Isotopically Labelled and Unlabelled β-Peptides with Geminal Dimethyl Substitution in 2-Position of Each Residue: Synthesis and NMR Investigation in Solution and in the Solid State

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Dedicated to Professor *Albert I. Meyers*, Colorado State University, on the occasion of his 70th birthday

The preparation of (*S*)-$\beta^{2,2,3}$-amino acids with two Me groups in the α-position and the side chains of Ala, Val, and Leu in the β-position (double methylation of Boc-β-HAla-OMe, Boc-β-Val-OMe, and Boc-β-Leu-OMe, *Scheme 2*) is described. These β-amino acids and unlabelled as well as specifically ^{13}C- and ^{15}N-labelled 2,2-dimethyl-3-amino acid ($\beta^{2,2}$-HAib) derivatives have been coupled in solution (*Schemes 1*, *3* and *4*) to give protected (*N*-Boc, *C*-OMe), partially protected (*N*-Boc/*C*-OH, *N*-H/*C*-OMe), and unprotected $\beta^{2,2}$- and $\beta^{2,2,3}$-hexapeptides, and $\beta^{2,2}$- and $\beta^{2,2,3}$-heptapeptides **1**–**7**. NMR Analyses in solution (*Tables 1* and *2*, and *Figs. 2–4*) and in the solid state (2D-MAS NMR measurements of the fully labelled Boc-($\beta^{2,2}$-HAib)$_6$-OMe ([$^{13}C_{30}$, $^{15}N_6$]-**1e**; *Fig. 5*), and TEDOR/REDOR NMR investigations of mixtures (*Fig. 6*) of the unlabelled Ac-($\beta^{2,2}$-HAib)$_7$-OMe (**4**) and of a labelled derivative ([$^{13}C_4$,$^{15}N_2$]-**5**; *Figs. 7–11,* and *19*), a molecular-modeling study (*Figs. 13–15*), and a search in the *Cambridge Crystallographic Data Base* (*Fig. 16*) allow the following conclusions: *i*) there is no evidence for folding (helix or turn) or for aggregation to sheets of the geminally dimethyl substituted peptide chains in solution; *ii*) there are distinct conformational preferences of the individual $\beta^{2,2}$- and $\beta^{2,2,3}$-amino acid residues: close to eclipsing around the C(O)–C(Me$_2$(CHR)) bond ($\tau_{1,2}$), almost perfect staggering around the C(2)–C(3) ethane bond ($\tau_{2,3}$), and antiperiplanar arrangement of H(C3) and H(N) ($\tau_{3,N}$; *Fig. 12*) in the solid state; *iii*) the $\beta^{2,2}$-peptides may be part of a turn structure with a ten-membered H-bonded ring; *iv*) the main structure present in the solid state of F$_3$CCO($\beta^{2,2}$-HAib)$_7$-OMe is a nonfolded chain (> 30 Å between the *termini* and > 20 Å between the *N-terminus* and the CH$_2$ group of residue 5) with all C=O bonds in a parallel

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[1]) Postdoctoral research at ETH Zürich (1998–2000) financed by the Swiss National Science Foundation, Grant No. 20-50674.97/1.

[2]) Part of the projected Ph.D. Theses of *D. J. B.* and *A. G.*, and of the Dissertation No. 14677 by *M. R.*, ETH-Zürich.

[3]) Gruppe für Informatikgestützte Chemie.

alignment (±10°). With these structural parameters, a simple modelling was performed producing three (maybe four) possible chain geometries: one fully extended, two with parallel peptide planes (with zick-zack and crankshaft-type arrangement of the peptide bonds), and (possibly) a fourth with meander-like winding (**D** – **G** in *Figs. 17* and *18*).

1. Introduction. – The influence of geminally disubstitued β-amino acid residues (*Fig. 1*) [1–6] on the secondary structure (*i.e.*, folding pattern) of β-peptides was first discussed in 1996, when we showed by circular-dichroism (CD) spectroscopy that a β^3-HAib residue in the central positon of a β^3-heptapeptide 'destroys' the 3_{14}-helical structure present in MeOH solutions of corresponding β^3-hexa- and β^3-heptapeptides lacking the geminal dimethyl groups [5]. Detailed analyses revealed that the β-peptidic 3_{14} helix does not tolerate non-H atoms[4]) or groups in axial positions due to steric hindrance[5]) in the *ca.* 5-Å pitches [3][6][7]. Most disturbingly, the CD spectra of β-hexapeptides consisting of $\beta^{2,2,3}$-trisubstituted amino acid moieties[6]) were essentially superimposable [3] on those of β-peptides, which clearly form 3_{14} helices [4–8]. Without structural assignments and with no synthetic details, these CD spectra have been published [3], and the CD 'puzzle' has led to an elaborate molecular dynamics (MD) study (with the GROMOS simulation package) and a theoretical calculation (according to the so-called matrix method [9]) of the CD spectrum of a β-hexapeptide built from trisubstituted β-amino acids[7]) in MeOH, including a comparison with the corresponding simple β^3-hexapeptide, the conclusion being that there is no preferred conformation (secondary structure) in MeOH solution of the chain carrying three substituents on each β-amino acid, in spite of very similar, distinct measured and calculated CD spectra [10]. On the other hand, CHARM m23.1 and *ab initio* calculations by *Hofmann* and co-workers of structures with simple geminally dimethyl-substituted $\beta^{2,2}$- and $\beta^{3,3}$-amino acid residues[8]) led to helical and β-strand-type secondary structures in the gas phase [11].

To obtain more experimental data about β-peptides with geminally-disubstituted residues, we have now synthesized derivatives **1** – **5** of specifically ^{13}C- and ^{15}N-labelled achiral $\beta^{2,2}$-HAib oligomers (for solid-state NMR analysis), and we describe here, in full detail, the preparation of the chiral precursors and the assembly of $\beta^{2,2,3}$-hexapeptides **6** and **7**, with and without terminal protection, as well as the results of extensive solution and solid-state-NMR investigations of these compounds[9]).

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4) Fluorine has, so far, not been tested; work on the synthesis of 3-amino-2-fluoro-acids and the derived β-peptides is underway in the group of *D.S.*

5) Like in α-peptides and proteins, geminal disubstitution of residues should also prevent pleated-sheet formation in β-peptides (see the discussion in [3]).

6) Even more irritating is that a β-peptide consisting of five (*S*)- and one (*R*)-3-amino-2,2-dimethylbutanoic acid building blocks (see **6c**, below) still gave rise to the same CD pattern [3], while incorporation of one 'wrong' or D-β^3-amino acid residue into the center of a chain consisting of six L-β^3-amino acid residues caused the characterisitic CD pattern of a 3_{14} helix to disappear [5].

7) *Cf.* the molecular formula **7** (below).

8) *Cf.* the molecular formulae **1** – **6** (below) and *Fig. 1*.

9) As mentioned above, the CD spectra have been published previously in this journal [3].

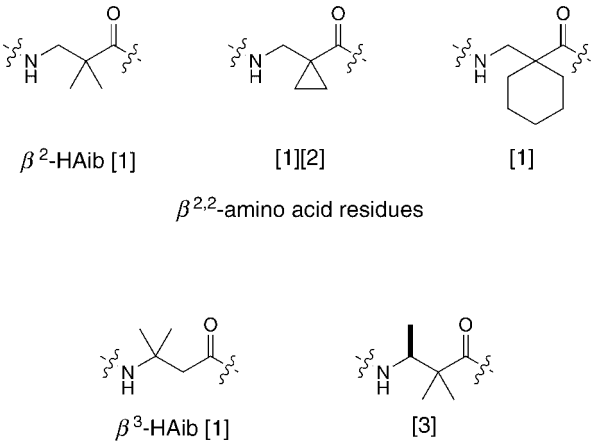

Fig. 1. *Geminally disubstituted β-amino-acid residues.* Structural information on the cyclopropane and cyclohexane derivatives has been obtained from single-crystal X-ray analysis; oligomers consisting of the 2,2-dimethylene residues fold to ribbon-type arrangements of eight-membered H-bonded rings [2], those with 2,2-pentamethylene substitution form a ten-membered H-bonded turn. No detailed structural information is available for the β-HAib oligomers and for those consisting of trisubstituted β-amino acid residues, except that the latter ones give rise to a pattern in the CD spectrum [3] identical to that of the nonmethylated analogs [4–6].

2. Synthesis of the Achiral $\beta^{2,2}$-Peptides 1–5. – The numerous methods for preparing geminally-disubstituted β-amino acids have been collected in a review article that covers the literature up to 1999 [12][10]). For the large-scale preparation of the unlabelled Boc-protected methyl 3-amino-2,2-dimethylpropanoate (Boc-$\beta^{2,2}$-HAib-OMe; **1a**), we used the method described previously (double methylation of Boc-β-HGly-OMe) [1]; we also followed procedures for the coupling and fragment coupling to oligomers **1**, **2**, and **3 b**–**f** reported previously [1][11]). The isotopically labelled compounds [^{13}C]-**1a** and [^{13}C,^{15}N]-**1a** were purchased[12]) and shown by NMR spectroscopy to contain >99% of the label(s) at the indicated positions. The fully ^{15}N- and ^{13}C-labelled $\beta^{2,2}$-HAib derivatives [$^{13}C_5$,^{15}N]-**1a** and [$^{13}C_{30}$,$^{15}N_6$]-**1e** had been prepared by us previously [1].

For application of the rotational-echo double-resonance (REDOR) NMR measurements in the solid state (*vide infra*, *Sect. 5.2*), which provide distances between magnetic nuclei, $\beta^{2,2}$-heptapeptides **4** and **5** were prepared, with positioning of the ^{19}F, ^{15}N, and ^{13}C labels in such a way that various intramolecular distances would result, which could be compared with various models of the $\beta^{2,2}$-peptide backbone (in these models the distances between certain nuclei would be different, *vide infra*, *Sect. 6*). To clearly distinguish between *intra*- and *inter*molecular distances by this method, the

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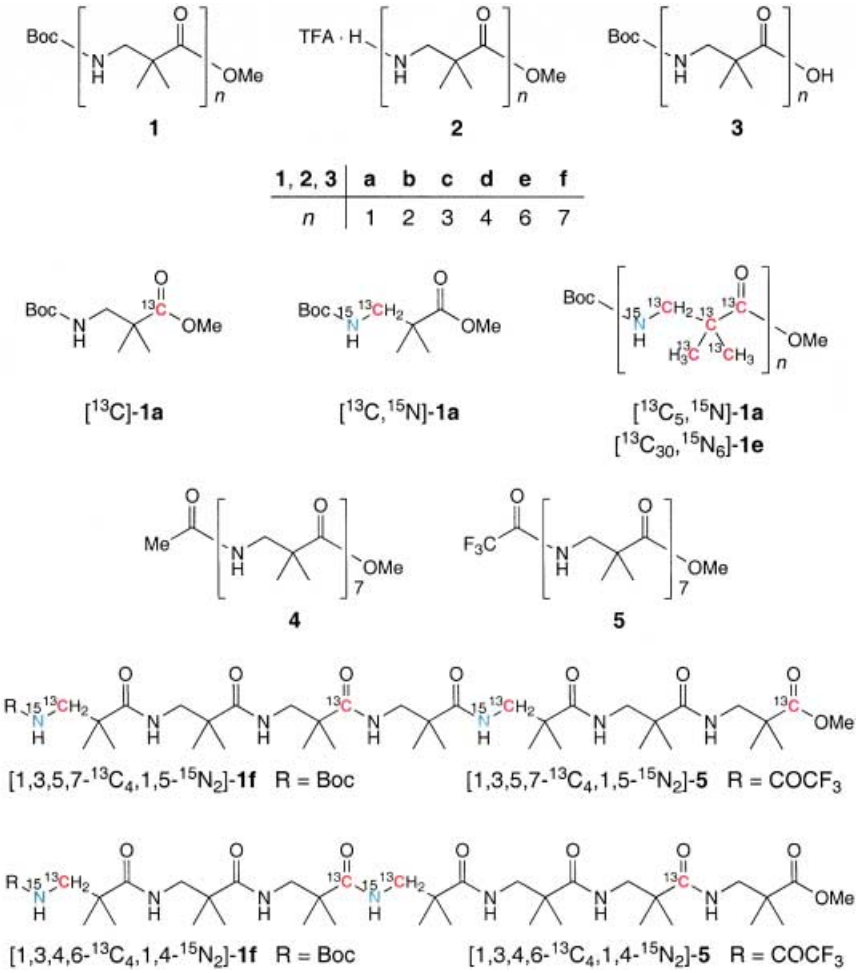
[10]) For recent papers on β-amino acids and β-peptides with geminal disubstitution, see [13]. For cryptophycin analogs containing $\beta^{2,2}$-amino-acid residues and for human chymase inhibitiors containing $\beta^{2,2,3}$-amino acid residues, see [14].

[11]) The $\beta^{2,2}$-HAib derivatives **1a**, **b**, **c**, **e**, and **2a**, **b**, and **3**, which have been characterized or used in our previous work [1], are not included in the *Exper. Part* of the present paper.

[12]) *Isotec Inc.*, 3858 Benner Road, Miamisburg, OH 45342, USA.

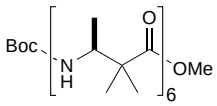


labelled materials [1,3,5,7-$^{13}C_4$, 1,5-$^{15}N_2$]-**5** and [1,3,4,6-$^{13}C_4$, 1,4-$^{15}N_2$]-**5** had to be diluted with unlabelled material to form solid solutions[13]), and, for this purpose, we also synthesized the $\beta^{2,2}$-heptapeptide ester **4** with an *N*-terminal Ac group.

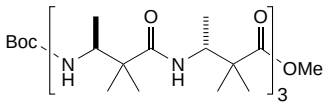
The assembly of the heptamers is outlined in *Scheme 1*. For peptide formation, the Boc-$\beta^{2,2}$-HAib methyl ester or the corresponding oligomers containing two, three, or four $\beta^{2,2}$-HAib units were deprotected at the *N*-terminus (with TFA[14]) in CH_2Cl_2) or at the C-terminus (with NaOH in MeOH/H_2O), and coupled with EDC/HOBt/Et_3N[14])

[13]) For an example of solid solutions formed by the dinitrobenzoates of ethyl (*S*)-3-hydroxybutanoate and ethyl (*R*)-4,4,4-trifluoro-3-hydroxybutanoate, see [15].

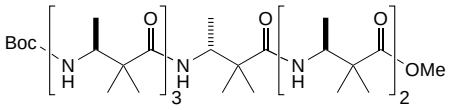
[14]) TFA = CF_3COOH; EDC = 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBt = 1-Hydroxy-1*H*-benzotriazole; TFAA = Trifluoroacetic acid anhydride; DIPEA = *Hünig* base = EtN(*i*-Pr)$_2$; for years, the common coupling reagent HATU was thought to exist as uronium salt (*N*,*N*,*N*,*N*-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate [16]); however, X-ray crystallographic analysis revealed its true structure as guanidinium salt (*N*-form) [17].



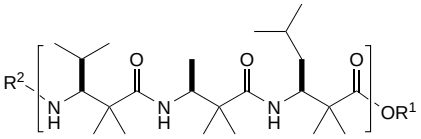
6a



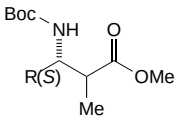
6b



6c

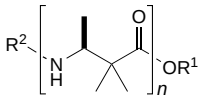


7a R^1 = Me, R^2 = Boc
7b R^1 = H, R^2 = Boc
7c R^1 = H, R^2 = H · TFA



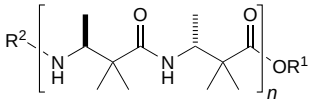
(*S*,*S*)-**8**-**10** (*l* or *syn* configuration)
(2*R*,3*S*)-**8**-**10** (*u* or *anti* configuration)

8 R = Me, **9** R = Me_2CH, **10** R = Me_2CHCH_2



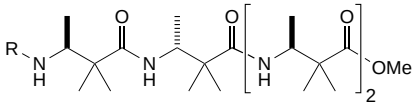
11 R^1 = Me, R^2 = Boc
12 R^1 = Me, R^2 = H · TFA
13 R^1 = H, R^2 = Boc

a *n* = 1
b *n* = 2
c *n* = 4



14 R^1 = Me, R^2 = Boc
15 R^1 = Me, R^2 = H · TFA
16 R^1 = H, R^2 = Boc

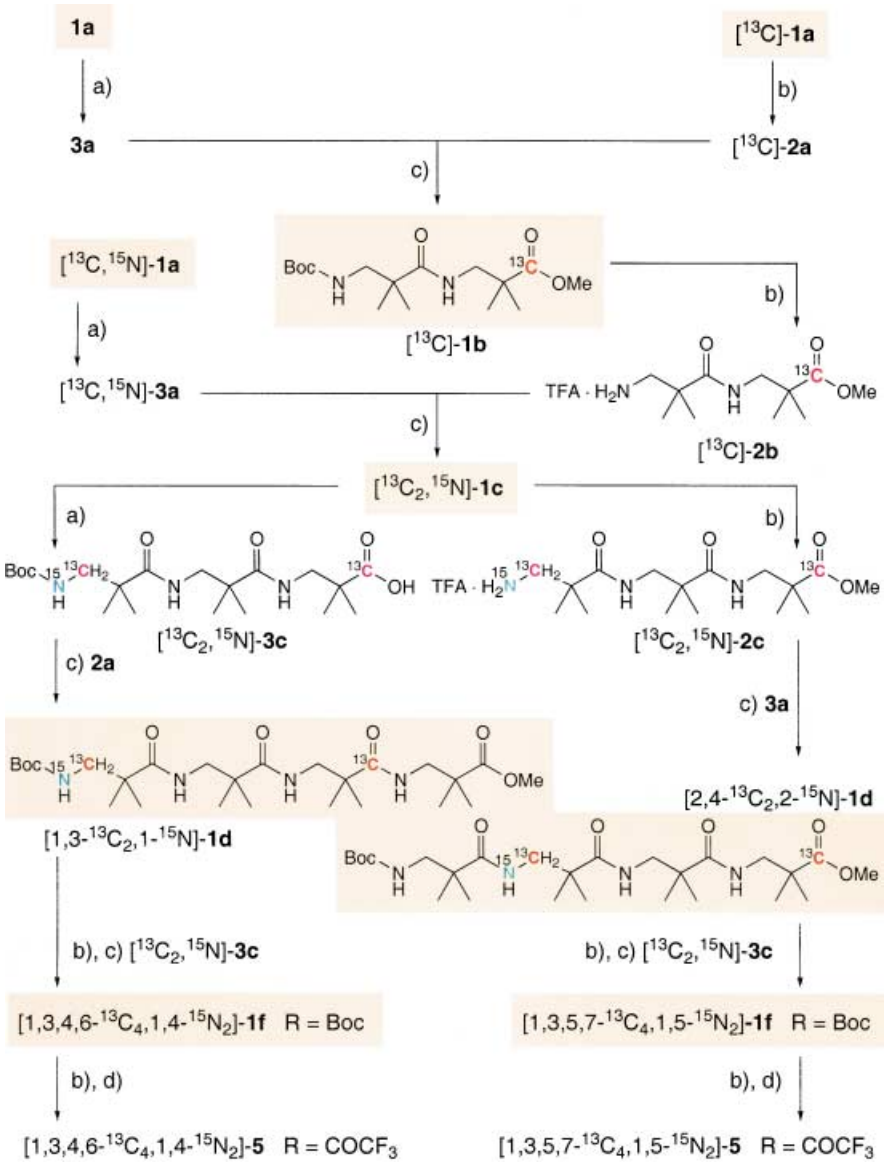
a *n* = 1, **b** *n* = 2



17 R = Boc
18 R = H·TFA

($CHCl_3$, room temperature, overnight); the CF_3CO ($\rightarrow$**5**) and the MeCO group ($\rightarrow$**4**) were introduced with the corresponding anhydrides[14]) and *Hünig*'s base[14]). All fully protected Boc-($\beta^{2,2}$-HAib)$_n$-OMe compounds were chromatographed, the Boc-($\beta^{2,2}$-HAib)$_n$-OH acids and the TFA salts of the *N*-deprotected esters were obtained as crude products in high purity (NMR analysis) and used as such for the coupling steps (for characterizations of the intermediates and of the heptapeptides, see the *Exper. Part*).

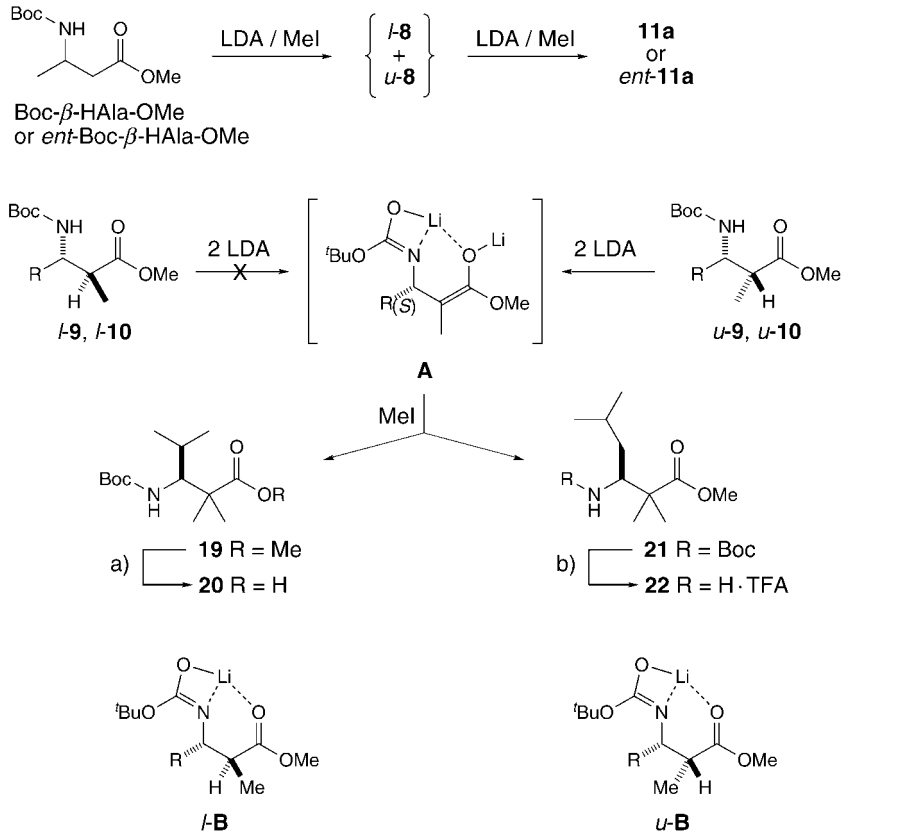
Scheme 1. *Assembly of $\beta^{2,2}$-HAib-Oligomers from Labelled and Unlabelled $\beta^{2,2}$-HAib Building Blocks*



a) NaOH, MeOH/H_2O, 0° → r.t. b) TFA/CH_2Cl_2, 0° → r.t. c) EDC, HOBt, Et_3N, $CHCl_3$, 0° → r.t. d) TFAA, DIPEA, CH_2Cl_2 (for abbreviations, see Footnote 14).

3. Synthesis of the $\beta^{2,2,3}$-Peptides 6 and 7. – For the β-hexapeptides **6** and **7** consisting of trisubstituted $\beta^{2,2,3}$-residues, we had to develop an easy preparative access to the corresponding 3-amino-2,2-dimethyl-alkanoate building blocks **11**–**13** and **19**–**22** (*Scheme 2*). Of the many methods available [12][13], we chose the methylation of

Scheme 2. *Preparation of the* N-*Boc- or* C-*OMe-Protected β-Amino-Acids H-β-HAla(αMe$_2$)-OH, H-β-HVal(αMe$_2$)-OH, and H-β-HLeu(αMe$_2$)-OH for Solution Synthesis of β^{2,2,3}-Peptides*



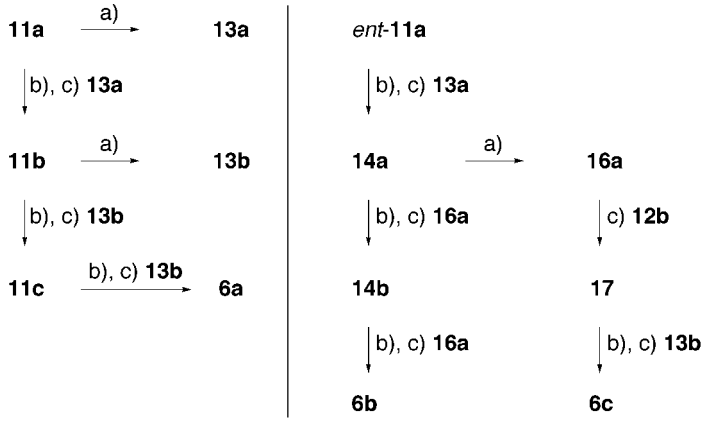
a) 10N NaOH, CF_3CH_2OH, 40°. b) TFA, CH_2Cl_2 (for abbreviations, see Footnote 14).

doubly lithiated β-amino acid derivatives Boc-NH-CHR-CH_2-CO_2Me [18], which we had used before for the preparation of *like*- and *unlike*-2-methyl-3-amino-alkanoates **8**, **9**, and **10** [7][18][19]. The protected β-amino acids, in turn, are readily prepared by *Arndt-Eistert* homologation[15]) of the α-amino acid derivatives Boc-Ala-OH, Boc-Val-OH, and Boc-Leu-OH, with rearranging decomposition of the intermediate diazo ketones in MeOH. The methylation of the doubly lithiated methyl *N*-Boc-β-amino-alkanoates with MeI was known to give mixtures of diastereoisomers of *l* and *u* configuration[16]). At first, we were confident that we could use these mixtures without separation of the stereoisomers for a subsequent second methylation to give the 2,2-dimethyl derivatives **11a**, **19**, and **21**, because, from both isomers, the same enolate **A**

15) For a review article with numerous original references, see [20].

