Synthesis and structural investigations of α-peptides containing β-aminocyclopropane dicarboxylic acids

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to my uncle Simone

Index

Abbreviations

Ac	Acetyl
Ala	Alanine
Alloc	Allyloxycarbonyl
Ar	Aryl
Bn	Benzyl
Boc	tert-Butyl
Bu	Butyl
CD	Circular Dichroism
COSY	Correlation Spectroscopy
DABCO	1,4-Diazabicyclo[2.2.2.]octane
DIC	Diisopropylcarbodiimide
DIPEA	Diisopropylethylamine
DKP	Diketopiperazine
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxyde
EDC	Ethyl-N,N-dimethyl-3-aminopropylcarbodiimide
Et	Ethyl
eq.	equivalents
Fmoc	9-Fluorenylmethoxycarbonyl
Gly	Glycine
h	hours
HBTU	O-benzotriazole-N,N,N',N' tetramethyluronium-hexafluoro-phosphate
HB	Hydrogen Bond
HFA	Hexafluoroacetone
HOBt	Hydroxybenzotriazole
IR	Infrared Spectroscopy
Me	Methanol
MeOH	Methyl
min.	minutes
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
PG	Protecting group
Ph	Phenyl
Phac	Phenylacetyl
Phe	Phenylalanine
ppb	Part per billion
Pro	Proline
Py	Pyridine
quant.	quantitative
RMSD	Root Mean Square Deviation
ROESY	Rotating Frame NOE spectroscopy
r.t.	room temperature
sat.	saturated
tert	tertiary
TFA	Trifluoro acetic acid
TFE	Trifluoroethanol
TOCSY	Total Correlation Spectroscopy
Xxx	General amino acid
UV	Utraviolet Spectroscopy

Introduction

"Virtually every life process involves peptides in some way"[1a]

Life is only possible if there is a high level of regulation to control every event in every living cell at any time. This regulation is achieved through the ability of the molecules of life to recognise and identify each other. A typical cell contains a number of molecules (receptors) exposed to the environment and in communication with it. Other molecules in the environment contain specific components called ligands.

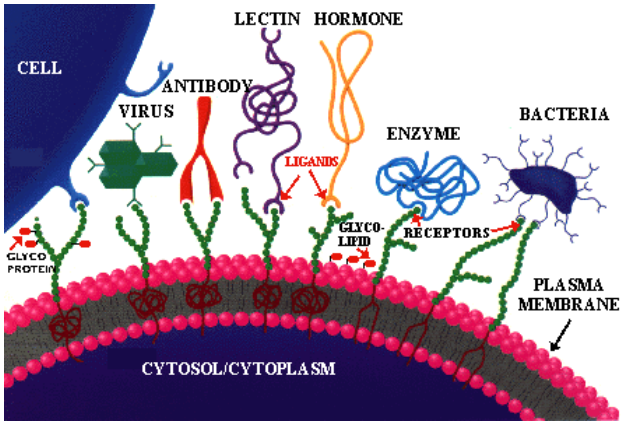


Figure 1. Molecular recognition at the cell surface. Cartoon reproduced from Sigma Chemical & Co.[1b]

Receptor-ligand interactions are recognised as the fundamental governing processes in biology and medicine, as they are responsible for transmitting informations about events inside and outside the cell and for prompting appropriate responses (Figure 1). Human diseases can be ultimately associated with receptor-ligand interactions, such as the altered function of a mutated receptor gene or the tropism of a virus for a cell surface.

The strong interaction between a ligand and its receptor depends on their complementarity in shape, surface potential and other non-covalent forces (hydrogen-bonds and hydrophobicity). Many ligands are peptides (examples are given in Table 1), and in this case the recognition requirements can be summarised in only three points: the primary, secondary, and tertiary ligand structure.

Table 1. A few examples of biologically active peptides.

Name	Sequence	Biological properties
Endomorphine-2	TPFF-NH_2	Endogenous ligand at the μ-opiate receptor. Analgesic.
Angiotensine II (human)	DRVYIHPF	Increases blood pressure (hypertensive agent).
Somatostatin	AGCKNFFWKTFTSC	Inhibits secretion of insulin, glucagon and gastrin.
Bombesin	Pyr-QRLGNQWAVGHLM-NH_2	Endogenous neurotransmitter in many animals including mammals. Affects vascular and other smooth muscle, gastric secretion and renal circulation and function.
Insuline	GIVEQCCTSIC SLYQLENYCNFVNQHLCGSH LVEALYLVCGERGFFYYPKT	Regulate glucose metabolism in tissues.

The central role played by peptides in the organism makes them a major target of investigation in pharmacology and drug design, and indeed a variety of peptide-based drugs are currently developed as therapeutics in the treatment of cancer,[2a] pain management,[2b] viral infections,[2c] and other diseases.[2d] However, the peculiar chemical and physical properties of peptides introduce additional difficulties over small-molecule drugs for their adoption as therapeutics in clinical practice. At least two basic issues have to be addressed in the development of a pharmacologically active peptide:

- the poor oral bio-availability due to the chemical degradation in the stomach and intestines, as well as the low absorption rate in the intestinal system. Moreover, peptides have often a short systemic half-life, because they are rapidly hydrolysed by proteolytic enzymes in blood and tissues. A further obstacle to the delivery of peptide drugs in the organism is constituted by the blood-brain-barrier.[1c]
- the complex elucidation of the conformation-activity relationship that is the key-point to be addressed in order to explain the mechanism of the biological action and eventually design analogues with improved pharmacological activity.

Many strategies have been developed in order to improve the systemic availability and stability of peptide drugs in the organism. For example, their inclusion into biodegradable

polymer micro-beads or their embedment into liposomes allows oral administration and controlled rate delivery of the drug.[3a-b]

A further promising approach to improve the stability of peptide drugs is the introduction of residues not recognised by peptidases in the primary peptides sequence. Insertion of non-natural residues in the primary sequence constitutes also a powerful tool for the inspection of peptides structure. In fact, the introduction of conformation stabilising building blocks can prevent the mutual exchange of conformers and possibly "freeze" the biologically active structure, which can be subsequently investigated by established spectroscopic techniques. Moreover, the synthesis of structurally defined building blocks which can be inserted as non-natural residues in a peptide, opens the exciting possibility of inducing a targeted modification into the secondary structure of a native peptide, thereby modulating their biological properties and activity.

Compounds that are able to mimic the critical features of the molecular recognition process of the parent peptide and thereby block or reproduce its action are named *peptidomimetics*.

I.1 α-Amino acids analogues as building blocks for peptidomimetics

A number of building blocks not belonging to the 20 proteinogenic amino acids have been devised as replacement for α-amino acids in a natural peptide sequence (Figure 2).

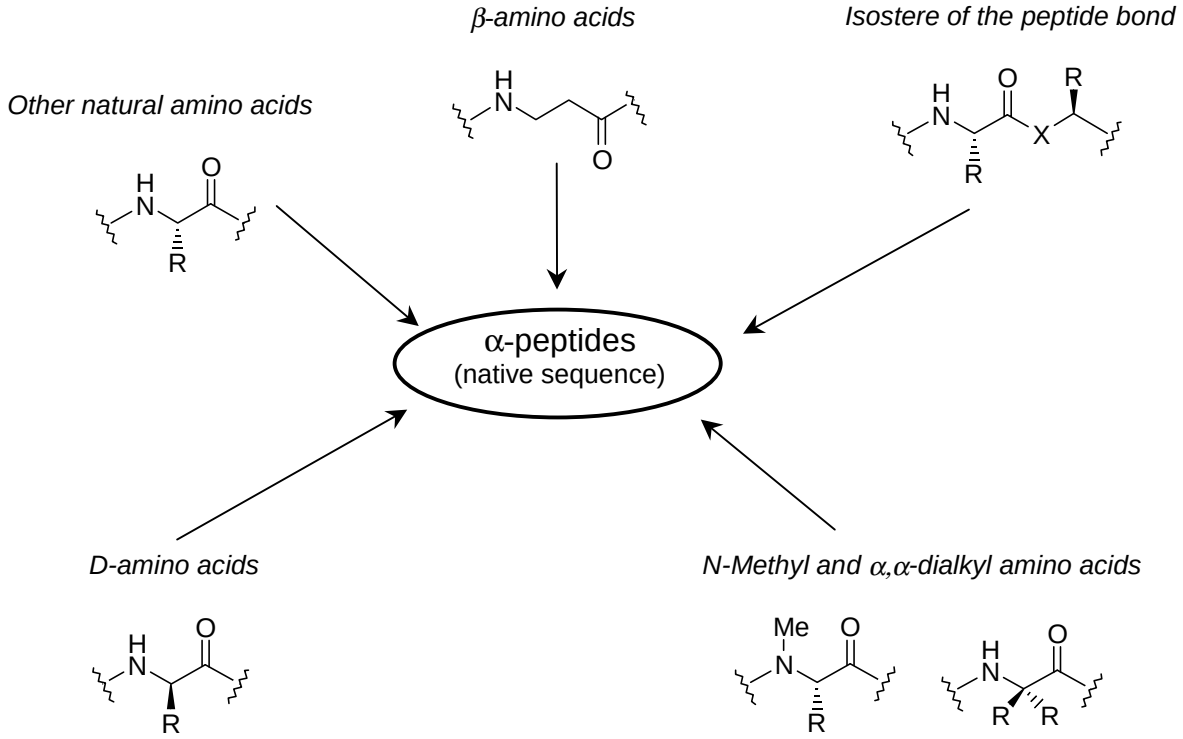


Figure 2. Some types of building blocks for amino acids substitution in the natural sequence.[4a,b]

Such units can stabilise and/or induce secondary structure motives in peptides, due to the lack of side chains (glycine, β-alanine), due to their chirality (D-amino acids), or due to the prolongation of the peptide back-bone (β-amino acids) or to some constraining features which can rigidify the peptide structure (α,α-dialkyl and cyclic amino acids). The application of some of these building blocks as unnatural constituents of peptides will be described in the following sections, in particular greater attention will be devoted to the effect of cyclopropane containing units.

I.1.1 α-Amino acids

The replacement of an amino acid of the original bioactive sequence with another natural amino acid is the most obvious way to investigate a peptide active conformation.[4c]
Among the 20 natural amino acids, in particular the incorporation of glycine and proline has a strong effect on peptides structure. Glycine, due to the lack of chirality at the α-carbon and the absence of a side chain, adopts conformations that are inaccessible to the other amino acids. Glycine is commonly found in β-sheets[4c] (Figure 3) or in turns (Figure 4) while it is known to terminate helices.[4d]

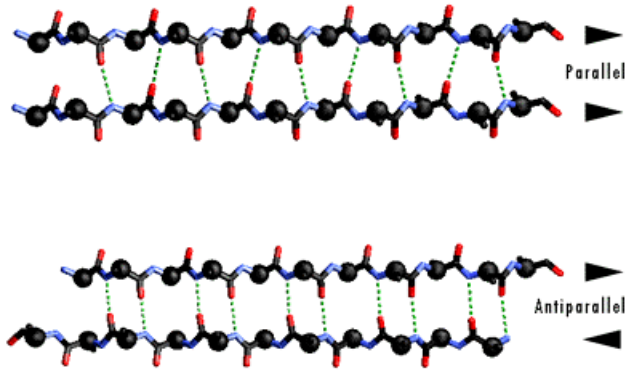
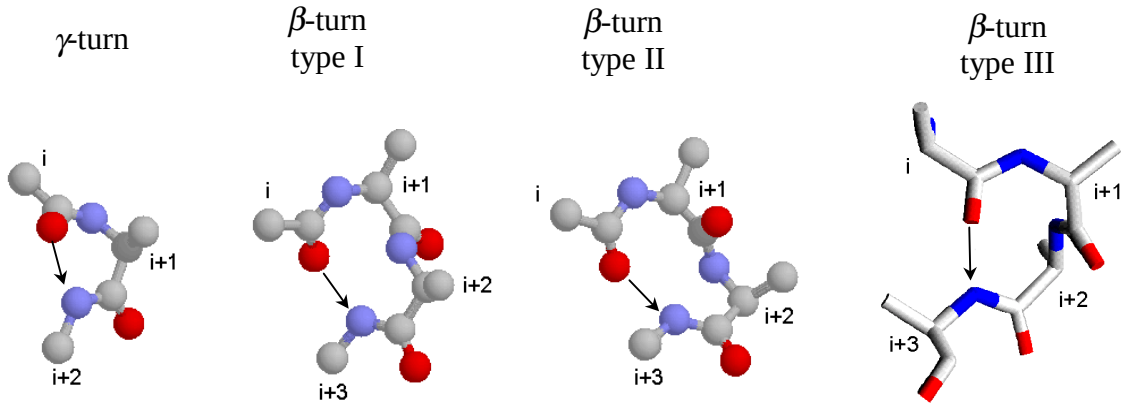


Figure 3. Parallel and antiparallel β-sheets.

Proline,[4c] in direct contrast to glycine, involves an additional constraint on the backbone caused by its cyclic structure. It can fit neither into helices nor into β-sheets because it has no NH available to take part in the hydrogen-bonding network. Proline is able, however, to act as turn inducing element[4e] due to its cyclic nature and to its unique capability to tolerate *cis* as well as *trans* peptide bonds.



	φ (i+1)	ψ (i+1)	φ (i+2)	ψ (i+2)
Type I	-60	-30	-90	0
Type II	-60	120	80	0
Type III	-60	-30	-60	-30

Figure 4. Turns: the type of turn is determined by the φ (rotation around the Cα-N bond) and ψ (rotation around the Cα-C=O bond) dihedral angles.

D-α-amino acids increase the proteolytic stability of peptides, and therefore play an important role in drug design. Moreover, D-amino acids favour reverse turns (Figure 4) but destabilise helices.[5a] Their unique ability is to induce a particular type of β-turn, so called type II, and its mirror image, so called type II' depending on whether it is found at the right or at the left corner of the turn (i.e. position i+2 or i+1). In contrast, the combination of two D-amino acids can induce so called type I β-turns.[5b] The ability to stabilise type II' β-turns is of particular interest since it does not often occur in L-peptides but can be decisive for the molecular recognition of some peptides toward their receptors. An example is the affinity of RGD peptides, containing the binding sequence Arg-Gly-Asp, toward $\alpha_v\beta_3$-integrines, implicated in human tumour metastasis and in angiogenesis.[5c]

I.1.2 N- Methyl and α,α-dialkyl amino acids

N-Methyl amino acids are commonly found in naturally occurring peptide antibiotics. They are generally not recognised by proteases since the lack of the NH in the backbone chain alters the hydrogen bonding pattern. Like proline, they also tolerate *cis* peptide bonds. They can be used as effective reverse-turn constraints when they are placed at the i+1 or i+2 position of a turn, especially when D-proline is occurring in the preceding position.[5d]

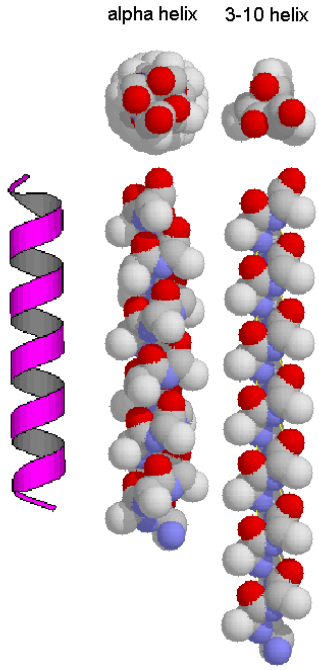
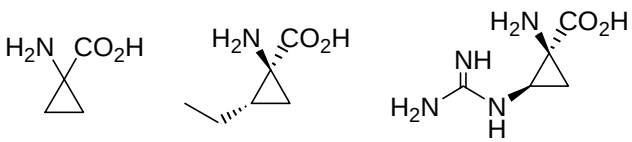


Figure 5. α- and 3_{10}-helices.

Prominent among the α,α-dialkyl amino acids is amino *iso* butyric acid (Aib) commonly found in a family of natural antibiotics produced by microbial sources. Tetrasubstitution of the α-carbon atom generates severe steric hindrance, therefore, as a consequence of the Torpe-Ingold effect,[6] the folding of Aib containing peptides in 3_{10}-, α-helices (Figure 5) and β-turns (Figure 7) is favoured compared to the extended structures.[7a,b] Analogous results were obtained with other types of α,α-dialkyl glycines.[7c-e]

I.1.2.1 α-Cyclopropyl amino acids

Among the α,α-disubstituted amino acids some cycloaliphatic residues[4a] were investigated. A particular role is played by 1-aminocyclopropane carboxylic acid (α-ACC) **1** (Figure 6).



1 **2** **3**

Figure 6. Naturally occurring α-ACCs.

α-ACC **1** is a natural amino acid which was first isolated from cider apples and perry pears and was identified as an intermediate in the biosynthesis of ethylene.[7e,g] Besides α-ACC **1**,

many analogues could be isolated from plants: Coronatine **2** and Carnosadine **3** are only two examples (Figure 6) and many other cyclopropane containing amino acids not occurring in nature were later synthesised.[7a-b]

Theoretical calculations[8a-c] showed that α-ACC, due to the additional constrain introduced by the cyclopropane ring, behaves quite differently from Aib since it favours folding of the peptide chain by formation of a C_7-helix or γ-turn[8a-d] (Figure 7). Peptides consisting of three or four α-ACC units have a propensity to fold into distorted type I β-bends and irregular 3_{10}-helices.[8e-g]

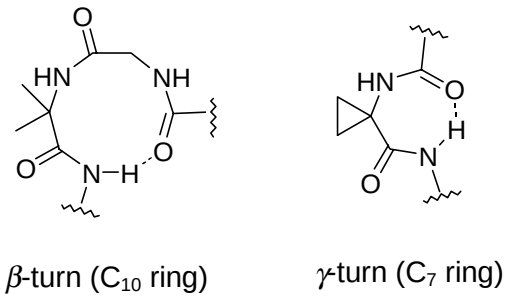


Figure 7. Aib favours β-turns, α-ACC γ-turns.

Many bioactive peptides containing α-ACC **1** have been so far synthesised: the aspartame analogue Asp-ACC-O^{n}Pr, which has 250-300 times higher sweetness potency than sucrose; N-benzoyl-ACC-Phe-OH and N-benzoyl-ACC-Pro-OH, which showed time-dependent inhibition of carboxypeptidase A; ACC^7-oxytocin, which has lower bioactivity than oxytocin, are only a few examples.[8h]

I.1.3 Peptide bond isosters

Many amide bond isosters have been devised (in Figure 8 only few examples are shown). These analogues resemble the peptide bond to varying degrees, but they are more resistant to proteolytic cleavage and, furthermore, they display improved passive diffusion across biological membranes.[4a,b]

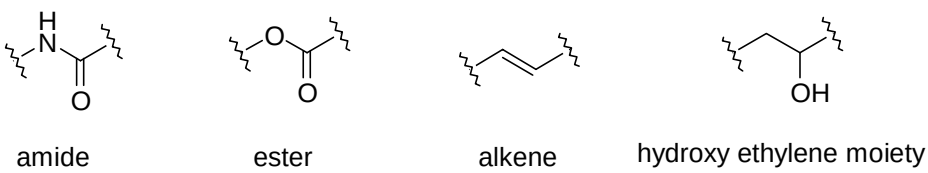
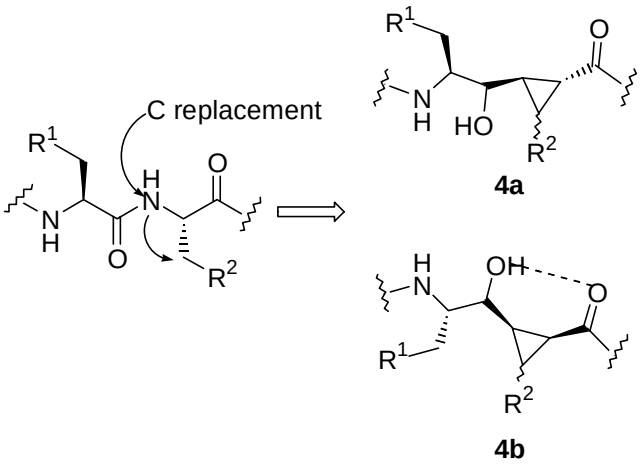


Figure 8. Amide bond isosters.

Depsipeptides are homologues of peptides in which some of the backbone amide linkages are replaced by ester bonds. The ester bond, among the amide bond isosters, is the one which can particularly well mimic the conformational properties of the parent amide bond: both groups are planar due to the electronic resonance, and although the ester bond lacks the proton of the amide NH to build hydrogen bonds, they are able to form loops. This feature can be useful to investigate the conformation preferences of short peptides avoiding the complication to find out which of the many NHs is hydrogen bonded.[9a,b]

Trans disubstituted alkene units can mimic *trans* configurated peptide bonds but are inert to peptidases. They are known to promote β-turns and β-hairpins,[10a,b] and are able to play an unusual role as hydrogen-bond acceptors.[10b]

Among the hydroxy ethylene amide bond isosters, the dipeptide mimics **4a** and **4b** (Scheme 1) are an interesting example of the ability of cyclopropane derivatives to control the backbone folding due to their constrained ring and to the forced *cis*- or *trans*- relationship between the substituents.[11a-d]



Scheme 1. The dipeptide mimics **4a** and **4b**.

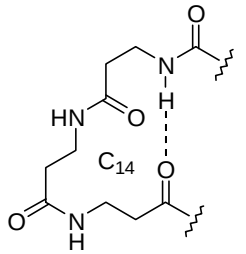
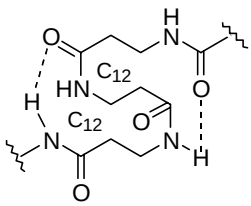
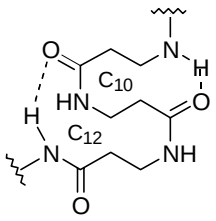
The *trans* **4a** proved to stabilise extended conformations in α-peptides, an important, although difficult task to accomplish, since structural elements mimicking β-strands are not so extensively investigated.[11a,d] The *cis* **4b** was designed to initiate a reverse turn which, however, was not formed in the solid state.[11d] Nevertheless both dipeptide mimics and related derivatives displayed interesting pharmacological properties as inhibitors of farnesyltransferase, as enkephalin analogues and as inhibitors of HIV-1 protease.[11a,c,d]

I.1.4 β-amino acids and β-peptides

β-amino acids are the higher homologues of α-amino acids having an additional methylene group which does not lead to decreased capability of folding but rather to new stable secondary structures occurring in much shorter oligomers than in α-peptides.[12a] β-amino acids can be Cα as well as Cβ substituted (and for each, obviously, L and D configurations are possible) thus providing a wide range of possible stereochemical combinations.[12b] As well as α-peptides, β-peptides are also able to fold into helical, sheet- and turn-like structures.

The most common secondary structures in β-peptides are helices, left (L) and right (R) handed, with various hydrogen bonding patterns (a brief overview is given in Table 2).[12a,c,d] In contrast to α-peptides requiring 12 to 15 residues to form stable helices in organic protic solvents, for β-peptides already six residues are sufficient.[12a,b,e] Some features in β-helices resemble those of α-helices: constrain on the side chain (due to the inclusion of the Cα-Cβ bond within a ring) favours the helix folding[12f] while incorporation of β-alanine (higher analogue of glycine) breaks the helices.[12g]

Table 2. Helical conformations in β-peptides. Helices 12/10/12 occur in mixed β-peptides containing Cα- and Cβ- monosubstituted β-amino acids.

hydrogen bonding pattern	helix type	hydrogen bond pattern
	L_{+2}, R_{+2}	i ← i+2
	L_{-3}, R_{-3}	i ← i-3
	12/10/12	Alternating C_{10} and C_{12}

The formation of sheet-like secondary structures is more difficult, however, great progress has already been accomplished toward this aim. Antiparallel β-sheet like arrangements were obtained by introduction of typical turn inducing moieties: α-depsipeptides (D-Pro-Lac[13a]), cyclisation,[13b] or β-nipecotic acid dimer.[13c] Parallel β-sheet like arrangement are on the other hand rarely encountered.[13d]

Besides all the β-amino acids which have been synthesised until now, β-alanine (**5**) deserves particular mentioning being an achiral β-amino acid which occurs in animal and plant kingdom.[14a]

β-Alanine lacks a side chain and therefore permits the folding of β-alanine containing peptides in a large number of well defined three-dimensional structures ranging from extended to folded β-alanine conformation (Figure 9).[14b]

5 extended conformation **5** folded conformation

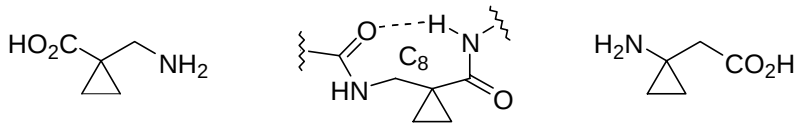
Figure 9. Extended and folded conformation of β-alanine are characterised by a value of the μ dihedral angle of ±180±20° and ±60±20° respectively.

The folded conformation of β-alanine has been observed in cyclic peptides[7a,15a-i] where β-alanine promotes β-turns[15b-d,g-h] or γ-turns.[15e,f] Nevertheless β-alanine has low propensity to be positioned at the corner of these turned structures unless other conformational constraints exist.[15i] In linear peptides on the other hand, the most favourable conformation is the extended one.[16a,b] Therefore β-alanine seems to have no intrinsic preference for the folded conformation but can surprisingly stabilise peptide conformations by mimicking fragments of various secondary structures, i.e. α-helix, β- and γ-turns.[17a-d]

I.1.4.1 β-ACCs

The effect of geminal substitution is interesting not only in α-peptides but also in β-peptides:[18a] the 1-(aminoethyl)-cyclopropanecarboxylic acid **6** (Figure 10), the β-analogue of α-ACC **1**, displays a completely new folding pattern,[18b] namely a stair-like structure **7** constituted of C_8 hydrogen bonded rings. The synthesis of **8** (the (1-aminocyclopropyl) acetic

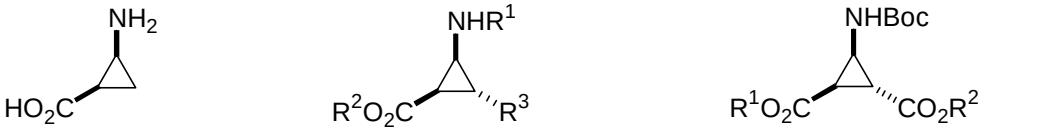
acid) and of its derivatives is already known[18d-f] but neither its oligomers nor otherwise **8** containing peptides have been yet synthesised.



6 **7** **8**

Figure 10. Geminal β-ACC.

Another way to introduce the cyclopropyl ring constrain into an amino acid is shown with the β-ACC **9** (Figure 11), a β-alanine analogue in which the rotation around the μ dihedral angle is not possible (fixed folded conformation) and the relationship between the amino and carboxy function is determined by the stereochemistry at the two stereocentres. As it has been illustrated so far, constrained α- and β- amino acids are efficient building blocks to rigidify peptides conformation and to induce their folding, and **9** in particular has attracted considerable attention as a potential turn inducer.[19a-d]



R^1= CO_2 Et, CO_2-Napht, Cbz, Boc
R^2= Me, Bn, Et
R^3= Me, H

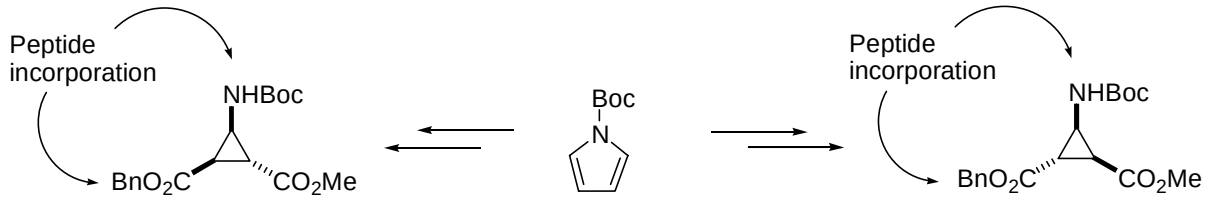
11 R^1= Bn, R^2= Me
12 R^1= Me, R^2= Bn

9 **10**

Figure 11. β-ACC **9** and related compounds **10**, **11** and **12**.

Unfortunately **9** and its derivatives are difficult to prepare due to the 1,2 donor-acceptor disubstitution which leads to rapid ring opening[20] in the absence of an electron withdrawing group on the amino function. Moreover, the control of the stereochemistry on the centres at the cyclopropane ring is an additional challenge.[19a] Therefore, only few syntheses of compounds with a *cis*-β-ACC substructure of type **9** (**10**) (Figure 11) have been developed.[19a-h] Despite many attempts toward this aim,[19b-d] only one general synthetic strategy is known that permits the introduction of β-ACC derivatives into a peptide chain[21a-d] in diastereomerically as well as enantiomerically pure form.[21d] This strategy makes use of the building blocks **11** and **12**, which can be obtained in five steps from N-Boc pyrrole (**13**),[21d-g]

having the N-Boc/CO_2Bn *cis* (**11**) or *trans* (**12**) to each other (Scheme 2). The additional carboxy group present in **11** and **12** allows, moreover, to introduce other functionalities imitating α-amino acids side chains.[22]

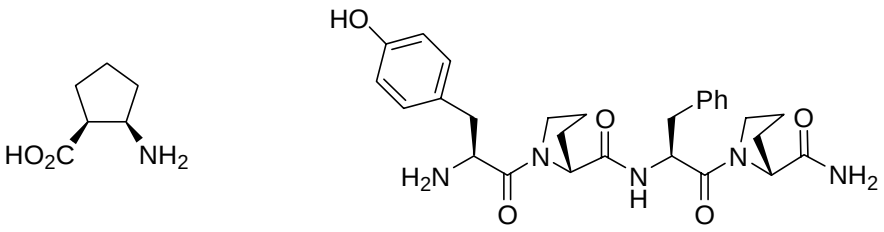


11 **13** **12**

Scheme 2. *Cis* β-ACC **11** and *trans* β-ACC **12**.

I.1.4.2 β-Amino acids and α-peptides

The interest in the role of β-amino acids with respect to conformational studies of peptides, is not limited to their oligomers, the so called β-peptides, but also applies to their effect on α-peptides. The backbone and the hydrogen bonding pattern of a peptide will be considerably modified upon incorporation of a β-amino acid.[23a,b] This may affect the overall conformation and the pharmacological properties of biologically active peptides. A successful example in this regard is the substitution of proline with its β-analogue, the 2-amino cyclopentane carboxylic acid (**14**), in morphecitine (**15**), one of the most selective agonists for the opioid μ-receptor (Figure 12).[24a-b]



14 **15**

Figure 12. 2-Aminocyclopentane carboxylic acid **14** and morphecitine **15**.

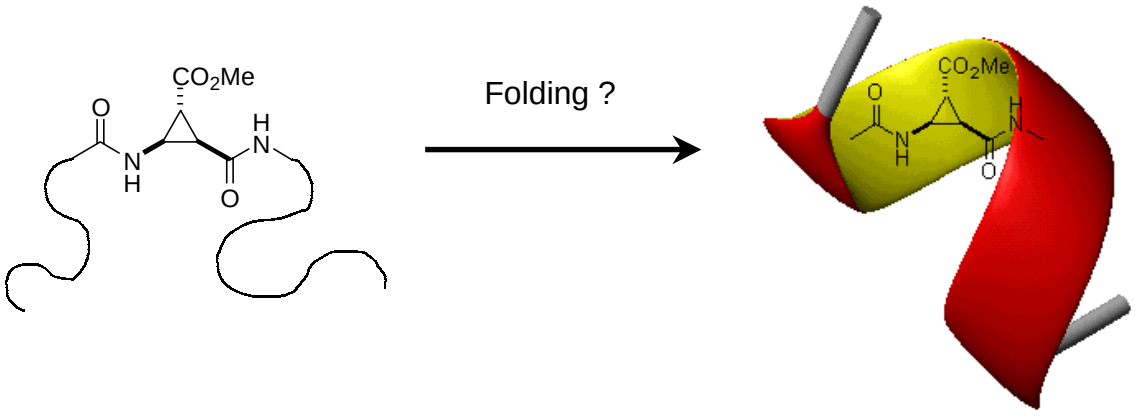
Apart from being useful tools to investigate the structure of α-peptides, β-amino acids have been used also in cyclic RGD peptides and their activity as agonists of blood platelet aggregation has been examined.[23a,b] It is believed that β-amino acids in cyclic peptides act as

pseudo γ-turn mimetics, thus giving the chance to regulate the orientation of the pharmacophoric groups.[23a]

I.2 Aim of this work

Compounds **11** and **12** are highly constrained cyclic β-amino acids, a property which promises interesting effects on the conformation of their derivatives.

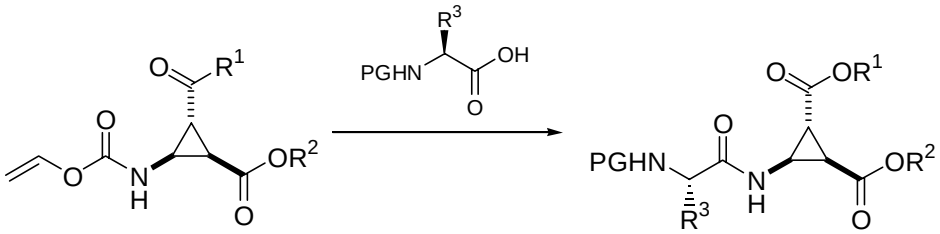
The introduction of the β-ACC **11** in Neuropeptide Y analogues resulted in increased selectivity[25,†] and bioactivity towards the different NPY receptors. Hence, the influence of **11** on the structure of short α-peptides has been addressed in the present work. The synthesis and the conformational studies on **11** derivatives (Scheme 3) will be presented.



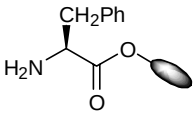
Scheme 3. β-ACC as possible folding-inducer in α-peptides.

The β-ACC containing α-peptides have been synthesised in solution, which allows to control each coupling step, monitor the presence of side products and optimise the reaction conditions. Nevertheless solid phase synthesis would produce longer peptides within shorter time. The most efficient and practicable solid phase synthesis is performed with the Fmoc strategy, but it has been already demonstrated that Fmoc is not a suitable protecting group for the β-ACC because of the deprotection conditions.[26] In contrast, preliminary acylation experiments on the N-allyloxycarbonyl (Alloc) protected β-ACC gave encouraging results,[26] therefore the peptide coupling of the N-Alloc β-ACC (**16a** and **17**) both in solution and on the solid phase has also been developed in the present work (Scheme 4).

† In collaboration with the research group of Prof. A. Beck-Sickinger, University of Leipzig. See Chapter 5.



16a R^1 = OMe, R^2 =Bn — synthesis in solution — **18**

17 R^1 =  R^2 =Me — synthesis on solid phase — **19**

Scheme 4. Coupling of the N-Alloc *β*-ACC derivatives: **16a** in solution and **17** on the solid phase.

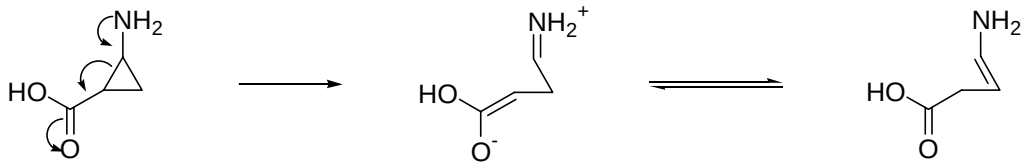
Chapter 1

1.1 Synthesis of peptides with one β-ACC unit

1.1.1 Coupling of the β-ACC at the N-terminus

The *cis* and *trans* β-ACC **11** and **12**, respectively, were synthesised in five steps from Boc-pyrrole either in racemic[21a-d] or enantiomerically pure form.[21d]

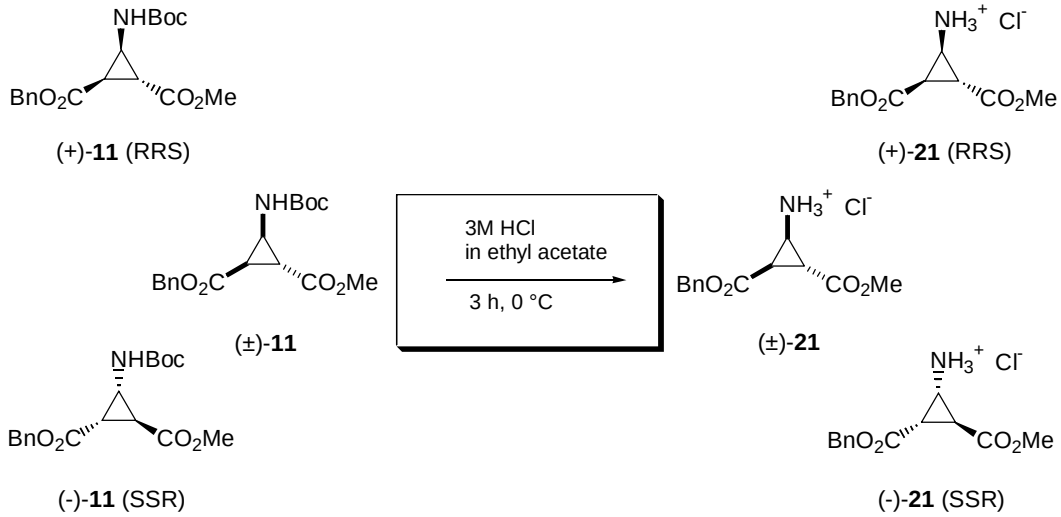
The coupling at the β-ACC N-*terminus* requires a particular protocol since the free amine on the cyclopropyl ring **9** is not tolerated in the presence of an electron withdrawing group in β-position which leads to ring opening products of the type **20a** and **20b** (Scheme 1).[21a-d]



9 **20a** **20b**

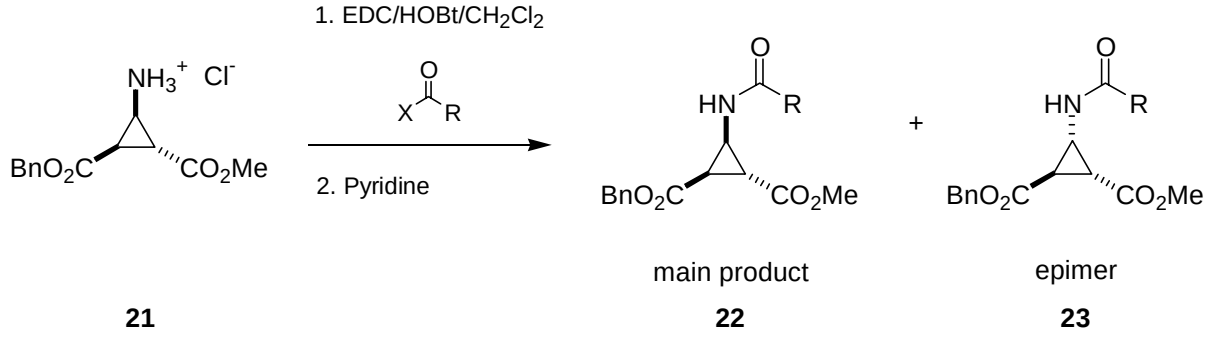
Scheme 1. Ring opening at the N-unprotected β-ACC **9**.

The protocol of choice[21a-d] to accomplish the coupling of β-ACC derivatives such as **11** is based on the *tert*-butoxycarbonyl deprotection by treatment with a saturated solution of HCl in ethyl acetate. The resulting racemic β-ACC ammonium salt (±)-**21** or the enantiomerically pure (+)-**21** and (-)-**21** (derived from (+)-**11** having the RRS configuration and from (-)-**11** having the SSR one) are stable (Scheme 2).



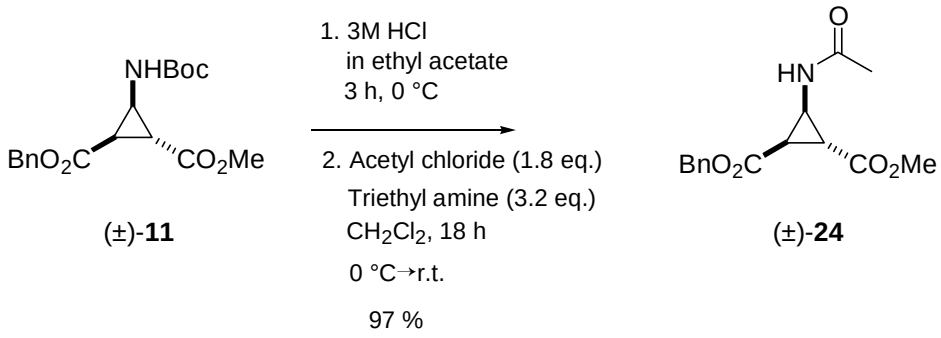
Scheme 2. Deprotection of the β-ACC at the N-*terminus*.

The salts **21** can then be liberated by the addition of a mild base in the presence of the preactivated amino acid or acid chloride to arrive at the corresponding coupling products **22** (Scheme 3). Under these conditions no ring opening products are formed but, depending on the reagents used in the coupling step, epimerisation at the cyclopropane ring leading to **23**, was observed (Scheme 3). The mechanism of this epimerisation and which particular stereocentre is involved is not as yet completely understood, but experimental evidence suggests that the cyclopropane stereocentre bearing the amino function could be labile.[27]



Scheme 3. General coupling at the *β*-ACC N-*terminus*.

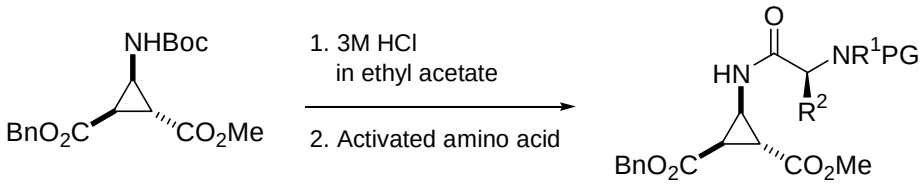
The coupling of the ammonium salt (±)-**21** in dry CH_2Cl_2 with acetyl chloride in the presence of triethyl amine afforded the N-acetyl-*β*-ACC (±)-**24** in high yields and without epimerisation (Scheme 4).



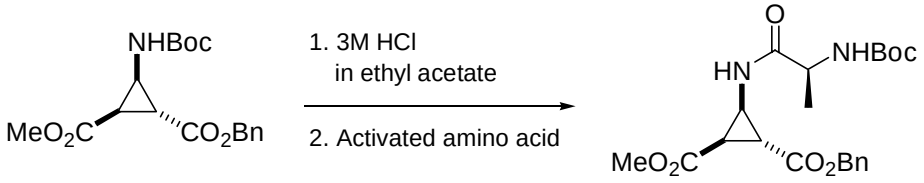
Scheme 4. Coupling of (±)-**11** with acetyl chloride.

Analogously, N-protected amino acids can be coupled with **11** or **12** to dipeptides **25a-f** and **26** under similar conditions (Table 1).

Table 1. Dipeptides containing the *β*-ACC building block (**11** or **12**).



11 **25a-f**



(±)–**12** (±)-**26**

Entry	R^1	R^2	PG	β-ACC	a.a. eq.	Activating reagents[a)]	Epimerisation	Yield	Product[b)]
1	H	Me	Boc	(±)-**11**	1.5	A	12 %	95 %	(±)-**25a**
2	H	Me	Boc	(±)-**11**	1.5	B	4 %	90 %	(±)-**25a**
3	H	Me	Fmoc	(+)-**11**	1.5	B	3 %	90 %	(+)-**25b**
4	Me	Me	Boc	(±)-**11**	1.7	A	10 %	88 %	(±)-**25c**
5	H	CH_2Ph	Fmoc	(±)-**11**	1.2	B	–	90 %	(±)-**25d**
6	H	$CH_2CO_2{}^tBu$	Fmoc	(±)-**11**	1.2	A	20 %	66 %	(±)-**25e**
7	H	$CH_2CO_2{}^tBu$	Fmoc	(±)-**11**	1.2	C	–	72 %	(±)-**25e**
8	H	H	Fmoc	(+)-**11**	1.5	B	–	77 %	(+)-**25f**
9	H	H	Fmoc	(-)-**11**	1.5	B	–	95 %	(-)-**25f**
10	H	Me	Boc	(±)-**12**	1.5	A	–	88 %	(±)-**26**

[a)] A: EDC (1.5 eq.), HOBt (1.5 eq.), NEt_3 (1.1 eq.); B: EDC (2 eq.), Py (2 eq.); C: EDC (2 eq.), HOBt (2 eq.), NEt_3 (1.1 eq.). [b)] (+) and (-) do not signify the optical rotation of the dipeptides, but rather the optical rotation of the *β*-ACC unit incorporated.

The use of HOBt should prevent the racemisation of the amino acid to be coupled and the reaction should be more efficient leading to better yields (Table 1, entry 1). It was observed, on the other hand, that in some cases the preactivation with HOBt leads to epimerisation (5-20%) of the *β*-ACC (entries 1, 4 and 6), which is suppressed or decreased in its absence (entries 2, 3, 5, 8-9). This effect seems to be caused by the larger steric hindrance of the

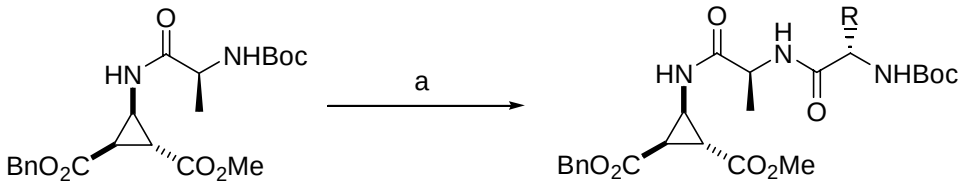
amino acid hydroxy-benzotriazol ester compared to the amino acid-EDC isourea. In the case of **25e** (entry 6) the steric bulk of the amino acid (Fmoc protected at the N-*terminus* and ^{t}Bu protected at the side chain) increased not only the epimerisation but decreased also the yield in the presence of HOBt. Surprisingly, the β-ACC **12** could be coupled with Boc-alanine without epimerisation despite activation with HOBt. In this case another effect must be considered, i.e. the steric bulk of the group *cis* to the reacting amino function. In **11** a benzyl ester will result in bigger steric hindrance compared to **12**, in which a methyl ester is in the *cis* position.

It is possible to completely separate the two diastereomers of **25a** by recrystallisation from ethyl acetate/hexanes. The stereochemistry of the two isomers was determined by converting the enantiomerically pure (+)-**11** to (+)-**25a** and comparing the ^{1}H NMR spectrum of (+)-**25a** with the spectra of the two diastereomers isolated by recrystallisation as described above. Separation of β-ACC containing dipeptides by recrystallisation was possible only with **25a**. All attempts to resolve the diastereomeric mixture of other dipeptides (even **26**, the *trans* analogue of **25a**) resulted only in the enrichment of one diastereomer.

Further coupling (under the EDC/HOBt activating conditions) at the N-*terminus* of some of the dipeptides listed in Table 1 afforded the corresponding tripeptides without epimerisation problems (Table 2).

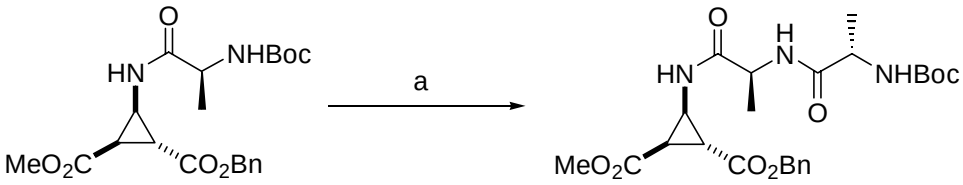
The tripeptide (-)-**27a** exhibits the same particular behaviour of (+)-**25a** toward recrystallisation: when a mixture of (+)-**27a** and (-)-**27a** is recrystallised from CH_2Cl_2/hexanes, (-)-**27a** only recrystallises.

Table 2. Tripeptides containing the β-ACC building block **11** or **12**.



(+)- and (-)-**25a** (+)- and (-)-**27a**

(+)- and (-)-**25a** (+)- and (-)-**27b**



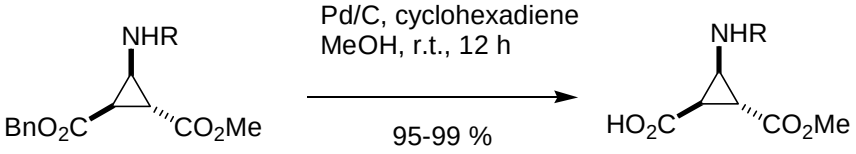
(±)-**26** (±)-**28**

Entry	R	Dipeptide	Yield	Product
1	CH_2Ph	(+)-**25a**	93 %	(+)-**27a**
2	CH_2Ph	(-)-**25a**	91 %	(-)-**27a**
3	H	(+)-**25a**	94 %	(+)-**27b**
4	H	(-)-**25a**	92 %	(-)-**27b**
5	CH_2Ph	(±)-**26**	94 %	(±)-**28**

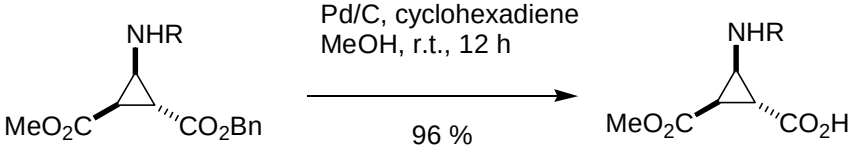
a) Coupling procedure: i. 3 M HCl in ethyl acetate, 3 h, 0 °C. ii. Amino acid (1.5 eq.), EDC (1.5 eq.), HOBt (1.5 eq.), NEt_3 (1.1 eq.), CH_2Cl_2, 18h, 0 °C→r.t..

1.1.2 Deprotection of the β-ACC C-terminus

The deprotection of the benzyl ester position at the C-*terminus* of the β-ACC ring to arrive at the free acid can be achieved by hydrogenolysis in methanol with Pd/C and a pentane solution of cyclohexadiene as the hydrogen source.[21d] The reaction proceeds overnight at room temperature in high yields for the N-acetyl β-ACC **24** as well as for the N-Boc protected derivatives **25a**, **25c**, **27a-b** and **28** (Scheme 5).



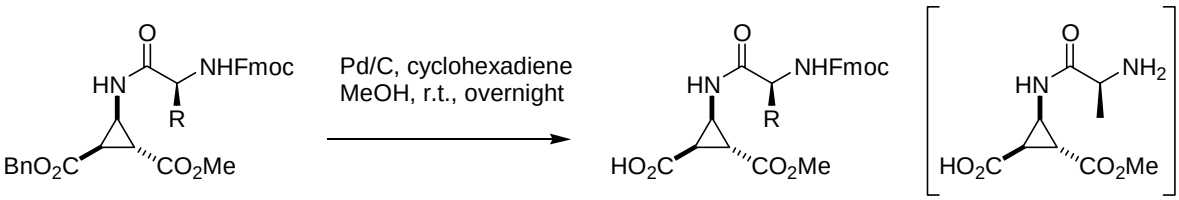
(±)-**24**	R = Ac	(±)-**29**
(+)- and (-)-**25a**	R = AlaNHBoc	(+)- and (-)-**30a**
(±)-**25c**	R = AlaNMeBoc	(±)-**30c**
(+)- and (-)-**27a**	R = Ala-PheNHBoc	(+)- and (-)-**31a**
(+)- and (-)-**27b**	R = Ala-GlyNHBoc	(+)- and (-)-**31b**



(±)-**28**	R = Ala-PheNHBoc	(±)-**32**

Scheme 5. Hydrogenolysis of the benzyl ester on the N-acetyl or N-Boc β-ACC derivatives.

Under the same reaction conditions N-Fmoc protected β-ACC derivatives (**25b**, **25e** and **25f**) gave lower yields or not pure products (**25d** and **25e**), and the problem was particularly severe for **25b** (Scheme 6).



(+)-**25b**	R = Me	40 %	(+)-**30b**	**33**
(±)-**25d**	R = CH_2Ph	99 %	(±)-**30d**	
(±)-**25e**	R = $CH_2CO_2{}^tBu$	86 %	(±)-**30e**	
(+)-**25f**	R = H	71 %	(+)-**30f**	
(-)-**25f**	R = H	62 %	(-)-**30f**	

Scheme 6. Hydrogenolysis of the benzyl ester on the N-Fmoc β-ACC derivatives.

The hydrogenation of **25b** required a longer reaction time than the other compounds, in fact, after 18 h, the starting material was not yet completely consumed but 48 h were necessary to

bring the reaction to completion (Table 3, entry 1). After filtration of the reaction mixture over a celite pad, **30b** was isolated together with the Fmoc deprotected dipeptide **33**. It is known that the Fmoc group undergoes hydrogenolysis but generally at a lower rate than *O*-benzyl systems.[28] In contrast, under the hydrogenolysis conditions described, the Fmoc cleavage occurs together with the benzyl cleavage. The reaction of **25b** was followed by TLC and the work up was done as soon as the starting material spot disappeared, nevertheless, **33** was found. Hydrogenation of **25b** at 1 atm of H_2, in methanol with Pd/C resulted in only 42 % yield of **30b** along with partial Fmoc deprotection (Table 3, entry 2).

Table 3. Various hydrogenolysis conditions tested on **25b**.

Entry	Hydrogen source	Solvent	Time	Yield of 30b	Product	Note
1	cyclohexadiene	MeOH	48 h	40 %	**30b** + **33**	
2	H_2 (1atm)	MeOH	18 h	42 %	**30b** + **33**	
3	HCO_2H (85 %)	MeOH	2 h	–	**33**	TLC monitoring difficult because of the high percentage of HCO_2H
4	HCO_2H (30 %)	MeOH/Benzene 1:1	1.5 h	98 %	**30b**	
5	HCO_2H (20 %)	MeOH/Benzene 1:1	1 h	94 %	**30b**	
6	HCO_2H (6 %)	MeOH/Benzene 1:2	2 h	85 %	**30b**	

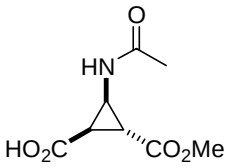
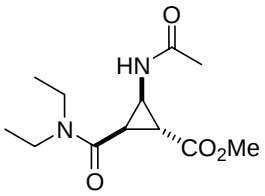
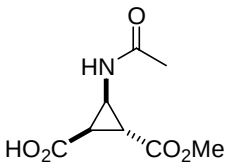
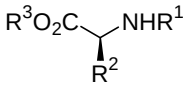
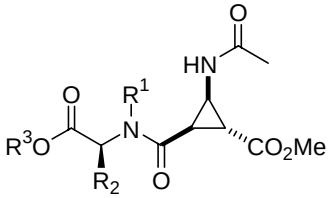
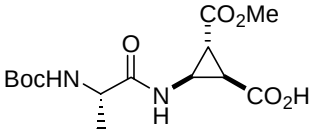
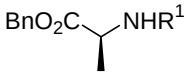
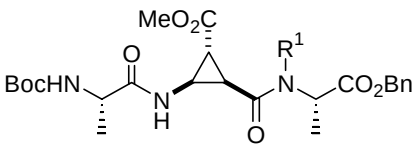
Hydrogenolysis of a benzyl ester in the presence of a N-Fmoc group can be also achieved in a 85 % solution of HCO_2H in methanol with Pd/C.[29] These conditions proved to be too drastic for **25b**: only Fmoc deprotection was observed. Therefore, different concentrations of HCO_2H were used to find the optimal methanol/HCO_2H ratio that would only affect the hydrogenolysis of the benzyl ester. By decreasing the HCO_2H concentration another problem arose: the low solubility of **25b** in methanol, therefore mixtures of methanol and benzene were used (Table 2, entries 4-6). The best results could be achieved for the conditions shown at entry 4.

1.1.3 Coupling at the β-ACC C-terminus

The coupling at the β-ACC C-*terminus* was performed under EDC/HOBt coupling conditions (Table 4), but the β-ACC derivatives were not preactivated to prevent a possible epimerisation of its benzotriazol-ester in the absence of the coupling partner.

When a secondary amine was coupled (leading to products **34**, **35c** and **36b**), epimerisation occurred to an extent ranging between 10 and 25 %. This coupling seems also to be sensitive to the steric hindrance of the partner, which reacts with the β-ACC derivatives. Only in the case of **34**, the epimer could be isolated by chromatography column and from the coupling constants at the cyclopropyl ring CHs (suggesting the presence of one proton *trans* related to the others) it can be reasonably stated that the epimerised centre is that bearing the amino function.

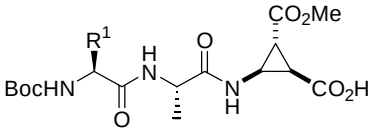
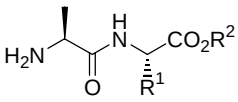
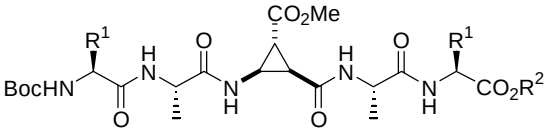
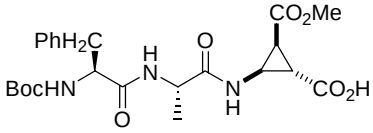
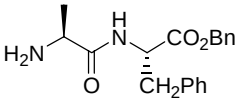
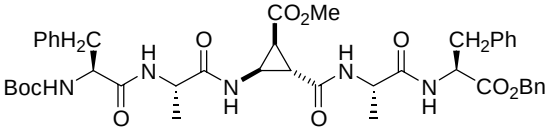
Table 4. Coupling at the β-ACC C-*terminus*. Reaction conditions: EDC/HOBt (1.5 eq.), amine (as ammonium salt, 1.5 eq), triethylamine (1.5 eq.), dry CH_2Cl_2, r.t., 18 h.

Starting material	Amine	Product	
 (±)-**29**	$HNEt_2$	 (±)-**34**	
 (±)-**29**		 (±)-**35a** (±)-**35b** (±)-**35c**	R^1= R^2= H, R^3= Me R^1= H, R^2= Me, R^3= Bn R^1= R^2= Me, R^3= Bn
 (+)- and (-)-**30a**		 (+)-**36a** and (-)-**36a** (+)-**36b** and (-)-**36b**	R^1= H R^1= Me

The presence of an isomeric by-product in the coupling of **29** and **30a** with a N-methyl amino acid could have been also caused by rotamers, generated at the N-methyl-C=O peptide bond. Nevertheless this possibility was excluded by NMR experiments performed on **35c** in DMSO-d_6 between r.t. and 140 °C, in which no coalescence was observed.

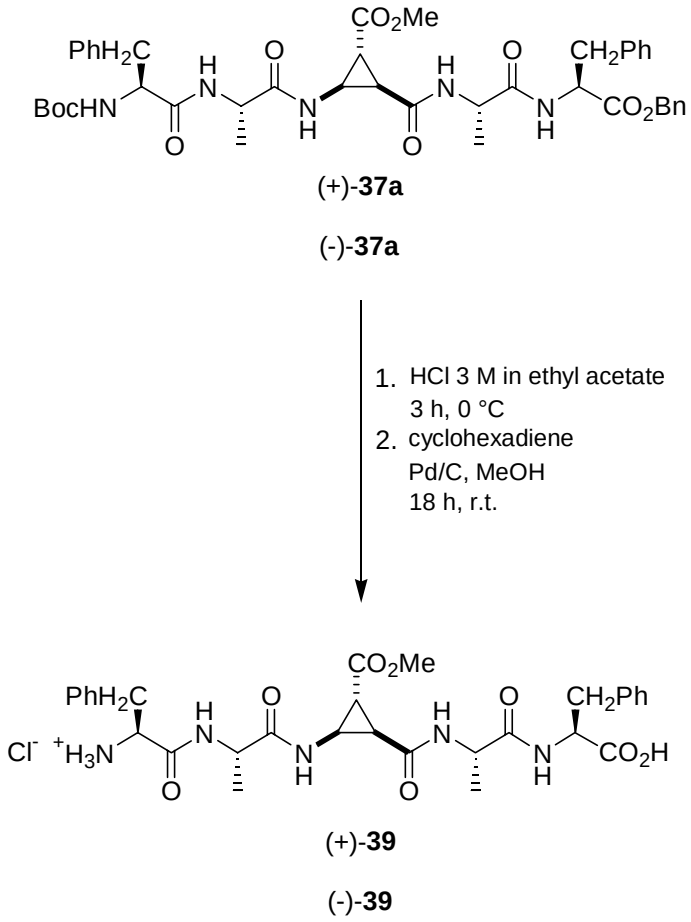
The coupling of the β-ACC acids **31a-b** and **32** (Table 5) with a dipeptide instead of an amino acid proceeded in high yields (85-98 %) without epimerisation, suggesting that the epimerisation determining factor is not the overall dimension of the amino partner but the steric hindrance directly at the reactive amino function.

Table 5. Coupling at the β-ACC acids C-*terminus*. Reaction conditions: EDC/HOBt (1.5 eq.), dipeptide amine (as ammonium salt, 1.5 eq.), triethylamine (1.5 eq.), dry CH_2Cl_2, r.t., 18 h.

Acid	Amine	Product
		
(+)-**31a** and (-)-**31a** R^1= CH_2Ph	R^2= Bn	(+)-**37a** and (-)-**37a**
(+)-**31b** and (-)-**31b** R^1= H	R^2= Me	(+)-**37b** and (-)-**37b**
		
(±)-**32**		(±)-**38**

Compound (±)-**38** is soluble only in DMSO, a solvent with a high hydrogen-bonding capability, which could disrupt the peptide conformation. Therefore DMSO is not suitable for structural investigations and (±)-**38** was not further taken into account.

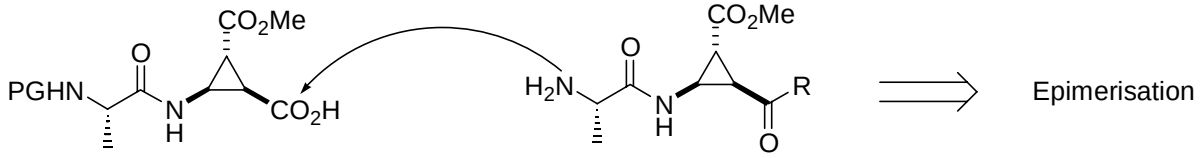
The pentapeptides **37a**, although not easily soluble in CH_2Cl_2 or MeOH, but only in a mixture of both solvents, were further investigated in their protected (**37a**) and unprotected (**39**) form (Scheme 7).



Scheme 7. The deprotection of the pentapeptides **37a** was achieved under acidic conditions to liberate the amino function and by hydrogenolysis to release the free acid.

1.2 Synthesis of multiple *β*-ACCs containing peptides

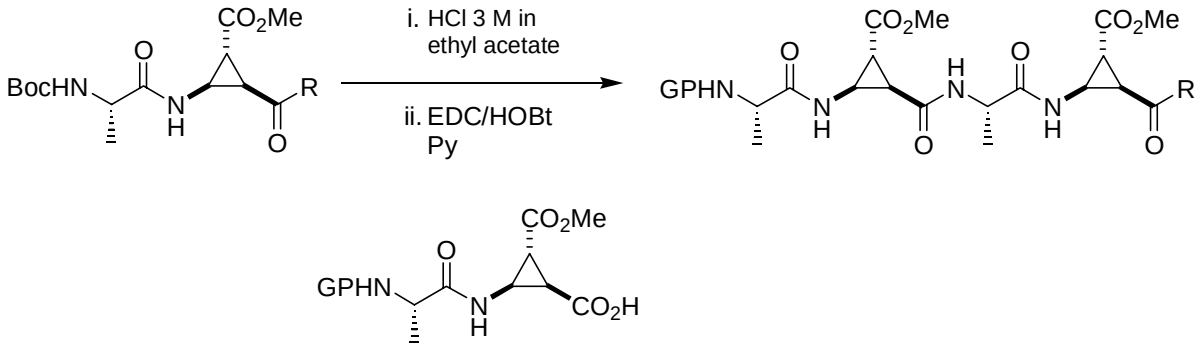
The epimerisation at the *β*-ACC ring becomes particularly severe when the coupling is performed at the cyclopropane carboxy function with a partner containing another *β*-ACC unit following the reactive residue (Scheme 8).



Scheme 8. Epimerisation occurs in particular when both coupling partners contain a *β*-ACC unit.

It was always possible to separate the products from their epimers by column chromatography, but the epimers were never isolated pure, therefore, there are no experimental data to support any hypothesis on which stereocentre epimerises.

Interestingly, the coupling of the tripeptides **36a** with the *β*-ACC acids **30a** proceeded in good yields (93-96 %) although 15 % epimerisation occurred. In contrast, the coupling of the *β*-ACC (+)-**25a** with (+)-**30b**, afforded consistently lower yields (71 %) and 22 % epimerisation (Scheme 9).



(+)-**36a**	R = AlaOBn	(+)-**30a**	PG = Boc	(+)-**40**	96 %	15 % epim.
(-)-**36a**	R = AlaOBn	(-)-**30a**	PG = Boc	(-)-**40**	97 %	15 % epim.
(+)-**25a**	R = OBn	(+)-**30b**	PG = Fmoc	(+)-**41**	71 %	22 % epim.

Scheme 9. Synthesis of peptides containing multiple *β*-ACCs.

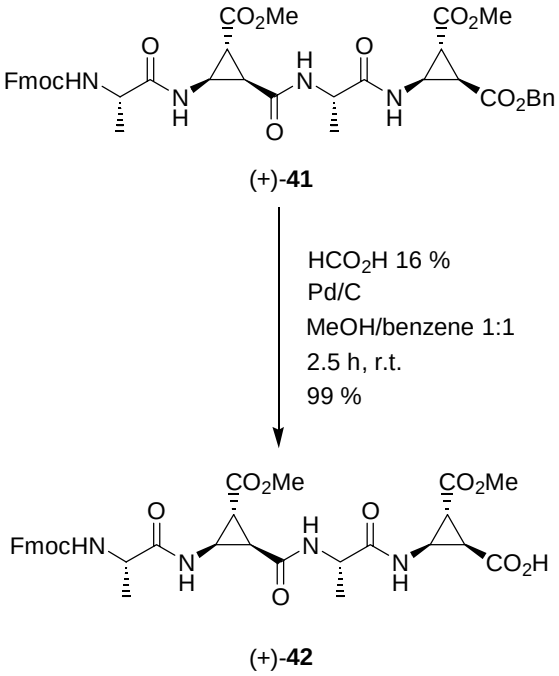
Other reaction conditions were tried to improve the yield of (+)-**41** (Table 6), but no substantial yield enhancement was observed.

Table 6. Reaction conditions for the coupling of (+)-**25a** and (+)-**30b** to afford (+)-**41**.

Reagents	Yield	Epimerisation
EDC/HOBt (1.5 eq.) Py (1.5 eq.)	71 %	22 %
HBTU (2 eq.) DIPEA (3 eq.)	55 %	15 %
EDC (2 eq.) Py (1.5 eq.)	68 %	12 %

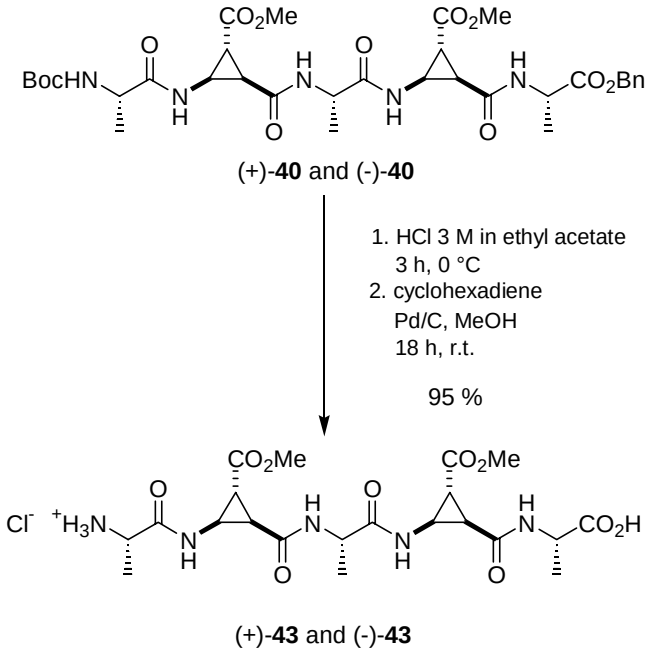
Activation of (+)-**30b** with HOBt resulted in the best yield but also in the highest amount of epimerisation. Less epimerisation was observed with HBTU as the activating agent, but at the cost of a lower yield. The best compromise between yield and degree of epimerisation was found with the EDC as the sole activation agent.

The free acid (+)-**42** (Scheme 10) was obtained by hydrogenolysis of (+)-**41** under the same conditions used for the Fmoc-protected β-ACC dipeptides.



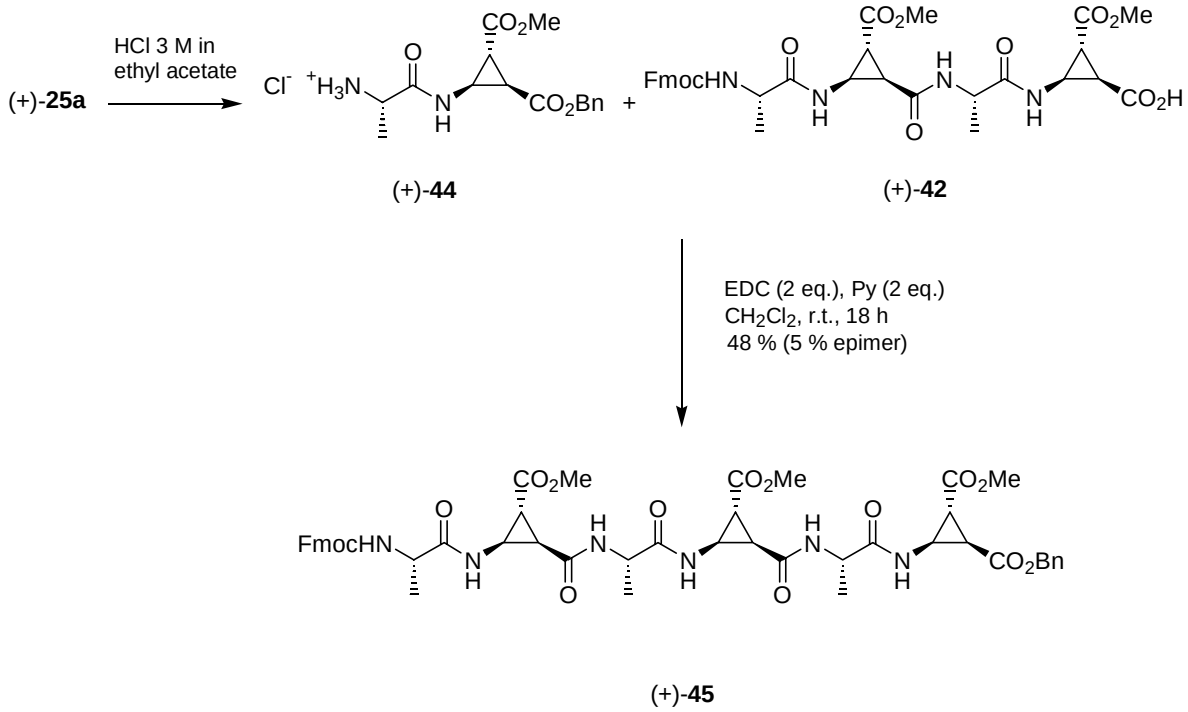
Scheme 10. Hydrogenolysis of the benzyl ester of tetrapeptide (+)-**41**.

Compounds (+)-**40** and (-)-**40** were completely deprotected under the same conditions used for (+)-**37a** and (-)-**37a** to arrive at the corresponding ammonium salts (+)-**43** and (-)-**43** (Scheme 11).



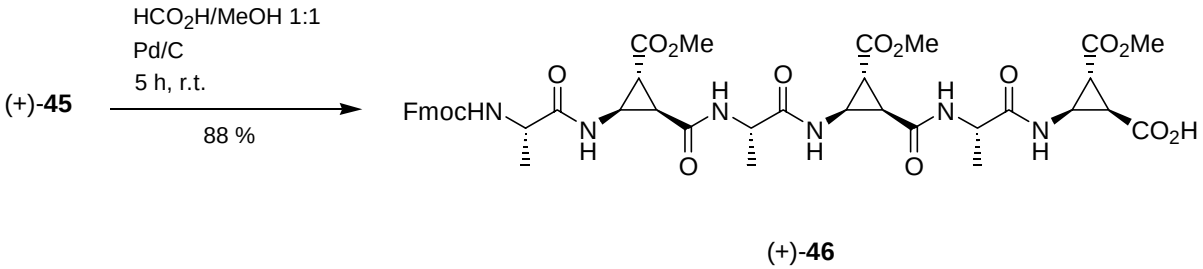
Scheme 11. Deprotection of pentapeptides (+)-**40** and (-)-**40**.

The acid (+)-**42** was subsequently coupled with the ammonium chloride (+)-**44** to afford the hexapeptide (+)-**45** (Scheme 12). The coupling worked in only 48 % yield but epimerisation had occurred to a lower extent compared to the previous couplings (Scheme 9).



Scheme 12. Synthesis of the hexamer (+)-**45**.

Finally the hexapeptide (+)-**45** was deprotected at the C-*terminus* to obtain the free acid (+)-**46** (Scheme 13) whose conformation is currently being investigated (see Chapter 5).



(+)-**46**

Scheme 13. Hydrogenolysis of hexapeptide (+)-**45**.

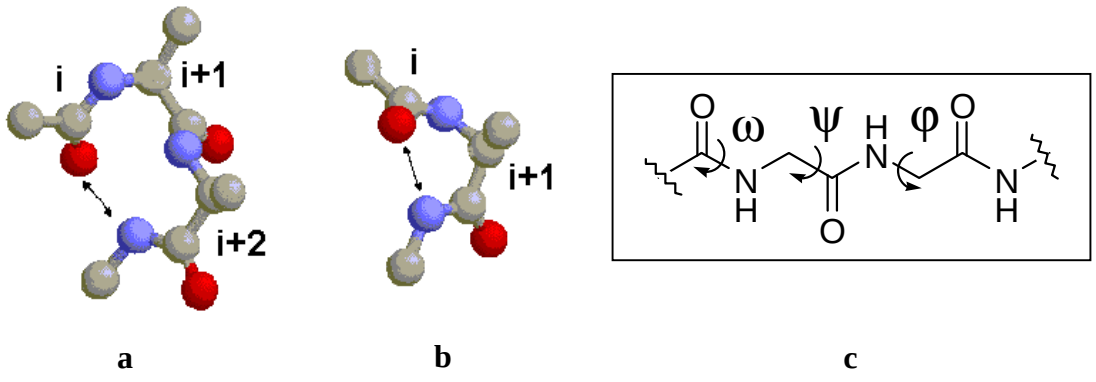
Chapter 2

2.1 Introduction

The three-dimensional structure of peptides and proteins is governed by several factors, among them, non-covalent forces such as hydrogen bonding, hydrophobic interactions, electrostatic interactions and van der Waals forces. Hydrogen bonding is one of the most important interactions related to protein folding. Although the energy associated to a hydrogen-bond is small compared to a covalent bond, a complex hydrogen-bonding network can have a great influence on protein structure.[30a]

The formation of secondary structural elements is mainly guided by hydrogen-bonding patterns. α-Helices are constituted of repeating $i \leftarrow i+4$ hydrogen bonds (the residue i acts as hydrogen bond donor involving its amide N-H, the residue $i+4$ acts as hydrogen bond acceptor involving its amide C=O). β-Sheets are stabilised by hydrogen bonds between two strands which can be aligned in the same (parallel sheets) or in opposite (antiparallel) N→C direction. These two types of secondary structure are regular and consist of a repetitive motif. There are other regular but not repetitive secondary structures: β- and γ-turns, in which the polypeptide chain reverses its overall direction (therefore they are also called reverse-turns) allowing for example the antiparallel β-sheet formation. Reverse turns play an important role in molecular recognition: in proteins the interaction with other molecules takes place on surfaces densely populated by turns which can expose and orient the pharmacophore to the receptor.[31a-c]

The three-dimensional arrangement of the polypeptide chain stabilised by a $i \leftarrow i+3$ hydrogen bond (forming a ten membered ring, also designated C_{10}) is called β-turn (Figure 1a).



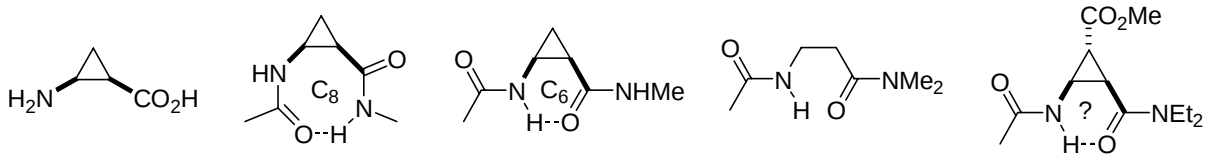
a **b** **c**

Figure 1. a: type I β-turn. **b**: direct γ-turn. **c**: Greek letters corresponding to dihedral angles.

There are also β-turns in which no hydrogen-bond occurs to hold together the chain direction inversion, so called "open reverse turn". A sequence of β-turns regularly following each other forms a 3_{10} helix.[31a] There are many different types of β-turns (the most common are type I, II and III right handed and type I', II', III' left handed, see Figure 4, Introduction) characterised by specific values of the dihedral angles φ and ψ (Figure 1c). γ-Turns, on the other hand, involve only three amino acids instead of four, and are stabilised by a *i*←*i*+2 hydrogen bond (Figure 1b).

The importance of turns for the structure of proteins and their biological activity has increased the interest to discover possibilities to induce their formation.

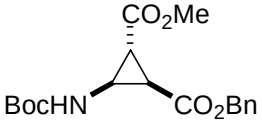
Among many others, the cyclopropane building block is supposed to be able to force turns formation.[11a-d] The potential structure inducing effect of β-ACC **9** has been already investigated by Molecular Modelling by *Rao et al.*.[19d] The N-acetyl methyl amide derivative **47** was found to have a global minimum stabilised by a 8-membered hydrogen-bonded ring (C_8, Figure 2), while a local minimum (Figure 2) is stabilised by a hydrogen-bonded 6-membered ring (C_6) and is 1.2 kcal/mol less stable than the C_8 conformation.



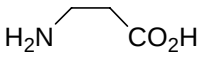
9 **47** **47** **48** **34**

Figure 2. Hydrogen bonding patterns. C_6 and C_8: six- or eight- membered ring closed by a hydrogen bond .

Unfortunately it is not yet possible to synthesise derivatives of **9** such as **47**, but its close analogue (±)-**11** has been already successfully built into peptide sequences.[21c] The β-ACC (±)-**11**, as well as **9**, can be regarded as a conformationally restricted β-alanine **5** (Figure 3) in which the folded conformation (Figure 9, Introduction) is blocked.



(±)-**11**



β-alanine **5**

Figure 3. β-Alanine (**5**) and β-ACC ((±)-**11**).

The C_6 hydrogen-bonded conformation is, on the other hand, not significantly populated in the analogue derivative of β-alanine **48** (Figure 2).[16b]

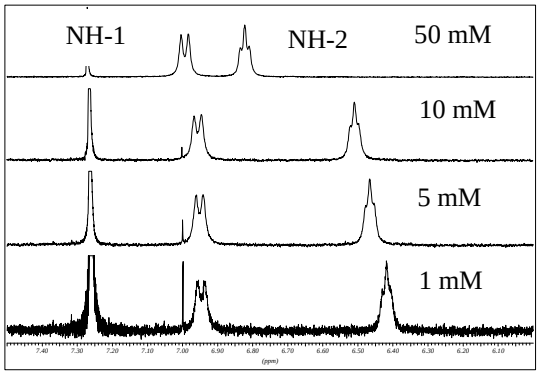
The preferred conformations of **11** derivatives were investigated starting from (±)-**34** (Figure 2) as model compound having only one possible hydrogen-bond. The results allowed to verify the molecular modelling calculation performed on **47** and to compare the IR results with those obtained on **48**.

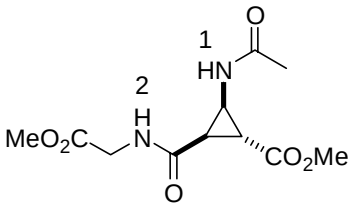
2.2 IR spectroscopy

In spite of some secondary structures lacking of hydrogen-bonding stabilisation[31a-c] hydrogen bond detection remains a powerful tool to investigate the structure of peptides and proteins. IR spectroscopy is particularly useful to examine intramolecular hydrogen-bonded and non hydrogen-bonded states. The equilibration between these two states is slow on the IR time scale, therefore distinct bands for hydrogen-bonded and non-hydrogen-bonded NHs can be observed. In a non polar solvent non hydrogen-bonded NHs absorb usually above 3400 cm^{-1}, while the hydrogen-bonded ones absorb below 3400 cm^{-1}.[32a-b]

The most suitable solvents to perform IR investigation in the amide A region (3500-3200 cm^{-1}) are CH_2Cl_2 and $CHCl_3$. These solvents have relatively low polarities (ε: 9.1 and 4.8 at r.t., respectively) and do not interact and/or disrupt hydrogen bonds. Acetonitrile is significantly more polar than either of the chlorocarbons, moreover the nitrile group is a moderate hydrogen-bond acceptor.[32b] DMSO, finally, is a powerful hydrogen-bond acceptor, therefore only the strongest intramolecular hydrogen-bonds persist in its solutions.

The concentration of the compounds under investigation is another factor to take into account. At high concentration, in fact, *inter*molecular hydrogen bonds can occur, therefore a calibration is necessary to determine the suitable concentration for the detection of *intra*molecular hydrogen-bonds. The calibration was performed by NMR spectroscopy on (±)-**35a** at 1 mM, 5 mM, 10 mM and 50 mM concentrations in $CDCl_3$ (Figure 4). The resonance of the NH protons is sensitive to hydrogen bonding: a proton resonates at lower field when hydrogen-bonded compared to its non bonded state. At 1 mM, 5 mM and 10 mM concentrations the resonance of the two NHs remains almost the same, while at 50 mM concentration NH-2 (the number following a functional group symbol identifies the residue counted starting from the N-*terminus*) shifts by 0.4 ppm down field due to the formation of a new intermolecular hydrogen bond. Interestingly NH-1 does not shift also at 50 mM concentration suggesting to be already intramolecularly hydrogen-bonded.

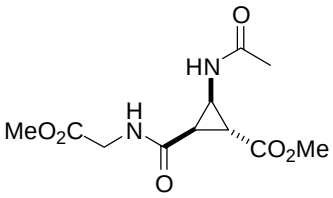




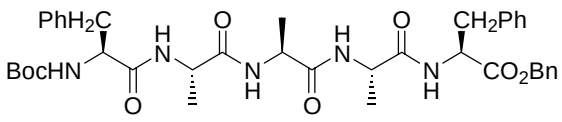
(±)-**35a**

Figure 4. NH resonances for (±)-**35a** at various concentrations in $CDCl_3$ (400 MHz).

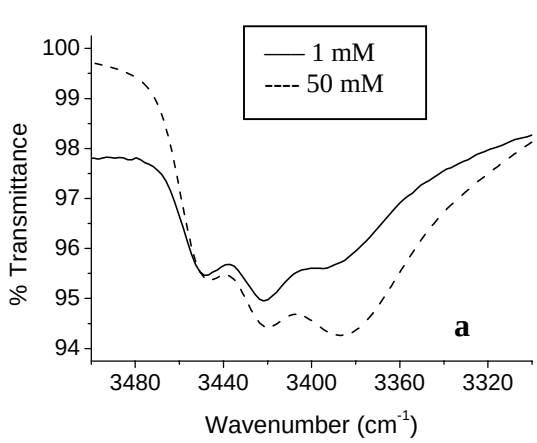
Further calibration experiments were done by means of IR spectroscopy on (±)-**35a** (Figure 5a) to confirm the NMR results and on **49** (Figure 5b) to evaluate the same problem on longer peptides.



