*= 15.1 Hz), 3.96 (t, 1H, *J*= 2.6 Hz), 3.89 (dd, 1H, J_2= 2.2 Hz, J_1= 12.7 Hz), 3.66 (dd, 1H, J_2= 3.4 Hz, J_1= 12.7 Hz), 3.30 (dd, 1H, J_2= 3.5 Hz, J_1= 17.5 Hz), 2.83 (dd, 1H, J_2= 9.0 Hz, J_1= 17.5 Hz),

^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*= 170.8, 167.0, 166.5, 135.6, 131.9, 129.4, 128.6, 128.4, 119.2, 66.2, 59.5, 52.0, 51.3, 48.2, 37.4



248

[(2S,5S)-4-Benzyl-5-(*tert*-butoxycarbonylamino-methyl)-3,6-dioxo-piperazin-2-yl]-acetic acid allyl ester (248)

To a solution of the azide **246** (269 mg, 0.75 mmol) in THF (2.5 mL), under nitrogen atmosphere and at -20ºC, was added Me_3P (828 µL of 1 M solution in THF, 0.83 mmol, 1.1 equiv) and 2-(*t*-butoxycarbonyloxyimino)-2-phenylacetronitrile (Boc-ON, 206 mg, 1.1 equiv). After stirring for 5 h at r.t., 60 mL of CH_2Cl_2 were added and the solution was extracted with H_2O (3×30 mL) and brine, the organic phase was dried over Na_2SO_4 and volatiles were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 99:1) to afford the desired product **248** as a white solid (253 mg, 78%).

Cis:

Rf (DCM/MeOH 95 :5)=0.43; Mp 57-58 ºC; $[\alpha]_D^{20}$=-123.7 ($CHCl_3$, *c*=1.00);

^{1}H NMR ($CDCl_3$, 400 MHz): *δ*= 7.28-7.36 (m, 5H), 7.06 (br s, 1H), 5.86-5.96 (m, 1H), 5.56 (d, 1H, *J*= 15.1 Hz), 5.25-5.36 (m, 3H), 4.60-4.69 (m, 2H), 4.48-4.51 (m, 1H), 4.09 (d, 1H, *J*= 15.1 Hz), 3.80-3.86 (m, 2H), 3.45-3.49 (m, 1H), 3.27 (dd, 1H, J_1= 17.6 Hz, J_2= 1.7 Hz), 2.85 (dd, 1H, J_1= 17.6 Hz, J_2= 11.1 Hz), 1.46 (s, 9H)

^{13}C NMR ($CDCl_3$, 100 MHz): *δ*= 171.5, 166.7, 164.9, 156.2, 135.6, 131.8, 129.4, 128.9, 128.5, 119.3, 80.8, 66.4, 59.2, 52.4, 47.2, 40.8, 40.6, 28.7

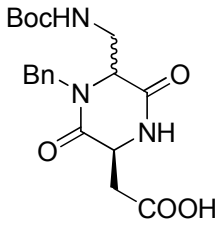
IR (Nujol) $\bar{\upsilon}$=3323, 3308, 1716, 1684, 1658, 1339, 1272, 1167, 1127.

MS (FAB$^+$) *m/z* (%) 432 (12) $[M+H]^+$, 376 (49), 332 (41), 302 (16), 91 (100); Elemental analysis calcd% for $C_{22}H_{29}N_3O_6$: C 61.24, H 6.77, N 9.74; found C 61.47, H 7.03, N 9.56.

Trans: **Rf** (DCM/MeOH 95:5) = 0.32; $[\alpha]_D^{20}$=+16.9 ($CHCl_3$, *c*=1.00)

^{1}H-NMR ($CDCl_3$, 400 MHz) *δ*= 7.27-7.37 (m, 5H), 5.92 (ddd, 1H, J_3= 5.8 Hz, J_2= 11.0 Hz, J_1= 16.3 Hz), 5.51 (d, 1H, *J*= 15.2 Hz), 5.32 (dd, 2H, J_2= 13.8 Hz, J_1= 26.0 Hz), 4.95 (m, 1H), 4.65 (d, 2H, *J*= 5.8 Hz), 4.49 (dd, 1H, J_2= 2.8 Hz, J_1= 9.6 Hz), 4.10 (d, 1H, *J*= 15.0 Hz), 3.56 (ddd, 1H, J_3= 2.2 Hz, J_2= 6.1 Hz, J_1= 14.5 Hz,), 3.37 (dd, 1H, J_2= 3.1 Hz, J_1= 17.6 Hz), 2.78 (dd, 1H, J_2= 9.7 Hz, J_1= 17.6 Hz), 1.46 (s, 9H)

^{13}C-NMR ($CDCl_3$, 100 MHz) = 171.0, 168.2, 165.7, 156.4, 135.9, 131.9, 129.3, 128.7, 128.3, 119.2, 80.5, 66.2, 59.8, 51.2, 47.4, 40.9, 37.9, 28.7



232

[(2*S*,5*S*)-4-benzyl-5-(*tert*-butoxycarbonylamino-methyl)-3,6-dioxo-piperazin-2-yl]-acetic acid (232)

To a solution of Boc-DKP-allyl **248** (242 mg, 0.56 mmol) in CH_2Cl_2 (3.0 mL), under nitrogen atmosphere and at 0 ºC, was added pyrrolidine (56 µL, 0.67 mmol, 1.2 equiv), PPh_3 (26 mg, 0.10 mmol, 0.18 equiv) and then $[Pd(PPh_3)_4]$ (24 mg, 0.02 mmol, 0.04 equiv). After stirring for 1 h at 0 ºC, EtOAc (25 mL) was added and the solution was extracted with aqueous $NaHCO_3$ (4×10 mL). The combined aqueous phases were acidified to pH 2 with a 1 M $KHSO_4$ solution and then extracted with CH_2Cl_2. The resulting organic phase was dried over Na_2SO_4 and the solvent evaporated to afford the desired product **232** as a fluffy white solid (209 mg, 95%).

Cis:

Mp 85-86 ºC; $[\alpha]_D^{20}$ = -69.9 (*c* 1.0, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 400 MHz, 50ºC) δ= 10.02 (br s, 1H), 8.05 (br s, 1H), 7.25-7.37 (m, 5H), 5.59 (d, 1H, *J*= 14.2 Hz), 5.36 (br s, 1H), 4.52 (d, 1H, *J*= 11.4 Hz), 4.03 (br s, 1H), 3.88 (s, 1H), 3.79-3.85 (m, 1H), 3.49-3.54 (m, 1H), 3.28 (dd, 1H, J_1= 17.7 Hz, J_2= 2.3 Hz), 2.74 (dd, 1H, J_1= 17.7 Hz, J_2= 11.4 Hz), 1.50 (s, 9H)

^{13}C NMR ($CDCl_3$, 100 MHz, 50ºC) δ= 175.1, 168.1, 164.9, 157.0, 135.4, 129.4, 128.8, 128.6, 81.4, 59.5, 52.4, 47.3, 40.9, 40.6, 28.7

IR (Nujol): $\bar{\upsilon}$= 3382, 3325, 3227, 1715, 1659, 1647, 1272, 1162, 1125

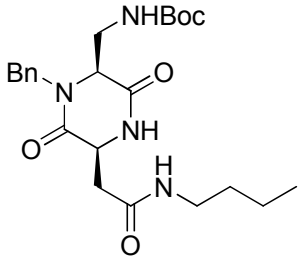
HRMS (ESI) m/z calcd for $[C_{19}H_{25}N_3NaO_6]^+$: 414.16356 $[M+Na]^+$; found: 414.16367.

Trans:

$[\alpha]_D^{20}$ =+5.42° ($CHCl_3$, *c*=1.00)

^{1}H-NMR ($CDCl_3$, 400 MHz) : δ= 7.23-7.37 (m, 5H), 5.49 (t, 1H, *J*= 13.8 Hz), 5.21-5.25 (m, 1H), 4.53 (t, 1H, *J*= 9.6 Hz), 4.11 (d, 1H, *J*= 17.1 Hz), 3.91 (d, 1H, *J*= 15.0 Hz), 3.61 (m, 1H), 3.83 (s, 1H), 3.41-3.50 (m, 1H), 2.64-2.75 (m, 1H), 1.45 (s, 9H)

4. Synthesis of peptides containing the diketopiperazine scaffold



251

tert-butyl ((2S,5S)-1-benzyl-5-(2-(butylamino)-2-oxoethyl)-3,6-dioxopiperazin-2-yl)methylcarbamate (251)

In a Schlenk tube under nitrogen containing 56 mg (0.14 mmol, 1 eq.) of *cis*-DKP **232cis** in 2 mL acetonitrile were added at 0°C 82 mg of HATU (0.21 mmol, 1.5 eq.) and 0.074 mL of DIPEA (0.43 mmol, 3 eq.). After stirring for 30 minutes, 0.021 mL of butylamine (0.21 mmol, 1.5 eq.) were added and the reaction mixture was stirred for 24 hours at room temperature. The reaction mixture was then diluted in EtOAc and washed with $KHSO_4$ 1 M, saturated $NaHCO_3$ and brine. The organic phase was then dried with Na_2SO_4 and filtered. The solvent was evaporated and the crude mixture was purified by flash chromatography (EA) affording 54 mg (0.12 mmol, 87%) of **251** as a colourless oil.

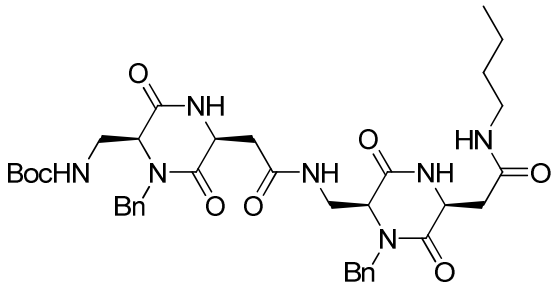
Rf (SiO_2, EA)= 0.32; $[\alpha]_D^{20}$ = -99.91° ($CHCl_3$, c=0.56)

^{1}H NMR ($CDCl_3$, 400 MHz): δ= 7.36-7.7.27 (m, 5H), 6.77 (bs, 1H), 6.32 (bs, 1H), 5.95 (bs, 1H), 5.54 (d, 1H, *J*= 15.1 Hz), 4.49 (m, 1H), 4.10 (d, 1H, *J*= 14.5 Hz), 3.87 (m, 1H), 3.75 (m, 1H), 3.27 (m, 2H), 3.09 (m, 1H), 2.66 (m, 1H), 1.49 (m, 2H), 1.46 (s, 9H), 1.35 (m, 2H), 0.94 (t, 3H, *J*= 7.2 Hz)

^{13}C NMR ($CDCl_3$, 100 MHz): δ= 170.5, 166.2, 165.2, 135.4, 129.5, 128.9, 128.6, 59.1, 52.9, 47.5, 47.1, 41.6, 40.9, 40.2, 39.8, 31.8, 28.8, 20.5, 14.1

IR (KBr): $\bar{\upsilon}$= 3427, 3321, 2962, 1649, 1541, 1452, 1385, 1367, 1336, 1273, 1168, 1062, 985, 846, 738, 702, 518, 420 cm^{-1}

MS (ESI): m/z (%): 446.2 (28) [M^+], 445.2 (100) [M^+-H], 328.2 (60)



252

Boc-(*cis*-DKP)$_2$-NHBu (252)

In 10 mL of DCM/TFA were added 57 mg (0.13 mmol, 1 eq.) Boc-*cis*-DKP-butylamide **251** and stirred for 30 minutes. Solvent was evaporated and the crude mixture was dried under vacuum. Washing with a few mL of Et_20 provoked the precipitation of the TFA salt. The solvent was removed with a Pasteur pipette capped with cotton wool and the salt was dried under vacuum affording the corresponding white TFA salt in a quantitative yield. In a Schlenk tube under nitrogen, 54 mg (0.13 mmol, 1 eq.) of Boc-*cis*-DKP-COOH **232cis** were solved in dry DMF and 58 mg of HATU (0.15 mmol, 1 eq.) and 0.074 mL of DIPEA (0.54 mmol, 3 eq.) were added at 0°C. After 30 minutes, the TFA salt solved in a few mL of DMF was added to the mixture and the reaction was let reacting for 24h at r.t. The reaction mixture was dissolved in Et_2O and H_20 and the phases were separated in a dropping funnel. The aqueous phase was extracted a second time with Et_2O and the organic phases were combined. The Et_2O phase was subsequently washed with aqueous $KHSO_4$ 1M, saturated $NaHCO_3$ solution and brine. The organic phase was then dried over Na_2SO_4 and concentrated with a rotary evaporator. The crude mixture was dried under vacuum and purified by a flash chromatography with EA/MeOH 95:5 as eluent affording the dimer of diketopiperazine **252** as a white solid in 54% yield (50 mg, 0.07 mmol).

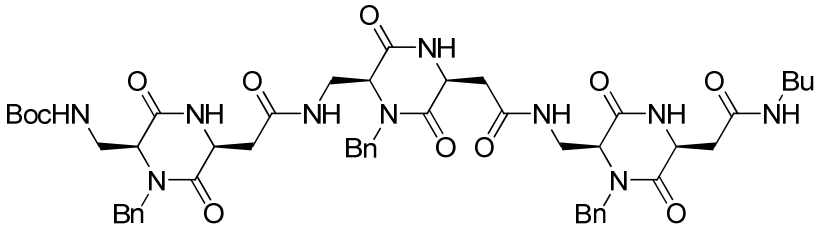
Rf (SiO_2, EA/MeOH 95:5) = 0.24, $[\alpha]_D^{20}$ = -95.4° ($CHCl_3$, c=0.23)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ= 8.28 (bs, 1H), 7.99 (bs, 1H), 7.80 (bs, 1H), 7.56 (bs, 1H), 7.56-7.22 (m, 10H), 6.00 (bs, 1H), 5.57 (d, 1H, *J*=15.4 Hz), 5.49 (d, 1H, *J*=15.3 Hz), 4.27 (m, 2H), 4.11 (d, 1H, *J*=15.4 Hz), 4.02 (d, 1H, *J*=15.3 Hz), 3.96 (m, 1H), 3.30-3.09 (m, 6H), 2.99 (m, 2H), 2.85 (m, 2H), 1.39 (m, 2H), 1.28 (m, 2H), 1.07 (s, 9H), 0.81 (t, 3H, *J*=7.2 Hz)

^{13}C NMR ($CDCl_3$, 100 MHz): δ= 172.5, 170.8, 166.8, 166.5, 166.2, 165.5, 168.2, 135.6, 135.4, 129.5, 129.4, 128.7, 128.3, 80.2, 60.8, 59.3, 58.4, 53.9, 52.7, 47.3, 46.6, 42.8, 42.4, 40.2, 39.5, 38.2, 31.7, 28.2, 20.2, 14.2

MS (ESI): m/z (%): 718.1 (100), 644.1 (44), 601.1 (44), 389.0 (37), 314.9 (12), 272.0 (5)

IR (KBr): $\bar{\upsilon}$= 3450, 3076, 2951, 2930, 2862, 1641, 1560, 1452, 1385, 1275, 1167, 1064, 700, 443 cm^{-1}



253

Boc-(cis-DKP)$_3$-NHBu (253)

In a Schlenk tube under nitrogen were dissolved 17 mg (0.042 mmol, 1.5 eq.) of Boc-*cis*-DKP-COOH **232cis** in acetonitrile and 16 mg (0.042 mmol, 1.5 eq.) of HATU and 0.015 mL of collidine (0.114 mmol, 4 eq.) were added at 0°C. After 30 minutes, 21 mg (0.029 mmol, 1 eq.) of the dimer **252** TFA salt were dissolved in a few mL of acetonitrile and added to the reaction mixture which was let at room temperature for 24 h. The reaction mixture was then dissolved in Et_2O and H_20 and the phases were separated in a dropping funnel. The aqueous phase was extracted a second time with Et_2O and the organic phases were subsequently washed with aqueous $KHSO_4$ 1M, saturated $NaHCO_3$ solution and brine. The organic phase was then dried over Na_2SO_4 and concentrated with a rotary evaporator. The crude mixture was dried under vacuum and purified by a flash chromatography with DCM/MeOH 95:5 as eluent affording the trimer of diketopiperazine **253** as a white solid in 80% yield (23 mg, 0.023 mmol).

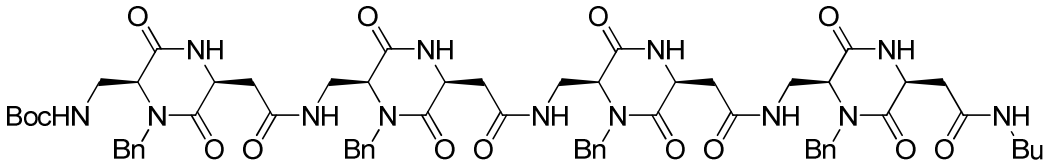
Rf (SiO_2, DCM/MeOH 9:1) = 0.46, $[\alpha]_D^{20}$ = -241.5° ($CHCl_3$, c=0.23)

^{1}H-NMR (DMSO-d_6, 400 MHz): δ= 8.40 (t, 1H, *J*=4.8Hz), 8.31 (t, 1H, *J*=5.6Hz), 8.25 (d, 1H, *J*=2.6Hz), 8.23 (d, 1H, *J*=1.7Hz), 8.09 (d, 1H, *J*=2.0Hz), 7.99 (t, 1H, *J*=5.4Hz), 7.36-7.25 (m, 15H), 7.04 (t, 1H, *J*=5.8Hz), 5.16 (d, 1H, *J*=6.9Hz), 5.13 (d, 1H, *J*=6.9Hz), 5.08 (d, 1H, *J*=15.4Hz), 4.31-4.23 (m, 3H), 4.20 (d, 1H, *J*=10.2Hz), 4.17 (d, 1H, *J*=9.9Hz), 4.05 (d, 1H, *J*=15.2Hz), 3.87-3.84 (m, 2H), 3.75-3.64 (m, 3H), 3.61-3.50 (m, 4H), 3.12-3.03 (m, 2H), 2.79-2.61 (m, 6H), 1.38 (m, 2H), 1.34 (s, 9H), 1.30-1.23 (m, 2H), 0.85 (t, 3H, *J*=7.3Hz)

^{13}C NMR (DMSO-d_6, 100 MHz): δ= 170.77, 170.64, 169.80, 166.85, 166.64, 166.38, 166.08, 165.96, 165.85, 137.42, 129.46, 128.58, 128.27, 79.07, 58.88, 58.69, 53.19, 52.74, 52.53, 47.58, 39.28, 31.96, 29.01, 20.46, 14.54,

MS (ESI): m/z (%): 991.3 (100), 939.3 (12), 684.2 (8)662.2 (6)

IR (KBr): = 3493, 3304, 3098, 2952, 1669, 1550, 1457, 1339, 1288, 1254, 1206, 1168, 1127, 736, 700



254

Boc-(cis-DKP)$_4$-NHBu (254)

In a Schlenk tube under nitrogen were dissolved 32 mg (0.082 mmol, 1.5 eq.) of *cis*-DKP **232cis** in acetonitrile and 31 mg (0.082 mmol, 1.5 eq.) of HATU and 0.024 mL of collidine (0.218 mmol, 4 eq.) were added at 0°C. After 30 minutes, 55 mg (0.055 mmol, 1 eq.) of the trimer **253** TFA salt were dissolved in a few mL of acetonitrile and added to the reaction mixture which was let at room temperature for 24 h. The reaction mixture was then dissolved in Et_2O and H_20 and the phases were separated in a dropping funnel. The aqueous phase was extracted a second time with Et_2O, reextracted with EA and the organic phases were subsequently washed with aqueous $KHSO_4$ 1M, saturated $NaHCO_3$ solution and brine. The organic phase was then dried over Na_2SO_4 and concentrated with a rotary evaporator. The crude mixture was dried under vacuum and purified by a flash chromatography with DCM/MeOH 95:5, then, the eluent polarity was increased using a mixture DCM/MeOH 9:1. Finally, the use of EA/MeOH 85/15 afforded the tetramer of diketopiperazine **254** as a white solid in 71% yield (49 mg, 0.039 mmol).

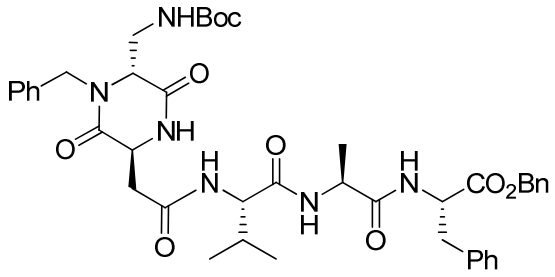
Rf (SiO_2, DCM/MeOH 95:5)= 0.32; $[\alpha]_D^{20}$ = -53.9° (MeOH, c=0.36)

^{1}H NMR (DMSO-d_6, 400 MHz): δ= 8.42 (t, 1H, *J*=5.4Hz) 8.32 (m, 2H), 8.29 (d, 1H, *J*=2.5Hz), 8.25 (bs, 1H), 8.23 (d, 1H, *J*=2.1Hz), 8.11 (bs, 1H), 8.00 (t, 1H, *J*=5.6Hz), 7.35-7.25 (m, 20H), 7.05 (t, 1H, *J*=6.1Hz), 5.16-5.06 (m, 4H), 4.31-4.12 (m, 7H), 4.05 (d, 1H, *J*=15.5Hz), 3.90-3.81 (m, 4H), 3.73-3.68 (m, 3H), 361-3.50 (m, 4H), 3.37-3.34 (m, 1H), 3.12-2.99 (m, 2H), 2.98 (bs, 1H), 2.91 (d, 1H, *J*=4.9Hz), 2.87 (bs, 1H), 2.74-2.63 (m, 5H), 1.42-1.36 (m, 2H), 1.34 (s, 9H), 1.25 (m, 2H), 0.85 (t, 3H, *J*=7.3Hz)

^{13}C NMR (DMSO-d_6, 100 MHz): δ= 170.33, 170.17, 169.75, 169.40, 166.55, 166.43, 166.31, 166.18, 165.98, 165.74, 165.54, 165.44, 165.36, 156.03, 137.02, 136.96, 129.04, 128.19, 128.15, 127.85, 78.66, 59.11, 58.46, 58.38, 58.22, 52.77, 52.36, 52.27, 52.12, 51.94, 47.28, 47.17, 41.73, 41.32, 41.06, 40.77, 38.88, 37.11, 35.44, 31.55, 28.61, 20.05, 14.13

MS (ESI): m/z (%) 1264.5 (100), 985.4 (17), 963.4 (12)

IR (KBr): $\bar{\upsilon}$= 3469, 3298, 3241, 3083, 2935, 2872, 1667, 1639, 1556, 1546, 1458, 1335, 1287, 1253, 1168, 1127, 1070, 736, 700



256

(S)-benzyl 2-((S)-2-((S)-2-(2-((2S,5R)-4-benzyl-5-((tert-butoxycarbonylamino)methyl)-3,6-dioxopiperazin-2-yl)acetamido)-3-methylbutanamido)propanamido)-3-phenylpropanoate (256)

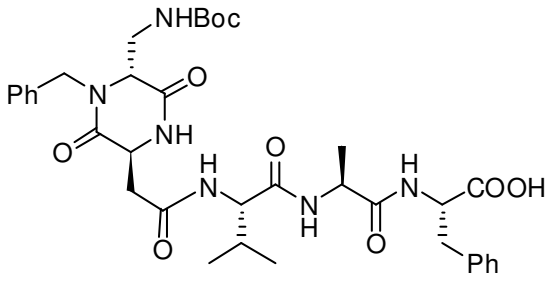
In a Schlenk tube under nitrogen were solved 97 mg (0.25 mmol, 1 eq.) of Boc-*trans*-DKP-COOH **232trans** in 2 mL acetonitrile. At 0°C were added 141 mg of HATU (0.37 mmol, 1.5 eq.) and 0.131 mL of collidine (0.99 mmol, 4 eq.) and the reaction was run for 30 minutes. Then, 200 mg of TFA•Val-Ala-Phe-OBn (0.37 mmol, 1.5 eq.) were dissolved in acetonitrile and DCM because of its low solubility and added to the reaction mixture. After two days, the reaction mixture was solved in EtOAc and washed successively by $KHSO_4$ 1M, saturated $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography with DCM/MeOH 97:3 as eluent affording 180 mg of the expected product **256** (0.225 mmol, 90%).

Rf (SiO_2, DCM/MeOH 95:5)=0.33

^{1}H NMR ($CDCl_3$, 400 MHz): δ= 7.70 (bs, 1H), 7.49 (bs, 1H), 7.36-7.00 (m, 17 H), 5.69 (bs, 1H), 5.43 (d, 1H, J=15.1 Hz), 5.13-5.02 (m, 2H), 4.83 (dd, 1H, J_1=6.7 Hz, J_2=13.4 Hz), 4.64 (m, 1H), 4.51-4.43 (m, 2H), 4.06 (d, 1H, J=15.0 Hz), 3.74-3.68 (m, 1H), 3.50-3.45 (m, 1H), 3.28-3.21 (m, 1H), 3.12-2.97 (m, 3H), 2.67 (m, 1H), 2.03 (m, 1H), 1.40 (s, 9H), 1.30 (d, 3H, J=7.0Hz), 0.89 (d, 6H, J= 6.8Hz)

^{13}C NMR ($CDCl_3$, 100 MHz): δ= 172.1, 171.1, 170.9, 170.7, 169.7, 167.5, 166.2, 156.1, 135.7, 135.3, 135.0, 129.3, 128.9, 128.6, 127.9, 127.0, 67.2, 59.9, 58.4, 53.5, 51.4, 48.7, 47.3, 37.7, 31.4, 28.3, 19.3, 18.3

MS (ESI): m/z (%) 821.4 (100) [M+Na^+], 524.4 (57)



257

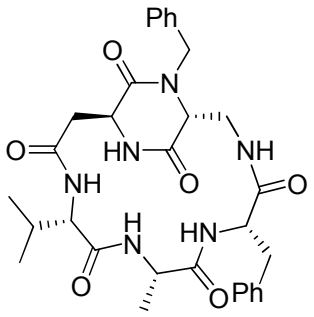
Boc-*trans*-DKP-Val-Ala-Phe-OH (257)

In 10 mL MeOH in a Schlenk tube under nitrogen were dissolved 170 mg (0.21 mmol) of Boc-*trans*-DKP-Val-Ala-Phe-Bn **256** and 17 mg (10 mass%) of $Pd(OH)_2$. The Schlenk tube was then put under vacuum and flushed 3 times by a sequence vacuum-hydrogen. The Schlenk tube was then filled with H_2 (1 atm.) and agitated for 24h. The solution was then filtered on a celite pad and washed with 200 mL MeOH. The solution was condensed under reduced pressure and dried under vacuum affording 101 mg (0.14 mmol, 68%) of the debenzylated product **257** as a white amorphous solid.

$[\alpha]_D^{20}$ = +19.23° (MeOH, *c*=0.60)

^{1}H NMR (CD_3OH, 400 MHz): δ= 8.26 (bs, 1H), 8.22 (d, 1H, *J*= 7.3 Hz), 8.11 (d, 1H, *J*= 8.2 Hz), 7.73 (d, 1H, *J*= 7.3 Hz), 7.35-7.16 (m, 10H), 7.02 (t, 1H, *J*= 6.1 Hz), 5.31 (d, 1H, *J*= 15.3 Hz), 4.55 (m, 1H), 4.38 (t, 1H, *J*= 7.1 Hz), 4.26 (dd, 1H, J_1= 6.1 Hz, J_2= 7.8 Hz), 4.00 (d, 1H, *J*= 15.4 Hz), 3.77-3.47 (m, 3H), 3.23-3.18 (m, 1H), 3.07-3.02 (m, 2H), 2.87 (ddd, 2H, J_1= 5.9 Hz, J_2= 15.4 Hz, J_3= 20.5 Hz), 2.22-2.14 (m, 1H), 1.43 (s, 9H), 1.32 (d, 3H, *J*= 7.0 Hz), 0.93 (dd, 1H, J_1= 6.8 Hz, J_2= 10.6 Hz)

^{13}C NMR (CD_3OH, 100 MHz): δ= 174.19, 172.95, 172.15, 170.95, 168.03, 167.42, 156.86, 137.22, 135.83, 129.23, 128.49, 127.90, 127.70, 127.46, 126.23, 79.30, 63.75, 59.90, 58.63, 54.73, 51.26, 49.32, 48.58, 40.95, 40.23, 38.79, 37.10, 31.23, 29.86, 27.37, 18.48, 16.65,



258

Cyclic *trans*-DKP-Val-Ala-Phe (258)

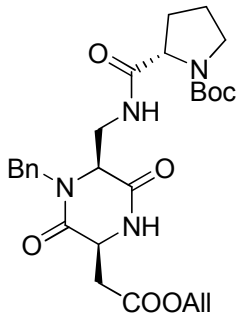
In a tarred flask were reacted 31 mg (0.044 mmol, 1 eq.) of Boc-trans-DKP-Val-Ala-Phe-OH **257** in 5 ml DCM/TFA 1:1 for 30 minutes. Once the deprotection completed, the solvent mixture was evaporated under reduced pressure and the flask was then dried under vacuum. A few mL of Et_2O were added resulting in the precipitation of the TFA salt. Et_2O was removed using a Pasteur pipette capped with cotton wool and the salt was dried under vacuum. After weighing to control whether the mass of TFA salt corresponded to a quantitative Boc deprotection, the flask was put under nitrogen and 22 mL of degassed DMF were added (substrate concentration 2 mM). The flask was put in an ice bath and 0.023 mL (0.175 mmol, 4 eq.) of collidine were added followed by addition of 33 mg (0.087 mmol, 2 eq.) of HATU. Two more equivalents of HATU were added after one day of reaction. Reaction was let at r.t. for 3 days. The reaction mixture was mixed to 50 mL of water and 150 mL of Et_2O, the organic phase was collected and the aqueous phase was extracted a second time with EtOAc. The organic phases were gathered and washed with $KHSO_4$ 1M, $NaHCO_3$ sat. and brine. The organic phase was dried with Na_2SO_4, filtered and concentrated under vacuum. The crude residue was purified by flash chromatography with DCM/MeOH 95:5 and afforded 8 mg (0.0135 mmol, 31%) of the cyclised product.

Rf (SiO_2, DCM/MeOH 9:1)=0.45

^{1}H NMR (CD_3OH, 400 MHz): δ= 8.83 (d, 0.23H, J= 5.4 Hz), 8.60 (dd, 1H, J_1= 3.8 Hz, J_2= 9.2 Hz), 8.53 (d, 0.77H, J= 6.1 Hz), 8.32 (s, 1H), 8.12 (d, 1H, J= 9.1 Hz), 7.52 (d, 1H, J= 6.8 Hz), 7.38-7.13 (m, 10H), 5.28 (d, 1H, J= 15.4 Hz), 4.78 (m, 1H), 4.48 (dd, 1H, J_1= 7.1 Hz, J_2= 9.3 Hz), 4.33-4.31 (m, 1H), 4.25 (dd, 1H, J_1= 7.8 Hz, J_2= 15.0 Hz), 4.10 (d, 1H, J= 15.7 Hz), 4.01 (dd, 1H, J_1= 6.2 Hz, J_2= 12.3 Hz), 3.81 (d, 1H, J= 5.2 Hz), 3.26-3.06 (m, 3H), 2.97 (dd, 1H, J_1= 9.7 Hz, J_2= 12.9 Hz), 2.37 (dd, 1H, J_1= 2.7 Hz, J_2= 13.0 Hz), 2.18 (m, 1H), 1.37 (d, 3H, J= 7.3 Hz), 1.04 (dd, 6H, J_1= 4.3 Hz, J_2= 6.9 Hz)

^{13}C NMR (CD_3OH, 100 MHz): δ= 173.24, 172.72, 172.21, 169.39, 169.12, 137.16, 135.93, 128.82, 128.57, 128.03, 127.60, 126.34, 70.13, 61.52, 59.21, 57.39, 52.14, 38.66, 38.33, 35.93, 28.89, 18.21, 16.93, 16.79,

5. Synthesis of organocatalysts containing the diketopiperazine scaffold



262

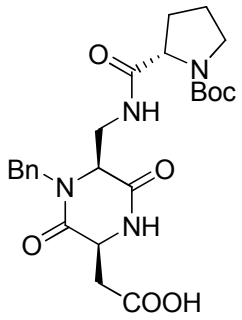
Boc-Pro-*cis*-DKP allyl ester (262)

In 10 mL of DCM/TFA were added 271 mg (0.63 mmol, 1 eq.) of Boc-*cis*-DKP-allyl ester **248** and stirred for 30 minutes. Solvent was evaporated and the crude mixture was dried under vacuum. Washing with a few mL of Et_20 provoked the precipitation of the TFA salt. The solvent was removed with a Pasteur pipette capped with cotton wool and the salt was dried under vacuum affording the corresponding white TFA salt in a quantitative yield. A Schlenk tube under nitrogen was put in an ice bath were added 195 mg (0.91 mmol, 1.5 eq.) of Boc-(S)-proline, 344 mg (0.91 mmol, 1.5 eq.) of HATU and 0.32 mL (2.42 mmol, 4 eq.) of collidine in 10 mL acetonitrile. The reaction was let running for 30 minutes and then the TFA salt was dissolved in a few mL of acetonitrile and added to the reaction mixture. After 24 h of reaction, the reaction mixture was solved in EtOAc and washed successively by $KHSO_4$ 1M, saturated $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography with DCM/MeOH 95:5 as eluent affording 302 mg of the expected product **262** (0.57 mmol, 95%).

