Diss. ETH No. 13203

Preparation of β-Amino Acids and Synthesis of β-Peptides with Novel Secondary Structures

A dissertation submitted to the
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Doctor of Natural Sciences

Presented by

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Accepted on the recommendation of

Prof. Dr. Dieter Seebach, examiner
Prof. Dr. Donald Hilvert, co-examiner

Zürich 1999

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For Isabelle

and my parents


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I am deeply indebted to my
'Doktorvater'

Prof. Dr. Dieter Seebach

for his encouraging guidance and generous support over the course of my thesis. The granted freedom for the design and execution of the projects, his confidence, as well as his constant interest in the progress of my work have made the past years a great and 'compact' experience.

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Parts of this thesis have been published:

Publications

G. Guichard, S. Abele, D. Seebach
'Preparation of *N*-Fmoc-Protected β^2- and β^3-Amino Acids and Their Use as Building Blocks for the Solid-Phase Synthesis of β-Peptides'
Helv. Chim. Acta **1998**, *81*, 187-206.

D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Jaun, J.L. Matthews, J.V. Schreiber, L. Oberer, U. Hommel, H. Widmer
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Helv. Chim. Acta **1998**, *81*, 2141-2156.

D. Seebach, S. Abele, T. Sifferlen, M. Hänggi, S. Gruner, P. Seiler
'Preparation and Structure of β-Peptides Consisting of Geminally Disubstituted $\beta^{2,2}$- and $\beta^{3,3}$-Amino Acids. A Turn Motif for β-Peptides'
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Angew. Chem. **1999**, *111*, 1700-1703;
'Pleated Sheets and Turns of β-Peptides with Proteinogenic Side Chains'
Angew. Chem. Int. Ed. **1999**, *38*, 1595-1597.

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Eur. J. Org. Chem. **1999**, in press.

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Helv. Chim. Acta **1999**, *82*, in press.

S. Abele, P. Seiler, D. Seebach
'Synthesis, Crystal Structures, and Modelling of β-Oligopeptides Consisting of 1-(Aminomethyl)cyclopropanecarboxylic Acid. Ribbon-Type Arrangement of Eight-Membered H-Bonded Rings'
Helv. Chim. Acta **1999**, *82*, in press.

M. Werder, S. Abele, H. Hauser, D. Seebach
'Inhibition of Cholesterol Uptake by β-Peptides'
in preparation.

Poster Presentations

T. Hintermann, S. Abele, B. Jaun, J.L. Matthews, J.V. Schreiber, L. Oberer, U. Hommel, H. Widmer, D. Seebach
'Synthesis of small β-peptides and investigation of their secondary structure', 36th IUPAC Congress, 17.-22.8.1997, Geneva, Switzerland.

S. Abele, K. Gademann, D. Seebach
'Searching for new secondary structures of β-peptides', 'Peptide and Protein Drugs' Meeting, 2.10.1997, Zürich, Switzerland; Herbstversammlung der Neuen Schweizerischen Chemischen Gesellschaft, 15.10.1997, Lausanne, Switzerland.

M. Werder, S. Abele, D. Seebach, H. Hauser
'Inhibition of cholesterol uptake in the small intestinal brush border membrane', D-BIOL Symposium ETH Zürich, 21.-23.5.1998, Davos, Switzerland.

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'New secondary structures of β-peptides. First animal experiments', 25th European Peptide Symposium, 30.8.-4.9.1998, Budapest, Hungary.


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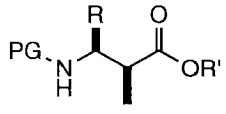
Contents

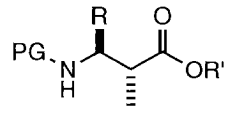
1 Zusammenfassung

Die vorliegende Arbeit befasst sich mit der Herstellung von β-Aminosäuren mit unterschiedlichen Substitutionsmustern sowie mit dem Entwurf und der Synthese von β-Peptiden mit neuen Sekundärstrukturen.

Im ersten Teil wurden β-Aminosäuren nach mehreren Methoden stereoselektiv hergestellt. Die $\beta^{2,3}$-Aminosäurederivate **I** und **II** mit *like*- bzw. *unlike*-Konfiguration wurden durch α-Methylierung doppelt lithiierter β^3-Aminosäureester synthetisiert. Die für die Herstellung der Boc-geschützten β^3-Aminosäuren erfolgreich benutzte *Arndt-Eistert* Homologisierung wurde ebenso für die Synthese der Fmoc-geschützten β^3-Aminosäuren **III** verwendet.



I



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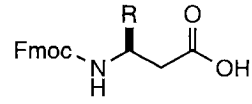
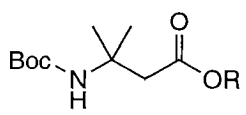
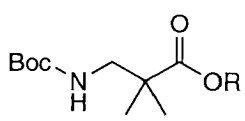


III

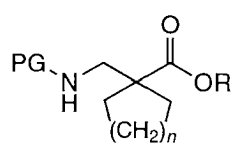
Geminal disubstituierte β-Aminosäuren wurden im Multigramm-Maßstab hergestellt. Die *Michael* Addition von Ammoniak an 3,3-Dimethylacrylsäure ergab die $\beta^{3,3}$-Aminosäurederivate **IV**, während die doppelte α-Methylierung von β-Aminopropionsäurederivaten die $\beta^{2,2}$-Aminosäuren **V** lieferte. Die Herstellung der 1-(Aminomethyl)cycloalkan-carbonsäure-derivate **VI** erfolgte mittels Dialkylierung von Cyanessigsäure-ester und anschliessender Hydrierung mit *Raney*-Nickel. Eine klassische Racematspaltung lieferte (*R*)- und (*S*)-Nipecotinsäureethylester (β^2-HPro); zur Bestimmung der Enantiomerenreinheit wurden beide Enantiomere des *N*-2,4-Dinitrophenylderivats **VII** mittels HPLC an chiraler stationärer Phase getrennt.



IV

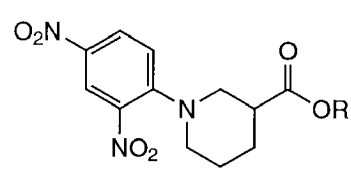


V



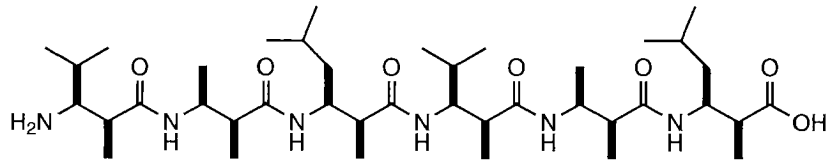
VI

$n = 0, 1, 2, 3$



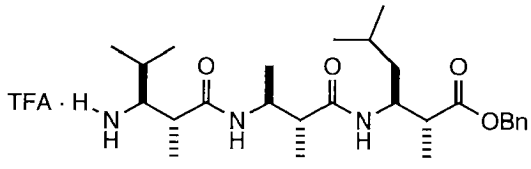
VII

Im zweiten Teil wurden die β-Aminosäuren für den Aufbau verschieden substituierter β-Peptide eingesetzt, deren Struktur mit CD- und NMR-Spektroskopie sowie Röntgenstrukturanalyse untersucht wurde. Für einen rascheren Zugang zu β-Peptiden wurde die Festphasensynthese neben der Synthese in Lösung benutzt. Die für das all-*like*-$\beta^{2,3}$-Hexapeptid **VIII** vorhergesagte 3_{14}-helikale Struktur wurde durch 2D-NMR Analyse bestätigt.

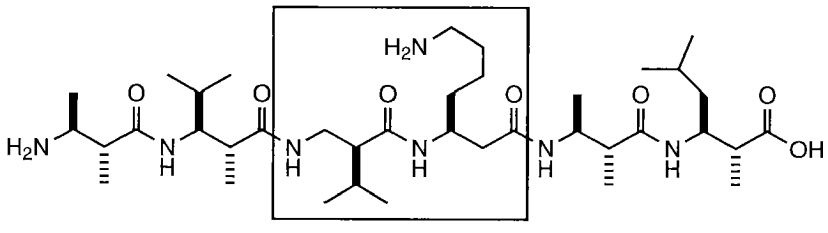


VIII

Zwei Sekundärstrukturelemente von β-Peptiden wurden auf der Grundlage von Röntgenstruktur- und NMR-Daten, sowie klassischer Konformationsanalyse entworfen. So bildet das all-*unlike*-$\beta^{2,3}$-Tripeptid-derivat **IX** gemäss der Kristallstruktur eine parallele Faltblattstruktur aus. Die antiparallele Faltblattstruktur wird von β-Heptapeptid **X** in Lösung eingenommen, wie durch 2D-NMR Spektroskopie gezeigt wurde. Weitere β-Peptide mit verschiedenen zentralen β-dipeptidischen Schleifenmotiven wurden synthetisiert und ergaben mögliche Substitutionsmuster für β-peptidische Schleifen.



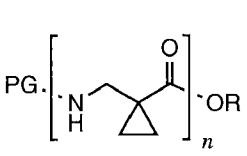
IX



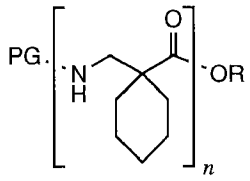
X

Zur Untersuchung der Sekundärstruktur von β-Peptiden aus geminal disubstituierten β-Aminosäuren wurden u.a. die β-Peptide **XI** und **XII** synthetisiert. Röntgenstrukturen der Di-, Tri- und Tetrapeptidderivate **XI** zeigen bandartige Strukturen mit *acht*gliedrigen H-Brücken-Ringen.

Dagegen wird eine *zehn*gliedrige H-Brücken-gebundene Schleife vom β-Tripeptid **XII** im Festkörper ausgebildet.

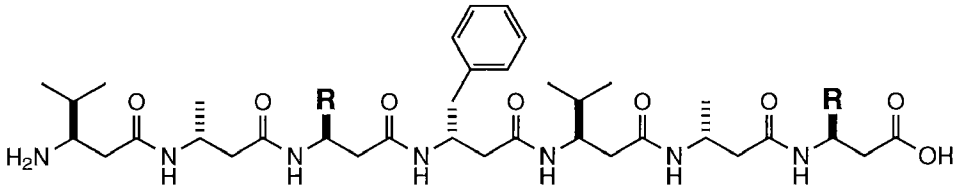


XI $n = 2, 3, 4, 6$



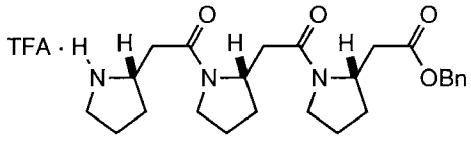
XII

Die β-Peptide **XIII** mit (*R*)/(*S*)-β³-Sequenz (R = Me_2CHCH_2, $H_2N(CH_2)_4$, $HOCH_2$) wurden an der Festphase synthetisiert. Sowohl CD- als auch NMR-Spektroskopie des β-Peptids mit Lysinseitenketten deuten auf eine neue Sekundärstruktur für dieses Substitutionsmuster hin, das mit keiner der bekannten β-Peptid-Sekundärstrukturen vereinbar ist.

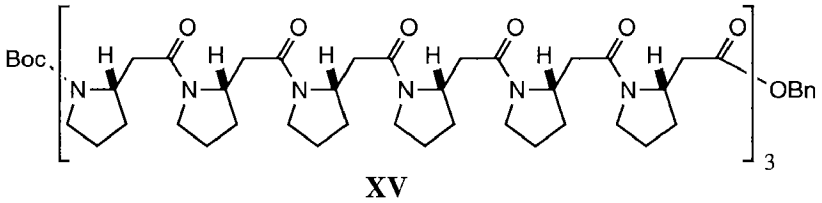


XIII

Für das bessere Verständnis der Rolle von H-Brücken bei der Ausbildung von β-peptidischen Sekundärstrukturen wurden β-Peptide aus β²- und β³-



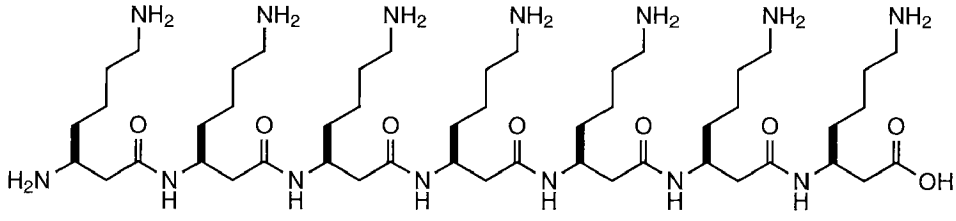
XIV



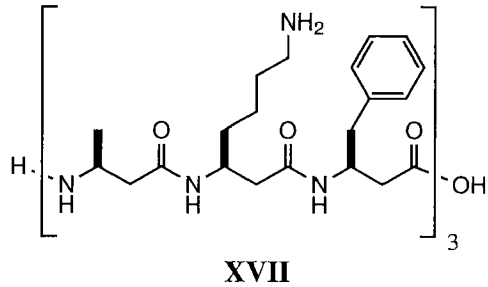
XV

Homoprolin hergestellt. Die Kristallstruktur vom Tripeptidderivat **XIV** sowie neuartige intensive CD-Spektren grösserer Oligomere wie z.B. des β-Octadecapeptids **XV** deuten darauf hin, dass sich stabile Konformationen bei β-Peptiden auch ohne H-Brücken ausbilden.

Im Rahmen einer Zusammenarbeit mit *Novartis Pharma* wurden erstmals *in vivo* pharmakokinetische Experimente mit β-Peptiden durchgeführt: die Lebensdauer im Rattenblut ist im Vergleich zu α-Peptiden deutlich verlängert. Die höhere metabolische Stabilität ist für die zukünftige Anwendung von β-Peptiden als Pharmazeutika bedeutsam. In einer weiteren Zusammenarbeit mit Prof. *Hauser* (ETH) wurden u.a. das polykationische β-Heptapeptid **XVI** und das β-Nonapeptid **XVII** als Inhibitoren der Cholesterinaufnahme in Membranen des Dünndarms getestet.



XVI

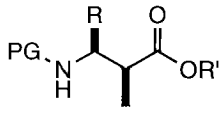


XVII

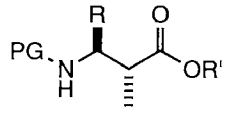
Summary

In this thesis, the preparation of various β-amino acids with different substitution patterns is presented. Moreover, the design, synthesis and structural investigation of β-peptides with novel secondary structures is described.

In the first part, β-amino acids were stereoselectively prepared by several methods. α-Methylation of Boc-protected β^3-amino acid esters through a doubly lithiated species provided the $\beta^{2,3}$-amino acid derivatives **I** and **II**. The *Arndt-Eistert*-homologation used for the synthesis of Boc-protected β^3-amino acid derivatives from the corresponding α-amino acids was, with slight modification, successfully applied to the preparation of Fmoc-protected β^3-amino acids **III**.



I



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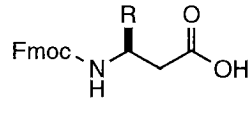
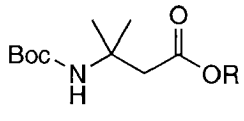


III

Geminally disubstituted β-amino acids were prepared by either *Michael*-addition of ammonia to 3,3-dimethylacrylic acid to provide the $\beta^{3,3}$-amino acid derivatives **IV** or by double α-methylation of β-aminopropanoic acid derivatives to afford the $\beta^{2,2}$-amino acid derivatives of type **V**. The 1-(aminomethyl)cycloalkanecarboxylic-acid derivatives **VI** were prepared by dialkylation of methyl cyanoacetate with various dibromides and subsequent hydrogenation using *Raney*-nickel. Classical resolution provided (*R*)- and (*S*)-ethyl nipecotate (β^2-HPro); a method for the determination of its enantiopurity was successfully developed using the *N*-2,4-dinitrophenyl-derivative **VII** for HPLC analysis on a chiral phase.



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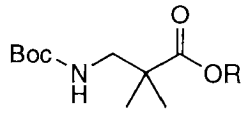

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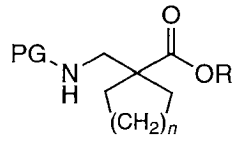
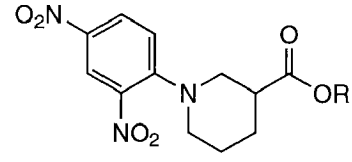


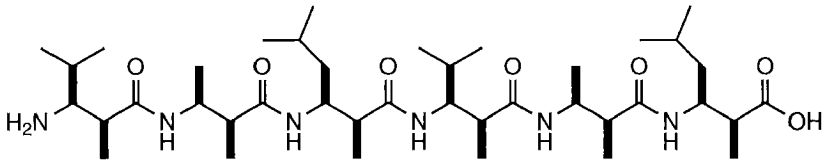
VI

$n = 0, 1, 2, 3$



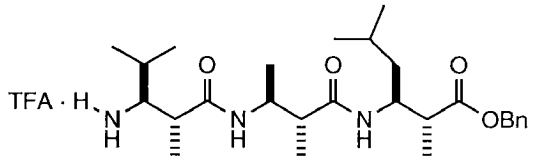
VII

In the second part, the β-amino acids were used for the construction of various β-peptides and their structure was analyzed by CD- and NMR spectroscopy, or X-ray analysis. The predicted well-known 3_{14}-helical conformation of the all-*like*-$\beta^{2,3}$-hexapeptide **VIII** was determined by NMR spectroscopy.

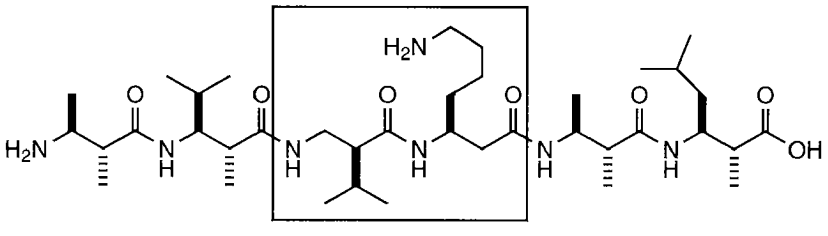


VIII

On the basis of X-ray and NMR data and by conformational analysis, two secondary structural elements of β-peptides with proteinogenic side chains were designed. The all-*unlike*-$\beta^{2,3}$-tripeptide derivative **IX** forms a parallel pleated sheet arrangement in the solid state, as determined by X-ray analysis. The antiparallel pleated sheet (hairpin) is adopted by β-hexapeptides **X** according to 2D-NMR spectroscopy. Solid phase synthesis was applied for the preparation of β-peptide **X** and derivatives thereof with various central turn motifs.

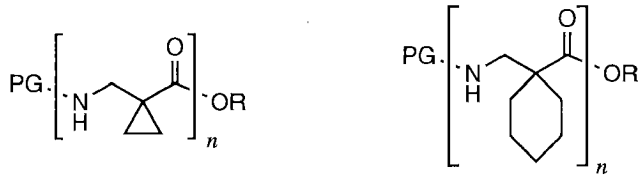


IX



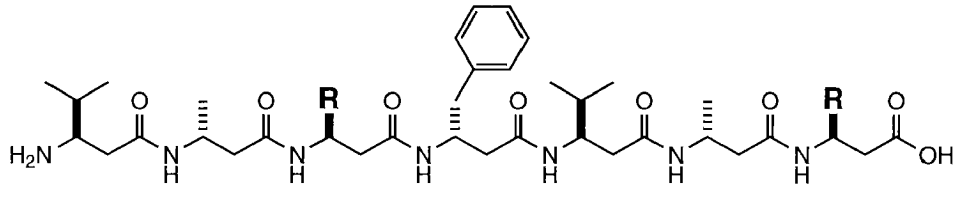
X

To investigate the structure of β-peptides consisting of geminally disubtituted β-amino acids, β-peptides **XI** and **XII** were prepared. X-Ray structure analysis revealed a ribbon-like structure with eight-membered H-bonded rings for cyclopropane derivatives **XI** (n = 2, 3, 4) and a ten-membered turn for cyclohexane tripeptide derivative **XII**.



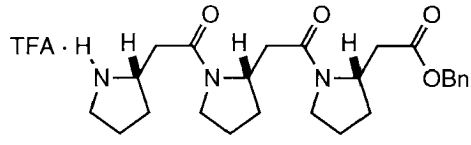
XI $n = 2, 3, 4, 6$ **XII**

The β-peptides **XIII** with alternating (*R*)/(*S*)-β^3-sequence (R = Me_2CHCH_2, $H_2N(CH_2)_4$, $HOCH_2$) were prepared on solid phase. CD and NMR spectroscopy of the β-peptide with lysine side chains indicate a novel secondary structure for this type of substitution pattern.

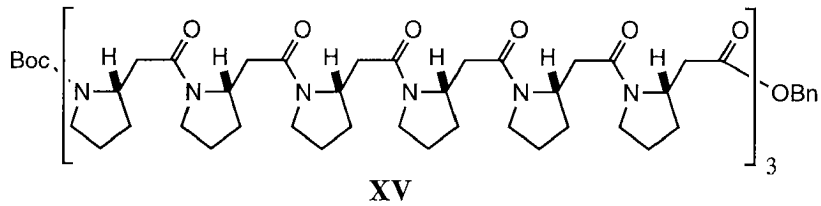


XIII

In another line of work addressing the role of H-bonds in stabilizing β-peptidic secondary structures, β-peptides consisting of β^3- and β^2-homoproline were synthesized. An X-ray structure of the TFA salt **XIV** and CD spectroscopy of higher oligomers such as the octadecapeptide **XV** suggest that these β-peptides, albeit devoid of H-bonds, fold into stable conformations.

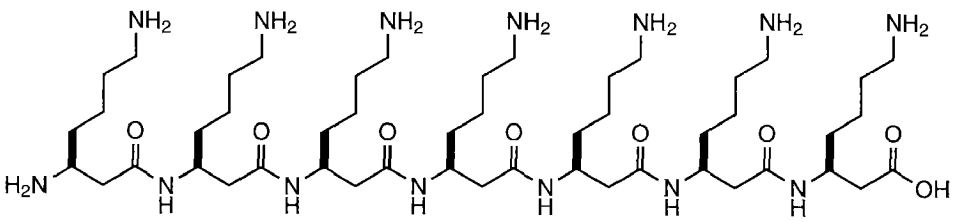


XIV

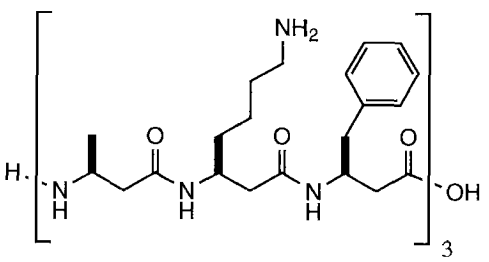


XV

In the final part of the thesis, *in vivo* pharmacokinetic studies – performed in collaboration with *Novartis Pharma* – showed that β-peptides are much more stable, as compared to α-peptides, towards metabolic processes. This result is of great importance for future applications of β-peptides as drugs. The polycationic β-heptapeptide **XVI** and the β-nonapeptide **XVII** were prepared on solid phase and were tested as inhibitors of cholesterol uptake in the small intestine (collaboration with Prof. *Hauser*, ETH).



XVI



XVII

2 Introduction

Open-chain and cyclic α,α-disubstituted or α-branched α-amino acids are among the most studied synthetic and naturally occurring non-coded amino acids. Their incorporation into peptides leads to restricted conformational flexibility [1], to stabilization of defined secondary structures in small peptides [2-5], to increased lipophilicity [6] and to higher resistance towards both enzymatic and chemical hydrolysis [7]. Moreover, some α,α-dialkylated α-amino acids are efficient enzyme inhibitors [8-12]. The preparation of enantiopure α,α-dialkylated α-amino acids has thus attracted considerable attention and has been described in several review articles [13-16].
In contrast, there is no compilation on the synthesis of chiral, geminally disubstituted β-amino acids. Since the far-reaching discovery that β-peptides form much more stable secondary structures in solution [17-19] than their α-peptidic natural counterparts, there is an ever-growing interest in the synthesis of β-amino acids with various substitution patterns. Due to the increased number of possible constitutional and configurational isomers of β-amino acids, many more synthetic strategies are applicable [20]. Stereoselective syntheses of β-amino acids have already been extensively reviewed [21-24]. Many methods for the synthesis of β-amino acids are listed in the book 'Methods of Non-α-Amino Acid Synthesis', edited by *Smith* [25]. The most recent and complete compilation of enantioselective syntheses of β-amino acids can be found in *Juaristi*'s book [26]. However, among all the methods for the preparation of β-amino acids (β^2- and β^3-amino acids[1]), only a few are suitable for the generation of a quaternary carbon at the α-position or of a tertiary carbon at the β-position. The following sections focus on methods for the preparation of both achiral and chiral *geminally disubstituted* β-amino acids. Furthermore, new strategies for the synthesis of enantiopure derivatives of this class of compounds are discussed.

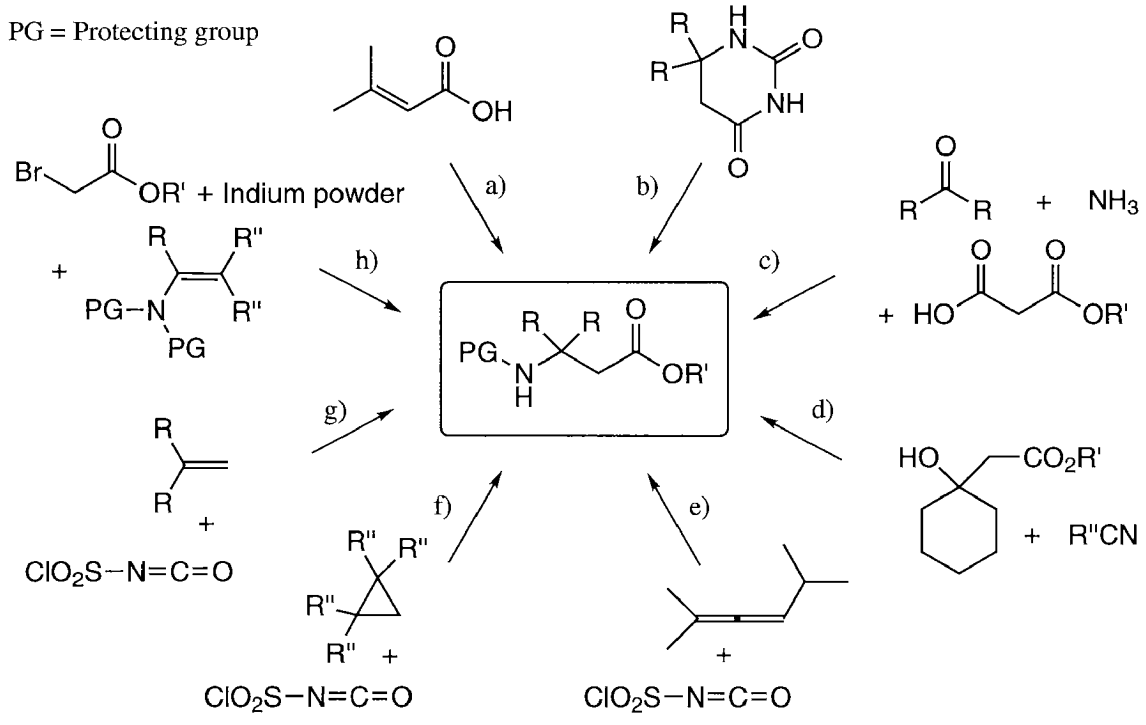
[1] The previously proposed nomenclature for β-amino acids [17,27,28] and β-peptides [29] is used.

2.1 Methods for the Synthesis of Achiral Geminally Disubstituted β-Amino Acids

2.1.1 *Achiral $\beta^{3,3}$-Amino Acids*

Some methods for the preparation of achiral β-amino acids, disubstituted at the β-carbon, are shown in *Scheme 1*.

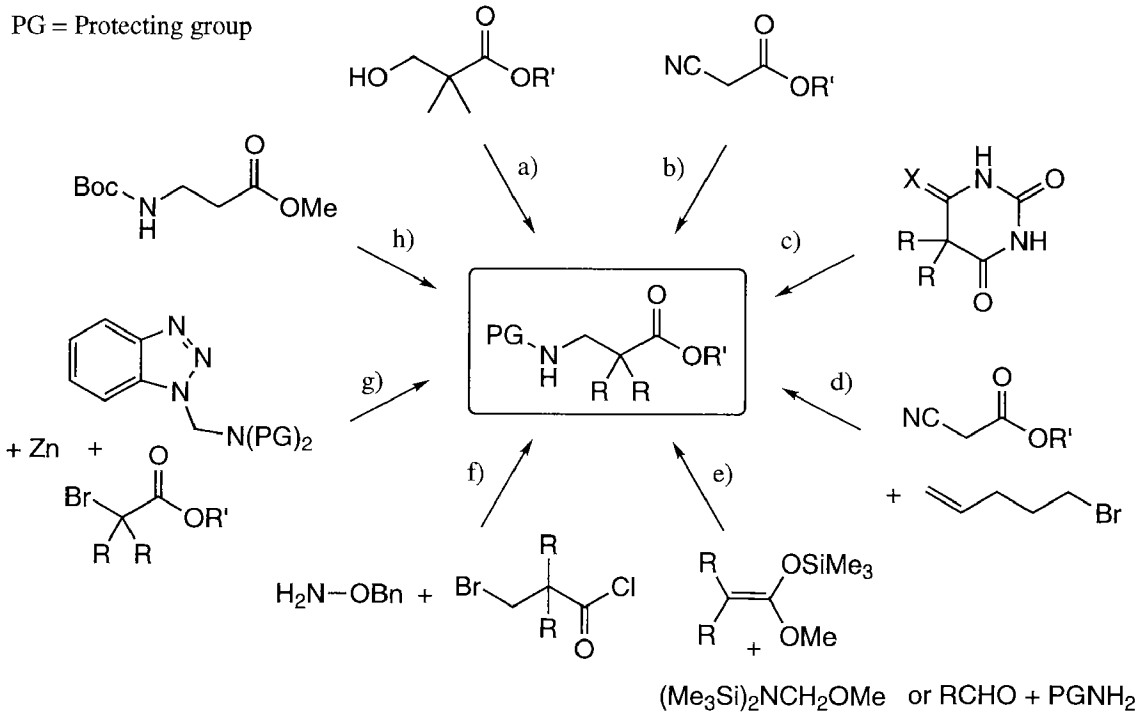
Scheme 1. Various methods for the preparation of achiral $\beta^{3,3}$-amino acids. a) *Michael* addition of ammonia to 3-methylbut-2-enoic acid derivatives (senecioic acid) [30-33]. b) Hydrolysis of 6,6-disubstituted dihydrouracils [34], followed by acidic work-up [35,36]. c) Three-component *Mannich* reaction of a ketone, NH_3, and a malonic-acid derivative [37,38]. d) *Ritter* transformation [39] of 3-hydroxy carboxylates with nitriles in the presence of conc. H_2SO_4 to give the *N*-acyl-β-amino acid [40,41]. e) Cycloaddition of chlorosulfonyl isocyanate with allenes to give an alkylidene β-lactam, which can be hydrolyzed and hydrogenated to give the corresponding β-amino acid [42]. f) Reaction of substituted cyclopropanes with chlorosulfonyl isocyanate to yield β-lactams which can be transformed as described in e) [43]. g) Cycloaddition of disubstituted alkenes and chlorosulfonyl isocyanate, reductive cleavage of the chlorosulfonyl group, followed by protection of the β-lactam *N*-atom and β-lactam opening [44-46], providing the desired β-amino acid [47,48]. h) Indium-mediated reaction of enamines with methyl bromoacetate in the presence of acid [49].



2.1.2 *Achiral $\beta^{2,2}$-Amino Acids*

Several methods for the preparation of achiral α,α-disubstituted β-amino acids are summarized in *Scheme 2*.

Scheme 2. Various methods for the preparation of achiral $\beta^{2,2}$-amino acids. a) Nucleophilic displacement of the hydoxy group in hydroxypivalic acid by PBr_5, followed by treatment with ethanolic NH_3 [50]. b) Dialkylation of methyl cyanoacetate by alkyl halides [51], or various dibromides [52,53] to yield α,α-disubstituted methyl cyanoacetates, that can alternatively be prepared by *Mitsunobu* reaction of diols with methyl cyanoacetate [54]. Selective reduction of the cyanoesters [32,33,55], *N*-protection and saponification of the ester group affords the $\beta^{2,2}$-amino acid derivatives. c) Hydrolytic cleavage of 5,5-diethylbarbituric acid [56,57], obtained by desulfurization [34] of 5,5-diethyl-4-thiobarbituric acid [58]. d) Alkylation of cyanoacetate by 5-bromo-1-pentene, followed by radical cyclization [59]. e) Aminomethylation of silyl ketene acetals with *N,N*-bis[(trimethylsilyl)-methoxy]-methylamine [60]; a one-pot *Mannich*-type condensation of aldehydes, primary amines, and silyl ketene acetals in H_2O in the presence of $InCl_3$ [61], or Ti-catalyzed reaction of phenethyl-protected imines with silyl ketene actals to give the β-lactam, which is hydrolyzed and hydrogenated to give the free β-amino acid [62]. f) Ring closure of benzyl-protected hydroxylamines with β-bromopropionyl chlorides to give β-lactams and subsequent hydrolysis [63]. g) *Reformatzky* reaction of an appropriate benzotriazol derivative with 2-bromoalkanoates [64]. h) Dialkylation of Boc-protected methyl 3-aminopropanoate via a doubly lithiated species [32].

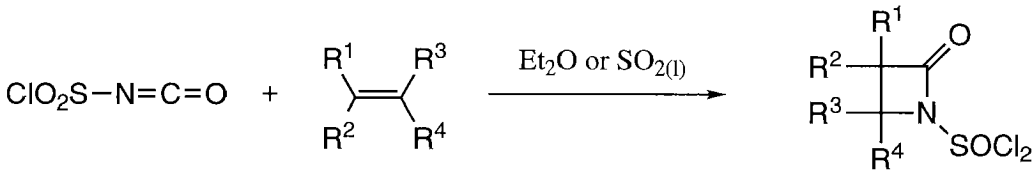


2.1.3 $\beta^{2,2,3,3}$-Amino Acids

Tetrasubstituted $\beta^{2,2,3,3}$-amino acids are interesting building blocks for testing the efficiency of coupling reagents in amide bond formation, since both the amino and carboxyl group are sterically hindered. Moreover, they could act as secondary-structure breakers if incorporated into β-peptides. Some of the methods presented above can also be applied to the synthesis of $\beta^{2,2,3,3}$-amino acids.

Tetramethyl- and dipentamethylene substituted β-lactams are prepared in quantitative yield by reaction of chlorosulfonyl isocyanate with tetrasubstituted olefins in diethyl ether or liquid SO_2 (*Scheme* 3) [47]. The chlorosulfonyl-β-lactams can easily be hydrolyzed to the corresponding $\beta^{2,2,3,3}$-amino acids [42,43,45]. Olefins bearing electron withdrawing substituents are not tolerated

Scheme 3. Reaction of chlorosufonylisocyanate with tetrasubstituted olefins according to *Graf*.



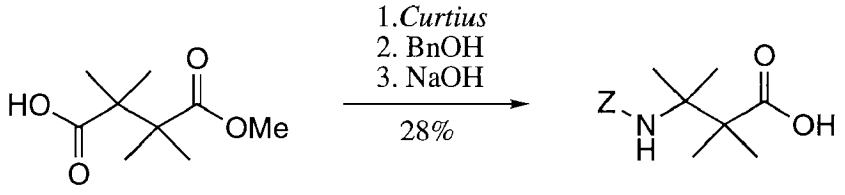
$R^1 = R^2 = R^3 = R^4 = Me$ (99%)

$R^1 = R^2 = -(CH_2)_5-$, $R^3 = R^4 = -(CH_2)_5-$ (99%)

Another approach uses methyl 2,2,3,3-tetramethylsuccinate[2] as the starting material for a *Curtius* rearrangement [69]. Treatment of the intermediate isocyanate with benzyl alcohol and hydrolysis with NaOH afforded the *N*-Z-protected 2,2,3,3-tetramethyl-β-alanine in 28% yield (*Scheme* 4).

[2] Tetramethyl succinate, the precursor for this transformation can be prepared a) by a *Wurtz*-type coupling of 2-bromo-2-methyl-propionates in the presence of 1.3 equivalents of Ag powder [65], b) by decomposition of acetyl peroxide in isobutyric acid [66], c) by dehydrodimerization of isobutyric acid via H atoms as abstractors, formed by Hg-photosensitized reaction with H_2 [67], or d) by oxidative coupling of dilithiated isobutyric acid with I_2 [68].

Scheme 4. Curtius rearrangement of methyl 2,2,3,3-tetramethylsuccinate, followed by treatment with benzyl alcohol and alkaline hydrolysis according to *Shadbolt*.



2.2 Methods for the Synthesis of Chiral Geminally Disubstituted β-Amino Acids

Asymmetric tertiary and quaternary carbons are often encountered in natural products [70-72]. The control of stereoselectivity in reactions generating these highly substituted stereogenic centers is still a synthetic challenge [73-75]. There are only few methods for the preparation of enantiomerically pure geminally disubstituted β-amino acids. The following sections are devoted to the enantioselective synthesis of geminally disubstituted β-amino acids[3].

2.2.1 Chiral $\beta^{3,3}$-Amino Acids

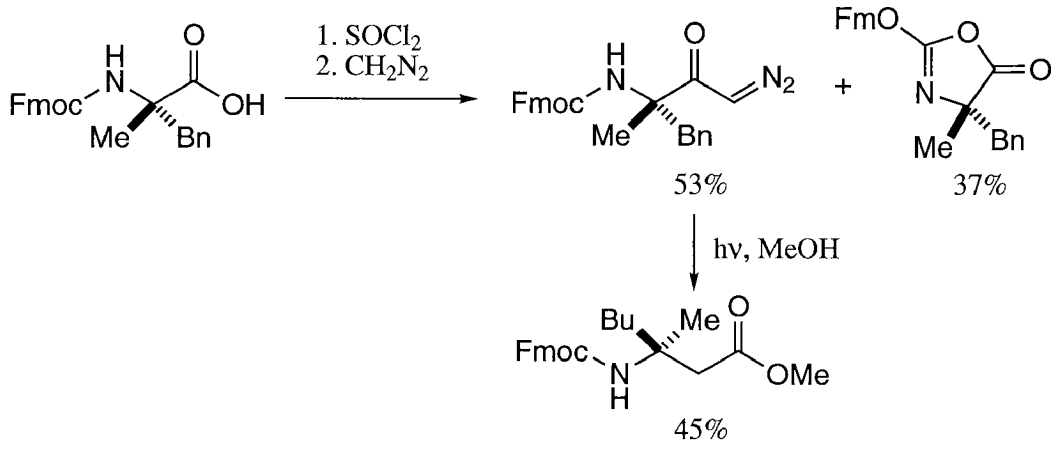
The *Arndt-Eistert* homologation, routinely applied in *Seebach's* group for the preparation of β^3-amino acids [85,86], appears to be the ideal method for the synthesis of chiral β,β-disubstituted β-amino acids from enantiopure α,α-disubstituted α-amino acids, since the latter are readily available (*vide supra*). However, both the preparation of the diazoketones and their subsequent homologation caused severe problems [87]. Treatment [88] of the acid chloride derived from an Fmoc-protected α,α-disubstituted α-amino acid [89,90] with diazomethane afforded a mixture (ca. 1.4 : 1) of a diazoketone and the corresponding oxazolone[4] (*Scheme 5*). These highly substituted diazoketones rearrange with poor yields in the presence of Ag^+/triethylamine in methanol. However, initiation of the rearrangement

[3] Methods for preparation of α-alkylated aspartic acid [76-84] which is both a α,α-disubstituted α-amino acid and a β,β-disubstituted β-amino acid, are not discussed here.

[4] The oxazolones can be recycled by hydrolysis to the corresponding Fmoc α-amino acids after separation by chromatography.

by photolysis in methanol yielded the Fmoc-protected $\beta^{3,3}$-amino acid in 45% yield (*Scheme 5*).

Scheme 5. Formation of the diazoketone from an enantiomerically pure benzyl methyl substituted α-amino acid and subsequent *Wolff* rearrangement upon irradiation, as described by *Seebach et al.*



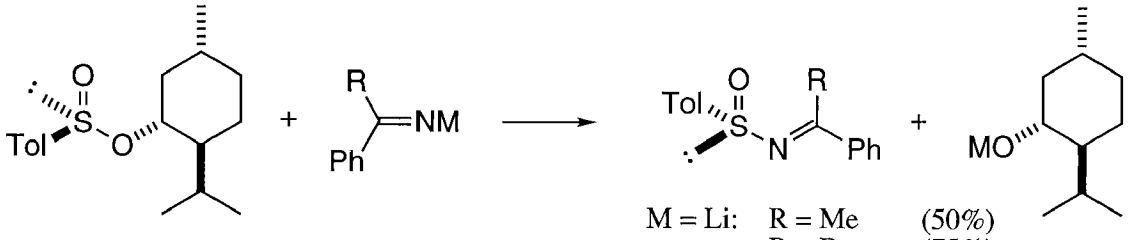
Recently, enantiopure *p*-toluenesulfinimines have been successfully used for the asymmetric synthesis of aziridines [91,92], α-branched amines [93,94] and β-amino acids [95-98]. *Davis*'s pioneering efforts on the study of *p*-toluenesulfinimines [99], demonstrate that the sulfinyl group serves as an ideal auxiliary because it activates the sulfinyl group for nucleophilic addition and provides high diastereofacial selectivity. Moreover, it is easily removed by treatment with mild acid.

Enantiomerically pure sulfinimines (also: thiooxime S-oxides) derived from ketones are versatile precursors for the synthesis of geminally disubstituted $\beta^{2,2}$-amino acids - after combination with an appropriate d^2 synthon [100]. A general approach to chiral sulfinimines is outlined in *Scheme 6*. It is based on the asymmetric iminolysis of the *Andersen* reagent [101-103] (*R*)- or (*S*)-menthyl *p*-toluenesulfinate[5]. The reaction is highly stereospecific[6].

[5] The prices of both enantiomeres (1*S*)-menthyl (*R*)-*p*-toluenesulfinate and (1*R*)-menthyl (*S*)-*p*-toluenesulfinate are comparable: 9.6 and 10.9 sFr/g respectively (*Fluka*-catalogue 1998).

[6] The molar activation enthalpy $\Delta H^{\neq}$ for pyramidal inversion of *sulfoxides* is 35-42 kcal/mol [104], accounting for their configurational stability at 25 °C. *p*-Toluene*sulfinates*, however, undergo slow acid-catalyzed mutarotation [105].

Scheme 6. Stereospecific (S_N2) displacement of metal menthoxide from the *Andersen* reagent by metal ketimines to provide enantiopure *p*-toluenesulfinimines according to *Hua* [96] and *Cinquini* [93].

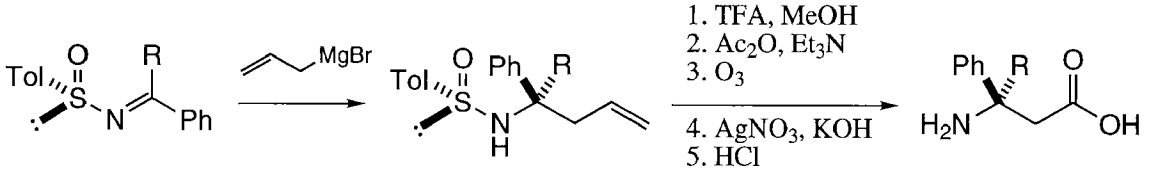


Andersen reagent

Metal ketimines[7] are obtained by the reaction of a Grignard reagent [93] or alkyllithium with benzonitrile [96]. Yields are much higher with lithium ketimines than with the imino Grignard derivatives. These *Andersen*-type syntheses [106] are limited to aromatic nitriles[8]. Alternatively, the sulfinimines can be prepared by asymmetric oxidation of racemic sulfenimines with chiral oxaziridines derived from camphor [107-109]. The e.e. (88-90%) of the enantiomerically enriched sulfinimines can reach up to > 97% after recrystallization from hexane. However, a major drawback of this procedure is the difficulty of improving the enantiopurity in the case of non-crystalline derivatives (i.e. most aliphatic compounds).

Chiral sulfinimines smoothly react with allylmagnesium bromide with high diastereoselectivity (*Scheme 7*). The diastereoisomers (R = Bu) are easily separated by chromatography and converted into the corresponding free $\beta^{3,3}$-amino acids in a five-step-sequence [96].

Scheme 7. Diastereoselective addition of allylmagnesium bromide to enantiopure sulfinimine according to *Hua*.



R = Me (98%, d.r. > 98 : 2)
R = Bu (92%, d.r. = 91 : 9)

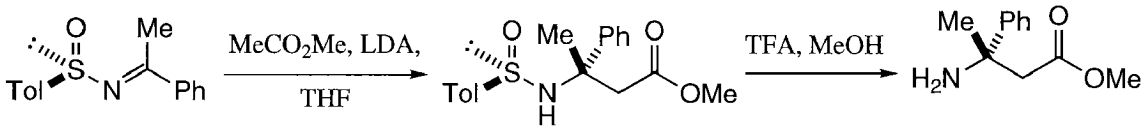
R = Me (50%)
R = Bu (52%)

7 The *E* configuration of the imine was proved by single-crystal X-ray analysis [96].

8 Aldehyde-derived sulfinimines can be prepared by an alternate procedure [107].

The number of steps to $\beta^{3,3}$-amino acids can be reduced by employing the lithium enolate of methyl acetate for the 1,2-addition reaction to enantiopure sulfinimines (*Scheme 8*). Mild hydrolysis of the sulfinamide S–N bond at 0 °C afforded chiral $\beta^{3,3}$-amino acid esters in high yield and e.e. [97].

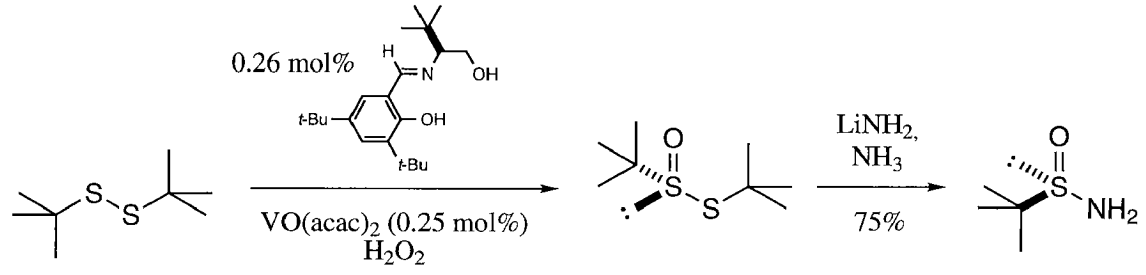
Scheme 8. Diastereoselective addition of enolates to enantiopure sulfinimines according to *Davis*.



90%, d.r. > 97 : 3 85%, e.e. > 95%

Recently, a new approach to enantiomerically pure sulfinimines has been developed by *Ellman et al.* [98]. The key step is an asymmetric catalytic oxidation of *tert*-butyl disulfide with a catalyst derived from *tert*-leucinol [110] to provide *tert*-butyl *tert*-butanethiosulfinate in high yield and enantioselectivity (*Scheme 9*). Optically pure *tert*-butanesulfinamides are then formed by addition of lithium amide to the chiral thiosulfinate [111].

Scheme 9. Asymmetric catalytic oxidation of *tert*-butyl disulfide, followed by addition of lithium amide to provide enantiopure *tert*-butanesulfinamide according to *Ellman*.



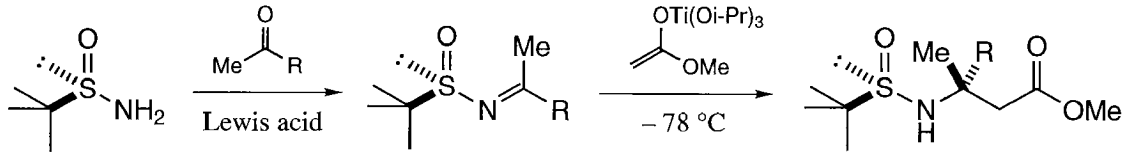
93%, e.e. = 91%

Enantiopure sulfinimines derived from *aliphatic* ketones are easily available from these sulfinamides (*Scheme 10*). Condensation of the *tert*-butanesulfinamide with aldehydes [111] or ketones [112] provides *tert*-butane-sulfinimines[9] in high yields. Addition of the titanium-enolate of methyl acetate, prepared by transmetallation of the corresponding Li-enolate

[9] Only the *E* isomer of sulfinyl ketimines was observed by ^{1}H-NMR.

with $ClTi(Oi\text{-}Pr)_3$, to enantiomerically pure *tert*-butanesulfinimines provides the $\beta^{3,3}$-amino esters [98][10].

Scheme 10. Preparation of enantiopure *tert*-butanesulfinimines for the asymmetric *Mannich* reaction with the Ti enolate of methyl acetate according to *Ellman et al.*



R = i-Pr (85%, d.r. = 99 : 1)
R = Ph (89%, d.r. = 98 : 2)

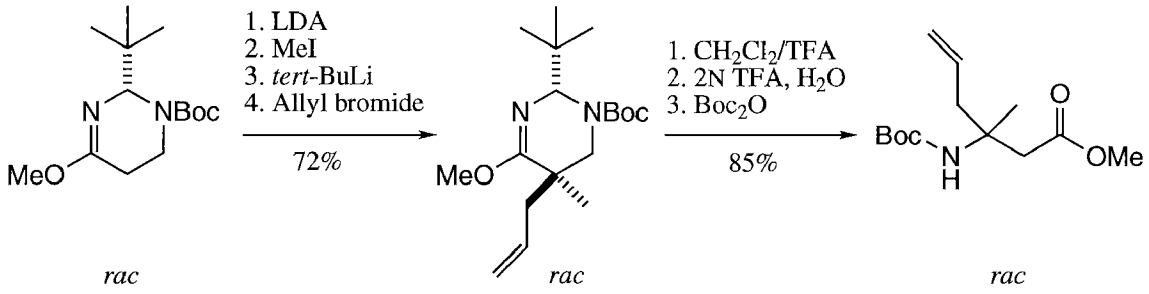
2.2.2 *Chiral $\beta^{2,2}$-Amino Acids*

The diastereoselective introduction of two substituents at the α-carbon of chiral 3-aminopropanoates seems to be the method of choice for the preparation of chiral α,α-disubstituted β-amino acids. A literature search (Beilstein Crossfire) revealed that there is no published procedure for the diastereoselective α-*di*alkylation of open-chain 3-aminopropanoic acid derivatives. However, better stereoselectivities are often obtained by the use of the corresponding cyclic derivatives, which allow for excellent differentiation of the diastereotopic faces of the enolate plane [14]. Thus, high diastereoselectivities are secured in two types of geminally disubstituted chiral cyclic derivatives (*Schemes 11* and *12*). *Scheme 11* outlines the application of chiral cyclic methyl iminoesters for the preparation of α,α-disubstituted β-amino acids [113,114]. Racemic[11] Boc-protected 2-*tert*-butyl-4-methoxytetrahydropyrimidine was easily prepared in three steps (56% yield) from Z-protected 3-amino propionic acid. The Li enaminate of this heterocycle reacted smoothly with methyl iodide and, after work-up and a second deprotonation, with allyl bromide to give the geminally disubstituted product. In both alkylation steps only *one* diastereoisomer is formed. A two-step cleavage under mild conditions converts the heterocycle to the corresponding *rac* or enantiopure $\beta^{2,2}$-amino acid methyl esters [115].

[10] The *tert*-butanesulfinyl group is cleaved at room temperature by brief treatment with ethanolic HCl [98].

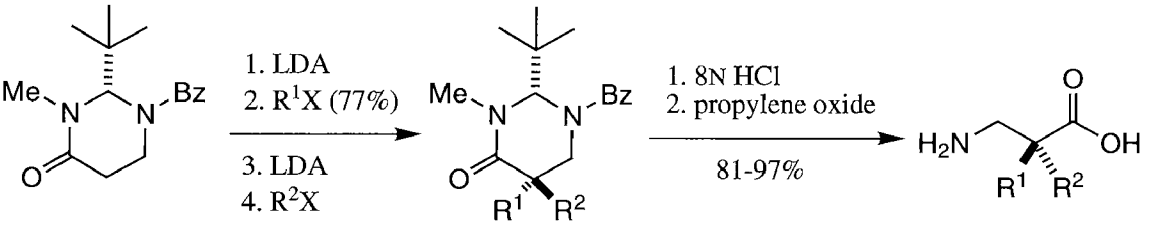
[11] Multi-gram amounts of non-racemic material were obtained by preparative chromatographic resolution of the pyrimidinone precursor on a chiral column [115].

Scheme 11. Highly diastereoselective dialkylation of *rac* 1-Boc-protected 2-*tert*-butyl-4-methoxytetrahydropyrimidine and subsequent hydrolysis to the $\beta^{2,2}$-amino acid methyl ester according to *Seebach* and *Boog*. Only one enantiomer is shown.



Another cyclic derivative of 3-amino propanoic acid, (*S*)- or (*R*)-1-benzoyl-2-*tert*-butyl-3-methylperhydropyrimidin-4-one[12], was dialkylated in comparable yields and diastereoselectivities (*Scheme 12*) [117].

Scheme 12. Diastereoselective dialkylation of (*S*)-1-benzoyl-2-*tert*-butyl-3-methylperhydropyrimidin-4-one and hydrolysis of the heterocycle affording the free enantiopure $\beta^{2,2}$-amino acids according to *Juaristi*.



R^1 = Me, R^2 = Bn (96%, d.r. > 95 : 5 (for 2nd alkylation step))
R^1 = Me, R^2 = *n*-Bu (81%, d.r. > 95 : 5 (for 2nd alkylation step))

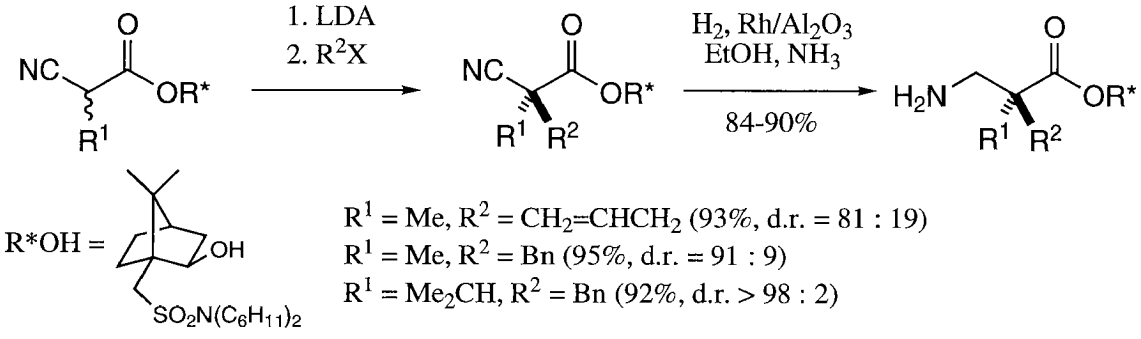
Whereas the dialkylation of 3-aminopropanoates has not been reported, the diastereoselective alkylation of chiral α-cyanoester- [118] or α-cyanocetamide-enolates [119] is ideally suited to prepare α,α-disubstituted α-cyanoacetic acid precursors which can be transformed into the corresponding $\beta^{2,2}$-amino acid derivatives by nitrile reduction.

An isoborneol derivative is the chiral auxiliary for the diastereoselective alkylation depicted in *Scheme 13*. The starting α-alkylated α-cyanoacetates are applied as a 1:1 mixture of their C(α)-epimers. Various substituents R^1 and R^2 are compatible with this method [118]. Reduction of the cyano group

[12] Enantiopure starting pyrimidinones were obtained from (*S*)- or (*R*)-asparagine in 25% yield in a five-step-sequence [116].

provides the diastereomerically pure $\beta^{2,2}$-amino esters[13]. With similar derivatives, *Cativiela* has also shown that the chiral auxiliary can be easily removed by saponification with 10% KOH in methanol [120,121].

Scheme 13. Diastereoselective alkylation of the enolate of (1*S*,2*R*,4*R*)-10-(dicyclohexylsulfamoyl)isobornyl-2-alkyl-2-cyanoacetates and hydrogenation of the nitrile according to *Cativiela*.



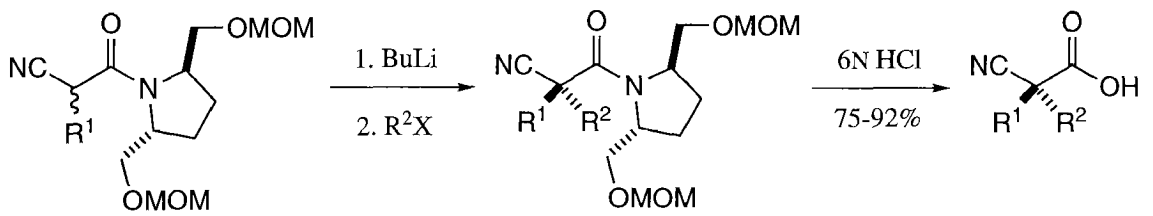
R^1 = Me, R^2 = CH_2=$CHCH_2$ (93%, d.r. = 81 : 19)
R^1 = Me, R^2 = Bn (95%, d.r. = 91 : 9)
R^1 = Me_2CH, R^2 = Bn (92%, d.r. > 98 : 2)

In *Katsuki*'s procedure, the chiral *trans*-2,5-bis(methoxymethoxymethyl)-pyrrolidine moiety accounts for high diastereoselectivity in the alkylation of the Li amide enolates[14] derived from α-alkylated α-cyanoacetic acid (*Scheme 14*) [119]. The chiral auxiliary was cleaved by treatment with 6N HCl to give enantiopure α,α-disubstituted α-cyanoacetic acid that could be transformed into the corresponding $\beta^{2,2}$-amino acid in one step by hydrogenation, following known procedures [33,118].

[13] In the published procedure [118], the $\beta^{2,2}$-amino esters were directly converted into the corresponding β-lactams by treatment with methylmagnesium bromide.

[14] It is noteworthy, that α-branched secondary amides have a low tendency to enolize due to $A^{1,3}$-strain [122-125]. A similar situation arises with α-branched ketocarboxamides with two substituents at the nitrogen atom ([126], and ref. cited therein). In the case shown above, the α-deprotonation was possible, probably due to small steric requirement of the cyano group.

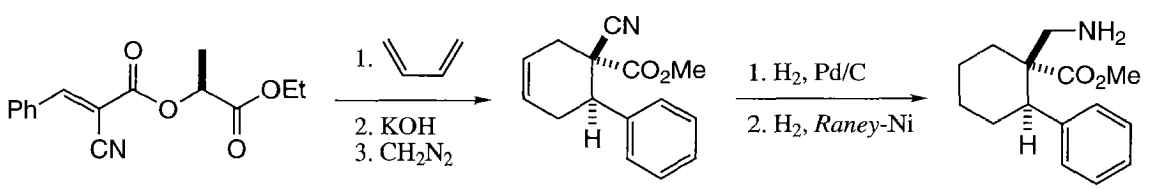
Scheme 14. Double alkylation of an α-branched cyanoacetamide enolate bearing the (2*R*,5*R*)-2,5-bis(methoxymethoxymethyl)pyrrolidine moiety as chiral auxiliary and acidic cleavage to give the enantiopure α,α-disubstituted α-cyanoacetic acid according to *Katsuki*.



R^1 = Me, R^2 = Et (96%, d.r. = 95 : 5)
R^1 = Me, R^2 = CH_2=$CHCH_2$ (96%, d.r. = 95 : 5)
R^1 = Me, R^2 = Bn (96%, d.r. = 92 : 8)

Another enantioselective synthesis of $\beta^{2,2}$-amino acids is shown in *Scheme 15*. A highly diastereoselective *Diels-Alder* reaction of 1,3-butadiene with *E*-2-cyanocinnamate of (*S*)-ethyl lactate [127] is the key step in this synthesis. After cleavage of the (*S*)-lactic acid moiety and formation of the methyl ester, the double bond was hydrogenated and the cyano group reduced with *Raney* nickel at room temperature and atmospheric pressure to give the corresponding $\beta^{2,2}$-amino acid methyl ester in enantiomerically pure form [128].

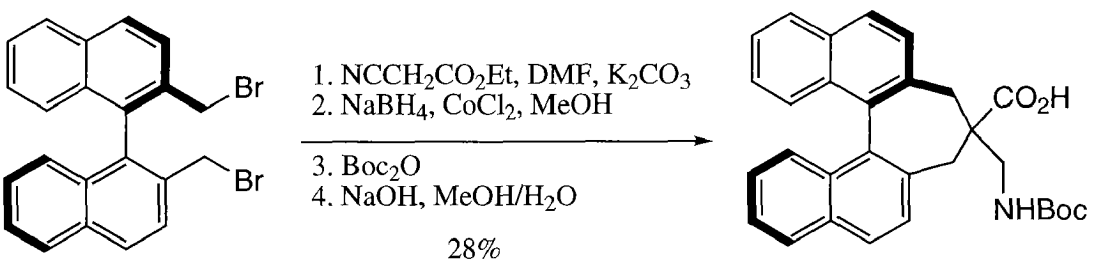
Scheme 15. Diels-Alder cycloaddition of a (*S*)-ethyl lactate with 1,3-butadiene and subsequent functional group transformations to give the enantiopure $\beta^{2,2}$-amino acid methyl ester according to *Cativiela*.



In the following synthesis (*Scheme 16*), enantiomerically[15] pure 2,2'-bis(bromomethyl)-1,1'-binaphthyl is the alkylating reagent for a double alkylation of ethyl cyanoacetate [131]. Selective cobalt-boride reduction [132,133] of the cyano group and some straightforward manipulations led to the $\beta^{2,2}$-amino acids containing a chirality axis.

[15] The starting dibromide did not racemize in boiling acetic acid for 10 minutes [129,130].

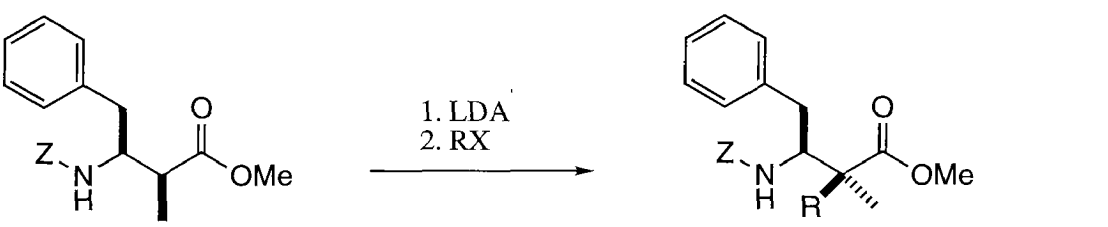
Scheme 16. Synthesis of α,α-disubstituted β-amino acids with a chirality axis, starting with dialkylation of ethyl cyanoacetate by enantiomerically pure 2,2'-bis(bromomethyl)-1,1'-binaphthyl according to *Mazaleyrat*.



2.2.3 *Chiral $\beta^{2,2,3}$-Amino Acids*

Chiral $\beta^{2,2,3}$-amino acids can be prepared either by α-alkylation of α-monoalkylated precursors or by a variety of *Mannich*-type reactions.
The *Seebach* method for α-alkylation of β-aminobutanoates[16] [134,135] was also applied for the alkylation of α,β-disubstituted β-amino acid esters with *lk*-1,2-induction [136] (*Scheme 17*) [85].

Scheme 17. Alkylation of α-methyl substituted β^3-homophenylalanine with ethyl iodide or allyl bromide to give the α,α,β-trisubstituted β-amino acid derivatives according to *Seebbach* and *Podlech*.



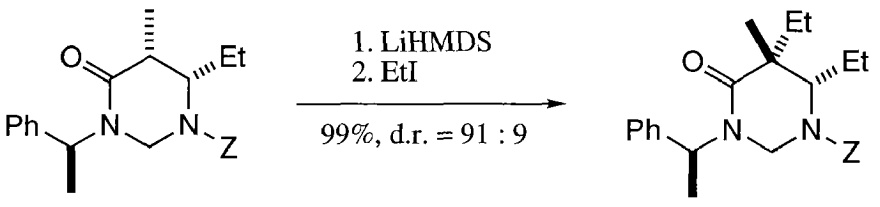
R = CH_2=$CHCH_2$ (33%, d.r. > 99 : 1)
R = Et (69%, d.r. = 5.2 : 1)

Davies obtained enantiopure $\beta^{2,2,3}$-amino acid *tert*-butyl esters by quenching the enolate derived from the conjugate addition of a chiral Li amide to an α,β-unsaturated ester with alkyl halides [137]. However, this reaction is very sensitive to steric hindrance (0% yield with EtI) and gives only moderate yields.

[16] The first example of the dialkylation of a racemic α,β-disubstituted amino acid methyl ester is also described in [134]. The d.r. was > 99 : 1.

Cardillo reported the alkylation of vicinally dialkylated perhydropyrimidin-4-ones (*Scheme 18*) [138]. The (*S*)-phenethyl-substituted perhydropyrimidin-4-one was ethylated with high yield and diastereoselectivity. Hydrolysis [139] of this *C*-trialkylated heterocycle should provide the corresponding free $\beta^{2,2,3}$-amino acid.

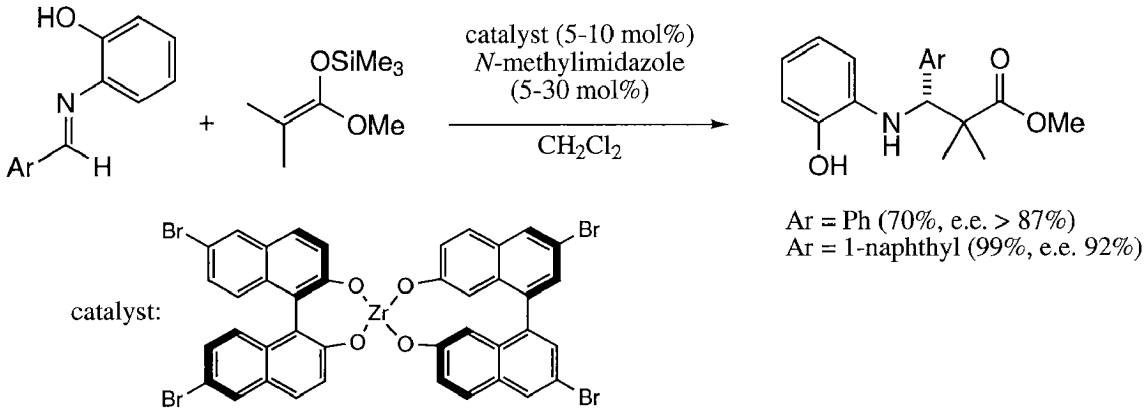
Scheme 18. Diastereoselective ethylation of a vicinally dialkylated perhydropyrimidin-4-one to give the direct precursor of an enantiopure $\beta^{2,2,3}$-amino acid according to *Cardillo*.



The following procedures for $\beta^{2,2,3}$-amino acid syntheses are *Mannich*-type reactions. Stereoselectivity arises either from a chiral catalyst or from the chirality of the substrate (for substrate-control, see [140-143]).
Recently, *Kobayashi* disclosed a catalytic enantioselective *Mannich*-type reaction of aldimines with silyl enol ethers, using a novel chiral Zr catalyst (*Scheme 19*) [144][17]. The catalyst was formed by treatment of two equivalents of (*R*)-6,6'-dibromo-1,1'-bi-2-naphthol with one equivalent of $Zr(O\textit{t}\text{-}Bu)_4$ and was applied in 5-10 mol%. Good yields and e.e.'s are obtained for imines derived from aromatic aldehydes. Removal of the 2-hydroxyphenyl group by methylation of the hydroxyl group and subsequent oxidative cleavage by cerium ammonium nitrate provides the corresponding $\beta^{2,2,3}$-amino acid methyl esters[18].

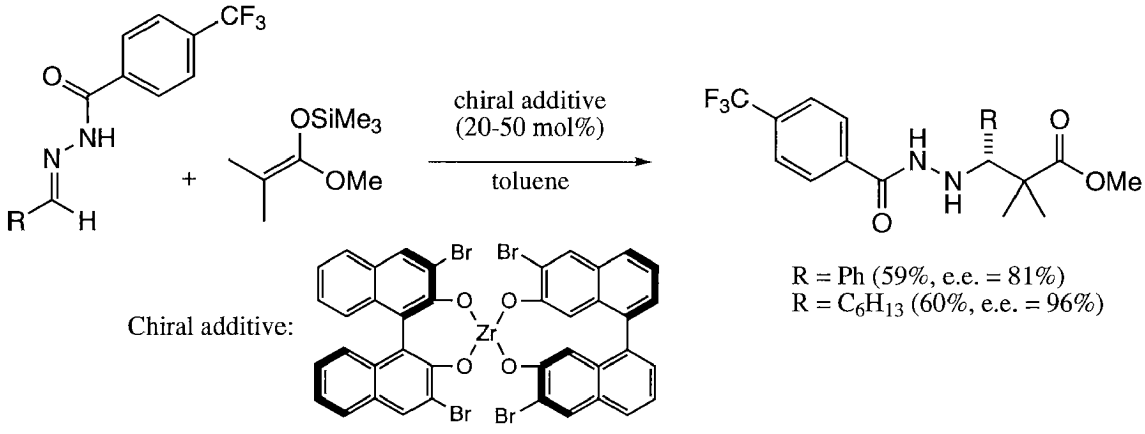
[17] *Rac*-$\beta^{2,2,3}$-amino acid derivatives were prepared by the reaction of *N*-trimethylsilylimines with ketene silyl acetals, catalyzed by tris(pentafluorophenyl)borane [145].
[18] This deprotection was described for Ar = Ph (83%) [144].

Scheme 19. Catalytic asymmetric *Mannich*-type reaction of the silyl ketene acetal derived from methyl isobutanoate with a suitably protected arylimine according to *Kobayashi*.



A variation of this method is the use of suitably protected acylhydrazones as electrophiles (*Scheme 20*) [146]. Moderate yields and good enantioselectivities are obtained with the catalyst (20 mol%) prepared from (*R*)-3,3'-dibromo-1,1'-bi-2-naphthol and Zr(O*t*-Bu)$_4$. Aliphatic imines are also tolerated. However, large amounts of catalyst are necessary; the reaction with the benzaldehyde derived imine required "50 mol% of catalyst". The $\beta^{2,2,3}$-amino acid methyl ester was liberated by reductive cleavage of the nitrogen-nitrogen bond of the hydrazino compound with samarium diiodide[19] [146].

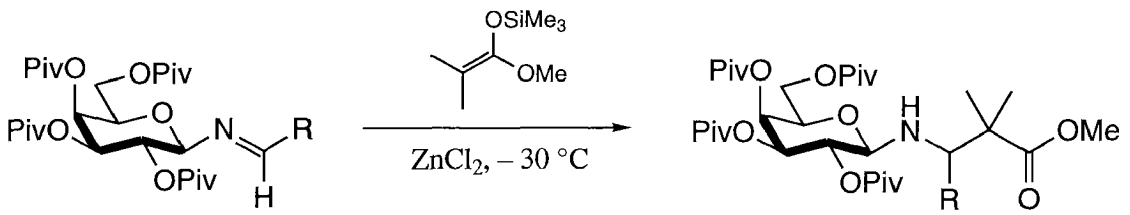
Scheme 20. Asymmetric *Mannich*-type reaction with substoichiometric amounts of a zirconate, using acylhydrazones as imine equivalents according to *Kobayashi*.



[19] Alternatively, reductive cleavage of the hydrazino moiety was successful using hydrogenation with *Raney* nickel [147].

Kunz et al. applied the same silyl ketene acetal for a highly diastereoselective *Mannich* reaction of aldimines of perpivaloylated β-D-galactopyranosylamine (*Scheme 21*) [148]. The β-amino acid esters[20] were released from the carbohydrate auxiliary in almost quantitative yield by treatment with methanolic HCl.

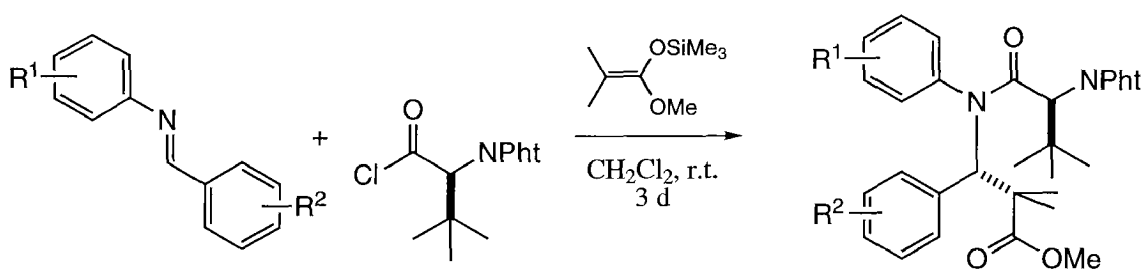
Scheme 21. Diastereoselective *Mannich* reaction of a β-D-galactosylamine derived aldimine according to *Kunz*.



R = Ph (89%, d.r. = 150 : 1)
R = Pr (83%, d.r. = 250 : 1)

In-situ generated chiral acyliminium ions derived from *N,N*-phthaloylamino acids were successfully used for the preparation of $\beta^{2,2,3}$-amino acid derivatives (*Scheme 22*) [150]. The reaction is highly diastereoselective, tolerating a wide range of aromatic substituents. A two-step procedure furnished the *N*-arylated $\beta^{2,2,3}$-amino acid ester: after removal of the phthaloyl group, the free amino group was subjected to an *Edman* degradation providing the *N*-arylated product [150].

Scheme 22. One-pot *Mannich* reaction according to *Waldmann et al.*.



$R^1 = R^2 = H$ (54%, d.r. = 93 : 7)
R^1 = 2-MeO, 6-Me, R^2 = 4-MeO (75%, d.r. > 99 : 1)

[20] Their configuration still remains to be assigned [149].

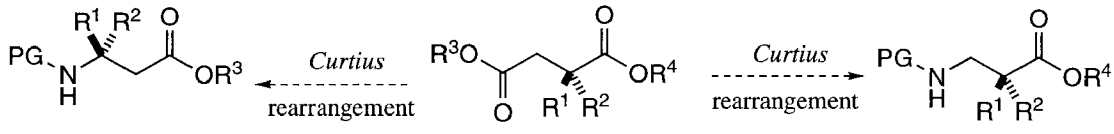
2.3 New Routes to Enantiomerically Pure $\beta^{2,2}$- and $\beta^{3,3}$-Amino Acids

Most of the methods for the enantioselective synthesis of geminally disubstituted β-amino acids presented so far are limited to specific residues and give either $\beta^{2,2}$- or $\beta^{3,3}$-amino acids. This inspired us to look for a more general approach for both substitution patterns. We envisaged an enantioselective route, the key step of which is the asymmetric synthesis of geminally disubstituted succinates. This approach is outlined below.

2.3.1 *Chiral 2,2-Dialkyl Succinates: Versatile Precursors to Chiral Geminally Disubstituted β-Amino Acids*

The solution to this problem emanates from research in *Seebach*'s laboratory dealing with the diastereoselective alkylation of β-heterosubstituted carbonyl compounds via dianion species [151-156]. Both β-hydroxycarboxylic esters [157-162] and β-aminocarboxylic esters [76,163] were alkylated with high diastereoselectivities[21]. During these studies, a convenient method for the synthesis of enantiopure geminally disubstituted malates and succinates was developed [159,163]. It seemed conceivable that these can be transformed into both $\beta^{2,2}$- and $\beta^{3,3}$-amino acids via a *Curtius* rearrangement, after liberating the free carboxy group regioselectively (*Scheme 23*).

Scheme 23. The concept: monoalkylesters of geminally dialkylated succinates as versatile precursors of both $\beta^{2,2}$- and $\beta^{3,3}$-amino acids.



R^3 = Alkyl, R^4 = H
$\beta^{3,3}$-amino acid derivative

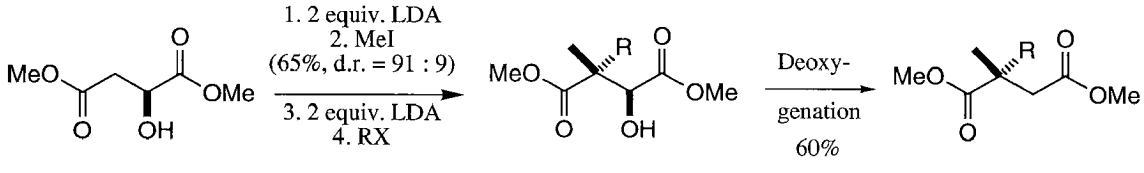
half-esters of 2,2-dialkylsuccinate

R^3 = H, R^4 = Alkyl
$\beta^{2,2}$-amino acid derivative

[21] For a recent review of the alkylation of ester enolates [164].

The synthesis of a dialkyl succinate begins with the highly diastereoselective alkylation of the enolate derived from dimethyl (*S*)-malate in 65% yield[22] (*Scheme 24*) [159,160]. A second alkylation step with either ethyl iodide or allyl bromide provided a geminally dialkylated dimethyl malate in excellent diastereoselectivity[23]. Both alkylation steps proceed with relative topicity *u l* [136]. The product can be deoxygenated either by reduction of the corresponding xanthate ester in 60% yield [159,165], or by hydroxyl/chloride exchange and reductive halide removal using zinc in acetic acid[24] [166]. Alkaline hydrolysis of the *gem*-disubstituted dimethyl succinate should give the half-ester containing an α,α-disubstituted ester preferentially, according to similar hydrolyses reported in the literature [167]. This half-ester is a direct precursor to $\beta^{2,2}$-amino acid derivatives as outlined in *Scheme 23*.

Scheme 24. Diastereoselective α-dialkylation of dimethyl (*S*)-malate with *ul* 1,2-induction, and subsequent deoxygenation to give enantiopure geminally disubstituted dimethyl succinates, according to *Seebach* and *Wasmuth*.



Dimethyl (*S*)-malate

R = Et (36%, d.r. = 95 : 5)
R = CH_2=$CHCH_2$ (74%, d.r. = 95 : 5)

A. I. Meyers devised an asymmetric synthesis of 2,2-dialkyl carboxylic esters [168-170] as shown in *Scheme 25*. The 2,2-dialkyl-4-oxo-carboxylic esters can be smoothly transformed into the corresponding succinates by ozonolysis, followed by basic work-up with hydrogen peroxide[25]. The synthesis starts with the alkylation of a chiral bicyclic lactam, which was prepared in 85% yield by condensing (*S*)-valinol with 3-benzoylpropionic acid. LDA enolate formation and addition of various alkyl halides gave good yields of the 2-alkyl derivatives. Treatment of this mixture of diastereoisomers with LDA and another alkyl halide provided the α,α-dialkylated lactam unit in good yields and good diastereoselectivities. Acidic hydrolysis gave the 2,2-dialkyl 4-oxo-carboxylates.

---

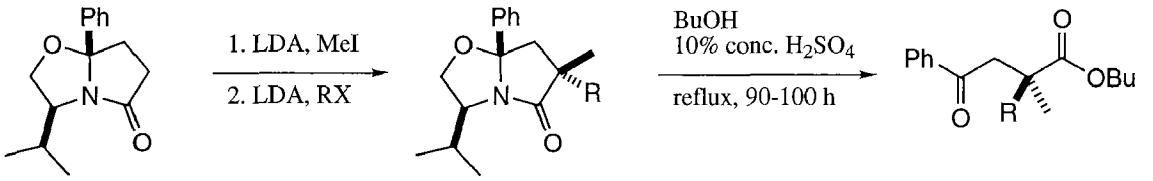
[22] The methylation of (*S*)-diethyl malate proceeds in 88% yield [157].

[23] It is essential to introduce the sterically less demanding substituent first: *ethyl*ation of dimethyl malate proceeds with 90% ds, but subsequent *methyl*ation provides the product as a 72 : 28 mixture [159].

[24] The authors [166] report a 60-70% overall yield on a multi-gram scale, starting from diethyl malate (for R = CH_2=$CHCH_2$).

[25] This was demonstrated for the methyl ethyl derivative (R = Et) [168].

Scheme 25. Diastereoselective dialkylation of a chiral bicyclic lactam and ensuing hydrolysis to 2,2-dialkylcarboxylates according to *Meyers*.



R = Bn (75%, d.r. = 42 : 1)
R = i-Pr (59%, d.r. = 3 : 1)
R = Et (80%, d.r. = 7.5 : 1)

R = Bn (79%)
R = Et (71%)

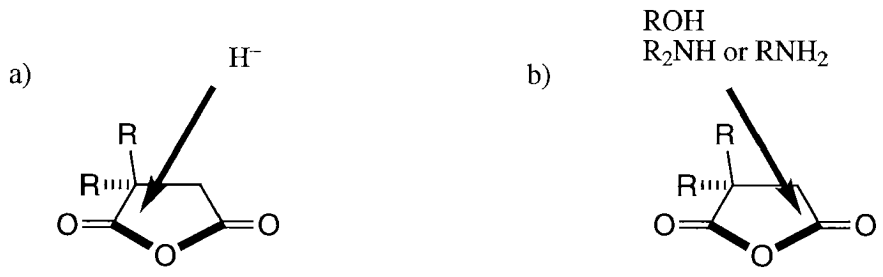
Ideally, both half-esters (see *Scheme 23*) should be available from the same precursor. There is literature precedent of regioselective nucleophilic ring-opening of unsymmetrical cyclic anhydrides (*Figure 1*). Hydride ions are reported to attack preferentially at the carbonyl adjacent to the disubstituted carbon [171]. However, *O*- [172-178] and *N*-nucleophiles [179,180] give rise to the regioisomeric products; heating cyclic geminally disubstituted anhydrides in absolute alcohol or in alcohol with catalytic amounts of concentrated sulfuric acid [167] gives the mono-ester resulting from attack at the carbonyl adjacent to the methylene group with high regioselectivity[26]; treatment of the cyclic anhydride with ammonia or an ethereal solution of the amine gives the α,α-disubstituted acid [179]. Ring opening by sodium methoxide is less regioselective, resulting in a mixture of both half-esters [181]. According to theoretical studies of the regioselectivity in metal hydride reductions of unsymmetrical cyclic anhydrides [171,182], the LUMO coefficient on the carbon of the carbonyl group next to the disubstituted carbon is considerably larger than the coefficient on the other carbonyl carbon atom[27] [171]. This is in agreement with experiment. This same type of regioselectivity was predicted before [183,184] on the basis of the nonperpendicular approach, along the *Bürgi-Dunitz* trajectory [185-188]. In contrast, the opposite regioselectivity observed for anhydride opening by alcohols or amines, is not yet understood[28].

[26] The minor regioisomer (α,α-disubstituted ester) could be separated by virtue of its greater solubility in petroleum ether [175].

[27] The authors note that other factors such as chelating effects by cations or protonation have to be taken into consideration.

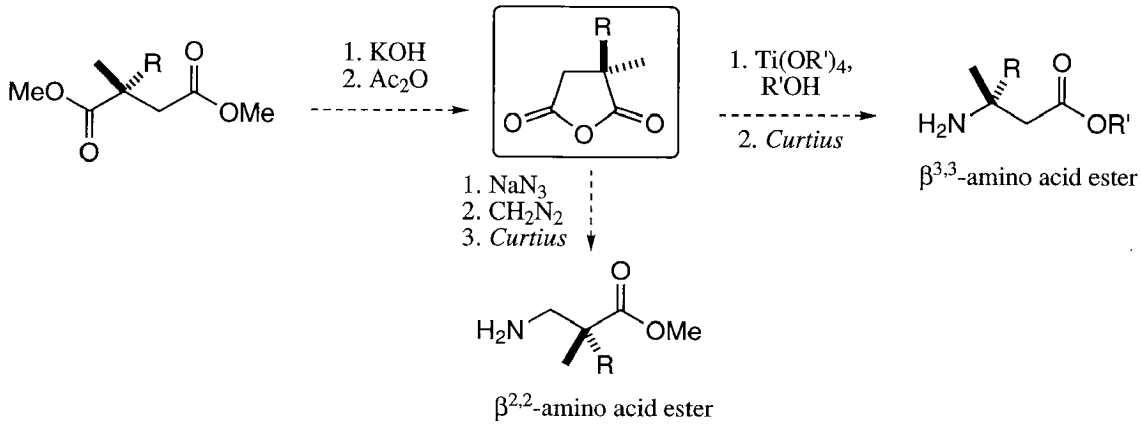
[28] Taking into account the *Bürgi-Dunitz* trajectory, the approach of the nucleophile to the carbonyl group adjacent to the methylene group (b) in *Figure 1*) should be strongly hindered.

Figure 1. Observed regioselectivity in nucleophilic ring opening of unsymmetrical cyclic anhydrides. Direction of the approach of hydride ions (a) and *O*- or *N*-nucleophiles (b).



On the basis of this precedent, the following path to both $\beta^{2,2}$- and $\beta^{3,3}$-amio acids can be envisaged (*Scheme 26*). Cyclization of the diester of chiral geminally disubstituted succinates (*Schemes 24*) should be possible by treatment with acetic anhydride [175,176], with acetyl chloride [189,190] or with thionyl chloride [167]. The regioselectivity of anhydride opening with alcohols should be improved by using titanates [191,192]. The free acid can then be subjected to a *Curtius* rearrangement to give the enantiopure $\beta^{3,3}$-amino acid ester. On the other hand, treatment of the chiral anhydride with azide should furnish the corresponding α,α-disubstituted acid, which can be esterified and rearranged to the enantiomerically pure $\beta^{2,2}$-amino acid ester.

Scheme 26. Chiral *gem* disubstituted cyclic anhydrides as versatile precursors of enantiopure $\beta^{2,2}$- and $\beta^{3,3}$-amio acids.



3 Project

". . . Science is not an abstract thing, but rather, as a product of human labor, is tightly bound in its development to the particularity and fate of the individuals who dedicate themselves to it."

Emil Fischer [193]

In March 1996, *Seebach* and *coworkers* disclosed that a β-hexapeptide consisting of Val-, Ala-, and Leu-homologues adopts a stable helical secondary structure in solution [17]. In the same year, *Gellman* showed that the same 3_{14} helix is adopted by a conformationally restricted β-hexapeptide in the solid state [19]. These results received all the more attention considering that conventional α-peptides are disordered below a chain length of 10-15 residues and that *Seebach*'s peptide was composed of β-amino acids bearing proteinogenic side chains which allow for free rotation around the C(α)–C(β) bond.

These results provided the starting point for this thesis. The goal was to dictate the folding propensities of β-peptides by incorporating the prerequisite β-amino acids with proteinogenic side chains. Thus, new β-peptidic secondary structures were expected.

The first objective was to create parallel pleated sheet structures, a major secondary structure of α-peptides from β-amino acid building blocks of suitable configuration. Second, the ability of β-peptidic segments to form reverse turns would be evaluated in order to construct an antiparallel sheet structure in attached β-peptide strands (hairpins). With suitably α,β-disubstituted β-amino acids at hand, another aim was to assess the stability of a 3_{14} helix with substituents at every backbone C-atom.

Encouraged by the high crystallinity of polypeptides from α-amino acids that are disubstituted at the C(α)-atom [3], β-peptides consisting of geminally disubstituted β-amino acids should provide suitable crystals for X-ray analysis to gain insight into their structural features. The fact that this substitution pattern did not fit in the secondary structures found to date, gave an additional stimulus for this project. Further targets were β-peptides composed of alternating (*S*)- and (*R*)-β^3-amino acids.

In another line of work, the synthesis and structural studies of β-peptides unable to form H-bonds was investigated to ascertain the role of H-bonds in stabilizing secondary β-peptide structures.

For further exploration of the structural versatility of β-peptides, and in order to provide rapid access to a larger and more diverse set of β-peptides, the solid-phase synthesis of β-peptides was evaluated and used for the preparation of β-peptides.

Potential applications of β-peptides as physiologically active agents will be favored by their resistance to enzymatic degradation. Therefore, *in vivo* studies with some of the synthesized β-peptides were expected to furnish the necessary pharmacokinetic parameters.

4 Preparation of β-Amino Acids

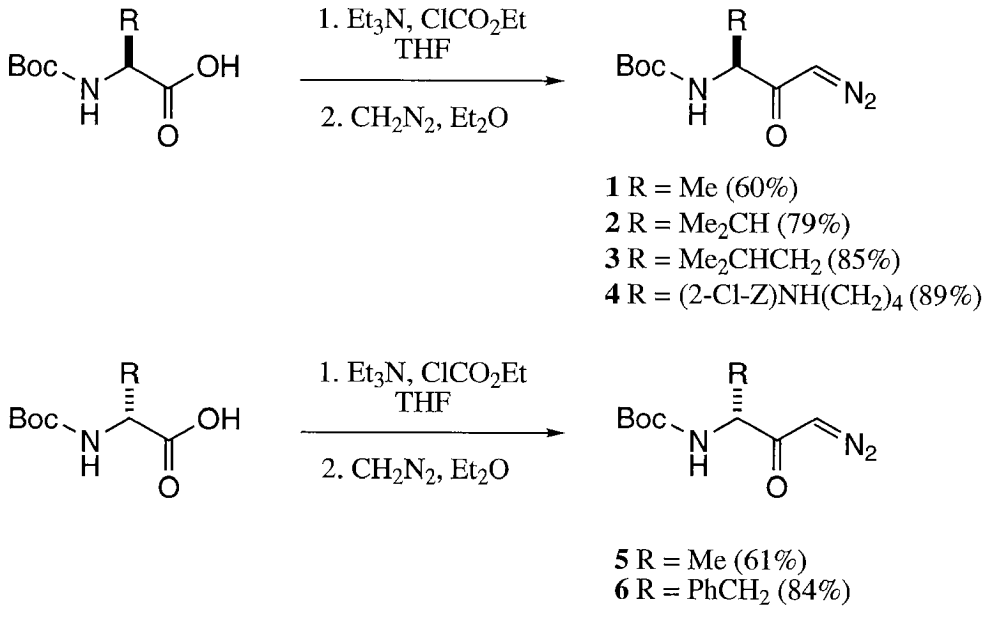
To study the folding of β-peptides into different secondary structures, a variety of β-amino acid building blocks was required. Thus, an arsenal of new β-amino acids with different substitution patterns was prepared to provide the prerequisite building blocks for both solution and solid-phase β-peptide coupling. Apart from the Boc- and Fmoc-protected β^3-amino acid derivatives, various α,β-disubstituted β-amino acids with *like-* (or *l*) and *unlike-* (or *u*)[29] configuration were synthesized. Straightforward methods supplied the geminally disubstituted β-amino acids and a resolution procedure gave the enantiomerically pure secondary β-amino acid β^2-homoproline.

4.1 Preparation of Boc-Protected β^3-Amino Acids

Boc-protected β-amino acids served as building blocks for β-peptide coupling in solution whereas Fmoc-protected compounds (*Chapter 4.2*) were used for coupling on solid phase.
Enantiomerically pure, naturally occurring α-amio acids served as starting materials ("pool of chiral building blocks" [196]) for the preparation of β^3-amino acid derivatives. Since the early 1950's, the *Arndt-Eistert* approach has been used for the synthesis of β^3-amino acids [197]. This protocol was adapted to Boc- and Z-protected amino acids [198,199]. By applying methods well-established in our group [85,86], the commercially available Boc-protected amino acids Boc-Ala-OH, Boc-Val-OH, Boc-Leu-OH, Boc-Lys(2-Cl-Z)-OH and Boc-D-Ala-OH and Boc-D-Phe-OH were converted in a one-pot reaction to the diazoketones **1-6** in good yields, via mixed anhydrides (using $Et_3N/ClCO_2Et$) (*Scheme 27*) [200].

[29] For stereochemical nomenclature see [136,194,195].

Scheme 27. Preparation of diazoketones **1-6** starting from *N*-Boc-protected α-amino acids.

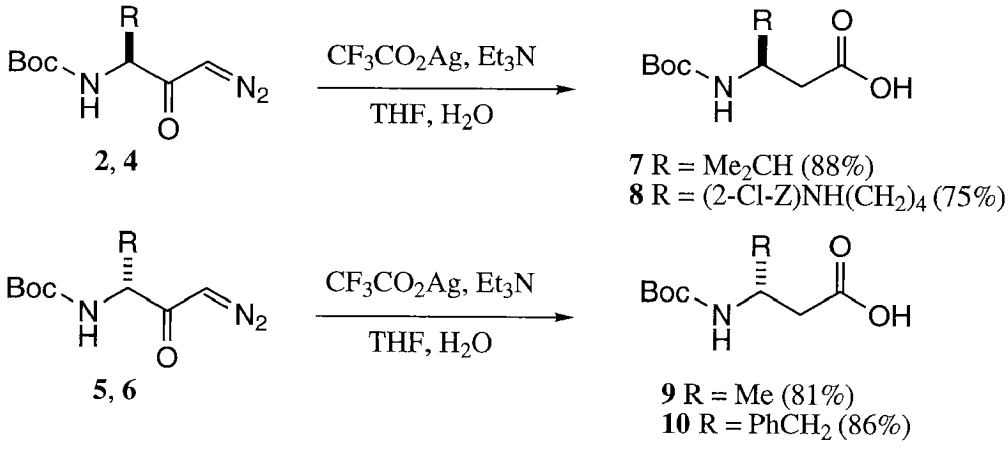


1 R = Me (60%)
2 R = Me_2CH (79%)
3 R = Me_2CHCH_2 (85%)
4 R = (2-Cl-Z)NH$(CH_2)_4$ (89%)

5 R = Me (61%)
6 R = $PhCH_2$ (84%)

The diazoketones of (*S*)- (**2** and **4**) and of (*R*)- (**5** and **6**) configuration were decomposed in a mixture of THF and H_2O, mediated by catalytic amounts of Ag^+ (CF_3CO_2Ag dissolved in Et_3N[30]) to give the Boc-protected β^3-amino acids β^3-(*R*)-HVal-OH (**7**), β^3-(*S*)-HLys(2-Cl-Z)-OH (**8**), β^3-(*R*)-HAla-OH (**9**) and β^3-(*R*)-HPhe-OH (**10**), in good yields after recrystallization (*Scheme 28*) [200].

Scheme 28. Wolff rearrangement of diazoketones **2, 4, 5** and **6** in aqueous THF to give the *N*-Boc-protected β^3-amino acids **7-10**.



2, 4

7 R = Me_2CH (88%)
8 R = (2-Cl-Z)NH$(CH_2)_4$ (75%)

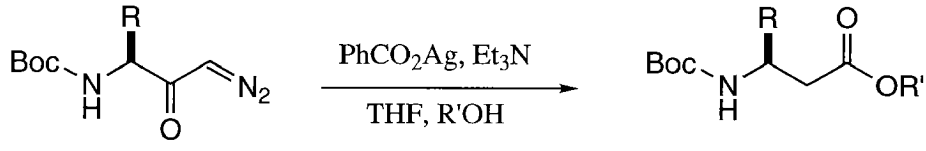
5, 6

9 R = Me (81%)
10 R = $PhCH_2$ (86%)

[30] The rearrangement to the free β-amino *acids* (**7-10** in *Scheme 28*) is preferentially carried out with CF_3CO_2Ag instead of the less expensive $PhCO_2Ag$ (see *Scheme 29*), otherwise isolation is hampered by the presence of benzoic acid.

Access to the β^3-amino acid esters **11-16** was secured by carrying out the *Wolff* rearrangement in the presence of the corresponding alcohol (MeOH for **11-14** and BnOH for **15** and **16**), catalyzed by $PhCO_2Ag$ (*Scheme 29*). The β^3-amino acid methyl esters **11-13** are oils [27], the β^3-HLys(2-Cl-Z)-methyl ester **14** and the benzyl esters **15** and **16** are crystalline solids. The esters were isolated in 70-95% yields after purification by chromatography.

Scheme 29. Wolff rearrangement of diazoketones **1-4** in THF/ROH to give the *N*-Boc-protected β^3-amino acid methyl (**11-14**) and benzyl (**15, 16**) esters.



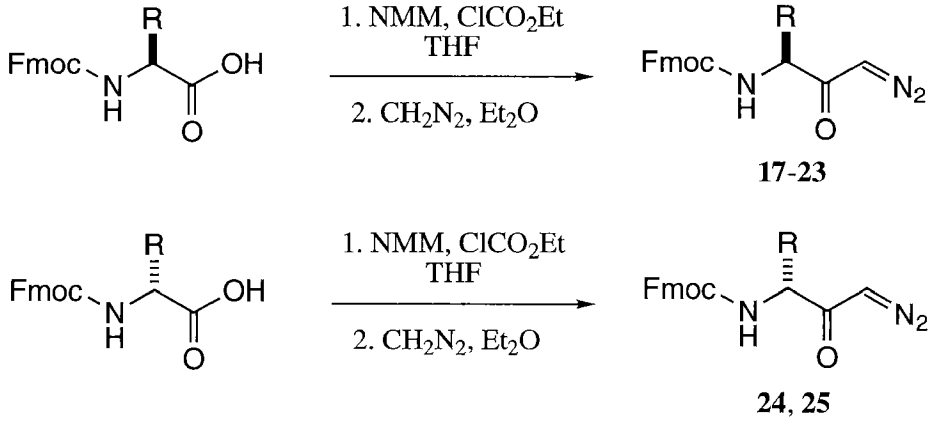
1-4

11 R = Me, R' = Me (88%)
12 R = Me_2CH, R' = Me (88%)
13 R = Me_2CHCH_2, R' = Me (95%)
14 R = (2-Cl-Z)NH$(CH_2)_4$, R' = Me (86%)
15 R = Me_2CH, R' = Bn (70%)
16 R = Me_2CHCH_2, R' = Bn (81%)

4.2 Preparation of Fmoc-Protected β^3-Amino Acids

Fmoc-protected β^3-amino acids were required for solid-phase β-peptide synthesis. The applicability of the *Arndt-Eistert* homologation procedure for Fmoc-protected amino acids had not yet been evaluated. It has been suggested that this procedure was not ideally suited to amino acids bearing Fmoc protecting groups, due to the sensitivity of the Fmoc group towards amines [201,202]. However, the synthesis of the Fmoc-protected diazoketones **17-25** was successfully achieved by exchanging Et_3N for the weaker base NMM (*Scheme 30*).

Scheme 30. Preparation of diazoketones **17-25** starting from *N*-Fmoc-protected α-amino acids.



	R	Diazoketone	Yield (%)	M.p. (°C)
1	Me_2CH	**17**	57[a)]	125-127
2	Me_2CHCH_2	**18**	87[a)]	90-91
3	*t*-$BuOCH_2$	**19**	93	Oil
4	*t*-$BuO_2C(CH_2)_2$	**20**	88	138.5-139.5
5	$BocNH(CH_2)_4$	**21**	89[a)]	117-118
6	$BocNH(CH_2)_3$	**22**	80	115[b)]
7	*t*-$BuO\text{-}C_6H_4\text{-}CH_2$	**23**	69	120.5-122.5
8	Me	**24**	71	116-117
9	$PhCH_2$	**25**	83	133-134

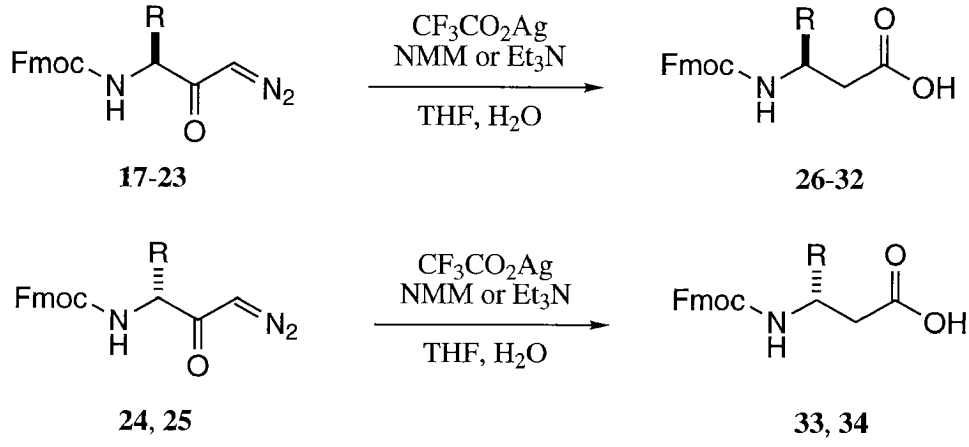
a) These diazoketones were first characterized by *G. Guichard* [14]. b) Sintering at 86 °C.