35a



49



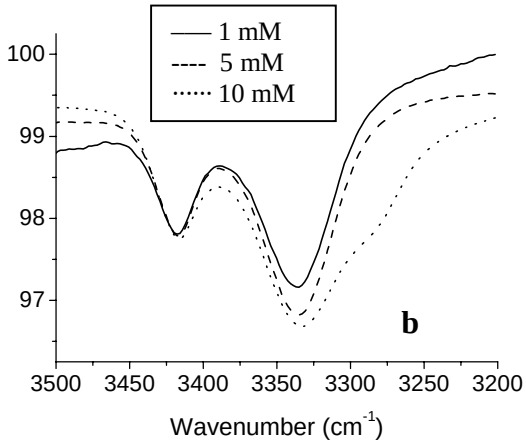


Figure 5. a: N-H stretching region FT-IR spectra for (±)-**35a** at 1 mM and 50 mM concentration at r.t. in CH_2Cl_2. **b**: N-H stretching region FT-IR spectra for **49** at 1 mM, 5 mM and 10 mM concentration at r.t. in CH_2Cl_2.

As already demonstrated by NMR spectroscopy, (±)-**35a** experiences more extensive hydrogen bonding at 50 mM than at 1mM concentration, on the other hand the pentapeptide **49** displays a significant enhancement of the hydrogen-bonding extent above 5 mM concentration. Nevertheless, it has been demonstrated that 1 mM concentration is suitable to detect *intra*molecular hydrogen-bonds without *inter*molecular contribution.

2.2.1 N-acetyl β-ACC derivatives

The carbamate and N-acetyl derivatives of β-ACC (±)-**11** were first analysed (Table 1) and the IR data were combined with molecular modelling calculation to get a first insight on the potential hydrogen bonding pattern. A *conformational search* was performed with the program Titan,[33a] in which the methods developed by Osawa and Montecarlo[33b,34] have been merged into a single simulated-annealing algorithm. The force field used was MMFF94 (Merck Molecular Force Field) parameterised especially for organic molecules and biopolymers.[33a,c] For each calculation the peptide bond dihedral angles were constrained to 180°. Only the minima occurring within 3 kcal/mol from the first minimum were taken into account. Hydrogen bonds were attributed for H--O distances shorter than 2.5 Å and N-H--O angles larger than 110°.[10a-b] The calculations were performed on (-)-**11** as well as on (+)-**11** derivatives, but when the hydrogen bonding pattern resulted to be the same, only one enantiomer/diastereomer is depicted in the following discussion. It should be stressed, however, that such molecular modelling calculations were performed in vacuum, therefore they do not take into account the solvent contribution, moreover the gain in energy due to the hydrogen bonds is probably overestimated.

Often more than one hydrogen-bonding pattern is possible in the molecules investigated. The discussion on each possibility should therefore take into account, besides the experimental data, two other factors: the aptitude of the hydrogen-bond acceptor (tertiary amides > secondary amides > esters ≥ carbamates > ketones)[35a-e] and the N-H--O angle which should be larger than 100° and ideally as close to 180° as possible.[30a-b]

The β-ACC (±)-**11** itself as well as (±)-**17** does not exist in any hydrogen-bonded conformation (N-H stretching occurring at 3422 and 3415 cm^{-1} respectively). In this case two factors contribute to disfavour the formation of the hydrogen bond: the mild hydrogen-bond acceptor capability of the ester carbonyl and the constraint N-H--O geometry (around 120°, Figure 7 for compound (±)-**34**). As soon as a keto function is introduced (compound (±)-**50**) affording a better hydrogen-bond geometry, about 50 % hydrogen-bond is observed

(3344 cm^{-1} absorption). The keto group is a weaker hydrogen bond acceptor than the ester but in this case the N-H--O angle is 150°, significantly close to linearity (Figure 6).

To confirm this hypothesis, molecular dynamics calculations were performed. The first minimum calculated on (+)-**50** uses the keto group as hydrogen-bond acceptor. The hydrogen bond closes the conformation in a nine membered (C_9) ring (Figure 6).

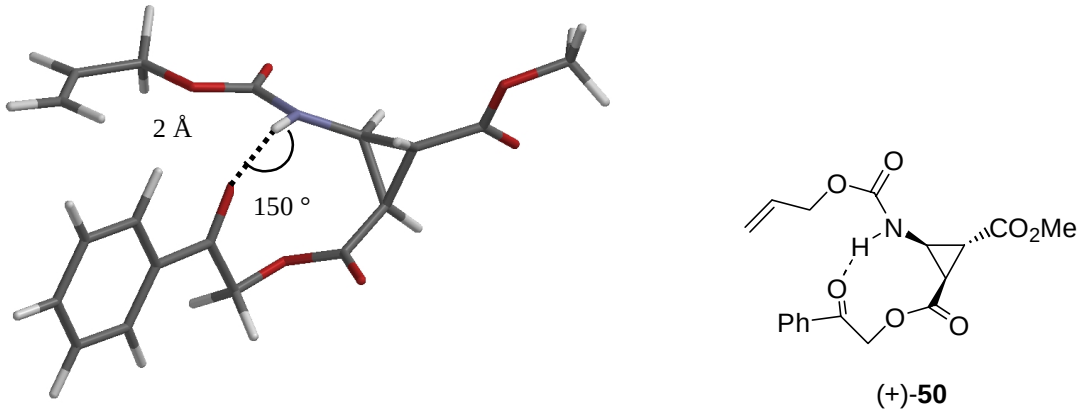


Figure 6. Lowest energy conformation for (+)-**50**.

To enhance the hydrogen donor and acceptor ability of the amino and carboxy functionalities respectively, the acetylamino diethyl amide β-ACC (±)-**34** (Figure 7) was investigated, being in close analogy to the β-alanine derivative **48** (Figure 2). The IR spectrum of **48** (1 mM in CH_2Cl_2) displays only one absorption at 3440 cm^{-1} at room temperature and at 3436 cm^{-1} at 205 K, **48**, therefore, does not experience any hydrogen-bond, it exists prevalently in the extended conformation.[16b] Compound (±)-**34**, in contrast, exists predominantly in the hydrogen-bonded conformation (main N-H absorption at 3364 cm^{-1} and only a shoulder at 3423 cm^{-1}).

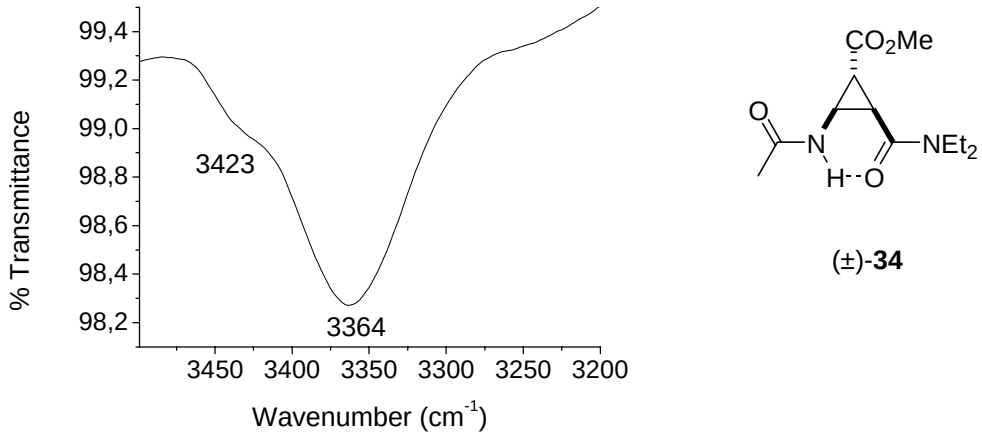


Figure 7. N-H stretching region FT-IR spectrum for (±)-**34** (1 mM in CH_2Cl_2 at r.t.).

In this case the strong hydrogen-bond acceptor capability of a tertiary amide carbonyl overcomes the not optimal N-H--O geometry. This result was confirmed by molecular modelling calculations suggesting that the most populated conformation is the hydrogen-bonded C_6 shown in Figure 8.

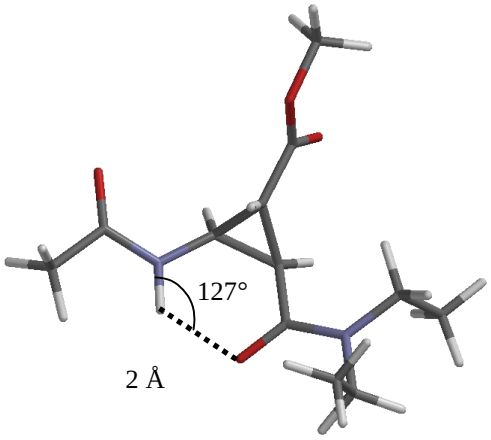


Figure 8. Conformational minimum for (+)-**34**.

Surprisingly the C_6 conformation is no longer favoured in the solid state. In the X-ray structure of (±)-**34** no *intra*molecular hydrogen-bond is found (Figure 9, left) but rather an *inter*molecular one, connecting the NH of a molecule with the sp^2 oxygen of the amide function of another symmetry related molecule leading to the formation of dimers (Figure 9, middle). The dimers crystall packing is shown in Figure 9, right side.

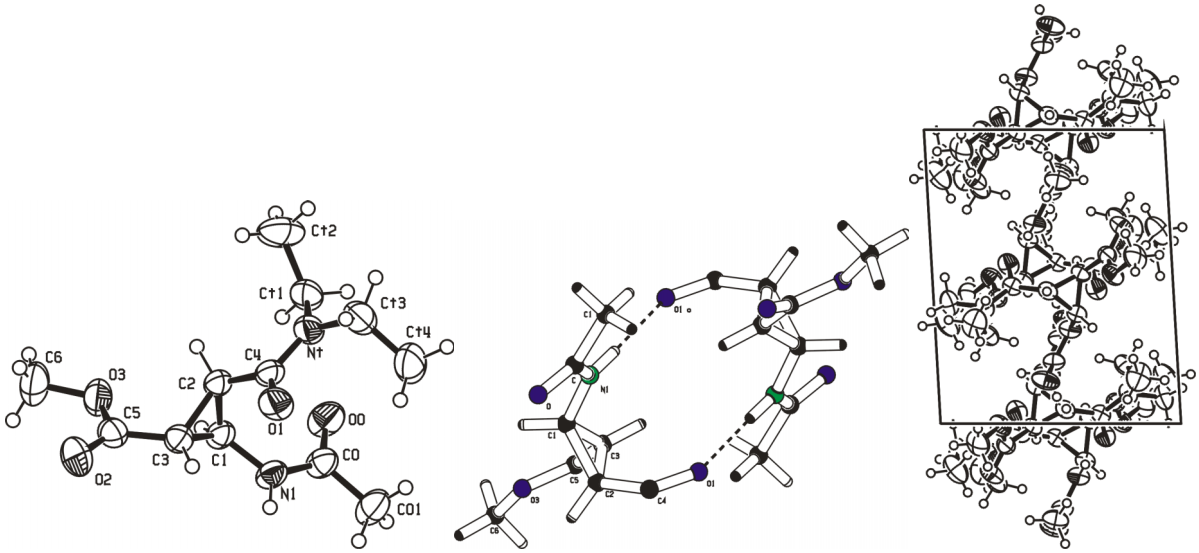


Figure 9. Left: crystall structure of (±)-**34**. Middle: Hydrogen-bonded dimer. Right: Crystall cell.

The glycine derivative (±)-**35a** shows three different absorptions in the N-H stretching region (3385, 3420 and 3428 cm^{-1}), two corresponding to non hydrogen-bonded and one to hydrogen-bonded values (Table 1). Since (±)-**35a** displays three absorptions of the same intensity but has only two NHs, it is clear that hydrogen-bonded conformations exchange with linear, non hydrogen-bonded ones.

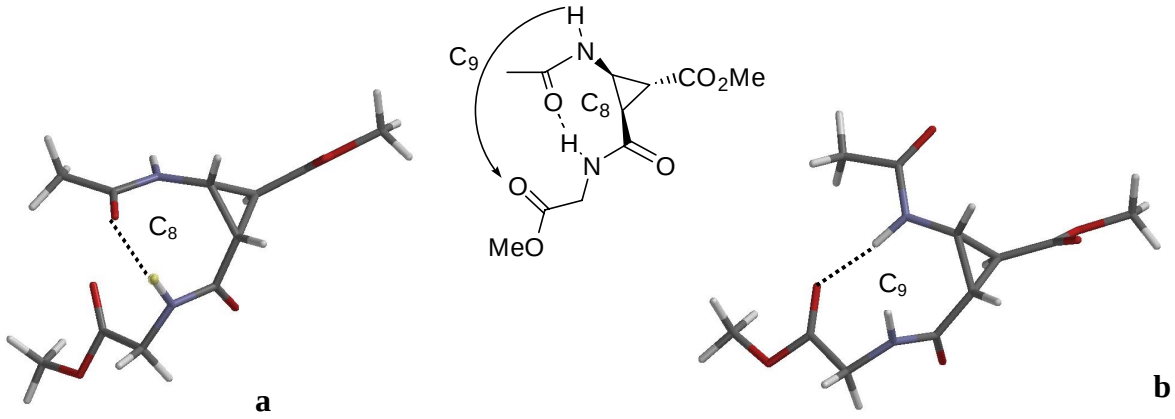
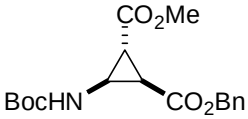
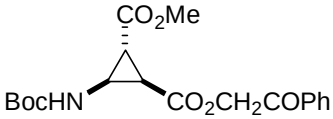
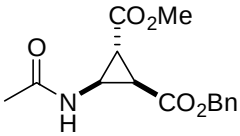
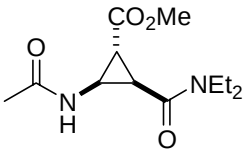
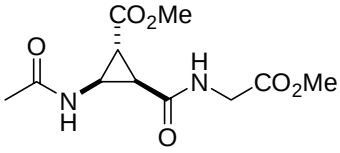
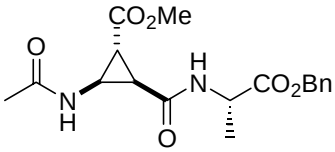
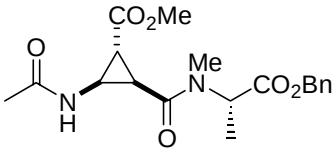


Figure 10. (+)-**35a**: (**a**) First energy minimum, C_8-hydrogen bonded ring. (**b**) Second energy minimum, C_9-hydrogen-bonded ring.

The energy minima for (+)-**35a** were investigated by a conformational search. The lowest minimum (E_{rel} = 0.0 kcal/mol) found contains a C_8-hydrogen bonded ring (Figure 10a), the second minimum (E_{rel} = 1.49 kcal/mol) has a C_9-hydrogen-bonded ring (Figure 10b) where the hydrogen acceptor function is the ester carbonyl.

In the alanine derivative (±)-**35b** two N-H absorptions of almost the same intensity can be observed at 3416 and 3383 cm^{-1}. It seems as if there is at least one hydrogen bond in each conformation equilibrating at r.t. in CH_2Cl_2. A conformational search on (+)-**35b** resulted in one predominantly populated C_8-conformation (E_{rel} = 0.0 kcal/mol) like the one shown for (+)-**35a** in Figure 10a and a C_6 as second, but not significant, minimum (E_{rel} = 2.51 kcal/mol).

Table 1. IR absorption in the amide A region. HB: hydrogen bonded.

		non HB N-H (cm^{-1})	**HB N-H** (cm^{-1})
	(±)-**11**	3422	
	(±)-**50**	3415	3344
	(±)-**24**	3415	
	(±)-**34**	3423 (shoulder)	3364
	(±)-**35a**	3428 3420	3385
	(±)-**35b**	3416	3383
	(±)-**35c**		3373

The N-methyl alanine derivative (±)-**35c** has only one absorption in the amide A IR region at 3373 cm^{-1}. The only N-H on (±)-**35c** should be therefore hydrogen-bonded. A tertiary amide carbonyl as hydrogen-bond acceptor is in fact a very good partner for hydrogen bonding.

Two possible hydrogen-bonding patterns were found for (+)-**35c** by a conformational search: the first minimum is a C_6 conformation (E_{rel} = 0.0 kcal/mol); the second minimum (E_{rel} = 0.13 kcal/mol) has an unusual hydrogen bond between the NH-1 and the π electrons of the phenyl ring (Figure 11). Hydrogen bonds to π-acceptors have already been widely investigated.[36a-g]

In proteins, on the other hand, they are rare since they are half as strong as a normal N-H--O=C hydrogen bond,[36h-i] therefore this second minimum should be considered with caution.

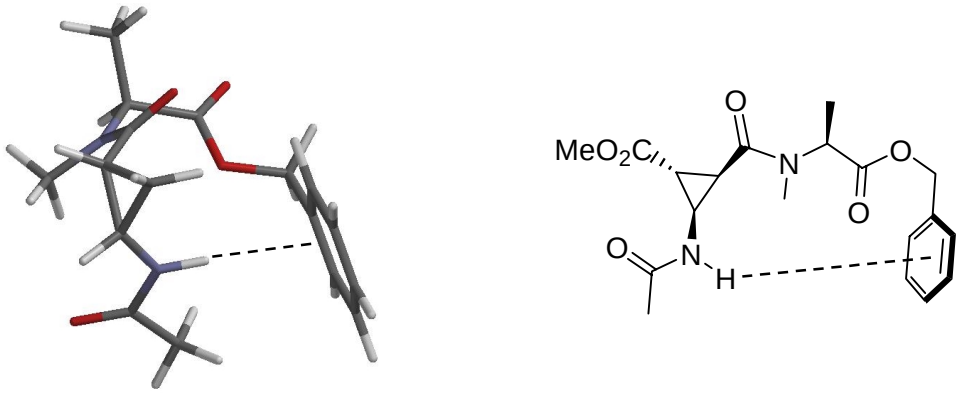


Figure 11. (+)-**35c**: Second energy minimum. For reasons of clarity the CO_2Me group on the cyclopropane ring is not displayed.

2.2.2 NMR: chemical shifts variations

The disadvantage of investigating the hydrogen-bonding pattern of molecules by IR spectroscopy is that there is no chance to know exactly which NH is the one involved in a hydrogen-bond and which is not, in contrast of NMR-techniques. Unfortunately, if a hydrogen atom is involved in a hydrogen-bond only in some of the conformations equilibrating in solutions, the corresponding resonance is a population-weighted average of the equilibrating states. In fact, the equilibrium between hydrogen-bonded and non hydrogen-bonded states is rapid on the NMR time scale.[10b]

For the N-acetyl β-ACC derivatives various hydrogen-bonded conformations were found to be possible, C_6, C_8, C_9. The C_6 seems to be favoured only in (±)-**34** and (±)-**35c**. When the bulkiness of the substituents at the N- and C-*termini* of the β-ACC ring and the length of the peptide increases, thereby allowing more hydrogen-bonding patterns, C_8 and C_9 conformations seem to be preferred over C_6 ones.

Besides the variation of the NHs chemical shift with the concentration (Figure 4), another NMR method to identify amide NHs engaged in hydrogen-bonds is the variation of the chemical shift with temperature and the H/D exchange rate.[7a] In both cases it is assumed that a hydrogen-bonded proton reacts slower than a non-hydrogen-bonded one to any perturbation affecting the NH bond. The effect of temperature on the NH chemical shift has been extensively investigated on proteins and peptides in H_2O and DMSO-d_6. Chemical shift temperature dependence (expressed in terms

of *reduced temperature coefficients* $\Delta\delta/\Delta T$) has been more rarely measured in non protic and relatively non polar solvents the such as CD_2Cl_2 and $CDCl_3$.[16b,37a-c] Temperature enhancement in these solvents will result in a large chemical shift change for NHs involved in hydrogen-bonds, which are no longer stable at higher kinetic energy values. Hydrogen-bonded NHs display a little chemical shift change when their environment remains constant against temperature (i.e. the hydrogen bond is not disrupted) and the same happens to completely solvent exposed NHs. There is an exception to this last situation: small $\Delta\delta/\Delta T$ values can be observed also in amide protons equilibrating between hydrogen-bonded and non-hydrogen-bonded states when the enthalpy difference between them is small.[32a] Conformation exchange unfortunately makes the interpretation of the results obtained difficult, because the NHs involved in a hydrogen bond could be different in different conformations. The H/D exchange experiment compares the time requested by different NHs in a molecule to disappear from a ^1H NMR spectrum in deuterated solvents (commonly D_2O or CD_3OD).[31a] In fact hydrogen-bonded NHs exchange their proton with deuterium slower than solvent exposed ones. When the hydrogen-bonds are not strong enough to survive in methanol (protic solvent, high polar) the same result can be obtained in an aprotic, apolar solvent such as CD_2Cl_2 or $CDCl_3$ by addition of a small quantity of CD_3OD.[37c]
The $\Delta\delta/\Delta T$ values for (±)-**34** and (±)-**35a** were measured in $CDCl_3$ at 1 mM concentration between 263 and 318 K (Figure 12).

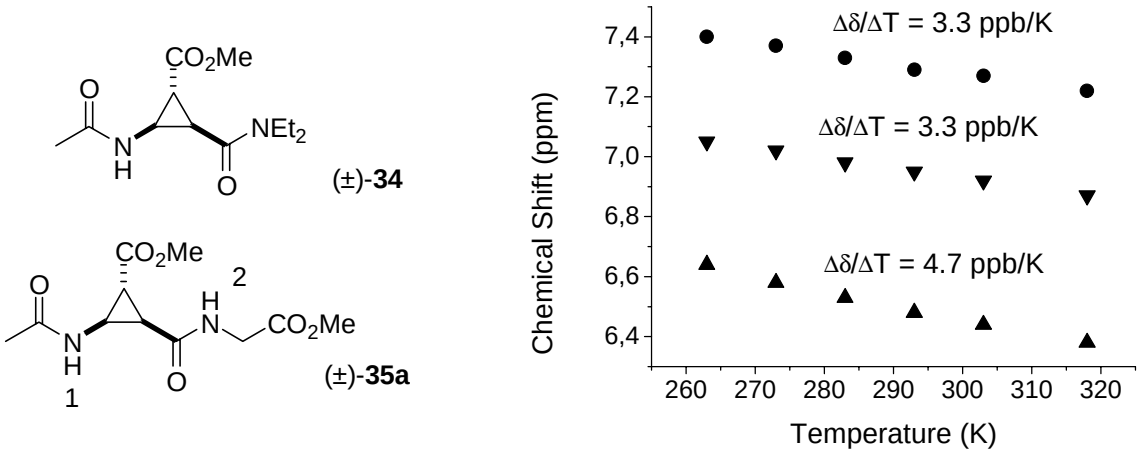


Figure 12. Chemical shift temperature dependence (in $CDCl_3$): • (±)-**34** N-H; ▼ (±)-**35a** NH-1; ▲ (±) **35a** NH-2.

The variation of the NH chemical shift with the temperature is linear in all cases. For (±)-**34** a $\Delta\delta/\Delta T$ value of 3.3 ppb/K corresponds to a hydrogen bonded NH (as demonstrate the NH absorption in the IR spectrum) stable in this range of temperatures. The coefficients found for the two NHs of (±)-**35a** are 3.3 ppb/K (NH-1) and 4.7 ppb/K (NH-2). The first value would be

compatible with both strong hydrogen-bonded and non hydrogen-bonded states, while the second value is indicative for a relative labile hydrogen-bond.

The H/D exchange for (±)-**35a** was measured in 0.2 M CD_3OD in $CDCl_3$ (Figure 13), but in this solvent mixture after 60 h both NHs experienced 64 % deuterium exchange. Then the solvent mixture was brought to 0.4 M CD_3OD and after 21 h the NH-2 signal disappeared while NH-1 exchanged 83 % of its proton with deuterium.

Finally, another factor suggests, for (±)-**35a**, NH-1 rather than NH-2 to be involved in a hydrogen-bond: NH-1 resonates at lower field than the NH of (±)-**17** (7.02 and 6.64 ppm respectively) which is definitely non-hydrogen-bonded. The chemical shift of hydrogen-bonded protons is, in general, higher than that of non-hydrogen-bonded ones.

NMR data would, therefore, support the hypothesis that (±)-**35a** exchanges between a C_9 hydrogen-bonded (involving NH-1) and a linear, non-hydrogen-bonded, conformation.

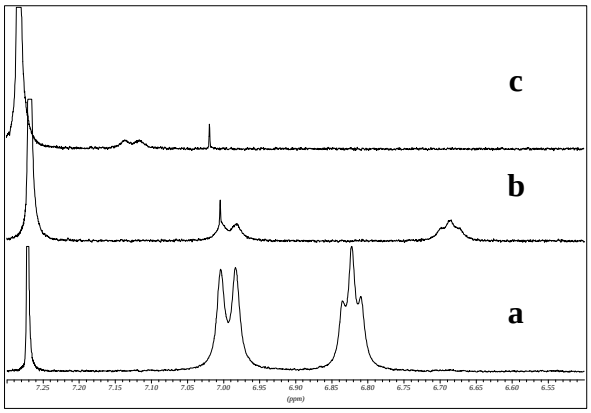


Figure 13. (±)-**35a** H/D exchange. (**a**) $CDCl_3$. (**b**) After 60 h in CD_3OD 0.2 M in $CDCl_3$. (**c**) After 60 h in CD_3OD 0.2 M in $CDCl_3$ and 21 h in CD_3OD 0.4 M in $CDCl_3$.

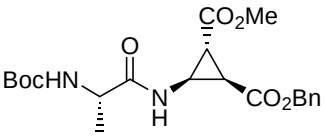
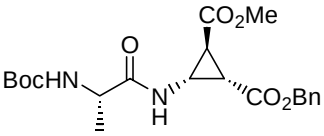
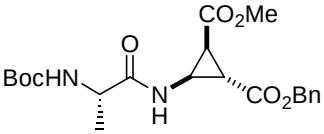
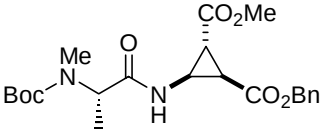
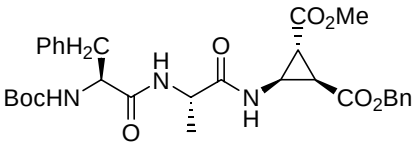
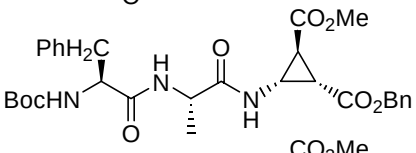
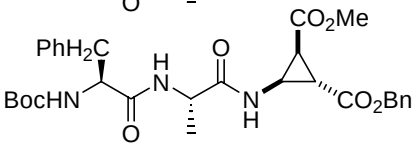
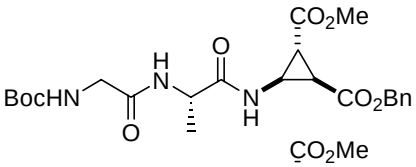
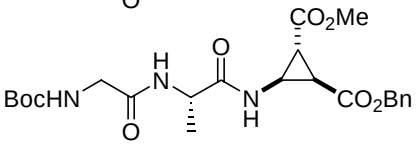
No additional informations could be obtained by NMR spectroscopy for the conformational preferences of (±)-**35b**, since the NH signals are hidden under the aromatic CH ones. The C_8 conformation (for (+)-**35a**, Figure 10) remains nevertheless most probable as suggested by the conformational search. Compound (±)-**35c** has only one NH group, therefore, NMR experiments would be not indicative for the elaboration of its conformation.

2.2.3 Oligopeptides containing β-ACC as the C-terminal amino acid

Several peptides were synthesised to gain further insight into the structural influence of the *β*-ACC building block at the C-*terminus* (Table 2).

The dipeptides **25a** and **25b** show about 50% hydrogen-bonding, while **25c** has only one absorption at 3391 cm^{-1}, its conformations are therefore all hydrogen-bonded.

Table 2. Derivatives of the *β*-ACC **11** and **12** at the N-*terminus*.

		non HB N-H (cm^{-1})	HB N-H (cm^{-1})
	(+)-**25a**	3430	3366
	(-)-**25a**	3431	3365
	(±)-**25b**	3429	3392
	(±)-**25c**		3391
	(+)-**27a**	3415	3342 (shoulder)
	(-)-**27a**	3417	3343 (shoulder)
	(±)-**28**	3416	3350 (shoulder)
	(+)-**28b**	3415	
	(-)-**28b**	3416	

Molecular modelling conformational searches were carried out in support of the experimental data obtained. The first minimum for (+)-**25a** and (+)-**25c** resulted a C_7-hydrogen-bonded ring corresponding to a *γ*-turn centred on the alanine residue (Figure 14a). The following minima found had higher energy values and often involved the sp^3 rather than the sp^2 oxygen atom of the ester group (an example is given in Figure 14b for (+)-**25a**). Usually esters, amides and carbamates act as hydrogen-bond acceptors at the sp^2 oxygen atom, therefore these higher energy minima should be carefully regarded.[35e]

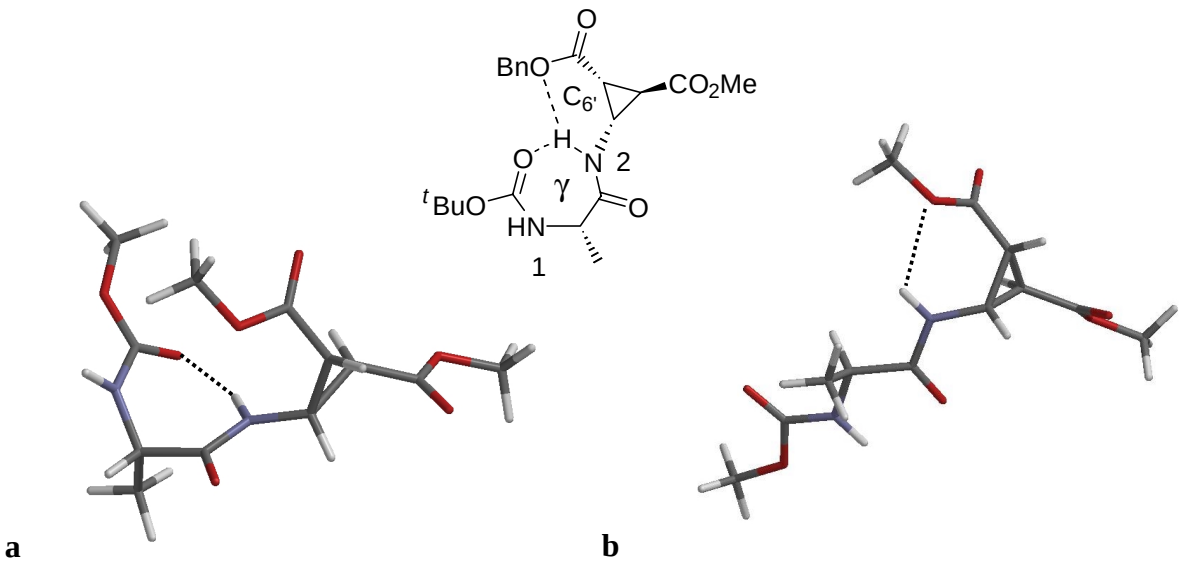


Figure 14. (+)-**25a**: (**a**) Global energy minimum, γ-turn (E_{rel} = 0.0 kcal/mol). (**b**) Local energy minimum (E_{rel} = 1.82 kcal/mol), type $C_{6'}$ hydrogen-bonded ring. For reasons of clarity the Boc and phenyl groups are not displayed.

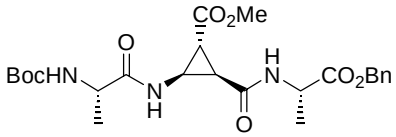
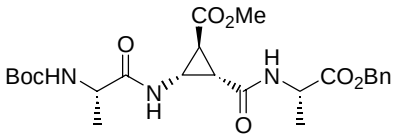
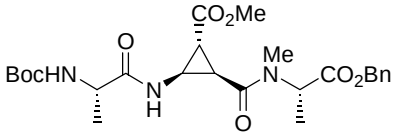
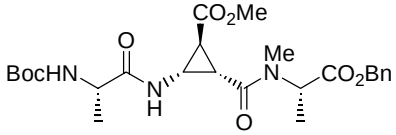
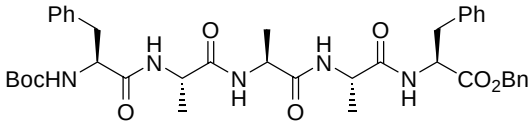
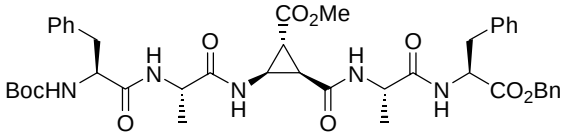
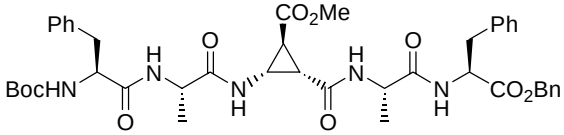
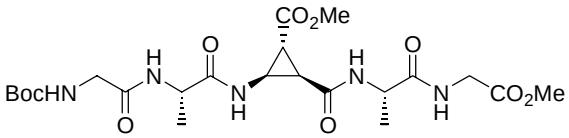
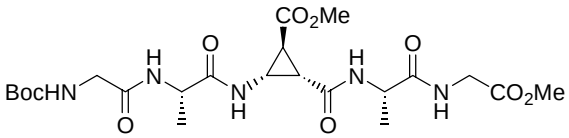
The conformational minima calculated for both **25b** isomers suggest, in spite of (+)-**25a**, the C_6 (E_{rel} = 0.0 kcal/mol) rather than the γ-turn conformation (E_{rel} = 0.84 kcal/mol) to be favourite.

The tripeptides (+)-**27a**, (-)-**27a** and (±)-**28** show only a shoulder corresponding to hydrogen-bonded NH. A linear, non hydrogen-bonded conformation is probably predominant. In the absence of any hydrogen-bond, the linear conformation is the only one possible for both **27b** isomers.

2.2.4 Oligopeptides containing the β-ACC as the central residue

More complex derivatives having the *β*-ACC building block in the middle are shown in Table 3. Compound **49** is the reference pentapeptide containing only natural amino acids. For these more complex products molecular modelling calculations led to a large number of possible conformations. NMR investigations on some of these products will be illustrated in Chapter 4.

Table 3. Tri- and penta- peptides containing the β-ACC as the central unit.

		non HB N-H (cm^{-1})	HB N-H (cm^{-1})	non HB/HB (at r.t.)
	(+)-**36a**	3426	3367	2:1
	(-)-**36a**	3426	3367	2:1
	(+)-**36b**	3426	3354	1:1
	(-)-**36b**	3426	3354	1:1
	**49**	3416	3335	1:4
	(+)-**37a**	3412	3309	2:3
	(-)-**37a**	3420	3364	2:5
	(+)-**37b**	3415	3334	2:3
	(-)-**37b**	3419	3361	>3:2

The ratio between the hydrogen-bonded (HB) and the non hydrogen-bonded (non HB) NHs (Table 3) was obtained by a gaussian fit based on a non linear simplex Nelder-Meade algorithm.[38] In Figure 15 the deconvolution of the IR amide A region is showed for (+)-**37a** and (-)-**37a**.

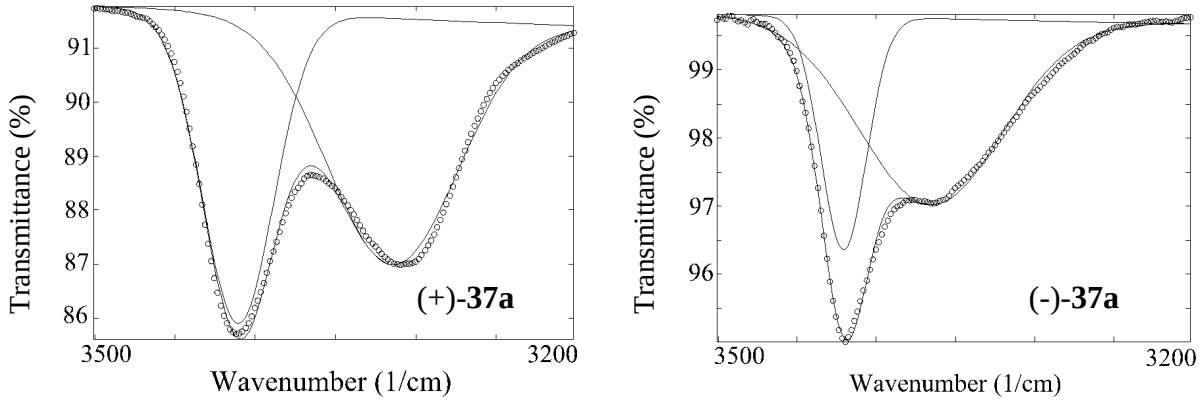


Figure 15. Deconvolution of the amide A IR region for (+)-**37a** (left) and for (-)-**37a** (right).

Compounds (+)-**36a** and (+)-**36b** have the same IR spectrum as the related diastereomers (-)-**36a** and (-)-**36b** respectively: the difference in chirality of the building block **11** produces no substantial effect on the hydrogen bonding extent of tripeptides. In contrast, for the pentapeptides **37**, it was observed that the *β*-ACC (-)-**11** promotes more extensive hydrogen-bonding than its enantiomer; the same effect as that of (-)-**11** was noted by replacement of *β*-ACC with L-alanine (compound **49**) in the same peptide sequence.

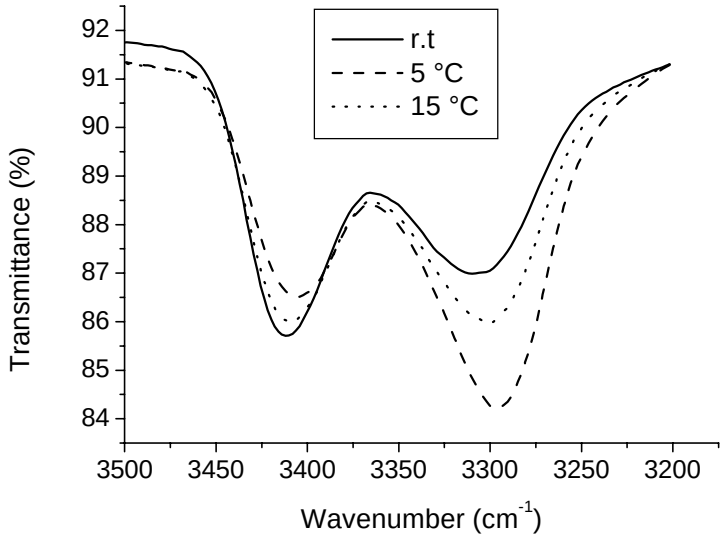


Figure 16. N-H stretching region FT-IR data for (+)-**37a** at 1 mM concentration in CH_2Cl_2 at r.t., 15 and 5 °C.

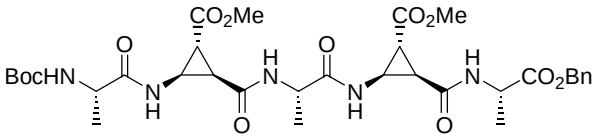
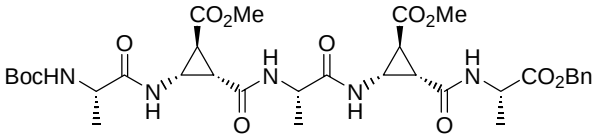
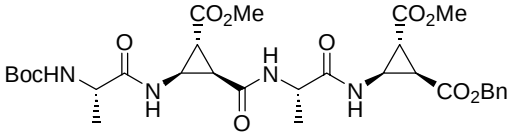
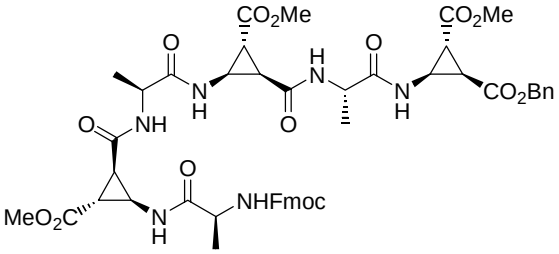
Interestingly (+)-**37a** is the only peptide in this series to display a temperature dependence of the NH hydrogen-bonding extent (Figure 16). At room temperature the non hydrogen-bonded NH absorption is more intense than the hydrogen-bonded one, at 15 °C they are of comparable intensity and at 5 °C the ratio is inverted. This suggests that (+)-**37a** folds into a

more hydrogen-bonded structure at lower temperatures. This feature was not observed in any other of the investigated compounds.

2.2.5 Oligopeptides containing multiple β-ACCs

Finally peptides containing two or three β-ACC building blocks were examined (Table 4).

Table 4. Penta- and hexa- peptides containing two or three β-ACC building blocks.

		non HB N-H (cm^{-1})	HB N-H (cm^{-1})	non HB/HB
	(+)-**40**	3421	3362	2:3
	(-)-**40**	3419	3350	1:4
	(+)-**41**	3418	3360	1:1
	(+)-**45**	3399	3301	1:5

As observed before, (+)-**11** promotes in (+)-**40** and (+)-**41** less hydrogen-bonding than (-)-**11** in (-)-**40**. In contrast, in (+)-**45**, the presence of three β-ACC (+)-**11** building blocks seems to exert a hydrogen-bonding enhancing effect. Five of the six NHs are hydrogen-bonded and the wavenumber of the sixth (3399 cm^{-1}) cannot be unambiguously attributed to a non hydrogen-bonded state. This feature suggests the presence of a regular secondary structure such as a helix, where each NH binds to the corresponding C=O at a definite residue distance.

2.3 IR spectra in acetonitrile

Acetonitrile is a suitable non protic solvent for circular dichroism (CD) spectroscopy, while CH_2Cl_2 and $CHCl_3$ are not. This is due to the self-absorption of the solvent which starts below 190 nm (acetonitrile), 205 nm (CH_2Cl_2) and 220 nm ($CHCl_3$). As it will be illustrated later on, it is very important to be able to measure the CD spectrum of a peptide below 200 nm.
Acetonitrile is a weak hydrogen-bond acceptor, therefore it could disrupt the hydrogen-bonds which resulted stable in CH_2Cl_2. In acetonitrile the solvent exposed NHs (engaged in a hydrogen-bond with the solvent) are expected to give a band between 3370 and 3400 cm^{-1},[12f] intramolecularly hydrogen-bonded NHs should absorb at the same wavelength as in CH_2Cl_2.
The main difficulty in the interpretation of IR spectra measured in acetonitrile has been the broadness of the NH signals which made difficult to guess how many peaks were really present and at which wavelength.

Table 5. Comparison between the NH absorptions in CH_2Cl_2 and acetonitrile.

	CH_2Cl_2 (cm^{-1})	**Acetonitrile** (cm^{-1})
(±)-**11**	3422	3409, 3376
(±)-**34**	3364	3368
(+)-**27a**	3417, 3343	3356
(+)-**36a**	3426, 3367	3362
(+)-**37a**	3412, 3309	3360

The first compound investigated was (±)-**11** (Table 5), which should serve as reference for the non hydrogen-bonded NHs in acetonitrile. Two peaks were observed at 3409 and 3376 cm^{-1}, the second absorption belonging to solvent hydrogen-bonded NH. Compound (±)-**34** gave, on the other hand, only one absorption at 3368 cm^{-1}. This value is only 4 cm^{-1} higher than in CH_2Cl_2, therefore it seems like (±)-**34** is keeping its C_6 conformation. On the other hand the absorption of (±)-**34** in acetonitrile is also at a border value between hydrogen-bonded and solvated NHs. Compound (+)-**27a** shows only one absorption at 3356 cm^{-1}, i.e. at lower values than normal ones for solvated NH in acetonitrile, nevertheless a higher hydrogen-bonding extent in acetonitrile than in dichlormethane does not seem reasonable. The same could be observed on (+)-**36a** which shows only one absorption at 3362 cm^{-1} and apparently

there are no more solvated NHs. Compound (+)-**37a** has only one broad absorption at 3360 cm^{-1}, 50 cm^{-1} higher than the hydrogen-bonded NH signal in CH_2Cl_2, probably the hydrogen-bonded NHs signal overlaps the non hydrogen-bonded one.

Acetonitrile does not seem to be the solvent of choice for hydrogen-bonding investigations on these peptides, in fact the solvated NH signal could coincide with the intramolecularly hydrogen-bonded one (they both show a band around 3360-3390 cm^{-1}).

2.4 Conclusions

The results obtained by IR spectroscopy on relatively small derivatives of the β-ACC (±)-**11** and (±)-**12** prove the capability of this building block to induce interesting hydrogen-bonding patterns, among them the C_6 and C_8 conformations which were already predicted by molecular modelling.[19d] The C_6 ring is thought to induce and stabilise β-turn-like conformations in α-peptides containing a β-amino acid.[38] Nevertheless, the C_6 conformation seems to be stabilised only by the presence of a tertiary amide as hydrogen-bond acceptor ((±)-**34** and (±)-**35c**), but becomes less favourable in longer peptides where better hydrogen-bonding geometries can be achieved in other conformations. Moreover, C_6 conformations seem to arrange the N- and C-*termini* into opposite directions in a sort of extended chain conformation (Figure 8) as a direct consequence of the dihedral angles requested to build the corresponding C_6 hydrogen-bond. This would disfavour the formation of turn-like conformations in the β-ACC ((±)-**11**) derivatives. On the contrary, a hydrogen-bonded C_8 ring would force the N- and C-*termini* close to each other, favouring a reverse turn like conformation. In dipeptides having the β-ACC unit at the C-*terminus*, a γ-turn conformation is preferred. Depending on its orientation, a γ-turn can allow the formation of other hydrogen-bonds supporting additional folding.

An influence of the β-ACC chirality on the hydrogen-bonding extent of its derivatives was observed only on pentapeptides. On (+)-**37a**, (+)-**37b** and (+)-**40**, (+)-**11** promotes less extensive hydrogen-bonding than (-)-**11** on (-)-**37a**, (-)-**37b** and (-)-**40**. In contrast, three (+)-**11** units are able to induce up to 80 % hydrogen-bonding.

Chapter 3

3.1 Circular Dichroism: an introduction

Circular dichroism (CD) spectroscopy is a powerful tool to detect the secondary structure of proteins and peptides.[31a-c,40] It is based on the property of asymmetric chromophores (or symmetric chromophores in asymmetric environments) to absorb differently right- and left-circular polarised light. Upon entrance into the absorbing sample, the two circular polarised components, R and L, of the plane-polarised light have the same intensity and phase. If one component has been absorbed to a grater degree than the other, the electric vector of the light describes no longer a circle, but an ellipse (Figure 1). When leaving the cell, the new elliptically polarised light is described by the angle θ, called ellipticity, which is proportional to the difference in absorption of the two original circular rays. CD spectra are commonly expressed in molar ellipticity (degree cm^2 $dmol^{-1}$) or mean residue ellipticity ($[\theta]_R$). In the latter case the molar ellipticity is divided by the number of residues forming the peptide, which allows direct comparison of the CD spectra of differently sized peptides.

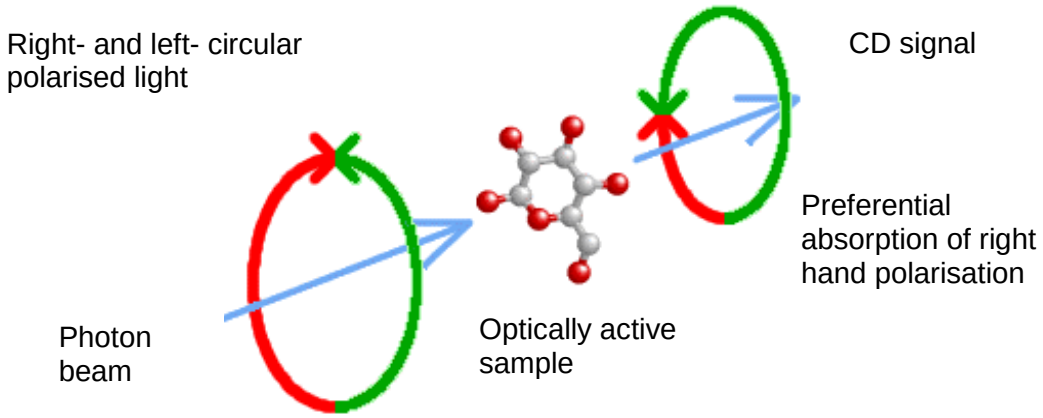


Figure 1. Origin of the CD signal.

The peptide bond is the main chromophore in peptides and proteins, besides the aromatic side chains. It is surrounded by an asymmetric environment due to the stereocentres of the amino acids but mainly due to the three-dimensional arrangement of the peptide backbone (φ and ψ dihedral angles, see Figure 1, Chapter 2). Therefore the CD absorption of the peptide bond is highly sensitive to the peptide secondary structure.

The range of frequencies where the peptide bond absorption can be observed is between 180 and 300 nm. The lowest energy transition of the peptide chromophore occurs between 210 and 220 nm and reflects the n→π* transition involving non-bonding electrons of the carbonyl group. The second transition is observed around 190 nm and describes the π→π* involving the π electrons of the carbonyl group. The intensity and energy of these transitions depend on the φ and ψ dihedral angles, thus relates to the secondary structure. In the case of a typical α-helix CD spectrum, the π→π* transition is split into two components, the parallel- and the perpendicular-polarised component with respect to the helical axis (Figure 2).

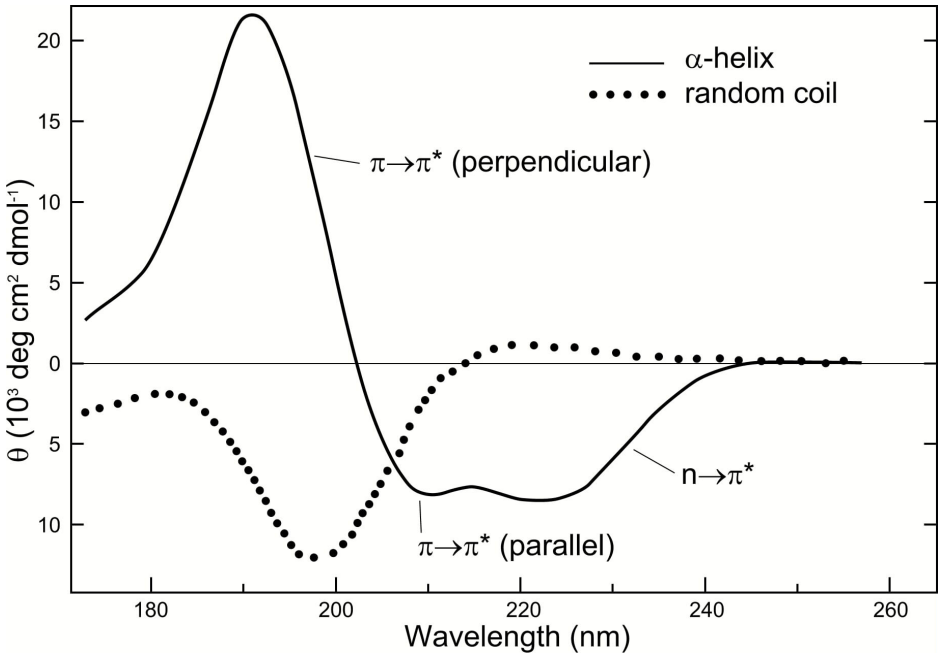


Figure 2. Typical α-helix and random coil CD spectra.

The CD spectrum is a weighed average of all conformers equilibrating in solution, therefore the typical CD pattern of a secondary structure can be observed only when the structure is reasonably populated. Nevertheless, the CD method is a fast application for the inspection of the conformational features of a peptide, whether any structural elements are present or not. Depending on the CD results, it can be interesting to further investigate the structure of a peptide with other spectroscopic methods.

3.1.1 CD and β-turns

The application of CD spectroscopy to the identification of peptides and proteins secondary structure is already well known.[31a-c,40] The main secondary structures (α-helices, β-sheets, 3_{10}–helices) have been extensively investigated and characterised. Turns have also been widely studied,[31c] but there are many difficulties to interpret their CD spectra. As already pointed out in the previous chapter, there are many types of turns, depending on the number of residues involved (β- and γ-turns) and on their three-dimensional arrangement (determined by the ψ and φ dihedral angles).

Table 1. Classification of β-turns CD spectra observed on linear peptides.[31c]

Class	Definition	Conformation
A	Negative band near 216 nm. Stroger positive band between 195 and 200 nm. Negative band near 175 nm.	β-sheet
B	Weak negative band between 220 and 230 nm. Stronger positive band between 200 and 210 nm. Strong negative band predicted between 180 and 190 nm.	β-turn type II
C	α-Helix like CD spectrum α-Helix like CD spectrum with low intensity, blue shifted bands	β-turns type I, II and II´ β-turns type I and III
C´	Positive shoulder above 220 nm. Positive band at 200 nm. Negative band below 190 nm.	L-a.a.-D-a.a. or L-a.a.-Gly sequence type II β-turns
D	Low intensity, redshifted class B spectrum	β-turns
U	Weak negative band or shoulder between 215 and 230 nm. Strong negative band near or below 200 nm.	aperiodic (unordered, random coil or irregular)

To achieve a pure turn CD spectrum, a short peptide is required that exists predominantly in only one turn type. Moreover, the CD spectrum can be unambiguously assigned to a specific turn type only when other data give strong evidence for it (X-ray, NMR, IR). Unfortunately,

short peptides are often a mixture of conformers equilibrating in solution, therefore a classification of CD spectra for each turn type can be achieved on cyclic peptides (where the conformational degrees of freedom are reduced) or on linear peptides containing strong turn-inducing sequences (i.e. one proline residue).[31c] Based on such investigation, typical CD patterns can be assigned to the different β-turns (Table 1). It should be stressed, however, that a particular CD pattern may correspond to more than one type of β-turn, and that the classification is achieved by statistical investigations, therefore a particular turn type may also have a not typical CD spectrum.

3.1.2 Choice of the solvent

Because of the $\pi \rightarrow \pi^*$ absorption frequency, the choice of the solvent is limited to those which absorb below 190 nm. Chloroform and dichlormethane are not suitable for CD spectroscopy because they absorb above 200 nm. This makes it difficult to correlate the informations obtained by IR spectroscopy in dichlormethane with those obtained by CD in other solvents. When IR spectra are measured in other solvents, only the C=O stretching absorption region gives informations on the peptide structure. However, in this case, detection of turns is difficult since no information on the hydrogen-bonding extent is obtainable.[41a-b]

Acetonitrile allows measurements above 195 nm in a 1 mm cell and above 190 nm in a 0.1 mm cell. It is a suitable non-protic solvent for CD measurements, but it is a weak hydrogen-bond acceptor, therefore it is not easy to correlate the hydrogen-bond extent detected in dichlormethane by IR spectroscopy with the CD observed in acetonitrile.

Suitable solvents, besides water, are alcohols such as methanol (absorption below 195 nm in a 1 mm cell and below 185 in a 0.1 mm cell) and particularly trifluoroethanol (TFE, absorption below 190 nm in a 1 mm cell and below 180 nm in a 0.1 mm cell). TFE is known to have a structure-stabilising effect, particularly on α-helices.[42] Compared to the non fluorinated analogue, TFE is 3-4 times more acidic, and therefore a good hydrogen-bond donor, but a poor hydrogen-bond acceptor (Figure 3). While water may disrupt hydrogen bonds and destabilise secondary structures, TFE, with its unique hydrogen-bonding properties, forms a bifurcated hydrogen-bond with the carbonyl oxygen without disrupting the intramolecular hydrogen-bond (Figure 3). The hydrogen-bonding network stabilising a secondary structure remains therefore intact in TFE.

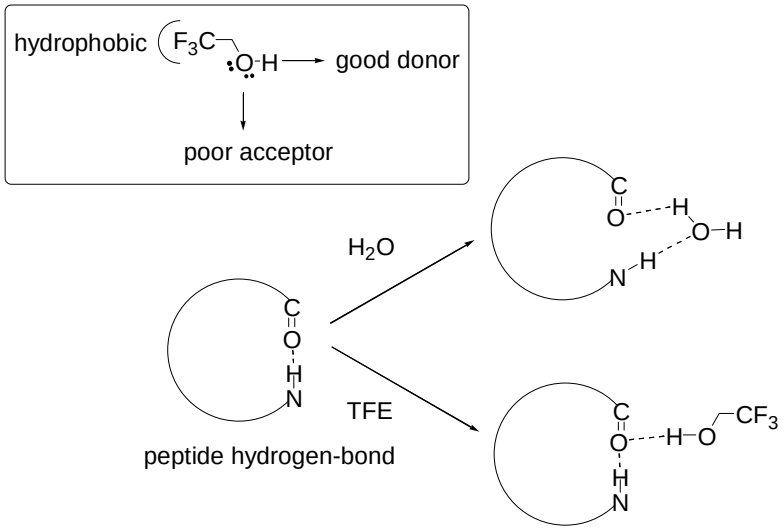


Figure 3. TFE: hydrogen-bonding ability and interaction mode with hydrogen-bonds in peptides compared to water.

Moreover, the high hydrophobicity of the CF_3 group allows TFE molecules to interact with hydrophobic side chains enhancing the hydrophobicity of the molecule which could favour a particular secondary structure.

Hexafluoroacetone (HFA) is also used in CD spectroscopy. The effect of this solvent on the peptide secondary structure is not clear, as it can cause loss of structure[43a-e] as well as induce and stabilise it.[43f-g] HFA is used in its hydrate form which has enhanced hydrogen-bond donor and poor acceptor ability at the oxygen. Moreover, the two trifluoromethyl groups contribute to the high hydrophobicity of this solvent and its water repelling properties (Figure 4, left). HFA in its hydrate form is thought to coat the peptide surface with the trifluoromethyl groups displacing the hydrogen-bond disrupting water molecules. On the other hand, its OH groups enhance the peptide solubility in water (Figure 4, right).

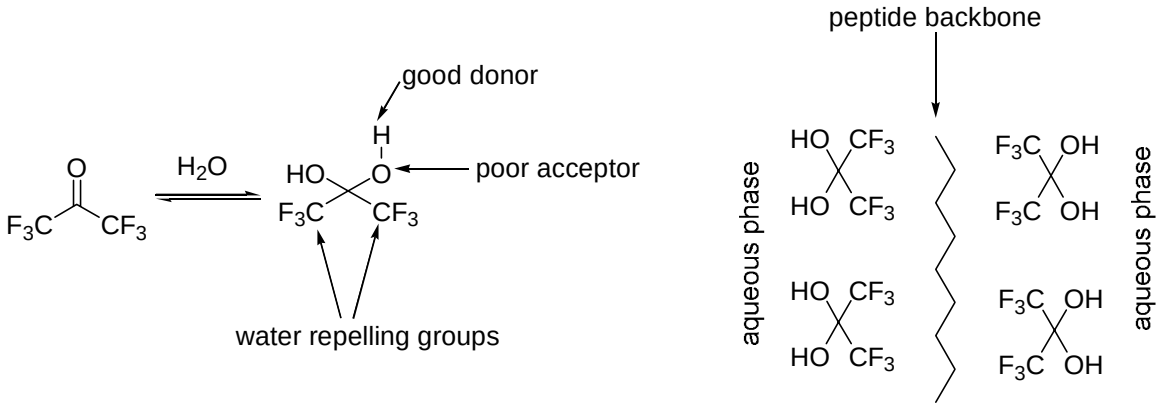


Figure 4. Left: HFA hydrogen-bonding properties. Right: mode of interaction with the peptide backbone resulting in structure stabilisation.

If the interaction with a peptide follows this scheme, HFA should stabilise its structure. On the other hand the peptide secondary structure could be broken if hexafluoroacetone builds stable hydrogen bonds with its backbone.[43b]

3.1.3 Sample concentration

The concentration of the sample, besides the choice of the solvent, is another determining factor to achieve a good CD spectrum. When the sample absorbs too much light in a certain UV region, the high tension (HT) voltage of the photomultiplier will be too high and the measurements are therefore not reliable. The optimal concentration to observe a good CD spectrum should not give HT values above 800 V. For the peptides investigated in the present work, a concentration around 10^{-3} M (in 0.1 mm cell) was found to give the best CD quality.

3.1.4 Contribution of aromatic groups

The UV spectra of the aromatic amino acids (phenylalanine, tyrosine and tryptophan) include bands in the near UV region (240-300 nm) as well as in the peptide bond region (185-240 nm). The absorption of the aromatic side chains at lower wavelengths can interfere with the secondary structure estimation if the chromophores are kept in an asymmetric environment produced by the secondary structure of the peptide.[31c] In fact aromatic side chains tend to give rise to positive absorptions in the 215-230 nm region.[44]

3.2 CD spectra of β-ACC containing peptides

In Figure 5 the CD spectrum of the building blocks (+)-**11** and (-)-**11** is shown. As expected, being enantiomers they display mirror image CD pattern having an intense maximum/minimum at 203 nm.

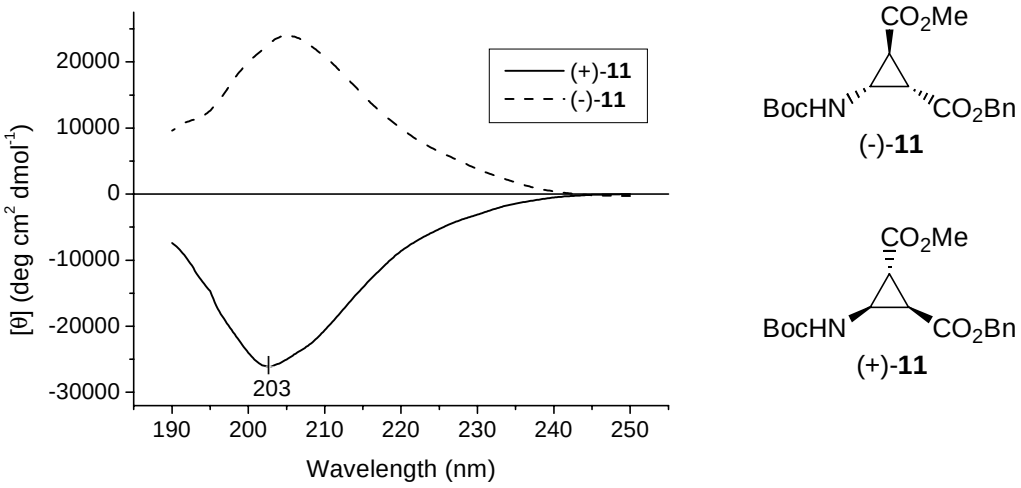


Figure 5. CD spectra of (+)- and (-)-**11** in methanol.

The CD spectra of (+)-**25a**, (-)-**25a**, (+)-**27b** and (-)-**27a** suggest that the peptides are structured (negative or positive absorption around 205 nm). Nevertheless, the strong intensity and the position of the band (similar to (+)-**11** and (-)-**11**), indicate a predominant effect of the chiroptical feature of the building block **11** (Figure 6).

The spectra of (+)-**25a** and (-)-**25a** have an additional shoulder around 220 nm which is more pronounced in the (-) diastereomer. This shoulder can be detected also in the case of (-)-**27a**, although its intensity is much lower compared to the band at 205 nm. Interestingly, no absorption at 220 nm was observed for (+)-**27a**. The additional absorption at 220 nm cannot be attributed to the contribution of **11**, but it should derive from presence of some structural elements, thus confirming the IR data which suggest that the dipeptide is conformationally better defined than the corresponding tripeptide.

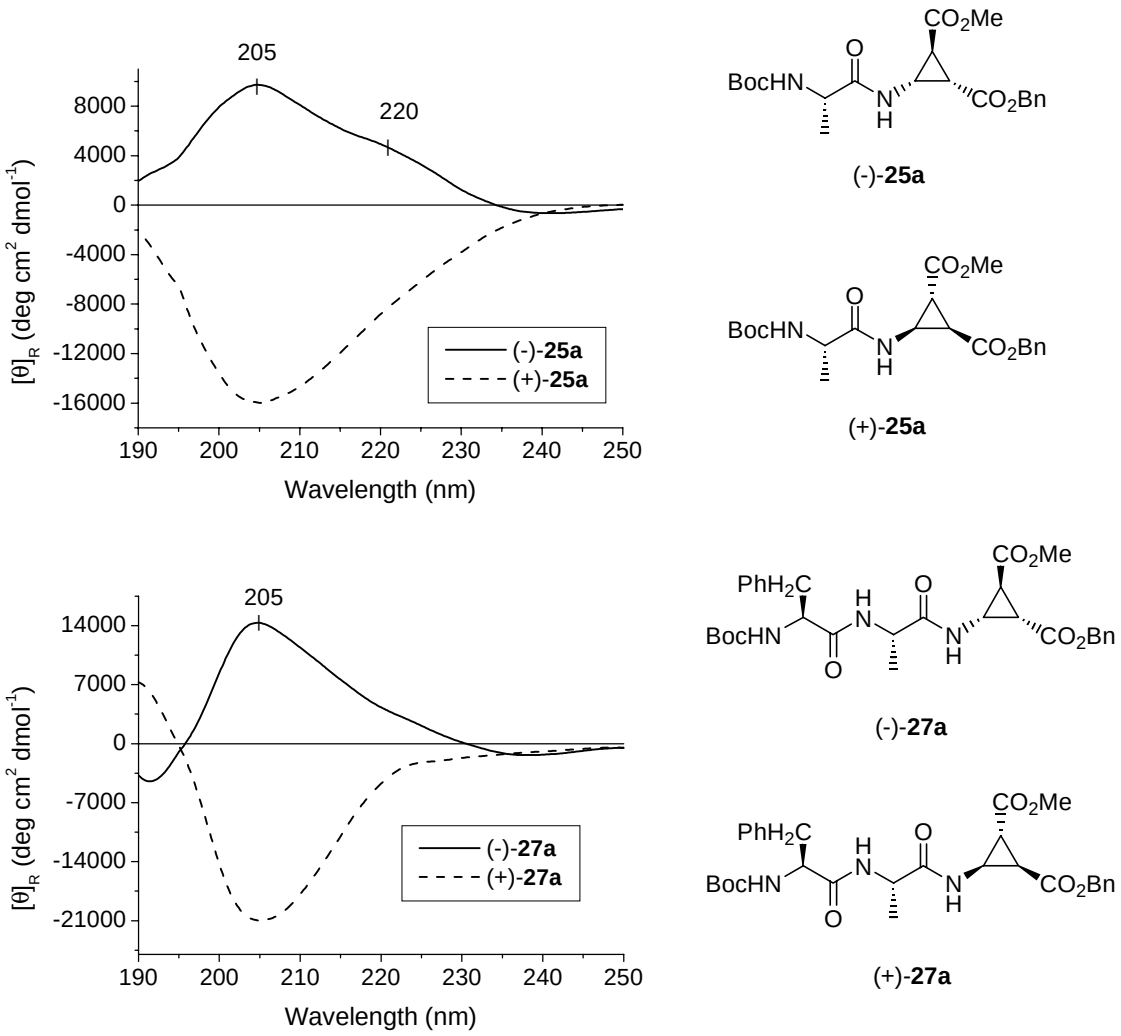


Figure 6. CD spectra of di- and tripeptides containing the β-ACC building block in methanol.

Comparing the CD spectra of (+)-**27a** and (+)-**27b** (Figure 7), the glycine analogue, in methanol, similar intensities are observed but the wavelength of (+)-**27b** transition is blue shifted to 202 nm. As already discussed in the previous chapter, in dichlormethane no hydrogen-bond was observed for (+)-**27b** and the CD pattern, with a prevalent class U (random coil) character, now confirms that (+)-**27b** has no well defined structure. In contrast, (-)-**27b** presents a low intensity class B spectrum in methanol (212 nm / θ_R +4800; 198 nm / θ_R -6250). Although no hydrogen-bond was found by IR spectroscopy, (-)-**27b** seems to be structured in methanol to some extent.

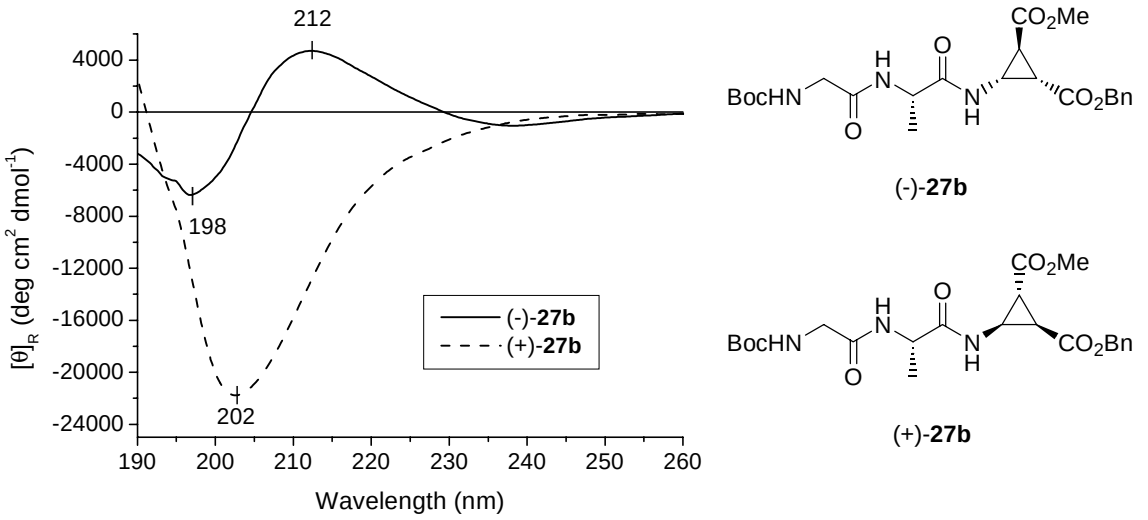


Figure 7. CD spectra of (+)-**27b** and (-)-**27b** in methanol.

The structure-inducing effect of the β-ACC building block (**11**) can be better appreciated on tripeptides having **11** as the central residue, where it can exert a greater conformation-directing effect.

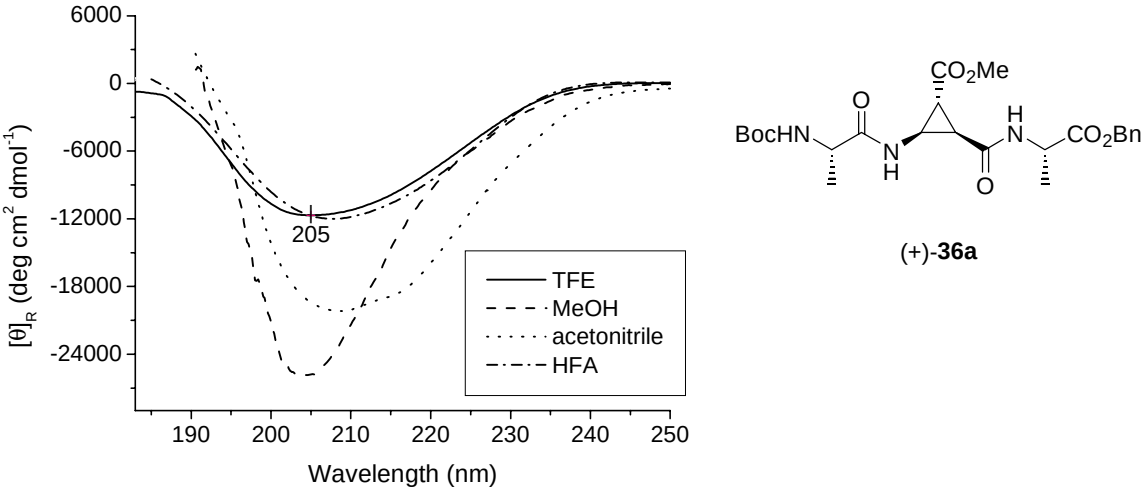
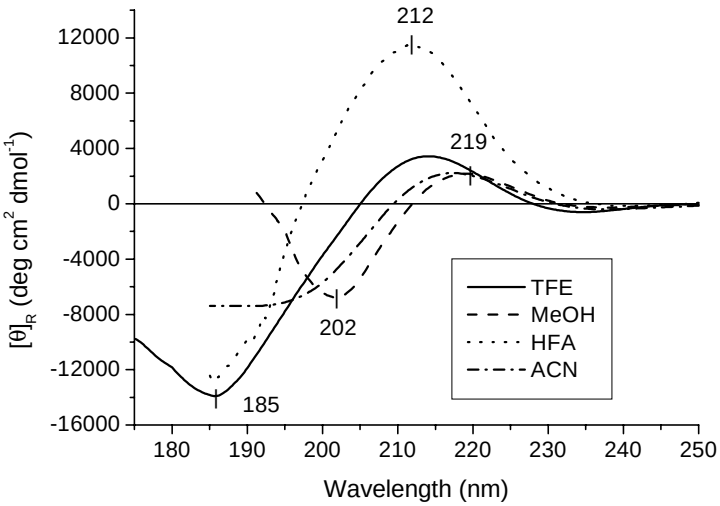
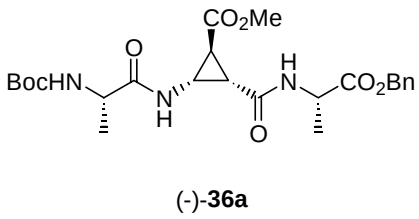


Figure 8. CD spectra of (+)-**36a** in various solvents.

The CD spectrum of (+)-**36a** was taken in TFE, acetonitrile, methanol and HFA (Figure 8). One minimum around 205 nm was found in each case. Such CD pattern can be assigned to class C turns.[45a-b] In TFE and HFA the CD band was less intensive and broader than in

methanol, due to the partial overlap of the n→π* with the π→π* transition. In acetonitrile a red-shift of the band was observed as well as the presence of a weak shoulder at 217 nm.





(-)-**36a**

Figure 9. CD spectra of (-)-**36a** in various solvents.

The CD pattern of (-)-**36a** (Figure 9) shows high solvent-dependence: in HFA it is a typical class B spectrum, having a maximum at 212 nm and a minimum at 185 nm. The CD profile in TFE is similar, but the maximum is much less intensive. In methanol and acetonitrile the n→π* and the parallel π→π* transitions are red-shifted (220 and 200 nm respectively) these spectra are characteristic for class D turns.

The N-methylation of the C-terminal alanine leads to a completely different CD spectrum compared to (+)-**36a** (Figure 10). In each solvent (+)-**36b** displays a helix-like spectrum with two negative bands at 222 and 201 nm and a positive one (visible only in TFE) at 183 nm.

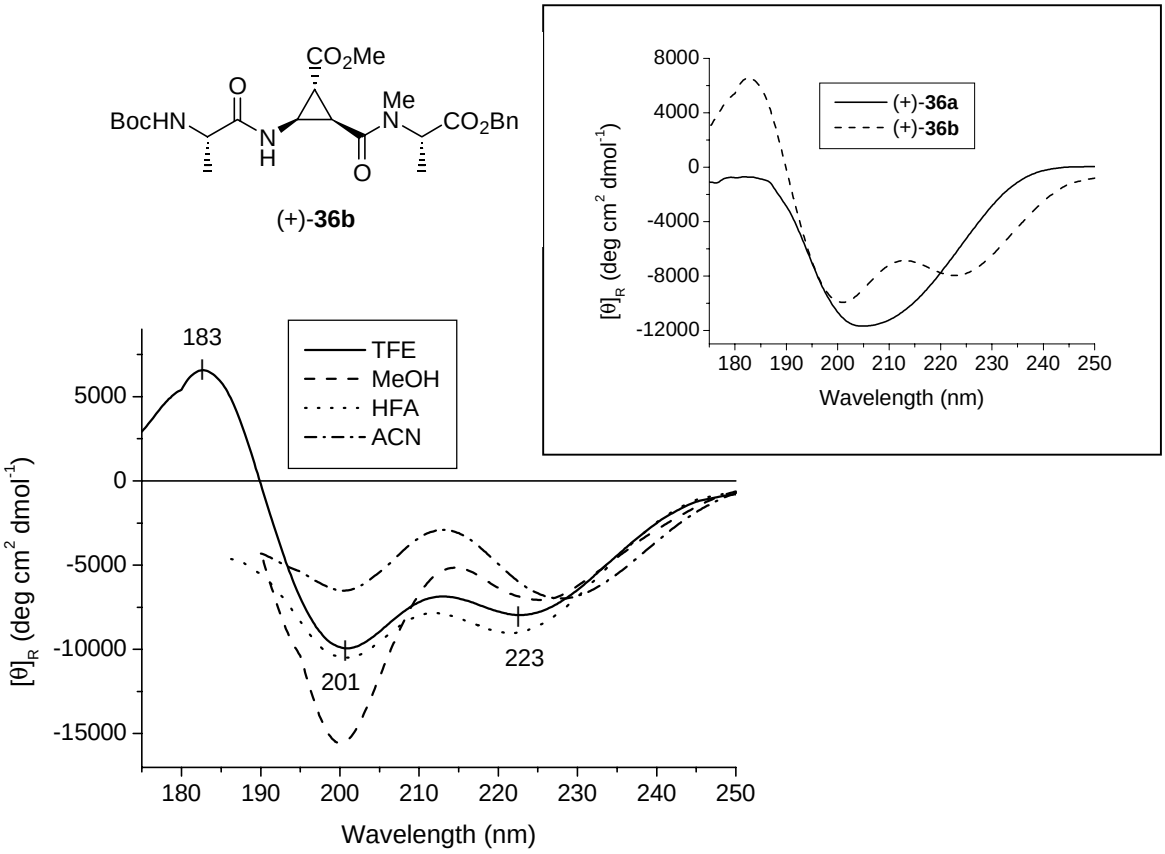


Figure 10. CD spectra of (+)-**36b** in various solvents. Inlet: (+)-**36a** compared to (+)-**36b** in TFE solution.

The difference observed between the various solvents is the ratio of the intensities corresponding to the n→π* and parallel π→π* transitions.

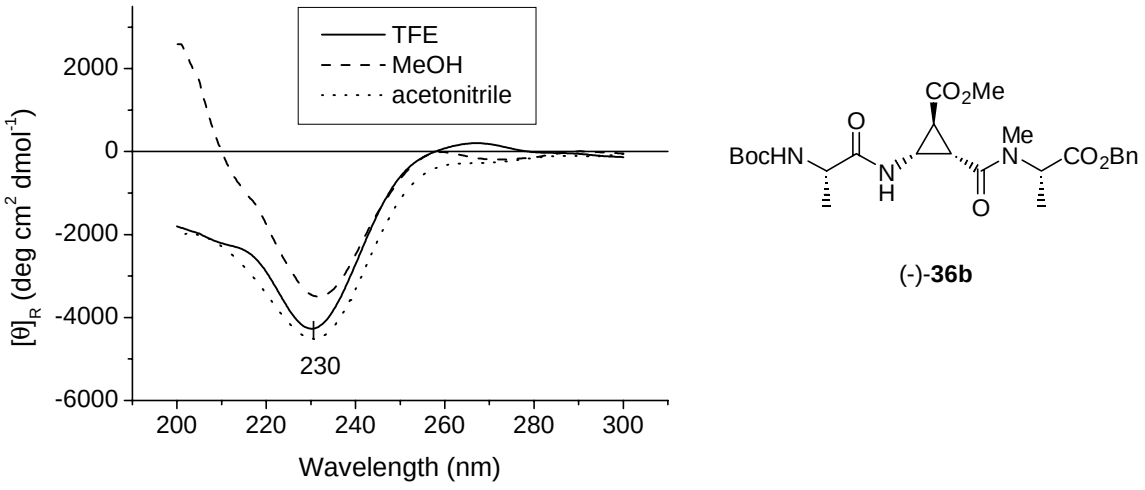
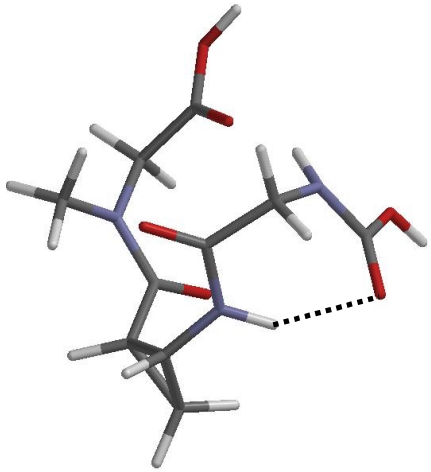


Figure 11. Compound (-)-**36b** CD spectra in various solvents.

The CD pattern of (-)-**36b** does not depend on the solvent used and has a minimum at 230 nm (Figure 11), typical for γ-turns.[31c, 46] Molecular modelling calculations (conformational search with the program Titan) confirmed these data, giving the γ-turn centred on alanine-1 as the most populated (Figure 12).



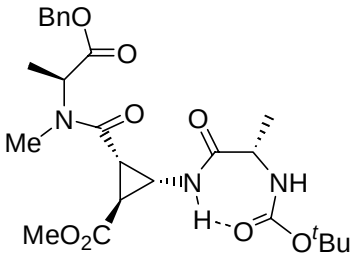


Figure 12. Compound (-)-**36b** first energy minimum. Protecting groups and side chains are not displayed for reasons of clarity.

In order to further investigate the effect of **11** on the conformation of α-peptides, longer peptides containing this building block were prepared. The α-peptide **49** was used as reference compound to be compared with the β-ACC containing α-peptides. In each solvent (acetonitrile, methanol, TFE and HFA) a minimum around 204 nm or below is present (Figure 13). In acetonitrile and in TFE there is a weak negative band around 240 nm and a stronger positive one at 223 nm, indicating the presence of turns. In methanol and HFA the CD spectrum experiences a blue shift and the band around 220 nm is much weaker than in acetonitrile or TFE. The broad intense minimum below 200 nm observed in HFA suggests the presence of a overwhelming unordered component.

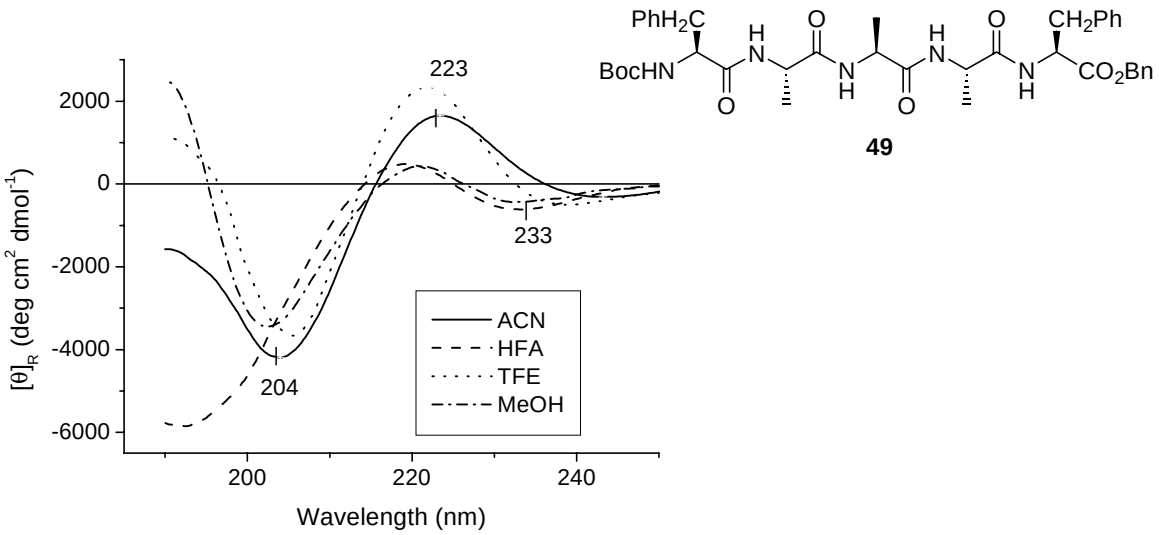


Figure 13. CD spectra of **49** in various solvents.

A comparison between the CD spectra of the two diastereomers of **37b** in TFE is shown in Figure 14. Same CD patterns were found in methanol, acetonitrile and HFA. The broad minimum at 205 nm and the maximum at 182 nm suggests a class C spectrum for (+)-**37b**,[45c] while the blue shift of the minimum below 200 nm observed for (-)-**37b** is indicative for a random coil. Interestingly, neither TFE nor HFA could exert any structurating effect on this diastereomer.