16) Addition of Li salts or of the co-solvent DMPU ('*N*,*N*′-dimethylpropylene urea') was found to give rise to high diastereoselectivities, which depend also upon the nature of the protecting group on the N-atom [18][21].

Scheme 3. *Coupling Steps Leading to the $\beta^{2,2,3}$-Hexapeptides* **6** *with Three Me Groups on Each Residue*



a) 10N NaOH, CF_3CH_2OH, 40°. b) TFA, CH_2Cl_2. c) EDC, HOBt, Et_3N, $CHCl_3$ (for abbreviations, see *Footnote* 14).

(*Scheme 2*)[17]) should be generated. This was indeed the case with the butanoic acid derivative Boc-NH-CHMe-CH_2-CO_2Me ($\rightarrow$ (*l*-**8** + *u*-**8**) $\rightarrow$ **A** (R = Me) $\rightarrow$ **11a**), so that the oligomers **6**, **11b** – **13b**, **c**, **14a** – **16a**, **16b**, **17**, and **18** could be prepared, with the same methods as for the simple $\beta^{2,2}$-HAib derivatives described in the previous section (see formulae **1** – **5** and *Scheme 1*), except that the saponification of the *C*-terminal methyl ester groups required much more drastic conditions (up to 100 equiv. NaOH in CF_3CH_2OH at 40 – 50°; see *Exper. Part* for details); a schematic presentation of the assembly of the Boc-$\beta^{2,2,3}$-hexapeptide esters **6** is given in *Scheme 3*. When we tried to apply the same procedure of introducing the second Me group to the *l*/*u* mixtures of the monomethylated β-HVal and β-HLeu derivatives **9** and **10**, only one of the diastereoisomers was actually methylated to give the desired products **19** and **21**, while the other diasteroisomer was recovered in very pure form; configurational assignment of the diastereoisomers by comparison with literature data [7] revealed that the *u* form had reacted, and the *l* form had remained unchanged[18]). To avoid tricky chromatographic separation of the mono- and dimethylated compounds *l*-**9**/**19** and *l*-**10**/**21**, we separated the monomethylated *l*/*u* isomers of **9** and **10**, and used the pure *u*-forms for the second methylation, which occurred in 75 – 80% yield (*Scheme 2*). The reason for the large rate difference for proton abstraction from the two diastereoisomers is obviously steric hindrance by the neighboring i-Pr and i-Bu groups *R* in the intermediate mono-Li derivative *l*-**B**, but not in *u*-**B** (*Scheme 2*).

With the Boc-$\beta^{2,2,3}$-amino acid esters **19** and **21** available, we could proceed with the solution synthesis – by stepwise and by fragment coupling – of the hexapeptide

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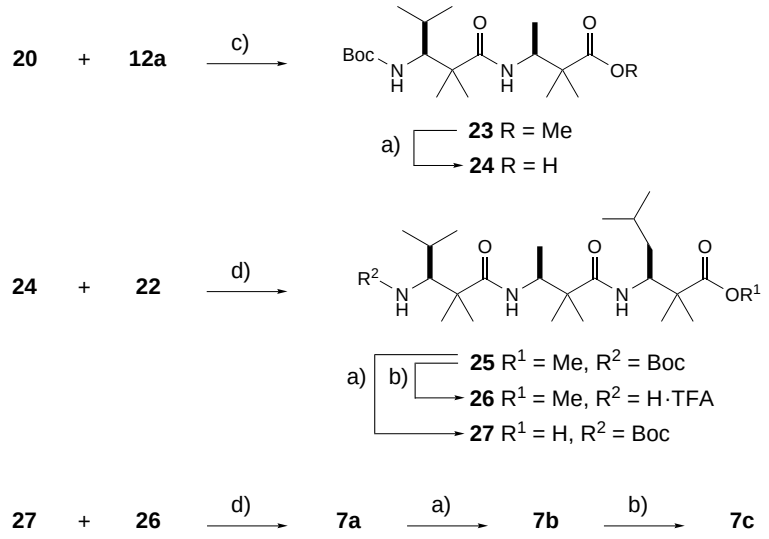
17) The structure of **A** in *Scheme 2* is presented as a Li enolate / Li azacarboxylate with complexation of Li on O and N, because this is the type of structure observed in crystal structures of lithiated carboxylic acid amides [22].

18) NMR Analysis of the crude product mixtures showed that, within detection limits, there was no trace of the *u* forms left.

Scheme 4. *Synthesis of the $\beta^{2,2,3}$-Hexapeptide* **7** *with Ala, Val, and Leu Side Chains in the 3-Position*



a), b) and c) as in *Scheme 3*. d) HATU/EtN(i-Pr)$_2$, $CHCl_3$ (for abbreviations, see *Footnote* 14).

derivatives **7** (by using the intermediate Boc-protected acids **20**, **24**, and **27**, and the methyl ester TFA salts **22** and **26**) through the fully protected di- and tripeptides **23** and **25**, respectively (see *Scheme 4*). Again, drastic conditions were required for saponification of methyl ester groups, and, for the last two coupling steps, activation by HATU/*Hünig* base[14] rather than by EDC/HOBt/Et_3N was necessary for achieving high yields of 79 and 75%, respectively. For characterization of the final products and of the intermediates involved, we refer to the *Exper. Part*.

The β-peptides **4**–**6** with their regular structures containing only one amino acid residue of a kind (without 'floppy side chains') had been chosen for the present study in the hope that we would obtain crystalline samples for single-crystal X-ray structure analysis. Unfortunately, none of them met our expectations, so far, inspite of numerous crystallization attempts by various co-workers over a period of several years. The β-hexapeptide **7** with three different residues was prepared for an NMR analysis in solution (see *Sect. 4*).

In contrast to previously prepared β^2- and β^3-peptides with aliphatic side chains on the amino acid residues, which become insoluble[19]) in H_2O and in most organic solvents with increasing chain lengths [4][5][7], the compounds described here are well soluble in organic media (not in H_2O!).

4. NMR Measurements with the $\beta^{2,2}$- and $\beta^{2,2,3}$-Hexapeptide 4, 6a, and 7c in Solution. – The 400-MHz ^{1}H-NMR spectrum of the *N*-acetyl-$\beta^{2,2}$-heptapeptide methyl ester **4** in $CDCl_3$ is shown in *Fig. 2*. The most remarkable feature of the spectrum is the

[19]) This is not true for the 'mixed' β^2/β^3-peptides, which are nonpolar compounds well-soluble in organic media [7][23].

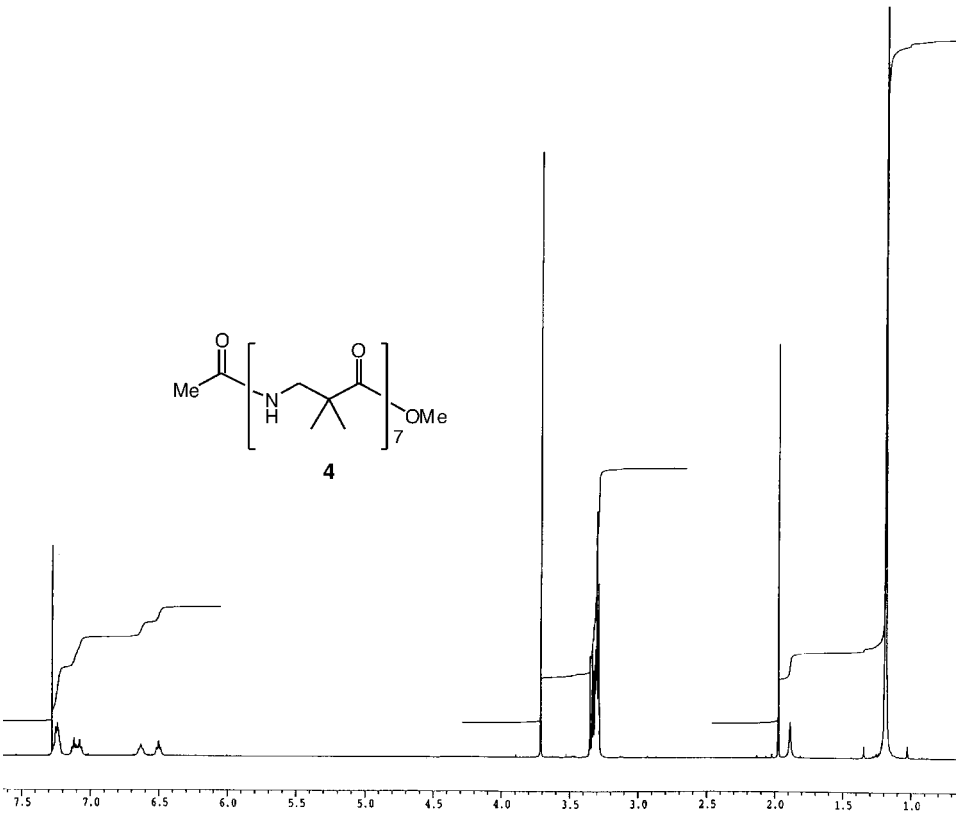


Fig. 2. *400-MHz-[1]H-NMR Spectrum of the* N-*Acetyl-β[2,2]-HAib-heptapeptide methyl ester* **4** *in* $CDCl_3$. Note the sharp signal for all Me_2C H-atoms and the rather small coupling constant $J(H(N)H(CH_2))$ (for details, see *Exper. Part*).

single signal, actually a *singlet*, from all 14 geminal Me groups at 1.19 ppm; the CH_2 groups give rise to a group of signals between 3.29 and 3.35 ppm; the NH H-atoms appear as five signals between 6.5 and 7.3 ppm. There is, of course, no chance of deriving a structure from this spectrum!

The 500-MHz [1]H-NMR spectrum of the *N*-Boc-$\beta^{2,2,3}$-hexapeptide methyl ester **6a** is shown in *Fig. 3*. In this case, the 18 backbone Me groups produce 14 clearly separated signals (theoretically 12 *singlets* and 6 *doublets*) between 1.08 and 1.30 ppm (see the inset in *Fig. 3*); the CH H-atoms give rise to three *multiplets* at 3.53, 3.81, and 3.98 ppm, with the one at lowest field as a *doublet* (*ca.* 9 Hz) of *quadruplet* (*ca.* 7 Hz), the NH *doublets* appear at 6.20, 7.10, 8.18, 8.30, 8.39, and 8.40 ppm ($J = 8.2$ to 9.4 Hz). A detailed NMR analysis – leading to a solution structure – does not appear to be impossible, especially with a higher-field spectrometer.

In the $\beta^{2,2,3}$-hexapeptide derivatives **7**, containing the side chains of Val, Ala, and Leu, the chance of arriving at full assignment of all signals and, thus, of determining the structure was much larger. Hence, it was of interest to examine the secondary structure

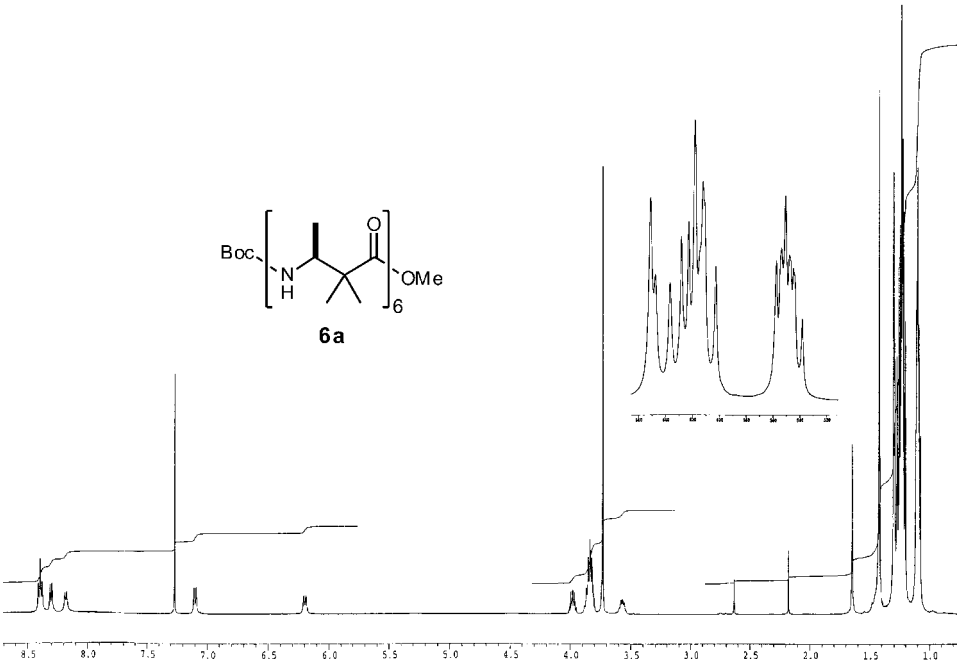


Fig. 3. *500-MHz-* ^{1}*H-NMR Spectrum of the* N-*Boc-(*$\beta^{2,2}$*-HAib(*β*-Me))*$_6$*-OMe* **6a** *consisting of (*S*)-residues.* The inset is an enlargement of the region containing the signals from the 18 MeC H-atoms (except for *t*-Bu). The NH signals are *doublets* of *ca.* 8.8 Hz. For details, see the accompanying text and the *Exper. Part.*

of the $\beta^{2,2,3}$-hexapeptide **7c** by means of high-resolution NMR spectroscopy in MeOH. The ^{1}H-NMR spectrum of the fully deprotected hexapeptide **7c**, recorded at 500 MHz, showed, indeed, good separation of the signals and allowed complete assignment of all resonances (including each of the 22 Me signals). By DQF-COSY, TOCSY, HSQC, and HMBC techniques, the amino acid spin systems were identified (*Fig. 4*).

The sequence assignment was then established by HMBC measurements and $d_{N,\alpha(Me)}(i, i-1)$ sequential NOEs (*Table 1*). The NH H-atoms of residues 2–6 show coupling constants J(NH,H–C(β)) in the range of 8–10 Hz, which correspond to an antiperiplanar arrangement of NH and H–C(β). To obtain more information about the three-dimensional structure in solution, ROESY spectra at different mixing times were acquired, and NOEs were extracted from the ROESY spectra with a mixing time of 100 ms (*Table 2*). Integration of the cross-peak volumes followed by calibration allowed the classification of the NOEs in three distance categories: strong, medium, and weak.

Qualitative inspection of the NOEs reveals that only *intra*residual and sequential NOEs are present, indicating an extended or unstructured conformation of **7c** in solution. The NOE data also give no evidence for a 3_{14}-helical structure as suggested by the CD measurements; in fact, there are no NOEs related to those observed for any of the helical or turn structures found in β-peptides (without cyclic residues) so far.

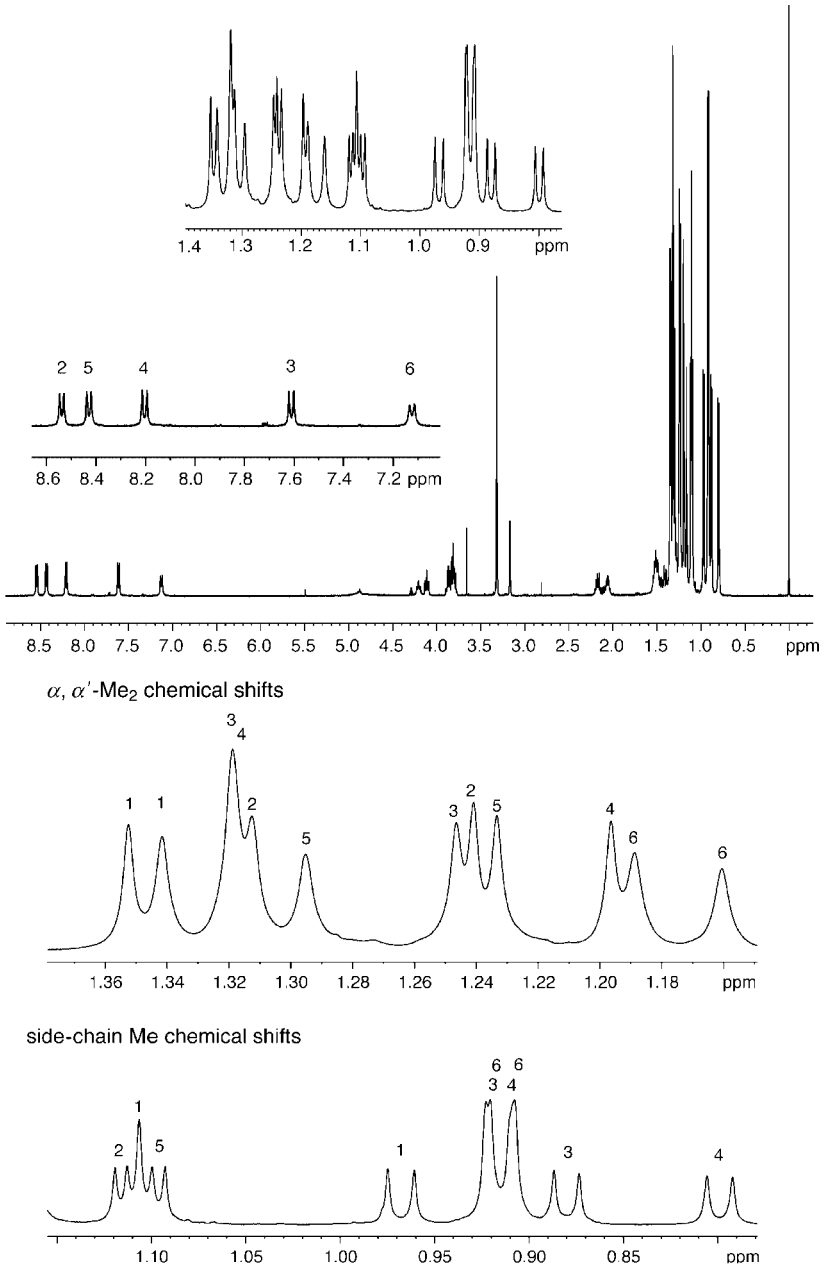


Fig. 4. *^{1}H-NMR Spectra of $\beta^{2,2,3}$-hexapeptide* **7c** *in MeOH solution*. Assignments of the H-atoms in the spectra are indicated by numbers 1–6, which correspond to residues 1–6 (residues 2 and 5 = β-HAla, residues 4 and 5 = β-HVal, residues 3 and 6 = β-HLeu).

Table 1. *^{1}H- and ^{13}C-NMR Chemical Shifts of the $\beta^{2,2,3}$-Hexapeptide* **7c**

β-Amino acid	NH (J(NH, Hβ) [Hz])	C=O	Me–C(α)	H–C(β)	H–C(γ), 2 H–C (γ), Me–C(γ)	H–C (δ), Me–C (δ)	Me–C (ε)
Val[1]	–	177.59	1.352/1.341 24.82/22.77	3.16 65.06	2.17 28.72	1.110/0.968 22.11/16.26	
Ala[2]	8.53 (8.86)	178.81	1.312/1.233 23.48/25.78	3.86 54.31	1.116 17.06		
Leu[3]	7.60 (9.72)	178.89	1.315/1.246 24.35/24.19	4.09 55.46	1.41/1.31 41.77	1.50 26.44	0.917/0.880 23.00/24.14
Val[4]	8.19 (9.35)	178.86	1.318/1.196 23.60/26.77	3.82 62.44	2.05 30.62	0.914/0.799 21.67/17.61	
Ala[5]	8.42 (8.61)	178.92	1.231/1.295 25.79/23.43	3.80 54.38	1.096 22.21		
Leu[6]	7.11 (9.84)	180.24	1.160/1.188 22.81/24.10	4.20 54.49	1.47/1.25 40.87	1.51 26.46	0.915/0.918 21.67/24.09

Table 2. *NOEs in the ROESY Spectra* (300 ms) *of* **7c** *in MeOH* (w = weak, m = medium, s = strong)

Atom	Residue	Atom	Residue	NOE	Atom	Residue	Atom	Residue	NOE
β	1	γ	1	m	β	3	ε	3	m
β	1	α(Me)	1	m	NH	4	β	3	m
β	1	δ	1	m	NH	4	β	4	m
β	1	δ	1	w	NH	4	γ	3	w
γ	1	α(Me)	1	m	NH	4	γ	4	m
γ	1	δ	2	m	NH	4	α(Me)	3	s
γ	1	δ	1	m	NH	4	α(Me)	3	s
NH	2	β	3	w	NH	4	δ	4	w
NH	2	β	2	m	NH	4	δ	4	m
NH	2	β	1	w	β	4	γ	4	m
NH	2	γ	1	w	β	4	α(Me)	4	s
NH	2	γ	3	w	β	4	α(Me)	4	s
NH	2	α(Me)	2	s	β	4	δ	4	m
NH	2	α(Me)	1	m	γ	4	α(Me)	3	m
NH	2	γ	1	m	γ	4	δ	4	m
NH	2	δ	1	w	γ	4	δ	4	w
β	2	α(Me)	2	m	NH	5	β	5	m
β	2	α(Me)	2	s	NH	5	γ	4	w
β	2	γ	2	m	NH	5	α(Me)	4	s
NH	3	β	3	m	NH	5	α(Me)	4	s
NH	3	β	2	m	NH	5	γ	5	m
NH	3	δ	3	m	NH	5	δ	4	w
NH	3	γ	3	s	NH	5	δ	4	w
NH	3	α(Me)	3	s	β	5	γ	5	m
NH	3	α(Me)	3	m	NH	6	β	5	m
NH	3	δ	2	w	NH	6	γ	6	m
NH	3	ε	3	w	NH	6	α(Me)	5	s
NH	3	ε	3	w	NH	6	α(Me)	5	m
β	3	δ	3	m	NH	6	α(Me)	6	w
β	3	γ	3	s	NH	6	α(Me)	6	w
β	3	γ	3	m	NH	6	γ	5	w
β	3	α(Me)	3	s	NH	6	ε	6	w
β	3	γ	2	w	β	6	γ	5	m
β	3	ε	3	s	β	6	ε	6	w

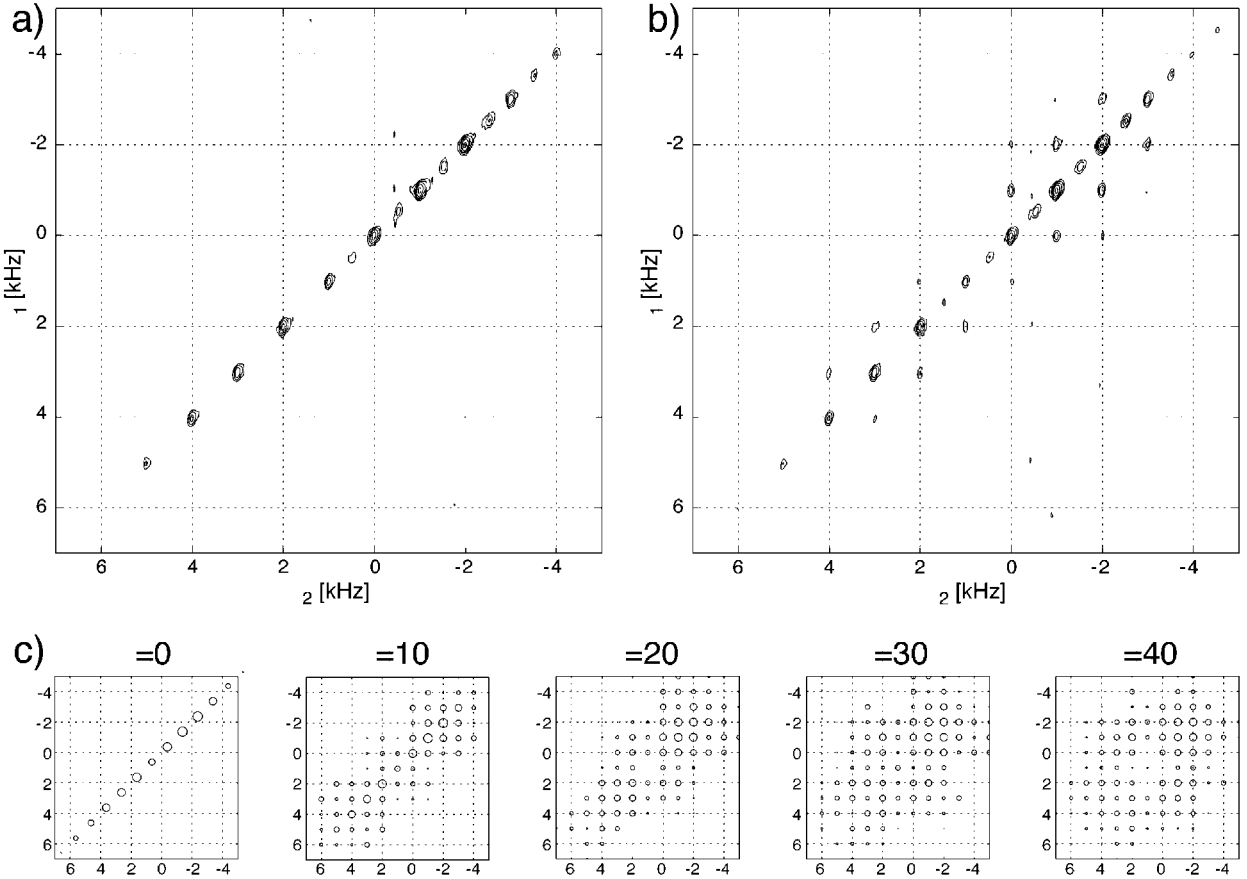


Fig. 5. *Two-dimensional ^{15}N-exchange spectra of the fully ^{13}C- and ^{15}N-labelled hexapeptide* **1e**. The spectra were recorded with an initial cross-polarization period from the protons to enhance signal intensity, and with proton-driven spin diffusion as a mixing sequence. The spinning frequency was set to 1000 Hz and stabilized to within 5 Hz. The spectrum with a mixing time of $\tau_m = 0$ s (*a*) shows, as expected, no cross-peaks, while the spectrum with a mixing time of $\tau_m = 1$ s (*b*) shows weak cross-peaks on the first and second side diagonal. Part *c* shows five simulated quasi-equilibrium two-dimensional exchange spectra with different angles β between the two axially symmetric N chemical-shielding tensors. For an angle $\beta = 0°$, as expected, no off-diagonal intensity is observed, while, for all other angles, significant off-diagonal cross-peaks are found.