The mixed anhydrides from commercially available Fmoc-protected α-amino acids Val, Leu, Ser(*t*-Bu), Glu(*t*-Bu), Lys(Boc), Orn(Boc), Tyr(*t*-Bu), D-

Ala and D-Phe were directly converted to the corresponding Fmoc-protected diazoketones **17-25** in good-to-excellent yields (*Scheme 30*) [200,203]. Except for the serine derivative **19** all diazoketones were yellow solids. Nevertheless, purification by chromatography was necessary to remove a major impurity (up to 15%)[31], formed by partial hydrolysis of the mixed anhydride [204] (moisture in the ethereal CH_2N_2 solution!).

Scheme 31 outlines the decomposition of the diazoketones **17-25** in THF containing 10% H_2O with catalytic amounts of CF_3CO_2Ag (dissolved in NMM or Et_3N).

Scheme 31. Wolff rearrangement of diazoketones **17-25** to give *N*-Fmoc-protected β³-amino acids **26-34**.



17-23 **26-32**

24, 25 **33, 34**

	R	Fmoc-β³-HXaa-OH	Yield[a)] (%)	M.p. (°C)
1	Me_2CH	**26**	75[b)]	158
2	Me_2CHCH_2	**27**	50[b)]	106-107
3	*t*-$BuOCH_2$	**28**	70 (63)	96-98
4	*t*-$BuO_2C(CH_2)_2$	**29**	71 (47)	58-60
5	$BocNH(CH_2)_4$	**30**	60[b)]	97
6	$BocNH(CH_2)_3$	**31**	63	104[c)]
7	*t*-$BuO\text{-}C_6H_4\text{-}CH_2$	**32**	74	190-191[c)]
8	Me	**33**	73	165-166.5[d)]
9	$PhCH_2$	**34**	39	186-190

a) Yield in brackets: Et_3N was used instead of NMM for the *Wolff* rearrangement. b) These amino acids were first characterized by *G. Guichard* [203]. c) Decomposition. d) Sintering at 125 °C.

[31] The formation of surprisingly large amounts of methyl ester has been reported in [202] (up to 38% starting from Fmoc-Ala-OH).

The Fmoc-protected β^3-amino acids **26-34** were isolated in good yields (*Scheme 31*). In some cases [203], a significant loss of the Fmoc group was observed under these conditions[32]. Therefore, milder conditions for the *Wolff* rearrangement were tested. The use of NMM led to significant improvements: diazoketone **19** and **20** were cleanly converted to **28** and **29** in ca. 70% yield [203]. After recrystallization, the Fmoc-β^3-amino acids could be directly used for solid-phase synthesis. Like the analogous α-amino acid derivatives [205], the Fmoc-protected β-amino acids **26-34** are poorly soluble. Their characterization required chromatographic purification (see *Exp. Part*). The *N,N'*-diphthalyl-[206], *N,N'*-dibenzyloxy- and *N,N'*-di-*tert*-butyloxycarbonyl [207] derivatives of **31** have already been synthesized in order to confirm the structure of "isolysine"[33], a hydrolysis product of several antibiotics. However, since orthogonality of the amino protecting groups in these compounds is not secured, the β-Fmoc-/ε-Boc-protected amino acid **31** that can be employed in Fmoc-coupling reactions was prepared. Like its α-analogues [205], the β^3-homotyrosine derivative **32** has a low solubility in common organic solvents (CH_2Cl_2, $CHCl_3$, AcOEt).

After this work was completed, several reports on the preparation of *N*-Fmoc-protected β^3-amino acids appeared. *Liguori et al.* published physical data of diazoketones and Fmoc-β^3-amino acids that strongly contradicted the data obtained independently by us [202]. *Marti et al.* used the crude Fmoc-diazoketones for homologation on solid support [208]. In addition, the preparation of Fmoc-diazoketones [209] and *N*-Fmoc-protected β^3-amino acids [210] were reported; in one case, the *Wolff* rearrangement was promoted by ultrasound [211].

[32] For a given reaction time, it was found that the extent of Fmoc loss was dependent upon the nature of the side chain. Especially, Fmoc-β^3-HGlu(*t*-Bu)-OH **29** was isolated in poor yield (47%), due to extensive Fmoc cleavage.

[33] 3,6-Diaminohexanoic acid, often called β-lysine, is a constitutional isomer of lysine. According to the nomenclature used for β-amino acids [27], this compound is β^3-HOrn.

4.3 Preparation of *like*- and *unlike*-$\beta^{2,3}$-Amino Acids

4.3.1 α-Alkylation of Boc-β³-Amino Acid Methyl Esters 11-13

Of the methods available for the EPC synthesis (syntheses of enantiomerically pure compounds [212,213]) of 2-substituted 3-amino-carboxylic acids, the α-methylation of Boc-protected β^3-amino acid esters through doubly lithiated species **XVIII** was chosen (*Scheme 32*). With 3-amino-*N*-*benzoyl*butanoates and in the presence of LiCl, the reaction is highly selective with relative topicity[34] *lk* [134,135]. The Boc-derivatives **11-13** which undergo methylation with poor selectivity were used for the simple reason that both epimers were needed. It is noteworthy that β^3-homoalanine derivatives **35** and **36** could not be separated by flash chromatography. By applying condition **A** (without Li salt) the diastereomer ratio, as determined by ^{1}H-NMR spectroscopy was ca. 2 : 1 in favour of *like*- (or *l*) compound **35**. Finally, **35** and **36** were separated by preparative HPLC (see *Exp. Part*) to provide enough material for peptide synthesis and characterization [18]. However, the epimeric products with the side chains of valine and leucine, **37/38** and **39/40**, could be isolated in pure form by flash chromatography. The diastereoselectivities in α-methylations of methyl esters **12** and **13** could be influenced by the addition of either LiX salts [214] or of the co-solvent DMPU [215]; *l*-$\beta^{2,3}$-amino acid esters **37** and **39** were obtained in 61 and 75% diastereoselectivity with Li salt addition[35]. Methylation in the presence of DMPU[36] gave the *unlike*- (or *u*) -derivatives **38** and **40** in 75 and 68% diastereoselectivity (*entries 2, 4*). All alkylation reactions gave yields between 75 and 90% on a 20 mmol scale[37].

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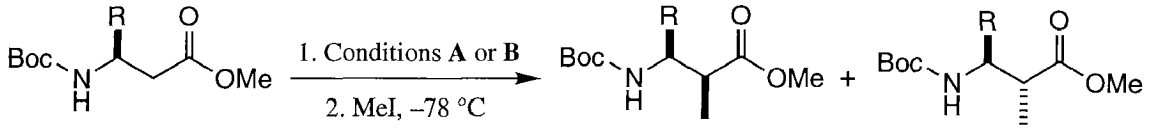
[34] For stereochemical nomenclature see [136,195].

[35] Without Li salts, the d.r. was only slightly different.

[36] DMPU has to be added before the addition of BuLi (condition **B**). Inversed order of addition resulted in a white precipitate that was reversibly dissolved and re-precipitated by warming up to – 10 °C and recooling to – 78 °C.

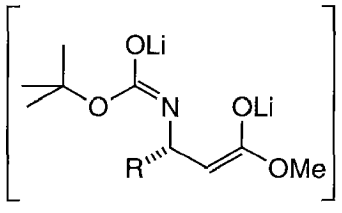
[37] It is essential to carry out the methylations below – 70 °C and to use exact amounts of BuLi (freshly titrated, see *Exp. Part*), otherwise *N*-methylated products were formed which could not be separated.

Scheme 32. Methylation of β³-amino acid methyl esters **11-13** through doubly lithiated derivatives **XVIII** to provide *like*- and *unlike*-β²,³-amino acid methyl esters. The configuration of the enolate and imino-carboxylate C=C and C=N bonds in **XVIII** is unknown and is drawn arbitrarily.



11-13 *like* (*l*) *unlike* (*u*)

35 R = Me **36** R = Me
37 R = Me_2CH **38** R = Me_2CH
39 R = Me_2CHCH_2 **40** R = Me_2CHCH_2



XVIII

Conditions **A**: 3 equiv. LiBr (R = Me_2CH) or LiCl (R = Me_2CHCH_2), 2.2 equiv. $(i\text{-}Pr)_2NH$, 2.2 equiv. BuLi, THF, – 78 °C.
Conditions **B**: 2.2 equiv. $(i\text{-}Pr)_2NH$, 4 equiv. DMPU, 2.2 equiv. BuLi, THF, – 78 °C.

	R	Yield[a)] (%)	Conditions	d.r.[b)] *u*:*l*[c)]
1	Me_2CH	90	**A**	**38** : **37** = 1.6 : 1
2	Me_2CH	90	**B**	**38** : **37** = 3 : 1
3	Me_2CHCH_2	75	**A**	**40** : **39** = 1 : 3
4	Me_2CHCH_2	83	**B**	**40** : **39** = 2.1 : 1

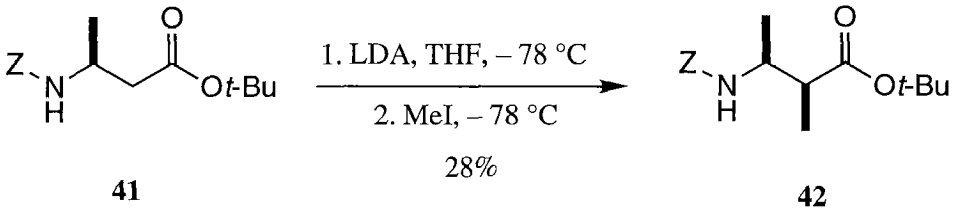
a) Isolated total yield of the two epimers. b) Determined by ^{1}H-NMR spectroscopy (200, 300 MHz). c) Epimers were separated by flash-chromatography.

It is interesting to note that there are only few reports on the synthesis of β²,³-amino acid derivatives of *unlike*-configuration[38]: enolate/imine reactions [218,219], reduction of oxime precursors [220] or β-enamio esters [221,222], the *Staudinger* reaction leading to *cis*-β-lactams [223-225], *Michael* addition followed by enolate trapping by electrophiles [226,227], and *Davies*' protocol of the conjugate addition to tiglic acid esters [137] have to be mentioned here.
Some β²,³-amino acids are found in bioactive compounds for example from marine microalgae [228] or marine sponges [229].

[38] Disregarding aspartate β-alkylations [76,216,217].

In order to check if other protecting groups facilitate the chromatographic separation of α-methylated β^3-homoalanine esters, **41**[39] was methylated to give the (2S,3S)-$\beta^{2,3}$-amino acid *tert*-butyl ester **42** after chromatographic purification (*Scheme 33*). However, the C(α)-epimer (not shown) has the same R_f value on thin layer chromatography (TLC).

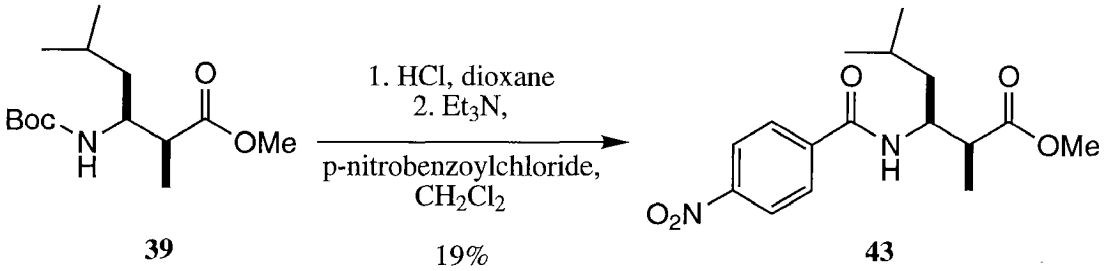
Scheme 33. α-Methylation of Z-(*S*)-β^3-HAla-O*t*-Bu **41** to give the *l*-$\beta^{2,3}$-amino acid derivative **42**.



41 **42**

The configuration of the $\beta^{2,3}$-homoalanine derivatives **35** and **36** was established by comparison with published compounds [18,134]. However, the relative configuration of $\beta^{2,3}$-amino acid esters **37/38** and **39/40** was not known. The exchange of the Boc- for acyl groups which are known to promote crystallization, was supposed to supply crystals for X-ray analysis which could unveil the relative configuration of epimeric pairs[40]. Thus, Boc-deprotection of **39** and acylation with *p*-nitrobenzoylchloride gave **43** as colorless needles[41], which were, unfortunately, not suitable for an X-ray crystal structure determination.

Scheme 34. Preparation of *p*-nitrobenzoylated $\beta^{2,3}$-amino acid methyl ester **43**.



39 **43**

In a series of experiments, α-methylation of β^3-amino acid benzyl esters **15** and **16** (see *Scheme 29*) was tried. In all cases, the conditions required for enolate formation led to extensive decomposition of the starting material.

---

[39] Compound **41** was generously provided by *A. Boog*.

[40] $\beta^{2,3}$-Amino acid methyl esters **37-40** are either oils or waxy solids.

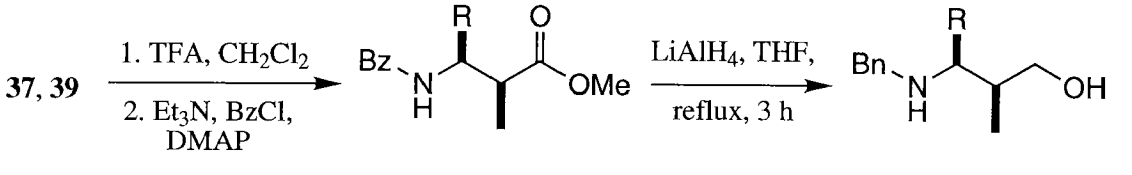
[41] The poor yield is caused by a competing acylation reaction of 2-(2-chloroethoxy)ethanol present in an old HCl/dioxane solution.

Otherwise, enolate formation was incomplete. Boc-β^3-HLys(2-Cl-Z)-OMe (**14**) was also examined for its use in the α-methylation with 3 equivalents of LDA; either enolate formation was not complete, as determined by enolate quenching with D_2O, or *N*-methylation occured. Therefore, all following methylations were carried out with methyl esters (*Scheme 32*).

4.3.2 Configurational Assignment of $\beta^{2,3}$-Amino Acid Esters

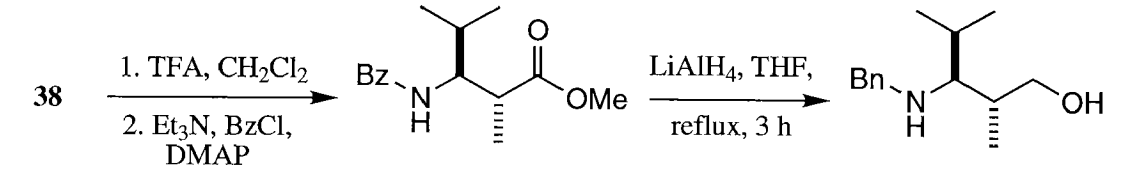
As suitable crystals could not be obtained from the acyclic $\beta^{2,3}$-amino acid derivatives **37-39**, they were converted to heterocycles (*Scheme 35, 36*). In the event, *l*-$\beta^{2,3}$-amino acid methyl esters **37** and **39** were Boc-deprotected and benzoylated to provide **44** and **45**, respectively, the ester and amide functions of which were successively reduced with $LiAlH_4$ in refluxing THF to give the *N*-benzylated $\beta^{2,3}$-amino alcohols **46** and **47** in good yields after aqueous work-up (*Scheme 35*). Similarly, the *u*-$\beta^{2,3}$-amino acid methyl ester **38** was smoothly transformed to the benzoylated $\beta^{2,3}$-amino acid methyl ester **48** which was reduced to the $\beta^{2,3}$-amino alcohol **49**.

Scheme 35. Preparation of *N*-benzylated $\beta^{2,3}$-amino alcohols **46**, **47** and **49**.



44 R = Me_2CH (93%)
45 R = Me_2CHCH_2 (72%)

46 R = Me_2CH (73%)
47 R = Me_2CHCH_2 (99%)

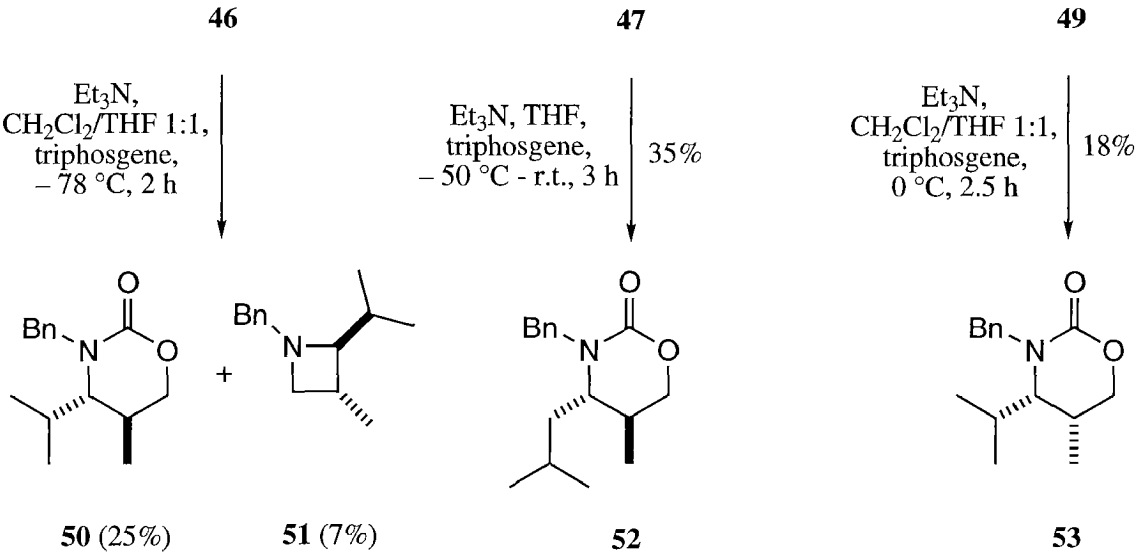


48 (92%)

49 (67%)

Crude *N*-benzylated $\beta^{2,3}$-amino alcohols **46**, **47** and **49** were treated with triphosgene[42] under various conditions (*Scheme 36*). Thus, **46**, triphosgene and Et_3N reacted at – 78 °C and quenching of the reaction mixture at this temperature gave the desired heterocycle **50** (50%), together with *N*-benzylated azetidine **51** (7%), resulting from attack of the secondary amine at the primary carbon of the intermediate (trichloromethyl)carbonate. Cyclization of **47** to **52** was carried out at higher temperatures; oxazinane-2-one **52** was isolated in 35% yield[43]. Action of triphosgene on $\beta^{2,3}$-amino alcohol **49** resulted in a 18% yield of purified oxazinan-2-one **53**.

Scheme 36. Cyclization of *N*-benzylated $\beta^{2,3}$-amino alcohols **46**, **47** and **49** with triphosgene under various reaction conditions to give the *N*-benzylated oxazinan-2-ones **50**, **52** and **53**.



Gratifyingly, both heterocycles **52** and **53** gave suitable crystals for X-ray crystal structure analysis, thus allowing for the assignment of relative (and absolute) configuration for both valine (**37**, **38**) and leucine (**39**, **40**) derived $\beta^{2,3}$-amino acid derivatives. The crystal structures of **52** and **53** are shown in *Figure 2*. Hence, the configuration of compounds **37-40** (see *Scheme 32*) was unambiguously proved[44]. The two heterocycles adopt the sofa-conformation

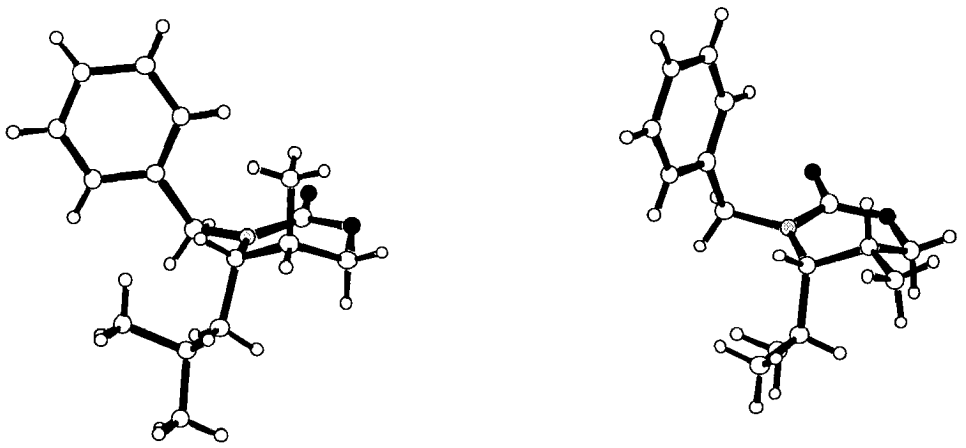
[42] For a recent review on the use of triphosgene in organic synthesis see [230]. For a ring closure of a β-amino alcohol derivative under *Schotten-Baumann* conditions with phosgene [231].

[43] Attempted cyclizations of $\beta^{2,3}$-amino alcohols with a free amino group were thwarted by isolation problems. By virtue of the *N*-benzyl group, the oxazinan-2-ones could be purified by flash chromatography.

[44] The *unlike*-compounds **38** and **40** correspond to the spot of smaller R_f value on TLC (see *Exp. Part*).

where C(5) is the out-of-plane atom. In analogy to *N*-benzoyl-2-(*tert*-butyl)tetrahydropyrimidin-4(1*H*)-ones [14,125], the *N*-atom of the cyclic carbamate is only slightly pyramidalized.

Figure 2. X-Ray crystal structures of *N*-benzylated oxazinan-2-ones **52** and **53**.

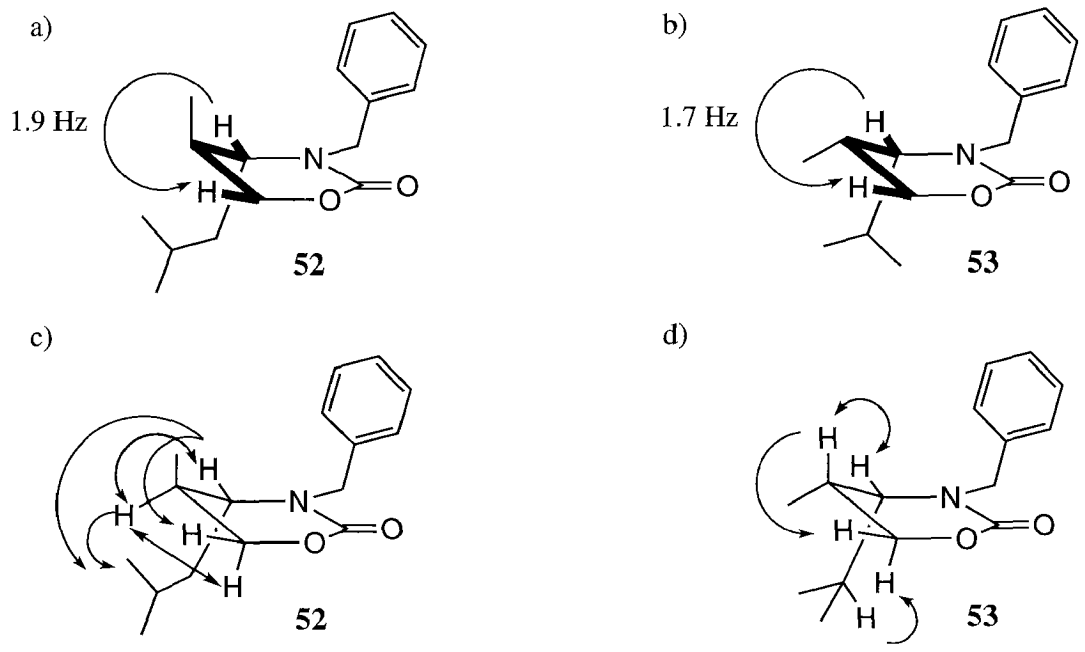


52 **53**

Interestingly, one of the OCH_2 protons in **52** and **53** couples with three protons, one of which has to be a proton involved in a long-range ^4J-coupling. The so-called "*W*"-coupling [232,233] between the indicated protons adjacent to the ring-*N*- and *O*-atoms (*Figure 3a, b*) accounts for this ^4J-coupling which is only possible if the four bonds connecting the two coupling nuclei are forced by a rigid molecular architecture to adopt a "*W*"-relationship (bold in *Figure 3a, b*). This long-range coupling alone is diagnostic of the conformation of the two *trans*- and *cis*-substituted heterocycles **52** and **53**. The configuration found by single crystal X-ray analysis (*Figure 2*) was further supported by NOE measurements. Both heterocycles **52** and **53** showed characteristic NOEs in their ^{1}H-NMR spectra (*Figure 3c, d*).

Oxazinan-2-ones are present in many biologically important natural products [234]. In addition, these cyclic carbamates exhibit a variety of biological activities [235] and served as key intermediates in the synthesis of several natural products [236,237].

Figure 3. ^4J-Coupling ("*W*"-coupling) between the specified H-atoms, observed in the 500 (**52**, a)) and 400 (**53**, b)) MHz ^{1}H-NMR spectra. Observed NOEs (300 MHz) in heterocycles **52** (c)) and **53** (d)). Arrows point from the sites of irradiation to the protons which showed a significant NOE enhancement.



4.3.3 Preparation of Boc- and Fmoc-Protected β^{2,3}-Amino Acids

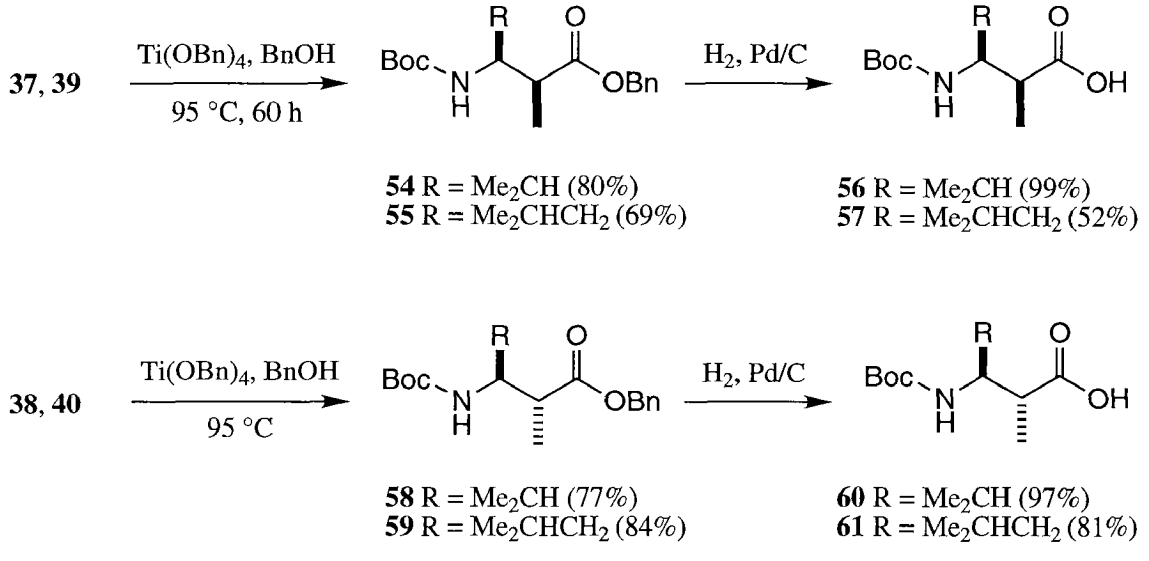
Having established the configuration of the β^{2,3}-amino acid methyl esters **37-40**, the stage was set to liberate the free β^{2,3}-amino acids for β-peptide coupling. However, straightforward basic hydrolysis of the α-substituted methyl esters was accompanied by considerable epimerization at the α-carbon. Changing the amount of base[45] (LiOH, NaOH, LiO_2H), the solvent ($MeOH/H_2O$, THF/H_2O mixtures), the temperature (room temperature or reflux) or the reaction time (with 2 equiv. LiOH the reaction was not complete in reaction times below 24 h!) did not reduce the amount of epimerized product below 1-2% (as determined by ^{1}H-NMR spectroscopy). This sluggish saponification is typical of α-substituted esters. Alternatively, S_N2-type ester cleavage [239-241] with LiCl or NaCN in refluxing pyridine led to decomposition of starting material.

Titanate-mediated transesterification was shown to be a mild method which is compatible with many functional groups, including the acid-labile Boc-

[45] After the present work had been completed, tetrabutylammonium hydroxide was used for the mild hydrolysis of non-polar α-peptide derivatives, with minimum racemization [238].

group [191,192,242]. With this procedure the methyl esters **37-40** could be transformed into the corresponding benzyl esters from which the free acids were smoothly liberated by hydrogenation as needed. These reactions are outlined in *Scheme 37*. Thus, titanate-mediated transesterification of *like*-$\beta^{2,3}$-amino acid methyl esters in BnOH afforded the diastereomerically pure[46] benzyl esters **54** and **55** in good yields[47] which were subsequently debenzylated by hydrogenation to give the free Boc-protected $\beta^{2,3}$-amino acids **56** and **57**. Similarly, the *unlike*-methyl esters **38** and **40** were transesterified (epimerization-free) to give the benzyl esters **58** and **59** which were deprotected to give the acids **60** and **61** in good overall yields[48].

Scheme 37. Transformations of the Boc-protected $\beta^{2,3}$-amino acid methyl esters **37-40** to the free Boc-protected $\beta^{2,3}$-amino acids **56, 57** and **60, 61** by a two-step procedure.



Although the transesterification method is generally considered safe concerning carbamate protection, traces of Z-protected benzyl esters were seen in the mass spectra of crude products. Recently a titanate-mediated protecting group manipulation was published [243]. The Boc/Z exchange can also be realized by other methods [244,245].

Exchange of the Boc-protecting group for the Fmoc-group acids **60** and **61** of *unlike*-configuration provided the Fmoc-protected $\beta^{2,3}$-amino acids **62** and **63**, the required building blocks for solid-phase β-peptide synthesis (*Scheme*

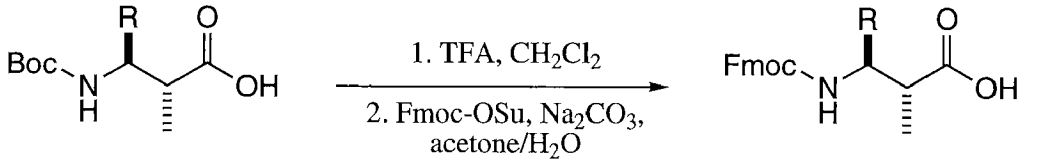
[46] In any case, ^{1}H-NMR spectra indicated the presence of the α-epimer.

[47] Since the benzyl esters have the same R_f value on TLC as the methyl esters, the reaction was monitored by ^{1}H-NMR spectroscopy of samples taken from the reaction mixture.

[48] After hydrogenating **58** in MeOH, the corresponding methyl ester **38** was also formed (5%). Therefore, ethyl acetate was used for hydrogenolyses of the benzyl esters (see *Exp. Part*).

38). These acids had to be purified by flash chromatography to remove small quantities of the corresponding Fmoc-dipeptide which may be formed via mixed anhydrides generated from the reaction of the formed Fmoc-$\beta^{2,3}$-amino acid with unreacted succinimide [246,247].

Scheme 38. Boc-Deprotection and ensuing Fmoc-protection of $\beta^{2,3}$-amino acids[49].



60, 61

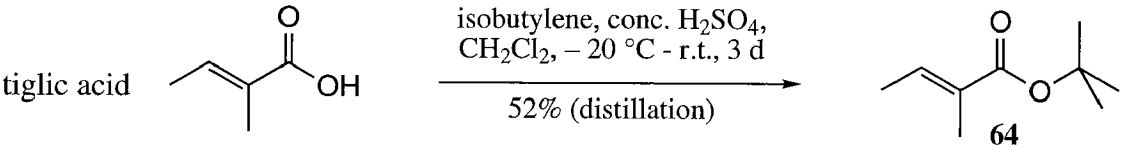
62 R = Me_2CH (86%)
63 R = Me_2CHCH_2 (87%)

4.3.4 Synthesis of u-*α-Methyl-Fmoc-β³-Homoalanine 68*

α-Methylation of Boc-protected β^3-homoalanine methyl ester **11** afforded a mixture of α-epimers (**35** and **36**, see *Scheme 32*) which could not be separated by simple flash chromatography. As larger amounts of the free acid **36** of *unlike*-configuration were needed, another method was employed for its synthesis.

High diastereoselectivities are obtained in the conjugate addition of chiral Li amides to α,β-unsaturated esters [248]. The appropriate *Michael* acceptor *tert*-butyl tiglate (**64**) was prepared by treatment of a CH_2Cl_2 solution of tiglic acid with isobutylene and a few drops of concentrated H_2SO_4 (*Scheme 39*). The product was purified by careful[50] distillation. The reaction was also carried out in *tert*-BuOH as solvent [249] or without solvent [250], but the yields were lower.

Scheme 39. Preparation of the *tert*-butyl ester (**64**) of tiglic acid.



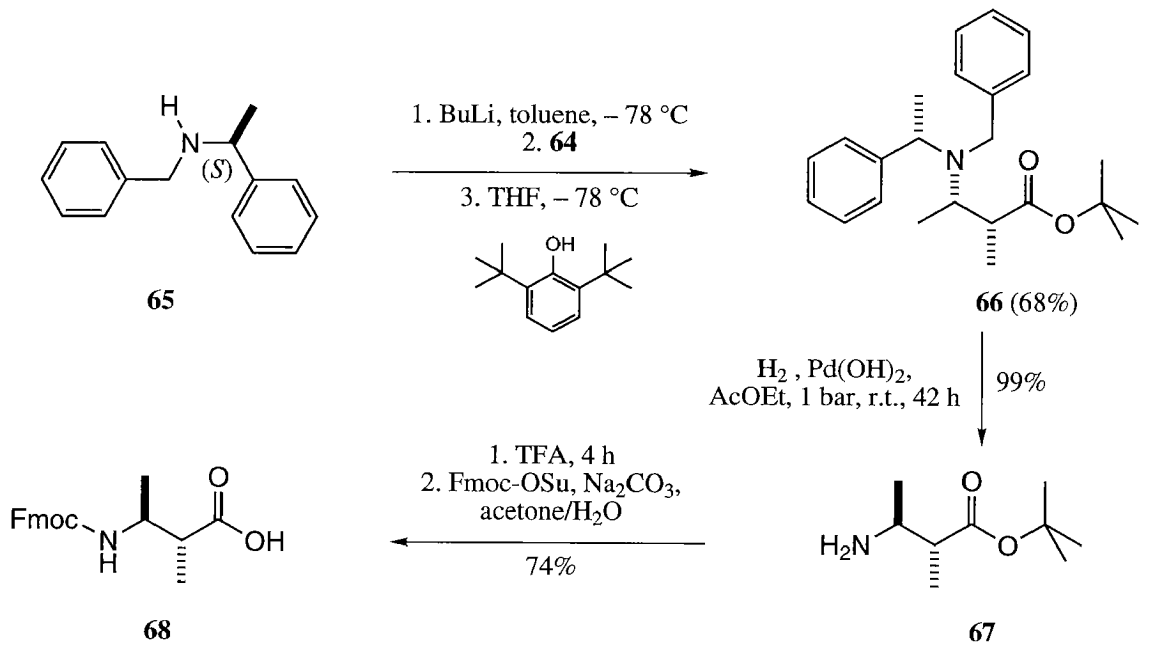
64

[49] The RP-HPLC purity (220 nm) was > 99%, the α-epimer was not detected.

[50] At elevated temperatures **64** is decomposed to tiglic acid which can not easily be separated by distillation!

Eanantiomerically pure (*S*)-*N*-benzyl-1-phenylethylamine **65** (*Scheme 40*), required for *Davies'* methodology, was prepared from enantiopure and inexpensive (*S*)-1-phenethyl amine[51] by benzylation in DMPU [252]. Following a published procedure [137], the Li amide derived from **65** was used for the conjugate addition to **64**. The intermediate enolate was subsequently quenched with 2,6-di-*tert*-butylphenol[52] to give in good yield fully protected **66** as single diastereoisomer on a 32 mmol scale[53]. A major drawback of the *Davies* method is the difficult *N*-deprotection. Thus, hydrogenation according to published protocols [248,254-256] gave only very low yields of the *N*-deprotected compound **67** [220]. However, if the hydrogenation was carried out in ethyl acetate under atmospheric pressure (!), the *N*-deprotection was quantitative to give the highly volatile aminoester **67** which was directly transformed to the Fmoc-(2*R*,3*S*)-α-methyl-β^3-homoalanine **68** by cleavage of the *tert*-butyl ester, followed by Fmoc-protection (*Scheme 40*).

Scheme 40. Synthesis of Fmoc-(2*R*,3*S*)-β^{2,3}-HAla(α-Me)-OH (**68**) by a three-step sequence.



[51] For a review on the application of 1-phenylethylamine in EPC [212] syntheses see [251].
[52] For a compilation of stereoselective protonations see [253].
[53] This method capitalizes on the individual assets of the two solvent systems by performing the conjugate addition in toluene, and then diluting the reaction with a greater volume of precooled THF before quenching with 2,6-di-*tert*-butylphenol.

4.3.5 *Characteristic Chemical Shifts of* like- *and* unlike-$\beta^{2,3}$-*Amino Acids and -Alcohols*

Several $\beta^{2,3}$-amino acid derivatives and $\beta^{2,3}$-amino alcohol derivatives have been synthesized. A comparison of the chemical shifts of corresponding protons in *l*- (**35**, **37**, **39**, **44** and **46**) or *u*-isomers (**36**, **38**, **40**, **48** and **49**) revealed some trends which are summarized in *Table 1*.

Table 1. Comparison of chemical shifts and coupling constants of *like*- and *unlike*-$\beta^{2,3}$-amino acid and *like*- and *unlike*-$\beta^{2,3}$-amino alcohol derivatives in the ^{1}H-NMR spectra (300 and 400 MHz). Only those protons are included the signals of which are well separated and correspond to the overall tendency.

	H-atoms and Me-groups	*like*-isomer	*unlike*-isomer
		35	**36**
1	C(α)-H	2.66, *m*	2.63, *m*
2	C(β)-H	3.78, *m*	3.88, *m*
3	β-Me	1.19, *d*, ^3J = 7.2	1.16, *d*, ^3J = 7.2
4	NH	5.06, br. *d*	4.86, br. *d*
		37	**38**
5	C(α)-H	2.77, *m*	2.60, *m*
6	α-Me	1.21, *d*, ^3J = 7.1	1.12, *d*, ^3J = 7.0
7	C(β)-H	3.43, *m*	3.80, *m*
8	NH	5.22, *d*, ^3J = 10.5	4.38, *d*, ^3J = 10.4
		39	**40**
9	C(α)-H	2.65, *m*	2.57, *m*
10	α-Me	1.20, *d*	1.14, *d*
11	C(β)-H	3.78, *m*	3.90, *m*
12	NH	5.02, *d*	4.62, *d*
		44	**48**
13	C(α)-H	2.96, *m*	2.78, *m*
14	α-Me	1.26, *d*, ^3J = 7.2	1.21, *d*, ^3J = 7.1
15	C(β)-H	4.01, *m*	4.38, *m*
16	NH	7.30, *d*, ^3J = 10.0	6.10, *d*, ^3J = 10.2
		46	**49**
17	C(α)-H	2.01, *m*	1.93, *m*
18	C(β)-H	2.39, *q*, *J* = 4.1/7.9	2.57, *q*, *J* = 2.9/7.1
19	NH/OH	4.21, br. *s*	3.56, br. *s*

For instance, in all cases the signals of the C(α)-H proton, the α-Me group, and the amide NH of *u*-isomers appear at higher field as compared to those of the *l*-epimers. However, the opposite is true for the C(β)-H atom. This will be useful for a first configurational assignment by analogy of new derivatives bearing other side chains.

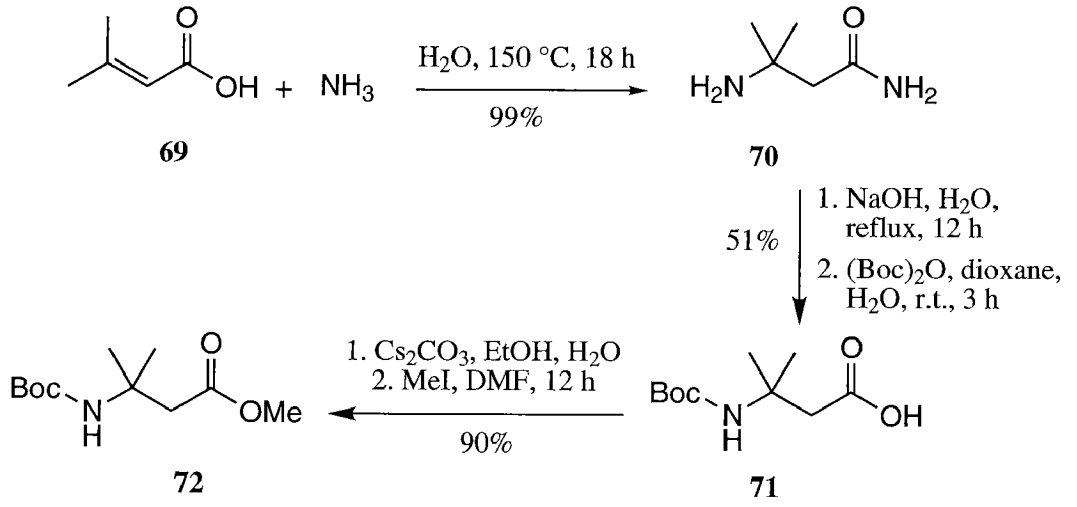
4.4 Preparation of Geminally Disubstituted $\beta^{3,3}$- and $\beta^{2,2}$-Amino Acids

Coupling of $\beta^{2,2}$- and $\beta^{3,3}$-amino acids in solution is desirable to β-peptides in quantities large enough for crystallization experiments. For multi-gram syntheses of geminally dimethylated $\beta^{3,3}$- and $\beta^{2,2}$-amino acids two straightforward methods were selected as outlined in *Schemes 41, 42* and *43*.

4.4.1 Preparation of Geminally Dimethylated $\beta^{3,3}$-Amino Acid Derivatives

The *Michael* addition of NH_3 to 3-methylbut-2-enoic-acid (senecioic acid, **69**) was chosen in view of a straightforward multi-gram synthesis of 3-amino-3-methylbutanoic-acid derivatives (β-aminoisovaleric acid or β^3-HAib residue; *Scheme 41*). Saponification of the amide **70**, obtained in quantitative yield[54], and Boc-protection yielded the $\beta^{3,3}$-amino acid derivative **71**, the methyl ester **72** of which (required for peptide coupling) was prepared by methylation of the Cs salt [257].

Scheme 41. Preparation of $\beta^{3,3}$-amino acid building blocks **71** and **72**.

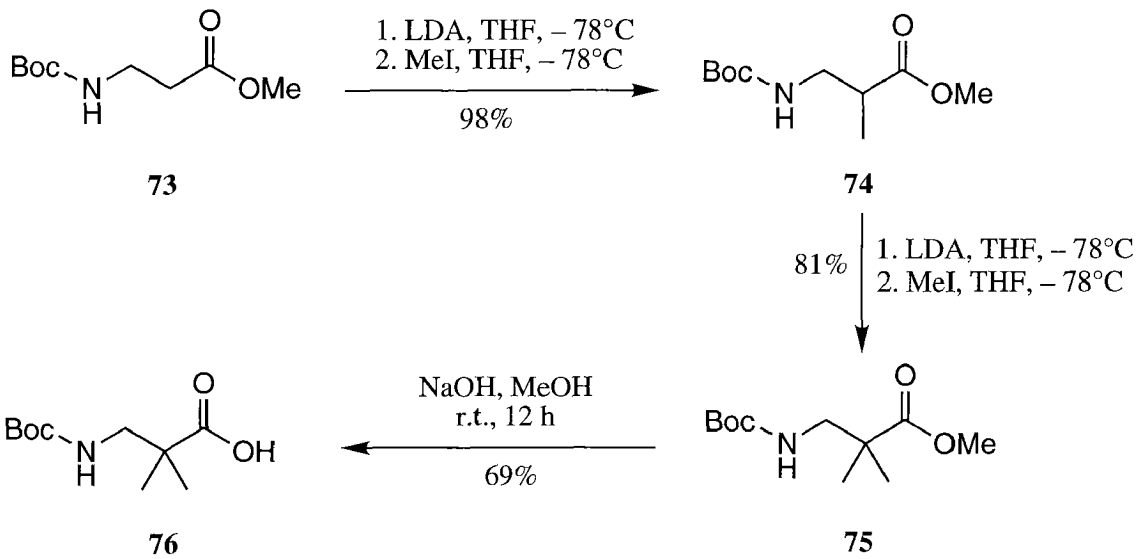


[54] In contrast to the results of this experiment, the corresponding carboxylic acid was reported as sole reaction product under the employed conditions [30].

4.4.2 *Preparation of Geminally Dimethylated $\beta^{2,2}$-Amino Acid Derivatives*

For the preparation of 3-amino-2,2-dimethylpropanoic-acid derivatives (β-aminopivalic acid or β^2-HAib; *Scheme 42*) a method developed in our group was chosen [27,134,135]: methylation of the Boc-protected methyl 3-aminopropanoate[55] **73** via a doubly lithiated species (see also *Scheme 32*). The monomethylated ester **74** underwent a second methylation to give the *N*-Boc-methyl ester **75** (80% from **73**), saponification of which with NaOH yielded the Boc-protected $\beta^{2,2}$-amino acid **76**[56].

Scheme 42. Preparation of $\beta^{2,2}$-amino acid building blocks **75** and **76**.



4.4.3 *Preparation of 1-(Aminomethylcycloalkane)carboxylic Acids*

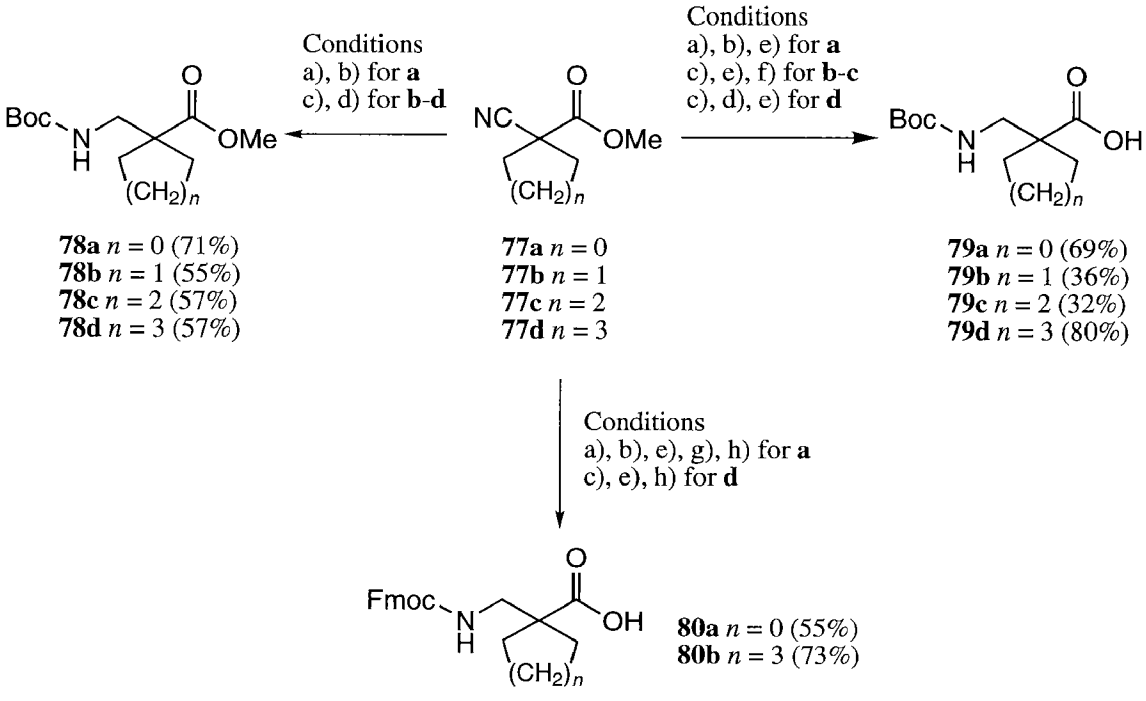
1-(Aminomethyl)cycloalkanecarboxylic-acid moieties are supposed to increase crystallizability, if incorporated into peptides, by introducing constraints that reduce the flexibility of the peptide backbone [5]. In order to study the folding propensities of β-peptides consisiting of $\beta^{2,2}$-amino acids, a couple of 1-(aminomethyl)cycloalkanecarboxylic-acid derivatives were prepared (see *Scheme 43*). Their preparation begins with the dialkylation of methyl 1-cyanoacetate by various dibromides to give methyl 1-

[55] Compound **73** was prepared from β-alanine in 60% yield [258].

[56] α,α-Disubstituted **75** undergoes saponification with a comparable rate as α-monosubstituted $\beta^{2,3}$-amino acid methyl esters (c.f. *Chapter 4.3.3*).

cyanocycloalkanecarboxylates **77** [53] following literature procedures[57]. Methyl 1-cyanocyclopropanecarboxylate **77a** was prepared by dialkylation of methyl cyanoacetate with 1,2-dibromoethane in DMF with K_2CO_3 [259] or by a phase transfer alkylation method [260,261]. The higher homologues **77b-d** were prepared by dialkylation of methyl cyanoacetate with the corrresponding dibromides with sodium methoxide in refluxing MeOH [52,262]. The cyanoesters **77** thus obtained were hydrogenated with *Raney*-Ni, and subsequent Boc-protection afforded the esters **78a-d** (details in *Scheme 43*). The free Boc-protected $\beta^{2,2}$-amino acids **79a-d** were prepared by a three-step sequence (with alternating order of hydrogenation/saponifi-cation/Boc-protection) as described in *Scheme 43*. Final Fmoc-protection provided the two Fmoc-protected cyclopropane- and cyclohexane building blocks **80a** and **80b** for solid-phase synthesis.

Scheme 43. Preparation of 1-(aminomethyl)cycloalkanecarboxylic-acid derivatives **78-80**.



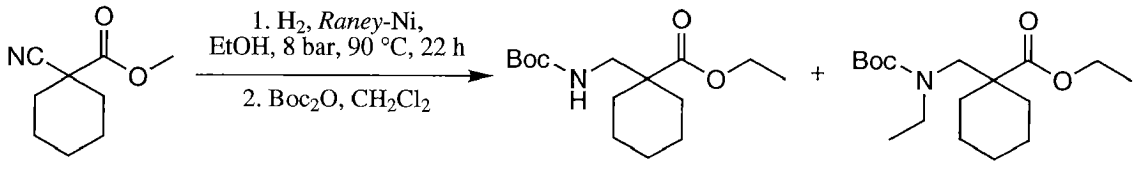
a) H_2, *Raney*-Ni, MeOH, 1 bar, r.t., 16 h; b) Boc_2O, MeCN, Et_3N, 0°, 16 h; c) H_2, *Raney*-Ni, MeOH, 4 bar, 40°, 22 h; d) Boc_2O, dioxane, CH_2Cl_2, r.t., 16 h; e) NaOH, MeOH, reflux, 5 h; f) Boc_2O, H_2O/dioxane; g) TFA, CH_2Cl_2; h) Fmoc-OSu, Na_2CO_3, acetone/H_2O.

[57] After the present work had been completed, a similar dialkylation was reported with enantiomerically pure 2,2'-bis(bromomethyl)-1,1'-binaphthyl as alkylating reagent [131].

It is interesting to note the mild conditions for the *Raney*-Ni reduction of the cyclopropane derivative **77a** (Cond. a) in *Scheme 43*). Higher pressure or temperature resulted in an increased amount of side products[58]. Compounds **77b-d** were hydrogenated at higher pressure (Cond. c) in *Scheme 43*), but still below the reported values for other nitrile hydrogenations [263-266]. Other catalysts, for instance PtO_2 [267] or Pd/C [268], were also tested for hydrogenation at 1 atm in EtOH/$CHCl_3$, but these reactions yielded only traces of reduced product [32].

Tests with freshly prepared [266] *Raney*-Ni were first done at elevated temperature and higher pressure (*Scheme 44*). The cyanoester **77d** was hydrogenated with *Raney*-Ni in EtOH, and the intermediate crude amine was subsequently Boc-protected to give the major product, ethyl ester **81**, in 51% yield together with 19% of a side product which was identified as the Boc-protected secondary amine[59] **82**. Therefore, the *Raney*-Ni hydrogenations (see *Scheme 43*) were carried out at 25-40 °C under low pressures to completely exclude *N*-alkylation.

Scheme 44. *N*-Alkylation as side reaction during *Raney*-Ni hydrogenation at elevated temperature and pressure.



77d **81** (51%) **82** (19%)

It is noteworthy that under the conditions outlined in *Scheme 43* the secondary amine, which could have been formed during *Raney*-Ni hydrogenation by trapping of the intermediate imine by the primary amino group of the product, was never detected. This is a known side reaction of *Raney*-Ni hydrogenations - especially without NH_3 added to the alcohol solution [265,266]. The undesired reaction prevailed during the cobalt-boride reduction of **77a** ($NaBH_4$ and $CoCl_6$ [132,133,270]; *Scheme 45*)[60]: the symmetrical derivative **83** was isolated in poor yield after Boc-protection.

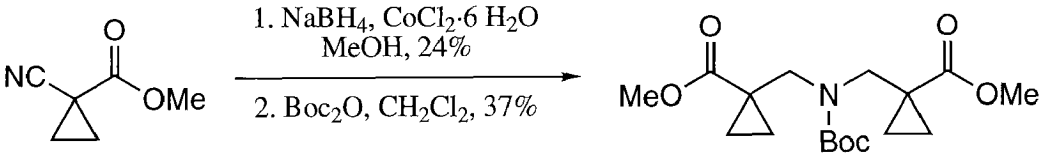
---

[58] The major side product was the dipeptide resulting from attack of the new primary amino group at the methyl ester of the starting material.

[59] Much more rigorous reaction conditions were reported to promote *N*-alkylation during *Raney*-Ni reductions of nitriles in alcohols [269].

[60] Among several methods for the reduction of nitriles [271], only few are suitable for the specific nitrile reduction in the presence of an ester.

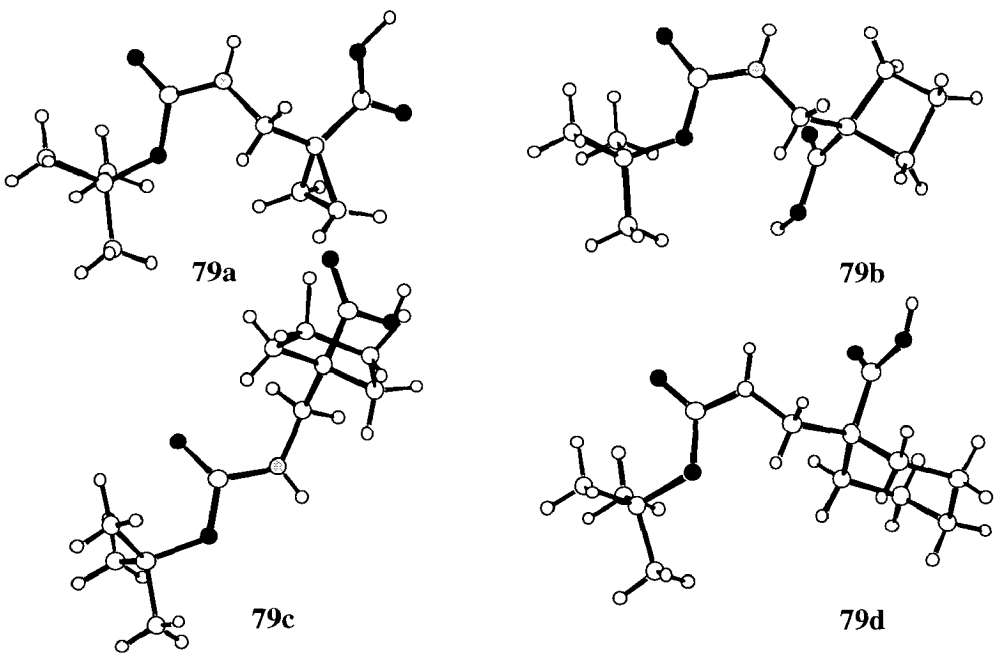
Scheme 45. Cobalt-boride reduction of cyanoester **77a**, followed by Boc-protection.



77a **83**

Single crystals suitable for X-ray diffraction were obtained of Boc-protected $\beta^{2,2}$-amino acids **79a**, **b**,**c**, and **d**. Their high crystallinity parallels the behavior of the α-amino acid analogues [3]. The conformation of these building blocks is of interest for studying the effect induced by the $\beta^{2,2}$-amino acids when incorporated into β-peptides. The crystal structures of the Boc-protected 1-(aminomethyl)cyclopropane-, cyclobutane-, cyclopentane-, and cyclohexanecarboxylic acids **79a-d**, respectively, are shown in *Figure 4*. In the structures of **79c** and **79d** the carboxy groups occupy axial positions[61] in the envelope and in the chair conformation, respectively.

Figure 4. X-Ray crystal structures of 1-(aminomethyl)cycloalkanecarboxylic-acid derivatives **79a-d**.



[61] In almost all crystal structures containing the α-analogues of **79c** [272] and **79d** [273], the amino group occupies the axial position [274]. The conformational preference of simple derivatives and peptides containing the α-analogues of **79a** [275] and **79b** [276] have been studied by X-ray diffraction as well.

Interestingly, the conformation of the urethane C–N bond of compounds **79a**, **79b**, and **79d** is *cis* (angle O–CO–N–C(β) is *sp*). Compound **79c** adopts the common *trans* conformation (angle O–CO–N–C(β) is *ap*). As expected for the three-membered ring on **79a**, the endocyclic angles have values close to 60°, but the CO–C(α)–C(β) angle (τ = 117.1°) is – expectedly so – greater than the tetrahedral angle. The angle τ is 106.5°, 107.0°, and 111.1° in the structures of compounds **79d**, **79c**, and **79b**, respectively. This substantial difference will result in specific conformations adopted by oligomers consisting of **79a** (see *Chapter 5.4.4*).

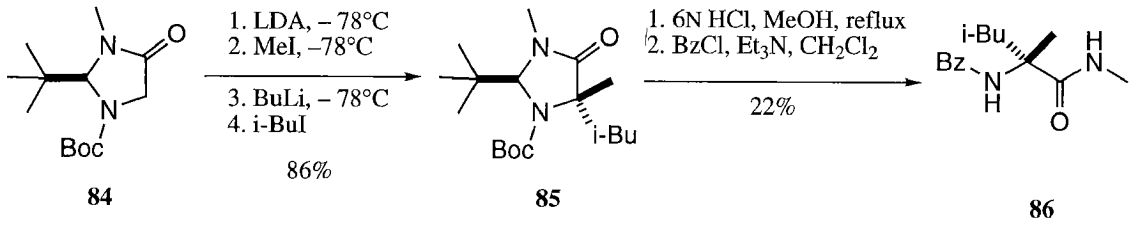
In the crystal packing, **79a-d** are very closely packed (not shown); the intermolecular H-bonds are short (1.63-1.99 Å). Carboxy/carbamate H-bonding patterns are formed by **79b** and **d** whereas **79a** and **c** are linked via carboxy/carboxy and carbamate/carbamate contacts.

As this work was nearing completion, different approaches to chiral *gem*-disubstituted $\beta^{2,2}$- and $\beta^{3,3}$-amino acids were envisaged (see *Chapter 2.3*). For a possible route to enantiopure $\beta^{3,3}$-amino acids, it was planned to homologate enantiomerically pure α,α-disubstituted α-amino acids either via *Arndt-Eistert* reaction (see *Scheme 5*) or following a sequence reported for the homologation of α-monosubstituted α-amino acids [277]: reduction to the β-amino alcohol, OH/I exchange, displacement of iodide by cyanide and subsequent hydrolysis to the β-amino acid. The required enantiopure α-branched α-amino acids were prepared by double alkylation of the readily accessible and commercially available[62] (S)-(–)-*tert*-butyl 2-*tert*-butyl-1,3-imidazolidin-4-one-1-carboxylate ((*S*)-(–)-Boc-BMI, **84**) [14,278,279]. Following published procedures [89], the chiral building block **84** was first methylated, then *i*-butylated in a one-pot procedure to give imidazolidinone **85** with excellent diastereoselectivity in 86% yield (*Scheme 46*). The hetereocycle **85** was then converted to the α-branched amide **86** according to *Studer* and *Seebach* [90][63]. Amide **86** was further hydrolyzed to the hydrochloride of the corresponding free α,α-disubstituted α-amino acid [280-282].

[62] (*R*)- or (*S*)-Boc-BMI were generously provided by *Degussa AG*, D-63457 Hanau.

[63] The analogous compounds bearing Bn-, Et-, and i-Pr-groups (already published [90]) in place of the i-Bu were also synthesized.

Scheme 46. Double alkylation of (*S*)-Boc-BMI **84** and cleavage of the heterocycle.



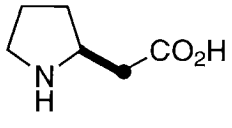
84 **85** **86**

The α-branched α-amino acids thus obtained could serve as starting material for future homologation experiments.

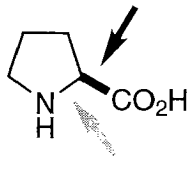
4.5 Preparation of β^2- and β^3-Homoproline

Proline, the only secondary proteinogenic amino acid with a secondary amino group, imparts specific restraints on the peptidic backbone by virtue of its pyrrolidine ring and of its fully substituted amide *N*-atom. This results in the unique structures of poly-proline [283a] and Pro-rich peptides (see collagen triple helix [284,285]). The β-homologues of proline should also enforce distinct backbone angles due to their constrained cyclic nature, and this might compensate for the lack of H-bonds. Thus, the interesting question of whether stable secondary β-peptidic structures without H-bonds are possible could be addressed.
A formal homologation of L-proline between the carbonyl group and the α-carbon atom gives (*S*)-β^3-homoproline while insertion of a methylene group between the α-carbon atom and the *N*-atom affords (*R*)-β^2-homoproline (nipecotic acid), see *Figure 5*. Whereas the former transformation can be realized by classical *Arndt-Eistert* homologation, the latter can not be effected in a simple way. This β-amino acid was therefore prepared by resolution.

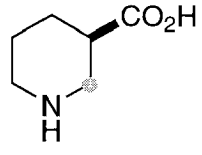
Figure 5. Two ways of homologating the α-amino acid proline for β^2- and β^3-HPro building blocks in β-peptides.



(*S*)-β^3-homoproline
(*S*)-H-β^3-HPro-OH



(*L*)- or (*S*)-proline
H-Pro-OH



(*R*)-β^2-homoproline
(*R*)-H-β^2-HPro-OH

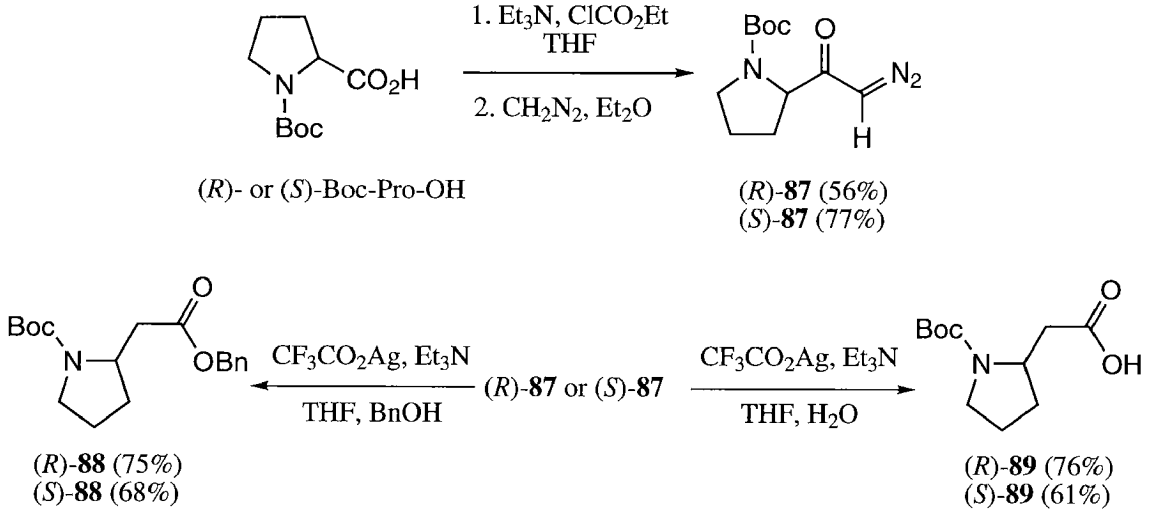
4.5.1 Preparation of β^3-Homoproline

Boc-protected H-(*S*)-β^3-HPro-OH was previously prepared by *Arndt-Eistert* homologation [286-288] and one-carbon homologation with cyanide [289] of L-Pro[64]. However, both the Boc-protected diazoketones derived from D- and L-Pro the and benzyl ester derivatives of β^3-HPro are new compounds.

[64] *Baláspiri et al.* reported the synthesis of H-(*S*)-β^3-HPro-OH by *Arndt-Eistert* homologation of Z-L-Pro. However the reported value and sign of the optical rotation is different from those reported by an industrial group [287] and others [290,291].

The classical *Arndt-Eistert* homologation was chosen for the preparation of the β^3-HPro derivatives. Commercially available Boc-protected (*R*)- (or D-) and (*S*)- (or L-) Pro-OH were converted to the corresponding diazoketones (*R*)-**87** and (*S*)-**87** in 56-77% yield (*Scheme 47*). The methyl ester (c.f. *Chapter 4.2*) was formed as a side product in 4-6% yield and was easily removed by flash chromatography. Classical Ag^+-catalyzed *Wolff* rearrangement in the presence of either BnOH or H_2O provided the benzyl esters (*R*)- and (*S*)- **88** and the acids (*R*)- or (*S*)- **89** in good yields, respectively (*Scheme 47*).

Scheme 47. Preparation of the Boc-protected building blocks of β^3-homoproline: the benzyl esters (*R*)- and (*S*)- **88** and the acids (*R*)- and (*S*)- **89**.



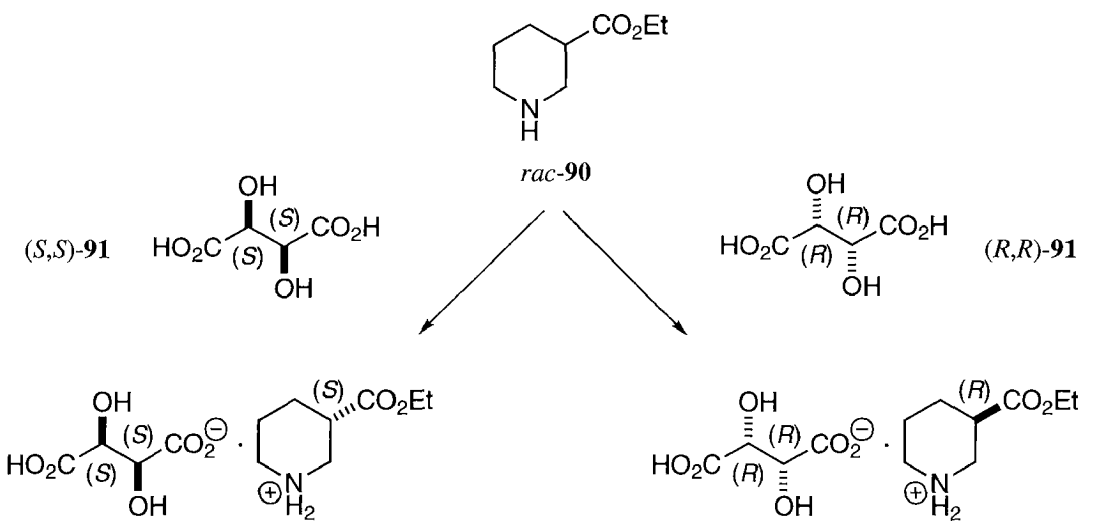
4.5.2 *Preparation of β^2-Homoproline by Resolution and Determination of the Enantiopurity*

As mentioned above, (*R*)- and (*S*)- ethyl nipecotate were prepared (following a literature procedure) by classical resolution [292] (*Scheme 48*)[65]. Commercially available *rac*-ethyl nipecotate **90** was treated with both enantiomers of tartaric acid ((*S,S*)-tartaric acid: (*S,S*)-**91**; (*R,R*)-tartaric acid: (*R,R*)-**91**) in EtOH which resulted in the precipitation of only one diastereomeric salt (the *like*-salt) **92**. After two or three recrystallizations, (*R,R,R*)-**92** and (*S,S,S*)-**92** were obtained in diastereomerically pure form, as determined by comparison of the optical rotation and the melting points

[65] An esterase-catalyzed hydrolysis of *rac-N*-acetyl methyl nipecotate provides the products in poor e.e.'s (22-24%) [293].

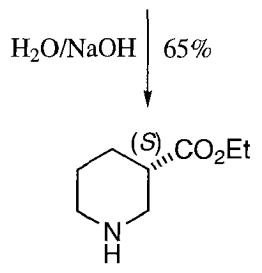
with literature values [292]. Basic (pH 13) extraction afforded (*R*)- and (*S*)-**90**[66]. This step was carried out rapidly at 0 °C and the pH was controlled because in the first batches complete saponification had occurred. Both enantiomers of ethyl nipecotate **90** showed optical rotations comparable to reported values.

Scheme 48. Resolution of *rac*-ethyl nipecotate **90** following a modified procedure [292]. The yields given for the *l*-salts **92** refer to starting *rac*-**90**.

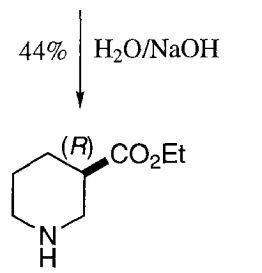


(*S*,*S*,*S*)-**92**
27% (recrystallized 3x)
m.p. = 156-157 °C ([292]: m.p. = 155-156 °C)
$[\alpha]_D^{r.t.} = -46.3$ ([292]: − 51.0)
(*c* = 2.0, 0.2% aq. $(NH_4)_6Mo_7O_{24}$-soln.)

(*R*,*R*,*R*)-**92**
25% (recrystallized 3x)
m.p. = 156-158 °C ([292]: m.p. = 155-156 °C)
$[\alpha]_D^{r.t.} = +52.3$ ([292]: + 51.0)
(*c* = 2.0, 0.2% aq. $(NH_4)_6Mo_7O_{24}$-soln.)



(*S*)-**90**
e.r. = 99.6 : 0.4
$[\alpha]_D^{r.t.} = +1.38$ (*c* = 5.0, H_2O)
([292]: $[\alpha]_D^{r.t.} = +1.6$ (*c* = 5.0, H_2O)



(*R*)-**90**
e.r. = 98.9 : 1.1
$[\alpha]_D^{r.t.} = -1.26$ (*c* = 5.0, H_2O)
([292]: $[\alpha]_D^{r.t.} = -1.8$ (*c* = 5.0, H_2O)

[66] The absolute configuration of (–)-nipecotic acid was established by CD-spectroscopy [294] and by chemical correlation [295].

However, even at high concentrations the value of the optical rotation ($[\alpha]_D^{r.t.} \approx 1.3$) is too small to allow for accurate determination of the enantiopurity[67]. Consequently, other methods for determining the enantiopurity were tested.

In a first approach, *rac*-**90** was acylated with (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) to give the corresponding diastereomeric amides, the so-called *Mosher* amides [300]. However, the signals of corresponding H- or F-atoms in the diastereomeric *Mosher* amides were not dispersed in the ^{19}F- and ^{1}H-NMR spectra.

α,α,α'α'-Tetraphenyl-1,3-dioxolane-4,5-dimethanol (TADDOL) was tested as chiral shift agent [301]; the ^{13}C-NMR spectrum (100 MHz) of a solution of *rac*-**90** and TADDOL (1:2) in $CDCl_3$ showed nonequivalence of two signals after several hours. The chemical-shift differences were ca. 24 and 45 ppm, respectively, but the peaks showed tailing preventing full base-line separation. This enantioselective shift effect allowed for a quick, but not accurate determination of enantiomer purity.

Compound *rac*-**90** was then acylated with pentafluoropropionyl chloride and treated with isopropanol to give the corresponding isopropyl *N*-pentafluoropropionyl-nipecotate suitable for GC-analysis[68]. However, with none of the tested chiral stationary phases (α-CD, β-CD, γ-CD and Chirasil-Val) was a separation of the enantiomers obtained. The *N*-pentafluoropropionyl derivative of *rac*-**90** was also injected onto an HPLC column packed with the chiral phase Chiralcel OD, but no separation ensued.

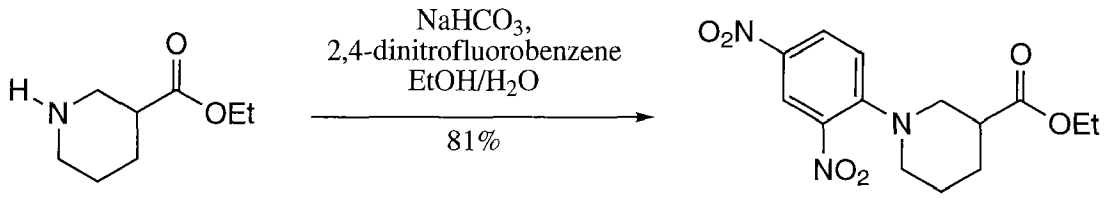
Next, another derivatization method was checked (*Scheme 49*). Compound *rac*-**90** was allowed to react with *Sanger*'s reagent (2,4-dinitrofluorobenzene, DNPF [305,306]) in alkaline solution to give the 2,4-dinitrophenyl (DNP)-derivative of ethyl nipecotate **93** in high yield.

---

[67] It is noteworthy that enantiopure nipecotic acid is often encountered in peptidomimetics [296-298]; in an independent work, (*R*)- and (*S*)-nipecotic acid were incorporated into a β-tetrapeptide [299]; the e.e. was always determined by polarimetry!

[68] The *N*-pentafluoropropionyl-isopropyl esters of α-amino acids are commonly used as volatile derivatives for GC analysis on a chiral phase [302-304].

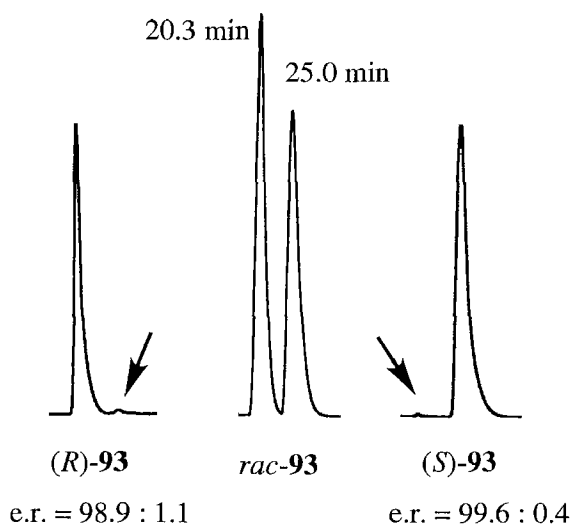
Scheme 49. Derivatization of **90** with *Sanger*'s reagent to give the 2,4-dinitrophenyl (DNP)-derivative **93**.



(*R*)-, (*S*)- or *rac*-**90** (*R*)-, (*S*)- or *rac*-**93**

Several chiral phases were tested for their ability to separate the enantiomers of **93**. No separation was observed with the following columns: DNBPG (*Baker*), Chiralpak AD (*Daicel*) and Chiraspher (*Merck*). Fortunately, with the Chiralcel-OD (*Daicel*)[69] column (*R*)- and (*S*)-**93** were separated (*Figure 6*); the (*R*)-enantiomer was eluted first (retention time, t_R = 20.3 min), the (*S*)-enantiomer had a retention time of 25.0 min. Integration of the peaks in the corresponding chromatograms revealed that (*R*)- and (*S*)-**90** had been obtained in an enantiomer ratio (e.r.) of 98.9 : 1.1 and 99.6 : 0.4, respectively[70].

Figure 6. HPLC Traces of *N*-2,4-dinitrophenyl-derivatives **93** (Chiralcel OD, mobile phase: i-PrOH/hexane 35:165; see *GP 17* in *Exp. Part*). The arrows indicate the signal of the minor enantiomer. The e.r. was determined by integration of the corresponding peak: (*R*)-**93**: 98.9 : 1.1; (*S*)-**93**: 99.6 : 0.4.



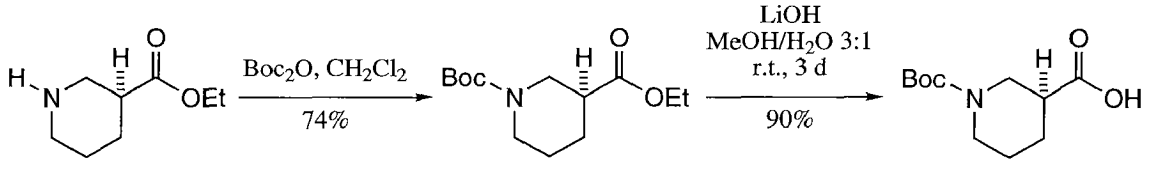
[69] Recently, *rac* ethyl 4-*hydroxy*piperidine-3-carboxylate was successfully separated as *N*-2,4-dinitrophenyl derivative on a Chiralpak AD HPLC column [307].

[70] The method has a high precision [308], reproducing the retention times with little deviations ($\Delta t_R \approx 0.8$ min).

With this method in hand, the diastereomer purity of the *l*-salts (*R,R,R*)-**92** and (*S,S,S*)-**92** (see *Scheme 48*) was determined by liberating a small quantity of ethyl nipecotate **90** and analysing the enantiopurity as outlined in *Scheme 49* and *Figure 6*[71]. It should be noted that polarimetry is also suited for a first determination of enantiopurity: the enantiomers of **93** have high values of optical rotation (+/– 165, c = 0, $CHCl_3$, see *Exp. Part*).

The following transformations were effected with the (*S*)-enantiomer of **90**: the (*S*)-form was Boc-protected to give the ester **94** as a colorless oil in 74% yield (after flash chromatography, see *Scheme 50*). The subsequent saponification step was considered crucial because there was the risk of racemization during alkaline hydrolysis (c.f. the epimerization of $\beta^{2,3}$-amino acid derivatives (*Chapter 4.3.3*) and of β^2-amino acids [203]). The mildest procedure for saponification was the hydrolysis with 2.5 equivalents of LiOH in a MeOH/H_2O solution at r.t. for 2-3 d providing, after recrystallization, Boc-protected (*S*)-nipecotic acid **95** in 90% yield (*Scheme 50*). The enantiopurity of **95** was determined by transforming it on a 1 mg-scale to the DNP-derivative **93** by a two-step sequence (see *Exp. Part*); (*S*)-**90** was used with an e.r. of 99.6 : 0.4. Assuming that the Boc-protection (without base) occurred without racemization, the e.r. is slightly decreased to 97.9 : 2.1 during saponification[72].

Scheme 50. Boc-Protection of enantiopure (*S*)-**90** to give **94** and subsequent saponification to provide Boc-protected nipecotic acid **95**.



(*S*)-**90** **94** **95**

[71] In the event, the enantiopurity of (*S,S,S*)-**92** was 97.0% after two recrystallizations. A third recrystallization increased it to 99.6%.

[72] Alternatively, saponification with the same amounts of base in refluxing MeOH/H_2O 3:1 for 3 h produced **95** in an e.r. of 97.3 : 2.7.

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5 Synthesis and Structural Analysis of β-Peptides

5.1 Introduction

Before *Seebach* and his group embarked on a project aimed at the synthesis of β-amino acid oligomers in 1995, the structure of monodisperse β-peptides had not been accurately established. Few and somewhat controversial reports on the structure of β-peptides and of β-amino acid polymers have appeared in the literature since the early 60's. Based on IR, CD, fiber X-ray, and NMR methods, a β-sheet conformation was assigned to several polydisperse poly(β-amino acid)s - the so-called nylon-3 derivatives[73] [310-318]. However, more recently it was shown from fiber X-ray scattering that poly(α-alkyl-β-L-aspartate)s adopt helical structures [319-322]. Poly-β-aspartates are biologically degradable polymers [323]. Recently, *Orgel* described the polymerization of β^3-homoasparagine ("β-glutamic acid") on hydroxylapatite, suggesting that β-peptides are promising candidates for a replicating system [324][74].

One impetus for the above-mentioned project came from research dealing with oligomers of (*R*)-3-hydroxybutanoates (oligo-HB) [325,326]. A left-handed 3_1 helix was modelled using X-ray structural parameters obtained from HB oligolides. Recognizing that the carbonyl O-atoms are close to the backbone O-atoms, the idea was born to replace the oxygens by NH groups in the hope that the helical structure would be stabilized by H-bonds[75]. Indeed, 2D-NMR spectroscopy revealed that **96**, the first of the β-peptides which was synthesized (from homologated valine, alanine, and leucine) formed a 3_{14} helix[76] in pyridine [17] and in methanol [18] (*Figure 7*). The CD spectra of β-peptide **96** are similar to those measured many years ago of poly[(*S*)-β-aminobutyric acid] [312], but the results had been interpreted as supporting the β-sheet conformation[77].

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[73] For a recent survey of *n*-nylons see [309].

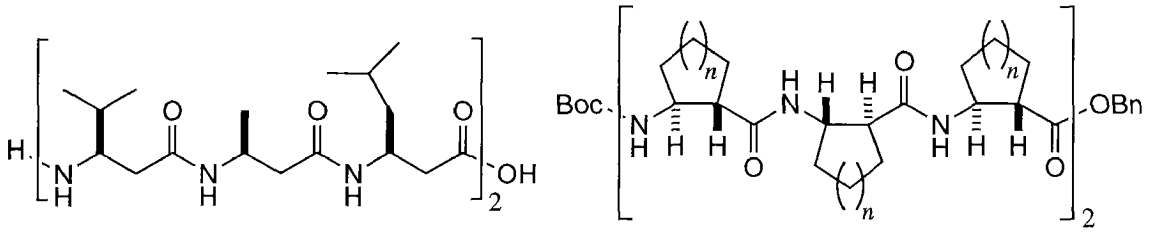
[74] Although not explicit, a pleated sheet of β-peptides was inferred on p. 256 in [324].

[75] For a more detailed presentation see [20].

[76] Peptide nomenclature is used here, designating a helix with three residues per turn; an H-bond between the backbone NH and C=O forms a14-membered H-bonded ring.

[77] This is a meaningful example of misleading conclusions due to a biased approach.

Figure 7. Seebach's β-hexapeptide **96** and *Gellman*'s restricted hexapeptide **97** both adopt the 3_{14} helix, in solution and in the solid state, respectively. A 2.5_{12} helix is formed by conformationally restricted **98**.



96

97 $n = 2$
98 $n = 1$

In an independent approach, *Gellman et al.* discovered that the hexamer **97** composed of *trans*-2-aminocyclohexanecarboxylic acid folds into the same 3_{14} helix in the solid state [19]. X-Ray analysis and 2D-NMR experiments revealed a second new helical structure - the 2.5_{12} helix - formed by β-peptides like **98** consisting of *trans*-2-aminocyclopentanecarboxylic acid [327]. A third type of helix, the 12/10/12 helix, was identified as the solution-conformation of a β-hexapeptide with alternating β^2- and β^3-amino acids [27,328]. Cyclic β-peptides adopt an infinite net of pleated-sheet type H-bonds with tubular stacking ("nanotubes") as determined from powder diffraction data [329,330] and their transmembrane ion channel-forming tendency was evaluated [331].

The properties of peptides and proteins depend on their three-dimensional structure which itself is determined by the sequence of the amino acids, i.e. the primary structure. The mechanisms of formation and the parameters determining the stability of secondary structures of proteins [332], comprising the helix, the pleated sheet and the turn, are not yet fully understood [283a][78]. In contrast, β-peptides adopt well-defined secondary structures that can also be predicted by calculations [335-337]! This holds for β-peptides the backbones of which are *not* conformationally restricted by cyclic residues. Recently, quantum mechanical calculations were used to evaluate the tendency of pleated sheet-, 3_{14}-, and 2.5_{12} helix formation [338] and the intrinsic preference of β-peptides to adopt either the 3_{14}- or the 12/10/10 helix [339][79].

[78] Recently, a 36-residue protein fragment was successfully folded by molecular dynamics simulation into a structure that resembles the native state [333,334].

[79] The conformational equilibria of β-alanine and related compounds were evaluated by NMR and IR spectroscopy [340-343] and by calculations [344].

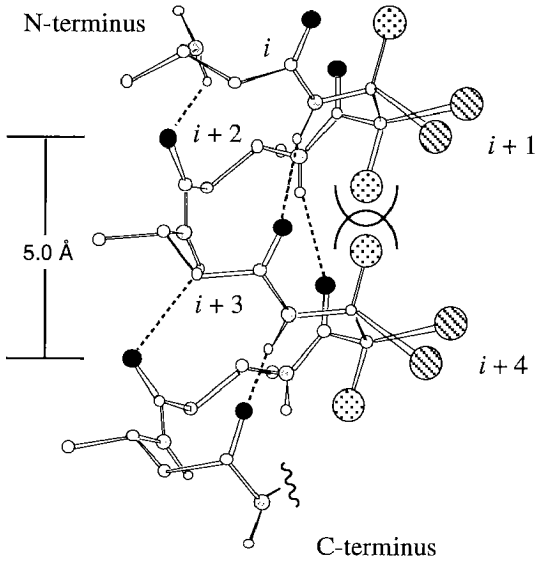
A further asset is that β-peptides are stable towards the most aggressive peptidases and proteases [345,346]. Thus, they are biologically inert with respect to proteolysis ("orthogonal to the α-peptide world"). Yet, biological activity can be achieved by the design of β-peptides mimicking α-peptidic hormones [347]. Moreover, the 3_{14} helix was shown to be stable (in methanol) in the temperature range between 298 and 393 K [348].

These far-reaching discoveries have brought about a surge of interest in the field of non-natural oligomers that adopt well-defined folding patterns; science journalists chose titles such as *'peptides do the twist'* [349] or *'β-Peptides: nature improved'* [350] and others [351,352] to describe these results, and several review articles on β-peptides have already been published [20,353-355].

5.2 Synthesis and Structure of Peptides Consisting of *like*-β^{2,3}-Amino Acids – The 3_{14} Helix

The model constructed from the NMR-data of β^3-peptide **96** displays two distinct substituent positions (*Figure 8*): There is a lateral (hatched large spheres) and an axial (dotted large spheres) position on each tetragonal C-atom along the backbone of the left-handed or (*M*)-3_{14} helix; non-H substituents are tolerated in the lateral but forbidden in the axial position.

Figure 8. Side-view of a section of a β-peptide (*M*)-3_{14} helix with a 5Å-pitch[80]. A lateral bond and substituent on C(β) of each β-amino acid is drawn, except on amino acids (*i* + 1) and (*i* + 4), where all four possible backbone-substituent positions are pictured (large spheres); sterically allowed substituent positions are represented as large hatched spheres in lateral positions, and large dotted spheres in axial positions indicate sterically forbidden positions.



To construct a β-peptide where all lateral positions are occupied by substituents, α,β-disubstitued β-amino acids with *like*-configuration have to be provided[81]. The resulting β-peptide 3_{14} helix should display a high stability in protic solvents due to the efficient "steric protection" of the peptide backbone by the many hydrophobic substituents. Moreover,

[80] MacMoMo (Prof. Dr. *M. Dobler*, ETH-Zürich) presentation; model constructed from data set of the NMR-structure determination of **96** as decribed in [17]).

[81] The (*M*)-3_{14} helix is compatible with β-amino acid residues of (2*S*,3*S*)-configuration. A β-peptide consisting of (2*R*,3*R*)-residues should fold into the right-handed or (*P*)-3_{14} helix.

structural studies could assess the degree of substitution, or steric crowding, that is tolerated by the 3_{14} helix.

*5.2.1 Synthesis of all-*like*-$\beta^{2,3}$-Peptides*

Peptide coupling was performed in solution by conventional methods with EDC/HOBt[82] [17,18]. Preliminary couplings were done with methyl ester derivatives (*Scheme 51*). Thus, Boc-protected $\beta^{2,3}$-amino acid methyl ester **39** was *N*-deprotected by treatment with TFA (CF_3CO_2H) in CH_2Cl_2. The resulting TFA salt was used for coupling with (2*S*,3*S*)-$\beta^{2,3}$-HAla(α-Me)-OH[83] **99** using EDC/HOBt-activation to yield the fully protected dipeptide **100**. An epimeric dipeptide, which was formed in a very small amount, was easily separated by flash chromatography. *N*-Deprotection and coupling with the Boc-protected $\beta^{2,3}$-amino acid **56** gave tripeptide methyl ester **101**. Due to complications arising during methyl ester saponification (epimerization!, see *Chapter 4.3.3*), the corresponding benzyl ester derivatives were employed for the following β-peptide syntheses. The reaction sequence was repeated with benzyl ester derivatives with minor alterations[84] (*Scheme 51*); coupling of the free amino ester of **55** with **99** led to the dipeptide **102** the amino group of which was attached to Boc-(2*S*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-OH **56** to give the fully protected $\beta^{2,3}$-tripeptide **103** in 85% yield.

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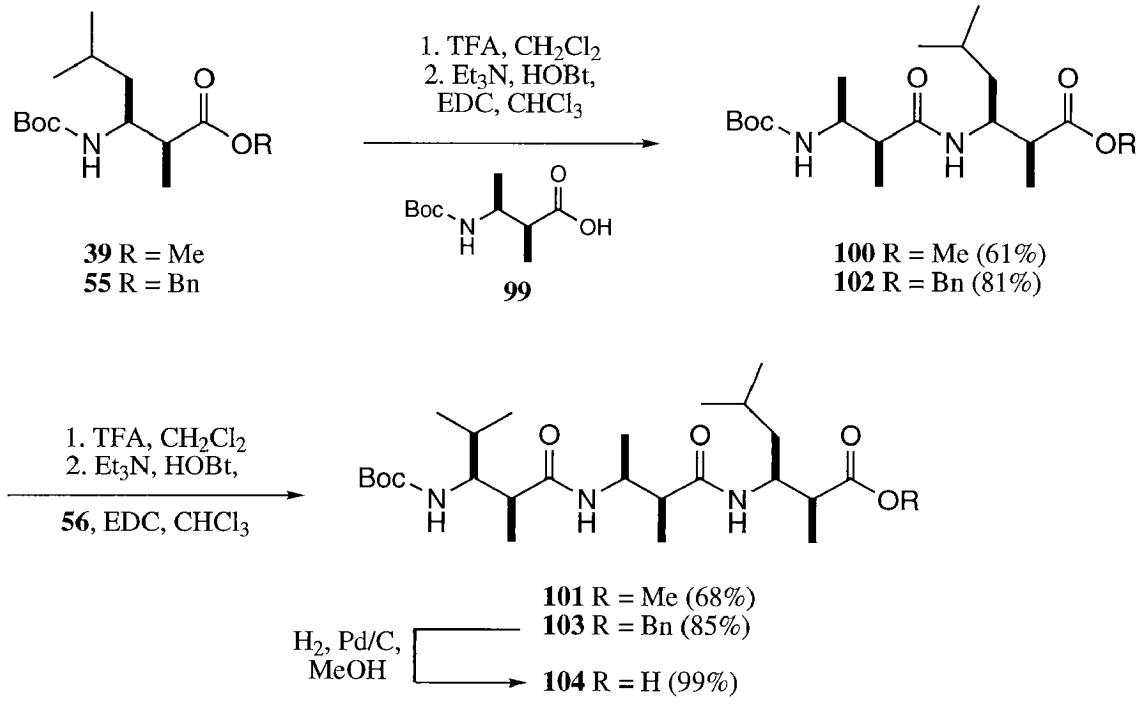
[82] For abbreviations, see *Exp. Part.*

[83] Compound **99** [18] was prepared by saponification of **35** with 2 equivalents of LiOH in refluxing MeOH/H_2O 3 : 1 for 65 min; diastereomer purity was > 95% according to ^{1}H-NMR spectroscopy.

[84] The yields of the coupling steps to $\beta^{2,3}$-di- and -tripeptides could be substantially increased by employing the free aminoester instead of the TFA salt in the coupling step.

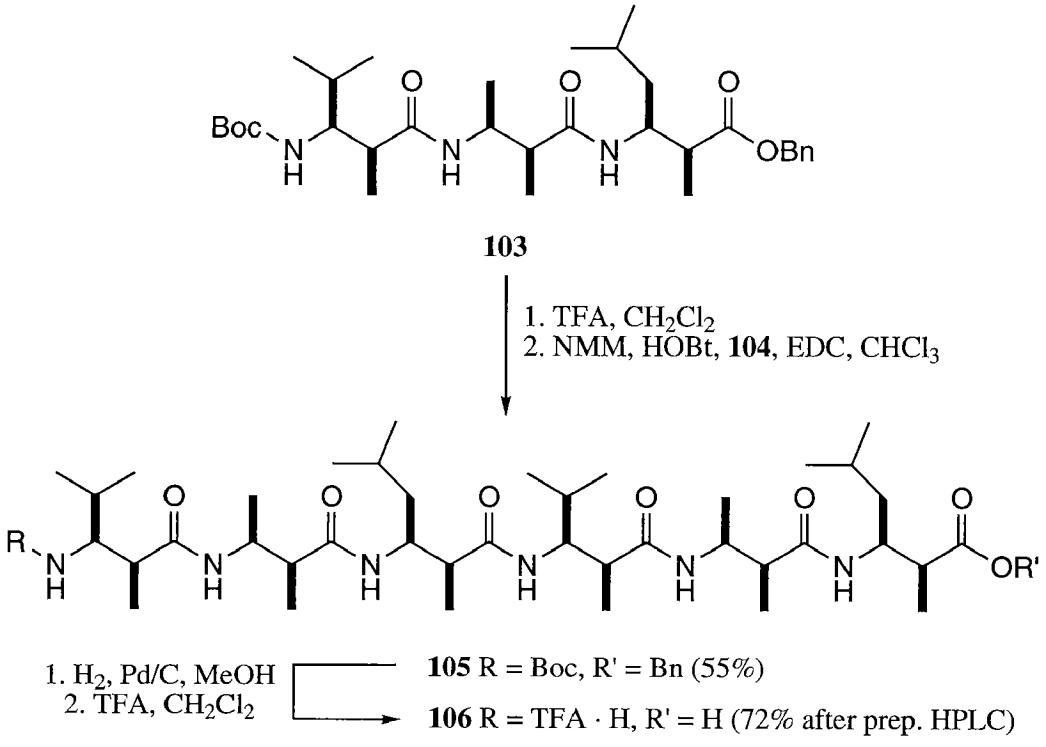
Scheme 51. Synthesis of $\beta^{2,3}$-tripeptide derivatives **101**, **103** and **104**.



Boc-protected $\beta^{2,3}$-tripeptide acid **104** needed for fragment coupling was quantitatively produced by hydrogenolysis (H_2, Pd/C). This β-peptide acid **104** was coupled with the TFA salt derived from $\beta^{2,3}$-tripeptide **103** to provide fully protected $\beta^{2,3}$-hexapeptide **105** in 55% yield after purification by flash chromatography (*Scheme 52*). Although Et_3N was replaced by the weaker base NMM to reduce epimerization, NMR analysis of the major impurity formed with **105** revealed that ca. 15% epimerization had taken place[85]. The benzyl ester group of **105** was cleaved by hydrogenolysis in MeOH (H_2, Pd/C), and the intermediate Boc-protected $\beta^{2,3}$-hexapeptide acid subsequently Boc-deprotected to give the free all-*l*-$\beta^{2,3}$-hexapeptide **106** in 72% yield after purification by preparative HPLC.

[85] An epimeric $\beta^{2,3}$-hexapeptide was isolated in 8% yield. Interestingly, this epimer precipitated on the wall of the test tubes as colorles *glass* after flash chromatography whereas the supposed 3_{14}-helix-forming **105** was precipitated as colorless *oil* just above the solution surface.

Scheme 52. Fragment coupling of **104** with *N*-deprotected **103** to the fully protected all-*l*-$\beta^{2,3}$-hexapeptide **105** and deprotection to give the free all-*l*-$\beta^{2,3}$-hexapeptide **106**.



5.2.2 *CD Spectroscopy*

Circular dichroism (CD) is extensively used to obtain information on the secondary structure of peptides and proteins in solution. For α-peptides and proteins, characteristic CD patterns can be assigned to β-sheet and α-helix structures [356-359]. However, in the world of β-peptides, the correlation between CD patterns and types of secondary structure is yet to be established. Results of efforts made with the goal to predict the CD spectra of poly-β-amino acids on the basis of theoretical calculations have been published [360,361].

β-Hexa- and β-heptapeptides which have been shown (by 2D-NMR spectroscopy) to adopt a (*M*)-3_{14} helix in solution, display a characteristic CD pattern with a broad minimum at ca. 216 nm and a more pronounced maximum at ca. 198 nm [17,18]. Since $\beta^{2,3}$-hexapeptides **105** and **106** comply with the configurational requirements for the formation of a 3_{14} helix (i.e. all side chains could occupy lateral positions on a 3_{14}-helical conformation, see *Figure 8*), we expected to see the typical CD pattern (extrema of opposite sign near 216 and near 200 nm). Indeed, the deprotected β-peptide **106** showed the

CD curve indicative of the 3_{14}-helical conformation (*Figure 9*)[86]. The intensity of the *Cotton* effect of the fully protected $\beta^{2,3}$-peptide **105** is drastically reduced[87], an effect often encountered with β^3-pepides [362].

Figure 9. CD spectra of $\beta^{2,3}$-peptide derivatives **105** and **106**, in comparison with the β^3-peptide **96**. Spectra were recorded at r.t. in MeOH (0.2 mM). [Θ] in 10 deg · cm^2 · mol^{-1}.

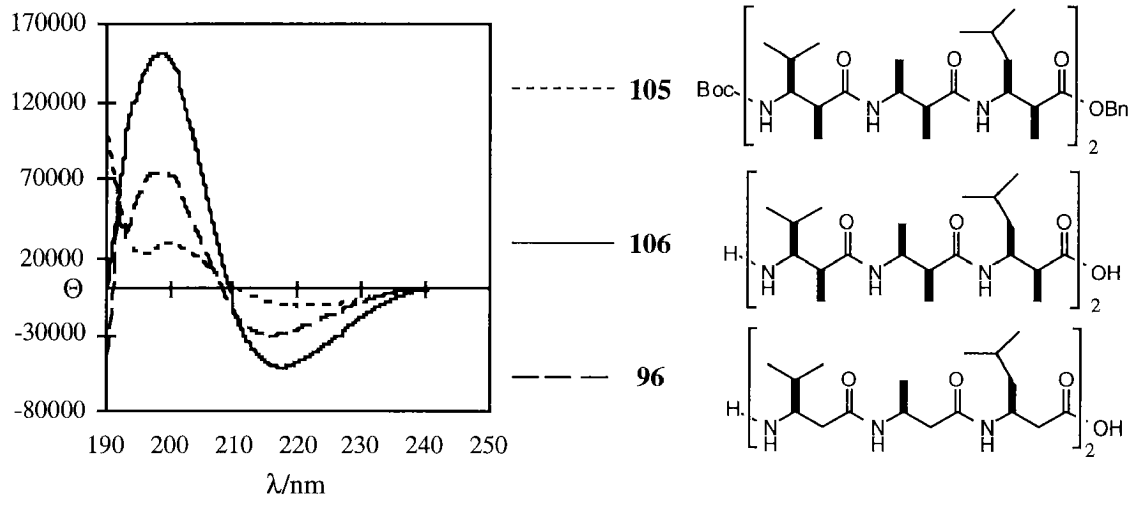


Figure 9 also shows the CD spectrum of β^3-hexapeptide **96**. At 198 nm its molar ellipticity is only ca. 50% of the molar ellipticity of fully substituted all-*l*-$\beta^{2,3}$-hexapeptide[88] **106**. This might reflect a higher 3_{14}-helical stability originating from the highly substituted backbone in **106** (see *Chapter 5.2.4*).