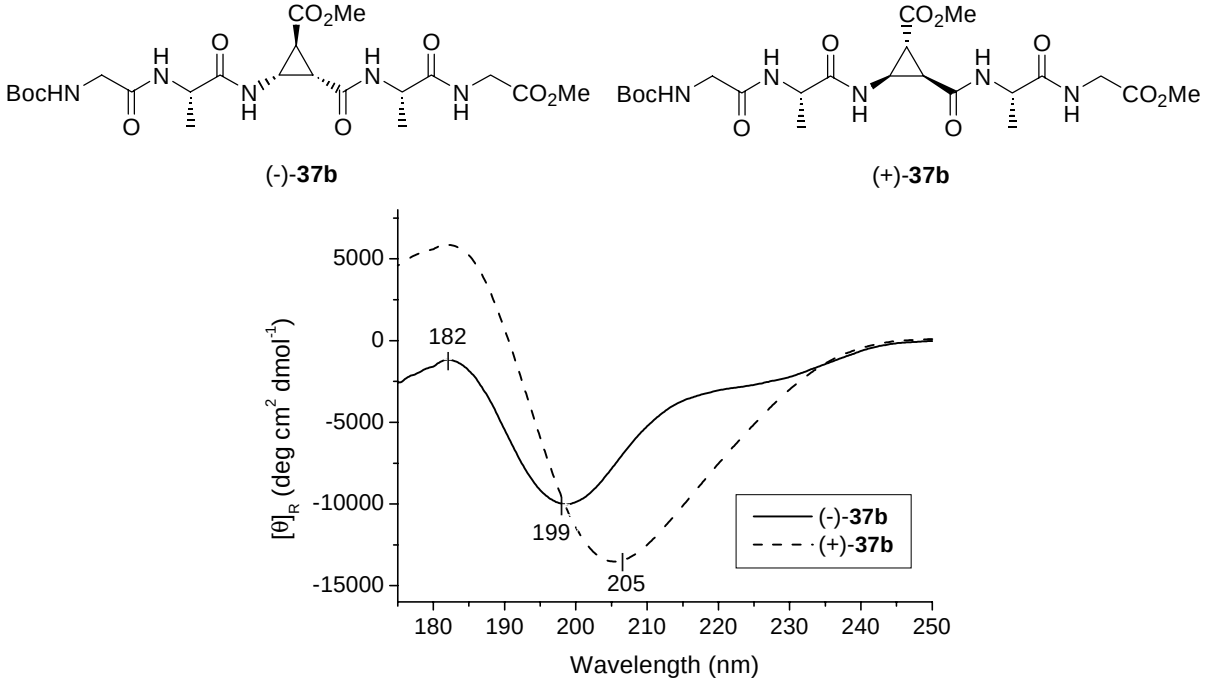


Figure 14. CD spectra of both diastereomers of **37b** in TFE.

Compound (+)-**37a**, analogous of (+)-**37b** but containing two phenylalanines instead of glycines, shows always class C spectra (228 nm / θ_R –5000, 210 nm / θ_R –12000, 190 nm / θ_R +4500 in TFE), where the n→π* transition results red shifted compared to the α-helix typical values, 222 nm (Figure 15). Such effect, although not typical, is not unusual in class C spectra.[45b] However, this red-shift could be also explained as a γ-turn CD contribution (230 nm). In fact, the amino acids adjacent to the β-ACC unit often prefer a γ-turn arrangement (see Chapter 2). The main feature of (+)-**37a** CD spectra is that they are not solvent dependent, suggesting a significant conformational stability of the peptide.

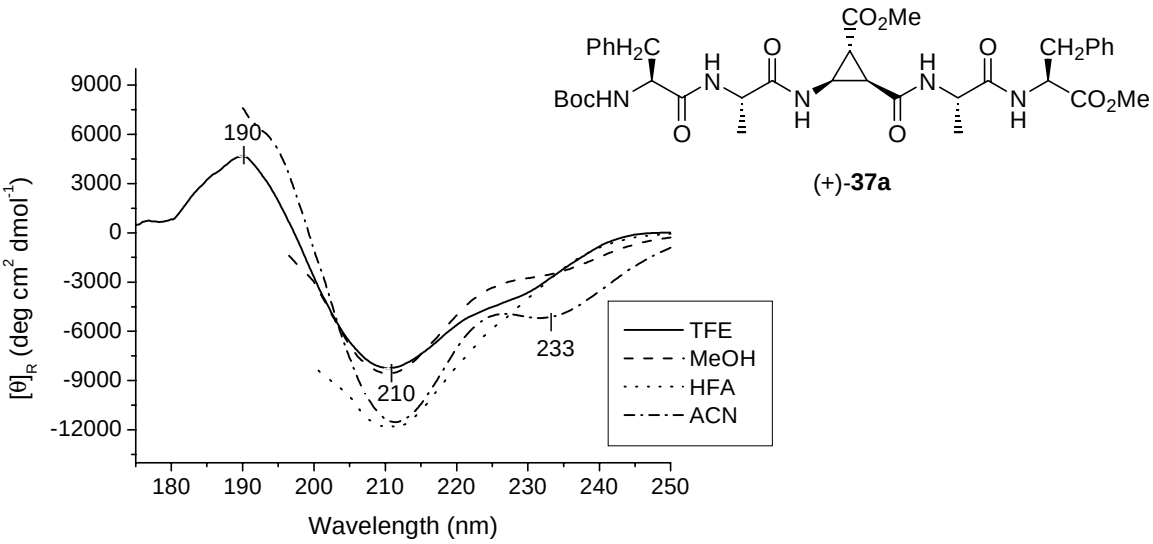


Figure 15. CD spectra of (+)-**37a** in various solvents.

Compound (-)-**37a** has CD patterns very sensitive to any change of solvent (Figure 16). In the structuring solvent TFE, a class C spectrum almost identical to that of the (+)-diastereomer can be observed. In HFA the peptide is unordered, while the CD spectra in methanol and in acetonitrile show a decrease in intensity as well as a red shift of the n→π* transition (235 nm) and a blue shift of the parallel π→π* transition (208 nm) compared to the spectrum taken in TFE and to those of (+)-**37a**. Moreover, the CD signal between the two minima is much less negative, probably due to an intrinsic conformational effect or to a positive contribution of the aromatic side chain of phenylalanine.[47]

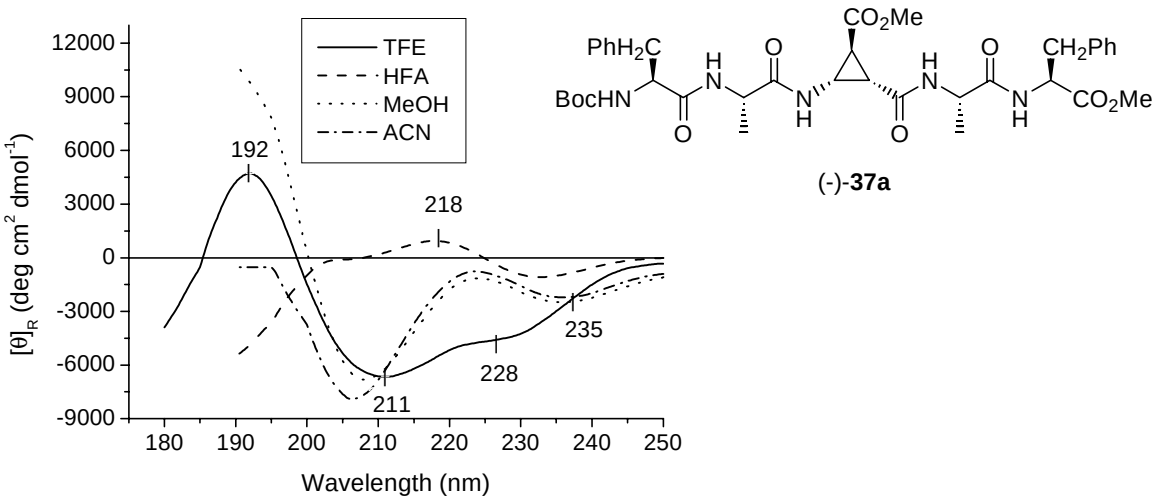


Figure 16. CD spectra of (-)-**37a** in various solvents.

The deprotected form of (+)-**37a**, (+)-**39**, shows CD spectra of class C (Figure 17). The n→π* transition is red shifted compared to the standard class C value and there is a positive contribution at 220 nm in methanol and HFA, which could be attributed to the phenylalanine side chain absorption.

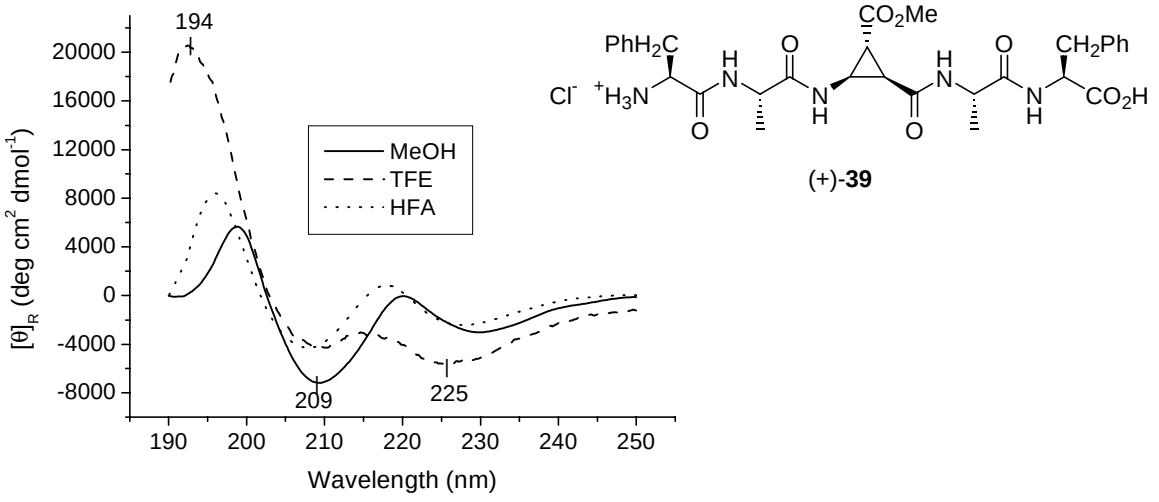


Figure 17. CD spectra of (+)-**39** in various solvents.

By comparison of the CD spectra of the protected and deprotected form of (+)-**37a** in TFE, an inversion of the ratio of the transition intensity at 211 *vs.* 225 nm can be observed.

The CD spectrum of (-)-**39** in TFE is similar to the (+)-**39** one, with two minima at 209 and 229 nm, and a positive band at 194 nm which is weaker in the case of the (-)-diastereomer

(θ_R 10000 instead of 20000). In methanol, the CD absorption between the two minima is positive, as already observed for (-)-**37a**. The CD spectrum in HFA has a maximum at 216 nm and a shoulder near 200 nm, suggesting the presence of β-turns (Figure 18).

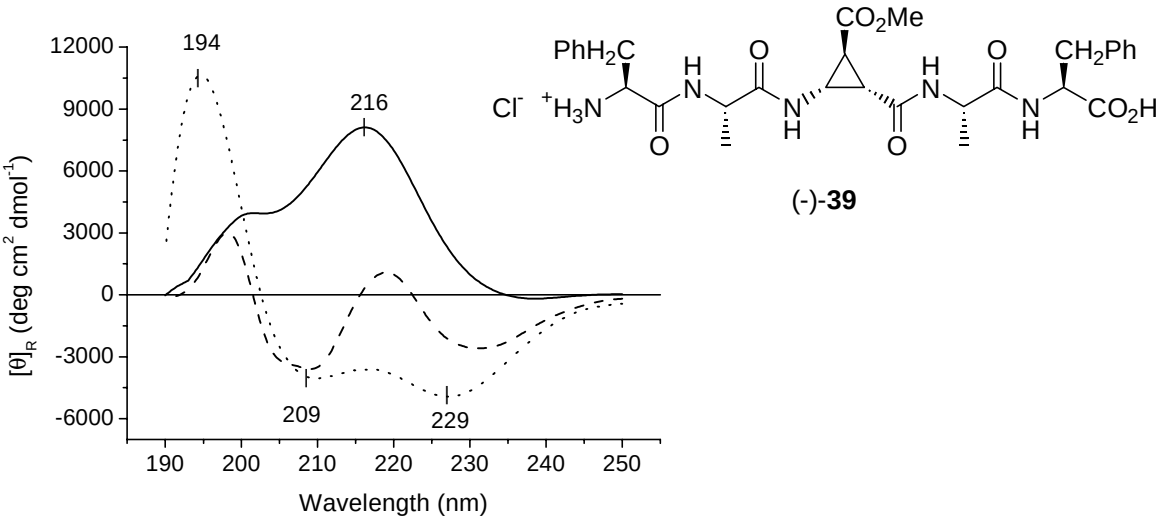


Figure 18. CD spectra of (-)-**39** in various solvents.

As already observed for the pair (+)-**37a**/(+)-**39**, an inversion of the ratio of the transition intensity at 211 *vs.* 225 nm is observed between the protected and deprotected form of (-)-**37a** in TFE.

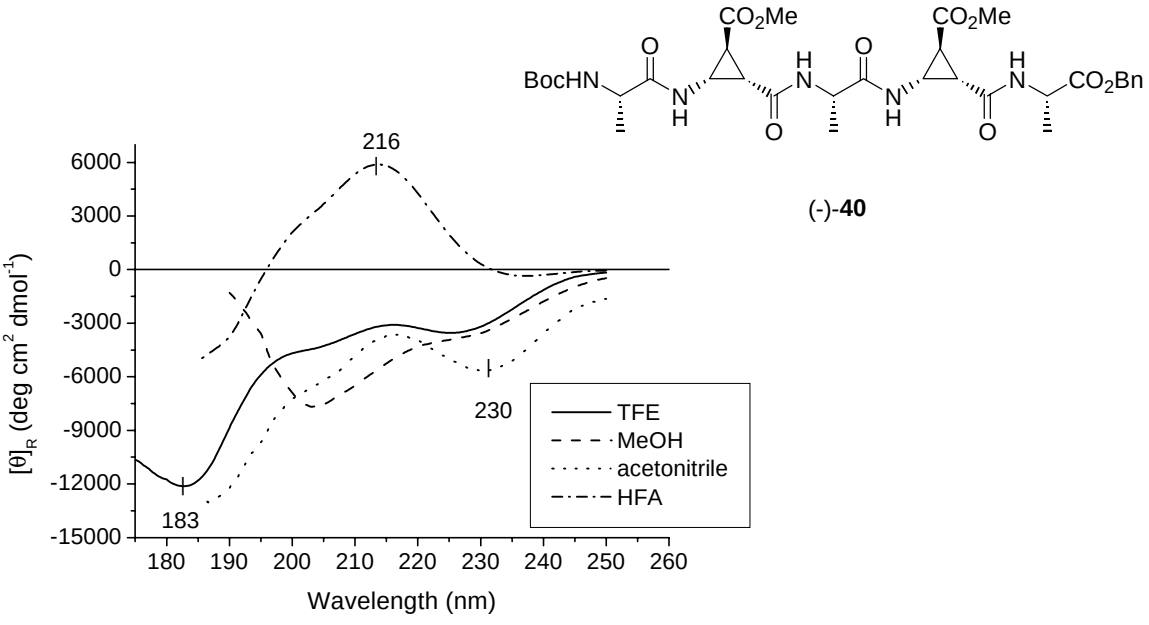


Figure 19. CD spectra of (-)-**40** in various solvents.

Compound (-)-**40** and the corresponding unprotected pentapeptide (-)-**43**, show CD spectra with the same profile in the same solvents (Figure 19, only (-)-**40** is shown), but the intensity is about 40% reduced in the case of the protected form. In acetonitrile (not used for (-)-**43**) and in TFE the pentapeptide is unordered (negative strong band at 183 nm). The broad positive band at 216 nm observed in HFA indicate the presence of β-turns. The same CD profile was found for the pentapeptide (-)-**39** containing only one β-ACC unit. In methanol a class C type CD spectrum is observed.

The peptides containing two or three (+)-β-ACC units ((+)-**41**, (+)-**42**, (+)-**40**, (+)-**43**, (+)-**45**, (+)-**46**) display very similar CD patterns, constant in acetonitrile, methanol and TFE (Table 2). In every case a broad minimum around 205 nm was observed, probably including the n→π* and the parallel π→π* transitions, while the perpendicular π→π* absorption falls around 185 nm, often weaker than the parallel component. Such spectra can be attributed to class C.

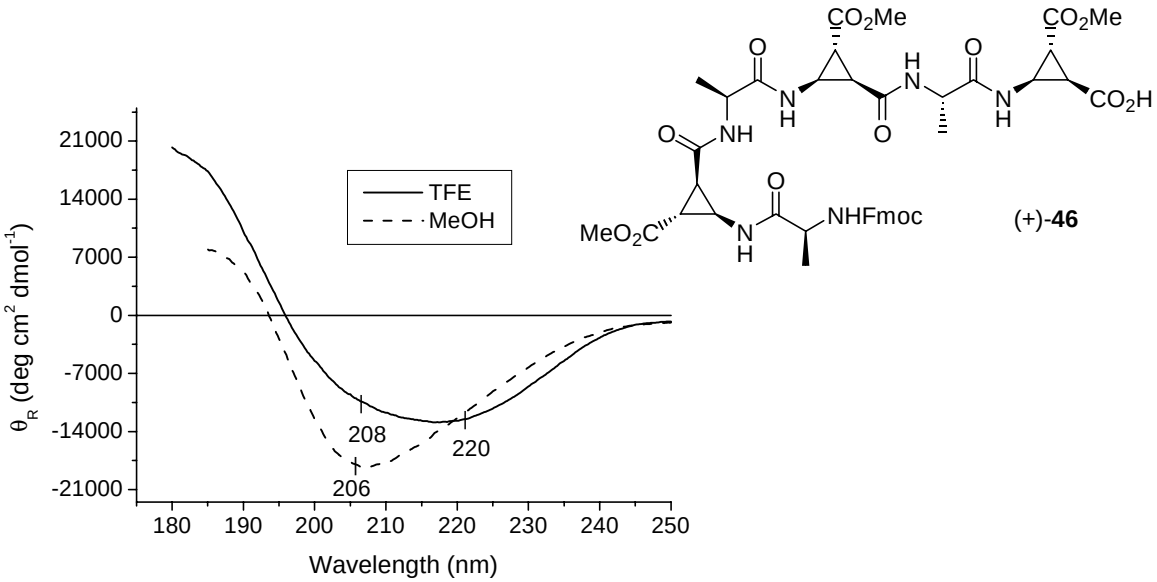
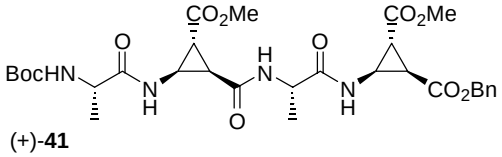
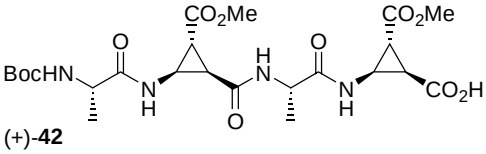
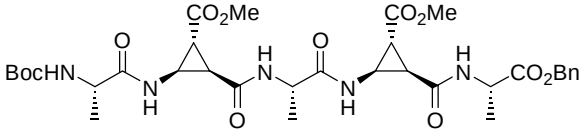
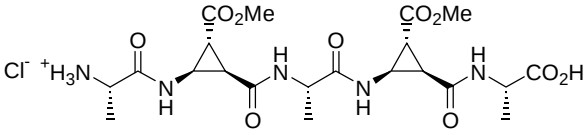
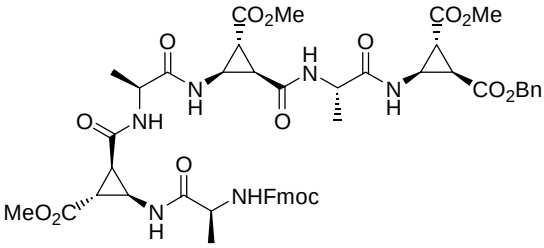
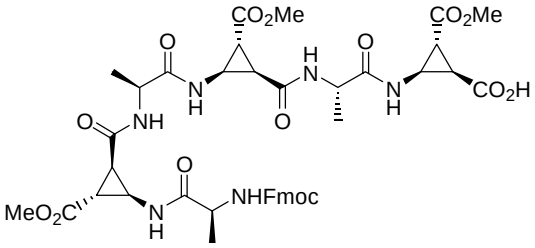


Figure 20. CD spectra of (+)-**46** in various solvents.

The overlap of the n→π* and the parallel π→π* transitions becomes evident comparing the CD spectra of (+)-**46** in TFE and methanol. The n→π* transition is weaker than the parallel π→π* in methanol but it turns stronger in TFE (Figure 20).

Table 2. CD pattern in TFE of compounds containing more than one (+)-*β*-ACC unit.

	parallel π-π* (nm)	perpendicular n-π* (nm)
 (+)-**41**	206 θ_R +29*10^3	<185 θ_R -15*10^3
 (+)-**42**	206 θ_R +24*10^3	<185 θ_R -15*10^3
 (+)-**40**	209 θ_R +20*10^3	186 θ_R -7.5*10^3
 (+)-**43**	209 θ_R +14*10^3	182 θ_R -10*10^3
 (+)-**45**	207 θ_R +30*10^3	<185 θ_R -20*10^3
 (+)-**46**	broad centred on 214 θ_R +14*10^3	<185 θ_R -21*10^3

3.3 Discussion

In this chapter the CD spectra of various derivatives of (-)-**11** and (+)-**11** have been shown. They proved to be often structured already at the tripeptide level displaying β-turn-like CD patterns.

A significant difference could be observed in the CD spectra of compounds differing only for the β-ACC configuration: the peptides containing (-)-**11** were unordered in several cases ((-)-**36a**, (-)-**37b**, (-)-**40**, (-)-**43**) while the derivatives of (+)-**11** were more structured. Moreover, the derivatives of (-)-**11** were very sensitive to any change in solvent, often exhibiting a solvent-induced conformation. HFA contributed to stabilise class B, D or C´ CD patterns, which seems to be due to its "teflon coating" effect.[43f-g] In some spectra positive components around 220 nm were observed, probably due to the contribution of the phenylalanine aromatic side chain. In contrast, the structure of peptides containing (+)-**11** was weakly or not at all solvent dependent and a positive contribution around 220 nm of the phenylalanine side chain was rarely observed ((+)-**39**) and, however, not as strong as on (-)-**11** derivatives. This could be explained by a predominant contribution of the peptide back-bone in this region, thus suggesting a well defined conformation. Finally, (-)-**11** containing peptides show CD spectra similar to those of the α-peptide **49**. In some solvents they displayed β-turns (class D) spectra and/or phenylalanine aromatic contribution. Similarities between (-)-**11** derivatives and **49** were already found on the hydrogen bonding extent measured by IR spectroscopy (Chapter 2). On the other hand, α-helix-like (class C) spectra were generally observed for derivatives of (+)-**11**.

Compounds containing multiple (+)-**11** displayed considerably constant CD patterns, without any solvent dependence. In the previous chapter it was suggested, on the base of the IR spectrum, that (+)-**45** could have a helix-like conformation. Based on the CD investigation, it can be stated that a helical turn conformation may be already present at the tetramer stage, i.e. (+)-**41**, suggesting that the introduction of two units of (+)-**11** is a powerful tool to induce and stabilise secondary structure elements in short peptides.

Chapter 4

4.1 General introduction

Several methods are available nowadays to determine the solution structure of peptides, e.g. circular dichroism (CD) and IR-spectroscopy. However, only NMR data may deliver structural information at atomic resolution. In addition, NMR allows to describe conformational equilibria in solution. While a complete structure determination based upon the methodology developed by *Wüthrich* and coworkers[48] offers the most comprehensive picture, a number of spectroscopic parameters exist to estimate whether a particular peptide is uniquely folded in solution or not.[31a-b]

Hydrogen-bonds can be identified by measuring for the amide proton signals: a) the reduced temperature coefficients ($\Delta\delta/\Delta T$), b) the solvent or concentration-dependence of the chemical shifts and c) the H/D exchange rate. As already pointed out in Chapter 2, all these methods are based upon the assumption that a hydrogen-bonded NH is less sensitive with respect to perturbations of environment variables (temperature, solvent, concentration...) than a fully solvent exposed amide proton. Substantial difficulties for interpretation in such experiments arise when conformational averaging occurs. Furthermore, solvent inaccessible NHs will experience little temperature or solvent chemical shift dependence similar to hydrogen-bonded NHs.[31a-b]

Since regular secondary structures are determined by well-defined φ and ψ dihedral angles, additional informations about the structure can be derived from a measurement of the magnitude of the scalar $^3J_{HN,H\alpha}$ coupling constants which have typical values for the common secondary structural elements.

Finally, the most useful information can be obtained from the nuclear Overhauser effect (NOE). A nucleus can relax through several mechanisms, and for protons the dipole-dipole magnetisation transfer with adjacent protons (closer than 5 Å)[49a-b] is the most efficient. The NOE is proportional to $1/r^6$ (r = distance between the two interacting nuclei), allowing to determine spatial proximities of protons which may then be translated into a three-dimensional model. The two dimensional NMR experiment that provides information about the NOE between protons is called NOESY. The NOE can be positive or negative depending on the frequency of overall tumbling of the molecule. It is positive for small molecules and negative for large ones. For peptides of 10-20 residues the NOE in aqueous solution can be very small. To avoid this problem the ROESY (rotating frame NOESY)

experiment has been developed. Therein, the signal decay occurs in the spin-locked state and the rotating frame NOE is always positive.

The principle of the reduced temperature coefficients in aprotic, apolar solvents have already been illustrated in Chapter 2. In the current chapter $\Delta\delta/\Delta T$ values have been measured also in protic (CD_3OH) or good hydrogen-bond acceptor (pyridine-d_5) solvents. The effect of such solvents on strongly or partially hydrogen-bonded NHs is the same as in CD_2Cl_2 or $CDCl_3$ (small *vs.* large variation of the chemical shift). However, in protic solvents, hydrogen-bonds are formed between the NHs and the solvent and the temperature dependence of the latter allows to distinguish them from intermolecular H-bonds.

4.2 The structure of β-ACC derivatives investigated by high resolution NMR

4.2.1 Temperature coefficients

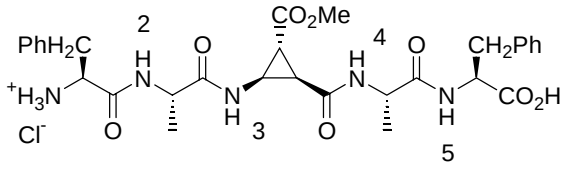
Although temperature coefficients have to be treated cautiously, they may be used to identify possible hydrogen bonding sites in the presence of further structural information. Values more positive than -6 ppb/K have been interpreted in literature to indicate that the corresponding amide proton is involved into a hydrogen bond in aqueous systems[50a] and similar values have been used by *Seebach*[50b] and *Gellman*[50c] for solutions in CD_3OH. For the peptides that have been structurally characterised in more detail by 2D NMR,[†] the temperature coefficients were measured in CD_3OH.

For the peptides that have been mainly characterised by IR and CD spectroscopy other solvents like pyridine or chloroform were used to solve problems such as occurrence of the NH signals in the aromatic region, low solubility of the peptides or broadening of the signals probably due to conformers exchange. However, in the investigation of (+)-**37a** and (-)-**37b**, the use of pyridine-d_5 always resulted in high $\Delta\delta/\Delta T$ values, thus proving the absence of hydrogen bonds in this solvent. The measurements performed in $CDCl_3$ ((+)-**37b**, (-)-**37a** and (-)-**40**) and in CD_3OH ((+)-**40**) often showed temperature coefficients with border values for hydrogen-bonding, but in the absence of further 2D NMR investigations they are not sufficient to prove the presence of hydrogen-bonds.

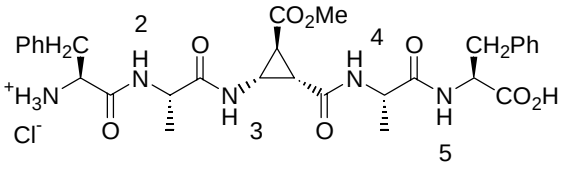
Detailed NMR studies were performed on peptides (+)-**39**, (-)-**39**, (+)-**43** or (-)-**43** containing one or two β-ACC units (Table 1).

[†] In collaboration with Dr. O. Zerbe, Department of Pharmacy, ETH Institute, Zürich, Switzerland.

Table 1. (+)-**39** (top left) and (-)-**39** (top right), (+)-**43** (bottom left) and (-)-**43** (bottom right) temperature coefficients measured at 500 MHz between 280 and 301 K, 10 mM in CD_3OH.

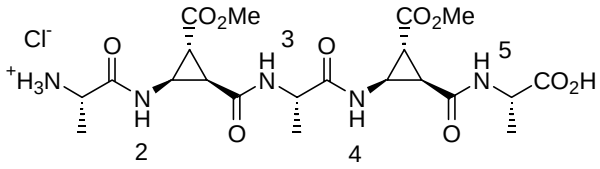


(+)-**39**

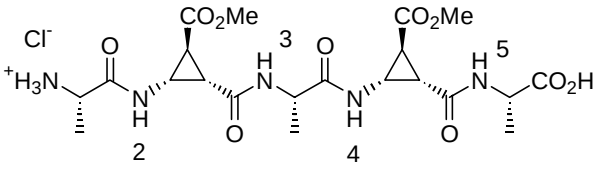


(-)-**39**

Amide NH	Δδ/ΔT (ppb/K)	Chemical shift (ppm) at 280 K	Δδ/ΔT (ppb/K)	Chemical shift (ppm) at 280 K
NH-2	-7.7	8.74	-6.0	8.75
NH-3	-7.7	8.39	-6.7	8.23
NH-4	-6.0	8.62	-7.3	8.81
NH-5	-9.4	8.08	-7.8	8.07



(+)-**43**



(-)-**43**

Amide NH	Δδ/ΔT (ppb/K)	Chemical shift (ppm) at 280 K	Δδ/ΔT (ppb/K)	Chemical shift (ppm) at 280 K
NH-2	-6.7	8.69	-4.0	8.38
NH-3	-6.7	8.78	-7.0	8.69
NH-4	-6.1	8.40	-4.7	8.00
NH-5	-6.9	8.81	-7.3	8.92

For the peptides (+)-**39**, (-)-**39** and (+)-**43** none of the values is low enough to unambiguously identify participation in hydrogen bonding. However, for (-)-**43** the amide protons of ACC-2 and ACC-4 display temperature coefficients indicative for hydrogen bonding.

When comparing the chemical shift of protons at the same position in the corresponding two diastereomers it is striking that the largest differences for the amide protons are encountered for residues ACC-2 and ACC-4 of (+)-**43** and (-)-**43**. Moreover, the temperature coefficients for the amide protons of only these two residues are significantly lowered in (-)-**43**. Since the involvement in a hydrogen bond would lead to a change in the chemical shift, these differences would be compatible with the findings from the temperature coefficients showing that only one of the two diastereomers of **43** forms hydrogen bonds.

Initially, the proton data were taken at concentrations of 10 mM in CD_3OH. At that high concentration aggregation has to be considered and therefore we repeated the measurements with 2 mM solutions for the two diastereomers of **39** and **43** (Table 2 and Table 3).

Table 2. (+)-**39** (left) and (-)-**39** (right) temperature coefficients measured at 500 MHz between 280 and 301 K, 2 mM in CD_3OH.

	(+)-**39**		(-)-**39**	
Amide NH	**Δδ/ΔT (ppb/K)**	**Chemical shift (ppm) at 280 K**	**Δδ/ΔT** [a] **(ppb/K)**	**Chemical shift (ppm)**[a] **at 280 K**
NH-2	-7.4	8.73	-	-
NH-3	-7.3	8.37	-6.4	8.23
NH-4	-5.8	8.62	-6.7	8.81
NH-5	-7.5	7.94	-7.0	8.04

[a] NH-2 exchanges rapidly with the solvent already at 280 K.

The values of the temperature coefficients for (+)-**39** at the two concentrations are very similar except for NH-5 (-9.4 and –7.5 ppb/K respectively). For this amide proton additionally the chemical shift experienced the most dramatic change. In total, these data may indicate that dimer association takes place involving NH-5 for (+)-**39**. For the corresponding diastereomer (-)-**39** the resonance of the amide proton of NH-5 displays similar Δδ/ΔT at both concentrations and the change in chemical shift is small. Furthermore, for both diastereomers the resonance of NH-2 is significantly broadened at 2 mM but not at 10 mM concentrations. This may also be due to exchange-broadening arising from monomer-dimer equilibria.

Table 3. (+)-**43** (left) and (-)-**43** (right) temperature coefficients measured at 500 MHz between 280 and 301 K, 2 mM in CD_3OH.

	(+)-**43**		(-)-**43**	
Amide NH	**Δδ/ΔT (ppb/K)**	**Chemical shift (ppm) at 280 K**	**Δδ/ΔT (ppb/K)**	**Chemical shift (ppm) at 280 K**
NH-2	-6.7	8.66	-3.9	8.37
NH-3	-6.1	8.78	-6.7	8.68
NH-4	-5.3	8.39	-3.9	8.01
NH-5	-6.2	8.79	-6.7	8.90

The concentration dependence of the temperature coefficients for the two-ACC residues containing peptides (+)-**43** and (-)-**43** was also measured (Table 3). In essence, the temperature dependence for the two diastereomers is similar at both concentrations. Furthermore, the chemical shifts of the amide protons do not vary significantly with changes in concentration so that dimerisation effect seems to play a much less pronounced role.

4.2.2 Chemical shift assignment derived from 2D NMR data

A more detailed picture of the solution conformation of peptides at atomic resolution can be gained from 2D NMR data. In a first step of the analysis, the sequence-specific resonance assignment has to be determined for all observable protons. In a second step, NOESY crosspeaks have to be completely assigned and the volume integrals translated into distance restraints. These restraints are then used in a molecular dynamics simulation to derive the structure in solution.

For the resonance assignment the combination of a DQF-COSY,[51a-b] a 80 ms TOCSY[51c-d] and a 500 ms ROESY[51e-f] experiments was used. Whereas the DQF-COSY displays crosspeaks for protons that are scalarly coupled, the TOCSY includes all (scalar) correlations with other protons that are in the same spin-system (Figure 1).

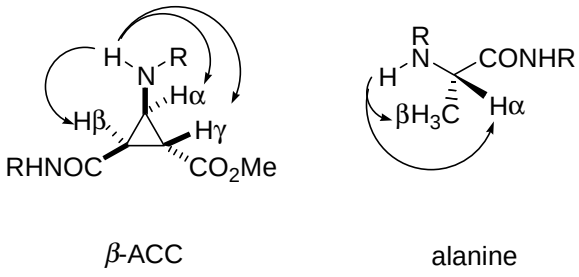


Figure 1. Correlations with NH proton visible in TOCSY for β-ACC and alanine

The spin-systems were identified from TOCSY and linked via sequential Hα/HN ROEs. The side chain assignment was then performed by combined use of the DQF-COSY and TOCSY data (Figure 2).

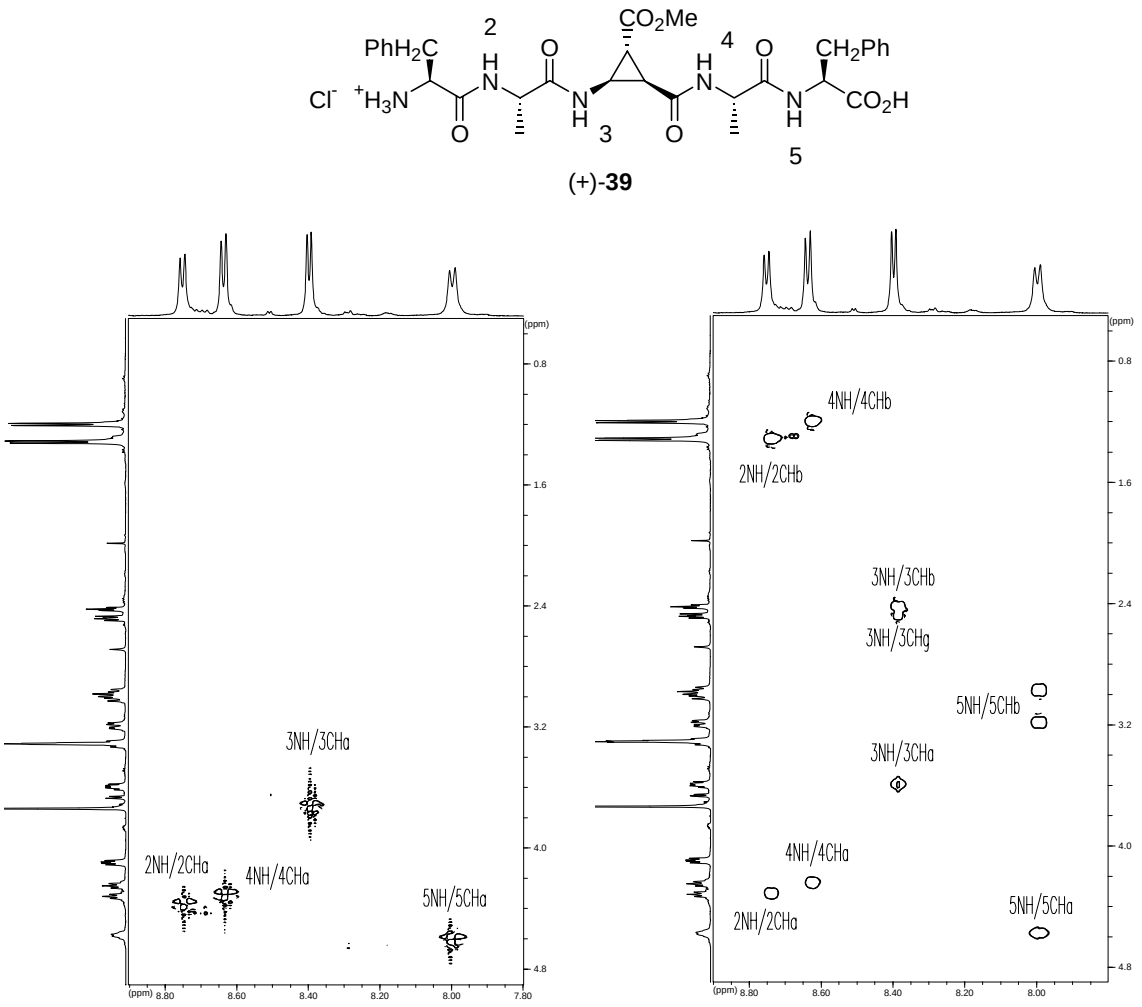
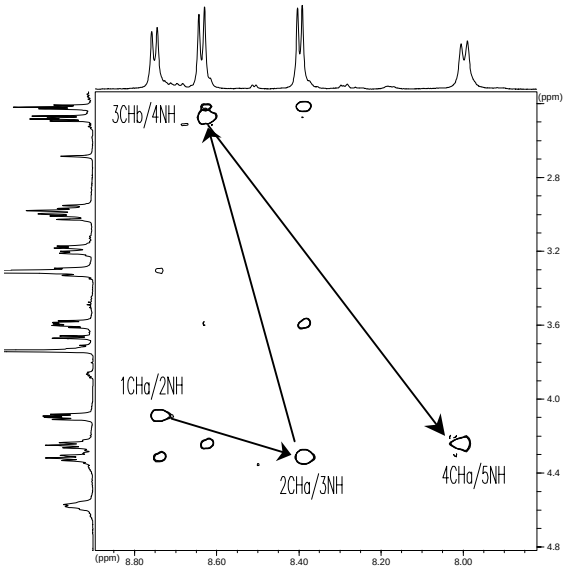
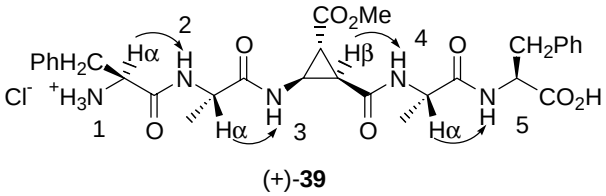


Figure 2. (+)-**39** COSY (left) and TOCSY (right) spectra taken at 500 MHz, 280 K, 10 mM in CD_3OH. a=α, b=β and g=γ.

After identification of the spin systems of the amino acids, their sequence positions were determined from the ROESY spectrum (Figure 3).

Neighbouring amino acids usually display $CH\alpha_i/NH_{i+1}$ cross peaks. For the β-ACC amino acid it was not possible to find a CHα (β-ACC)/NH (β-ACC+1) cross peak because in a β-amino acid the CHα is too far away from the NH of the following residue. Instead, a CHβ (β-ACC)/NH (Ala-4) was found.





(+)-**39**

Figure 3. (+)-**39** ROESY NH/CHα region showing the $NH_i/CH\alpha_{i+1}$ cross peaks connectivity. Spectrum measured at 500 MHz, 280 K, 10 mM in CD_3OH.

The analysis of the spectra yielded the assignments illustrated in Table 4.

Table 4. Assignments of the signal in the ^1H NMR spectrum of (+)-**39** and (-)-**39**, (+)-**43** and (-)-**43**, 500 MHz, 280 K, 10 mM in CD_3OH.

(+)-**39**	HN	Hα	Hβ	Others
Phe-1	-	4.08	3.00, 3.30	δH 7.30, 7.30 εH 7.34, 7.34 ζH -
Ala-2	8.74	4.31	1.31	
β-ACC-3	8.39	3.59	2.47	γH 2.41 Me 3.73
Ala-4	8.62	4.24	1.19	
Phe-5	8.01	4.58	2.98, 3.18	δH 7.18, 7.18 εH 7.22, 7.22 ζH -

(-)-**39**	HN	Hα	Hβ	Others
Phe-1	-	4.13	2.94, 3.44	δH 7.30, 7.30 εH 7.35, 7.35 ζH -
Ala-2	8.75	4.43	1.31	
β-ACC-3	8.23	3.53	2.50	γH 2.24 Me 3.71
Ala-4	8.81	4.29	1.24	
Phe-5	8.07	4.61	3.11, 3.23	δH 7.26, 7.26 εH 7.21, 7.21 ζH -

(+)-**43**	HN	Hα	Hβ	Others
Ala-1	-	3.85	1.38	
β-ACC-2	8.69	3.51	2.53	γH 2.47 Me ?
Ala-3	8.78	4.18	1.30	
β-ACC-4	8.40	3.73	2.48	γH 2.34 Me ?
Ala-5	8.81	4.40	1.39	

(-)-**43**	HN	Hα	Hβ	Others
Ala-1	-	3.92	1.48	
β-ACC-2	8.38	3.42	2.47	γH 2.38 Me 3.71
Ala-3	8.69	4.25	1.34	
β-ACC-4	8.00	3.56	2.54	γH 2.34 Me 3.71
Ala-5	8.92	4.36	1.43	

4.2.3 Structure calculation from NOE data

After integration of the peaks, the corresponding interproton distances were calibrated from the volume integrals by DYANA, a program dedicated to NMR structure calculation.[52] The volume (V) of the peak corresponding to a NOE between two protons is proportional to the sixth power of their distance (r):

$$V = k/r^6$$

were k is a calibration constant. A known distance between two protons may serve to calibrate k, then all the other distances can be calculated. In the current calculations k was derived according to the known distance for the cyclopropylic Hα/Hβ of 2.4-2.5 Å.

4.2.3.1 The solution structures of pentapeptides containing one β-ACC unit

The number of restraints per residue and the number of long-range restraints indicate how well a structure is defined. For the peptides (+)-**39** and (-)-**39**, both values were comparably high for a small peptide.

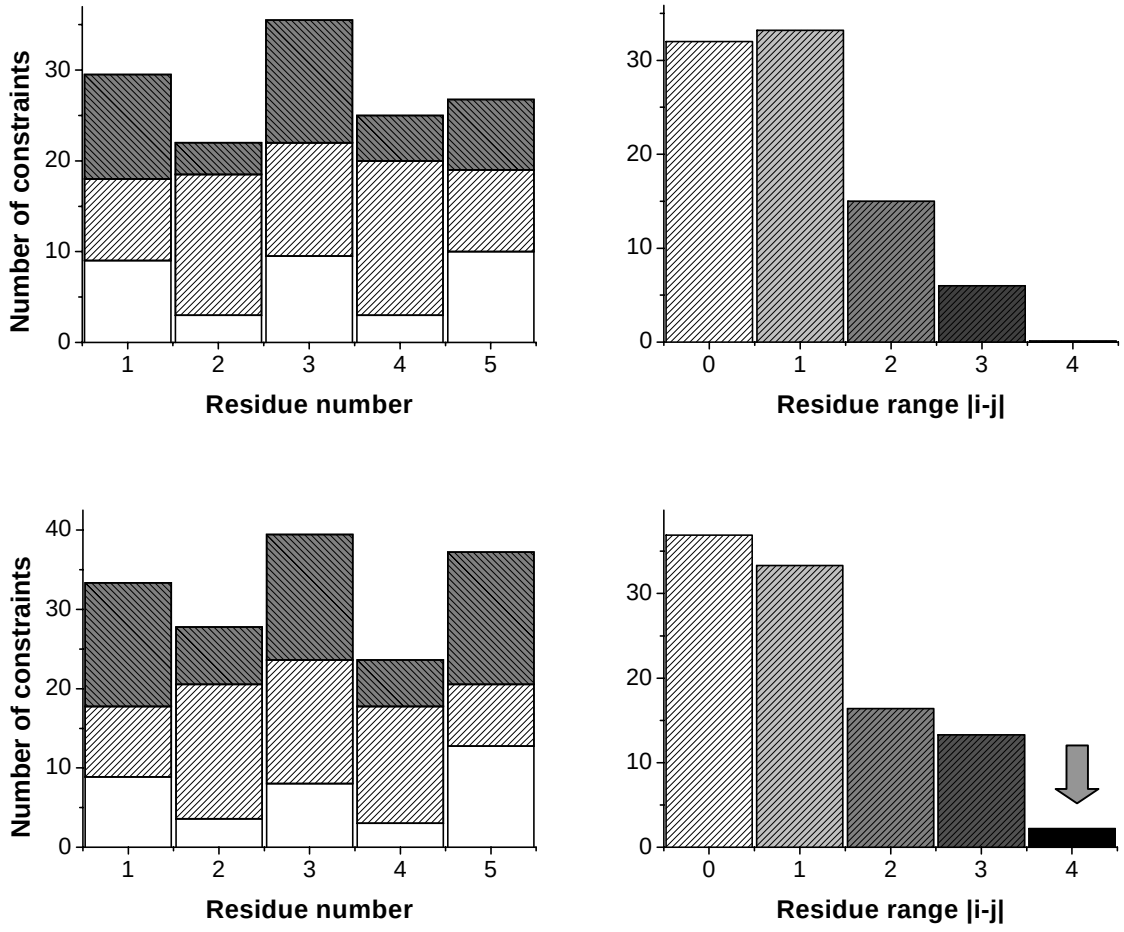


Figure 4. (+)-**39** (top) and (-)-**39** (bottom) distance restraints. Left: for each residue white = intraresidual; pale grey = i-i+1; dark grey = long range. Right: restraints for the whole molecule *vs.* the distance between the interacting residues.

For (+)-**39** as well as for (-)-**39** a relatively large number of long range (≥ i-i+2) restraints were found suggesting that the two peptides are at least partially well structured. An important difference observed between (+)-**39** and (-)-**39** (Figure 4) distance restraints is that (+)-**39** has no i-i+4 long range restraints (i.e. between the first and the last residue), while (-)-**39** has two of them (Figure 5).

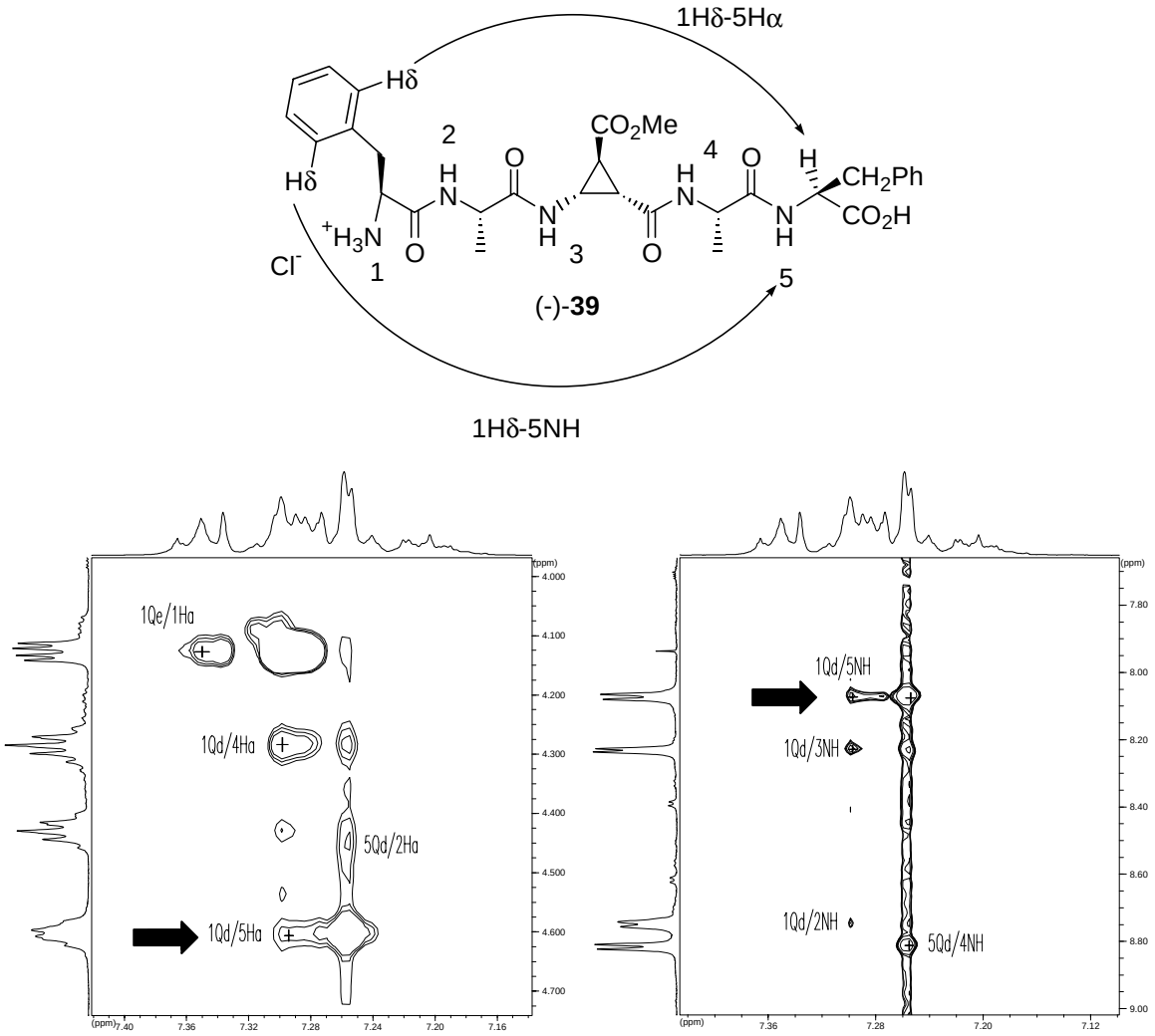


Figure 5. (-)-**39**: two ROESY expansions showing the two i-i+4 NOEs. a = α, d = δ, e = ε, Q indicates the pseudoatoms.

The non-sequential distance restraints used in the calculations for (+)-**39** and (-)-**39** are shown in Table 5.

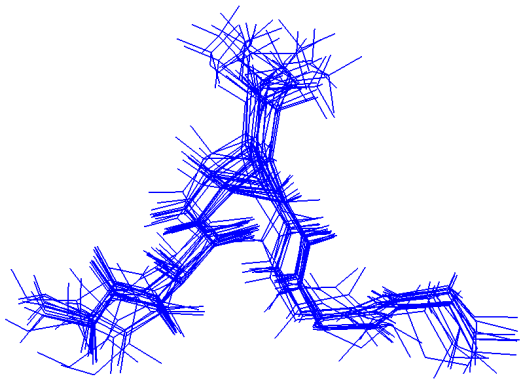
Table 5. Non-sequential distance restraints for (+)-**39** and (-)-**39**.

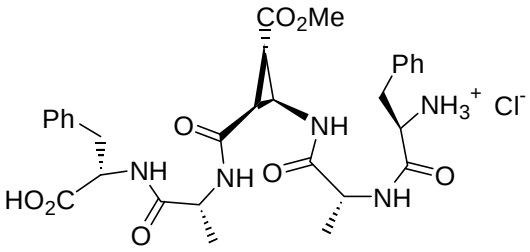
(+)-**39**	
HB3(1)-HN(3)	HB(3)-QE(5)
HB3(1)-HA(4)	HG(3)-QD(5)
QD(1)-HN(3)	
QD(1)-HA(3)	
QD(1)-HG(3)	
QD(1)-HA(4)	
QD(1)-QB(4)	
QE(1)-HA(3)	
HG(3)-QE(5)	
QE(1)-HG(3)	
QE(1)-QB(4)	
HA(2)-QD(5)	
QB(2)-HA(4)	
QB(2)-QD(5)	
HB(3)-QD(5)	

(-)-**39**	
HB2(1)-HA(4)	QD(1)-HG
HB3(1)-HN(3)	QD(1)-HA(4)
HB3(1)-HA(4)	QD(1)-QB(4)
QD(1)-HN(3)	QD(1)-HN(5)
QD(1)-HA(3)	QD(1)-HA(5)
QD(1)-HB(3)	QE(1)-HA(3)
QE(1)-HG(3)	QB(2)-HA(5)
QE(1)-HA(4)	QB(2)-HB2(5)
QE(1)-QB(4)	QB(2)-HB3(5)
HN(2)-HN(5)	QB(2)-QD(5)
HA(2)-QD(5)	QB(2)-QE(5)
HN(3)-HN(5)	HB(3)-HN(5)
HN(3)-QD(5)	HB(3)-QD(5)
HA(3)-QD(5)	HB(3)-QE(5)
HA(3)-QE(5)	HG(3)-QE(5)

Then, 40 randomized conformations were generated by DYANA and minimised in a simulated annealing protocol by molecular dynamics in torsion angle space. DYANA uses the NMR restraints as an additional energy contribution to the force field potential used in the simulated annealing. This ensures that the final minimised structures are compatible with the NMR restraints. Besides distance restraints, dihedral angles restraints can be implemented in the DYANA calculation. Unfortunately, all the peptides investigated had $^{3}J_{HN,H\alpha}$ around 7 Hz, which is the typical value for rotational averaged backbone angles.[31a-b] The peptide bond (dihedral angle ω) is normally fixed to 180°, the standard value in peptide and proteins except for N-methyl amino acids and proline.

After the calculation, the target functions (that reflects how well NOEs and the standard force-field parameters are satisfied) among the best 20 conformations should be reasonably small but not too similar. After superimposition of the backbone atoms of well-structured parts the root mean square deviation (RMSD) among the best 20 conformations should be < 0.5 Å for the backbone and < 1 Å for the side chains, and there should be no consistent restraint violations.





(+)-**39**

Figure 6. Superimposition of the best 20 conformations calculated for (+)-**39** visualised in the program MOLMOL. Side chains are omitted for reasons of clarity.

For (+)-**39** and (-)-**39** the RMSD for the backbone among the best 20 structures calculated was good (0.59 Å and 0.63 Å, respectively) as it can be observed in Figure 6 for (+)-**39**. Only few and not significant violations were found (Table 6) and the mean target function was 0.16 $\pm$ 4.10 * 10^{-2} for (+)-**39** and 7.06 * 10^{-2} $\pm$ 4.37 * 10^{-3} for (-)-**39**.

Table 6. Distance restraints violations for (+)-**39** and (-)-**39**.

Constraint	mean violation	number of violated structures
	(+)-**39**	
2Qβ/3Hβ	0.09	2
3Hα/4Hα	0.21	18
3Hβ/4HN	0.07	2
	(-)-**39**	
3Hα/3Hβ	0.23	20

One violation, however occurred on (+)-**39**: the sequential ROE between the alpha-protons from β-ACC-3 and Ala-4 indicate that a portion of conformers displays a *cis*-peptide bond between these two residues. Such a NOE between 3-CHα and 4-CHα would be possible if the corresponding 3/4 peptide bond would be *cis*, because only in this case the corresponding proton-proton distance would be below 5 Å (Figure 7). *Cis*-peptide bonds are rarely encountered in peptides. However, for a Xxx-Pro peptide bond there is usually a low (< 10 %) amount of the *cis* conformer. Whereas the *cis-trans* isomerisation for the Xxx-Pro bond is

slow on the NMR time scale, the absence of a second set of peaks due to the *cis* form indicates that the interconversion is fast for a Xxx-ACC peptide bond.

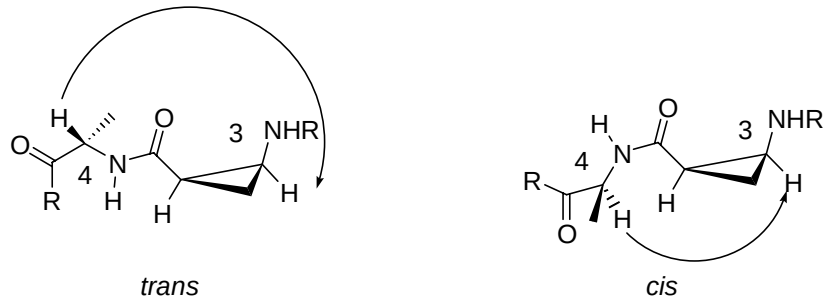


Figure 7. Distance between 3-CHα and 4-CHα in the *trans* and *cis* configuration of the 3/4 peptide bond. The carbomethoxy group on the β-ACC is omitted for reasons of clarity.

The conformations minimised by DYANA can be visualised and elaborated by MOLMOL,[53] a program devoted to manipulate the three-dimensional structure of biological macromolecules. On both peptides the influence of the cyclopropane-amino acid is to revert the chain direction by a sharp turn. The main difference between the two diastereomers lies in the relative orientation of the N- and C-*termini* (Figure 8).

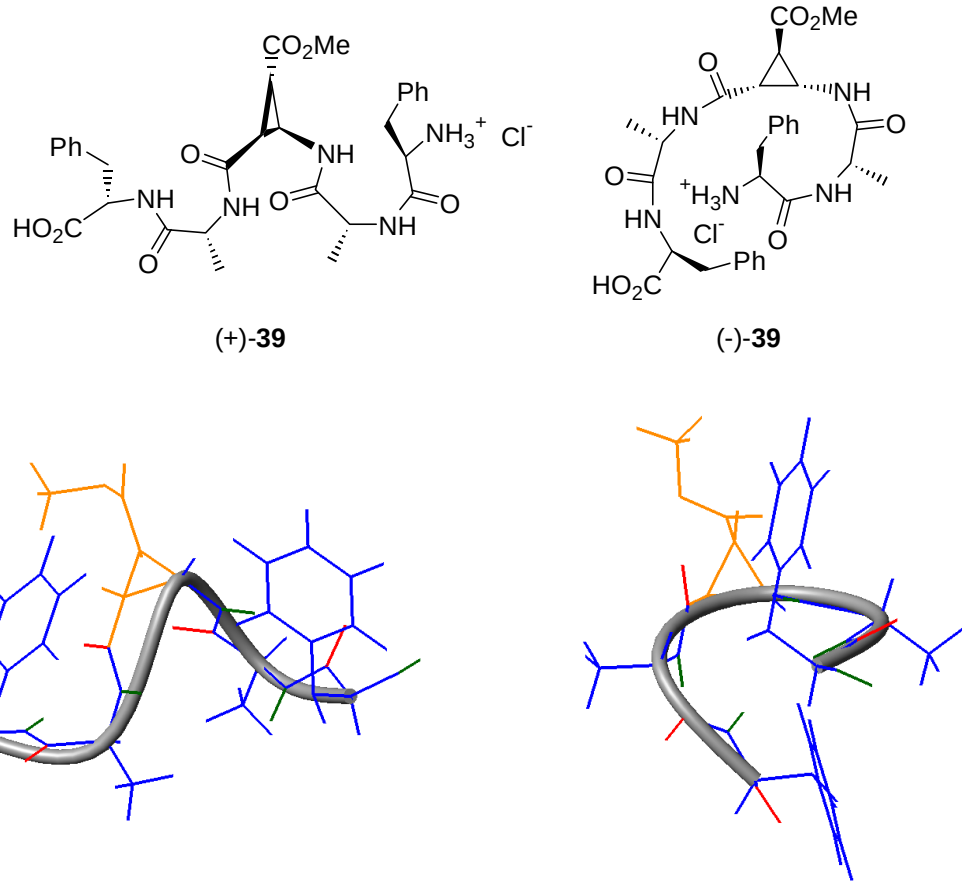


Figure 8. Lowest energy structure for (+)-**39** (left) and (-)-**39** (right) visualised in the program MOLMOL.

Compound (+)-**39** has an opened turned structure (Figure 8) sharper than (-)-**39**, where the backbone of residues 1 and 5 extends into opposite directions giving the structure a distorted Ω shape. On the other hand, (-)-**39** has a real turn like pattern with an helical outlook (Figure 8). Worth to note that the alanine side chains orientation in the sharper (+)-**39** turn (i.e. the side chains belonging to the direct neighbours of the β-ACC) are directed perpendicularly to the loop plane, while in (-)-**39** the alanine side chains lie on the turn plane and are directed outside it.

Small linear peptides are usually unstructured or exists as mixtures of conformers. Particular care must therefore be taken in order to prove that the conformers derived from the DYANA calculations are indeed physically relevant and do not represent artefacts from the structure calculation. It is clear that the presented structures are in exchange with differently folded conformers as evident from the $^{3}J_{HN,H\alpha}$ scalar couplings. However, the NMR data bear evidence that they exist in solution to a large extent. In fact, the NOEs have been calibrated so that the fixed distance between Hα and Hβ of ACC corresponds to the correct value. All NOEs have been assigned and used in the structure calculation. The target function was reasonably low indicating that all NOEs (except the sequential Hα/Hα NOE) can be fitted onto a single structure. Nevertheless, for a structured peptide in fast exchange with an unstructured (random coil) form, the ROEs may still be compatible with a single structure (but need not necessarily be). This situation was encountered for both diastereomers of **39** and for (-)-**43** (paragraph 4.2.3.2). On the other hand, when two or more conformational distinct species are in exchange, large violations in the structure calculations are expected, since the ROEs can not be fitted onto a single structure. This was indeed observed for (+)-**43** (paragraph 4.2.3.2).

Another question is whether the structural difference between the two diastereomers is really significant or due to a particular, wrongly assigned NOE in one of the two molecules. It was therefore checked, how many NOEs support the structures and whether these NOEs may be differently assigned. One of the two 1-$CH_2\beta$ (Phe)→4-CHα (Ala) NOEs is strong in (-)-**39**, while the other 1-$CH_2\beta$ (Phe)→4-CHα (Ala) is weak. Both NOEs are weak in (+)-**39**. Furthermore, the correlations encountered between the methyl group of Ala-2 and the methylene group of Phe-5 (2-$CH_3\beta$ (Ala)→5-$CH_2\beta$ (Phe)) is only seen in (-)-**39**. Both NOE support the type of turn that is found in the structure calculation for (+)-**39**. The NOEs occur in non-crowded regions of the spectrum and cannot be differently assigned. In contrast, (+)-**39** displays a 2-$CH_3\beta$ (Ala)→4-CHα (Ala) NOE not visible in the other diastereomer.

No hydrogen-bonds were found in the ensemble of NMR-derived structures, compatible with the data gained from the temperature coefficients that could not reveal their presence.

Finally, a ROESY at 2 mM concentration of (+)-**39** was recorded in order to exclude that the observed ROEs are *inter*molecular. In fact, in section 4.2.1, it has been noted that the temperature coefficient of NH-5 of (+)-**39** changed from -9.4 ppb at 10 mM concentration to -7.5 ppb at 2 mM. Such effect can be due to a change in the state of aggregation. However, the chemical shifts at 2 mM and 10 mM concentration are very similar and essentially the same. Moreover, the same ROEs were observed upon dilution indicating that the structure is concentration-independent. In particular, long-range contacts (i-i+3) such as 2-CHα (Ala)→5-CQδ (Phe) (Q = pseudo hydrogen atom) were visible at both concentrations.

Spectra of all peptides in aqueous solution (10 mM, pH = 3.1, acetate buffer) have been also recorded. The signal dispersion was slightly poorer in that solvent for both diastereomers of **39** and many structurally important long-range ROEs were placed in overlapped regions of the spectra. The clearly observed ROEs are similar in strength and hence, the peptides are expected to have similar structures in water and in methanol. However, due to the problems in assignment and integration in overlapping regions, no structure calculations were performed for that solvent system.

4.2.3.2 The solution structures of peptides containing two β-ACC units

For the peptides (+)-**43** and (-)-**43** the number of distance restraints found in the NOESY spectrum is large as in the previous case. As already observed for the diastereomer pair (-)-**39** and (+)-**39**, (-)-**43** presents i-i+4 restraints which are absent in (+)-**43** (Figure 9).

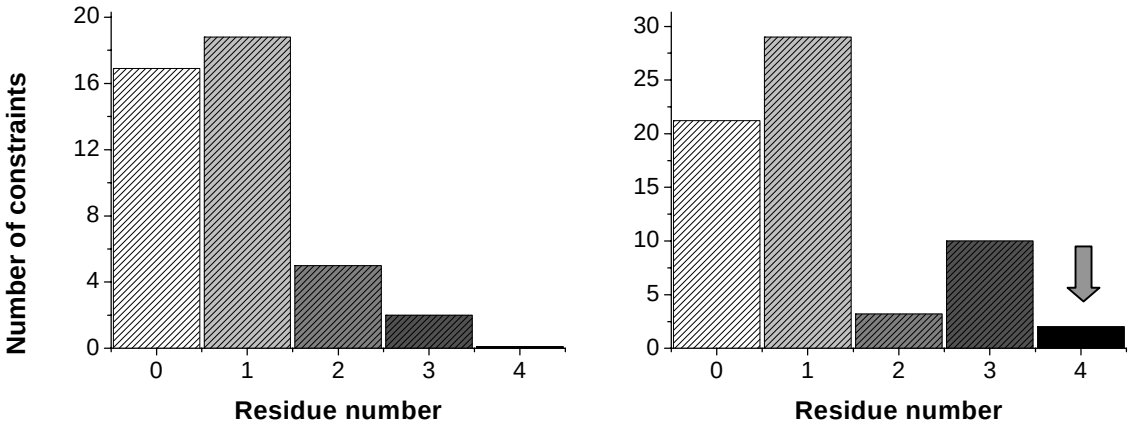


Figure 9. Number of distance restraints for the whole molecule *vs.* the distance between the interacting residues. Left: (+)-**43**; right: (-)-**43**.

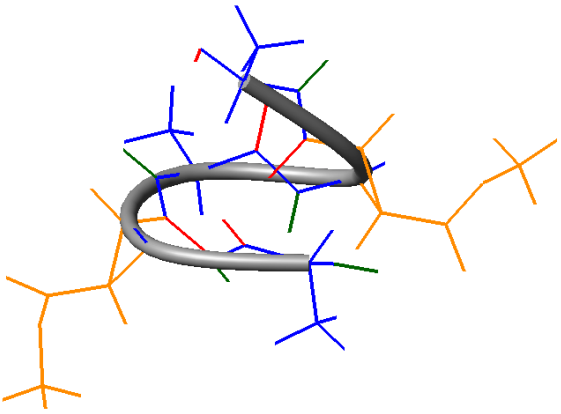
The upper limit (non-sequential) restraints used for the DYANA calculations are listed in Table 7.

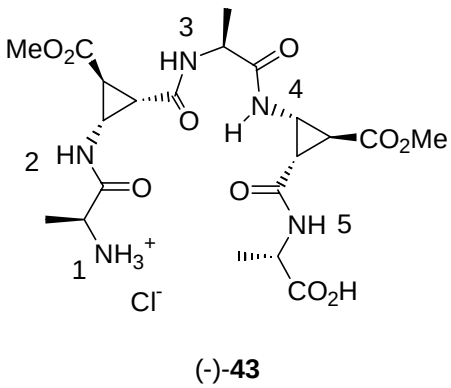
Table 7. Non-sequential distance restraints for (+)-**43** and (-)-**43**.

(+)-**43**	(-)-**43**	
QB(1)-HA(3)	HA(1)-HA(4)	QB(1)-HA(4)
HA(2)-HA(4)	HA(1)-HB(4)	QB(19-HG(4)
HB(2)-HN(4)	HA(1)-HG(4)	HN(2)-HN(4)
HB(2)-HA(4)	HA(1)-HN(5)	HN(2)-HA(5)
HB(2)-HA(5)	HA(1)-HA(5)	HA(2)-HA(5)
HG(2)-HA(5)	QB(1)-HA(3)	HA(2)-QB(5)
QB(3)-HA(5)	QB(1)-HN(4)	HB2-HN(4)
	HB(2)-HA(5)	

The restrained molecular dynamics calculations performed by DYANA on (-)-**43** gave reasonable results, with a low mean target function (0.18 ± 0.18) and few violations of the restraints. The RMSD for the backbone was higher than for (-)-**39** (1.48 Å). However, there are fewer protons in **43** than in **39**, due to the absence of the phenylalanine side chains, thereby reducing the number of cross peaks used to define the peptide structure. The calculations converged to a helix-like structure tighter than that found for (-)-**39** (Figure 10).

When looking at the primary sequence of **43** it is obvious that the molecule contains two Ala-ACC-Ala segments. In (-)-**39** this segment produced a turn. Fusing two of these segments together would produce a helical-type fold similar to the structure calculated for (-)-**43**. Based on the occurrence of the same 1→5 cross peaks on (-)-**40** (the corresponding Boc/Bn protected pentapeptide), this structure could also apply to (-)-**40**.





(-)-**43**

Figure 10. Lowest energy structure for (-)-**43** visualised in the program MOLMOL.

Applying the suitable MOLMOL option, no hydrogen bond could be found in (-)-**43** structure. In contrast, the reduced temperature coefficients indicate that NH-2 and NH-4 are probably hydrogen bonded while the $\Delta\delta/\Delta T$ values measured for (+)-**43** are all typical for solvated NHs (Table 1).

Looking for additional hydrogen-bonds compatible with the observed reduced temperature coefficients, the best 20 conformations of (-)-**43** were minimised with the program Spartan. In each minimised structure a hydrogen bond closing a 13-membered ring between 2-NH (ACC) and 4-C=O (ACC) was observed (Figure 11). The H--O distance was 2.6 Å before and 1.6 Å after minimisation, the N-H--O angle was 86° before 137° after minimisation. Moreover, a γ-turn centred on alanine-3 (hydrogen bond between 4-NH (ACC) and 2-C=O (ACC)), obtainable by simple rotation of about 15° of the φ angle of the alanine-3, yielded a hydrogen bond. The H--O distance was 2.6 Å before and 2.2 Å after minimisation, the N-H--O angle was 96° before 145° after minimisation. The minimisation led to considerable energy decrease compared to the starting conformations calculated by DYANA, but did not alter the overall shape of (-)-**43** structure. These data would confirm the $\Delta\delta/\Delta T$ values measured for (-)-**43**. However, since the calculation were performed in *vacuum*, solvation of amide functions is absent and hydrogen-bonded structures are likely to be overestimated. In solution interconversion between structures containing the corresponding hydrogen-bonds and solvated forms most likely exist.

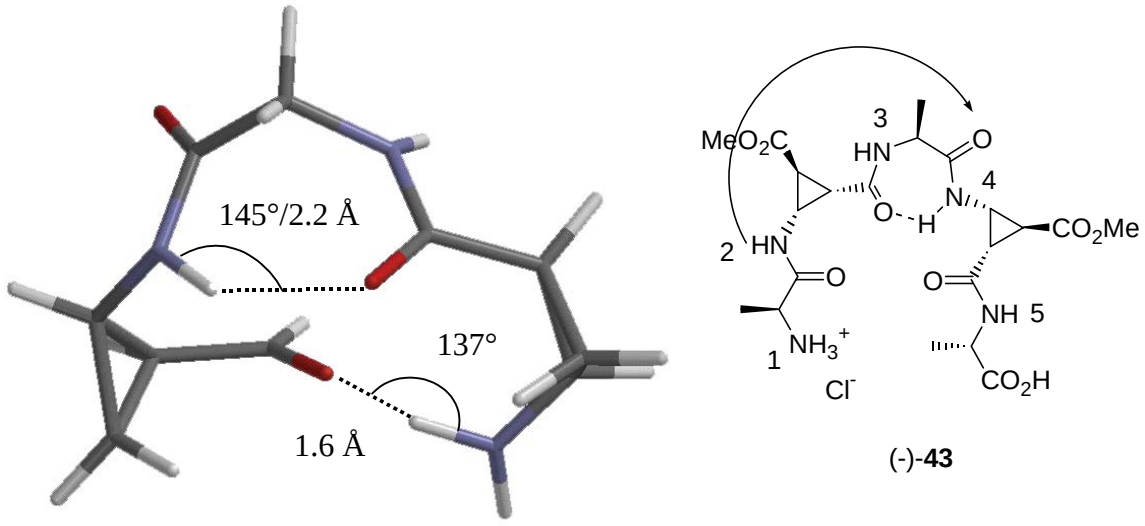


Figure 11. First DYANA structure after minimisation with the program Spartan. Side chains, Ala-1 and Ala-5 are nor displayed for reasons of clarity.

Unfortunately, the calculations performed on (+)-**43** did not converge to a distinct structure and the target function calculated by DYANA was still high, indicating that more than one conformer is significantly populated. Many restraint violations were found, in all the best 20 structures. Particularly one of them was severe, the 4CHα/5CHα (Table 8). As already illustrated for (+)-**39** (Figure 7), such NOE is indicative for a *cis* peptide bond between the 4th and the 5th residue.

Table 8. Distance restraints violations on (+)-**43**.

Constraint	mean violation	number of violated structures
	(+)-**43**	
1Qβ/3Hα	0.69	20
2Hα/4Hα	0.68	20
2Hβ/4Hα	0.86	20
2Hβ/5Hα	0.53	20
4Hα/5Hα	1.67	20
4Hβ/5HN	0.43	20

Indeed the DYANA calculations are usually performed with the ω angle constrained to 180°. Therefore the DYANA computations were repeated on (+)-**39**, (-)-**39**, (+)-**43** and (-)-**43** allowing ω to adopt values of zero or 180°. Table 9 indicates how often a *cis* bond is encountered in the 20 lowest energy conformers.

On (+)-**39**, (-)-**39** and (-)-**43** few *cis* bonds were found, and occur principally in the highest energy conformations. On the other hand, in (+)-**43** a 4/5 *cis* bond occurred in any of 20 lowest structures and a 3/4 *cis* bond, although present in only 11 structures, was found in the first 7 conformations (Table 9).

The DYANA calculations on (+)-**43** were repeated with the 4/5 peptide bond fixed at 0°. Unfortunately, although the violation 4CHα/5CHα disappeared, new strong violations occurred. It is obvious that at least two conformations rapidly exchange in solution, one of them has probably a *cis* peptide bond between the 4th and the 5th residue. Hence, the ensemble of observed NOEs may not be fitted onto a single structure, and the calculations should therefore be evaluated with great care.

Table 9. *Cis* peptide bond occurrences in DYANA conformers.
: indicates the presence of a *cis* peptide bond in the n^{th} conformation.

***cis* peptide bond**	**Conformations**				
	1	**5**	**10**	**5**	**20**
	(+)-**39**				
1/2			###0###	5	##0
3/4				# ## #	
4/5				5# # 0	
	(-)-**39**				
1/2			#	5# #	
2/3				#	
3/4				#	## 0
4/5					##

***cis* peptide bond**	**Conformations**				
	1	**5**	**10**	**5**	**20**
	(+)-**43**				
1/2			####	##5 ##	
2/3		5	## 0##	5 ### 0	
3/4		####5##		##5#	
4/5		####5####0####5#### 0			
	(-)-**43**				
1/2			0	#5#	
2/3				#	
3/4					#
4/5				#	####

In this chapter it could be shown that short α-peptides containing one or two β-ACC units have surprisingly well-defined structures in CD_3OH. The constrained β-amino acid is capable to induce turn-like structures: (-)-**11** influence drives the turn toward a helix evolution ((-)-**39** and (-)-**43**), while the (+)-**11** generates in (+)-**39** a **Ω** loop like divergence of the N- and C-*termini*. Surprisingly, neither (+)-**39** nor (-)-**39** structures are hold together by any hydrogen bond, in contrast, (-)-**43** presents two hydrogen bonds. The presence of two (-)-β-ACC units has a stronger stabilising effect on the peptide conformation. No convergence was obtained on the (+)-**43** structure calculations although a large number of distance constraints was found.

Chapter 5

Neuropeptide Y (NPY) is a 36 amino acid polypeptide hormone and neurotransmitter and is expressed in the central and peripheral nervous system. NPY is involved in the regulation of food intake, peripheral vascular resistance, sexual functioning, anxiety and stress response.[54] Five different G protein coupled receptors (Y_1, Y_2, Y_4, Y_5 and y_6) have been isolated,[55] but the exact role played by each of them in the various NPY activated bioprocesses is still object of investigations. The identification of the biologically active NPY conformations is another difficult but important target to achieve. NPY is a polypeptide having an α-helical structure between the 15th and the 31st amino acid but the C-*terminus* (residues 32-36), which mainly interacts with the receptor binding site, has no regular structure (Figure 1).[56] The synthesis of NPY analogues with a stabilised secondary structure at the C-*terminus* can clarify the role of the NPY receptors and the bioactive conformation of NPY. If the analogue fits into only one receptor, only the bioprocesses correlated to this receptor will be activated. On the other hand, if the affinity of an analogue is higher than that of the natural sequence, its conformation should be very close to the bioactive one.

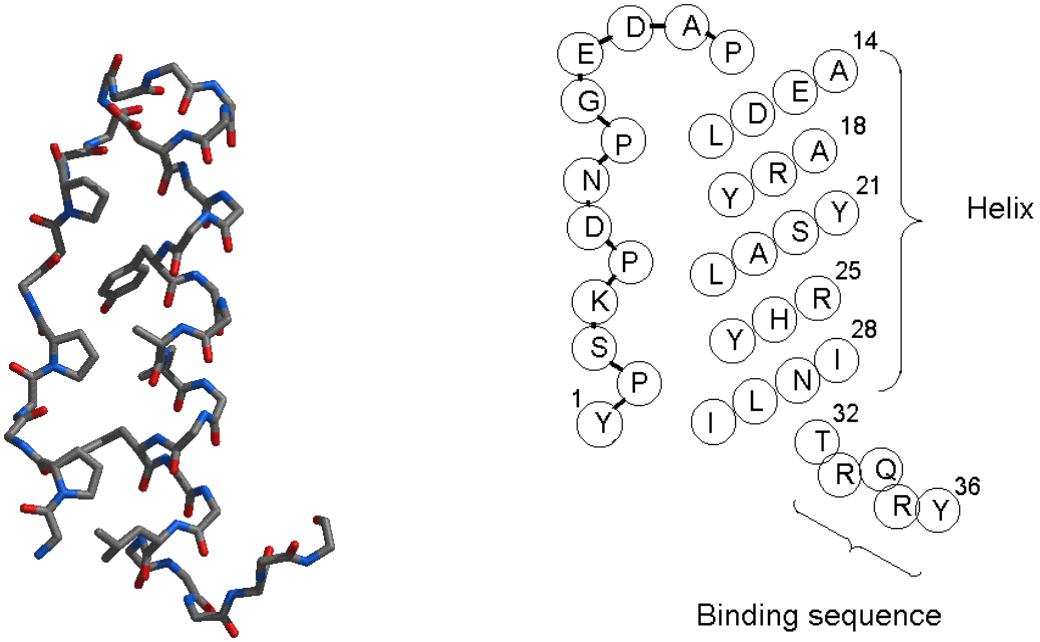
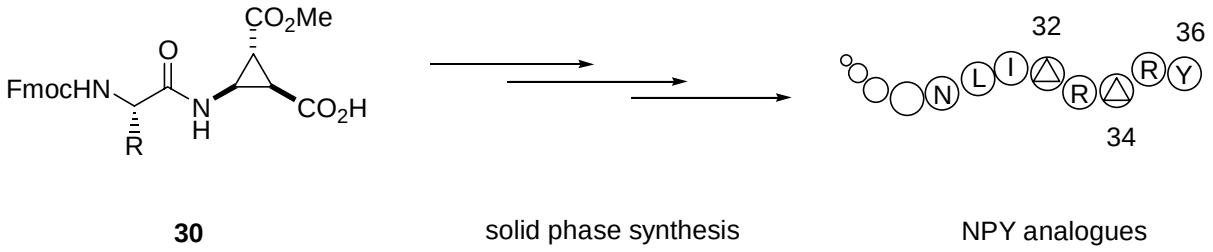


Figure 1. Three-dimensional model of NPY derived from pancreatic polypeptide (PP) crystal structure.

In collaboration with the research group of Prof. A. Beck-Sickinger dipeptides containing the β-ACC residue were built into the NPY (or truncated NPY) sequence at the position 32 and/or

34, the only choice at the C-*terminus*, since arginines at position 33 and 35 proved to be essential for binding (Scheme 1).



30 solid phase synthesis NPY analogues

Scheme 1. Synthesis of NPY analogues starting from *β*-ACC containing dipeptides **30**.

In Table 1 the investigated[57] NPY analogues containing the Ile-*β*-ACC,[58] Arg-*β*-ACC[58] and Gly-*β*-ACC ((+)-**30f**, see Chapter 1) dipeptides are shown.[†] They are all truncated NPY analogues (12 or 14 peptides) whose activity toward Y_1, Y_2 and Y_5 receptors was compared with the natural truncated sequence (cR35, Table 1).

As already pointed out in previous studies[25] the influence of (+)-**11** on the bioactivity of the NPY analogues is positive, leading to biologically active ligands selective toward Y_1, while its enantiomer (-)-**11** leads to inactive ligands.

In the series presented here (Table 1) once more the inefficacy of (-)-**11** to produce active ligands upon its incorporation into NPY analogues can be observed (*cf*. bV19 and bV20). The (+)-**11** building block incorpored at position 34 (cT5) seems to induce a good Y_1 selectivity while at position 32 (cQ33) no binding at all is encountered. This effect is unexpected since the corresponding not truncated NPY analogues ([R/S-ACC[32]]-NPY, Table 1) gave good binding values toward Y_5.[25] Therefore the dipeptide Gly-ACC ((+)-**30f**) was built in the 28-29 and 31-32 position (cR39). With glycine a greater degree of freedom was introduced aiming to favour the formation of a *β*-turn interacting with the receptor. Unfortunately, the ligand cR39 gave no relevant affinity to NPY receptors. On the other hand, two (+)-**11** residues in the 32 and 34 position bind very efficiently to Y_1 and moderately to Y_5. The selectivity and affinity of these truncated NPY analogues are distinctively higher than the truncated natural sequence (cR35).

In the positions 32 and 34 proline (cR43) and *β*-HGln (cT9) were also introduced to verify whether the effect observed with *β*-ACC derivatives is only due to the presence of a cyclic amino acid like proline or to the *β*-amino acid additional methylene group (*β*-HGln). In both

[†] Solid phase synthesis and biological tests performed by Norman Koglin, research group of Prof. A. Beck-Sickinger, Leipzig.

cases (cT9 and cR43) no binding was observed thus confirming that the influence of *β*-ACC on the structure and biological activity of the NPY analogues is due to its specific structural features.

Table 1. Bioactivity of truncated NPY-analogues on the human (h) Y_1- and Y_2-receptors and on rat (r) Y_5-receptor. Substitutions on the natural sequence are bold.
▲ : (RRR)-*β*-ACC, ▼ : (SSS)-*β*-ACC, **q**: *β*-HGln.
- = 0-25 %, + = 25-50 %, ++ = > 50 % change in specific binding.