Rf (DCM/MeOH 95:5)= 0.36, $[\alpha]_D^{20}$ = -120.11 ($CHCl_3$, *c*=1.00)

^{1}H NMR ($CDCl_3$, 400 MHz): δ= 7.36-7.30 (m, 6H), 6.71 (bs, 1H), 5.92 (tdd, 1H, J1= 5.9 Hz, J2= 10.5 Hz, J3= 16.4 Hz), 5.55 (d, 1H, J= 14.2 Hz), 5.32 (dd, 2H, J1= 13.8 Hz, J2= 25.6 Hz), 4.65 (dq, 2H, J1= 6.0 Hz, J2= 13.2 Hz), 4.48 (td, 1H, J1= 2.5 Hz, J2= 11.0 Hz), 4.30 (bs, 1H), 4.13 (d, 1H, J= 15.0 Hz), 3.94 (m, 1H), 3.84 (bs, 1H), 3.67-3.62 (m, 1H), 3.50-3.21 (m, 4H), 3.08-2.99 (m, 1H), 2.37 (bs, 1H), 1.88 (bs, 2H), 1.45 (s, 9H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ= 172.9, 165.5, 164.4, 135.1, 131.4, 128.9, 128.6, 128.1, 119.1, 80.7, 66.1, 60.1, 57.8, 52.0, 47.3, 46.9, 39.5, 38.7, 28.4, 28.2, 24.5



263

Boc-Pro-cis-DKP-COOH (263)

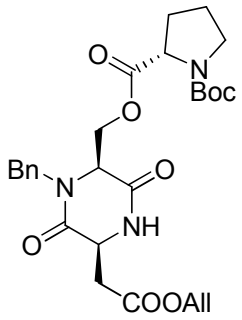
In a Schlenk tube under nitrogen were dissolved 300 mg (0.57 mmol, 1 eq.) of Boc-Pro-*cis*-DKP-COOAllyl **262** in 4 mL of DCM. At 0°C were added subsequently 0.056 mL (0.68 mmol, 1.2 eq.) of pyrrolidine, 27 mg (0.102 mmol, 0.18 eq.) of PPh_3 and 26 mg (0.023 mmol, 0.04 eq.) of $Pd(PPh_3)_4$. Reaction was run for 2 h and the reaction mixture was then dissolved in 50 mL EtOAc and was extracted twice with a saturated solution of $NaHCO_3$. The aqueous phase was washed a second time with EtOAc and acidified to pH=2 with $KHSO_4$ 1M. The aqueous phase was then extracted with 100 mL DCM (3 times). The organic phase was then dried with Na_2SO_4, filtered and condensed under reduced pressure. The expected product **263** could be obtained after drying under vacuum as a white solid (256 mg, 0.54 mmol, 95%).

$[\alpha]_D^{20}$ = -48.22 (MeOH, *c*=1.00)

^{1}H NMR (DMSO-d_6, 400 MHz): δ= 12.65 (s, 1H), 8.28 (dd, 1H, J_1= 1.9 Hz, J_2= 13.4 Hz), 8.15 (bs, 1H), 7.35-7.26 (m, 5H), 5.05 (d, 1H, *J*= 15.2 Hz), 4.29 (m, 1H), 4.15 (d, 1H, *J*= 15.0 Hz), 4.11-4.04 (m, 1H), 3.79-3.65 (m, 2H), 3.43-3.38 (m, 3H), 2.74 (m, 2H), 2.06-2.01 (m, 1H), 1.86-1.67 (m, 3H), 1.28 (s, 9H)

^{13}C NMR (DMSO-d_6, 100 MHz): 174.97, 172.83, 166.72, 166.19, 154.54, 135.97, 128.49, 127.77, 127.49, 80.07, 60.13, 58.17, 51.91, 46.45, 39.77, 38.04, 31.09, 27.24, 23.24

MS (ESI): m/z (%) 999.0 (100) [2M+Na^+], 511.2 (88) [M+Na^+], 303.4 (40)



264

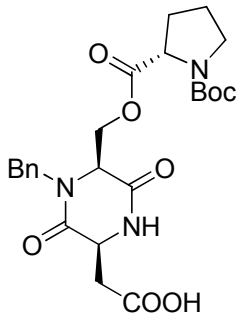
Boc-Pro-COO-cis-DKP allyl ester (264)

In a Schlenk tube under nitrogen at 0°C were dissolved in DCM/THF 271 mg (1.26 mmol, 1.5 eq.) of Boc-(*S)*-Proline, 260 mg (1.26 mmol, 1.5 eq.) of DCC, 170 mg (1.26 mmol, 1.5 eq.) of HOBt and 308 mg (2.52 mmol, 3 eq.) of DMAP. After 30 minutes were added 279 mg (0.84 mmol, 1 eq.) of HO-DKP-COOAllyl **245** and the reaction was run for two days. The reaction mixture was solved in EtOAc and washed successively by $KHSO_4$ 1M, saturated $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography with DCM/MeOH 95:5 as eluent affording 225 mg of the expected product **264** (0.42 mmol, 50%).

Rf (DCM/MeOH 95 :5)= 0.40, $[\alpha]_D^{20}$ = -87.29 ($CHCl_3$, c=1.13)

^{1}H NMR ($CDCl_3$, 400 MHz): δ= 7.35-7.19 (m, 5H), 6.97 (bd, 1H), 5.94-5.84 (m, 1H), 5.34-5.23 (m, 3H), 4.68-4.57 (m, 3H), 4.52 (d, 1H, *J*= 9.4 Hz), 4.33 (m, 2H), 4.15 (dd, 1H, J_1= 3.4 Hz, J_2= 8.5 Hz), 4.04-3.94 (m, 2H), 3.53-3.34 (m, 2H), 3.27-3.23 (m, 1H), 2.87 (ddd, 1H, J_1= 10.9 Hz, J_2= 17.3 Hz, J_3= 27.9 Hz), 2.19 (m, 1H), 1.89 (m, 2H), 1.43 (s, 9H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ= 172.6, 170.6, 165.3, 164.4, 154.3, 153.5, 134.6, 131.5, 129.13, 128.30, 119.4, 80.3, 66.2, 62.3, 58.7, 57.5, 52.1, 47.3, 46.6, 40.2, 30.9, 29.9, 28.4, 24.4, 23.7



265

Boc-Pro-COO-cis-DKP-COOH (265)

In a Schlenk tube under nitrogen were dissolved 225 mg (0.57 mmol, 1 eq.) of Boc-Pro-COO-*cis*-DKP-COOAllyl **264** in 3 mL of DCM. At 0°C were added subsequently 0.042 mL (0.51 mmol, 1.2 eq.) of pyrrolidine, 20 mg (0.076 mmol, 0.18 eq.) of PPh_3 and 20 mg (0.017 mmol, 0.04 eq.) of $Pd(PPh_3)_4$. Reaction was run for 2 h and the reaction mixture was then dissolved in 50 mL EtOAc and was extracted twice with a saturated solution of $NaHCO_3$. The aqueous phase was washed a second time with EtOAc and acidified to pH=2 with $KHSO_4$ 1M. The aqueous phase was then extracted with 100 mL DCM (3 times). The organic phase was then dried with Na_2SO_4, filtered and condensed under reduced pressure. The expected product **265** could be obtained after drying under vacuum as a white solid (177 mg, 0.36 mmol, 86%).

$[\alpha]_D^{20}$ = -20.87 (MeOH, *c*=1.00)

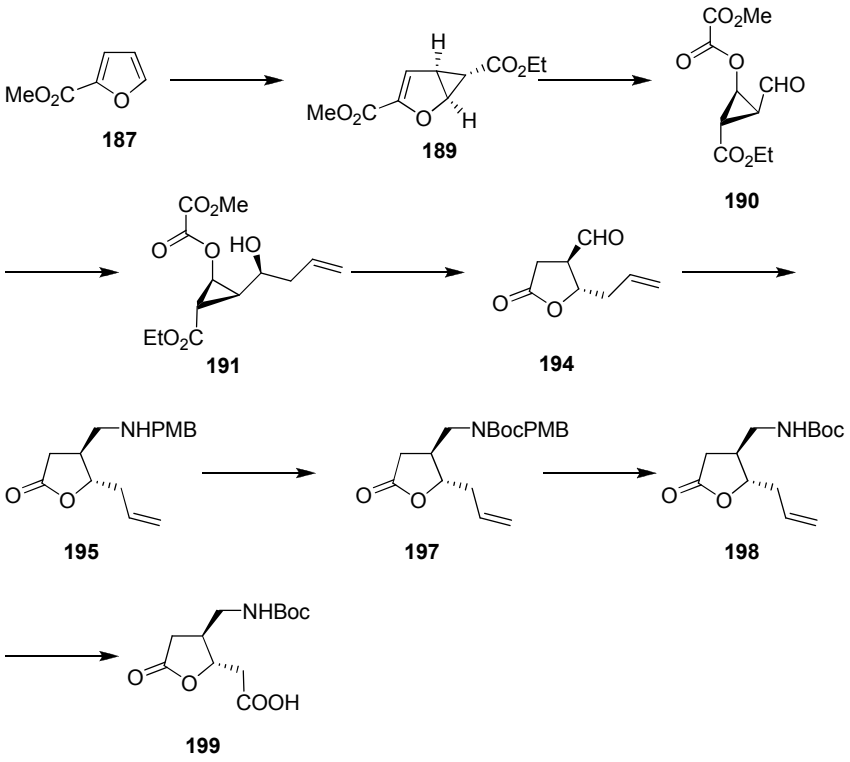
^{1}H-NMR (CD_3OH, 400 MHz): δ= 8.29 (s, 1H), 7.35-7.28 (m, 5H), 4.81 (bs, 1H), 4.62 (d, 1H, *J*= 15.2 Hz), 4.52-4.47 (m, 2H), 4.41 (dq, 1H, J_1= 2.8 Hz, J_2= 12.0 Hz), 4.28-4.20 (m, 2H), 3.49-3.44 (m, 1H), 3.41-3.35 (m, 1H), 3.04 (ddd, 1H, J_1= 4.0 Hz, J_2= 8.8 Hz, J_3= 16.6 Hz), 2.91 (ddd, 1H, J_1= 5.2 Hz, J_2= 8.9 Hz, J_3= 16.5 Hz), 2.31-2.21 (m, 1H), 1.91-1.82 (m, 3H), 1.36 (s, 9H)

^{13}C-NMR (CD_3OH, 100 MHz): δ= 172.50, 172.17, 166.80, 165.33, 154.19, 136.22, 128.54, 127.57, 80.35, 63.22, 59.26, 58.78, 52.36, 46.04, 40.32, 30.26, 27.28, 27.14, 23.10

MS (ESI): m/z (%) 1001.0 (25) [2M+Na$^+$], 512.2 (100) [M+Na$^+$], 456.2 (11), 303.4 (33)

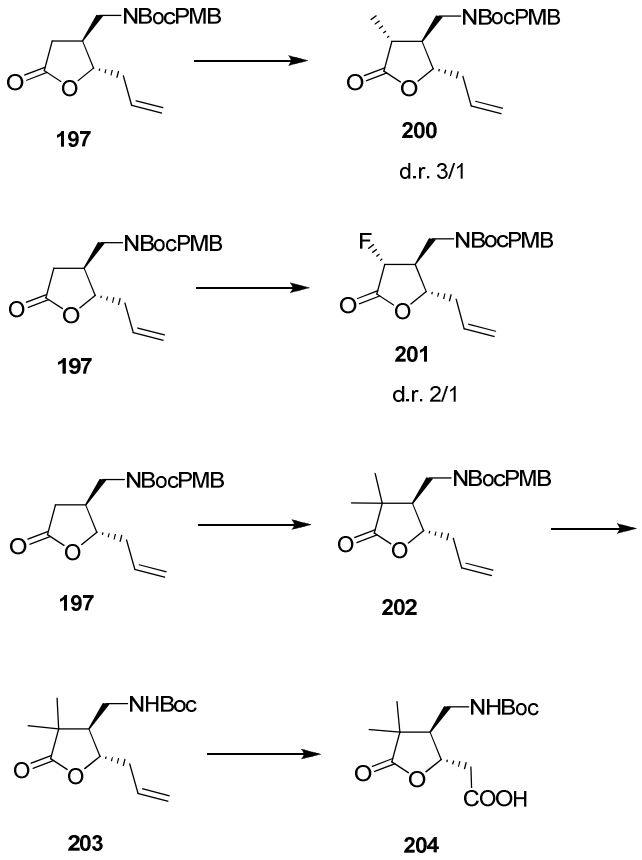
Summary

The efficient synthesis of a γ-butyrolactone based δ-amino acid was realised starting from furan methyl ester. Its enantioselecive cyclopropanation by copper triflate and a chiral bis-oxazoline led to the formation of an enantiopure cyclopropanated furan product which could be subjected to ozonolysis affording a cyclopropane aldehyde having three chiral centers. A Sakurai allylation was performed efficiently in high d.r. The lactonisation of the allylated cyclopropane alcohol could be realised with barium hydroxide. This reaction could be scaled up using triethylamine in the same conditions. The reductive amination of the resulting lactonaldehyde could be realised in good yields in mild conditions. The Boc protection of the secondary amine and the PMB cleavage were performed affording the lactone with a protected primary amine. The allylic double bond could be oxidised by ruthenium chloride and sodium periodate affording the δ-amino acid **199** in good yields (Scheme 85).



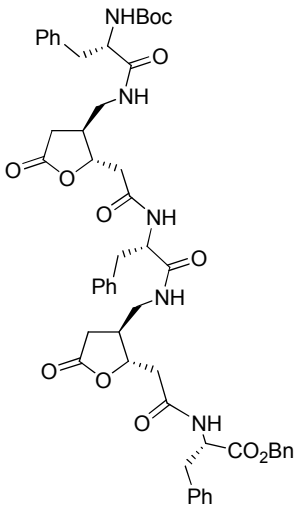
Scheme 85: Synthesis of the δ-amino acid

Modification of the lactone by α-substitution was investigated to obtain a better NMR dispersion. The α-methylation with LDA and methyl iodide led to poor diastereoselectivities (d.r. 3/1). The α-fluorination was not diastereoselective as well (d.r. 2/1) and yields were poor. The α-dimethylation of the lactone could be performed in good yields and the resulting δ-amino acid showed an excellent NMR dispersion for further foldamers studies (Scheme 86).



Scheme 86: α-substitution of the lactone ring and new δ-amino acid synthesis

The liquid-phase synthesis of the desired tetramer of dimethylated δ-amino acid could not be performed, even by using efficient coupling reagents. The synthesis of mixed α-δ-peptides with the non-methylated building block was realised. First, an alternated α-δ-pentapeptide containing L-Phe and the lactone building block was efficiently synthesised and its secondary structure analysed by various techniques. Despite overlapping of some protons in NMR spectroscopy, some NOE contacts could be observed and allowed computational studies using NOE constraints. The calculations predicted an extended loop conformation of the pentapeptide and other analyses such as IR in solution or CD spectroscopy are in favour of intramolecular hydrogen bonding. CD spectra refences missing for that type of alternated peptide, no comparison could be done with similar peptides. The secondary structure could not be determined with certainty and attemps to crystallise the peptide in a stable form failed (Scheme 87).



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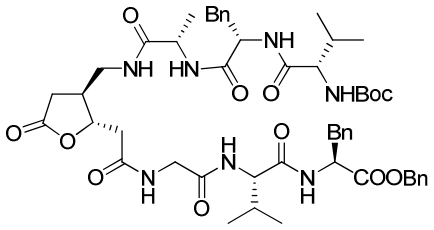
Scheme 87: Alternated α-δ-peptide

Secondly, a heptapeptide containing only one δ-amino acid was synthesised in order to know whether this building block could induce hairpin secondary structures. IR in solution revealed the presence of intramolecular hydrogen bonding and CD spectroscopy showed interesting features suggesting the presence of a stable secondary structure although its CD spectrum looked a little disorganised. Its 2D-NMR analysis revealed interstrand contacts and showed that this heptapeptide was forming, among other things, a 13-membered hydrogen-bonded ring. As a consequence, we came to the conclusion that it did not adopt a hairpin conformation but a disorganised helicoidal structure (Scheme 88).



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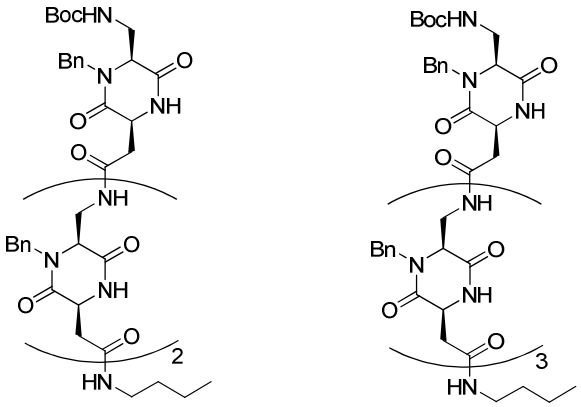
Scheme 88: Heptapeptide containing one δ-amino acid scaffold

For the second part of this project, the ability of *cis*-DKP in β-bend ribbon structure was investigated. The trimer of DKP could be synthesised and fully characterised and revealed a partial β-bend ribbon conformation. The tetramer, on the other hand, could not be fully characterised by 2D-NMR, but its CD spectrum showed the presence of a stable secondary structure and comparison with other β-bend ribbon CD spectra in the literature showed a similar behaviour in terms of shapes and intensities. The CD data collected, in addition to proton-deuterium exchange analysis are in favour of a stable β-bend ribbon secondary structure (Scheme 89).



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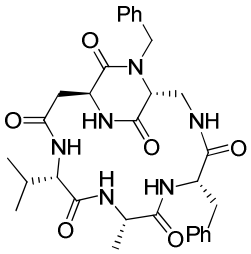
Scheme 89: Trimer and tetramer of the diketopiperazine scaffold

A short cyclic peptide based on a *trans*-DKP scaffold was successfully synthesised and fully characterised by 2D NMR spectroscopy. The presence of a γ-turn in the peptide was detected and confirmed preliminary computational studies realised on a similar peptide. It is very probable that a pentapeptide containing a RGD sequence and the *trans*-DKP scaffold would as well induce a γ-turn, whose presence is primordial for its activity against integrins $\alpha_v\beta_3$ (Scheme 90).



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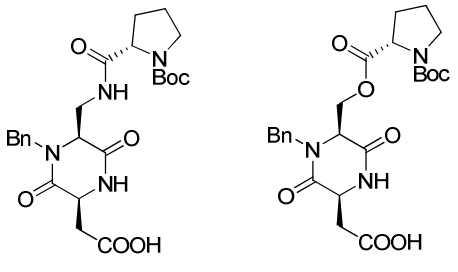
Scheme 90: Cyclic peptide containing a *trans*-DKP building block

Finally, two potential tripeptidic organocatalysts were synthesised, each of them containing a *cis*-DKP and being linked by an amide or an ester bond to a proline. Their investigation in organocatalysed aldol reaction and Michael addition is now in progress (Scheme 91).



263 265

Scheme 91: Potential organocatalysts containing the *cis*-DKP scaffold

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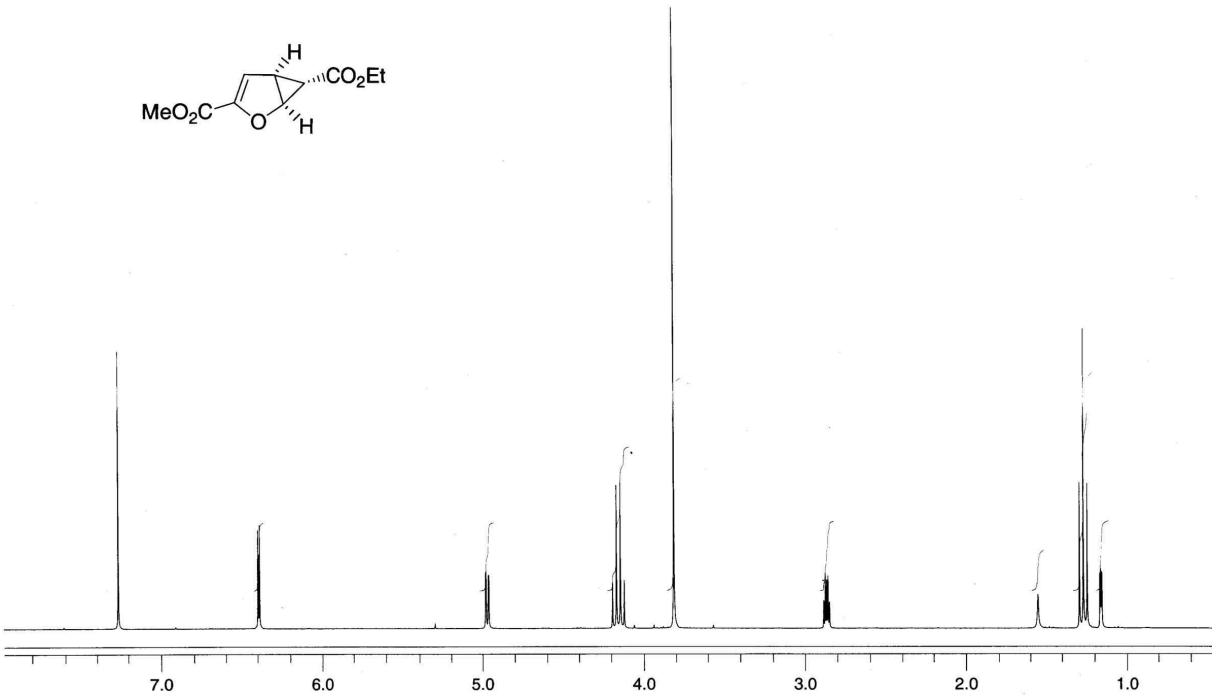
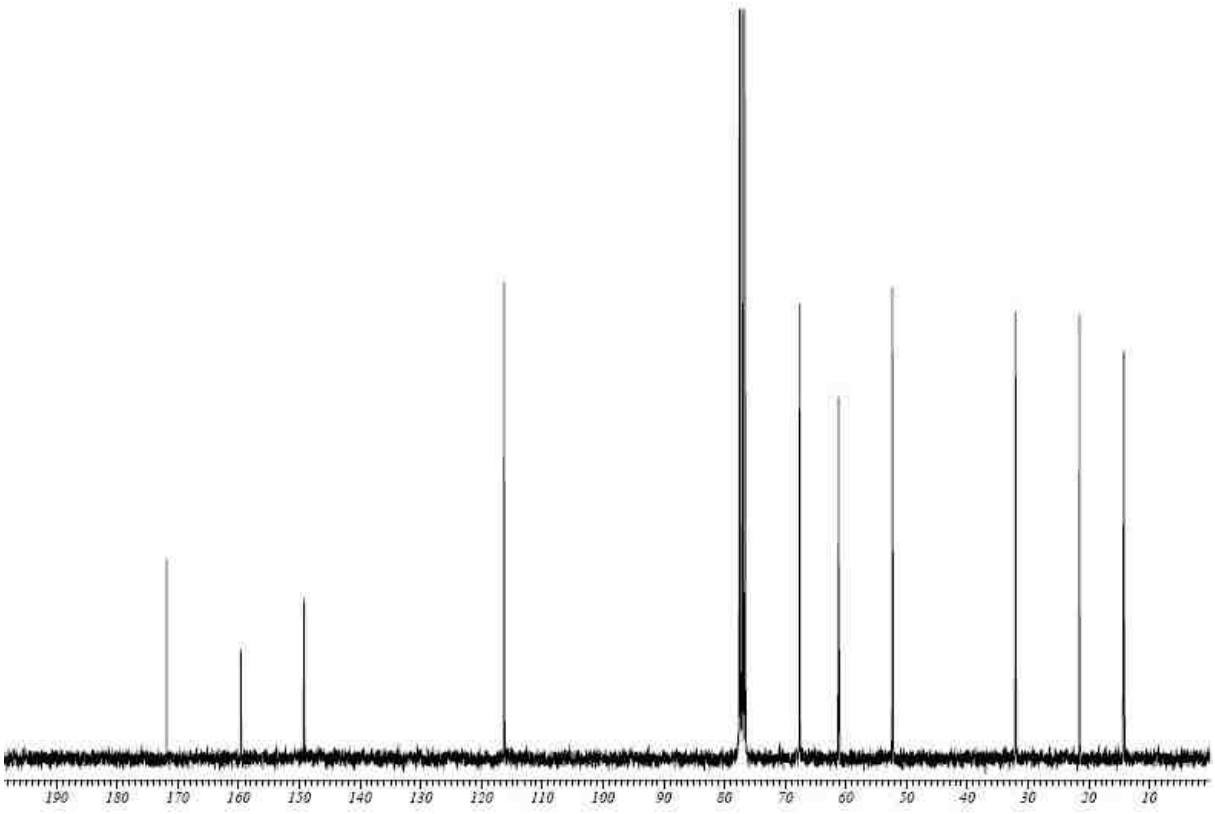
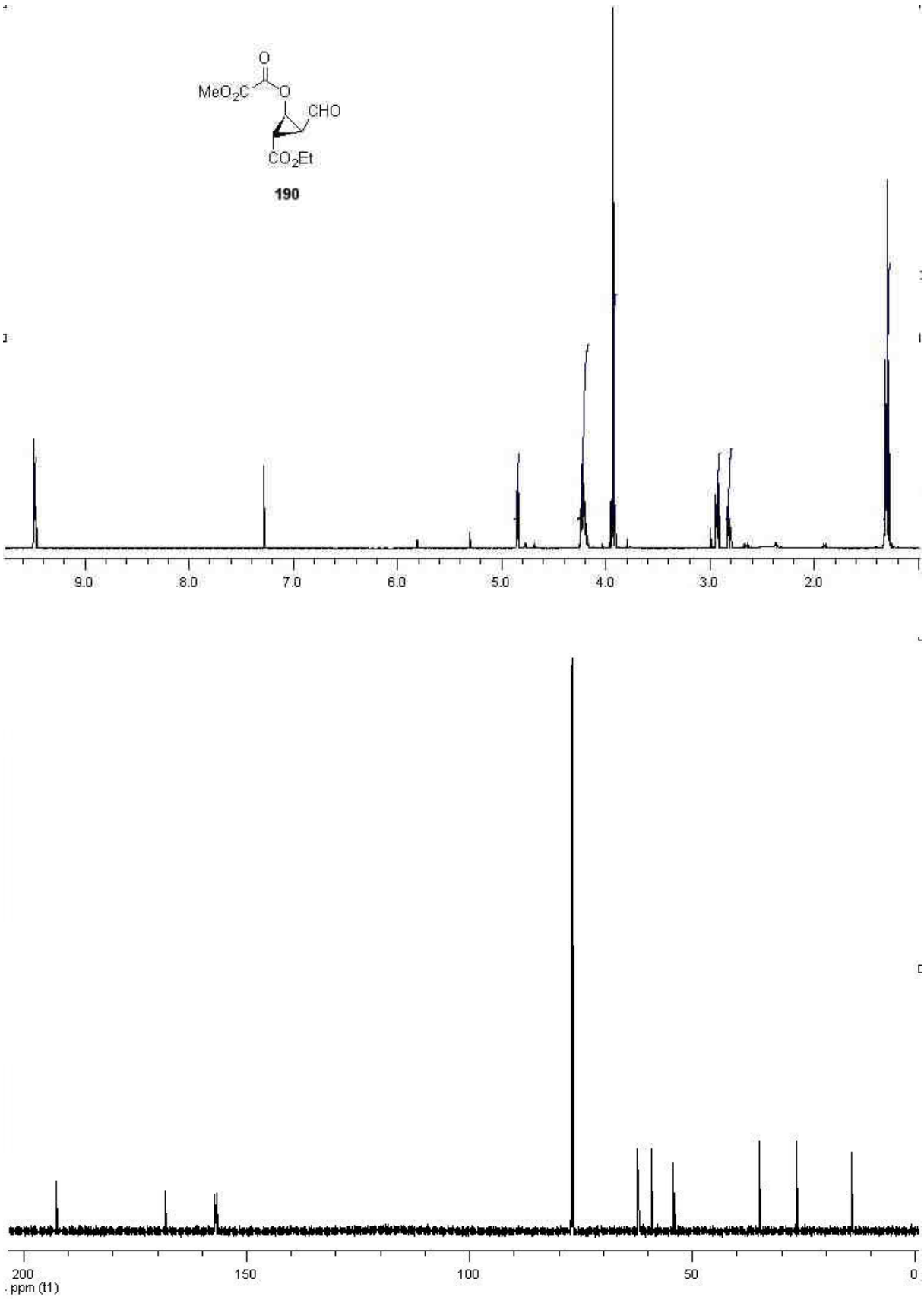
Appendix of NMR

(1S,5S,6S)-(-)-2-Oxabicyclo[3.1.0]hex-3-ene-3,6-dicarboxylic 6-ethylester-3-methyl ester (189) in $CDCl_3$

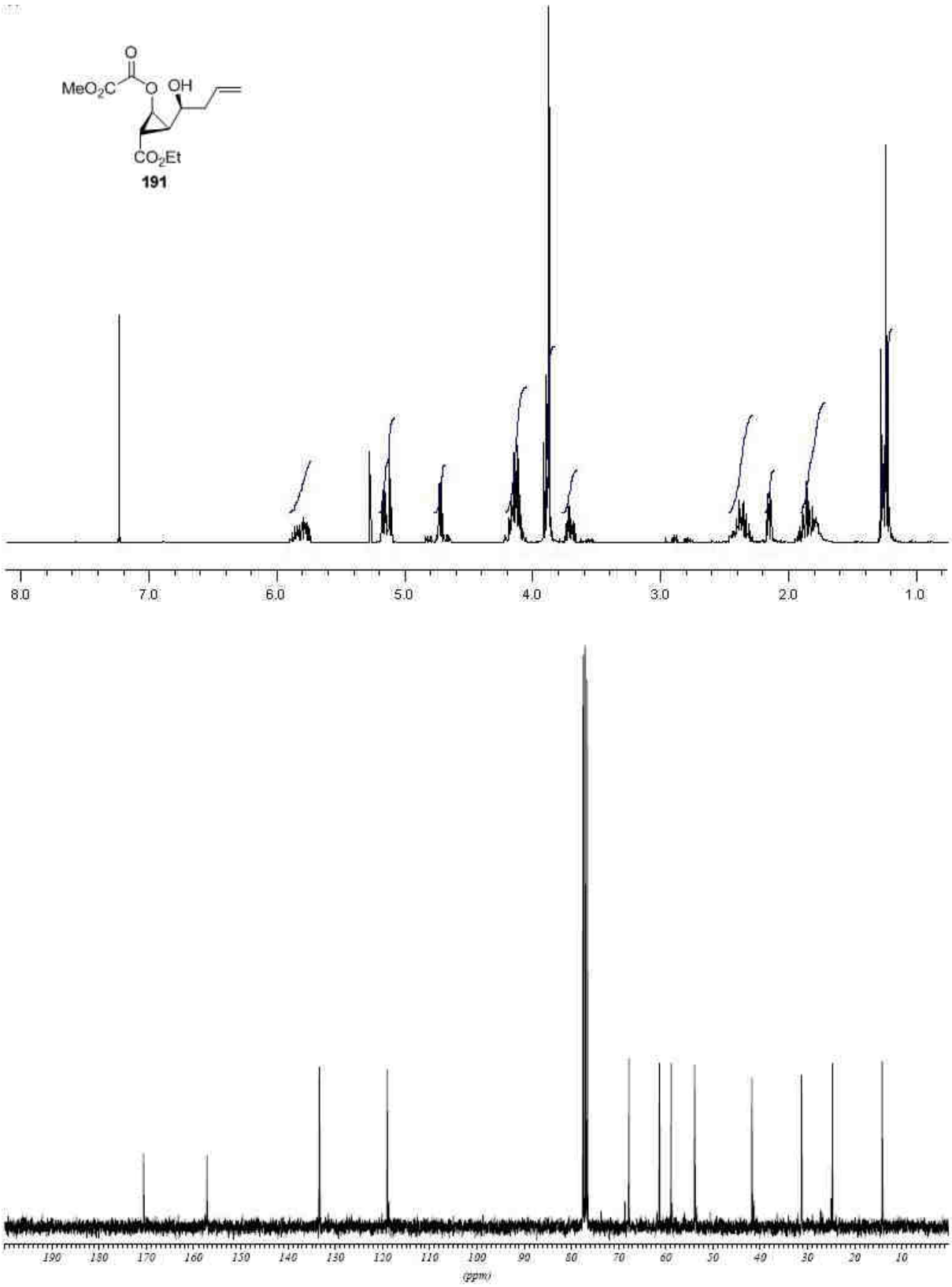




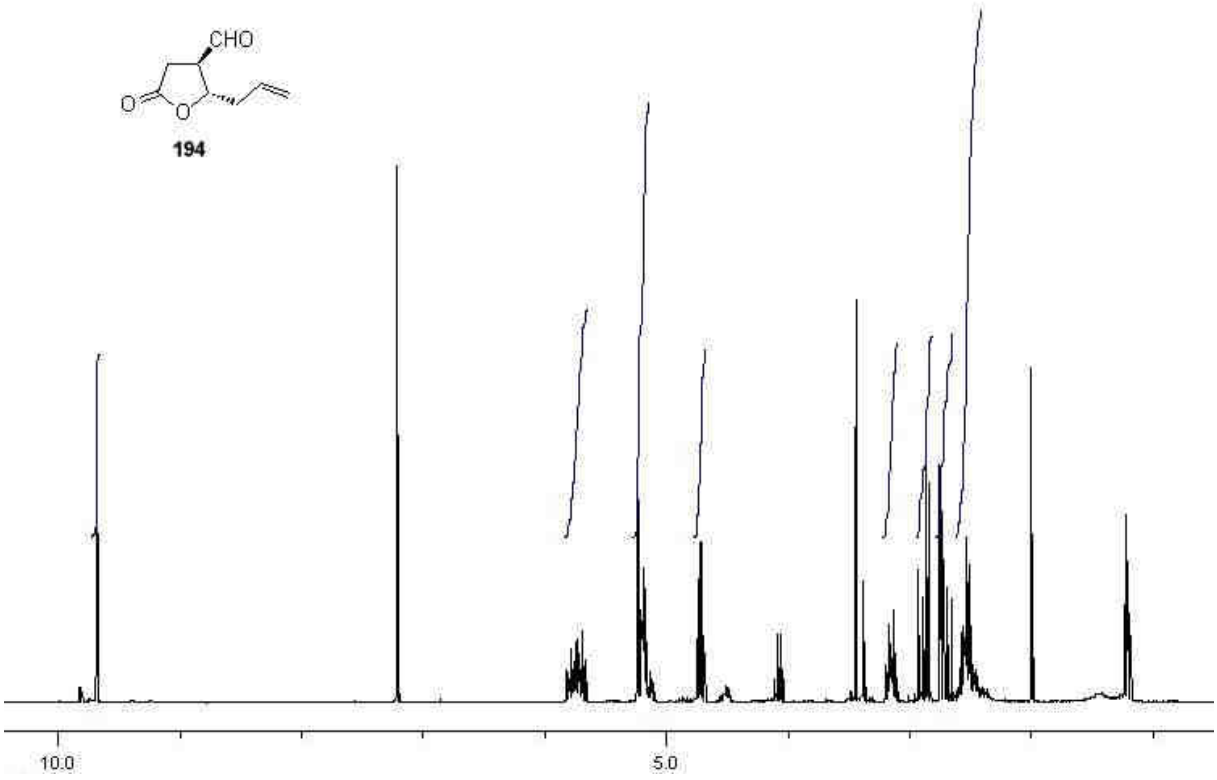
(1S,2R,3S)-2-(ethoxycarbonyl)-3-formylcyclopropyl methyl oxalate (190) in $CDCl_3$