As reported previously, the CD spectra of *β*-peptides were found to be concentration-independent within the range studied. However, the highly substituted $\beta^{2,3}$-peptide **106** gave rise to concentration-dependent CD spectra. The molar ellipticity at 198 nm proved to be very sensitive to concentration, indicative of an aggregation phenomenon; amazingly, the intensity was increased when the concentration of the MeOH solutions was decreased from 0.2 to 0.01 mM.

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[86] All CD measurements were performed with lyophilized samples. The molecular mass corresponds to the TFA salts of the corresponding β-peptides (see *Exp. Part*).

[87] The fully protected **96** (Boc-protection, methyl ester) has actually no distinct CD pattern [362].

[88] In fact, the record molar ellipticity of $1.5 \cdot 10^5$ at 198 nm for a 3_{14}-helix-forming β-hexapeptide was measured with **106**.

5.2.3 Amide Proton Exchange Kinetics

The kinetics of amide proton H/D exchange in α-peptides and proteins [363,364] can give useful information concerning the solvent accessibility of the amide protons and the dynamics of protein folding. Amide proton exchange rates have been used to evaluate the role of NH protons of peptidic inhibitors H-bonding to an enzyme [365] or to obtain information on the spatial distribution of internal motions in the inhibitor structure [366]. Moreover, NH/ND-exchange kinetics have helped in structure determination of proteins [367,368]. Recently, the α-helix-stabilizing effect of salt bridges was investigated by measuring amide H/D exchange rates along dodecapeptides [369]. In native proteins, amide protons found in α-helices and β-sheets are likely to be strongly protected against H/D exchange.
The exchange rates of the amide protons of $\beta^{2,3}$-peptides **105** and **106** were measured in CD_3OD (see *Exp. Part*); their half-lives ($\tau_{1/2}$) are presented in *Table 2*. The most perseverant proton NH(3) in **106** (M_r 752.58) has a half-life value (24 days!) which is normally characteristic of large proteins with much higher molecular mass (> 4000). However, peptides and proteins have been measured in D_2O [366-369] or DMSO/D_2O [364,365], so a direct comparison is not possible.

Table 2. Half-life values $\tau_{1/2}$ (min) of NH protons in $\beta^{2,3}$-peptides **105** and **106** at 25 °C in CD_3OD. Compound **106** was measured as the TFA salt. The amide protons of **105** were not assigned. For **106**, amide NH signals were assigned to specific amino acids by 2D-NMR techniques (see *Chapter 5.2.4*).

Peptide	NH(1)	NH(2)	NH(3)	NH(4)	NH(5)	NH(6)
105[a)]	507	69	27	308	1439	-[b)]
106[c)]	-	60	ca. 24 d	796	45	54

a) NH protons of **105** are numbered according to the position of the chemical shifts in the ^{1}H-NMR, starting from the low field side. b) $\tau_{1/2}$ < 5 min. c) NH protons were assigned for β-peptide **106**: numbering according to the position in the sequence starting from the *N*-terminus.

The half-life values of the amide protons in **106** are higher than the values measured in analogous β^2- or β^2/β^3-peptides [18,27]. Indeed, the half-lives are surprisingly high for these rather short β-peptides. Even the *fully protected* **105** has half-life values of up to one day (NH(5))! This correlates with the presence of a distinct *Cotton* effect in its CD spectrum (*Scheme 9*). In **106** (where the amide NH signals were assigned to specific amino acids by 2D-NMR techniques (see *Chapter 5.2.4*)) the amide protons in the middle of the

β-peptide have longer half-lives than those located near the *C*- and *N*-terminus. As expected, the α- and β-substituents in **105** and **106** are very effective in shielding the β-peptide backbone from solvent molecules. This local shielding effect adds to the intrinsic shielding due to the 3_{14} helix.

5.2.4 *2D-NMR Analysis of $\beta^{2,3}$-Hexapeptide* **106**

A detailed NMR investigation in CD_3OH was carried out with $\beta^{2,3}$-hexapeptide **106**[89]. So far, only $\beta^{2,3}$-peptides built from *trans*-2-aminocyclohexane- and *trans*-2-aminocyclopentanecarboxylic acids, i.e. with conformationally fixed C(α)–C(β) bond, have been investigated (see **97** and **98** in *Figure 7*) [19,327]). DQF-COSY[90] [370,371] and HSQC [372-374] techniques allowed the assignment of all resonances in the ^{1}H-spectrum as well as of all H-bearing C-atoms in the ^{13}C-spectrum. An HMBC [370,375,376] experiment was performed in order to assign not only all C=O resonances, but also to determine the sequence through C,H long-range correlations across the peptide bond. The ^{1}H-chemical shifts and coupling constants together with the ^{13}C-NMR chemical shifts for **106** are listed in the *Exp. Part.* The dispersion of the chemical shifts for the NH, C(α)-H and C(β)-H protons is very large. Together with the coupling constants for the backbone, they indicate a secondary structure of great stability, as suggested by CD-spectroscopy and amide proton exchange kinetics (*Chapters 5.2.2-3*). The small coupling constants (2.7 Hz and 2.9 Hz) between the γ-CH and β-CH protons of the $\beta^{2,3}$-valine residue, however, show that the side-chain is not freely rotating around the C(β)-C(γ) bond. This is an indication for steric hindrance between the $CH(Me)_2$-side chain and the adjacent Me group which could destabilize the secondary structure[91].

ROESY [378] spectra with three different mixing times (50, 100, and 150 ms) were measured, and the resulting NOE cross peaks are presented in *Table 3*. These NOEs for **106** are weaker in intensity compared to other β-hexa- [17] or -heptapeptides [27]. Due to the substituents in 2- *and* 3-position of the β-

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[89] The NMR analysis and the MD simulations were performed by *K. Gademann*.

[90] HSQC: 'heteronuclear single-quantum coherence spectroscopy'; HMBC: 'two-dimensional heteronuclear multiple-bond correlation spectroscopy; DQF-COSY: 'double-quantum-filtered chemical shift-correlation spectroscopy'; ROESY: 'rotating-frame nuclear Overhauser enhancement and exchange spectroscopy'.

[91] β-Branched α-amino acids, such as valine, are known to destabilize α-helical conformations due to entropic effects [377].

amino acids in **106,** fewer interresidue NOEs were observed than with β-peptides with not more than one $\beta^{2,3}$-amino acid. However, the overall pattern of NOEs observed with **106** is in full agreement with a high proportion of 3_{14}-helical conformations in MeOH at room temperature.

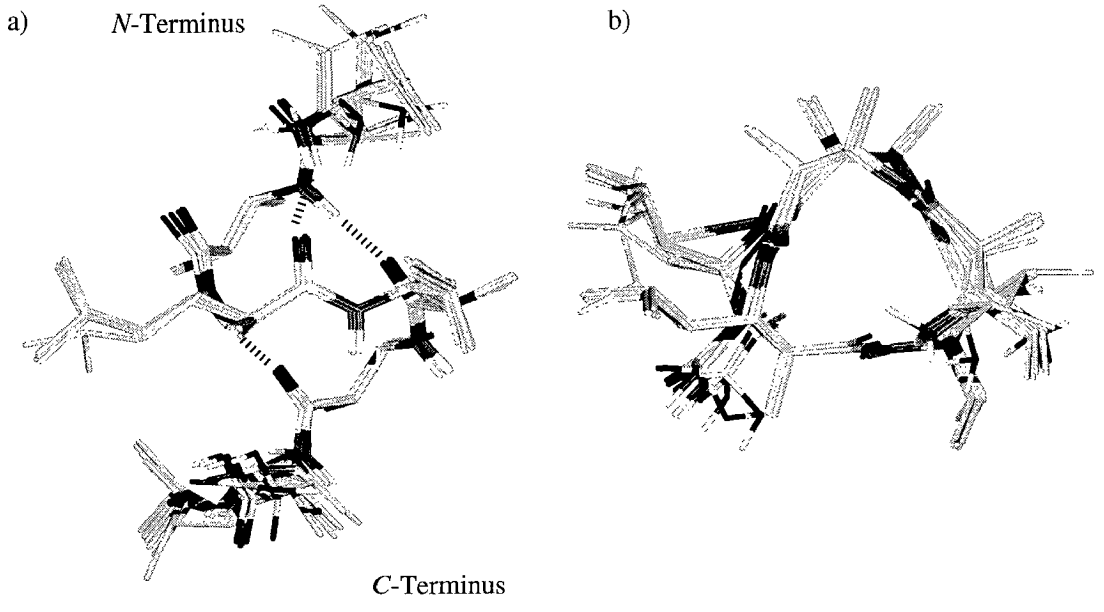
Table 3. NOEs observed for the backbone protons in the 150ms-ROESY NMR spectrum of $\beta^{2,3}$-hexapeptide **106** in CD_3OH (500 MHz). The NOEs were classified in three distance categories: s (strong, < 3 Å), m (medium, < 3.5 Å) and w (weak, < 4.5 Å).

Residue	H-Atom	Residue	H-Atom	NOE
$\beta^{2,3}$-HVal(αMe)(1)	α	$\beta^{2,3}$-HVal(αMe)(4)	β	s
$\beta^{2,3}$-HVal(αMe)(1)	NH	$\beta^{2,3}$-HVal(αMe)(1)	α	m
$\beta^{2,3}$-HVal(αMe)(1)	NH	$\beta^{2,3}$-HAla(αMe)(2)	NH	w
$\beta^{2,3}$-HVal(αMe)(1)	NH	$\beta^{2,3}$-HAla(αMe)(2)	β	w
$\beta^{2,3}$-HVal(αMe)(1)	NH	$\beta^{2,3}$-HLeu(αMe)(3)	β	w
$\beta^{2,3}$-HVal(αMe)(1)	NH	$\beta^{2,3}$-HVal(αMe)(4)	β	w
$\beta^{2,3}$-HAla(αMe)(2)	α	$\beta^{2,3}$-HAla(αMe)(5)	β	s
$\beta^{2,3}$-HAla(αMe)(2)	NH	$\beta^{2,3}$-HVal(αMe)(1)	β	w
$\beta^{2,3}$-HAla(αMe)(2)	NH	$\beta^{2,3}$-HVal(αMe)(1)	α	s
$\beta^{2,3}$-HAla(αMe)(2)	NH	$\beta^{2,3}$-HAla(αMe)(2)	α	s
$\beta^{2,3}$-HAla(αMe)(2)	NH	$\beta^{2,3}$-HVal(αMe)(4)	β	m
$\beta^{2,3}$-HAla(αMe)(2)	NH	$\beta^{2,3}$-HAla(αMe)(5)	β	m
$\beta^{2,3}$-HLeu(αMe)(3)	α	$\beta^{2,3}$-HLeu(αMe)(6)	β	m
$\beta^{2,3}$-HLeu(αMe)(3)	NH	$\beta^{2,3}$-HAla(αMe)(2)	α	s
$\beta^{2,3}$-HLeu(αMe)(3)	NH	$\beta^{2,3}$-HLeu(αMe)(3)	α	s
$\beta^{2,3}$-HLeu(αMe)(3)	NH	$\beta^{2,3}$-HVal(αMe)(4)	NH	w
$\beta^{2,3}$-HLeu(αMe)(3)	NH	$\beta^{2,3}$-HLeu(αMe)(6)	β	m
$\beta^{2,3}$-HVal(αMe)(4)	NH	$\beta^{2,3}$-HLeu(αMe)(3)	α	s
$\beta^{2,3}$-HVal(αMe)(4)	NH	$\beta^{2,3}$-HVal(αMe)(4)	α	s
$\beta^{2,3}$-HVal(αMe)(4)	NH	$\beta^{2,3}$-HLeu(αMe)(6)	β	w
$\beta^{2,3}$-HAla(αMe)(5)	NH	$\beta^{2,3}$-HVal(αMe)(4)	α	s
$\beta^{2,3}$-HAla(αMe)(5)	NH	$\beta^{2,3}$-HAla(αMe)(5)	α	s
$\beta^{2,3}$-HAla(αMe)(5)	NH	$\beta^{2,3}$-HLeu(αMe)(6)	NH	w
$\beta^{2,3}$-HLeu(αMe)(6)	NH	$\beta^{2,3}$-HAla(αMe)(5)	α	s
$\beta^{2,3}$-HLeu(αMe)(6)	NH	$\beta^{2,3}$-HLeu(αMe)(6)	α	m

The experimental data (25 NOE restraints and 13 torsion angle restraints derived from the ^3J-coupling constants via a modified *Karplus* equation [379-381]) were used as restraints in *simulated annealing* [382] using the Amber* force field and molecular model [383-386][92]. The structural bundle consisting of the six conformers lowest in energy is depicted in *Figure 10.* The 3_{14} helix is very well defined for residues 2 to 6, with slightly greater structural variance for the *N*-terminal residue. This is also reflected by the H/D exchange half-life values of the amide protons (see *Table 2*).

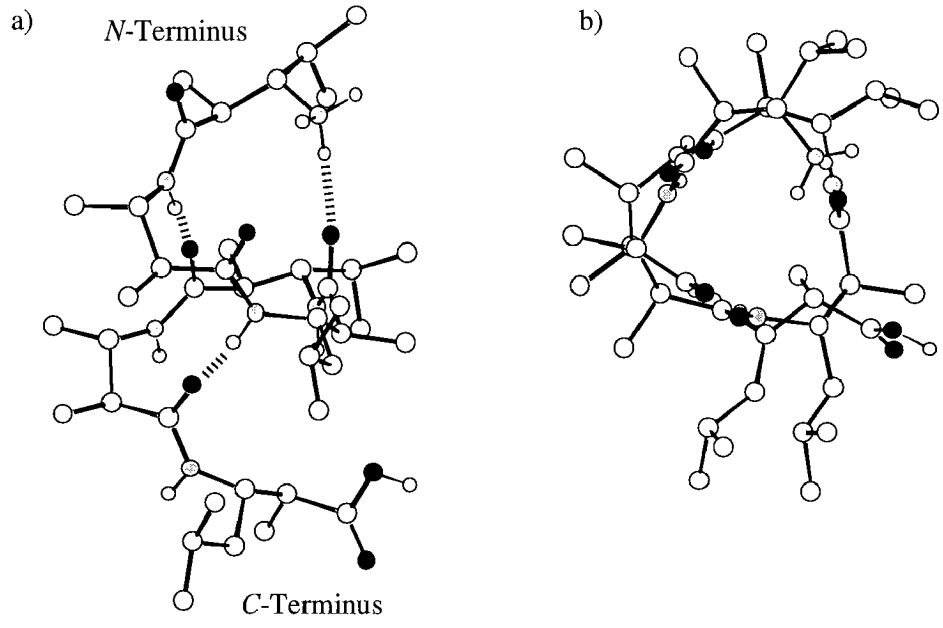
[92] Details will be given in the Ph. D. thesis of *K. Gademann.*

Figure 10. NMR solution structure of $\beta^{2,3}$-hexapeptide **106** in CD_3OH. a) Side and b) top view. Bundle of the six conformers showing no significant violation of the experimental restraints (NOE and *J*-values). All C-bound H-atoms have been omitted for clarity. Code: carbonyl O-atoms *black*, amide N-atoms *grey*, C-atoms *light-grey*. The figure was generated by MolMol [387].



One conformer of this bundle is shown in *Figure 11*. H-Bond lengths were found to range for N(1)-H · · · O=C(3) from 1.8 to 2.0 Å, for N(2)-H · · · O=C(4) from 1.6 to 1.9 Å and for N(3)-H · · · O=C(5) from 1.8 to 2.1 Å, while no H-bond results from N-H(4) to the terminal carboxy O-atom. From the top view (*Figure 11b*), "steric protection" of the peptide backbone by the many (hydrophobic) substituents is clearly evident. This "protection" is probably causing the very slow exchange of the NH protons of the $\beta^{2,3}$-hexapeptide **106** (see *Chapter 5.2.3*). On the other hand and as mentioned above, steric crowding may be a destabilizing contribution (unwinding of the helix). A consequence of side-chain repulsion is visible in the top view of the 3_{14} helix (*Figure 11b*); there is a twist, so that precise juxtaposition of Me_2CH and Me_2CHCH_2 is avoided. Another contribution to this deviation from the ideal 3_{14}-helical geometry may come from the Me groups of the Me_2CH side chains of residues 1 and 4 that are located in a plane approximately parallel to the helix axis.

Figure 11. a) Side and b) top view of one low-energy model of $\beta^{2,3}$-hexapeptide **106**. H-bonds are indicated. All C-bound H-atoms have been omitted for clarity. The figure was generated with MacMoMo (program by Prof. Dr. *M. Dobler*, ETH-Zürich).



While the NH/D exchange rates in the central part of the $\beta^{2,3}$-peptide **106** (*Table 2*) are the largest of all β-hexa- and β-heptapeptides measured so far [27], and while the *Cotton* effect of its CD spectrum is very large (*Figure 9*), the intensity of the cross-peaks in its ROESY spectrum (*Table 3*) is weaker than with analogous β^3-hexapeptides [18] lacking the α-Me groups (less highly populated helix form in MeOH). This may be due to a helix destabilization by steric repulsion between side chains, as evident from the non-ideal juxtaposition of side chains (*Figures 10b* and *11b*)[93].

[93] The cyclic nature of the $\beta^{2,3}$-amino-acid residues in *Gellman*'s β-peptide **97** not only locks the conformation around the C(2)–C(3) bond but also prevents the kind of steric crowding present in the helix of **106**!

5.3 Synthesis and Structure of Peptides Containing *unlike*-$\beta^{2,3}$-Amino Acids – The Parallel and Antiparallel Pleated Sheet

There are many proteins in which β-sheets play an important functional role. β-Sheets can provide the key element in protein-DNA [388], protein-RNA [389], and protein-protein recognition [390]. Several of these interactions are based upon direct, edge-on β-sheet contacts, which can often be mimicked by peptides, for example, the dimerization of HIV protease [391]. The behavior of the hormone erythropoetin can be mimicked by disulfide-linked β-hairpin peptides [392]. Formation of amyloid fibrils mediated by the interaction of β-strands is thought to be a crucial event in the progression of a variety of pathological disorders, ranging from Alzheimer's disease to spongiform encephalopathies [393-395] and has recently been judged to be an inherent ability of most proteins [396]. The conversion of α-helices to larger β-sheet aggregates [397] causes BSE/Creutzfeld-Jakob and other prion deseases [398-400].

Despite the importance of β-sheet structures as regular secondary structural elements in proteins, the principles underlying their formation are not well understood. This contrasts with the growing number of short peptides [401-406] or proteins [407-409] that form monomeric α-helices in solution[94]. The major problem in β-sheet design is the limited solubility as a result of the pronounced tendency of extended peptide strands to aggregate, and thus separate from solution. Thus, the synthesis of small, soluble model compounds is of high interest [412-417]. Recently, several water-soluble hairpin-forming peptides that contain mainly natural amino acids have been published [418-422][95]; three-stranded hairpins have been designed and prepared, and their structure was established in aqueous solution [424-426], aqueous MeOH [427,428], or $CHCl_3$ [429]. Water-soluble *single-stranded* 16- and 17-residue peptides have also been studied as models for self-association to pleated sheet structures [423,430,431].

β-Peptidic pleated sheet structures should display similar properties. A pleated sheet structure was assigned to poly(β-aminobutyric acid)s based on CD, powder and fiber X-ray and IR studies [311,312,432,433]. But, as

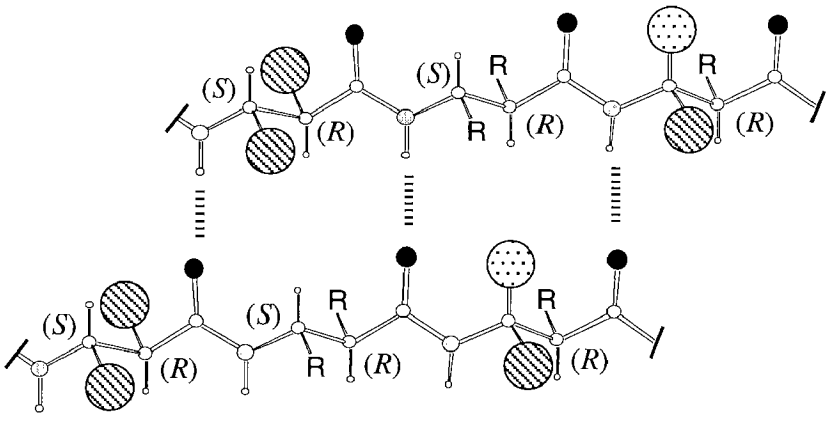
[94] However, the α-helical conformation of short α-peptides (5-9 residues) is not very stable in protic solvents, as first noted by *Goodman* and *Schmitt* [410,411].

[95] For a review see [423].

mentioned above, the polymeric material was examined by low-resolution methods. Recently, monodisperse oligomers of (*S*)-β^3-homoalanine ((*S*)-β-aminobutyric acid) were synthesized; however, structural studies by CD or NMR spectroscopy were thwarted by insolubility [434]. Homooligopeptides (three to eight residues) of β-alanine (β^3-HGly) were suggested to adopt β-sheet structures on the basis of IR spectroscopy and solubility properties [435]. This was also concluded from IR-studies of β-alanine derivatives by *Gung* [341,342]. The β^3-tripeptide Boc-β^3-HVal-β^3-HAla-β^3-HLeu-OMe adopts a β-sheet-type arrangement in the solid state with characteristic 14-membered H-bonded rings [17]. However, the structure also displays the initial turn leading to a 3_{14} helix.

We can now address the design of *parallel* pleated sheet structures of β-peptides with proteinogenic side chains which was inspired by X-ray data and classical conformational analysis, as presented in *Figure 12*. β^{2,3}-Amino-acid building blocks with *unlike*-configuration (the (2*R*,3*S*)-configuration is drawn) fit into the extended conformation. The α- and β-substituents alternatingly occupy both sides of the plane formed by the parallel amide groups. Intermolecular H-bonds, and thus sheet arrangement is hampered by chains consisting of building blocks with *like*-configuration (one substituent would be parallel to the C=O or NH groups).

Figure 12. Model of two fully extended β-peptidic strands in a parallel pleated sheet arrangement (model constructed from the extended section of Boc-β^3-HVal-β^3-HAla-β^3-HLeu-OMe [17] by MacMoMo). Hatched large spheres represent allowed backbone substituent positions, dotted large spheres indicate forbidden substituent positions. With (2*R*,3*S*)-β^{2,3}-amino-acid building blocks both alkyl substituents R and HN are antiperiplanar as are HN and C=O. Geminal disubstitution prevents the aggregation to a pleated sheet.



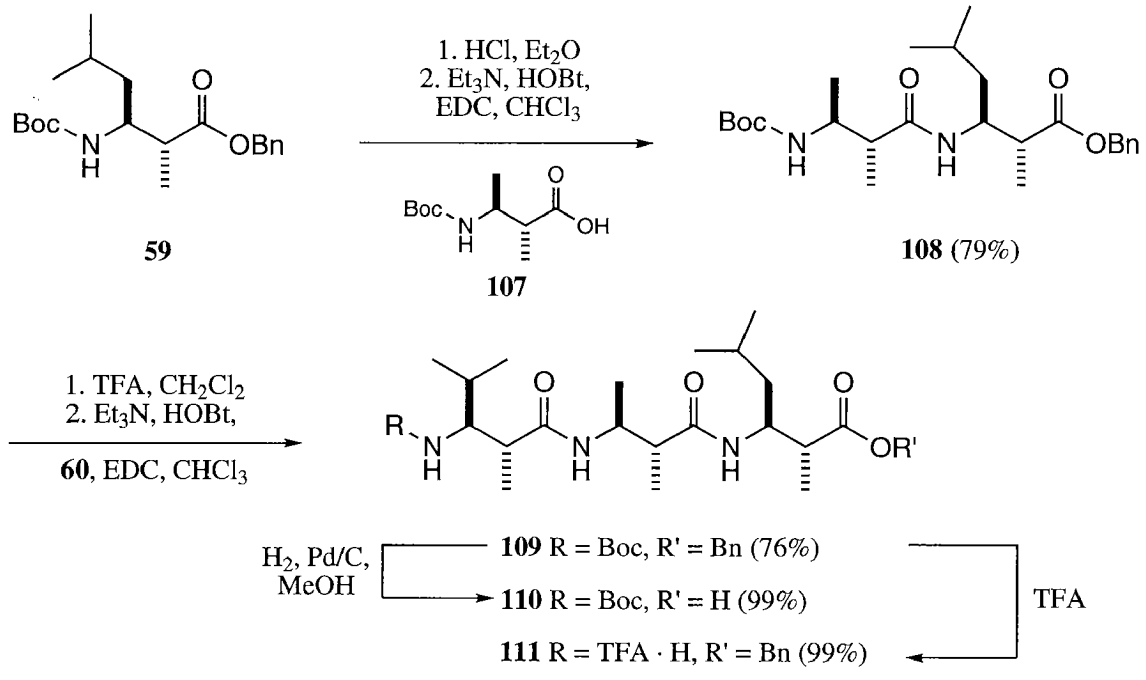
Thus, β-peptides consisting of *u*-$\beta^{2,3}$-amino acids had to be prepared to enforce the parallel pleated-sheet arrangement. There are big differences between *polymers* (nylon-3 polymers, *vide infra*) of *l*- or *u*-α-methyl β^3-homoalanine [311,433]: *i*) the polymeric material composed of the *u*-building blocks was insoluble in all solvents except for concentrated H_2SO_4 and liquid $SbCl_3$, whereas the polymer composed of the *l*-building blocks was well soluble in solvents commonly used for polyamides. *ii*) The melting point of the all-*u*-polymer is ca. 50 °C higher than that of the polymer with *l*-components. *iii*) The all-*u*-polymer was characterized as pleated sheet structure (cf. *Figure 12*). The measurement of the optical rotation of the all-*l*-polymer as a function of the optical rotation of the *l*-monomer revealed non-linearity. The authors suggested that the *l*-configuration precludes the sheet arrangement, but enforces a distinct asymmetric conformation[96] with a further contribution to the overall optical rotation [311].

[96] Given our knowledge on β-peptide structure, we would assign the (*M*)-3_{14} helix to this (–)-all-*l*-polymer.

5.3.1 *Synthesis of all-unlike-$\beta^{2,3}$-Peptides*

The synthesis of all-*u*-$\beta^{2,3}$-peptides is outlined in *Scheme 53*. The coupling of the TFA salt derived from **59** with Boc-(2*R*,3*S*)-$\beta^{2,3}$-HAla(α-Me)-OH[97] **107** proceeded smoothly as in the case of the *l*-compounds (see *Scheme 51*) to give $\beta^{2,3}$-dipeptide **108**; compound **107** was contaminated with small amounts of the α-epimer **99**, the corresponding diastereoisomeric dipeptide (ca. 4%) was easily removed by flash chromatography. Boc-Deprotection of compound **108** and coupling with Boc-$\beta^{2,3}$-amino acid **60** gave the fully protected all-*u*-$\beta^{2,3}$-tripeptide **109** the benzyl ester group of which was cleaved by hydrogenolysis (H_2, Pd/C) in a 13 mM MeOH solution to quantitatively provide **110**. Boc-deprotection of **109** was performed in pure TFA to give the TFA salt **111**.

Scheme 53. Synthesis of all-*u*-$\beta^{2,3}$-tripeptide derivatives **109**, **110** and **111**.



These all-*u*-$\beta^{2,3}$-tripeptide derivatives displayed very poor solubility: fully protected all-*u*-$\beta^{2,3}$-tripeptide **109** was only soluble in protic sovents (MeOH). The solubility of benzyl-ester-deprotected tripeptide acid **110** was even

[97] Compound **107** [18] was prepared by saponification of **36** with 2 equivalents of LiOH in refluxing MeOH/H_2O 3 : 1 for 50 min; diastereomer purity was > 95% according to ^{1}H-NMR spectroscopy.

poorer, it was soluble in hot MeOH only (*Table 4*). The Boc-deprotected TFA salt **111** displayed slightly better solubility.

Table 4. Solubility of all-*u*-$\beta^{2,3}$-tripeptide derivatives **110** and **111** in various solvents.

	Solvent	Solubility of **110** (mg/ml)	Solubility of **111** (mg/ml)
1	MeOH	5-6[a)]	> 10
2	$CHCl_3$	< 1	3
3	CCl_4	< 0.5	-[b)]
4	$ClCH_2CH_2Cl$	< 0.5	-[b)]
5	CF_3CH_2OH	< 1	-[b)]
6	NMP[c)]	< 0.5	-[b)]
7	DMF	1	-[b)]
8	CH_3CN	0.5	10
9	THF	< 4[d)]	-[b)]

a) In hot MeOH (50 °C). b) Not examined. c) 1-Methyl-2-pyrrolidinone. d) With 3-9 equiv. LiCl, according to ^{1}H-NMR in D_8-THF [115].

Thus, dimerizing coupling of **110** with **111** in various solvent systems[98] was unsuccessful because of unsurmountable solubility problems. Another strategy had to be devised for the construction of higher oligomers of **109** (*Chapter 5.3.3*).

5.3.2 X-Ray Crystal Structure of a β-Peptidic Pleated Sheet

There are few methods to prove the parallel arrangement of the all-*u*-$\beta^{2,3}$-tripeptide derivatives. A detailed NMR study necessitates a ca. 2 mM solution. However, the protic solvent would disrupt the interstrand H-bonds that are characteristic of the pleated sheet structure. Thus, X-ray and IR spectroscopy appear to be the only methods to elucidate the structure of these highly insoluble compounds.

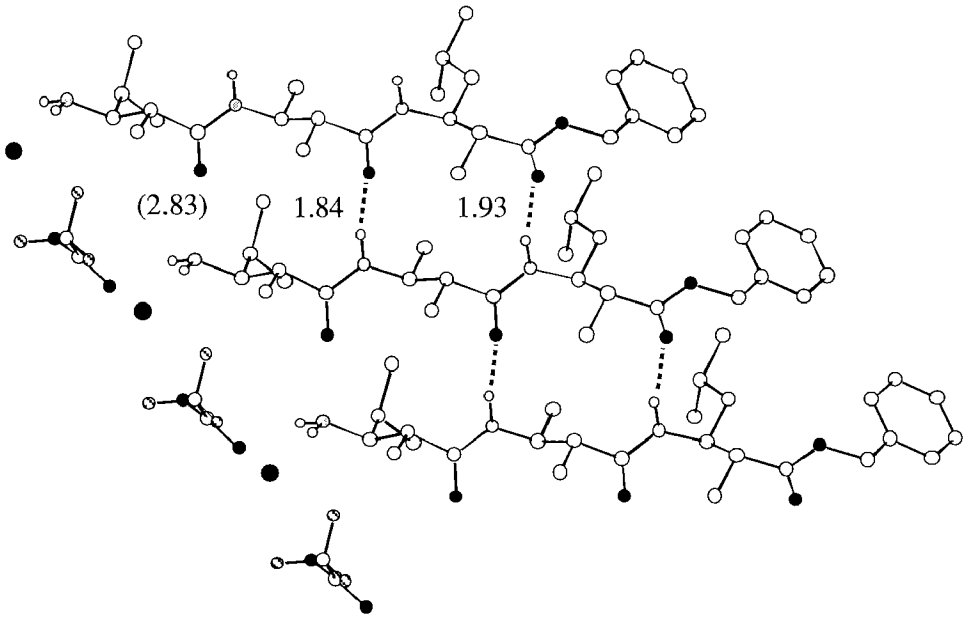
Gratifyingly, **111** gave crystals suitable for single-crystal X-ray analysis[99]. The crystal packing of **111**, which indeed forms sheets, is shown in *Figure 13*. The parallel amide planes in the individual strands are connected via the -CHR-

[98] Tested solvents: a) CF_3CH_2OH, b) $CHCl_3$/DMF, c) DMF, d) $CHCl_3$/DMF, LiCl.

[99] Fully protected **109** was also examined by powder X-ray techniques by *S. Brenner* from the Laboratorium für Kristallographie of the ETH Zürich. The structure indicates the aggregation to parallel pleated sheets albeit with less accuracy. For a recent impressive application (inorganic compounds) of their method, see [436,437].

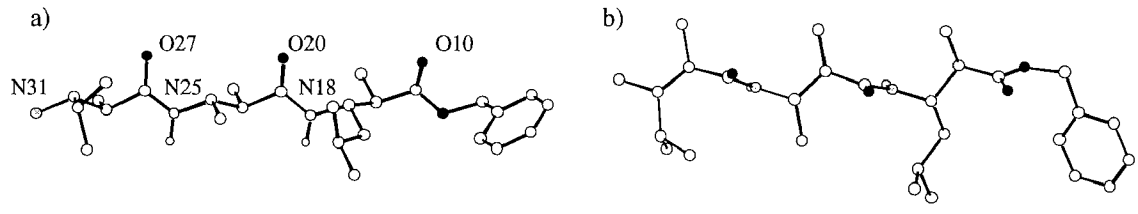
CHMe ethane moieties, with *antiperiplanar* R/Me and HN/CO; 14-membered H-bonded rings connect the strands in the *parallel* pleated sheet structure. In contrast to α-peptidic pleated sheets, where neighboring O=C-bonds point in opposite directions [438], these same bonds are unidirectional in the β-peptide structure, leading to a polar packing that might be an additional reason for the low solubility of compounds of this type (cf. cyclo-β-tetrapeptides [329,330,347]).

Figure 13. Parallel pleated sheet structure of $\beta^{2,3}$-tripeptide ester **111**·H_2O. The angles N–H···O are 159.7 resp. 165.6°; the distance N···O is shown for the *N*-terminal H-bond. The TFA salt **111** crystallizes with a molecule of H_2O. The X-ray structure analysis was performed by Dr. *P. Seiler*.



In *Figure 14a* the similarity of the crystal structure of **111** and the model (see *Figure 12*) is obvious. The fully extended conformation of the backbone and of the antiperiplanar alkyl substituents at C(α) and C(β) are clearly visible in *Figure 14b*.

Figure 14. Two views of the X-ray crystal structure of $\beta^{2,3}$-tripeptide benzyl ester **111**. a) View perpendicular to the amide planes with numbering of atoms. b) View along the parallel amide planes.



The interstrand H-bond distances and the H-bond angles are summarized in *Table 5*. The strands are held together by relatively short H-bonds[100].

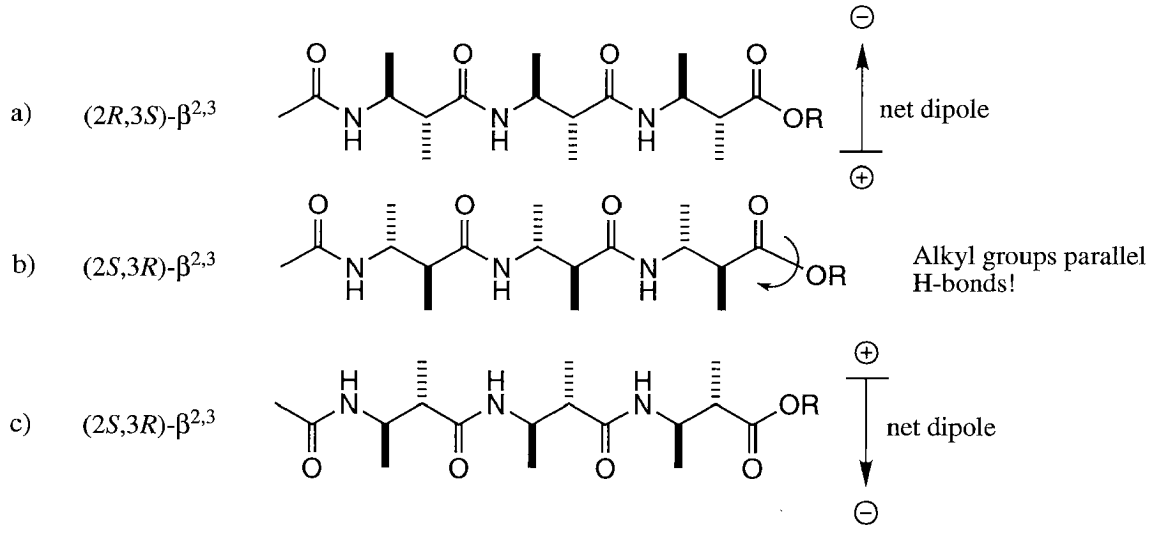
Table 5. Intermolecular H-bond distances and angles in the crystal structure of $\beta^{2,3}$-tripeptids **111** (N–H bond length 1.02 Å).

Atoms[a)]	Distance N · · · O [Å]	Distance H · · · O [Å]	Angle N–H · · · O [°]
N18 · · · O10	2.91	1.93	159.7
N25 · · · O20	2.84	1.84	165.6
N31 · · · O27	2.83	-	-

a) Numbering of atoms, as specified in *Figure 14a)*.

The well-defined polar structure of these pleated sheets implies the following: if the peptide chain is attached to a solid support the absolute configuration of the *u*-$\beta^{2,3}$-amino acids will determine the direction of the net dipole (*Figure 15*). Thus enantiomorph macroscopic sheet structures with defined sense of the macroscopic dipole should be conceivable.

Figure 15. Models of all-*u*-$\beta^{2,3}$-peptides attached to a solid phase (represented by R). If the configuration of the $\beta^{2,3}$-amino acids is changed from (2*R*,3*S*) (a) to (2*S*,3*R*) (b), all α- and β-substituents that were in allowed positions (cf. *Figure 12*) will be in forbidden substituent positions, i.e. parallel to the interstrand H-bonds. A 180°-rotation of the peptide chain decreases this steric repulsion and enables the parallel pleated sheet arrangement (c). During this process (a→c), the net dipole is inversed.



100 For reviews on the H-bond geometry in organic crystals see [439,440].

5.3.3 *Solid-Phase Synthesis of β-Peptides*

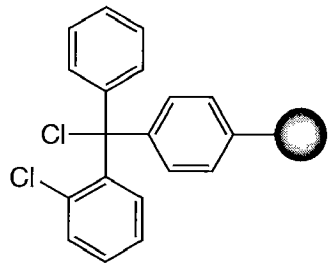
Solid-phase β-peptide synthesis was thought to be ideally suited for the rapid synthesis of a large variety of β-peptides as well as for new lead discovery and further exploration of the structural versatility of β-peptides. Moreover, the solubility problems arising with u-β^{2,3}-sequences (see *Chapter 5.3.1*) can be circumvented by synthesizing the β-peptide on solid support; the difficult purification procedures are applied to only one peptide.

The synthesis of α-peptides on an insoluble polymeric support is now a standard method in peptide synthesis, allowing for rapid chain elongation processes which can be automated. Several reviews have appeared on solid-phase peptide synthesis (SPPS) [205,441-443][101].

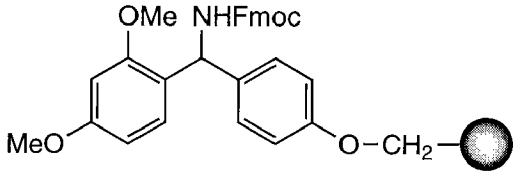
β-Peptides were coupled in the solid phase for the first time in our group [200,203,444][102]. Two types of linkers were chosen to provide access to both peptide acids and amides. In this chapter the methods used for solid-phase synthesis of β-peptides are presented; details will be discussed with the corresponding β-peptides. The highly acid-labile *ortho*-chlorotrityl-chloride resin [445,446] was first used for the synthesis of β-peptide acids (*Figure 16a*). β-Peptide amides were obtained by cleavage of the corresponding β-peptide *Rink* amide resin [447-449] (*Figure 16b*).

Figure 16. Linkers used for solid-phase synthesis of β-peptides. a) Hyper-acid labile *ortho*-chlorotrityl-chloride resin. b) Acid-labile *Rink* amide resin. The spheres represent the polymer support (polystyrene matrix, cross-linked with 1% 1,3-divinylbenzene).

a) *ortho*-chlorotrityl-chloride resin



b) Fmoc-protected *Rink* amide resin



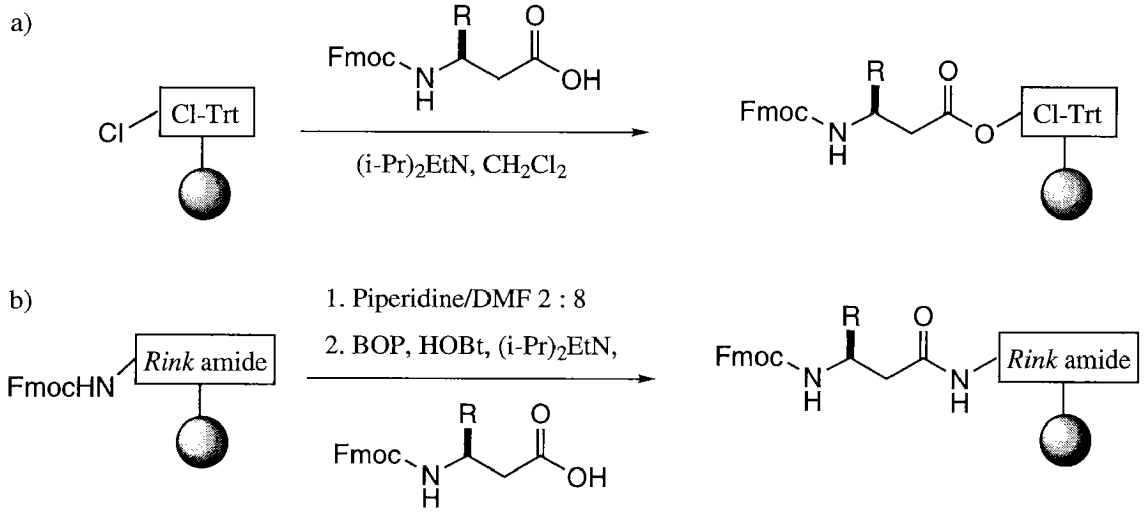
Two methods were used for anchoring of the first β-amino acid to the resins. In *Figure 17a*, the *ortho*-chlorotrityl-chloride resin (initial loading: 1.05 or 1.3

[101] A good compilation of resins, reagents and methods for SPS is given in the *Calbiochem/Novabiochem* 'Catalog & Peptide Synthesis Handbook', 1999, p. S1-S85.

[102] Independent of our work, there was a report on solid-phase β-peptide synthesis by an industrial group [208].

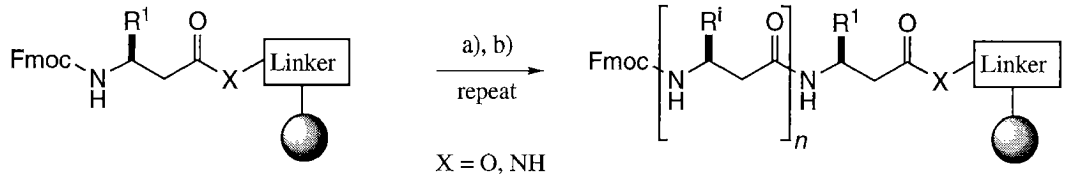
mmol Cl/g) was esterified for 4 h with an Fmoc-protected β-amino acid (0.7-0.9 equiv.) in the presence of *Hünig*'s base (4 equiv. with respect to the amino acid), as previously described for α-amino acids [445,446,450]. Unreacted chloride was neutralized by addition of MeOH ("capping"), and the resin loading was determined, after treatment with 20% piperidine in DMF, by measuring the absorbance of the dibenzofulvene-piperidine adduct at 300, 289, and 266 nm [451-453]. Anchoring of an Fmoc-protected β-amino acid to the *Rink* amide resin (initial loading of 0.45 and 1.00 mmol/g) was achieved as described in *Figure 17b*. No capping step was necessary as the corresponding deletion sequences were not detected; thus the anchoring step was actually quantitative.

Figure 17. Methods used to anchor the first β-amino acid to the resin, as shown for β^3-amino acids. a) Standard coupling of the *ortho*-chlorotrityl-chloride resin with Fmoc-protected β-amino acids. b) Fmoc-Deprotection of the *Rink* amide resin and coupling with Fmoc-protected β-amino acids using BOP, HOBt and *Hünig*'s base. For abbreviations, see *Exp. Part*.



Chain elongation on solid phase was carried out as outlined in *Figure 18*. Fmoc-β-amino acids (2.5-6 equiv. with respect to the resin loading) were activated using BOP/HOBT/(*i*-Pr)$_2$EtN and coupling reactions were performed in DMF at r.t. for 15-60 min. It is important to mention that the *Kaiser* ninhydrin test [454] of Fmoc-deprotected β^3-peptide-resins fails to work. Hence, coupling reactions were monitored using 2,4,6-trinitrobenzenesulfonic acid (TNBS) [455]. After removal of the last Fmoc protecting group, peptide-resins were cleaved with TFA in various "cleavage-cocktails" (see *Exp. Part*) to afford either the peptide acids (*ortho*-chlorotrityl-chloride resin) or the peptide amides (*Rink* amide resin).

Figure 18. General procedure for the solid-phase synthesis of β-peptides on *ortho*-chlorotrityl-chloride (X = O) or *Rink* amide resin (X = NH), as shown for β^3-amino acids. *ortho*-Chlorotrityl-chloride resin (X = O): cleavage by 0.5% TFA in CH_2Cl_2 provides peptide acids; *Rink* amide resin (X = NH): cleavage by 10% TFA in CH_2Cl_2 (flow method, see *Exp. Part*) provides peptide amides.

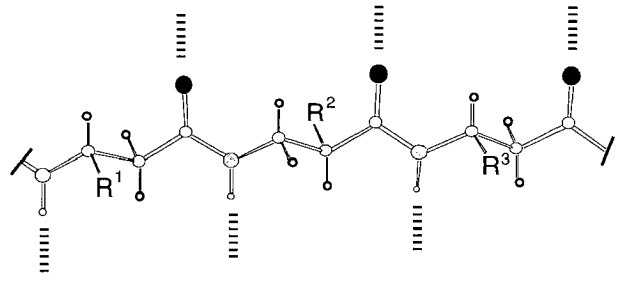


a) 20% piperidine in DMF or DBU/piperidine/DMF 1 : 1 : 48; b) Fmoc-(*S*)-β^3-HXaa-OH, BOP, HOBT, (*i*-Pr)$_2$EtN

5.3.4 Solid-Phase Synthesis of β-Peptide **112**

The insolubility of the $\beta^{2,3}$-tripeptide derivatives **109-111** led to the design of a second type of pleated sheet-forming β-peptide (*Figure 19*). Alternatingly, the α- and β-substituents of the α,β-disubstituted residues are left out (see *Figure 12*). This lower degree of substitution was expected to increase solubility (only one hydrophobic side chain per residue) while maintaining the tendency to form the parallel pleated sheet structure.

Figure 19. Model of a β-peptidic strand in a pleated sheet structure. The indicated β-amino acid sequence with R^1, R^2, $R^3 \neq H$ (-(*R*)-β^2/(*S*)-β^3-) should also enforce the linear arrangement; all side chains are in allowed positions. A 3_{14} helix can not be formed.

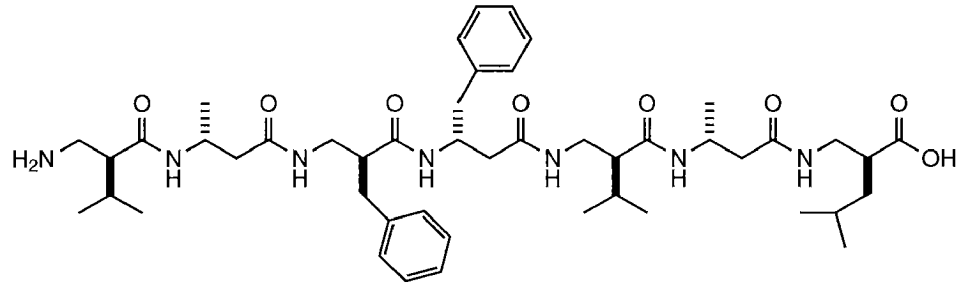


The β-heptapeptide **112** consisting of (*S*)-β^2/(*R*)-β^3-residues (*Scheme 20*) was synthesized on *ortho*-chlorotrityl-chloride resin[103]. Its configuration corresponds to the mirror image of the model peptide shown in *Figure 19*.

[103] The β^2-amino acids Fmoc-(*S*)-β^2-HLeu-OH, Fmoc-(*S*)-β^2-HVal-OH, and Fmoc-(*S*)-β^2-HPhe-OH were generously provided by Dr. *G. Guichard*. As determined by GC on a chiral phase (Prof. Dr. *P. Fischer*, Stuttgart), the enantiopurity was between 92.3 and 96.5%.

Figure 20. β-Peptide **112** with (*S*)-β^2/(*R*)-β^3-sequence synthesized on *ortho*-chlorotrityl-chloride resin.

112



A satisfactory esterification yield (74%, corresponding to a substitution of 0.47 mmol/g resin) was monitored for anchoring the first β-amino acid to the resin. Peptide synthesis proceeded smoothly to give crude **112** in 99% yield after cleavage from the resin by treatment with 5% TFA in CH_2Cl_2 (Table 6). However, **112** was contaminated with epimeric β-heptapeptides, resulting in a low HPLC purity. Still, **112** could be isolated in pure form by preparative reversed-phase (RP)-HPLC.

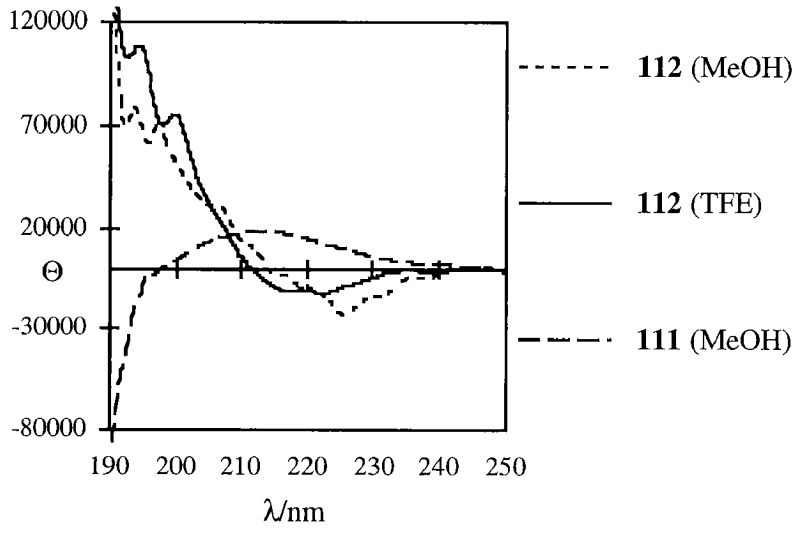
Table 6. Isolation and characterization by HPLC and MS of β-peptide **112**.

β-Peptide	Yield (%)[a]	Purity[b] (%)	t_R (min)[c]	FAB-MS
112	99	64	8.8[d]	864.8 ([*M* + 1]$^+$)

a) % Mass recovered based on polymer loading. b) HPLC purity (220 nm) of the crude product. c) Retention time in the HPLC (linear gradient of *A* (0.1% TFA in H_2O) and *B* (MeCN); see *GP 27* in *Exp. Part.* d) 30-90% *B* in 20 min (C_{18}).

However, β-heptapeptide **112** was only soluble, to some extent, in HOAc or TFA after ultrasonication or heating. The ^{1}H-NMR spectrum was measured as a turbid suspension of a ca. 2 mg-sample in 0.7 ml CD_3CO_2D, and CD spectroscopy was possible, because low concentrations are sufficient for measurement. The CD spectra of **112** in MeOH and CF_3CH_2OH (TFE) are compared with the CD spectrum of the parallel-pleated-sheet-forming *u*-$\beta^{2,3}$-tripeptide **111** in *Figure 21.* There are no common features between **111** and **112**: tripeptide **111** displays a *Cotton* effect which is typical of a disordered β-peptide [17,18] and β-heptapeptide **112** shows a new type of CD pattern in both solvents with a trough at ca. 220 nm. It is evident, that the aggregation of **111** (see *Figure 13*) is disturbed in protic solvents. Thus, the CD spectrum of a β-peptide adopting the parallel pleated structure remains unknown.

Figure 21. CD spectra of *u*-$\beta^{2,3}$-tripeptide **111** and β-heptapeptide **112**. Spectra were recorded at r.t. The concentration was 0.2 mM in MeOH and CF_3CH_2OH (TFE). [Θ] in 10 deg · cm^2 · mol^{-1}.

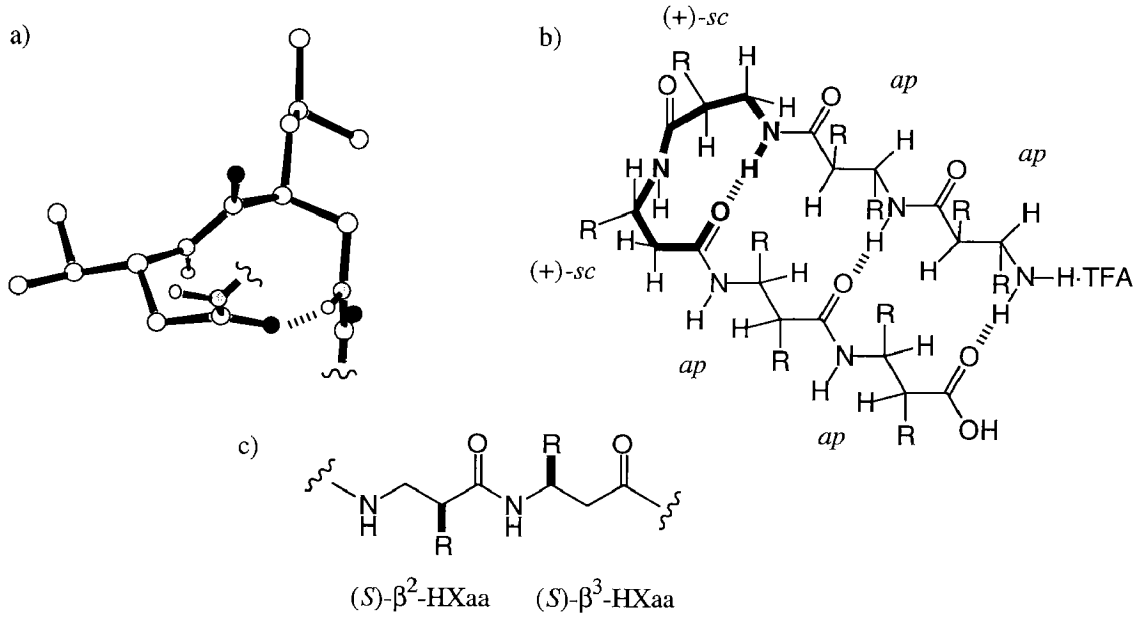


In summary, the structure of β-heptapeptide **112** is still to be established; its insolubility, however, suggests aggregation to pleated sheets as in the case of **111**.

5.3.5 *Solid-Phase Synthesis of Hairpin-Forming β-Peptides*

To secure access to larger sheet-forming β-peptides, formation of insoluble aggregates has to be prevented. Possible strategies include *N*-methylation, as described for an α-peptide [424] or connection of two β-peptidic strands with an appropriate turn element, to form an *antiparallel* pleated sheet arrangement, i.e. a hairpin. In the meantime, a 12/10/12 helix was discovered in our group [27,328]; the central 10-membered H-bonded turn of which was identified as a possible generic motif for forming β-peptidic hairpins (*Figure 22*). The required substitution pattern is shown in *Figure 22c*. Two dipeptide segments of *u*-$\beta^{2,3}$-amino acids, which enforce an extended conformation, can be attached on each end of this unit and should be able to form *intra-* rather than *inter*molecular H-bonds with each other.

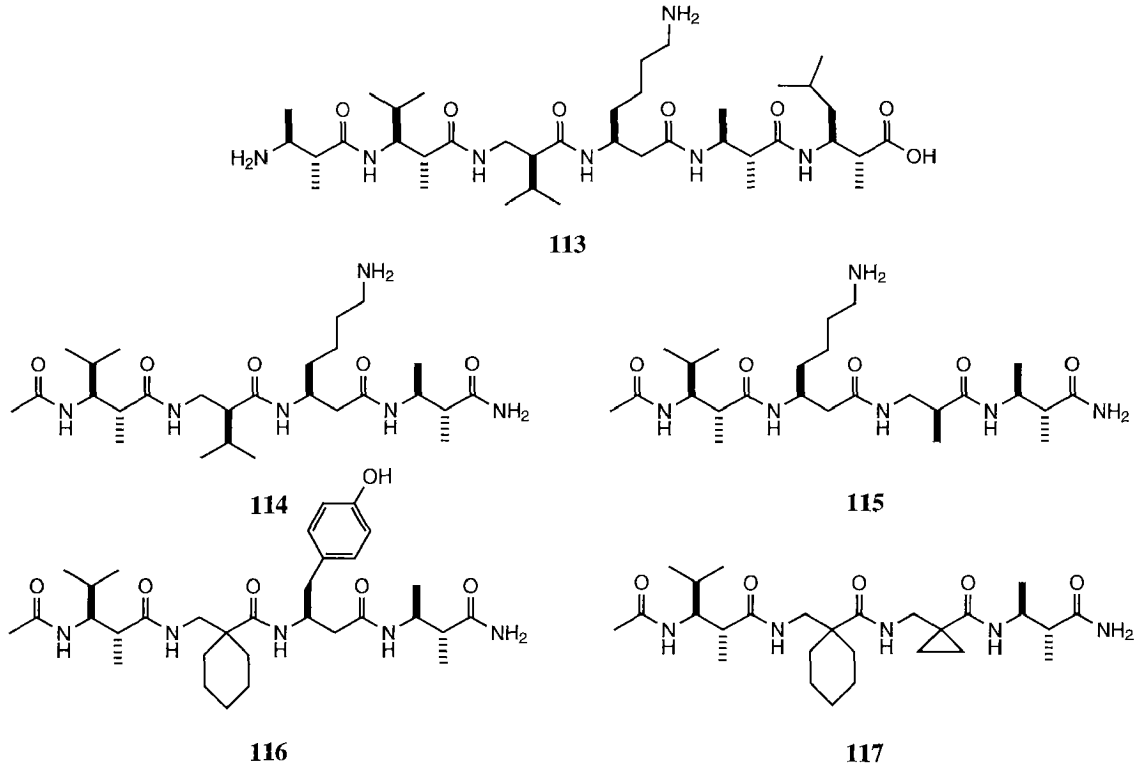
Figure 22. Design of a β-peptidic hairpin. a) Ten-membered H-bonded turn, found in the β^2/β^3-segment of the 12/10/12 helix [27,328]. b) Model of two antiparallel *u*-residue β-peptide strands held together by a β-dipeptide-turn segment built of an (*S*)-β^2- and an (*S*)-β^3-amino acid. The torsion angle Θ (NH–C(β)–C(α)-CO) is indicated for each residue. c) Turn segment which should link the adjacent β-peptide strands in a β-peptide hairpin.



The first β-peptide synthesized for this purpose (**113**) bears the side chains of valine and lysine in the turn segment (*Figure 23*). To study the hairpin-forming propensity of β-peptides further, and to find other possible turn

segments, β-tetrapeptides **114-117** with different substitution patterns in the turn section were also prepared on solid phase.

Figure 23. β-Peptides which were designed to promote hairpin formation. **113-117** were synthesized on solid phase.



113

114

115

116

117

Yields and purities of the crude peptides are given in *Table 7*. All β-peptides were synthesized using BOP/HOBt/(i-Pr)$_2$EtN activation of the corresponding Fmoc-protected β-amino acids. Fmoc-Protected β^{2,3}-amino acid **62** was not soluble in DMF or DMF/CH_2Cl_2[104]. However, it turned out that a suspension of **62** in DMF could also be used with the same coupling efficiency. The yields of crude products were good, indicating nearly quantitative yields for the coupling and deprotection steps.

β-Hexapeptide **113** was synthesized on *ortho*-chlorotrityl-chloride resin. The Fmoc-protected β^{2,3}-amino acid **63** was attached to the resin in 85% yield (loading 0.53 mmol/g). Elongation of the peptide chain was achieved uneventfully. Treatment of the peptide-resin with TFA/H_2O/(i-Pr)$_3$SiH 95 : 2.5 : 2.5 afforded peptide **113** in low purity. This was due to the presence of an

[104] Attempts to solubilize it in so-called "magic-mixture" (CH_2Cl_2/DMF/NMP 1 : 1 : 1 with 1% Triton X100 and 2M ethylenecarbonate) were unsuccessful.

epimeric β-heptapeptide[105] which could, however, be removed by preparative RP-HPLC by applying an appropriate gradient. The final purity, as determined by RP-HPLC, was > 98%. All deprotection steps were performed using 20% piperidine in DMF. Peptides **114-117** were prepared on the *Rink* amide resin. The Fmoc group was removed using DBU/piperidine/DMF 1 : 1 : 48, because incomplete Fmoc removal occurred in the last steps of some syntheses (*vide supra*). After removal of the last Fmoc-group, the *N*-terminus of these β-peptides was acylated on the solid support with $Ac_2O/(i\text{-}Pr)_2EtN$ for 10 min[106]. Monitoring of the coupling rate during the synthesis of β-tetrapeptide **114** revealed that some coupling steps were finished after as little as 10-15 min. Cleavage of the peptide–resin bond was achieved by successive treatment with 5- and 10% TFA in CH_2Cl_2 ("flow method", see *GP 26* in *Exp. Part*) affording three fractions of crude peptide with HPLC purities between 57 and 59%. Two fractions of β-peptide **115** were prepared by the same procedure. The crude product was of high purity. Final HPLC purities of **114** and **115** were > 96%. β-Tetrapeptides **116** and **117** contain the geminally disubstituted $\beta^{2,2}$-amino acids **80a** and **80b**; the latter was not soluble in DMF/CH_2Cl_2 which is normally used as solvent for coupling on *Rink* amide resin. Instead, **80b** was added as a solution in DMF/NMP.

Table 7. Isolation and characterization by HPLC and MS of β-peptides **113-117**.

	β-Peptide	Yield (%)[a)]	Purity[b)] (%)	t_R (min)[c)]	FAB-MS
1	**113**	90	57	13.0[d)]	740.5 (M^+)
2	**114**	85	57	12.0[e)]	541.3 ($[M + 1]^+$)
3	**115**	97	82	11.0[f)]	513.6 ($[M + 1]^+$)
4	**116**	99	93	11.9[g)]	602.4 ($[M + 1]^+$)
5	**117**	80	83	11.1[g)]	522.4 ($[M + 1]^+$)

a) % Mass recovered based on polymer loading. b) HPLC purity (220 nm) of the crude product. c) Retention time in the HPLC (linear gradient of *A* (0.1% TFA in H_2O) and *B* (MeCN); see *GP 27* in *Exp. Part*. d) 5-30% *B* in 10 min, then, 30-40% *B* in 10 min (C_8). e) 2-50% *B* in 20 min (C_{18}). f) 2-20% *B* in 10 min, then 20-30% *B* in 15 min (C_{18}). g) 20-50% *B* in 20 min (C_8).

β-Peptides **113-115** are very soluble in H_2O and MeOH. Obviously, the lysine side chain is very effective in rendering β-peptides rich in hydrophobic side chains water-soluble. However, these β-peptides can not be solubilized in aprotic solvents such as $CHCl_3$ or CCl_4; thus, IR-studies of the H-bonding

105 NMR-analysis suggested that the configuration at the C(α) of the third residue was inverted.

106 *N*-Acetylation was thought to increase the crystallinity.

pattern were not possible [340,342,456-460]. A β^3-homotyrosine residue in the β-tetrapeptide **116** does not result in better water-solubility; actually, **116** is insoluble in H_2O, MeCN and $CHCl_3$. It is poorly soluble in MeOH. β-Peptide **117** is the only β-peptide among the compounds **113-117** which is soluble in $CHCl_3$ with concomitant loss of solubility in H_2O.

5.3.6 *2D-NMR Analysis of the Hairpin-Forming β-Peptide* **113**

The structure of hexapeptide **113** in CD_3OH was determined by 2D-NMR spectroscopy[107]. All resonances were assigned unequivocally by the evaluation of DQF-COSY and TOCSY measurements. The coupling constants between the protons of the peptide backbone are listed in *Table 21* in the *Exp. Part.* The *J*-values of the backbone protons of amino acids 2 and 5 are large, implying an *antiperiplanar* arrangement of the corresponding NH and C(β)-H, as well as C(β)-H and C(α)-H protons; more than one preferred conformation of the terminal amino acids 1 and 6 is suggested by the smaller *J*-values of the corresponding protons. However, the values observed for the central amino acids 3 and 4 indicate a completely different, non-extended conformation, and they resemble those found for the 10-membered H-bonded ring of the 12/10/12 helix [27,328]. ROESY measurements were used to obtain information about the distances between the protons, and the volumina of 20 NOE cross peaks were classified in three distance categories according to their intensities (*Table 8*).

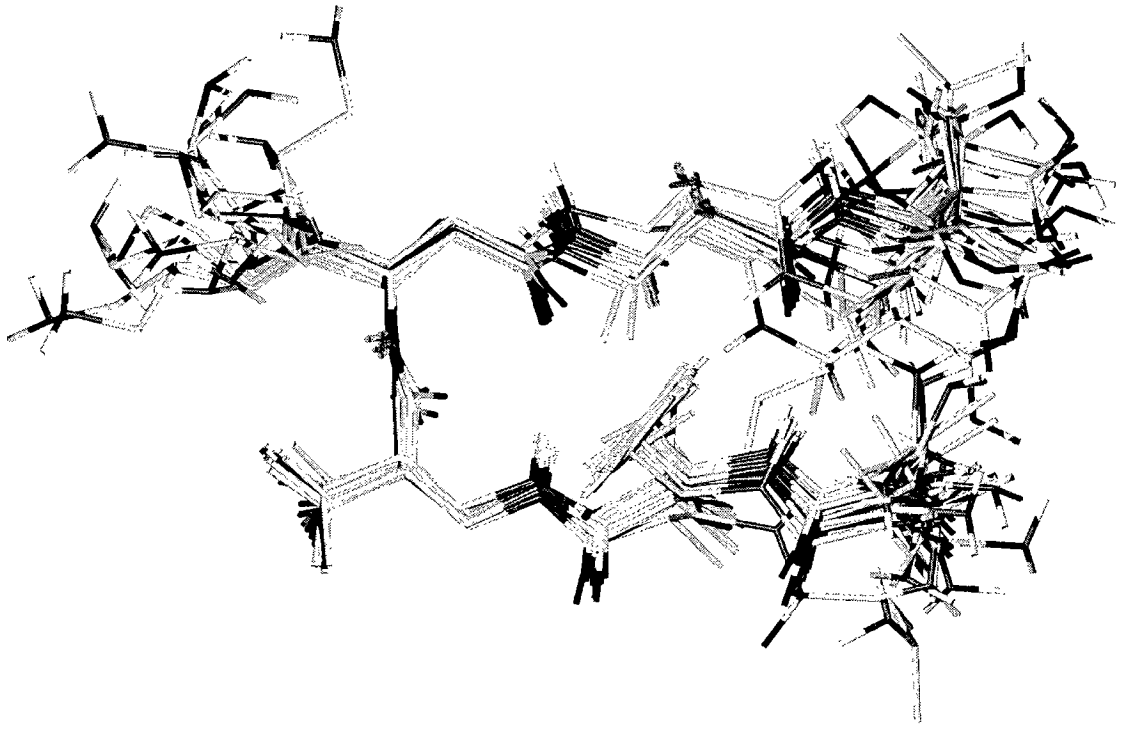
Table 8. NOEs of β-hexapeptide **113** (CD_3OH, 500 MHz) extracted from the 150ms-ROESY spectrum. The NOEs were classified in three distance categories: s (strong, < 3 Å), m (medium, < 3.5 Å) and w (weak, < 4.5 Å). Numbering of backbone atoms starts from the *N*-terminus.

Residue	H-Atom	Residue	H-Atom	NOE
$\beta^{2,3}$-HVal(αMe)(2)	NH	$\beta^{2,3}$-HVal(αMe)(2)	C*H*Me$_2$	m
$\beta^{2,3}$-HVal(αMe)(2)	NH	$\beta^{2,3}$-HVal(αMe)(2)	α	m
$\beta^{2,3}$-HVal(αMe)(2)	NH	$\beta^{2,3}$-HAla(αMe)(1)	β	w
$\beta^{2,3}$-HVal(αMe)(2)	NH	$\beta^{2,3}$-HAla(αMe)(1)	α	s
β^{2}-HVal(3)	β-H^{Si}	β^{2}-HVal(3)	C*H*Me$_2$	m
β^{2}-HVal(3)	β-H^{Si}	β^{2}-HVal(3)	α	m
β^{2}-HVal(3)	NH	$\beta^{2,3}$-HVal(αMe)(2)	β	m
β^{2}-HVal(3)	NH	$\beta^{2,3}$-HVal(αMe)(2)	α	s
β^{3}-HLys(4)	NH	β^{3}-HLys(4)	α-H^{Si}	w
β^{3}-HLys(4)	NH	β^{3}-HLys(4)	α-H^{Re}	s
$\beta^{2,3}$-HAla(αMe)(5)	NH	$\beta^{2,3}$-HAla(αMe)(5)	α-Me	w
$\beta^{2,3}$-HAla(αMe)(5)	NH	$\beta^{2,3}$-HAla(αMe)(5)	β-Me	m
$\beta^{2,3}$-HAla(αMe)(5)	NH	β^{3}-HLys(4)	α-H^{Si}	m
$\beta^{2,3}$-HAla(αMe)(5)	NH	$\beta^{2,3}$-HAla(αMe)(5)	α	m
$\beta^{2,3}$-HAla(αMe)(5)	NH	β^{3}-HLys(4)	β	m
$\beta^{2,3}$-HLeu(αMe)(6)	NH	$\beta^{2,3}$-HLeu(αMe)(6)	α	m
$\beta^{2,3}$-HLeu(αMe)(6)	NH	$\beta^{2,3}$-HLeu(αMe)(6)	C*H*Me$_2$	m
$\beta^{2,3}$-HAla(αMe)(1)	α	$\beta^{2,3}$-HLeu(αMe)(6)	β	w
$\beta^{2,3}$-HVal(αMe)(2)	α	$\beta^{2,3}$-HAla(αMe)(5)	β	m
$\beta^{2,3}$-HVal(αMe)(2)	δ-Me	$\beta^{2,3}$-HAla(αMe)(5)	α-Me	w

[107] The 2D-NMR-analysis and MD-simulation were performed by *K. Gademann.*

Long-range NOEs between amino acids 1 and 6, as well as 2 and 5 are especially significant. An extended section of the structure is suggested by NOEs between NH(3), C(α)-H(2) and NH(2), as well as between NH(6), C(α)-H(5) and NH(5). Twenty NOEs and twelve *J*-values were used as distance and torsion-angle restraints in molecular dynamics simulations, using simulated annealing (program X-PLOR). The resulting conformations converged to a unique structure of peptide **113** (*Figure 24*): obviously, this β-peptide adopts a so-called *hairpin* arrangement, a secondary structural element often responsible for interactions of α-peptides with receptors[108]. Hitherto, turns of β-peptides were only achieved by incorporating "unnatural" building blocks with cyclic α- and β-amino acids, as e.g. -*D*-Pro-Xxx- [461] or (*R*)-β²-HPro-(*S*)-β²-HPro [299].

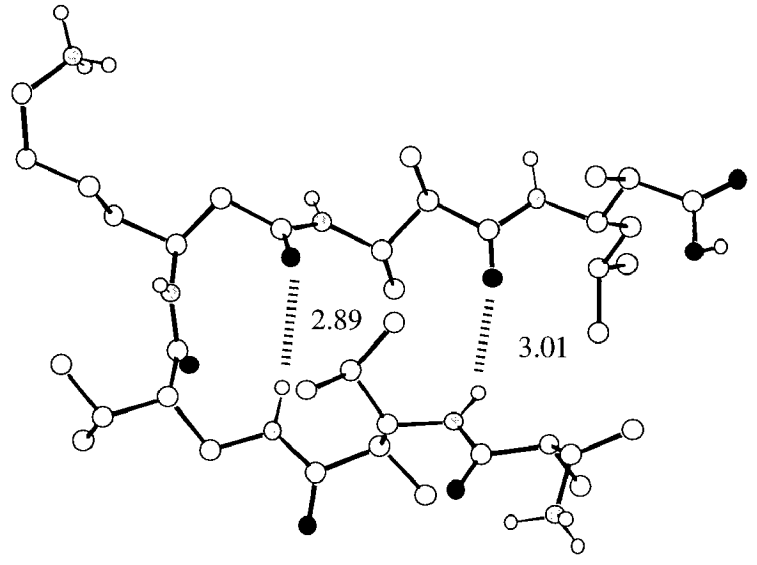
Figure 24. Backbone traces of the 15 lowest-energy conformers of β-peptide **113** showing no significant violation of the experimental NMR restraints in CD_3OH. Code: carbonyl O-atoms *black*, amide N-atoms *grey*, C-atoms *light-grey*. According to this analysis, the *C*- and *N*-terminal amino acids 1 and 6 are rather flexible, and two conformations are observed for 6, which are both in agreement with the experimental data. The figure was generated by MOLMOL [387].



[108] For the first example of a pharmacologically active β-peptide with affinity to a human receptor see [347].

Figure 25 shows one low-energy conformer of **113**. The four C=O bonds associated with the H-bonding 14-ring are orientated in approximately the same direction.

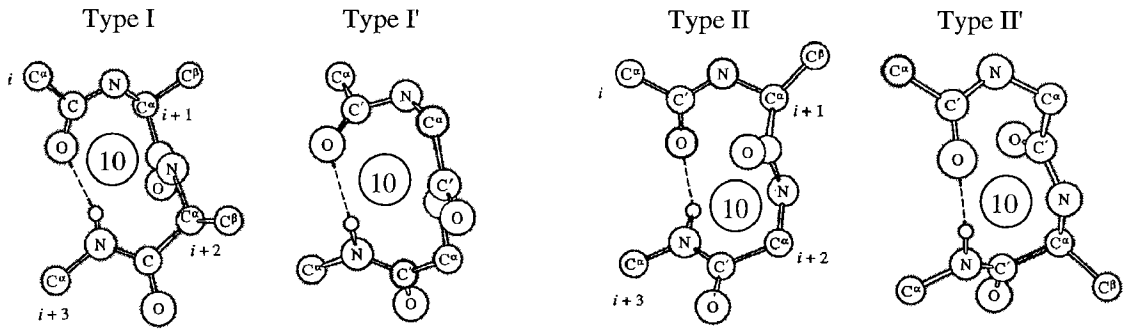
Figure 25. Top view of a low-energy model of β-hexapeptide **113**. Two cross-strand H-bonds (N–H · · · O in [Å]) are indicated; assumed N–H bond length: 1.00 Å. All CH hydrogens have been omitted for clarity. The figure was generated by MacMoMo (program by Prof. Dr. *M. Dobler*, ETH-Zürich).



Especially in nonpolar solvents, where intermolecular H-bonds are favorable, it is important to show that the detected H-bonds are clearly intramolecular. The structure of β-peptide **113** was determined in the protic solvent CD_3OH, and concentration-independent ^{1}H-NMR shift values confirmed the monomeric form of **113**. NMR analysis provided no evidence for secondary structure in H_2O/D_2O.

Reverse turns of α-proteins are often stabilized by interaction with the rest of the molecule; the restrictions on the flexibility of the peptide backbone are not very stringent due to a great Φ and Ψ range of the β turn region in the *Ramachandran* plot [283a]; in particular, an H-bond between residues *i* and (*i* + 3) in the β-turn is often missing. The structures of the most common β-turns are illustrated in *Figure 26*. The β-turn propensities of the natural α-amino acids are listed in the literature [462-465]. The mirror images of turns I and II (of the backbone, but not the side chains, of course) are the primed variants I' and II'. Type I β-turns occur most frequently, two to three times more frequently than type II.

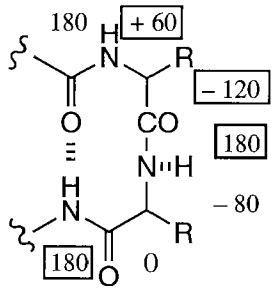
Figure 26. The most common β-turns connecting adjacent strands of an antiparallel β-sheet in α-peptides and proteins in ten-membered H-bonded rings. The first of the four residues that are considered to define the turns is designated *i*. C(β)-Atoms are shown only in positions where non-Gly residues occur frequently. The H-bond of the β-sheet is shown as a dashed line (from [466]).



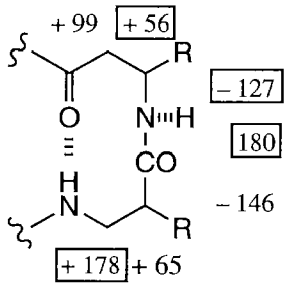
Comparison of the turn segment of β-peptide **113** with these α-peptidic β-turns reveales that the type II' turn looks like the ten-membered β-peptide turn (*Figure 27*); of six torsion angles defining both structures, four are of comparable size (numbers in frames in *Figure 27*).

Figure 27. Comparison of type II' β-turn (a) with the turn segment of the hairpin-forming β-peptide **113** (b). The dihedral angles in [°] are indicated.

a) 10-Membered ring of a type II' β-turn

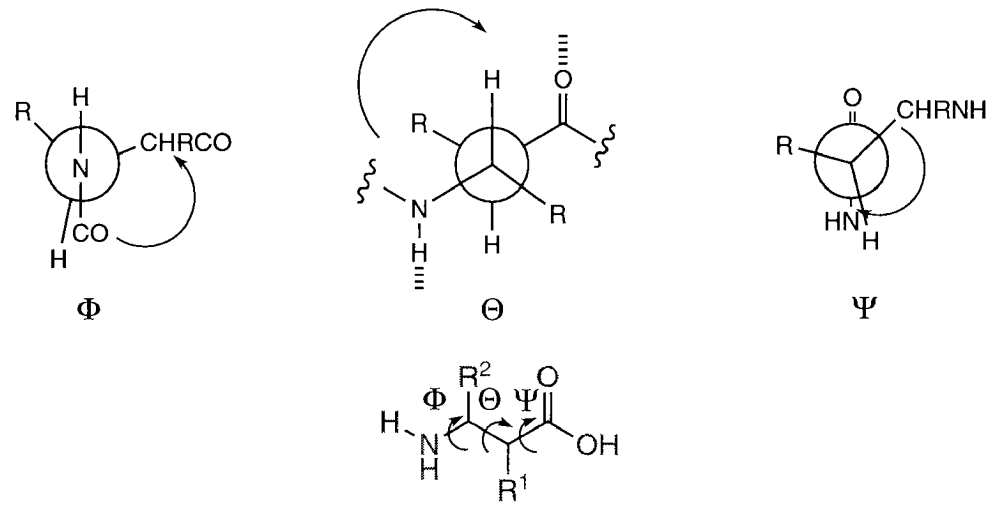


b) 10-Membered ring in the hairpin structure of **113**



With the X-ray structure of **111** and the NMR-structure of **113**, two β-peptidic pleated sheets have now been characterized. The sheet-forming *u*-residues in **113** adopt the typical values of the extended conformation, as do the $\beta^{2,3}$-amino acids in **111**. Following a proposal by *Balaram*, the torsion angles in β-amino acids are specified as depicted in *Figure 28*.

Figure 28. Torsion angles in β-amino acid residues, according to [467]. The ideal values characteristic of a pleated sheet are drawn: Φ = – 120°, Θ = 180° and Ψ = + 120°).



In *Table 9* the dihedral angles of both, the residues in the parallel pleated sheet **111** and the two $\beta^{2,3}$-amino acids attached to the central β^2-β^3-segment of the antiparallel pleated sheet **113** are compared. The observed values are close to the ideal values (cf. *Figure 28).*

Table 9. Torsion angles Φ, Θ, and Ψ of $\beta^{2,3}$-tripeptide benzyl ester **111** and β-hexapeptide **113**. Residues are numered starting from the *N*-terminus.

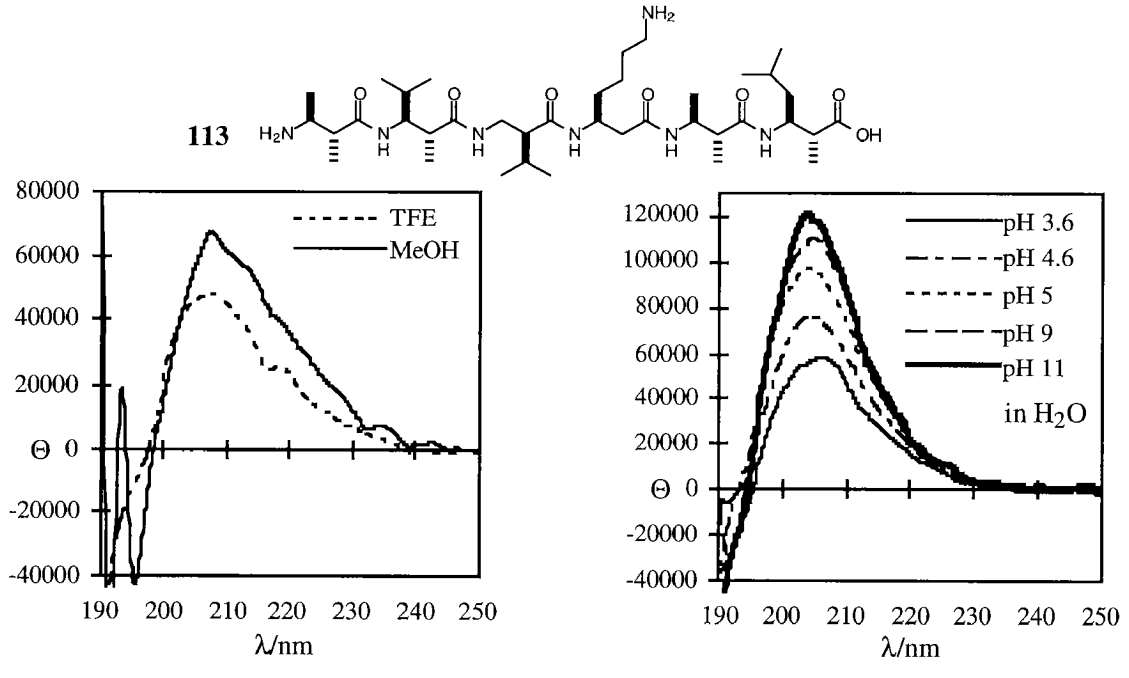
111		**113**	
Torsion angles	[°]	Torsion angles[a)]	[°]
Φ(1)	– 106.47	Φ(2)	– 118.9
Θ(1)	+ 177.06	Θ(2)	+ 160.0
Ψ(1)	+ 127.21	Ψ(2)	+ 66.0
Φ(2)	– 115.73	Φ(5)	– 110.4
Θ(2)	+ 177.67	Θ(5)	+ 172.6
Ψ(2)	+ 127.03	Ψ(5)	+ 147.9
Θ(3)	+ 174.32		-
Ψ(3)	+ 125.41		-

a) Torsion angles of sheet-forming residues 2 and 5, that are attached to the central turn segment.

5.3.7 CD Spectra of Hairpin-Forming β-Peptides and Discussion of the Different Turn Segments

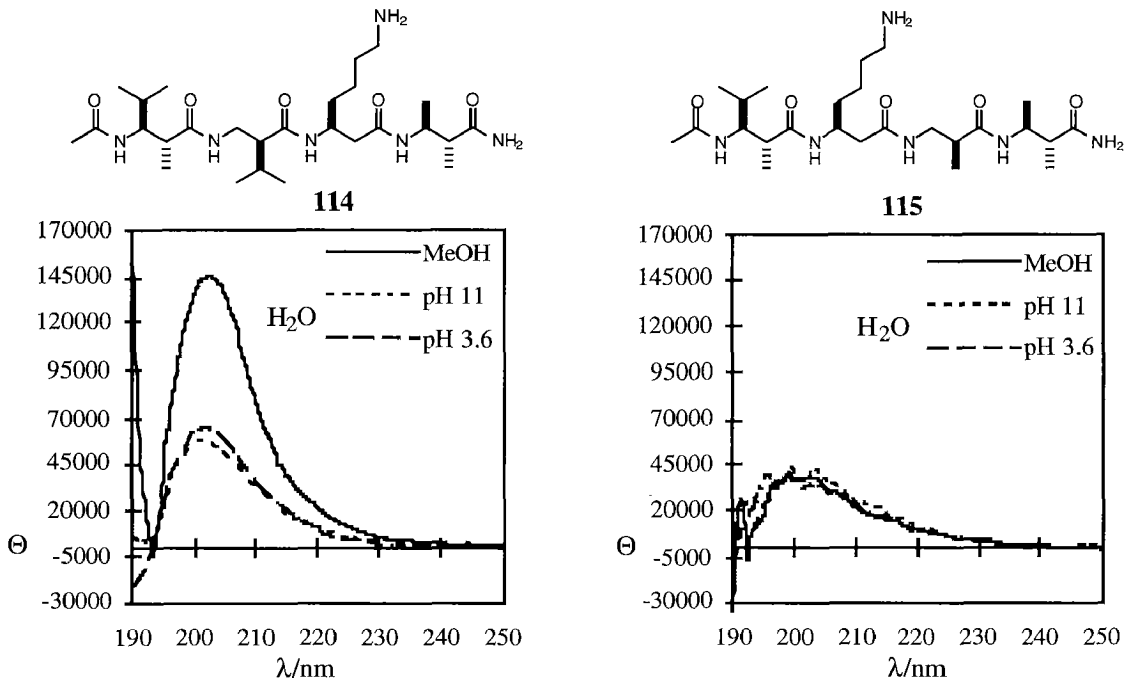
It is of great interest to see whether the hairpin secondary structure of β-peptides is also associated with a specific CD pattern. The CD spectra of **113** in MeOH and TFE (CF_3CH_2OH) are quite similar, with a maximum at ca. 210 nm (*Figure 29*). Very intense *Cotton* effects were measured in buffered aqueous solution; indeed, the molar ellipticity of over $1.2 \cdot 10^5$ (at pH 11) for a β-peptide in aqueous solution is unrivaled. Furthermore, the CD spectra of **113** are pH-dependent. The *Cotton* effect decreases with pH; the lowest molar ellipticity (ca. $5.85 \cdot 10^4$) was recorded at pH 3.6. At pH 12 (not shown), the CD spectrum was virtually the same as at pH 11. The maxima of these CD curves are centered roughly at 205 nm, similar to the "mixed" β^2/β^3-peptides which fold into the 12/10/12-helical structure [27,328]. The ten-membered H-bonded turn is the common feature of the hairpin structure and of this helix type. It appears likely that the *Cotton* effect is mainly due to the relative orientation of the carbonyl chromophores in this turn geometry.

Figure 29. CD spectra of β-hexapeptide **113** which folds into a hairpin structure in CD_3OH-solution as determined by NMR. The curves specified by pH values all refer to aqueous solutions (see *Exp. Part*). Spectra were recorded at r.t. The concentration was 0.2 mM. [Θ] in 10 deg · cm^2 · mol^{-1}.



This idea is further supported by the following CD spectra: β-peptide **114** corresponds to the central tetrapeptide segment of **113**, the terminal β-amino acid residues are cut off. As suggested by the CD spectrum in MeOH, the ten-membered H-bonded ring is populated to an even higher degree (*Figure 30*):

Figure 30. CD spectra of β-tetrapeptides **114** and **115** differing only in the substitution pattern of the central dipeptide sequence. Spectra were recorded at r.t. The concentration was 0.2 mM. [Θ] in 10 deg · cm^2 · mol^{-1}.

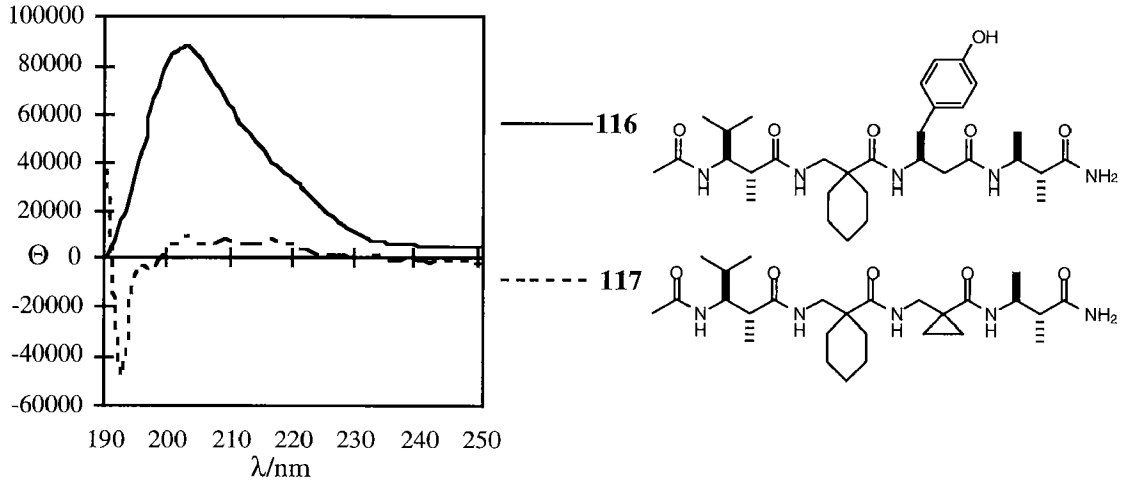


The mean residue molar ellipticity of β-*tetra*peptide **114** is more than three times larger than that of β-*hexa*peptide **113** ($3.83 \cdot 10^4$ vs. $1.12 \cdot 10^4$). Either the turn conformation of **114** is populated more highly under these conditions (in this case, the pleated sheet would have a minor contribution to the overall *Cotton* effect) or the intrinsic *Cotton* effect of the pleated sheet is of opposite sign as compared to that of the ten-membered turn motif. In contrast to **113**, β-peptide **114** does not give rise to pH dependent CD spectra. The CD spectra of β-tetrapeptide **115** in MeOH ($4.02 \cdot 10^4$, 200 nm) and in buffered aqueous solutions are less intense, suggesting a low propensity for this sequence with a central $-CH_2-CO-NH-CH_2-$ section to fold into the ten-membered turn. In the 12/10/12 helix, the same section is part of the twelve-membered H-bonded ring. The CD spectra of **114** and **115** nicely complement the notion [27] that there is a tendency to form the ten-membered turn in the presence of substituents on both sides of the amide group of β-peptide residues (–CHR–CO–NH–CHR–; "flanked amide

groups") and that the twelve-membered turn is favored in the absence of substituents on the positions surrounding the central amide group (–CH_2–CO–NH–CH_2–)[109].

β-Tetrapeptides **116** and **117** were designed after an X-ray structure had shown that a $\beta^{2,2}$-tripeptide composed of the amino acid **79d** forms the same ten-membered turn as does **113** (see *Figure 40b*). In fact, exchange of the β^2-homovaline residue in **114** for an achiral 1-(aminomethyl)cyclohexanecarboxylic-acid residue in **116** does not change the typical CD pattern (*Figure 31*).

Figure 31. Overlay of CD spectra of β-tetrapeptides **116** and **117** which contain α,α-disubstituted β-amino acids in their central part. Spectra were recorded in MeOH at r.t. The concentration was 0.2 mM. [Θ] in 10 deg · cm^2 · mol^{-1}.



The curve in MeOH is even more intense (8.82 · 10^4, 203 nm) than the curve of β-hexapeptide **113** (see *Figure 29*). This shows that the ten-membered turn can also be built by incorporating readily available achiral $\beta^{2,2}$-amino acid residues in place of the chiral β^2-amino acids. However, CD measurements with β-peptide **117**, the turn segment of which would consist of the $\beta^{2,2}$-amino acid derivatives **79a** and **d**, show no *Cotton* effect. Homopeptides composed of the β-amino acid **79a** fold into ribbon-like structures which are stabilized by eight-membered H-bonded rings (see *Figure 33* below). The propensity to form such an eight-membered ring could preclude β-peptide **117** from adopting the hairpin structure. However, another possibility

[109] This bias could be due to the different steric crowding in the turn structures; in the twelve-membered turn the flanking substituents (H-atoms) are "eclipsed", while in the ten-membered turn they are "staggered" (see Figure 12 in [27]).

should also be considered: the central turn segment in **117** is achiral; thus, there is no intrinsic bias for a right- or left-handed turn. The coexistence of these diastereomeric conformations could, in principle, be responsible for the absence of a *Cotton* effect.

5.4 Synthesis and Structure of β-Peptides Consisting of Geminally Disubstituted Amino Acids

5.4.1 Introduction

The design of geminally disubstituted β-amino acids was encouraged by the high crystallinity of polypeptides containing α,α-disubstituted α-amino acids[110] [3,472]. In addition, it was interesting to compare the β-analogues of Aib (α-aminoisobutyric acid) peptides with Aib peptides themselves which are known to adopt the 3_{10} helix (beginning at the trimer level [4]); Aib is the strongest β-bend and helix promotor, when incorporated into peptides [2,4]. The 1-(aminomethyl)cyclohexanecarboxylic-acid moiety[111] **79d** was expected to increase crystallinity by introducing constraints that reduce the flexibility of the peptide backbone [5]. It was also of interest to learn about the structure of $\beta^{2,2}$-homopeptides built from the cyclopropane carboxylic acid derivative **79a**. This residue could significantly restrict the peptide backbone due to an enlarged exocyclic bond angle C(β)–C(α)–CO and the "bisecting" conformational restriction (see *Figure 38*).

Many years ago, *Drey et al.* have synthesized a tripeptide consisting of 3-amino-3-methylbutanoic acid (β-aminoisovaleric acid, β^3-HAib) [473,474] and a hexapeptide[112] consisting of 3-amino-2,2-dimethylpropanoic acid (β-aminopivalic acid, β^2-HAib) [475]. The primary goal of their study was to test the coupling of sterically demanding amino acids which could not be efficiently coupled by conventional methods[113] [474]. However, the structures of these compounds were not elucidated.

The substitution pattern of a *gem.* dialkylated β-amino acid does not fit into the secondary structures found to date (geminally disubstituted β-amino acids are helix- and pleated sheet breaking residues in β-peptides; see the analysis presented in [27]). Can yet another helix be formed by $\beta^{2,2}$- or $\beta^{3,3}$-

---

110 The number of solved structures of Aib homopeptides is rapidly increasing, much more so than that of any other amino acid derivatives [3]; see also the structures of oligo-Iva-peptides consisting of enantiopure 2-amino-2-methyl-butanoic-acid residues [468-471].

111 The X-ray structures of protected dimers, trimers and tetramers of the α-amino acid analogue have been reported (review: [3]).

112 Cf. compound **128** in this thesis.

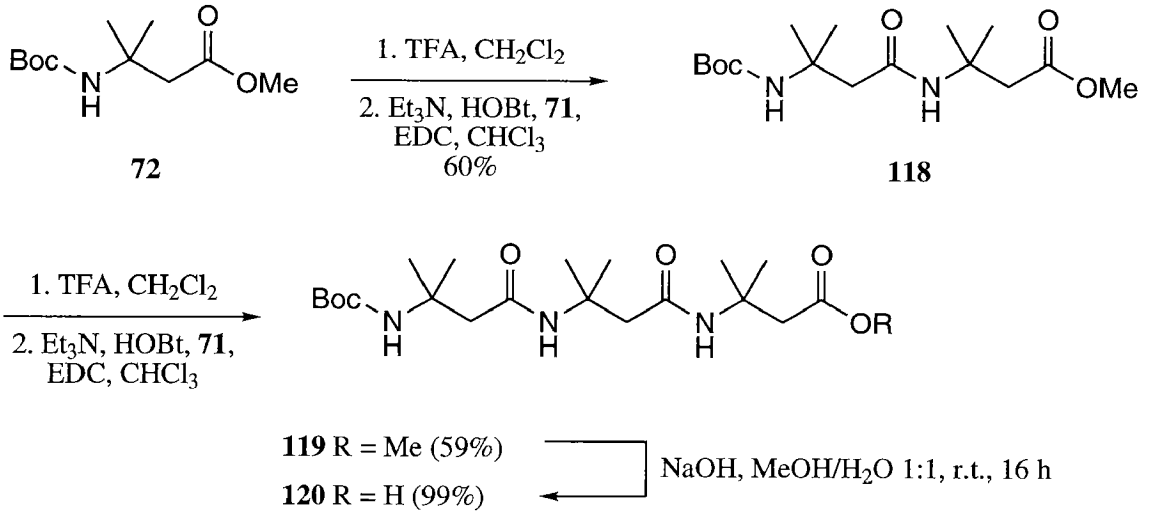
113 Eventually, the synthesis of the trimer of β-aminoisovaleric acid (half the chain length of compound **122** described here) required the aminolysis of the corresponding dihydro-1,3-oxazinones [474], whereas the synthesis of the hexamer of β-aminopivalic acid could be achieved with DCC and HOBt [475].

homopeptides, other than the known 3_{14}-, the 12/10/12-, and the 2.5_{12} helices, or are novel secondary structures formed?
The β-peptide derivatives were synthesized by both, solution- and solid-phase synthetic procedures. Classical coupling in solution yielded β-peptides in quantities large enough for crystallization experiments, whereas the flexible solid-phase method, successfully introduced in our laboratory for the synthesis of β-peptides [200,203,444], was chosen for the synthesis of β-peptides with varying *C*- and *N*-terminal protecting groups.

5.4.2 Synthesis of $\beta^{3,3}$-Peptides

The α,α- or β,β-disubstituted β-amino acids were coupled by conventional methods (EDC/HOBt), without encountering the type of complications known for sterically congested α,α-disubstituted α-amino acids [476-478]. Monomer **72** was Boc-deprotected with TFA/CH_2Cl_2 and coupled with the Boc-protected amino acid **71** to yield the dipeptide ester **118** (*Scheme 54*). *N*-Deprotection of **118** with TFA/CH_2Cl_2 and coupling with the Boc-protected acid **71** provided the tripeptide derivative **119**, the *C*-terminus of which was deprotected by saponification (NaOH/H_2O/MeOH) to yield the free peptide acid **120**, necessary for fragment coupling.