Code	Sequence	hY_1	hY_2	rY_5
NPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLITRQRY-NH_2	++	++	++
cR35	Ac-RHYINLITRQRY-NH_2	-	+	-
[R/S-ACC^{32}]-NPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLI ⬍ RQRY-NH_2	>1000	>1000	43[a)]
bV19	Ac-RHYINLIT**R**▼R▼RY-NH_2	-	-	-
bV20	Ac-RHYINLIT**R**▲R▲RY-NH_2	++	-	+
cP8	Ac-RHYINLI▲R▲RY-NH_2	++	-	+
cT5	Ac-RHYINLITR▲RY-NH_2	++	-	-
cQ33	H-RHYINLI▲RQRY-NH_2	-	-	-
cR39	Ac-RHY**G**▲L**G**▲RQRY-NH_2	-	-	-
cT9	Ac-RHYINLITR**q**RY-NH_2	-	-	+
cR43	Ac-RHYINLI**P**R**P**RY-NH_2	-	-	-

a) IC_{50} [nM]. Synthesis and biological tests by Dr. Chiara Cabrele.[25]

These results confirm that the (+)-**11** building block exerts a significant influence on the structure of *α*-peptides, particularly if two (+)-*β*-ACC units are present at position 32 and 34. Interestingly the CD spectra of cP8 in buffer with or without the addition of TFE (30 %) are identical and have a minimum around 206 nm. The same minimum and behaviour toward TFE was observed in the present work on the peptides containing two or three (+)-*β*-ACC units. On the contrary, with cT5 as well as with (+)-**37a** and (+)-**39** (incorporating only one (+)-**11** unit) some helix-induction was observed upon addition of TFE. This suggests that the structure-inducing effect of (+)-**11** is manifested already on short peptides (5-6 residues) and leads to the same kind of structure as that of longer peptides.

The peptides (+)-**42** and (+)-**46** containing two and three *β*-ACC units respectively, have been synthesised as building blocks for other NPY analogues, but not yet built in the sequence and biologically tested.

Chapter 6

The interesting results on the structural properties of *β*-ACC containing peptides rise the problem that the synthesis of longer peptides is not practicable in solution. A method which allows the coupling of the *β*-ACC on the solid phase should be developed.

The solid phase synthesis can be performed by two alternative protecting group (PG) strategies: Boc (temporary PG)/Bzl (permanent PG) and Fmoc (temporary PG)/ ^{t}Bu (permanent PG). The Boc strategy implies the use of Merrifield linker (Figure 1) requiring liquid HF for the cleavage from the resin, highly acidic conditions which need a specialised apparatus and catalyse several possible rearrangements. In contrast, in the Fmoc strategy the cleavage of the peptide from the resin occurs under milder acidic conditions (TFA 50 % in CH_2Cl_2 for the Wang linker), therefore Fmoc protection is widely used. Another kind of linker has been developed to introduce extremely mild cleaving conditions, it is called Sasrin^{TM} (super acid labile resin), it is highly labile to acids (1% TFA in CH_2Cl_2) and therefore suitable for combinatorial chemistry and in peptide synthesis, for example, it allows the cleavage of side chain protected peptides.

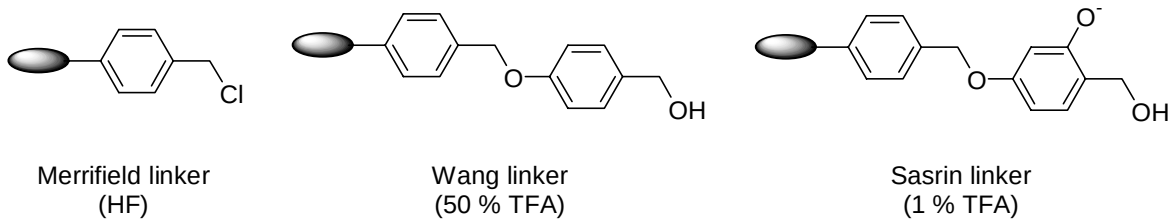
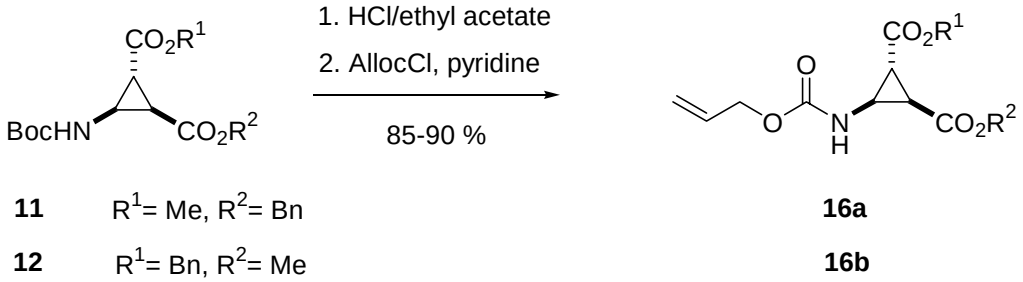


Figure 1. Various types of linkers for solid phase synthesis (cleaving conditions).

To make use of the *β*-ACC on acid labile resins, a N-protecting group is requested to be cleaved under neutral or weakly basic conditions. The protecting group should also allow *in situ* deprotection/coupling to prevent ring opening. The N-Fmoc protected *β*-ACC has been already synthesised but the coupling with acyl chlorides was unsuccessful, resulting only in ring-opening products.[26]

Among the many other N-protecting groups known in the peptide chemistry, the allyl carbamate (Alloc) seemed to be the most promising for the *β*-ACC unit. In fact, the deprotection is achieved under non-acidic conditions by catalytic amounts of palladium (0) in the presence of a nucleophile acting as allyl scavenger to avoid allylation at the free amine after deprotection to give **52** (Scheme 2).[59a] N-Alloc-*β*-ACC **16a** is already known, it can be synthesised in high yield and without epimerisation and it has been coupled with various acyl

chlorides giving 50 up to 70 % yields.[26] Compound **16b** could be obtained under the same conditions.



11 R^1= Me, R^2= Bn **16a**

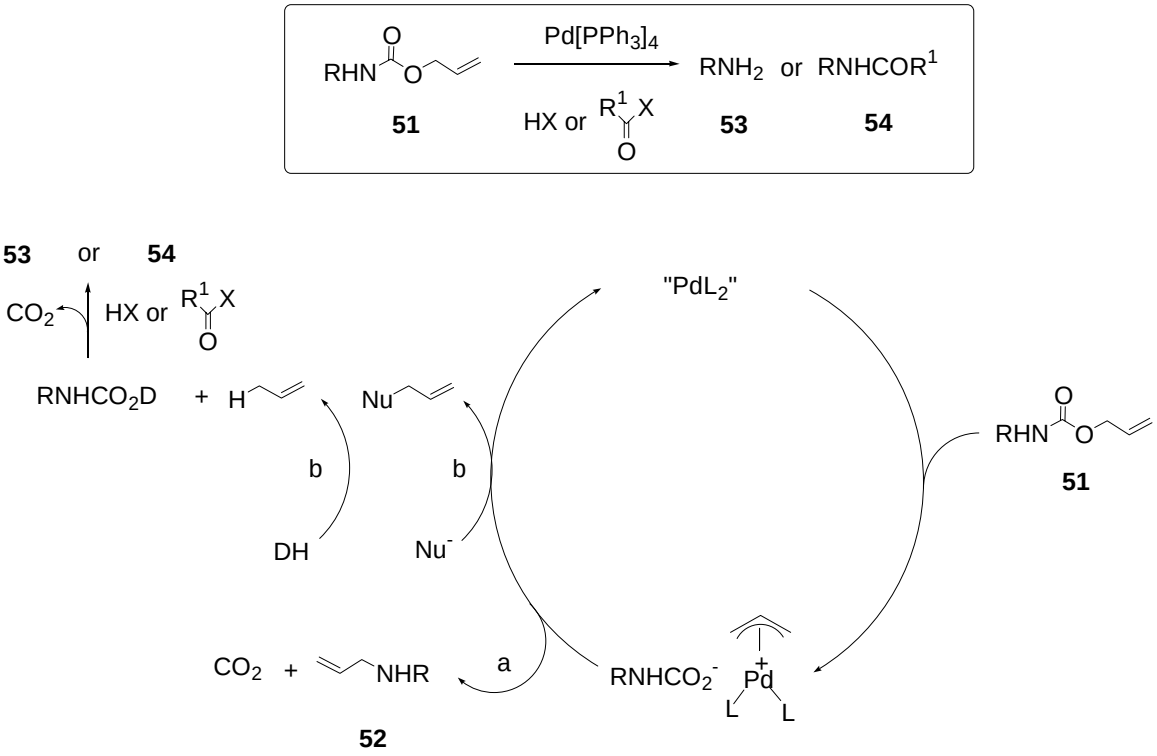
12 R^1= Bn, R^2= Me **16b**

Scheme 1. Synthesis of N-Alloc β-ACCs (**16a** and **16b**).

6.1 Tandem deprotection/coupling of N-Alloc protected amino acids in solution

In the present work the reaction conditions for one pot deprotection/coupling of **16a** to amino acids (Boc and Fmoc protected) has been optimised in solution and applied to the solid phase.
The choice of the allyl scavenger has been a critical point. In fact, non acidic deprotection conditions are requested for the Fmoc strategy and strong nucleophile scavengers (β-dicarbonyl compounds[59a] or secondary amines[59b-f]) capable to react with the preactivated amino acids are also not suitable. Only two categories of allyl scavengers fit these requirements: tertiary amines and some hydrides (Scheme 2).
Among the tertiary amines which have been reported as allyl scavengers there are pyridine,[60] triethylamine,[60] and N-methylmorpholine, but the latter is normally used in the presence of an acid (HCl or acetic acid).[59a] Pyridine and triethylamine have been rarely used in Alloc deprotection and, moreover, with low yields.[60]
Among the hydrides tributyltin hydride, sodium borohydride and phenylsilane have been successfully applied as allyl scavengers to the Alloc-peptide synthesis. The use of a hydride as allyl scavenger requires an acidic treatment after deprotection to isolate the free amine (**53**), while direct coupling with activated amino acids avoids the employment of acids (Scheme 2, b). Using tributyltin hydride the yields are good to excellent but the reactant is highly toxic.[61a,b] Sodium borohydride leads to significant amounts of the N-allylated amine (**52**, Scheme 2, a) in the only deprotection step, while in the one pot deprotection/coupling procedure it gives high yields (68-84 %) of **54** (Scheme 2). The deprotection/coupling protocol can be successfully applied to EDC/HOBt activated amino acids but fails with pentafluorophenyl activated amino acids which are reduced by $NaBH_4$ to the corresponding amino alcohols.[61c] The most efficient allyl scavenger, widely used in peptide chemistry, is

phenylsilane. It has been used to obtain free amines and for one pot deprotection/coupling with excellent yields, in solution as well as on solid phase. For the one pot deprotection/coupling protocol, many carboxy activated species (pentafluorphenyl esters, N-hydroxysuccinimmide esters, acyl fluorides and N-urethane-N-carboxy anhydrides) but so far no EDC/HOBt preactivated amino acids were used.[61d-g]



Nu$^-$ = amines, thiols, carboxylates, β-dicarbonyl compounds

D (hydride donor) = Bu_3Sn, $NaBH_3$, $PhSiH_2$

Scheme 2. Cycle of the palladium (0) catalyst in Alloc deprotection reactions: a) N-allylation side reaction; b) in the presence of nucleophiles or hydride donors as allyl scavengers.

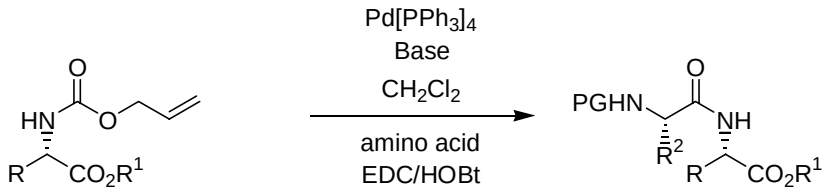
The first attempt to couple **16a** to an activated amino acid was performed with $PhSiH_3$. Compound **16a** was coupled with Fmoc-alanine in high yields (90 %) but, unfortunately, also 5 % ring opening was observed (Table 2, entry 7), which made the purification of the product a difficult task. Moreover, the use of phenylsilane (as any hydride) is complicated by the requirement of rigorously anhydrous conditions and by some difficulties in its storage for a prolonged period.

Therefore, the efficiency of pyridine as allyl scavenger was investigated. The coupling of N-Alloc natural amino acids was performed as test reaction (Table 1). Indeed, the dipeptides **56** were obtained in high yields (94-99 %) and N-Boc as well as N-Fmoc protected amino

acids were found to be compatible with the reaction conditions. Nevertheless, the reactions proceeded slowly (Table 1, entry 1) making 20 mol % of palladium (0) catalyst necessary to stay within acceptable reaction times (Table 1, entries 2-4).

Among the amines used as allyl scavengers pyridine is the less basic and the less nucleophilic (pK_a (piperidine, triethyl- and diethylamine) > 10; pK_a (morpholine) = 8.7; pK_a (pyridine) = 5.3). DABCO is a tertiary amine which has not yet been used as allyl scavenger in Alloc deprotection reactions, it is a stronger base (pK_a = 8.9)[62a] than pyridine and a good nucleophile.[62b] Indeed, the rate of the deprotection/coupling reactions performed with DABCO as allyl scavenger (Table 1, entries 5-8) dramatically accelerated, giving rise to **56a-e** after a reaction time of only 10-20 minutes when 10 mol % of palladium (0) were employed.

Table 1. Peptide coupling of Alloc protected amino acids with preactivated Boc and Fmoc amino acids.



55 **56**

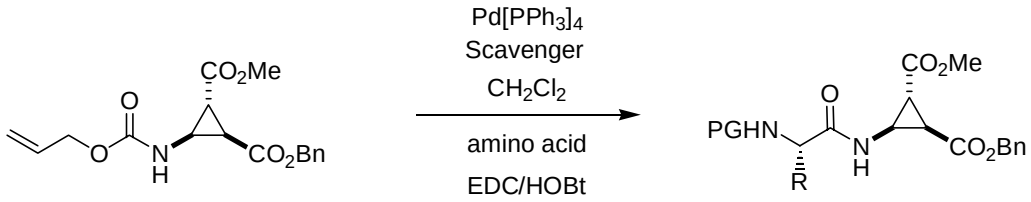
entry	R	R^1	R^2	PG		eq. activated a.a.	base[a)]	$Pd[PPh_3]_4$ [mol %]	time	yield [%]
1	Me	Bn	Me	Boc	**56a**	3	Py	10	15 h	94
2	Me	Bn	Bn	Boc	**56b**	3	Py	20	1.5 h	99
3	Me	Bn	Me	Fmoc	**56c**	1.5	Py	20	1.5 h	94
4	$CHMe_2$	Me	Me	Fmoc	**56d**	1.5	Py	20	1.5 h	98
5	Me	Bn	Me	Boc	**56a**	3	DABCO	10	10 min	99
6	Me	Bn	Bn	Boc	**56b**	3	DABCO	10	10 min	99
7	$CHMe_2$	Me	Me	Boc	**56e**	3	DABCO	10	20 min	97
8	$CHMe_2$	Me	Me	Fmoc	**56d**	1.5	DABCO	10	20 min	87

[a)] 20 eq. of pyridine or 5 eq. of DABCO were employed.

Most notably, under these reaction conditions N-Fmoc protected amino acids (Table 1, entries 3, 4 and 8) are tolerated as coupling partners, so that the synthesis of peptide fragments with the Fmoc strategy is possible.

Both pyridine and DABCO deprotection/coupling protocols were applicable to **16a**: the dipeptides **25** could be obtained in high yields (92-97 %) (Table 2). Again the use of DABCO resulted in considerable shortened reaction times, but another advantage of this procedure in comparison to the use of pyridine became apparent: although ring opening side products formation could be suppressed in both cases, epimerisation was observed to a substantial extent (up to 20 %) when pyridine was employed. In contrast, epimerisation was minimal (< 3 %) when DABCO was used, indicating that this reagent is not responsible solely for the cleavage of the allyl group but might also activate the HOBt ester of the amino acid used as the coupling partner. Again, N-Boc and N-Fmoc protected amino acids could be used as substrates.

Table 2. Peptide coupling of **16a** with preactivated Boc and Fmoc amino acids.



16a **25a**-**b** and **25g**-**h**

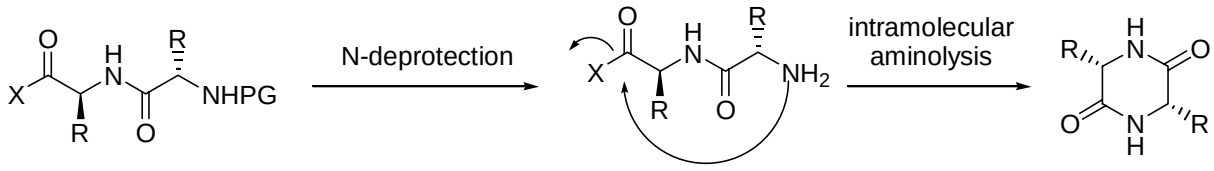
entry	R	PG		eq. activated a.a.	scavenger[a)]	$Pd[PPh_3]_4$ [mol %]	time	yield [%]
1	H	Boc	**25g**	3	Py	20	2 h	93
2	Me	Boc	**25a**	3	Py	10	15 h	95
3	Bn	Boc	**25h**	3	DABCO	10	15 min	96
4	Me	Fmoc	**25b**	1.5	DABCO	10	15 min	93
5	H	Boc	**25g**	3	DABCO	10	15 min	92
6	Me	Boc	**25a**	3	DABCO	10	15 min	97
7	Me	Fmoc	**25b**	1.5	$PhSiH_3$	10	15 min	90 [b)]

[a)] 10 eq. of pyridine or 5 eq. of DABCO or $PhSiH_3$ were employed.

[b)] Contaminated with 5 % ring opening product.

6.2 Suppression of DKP formation by DABCO protocol

Several residue- or sequence-specific side reactions can occur during the solid phase peptide synthesis (SPPS). One of the most important is the formation of diketopiperazines (DKP, **59**) as side products in peptide chemistry (Scheme 3).[63a] Due to the presence of particular sequences prone to DKP formation, when the terminal amino group is liberated (**58**) an intramolecular aminolysis at the i-1 peptide bond occurs with formation of the corresponding cyclic dipeptide (DKP) and interruption of the chain elongation.[63b]

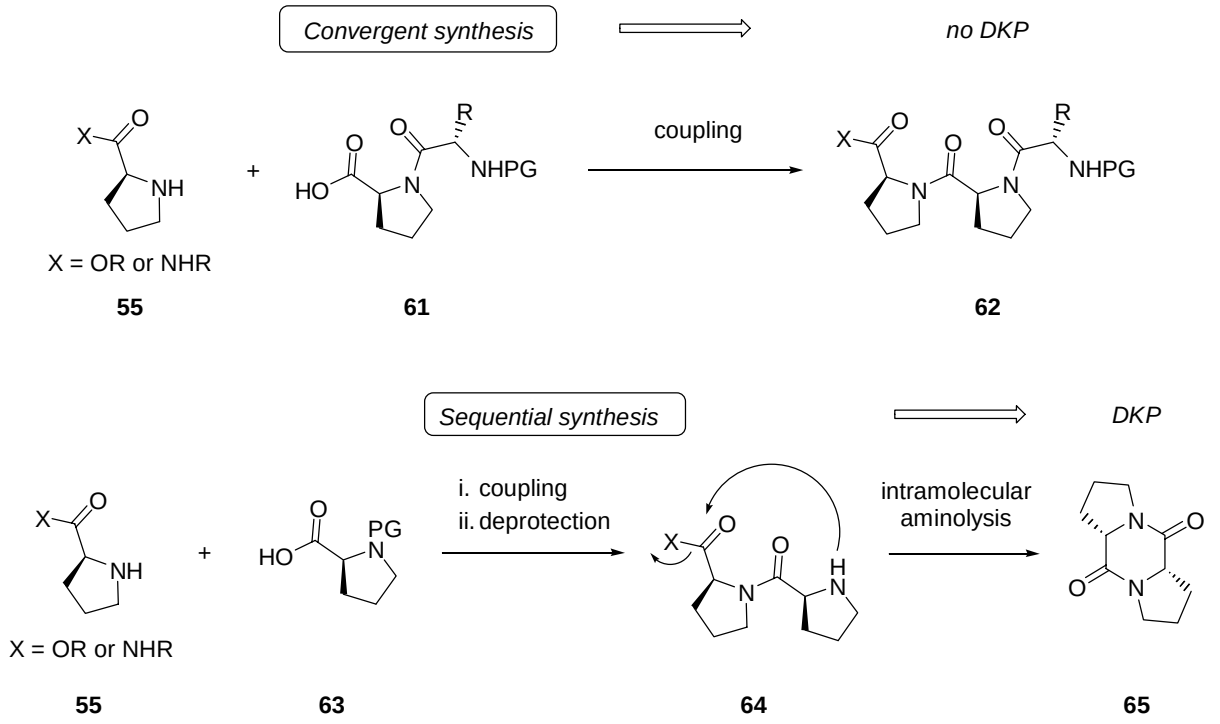


Scheme 3. DKP side-reaction.

Sequences containing amino acids able to reverse the chain direction, and thus bringing in **58** the free amino group near to the i-1 peptide bond, are particularly prone to DKP formation. Proline and N-methyl amino acids can adopt *cis* peptide bond conformations, glycine favours any type of turn, due to the lack of a side chain. These amino acids in either the first or the second position of the elongating chain produce substantial amounts of DKP during the deprotection step. The cyclisation can be hardly avoided when a strong turn forming sequence involves the first and the second residue of the peptide. A combination of a D- and a L-amino acids is very unfavourable and the presence of two adjacent prolines is particularly troublesome.

On the solid phase the DKP problem is relevant at the third coupling cycle, because the ester bond between the peptide and the linker is prone to intramolecular aminolysis.

Various solutions have been proposed to avoid this undesired side-reaction: the use of a sterically hindered linkers (trytil-resins)[63b] preventing a nucleophilic attack at the ester bond; the use of the Boc/Bzl specially designed coupling protocols involving *in situ* neutralisation[63b] and some other particular Fmoc/^{t}Bu protocols. Nevertheless, these solution can be applied to solve specific problems but are not of general use. Moreover, the incorporation of a Pro-Pro sequence in a peptide remains a difficult task which is often resolved by the incorporation of the second and third amino acid as the protected dipeptide **61** (Scheme 4).



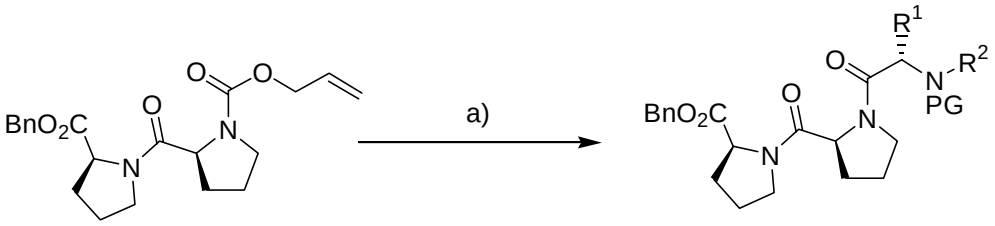
Scheme 4. Convergent synthesis (top) to prevent DKP formation (bottom) in the presence of the Pro-Pro sequence.

An efficient way to suppress DKP formation in solution and on the solid phase is the entrapment of the free amine *in situ* by acylation prior to intramolecular aminolysis. An early example is the Cbz deprotection/coupling in the presence of Pd/C and activated Boc- or Teoc-amino acids.[64] This strategy is not very efficient since it is not compatible with Fmoc chemistry and makes use of a heterogeneous catalyst, which would not be washed out during the solid phase synthesis.

The Alloc-protection followed by the Pd (0)/$PhSiH_3$ tandem deprotection/coupling reaction already proved to prevent DKP formation.[61f] Therefore the efficiency of the deprotection/coupling protocol using DABCO as allyl scavenger was tested. The coupling of a peptide containing two prolines at the N-*terminus* with a further amino acid is one of the most DKP sensitive sequences, thus the dipeptide **66** chain elongation was taken as a representative example (Table 3).

Indeed, the coupling successfully afforded the tripeptides **67** in high yields and short reaction times, moreover, no DKP **65** was observed.

Table 3. Suppression of DKP formation with the Alloc/DABCO tandem deprotection/ coupling protocol.



66 **67**

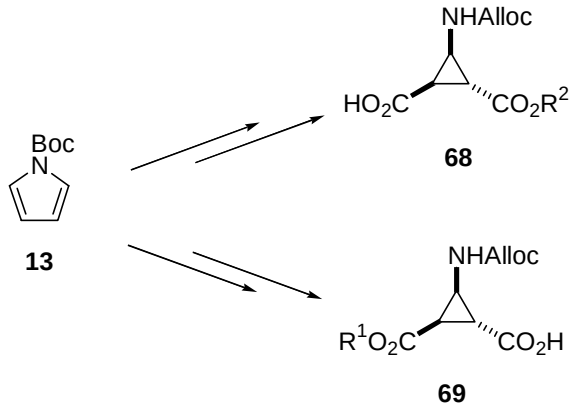
entry	activated a.a.	coupling product		time	yield [%]
1	N-Boc-Gly-OH	BnO-Pro-Pro-Gly-NHBoc	**67a**	15 min.	90
2	N-Fmoc-Ala-OH	BnO-Pro-Pro-Ala-NHFmoc	**67b**	15 min.	94
3	N-Fmoc-Pro-OH	BnO-Pro-Pro-Pro-NFmoc	**67c**	30 min.	96

[a] Reaction conditions: $Pd[PPh_3]_4$ (10 mol %), DABCO (5 eq.), EDC/HOBt/amino acid (3 eq.), CH_2Cl_2, room temperature.

6.3 Application of the DABCO protocol to the solid phase synthesis

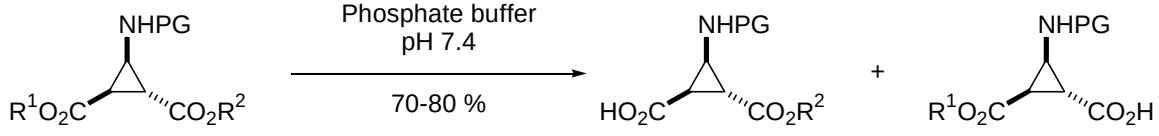
The efficiency of the DABCO protocol was then tested on the solid phase.

The first problem which arises is the synthesis of the free acids **68** or **69** (Scheme 5) to be coupled on the resin. The strategy so far employed to arrive at free carboxylic acids of the β-ACC structure has been the hydrogenolysis of the corresponding benzyl esters such as **24** or **25**. However, the hydrogenolysis of the benzyl ester would also affect the Alloc protecting group. At the present there is only one synthesis developed affording selectively **68** and **69**, but it requires many steps on the N-Boc pyrrole **13** (Scheme 5).[65]



Scheme 5. Synthesis of N-Alloc β-ACC free acids **68** and **69**.

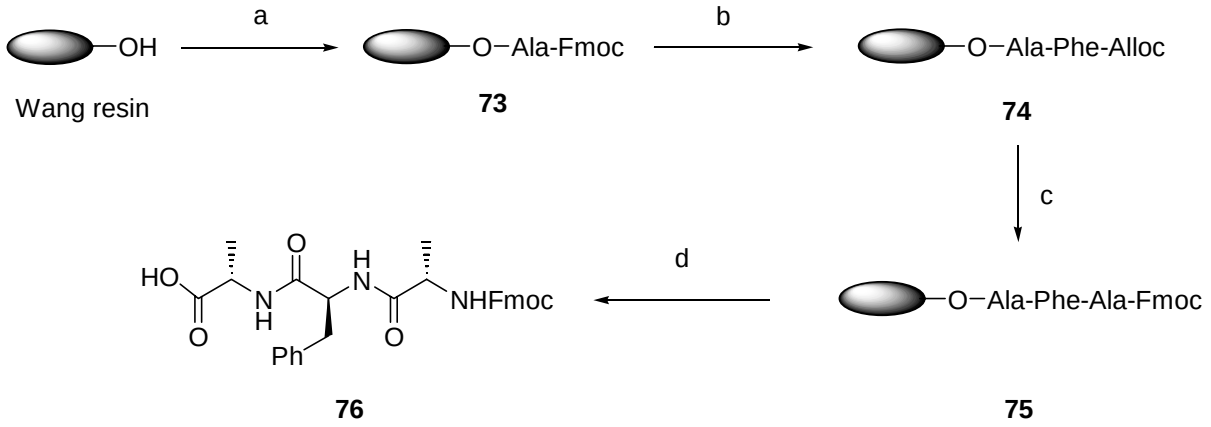
Another efficient possibility to arrive at **68** or **69** acids would be to cleave one of the two ester groups on **16** selectively by enzymatic hydrolysis. The enzymatic resolution of racemic cyclopropanes bearing two methyl ester groups has been already successfully carried out.[66] The enantiomeric mixture of the cyclopropanated Boc-pyrrole **13** has been also enzymatically resolved,[21d] while no success was obtained on the enantiomeric mixture of **11** analogue having two methyl ester functions. Nevertheless, only a regioselective hydrolysis of **16a** is required to achieve the synthesis of **68a** or **69a**. Such a strategy would permit to obtain the free acids in only two steps starting from the well known and efficient synthesis of **11** and **12**. Three different β-ACC derivatives (**16a-b** and **70**) were hydrolysed by treatment with PLE-E1 (Pig Liver Esterase), to investigate the dependence of the enzymatic regioselectivity on the ester nature and on its stereochemistry (Scheme 6). It could be observed that the benzyl and the phenacetyl (Phac) esters are more efficiently hydrolysed compared to the methyl ester, probably due to the better interaction of the more lipophylic aromatic group with the enzyme binding site. On the other hand the stereochemistry plays also an important role, in fact, the benzyl ester group is more extensively hydrolysed when it occurs in the *trans* position with respect to the amino group (*cf.* **16a** and **16b**).



16a	R^1= Bn, R^2= Me, PG = Alloc	PLE-E1	**68a**	2:1	**69a**
70	R^1= Phac, R^2= Me, PG = Boc	PLE-E1	**71**	5:1	**72**
16b	R^1= Me, R^2= Bn, PG = Alloc	PLE-E1	**68b**	1:8	**69b**

Scheme 6. Enzymatic hydrolysis of β-ACC derivatives **16a**, **70** and **16b**.

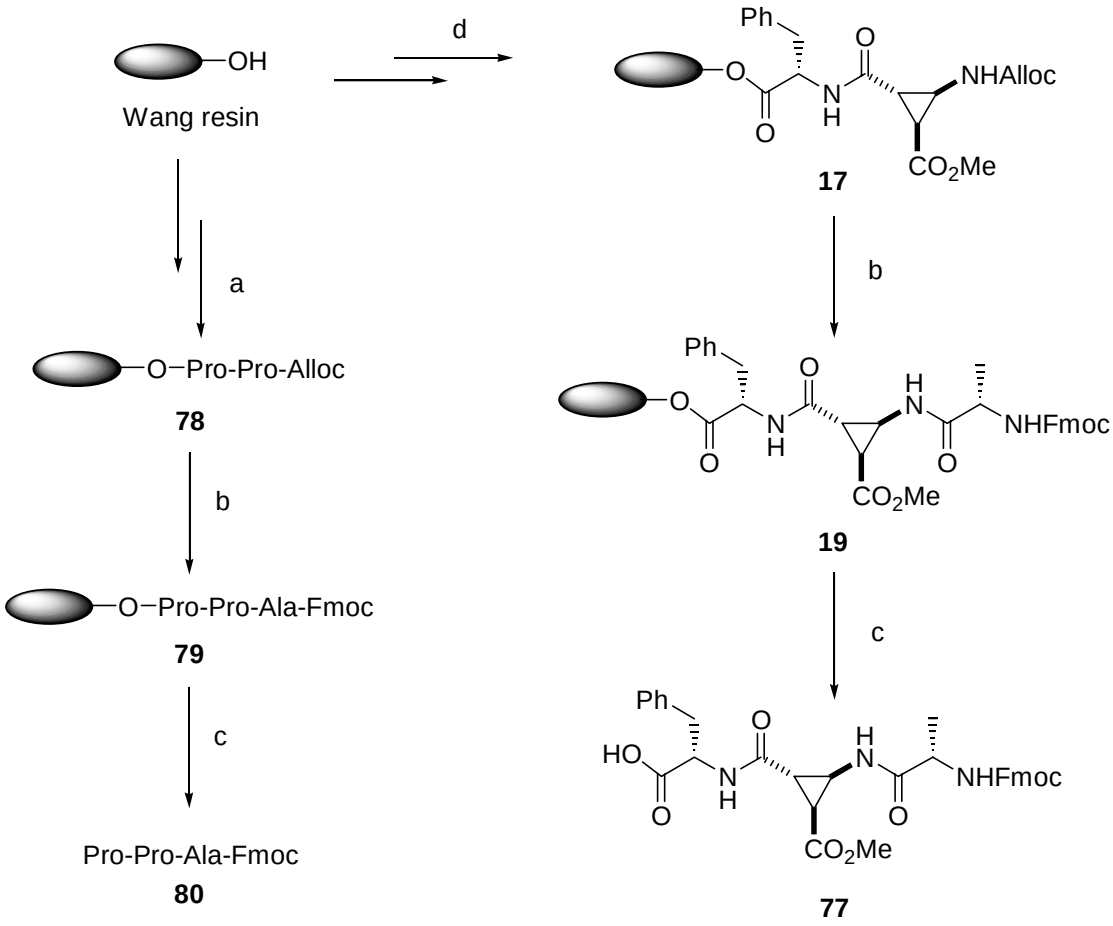
The hydrolysis of **70** and **16b** gave the best enrichment in only one free acid (the *cis* and *trans* respectively as racemate); **69b** could be isolated pure by recrystallisation and was then employed as building block in the solid phase synthesis.



Scheme 7. *a*: Fmoc-Ala-OH/DIC/HOBt (3 eq.), DMAP (0.1 eq.), DMF. *b*: i. piperidine/DMF. ii. Alloc-Phe-OH/DIC/HOBt (3 eq.), DMF. *c*: Fmoc-Ala-OH/EDC/HOBt (15 eq.), $Pd[PPh_3]_4$ (0.2 eq.), DABCO (18 eq.),CH_2Cl_2, 2 h, r.t.. *d*: TFA/CH_2Cl_2 2:1. Yield: 99 %.

First, the Alloc deprotection/coupling protocol with DABCO as allyl scavenger was brought on the solid phase for the synthesis of a non critical sequence to prove its applicability (Scheme 7). The tripeptide Fmoc-Ala-Phe-Ala **76** was obtained in high yields confirming the quality of the coupling method also on the solid phase.

The coupling conditions were then optimised and applied to the troublesome synthesis of the sequence Fmoc-Ala-Pro-Pro-OH (**80**) and of a tripeptide containing the β-ACC **69b** (Scheme 8). For a direct comparison of the results, the reactions on the solid phase were performed with both DABCO and phenylsilane protocols. DABCO resulted of comparable efficiency with phenylsilane, affording high yields and short reaction times. The advantage of DABCO on phenylsilane is the stability of the reagent to moisture[67] which allows an easy application to the automated synthesis.



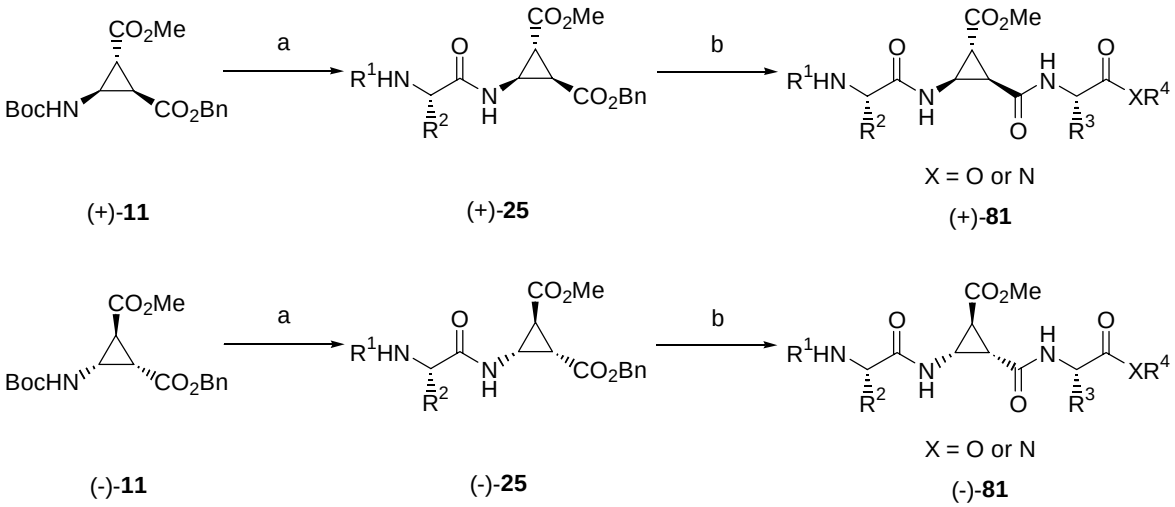
DABCO protocol	99 % [e]	DABCO protocol	85 % [f]
$PhSiH_3$ protocol	99 % [e]	$PhSiH_3$ protocol	83 % [f]

Scheme 8. *a*: i. Fmoc-Pro-OH/DIC/HOBt (3 eq.), DMAP (0.1 eq.), DMF. Loading 0.85 mmol/g. ii. piperidine/DMF. iii. Alloc-Pro-OH/DIC/HOBt (3 eq.), DMF. *b*: Fmoc-Ala-OH/EDC/HOBt (6 eq.), $Pd[PPh_3]_4$ (0.2 eq.), Allyl scavenger (12 eq.), CH_2Cl_2, 2 h, r.t.. *c*: TFA/CH_2Cl_2 2:1. *d*: i. Fmoc-Phe-OH/DIC/HOBt (3 eq.), DMAP (0.1 eq.), DMF. Loading 0.6 mmol/g. ii. piperidine/DMF. iii. **69b**/DIC/HOBt (1.5 eq.), DMF. *e*: Without further purification after cleavage from the resin. *f*: The product was purified by column chromatography after cleavage from the resin.

In conclusion the coupling of the N-Alloc protected β-ACCs was developed and optimised in solution (**16a**) and on the solid phase (**69b**). A new tandem deprotection/coupling protocol was applied on N-Alloc protected amino acids (or peptides) making use of Pd(0) as catalyst, DABCO as allyl scavenger and EDC/HOBt preactivated Boc- and Fmoc-amino acids. This protocol affords good to excellent yields within short reaction times. DABCO was found to be an allyl scavenger as effective as phenylsilane, but cheaper, easier to store and to employ.

Summary

In this work the synthesis of α-peptides containing the β-ACC building blocks (+)-**11** and (-)-**11** was developed and their structure was investigated by IR, CD and NMR spectroscopy. The synthesis of β-ACC containing peptides was achieved in solution by Boc/Bn protection strategy (Scheme 1).



Scheme 1. *a*: i. HCl/ethyl acetate. ii. N-protected amino acid/EDC/HOBt, pyridine. *b*: i. Pd/C, cyclohexadiene or formic acid. ii. EDC/HOBt, C-protected amino acid, pyridine.

By means of IR spectroscopy, the extent of the hydrogen-bonding network in derivatives of β-ACC **11** could be estimated. Molecular modelling calculations assisted to give reasonable hypotheses for the location of the hydrogen-bonds, however, only calculation in vacuum have been carried out, therefore the hydrogen-bond contribution is overestimated. It could be proved that the β-ACC unit can force hydrogen-bonded sharp turns forming 6-membered (C_6), 7-membered (C_7), 8-membered (C_8) or 9-membered (C_9) rings.

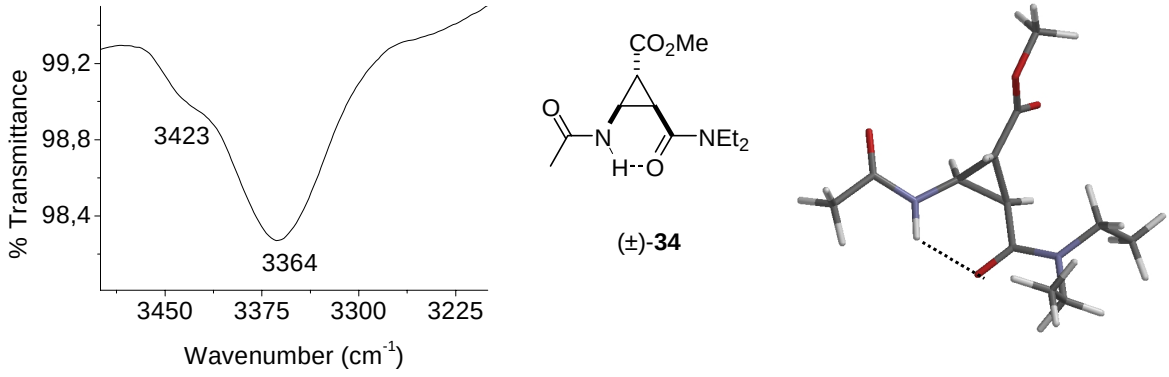


Figure 1. Left: IR amide A region of (±)-**34**, right: C_6 global minimum calculated for (+)-**34**.

The 6-membered conformation is the most constrained (Figure 1). It has been encountered only in **34** and **35c** (Figure 2) with only the cyclopropyl NH free for binding and containing a tertiary amide group at the β-ACC C-*terminus*, which makes the carboxyl amide group a particularly good hydrogen-bond acceptor. If other hydrogen-bonding patterns were possible, C_8 and C_9 rings resulted to be more stable (Figure 2). Moreover, the presence of an amino acid at the β-ACC N-*terminus* seems to favour a γ-turn structure.

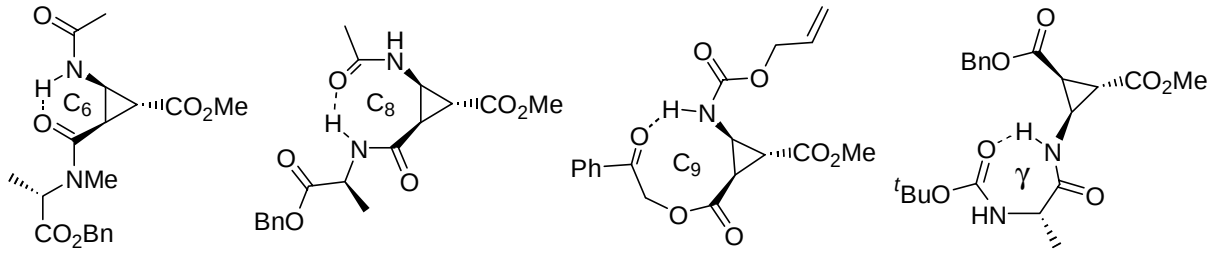
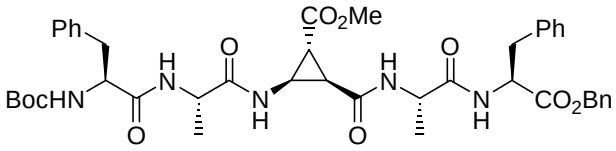
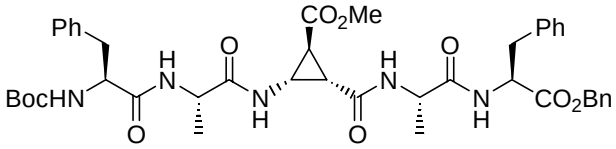
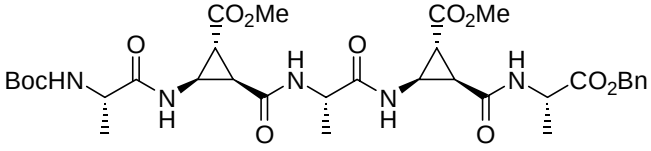
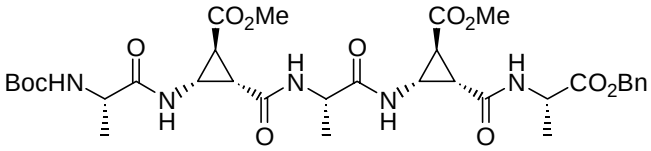


Figure 2. C_8 hydrogen bonded structure calculated for (+)-**35b**, C_9 for (+)-**50** and γ-turn for (+)-**25a**.

The mixture of the two diastereomers of the dipeptide Boc-Ala-β-ACC-OBn (**25a**) could be resolved by recrystallisation allowing an easy access to dia- and enantiomerically pure peptides containing the unit Ala-β-ACC. Circular dichroism (CD) measurements for oligomers incorporating (-)-**11** showed often β-turn like CD patterns, while the derivatives of (+)-**11** displayed helix-like CD patterns.

A detailed picture of the conformations of (+)-**39**, (-)-**39**, (+)-**43** and (-)-**43** in solution (Figure 3) could be achieved by CD and 2D NMR spectroscopy. Since **39** and **43** are unprotected peptides, they are not soluble in CH_2Cl_2 and IR measurements were performed only on the corresponding protected forms (+)-**37a**, (-)-**37a**, (+)-**40** and (-)-**40** (Table 1). The IR spectra of **37a** and **40** diastereomers showed a substantial difference in the hydrogen-bonding extent stabilised by the two β-ACC enantiomers, demonstrating the influence of the chirality of the β-ACC building block when combined with α-amino acids. It was found that (+)-**11** promotes the formation of fewer hydrogen-bonds than (-)-**11** (Table 1). This effect was surprisingly not observed on the hexapeptide (+)-**45** containing three (+)-**11** units, in which five of the six NHs are hydrogen-bonded, suggesting some regular structure to occur.

Table 1. Extent of the hydrogen-bonding network on pentapeptides measured by IR spectroscopy in CH_2Cl_2.

		non HB N-H (cm^{-1})	HB N-H (cm^{-1})	non HB/HB
	(+)-**37a**	3412	3309	2:3
	(-)-**37a**	3420	3364	2:5
	(+)-**40**	3421	3362	2:3
	(-)-**40**	3419	3350	1:4

The CD spectra of the protected (**37a** and **40**) and unprotected (**39** and **43**) diastereomers displayed similar patterns suggesting that the protecting groups do not substantially influence their conformation. Once again, it was striking the difference in the influence of the two *β*-ACC enantiomers on the peptides structure. The CD spectra of (-)-**11** containing peptides were usually solvent dependent (Figure 3, (-)-**39** and (-)-**43**, right) and occasionally typical random coil CD spectra were observed ((-)-**43** in TFE, Figure 3). In contrast, the (+)-**11** unit induced helix-like CD patterns (Figure 3, (+)-**39** and (+)-**43**, left) which showed little solvent dependence. Moreover, the introduction of two or three (+)-**11** units resulted in very similar CD spectra, so stable to any change of solvent that even TFE could exert no structuring effect (*cf.* (+)-**43** in methanol and in TFE, Figure 3).

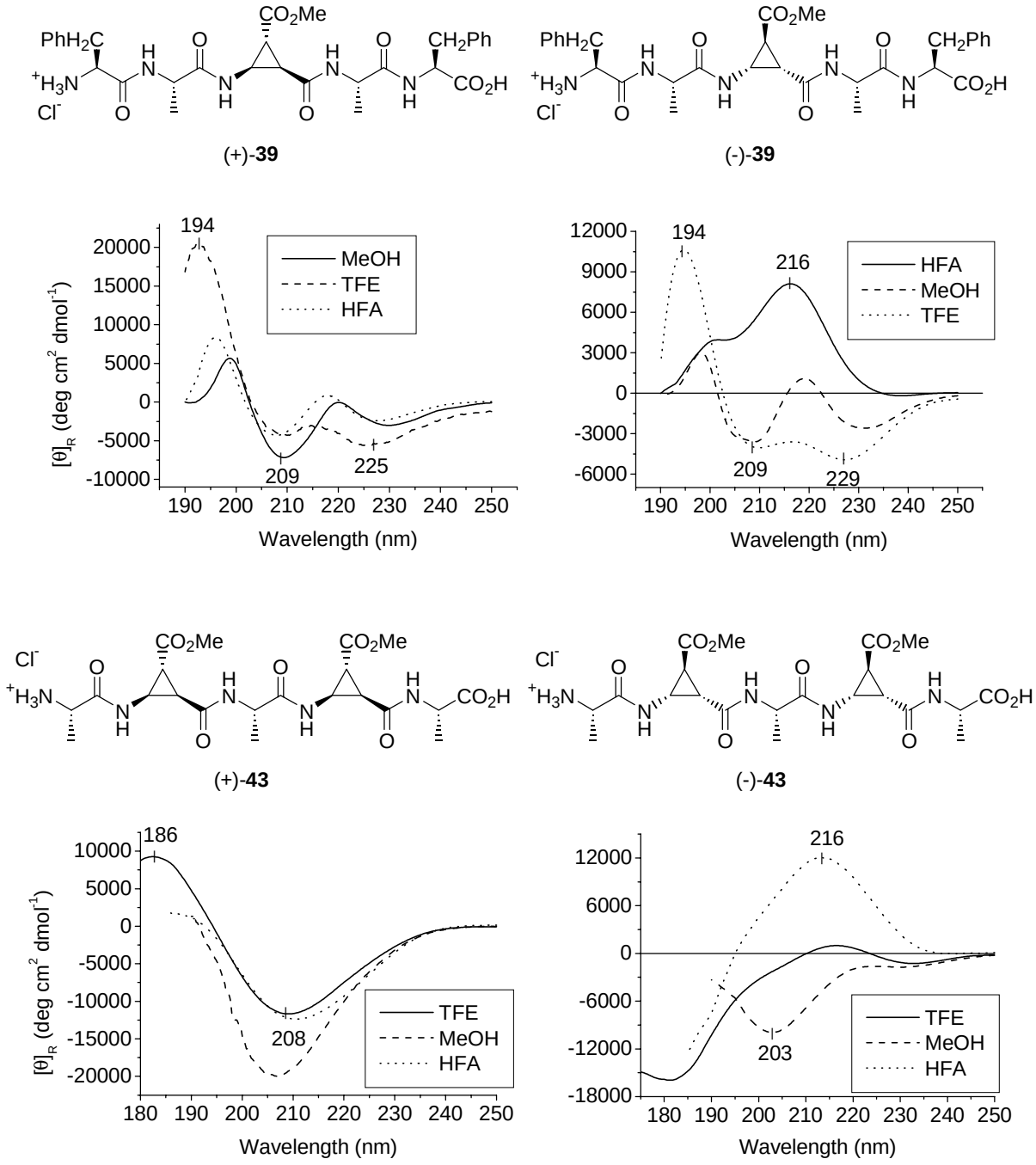


Figure 3. CD spectra of (+)-**39** and (+)-**43** (left) and (-)-**39** and (-)-**43** (right) in various solvents.

Finally, by means of high resolution NMR spectroscopy the conformation of the pentapeptides **39** and **43** in methanol-d_3 could be more precisely depicted. The temperature coefficients were indicative for hydrogen-bonding only in the case of (-)-**43**. Successively, ROESY spectra provided distance restraints for molecular dynamics calculations. The number of interproton distances obtained for each oligomer by ROESY spectra, resulted to be high for so small peptides, suggesting that their structure should be well defined. The molecular

dynamics calculation did not converge for (+)-**43**, probably due to the presence of at least two different conformations differing for the *cis*/*trans* configuration at the peptide bond between the 4th and 5th residue. In contrast, (+)-**39**, (-)-**39** and (-)-**43** calculations converged to turn like structures (Figure 4): (+)-**39** turn is the sharpest, having an **Ω** like N- and C-*terminus* divergence; (-)-**39** and (-)-**43** turns are both helix-like structured but in (-)-**43** it seems to be sharper.

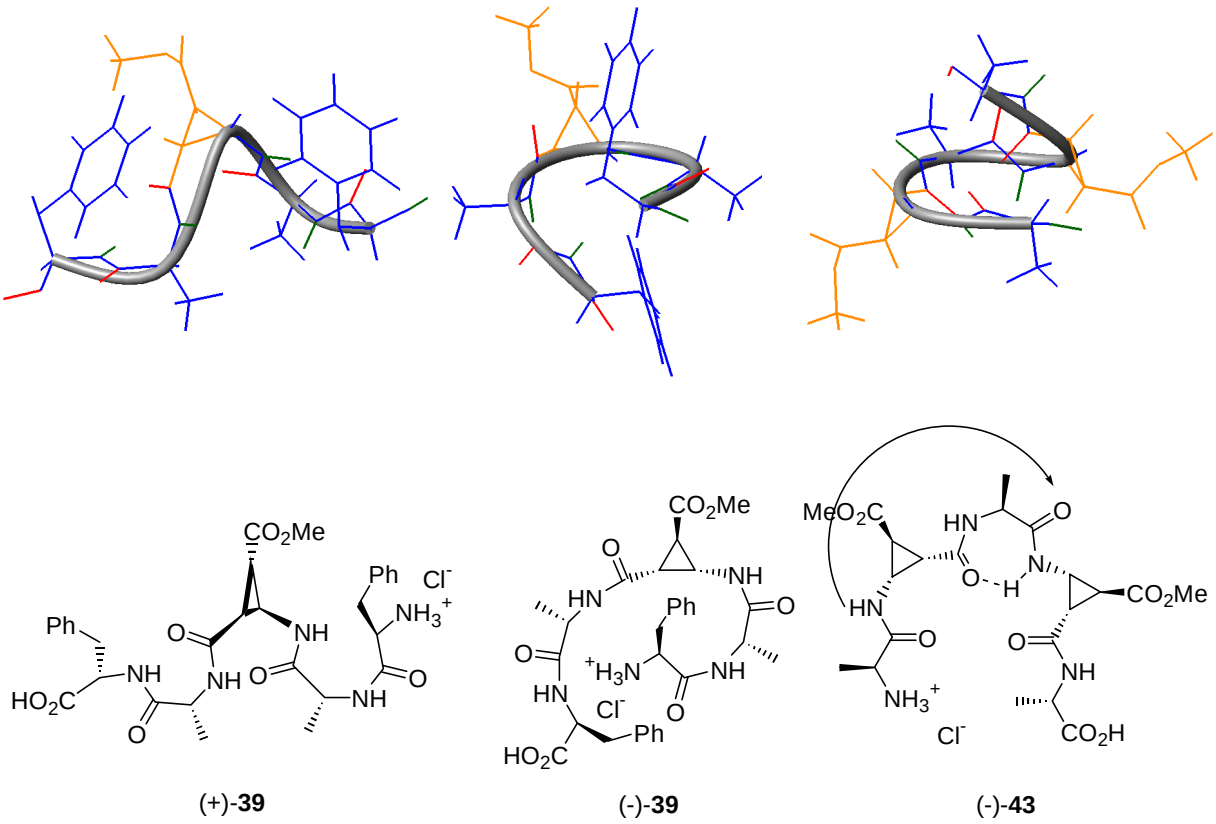
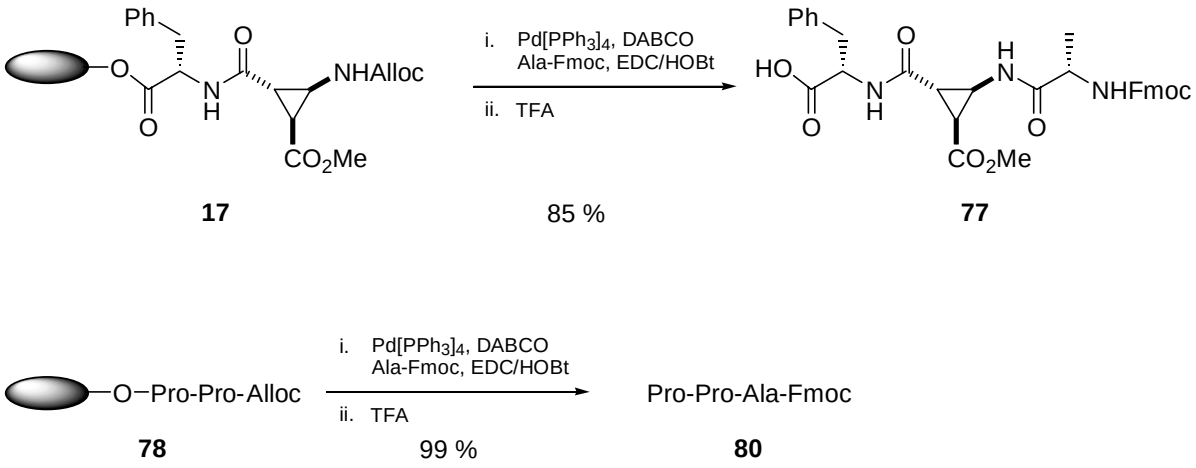


Figure 4. Structures calculated for (+)-**39**, (-)-**39** and (-)-**43**.

A further aim of this work was the design of a synthetic procedure for the incorporation of the *β*-ACC unit in peptides making use of the solid phase technique. Compatibility with the common Fmoc-coupling strategy could be achieved by protection of the *β*-ACC N-*terminus* with the allyloxycarbonyl (Alloc) group, which can be removed by treatment with palladium (0) as the catalyst in the presence of an allyl scavenger. To avoid ring opening under deprotection conditions, an *in situ* deprotection/coupling protocol was developed.

DABCO was found to be an especially suitable allyl scavenger for the coupling of *β*-ACC derivatives (Scheme 2), affording high yields, short reaction times and preventing ring

opening and epimerisation, in solution as well as on the solid phase as depicted with the synthesis of **77**.



17 85 % **77**

78 99 % **80**

Scheme 2. Two examples of the application of the Alloc/DABCO protocol to the synthesis of **77** and **80** on the solid phase.

The Alloc/DABCO protocol was also successfully applied to the chain-elongation of the Pro-Pro dimer (in solution and on the solid phase), being a sequence particularly prone to intramolecular aminolysis with consequent interruption of the chain elongation. For instance **78** could be coupled with Fmoc-Ala giving rise to the tripeptide **80** in almost quantitative yield.

Experimental part

1. Instruments and general techniques

^{1}H NMR Bruker ARX 400 (400 MHz), Bruker AC 250 (250 MHz). The chemical shifts are reported in δ (ppm) relative to chloroform ($CDCl_3$, 7.26 ppm), dymethylsulfoxide (DMSO-d_6, 2.49 ppm), methanol (CD_3OD, 3.34 ppm) and tetramethylsilane (TMS, 0 ppm). The spectra were analysed by first order, the coupling constants are reported in Hertz (Hz). Characterisation of signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, s br = broad singlet, dd = double douplet, dt = double triplet, dq = double quartet, ddd = double double doublet. Integration is determined as the relative number of atoms. Diastereomeric ratios were determined by comparing the integrals of corresponding protons in the ^{1}H NMR spectra.

^{13}C NMR Bruker ARX 400 (100.6 MHz), Bruker AC 250 (62.9 MHz). The chemical shifts are reported in δ (ppm) relative to chloroform ($CDCl_3$, 77.0 ppm), dymethylsulfoxide (DMSO-d_6, 36.9 ppm), methanol (CD_3OD, 49.0 ppm) and tetramethylsilane (TMS, 0 ppm). ^{13}C NMR resonance assignment were aided by the use of the DEPT 135 (DEPT = distortionless enhancement by polarisation transfer) technique to determine the number of hydrogens attached to each carbon atom and is declared as: + = primary or tertiary (positive DEPT signal intensity), - = secondary (negative DEPT signal) and quat = quaternary (no DEPT signal intensity) carbon atoms. In some cases DEPT 90 spectra were recorded to distinguish between primary and tertiary carbon atoms. This is marked with the CH or CH_3 notation at the corresponding signal.

2D-NMR Bruker DRX-500 spectrometer operating at a basic ^{1}H frequency of 500 MHz at 298 K. The spectra were recorded at 280 K, using the solvent line (CD_3OH, ^{1}H δ 3.31) for referencing. DQF-COSY, 80 ms-TOCSY and 500 ms-ROESY standard experiments were performed with suppression of the methanol OH line by low-power presaturation.

IR-spectra were recorded with an ATI Mattson Genesis Series FT-IR or a Bio-Rad Excalibur series FT-IR.

MS-spectra: masspectroscopy department of the University of Regensburg, Varian Mat 311 A.

Elemental analysis: microanalytical department of the University of Regensburg.

Melting points (m p) were determined with a Buchi SMP 20 and are uncorrected.

Thin layer chromatography (TLC) was performed on alumina plates coated with silica gel (Merck silica gel 60 F 254, layer thickness 0.2 mm). Visualisation was accomplished by UV-light (wavelenght λ = 254 nm) and a vanillin/sulphuric acid solution.

Optical rotations were measured on a Perkin-Elmer-Polarimeter 241 with sodium lamp at 589 nm in the specified solvent. The optical rotation was calculated with the following formula:

$$[\alpha]_D^{\vartheta} = ([\alpha]_{exp} \times 100)/ (c \times d)$$

θ = temperature (°C)
$[\alpha]_{exp}$ = measured value
c = concentration (g/100 ml)
d = length of the cuvette

CD spectra were measured on a JASCO model J-710/720 at the Institut für Bioanalytic und Sensorik of the University of Regensburg (research group of Prof. Dr. O. Wolfbeis) at 21° C between 300 and 180 nm in the specified solvent, the number of scans ranging between 10 and 50. The length of the cylindrical cuvettes was 1 or 0.1 mm, the resolution was 0.2 nm, the band width 1.0 nm, the sensitivity 100 mdeg, the response 0.25 s, the speed 50 nm/min. The background was subtracted to each spectrum. The absorption value is measured as Molar Ellipticity per Residue (deg cm^2 $dmol^{-1}$). The spectra were smoothed by the adjacent averaging algorithm with the Origin 6.0 program.

Column chromatography was performed on silica gel Geduran SI 60 (70-230 mesh) purchased from Merck and flash chromatography on flash-silica gel 60 (230-400 mesh ASTM) purchased from Merck.

Solvents were purified according to standard laboratory methods. THF, diethyl ether and toluene were distilled over sodium/benzophenone before use. Dichlormethane, DMSO and DMF were distilled over calcium hydride and acetonitrile over P_2O_5. Methanol was refluxed 2 h over magnesia, distilled and stored under nitrogen over 4 Å molecular sieves. The hexanes used had a boiling point of 40-60 °C. All solvents were distilled before use. Other chemicals were purchased from commercial suppliers and used as received.

All reactions with oxygen or moisture sensitive reactants were performed under nitrogen atmosphere.

For some peptide coupling the peptide synthesiser ACT90 of Advanced ChemTech was used.

2 Synthesis of compounds

2.1 General procedures

General procedure for solid phase loading. GP1

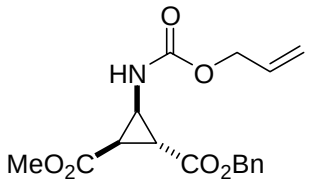
The resin (Wang resin) was swelled in CH_2Cl_2 for 20 min.. The mixture was drained and N-Fmoc amino acid (3 eq.), DIC (3 eq.); HOBt (3 eq.) and DMAP (0.1 eq.) in DMF (12 ml/g of resin) were added. The resin was agitated at room temperature overnight. The mixture was drained and the resin washed with DMF (5 x 15 ml/g of resin), CH_2Cl_2 (5 x 15 ml/g of resin), methanol (5 x 15 ml/g of resin) and diethyl ether (5 x 15 ml/g of resin). The resin was dried under vacuum at room temperature overnight. The loading of the resin was estimated from the weight gain of the resin; spectrophotometrically from the amount of Fmoc released from a weighed sample of resin; by elemental analysis and by estimation of the amount of amino acid released by cleavage from the resin (about 200 mg).

General procedure for solid phase coupling of Fmoc/Alloc-protected amino acids on Fmoc-protected amino acids loaded on a resin. GP2

The resin was swelled in CH_2Cl_2 (15 ml/g of resin) for 20 min.. The mixture was drained and piperidine 20 % in DMF (15 ml/g of resin) was added. The resin was agitated for 20-40 min.. The mixture was drained, the resin was washed 10 x with DMF and a solution of N-Fmoc/Alloc amino acid (3 eq.), DIC (3 eq.) and HOBt (3eq.) in DMF (12 ml/g of resin) was added. The resin was agitated 2 h at room temperature. The solution was filtrated and the resin was washed with DMF (5 x 15 ml/g of resin), CH_2Cl_2 (5 x 15 ml/g of resin), methanol (5 x 15 ml/g of resin) and diethyl ether (5 x 15 ml/g of resin). The coupling yield was verified by cleavage of the dipeptide from the resin (about 200 mg), or by the Kaiser test.
The number of equivalents of amino acid, DIC and HOBt used when the reactions were performed on the synthesiser ACT90 of Advanced ChemTech was increased three times.

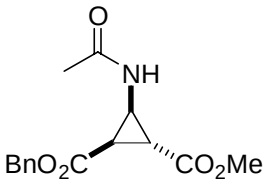
2.2 Preparation of compounds

2.2.1 Coupling at the N-terminus



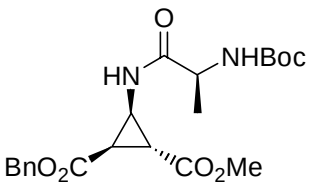
(±)-**16b**

(1R*, 2R*, 3R*) 3-Allyloxycarbonylamino-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((±)-**16b):** A solution of (±)-**11** (209 mg, 0.60 mmol) in HCl 3 M in ethyl acetate (4 ml) was stirred at 0 °C for 3 h. The solution was concentrated in vacuum and the salt resuspended in CH_2Cl_2 (20 ml). Allyloxycarbonyl chloride (64 µl, 0.6 mmol, 1 eq.) was added, then the mixture was cooled down at 0 °C and pyridine (97 µl, 1.2 mmol, 2 eq.) was added dropwise. The mixture was stirred overnight at room temperature. The solvent was evaporated and the product purified by chromatography (ethyl acetate/hexanes 1:2) to afford a yellow oil (188 mg, 95 %). - R_f ((±)-**16b**): 0.21. - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.33 (dd, *J* = 4.9, 4.9 Hz, 1H, cyclopropyl-CH), 2.50 (dd, *J* = 3.1, 8.3 Hz, 1H, cyclopropyl-CH), 3.74 (s, 3H, CH_3O), 3.86-4.03 (m, 1H, cyclopropyl-CHN), 4.57-4.59 (m, 2H, $=CH_2$), 5.08-5.16 (m, 2H, $PhCH_2O$), 5.15-5.34 (m, 2H, OCH_2CH), 5.76 (s br, 1H, NHAlloc), 5.78-5.97 (m, 1H, $CH=CH_2$), 7.30-7.38 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 26.1 (+, cyclopropyl-CH), 28.8 (+, cyclopropyl-CH), 37.6 (+, cyclopropyl-CHN), 52.6 (+, CH_3O), 66.1 (-, CH_2O), 67.3 (-, CH_2O), 118.0 (-, $=CH_2$), 128.4 (+, Ph-CH, 2C), 128.5 (+, Ph-CH), 128.7 (+, Ph-CH, 2C), 132.5 (+, =CH), 135.2 (C_{quat}, Ph-C), 155.9 (C_{quat}, N(CO)O), 169.5 (C_{quat}, C=O), 170.3 (C_{quat}, CO). - MS CI (NH_3) *m/z* (%) 351 (MNH_4^+, 100), 334 (MH^+, 7). - IR (film) 3360, 3067, 3033, 2954, 1727, 1522, 1428, 1356 cm^{-1}. - Anal. Calcd for $C_{17}H_{19}NO_6$ (333.34): C, 61.25; H, 5.74; N, 4.20. Found: C, 61.36; H, 5.96; N, 4.10.

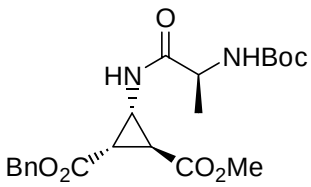


(±)-**24**

(1R*, 2R*, 3S*) 3-Acetylamino-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((±)-**24):** A solution of (±)-**11** (470 mg, 1.35 mmol) in HCl 3 M in ethyl acetate (4 ml) was stirred at 0 °C for 3 h. The solution was concentrated in vacuum and the salt resuspended in CH_2Cl_2 (20 ml). Acetyl chloride (173 µl, 2.4 mmol, 1.8 eq.) was added, then the solution was cooled down at 0 °C and triethylamine (598 µl, 4.3 mmol, 3.2 eq.) was added dropwise. The mixture was stirred overnight at room temperature. The solvent was evaporated and the product purified by chromatography ($CHCl_3$/MeOH 60:1) to afford a white solid (380 mg, 97 %). - R_f ((±)-**24**): 0.21. - mp 68-70 °C. - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.92 (s, 3H, CH_3C=O), 2.28 (dd, *J* = 5.0, 5.0 Hz, 1H, cyclopropyl-CH), 2.54 (dd, *J* = 5.2, 8.3 Hz, 1H, cyclopropyl-CH), 3.66 (s, 3H, CH_3O), 4.07 (ddd, J = 4.8, 8.1, 8.1 Hz, 1H, cyclopropyl-CHN), 5.09 (d, *J* = 12.2 Hz, 1H, CH_2O), 5.19 (d, *J* = 12.2 Hz, 1H, CH_2O), 6.64 (d, *J* = 7.6 Hz, 1H, NH), 7.31-7.39 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 23.0 (+, *C*H_3C=O), 26.2 (+, cyclopropyl-CH), 28.6 (+, cyclopropyl-CH), 36.2 (+, cyclopropyl-CHN), 52.3 (+, CH_3O), 67.4 (-, CH_2O), 128.3 (+, Ph-CH, 2C), 128.5 (+, Ph-CH, 2C), 128.7 (+, Ph-CH), 135.1 (C_{quat}, Ph-C), 169.8 (C_{quat}, C=O), 170.0 (C_{quat}, C=O), 170.6 (C_{quat}, C=O). - MS CI (NH_3) *m/z* (%) 309 (MNH_4^+, 100), 292 (MH^+, 18), 274 (20), 257 (17), 201 (21). - IR (KBr) 3332, 3063, 2959, 1728, 1654, 1525, 1453, 1385, 1317 cm^{-1}. - Anal. Calcd for $C_{15}H_{17}NO_5$ (292.30): C, 61.85; H, 5.88; N, 4.81. Found: C, 61.47; H, 5.88; N, 4.71.



(+)-**25a**

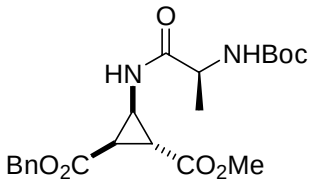


(-)-**25a**

(1R, 2R, 3S) and (1S, 2S, 3R) 3-(2S-*tert*-Butoxycarbonylamino-propionylamino)-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((+)-**25a** and (-)-**25a):** A solution of (±)–**11** (1.170 g, 3.35 mmol) in HCl 3 M in ethyl acetate (11 ml) was stirred at 0 °C for 3 h. The solvent was evaporated and a solution of Boc-alanine (950 mg, 5.02 mmol,

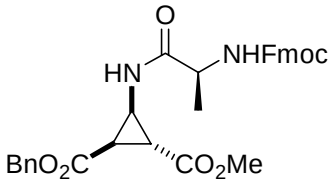
1.5 eq.), EDC (964 mg, 5.02 mmol, 1.5 eq) and HOBt (677 mg, 5.02 mmol, 1.5 eq.) in CH_2Cl_2 (250 ml) (previously stirred 1 h at 0 °C and 1 h at room temperature) was added. Triethylamine (510 µl, 3.64 mmol, 1.1 eq.) was then added dropwise at 0 °C. The mixture was stirred overnight at room temperature. The solution was washed with saturated $NaHCO_3$ (200 ml), 1 M $KHSO_4$ (200 ml) and saturated $NaHCO_3$ (200 ml). The organic phase was dried over $MgSO_4$ and concentrated. The mixture of the two diastereomers was purified by chromatography (CH_2Cl_2/MeOH 40:1).Yield: 1.34 g (95 %). 5-12 % epimerisation was observed. (+)-**25a** was isolated as a white solid by recrystallization from ethyl acetate/hexanes. (-)-**25a** was obtained pure as a white solid by recrystallization of the mother liquor. (+)-**25a** - R_f ((+)-**25a**): 0.17. - mp 148-150 °C. - $[\alpha]_D^{21}$ -33.8 (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.31 (d, *J*= 7.1 Hz, 3H, C*H*$_3$CH), 1.46 (s, 9H, $(CH_3)_3C$), 2.29 (dd, *J* = 5.0, 4.9 Hz, 1H, cyclopropyl-CH), 2.56 (dd, *J* = 5.2, 8.3 Hz, 1H, cyclopropyl-CH), 3.70 (s, 3H, CH_3O), 4.01-4.18 (m, 2H, cyclopropyl-CHN + Ala-CHN), 4.88 (s br, 1H, NHBoc), 5.11 (d, *J* = 12.1 Hz, 1H, CH_2O), 5.20 (d, *J* = 12.1 Hz, 1H, CH_2O), 7.16 (d, *J* = 8.1 Hz, 1H, NH), 7.31-7.39 (m, 5H. Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 18.4 (+, *C*H_3CH), 26.1 (+, cyclopropyl-CH), 28.3 (+, (*C*H_3)$_3$C, 3C), 28.7 (+, cyclopropyl-CH)), 36.0 (+, cyclopropyl-CHN), 50.4 (+, CHN), 52.3 (+, CH_3O), 67.4 (-, CH_2O), 80.2 (C_{quat}, $(CH_3)_3C$), 128.4 (+, Ph-CH, 2C), 128.6 (+, Ph-CH, 2C), 128.7 (+, Ph-CH), 135.1 (C_{quat}, Ph-C), 155.2 (C_{quat}, N(CO)O), 167.8 (C_{quat}, C=O), 169.9 (C_{quat}, C=O), 173.2 (C_{quat}, C=O). - MS CI (NH_3) *m/z* (%) 858 ($2MNH_4^+$, <1), 841 ($2MH^+$, <1), 741 ($2MH^+$-Boc, 7), 438 (MNH_4^+, 100), 421 (MH^+, 82), 382 (MNH_4^+-^{t}Bu, 44), 365 (MH^+-^{t}Bu, 9), 338 (MNH_4^+-Boc, 2), 321 (MH^+-Boc, 54). - IR (KBr) 3348, 3316, 2987, 1739, 1687, 1495 cm^{-1}. - Anal. Calcd for $C_{21}H_{28}N_2O_7$ (420.46): C, 59.99; H, 6.71; N, 6.66. Found: C, 60.06; H, 6.73; N, 6.56. (-)-**25a** - R_f ((-)-**25a**): 0.17. - mp 93-95 °C. - $[\alpha]_D^{21}$ -12.0 (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.30 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 1.46 (s, 9H, $(CH_3)_3C$), 1.29 (dd, *J* = 5.0, 4.9 Hz, 1H, cyclopropyl-CH), 2.57 (dd, *J* = 5.2, 8.3 Hz, 1H, cyclopropyl-CH), 3.71 (s, 3H, CH_3O), 4.02-4.18 (m, 2H, cyclopropyl-CHN + Ala-CHN), 4.78 (s br, 1H, NHBoc), 5.13 (d, *J* = 12.2 Hz, 1H, CH_2O), 5.20 (d, *J* = 12.2 Hz, 1H, CH_2O), 7.08 (d, *J* = 7.7 Hz, 1H, NH), 7.36-7.40 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 18.1 (+, *CH*$_3$CH), 26.3 (+, cyclopropyl-CH), 28.3 (+, (*CH*$_3$)$_3$C, 3C), 28.6 (+, cyclopropyl-CH), 36.0 (+, cyclopropyl-CHN), 50.3 (+, Ala-CHN), 52.3 (+, CH_3O), 67.4 (-, CH_2O), 80.23 (C_{quat}, *C*$(CH_3)_3$), 128.4 (+, Ph-CH, 2C), 128.5 (+, Ph-CH, 2C), 128.6 (+, Ph-CH), 135.2 (C_{quat}, Ph-C), 155.3 (C_{quat}, C=O Boc), 169.7 (C_{quat}, C=O), 169.8 (C_{quat}, C=O), 173.4 (C_{quat}, C=O). - MS CI (NH_3) *m/z* (%) 859 ($2MNH_4^+$, <1), 841 ($2MH^+$, <1), 741 (3), 438 (MNH_4^+, 100), 421(30), 382 (25), 321 (20). - IR (KBr) 3359, 3058, 2982, 1727, 1690, 1667,

1519, 1435, 1311 cm^{-1}. - Anal. Calcd for $C_{21}H_{28}N_2O_7$ (420.46): C, 59.99; H, 6.71; N, 6.66. Found: C, 59.89; H, 6.72; N, 6.53.



(±)-**25a**

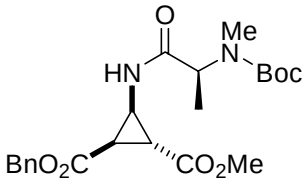
(1R*, 2R*, 3S*) 3-(2S-*tert*-Butoxycarbonylamino-propionylamino)-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((±)-**25a):** Boc-alanine (170 mg, 0.9 mmol, 3 eq.) was preactivated with EDC (174 mg, 0.9 mmol, 3 eq.) and HOBt (121 mg, 0.9 mmol, 3 eq.) in dry CH_2Cl_2 (5 ml) and under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. This solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (35 mg, 0.03 mmol, 0.1 eq.), then (±)-**16a** (100 mg, 0.3 mmol) and finally DABCO (168 mg, 1.5 mmol, 5 eq.) or pyridine (242 µl, 3 mmol, 10 eq.) were added. The solution was stirred 10 min. (DABCO) or 15 h (pyridine) at room temperature, then CH_2Cl_2 (10 ml) was added and the organic phase was washed with saturated $NaHCO_3$ (10 ml), 1 M $KHSO_4$ (10 ml) and saturated $NaHCO_3$ (10 ml) . The organic phase was dried over $MgSO_4$ and concentrated. The mixture of the two diastereomers was purified by chromatography (ethyl acetate/hexanes 1:1) to yield the product as a white solid (DABCO: 122 mg, 97 %, pyridine: 120 mg, 95 %) with 4 % (DABCO) or 20 % (pyridine) epimerization.



(±)-**25b**

(1R*, 2R*, 3S*) 3-[2S-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionylamino]-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((±)-**25b):** Fmoc-alanine (168 mg, 0.54 mmol, 1.5 eq.) was preactivated with EDC (104 mg, 0.54 mmol, 1.5 eq.) and HOBt (73 mg, 0.54 mmol, 1.5 eq.) in dry CH_2Cl_2 (5 ml) and under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. This solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (41 mg, 0.036 mmol, 0.1 eq.), then (±)-**16a** was added (120 mg, 0.36 mmol) and

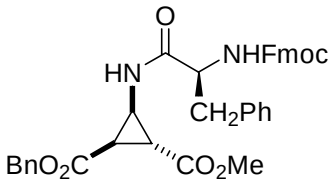
finally DABCO (201 mg, 1.8 mmol, 5 eq.) or, in a parallel experiment, phenylsilane (221 µl, 1.8 mmol, 5 eq.). The solution was stirred for 20 min. at room temperature, then CH_2Cl_2 (10 ml) was added and the organic phase was washed with saturated $NaHCO_3$ (10 ml), 1 M $KHSO_4$ (10 ml) and saturated $NaHCO_3$ (10 ml). The organic phase was dried over $MgSO_4$ and concentrated. The product (mixture of two diastereomers) was purified by chromatography (ethyl acetate/hexanes 1:1) to afford a white solid (181 mg, 93 % with DABCO-protocol; 175 mg, 90 % contaminated with 5 % of ring opening product with the phenylsilane-protocol). - R_f ((±)-**25b**): 0.37. - mp 65-67 °C. - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.32 (d, *J* = 6.8 Hz, 3H, C*H*$_3$CH, 1 diast.), 1.34 (d, *J* = 6.2 Hz, 3H, C*H*$_3$CH, 1 diast.), 2.31 (dd, *J* = 4.7, 4.7 Hz, 1H, cyclopropyl-CH), 2.56 (dd, *J* = 5.2, 8.2 Hz, 1H, cyclopropyl-CH), 3.69 (s, 3H, CH_3O, 1 diast.), 3.70 (s, 3H, CH_3O, 1 diast.), 4.03-4.07 (m, 1H, cyclopropyl-CHN, 1 diast.), 4.10 (ddd, *J* = 4.7, 8.1, 8.1 Hz, 1H, cyclopropyl-CHN, 1 diast.), 4.18-4.25 (m, 2H, Fmoc-CH + CHN), 4.33-4.48 (m, 2H, Fmoc-CH_2), 5.04 (d, *J* = 12.2 Hz, 1H, CH_2O, 1 diast.), 5.08-5.12 (m, 2H, CH_2O, 1 diast.), 5.13 (d, *J* = 12.2 Hz, 1H, CH_2O, 1 diast.), 5.18 (d, J = 6.6 Hz, 1H, NH, 1 diast.), 5.35 (d, J = 7.0 Hz, 1H, NH, 1 diast.), 7.06 (d, *J* = 7.9 Hz, 1H, NH, 1 diast.), 7.12 (d, *J* = 7.9 Hz, 1H, NH, 1 diast.), 7.28-7.41 (m, 9H, Ar-CH), 7.51-7.59 (m, 2H, Fmoc-Ar-CH), 7.75-7.77 (m, 2H, Fmoc-Ar-CH). – ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 18.3 (+, *C*H_3CH, 1 diast.), 18.5 (+, *C*H_3CH, 1diast.), 26.0 (+, cyclopropyl-CH, 1 diast.), 26.2 (+, cyclopropyl-CH, 1 diast.), 28.6 (+, cyclopropyl-CH, 1 diast.), 28.7 (+, cyclopropyl-CH, 1 diast.), 35.9 (+, cyclopropyl-CHN), 47.1 (+, Fmoc-CH), 50.5 (+, Ala-CHN, 1 diast.), 50.6 (+, Ala-CHN, 1 diast.), 52.4 (+, CH_3O), 67.06 (-, CH_2O, 1 diast.), 67.13 (-, CH_2O, 1 diast.), 67.4 (-, Fmoc-CH_2), 119.9-128.6 (+, Ar-CH, 13C), 134.9 (C_{quat}, Ph-C, 1 diast.), 135.0 (C_{quat}, Ph-C, 1 diast.), 141.22 (C_{quat}, Fmoc-Ar-C, 1 diast.), 141.25 (C_{quat}, Fmoc-Ar-C, 1 diast.), 141.27 (C_{quat}, Fmoc-Ar-C, 1 diast., 2C), 143.64 (C_{quat}, Fmoc-Ar-C, 1 diast.), 143.68 (C_{quat}, Fmoc-Ar-C, 1 diast.), 143.8 (C_{quat}, Fmoc-Ar-C, 1 diast., 2C), 155.8 (C_{quat}, N(CO)O, 1 diast.), 155.9 (C_{quat}, N(CO)O, 1 diast.), 169.73 (C_{quat}, C=O, 1 diast.), 169.75 (C_{quat}, C=O, 1 diast.), 169.8 (C_{quat}, C=O, 1 diast.), 169.9 (C_{quat}, C=O, 1 diast.), 172.7 (C_{quat}, C=O, 1 diast.), 172.8 (C_{quat}, C=O, 1 diast.). - MS FAB (NH_3) *m/z* (%) 543 (MH^+, 9), 307 (100). - IR (KBr) 3322, 3064, 2953, 1724, 1678, 1524, 1450, 1311 cm^{-1}. - Anal. Calcd for $C_{31}H_{30}N_2O_7$ (542.59): C, 68.62; H, 5.57; N, 5.16. Found: C, 68.31; H, 5.61; N, 5.08.