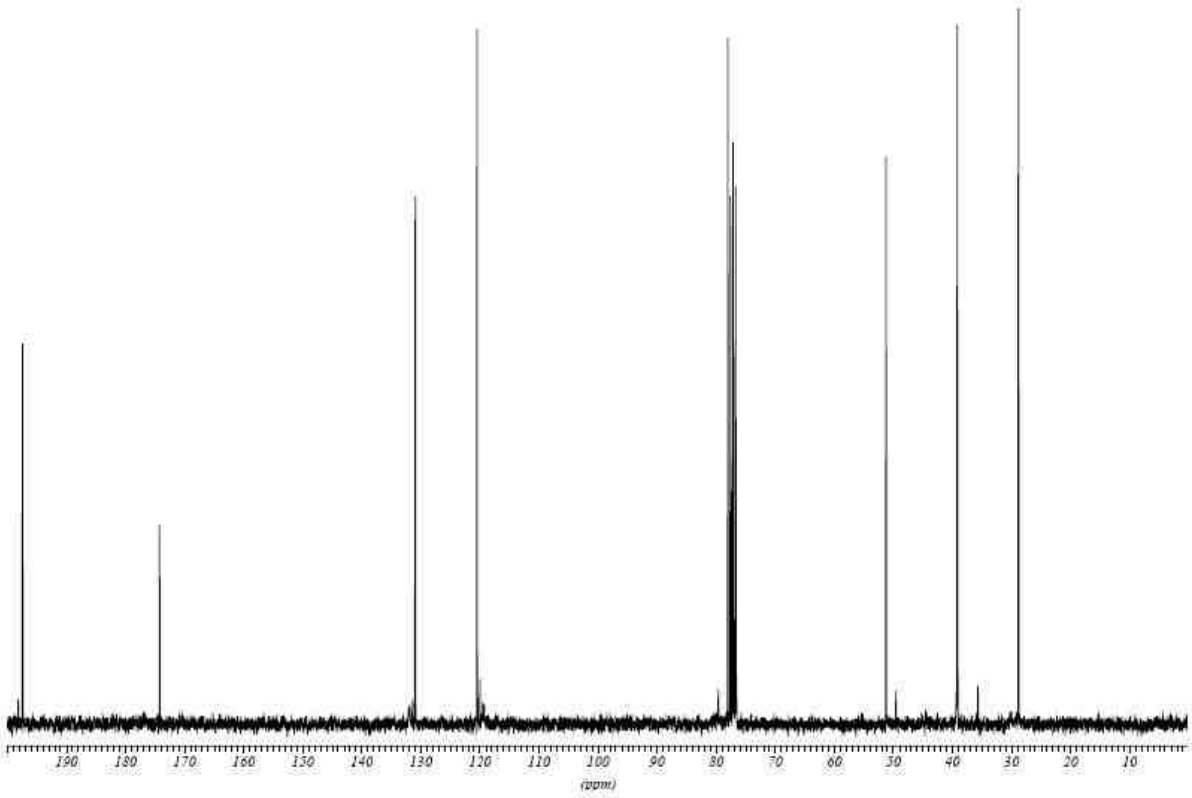


(1S,2R,3R)-2-(ethoxycarbonyl)-3-((S)-1-hydroxybut-3-enyl)cyclopropyl methyl oxalate (191) in $CDCl_3$

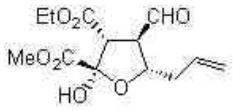


(2S/R,3S)-2-Allyl-5-oxotetrahydrofuran-3-carbaldehyde (194) in $CDCl_3$

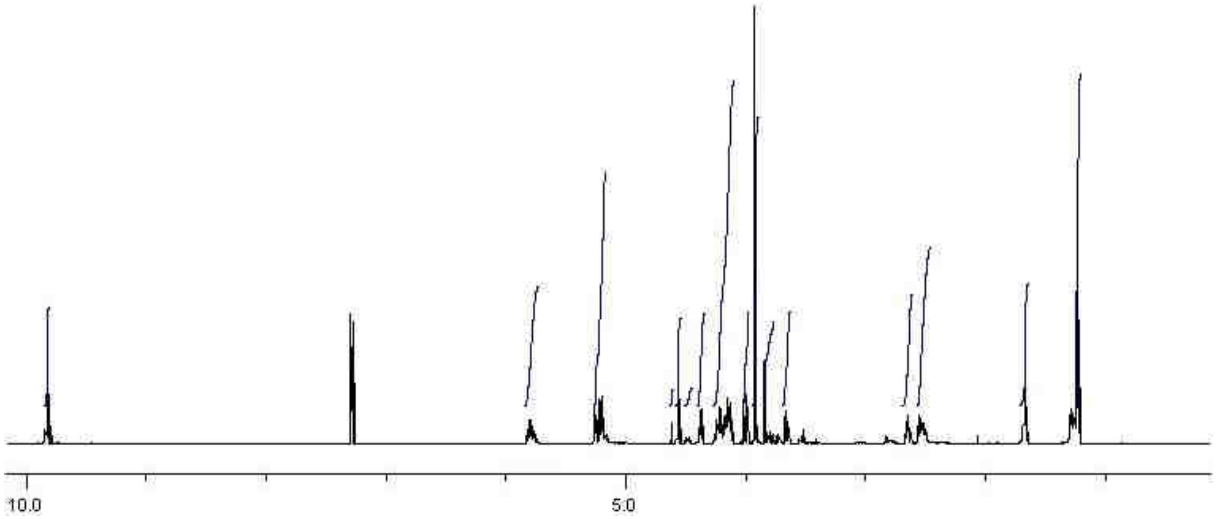


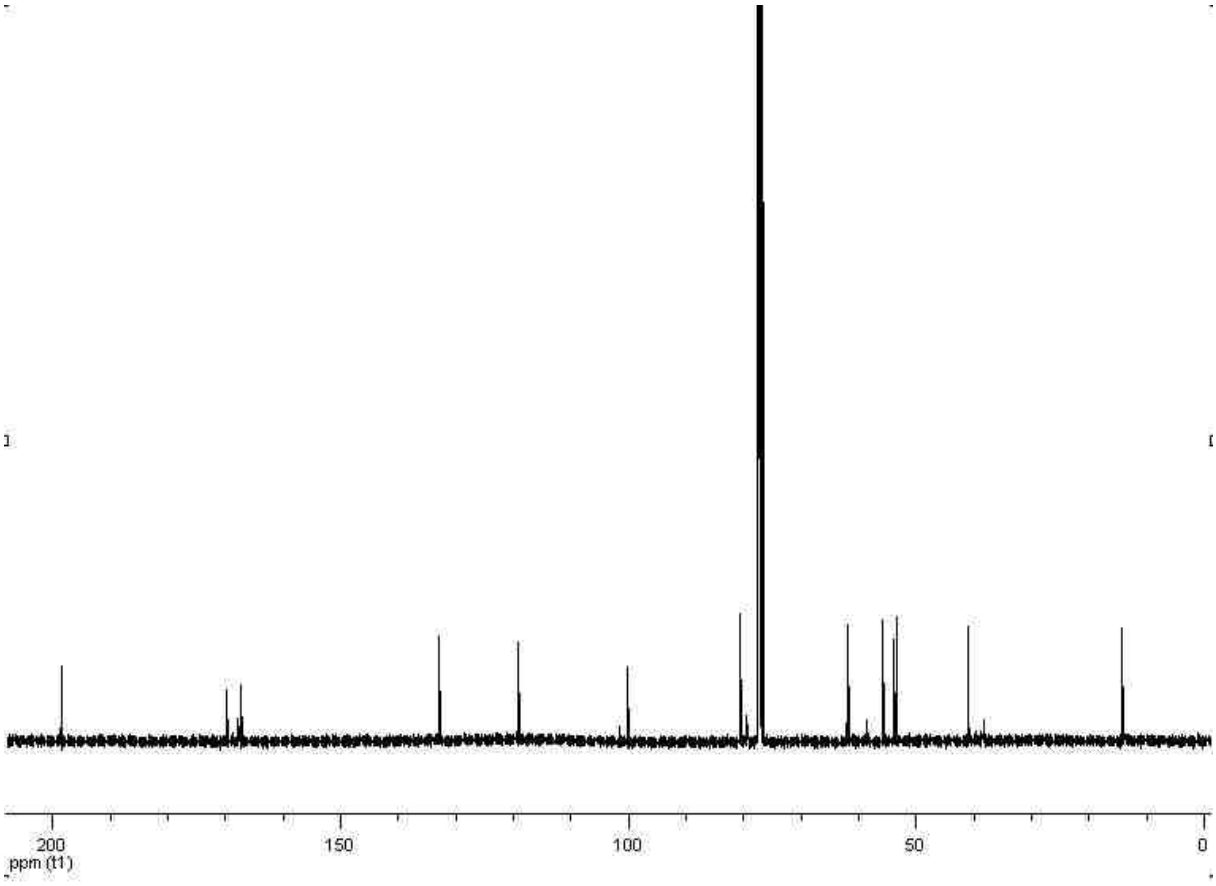


(2R/S, 3S, 4S, 5S)-5-Allyl-4-formyl-2-hydroxy-tetrahydro-furan-2,3-dicarboxylic acid 3-ethyl ester 2-methyl ester (193) in $CDCl_3$

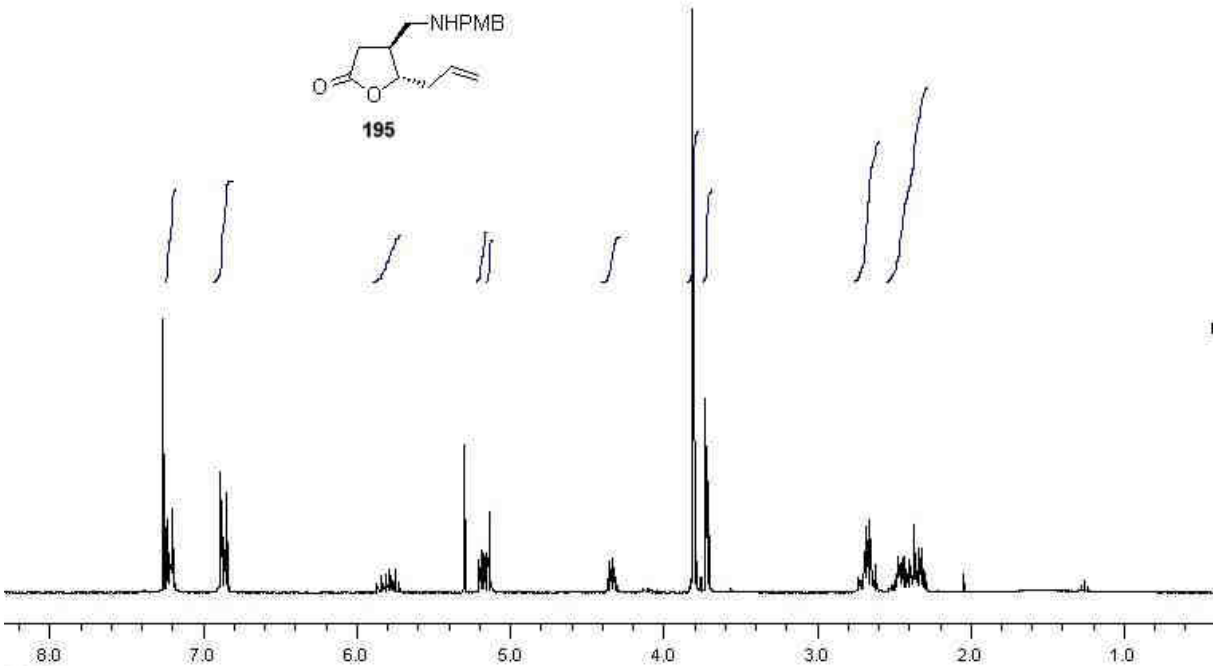


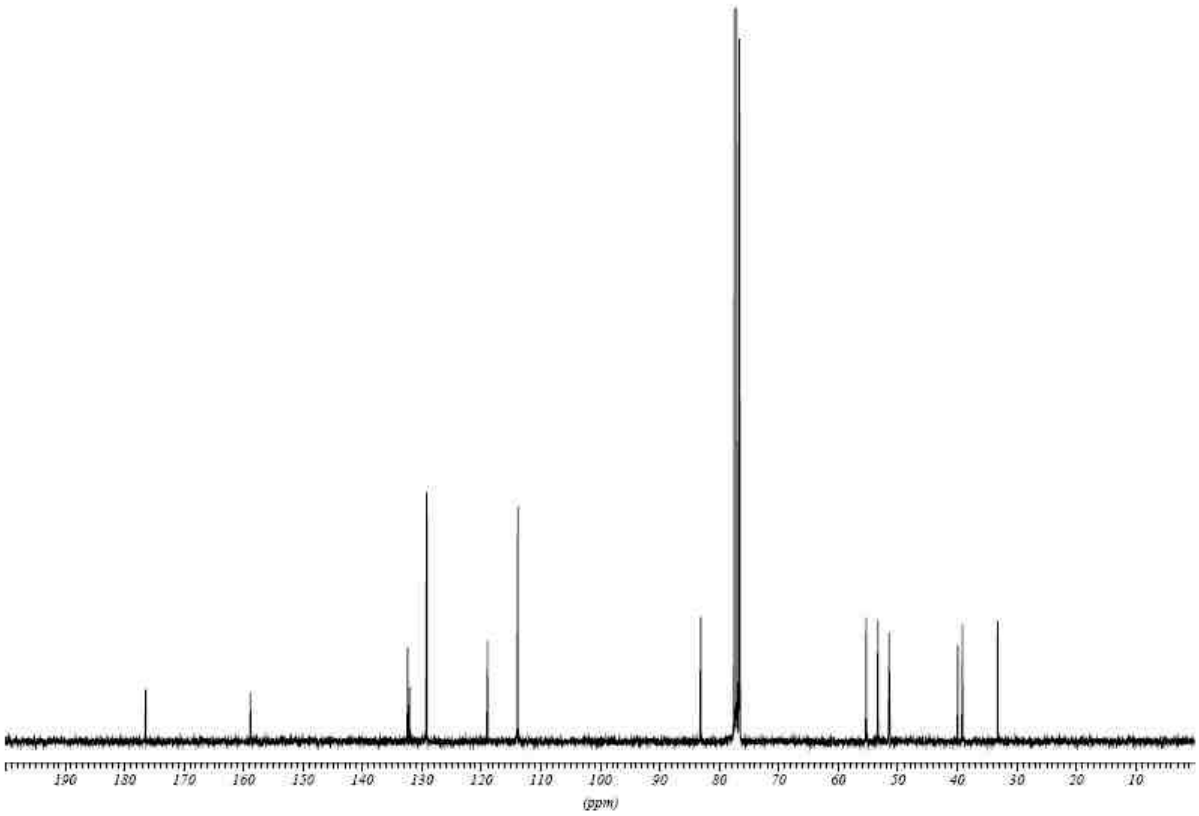
193



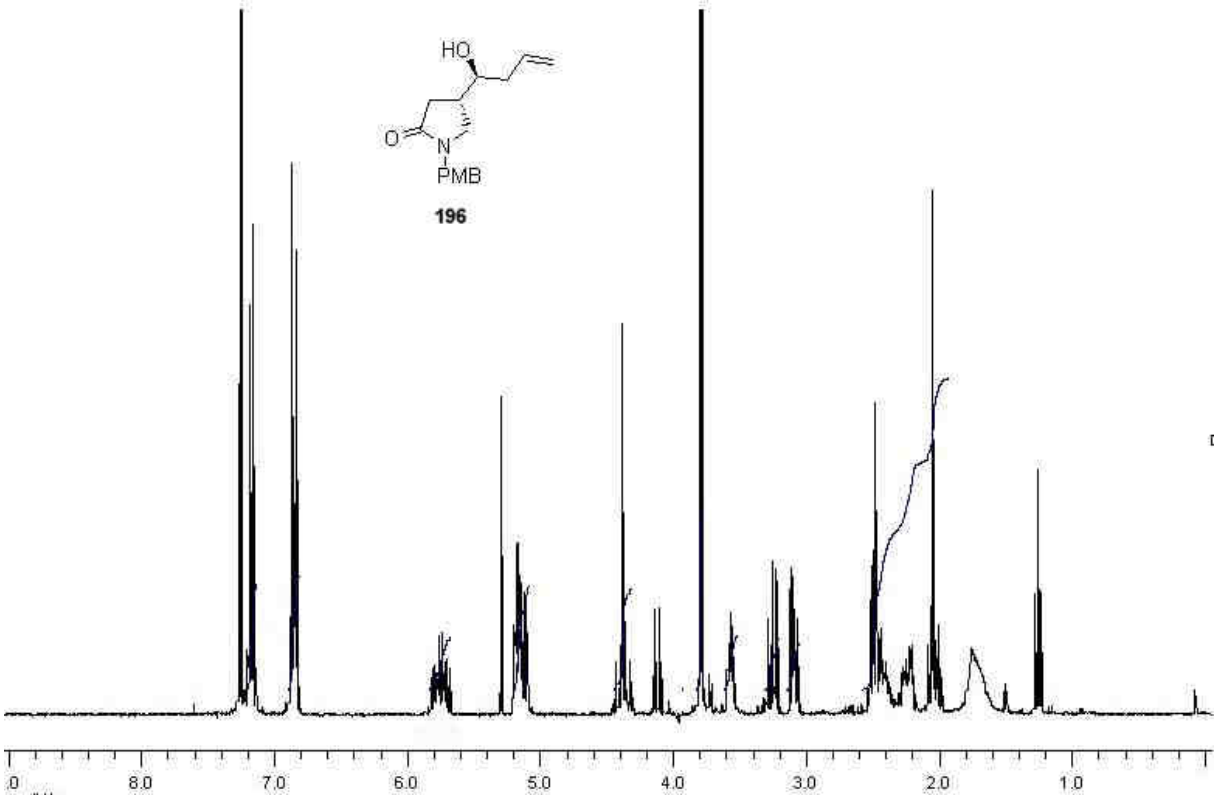


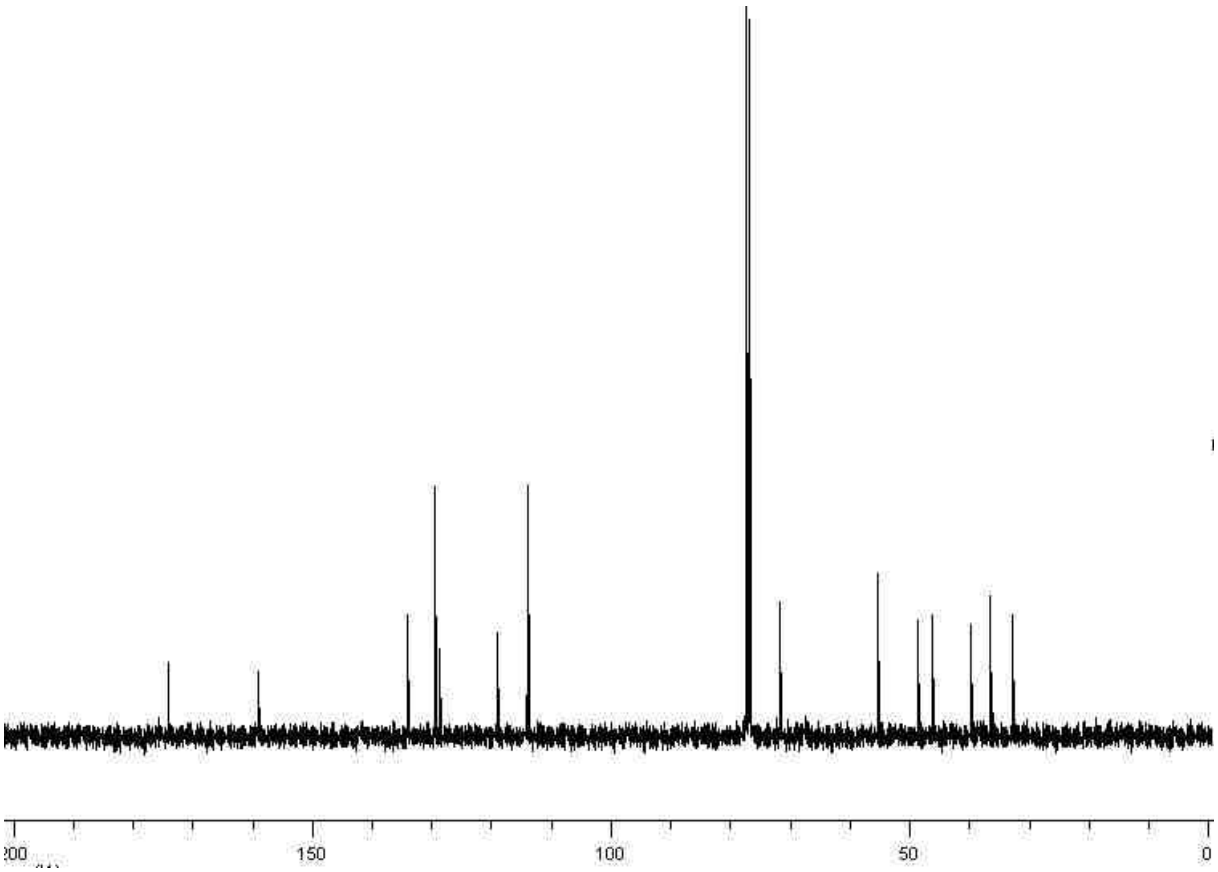
(4S,5S)-5-Allyl-4-[(4-methoxy-benzylamino)-methyl]-dihydro-furan-2-one (195) in $CDCl_3$



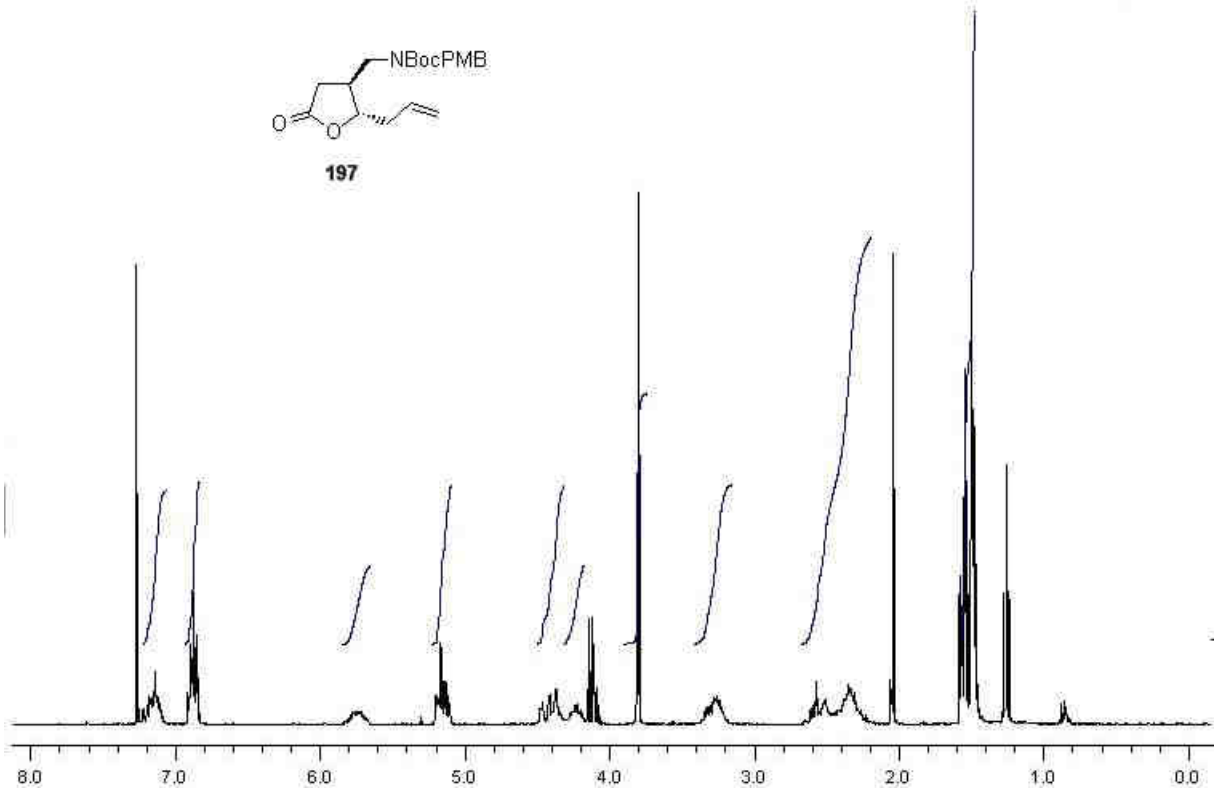


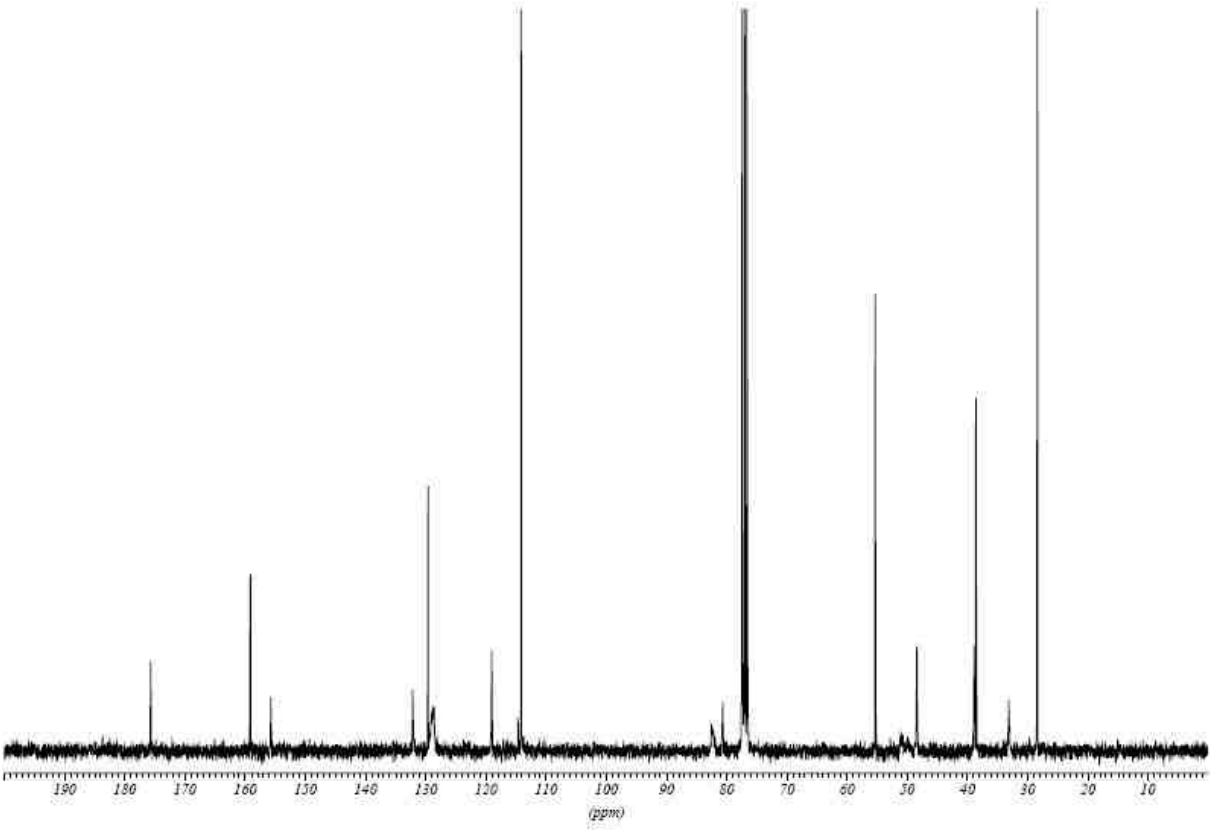
4-(1'-Hydroxy-but-3'-enyl)-1-(para-methoxy-benzyl)-pyrrolidin-2-one (196) in $CDCl_3$



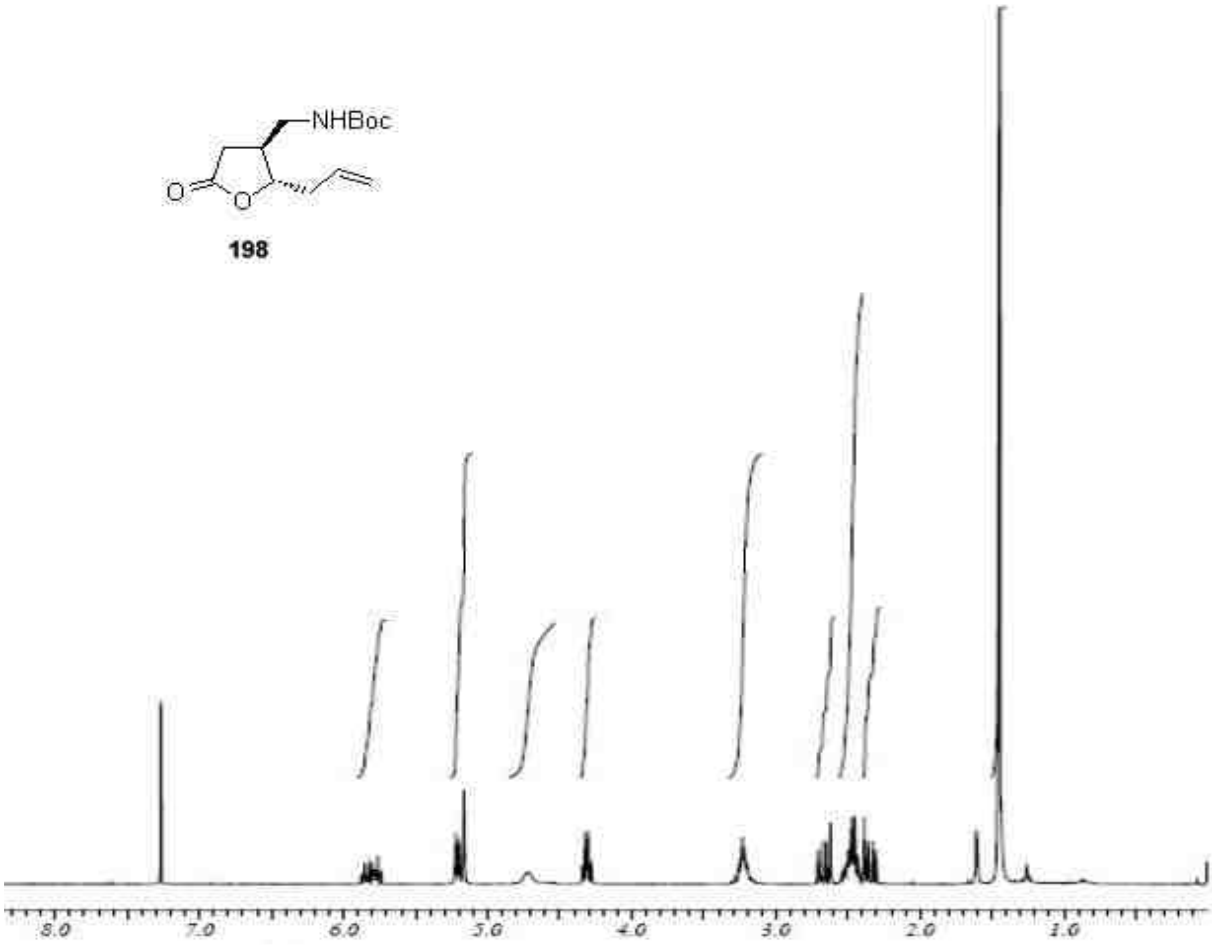


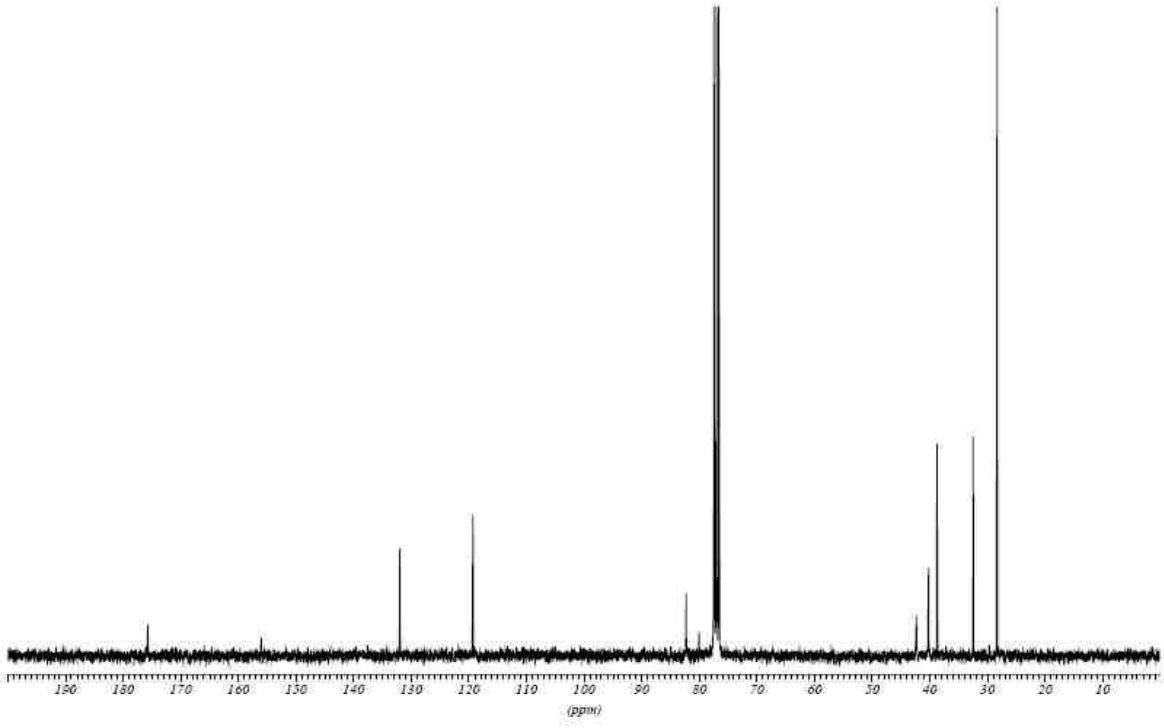
(2S,3S)-(2-Allyl-5-oxo-tetrahydro-furan-3-ylmethyl)-(4-methoxy-benzyl)-carbamic acid tert-butyl ester (197) in $CDCl_3$