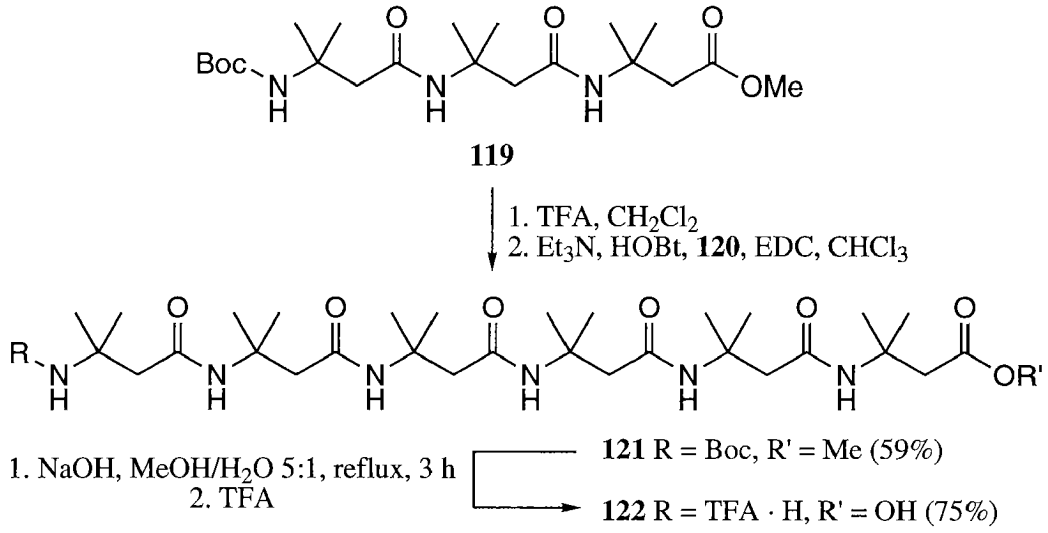
Scheme 54. Synthesis of $\beta^{3,3}$-di- and tripeptides consisting of β^3-HAib.



The peptide acid **120** was coupled with the peptide ester from **119** to give the fully protected $\beta^{3,3}$-hexapeptide **121** in 59% yield (*Scheme 55*). Successive deprotection of the *C*-terminus by saponification (NaOH, MeOH/H_2O,

reflux) and of the *N*-terminus by treatment with TFA gave $\beta^{3,3}$-hexapeptide **122**.

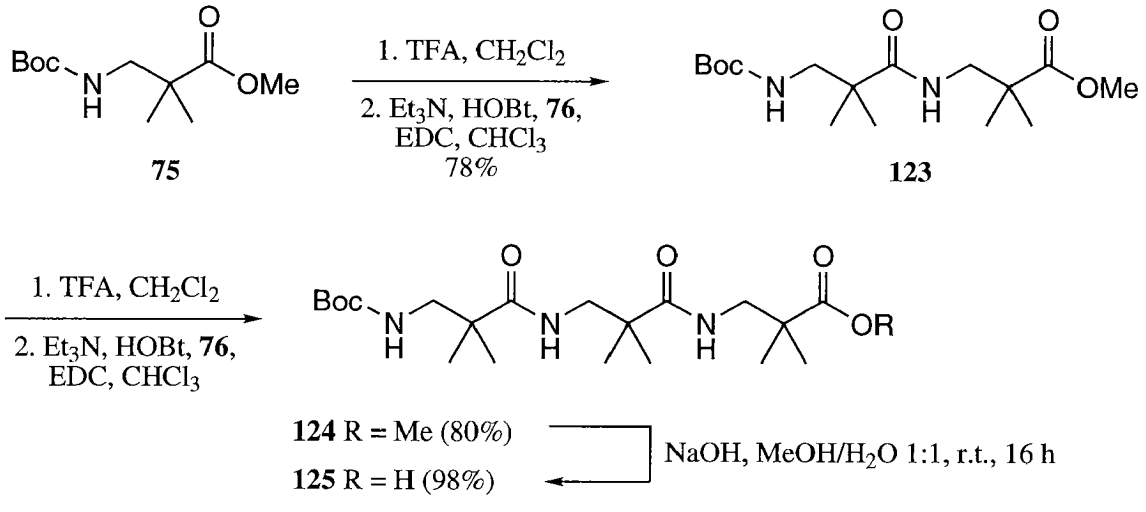
Scheme 55. Synthesis of the $\beta^{3,3}$-hexapeptide derivatives **121** and **122** consisting of β^3-HAib.



5.4.3 *Synthesis of $\beta^{2,2}$-Peptides*

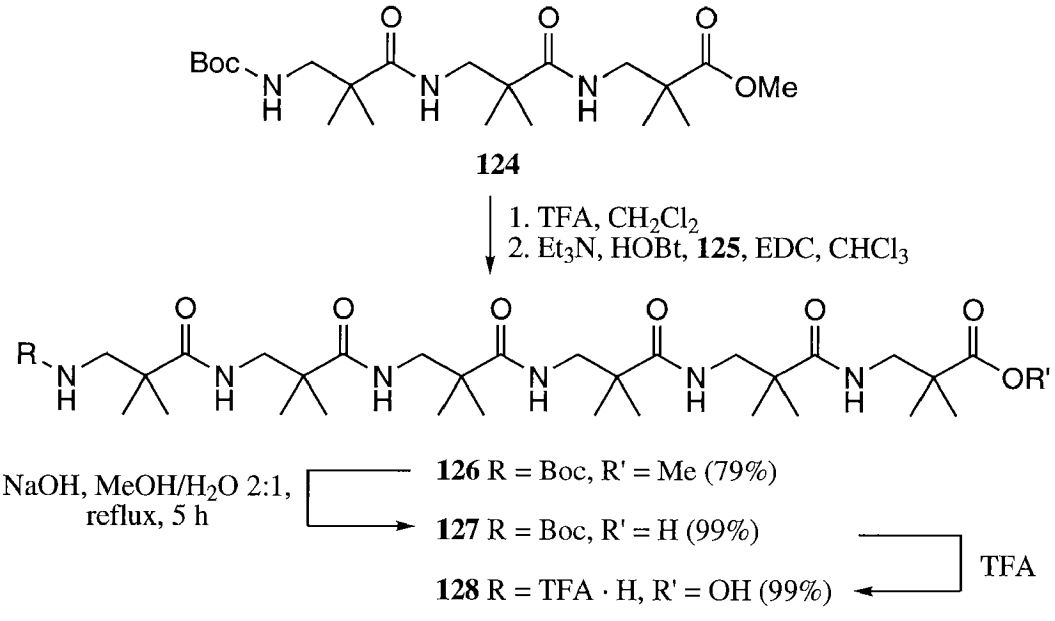
The synthesis of the α,α-disubtituted derivatives was carried out in an analogous manner. Thus, $\beta^{2,2}$-dipeptide **123** was obtained from **75** in 78% yield (*Scheme 56*). Further coupling with the acid **76** yielded fully protected $\beta^{2,2}$-tripeptide **124** which was prepared for fragment coupling by saponification to the tripeptide acid **125**.

Scheme 56. Synthesis of $\beta^{2,2}$-di- and tripeptide derivatives consisting of β^2-HAib.



Fragment coupling of **125** with the TFA salt of derived from **124** proceeded in high yield providing fully protected $\beta^{2,2}$-hexapeptide **126** (*Scheme 57*). Saponification required heating at reflux for several hours; the free acid **127** was isolated in quantitative yield. Its *N*-terminus was finally deprotected to give $\beta^{2,2}$-hexapeptide **128**.

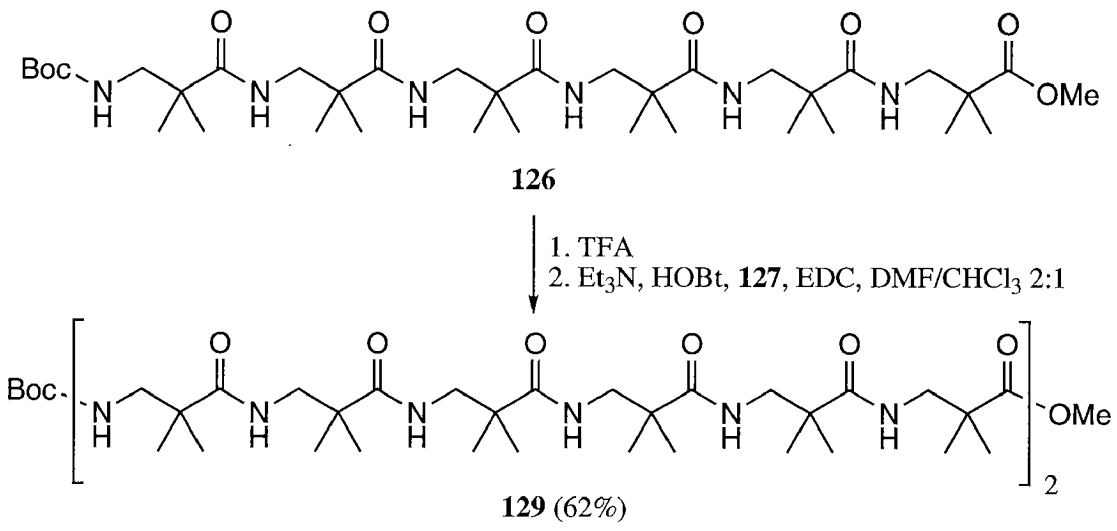
Scheme 57. Synthesis of $\beta^{2,2}$-hexapeptide derivatives **126**, **127** and **128** consisting of β^2-HAib.



The TFA salt derived from $\beta^{2,2}$-hexapeptide from **126** was used for coupling with **127** to provide the $\beta^{2,2}$-dodecapeptide **129** in an acceptable yield (*Scheme 58*). Like all other *gem.*-disubstituted, protected β-hexapeptides, the rather hydrophobic compound **129** (M_r 1321.8) is quite soluble[114] in protic and aprotic solvents (MeOH, $CHCl_3$, CH_2Cl_2).

[114] Normally, β^2- and β^3-peptides with alkyl side chains become more and more insoluble in common organic solvents with increasing chain length [17,27].

Scheme 58. Synthesis of $\beta^{2,2}$-dodecapeptide derivative **129**.

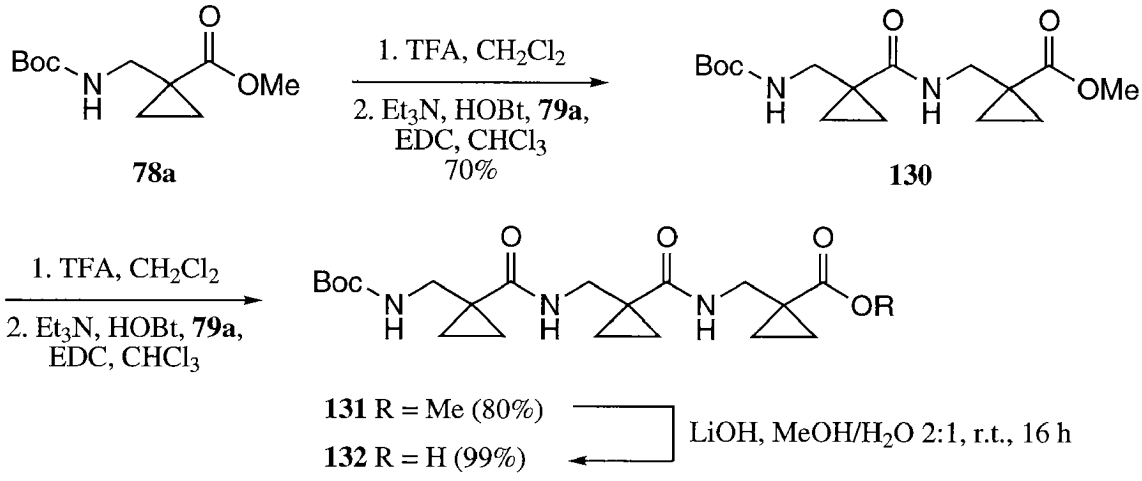


It is interesting to note that the yields of the coupling steps involving $\beta^{2,2}$-peptides (*ca.* 80%) always exceeded those obtained with the corresponding $\beta^{3,3}$-peptides (*ca.* 60%). This result is reminiscent of the difficulties encountered in *N*-acylations of (Aib) and other α,α-dialkylated glycine derivatives [479,480][115].

The 1-(aminomethyl)cyclopropane- and 1-(aminomethyl)cyclohexane-carboxylic-acid containing β-peptides were synthesized by the same straightforward methodology as the non-cyclic compounds (*Schemes 59-62*). The $\beta^{2,2}$-amino acid hydrogen trifluoroacetate derived from methyl ester **78a** was used for coupling with the Boc-protected $\beta^{2,2}$-amino acid **79a** (*Scheme 59*). The $\beta^{2,2}$-dipeptide derivative **130**, obtained in 70% yield on a six-gram scale, was Boc-deprotected and coupled once more with **79a** to yield the $\beta^{2,2}$-tripeptide derivative **131** (80% after flash chromatography) which was saponified under mild conditions to give tripeptide acid **132**.

[115] Coupling of α,α-diphenylglycine (Dph) with alanine by the EDC/HOBt method proceeded with high yields at the *C*-terminus but with very low yields at the *N*-terminus of Dph [479].

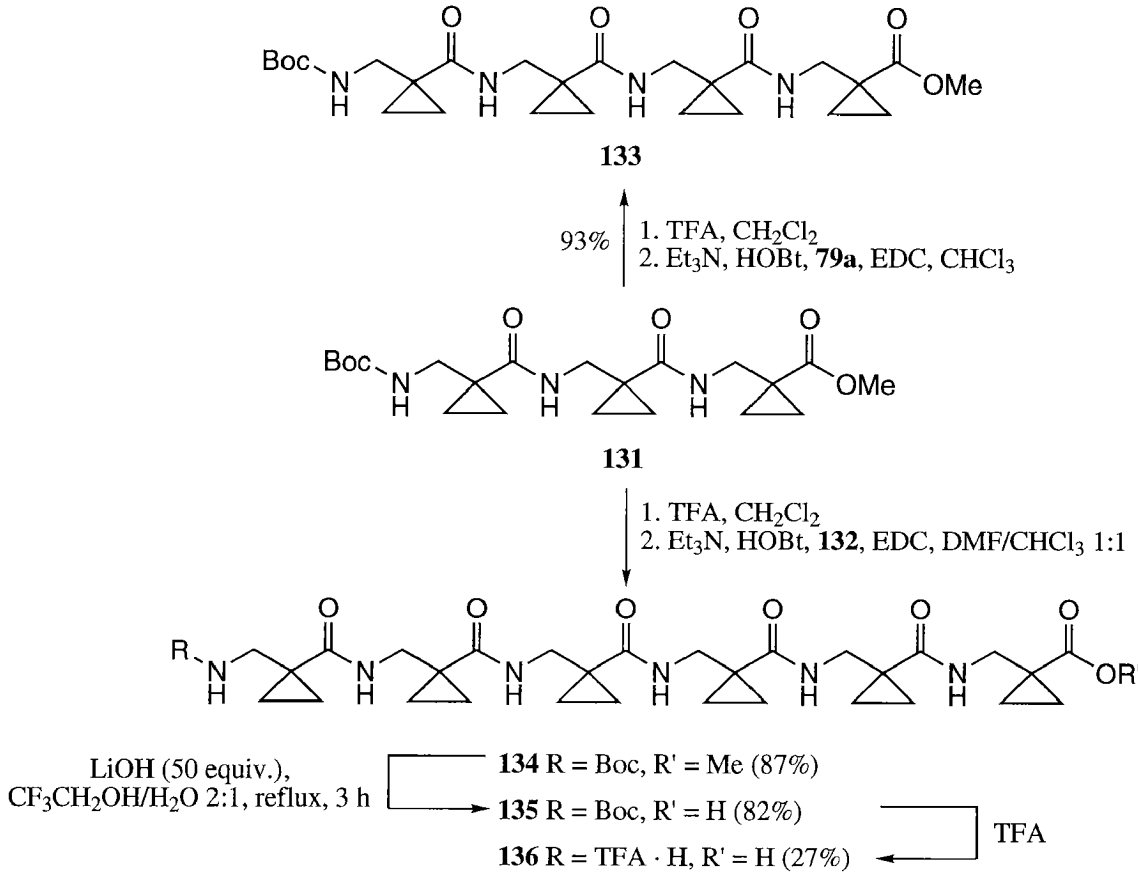
Scheme 59. Synthesis of $\beta^{2,2}$-di- and -tripeptides composed of 1-(aminomethyl)cyclopropanecarboxylic-acid residues (β^{2}-HAc_3c).



The idea was to overcome the lack of formation of suitable single crystals from hexameric β-peptides (excluding conformationally constrained compounds [19,327]) by preparing smaller oligomers. Thus, the TFA salt derived from **131** was coupled with monomer **79a** or tripeptide acid **132** to give protected $\beta^{2,2}$-tetrapeptide **133** and $\beta^{2,2}$-hexapeptide **134**, respectively, in excellent yield (*Scheme 60*). Saponification of **134** required heating with NaOH at reflux in CF_3CH_2OH to give, after recrystallization, $\beta^{2,2}$-hexapeptide acid **135**, the *N*-terminus of which was deprotected by TFA to yield fully deprotected $\beta^{2,2}$-hexapeptide **136**[116].

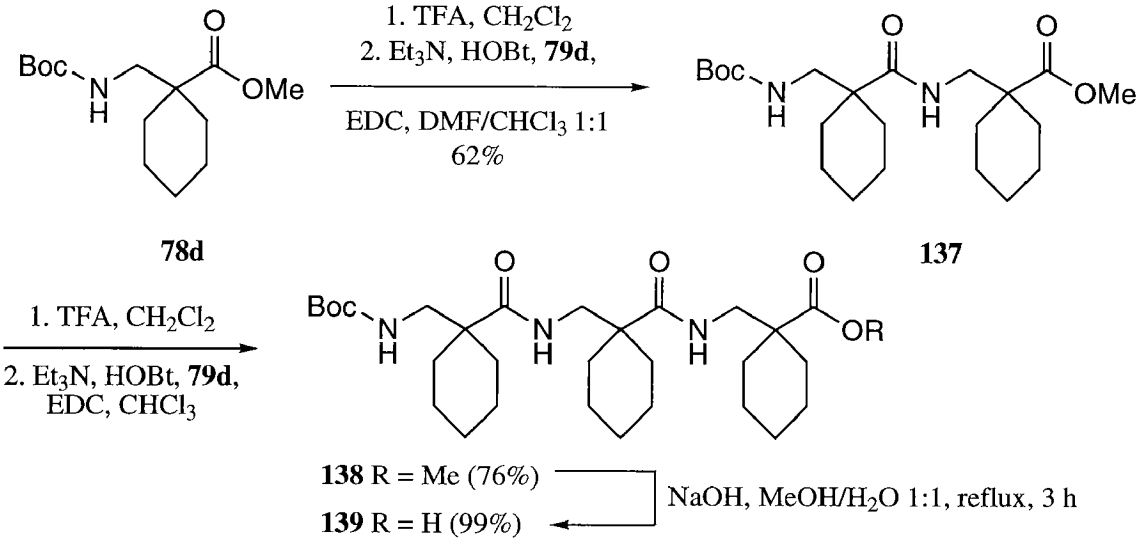
[116] The poor yield is due to complications during isolation; **136** has very low solubility.

Scheme 60. Synthesis of $\beta^{2,2}$-tetrapeptide **133** and $\beta^{2,2}$-hexapeptide derivatives **134**, **135**, and **136**.



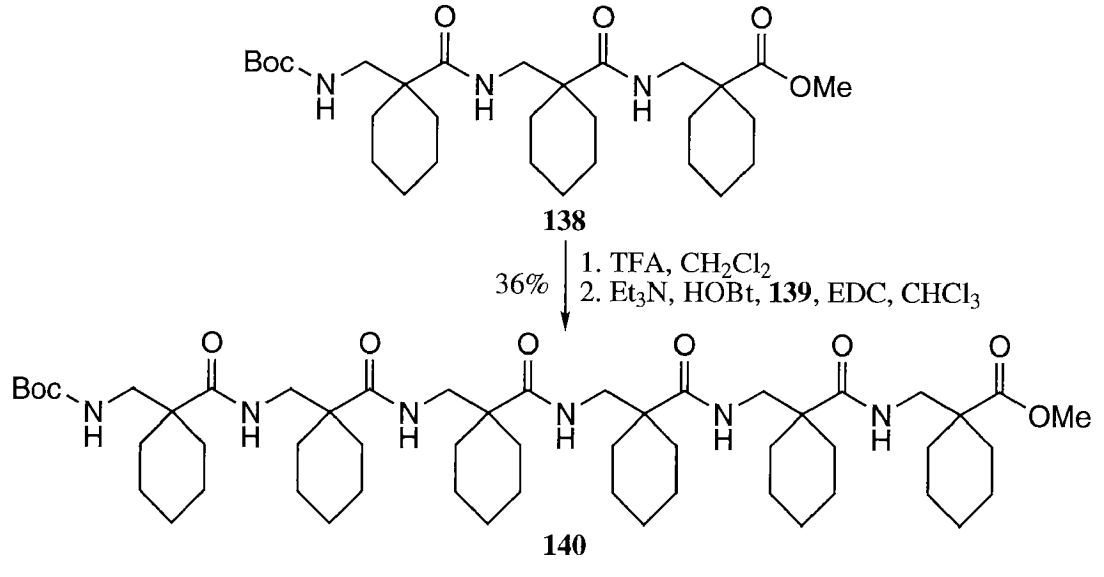
Then, the TFA salt derived from Boc-protected $\beta^{2,2}$-cyclohexane amino acid ester **78d** was coupled with **79d** to give dipeptide derivative **137** the TFA salt of which was again coupled with **79d** to yield the $\beta^{2,2}$-tripeptide derivative **138** (*Scheme 61*).

Scheme 61. Synthesis of $\beta^{2,2}$-di- and tripeptide derivatives consisting of 1-(aminomethyl)cyclohexanecarboxylic-acid residues (β^2-HAc$_6$c).



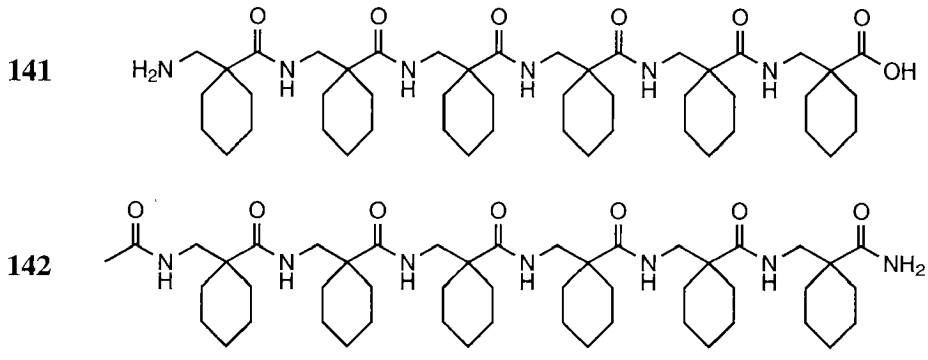
For the following fragment coupling step, a portion of the **138** sample was deprotected at the *C*-terminus to give $\beta^{2,2}$-tripeptide acid **139** (*Scheme 61*) and another portion at the *N*-terminus, and the two $\beta^{2,2}$-tripeptide units were coupled to give the protected $\beta^{2,2}$-hexapeptide **140** in 36% yield (*Scheme 62*). It is noteworthy that **140** was precipitated from pentane/Et_2O 95 : 5. All $\beta^{2,2}$-cyclopropane β-peptides had higher melting points than the corresponding $\beta^{2,2}$-cylohexane derivatives (fully protected).

Scheme 62. Fragment coupling of the TFA salt derived from **138** with **139** to give protected $\beta^{2,2}$-hexapeptide **140**.



The $\beta^{2,2}$-hexapeptides **141** and **142** were synthesized on solid support (*Figure 32*).

Figure 32. $\beta^{2,2}$-Hexapeptides consisting of $\beta^{2,2}$-HAc$_6$c prepared on *ortho*-chlorotrityl-chloride resin (**141**) and on *Rink* amide resin (**142**).



Yields and purities of crude peptides are given in *Table 10*. Hexapeptide **141** was prepared on *ortho*-chlorotrityl-chloride resin, following the reported procedure (activation of the $\beta^{2,2}$-amino acids **80b** with BOP/HOBt/(i-Pr)$_2$EtN)[203,444]. Anchoring yield (55%) and HPLC-purity (65-95%) of the crude peptide were comparable to the reported values for β^3-peptides, however, the cleavage yield (47%) was substantially lower for this sterically more demanding $\beta^{2,2}$-amino acid derivative. The *Rink* amide resin was chosen for the solid phase synthesis of peptide amide **142**. After *N*-acetylation of the *N*-terminus and cleavage from the resin the crude β-peptide was recovered in good yields and with a purity of 77%, as determined by reversed-phase HPLC. It is noteworthy that these α,α-disubstituted β-amino acids could be effectively coupled on both resin types under essentially the same conditions that had been used for sterically less crowded β-amino acids, as indicated by both reaction time (15-60 min) and the purity of the crude products.

Table 10. Yield and purity of crude $\beta^{2,2}$-peptides **141** and **142**.

β-Peptide	Yield (%)[a]	Purity[b] (%)	t_R (min)[c]	FAB-MS
141	47	65	14.3[d]	853.8 (M^+)
142	99	77	4.9[f]	894.4 ([M + 1]$^+$)

a) % Mass recovered based on polymer loading. b) HPLC purity (220 nm) of the crude product. c) Retention time in the HPLC (linear gradient of *A* (0.1% TFA in H_2O) and *B* (MeCN); see *GP 27* in *Exp. Part.* d) 30-90% *B* in 20 min (C_{18}). e) 75% isocratic *B* in 20 min (C_8).

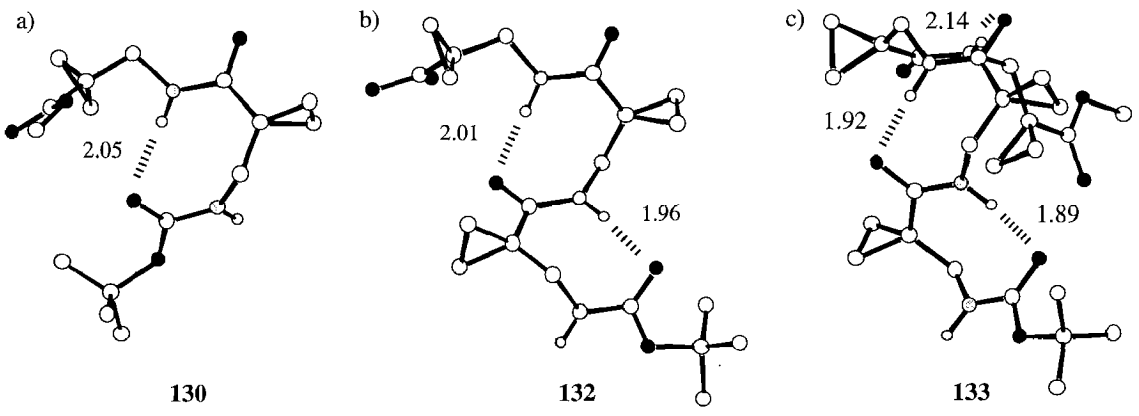
5.4.4 *Secondary Structure Analysis of Geminally Disubstituted β-Peptides*

All attempts to crystallize the dimethylated peptides led to amorphous powders. The ^{1}H-NMR spectra of **121** and **126** display nicely separated amide proton signals. It was not possible to elucidate the 3D structure of this β-homopeptide by standard 2D-NMR methods because of extensively overlapping CH_2 and Me signals[117]. No structures containing the N–CH_2–CMe_2–CO unit were found in the Cambridge Crystallographic Data Centre.

5.4.4.1 *X-Ray Crystal Structures of $\beta^{2,2}$-Peptides* **130**, **132**, **133** *and* **138**

The X-ray crystal structures of Boc-protected $\beta^{2,2}$-amino acid derivatives **130**, **132**, and **133** reveal a secondary structure that is unprecedented in the α-peptide world [332]. The structures are characterized by eight-membered H-bonded rings[118]. Inspection of the structures of the $\beta^{2,2}$-di- and tripeptide derivatives **130** and **132** led to the expectation that the $\beta^{2,2}$-tetrapeptide derivative **133** would also adopt this fold which is essentially a flat ribbon. The same H-bonding pattern is observed in **133**, but, the ribbon-like structure is not flat but rather forms a bend (*Figure 33c*); the first two eight-membered H-bonded rings display a geometry similar to those in $\beta^{2,2}$-dipeptide acid **132**.

Figure 33. X-ray crystal structures of the $\beta^{2,2}$-peptide derivatives **130**, **132**, and **133** (N–H bond length 1.00 Å). X-Ray by Dr. *P. Seiler*.



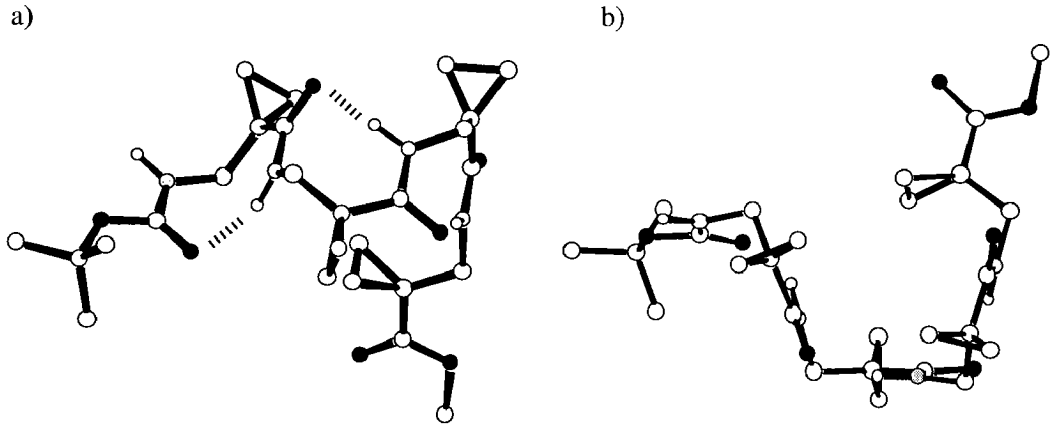
130 **132** **133**

117 For combined liquid- and solid-state NMR studies, $\beta^{2,2}$-peptide **126** was also prepared as fully $^{13}C/^{15}N$-labeled derivatived by Dr. *T. Sifferlen* [33]: however, these modifications did not reveal its structure.

118 Comparable eight-membered rings were observed in the structure of a protected chiral α-aminoxy acid [481,482].

The third residue, however, does not continue the regular pleated ribbon structure of the first two residues, as depicted in *Figure 34*. It is not clear why the tetrapeptide **133** does not follow the folding pattern initiated by **130** and **132**.

Figure 34. Two different views of the X-ray crystal structure of $\beta^{2,2}$-tetrapeptide derivative **133**. a) With and b) without H-bonds. In b) the initial folding of the first two residues into a pleated ribbon is evident (cf. also *Figure 35a*).



The parameters for intramolecular H-bonding in the crystals of **130**, **132**, and **133** are listed in *Table 11*. The H-bond donor and acceptor atoms are typically ca. 2.9 Å apart [283a].

Table 11. Intramolecular H-bond parameters for the $\beta^{2,2}$-homopeptides **130**, **132** and **133** with an assumed N–H bond length of 1.00 Å.

β-Pepide	Atoms[a)]	Distance N · · · O [Å]	Angle NH · · · O [°]
130	H–N(2) · · · O(Boc)	3.02	+ 164.1
132	H–N(2) · · · O(Boc)	2.90	+ 156.6
	H–N(3) · · · O(1)	2.99	+ 166.9
133	H–N(2) · · · O(Boc)	2.80	+ 149.4
	H–N(3) · · · O(1)	2.89	+ 163.7
	H–N(4) · · · O(2)	3.14	+ 178.5

a) Donor group (H–N) and acceptor group (carbonyl O-atom) of the corresponding residues or of the Boc group. Residues are numbered starting from the *N*-terminus.

The torsion angles in the crystal structures of the oligomers **130**, **132**, and **133** are summarized in *Table 12*. Interestingly, in the di- and tetrapeptide derivatives **130** and **133** most angles are similar to those found in the tripeptide acid **132** but of opposite sign.

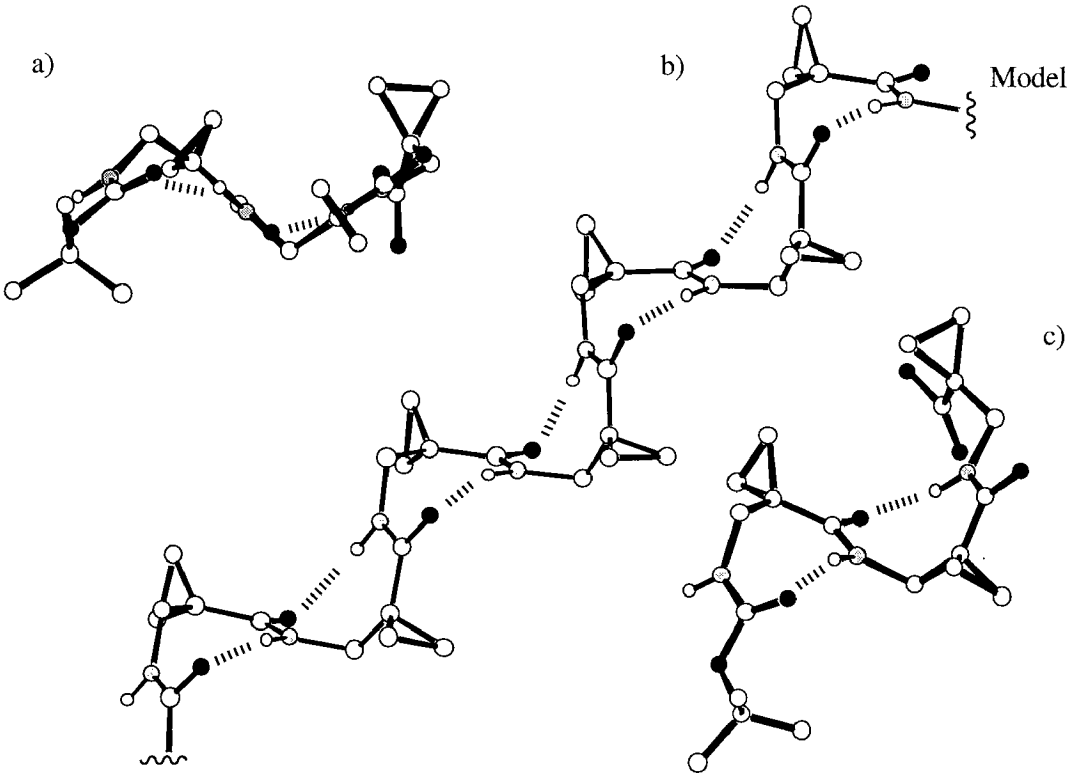
Table 12. Torsion angles in crystal structures of **130**, **132** and **133**. Angles Φ, Θ, and Ψ are defined in *Figure 28* (in [°]).

Torsion angles[a)]	**130**	**132**	**133**
Φ(1)	− 116.5	+ 110.5	− 105.5
Θ(1)	+ 70.0	− 65.4	+ 71.3
Ψ(1)	+ 2.6	− 8.8	+ 1.7
Φ(2)	+ 147.5	+ 111.7	− 110.8
Θ(2)	+ 72.7	− 73.4	+ 72.6
Ψ(2)	+ 2.6	− 2.0	+ 1.8
Φ(3)	-	− 151.4	+ 118.0
Θ(3)	-	− 71.6	− 69.5
Ψ(3)	-	-	+ 4.6
Φ(4)	-	-	− 92.9
Θ(4)	-	-	+ 177.5
Ψ(4)	-	-	+ 3.3

a) The residues are numbered starting from the *N*-terminus of the peptide. The torsion angles are defined as shown in *Figure 28*.

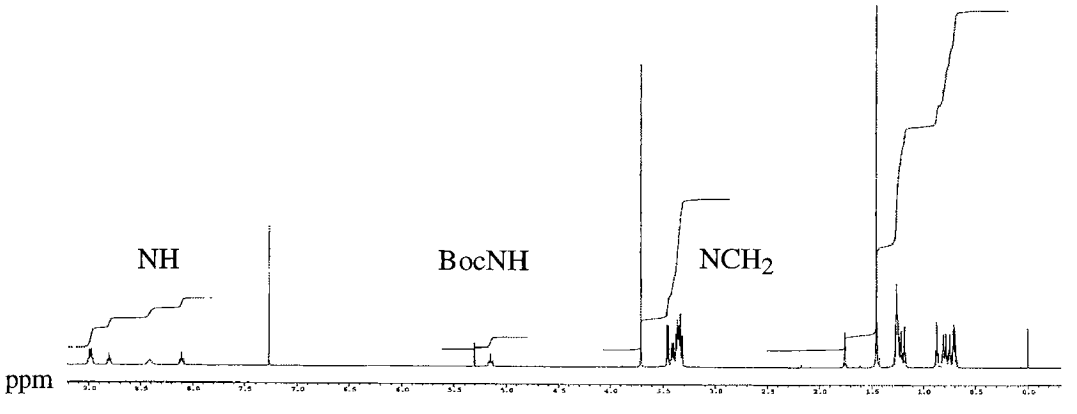
The regularity of the eight-membered turn motif stimulated the design of a model β-peptide consisting of 1-(aminomethyl)cyclopropanecarboxylic-acid building blocks (*Figure 35*). "Ideal" values of the torsion angles were assumed; the resulting structure is a pleated ribbon or a stair-like structure.

Figure 35. Model of a pleated ribbon. a),c) Different views of the crystal structure of $\beta^{2,2}$-tripeptide acid **132** to underline the similarity to the model. b) Model constructed from the X-ray crystal structure of **132** with slightly modified torsion angles (Φ = + 120, Θ = – 72, Ψ = 0, and ω = 180°). This figure was generated with MacMoMo (Prof. Dr. *M. Dobler*, ETH-Zürich).



The ^{1}H-NMR spectra of these α,α-disubstituted β-peptides display nicely separated amide NH signals. A typical spectrum (of **133**) is shown in *Figure 36*. In many instances, the NH signals are triplets with coupling constants of 5-7 Hz.

Figure 36. 400-MHz ^{1}H-NMR spectrum of $\beta^{2,2}$-tetrapeptide derivative **133** in $CDCl_3$.



However, for a triplet to occur, the coupling constants between the corresponding proton and the two adjacent H-atoms (NCH_2) must be essentially the same. Similar coupling constants generally imply similar torsion angles between the methylene protons and the NH proton. The torsion angles in the crystal structures of $\beta^{2,2}$-peptide derivatives **130**, **132** and **133** are collected in *Table 13*; the dihedral angles between the stereoheterotopic methylene protons (they are *enantiotopic* in the case of a fully extended peptide chain) and the amide proton are quite different! In such a setting, the NH protons should display a doublet of doublets. However, by virtue of its achiral nature, **133** (like the other oligomers) may be subject to rapid (on the NMR time scale) interconversion between a left- and a right-handed turn structure.

Table 13. Dihedral angles Φ' (H–N–C(β)–C(β)–H^{Re} and –H^{Si} in [°]) extracted from the X-ray structures of $\beta^{2,2}$-peptides **130**, **132** and **133**. The enantiotopic C(β)-H atoms are designated H^{Re} and H^{Si}.

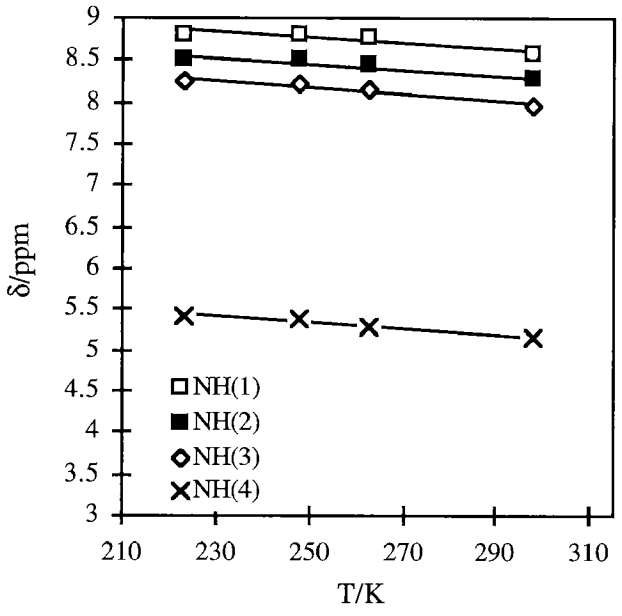
Peptide	**130**		**132**		**133**	
Residue[a)]	H^{Re}	H^{Si}	H^{Re}	H^{Si}	H^{Re}	H^{Si}
1	− 176.5	− 57.6	+ 50.9	+ 170.0	− 165.0	− 46.0
2	+ 87.5	− 152.4	+ 51.9	+ 171.5	− 170.4	− 51.1
3	-	-	+ 149.0	− 91.8	+ 58.4	+ 177.5
4	-	-	-	-	− 152.4	− 33.3

a) Numbering starting from the *N*-terminus.

At low temperatures the rate of this conversion may be slow on the time scale of NMR spectroscopy, so that diastereotopic protons give rise to different chemical shifts. Temperature-dependent ^{1}H-NMR measurements with **133** in CD_2Cl_2 (at 25, − 10, − 25, and − 50 °C) showed that the triplet is retained for all NH protons at − 25°C. At − 50 °C the NH signals are broad singlets.

However, the temperature coefficients determined over the range of 75 K (they range from − 3.2 to − 4.0 ppb/K) are all negative, corresponding to the observed values for the NH signals in proteins. In general, values more positive than − 6 ppb/K reflect intramolecular H-bonded NH protons in H_2O [348,483]. This suggests that the NH protons are strongly intramolecularly H-bonded at this rather high concentration.

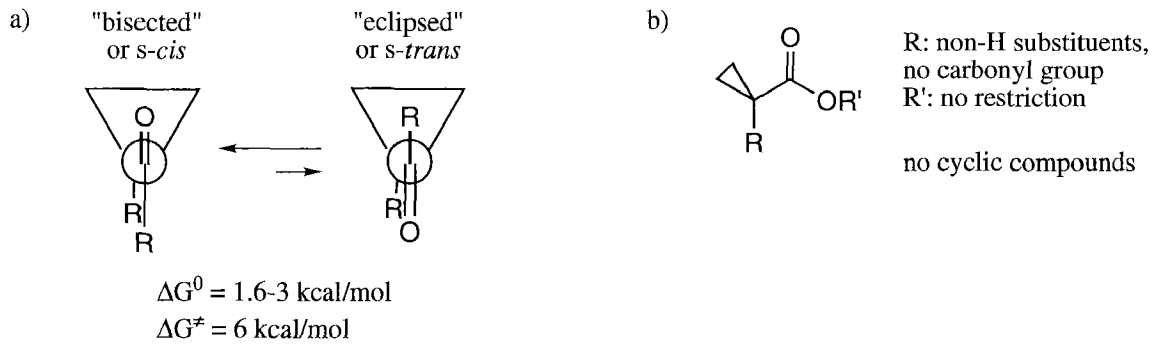
Figure 37. Temperature coefficients for the NH-proton chemical shifts of the $\beta^{2,2}$-peptide derivative **133** calculated over a range of 75 K by linear regression. The NH protons are numbered according to their decreasing chemical shift. 300 MHz ^{1}H-NMR spectra were recorded at 25 mM in CD_2Cl_2.



The conformation around the C(α)–CO bond is the same in cyclopropanecarboxylic-acid derivatives **130**, **132**, and **133**; the so-called "bisected" conformation is adopted (*Figure 38a*). Another conformation, the "eclipsed" one, would also allow favorable overlap of the electron-rich cyclopropane Walsh orbitals (C,C-σ) with the p-orbitals of the carbonyl group [484-487]. A search in the Cambridge Crystallographic Data Center (CCDC) for structures as defined in *Figure 38b* provided eleven X-ray structures. Among these, seven showed the bisected and three the eclipsed conformation. Only one structure displayed an angle O=C–C(α)–R (– 129°) which did not fit into either of the two categories. Thus, there is a preference for the bisected conformation. This is also indicated by computational [488,489], NMR [485,490] and CD [484] studies: the rotational barrier for the interconversion of the s-*cis*-and s-*trans* conformers of cyclopropylmethylketones is ca. 6 kcal/mol[119].

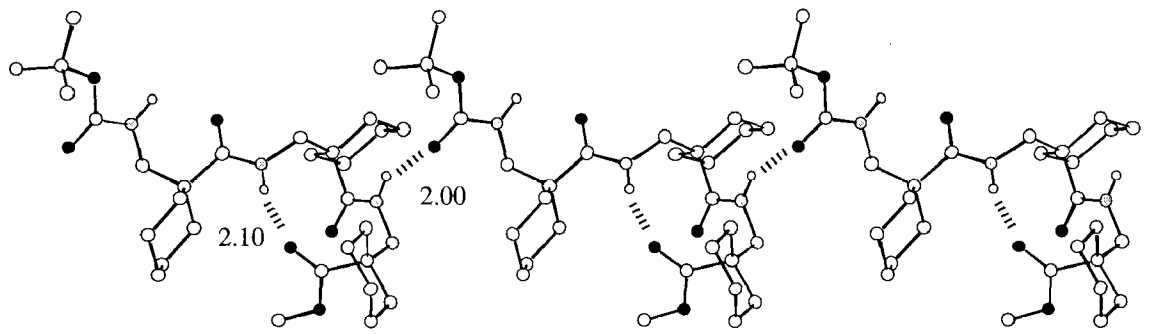
[119] Recently, the diastereoselective hydroboration of isopropenylcyclopropanes was rationalized as occurring via the more reactive s-*cis*-conformation [491]. For a diastereoselective reduction of cyclopropyl ketones with $LiAlH_4$ see [492].

Figure 38. a) Distinct conformations ("bisecting" and "eclipsing") around the C(α)–CO bond in cyclopropanecarboxylic-acid derivatives. The rotational barrier ($\Delta G^{\neq}$) is ca. 6 kcal/mol for cyclopropylmethylketones, ΔG^0 is ca. 1.6-3 kcal/mol [488]. b) Starting structures for CCDC search with excluded substituents.



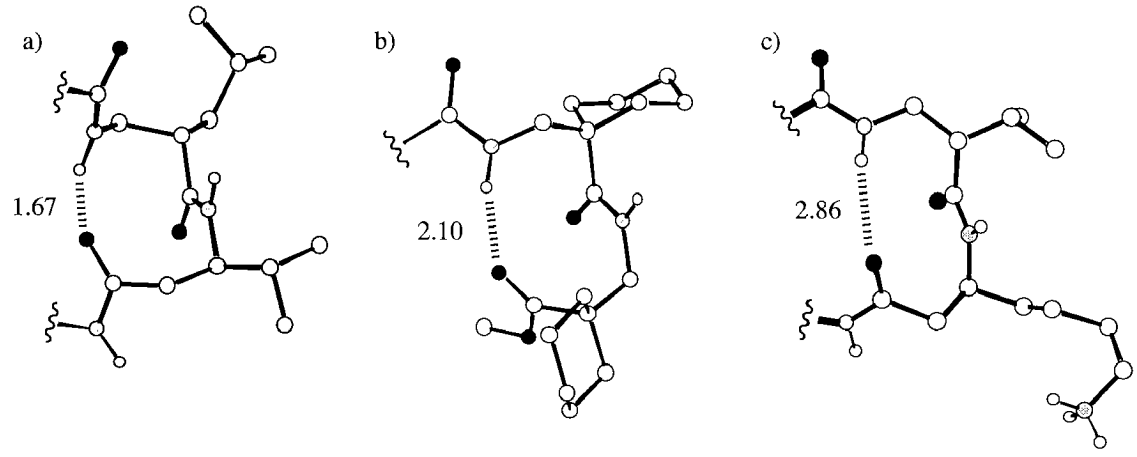
Changing from the 1-(aminomethyl)cyclo*propane*- to the 1-(aminomethyl)-cyclo*hexane*carboxylic-acid derivatives has significant implications for the folding geometry in the crystal structures: The structure of β-tripeptide **138** is characterized by a ten-membered H-bonded ring between the *N*-terminal carboxy group and the amide NH of the second amino acid (*Figure 39*). In the crystal lattice there are intermolecular H-bonds between the carbonyl O-atom of the Boc group of one molecule and the amide NH of the *C*-terminal amino acid of the neighboring molecule. This head to tail arrangement leads to infinite chains.

Figure 39. Crystal packing of $\beta^{2,2}$-tripeptide derivative **138**, showing intramolecular (2.1 Å) and intermolecular (2.0 Å) H-bonds (N–H bond 1.03 Å). Only the amide protons are shown.



This ring is quite similar to the central ten-membered H-bonded ring of the 12/10/12 helix of a β^2/β^3-hexapeptide [27], and it actually provides a turn! *Figure 40* shows the three turns which have been characterized to date.

Figure 40. Comparison of the three ten-membered H-bonded turns. a) Central ten-membered turn of the 12/10/12 helix (structure determined in CD_3OH by 2D-NMR spectroscopy [27]). b) Turn formed by the two C-terminal residues of $\beta^{2,2}$-tripeptide **138**. c) Turn connecting adjacent β-peptide strands in the hairpin **113** (structure determined in CD_3OH by 2D-NMR spectroscopy). Only the amide protons are shown; H-bond lengths are indicated with an assumed standard N–H bond length of 1.03 Å.



The torsion angles of these types of turns are summarized in *Table 14*. Exluding the second residue of **138** the central torsion angle Θ has comparable values (ca. 60°) in all three structures.

Table 14. Torsion angles in the ten-membered H-bonded rings found in the hairpin structure (**113**), in the loop structure (**138**), and in the 12/10/12 helix [27] (see *Figure 40*). Angles Φ, Θ, and Ψ are defined in *Figure 28* and are depicted in [°]). Numbering of atoms starting from the *N*-terminus.

12/10/12 helix[a)]		**138**		**113**	
Φ(3)	+ 89	Φ(2)	− 101.9	Φ(3)	+ 177.3
Θ(3)	+ 65	Θ(2)	− 59.6	Θ(3)	+ 64.6
Ψ(3)	+ 71	Ψ(2)	− 75.9	Ψ(3)	− 145.8
Φ(4)	− 103	Φ(3)	− 64.8	Φ(4)	− 127.2
Θ(4)	+ 55	Θ(3)	+ 64.8	Θ(4)	+ 56.1
Ψ(4)	− 83	Ψ(3)	+ 81.7	Ψ(4)	+ 99.1

a) Torsion angles of the residues 3 ((*S*)-β^3-HLeu) and 4 ((*S*)-β^2-HVal) of the β^2/β^3-hexapeptide in [27].

In summary, we have found two substituent-controlled turn-forming motifs for β-peptides. The eight- and ten-membered H-bonded ring is adopted by α,α-cyclopropane- and α,α-cyclohexane β-amino acid residues, respectively, in the corresponding β-peptides. The preference for the bisected conformation of the 1-(aminomethyl)cyclo*propane*carboxylic-acid residues

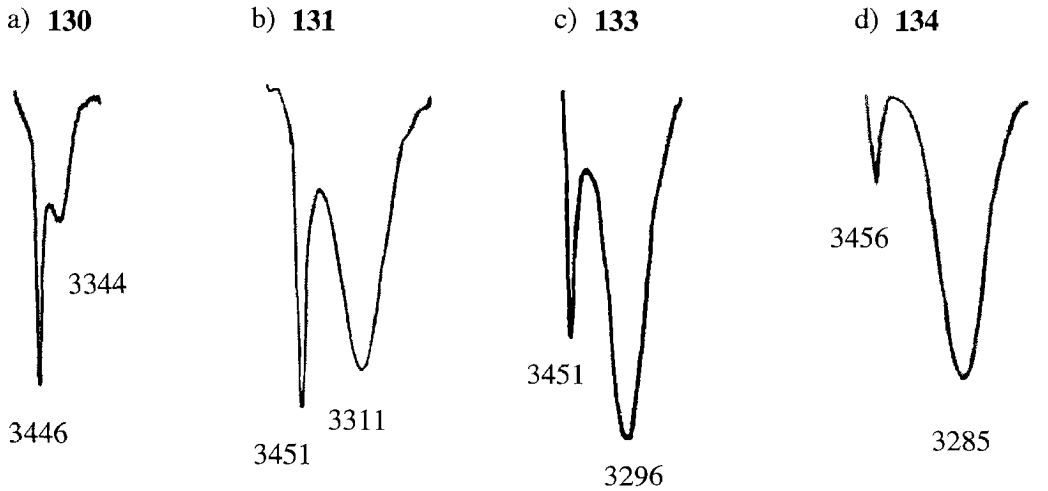
(cf. *Figure 38*) is a further constraint on the Ψ torsion angle (in addition to the stabilizing effect produced by the preferred conformation around the C(α)–CO bond, operating in both derivatives). An additional constraint stems from the enlarged exocyclic bond angle τ (C(β)–C(α)–CO) in the structures of the cyclopropane derivatives **130**, **132**, and **133**; the angle τ is ca. 120° as compared to ca. 107° in the structures of the cyclohexane derivative **138**. These "ordering elements" may account for the clear-cut difference between these building blocks and the 1-(aminomethyl)cyclo*hexane*-carboxylic-acid residues which lack this additional conformational restriction.

5.4.4.2 IR Analysis of the NH-Stretch Region of $\beta^{2,2}$-Peptides

The solubility of the fully protected $\beta^{2,2}$-peptides in solvents of low polarity allowed for FT-IR measurements in $CHCl_3$. In dilute solution, inter- and intramolecular H-bonding is directly detectable by analysis of the N–H stretch region in the IR spectra [456,493,494]. It was to be expected that an increasing number of NH groups is intramolecularly H-bonded with increasing chain length of the $\beta^{2,2}$-peptide derivatives (cf. *Figure 33*). The IR spectra of the fully protected $\beta^{2,2}$-di-, tri-, tetra- and hexapeptides **130**, **131**[120], **133** and **134** indeed display the expected tendency (*Figure 41*). Assignment of the corresponding bands was aided by IR data of β-alanine derivatives [340-343,495,496]. The dipeptide derivative **130** shows little H-bonded N–H stretching at 3344 cm^{-1}; a much higher population of intramolecularly H-bonded NH groups is suggested by the relative intensity of two bands found for hexapeptide **134** (see the intensive broad peak at 3285 cm^{-1} in *Figure 41d*). This correlates with the crystal structures of **130**, **132**, and **133** (see *Figure 33*): in these structures one amide N–H is not engaged in intramolecular H-bonds. Thus, the ratio of bonded to non-bonded NH groups increases gradually with chain length.

[120] The peptide acid **132** is insoluble in $CHCl_3$, therefore the methyl ester derivative **131** was used for this comparative study.

Figure 41. NH Stretch region of the IR spectra of oligomers of 1-(aminomethyl)cyclopropanecarboxylic acid **130**, **131**, **133** and **134** at 25 °C. Concentration ca. 5 mM in $CHCl_3$; wavenumbers in [cm^{-1}]. The sharp band at 3446-3456 cm^{-1} is assigned to the free N–H stretch and the broad band at 3285-3344 cm^{-1} to the amide NH intramolecularly H-bonded. Data obtained from concentration-dependent ^{1}H-NMR experiments indicate that there is little or no aggregation in a 5 mM $CHCl_3$ solution.



When studying intramolecular H-bonding by this method one must exclude the existence of intermolecular aggregates. As a representative example, **133** was used for variable-concentration ^{1}H-NMR experiments which showed that no intermolecular aggregation occurred in the concentration range of 2-20 mM[121]. Thus, the observed IR bands at the lower frequency are mainly produced by intramolecularly bonded amide N–H groups. The concentration-independent δ(NH) values for all four amide protons of **133** are consistent with the intramolecular H-bonding pattern observed in the solid state.

This clear-cut preference of β-peptides of 1-(aminomethyl)cyclopropane-carboxylic-acid building blocks to form eight-membered H-bonded rings between nearest neighbor amide groups sharply contrasts with the folding preferences of unsubstituted β-amino acid analogues: *Gellman* [496] and *Gung* [341,343] studied β-alanine (β-HGly) derivatives by FT-IR spectroscopy and have found that intramolecular H-bonds are unfavorable between

[121] The largest Δδ (0.06 ppm) was measured for the NH(1), the Boc-NH proton which is not involved in intramolecular H-bonding in the crystal structures. The Δδ of the other three NH protons was 0.01-0.02 ppm. This is further proof that **133** adopts the "double-turn" in solution as well.

neighboring amide groups[122]. Thus, a distinct substituent effect on intramolecular H-bonding is apparent.

[122] Only β-alanine derivatives with a tertiary amide group were found to fold into the eight-membered ring [496].

5.5 Synthesis and Structure of β-Peptides with (*R*)-β³/(*S*)-β³-Sequence

5.5.1 Introduction

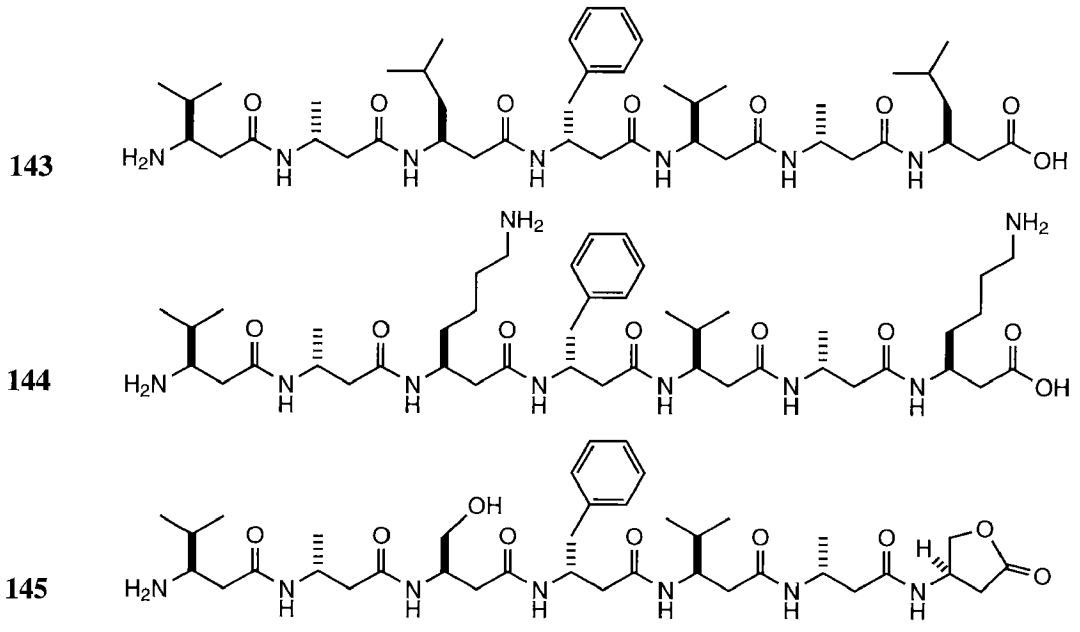
The conformation of α-peptides with strictly repeating L- and D-residues differs from peptides with homochiral residues [497]. The most thoroughly studied of these are poly(γ-benzyl-D,L-glutamate)s [498-501]. *Lorenzi et al.* have studied octapeptides consisting of alternating D-and L-valine [502] or phenylalanine residues [503]. These alternating D,L-peptides adopt the so-called $\pi_{(L,D)}$ (or $\beta_{(L,D)}$) helix or double stranded antiparallel β-helices ($\uparrow\downarrow\beta^{5.6}$) [503,504]. The biological interest in these structures stems from their possible role as transducting channels for alkali ions through lipidic membranes. The pentadecapeptide gramicidin A, a natural antibiotic with alternating L- and D-residues forms specific ion-conducting channels across natural and synthetic membranes [505]. The ion-conducting transmembrane channel originates from dimerization of nonconducting species constituted by β-helices[123] [509-511].
The conformational preferences of β-peptides with repeating (*R*)- and (*S*)-residues, an array which can form neither a 3_{14}-helical nor a sheet-like secondary structure (of **111**), were consequently intriguing.

5.5.2 Synthesis of β-Peptides **143, 144,** *and* **145**

The target β-heptapeptides **143**, **144**, and **145** (*Figure 42*) contain an alternating sequence of heterochiral [195] β³-amino acid residues. They differ in the side chains of residues 3 and 7: **143**, **144**, and **145** contain the isobutyl, ω-aminobutyl and hydroxymethyl group, respectively, in these positions. By varying only these side chains, the influence of substituents with different hydrophobicity on the solubilities and folding propensities of β-peptides with (*R*)-β³/(*S*)-β³-sequence was studied.

[123] Recently, the structure of gramicidin A in the solid state [506] and in solution [507,508] has been studied by NMR spectroscopy

Figure 42. β-Heptapeptides **143**, **144**, and **145** (with alternating[124] (*R*)- and (*S*)-β^3-amino acid building blocks) synthesized on *ortho*-chlorotrityl-chloride resin.



The synthesis of these β-peptides was performed on *ortho*-chlorotrityl-chloride resin (*Table 15*). The anchoring of the first β^3-amino acid on the resin proceeded in 54 (**143**), 45 (**144**), and 77% (**145**) yield, respectively. The synthesis was performed as described in *Chapter 5.3.3*. The yields of crude products after cleavage from the resin were quantitative. Surprisingly, the purities of crude products were substantially different. This was due to incomplete Fmoc deprotection in the case of **144**, as will be discussed later. β-Peptide **143** was cleaved from the resin by 2% TFA in CH_2Cl_2; TFA/H_2O/(i-Pr)$_3$SiH 95:3:2 was used for cleaving **144** and **145** containing acid-labile protecting groups in their functionalized side chains. After purification by preparative RP-HPLC, MALDI mass spectoscopy and the ^{1}H-NMR spectrum of β-peptide **145** revealed that lactonization (→ γ-lactone) of the *C*-terminus had occurred, probably during TFA cleavage from the resin. Such γ-lactones have previously been reported in the literature [512].

[124] The CIP priority is inversed when going from valine to β^3-homovaline, so that (*S*)-Val is converted to (*R*)-β^3-HVal upon homologation with retention of configuration.

Table 15. Isolation and characterization by HPLC and MS of (*R*)/(*S*)-β³-heptapeptides **143**, **144**, and **145**.

β-Peptide	Yield (%)[a]	Purity[b] (%)	t_R (min)[c]	FAB-MS
143	99	80	9.3[d]	830.5 (M^+)
144	99	50	11.4[e]	860.1 (M^+)
145	99	74	12.8[f]	760.6 ([M + 1]$^+$)

a) % Mass recovered based on polymer loading. b) HPLC purity (220 nm) of the crude product. c) Retention time in the HPLC (linear gradient of *A* (0.1% TFA in H_2O) and *B* (MeCN); see *GP 27* in *Exp. Part*). d) 30-90% *B* in 20 min (C_8). e) 5-65% *B* in 20 min (C_8). f) 5-65% *B* in 20 min (C_{18}).

β-Peptide **143** displayed poor solubility (comparable to that of β-peptide **112**): it was only soluble in pentafluorophenol, 1,1,1,3,3,3-hexafluoro-2-propanol (ca. 5 mg/ml), hot HOAc[125], and MeOH. β-Peptide **143** was insoluble in H_2O, DMSO (according to the ^{1}H-NMR spectrum in CD_3SOCD_3), pyridine, THF with LiCl addition [513], and even in trifluoroethanol. However, β-peptide **144** in which the leucine side chains have been replaced by lysine side chains is quite soluble in protic solvents such as H_2O and MeOH, as compared to **143**, but virtually insoluble in $CHCl_3$. The ω-aminobutyl side chain has a much higher potential for solubilizing a β-peptide in protic solvents than the hydroxymethyl side chain: β-peptide **145** is poorly soluble in MeOH (it forms a gel at high concentrations) but insoluble in H_2O.

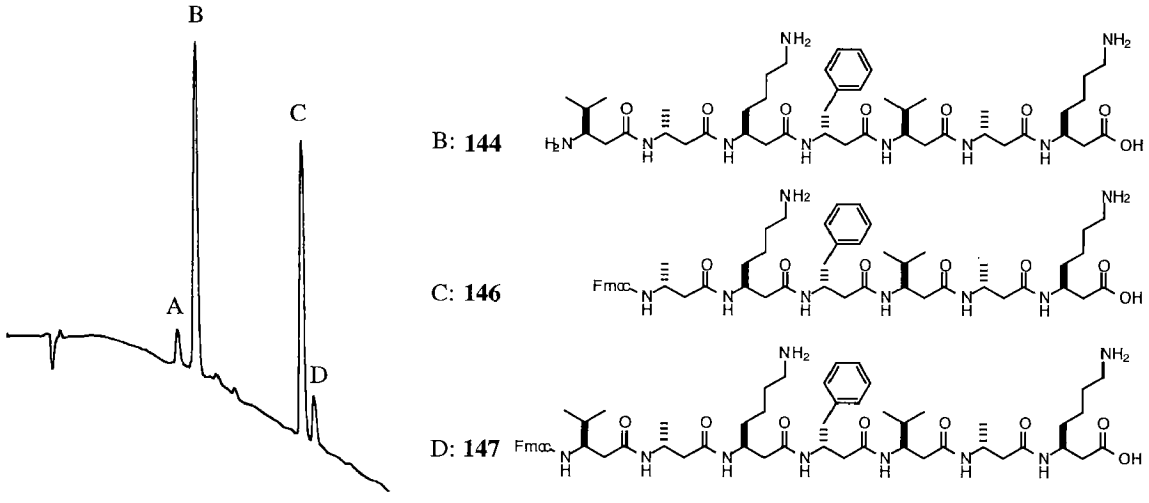
In the case of β-peptide **144**, identification of the two main impurities revealed incomplete Fmoc deprotection during the last two steps in the synthesis. Incomplete Fmoc removal was previously observed in the solid phase peptide synthesis of long-chain peptides [514]. In the present case, the use of higher concentrations of piperidine in DMF or longer deprotection times did not lead to any improvement. However, switching to a stronger base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [515] gave more satisfactory results, and the formation of the impurity resulting from incomplete Fmoc removal in the last stage of the synthesis could be suppressed. *Figure 43* shows an HPLC trace of crude **144** which was synthesized using 20% piperidine for Fmoc deprotection. Fmoc-protected β-peptide derivatives **146** and **147** correspond to peaks C and D, respectively[126], proving that the Fmoc group was not completely removed. The DBU

[125] A dilute CD_3CO_2D solution of **143** was used for ^{1}H-NMR spectroscopy. **143** was solubilized to some extend by ultrasonication before measurement.

[126] The corresponding β-peptide derivatives were isolated by RP-HPLC and identified by MS and NMR spectroscopy.

deprotection protocol (see *Exp. Part*) reduced the amount of these side products to a fraction half as small as in the batches where the piperidine deprotection protocol was applied.

Figure 43. HPLC trace of crude **144** after synthesis using 20% piperidine in DMF for Fmoc deprotection; the main impurities were β-peptide derivatives **146** and **147**. Composition of crude product: A (4%), B (47%), C (42%), and D (6%). Impurity A: not identified. Parameters: gradient: 5-65% MeCN in H_2O (0.1% TFA) in 20 min; C_8 column; UV detection at 220 nm.



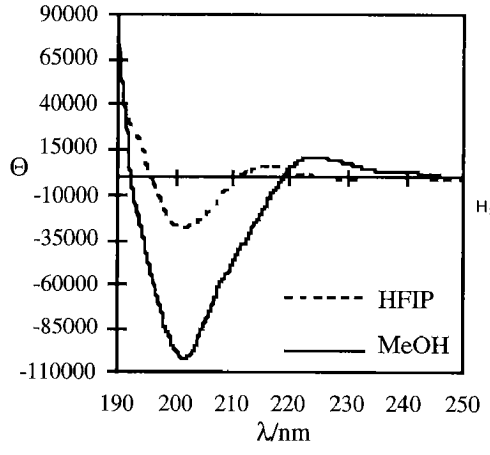
Amide proton exchange kinetics were measured for β-peptide **144** in CD_3OD. The overall half-life ($\tau_{1/2}$) was determined to be 50-70 min for the amide protons. Thus, the NH/ND exchange rate is substantially higher as compared to the 3_{14}-helix forming $\beta^{2,3}$-peptide **106** (see *Chapter 5.2.3*).

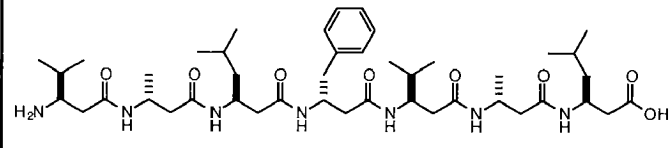
5.5.3 CD Spectroscopy

As usual, CD spectroscopy can provide evidence for secondary structure. The β-peptides **143**, **144**, and **145** displayed a unique CD signature in fluorinated solvents (*Figures 44* and *45*). This is the first indication that these β-peptides share a common secondary structure. The CD spectra are discussed in detail below.

β-Peptide **143** displays a much more intense negative *Cotton* effect at 202 nm in MeOH ($-1.02 \cdot 10^5$) than in 1,1,1,3,3,3-hexafluoro-2-propanol ($-2.90 \cdot 10^4$), see *Figure 44*. Due to its poor solubility, **143** could only be measured in these solvents. The CD spectrum in MeOH was recorded immediately after ultrasonication of the 0.2 mM solution; a time-dependent decrease of the *Cotton* effect was observed due to precipitation of **143** after 5-10 minutes. This CD spectrum does not correspond to the spectrum assigned to any known secondary structure.

Figure 44. Overlay of the CD spectra of β-peptide **143** recorded in MeOH and HFIP (1,1,1,3,3,3-hexafluoro-2-propanol). Spectra were recorded at r.t. The concentration was 0.2 mM. [Θ] in 10 deg · cm^2 · mol^{-1}.

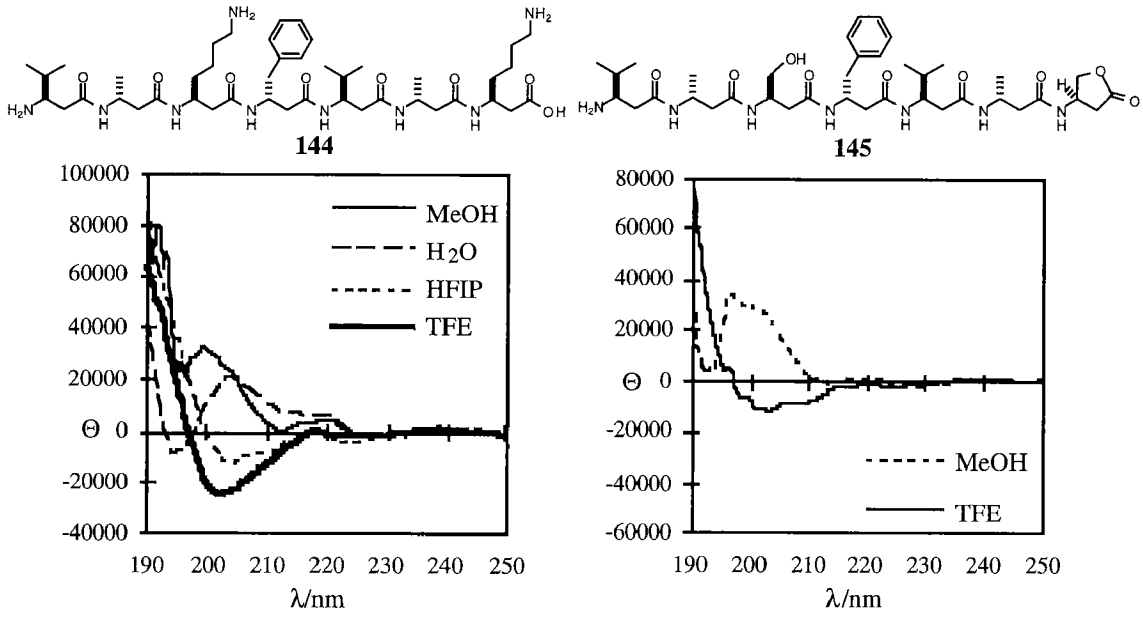




143

A similar CD spectrum is displayed by β-heptapeptide **144** in 2,2,2-trifluoroethanol and HFIP (*Figure 45*), albeit with much lower intensity ($-2.54 \cdot 10^4$ and $-1.22 \cdot 10^4$, respectively). Due to the ω-aminobutyl groups β-heptapeptide **144** is quite soluble in H_2O. However, H_2O solutions of **144** feature a different CD spectrum with a maximum at ca. 203 nm ($+2.07 \cdot 10^4$) and a shoulder at ca. 220 nm ($+7.71 \cdot 10^3$). In MeOH the molar ellipticities are $+3.30 \cdot 10^4$ at 199 nm and $+4.40 \cdot 10^3$ at 220 nm. This solvent dependence is indicative of different conformations in protic and aprotic solvents. The same trend is found with β-peptide **145**.

Figure 45. CD spectra of β-peptides **144** and **145**. Spectra were recorded at r.t. The concentration was 0.2 mM. [Θ] in 10 deg · cm^2 · mol^{-1}. HFIP: 1,1,1,3,3,3-hexafluoro-2-propanol; TFE: 2,2,2-trifluoroethanol.

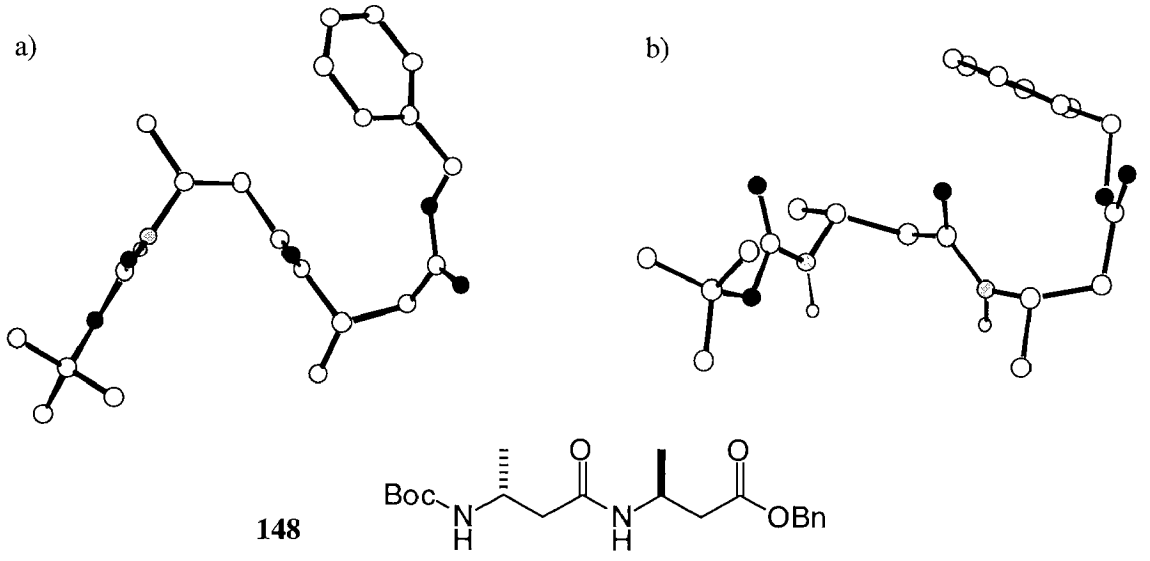


1.1.1 X-Ray Structure of (R)-β^3/(S)-β^3-*Dipeptide* 148

The X-ray crystal structure of β-dipeptide[127] **148** constructed from β-amino acids of alternating configuration provided a first step towards the elucidation of the (*R*)/(*S*)-β^3-peptide structure (*Figure 46*). *Figure 46a* shows the orientation of the methyl substituents along the peptide backbone and in *Figure 46b* the colinear arrangement of the carbonyl groups is evident.

127 I am grateful to Dr. *M. Oberhoff* for providing compound **148**.

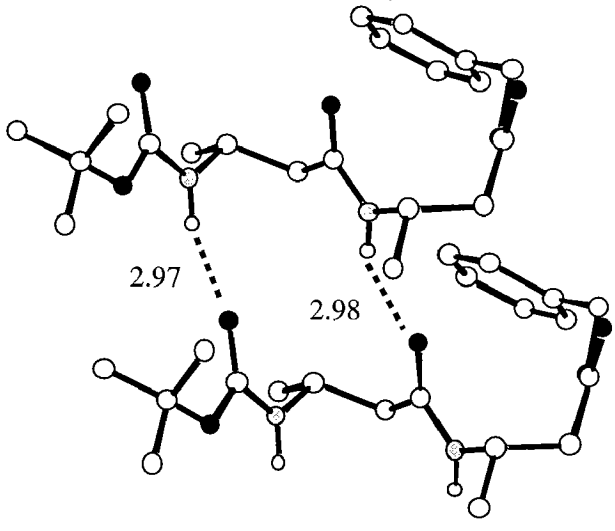
Figure 46. X-Ray crystal structure of (*R*)/(*S*)-β^3-peptide **148**. X-Ray analysis by Dr. *P. Seiler*.



148

This arrangement implies a polar structure that could foster intermolecular aggregation via H-bonding between amide groups of neighboring molecules (cf. pleated sheet structures, *Chapter 5.3.2*). Indeed, the crystal packing of **148** clearly shows intermolecular H-bonds holding neighboring molecules in a parallel arrangement (*Figure 47*).

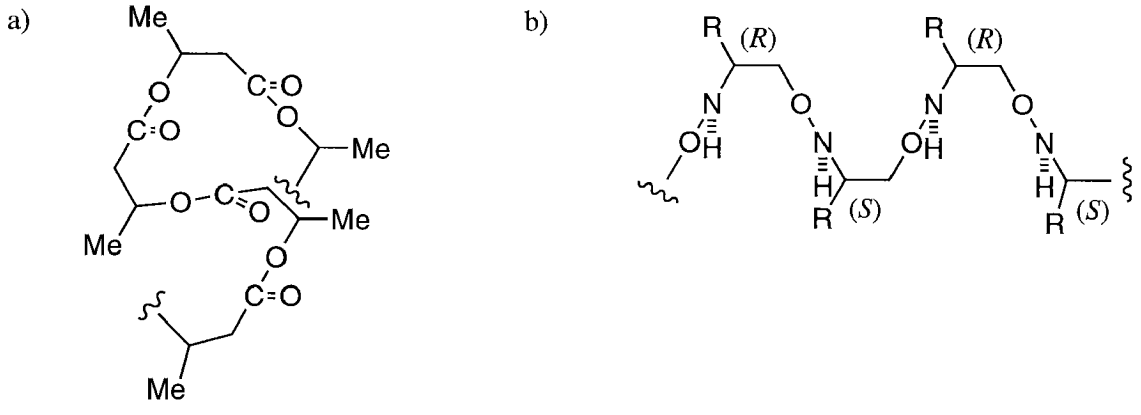
Figure 47. Crystal packing of β-dipeptide derivative **148** showing two neighboring molecules . The N · · O distance is given in [Å]. The angles N–H · · · O are + 157.6 and + 148.7° for NH(1) and NH(2). The phenyl rings are ca. 5 Å apart.



There is a striking analogy between the crystal structures of oligomers from (*R*)-3-hydroxybutanoic (HB) acid [516] and the structure of **148** (*Figure 48*). In

Figure 48b the initial two turn segments present in the structure of **148** were taken to construct a potential structure of higher oligomers.

Figure 48. a) Schematic representation of the structure of oligomers of (*R*)-3-hydroxybutanoic acid. b) Model constructed from the X-ray crystal structure of **148**. All C=O bonds point to the same direction (up), the N–H bonds to the opposite direction (down).



A comparison of the torsion angles provides further evidence for this analogy (*Table 16*). In **148** the torsion angles in the two neighboring turns have opposite signs which accounts for the linear structure.

Table 16. Torsion angles in the crystal structure of (*R*)/(*S*)-β³-dipeptide derivative **148** in comparison to the averaged torsion angles from crystal structures of oligomers from (*R*)-3-hydroxybutanoic (HB) acid (from [517]). Dihedral angles Φ, Θ, and Ψ as defined in *Figure 28*.

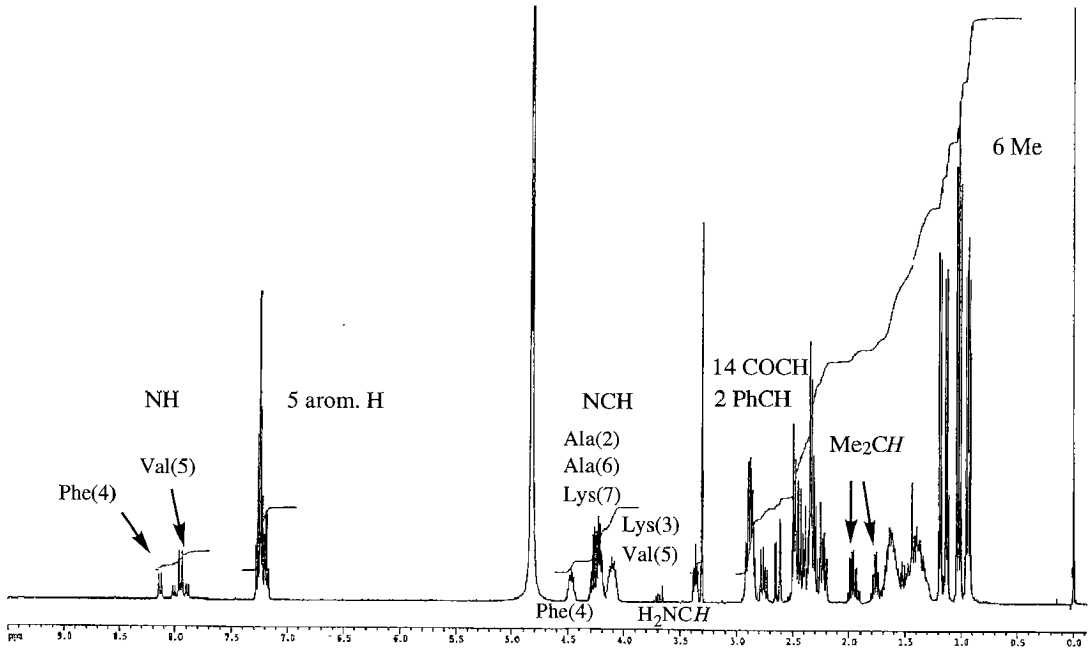
	Residue[a)]	Φ	Θ	Ψ
148	1	+ 134.0	− 60.4	+ 135.8
148	2	− 104.9	+ 70.4	− 90.8
HB-oligomer	S fragment[b)]	+ 150	− 56	− 43
HB-oligomer	Δ fragment[b)]	+ 142	− 62	+ 151

a) Numbering starting from the *N*-terminus. b) The S and Δ fragments occur in the crystal structures of the HB-oligomers, as defined in [516]. Angles Φ, Θ, and Ψ correspond to τ_1, τ_2, and τ_3 (used in [518]).

5.5.5 *NMR Analysis of* (R)-β^3/(S)-β^3-*Heptapeptide* **144**

The ^{1}H-NMR spectrum of β-heptapeptide **144** is shown in *Figure 49*. The NH signals are well separated. The half-life of NH/ND exchange are ca. 46 min for the NH of the (*R*)-β^3-HPhe- and ca. 70 min for the NH of the (*R*)-β^3-HVal residue (cf. *Chapter 5.5.2*). An antiperiplanar arrangement of the NH and the C(β)-H protons is suggested by the large coupling constants ($^3J_{NH,C(\beta)-H}$ = 8-9 Hz) [379-381].

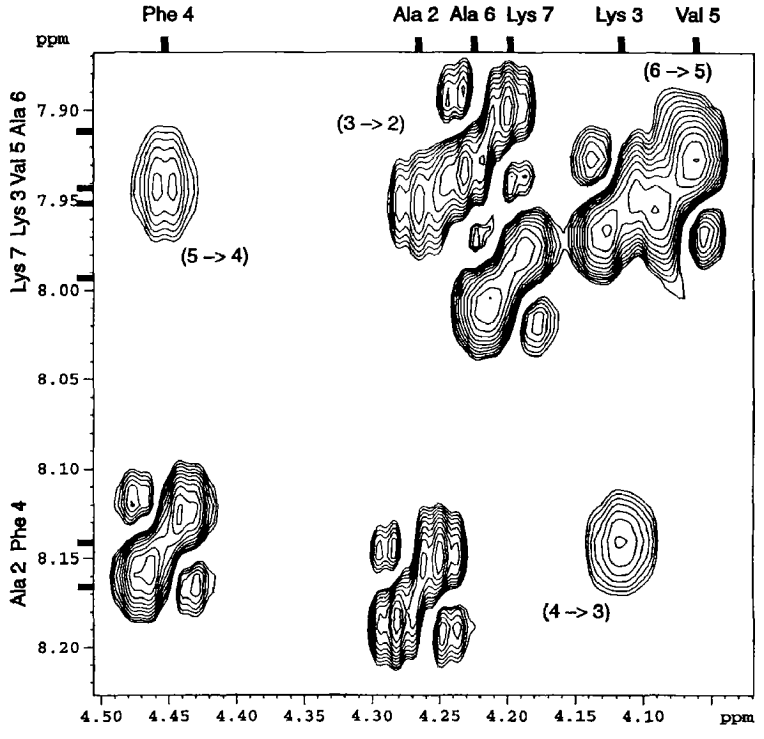
Figure 49. 400 MHz ^{1}H-NMR spectrum of **144** (16 mM in CD_3OD).



All protons were assigned by detailed NMR analysis[128]. The well resolved NH/C(β)-H region of the ROESY spectrum is shown in *Figure 50*.

[128] The 2D-NMR experiments were carried out by *K. Gademann*.

Figure 50. Part (NH/C(β) region) of the 150 ms ROESY spectrum of **144** in CD_3OH. NOEs from NH of residue *i* to C(β)-H of residue (*i* – 1) are indicated. The chemical shifts of the residues are marked by Xaa, representing the β^3-HXaa residues.



The relevant NOE cross peaks are collected in *Table 17.* NOEs were detected from the NH proton of residue *i* to the C(β)-H proton of residue (*i* – 1). These NOEs are not compatible with any known β-peptide secondary structures, i.e. the 3_{14}-, 2.5_{12}-, or 12/10/12 helix, or the pleated sheet. The next step in structure determination will be to verify whether these NOEs originate from a random coil structure or whether they are characteristic of a new secondary structure. The data from the NMR measurements will be used as distance restraints in a simulated annealing protocol.

Table 17. NOEs of β-heptapeptide **144** extracted from the 150ms-ROESY spectrum (CD_3OH, 500 MHz).

NH (residue)	H Atom	Residue	NH (residue)	H Atom	Residue
Phe(4)	β	Lys(3)	Ala(2)	α'	Ala(2)
Val(5)	β	Phe(4)	Ala(2)	α'	Val(1)
Lys(3)	β	Ala(2)	Phe(4)	α	Lys(3)
Ala(6)	β	Val(5)	Lys(7)	α	Lys(7)
Phe(4)	γ	Phe(4)	Lys(7)	α'	Lys(7)
Phe(4)	γ'	Phe(4)	Lys(7)	α	Ala(6)
Ala(2)	α	Val(1)	Lys(7)	α'	Ala(6)
Phe(4)	α	Phe(4)	Ala(6)	α	Val(5)
Phe(4)	α'	Phe(4)	Ala(6)	α'	Val(5)
Ala(2)	α	Ala(2)			

5.6 Synthesis and Structure of β-Peptides Consisting of β^2- or β^3-Homoproline

5.6.1 Introduction

The following question arose in the course of rational β-peptide design: do β-peptides without any backbone H-bonds form stable secondary structures? Considering the importance of H-bonds in stabilizing α-peptidic structures [438], one might be inclined to answer in the negative. However, several precedents suggest that stable secondary β-peptidic structures may be possible without H-bonds: *i*) The preferred backbone conformation around the central C(α)–C(β))-bond has been identified as a major contributor to the stabilization of β-peptidic helical and pleated sheet structures [27]; the lack of a melting point in temperature-dependent NMR and CD spectra of MeOH solutions of β-peptides [348] is compatible with this non-cooperative source of stability. In *N*-alkylated β-peptides the staggering effect must still be present. *ii*) Recent molecular dynamics simulations suggest that spontaneous folding into the 3_{14} helix is possible even if the corresponding H-bonds[129] are not present [335]. *iii*) Peptoids [518] (oligomers of *N*-substituted glycine residues) containing chirality centers at the α-position of their *N*-substituents can form stable structures in solution[130] despite their lack of amide protons and inability to form H-bonds within the backbone [519-521].

A fully *N*-methylated β-hexapeptide consisting of *N*-methyl-β-homoalanine has already been synthesized, but it is not crystalline as a consequence of multiple rotamers, thwarting structure analysis [434]. The homologues of proline were chosen to solve this problem: the constraints that determine the allowed values of the backbone dihedral angles for β-peptides consisiting of β^2- or β^3-homoproline, i.e. β-peptides with tertiary amide groups, should be substantially different from those operating in β-peptides composed of β-amino acids with a primary amino group. These β-amino acids should enforce distinct backbone torsion angles Φ, Θ and Ψ (*Figure 28*), due to their constrained cyclic structure, and this might compensate for the lack of amide

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[129] There is only a small energetic difference between an intramolecular and an intermolecular H-bond in MeOH! [348].

[130] NMR-analysis suggested that the major conformation of a peptoid pentamer in MeOH is a (*P*)-3_{14} helix [519].

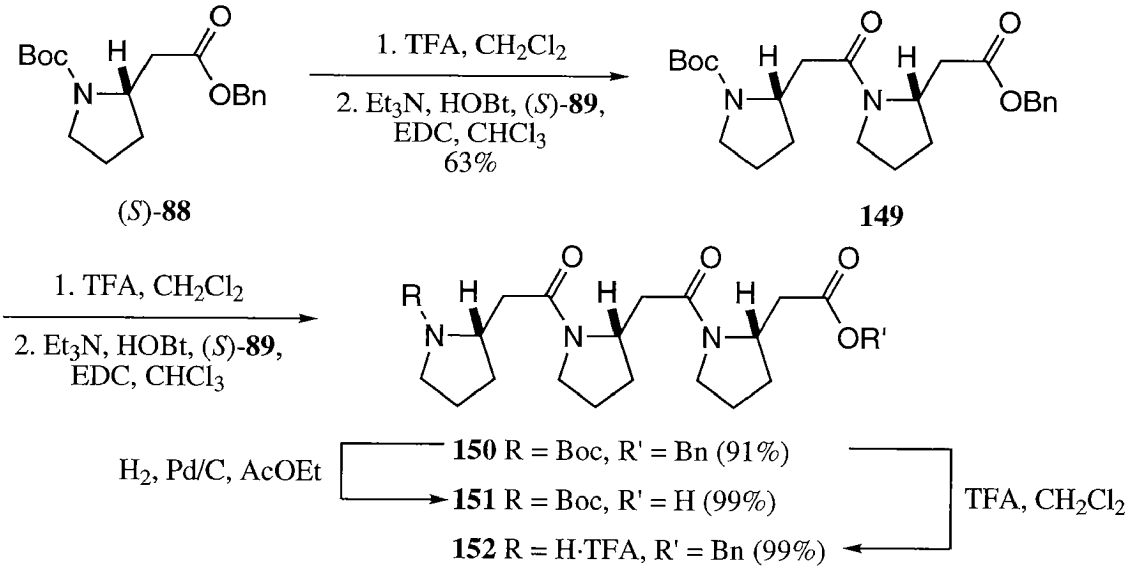
protons. Proline, the only secondary proteinogenic amino acid, imparts special conformations to a peptidic backbone by virtue of its pyrrolidine ring and of its fully substituted amide *N*-atom. As a consequence, the energetic difference between the *cis*- and *trans* form of the prolyl–peptidyl-bond is decreased, accounting for 10-30% of *cis* amide bond (ω = 0°, or *ap* conformation) in Pro-containing peptides [438]. This leads to unique structures of polyproline [283a] and of Pro-rich proteins (see collagen triple helix [284,285,522]).

5.6.2 *Synthesis of β³-HPro-Peptides*

Both all-(*S*)-β-peptides (isotactic) and the β-peptides containing alternating sequences of (*S*)- and (*R*)-β³-homoproline (syndiotactic) were prepared in solution, using the EDC/HOBt procedure. First, the peptides with homochiral building blocks are presented.

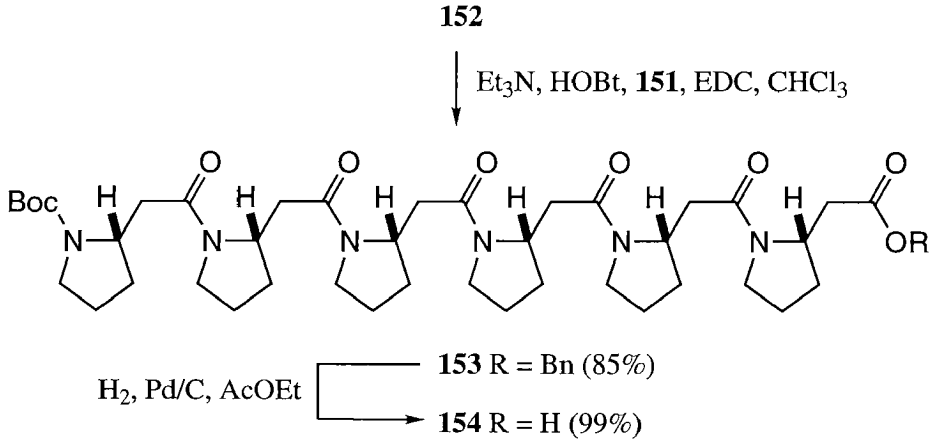
The benzyl ester derivative (*S*)-**88** was Boc-deprotected (TFA, CH_2Cl_2) and the resulting TFA salt was employed for coupling with the Boc-protected amino acid (*S*)-**89** to give the fully protected dipeptide derivative **149** (*Scheme 63*). After *N*-deprotection another coupling step with (*S*)-**88** followed to give the protected β-tripeptide derivative **150** as a white foam in 89% yield. Benzyl ester cleavage (H_2, Pd/C) provided the β-tripeptide acid **151** and Boc-deprotection yielded the TFA salt **152**.

Scheme 63. Synthesis of all-(*S*)-β-tripeptide derivatives **150**, **151**, and **152**, consisting of (S)-β³-HPro.



Fragment condensation of the tripeptide derivatives **152** and **151** then furnished protected β-hexapeptide **153** which was converted to the peptide acid **154** by hydrogenation (*Scheme 64*). The fully protected peptide **153** was quite soluble in various protic and aprotic solvents ($CHCl_3$, CH_2Cl_2, MeOH, AcOEt, Et_2O). The white foam obtained after evaporation was even soluble in H_2O! However, once precipitated, compound **153** displayed much poorer solubility.

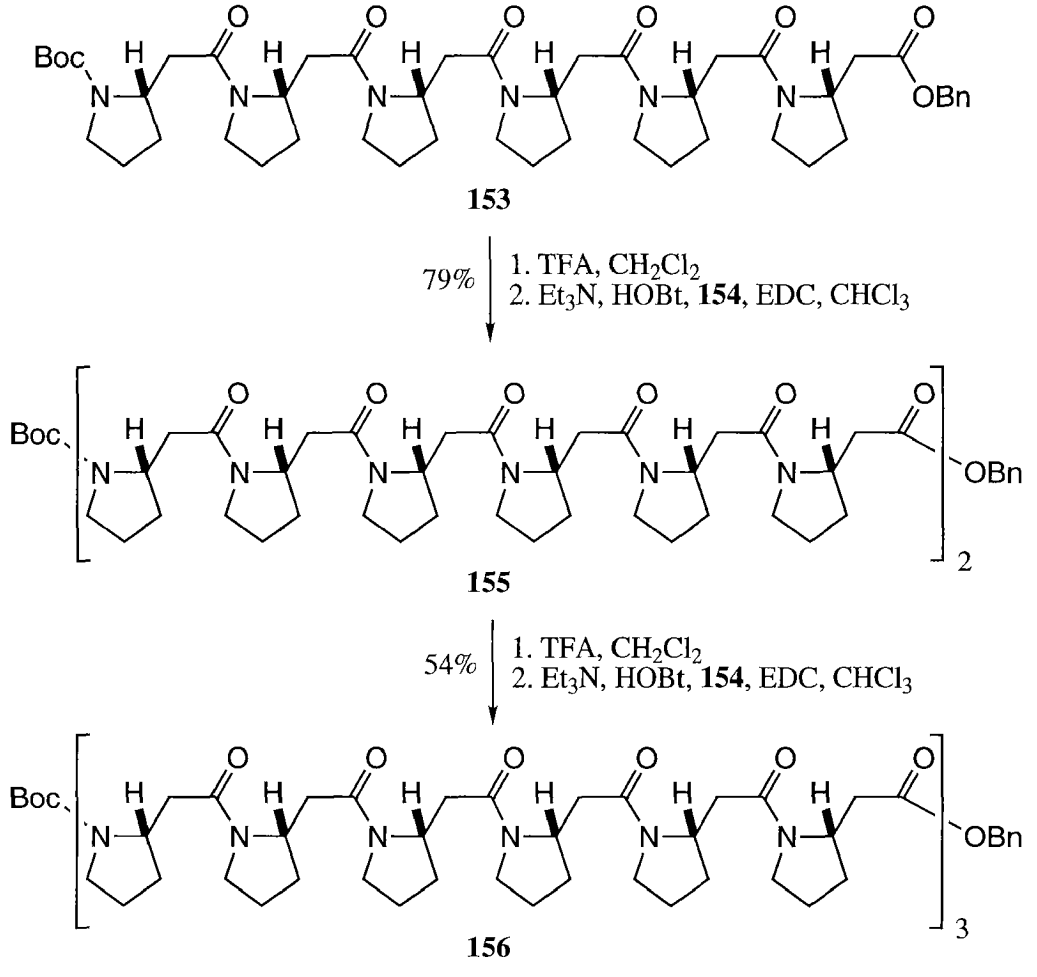
Scheme 64. Synthesis of the all-(*S*)-β-hexapeptide derivatives **153** and **154**.



The good solubility of hexapeptide derivative **153** and the high yields in the coupling steps provided motivation for the synthesis of higher oligomers. The TFA salt obtained from hexapeptide **153** was used for coupling with hexapeptide acid **154** to give the fully protected β-dodecapeptide **155** as a white foam in 79% yield after purification by precipitation from CH_2Cl_2/hexane (*Scheme 65*). After Boc-deprotection, this dodecamer was further coupled with the peptide acid **154** to give the fully protected β-octadecapeptide **156** as a white powder. This is the longest β-peptide synthesized to date[131].

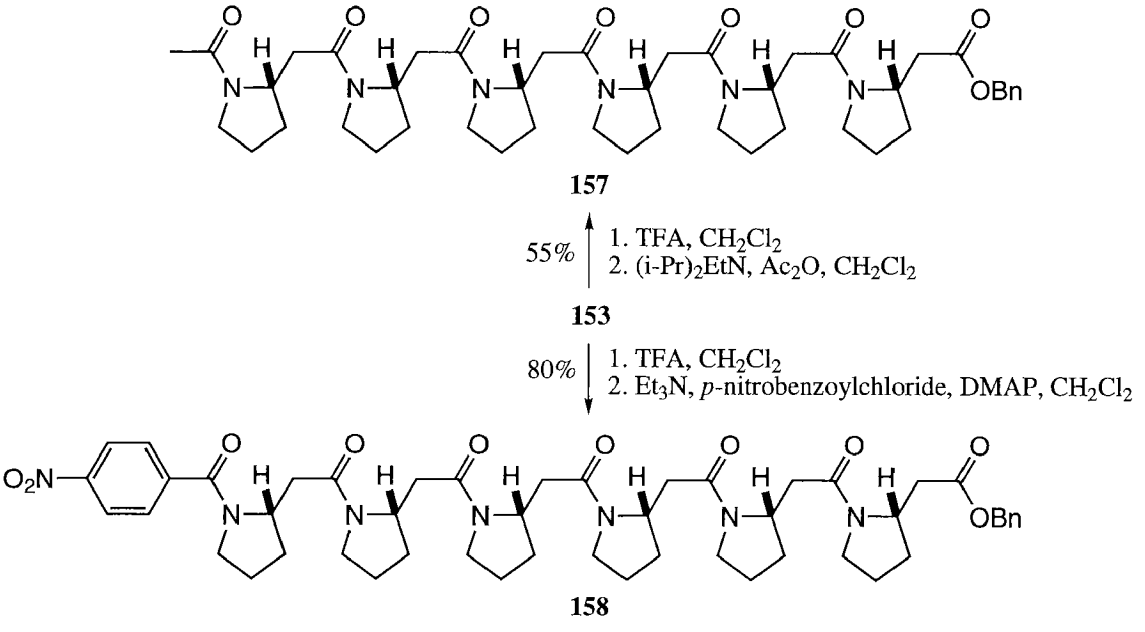
[131] A highly insoluble β-pentadecapeptide was reported in [203].