5. Solid-State NMR Investigations. – 5.1. *Magic-Angle Spinning (MAS) Double Resonance of the Fully ^{15}N- and ^{13}C-Labelled Hexapeptide* **1e**. Two-dimensional exchange spectra [24] under slow magic-angle spinning (MAS) conditions allow determination of the relative orientation of chemical-shielding tensors in a molecule [25]. Such an experiment follows the general scheme of two-dimensional NMR spectroscopy. During the time t_1, the polarization is frequency-labelled with the resonance frequency of the first spin, then transferred to a second spin, and during t_2 detected with the resonance frequency of the second spin [26]. Under MAS, we obtain a side-band spectrum of [$^{13}C_{30}$,$^{15}N_6$]-**1e**, which is characteristic for the relative orientation of the two chemical-shielding tensors. *Fig. 5* shows two-dimensional ^{15}N-exchange spectra of the fully labelled hexapeptide **1e** under slow magic-angle spinning (MAS) ($\nu_r = 1000$ Hz) for mixing times $\tau_m = 0$ (*Fig. 5,a*) and $\tau_m = 1$ s (*Fig. 5,b*).

As expected, the spectrum with a vanishing mixing time (*Figr. 5, a*) shows no cross-peaks and two sets of diagonal-peak families spaced by the spinning frequency. The weaker side-band family with the centerband at -1.54 kHz arises from the *N*-terminal Boc-protected amino group. All the other five amide N-atoms contribute to the stronger side-band family with the center band at 0 Hz. The full width at half height of the lines is *ca.* 140 Hz, indicating a strong structural similarity between the five amide N-atoms. The spectrum with a mixing time (*Fig. 5,b*) shows only weak cross-peaks along the first and second side diagonals. Numerical simulations of the tensor-correlation spectra show that such a spectrum is a clear indication that the symmetry axes of the two axially symmetric tensors are parallel or antiparallel to within 10°, as can be seen from *Fig. 5,c*. Assuming that the symmetry axis of the chemical-shielding tensors is aligned along the N–H bond direction, this implies that the N–H bonds are parallel or antiparallel to within 10°. Very similar experimental results were obtained for ^{13}C 2D exchange spectra of [$^{13}C_{30}$,$^{15}N_6$]-**1e** by measuring the relative orientation of the C=O chemical-shielding tensors (data not shown). At a mixing time of $\tau_m = 100$ ms, intense cross-peaks between the C=O C-atom and all other C-atoms could be seen due to the fast polarization transfer across the one-bond dipolar couplings. However, there were only weak cross-peaks visible along the first side diagonal within the side-band family of the C=O tensor even at mixing times as long as $\tau_m = 1$ s. Numerical simulations of the tensor-correlation spectra indicate that the two tensors are parallel or antiparallel to within less than 10°. Since the C=O tensor is not axially symmetric, this implies that the two peptide planes are either parallel or antiparallel to within 10°, and that the N–H bond as well as the C=O bond are also parallel or antiparallel to within 10°.

5.2. *REDOR and TEDOR-REDOR Spectra.* As mentioned in *Sect. 2*, this solid-state NMR technique requires 'dilution' of the specifically labelled compound with the unlabelled one to form a so-called solid solution. While this is no problem with the isotopic labels ^{13}C and ^{15}N, CF_3 in the heptapeptide derivatives **5** had to be 'diluted' with CH_3[13]). To our surprise, solutions containing 16–50% of compound **4** (with *N*-Ac) and 84–50% of compound **5** (with *N*-CF_3CO) gave, upon solvent evaporation, solid samples, in which the two compounds appeared to have been separated from each other: upon heating in a melting-point tube, there was melting between 156 and 160°, and resolidification and melting again above 166° (see *Fig. 6*); the samples obtained

from the melt upon solidification did not show the two-stage melting behavior any more. Thus, it looks like the difference between CH_3CO and CF_3CO in a peptide of molecular weight 734 is sufficient for a phase separation! This demixing was confirmed by initial REDOR measurements, and the solid solutions used in the subsequent experiments had to be prepared carefully (see *Exper. Part*).

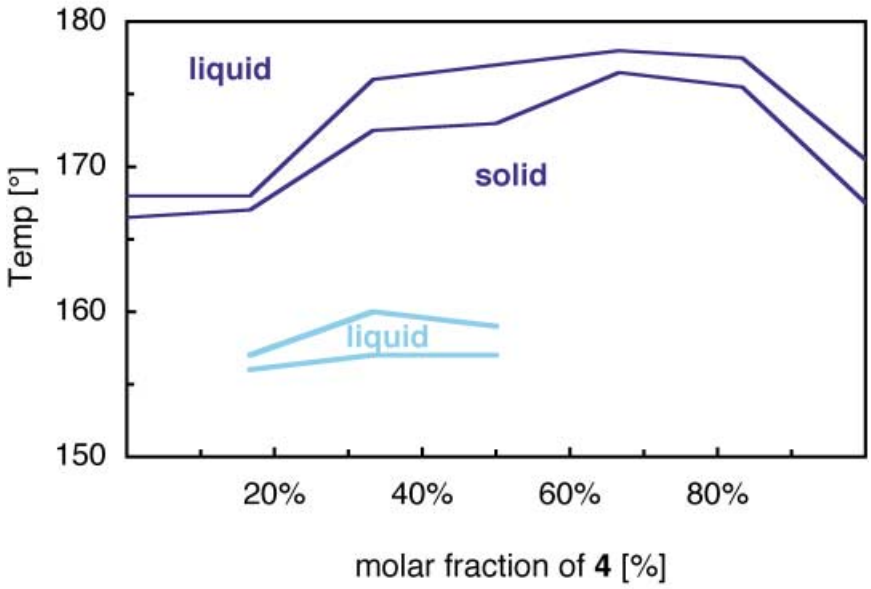


Fig. 6. *Melting behavior of mixtures of the* N-*acetyl- and* N-*trifluoroacetyl-*$\beta^{2,2}$*-heptapeptide methyl esters* **4** *and* **5**. As an example, the 33 : 67 mixture melts between 157 and 160°, solidifies, and melts again between 173 and 176°. The mixtures were prepared by precipitation with hexane from solutions in CH_2Cl_2.

The cross-polarization magic-angle-spinning (CPMAS) 50.3-MHz ^{13}C-NMR spectrum of [1,3,5,7-$^{13}C_4$, 1,5-$^{15}N_2$]-**5**, (R = $COCF_3$), diluted tenfold by **4**, shows peaks near 180, 45, and 25 ppm (*Fig. 7,a*), which are due to ^{13}C-labelled and natural-abundance C=O, CH_2, and Me C-atoms, respectively. Shift resolution of site differences within the β-heptapeptide is poor. The $^{15}N \rightarrow {}^{13}C$ TEDOR spectrum of this blend is simpler but not any better resolved (*Fig. 7,b*). Nevertheless, an expansion of the C=O C-atom region of the rotational-echo double-resonance (REDOR) spectrum of the same labelled version of **5**, but now diluted 50-fold by **4**, shows the presence of two overlapping peaks (*Fig. 8,c*). The low-field peak of this pair is not observed in spectra of [1,3,4,6-$^{13}C_4$, 1,4-$^{15}N_2$]-**5** (R = $COCF_3$ (not shown)), a result that allows an assignment of the low-field peak (178 ppm) to the terminal labelled C=O C-atom in residue 7 (see formula in *Fig. 7*).

The combination of transferred-echo double-resonance (TEDOR) with REDOR within the same pulse sequence is called TEDOR-REDOR. The $^{15}N \rightarrow {}^{13}C\{^{19}F\}$ TEDOR-REDOR full-echo spectrum of the CH_2 C-atom region of [1,3,5,7-$^{13}C_4$, 1,5-$^{15}N_2$]-**5** (R = $COCF_3$), diluted 50-fold by **4**, has only a single, relatively broad peak centered near 45 ppm, with just a hint of a shoulder toward low field (*Fig. 8,d*). Only

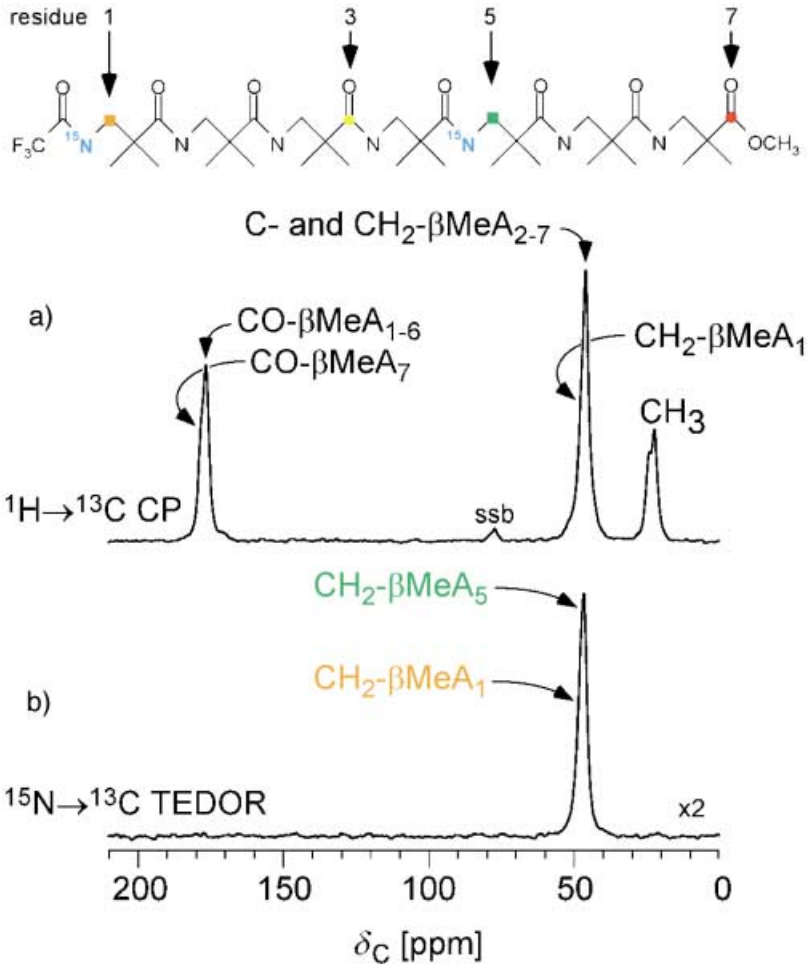


Fig. 7. *CPMAS ^{13}C-NMR Spectrum* (*a*) *and ^{15}N→^{13}C TEDOR spectrum* (*b*) *of a homogeneous blend of 1 part [^{13}C,^{15}N,^{19}F]-labelled β-heptapeptide [1,3,5,7-$^{13}C_4$, 1,5-$^{15}N_2$]-***5*** (R = COCF$_3$) and 10 parts unlabelled β-heptapeptide* **4**. The blend was precipitated from CH_2Cl_2/pentane. The TEDOR selection was adjusted so that only ^{13}C directly bonded to ^{15}N was observed.

the labelled CH_2 C-atoms in residues 1 and 5 are directly bonded to ^{15}N and so contribute to this spectrum. The corresponding REDOR difference is also a single peak, but the center of this peak is shifted downfield from the center of the full-echo peak by several ppm (*Fig. 8*, *b* and *d*). Curve fitting with overlapping Gaussian peaks results in a deconvolution that is consistent with a much stronger ^{13}C, ^{19}F dipolar coupling (larger REDOR difference) for the weaker, low-field full-echo peak. The same strong ^{13}C{^{19}F} dephasing was observed following a ^{1}H-^{19}F-^{13}C double-cross-polarization preparation of carbon magnetization (not shown). Thus, we assign this low-field peak (48 ppm) to the labelled CH_2 C-atom in residue 1 (orange in the formula in *Fig. 7*), which is close to the CF_3 group, and the stronger, high-field full-echo peak

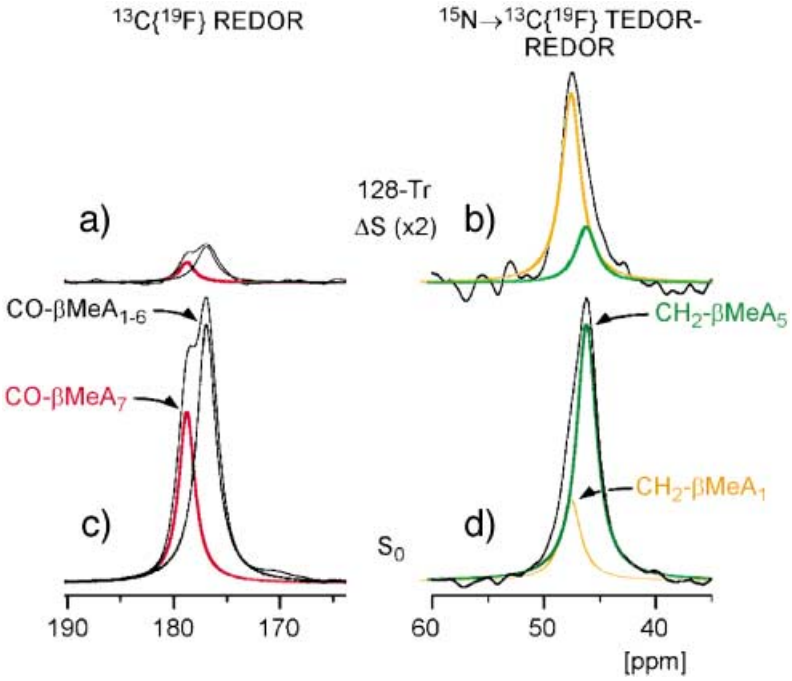


Fig. 8. *Deconvolution of $^{13}C\{^{19}F\}$ REDOR spectra* (*a and c*) *and $^{15}N\rightarrow{}^{13}C\{^{19}F\}$ TEDOR-REDOR spectra* (*b and d*) *of a homogeneous blend of 1 part [^{13}C,^{15}N,^{19}F]*-labelled β-heptapeptide [1,3,5,7-$^{13}C_4$, 1,5-$^{15}N_2$]-**5** (R = $COCF_3$; see *formula* in *Fig. 7*) *and 50 parts unlabelled β-heptapeptide* **4**. The REDOR differences are shown in *a* and *b* and the full echoes in *c* and *d*. An intensive low-field peak (red) near 180 ppm in the REDOR spectra was only observed in samples containing a ^{13}C-labelled C=O C-atom in residue 7.

(46 ppm) to the more distant labelled CH_2 C-atom in residue 5 (green). We attribute the differences in full-echo intensities after 128 rotor cycles to differences in T_2 values for the end and middle of the β-heptapeptide. The $^{13}C\{^{19}F\}$ $\Delta S/S_0$ for the CH_2 C-label in residue 1 suggests a *ca.* 5-Å distance to the terminal CF_3 (*Fig. 9*), which is plausible for a three-bond distance [27].

The $^{13}C\{^{19}F\}$ $\Delta S/S_0$ for the C=O C-label in residue 7 (*Fig. 10*) and the CH_2 C-label in residue 5 (*Fig. 11*) is between 6 and 10% after 25 ms of dipolar evolution. For dephasing as small as this, the possibility exists of contributions to the REDOR difference from natural-abundance ^{13}C proximate to ^{19}F and with the same shift as one of the labels. The small but discernable shift resolution of the terminal C=O C-atom in residue 7 (see *Fig. 8,c*) allowed estimation of this natural-abundance contribution to the dephasing. We determined that the $^{13}C\{^{19}F\}$ $\Delta S/S_0$ (for the low-field peak at 178 ppm) for a homogeneous blend of 1 part of **5** (no C-labels) and 10 parts of **4** was $12 \pm 2\%$ after 128 rotor cycles, and for a blend of 1 part of **5** and 50 parts of **4,** only 2%. Thus, some 70–80% of the dephasing of *Figs. 10* and *11* (solid lines) is due to β-heptapeptide *intramolecular* ^{13}C, ^{19}F coupling. This means that a fraction of the β-heptapeptides exist in a bent or kinked conformation that brings the two ends of the peptide close to one another. The dephasing simulations [28] shown in the insets to

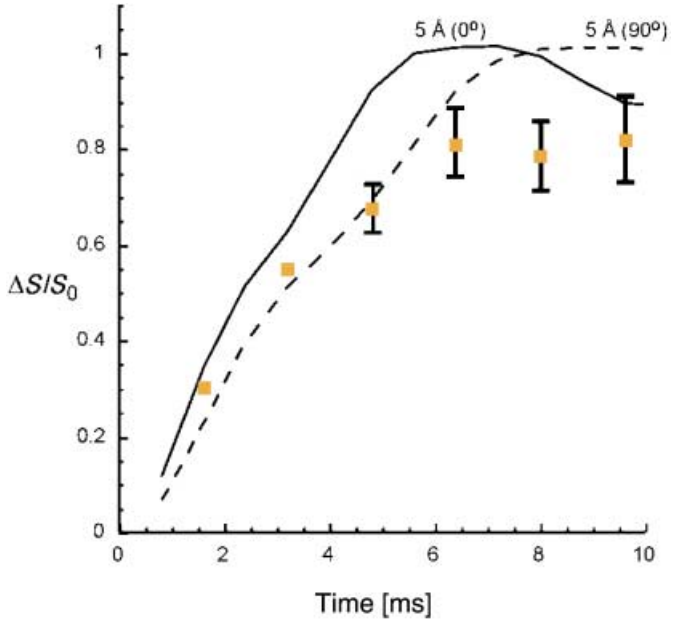


Fig. 9. *$^{13}C\{^{19}F\}$-Dephasing* ($\Delta S/S_0$) *for the labelled CH_2 C-atom in residue 1* (orange) *of the β-heptapeptide sample of* Fig. 7. The solid and dotted lines show the calculated dephasing, assuming a 5-Å distance from the ^{13}C label to the center of the F_3 triangle and a $^{13}C \rightarrow C(F_3)$ vector parallel (0°) or perpendicular (90°) to the plane of the three F-atoms.

Figs. 10 and *11* suggest that *ca.* 5–10% of the β-heptapeptides are kinked and that 90–95% are fully extended in the solid state.

6. Discussion and Conclusions. – 6.1 *Solution Structures.* As pointed out in *Sect. 4*, the NMR spectra in MeOH solution could either not be analyzed in detail (**4** and **6a**) or led to the conclusion that there is no folded secondary structure, which would produce long- or medium-range *inter*-residual NOEs, on the NMR time scale (**7c**). This conclusion about the structure of the β-hexapeptide **7c** (carrying three side chains on each residue) is in agreement with the results of long (100 ns) MD simulations, performed in MeOH at 298 and 340 K, by which a myriad of different conformers was found [10][20]). The preferred conformations around the $C(Me_2)-CO$ ($\tau_{1,2}$), $C(HR)-C(Me_2)$ ($\tau_{2,3}$), $C(HR)-N(HCO)$ ($\tau_{3,N}$), and $C(O)-N(H)$ ($\tau_{1,N}$) bonds in the backbone of β-hexapeptide **7c** are shown in *Figs. 12–15*; besides the peptide bond ($\tau_{1,N}$) the $N(H)-C(HR)$ bond has a single conformation at 298 *and* 340 K in five amino acid residues of **7c** with N(H) and HC(R) in an antiperiplanar disposition ($\tau_{3,N}$,

[20]) This is in sharp contrast to the situation with geminally disubstituted α-peptides, such as oligomers of Aib or Iva, which form helices with as few as three residues! For a structural analysis (NMR and X-ray) of oligomers consisting of Iva residues, Boc(NH-CMeEt-CO)$_n$-OMe, see [29] and refs. cit. therein, as well as papers by *Toniolo*, *Balaram*, and *Karle* with their respective co-workers [30].

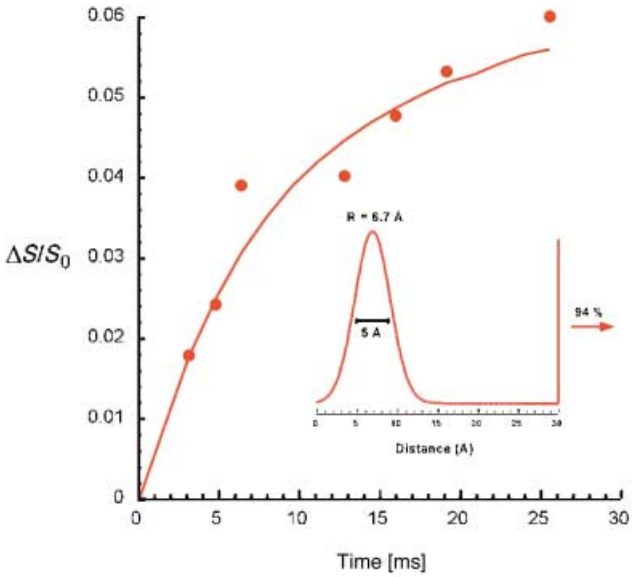


Fig. 10. *$^{13}C[^{19}F]$-Dephasing ($\Delta S/S_0$) for the labelled C=O C-atom in residue 7* (red) *of the β-heptapeptide* (shown in *Fig. 7*). The solid line in the inset shows the calculated dephasing, assuming a Gaussian distribution of distances (centered at 6.7 Å with a 5-Å width) from the ^{13}C label to the CF_3-dephasing center for 6% of the labelled β-heptapeptides, and a separation of more than 30 Å for 94% of the labelled β-heptapeptides.

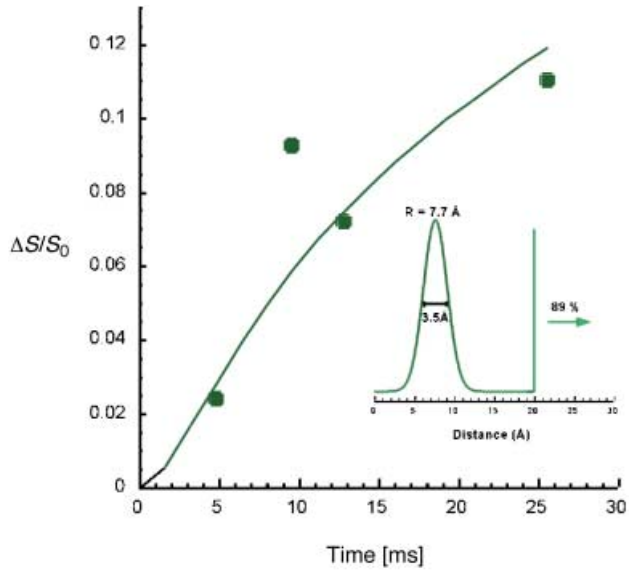


Fig. 11. *$^{13}C[^{19}F]$-Dephasing ($\Delta S/S_0$) for the labelled CH_2 C-atom in residue 5* (green) *of the β-heptapeptide* (formula in *Fig. 7*). The solid line in the inset shows the calculated dephasing assuming a Gaussian distribution of distances (centered at 7.7 Å with a 3.6-Å width) from the ^{13}C label to the CF_3 dephasing center for 11% of the labelled β-heptapeptides, and a separation of more than 20 Å for 89% of the labelled β-heptapeptides.