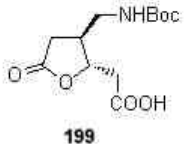


(2S,3S)-(-)-(2-Allyl-5-oxo-tetrahydro-furan-3-ylmethyl)-carbamic acid tert-butyl ester (198) in $CDCl_3$

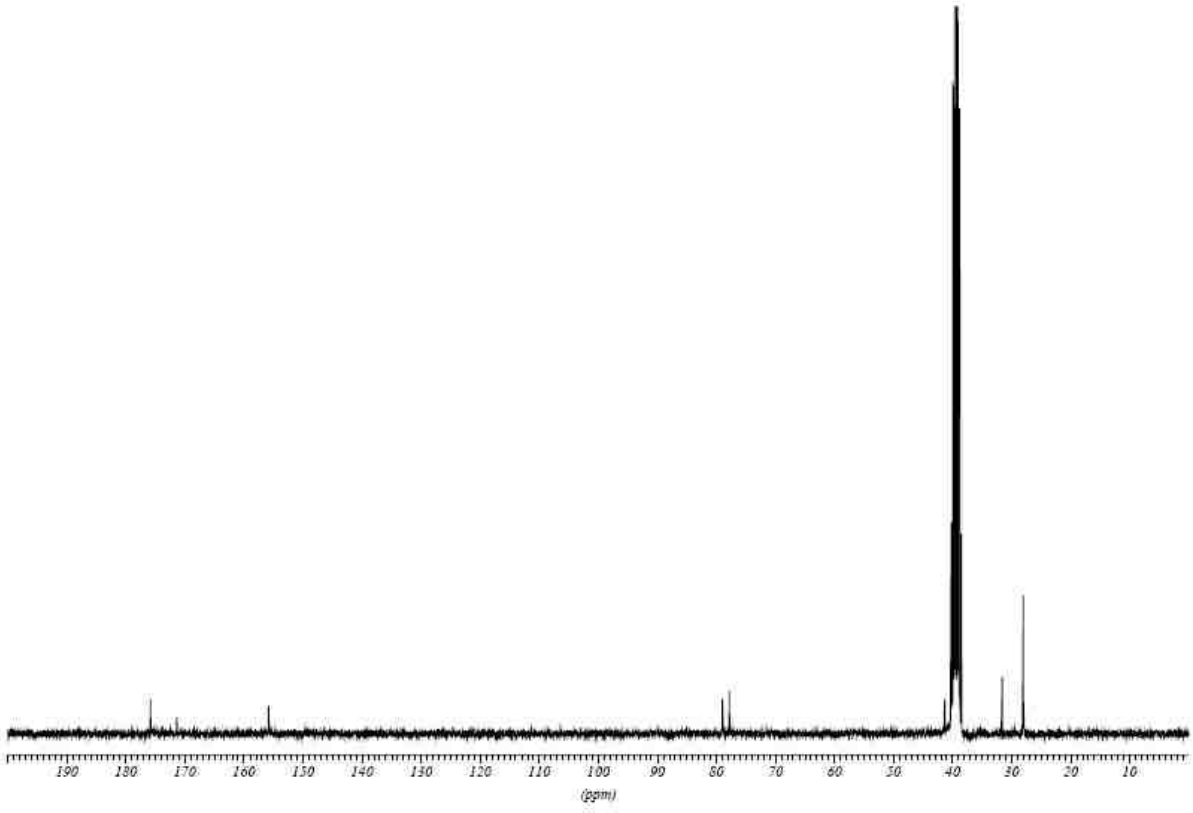




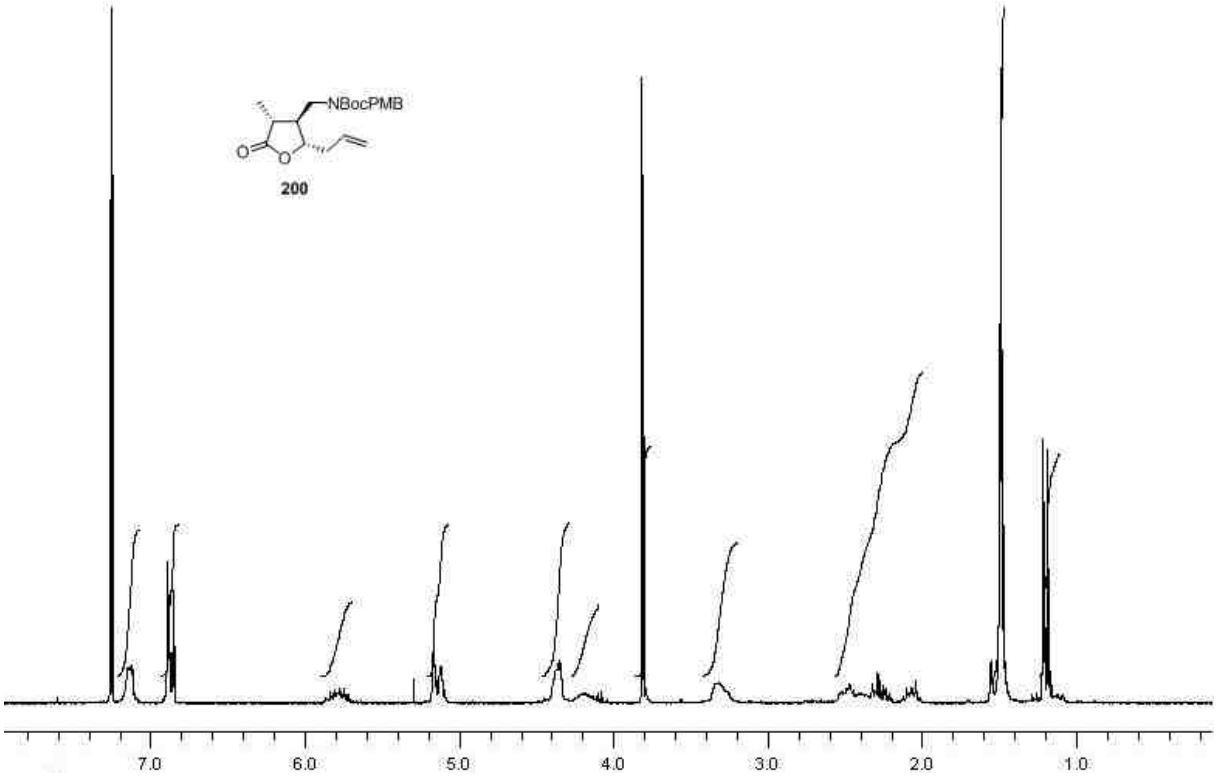
(-)-(2S,3S)-[3-(tert-Butoxycarbonylamino-methyl)-5-oxo-tetrahydro-furan-2-yl]-acetic acid (200) in DMSO-d_6



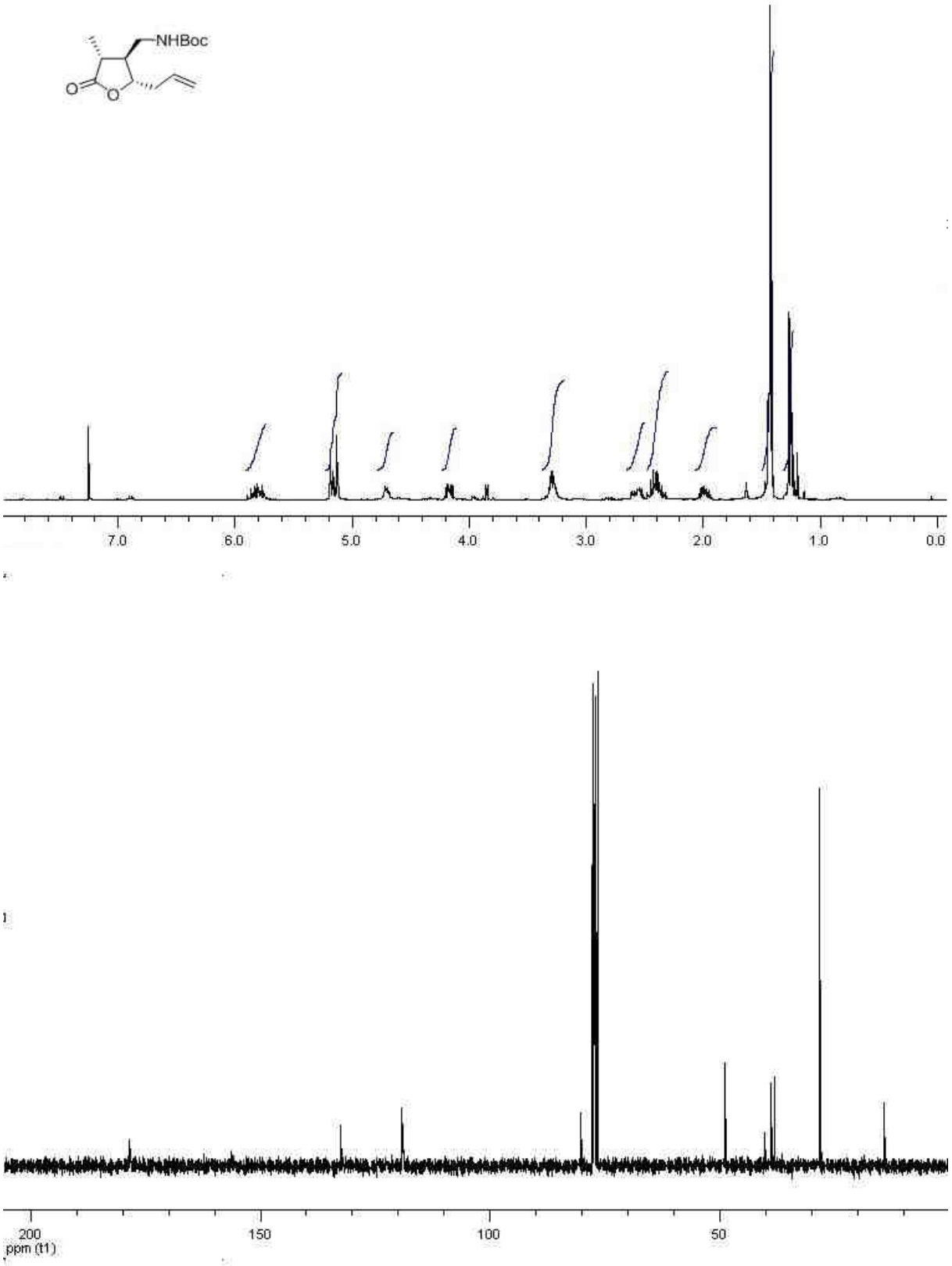
199



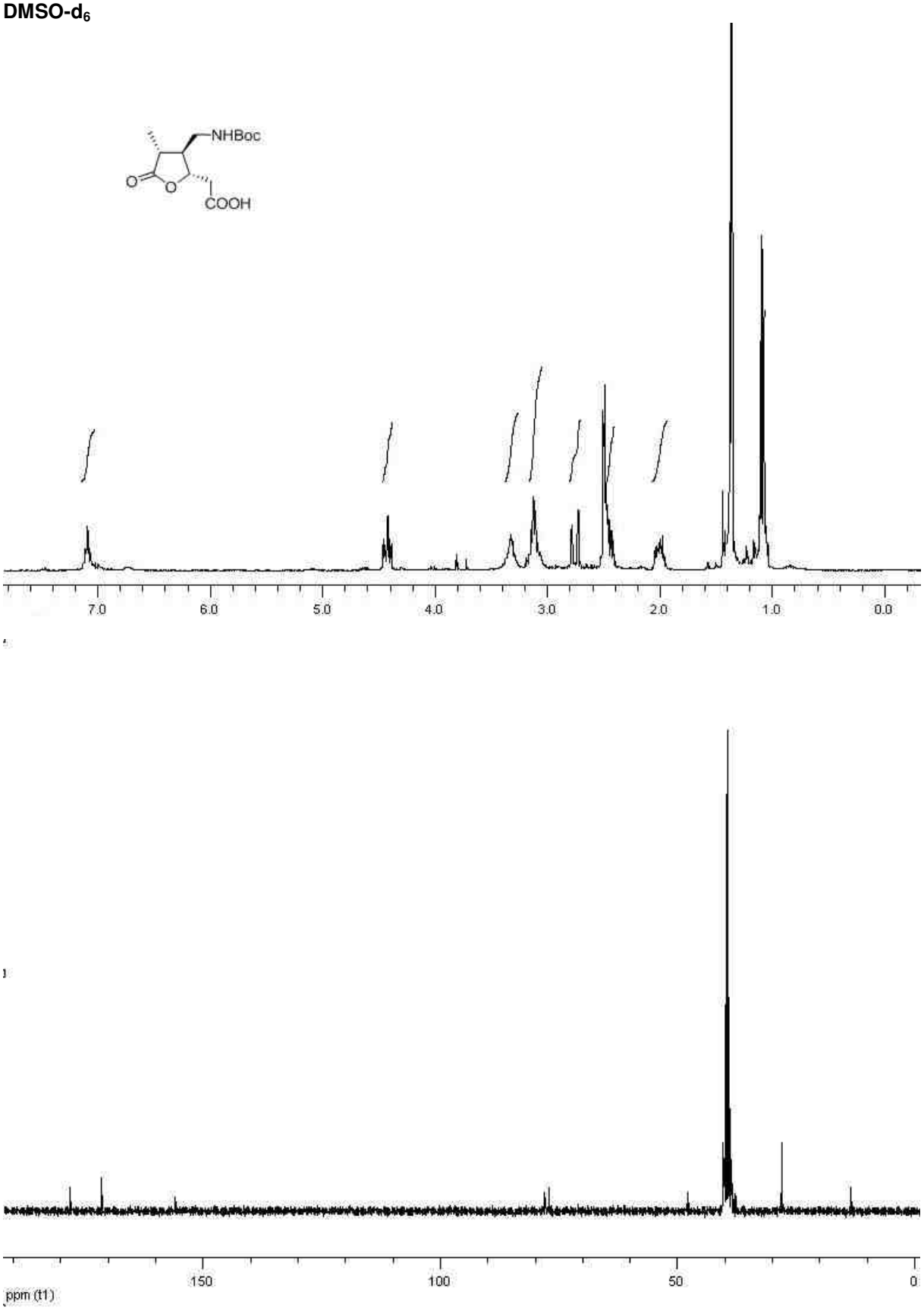
tert-butyl-((2S,3S,4R)-2-allyl-4-methyl-5-oxotetrahydrofuran-3-yl)methyl(4-methoxybenzyl) carbamate (200) in $CDCl_3$



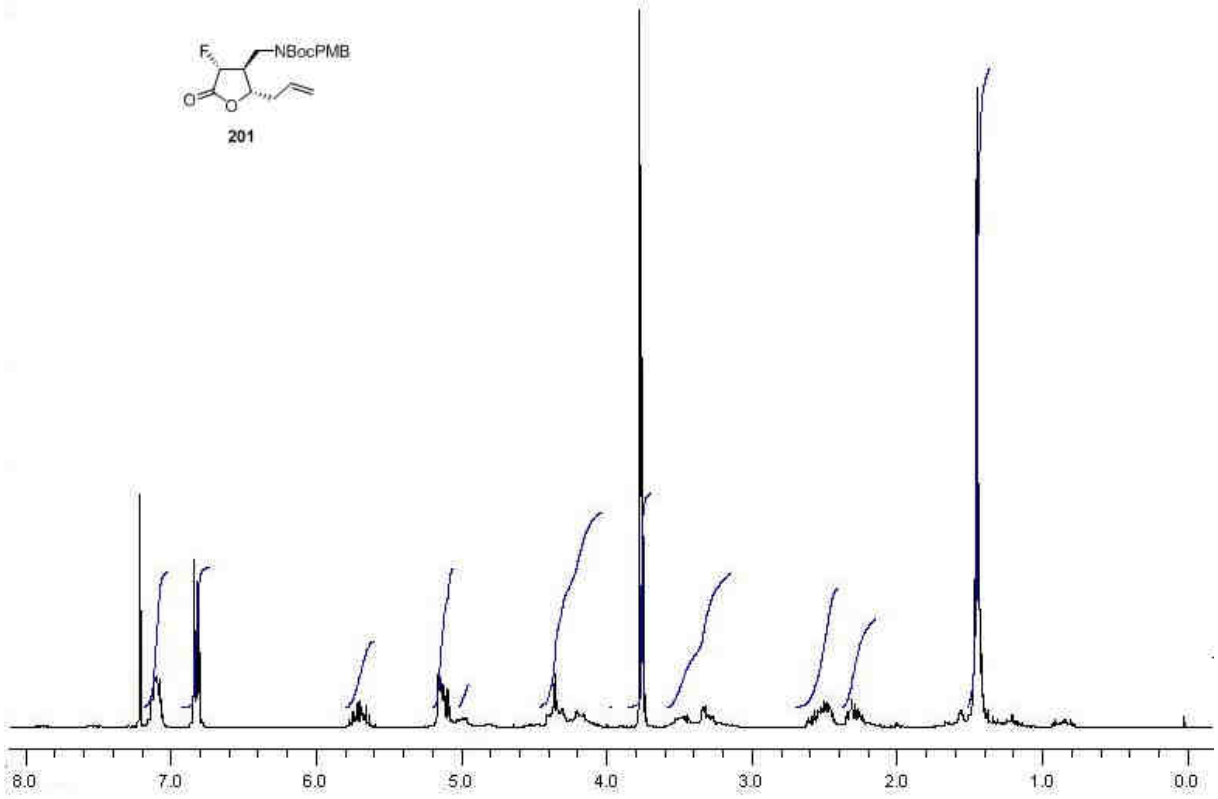
tert-butyl ((2S,3S,4R)-2-allyl-4-methyl-5-oxotetrahydrofuran-3-yl)methylcarbamate in $CDCl_3$

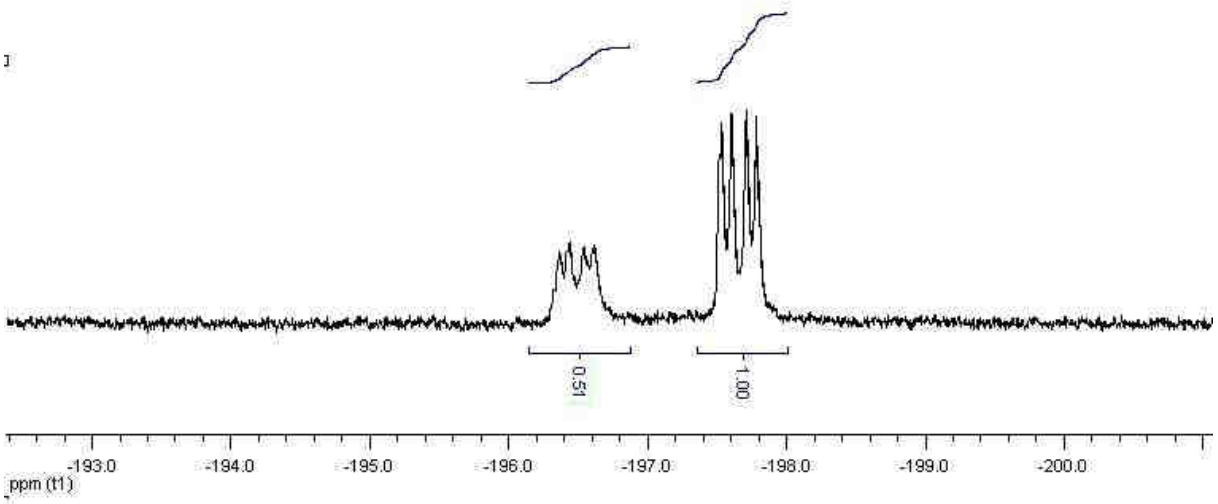


2-((2S,3S,4R)-3-((tert-butoxycarbonylamino)methyl)-4-methyl-5-oxotetrahydrofuran-2-yl)acetic acid in DMSO-d_6

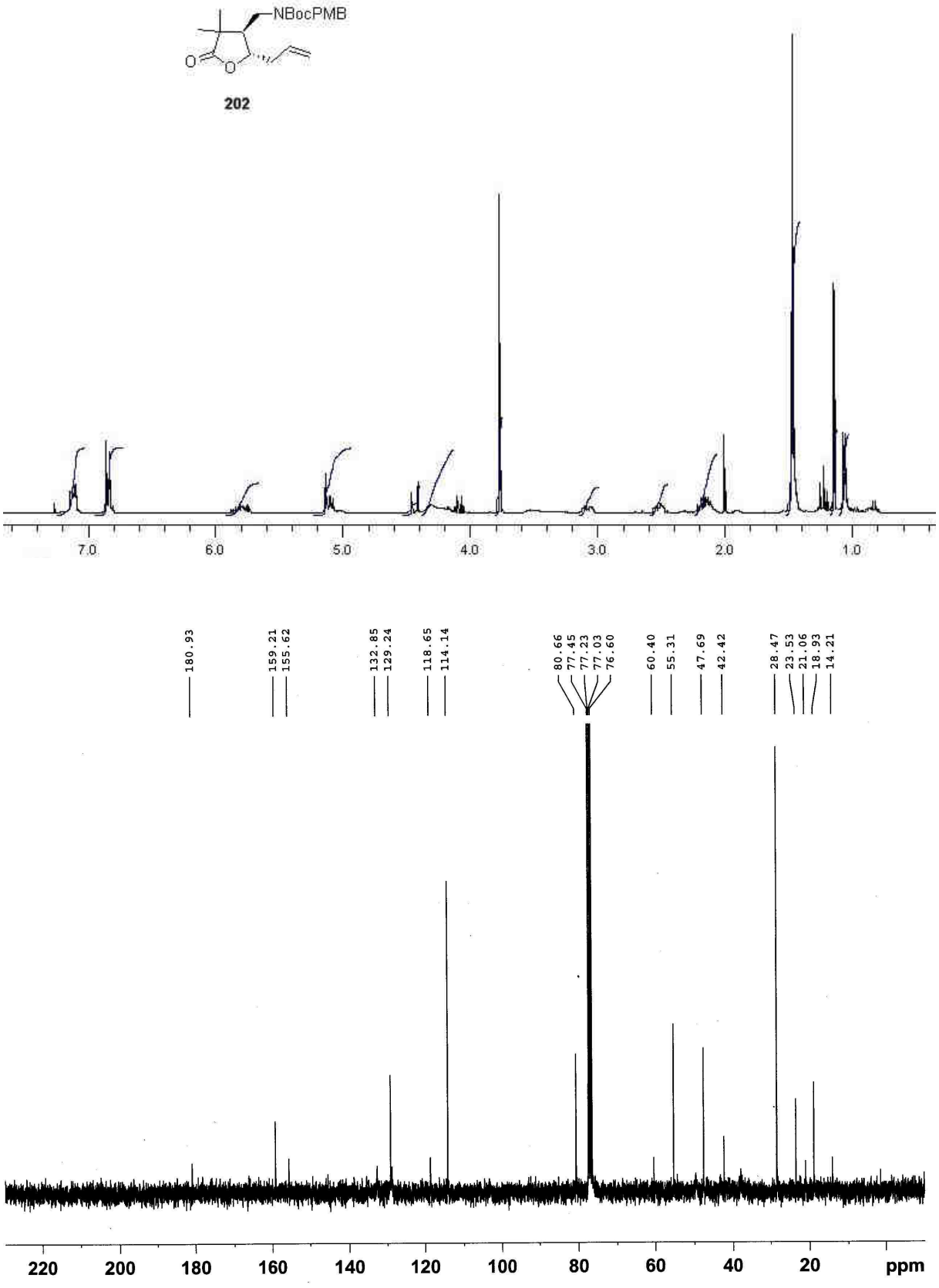


tert-butyl ((2S,3S,4R)-2-allyl-4-fluoro-5-oxotetrahydrofuran-3-yl)methyl(4-methoxybenzyl) carbamate (201) in $CDCl_3$: ^1H and ^{19}F NMR

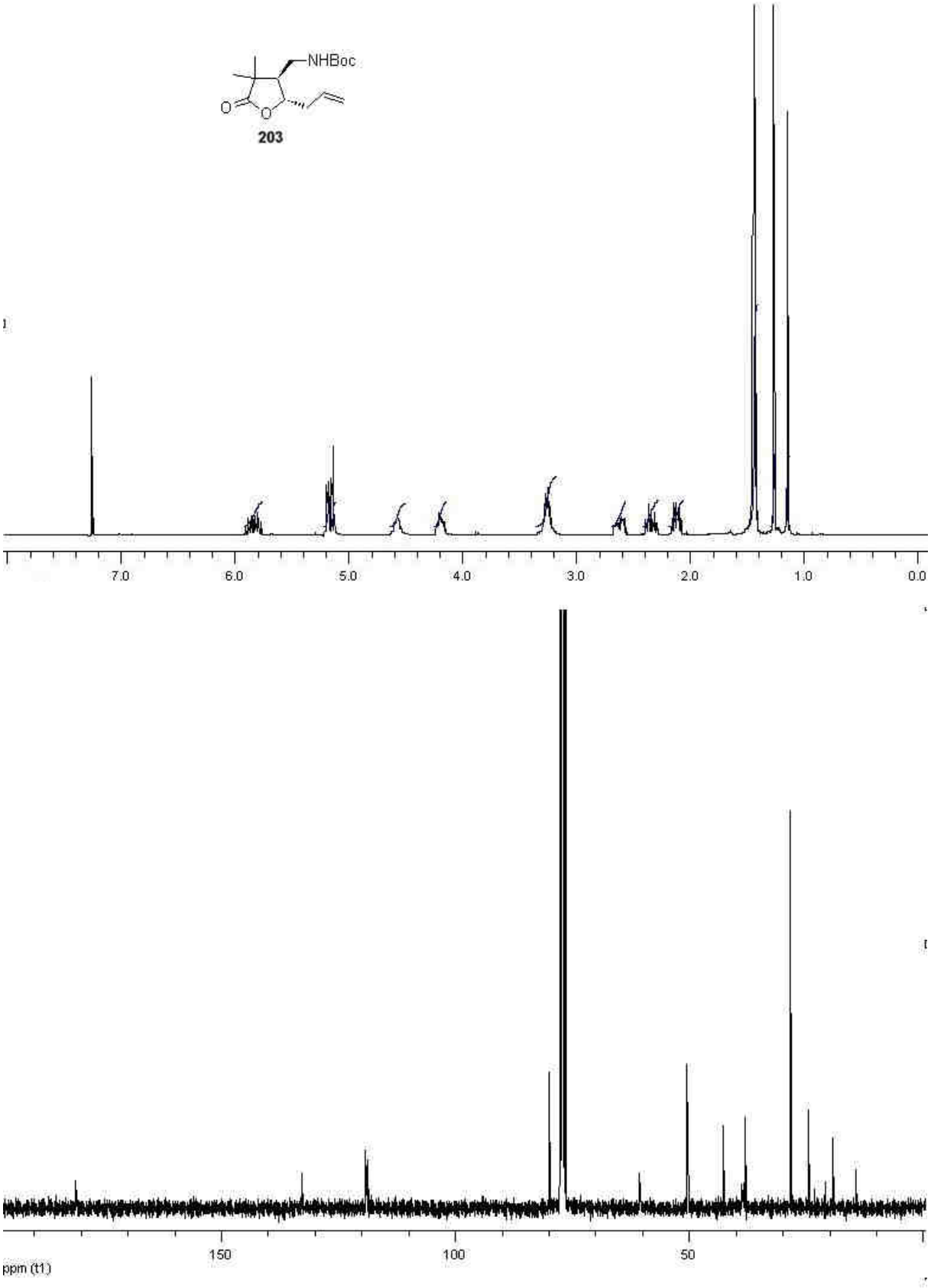




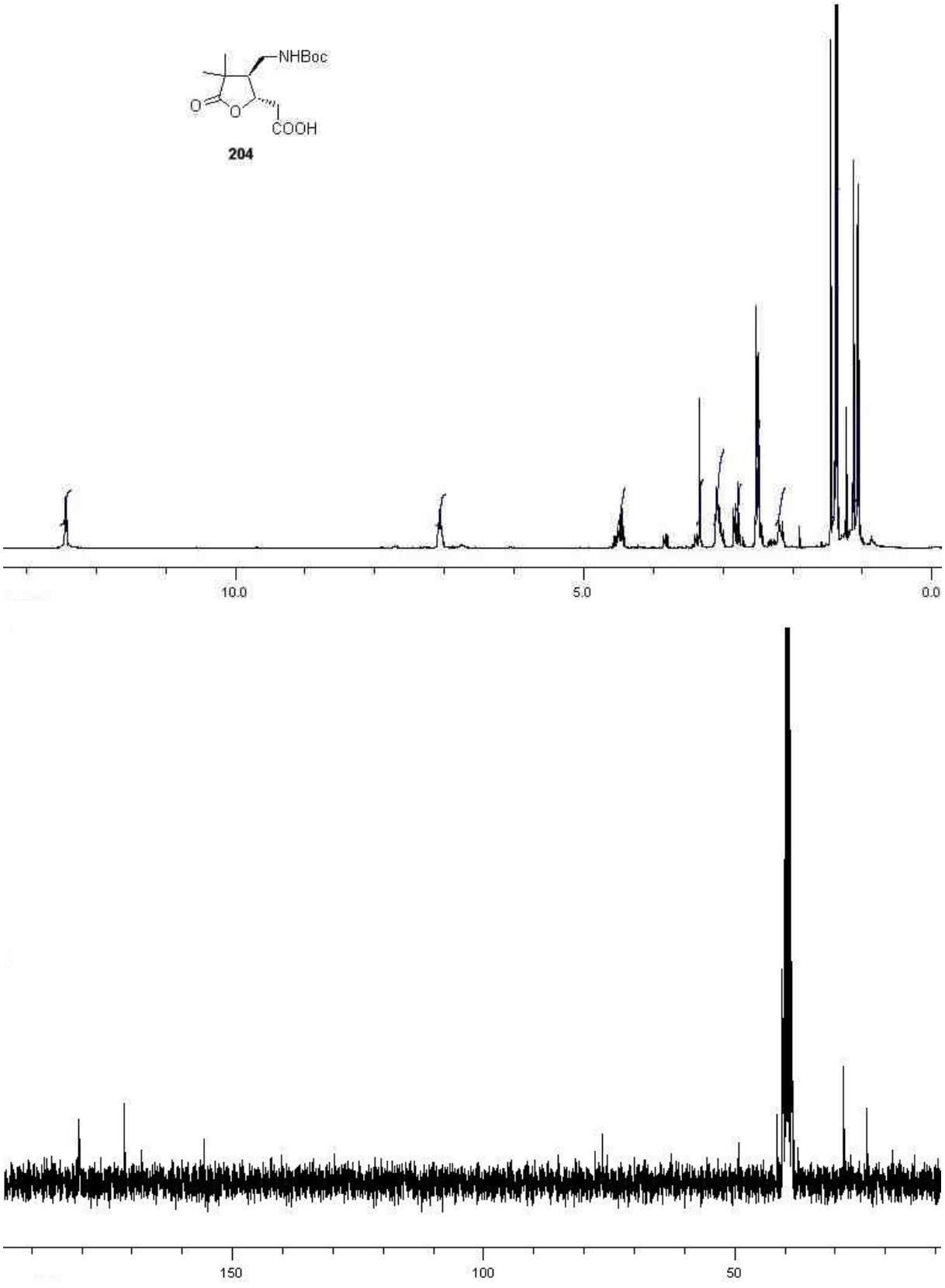
(2S, 3S)-(2-Allyl-4,4-dimethyl-5-oxo-tetrahydro-furan-3-ylmethyl)-(4-methoxy-benzyl)-carbamic acid tert-butyl ester (202) in $CDCl_3$