(±)-**25c**

(1R*, 2R*, 3S*) 3-(2S-*tert*-Butoxycarbonyl-methyl-amino-propionylamino)-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((±)-**25c):** A solution of (±)–**11** (500 mg, 1.43 mmol) in HCl 3 M in ethyl acetate (5 ml) was stirred at 0 °C for 3 h. The solvent was then evaporated and a solution of N-methyl-Boc-alanine (500 mg, 2.46 mmol, 1.7 eq.), EDC (411 mg, 2.04 mmol, 1.5 eq), HOBt (289 mg, 2.04 mmol, 1.5 eq.) in CH_2Cl_2 (100 ml) (previously stirred 1 h at 0 °C and 1 h at room temperature) was added. Triethylamine (200 µl, 1.43 mmol, 1 eq.) was then added dropwise at 0 °C. The mixture was stirred at room temperature overnight. The solution was washed with saturated $NaHCO_3$ (100 ml), 1 M $KHSO_4$ (100ml) and then saturated $NaHCO_3$ (100 ml). The organic phase was dried over $MgSO_4$ and concentrated. The mixture of the two diastereomers (at least one of them exists as a mixture of two rotamers) was obtained as a colourless oil (546 mg, 88 %) by chromatography (ethyl acetate/hexanes 1:2). 10 % epimerization was observed. - R_f ((±)-**25c**): 0.24. - ^{1}H NMR (measured on a mixture of diastereomers enriched in one of them): ($CDCl_3$, 250 MHz) δ 1.31 (d, *J* = 6.7 Hz, 3H, C*H*$_3$CH, major diast.), 1.34 (d, *J* = 6.9 Hz, 3H, C*H*$_3$CH, minor diast.), 1.47 (s, 9H, (C*H*$_3$)$_3$C, minor diast.), 1.49 (s, 9H, $(CH_3)_3C$, major diast.), 2.22 (dd, *J* = 5.0, 5.0 Hz, 1H, cyclopropyl-CH, major diast.), 2.28 (dd, *J* = 4.7, 4.7 Hz, 1H, cyclopropyl-CH, 1 minor diast.), 2.31 (dd, *J* = 5.0, 5.0 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.29-2.32 (m, 1H, cyclopropyl-CH, minor diast.), 2.63 (s br, 3H, CH_3N, major diast. 1 epimer), 2.71 (s br, 3H, CH_3N, major diast., 1 epimer), 2.78 (s, CH_3N, minor diast.), 3.70 (s, 3H, CH_3O, major diast.), 3.71 (s, 3H, CH_3O, minor diast.), 4.05-4.17 (m, 1H, cyclopropyl-CHN), 4.62-4.83 (m, 1H, CHN), 5.13-5.20 (m, 2H, CH_2O), 7.10 (d, *J* = 7.1 Hz, 1H, NH, major diast.), 7.19 (s br, 1H, NH, minor diast.), 7.32-7.41 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 13.6 (+, *C*H_3CH, 1 diast.), 14.2 (*C*H_3CH, 1 diast.), 25.9 (+, cyclopropyl-CH, 1 diast.), 26.2 (+, cyclopropyl-CH, 1 diast.), 28.4 (+, (*C*H_3)$_3$C + cyclopropyl-CH, 4C, 1 diast.), 28.7(+, cyclopropyl-CH, 1 diast.), 29.1 (+, cyclopropyl-CH, 1 diast. 1 epimer), 29.9 (+, CH_3N, 1 diast.), 36.09 (+, CH_3N, 1 diast.), 36.13 (+, cyclopropyl-CHN), 52.4 (+, CH_3O), 53.9 (+, CHN), 67.3 (-, CH_2O, 1 diast.), 67.4 (-, CH_2O, 1 diast.), 80.7 (C_{quat}, *C*$(CH_3)_3$), 128.4 (+, Ph-CH), 128. 5 (+, PH-CH), 128.57 (+, Ph-CH), 128.62 (+, Ph-CH), 128.68 (+, Ph-CH), 135.0 (C_{quat}, Ph-CH, 1 diast.), 135.2 (C_{quat}, Ph-C, 1 diast.), 155.9 (C_{quat}, N(C=O)O), 169.4

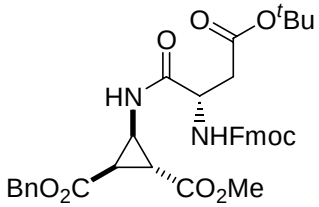
(C_{quat}, C=O, 1 diast.), 169.9 (C_{quat}, C=O, 1 diast.), 169.91 (C_{quat} , C=O), 1 diast.), 170.1 (C_{quat}, C=O, 1 diast.), 172.3 (C_{quat}, C=O). - MS CI (NH_3) *m/z* (%) 886 ($2MNH_4^+$, <1), 769 ($2MH^+$-Boc, 16), 452 (MNH_4^+, 78), 435 (MH^+, 100), 396 ($2MH^+$-^{t}Bu, 13), 335 (MH^+-Boc, 40), 267 (12). - IR (CH_2Cl_2) 3394, 1722, 1687, 1606, 1515 cm^{-1}. - Anal. Calcd for $C_{22}H_{30}N_2O_7$ (434.488): C, 60.82; H, 6.96; N, 6.45. Found: C, 60.36; H, 6.91; N, 6.41.



(±)-**25d**

(1R*, 2R*, 3S*) 3-[2S-(9*H*-fluoren-9-ylmethoxycarbonylamino)-phenyl-propionylamino]-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((±)-25d): A solution of (±)-**11** (300 mg, 0.86 mmol) in HCl 3 M in ethyl acetate (4 ml) was stirred at 0 °C for 3 h. The solution was then evaporated, the salt was resuspended in CH_2Cl_2 (10 ml) and Fmoc-phenylalanine (399 mg, 1.03 mmol, 1.2 eq.), EDC (330 mg, 1.72 mmol, 2 eq), and pyridine (83 µl, 1.03 mmol, 1.2 eq.) were added. The mixture was stirred overnight at room temperature. The solution was washed with saturated $NaHCO_3$ (50 ml), 1 M $KHSO_4$ (50 ml) and then saturated $NaHCO_3$ (50 ml). The organic phase was dried over $MgSO_4$ and concentrated. The mixture of the two diastereomers was purified by chromatography (CH_2Cl_2/MeOH 50:1) to give a white solid (477 g, 88 %). No epimerization was observed. - R_f ((±)-**25d**): 0.30. - mp 164-166 °C. - ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.97 (dd, *J* = 5.0, 5.8 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.20 (dd, *J* = 4.6, 4.6 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.49 (dd, *J* = 5.2, 8.3 Hz, 1H, cyclopropyl-CH), 3.00-3.09 (m, 2H, CH_2Ph), 3.68 (s, 3H, CH_3O, 1 diast.), 3.70 (s, 3H, CH_3O, 1 diast.), 4.02-4.10 (m, 1H, cyclopropyl-CHN), 4.17-4.22 (m, 1H, Fmoc-CH), 4.33-4.39 (m, 3H, Fmoc-CH_2 + CHN), 4.97-5.12 (m, 2H, Ph-CH_2O), 5.27 (s br, 1H, NHFmoc), 6.76 (d, *J* = 7.3 Hz, 1H, NH, 1 diast.), 7.00 (d, *J* = 8.3 Hz, NH, 1 diast.), 7.18-7.42 (m, 14H, Ar-CH), 7.50-7.56 (m, 2H, Fmoc-Ar-CH), 7.75 (pseudo d, *J* = 7.3 Hz, 2H, Fmoc -Ar-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 25.7 (+, cyclopropyl-CH, 1 diast.), 25.8 (+, cyclopropyl-CH, 1 diast.), 28.6 (+, cyclopropyl-CH, 1 diast.), 28.8 (+, cyclopropyl-CH, 1 diast.), 35.7 (+, cyclopropyl-CHN, 1 diast.), 35.9 (+, cyclopropyl-CHN, 1 diast.), 38.5 (-, CH_2Ph, 1 diast.), 38.7 (-, CH_2Ph, 1 diast.), 47.1 (+, Fmoc-CH), 52.5 (+, CH_3O), 56.3 (+, CHN), 67.2 (-, CH_2O, 1 diast.), 67.3 (-, CH_2O, 1 diast.), 67.5 (-, CH_2O), 120.0 (+, Fmoc-Ar-CH, 2C), 125.1 (+, Fmoc-Ar-CH, 2C), 127.1 (+, Ar-CH, 3C), 127.8 (+,

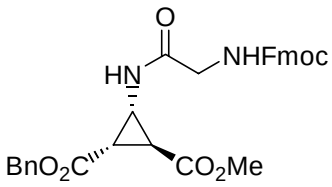
Ar-CH, 2C), 128.3 (+, Ar-CH), 128.4 (Ar-CH), 128.6 (+, Ar-CH), 129.3 (+, Ar-CH, 2C), 134.9 (C_{quat}, Ph-C, 1 diast.), 135.0 (C_{quat}, Ph-C, 1 diast.), 136.16 (C_{quat}, Ph-C, 1 diast.), 136.24 (C_{quat}, Ph-C, 1 diast.), 141.30 (C_{quat}, Fmoc-Ar-C, 1 diast.), 141.33 (C_{quat}, Fmoc-Ar-C, 1 diast.), 141.34 (C_{quat}, Fmoc-Ar-C), 143.68 (C_{quat}, Fmoc-Ar-C, 1 diast.), 143.74 (C_{quat}, Fmoc-Ar-C, 1 diast.), 143.8 (C_{quat}, Fmoc-Ar-C), 155.8 (C_{quat}, N(CO)O), 169.7 (C_{quat}, C=O, 1 diast.), 169.77 (C_{quat}, C=O, 1 diast.), 169.84 (C_{quat}, C=O, 1 diast.), 170.0 (C_{quat}, C=O, 1 diast.), 171.43 (C_{quat}, C=O, 1 diast.), 171.45 (C_{quat}, C=O, 1 diast.). - MS FAB (MeOH/Glycerine) *m/z* (%) 619 (MH^+, 100), 482 (24), 397 (MH^+-Fmoc, 40). - IR (KBr) 3212, 3062, 3033, 2953, 1727, 1692, 1662, 1532, 1449, 1309, 1259 cm^{-1}. - Anal. Calcd for $C_{37}H_{34}N_2O_7 \cdot 0.7\ H_2O$ (631.30): C, 70.39; H, 5.65; N, 4.43. Found: C, 70.09; H, 5.38; N, 4.17.



(±)-**25e**

(1R*, 2R*, 3S*) 3-[4-*tert*-Butoxycarbonyl-2S-(9*H*-fluoren-9-ylmethoxycarbonylamino)-butyrylamino]-cyclopropane-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester ((±)-**25e):** A solution of (±)-**11** (300 mg, 0.86 mmol) in HCl 3 M in ethyl acetate (4 ml) was stirred at 0 °C for 3 h. The mixture was evaporated, the salt was resuspended in CH_2Cl_2 (10 ml) and Fmoc-Asp(O^{t}Bu) (534 mg, 1.3 mmol, 1.5 eq.), EDC (336 mg, 1.7 mmol, 2 eq), and pyridine (83 µl, 1.03 mmol, 1.2 eq.) were added. The mixture was stirred overnight at room temperature. The solution was washed with saturated $NaHCO_3$ (50 ml), 1 M $KHSO_4$ (50 ml) and saturated $NaHCO_3$ (50 ml). The organic phase was dried over $MgSO_4$ and concentrated. The mixture of the two diastereomers was purified by chromatography (ethyl acetate/hexanes 1:3) to afford the product as a white solid (400 g, 72 %). No epimerization was observed. - R_f ((±)-**25e**): 0.23. - mp 55-57 °C. - ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.46 (s, 9H, $(CH_3)_3C$, 1 diast.), 1.47 (s, 9H, $(CH_3)_3C$, 1 diast.), 2.29-2.33 (m, 1H, cyclopropyl-CH), 2.55-2.65 (m, 2H, cyclopropyl-CH + $CH_2CO_2{}^t$Bu), 2.86-2.95 (m, 1H, $CH_2CO_2{}^t$Bu), 3.70 (s, 3H, CH_3O), 3.98-4.10 (m, 1H, cyclopropyl-CHN), 4.16-4.40 8m, 2H, Fmoc-CH + CHN), 4.45-4.54 (m, 2H, CH_2Fmoc), 5.00-5.16 (m, 2H, CH_2O), 5.84 (d, *J* = 8.7 Hz, 1H, NH, 1 diast.), 5.92 (d, *J* = 7.0 Hz, 1H, NH, 1 diast.), 7.28-7.40 (m, 10H, Ar-CH + NH), 7.52-7.63 (m, 2H, Fmoc-Ar-CH), 7.75-7.77 (m, 2H, Fmoc-Ar-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ

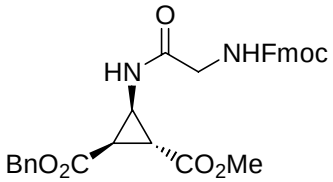
18.2 (+, (CH_3)$_3$C, 3C), 26.2 (+, cyclopropyl-CH), 28.6 (+, cyclopropyl-CH, 1 diast.), 28.7 (+, cyclopropyl-CH, 1 diast.), 36.0 (+, cyclopropyl-CHN, 1 diast.), 36.1 (+, cyclopropyl-CHN, 1 diast.), 36.9 (-, $CH_2CO_2{}^t$Bu, 1 diast.), 37.2 (-,$CH_2CO_2{}^t$Bu, 1 diast.), 47.2 (+, Fmoc-CH), 51.4 (+, CHN), 52.5 (+, CH_3O, 1 diast.), 52.6 (+, CH_3O, 1 diast.), 67.4 (-, CH_2O), 67.5 (-, CH_2O), 81.9 (C_{quat}, $C(CH_3)_3$, 1 diast.), 82.0 (+, $C(CH_3)_3$, 1 diast.), 120.0 (+, Fmoc-Ar-CH, 2C), 125.2 (+, Fmoc-Ar-CH, 2C), 127.1 (+, Ar-CH, 2C), 127.8 (+, Ar-CH, 2C), 128.4 (+, Ar-CH), 128.46 (+, Ar-CH), 128.53 (+, Ar-CH), 128.6 (+, Ar-CH, 2C), 135.0 (C_{quat}, Ph-C, 1 diast.), 135.1 (C_{quat}, Ph-C, 1 diast.), 141.29 (C_{quat}, Fmoc-Ar-C, 1 diast.), 141.35 (C_{quat}, Fmoc-Ar-C, 3C, 1 diast.), 143.6 (C_{quat}, Fmoc-Ar-C, 1 diast.), 143.7 (C_{quat}, Fmoc-Ar-C, 1 diast.), 143.9 (C_{quat}, Fmoc-Ar-C), 156.2 (C_{quat}, N(CO)O), 160.7 (C_{quat}, C=O, 1 diast.), 169.8 (C_{quat}, C=O, 1 diast.), 169.9 (C_{quat}, C=O), 171.2 (C_{quat}, C=O, 2C). - MS FAB (MeOH/Glycerine) *m/z* (%) 643 (MH^+, 80), 587 (100). - IR (KBr) 3348, 3065, 2955, 1727, 1526 cm^{-1}. - Anal. Calcd for $C_{36}H_{38}N_2O_9$ (642.70): C, 67.28; H, 5.96; N, 4.36. Found: C, 67.06; H, 5.96; N, 3.92.



(-)-**25f**

(1S, 2S, 3R) 3-[(9*H*-fluoren-9-ylmethoxycarbonylamino)-acetylamino]-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((-)-25f): A solution of (–)–**11** (200 mg, 0.57 mmol) in HCl 3 M in ethyl acetate (4 ml) was stirred at 0 °C for 3 h. The solution was then evaporated, the salt was resuspended in CH_2Cl_2 (10 ml) and Fmoc-glycine (252 mg, 0.85 mmol, 1.5 eq.), EDC (219 mg, 1.14 mmol, 2 eq), and pyridine (91 µl, 1.14 mmol, 2 eq.) were added. The mixture was stirred overnight at room temperature. The solution was washed with saturated $NaHCO_3$ (50 ml), 1 M $KHSO_4$ (50 ml) and saturated $NaHCO_3$ (50 ml). The organic phase was dried over $MgSO_4$ and concentrated. The product was purified by chromatography (CH_2Cl_2/MeOH 40:1). Yield: 284 g (95 %). No epimerization was observed. - R_f ((-)-**25f**): 0.31. - mp 57-58 °C. - $[\alpha]_D^{21}$ +22.8 (c 0.5, MeOH). - ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.31 (dd, *J* = 4.9, 4.9 Hz, 1H, cyclopropyl-CH), 2.56 (dd, *J* = 5.2, 8.3 Hz, 1H, cyclopropyl-CH), 3.69 (s, 3H, CH_3O), 3.81-3.83 (m, 2H, CH_2N), 4.10 (ddd, *J* = 4.7, 8.1, 8.1 Hz, 1H, cyclopropyl-CHN), 4.21-4.27 (m, 1H, Fmoc-CH), 4.41-4.43 (m, 2H, Fmoc-CH_2), 5.06-5.15 (m, 2H, CH_2O), 5.35 (s br, 1H, NHFmoc), 7.09 (d, *J* = 7.9 Hz, 1H, NH), 7.25-7.42 (m, 9H, Ar-CH), 7.58-7.61 (m, 2H, Fmoc-Ar-CH), 7.74-7.77 (m, 2H, Fmoc-Ar-CH). -

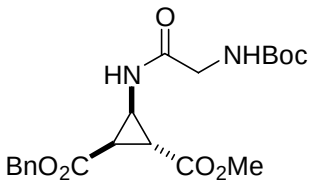
^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 26.0 (+, cyclopropyl-CH), 28.6 (+, cyclopropyl-CH), 35.8 (+, cyclopropyl-CHN), 44.6 (-, CH_2N), 47.1 (+, Fmoc-CH), 52.5 (+, CH_3O), 67.4 (-, CH_2O), 67.5 (-, CH_2O), 120.0 (+, Fmoc-Ar-CH, 2C), 125.1 (+, Fmoc-Ar-CH), 127.1 (+, Ar-CH, 2C), 127.8 (+, Ar-CH, 2C), 128.4 (+, Ar-CH, 2C), 128.6 (+, Ar-CH, 2C), 128.7 (+, Ar-CH, 2C), 135.0 (C_{quat}, Ph-C), 141.3 (C_{quat}, Fmoc-Ar-C, 2C), 143.75 (C_{quat}, Fmoc-Ar-C), 143.78 (C_{quat}, Fmoc-Ar-C), 156.5 (C_{quat}, N(CO)O), 169.5 (C_{quat}, C=O), 169.7 (C_{quat}, C=O), 170.0 (C_{quat}, CO). - MS FAB (MeOH/Glycerine) *m/z* (%) 1057 ($2MH^+$, 8), 529 (MH^+, 100), 307 (MH^+-NHFmoc, 31). - IR (KBr) 3245, 3056, 2803, 1708, 1638, 1505, 1427, 1303 cm^{-1}. - Anal. Calcd for $C_{30}H_{28}N_2O_7 \cdot 0.3\ H_2O$ (533.965): C, 67.48; H, 5.39; N, 5.25. Found: C, 67.57; H, 5.49; N, 4.88.



(+)-**25f**

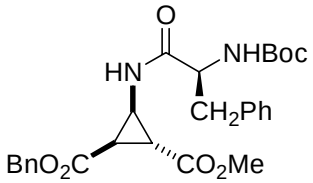
(1R, 2R, 3S) 3-[(9*H*-Fluoren-9-ylmethoxycarbonylamino)-acetylamino]-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((+)-**25f):** A solution of (+)–**11** (200 mg, 0.57 mmol) in HCl 3 M in ethyl acetate (4 ml) was stirred at 0 °C for 3 h. The solution was then evaporated, the salt was resuspended in CH_2Cl_2 (10 ml) and Fmoc-glycine (252 mg, 0.85 mmol, 1.5 eq.), EDC (219 mg, 1.14 mmol, 2 eq), and pyridine (91 µl, 1.14 mmol, 2 eq.) were added. The mixture was stirred overnight at room temperature. The solution was washed with saturated $NaHCO_3$ (50 ml), 1 M $KHSO_4$ (50 ml) and saturated $NaHCO_3$ (50 ml). The organic phase was dried over $MgSO_4$ and concentrated. The product was purified by chromatography (CH_2Cl_2/MeOH 40:1). Yield: 231 g (77 %). No epimerization was observed. - R_f ((+)-**25f**): 0.31. - mp 56-58 °C. - $[\alpha]_D^{21}$ -21.1 (c 0.5, MeOH). - ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.31 (dd, *J* = 4.8, 4.8 Hz, 1H, cyclopropyl-CH), 2.56 (dd, *J* = 5.3, 8.3 Hz, 1H, cyclopropyl-CH), 3.70 (s, 3H, CH_3O), 3.81-3.83 (m, 2H, CH_2N), 4.10 (ddd, *J* = 4.7, 8.1, 8.1 Hz, 1H, cyclopropyl-CHN), 4.21-4.27 (m, 1H, Fmoc-CH), 4.41-4.44 (m, 2H, Fmoc-CH_2), 5.06-5.14 (m, 2H, CH_2O), 5.35 (s br, 1H, NHFmoc), 7.09 (d, *J* = 7.9 Hz, 1H, NH), 7.25-7.42 (m, 9H, Ar-CH), 7.58-7.63 (m, 2H, Ar-CH), 7.74-7.78 (m, 2H, Ar-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 26.0 (+, cyclopropyl-CH), 28.6 (+, cyclopropyl-CH), 35.8 (+, cyclopropyl-CHN), 44.5 (-, CH_2N), 47.1 (+, Fmoc-CH), 52.5 (+, CH_3O), 67.4 (-, CH_2O), 67.5 (-, CH_2O), 120.0 (+,Fmoc-Ar-CH, 2C), 125.1 (+, Fmoc-Ar-CH), 127.2 (+, Ar-CH, 2C), 127.8 (+, Ar-CH,

2C), 128.4 (+, Ar-CH, 2C), 128.6 (+, Ar-CH, 2C), 128.7 (+, Ar-CH, 2C), 135.0 (C_{quat}, Ph-C), 141.3 (C_{quat}, Fmoc-Ar-C, 2C), 143.75 (C_{quat}, Fmoc-Ar-C), 143.78 (C_{quat}, Fmoc-Ar-C), 156.5 (C_{quat}, N(CO)O), 169.5 (C_{quat}, C=O), 169.7 (C_{quat}, C=O), 170.0 (C_{quat}, CO). - MS FAB (MeOH/Glycerine) *m/z* (%) 1057 ($2MH^+$, 8), 529 (MH^+, 100), 307 (MH^+-NHFmoc, 30). - IR (KBr) 3247, 3054, 2801, 1710, 1635, 1507, 1427, 1303 cm^{-1}. - Anal. Calcd for $C_{30}H_{28}N_2O_7 \cdot 0.6\ H_2O$ (539.369): C, 66.81; H, 5.46; N, 5.19. Found: C, 66.74; H, 5.17; N, 5.02.



(±)-**25f**

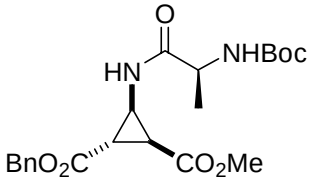
(1R*, 2R*, 3S*) 3-[(*tert*-Butoxycarbonylamino]-acetylamino)-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((±)-**25f):**[68] Boc-glycine (87 mg, 0.5 mmol, 3 eq.) was preactivated with EDC (95 mg, 0.5 mmol, 3 eq.) and HOBt (67 mg, 0.5 mmol, 3 eq.) in dry CH_2Cl_2 (4 ml) and under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. This solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (19 mg, 0.016 mmol, 0.1 eq. for DABCO protocol, 38 mg, 0.032 mmol, 0.2 eq. for pyridine protocol), then (±)-**16a** (57 mg, 0.17 mmol) and finally DABCO (94 mg, 0.85 mmol, 5 eq.) or pyridine (137 µl, 1.7 mmol, 10 eq.) were added. The solution was stirred 10 min. (DABCO) or 2 h (pyridine) at room temperature, then CH_2Cl_2 (10 ml) was added and the organic phase was washed with saturated $NaHCO_3$ (10 ml), 1 M $KHSO_4$ (10 ml) and of saturated $NaHCO_3$ (10 ml) . The organic phase was dried over $MgSO_4$ and concentrated. The mixture of the two diastereomers was purified by chromatography (ethyl acetate/hexanes 1:1) to yield the product as a white solid (DABCO: 63 mg, 92 %, 4 % epimerisation; pyridine: 64 mg, 93 %, 15 % epimerisation). R_f ((±)-**25f**): 0.36. - ^1H NMR ($CDCl_3$, 250 MHz) δ 1.44 (s, 9H, $(CH_3)_3C$), 2.30 (dd, *J* = 5.2; 4.7 Hz, 1H, cyclopropyl-CH), 2.55 (dd, *J* = 8.3; 5.2 Hz, 1H, cyclopropyl-CH), 3.67 (s, 3H, CH_3O), 3.74 (m, 2H, CH_2N), 4.08 (ddd, *J* = 8.1; 8.1; 4.7 Hz, 1H, cyclopropyl-CH), 5.08 (d, *J* = 12.1 Hz, 1H, CH_2O), 5.17 (d, *J* = 12.1 Hz, CH_2O), 5.20 (t, *J* = 5.8 Hz, 1H, NHBoc), 7.24 (d, *J* = 8.0 Hz, 1H, NH), 7.33-7.35 (m, 5H, Ph-CH).



(±)-**25h**

(1R*, 2R*, 3S*) 3-(2S-*tert*-Butoxycarbonylamino-phenyl-propionylamino)-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((±)-25h): Boc-phenylalanine (444 mg, 1.67 mmol, 3.1 eq.) was preactivated with EDC (305 mg, 1.59 mmol, 3 eq.) and HOBt (215 mg, 1.59 mmol, 3 eq.) in dry CH_2Cl_2 (5 ml) and under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. This solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (61 mg, 0.053 mmol, 0.1 eq.), then (±)-**16a** (177 mg, 0.53 mmol) and finally DABCO (314 mg, 2.8 mmol, 5.3 eq.) were added. The solution was stirred 10 min. at room temperature, then CH_2Cl_2 (10 ml) was added and the organic phase was washed with saturated $NaHCO_3$ (10 ml), 1 M $KHSO_4$ (10 ml) and of saturated $NaHCO_3$ (10 ml). The organic phase was dried over $MgSO_4$ and concentrated. The mixture of the two diastereomers was purified by chromatography (ethyl acetate/hexanes 1: 1) to yield the product as a white solid (257 mg, 96 %) with 4-5 % epimerization. - R_f ((±)-**25h**): 0.33. - mp 138-140 °C. - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.41 (s, 9H, $(CH_3)_3C$, 1 diast.), 1.42 (s, 9H, $(CH_3)_3C$, 1 diast.), 1.95-1.97 (m, 1H, cyclopropyl-CH, 1 diast.), 2.18 (dd, *J* = 5.0, 5.0 Hz, 1 H, cyclopropyl-CH, 1 diast.), 2.47-2.52 (m, 1H, cyclopropyl-CH), 2.99-3.05 (m, 2H, CH_2Ph), 3.69 (s, 3H, CH_3O, 1 diast.), 3.70 (s, 3H, CH_3O, 1 diast.), 4.03 (ddd, *J* = 4.5, 8.1,8.1 Hz, 1H, cyclopropyl-CHN, 1 diast.), 4.11 (ddd, *J* = 4.7, 8.3, 8.3 Hz, 1H, cyclopropyl-CHN, 1 diast.), 4.28-4.39 (m, 1H, CHN), 4.93 (s br, 1H, NH), 5.01-5.16 (m, 2H, CH_2O), 6.74 (d, *J* = 7.6 Hz, 1H, NH, 1 diast.), 7.04 (d, *J* = 8.3 Hz, 1H, NH, 1 diast.), 7.16-7.37 (m, 10H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 25.66 (+, cyclopropyl-CH, 1 diast.), 25.74 (+, cyclopropyl-CH, 1 diast.), 28.3 (+, $(CH_3)_3C$, 3C), 28.6 (+, cyclopropyl-CH, 1 diast.), 28.9 (+, cyclopropyl-CH, 1 diast.), 35.7 (+, cyclopropyl-CHN, 1 diast.), 35.9 (+, cyclopropyl-CHN, 1 diast.), 38.4 (-, CH_2Ph, 1 diast.), 38.7 (-, CH_2Ph, 1 diast.), 52.5 (+, CH_3O), 55.9 (+, CHN, 1 diast.), 56.1 (+, CHN, 1 diast.), 67.4 (-, CH_2O, 1 diast.), 67.5 (-, CH_2O, 1 diast.), 80.3 (C_{quat}, $(CH_3)_3C$), 126.98 (+, Ph-CH, 1 diast.), 127.03 (+, Ph-CH, 1 diast.), 128.4 (+, Ph-CH, 2C, 1 diast.), 128.5 (+, Ph-CH, 2C, 1 diast.), 128.64 (+, Ph-CH, 1 diast.), 128.67 (+, Ph-CH, 1 diast.), 128.69 (+, Ph-CH, 2C, 1 diast.), 128.71 (+, Ph-CH, 2C, 1 diast.), 128.78 (+, Ph-CH, 2C, 1 diast.), 128.8 (+, Ph-CH, 2C, 1 diast.), 129.2 (+, Ph-CH, 2C, 1 diast.), 129.3 (+, Ph-CH, 2C, 1 diast.), 134.9 (C_{quat}, Ph-C, 1 diast.), 135.0 (C_{quat}, Ph-C, 1 diast.), 136.4 (C_{quat}, Ph-C, 1 diast.), 136.5 (C_{quat}, Ph-C, 1 diast.),

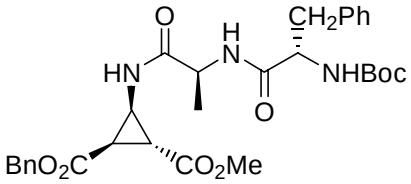
155.2 (C_{quat}, N(CO)O), 169.7 (C_{quat}, C=O, 1 diast.), 169.78 (C_{quat}, C=O, 1 diast.), 169.82 (C_{quat}, C=O, 1 diast.), 170.0 (C_{quat}, C=O, 1 diast.), 171.8 (C_{quat}, C=O, 1 diast.), 171.9 (C_{quat}, C=O, 1 diast.). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 993 ($2MH^+$, 16), 497 (MH^+, 91), 441 (93), 397 (MH^+-Boc, 100). - IR (KBr) 3350, 3325, 2955, 1726, 1691, 1664, 1455, 1309, 1170 cm^{-1}. - Anal. Calcd for $C_{27}H_{32}N_2O_7$ (496.563): C, 65.31; H, 6.49; N, 5.64. Found: C, 65.15; H, 6.53; N, 5.55.



(±)-**26**

(1R*, 2R*, 3R*) 3-(2S-*tert*-Butoxycarbonylamino-propionylamino)-cyclopropane-1,2-dicarboxylic acid 1-bezyl ester 2-methyl ester ((±)-26): A solution of (±)–**12** (2.00 g, 5.73 mmol) in HCl 3 M in ethyl acetate (20 ml) was stirred at 0 °C for 3 h. The solvent was evaporated and a solution of Boc-alanine (1.62 mg, 8.59 mmol, 1.5 eq.), EDC (1.65 g, 8.59 mmol, 1.5 eq) and HOBt (1.16 g, 8.59 mmol, 1.5 eq.) in CH_2Cl_2 (250 ml) (previously stirred 1 h at 0 °C and 1 h at room temperature) was added. Triethylamine (797 µl, 5.73 mmol, 1 eq.) was then added dropwise at 0°C. The mixture was stirred at room temperature overnight. The solution was washed with saturated $NaHCO_3$ (200 ml), 1 M $KHSO_4$ (200ml) and then saturated $NaHCO_3$ (200 ml). The organic phase was dried over $MgSO_4$ and concentrated. The mixture of the two diastereomers was purified by chromatography (ethyl acetate/hexanes 1:2). Yield: 2.12 g (88 %). Any attempt to separate the two diastereomers by recrystallization failed. - R_f ((±)-**26**): 0.10. - mp 75-77 °C. - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.33 (d, *J* = 7.1 Hz, 3H, *CH*$_3$CH), 1.42 (s, 9H, $(CH_3)_3C$, 1 diast.), 1.43 (s, 9H, $(CH_3)_3C$, 1 diast.), 2.31 (dd, *J* = 4.9, 4.9 Hz, 1H, cyclopropyl-CH), 2.55 (dd, *J* = 5.2, 8.3 Hz, 1H, cyclopropyl-CH), 3.71 (s, 3H, CH_3O, 1 diast.), 3.72 (s, 3H, CH_3O, 1 diast.), 4.03-4.17 (m, 2H, cyclopropyl-CHN + Ala-CHN), 4.98 (d, *J* = 7.33 Hz, 1H, NH), 5.07-5.18 (m, 2H, CH_2O), 7.13 (d, *J* = 7.6 Hz, 1H, NH, 1 diast.), 7.23 (d, *J* = 8.2 Hz, 1H, 1diast.), 7.29-7.36 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 18.2 (+, *C*H_3CH, 1 diast.), 18.3 (+, *C*H_3CH, 1 diast.), 26.10 (+, cyclopropyl-CH, 1 diast.), 26.14 (+, cyclopropyl-CH, 1 diast.), 28.3 (+, $(CH_3)_3C$, 3C), 28.77 (+, cyclopropyl-CH, 1 diast.), 28.83 (+, cyclopropyl-CH, 1 diast.), 36.0 (+, cyclopropyl-CHN), 50.4 (+, CHN), 52.4 (+, CH_3O), 67.3 (-, CH_2O), 80.2 (C_{quat}, $C(CH_3)_3$), 128.4 (+, Ph-CH, 2C), 128.5 (+, Ph-CH, 2C), 128.6 (+, Ph-CH), 135.2 (C_{quat},

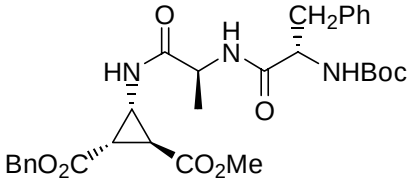
Ph-C), 155.3 (C_{quat}, C=O), 169.3 (C_{quat}, C=O), 170.3 (C_{quat}, C=O), 173.3 (C_{quat}, C=O, 1diast.), 173.4 (C_{quat}, C=O, 1 diast.). - MS CI (NH_3) *m/z* (%) 858 ($2MNH_4^+$, <1), 841 ($2MH^+$, <1), 741 ($2MH^+$-Boc, 11), 438 (MNH_4^+, 100), 421 (MH^+, 26), 382 (23), 321 (MH^+-Boc, 9). - IR (KBr) 3345, 2980, 2929, 1722, 1688, 1666, 1523, 1451 cm^{-1}. - Anal. Calcd for $C_{21}H_{28}N_2O_7$ (420.461): C, 59.99; H, 6.71; N, 6.66. Found: C, 60.05; H, 6.76; N, 6.43.



(+)-**27a**

(1R, 2R, 3S) 3-[2S-(2S-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-propionylamino] cyclopropane-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester ((+)-**27a):** Recrystallized (+)-**25a** (310 mg, 0.74 mmol) was stirred in HCl 3 M in ethyl acetate (11 ml) at 0 °C for 3 h. The solvent was then evaporated and a solution of Boc-phenylalanine (303 mg, 1.14 mmol, 1.5 eq.), EDC (219 mg, 1.14 mmol, 1.5 eq) and HOBt (153 mg, 1.14 mmol, 1.5 eq.) in CH_2Cl_2 (12 ml) (previously stirred 1 h at 0 °C and 1 h at room temperature) was added. Finally triethylamine (103 µl, 0.74 mmol, 1 eq.) was added dropwise. The mixture was stirred at room temperature overnight. The solution was washed with saturated $NaHCO_3$ (15 ml), 1 M $KHSO_4$ (15 ml) and then saturated $NaHCO_3$ (15 ml). The organic phase was dried over $MgSO_4$ and concentrated. The crude was purified by chromatography (ethyl acetate/hexanes 1:1) to afford a white solid (390 mg, 93 %). - R_f ((+)-**27a**): 0.16. - mp 73-75 °C. - $[\alpha]_D^{21}$ -24.9 (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.24 (d, *J* = 7.1 Hz, 3H, *CH*$_3$CH), 1.40 (s, 9H, $(CH_3)_3C$), 2.37 (dd, *J* = 5.0, 5.1 Hz, 1H, cyclopropyl-CH), 2.56 (dd, *J* = 5.2, 8.3 Hz, 1H, cyclopropyl-CH), 3.05-3.16 (m, 2H, CH_2Ph), 3.71 (s, 3H, CH_3O), 4.00-4.06 (m, 1H, cyclopropyl-CHN), 4.28-4.40 (m, 2H, 2 CHN), 4.95 (s br, 1H, NHBoc), 5.11-5.22 (m, 2H, CH_2O), 6.40 (d, *J* = 7.2 Hz, 1H, NH), 7.05 (d, *J* = 7.5 Hz, 1H, NH), 7.20-7.36 (m, 10H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 18.1 (+, cyclopropyl-CH), 26.8(+, cyclopropyl-CH), 28.1 (+, $(CH_3)_3C$, 3C), 36.01 (+, cyclopropyl-CHN), 38.3 (-, CH_2Ph), 48.9 (+, CHN), 52.34 (CH_3O), 55.9 (+, CHN), 67.3 (-, CH_2O), 80.2 (C_{quat}, $C(CH_3)_3$), 126.9 (+, Ph-CH, 2C), 128.3 (+, Ph-CH, 2C), 128.5 (+, Ph-CH, 2C), 128.6 (+, Ph-CH, 2C), 129.3 (+, Ph-CH, 2C), 135.2 (C_{quat}, Ph-C), 136.6 (C_{quat}, Ph-C), 155.5 (C_{quat}, C=O Boc), 169.2 (C_{quat}, C=O), 169.9 (C_{quat}, C=O), 171.4 (C_{quat}, C=O), 172.5 (C_{quat}, C=O). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 1136 ($2MH^+$, 17, 568 (MH^+, 100), 512 (MH^+-^{t}Bu, 42), 468 (MH^+-

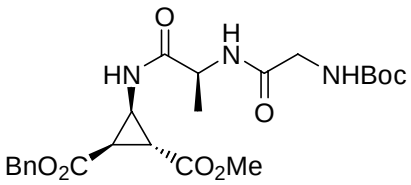
Boc, 30), 307 (34), 263 (32), 154 (100). - IR (KBr) 3296, 3065, 2979, 1729, 1693, 1648, 1533, 1454, 1388 cm^{-1}. - Anal. Calcd for $C_{30}H_{37}N_3O_8$ (567.64): C, 63.48; H, 6.57; N, 7.40. Found: C, 63.21; H, 6.62; N, 7.64.



(-)-**27a**

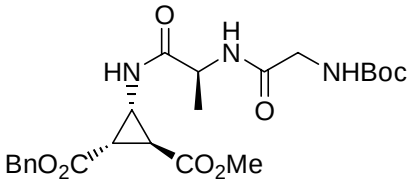
(1S, 2S, 3R) 3-[2S-(2S-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-propionylamino] cyclopropane-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester ((-)-**27a):** A recrystallisation fraction of (±)-**25a** enriched in the (-)-**25a** diastereomer (418 mg, 0.99 mmol) was stirred in HCl 3 M in ethyl acetate (20 ml) at 0 °C for 3 h. The solvent was then evaporated and a solution of Boc-phenylalanine (398 mg, 1.5 mmol, 1.5 eq.), EDC (288 mg, 1.5 mmol, 1.5 eq) and HOBt (270 mg, 2 mmol, 2 eq.) in CH_2Cl_2 (12 ml) (previously stirred 1 h at 0 °C and 1 h at room temperature) were added. Finally triethylamine (140 µl, 1.0 mmol, 1 eq.) was added dropwise. The mixture was stirred at room temperature overnight. The solution was washed with saturated $NaHCO_3$ (15 ml), 1 M $KHSO_4$ (15 ml) and then saturated $NaHCO_3$ (15 ml). The organic phase was dried over $MgSO_4$ and concentrated. The crude was purified by chromatography (ethyl acetate/hexanes 1:1) to give a white solid (510 mg, 91 %). Diastereomerically pure (-)-**27a** was obtained by recrystallization by CH_2Cl_2/ hexanes. - R_f ((-)-**27a**): 0.16. - mp 180-183 °C. - $[\alpha]_D^{21}$ -4.2 (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.27 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 1.41 (s, 9H, $(CH_3)_3C$), 2.30 (dd, *J* = 5.0, 4.9 Hz, 1H, cyclopropyl-CH), 2.56 (dd, *J* = 5.2, 8.2 Hz, 1H, cyclopropyl-CH), 3.05-3.15 (m, 2H, CH_2Ph), 3.71 (s, 3H, CH_3O), 3.88-3.96 (m, 1H, cyclopropyl-CHN), 4.32-4.43 (m, 2H, CHN), 4.86 (s br, 1H, NH Boc), 5.12 (d, *J* = 12.2 Hz, 1H, CH_2O), 5.20 (d, *J* = 12.2 Hz, 1H, CH_2O), 6.38 (d, *J* = 7.4 Hz, 1H, NH), 6.95 (d, *J* = 6.9 Hz, 1H, NH), 7.18-7.27 (m, 5H, Ph-CH), 7.30-7.39 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 17.7 (+, *C*H$_3$CH), 26.5 (+, cyclopropyl-CH), 28.3 (+, (*C*H$_3$)$_3$C, 3C), 28.5 (+, cyclopropyl-CH), 35.93 (+, cyclopropyl-CHN), 38.0 (-, CH_2Ph), 48.9 (+, CHN), 52.4 (+, CH_3O), 56.0 (+, CHN), 67.4 (-, CH_2O), 80.5 (C_{quat}, $(CH_3)_3C$), 127.0 (+, Ph-CH, 2C), 128.3 (+, Ph-CH, 2C), 128.5 (+, Ph-CH, 2C), 128.6 (+, Ph-CH, 2C), 129.3 (+, Ph-CH, 2C), 135.2 (C_{quat}, Ph-C), 136.5 (C_{quat}, Ph-C), 155.6 (C_{quat}, C= Boc), 169.5 (C_{quat}, C=O), 169.8 (C_{quat}, C=O), 171.2 (C_{quat}, C=O), 172.5 (C_{quat}, C=O). – MS CI (NH_3) *m/z* (%) 1152 ($2MNH_4^+$, 9), 1135 ($2MH^+$, 4), 1035 ($2MH^+$-Boc, 2), 585

(MNH_4^+, 100), 568 (MH^+, 22). - IR (KBr) 3291, 2980, 1728, 1675, 1638, 1534 cm^{-1}. - Anal. Calcd for $C_{30}H_{37}N_3O_8$ (567.64): C, 63.48; H, 6.57; N, 7.40. Found: C, 63.25; H, 6.56; N, 7.31.



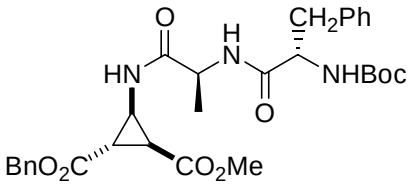
(+)-**27b**

(1R, 2R, 3S) 3-[2S-(*tert*-Butoxycarbonylamino-acetylamino)-propionylamino] cyclopropane-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester ((+)-**27b):** (+)-**25a** (600 mg, 1.43 mmol) was stirred in HCl 3 M in ethyl acetate (15 ml) at 0 °C for 3 h. The solvent was then evaporated and a solution of Boc-glycine (375 mg, 2.14 mmol, 1.5 eq.), EDC (411 mg, 2.14 mmol, 1.5 eq) and HOBt (289 mg, 2.14 mmol, 1.5 eq.) in CH_2Cl_2 (20 ml) (previously stirred 1 h at 0 °C and 1 h at room temperature) was added. Finally triethylamine (200 µl, 1.43 mmol, 1 eq.) was added dropwise. The mixture was stirred at room temperature overnight. The solution was washed with saturated $NaHCO_3$ (20 ml), 1 M $KHSO_4$ (20 ml) and saturated $NaHCO_3$ (20 ml). The organic phase was dried over $MgSO_4$ and concentrated. The crude product was purified by chromatography (ethyl acetate/hexanes 2:1) to afford a white solid (645 mg, 94 %). - R_f ((+)-**27b**): 0.30. - mp 44-46 °C. - $[\alpha]_D^{21}$ -23.2 (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.31 (d, *J* = 7.0 Hz, 3H, C*H*$_3$CH), 1.45 (s, 9H, $(CH_3)_3C$), 2.36 (dd, *J* = 5.0, 5.0 Hz, 1H, cyclopropyl-CH), 2.56 (dd, *J* = 5.2, 8.1 Hz, 1H, cyclopropyl-CH), 3.69 (s, 3H, CH_3O), 3.80-3.85 (m, 2H, CH_2N), 4.01 (ddd, *J* = 4.8, 7.9, 7.9 Hz, 1H, cyclopropyl-CHN), 4.43-4.50 (m, 1H, CHN), 5.14-5.21 (m, 2H, CH_2O), 5.32 (s br, 1H, NH), 6.81 (d, *J* = 6.4 Hz, 1H, NH), 7.16 (d, *J* = 5.9 Hz, 1H, NH), 7.30-7.39 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 18.0 (+, *C*H_3CH), 26.5 (+, cyclopropyl-CH), 28.3 (+,(CH$_3$)$_3$C, 3C), 28.4 (+, cyclopropyl-CH), 35.9 (+, cyclopropyl-CHN), 44.4 (-, CH_2N), 48.9 (+, CHN), 52.5 (+, CH_3O), 67.5 (-, CH_2O), 80.4 (C_{quat}, $(CH_3)_3C$), 128.4 (+, Ph-CH, 2C), 128.6 (+, Ph-CH), 128.7 (+, Ph-CH, 2C), 135.1 (C_{quat}, Ph-C), 156.1 (C_{quat}, N(CO)O), 169.5 (C_{quat}, C=O), 169.7 (C_{quat}, C=O), 169.8 (C_{quat}, C=O), 172.6 (C_{quat}, C=O). - MS CI (NH_3) *m/z* (%) 495 (MNH_4^+, 100), 478 (MH^+, 23), 439 (20), 252 (36), 189 (29). - IR (KBr) 3303, 2980, 1726, 1661, 1524, 1173 cm^{-1}. - Anal. Calcd for $C_{23}H_{31}N_3O_8$(477.513): C, 57.85; H, 6.54; N, 8.80. Found: C, 57.24; H, 6.74; N, 8.39. - HR MS calcd for $C_{23}H_{31}N_3O_8$ + H 478.21894, found 478.21845.



(-)-**27b**

(1S, 2S, 3R) 3-[2S-(*tert*-Butoxycarbonylamino-acetylamino)-propionylamino] cyclopropane-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester ((-)-**27b):** (-)-**25a** (920 mg, 2.19 mmol) was stirred in HCl 3 M in ethyl acetate (14 ml) at 0 °C for 3 h. The solvent was evaporated and a solution of Boc-glycine (574 mg, 3.28 mmol, 1.5 eq.), EDC (631 mg, 3.28 mmol, 1.5 eq.) and HOBt (443 mg, 3.28 mmol, 1.5 eq.) in CH_2Cl_2 (50 ml) (previously stirred 1 h at 0 °C and 1 h at room temperature) was added. Triethylamine (300 µl, 2.19 mmol, 1 eq.) was finally added dropwise. The mixture was stirred at room temperature overnight. The solution was washed with saturated $NaHCO_3$ (50 ml), 1 M $KHSO_4$ (50 ml) and then saturated $NaHCO_3$ (50 ml). The organic phase was dried over $MgSO_4$ and concentrated. The crude product was purified by chromatography ($CHCl_3$/MeOH 80:1) to afford a white solid (966 mg, 92 %). - R_f ((-)-**27b**): 0.05. - mp 55-57°C. - $[\alpha]_D^{21}$ -17.9 (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.33 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 1.45 (s, 9H, $(CH_3)_3$C), 2.37 (dd, *J* = 4.9, 4.9 Hz, 1H, cyclopropyl-CH), 2.56 (dd, *J* = 5.4, 8.1 Hz, 1H, cyclopropyl-CH), 3.70 (s, 3H, CH_3O), 3.78-3.84(m, 2H, CH_2N), 3.88-3.96 (ddd, *J* = 4.8, 7.9, 7.9 Hz, 1H, cyclopropyl-CHN), 4.48 (dq, *J* = 3.6, 3.6 Hz, 1H, CHN), 5.12-5.21 (m, 2H, CH_2O), 5.37 (s br, 1H, NH), 6.68 (d, *J* = 7.4 Hz, 1H, NH), 7.28 (d, *J* = 4.9 Hz, 1H, NH), 7.30-7.37 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 17.6 (+, *C*H_3CH), 26.7 (+, cyclopropyl-CH), 28.3 (+, (*C*H_3)$_3$C, 3C), 28.4 (+, cyclopropyl-CH), 35.9 (+, cyclopropyl-CHN), 48.7 (-, CH_2N), 48.9 (+, CHN), 52.5 (+, CH_3O), 67.4 (-, CH_2O), 80.5 (C_{quat}, $(CH_3)_3C$), 128.3 (+, Ph-CH, 2C), 128.5 (+, Ph-CH), 128.7 (+, Ph-CH, 2C), 135.2 (C_{quat}, Ph-C), 156.2 (C_{quat}, N(CO)O), 169.6 (C_{quat}, C=O), 169.7 (C_{quat}, C=O), 169.9 (C_{quat}, C=O), 172.9 (C_{quat}, C=O). - MS CI (NH_3) *m/z* (%) 495 (MNH_4^+, 100), 478 (MH^+, 27), 439 (18), 263 (19), 252 (95), 189 (88). - IR (KBr) 3319, 3268, 3063, 2979, 1727, 1675, 1649, 1539, 1173 cm^{-1}. - Anal. Calcd for $C_{23}H_{31}N_3O_8$ (477.513): C, 57.85; H, 6.54; N, 8.80. Found: C, 57.27; H, 6.60; N, 8.57. HR MS calcd for $C_{23}H_{31}N_3O_8$ + H 478.21894, found 478.21834.

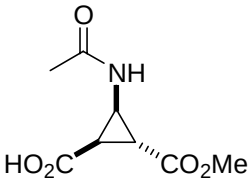


(±)-**28**

(1S*, 2S*, 3S*) 3-[2S-(2S-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-propionylamino] cyclopropane-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester ((±)-**28):** (±)-**26** (1.130 g, 2.69 mmol) was stirred in HCl 3 M in ethyl acetate (11 ml) at 0 °C for 3 h. The solvent was evaporated and a solution of Boc-phenylalanine (1.015 g, 3.83 mmol, 1.4 eq.), EDC (723 mg, 3.77 mmol, 1.4 eq) and HOBt (508 mg, 3.77 mmol, 1.4 eq.) in CH_2Cl_2 (30 ml) (previously stirred 1 h at 0 °C and 1 h at room temperature) was added. Triethylamine (374 µl, 2.69 mmol, 1 eq.) was finally added dropwise. The mixture was stirred at room temperature overnight. The solution was washed with saturated $NaHCO_3$ (50 ml), 1 M $KHSO_4$ (50 ml) and then saturated $NaHCO_3$ (50 ml). The organic phase was dried over $MgSO_4$ and concentrated. The crude product was purified by chromatography (ethyl acetate/hexanes 1:1) to give a white solid (1.44 g, 94 %). - R_f ((±)-**28**): 0.16. - mp 71-73 °C. - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.25 (d, *J* = 7.0 Hz, 3H, C*H*$_3$CH, 1 diast.), 1.29 (d, *J* = 7.0 Hz, 3H, C*H*$_3$CH, 1 diast.), 1.37 (s, 9H, $(CH_3)_3C$, 1 diast.), 1.39 (s, 9H, $(CH_3)_3C$, 1 diast.), 2.33 (dd, *J* = 5.1, 5.1 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.40 (dd, *J* = 5.0, 5.0 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.53 (dd, *J* = 5.5, 8.3 Hz, 1H, cyclopropyl-CH), 3.03-3.12 (m, 2H, CH_2Ph), 3.72 (s, 3H, CH_3O, 1 diast.), 3.73 (s, 3H, CH_3O, 1 diast.), 3.88-3.97 (m, 1H, cyclopropyl-CHN , 1 diast.), 3.99-4.06 (m, 1H, cyclopropyl-CH, 1 diast.), 4.32-4.45 (m, 2H, CHN), 4.91 (d, *J* = 7.6 Hz, 1H, NHBoc, 1 diast.), 4.95 (d, *J* = 8.4 Hz, 1H, NHBoc, 1 diast.), 5.14 (s, 2H, CH_2O), 6.44 (d, *J* = 7.3 Hz, 1H, NH, 1 diast.), 6.46 (d, *J* = 7.3 Hz, 1H, NH, 1 diast.), 6.96 (d, *J* = 6.8 Hz, 1H, NH, 1 diast.), 7.11 (d, *J* = 7.6 Hz, 1H, NH, 1 diast.). 7.17-7.32 (m, 5H, Ph-CH), 7.32-7.40 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 17.7 (+, *C*H_3CH, 1 diast.), 17.8 (+, *C*H_3CH, 1 diast.), 26.5 (+, cyclopropyl-CH), 28.2 (+, $(CH_3)_3C$, 3C), 28.3 (+, cyclopropyl-CH, 1 diast.), 28.5 (+, cyclopropyl-CH, 1 diast.), 35.8 (+, cyclopropyl-CHN, 1 diast.), 35.9 (+, cyclopropyl-CHN, 1 diast.), 38.0 (-, CH_2Ph), 48.9 (+, CHN), 52.3 (+, CH_3O), 55.9 (+, CHN), 67.3 (-, CH_2O), 80.5 ($C_{quat.}$, $C(CH_3)_3$, 1 diast.), 80.6 ($C_{quat.}$, $C(CH_3)_3$, 1 diast), 126.98 (+, Ph-CH, 1 diast.), 127.0 (+, Ph-CH, 1 diast.), 128.39 (+, Ph-CH, 1 diast.), 128.42 (+, Ph-CH, 1 diast.), 128.48 (+, Ph-CH, 2C), 128.60 (+, Ph-CH, 2C), 128.63 (+, Ph-CH), 128.64 (+, Ph-CH), 129.23 (+, Ph-CH); 129.24 (+, Ph-CH), 135.2 ($C_{quat.}$, Ph-C), 136.5 ($C_{quat.}$, Ph-C), 155.5 ($C_{quat.}$, N(C=O)O), 169.3 ($C_{quat.}$, C=O), 169.9 ($C_{quat.}$, C=O), 171.2 ($C_{quat.}$, C=O), 172.3 ($C_{quat.}$,

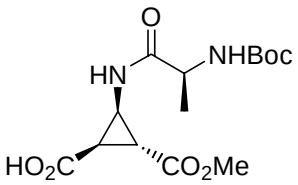
C=O, 1 diast.), 172.5 ($C_{quat.}$, C=O, 1 diast.). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 1135 ($2MH^+$, 7), 568 (MH^+, 100), 512 (MH^+-^{t}Bu, 42), 468 (MH^+-Boc, 37), 307 (66), 263 (38). - IR (KBr) 3288, 3065, 2979, 1733, 1696, 1649, 1520, 1455, 1367 cm^{-1}. - Anal. Calcd for $C_{30}H_{37}N_3O_8$ (567.64): C, 63.48; H, 6.57; N, 7.40. Found: C, 63.37; H, 6.60; N, 7.33.

2.2.2 Deprotection of the C-terminus

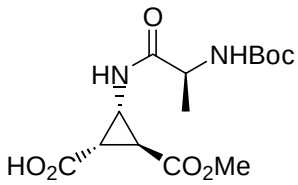


(±)-**29**

(1R*, 2R*, 3R*) 3-(2S-*tert*-Butoxycarbonylamino-propionyl)-cyclopropane-1,2-dicarboxylic acid mono methyl ester ((±)-**29):** (±)-**24** (438 mg, 1.5 mmol) was dissolved under nitrogen atmosphere in MeOH (10 ml), then 1,4-cyclohexadiene (3.5 ml, 40 % in pentane) and of Pd/C 10 % (85 mg) were added. The reaction mixture was stirred under inert atmosphere overnight, then it was filtrated on a 2 cm celite pad and concentrated to afford the product as a white solid (300 mg, 99 %). - mp 62-64 °C. - ^{1}H NMR (CD_3OD, 250 MHz) δ 1.93 (s, 3H, CH_3C=O), 2.31-2.50 (m, 2H, cyclopropyl-CH), 3.53-3.58 (m, 1H, cyclopropyl-CHN), 3.72 (s, 3H, CH_3O). - ^{13}C NMR (CD_3OD, 62.9 MHz) δ 22.3 (+, *C*H_3C=O), 28.1 (+, cyclopropyl-CH), 28.2 (+, cyclopropyl-CH), 36.9 (+, cyclopropyl-CHN), 52.9 (+, CH_3O), 172.1 (C_{quat}, C=O, 2C), 174.4 (C_{quat}, C=O). - MS EI (70 eV) *m/z* (%) 201 (M^+, 3), 183 (MH^+-H_2O, 9), 169 (14), 158 (M^+-acetyl, 16), 141 (38), 114 (47), 100 (86), 82 (56), 43 (100). - IR (KBr) 3358, 3266, 3071, 2657, 1727, 1663, 1638, 1541, 1439, 1312 cm^{-1}. - HR MS calcd for $C_8H_{10}NO_5$ 201.06372, found 201.06365.



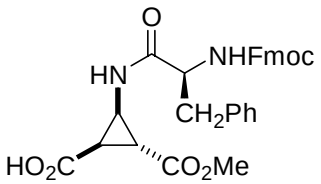
(+)-**30a**



(-)-**30a**

(1R, 2R, 3R) and (1S, 2S, 3S) 3-(2S-*tert*-Butoxycarbonylamino-propionylamino)-cyclopropane-1,2-dicarboxylic acid mono methyl ester ((+)-**30a** and (-)-**30a):** (±)-**25a** (819 mg, 1.95 mmol) was dissolved under nitrogen atmosphere in MeOH (100 ml), then 1,4-

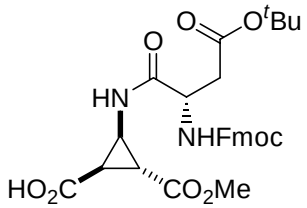
cyclohexadiene (4.5 ml, 40 % in pentane) and Pd/C 5 % (100 mg) were added. The reaction mixture was stirred under inert atmosphere overnight, then it was filtrated on a 2 cm celite pad and concentrated to afford the product as a white solid (640 mg, 99 %). The two diastereomers can be obtained diastereomerically pure from the corresponding dipeptides. - (+)-**30a**- mp 70-72 °C. - $[\alpha]_D^{21}$ -72.0° (c 1, CH_3OH). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.28 (d, *J* = 6.9 Hz, 3H, C*H*$_3$CH), 1.43 (s, 9H, $(CH_3)_3C$), 2.32 (dd, *J* = 4.7, 4.7 Hz, 1H, cyclopropyl-CH), 2.53 (dd, *J* = 5.2, 8.3 Hz, 1H, cyclopropyl-CH), 3.73 (s, 3H, CH_3O), 4.13-4.21 (m, 1H, cyclopropyl-CHN), 4.51-4.57(m, 1H, CHN), 5.43 (d, *J* = 5.3 Hz, 1H, NH), 7.54 (d, *J* = 8.3 Hz, 1H, NH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 19.3 (+, *C*H_3CH), 26.0 (+, cyclopropyl-CH), 28.3 (+,(*C*H_3)$_3$C, 3C), 28.6 (+, cyclopropyl-CH), 36.1 (+, cyclopropyl-CHN), 49.6 (+, CHN), 52.5 (+, CH_3O), 81.0 (C_{quat}, $(CH_3)_3$*C*), 156.0 (C_{quat}, N(CO)O), 170.0 (C_{quat}, C=O), 172.6 (C_{quat}, C=O), 173.7 (C_{quat}, C=O). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 331 (MH^+, 29), 275 (MH^+-^{t}Bu, 35), 154 (100). - IR (KBr) 3352, 2981, 1718, 1526, 1306 cm^{-1}. - HR MS calcd for 331.15053, found 331.14958. - (-)-**30a**- mp 78-80 °C. - $[\alpha]_D^{21}$ -16.5° (c 1, CH_3OH). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.33 (d, *J* = 6.6 Hz, 3H, C*H*$_3$CH), 1.44 (s, 9H, $(CH_3)_3C$), 2.30-2.38 (m, 1H, cyclopropyl-CH), 2.53 (dd, *J* = 5.2, 8.2 Hz, 1H, cyclopropyl-CH), 3.73 (s, 3H, CH_3O), 4.02-4.07 (m, 1H, cyclopropyl-CHN), 4.37-4.41(m, 1H, CHN), 5.37 (d, *J* = 5.3 Hz, 1H, NH), 7.36 (d, *J* = 8.3 Hz, 1H, NH), 9.05 (s br, 1H, COOH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 18.4 (+, *C*H_3CH), 26.2 (+, cyclopropyl-CH), 28.3 (+,(*C*H_3)$_3$C, 3C), 28.7 (+, cyclopropyl-CH), 36.0 (+, cyclopropyl-CHN), 50.0 (+, CHN), 52.5 (+, CH_3O), 80.8 (C_{quat}, $(CH_3)_3$*C*), 156.0 (C_{quat}, N(CO)O), 170.1 (C_{quat}, C=O), 172.2 (C_{quat}, C=O), 174.3 (C_{quat}, C=O). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 353 (MNa^+, 27), 331 (MH^+, 100), 275 (MH^+-^{t}Bu, 123), 231 (MH^+-Boc, 49). - IR (KBr) 3352, 2982, 1719, 1526, 1305 cm^{-1}. - HR MS calcd for $C_{14}H_{22}N_2O_5$ + H 331.15053, found 331.14943.



(±)-**30d**

(1R*, 2R*, 3R*) 3-[2S-(9*H*-fluoren-9-ylmethoxycarbonylamino)-phenyl-propionylamino]-cyclopropane-1,2-dicarboxylic acid mono methyl ester ((±)-**30d):** (±)-**25d** (400 mg, 0.65 mmol) was dissolved under nitrogen atmosphere in a mixture 2:1 of

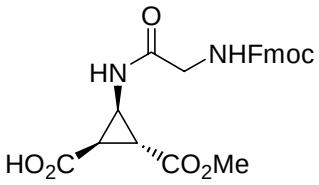
THF and MeOH (50 ml), then 1,4-cyclohexadiene (4 ml, 40 % in pentane) and Pd/C 10 % (180 mg) were added. The reaction mixture was stirred under inert atmosphere 24 h, then it was filtrated on a 2 cm Celite pad and concentrated to afford the product as a white solid (335 mg, 98 %). - mp 150 °C decomp. - ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.04 (dd, *J* = 4.3, 4.3 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.20 (dd, *J* = 4.6, 4.6 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.24-2.29 (m, 1H, cyclopropyl-CH), 2.78-2.86 (m, 1H, CH_2Ph), 3.12-3.22 (m, 1H, CH_2Ph), 3.64 (s, 3H, CH_3O), 3.67-3.77 (m, 1H, cyclopropyl-CHN), 3.98-4.15 (m, 2H, Fmoc-CH + CHN), 4.24-4.29 (m, 2H, Fmoc-CH_2), 6.20 (d, *J* = 9.7 Hz, 1H, NH, 1 diast.), 6.22 (d, *J* = 9.8 Hz, 1H, NH, 1 diast.), 7.14-7.23 (m, 7H, Ar-CH), 7.24-7.34 (m, 2H, Ar-CH), 7.43-7.51 (m, 2H, Fmoc-Ar-CH), 7.71-7.73 (m, 2H, Fmoc-Ar-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 28.8 (+, cyclopropyl-CH, 1 diast.), 28.9 (+, cyclopropyl-CH, 1 diast.), 30.06 (+, cyclopropyl-CH, 1 diast.), 30.12 (+, cyclopropyl-CH, 1 diast.), 36.3 (+, cyclopropyl-CHN, 1 diast.), 36.4 (+, cyclopropyl-CHN, 1 diast.), 38.6 (-, CH_2Ph, 1 diast.), 38.8 (-, CH_2Ph, 1 diast.), 48.2 (+, Fmoc-CH), 52.6 (+, CH_3O), 58.0 (+, CHN, 1 diast.), 58.2 (+, CHN, 1 diast.), 67.6 (-, Fmoc-CH_2, 1 diast.), 67.8 (-, Fmoc-CH_2, 1 diast.), 120.9 (+, Fmoc-Ar-CH, 2C), 126.2 (+, Ar-CH), 126.29 (+, Ar-CH, 1 diast.), 126.33 (+, Ar-CH, 1 diast.), 127.7 (+, Ar-CH, 1 diast.), 127.8 (+, Ar-CH, 1 diast.), 128.1 (+, Ar-CH, 2C), 128.7 (+, Ar-CH, 2C), 129.47 (+, Ar-CH), 129.52 (+, Ar-CH), 130.3 (+, Ar-CH, 2C), 138.5 (C_{quat}, Ph-C, 1 diast.), 138.6 (C_{quat}, Ph-C, 1 diast.), 142.4 (C_{quat}, Fmoc-Ar-C, 2C), 145.1 (C_{quat}, Fmoc-Ar-C, 2C), 158.2 (C_{quat}, N(CO)O, 1 diast.), 158.3 (C_{quat}, N(CO)O, 1 diast.), 172.9 (C_{quat}, C=O), 174.5 (C_{quat}, C=O, 1 diast.), 174.6 (C_{quat}, C=O, 1 diast.), 176.5 (C_{quat}, CO). - MS FAB (MeOH/Glycerine) *m/z* (%) 529 (MH^+, 100). - IR (KBr) 3387, 3065, 2929, 1723, 1669, 1444, 1320 cm^{-1}. - HR MS calcd for $C_{30}H_{28}N_2O_7$ + H 529.19748, found 529.19750.



(±)-**30e**

(1R*, 2R*, 3R*) 3-[4-*tert*-Butoxycarbonyl-2S-(9*H*-fluoren-9-ylmethoxycarbonylamino)-butyrylamino]-cyclopropane-1,2-dicarboxylic acid mono methyl ester ((±)-**30e):** (±)-**25e** (345 mg, 0.54 mmol) was dissolved under nitrogen atmosphere in MeOH (25 ml), then 1,4-cyclohexadiene (2.5 ml, 40 % in pentane) and Pd/C 10 % (250 mg) were added. The reaction

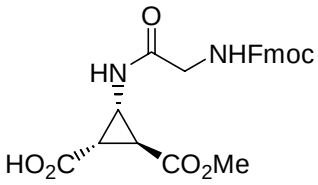
mixture was stirred under inert atmosphere 24 h, then it was filtrated on a 2 cm celite pad and concentrated to afford the product as a white solid (256 mg, 86 %). - mp 74-76 °C. - ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.42 (s, 9H, $(CH_3)_3C$), 2.34-2.38 (m, 1H, cyclopropyl-CH), 2.45-2.49 (m, 1H, cyclopropyl-CH), 2.55-2.77 (m, 2H, $CH_2CO_2{}^{t}Bu$), 3.69 (s, 3H, CH_3O), 3.87-4.03 (m, 1H, cyclopropyl-CHN), 4.16-4.20 (m, 1H, Fmoc-CH), 4.34-4.40 (m, 2H, Fmoc-CH_2), 4.56-4.63 (m, 1H, CHN), 6.20 (d, *J* = 9.7 Hz, 1H, NH, 1 diast.), 6.23 (d, *J* = 9.6 Hz, 1H, NH, 1diast.), 7.26-7.40 (m, 4H, Ar-CH), 7.55-7.64 (m, 3H, Fmoc-Ar-CH + NH), 7.73-7.76 (m, 2H, Fmoc-Ar-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 26.4 (+, cyclopropyl-CH), 27.9 (+, $(CH_3)_3C$, 3C), 28.4 (+, cyclopropyl-CH, 1 diast.), 28.5 (+, cyclopropyl-CH, 1 diast.), 36.1 (+, cyclopropyl-CHN, 1 diast.),36.2 (+, cyclopropyl-CHN, 1 diast.), 37.6 (-, $CH_2CO_2{}^{t}Bu$, 1 diast.), 37.7 (-, $CH_2CO_2{}^{t}Bu$, 1 diast.), 46.9 (+, Fmoc-CH), 51.2 (+, CHN, 1 diast.), 51.4 (+, CHN, 1 diast.), 52.4 (+, CH_3O), 67.5 (-, CH_2O), 82.0 (C_{quat}, $(CH_3)_3C$, 1 diast.), 82.1 (C_{quat}, $(CH_3)_3C$, 1 diast.), 120.0 (+, Fmoc-Ar-CH, 2C), 125.1 (+, Fmoc-Ar-CH, 2C), 126.9 (+, Fmoc-Ar-CH), 127.1 (+, Fmoc-Ar-CH), 127.7 (+, Fmoc-Ar-CH, 2C), 141.21 (C_{quat}, Fmoc-ArC), 141.23 (C_{quat}, Fmoc-Ar-C), 143.5 (C_{quat}, Fmoc-Ar-C), 143.7 (C_{quat}, Fmoc-Ar-C), 156.2 (C_{quat}, N(CO)O, 1 diast.), 156.3 (C_{quat}, N(CO)O, 1 diast.), 169.96 (C_{quat}, C=O, 1 diast.), 170.0 (C_{quat}, C=O, 1 diast.), 171.6 (C_{quat}, C=O), 171.7 (C_{quat}, C=O), 172.5 (C_{quat}, CO). - MS FAB (MeOH/Glycerine) *m/z* (%) 553 (MH^+, 12), 497 (MH^+-^{t}Bu, 62), 275 (100). - IR (KBr) 3337, 3066, 2979, 1724, 1530, 1449, 1370, 1302 cm^{-1}. - HR MS calcd for $C_{29}H_{32}N_2O_9$ + H 553.21861, found 553.21846.



(+)-**30f**

(1R, 2R, 3R) 3-[(9*H*-fluoren-9-ylmethoxycarbonylamino)-ethylamino]-cyclopropane-1,2-dicarboxylic acid mono methyl ester ((+)-**30f):** (+)-**25f** (210 mg, 0.40 mmol) was dissolved under nitrogen atmosphere in MeOH (15 ml), then 1,4-cyclohexadiene (2 ml, 40 % in pentane) and Pd/C 10 % (100 mg) were added. The reaction mixture was stirred under inert atmosphere 24 h, then filtrated on a 2 cm celite pad and concentrated to afford the product as a white solid (123 mg, 71 %). - mp 78-80 °C. - $[\alpha]_D^{21}$ -22.4 (c 0.5, MeOH). - ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.24-2.40 (m, 1H, cyclopropyl-CH), 2.50-2.53 (m, 1H, cyclopropyl-CH), 3.67 (s, 3H, CH_3O), 3.86-4.03 (m, 3H, CH_2N + cyclopropyl-CHN), 4.16 (dd, *J* = 6.8, 7.0 Hz, 1H,

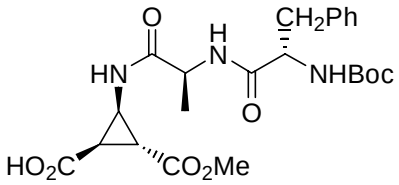
Fmoc-CH), 4.32-4.35 (m, 2H, Fmoc-CH_2), 6.05 (s br, 1H, NHFmoc), 7.23-7.39 (m, 5H, Ar-CH + NH), 7.49-7.56 (m, 2H, Ar-CH), 7.70-7.77 (m, 2H, Ar-CH), 8.64 (s br, CO_2H). - ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 26.5 (+, cyclopropyl-CH), 28.3 (+, cyclopropyl-CH), 35.9 (+, cyclopropyl-CHN), 44.3 (-, CH_2N), 47.0 (+, Fmoc-CH), 52.6 (+, CH_3O), 67.5 (-, CH_2O), 120.0 (+, Ar-CH, 2C), 125.1 (+, Ar-CH, 2C), 127.1 (+, Ar-CH, 2C), 127.8 (+, Ar-CH, 2C), 141.3 (C_{quat}, ArC, 2C), 143.6 (C_{quat}, ArC, 2C), 157.1 (C_{quat}, N(CO)O), 170.0 (C_{quat}, C=O), 170.9 (C_{quat}, C=O), 172.2 (C_{quat}, CO). - MS FAB (MeOH/Glycerine) *m/z* (%) 877 ($2MH^+$, 2), 715 ((MH + 3 Glyc.)$^+$, <1), 623 (MH^++2Glyc., <1), 531 (MH^+ + Glyc., 2), 439 (MH+, 23). - IR ($CDCl_3$) 3431, 3377, 3070, 2956, 1725, 1603, 1516, 1445 cm^{-1}. - HR MS calcd for $C_{23}H_{22}N_2O_7$ + H 439.15053, found 439.15008.



(-)-**30f**

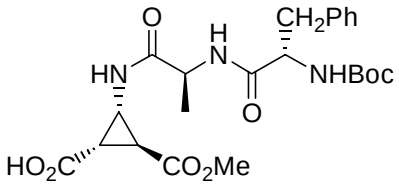
(1S, 2S, 3S) 3-[(9*H*-fluoren-9-ylmethoxycarbonylamino)-ethylamino]-cyclopropane-1,2-dicarboxylic acid mono methyl ester ((-)-**30f):** (-)-**25f** (200 mg, 0.38 mmol) was dissolved under nitrogen atmosphere in MeOH (15 ml), then 1,4-cyclohexadiene (2 ml, 40 % in pentane) and Pd/C 10 % (100 mg) were added. The reaction mixture was stirred under inert atmosphere 24 h, then filtrated on a 2 cm celite pad and concentrated to afford the product as a white solid (100 mg, 60 %). - mp 77-79 °C. - $[\alpha]_D^{21}$ +18.2 (c 0.5, MeOH). - ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.23-2.40 (m, 1H, cyclopropyl-CH), 2.50-2.54 (m, 1H, cyclopropyl-CH), 3.67 (s, 3H, CH_3O), 3.86-4.02 (m, 3H, CH_2N + cyclopropyl-CHN), 4.16 (dd, *J* = 6.7, 7.0 Hz, 1H, Fmoc-CH), 4.31-4.35 (m, 2H, Fmoc-CH_2), 6.05 (s br, 1H, NHFmoc), 7.23-7.39 (m, 5H, Fmoc-Ar-CH + NH), 7.49-7.55 (m, 2H, Fmoc-Ar-CH), 7.70-7.77 (m, 2H, Fmoc-Ar-CH), 8.60 (s br, CO_2H). - ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 26.5 (+, cyclopropyl-CH), 28.3 (+, cyclopropyl-CH), 35.9 (+, cyclopropyl-CHN), 44.2 (-, CH_2N), 47.0 (+, Fmoc-CH), 52.6 (+, CH_3O), 67.4 (-, CH_2O), 120.0 (+, Fmoc-Ar-CH, 2C), 125.1 (+, Fmoc-Ar-CH, 2C), 127.2 (+, Fmoc-Ar-CH, 2C), 127.8 (+, Fmoc-Ar-CH, 2C), 141.3 (C_{quat}, Fmoc-Ar-C, 2C), 143.6 (C_{quat}, Fmoc-Ar-C, 2C), 157.0 (C_{quat}, N(CO)O), 170.0 (C_{quat}, C=O), 170.9 (C_{quat}, C=O), 172.2 (C_{quat}, CO). - MS FAB (MeOH/Glycerine) *m/z* (%) 877 ($2MH^+$, 3), 715 ((MH + 3 Glyc.)$^+$, <1), 623 (MH^++2Glyc., <1), 531 (MH^+ + Glyc., 1), 439 (MH+, 25). - IR ($CDCl_3$) 3434, 3380, 3068,

2958, 1726, 1602, 1517, 1445 cm^{-1}. - HR MS calcd for $C_{23}H_{22}N_2O_7$ + H 439.15053, found 439.15008.



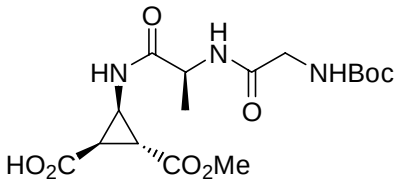
(+)-**31a**

(1R*, 2R*, 3R*) 3-[2S-(2S-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-propionylamino] cyclopropane-1,2-dicarboxylic acid mono methyl ester ((+)-**31a):** (+)-**27a** (200 mg, 0.35 mmol) was dissolved under nitrogen atmosphere in MeOH (10 ml), then 1,4-cyclohexadiene (0.5 ml, 40 % in pentane) and Pd/C 5 % (100 mg) were added. The reaction mixture was stirred under inert atmosphere overnight, then it was filtrated on a 2 cm celite pad and concentrated to afford the product as a white solid (160 mg, 96 %). mp 101-103 °C. - $[\alpha]_D^{21}$ -19.6 (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.15 (d, *J* = 7.0 Hz, 3H, *CH*$_3$CH), 1.27 (s, 9H, $(CH_3)_3C$), 2.22 (dd, *J* = 5.5, 8.0 Hz, 1H, cyclopropyl-CH), 2.34 (dd, *J* = 5.1, 5.1 Hz, 1H, cyclopropyl-CH), 2.68 (dd, *J* = 11.1, 13.5 Hz, 1H, CH_2Ph), 2.97 (dd, *J* = 3.4, 13.7 Hz, 1H, CH_2Ph), 3.37-3.44 (m, 1H, cyclopropyl-CHN), 3.65 (s, 3H, CH_3O), 4.09-4.25 (m, 2H, CHN), 6.90 (d, *J* = 8.6 Hz, 1H, NH), 7.14-7.26 (m, 5H, Ph-CH), 8.03 (d, *J* = 7.3 Hz, 1H, NH), 8.37 (d, *J* = 4.7 Hz, 1H, NH), 12.69 (s br, 1H, COOH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 18.6 (+, *C*H_3CH), 26.4 (+, cyclopropyl-CH), 28.2 (+, cyclopropyl-CH), 28.3 (+, (*C*H_3)$_3$C, 3C), 36.1 8+, cyclopropyl-CHN), 38.4 (-, CH_2Ph), 48.8 (+, CHN), 52.4 (+, CH_3O), 55.9 (+, CHN), 80.7 (C_{quat}, *C*$(CH_3)_3$), 127.0 (+, Ph-CH), 128.6 (+, Ph-CH, 2C), 129.3 (+, Ph-CH, 2C), 136.3 (C_{quat}, Ph-C), 155.7 (C_{quat}, N(CO)O), 170.0 (C_{quat}, C=O), 171.6 (C_{quat}, C=O), 171.9 (C_{quat}, C=O), 172.9 (C_{quat}, C=O). - MS FAB (Glycerin/MeOH) *m/z* (%) 977 ($2MNa^+$, 2), 955 ($2MH^+$, 2), 855 ($2MH^+$-Boc), 500 (MNa^+, 3), 478 (MH^+, 17), 422 (MH^+-*t*-Bu, 37), 378 (MH^+-Boc, 46), 263 (48), 120 (100). - IR (KBr) 3311, 3065, 2980, 1721, 1656, 1526, 1453 cm^{-1}. - HR MS calcd for $C_{23}H_{31}N_3O_8$ + H 478.2189, found 478.22038.



(-)-**31a**

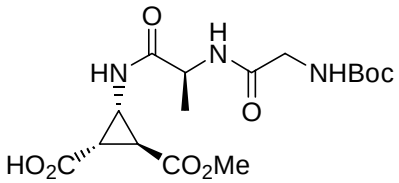
(1R*, 2R*, 3R*) 3-[2S-(2S-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-propionylamino] cyclopropane-1,2-dicarboxylic acid mono methyl ester ((-)-**31a):** (-)-**27a** (1.6 g, 2.82 mmol) was dissolved under nitrogen atmosphere in MeOH (300 ml), then 1,4-cyclohexadiene (4 ml, 40 % in pentane) and Pd/C 10 % (100 mg) were added. The reaction mixture was stirred under inert atmosphere overnight, then it was filtrated on a 2 cm celite pad and concentrated to afford the product as a white solid (1.34 g, 99 %). - mp 108-110 °C. - $[\alpha]_D^{21}$ -4.9 (c 1, $CHCl_3$). - ^{1}H NMR (DMSO-d_6, 250 MHz) δ 1.23 (d, *J* = 7.0 Hz, 3H, C*H*$_3$CH), 1.32 (s, 9H, $(CH_3)_3C$), 2.18-2.22 (m, 2H, cyclopropyl-CH), 2.80 (dd, *J* = 9.3, 14.0 Hz, 1H, CH_2Ph), 3.07 (dd, *J* = 4.76, 14.0 Hz, 1H, CH_2Ph), 3.51, (dd, *J* = 4.7, 8.0 Hz, 1H, cyclopropyl-CHN), 3.66 (s, 3H, CH_3O), 4.19-4.27 (m, 2H, CHN), 6.31 (d, *J* = 8.7 Hz, 1H, NH), 7.15-7.22 (m, 1H, Ph-CH), 7.23-7.24 (m, 4H, Ph-CH), 7.67 (d, *J* = 7.6 Hz, 1H, NH), 8.03 (s br, 1H, NH). - ^{13}C NMR (DMSO-d_6, 62.9 MHz) δ 17.8 (+, *C*H_3CH), 27.1 (+, cyclopropyl-CH), 28.1 (+, cyclopropyl-CH), 28.3 (+, (*C*H_3)$_3$C), 36.1 (+, cyclopropyl-CHN), 38.2 (-, CH_2Ph), 49.0 (+, CHN), 52.4 (+, CH_3O), 55.9 (+, CHN), 81.0 (C_{quat}, *C*$(CH_3)_3$), 127.2 (+, Ph-CH), 128.5 (+, Ph-CH, 2C), 128.7 (+, Ph-CH, 2C), 136.3 (C_{quat}, Ph-C), 156.0 (C_{quat}, N(CO)O), 170.2 (C_{quat}, C=O, 2C), 172.0 (C_{quat}, C=O), 173.1 (C_{quat}, C=O). - MS FAB (Glycerin/MeOH) *m/z* (%) 977 ($2MNa^+$, 7), 955 ($2MH^+$, 2), 500 (MNa^+, 10), 478 (MH^+, 16), 422 (MH^+-*t*-Bu, 24), 378 (MH^+-Boc, 23), 263 (35), 219 (30). - IR (KBr) 3319, 3063, 2980, 1721, 1711, 1662, 1528, 1453 cm^{-1}. - HR MS calcd for $C_{23}H_{31}N_3O_8$ + H 478.2189, found 478.22043.



(+)-**31b**

(1R*, 2R*, 3R*) 3-[2S-(*tert*-Butoxycarbonylamino-ethylamino)-propionylamino] cyclopropane-1,2-dicarboxylic acid mono methyl ester ((+)-**31b):** (+)-**27b** (490 mg, 1.03 mmol) was dissolved under nitrogen atmosphere in 30 ml of MeOH, then 2.5 ml of 1,4-

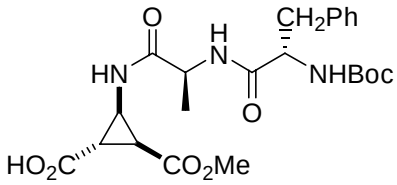
cyclohexadiene (40 % in pentane) and 60 mg of Pd/C 5 % were added. The reaction mixture was stirred under inert atmosphere overnight, then it was filtrated on a 2 cm celite pad and concentrated to afford 402 mg (>99 %) of product as a white solid. - mp 112-115 °C. - $[\alpha]_D^{21}$ -23.7 (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.34 (d, *J* = 6.6 Hz, 3H, C*H*$_3$CH), 1.45 (s, 9H, $(CH_3)_3C$), 2.39-2.40 (m, 1H, cyclopropyl-CH), 2.43-2.52 (m, 1H, cyclopropyl-CH), 3.71 (s, 3H, CH_3O), 3.81-3.94 (m, 3H, cyclopropyl-CHN + CH_2N), 4.57-4.70 (m, 1H, CHN), 5.78 (s, br, 1H, NH), 7.46 (d, *J* = 7.5 Hz, 1H, NH), 7.73 (s br, 1H, NH), 9.30 (s br, 1H, COOH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 18.0 (+, *C*H_3CH), 26.8 (+, cyclopropyl-CH), 28.3 (+,(*C*H_3)$_3$C + cyclopropyl-CH, 4C), 35.9 (+, cyclopropyl-CHN), 44.1 (-, CH_2N), 49.0 (+, CHN), 52.5 (+, CH_3O), 80.6 (C_{quat}, $(CH_3)_3$*C*), 156.6 (C_{quat}, N(CO)O), 170.2 (C_{quat}, C=O), 170.6 (C_{quat}, C=O), 172.2 (C_{quat}, C=O), 173.5 (C_{quat}, C=O). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 775 ($2MH^+$, 2), 388 (MH^+, 24), 332 (15), 307 (32), 289 (15), 154 (100). - IR (KBr) 3317, 2981, 1721, 1669, 1527, 1302, 1252 cm^{-1}. - HR MS calcd for $C_{16}H_{25}N_3O_8$ + H 388.17199, found 388.17042.