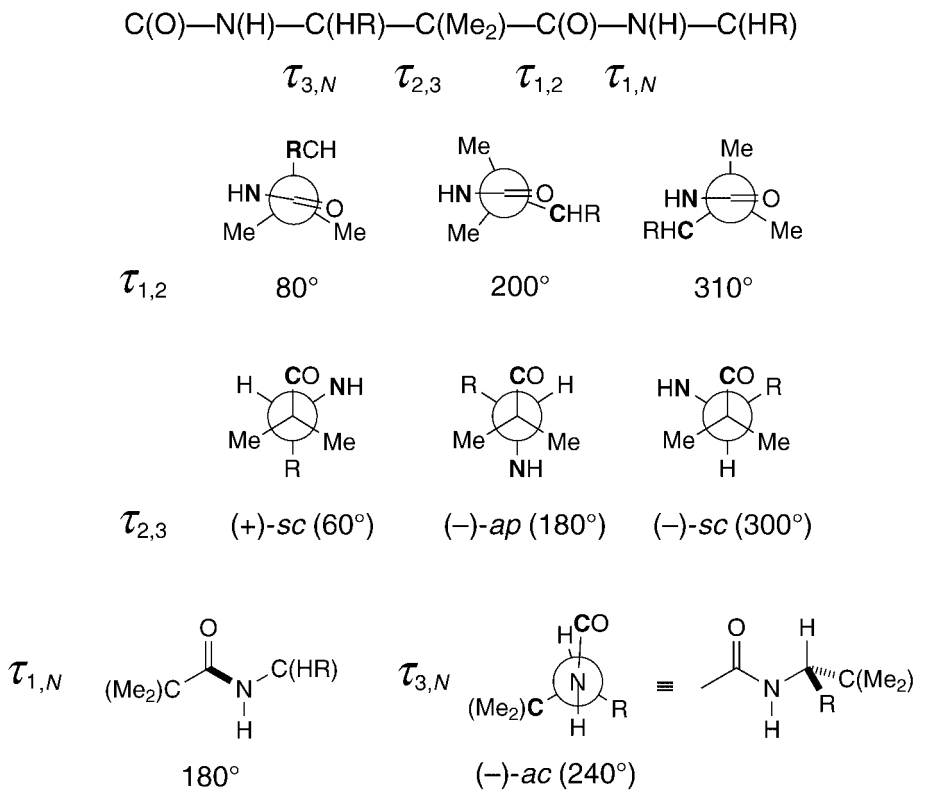


Fig. 12. *Definition of the dihedral angles τ in the back bone of $\beta^{2,2}$- and $\beta^{2,2,3}$-peptides and preferred angles $\tau_{1,2}$, $\tau_{2,3}$, $\tau_{1,N}$, and $\tau_{3,N}$ found by MD studies of* **7c** *with the GROMOS simulation package* (*cf. Figs. 13–16*) [10]. The atoms between which the dihedral angles are defined are printed in bold in the *Newman* projections. For $\tau_{1,2}$ see *Fig. 13*, for $\tau_{2,3}$ see *Fig. 14*, for $\tau_{3,N}$ see *Fig. 15*. The torsion angle $\tau_{1,N}$ around the amide (peptide) bond is close to 180° in all conformations of all residues! *ap* = antiperiplanar, *sc* = synclinal, *ac* = anticlinal.

Fig. 15), in agreement with the large coupling constant between these two H-atoms in the NMR spectrum of **7c**[21]).

6.2 *Solid-State Structures.* For proposing a solid-state structure of [$^{13}C_{30}$,$^{15}N_6$]-**1e** and **5**, we feel like pupils, having to determine *n* unknowns with fewer than *n* equations.

Disregarding the < 10% turn content[22]) of **5** and assuming that the $\beta^{2,2}$-hexa- and the $\beta^{2,2}$-heptapeptide derivatives [$^{13}C_{30}$,$^{15}N_6$]-**1e** and **5** have the same backbone arrangements, we consider the following structural features: *i*) all C=O bonds are parallel (or antiparallel), *ii*) the distance between the *termini* of **5** is > 30 Å; *iii*) the distance between the N-terminus of **5** and the $^{13}CH_2$ of residue 5 is > 20 Å; *iv*) the

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[21]) We also observed these large coupling constants in the NMR spectrum of the β-peptide **6a** (see *Sect. 4* and *Fig. 3*) with three Me groups on each residue.

[22]) $\beta^{2,2}$-Amino acid residues can be part of a turn formed by a ten-membered H-bonded ring [2]. It is probably possible to have such a turn or kink in part of the molecules in the solid state of **5** and arrive at a distance of *ca.* 7 Å between the N-terminus and the ^{13}CO of residue 7.

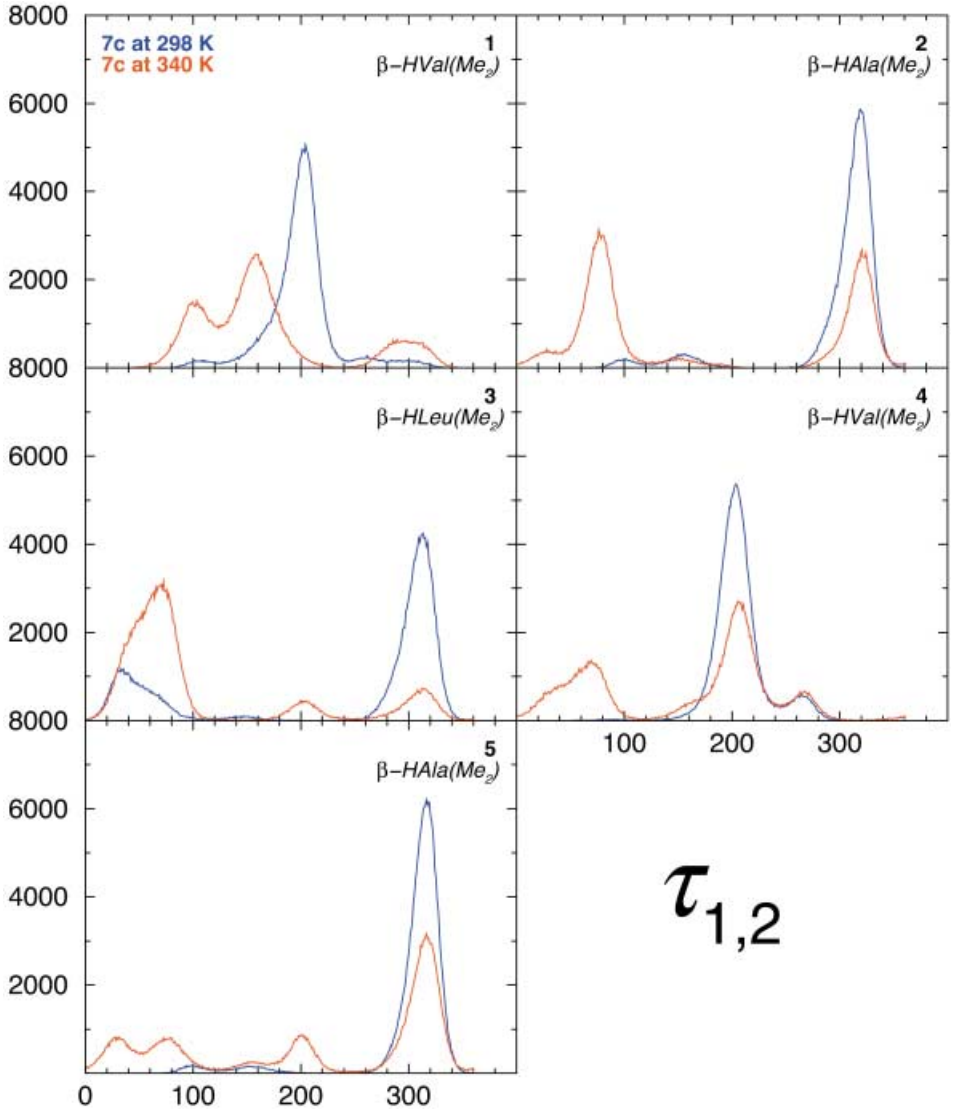

Fig. 13. *Distribution of dihedral angles C(HR)–C(Me$_2$)–C(O)–N(H) ($\tau_{1,2}$) of the residues 1–5 in the $\beta^{2,2,3}$-hexapeptide* **7c** *observed in 100-ns MD simulations in MeOH at 25 and 67°*. The dihedral angles were calculated for over 200 000 structures extracted at 0.5-ps intervals from the simulations [10]. *x*-Axis: angles [°], *y*-axis: number of conformations having a given dihedral angle in the corresponding amino acid residue 1–5. *Blue:* 298 K, *red:* 340 K. As can be seen, $\tau_{1,2}$ is *ca.* 200° in the two β-HVal(Me$_2$) and *ca.* 300° in the other three residues at room temperature. At higher temperature, other $\tau_{1,2}$ angles are found (30, 80, 100, 160, and 270°). The most prominent angles are shown as *Newman* projections in *Fig. 12*. Clearly, there is a rather flat conformational energy profile around $\tau_{1,2}$, with less sharp preferences than found for $\tau_{2,3}$, $\tau_{3,N}$, and $\tau_{1,N}$ (*cf. Figs. 12* and *14–16*).

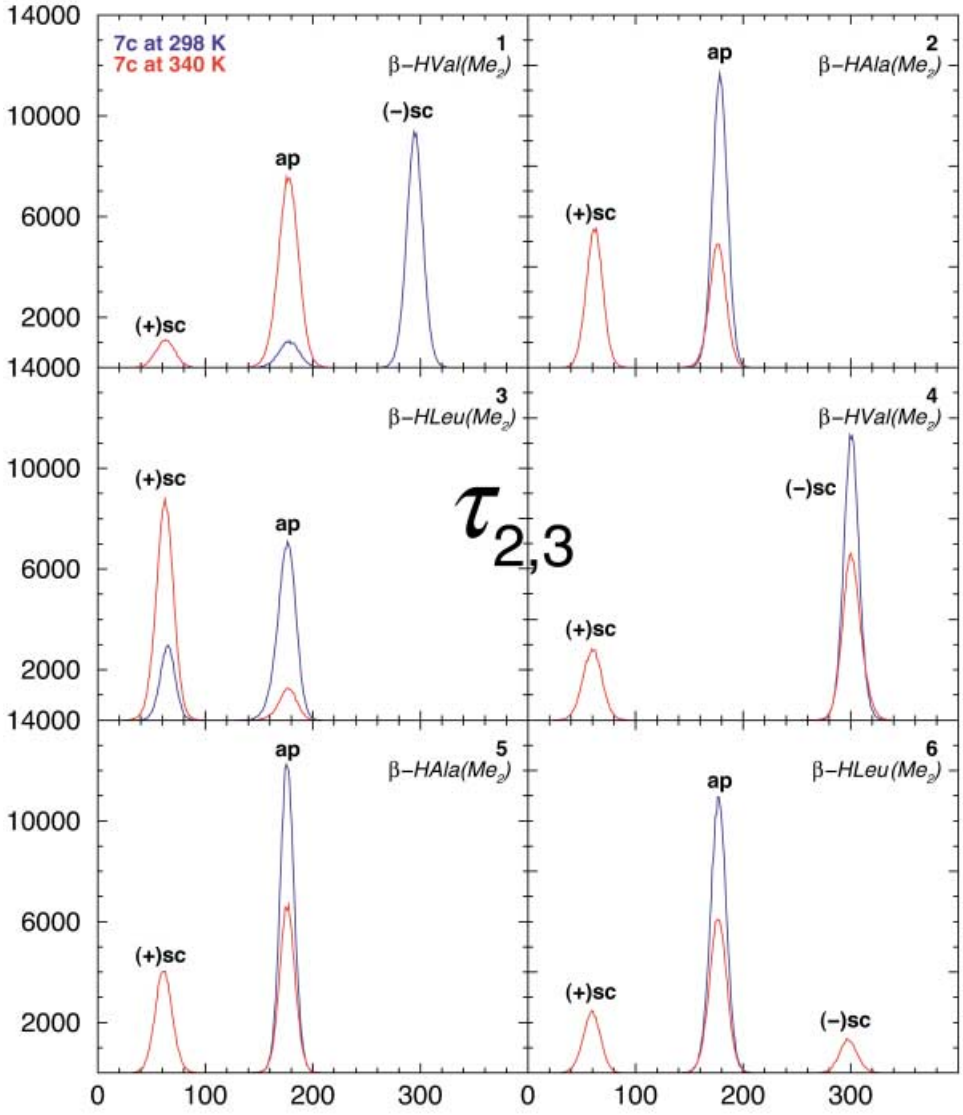

Fig. 14. *Distribution of dihedral angles N(H)–C(HR)–C(Me$_2$)–CO* ($\tau_{2,3}$) *of the residues 1–6 in the* $\beta^{2,2,3}$*-hexapeptide* **7c** *observed in 100-ns MD simulations in MeOH at 25 and 67°* [10]. For details and definitions, see caption of *Fig. 13*, and for *Newman* projections of the preferred conformations, see *Fig. 12*. All $\tau_{1,2}$ angles (*ca.* 60, 180, and 300°) are those of staggered ethane moieties, and, at room temperature, the $\beta^{2,2,3}$-amino acid residues with Ala and Leu side chains (No. 2, 3, 5 and 6) are mostly in an *ap* conformation (*ca.* 180°), while the residues (No. 1 and 4) with Val side chains are in a (–)-*sc* conformation (*ca.* 300°). At 67°, all residues show a shift of the conformational equilibrium towards *sc* ('*gauche*'), while $\beta^{2,2,3}$-HVal(Me$_2$) (No. 1) is mainly in the *ap* ('*trans*') conformation.

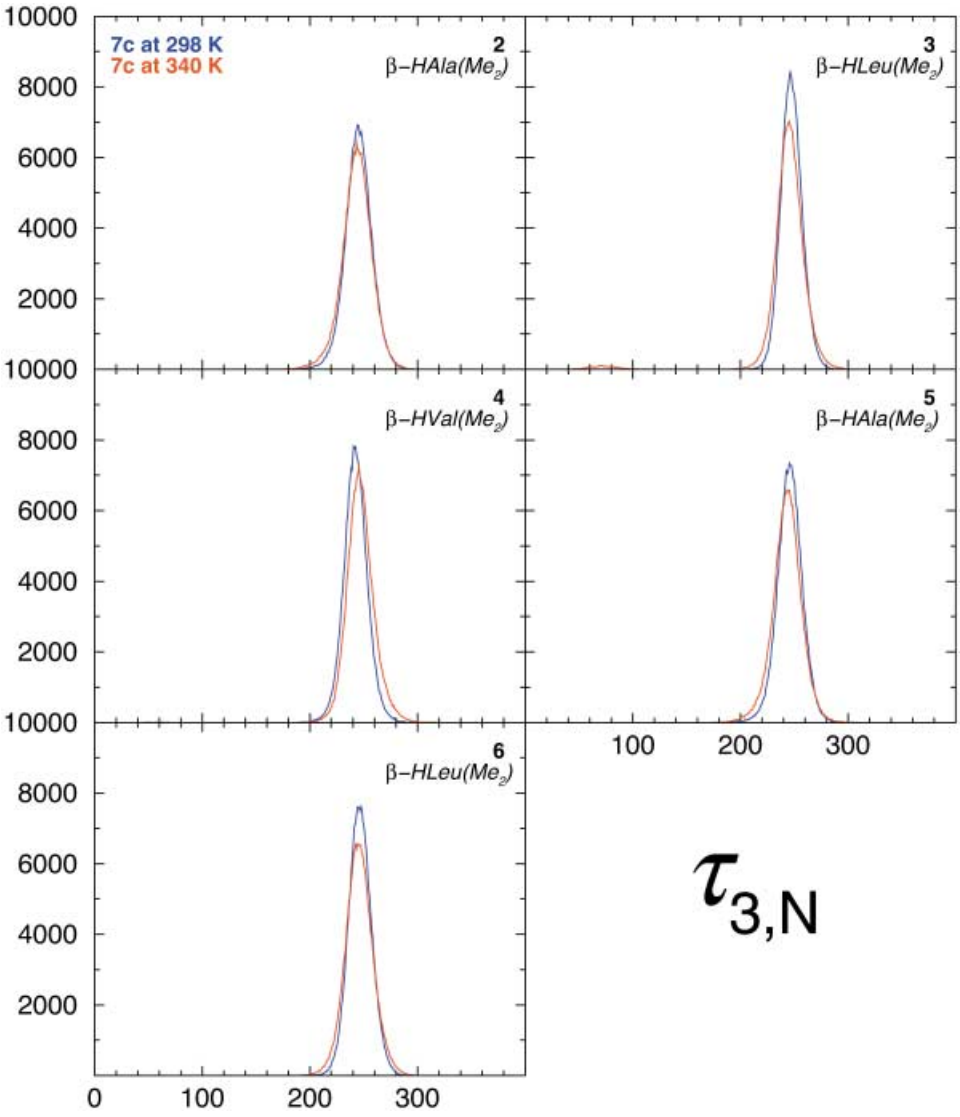

Fig. 15. *Dihedral angle C(O)–N(H)–C(HR)–C(Me$_2$) ($\tau_{3,N}$) of the residues 2–6 in the $\beta^{2,2,3}$-hexapeptide* **7c** *observed in 100-ns MD simulations in MeOH at 25 and 67°* [10]. For details and definitions, see caption of *Fig. 13*, and, for presentations (*Newman* and 'flying wedge' formulae), see *Fig. 12*. Clearly, the angle $\tau_{3,N}$ is fixed near 245°, *i.e.* (–)-*ac*, with the C=O O-atom and the C(R,CMe$_2$)H H-atom in a 1,5-coplanar arrangement (no *Newman* strain! [52]) and the NH H-atom staggering between the two large groups R and C(Me$_2$(CO)).

amide group is planar, placing $C(Me_2)$, C(O), O, N, H, and $C(H_2)$ in one plane; *v*) the corresponding dihedral angles O=C–N–H and $C(Me_2)$–C(O)–N(H)–$C(H_2)$ are *ca*. 180°; and *vi*) the ethane bond $C(H_2)$–$C(Me_2)$ has a staggered, rather than an eclipsed conformation. With these structural features, there are still numerous possibilities for the backbone arrangements of **5**.

To obtain more structural information, a search in the Cambridge file[23]) for the substructures of (C_3)C–C(O)–N(H)–$C(H_2)$–$C(C_3)$[24]) was undertaken; the results are collected in *Fig. 16*. Not surprisingly, the structural features defined in *iv* – *vi* above are confirmed. In addition, there is a preference for a dihedral angle near 180° of H–N–C–H (*Fig. 16,c*; structural feature *vii*; *cf.* $\tau_{3,N}$, *Figs. 12* and *15*).

Simple computer modelling for the *N*-acetyl-β-heptapeptide ester **4**, with the structural prerequisites *i* and *iv* – *vii* above, produced a number of arrangements of $\beta^{2,2}$-peptide chains, including wide helical [11] and meander-type structures. The four structures **D** – **G**, which are also compatible with the conclusions *ii* and *iii* from the REDOR spectra, are shown schematically in *Fig. 17* and as rendered 3D presentations in *Fig. 18*. All models have the C=O groups pointing in the same direction, and they are all unable to form sheets due to steric hindrance (by Me groups) of *inter*molecular H-bonding[25]). The fully extended conformation **D** (in-line amide and C–C bonds) has a length well above 30 Å (as deduced from the REDOR measurements), and it is the only one in which H(N) and H(CH) are not antiperiplanar ($\tau_{3,N}$); the pairs of geminal Me groups are equivalent in this conformation. Backbone arrangement **E** is the one found in β-peptide sheets [4][19][32] with parallel displaced amide planes. In **F**, the peptide bonds are parallel to the chain axis. In the somewhat short model **G**, there is a meander-type winding of the chain, which consists of elements found in cyclo-β-tripeptides [33], and also in the β-peptide 3_1 or 3_{14} helix [7]. There is no way, at this point[26]), to distinguish between the models, or to be sure that there are not alternative arrangements[27]).

In summary, the solution- and solid-state-NMR investigations of the labelled and unlabelled β-peptides with geminal dimethyl substitution in the 2-position, combined with simple modelling, Cambridge file searches, and MD calculations [10], have provided intriguing details about the structure and dynamic behavior of this type of β-peptides. Unlike in α-peptides[20]), the geminal disubstitution leads to a much more flexible structure of β-peptides in solution, with no evidence for folding to a helix. Like geminally disubstituted α-amino acid residues, the $\beta^{2,2}$-analogs may be part of a turn

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23) *Cambridge Structural Data Base*, CSD ConQuest software; search of July 2002.

24) There are quite a few more structures in the CSD now, as compared to the early eighties, when *Dunitz et al*. performed their classical analysis of amide and ester crystal structures [31].

25) With antiparallel C=O groups, it is difficult to have staggering of the ethane moieties and to arrive at large enough distances (conditions *ii*, *iii*, and *vi*).

26) Powder X-ray measurements with **1f** and **5** produced no suitable diffraction patterns.

27) When the coordinates of the models **D**, **E**, and **F**, as shown in *Fig. 18*, are loaded into the program SPARTAN SGI Version 5.1.3 – with constraints for parallel C=O bonds – and subjected to MD or semi-empirical energy minimization, the models **D**, **E**, and **F** interchange, depending on the method of optimization. Furthermore, all three models **D**, **E**, and **F** wind up with a flat curvature rather than as straight chains. This is mainly caused by an enlargement of the angle Me–C(Me)–C(O) above 109° (*cf. Fig. 16*), with simultaneous slight rotation around the $C(Me_2)$–C(O) bond, such that the distance between the NH H-atom and the neighboring Me group increases.

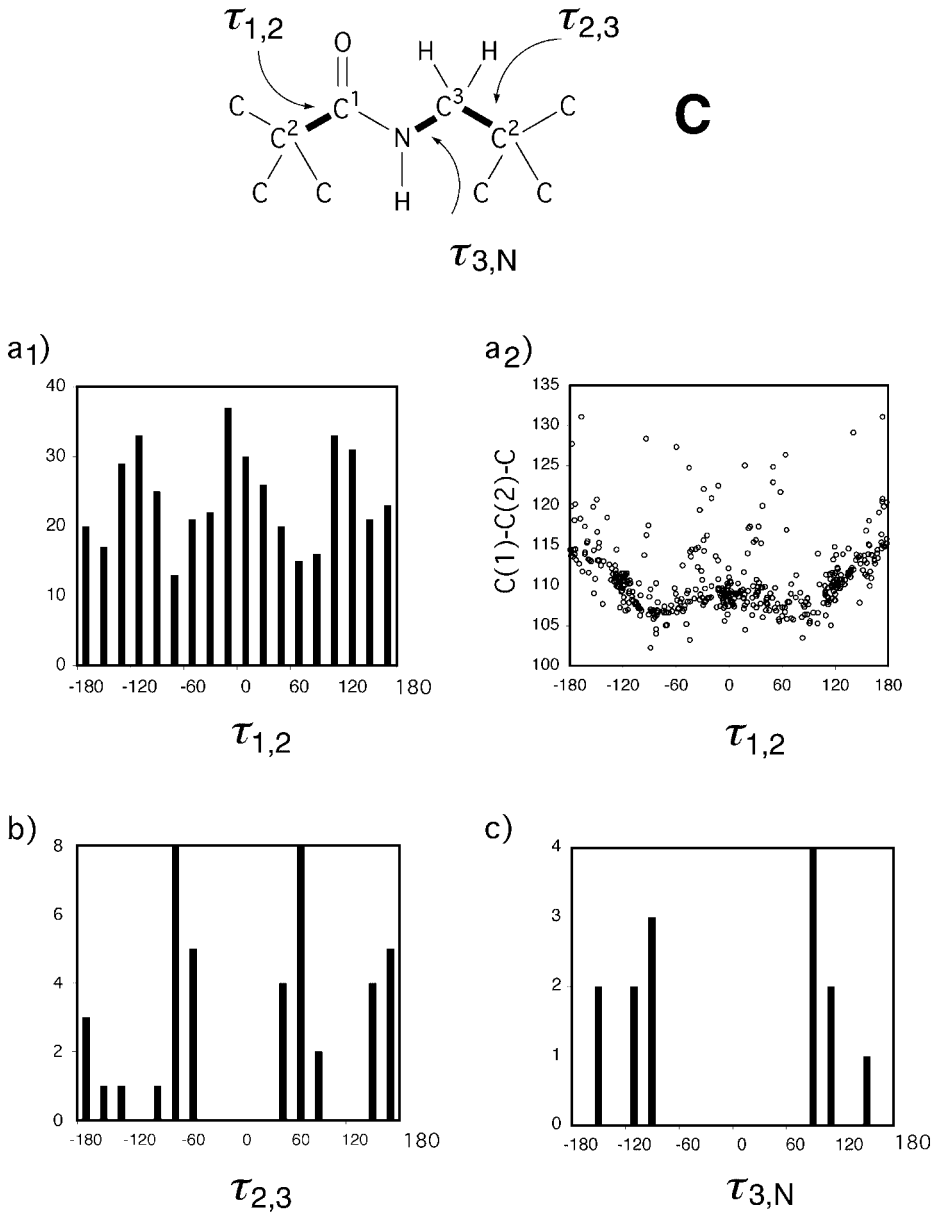
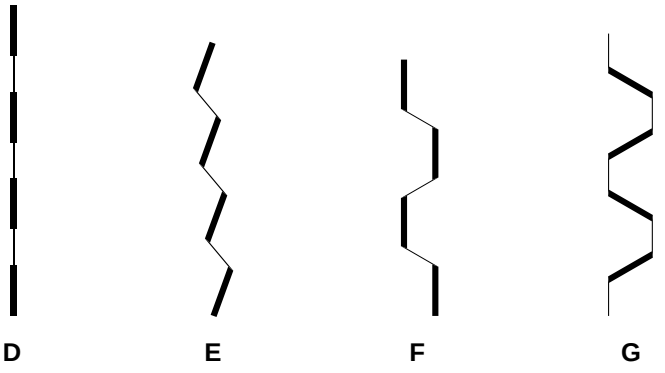


Fig. 16. *Results of a CSD search for the substructures of* **C**. C(2) was chosen to have any three C substituents. The angles τ are as in *Fig. 12*. Conditions for structures included: *R* value <0.07 Å, no errors, no disorder, polymers allowed, ionic species allowed. a_1) Angles $\tau_{1,2}$ vary widely from 0 to $\pm 180°$ (*cf.* the results of the MD study in *Fig. 13*); a_2) as 'we rotate' around the C(1)–C(2) bond, there is widening of the C(1)–C(2)–C angle from tetrahedral to *ca.* 114°, as we approach coplanarity of the NH H-atom with a C substituent at C(2). *b*) The sp^3–sp^3 bond between C(2) and C(3) is staggered ($\tau_{2,3}$ angles $\pm$ 60 and 180°). *c*) The quaternary C(2)-atom is out-of-plane with respect to the amide plane, *i.e.*, one H(3) and the H(N) are approximately antiperiplanar (*ap*), avoiding O, C(C_3) 1,5-repulsion (*Newman* strain [52]; *cf.* the MD conclusion, *Fig. 15*): $\tau_{3,N}$ angles are centered near $\pm 100°$.