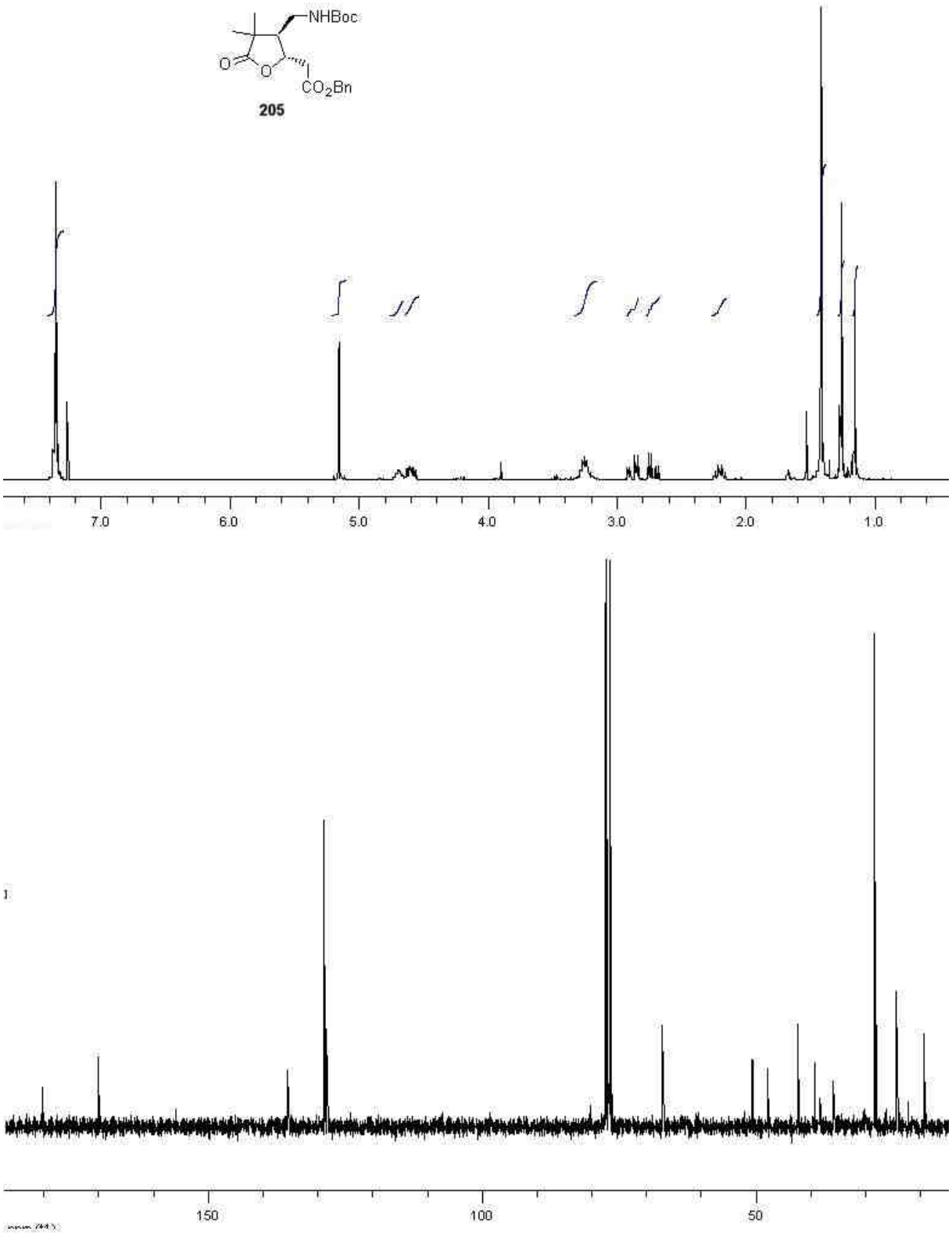
(2S, 3S)-(2-Allyl-4,4-dimethyl-5-oxo-tetrahydro-furan-3-ylmethyl)-carbamic acid tert-butyl ester (203) in $CDCl_3$



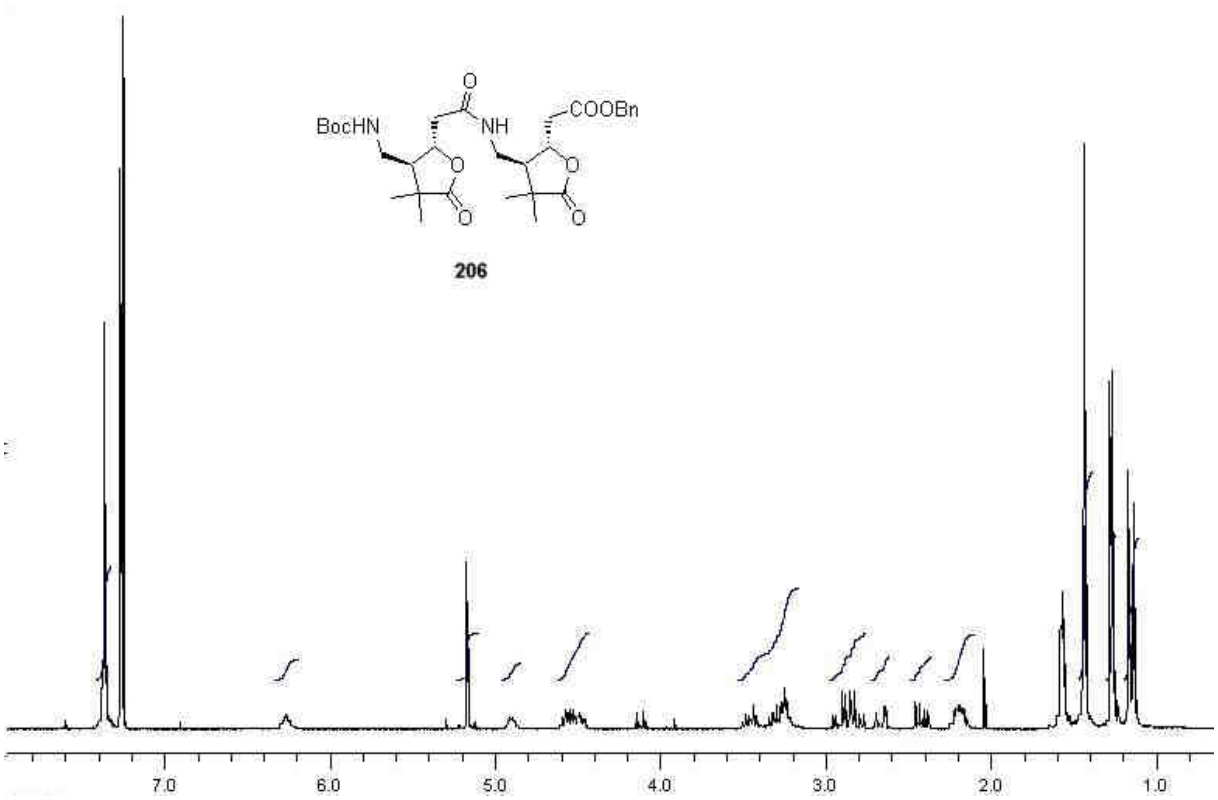
(2S, 3S)-[3-(tert-Butoxycarbonylamino-methyl)-4,4-dimethyl-5-oxo-tetrahydro-furan-2-yl]-acetic acid (204) in DMSO-d_6



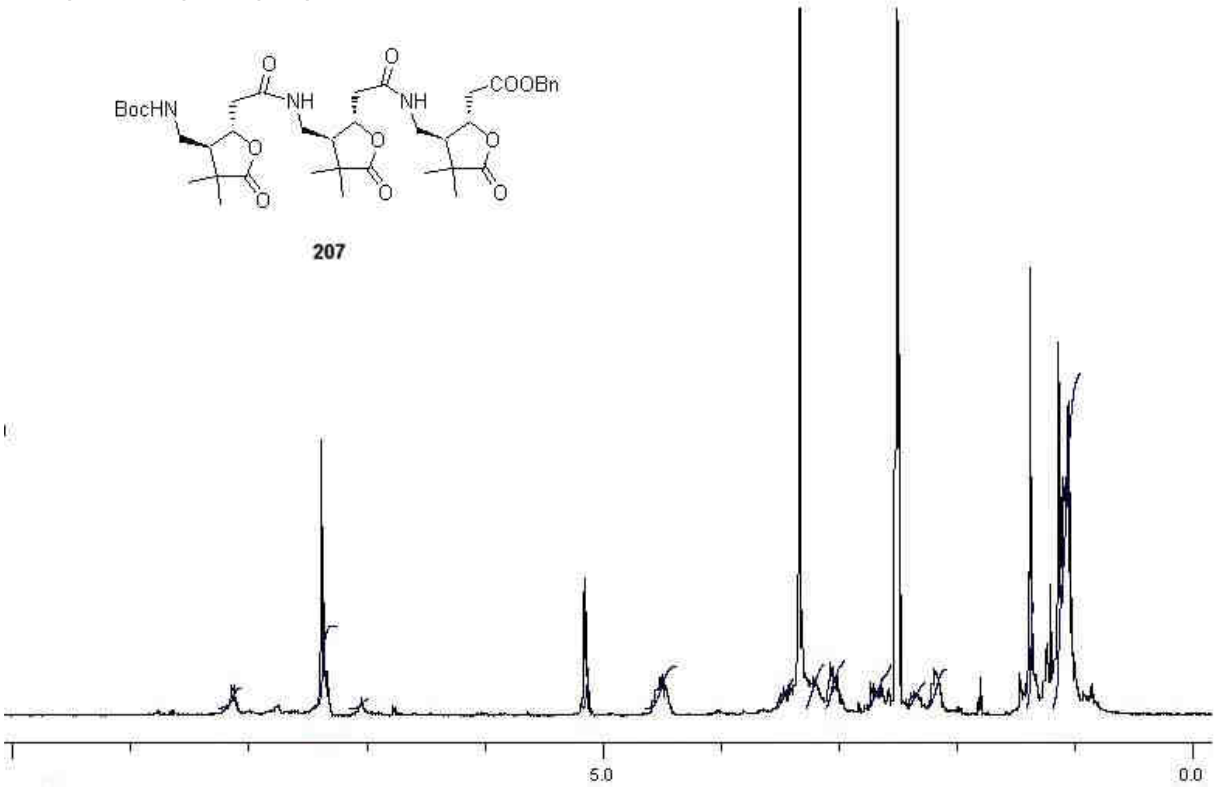
[3-(tert-Butoxycarbonylamino-methyl)-4,4-dimethyl-5-oxo-tetrahydro-furan-2-yl]-acetic acid benzyl ester (205) in $CDCl_3$



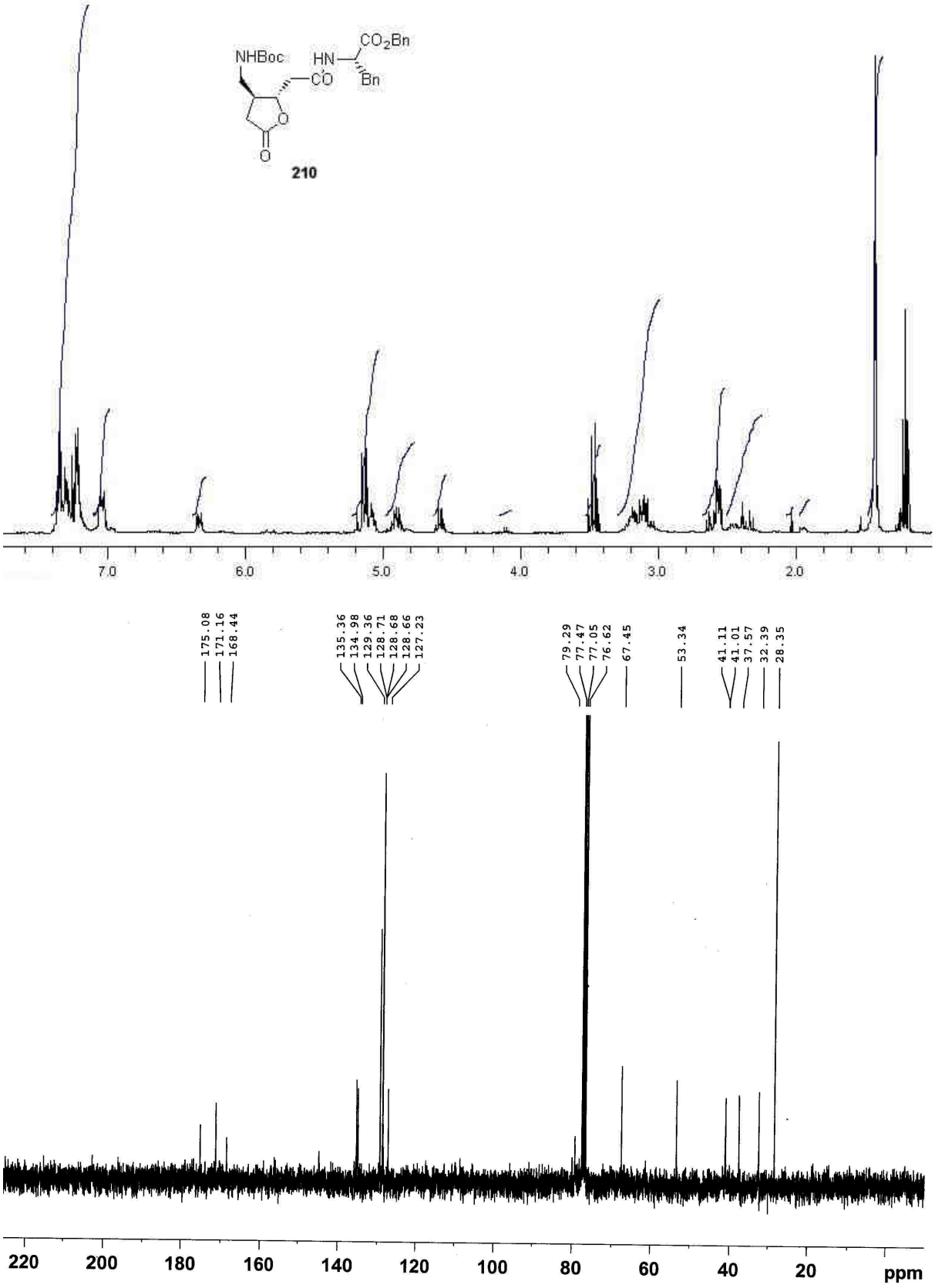
Boc-(DiMeLac)$_2$-Bn (206) in $CDCl_3$



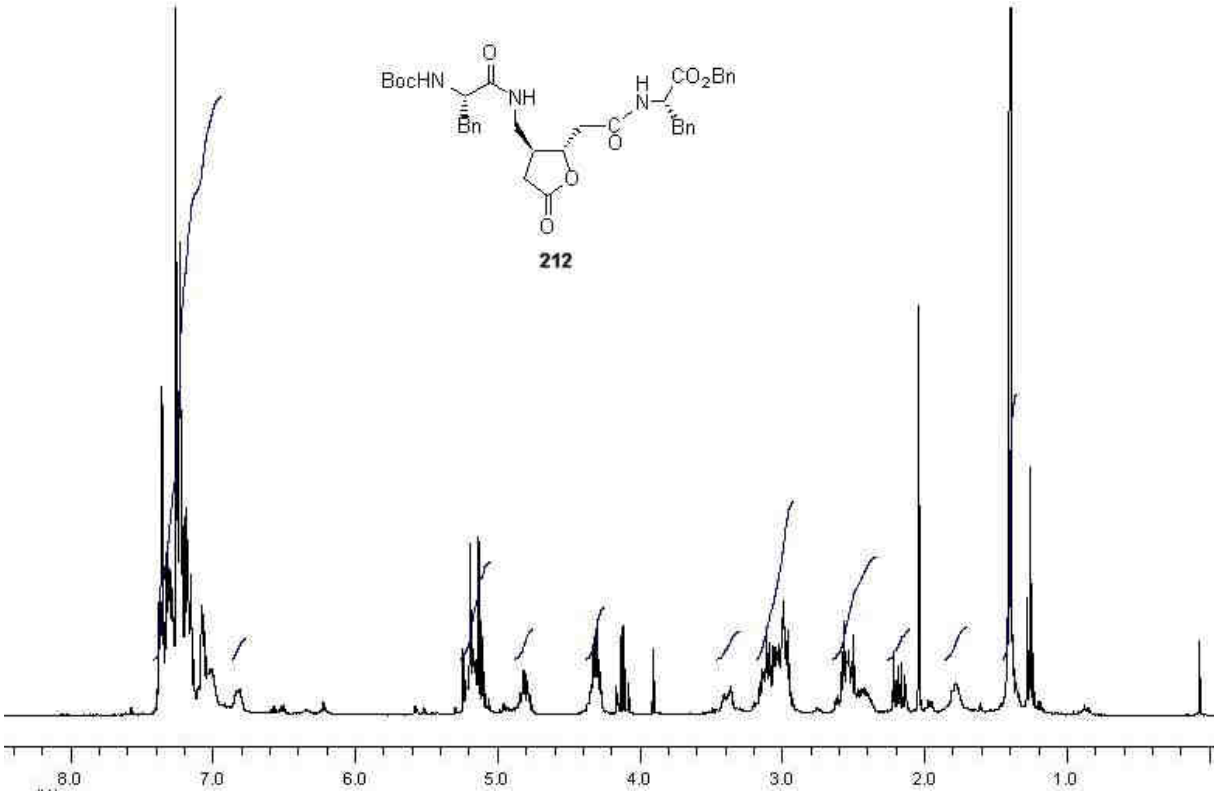
Boc-(DiMeLac)$_3$-Bn (207) in DMSO-d_6



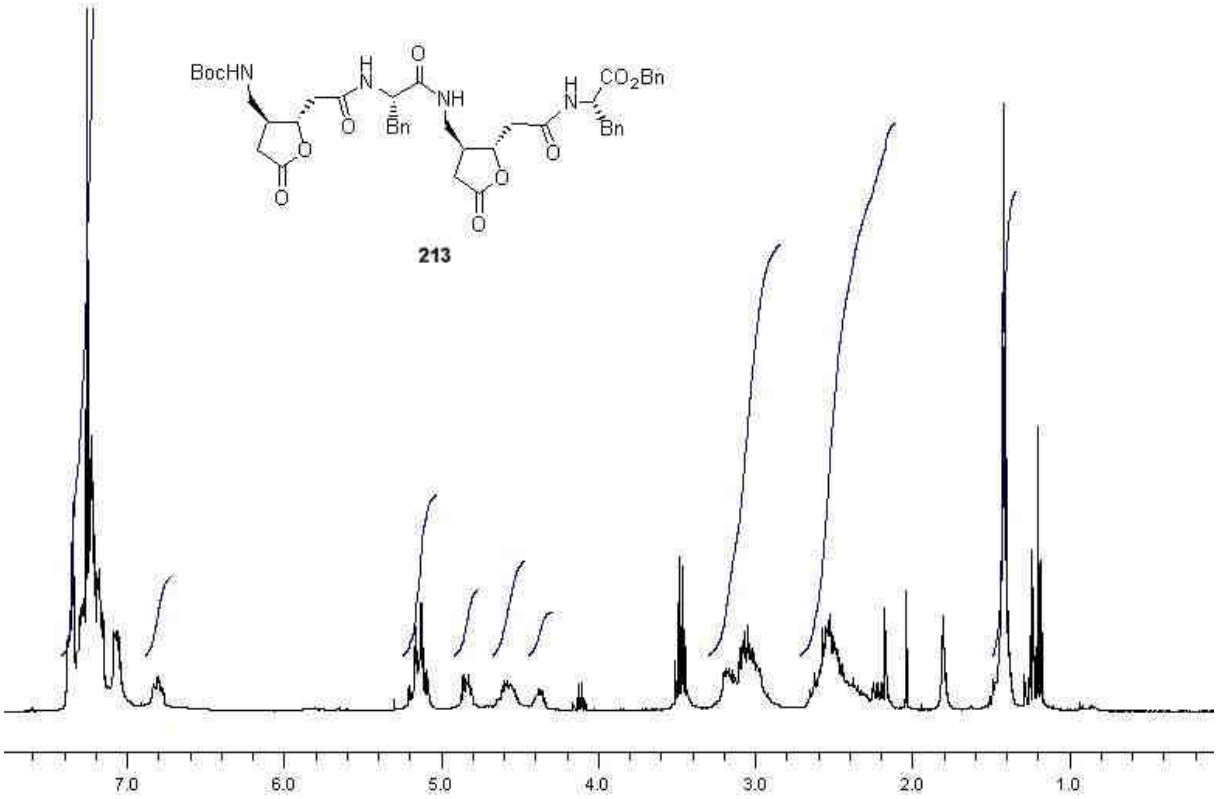
Boc-Lac-Phe-Bn (210) in $CDCl_3$



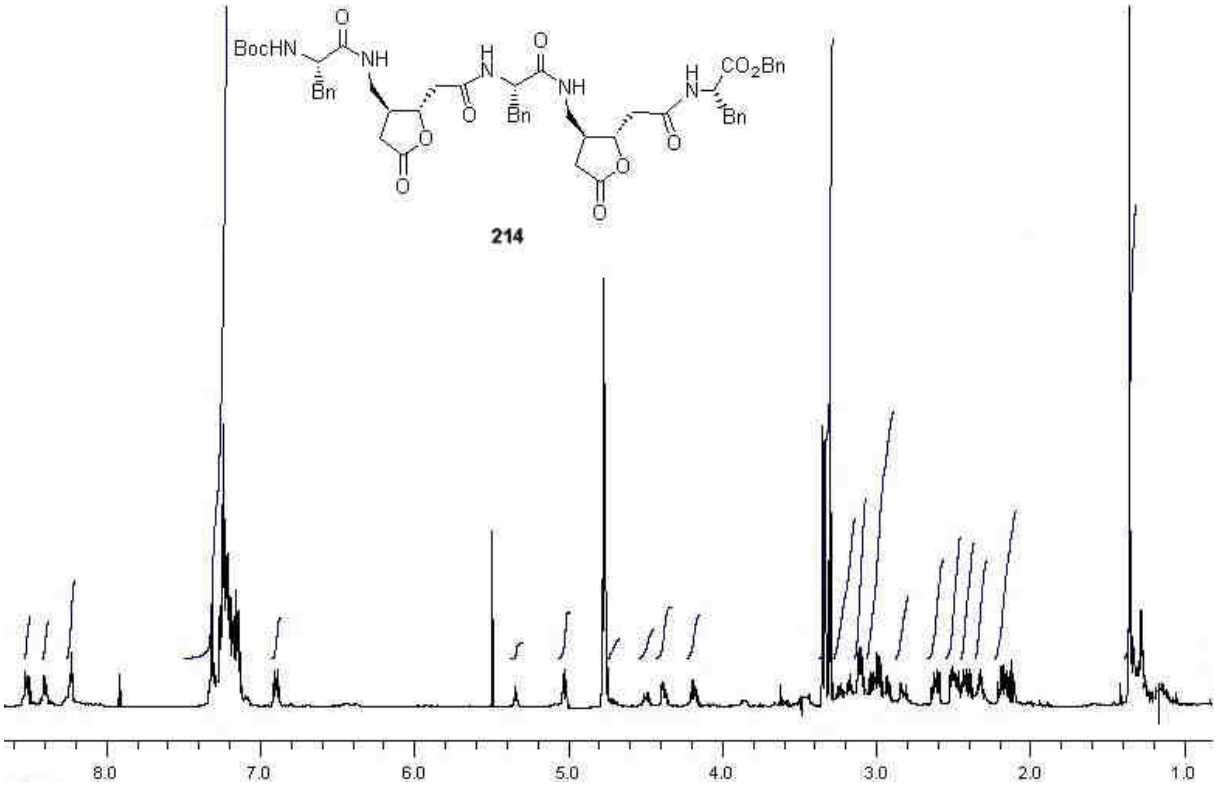
Boc-Phe-Lac-Phe-Bn (212) in $CDCl_3$



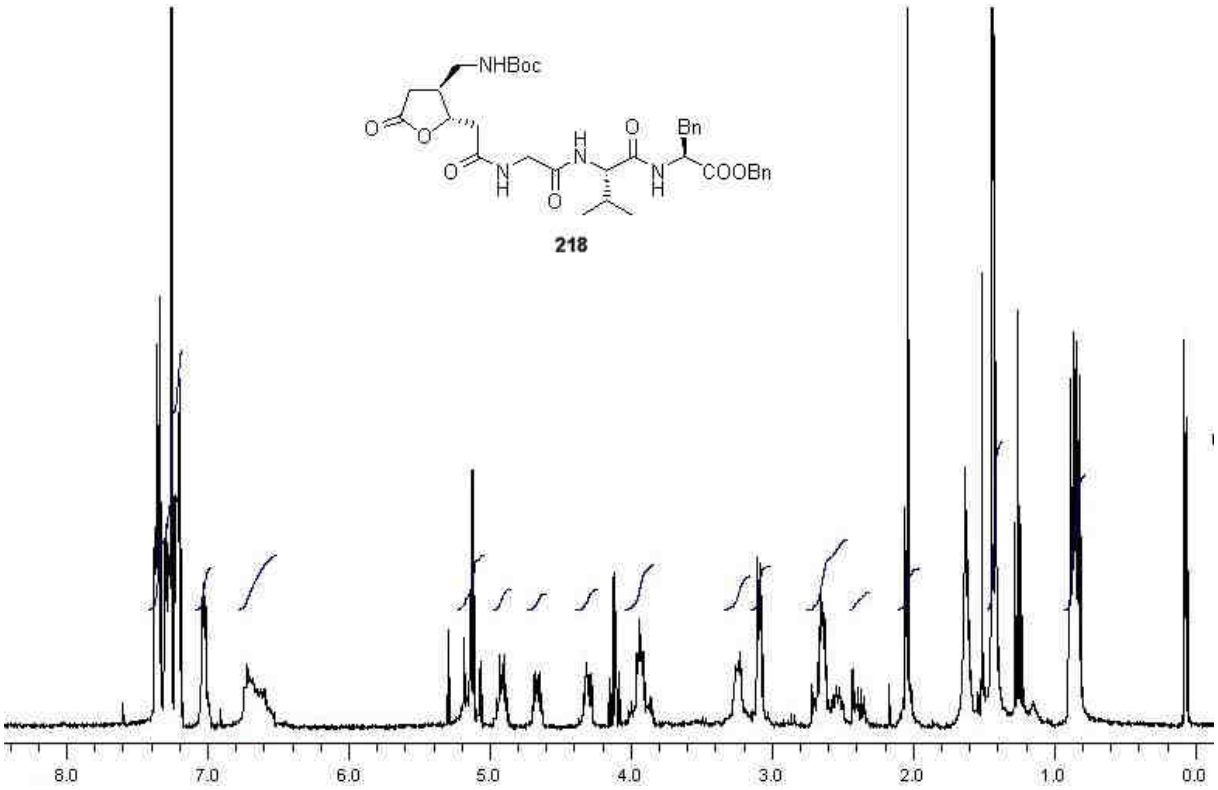
Boc-Lac-Phe-Lac-Phe-Bn (213) in $CDCl_3$



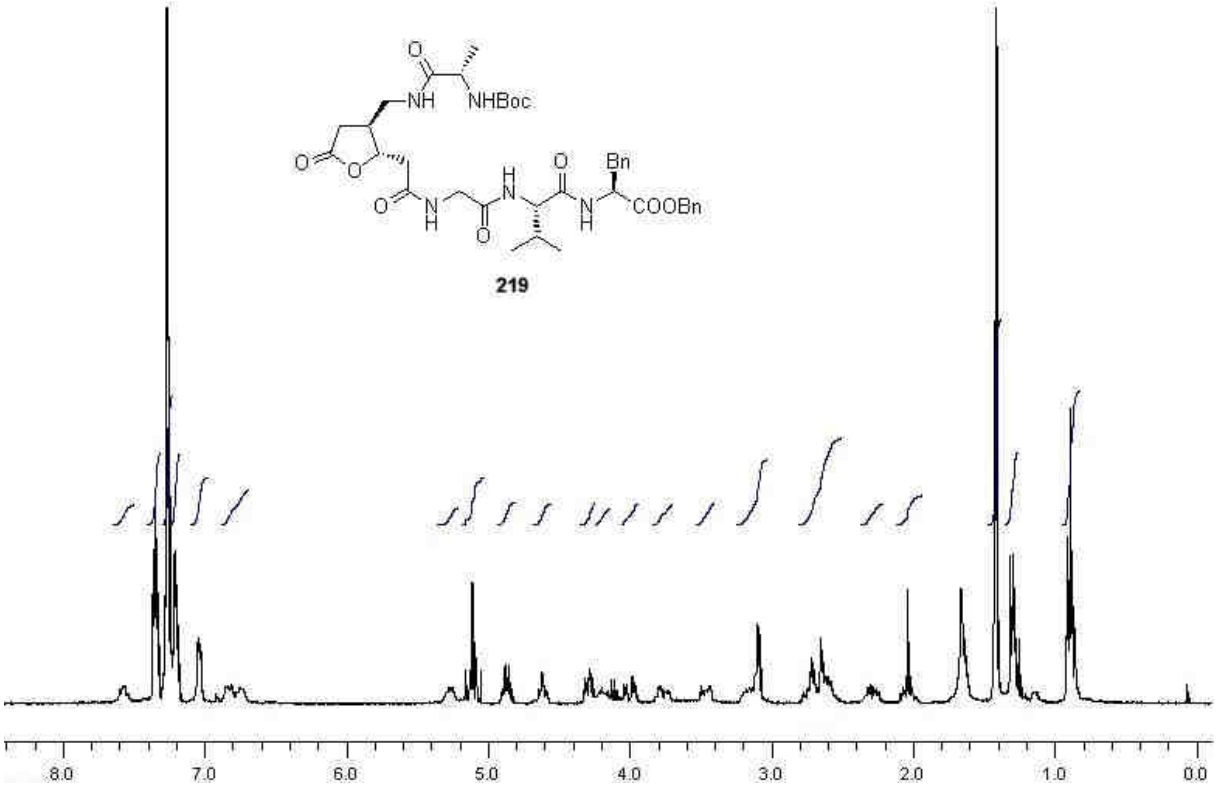
Boc-Phe-Lac-Phe -Lac-Phe-Bn (214) in CD_3OH



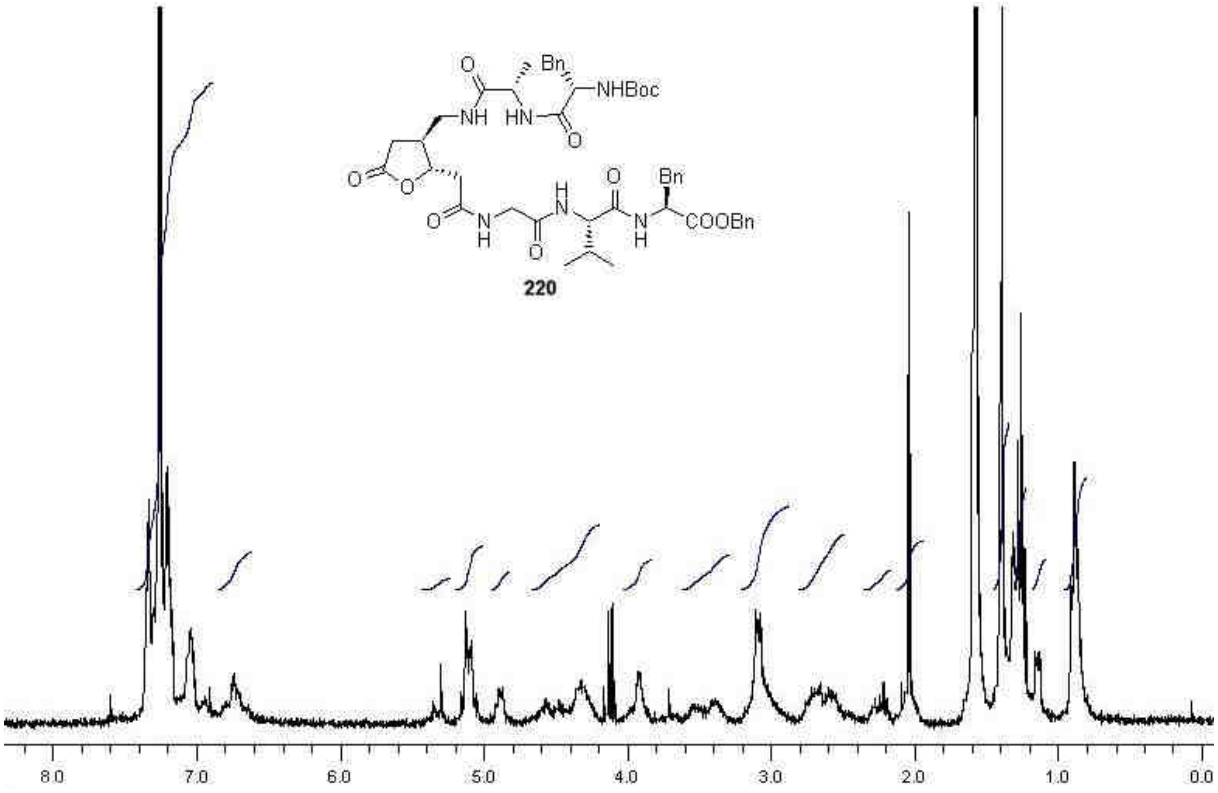
Boc-Lac-Gly-Val-Phe-Bn (218) in $CDCl_3$