(-)-**31b**

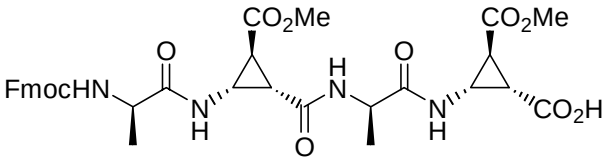
(1S*, 2S*, 3S*) 3-[2S-(*tert*-Butoxycarbonylamino-ethylamino)-propionylamino] cyclopropane-1,2-dicarboxylic acid mono methyl ester ((-)-**31b):** (-)-**27b** (915 mg, 1.92 mmol) was dissolved under nitrogen atmosphere in MeOH (100 ml), then 1,4-cyclohexadiene (4.5 ml, 40 % in pentane) and Pd/C 5 % (110 mg) were added. The reaction mixture was stirred under inert atmosphere overnight, then it was filtrated on a 2 cm celite pad and concentrated to afford the product as a white solid (765 mg, >99 %). - mp 69-72 °C. - $[\alpha]_D^{21}$ -13.7 (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.34 (d, *J* = 4.8 Hz, 3H, C*H*$_3$CH), 1.43 (s, 9H, $(CH_3)_3C$), 2.36-2.51 (m, 2H, cyclopropyl-CH), 3.70 (s, 3H, CH_3O), 3.81-3.99 (m, 3H, cyclopropyl-CHN + CH_2N), 4.57-4.61 (m, 1H, CHN), 5.77 (s, br, 1H, NH), 7.56 (s br, 1H, NH), 7.97 (s br, 1H, NH), 10.78 (s br, 1H, COOH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 17.5 (+, *C*H_3CH), 27.5 (+, cyclopropyl-CH), 28.3 (+, (*C*H_3)$_3$C) + cyclopropyl-CH, 4C), 36.9 (+, cyclopropyl-CHN), 44.0 (-, CH_2N), 48.8 (+, CHN), 52.5 (+, CH_3O), 80.6 (C_{quat}, $(CH_3)_3$*C*)), 156.6 (C_{quat}, N(CO)O), 170.2 (C_{quat}, C=O), 171.0 (C_{quat}, C=O), 172.0 (C_{quat}, C=O), 173.9 (C_{quat}, C=O). - MS FAB (Glycerin/MeOH) *m/z* (%) 775 ($2MH^+$, 11), 388 (MH^+, 60),

332 (100). - IR (KBr) 3337, 2982, 1721, 1669, 1533 cm^{-1}. - HR MS calcd for $C_{16}H_{25}N_3O_8$ + H 388.17199, found 388.17119.



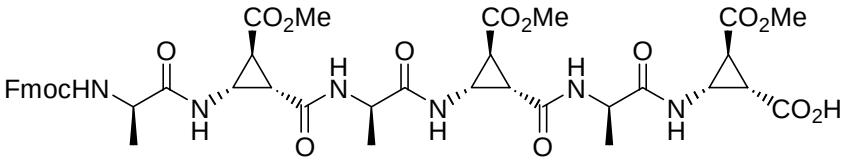
(±)-**32**

(1S*, 2S*, 3R*) 3-[2S-(2S-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-propionylamino] cyclopropane-1,2-dicarboxylic acid mono methyl ester ((±)-32): (±)-**28** (230 mg, 0.40 mmol) was dissolved under nitrogen atmosphere in MeOH (10 ml), then 1,4-cyclohexadiene (3 ml, 40 % in pentane) and Pd/C 10 % (50 mg) were added. The reaction mixture was stirred under inert atmosphere overnight, then it was filtrated on a 2 cm celite pad and concentrated to afford the product as a white solid (188 mg, 99%). - mp 105-107 °C. - ^{1}H NMR (CD_3OD, 250 MHz) δ 1.28 (d, *J* = 6.6 Hz, 3H, C*H*$_3$CH), 1.35 (s, 9H, $(CH_3)_3C$), 2.31-2.45 (m, 2H, cyclopropyl-CH), 2.76-2.93 (m, 1H, CH_2Ph), 3.14 (ddd, *J* = 2.3, 2.3, 11.8 Hz, 1H, CH_2Ph), 3.49-3.63 (m, 1H, cyclopropyl-CHN), 3.66 (s, 3H, CH_3O, 1 diast.), 3.67 (s, 3H, CH_3O, 1 diast.), 4.23-4.42 (m, 2H, CHN), 7.12-7.34 (m, 5H, Ph-CH). - ^{13}C NMR (CD_3OD, 62.9 MHz) δ 18.1 (+, *C*H_3CH, 1 diast.), 18.2 (+, CH_3CH, 1 diast.), 28.5 (+, cyclopropyl-CH), 28.7 (+, (*C*H_3)$_3$C, 3C), 36.6 (+, cyclopropyl-CH), 39.1 (-, CH_2Ph), 50.4 (+, CHN), 52.8 (+, CHN), 52.9 (+, CH_3O, 1 diast.), 53.0 (+, CH_3O, 1 diast.), 57.4 (+, CHN), 80.5 ($C_{quat.}$, *C*$(CH_3)_3$, 1 diast.), 80.7 ($C_{quat.}$, *C*$(CH_3)_3$, 1 diast.), 127.7 (+, Ph-CH, 1 diast.), 127.8 (+, Ph-CH, 1 diast.), 129.4 (+, Ph-CH, 2C, 1 diast.), 129.5 (+, Ph-CH, 2C, 1 diast.), 130.3 (+, Ph-CH, 2C, 1 diast.), 130.5 (+, Ph-CH, 2C, 1 diast.), 138.4 ($C_{quat.}$, Ph-C, 1 diast.), 138.7 ($C_{quat.}$, Ph-C, 1 diast.), 157.7 ($C_{quat.}$, N(C=O)O), 170.5 ($C_{quat.}$, C=O), 170.7 ($C_{quat.}$, C=O), 174.1 ($C_{quat.}$, C=O), 175.8 ($C_{quat.}$, C=O). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 978 ($2MNa^+$, 27), 500 (MNa^+, 25), 478 (MH^+, 100), 422 (MH^+-^{t}Bu, 84), 378 (MH^+-Boc, 51), 277 (46), 263 (82), 219 (46). - IR (KBr) 3336, 3065, 2980, 1717, 1653, 1540, 1455, 1368 cm^{-1}. - HR MS calcd for $C_{23}H_{31}N_3O_8$ + H 478.2189, found 478.22002.



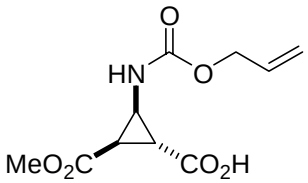
(+)-**42**

(1R, 2R, 3R) 3-[2S-({2R-[2S-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3R-methoxycarbonyl-cyclopropane-1R-carbonyl}-amino)-propionylamino]-cyclopropane-1, 2-dicarboxylic acid mono methyl ester ((+)-**42):** (+)-**41** (200 mg, 0.26 mmol) was dissolved under nitrogen atmosphere in a mixture MeOH/Benzene/HCO_2H 5/5/2 (15 ml), then Pd/C 10 % (180 mg) was added. The reaction mixture was stirred under inert atmosphere 2.5 h, then it was filtrated on a 2 cm celite pad and concentrated to afford the product as a white solid (175 mg, 99 %). - mp 220 °C decomp. - $[\alpha]_D^{21}$ -70.8 (c 0.5, MeOH). - ^{1}H NMR (CD_3OD, 400 MHz): δ 1.22 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 1.28 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 2.31-2.42 (m, 3H, cyclopropyl-CH), 2.45-2.51 (m, 1H, cyclopropyl-CH), 3.51-3.62 (m, 2H, cyclopropyl-CHN), 3.67 (s, 3H, CH_3O), 3.69 (s, 3H, CH_3O), 4.04-4.09 (m, 1H, CHN), 4.18-4.23 (m, 2H, CHN + Fmoc-CH), 4.28-4.38 (m, 2H, Fmoc-CH_2), 7.28-7.31 (m, 2H, Fmoc-Ar-CH), 7.35-7.39 (m, 2H, Fmoc-Ar-CH), 7.63-7.69 (m, 2H, Fmoc-Ar-CH), 7.76-7.78 (m, 2H, Fmoc-Ar-CH). - ^{13}C NMR (CD_3OD, 62.9 MHz): δ 17.9 (+, *C*H$_3$CH), 18.0 (+, *C*H$_3$CH), 28.1 (+, cyclopropyl-CH), 28.5 (+, cyclopropyl-CH), 28.6 (+, cyclopropyl-CH), 29.0 (+, cyclopropyl-CH), 36.6 (+, cyclopropyl-CHN, 2C), 48.4 (+, Fmoc-CH), 50.4 (+, CHN), 52.0 (+, CHN), 52.8 (+, CH_3O), 52.9 (+, CH_3O), 68.0 (-, Fmoc-CH_2), 120.9 (+, Fmoc-Ar-CH, 2C), 126.2 (+, Fmoc-Ar-CH), 126.4 (+, Fmoc-Ar-CH), 128.2 (+, Fmoc-Ar-CH, 2C), 128.8 (+, Fmoc-Ar-CH, 2C), 142.5 (C_{quat}, Fmoc-Ar-C), 142.6 (C_{quat}, Fmoc-Ar-C), 145.1 (C_{quat}, Fmoc-Ar-C), 145.4 (C_{quat}, Fmoc-Ar-C), 158.2 (C_{quat}, N(CO)O), 169.4 (C_{quat}, C=O), 171.6 (C_{quat}, C=O), 171.9 (C_{quat}, C=O), 172.4 (C_{quat}, C=O), 175.9 (C_{quat}, C=O), 176.5 (C_{quat}, C=O). – MS FAB (MeOH/Glycerine) *m/z* (%) 665 (MH^+, 6), 179 (Fluorenyl cation, 100). - IR (KBr) 3298, 3066, 2955, 1724, 1664, 1529, 1448, 1312 cm^{-1}. - HR MS calcd for $C_{33}H_{36}N_4O_{11}$ + H 665.24588, found 665.24705.



(+)-**46**

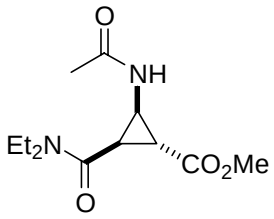
(1R, 2R, 3R) 3-{2S-[2R-(2S-{2R-[2S-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3R-methoxycarbonyl-cyclopropane-1R-carbonylamino}-propionylamino)-3R-methoxycarbonyl-cyclopropane-1R-carbonylamino]-propionylamino}-cyclopropane-1, 2-dicyrboxylic acid mono methyl ester ((+)-**46):** (+)-**45** (200 mg, 0.21 mmol) was dissolved under nitrogen atmosphere in a mixture MeOH/HCO_2H 1/1 (25 ml), then Pd/C 10 % (180 mg) was added. The reaction mixture was stirred under inert atmosphere 5 h, then it was filtrated on a 2 cm celite pad and concentrated to afford the product as a white solid (160 mg, 88 %). - mp 180 °C decomp. - $[\alpha]_D^{21}$ -48.7 (c 0.5, MeOH) - ^{1}H NMR (CD_3OD, 250 MHz): δ 1.25 (d, *J* = 7.7 Hz, 3H, C*H*$_3$CH), 1.29(d, J =7.2 Hz, 6H, C*H*$_3$CH), 2.29-2.32 (m, 2H, cyclopropyl-CH), 2.44-2.80 (m, 4H, cyclopropyl-CH), 3.58-3.69 (m, 3H, cyclopropyl-CHN), 3.66 (s, 6H, CH_3O), 3.69 (s, 3H, CH_3O), 4.07-4.36 (m, 6H, 3 CHN + Fmoc-CH + Fmoc-CH_2), 7.25-7.39 (m, 4H, Fmoc-Ar-CH), 7.62-7.68 (m, 2H, Fmoc-Ar-CH), 7.75-7.78 (m, 2H, Fmoc-Ar-CH). - ^{13}C NMR (CD_3OD, 62.9 MHz): δ 17.8 (+, *C*H_3CH), 18.0 (+, *C*H_3CH), 18.1 (+, *C*H_3CH), 27.5 (+, cyclopropyl-CH), 27.6 (cyclopropyl-CH, 2C), 28.6 (cyclopropyl-CH), 29.1 (cyclopropyl-CH, 2C), 36.5 (+, cyclopropylCHN), 36.6 (cyclopropyl-CHN, 2C), 48.4 (+, Fmoc-CH), 50.06 (+, CHN), 50.13 (+, CHN), 52.1 (+, CHN), 52.9 (+, CH_3O), 53.0 (+, CH_3O), 53.1 (+, CH_3O), 68.1 (-, Fmoc-CH_2), 121.0 (+, Fmoc-Ar-CH, 2C), 126.3 (+, Fmoc-Ar-CH), 126.4 (+, Fmoc-Ar-CH), 128.3 (+, Fmoc-Ar-CH, 2C), 128.9 (+, Fmoc-Ar-CH, 2C), 142.55 (C_{quat}, Fmoc-Ar-C), 142.60 (C_{quat}, Fmoc-Ar-C), 145.1 (C_{quat}, Fmoc-Ar-C), 145.4 (C_{quat}, Fmoc-Ar-C), 158.3 (C_{quat}, N(CO)O), 169.5 (C_{quat}, C=O), 169.6 (C_{quat}, C=O), 172.48 (C_{quat}, C=O), 172.54 (C_{quat}, C=O), 172.7 (C_{quat}, C=O), 173.8 (C_{quat}, C=O), 175.5 (C_{quat}, C=O), 175.7 (C_{quat}, C=O), 176.6 (C_{quat}, C=O). - MS NI-FAB (MeOH/Glycerine) *m/z* (%) 875 ($(M-H)^-$, 100). - IR (KBr) 3320, 3065, 2955, 1729, 1656, 1602, 1447, 1317 cm^{-1}. - HR MS calcd for $C_{42}H_{48}N_6O_{15}$ – H 875.30994, found 875.30801.



(±)-**69b**

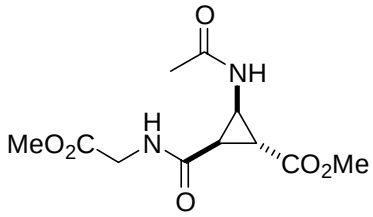
(1R*, 2R*, 3S*) 3-Allyloxycarbonyl-cyclopropane-1,2-dicarboxylic acid mono methyl ester ((±)-**69b):** (±)-**16b** (1.353 g, 4.06 mmol) was dissolved in acetone (13 ml). The solution was added dropwise to a 0.1 M (pH = 7.4) phosphate buffer (170 ml) in an ultrasonic bad. To this suspension PLE (300 mg, 50 U/mg) was added and the reaction mixture was stirred at room temperature and at constant pH by addition of a 0.1 M solution of NaOH. After 4 h the pH was brought to 8 by addition of a 0.1 M NaOH solution and the water solution was extracted with diethyl ether (100 ml), then the pH of the water phase was brought to 2 by addition of a 1 M solution of H_3PO_4, saturated with NaCl and extracted twice with diethyl ether (150 ml). The organic phase was dried over Na_2SO_4 and concentrated to yield a mixture 8:1 of the hydrolysed benzyl ester and hydrolysed methyl ester (770 mg, 78 %). (±)-**69b** (550 mg, 56 %) could be isolated pure by recrystallisation from diethyl ether/hexanes. - mp 100-101 °C. - ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.24-2.28 (m, 1H, cyclopropyl-CH), 2.53 (dd, *J* = 5.2, 8.5 Hz, 1H, cyclopropyl-CH), 3.77 (s, 3H, CH_3O), 3.91-4.06 (m, 1H, cyclopropyl-CHN), 4.59-4.61 (m, 2H, CH_2=), 5.21-5.35 (m, 2H, CH_2O), 5.83-5.98 (m, 1H, C*H*=CH_2), 6.21 (s br, 1H, NHAlloc), 10.48 (s br, 1H, CO_2H). - ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 26.0 (+, cyclopropyl-CH), 28.7 (+, cyclopropyl-CH), 37.6 (+, cyclopropyl-CHN), 52.7 (+, CH_3O), 66.3 (-, $PhCH_2O$), 118.5 (-, CH_2=), 132.2 (+, *C*H=CH_2), 156.5 (C_{quat}, N(CO)O), 170.4 (C_{quat}, C=O), 174.0 (C_{quat}, C=O). - MS PI-EI (70 eV) *m/z* (%) 243 (M^+, 1), 225 (M^+-H_2O, 3), 211 (M^+-CH_3OH, 3), 202 (M^+-C_3H_5, 3), 158 (M^+-Alloc, 27), 140 (22), 114 (23), 82 (23), 41 ($C_3H_5^+$, 100). - IR (KBr) 3354, 3069, 3022, 2890, 1737, 1700, 1516, 1441, 1316, 1236 cm^{-1}. - HR MS calcd for $C_{10}H_{13}NO_6$ 243.07429, found 243.07441.

2.2.3 Coupling at the C-terminus



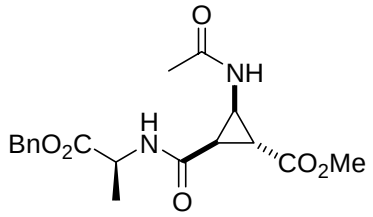
(±)-**34**

(1R*, 2R*, 3R*) 2-acetylamino-3-diethyl-carbamoyl-cyclopropane carboxylic acid methyl ester ((±)-**34):** (±)-**29** (200 mg, 0.99 mmol) was dissolved in CH_2Cl_2 (60 ml) and DMF to ensure solubilisation (3 ml). To this solution EDC (190 mg, 0.99 mmol, 1 eq.) and HOBt (133 mg, 0.99 mmol, 1 eq.) were added. The mixture was cooled in an ice bath, then diethylamine (156 µl, 109 mg, 1.5 mmol, 1.5 eq.) was added. The mixture was stirred overnight at room temperature. The solution was then concentrated in vacuum and the product was isolated by chromatography ($CHCl_3$/MeOH 50:1) as a white solid (165 mg, 65 %). The reaction resulted in 25 % epimerization, but the epimer could be easily separated by chromatography. - R_f ((±)-**34**): 0.18. - mp 97-98 °C. - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.06 (t, *J* = 7.1 Hz, 3H, C*H*$_3$CH$_2$), 1.17 (t, *J* = 7.1 Hz, 3H, C*H*$_3$CH$_2$), 1.89 (s, 3H, CH_3C=O), 2.28 (dd, *J* = 5.1, 5.1 Hz, 1H, cyclopropyl-CH), 2.48 (dd, *J* = 5.2, 8.3 Hz, 1H, cyclopropyl-CH), 3.27-3.54 (m, 4H, C*H*$_2$CH$_3$), 3.64 (s, 3H, CH_3O), 3.98 (ddd, *J* = 4.4, 8.1, 8.1 Hz, 1H, cyclopropyl-CHN), 7.35 (d, *J* = 7.7 Hz, 1H, NH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 12.9 (+, *C*H$_3$CH$_2$), 14.6 (+, *C*H$_3$CH$_2$), 23.1 (+, CH_3C=O), 24.7 (+, cyclopropyl-CH), 27.6 (+, cyclopropyl-CH), 36.6 (+, cyclopropyl-CHN), 41.1 (-, CH_2), 42.6 (-, CH_2), 52.1 (+, CH_3O), 167.7 (C_{quat}, C=O), 170.6 (C_{quat}, C=O), 171.1 (C_{quat}, C=O). - MS CI(NH_3) *m/z* (%) 513 ($2MH^+$, 12), 272 (MNH_4^+, 4), 257 (MH^+, 100). - IR (KBr) 3297, 3035, 2973, 1732, 1677, 1626, 1534, 1446, 1314 cm^{-1}. - Anal. Calcd for $C_{12}H_{20}N_2O_4$ (256.301): C, 56.24; H, 7.86; N, 10.93. Found: C, 56.25; H, 7.84; N, 10.76.



(±)-**35a**

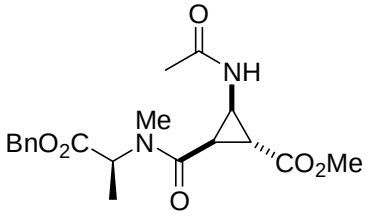
(1R*, 2R*, 3R*) 2-acetylamino-3-(methoxycarbonyl-methyl-carbamoyl)-cyclopropane carboxylic acid methylester ((±)-**35a):** Glycine methylester hydrochloride (328 mg, 2.61 mmol, 1.5 eq.) was suspended in CH_2Cl_2 (50 ml) and triethylamine (363 µl, 2.61 mmol, 1.5 eq.) was added. To this solution EDC (334 mg, 1.74 mmol, 1 eq.), HOBt (234 mg, 1.74 mmol, 1 eq.) and (±)-**29** (350 mg, 1.74 mmol, 1 eq.) were added at 0 °C. The mixture was stirred overnight at room temperature. The solution was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 60:1) as a white solid (459 mg, 97 %). - R_f ((±)-**35a**): 0.05. - mp 125-126 °C. - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.98 (s, 3H, CH_3C=O), 2.33 (dd, *J* = 5.0,5.0 Hz, 1H, cyclopropyl-CH), 2.46 (dd, *J* = 5.1, 8.3 Hz, 1H, cyclopropyl-CH), 3.70 (s, 3H, CH_3O), 3.77 (s, 3H, CH_3O), 4.03-4.12 (m, 3H, cyclopropyl-CHN + CH_2N), 6.90 (t, *J* = 4.9 Hz, 1H, NH), 7.00 (d, *J* = 8.3 Hz, 1H, NH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 23.2 (+, *C*H_3C=O), 27.1 (+, cyclopropyl-CH), 27.8 (+, cyclopropyl-CH), 36.3 (+, cyclopropyl-CHN), 41.5 (-, CH_2N), 52.3 (+, CH_3O), 52.5 (+, CH_3O), 169.1 (C_{quat}, C=O), 169.7 (C_{quat}, C=O), 170.58 (C_{quat}, C=O), 170.65 (C_{quat}, C=O). - MS CI (NH_3) *m/z* (%) 290 (MNH_4^+, 18), 273 (MH^+, 12), 258 (MH^+-CH_3, 100), 241 (MH^+-CH_3O), 216 (6), 201 (8). - IR (KBr) 3315, 3248, 3069, 2955, 1760, 1731,. 1670, 1649, 1565, 1547, 1445, 1375, 1319 cm^{-1}. - Anal. Calcd for $C_{11}H_{16}N_2O_6$ (272.26): C, 48.53; H, 5.92; N, 10.29. Found: C, 48.25; H, 5.88; N, 10.10.



(±)-**35b**

(1R*, 2R*, 3R*) 2-acetylamino-3 (1S-benzyloxycarbonyl-ethylcarbamoyl)-cyclopropane carboxylic acid methylester ((±)-**35b)**: The Boc-alanine benzyl ester (474 mg, 1.7 mmol, 1.5 eq.) was deprotected by treatment with HCl 3 M in ethyl acetate (15 ml) for 3 h at 0 °C. The solution was then concentrated in vacuum, the salt resuspended in CH_2Cl_2 (50 ml) and

triethylamine (236 µl, 1.7 mmol, 1.5 eq.) was added dropwise. To this solution EDC (219 mg, 1.14 mmol, 1 eq.) and HOBt (154 mg, 1.14 mmol, 1 eq.) were added. The mixture was cooled in an ice bath, then (±)-**29** (230 mg, 1.14 mmol) dissolved in CH_2Cl_2 (20 ml) was added. The mixture was stirred at room temperature overnight. The solution was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 60:1) as a white solid (285 mg, 69 %). - R_f ((±)-**35b**): 0.06. - mp 106-108 °C. - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH, 1 diast.), 1.44 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH, 1 diast.), 1.93 (s, 3H, CH_3C=O, 1 diast.), 1.96 (s, 3H, CH_3O, 1 diast.), 2.29 (dd, *J* = 5.2, 9.9 Hz, 1H, cyclopropyl-CH), 2.37-2.44 (m, 1H, cyclopropyl-CH), 3.68 (s, 3H, CH_3O, 1 diast.), 3.69 (s, 3H, CH_3O, 1 diast.), 4.03-4.12 (m, 1H, cyclopropyl-CHN), 4.55-4.63 (m, 1H, Ala-CHN), 5.14-5.23 (m, 2H, CH_2O), 7.04 (d, *J* = 8.3 Hz, 1H, NH, 1 diast.), 7.11-7.12 (m, 3H, NH, 2 NH of one diast. + 1 NH of the other), 7.32-7.39 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 17.9 (+, *C*H$_3$CH, 1 diast.), 18.0 (+, *C*H$_3$CH, 1 diast.), 23.20 (+, CH_3C=O, 1 diast.), 23.22 (+, CH_3C=O, 1 diast.), 26.90 (+, cyclopropyl-CH, 1 diast.), 26.94 (+, cyclopropyl-CH, 1 diast.), 27.56 (+, cyclopropyl-CH, 1 diast.), 27.61 (+, cyclopropyl-CH, 1 diast.), 36.18 (+, cyclopropyl-CHN, 1 diast.), 36.20 (+, cyclopropyl-CHN, 1 diast.), 48.49 (+, CHN, 1 diast.), 48.54 (+, CHN, 1 diast.), 52.29 (+, CH_3O, 1 diast.), 52.31 (+, CH_3O, 1 diast.), 67.2 (-, CH_2O, 1 diast.), 67.3 (-, CH_2O, 1 diast.), 128.06 (+, Ph-CH, 1 diast., 2C), 128.12 (+, Ph-CH, 1 diast., 2C), 128.4 (+, Ph-CH), 128.6 (+, Ph-CH, 2C), 135.1 (C_{quat}, Ph-C), 168.4 (C_{quat}, C=O, 1 diast.), 168.5 (C_{quat}, C=O, 1 diast.), 170.71 (C_{quat}, C=O, 1 diast.), 170.76 (C_{quat}, C=O, 1 diast.), 170.79 (C_{quat}, C=O, 1 diast.), 170.81 (C_{quat}, C=O, 1 diast.), 172.26 (C_{quat}, C=O, 1 diast.), 172.31 (C_{quat}, C=O, 1 diast). - MS CI (NH_3) *m/z* (%) 725 ($2MH^+$, 6), 380 (MNH_4^+, 100), 363 (MH^+, 45), 348 (14). - IR (KBr) 3297, 3059, 2980, 2935, 1734, 1654, 1546, 1450, 1370, 1319, 1213 cm^{-1}. - Anal. Calcd for $C_{18}H_{22}N_2O_6$ (362.38): C, 59.66; H, 6.12; N, 7.73. Found: C, 59.55; H, 6.12; N, 7.67.

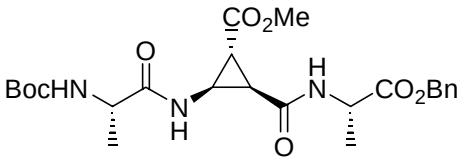


(±)-**35c**

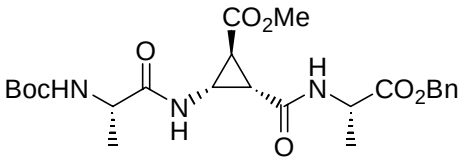
(1R*, 2R*, 3R*) 2-acetylamino-3-(1S-benzyloxycarbonyl-ethyl-methylcarbamoyl)-cyclopropane carboxylic acid methylester ((±)-**35c)**: N-methyl Boc-alanine benzyl ester (589 mg, 2.01 mmol, 1.5 eq.) was deprotected by treatment with HCl 3 M in ethyl acetate

(8 ml) for 3 h at 0 °C. The solution was then concentrated in vacuum, the salt was resuspended in CH_2Cl_2 (50 ml) and triethylamine (280 µl, 2.01 mmol, 1.5 eq.) was added dropwise. To this solution EDC (257 mg, 1.34 mmol, 1 eq.) and HOBt (181 mg, 1.34 mmol, 1 eq.) were added. The mixture was cooled in an ice bath and (±)-**29** (270 mg, 1.34 mmol) dissolved in CH_2Cl_2 (20 ml) was added. The mixture was stirred overnight at room temperature. The solution was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 80:1) as a colourless oil (488 mg, 97 %). 10 % epimerisation was observed. - R_f ((±)-**35c**): 0.07. - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.43 (d, *J* = 5.7 Hz, C*H*$_3$CH, 3H, 1 diast.), 1.46 (d, *J* = 5.8 Hz, 3H, C*H*$_3$CH, 1 diast.), 1.52 (d, *J* = 5.7 Hz, 3H, C*H*$_3$CH, 1 diast. epimer), 1.54 (d, *J* = 5.7 Hz, 3H, C*H*$_3$CH, 1 diast. epimer), 1.90 (s, 3H, CH_3C=O, 1 diast. epimer), 1.91 (s, 3H, CH_3C=O, 1 diast. epimer), 1.94 (s, 3H, CH_3C=O, 1 diast.), 1.95 (s, 3H, CH_3C=O, 1 diast.), 2.26 (dd, *J* = 4.9, 4.9 Hz, 1H, cyclopropyl-CH 1 diast. epimer), 2.34 (dd, *J* = 4.6, 5.2 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.39 (dd, *J* = 4.5, 5.1 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.52 (dd, *J* = 5.2, 8.4 Hz, 1H, cyclopropyl-CH, 1 diast. epimer), 2.56 (dd, *J* = 5.3, 8.3 Hz, 1H, cyclopropyl-CH, 1 diast. epimer), 2.62 (dd, *J* = 4.9, 4.9 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.66 (dd, *J* = 4.9, 4.9 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.85 (s, 3H, CH_3N, 1 diast. epimer), 2.86 (s, 3H, CH_3N, 1 diast. epimer), 3.08 (s, 3H, CH_3N, 1 diast.), 3.09 (s, 3H, CH_3N, 1 diast.), 3.71 (s, 3H, CH_3O, 1 diast. epimer + 1 diast.), 3.72 (s, 3H, CH_3O, 1 diast.), 3.76 (s, 3H, CH_3O, 1 diast. epimer), 3.99-4.08 (m, 1H, cyclopropyl-CHN), 5.07-5.20 (m, 3H, CH_2O + CHN), 6.58 (d, *J* = 7.5 Hz, 1H, NH, 1 diast. epimer), 6.88 (d, *J* = 7.3 Hz, 1H, NH, 1 diast.), 7.00 (d, *J* = 7.5 Hz, 1H, NH, 1 diast. epimer), 7.11 (d, *J* = 7.2 Hz, 1H, NH, 1 diast.), 7.29-7.38 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 14.3 (+, *C*H_3CH, 1 diast.), 14.5 (+, *C*H_3CH, 1 diast.), 15.6 (+, *C*H_3CH, 1 diast. epimer), 23.1 (+, CH_3C=O, 1 diast. + epimer), 23.2 (+, CH_3C=O, 1 diast. + epimer), 24.8 (+, cyclopropyl-CH, 1 diast. epimer), 24.9 (+, cyclopropyl-CH, 1 diast.), 25.4 (+, cyclopropyl-CH, 1 diast. epimer), 25.8 (+, cyclopropyl-CH, 1 diast.), 27.6 (+, cyclopropyl-CH, 1 diast.), 27.9 (+, cyclopropyl-CH, 1 diast.), 28.0 (+, cyclopropyl-CH, 1 diast. epimer), 28.8 (+, cyclopropyl-CH, 1 diast. epimer), 29.3 (+, CH_3N, 1 diast. epimer), 29.5 (+, CH_3N, 1 diast. epimer), 32.2 (+, CH_3N,1 diast.), 32.5 (+, CH_3N,1 diast.), 36.1 (+, cyclopropyl-CHN, 1 diast. epimer), 36.4 (+, cyclopropyl-CHN, 1 diast.), 36.6 (+, cyclopropyl-CHN, 1 diast. epimer), 36.7 (+, cyclopropyl-CHN, 1 diast.), 52.3 (+, CH_3O, 1 diast. + epimer), 52.37 (+, CH_3O, 1 diast. + 1 diast. epimer), 53.18 (+, CHN, 1 diast), 53.22 (+, CHN, 1 diast.), 55.5 (+, CHN, 1 diast. epimer), 55.6 (+, CHN, 1 diast. epimer), 67.06 (-, CH_2O, 1 diast.), 67.15 (-, CH_2O, 1 diast.), 67.5 (-, CH_2O, 1 diast. epimer), 67.6 (+, CH_2O, 1 diast. epimer), 128.1-135.5

(Aromatics CH and C_{quat}), 168.8 (C_{quat}, C=O, 1 diast. + 1 diast. epimer), 169.2 (C_{quat}, C=O, 1 diast. + 1 diast. epimer), 170.6 (C_{quat}, C=O, 1 diast. + 1 diast. epimer), 170.91 (C_{quat}, C=O, 1 diast. + 1 diast. epimer), 170.97 (C_{quat}, C=O, 1 diast. + 1 diast. epimer), 171.0 (C_{quat}, C=O, 1 diast. + epimer), 171.1 (C_{quat}, C=O, 1 diast. + 1 diast. epimer), 171.3 (C_{quat}, C=O, 1 diast. + 1 diast. epimer). - MS CI (NH_3) *m/z* (%) 753 ($2MH^+$, 4), 394 (MNH_4^+, 53), 377 (MH^+, 100), 301 (MH^+-Phenyl, 6). - IR (CH_2Cl_2) 3373, 1732, 1679, 1634, 1606 cm^{-1}. - Anal. Calcd for $C_{19}H_{24}N_2O_6 \cdot 0.33\ H_2O$ (381.81): C, 59.77; H, 6.49; N, 7.34. Found: C, 59.76; H, 6.51; N, 7.34. - HR MS calcd for $C_{19}H_{24}N_2O_6$ 376.16344, found 376.16285.



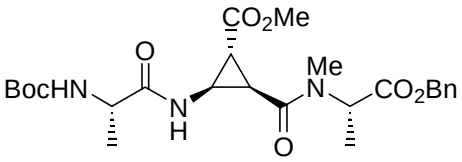
(+)-**36a**



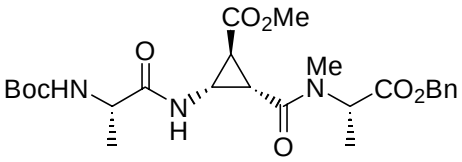
(-)-**36a**

(1R, 2R, 3R) and (1S, 2S, 3S) 2-(1S-Benzyloxycarbonyl-ethylcarbamoyl)-3-(2S-*tert*-butyloxycarbonylamino-propionyl)-cyclopropane carboxylic acid methyl ester ((+)-**36a** and (-)-**36a):** The N-Boc alanine benzyl ester (381 mg, 1.36 mmol, 1.5 eq.) was deprotected by treatment with HCl 3 M in ethyl acetate (4 ml) for 3 h at 0 °C. The solution was then concentrated in vacuum, the salt resuspended in CH_2Cl_2 (50 ml) and triethylamine (190 µl, 1.36 mmol, 1.5 eq.) was added dropwise. To this mixture EDC (175 mg, 0.91 mmol, 1 eq.) and HOBt (123 mg, 0.91 mmol, 1 eq.) were added. The mixture was cooled in an ice bath, then (±)-**30a** (300 mg, 0.91 mmol) dissolved in CH_2Cl_2 (20 ml) was added. The mixture was stirred overnight at room temperature. The solution was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 40:1) as a white solid (410 mg, 92 %, a mixture of two diastereomer). The two diastereomers can be separated by recrystallization from ethyl acetate/hexanes. The pure diastereomers can be obtained with the same procedure from diastereomerically pure **30a** (yield 92 % for (+)-**36a** and 90 % for (-)-**36a**). – (+)-**36a:** R_f ((+)-**36a**): 0.17. - mp 157-159 °C. - $[\alpha]_D^{21}$ -18.7 ° (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.32 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 1,40 (d, *J* = 7.5 Hz, 3H, C*H*$_3$CH), 1.43 (s, 9H, $(CH_3)_3C$), 2.31 (dd, *J* = 4.8, 4.9 Hz, 1H, cyclopropyl-CH), 2.39 (dd, *J* = 5.2, 8.2 Hz, 1H, cyclopropyl-CH), 3.69 (s, 3H, CH_3O), 4.01-4.14 (m, 2H, CHN), 4.59 (dq, *J* = 7.2, 7.2 Hz, 1H, CHN), 5.06 (d, *J* = 7.3 Hz, 1H, NHBoc), 5.12-5.23 (m, 2H, CH_2O), 6.90 (d, *J* = 7.3 Hz, 1H, NH), 7.33-7.37 (m, 5H, Ph-CH), 7.61 (d, *J* = 8.2 Hz, 1H, NH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 18.2 (+, *C*H_3CH), 18.8 (+, *C*H_3CH), 27.2 (+, cyclopropyl-CH), 27.6 (+,

cyclopropyl-CH), 28.3 (+, (CH_3)$_3$C, 3C), 36.1 (+, cyclopropyl-CHN), 48.5 (+, CHN), 50.3 (+, CHN), 52.4 (+, CH_3O), 67.4 (-, CH_2O), 80.0 (C_{quat}, (CH_3)$_3$*C*), 128.2 (+, Ph-CH, 2C), 128.5 (+, Ph-CH), 128.7 (+, Ph-CH, 2C), 135.2 (C_{quat}, Ph-C), 155.2 (C_{quat}, N(CO)O), 168.2 (C_{quat}, C=O), 170.7 (C_{quat}, C=O), 172.5 (C_{quat}, C=O), 173.4 (C_{quat}, C=O). - MS CI (NH_3) *m/z* (%) 509 (MNH_4^+, 50), 492 (MH^+, 78), 392 (MH^+-Boc, 30), 293 (37), 279 (100), 182 (44). - IR (KBr) 3331, 3294, 2983, 1739, 1657, 1538, 1451 cm^{-1}. - Anal. Calcd for $C_{24}H_{33}N_3O_8$ (491.54): C, 58.64; H, 6.77; N, 8.55. Found: C, 58.70; H, 6.74; N, 8.44. – (-)-**36a:** R_f ((-)-**36a**): 0.17. - mp 65-67 °C. - $[\alpha]_D^{r.t.}$ -21.6 ° (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.30 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 1,43 (d, *J* = 7.0 Hz, 3H, C*H*$_3$CH), 1.42 (s, 9H, (CH_3)$_3$C), 2.32 (dd, *J* = 4.8, 4.8 Hz, 1H, cyclopropyl-CH), 2.42 (dd, *J* = 5.1, 8.3 Hz, 1H, cyclopropyl-CH), 3.70 (s, 3H, CH_3O), 4.01 (ddd, *J* = 4.5, 8.0, 8.0 Hz, 1H, cyclopropyl-CHN), 4.07-4.16 (m, 1H, CHN), 4.57 (dq, *J* = 7.2, 7.2 Hz, 1H, CHN), 5.04 (s br, 1H, NH), 5.13-5.23 (m, 2H, CH_2O), 6.85 (d, *J* = 7.2 Hz, 1H, NH), 7.29-7.36 (m, 6H, Ph-CH + NH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 17.9 (+, *C*H_3CH), 18.6 (+, *C*H_3CH), 27.4 (+, cyclopropyl-CH), 27.5 (+, cyclopropyl-CH), 28.3 (+, (CH_3)$_3$C, 3C), 36.0 (+, cyclopropyl-CHN), 48.5 (+, CHN), 48.7 (+, CHN), 52.4 (+, CH_3O), 67.4 (-, CH_2O), 80.0 (C_{quat}, (CH_3)$_3$*C*), 128.2 (+, Ph-CH, 2C), 128.6 (+, Ph-CH), 128.7 (+, Ph-CH, 2C), 135.2 (C_{quat}, Ph-C), 155.2 (C_{quat}, N(CO)O), 168.0 (C_{quat}, C=O), 170.8 (C_{quat}, C=O), 172.4 (C_{quat}, C=O), 173.0 (C_{quat}, C=O). - MS CI (NH_3) *m/z* (%) 1000 ($2MNH_4^+$, <1), 983 ($2MH^+$, <1), 883 ($2MH^+$-Boc, <1), 509 (MNH_4^+, 70), 492 (MH^+, 100), 477 (88), 392 (MH^+-Boc, 69), 279 (82). - IR (KBr) 3321, 3281, 2983, 1739, 1664, 1539, 1520, 1457 cm^{-1}. - Anal. Calcd for $C_{24}H_{33}N_3O_8$ (491.54): C, 58.64; H, 6.77; N, 8.55. Found: C, 58.36; H, 6.67; N, 8.49.



(+)-**36b**

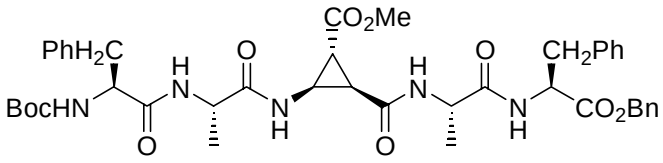


(-)-**36b**

(1R, 2R, 3R) and (1S, 2S, 3S) 2-[(1S-Benzyloxycarbonyl-ethyl)-methyl-carbamoyl]-3-(2S-*tert*-butyloxycarbonylamino-propionyl)-cyclopropane carboxylic acid methyl ester ((+)-**36b** and (-)-**36b):** N-methyl Boc-alanine benzyl ester (510 mg, 1.74 mmol, 1.5 eq.) was deprotected by treatment with HCl 3 M in ethyl acetate (10 ml) for 3 h at 0 °C. Then the solution was concentrated in vacuum, the salt was resuspended in CH_2Cl_2 (50 ml) and triethylamine (201 µl, 1.4 mmol, 1.7 eq.) was added dropwise. To this mixture EDC (180 mg,

0.93 mmol, 1.1 eq.) and HOBt (125 mg, 0.93 mmol, 1.1 eq.) were added. The mixture was cooled in an ice bath, then (±)-**30a** (280 mg, 0.85 mmol) dissolved in CH_2Cl_2 (20 ml) was added. The mixture was stirred overnight at room temperature. The solution was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 60:1) as a white solid (337 mg, 78 %, a mixture of two diastereomer). The two diastereomers can be obtained pure by reacting the diastereomerically pure acid with the same procedure described before and with the same yields. - R_f (**36b**): 0.16. – (+)-**36b:** mp 52-54 °C. - $[\alpha]_D^{21}$ -59.3 ° (c 1, MeOH). - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.32 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 1.40 (d, *J* = 7.4 Hz, 3H, C*H*$_3$CH), 1.43 (s, 9H, (C*H*$_3$)$_3$C), 2.36 (dd, *J* = 4.9, 4.9 Hz, 1H, cyclopropyl-CH), 2.62 (dd, *J* = 5.3, 8.3 Hz, 1H, cyclopropyl-CH), 3.05 (s, 3H, CH_3N), 3.72 (s, 3H, CH_3O), 4.03 (ddd, *J* = 4.4, 8.0, 8.0 Hz, 1H, cyclopropyl-CHN), 4.09-4.15 (m, 1H, CHN), 4.99 (d, *J* = 7.0 Hz, 1H, NH), 5.16 (s, 2H, CH_2O), 5.19-5.23 (m, 1H, CHN), 7.33-7.36 (m, 5H, Ph-CH), 7.64 (d, J = 7.5 Hz, 1H, NH). - ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 14.5 (+, *C*H$_3$CH), 18.8 (+, *C*H$_3$CH), 24.7 (+, cyclopropyl-CH), 28.0 (+, cyclopropyl-CH), 28.3 (+, (*C*H$_3$)$_3$C, 3C), 32.0 (+, CH_3N), 36.6 (+, cyclopropyl-CHN), 52.4 (+, CHN), 52.6 (+, CH_3O), 55.6 (+, CHN), 67.1 (-, CH_2O), 80.0 (C_{quat}, *C*(CH$_3$)$_3$), 128.1 (+, Ph-CH, 2C), 128.4 (+, Ph-CH), 128.6 (+, Ph-CH, 2C), 135.5 (C_{quat}, Ph-C), 155.1 (C_{quat}, N(C=O)O), 169.1(C_{quat}, C=O), 170.9 (C_{quat}, C=O), 171.2 (C_{quat}, C=O), 173.4 (C_{quat}, C=O). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 1011 ($2M^+$, 9), 506 (MH^+, 100), 317 (32), 307 (37). - IR (CH_2Cl_2) 3322,2980, 1737, 1676, 1640, 1519 cm^{-1}. - Anal. Calcd for $C_{25}H_{35}N_3O_8$ (505.57): C, 59.39; H, 6.98; N, 8.31. Found: C, 59.14; H, 7.11; N, 8.19. – (-)-**36b:** mp not measurable, very hygroscopic. - $[\alpha]_D^{21}$ -52.5 (c 1, MeOH). - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.30 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 1.43-1.46 (d, *J* = not measurable: it lays under the Boc signal, 3H, C*H*$_3$CH), 1.43 (s, 9H, (C*H*$_3$)$_3$C), 2.43-2.45 (m, 1H, cyclopropyl-CH), 2.66 (dd, *J* = 5.2, 8.4 Hz, 1H, cyclopropyl-CH), 3.07 (s, 3H, CH_3N), 3.72 (s, 3H, CH_3O), 3.99 (ddd, *J* = 4.3, 8.0, 8.0 Hz, 1H, cyclopropyl-CHN), 4.16-4.18 (m, 1H, CHN), 5.09-5.21 (m, 4H, CH_2O + CHN + NH), 7.18 (d, *J* = 6.3 Hz, 1H, NH), 7.34-7.36 (m, 5H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 14.3 (+, *C*H$_3$CH), 18.5 (+, *C*H$_3$CH), 26.5 (+, cyclopropyl-CH), 27.2 (+, cyclopropyl-CH), 28.3 (+, (*C*H$_3$)$_3$C, 3C), 31.9 (+, CH_3N), 36.1 (+, cyclopropyl-CHN), 52.4 (+, CHN), 53.0 (+, CH_3O), 55.5 (+, CHN), 67.2 (-, CH_2O), 79.9 (C_{quat}, *C*(CH$_3$)$_3$), 128.1 (+, Ph-CH, 2C), 128.5 (+, Ph-CH), 128.8 (+, Ph-CH, 2C), 135.3 (C_{quat}, Ph-C), 155.3 (C_{quat}, N(C=O)O), 168.4 (C_{quat}, C=O), 170.9 (C_{quat}, C=O), 171.6 (C_{quat}, C=O), 173.7 (C_{quat}, C=O). - MS FAB (MeOH/Glycerine) *m/z* (%) 506 (MH^+, 100), 450 (MH^+-^{t}Bu, 42), 407 (MH^+-Boc, 75), 317 (69). - IR ($CDCl_3$) 3431, 3370, 2982,

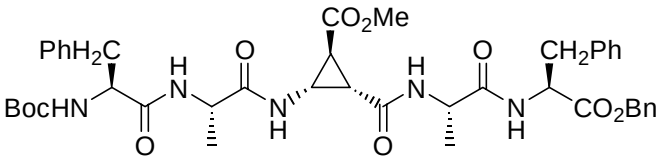
1727, 1685, 1639, 1494 cm^{-1}. - HR MS calcd for $C_{25}H_{35}N_3O_8$ + H506.25024, found 506.24853.



(+)-**37a**

(1R, 2R, 3R) 2-[1S-(1S-Benzyloxycarbonyl-2-phenyl-ethylcarbamoyl)-ethylcarbamoyl]-3-[2S-(2S-*tert*-butoxycarbonylamino-3-phenyl-propionylamino)-propionylamino]-cyclopropane carboxylic acid methyl ester ((+)-**37a):** Boc-phenylalanylalanine benzyl ester (492 mg, 1.15 mmol, 1.1 eq.) was deprotected by treatment with HCl 3 M in ethyl acetate (20 ml) for 3 h at 0 °C. The solution was concentrated in vacuum, the salt was resuspended in CH_2Cl_2 (50 ml) and triethylamine (160 µl, 1.15 mmol, 1.1 eq.) was added dropwise. To this mixture EDC (201 mg, 1.05 mmol, 1 eq.) and HOBt (142 mg, 1.05 mmol, 1 eq.) were added. The mixture was cooled in an ice bath, then (+)-**31a** acid (500 mg, 1.05 mmol) dissolved in CH_2Cl_2 (20 ml) was added. The mixture was stirred overnight at room temperature. The solution was concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 20:1) as a white solid (820 mg, 99 %). - R_f ((+)-**37a**): 0.27. - mp 185-186 °C. - $[\alpha]_D^{r.t.}$ -56.0 (c 1, MeOH). - ^{1}H NMR (CD_3OD, 250 MHz) δ 1.19 (d, *J* = 7.1 Hz, 3H, *CH*$_3$CH), 1.29 (d, *J* = 7.1 Hz, 3H, *CH*$_3$CH), 1.34 (s, 9H, $(CH_3)_3C$), 2.38-2.48 (m, 2H, cyclopropyl-CH), 2.80 (dd, *J* = 13.7, 9.7 Hz, 1H, CH_2Ph), 2.98 (dd, *J* = 13.8, 8.0 Hz, 1H, CH_2Ph), 3.08-3.18 (m, 2H, CH_2Ph), 3.61 (dd, *J* 8.4, 4.7 Hz, 1H, cyclopropyl-CHN), 3.73 (s, 3H, CH_3O), 4.25-4.33 (m, 3H, CHN), 4.65 (dd, *J* = 7.9, 6.2 Hz, 1H, CHN), 5.10 (s, 2H, CH_2O), 7.09-7.36 (m, 15H, Ph-CH). - ^{13}C NMR (CD_3OD, 62.9 MHz) δ 17.8 (+, *C*H_3CH), 18.0 (+, *C*H_3CH), 27.4 (+, cyclopropyl-CH), 28.6 (+, (*C*H_3)$_3$C, 3C), 29.3 (+, cyclopropyl-CH), 36.7 (+, cyclopropyl-CHN), 38.3 (-, CH_2Ph), 39.1 (-, CH_2Ph), 50.3 (+, CHN), 50.4 (+, CHN), 55.3 (+, CHN), 57.2 (+, CHN), 68.1 (-, CH_2O), 80.7 (C_{quat}, *C*$(CH_3)_3$), 127.7 (+, Ph-CH), 127.8 (+, Ph-CH), 129.37 (+, Ph-CH), 129.42 (+, Ph-CH, 2C) 129.47 (+, Ph-CH, 2C), 129.49 (+, Ph-CH, 2C), 129.6 (+, Ph-CH, 2C), 130.4 (+, Ph-CH, 2C), 130.5 (+, Ph-CH, 2C), 137.0 (C_{quat}, Ph-C), 137.9 (C_{quat}, Ph-C), 138.7 (C_{quat}, Ph-C), 157.6 (C_{quat}, C=O Boc), 169.1 (C_{quat}, C=O), 172.4 (C_{quat}, C=O), 172.6 (C_{quat}, C=O), 174.1 (C_{quat}, C=O), 174.5 (C_{quat}, C=O), 175.6 (C_{quat}, C=O). - MS FAB (Glycerin/MeOH) *m/z* (%) 786 (MH^+, 40), 686 (MH^+-Boc). - IR (KBr) 3302, 3064, 2978,

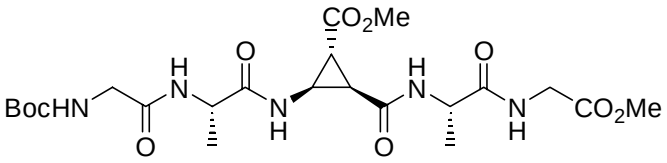
1734, 1649, 1526, 1453 cm^{-1}. - Anal. Calcd for $C_{42}H_{51}N_5O_{10}$ (785.89): C, 64.19; H, 6.54; N, 8.91. Found: C, 64.09; H, 6.54; N, 8.88.



(-)-**37a**

(1S, 2S, 3S) 2-[1S-(1S-Benzyloxycarbonyl-2-phenyl-ethylcarbamoyl)-ethylcarbamoyl]-3-[2S-(2S-*tert*-butoxycarbonylamino-3-phenyl-propionylamino)-propionylamino]-cyclopropane carboxylic acid methyl ester ((-)-37a) : Boc-phenylalanylalanine benzyl ester (492 mg, 1.15 mmol, 1 eq.) was deprotected by treatment with HCl 3 M in ethyl acetate (15 ml) for 3 h at 0 °C. Then the solution was concentrated in vacuum, the salt resuspended in CH_2Cl_2 (50 ml) and triethylamine (160 µl, 1.15 mmol, 1.1 eq.) was added dropwise. To this mixture EDC (201 mg, 1.05 mmol, 1 eq.) and HOBt (142 mg, 1.05 mmol, 1 eq.) were added. The mixture was cooled in an ice bath, then (-)-**31a** (500 mg, 1.05 mmol) dissolved in CH_2Cl_2 (20 ml) was added. The mixture was stirred overnight at room temperature. The solution was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 25:1) as a white solid (700 mg, 85 %). - R_f ((-)-**37a**): 0.31. - mp 52-54 °C. - $[\alpha]_D^{r.t.}$ -34.0 ° (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (d, *J* = 7.0 Hz, 3H, *CH*$_3$CH), 1.30 (d, *J* = 7.0 Hz, 3H, *CH*$_3$CH), 1.36 (s, 9H, $(CH_3)_3C$), 2.32-2.33 (m, 2H, cyclopropyl-CH), 2.99-3.08 (m, 1H, CH_2Ph), 3.10-3.15, m, 2H, CH_2Ph), 3.21 (dd, *J* = 5.8, 13.6, 1H, CH_2Ph), 3.71 (s, 3H, CH_3O), 3.78 (dd, *J* = 6.3, 12.2 , 1H, cyclopropyl-CHN), 4.40-4.49 (m, 3H, CHN), 4.86 (dd, *J* = 6.4, 13.7 , 1H, CHN), 5.03 (d, *J* = 7.8 Hz, 1H, NH), 5.14 (d, *J* = 12.2 Hz, 1H, CH_2O), 5.20 (d, *J* = 12.2 Hz, 1H, CH_2O), 6.49 (d, *J* = 7.3 Hz, 1H, NH), 6.83 (d, *J* = 6.2 Hz, 1H, NH), 6.92 (d, *J* = 7.5 Hz, 1H, NH), 7.06 (s br, 1H, NH), 7.17-7.38 (m, 15H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 17.7 (+, *C*H_3CH), 18.0 (+, *C*H_3CH), 27.5 (+, cyclopropyl-CH), 27.9 (+, cyclopropyl-CH), 28.2 (+, $(CH_3)_3C$, 3C), 35.7 (+, CHN), 37.3 (-, CH_2Ph), 38.2 (-, CH_2Ph), 48.7 (+, CHN), 49.0 (+, CHN), 52.4 (+, CH_3O), 53.3 (+, CHN), 53.6 (+, CHN), 67.6 (-, CH_2O), 80.0 (C_{quat}, $C(CH_3)_3$), 126.9 (+, Ph-CH), 127.2 (+, Ph-CH), 128.51 (+, Ph-CH, 2C), 128.53 (+, Ph-CH, 2C), 128.55 (+, Ph-CH), 128.56 (+, Ph-CH, 2C), 128.6 (+, Ph-CH, 2C), 129.31 (+, Ph-CH, 2C), 129.35 (+, Ph-CH, 2C), 134.9 (C_{quat}, Ph-C), 135.7 (C_{quat}, Ph-C), 136.7 (C_{quat}, Ph-C), 155.4 (C_{quat}, C=O Boc), 167.7 (C_{quat}, C=O), 170.7 (C_{quat}, C=O), 171.31 (C_{quat}, C=O), 171.34 (C_{quat}, C=O), 171.7 (C_{quat}, C=O), 172.9 (C_{quat}, C=O). - MS FAB

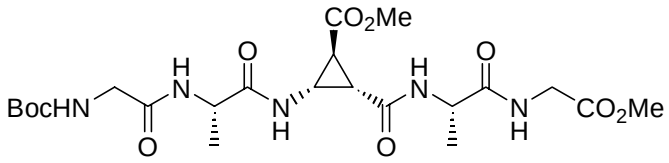
(NBA/CH_2Cl_2) *m/z* (%) 1571 ($2MH^+$, 5), 786 (MH^+, 82), 686 (M^+-Boc, 100), 450 (19), 307 (59), 289 (24). - IR (KBr) 3300, 3062, 2978, 1727, 1659, 1641, 1529, 1170 cm^{-1}. - Anal. Calcd for $C_{42}H_{51}N_5O_{10}$. $0.5H_2O$ (794.90): C, 63.46; H, 6.59; N, 8.81. Found: C, 63.49; H, 6.61; N, 8.75.



(+)-**37b**

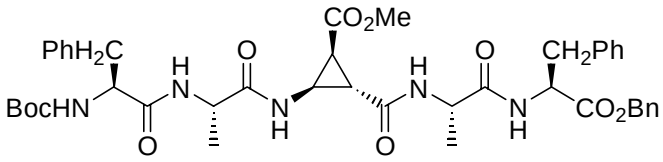
(1R, 2R, 3R) 2-[1S-(Methyloxycarbonyl-methyl-carbamoyl)-ethylcarbamoyl]-3-[2S-(*tert*-butoxycarbonylamino-acetylamino)-propionylamino]-cyclopropane carboxylic acid methyl ester ((+)-**37b):** Boc-glycylalanine methyl ester (227 mg, 1.16 mmol, 1.5 eq.) was deprotected by treatment with HCl 3 M in ethyl acetate (10 ml) for 3 h at 0 °C. The solution was concentrated in vacuum, the salt was resuspended in CH_2Cl_2 (50 ml) and triethylamine (161 µl, 1.16 mmol, 1.5 eq.) was added dropwise. To this mixture EDC (163 mg, 0.85 mmol, 1.1 eq.) and HOBt (114 mg, 0.85 mmol, 1.1 eq.) were added. The mixture was cooled in an ice bath, then (+)-**31b** (300 mg, 0.77 mmol, 1 eq.) dissolved in CH_2Cl_2 (30 ml) was added. The addition of DMF (6 ml) ensured solubilisation. The mixture was stirred overnight at room temperature. The solution was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 20:1) as a white solid (328 mg, 80 %). The product could be recrystallized from ethyl acetate/MeOH/hexanes. - R_f ((+)-**37b**): 0.05. - mp 180-182 °C. - $[\alpha]_D^{21}$ -76.7 (c 1, CH_3OH). - ^{1}H NMR (CD_3OD, 250 MHz) δ 1.31 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 1.36 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 1.45 (s, 9H, (C*H*$_3$)$_3$C), 2.41 (dd, *J* = 5.1, 5.1 Hz, 1H, cyclopropyl-CH), 2.52 (dd, *J* = 5.4, 8.2 Hz, 1H, cyclopropyl-CH), 3.65-3.78 (m, 3H, cyclopropyl-CHN + CH_2N), 3.70 (s, 3H, CH_3O), 3.71 (s, 3H, CH_3O), 3.94-3.97 (m, 2H, CH_2N), 4.32 (q, *J* = 7.2 Hz, 1H, CHN), 4.39 (q, *J* = 7.2 Hz, 1H, CHN). - ^{13}C NMR (CD_3OD, 62.9 MHz) δ 18.0 (+, *C*H_3CH), 18.1 (+, *C*H_3CH), 27.4 (+, cyclopropyl-CH), 28.8 (+, (*C*H_3)$_3$C, 3C), 29.3 (+, cyclopropyl-CH), 36.6 (+, cyclopropyl-CHN), 41.9 (-, CH_2N), 44.6 (-, CH_2N), 50.4 (+, CHN), 50.6 (+, CHN), 52.7 (+, CH_3O), 52.9 (+, CH_3O), 80.8 (C_{quat}, (CH$_3$)$_3$*C*), 158.4 (C_{quat}, N(CO)O), 169.4 (C_{quat}, C=O), 171.7 (C_{quat}, C=O), 172.3 (C_{quat}, C=O), 172.4 (C_{quat}, C=O), 175.6 (C_{quat}, C=O), 175.7 (C_{quat}, C=O). - MS FAB (Glycerin/MeOH) *m/z* (%) 1060 ($2MH^+$, 7), 530 (MH^+, 71), 430 (MH^+-Boc, 80), 285 (100). - IR (KBr) 3300, 3079,

2981, 1734, 1654, 1532, 1449 cm^{-1}. - Anal. Calcd for $C_{22}H_{35}N_5O_{10}$ (529.546): C, 49.90; H, 6.66; N, 13.20. Found: C, 49.55; H, 6.59; N, 13.02.

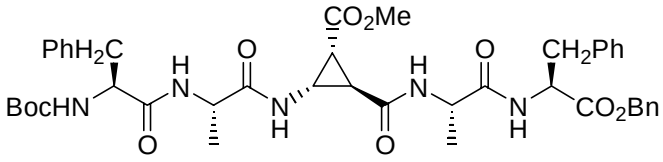


(-)-**37b**

(1S, 2S, 3S) 2-[1S-(Methyloxycarbonyl-methyl-carbamoyl)-ethylcarbamoyl]-3-[2S-(*tert*-butoxycarbonylamino-acetylamino)-propionylamino]-cyclopropane carboxylic acid methyl ester ((-)-**37b):** Boc-glycylalanine benzyl ester (253 mg, 1.58 mmol, 1.4 eq.) was deprotected by treatment with HCl 3 M in ethyl acetate (5 ml) for 3 h at 0 °C. Then the solution was concentrated in vacuum, the salt was resuspended in CH_2Cl_2 (50 ml) and triethylamine (220 µl, 1.58 mmol, 1.4 eq.) was added dropwise. To this mixture EDC (303 mg, 1.58 mmol, 1.4 eq.) and HOBt (213 mg, 1.58 mmol, 1.4 eq.) were added. The mixture was cooled in an ice bath and (-)-**31b** (442 mg, 1.14 mmol, 1 eq.) dissolved in CH_2Cl_2 (30 ml) was added. The addition of DMF (4 ml) ensured solubilisation. The mixture was stirred at room temperature overnight. The solution was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 20:1) as a white solid (560 mg, 93 %). - R_f ((-)-**37b**): 0.06. - mp 88-90 °C. - $[\alpha]_D^{21}$ -64.8 (c 1, CH_3OH). - ^{1}H NMR (CD_3OD, 250 MHz) δ 1.29 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 1.38 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 1.44 (s, 9H, (C*H*$_3$)$_3$C), 2.36 (dd, *J* = 4.8, 4.8 Hz, 1H, cyclopropyl-CH), 2.51 (dd, *J* = 5.3, 8.1 Hz, 1H, cyclopropyl-CH), 3.51 (dd, *J* = 4.2, 8.0 Hz, 1H, cyclopropyl-CHN), 3.70 (s, 3H, CH_3O), 3.72 (s, 3H, CH_3O), 3.70-3.72 (m, 2H, CH_2N), 3.93-4.00 (m, 2H, CH_2N), 4.33 (q, *J* = 7.3 Hz, 1H, CHN), 4.40 (q, *J* = 7.3 Hz, 1H, CHN). - ^{13}C NMR (CD_3OD, 62.9 MHz) δ 17.6 (+, *C*H_3CH), 17.8 (+, *C*H_3CH), 27.8 (+, cyclopropyl-CH), 28.7 (+, (*C*H$_3$)$_3$C, 3C), 29.2 (+, cyclopropyl-CH), 36.4 (+, cyclopropyl-CHN), 41.9 (-, CH_2N), 44.8 (-, CH_2N), 50.46 (+, CHN), 50.53 (+, CHN), 52.8 (+, CH_3O), 52.9 (+, CH_3O), 80.8 (C_{quat}, (CH$_3$)$_3$*C*), 158.1 (C_{quat}, N(CO)O), 169.4 (C_{quat}, C=O), 171.9 (C_{quat}, C=O), 172.4 (C_{quat}, C=O), 172.5 (C_{quat}, C=O), 175.6 (C_{quat}, C=O), 176.1 (C_{quat}, C=O). - MS FAB (Glycerin/MeOH) *m/z* (%) 1060 ($2MH^+$, 9), 530 (MH^+, 82), 430 (MH^+-Boc, 45), 153 (100). - IR (KBr) 3319, 2982, 1734, 1662, 1654, 1539, 1449 cm^{-1}. - HR MS calcd for $C_{22}H_{35}N_5O_{10}$ + H 530.24622, found 530.24923.



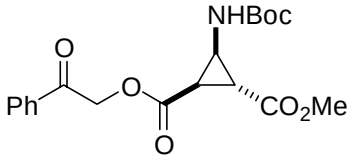
(+)-**38**



(-)-**38**

(1R*, 2R*, 3S*) 2-[1S-(1S-Benzyloxycarbonyl-2-phenyl-ethylcarbamoyl)-ethylcarbamoyl]-3-[2S-(2S-*tert*-butoxycarbonylamino-3-phenyl-propionylamino)-propionylamino]-cyclopropane carboxylic acid methyl ester ((±)-**38):** Boc-phenylalanylalanine benzyl ester (552 mg, 1.3 mmol, 1.1 eq.) was deprotected by treatment with HCl 3 M in ethyl acetate (5 ml) for 3h at 0 °C. Then the solution was concentrated in vacuum, the salt was resuspended in CH_2Cl_2 (50 ml) and triethylamine (180 µl, 1.3 mmol, 1 eq.) was added dropwise. To this mixture EDC (226 mg, 1.18 mmol, 1 eq.) and HOBt (159 mg, 1.18 mmol, 1 eq.) were added. The solution was cooled in an ice bath, then (±)-**32** (567 mg, 1.18 mmol, 1 eq.) dissolved in CH_2Cl_2 (20 ml) was added. The mixture was stirred at room temperature overnight. The solution was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 25:1) as a white solid (830 mg, 90 %). The two diastereomers can be almost completely separated by several recrystallizations performed by cooling down their solution in $CHCl_3$/MeOH at -18 °C overnight. – **Recrystallized isomer** - R_f : 0.30. - mp 300 °C decomp. - $[\alpha]_D^{r.t.}$ not measurable because of solubility problems. - ^{1}H NMR (DMSO-d_6, 250 MHz) δ 1.13 (d, *J* = 7.0 Hz, 3H, C*H*$_3$CH), 1.19 (d, *J* = 6.7 Hz, 3H, C*H*$_3$CH), 1.27 (s, 9H, $(CH_3)_3C$), 2.11 (dd, *J* = 5.2, 7.9 Hz, 1H, cyclopropyl-CH), 2.68 (dd, *J* = 10.6, 13.7 Hz, 1H, cyclopropyl-CH), 2.96-3.04 (m, 4H, CH_2Ph), 3.23-3.40 (m , 1H, cyclopropyl-CHN), 3.57 (s, 3H, CH_3O), 4.12-4.18 (m, 1H, CHN), 4.27-4.36 (m , 1H, CHN), 4.48 (dd, *J* = 7.2, 14.2 Hz, 1H, CHN), 5.04 (dd, *J* = 7.2, 14.2 Hz, 1H, CHN), 4.94-5.09 (m, 2H, CH_2O), 6.92 (d, *J* = 8.6 Hz, 1H, NH), 7.16-7.25 (m, 12H, Ph-CH), 7.30-7.33 (m, 3H, Ph-CH), 7.99 (d, *J* = 7.5 Hz, 1H, NH), 8.16 (d, *J* = 4.8 Hz, 1H, NH), 8.42 (d, *J* = 7.3 Hz, 1H, NH), 8.62 (d, *J* = 7.6 Hz, 1H, NH). - ^{13}C NMR (DMSO-d_6, 250 MHz) δ 18.1 (+, *C*H_3CH), 18.4 (+, *C*H_3CH), 26.3 (+, cyclopropyl-CH), 27.6 (+, cyclopropyl-CH), 28.1 (+, $(CH_3)_3C$, 3C), 34.7 (+, CHN), 36.5 (-, CH_2Ph), 37.3 (-, CH_2Ph), 48.0 (+, CHN, 2C), 51.8 (+, CH_3O), 53.7 (+, CHN), 55.6 (+, CHN), 66.0 (-, CH_2O), 78.1 ($C_{quat.}$, $C(CH_3)_3$),

126.1 (+, Ph-CH), 126.5 (+, Ph-CH), 127.8 (+, Ph-CH, 2C), 127.9 (+, Ph-CH, 2C), 128.0 (+, Ph-CH), 128.2 (+, Ph-CH, 2C), 128.3 (+, Ph-CH, 2C), 129.0 (+, Ph-CH, 2C), 129.2 (+, Ph-CH, 2C), 135.7 ($C_{quat.}$, Ph-C), 136.9 ($C_{quat.}$, Ph-C), 138.2 ($C_{quat.}$, Ph-C), 155.2 ($C_{quat.}$, N(CO)O), 167.4 ($C_{quat.}$, C=O), 168.7 ($C_{quat.}$, C=O), 171.1 (C=O), 171.2 ($C_{quat.}$, C=O), 172.1 ($C_{quat.}$, C=O), 173.2 ($C_{quat.}$, C=O). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 1572 ($2MH^+$, <1), 787 (MH^+, 9), 687 (MH^+-Boc, 19), 307 (32), 154 (100). - IR (KBr) 3329, 3286, 3061, 2977, 1754, 1717, 1689, 1640, 1530, 1453, 1368 cm^{-1}. - Anal. Calcd for $C_{42}H_{51}N_5O_{10}$ (785.89): C, 64.19; H, 6.54; N, 8.91. Found: C, 63.99; H, 6.58; N, 8.83. – **Isomer isolated in the mother liquor** - R_f : 0.30. - mp 207-209 °C. - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.13 (d, *J* = 7.0 Hz, 6H, *CH*$_3$CH), 1.27 (s, 9H, $(CH_3)_3C$), 2.08 (dd, *J* = 5.2, 7.9 Hz, 1H, cyclopropyl-CH), 2.55 (dd, *J* = 5.1, 5.1 Hz, 1H, cyclopropyl-CH), 2.68 (dd, *J* = 11.1, 13.1 Hz, 1H, CH_2Ph), 2.89-3.07 (m, 3H, CH_2Ph), 3.30-3.40 (m, 1H, cyclopropyl-CHN), 3.57 (s, 3H, CH_3O), 4.10-4.17 (m, 1H, CHN), 4.23-4.35 (m, 2H, CHN), 4.46 (dd, *J* = 7.9, 14.2 Hz, 1H, CHN), 5.02 (d, J = 15.9 Hz, 1H, CH_2O), 5.07 (d, J = 15.9 Hz, 1H, CH_2Ph), 6.91 (d, *J* = 8.6 Hz, 1H, NH), 7.17-7.23 (m, 15H, Ph-CH), 7.99 (d, *J* = 7.6 Hz, 1H, NH), 8.30 (d, *J* = 4.7 Hz, 1H, NH), 8.40 (d, *J* = 7.5 Hz, 1H, NH), 8.66 (d, *J* = 7.8 Hz, 1H, NH). - ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 18.1 (+, *C*H_3CH), 18.4 (+, *C*H_3CH), 26.7 (+cyclopropyl-CH), 27.2 (+, cyclopropyl-CH), 28.1 (+, (*C*H_3)$_3$C, 3C), 34.4 (+, cyclopropyl-CHN), 36.4 (-, CH_2Ph), 37.3 (-, CH_2Ph), 47.7 (+, CHN), 47.9 (+, CHN), 51.7 (+, CH_3O), 53.7 (+, CHN), 55.6 (+, CHN), 65.9 (-, CH_2O), 78.0 (C_{quat}, *C*$(CH_3)_3$), 126.1 (+, Ph-CH), 126.5 (+, Ph-CH), 127.8 (+, Ph-CH, 2C), 127.9 (+, Ph-CH, 2C), 128.0 (+, Ph-CH), 128.2 (+, Ph-CH, 2C), 128.3 (+, Ph-CH, 2C), 129.1 (+, Ph-CH, 2C), 129.2 (+, Ph-CH, 2C), 135.7 (C_{quat}, Ph-C), 137.0 (C_{quat}, Ph-C), 138.3 (C_{quat}, Ph-C), 155.24 (C_{quat}, N(C=O)O), 167.4 (C_{quat}, C=O), 168.5 (C_{quat}, C=O), 171.1 (C_{quat}, C=O), 171.3 (C_{quat}, C=O), 172.2 (C_{quat}, C=O), 173.5 (C_{quat}, C=O). – MS FAB (Glycerin/DMSO) *m/z* (%)1572 (2M H^+, <1), 787 (MH^+, 11), 687 (MH^+-Boc, 100), 596 (14), 451 (16), 327 (12), 256 (24). - IR (KBr) 3310, 2977, 1721, 1689, 1643, 1530, 1449, 1353 cm^{-1}. - HR MS calcd for $C_{42}H_{51}N_5O_{10}$ 786.371, found 786.3701.

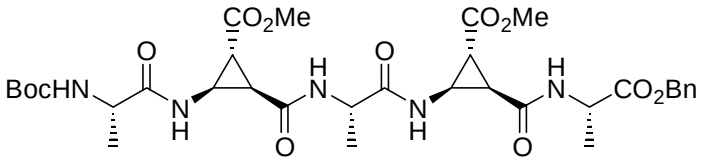


(±)-**50**

(1R*, 2R*, 3S*) 3-(*tert*-Butylcarbonylamino)-cyclopropane-1, 2-dicarboxylic acid 1-phenacil ester 2-methyl ester ((±)-**50):** Phenacylbromid (388 mg, 1.95 mmol, 5 eq.) and

potassiumfluorid (49 mg, 0.85 mmol, 2.2 eq.) were dissolved in DMF (4 ml) and stirred 1 min., then (1R*, 2R*, 3R*)-N-*tert*-Butoxycarbonyl-3-aminocyclopropane-1,2-dicarboxylic acid monomethyl ester (100 mg, 0.39 mmol) was added. After 5 h, diethyl ether (20 ml) was added and the organic phase was washed with a water solution of 1 M $KHSO_4$ (15 ml). The organic phase was dried over Na_2SO_4 and evaporated. The product was purified by chromatography (ethyl acetate/hexanes 2:3). Yield: 140 mg (96 %). – R_f ((±)-**50**): 0.35. - mp 129-131 °C. - ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.46 (s, 9H, $(CH_3)_3C$), 2.38-2.41 (m, 1H, cyclopropyl-CH), 2.68 (dd, *J* = 5.3, 8.6 Hz, 1H, cyclopropyl-CH), 3.73 (s, 3H, CH_3O), 3.87-3.91 (m, 1H, cyclopropyl-CHN), 5.35 (d, *J* = 16.4 , 1H, CH_2O), 5.52 (d, *J* = 16.4 Hz, 1H, CH_2O), 5.81 (s br, 1H, NHBoc), 7.49-7.53 (m, 2H, Ph-CH), 7.61-7.64 (m, 1H, Ph-CH), 7.90-7.93 (m, 1H, Ph-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 26.3 (+, cyclopropyl-CH), 28.3 (+, $(CH_3)_3C$, 3C), 28.8 (+, cyclopropyl-CH), 37.6 (+, cyclopropyl-CHN), 52.3 (+, CH_3O), 66.7 (-, CH_2O), 80.2 (C_{quat}, $(CH_3)_3C$), 127.8 (+, Ph-CH, 2C), 128.9 (+, Ph-CH, 2C), 133.7 (C_{quat}, Ph-C), 134.2 (+, Ph-CH), 155.6 (C_{quat}, N(CO)O), 168.9 (C_{quat}, C=O), 170.0 (C_{quat}, C=O), 191.4 (C_{quat}, Ph(C=O)CH_2). - MS CI (NH_3) *m/z* (%) 395 (MNH_4^+, 52), 378 (MH^+, 2), 339 (MH^+-^{t}Bu, 100), 295 (MH^+-Boc, 14), 277 (33), 221 (34). - IR (KBr) 3370, 3087, 3066, 2986, 2952, 1731, 1708, 1689, 1510,1323 cm^{-1}. - Anal. Calcd for $C_{19}H_{23}NO_7$ (377.393): C, 60.47; H, 6.14; N, 3.71. Found: C, 60.45; H, 6.23; N, 3.60.

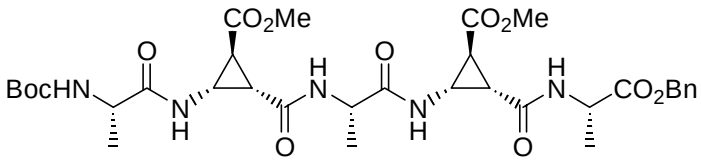
2.2.4 Compounds containing multiple β-ACCs



(+)-**40**

(1R, 2R, 3R) 2-(2S-Benzyloxycarbonyl-ethylcarbamoyl)-3-{2S-[2R-(2S-*tert*-butylcarbonylamino-propionylamino)-3R-methoxycarbonyl-cyclopropane-1R-carbonyl-amino]-propionylamino}-cyclopropane carboxylic acid methyl ester ((+)-**40):** (+)-**36a** (541 mg, 1.10 mmol) was deprotected by treatment with HCl 3 M in ethyl acetate (7 ml) for 3 h at 0 °C. The solution was concentrated in vacuum, the salt was resuspended in CH_2Cl_2 (50 ml) and triethylamine (168 µl, 1.21 mmol, 1.1 eq.) was added dropwise. To this mixture EDC (232 mg, 1.21 mmol, 1.1 eq.) and HOBt (163 mg, 1.21 mmol, 1.1 eq.) were added. The mixture was cooled in an ice bath and (+)-**30a** (375 mg, 1.13 mmol, 1.03 eq.) dissolved in

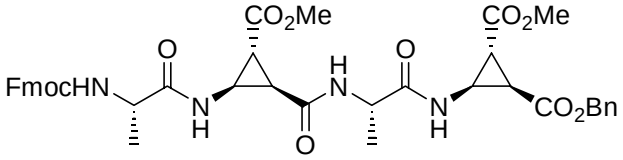
CH_2Cl_2 (20 ml) was added. The mixture was stirred at room temperature 60 h. The solution was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 20:1) as a white solid (741 mg, 96 %). 15 % epimerisation was observed. - R_f ((+)-**40**): 0.23. - mp 134-136 °C. - $[\alpha]_D^{21}$ -86.2 (c 1, CH_3OH). - ^{1}H NMR (CD_3OD, 250 MHz) δ 1.24 (d, *J* = 7.3 Hz, 3H, C*H*$_3$CH), 1.29 (d, *J* = 7.3 Hz, 3H, C*H*$_3$CH), 1.39 (d, *J*= 7.4 Hz, 3H, C*H*$_3$CH), 1.42 (s, 9H, $(CH_3)_3C$), 2.33-2.38 (m, 2H, cyclopropyl-CH), 2.49-2.54 (m, 2H, cyclopropyl-CH), 3.64 (s, 3H, CH_3O), 3.70 (s, 3H, CH_3O), 3.68-3.77 (m, 2H, cyclopropyl-CHN), 3.94-4.03 (m, 1H, Ala-CHN), 4.24 (q, *J* = 7.3 Hz, 1H, Ala-CHN), 4.50 (q, *J* = 7.3 Hz, 1H, Ala-CHN), 5.11 (d, J = 12.3 Hz, 1H, CH_2O), 5.19 (d, *J* = 12.3 Hz, 1H, CH_2O), 7.33-7.37 (m, 5H, Ph-CH). - ^{13}C NMR (CD_3OD, 62.9 MHz) δ 17.6 (+, *C*H_3CH), 17.7 (+, *C*H_3CH), 18.2 (+, *C*H_3CH), 27.6 (+, cyclopropyl-CH), 28.5 (+, cyclopropyl-CH), 28.7 (+, $(CH_3)_3C$, 3C), 28.9 (+, cyclopropyl-CH, 2C), 36.5 (+, cyclopropyl-CHN), 36.7 (+, cyclopropyl-CHN), 49.6 (+, CHN), 50.8 (+, CHN), 51.6 (+, CHN), 52.9 (+, CH_3O, 2C), 68.0 (-, CH_2O), 80.7 (C_{quat}, $(CH_3)_3C$), 129.2 (+, Ph-CH, 2C), 129.4 (+, Ph-CH), 129.6 (+, Ph-CH, 2C), 137.3 (C_{quat}, Ph-C), 157.6 (C_{quat}, N(CO)O), 169.68 (C_{quat}, C=O), 169.73 (C_{quat}, C=O), 172.3 (C_{quat}, C=O), 172.4 (C_{quat}, C=O), 173.9 (C_{quat}, C=O), 175.5 (C_{quat}, C=O), 176.8 (C_{quat}, C=O). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 1408 ($2MH^+$, 2), 704 (MH^+, 58), 604 (MH^+-Boc, 79), 307 (100). - IR (KBr) 3310, 2982, 1736, 1660, 1521, 1451, 1314 cm^{-1}. - Anal. Calcd for $C_{33}H_{45}N_5O_{12}$ (703.745): C, 56.32; H, 6.44; N, 9.95. Found: C, 55.77; H, 6.54; N, 0.37.



(-)-**40**

(1S, 2S, 3S) 2-(2S-Benzyloxycarbonyl-ethylcarbamoyl)-3-{2S-[2S-(2S-*tert*-butylcarbonylamino-propionylamino)-3S-methoxycarbonyl-cyclopropane-1S-carbonyl-amino]-propionylamino}-cyclopropane carboxylic acid methyl ester ((-)-40): (-)-**36a** (750 mg, 1.53 mmol) was deprotected by treatment with HCl 3 M in ethyl acetate (10 ml) for 3 h at 0 °C. Then the solution was concentrated in vacuum, the salt resuspended in CH_2Cl_2 (50 ml) and triethylamine (230 µl, 1.68 mmol, 1.1 eq.) was added dropwise. To this mixture EDC (323 mg, 1.68 mmol, 1.1 eq.) and HOBt (227 mg, 1.68 mmol, 1.1 eq.) were added. The mixture was cooled in an ice bath and the (-)-**30a** (503 mg, 1.53 mmol, 1.0 eq.) dissolved in CH_2Cl_2 (30 ml) was added. The mixture was stirred 60 h at room temperature. The solution

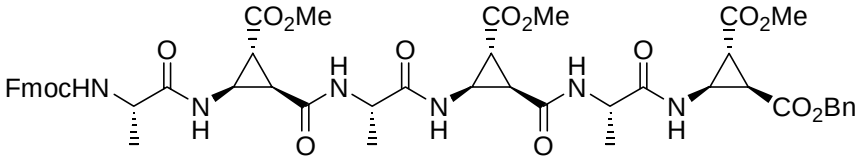
was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 20:1) as a white solid (1.052 g, 97 %). 15 % epimerisation was observed. - R_f ((-)-**40**): 0.24. - mp 103-105 °C. - $[\alpha]_D^{21}$ -117.3 (c 1, CH_3OH). - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.21 (d, *J* = 6.7 Hz, 3H, C*H*$_3$CH), 1.33 (d, *J* = 7.0 Hz, 3H, C*H*$_3$CH), 1.44 (d, *J*= 6.9 Hz, 3H, C*H*$_3$CH), 1.44 (s, 9H, $(CH_3)_3C$), 2.29 (dd, J = 5.1, 5.1 Hz, 1H, cyclopropyl-CH), 2.42-2.45 (m, 3H, cyclopropyl-CH), 3.69 (s, 3H, CH_3O), 3.70 (s, 3H, CH_3O), 3.85 (dd, J = 6.6, 6.6 Hz, 1H, cyclopropyl-CHN), 3.96-3.98 (m, 1H, cyclopropyl-CHN), 4.35 (dq, *J* = 7.2, 7.2 Hz, 1H, CHN), 4.43 (dq, *J* = 7.0, 7.0 Hz, 1H, CHN), 4.51 (dq, *J* = 7.1, 7.1 Hz, 1H, CHN), 5.17 (d, J = 12.2 Hz, 1H, CH_2O), 5.28 (d, *J* = 12.2 Hz, 1H, CH_2O), 5.48 (d, *J* = 8.3 Hz, 1H, NH), 6.52 (s br, 1H, NH), 7.31-7.38 (m, 6H, Ph-CH + NH), 7.48 (d, *J* = 6.3 Hz, 1H, NH), 7.64 (d, *J* = 7.2 Hz, 1H, NH). - ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 16.4 (+, *C*H$_3$CH), 17.2 (+, *C*H$_3$CH), 19.3 (+, *C*H$_3$CH), 26.9 (+, cyclopropyl-CH), 27.3 (+, cyclopropyl-CH), 27.9 (+, cyclopropyl-CH), 28.3 (+, cyclopropyl-CH, + (*C*H$_3$)$_3$C, 4C), 35.8 (+, cyclopropyl-CHN), 36.0 (+, cyclopropyl-CHN), 48.5 (+, CHN), 48.7 (+, CHN), 49.7 (+, CHN), 52.2 (+, CH_3O), 52.3 (+, CH_3O), 67.3 (-, CH_2O), 79.9 (C_{quat}, $(CH_3)_3C$), 128.1 (+, Ph-CH, 2C), 128.4 (+, Ph-CH), 128.6 (+, Ph-CH, 2C), 135.3 (C_{quat}, Ph-C), 155.4 (C_{quat}, N(CO)O), 167.7 (C_{quat}, C=O), 168.0 (C_{quat}, C=O), 170.7 (C_{quat}, C=O), 170.9 (C_{quat}, C=O), 172.7 (C_{quat}, C=O), 172.8 (C_{quat}, C=O), 173.6 (C_{quat}, C=O). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 1408 ($2MH^+$, 7), 704 (MH^+, 55), 604 (MH^+-Boc, 100), 307 (27). - IR (KBr) 3325, 2982, 1734, 1663, 1528, 1450, 1315 cm^{-1}. - Anal. Calcd for $C_{33}H_{45}N_5O_{12}$ (703.745): C, 56.32; H, 6.44; N, 9.95. Found: C, 55.77; H, 6.54; N, 9.58.