	D	E	F	G
N to *C* terminus	36 Å	35 Å	34 Å	27 Å
N-terminus to residue 5	24 Å	23 Å	23Å	18 Å

REDOR distance *N* to *C* terminus >30 Å

N terminus to residue 5 > 20 Å

Fig. 17. *Schematic representations of four possible back-bone structures* **D** – **G** *of the* $\beta^{2,2}$*-heptapeptide derivative* **4** (and **5**) *in the solid state*. The bold lines represent the amide planes C–CO–NH–C, the thin lines the sp^3–sp^3 C,C bonds. The distances between the *N*-terminal **C**H_3 (**C**F_3) Ac C-atom and the *C*-terminal C=O C-atom (**C**O_2Me) or the **C**H_2 C-atom of residue 5 in these models are given in the table and are compared with the distances derived from REDOR measurements. Except for **G**, these models have chain lengths within the limits from the REDOR NMR determination. For ball-and-stick models of the four conformations, see *Fig. 18*.

structure (ten-membered H-bounded ring; *cf. Fig. 1* [1][22]), and both prevent sheet formation by steric hindrance of the NH H-bond donor; the models in *Fig. 18* nicely show the shielding of the NH H-atoms by the 'parallel' C-CH_3 groups, especially in **E** – **G**; it is also evident that the chains in *Fig. 18* are still able to act as H-bond acceptors, because access to the C=O O-atom is not blocked – a chain like **E** could be used for 'capping' a β-peptide sheet [19]. Thus, geminally dimethyl-substituted $\beta^{2,2}$-peptide sections may be useful components in our ongoing construction of more complex β-peptides and in our search for tertiary structures of β-peptides.

We gratefully acknowledge the financial support by the *Swiss National Science Foundation*[1]) and by *Novartis*. This work was also supported, in part, by *NIH* grant GM40634 (*J. S.*).

Experimental Part

1. *General. Abbreviations:* DIPA: $(i\text{-Pr})_2NH$, DIPEA: $(i\text{-Pr})_2NEt$, EDC: 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, FC: flash chromatography, HATU: *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate[14]), HOBt: 1-hydroxy-1*H*-benzotriazole, h.v.: high vacuum, 0.01 – 0.1

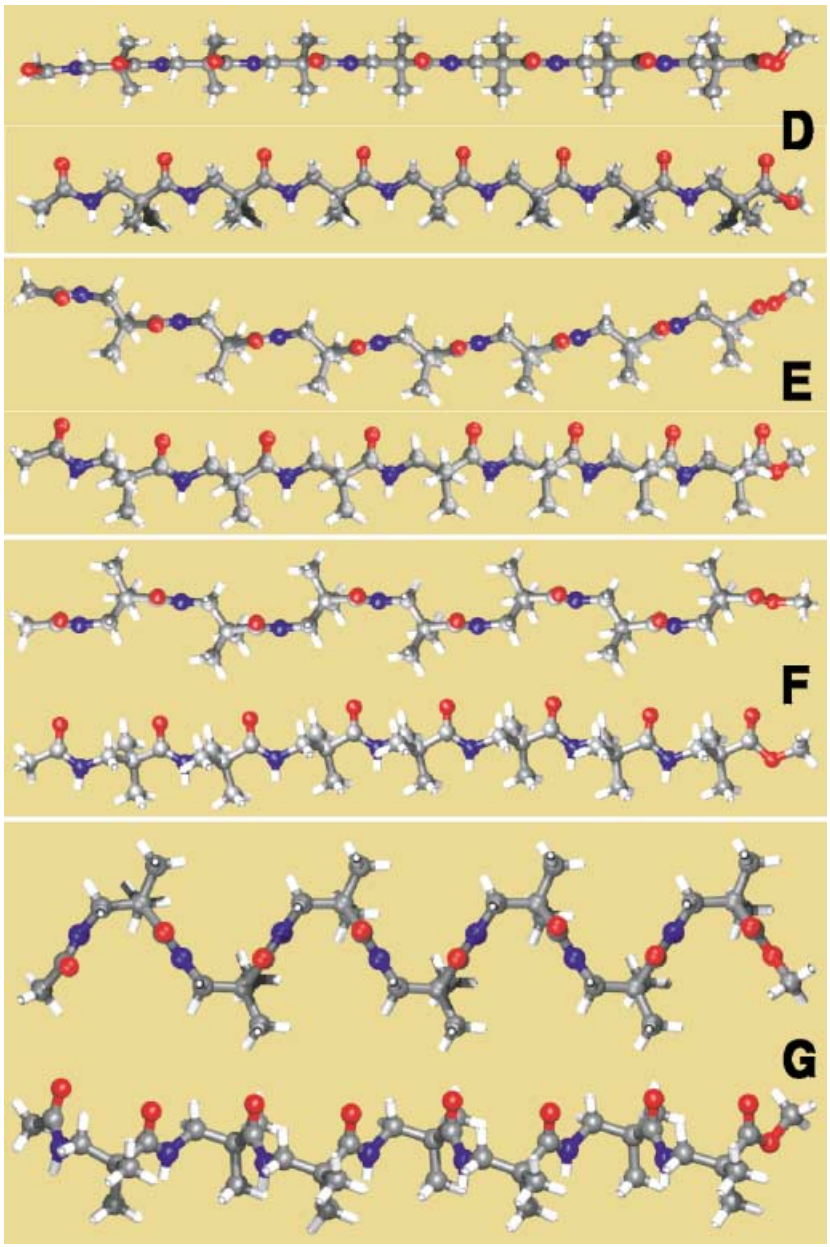


Fig. 18. *Ball-and-stick models of four conformations of Ac-($\beta^{2,2}$-HAib)$_7$-OMe, fulfilling* (**D**–**F**) *or almost fulfilling* (**G**) *the structural conditions* i–vii *given in the accompanying text*. For a schematic presentation of the backbones, see *Fig. 17*. The models were produced with the program MOLMOL 2.6 and rendered with POV-Ray 3.5 for Mac OS.

Torr. β-HXxx: β-homoamino acid [4][5], TFA: CF_3CO_2H, TFAA: $(CF_3CO)_2O$, TFE: CF_3CH_2OH. $CHCl_3$ employed for the coupling reactions was filtered over Al_2O_3 (Alumina *Woelm N* and *ICN Alumina B*, Akt. I) to remove EtOH. THF was freshly distilled over K/benzophenone under Ar before use. Et_3N was distilled from CaH_2 and stored under Ar. DIPA and DIPEA were freshly distilled over CaH_2. Solvents for chromatography and workup were distilled from *Sikkon* (anh. $CaSO_4$: *Fluka*; AcOEt, pentane), P_2O_5 (CH_2Cl_2) or $FeSO_4$/KOH (Et_2O). Amino acid derivatives were purchased from *Bachem*, *Senn*, or *Degussa*. The labelled β-amino acids were purchased from *ISOTEC Inc.* All other reagents were used as received from *Fluka*. The unlabelled $\beta^{2,2}$-HAib derivatives up to the tripeptide were synthesized according to [1]. For the synthesis of the β^3-amino acids and the α-methyl β^3-amino acids *l*-**8**/*u*-**8**, *ent*-*l*-**8l**/*ent*-*u*-**8**, *u*-**9**, *u*-**10**, see [4][5][7][18][19]. Flasks and stirring bars for the alkylations were dried for *ca.* 12 h at 120° and allowed to cool in a dessicator over silica gel. All indicated temps. were monitored with an internal thermometer (*Ebro TTX-690* digital thermometer). TLC: *Merck* silica gel 60 F_{254} plates with UV, anisaldehyde (9.2 ml of anisaldehyde, 3.75 ml of AcOH, 12.5 ml of conc. H_2SO_4, 350 ml of EtOH) or $KMnO_4$ soln. (0.5 g of $KMnO_4$, 100 ml of 1M NaOH). FC: *Fluka* silica gel *60* (40–63 μm); at *ca.* 0.3 bar. Anal. HPLC: *Knauer* HPLC system *K 1000*, pump type *64*, *EuroChrom 2000* integration package, degaser, UV detector *K 2000* (variable-wavelength monitor), *Macherey-Nagel* C_{18} column: *Nucleosil 100-5* C_{18} (250 × 4 mm). Prep. HPLC: *Knauer* HPLC system: pump type *64*, programmer *50*, UV detector (variable-wavelength monitor), *Macherey-Nagel* C_{18} column: *Nucleosil 100-7* C_{18} (250 × 21 mm). M.p.: *Büchi* 510 apparatus; uncorrected. Optical rotations: *Perkin-Elmer 241* polarimeter (10 cm, 1-ml cell) at r.t. Circular dichroism (CD) spectra: *Jasco J-710*, recording from 190 to 250 nm at 20°; 1-mm rectangular cell; average of five scans, corrected for the baseline; peptide concentration 0.2 mM in MeOH; molar ellipticity $[\theta]$ in $deg \cdot cm^2 \cdot mol^{-1}$ (λ in nm); smoothing by *Jasco* software. IR Spectra: *Perkin-Elmer 782* spectrophotometer. NMR Spectra: *Bruker AMX-500* (^1H: 500 MHz, ^{13}C: 125 MHz), *AMX-400* (^1H: 400 MHz, ^{13}C: 100 MHz): chemical shifts δ in ppm downfield from internal Me_4Si (= 0 ppm); *J* values in Hz; signals for rotamers are underlined; signals for labelled C-atoms are in *italics*. MS: *Hitachi-Perkin-Elmer RHU-6M* (FAB), *Finnigan MAT TSQ-7000* (ESI) spectrometer and *IonSpec Ultima FTMS-Spectrometer* (HR-MALDI); in *m/z* (% of basis peak). Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

2. *Boc Deprotection: General Procedure 1* (*GP 1*). Similarly to the procedures reported in [1][4][5][7], the Boc-protected amino acid was dissolved in CH_2Cl_2 (0.23–0.8M) and cooled to 0°. An equal volume of TFA was added, and the mixture was allowed to warm to r.t., and stirring continued for further 1 to 1.5 h. Concentration under reduced pressure, co-evaporation with CH_2Cl_2, and drying of the residue under h.v. yielded the crude TFA salt, which was identified by NMR and used without further purification.

3. *Methyl Ester Hydrolysis: General Procedure 2* (*GP 2*). *GP 2a:* Similarly to the procedure reported in [34], a soln. of the protected amino acid or β-peptide in MeOH (1.2M) was treated with 1N NaOH (1.2 equiv.) at 0°. The soln. was allowed to warm to r.t., and stirring was continued for 16 h at r.t. After dilution with H_2O, the soln. was adjusted to pH 2 with 1N $NaHSO_4$ 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 dried under h.v. and used in the next step without further purification.

GP 2b: The protected amino acid or β-peptide was dissolved in TFE (1.2M), treated with 10N NaOH (20–30 equiv.), and heated at 40°. After completion of the reaction (TLC), the mixture was diluted with H_2O, and the pH was adjusted to 2–3 with 1N HCl (0°). After extraction with AcOEt, the org. phase was washed with sat. aq. NaCl soln., dried ($MgSO_4$), and concentrated under reduced pressure. The acid was dried under h.v. and used in the next step without further purification.

4. *Peptide Coupling with EDC: General Procedure 3* (*GP 3*). According to [1][35], the appropriate TFA salt was dissolved in $CHCl_3$ or CH_2Cl_2 (0.5M) and cooled to 0°. This soln. was treated successively with Et_3N, HOBt (1.2 equiv.), a soln. of the Boc-protected fragment (1 equiv.) in $CHCl_3$, CH_2Cl_2, or DMF (0.25M), and EDC (1.2 equiv.). The mixture was allowed to warm to r.t., and stirring was continued for 15 h with exclusion of light. Subsequent dilution with $CHCl_3$ was followed by thorough washing with 1N HCl soln. (3×) and sat. aq. $NaHCO_3$ (2×) and NaCl solns. (1×). The org. phase was dried ($MgSO_4$) and then concentrated under reduced pressure. FC yielded the pure peptide.

5. *Preparation of the Trifluoroacetamide Compounds: General Procedure 4* (*GP 4*). Similarly to the procedure reported in [36], a CH_2Cl_2 soln. of the TFA salt (0.1M) of the appropriate peptide was cooled to 0° and treated with DIPEA (4–5 equiv.) and TFAA (1.5 equiv.). After 60 min at 0°, the reaction soln. was allowed to warm to r.t., and stirring was continued for another 30 min. Subsequent dilution with $CHCl_3$ was followed by thorough washing with 1N $NaHSO_4$ soln. (3×) and sat. aq. $NaHCO_3$ (3×), and NaCl solns. (2×). The org. phase was dried ($MgSO_4$) and then concentrated under reduced pressure. FC yielded the pure trifluoroacetamide.

6. *Alkylation of the $\beta^{2,3}$-Amino Acid Derivatives: General Procedure 5* (*GP 5*). According to [5], BuLi (2.2 equiv.) was added to a soln. of DIPA (2.2 equiv.) in THF (1.42M) at $-78°$. After 1 h, a soln of the $\beta^{2,3}$-amino acid derivative (1 equiv.) in THF (0.40M) was added, and stirring was continued for 1.5 h at $-78°$. MeI (4 equiv.) was then added slowly, and the mixture was stirred for 5 h at this temp., subsequently hydrolyzed with a 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 then concentrated under reduced pressure. FC yielded the pure $\beta^{2,2,3}$-amino acid.

7. *Peptide Coupling with HATU: General Procedure 6* (*GP 6*). The appropriate TFA salt, HATU (1.2 equiv.) and the Boc-protected fragment (1 equiv.) were dissolved in $CHCl_3$ (0.4M), and the soln. was treated with DIPEA (5 equiv.). The resulting mixture was stirred under Ar at r.t., and the reaction was monitored by TLC. After completion of the reaction, the mixture was diluted with $CHCl_3$, followed by thorough washing with 1N HCl (4×), sat. aq. $NaHCO_3$ soln. (3×), and sat. aq. NaCl soln. (1×). The org. phase was dried ($MgSO_4$) and then concentrated under reduced pressure. FC yielded the pure peptide.

Boc-$\beta^{2,2}$-HAib-$\beta^{2,2}$-HAib(^{13}CO)-OMe ([^{13}C]-**1b**). Amino acid [^{13}C]-**1a** (502 mg, 2.04 mmol, labelling *ca.* 99%, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (4.1 ml), and treated with Et_3N (1.28 ml, 9.18 mmol, 4.5 equiv.), HOBt (400 mg, 2.96 mmol, 1.45 equiv.), acid **3a** (532 mg, 2.45 mmol, 1.2 equiv.) dissolved in $CHCl_3$ (8.2 ml), and EDC (626 mg, 3.26 mmol, 1.6 equiv.) according to *GP 3*. FC (Et_2O/pentane 3:2) yielded [^{13}C]-**1b** (622 mg, 1.88 mmol, 92%). White powder. R_f (Et_2O/pentane 3:2) 0.31. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.18–1.19 (*m*, 4 Me); 1.42 (*s*, *t*-Bu); 3.21 (*d*, ^3J(H,NH) = 6.5, CH_2); 3.33–3.36 (*m*, CH_2); 3.71 (*d*, ^3J(H,^{13}C) = 3.8, MeO); 5.20 (br., NH); 6.41 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.13, 23.65, 28.38 (Me); 42.93, 43.38, 43.49 (C); 46.67, 48.89 (CH_2); 52.15, 52.18 (Me); 79.02 (C); 83.10 (CH_2); 156.41, 177.00, 177.61, *177.89* (C).

Boc-$\beta^{2,2}$-HAib[^{13}CH$_2$,^{15}N]-$\beta^{2,2}$-HAib-$\beta^{2,2}$-HAib[^{13}CO]-OMe ([^{13}C$_2$,^{15}N]-**1c**). Dipeptide [^{13}C]-**1b** (812 mg, 2.45 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (4.5 ml), and treated with Et_3N (1.88 ml, 13.5 mmol, 5.5 equiv.), HOBt (463 mg, 3.43 mmol, 1.4 equiv.), acid [^{13}C,^{15}N]-**3a** (630 mg, 2.87 mmol, 1.2 equiv.) in $CHCl_3$ (8 ml), and EDC (751 mg, 3.92 mmol, 1.6 equiv.) according to *GP 3*. FC (AcOEt/pentane 3:2) yielded [^{13}C$_2$,^{15}N]-**1c** (961 mg, 2.22 mmol, 91%). White powder. R_f (AcOEt/pentane 3:2) 0.22. IR ($CHCl_3$): 3446*m*, 3007*m*, 2977*m*, 2933*m*, 2872*w*, 1703*s*, 1652*s*, 1514*s*, 1490*s*, 1472*s*, 1392*m*, 1368*m*, 1310*m*, 1166*s*, 1144*s*, 916*w*, 858*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.17–1.19 (*m*, 6 Me); 1.42 (*s*, *t*-Bu); 3.03 (*d*, ^3J(H,NH) = 5.7, 0.5 CH_2); 3.31–3.39 (*m*, 2.5 CH_2); 3.71 (*d*, ^3J(H,^{13}C) = 3.8, MeO); 5.25 (br., ^1J(H,^{15}N) = 91.1, NH); 6.47 (br., NH); 6.88 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.10, 23.11, 23.62, 23.80, 28.40 (Me); 42.55, 42.90, 43.02, 43.39, 43.46 (C); 46.71, 47.36, *48.89*, *49.00*, 50.29 (CH_2); 52.19, 52.22 (Me); 78.88 (C); 83.23 (CH_2); 156.28, 156.54, 177.26, 177.56, 177.62, *177.90*, 178.18 (C).

Boc-$\beta^{2,2}$-HAib-$\beta^{2,2}$-HAib-$\beta^{2,2}$-HAib-$\beta^{2,2}$-HAib-OMe (**1d**). Tripeptide **1c** (3.71 g, 8.64 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in CH_2Cl_2 (25 ml), and treated with Et_3N (6.0 ml, 43 mmol, 5 equiv.), HOBt (1.40 g, 10.3 mmol, 1.2 equiv.), **3a** (1.91 g, 8.79 mmol, 1 equiv.) in CH_2Cl_2 (15 ml), and EDC (1.98 g, 10.3 mmol, 1.2 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 40:1) yielded **1d** (3.38 g, 6.39 mmol, 74%). White powder. M.p. 135.0–136.0°. R_f (CH_2Cl_2/MeOH 40:1) 0.15. IR ($CHCl_3$): 3451*m*, 3007*m*, 2972*m*, 2933*w*, 2872*w*, 1710*s*, 1649*s*, 1507*s*, 1474*m*, 1392*w*, 1367*m*, 1312*m*, 1158*s*, 990*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.18 (*s*, 2 Me); 1.19 (*s*, 6 Me); 1.42 (*s*, *t*-Bu); 3.20 (*d*, ^3J(H,NH) = 6.4, CH_2); 3.30-3.35 (*m*, 3 CH_2); 3.71 (*s*, MeO); 5.30 (br., NH); 6.50 (br., NH); 7.00–7.05 (br., 2 NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.09, 23.61, 23.73, 23.75, 28.39 (Me); 42.35, 42.41, 43.15 (C); 46.72, 47.38, 47.41, 48.95 (CH_2); 52.23 (Me); 78.82, 156.42, 177.20, 177.48, 177.68, 177.91 (C). FAB-MS: 551.3 (7.5, $[M+Na]^+$), 529.3 (100.0, M^+), 429.2 (69.4), 399.2 (10.3), 231.2 (11.2). Anal. calc. for $C_{26}H_{48}N_4O_7$ (528.69): C 59.07, H 9.15, N 10.60; found: C 59.19, H 9.12, N 10.48.

Boc-$\beta^{2,2}$-HAib[^{15}N,^{13}CH$_2$]-$\beta^{2,2}$-HAib-$\beta^{2,2}$-HAib[^{13}CO]-$\beta^{2,2}$-HAib-OMe ([1,3-^{13}C$_2$, 1-^{15}N]-**1d**). Amino acid **1a** (150 mg, 648 µmol, 1.3 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (1 ml), and treated with Et_3N (383 µl, 2.74 mmol, 5.5 equiv.), HOBt (105 mg, 773 µmol, 1.55 equiv.), [^{13}C$_2$,^{15}N]-**3c** (209 mg, 499 µmol, 1 equiv.) in $CHCl_3$ (2 ml), and EDC (172 mg, 898 µmol, 1.8 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 40:1) yielded [1,3-^{13}C$_2$, 1-^{15}N]-**1d** (251 mg, 472 µmol, 95%). White powder. R_f (CH_2Cl_2/MeOH 40:1) 0.15. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.17–1.19 (*m*, 8 Me); 1.42 (*s*, *t*-Bu); 3.06 (*d*, ^3J(H,NH) = 5.8, 0.5 CH_2); 3.30–3.35 (*m*, 3.5 CH_2); 3.71 (*s*, MeO); 5.29 (br., ^1J(^{15}N,H) = 91.1, NH); 6.48 (br., NH); 7.00–7.06 (br., 2 NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 23.10, 23.63, 23.74, 23.77, 28.40 (Me); 42.19, 42.35, 42.58, 43.01, 43.16, 43.30 (C); 46.71, 47.39, 47.42, *48.90*, *48.99*, 50.30 (CH_2); 52.24 (Me); 78.82(C); 83.23 (CH_2); 156.31, 156.52, 177.20, 177.28, *177.48*, 177.68, 177.77, 177.92(C).

Boc-$\beta^{2,2}$-HAib-$\beta^{2,2}$-HAib[^{15}N,^{13}CH$_2$]-$\beta^{2,2}$-HAib-$\beta^{2,2}$-HAib[^{13}CO]-OMe ([2,4-^{13}C$_2$, 2-^{15}N]-**1d**). Tripeptide [^{13}C$_2$,^{15}N]-**1c** (206 mg, 476 µmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (1 ml), and treated with Et_3N (366 µl, 2.63 mmol, 5.5 equiv.), HOBt (100 mg, 740 µmol, 1.55 equiv.), **3a** (135 mg,

621 µmol, 1.3 equiv.) in $CHCl_3$ (2 ml), and EDC (165 mg, 861 µmol, 1.8 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 40:1) yielded [2,4-$^{13}C_2$, 2-^{15}N]-**1d** (239 mg, 450 µmol, 95%). White powder. R_f (CH_2Cl_2/MeOH 40:1) 0.17. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.18–1.19 (*m*, 8 Me); 1.42 (*s*, *t*-Bu); 3.20 (*d*, ^3J(H,NH) = 6.4, CH_2); 3.30 (*dd*, ^1J(H,^{13}C) = 140.0, ^3J(H,NH) = 5.4, CH_2); 3.31–3.37 (*m*, 2 CH_2); 3.71 (*d*, ^3J(H,^{13}C) = 3.8, MeO); 5.29 (br., NH); 7.00 (br., ^1J(H,^{15}N) = 91.0, NH); 7.04 (br., NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 23.10, 23.63, 23.74, 28.40 (Me); 42.19, 42.0, 42.48, 42.92, 43.14, 43.19, 43.36 (C); 46.72, *47.36*, *47.45*, 47.69, 47.77, 48.96 (CH_2); 52.22, 52.25 (Me); 78.82 (C); 83.29 (CH_2); 156.43, 177.13, 177.24, 177.48, 177.70, *177.92*, 178.15, 178.21 (C).