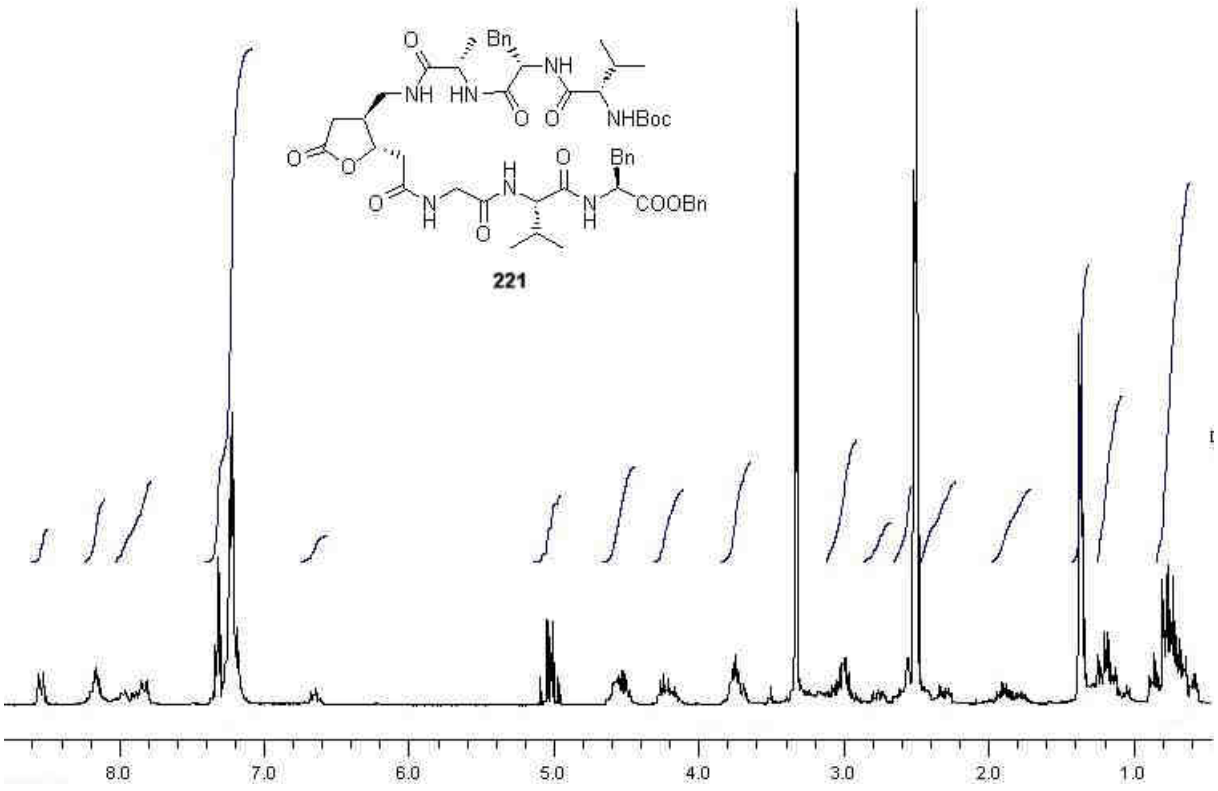
Boc-Ala-Lac-Gly-Val-Phe-Bn (219) in $CDCl_3$



Boc-Phe-Ala-Lac-Gly-Val-Phe-Bn (220) in $CDCl_3$

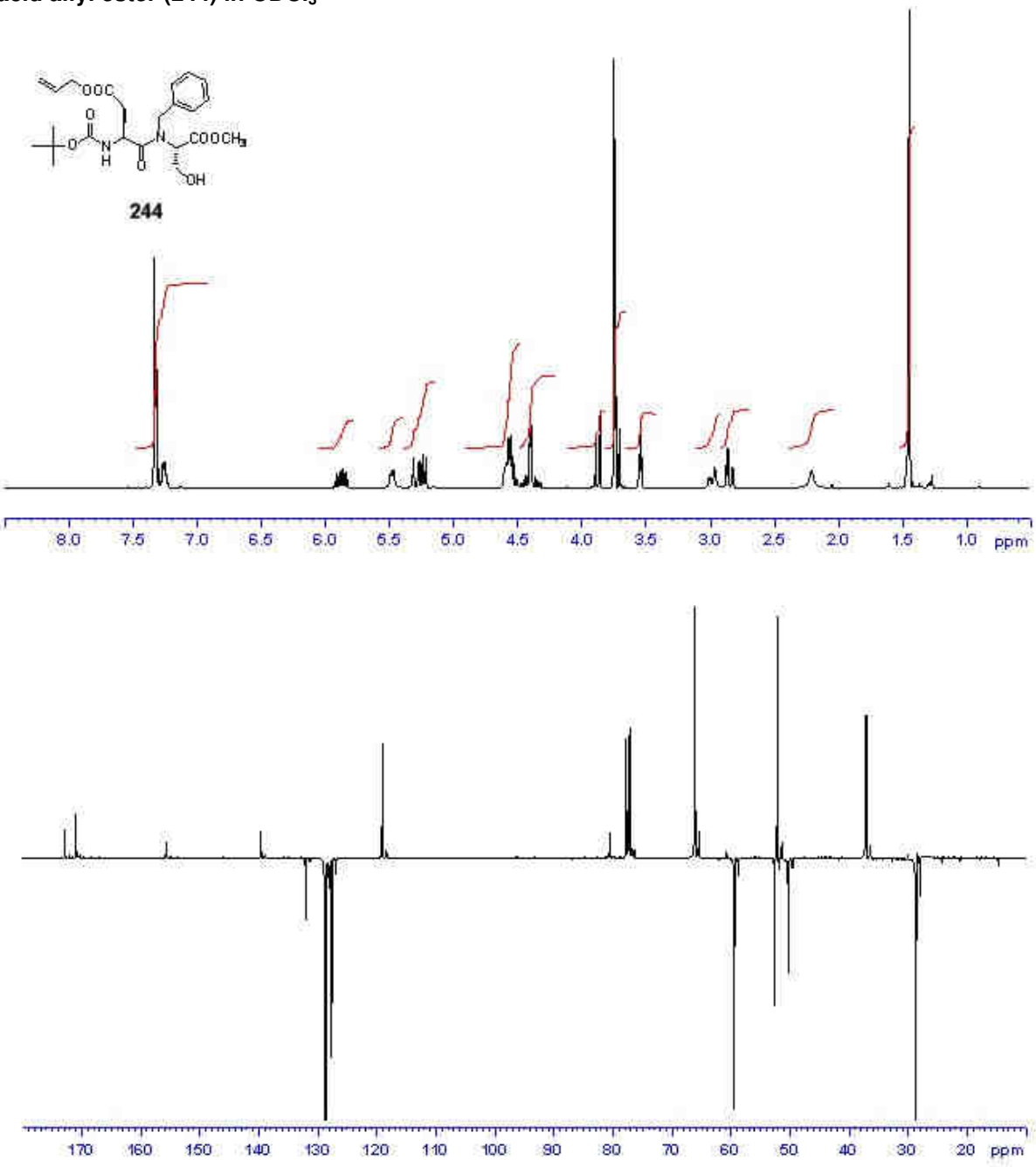


Boc-Val-Phe-Ala-Lac-Gly-Val-Phe-Bn (221) in CD_3OH

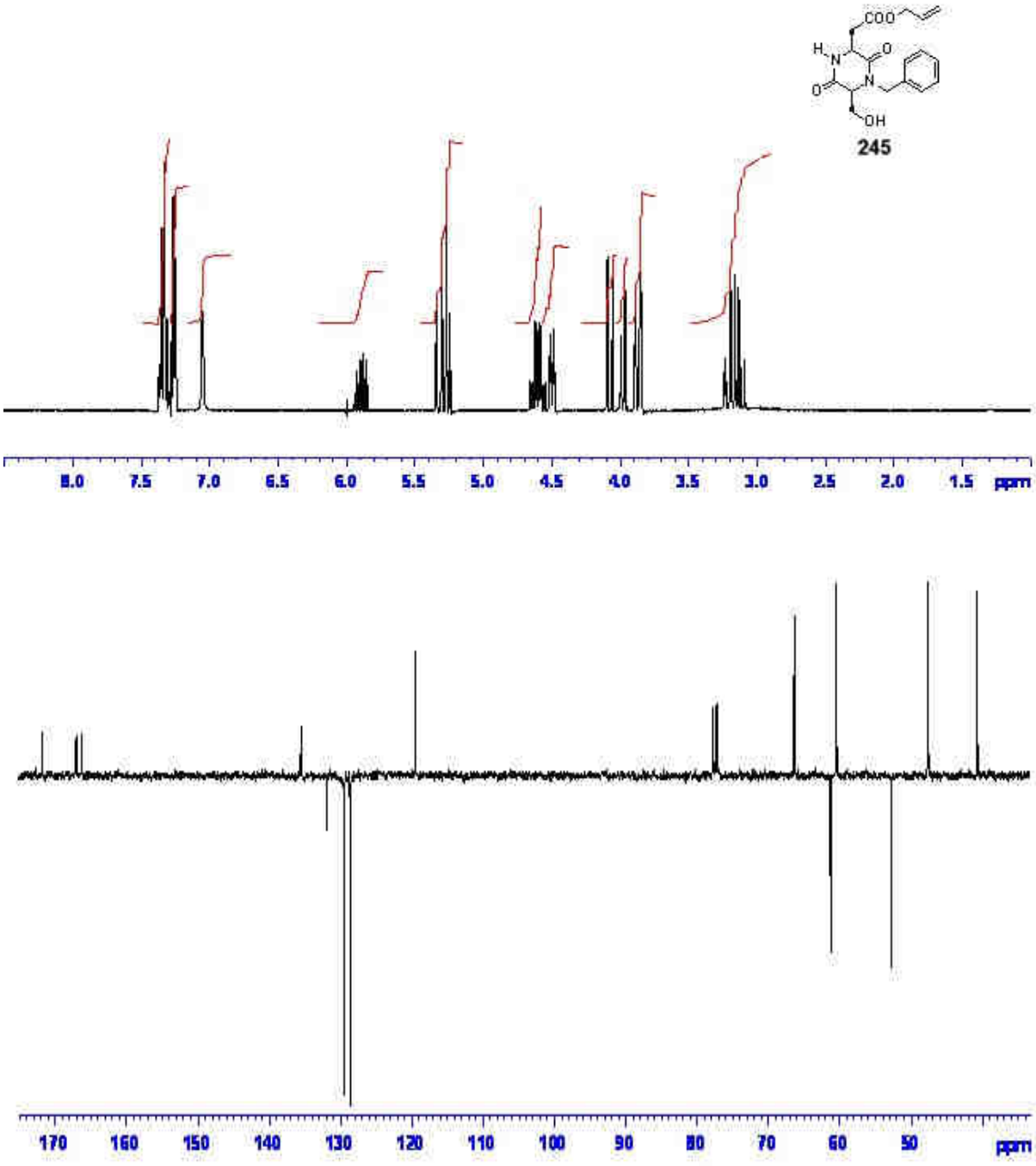


221

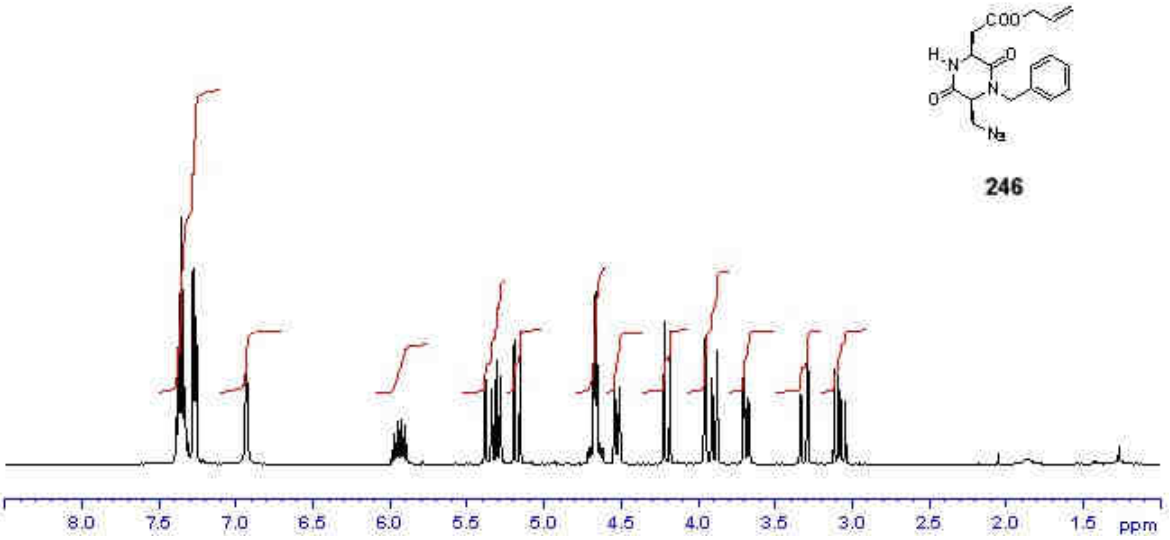
(S)-*N*-Benzyl-3-*tert*-butoxycarbonylamino-*N*-[(S)-2-hydroxy-1-methoxycarbonyl-ethyl]-succinamic acid allyl ester (244) in $CDCl_3$

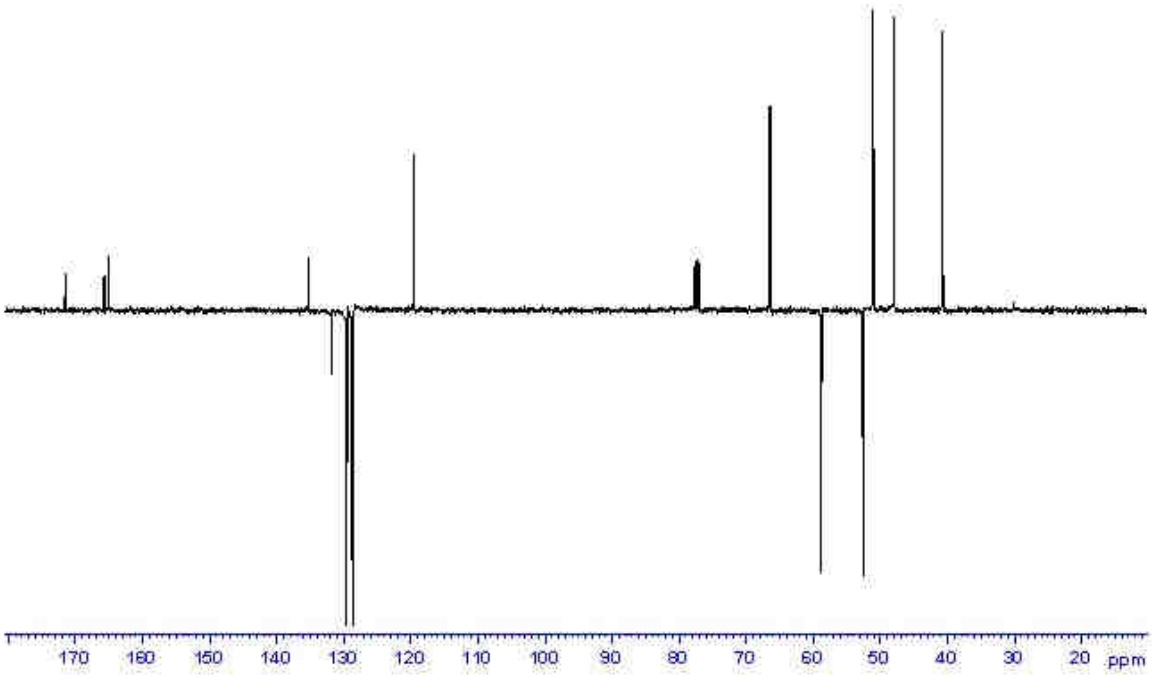


[(2S,5S)-4-Benzyl-5-hydroxymethyl-3,6-dioxo-piperazin-2-yl]-acetic acid allyl ester (245) in $CDCl_3$

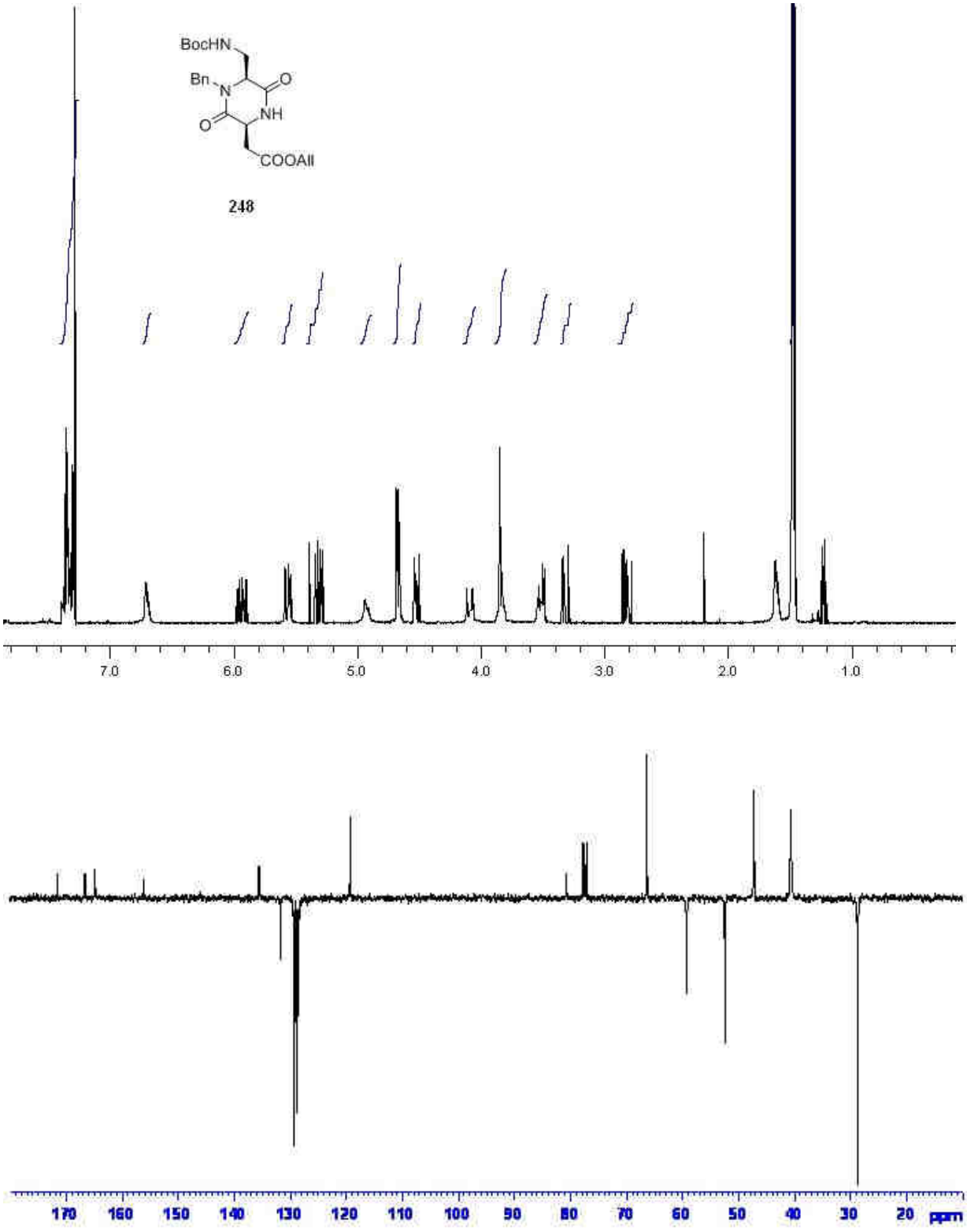


[(2S,5S)-5-Azidomethyl-4-benzyl-3,6-dioxo-piperazin-2-yl]-acetic acid allyl ester(246) in $CDCl_3$

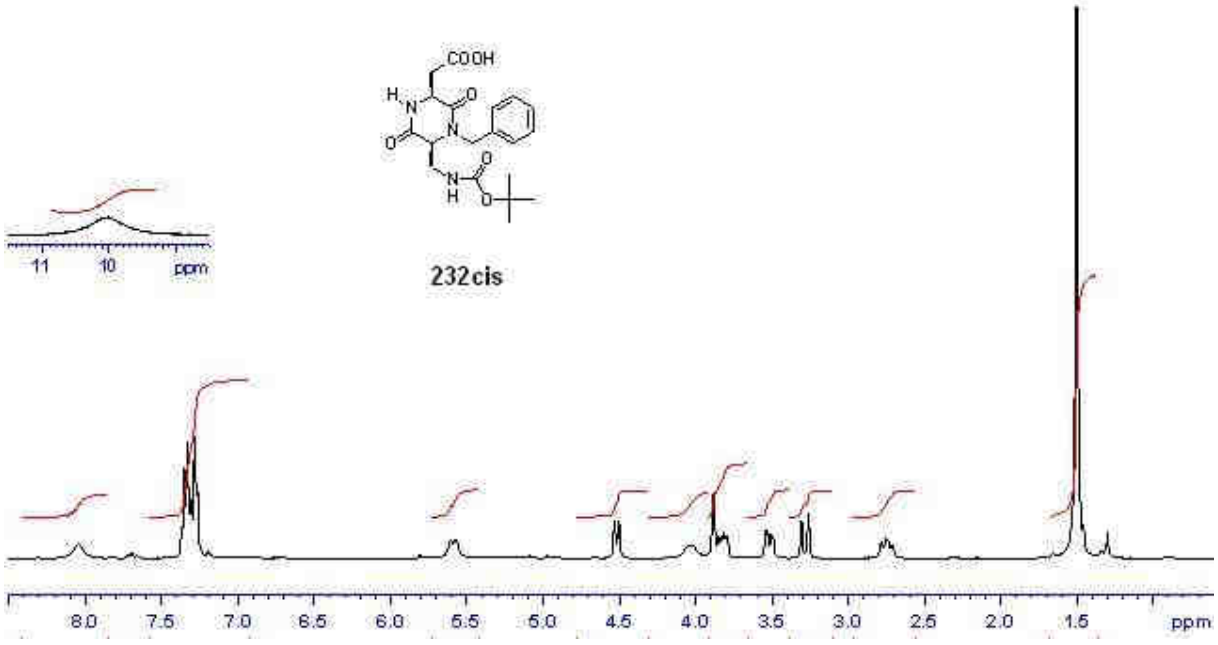


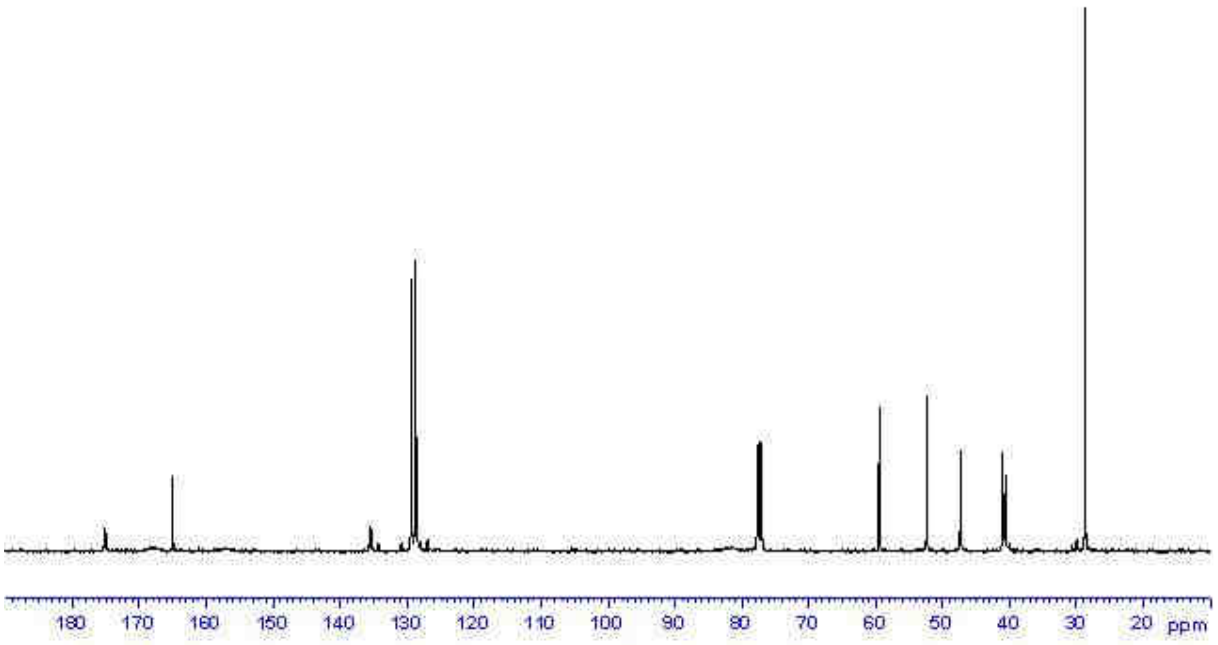


[(2S,5S)-4-Benzyl-5-(*tert*-butoxycarbonylamino-methyl)-3,6-dioxo-piperazin-2-yl]-acetic acid allyl ester (248) in $CDCl_3$

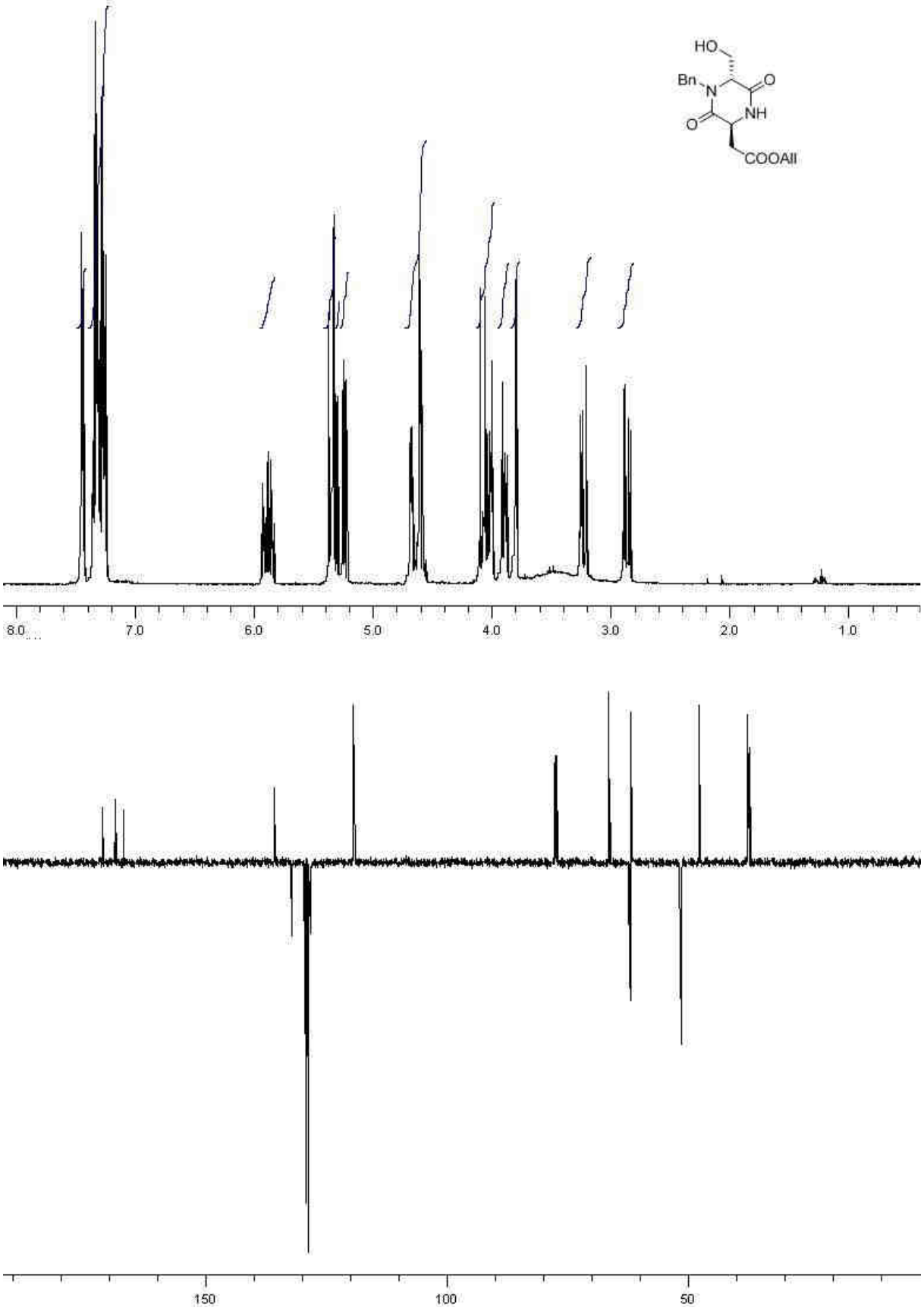


[(2*S*,5*S*)-4-benzyl-5-(*tert*-butoxycarbonylamino-methyl)-3,6-dioxo-piperazin-2-yl]-acetic acid (232cis) in $CDCl_3$ at 50℃

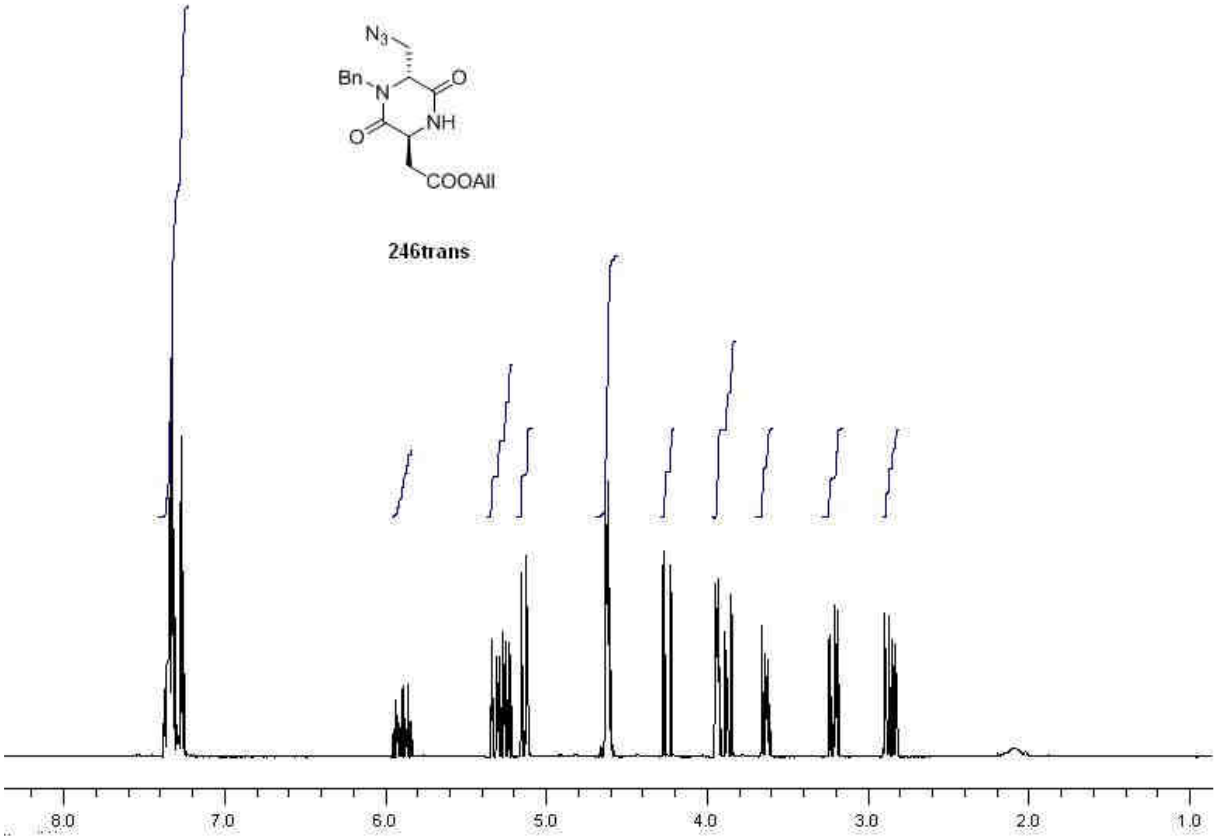


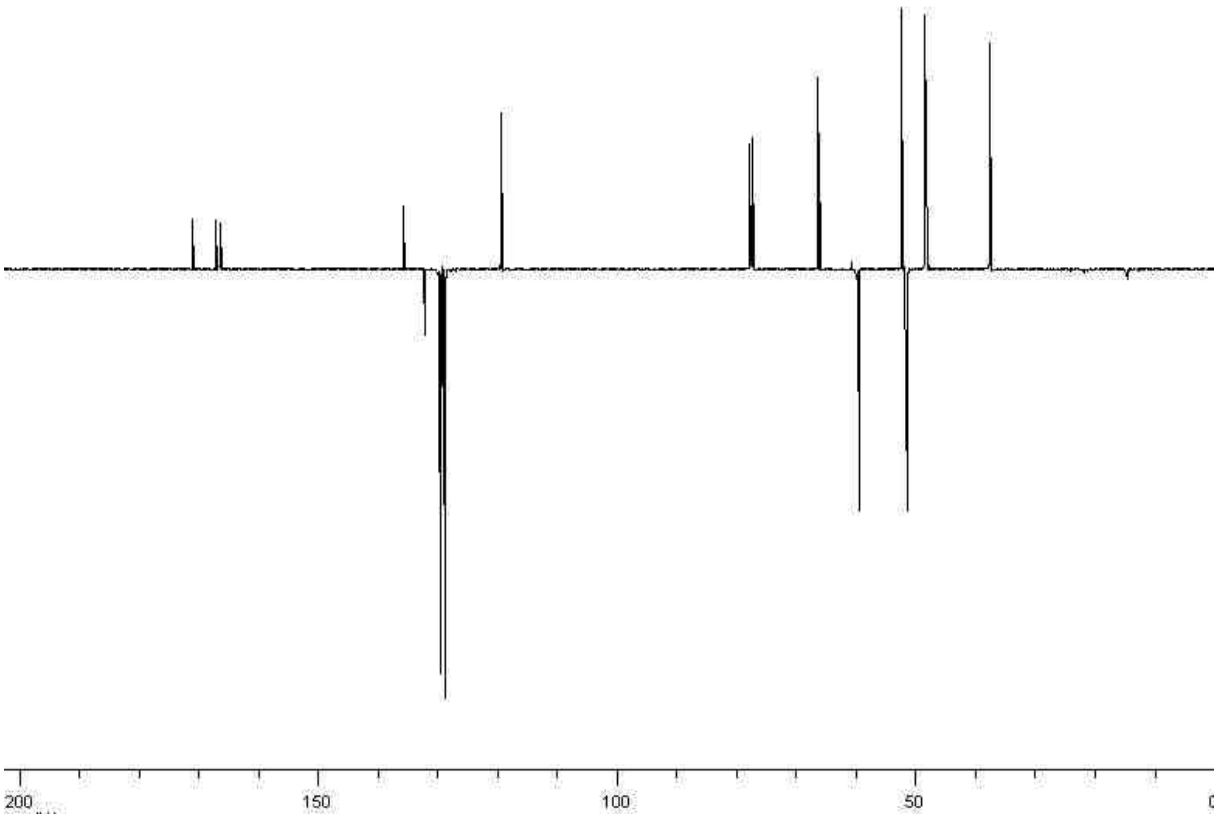


[(2S,5R)-4-Benzyl-5-hydroxymethyl-3,6-dioxo-piperazin-2-yl]-acetic acid allyl ester (245) in $CDCl_3$