(+)-**41**

(1R, 2R, 3R) 3-[2S-({2R-[2S-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3R-methoxycarbonyl-cyclopropane-1R-carbonyl}-amino)-propionylamino]-cyclopropane-1, 2-dicarboxylic acid 1-benzy ester 2-methyl ester ((+)-**41):** (+)-**25a** (40 mg, 0.095 mmol) was (2 ml) at 0 °C for 3 h. The solution was concentrated in vacuum, the salt was resuspended in CH_2Cl_2 (5 ml), then (+)-**30b** (43 mg, 0.095 mmol, 1 eq.), EDC (36 mg, 0.19 mmol, 2 eq), and pyridine (11 µl, 0.14 mmol, 1.5 eq.) were added. The mixture was stirred at room temperature overnight. The solution was washed with saturated $NaHCO_3$ (8 ml), 1 M $KHSO_4$ (8 ml) and saturated $NaHCO_3$ (8 ml). The organic phase was dried over $MgSO_4$ and concentrated. The

product was purified by chromatography (CH_2Cl_2/MeOH 40:1). Yield: 49 mg (68 %). 10 to 15 % epimerization was observed. - R_f ((+)-**41**): 0.15. - mp 188-189 °C. - $[\alpha]_D^{21}$ -58.1 (c 0.5, MeOH/$CHCl_3$ 1:1). - ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.16 (d, *J*= 6.7 Hz, 3H, C*H*$_3$CH), 1.34 (d, *J* = 7.0 Hz, 3H, C*H*$_3$CH), 2.32-2.47 (m, 3H, cyclopropyl-CH), 2.56 (dd, *J* = 5.3, 8.0 Hz, 1H, cyclopropyl-CH), 3.61 (s, 3H, CH_3O), 3.68 (s, 3H, CH_3O), 3.89-3.96 (m, 1H, cyclopropyl-CHN), 4.02-4.10 (m, 1H, cyclopropyl-CHN), 4.17-4.22 (m, 1H, Fmoc-CH), 4.26-4.48 (m, 4H, Fmoc-CH_2 + 2 CHN), 5.05-5.15 (m, 2H, CH_2O), 5.77 (d, *J* = 7.5 Hz, 1H, NHFmoc), 7.11 (d, *J* = 7.3 Hz, 1H, NH), 7.23-7.41 (m, 10H, Ar-CH + NH), 7.57-7.60 (m, 2H, Ar-CH), 7.73-7.76 (m, 3H, Ar-CH + NH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ 18.9 (+, *C*H_3CH), 19.3 (+, *C*H_3CH), 26.7 (+, cyclopropyl-CH), 27.0 (+, cyclopropyl-CH), 27.9 (+, cyclopropyl-CH), 28.1 (+, cyclopropyl-CH), 35.9 (+, cyclopropyl-CHN), 36.1 (+, cyclopropyl-CHN), 47.1 (+, Fmoc-CH), 49.0 (+, CHN), 50.5 (+, CHN), 52.4 (+, CH_3O, 2C), 67.2 (-, CH_2O), 67.3 (-, CH_2O), 120.0 (+, Fmoc-Ar-CH, 2C), 125.2 (+, Fmoc-Ar-CH, 2C), 127.1 (+, Ar-CH, 2C), 127.7 (+, Ar-CH, 2C), 128.3 (+, Ar-CH, 2C), 128.5 (+, Ar-CH), 128.6 (+, Ar-CH, 2C), 135.1 (C_{quat}, Ph-C), 141.3 (C_{quat}, Fmoc-Ar-C, 2C), 143.8 (C_{quat}, Fmoc-Ar-C), 143.9 (C_{quat}, Fmoc-Ar-C), 155.9 (C_{quat}, N(CO)O), 167.8 (C_{quat}, C=O), 169.3 (C_{quat}, C=O), 169.7 (C_{quat}, C=O), 170.6 (C_{quat}, C=O), 172.5 (C_{quat}, C=O), 173.2 (C_{quat}, CO). - MS FAB (CH_2Cl_2/NBA) *m/z* (%) 755 (MH^+, 91), 444 (19), 179 (Fluorenyl cation, 100). - IR (KBr) 3418, 3345, 1728, 1685, 1504 cm^{-1}. - Anal. Calcd for $C_{40}H_{42}N_4O_{11} \cdot 2\ H_2O$ (790.822): C, 60.75; H, 5.86; N, 7.08. Found: C, 60.56; H, 5.41; N, 6.98. - HR MS calcd for 755.29283, found 755.29165.

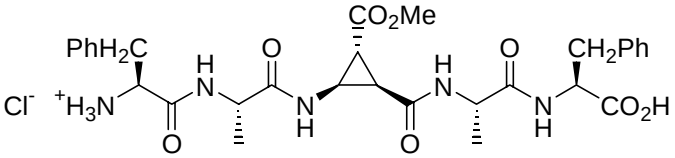


(+)-**45**

(1R, 2R, 3R) 3-{2S-[2R-(2S-{2R-[2S-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3R-methoxycarbonyl-cyclopropane-1R-carbonylamino}-propionylamino)-3R-methoxycarbonyl-cyclopropane-1R-carbonylamino]-propionylamino}-cyclopropane-1, 2-dicarboxylic acid 1-benzyl ester 2-methyl ester ((+)-**45):** (+)-**25b** (72 mg, 0.22 mmol, 1.5 eq.) was deprotected by treatment with HCl 3 M in ethyl acetate (10 ml) for 3 h at 0 °C. The solution was concentrated in vacuum, the salt was resuspended in CH_2Cl_2 (5 ml) and (+)-**42** (100 mg, 0.15 mmol, 1 eq.), EDC (58 mg, 0.30 mmol, 2 eq) and pyridine (24 µl,

0.30 mmol, 2 eq.) were added. The mixture was stirred at room temperature overnight. The product was purified by chromatography (CH_2Cl_2/MeOH 40:1). Yield: 70 mg (48 %). 5 to 10 % epimerization was observed. - R_f ((+)-**45**): 0.18. - mp 174-176 °C. - $[\alpha]_D^{21}$ -77.1 (c 0.5, MeOH/$CHCl_3$ 1:1). - ^{1}H NMR ($CDCl_3$/CD_3OD 1:1, 250 MHz): δ 1.26 (d, *J* = 7.1 Hz, 6H, C*H*$_3$CH), 1.34 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 2.31-2.41 (m, 2H, cyclopropyl-CH), 2.45-2.57 (m, 4H, cyclopropyl-CH), 3.68 (s, 3H, CH_3O), 3.71 (s, 3H, CH_3O), 3.72 (s, 3H, CH_3O), 3.80-3.88 (m, 3H, cyclopropyl-CHN), 4.12-4.41 (m, 6H, 3 CHN + Fmoc-CH_2 + Fmoc-CH), 5.09-5.20 (m, 2H, CH_2O), 7.31-7.39 (m, 9H, Ar-CH), 7.60-7.68 (m, 2H, Fmoc-Ar-CH), 7.76-7.78 (m, 2H, Fmoc-Ar-CH), 8.02 (s br, 1H, NH), 8.20 (s br, 1H, NH), 8.46 (s br, 2H, NH). - ^{13}C NMR ($CDCl_3$/CD_3OD 1:1, 62.9 MHz): δ 17.6 (+, *C*H_3CH), 17.7 (+, *C*H_3CH), 18.2 (+, *C*H_3CH), 27.38 (+, cyclopropyl-CH), 27.43 (cyclopropyl-CH), 27.8 (cyclopropyl-CH), 28.0 (cyclopropyl-CH, 2C), 28.2 (cyclopropyl-CH), 36.2 (+, cyclopropyl-CHN, 2C), 36.3 (+, cyclopropyl-CHN), 47.7 (+, Fmoc-CH), 49.7 (+, CHN), 50.0 (+, CHN), 51.4 (+, CHN), 52.7 (+, CH_3O), 52.8 (CH_3O), 52.9 (CH_3O), 67.6 (-, CH_2O), 67.8 (-, CH_2O), 120.4 (+, Fmoc-Ar-CH, 2C), 125.6 (+, Fmoc-Ar-CH), 125.8 (+, Fmoc-Ar-CH), 127.7 (+, Ar-CH, 2C), 128.3 (+, Ar-CH, 2C), 128.8 (+, Ar-CH, 2C), 129.0 (+, Ar-CH), 129.1 (+, Ar-CH, 2C), 136.0 (C_{quat}, Ph-C), 141.87 (C_{quat}, Fmoc-Ar-C), 141.90 (C_{quat}, Fmoc-Ar-C), 144.3 (C_{quat}, Fmoc-Ar-C), 144.6 (C_{quat}, Fmoc-Ar-C), 157.4 (C_{quat}, N(CO)O), 169.03 (C_{quat}, C=O), 169.06 (C_{quat}, C=O), 169.5 (C_{quat}, C=O), 171.0 (C_{quat}, C=O), 171.7 (C_{quat}, C=O), 171.9 (C_{quat}, C=O), 174.4 (C_{quat}, C=O), 174.6 (C_{quat}, C=O), 175.4 (C_{quat}, C=O). - MS FAB (CH_2Cl_2/NBA) *m/z* (%) 967 (MH^+, 100). - IR ($CDCl_3$) 3426, 3335, 3070, 2984, 1726, 1678, 1505, 1450, 1314 cm^{-1}. - HR MS calcd for $C_{49}H_{54}N_6O_{15}$ + H 967.37254, found 967.37171.

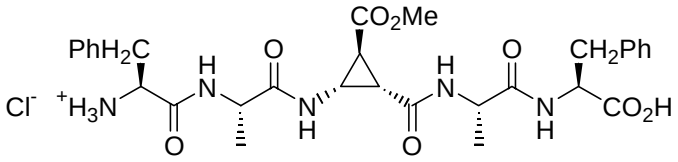
2.2.5 Completely deprotected peptides



(+)-**39**

(1R, 2R, 3R) 2-[2S-(2S-Amino-3-phenyl-propionylamino)-propionylamino]-3-[1S-(1S-carboxy-2-phenyl-ethylcarbamoyl)-ethylcarbamoyl]-cyclopropane carboxylic acid methyl ester; HCl salt ((+)-**39):** (+)-**37a** (390 mg, 0.50 mmol) was stirred in HCl 3 M in ethyl acetate (5 ml) at 0°C for 3 h. The solution was concentrated in vacuum and the salt

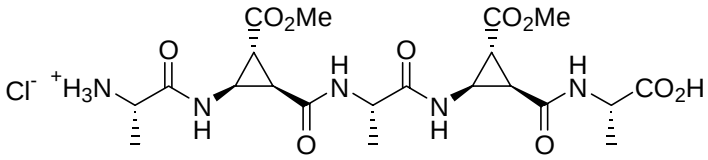
redissolved in methanol (25 ml). Then Pd/C 10 % (100 mg) and cyclohexadiene (1.1 ml, 40 % in pentane) were added. The reaction mixture was stirred overnight, filtrated over a celite pad and concentrated in vacuum to afford the deprotected peptide as a white solid (295 mg, 94 %). - mp 260 °C decomp. - $[\alpha]_D^{21}$ -39.9° (c 1, MeOH). - ^{1}H NMR (CD_3OD, 250 MHz) δ 1.22 (d, *J* = 6.1 Hz, 3H, C*H*$_3$CH), 1.32 (d, *J* = 6.6 Hz, 3H, C*H*$_3$CH), 2.41 (dd, *J* = 5.2, 5.2 Hz, 1H cyclopropyl-CH), 2.47 (dd, *J* = 5.4, 8.2 Hz, 1H, cyclopropyl-CH), 2.93-3.10 (m, 2H, CH_2Ph), 3.20 (dd, *J* = 5.2, 13.9 Hz, 1H, CH_2Ph), 3.59-3.71 (m, 2H, cyclopropyl-CHN + CH_2Ph), 3.74 (s, 3H, CH_3O), 4.10 (dd, J = 5.4, 8.8 Hz, 1H, Phe-CHN), 4.22-4.36 (m, 2H, Ala-CHN), 4.62 (dd, *J* = 5.2, 8.2 Hz, 1H, CHN), 7.17-7.24 (m, 5H, Ph-CH), 7.29-7.36 (m, 5H, Ph-CH). - ^{13}C NMR (CD_3OD, 100.6 MHz) δ 17.8 (+, *C*H$_3$CH), 18.0 (+, *C*H$_3$CH), 27.4 (+, cyclopropyl-CH), 29.3 (+, cyclopropyl-CH), 36.6 (+, cyclopropyl-CHN), 38.2 (-, CH_2Ph), 38.5 (-, CH_2Ph), 50.49 (+, CHN), 50.53 (+, CHN), 52.9 (+, CH_3O), 55.1 (+, CHN), 55.6 (+, CHN), 127.7 (+, Ph-CH), 128.8 (+, Ph-CH), 129.4 (+, Ph-CH, 2C), 130.1 (+, Ph-CH, 2C), 130.4 (+, Ph-CH, 2C), 130.6 (+, Ph-CH, 2C), 135.6 (C_{quat}, Ph-CH), 138.2 (C_{quat}, Ph-C), 169.1 (C_{quat}, C=O), 169.5 (C_{quat}, C=O), 172.4 (C_{quat}, C=O), 174.5 (C_{quat}, C=O, 2C), 175.1 (C_{quat}, C=O). – MS FAB (Glycerin/MeOH) *m/z* (%) 1191 ($2MH^+$, 3), 597 (MH^+, 100), 361 (28), 219 (20). - IR (KBr) 3318, 3297, 3063, 2933, 1732, 1654, 1540 cm^{-1}. - HR MS calcd for $C_{30}H_{38}N_5O_8$ 596.27204, found 596.27248.



(-)-**39**

(1S, 2S, 3S) 2-[2S-(2S-Amino-3-phenyl-propionylamino)-propionylamino]-3-[1S-(1S-carboxy-2-phenyl-ethylcarbamoyl)-ethylcarbamoyl]-cyclopropane carboxylic acid methyl ester; HCl salt ((-)-**39):** (-)-**37a** (400 mg, 0.51 mmol) was stirred in HCl 3 M in ethyl acetate (5 ml) at 0°C for 3 h. The solution was then concentrated in vacuum and the salt redissolved in methanol (25 ml). Then Pd/C 10 % (200 mg) and cyclohexadiene (1.2 ml, 40 % in pentane) were added. The reaction mixture was stirred overnight, filtrated over a celite pad and concentrated in vacuum to afford (321 mg, >99 %) of the unprotected peptide as a white solid. - mp 115-120 °C. - $[\alpha]_D^{21}$ -43.7 (c 1, MeOH). - ^{1}H NMR (CD_3OD, 250 MHz) δ 1.25 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 1.32 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 2.26 (dd, *J* = 5.2, 5.2 Hz, 1H cyclopropyl-CH), 2.52 (dd, *J* = 5.5, 8.3 Hz, 1H, cyclopropyl-CH), 2.97-3.14 (m, 2H, CH_2Ph),

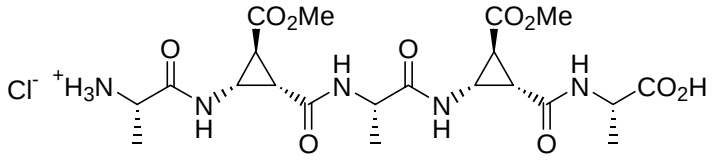
3.20-3.44 (m, 2H, CH_2Ph), 3.56 (dd, *J* = 4.4, 8.3 Hz, 1 H, cyclopropyl-CHN), 3.72 (s, 3H, CH_3O), 4.17 (dd, J = 4.8, 9.5 Hz, 1H, Phe-CHN), 4.31 (q, *J* = 7.1 Hz, 1H, Ala-CHN), 4.41 (q, *J* = 6.7 Hz, 1H, Ala-CHN), 4.60-4.69 (m, 1H, Phe-CHN), 7.19-7.35 (m, 10H, Ph-CH). - ^{13}C NMR (CD_3OD, 100.6 MHz) δ 17.4 (+, *C*H_3CH), 18.2 (+, *C*H_3CH), 28.3 (+, cyclopropyl-CH), 28.8 (+, cyclopropyl-CH), 36.5 (+, cyclopropyl-CHN), 38.1 (-, CH_2Ph), 38.6 (-, CH_2Ph), 50.6 (+, CHN), 52.9 (+, CH_3O), 55.5 (+, CHN), 55.6 (+, CHN), 55.9 (+, CHN), 127.9 (+, Ph-CH), 128.8 (+, Ph-CH), 129.5 (+, Ph-CH, 2C), 130.2 (+, Ph-CH, 2C), 130.5 (+, Ph-CH, 2C), 130.7 (+, Ph-CH, 2C), 135.9 (C_{quat}, Ph-CH), 138.4 (C_{quat}, Ph-C), 169.6 (C_{quat}, C=O), 172.3 (C_{quat}, CO, 3C), 174.9 (C_{quat}, CO, 2C). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 597 (MH^+, 15), 460 (8), 307 (55), 154 (100). - IR (KBr) 3318, 3063 2954, 1727, 1661, 1527, 1453, 1316 cm^{-1}. - HR MS calcd for $C_{30}H_{38}N_5O_8$ 596.27204, found 596.27085.



(+)-**43**

(1R, 2R, 3R) 2-(2S-Carboxyl-ethylcarbamoyl)-3-{2S-[2R-(2S-amino-propionylamino)-3R-methoxycarbonyl-cyclopropane-1R-carbonyl-amino]-propionylamino}-cyclopropane carboxylic acid methyl ester; HCl salt ((+)-**43):** (+)-**40** (300 mg, 0.43 mmol) was stirred in HCl 3 M in ethyl acetate (5 ml) at 0 °C for 3 h. The solution was concentrated in vacuum and the salt redissolved in methanol (25 ml). Then Pd/C 10 % (150 mg) and cyclohexadiene (1.2 ml, 40 % in pentane) were added. The reaction mixture was stirred overnight, filtrated over a celite pad and concentrated in vacuum to afford the unprotected peptide as a white solid (218 mg, 92 %). - mp 193-195 °C. - $[\alpha]_D^{21}$ -72.4 (c 0.5, MeOH). - ^{1}H NMR (CD_3OD, 400 MHz) δ 1.30 (d, *J* = 7.3 Hz, 3H, C*H*$_3$CH), 1.38 (d, *J* = 7.3 Hz, 3H, C*H*$_3$CH), 1.39 (d, *J* = 7.1 Hz, 3H, C*H*$_3$CH), 2.34 (dd, *J* = 4.5, 5.2 Hz, 1H, cyclopropyl-CH), 2.47 (dd, *J* = 5.0, 5.0 Hz, 1H, cyclopropyl-CH), 2.49 (dd, *J* = 5.2, 8.3 Hz, 1H, cyclopropyl-CH), 2.54 (dd, *J* = 5.4, 7.9 Hz, 1H, cyclopropyl-CH), 3.52 (dd, *J* = 4.6, 8.0 Hz, 1H, cyclopropyl-CHN), 3.70 (s, 3H, CH_3O), 3.72-3.75 (m, 1H, cyclopropyl-CHN), 3.73 (s, 3H, CH_3O), 3.88 (q, *J* = 7.0 Hz, 1H, CHN), 4.20 (q, *J* = 7.2 Hz, 1H, CHN), 4.41 (q, *J* = 7.2 Hz, 1H, CHN). - ^{13}C NMR (CD_3OD, 100.6 MHz) δ 17.6 (+, *C*H_3CH), 17.7 (+, *C*H_3CH), 17.8 (+, *C*H_3CH), 26.9 (+, cyclopropyl-CH), 27.5 (+, cyclopropyl-CH), 28.6 (+, cyclopropyl-CH), 29.9 (+, cyclopropyl-CH), 36.3 (+, cyclopropyl-CHN), 36.5 (+, cyclopropyl-CHN), 49.3 (+, Ala-CHN), 50.1 (+,

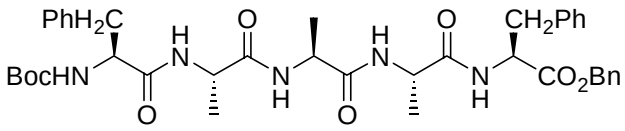
Ala-CHN), 50.9 (+, Ala-CHN), 52.9 (+, CH_3O), 53.0 (+, CH_3O), 168.7 (C_{quat}, C=O), 169.5 (C_{quat}, C=O), 172.1 (C_{quat}, C=O), 172.27 (C_{quat}, C=O), 172.33 (C_{quat}, C=O), 175.6 (C_{quat}, C=O), 175.8 (C_{quat}, C=O). - MS FAB (HCO_2H/MeOH/Glycerin) *m/z* (%) 514 (M^+, 27), 369 (32), 277 (100). - IR (KBr) 3326, 3061, 1731, 1538, 1452, 1351, 1211, 1173 cm^{-1}. - HR MS calcd for $C_{21}H_{32}N_5O_{10}$ 514.21492, found 514.21296.



(-)-**43**

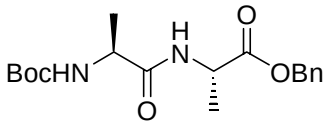
(1S, 2S, 3S) 2-(2S-Carboxy-ethylcarbamoyl)-3-{2S-[2S-(2S-amino-propionylamino)-3S-methoxycarbonyl-cyclopropane-1S-carbonyl-amino]-propionylamino}-cyclopropane carboxylic acid methyl ester; HCl salt ((-)-**43):** (-)-**40** (357 mg, 0.51 mmol) was stirred in HCl 3 M in ethyl acetate (5 ml) at 0°C for 3 h. The solution was then concentrated in vacuum and the salt redissolved in methanol (25 ml). Then Pd/C 10 % (60 mg) and cyclohexadiene (1.2 ml, 40 % in pentane) were added. The reaction mixture was stirred overnight, filtrated over a celite pad and concentrated in vacuum to afford the deprotected peptide as a white solid (270 mg, 96 %). - mp 195-197 °C. - $[\alpha]_D^{21}$ -116.6 (c 1, MeOH). - ^{1}H NMR (CD_3OD, 250 MHz) δ 1.35 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 1.44 (d, *J* = 7.3 Hz, 3H, C*H*$_3$CH), 1.51 (d, *J* = 7.0 Hz, 3H, C*H*$_3$CH), 2.35 (dd, *J* = 4.8, 4.8 Hz, 1H, cyclopropyl-CH), 2.42 (d, *J* = 4.9, 4.9 Hz, 1H, cyclopropyl-CH), 2.52 (dd, *J* = 5.5, 8.0 Hz, 1H, cyclopropyl-CH), 2.60 (dd, *J* = 5.4, 6.0 Hz, 1H, cyclopropyl-CH), 3.45 (dd, *J* = 4.5, 8.0 Hz, 1H, cyclopropyl-CHN), 3.59 (dd, *J* = 4.4, 8.3 Hz, 1H, cyclopropyl-CHN), 3.71 (s, 6H, CH_3O), 3.97 (q, *J* = 7.1 Hz, 1H, CHN), 4.27 (q, *J* = 7.1 Hz, 1H, CHN), 4.38 (q, *J* = 7.2 Hz, 1H, CHN), 7.93 (d, *J* = 5.4 Hz, 1H, NH), 8.32 (d, *J* = 3.5 Hz, 1H, NH), 8.55 (d, *J* = 5.9 Hz, 1H, NH), 8.82 (d, *J* = 6.8 Hz, 1H, NH). - ^{13}C NMR (CD_3OD, 62.9 MHz) δ 17.3 (+, *C*H$_3$CH), 17.4 (+, *C*H$_3$CH), 17.5 (+, *C*H$_3$CH), 27.7 (+, cyclopropyl-CH, 2C), 28.9 (+, cyclopropyl-CH), 29.6 (+, cyclopropyl-CH), 36.2 (+, cyclopropyl-CHN), 36.6 (+, cyclopropyl-CHN), 50.0 (+, CHN), 50.4 (+, CHN), 51.0 (+, CHN), 52.92 (+, CH_3O), 52.93 (+, CH_3O), 168.9 (C_{quat}, C=O), 169.4 (C_{quat}, C=O), 172.29 (C_{quat}, C=O), 172.35 (C_{quat}, C=O, 2C), 176.0 (C_{quat}, C=O), 176.1 (C_{quat}, C=O). - MS FAB (NBA/CH_2Cl_2) *m/z* (%) 514 (M^+, 23), 443 (3). - IR (KBr) 3449, 3307, 3061, 1742, 1660, 1542, 1451, 1313, 1173 cm^{-1}. - HR MS calcd for $C_{21}H_{32}N_5O_{10}$ 514.21492, found 514.2144.

2.2.6 Synthesis of α-peptides in solution



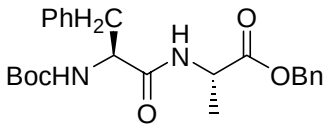
49

2S-(2S-{2S-[2S-(2S-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-propionylamino]-propionylamino}-propionylamino)-3-phenyl-propionic acid benzyl ester (49): Boc-alanylalanylalanylphenylalanine benzyl ester (198 mg, 0.35 mmol, 1 eq.) was deprotected by treatment with HCl 3 M in ethyl acetate (3 ml) for 3 h at 0 °C. The solution was concentrated in vacuum, the salt was resuspended in CH_2Cl_2 (20 ml) and triethylamine (56 µl, 0.4 mmol, 1.1 eq.) was added dropwise. To this mixture EDC (115 mg, 0.6 mmol, 1.7 eq.), HOBt (81 mg, 0.6 mmol, 1.7 eq.) and Boc-phenylalanine (159 mg, 0.6 mmol, 1.7 eq.) were added. The mixture was stirred at room temperature overnight. The solution was then concentrated in vacuum and the product was obtained by chromatography ($CHCl_3$/MeOH 20:1) as a white solid (225 mg, 90 %). - R_f (**49**): 0.12. - mp 217-218 °C. - $[\alpha]_D^{21}$ -43.2 (c 0.5, $CHCl_3$). - ^{1}H NMR ($CDCl_3$/CD_3OD 1:1, 400 MHz) δ 1.32 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 1.36 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 1.37 (d, *J* = 6.9 Hz, 3H, C*H*$_3$CH), 1.40 (s, 9H, $(CH_3)_3C$), 2.90 (dd, *J* = 8.6, 13.7 Hz, 1H, CH_2Ph), 3.04-3.18 (m, 3H, CH_2Ph), 4.19-4.25 (m, 1H, CHN), 4.26-4.32 (m, 2H, CHN), 4.36 (q, *J* = 7.2 Hz, 1H, CHN), 4.76 (dd, *J* = 6.2, 7.5 Hz, 1H, CHN), 5.09-5.15 (m, 2H, CH_2O), 7.12-7.37 (m, 15H, Ph-CH). - ^{13}C NMR ($CDCl_3$/CD_3OD 1:1, 100.6 MHz) δ 17.3 (+, *C*H$_3$CH), 17.4 (+, *C*H$_3$CH), 17.6 (+, *C*H$_3$CH), 28.4 (+, $(CH_3)_3C$, 3C), 37.9 (-, CH_2Ph), 38.1 (-, CH_2Ph), 49.2 (+, CHN), 49.8 (+, CHN), 50.4 (+, CHN), 54.1 (+, CHN), 56.8 (+, CHN), 67.4 (-, CH_2O), 80.9 (C_{quat}, $(CH_3)_3C$), 127.2 (+, Ph-CH), 127.3 (+, Ph-CH), 128.7 (+, Ph-CH, 3C), 128.78 (+, Ph-CH, 2C), 128.83 (+, Ph-CH, 2C), 128.9 (+, Ph-CH, 2C), 129.5 (+, Ph-CH, 2C), 129.6 (+, Ph-CH, 2C), 135.6 (C_{quat}, Ph-C), 136.6 (C_{quat}, Ph-C), 136.8 (C_{quat}, Ph-C), 156.8 (C_{quat}, N(CO)O), 171.7 (C_{quat}, CO, 2C), 173.2 (C_{quat}, C=O), 173.5 (C_{quat}, C=O), 173.7 (C_{quat}, C=O). - MS FAB (NBA/MeOH/CH_2Cl_2) *m/z* (%) 1432 ($2MH^+$, <1), 717 (MH^+, 17), 616 (MH^+-Boc, 3), 154 (100). - IR (KBr) 3382, 3287, 2978, 2933, 1710, 1636, 1525, 1451 cm^{-1}. - Anal. Calcd for $C_{39}H_{49}N_5O_8$ (715.845): C, 65.44; H, 6.90; N, 9.78, found: C, 65.30; H, 6.92; N, 9.77.



56a

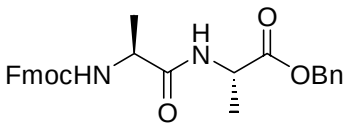
2S-(2S-*tert*-Butoxycarbonylamino-propionylamino)-propionic acid benzyl ester (56a):[69] N-Boc-alanine (144 mg, 0.76 mmol, 2 eq.) was preactivated by stirring with EDC (146 mg, 0.76 mmol, 2 eq.) and HOBt (103 mg, 0.76 mmol, 2 eq.) in dry CH_2Cl_2 (5 ml), under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. The solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (44 mg, 0.038 mmol, 0.1 eq.), then **55a** (100 mg, 0.38 mmol, 1 eq.) and finally DABCO (191 mg, 1.71 mmol, 4.5 eq.) were added. The solution was stirred 10 min. at room temperature, then CH_2Cl_2 (10 ml) was added and the solution was washed with saturated $NaHCO_3$ (10 ml), 1 M $KHSO_4$ (10 ml) and saturated $NaHCO_3$ (10 ml). The organic phase was dried over $MgSO_4$ and concentrated. The product was purified by chromatography (ethyl acetate/hexanes 2:3) to afford a white solid (131 mg, 99 %). - R_f (**56a**): 0.15. - mp 65-67 °C. - $[\alpha]_D^{21}$ -59.4 (c 0.5, MeOH). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.24 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 1.41 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 1.44 (s, 9H, $(CH_3)_3C$), 4.10-4.17 (m, 1H, CHN), 4.61 (dq, *J* = 7.3, 7.3 Hz, 1H, CHN), 5.04 (s br, 1H, NHBoc), 5.22-5.22 (m, 2H, CH_2O), 6.69 (d, J = 6.9 Hz, 1H, NH), 7.32-7.36 (m, 5h, Ph-CH).



56b

2S-(2S-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-propionic acid benzyl ester (56b):[70] N-Boc-phenylalanine (263 mg, 0.97 mmol, 2.7 eq.) was preactivated by stirring with EDC (187 mg, 0.97 mmol, 2.7 eq.) and HOBt (132 mg, 0.97 mmol, 2.7 eq.) in dry CH_2Cl_2 (5 ml), under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. The solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (40 mg, 0.036 mmol, 0.1 eq.), then **55b** (96 mg, 0.36 mmol, 1 eq.) and finally DABCO (190 mg, 1.7 mmol, 4.7 eq.) were added. The solution was stirred 10 min. at room temperature, then CH_2Cl_2 (10 ml) was added and the solution was washed with saturated $NaHCO_3$ (10 ml), 1 M $KHSO_4$ (10 ml) and saturated $NaHCO_3$ (10 ml). The organic phase was dried over $MgSO_4$ and concentrated. The product was purified by chromatography (ethyl acetate/hexanes 2:3) to afford a white solid (155 mg,

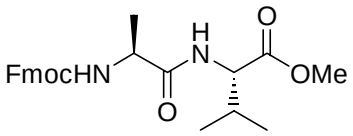
quantitative). - R_f (**56b**): 0.45. - mp 93-95 °C. - $[\alpha]_D^{21}$ -19.4 (c 1, MeOH). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.35 (d, *J* = 7.2 Hz, 3H, CH_3CH), 1.39 (s, 9H, $(CH_3)_3C$), 3.03-3.06 (m, 2H, CH_2Ph), 4.35-4.38 (m, 1H, CHN), 4.56 (dq, *J* = 7.2, 7.2, 1H, CHN), 5.08 (s br, 1H, NH), 5.14 (s, 2H, CH_2O), 6.52 (d, *J* = 7.1 Hz, 1H, NH), 7.16-7.39 (m, 10H, Ph-CH).



56c

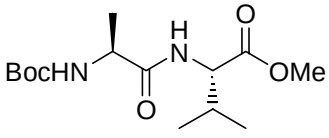
2S-[2S-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-propionic acid methyl ester (56c): N-Fmoc-alanine (177 mg, 0.57 mmol, 3 eq.) was preactivated by stirring with EDC (109 mg, 0.57 mmol, 3 eq.) and HOBt (77 mg, 0.57 mmol, 3 eq.) in dry CH_2Cl_2 (5 ml), under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. The solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (22 mg, 0.019 mmol, 0.1 eq.), then **55c** (50 mg, 0.19 mmol, 1 eq.) and finally DABCO (106 mg, 0.95 mmol, 5 eq.) were added. The solution was stirred 10 min. at room temperature, then CH_2Cl_2 (10 ml) was added and the solution was washed with saturated $NaHCO_3$ (10 ml), 1 M $KHSO_4$ (10 ml) and saturated $NaHCO_3$ (10 ml). The organic phase was dried over $MgSO_4$ and concentrated. The product was purified by chromatography (ethyl acetate/hexanes 2:3) to afford a white solid (85 mg, 94 %). - R_f (**56c**): 0.17. - mp 157-158 °C. - $[\alpha]_D^{21}$ -48.2 (c 1, MeOH). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.37 (d, *J* = 4.6 Hz, 3H, CH_3CH), 1.40 (d, *J* = 7.1 Hz, 3H, CH_3CH), 4.20 (t, *J* = 7.0 Hz, 1H, Fmoc-CH), 4.26-4.32 (m, 1H, CHN), 4.38 (d, *J* = 7.0 Hz, 2H, Fmoc-CH_2), 4.61 (dq, *J* = 7.2, 7.2 Hz, 1H, CHN), 5.09-5.21 (m, 2H, CH_2O), 5.49 (d, *J* = 6.5 Hz, 1H, NHFmoc), 6.64 (d, *J* = 6.0 Hz, 1H, NH), 7.25-7.41 (m, 9H, Ar-CH), 7.56-7.59 (m, 2H, Fmoc-Ar-CH), 7.74-7.77 (m, 2H, Fmoc-Ar-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 18.3 (+, CH_3CH), 18.9 (+, CH_3CH), 47.1 (+, Fmoc-CH), 48.3 (+, CHN), 50.4 (+, CHN), 67.1 (-, CH_2O), 67.3 (-, CH_2O), 120.0 (+, Fmoc-Ar-CH, 2C), 125.1 (+, Fmoc-Ar-CH, 2C), 127.1 (+, Ar-CH, 2C), 127.8 (+, Ar-CH, 2C), 128.2 (+, Ar-CH, 2C), 128.5 (+, Ar-CH), 128.7 (+, Ar-CH, 2C), 135.3 (C_{quat}, Ph-C), 141.3 (C_{quat}, Fmoc-Ar-C, 2C), 143.81 (C_{quat}, Fmoc-Ar-C), 143.84 (Fmoc-C_{quat}-, ArC), 155.9 (C_{quat}, N(CO)O), 171.9 (C_{quat}, C=O), 172.5 (C_{quat}, C=O). - MS CI (NH_3) *m/z* (%) 490 (MNH_4^+, 31), 473 (MH^+, 2), 400 (10), 294 (15), 251 (15), 214 (100). - IR (KBr) 3304, 3065, 2979, 1737, 1690, 1654, 1537, 1450 cm^{-1}. - Anal. Calcd for $C_{28}H_{28}N_2O_5$.0.3 H_2O (477.944): C, 70.36; H,

6.03; N, 5.86. Found: C, 70.35; H, 5.99; N, 5.82. - HR MS calcd for $C_{28}H_{28}N_2O_5$ 472.19982, found 472.20010.



56d

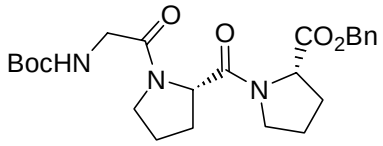
2S-[2S-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]3-methyl-butyric acid methyl ester (56d):[71] N-Fmoc-alanine (130 mg, 0.42 mmol, 1.5 eq.) was preactivated by stirring with EDC (81 mg, 0.42 mmol, 1.5 eq.) and HOBt (57 mg, 0.42 mmol, 1.5 eq.) in dry CH_2Cl_2 (5 ml), under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. The solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (32 mg, 0.028 mmol, 0.1 eq.), then **55d** (60 mg, 0.28 mmol, 1 eq.) and finally DABCO (110 mg, 0.98 mmol, 3.5 eq.) were added. The solution was stirred 10 min. at room temperature, then CH_2Cl_2 (10 ml) was added and the solution was washed with saturated $NaHCO_3$ (10 ml), 1 M $KHSO_4$ (10 ml) and saturated $NaHCO_3$ (10 ml). The organic phase was dried over $MgSO_4$ and concentrated. The product was purified by chromatography (ethyl acetate/hexanes 2:3) to afford a white solid (103 mg, 87 %). - R_f (**56d**): 0.21. - mp 151-153 °C. - $[\alpha]_D^{21}$ -33.9 (c 0.5, MeOH). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.88 (t, *J* = 6.9 Hz, 3H, $(CH_3)_2CH$), 0.91 (t, *J* = 6.9 Hz, 3H, $(CH_3)_2CH$), 1.40 (d, J = 7.0 Hz, 3H, CH_3CH), 2.09-2.20 (m, 1H, $(CH_3)_2CH$), 3.72 (s, 3H, CH_3O), 4.18-4.23 (m, 1H, Fmoc-CH), 4.31-4.40 (m, 3H, Fmoc-CH_2 + Ala-CHN), 4.54 (dd, J = 4.9, 8.8 Hz, 1H, Val-CHN), 5.49 (d, J = 7.6 Hz, 1H, NHFmoc), 6.61 (d, J = 8.3 Hz, 1H, NH), 7.25-7.42 (m, 4H, Fmoc-Ar-CH); 7.56-7.59 (m, 2H, Fmoc-Ar-CH), 7.74-7.77 (m, 2H, Fmoc-Ar-CH).



56e

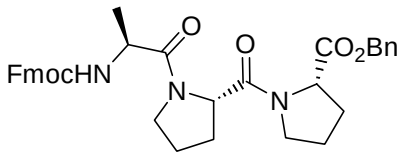
2S-(2S-*tert*-Butoxycarbonylamino-propionylamino)-3-methyl-butyric acid methyl ester (56e):[72] N-Boc-alanine (261 mg, 1.38 mmol, 3 eq.) was preactivated by stirring with EDC (265 mg, 1.38 mmol, 3 eq.) and HOBt (186 mg, 1.38 mmol, 3 eq.) in dry CH_2Cl_2 (5 ml), under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. The solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (50 mg, 0.046 mmol, 0.1 eq.), then **55e** (107 mg,

0.46 mmol, 1 eq.) and finally DABCO (283 mg, 2.53 mmol, 5.5 eq.) were added. The solution was stirred 10 min. at room temperature, then CH_2Cl_2 (10 ml) was added and the solution was washed with saturated $NaHCO_3$ (10 ml), 1 M $KHSO_4$ (10 ml) and saturated $NaHCO_3$ (10 ml). The organic phase was dried over $MgSO_4$ and concentrated. The product was purified by chromatography (ethyl acetate/hexanes 2:3) to afford a white solid (145 mg, 97 %). - R_f (**56e**): 0.18. - mp 82-84 °C. - $[\alpha]_D^{21}$ -49.8 (c 0.5, MeOH). - ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.90 (d, *J* = 7.0 Hz, 3H, $(CH_3)_2CH$), 0.93 (d, *J* = 7.0 Hz, 3H, $(CH_3)_2CH$), 1.36 (d, *J* = 7.0 Hz, 3H, CH_3CH), 1.45 (s, 9H, $(CH_3)_3C$), 2.11-2.21 (m, 1H, $(CH_3)_2CH$), 3.74 (s, 3H, CH_3O), 4.13-4.22 (m, 1H, Ala-CHN); 4.53 (dd, *J* = 4.9, 13.8 Hz, 1H, Val-CHN), 5.04 (d, *J* = 6.7 Hz, 1H, NHBoc), 6.69 (s br, 1H, NH).



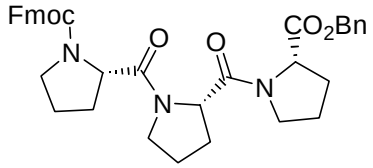
67a

1-{1-[(*tert*-Butoxycarbonylamino)-acetylamino]-pyrrolidine-2S-carbonyl}-pyrrolidine-2S-carboxylic acid benzyl ester (67a):[73] N-Boc-glycine (272 mg, 1.55 mmol, 3eq.) was preactivated by stirring with EDC (298 mg, 1.55 mmol, 3 eq.) and HOBt (209 mg, 1.55 mmol, 3 eq.) in dry CH_2Cl_2 (8 ml), under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. The solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (60 mg, 0.05 mmol, 0.1 eq.), then **66** (200 mg, 0.52 mmol, 1 eq.) and finally DABCO (349 mg, 3.1 mmol, 6 eq.) were added. The solution was stirred 20 min. at room temperature, then CH_2Cl_2 (10 ml) was added and the solution was washed with saturated $NaHCO_3$ (15 ml), 1 M $KHSO_4$ (10 ml) and saturated $NaHCO_3$ (15 ml). The organic phase was dried over $MgSO_4$ and concentrated. The product was purified by chromatography ($CHCl_3$/MeOH 40:1) to afford a white solid (215 mg, 90 %). - R_f (**67a**): 0.6. - mp 58-60 °C. - $[\alpha]_D^{21}$ -105.7 (c 1, $CHCl_3$). - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (s, 9H, $(CH_3)_3C$), 2.01-2.24 (m, 8H, CH_2-CH_2), 3.40-3.49 (m, 1H, proline-CH_2N), 3.54-3.65 (m, 2H, proline-CH_2N), 3.81-3.90 (m, 2H, proline-CH_2N + glycine CH_2N), 4.03 (dd, *J* = 5.4; 17.1 Hz, 1H, gycine-CH_2N), 4.59-4.69 (m, 2H, proline-CHN), 5.06 (d, *J* = 12.3 Hz, 1H, CH_2O), 5.22 (d, *J* = 12.3 Hz, CH_2O), 5.30 (s br, 1H, NH), 7.32-7.34 (m, 5H, Ph-CH).



67b

1-{1-[2S-(9*H*-Fluoren-9-ylmethoxycarbonyl-amino)-propionyl]-pyrrolidine-2S-carbonyl}-pyrrolidine-2S-carboxylic acid benzyl ester (67b): N-Fmoc-alanine (805 mg, 2.59 mmol, 4 eq.) was preactivated by stirring with EDC (497 mg, 2.59 mmol, 4 eq.) and HOBt (349 mg, 2.59 mmol, 4 eq.) in dry CH_2Cl_2 (10 ml), under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. The solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (99 mg, 0.086 mmol, 0.13 eq.), then **66** (247 mg, 0.64 mmol, 1 eq.) and finally DABCO (532 mg, 4.75 mmol, 7.5 eq.) were added. The solution was stirred 20 min. at room temperature, then CH_2Cl_2 (10 ml) was added and the solution was washed with saturated $NaHCO_3$ (15 ml), 1 M $KHSO_4$ (10 ml) and saturated $NaHCO_3$ (15 ml). The organic phase was dried over $MgSO_4$ and concentrated. The product was purified by chromatography ($CHCl_3$/MeOH 90:1) to afford a white solid (375 mg, 98 %). - R_f (**67b**): 0.14. - mp 58-60 °C. - $[\alpha]_D^{21}$ -127.1 (c 0.5, MeOH). - ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (d, *J* = 6.9 Hz, 3H, C*H*$_3$CH), 1.93-2.06 (m, 5H, $CH_2C\mathit{H}_2CH_2$), 2.08-2.24 (m, 3H, $CH_2C\mathit{H}_2CH_2$), 3.57-3.70 (m, 3H, CH_2N), 3.77-3.81 (m, 1H, CH_2N), 4.20 (dd, *J* = 7.2, 7.2 Hz, 1H, Fmoc-CH), 4.34 (d, *J* = 7.1 Hz, 2H, Fmoc-CH_2), 4.54 (pseudo p, *J* = 7.6 Hz, 1H, Ala-CHN), 4.66 (dd, *J* = 3.9, 9.1 Hz, 1H, Pro-CHN), 4.68-4.70 (m, 1H, Pro-CHN), 5.04 (d, J = 12.3 Hz, 1H, CH_2O), 5.24 (d, J = 12.3 Hz, 1H, CH_2O), 5.69 (d, *J* = 8.0 Hz, 1H, NH), 7.30-7.40 (m, 9H, Ar-CH), 7.57-7.60 (m, 2H, Fmoc-Ar-CH), 7.74-7.76 (m, 2H, Fmoc-Ar-CH). - ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 18.2 (+, *C*H_3CH), 24.77 (-, CH_2), 24.81 (-, CH_2), 28.0 (-, CH_2), 28.7 (-, CH_2), 46.6 (-, CH_2N), 47.06 (+, Fmoc-CH), 47.10 (-, CH_2N), 48.3 (+, CHN), 57.8 (+, CHN), 58.7 (+, CHN), 66.8 (-, CH_2O), 66.9 (-, CH_2O), 119.9 (+, Fmoc-Ar-CH, 2C), 125.1 (+, Fmoc-Ar-CH, 2C), 127.0 (+, Ar-CH, 2C), 127.6 (+, Ar-CH, 2C), 128.1 (+, Ar-CH, 2C), 128.2 (+, Ar-CH), 128.5 (+, Ar-CH, 2C), 135.5 (C_{quat}, Ph-C), 141.19 (C_{quat}, Fmoc-Ar-C), 141.20 (C_{quat}, Fmoc-Ar-C), 143.8 (C_{quat}, Fmoc-Ar-C), 143.9 (C_{quat}, Fmoc-Ar-C), 155.6 (C_{quat}, N(CO)O), 170.2 (C_{quat}, C=O), 171.0 (C_{quat}, C=O), 171.9 (C_{quat}, C=O). - MS FAB (MeOH/Glycerin) *m/z* (%) 596 (MH^+, 25), 391 (9), 303 (23), 179 (100). - IR (KBr) 3388, 3209, 2942, 2786, 1739, 1700, 1609, 1521, 1420 cm^{-1}. - HR MS calcd for $C_{35}H_{37}N_3O_6$ + H 596.27606, found 596.27622.

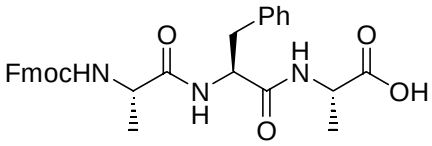


67c

1-{1-[1-(9*H*-Fluoren-9-ylmethoxycarbonyl)-pyrrolidine-2S-carbonyl]-pyrrolidine-2S-carbonyl}-pyrrolidine-2S-carboxylic acid benzyl ester (67c): N-Fmoc-proline (526 mg, 1.56 mmol, 3 eq.) was preactivated by stirring with EDC (299 mg, 1.56 mmol, 3 eq.) and HOBt (210 mg, 1.56 mmol, 3 eq.) in dry CH_2Cl_2 (10 ml), under nitrogen atmosphere 1 h at 0 °C and 1 h at room temperature. The solution was added under nitrogen atmosphere to $Pd[PPh_3]_4$ (60 mg, 0.052 mmol, 0.1 eq.), then **66** (200 mg, 0.52 mmol, 1 eq.) and finally DABCO (323 mg, 2.86 mmol, 5.5 eq.) were added. The solution was stirred 20 min. at room temperature, then CH_2Cl_2 (10 ml) was added and the solution was washed with saturated $NaHCO_3$ (15 ml), 1 M $KHSO_4$ (10 ml) and saturated $NaHCO_3$ (15 ml). The organic phase was dried over $MgSO_4$ and concentrated. The product was purified by chromatography ($CHCl_3$/MeOH 90:1) to afford a mixture of two rotamers 70:30 (310 mg, 96 %). - R_f (**67c**): 0.13. - mp 74-76 °C. - $[\alpha]_D^{21}$ -140.0 (c 0.5, MeOH). - ^{1}H NMR ($CDCl_3$, 62.9 MHz) δ 1.79-2.25 (m, 12H, CH_2), 3.19-3.22 (m, 1H, CH_2N, minor rotamer), 3.43-3.82 (m, 6H, CH_2N, major rotamer + 5H minor rotamer), 4.14-4.46 (m, 3H, Fmoc-CH_2 + Fmoc-CH), 4.48-4.65 (m, 2H, CHN, major rotamer + 3H minor rotamer), 4.76 (dd, *J* = 3.6, 7.7 Hz, 1H, CHN, major rotamer), 5.01 (d, *J* = 12.3 Hz, 1H, CH_2O, minor rotamer), 5.02 (d, *J* = 12.3 Hz, 1H, CH_2O, major rotamer), 5.23 (d, *J* =12.3 Hz, 1H, CH_2O, minor rotamer + 1H major rotamer), 7.29-7.41 (m, 9H, Ar-CH), 7.54-7.64 (m, 2H, Fmoc-Ar-CH), 7.74-7.77 (m, 2H, Fmoc-Ar-CH). - ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ **major rotamer**: 23.4 (-, CH_2), 24.9 (-, CH_2, 2C), 27.9 (-, CH_2), 28.8 (-, CH_2), 29.1 (-, CH_2), 46.6 (-, CH_2N), 46.89 (-, CH_2N), 46.94 (-, CH_2N), 47.3 (+, Fmoc-CH), 57.9 (+, CHN), 58.3 (+, CHN), 58.8 (+, CHN), 66.8 (-, CH_2O), 67.4 (-, CH_2O), 119.9 (+, Fmoc-Ar-CH, 2C), 125.2 (+, Fmoc-Ar-CH), 125.3 (+, Ar-CH), 127.0 (+, Ar-CH), 127.1 (+, Ar-CH), 127.64 (+, Ar-CH), 127.65 (+, Ar-CH), 128.20 (+, Ar-CH, 2C), 128.3 (+, Ar-CH), 128.6 (+, Ar-CH, 2C), 135.7 (C_{quat}, Ph-C), 141.28 (C_{quat}, Fmoc-Ar-C), 141.31 (C_{quat}, Fmoc-Ar-C), 144.0 (C_{quat}, Fmoc-Ar-C), 144.3 (C_{quat}, Fmoc-Ar-C), 155.0 (C_{quat}, N(CO)O), 170.68 (C_{quat}, C=O), 170.71 (C_{quat}, C=O), 172.1 (C_{quat}, C=O). **Minor rotamer**: 23.2 (-, CH_2), 24.8 (-, CH_2), 24.9 (-, CH_2N), 27.8 (-, CH_2), 28.8 (-, CH_2), 29.9 (-, CH_2), 46.5 (-, CH_2N), 46.6 (-, CH_2N), 47.3 (-, CH_2N), 47.7 (+, Fmoc-CH), 57.69 (+, CHN), 57.73 (+, CHN), 58.7 (+, CHN), 66.3 (-, CH_2O), 66.8 (-, CH_2O), 119.8 (+, Fmoc-Ar-CH, 2C), 124.9 (+, Fmoc-Ar-CH),

125.0 (+, Fmoc-Ar-CH), 126.8 (+, Ar-CH), 127.0 (+, Ar-CH), 127.4 (+, Ar-CH), 127.6 (+, Ar-CH), 128.18 (+, Ar-CH, 2C), 128.3 (+, Ar-CH), 128.6 (+, Ar-CH, 2C), 135.68 (C_{quat}, Ph-C), 141.26 (C_{quat}, Fmoc-Ar-C), 141.35 (C_{quat}, Fmoc-Ar-C), 143.9 (C_{quat}, Fmoc-Ar-C), 144.6 (C_{quat}, Fmoc-Ar-C), 154.3 (C_{quat}, N(CO)O), 170.5 (C_{quat}, C=O), 170.6 (C_{quat}, C=O), 172.0 (C_{quat}, C=O). - MS FAB (MeOH/Glycerine) *m/z* (%) 1243 ($2MH^+$, <1), 622 (MH^+, 28), 179 (Fuorenyl cation, 100). - IR (KBr) 2971, 2875, 1742, 1702, 1649, 1421 cm^{-1}. - HR MS calcd for $C_{37}H_{39}N_3O_6$ + H 622.29171, found 622.29072.

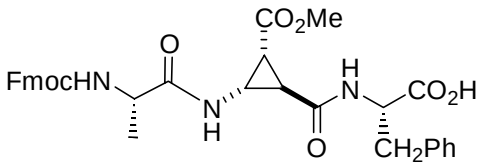
2.2.7 Solid-phase synthesis



76

2S-{2S-[2S-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3-phenyl-propionylamino}-propionic acid (76): The solid phase synthesis was performed on a Wang resin. The loading of N-Fmoc-alanine (0.35 mmol/g) on the resin was performed following the Advanced ChemTech catalogue protocol (GP1). Fmoc group removal was carried out with 20 % piperidine in DMF (20 min.). The coupling of N-Alloc-phenylalanine was achieved following the Advanced ChemTech Synthesiser (Model 90) manual protocol (GP2) for HOBt/DIC/amino acid method. Then the resin (495 m, 0.15 mmol) was swelled in CH_2Cl_2 (10 ml) for 25 min.. The resin was drained and a solution (previously stirred 1 h at 0 °C and 1 h at room temperature for preactivation) of N-Fmoc-alanine (700 mg, 2.25 mmol, 15 eq.), EDC (432 mg, 2.25 mmol, 15 eq.) and HOBt (304 mg, 2.25 mmol, 15 eq.) in freshly distilled CH_2Cl_2 (8 ml) was added under nitrogen atmosphere. $Pd[PPh_3]_4$ (34 mg, 0.03 mmol, 0.2 eq.) and finally DABCO (302 mg, 2.7 mmol, 18 eq.) were added. The reaction mixture was agitated 2 h, the resin was drained and washed with CH_2Cl_2 (10 x 10 ml). The product was cleaved from the resin by stirring for 1 h with a 2:1 mixture of TFA/CH_2Cl_2 (10 ml). After solvent evaporation the pure product was isolated as a white solid (78 mg, 99 %). - mp 176-178 °C. - $[\alpha]_D^{21}$ -37.3 (c 0.5, MeOH). - ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.11 (d, *J* = 7.0 Hz, 3H, C*H*$_3$CH), 1.27 (d, *J* = 7.2 Hz, 3H, C*H*$_3$CH), 2.78 (dd, *J* = 9.3, 13.8 Hz, 1H, CH_2Ph), 3.03 (dd, *J* = 4.0, 13.8 Hz, 1H, CH_2Ph), 3.95-4.03 (m, 1H, Fmoc-CH), 4.16-4.21 (m, 4H, Fmoc-CH_2 + 2 CHN), 4.27-4.51 (m, 1H, CHN), 7.19-7.48 (m, 10H, Ar-CH + NH), 7.69-7.71 (m, 2H, Fmoc-Ar-CH), 7.87-7.89 (m, 3H, Fmoc-Ar-CH + NH), 8.22 (d, *J* = 7.0 Hz, 1H, NH),

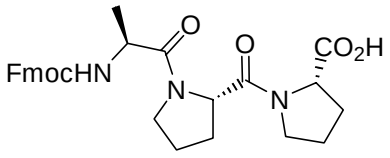
12.52 (s br, 1H, CO_2H). - ^{13}C NMR (DMSO-d_6, 62.9 MHz): δ 17.2 (+, *C*H_3CH), 18.1 (+, *C*H_3CH), 37.3 (-, CH_2Ph), 46.6 (+, Fmoc-CH), 47.5 (+, CHN), 50.2 (+, CHN), 53.3 (+, CHN), 65.6 (-, CH_2O), 120.1 (+, Fmoc-Ar-CH, 2C), 125.3 (+, Fmoc-Ar-CH, 2C), 126.2 (+, Ar-CH), 127.1 (+, Ar-CH, 2C), 127.6 (+, Ar-CH, 2C), 127.9 (+, Ar-CH, 2C), 129.3 (+, Ar-CH, 2C), 137.6 (C_{quat}, Ph-C), 140.7 (C_{quat}, Fmoc-Ar-C, 2C), 143.7 (C_{quat}, Fmoc-Ar-C), 143.8 (C_{quat}, Fmoc-Ar-C), 155.6 (C_{quat}, N(CO)O), 170.6 (C_{quat}, C=O), 172.2 (C_{quat}, C=O), 173.9 (C_{quat}, C=O). - MS FAB (MeOH/Glycerine) *m/z* (%) 530 (MH^+, 13), 441 (MH^+-Alanine, 8), 237 (7), 179 (Fluorenyl cation, 100). - IR (KBr) 3298, 3066, 1711, 1688, 1645, 1536, 1450 cm^{-1}. - HR MS FAB (MeOH/Glycerine) calcd for $C_{30}H_{31}N_3O_6$ + H 530.22911, found 596.27622.



77

(1R*, 2R*, 3S*) 2-(1S-Carboxy-2-phenyl-ethylcarbamoyl)-3-[2S-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionylamino]-cyclopropane carboxylic acid methyl ester (77): The solid phase synthesis was performed on a Wang resin. The loading of Fmoc-phenylalanine (0.85 mmol/g) on the resin was performed following the Advanced ChemTech catalogue protocol (GP1). Fmoc group removal was carried out with 20 % piperidine in DMF (20 min.). The coupling of (±)-**69b** was achieved following the Advanced ChemTech Synthesiser (Model 90) manual protocol (GP2) for HOBt/DIC/amino acid method. Then the resin (200 mg, 0.13 mmol) was swelled in CH_2Cl_2 (10 ml) for 25 min.. The resin was drained and a solution (previously stirred 1 h at 0 °C and 1 h at room temperature for preactivation) of N-Fmoc-alanine (248 mg, 0.80 mmol, 6 eq.), EDC (154 mg, 0.80 mmol, 6 eq.) and HOBt (108 mg, 0.80 mmol, 6 eq.) in freshly distilled CH_2Cl_2 (4 ml) was added under nitrogen atmosphere. $Pd[PPh_3]_4$ (30 mg, 0.026 mmol, 0.2 eq.) and finally DABCO (175 mg, 1.56 mmol, 12 eq.) were added. A parallel experiment was performed under the same conditions but with the addition of $PhSiH_3$ (192 µl, 1.56 mmol, 12 eq.) instead of DABCO. The reaction mixture was agitated 2 h, the resin was then drained and washed CH_2Cl_2 with (10 x 10 ml). The product was cleaved from the resin by stirring 1 h with a 2:1 mixture of TFA/CH_2Cl_2 (10 ml). After solvent evaporation the product (as a mixture of two diastereomers) was purified by chromatography ($CHCl_3$/MeOH 15:1 + 1 % AcOH) as a white solid (66 mg, 85 % for DABCO protocol, 62 mg, 82 % for $PhSiH_3$ protocol). - R_f (**77**): 0.3 - mp 200 °C decomp. - ^1H NMR (CD_3OD, 400 MHz): δ 1.26-1.29 (m, 3H, C*H*$_3$CH),

2.19 (dd *J* = 5.4, 7.9 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.32 (dd, *J* = 5.4, 7.8 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.42 (dd, *J* = 5.1, 5.1 Hz, 1H, cyclopropyl-CH, 1 diast.), 2.46-2.49 (m, 1H, cyclopropyl-CH, 1 diast.), 2.90-2.97 (m, 1H, $PhCH_2$), 3.17-3.22 (m, 1H, $PhCH_2$), 3.45-3.50 (m, 1H, cyclopropyl-CHN), 3.59 (s, 3H, CH_3O, 1 diast.), 3.61 (s, 3H, CH_3O, 1 diast.), 4.04-4.09 (m, 1H, Ala-CHN), 4.19-4.2 (m, 1H, Fmoc-CH), 4.29-4.39 (m, 2H, Fmoc-CH_2), 4.62-4.67 (m, 1H, Phe-CHN), 7.13-7.39 (m, 9H, Ar-CH), 7.64-7.68 (m, 2H, Fmoc-Ar-CH), 7.77-7.79 (m, 2H, Fmoc-Ar-CH). - ^{13}C NMR (CD_3OD, 100.6 MHz): δ 18.0 (+, *C*H_3CH, 1 diast.), 18.1 (+, *C*H_3CH, 1 diast.), 27.6 (+, cyclopropyl-CH, 1 diast.), 27.7 (+, cyclopropyl-CH, 1 diast.), 29.4 (+, cyclopropyl-CH, 1 diast.), 29.7 (+, cyclopropyl-CH, 1 diast.), 35.7 (+, cyclopropylCHN, 1 diast.), 35.9 (+, cyclopropyl-CHN, 1 diast.), 38.4 (-, CH_2Ph, 1 diast.), 38.6 (-, CH_2Ph, 1 diast.), 48.4 (+, Fmoc-CH), 51.9 (+, CH_3O, 1 diast.), 52.0 (+, CH_3O, 1 diast.), 52.7 (+, CHN, 1 diast.), 52.8 (+, CHN, 1 diast.), 55.4 (+, CHN, 1 diast.), 55.5 (+, CHN, 1 diast.), 68.0 (-, Fmoc-CH_2), 120.9 (+, Fmoc-Ar-CH, 2C), 126.2 (+, Ar-CH, 2C, 1 diast.), 126.3 (+, Ar-CH, 2C, 1 diast.), 127.78 (+, Ar-CH, 1 diast.), 127.81 (+, Ar-CH, 1 diast.), 128.14 (+, Ar-CH, 2C, 1 diast.), 128.18 (+, Ar-CH, 2C, 1 diast.), 128.8 (+, Ar-CH, 2C), 129.4 (+, Ar-CH, 2C, 1 diast.), 129.5 (+, Ar-CH, 2C, 1 diast.), 130.28 (+, Ar-CH, 2C, 1 diast.), 130.31 (+, Ar-CH, 2C, 1 diast.), 138.2 (C_{quat}, Ph-C, 1 diast.), 138.3 (C_{quat}, Ph-C, 1 diast.), 142.57 (C_{quat}, Fmoc-Ar-C, 2C, 1 diast.), 142.59 (C_{quat}, Fmoc-Ar-C, 2C, 1 diast.), 145.51 (C_{quat}, Fmoc-Ar-C, 1 diast.), 145.2 (C_{quat}, Fmoc-Ar-C, 1 diast.), 145.4 (C_{quat}, Fmoc-Ar-C), 158.2 (C_{quat}, N(CO)O), 170.5 (C_{quat}, C=O, 1 diast.), 170.6 (C_{quat}, C=O, 1 diast.), 170.8 (C_{quat}, C=O, 1 diast.), 170.9 (C_{quat}, C=O, 1 diast.), 174.56 (C_{quat}, C=O, 1 diast.), 174.65 (C_{quat}, C=O, 1 diast.), 176.6 (C_{quat}, C=O). - MS FAB (MeOH/Glycerine) *m/z* (%) 600 (MH^+, 100), 442 (18). - IR (KBr) 3500-277 (OH broad signal), 3292, 3047, 2961, 1719, 1649, 1529, 1439, 1247 cm^{-1}. - HR MS calcd for $C_{33}H_{33}N_3O_8$ + H 600.23459, found 600.23475.



80

1-{1-[2S-(9*H*-Fluoren-9-ylmethoxycarbonyl-amino)-propionyl]-pyrrolidine-2S-carbonyl}-pyrrolidine-2S-carboxylic acid (80): The solid phase synthesis was performed on a Wang resin. The loading of N-Fmoc-proline (0.6 mmol/g) on the resin was performed following the Advanced ChemTech catalogue protocol (GP1). Fmoc group removal was carried out with 20 % piperidine in DMF (20 min.). The coupling of N-Alloc-proline was achieved following

the Advanced ChemTech Synthesiser (Model 90) manual protocol (GP2) for HOBt/DIC/amino acid method. Then the resin (300 mg, 0.15 mmol) was swelled in CH_2Cl_2 (10 ml) for 25 min.. The resin was drained and a solution (previously stirred 1 h at 0 °C and 1 h at room temperature for preactivation) of N-Fmoc-alanine (299 mg, 0.96 mmol, 6 eq.), EDC (184 mg, 0.96 mmol, 6 eq.) and HOBt (130 mg, 0.96 mmol, 6 eq.) in freshly distilled CH_2Cl_2 (4 ml) was added under nitrogen atmosphere. $Pd[PPh_3]_4$ (34 mg, 0.03 mmol, 0.2 eq.) and finally DABCO (201 mg, 1.8 mmol, 12 eq.) were added. A parallel experiment was performed under the same conditions but with the addition of $PhSiH_3$ (221 µl, 1.8 mmol, 12 eq.) instead of DABCO. The reaction mixture was agitated 2 h, the resin was then drained and washed with CH_2Cl_2 (10 x 10 ml). The product was cleaved from the resin by stirring 1 h with a 2:1 mixture of TFA/CH_2Cl_2 (10 ml). After solvent evaporation the pure product was isolated as a white solid (77 mg, quantitative for DABCO protocol, 76 mg, 99 % for $PhSiH_3$ protocol). - mp 96-99 °C. - $[\alpha]_D^{21}$ -107.5 (c 0.5, MeOH). - ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.38 (d, *J* = 6.8 Hz, 3H, C*H*$_3$CH), 1.97-2.06 (m, 4H, $CH_2CH_2CH_2$), 2.09-2.20 (m, 4H, $CH_2CH_2CH_2$), 3.54-3.80 (m, 4H, CH_2N), 4.20 (dd, *J* = 7.4, 7.4 Hz, 1H, Fmoc-CH), 4.33 (d, *J* = 6.8 Hz, 2H, Fmoc-CH_2), 4.54 (dq, *J* = 7.3, 7.3 Hz, 1H, Ala-CHN), 4.60 (dd, *J* = 4.2, 7.7 Hz, 1H, CHN), 4.67 (dd, *J* = 4.6, 7.9 Hz, 1H, CHN), 5.82 (d, *J* = 8.3 Hz, 1H, NH), 7.26-7.31 (m, 2H, Fmoc-Ar-CH), 7.37-7.41 (m, 2H, Fmoc-Ar-CH), 7.57-7.70 (m, 2H, Fmoc-Ar-CH), 7.74-7.76 (m, 2H, Fmoc-Ar-CH), 9.23 (s br, 1H, CO_2H). - ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 17.9 (+, *C*H$_3$CH), 24.9 (-,$CH_2CH_2CH_2$), 28.0 (-,$CH_2CH_2CH_2$), 28.1 (-,$CH_2CH_2CH_2$), 29.6 (-,$CH_2CH_2CH_2$), 47.09 (+, Fmoc-CH), 47.12 (-, CH_2N), 47.2 (-, CH_2N), 48.3 (+, CHN), 57.9 (+, CHN), 59.4 (+, CHN), 67.0 (-, CH_2O), 119.9 (+, Fmoc-Ar-CH, 2C), 125.1 (+, Fmoc-Ar-CH, 2C), 127.0 (+, Fmoc-Ar-CH, 2C), 127.6 (+, Fmoc-Ar-CH, 2C), 141.2 (C_{quat}, Fmoc-Ar-C, 2C), 143.8 (C_{quat}, Fmoc-Ar-C), 143.9 (C_{quat}, Fmoc-Ar-C), 155.8 (C_{quat}, N(CO)O), 171.6 (C_{quat}, C=O), 171.8 (C_{quat}, C=O), 173.9 (C_{quat}, C=O). - MS FAB (MeOH/Glycerine) *m/z* (%) 506 (MH^+, 17), 319 (MH^+-Pro, 9), 213 (25), 179 (Fluorenyl cation, 100). - IR (KBr) 3414, 3308, 2978, 1715, 1637, 1449, 1248 cm^{-1}. - HR MS calcd for $C_{28}H_{31}N_3O_6$ + H 506.22911, found 506.2293.

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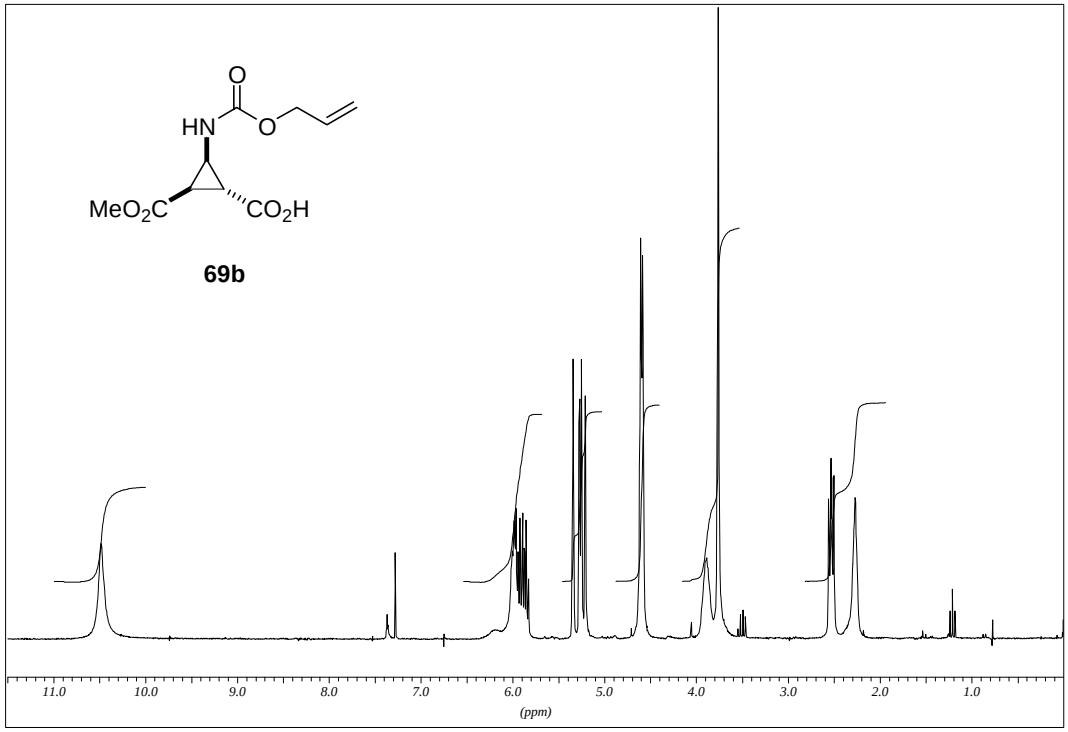
Appendix of NMR and X-Ray Data

NMR

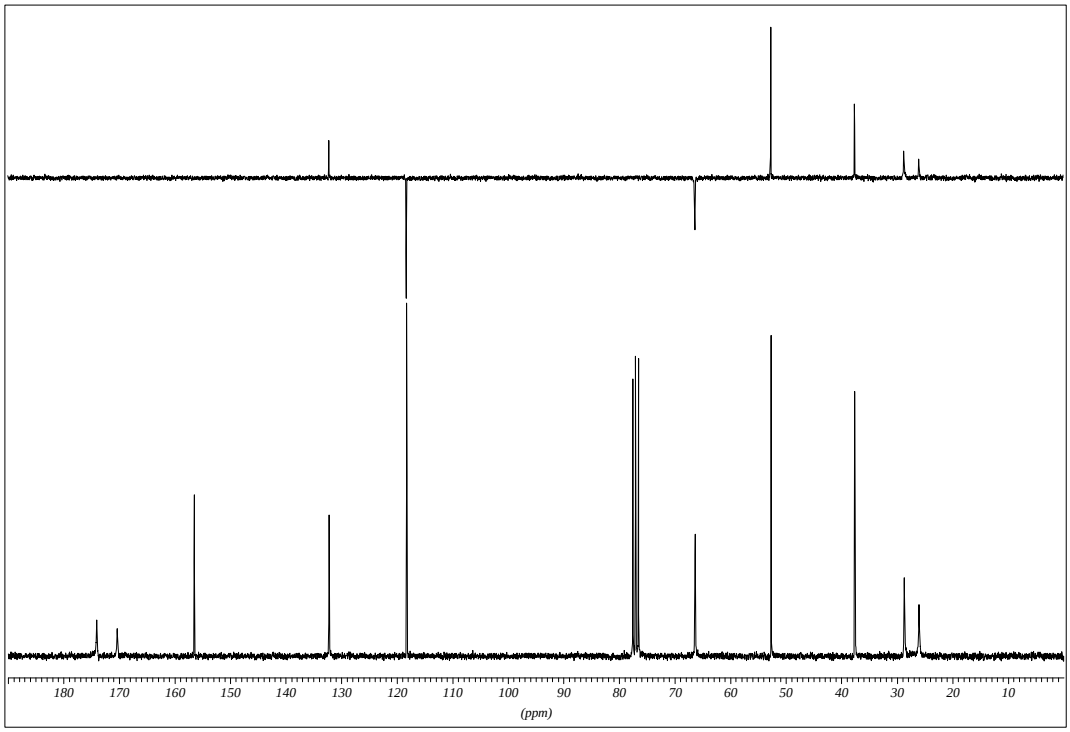
^{1}H-Spectra (top of the page)

^{13}C-Spectra (DEPT) (bottom of the page)

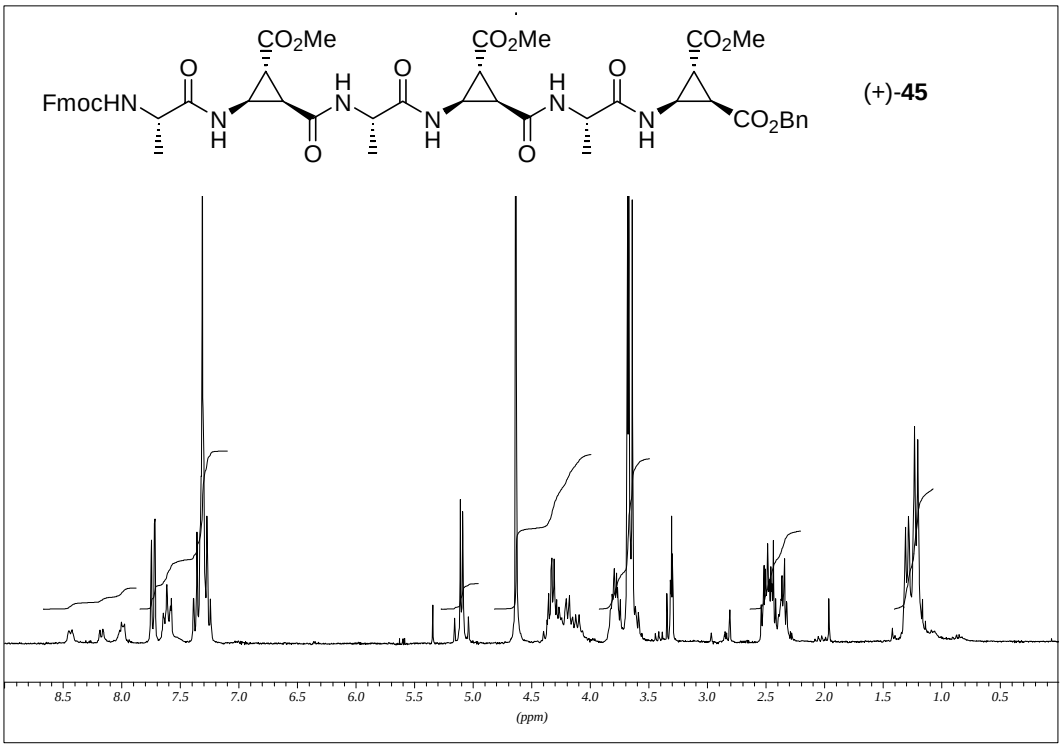
In some cases DQF-COSY, 80 ms TOCSY and 500 ms ROESY are given in this order after the ^{13}C spectrum.



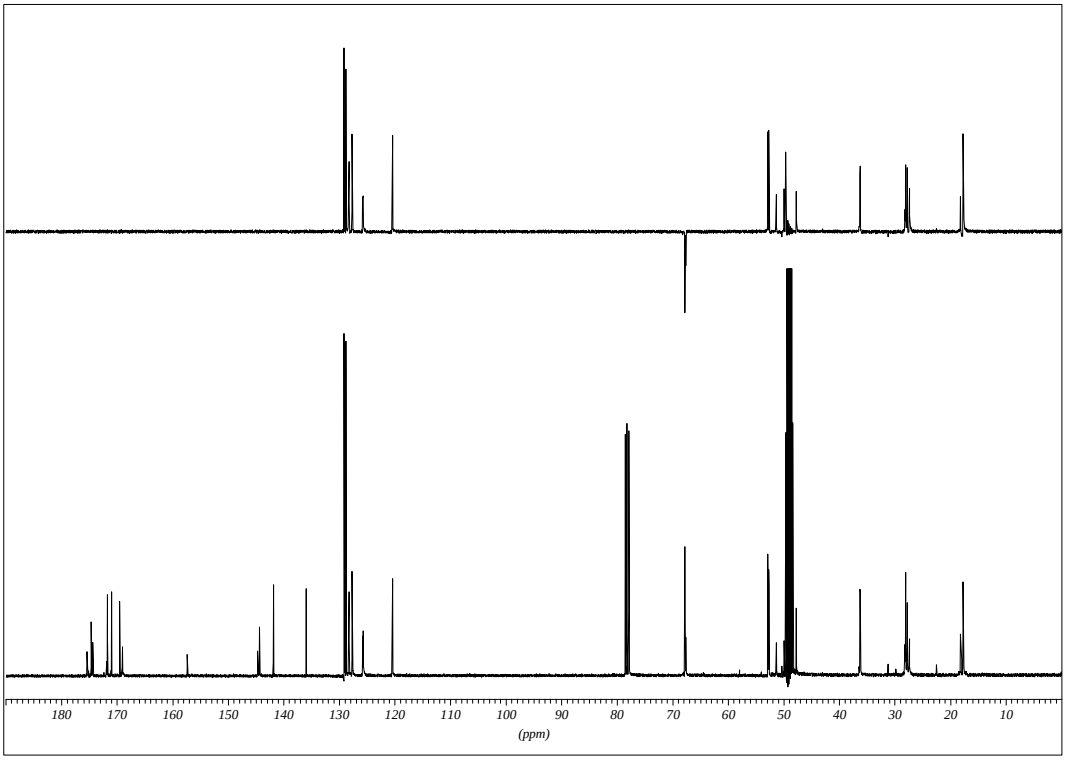
(1R*, 2R*, 3S*) 3-Allyloxycarbonyl-cyclopropane-1,2-dicarboxylic acid mono methyl ester, 250 MHz, $CDCl_3$.



(1R*, 2R*, 3S*) 3-Allyloxycarbonyl-cyclopropane-1,2-dicarboxylic acid mono methyl ester, 62.9 MHz, $CDCl_3$.



(1R, 2R, 3R) 3-{2S-[2R-(2S-{2R-[2S-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-propionylamino]-3R-methoxycarbonyl-cyclopropane-1R-carbonylamino}-propionylamino)-3R-methoxycarbonyl-cyclopropane-1R-carbonylamino]-propionylamino}-cyclopropane-1, 2-dicarboxylic acid 1-benzyl ester 2-methyl ester, 250 MHz, $CDCl_3/CD_3OD$ 1:1.



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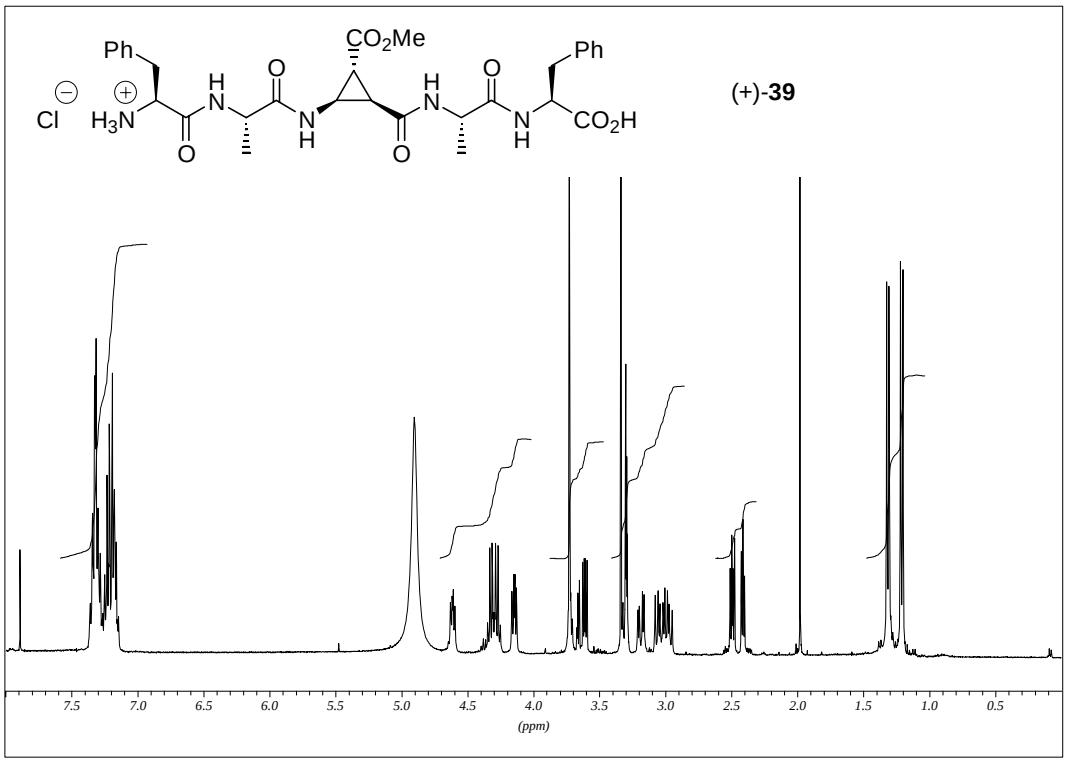
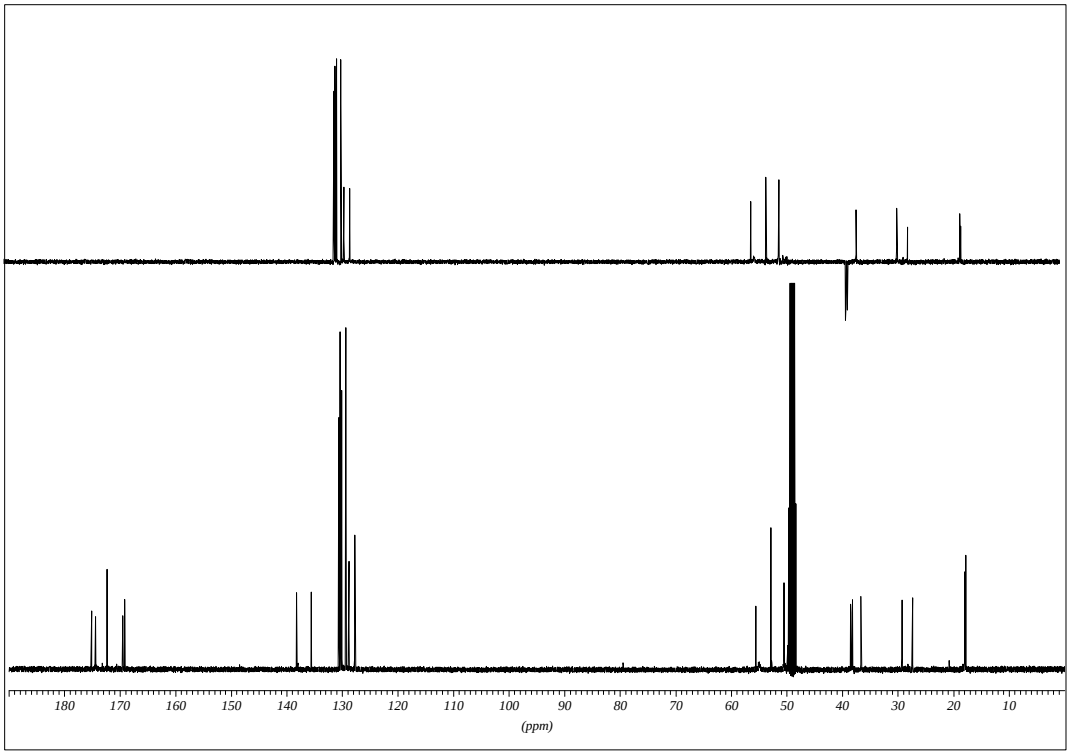


(1R, 2R, 3R) 2-[2S-(2S-Amino-3-phenyl-propionylamino)-propionylamino]-3-[1S-(1S-carboxy-2-phenyl-ethylcarbamoyl)-ethylcarbamoyl]-cyclopropane carboxylic acid methyl ester, HCl salt, 400 MHz, CD_3OD.