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-OMe (**1f**). Tetrapeptide **1d** (513 mg, 0.95 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in CH_2Cl_2 (1.5 ml), and treated with Et_3N (662 µl, 4.73 mmol, 5 equiv.), HOBt (153 mg, 1.14 mmol, 1.2 equiv.), **3c** (393 mg, 0.95 mmol, 1 equiv.) in CH_2Cl_2 (3.5 ml), and EDC (218 mg, 1.14 mmol, 1.2 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 25:1) yielded **1f** (539 mg, 0.65 mmol, 68%). White powder. M.p. 177.5–179.0°. R_f (CH_2Cl_2/MeOH 25:1) 0.16. IR ($CHCl_3$): 3448*w*, 3007*m*, 2970*m*, 2930*w*, 2869*w*, 1710*m*, 1647*s*, 1506*s*, 1474*m*, 1392*w*, 1368*m*, 1312*m*, 1159*m*, 1046*w*, 988*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.18–1.19 (*m*, 14 Me); 1.41 (*s*, *t*-Bu); 3.20 (*d*, ^3J(H,NH) = 6.4, CH_2); 3.29–3.32 (*m*, 5 CH_2); 3.34 (*d*, ^3J(H,NH) = 6.2, CH_2); 3.71 (*s*, MeO); 5.31 (br., NH); 6.50 (br., NH); 7.05–7.07 (br., NH); 7.12 (br., NH); 7.19–7.25 (br., 3 NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.09, 23.62, 23.73, 23.77, 28.40 (Me); 42.10, 42.12, 42.14, 42.27, 43.13 (C); 46.72, 47.43, 47.48, 48.95 (CH_2); 52.25 (Me); 78.79, 156.43, 177.21, 177.50, 177.62, 177.63, 177.67, 177.93 (C). FAB-MS: 848.3 (39.2, $[M+Na]^+$), 826.3 (72.8, M^+), 726.3 (100), 696.2 (5.1). Anal. calc. for $C_{41}H_{75}N_7O_{10}$ (826.08): C 59.61, H 9.15, N 11.87; found: C 59.49, H 9.22, N 11.69.

Boc-β^{2,2}-HAib[^{15}N,^{13}CH$_2$]-β^{2,2}-HAib-β^{2,2}-HAib[^{13}CO]-β^{2,2}-HAib[^{15}N,^{13}CH$_2$]-β^{2,2}-HAib-β^{2,2}-HAib[^{13}CO]-β^{2,2}-HAib-OMe ([1,3,4,6-$^{13}C_4$, 1,4-$^{15}N_2$]-**1f**). Tetraptide [1,3-$^{13}C_2$,1-^{15}N]-**1d** (236 mg, 444 µmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (0.9 ml), and treated with Et_3N (340 µl, 2.44 mmol, 5.5 equiv.), HOBt (93 mg, 688 µmol, 1.55 equiv.), [$^{13}C_2$,^{15}N]-**3c** (180 mg, 430 µmol, 1 equiv.) in $CHCl_3$ (1.6 ml) and EDC (153 mg, 799 µmol, 1.8 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 25:1) yielded [1,3,4,6-$^{13}C_4$, 1,4-$^{15}N_2$]-**1f** (299 mg, 359 µmol, 84%). White powder. R_f (CH_2Cl_2/MeOH 25:1) 0.15. IR ($CHCl_3$): 3444*m*, 3007*m*, 2972*s*, 2933*m*, 2873*w*, 1710*s*, 1645*s*, 1509*s*, 1393*w*, 1368*m*, 1310*m*, 1158*s*, 989*w*, 889*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.17–1.19 (*m*, 14 Me); 1.41 (*s*, *t*-Bu); 3.02 (*d*, ^3J(H,NH) = 6.1, 0.5 CH_2); 3.29 (br., ^1J(H,^{13}C) = 138.0, CH_2); 3.29–3.38 (*m*, 5.5 CH_2); 3.71 (*s*, MeO); 5.30 (br., ^1J(H,^{15}N) = 91.5, NH); 6.48 (br., NH); 7.12–7.36 (br., 5 NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.10, 23.64, 23.74, 23.79, 28.41 (Me); 41.86, 41.92, 42.02, 42.14, 42.27, 42.34, 42.41, 42.51, 42.96, 43.14, 43.33 (C); 46.40, 46.50, 46.74, 47.26, *47.44*, *47.54*, 47.71, 47.80, 48.23, *48.91*, *49.02*, 50.30 (CH_2); 52.25 (Me); 78.79 (C); 83.32 (CH_2); 156.29, 156.55, 177.21, 177.26, 177.30, *177.51*, *177.55*, 177.63, *177.69*, 177.93 (C). HR-MALDI-MS: 854.6 (11.9, $[M+Na]^+$), 754.5 (100), 732.5 (18.8), 532.4 (11.3), 501.4 (8.0), 432.3 (11.8), 402.3 (10.7), 384.3 (8.2), 331.2 (6.9), 283.2 (6.8), 251.5 (10.2).

Boc-β^{2,2}-HAib[^{15}N,^{13}CH$_2$]-β^{2,2}-HAib-β^{2,2}-HAib[^{13}CO]-β^{2,2}-HAib-β^{2,2}-HAib[^{15}N,^{13}CH$_2$]-β^{2,2}-HAib-β^{2,2}-HAib[^{13}CO]-OMe ([1,3,5,7-$^{13}C_4$, 1,5-$^{15}N_2$]-**1f**). Tetraptide [2,4-$^{13}C_2$, 2-^{15}N]-**1d** (225 mg, 423 µmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (0.9 ml), and treated with Et_3N (324 µl, 2.33 mmol, 5.5 equiv.), HOBt (89 mg, 656 µmol, 1.55 equiv.), [$^{13}C_2$,^{15}N]-**3c** (182 mg, 423 µmol, 1 equiv.) in $CHCl_3$ (1.5 ml), and EDC (146 mg, 761 µmol, 1.8 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 25:1) yielded [1,3,5,7-$^{13}C_4$, 1,5-$^{15}N_2$]-**1f** (268 mg, 322 µmol, 76%). White powder. R_f (CH_2Cl_2/MeOH 25:1) 0.13. IR ($CHCl_3$): 3445*m*, 3008*m*, 2971*m*, 2933*m*, 2873*w*, 1702*m*, 1646*s*, 1497*s*, 1392*w*, 1368*m*, 1310*m*, 1170*m*, 987*w*, 889*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.17–1.19 (*m*, 14 Me); 1.41 (*s*, *t*-Bu); 3.02 (*d*, ^3J(H,NH) = 5.8, 0.5 CH_2); 3.28–3.38 (*m*, 5.5 CH_2); 3.29 (*dd*, ^1J(H,^{13}C) = 140.1; ^3J(H,NH) = 5.3, CH_2); 3.71 (*d*, ^3J(H,^{13}C) = 3.8, MeO); 5.30 (br., J(^{15}N,H) = 90.9, NH); 6.48 (br., NH); 7.05–7.34 (br., 5 NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.10, 23.64, 23.75, 23.79, 28.41 (Me); 41.88, 41.93, 42.07, 42.13, 42.27, 42.37, 42.84, 42.95, 43.32, 43.40 (C); 46.40, 46.74, 47.26, *47.44*, *47.54*, 47.72, 48.24, *48.91*, *49.03*, *50.29* (CH_2); 52.22, 52.25 (Me); 78.77 (C); 83.33 (CH_2); 156.28, 156.54, 177.20, 177.38, 177.46, 177.49, *177.62*, *177.92*, 178.19 (C). HR-MALDI-MS: 854.6 (20.4, $[M+Na]^+$), 754.5 (100), 732.5 (33.3), 532.4 (16.7), 501.4 (12.4), 432.3 (17.5), 400.3 (18.2), 382.3 (12.3), 333.2 (9.4), 301.2 (5.9), 251.5 (11.2).

Boc-β^{2,2}-HAib[^{15}N,^{13}CH$_2$]-OH ([^{13}C,^{15}N]-**3a**). Amino acid [^{13}C,^{15}N]-**1a** (690 mg, 2.96 mmol, labelling *ca.* 99.5%) was saponified according to *GP 2a* and yielded [^{13}C,^{15}N]-**3a** (640 mg, 2.92 mmol, 99%). White solid. R_f (Et_2O/pentane 1:2) 0.32. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.23 (*d*, ^3J(H,^{13}C) = 4.6, 2 Me); 1.44, 1.46 (*s*, *t*-Bu); 3.04–3.09 (*m*, 0.5 CH_2); 3.39–3.44 (*m*, 0.5 CH_2); 5.02, 6.35 (*dm*, ^1J(H,^{15}N) = 90.9, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.90, 28.37 (Me); 43.40, 43.77, 44.20 (C); *47.92, 48.03, 49.51, 49.61* (CH_2); 79.40, 80.89, 156.12, 156.38, 181.22, 182.90 (C).

Boc-β^{2,2}-HAib[^{15}N,^{13}CH$_2$]-β^{2,2}-HAib-β^{2,2}-HAib[^{13}CO]-OH ([$^{13}C_2$,^{15}N]-**3c**). Tripeptide [$^{13}C_2$,^{15}N]-**1c** (226 mg, 523 µmol) was saponified according to *GP 2a* and yielded [$^{13}C_2$,^{15}N]-**3c** (222 mg, quant.). White solid. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.17–1.23 (*m*, 6 Me); 1.42 (*s*, *t*-Bu); 1.67 (*s*, *t*-Bu); 3.04–3.05 (br., 0.5 CH_2); 3.32–3.39 (*m*,

2.5 CH_2); 5.24 (br., $^1J(H,^{15}N) = 91.2$, NH); 6.16–6.87 (br., 2 NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.15, 23.42, 23.74, 28.39 (Me); 42.64, 42.79, 43.18, 43.51 (C); 46.77, 47.49, 47.93, 48.13, *48.84*, *48.96*, *50.36*, *50.46* (CH_2); 79.34 (C); 83.23 (CH_2); 177.41, *180.07* (C).

Ac-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 (**4**). [36] Heptapeptide **1f** (830 mg, 1 mmol) was Boc-deprotected according to *GP 1*, dissolved in CH_2Cl_2 (10 ml), and cooled to 0°. The cooled soln. was treated with DIPEA (685 µl, 4 mmol, 4 equiv.) and Ac_2O (142 µl, 1.5 mmol, 1.5 equiv.). After 70 min at 0°, the soln. was allowed to warm to r.t., and stirring was continued for another 90 min. Subsequent dilution with $CHCl_3$ was followed by thorough washing with 1N $NaHSO_4$ soln. (3×), and sat. aq. $NaHCO_3$ (3×) and NaCl solns. (2×). The org. layer was dried ($MgSO_4$) and then concentrated under reduced pressure. FC (CH_2Cl_2/MeOH 18:1) and precipitation from CH_2Cl_2/hexane yielded **4** (736 mg, 0.96 mmol, 96%). White powder. M.p. 168.0–170.5°. R_f (CH_2Cl_2/MeOH 18:1) 0.12. IR ($CHCl_3$): 3443*w*, 3004*m*, 2971*m*, 2876*w*, 1714*w*, 1649*s*, 1507*s*, 1474*m*, 1392*w*, 1369*w*, 1312*w*, 1157*m*, 988*w*, 890*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.19 (*s*, 14 Me); 1.97 (*s*, MeCON); 3.29–3.35 (*m*, 7 CH_2); 3.71 (*s*, MeO); 6.50 (br., NH); 6.63 (br., NH); 7.07–7.13 (br., 2NH); 7.23–7.27 (br., 3 NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.09, 23.38, 23.73, 23.75, 23.77 (Me); 42.09, 42.12, 42.26, 42.27, 42.57, 43.13 (C); 46.73, 47.43, 47.50, 47.61 (CH_2); 52.25 (Me); 170.29, 177.52, 177.61, 177.63, 177.68, 177.93 (C). FAB-MS: 790.4 (22.6, $[M+Na]^+$), 768.4 (100), 439.2 (5.2), 340.2 (7.1), 241.2 (9.7). Anal. calc. for $C_{38}H_{69}N_7O_9$ (768.00): C 59.43, H 9.06, N 12.77; found: C 59.22, H 9.02, N 12.70.

CF$_3$CO-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 (**5**). Heptapeptide **1f** (200 mg, 242 µmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in CH_2Cl_2 (2.4 ml), and treated with DIPEA (166 µl, 970 µmol, 4 equiv.) and TFAA (50 µl, 360 µmol, 1.5 equiv.) according to *GP 4*. FC (CH_2Cl_2/MeOH 25:1) and precipitation from CH_2Cl_2/hexane yielded **5** (168 mg, 204 µmol, 84%). White powder. M.p. 166.0–168.0°. R_f (CH_2Cl_2/MeOH 25:1) 0.08. IR ($CHCl_3$): 3442*w*, 3008*m*, 2971*m*, 2872*w*, 1720*m*, 1647*s*, 1508*s*, 1474*m*, 1394*w*, 1369*w*, 1311*m*, 1160*m*, 986*w*, 892*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.19 (*s*, 12 Me); 1.23 (*s*, 2 Me); 3.29–3.32 (*m*, 5 CH_2); 3.34 (*d*, $^3J(H,NH) = 6.2$, CH_2); 3.40 (*d*, $^3J(H,NH) = 5.9$, CH_2); 3.71 (*s*, MeO); 6.49 (br., NH); 7.12 (br., NH); 7.22–7.26 (br., 4 NH); 7.94 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.09, 23.55, 23.73 (Me); 42.02, 42.06, 42.07, 42.11, 42.26, 43.13 (C); 46.72, 47.43, 47.51, 47.76 (CH_2); 52.25 (Me); 114.62, 117.48, 120.34, 156.88, 157.25, 157.61, 157.98, 177.22, 177.51, 177.62, 177.68, 177.94 (C). FAB-MS: 844.4 (33.2, $[M+Na]^+$), 822.5 (100, $[M+1]^+$), 493.2 (6.6), 394.2 (7.0), 295.1 (7.2). Anal. calc. for $C_{38}H_{66}F_3N_7O_9$ (821.98): C 55.53, H 8.09, N 11.93; found: C 55.40, H 8.09, N 11.82.

CF$_3$CO-β^{2,2}-HAib[^{15}N,^{13}CH$_2$]-β^{2,2}-HAib-β^{2,2}-HAib[^{13}CO]-β^{2,2}-HAib[^{15}N,^{13}CH$_2$]-β^{2,2}-HAib-β^{2,2}-HAib-[^{13}CO]-β^{2,2}-HAib-OMe (**5**). Heptapeptide [1,3,4,6-$^{13}C_4$, 1,4-$^{15}N_2$]-**1f** (50 mg, 60 µmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in CH_2Cl_2 (0.6 ml), and treated with DIPEA (51 µl, 298 µmol, 5 equiv.) and TFAA (12 µl, 90 µmol, 1.5 equiv.) according to *GP 4*. FC (CH_2Cl_2/MeOH 30:1) and precipitation from CH_2Cl_2/hexane yielded [1,3,4,6-$^{13}C_4$, 1,4-$^{15}N_2$]-**5** (45 mg, 54 µmol, 90%). White powder. R_f (CH_2Cl_2/MeOH 30:1) 0.08. IR ($CHCl_3$): 3440*w*, 3007*m*, 2971*m*, 2933*w*, 2872*w*, 1718*m*, 1645*s*, 1509*s*, 1474*s*, 1395*w*, 1369*w*, 1309*m*, 1162*m*, 1044*w*, 990*w*, 887*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.17–1.22 (*m*, 14 Me); 3.28 (br., $^1J(H,^{13}C) = 136.8$, CH_2); 3.38 (*dd*, $^1J(H,^{13}C) = 141.2$, $^3J(H,NH) = 5.6$, CH_2); 3.24–3.34 (*m*, 5 CH_2); 3.70 (*s*, MeO); 6.46 (br., NH); 7.08–7.36 (br., 5 NH); 7.91 (br., $^1J(H,^{15}N) = 93.1$, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.10, 23.74 (Me); 41.81, 41.87, 42.00, 42.05, 42.11, 42.18, 42.24, 42.33, 42.49, 43.14 (C); 46.74, *47.47*, *47.57*, *47.72*, *47.82*, 48.00 (CH_2); 52.25 (Me); 177.22, 177.26, 177.51, 177.66, 177.75, 177.93 (C). HR-MALDI-MS: 850.5 (100, $[M+Na]^+$), 828.4 (2.3), 697.4 (8.1), 597.3 (13.2), 532.4 (2.7), 498.3 (10.7), 432.3 (10.2), 425.7 (2.6), 425.2 (5.8), 397.2 (9.0), 331.2 (14.6), 283.5 (12.8).

CF$_3$CO-β^{2,2}-HAib[^{15}N,^{13}CH$_2$]-β^{2,2}-HAib-β^{2,2}-HAib[^{13}CO]-β^{2,2}-HAib-β^{2,2}-HAib[^{15}N,^{13}CH$_2$]-β^{2,2}-HAib-β^{2,2}-HAib[^{13}CO]-OMe ([1,3,5,7-$^{13}C_4$, 1,5-$^{15}N_2$]-**5**). Heptapeptide [1,3,5,7-$^{13}C_4$, 1,5-$^{15}N_2$]-**1f** (50 mg, 60 µmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in CH_2Cl_2 (0.6 ml), and treated with DIPEA (51 µl, 298 µmol, 5 equiv.) and TFAA (12 µl, 90 µmol, 1.5 equiv.) according to *GP 4*. FC (CH_2Cl_2/MeOH 30:1) and precipitation from CH_2Cl_2/hexane yielded [1,3,5,7-$^{13}C_4$, 1,5-$^{15}N_2$]-**5** (46 mg, 56 µmol, 93%). White powder. R_f (CH_2Cl_2/MeOH 30:1) 0.07. IR ($CHCl_3$): 3442*w*, 3007*m*, 2971*m*, 2933*w*, 2872*w*, 1719*m*, 1646*s*, 1506*s*, 1474*m*, 1395*w*, 1369*w*, 1310*w*, 1167*m*, 985*w*, 885*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.18–1.23 (*m*, 14 Me); 3.29 (*dd*, $^1J(H,^{13}C) = 140.1$, $^3J(H,NH) = 5.2$, CH_2); 3.28–3.36 (*m*, 5 CH_2); 3.39 (*dd*, $^1J(H,^{13}C) = 141.4$, $^3J(H,NH) = 5.8$, CH_2); 3.71 (*d*, $^3J(H,^{13}C) = 3.8$, MeO); 6.48 (br. NH); 7.10–7.35 (br., 5 NH); 7.93 (br., $^1J(H,^{15}N) = 98.2$, 5 NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.09, 23.74 (Me); 41.82, 41.92, 42.06, 42.11, 42.20, 42.27, 42.31, 42.84, 43.40 (C); 46.74, *47.44*, *47.54*, *47.71*, *47.81* (CH_2); 52.23, 52.26 (Me); 83.33 (CH_2); 177.21, 177.36, 177.51, *177.61*, 177.85, *177.93*, 178.21 (C). HR-MALDI-MS: 850.5 (100, $[M+Na]^+$), 828.5 (4.0), 696.4 (7.3), 597.3 (13.7), 532.4 (2.4), 496.3 (5.4), 432.3 (9.1), 425.2 (5.7), 397.2 (3.7), 333.2 (14.0), 283.5 (12.6).

Boc-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-OMe (**6a**). Compound **11c** (280 mg, 0.47 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (0.6 ml), treated with Et_3N (0.5 ml, 3.58 mmol, 7.6 equiv.), HOBt (78 mg, 0.57 mmol, 1.2 equiv.), **13b** (170 mg, 0.49 mmol, 1 equiv.) in $CHCl_3$ (2 ml), and EDC (110 mg, 0.57 mmol, 1.2 equiv.) according to *GP 3*. 2 × FC (CH_2Cl_2/MeOH 18:1) yielded **6a** (197 mg, 0.24 mmol, 51%). Colorless solid. M.p. 214–216°. R_f (CH_2Cl_2/MeOH 18:1) 0.26. $[\alpha]_D^{r.t.} = +3.8$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3356*m*, 2978*m*, 2878*w*, 1717*s*, 1639*s*, 1506*s*, 1456*s*, 1394*w*, 1372*m*, 1344*w*, 1306*w*, 1278*w*, 1244*w*, 1172*m*, 1128*m*, 1117*w*, 1050*w*, 944*w*, 917*w*, 889*w*, 772*w*, 644*w*, 594*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.02–1.16 (*m*, 6 Me); 1.17–1.38 (*m*, 12 Me); 1.42 (*s*, *t*-Bu); 3.52–3.61 (*m*, CH); 3.73 (*s*, MeO); 3.78–3.90 (*m*, 4 CH); 3.93–4.03 (*m*, CH); 6.20 (*d*, ^3J(H,CH) = 9.2, NH); 7.10 (*d*, ^3J(H,CH) = 9.4, NH); 8.18 (*d*, ^3J(H,CH) = 8.4, NH); 8.30 (*d*, ^3J(H,CH) = 8.6, NH); 8.39 (*d*, ^3J(H,CH) = 8.2, NH); 8.40 (*d*, ^3J(H,CH) = 8.2, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 17.0, 17.1, 17.1, 17.2, 17.5, 22.8, 22.8, 22.8, 22.9, 23.0, 23.3, 24.8, 25.0, 25.8, 25.9, 26.0, 26.0, 28.5, 29.3 (Me); 44.0, 44.1, 45.1, 45.4 (C); 51.8 (CH); 52.2 (Me); 52.7, 52.8, 52.9, 54.1 (CH); 78.4, 156.0, 176.0, 176.3, 176.4, 176.4, 177.6 (C). FAB-MS: 848 (21, $[M + K - 2]^+$), 836 (5, $[M + Na + 2]^+$), 835 (18, $[M + Na + 1]^+$), 833 (100, $[M + Na - 1]^+$), 831 (4, $[M + Na - 3]^+$), 814 (4, $[M + 3]^+$), 813 (23, $[M + 2]^+$), 811 (69, $[M]^+$), 809 (3, $[M - 2]^+$), 714 (5, $[M - Boc + 4]^+$), 713 (28, $[M - Boc + 3]^+$), 711 (85, $[M - Boc + 1]^+$). ESI-MS (pos.): 834 (100, $[M + Na]^+$), 835 (46, $[M + Na + 1]^+$).

Boc-(3S)-β²,²,³-HAla(α-Me₂)-(3R)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3R)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3R)-β²,²,³-HAla(α-Me₂)-OMe (**6b**). Compound **14b** (172 mg, 0.29 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (0.5 ml), and treated with Et_3N (0.25 ml, 1.79 mmol, 6.2 equiv.), HOBt (47 mg, 0.35 mmol, 1.2 equiv.), **16a** (101 mg, 0.29 mmol, 1 equiv.) in $CHCl_3$ (1.3 ml) and EDC (68 mg, 0.35 mmol, 1.2 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 24:1) yielded **6b** (152 mg, 0.18 mmol, 64%). Colorless solid. M.p. 244–246° (dec.). R_f (CH_2Cl_2/MeOH 18:0.75) 0.24. $[\alpha]_D^{r.t.} = -0.4$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3359*m*, 2967*m*, 2879*w*, 1711*s*, 1639*s*, 1511*s*, 1462*s*, 1393*w*, 1371*m*, 1366*m*, 1347*w*, 1309*w*, 1281*w*, 1239*w*, 1170*m*, 1127*m*, 1091*w*, 1050*w*, 1002*w*, 945*w*, 918*w*, 890*w*, 776*w*, 677*w*, 605*w*, 534*w*, 463*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.04–1.16 (*m*, 6 Me); 1.18–1.34 (*m*, 12 Me); 1.42 (*s*, *t*-Bu); 3.51–3.61 (*m*, CH); 3.73 (*s*, MeO); 3.78–3.89 (*m*, 4 CH); 3.95–4.03 (*m*, CH); 6.15 (*d*, ^3J(H,CH) = 9.3, NH); 7.11 (*d*, ^3J(H,CH) = 9.4, NH); 8.14 (*d*, ^3J(H,CH) = 8.5, NH); 8.26 (*d*, ^3J(H,CH) = 8.6, NH); 8.32–8.40 (*m*, 2 NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 17.1, 17.2, 17.3, 17.3, 22.7, 22.8, 22.9, 23.0, 23.3, 24.7, 24.8, 25.7, 25.7, 28.5 (Me); 44.2, 44.2, 44.3, 44.3, 45.1, 45.7 (C); 51.7 (CH); 52.1 (Me); 52.8, 52.8, 52.9, 53.0, 54.1 (CH); 78.4, 156.0, 175.9, 176.3, 176.3, 177.6 (C). ESI-MS (pos.): 834 (100, $[M + Na]^+$), 835 (47, $[M + Na + 1]^+$).