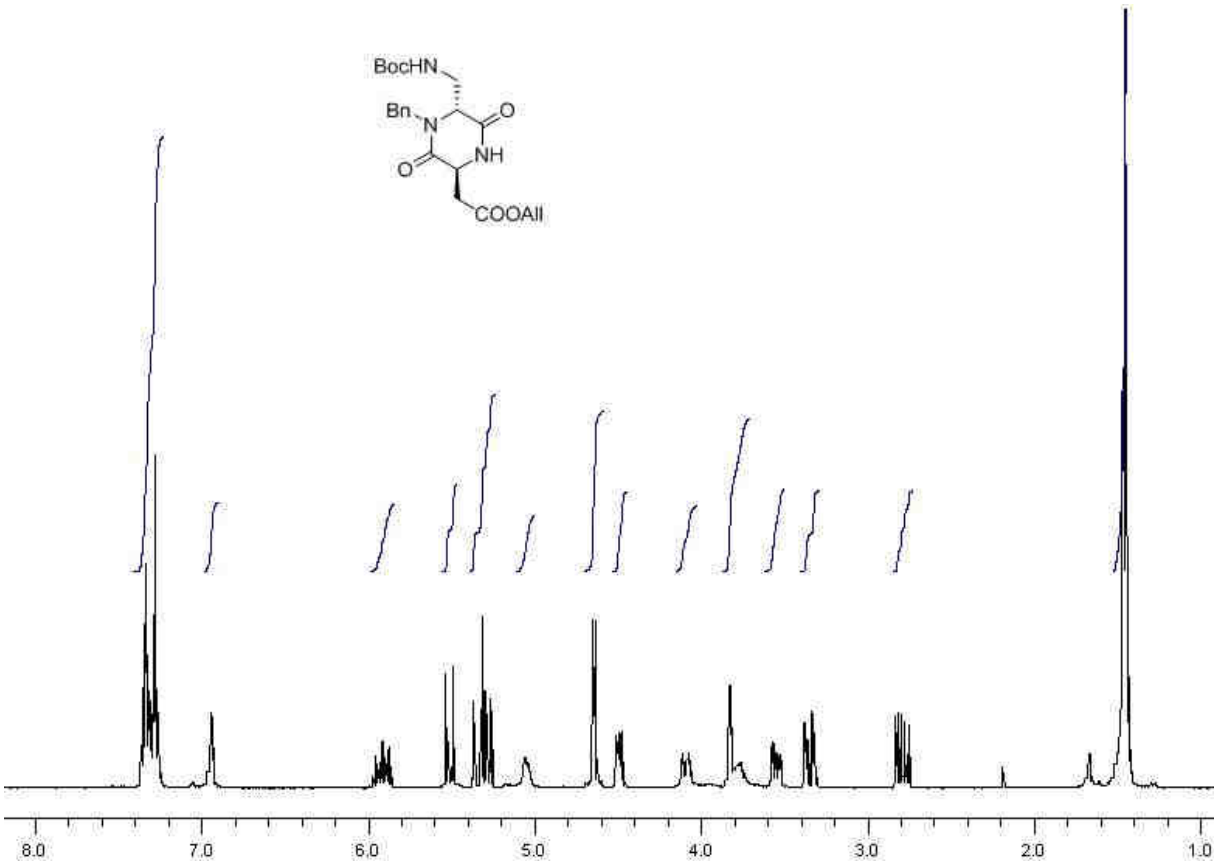


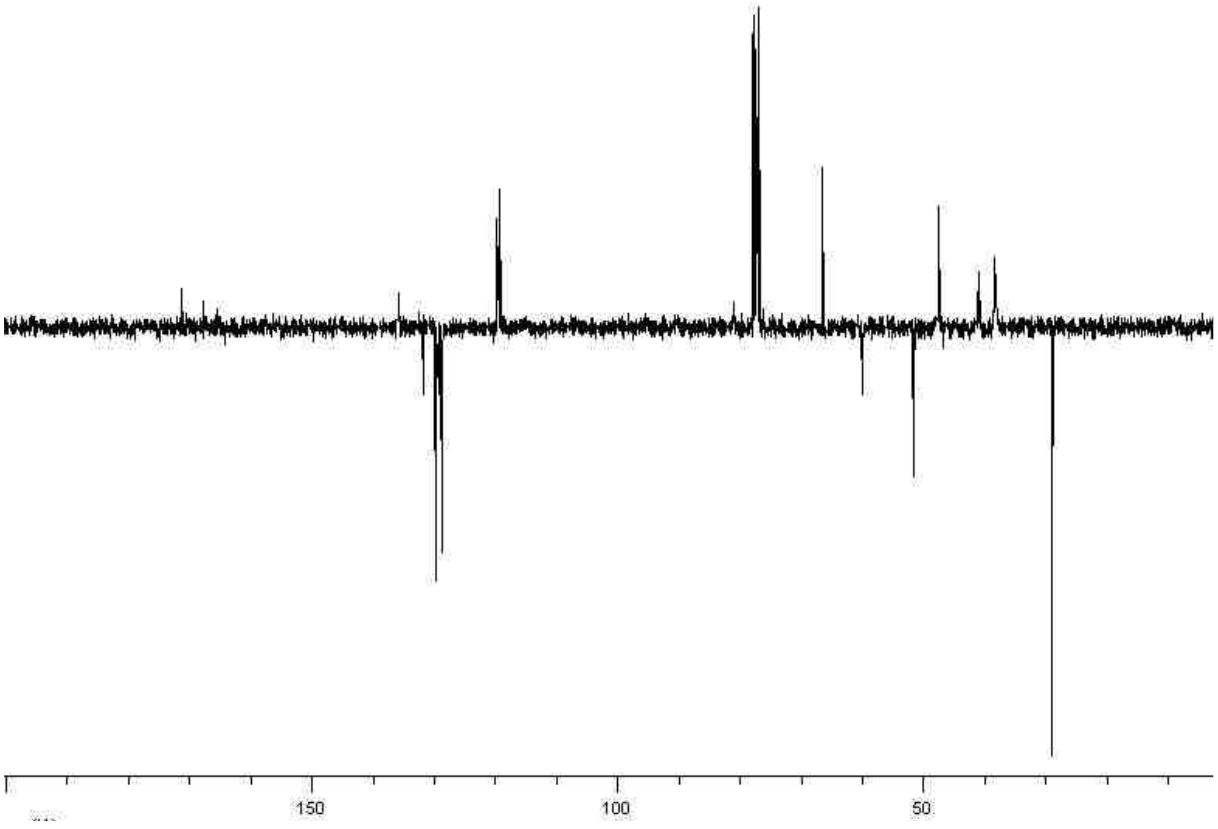
Allyl 2-((2S,5R)-5-(azidomethyl)-4-benzyl-3,6-dioxopiperazin-2-yl)acetate (246trans) in $CDCl_3$



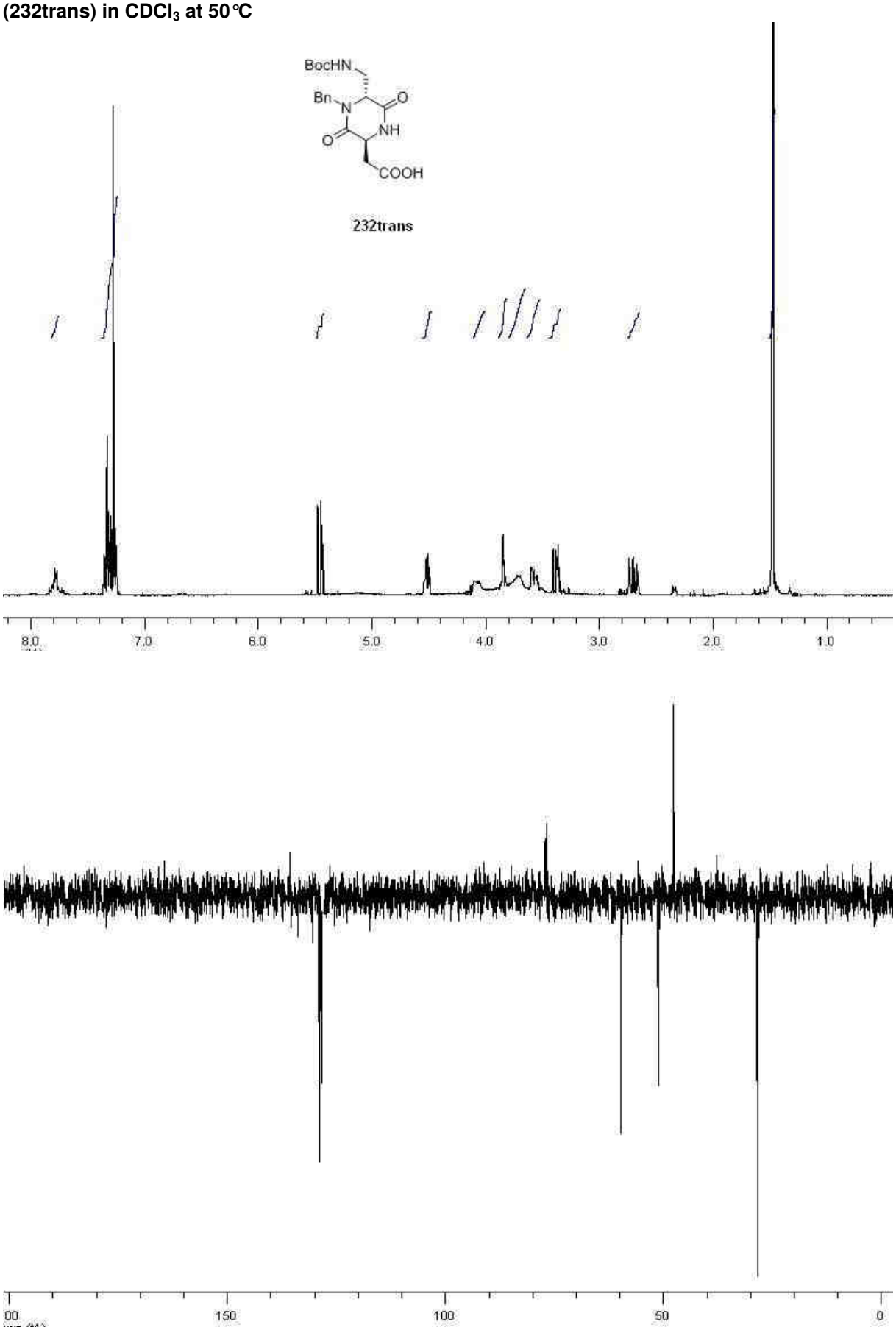


[(2S,5R)-4-Benzyl-5-(*tert*-butoxycarbonylamino-methyl)-3,6-dioxo-piperazin-2-yl]-acetic acid allyl ester (248) in $CDCl_3$

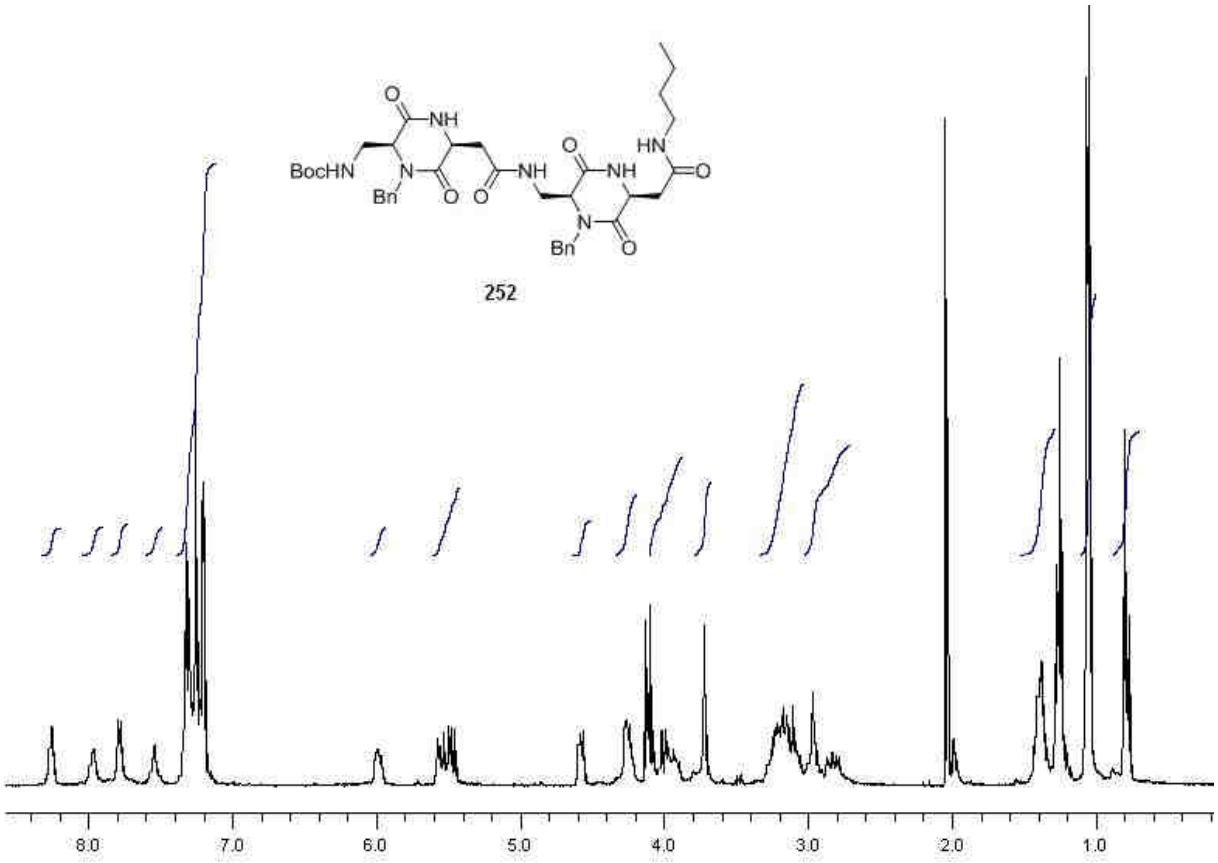


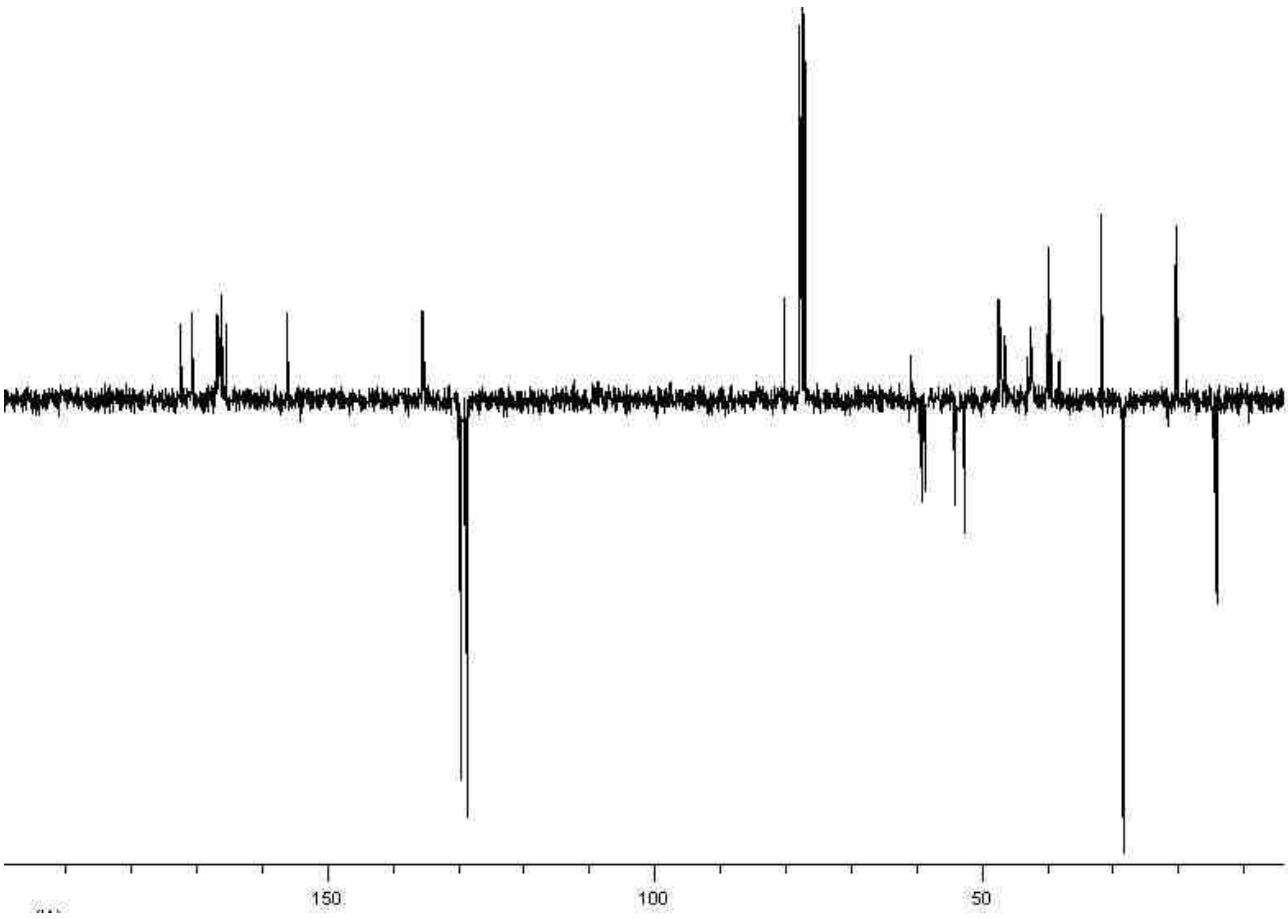


2-((2S,5R)-4-benzyl-5-((tert-butoxycarbonylamino)methyl)-3,6-dioxopiperazin-2-yl)acetic acid (232trans) in $CDCl_3$ at 50℃

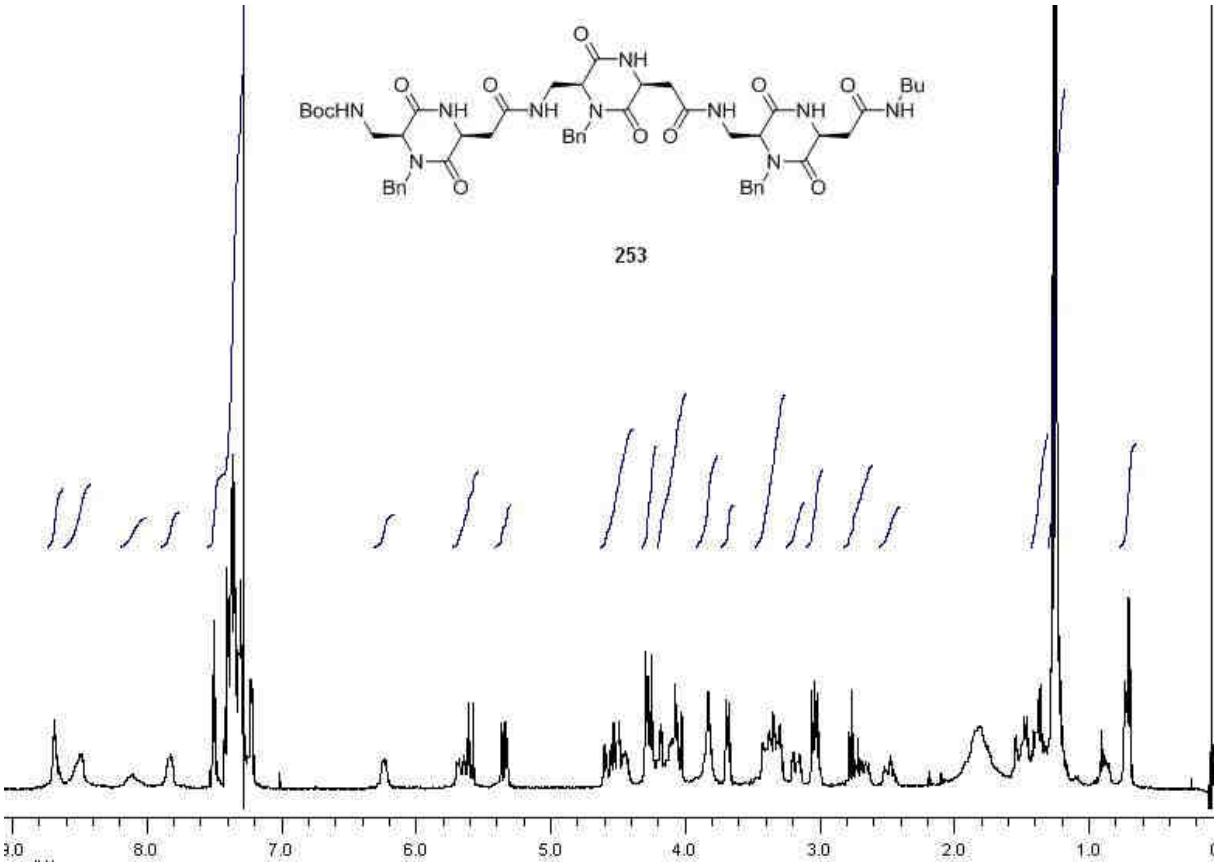


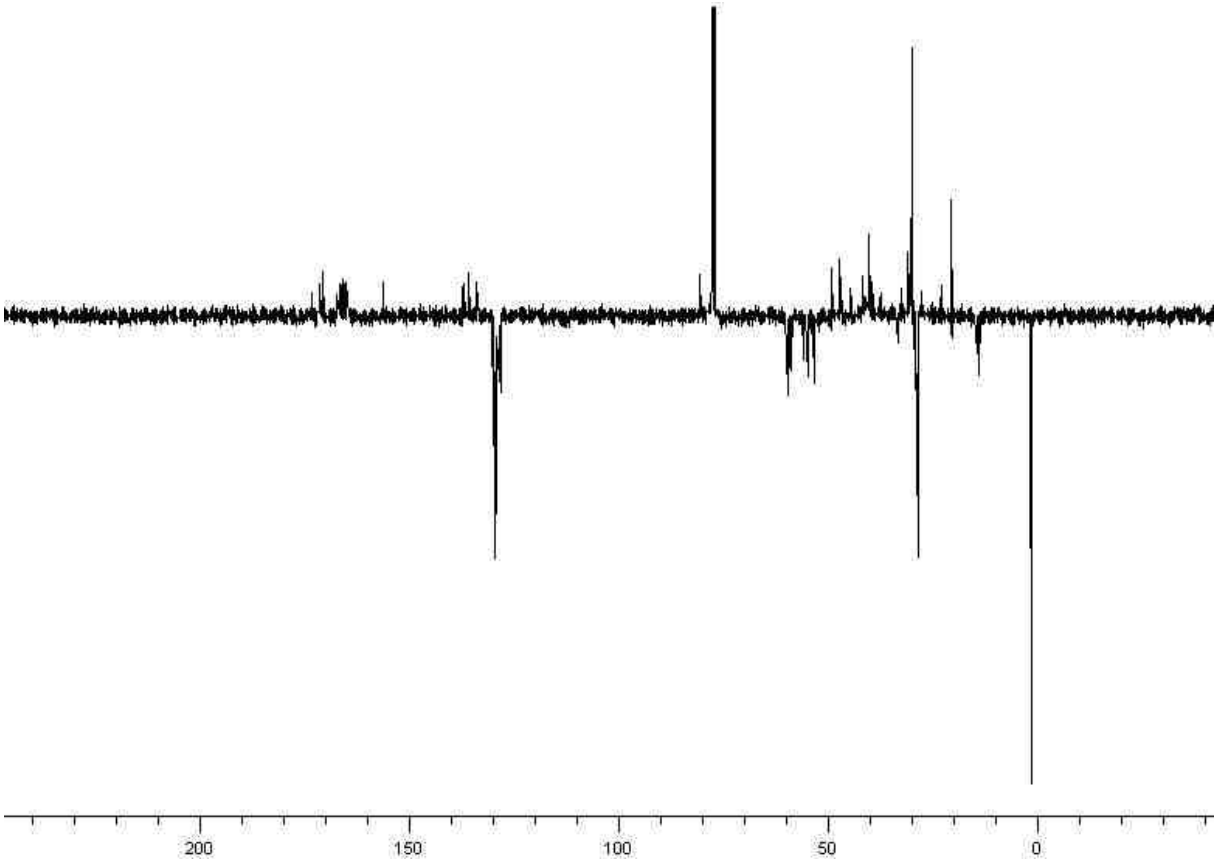
Boc-(*cis*-DKP)$_2$-NHBu (252) in $CDCl_3$



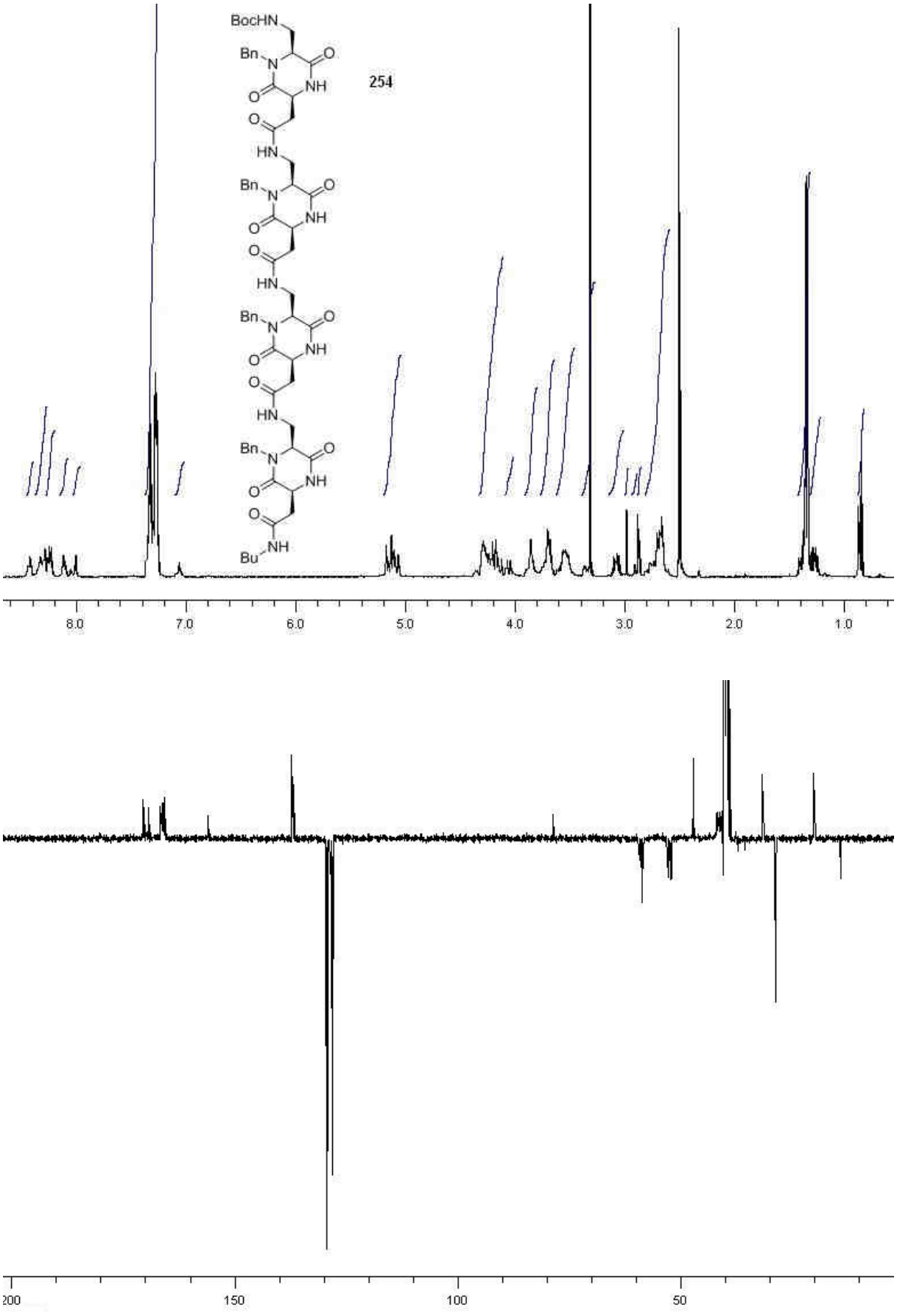


Boc-(*cis*-DKP)$_3$-NHBu (253) in $CDCl_3$

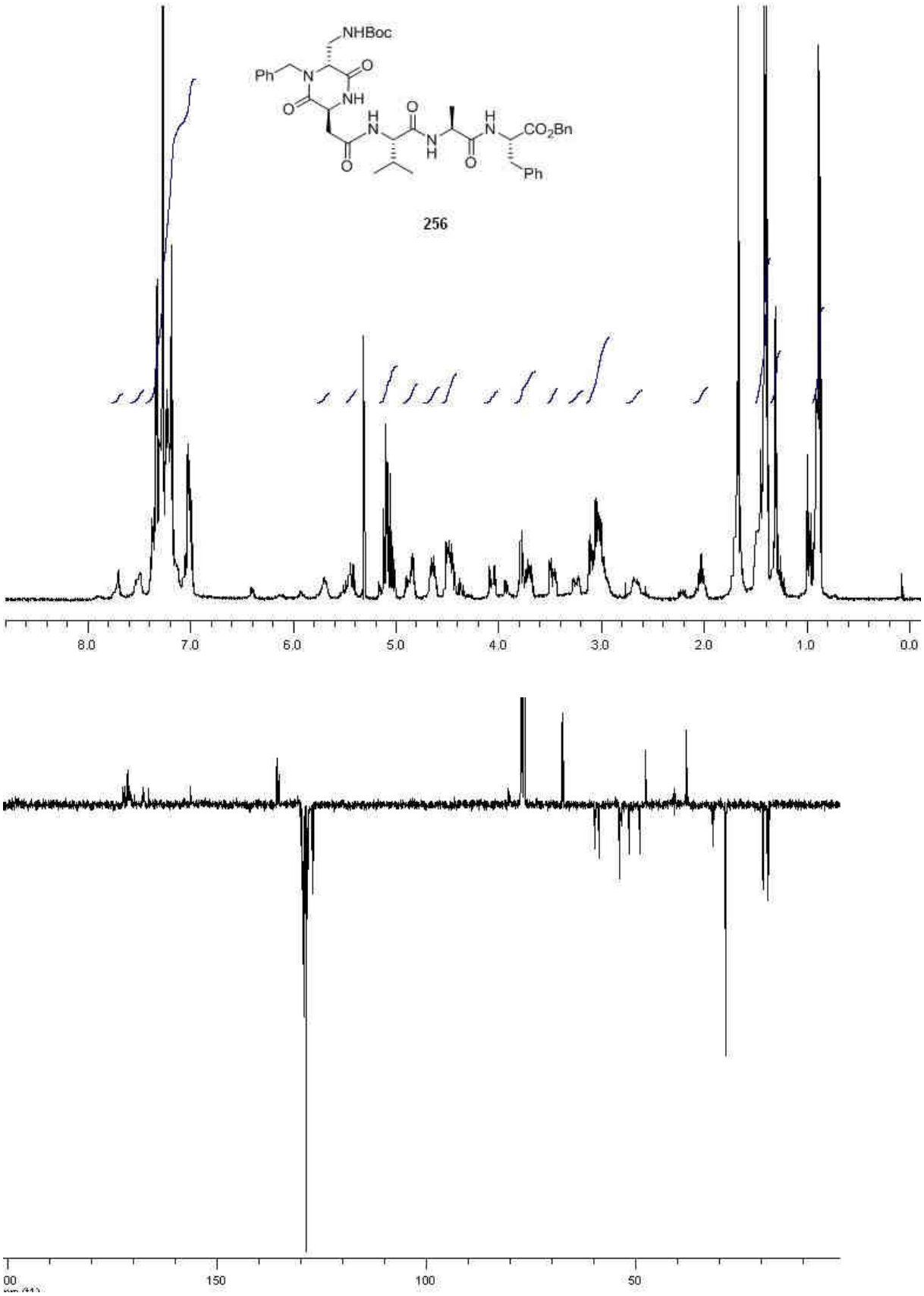




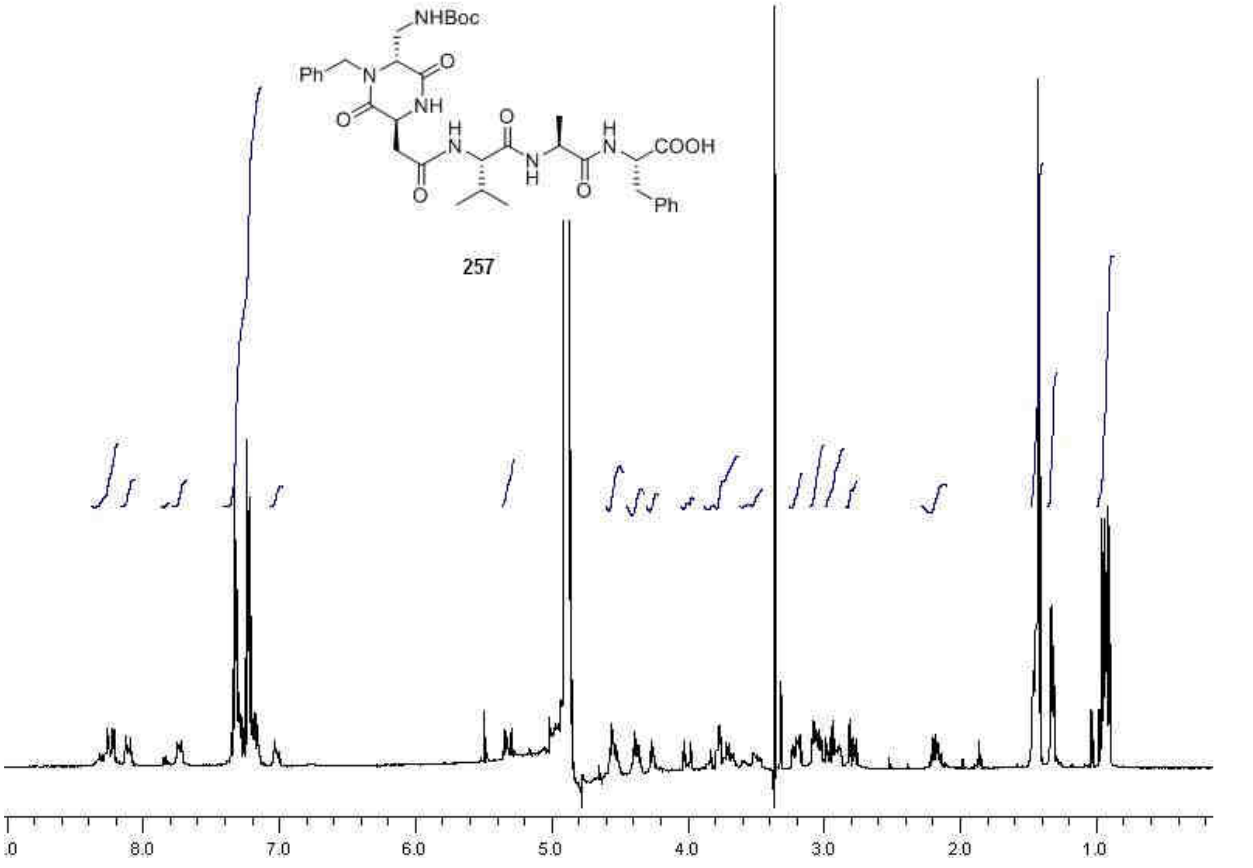
Boc-(*cis*-DKP)$_4$-NHBu (254) in DMSO-d$_6$