Scheme 65. Two fragment condensations leading to the fully protected β-dodeca- and β-octadecapeptides **155** and **156**.



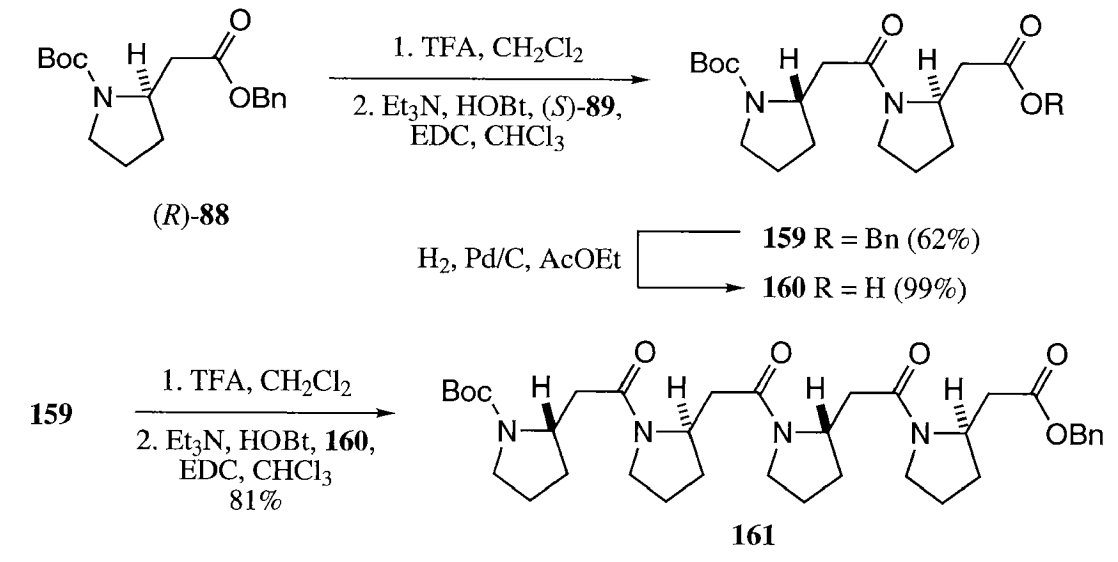
In an effort to increase the crystallinity of the β-peptides, the acetyl and *p*-nitrobenzoyl group were introduced at the *N*-terminus of β-hexapeptide **153** (*Scheme 66*). The TFA salt from **153** was used for acylation with either acetic anhydride or *p*-nitrobenzoylchloride to provide β-peptide derivatives **157** and **158** as a white and yellow foam, respectively.

Scheme 66. Protecting group exchange at the *N*-terminus of β-hexapeptide **153** to give the *N*-acetyl- and *p*-nitrobenzoyl-derivatives **157** and **158**.



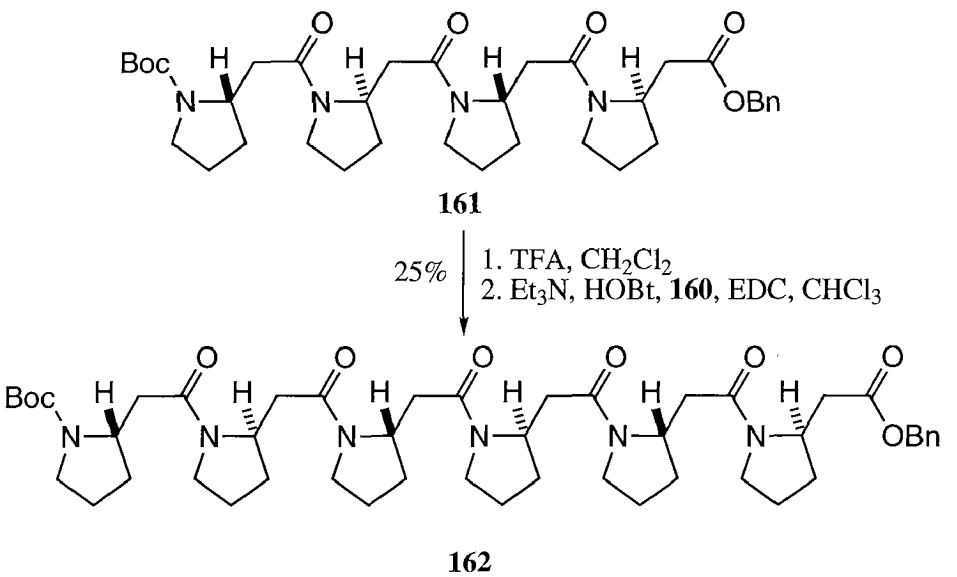
The synthesis of the (*S*)/(*R*)-β-peptides involves the fragment coupling of (*S*)/(*R*)-β-dipeptidic building blocks. Thus, dipeptide derivative **159** was prepared from the TFA salt obtained from (*R*)-**88** and the Boc-protected amino acid (*S*)-**89** (*Scheme 67*). For fragment coupling, the benzyl ester group of **159** was deprotected (H_2, Pd/C) to give the β-dipeptide acid **160** which was coupled with the Boc-deprotected dipeptide benzyl ester **159** to provide the syndiotactic β-tetrapeptide derivative **161** as white powder in 81% yield.

Scheme 67. Synthesis of the (*S*)/(*R*)-β-tetrapeptide derivative **161**.



A second fragment coupling step between *N*-deprotected **161** and the peptide acid **160** furnished the fully protected (*S*)/(*R*)-β-hexapeptide **162** as a white powder (*Scheme 68*).

Scheme 68. Synthesis of the fully protected syndiotactic β-hexapeptide **162**.



In general, the all-(*S*)-derivatives have higher solubility than peptides with alternating heterochiral building blocks. Crystallization experiments were performed in CH_2Cl_2, $CHCl_3$, and MeOH with all the HPro-hexapeptides. However, crystals suitable for X-ray analysis have not yet been obtained[132]. As in the case of the *N*-methyl β-peptides [434], the NMR spectra of these β-peptides show that multiple rotamers are present.

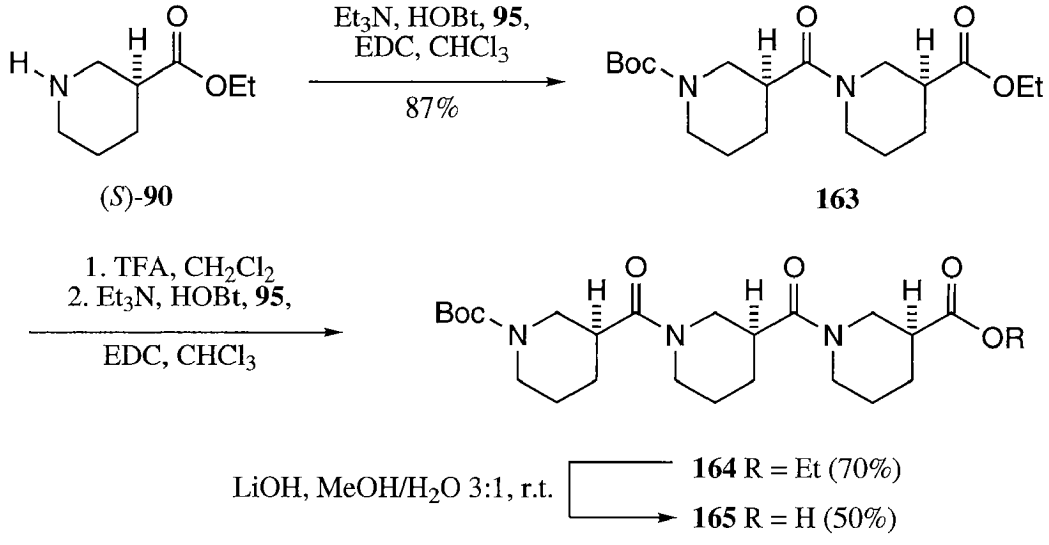
5.6.3 Synthesis of β²-HPro-Peptides

The enantiopure ethyl nipecotate (*S*)-**90**, obtained from resolution (see *Chapter 4.5.2*) was acylated with Boc-protected (*S*)-β²-homoproline **95** to give the dipeptide derivative **163** as a waxy solid (*Scheme 69*). After Boc-deprotection, a further coupling with the β-amino acid **95** gave fully protected β-tripeptide **164** as a white waxy solid. The following saponification was performed using the same mild procedure as for the saponification of the monomeric building block **94**. Thus, treatment of **164**

[132] The all-(*S*)-hexapeptide **153** was obtained from Et_2O or AcOEt as fine moss-like needles.

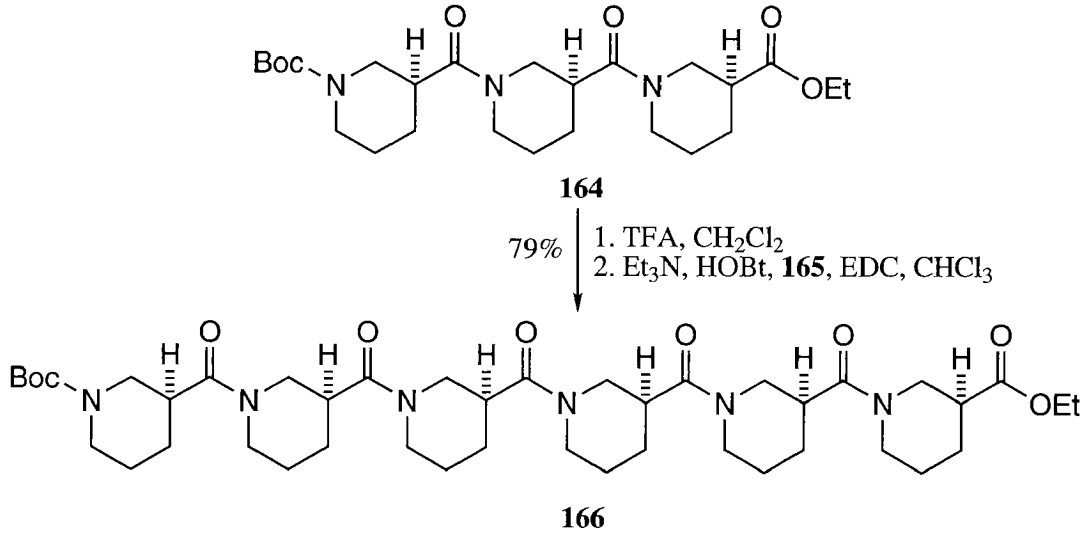
with LiOH in a MeOH/H_2O solution provided the tripeptide acid **165** after precipitation from AcOEt/pentane.

Scheme 69. Synthesis of the all-(*S*)-β-tripeptide derivatives **164** and **165**.



Final fragment coupling, using the peptide acid **165** and the TFA salt derived from **164**, gave the fully protected β-hexapeptide **166** as a colorless "glass" in 79% yield (*Scheme 70*).

Scheme 70. Synthesis of the fully protected all-(*S*)-β-hexapeptide **166**.



The β-peptide derivatives (starting from the dipeptide **163**) display a ca. 1:1 ratio of rotamers in $CDCl_3$, according to their ^{1}H-NMR spectra. This may

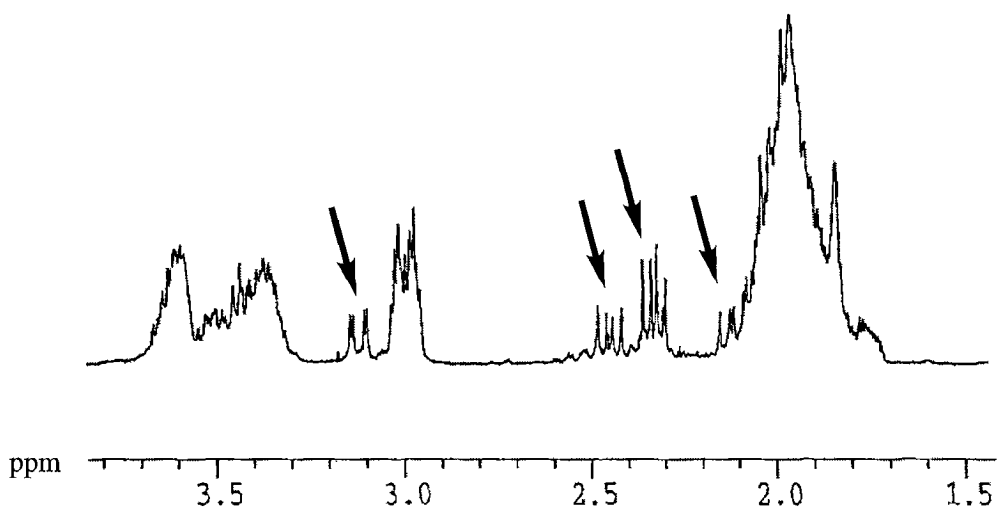
indicate that both, *cis*- and *trans*-amide bonds are present, similar to the situation with proline-containing α-peptides [283].

5.7 Secondary Structure Analysis

5.7.1 NMR Spectroscopy

Most of the ^{1}H-NMR spectra of β-peptides containing β^3-HPro show the presence of rotamers. They also display nicely separated double doublets (*Figure 51*) arising from the C(α)-protons (COCH), with a vicinal coupling constants (^3J) of ca. 8 Hz. A geometry in which the C(β)-H atom and one of the C(α)-H atom have an antiperiplanar arrangement is consistent with this coupling constant [379,523]; the rotation around the C(β)–C(α) bond is restricted. Moreover, there is a large dispersion (ca. 1 ppm) of the chemical shifts suggesting that a stable secondary secondary structure is present in solution.

Figure 51. Part of the 400 MHz ^{1}H-NMR spectrum of β^3-HPro-hexapeptide **158** in $CDCl_3$. Arrows indicate the double doublets of the COCH hydrogens.

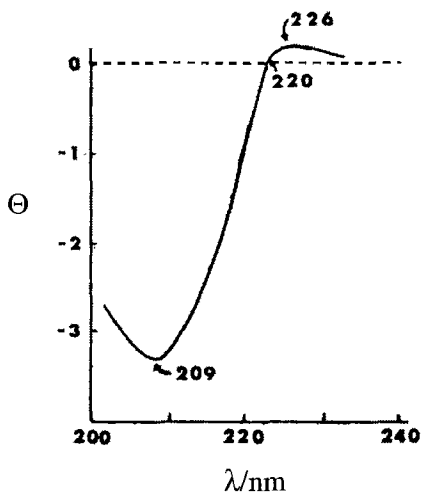


5.7.2 CD Spectroscopy

CD spectroscopy proved useful for characterizing these β-peptides at lower resolution. The polypeptide containing "normal" or "α-proline", i.e. poly-L-proline, forms a left-handed 3_1 helix, the CD spectrum of which is shown in

Figure 52[133]. There is a minimum at 209 nm and a less intense maximum at 226 nm. This α-homopeptide adopts a unique structure and, therefore, displays a CD spectrum that is quite different than that of α-helix-, β-sheet-, or coiled-coil-peptides [283a].

Figure 52. CD spectrum of polyproline II in aqueous solution (Fig. 9.11 in [497]). Molar ellipticity [Θ] in 10^4 deg · cm^2 · $dmol^{-1}$.

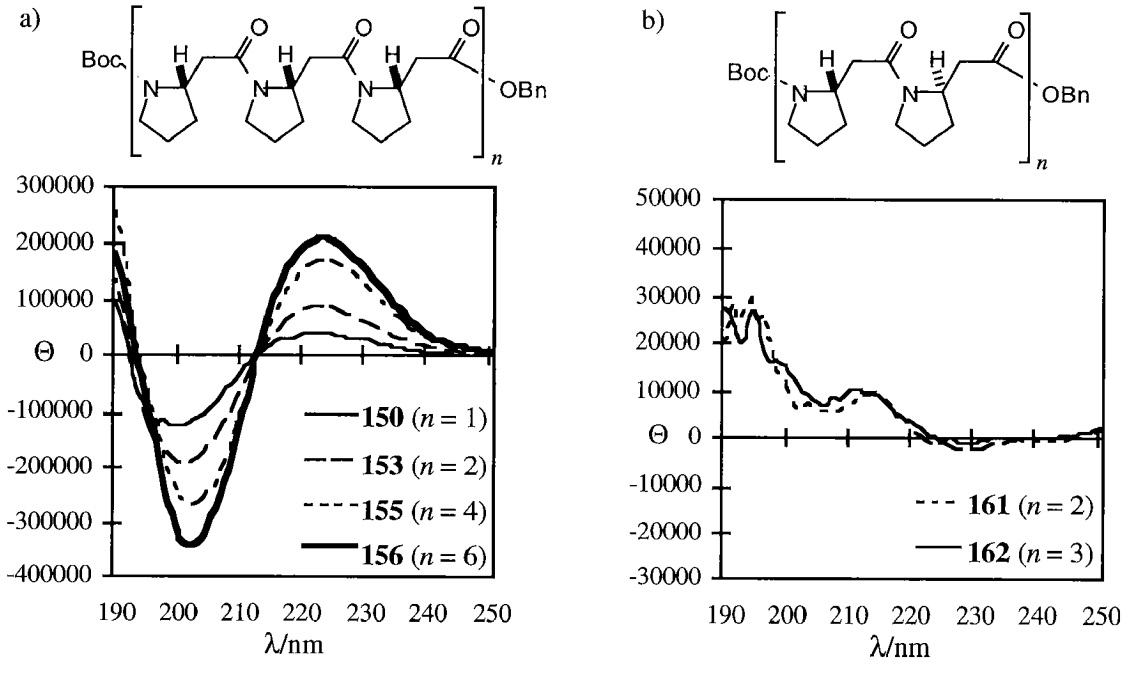


The CD spectra of the all-(*S*)-β^3-HPro-peptides have a characteristic, very intensive minimum at 202 nm and a maximum at 223 nm, with a zero cross-over at 212 nm (*Figure 53a*). The absolute mean residue molar ellipticity at 202 nm decreases with growing chain length (**150** with 3 residues: – 4.40 · 10^4 vs. **156** with 18 residues: – 1.92 · 10^4). The same is true for the mean residue molar ellipticity at 223 nm. This suggests that the secondary structure of longer peptide chains of this type is destabilized. However, the high molar ellipticities (for instance – 3.46 · 10^5 for **156** at 202 nm) still imply that a secondary structure is present in MeOH. The fully protected hexapeptide **153** featured the same CD signal in CF_3CH_2OH and in aqueous buffered solution (pH 5.7), albeit with lower intensity as compared to the CD spectra measured in MeOH. In sharp contrast to the CD spectra of these homochiral [195] β-peptides, the CD spectra of the β-peptides consisting of alternating (*S*)- and (*R*)- building blocks show virtually no *Cotton* effect (*Figure 53b*), suggesting that these compounds are devoid of an ordered

[133] Collagen, by comparison, consists of a three-chain coiled-coil, each of which has a distorted polyproline II helix.

secondary structure[134][135]. The CD spectra did not change substantially with different *C*- and *N*-terminal end groups for either substitution patterns.

Figure 53. CD spectra of β-peptides at 0.2 mM in MeOH at room temperature. a) Overlay of the CD spectra of all-(*S*)-β-peptides **150, 153, 155,** and **156.** b) Overlay of the CD spectra of (*S*)/(*R*)-β-peptides **161** and **162.** Molar ellipticity [Θ] in 10 deg · cm^2 · mol^{-1}.



The β-peptides composed of (*S*)-nipecotic acid building blocks show weaker *Cotton* effects but the overall CD spectrum is similar to that of the β^3-HPro-peptides (*Figure 54*). Here, the mean residue molar ellipticity of hexapeptide derivative **166** (+ 4.07 · 10^3) is nearly three times larger than that of tripeptide derivative **164** (+ 1.38 · 10^3) at ca. 229 nm. Thus, the secondary structure may be stabilized by a longer β-peptidic chains in this case. The CD curve of non-structured protected β-dipeptide **163** is included to show that the measured *Cotton* effects of the higher oligomers are really due to an ordered structure.

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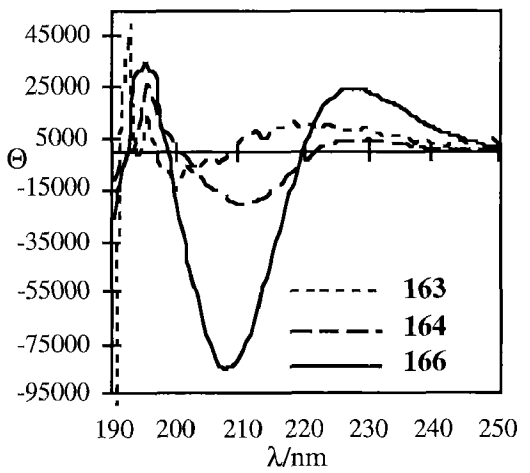
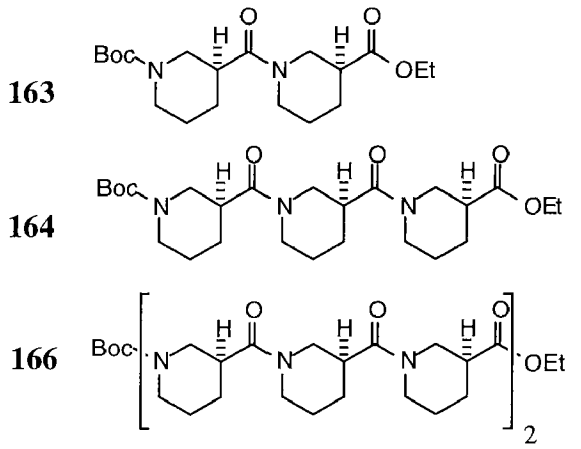
134 The absence of a *Cotton* effect does *not* preclude the existence of a stable secondary structure *a priori*; for instance, γ-peptides which adopt a helical structure according to 2D-NMR analysis do not show any *Cotton* effect [524].

135 Candidates for random coils in the world of β-peptides?

Figure 54. CD spectra of all-(*S*)-β-peptides **163, 164,** and **166** at 0.2 mM in MeOH a t room temperature. Molar ellipticity [Θ] in 10 deg · cm^2 · mol^{-1}.



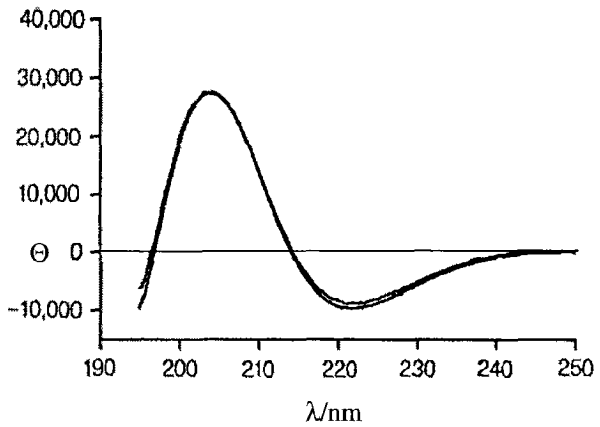


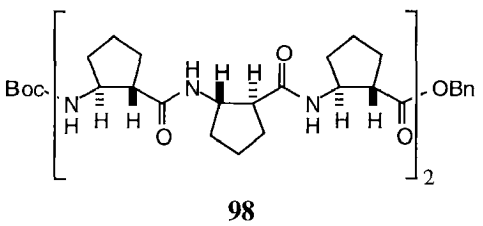
It is noteworthy that a distinctive CD spectrum is observed with β-HPro peptides containing as few as three residues. Normally, β-tripeptides show no *Cotton* effect [17], including β-peptides **101, 103, 109,** and **111** (see *Figure 21*) discussed in this dissertation. It is especially remarkable that the *N*-deprotected β^3-HPro-tripeptide **152**, for which an X-ray crystal structure could be determined (*vide infra*), gives rise to the same, but less intense, CD spectrum as the fully protected **150** (cf. *Figure 53* and *Exp. Part*).

There is a conspicuous (mirror-image-type) analogy between the CD spectra of our β-HPro-containing β-peptides and the CD spectra of *Gellman*'s $\beta^{2,3}$-hexapeptide derivative consisting of *trans*-2-aminocyclopentanecarboxylic-acid building blocks such as **98** (*Figure 55*): they show a maximum at 204 nm, a zero crossing at 214 nm, and a minimum at 221 nm [327]. β-Peptide **98** adopts a 2.5_{12}-helical structure in solution and in the solid state. However, we think that it is highly unlikely that our β-HPro-peptides adopt the same conformation as the cyclopentane derivatives such as **98**[136].

[136] Steric strain is apparent from inspection of a model of the 2.5_{12} helix built with β^2- or β^3-homoproline.

Figure 55. CD spectrum of 2.5_{12}-helix-forming $\beta^{2,3}$-hexapeptide derivative **98** at 2.0 mM and 0.1 mM in MeOH at 20 °C (from [327]). Molar ellipticity [Θ] in 10 deg · cm^2 · mol^{-1}.



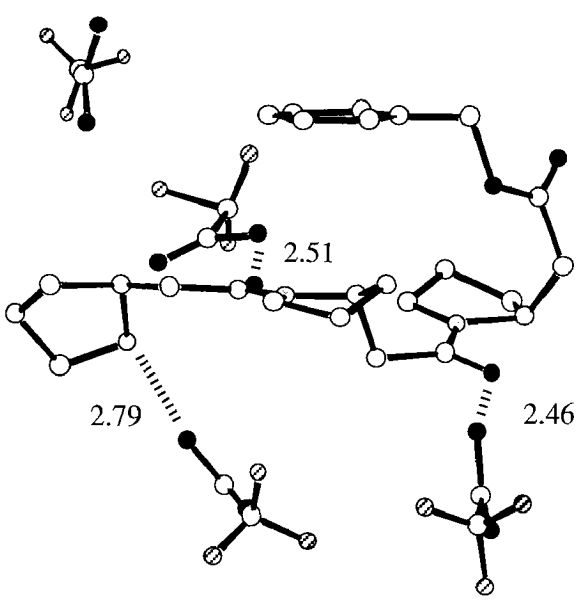


98

5.7.3 *X-Ray Crystal Structure of β^3-HPro-Tripeptide Derivative* 152

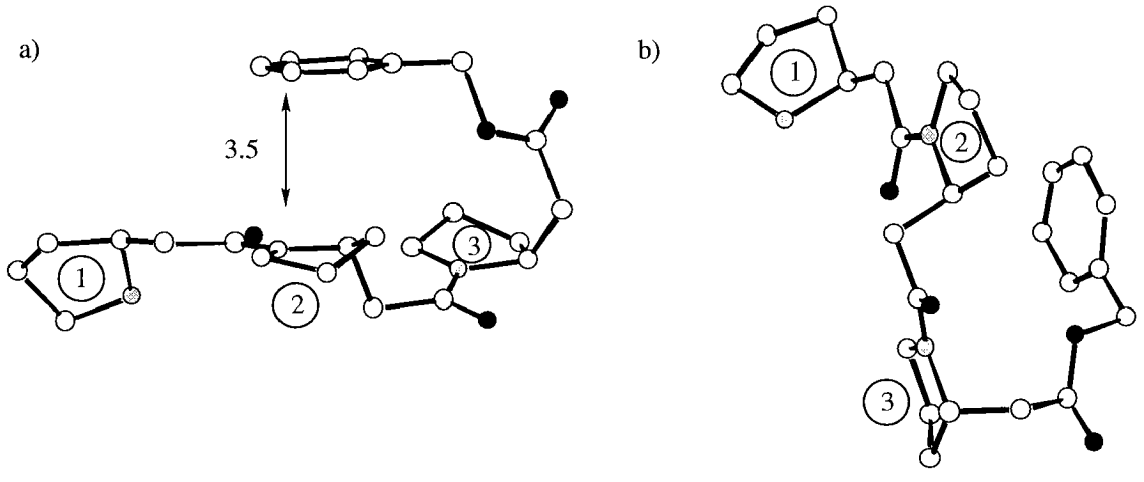
The TFA salt **152** which had been obtained as a colorless oil solidified after two weeks at – 25 °C. Suitable crystals were separated and the structure was solved (*Figure 56*). Four molecules of CF_3CO_2H are incorporated in the crystal.

Figure 56. X-Ray structure of β-tripeptide derivative **152.** The peptide crystallized with four CF_3CO_2H molecules. Two of these form H-bonds with amide carbonyl oxygens, and one CF_3CO_2H molecule is protonating the terminal nitrogen; the distance O · · · O is indicated in [Å]. H-Atoms have been omitted for clarity.



Inspection of this structure reveals some interesting features (*Figure 57*): *i*) The phenyl is parallel (at van-der-Waals distance) to the plane formed by the amide group of the first two β-amino acids[137]. *ii*) The substituents at C(2) of the second and third pyrrolidine ring are in pseudo axial position, a direct consequence of allylic 1,3-strain ($A^{1,3}$): the exocyclic amide group pushes the neighboring substituent out of its plane into an axial position of the ring. This is in agreement with a variety of X-ray structures of *N*-acylated five-membered heterocycles [125]. *iii*) The pyrrolidine rings exhibit the twist conformation[138]. The N-atoms are not pyramidalized.

Figure 57. Two views of the crystal structure of the TFA salt **152**. a) Projection which shows the quasi parallel arrangement of the phenyl ring with the amide plane. The distance is indicated in [Å]. b) In this view, the two fully extended segments are visible in the backbone.



Torsion angles are summarized in *Table 18*. The angle Φ is dictated by the pyrrolidine ring. The C(α)–C(β) bond of the central residue exhibits the *ap* conformation. The *C*-terminal residue of the β-tripeptide can not adopt this geometry because of the benzyl-ester group; here, the C(α)–C(β) bond adopts a (–)-*sc* conformation which requires rotation around the C(α)–CO bond (Ψ = + 88°), otherwise the benzyl ester C=O groups would be orientated towards the pyrrolidine ring. The *N*-terminal residue displays a (+)-*sc* conformation around the central bond. This is consistent with the free secondary amino group; this geometry would be precluded in an *N*-acylated terminus because of 1,5-strain (or *Newman* strain [438]) between neighboring C=O groups.

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137 A similar, more twisted geometry of an α-dipeptide derivative has been reported [525]. For α-peptides with amide protons, various examples are known where N–H bonds point to the center of the aromatic π-system of phenyl groups (π-type interaction) [526,527].

138 For a definition of five-ring twist and envelope see [528-530].

Table 18. Torsion angles in the crystal structure of β-tripeptide derivative **152**. Torsion angles as defined in *Figure 28.*

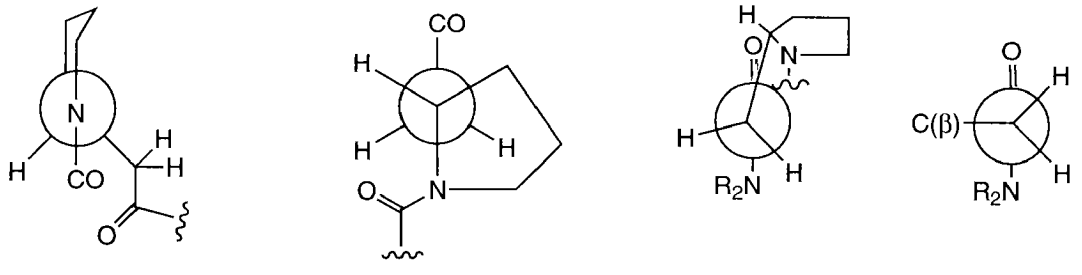
Residue[a)]	Φ [°]	Θ [°]	Ψ [°]
1	-	+ 59.2	– 175.6
2	– 73.6	+ 171.9	– 82.9
3	– 71.6	– 66.8	+ 88.2

a) Numbering starting from the *N*-terminus.

5.7.4 *Conformational Analysis of β-Peptides Consisting of (S)-β³-HPro*

The X-ray structure of **152** and the conformational constraints derived from the *J*-values of the ^{1}H-NMR spectra provided guidance for the construction of a model of the structure of β-peptides composed of (*S*)-β³-homoproline. The angle Φ is enforced by the pyrrolidine ring and is in the range of – 60 to – 72° (*Figure 58*). The ^{1}H-NMR spectrum of **152** shows three well separated double doublets for the COCH protons with vicinal coupling constants of 7-8 Hz (cf. *Figure 51*). This suggests an *ap* conformation around the C(α)–C(β) bond (Θ = 180°), corresponding to the angle Θ of the central β-amino acid in the crystal structure of **152**. Moreover, the amide bond was fixed in the normal *trans* configuration. Thus, the following two conformations **XIX** and **XX** differ in the angle Ψ as shown in *Figure 58*:

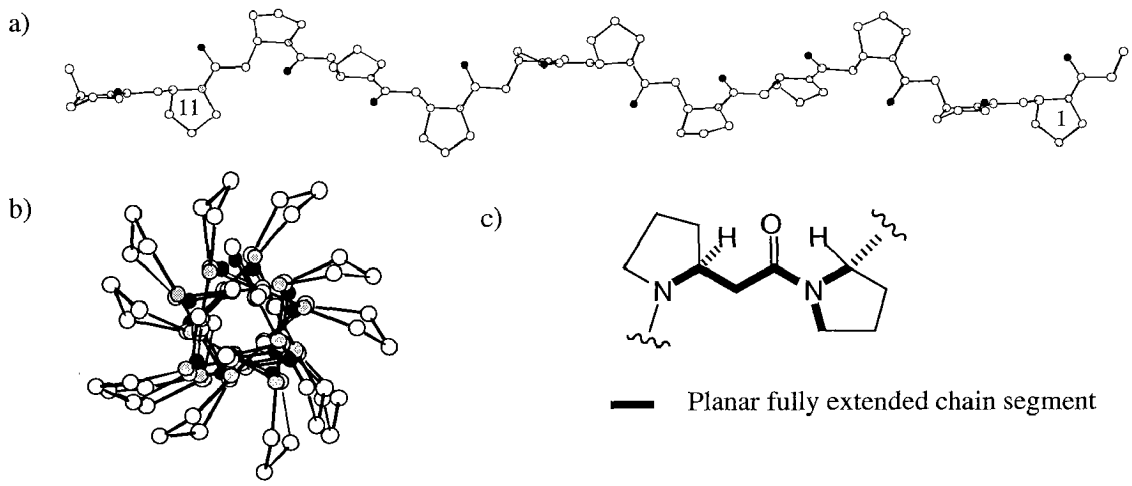
Figure 58. Newman projections showing the torsion angles in possible structures **XIX** and **XX** differing in the dihedral angles Ψ.



Φ (**XIX** and **XX**) = – 72° Θ (**XIX** and **XX**) = 180° Ψ (**XIX**) = 180° Ψ (**XX**) = – 90°

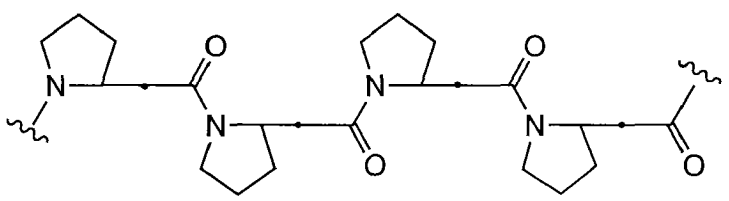
In structure **XIX** (*Figure 59*), the angle Ψ was chosen to be 180° so that the large substituents at the carbonyl C-atom and at C(α) are antiperiplanar. The C=O group lies between the γ-methylene group and the C(β)-H ("staggered", see *Figure 58*). The resulting structure is a right-handed 10_3 helix with three pitches to bring residue (*i* + 10) above residue *i*. The model involves consecutive and fully extended chain segments (N-C(β)–C(α)–CO–N) which are twisted by – 72°.

Figure 59. Model of structure **XIX** consisting of (*S*)-β^3-HPro, constructed with the torsion angles in *Figure 58*. a) Side view of a 10_3 helix. b) Top view of a 10_3 helix; two of the pyrrolidine rings are in juxtaposition. c) Characteristic fully extended chain segment (N-C(β)–C(α)–CO–N) identified in this structure **XIX**. Model constructed with MacMoMo (Prof. Dr. *M. Dobler*, ETH Zürich).



The conformation of the central amino acid in the crystal structure of **152** which is less influenced by the *C*- and *N*-terminal groups provided the basis for the proposal of structure **XX** (*Figure 60*). Thus, the dihedral angle Ψ was fixed at – 90° (cf. *Figure 58*). These angle constraints result in a band structure (*Figure 60*). The amide planes are parallel, as are the planes formed by the pyrrolidine rings. C(β)–N–CO–C(α) segments are joined alternately in two parallel planes. Like structure **XIX**, this conformation does not violate conformational preferences. Structure **XX** is non polar, whereas the helix of structure **XIX** has a net dipole moment as all C=O groups point in the same direction.

Figure 60. Schematic representation of the model of structure **XX** consisting of (*S*)-β^3-HPro, constructed with the torsion angles in *Figure 58*. The black dots represent the C(β) methylene groups which are consecutively above and below the paper plane.



6 Pharmacokinetic and Biological Studies with β-Peptides

6.1 Pharmacokinetic Studies with β-Peptides

6.1.1 Introduction

Many β-amino acids are building blocks for peptides and antibiotics [531,532] which were isolated from plants and, more often, from marine microorganisms [22,228,229][139]. However, relatively few β-amino acids are found in mammals [534]. Their metabolism has been examined in some cases [534-536]. Early studies by *Abderhalden*[140], for example, suggested that peptide bonds involving β-amino acids are resistant to enzymatic hydrolysis [538,539]: "Wir liessen ferner auf Glycyl-β-alanin und dl-Leucyl-β-alanin und endlich auf die Phenylisocyanat-Verbindung der letzteren Verbindung Erepsin und Trypsin-Kinase einwirken. Es blieb in allen Fällen eine Spaltung aus" [538]. Certain β-amino acids have been incorporated into naturally occurring peptides with important pharmacological properties to improve resistance against degradation [297,540-547].

Potential applications of β-peptides in biological systems should be favored by their resistance to enzymatic degradation. β-Peptides are expected to be more stable towards enzymatic hydrolysis in stomach and intestine than their α-peptidic counterparts. Initially, the stability of β-peptides in the presence of a variety of peptidases has been studied [345]. Subsequently, several water-soluble β-peptides were subjected to systems of increasing complexity. β-Amino acids and β-oligopeptides (up to nine residues, carrying the side-chains of Ala, Val, Leu, Ile, Phe, Ser, Lys and Hop) proved resistance to diverse and highly potent peptidases (pronase, proteinase K, 20S proteasome) and to microorganisms (*Pseudomonas aeruginosa* and *Pseudomonas putida*) over 24 h. These enzymes were not inhibited by β-peptides either [346]. Moreover, neither free, nor *N*-acetyl β-amino acids, nor β-peptides (offered as sole N and C source) led to growth of two bacteria tested, although certain 3-amino-butanoic acid (β-HAla) derivatives could

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[139] Recently, the biosynthesis of the phenylisoserine side chain (an α-hydroxy β-amino acid) of paclitaxel (Taxol®) was studied [533].

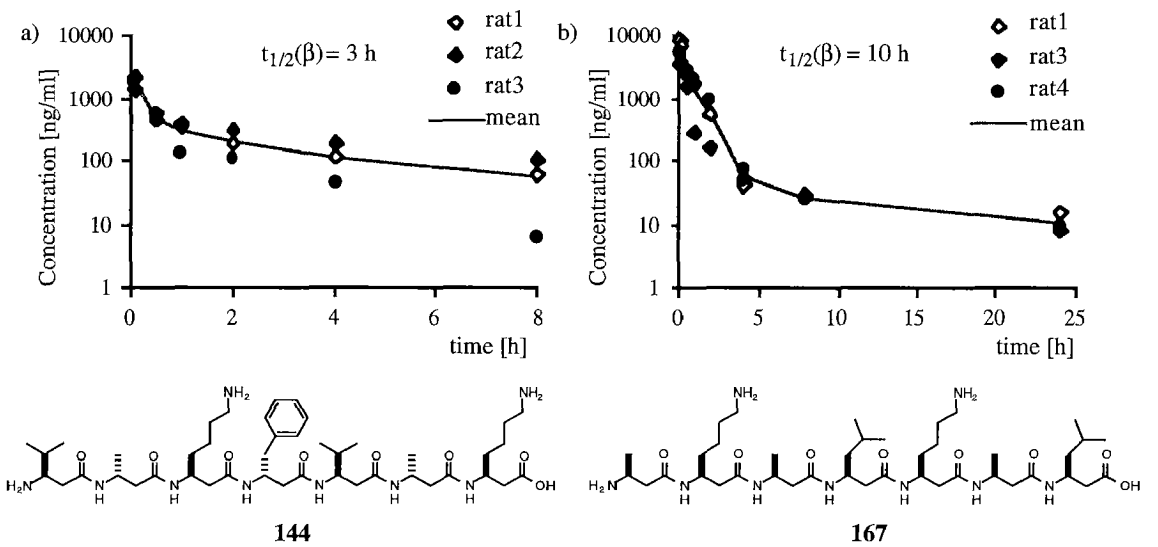
[140] *M. Bergmann, E. Abderhalden,* and *H. Leuchs* were the only students of *E. Fischer*'s group to continue the research on peptide and protein chemistry. For an excellent review on the history of early peptide synthesis see [537].

apparently be metabolized. The latter result suggests that there might be interactions (by hitherto unknown mechanisms) between the worlds of α- and β-peptides. These results are relevant to the question whether the α-peptidic world is orthogonal to the β-peptidic one [17].

6.1.2 *Pharmacokinetic Studies*[141]

Next, the question was addressed as to whether β-peptides also display high metabolic stability *in vivo*. Thus, β-peptides **144** and **167** [548] were administered intravenously (*iv.*) to rats. The results are outlined in *Figure 61*. Peptide concentrations in blood were measured at different times (for experimental details see *Exp. Part* in [346]). The elimination half-life values $t_{1/2}(\beta)$ were 3.1 hours (**144**) and 10.1 hours (**167**). After a rapid decrease of peptide concentration in the first hour, the concentration remained almost constant at a ca. 100 ng/ml and 30 ng/ml level, respectively.

Figure 61. Concentrations of β-peptide **144** (a) and **167** (b) in blood, measured after 4.0-mg/kg *iv.* administration.



Pharmacokinetic and metabolic properties of α-peptides have been compiled [549-551]. As reviewed [552], determination of peptide metabolic stability *in vivo* is experimentally complex, and the cooperative analysis of half-lives of natural and synthetic peptides in serum, plasma, or blood is further

[141] These experiments were performed by Dr. *R. Wössner* and Dr. *F. Bitsch* of the *Novartis Pharma AG*, Basel.

complicated because experimental methods often differ among investigators. Half-lives of α-peptidic drugs, administered intravenously, are on the order of several minutes[142] (Table 3 in [551]). β-Peptides **144** and **167** thus display much higher stability towards metabolic processes in rodent blood.

β-Peptides **144** and **167** differ in their secondary structures: The configurational pattern of β-peptide **144** does not allow for the formation of a 3_{14} helix [27,203] (see *Chapter 5.5*) whereas β-peptide **167** adopts a 3_{14}-helical structure according to the CD spectrum [548]. Whether or not the higher level of β-peptide **144** in blood after 8 h, as compared to **167**, is caused by its different structure can not be answered at this point. Metabolic stability of α-peptides is no guarantee of either oral activity or sustained biological activity because absorption barriers (e.g., intestinal, nasal, and buccal) and hepatobiliary excretion mechanisms may severely compromise the therapeutic potential of a peptide or peptidomimetic drug, thereby requiring acute or chronic intravenous administration. It will be of interest to study the differing catabolism of β-peptides that account for the rapidly decreasing β-peptide concentrations in the first 30 minutes and to localize the β-peptide in the animals with radioactively labeled β-peptides ("secretion" or "slow-release"?).

6.2 Inhibition of Cholesterol Uptake by β-Peptides and Synthesis of a Polycationic β-Peptide (168)

6.2.1 Introduction

The excess of lipids in the circulatory system is responsible for widespread diseases such as atherosclerosis [553] and obesity[143] [555,556]. An immense effort is devoted to the reduction of cholesterol blood levels [557].
In 1990, *Hauser* and coworkers reported that the transport of free and esterified cholesterol from either small unilamellar vesicles (SUV) or mixed bile salt micelles (as donor system) to the small-intestinal brush border

[142] For example, angiotensin II, an eight-residue peptide, has a half-life of less than 1 minute after *iv.* administration.

[143] Atherosclerosis [553] and obesity [554] are the leading causes of death in the so-called developed societies.

membranes (BBMV) (as acceptor system) is protein-mediated. This was shown for rabbit [558,559], pig and human BBMV, and for Caco-2 cells[144] [561]. These results challenge the generally accepted view that dietary lipid uptake occurs by the passive diffusion mechanism presented in textbooks [562,563].

Recently, *Hauser et al.* showed that serum apolipoproteins and amphipathic α-helical peptides (amphipathic 18-residue peptides) inhibit sterol uptake by BBMV [564]. Experiments with reconstituted membrane systems further suggest that the sterol transporter is indeed an integral protein of the BBMV [565,566].

A drug designed to inhibit cholesterol uptake in the small intestine could also be effective in lowering triacylglycerol uptake as the very same protein might be responsible for the uptake of several classes of lipids. This would provide a potential treatment for obesity. The first representative of a new class of drugs designed for this purpose was recently introduced (September 1998) in several European countries, including Switzerland[145]. The drug orlistat (tradename Xenical®, Hoffman-La Roche[146]) was designed to inhibit lipase activity in the gastrointestinal tract[147]. However, this drug is based on the idea that hydrolysis is an obligatory step for lipid absorption. *Hauser*'s results show that this view does not hold and explain the limited effectiveness of this drug [569].

By virtue of their metabolic stability [345,346], β-peptides are promising candidates for the inhibition of cholesterol uptake in the small intestine. For this purpose, β-hepta- and β-nonapaptide **168** and **169** were synthesized on the *ortho*-chlorotrityl-chloride resin (*Figure 62*). Both β-peptides are expected to adopt the (*M*)-3_{14} helical conformation.

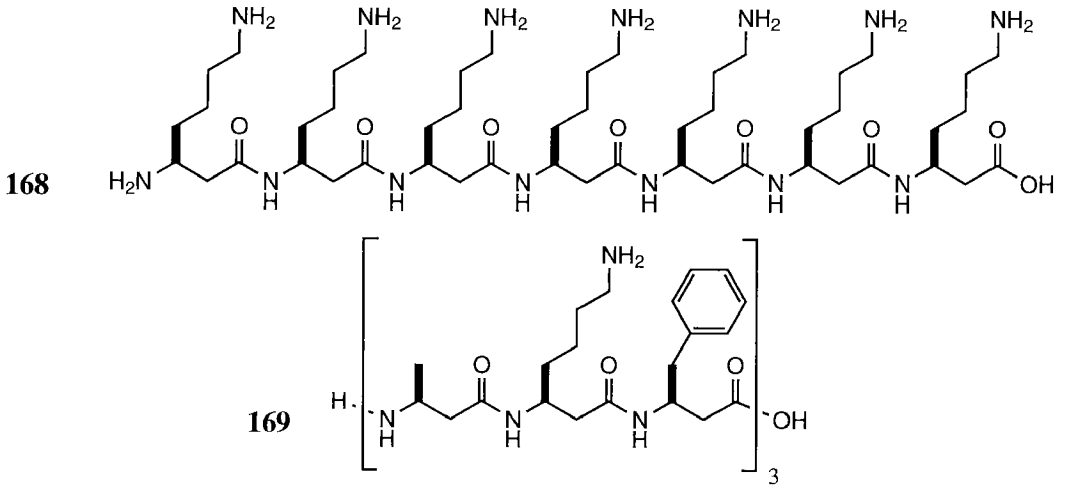
144 The Caco-2 cell line is derived from a human colon adenocarcinoma [560] and resembles the small intestinal enterocytes in morphology and biochemical activity.

145 It was followed in 1999 by sibutramine (tradename reductil®, BASF).

146 A concise description and leading references to Xenical® are compiled in 'Xenical®, orlistat: Produkt Monographie', Roche Pharma (Schweiz) AG, 1998.

147 Xenical® is reported to inhibit triacylglycerol uptake by 30% and to lead to an average weight loss of 4 kg per year [567,568].

Figure 62. β^3-Peptides **168** and **169** synthesized on *ortho*-chlorotrityl-chloride resin.



Compound **168** can be considered as a section of the β-peptide analogue of poly-α-lysine. Poly-α-lysine is known to adopt all of the three major conformations, i.e., α-helix, random coil, and β-sheet, depending on temperature and pH [570-572]. Due to its polycationic nature it has been widely used to explore complexation with anionic partners such as polynucleotides [573], DNA [574-576], bilirubin [577], or acidic proteoglycans such as heparin [578,579]. Moreover, polylysine has been covalently bound to gadolinium complexes [580] to provide high molecular contrast agents for visualization of veins in the body (NMR-imaging [581]).

6.2.2 Synthesis of β-Peptides

The solid phase synthesis of β-peptides **168** and **169** proceeded smoothly (*Table 19*)[148]; the anchoring yields of the first β-amino acids were 57 and 65%, respectively (corresponding to ca. 60 μmol anchored β-amino acid). Deprotection was performed with the DBU/piperidine/DMF mixture (1:1:48). FAB-MS analysis of the major impurities isolated by preparative RP-HPLC revealed that incomplete Fmoc removal reduced the purity of **169**. It seems that this complication may arise during synthesis of β^3-HLys-containing β-peptides exceeding seven residues (cf. *Chapter 5.5.2*). A non-polar gradient had to be used on RP-HPLC purification of the polycationic **168**. Both β-peptides are quite soluble in MeOH and H_2O (ca. 20 mg/ml). β-

[148] The building blocks Fmoc-(*S*)-β^3-HAla-OH and Fmoc-(*S*)-β^3-HPhe-OH were prepared according to [203].

Peptide **169** is a white powder, whereas polycationic **168** was obtained as a colorless highly viscous oil.

Table 19. Isolation and characterization by HPLC and MS of β-peptides **168** and **169** .

β-Peptide	Yield (%)[a)]	Purity[b)] (%)	t_R (min)[c)]	FAB-MS
168	91	79	10.0[d)]	1014.9 ([*M* + 1]$^+$)
169	99	49	11.7[e)]	1184.7 ([*M* + 1]$^+$)

a) % Mass recovered based on polymer loading. b) HPLC purity (220 nm) of the crude product. c) Retention time in the HPLC (linear gradient of *A* (0.1% TFA in H_2O) and *B* (MeCN); see *GP 27* in *Exp. Part.* d) 0-25% *B* in 20 min (C_{18}). e) 15-65% *B* in 20 min (C_{18}).

An elemental analysis was performed with β^3-heptahomolysine in order to determine the amount of CF_3CO_2H (TFA) present after purification and lyophilization. The experimental values correspond to the heptapeptide with eight molecules of TFA. This result is of importance for other β-peptides containing free amino groups[149]. It shows that the free amino groups remain as TFA ammonium salts during lyophilization.

6.2.3 CD Spectroscopy

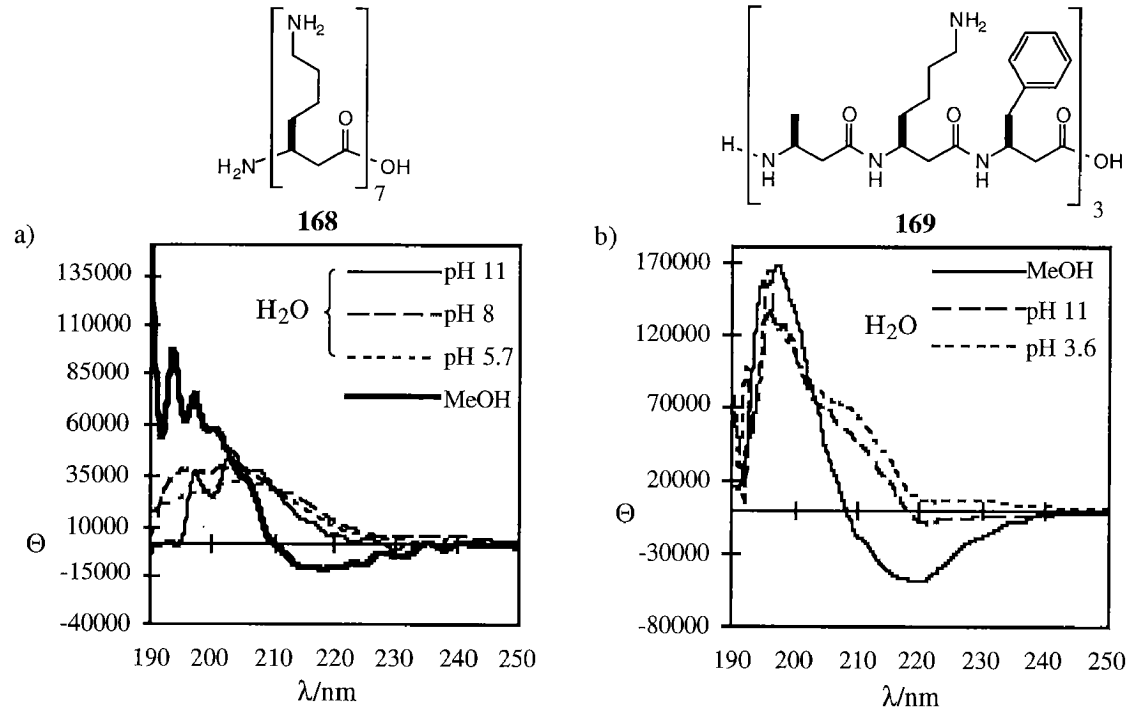
CD spectra of β-peptides **168** and **169** were measured in MeOH and in buffered aqueous solutions at different pH values (*Figure 63*). In MeOH, β-peptide **168** showed the typical CD pattern (positive *Cotton* effect at 200 nm, negative *Cotton* effect at 215-220 nm) that was assigned to the (*M*)-3_{14} helix [27]. However, the *Cotton* effect is very weak ($-1.2 \cdot 10^4$ at 219 nm) suggesting that the large number of neighboring cationic side chains destabilizes the secondary structure. The CD curve of β-nonapeptide **169** in MeOH is much more intense (ca. $+1.7 \cdot 10^5$ at 200 nm; $-4.8 \cdot 10^4$ at 219 nm) indicating that the 3_{14}-helical conformation is more highly populated. In aqueous solution, the molar ellipticity of both β-peptides at 215 nm is only sligthly negative, or even positive, in contrast to the typical spectrum expected for a 3_{14}-helical structure. This may be due to destabilization of the helix caused by partial or total disruption of the H-bonded network[150]. Surprisingly, in contrast to its

149 The molar ellipticity Θ can only be determined accurately from the CD spectra if the exact mass of the sample is known.

150 This effect is well-known for α-helices which are more stable in MeOH and TFE than in H_2O.

α-peptidic analogue [570-572], the CD spectrum of β-peptide **168** did not show any significant pH-dependence.

Figure 63. CD spectra of β^3-peptides **168** (a) and **169** (b). Spectra were recorded at 0.2 mM in MeOH and aqueous buffered solutions at room temperature. Molar ellipticity [Θ] in 10 deg · cm^2 · mol^{-1} (not normalized, as in all other cases described in this thesis).



An interesting feature of β-peptide **169** (and of several other water-soluble β-peptides reported recently [200]) is the occurrence of a positive shoulder at 205-210 nm in aqueous solution. This might reflect either a single new conformation or the co-existence of several conformational isomers.

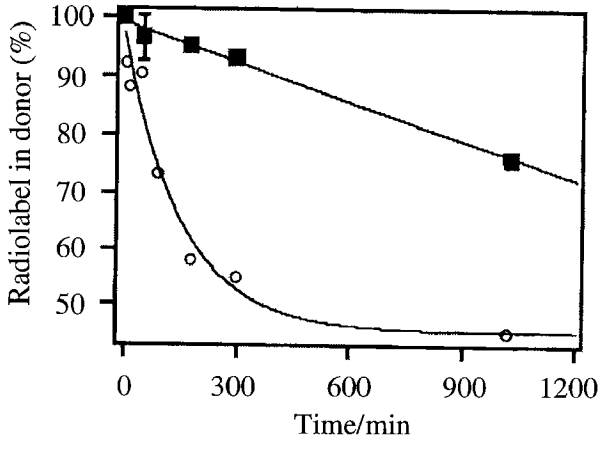
Alternative strategies that could be used to achieve 3_{14}-helix stabilization in aqueous solution would include: *i*) The creation of ion pairs or salt bridges between side chains of two β-amino-acid residues *i* and (*i* + 3) (i.e. side chains of Glu and Lys). *ii*) Since the 3_{14} helix has a resulting dipole moment with the positive pole near the *C*- and the negative near the *N*-terminus [27], the ideal situation would be to insert the negatively charged side chains near the *C*- and the positively charged ones near the *N*-terminus. *iii*) The design of covalent macrocycles (for instance via a disulfide bridge [582]) between *i* and (*i* + 3) side chains. *iv*) The design of water-soluble β-peptides built completely from *like*-$\beta^{2,3}$-amino acids (all-*like*-$\beta^{2,3}$-peptides have very slow

NH/ND exchange kinetics, probably due to the diminished solvent accessibility of the NH protons, see *Chapter 5.2.3*). *v*) The design of water-soluble long-chain β-peptides [203].

6.2.4 Inhibition of Cholesterol Uptake by β-Peptides[151]

Two brush border membrane models were used as acceptors for the study of cholesterol uptake: brush border membrane vesicles (BBMV) and CaCo-2 cells. The former are a simple and commonly used model of the apical part of the plasma membrane of enterocytes. Caco-2 cells are often used to study drug absorption through the small intestinal epithelium. Reconstituted high-density lipoprotein (HDL) or small unilamellar vesicles (SUV) were employed as donors. *Figure 64* shows the typical time dependent decrease of radiolabeled HDL donor measured at the apical side of the CaCo-2-cell layer in the presence and in the absence of β-peptide **169**.

Figure 64. Inhibition of cholesterol uptake by β-peptide **169**. The uptake of free cholesterol from reconstituted HDL consisting of 0.15 mg/ml apo A-I and 0.3 mg/ml total lipid (95 mol% DMPC and 5 mol% radiolabeled free cholesterol) was measured at 37 °C in the presence (■) an in the absence (○) of 1.0 mg/ml of β-peptide **169**. Acceptor: Caco-2 cells. Error bars are not shown for standard deviation < 5%. DMPC: dimyristoylphosphatidylcholine.

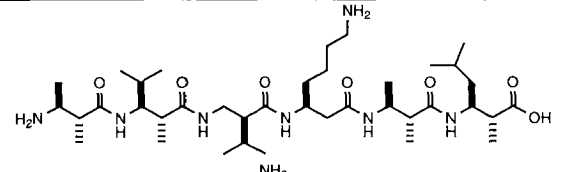
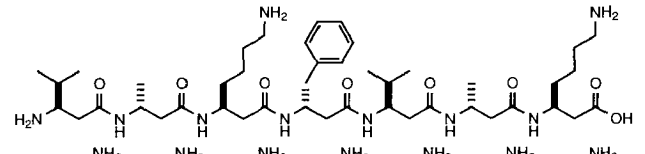
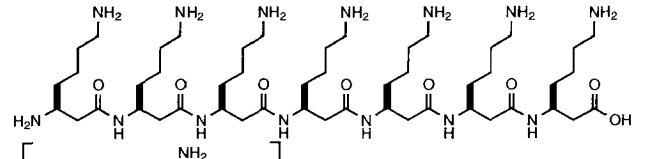
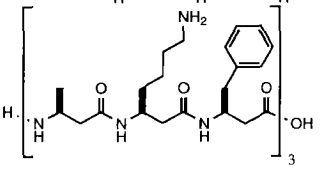


Inhibition is normalized as follows: the rate of sterol uptake of the acceptor measured in the absence of inhibitor was taken as 100%. The loss in activity observed in the presence of a β-peptide is expressed as percent inhibition.

[151] The inhibition experiments were performed by *M. Werder* at ETH-Zürich.

The results of the inhibition experiments (experimental details in [566]) performed with β-peptides **113**, **144**, **168** and **169** are summarized in *Table 20*.

Table 20. Inhibition of cholesterol uptake by various β-peptides. Only β-peptide **169** which forms an *amphipathic* (*M*)-3_{14} helix is active. The β-amino acids around the schematic "helical wheel representations" are abbreviated with single letters (A: β^3-HAla, F: β^3-HPhe, K: β^3-HLys). β-Peptide concentrations were 0.6-1.35 mg/ml.

β-Peptides		Acceptor	Inhibition (%)
113		BBMV	0
144		BBMV	0
168		BBMV	0
169		BBMV Caco-2	70 50

Only β-peptide **169**, which according to its CD spectrum (see *Figure 69*) forms a 3_{14} helix, was effective in inhibiting cholesterol uptake with BBMV and CaCo-2 cells as acceptor. β-Peptide **168** which could form a (*M*)-3_{14} helix was inactive. The difference probably stems from the amphipathic character of the helix of **169**. Inspection of the "helical wheels" of these β-peptides (*Figure 65*) shows that one side is polar (positively charged lysine side chains) whereas the other side is hydrophobic. The presumed 3_{14} helix of **168** is devoid of such an amphipathic character. These results are consistent with recent experiments performed with two 18-residue α-peptides both forming an α-helix, but only one of which was amphipathic; the experiments demonstrated that the amphipathic α-helix is a necessary structural principle underlying the inhibition [564]. β-Peptides **113** and **144** did not inhibit cholesterol uptake at all. Neither β-peptide adopts the 3_{14}-helical structure; β-peptide **113** folds into a hairpin (see *Chapter 5.3.5-7*) and the structure of β-peptide **144** still remains to be established.

These results show that β-peptides can interact with naturally occurring proteins. On the one hand, the α- and β-peptidic worlds appear to be orthogonal in terms of proteolysis but on the other hand, interactions of β-peptides with α-peptides and proteins can conceivably be realized due to the predictability of β-peptidic secondary structures. *Seebach* and coworkers showed that conjugates of α- and β-peptides (containing up to four β-amino acids) bind to a major histocompatibility protein (MHC) [583]. Moreover, a cyclic β-tetrapeptide displayed affinity to human receptors [347]. By virtue of these striking features, we see a bright future for β-peptides in large structures (β-proteins, β-enzymes) and in β-peptidic drugs.

7 Experimental Part

"If you can't stand the heat, get out of the kitchen."
Harry S. Truman

Den Mitarbeitern der analytischen Abteilung des Laboratoriums für Organische Chemie danke ich für ihre Unterstützung: Prof. Dr. *B. Jaun, B. Brandenberger, M. Hemmi, P. Zumbrunnen* (NMR); *M. Schneider, D. Manser* (EA); Dr. *W. Amrein, O. Greter, R. Häfliger, H. U. Hediger* (MS). Mein Dank gilt auch *J. Meienberger* (Bibliothek) und Dr. *E. Zass* (Literaturrecherchen) und allen übrigen Angestellten des Instituts.

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Für die speditive und kritische Durchsicht des Manuskripts bin ich Dr. *Armido Studer* und *Karl Gademann* dankbar.

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Ohne anregende Erholung wäre die Arbeit in diesem Umfang nicht möglich gewesen; in spezieller Weise bin ich meinen Freunden verbunden, die mit mir unzählige sportliche und musikalische Stunden verbracht haben. Mein grösster Dank gilt Isabelle, die mich in allen Belangen unterstützte.

7.1 Abbreviations

AcOEt	Ethyl acetate	IR	Infrared spectroscopy
aq.	Aqueous	LDA	Lithium diisopropylamide
arom.	Aromatic	M	Molecule peak (MS)
Bn	Benzyl	min	Minute(s)
Boc	*tert*-Butoxycarbonyl	m.p.	Melting point
Boc_2O	Di-*tert*-butyl dicarbonate	MS	Mass spectroscopy
BOP	(Benzotriazol-1-yloxy)-tris-(dimethylamino)-phosphonium-hexafluorophosphate	NMM	*N*-Methylmorpholine
b.p.	Boiling point	NMR	Nuclear Magnetic Resonance Spectroscopy
BuLi	Butyllithium	org.	Organic
Bz	Benzoyl	Pd/C	Palladium on charcoal
d	Day(s)	Ph	Phenyl
conc.	Concentrated	prep.	Preparative
DBU	(1,8-Diazabicyclo[5.4.0]undec-7-en	*rac*	Racemic
DCC	1,3-Dicyclohexylcarbodiimide	R_f	Retention factor
DIPA	Diisopropylamine	r.t.	Room temperature
d.r.	Diastereomer ratio	RV	Rotatory evaporator
dist.	Distillation	sat.	Saturated
DMAP	4-(Dimethylamino)pyridine	soln.	Solution
DMF	Dimethylformamide	solv.	Solvent
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydropyrimidin-2(1*H*)-one	temp.	Temperature
equiv.	Equivalent(s)	TFA	Trifluoroacetic acid
e.r.	Enantiomer ratio	TFE	2,2,2-Trifluoroethanol
FC	Flash-chromatography	TLC	Thin layer chromatography
Fmoc	9-Fluorenylmethoxycarbonyl	TNBS	2,4,6-Trinitrobenzolsulfonic acid
Fmoc-OSu	*N*-(9-Fluorenylmethoxycarbonyl-oxy)succinimide	TosOH	*p*-Toluolsulfonic acid
h	Hour(s)	Z	Benzyloxycarbonyl
HFIP	1,1,1,3,3,3-Hexafluoro-2-propanol		
HOBT	1-Hydroxy-1*H*-benzotriazol		
HPLC	High Performance Liquid Chromatography		
HV	High vacuum (0.01-0.1 Torr)		

7.2 Materials and Methods

Solvents: THF and toluene were freshly distilled over Na/benzophenone under Ar before use. $CHCl_3$ employed for the coupling reactions was filtered over basic Al_2O_3 (Alumina *Woelm N*, activity I) to remove EtOH. Solvents for chromatography and workup procedures were distilled from Sikkon (pentane, ethyl acetate, MeOH), P_2O_5 (CH_2Cl_2) and KOH/$FeSO_4$ (Et_2O).

Reagents and methods: Et_3N, $(i\text{-}Pr)_2NH$ and DBU were distilled from CaH_2 and stored under Ar. BnOH was distilled over $CaSO_4$ and stored under Ar. $ClCO_2Et$ was distilled and stored at + 4 °C under Ar. The BuLi employed (ca. 1.6M soln. in hexane) was titrated before use according to the method of *Juaristi et al.* [584]. Alkyl halides were filtrated over Al_2O_3 (Alumina *Woelm* N, activity I) before use. LiCl and LiBr were dried at 150 °C under h.v. for 16 h. *Raney*-Ni was activated according to [266]. Amino acids were purchased from *Senn*. *Ortho*-chlorotrityl-chloride and *Rink* amide resins were purchased from *Novabiochem*. All other reagents were used as received from *Fluka*.

Flasks and stirring bars for the alkylations were dried for ca. 16 h at 120 °C and allowed to cool in a desiccator over silica gel (Blaugel). All reaction were carried out under Ar (*PanGas*). Indicated temperatures were monitored with an internal thermometer (*Ebro TTX 690*).

High pressure reactions were carried out in *Büchi* glass autoclaves or high-grade steel autoclaves (p_{max} 200 bar) in the high pressure laboratory of the ETH.

Thin-layer chromatography: *Merck* silica gel *60* F_{254} or *Macherey-Nagel* Durasil-25 UV_{254} plates; detection with UV and dipping into a soln. of I_2 (30 g I_2, 20 g, KI, 200 ml EtOH, 200 ml H_2O), anisaldehyde (9.2 ml anisaldehyde, 3.75 ml HOAc, 12.5 ml conc. H_2SO_4, 350 ml EtOH), ninhydrine (0.6 g ninhydrine, 2 ml HOAc, 13 ml H_2O, 285 ml butanol), "Mo-stain" (25 g phosphomolybdic acid, 10 g, $Ce(SO_4)_2 \cdot H_2O$, 60 ml conc. H_2SO_4 and 940 ml H_2O), $KMnO_4$-soln. (12 g NaOH, 1.5 g $KMnO_4$, 300 ml H_2O), bromocresol green soln. [585] (0.3% bromocresol green soln. in MeOH/H_2O 20:80 with 24 drops 10% NaOH soln.) or hydroxylamine/$FeCl_3$ reagent [266], followed by heating with a hair dryer.

Flash chromatography: *Fluka* silica gel *60* (40-63 μm) at r.t. with a pressure of *ca.* 0.3 bar. Eluents are indicated.

Melting points were measured in open end glass capillary tubes on a *Büchi 510* apparatus and are uncorrected.

IR Spectra: Measured as film, 1-2% $CHCl_3$-soln. or KBr-pellet on a *Perkin-Elmer-782* spectrophotometer. The maxima are classified in three intensities: *s* (strong), *m* (medium) and *w* (weak) and are reported in cm^{-1}.

NMR Spectra: ^{1}H-NMR spectra were recorded on a *Bruker AMX 500* (500 MHz), *AMX 400* (400 MHz), *ARX 300* (300 MHz), *Varian Gemini 300* (300 MHz), or *Varian Gemini 200* (200 MHz). ^{13}C-NMR spectra were recorded on a *Bruker AMX 500* (125 MHz), *AMX 400* (100 MHz), *Varian Gemini 300* (75 MHz) or *Varian Gemini 200* (50 MHz). Chemical shifts δ in ppm downfield from internal standard Me_4Si (δ = 0 ppm); *J* values in Hz; some compounds show the presence of rotamers: the chemical shifts are reported and the intensities of rotamers is calculated where the signal of rotamers could be assigned unequivocally. The multiplicities were classified by the following symbols: *s* (singulet), *d* (doublet), *t* (triplet), *q* (quadruplet), *m* (multiplet or more overlapping signals), *br.* (broad signal).

Mass spectra: *VG Tribrid* (EI), *ZAB2 SEQ* (FAB, in a 3-nitrobenzyl-alcohol matrix), *Bruker Reflex* (MALDI, matrix is indicated) or *Finnigan MAT TSQ 7000* (ESI) spectrometer; in *m/z* (% of basis peak).

Optical rotations: $[\alpha]_D^{r.t.}$ was measured on a *Perkin-Elmer 241* polarimeter (10 cm, 1 ml cell) at r.t. The solvent and the concentration (in g/100 ml) are indicated.

Circular dichroism (CD) spectra: CD spectra were recorded on a *Jasco J-710* spectropolarimeter from 190 to 250 nm at r.t. in 1-mm rectangular cells. The optical system was flushed with N_2 at a flow rate of ca. 10 l/min. Parameters: band width 1.0 nm, resolution 0.2-1 nm, sensitivity 100 mdeg, response 0.5 s, speed 50 nm/min, 5 accumulations. All spectra were corrected for the corresponding solvent spectrum. Peptide concentration 0.2 mM. The molar ellipticity Θ in 10 $deg \cdot cm^2 \cdot mol^{-1}$ (λ in nm) is calculated for the corresponding peptide (not normalized), taking into account the mass of TFA for each free amino group. Smoothing was done by *Jasco* software. Solvents: MeOH (HPLC grade), TFE (puriss. ≥ 99.5% GC), 1,1,1,3,3,3-hexafluoro-2-propanol (purum >99% GC), aq. buffers: pH 3.6 and 4.6: 0.1M NaOAc/HOAc, pH 5.7 and 8.0: 0.1M NaH_2PO_4/Na_2HPO_4, prepared according to [586]; pH 9.6, 10.0, 11.0: 0.05M $NaHCO_3$/NaOH, pH 12.0: 0.05M Na_2HPO_4/NaOH, prepared according to [587].

UV spectra were measured on a *Uvikon 860 Kontron Instruments* at r.t. in 1-cm rectangular cells. λ_{max} in nm.

Anal. HPLC: *Knauer* HPLC system (pump type *64*, *EuroChrom 2000* integration package, degaser, UV detector (variable-wavelength monitor)), or *Waters* HPLC system (pump type *515*, automated gradient controller type *680*, data module type *746*, tunable absorbance detector type *484*). *Macherey-Nagel* C_8-column (*Nucleosil 100-5 C_8* (250 × 4 mm)) or *Macherey-Nagel* C_{18}-column (*Nucleosil 100-5 C_{18}* (250 × 4 mm)). TFA for anal. HPLC was used as UV grade quality (> 99% GC).

Prep. HPLC: *Knauer* HPLC system (pump type *64*, programmer *50*, UV detector (variable-wavelength monitor)), *Macherey-Nagel* C_8-column (*Nucleosil 100-7 C_8* (250 × 21 mm)) or *Macherey-Nagel* C_{18}-column (*Nucleosil 100-7 C_{18}* (250 × 21 mm)).

Lyophilization: *Hetosicc* cooling condenser with h.v. pump. Solvents are specified.

Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

β-Peptides with free amino groups: All free amino groups form TFA salts, also after lyophilization. These β-peptides are specified without TFA. The molecular mass (MS) corresponds to the β-peptide without TFA.

X-Ray analyes: The crystal structures of compounds **52**, **79a**, **79b**, **79d**, **111**, **130**, **132**, **133**, **138**, **148** and **152** were determined by Dr. *P. Seiler*; Dr. *W. B. Schweizer* provided the structure of **79c** (X-ray service, Laboratorium für Organische Chemie, ETH-Zürich). The structure of compound **53** was determined as part of the requirements in the crystallographic laboratory course of ETH under supervision of Dr. *V. Gramlich*.

Boc-β-HGly-OMe (**73**) was prepared from β-alanine by esterification, followed by Boc-protection in analogy to [258]. Methyl 1-cyanocyclopropane-1-carboxylate (**77a**) was pepared according to [259], and methyl 1-cyanocyclobutane- (**77b**), -cyclopentane- (**77c**) and -cyclohexane-1-carboxylate (**77d**) were synthesized by dialkylation of methyl cyanoacetate, similarly to [52]. *K. Gademann* is thanked for providing the elemental analysis of Boc-(*S*)-Lys(2-Cl-Z)-CHN_2 (**4**). I acknowledge the donation of 170 mg of Z-(*S*)-β^3-HAla-O*t*-Bu (**41**) by *A. Boog* and the generous supply of the following β^2-amino acids by Dr. *G. Guichard*: Fmoc-(*S*)-β^2-HAla-OH, Fmoc-(*S*)-β^2-HVal-OH, Fmoc-(*S*)-β^2-HLeu-OH and Fmoc-(*S*)-β^2-HPhe-OH. The β-dipeptide **148** was prepared by Dr. *M. Oberhoff*.

7.3 Preparation of β-Amino Acids

7.3.1 Preparation of N-Boc-Protected β³-Amino Acid Derivatives

Synthesis of *N*-Boc-Protected Diazo Ketones: General Procedure 1 (*GP 1*). Similar to the reported procedure [85], the *N*-Boc-protected amino acid was dissolved in THF (0.2M) under Ar and cooled to – 20 °C. After addition of $ClCO_2Et$ (1.05 equiv.) and Et_3N (1.05 equiv.), the mixture was stirred at – 20 °C for 20 min. The resulting white suspension was allowed to warm up to – 5 °C and a soln. of CH_2N_2 in Et_2O was added until the rich yellow colour persisted. Stirring was continued for 4 h as the mixture was allowed to warm to r.t. Excess CH_2N_2 was destroyed by the addition of a few drops of HOAc. The mixture was then diluted with Et_2O and washed with sat. NH_4Cl, $NaHCO_3$, and sat. NaCl solns. The org. phase was dried ($MgSO_4$) and concentrated under reduced pressure. FC and/or recrystallization afforded the pure diazo ketone.

Rearrangement of α-Diazoketones to *N*-Boc-protected β³-Amino Acids: General Procedure 2 (*GP 2*). ***GP 2a:*** Similar to the reported procedure [85], the diazoketone was dissolved in THF (0.25M) containing 10% H_2O and then cooled to – 25 °C under Ar with the exclusion of light. A soln. of CF_3CO_2Ag (0.11 equiv.) in Et_3N (2.8 equiv.) was added and the resulting mixture was allowed to warm to r.t. in 4-5 h in the dark. After evaporation of the bulk of THF, the mixture was diluted with sat. aq. $NaHCO_3$ soln. and extracted with Et_2O. The aq. phase was then carefully adjusted to pH 2-3 at 0°C with 1N HCl and extracted with AcOEt. The org. layer was dried ($MgSO_4$) and concentrated under reduced pressure. FC and/or recrystallization afforded the pure *N*-Boc-protected β³-amino acid.

***GP 2b*:** As in *GP 2a*, except that NMM (2.5 equiv.) was used instead of Et_3N.

Rearrangement of α-Diazoketones to *N*-Boc-protected β³-Amino Acid Methyl Ester Derivatives: General Procedure 3 (*GP 3*). Similar to the reported procedure [85], the diazoketone was dissolved in MeOH (0.25M) containing 10% H_2O and then cooled to – 25 °C under Ar with the exclusion of light. A soln. of $PhCO_2Ag$ (0.11 equiv.) in Et_3N (2.8 equiv.) was added and the resulting mixture was allowed to warm to r.t. in 4-5 h in the dark. The solv.

was removed under reduced pressure and the residue was taken up in Et_2O. Workup by extraction with sat. aq. NH_4Cl, $NaHCO_3$ solns., drying ($MgSO_4$), concentration under reduced pressure and FC afforded the pure *N*-Boc-protected β^3-amino acid methyl ester.

Rearrangement of α-Diazoketones to *N*-Boc-protected β^3-Amino Acid Benzyl Ester Derivatives: General Procedure 4 (*GP 4*). Similar to the reported procedure [434], the diazoketone was dissolved in THF (0.25M) containing 15% (v/v) BnOH and then cooled to – 25 °C under Ar with the exclusion of light. A soln. of $PhCO_2Ag$ (0.11 equiv.) in Et_3N (2.8 equiv.) was added and the resulting mixture was allowed to warm to r.t. in 4-5 h in the dark. The mixture was fitrated throgh *Celite*, concentrated under reduced pressure and taken up in AcOEt (0.25M). After workup by extraction with sat. aq. $Na_2S_2O_3$, $NaHCO_3$, NH_4Cl and NaCl solns., drying ($MgSO_4$), excess BnOH was removed by distillation (57 °C, 0.1 Torr). The resulting crude product was purified by FC.

(*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-1-diazo-2-butanone (Boc-(*S*)-Ala-CHN_2; 1). Transformation of Boc-L-Ala-OH (30.3 g, 0.16 mol) according to *GP 1* and FC (AcOEt/pentane 1:2) yielded **1** (17.1 g, 50%). Yellow crystals. Spectroscopic data: corresponding to [588].

(*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-1-diazo-4-methyl-2-pentanone (Boc-(*S*)-Val-CHN_2; 2). Transformation of Boc-L-Val-OH (13.4 g, 61.7 mmol) according to *GP 1* and FC (Et_2O/pentane 1:1) yielded **2** (11.6 g, 79%). Yellow solid. Spectroscopic data: corresponding to [588].

(*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-1-diazo-5-methyl-2-hexanone (Boc-(*S*)-Leu-CHN_2; 3). Transformation of Boc-L-Leu-OH (12.5 g, 50.0 mmol) according to *GP 1* and FC (Et_2O/pentane 1:3) yielded **3** (10.9 g, 85%). Yellowish crystals. Spectroscopic data: corresponding to [589,590].

(*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-7-{[(2-chlor-benzyloxy)carbonyl]amino}-1-diazoheptan-2-one (Boc-(*S*)-Lys(2-Cl-Z)-CHN_2; 4). Boc-L-Lys(2-Cl-Z)-OH (30.0 g, 72.0 mmol) was transformed according to *GP 1*. FC (AcOEt/pentane 2:3) yielded **4** (28.09 g, 89%). Yellow solid. M.p. 72-74 °C. R_f 0.29 (AcOEt/pentane 2:3). $[\alpha]_D^{r.t.}$ = – 18.0 (*c* = 1.03, $CHCl_3$). IR ($CHCl_3$): 3446*w*, 3008*w*, 2960*w*, 2940*w*, 2110*s*, 1800*w*, 1711*s*, 1642*m*, 1500*m*, 1446*w*, 1368*m*,

1248*w*, 1163*w*, 1042*w*, 859*w*, 630*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.36-1.69 (*m*, *t*-Bu, 5 CH); 1.74-1.83 (*m*, CH); 3.17-3.23 (*m*, CH_2); 4.14 (*br.*, NCH), 4.91 (*br.*, NH); 5.21 (br. *s*, CH_2, NH); 5.45 (br. *s*, N_2CH); 7.23-7.29 (*m*, 2 arom. H); 7.35-7.43 (*m*, 2 arom. H). ^{13}C-NMR (76 MHz, $CDCl_3$): 22.33, 28.36, 29.55, 32.10, 40.56, 53.97, 57.34, 64.05, 80.21, 127.11, 129.51, 129.61, 129.77, 130.01, 133.83, 134.57, 155.86, 156.62, 193.48. FAB-MS: 877 (1.7, $[2M]^+$), 439 (23.1, $[M + 1]^+$), 355 (100), 339 (24.7), 311 (27.8), 252 (50.2), 154.1 (23.8), 127 (33.8). Anal. calc. for $C_{20}H_{27}ClN_4O_5$ (438.91): C 54.73, H 6.20, N 12.76; found: C 54.65, H 6.17, N 12.50.

(*R*)-3-{[(*tert*-Butoxy)carbonyl]amino}-1-diazo-2-butanone (Boc-(*R*)-Ala-CHN$_2$; 5). Transformation of Boc-D-Ala-OH (7.57 g, 40.0 mmol) according to *GP 1* and FC (AcOEt/pentane 1:3 → 1:2) yielded **5** (5.17 g, 61%). Yellow crystals. Spectroscopic data: corresponding to *ent*-**5** in [588].

(*R*)-3-{[(*tert*-Butoxy)carbonyl]amino}-1-diazo-4-phenyl-2-butanone (Boc-(*R*)-Phe-CHN$_2$; 6). Transformation of Boc-D-Phe-OH (10.56 g, 40.0 mmol) according to *GP 1* and FC (AcOEt/pentane 1:3) yielded **6** (9.71 g, 84%). Yellow crystals. Spectroscopic data: corresponding to *ent*-**6** in [199,590].

(*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-4-methylpentanoic Acid (Boc-(*S*)-β^3-HVal-OH; 7). Rearrangement of diazoketone **2** (2.44 g, 10.1 mmol) according to *GP 2a*, FC (CH_2Cl_2/MeOH 10:1) and recrystallization (dioxane/hexane/AcOEt) yielded **7** (2.1 g, 88%). White powder. Spectroscopic data: corresponding to [17].

(*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-7-{[(2-chlor-benzyloxy)carbonyl]amino}-heptanoic Acid (Boc-(*S*)-β^3-HLys(2-Cl-Z)-OH; 8). Diazoketone **4** (6.85 g, 15.61 mmol) was transformed according to *GP 2a*. Recrystallization (CH_2Cl_2/pentane) yielded **8** (5.05 g, 75%). White powder. M.p. 72-78 °C. R_f 0.3 (CH_2Cl_2/MeOH 12:1). $[\alpha]_D^{r.t.}$ = − 11.43 (*c* = 1.19, $CHCl_3$). IR ($CHCl_3$): 3448*w*, 3326*w*, 2981*m*, 2941*m*, 2859*w*, 1709*s*, 1597*w*, 1506*s*, 1445*m*, 1393*m*, 1368*m*, 1167*s*, 1039*m*, 867*w*. ^{1}H-NMR (400 MHz, CD_3COCD_3): 1.39 (*s*, *t*-Bu); 2.45 (*dd*, *J* = 15.4, 6.7, COC*H*H); 2.54 (*dd*, *J* = 15.4, 6.0, COCH*H*); 3.14-3.19 (*m*, NCH_2); 3.91 (*br.*, NCH); 5.16 (*s*, $PhCH_2$); 5.86 (*br. s*, NH); 6.43 (*br. s*, NH); 7.32-7.37 (*m*, 2 arom. H); 7.40-7.44 (*m*, 1 arom. H); 7.48-7.50 (*m*, 1 arom. H); 10.74 (*br.*, COOH). ^{13}C-NMR (100 MHz, CD_3COCD_3): 23.92 (CH_2); 28.64 (Me); 30.42, 35.00, 40.13, 41.45 (CH_2); 48.50 (CH); 63.63 (CH_2); 78.60 (C); 127.99, 130.13, 130.20, 130.33 (CH); 133.57, 136.05, 156.22, 156.91, 172.94 (C). FAB-MS: 857 (5.2, $[2M]^+$), 451

(10.4, [*M* + Na]$^+$), 429 (20.8, M^+), 329 (100), 125 (60.0). Anal. calc. for $C_{20}H_{29}ClN_2O_6$ (428.91): C 56.01, H 6.81, N 6.53; found: C 56.02, H 6.79, N 6.36.

(*R*)-3-{[(*tert*-Butoxy)carbonyl]amino}butanoic Acid (Boc-(*R*)-β^3-HAla-OH; 9). Rearrangement of diazoketone **5** (4.77 g, 22.0 mmol) according to *GP 2a* and recrystallization (AcOEt/hexane) yielded 9 (3.37 g, 81%). White powder. Spectroscopic data: corresponding to *ent*-**9** in [588].

(*R*)-3-{[(*tert*-Butoxy)carbonyl]amino}-4-phenylbutanoic Acid (Boc-(*R*)-β^3-HPhe-OH; 10). Rearrangement of diazoketone **6** (9.3 g, 32.0 mmol) according to *GP 2a* and recrystallization (AcOEt/hexane) yielded **10** (7.66 g, 86%). White powder. Spectroscopic data: corresponding to [590,591].

Methyl (*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}butanoate (Boc-(*S*)-β^3-HAla-OMe; 11). Rearrangement of diazoketone **1** (12.65 g, 59.0 mmol) according to *GP 3* and FC (AcOEt/pentane 1:5) yielded **10** (11.25 g, 88%). Colorless oil which solidified upon refrigeration at – 4 °C. Spectroscopic data: corresponding to [592].

Methyl (*R*)-3-{[(*tert*-Butoxy)carbonyl]amino}-4-methylpentanoate (Boc-(*R*)-β^3-HVal-OMe; 12). Rearrangement of diazoketone **2** (8.74 g, 36.2 mmol) according to *GP 3* and FC (AcOEt/pentane 1:7) yielded **12** (7.81 g, 88%). Colorless oil which solidified upon refrigeration at – 4 °C. R_f 0.35 (AcOEt/pentane 1:7). $[\alpha]_D^{r.t.} = -28.8$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3440*m*, 3008*m*, 2972*m*, 2931*m*, 2873*w*, 1731*s*, 1709*s*, 1503*s*, 1439*m*, 1392*m*, 1368*m*, 1168*s*, 1110*m*, 1048*w*, 1019*w*, 858*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*d*, *J* = 6.8, 2 Me); 1.43 (*s*, *t*-Bu); 1.71-1.84 (*m*, 2 Me); 2.45-2.54 (*m*, CH_2CO); 3.68 (*s*, OMe); 3.72-3.79 (*m*, NCH); 4.86 (*bd*, *J* = 8.4, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 18.5, 19.3, 28.4 (Me); 31.8 (CH); 37.2 (CH_2); 51.7 (Me); 53.0 (CH); 79.1, 155.6, 172.4 (C). EI-MS: 246 (< 1, [*M* + 1]+), 202 (20), 190 (12), 172 (9), 158 (16), 146 (55), 130 (15), 116 (13), 102 (100), 74 (3), 57 (9). Anal. calc. for $C_{12}H_{23}NO_4$ (245.32): C 58.75, H 9.45, N 5.71; found: C 58.72, H 9.37, N 5.82.

Methyl (*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-5-methylhexanoate (Boc-(*S*)-β^3-HLeu-OMe; 13). Rearrangement of diazoketone **3** (9.0 g, 35.0 mmol) according to *GP 3* and FC (AcOEt/pentane 1:7) yielded **13** (8.63 g, 95%). Yellowish oil. Spectroscopic data: corresponding to [589,590].

Methyl (*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-7-{[(2-chlor-benzyloxy)carbonyl]-amino}heptanoate (Boc-(*S*)-β³-HLys(2-Cl-Z)-OMe; 14). Rearrangement of diazoketone **4** (14.6 g, 33.3 mmol) according to *GP 3* and recrystallization (CH_2Cl_2/hexane) gave **14** (12.7 g, 86%). White powder. M.p. 77.5-79°C. R_f 0.42 (AcOEt/pentane 1:2). $[\alpha]_D^{r.t.} = -15.5$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3447*w*, 3008*w*, 2945*w*, 2871*w*, 1726*s*, 1713*s*, 1505*s*, 1456*w*, 1439*m*, 1410*w*, 1392*w*, 1369*m*, 1164*m*, 1056*w*, 877*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.35-1.60 (*m*, 3 CH_2, *t*-Bu); 2.46-2.56 (*m*, $COCH_2$); 3.20 (*q*, *J* = 12.9, 6.6, NCH_2); 3.68 (*s*, Me); 3.86-3.92 (*m*, NCH); 4.91 (*br.*, NH); 4.97 (*br. d*, *J* = 8.3, NH); 5.21 (*s*, $PhCH_2$); 7.22-7.28 (*m*, 2 arom. H); 7.25-7.44 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.12 (CH_2); 28.38 (Me); 29.45, 34.18, 39.25, 40.79 (CH_2); 47.28 (CH); 51.67 (Me); 63.86 (CH_2); 79.36 (C); 126.85, 129.30, 129.49, 129.75 (CH); 133.55, 134.41, 155.51, 156.24, 172.08 (C). FAB-MS: 886 (1.8, $[2M]^+$), 443 (12.0, $[M + 1]^+$), 343 (100), 125 (45.2). Anal. calc. for $C_{21}H_{31}ClN_2O_6$ (442.94): C 56.94, H 7.05, N 6.32; found: C 56.89, H 6.96, N 6.29.

Benzyl (*R*)-3-{[(*tert*-Butoxy)carbonyl]amino}-4-methylpentanoate (Boc-(*R*)-β³-HVal-OBn; 15). Rearrangement of diazoketone **2** (6.45 g, 26.7 mmol) according to *GP 4*, FC (Et_2O/pentane 1:4) and recrystallization (pentane) yielded **15** (6.01 g, 70%). Colorless crystals. M.p. 69-70 °C. R_f 0.20 (Et_2O/pentane 1:4). $[\alpha]_D^{r.t.} = -21.5$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3676*w*, 3441*w*, 3007*m*, 2972*m*, 2932*m*, 1708*s*, 1501*s*, 1456*m*, 1392*m*, 1368*m*, 1167*s*, 1107*m*, 1047*m*, 860*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90 (*d*, *J* = 6.78, 2 Me); 1.43 (*s*, *t*-Bu); 1.75-1.84 (*m*, Me_2CH); 2.49-2.59 (*m*, $COCH_2$); 3.73-3.82 (*m*, NCH); 4.86 (*d*, *J* = 9.1, NH); 5.09 (*d*, *J* = 12.3, PhC*H*H); 5.13 (*d*, *J* = 12.3, PhCH*H*); 7.31-7.39 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 18.46, 19.30, 28.38 (Me); 31.81 (CH); 37.29 (CH_2); 53.04 (CH); 66.46 (CH_2); 79.13 (C); 128.26, 128.28, 128.58 (CH); 135.78, 155.51, 171.74 (C). FAB-MS: 665 (10.2, $[2M + Na]^+$), 643 (24.0, $[2M + 1]^+$), 344 (25.0, $[M + Na]^+$), 322 (61.1, $[M + 1]^+$), 266 (100), 222 (83.9). Anal. calc. for $C_{18}H_{27}NO_4$ (321.42): C 67.26, H 8.47, N 4.36; found: C 67.21, H 8.26, N 4.36.

Benzyl (*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-5-methylhexanoate (Boc-(*S*)-β³-HLeu-OBn; 16). Rearrangement of diazoketone **3** (7.57 g, 29.6 mmol) according to *GP 4*, FC (Et_2O/pentane 1:4) yielded **16** (8.03 g, 81%). Colorless waxy solid. $[\alpha]_D^{r.t.} = -31.2$ (c = 1.0, $CHCl_3$). Other spectroscopic data: corresponding to [27].

7.3.2 Preparation of N-Fmoc-Protected β³-Amino Acids

Synthesis of *N*-Fmoc-Protected Diazo Ketones: General Procedure 5 (*GP 5*). Similar to the reported procedure [85,203], the *N*-Fmoc-protected amino acid was dissolved in THF (0.35M) under Ar and cooled to −20 °C. After addition of $ClCO_2Et$ (1.05 equiv.) and NMM (1.05 equiv.), the mixture was stirred at − 20 °C for 20 min. The resulting white suspension was allowed to warm up to − 5 °C and a soln. of CH_2N_2 in Et_2O was added until the rich yellow colour persisted. Stirring was continued for 4 h as the mixture was allowed to warm to r.t. Excess CH_2N_2 was destroyed by vigorous stirring or by the addition of a few drops of HOAc. The mixture was then diluted with Et_2O and washed with sat. $NaHCO_3$, 1N HCl, and sat. NaCl solns. The org. phase was dried ($MgSO_4$) and concentrated under reduced pressure. FC and/or recrystallization afforded the pure diazo ketone.

Rearrangement of α-Diazoketones to *N*-Fmoc-protected β³-Amino Acids: General Procedures 6 (*GP 6*). ***GP 6a:*** Similar to the reported procedures [85], the diazoketone was dissolved in THF (0.25M) containing 10% H_2O and then cooled to − 25 °C under Ar with the exclusion of light. A soln. of CF_3CO_2Ag (0.11 equiv.) in Et_3N (2.8 equiv.) was added and the resulting mixture was allowed to warm to r.t. in 4-5 h in the dark. After removing the bulk of THF under reduced pressure, the mixture was taken up in aq. sat. $NaHCO_3$ soln. and extracted with Et_2O. In the case of poor phase separation, the Et_2O phase was passed through a folded paper filter to remove the voluminous precipitate (Ag-salts). The precipitate was washed with sat. aq. $NaHCO_3$ soln. The combined aq. phase was then carefully adjusted to pH 2-3 at 0 °C with 1N HCl and extracted with AcOEt (3×). The org. phase was dried ($MgSO_4$) and concentrated under reduced pressure. FC and/or recrystallization afforded the pure *N*-Fmoc-protected β³-amino acids.

***GP 6b*:** As in *GP 6a*, except that NMM (2.5 equiv.) was used instead of Et_3N.

(*S*)-1-Diazo-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-methylpentan-2-one (Fmoc-(*S*)-Val-CHN$_2$; 17). Fmoc-L-Val-OH (11.9 g, 35.0 mmol) was transformed according to *GP 5*. FC (AcOEt/pentane 1:4) yielded **17** (7.25 g, 57%). Yellowish crystals. Spectroscopic data: corresponding to [203].

(*S*)-1-Diazo-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-5-methylhexan-2-one (Fmoc-(*S*)-Leu-CHN$_2$; 18). Fmoc-L-Leu-OH (12.6 g, 36.0 mmol) was transformed according to *GP 5*. FC (AcOEt/pentane 1:4) yielded **18** (11.88 g, 87%). Yellow solid. Spectroscopic data: corresponding to [203].

(*S*)-4-(*tert*-Butoxy)-1-diazo-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}butan-2-one (Fmoc-(*S*)-Ser(*t*-Bu)-CHN$_2$; 19). Fmoc-L-Ser(*t*-Bu)-OH (15.0 g, 39 mmol) was transformed according to *GP 5*. FC (AcOEt/pentane 1:3) yielded **19** (14.85 g, 93%). Viscous yellow oil. R_f 0.29 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.} = -12.8$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3432*w*, 3008*w*, 2976*m*, 2877*w*, 2112*s*, 1719*s*, 1638*m*, 1500*s*, 1450*m*, 1365*s*, 1260*m*, 1150*m*, 1059*m*, 1010*m*, 876*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.16 (*s*, *t*-Bu); 3.45 (*dd*, *J* = 5.8, 2.7, OC*H*H); 3.76-3.77 (*m*, OCH*H*); 4.22 (*t*, *J* = 6.6, OCH_2CH); 4.28 (br. *s*, COCH); 4.41-4.56 (*m*, OCH_2CH); 5.39 (*s*, N_2CH); 5.62 (br. *d*, *J* = 7.3, NH); 7.30-7.34 (*m*, 2 arom. H); 7.38-7.43 (*m*, 2 arom. H); 7.51-7.62 (*m*, 2 arom. H); 7.77 (*d*, *J* = 7.6, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 27.3 (Me); 47.3, 54.2, 58.4 (CH); 61.7, 66.8 (CH_2); 73.8 (C); 120.0, 125.0, 125.2, 127.1, 127.7, 127.8 (CH); 141.4, 143.7, 143.8 (C); 156.0, 192.8 (C). FAB-MS: 408 (12.3, $[M + 1]^+$), 380 (18.0), 179 (100), 146 (33.8).

(*S*)-5-[(*tert*-Butoxy)carbonyl]-1-diazo-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]-amino}pentan-2-one (Fmoc-(*S*)-Glu(*t*-Bu)-CHN$_2$; 20). Fmoc-L-Glu(*t*-Bu)-OH·H_2O (17.74 g, 40 mmol) was transformed according to *GP 5*. FC (AcOEt/pentane 1:3) and recrystallization ($CHCl_3$/hexane) yielded **20** (15.75 g, 88%). Yellowish solid. M.p. 138.5-139.5 °C. R_f 0.29 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.} = -25.6$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3425*w*, 3004*w*, 2978*w*, 2112*s*, 1720*s*, 1643*m*, 1506*m*, 1450*m*, 1368*s*, 1248*m*, 1153*s*, 1081*w*, 1041*m*, 844*w*, 650*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.44 (*s*, *t*-Bu); 1.79-1.88 (*m*, CH); 2.08-2.15 (*m*, CH); 2.24-2.41 (*m*, $COCH_2$); 4.20 (*t*, *J* = 6.7, OCH_2CH); 4.26 (br. *s*, COCH); 4.37-4.49 (*m*, OC*H*HCH); 4.45-4.49 (*m*, OCH*H*CH); 5.40 (*s*, N_2CH); 5.61 (*d*, *J* = 7.8, NH); 7.29-7.33 (*m*, 2 arom. H); 7.38-7.42 (*m*, 2 arom. H); 7.52 (*m*, 2 arom. H); 7.76 (*d*, *J* = 7.5, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 27.6 (CH_2); 28.1 (Me); 31.3 (CH_2); 47.3, 54.1, 57.4 (CH); 66.9 (CH_2); 81.0 (C); 120.0, 125.0, 125.1, 127.1, 127.7, 127.8 (CH); 141.4, 143.8 (C); 156.1, 172.3, 193.0 (C). FAB-MS: 450 (26.5, $[M + 1]^+$), 422 (47.1), 366 (15.4), 324 (49.0), 307 (34.6), 289 (23.7), 188 (62.6), 179 (100). Anal. calc. for $C_{25}H_{27}N_3O_5$ (449.51): C 66.80, H 6.05, N 9.35; found C 66.86, H 5.79, N 9.35.

(*S*)-7-{[(*tert*-Butoxy)carbonyl]amino}-1-diazo-3-{[(9*H*-fluoren-9-ylmethoxy)-carbonyl]amino}heptane-2-one (Fmoc-(*S*)-Lys(Boc)-CHN$_2$; 21). Fmoc-L-Lys(Boc)-OH (8.68 g, 18.5 mmol) was transformed according to *GP 5*. FC

(CH_2Cl_2/Et_2O 6:1 → 1:1) yielded **21** (8.08 g, 89%). Yellowish solid. Spectroscopic data: corresponding to [203].