Boc-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3R)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-OMe (**6c**). Compound **17** (230 mg, 0.39 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (0.5 ml) and treated with Et_3N (0.3 ml, 2.15 mmol, 5.5 equiv.), HOBt (63 mg, 0.47 mmol, 1.2 equiv.), **13b** (136 mg, 0.39 mmol, 1 equiv.) in $CHCl_3$ (1.7 ml), and EDC (91 mg, 0.47 mmol, 1.2 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 24:1) yielded **6c** (211 mg, 0.26 mmol, 66%). Colorless solid. M.p. 224–225°. R_f (CH_2Cl_2/MeOH 18:1) 0.35. $[\alpha]_D^{r.t.} = +2.9$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3364*m*, 2975*m*, 1713*s*, 1639*s*, 1501*s*, 1392*w*, 1374*m*, 1341*w*, 1171*m*, 1128*m*, 1049*w*, 1004*w*, 945*w*, 920*w*, 890*w*, 775*w*, 670*w*, 605*w*, 458*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.04–1.16 (*m*, 6 Me); 1.18–1.35 (*m*, 12 Me); 1.42 (*s*, *t*-Bu); 3.52–3.62 (*m*, CH); 3.73 (*s*, MeO); 3.78–3.91 (*m*, 4 CH); 3.92–4.03 (*m*, CH); 6.20 (*d*, ^3J(H,CH) = 9.4, NH); 7.10 (*d*, ^3J(H,CH) = 9.4, NH); 8.18 (*d*, ^3J(H,CH) = 8.3, NH); 8.30 (*d*, ^3J(H,CH) = 8.6, NH); 8.32–8.43 (*m*, 2 NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 16.9, 17.0, 17.1, 17.2, 17.4, 17.5, 22.7, 22.8, 22.8, 22.9, 23.0, 23.2, 24.8, 25.0, 25.6, 25.7, 25.8, 26.1, 28.5 (Me); 43.9, 44.1, 44.2, 44.4, 45.1, 45.4 (C); 51.7 (CH); 52.2 (Me); 52.7, 52.8, 52.9, 53.0, 54.0 (CH); 78.4, 156.0, 176.0, 176.3, 176.3, 176.3, 176.4, 177.6 (C). ESI-MS (pos.): 834 (100, $[M + Na]^+$), 835 (47, $[M + Na + 1]^+$).

Boc-(3S)-β²,²,³-HVal(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HLeu(α-Me₂)-(3S)-β²,²,³HVal(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HLeu(α-Me₂)-OMe (**7a**). Compound **25** (101 mg, 0.18 mmol, 1 equiv.), was Boc-deprotected according to *GP 1,* treated with **27** (98 mg, 0.18 mmol, 1 equiv.), HATU (85 mg, 0.22 mmol, 1.2 equiv.) and DIPEA (0.16 ml, 0.93 mmol, 5 equiv.) in $CHCl_3$ (1 ml) according to *GP 6* (reaction time 2.5 days). FC (CH_2Cl_2/MeOH 36:1) yielded **7a** (133 mg, 0.13 mmol, 75%). Colorless solid. $[\alpha]_D^{r.t.} = -17.1$ ($c = 1.0$, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): 0.75 (*d*, ^3J(H,CH) = 6.9, Me); 0.77 (*d*, ^3J(H,CH) = 6.9, Me); 0.84 (*d*, ^3J(H,CH) = 6.6, Me); 0.87–0.97 (*m*, 7 Me); 1.09 (*s*, Me); 1.10 (*s*, Me); 1.13–1.41 (*m*, 10 Me, 2 CH_2); 1.43 (*s*, *t*-Bu); 1.41–1.57 (*m*, 2 CH); 1.90–2.08 (*m*, 2 CH); 3.42 (*dd*, ^3J(H,CH) = 2.7, ^3J(H,NH) = 10.0, CH); 3.71 (*s*, MeO); 3.68–3.82 (*m*, 3 CH); 3.84–3.93 (*m*, CH); 3.96–4.05 (*m*, CH); 6.42 (*d*, ^3J(H,CH) = 10.0, NH); 6.57 (*d*, ^3J(H,CH) = 10.0, NH); 7.77 (*d*, ^3J(H,CH) = 9.6, NH); 8.27–8.36 (*m*, 2 NH); 8.38 (*d*, ^3J(H,CH) = 8.4, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 16.8, 16.8, 17.0, 17.1, 21.5, 21.7, 22.9, 22.9, 23.0, 23.2, 23.3, 23.4, 23.5, 23.9, 24.0 (Me); 24.6, 25.2 (CH);

25.3, 26.5, 26.7, 26.8, 27.6, 28.5 (Me); 29.1, 29.4 (CH); 40.6, 41.3 (CH_2); 52.0 (Me); 53.0, 53.8, 54.8, 61.0, 63.0 (CH); 78.1, 157.0, 176.7, 176.8, 176.9, 177.2, 177.5, 177.6 (C). HR-MALDI-MS (pos.): 976 (1, $[M+Na+2]^+$), 975 (4, $[M+Na+1]^+$), 974 (7, $[M+Na]^+$), 876 (15, $[M-Boc+Na+3]^+$), 875 (52, $[M-Boc+Na+2]^+$), 874 (100, $[M-Boc+Na+1]^+$), 734 (40, $[M-217]^+$), 733 (93, $[M-218]^+$).

TFA · H-(3S)-β²,²,³-HVal(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HLeu(α-Me₂)-(3S)-β²,²,³HVal(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HLeu(α-Me₂)-OH (**7c**). Compound **7a** (90 mg, 94 μmol, 1 equiv.) was dissolved in TFE (1 ml) and treated with 10N NaOH (0.5 ml, 5 mmol, 50 equiv.) according to *GP 2b* (heating at 50°). After 4 d, the mixture was again treated with 10N NaOH (0.5 ml, 5 mmol, 50 equiv.) and heated at 50° for 8 d, until completion of the reaction. Workup gave the carboxylic acid **7b** (88 mg, 93 μmol, 99%), which was Boc-deprotected according *to GP 1*. RP-HPLC Analysis was performed with 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. The crude product was purified by prep. RP-HPLC with a gradient of *A* and *B* (30 to 70% *B* in 20 min) at a flow rate of 20 ml/min. The pure peptide was lyophilized, to yield **7c** (52 mg, 54 μmol, 58%). Voluminous colorless solid. For NMR measurements, see Sect. 4.

Boc-(3S)-β²,²,³-HAla(α-Me₂)-OMe (**11a**). Boc-(3*S*)-β²,³-HAla(α-Me)-OMe (*l*-**8**/*u*-**8**; 7.48 g, 32.3 mmol) was alkylated according to *GP 5*. FC (Et_2O/pentane 1:5) yielded **11a** (5.89 g, 24.0 mmol, 74%). Colorless crystals. M.p. 71–73°. R_f (Et_2O/pentane 1:4) 0.31. $[\alpha]_D^{r.t.} = -3.5$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3311*m*, 2978*m*, 1733*s*, 1694*s*, 1544*s*, 1456*m*, 1394*m*, 1367*m*, 1272*s*, 1178*s*, 1139*s*, 1106*s*, 1056*s*, 1006*m*, 939*w*, 872*m*, 783*w*, 772*w*, 689*w*, 550*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.09 (*d*, ^3J(H,CH) = 6.8, Me); 1.19 (*s*, Me); 1.20 (*s*, Me); 1.44 (*s*, *t*-Bu); 3.69 (*s*, MeO); 3.74–3.84 (*m*, CH); 5.03 (*d*, ^3J(H,CH) = 10.0, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.2, 22.8, 22.9, 28.4 (Me); 46.5 (C); 51.8 (Me); 52.6 (CH); 79.1, 155.6, 177.2 (C). FAB-MS: 513 (7, $[2M+Na]^+$), 491 (5, $[2M+1]^+$), 268 (39, $[M+Na]^+$), 247 (7, $[M+2]^+$), 246 (48, $[M+1]^+$), 190 (100, $[M-55]^+$), 146 (55, $[M-Boc+1]^+$). Anal. calc. for $C_{12}H_{23}NO_4$ (245.32): C 58.75, H 9.45, N 5.71; found: C 58.89, H 9.50, N 5.67.

Boc-(3R)-β²,²,³-HAla(α-Me₂)-OMe (*ent*-**11a**). *Boc-(3R)-β²,³-HAla(α-Me)-OMe (ent-u-***8***/ent-l-***8** (4.29 g, 18.5 mmol) was alkylated according to *GP 5*. FC (Et_2O/pentane 1:5) yielded *ent*-**11a** (3.18 g, 13.0 mmol, 70%). Colorless crystals. M.p. 71–73°. R_f (Et_2O/pentane 1:4) 0.31. $[\alpha]_D^{r.t.} = +3.3$ ($c = 1.0$, $CHCl_3$). NMR Data in agreement with those obtained for **11a**.

Boc-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-OMe (**11b**). Compound **11a** (2.02 g, 8.23 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (9 ml), and treated with Et_3N (4 ml, 28.7 mmol, 3.5 equiv.), HOBt (1.11 g, 8.24 mmol, 1 equiv.), **13a** (1.90 g, 8.21 mmol) in $CHCl_3$ (27 ml), and EDC (1.89 g, 9.89 mmol, 1.2 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 72:1) yielded **11b** (2.70 g, 7.53 mmol, 91%). Colorless oil. R_f (CH_2Cl_2/MeOH 72:1) 0.19. $[\alpha]_D^{r.t.} = -3.3$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3682*w*, 3436*w*, 3036*w*, 3005*m*, 2985*m*, 1703*s*, 1641*m*, 1497*s*, 1456*m*, 1364*m*, 1344*w*, 1277*w*, 1164*s*, 1051*m*, 892*w*, 861*w*, 600*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.08 (*d*, ^3J(H,CH) = 6.8, Me); 1.10 (*d*, ^3J(H,CH) = 6.7, Me); 1.21 (*s*, 2 Me); 1.23 (*s*, 2 Me); 1.43 (*s*, *t*-Bu); 3.58–3.67 (*m*, CH); 3.72 (*s*, MeO); 3.96–4.06 (*m*, CH); 5.88 (br. *d*, ^3J(H,CH) = 9.2, NH); 6.80 (*br. d*, ^3J(H,CH) = 9.1, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 16.9, 17.4, 22.8, 22.9, 24.5, 24.6, 28.4 (Me); 45.5, 45.6 (C); 51.3 (CH); 52.1 (Me); 53.7 (CH); 78.7, 155.9, 176.0, 177.5 (C). FAB-MS: 740 (10, $[2M+Na+1]^+$), 739 (31, $[2M+Na]^+$), 717 (13, $[2M+1]^+$), 382 (11, $[M+Na+1]^+$), 381 (55, $[M+Na]^+$), 360 (20, $[M+2]^+$), 359 (100, $[M+1]^+$), 358 (2, M^+), 357 (3, $[M-1]^+$), 303 (13, $[M-55]^+$), 260 (18, $[M-Boc+3]^+$), 259 (99, $[M-Boc+2]^+$). Anal. calc. for 0.75 $[C_{18}H_{34}N_2O_5]\cdot 0.25\ CHCl_3$ (388.32): C 55.29, H 8.69, N 7.03; found: C 55.73, H 8.49, N 7.03.

Boc-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-OMe (**11c**). Compound **11b** (309 mg, 0.86 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (1 ml), and treated with Et_3N (0.5 ml, 3.58 mmol, 4.2 equiv.), HOBt (140 mg, 1.03 mmol, 1.2 equiv.), **13b** (297 mg, 0.86 mmol, 1 equiv.) in $CHCl_3$ (3 ml), and EDC (199 mg, 1.03 mmol, 1.2 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 36:1) yielded **11c** (401 mg, 0.68 mmol, 80%). Colorless solid. M.p. 137–139°. R_f (CH_2Cl_2/MeOH 36:1) 0.16. $[\alpha]_D^{r.t.} = +1.6$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3433*m*, 3367*m*, 2978*m*, 1717*s*, 1644*s*, 1517*s*, 1494*s*, 1461*m*, 1394*m*, 1378*m*, 1344*m*, 1306*m*, 1278*m*, 1250*m*, 1206*m*, 1178*m*, 1161*m*, 1128*m*, 1100*w*, 1050*m*, 978*w*, 894*w*, 778*w*, 640*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.05–1.14 (*m*, 4 Me); 1.19–1.32 (*m*, 8 Me); 1.42 (*s*, *t*-Bu); 3.52–3.63 (*m*, CH); 3.73 (*s*, MeO); 3.78–3.92 (*m*, 2 CH); 3.94–4.04 (*m*, CH); 6.19 (*d*, ^3J(H,CH) = 9.4, NH); 7.09 (*d*, ^3J(H,CH) = 9.3, NH); 8.15 (*d*, ^3J(H,CH) = 8.3, NH); 8.26 (*d*, ^3J(H,CH) = 8.6, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.0, 17.1, 17.5, 22.8, 22.8, 23.0, 23.2, 24.7, 25.0, 25.8, 26.0, 28.5 (Me); 44.1, 45.1, 45.4 (C); 51.7 (CH); 52.2 (Me); 52.7, 52.7, 54.0 (CH); 78.4, 156.0, 176.0, 176.4, 176.4, 177.6 (C). FAB-MS: 1192 (6, $[2M+Na]^+$), 609 (4, $[M+Na+2]^+$), 608 (19, $[M+Na+1]^+$), 607 (78, $[M+Na]^+$), 587 (6, $[M+3]^+$), 586 (31, $[M+2]^+$), 585 (100, $[M+1]^+$), 584 (1, M^+), 583 (3, $[M-1]^+$), 486 (20, $[M-Boc+3]^+$), 485 (71, $[M-Boc+2]^+$).

Boc-(3S)-β²,²,³-HAla(α-Me₂)-OH (**13a**). Compound **11a** (3.14 g, 12.8 mmol, 1 equiv.) was dissolved in TFE (11 ml) and treated with 10N NaOH (25.6 ml, 256 mmol, 20 equiv.) according to *GP 2b* (reaction time 8 h) to yield **13a** (2.92 g, 12.6 mmol, 99%). Colorless solid. M.p. 165–167° (dec.). $[\alpha]_D^{r.t.} = -2.2$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3300*m*, 3244*m*, 3089*w*, 2978*m*, 2544*w*, 1694*s*, 1644*s*, 1456*m*, 1406*s*, 1367*m*, 1283*m*, 1167*m*, 1144*m*, 1094*m*, 1056*m*, 972*w*, 861*w*, 844*w*, 778*w*, 689*w*, 633*w*, 578*w*, 517*w*, 450*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.16 (*d*, ^3J(H,CH) = 6.8, Me); 1.22 (*s*, Me); 1.25 (br., Me); 1.45 (*s*, *t*-Bu); 3.74–3.85 (*m*, CH); 5.08 (*d*, ^3J(H,CH) = 9.6, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.2, 22.9, 23.1, 28.4 (Me); 46.4 (C); 52.5 (CH); 79.3, 155.7, 182.3 (C). FAB-MS: 485 (15, $[2M + Na]^+$), 463 (25, $[2M + 1]^+$), 254 (45, $[M + Na]^+$), 232 (57, $[M + 1]^+$), 176 (100, $[M - 55]^+$), 132 (24, $[M - Boc + 2]^+$), 130 (21, $[M - Boc]^+$).

Boc-(3S)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-OH (**13b**). Compound **11b** (1.59 g, 4.43 mmol, 1 equiv.) was dissolved in TFE (3.7 ml) and treated with 10N NaOH (8.9 ml, 89 mmol, 20 equiv.) according to *GP 2b* (reaction time: 6.5 h) to yield **13b** (1.57 g, 4.09 mmol, 98%). Colorless solid. $[\alpha]_D^{r.t.} = +1.9$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3411*w*, 2978*m*, 2933*w*, 1700*s*, 1644*s*, 1522*s*, 1456*m*, 1394*m*, 1367*m*, 1250*m*, 1167*s*, 1056*m*, 894*w*, 867*w*, 756*w*, 600*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.05–1.30 (*m*, 6 Me); 1.38–1.52 (*m*, *t*-Bu); 3.60–4.19 (*m*, 2 CH); 5.71–5.89 (*m*, 0.6 NH); 6.45–6.67 (*m*, 0.5 NH); 6.70–6.88 (*m*, 0.6 NH); 7.30–7.40 (*m*, 0.3 NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 16.1, 16.6, 16.9, 17.2, 17.3, 22.6, 22.8, 23.2, 24.0, 24.4, 25.4, 28.3, 28.4, 28.5 (Me); 45.0, 45.4, 45.6, 46.1 (C); 51.1, 51.7, 52.9, 53.5 (CH); 79.0, 81.3, 156.0, 157.9, 175.2, 176.1, 180.8, 182.2 (C). FAB-MS: 711 (11, $[2M + Na]^+$), 368 (17, $[M + Na + 1]^+$), 367 (86, $[M + Na]^+$), 346 (19, $[M + 2]^+$), 345 (100, $[M + 1]^+$), 344 (3, M^+), 343 (7, $[M - 1]^+$), 289 (23, $[M - 55]^+$), 246 (9, $[M - Boc + 3]^+$), 245 (56, $[M - Boc + 2]^+$).

Boc-(3S)-β²,²,³-HAla(α-Me₂)-(3R)-β²,²,³-HAla(α-Me₂)-OMe (**14a**). Compound *ent*-**11a** (884 mg, 3.60 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (4 ml), and treated with Et_3N (1.6 ml, 11.47 mmol, 3.2 equiv.), HOBt (584 mg, 4.32 mmol, 1.2 equiv.), **13a** (833 mg, 3.60 mmol, 1 equiv.) in $CHCl_3$ (18 ml) and EDC (829 mg, 4.32 mmol, 1.2 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 72:1) yielded **14a** (996 mg, 2.77 mmol, 77%). Colorless solid. M.p. 88–90°. R_f (CH_2Cl_2/MeOH 72:1) 0.15. $[\alpha]_D^{r.t.} = +4.8$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3364*m*, 3304*m*, 2984*m*, 1736*s*, 1691*s*, 1638*s*, 1526*s*, 1477*m*, 1458*m*, 1403*w*, 1362*m*, 1272*m*, 1174*m*, 1148*m*, 1121*m*, 1090*m*, 1053*m*, 1028*w*, 1009*w*, 954*w*, 918*w*, 898*w*, 870*w*, 850*w*, 811*w*, 788*w*, 758*w*, 663*w*, 637*w*, 460*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.09 (*d*, ^3J(H,CH) = 6.7, Me); 1.10 (*d*, ^3J(H,CH) = 6.8, Me); 1.20 (*s*, Me); 1.21–1.26 (*m*, 3 Me); 1.43 (*s*, *t*-Bu); 3.56–3.65 (*m*, CH); 3.72 (*s*, MeO); 3.98–4.07 (*m*, CH); 5.88 (br. *d*, ^3J(H,CH) = 9.2, NH); 6.76 (br. *d*, ^3J(H,CH) = 9.1, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 17.1, 17.2, 22.7, 23.2, 24.3, 24.5, 28.5 (Me); 45.4, 45.9 (C); 51.2 (CH); 52.1 (Me); 53.8 (CH); 78.7, 155.9, 175.8, 177.5 (C). FAB-MS: 717 (9, $[2M]^+$), 382 (4, $[M + Na + 1]^+$), 381 (20, $[M + Na]^+$), 360 (22, $[M + 2]^+$), 359 (100, $[M + 1]^+$), 358 (2, M^+), 303 (15, $[M - 55]^+$), 260 (17, $[M - Boc + 3]^+$), 259 (99, $[M - Boc + 2]^+$). Anal. calc. for $C_{18}H_{34}N_2O_5$ (358.47): C 60.31, H 9.56, N 7.81; found: C 60.25, H 9.51, N 7.77.

Boc-(3S)-β²,²,³-HAla(α-Me₂)-(3R)-β²,²,³-HAla(α-Me₂)-(3S)-β²,²,³-HAla(α-Me₂)-(3R)-β²,²,³-HAla(α-Me₂)-OMe (**14b**). Compound **14a** (180 mg, 0.50 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (0.6 ml), and treated with Et_3N (0.35 ml, 2.51 mmol, 5 equiv.), HOBt (81 mg, 0.60 mmol, 1.2 equiv.), **16a** (173 mg, 0.50 mmol, 1 equiv.) in $CHCl_3$ (1.75 ml), and EDC (115 mg, 0.60 mmol, 1.2 equiv.) according to *GP 3*. FC (CH_2Cl_2/MeOH 36:1) yielded **14b** (219 mg, 0.37 mmol, 75%). Colorless solid. M.p. 174–176°. R_f (CH_2Cl_2/MeOH 36:1) 0.17. $[\alpha]_D^{r.t.} = -1.2$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3462*m*, 3368*s*, 2972*s*, 1743*s*, 1713*s*, 1643*s*, 1506*s*, 1391*m*, 1373*m*, 1338*m*, 1313*m*, 1275*m*, 1231*m*, 1157*s*, 1128*m*, 1098*m*, 1048*m*, 1004*w*, 986*w*, 945*w*, 920*w*, 897*w*, 856*w*, 832*w*, 766*w*, 668*m*, 603*m*, 461*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.07 (*d*, ^3J(H,CH) = 6.5, Me); 1.09 (*d*, ^3J(H,CH) = 7.0, Me); 1.10 (*d*, ^3J(H,CH) = 6.7, Me); 1.13 (*d*, ^3J(H,CH) = 6.8, Me); 1.19–1.31 (*m*, 8 Me); 1.42 (*s*, *t*-Bu); 3.51–3.62 (*m*, CH); 3.73 (*s*, MeO); 3.77–3.90 (*m*, 2 CH); 3.94–4.05 (*m*, CH); 6.12 (*d*, ^3J(H,CH) = 9.5, NH); 7.08 (*d*, ^3J(H,CH) = 9.3, NH); 8.08 (*d*, ^3J(H,CH) = 8.4, NH); 8.18 (*d*, ^3J(H,CH) = 8.5, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.1, 17.2, 17.3, 22.7, 22.8, 23.0, 23.3, 24.6, 24.8, 25.7, 25.7, 28.5 (Me); 44.3, 44.4, 45.1, 45.7 (C); 51.6 (CH); 52.1 (Me); 52.7, 52.8, 54.1 (CH); 78.4, 156.0, 175.9, 176.4, 177.6 (C). FAB-MS: 608 (12, $[M + Na + 1]^+$), 607 (39, $[M + Na]^+$), 587 (6, $[M + 3]^+$), 586 (32, $[M + 2]^+$), 585 (100, $[M + 1]^+$), 584 (2, M^+), 583 (3, $[M - 1]^+$), 487 (4, $[M - Boc + 4]^+$), 486 (24, $[M - Boc + 3]^+$), 485 (83, $[M - Boc + 2]^+$), 441 (26, $[M - 143]^+$).

Boc-(3S)-β²,²,³-HAla(α-Me₂)-(3R)-β²,²,³-HAla(α-Me₂)-OH (**16a**). Compound **14a** (593 mg, 1.65 mmol, 1 equiv.) was dissolved in TFE (1.5 ml) and treated with 10N NaOH (3.3 ml, 33 mmol, 20 equiv.) according to *GP 2b* (reaction time: 2.5 h) to yield **16a** (542 mg, 1.57 mmol, 95%). Colorless solid. M.p. 146–149°. $[\alpha]_D^{r.t.} = +4.6$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3430*m*, 3315*w*, 2982*s*, 1740*s*, 1666*s*, 1638*s*, 1535*s*, 1476*w*, 1449*w*, 1400*w*, 1365*m*, 1320*w*, 1276*m*, 1255*w*, 1225*m*, 1156*s*, 1095*w*, 1059*m*, 921*w*, 894*w*, 864*w*, 805*w*, 786*w*, 740*w*, 721*w*, 632*w*, 584*w*, 451*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.01–1.31 (*m*, 6 Me); 1.36–1.55 (*m*, *t*-Bu); 3.56–3.70 (*m*, 0.67 CH); 3.91–4.14 (*m*, 1.33 CH); 5.83 (*d*, ^3J(H,CH) = 8.8, 0.67 NH); 6.27–6.42 (*m*, 0.33 NH); 6.73 (*d*, ^3J(H,CH) = 8.2, 0.67 NH); 7.10–7.21 (*m*, 0.33 NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.1, 22.8, 23.0, 24.1, 24.3, 28.4 (Me); 45.5, 45.6 (C); 51.0, 53.7

(CH); 78.9, 156.0, 176.0, 180.9 (C). FAB-MS: 712 (5, $[2M+Na+1]^+$), 711 (15, $[2M+Na]^+$), 689 (6, $[2M]^+$), 368 (9, $[M+Na+1]^+$), 367 (45, $[M+Na]^+$), 346 (21, $[M+2]^+$), 345 (85, $[M+1]^+$), 344 (2, M^+), 343 (3, $[M-1]^+$), 289 (25, $[M-55]^+$), 246 (17, $[M-Boc+3]^+$), 245 (100, $[M-Boc+2]^+$), 243 (6, $[M-Boc]^+$).