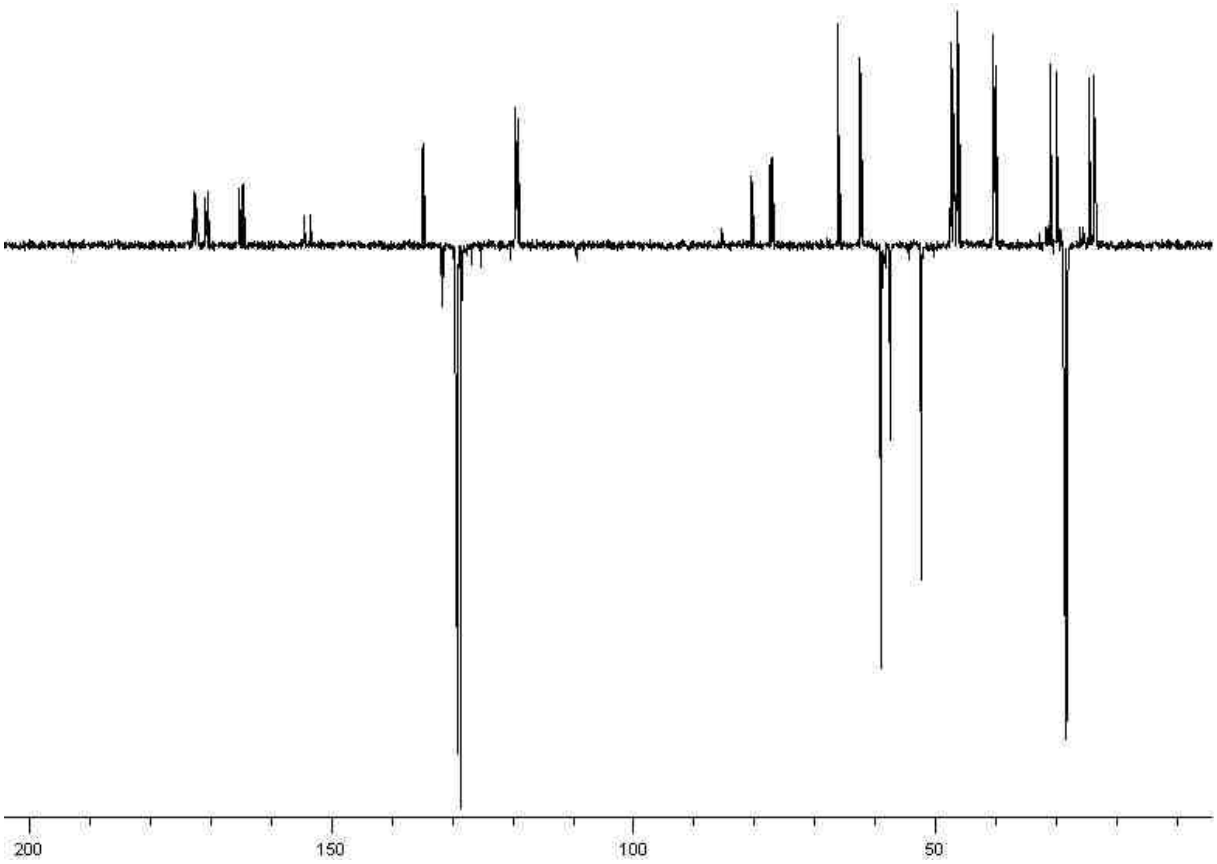


Boc-*trans*-DKP-Val-Ala-Phe-Bn (256) in $CDCl_3$

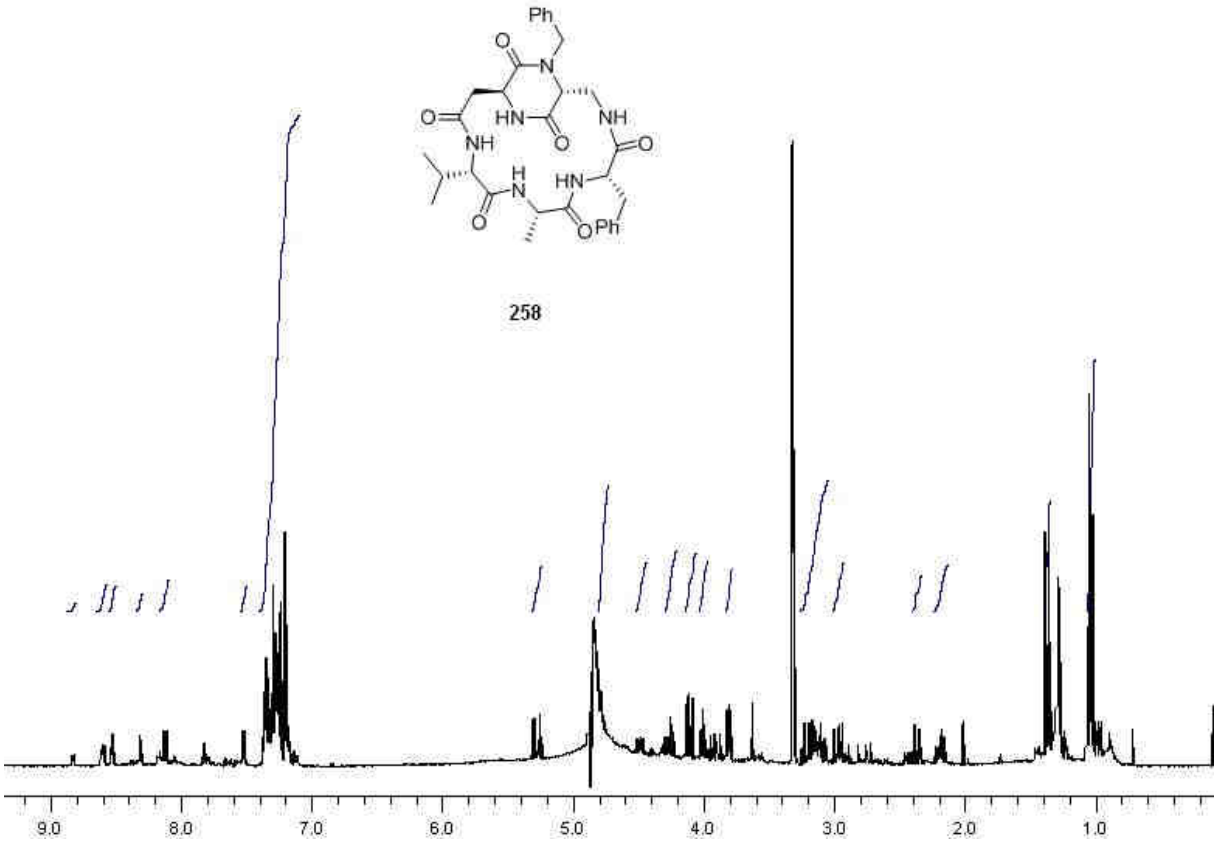


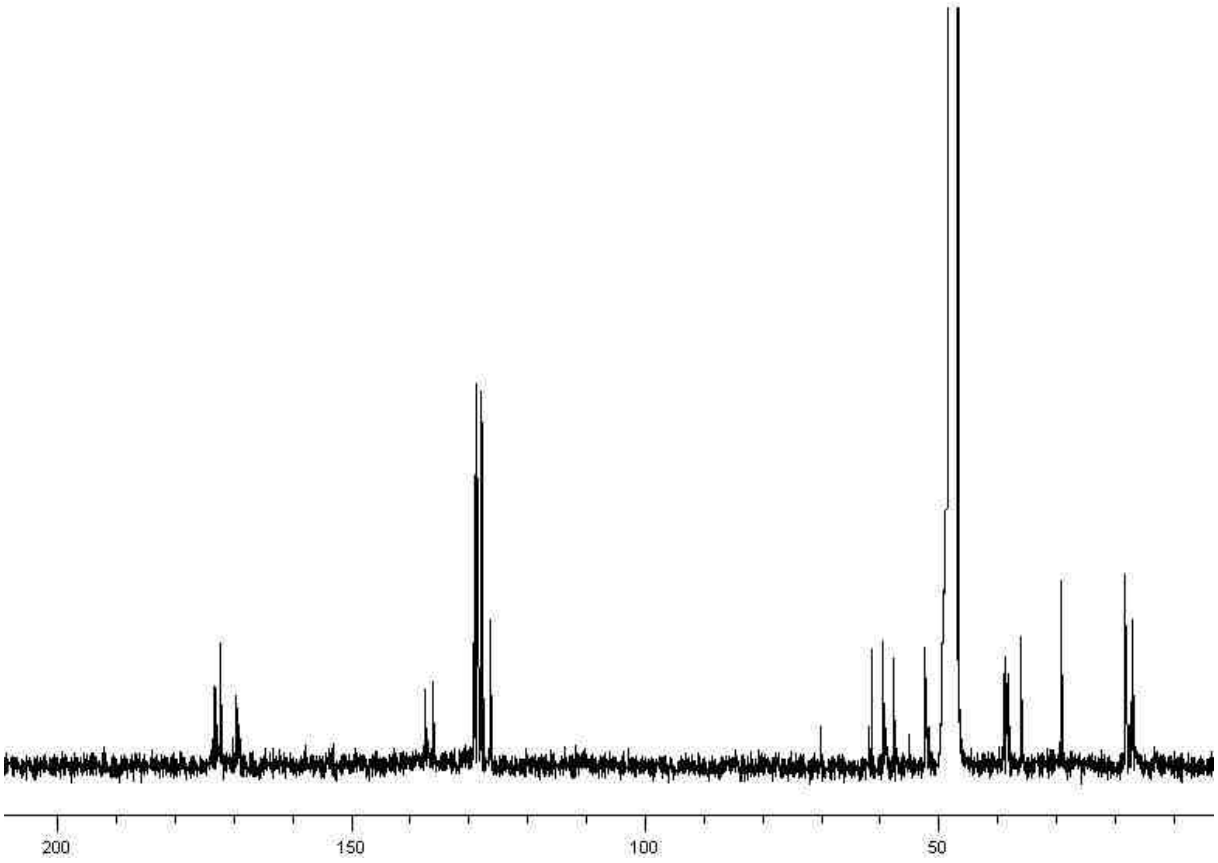
Boc-*trans*-DKP-Val-Ala-Phe-OH (257) in CD_3OH



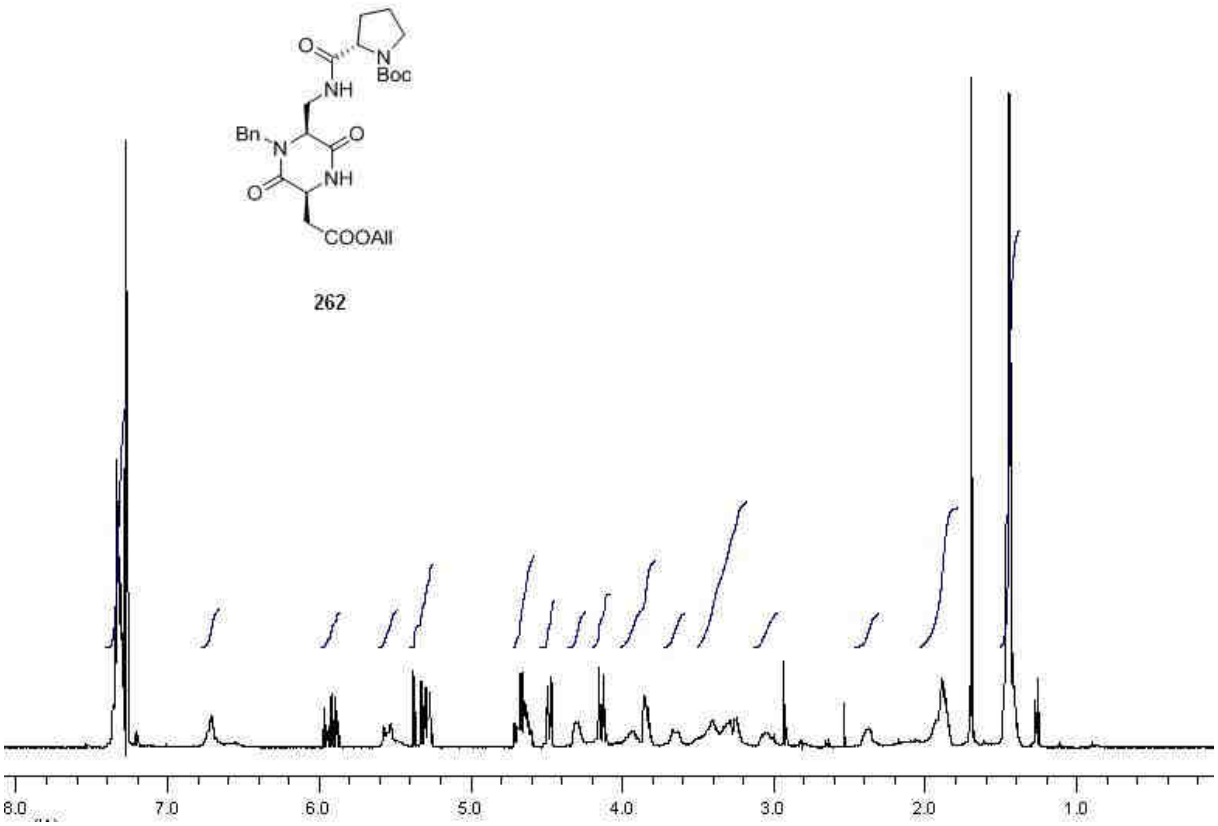


***Cyclo*[*trans*-DKP-Val-Ala-Phe] (258) in CD_3OH**

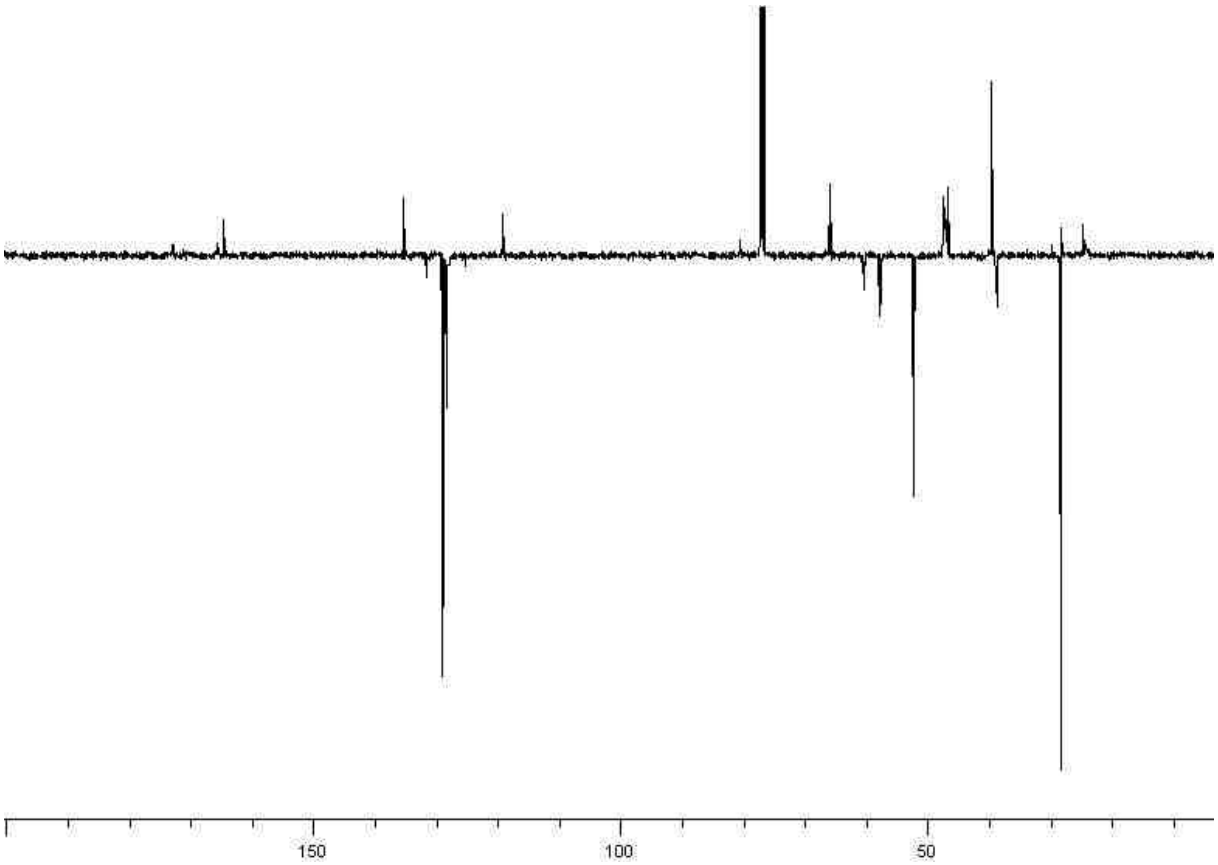




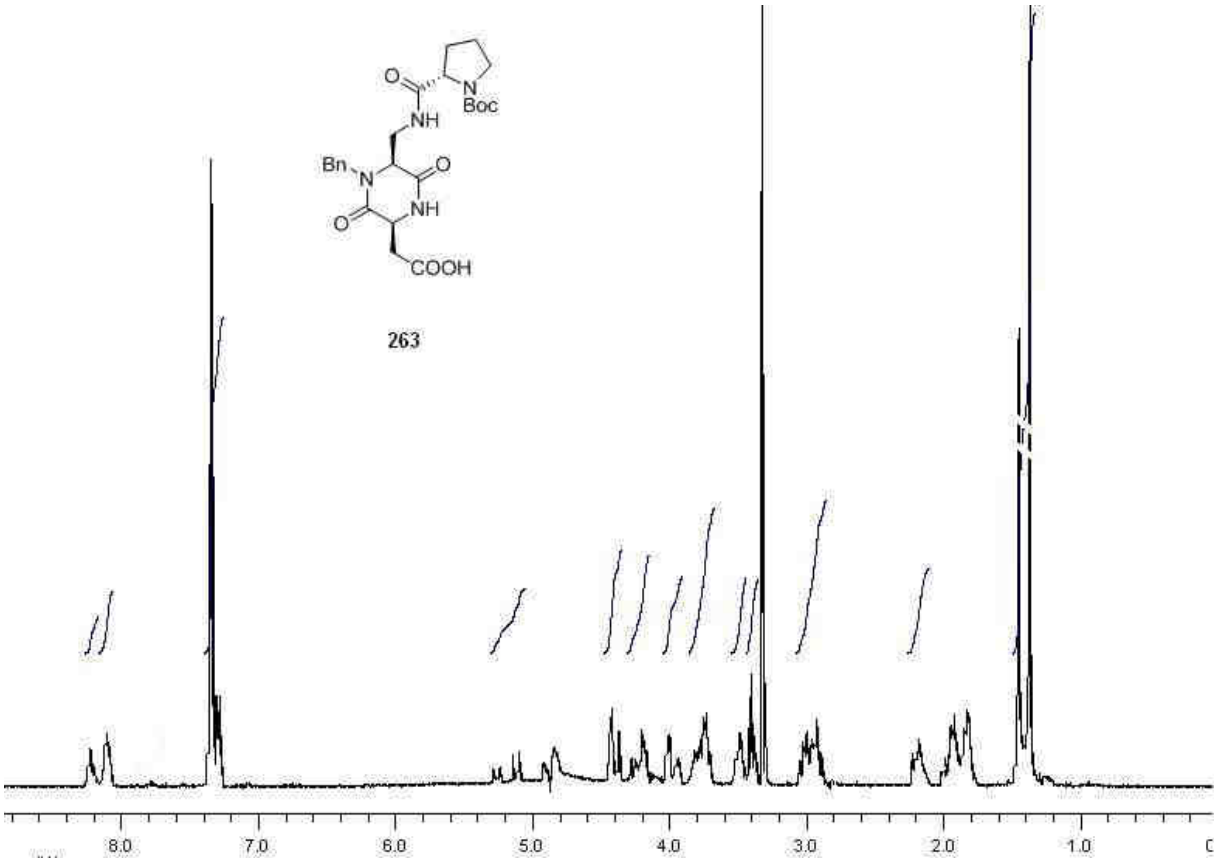
Boc-Pro-*cis*-DKP allyl ester (262) in $CDCl_3$

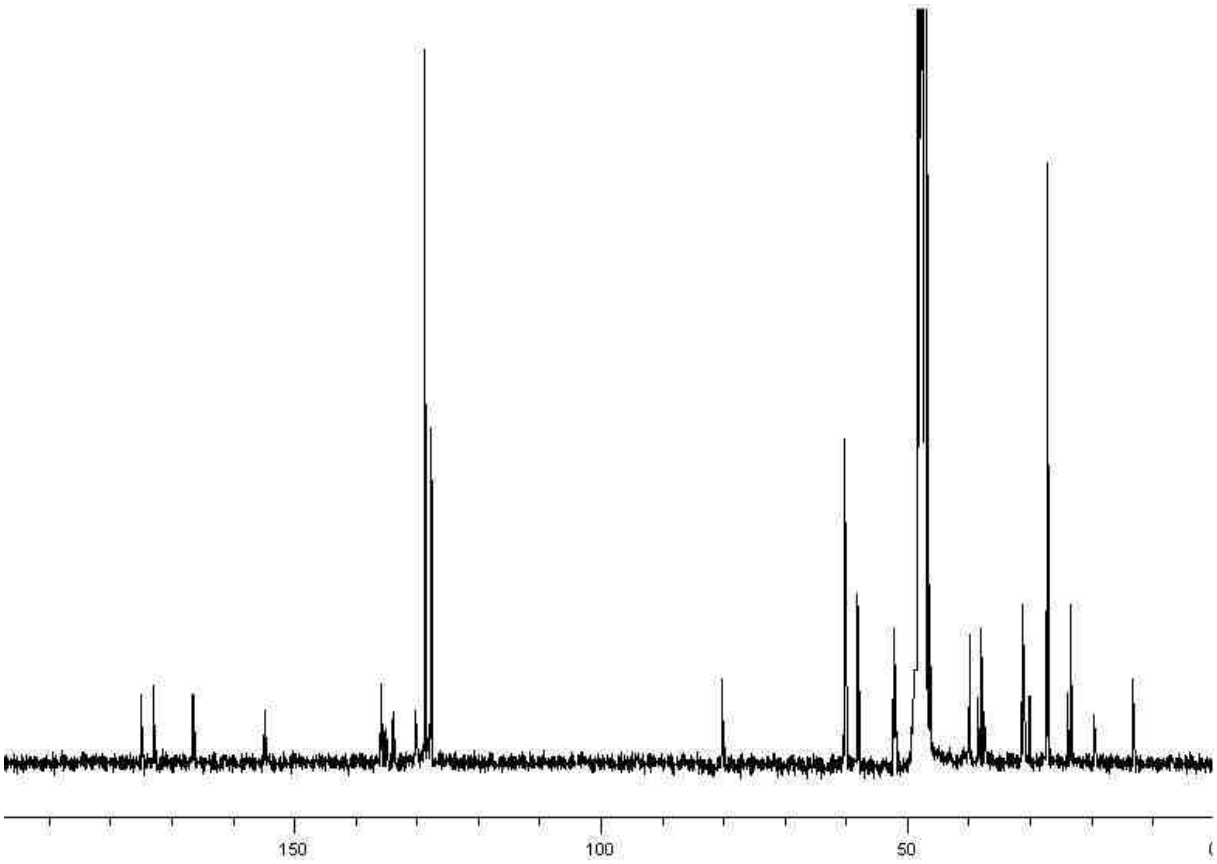


262

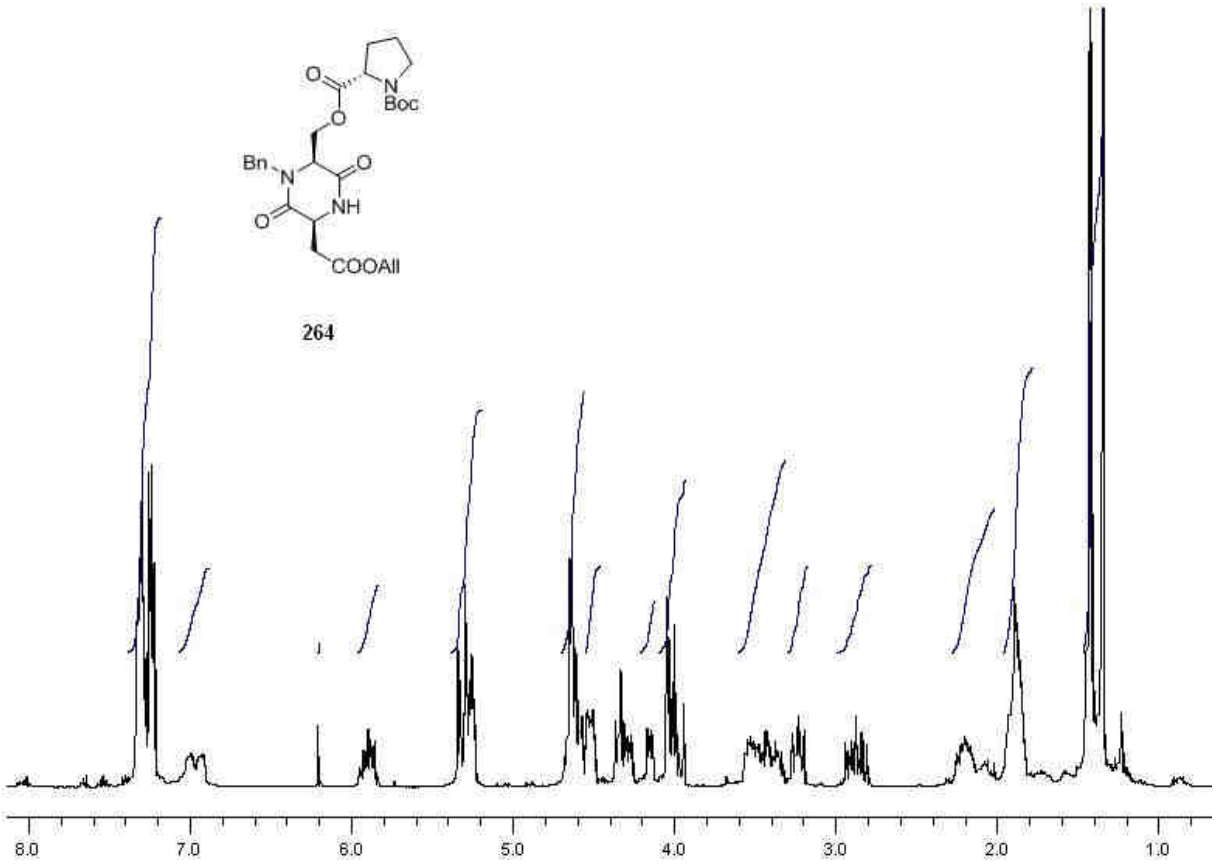


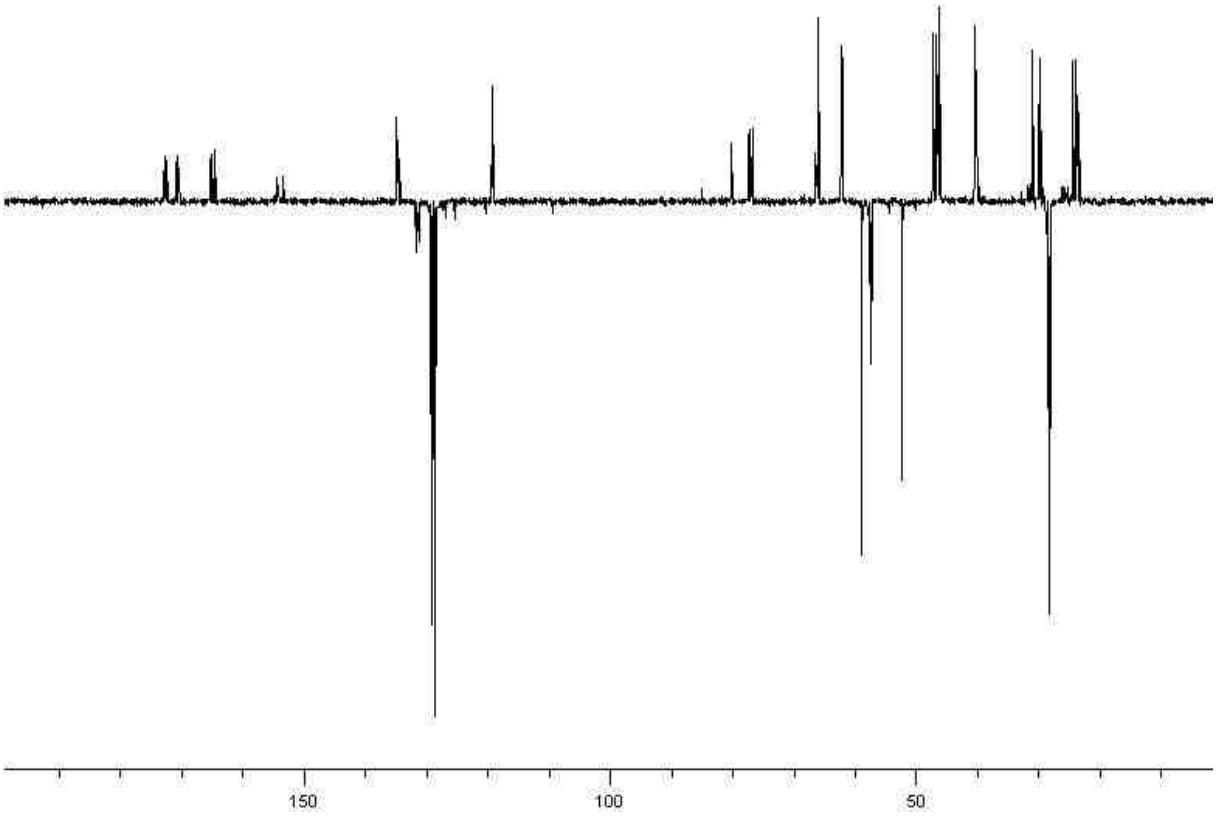
Boc-Pro-*cis*-DKP-COOH (263) in CD_3OH





Boc-Pro-COO-*cis*-DKP allyl ester (264) in $CDCl_3$





Boc-Pro-COO-*cis*-DKP-COOH (265) in CD_3OH