(*S*)-6-{[(*tert*-Butoxy)carbonyl]amino}-1-diazo-3-{[(9*H*-fluoren-9-ylmethoxy)-carbonyl]amino}hexan-2-one (Fmoc-(*S*)-Orn(Boc)-CHN_2; 22). Fmoc-L-Orn(Boc)-OH (5.0 g, 11.0 mmol) was transformed according to *GP 5*. FC (Et_2O/CH_2Cl_2 1:8) and recrystallization (AcOEt/hexane) yielded **22** (3.18 g, 60%). Bright yellow powder. M.p. 115 °C (dec., sintering at 86 °C). R_f 0.09 (Et_2O/CH_2Cl_2 1:8). $[\alpha]_D^{r.t.}$ = – 24.0 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3453*w*, 3007*w*, 2111*m*, 1712*s*, 1641*m*, 1507*s*, 1450*m*, 1391*m*, 1367*s*, 1166*m*, 1044*w*, 867*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.44 (*s*, *t*-Bu); 1.47-1.60 (*m*, 3 CH); 1.81-1.87 (*m*, 1 CH); 3.13-3.16 (*m*, NCH_2); 4.21 (*t*, *J* = 7.0, OCH); 4.27 (br. *s*, NH); 4.39-4.49 (*m*, OCH_2); 4.60 (br. *s*, NH); 5.43 (br. *s*, N_2CH); 5.62 (br. *d*, *J* = 7.0, NH); 7.30-7.34 (*m*, 2 arom. H); 7.38-7.43 (*m*, 2 arom. H); 7.59-7.62 (*m*, 2 arom. H); 7.77 (*d*, *J* = 7.5, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 26.25 (CH_2); 28.42 (Me); 29.56, 39.74 (CH_2); 47.29, 54.02, 57.35 (CH); 66.77 (CH_2); 79.36 (C); 120.0, 124.71, 125.06, 125.13, 127.10, 127.73 (CH); 141.37, 143.76, 156.05, 156.20, 193.37 (C). FAB-MS: 479 (3.2, $[M + 1]^+$), 178 (78.0), 165 (34.3), 132 (100). Anal. calc. for $C_{26}H_{30}N_4O_5$ (478.55): C 65.26, H 6.32, N 11.71; found: C 65.20, H 6.42, N 11.52.

(*S*)-4-[4-(*tert*-Butoxy)phenyl]-1-diazo-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]-amino}butan-2-one (Fmoc-(*S*)-Tyr(*t*-Bu)-CHN_2; 23). Fmoc-L-Tyr(*t*-Bu)-OH (20.0 g, 43.5 mmol) was transformed according to *GP 5*. FC (AcOEt/pentane 1:3) and recrystallization (CH_2Cl_2/hexane) yielded **23** (14.4 g, 69%). Yellow powder. M.p. 120.5-121.5 °C. R_f 0.18 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.}$ = + 3.5 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3426*w*, 3005*w*, 2985*m*, 2113*s*, 1718*s*, 1641*m*, 1503*s*, 1451*m*, 1390*m*, 1364*s*, 1318*m*, 1159*m*, 1108*w*, 1082*w*, 1041*w*, 918*w*, 897*m*, 851*w*, 826*w*. ^{1}H-NMR (400 MHz, CD_3COCD_3, signals of rotamers in italics): 1.25 (*s*, *t*-Bu); 2.86 (*dd*, *J* = 14.0, 9.6, PhC*H*H); 3.14 (*dd*, *J* = 14.0, 4.9, PhCH*H*); 4.16 (*t*, *J* = 6.9, OCH); 4.31 (*d*, *J* = 7.0, OCH_2); 3.32-4.44 (*m*, NCH); 5.86, *6.16* (*s*, N_2CH); 6.80 (*d*, *J* = 8.4, NH); 6.88 (*d*, *J* = 8.4, 2 arom. H); *7.09*, 7.19 (*d*, *J* = 8.3, 2 arom. H); 7.30-7.34 (*m*, 2 arom. H); 7.39-7.43 (*m*, 2 arom. H); 7.65 (*d*, *J* = 7.3, 2 arom. H); 7.85 (*d*, *J* = 8.5, 2 arom. H). ^{13}C-NMR (100 MHz, CD_3COCD_3, signals of rotamers in italics): 29.11 (Me); 37.31, 48.07 (CH_2); 53.71, 60.81, 67.01 (CH); 78.41 (C); 120.79, 124.66, 126.11, 126.14, 127.93, 128.51, 130.61 (CH); 133.12, 142.15, 142.16, 144.92, 145.07, 155.23, *156.74*, 194.59 (C). FAB-MS: 967 (8.6, $2M^+$), 484 (40.6, $[M + 1]^+$), 456 (41.4), 399 (13.5), 222 (83.5), 179 (100), 165 (14.7), 154 (53.1), 136 (34.4). Anal. calc. for $C_{29}H_{29}N_3O_4$ (483.57): C 72.03, H 6.04, N 8.69; found: C 72.11, H 6.17, N 8.58.

(*R*)-1-Diazo-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}butan-2-one (Fmoc-(*R*)-Ala-CHN$_2$; 24). Fmoc-D-Ala-OH·H_2O (13.17 g, 40 mmol) was transformed according to *GP 5*. FC (CH_2Cl_2/Et_2O 20:1) yielded **24** (9.5 g, 71%). Yellowish solid. M.p. 116-117 °C. R_f 0.38 (CH_2Cl_2/Et_2O). $[\alpha]_D^{r.t.}$ = + 42.1 (*c* = 1.0, $CHCl_3$). Other spectroscopic data: corresponding to *ent*-**24** in [203].

(*R*)-1-Diazo-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-phenylbutan-2-one (Fmoc-(*R*)-Phe-CHN$_2$; 25). Fmoc-D-Phe-OH (15.5 g, 40 mmol) was transformed according to *GP 5*. Recrystallization (CH_2Cl_2/pentane) yielded **25** (13.72 g, 83%). Yellowish crystals. M.p. 133-134 °C. R_f 0.33 (AcOEt/pentane). $[\alpha]_D^{r.t.}$ = + 16.5 (*c* = 1.0, $CHCl_3$). Other spectroscopic data: corresponding to *ent*-**25** in [203].

(*R*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-4-methylpentanoic Acid (Fmoc-(*R*)-β^3-HVal-OH; 26). Diazoketone **17** (7.06 g, 19.4 mmol) was transformed according to *GP 6a*. FC (AcOEt/pentane/HOAc 1:3:0.2) and recrystallization ($CHCl_3$/hexane) yielded **26** (5.06 g, 75%). White solid. Spectroscopic data: corresponding to [203].

(*S*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-5-methylhexanoic Acid (Fmoc-(*S*)-β^3-HLeu-OH; 27). Diazoketone **18** (9.44 g, 25.0 mmol) was transformed according to *GP 6a*. FC (CH_2Cl_2/MeOH 20:1) and recrystallization ($CHCl_3$/hexane) yielded **27** (4.60 g, 50%). White solid. Spectroscopic data: corresponding to [203].

(*S*)-4-(*tert*-Butoxy)-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-butanoic Acid (Fmoc-(*R*)-β^3-HSer(*t*-Bu)-OH; 28). *Method A*: Diazoketone **19** (2.0 g, 4.91 mmol) was transformed according to *GP 6a*. Recrystallization (CH_2Cl_2/pentane) yielded **28** (1.23 g, 63%). *Method B*: Diazoketone **19** (4.26 g, 10.4 mmol) was transformed according to *GP 6b*. FC (AcOEt/pentane/AcOH 5:5:0.1) and recrystallization (CH_2Cl_2/pentane) yielded **28** (2.89 g, 70%). White solid. M.p. 96-98 °C. R_f 0.23 (AcOEt/pentane/AcOH 5:5:0.1). $[\alpha]_D^{r.t.}$ = + 15.7 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3435*w*, 2978*m*, 1717*s*, 1509*s*, 1450*m*, 1365*m*, 1082*m*, 872*w*, 620*w*. ^{1}H-NMR (400 MHz, CD_3COCD_3): 1.17 (*s*, *t*-Bu); 2.55 (*dd*, *J* = 15.9, 7.2, COC*H*H); 2.68 (*dd*, *J* = 16.0, 6.1, COCH*H*); 3.38-3.41 (*m*, OC*H*H); 3.48-3.52 (*m*, OCH*H*); 4.06-4.14 (*m*, NCH); 4.21-4.24 (*m*, OCH_2CH); 4.32-4.33 (*m*, OCH_2CH); 6.35 (br. *d*, *J* = 8.4, NH); 7.30-7.34 (*m*, 2 arom. H); 7.39-7.43 (*m*, 2 arom. H); 7.69 (*d*, *J* = 7.3, 2 arom. H); 7.84 (*d*, *J* = 7.3, 2 arom. H). ^{13}C-NMR (100 MHz, CD_3COCD_3): 27.7 (Me); 36.5 (CH_2); 48.1 (CH); 49.6 (CH); 63.7 (CH_2); 66.9

(CH_2); 73.4 (C); 120.8, 126.1, 127.9, 128.5 (CH); 142.1, 145.1, 145.2 (C); 156.5 (C); 172.9 (C). FAB-MS: 795 (14.8, $[2M]^+$), 420 (20.9, $[M + Na]^+$), 398 (100, M^+), 342 (45.7), 178 (62.1). Anal. calc. for $C_{23}H_{27}NO_5$ (397.47): C 69.50, H 6.85, N 3.52; found: C 69.54, H 7.03, N 3.50.

6-(*tert*-Butyl) Hydrogen (*S*)-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-hexanedioate (Fmoc-(*S*)-β³-HGlu(*t*-Bu)-OH; 29). *Method A*: Diazoketone **20** (2.0 g, 4.45 mmol) was transformed according to *GP 6a*. FC (CH_2Cl_2/MeOH) yielded **29** (0.91 g, 47%). *Method B*: Diazoketone **20** (7.0 g, 15.6 mmol) was transformed according to *GP 6b*. FC (CH_2Cl_2/MeOH 10:1) and recrystallization (CH_2Cl_2/pentane) yielded **29** (4.7 g, 71%). White solid. M.p. 58-60 °C. R_f 0.33 (CH_2Cl_2/MeOH 10:1). $[\alpha]_D^{r.t.}$ = – 11.4 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3430*w*, 3008*w*, 2982*w*, 1720*s*, 1510*m*, 1450*w*, 1369*w*, 1248*m*, 1154*m*, 1046*w*, 658*w*, 630*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, signals of rotamers in italics): 1.44 (*s*, *t*-Bu); 1.74-1.88 (*m*, CH_2); 2.16-2.31 (*m*, $COCH_2$); 2.62 (*d*, *J* = 5.1, $COCH_2$); *3.76*, 3.79 (m, NCH); 4.20 (*t*, *J* = 6.8, OCH_2CH); 4.34-4.43 (*m*, OCH_2CH); 5.35, *5.58* (*d*, *J* = 9,0, NH); 7.28-7.32 (*m*, 2 arom. H); 7.36-7.40 (*m*, 2 arom. H); 7.57 (*d*, *J* = 7.5, 2 arom. H); 7.75 (*d*, *J* = 7.5, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 28.1 (Me); 29.1, 32.3, 38.9 (CH_2); 47.3, 47.8 (CH); 66.7 (CH_2); 80.9 (C); 120.0, 125.1, 127.1, 127.7 (CH); 141.3, 143.8, 143.9 (C); 156.0, 172.8, 175.6 (C). FAB-MS: 917 (3.3, $[2M + K]^+$), 902 (0.7, $[2M + Na]^+$), 880 (3.6, $[2M + 1]^+$), 879 (6.5, $[2M]^+$), 478 (1.4, $[M + K]^+$), 462 (7.0, $[M + Na]^+$), 441 (10.1, $[M + 1]^+$), 440 (33.9, M^+), 384 (100), 178 (32.7). Anal. calc. for $C_{25}H_{29}NO_6$ (439.51): C 68.32, H 6.65, N 3.19; found: C 68.28, H 6.73, N 3.15.

(*S*)-7-{[(*tert*-Butoxy)carbonyl]amino}-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-amino}heptanoic Acid (Fmoc-(*S*)-β³-HLys(Boc)-OH; 30). Diazoketone **21** (7.40 g, 15.0 mmol) was transformed according to *GP 6b*. FC (AcOEt/pentane 1:1, 1% HOAc) and recrystallization ($CHCl_3$/hexane) yielded **30** (4.33 g, 60%). White solid. Spectroscopic data: corresponding to [203].

(*S*)-6-{[(*tert*-Butoxy)carbonyl]amino}-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]-amino}hexanoic Acid (Fmoc-(*S*)-β³-HOrn(Boc)-OH; 31). Diazoketone **22** (2.65 g, 5.54 mmol) was transformed according to *GP 6b*. Recrystallization ($CHCl_3$/hexane) yielded **31** (1.63 g, 63%). White powder. M.p. 104 °C (dec.). R_f 0.27 (MeOH/CH_2Cl_2 1:9). $[\alpha]_D^{r.t.}$ = – 8.0 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3436*w*, 3008*w*, 2981*w*, 1710*s*, 1511*m*, 1450*m*, 1406*w*, 1367*w*, 1169*m*, 1107*w*, 1082*w*, 1046*w*, 872*w*. ^{1}H-NMR (400 MHz, CD_3COCD_3; signals of rotamers in italics): 1.40 (*s*, *t*-Bu); 1.52-1.66 (*m*, 2 CH_2); 2.51 (*dd*, *J* = 15.6, 6.5, CHCO); 2.58 (*dd*, *J* =

15.6, 6.6, COCH); 3.09-3.18 (*m*, NCH_2); 3.95-4.05 (*m*, NCH); 4.22 (*t*, *J* = 7.1, OCH); 4.29-4.40 (*m*, OCH_2); *5.58*, 5.95 (*br.*, NH); 6.46 (*d*, *J* = 8.7, NH); 7.30-7.34 (*m*, 2 arom. H); 7.39-7.43 (*m*, 2 arom. H); 7.60-7.70 (*m*, 2 arom. H); 7.86 (*d*, *J* = 7.5, 2 arom. H). ^{13}C-NMR (100 MHz, CD_3COCD_3; signals of rotamers in italics): *14.34*, 28.67 (Me); 23.28, 27.55, 32.58, 40.17, 40.90 (CH_2); 48.16, 49.10 (CH); 66.72 (CH_2); 78.38 (C); 120.79, 126.13, 126.16, 127.93, 128.48 (CH); 142.11, 145.12, 145.23, 156.65, 156.71, 172.79 (C). FAB-MS: 938 (6.2, [2*M* + 1]$^+$), 491 (4.9, [*M* + Na]$^+$), 469 (30.3, [*M* + 1]$^+$), 369 (50.9), 307 (47.0), 289 (24.8), 178 (100). Anal. calc. for $C_{26}H_{32}N_2O_6$ (468.55): C 66.65, H 6.88, N 5.98; found: C 66.53, H 6.79, N 5.84.

(*S*)-4-[4-(*tert*-Butoxy)phenyl]-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-butanoic Acid (Fmoc-(*S*)-β^3-HTyr(*t*-Bu)-OH; 32). Diazoketone **23** (8.0 g, 16.5 mmol) was transformed according to *GP 6a*. FC (CH_2Cl_2/MeOH 15:1 → 6:1) and recrystallization (AcOEt/pentane) yielded **32** (5.81 g, 74%). White powder. M.p. 190-191 °C (dec.). R_f 0.44 (CH_2Cl_2/MeOH 10:1). $[\alpha]_D^{r.t.}$ = – 20.8 (*c* = 0.5, DMF). IR (KBr): 3500-2700*br.*, 1695*s*, 1656*m*, 1606*w*, 1562*m*, 1534*m*, 1506*s*, 1451*m*, 1367*m*, 1262*m*, 1234*s*, 1162*m*, 1106*m*, 1084*m*, 1045*m*, 901*m*, 851*w*, 756*m*, 734*m*, 623*w*, 573*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, signals of rotamers in italics): *1.25*, 1.30 (*s*, *t*-Bu); 2.10-2.95 (*m*, 2 COCH, $PhCH_2$); *3.90*, 4.16-4.20 (*m*, OCH, NCH); 4.30-4.51 (m, OCH_2); 5.25, *5.79* (*br.*, NH); 6.89 (*d*, *J* = 7.5, 2 arom. H); 7.00-7.10 (*m*, 2 arom. H); 7.28-7.31 (*m*, 2 arom. H); 7.36-7.40 (*m*, 2 arom. H); 7.55 (*d*, *J* = 7.4, 2 arom. H); 7.74 (*d*, *J* = 7.5, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$, signals of rotamers in italics): 28.82, *29.70* (Me); 37.37, 39.51 (CH_2); 47.25, *49.25* (CH); 66.72 (CH_2); 78.42, 119.98, 124.26, 125.05, 127.07, 127.70, 129.77, 132.03 (CH); 141.33, 143.87, 154.17, 155.74, 176.10 (C). FAB-MS: 1497 (41.4, [3*M* – 1 + 2 K]$^+$), 985 (8.8, [2*M* + K]$^+$), 512 (17.9, [*M* + K]$^+$), 496 (43.8, [*M* + Na]$^+$), 474 (78.3, [*M* + 1]$^+$), 307 (22.3), 179 (100). Anal. calc. for $C_{29}H_{31}NO_5 \cdot 0.75\ H_2O$ (487.09): C 71.51, H 6.73, N 2.88; found: C 71.54, H 6.81, N 2.92.

(*R*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}butanoic Acid (Fmoc-(*R*)-β^3-HAla-OH; 33). Diazoketone **24** (9.34 g, 27.8 mmol) was transformed according to *GP 6a*. FC (CH_2Cl_2/MeOH 10:1) and recrystallization (CH_2Cl_2/hexane) yielded **33** (6.44 g, 73%). White solid. M.p. 165-166.5 °C (sintering at 125 °C). R_f 0.30 (CH_2Cl_2/MeOH 10:1). $[\alpha]_D^{r.t.}$ = – 7.4 (*c* = 1.0, MeOH). Other spectroscopic data: corresponding to [203].

(*R*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-4-phenylbutanoic Acid (Fmoc-(*R*)-β^3-HPhe-OH; 34). Diazoketone **25** (13.6 g, 33 mmol) was

transformed according to *GP 6a*. Recrystallization (CH_2Cl_2/hexane) yielded **34** (5.19 g, 39%). White solid. M.p. 186-190 °C. R_f 0.36 (CH_2Cl_2/MeOH 10:1). $[\alpha]_D^{r.t.}$ = + 23.9 (*c* = 1.0, $CHCl_3$). Other spectroscopic data: corresponding to [203].

7.3.3 Preparation of like- *and* unlike-$\beta^{2,3}$-*Amino Acid Derivatives*

Synthesis of *unlike*-$\beta^{2,3}$-Amino Acid Methyl Ester Derivatives by Alkylation: General Procedure 7 (*GP 7*). $(i\text{-}Pr)_2NH$ (2.2 equiv.) and DMPU (4 equiv.) was dissolved in THF (0.3M). At – 78 °C, BuLi (2.2 equiv.) was added. After 15 min, a soln. of the Boc-protected (*S*)-β^3-amino acid methyl ester in THF (1M) was added to the clear yellow soln. during 10 min and the mixture stirred for 1 h at – 78 °C. MeI (4 equiv.) was then added slowly (temp. at – 78 °C), and the mixture was stirred for 3-4 h at this temp., subsequently hydrolyzed with sat. NH_4Cl soln., diluted with Et_2O, and extracted with sat. $NaHCO_3$, NH_4Cl and NaCl solns. The org. layer was dried ($MgSO_4$) and evaporated. FC yielded both C(2)-epimers.

Transesterification of β-Amino Acid Derivatives: General Procedure 8 (*GP 8*). The appropriate methyl ester was dissolved in BnOH (0.5M). A soln. of $Ti(OBn)_4$ in BnOH (0.7-4 equiv., 0.58M) and molecular sieves (4 Å) was added. This mixture was heated at 95 °C for 40-60 h (NMR control). After filtration over *Celite* and dilution with Et_2O the org. phase was washed thoroughly with aq. KF (pH 1), sat. aq. $NaHCO_3$ and NaCl solns. and then dried ($MgSO_4$). The solvent was removed at RV and excess BnOH was removed by bulb-to-bulb dist. (100 °C, 0.1 Torr). The resulting crude product was purified by FC.

Benzyl Ester Deprotection: General Procedure 9 (*GP 9*). The benzyl ester was dissolved in the appropriate solvent (0.1M) and *ca.* 10% (m/m) Pd/C (10%) was added. The apparatus was evacuated and flushed with H_2 (3×) and the mixture was stirred under an atmosphere of H_2 (1 bar) for 18 h. Subsequent filtration through *Celite* and concentration under reduced pressure yielded the crude carboxylic acid which was further purified by FC and/or recrystallization.

Boc-Deprotection: General Procedures 10 (*GP 10*). ***GP 10a:*** Similarly to the reported procedure [17], the Boc-protected amino acid was dissolved in

CH_2Cl_2 (0.5M) and cooled to 0 °C. An equal volume of TFA was added and the mixture was allowed to slowly warm to r.t. and then stirred for further 1.5 h. Concentration under reduced pressure and drying of the residue under h.v. yielded the crude TFA salt, which was identified by NMR and MS and used without further purification.

GP 10b: The Boc-protected amino acid was dissolved in cold TFA (4 °C) (0.15M). After stirring at r.t. for 2 h, concentration under reduced pressure, coevaporation with CH_2Cl_2 (2×) and drying under h.v. yielded the crude TFA salt,which was identified by NMR and MS and used without further purification.

***N*-Fmoc-Protection of $\beta^{2,3}$-Amino Acids: General Procedure 11 (*GP 11*).** A stirred soln. of the TFA salt of the β-amino acid in 0.6M aq. Na_2CO_3 (3 equiv.) was treated with a soln. of Fmoc-OSu (1.1 equiv.) in acetone (0.2M). If necessary, the pH was readjusted to 9-10 with additional Na_2CO_3. After 5 h, the mixture was diluted with H_2O and extracted with Et_2O. The aq. phase was carefully adjusted to pH 1-2 at 0 °C with 1N HCl and extracted with AcOEt (3×). The org. layer was washed with H_2O, dried ($MgSO_4$) and concentrated under reduced pressure. FC and/or recrystallization afforded the pure *N*-Fmoc-protected $\beta^{2,3}$-amino acids.

Methyl (2*S*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2-methylbutanoate (Boc-(2*S*,3*S*)-$\beta^{2,3}$-HAla(α-Me)-OMe; 35) and **Methyl (2*R*,3*S*)-3-{[(*tert*-Butoxy)-carbonyl]amino}-2-methylbutanoate (Boc-(2*R*,3*S*)-$\beta^{2,3}$-HAla(α-Me)-OMe; 36).** Methyl ester **11** (5.04 g, 23.0 mmol) was alkylated as described in [18]. FC (Et_2O/pentane 1:4) yielded the two unseparable C(2)-epimers **35** and **36** (5.15 g, 97%). Colorless oil; **35** : **36** = 2 : 1 as determined by ^{1}H-NMR spectroscopy. Separation by prep. HPLC (21 injections of *ca.* 50 mg epimer mixture, eluent: Et_2O/hexane 2:8, Silicagel 20×250 mm column, flow 10 ml/min, detection by RID) yielded **35** (0.54 g, 10%) and **36** (0.565 g, 11%).

Data of **35**: Colorless needles. M.p. 54-55 °C ([18]: 49-50 °C). R_f 0.46 (Et_2O/pentane 1:3, 2×). $[\alpha]_D^{r.t.} = -9.8$ (c = 1.0, $CHCl_3$) ([18]: $[\alpha]_D^{r.t.} = -5.2$ (c = 1.0, $CHCl_3$)). ^{1}H-NMR (400 MHz, $CDCl_3$): 1.13 (*d*, *J* = 6.8, Me); 1.19 (*d*, *J* = 7.2, Me); 1.44 (*s*, *t*-Bu); 2.60-2.71 (*m*, COCH); 3.69 (*s*, OMe); 3.70-3.87 (*m*, NCH); 5.06 (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.09, 19.26, 28.42 (Me); 44.16, 48.28 (CH); 51.64 (Me); 79.15, 155.57, 175.64 (C). Anal. calc. for $C_{11}H_{21}NO_4$ (231.29): C 57.12, H 9.15, N 6.06; found: C 57.09, H 9.28, N 6.01.

Data of **36**: Colorless oil that solidifies at 4 °C to give a colorless waxy solid. R_f 0.40 (Et_2O/pentane 1:3, 2×). $[\alpha]_D^{r.t.} = -26.6$ (c = 1.0, $CHCl_3$) ([18]: $[\alpha]_D^{r.t.} = -37.3$ (c

= 1.0, $CHCl_3$)). ^{1}H-NMR (400 MHz, $CDCl_3$): 1.12 (*d*, *J* = 6.8, Me); 1.16 (*d*, *J* = 7.2, Me); 1.44 (*s*, *t*-Bu); 2.62-2.65 (*m*, COCH); 3.70 (*s*, OMe); 3.80-3.91 (*m*, NCH); 4.86 (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 13.55, 17.41, 28.41 (Me); 44.56, 48.48 (CH); 51.68 (Me); 79.27, 155.19, 174.82 (C). Anal. calc. for $C_{11}H_{21}NO_4$ (231.29): C 57.12, H 9.15, N 6.06; found: C 57.25, H 9.28, N 5.86.

Methyl (2*S*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,4-dimethylpentanoate (Boc-(2*S*,3*S*)-β^{2,3}-HVal(α–Me)-OMe; 37). LiBr (0.4 g, 4.59 mmol) was suspended in THF (5 ml). After cooling to – 78 °C, $(i\text{-}Pr)_2NH$ (0.48 ml, 3.37 mmol) and BuLi (2.25 ml, 3.37 mmol) were added. After 15 min, a soln. of **12** (0.375 g, 1.53 mmol) in THF (5 ml) was added during 10 min (clear soln.) and the mixture stirred for 2 h at – 78 °C. MeI (0.38 ml, 6.12 mmol) was then added slowly (temp. at – 78 °C), and the mixture was stirred for 4 h at this temp., subsequently hydrolyzed with sat. NH_4Cl soln., diluted with Et_2O, and extracted with sat. $NaHCO_3$, NH_4Cl and NaCl solns. The org. layer was dried ($MgSO_4$) and evaporated. FC (Et_2O/pentane 2:7) yielded the diastereomer **37** as a colorless oil (0.136 g, 34%), the diastereomer **38** as a colorless solid (0.22 g, 55%).

Data of **37**: R_f 0.23 (Et_2O/pentane 2:7). $[\alpha]_D^{r.t.}$ = – 34.9 (*c* = 1.0, $CHCl_3$). IR (film): 3436*w*, 2968*s*, 2876*w*, 1720*s*, 1503*s*, 1461*m*, 1390*m*, 1366*m*, 1307*m*, 1237*m*, 1170*s*, 1100*m*, 1074*m*, 1041*m*, 985*w*, 914*w*, 870*w*, 831*w*, 759*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*t*, *J* = 6.5, 2 Me); 1.21 (*d*, *J* = 7.1, Me); 1.44 (*s*, 8.1 H, *t*-Bu, rotamer); 1.45 (*s*, 0.9 H, *t*-Bu, rotamer); 1.62-1.72 (*m*, Me_2CH); 2.71-2.82 (*m*, COCH); 3.38-3.49 (*m*, NCH); 3.67 (*s*, OMe); 4.80 (*d*, *J* = 10.5, 0.1 H, NH, rotamer); 5.22 (*d*, *J* = 10.5, 0.9 H, NH, rotamer). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.7, 19.1, 19.9, 28.4 (Me); 31.8, 40.6 (CH); 51.6 (Me); 58.6 (CH); 78.8, 156.4, 176.2 (C). EI-MS: 260 (13, [*M* + 1]1$^+$), 246 (1), 216 (33), 204 (64), 186 (12), 172 (21), 160 (77), 130 (10), 116 (100), 84 (7), 72 (9), 57 (25), 41 (4). Anal. calc. for $C_{13}H_{25}NO_4$ (259.34): C 60.21, H 9.72, N 5.40; found: C 60.07, H 9.90, N 5.58.

Methyl (2*R*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,4-dimethyl-pentanoate (Boc-(2*R*,3*S*)-β^{2,3}-HVal(α–Me)-OMe; 38). Methyl ester **12** (5.0 g, 20.4 mmol) was alkylated according to *GP 7*. FC (Et_2O/pentane 1:5) yielded the major diastereomer **38** as a colorless solid (2.99 g, 58%), the minor diastereomer **37** as a colorless oil (1.11 g, 21%) and mixed fractions (0.58 g, 11%). Total yield: 4.68 g (90%).

Data of **38**: M.p. 47-50 °C. R_f 0.12 (Et_2O/pentane 2:7). $[\alpha]_D^{r.t.}$ = – 16.3 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3446*w*, 2970*m*, 1723*s*, 1713*s*, 1503*s*, 1456*m*, 1436*w*, 1392*m*, 1365*s*, 1179*s*, 1063*w*, 991*w*, 865*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.89 (*d*, *J* = 6.8,

Me); 0.94 (*d*, *J* = 6.7, Me); 1.12 (*d*, *J* = 7.0, Me); 1.43 (*s*, 7.6 H, *t*-Bu, rotamer); 1.47 (*s*, 1.4 H, *t*-Bu, rotamer); 1.63-1.71 (*m*, Me_2CH); 2.54-2.66 (*m*, COCH); 3.67 (*s*, OMe); 3.76-3.85 (*m*, NCH); 4.38 (*d*, *J* = 10.4, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.2, 17.5, 20.2, 28.4 (Me); 30.4, 42.3 (CH); 51.8 (Me); 57.4 (CH); 79.2, 155.9, 175.3 (C). EI-MS: 260 (7, $[M + 1]^+$), 216 (42), 204 (34), 186 (12), 172 (29), 160 (71), 130 (11) 116 (100), 72 (23), 57 (57), 41 (9). Anal. calc. for $C_{13}H_{25}NO_4$ (259.34): C 60.21, H 9.72, N 5.40; found: C 60.01, H 9.82, N 5.38.

Methyl (2*S*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,5-dimethylhexanoate (Boc-(2*S*,3*S*)-β^{2,3}-HLeu(α–Me)-OMe; 39). LiCl (0.29 g, 6.87 mmol) was suspended in THF (7 ml). After cooling to – 78 °C, $(i\text{-}Pr)_2NH$ (0.72 ml, 5.04 mmol) and BuLi (3.36 ml, 5.04 mmol) were added. After 15 min, a soln. of **13** (0.593 g, 2.29 mmol) in THF (5 ml) was added during 20 min (clear soln.) and the mixture stirred for 1 h at – 78 °C. MeI (0.57 ml, 9.16 mmol) was then added slowly (temp. at – 78 °C), and the mixture was stirred for 18 h allowing to reach – 56 °C, subsequently hydrolyzed with sat. NH_4Cl soln., diluted with Et_2O, and extracted with sat. $NaHCO_3$, NH_4Cl and NaCl solns. The org. layer was dried ($MgSO_4$) and evaporated. FC (AcOEt/pentane 1:5) yielded the major diastereomer **39** as a colorless oil (0.348 g, 56%), the minor diastereomer **40** as a colorless waxy solid (0.117 g, 19%).
Data of **39**: R_f 0.49 (AcOEt/pentane 1:5). $[\alpha]_D^{r.t.}$ = – 43.0 (*c* = 1.0, $CHCl_3$). IR (film): 3369*m*, 2956*s*, 2871*m*, 1740*s*, 1702*s*, 1523*s*, 1456*m*, 1436*m*, 1390*m*, 1366*s*, 1324*m*, 1301*m*, 1251*s*, 1174*s*, 1102*m*, 1072*m*, 1038*m*, 1023*m*, 999*m*, 948*w*, 914*w*, 873*w*, 757*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.91 (*t*, *J* = 6.0, 2 Me); 1.13-1.21 (*m*, CH, Me); 1.31-1.38 (*m*, CH); 1.44 (*s*, 8 H, *t*-Bu, rotamer); 1.47 (*s*, 1 H, *t*-Bu, rotamer); 1.60-1.70 (*m*, CH); 2.59-2.69 (*m*, COCH); 3.68 (*s*, OMe); 3.70-3.87 (*m*, NCH); 4.70 (*d*, *J* = 9.8, 0.1 H, NH, rotamer); 5.02 (*d*, *J* = 9.8, 0.9 H, NH, rotamer). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.3, 22.1, 23.1 (Me); 24.9 (CH); 28.4 (Me); 43.0 (CH_2); 43.3, 50.7 (CH); 51.5 (Me); 78.9, 155.9, 175.8 (C). EI-MS: 273 (1, M^+), 216 (15), 200 (15), 186 (38), 172 (5), 160 (41), 144 (34), 130 (74), 116 (99), 100 (15), 86 (100), 70 (6), 57 (21). Anal. calc. for $C_{14}H_{27}NO_4$ (273.37): C 61.51, H 9.95, N 5.12; found: C 61.66, H 9.74, N 5.08.

Methyl (2*R*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,5-dimethylhexanoate (Boc-(2*R*,3*S*)-β^{2,3}-HLeu(α–Me)-OMe; 40). Methyl ester **13** (4.6 g, 17.7 mmol)was alkylated according to *GP 7*. FC (Et_2O/pentane 1:6) yielded the major diastereomer **40** as a colorless solid (2.72 g, 57%), the minor diastereomer **39** as a colorless oil (1.29 g, 27%). Total yield: 4.01 g (84%).

Data of **40**: M.p. 41-43 °C. R_f 0.40 (AcOEt/pentane 1:5). $[\alpha]_D^{r.t.} = -46.1$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3443*w*, 2958*m*, 1708*s*, 1503*s*, 1456*m*, 1436*w*, 1392*m*, 1368*s*, 1171*s*, 1101*w*, 909*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.91 (2 *s*, J = 6.7, 6.5, 2 Me); 1.14 (*d*, J = 7.2, Me); 1.18-1.35 (*m*, CH_2); 1.43 (*s*, *t*-Bu); 1.59-1.68 (Me_2CH); 2.50-2.64 (*m*, COCH); 3.69 (*s*, OMe); 3.87-3.94 (*m*, NCH); 4.62 (*d*, J = 9.5, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.9, 21.7, 23.5 (Me); 25.0 (CH); 28.4 (Me); 41.2 (CH_2); 44.3, 50.8 (CH); 51.7 (Me); 79.2, 155.5, 175.1 (C). EI-MS: 274 (3, $[M + 1]^+$), 218 (31), 200 (10), 186 (37), 174 (23), 160 (19), 144 (21), 130 (100), 116 (51), 97 (13), 88 (30), 86 (97), 57 (42). Anal. calc. for $C_{14}H_{27}NO_4$ (273.37): C 61.51, H 9.95, N 5.12; found: C 61.47, H 10.02, N 5.23.

***tert*-Butyl (2*S*,3*S*)-3-{[(Benzyloxy)carbonyl]amino}-2-methylbutanoate (Z-(2*S*,3*S*)-β^{2,3}-HAla(α-Me)-O*t*-Bu; 42).** Compound **41** (0.17 g, 0.58 mmol) was alkylated according to *GP 7*, except that DMPU was omitted. FC (Et_2O/pentane 1:4) yielded one C(2)-epimer **42** (50 mg, 28%). The other C(2)-epimer with lower R_f was present < 5% (according to ^{1}H-NMR) was not characterized. White waxy solid. R_f 0.22 (Et_2O/pentane 1:4). $[\alpha]_D^{r.t.} = -0.70$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3427*w*, 2975*m*, 2934*w*, 2883*w*, 1713*s*, 1508*s*, 1452*m*, 1385*w*, 1370*m*, 1154*s*, 1108*m*, 1088*m*, 1021*w*, 995*w*, 847*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.16 (*d*, J = 7.1, Me); 1.17 (*d*, J = 6.7, Me); 1.44 (*s*, *t*-Bu); 2.46-2.52 (*m*, COCH); 3.83-3.94 (*m*, NCH); 5.10 (*s*, $PhCH_2$); 5.51 (*d*, J = 8.3, NH); 7.30-7.38 (*m*, 5 arom. H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.37, 19.36, 27.93 (Me); 44.75, 49.07 (CH); 66.43 (CH_2); 80.94 (C); 128.01, 128.52 (CH); 136.77, 156.10, 174.70 (C). EI-MS: 308 (23.7, $[M + 1]^+$), 251 (100), 234 (37.8), 91 (69.8). Anal. calc. for $C_{17}H_{25}NO_4$ (307.39): C 66.43, H 8.20, N 4.56; found: C 66.46, H 8.42, N 4.48.

Methyl (2*S*,3*S*)-3-[(4-nitro-benzoyl)amino]-2,5-dimethyl-hexanoate (4-NO_2-Bz-(*S*,*S*)-β^{2,3}-HLeu(α-Me)-OMe; 43). Compound **39** (93 mg, 0.34 mmol) was dissolved in sat. HCl/dioxane (2 ml) and stirred at r.t. overnight. Concentration under reduced pressure and drying under h.v. yielded the crude HCl salt (71 mg, quant.), that was dissolved in CH_2Cl_2 (3 ml) and treated with Et_3N (0.1 ml, 0.68 mmol) and a soln. of p-nitrobenzoylchloride (0.126 g, 0.68 mmol) in CH_2Cl_2 (1 ml) at r.t. After 1 h, the yellow soln. was washed with sat. aq. NH_4Cl soln. (2×), dried ($MgSO_4$) and evaporated at RV. 2× FC (AcOEt/pentane 1:4) yielded **43** (21 mg, 19%). Colorless needles after recrystallization (pentane). M.p. 90-91 °C. R_f 0.47 (AcOEt/pentane 1:4). $[\alpha]_D^{r.t.} = -34.4$ (c = 0.76, $CHCl_3$). IR ($CHCl_3$): 3610*w*, 3415*w*, 3005*w*, 2964*m*, 2923*s*, 2851*m*, 1723*m*, 1667*s*, 1605*w*, 1528*s*, 1487*m*, 1462*m*, 1385*w*, 1349*s*, 1174*m*, 1046*w*, 872*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.93 (*d*, J = 6.7, Me); 0.98 (*d*, J = 6.5,

Me); 1.27 (*d*, *J* = 7.3, Me); 1.31-1.36 (*m*, CH); 1.50-1.56 (*m*, CH); 1.60-1.72 (*m*, Me_2CH); 2.78 (*dq*, *J* = 7.2, 3.2, COCH); 3.75 (*s*, OMe); 4.36-4.43 (*m*, NCH); 7.28 (*d*, *J* = 11.0, NH); 7.97-8.00 (*m*, 2 arom. H); 8.28-8.32 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.75, 22.26, 23.02 (Me); 25.16, 42.72 (CH); 43.62 (CH_2); 50.29 (CH); 51.94 (Me); 123.86, 128.12 (CH); 140.18, 149.64, 165.04, 176.97 (C). EI-MS: 323 (100, $[M + 1]^+$), 291 (19.3), 265 (83.1), 235 (86.4), 150 (82.2), 84 (15.6), 49 (21.5).

Benzyl (2*S*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,4-dimethylpentanoate (Boc-(2*S*,3*S*)-β^{2,3}-HVal(α–Me)-OBn; 54). Methyl ester **37** (0.90 g, 3.47 mmol) was transesterified with $Ti(OBn)_4$ (0.7 equiv.) for 40 h according to *GP 8*. FC (Et_2O/pentane 1:5) yielded **54** (0.93 g, 80%). Colorless oil. R_f 0.32 (Et_2O/pentane 1:5). $[\alpha]_D^{r.t.} = -30.5$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3435*w*, 2974*m*, 2933*m*, 2875*w*, 1720*s*, 1707*s*, 1502*s*, 1455*m*, 1392*m*, 1368*m*, 1165*s*, 1019*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90 (*t*, *J* = 6.9, 2 Me); 1.23 (*d*, *J* = 7.1, Me); 1.43 (*s*, *t*-Bu); 1.59-1.70 (*m*, CH); 2.73 (*m*, 0.1 H, COCH, rotamer); 2.80-2.87 (*m*, 0.9 H, COCH, rotamer); 3.33-3.39 (*m*, 0.1 H, NCH, rotamer); 3.40-3.44 (*m*, 0.9 H, NCH, rotamer); 4.84 (*d*, *J* = 10.6, 0.1 H, NH, rotamer); 5.09 (*d*, *J* = 12.4, 1 H, $PhCH_2$); 5.13 (*d*, *J* = 12.4, 1 H, $PhCH_2$); 5.24 (*d*, *J* = 10.4, 0.9 H, NH, rotamer); 7.30-7.39 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.70, 19.17, 19.94, 28.42 (Me); 31.85, 40.60, 58.69 (CH); 66.30 (CH_2); 78.79 (C); 128.10, 128.31, 128.61 (CH); 135.79, 156.39, 175.61 (C). EI-MS: 336 (3, $[M + 1]^+$), 326 (2), 292 (14), 262 (5), 248 (2), 236 (48), 186 (1), 172 (6), 116 (33), 91 (100), 83 (4), 72 (9), 57 (12). Anal. calc. for $C_{19}H_{29}NO_4$ (335.44): C 68.03, H 8.71, N 4.18; found: C 68.18, H 8.55, N 4.14.

Benzyl (2*S*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,5-dimethylhexanoate (Boc-(2*S*,3*S*)-β^{2,3}-HLeu(α-Me)-OBn; 55). Methyl ester **39** (59 mg, 0.21 mmol) was transesterified with of $Ti(OBn)_4$ (4 equiv.) for 4 d according to *GP 8*. FC (Et_2O/pentane 1:6 to 1:3) and recrystallization (pentane) yielded **55** (51 mg, 69%). Colorless solid. M.p. 43-44 °C. R_f 0.22 (Et_2O/pentane 1:6). $[\alpha]_D^{r.t.} = -24.9$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3436*w*, 2961*m*, 1706*s*, 1503*s*, 1456*m*, 1392*m*, 1367*m*, 1164*s*, 1116*w*, 1028*w*, 626*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86 (*d*, *J* = 6.7, 2 Me); 1.09-1.18 (*m*, CH); 1.21 (*d*, *J* = 7.2, Me); 1.26-1.38 (*m*, CH); 1.43 (*s*, *t*-Bu); 1.58-1.65 (*m*, CH); 2.59-2.73 (*m*, COCH); 3.74-3.88 (*m*, NCH); 5.02 (*d*, *J* = 10.0, NH); 5.08 (*d*, *J* = 12.3, PhC*H*H); 5.16 (*d*, *J* = 12.3, PhCH*H*). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.4, 22.1, 23.0 (Me); 24.9 (CH); 28.4 (Me); 43.0 (CH_2); 43.2, 50.7 (CH); 66.2 (CH_2); 78.9 (C); 128.2, 128.3, 128.6 (CH); 135.9, 155.9, 175.2 (C). EI-MS: 340 (<1, $[M + 1]^+$), 292 (2), 276 (1), 250 (2), 236 (7), 192 (24), 169 (4), 158 (2), 144 (5), 130 (46), 107 (13), 91 (100), 57 (8). Anal. calc. for $C_{20}H_{31}NO_4$ (349.47): C 68.74, H 8.94, N 4.01; found: C 68.65, H 8.83, N 3.97.

(2*S*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,4-dimethylpentanoic Acid (Boc-(2*S*,3*S*)-β^{2,3}-HVal(α-Me)-OH; 56). Benzyl ester **54** (0.476 g, 1.42 mmol) was debenzylated in MeOH according to *GP 9*. **56** (0.347 g, 99%). Colorless needles. M.p. 88-90 °C. R_f 0.42 ($CHCl_3$/MeOH 9:1). $[\alpha]_D^{r.t.}$ = – 19.0 (*c* = 1.0, MeOH). IR ($CHCl_3$): 3438*w*, 2981*m*, 2926*w*, 2873*w*, 2672*w*, 1706*s*, 1502*m*, 1461*w*, 1413*w*, 1392*m*, 1368*m*, 1306*w*, 1172*m*, 974*w*, 897*w*, 868*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.88 (*d*, *J* = 6.7, Me); 0.94 (*d*, *J* = 6.7, Me); 1.16 (*d*, *J* = 7.1, Me); 1.44 (*s*, *t*-Bu); 1.72-1.82 (*m*, CH); 2.63-2.74 (*m*, COCH); 3.38-3.43 (*m*, NCH). ^{13}C-NMR (100 MHz, CD_3OD): 15.82, 18.75, 20.50, 28.80 (Me); 32.11, 42.45, 59.73 (CH); 79.99, 158.51, 179.11 (C). FAB-MS: 513 (6, [2*M* + Na]$^+$), 491 (33, [2*M* + 1]$^+$), 268 (12), 246 (38, [*M* + 1]$^+$), 202 (6), 190 (100), 172 (33), 154 (11), 146 (27), 136 (12), 116 (19), 101 (10), 91 (15).

(2*S*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,5-dimethyl-hexanoic Acid (Boc-(2*S*,3*S*)-β^{2,3}-HLeu(α-Me)-OH; 57). Benzyl ester **55** (0.466 g, 1.33 mmol) was debenzylated in AcOEt according to *GP 9* to yield quantitatively crude **57**. FC (CH_2Cl_2/MeOH 12:1) yielded **57** (0.179 g, 52%). Colorless oil. R_f 0.36 (CH_2Cl_2/MeOH 12:1). $[\alpha]_D^{r.t.}$ = – 24.7 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3436*w*, 2964*m*, 2677*w*, 1708*s*, 1503*m*, 1462*w*, 1395*m*, 1369*m*, 1169*m*, 1118*w*, 974*w*, 949*w*, 898*w*, 872*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, signals of rotamers in italics): 0.91 (*d*, *J* = 6.7, 2 Me); 1.25 (*d*, *J* = 7.1, Me); 1.44, *1.48* (*s*, *t*-Bu); 1.63-1.73 (*m*, Me_2CH); 2.61-2.71 (*m*, COCH); 3.77-3.88 (*m*, NCH); 5.01, *5.77* (*d*, *J* = 9.9, NH); 8.71 (*br. s*, CO_2H). ^{13}C-NMR (100 MHz, $CDCl_3$): *14.05*, 14.44, *21.54*, 22.04, 23.11, *23.36*, 28.39 (Me); *42.36*, 43.00 (CH_2); 43.34, *43.92*, 50.51, *52.07* (CH); 79.36, 156.10, *157.49*, *179.49*, 180.63. FAB-MS: 298 (3.6, [*M* + K]$^+$), 282 (87.9, [*M* + K]$^+$), 260 (58.5, [*M* + 1]$^+$), 204 (100), 186 (53.1), 160 (87.4), 130 (44.3), 107 (30.3).

Benzyl (2*R*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,4-dimethyl-pentanoate (Boc-(2*R*,3*S*)-β^{2,3}-HVal(α-Me)-OBn; 58). Methyl ester **38** (1.26 g, 5.12 mmol) was transesterified with $Ti(OBn)_4$ (0.68 equiv.) for 37 h according to *GP 8*. FC (Et_2O/pentane 1:6 → 1:5) yielded **58** (1.33 g, 77%). Colorless waxy solid. M.p. 59.5-61.5 °C. R_f 0.15 (Et_2O/pentane 1:6). $[\alpha]_D^{r.t.}$ = + 3.8 (*c* = 1.34, $CHCl_3$). IR ($CHCl_3$): 3446*w*, 3036*w*, 3005*w*, 2974*m*, 2923*m*, 2882*w*, 1713*s*, 1503*s*, 1456*m*, 1390*m*, 1369*s*, 1303*m*, 1169*s*, 1097*w*, 1072*w*, 1046*w*, 903*w*, 867*w*, 626*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; signals of rotamers in italics): 0.86 (*d*, *J* = 6.8, Me); 0.90 (*d*, *J* = 6.7, Me); 1.15 (*d*, *J* = 7.0, Me); 1.43 (*s*, *t*-Bu); 1.61-1.69 (*m*, Me_2CH); 2.56-2.67 (*m*, COCH); , 3.79-3.85 (*m*, NCH); *4.06*, 4.38 (*d*, *J* = 10.6, NH); 5.06 (*d*, *J* = 12.3, PhC*H*H); 5.14 (*d*, *J* = 12.3, PhCH*H*); 7.29-7.56 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.58, *13.78*, *16.18*, 17.21, 20.21, 28.37 (Me); 30.34, *30.61*, 42.62,

43.13, 57.37, *58.83* (CH); 66.47 (CH_2); 79.16 (C); 128.18, 128.32, 128.53 (CH); 135.97, 155.91, 174.65 (C). FAB-MS: 671 (5.4, $[2M]^+$), 336 (67.3, $[M + 1]^+$), 280 (100), 236 (80.5), 192 (22.7), 172 (32.8), 116 (30.0). Anal. calc. for $C_{19}H_{29}NO_4$ (335.44): C 68.03, H 8.71, N 4.18; found: C 68.10, H 8.55, N 4.11.

Benzyl (2*R*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,5-dimethyl-hexanoate (Boc-(2*R*,3*S*)-β^{2,3}-HLeu(α-Me)-OBn; 59). Methyl ester **40** (2.42 g, 8.85 mmol) was transesterified with $Ti(OBn)_4$ (1.5 equiv.) for 45 h according to *GP 8*. FC (Et_2O/pentane 1:5) yielded **59** (2.59 g, 84%). White waxy solid. R_f 0.26 (Et_2O/pentane 1:5). $[\alpha]_D^{r.t.} = -37.4$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3443*w*, 3005*m*, 2964*m*, 2872*w*, 1708*s*, 1503*s*, 1456*m*, 1390*m*, 1369*s*, 1174*s*, 1103*m*, 1041*w*, 908*w*, 872*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; signals of rotamers in italics): 0.92 (*d*, *J* = 6.7, 2 Me); 0.86 (*d*, *J* = 6.6, Me); 0.87 (*d*, *J* = 6.4, Me); 1.11-1.26 (*m*, CH_2, Me); 1.42, *1.64* (s, *t*-Bu); 1.69-1.65 (*m*, Me_2CH); *2.48-2.57*, 2.63-2.69 (*m*, COCH); *3.70-3.79*, 3.84-3.91 (m, NCH); *4.19* (*br.*, NH); 4.57 (*d*, *J* = 9.5, NH); 5.10 (*d*, *J* = 12.3, PhC*H*H); 5.14 (*d*, *J* = 12.3, PhCH*H*); 7.30-7.56 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 13.17, 21.60, 23.48 (Me); 24.91 (CH); 28.38 (Me); 41.05 (CH_2); 44.52, 51.02 (CH); 66.31 (CH_2); 79.13 (C); 128.22, 128.28, 128.57 (CH); 136.02, 155.50, 174.39 (C). EI-MS: 350 (0.7, M^+), 192 (22.9), 186 (31), 130 (81.4), 91 (100). Anal. calc. for $C_{20}H_{31}NO_4$ (349.47): C 68.74, H 8.94, N 4.01; found: C 68.78, H 8.84, N 3.96.

(2*R*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,4-dimethyl-pentanoic Acid (Boc-(2*R*,3*S*)-β^{2,3}-HVal(α-Me)-OH; 60). Benzyl ester **58** (2.27 g, 7.98 mmol) was debenzylated in MeOH (40 ml) according to *GP 9*. Recrystallization (CH_2Cl_2/hexane) yielded **60** (1.90 g, 97%). White powder. M.p. 113-114 °C. R_f 0.48 (CH_2Cl_2/MeOH 12:1). $[\alpha]_D^{r.t.} = +13.3$ (*c* = 1.0, MeOH). IR ($CHCl_3$): 3446*w*, 2980*m*, 2931*m*, 2875*s*, 1714*s*, 1504*s*, 1456*m*, 1392*m*, 1368*m*, 1170*s*, 1092*w*, 1043*w*, 986*w*, 868*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; signals of rotamers in italics): 0.89 (*d*, *J* = 6.8, Me); 0.95 (*d*, *J* = 6.6, Me); 1.17 (*d*, *J* = 7.0, Me); 1.43, *1.45* (*s*, *t*-Bu); 1.59-1.83 (*m*, Me_2CH); 2.57-2.64 (*m*, COCH); *3.68-3.72*, 3.80-3.84 (*m*, NCH); 4.45, *5.57* (*d*, *J* = 10.5, NH); 10.6 (*br.*, COOH). ^{13}C-NMR (100 MHz, $CDCl_3$; signals of rotamers in italics): *12.77*, 12.95, *16.88*, 17.10, 20.30, *28.28*, 28.35 (Me); 30.38, *30.65*, *42.40*, 42.60, 57.20, *58.71* (CH); 79.38, *80.71*, 156.12, *157.71*, 180.07, *180.57* (C). EI-MS: 246 (0.5, $[M + 1]^+$), 202 (41.4), 172 (25.2), 146 (58.9), 116 (24.2), 102 (100), 84 (31.5), 74 (21.4), 72 (21.1), 57 (26.7). Anal. calc. for $C_{12}H_{23}NO_4$ (245.32): C 58.75, H 9.45, N 5.71; found: C 58.64, H 9.37, N 5.70.

(2*R*,3*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-2,5-dimethyl-hexanoic (Boc-(2*R*,3*S*)-β^{2,3}-HLeu(α-Me)-OH; 61). Benzyl ester **59** (2.59 g, 7.42 mmol) was debenzylated in AcOEt (37 ml) according to *GP 9*. FC (CH_2Cl_2/MeOH 12:1) yielded **61** (1.56 g, 81%). White foam. R_f 0.36 (CH_2Cl_2/MeOH 12:1). $[\alpha]_D^{r.t.}$ = – 44.4 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3443*w*, 3200-2850*br*, 1707*s*, 1505*s*, 1469*m*, 1392*m*, 1368*s*, 1168*s*, 1103*w*, 1046*w*, 1007*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; signals of rotamers in italics): 0.92 (*d*, *J* = 6.7, 2 Me); 1.16 (*d*, *J* = 7.1, Me); 1.24-1.37 (*m*, CH_2); 1.44, *1.48* (*s*, *t*-Bu); 1.60-1.67 (*m*, Me_2CH); *2.49-2.54*, 2.63-2.66 (*m*, COCH); 3.84-3.92 (*m*, NCH); 4.76 (br. *d*, *J* = 9.0, NH); *5.50* (br. *s*, NH); 7.52 (br., COOH). ^{13}C-NMR (100 MHz, $CDCl_3$): 13.12, 21.59, 23.50 (Me); 24.95 (CH); 28.37 (Me); 40.76 (CH_2); *41.92*, 44.27, 50.78, *51.74* (CH); 79.36; 155.66, 179.89 (C). FAB-MS: 541 (10.2, [2*M* + Na]$^+$), 282 (45.7, [*M* + Na]$^+$), 204 (100), 130 (63.8). Anal. calc. for $C_{13}H_{25}NO_4$ (259.34): C 60.21, H 9.72, N 5.40; found: C 60.20, H 9.64, N 5.23.

(2*R*,3*S*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-2,4-dimethyl-pentanoic Acid (Fmoc-(2*R*,3*S*)-β^{2,3}-HVal(α-Me)-OH; 62). β^3-Amino acid **61** (0.308 g, 1.26 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was transformed according to *GP 11*. Recrystallization (CH_2Cl_2/hexane) yielded **62** (0.397 g, 86%). White powder. RP-HPLC according to *GP 27* (20-80% *B* in 20 min; C_8) t_R 10.6, purity > 99%. M.p. 176.5-177.5 °C. R_f 0.37 (CH_2Cl_2/MeOH 10:1). $[\alpha]_D^{r.t.}$ = + 3.50 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3440*w*, 3150-2860*br*, 1724*s*, 1513*s*, 1451*m*, 1302*w*, 1095*w*, 1045*w*, 909*w*, 620*w*. ^{1}H-NMR (400 MHz, CD_3COCD_3, signals of rotamers in italics): 0.93 (*d*, *J* = 6.8, Me); 0.93 (*d*, *J* = 6.7, Me); 1.14 (*d*, *J* = 7.0, Me); 1.80-1.90 (*m*, Me_2CH); 2.66-2.71 (*m*, COCH); 3.84-3.90 (*m*, NCH); 4.22 (*t*, *J* = 7.0, OCH); 4.31-4.41 (*m*, OCH_2); *3.65*, 6.24 (*d*, *J* = 10.3, NH); 7.30-7.39 (*m*, 2 arom. H); 7.39-7.43 (*m*, 2 arom. H); 7.68-7.71 (*m*, 2 arom. H); 7.86 (*d*, *J* = 7.5, 2 arom. H). ^{13}C-NMR (100 MHz, CD_3COCD_3): 14.22, 17.12, 20.83 (Me); 31.34, 42.89, 48.24, 58.97 (CH); 66.69 (CH_2); 120.79, 126.08, 126.15, 127.89, 127.91, 128.48 (CH); 142.15, 145.18, 145.23, 157.64, 176.41 (C). FAB-MS: 735 (2.0, [2*M*]$^+$), 368 (38.8, [*M* + 1]$^+$), 178 (100), 165 (23.2). Anal. calc. for $C_{22}H_{25}NO_4 \cdot 0.5\ H_2O$ (376.46): C 70.19, H 6.96, N 3.72; found: C 70.20, H 6.85, N 3.74.

(2*R*,3*S*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-2,5-dimethyl-hexanoic Acid (Fmoc-(2*R*,3*S*)-β^{2,3}-HLeu(α-Me)-OH; 63). β-Amino acid **61** (0.87 g, 3.35 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was transformed according to *GP 11*. FC (CH_2Cl_2/MeOH 20:1 → 10:1) and recrystallization (CH_2Cl_2/pentane) yielded **63** (1.12 g, 87%). White powder. RP-HPLC according to *GP 27* (20-80% *B* in 20 min; C_8) t_R 13.2, purity > 99%.

M.p. 184-186 °C (dec.). R_f 0.39 (CH_2Cl_2/MeOH 10:1). $[\alpha]_D^{r.t.}$ = – 28.4 (*c* = 0.68, $CHCl_3$). IR ($CHCl_3$): 3436*w*, 3100-2850*br*, 1716*s*, 1513*s*, 1450*m*, 1331*w*, 1105*w*, 600*w*. ^{1}H-NMR (400 MHz, CD_3COCD_3): 0.90 (2 *d*, *J* = 6.7, 6.5, 2 Me); 1.14 (*d*, *J* = 7.1, Me); 1.20-1.29 (*m*, *CH*H); 1.47-1.54 (*m*, CH*H*); 1.63-1.71 (*m*, Me_2CH); 2.52 (*quintett*, *J* = 7.2, COCH); 3.92-4.05 (*m*, NCH); 4.22 (*t*, *J* = 7.0, OCH); 4.38 (*d*, *J* = 6.9, OCH_2); 6.26 (*d*, *J* = 9.0, NH); 7.29-7.33 (*m*, 2 arom. H); 7.38-7.42 (*m*, 2 arom. H); 7.67-7.70 (*m*, 2 arom. H); 7.85 (*d*, *J* = 7.5, 2 arom. H). ^{13}C-NMR (100 MHz, CD_3COCD_3): 14.26, 21.81, 24.00 (Me); 25.62 (CH); 42.97 (CH_2); 45.76, 48.25, 52.30 (CH); 66.59 (CH_2); 120.77, 126.07, 126.12, 127.86, 127.89, 128.46 (CH); 142.15, 145.11, 145.24, 157.17, 172.20 (C). FAB-MS: 2021 (34.9, [5*M* – 2 H + 3 K]$^+$), 1221 (36.5, [3*M* – 1 H + 2 K]$^+$), 801 (8.5, [2*M* + K]$^+$), 420 (18.4, [*M* + K]$^+$), 404 (15.1, [*M* + Na]$^+$), 382 (29.6, [*M* + 1]$^+$), 178 (100). Anal. calc. for $C_{23}H_{27}NO_4$ (381.47): C 72.42, H 7.13, N 3.67; found: C 72.45, H 7.25, N 3.62.

***tert*-Butyl (*E*)-2-Methylbut-2-enoate (64).** Isobutylene (41 g, 0.75 mol) was condensed into a 500 ml-round-bottom flask containing a soln. of tiglic acid ((*E*)-2-methylbut-2-enoic acid; 15.0 g, 0.15 mol) in CH_2Cl_2 (100 ml) at – 20 °C. Conc. H_2SO_4 (0.8 ml) was added and the soln. was stirred at r.t. for 56 h. Before opening, the mixture was cooled to – 4 °C. It was quenched with sat. aq. $NaHCO_3$ soln. and stirred vigorously to evaporate excess isobutylene. After drying ($MgSO_4$) the crude product was distillated (90-95 °C, 79 Torr) to yield **64** (12.28 g, 52%). Colorless oil. B.p. 95 °C (78 Torr). R_f 0.65 (Et_2O/pentane 1:9). Spectroscopic data: corresponding to [250].

(*S*)-*N*-Benzyl-1-phenylethylamine (65). (*S*)-1-Phenylethylamine (15.9 ml, 0.125 mol) was benzylated acccording to [252]. Distillation (122 °C, 0.12 Torr) yielded **65** (19.74 g, 75%). Colorless oil. B.p. and spectroscopic data: corresponding to [252].

***tert*-Butyl (2*R*,3*S*,α*S*)-3-(*N*-benzyl-*N*-α-methylbenzylamino)-2-methylbutyrate (66).** *tert*-Butyltiglate **64** (5.0 g, 32 mmol) was transformed with the Li amide derived from **65** (10.82 g, 51.2 mmol) according to [137]. FC (Et_2O/pentane 1:50) yielded **66** (7.95 g, 68%). Colorless oil. R_f 0.26 (Et_2O/pentane 1:50). Spectroscopic data: corresponding to [137].

***tert*-Butyl (2*R*,3*S*)-3-Amino-2-methylbutanoate ((H-(2*R*,3*S*)-β^{2,3}-HAla(α-Me)-O*t*-Bu; 67).** Compound **66** (3.42 g, 9.31 mmol) was dissolved in AcOEt (60 ml) and $Pd(OH)_2$ (0.68 g) was added. The flask was evacuated (3×) and flushed with H_2 (3×), and the mixture was stirred under an atmosphere of H_2 (2

balloons) at. r.t. for 42 h. The mixture was fitrated through *Celite* and evaporated under reduced pressure (45 °C, 85 mbar). Crude **67** (1.61 g, 99%). Yellowish crystals, used directly in the next step. R_f 0.78 ($EtOH/NH_3/H_2O$ 7:1:1). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.14 (*d*, *J* = 6.2, 2 Me); 1.46 (*s*, *t*-Bu); 2.29-2.42 (*m*, COCH); 2.84 (br. *s*, NH_2); 3.13-3.26 (*m*, NCH).

(2*R*,3*S*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-2-methyl-butanoic Acid (Fmoc-(2*R*,3*S*)-β^{2,3}-HAla(α-Me)-OH; 68). Amine **67** (1.61 g, 9.29 mmol) was dissolved in TFA (10 ml) and stirred for 3 h at r.t. Evaporation yielded the crude amino acid that was Fmoc-protected according to *GP 11*. FC (Et_2O/pentane/AcOH 6:4:0.1) and recrystallization (AcOEt/hexane) gave **68** (2.32 g, 74%). White powder. M.p. 205-205.5 °C. R_f 0.19 (Et_2O/pentane/AcOH 6:4:0.1). $[\alpha]_D^{r.t.}$ = + 7.79 (*c* = 0.68, acetone). IR (KBr): 3327*s*, 3066*m*, 2976*s*, 2889*m*, 2622*w*, 1685*s*, 1544*s*, 1450*s*, 1420*m*, 1380*m*, 1333*m*, 1284*s*, 1256*s*, 1217*s*, 1150*m*, 1107*s*, 1089*s*, 1028*s*, 976*m*, 928*m*, 880*m*, 795*w*, 779*w*, 757*m*, 737*s*, 669*m*, 622*m*, 588*w*, 547*w*, 502*w*, 424*m*. ^{1}H-NMR (400 MHz, CD_3COCD_3): 1.16 (*d*, *J* = 7.1, Me); 1.19 (*d*, *J* = 6.7, Me); 2.58 (*quintett*, *J* = 7.2, COCH); 2.85-3.94 (*m*, NCH); 4.21-4.24 (*m*, OCH); 4.28-4.33 (*m*, OCH); 4.37-4.41 (*m*, OCH); 6.37 (*d*, *J* = 7.9, NH); 7.30-7.34 (*m*, 2 arom. H); 7.39-7.50 (*m*, 2 arom. H); 7.69 (*d*, *J* = 7.5, 2 arom. H); 7.86 (*d*, *J* = 7.5, 2 arom. H); 10.73 (br., COOH). ^{13}C-NMR (100 MHz, CD_3COCD_3): 14.67, 19.08 (Me); 45.80, 48.18, 49.98 (CH); 66.71 (CH_2); 120.81, 126.06, 126.12, 127.90, 127.92, 128.49 (CH); 142.14, 145.17, 145.21, 156.65, 176.07 (C). FAB-MS: 679 (4.7, [2*M*]$^+$), 340 (100, [*M* + 1]$^+$). Anal. calc. for $C_{20}H_{21}NO_4$ (339.39): C 70.78, H 6.24, N 4.13; found: C 70.65, H 6.44, N 4.10.

7.3.4 *Experiments for Configurational Assignment of β^{2,3}-Amino Acid Derivatives*

Benzoylation of β^{2,3}-Amino Acid Methyl Ester Derivatives: General Procedure 12 (*GP 12*). The corresponding (*S*)-Boc-β^{2,3}-HXaa-OMe was Boc-deprotected according to *GP 10a*. The resulting TFA salt was dissolved at 0 °C in CH_2Cl_2 (0.2M) and treated with Et_3N (5 equiv.), BzCl (1.2 equiv.) and a catalytic amount of DMAP. The mixture was stirred for 16 h, diluted with CH_2Cl_2 and washed with sat. aq. NH_4Cl and NaCl solns. The org. phase was dried ($MgSO_4$) and evaporated. FC yielded the pure product.

Reduction of *N*-Benzoylated β^{2,3}-Amino Acid Methyl Ester Derivatives: General Procedure 13 (*GP 13*). A soln. of (*S*)-Bz-β^{2,3}-HXaa-OMe in THF (0.25M) was added under Ar to a suspension of $LiAlH_4$ (3.5 equiv.) in THF (0.8M). The resulting light yellow soln. was heated to reflux for 3 h and hydrolyzed subsequently with H_2O (5 ml). After filtration through *Celite* it was extracted with AcOEt and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield the crude product which was characterized by NMR and MS and directly used in the cyclization step.

Methyl (2*S*,3*S*)-3-(Benzoylamino)-2,4-dimethylpentanoate (Bz-(*S*,*S*)-β^{2,3}-HVal(α-Me)-OMe; 44). Compound **37** (0.275 g, 1.06 mmol) was transformed according to *GP 12*. FC (AcOEt/pentane 1:4) yielded **44** (0.261 g, 93 %). Colorless waxy solid. M.p. 39-40 °C. R_f 0.27 (AcOEt/pentane 1:4) $[\alpha]_D^{r.t.} = -74.3$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3424*w*, 3007*m*, 2962*m*, 2877*w*, 1717*s*, 1654*s*, 1602*w*, 1580*w*, 1520*s*, 1488*s*, 1461*m*, 1437*w*, 1383*w*, 1364*w*, 1259*w*, 1177*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*d*, *J* = 6.7, Me), 1.00 (*d*, *J* = 6.7, Me), 1.26 (*d*, *J* = 7.2, Me), 1.71-1.83 (*m*, CH), 2.92-3.00 (*m*, COCH), 3.72 (*s*, OMe), 4.01 (*ddd*, *J* = 9.8, 8.9, 3.4, NCH), 7.39 (*d*, *J* = 10.0, NH), 7.41 (*m*, 3 arom. H), 7.84-7.87 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 16.3; 19.7; 19.8; 32.2; 39.9; 51.9; 57.5; 126.9; 128.6; 131.4; 134.7; 167.4; 177.1. EI-MS: 264 (4, $[M + 1]^+$), 220 (100). Anal. calc. for $C_{15}H_{21}NO_3$ (263.34): C 68.42, H 8.04, N 5.32; found: C 68.48, H 8.03, N 5.31.

Methyl (2*S*,3*S*)-3-(Benzoylamino)-2,5-dimethyl-hexanoate (Bz-(*S*,*S*)-β^{2,3}-HLeu(α-Me)-OMe; 45). Compound **39** (0.237 g, 0.87 mmol) was transformed according to *GP 12*. FC (AcOEt/pentane 1:4) and recrystallization (pentane) yielded **45** (0.175 g, 72%). Colorless crystals. M.p. 84-86 °C. R_f 0.27 (AcOEt/pentane 1:4). $[\alpha]_D^{r.t.} = -59.1$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3427*w*, 3004*m*, 3957*m*, 2871*w*, 1718*s*, 1655*s*, 1580*w*, 1520*s*, 1487*s*, 1462*m*, 1437*w*, 1384*w*, 1366*w*, 1178*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*d*, *J* = 6.7, Me); 0.98 (*d*, *J* = 6.5, Me); 1.26 (*d*, *J* = 7.2, Me); 1.28-1.34 (*m*, CH); 1.47-1.54 (*m*, CH); 1.62-1.75 (*m*, CH); 2.77 (*dq*, *J* = 14.4, 7.2, 3.5, COCH); 3.73 (*s*, OMe); 4.38-4.45 (*m*, *J* = 9.7, 4.9, 3.5, NCH); 7.06 (*d*, *J* = 9.5, NH); 7.42-7.52 (*m*, 3 arom. H); 7.79-7.84 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.5, 22.3, 23.1 (Me); 25.1, 43.0 (CH); 43.6 (CH_2); 49.7 (CH); 51.8 (Me); 126.9, 128.6, 131.4 (CH); 134,7, 167.1, 176.8 (C). EI-MS: 278 (16, $[M + 1]^+$), 190 (49), 105 (100). Anal. calc. for $C_{16}H_{23}NO_3$ (277.36): C 69.29, H 8.36, N 5.05; found: C 69.24, H 8.33, N 5.09.

(2*S*,3*S*)-3-(Benzoylamino)-2,4-dimethylpentan-1-ol (46). **44** (0.186 g, 0.71 mmol) was reduced according to *GP 13*. **46** (0.114 g, 73%). Colorless oil. R_f 0.34

(AcOEt/pentane 1:1). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.89 (*d*, *J* = 7.1, Me); 0.92 (*d*, *J* = 7.1, Me); 1.05 (*d*, *J* = 7.1, Me); 1.77-1.82 (*m*, Me_2CH); 1.97-2.06 (*m*, COCH); 2.36-2.42 (*m*, NCH); 3.58 (*dd*, *J* = 10.8, 7.9, OC*H*H); 3.71 (*dd*, *J* = 10.8, 3.3, OCH*H*); 3.88 (*s*, $PhCH_2$); 4.21 (*br. s*, NH, OH); 7.27-7.35 (*m*, 5 arom. H). EI-MS: 222 (2.9, $[M + 1]^+$), 178 (100), 160 (47.3), 91 (21.9).

(2*S*,3*S*)-3-(Benzylamino)-2,5-dimethylhexan-1-ol (47). **45** (0.124 g, 0.45 mmol) was reduced according to *GP 13*. **47** (0.106 g, quant.). Colorless oil. R_f 0.30 ($CHCl_3$/MeOH/Et_3N 9:1:0.5). ^{1}H-NMR (200 MHz, $CHCl_3$): 0.95 (*m*, 3 Me), 1.46-1.86 (*m*, CH_2, Me_2CH, MeC*H*), 2.72 (*dd*, *J* = 13.3, 6.2, NCH), 3.54 (*dd*, *J* = 10.8, 7.5, OC*H*H), 3.81 (*d*, *J* = 12.5, PhC*H*H), 3.82 (*dd*, *J* = 10.8, 3.3, OCH*H*), 3.96 (*d*, *J* = 12.5, PhCH*H*), 4.44 (br. *s*, NH, OH), 7.29-7.37 (*m*, 5 arom. H). EI-MS: 236 (1, $[M + 1]^+$), 176 (100), 91 (28).

Methyl (2*R*,3*S*)-3-(Benzoylamino)-2,4-dimethylpentanoate (Bz-(2*R*,3*S*)-β^{2,3}-HVal(α-Me)-OMe; 48). Compound **38** (0.309 g, 1.19 mmol) was transformed according to *GP 12*. FC (AcOEt/pentane 1:3) yielded **48** (0.286 g, 92%). White solid. M.p. 89-90°. R_f 0.36 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.} = -16.6$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3442*w*, 3007*m*, 2969*m*, 1732*s*, 1665*s*, 1602*w*, 1580*w*, 1514*s*, 1487*s*, 1436*w*, 1311*w*, 1269*m*, 1141*w*, 1063*w*, 1029*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.96 (*d*, *J* = 6.8, Me); 1.01 (*d*, *J* = 6.7, Me); 1.21 (*d*, *J* = 7.1, Me); 1.79-1.90 (*m*, Me_2CH); 2.78 (*q*, *J* = 7.0, COCH); 3.70 (*s*, OMe); 4.36-4.41 (*m*, NCH); 6.10 (*bd*, *J* = 10.2, NH); 7.41-7.56 (*m*, 3 arom. H); 7.74-7.96 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.5, 17.7, 20.4 (Me); 30.3, 42.1 (CH); 51.9 (Me); 56.1, 126.9, 128.7, 131.4 (CH); 135.0, 167.5, 175.2 (C). EI-MS: 263 (<1, $[M + 1]^+$), 220 (95), 176 (36), 105 (100). Anal. calc. for $C_{15}H_{21}NO_3$ (263.34): C 68.42, H 8.04, N 5.32; found: C 68.50, H 7.92, N 5.37.

(2*R*,3*S*)-3-(Benzylamino)-2,4-dimethylpentan-1-ol (49). **48** (0.229 g, 0.87 mmol), was reduced according to *GP 13*. **49** (0.123 g, 67%). Yellowish waxy solid. R_f 0.21 ($CHCl_3$/MeOH/Et_3N 18:1:0.1). ^{1}H-NMR (200 MHz, $CHCl_3$): 0.94 (*d*, *J* = 3.2, Me); 0.98 (*d*, *J* = 3.3, Me); 1.07 (*d*, *J* = 6.6, Me); 1.86-2.01 (*m*, MeC*H*, Me_2CH); 2.57 (*dd*, *J* = 7.1, 2.9, NCH); 3.7 (br. *s*, NH, OH); 3.70 (*dd*, *J* = 10.4, 5.8, OC*H*H); 3.81 (*dd*, *J* = 10.4, 2.9, OCH*H*); 3.87 (*s*, $PhCH_2$); 7.26-7.34 (*m*, 5 arom. H). EI-MS: 222 (3, $[M + 1]^+$), 162 (40), 91 (19).

(4*S*,5*S*)-3-Benzyl-4-isopropyl-5-methyl-1,3-oxazinan-2-one (50) and **(2*S*,3*R*)-*N*-Benzyl-2-isopropyl-3-methyl-azetidine (51).** To a soln. of **46** (0.114 g, 0.515 mmol) in CH_2Cl_2/THF 1:1 (5 ml) was added Et_3N (150 µl, 1.1 mmol). The

mixture was cooled to – 78 °C and a soln. of triphosgene (59 mg, 0.198 mmol) in THF (2 ml) was added. After 2 h at – 78 °C the mixture was diluted with Et_2O. The salts were filtered off and it was evaporated to yield a yellow oil. 2× FC (AcOEt/pentane 1:3) yielded **50** (31 mg, 25%) and **51** (7.0 mg, 7%).

Data of **50**: Colorless oil. R_f 0.30 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.}$ = – 11.8 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3007*m*, 2988*m*, 2934*w*, 2878*w*, 1678*s*, 1485*m*, 1474*m*, 1450*m*, 1436*m*, 1392*m*, 1248*w*, 1154*m*, 1080*w*, 820*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.83 (*d*, *J* = 7.0, Me), 0.94 (*d*, *J* = 6.9, Me), 1.00 (*d*, *J* = 7.0, Me), 2.05-2.16 (*m*, MeC*H*, Me_2CH), 2.76-2.78 (*m*, NCH), 3.84 (*ddd*, *J* = 11.0, 4.8, 1.0, OC*H*H), 3.93 (*d*, *J* = 14.9, PhC*H*H), 4.28 (*dd*, *J* = 11.0, 4.3, OCH*H*), 5.26 (*d*, *J* = 14.9, PhCH*H*), 7.26-7.35 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.69; 17.71; 19.62; 27.36; 30.77; 50.91; 64.36; 69.60; 127.70; 128.59; 128.72; 137.07; 155.21. EI-MS: 247.1 (10, M^+), 204.0 (100), 91.0 (70). NOE (300 MHz, $CDCl_3$): Irradiation at 0.83: strong positive NOE at 2.77 and NOE at 3.84 and 2.11, irradiation at 2.77: strong positive NOE at 0.83.

Data of **51**: Colorless oil. R_f (0.38 (AcOEt/pentane 1:3). ^{1}H-NMR (200 MHz, $CDCl_3$): 0.86 (*d*, *J* = 6.6, Me); 0.93 (*d*, *J* = 6.6, Me); 1.09 (*d*, *J* = 6.2, Me); 1.73-1.80 (*m*, Me_2CH); 2.17-2.50 (*m*, MeC*H*, 2 NCH); 3.35 (*d*, *J* = 12.5, PhC*H*H); 3.41 (*t*, *J* = 7.1, NCH); 3.90 (*d*, *J* = 12.9, PhCH*H*); 7.22-7.31 (*m*, 5 arom. H). ^{13}C-NMR (25 MHz, $CDCl_3$): 17.99, 19.48, 20.10 (Me); 30.52, 34.27 (CH); 59.16, 64.43, 80.52 (CH_2); 126.81, 128.21, 128.78 (CH). EI-MS: 204 (100, $[M + 1]^+$), 190 (19.9), 176 (26.7), 162 (47.1), 120 (32.3), 91 (34.7).

(4*S*,5*S*)-3-Benzyl-4-isobutyl-5-methyl-1,3-oxazinan-2-one (52). To a soln. of **47** (44 mg, 0.19 mmol) in CH_2Cl_2/THF 1:1 (3 ml) was added Et_3N (52 µl, 0.37 mmol). The mixture was cooled to – 50 °C and a soln. of triphosgene (18 mg, 0.062 mmol) in THF (1.5 ml) was added. The mixture was allowed to warm to r.t. within 3 h and diluted with Et_2O. The salts were filtered off and it was evaporated to yield a yellow oil. FC (AcOEt/pentane 1:3 → 1:1) yielded **52** (16.9 mg, 35%). Colorless solid. Crystallization from pentane gave single crystals suitable for X-ray analysis. M.p. 95-96 °C. R_f 0.13 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.}$ = – 40.8 (*c* = 0.5, $CHCl_3$). IR ($CHCl_3$): 3008*m*, 2961*m*, 2932*m*, 2872*w*, 1676*s*, 1484*m*, 1451*m*, 1370*w*, 1311*w*, 1256*m*, 1152*m*, 1080*w*, 1011*w*, 960*w*, 829*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.86 (*d*, *J* = 6.1, Me); 0.89 (*d*, *J* = 7.1, Me); 0.94 (*d*, *J* = 6.3 , Me); 1.44-1.59 (*m*, CH_2, Me_2CH); 1.85-1.91 (*m*, MeC*H*); 2.90-2.92 (*m*, NCH); 3.90 (*d*, *J* = 14.9, PhC*H*H); 3.94 (*dt*, *J* = 11.2, 1.9, OC*H*H); 4.42 (*dd*, *J* = 11.1, 3.0, OCH*H*); 10.43 (*d*, *J* = 14.9, PhCH*H*); 7.26 (*m*, 5 arom. H). ^{13}C-NMR (126 MHz, $CDCl_3$): 16.21, 21.64, 23.76 (Me); 25.20, 28.64 (CH); 42.26, 50.13 (CH_2); 57.41 (CH); 67.80 (CH_2); 127.69, 128.56, 128.61 (CH); 137.31, 153.63 (C). NOE (300 MHz,

$CDCl_3$): Irradiation at 1.88: strong positive NOE at 0.9 and positive NOE at 2.91, 3.94 and 4,42, irradiation at 2.91: strong positive NOE at 0.9 and positive NOE at 3.94 and 1.88, irradiation at 4.42: strong positive NOE at 3.94 and positive NOE at 1.88. EI-MS: 261 (20, M^+), 204 (70), 91 (100).

(4*S*,5*R*)-3-Benzyl-4-isopropyl-5-methyl-1,3-oxazinan-2-one (53). To a soln. of **49** (60 mg, 0.27 mmol) in CH_2Cl_2/THF 1:1 (2 ml) at 0 °C was added Et_3N (75 µl, 0.54 mmol) followed by a soln. of triphosgene (30 mg, 0.1 mmol) in THF (1 ml). The mixture was stirred for 2.5 h at 0 °C, then diluted with Et_2O, filtered and evaporated. The resulting yellow oil was dissolved in AcOEt, washed with citric acid soln. (pH 2.5) and sat. NaCl soln., dried ($MgSO_4$) and evaporated. FC (AcOEt/pentane 2:3) yielded **53** (24 mg, 18%). Colorless solid. Crystallization from hexane gave single cristals suitable for X-ray analysis. M.p. 94-95 °C. R_f 0.29 (AcOEt/pentane 2:3). $[\alpha]_D^{r.t.} = -50.0$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3007*m*, 2968*m*, 1676*s*, 1515*w*, 1484*s*, 1451*s*, 1394*w*, 1359*w*, 1248*m*, 1153*m*, 1080*w*, 1034*w*, 967*w*, 879*w*, 658*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*d*, *J* = 7.0, Me); 1.06 (*d*, *J* = 7.1, Me); 1.11 (*d*, *J* = 7.1, Me); 1.96-2.08 (*m*, Me_2CH); 2.17-2.28 (*m*, MeC*H*); 3.02-3.04 (*m*, NCH); 3.82 (*d*, *J* = 15.2, PhC*H*H); 4.07 (*t*, *J* = 11.5, OC*H*H); 4.16 (*ddd*, *J* = 11.0, 5.5, 1.7, OCH*H*); 5.43 (*d*, *J* = 15.2, PhCH*H*); 7.26-7.36 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.93, 19.59, 24.61 (Me); 28.04, 32.36 CH); 53.19 (CH_2); 61.96 (CH); 69.84 (CH_2); 127.62, 127.99, 128.70 (CH); 136.98, 153.93 (C). NOE (300 MHz, $CDCl_3$): Irradiation at 2.04: strong positive NOE at 4.07, 1.06 and 1.11, irradiation at 2.23: strong positive NOE at 3.03 and 4.16, irradiation at 3.03: positive NOE at 2.23. EI-MS: 247 (2, M^+), 204 (18), 160 (3), 117 (2), 104 (2), 91 (100), 77 (2), 65 (6), 56 (2). Anal. calc. for $C_{15}H_{21}NO_2$ (247.34): C 72.84, H 8.56, N 5.66; found: C 72.66, H 8.63, N 5.71.

7.3.5 Preparation of Geminally Disubstituted β-Amino Acid Derivatives

Alkylation of β-Alanine Derivatives: General Procedure 14 (*GP 14*). BuLi (1 equiv.) was added to a soln. of $(i\text{-}Pr)_2NH$ in THF (0.5M) at – 78 °C. After 20 min at – 78 °, a soln. of the *β*-alanine derivative in THF (0.8mM) was added during 10 min and the mixture stirred for 20 min at – 78 °C. MeI (4 equiv.) was then added slowly (temp. < – 65 °C), and the mixture was stirred for 15 min at this temp., subsequently hydrolyzed with sat. NH_4Cl soln., diluted with Et_2O, and washed with sat. $NaHCO_3$, NH_4Cl and NaCl solns.

The org. layer was dried ($MgSO_4$) and evaporated. FC yielded the pure product.

Ester Hydrolysis: General Procedures 15 (*GP 15*). *GP 15a:* Similarly to the reported procedure [593] a soln. of the fully protected amino acid in MeOH (1.2M) was treated with 1N NaOH (1.2 equiv.) at r.t. After stirring for 16 h the mixture was diluted with H_2O (in the case of small scale reactions) and extracted with pentane (2×). The soln. was adjusted to pH 2 with 1N HCl and extracted with AcOEt (3×). The org. phase was washed with sat. aq. NaCl soln., dried ($MgSO_4$) and concentrated under reduced pressure. The acid was either recrystallized for analytical purpose or used in the next step without further purification.

***GP 15b*:** As *GP 15a* except that the reaction mixture (1.5 equiv. NaOH) was refluxed for 2-4 h.

Reduction of Nitriles 77 and Subsequent *N*-Boc-Protection: General Procedures 16 (*GP 16*). *GP 16a:* Freshly prepared *Raney*-Nickel (ca. 100 g alloy/mol) [266] was added to a soln. of the nitrile in MeOH (0.25M). This mixture was stirred for 24-36 h at 40 °C under H_2 (4 bar) in a glass autoclave. Excess H_2 was removed by bubbling Ar through the mixture. After filtration through *Celite* and evaporation (30 °C, 150 mbar) the crude amine methyl ester was obtained as yellowish oil in quantitative yield. This crude product was identified by ^{1}H-NMR and immediately used for the Boc-protection step: To a stirred soln. of the free amine in CH_2Cl_2 (0.5M) a soln. of Boc_2O (1.1 equiv.) in dioxane (0.5M) was added at 0 °C. The mixture was alowed to warm to r.t., and stirring was continued for 2-12 h. After evaporation the residue was dissolved in AcOEt. The org. phase was washed with sat. aq. NH_4Cl, $NaHCO_3$ and NaCl solns., dried ($MgSO_4$) and evaporated. FC yielded the pure product.

***GP 16b*:** As in *GP 16a*, except that the hydrogenation was carried out at r.t. and 1 bar (H_2-balloon).

***GP 16c*:** The nitrile was reduced as described in *GP 16a*. The resulting amino-ester was saponified by refluxing it in MeOH (1M) with 1N NaOH (1.5 equiv.) for 5 h. After evaporation, the free amino acid was dissolved in H_2O (0.5M) and treated with a soln. of Boc_2O (1.1 equiv.) in dioxane (0.6M). The mixture was stirred for 24 h at r.t. and extracted with pentane. The aq. phase was adjusted to pH 2 with 1N HCl and extracted with AcOEt (3×). The org. phase was washed with sat. aq. NaCl soln., dried ($MgSO_4$) and concentrated

under reduced pressure. The acid was recrystallized after refluxing the AcOEt soln. with charcoal and filtration of the hot soln. through *Celite*.

3-Amino-3-methylbutanamide (70). Similarly to [30], 3,3-dimethylacrylic acid (**69**; 50.0 g, 0.50 mol) in aq. NH_3 soln. (24%, 550 ml) was heated at 150 °C for 18 h in an autoclave. After cooling to r.t. the green soln. was refluxed for 3.5 h with $Ba(OH)_2$ (15.0 g, 97.2 mmol). The pH of the cooled suspension was adjusted to 3-4 with conc. H_2SO_4. This suspension was refluxed in the presence of charcoal for 15 min. The filtrate was concentrated to dryness and dried under h.v. The crude product was washed with cold EtOH to yield **70** (58.0 g, 99%). White powder. M.p. 230 °C. R_f 0.54 (EtOH/NH_3/H_2O 7:1:1). ^{1}H-NMR (200 MHz, D_2O): 1.45 (*s*, 2 Me); 2.75 (*s*, CH_2). ^{13}C-NMR (50 MHz, D_2O): 28.03 (Me); 45.84 (CH_2); 55.20, 177.36 (C).

3-{[(*tert*-Butoxy)carbonyl]amino}-3-methyl-butanoic Acid (Boc-β^{3,3}-HAib-OH; 71). Similarly to [266,593] amide **70** (17.86 g, 154.0 mmol) was refluxed in aq. NaOH (25%, 35 ml) for 24 h. The mixture was cooled to r.t. and diluted with H_2O (235 ml) and dioxane (280 ml). At 0 °C, Boc_2O (33.6 g, 0.154 mol) was added. After stirring at r.t. for 12 h dioxane was evaporated. The basic aq. soln. was extracted with pentane (1×) and adjusted to pH 2 with aq. HCl (10%). The aq. phase was extracted with AcOEt (3×). The AcOEt phases were washed with sat. aq. NaCl soln., dried ($MgSO_4$) and evaporated. Recrystallization (AcOEt/pentane) yielded **70** (16.92 g, 51%). White powder. M.p. 98-99 °C. R_f 0.09 (Et_2O/pentane 1:2). ^{1}H-NMR: in agreement with [48].

Methyl 3-{[(*tert*-Butoxy)carbonyl]amino}-3-methyl-butanoate (Boc-β^{3,3}-HAib-OMe; 72). Similar to [257], Boc-protected acid **71** (6.50 g, 29.9 mmol) was dissolved in EtOH/H_2O 10:1 (120 ml). The pH was adjusted to 7 with aq. 10% Cs_2CO_3 soln. The mixture was evaporated and dried under h.v. The residue was dissolved in DMF (58 ml) and MeI (7.22 ml, 0.116 mol) was added at 0 °C. After stirring for 24 h at r.t., excess MeI was destroyed with a few ml 1N NaOH. Upon removal of the solv., the residue was taken up in AcOEt, washed with sat. aq. $NaHCO_3$ and NaCl solns., and evaporated. FC (Et_2O/pentane 1:4) yielded **72**(6.20 g, 90%). Colorless oil. R_f 0.27 (Et_2O/pentane 1:4). IR ($CHCl_3$): 3444*w*, 2978*m*, 1716*s*, 1502*s*, 1454*m*, 1438*m*, 1392*m*, 1368*s*, 1289*m*, 1166*s*, 1081*m*, 1013*w*, 864*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.38 (*s*, 2 Me); 1.43 (*s*, *t*-Bu); 2.70 (*s*, CH_2); 3.67 (*s*, OMe); 4.87 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 27.50, 28.42 (Me); 44.15 (CH_2); 51.11 (C); 51.43 (Me); 79.04, 154.63, 171.82 (C). EI-MS: 231 (0.1, M^+), 158 (34.5), 144 (24.9), 116 (62.0), 102 (51.3), 84 (85.9), 57

(100.0). Anal. calc. for $C_{11}H_{21}NO_4$ (231.29): C 57.12, H 9.15, N 6.06, O 27.67; found: C 57.05, H 9.06, N 6.05, O 27.70.

Methyl 3-{[(*tert*-Butoxy)carbonyl]amino}-2-methylpropanoate (Boc-β-HGly(α-Me)-OMe; 74). Methyl ester **73** (12.00 g, 58.9 mmol), prepared according to [258], was alkylated according to *GP 14* to yield **74** (12.66 g, 98%) as a clear orange oil which was used in the following step without further purification. R_f 0.29 (Et_2O/pentane 1:2). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.17 (*d*, *J* = 7.1, Me); 1.44 (s, *t*-Bu); 2.63-2.73 (*m*, CH); 3.20-3.38 (*m*, CH_2); 3.70 (*s*, OMe); 4.98 (br. *s*, NH).

Methyl 3-{[(*tert*-Butoxy)carbonyl]amino}-2,2-dimethylpropanoate (Boc-β^{2,2}-HAib-OMe; 75). Ester **74** (12.66 g, 58.1 mmol) was alkylated acording to *GP 14*. FC (Et_2O/pentane 1:3) yielded **75** (10.84 g, 81%). Yellowish oil. R_f 0.31 (Et_2O/pentane 1:3). IR ($CHCl_3$): 3456*m*, 2981*m*, 1715*s*, 1509*s*, 1474*m*, 1453*m*, 1393*m*, 1368*m*, 1313*m*, 1155*s*, 1048*w*, 984*w*, 932*w*, 856*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.19 (*s*, 2 Me); 1.43 (*s*, *t*-Bu); 3.23 (*d*, *J* = 6.6, CH_2); 3.69 (*s*, OMe); 4.96 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.02, 28.38 (Me); 43.69 (C); 48.32 (CH_2); 52.00 (Me); 79.17, 156.18, 177.65 (C). EI-MS: 253 (3.3, $[M + Na]^+$), 231 (0.7, M^+), 130 (45.7), 102 (100.0). Anal. calc. for $C_{11}H_{21}NO_4$ (231.29): C 57.12, H 9.15, N 6.06; found: C 57.02, H 9.15, N 6.08.

3-{[(*tert*-Butoxy)carbonyl]amino}-2,2-dimethylpropanoic Acid (Boc-β^{2,2}-HAib-OH; 76). Ester **75** (2.00 g, 8.6 mmol) was saponified according to *GP 15a*. Recrystallization (AcOEt/pentane) yielded **76** (1.28 g, 69%). White powder. M.p. 114-115 °C. R_f 0.34 (Et_2O/pentane 1:2). IR ($CHCl_3$): 3456*w*, 2984*m*, 2933*w*, 1708*s*, 1508*s*, 1476*m*, 1456*w*, 1410*w*, 1395*w*, 1369*m*, 1308*w*, 1169*s*, 1041*w*, 933*w*, 856*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, signals of rotamers in italics): 1.23 (*s*, 2 Me); 1.44 (*s*, *t*-Bu); 3.22-3.27 (*m*, CH_2); 5.02, *6.37* (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.90, 28.37 (Me); 43.61 (C); 47.98 (CH_2); 79.40, 156.27, 183.12 (C). EI-MS: 161 (9.1), 144 (7.2), 130 (4.6), 116 (5.6), 98 (11.4), 88 (42.0), 70 (24.3), 57 (100.0), 41 (30.7), 30 (30.8). Anal. calc. for $C_{10}H_{19}NO_4$ (217.26): C 55.28, H 8.81, N 6.45; found: C 55.07, H 8.91, N 6.51.

Methyl 1-Cyanocyclopropane-1-carboxylate (77a). According to [259] 1,2-dibromoethane (113.5 g, 0.604 mol) was added to a mixture of methyl cyanoacetate (42.7 g, 0.431 mol), K_2CO_3 (131.1 g, 0.949 mol) in DMF (500 ml). The suspension was stirred for 20 h at r.t. After removal of the yellow solid by filtration, the filtrate was diluted with Et_2O (*ca.* 300 ml) and washed with

H_2O (3 × 200 ml). The ethereal phase was dried ($MgSO_4$), evaporated under reduced pressure to yield the crude product (24.5 g, 45%) that was further purified by distillation (b.p. 90°C, 750 Torr) to yield **77a** (21.5 g, 38%). Colorless oil. R_f 0.48 (Et_2O/pentane 1:1). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.63-1.71 (*m*, 2 CH_2); 3.83 (*s*, CO_2Me).

Methyl 1-({[(*tert*-Butoxy)carbonyl]amino}methyl)cyclopropane-1-carboxylate (Boc-β^{2,2}-HAc$_3$c-OMe; 78a)[152]. Nitrile **77a** (12.40 g, 97.6 mmol) was transformed according to *GP 16b*. FC (Et_2O/pentane 1:2) yielded **78a** (15.88 g, 71%). Colorless oil. A second batch yielded **78a** (10.45 g, 69%). B.p. 77 °C/0.3 Torr. R_f 0.32 (Et_2O/pentane 1:2). IR ($CHCl_3$): 3450*w*, 3008*m*, 2980*w*, 1709*s*, 1507*m*, 1439*m*, 1392*w*, 1367*m*, 1160*s*, 939*w*, 854*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.95-0.98 (*m*, 2 CH); 1.21-1.27 (*m*, 2 CH); 1.44 (*s*, *t*-Bu); 3.28 (*d*, *J* = 6.4, CH_2N); 3.68 (*s*, OMe); 5.18 (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.70 (CH_2); 24.96 (C); 28.38 (Me); 43.97 (CH_2); 51.98 (Me); 79.22, 156.17, 175.32 (C). FAB-MS: 459 (12.1, [2 *M* + 1]$^+$), 230 (69.4, [*M* + 1]$^+$), 229 (3.1, *M*$^+$), 174 (100), 130 (56.9). Anal. calc. for $C_{11}H_{19}NO_4$ (229.28): C 57.63, H 8.35, N 6.11; found: C 57.61, H 8.08, N 6.05.

Methyl 1-({[(*tert*-Butoxy)carbonyl]amino}methyl)cyclobutane-1-carboxylate (Boc-β^{2,2}-HAc$_4$c-OMe; 78b). Nitrile **77b** [53] (2.20 g, 15.8 mmol) was transformed according to *GP 16a*. FC (Et_2O/pentane 1:3) yielded **78b** (2.10 g, 55%). Colorless oil. R_f 0.33. IR ($CHCl_3$): 3453*m*, 3026*m*, 3016*m*, 2981*m*, 2954*m*, 2874*w*, 1712*s*, 1507*s*, 1436*m*, 1393*m*, 1368*m*, 1333*m*, 1250*s*, 1236*m*, 1167*s*, 1128*s*, 1006*w*, 981*w*, 946*w*, 860*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.44 (*s*, *t*-Bu); 1.84-2.12 (*m*, 4 CH); 2.34-2.44 (*m*, 2 CH); 3.50 (*d*, *J* = 6.3, NCH_2); 3.73 (*s*, CO_2Me); 4.94 (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.65, 27.64 (CH_2); 28.37 (Me); 45.36 (CH_2); 47.36 (C); 52.06 (Me); 79.26, 156.41, 176.58 (C). EI-MS: 244 (3.2, [*M* + 1]$^+$), 188 (43.8), 170 (34.8), 156 (38.1), 138 (32.8), 126 (61.1), 114 (100), 57 (95.8). Anal. calc. for $C_{12}H_{21}NO_4$ (243.30): C 59.24, H 8.70, N 5.76; found: C 59.33, H 8.62, N 5.70.

Methyl 1-({[(*tert*-Butoxy)carbonyl]amino}methyl)cyclopentane-1-carboxylate (Boc-β^{2,2}-HAc$_5$c-OMe; 78c). Nitrile **77c** [53] (4.11 g, 26.8 mmol) was transformed according to *GP 16a*. FC (Et_2O/pentane 1:5) yielded **78c** (3.93 g, 57%). Colorless oil. R_f 0.33 (Et_2O/pentane 1:5). IR ($CHCl_3$): 3452*w*, 3146*br*, 2982*m*, 2933*m*, 2862*w*, 1705*s*, 1507*s*, 1455*w*, 1393*w*, 1368*m*, 1326*w*, 1166*s*,

[152] The nomenclature of the 1-(amino*methyl*cycloalkane)carboxylic-acid derivatives is proposed in analogy to the corresponding 1-(aminocycloalkane)carboxylic-acid derivatives [3].

1128*m*, 1039*w*, 955*w*, 863*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.43 (*s*, *t*-Bu); 1.55-1.79 (*m*, 6 CH); 1.91-2.00 (*m*, 2 CH); 3.27 (*d*, *J* = 6.5, NCH_2); 3.70 (*s*, CO_2Me); 5.04 (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 25.57 (CH_2); 28.40 (Me); 34.45, 46.13 (CH_2); 52.05 (Me); 54.37, 79.17, 156.34, 178.24 (C). EI-MS: 279 (<1, $[M + Na]^+$), 257 (<1, M^+), 128 (100), 57 (37.3). Anal. calc. for $C_{13}H_{23}NO_4$ (257.33): C 60.68, H 9.01, N 5.44; found: C 60.79, H 9.08, N 5.38.

Methyl 1-({[(*tert*-Butoxy)carbonyl]amino}methyl)cyclohexane-1-carboxylate (Boc-β^{2,2}-HAc$_6$c-OMe; 78d). Nitrile **77d** (15.0 g, 88.0 mmol) was transformed according to *GP 16a*. FC (Et_2O/pentane 1:7 → 2:7) yielded **78d** (13.59 g, 57%). Colorless oil. R_f 0.26 (Et_2O/pentane 1:7). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.21-1.70 (*m*, *t*-Bu, 8 CH); 1.90-2.05 (*m*, 2 CH); 3.27 (*d*, *J* = 6.7, NCH_2); 3.70 (*s*, OMe); 4.90 (br. *s*, NH). **78d** was fully characterized as ethyl ester derivative **81**.