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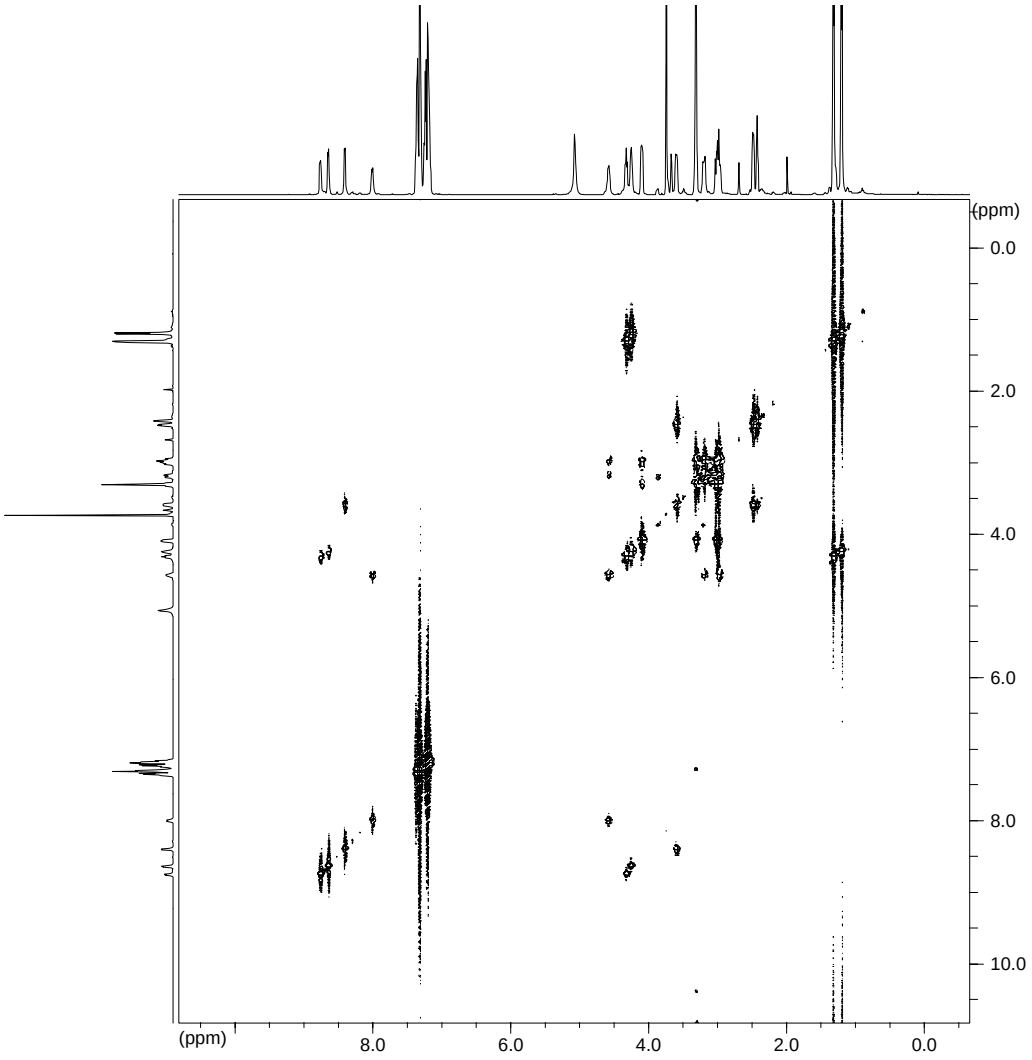
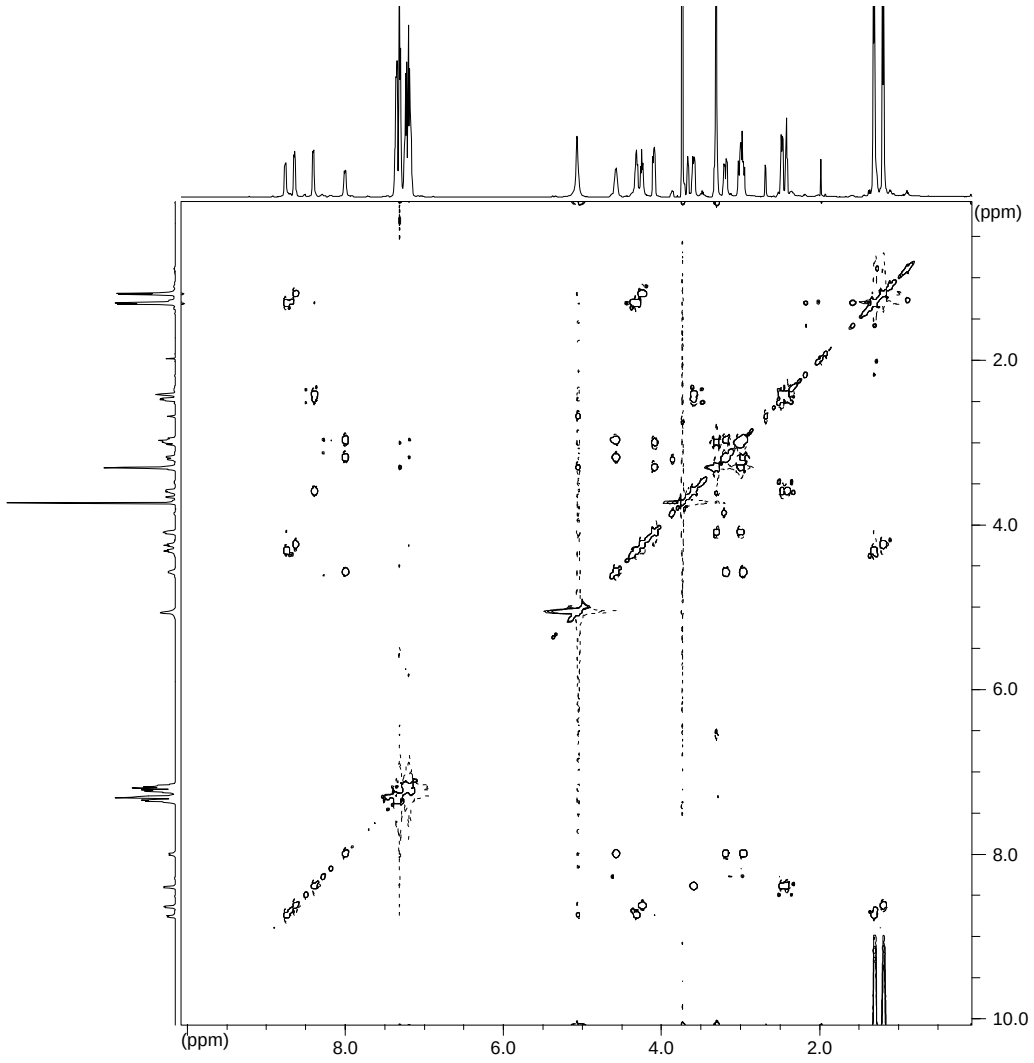


(1R, 2R, 3R) 2-[2S-(2S-Amino-3-phenyl-propionylamino)-propionylamino]-3-[1S-(1S-carboxy-2-phenyl-ethylcarbamoyl)-ethylcarbamoyl]-cyclopropane carboxylic acid methyl ester HCl salt. COSY: 500 MHz, 280 K, CD_3OH.



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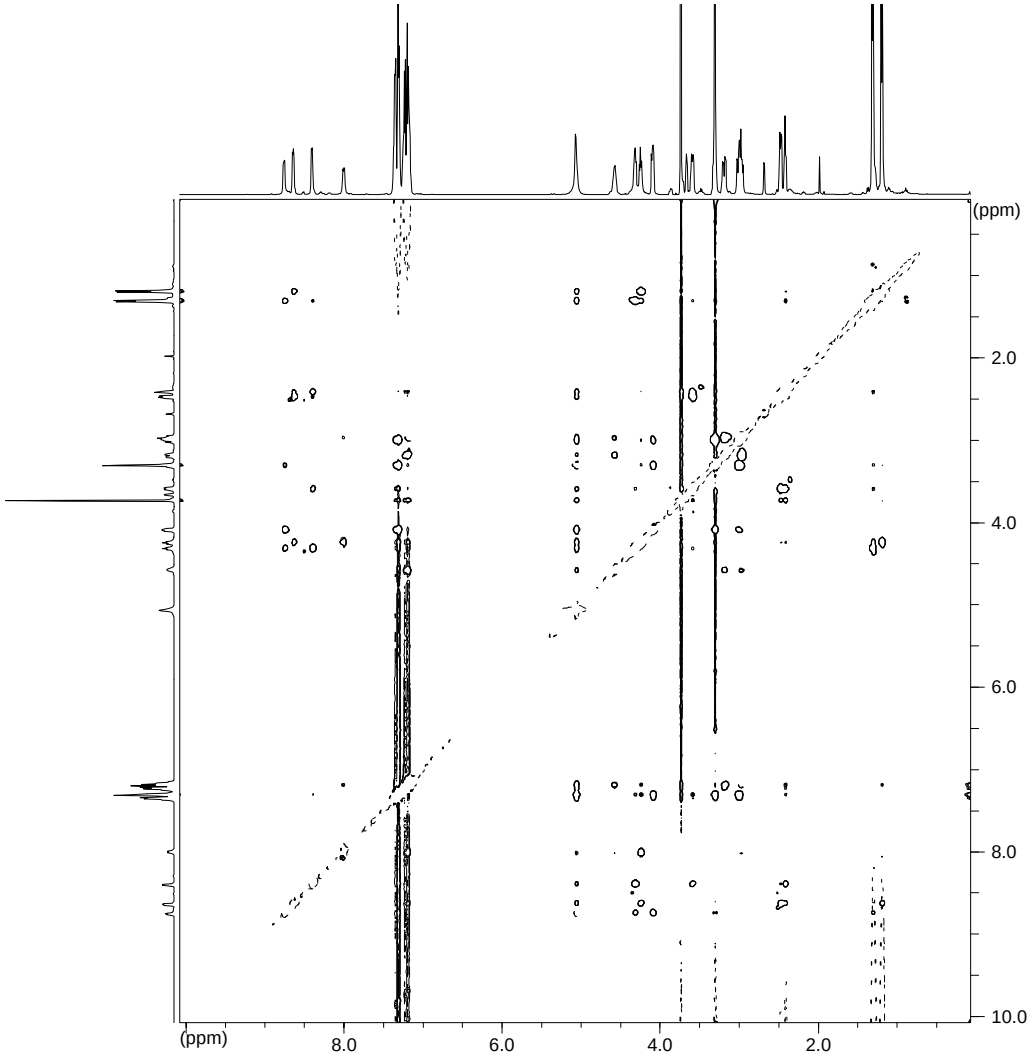
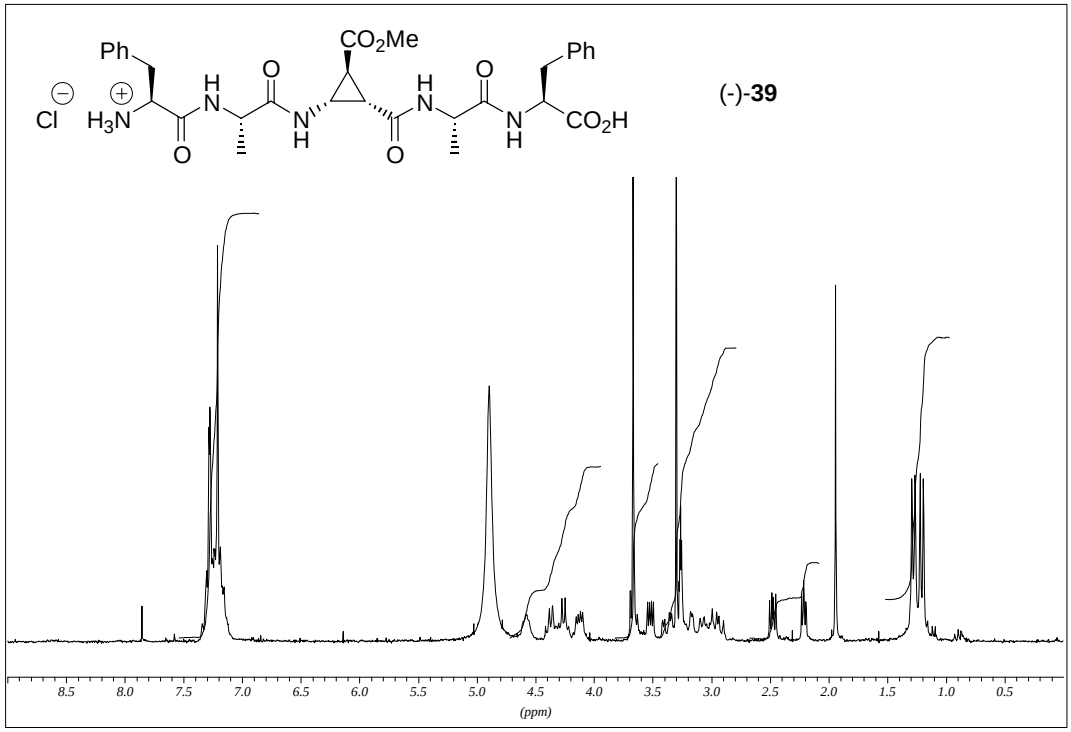


(1R, 2R, 3R) 2-[2S-(2S-Amino-3-phenyl-propionylamino)-propionylamino]-3-[1S-(1S-carboxy-2-phenyl-ethylcarbamoyl)-ethylcarbamoyl]-cyclopropane carboxylic acid methyl ester HCl salt. ROESY: 500 MHz, 500 ms spin lock, 280 K, CD_3OH.



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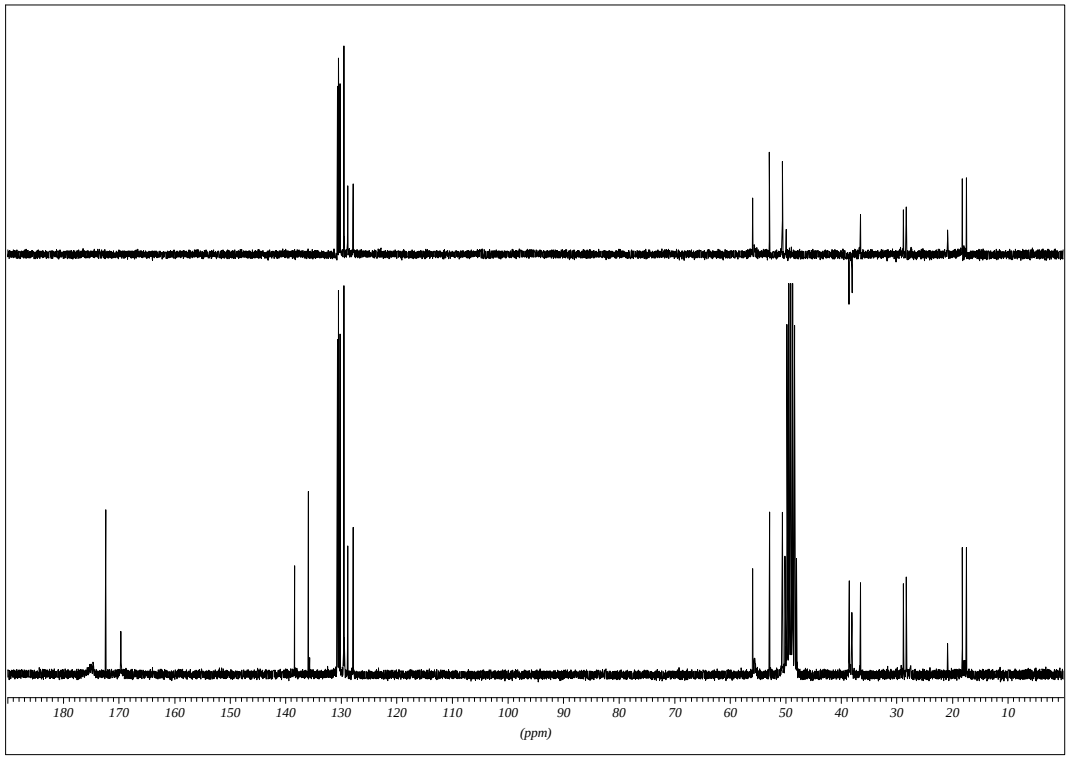

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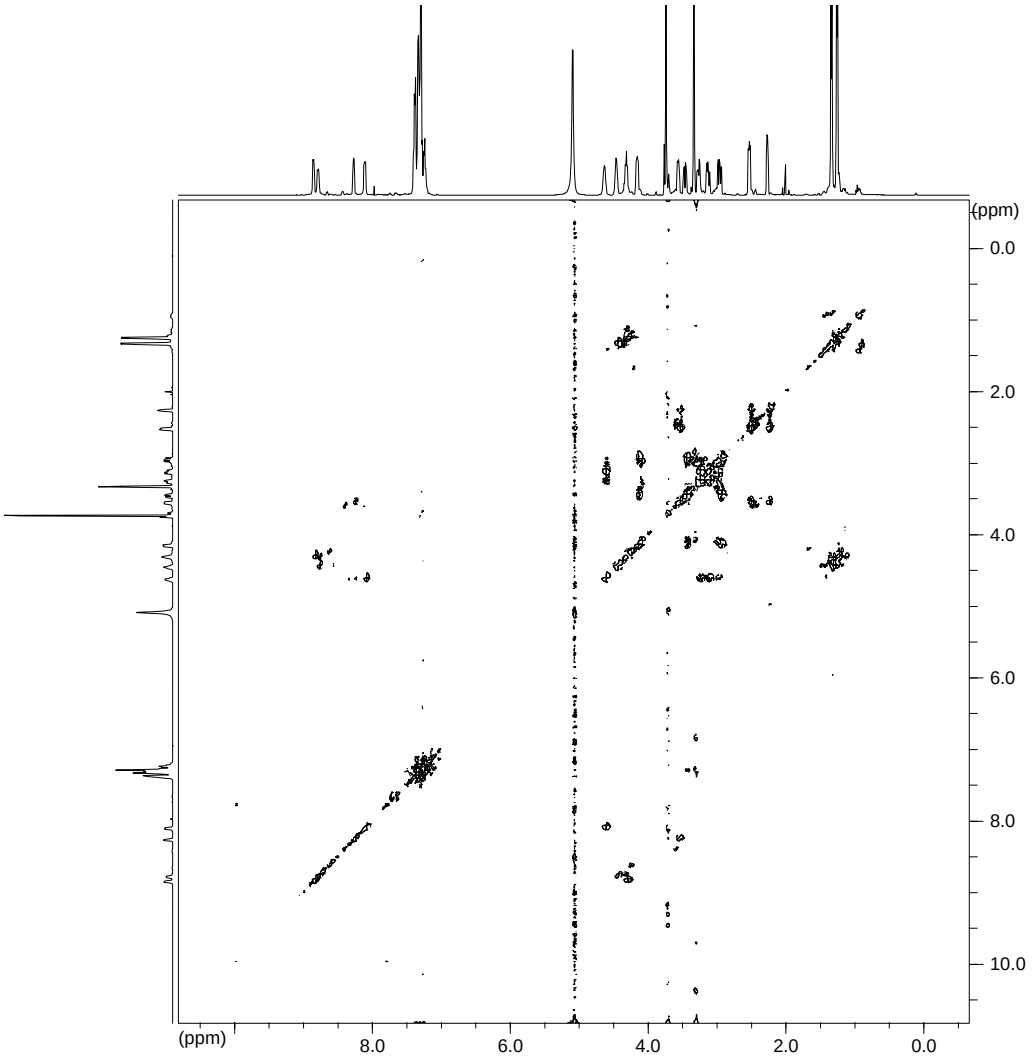

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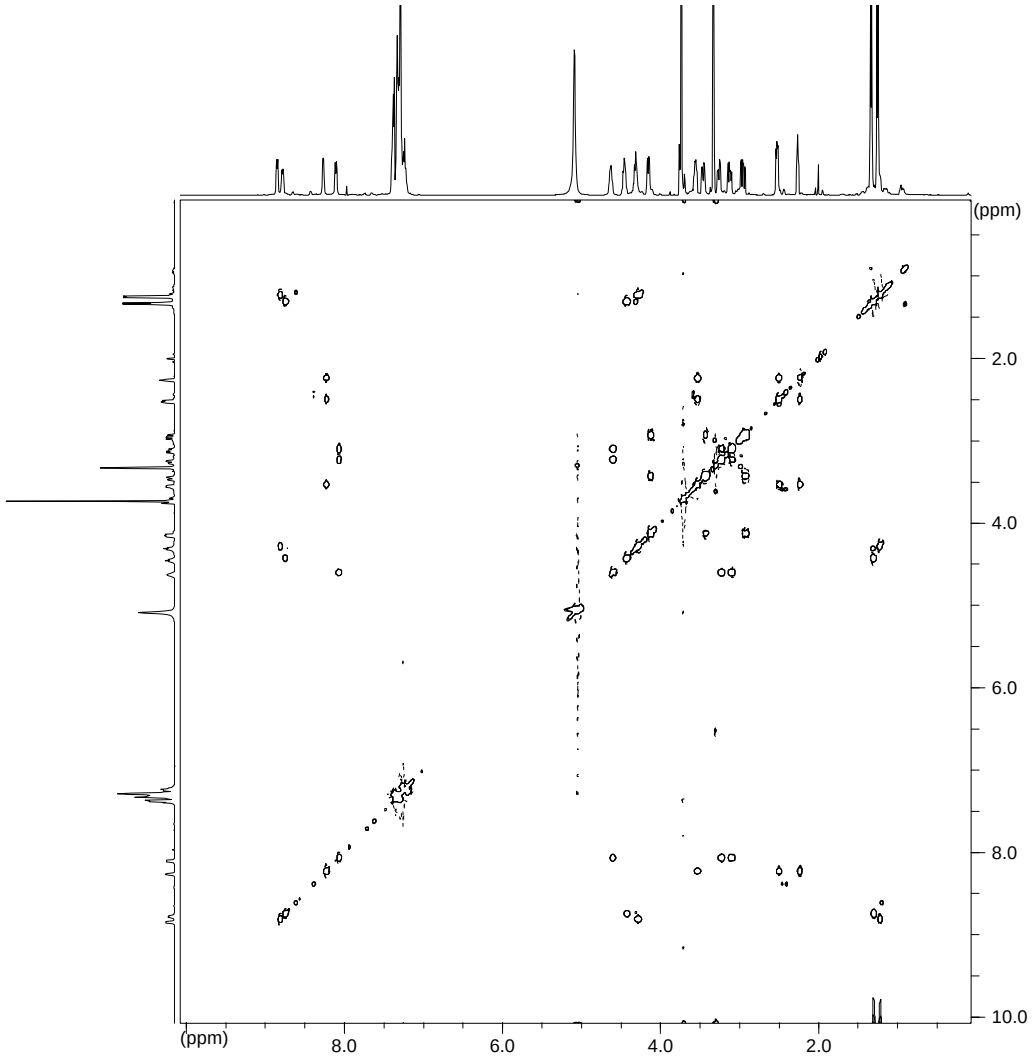

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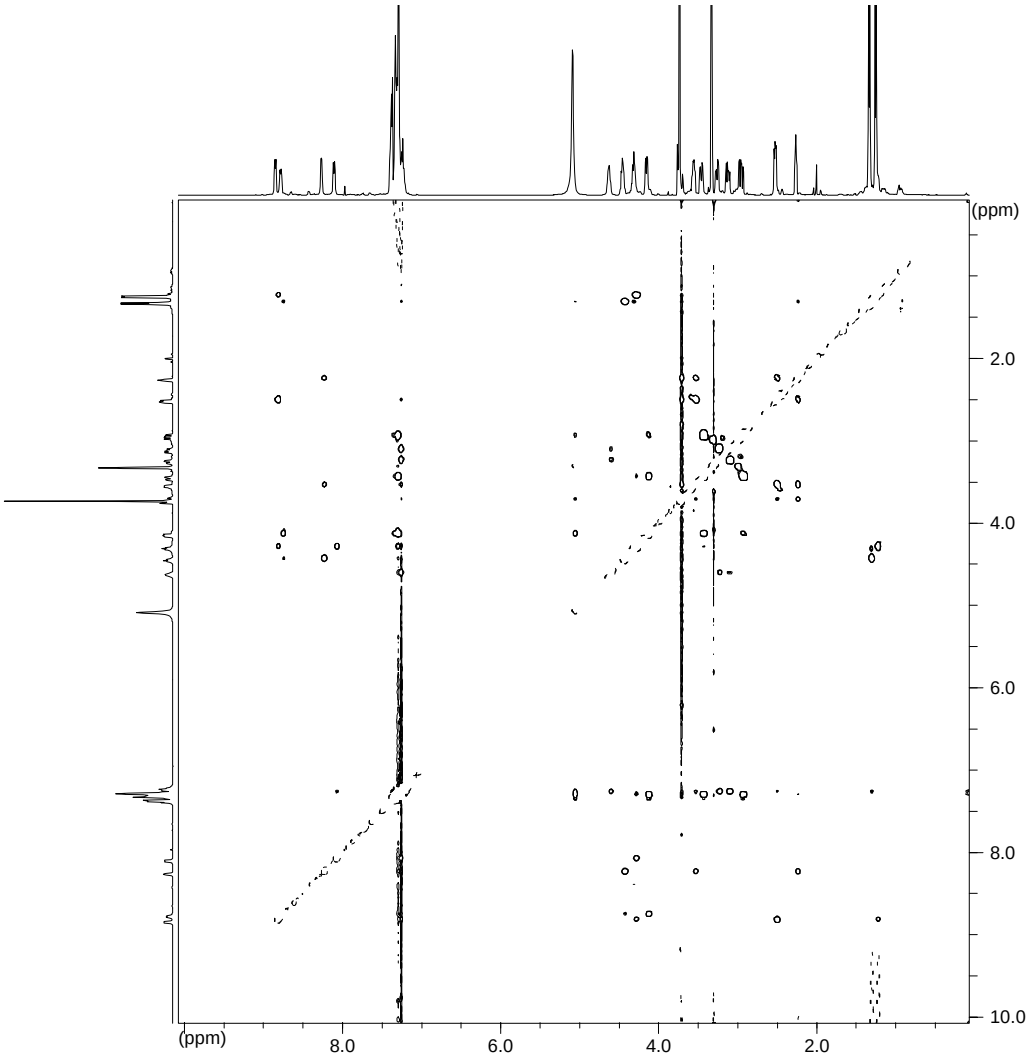

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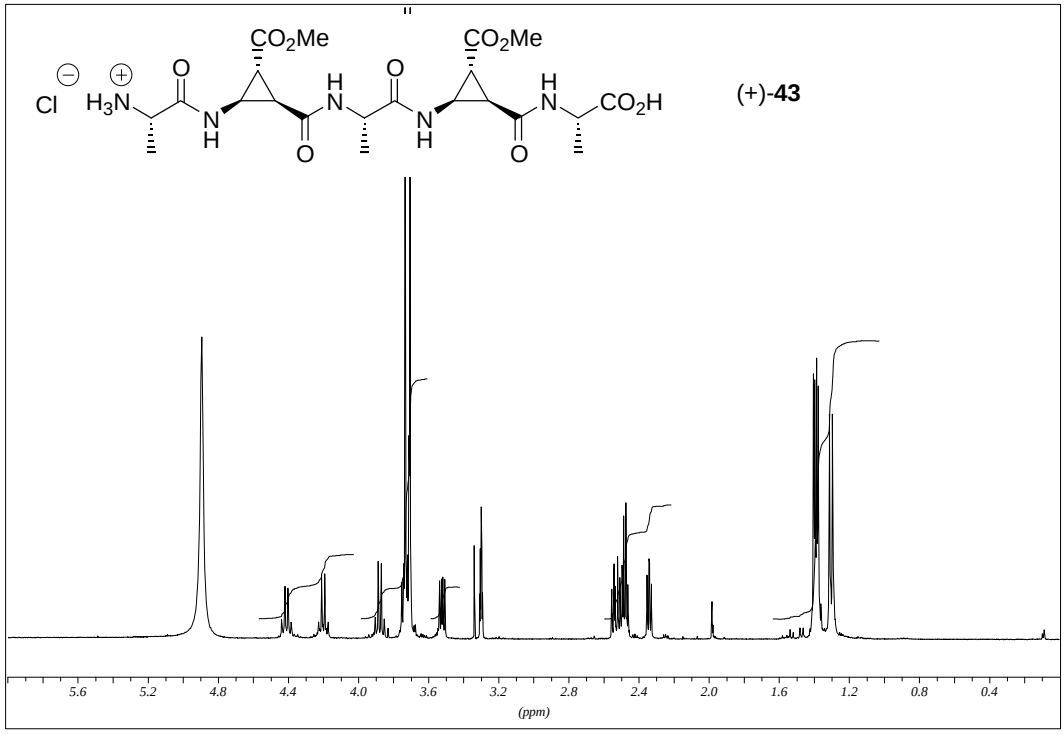
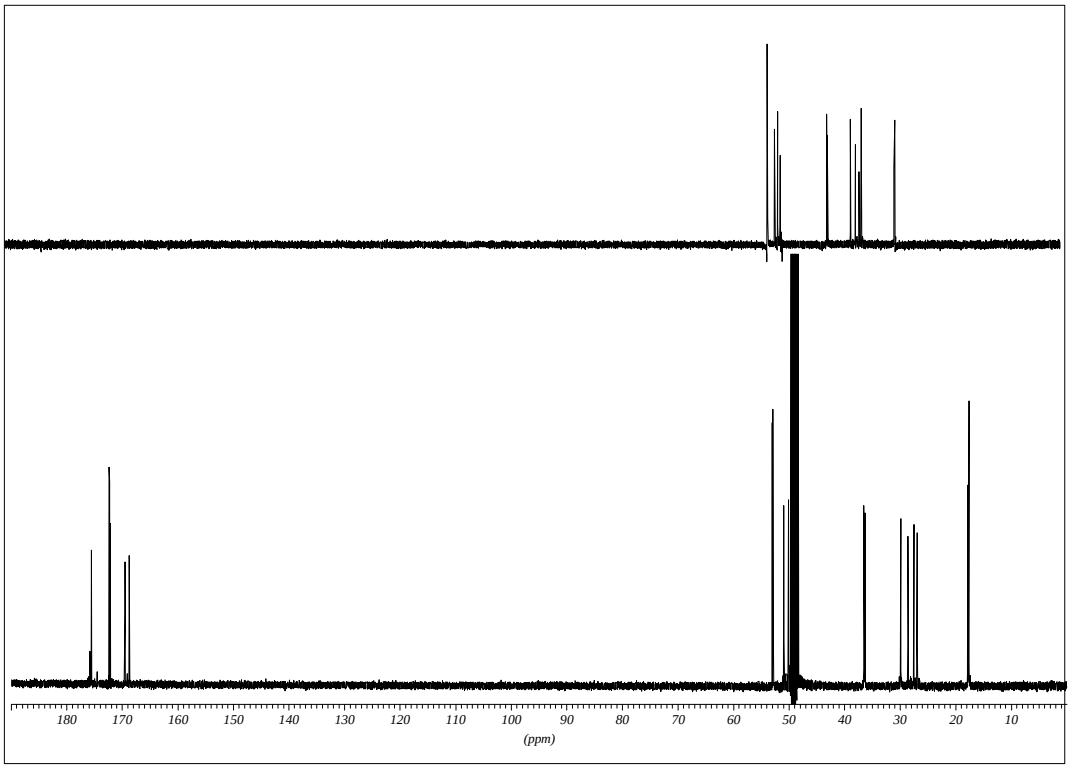


(1R, 2R, 3R) 2-(2S-Carboxyl-ethylcarbamoyl)-3-{2S-[2R-(2S-amino-propionylamino)-3R-methoxycarbonyl-cyclopropane-1R-carbonyl-amino]-propionylamino}-cyclopropane carboxylic acid methyl ester, HCl salt, 400 MHz, CD_3OD.



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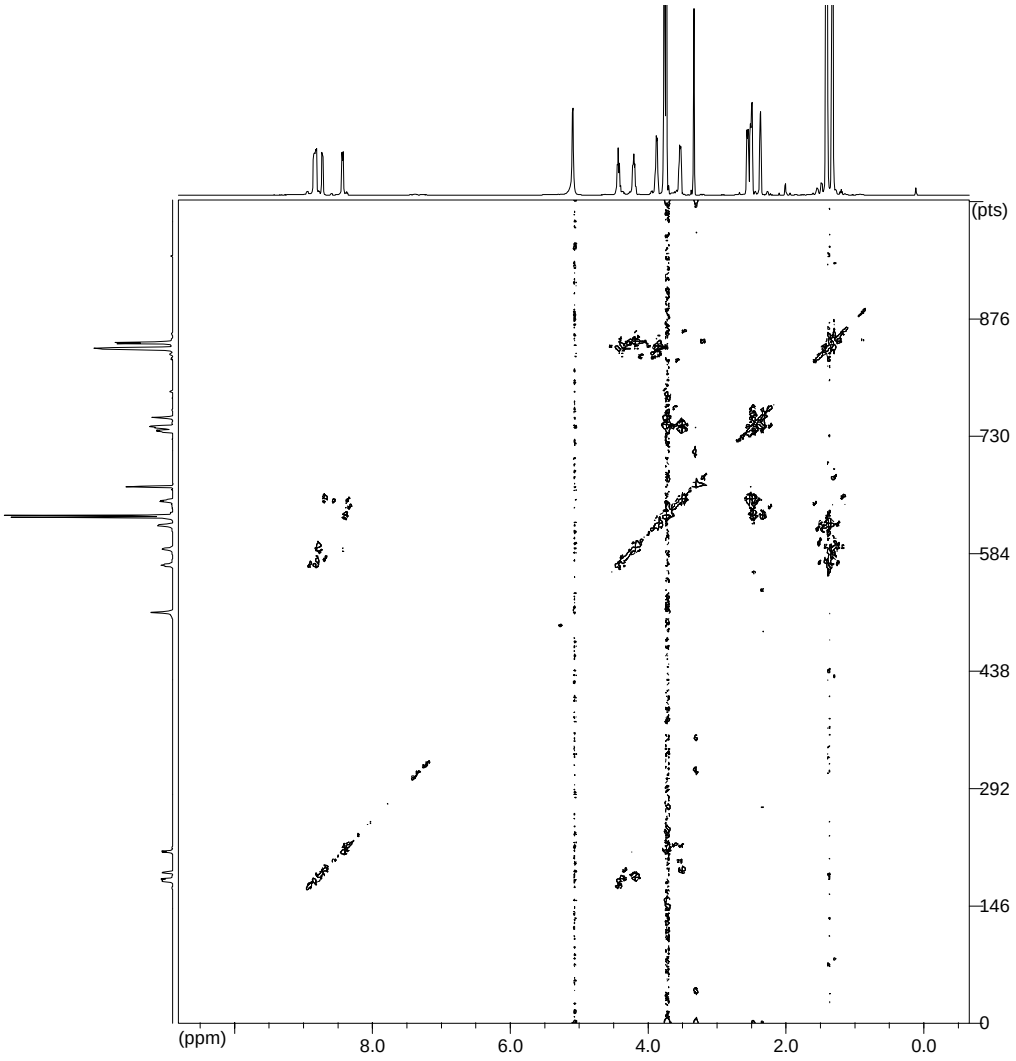

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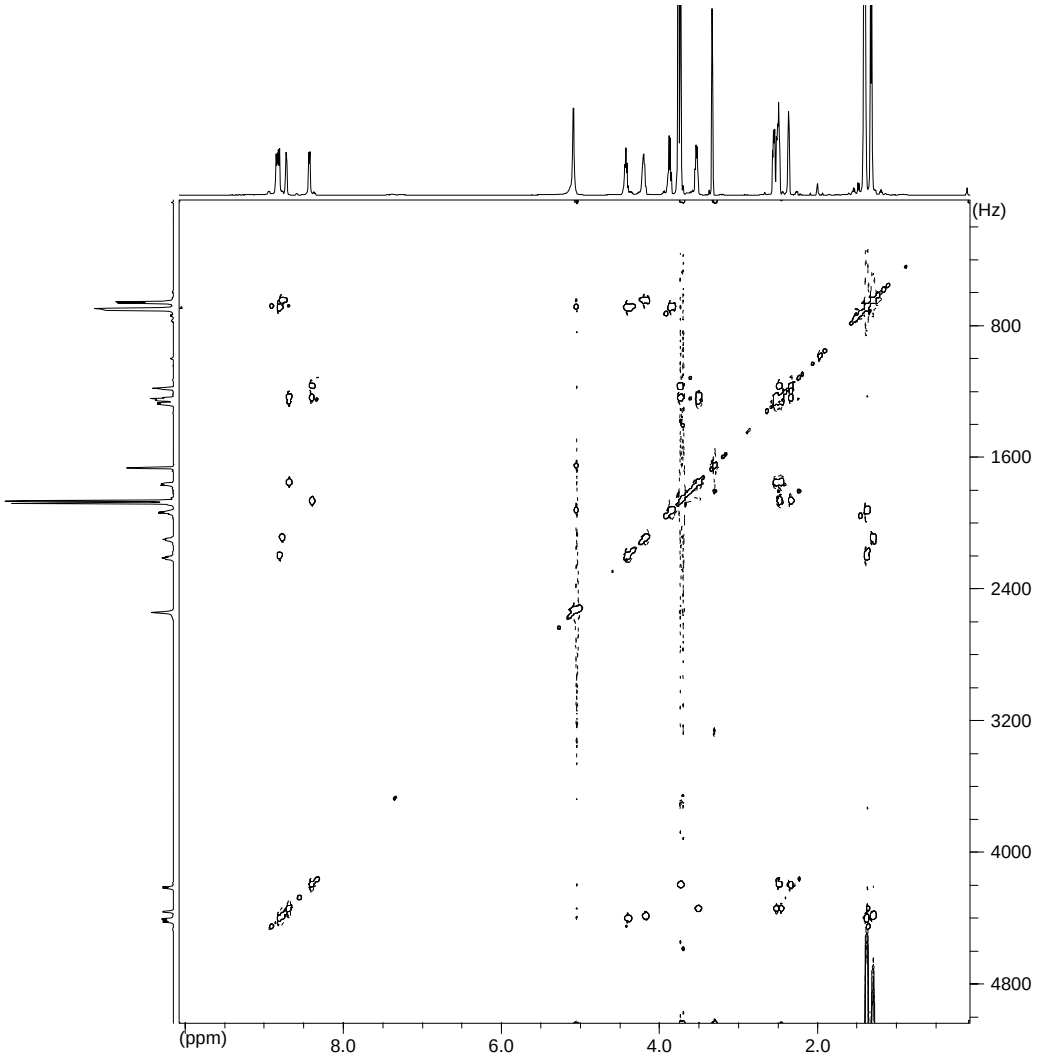

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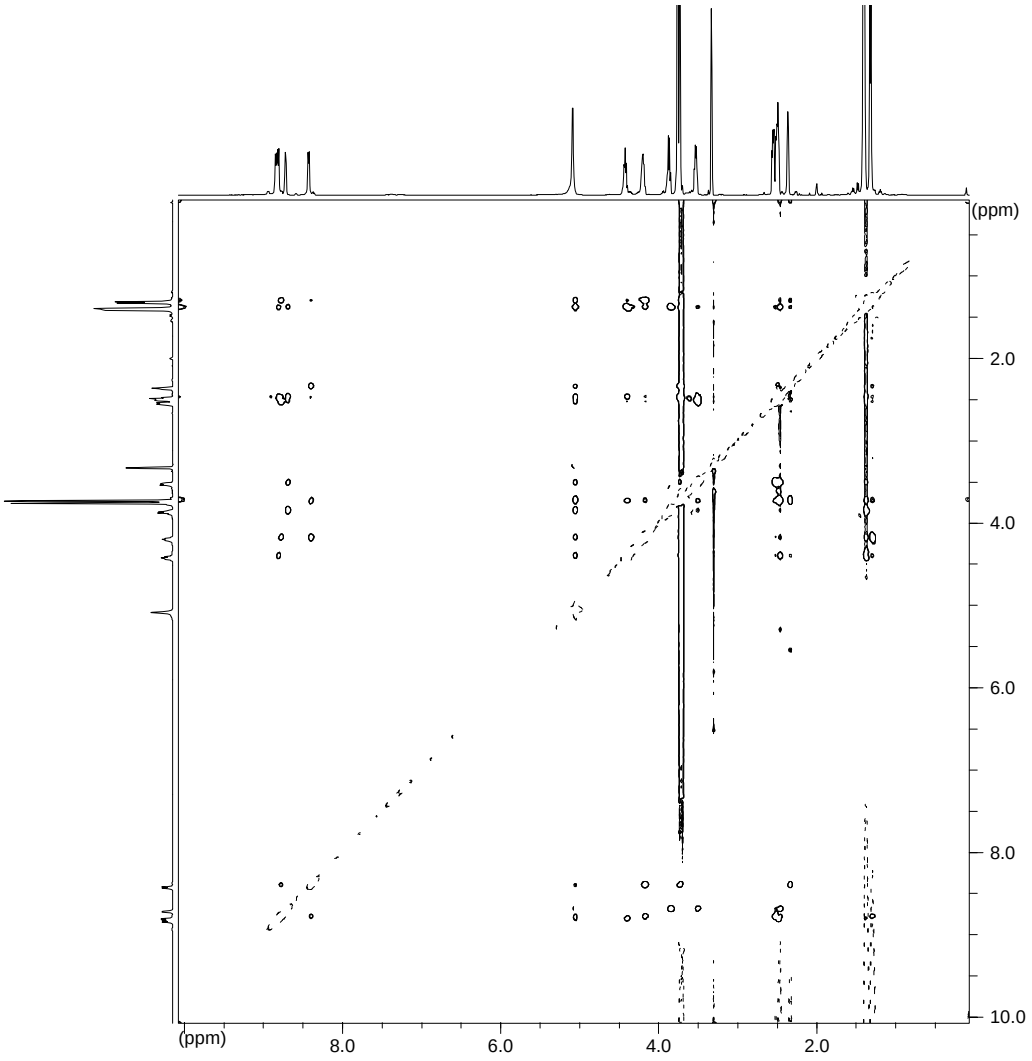

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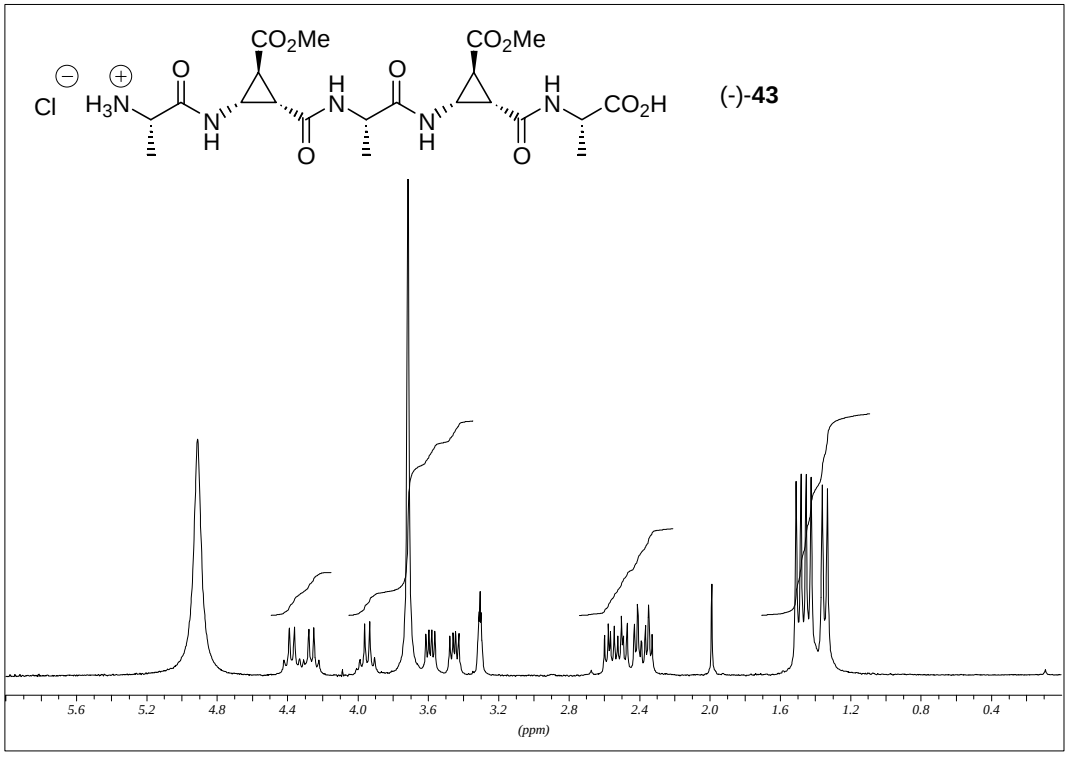

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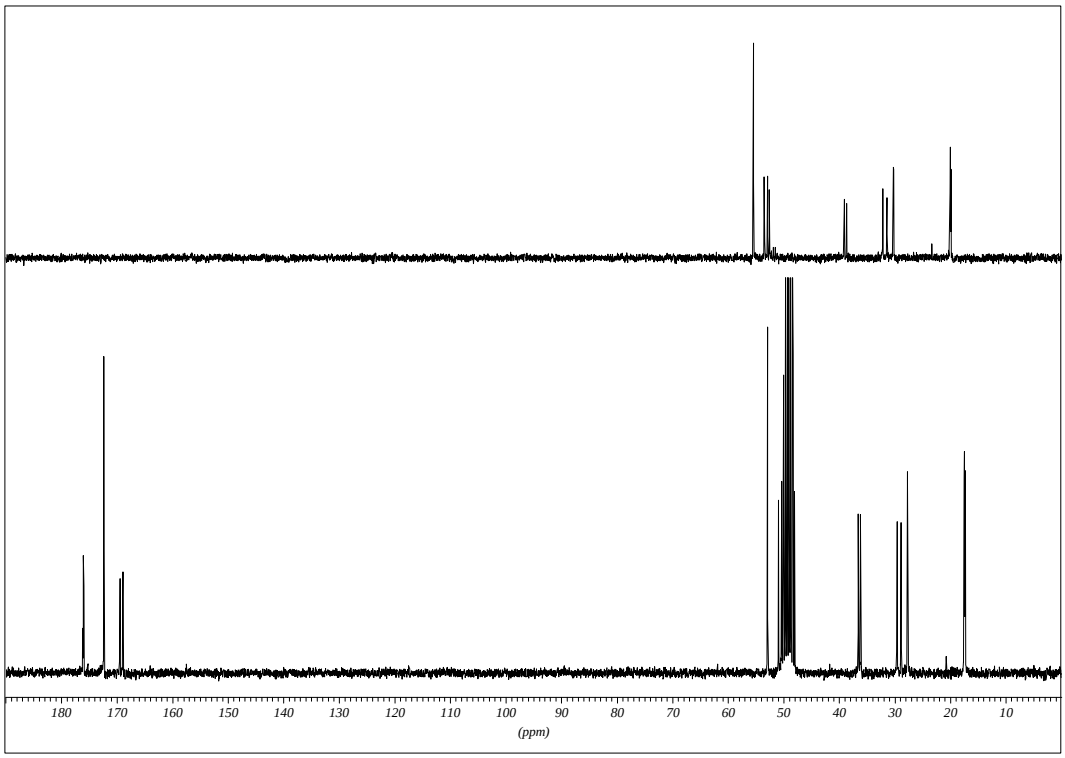

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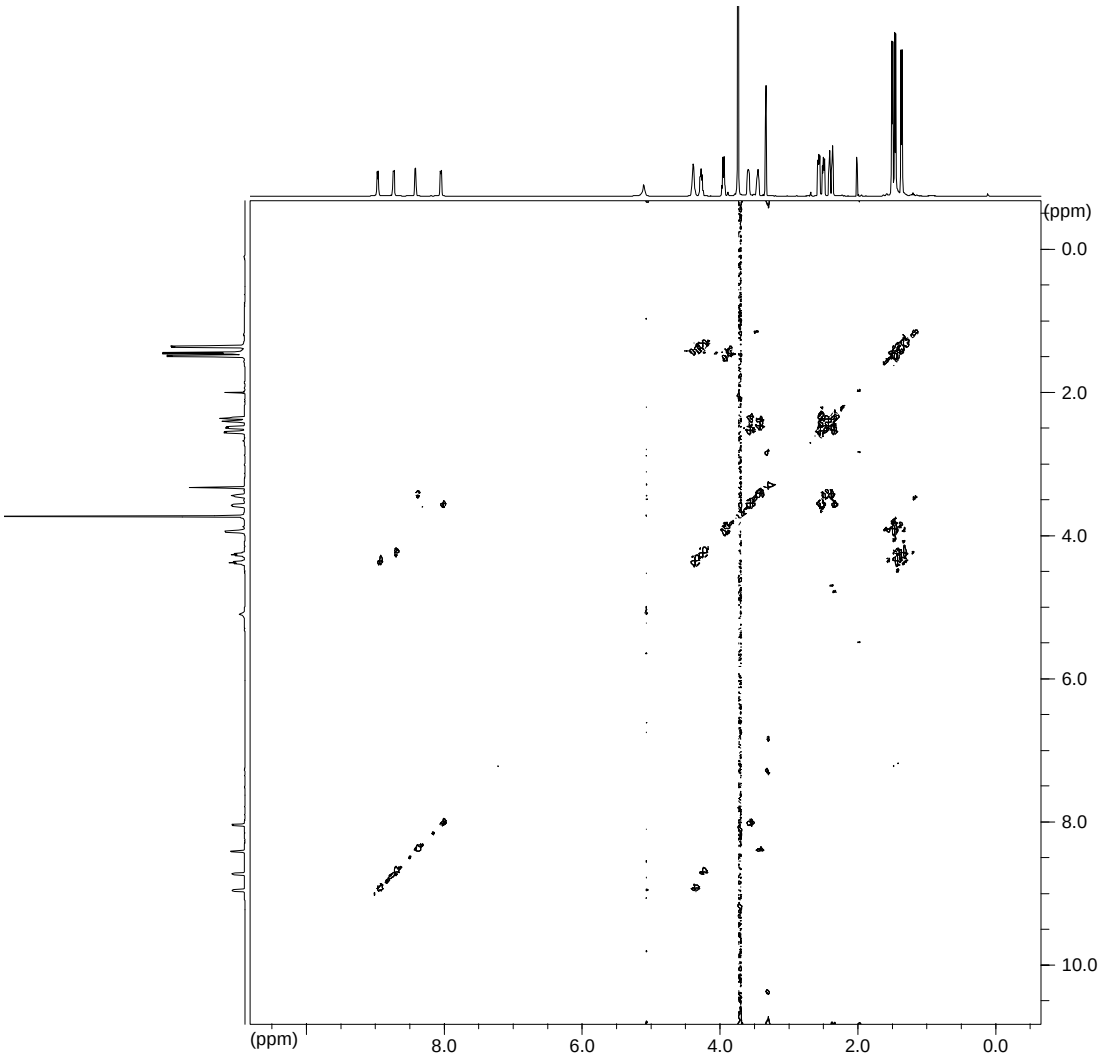

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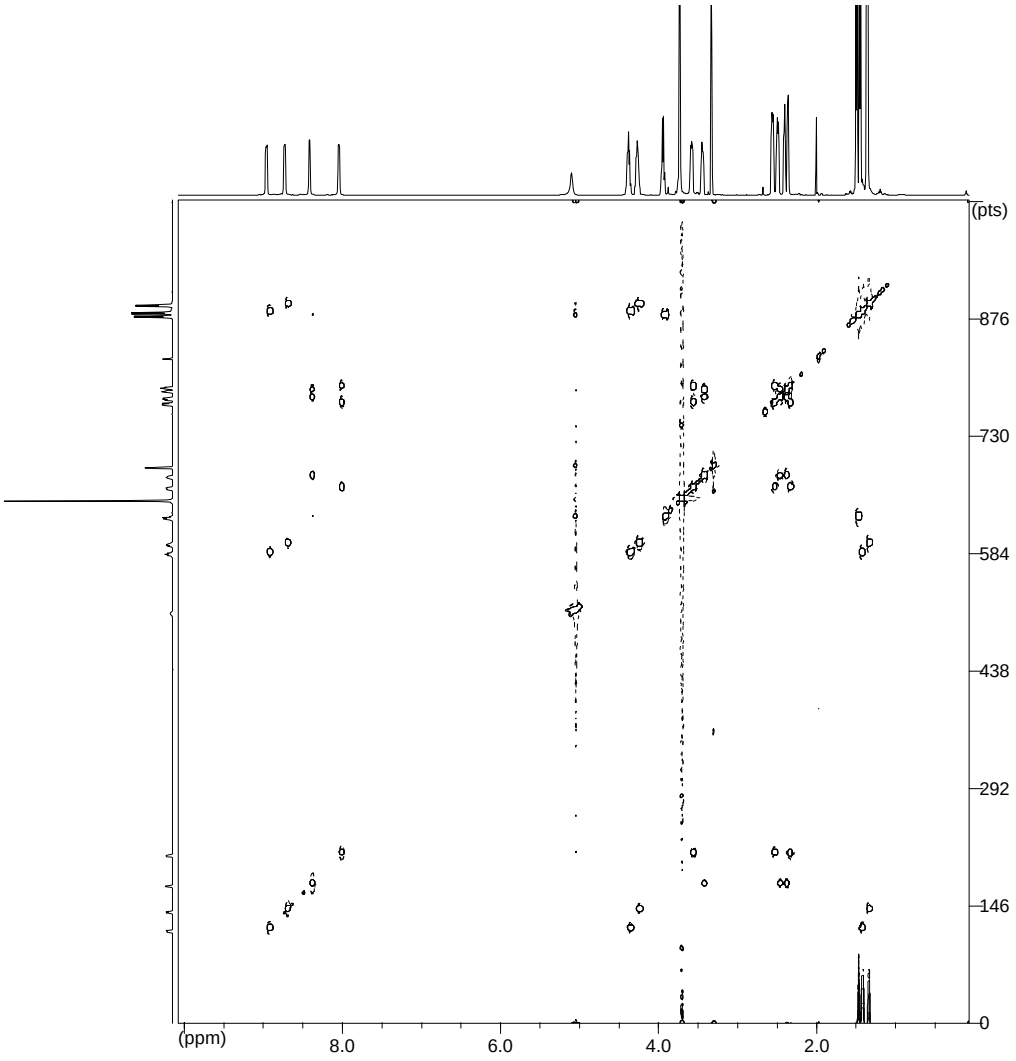

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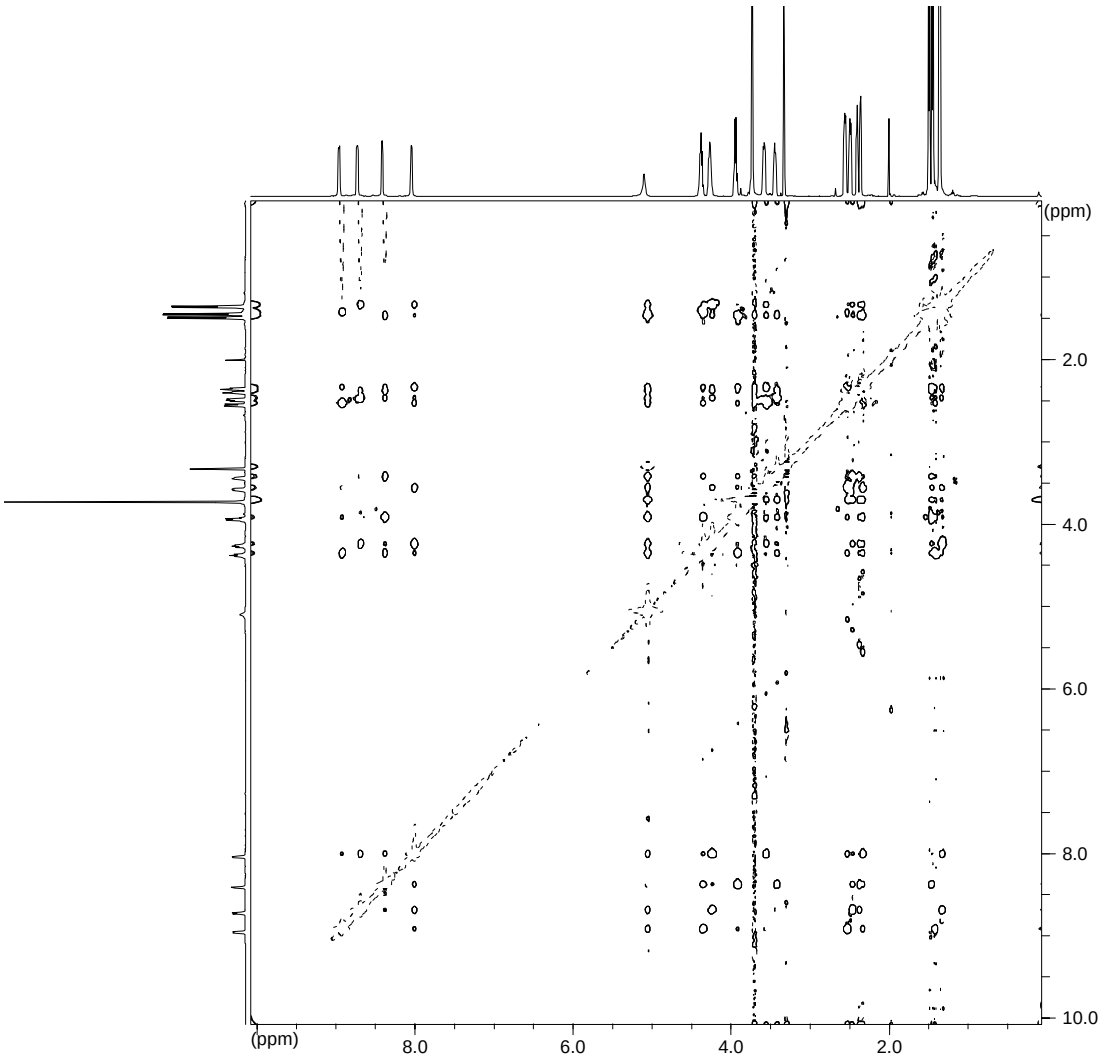

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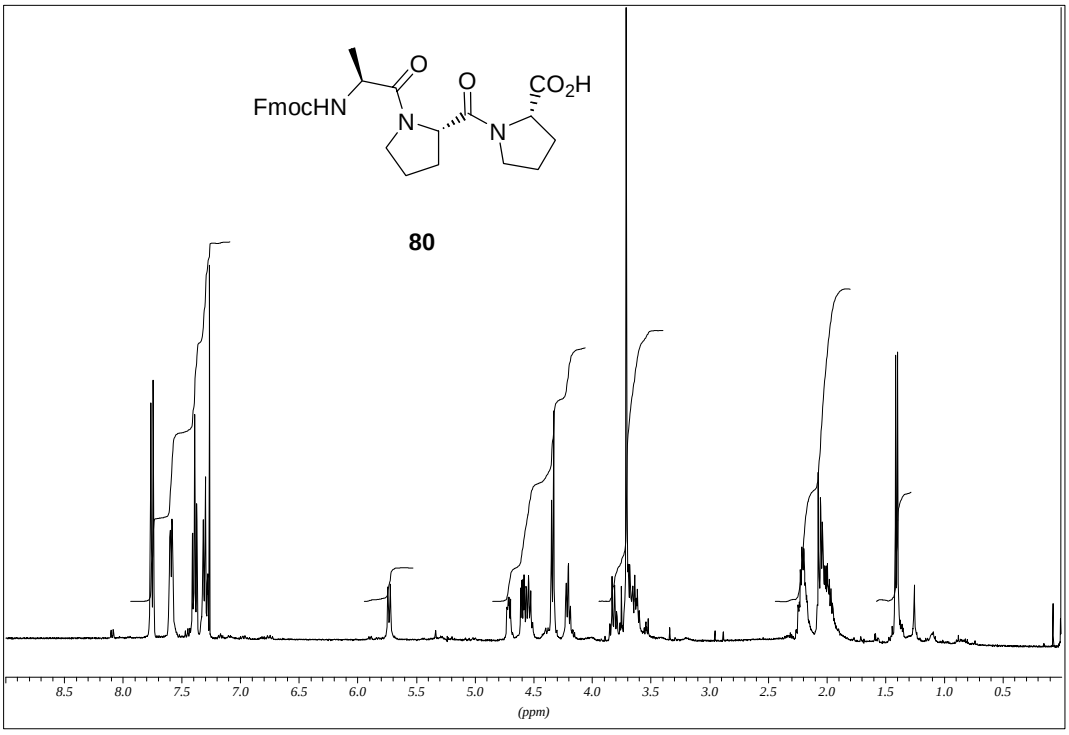
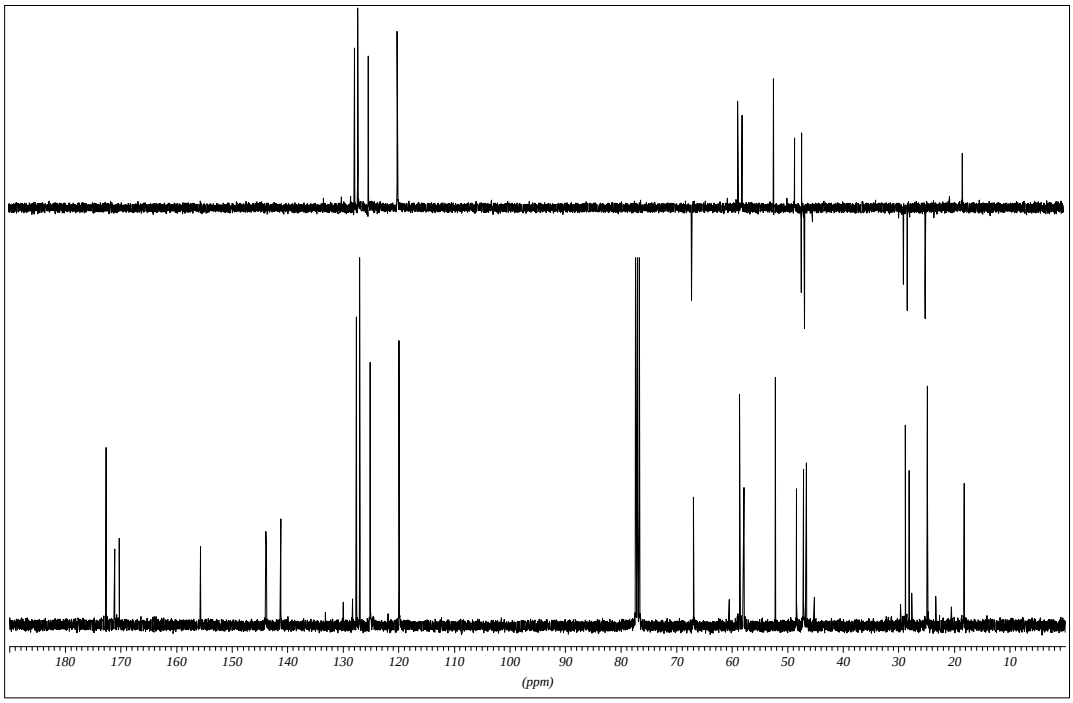


1-{1-[2S-(9*H*-Fluoren-9-ylmethoxycarbonyl-amino)-propionyl]-pyrrolidine-2S-carbonyl]-pyrrolidine-2S-carboxylic acid, 400 MHz, $CDCl_3$.



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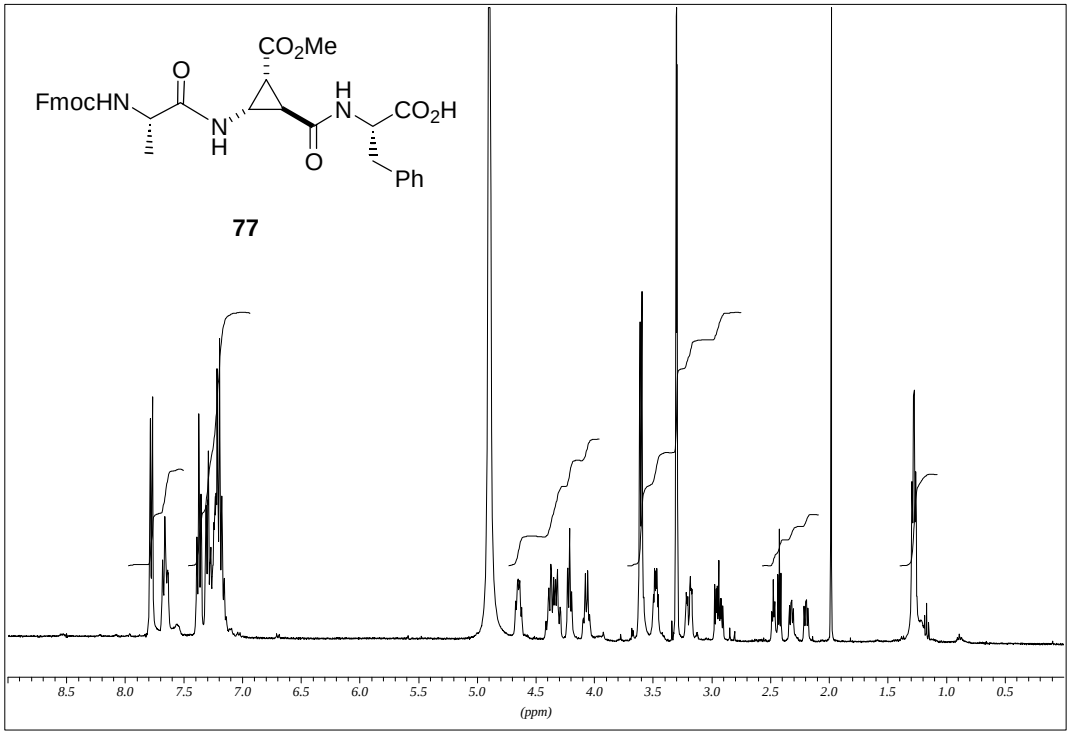
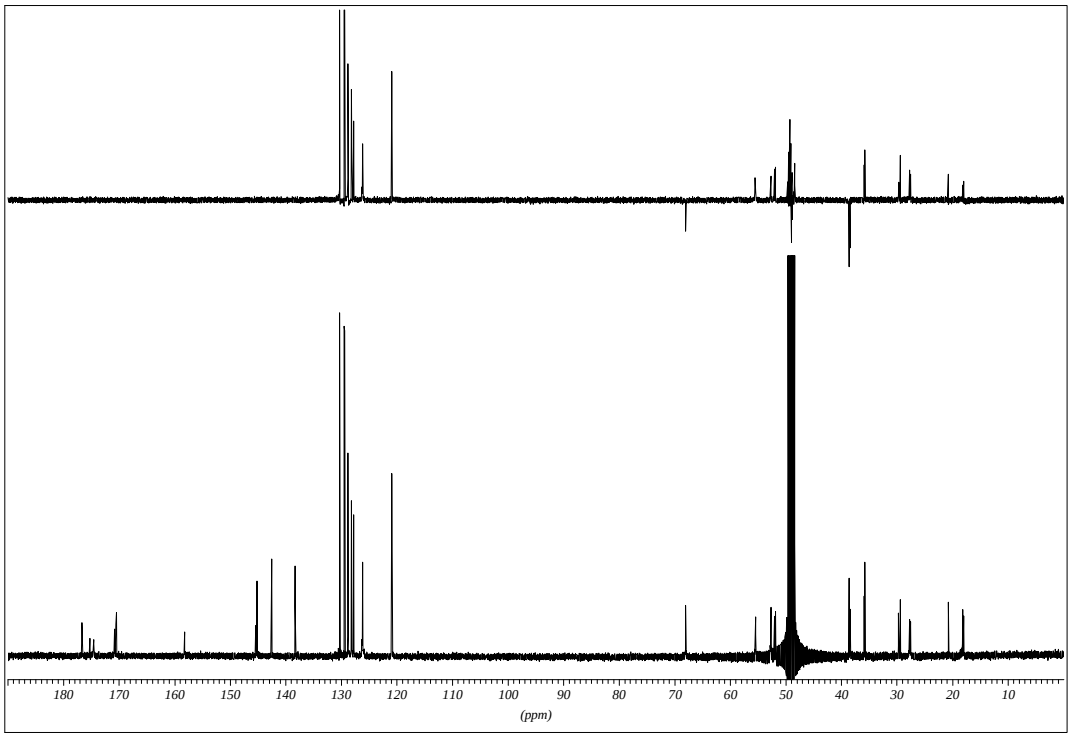


(1R*, 2R*, 3S*) 2-(1S-Carboxy-2-phenyl-ethylcarbamoyl)-3-[2S-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionylamino]-cyclopropane carboxylic acid methyl ester, 400 MHz, CD_3OD.



(1R*, 2R*, 3S*) 2-(1S-Carboxy-2-phenyl-ethylcarbamoyl)-3-[2S-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionylamino]-cyclopropane carboxylic acid methyl ester, 100.6 MHz, CD_3OD.

X-ray data of compound (±)-34

(1R*, 2R*, 3R*) 2-acetylamino-3-diethyl-carbamoyl-cyclopropane carboxylic acid methyl ester ((±)-34)

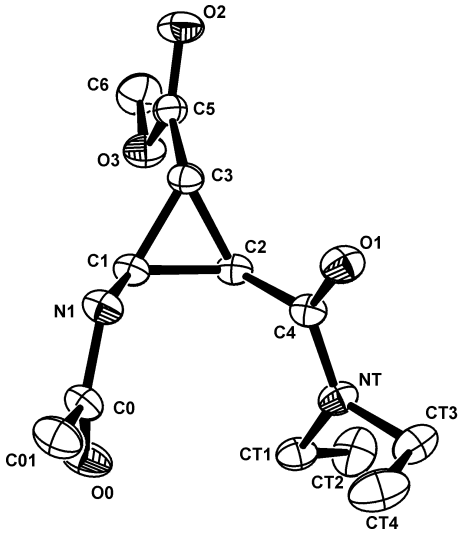


Table 1. Crystal data and structure refinement for **(±)-34**.

Identification code	mc11b
Empirical formula	C_{12} H_{20} N_2 O_4
Formula weight	256.30
Temperature	293(2) K
Wavelength	1.54178 Å
Crystal system, space group	Monoclinic, P2(1)/c
Unit cell dimensions	a = 8.873(3) Å alpha = 90 deg.
	b = 14.219(4) Å beta = 93.28(5) deg.
	c = 10.839(4) Å gamma = 90 deg.
Volume	1365.3(8) $Å^3$
Z, Calculated density	4, 1.247 Mg/m^3
Absorption coefficient	0.778 mm^{-1}
F(000)	552
Crystal size	0.60 x 0.60 x 0.40 mm
Theta range for data collection	5.14 to 59.99 deg.
Limiting indices	-9<=h<=9, -1<=k<=15, 0<=l<=12
Reflections collected / unique	2323 / 2013 [R(int) = 0.0289]

Completeness to theta = 59.99	99.7 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2013 / 0 / 164
Goodness-of-fit on F^2	1.097
Final R indices [I>2sigma(I)]	R1 = 0.0582, wR2 = 0.1552
R indices (all data)	R1 = 0.0595, wR2 = 0.1569
Extinction coefficient	0.074(5)
Largest diff. peak and hole	0.258 and -0.319 e. $Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for **(±)-34**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U (eq)
C01	5453(3)	5423(2)	3276(2)	65(1)
C0	5132(2)	4426(1)	2888(2)	48(1)
O0	5526(2)	3757(1)	3531(1)	68(1)
N1	4385(2)	4323(1)	1789(1)	47(1)
C1	4071(2)	3416(1)	1261(2)	43(1)
C2	5225(2)	2967(1)	484(2)	42(1)
C3	3806(2)	3391(1)	-120(2)	44(1)
C4	6690(2)	3454(1)	329(2)	43(1)
O1	6799(2)	4105(1)	-414(1)	58(1)
NT	7878(2)	3154(1)	1029(2)	49(1)
CT1	7799(2)	2435(1)	1994(2)	55(1)
CT2	8357(3)	1490(2)	1589(3)	84(1)
CT3	9340(2)	3607(2)	893(2)	64(1)
CT4	9478(3)	4536(2)	1557(3)	84(1)
C5	2671(2)	2757(1)	-726(2)	49(1)
O2	2005(2)	2922(1)	-1698(1)	71(1)
O3	2479(2)	1978(1)	-73(1)	61(1)
C6	1515(3)	1278(2)	-660(3)	80(1)

Table 3. Bond lengths [Å] and angles [deg] for **(±)-34**.

C01-C0	1.500(3)	N1-C1-C3	115.82(15)
C0-O0	1.219(2)	N1-C1-C2	118.88(16)
C0-N1	1.338(3)	C3-C1-C2	60.32(12)
N1-C1	1.432(2)	C4-C2-C1	119.45(15)
C1-C3	1.502(3)	C4-C2-C3	118.45(15)
C1-C2	1.505(3)	C1-C2-C3	59.75(12)
C2-C4	1.491(3)	C5-C3-C1	121.42(17)
C2-C3	1.511(2)	C5-C3-C2	118.62(16)
C3-C5	1.478(3)	C1-C3-C2	59.92(12)
C4-O1	1.235(2)	O1-C4-NT	121.52(17)
C4-NT	1.333(2)	O1-C4-C2	121.53(16)
NT-CT3	1.464(3)	NT-C4-C2	116.95(15)
NT-CT1	1.466(2)	C4-NT-CT3	118.57(16)
CT1-CT2	1.506(3)	C4-NT-CT1	124.12(16)
CT3-CT4	1.506(4)	CT3-NT-CT1	117.19(16)
C5-O2	1.201(2)	NT-CT1-CT2	112.60(19)
C5-O3	1.331(3)	NT-CT3-CT4	112.7(2)
O3-C6	1.437(3)	O2-C5-O3	123.91(18)
O0-C0-N1	122.35(18)	O2-C5-C3	123.99(19)
O0-C0-C01	122.24(17)	O3-C5-C3	112.10(15)
N1-C0-C01	115.41(17)	C5-O3-C6	115.62(17)
C0-N1-C1	121.98(15)		

Table 4. Anisotropic displacement parameters ($Å^2$ x 10^3) for **(±)-34**. The anisotropic displacement factor exponent takes the form: $-2\ pi^2\ [\ h^2\ a^{*2}\ U11 + ... + 2\ h\ k\ a^*\ b^*\ U_{12}]$

U11	U22	U33	U23	U13	U12	
C01	95(2)	47(1)	54(1)	-9(1)	-5(1)	-1(1)
C0	65(1)	41(1)	39(1)	-1(1)	1(1)	4(1)
O0	106(1)	48(1)	48(1)	5(1)	-16(1)	7(1)
N1	67(1)	32(1)	42(1)	1(1)	-4(1)	5(1)
C1	54(1)	34(1)	41(1)	2(1)	-3(1)	1(1)
C2	51(1)	31(1)	44(1)	1(1)	-6(1)	3(1)
C3	50(1)	40(1)	42(1)	1(1)	-4(1)	4(1)
C4	52(1)	31(1)	45(1)	2(1)	-3(1)	4(1)
O1	60(1)	48(1)	65(1)	21(1)	-5(1)	-1(1)
NT	47(1)	39(1)	59(1)	10(1)	-7(1)	0(1)
CT1	58(1)	48(1)	58(1)	13(1)	-10(1)	3(1)
CT2	87(2)	51(1)	114(2)	27(1)	13(2)	21(1)
CT3	49(1)	56(1)	85(2)	14(1)	-9(1)	-3(1)
CT4	81(2)	57(1)	110(2)	10(1)	-31(2)	-17(1)
C5	47(1)	54(1)	45(1)	-6(1)	-3(1)	7(1)
O2	71(1)	83(1)	56(1)	-4(1)	-23(1)	1(1)
O3	62(1)	54(1)	64(1)	-3(1)	-13(1)	-13(1)
C6	71(2)	74(2)	93(2)	-17(1)	-9(1)	-25(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for **(±)-34**.

	U11	U22	U33	U23	U13	U12
C01	95(2)	47(1)	54(1)	-9(1)	-5(1)	-1(1)
C0	65(1)	41(1)	39(1)	-1(1)	1(1)	4(1)
O0	106(1)	48(1)	48(1)	5(1)	-16(1)	7(1)
N1	67(1)	32(1)	42(1)	1(1)	-4(1)	5(1)
C1	54(1)	34(1)	41(1)	2(1)	-3(1)	1(1)
C2	51(1)	31(1)	44(1)	1(1)	-6(1)	3(1)
C3	50(1)	40(1)	42(1)	1(1)	-4(1)	4(1)
C4	52(1)	31(1)	45(1)	2(1)	-3(1)	4(1)
O1	60(1)	48(1)	65(1)	21(1)	-5(1)	-1(1)
NT	47(1)	39(1)	59(1)	10(1)	-7(1)	0(1)
CT1	58(1)	48(1)	58(1)	13(1)	-10(1)	3(1)
CT2	87(2)	51(1)	114(2)	27(1)	13(2)	21(1)
CT3	49(1)	56(1)	85(2)	14(1)	-9(1)	-3(1)
CT4	81(2)	57(1)	110(2)	10(1)	-31(2)	-17(1)
C5	47(1)	54(1)	45(1)	-6(1)	-3(1)	7(1)
O2	71(1)	83(1)	56(1)	-4(1)	-23(1)	1(1)
O3	62(1)	54(1)	64(1)	-3(1)	-13(1)	-13(1)
C6	71(2)	74(2)	93(2)	-17(1)	-9(1)	-25(1)

Table 6. Torsion angles [deg] for **(±)-34**.

O0-C0-N1-C1	-3.6(3)	C3-C2-C4-NT	169.40(15)
C01-C0-N1-C1	176.40(18)	O1-C4-NT-CT3	-0.8(3)
C0-N1-C1-C3	-156.56(17)	C2-C4-NT-CT3	179.29(17)
C0-N1-C1-C2	-87.7(2)	O1-C4-NT-CT1	175.06(18)
N1-C1-C2-C4	2.7(2)	C2-C4-NT-CT1	-4.9(3)
C3-C1-C2-C4	107.70(18)	C4-NT-CT1-CT2	102.5(2)
N1-C1-C2-C3	-104.96(17)	CT3-NT-CT1-CT2	-81.6(2)
N1-C1-C3-C5	-142.86(17)	C4-NT-CT3-CT4	79.1(3)
C2-C1-C3-C5	107.15(19)	CT1-NT-CT3-CT4	-97.0(2)
N1-C1-C3-C2	109.99(18)	C1-C3-C5-O2	151.01(19)
C4-C2-C3-C5	138.91(17)	C2-C3-C5-O2	-138.6(2)
C1-C2-C3-C5	-111.74(19)	C1-C3-C5-O3	-29.4(2)
C4-C2-C3-C1	-109.36(18)	C2-C3-C5-O3	41.0(2)
C1-C2-C4-O1	-79.9(2)	O2-C5-O3-C6	6.0(3)
C3-C2-C4-O1	-10.5(3)	C3-C5-O3-C6	-173.57(18)
C1-C2-C4-NT	100.0(2)		

Table 7. Hydrogen bonds for **(±)-34** [Å and deg.].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N1-H1...O1#1	0.86	2.00	2.853(2)	174.4

Symmetry transformations used to generate equivalent atoms: #1 -x+1,-y+1,-z.

Publications

- Zorn, C.; Goti, A.; Brandi, A.; Johnsen, K.; Kozhushkov, S.I.; de Meijere, A. “A new cascade ring enlargement of isoxazolidines formed from 2-chloro-2-cyclopropylideneacetates“ *Chem. Commun.* **1998**, 903-904.

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- Guarna, A.; Occhiato E.G.; Scarpi, D.; Zorn, C.; Danza, G.; Comerci, A.; Mancina R.; Serio M. “Synthesis of 8-chloro-benzo[c]quinolizin-3-ones as potent and selective inhibitors of human steroid 5α-reductase 1“ *Bioorg. Med. Chem. Lett.* **2000**, *10*, 353-356.

- Zorn, C.; Gnad, F.; Salmen, S.; Herpin, T.; Reiser, O. “Deprotection on N-Alloc amines by Pd(0)/DABCO-an efficient method for *in situ* peptide coupling of labile amino acids“ *Tet. Lett.* **2001**, *42*, 7049-7053.

Poster presentations

- Zorn, C.; Raach, A.; Reiser, O., 37° IUPAC International Congress **1999**, Berlin: “The Effect of β-ACC on the Secondary Structure of α- and β–Peptides“.

- Zorn, C.; Gnad, F.; Salmen, S.; Reiser, O. 5° German Peptide Symposium **2001**, Bielefeld: “*In situ* Peptide Coupling Methods of Amino Acids“.

- Poleschak, M.; Raach, A.; Zerbe, O.; Zorn, C.; Reiser, O. Annual Chemistry GDCh Congress **2001**, Würzburg: “Structural Studies on Oligopeptides with Multiple β-ACCs”.

Short Lecture

- Zorn, C.; Zerbe, O.; Reiser, O., 5° German Peptide Symposium **2001**, Bielefeld: “β-ACC as Conformation Stabilising Building Block in α-Peptides“.

Curriculum vitae

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Education

1986-1991	Liceo Classico (Humanistic High School) in Florence (Italy)
08/1991-02/1996	Chemistry studies
03/1996-07/1997	Diploma thesis in the research group of Prof. A. Brandi "Synthesis of Azaheterocycles with DNA alkylating properties"
07/1997	Laurea (diploma) in Chemistry
10/1998-present	PhD work in the research group of Prof. Dr. O. Reiser "Synthesis and structural investigation on α-peptides containing β-aminocyclopropane carboxylic acids"

Further educational and professional experiences

08-10/1996	ERASMUS EU studentship. (Synthesis training in Prof. Dr. A. de Meijere´s research group, University of Göttingen, Germany).
09/1997-07/1998	Research position in a joint project between ARES-SERONO (Genève) and the University of Florence, Italy (Prof. Dr. A. Guarna, Dept. of Organic Chemistry).
09/2000-09/2001	Teaching assistant for the practical training of undergraduate and graduated students at the University of Regensburg (Germany).

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