1-({[(*tert*-Butoxy)carbonyl]amino}methyl)cyclopropane-1-carboxylic Acid (Boc-β^{2,2}-HAc$_3$c-OH; 79a). Methyl ester **78a** (7.64 g, 33.0 mmol) was saponified according to *GP 15b*. Recrystallization (CH_2Cl_2/pentane or AcOEt/pentane) at 0°C yielded **79a** (6.93, 97%). Colorless needles, suitable for X-ray analysis. M.p. 121-122 °C. R_f 0.18 (CH_2Cl_2/MeOH 15:1). IR ($CHCl_3$): 3454*w*, 2974*br*, 1705*s*, 1508*m*, 1451*w*, 1395*w*, 1367*w*, 1169*w*, 1046*w*, 939*w*, 850*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88-1.02 (*m*, 2 CH); 1.24-1.30 (*m*, 2 CH); 1.44 (*s*, *t*-Bu); 3.28 (br. *d*, *J* = 5.6, NCH_2); 5.26, *6.11* (*br.*, NH) .^{13}C-NMR (100 MHz, $CDCl_3$): 15.44 (CH_2); 24.99 (C); 28.40 (Me); 43.69 (CH_2); 79.43, 156.32, 181.34 (C). FAB-MS: 216 (42.7, $[M + 1]^+$), 160 (100), 116 (35.6). Anal. calc. for $C_{10}H_{17}NO_4$ (215.25): C 55.80, H 7.96, N 6.51; found: C 55.32, H 7.52, N 6.27.

1-{{[(*tert*-Butoxy)carbonyl]amino}methyl}-cyclobutane-1-carboxylic Acid (Boc-β^{2,2}-HAc$_4$c-OH; 79b). Nitrile **77b** [53] (4.29 g, 30.8 mmol) was transformed according to *GP 16c*. Recrystallization (AcOEt/pentane) yielded **79b** (2.57 g, 36%). Colorless crystals, suitable for X-ray analysis. M.p. 94.2-95.2 °C. R_f 0.67 (MeOH/CH_2Cl_2 1:9). IR ($CHCl_3$): 3453*w*, 2978*m*, 2922*m*, 1706*s*, 1506*s*, 1450w, 1394*w*, 1361*m*, 1322*w*, 1250*m*, 1167*m*, 1128*w*, 1039*w*, 1006*w*, 956*w*, 917*w*, 861*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, signals of rotamers in italics): 1.44, *1.49* (*s*, *t*-Bu); 1.96-2.18 (*m*, 4 CH); 2.41-2.47 (*m*, 2 CH); 3.53 (br. *d*, *J* = 6.3, NCH_2); 5.02, *6.22* (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$, signal of rotamers in italics): *14.13*, 15.66, *22.66*, 27.59 (CH_2); 28.36, *31.60* (Me); 45.03, *46.50* (CH_2); 47.25, *47.81*, 79.46, *80.88*, 156.53, *157.71*, *180.41*, 182.01 (C). FAB-MS: 459 (11.3, $[2M + 1]^+$), 230 (49.6, $[M + 1]^+$), 174 (100). Anal. calc. for $C_{11}H_{19}NO_4$ (229.28): C 57.63, H 8.35, N 6.11; found: C 57.73, H 8.33, N 6.18.

1-({[(*tert*-Butoxy)carbonyl]amino}methyl)cyclopentane-1-carboxylic Acid (Boc-β^{2,2}-HAc$_5$c-OH; 79c). Nitrile **77c** [53] (13.79 g, 90.0 mmol) was transformed according to *GP 16c*. Recrystallization (AcOEt/hexane) yielded **79c** (7.03 g, 32%). Colorless crystals, suitable for X-ray analysis. M.p. 124.5-125.5 °C. R_f 0.45 (MeOH/CH_2Cl_2 1:9). IR ($CHCl_3$): 3444*w*, 2967*m*, 2867*w*, 1700*s*, 1506*s*, 1450*w*, 1394*m*, 1367*m*, 1167*s*, 1039*w*, 906*w*, 856*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, signals of rotamers in italics): 1.44, *1.47* (*s*, *t*-Bu); 1.60-1.76 (*m*, 6 CH); 2.00-2.11 (*m*, 2 CH); 3.28 (*d*, *J* = 6.5, NCH_2); 5.11, *6.33* (br. *t*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$, signals of rotamers in italics): *25.33*, 25.70 (CH_2); 28.38 (Me); *34.15*, 34.62, 45.75, *46.88* (CH_2); 54.24, *54.70* (C); 79.36, *80.77*, 156.43, *157.74*, *181.86*, 183.97 (C). FAB-MS: 768 (8.7, [3*M* + K]$^+$), 525 (12.3, [2*M* + K]$^+$), 509 (10.8, [2*M* + Na]$^+$), 487 (16.3, [2*M* + 1]$^+$), 266 (25.5, [*M* + Na]$^+$), 244 (11.6, [*M* + 1]$^+$), 188 (61.0), 170 (100), 142 (50.0). Anal. calc. for $C_{12}H_{21}NO_4$ (243.30): C 59.24, H 8.70, N 5.76; found: C 59.16, H 8.60, N 5.79.

1-({[(*tert*-Butoxy)carbonyl]amino}methyl)cyclohexane-1-carboxylic Acid (Boc-β^{2,2}-HAc$_6$c-OH; 79d). Methyl ester **78d** (3.20 g, 11.8 mmol) was saponified according to *GP 15b*. Recrystallization (CH_2Cl_2/AcOEt/hexane) yielded **79d** (2.44 g, 80%). Colorless crystals, suitable for X-ray analysis. M.p. 156-158 °C. R_f 0.29 (MeOH/CH_2Cl_2 1:20). IR (KBr): 3318*m*, 3260*m*, 3107*w*, 2982*m*, 2954*m*, 2867*m*, 2550*w*, 1894*w*, 1706*s*, 1656*s*, 1483*m*, 1449*s*, 1411*s*, 1367*s*, 1330*m*, 1319*m*, 1237*m*, 1204*m*, 1151*s*, 1140*s*, 1102*m*, 1082*w*, 1047*m*, 1027*m*, 980*m*, 950*w*, 934*w*, 921*w*, 881*w*, 849*w*, 822*w*, 784*w*, 767*w*, 749*w*, 687*w*, 659*w*, 590*w*, 554*w*, 536*w*, 446*w*, 410*w*. ^{1}H-NMR (400 MHz, $(CD_3)_2NCDO$, signals of rotamers in italics): 1.19-1.39 (*m*, *t*-Bu, 5 CH); 1.51-1.59 (*m*, 3 CH); 1.91-1.99 (*m*, 2 CH); 3.21 (*d*, *J* = 6.5, CH_2N); *6.21*, 6.51 (*br.*, NH). ^{13}C-NMR (100 MHz, $(CD_3)_2NCDO$, signals of rotamers in italics): 23.41, 26.19 (CH_2); 28.46 (Me); 32.00, 48.56, *48.76* (CH_2); 78.62, 157.04, 177.77 (C). FAB-MS: 537 (7.1, [2*M* + Na]$^+$), 515 (13.5, [2*M* + 1]$^+$), 280 (21.4, [*M* + Na]$^+$), 258 (33.3, [*M* + 1]$^+$), 202 (100), 184 (87.0), 156 (60.3). Anal. calc. for $C_{13}H_{23}NO_4$ (257.33): C 60.68, H 9.01, N 5.44; found: C 60.41, H 8.96, N 5.42.

1-({[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}methyl)cyclopropane-1-carboxylic Acid (Fmoc-β^{2,2}-HAc$_3$c-OH; 80a). β-Amino acid **79a** (0.5 g, 2.32 mmol) was Boc-deprotected according to *GP 10a* to yield the corresponding TFA salt that was Fmoc-protected according to *GP 11*. FC (CH_2Cl_2/MeOH 9:1) and recrystallization (Et_2O/pentane) yielded **80a** (0.626 g, 80%). White powder. M.p. 156-157.5 °C. R_f 0.36 (CH_2Cl_2/MeOH 15:1). IR ($CHCl_3$): 3453*m*, 3006*m*, 2961*m*, 1716*s*, 1513*s*, 1478*w*, 1450*m*, 1336*w*, 1261*s*, 1102*s*, 1034*m*, 1009*s*,

917*w*, 862*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, signals of rotamer (ca. 17%) in italics): *0.54* (br. *s*, CH_2); 1.07-1.10 (*m*, 2 CH); *1.14* (br. *s*, CH_2); 1.32-1.39 (*m*, 2 CH); *3.05* (br. *d*, NCH_2); 3.36 (*d*, *J* = 6.4, NCH_2); 4.19-4.23 (m, OCH); 4.39 (*d*, *J* = 7.0, OCH_2); *4.54* (br. *d*, OCH_2); 5.47 (*t*, *J* = 6.2, NH); *5.96* (*br.*, NH); 7.29-7.33 (*m*, 2 arom. H); 7.39 (*t*, *J* = 7.4, 2 arom. H); 7.51-7.60 (*m*, 2 arom. H); 7.76 (*d*, *J* = 7.5, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$, signals of rotamer (ca. 17%) in italics): 15.61 (2 CH_2); 24.82 (C); 44.12, *44.65* (CH_2); 47.26 (CH); *65.87*, 66.81 (CH_2); 119.99, 124.64, 125.06, 127.05, 127.70 (CH); 141.33, 143.93, 156.81, *180.02*, 181.11 (C). FAB-MS: 697 (5.9, [2*M* + Na]$^+$), 675 (42.5, 2*M*$^+$), 360 (11.8, [*M* + Na]$^+$), 338 (61.9, [*M* + 1]$^+$), 178 (100), 165 (23.3), 142 (22.4).

1-({[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}methyl)cyclohexane-1-carboxylic Acid (Fmoc-β^{2,2}-HAc$_6$c-OH; 80b). Nitrile **77d** (3.0 g, 18.1 mmol) was reduced and saponified as described in *GP 16c*, but, instead of Boc-protection, the crude free amino acid was Fmoc-protected according to *GP 11*. Recrystallization (CH_2Cl_2) gave **80b** (5.00 g, 73%). Crystalline solid (needles). M.p. 175-185 °C. R_f 0.56 (CH_2Cl_2/MeOH 9:1). IR ($CHCl_3$): 3677*w*, 3448*w*, 3032*w*, 3012*m*, 2938*m*, 2860*w*, 1717*s*, 1517*s*, 1451*m*, 1228*s*, 1220*s*, 1204*s*, 1137*w*, 1106*w*, 1040*w*, 880*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88-2.17 (*m*, 10 CH); 3.13 (br. *s*, 0.2 H, NCH_2, rotamer); 3.36 (*d*, *J* = 6.6, 1.8 H, NCH_2, rotamer); 4.08-4.23 (*m*, 1 CH); 4.31 (*d*, *J* = 7.2, 1.8 H, OCH_2, rotamer); 4.47 (br. *s*, 0.2 H, OCH_2); 5.78 (br. *s*, 0.1 H, NH, rotamer); 6.41 (*s*, 0.9 H, NH, rotamer); 7.32 (*d*, *J* = 7.5, 2 arom. H); 7.39 (*d*, *J* = 7.5, 2 arom. H); 7.69 (*d*, *J* = 7.7, 2 arom. H); 7.85 (*d*, *J* = 7.6, 2 arom. H); 10.79 (br. *s*, COOH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.55, 26.46, 29.83, 32.23 (CH_2); 48.08 (CH); 48.74 (CH_2); 49.24 (C); 66.98 (CH_2); 120.77, 126.15, 127.90, 128.47 (CH); 142.08, 145.14, 157.43, 177.02 (C). FAB-MS: 759 (3.0, [2*M* + 1]$^+$), 380 (50.6, [*M* + 1]$^+$), 289 (43.4). Anal. calc. for $C_{23}H_{25}NO_4$ (379.45): C 72.80, H 6.64, N 3.69; found: C 72.64, H 6.69, N 3.66.

Ethyl 1-({[(*tert*-Butoxy)carbonyl]amino}methyl)cyclohexane-1-carboxylate (Boc-β^{2,2}-HAc$_6$c-OEt; 81) and **Ethyl 1-({[(*tert*-Butoxy)carbonyl]-ethyl-amino}methyl)cyclohexane-1-carboxylate (Boc-β^{2,2}-NEt–HAc$_6$c-OEt; 82).** Methyl 1-cyanocyclohexane-1-carboxylate **77d** (5.63 g, 33.7 mmol) was transformed according to *GP 16a*, except that EtOH was used instead of MeOH (8 bar, 90° C). FC (AcOEt/pentane 1:12) yielded **81** (4.90 g, 51%) and **82** (2.03 g, 19%).

Data of **81**: Colorless oil. R_f 0.21 (AcOEt/pentane 1:12). IR ($CHCl_3$): 3451*w*, 2983*w*, 2936*m*, 2861*w*, 1711*s*, 1509*s*, 1454*m*, 1393*w*, 1368*m*, 1165*s*, 1137*m*, 1102*w*, 1022*w*, 967*w*, 859*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.27 (*t*, *J* = 7.1, Me);

1.42 (*s*, *t*-Bu); 1.25-1.64 (*m*, 4 CH_2); 1.96-2.01 (*m*, CH_2); 3.27 (*d*, *J* = 6.4, NCH_2); 4.16 (*q*, *J* = 7.1, OCH_2); 4.76 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.24 (Me); 22.51, 25.69 (CH_2); 28.37 (Me); 31.46, 47.37 (CH_2); 47.70 (C); 60.60 (CH_2); 79.18, 155.99, 175.99 (C). EI-MS: 286 (3.6, $[M + 1]^+$), 230 (31.7), 212 (11.7), 184 (39.9), 156 (100.0), 138 (19.0), 128 (29.4), 110 (10.5), 95 (26.1), 81 (28.5), 74 (8.9), 67 (14.0), 57 (80.4), 41 (24.4), 30 (16.1). Anal. calc. for $C_{15}H_{27}NO_4$ (285.38): C 63.13, H 9.54, N 4.91; found: C 63.34, H 9.52, N 5.07.

Data of **92**: R_f 0.37 (AcOEt/pentane 1:12). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.03 (*t*, *J* = 7.1, Me); 1.15-1.31 (*m*, NCH_2CH_3, 4 CH); 1.45 (*s*, *t*-Bu); 1.53-1,60 (*m*, 4 CH); 2.06-2.12 (*m*, 2 CH); 3.09-3.12 (*m*, NCH_2CH_3); 3.69 (*s*, NCH_2); 4.14 (*q*, *J* = 7.1, OCH_2). ^{13}C-NMR (50 MHz, $CDCl_3$): 12.63, 13.61 (Me); 22.60, 25.23 (CH_2); 27.84 (Me); 31.99, 42.95 (CH_2); 48.08 (C); 54.92, 59.87 (CH_2); 78.63, 155.55, 175.17 (C). EI-MS: 313 (4.2, $[M + 1]^+$), 158 (100).

Methyl 1- ({[(*tert*-Butoxy)carbonyl]((1-(methoxycarbonyl)cyclopropyl)methyl)-amino}methyl)cyclopropane-1-carboxylate (83). Similarly to [132], nitrile **77a** (0.57 g, 4.56 mmol) and $CoCl_2 \cdot 6\ H_2O$ (2.17 g, 9.1 mmol) were dissolved in MeOH (23 ml). At 0 °C, $NaBH_4$ (1.73 g, 45.6 mmol) was added in portions (vigorous gas development) to the deep blue soln. After 1 h, the black precipitate was dissolved by the addition of 10% HCl (25 ml) at 0 °C. After evaporation of MeOH at RV, it was extracted with pentane (2×), 30% NH_3-soln. and 1N NaOH and extracted with Et_2O (3×). The ethereal phase was washed with sat. aq. NaCl-soln., dried ($MgSO_4$) and evaporated at RV (40 °C, 750 mbar). The crude secondary amine (0.14 g, 24%) and Boc_2O (0.25 g, 1.13 mmol) were dissolved in CH_2Cl_2 (3 ml) and stirred for 12 h. After dilution with CH_2Cl_2 (10 ml) and aqueous work-up with sat. aq. NH_4Cl-, $NaHCO_3$-, and NaCl-solns., the org. phase was dried ($MgSO_4$) and evaporated. FC (Et_2O/pentane 1:2) yielded **83** (85 mg, 37%). White solid. M.p. 72.74.5 °C. R_f 0.28 (Et_2O/pentane 1:2). IR ($CHCl_3$): 3008*w*, 2954*w*, 1716*s*, 1682*s*, 1603*w*, 1437*m*, 1368*m*, 1309*m*, 1152*s*, 934*w*, 903*w*, 862*w*. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.91-1.08 (*br.* , 4 CH), 1.18-1.26 (*m*, 4 CH); 1.44 (*s*, *t*-Bu); 3.66 (*s*, 2 OMe): 3.75 (br. *s*, 2 NCH_2). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.22 (CH_2); 23.33 (C); 27.87 (Me); 47.87 (CH_2); 51.45 (Me); 79.42, 156.06, 174.63 (C). EI-MS: 341 (<1, M^+), 285 (3.4), 254 (6.4), 240 (15.2), 226 (83.1), 142 (47.0), 128 (100).

***tert*-Butyl (2*S*,5*S*)-2-(*tert*-Butyl)-5-isobutyl-3,5-dimethyl-4-oxo-1-imidazolidin-carboxylate (85).** According to [470], a soln. of $(i\text{-}Pr)_2EtN$ (14 ml, 98.9 mmol) in THF (152 ml) was cooled to – 78 °C, followed by the dropwise addition of BuLi (65 ml, 104 mmol). The soln. was kept at 0 °C for 30 min and recooled

to – 78 °C. (*S*)-Boc-BMI (**84**; 23.4 g, 91.43 mmol) in THF (76 ml) was slowly added while maintaining the internal temp. below – 65 °C. Stirring was continued for 30 min before MeI (6.1 ml, 98.8 mmol) was added in one portion. After 1 h at – 78 °C BuLi (60.7 ml, 92 mmol) was added to the white suspension (T < – 70 °C). After further 30 min i-BuI (52.5 ml, 0.453 mol) was added in one portion and the reaction was allowed to warm up to r.t. overnight. The clear yellow soln. was quenched with 1N HCl (140 ml) and diluted with AcOEt. The org. phase was washed with sat. aq. $Na_2S_2O_3$, $NaHCO_3$ and NaCl solns., dried ($MgSO_4$) and evaporated under reduced pressure. FC (AcOEt/pentane 1:5 1:1) yielded **85** (25.88 g, 86%). White waxy solid. M.p. 57-58 °C. R_f 0.35 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.}$ = – 43.0 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 2980*m*, 1693*s*, 1482*m*, 1434*w*, 1407*m*, 1362*s*, 1306*m*, 1256*m*, 1171*m*, 1115*w*, 1047*w*, 985*w*, 906*w*, 884*w*, 857*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, only main rotamer): 0.70 (*d*, *J* = 6.5, Me); 0.85 (*d*, *J* = 6.7, Me); 1.00 (*s*, *t*-Bu); 1.31-1.40 (*m*, Me_2CH); 1.49 (*s*, Me); 1.49 (*s*, *t*-Bu); 1.41 (*dd*, *J* = 14.4, 9.2, CHH); 2.15 (*dd*, *J* = 14.4, 3.9, CHH); 2.98 (*s*, NMe); 5.04 (s, NCH). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.68 (Me); 24.06 (CH); 27.08, 28.31, 31.70 (Me); 39.07 (C); 46.61 (CH_2); 63.64 (Me); 80.34 (C); 80.55 (CH); 154.91, 174.29 (C). FAB-MS: 677 (<1, [2*M* + Na]$^+$), 654 (4.4, [2*M* + 1]$^+$), 349 (1.5, [*M* + Na]$^+$), 327 (46.0, [*M* + 1]$^+$), 213 (100), 169 (24.0). Anal. calc. for $C_{18}H_{34}N_2O_3$ (326.48): C66.22, H 10.50, N 8.59; found: C 66.26, H 10.44, N 8.58.

(*S*)-*N*-Benzoyl-2-methyl-leucine-methylamide (86). **85** (12.7 g, 37.3 mmol) was hydrolyzed as described in [470]. The intermediate HCl salt (7.31 g, 92%) was benzoylated according to [90]. FC (AcOEt/pentane 1:1) yielded **86** (3.5 g, 24%). Colorless glass. R_f 0.18 (AcOEt/pentane 1:1). $[\alpha]_D^{r.t.}$ = + 30.8 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3467*w*, 3377*m*, 3007*m*, 2961*m*, 2871*w*, 1651*s*, 1602*w*, 1579*m*, 1511*s*, 1484*s*, 1440*m*, 1415*m*, 1378*w*, 1315*w*, 1030*w*, 969*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.85 (*d*, *J* = 6.5, Me); 0.90 (*d*, *J* = 6.6, Me); 1.57-1.71 (*m*, Me_2CH, CHH); 1.72 (*s*, Me); 2.69 (*dd*, *J* = 14.0, 5.1, CHH); 2.88 (*d*, *J* = 4.8, NH*Me*); 6.35 (br. *d*, *J* = 4.1, NHMe); 7.41-7.52 (*m*, 3 arom. H); 7.78 (*s*, NH); 7.80-7.89 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.11, 23.87, 24.89 (Me); 24.95 (CH); 26.77 (Me), 45.26 (CH_2), 60.20 (C); 126.87, 126.59, 131.43 (CH); 135.25, 166.34, 175.51 (C). FAB-MS: 548 (< 1, [2*M* + Na]$^+$), 525 (19.9 [2*M*]$^+$), 285 (4.0, [*M* + Na]$^+$), 263 (100, [*M* + 1]$^+$), 232 (41.5), 204 (39.7), 104.9 (49.3). Anal. calc. for $C_{15}H_{22}N_2O_2$ (262.36): C 68.67, H 8.45, N 10.68; found: C 68.33, H 8.60, N 10.44.

7.3.6 Preparation of β²- and β³-Homoproline

HPLC Analysis of (*R*)-, (*S*)-, or *rac*-93: General Procedures 17 (*GP 17*). Derivatization of 90 With 2,4-Dinitrofluorobenzene: General Procedure 17a (*GP 17a*). To a soln. of **90** in H_2O (0.5M) was added $NaHCO_3$ (1.2 equiv.) and a soln. of 2,4-dinitrofluorobenzene (1.2 equiv.) in EtOH (0.35M) at 0 °C. After 1 h, EtOH was evaporated and the pH adjusted to 2 with 1N HCl and extracted with Et_2O (2×). The Et_2O phase was filtrated through a Buchner funnel packed with silica gel on a $MgSO_4$-layer and evaporated to yield crude **93**. The yellow oil was dissolved in i-PrOH/hexane 35:165 (1 mg/ml) and injected onto the HPLC system according to *GP 17c*.

b) Derivatization of 95: General Procedure 17b (*GP 17b*). A soln. of **95** (5 mg, 0.022 mmol) in HCl/EtOH[153] (1 ml, 4M) was heated to 110 °C for 1.5 h in a *Wheats V-Vial* (with teflon-faced rubber septum) to yield HCl·**90**. The HCl salt was further derivatized according to *GP 17a*.

c) HPLC Conditions for Determination of Enantiomer Ratio of 93: General procedure 17c (*GP 17c*). HPLC Analysis was performed on a *Daicel Chiralcel OD* column (4.6 × 250 mm) by using an isocratic eluent of i-PrOH/hexane 35:165 at a flow rate of 1 ml/min with UV detection at 390 nm at 25 °C. t_R in min.

Ester Hydrolysis: General Procedures 18 (*GP 18*). A soln. of the fully protected amino acid was treated with LiOH (2.5 equiv.) in MeOH/H_2O 3:1 (0.15M) at r.t. After stirring at r.t. for 1-3 d the mixture was diluted with H_2O (in the case of small scale reactions) and extracted with Et_2O (2×). The soln. was adjusted to pH 2 at 0 °C with 10% HCl and extracted with Et_2O (3×). The org. phase was washed with H_2O, dried ($MgSO_4$) and concentrated under reduced pressure.

***tert*-Butyl (*R*)-2-Diazoacetyl-1-pyrrolidine-1-carboxylate (Boc-(*R*)-Pro-CHN$_2$; (*R*)-87).** Boc-D-Pro-OH (13.99 g, 65.0 mmol) was transformed according to *GP 1*. FC (AcOEt/pentane 1:3) yielded (*R*)-**87** (8.70 g, 56%). Yellow waxy solid. M.p. 47-48 °C. R_f 0.25 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.}$ = + 146 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3120*w*, 3008*m*, 2980*m*, 2881*w*, 2110*s*, 1690*s*, 1646*m*, 1477*w*, 1454*w*, 1394*s*, 1367*s*, 1323*m*, 1163*m*, 1123*m*. ^{1}H-NMR (400 MHz, $CDCl_3$, rotamers in italics): 1.44, *1.48* (*s*, *t*-Bu); 1.84-2.27 (*m*, CH_2CH_2); 3.36-3.56 (*m*, NCH_2); 4.24

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[153] Freshly prepared according to [303] by slow addition of EtOH (0.5 ml) to AcCl (0.85 ml) at 0°C and dilution to 1 ml.

(*br.*, NCH); 5.44 (*br.*, N_2CH). ^{13}C-NMR (100 MHz, $CDCl_3$, rotamers in italics): 23.71, *24.41* (CH_2); 28.38, *28.44* (Me); *29.67*, 31.27, 46.79, *47.10* (CH_2); 52.01, *53.11*, *63.60*, 64.48 (CH), *80.13*, 80.45, 109.16, *154.14*, 154.82, *195.09*, 196.06 (C). EI-MS: 170 (16.2), 114 (52.4), 70 (93.8), 57 (100). Anal. calc. for $C_{11}H_{17}N_3O_4$ (239.27): C 55.22, H 7.16, N 17.56; found: C 55.38, H 7.22, N 17.36.

***tert*-Butyl (*S*)-2-Diazoacetyl-1-pyrrolidine-1-carboxylate (Boc-(*S*)-Pro-CHN$_2$; (*S*)-87).** L-Boc-Pro-OH (26.9 g, 125 mmol) was transformed according to *GP 1*. FC (AcOEt/pentane 1:3) yielded (*S*)-**87** (22.9 g, 77%). Yellow waxy solid. R_f 0.25 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.} = -145$ (c = 1.0, $CHCl_3$). Other spectroscopic data: corresponding to (*R*)-**87**.

Benzyl (*S*)-1-{[(*tert*-Butoxy)carbonyl]-2-pyrrolidin-2-yl}acetate (Boc-(*S*)-β^3-HPro-OBn; (*S*)-88). (*S*)-**87** (6.00 g, 25.0 mmol) was transformed according to *GP 4*, using CF_3CO_2Ag (10%). FC (Et_2O/pentane 1:2) yielded (*S*)-**88** (5.40 g, 68%). Colorless oil. R_f 0.32 (Et_2O/pentane 1:2). $[\alpha]_D^{r.t.} = -35.7$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3007*w*, 2977*m*, 2882*w*, 1730*m*, 1684*s*, 1477*w*, 1455*w*, 1403*s*, 1367*m*, 1304*w*, 1166*m*, 1125*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.45 (*s*, *t*-Bu); 1.70-1.89 (*m*, 3 CH); 2.00-2.09 (*m*, 1 CH); 2.36 (*dd*, *J* = 15.1, 9.8, COCH); 2.82-3.04 (br., COCH), 3.30-3.34 (*br.*, NCH_2); 4.12, *4.20* (*s*, NCH); 5.12 (*s*, $PhCH_2$); 7.35 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$, rotamers in italics): 22.80, *23.53* (CH_2); 28.50 (Me); *30.56*, 31.27, *38.53*, 39.37, 46.20, *46.59* (CH_2); 54.05 (CH); 66.21 (CH_2); *79.27*, 79.62 (C); 128.21, 128.54 (CH); 135.92, 154.27, 171.36 (C). EI-MS: 320 (<1, $[M + 1]^+$), 319 (<1, M^+), 21 (100), 128 (67.2), 91 (89.0), 70 (45.3). Anal. calc. for $C_{18}H_{25}NO_4$ (319.40): C 67.69, H 7.89, N 4.39; found: C 67.83, H 7.89, N 4.42.

Benzyl (*R*)-1-{[(*tert*-Butoxy)carbonyl]-2-pyrrolidin-2-yl}acetate (Boc-(*R*)-β^3-HPro-OBn; (*R*)-88). (*R*)-**87** (3.00 g, 12.5 mmol) was transformed according to *GP 4*. FC (Et_2O/pentane 1:2) yielded (*R*)-**88** (2.99 g, 75%). Colorless oil. $[\alpha]_D^{r.t.} = +36.3$ (c = 1.0, $CHCl_3$). Other spectroscopic data: corresponding to (*S*)-**88**.

(*R*)-1-{[(*tert*-Butoxy)carbonyl]-2-pyrrolidin-2-yl}acetic Acid (Boc-(*R*)-β^3-HPro-OH; (*R*)-89). (*R*)-**87** (5.20 g, 21.7 mmol) was transformed according to *GP 2a*. Recrystallization (CH_2Cl_2/hexane) yielded (*R*)-**89** (3.76 g, 76%). White powder. M.p. 99-100 °C. R_f 0.30 (CH_2Cl_2/MeOH 20:1). $[\alpha]_D^{r.t.} = +40.6$ (c = 1.9, DMF). IR ($CHCl_3$): 2980*m*, 2881*w*, 1711*s*, 1684*s*, 1477*w*, 1403*s*, 1368*m*, 1286*w*, 1168*s*, 1127*m*, 927*w*, 860*w*. ^{1}H-NMR (400 MHz, CD_3OCD_3): 1.44 (*s*, *t*-Bu); 1.77-1.96 (*m*, 3 CH); 2.01-2.10 (*m*, 1 CH); 2.26-2.37 (*m*, COCH); 2.75-2.95 (*m*, COCH); 3.31 (*m*, NCH_2); 4.05-4.11 (*m*, NCH). ^{13}C-NMR (100 MHz, CD_3OCD_3, rotamers

in italics): *28.28*, 24.06 (CH_2); 28.63 (Me); 31.19, *32.00*, 38.48, *39.55*, *46.90*, 47.25 (CH_2); 54.91 (CH); 79.28, *154.45*, 154.76, 172.85 (C). FAB-MS: 481 (13.4, $[2M + Na]^+$), 459 (14.7, $[2M + 1]^+$), 252 (28.6, $[M + Na]^+$), 230 (100, $[M + 1]^+$), 174 (92.1), 130 (55.6). Anal. calc. for $C_{11}H_{19}NO_4$ (229.28): C 57.63, H 8.35, N 6.11; found: C 57.51, H 8.34, N 6.04.

(*S*)-1-{[(*tert*-Butoxy)carbonyl]-2-pyrrolidin-2-yl}acetic Acid (Boc-(*S*)-β³-HPro-OH; (*S*)-89). (*S*)-**87** (20.34 g, 85.0 mmol) was transformed according to *GP 2a*. Recrystallization (CH_2Cl_2/hexane) yielded (*S*)-**89** (11.86 g, 61%). White powder. $[\alpha]_D^{r.t.} = -40.5$ (*c* = 1.9, DMF) ([286]: $[\alpha]_D^{r.t.} = -41.6$ (*c* = 1.9, DMF); [287]: $[\alpha]_D^{r.t.} = -39.5$ (*c* = 1.9, DMF)). Other spectroscopic data: corresponding to (*R*)-**89**.

Ethyl (*S*)-Piperidine-3-carboxylate (*S,S*)-Hydrogen Tartrate ((*S*)-Ethyl Nipecotate (*S,S*)-Hydrogen Tartrate; (*S,S,S*)-92). According to [292] *rac* ethyl nipecotate (*rac*-**90**; 28.0 g, 0.178 mol) was resolved with (*S,S*)-tartaric acid ((*S,S*)-**91**; 26.7 g, 0.178 mol) to yield the hydrogen tartrate (*S,S,S*)-**92** (15.04 g, 27%) after recrystallization from EtOH (3×). M.p. 156-157 °C ([292]: M.p. 155-156 °C). $[\alpha]_D^{r.t.} = -46.3$ (*c* = 2.0, $(NH_4)_6Mo_7O_{24}$ (0.2% aq. soln.)) ([292]: $[\alpha]_D^{r.t.} = -51.0$ (*c* = 2.0, $(NH_4)_6Mo_7O_{24}$ (0.2% aq. soln.))).

Ethyl (*R*)-Piperidine-3-carboxylate (*R,R*)-Hydrogen Tartrate ((*R*)-Ethyl Nipecotate (*R,R*)-Hydrogen Tartrate; (*R,R,R*)-92). According to [292] *rac* ethyl nipecotate (*rac*-**90**; 11.28 g, 71.6 mmol) was resolved with (*R,R*)-tartaric acid ((*R,R*)-**91**; 14.5 g, 67.0 mmol) to yield the hydrogen tartrate (*R,R,R*)-**92** (5.44 g, 25%) after recrystallization from EtOH (3×). M.p. 156-158 °C ([292]: M.p. 155-156 °C). $[\alpha]_D^{r.t.} = +52.3$ (*c* = 2.0, $(NH_4)_6Mo_7O_{24}$ (0.2% aq. soln.)) ([292]: $[\alpha]_D^{r.t.} = +51.0$ (*c* = 2.0, $(NH_4)_6Mo_7O_{24}$ (0.2% aq. soln.))).

Ethyl (S)-Piperidine-3-carboxylate ((*S*)-Ethyl Nipecotate; (*S*)-β²-HPro-OEt; (*S*)-90). Similarly to [292], (*S,S,S*)-**92** (30 g, 97.6 mmol) was dissolved at 0 °C in sat. aq. NaCl soln. (50 ml). At this temp., the pH was carefully adjusted to 13 and the aq. phase was extracted rapidly with Et_2O (3×). The ethereal phases were washed with H_2O, dried ($MgSO_4$) and evaporated to yield (*S*)-**90** (10.0 g, 65%). Yellowish oil. $[\alpha]_D^{r.t.} = +1.38$ (*c* = 5.0, H_2O) ([292]: $[\alpha]_D^{r.t.} = +1.6$ (*c* = 5.0, H_2O)). The e.r. was determined by derivatization according to *GP 17a* and subsequent HPLC analysis according to *GP 17c*: e.r. 99.6 : 0.4.

Ethyl (*R*)-Piperidine-3-carboxylate ((*R*)-Ethyl Nipecotate; (*R*)-β²-HPro-OEt; (*R*)-90). Similarly to [292], (*R,R,R*)-**92** (19.5 g, 64 mmol) was dissolved at 0 °C in sat. aq. NaCl soln. (30 ml). At this temp., the pH was carefully adjusted to 13 and the aq. phase was extracted rapidly with Et_2O (3×). The ethereal phases were washed with H_2O, dried ($MgSO_4$) and evaporated to yield (*R*)-**90** (4.5 g, 45%). Yellowish oil. $[\alpha]_D^{r.t.} = -1.26$ (*c* = 5.0, H_2O) ([292]: $[\alpha]_D^{r.t.} = -1.8$ (*c* = 5.0, H_2O)). The e.r. was determined by derivatization according to *GP 17a* and subsequent HPLC analysis according to *GP 17c*: e.r. 98.9 : 1.1.

Ethyl *rac-N*-(2,4-Dinitrophenyl)piperidine-3-carboxylate (*rac*-93). Similarly to [306], to a soln. of *rac* ethyl nipecotate (*rac*-**90**; 0.671 g, 4.27 mmol) and $NaHCO_3$ (0.43 g, 5.12 mmol) in H_2O (8.5 ml) was added a soln. of 2,4-dinitrofluorobenzene (0.95 g, 5.12 mmol) in EtOH (16 ml) at 0 °C. After 5 h, the soln. was diluted with Et_2O and washed with 1N HCl (1×) and sat. NH_4Cl-soln. (3×), dried ($MgSO_4$) and evaporated. FC (Et_2O/pentane 1:1) yielded *rac*-**93** (1.123 g, 81%). Orange sirup. R_f 0.31 (Et_2O/pentane 1:1). IR ($CHCl_3$): 3091*w*, 2961*w*, 2864*w*, 1726*m*, 1606*s*, 1530*s*, 1447*w*, 1336*s*, 1262*m*, 1178*m*, 1150*w*, 1097*m*, 1067*w*, 1030*m*, 965*w*, 943*w*, 916*w*, 858*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.25 (*t*, *J* = 7.1, Me); 1.71-1.91 (*m*, 3 CH); 2.10-2.17 (*m*, 1 CH); 2.72-2.78 (*m*, COCH); 3.07-3.13 (*m*, NCH); 3.30-3.39 (*m*, 2 NCH); 3.57-3.62 (*m*, NCH); 4.15 (*q*, *J* = 7.1, OCH_2); 7.19 (*d*, *J* = 9.4, 1 arom. CH); 8.25 (*dd*, *J* = 9.3, 2.7, 1 arom. CH); 8.70 (*d*, *J* = 2.7, 1 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.16 (Me); 23.93, 26.34 (CH_2); 40.95 (CH); 51.50, 52.52, 60.99 (CH_2); 119.95, 123.76, 128.17 (CH); 138.17, 138.30, 149.74, 172.61 (C). FAB-MS: 323 (7.1, M^+), 306 (100), 278 (29.7), 260 (25.5), 232 (59.9), 216 (45), 203 (67.7), 180 (45.2), 157 (29.4). Anal. calc. for $C_{14}H_{17}N_3O_6$ (323.30): C 52.01, H 5.30, N 13.00; found: C 52.27, H 5.52, N 12.85.

Ethyl (*R*)-*N*-(2,4-Dinitrophenyl)piperidine-3-carboxylate (*R*)-93. Prepared according to *GP 17a*. Purification by FC (Et_2O/pentane 1 : 1). HPLC according to *GP 17c*: t_R 20.5. $[\alpha]_D^{r.t.} = -165.0$ (*c* = 0.6, $CHCl_3$). Other spectroscopic data: corresponding to *rac*-**93**.

Ethyl (*S*)-*N*-(2,4-Dinitrophenyl)piperidine-3-carboxylate (*S*)-93. Prepared according to *GP 17a*. Purification by FC (Et_2O/pentane 1 : 1). HPLC according to *GP 17c*: t_R 25.5. $[\alpha]_D^{r.t.} = +164.8$ (*c* = 0.6, $CHCl_3$). Other spectroscopic data: corresponding to *rac*-**93**.

Ethyl (*S*)-1-[(*tert*-Butoxy)carbonyl]piperidine-3-carboxylate (Boc-(*S*)-β²-HPro-OEt; 94). (*S*)-**90** (6.5 g, 41.3 mmol, e.r. 99.6:0.4) and Boc_2O (9.5 g, 43.5 mmol)

were dissolved in CH_2Cl_2 (80 ml). After stirring at r.t. for 16 h, the soln. was washed with sat. aq. NH_4Cl and NaCl solns., dried ($MgSO_4$) and evaporated at RV. FC (Et_2O/pentane 1:6 → 1:1) yielded **94** (7.9 g, 74%). Colorless oil. R_f 0.29 (Et_2O/pentane 1:6). $[\alpha]_D^{r.t.}$ = + 50.7 (*c* = 0.95, $CHCl_3$). IR ($CHCl_3$): 3008*m*, 2979*m*, 1725*s*, 1683*s*, 1476*w*, 1426*s*, 1393*w*, 1367*m*, 1170*s*, 1151*s*, 1043*w*, 928*w*, 880*w*, 856*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.26 (*t*, *J* = 7.1, Me); 1.46-1.55 (*m*, *t*-Bu, CH); 1.56-1.74 (*m*, 3 CH); 2.01-2.07 (*m*, CH); 2.39-2.46 (*m*, CH); 2.80 (*ddd*, *J* = 13.3, 11.3, 3.1, NCH); 2.97 (*br.*, NCH); 3.92 (br. *d*, *J* = 12.9, NCH); 3.94-4.35 (*m*, NCH); 4.13 (*q*, *J* = 7.1, OCH_2). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.20 (Me); 24.30, 24.37 (CH_2); 28.43 (Me); 41.46 (CH); 44.00, 45.65, 60.50 (CH_2); 79.67, 154.70, 173.49 (C). FAB-MS: 281 (<1, $[M + Na]^+$), 257 (<1, M^+), 200 (18.2), 156 (38.1), 128 (38.1), 86 (51.8), 84 (100), 57 (27.1), 49 (44.3). Anal. calc. for $C_{13}H_{23}NO_4$ (257.33): C 60.68, H 9.01, N 5.44; found: C 60.71, H 8.98, N 5.45.

(*S*)-1-[(*tert*-Butoxy)carbonyl]piperidine-3-carboxylic Acid (Boc-(*S*)-β²-HPro-OH; 95). **94** (6.2 g, 24.1 mmol) was saponified according to *GP 18* with LiOH (1.44 g, 60.25 mmol) in MeOH (130 ml) and H_2O (43 ml) for 3 d at r.t. Recrystallization (Et_2O/pentane) yielded **95** (5.01 g, 90%). Derivatization according to *GP 17b* and HPLC according to *GP 17c*: e.r. 97.9 : 2.1. White powder. M.p. 165-167 °C. R_f 0.29 (MeOH/CH_2Cl_2 1:10). $[\alpha]_D^{r.t.}$ = + 50.5 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 2980*w*, 2865*m*, 1709*s*, 1684*s*, 1467*w*, 1426*m*, 1367*m*, 1269*m*, 1173*m*, 1150*s*, 1040*w*, 1003*w*, 936*w*, 873*w*, 858*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.41-1.56 (*m*, *t*-Bu, CH); 1.60-1.76 (*m*, 2 CH); 2.05-2.10 (*m*, CH); 2.45-2.53 (*m*, COCH); 2.83-2.89 (*m*, NCH); 3.05 (*br.*, NCH); 3.86-3.91 (*m*, NCH); 4.12 (*br.*, NCH); 7.27 (*br.*, CO_2H). ^{13}C-NMR (100 MHz, $CDCl_3$): 24.12, 27.18 (CH_2); 28.40 (Me); 41.07 (CH); 43.83, 45.50, (CH_2); 79.92, 154.72, 178.88 (C). FAB-MS: 688 (9.6, $[3M + 1]^+$), 459 (16.4, $[2M + 1]^+$), 230 (15.1, $[M + 1]^+$), 174 (100), 156 (34.9), 154 (25.0), 136 (21.5), 128 (24.9). Anal calc. for $C_{11}H_{19}NO_4$ (229.28): C 57.63, H 8.35, N 6.11. found: C 57.50, H 8.16, N 5.97.

7.4 Synthesis of β-Peptides

Peptide Coupling with EDC: General Procedures 19 (*GP 19*). *GP 19a*: The appropriate TFA salt was dissolved in $CHCl_3$ (0.5M) and cooled to 0 °C. This was treated successively with Et_3N (5 equiv.), HOBt (1.2 equiv.), a soln. of the Boc-protected fragment (1 equiv.) in $CHCl_3$ (0.25M) and EDC (1.2 equiv). The mixture was allowed to warm to r.t. and then stirred until TLC indicated complete reaction. Subsequent dilution with $CHCl_3$ was followed by thorough washing with 1M HCl and sat. aq. $NaHCO_3$ and NaCl solns. The org. phase was dried ($MgSO_4$) and then concentrated under reduced pressure. FC or recrystallization yielded the pure peptide.

GP 19b: The appropriate TFA salt was dissolved in $CHCl_3$ and washed with 1N NaOH (3×). The H_2O phase was extracted with $CHCl_3$. The combined org. phase was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting free amino compound was coupled with the Boc-protected fragment according to *GP 19a*.

Anchoring of *N*-Fmoc-Protected β-Amino Acids on *ortho*-Chlorotrityl-Chloride Resin and Determination of Substitution of β-Amino Acid-Resin Esters: General Procedure 20 (*GP 20*). Esterification of the Fmoc-protected β-amino acid with the *ortho*-chlorotrityl-chloride resin was performed according to [446,450]. The resin (initial loading: 1.00 mmol Cl/g) was dried under h.v. for 20 min and swelled in CH_2Cl_2 (20 ml/mmol) for 10 min. A soln. of Fmoc-protected β-amino acid (0.7-0.9 equiv.) in CH_2Cl_2 (10 ml/mmol) and $(i\text{-}Pr)_2EtN$ (2.8 equiv.) were then added successively and the suspension was mixed by Ar bubbling for 4 h. Subsequently, the resin was filtered, washed (20 ml/mmol) with CH_2Cl_2/MeOH/$(i\text{-}Pr)_2EtN$ 17:2:1 (3 × 3 min), CH_2Cl_2 (3 × 3 min), DMF (2 × 3 min), CH_2Cl_2 (3 × 3 min), MeOH (2 × 3 min) and finally dried under h.v. for 12 h. The resin substitution was determined by measuring the absorbance of the dibenzofulvene piperidine adduct:

An aliquot (5-10 mg) of the Fmoc-amino acid resin was washed with MeOH and Et_2O in a small glass tube ("Glühröhrchen"), dried under h.v. for 20-30 min and weighed exactly (m_{resin}). 20% Piperidine in DMF (2 ml) was added. After 20 min this soln. was diluted with DMF to 25 ml in a graduated cylinder. The obtained soln. was dispensed in a UV cell, and DMF in another UV cell (blank) and the absorbance (A) was measured at 300, 289, and 266 nm

[451-453]. The loading (Subst) was calculated for each of the three values according to *Equation 1*.

$$\text{Subst (mmol/g resin)} = 25000 \cdot A / (\varepsilon \cdot m_{resin}) \quad (1)$$

Extinction coefficients of the dibenzofulvene piperidine adduct: ε(300 nm) = 7800; ε(289 nm) = 5800; ε(266 nm) = 17500; m_{resin} in mg.

The theoretical substitution of the *ortho*-chlorotrityl-chloride resin ($Subst_{theor.}$), which corresponds to 100% esterification, is given by *Equation 2* [446][154].

$$Subst_{theor.}\ \text{(mmol/g resin)} = n / [1 + 0.001 \cdot n(MW - 36.5)] \quad (2)$$

n = mmol of Fmoc-protected β-amino acid used for esterification per 1 g resin; MW = molecular weight of the Fmoc-protected β-amino acid.

The yield for the attachment to the resin (loading yield) was determined by *Equation 3*.

$$\text{Loading yield} = \text{Subst} / Subst_{theor.} \quad (3)$$

Anchoring of *N*-Fmoc-Protected β-Amino Acids on *Rink* Amide Resin: General Procedure 21 (*GP 21*). *Rink* amide resin [447,449] (loading 0.45 mmol/g) was swelled in DMF/CH_2Cl_2 1:1 (20 ml/mmol) for 30 min and Fmoc deprotected using 20% piperidine in DMF (30 ml/mmol, 2 × 15 min) under Ar bubbling. A soln. of Fmoc-protected β-amino acid (3 equiv.), BOP (3 equiv.) and HOBt (3 equiv.) in DMF (2 ml) and $(i\text{-Pr})_2EtN$ (9 equiv.) were added successively to the resin and the suspension was mixed for 12-60 min by Ar bubbling. Monitoring of the coupling was performed with TNBS [455]. The resin was then filtered and washed (60 ml/mmol) with DMF/CH_2Cl_2 1:1 (3 × 3 min) prior to the following Fmoc deprotection step. The initial loading of the *Rink* amide resin was used to calculate the amount of the first β-amino acid attached to the resin.

β-Peptide on *ortho*-Chlorotrityl-Chloride Resin: General Procedures 22 (*GP 22*). ***GP 22a:*** The Fmoc group of the first amino acid attached to the *ortho*-chlorotrityl-chloride resin was removed using 20% piperidine in DMF

[154] This formula does not take into account the small difference in weight between the substituted chloride by methoxide; the latter is formed on the resin during the capping step.

(30 ml/mmol, 2 x 15 min) under Ar bubbling. The resin was then filtered and washed with DMF (30 ml/mmol, 6 × 3 min). For each coupling step, a soln. of the Fmoc-β-amino acid (3 equiv.), BOP (3 equiv.) and HOBt (3 equiv.) in DMF (2 ml) and $(i\text{-}Pr)_2EtN$ (9 equiv.) were added successively to the resin and the suspension was mixed by Ar bubbling for 15-60 min. Monitoring of the coupling reaction was performed with TNBS [455]. In case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react further for 15-60 min. The resin was then filtered and washed (30 ml/mmol) with DMF (3 × 3 min) prior to the following Fmoc deprotection step. After the removal of the last Fmoc protecting group, the resin was washed (30 ml/mmol) with DMF (6 × 3 min), CH_2Cl_2 (3 × 3 min), Et_2O (5 × 1 min) and dried under h.v. for 12 h.

GP 22b: As in *GP 22a*, except that the Fmoc group is removed using DBU/piperidine/DMF (30 ml/mmol); 1:1:48, 1 × 5 min, 1 × 10 min) under Ar bubbling.

β-Peptide on *Rink* Amide Resin: General Procedures 23 (*GP 23*). ***GP 23a:*** The Fmoc group of the first β-amino acid attached to the *Rink* amide resin was removed using 20% piperidine in DMF (30 ml/mmol, 2 x 20 min) under Ar bubbling. The resin was then filtered and washed with DMF/CH_2Cl_2 1:1 (50 ml/mmol, 6 × 3 min). For each coupling step, a soln. of the Fmoc β-amino acid (3 equiv.), BOP (3 equiv.) and HOBt (3 equiv.) in DMF (2 ml) and $(i\text{-}Pr)_2EtN$ (9 equiv.) were added successively to the resin and the suspension was mixed by Ar bubbling for 15-60 min. Monitoring of the coupling reaction was performed with TNBS [455]. In case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react further for 15-60 min with an additional equiv. of Fmoc β-amino acid and coupling reagents. The resin was then filtered and washed (50 ml/mmol) with DMF/CH_2Cl_2 1:1 (3 × 3 min) prior to the following Fmoc deprotection step. After the removal of the last Fmoc protecting group, the resin was acetylated at the *N*-terminus according to *GP 24* prior to the cleaveage procedure.

GP 23b: As in *GP 23a*, except that the Fmoc group is removed using DBU/piperidine/DMF (50 ml/mmol); 1:1:48, 2 × 10 min) under Ar bubbling.

Acetylation of Peptides on Solid Support: General Procedure 24 (*GP 24*): The Fmoc-deprotected peptide-resin was washed (30 ml/mmol) with DMF/CH_2Cl_2 1:1 (5 × 3 min) and treated successsively with $(i\text{-}Pr)_2EtN$ (15-20 equiv.), Ac_2O (10 equiv.) in DMF/CH_2Cl_2 1:1 (2 ml) under Ar bubbling for 10-

15 min. Monitoring of the acetylation was performed with TNBS [455]. The resin was then washed (30 ml/mmol) with DMF (6 × 3 min), CH_2Cl_2 (3 × 3 min), Et_2O (5 × 1 min) and dried under h.v. for 12 h.

***ortho*-Chlorotrityl-Chloride Resin Cleavage and Final Deprotection: General Procedures 25 (*GP 25*).** ***GP 25a*:** The dry Fmoc-deprotected peptide-resin was treated with 2% TFA in CH_2Cl_2 (2 ml, 5 x 15 min) under Ar bubbling. The resin was removed by filtration and the combined organic phases containing the peptide were concentrated under reduced pressure. The precipitate which formed upon addition of cold Et_2O to the oily residue was collected by filtration or centrifugation. The solid was then dissolved (at least partially) in H_2O (containing 5% HOAc in the case of insoluble material) or in 1,4-dioxane and lyophilized to afford a crude product which was analysed and purified by RP-HPLC.

***GP 25b*:** The dry Fmoc-deprotected peptide-resin was treated for 2 h with 10 ml of a TFA/H_2O/(i-Pr)$_3$SiH (95:2.5:2.5) soln. The resin was removed by filtration washed with TFA and the organic phase containing the peptide was concentrated under reduced pressure. The oily residue was then treated as in *GP 25a* to give the crude β-peptide which analysed and purified by HPLC.

***Rink* Amide Resin Cleavage and Final Deprotection: General Procedure 26 (*GP 26*).** The dry Fmoc-deprotected *Rink* amide peptide-resin was first treated with a mixture of CH_2Cl_2/TFA/(i-Pr)$_3$SiH 90:9:1 (20 ml/mmol, 3 × 2 ml), then with a mixture of CH_2Cl_2/TFA/(i-Pr)$_3$SiH 95:4:1 (20 ml/mmol, 3 × 2 ml), allowing the solvent to pass through the resin bed slowly. Excess TFA/CH_2Cl_2 was evaporated and deprotection was completed by stirring the oily residue in 95% TFA in CH_2Cl_2 for 1 h. The solvent was evaporated, coevaporated with CH_2Cl_2, dried under h.v. and the oily residue treated with Et_2O as described in *GP 25a*. Repeated treatment of the resin as above yielded an additional fraction of the crude peptide.

HPLC Analysis and Purification of β-Peptides: General Procedure 27 (*GP 27*). RP-HPLC analysis was performed on a *Macherey-Nagel* C_8 column/*Nucleosil 100*-5 C_8 (250×4 mm) or *Macherey-Nagel* C_{18} column/*Nucleosil 100*-5 C_{18} (250×4 mm) by using a linear gradient of *A*: 0.1% TFA in H_2O and *B*: MeCN at a flow rate of 1 ml/min with UV detection at 220 nm. t_R in min. Crude products were purified by prep. RP-HPLC on a *Macherey-Nagel* C_8 column/*Nucleosil 100*-7 C_8 (250×21 mm) or

Macherey-Nagel C_{18} column/*Nucleosil 100-7* C_{18} (250×21 mm) using gradient of *A* and *B* at a flow rate of 4 ml/min with UV detection at 214 nm and then lyophilized.

7.4.1 Synthesis of β-Peptides Consisting of like-$\beta^{2,3}$-*Amino Acids*

Boc-(2*S*,3*S*)-$\beta^{2,3}$-HAla(α-Me)-(2*S*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OMe (100). Compound **39** (0.191 g, 0.7 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with **99** (prepared by saponification of **35** with 2 equiv. of LiOH in refluxing MerOH/H_2O 3 : 1 for 65 min; diastereomer purity was ca. 95% according to ^{1}H-NMR spectroscopy; 0.152 g, 0.7 mmol) according to *GP 19a* for 24 h. FC (AcOEt/pentane 1:5 → 1:3) yielded **100** (0.158 g, 61%). Colorless solid. M.p. 168-170 °C. R_f 0.35 (AcOEt/pentane 1:3). $[\alpha]_D^{r.t.} = -12.8$ (*c* = 0.47, $CHCl_3$). IR ($CHCl_3$): 3418*w*, 3007*m*, 2974*w*, 1701*s*, 1659*m*, 1495*s*, 1367*m*, 1169*m*, 1088*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90 (*d*, *J* = 6.7, Me); 0.92 (*d*, *J* = 6.5, Me); 1.17 (*d*, *J* = 6.7, Me); 1.20 (*d*, *J* = 7.2, Me); 1.21 (*d*, *J* = 7.0, Me); 1.24-1.28 (*m*, CH); 1.34-1.41 (*m*, CH); 1.43 (s, *t*-Bu); 1.52-1.62 (*m*, Me_2CH); 2.31 (*dq*, *J* = 7.0, 4.2, COCH); 2.67 (*dq*, *J* = 7.2, 3.6, COCH); 3.70 (*s*, OMe); 3.72-3.77 (*m*, NCH); 4.13-4.20 (*m*, NCH); 5.87 (*d*, *J* = 7.9, NH); 6.30 (*d*, *J* = 9.6, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.33, 15.83, 20.10, 22.16, 23.04 (Me); 25.02 (CH); 28.46 (Me); 42.49 (CH); 43.34 (CH_2); 45.62, 48.87, 48.95 (CH); 51.74 (Me); 78.70, 155.99, 175.12, 176.29 (C). FAB-MS: 767 (64.2, [2*M* + Na]$^+$), 745 (17.2, [2*M*]$^+$), 395 (96.7, [*M* + Na]$^+$), 373 (100, M^+), 317 (24.0), 295 (18.0), 273 (96.6).

Boc-(2*S*,3*S*)-$\beta^{2,3}$-HVal(α-Me)-(2*S*,3*S*)-$\beta^{2,3}$-HAla(α-Me)-(2*S*,3*S*)-$\beta^{2,3}$-HLeu(α-Me)-OMe (101). Compound **100** (89.8 mg, 0.268 mmol) Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with **56** (66 mg, 0.268 mmol) according to *GP 19a* for 25 h. FC (AcOEt/pentane 1:1) and recrystallization (MeOH) yielded **101** (91 mg, 68%). Colorless needles. M.p. 191-192 °C. R_f 0.32 (AcOEt/pentane 1:1). $[\alpha]_D^{r.t.} = -27.9$ (*c* = 0.56, $CHCl_3$). IR ($CHCl_3$): 3412*w*, 2970*m*, 1703*m*, 1653*m*, 1494*s*, 1462*w*, 1390*w*, 1367*m*, 1292*w*, 1174*m*, 1077*m*, 974*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.91 (*d*, *J* = 6.7, Me); 0.92 (*d*, *J* = 6.5, Me); 0.94 (*d*, *J* = 6.7, Me); 0.97 (*d*, *J* = 6.7, Me); 1.15 (*d*, *J* = 6.7, Me); 1.19-1.26 (*m*, 3 Me, CH); 1.35-1.41 (*m*, CH); 1.42 (*s*, *t*-Bu); 1.52-1.70 (*m*, 2 Me_2CH); 2.31 (*dq*, *J* = 7.0, 3.3, COCH); 2.54 (*dq*, *J* = 7.0, 3.8, COCH); 2.69 (*dq*, *J* = 7.2, 3.5, COCH); 3.28 (*dt*, *J* = 9.5, 3.8, NCH); 3.70 (*s*, OMe); 4.01-4.08 (*m*, NCH); 4.11-4.18 (*m*, NCH); 6.05 (*d*, *J* = 10.0, NH); 6.38 (*d*, *J* = 9.7, NH); 7.41 (*d*, *J* = 8.5, NH). ^{13}C-

NMR (100 MHz, $CDCl_3$): 15.43, 16.56, 16.66, 19.72, 19.97, 20.29, 22.16, 22.95 (Me); 25.06 (CH); 28.47 (Me); 32.35, 41.34, 42.29 (CH); 43.27 (CH_2); 44.69, 47.28, 49.16 (CH); 51.80 (Me); 59.29 (CH); 78.25, 156.79, 175.38, 175.48, 176.29 (C). FAB-MS: 1022 (13.5, [2*M* + Na]$^+$), 522 (100, [*M* + Na]$^+$), 500 (52.4, [*M* + 1]$^+$), 400 (73.7). Anal. calc. for $C_{26}H_{49}N_3O_6$ (499.69): C 62.50, H 9.88, N 8,41; found: C 62.49, H 9.83, N 8.26.

Boc-(2*S*,3*S*)-β^{2,3}-HAla(α-Me)-(2*S*,3*S*)-β^{2,3}-HLeu(α-Me)-OBn (102). Compound **55** (0.743 g, 2.13 mmol) was Boc-deprotected according to *GP 10a*. The resulting crude TFA salt was coupled with Boc-(2*S*,3*S*)-β^{2,3}-HAla(α-Me)-OH **99** (0.463 g, 2.13 mmol) according to *GP 19b* for 13 h. FC (AcOEt/pentane 2:7) yielded **102** (0.758 g, 81%). Colorless solid. M.p. 156-158 °C. R_f 0.29 (AcOEt/pentane 2:7). $[\alpha]_D^{r.t.} = -19.8$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3419*w*, 3008*m*, 2988*m*, 2931*m*, 2871*w*, 1702*s*, 1660*m*, 1496*s*, 1456*m*, 1392*m*, 1368*m*, 1347*m*, 1170*s*, 1106*w*, 992*w*, 624*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.87 (*d*, *J* = 6.6, 2 Me); 1.14-1.22 (*m*, 10 H, 3 Me, CH); 1.31-1.38 (*m*, CH); 1.42 (*s*, *t*-Bu); 1.51-1.59 (*m*, CH); 2.23-2.29 (*m*, COCH); 2.63-2.75 (*m*, COCH); 3.73-3.74 (*m*, NCH); 4.13-4.20 (*m*, NCH); 5.10 (*d*, *J* = 12.2, PhC*H*H); 5.17 (*d*, *J* = 12.2, PhCH*H*); 5.85 (*d*, *J* = 7.5, NH); 6.25 (*d*, *J* = 9.6, NH); 7.31-7.40 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.26, 15.81, 20.12, 22.15, 22.90 (Me); 24.98 (CH); 28.46 (Me); 42.49 (CH); 43.26 (CH_2); 45.60, 48.85, 48.99 (CH); 66.46 (CH_2); 78.88 (C); 128.24, 128.49, 128.69 (CH); 135.65, 156.00, 175.01, 175.55 (C). EI-MS: 920 (93, [2*M* + Na]$^+$), 898 (14), 471 (100), 449 (52), 371 (24), 349 (57), 90 (31). Anal. calc. for $C_{25}H_{40}N_2O_5$ (448.60): C 66.91, H 8.99, N 6.24; found: C 66.91, H 8.79, N 6.14.

Boc-(2*S*,3*S*)-β^{2,3}-HVal(α-Me)-(2*S*,3*S*)-β^{2,3}-HAla(α-Me)-(2*S*,3*S*)-β^{2,3}-HLeu(α-Me)-OBn (103). Compound **102** (0.526 g, 1.17 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with **56** (0.287 g, 1.17 mmol) according to *GP 10b*. FC (CH_2Cl_2/Et_2O 4:1) yielded **103**. White powder (0.569 g, 85 %). M.p. 179.5-181 °C. R_f 0.19 (CH_2Cl_2/Et_2O 4:1). $[\alpha]_D^{r.t.} = -32.3$ (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3412*w*, 3008*m*, 2970*m*, 2936*m*, 2923*m*, 2872*w*, 1703*m*, 1653*m*, 1494*s*, 1456*m*, 1390*w*, 1367*m*, 1174*s*, 1018*w*, 616*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86 (*d*, *J* = 3.4, Me); 0.88 (*d*, *J* = 3.6, Me); 0.93 (*d*, *J* = 6.7, Me); 0.97 (*d*, *J* = 6.7, Me); 1.13 (*d*, *J* = 6.7, Me); 1.15-1.25 (*m*, 3 Me, CH); 1.29-1.38 (*m*, CH); 1.42 (*s*, *t*-Bu); 1.49-1.71 (*m*, 2 CH); 2.22-2.28 (*m*, COCH); 2.50-2.56 (*m*, COCH); 2.70-2.76 (*m*, COCH); 3.28 (*dt*, *J* = 9.6, 3.7, BocNHC*H*); 3.98-4.06 (*m*, NCH); 4.11-4.18 (*m*, NCH); 5.1 (*d*, *J* = 12.2, PhC*H*H); 5.17 (*d*, *J* = 12.2, PhCH*H*); 6.05 (*d*, *J* = 9.9; NH); 6.33 (*d*, *J* = 9.8, NH); 7.31-7.40 (*m*, NH, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.38, 16.53, 16.66, 19.73, 19.97, 20.30, 22.16, 22.80 (Me); 25.02 (CH); 28.47

(Me); 32.36, 41.31, 42.27 (CH); 43.19 (CH_2); 44.66, 47.26, 49.20, 59.29 (CH); 66.53 (CH_2); 78.24 (C); 128.22, 128.54, 128.71 (CH); 135.58, 156.78, 175.33, 175.47, 175.55 (C). FAB-MS: 1750 (9, $[3M + Na]^+$), 1728 ($3M + 1]^+$), 1174 (100, $[2M + Na]^+$), 1152 (33, $[2M + 1]^+$), 598 (<1, $[M + Na]^+$). Anal. calc. for $C_{32}H_{53}N_3O_6$ (575.79): C 66.75, H 9.28, N 7.30; found: C 66.66, H 9.21, N 7.23.

Boc-(2*S*,3*S*)-β^{2,3}-HVal(α-Me)-(2*S*,3*S*)-β^{2,3}-HAla(α-Me)-(2*S*,3*S*)-β^{2,3}-HLeu(α-Me)-OH (104). Compound **103** (0.188 g, 0.327 mmol) was debenzylated in MeOH according to *GP 9*. **104** (0.16 g, 99%). M.p. 191-193 °C. R_f 0.38 ($CHCl_3$/MeOH 9:1). $[\alpha]_D^{r.t.} = -9.7$ (*c* = 0.37, MeOH). IR ($CHCl_3$): 3411*w*, 2966*s*, 2934*m*, 2874*m*, 1702*s*, 1652*s*, 1496*s*, 1463*m*, 1391*m*, 1368*m*, 1295*m*, 1172*s*, 1100*w*, 1076*w*, 1040*w*, 975*w*, 889*w*, 864*w*, 652*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.85-0.97 (*m*, 4 Me); 1.13-1.36 (*m*, CH, 4 Me); 1.42 (*s*, 6 H, *t*-Bu, rotamer); 1.45 (*s*, 3 H, *t*-Bu, rotamer); 1.58-1.60 (*m*, Me_2CH); 1.69-1.74 (*m*, Me_2CH); 2.31-2.33 (*m*, COCH); 2.48-2.53 (*m*, COCH); 2.64-2.67 (*m*, COCH); 3.37-3.41 (*m*, BocNHCH); 4.03-4.16 (*m*, 2 NCH); 5.84 (*d*, *J* = 9.6, NH); 6.51 (br. *d*, *J* = 8.5, NH); 7.37 (*d*, *J* = 8.6, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.52, 16.21, 16.36, 18.61, 19.63, 20.25, 22.12, 23.05 (Me); 25.04 (CH); 28.45 (Me), 31.47, 42.14, 42.97 (CH); 43.13 (CH_2); 44.80, 47.50, 49.28, 58.91 (CH); 78.70 (C); 156.78, 175.14, 175.58, 178.2 (C). FAB-MS: 1009 (4, $[2M + K]^+$), 995 (31, $[2M + Na]^+$), 509 (100, $[M + Na]^+$), 487 (20, $[M + 1]^+$), 409 (16), 387 (66), 259 (12), 154 (23), 137 (13).

Boc-(2*S*,3*S*)-β^{2,3}-HVal(α-Me)-(2*S*,3*S*)-β^{2,3}-HAla(α-Me)-(2*S*,3*S*)-β^{2,3}-HLeu(α-Me)-(2*S*,3*S*)-β^{2,3}-HVal(α-Me)-(2*S*,3*S*)-β^{2,3}-HAla(α-Me)-(2*S*,3*S*)-β^{2,3}-HLeu(α-Me)-OBn (105). Compound **103** (86.7 mg, 0.15 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with **104** (73 mg, 0.15 mmol) according to *GP 19a* for 16 h. FC (CH_2Cl_2/MeOH 25:1 to 10:1) yielded **105** (77.7 mg, 55%) and an epimeric, fully protected β^{2,3}-hexapeptide (11 mg, 8%). Colorless glass which gave a voluminous colorless powder after lyophilization from dioxane. M.p. 222-223.5 °C. R_f 0.21 ($CHCl_3$/MeOH 10:1). $[\alpha]_D^{r.t.} = -34.5$ (*c* = 1.0, $CHCl_3$). CD (0.2 mM in MeOH): + $2.87 \cdot 10^4$ (199 nm), − $1.13 \cdot 10^4$ (220 nm). IR ($CHCl_3$): 3386*w*, 3007*m*, 2968*m*, 2934*m*, 2875*w*, 1702*m*, 1649*s*, 1494*s*, 1456*m*, 1389*m*, 1368*m*, 1290*m*, 1174*m*, 1047*w*, 977*w*, 648*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86-1.00 (*m*, 8 Me); 1.14-1.25 (*m*, 8 Me); 1.27-1.42 (*m*, *t*-Bu, 3 CH); 1.50-1.70 (*m*, 5 CH); 2.24-2.35 (*m*, 2 COCH); 2.42 (*dq*, *J* = 7.0, 3.1, COCH); 2.51-2.62 (*m*, 2 COCH); 2.73 (*dq*, *J* = 7.2, 3.5, COCH); 3.27 (*dt*, *J* = 9.5, 3.6, NCH); 3.59 (*dt*, *J* = 9.4, 2.9, NCH); 3.96-4.06 (*m*, 2 NCH); 4.10-4.17 (*m*, 2 NCH); 5.11 (*d*, *J* = 12.2, PhCHH); 5.17 (*d*, *J* = 12.2, PhCHH); 6.12 (*d*, *J* = 9.9, NH); 6.39 (*d*, *J* = 9.8, NH); 7.32-7.40 (*m*, 5 arom. H); 7.56 (*d*, *J* = 8.9, 2 NH); 7.61-7.64 (*m*, 2

NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.47, 16.69, 16.71, 16.96, 16.99, 19.77, 19.93, 20.04, 20.09, 20.19, 20.32, 22.14, 22.49, 22.57, 22.79, 25.03 (Me); 25.05 (CH); 28.48 (Me); 29.71, 32.39, 32.45, 40.13, 41.24, 42.20, 42.92 (CH); 43.23; 44.00 (CH_2); 44.49, 44.54, 47.42, 47.52, 49.29, 49.78, 57.74, 59.35, 66.58 (CH_2); 78.16 (C); 128.21, 128.57, 128.72 (CH); 135.53, 156.82, 175.32, 175.46, 175.59, 175.90 (C). FAB-MS: 981 (66, $[M + K]^+$), 966 (100, $[M + Na]^+$), 875 (6), 844 (63). Anal. calc. for $C_{52}H_{90}N_6O_9$ (943.32): C 66.21, H 9.62, N 8.91; found: C 65.97, H 9.90, N 8.62.

H-(2*S*,3*S*)-β^{2,3}-HVal(α-Me)-(2*S*,3*S*)-β^{2,3}-HAla(α-Me)-(2*S*,3*S*)-β^{2,3}-HLeu(α-Me)-(2*S*,3*S*)-β^{2,3}-HVal(α-Me)-(2*S*,3*S*)-β^{2,3}-HAla(α-Me)-(2*S*,3*S*)-β^{2,3}-HLeu(α-Me)-OH (106). Compound **105** (46.5 mg, 0.049 mmol) was debenzylated in MeOH according to *GP 9* affording the corresponding acid (45 mg, 99%) which was Boc-deprotected according to *GP 10a* to give a colorless glass, TFA salt of **106**. The peptide was purified by prep. RP-HPLC (MeCN (0.08 % TFA)/H_2O (0.1 % TFA) 1:1; C_8). The product was obtained as a voluminous colorless powder after lyophylization from dioxan (31.4 mg, 72% after HPLC). RP-HPLC (60-97% *B* in 30 min; C_8) t_R 21.8, purity > 97%. M.p. 240 °C (dec.). R_f 0.38 (CH_2Cl_2/MeOH 13:1, 1 % AcOH). $[\alpha]_D^{r.t.}$ = + 14.2 (*c* = 0.6, MeOH). CD (0.2 mM in MeOH): + 1.52·10^5 (198 nm), − 5.16·10^4 (217 nm). IR (KBr): 3286*s*, 3086*m*, 2969*s*, 2936*s*, 2879*m*, 1717*s*, 1653*s*, 1557*s*, 1545*s*, 1458*m*, 1384*m*, 1368*m*, 1261*w*, 1241*m*, 1194*s*, 1138*s*, 974*w*, 947*w*, 931*w*, 913*w*, 847*w*, 831*w*, 800*w*, 720*m*. ^{1}H-NMR (500 MHz, CD_3OH): 0.85 (*d*, *J* = 6.9, Val(4)-δ–Me); 0.88 (*d*, *J* = 7.0, Val(4)-δ-Me'); 0.87 (*d*, *J* = 7.0, Leu(3)-ε–Me); 0.94 (2*d*, *J* = 6.7, 6.5, Leu(6)-ε–Me, -ε-Me'); 0.96 (*d*, *J* = 6.3, Leu(3)-ε-Me'); 1.09 (*d*, *J* = 7.0, Val(1)-δ-Me); 1.10 (*d*, *J* = 7.0, Ala(5)-β-Me, Val(4)-α-Me); 1.16 (*d*, *J* = 7.0, Val(1)-δ-Me, Ala(2)-α-Me); 1.18 (*m*, Val(1)-α-Me, Ala(5)-α-Me); 1.19 (*d*, *J* = 7.0, Ala(2)-β-Me, Leu(3)-α-Me); 1.22 (*m*, Leu(6)-α-Me); 1.30 (*m*, Leu(3)-γ-C*H*H); 1.34 (*m*, Leu(3)-γ-CH*H*); 1.38 (*m*, Leu(6)-γ-C*H*H); 1.40 (*m*, Leu(6)-γ-CH*H*); 1.55 (*m*, Leu(3)-δ-CH); 1.62 (*m*, Leu(6)-δ-CH); 1.93 (*m*, Val(4)-γ-CH, *J*(γ-CH, β-CH) = 2.9); 2.22 (*m*, Val(1)-γ-CH, *J*(γ-CH, β-CH) = 2.7); 2.30 (*m*, Ala(5)-α-CH_{ax}, *J*(α,β'-Me) = 6.9); 2.49 (*m*, Val(4)-α-CH_{ax}, *J*(α,β'-Me) = 6.9); 2.55 (*m*, Leu(6)-α-CH_{ax}, *J*(α,β'-Me) = 6.7); 2.69 (*m*, Leu(3)-α-CH_{ax}, *J*(α,β'-Me) = 7.0); 2.90 (*m*, Val(1)-α-CH_{ax}, *J*(α,β'-Me) = 6.9); 3.04 (*m*, Ala(2)-α-CH_{ax}, *J*(α,β'-Me) = 6.9); 3.39 (*m*, Val(1)-β-CH_{ax}, *J*(β_{ax},α_{ax}) = 10.8); 3.99 (*m*, Leu(3)-β-CH_{ax}, *J*(β_{ax},α_{ax}) = 10.6); 4.03 (*m*, Val(4)-β-CH_{ax}, *J*(β_{ax},α_{ax}) = 11.3); 4.13 (*m*, Ala(5)-β-CH_{ax}, *J*(β_{ax},α_{ax}) = 10.9, Leu(6)-β-CH_{ax}, *J*(β_{ax},α_{ax}) = 9.3); 4.19 (*m*, Ala(2)-β-CH_{ax}, *J*(β_{ax},α_{ax}) = 11.1); 7.33 (*d*, *J* = 9.2, Ala(5)-NH); 7.42 (*d*, *J* = 10.0, Val(4)-NH); 7.52 (br. *d*, Val(1)-NH_3); 7.96 (*d*, *J* = 9.9, Leu(6)-NH); 8.50 (*d*, *J* = 9.6, Leu(3)-NH); 8.62 (*d*, *J* = 9.3, Ala(2)-NH). ^{13}C-NMR (125 MHz, CD_3OH): 15.1 (β'-Me(1)), 15.4 (β'-C(6)), 15.7 (δ-Me'(1), ε-Me'(3)), 16.5 (β'-Me(4)), 16.9 (β'-

Me(2)), 17.5 (β'-Me(4)), 17.9 (β'-Me(3)), 18.3 (γ-Me(5)), 18.5 (γ-Me(2)), 20.1 (δ-Me(1)), 22.2 (ε-Me(3)), 20.8 (δ-Me'(4)), 22.4 (ε-Me'(6)), 24.1 (ε-Me(6)), 24.4 (δ-Me(4)), 25.5 (δ-Me(6)), 25.9 (δ-Me(3)), 27.6 (γ-CH(4)), 29.6 (γ-CH(1)), 43.2 (γ-CH_2(6)), 43.3 (α-C(1)), 44.0 (α-C(4)), 44.4 (γ-CH_2(3)), 47.1 (α-C(6)), 47.2 (α-C(3)), 47.6 (α-C(2)), 47.7 (α-CH(5)), 48.0 (β-C(2), -C(5)), 49.9 (β-C(3)), 55.7 (β-C(4)), 61.1 (β-C(1)), 174.9 (CO(1)), 175.6 (CO(4)), 175.7 (CO(5)), 177.3 (CO(3)), 177.9 (CO(2)), 178.4 (CO(6). FAB-MS: 1546 (26, $[2M + K]^+$), 1508 (6, $[2M + 1]^+$), 792 (94, $[M + K]^+$), 777 (40, $[M + Na]^+$), 755 (100, $[M + 1]^+$).

7.4.2 Synthesis of β-Peptides Containing unlike-$\beta^{2,3}$-*Amino Acids and Synthesis of β-Peptide* 112

Boc-(2*R*,3*S*)-β^{2,3}-HAla(α-Me)-(2*R*,3*S*)-β^{2,3}-HLeu(α-Me)-OBn (108). Compound **59** (0.363 g, 1.04 mmol) was dissolved in sat. HCl/dioxane (4.5 ml) and stirred for 1.5 h at r.t. Concentration at RV and drying under h.v. yielded the crude HCl salt, that was coupled with **107** (prepared by saponification of **36** with 2 equiv. of LiOH in refluxing MeOH/H_2O 3: 1 for 50 min; diastereomer purity was ca. 95% according to ^{1}H-NMR spectroscopy; 0.226 g, 1.04 mmol) according to *GP 19a*. 2 × FC (Et_2O/CH_2Cl_2 1:10) yielded **109** (0.367 g, 79%). Colorless needles. M.p. 164-166 °C (recrystallized from MeOH). R_f 0.17 (Et_2O/CH_2Cl_2 1:10). $[\alpha]_D^{r.t.} = -49.8$ (c = 1.04, $CHCl_3$). IR ($CHCl_3$): 3436*m*, 3005*m*, 2974*m*, 2872*w*, 1703*s*, 1497*s*, 1466*m*, 1390*m*, 1369*m*, 1169*s*, 1103*w*, 1077*w*, 1062*w*, 867*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.87 (*d*, *J* = 6.6, 2 Me); 1.07 (*d*, *J* = 7.1, Me); 1.11 (*d*, *J* = 6.8, Me); 1.17 (*d*, *J* = 7.2, Me); 1.21-1.26 (*m*, CH_2); 1.43 (*s*, *t*-Bu); 1.49-1.59 (*m*, Me_2CH); 2.43 (*br.*, COCH); 2.64-2.70 (*m*, COCH); 3.67-3.75 (*m*, NCH); 4.17-4.24 (*m*, NCH); 4.94 (*br.*, NH); 5,12 (*s*, $PhCH_2$); 5.82 (br. *d*, *J* = 8.7, NH); 7.31-7.40 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 13.32, 14.34, 17.21, 21.42, 23.57 (Me); 25.01 (CH); 28.43 (Me); 39.98 (CH_2); 44.02, 45.45, 49.14 (CH); 66.52 (CH_2); 79.21 (C); 128.43, 128.48, 128.66 (CH); 135.85, 155.41, 173.45, 174.17 (C). FAB-MS: 920 (57.0, $[2M + Na]^+$), 898 (24.0, $[2M]^+$), 471 (98.5, $[M + Na]^+$), 449 (100, M^+), 393 (20.2), 349 (83.3). Anal. calc. for $C_{25}H_{40}N_2O_5$ (448.60): C 66.94, H 8.99, N 6.24; found: C 66.92, H 8.87, N 6.20.

Boc-(2*R*,3*S*)-β^{2,3}-HVal(α-Me)-(2*R*,3*S*)-β^{2,3}-HAla(α-Me)-(2*R*,3*S*)-β^{2,3}-HLeu(α-Me)-OBn (109). Fully protected dipeptide **108** (0.215 g, 0.48 mmol) was Boc-deprotected according to *GP 10a*. The resulting crude TFA salt was coupled with **60** (0.13 g, 0.53 mmol) according to *GP 19a*. 2 × FC ($CHCl_3$/MeOH 10:0.25)

yielded **109** (0.211 g, 76%). White glass. For analytical purposes, **109** was crystallized from MeOH. Colorless needles suitable for powder X-ray analysis. M.p. 245-245.5 °C. R_f 0.34 ($CHCl_3$/MeOH 10:0.25). CD (0.2 mM in MeOH): – $1.57 \cdot 10^4$ (193.2 nm), + $2.31 \cdot 10^4$ (213 nm). IR (KBr): 3344*s*, 3311*s*, 3067*w*, 2978*m*, 2922*m*, 2878*m*, 2489*w*, 1733*s*, 1663*s*, 1644*s*, 1539*s*, 1456*s*, 1422*m*, 1389*m*, 1367*s*, 1311*s*, 1278*w*, 1250*m*, 1211*m*, 1178*s*, 1122*m*, 1078*w*, 1044*w*, 1006*m*, 978*w*, 920*w*, 902*w*, 871*w*, 780*w*, 737*m*, 695*m*, 689*w*, 470*w*. ^{1}H-NMR (500 MHz, CD_3OD): 0.82 (*d*, *J* = 6.5, Me); 0.85 (*t*, *J* = 6.4, 2 Me); 0.89 (*d*, *J* = 6.8, Me); 1.09 (*t*, *J* = 6.8, 2 Me); 1.10 (*d*, *J* = 6.9, Me); 1.12 (*d*, *J* = 7.0, Me); 1.39-1.44 (*m*, CH_2, *t*-Bu); 1.52-1.59 (*m*, Me_2CH); 1.70-1.76 (*m*, Me_2CH); 2.30-2.36 (*m*, COCH); 2.39-2.45 (*m*, COCH); 2.50-2.56 (*m*, COCH); 3.67-3.71 (*m*, NCH); 4.01-4.07 (*m*, NCH); 4.20-4.24 (*m*, NCH); 5.05 (*d*, *J* = 12.2, PhC*H*H); 5.65 (*d*, *J* = 12.2, PhCH*H*); 6.26 (*d*, *J* = 10.6, NH); 7.29-7.40 (*m*, 5 arom.). ^{13}C-NMR (125 MHz, CD_3OD): 13.61, 15.49, 16.18, 16.65, 19.61, 21.00, 21.63, 23.95 (Me); 26.13 (CH); 28.85 (Me); 31.47 (CH); 42.67 (CH_2); 45.10, 46.23, 48.30, 48.45, 50.37, 58.85 (CH); 67.56 (CH_2); 79.80 (C); 129.33, 129.59, 129.67 (CH); 137.62, 158.95, 176.07, 177.18 (C). FAB-MS: 576 (100, $[M + 1]^+$), 476 (36.4), 91 (37.9). Anal. calc. for $C_{32}H_{53}N_3O_6$ (575.79): C 66.75 H 9.28, H 7.30; found: C 66.60, H 9.53, H 7.27.

Boc-(2*R*,3*S*)-β^{2,3}-HVal(α-Me)-(2*R*,3*S*)-β^{2,3}-HAla(α-Me)-(2*R*,3*S*)-β^{2,3}-HLeu(α-Me)-OH (110). Fully protected tripeptide **109** (95 mg, 0.165 mmol) was debenzylated according to *GP 9* in MeOH (12 ml) affording **110** (80 mg, quant.). White powder, extremely insoluble. M.p. 250 °C (dec.). IR (KBr): 3344*m*, 3506*m*, 2975*m*, 2878*m*, 1684*s*, 1643*s*, 1533*s*, 1458*m*, 1389*m*, 1368*m*, 1314*m*, 1278*m*, 1250*m*, 1212*m*, 1175*m*, 1139*m*, 1122*m*, 1079*w*, 1044*w*, 1006*m*, 950*w*, 918*w*, 868*w*, 783*w*, 711*w*, 668*w*, 504*w*. ^{1}H-NMR (200 MHz, CD_3OD): 0.83-0.93 (*m*, 4 Me); 1.09-1.30 (*m*, 4 Me, CH_2); 1.44 (*s*, *t*-Bu); 1.53-1.75 (*m*, 2 Me_2CH); 2.31-2.45 (*m*, 3 COCH); 3.66-3.76 (*m*, NCH); 4.05-4.18 (*m*, 2 NCH); 6.28 (*d*, *J* = 10.4, NH); 7.75-7.84 (*m*, 2 NH). FAB-MS: 1016 (2.1, $[2M - 1 + 2\ Na]^+$), 995 (3.1, $[2M + Na]^+$), 530 (23.7, $[M - 1 + 2\ Na]^+$), 524 (3.6, $[M + K]^+$), 508 (100, $[M + Na]^+$), 486 (68, M^+), 386 (25).

TFA·H-(2*R*,3*S*)-β^{2,3}-HVal(α-Me)-(2*R*,3*S*)-β^{2,3}-HAla(α-Me)-(2*R*,3*S*)-β^{2,3}-HLeu(α-Me)-OBn (111). Boc-deprotection of compound **109** (52.0 mg, 90 μmol) according to *GP 10b* gave the TFA salt **111** (53 mg, 99%). Colorless glass. Crystallization from MeOH (0.2 mM) gave single crystals suitable for X-ray analysis. M.p. 154 °C (dec.). CD (0.2 mM in MeOH): + $1.79 \cdot 10^4$ (213.8 nm). IR (KBr): ^{1}H-NMR (200 MHz, CD_3OD): 0.84 (*t*, *J* = 6.6, 2 Me); 1.00-1.60 (*m*, 6 Me, Me_2CH, CH_2); 1.83-1.97 (*m*, Me_2CH); 2.26-2.37 (*m*, COCH); 2.41-2.60 (*m*,

COCH); 2.75-2.88 (*m*, COCH); 3.05-3.11 (*m*, NH_2CH); 4.03-4.29 (*m*, 2 NCH); 5.06 (*d*, *J* = 12.5, PhC*H*H); 5.15 (*d*, *J* = 12.0, PhCH*H*); 7.27-7.39 (*m*, 5 arom. H); 7.86 (*d*, *J* = 10.8, NH). FAB-MS: 952 (<1, $[2M + 1]^+$), 476 (100, $[M + 1]^+$); 338 (41.0), 284 (42.1), 256 (28.7), 154 (31.6).

H-(*S*)-β²-HVal-(*R*)-β³-HAla-(*S*)-β²-HPhe-(*S*)-β²-HVal-(*R*)-β³-HAla-(*S*)-β²-HLeu-OH (112). According to *GP 20* the *ortho*-chlorotrityl-chloride resin (182 mg, 1.00 mmol Cl/g) was esterified with (*S*)-2-({[(9*H*-fluoren9-ylmethoxy)carbonyl]amino}methyl)-4-methylpentanoic acid [203] (Fmoc-(*S*)-β²-HLeu-OH; 54 mg, 0.146 mmol). Loading 0.47 mmol/g (74%), corresponding to 85.4 μmol of anchored Fmoc-(*S*)-β²-HLeu-OH. Synthesis according to *GP 22a* and cleavage from the resin according to *GP 25a* afforded crude **112** as TFA salt (80 mg, 99%), purity 64% (RP-HPLC). Purification by RP-HPLC (20-80% *B* in 40 min; C_8) according to *GP 27* yielded the TFA salt of **112** (19.7 mg, 24%). White solid. RP-HPLC (30-90% *B* in 20 min; C_{18}) t_R 8.8 min, 17.2 mg: purity >88%; 2.5 mg: purity 98%. M.p. 284 °C (dec.). CD (0.2 mM in MeOH): – $2.24 \cdot 10^4$ (225 nm). IR (KBr): 3281*m*, 3089*w*, 2961*m*, 2930*m*, 2878*w*, 1716*m*, 1658*s*, 1552*m*, 1454*m*, 1389*w*, 1255*w*, 1202*m*, 1140*w*, 798*w*, 699*w* ^{1}H-NMR (200 MHz, CD_3COOD, poorly soluble): 0.80-0.90 (*m*, 5 Me); 1.00-1.80 (*m*, 3 Me, 2 CH, 3 Me_2CH); 2.05-2.85 (*m*, 10 COCH, 2 $PHCH_2$); 3.08-3.45 (*m*, 8 NCH); 4.00-4.20 (*m*, 3 NCH); 7.04-7.15 (*m*, 10 arom. H). FAB-MS: 903 (4.9, $[M + K]^+$), 887 (8.5, $[M + Na]^+$), 865 (100, $[M + 1]^+$).

H-(2*R*,3*S*)-β²,³-HAla(α-Me)-(2*R*,3*S*)-β²,³-HVal(α-Me)-(*S*)-β²-HVal-(*S*)-β³-HLys-(2*R*,3*S*)-β²,³-HAla-(2*R*,3*S*)-β²,³-HLeu(α-Me)-OH (113). According to *GP 20* the *ortho*-chlorotrityl-chloride resin (210 mg, 1.00 mmol Cl/g) was esterified with acid **63** (64.0 mg, 0.168 mmol). Loading 0.53 mmol/g (85%), corresponding to 112 μmol of anchored **63**. Synthesis according to *GP 22a* and cleavage from the resin according to *GP 25b* afforded crude **113** as TFA salt (97.2 mg, 90%), purity 57% (RP-HPLC). Purification by RP-HPLC (5–18% *B* in 10 min, then 18-30% *B* in 25 min; C_8) according to *GP 27* yielded the TFA salt of **113** (18.7 mg, 17%). White solid. RP-HPLC (5–30% *B* in 10 min, then 30-40% *B* in 10 min; C_8) t_R 13.0 min, purity > 98%. M.p. <250 °C (dec.). CD (0.2 mM in MeOH): + $6.73 \cdot 10^4$ (208 nm), (0.2 mM pH 11): + $1.21 \cdot 10^5$ (204 nm), (0.2 mM pH 3.6): + $5.85 \cdot 10^4$ (206 nm). IR (KBr): 3600-2600*br*, 1654*s*, 1541*s*, 1458*m*, 1388*w*, 1304*w*, 1271*w*, 1202*s*, 1176*s*, 1138*s*, 836*w*, 799*w*, 722*w*, 668*w*. ^{1}H-NMR (400 MHz, D_2O): 0.80-0.87 (*m*, 4 Me); 0.92-1.05 (*m*, 6 Me); 1.21-1.80 (*m*, 17 H, 2 Me, 11 CH); 2.11-2.18 (*m*, COCH); 2.29-2.40 (*m*, 3 COCH); 2.49-2.64 (*m*, 3 COCH); 2.91 (*t*, *J* = 7.8, NCH_2); 3.23 (*dd*, *J* = 13.7, 9.5, NCH); 3.40-3.51 (*m*, 2

NCH); 3.86-3.95 (*m*, 2 NCH); 4.11-4.16 (*m*, 2 NCH); 8.00 (*d*, *J* = 9.5, NH); 8.00-8.02 (*m*, NH); 8.06 (*d*, *J* = 8.7 , NH). ^{13}C-NMR (100 MHz, D_2O): 14.60, 16.09, 16.11, 17.32, 18.88, 19.48, 21.10, 22.27, 22.52, 22.57, 23.23 (Me); 25.04 (CH_2); 25.42 (Me); 27.16 (CH); 29.03 (CH_2); 31.21, 32.30 (CH); 35.44, 41.94, 42.17, 43.42, 44.46 (CH_2); 45.23, 46.69, 47.30, 49.25, 59.71, 50.29, 51.91, 52.03 (CH); 55.79, 59.09 (CH); 175.15, 178.48, 178.93, 179.53, 179.95, 182.31 (C). FAB-MS: 763 (27.8, [*M* + Na]$^+$), 741 (100, M^+).

Table 21. Coupling constants for β-hexapeptide **113** (CD_3OH, 500 MHz). The backbone CH_2 protons of amino acids 3 and 4 were assigned stereospecifically. The large values for the amino acids 2 and 5 suggest an extended conformation, whereas the *J*-values for 1 and 6 indicate several preferred conformations. The coupling constants in residues 3 and 4 are in agreement with a well defined, not extended conformation.

Amino acid	*J*(NH, C(β)-H)	*J*(C(α)-H, C(β)-H)
1	-	7
2	10.1	9.7
3	*J*(NH, C(β)-H^{Si}) 7.4 *J*(NH, C(β)-H^{Re}) 3.8	*J*(C(β)-H^{Si}, C(α)-H) 3.8 *J*(C(β)-H^{Re}, C(α)-H) 10.7
4	9.0	*J*(C(β)-H, C(α)-H^{Si}) 4.1 *J*(C(β)-H, C(α)-H^{Re}) 9.6
5	10.6	9.2
6	7	7

Ac-(2*R*,3*S*)-β^{2,3}-HVal(α-Me)-(*S*)-β^2-HVal-(*S*)-β^3-HLys-(2*R*,3*S*)-β^{2,3}-HAla-NH$_2$ (114). According to *GP 21* the *Rink* amide resin (181 mg, 1.00 mmol/g) was coupled with **68** (83.0 mg, 0.244 mmol). Synthesis according to *GP 23b*, final acetylation according to *GP 24* and cleavage from the resin according to *GP 26* afforded a first fraction of crude **114** as TFA salt (41 mg, 77%, 58% HPLC-purity) and a second fraction (4.5 mg, 8%, 57% HPLC-purity). Purification by RP-HPLC (2–40% *B* in 30 min; C_8) according to *GP 27* yielded the TFA salt of **114** (23.9 mg, 45%) . White solid. RP-HPLC (2–50% *B* in 20 min; C_{18}) t_R 12.0, purity > 97%. M.p. 267 °C (dec.). CD (0.2 mM in MeOH): + $1.53 \cdot 10^5$ (202 nm), (0.2 mM pH 11): + $6.76 \cdot 10^4$ (201 nm), (0.2 mM pH 3.6): + $6.56 \cdot 10^4$ (202 nm). IR ($CHCl_3$): 3634*m*, 3437*br*, 3007*w*, 2945*m*, 2838*w*, 1713*w*, 1601*w*, 1467*w*, 1333*w*, 1261*m*, 1098*m*, 1016*s*. ^{1}H-NMR (500 MHz, D_2O): 0.84 (*d*, *J* = 6.8, Me); 0.86 (*d*, *J* = 6.8, Me); 0.89 (*d*, *J* = 6.7, Me); 0.97 (*d*, *J* = 6.7, Me); 1.02 (*d*, *J* = 6.9, Me); 1.09 (*d*, *J* = 7.0, Me); 1.12 (*d*, *J* = 6.7, Me); 1.33-1.70 (*m*, 7 H, CH, CH_2); 1.71-1.82 (*m*, CH); 2.02 (*s*, COMe); 2.12-2.16 (*m*, COCH); 2.37-2.47 (*m*, 3 COCH); 2.56-2.61 (*m*, COCH); 2.95 (*t*, *J* = 7.5, NCH_2); 3.21 (*dd*, *J* = 13.7, 9.6, C*H*HN); 3.50 (*dd*, *J* = 13.7, 4.2, CH*H*N); 3.83-3.88 (*m*, NCH); 3.95-4.01 (*m*, NCH); 4.16-4.17 (*m*, NCH); 7.82 (*d*, *J* = 10.4, NH); 8.04 (*t*, *J* = 5.4, NH); 8.11 (*d*, *J* = 8.9, NH). ^{13}C-NMR (125 MHz,

D_2O): 15.36, 16.46, 19.06, 20.56, 22.19, 22.23, 22.59, 24.50, (Me); 24.50, 28.99 (CH_2); 31.02, 32.36 (CH); 35.40, 41.90, 42.14, 44.54 (CH_2); 45.04, 48.23, 49.68, 50.31, 55.97, 59.48 (CH); 175.09, 176.87, 178.62, 180.18, 182.87 (C). FAB-MS: 541 (100, [*M* + 1]$^+$).

Ac-(2*R*,3*S*)-β^{2,3}-HVal(α-Me)-(*S*)-β^3-HLys-(*S*)-β^2-HAla-(2*R*,3*S*)-β^{2,3}-HAla-NH$_2$ (115). According to *GP 21* the *Rink* amide resin (202 mg, 0.45 mmol/g) was coupled with **68** (93.0 mg, 0.274 mmol). Synthesis according to *GP 23b*, final acetylation according to *GP 24* and cleavage from the resin according to *GP 26* afforded a first fraction of crude **115** as TFA salt (44 mg, 77%, 82% HPLC purity) and a second fraction (11.6 mg, 20%, 82% HPLC purity) . Purification by RP-HPLC (2–8% *B* in 10 min, then 8-15% *B* in 15 min; C_{18}) according to *GP 27* yielded the TFA salt of **115** (9.0 mg, 16%). White solid. RP-HPLC (2–20% *B* in 10 min, then 20-30% *B* in 15 min; C_{18}) t_R 11.0, purity > 96%. M.p. 250 °C (dec.). CD (0.2 mM in MeOH): + $4.02 \cdot 10^4$ (199 nm), (0.2 mM pH 11): + $3.48 \cdot 10^4$ (198.7 nm), (0.2 mM pH 3.6): + $4.80 \cdot 10^4$ (202.3 nm). IR (KBr): 3400*m*, 3294*m*, 3220*w*, 3110*w*, 2978*m*, 2933*m*, 1750-1620*br*, 1560*s*, 1541*s*, 1458*m*, 1378*w*, 1317*w*, 1211*m*, 1183*m*, 1140*s*, 1067*w*, 978*w*, 950*w*, 922*w*, 839*w*, 800*w*, 723*m*. ^{1}H-NMR (500 MHz, D_2O): 0.83 (*d*, *J* = 6.8, Me); 0.84 (*d*, *J* = 6.7, Me); 1.02 (*d*, *J* = 6.9, Me); 1.07 (*d*, *J* = 6.9, Me); 1.08 (*d*, *J* = 6.9, Me); 1.17 (*d*, *J* = 6.7, Me); 1.31-1.42 (*m*, 2 CH); 1.47-1.70 (*m*, 5 CH); 2.02 (*s*, COMe); 2.32 (*dd*, *J* = 14.1, 8.9, COCH); 2.40-2.53 (*m*, 3 COCH); 2.56-2.63 (*m*, COCH); 2.91-3.00 (*m*, NCH_2); 3.19 (*dd*, *J* = 13.7, 8.6, NCH); 3.26 (*dd*, *J* = 13.7, 5.7, NCH); 3.86 (*dd*, *J* = 9.4, 4.2, NCH); 3.97-4.02 (*m*, NCH); 4.12-4.18 (*m*, NCH); 7.79 (*d*, *J* = 10.4, NH); 8.05 (*d*, *J* = 9.0, NH). ^{13}C-NMR (125 MHz, D_2O): 16.33, 16.48, 17.18, 18.11, 20.56, 22.31, 24.48 (Me); 24.94, 28.97 (CH_2); 32.43 (CH); 35.79, 41.95 (CH_2); 43.16 (CH); 43.95, 45.08 (CH_2); 45.67, 48.04, 49.34, 50.16, 59.07 (CH); 176.00, 177.03, 179.46, 179.61, 183.00 (C). FAB-MS: 1024 (1.4, [2*M*]$^+$], 652 (< 1, [M + *K*]$^+$), 536 (47.4, [*M* + Na]$^+$), 514 (100, [*M* + 1]$^+$).

Ac-(2*R*,3*S*)-β^{2,3}-HVal-β^{2,2}-HAc$_6$c-(*S*)-β^3-HTyr-(2*R*,3*S*)-β^{2,3}-HAla-NH$_2$ (116). According to *GP 21* the *Rink* amide resin (207.8 mg, 0.45 mmol/g) was coupled with **68** (95.2 mg, 0.281 mmol). Synthesis according to *GP 23b*, final acetylation according to *GP 24* and cleavage from the resin according to *GP 26* afforded crude **116** (69 mg, quant., 93% HPLC purity). Purification by RP-HPLC (10–40% *B* in 20 min; C_8) according to *GP 27* yielded **116** (15.7 mg, 28%). White solid. RP-HPLC (20–50% *B* in 20 min, C_8) t_R 11.8, purity > 99%. CD (0.2 mM in MeOH): + $8.8 \cdot 10^4$ (203 nm). M.p. 255 °C (dec.). IR (KBr): 3297*br*., 2966*m*, 2931*m*, 2856*w*, 1652*s*, 1540*s*, 1516*s*, 1456*m*, 1372*m*, 1339*w*, 1310*w*, 1233*m*,

1202*m*, 1158*w*, 1139*m*, 985*w*, 927*w*, 723*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88 (*d*, *J* = 6.7, Me);0.91 (*d*, *J* = 6.8, Me); 1.05 (*d*, *J* = 6.9, Me); 1.08-1.44 (*m*, 3 Me, 8 CH); mit 1.13 (2 *d*, *J* = 6.9, *J* = 6.6, 2 Me); 1.68-1.78 (*m*, Me_2CH, CH); 1.85-1.99 (*m*, CH); 2.00 (*s*, COMe); 2.31-2.39 (*m*, 2 COCH); 2.50 (*dd*, *J* = 13.3, 3.9, COCH); 2.65-2.85 (*m*, 1 COCH, 2 NCH); 3.16 (*d*, *J* = 13.5, $PhCH_2$); 3.21 (*d*, *J* = 13.5, $PhCH_2$); 4.06-4.19 (*m*, 2 NCH); 4.50-4.57 (*m*, NCH); 6.68 (*d*, *J* = 8.6, 2 arom. H); 7.06 (*d*, *J* = 8.5, 2 arom. H); 7.67 (*d*, *J* = 10.2, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.51, 15.79, 17.63, 19.38, 20.88, 22.73 (Me); 23.63, 23.72, 26.89 (CH_2); 31.60 (CH); 32.83, 33.03, 41.26, 42.34 (CH_2); 44.04, 47.63 (CH); 48.19 (CH_2); 50.50, 57.37, 116.19 (CH); 130.41 (C); 131.38 (CH); 157.12, 173.57, 173.79, 176.29, 177.59, 180.15 (C). FAB-MS: 624 (39.1, $[M + Na]^+$), 602 (100, $[M + 1]^+$), 460 (33.6).

Ac-(2*R*,3*S*)-β^{2,3}-HVal-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_6$c-(2*R*,3*S*)-*β*^{2,3}-HAla-NH$_2$ (117). According to *GP 21* the *Rink*-amide resin (203.3 mg, 0.45 mmol/g) was coupled with **68** (93.0 mg, 0.274 mmol). Synthesis according to *GP 23b*, final acetylation according to *GP 24* and cleavage from the resin according to *GP 26* afforded crude **117** (38 mg, 80%, 83% HPLC purity). Purification by RP-HPLC (10–40% *B* in 20 min; C_8) according to *GP 27* yielded **117** (22.3 mg, 47%). White solid. RP-HPLC (20–50% *B* in 20 min, C_8) t_R 11.1, purity > 99%. M.p. 220 °C. IR ($CHCl_3$, 19.2 mM): 3460*w*, 3325*m*, 2964*m*, 2935*m*, 2861*w*, 1779*w*, 1655*s*, 1513*s*, 1457*m*, 1374*m*, 1332*w*, 1261*s*, 1170*s*, 1096*s*, 1015*s*, 865*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.72-0.81 (*m*, 2 CH); 0.92 (*d*, *J* = 6.7, Me); 0.95 (*d*, *J* = 6.7, Me); 1.07-1.42 (*m*, 3 Me, 8 CH): 1.10 (*d*, *J* = 7.1, Me); 1.16 (*d*, *J* = 7.1, Me); 1.20 (*d*, *J* = 6.9, Me); 1.57-1.77 (*m*, Me_2CH, 3 CH); 1.81-1.90 (*m*, CH); 2.02 (*s*, COMe); 2.60-2.69 (*m*, COCH); 2.72-2.79 (*m*, COCH); 3.27-3.42 (*m*, 3 NCH); 3.46 (*dd*, *J* = 15.1, 6.3, NCH); 4.12-4.26 (*m*, 2 NCH); 5.87 (*d*, *J* = 10.3, CHN*H*); 6.26 (*d*, *J* = 8.5, CHN*H*); 6.46 (br. *s*, N*H*H); 7.23 (br. *s*, NH*H*); 7.36 (*t*, *J* = 6.2, CH_2NH); 7.69 (*t*, *J* = 5.7, CH_2NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 11.57, 13.0 (Me); 14.01 (2 CH_2); 16.50, 18.43, 19.21, 19.85 (Me); 22.50, 22.72 (CH_2); 23.10 (Me); 25.50 (CH_2); 25.65 (C); 30.37 (CH); 32.07, 32.64 (CH_2); 42.21, 43.32 (CH); 43.49 (CH_2); 47.34 (CH); 47.61 (C); 48.08 (CH_2); 56.51 (CH); 171.40, 173.44, 173.89, 175.62, 178.00 (C). FAB-MS: 544 (19.5, $[M + Na]^+$), 522 (100, $[M + 1]^+$).

7.4.3 Synthesis of β-Peptides Consisting of Geminally Disubstituted Amino Acids

Methyl 3-[(3-{[(*tert*-Butoxy)carbonyl]amino}-3-methylbutanoyl)amino]-3-methylbutanoate (Boc-$\beta^{3,3}$-HAib-$\beta^{3,3}$-HAib-OMe; 118). Compound **72** (4.50 g, 19.5 mmol) was Boc-deprotected according to *GP 10a* and coupled with acid **71** (4.23 g, 19.5 mmol) according to *GP 19a* for 12 h. FC (Et_2O/pentane 1:1 → Et_2O) yielded **118** (3.87 g, 60%). White powder. M.p. 76-77 °C. R_f 0.14 (Et_2O/pentane 1:1). IR ($CHCl_3$): 3441*w*, 2978*m*, 1705*s*, 1666*s*, 1501*s*, 1454*m*, 1391*m*, 1368*m*, 1165*s*, 1081*m*, 1011*w*, 866*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.37 (*s*, 2 Me); 1.41 (*s*, 2 Me); 1.44 (*s*, *t*-Bu); 2.45 (*s*, CH_2); 2.79 (*s*, CH_2); 3.66 (*s*, OMe); 5.17 (*s*, OC(O)NH); 5.94 (*s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 27.19, 27.74, 28.52 (Me); 43.45, 47.45 (CH_2); 51.45 (C); 51.76 (Me); 52.00, 79.02, 155.06, 170.57, 171.75 (C). EI-MS: 331 (1.6, $[M + 1]^+$), 330 (5.8, M^+), 215 (36.2), 173 (33.9), 158 (51.8), 143 (45.4), 116 (100.0), 102 (50.2). Anal. calc. for $C_{16}H_{30}N_2O_5$ (330.42): C 58.16, H 9.15, N 8.48; found: C 58.03, H 9.22, N 8.45.

Boc-$\beta^{3,3}$-HAib-$\beta^{3,3}$-HAib-$\beta^{3,3}$-HAib-OMe (119). Dipeptide **118** (3.50 g, 10.6 mmol) was Boc-deprotected according to *GP 10a* and coupled with acid **71** (2.30 g, 10.6 mmol) according to *GP 19a* for 60 h. Recrystallization (AcOEt/pentane) and FC (AcOEt/pentane 1:1) yielded **119** (2.69 g, 59%). White powder. M.p. 105-106 °C. R_f 0.15 (AcOEt/pentane 1:1). IR ($CHCl_3$): 3439*w*, 3005*m*, 2974*m*, 1707*s*, 1665*s*, 1501*s*, 1453*m*, 1390*m*, 1368*m*, 1165*s*, 1080*m*, 1047*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.38 (*s*, Me); 1.40 (*s*, Me); 1.41 (*s*, Me); 1.44 (*s*, *t*-Bu); 2.42 (*s*, CH_2); 2.47 (*s*, CH_2); 2.76 (*s*, CH_2); 3.66 (*s*, OMe); 5.38 (*s*, OC(O)NH); 6.03 (*s*, NH); 6.54 (*s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 27.13, 27.21, 27.56, 28.52 (Me); 43.55, 47.42, 47.88 (CH_2); 51.48 (Me); 51.76, 52.09, 52.77, 78.95, 155.08, 170.54, 171.12, 171.75 (C). FAB-MS: 452 (1.2, $[M + Na]^+$), 430 (100.0, M^+), 330 (72.9). Anal. calc. for $C_{21}H_{39}N_3O_6$ (429.56): C 58.71, H 9.15, N 9.78; found: C 58.78, H 9.05, N 9.82.

Boc-$\beta^{3,3}$-HAib-$\beta^{3,3}$-HAib-$\beta^{3,3}$-HAib-OH (120). Methyl ester **119** (1.10 g, 2.6 mmol) was saponified according to *GP 15a* to yield **120** (1.06 g, quant.). White powder. Acid **120** was used in the next step without further purification. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.36 (*s*, Me); 1.40 (*s*, Me); 1.42 (*s*, Me), 1.45 (*s*, *t*-Bu); 2.50 (*s*, CH_2); 2.54 (*s*, CH_2); 2.81 (*s*, CH_2); 5.29 (br. *s*, OC(O)NH); 6.20 (*s*, NH); 6.42 (*s*, NH).

Boc-β^{3,3}-HAib-β^{3,3}-HAib-β^{3,3}-HAib-β^{3,3}-HAib-β^{3,3}-HAib-β^{3,3}-HAib-OMe (121). Tripeptide **119** (1.10 g, 2.6 mmol) was Boc-deprotected according to *GP 10a* and coupled with tripeptide acid **120** (1.06 g, 2.6 mmol) according to *GP 19a*. The cloudy viscous mass obtained after 15 h was dissolved in a few ml of $CHCl_3$. After addition of further HOBt (0.18 g, 1.3 mmol) and EDC (0.25 g, 1.3 mmol) the mixture was stirred for 24 h. FC ($MeOH/CH_2Cl_2$ 1:18) yielded **121** (2.69 g, 59%). White powder. M.p. 199-200 °C. R_f 0.11 ($MeOH/CH_2Cl_2$ 1:18). IR ($CHCl_3$): 3439*w*, 3336*w*, 3006*m*, 2974*m*, 1706*m*, 1659*s*, 1506*s*, 1453*m*, 1390*w*, 1367*m*, 1261*m*, 1168*m*, 1080*w*, 1015*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.38 (*s*, 2 Me); 1.42-1.43 (*m*, 2 Me, *t*-Bu); 2.30 (*s*, CH_2); 2.31 (*s*, CH_2); 2.33 (*s*, CH_2); 2.34 (*s*, CH_2); 2.35 (*s*, CH_2); 2.73 (*s*, CH_2); 3.67 (*s*, OMe); 5,74 (*s*, OC(O)NH); 6.39 (*s*, NH); 7.13 (*s*, NH); 7.19 (*s*, NH); 7.32 (*s*, NH); 7.36 (*s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 26.44, 26.58, 26.63, 27.02, 27.15, 28.53 (Me); 43.68, 47.96, 48.34, 48.42, 48.46 (CH_2); 51.51 (Me); 51.72, 52.41, 52.82, 53.93, 53.01, 53.03, 78.69, 155.07, 170.75, 170.84, 170.86, 170.91, 170.94, 171.84 (C). FAB-MS: 749 (6.4, [*M* + Na]$^+$), 727 (100.0, M^+), 627 (31.9). Anal. calc. for $C_{36}H_{66}N_6O_9$ (726.95): C 59.48, H 9.15, N 11.56; found: C 59.46, H 8.98, N 11.31.

H-β^{3,3}-HAib-β^{3,3}-HAib-β^{3,3}-HAib-β^{3,3}-HAib-β^{3,3}-HAib-β^{3,3}-HAib-OH (122). Hexapeptide **121** (267 mg, 0.37 mmol) was saponified according to *GP 15b* with 6 equiv. NaOH in $MeOH/H_2O$ 5:1 (0.03M) for 3 h. The crude acid (196 mg, 75%) was Boc-deprotected according to *GP 10b*. TFA salt **122** (200 mg, quant.). White powder. RP-HPLC (5-65% B in 20 min; C_8) t_R 13.3 min, purity > 99%. M.p. 50-62 °C. IR (KBr): 3334*m*, 3081*m*, 2978*m*, 1654*s*, 1540*s*, 1457*m*, 1368*m*, 1252*m*, 1202*s*, 1138*s*, 928*w*, 874*m*, 834*w*, 800*m*, 722*m*, 706*w*, 595*w*. ^{1}H-NMR (400 MHz, CD_3OD): 1.39-1.41 (*m*, 12 Me); 2.46 (*s*, CH_2); 2.49 (*s*, CH_2); 2.50 (*s*, 2 CH_2); 2.60 (*s*, CH_2); 2.79 (*s*, CH_2). ^{13}C-NMR (100 MHz, CD_3OD): 26.29, 27.29, 27.33, 27.37, 27.52, 27.60 (Me); 44.20, 45.24, 46.69, 47.45, 47.77, 47.79 (CH_2); 53.01, 53.87, 53.89, 53.91, 54.07, 54.10, 171.69, 172.80, 172.83, 172.85, 172.87, 174.65 (C). FAB-MS: 1286 (1.3, [2*M* + Na + K - 2]$^+$), 652 (36.8, [*M* + K]$^+$), 636 (50.4, [*M* + Na]$^+$), 614 [*M* + 1]$^+$).

Methyl 3-[(3-{[(*tert*-Butoxy)carbonyl]amino}-2,2-dimethylpropanoyl)amino]-2,2-dimethylpropanoate (Boc-β^{2,2}-HAib-β^{2,2}-HAib-OMe; 123). Compound **75** (3.60 g, 15.5 mmol) was Boc-deprotected according to *GP 10a* and coupled with acid **76** (3.37 g, 15.5 mmol) according to *GP 19a*. FC (Et_2O/pentane 3:2) yielded **123** (3.98 g, 78%). White powder. M.p. 66-67 °C. R_f 0.33 (Et_2O/pentane 3:2). IR ($CHCl_3$): 3453*m*, 3006*m*, 2975*m*, 1710*s*, 1656*m*, 1506*s*, 1474*m*, 1392*w*, 1367*m*, 1312*m*, 1158*s*, 1046*w*, 930*w*, 863*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.18 (*s*,

2 Me); 1.19 (*s*, 2 Me); 1.42 (*s*, *t*-Bu); 3.21 (*d*, *J* = 6.5, CH_2); 3.35 (*d*, *J* = 6.3, CH_2); 3.71 (*s*, OMe); 5.20 (*br.*, NH); 6.40 (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.14, 23.65, 28.39 (Me); 43.25, 43.40 (C); 46.71, 48.92 (CH_2); 79.01, 156.42, 177.00, 177.88 (C). EI-MS: 330 (0.3, M^+), 201 (61.9), 169 (30.8), 155 (52.7), 98 (100.0). Anal. calc. for $C_{16}H_{30}N_2O_5$ (330.42): C 58.16, H 9.15, N 8.48; found: C 58.06, H 9.27, N 8.45.

Boc-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-OMe (124). Dipeptide **123** (3.40 g, 10.3 mmol) was Boc-deprotected according to *GP 10a* and coupled with **76** (2.24 g, 10.3 mmol) according to *GP 19a* for 14 h. FC (AcOEt/pentane 3:2) yielded **124** (3.55 g, 80%). White powder. M.p. 107-108 °C. R_f 0.22 (AcOEt/pentane 3:2). IR ($CHCl_3$): 3451*m*, 3006*m*, 2972*m*, 1711*s*, 1652*s*, 1505*s*, 1474*m*, 1392*w*, 1368*m*, 1312*m*, 1158*s*, 932*w*, 860*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.17 (*s*, 2 Me); 1.18 (*s*, 2 Me); 1.19 (*s*, 2 Me); 1.42 (*s*, *t*-Bu); 3.21 (*d*, *J* = 6.4, CH_2); 3.32 (*d*, *J* = 5.9, CH_2); 3.35 (*d*, *J* = 6.3, CH_2); 3.71 (*s*, OMe); 5.26 (*br.*, NH); 6.46 (*br.*, NH); 6.88 (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.11, 23.62, 23.80 , 28.41 (Me); 42.55, 43.21 (C); 46.71, 47.36, 48.97 (CH_2); 52.21 (Me); 78.88, 156.42, 177.26, 177.46, 177.90 (C). FAB-MS: 452 (13.9, [*M* + Na]$^+$), 430 (100.0, M^+), 330 (75.9). Anal. calc. for $C_{21}H_{39}N_3O_6$ (429.56): C 58.72, H 9.15, N 9.78; found: C 58.52, H 9.05, N 9.76.

Boc-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-OH (125). Tripeptide **124** (1.61 g, 3.8 mmol) was saponified according to *GP 15a*. **125** (1.53 g, 98%). White powder. **125** was used in the next step without further purification. R_f 0.29 (MeOH/CH_2Cl_2 1:9). ^{1}H-NMR (200 MHz, CD_3OD): 1.15 (*s*, 2 Me); 1.17 (*s*, 4 Me); 1.43 (*s*, *t*-Bu); 3.18 (*s*, CH_2); 3.29-3.35 (*m*, 2 CH_2).

Boc-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-OMe (126). Fully protected tripeptide **124** (1.61 g, 3.8 mmol) was Boc-deprotected according to *GP 10a* and coupled with tripeptide acid **125** (1.53 g, 3.7 mmol) according to *GP 19a* for 15 h. 2 × FC (MeOH/CH_2Cl_2 1:18) yielded **126** (2.07 g, 79%). White powder. M.p. 168-170 °C. R_f 0.23 (MeOH/CH_2Cl_2 1:18). IR ($CHCl_3$): 3436*w*, 3323*w*, 3005*m*, 2975*m*, 2923*w*, 2872*w*, 1713*m*, 1656*s*, 1503*s*, 1451*m*, 1390*w*, 1369*m*, 1169*m*, 1077*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.17 (*s*, 2 Me); 1.18 (*s*, 4 Me); 1.19 (*s*, 6 Me); 1.41 (*s*, *t*-Bu); 3.20 (*d*, *J* = 6.4, CH_2); 3.29-3.32 (*m*, 4 CH_2); 3.34 (*d*, *J* = 6.2, CH_2); 3.71 (*s*, OMe); 5.30 (*br.*, NH); 6.49 (*br.*, NH); 7.05 (*br.*, NH); 7.10 (br. *t*, *J* = 5.6, NH); 7.20 (*br.*, 2 NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.10, 23.64, 23.74, 23.78, 28.41 (Me); 42.16, 42.18, 42.27, 42.30, 43.15 (C); 46.74, 47.47, 49.00 (CH_2); 52.25 (Me); 78.80, 156.43, 177.22, 177.51, 177.62, 177. 68, 177.93 (C). FAB-MS: 749 (28.8, [*M* + Na]$^+$), 727 (51.4, M^+), 627 (100.0). Anal. calc.

for $C_{36}H_{66}N_6O_9$ (726.95): C 59.48, H 9.15, N 11.56; found: C 59.20, H 8.92, N 11.40.

Boc-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-OH (127). Fully protected hexapeptide **126** (520 mg, 0.72 mmol) was dissolved in MeOH (4 ml), treated with 1N NaOH (3.6 ml) and stirred for 15 h at r.t. The cloudy mixture was solubilized with MeOH (8 ml) and 1N NaOH (3.6 ml) which was further heated at 50 °C for 5 h. MeOH was evaporated and the pH was adjusted to 1N HCl. The crude product was extracted with $CHCl_3$ (4×). The combined org. phases were washed with sat. aq. NaCl soln. (1×) dried ($MgSO_4$) and evaporated. **127** (509 mg, quant.). Yellowish powder. M.p. 64-65 °C. R_f 0.06 (MeOH/CH_2Cl_2 1:9). IR ($CHCl_3$): 3447*w*, 3008*m*, 2972*m*, 2936*w*, 1706*m*, 1648*s*, 1509*s*, 1747*m*, 1394*w*, 1368*m*, 1310*w*, 1170*m*, 989*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.17 (*s*, 6 Me); 1.18 (*s*, 4 Me); 1.21 (*s*, 2 Me); 1.42 (*s*, *t*-Bu); 3.22 (*d*, *J* = 6.5, CH_2); 3.27-3.35 (*m*, 5 CH_2); 5.33 (*br.*, NH); 6.55 (*br.*, NH); 6.85 (*br.*, NH); 7.05 (*br.*, 2 NH); 7.15 (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.22, 23.52, 23.67, 23.77, 28.42 (Me); 42.29, 42.53, 42.60, 42.81, 42.86, 43.34 (C); 46.77, 47.38, 47.44, 47.55, 47.65, 48.88 (CH_2); 79.00, 156.55, 177.31, 177.51, 177.73, 179.58 (C). FAB-MS: 736 (18.7, [*M* + Na]$^+$), 714 (83.8, [*M* + 1]$^+$), 614 (100.0).

H-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-OH (128). Hexapeptide acid **127** (200 mg, 0.28 mmol) was Boc-deprotected according to *GP 10b*. TFA salt of **128** (251 mg, quant.). Clear colorless oil. RP-HPLC (5-65% *B* in 20 min; C_8) t_R 12.6 min, purity 98.5%. IR (KBr): 3375*m*, 2973*m*, 2936*m*, 1654*s*, 1522*s*, 1475*m*, 1399*w*, 1368*w*, 1313*w*, 1202*s*, 1181*s*, 1139*s*, 1025*w*, 991*w*, 874*w*, 798*w*, 721*w*. ^{1}H-NMR (400 MHz, CD_3OD): 1.17 (*s*, 10 Me); 1.31 (*s*, 2 Me); 3.02 (*s*, CH_2); 3.30-3.34 (*m*, 5 CH_2). ^{13}C-NMR (100 MHz, CD_3OD): 23.73, 23.94, 23.98 (Me); 41.54, 44.26, 44.44, 44.48, 44.51, 44.53 (C); 48.03, 48.56, 48.67 (CH_2); 178.26, 179.29, 179.30, 179.35, 179.40, 180.42 (C). FAB-MS: 1416 (1.5), 670 (2.8), 652 (55.6, [*M* + K]$^+$), 636 (12.1, [*M* + Na]$^+$), 614 (100.0, [*M* + 1]$^+$).

Boc-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-β^{2,2}-HAib-OMe (129). Hexapeptide **126** (120 mg, 0.165 mmol) was Boc-deprotected according to *GP 10b* and coupled with acid **127** (120 mg, 0.165 mmol) according to *GP 19a* for 18 h, except that the acid **127** was dissolved in DMF (2 ml) instead of $CHCl_3$. FC (MeOH/CH_2Cl_2 1:14) gave **129** (135 mg. 62%). Glass. R_f 0.19 (MeOH/CH_2Cl_2 1:14). IR ($CHCl_3$): 3440*w*, 3336*w*, 3007*m*, 2972*m*, 2931*w*, 2870*w*, 1711*w*, 1649*s*, 1506*s*, 1475*m*, 1452*m*, 1388*w*, 1367*m*, 1314*w*, 1260*m*, 1174*m*, 987*w*, 911*w*. ^{1}H-

NMR (400 MHz, $CDCl_3$): 1.16-1.19 (*m*, 12 Me); 1.40-1.43 (*m*, 12 Me, *t*-Bu); 2.23-2.31 (*m*, 6 CH_2); 3.20 (*d*, *J* = 6.4, CH_2); 3.27-3.30 (*m*, 5 CH_2); 3.67 (*s*, OMe); 5.30 (*br.*, NH); 6.16 (*s*, NH); 7.06 (*m*, 2 NH); 7.21 (br. *t*, *J* = 5.7, NH); 7.25 (*br.*, NH); 7.30 (br. *t*, *J* = 5.7, NH); 7.36 (*s*, NH); 7.44 (*s*, NH); 7.61 (*s*, NH); 7.69 (*s*, NH); 7.78 (*s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 20.95, 23.65, 23.72, 23.76, 23.79, 26.03, 26.12, 26.21, 26.38, 26.42, 27.01, 28.42 (Me); 42.08, 42.12, 42.25, 42.28, 43.09, 43.17 (C); 47.47, 47.50, 47.54, 47.73, 48.13, 48.17, 48.62, 48.71, 48.92, 48.99 (CH_2); 51.64 (Me); 52.50, 53.08, 53.10, 53.12, 78.78, 128.92, 134.68, 156.44, 170.80, 170.84, 170.91, 170.95, 171.85, 176.93, 177.22, 177.64, 177.69 (C). FAB-MS: 1322 (100.0, M^+), 1222 (68.8).

Boc-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-OMe (130). Compound **78a** (6.00 g, 26.2 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with Boc-β^{2,2}-HAc$_3$c-OH (**79a**; 5.63 g, 26.2 mmol) according to *GP 19a* for 62 h. Recrystallization (Et_2O/pentane) yielded **130** (5.98 g, 70 %). Colorless crystals, suitable for X-ray analysis. M.p. 121.5-122.5 °C. R_f 0.54 (AcEt/pentane 2:1). IR ($CHCl_3$): 3446*m*, 3344*m*, 3007*s*, 2473*w*, 1709*s*, 1648*s*, 1515*s*, 1439*s*, 1392*m*, 1367*s*, 1058*w*, 1034*m*, 979*m*, 863*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.69-0.72 (*m*, 2 CH); 0.93-0.96 (*m*, 2 CH); 1.17-1.23 (*m*, 4 CH); 1.45 (*s*, *t*-Bu); 3.30 (*d*, *J* = 6.3, NCH_2); 3.43 (*d*, *J* = 5.9, NCH_2); 3.73 (*s*, OMe); 4.97 (*br.*, NH); 7.27 (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.02, 14.82 (CH_2); 24.28, 25.20 (C); 28.35 (Me); 42.71, 44.87 (CH_2); 52.01 (Me); 79.90 (C); 156.48, 172.91, 175.10 (C). EI-MS: 326 (<1, M^+), 224 (82.7), 193 (100), 124 (82.2). Anal. calc. for $C_{16}H_{26}N_2O_5$ (326.39): C 58.88, H 8.03, N 8.58; found: C 58.90, 7.92, N 8.45.

Boc-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-OMe (131). Dipeptide ester **130** (5.95 g, 18.3 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with Boc-β^{2,2}-HAc$_3$c-OH (**79a**; 3.93 g, 18.3 mmol) according to *GP 19a* overnight. Recrystallization (Et_2O/AcOEt/pentane 5:1:10) yielded **131** (6.27 g, 80%). White powder. M.p. 131-133 °C. R_f 0.37 (AcOEt/pentane 2:1). IR ($CHCl_3$): 3451*w*, 3311*w*, 3087*w*, 3005*m*, 2451*w*, 1696*s*, 1642*s*, 1561*m*, 1516*s*, 1439*m*, 1367*m*, 1162*s*, 1034*w*, 980*w*, 940*w*, 860*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.66-0.69 (*m*, 2 CH); 0.72-0.75 (*m*, 2 CH); 0.88-0.91 (*m*, 2 CH); 1.18-1.28 (*m*, 6 CH); 1.44 (*s*, *t*-Bu); 3.32 (*d*, *J* = 6.8, NCH_2); 3.42 (*d*, *J* = 6.2, NCH_2); 3.45 (*d*, *J* = 5.6 NCH_2); 3.71 (*s*, OMe); 5.04 (br. *t*, *J* = 6.8, NH), 7.84 (*br.*, NH); 7.92 (*br.*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.56, 14.60, 14.64 (CH_2); 23.94, 25.07, 25.44 (C); 28.36 (Me); 42.55, 43.82, 44.76 (CH_2); 51.97 (Me); 80.55, 157.11, 172.78, 173.65, 174.96 (C). FAB-MS: 848 (<1, $[2M+1]^+$), 847 (1.8, $[2M]^+$), 425 (27.3, $[M+1]^+$), 424 (100,

M^+). Anal. calc. for $C_{21}H_{33}N_3O_6$ (423.51): C 59.56, H 7.85, N 9.92; found: C 59.54, H 7.79, N 9.89.

Boc-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-OH (132). Fulla protected tripeptide **131** (3.19 g, 7.53 mmol) was saponified according to *GP 18*. Recrystallization (CH_2Cl_2) and drying under h.v. over P_2O_5 yielded **132** (2.44 g, 79%). M.p. 184-185.5 °C. R_f 0.48 (MeOH/CH_2Cl_2 1:9). IR ($CHCl_3$): 3450*w*, 3303*w*, 3008*m*, 1695*s*, 1638*m*, 1569*m*, 1517*m*, 1369*m*, 1041*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.77-0.84 (*m*, 4 CH); 0.88-0.96 (*m*, 2 CH); 1.12-1.21 (*m*, 6 CH); 1.44 (*s*, *t*-Bu); 3.28 (*s*, NCH_2); 3.40-3.44 (*m*, 2 NCH_2); 8.09 (*br.*, NH); 8.40 (*br.*, NH). ^{13}C-NMR (100 MHz, CD_3OD, rotamers!): 14.72, 14.90, 14.96 (CH_2); 24.23, 26.06, 26.75, 26.78 (C); 28.83 (Me); 44.00, 44.14, 44.48, 44.60, 45.16 (CH_2); 80.71, 159.27, 175.31, 176.51, 176.59, 177.89 (C). FAB-MS: 857 (<1, [2*M* + K]$^+$), 841 (9.2, [2 *M*+Na]$^+$), 819 (5.4, [2*M*]$^+$), 432 (31.9, [*M*+Na]$^+$), 411 (28.8, [*M*+1]$^+$), 410 (100, M^+). Anal. calc. for $C_{20}H_{31}N_3O_6$ (409.48): C 58.66, H 7.63, N 10.26; found: C 58.59, H 7.70, N 10.24.

Boc-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$-OMe (133). Fully protected tripeptide **131** (150 mg, 0.35 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with acid **79a** (75 mg, 0.35 mmol) according to *GP 19a* for 20 h. Recrystallization (AcOEt) yielded **133** (170 mg, 93%). Colorless crystals, suitable for X-ray analysis. M.p. 145 °C. R_f 0.33 (CH_2Cl_2/MeOH 15:1). IR ($CHCl_3$): 3451*w*, 3296*m*, 3092*w*, 3007*m*, 1692*s*, 1634*s*, 1573*s*, 1517*s*, 1439*m*, 1368*s*, 1163*s*, 1051*w*, 1035*w*, 980*w*, 943*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.63-0.74 (*m*, 4 CH); 0.76 (*dd*, *J* = 6.8, 3.9, 2 CH); 0.86-0.93 (*m*, 2 CH); 1.18-1.24 (*m*, 4 CH); 1.26-1.29 (*m*, 4 CH); 1.45 (*s*, *t*-Bu); 3.32 (*d*, *J* = 7.0, NCH_2); 3.38 (*d*, *J* = 6.3, NCH_2); 3.40 (*d*, *J* = 6.6, NCH_2); 3.46 (*d*, *J* = 5.6, NCH_2); 3.71 (*s*, OMe); 5.06 (*t*, *J* = 7.0, NH); 8.07 (*t*, *J* = 5.4, NH); 8.31 (*br.*, NH); 8.62 (br. *t*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.47, 14.77, 14.97, 15.03 (CH_2); 23.82, 25.25, 25.46, 25.52 (C); 28.35 (Me); 42.30, 43.69, 43.90, 44.74 (CH_2); 51.97 (Me); 80.79, 157.40, 173.03, 173.69, 174.34, 174.90 (C). FAB-MS: 543 (2.7, [*M* + Na]$^+$), 521 (100, M^+). Anal. calc. for $C_{26}H_{40}N_4O_7$ (520.62): C 59.98, H 7.74, N 10.76; found: C 59.99, H 7.68, N 10.68.

Boc-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$-OMe (134). Fully protected tripeptide **131** (2.413 g, 5.7 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled overnight with **132** (2.34 g, 5.7 mmol) according to *GP 19a*, except that **132** was dissolved in DMF (10 ml) instead of $CHCl_3$ before addition. Recrystallization (CH_2Cl_2/pentane) yielded **134** (3.56 g, 87%). White powder. M.p. 190 °C (sintering at 118-120 °C).

R_f 0.55 (MeOH/CH_2Cl_2 1:10). IR ($CHCl_3$): 3456*w*, 3285*m*, 3086*w*, 3004*m*, 1689*m*, 1630*s*, 1582*m*, 1516*m*, 1439*m*, 1367*m*, 1163*m*, 1036*w*, 980*w*, 945*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.68-0.82 (*m*, 10 CH); 0.86-0.90 (*m*, 2 CH); 1.18-1.29 (*m*, 12 CH); 1.45 (*s*, *t*-Bu); 3.32-3.46 (*m*, 6 NCH_2); 3.70 (*s*, OMe); 5.15 (br. *t*, *J* = 6.9, NH); 8.10 (br. *t*, *J* = 5.4, NH); 8.41 (*br.*, NH); 8.80 (br. *t*, *J* = 6.2, NH); 8.96-9.01 (*m*, 2 NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.49, 14.76, 15.09, 15.37, 15.43 (CH_2); 23.81, 25.30, 25.52, 25.64, 25.66, 25.72 (C); 28.36 (Me); 42.28, 43.60, 43.78, 43.89, 43.94, 44.73 (CH_2); 51.96 (Me); 80.84, 157.51, 173.10, 174.05, 174.61, 174.64, 174.93 (C). FAB-MS: 737 (5.6, [*M* + Na]$^+$), 715 (100, [*M* + 1]$^+$). Anal. calc. for $C_{36}H_{54}N_6O_9$ (714.86): C 60.49, H 7.61, N 11.76; found: C 60.41, H 7.62, N 11.73.

Boc-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$-OH (135). A soln. of hexapeptide **134** (0.125 g, 0.175 mmol) in TFE (0.9 ml) was heated under reflux with a soln. of LiOH (0.20 g, 8.75 mmol) in H_2O (0.4 ml) for 6 h. The product was precipitated by adding 1N HCl to the soln. at 0 °C. After evaporation of TFE under reduced pressure, crude **135** was filtrated and washed intensively with H_2O. Recrystallization (CH_2Cl_2/pentane, – 20 °C) yielded **135** (100 mg, 82%). White powder. M.p. 198 °C. R_f 0.31 (MeOH/CH_2Cl_2 1:9). IR ($CHCl_3$): 3686*w*, 3448*m*, 3281*m*, 3082*m*, 3008*m*, 2933*m*, 169*m*, 1628*s*, 1518*m*, 1440*m*, 1369*m*, 1164*m*, 1037*w*, 946*m*. ^{1}H-NMR (400 MHz, CD_3OD): 0.78-0.90 (*m*, 12 CH); 1.10-1.20 (*m*, 12 CH); 1.44 (*s*, *t*-Bu); 3.27 (*s*, NCH_2); 3.40-3.42 (*m*, 5 NCH_2); 8.55-8.58 (br. *t*, *J* = 6.0, NH). ^{13}C-NMR (100 MHz, CD_3OD): 14.74, 14.91, 15.17 (CH); 24.21, 26.06, 26.40, 26.43, 26.47, 26.77 (C); 28.83 (Me); 43.93,44.43, 44.55, 45.08 (CH_2); 80.76 (C); 159.30, 175.33, 176.12, 176.23, 176.29, 176.74, 177.86 (C). FAB-MS: 739 (1.4, [*M* + K]$^+$), 723 (16.1, [*M* + Na]$^+$), 702 (36.2, [*M* + 1]$^+$), 701 (100, *M*$^+$).

H-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$-β^{2,2}-HAc$_3$c-β^{2,2}-HAc$_3$-OH (136). Acid **135** (98 mg, 0.136 mmol) was Boc-deprotected according to *GP 10b*. The crude product was precipitated with $CHCl_3$/PE to yield the TFA salt of **136** (22 mg, 27%). White solid. M.p. 203 °C. IR (KBr): 3274*s*, 3080*s*, 3000*s*, 2934*s*, 1718*s*, 1636*s*, 1439*s*, 1362*s*, 1272*s*, 1204*s*, 1036*s*, 947*s*, 703*m*. ^{1}H-NMR (400 MHz, CD_3OD): 0.80-0.90 (*m*, 10 CH); 1.09-1.21 (*m*, 12 CH); 1.28-1.32 (*m*, 2 CH); 3.10 (*s*, NCH_2); 3.40 (*m*, 10 NCH); 8.06-8.08 (*m*, NH); 8.48 (*t*, *J* = 5.8, NH); 8.59 (*t*, *J* = 5.8, NH). ^{13}C-NMR (100 MHz, CD_3OD): 14.544, 14.88, 15.00 (CH_2); 23.42, 24.24, 26.15, 26.41, 26.47, 26.54 (C); 43.91, 44.11, 44.43, 44.48, 45.82 (CH_2); 175.37, 176.08, 176.25, 176.31, 176.44, 177.90 (C). FAB-MS: 602 (28.3, [*M* + 1]$^+$), 601 (75.1, *M*$^+$).

Boc-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-OMe (137). Compound **78d** (1.31 g, 4.6 mmol) was Boc-deprotected according to *GP 10a* and coupled with the Boc-protected acid **79d** (1.19 g, 4.6 mmol) according to *GP 19a* for 13 h, except that the acid **79d** was dissolved in DMF (0.25M) instead of $CHCl_3$. The crude product **137** was purified by FC (AcOEt/pentane 1:2) yielding **137** (1.169 g, 62%). Colorless oil. R_f 0.19 (Et_2O/pentane 1:1). IR ($CHCl_3$): 3457*w*, 3364*w*, 3007*m*, 2934*s*, 2859*m*, 1707*s*, 1660*m*, 1510*s*, 1454*m*, 1392*w*, 1367*m*, 1323*w*, 1246*m*, 1163*s*, 1140*m*, 1105*w*, 1045*w*, 964*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.16-1.65 (*m*, *t*-Bu, 16 CH); 1.79-1.83 (*m*, 2 CH); 2.04-2.08 (*m*, 2 CH); 3.19 (*d*, *J* = 6.1, NCH_2); 3.40 (*d*, *J* = 6.2, NCH_2); 3.72 (*s*, OMe); 5.30 (*s*, NH); 6.14 (*t*, *J* = 5.7, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.49, 25.65 (CH_2); 28.41 (Me); 31.83 (CH_2); 46.40 (CH_2); 47.60 (C); 48.00 (CH_2); 52.25 (Me); 77.04 (C); 156.38 (C); 175.00, 176.70 (C). FAB-MS: 843 (1.4, [2*M* + Na]$^+$), 821 (1.2, 2*M*$^+$), 433 (11.9, [*M* + Na]$^+$), 411 (67.5, [*M* + 1]$^+$) 311 (100). Anal. calc. for $C_{22}H_{38}N_2O_5$ (410.55): C 64.36, H 9.33, N 6.82; found: C 64.34, H 9.11, N 6.60.

Boc-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-OMe (138). Dipeptide **137** (1.145 g, 2.79 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with acid **79d** (0.719 g, 2.79 mmol) according to *GP 19a* for 16 h. Recrystallization (CH_2Cl_2/hexane) afforded **138** (1.163 g, 76%). Crystalline solid. M.p. 98-101 °C. R_f 0.20 (AcOEt/pentane 1:2). IR ($CHCl_3$): 3453*m*, 3007*m*, 2935*s*, 2859*m*, 1707*s*, 1646*s*, 1511*s*, 1455*m*, 1392*w*, 1367*m*, 1248*m*, 1166*m*, 1140*w*, 1047*w*, 874*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.26-1.64 (*m*, *t*-Bu, 24 CH); 1.78-1.86 (*m*, 4 CH); 2.02-2.05 (*m*, 2 CH); 3.24 (*d*, *J* = 6.1, NCH_2); 3.37 (*d*, *J* = 5.8, NCH_2); 3.40 (*d*, *J* = 6.1, NCH_2); 3.70 (*s*, OMe); 5.20 (*s*, NH); 6.28 (*s*, NH); 6.68 (*s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.35, 22.43, 22.54, 22.66, 25.60, 25.77, 25.81 (CH_2); 28.42 (Me); 31.71, 31.84, 31.92 (CH_2); 46.10, 46.18, 46.55, 47.00 (CH_2); 47.39 (C); 47.51 (CH_2); 52.22 (C); 53.43 (Me); 76.78, 77.03, 77.28 (C); 78.88 (C). FAB-MS: 1010 (7.9, [2*M* + 1]$^+$), 550 (100, [*M* + 1]$^+$), 450 (84.8). Anal. calc. for $C_{30}H_{51}N_3O_6$ (549.76): C 65.54, H 9.35, N 7.64; found: C 65.55, H 9.39, N 7.28.

Boc-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-OH (139). Fully protected tripeptide **138** (1.10 g, 2 mmol) was saponified according to *GP 15b* to afford the tripeptide acid **139** (1.056 g, 99%). White powder. M.p. 172-174 °C. R_f 0.18 (AcOEt/pentane 1:2). IR ($CHCl_3$): 3454*m*, 3383*m*, 3007*m*, 2932*s*, 2861*m*, 1706*s*, 1652*s*, 1515*s*, 1455*s*, 1404*m*, 1368*m*, 1318*w*, 1252*m*, 1165*s*, 1045*w*, 1024*w*, 959 *w*, 908*m*, 849*w*, 652*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.20-2.25 (*m*, *t*-Bu, 30 CH); 3.17 (*d*, *J* = 6.8, 1.4 H, CH_2N, rotamer); 3.21 (*d*, *J* = 6.1, 0.6 H, NCH_2, rotamer); 3.33-3.37 (*m*, NCH_2); 3.41-3.43 (*m*, NCH_2); 5.23 (br. *s*, 0.3 H, NH, rotamer); 6.01 (*t*, *J*

= 6.4, 0.7 H, NH, rotamer); 6.34 (br. *s*, 0.3 H, NH, rotamer); 6.45 (*t*, *J* = 6.6, NH); 6.65 (br. *s*, 0.7 H, NH, rotamer). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.49, 22.64, 22.76, 22.97, 23.05, 25.79, 25.86, 26.08 (CH_2); 32.14 (Me); 31.58, 31.83, 31.97, 32.14, 46.46, 46.64, 47.04 (CH_2); 47.17 (C); 47.27, 47.36, 47.47, 47.85 (CH_2); 48.21 (C); 48.41, 49.84 (CH_2); 76.72, 77.04, 77.24, 77.35, 79.75, 81.38, 156.68, 158.31, 174.97, 175.40, 175.75, 177.95, 178.40 (C). FAB-MS: 1094 (1.1, $[2M + Na]^+$), 1072 (0.9, $[2M + 1]^+$), 575 (1.1, $[M + K]^+$), 559 (8.0, $[M + Na]^+$), 537 (100.0, $[M + 1]^+$).

Boc-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-OMe (140). Tripeptide **138** (1.028 g, 1.87 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with tripeptide acid **139** (1.00 g, 1.87 mmol) according to *GP 19a* for 16 h. FC (AcOEt/pentane 3:2), followed by recrystallization (Et_2O/pentane 5:95) yielded **140** (0.651 g, 36%). White powder. M.p. 94-96 °C. R_f 0.29 (AcOEt/pentane 3:2). IR ($CHCl_3$): 3446*w*, 3378*w*, 3005*m*, 2933*s*, 2859*m*, 1708*m*, 1641*s*, 1509*s*, 1455*m*, 1252*w*, 1166*w*, 654*w*, 600*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.16-1.54 (*m*, *t*-Bu, 48 CH); 1.84-1.94 (*m*, 10 CH); 2.03-2.13 (*m*, 2 CH); 3.22 (*d*, *J* = 6.2, NCH_2); 3.32-3.39 (*m*, 5 NCH_2); 5.46 (*t*, *J* = 5.8, NH); 6.44 (*t*, *J* = 6.0, NH); 6.84 (br. *s*, NH); 6.95 (*t*, *J* = 5.7, NH); 7.03 (br. *s*, NH); 7.08 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.34, 22.38, 22.45, 22.60, 23.73, 25.61, 25.70, 25.75, 25.83 (CH_2); 28.43 (Me), 30.65, 31.68, 31.89, 31.92, 31.98, 34.13, 45.89, 46.09 (CH_2); 46.15 (C); 46.20, 46.27, 46.50, 46.59, 46.68, 47.07, 47.39 (CH_2); 47.57 (C); 52.27 (Me); 78.78, 156.40, 175.76, 175.80, 176.09, 176.25, 176.31, 176.64 (C). FAB-MS: 990 (15.0, $[M + Na]^+$), 968 (72.3, $[M + 1]^+$), 868 (100.0).

H-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-OH (141). According to *GP 20*, the *ortho*-chlorotrityl-choride resin (381 mg, 1 mmol/g) was esterified with acid **80b** (116 mg, 305 µmol). Loading 0.35 mmol/g (55%), corresponding to 132 µmol of anchored **80b**. Synthesis according to *GP 22a* and cleavage from the resin according to *GP 25a* afforded the crude TFA salt of **141** (55.6 mg, 47%) purity 65-95% (RP-HPLC), depending on the cleavage fraction (purity of first cleavage fraction: 65 %; purity of the following cleavage fractions up to 95% (HPLC)). Preparative RP-HPLC (20-90% *B* in 45 min) according to *GP 27* yielded the TFA salt of **141** (30.7 mg, 26%). White powder. Colorless crystals were obtained from $CDCl_3$ by slow evaporation at r.t. RP-HPLC (30-90 % *B* in 20 min; C_{18}) t_R 14.26. M.p. 140 °C (dec., sintering at 100 °C). R_f 0.03 (AcOEt). IR ($CHCl_3$): 3456*w*, 3364*w*, 3056*w*, 2935*s*, 2851*m*, 2236*w*, 1780*w*, 1643*s*, 1523*s*, 1251*w*, 1168*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.20-1.38 (*m*, 48 CH); 1.51-1.92 (*m*, 8 CH); 2.07-2.23 (*m*, 4 CH); 3.05 (br. *s*, NCH_2); 3.28-3.40 (*m*, 5 NCH_2); 5.15 (br. *s*, NH_3^+); 6.44 (*s*, NH);

6.67 (*s*, NH); 6.90 (*s*, NH); 7.04 (*s*, NH); 7.52 (*s*, NH); 8.16 (br. *s*, COOH). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.11, 22.47, 22.78, 25.30, 25.61, 29.71, 31.41, 32.03 (CH_2); 44.85 (C); 46.91, 47.26, 47.68 (CH_2); 77.02, 174.57, 175.96, 176.11, 176.43, 178.27 (C). FAB-MS: 892 (1.4, $[M + K]^+$), 876 (8.5, $[M + Na]^+$), 854 (100.0, $[M + 1]^+$).

Ac-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-β^{2,2}-HAc$_6$c-NH$_2$ (142). According to *GP 21*, the *Rink* amide resin (195.6 mg, 0.45 mmol/g) was loaded with acid **80b** (105 mg, 277 μmol). Synthesis according to *GP 23a*, acetylation according to *GP 24* and cleavage from the resin according to *GP 26* afforded the crude peptide **142** (87.6 mg, 105%), purity 77% (RP-HPLC). The peptide was purified by RP-HPLC (35-80% *B* in 20 min) according to *GP 27*: **142** (52 mg, 63%). White fluffy solid. RP-HPLC (75% *B* isocratic in 20 min; C_8) t_R 4.87. M.p. 99-102 °C. IR ($CHCl_3$): 3353*w*, 3008*m*, 2933*s*, 2859*m*, 1645*s*, 1516*s*, 1464*w*, 1448*s*, 1252*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.26-1.60 (*m*, 48 CH); 1.80-1.85 (*m*, 2 CH); 1.92-2.10 (*m*, 10 CH); 2.11 (*s*, COMe); 3.26-3.41 (*m*, 6 NCH_2); 3.90 (br. *s*, NH_2); 6.57-6.60 (*m*, 2 NH); 6.91 (*s*, NH); 7.16 (*s*, NH); 7.29 (*s*, NH); 7.62 (*s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.33, 22.38 (CH_2); 22.49 (Me); 22.53, 25.67, 31.67, 31.90, 32.12, 32.36 (CH_2); 46.69 (C); 46.83, 46.88, 47.04, 47.15, 47.42, 47.72 (CH_2); 76.70, 77.22, 77.34, 172.70, 175.46, 176.03, 176.13, 180.34 (C). FAB-MS: 916 (2.34, $[M + Na]^+$), 894 (100.0, $[M + 1]^+$).

7.4.4 Solid-Phase Synthesis of β-Peptides with (R)/(S)-β^3-Sequence

H-(*R*)-β^3-HVal-(*R*)-β^3-HAla-(*S*)-β^3-HLeu-(*R*)-β^3-HPhe-(*R*)-β^3-HVal-(*R*)-β^3-HAla-(*S*)-β^3-HLeu-OH; 143). According to *GP 20*, the *ortho*-chlorotrityl-chloride resin (140 mg, 1.3 mmol Cl/g) was esterified with acid **27** (47 mg, 128 μmol). Loading 0.38 mmol/g (54%), corresponding to 53 μmol of anchored **27**. Synthesis according to *GP 22a* and cleavage from the resin according to *GP 25a* afforded crude **143** as TFA salt (49.5 mg, 99%). Purity of the crude product: 80% (RP-HPLC). The peptide was purified by RP-HPLC (20-80% B in 40 min; C_8) according to *GP 27*: TFA salt of **143** (26.2 mg, 53%). White solid. HPLC (30-90% B, 20 min; C_8) t_R 9.52. M.p. 320 °C (dec.). CD (0.2mM in MeOH): + $1.02 \cdot 10^4$ (224 nm), – $1.02 \cdot 10^5$ (202 nm). IR (KBr): 3430*w*, 3278*w*, 3088*w*, 2966*w*, 1718*s*, 1654*m*, 1577*w*, 1560*w*, 1541*w*, 1506*w*, 1458*w*, 1410*m*, 1374*w*, 1341*s*, 1246*s*, 1124*s*, 1098*s*, 1017*s*, 971*w*, 872*m*, 789*w*, 725*s*. ^{1}H-NMR (200 MHz, CD_3CO_2D): 0.84-0.94 (*m*, 6 Me); 1.01 (*d*, *J* = 7.2, Me); 1.04 (*d*, *J* = 6.9, Me); 1.15 (*d*,

J = 6.9, Me); 1.20 (*d*, *J* = 6.5, Me); 1.23-1.41 (*m*, 3 CH); 1.43-1.64 (*m*, 4 CH); 1.79-1.81 (*m*, CH); 2.24-2.91 (*m*, 7 $COCH_2$, $PhCH_2$); 3.42-3.51 (*m*, NCH); 4.12-4.20 (*m*, NCH); 4.21-4.41 (*m*, 4 NCH); 4.42-4.59 (*m*, NCH); 7.20-7.30 (*m*, 5 arom. H); 7.45-8.15 (*m*, 7 NH). FAB-MS: 968 (9.0, $[M + Na]^+$), 830 (100, M^+).

H-(*R*)-β³-HVal-(*R*)-β³-HAla-(*S*)-β³-HLys-(*R*)-β³-HPhe-(*R*)-β³-HVal-(*R*)-β³-HAla-(*S*)-β³-HLys-OH; 144). According to *GP 20*, the *ortho*-chlorotrityl-chloride resin (162 mg, 1.3 mmol Cl/g) was esterified with acid **30** (92 mg, 190 μmol). Loading 0.35 mmol/g (45%), corresponding to 56 μmol of anchored **30**. Synthesis according to *GP 22a* and cleavage from the resin according to *GP 25b* afforded crude **144** as TFA salt (67 mg, 99%). Purity of the crude product: 50% (RP-HPLC). The peptide was purified by RP-HPLC (5-30% B in 60 min; C_8) according to *GP 27*: TFA salt of **144** (39.2 mg, 56%). White solid. HPLC (5-65% B in 20 min; C_8) t_R 11.43. M.p. 200 °C (dec.). $[\alpha]_D^{r.t.}$ = + 11.4 (*c* = 0.465, MeOH). CD (0.2mM in TFE): – $2.54 \cdot 10^4$ (202 nm), + 257 (218 nm). IR (KBr): 3288*m*, 3087*m*, 2970*m*, 2933*m*, 2872*m*, 1654*s*, 1560*s*, 1542*s*, 1508*m*, 1438*w*, 1376*w*, 1204*s*, 1182*s*, 1135*s*, 836*w*, 800*w*, 722*m*, 700*w*, 598*w*, 518*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.93 (*d*, *J* = 6.8, Me); 0.95 (*d*, *J* = 6.8, Me); 1.01 (*d*, *J* = 6.9, Me); 1.03 (*d*, *J* = 6.9, Me), 1.13 (*d*, *J* = 6.7, Me); 1.19 (*d*, *J* = 6.7, Me); 1.31-1.70 (*m*, 12 CH); 1.72-1.81 (*m*, Me_2CH); 1.92-2.00 (*m*, Me_2CH); 2.19-2.54 (*m*, 13 CH); 2.63 (*dd*, *J* = 16.2, 3.9, CH); 2.76 (*dd*, *J* = 13.7, 8.6, CH); 2.82-2.95 (*m*, 5 CH); 3.34-3.39 (*m*, NCH); 4.06-4.13 (*m*, 2 NCH); 4.18-4.32 (*m*, 3 NCH); 4.45-4.50 (*m*, NCH); 7.17-7.29 (*m*, 5 arom. H); 7.89 (*d*, *J* = 8.1, NH); 7.95 (*d*, *J* = 9.3, NH); 8.01 (*d*, *J* = 8.5, NH); 8.13 (*d*, *J* = 8.9, NH). ^{13}C-NMR (100 MHz, CD_3OD): 18.2, 18.7, 18.8, 20.0, 20.3, 20.6 (CH_3); 24.0, 24.1, 28.2, 28.2 (CH_2); 31.8, 33.3 (CH), 34.5, 35.0, 35.3, 40.2, 40.4, 40.6, 40.6, 41.4, 41.6, 42.3, 43.7, 43.8 (CH_2); 44.3, 44.6, 47.4, 48.1, 50.0, 53.8, 55.8 (CH); 127.6, 127.8, 128.7, 129.5, 130.1, 130.4 (CH); 139.8 (C); 171.6, 172.4, 172.7, 172.8, 172.8, 172.8, 174.9 (C). MALDI-MS: 899 ($[M + K]^+$), 883 ($[M + Na]^+$), 860 (M^+). FAB-MS: 1720 (2.1, $[2M]^+$), 882 (21.7, $[M + Na]^+$), 860 (100, M^+).

H-(*R*)-β³-HVal-(*R*)-β³-HAla-(*R*)-β³-HSer-(*R*)-β³-HPhe-(*R*)-β³-HVal-(*R*)-β³-Ala-(*R*)-β³-HSer-γ-lactone (145). According to *GP 20*, the *ortho*-chlorotrityl-chloride resin (169.0 mg, 1.00 mmol Cl/g) was esterified with Fmoc-β³-(*R*)-HSer-OH (**28**, 54.0 mg, 0.135 mmol). Loading 0.47 mmol/g (77%) corresponding to 80 μmol of anchored **28**. Synthesis according to *GP 22b* and cleavage from the resin according to *GP 25b* afforded crude **145** as TFA salt (61 mg, 99%), purity 74% (RP-HPLC). The peptide was purified by RP-HPLC (5-65% *B* in 20 min; C_{18}) according to *GP 27* and lyophilized from dioxane containing 10% H_2O. TFA salt of **145** (16 mg, 26%). White solid. HPLC (5-65%

B in 20 min; C_{18}) t_R 12.7 min, purity 90%. M.p. 214 °C (dec.). CD (0.2 mM in MeOH): + $3.04 \cdot 10^4$ (200 nm); CD (0.2 mM in TFE): – $1.15 \cdot 10^4$ (203 nm). IR (KBr): 3290*s*, 3078*m*, 2970*m*, 2933*m*, 2878*m*, 1774*m*, 1654*s*, 1543*s*, 1499*m*, 1422*m*, 1376*m*, 1306*m*, 1261*w*, 1200*s*, 1170*w*, 1133*m*, 1056*w*, 1033*w*, 833*w*, 734*w*, 717*w*, 700*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.92-0.97 (*m*, 2 Me); 1.01 (*d*, *J* = 6.9, Me); 1.03 (*d*, *J* = 6.9, Me); 1.15 (*d*, *J* = 6.8, Me); 1.20 (*d*, *J* = 6.7, Me); 1.31 (*t*, *J* = 7.3, OH); 1.34-1.40 (*m*, Me_2CH); 1.60-1.68 (m, Me_2CH); 1.73-1.81 (*m*, COCH); 1.91-1.99 (*m*, COCH); 2.21-2.30 (*m*, 2 COCH); 2.32-2.51 (*m*, 10 COCH); 2.64 (*dd*, *J* = 16.1, 3.6, CHH);); 2.77 (*dd*, *J* = 13.7, 8.6, CHH); 2.85-2.92 (*m*, CH_2); 3.33-3.38 (*m*, CHH); 3.42-3.50 (*m*, CHH); 4.00-4.05 (*m*, CHH); 4.15 (*dd*, *J* = 9.4, 2.8, CHH); 4.18-4.30 (*m*, 3 NCH); 4.40-4.52 (*m*, 3 NCH); 7.16-7.28 (*m*, 5 arom. H); 7.95 (*d*, *J* = 9.6, NH). ^{13}C-NMR (100 MHz, CD_3OD): 18.17, 18.77, 18.96, 20.00, 20.35, 20.69 (Me); 31.84, 33.17 (CH); 35.12, 35.16, 39,04, 40.03, 41.21, 41.58, 43.30, 43.74 (CH_2); 44.25, 44.61, 48.09, 50.07, 50.50, 54.04, 55.81 (CH); 64.38, 74.81 (CH_2); 127.57, 129.47, 130.45 (CH); 139.85, 171.72, 172.46, 172.66, 172.88, 173.02, 173.45, 178.16 (C). FAB-MS: 783 (20.0, $[M + Na]^+$), 761 (100, $[M + 1]^+$), 401 (21), 289 (52.5). MALDI (calc. for M^+: 759.96; matrix: 2,5-dihydroxy-benzoic acid): 782.6 ($[M + Na]^+$), 760.3 ($[M + 1]^+$).

7.4.5 Synthesis of β-Peptides Consisting of β²- and β³-Homoproline

Boc-(*S*)-β³-HPro-(*S*)-β³-HPro-OBn (149). (*S*)-**88** (0.459 g mg, 2.0 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with (*S*)-**89** (459 mg, 2.0 mmol) according to *GP 19a* for 2.5 d. FC (AcOEt/pentane 1:1) yielded **149** (540 mg, 63%). White waxy solid. M.p. 68-69 °C. R_f 0.31 (AcOEt/pentane 1:1). $[\alpha]_D^{r.t.}$ = – 60.8 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3007*m*, 2978*m*, 2880*w*, 1730*m*, 1680*s*, 1634*m*, 1454*m*, 1401*s*, 1366*m*, 1305*w*, 1168*m*, 1124*w*, 907*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.46 (*s*, *t*-Bu); 1.81-2.55 (*m*, 8 CH, 2 COCH); 2.98-3.03 (*m*, 2 COCH); 3.31-3.68 (*m*, 4 NCH); 4.05-4.13 (*m*, NCH); 4.39-4.45 (*m*, NCH); 5.07-5.14 (*m*, $PhCH_2$); 7.30-7.39 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$, rotamers in italics): *21.54*, 23.47, 24.00 (CH_2); 28.58 (Me); 29.91, 30.12, *31.20*, 37.55, 39.24, 45.23, 46.28 (CH_2); 53.74, *54.24*, 54.57 (CH); 66.25, *66.63* (CH_2); 79.10, *79.51* (C); 128.20, 128.32, 128.54, 128.67 (CH); 135.92, 154.40, 169.89, 171.31 (C). FAB-MS: 453 (5.9, $[M + Na]^+$), 431 (66.5, $[M + 1]^+$), 331 (100), 329 (35.0). Anal. calc. for $C_{24}H_{34}N_2O_5$ (430.54): C 66.95, H 7.96, N 6.51; found: C 66.76, H 7.88, N 6.56.

Boc-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-OBn (150). Fully protected dipeptide **149** (5.73 g, 13.3 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with (*S*)-**89** (3.05 g, 13.3 mmol) according to *GP 19a* for 16 h. FC (MeOH/CH_2Cl_2 1:22 → 1:10) yielded **150** (6.58 g, 91%). Colorless highly viscous oil. R_f 0.29 (CH_2Cl_2/MeOH 22:1). $[\alpha]_D^{r.t.} = -69.6$ (*c* = 1.0, $CHCl_3$). UV (0.2 mM, MeOH): λ_{max} = 213 nm. CD (0.2 mM, MeOH): − 1.32 · 10^5 (202 nm), + 4.09 · 10^4 (222 nm). IR ($CHCl_3$): 3007*m*, 2977*m*, 2879*w*, 1729*m*, 1681*s*, 1632*s*, 1402*s*, 1366*m*, 1168*m*, 1124*w*, 907*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.46 (*s*, *t*-Bu); 1.74-2.36 (*m*, 16 CH); 2.94-3.02 (*m*, 2 COCH); 3.32-3.68 (*m*, 6 NCH); 4.13-4.15 (*m*, NCH); 4.27-4.30 (*m*, NCH); 4.38-4.45 (*m*, NCH); 5.07-5.14 (*m*, PhCH_2); 7.29-7.36 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$, rotamers in italics): 23.82, 23.93, 24.02 (CH_2); 28.57 (Me); 29.36, 29.92, 30.57, 37.61, 38.30, 39.17, 46.64, 47.18, 47.29 (CH_2); 53.72, 54.13 (CH); 66.24, *66.57* (CH_2); 79.13, *79.47* (C); 128.17, 128.32, 128.52, 128.62 (CH); 135.93, 154.39, 169.60, 169.75, 171.30 (C). FAB-MS: 542 (42.9, [*M* + 1]$^+$), 443 (30.5), 442 (100), 91 (100). Anal. calc. for $C_{30}H_{43}N_3O_6$ (541.69): C 66.52, H 8.00, N 7.76; found: C 66.66, H 7.86, N 7.63.

Boc-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-OH (151). Fully protected tripeptide **150** (3.20 g, 5.9 mmol) was debenzylated in AcOEt according to *GP 9* to yield **151** (2.65 g, quant.). White powder. M.p. 63-68 °C. R_f 0.28 (CH_2Cl_2/MeOH 12:1). $[\alpha]_D^{r.t.} = -56.5$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3007*m*, 2979*m*, 2880*w*, 1728*m*, 1681*s*, 1629*s*, 1451*m*, 1401*s*, 1367*m*, 1170*m*, 1125*w*, 907*w*. ^{1}H-NMR (400 MHz, CD_3OD): 1.45 (*s*, *t*-Bu); 1.80-2.11 (*m*, 12 CH); 2.22-2.43 (*m*, 3 COCH); 2.77-3.03 (*m*, 3 COCH); 3.28-3.66 (*m*, 6 NCH); 4.10-4.15 (*m*, NCH); 4.30-4.35 (*br.*, 2 NCH). ^{13}C-NMR (100 MHz, CD_3OD, rotamers in italics): 22.41, 24.54, 24.67 (CH_2); 28.85 (Me); 30.81, 30.99, 32.17, 38.03, 39.02, 40.03, 46.50, 47.33, 47.72 (CH_2); 55.45, 55.52, 55.59 (CH); 73.93, 80.70, *81.36*, 156.16, 171.92, 175.04 (C). FAB-MS: 474 (16.2, [*M* + Na]$^+$), 452 (24.4, [*M* + 1]$^+$), 352 (100), 241 (48.0). Anal. calc. for $C_{23}H_{37}N_3O_6$ (451.56): C 61.18, H 8.26, N 9.31; found: C 61.09, H 8.16, N 9.11.

TFA·H-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-OBn (152). Fully protected tripeptide **150** (2.59 g, 4.8 mmol) was Boc-deprotected according to *GP 10a* to yield the TFA salt **152** (3.41 g, quant.). Colorless oil, that crystallized after 15 d upon storage at − 20 °C, colorless crystals, suitable for X-ray analysis. CD (0.2 mM, MeOH): − 2.83 · 10^4 (203 nm), 8.80 · 10^3 (223 nm). ^{1}H-NMR (400 MHz, $CDCl_3$): 1.78-2.26 (*m*, 12 CH); 2.43 (*dd*, *J* = 14.3, 7.4, COCH); 2.51 (*dd*, *J* = 15.5, 8.4, COCH); 2.70 (*dd*, *J* = 14.3, 6.8, COCH); 2.83-2.92 (*m*, 3 COCH); 3.35-3.61 (*m*, 6 NCH); 3.88-3.94 (*m*, NCH); 4.33-4.38 (*m*, NCH); 4.49-4.52 (*m*, NCH); 5.10-5.17 (*m*, PhCH_2); 7.33-7.42 (*m*, 5 arom. H); 8.55 (*br.*, NH); 8.91 (*br.*, NH). ^{13}C-NMR

(100 MHz, $CDCl_3$, rotamers in italics): 23.23, 23.36, 23.61, 29.25, 30.07, 30.19, 34.23, 37.37, 38.25, 45.58, 47.17, 47.89 (CH_2); 53.92, 55.32, 57.57 (CH); 66.78, *67.03* (CH_2); 115.23 (*q*, *J* = 282.9, CF_3); 128.25, 128.34, 128.42, 128.62, 128.71 (CH); *135.20*, 135.52 (C); 160.20 (*q*, *J* = 40.0, CCF_3); 169.90, 171.28, 171.31 (C). FAB-MS: 883 (12.1, $[2M]^+$), 443 (36.1, $[M + 1]^+$), 442 (100, M^+).

Boc-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-OBn (153). Fully protected tripeptide **150** (2.60 g, 4.8 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with **151** (2.16 g, 4.8 mmol) according to *GP 19a* for 2.5 d. FC (MeOH/CH_2Cl_2 1:15) yielded **153** (3.54 g, 85%). White foam. M.p. 65-68 °C. R_f 0.28 (CH_2Cl_2/MeOH 15:1). $[\alpha]_D^{r.t.} = -84.8$ (*c* = 1.0, $CHCl_3$). UV (0.2 mM, MeOH): λ_{max} = 217 nm. CD (0.2 mM, MeOH): $-1.90 \cdot 10^5$ (202 nm), $+9.02 \cdot 10^4$ (222 nm). IR ($CHCl_3$): 3007*m*, 2978*m*, 2879*w*, 1730*w*, 1680*m*, 1632*s*, 1425*s*, 1366*w*, 1170*m*, 1123*w*, 1097*w*, 907*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.46 (*s*, *t*-Bu); 1.72-2.15 (*m*, 30 CH); 2.33 (*dd*, *J* = 15.4, 9.5, COCH); 2.96-3.05 (*m*, 5 COCH); 3.36-3.66 (*m*, 12 NCH); 4.11-4.18 (*m*, NCH); 4.29-4.44 (*m*, 5 NCH); 5.07-5.30 (*m*, PhCH_2); 7.29-7.37 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$, rotamers in italics): 21.57, 23.48, 23.80, 23.93, 24.01 (CH_2); 28.58 (Me); 29.40, 29.83, 29.86, 29.92, 30.61, 37.62, 38.11, 38.17, 38.31, 39.11, 45.25, 46.65, 47.11, 47.15, 47.23, 47.28, *47.37* (CH_2); 53.71, 54.03, 54.06, 54.11, 54.25, 54.49 (CH); 66.22, *66.55* (CH_2); 79.14, *79.48* (C); 128.15, 128.31, 128.38, 128.52, 128.60 (CH); *135.68*, 135.96, 154.42, 169.51, 169.62, 169.71, 169.74, 171.32 (C). FAB-MS: 898 (13.1, $[M + Na]^+$), 876 (27.1, $[M + 1]^+$), 875 (52.9, M^+), 775 (100). Anal. calc. for $C_{48}H_{70}N_6O_9$ (875.12): C 65.43, H 8.69, N 9.54; found: C 65.47, H 8.87, N 9.45.

Boc-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-OH (154). 153 (1.01 g, 1.2 mmol) was debenzylated in AcOEt according to *GP 9* to yield **154** (1.02 g, quant.). White foam. M.p. 69-73 °C. R_f 0.24 (CH_2Cl_2/MeOH 7:1). $[\alpha]_D^{r.t.} = -67.3$ (*c* = 1.0, $CHCl_3$). CD (0.2 mM, MeOH): $-8.14 \cdot 10^4$ (204 nm), $+5.20 \cdot 10^4$ (224 nm). IR ($CHCl_3$): 3307*m*, 2979*m*, 2879*w*, 1728*w*, 1681*m*, 1630*s*, 1423*s*, 1366*w*, 1173*w*, 1048*w*, 881*w*. ^{1}H-NMR (400 MHz, CD_3OD): 1.45 (*s*, *t*-Bu); 1.83-2.16 (*m*, 24 CH); 2.23-2.38 (*m*, 6 COCH); 2.46-2.59 (*m*, COCH); 2.77-3.03 (*m*, 5 COCH); 3.30-3.71 (*m*, 12 NCH); 4.09-4.14 (*m*, NCH); 4.31-4.49 (*m*, 5 NCH). ^{13}C-NMR (100 MHz, CD_3OD, rotamers in italics): 22.41, 24.33, 24.55, 24.67 (CH_2); 28.78, 28.86 (Me); 30.85, 31.01, 31.42, 31.69, 32.16, 38.07, 39.01, 40.03, 46.50, 47.36, 47.72 (CH_2); 55.45, 55.51, 55.60, 56.11, 56.22, 57.81 (CH); 80.69, *81.35*, 156.16, 171.02, 171.14, 171.92, 172.03, 174.40, 175.11 (C). FAB-MS: 808 (39.1, $[M + Na]^+$), 807 (91.7), 786 (42.1, $[M + 1]^+$), 785 (100, M^+), 685 (60.5).

Boc-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-OBn (155). Hexapeptide **153** (166 mg, 0.19 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with **154** (150 mg, 0.19 mmol) according to *GP 19a* for 40 h. Recrystallization (CH_2Cl_2/hexane) yielded **155** (233 mg, 79%). White foam. M.p. 95 °C (dec.). R_f 0.30 (CH_2Cl_2/MeOH 9:1). $[\alpha]_D^{r.t.} = -87.4$ (c = 1.0, $CHCl_3$). CD (0.1 mM, MeOH): – 2.63 · 10^5 (203 nm), + 1.82 · 10^5 (223 nm). IR ($CHCl_3$): 3005*m*, 2878*w*, 1729*m*, 1631*s*, 1525*m*, 1426*s*, 1352*m*, 857*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.46 (*s*, *t*-Bu); 1.74-2.17 (*m*, 60 CH); 2.33 (*dd*, *J* = 15.3, 9.5, COCH); 2.96-3.02 (*m*, 11 COCH); 3.36-3.67 (*m*, 24 NCH); 4.15-4.49 (*m*, 12 NCH); 5.07-5.14 (*m*, $PhCH_2$); 7.28-7.38 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.53, 22.66, 23.78, 24.01 (CH_2); 28.58 (Me); 29.39, 29.71, 29.88, 29.91, 31.59, 37.61, 38.10, 38.16, 38.30, 47.12, 47.28 (CH_2); 53.44, 53.71, 54.05, 54.10, 54.23, 54.52 (CH); 66.23 (CH_2); 79.16 (C); 128.16, 128.32, 128.40, 128.52, 128.61 (CH); 135.95, 154.44, 168.64, 169.32, 169.64, 169.74, 171.35 (C). FAB-MS: 1565 (4.1, $[M + Na]^+$), 1543 (100, $[M + 1]^+$). ESI-MS (pos. mode): 1580.3 ($[M + K]^+$), 1564.4 ($[M + Na]^+$), 1542.3 ($[M + 1]^+$).

Boc-(S)-β³-HPro-OBn (156). 155 (62 mg, 0.040 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with **154** (31 mg, 0.040 mmol) according to *GP 19a* for 3 d. Recrystallization (CH_2Cl_2/pentane) yielded **156** (48 mg, 54%). White powder. M.p. 228 °C (dec.). R_f 0.22 (CH_2Cl_2/MeOH 9:1). $[\alpha]_D^{r.t.} = -74.0$ (c = 1.0, $CHCl_3$). CD (0.2 mM, MeOH): – 3.46 · 10^5 (202 nm), + 2.09 · 10^5 (223 nm). IR ($CHCl_3$): 3006*m*, 2878*w*, 1728*w*, 1680*w*, 1632*s*, 1425*m*, 1360*w*, 1323*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.46 (*s*, *t*-Bu); 1.83-2.18 (*m*, 90 CH); 2.33 (*dd*, *J* = 15.4, 9.5, COCH); 2.92-3.02 (*m*, 17 COCH); 3.33-3.65 (*m*, 36 NCH); 4.12-4.52 (*m*, 18 NCH); 5.07-5.13 (*m*, $PhCH_2$); 7.29-7.37 (*m*, 5 arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$): 23.78, 24.01 (CH_2); 28.60 (Me); 29.42, 29.89, 37.62, 38.12, 38.40, 47.13, 47.29 (CH_2); 53.44, 53.73, 54.06, 54.12, 54.47 (CH); 66.24 (C); 128.16, 128.31, 128.52 (CH); 135.96, 154.45, 169.67, 169.76, 171.34 (C). FAB-MS: 2208 (100, M^+), 1104 (52.2). ESI-MS (pos. mode): 2231.8 ($[M + Na]^+$),

Ac-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-(*S*)-β³-HPro-OBn (157). 153 (0.197 g, 0.225 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was dissolved in CH_2Cl_2 (2.3 ml) and treated at 0 °C with Ac_2O (0.03 ml, 0.293 mmol) and $(i\text{-}Pr)_2EtN$ (0.12 ml, 0.675 mmol). After

2.5 h the soln. was diluted with CH_2Cl_2 and washed with 1N HCl, sat. aq. $NaHCO_3$ and NaCl solns. The org. phase was dries ($MgSO_4$) and evaporated under reduced pressure. FC (MeOH/CH_2Cl_2 1:12) yielded **157** (101 mg, 55%). White foam. M.p. 63-67 °C. R_f 0.26 (CH_2Cl_2/MeOH 12:1). $[\alpha]_D^{r.t.} = -77.9$ (c = 1.0, $CHCl_3$). CD (0.2 mM, MeOH): $-1.14 \cdot 10^5$ (203 nm), $+6.42 \cdot 10^4$ (223 nm). IR ($CHCl_3$): 3004*m*, 2878*w*, 1730*w*, 1631*s*, 1503*w*, 1422*m*, 1359*w*, 1325*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.73-2.18 (*m*, 33 CH); 2.32 (*dd*, *J* = 15.4, 9.5, COCH); 2.96-3.09 (*m*, 5 COCH); 3.29-3.73 (*m*, 12 NCH); 4.29-4.44 (*m*, 6 NCH); 5.07-5.14 (*m*, $PhCH_2$); 7.28-7.37 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$, rotamers in italics): 14.05 (C); 22.21, 22.33 (CH_2); 23.03 (Me); 23.76, 23.90, 23.99, 29.38, 29.72, 29.81, 29.84, 29.90, 30.04, *31.63*, 37.59, 37.94, 38.11, 38.15, 38.29, 39.63, 45.25, 45.47, 47.13, 47.21, 47.27, 47.88 (CH_2); 53.70, 54.01, 54.05, 54.09, 54.16, 54.39, *54.48*, *55.32* (CH); 66.22 (CH_2); 128.15, 128.30, 128.38, 128.51, 128.60 (CH); 135.94, *168.64*, *169.33*, 169.57, 159.59, 169.61, 169.64, 169.73, 171.32 (C). FAB-MS: 839 (11.8, $[M + Na]^+$), 818 (45, $[M + 1]^+$), 817 (100, M^+).

(4-$NO_2C_6H_5$)CO-(*S*)-β^3-HPro-(*S*)-β^3-HPro-(*S*)-β^3-HPro-(*S*)-β^3-HPro-(*S*)-β^3-HPro-(*S*)-β^3-HPro-OBn (158). **153** (0.197 g, 0.225 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was dissloved in CH_2Cl_2 (1 ml) and treated at 0 °C with Et_3N (0.28 ml, 0.675 mmol), *p*-nitrobenzoylchloride (50 mg, 0.270 mmol) and DMAP (2.8 mg, 0.023 mmol). The yellow suspension was stirred for 13 h at r.t. After dilution with CH_2Cl_2, the mixture was washed with sat. aq. NH_4Cl, $NaHCO_3$ and NaCl solns. The org. phase was dries ($MgSO_4$) and evaporated under reduced pressure. FC (MeOH/CH_2Cl_2 1:12) yielded **158** (166 mg, 80%). Yellow foam. M.p. 148 °C (dec.). R_f 0.34 (CH_2Cl_2/MeOH 12:1). $[\alpha]_D^{r.t.} = -44.1$ (c = 1.0, $CHCl_3$). CD (0.2 mM, MeOH): $-1.23 \cdot 10^5$ (204 nm), $+6.06 \cdot 10^4$ (225 nm). IR ($CHCl_3$): 3005*m*, 2878*w*, 1730*w*, 1632*s*, 1525*m*, 1426*s*, 1352*m*, 1045*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.73-2.08 (*m*, 27 CH); 2.12 (*dd*, *J* = 15.0, 10.5, COCH); 2.33 (*dd*, *J* = 15.4, 9.4, COCH); 2.45 (*dd*, *J* = 15.1, 9.4, COCH); 2.96-3.03 (*m*, 5 COCH); 3.12 (*dd*, *J* = 15.1, 3.1, COCH); 3.35-3.67 (*m*, 12 NCH); 4.37-4.56 (*m*, 6 NCH); 5.07-5.14 (*m*, $PhCH_2$); 7.28-7.38 (*m*, 5 arom. H); 7.67-7.72 (*m*, 2 arom. H); 8.25-8.29 (*m*, 2 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$, rotamers in italics): 21.54, 23.77, 23.79, 23.99, 24.92, 29.38, 29.69, 29.83, 29.90, 29.94, 30.40, 37.59, 38.01, 38.08, 38.14, 38.22, 38.29, 47.11, 47.14, 47.21, 47.26, 50.23 (CH_2); 53.70, 54.02, 54.10, 54.18, 54.98 (CH); 66.22 (CH_2); 123.63, 123.71, 128.15, 128.23, 128.30, 128.38, 128.51, *128.60* (CH); 135.94, 143.07, 148.53, *167.58*, *169.09*, 169.47, 169.47, 169.56, 169.61, 169.71, 171.33 (C). FAB-MS: 946 (17.4, $[M + Na]^+$), 925 (49.0, $[M + 1]^+$), 924 (100, M^+).

Boc-(*S*)-β³-HPro-(*R*)-β³-HPro-OBn (159). (*R*)-**88** (3.23 g, 10.1 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with (*S*)-**89** (2.42 g, 10.1 mmol) according to *GP 19a* for 2 d. FC (AcOEt/pentane 1:1) yielded **159** (2.82 g, 62%). Colorless oil. R_f 0.26 (AcOEt/pentane 1:1). $[\alpha]_D^{r.t.}$ = – 6.02 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3007*m*, 2978*m*, 2879*w*, 1730*m*, 1681*s*, 1635*m*, 1456*m*, 1401*s*, 1367*m*, 1168*m*, 1125*w*, 907*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, rotamers in italics): 1.43, *1.46* (*s*, *t*-Bu); 1.81-2.21 (*m*, 10 CH); 2.72-3.04 (*m*, 2 COCH); 3.32-3.57 (*m*, 4 NCH); 4.15 (*m*, NCH); 4.41 (*m*, NCH); 5.07-5.14 (*m*, $PhCH_2$); 7.30-7.38 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$, rotamers in italics): 23.52, 23.95 (CH_2); 28.59 (Me); 30.08, 31.26, 37.69, 39.00, 45.42, 47.19 (CH_2); 53.82, 54.28 (CH); 66.25 (CH_2); 79.09, *79.49* (C); 128.21, 128.28, 128.53 (CH); 135.94, 154.41, 169.73, *171.30* (C). FAB-MS: 430 (3.8, M^+), 329 (64.0) 91 (100). Anal. calc. for $C_{24}H_{34}N_2O_5$ (430.54): C 66.95, H 7.96, N 6.51; found: C 66.88, H 7.86, N 6.56.

Boc-(*S*)-β³-HPro-(*R*)-β³-HPro-OH (160). 159 (3.30 g, 7.7 mmol) was debenzylated in AcOEt according to *GP 9* to yield **160** (2.65 g, quant.). White powder. M.p. 140-141 °C. R_f 0.35 (CH_2Cl_2/MeOH 9:1). $[\alpha]_D^{r.t.}$ = – 38.4 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3007*m*, 2980*m*, 2880*w*, 1729*m*, 1681*s*, 1627*m*, 1455*m*, 1401*s*, 1367*m*, 1169*m*, 1126*w*, 904*w*. ^{1}H-NMR (400 MHz, CD_3OD): 1.46 (*s*, *t*-Bu); 1.83-2.13 (*m*, 8 CH); 2.34-2.51 (*m*, 2 COCH); 2.68-2.89 (*m*, 2 COCH); 3.28-3.60 (*m*, 4 NCH); 4.10-4.22 (*m*, NCH). ^{13}C-NMR (100 MHz, CD_3OD, rotamers in italics): 22.42, 24.64 (CH_2); 28.84 (Me); 31.12, 38.12, *39.86*, *40.37*, 46.60, *47.37*, 47.76 (CH_2); 55.51, 56.35 (CH); 80.74, *81.35*, 156.22, 172.02, 175.08 (C). FAB-MS: 703 (3.8, [*M* + Na]$^+$), 681 (7.7, [2*M* + 1]$^+$), 363 (11.4, [*M* + Na]$^+$), 341 (100, [*M* + 1]$^+$), 241 (95.8). Anal. calc. for $C_{17}H_{28}N_2O_5$ (340.42): C 59.98, H 8.29, N 8.23; found: C 60.10, H 8.12, N 8.22.

Boc-(*S*)-β³-HPro-(*R*)-β³-HPro-(*S*)-β³-HPro-(*R*)-β³-HPro-OBn (161). 159 (1.68 g, 3.9 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with **160** (1.33 g, 3.9 mmol) according to *GP 19a* for 16 h. FC (MeOH/CH_2Cl_2 1:17) yielded **161** (2.08 g, 81%). White solid. M.p. 156-159 °C. R_f 0.30 (CH_2Cl_2/MeOH 17:1). $[\alpha]_D^{r.t.}$ = – 2.12 (*c* = 1.0, $CHCl_3$). CD (0.2 mM, MeOH): + 9.86 · 10^3 (214 nm), – 2.07 · 10^3 (228 nm). IR ($CHCl_3$): 3007*m*, 2979*m*, 2879*w*, 1730*m*, 1681*m*, 1631*s*, 1495*s*, 1366*m*, 1169*m*, 1124*w*, 1103*w*, 907*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, rotamers in italics): *1.42*, 1.46 (*s*, *t*-Bu); 1.70-2.21 (*m*, 19 CH); 2.40 (*dd*, *J* = 15.3, 9.2, COCH); 2.71-3.02 (*m*, 4 COCH); 3.35-3.66 (*m*, 8 NCH); 4.07-4.18 (*m*, NCH); 4.31-4.41 (*m*, 3 NCH); 5.07-5.15 (*m*, $PhCH_2$); 7.29-7.38 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$, rotamers in italics): 21.65,

23.83, 23.91, 23.98 (CH_2); 29.59 (Me); *29.71*, 29.76, 29.84, *30.07*, 30.12, *30.61*, 31.59, *37.03*, *37.63*, 37.69, 37.83, 38.18, 38.40, *39.46*, 45.57, 47.05, 47.17, 47.26 (CH_2); 53.87, *53.99*, 54.13, 54.22, 54.57 (CH); 66.26, 66.37 (CH_2); 79.16, *79.59*, 128.20, 128.24, 128.28, *128.38*, 128.54, 128.57, *128.62*, 135.94, 154.42, 169.61, 171.36 (C). FAB-MS: 653 (100, $[M+1]^+$), 554 (21), 553 (57.4). Anal. calc. for $C_{36}H_{52}N_4O_7$ (652.83): C 66.23, H 8.03, N 8.58; found: C 66.05, H 7.96, N 8.60.

Boc-(*S*)-β³-HPro-(*R*)-β³-HPro-(*S*)-β³-HPro-(*R*)-β³-HPro-(*S*)-β³-HPro-(*R*)-β³-HPro-OBn (162). 161 (1.89 g, 2.9 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with **160** (0.98 g, 2.9 mmol) according to *GP 19a* for 3 d. FC ($MeOH/CH_2Cl_2$ 1:15) yielded **162** (632 mg, 25%). White powder. M.p. 246 °C (dec.). R_f 0.29 (CH_2Cl_2/MeOH 17:1). $[\alpha]_D^{r.t.}$ = – 1.68 (*c* = 1.0, $CHCl_3$). CD (0.2 mM, MeOH): + 1.06 · 10^4 (210 nm). IR ($CHCl_3$): 3004*m*, 2976*m*, 2880*w*, 1730*w*, 1682*m*, 1631*s*, 1421*s*, 1366*w*, 1171*m*, 1123*w*, 1103*w*, 906*w*. ^{1}H-NMR (500 MHz, $CDCl_3$, rotamers in italics): *1.42*, 1.46 (*s*, *t*-Bu); 1.73-2.19 (*m*, 30 CH); 2.39 (*dd*, *J* = 15.3, 9.2, COCH); 2.75-3.03 (*m*, 6 COCH); 3.36-3.65 (*m*, COCH); 4.08-4.18 (*m*, NCH); 4.29-4.41 (*m*, 5 NCH); 5.07-5.15 (*m*, $PhCH_2$); 5.07-5.14 (*m*, 5 arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$, rotamers in italics): 21.63, *21.66*, *23.75*, 23.83, 23.85, 23.89, 23.92, 24.01 (CH_2); 28.60 (Me); 29.71, 29.75, 29.82, 30.14, *30.65*, 30.69, 31.62, 37.08, *37.64*, 37.69, *38.16*, 38.22, 38.41, 38.46, 39.49, 46.55, 47.01, 47.11, 47.17, 47.22, 47.26 (CH_2); 53.85, *53.97*, 54.13, 54.20, 54.22, 54.51, 54.63 (CH); 66.22, 66.33 (CH_2); 79.10, *79.56* (C); 128.18, 128.23, 128.26, 128.52, 128.56 (CH); 135.97, 154.39, 169.56, 171.33 (C). FAB-MS: 898 (14.2, $[M + Na]^+$), 877 (49.3, $[M + 1]^+$), 876 (100, M^+). Anal. calc. for $C_{48}H_{70}N_6O_9$ (875.12): C 65.88, H 8.06, N 9.60; found: C 65.62, H 8.01, N 9.58.

Boc-(*S*)-β²-HPro-(*S*)-β²-HPro-OEt (163). (*S*)-**90** (1.37 g, 8.72 mmol) was coupled with **95** (2 g, 8.72 mmol) according to *GP 19a* for 16 h. FC (AcOEt/pentane 1:2) yielded **163** (2.8 g, 87%). For analytical purposes, **163** was dried under h.v. at 35 °C overnight. Yellowish waxy solid. M.p. 71-72 °C. R_f 0.25 (AcOEt/pentane 1:2). $[\alpha]_D^{r.t.}$ = + 61.1 (*c* = 0.365, $CHCl_3$). IR ($CHCl_3$): 3007*w*, 2945*w*, 2866*w*, 1726*m*, 1681*s*, 1628*s*, 1468*w*, 1444*m*, 1425*m*, 1367*w*, 1306*w*, 1265*m*, 1177*m*, 1150*s*, 1031*w*, 856*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, rotamers!): 1.23-1.30 (*m*, Me); 1.46-1.51 (*m*, *t*-Bu, CH); 1.62-1.88 (*m*, 6 CH); 2.05-2.08 (*m*, CH); 2.43-3.12 (*m*, 4 NCH, 2 COCH); 3.39 (*br.*, 0.5 H, NCH); 3.73-4.21 (*m*, 5.5 H, NCH, OCH_2); 4.59 (br. *d*, *J* = 10.3, 0.5 H, NCH). ^{13}C-NMR (100 MHz, $CDCl_3$, rotamers!): 14.19 (Me); 23.98, 24.71, 25.41, 27.35, 27.46, 27.73 (CH_2); 28.49 (Me); 38.97, 41.27, (CH); 42.02, 43.65, 45.75, 47.19, 60.62, *60.90*, (CH_2); 72.51 (CH); 79.63, 154.66, 171.67, *171.99*, 172.70, *173.19* (C). FAB-MS: 369 (24.2, $[M+1]^+$), 327 (18.8); 313 (100), 295 (78.8), 269

(63.9), 267 (64.1), 156 (70.1), 154 (49.6), 147 (76.1), 136 (96.1). Anal. calc. for $C_{19}H_{32}N_2O_5$ (368.47): C 61.93, H 8.75, N 7.60; found: C 61.89, H 8.74, N 7.53.

Boc-(*S*)-β²-HPro-(*S*)-β²-HPro-(*S*)-β²-HPro-OEt (164). 163 (2.53 g, 6.86 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with **95** (1.57 g, 6.86 mmol) according to *GP 19a* for 16 h. FC ($MeOH/CH_2Cl_2$ 1:15) yielded **164** (2.31 g, 70 %). White waxy solid. M.p. 80 °C (sintering at 50-55 °C). R_f 0.33 ($MeOH/CH_2Cl_2$ 1:15). $[\alpha]_D^{r.t.}$ = + 67.8 (*c* = 0.515, $CHCl_3$). CD (0.2 mM in MeOH): – $2.09 \cdot 10^4$ (211 nm), + $4.15 \cdot 10^3$ (230 nm). IR ($CHCl_3$): 3008*m*, 2943*w*, 2865*w*, 1726*m*, 1675*m*, 1631*s*, 1443*m*, 1367*w*, 1150*m*, 855*w*. ^{1}H-NMR (500 MHz, $CDCl_3$, rotamers!): 1.23-1.30 (*m*, Me); 1.44-2.17 (*m*, *t*-Bu, 12 CH); 2.40-3.14 (*m*, 3 COCH, 6 NCH); 3.33-4.21 (*m*, OCH_2, 5 NCH); 4.49-5.30 (*m*, NCH). ^{13}C-NMR (125 MHz, $CDCl_3$, rotamers!): 14.19, 23.54, 24.00, 24.65, 25.31, 26.06, 27.05, 27.19, 27.44, 27.63, 27.76, 28.20, 28.46, 28.50, 28.61, 31.44, 36.48, 38.55, 39.04, 39.73, 41.06, 41.27, 42.01, 42.05, 42.50, 43.69, 44.49, 44.79, 45.86, 46.07, 46.78, 47.31, 48.06, 60.59, 60.70, 60.91, 60.59; 79.66, 154.63, 162.52, 171.26, 171.60, 171.69, 171.84, 172.03, 172.68, 172.95, 173.21 (C). FAB-MS: 502 (2.0, [*M* + Na]$^+$), 480 (62.2, M^+), 380 (100), 269 (30.0).

Boc-(*S*)-β²-HPro-(*S*)-β²-HPro-(*S*)-β²-HPro-OH (165). 164 (1.22 g, 2.54 mmol) was saponified according to *GP 18*. Recrystallization (AcOEt/pentane) yielded **165** (0.574 g, 50%). White powder. M.p. 175-177 °C. R_f 0.25 ($MeOH/CH_2Cl_2$ 1:10). $[\alpha]_D^{r.t.}$ = + 52.6 (*c* = 0.50, $CHCl_3$). IR ($CHCl_3$): 3006*w*, 2944*w*, 2863*w*, 1719*w*, 1680*m*, 1625*s*, 1444*m*, 1368*w*, 1152*m*, 855*w*. ^{1}H-NMR (500 MHz, $CDCl_3$, rotamers!): 1.44-2.17 (*m*, *t*-Bu, 12 H); 2.46-4.21 (*m*, 3 COCH, 11 NCH); 4.49-4.63 (*m*, NCH); 5.96 (br. *s*, CO_2H). ^{13}C-NMR (125 MHz, $CDCl_3$): not shown because of rotamers. FAB-MS: 474 (18.5, [*M* + Na]$^+$), 452 (100, [*M* + 1]$^+$), 352 (71.2), 241 (44.7). Anal. calc. for $C_{23}H_{37}N_3O_6$ (451.56): C 61.18, H 8.26, N 9.31; found: C 60.99, H 8.17, N 9.13.

Boc-(*S*)-β²-HPro-(*S*)-β²-HPro-(*S*)-β²-HPro-(*S*)-β²-HPro-(*S*)-β²-HPro-(*S*)-β²-HPro-OEt (166). 164 (0.63 g, 1.31 mmol) was Boc-deprotected according to *GP 10a*. The resulting TFA salt was coupled with **165** (0.474 g, 1.048 mmol) according to *GP 19a* for 16 h. FC (CH_2Cl_2/MeOH 9:1) yielded **166** (681 mg, 79%). Colorless glass. M.p. 116 °C (sintering at 104 °C). R_f 0.35 (CH_2Cl_2/MeOH 9:1). $[\alpha]_D^{r.t.}$ = + 96.3 (*c* = 0.325, $CHCl_3$). CD (0.2 mM in MeOH): – $8.54 \cdot 10^4$ (208 nm), + $2.44 \cdot 10^4$ (228 nm). IR ($CHCl_3$): 3007*m*, 2946*w*, 2860*w*, 1726*w*, 1682*m*, 1631*s*, 1442*m*, 1367*w*, 1149*m*, 856*w*. ^{1}H-NMR (500 MHz, $CDCl_3$, rotamers!): 1.23-1.29 (*m*, Me); 1.39-2.17 (*m*, *t*-Bu, 24 CH); 2.40-4.19 (*m*, 6 COCH, 20 NCH,

OCH_2); 4.60 (*br.*, 4 NCH). FAB-MS: 1649 (12.8, [2 *M* + Na]$^+$), 1627 (19.4, [2*M*]$^+$), 835 (15.9, [*M* + Na]$^+$), 813 (37.9, [*M* + 1]$^+$), 713 (100), 306 (26.1), 195 (34.7).

7.4.6 Solid Phase Synthesis of β-Peptides **168** *and* **169**

H-(*S*)-β^3-HLys-(*S*)-β^3-HLys-(*S*)-β^3-HLys-(*S*)-β^3-HLys-(*S*)-β^3-HLys-(*S*)-β^3-HLys-(*S*)-β^3-HLys-OH·8 TFA (168). According to *GP 20* the *ortho*-chlorotrityl-chloride resin (189 mg, 1.00 mmol Cl/g) was esterified with Fmoc-(*S*)-β^3-HLys(Boc)-OH (**30**, 64.0 mg, 0.168 mmol). Loading 0.338 mmol/g (57%) corresponding to 64 μmol of anchored **30**. Synthesis according to *GP 22b* and cleavage from the resin according to *GP 25b* afforded crude **168** as TFA salt (112 mg, 91%), purity 79% (RP-HPLC). The peptide was purified by prep. RP-HPLC (2–30% *B* in 20 min; C_8) according to *GP 27*: TFA salt of **168** (67.8 mg, 55%). Colorless high viscous liquid. RP-HPLC (0–25% *B* in 20 min; C_{18}) t_R 10.0 min, purity > 99%. M.p. 235 °C (dec., sintering at 200 °C). CD (0.2 mM in MeOH): – $1.2 \cdot 10^4$ (219 nm). IR (KBr): 3600-3000 (br.), 2956*m*, 2867*m*, 2362*s*, 2340*s*, 1850-1350*br*, 1206*m*, 1172*m*, 1128*m*, 839*w*, 800*w*, 722*w*, 666*m*, 606*w*, 522*w*. ^{1}H-NMR (400 MHz, D_2O): 1.29-1.70 (*m*, 21 CH_2); 2.28-2.46 (m, 11 COCH); 2.50-2.66 (m, 3 COCH); 3.55-3.59 (m, NCH); 4.07-4.17 (m, 6 NCH). ^{13}C-NMR (100 MHz, D_2O): 24.34, 24.89, 29.07, 29.13, 29.17, 34.35, 35.69, 35.81, 39.68, 41.77, 41.88, 41.97, 43.73, 43.79, 43.93 (CH_2); 49.02, 49.70, 49.75, 51.43 (CH); 119.07 (*q*, *J* = 583.5, 291.6, CF_3); 165.61 (*q*, *J* = 70.7, 35.1, CF_3CO); 173.85, 175.14, 175.20, 175.27, 178.05 (C). FAB-MS: 1052 (4.9, [*M* + K]$^+$), 1036 (14.9, [*M* + Na]$^+$), 1015 (100, [*M* + 1]$^+$), 307 (19), 137 (63.5), 84 (62.5). Anal. calc. for $C_{49}H_{100}N_{14}O_8 \cdot 8\ CF_3CO_2H$ (1925.61): C 40.54, H 5.65, N 10.18; found: C 39.42, H 5.39, N 9.82.

H-(*S*)-β^3-HAla-(*S*)-β^3-HLys-(*S*)-β^3-HPhe-(*S*)-β^3-HAla-(*S*)-β^3-HLys-(*S*)-β^3-HPhe-(*S*)-β^3-HAla-(*S*)-β^3-HLys-(*S*)-β^3-HPhe-OH (169). According to *GP 20*, the *ortho*-chlorotrityl-chloride resin (158.6 mg, 1.00 mmol Cl/g) was esterified with Fmoc-(*S*)-β^3-HPhe-OH [203] (48 mg, 0.12 mmol). Loading 0.38 mmol/g (65%) corresponding to 60 μmol of anchored Fmoc-(*S*)-β^3-HPhe-OH. Synthesis according to *GP 22b* and cleavage from the resin according to *GP 25b* afforded crude **169** as TFA salt (99 mg, quant.), purity 49% (RP-HPLC). The peptide was purified by RP-HPLC (10-35% *B* in 20 min, then 35-45% *B* in 15 min; C_8) according to *GP 27*: TFA salt of **169** (25.7 mg, 26%). White solid. HPLC (15-65% *B* in 20 min; C_8) t_R 11.7 min, purity > 95%. M.p. 137 °C (dec.). CD (0.2 mM in MeOH): – $4.8 \cdot 10^4$ (219 nm). IR (KBr): 3293*br*, 3096*m*, 2974*m*, 2947*m*, 1676*s*,

1654*s*, 1560*m*, 1541*m*, 1437*w*, 1265*w*, 1205*s*, 1136*m*, 1031*w*, 838*w*, 800*w*, 723*m*, 701*w*. ^{1}H-NMR (400 MHz, CD_3OD): 1.12 (*d*, *J* = 6.7, Me); 1.14 (*d*, *J* = 6.6, Me); 1.29-1.76 (*m*, Me, 9 CH_2); 2.25-2.70 (*m*, 9 $COCH_2$); 2.74-2.96 (*m*, 3 PhCH_2, 3 NCH_2); 3.69-3.75 (*m*, NCH); 4.38-4.68 (*m*, 8 NCH); 7.16-7.28 (*m*, 15 arom. H); 7.77 (*d*, *J* = 9.0, NH); 8.16 (*d*, *J* = 9.8, NH); 8.23 (*d*, *J* = 8.8, NH); 8.41-8.44 (m, NH). ^{13}C-NMR (100 MHz, CD_3OD): 18.84, 20.93, 21.56 (Me); 23.90, 24.09, 24.45, 28.03, 28.73, 28.82, 35.89, 36.54, 40.64, 40.67, 40.70, 41.11, 41.57, 41.65, 42.25, 43.23, 43.37 (CH); 43.49, 43.64, 43.81, 46.67, 46.99, 47.26, 47.41, 127.70, 129.50, 129.57, 130.52, 130.62, 130.65 (CH); 139.40, 139.52, 139.66, 171.43, 171.46, 171.64, 171.75, 171.91, 172.13, 172.21, 173.11. FAB-MS: 1222 (18.0, $[M + K]^+$), 1206 (39.8, $[M + Na]^+$), 1184 (100, $[M + 1]^+$), 1042 (13.4).

7.5 Exchange Kinetics of Amide Protons of $\beta^{2,3}$-Hexapeptides 105 and 106.

^{1}H-NMR, 200 (**106**) or 300 MHz (**105**), 24.5 °C: The sample was either evaporated to dryness under h.v. or lyophilized (from H_2O and dioxane, respectively) before dissolving it in CD_3OD. The concentrations were 12.5 mg (**106**) and 15 mg (**105**) in CD_3OD (0.7 ml). ^{1}H-NMR spectra were taken at different times, covering 2 to 3 times the half-life of the corresponding amide proton. The intensity of each NH-signal was normalized relative to the corresponding value for a nonexchangeable peak for each data set. First-order rate constants, k, were calculated from the slope of the plot of $\ln[I(NH_{exchangeable})/I(H_{nonexchangeable})]$ *vs.* time.

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During my Ph.D. thesis, I was responsible for the training and supervision of two apprentices in organic chemistry. In addition to supervising two organic laboratory classes for first and second year students, I was also an assistant in two organic chemistry laboratory classes for final year students. In the last year, I was a teaching assistant for Prof. *Seebach*'s Organic Chemistry III lecture course. Moreover, I had the opportunity to supervise three diploma theses in the *Seebach* group.

Zürich, June 1999 Stefan Abele