*Boc-(3*S*)-β^{2,2,3}-HAla(α-Me$_2$)-(3*R*)-β^{2,2,3}-HAla(α-Me$_2$)-(3*S*)-β^{2,2,3}-HAla(α-Me$_2$)-(3*S*)-β^{2,2,3}-HAla(α-Me$_2$)-OMe* (**17**). Compound **11b** (310 mg, 0.86 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (1 ml), and treated with Et_3N (0.6 ml, 4.29 mmol, 5 equiv.), HOBt (50 mg, 0.36 mmol, 0.4 equiv.), **16a** (297 mg, 0.86 mmol, 1 equiv.) in $CHCl_3$ (3 ml) and EDC (199 mg, 1.03 mmol, 1.2 equiv.) according to *GP 3*. 2 × FC (CH_2Cl_2/MeOH 36:1) yielded **17** (302 mg, 0.51 mmol, 60%). Colorless solid. M.p. 168–170°. R_f (CH_2Cl_2/MeOH 36:1) 0.18. $[\alpha]_D^{r.t.} = -3.5$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3390*m*, 2976*m*, 1712*s*, 1643*s*, 1508*s*, 1457*s*, 1392*m*, 1374*m*, 1338*m*, 1312*m*, 1271*m*, 1171*m*, 1129*m*, 1098*w*, 1050*m*, 1002*w*, 948*w*, 920*w*, 898*w*, 774*w*, 651*w*, 604*w*, 451*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.04–1.14 (*m*, 4 Me); 1.19–1.31 (*m*, 8 Me); 1.42 (*s*, *t*-Bu); 3.52–3.62 (*m*, CH); 3.73 (*s*, MeO); 3.78–3.91 (*m*, 2 CH); 3.94–4.04 (*m*, CH); 6.12 (*d*, ^3J(H,CH) = 9.3, NH); 7.07 (*d*, ^3J(H,CH) = 9.2, NH); 8.09 (*d*, ^3J(H,CH) = 8.6, NH); 8.22 (*d*, ^3J(H,CH) = 8.7, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.0, 17.1, 17.3, 17.4, 22.7, 22.8, 23.0, 23.3, 24.8, 25.0, 25.6, 25.7, 28.5 (Me); 44.3, 44.5, 45.1, 45.4 (C); 51.7 (CH); 52.1 (Me); 52.7, 54.0 (CH); 78.4, 156.0, 175.9, 176.3, 176.5, 177.6 (C). FAB-MS: 608 (7, $[M+Na+1]^+$), 607 (22, $[M+Na]^+$), 587 (7, $[M+3]^+$), 586 (33, $[M+2]^+$), 585 (100, $[M+1]^+$), 584 (2, M^+), 583 (3, $[M-1]^+$), 486 (19, $[M-Boc+3]^+$), 485 (65, $[M-Boc+2]^+$).

*Boc-(3*S*)-β^{2,2,3}-HVal(α-Me$_2$)-OMe* (**19**). Amino acid derivative **9** (2.19 g, 8.44 mmol) was alkylated according to *GP 5*. FC (Et_2O/pentane 1:4) and (CH_2Cl_2/Et_2O 30:1) yielded **19** (1.82 g, 6.65 mmol, 79%). Colorless oil. R_f (CH_2Cl_2/Et_2O 30:1) 0.35. $[\alpha]_D^{r.t.} = -9.6$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3446*w*, 2974*m*, 1708*s*, 1503*s*, 1390*m*, 1369*m*, 1318*w*, 1159*s*, 1092*w*, 1026*w*, 985*w*, 903*w*, 867*w*, 826*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 2 rotamers (ratio 9:1) 0.72 (*d*, ^3J(H,CH) = 6.8, Me); 0.75 (*d*, ^3J(H,CH) = 6.8, Me); 0.90 (*d*, ^3J(H,CH) = 6.8, Me); 0.91 (*d*, ^3J(H,CH) = 6.8, Me); 1.18 (*s*, Me); 1.19 (*s*, Me); 1.20 (*s*, Me); 1.21 (*s*, Me); 1.44 (*s*, *t*-Bu); 1.46 (*s*, *t*-Bu); 1.79–1.97 (*m*, CH + CH); 3.52 (*dd*, ^3J(H,CH) = 3.8, ^3J(H,NH) = 10.9, CHN); 3.61 (*dd*, ^3J(H,CH) = 3.7, ^3J(H,NH) = 10.8, CHN); 3.67 (*s*, MeO); 4.79 (*d*, ^3J(H,CH) = 10.0, NH); 5.17 (*d*, ^3J(H,CH) = 10.6, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 16.9, 22.1, 23.1, 23.8, 24.0, 24.3, 28.4, 28.4 (Me); 29.2, 29.5 (CH); 45.6 (C); 51.8 (Me); 61.5, 62.8 (CH); 78.9, 79.7, 156.6, 177.8 (C). FAB-MS: 547 (27, $[2M+1]^+$), 296 (6, $[M+Na]^+$), 275 (10, $[M+2]^+$), 274 (58, $[M+1]^+$), 218 (100, $[M-55]^+$), 174 (39, $[M-Boc+2]^+$). Anal. calc. for $C_{14}H_{27}NO_4$ (273.37): C 61.51, H 9.95, N 5.12; found: C 61.36, H 9.92, N 5.03.

*Boc-(3*S*)-β^{2,2,3}-HVal(α-Me$_2$)-OH* (**20**). Compound **19** (1.14 g, 4.17 mmol, 1 equiv.) was dissolved in TFE (3.5 ml) and treated with 10N NaOH (12.6 ml, 126 mmol, 30 equiv.) according to *GP 2b* (reaction time: 24 h) to yield **20** (1.06 g, 4.09 mmol, 98%). Colorless solid. M.p. 161–163°. $[\alpha]_D^{r.t.} = -14.6$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3299*s*, 2979*s*, 2658*w*, 1690*s*, 1674*s*, 1539*s*, 1478*m*, 1458*m*, 1406*m*, 1389*m*, 1367*m*, 1349*w*, 1297*s*, 1249*s*, 1174*s*, 1120*w*, 1040*w*, 1018*m*, 986*m*, 947*w*, 880*m*, 782*w*, 764*w*, 730*w*, 677*w*, 657*w*, 582*w*, 548*w*, 438*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 2 rotamers (ratio 85:15) 0.80 (*d*, ^3J(H,CH) = 6.8, Me); 0.86 (*d*, ^3J(H,CH) = 6.5, Me); 0.94 (*d*, ^3J(H,CH) = 6.8, Me); 1.18–1.28 (*m*, 2 Me); 1.42–1.50 (*m*, *t*-Bu); 1.80–1.92 (*m*, CH); 1.92–2.04 (*m*, CH); 3.63 (*dd*, ^3J(H,CH) = 3.5, ^3J(H,NH) = 10.8, CHN); 5.22 (*d*, ^3J(H,CH) = 10.7, NH); 5.70 (*d*, ^3J(H,CH) = 10.7, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.2, 15.6, 16.8, 17.6, 22.2, 23.6, 24.2, 28.4 (Me); 29.2, 29.9 (CH); 45.4 (C); 61.3, 62.3 (CH); 79.1, 80.4, 156.6, 183.3 (C). FAB-MS: 541 (4, $[2M+Na]^+$), 519 (12, $[2M+1]^+$), 282 (17, $[M+Na]^+$), 260 (32, $[M+1]^+$), 204 (100, $[M-55]^+$), 160 (19, $[M-Boc+2]^+$).

*Boc-(3*S*)-β^{2,2,3}-HLeu(α-Me$_2$)-OMe* (**21**). Amino acid derivative **10** (682 mg, 2.49 mmol) was alkylated according to *GP 5*. FC (Et_2O/pentane 1:6) yielded **21** (542 mg, 1.88 mmol, 75%). Colorless oil. R_f (Et_2O/pentane 1:6) 0.35. $[\alpha]_D^{r.t.} = -25.7$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3443*w*, 2956*m*, 2870*w*, 1710*s*, 1504*s*, 1470*w*, 1435*w*, 1392*m*, 1368*m*, 1292*w*, 1162*s*, 1111*w*, 1049*w*, 1005*w*, 880*w*, 658*w*, 626*w*, 600*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 2 rotamers (ratio 85:15) 0.89 (*d*, ^3J(H,CH) = 6.7, Me); 0.92 (*d*, ^3J(H,CH) = 6.5, Me); 1.17 (*s*, Me); 1.19 (*s*, Me); 1.13–1.22 (*m*, CH_2); 1.43 (*s*, *t*-Bu); 1.47 (*s*, *t*-Bu); 1.58–1.70 (*m*, CH); 3.68 (*s*, MeO); 3.72–3.80 (*m*, CHN); 4.39 (*d*, ^3J(H,CH) = 11.0, NH); 4.73 (*d*, ^3J(H,CH) = 10.5, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 21.0, 21.4, 22.2, 23.1, 23.6, 23.9, 24.8 (Me); 25.2 (CH); 28.4 (Me); 40.8, 41.2 (CH_2); 46.8 (C); 51.7 (Me); 55.0, 56.1 (CH); 78.9, 79.7, 156.0, 177.0, 177.3 (C). FAB-MS: 597 (6, $[2M+Na]^+$), 575 (10, $[2M+1]^+$), 310 (29, $[M+Na]^+$), 289 (13, $[M+2]^+$), 288 (50, $[M+1]^+$), 233 (20, $[M-54]^+$), 232 (100, $[M-55]^+$), 188 (68, $[M-Boc+2]^+$), 186 (38, $[M-Boc]^+$). Anal. calc. for $C_{15}H_{29}NO_4$ (287.40): C 62.69, H 10.17, N 4.87; found: C 62.53, H 9.97, N 4.67.

*Boc-(3*S*)-β^{2,2,3}-HVal(α-Me$_2$)-(3*S*)-β^{2,2,3}-HAla(α-Me$_2$)-OMe* (**23**). Compound **11a** (588 mg, 2.39 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, dissolved in $CHCl_3$ (5.5 ml), and treated with Et_3N (1.1 ml, 7.89 mmol, 3.3 equiv.), HOBt (389 mg, 2.87 mmol, 1.2 equiv.), **20** (622 mg, 2.39 mmol, 1 equiv.) in DMF (5.5 ml), and EDC (552 mg, 2.87 mmol, 1.2 equiv.) according to *GP 3*. 2 × FC (CH_2Cl_2/MeOH 72:1) yielded **23** (696 mg, 1.80 mmol, 75%). Colorless oil. R_f (CH_2Cl_2/MeOH 72:1) 0.19. $[\alpha]_D^{r.t.} = -2.8$ ($c = 1.0$, $CHCl_3$). IR

($CHCl_3$): 3422*m*, 2978*m*, 1702*s*, 1643*m*, 1495*s*, 1392*m*, 1366*m*, 1318*w*, 1162*s*, 1090*m*, 1040*w*, 1021*w*, 981*w*, 911*w*, 862*w*, 657*w*, 650*w*, 606*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.77 (*d*, ^3J(H,CH) = 6.8, Me); 0.95 (*d*, ^3J(H,CH) = 6.8, Me); 1.07 (*d*, ^3J(H,CH) = 6.7, Me); 1.22 (*s*, Me); 1.23 (*s*, 2 Me); 1.25 (*s*, Me); 1.44 (*s*, *t*-Bu); 1.86 – 1.99 (*m*, CH); 3.46 (*dd*, ^3J(H,CH) = 2.9, ^3J(H,NH) = 10.2, CH); 3.73 (*s*, MeO); 3.87 – 3.98 (*m*, CH); 6.10 (br. *d*, ^3J(H,CH) = 10.2, NH); 6.93 (br. *d*, ^3J(H,CH) = 9.1, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 16.9, 17.0, 22.8, 22.9, 23.4, 24.8, 26.2, 28.5 (Me); 29.2 (CH); 44.7, 45.6 (C); 51.7 (CH); 52.1 (Me); 62.6 (CH); 78.4, 156.9, 176.8, 177.5 (C). FAB-MS: 773 (9, $[2M+1]^+$), 410 (6, $[M+Na+1]^+$), 409 (28, $[M+Na]^+$), 389 (5, $[M+3]^+$), 388 (31, $[M+2]^+$), 387 (100, $[M+1]^+$), 386 (3, M^+), 385 (5, $[M-1]^+$), 331 (11, $[M-55]^+$), 289 (4, $[M-Boc+4]^+$), 288 (19, $[M-Boc+3]^+$), 287 (76, $[M-Boc+2]^+$). Anal. calc. for $C_{20}H_{38}N_2O_5$ (386.51): C 62.15, H 9.91, N 7.25; found: C 62.29, H 9.80, N 7.21.

*Boc-(3*S*)-β^{2,2,3}-HVal(α-Me$_2$)-(3*S*)-β^{2,2,3}-HAla(α-Me$_2$)-OH* (**24**). Compound **23** (668 mg, 1.72 mmol, 1 equiv.) was dissolved in TFE (1.5 ml) and treated with 10N NaOH (3.45 ml, 34.5 mmol, 20 equiv.) according to *GP 2b* (reaction time: 10 h) to yield **24** (640 mg, 1.71 mmol, 99%). Slightly yellow oil. $[\alpha]_D^{r.t.} = -1.8$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3428*m*, 2979*m*, 1694*s*, 1641*m*, 1496*s*, 1392*m*, 1367*m*, 1168*s*, 1123*w*, 1089*m*, 1040*w*, 1022*w*, 998*w*, 909*w*, 843*w*, 637*w*, 600*w*. ^{1}H-NMR (500 MHz, $CDCl_3$): 2 rotamers (ratio 50:50) 0.78 (*d*, ^3J(H,CH) = 6.8, Me); 0.87 (*d*, ^3J(H,CH) = 6.7, Me); 0.94 (*d*, ^3J(H,CH) = 6.8, Me); 0.98 (*d*, ^3J(H,CH) = 6.8, Me); 1.11 (*d*, ^3J(H,CH) = 6.7, Me); 1.13 (*d*, ^3J(H,CH) = 6.7, Me); 1.19 – 1.27 (*m*, 8 Me); 1.44 (*s*, *t*-Bu); 1.46 (*s*, *t*-Bu); 1.87 – 1.98 (*m*, CH); 3.49 (*dd*, ^3J(H,CH) = 2.9, ^3J(H,NH) = 10.3, 0.5 CHN); 3.88 – 4.01 (*m*, 1.5 CHN); 6.04 (*d*, ^3J(H,CH) = 10.1, 0.5 NH); 6.73 – 6.83 (*m*, 0.5 NH); 6.93 (*d*, ^3J(H,CH) = 9.3, 0.5 NH); 7.39 (*d*, ^3J(H,CH) = 9.5, 0.5 NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 16.8, 17.1, 18.1, 22.9, 22.9, 23.3, 23.3, 23.7, 23.9, 24.7, 25.6, 25.9, 28.3, 28.5 (Me); 29.2 (CH); 44.9, 45.0, 45.3, 47.1 (C); 51.5, 51.7, 61.3, 62.4 (CH); 78.7, 81.2, 157.0, 158.7, 175.3, 176.8, 180.6, 182.3 (C). FAB-MS: 746 (4, $[2M+2]^+$), 745 (11, $[2M+1]^+$), 396 (8, $[M+Na+1]^+$), 395 (32, $[M+Na]^+$), 375 (5, $[M+3]^+$), 374 (32, $[M+2]^+$), 373 (100, $[M+1]^+$), 372 (3, $[M]^+$), 317 (19, $[M-55]^+$), 274 (22, $[M-Boc+3]^+$), 273 (99, $[M-Boc+2]^+$), 271 (5, $[M-Boc]^+$).

*Boc-(3*S*)-β^{2,2,3}-HVal(α-Me$_2$)-(3*S*)-β^{2,2,3}-HAla(α-Me$_2$)-(3*S*)-β^{2,2,3}-HLeu(α-Me$_2$)-OMe* (**25**). Compound **21** (332 mg, 1.15 mmol, 1 equiv.) was Boc-deprotected according to *GP 1*, treated with **24** (431 mg, 1.15 mmol, 1 equiv.), HATU (528 mg, 1.38 mmol, 1.2 equiv.), and DIPEA (0.99 ml, 5.78 mmol, 5 equiv.) in $CHCl_3$ (2.5 ml) according to *GP 6* (reaction time 2.5 d). FC (CH_2Cl_2/MeOH 36:1) yielded **25** (493 mg, 0.90 mmol, 79%). Colorless solid. $[\alpha]_D^{r.t.} = -17.1$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3456*m*, 3366*m*, 2972*s*, 1716*s*, 1646*s*, 1498*s*, 1391*m*, 1365*s*, 1315*m*, 1261*m*, 1175*s*, 1086*m*, 1039*w*, 1023*w*, 1001*w*, 933*w*, 911*w*, 862*w*, 772*w*, 624*w*, 454*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.77 (*d*, ^3J(H,CH) = 6.8, Me); 0.90 (*d*, ^3J(H,CH) = 6.8, Me); 0.91 (*d*, ^3J(H,CH) = 6.6, Me); 0.94 (*d*, ^3J(H,CH) = 6.8, Me); 1.08 (*d*, ^3J(H,CH) = 6.6, Me); 1.17 – 1.36 (*m*, 2 CH); 1.20 – 1.30 (*m*, 6 Me); 1.44 (*s*, *t*-Bu); 1.40 – 1.54 (*m*, CH); 1.90 – 2.01 (*m*, CH); 3.43 (*dd*, ^3J(H,CH) = 2.8, ^3J(H,NH) = 10.1, CH); 3.71 (*s*, MeO); 3.73 – 3.82 (*m*, CH); 3.97 – 4.06 (*m*, CH); 6.32 (br. *d*, ^3J(H,CH) = 10.0, NH); 6.51 (br. *d*, ^3J(H,CH) = 10.0, NH); 8.14 (br. *d*, ^3J(H,CH) = 8.3, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 16.9, 17.0, 21.6, 22.9, 23.0, 23.3, 23.5, 23.9, 24.5 (Me); 25.3 (CH); 26.4, 26.5, 28.5 (Me); 29.2 (CH); 40.6 (CH_2); 52.0 (Me); 52.8, 53.6, 62.9 (CH); 78.2, 157.0, 176.8, 176.9, 177.6 (C). HR-MALDI-MS (pos.): 581 (4, $[M+K+1]^+$), 580 (12, $[M+K]^+$), 565 (12, $[M+Na+1]^+$), 564 (38, $[M+Na]^+$), 443 (8, $[M-Boc+3]^+$), 442 (33, $[M-Boc+2]^+$), 413 (12, $[M-128]^+$), 394 (11, $[M-147]^+$), 371 (61, $[M-170]^+$). Anal. calc. for $C_{29}H_{55}N_3O_6$ (541.77): C 64.29, H 10.23, N 7.76; found: C 64.06, H 10.04, N 7.81.

*Boc-(3*S*)-β^{2,2,3}-HVal(α-Me$_2$)-(3*S*)-β^{2,2,3}-HAla(α-Me$_2$)-(3*S*)-β^{2,2,3}-HLeu(α-Me$_2$)-OH* (**27**). Compound **25** (267 mg, 0.49 mmol, 1 equiv.) was dissolved in TFE (0.7 ml) and treated with 10N NaOH (0.99 ml, 9.9 mmol, 20 equiv.) according to *GP 2b* (reaction time 24 h) to yield **27** (258 mg, 0.48 mmol, 99%). Colorless solid.

9. *Preparation of the Ac- and CF$_3$CO-Peptide Mixtures.* Mixtures for the melting curve (*Fig. 6*): 6 mg of a mixture **4**/**5** in the desired ratio were dissolved in 0.4 ml of CH_2Cl_2 and 0.6 ml hexane. The solvent was allowed to evaporate under laboratory conditions (several days) to yield a white powder, which was dried under h.v. Mixtures (solid solutions) for TEDOR-REDOR-NMR measurements: the appropriately labelled CF_3CO-heptapeptide **5** was mixed in the desired ratio with **4** and dissolved in CH_2Cl_2; pentane was added to generate a suspension, which was immediately evaporated to dryness (10 Torr); the residue was then put under h.v. to yield a white powder.

10. *NMR Measurements in Solution: NMR Spectroscopy of β-hexapeptide* **7c** *in MeOH* Sample: 7 mg were dissolved in 0.6 ml of CD_3OH. 1D-NMR (DRX500): ^{1}H-NMR (500 MHz): suppression of the $CD_3O\mathit{H}$ signal by presaturation; 90-K data points, 128 scans, 5.6-s acquisition time. {^{1}H}-BB-decoupled-^{13}C-NMR (125 MHz): 80-K data points, 20-K scans, 1.3-s acquisition time, 1-s relax. delay 45° excitation pulse. Processed with 1.0-Hz exponential line broadening. 2D-NMR: All with solvent suppression by presat. DQF.COSY (500 MHz, CD_3OH) with pulsed field gradients (PFG) for coherence pathway selection [37]: *Acquisition:* 2K(t_2) × 512 (t_1)

data points. 10 scans per t_1 increment, 0.17-s acquisition time in t_2; relaxation delay 2.0-s. TPPI quadrature detection in w_1. *Processing:* zero filling and FT to 1K × 1K real/real datapoints after multiplication with $\sin^2$ filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. HSQC with PFG [38] (500, 125 MHz, CD_3OH): *Acquisition:* 2K(t_2) × 512 (t_1) data points, 48 scans per t_1 increment. ^{13}C-GARP decoupling during t_2. 0.17-s acquisition time in t_2. *Processing:* zero filling and FT to 1K × 1K real/real data points after multiplication with $\sin^2$ filter shifted by $\pi/2$ in ω_2 and sin filter shifted by $\pi/2$ in ω_1. HMBC with PFG [39] (500, 125 MHz, CD_3OH): *Acquisition:* delay for evolution of long-range antiphase magn. 50 ms. No ^{13}C-decoupling, otherwise identical to parameters for HSQC. *Processing:* zero filling and FT to 1K × 1K after multiplication with $\cos^2$ filter in ω_2 and gaussian filter in ω_1; power spectrum in both dimensions. ROESY [40] (500 MHz, CD_3OH; see *Table 2)*. *Acquisition:* 2 ROESY spectra with mixing times of 100 and 300 ms were acquired. CW-spin lock (2.7 kHz) between trim pulses, 2K(t_2) × 512 (t_1) data points, 64 scans per t_1 increment. 0.17-s acquisition time in t_2, other parameters identical to DQF.COSY. *Processing:* zero filling and FT to 1K × 512K real/real datapoints after multiplication by $\cos^2$ filter in ω_2 and ω_1. Baseline correction with 3rd-degree polynomial in both dimensions.

11. *MAS Double-Resonance Measurements.* All spectra were recorded on a *Bruker Avance-500* spectrometer equipped with a *Bruker* 4-mm outer diameter magic-angle spinning (MAS) double-resonance probe. All measurements were carried out on a sample of fully ^{13}C/^{15}N-labelled hexapeptide, which was diluted 1:10 in natural-abundance hexapeptide to avoid intermolecular contacts. To obtain the values and the orientations of the ^{15}N chemical-shielding tensor, 1D-NMR spectra were recorded at a spinning frequency of 1 kHz. The spectra were fitted by means of the GAMMA simulation environment. The ^{15}N chemical-shielding tensor was found to be axially symmetric with $\Delta\sigma = -146 \pm 2$ ppm. The orientation of the principal-axis system was assumed such that the symmetry axis of the tensor was aligned along the N–H bond vector [41][42], and the bond geometry was assumed to be planar with angles of 120° between the bonds. The principal values of the C=O chemical shielding tensor were found to be $\sigma_{xx} = 70 \pm 1$, $\sigma_{yy} = 10 \pm 0.5$, and $\sigma_{zz} = -80 \pm 1.5$ ppm. The tensor was assumed to be aligned such that the σ_{zz} axis points out of the peptide plane and the σ_{yy} axis points along the C=O bond. It was found that a deviation of the σ_{yy} axis from the C=O bond direction [41] did not change the tensor values significantly. Two-dimensional exchange spectra [24] were recorded by cross polarization from the protons to enhance the initial polarization. The mixing time in the exchange spectra was implemented rotor synchronized allowing pure absorption mode peaks [43] and with proton-driven spin diffusion as a transfer mechanism.

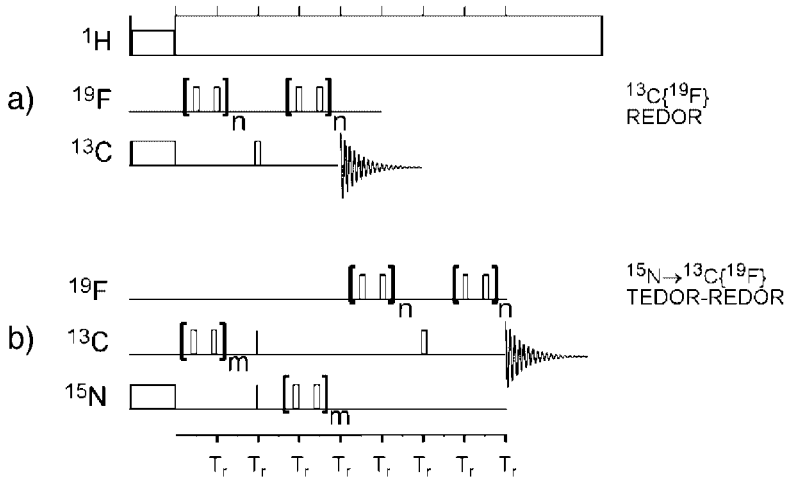


Fig. 19. *Outline of the pulse sequences for $^{13}C\{^{19}F\}$-REDOR* (*a*) *and $^{15}N \rightarrow {}^{13}C\{^{19}F\}$-TEDOR-REDOR* (*b*), *initiated by a cross-polarization transfer from the protons.* Only a single ^{13}C-refocusing π pulse was used in both sequences to minimize possible interference from ^{13}C, ^{13}C couplings in a multi-labelled system. The coherence transfer in the TEDOR part of the bottom sequence was achieved after *m* rotor cycles with coincident ^{15}N and ^{13}C $\pi/2$ pulses. Timing of the ^{1}H pulses is the same in both sequences.

12. *REDOR and TEDOR-REDOR Measurements.* REDOR was used to restore the dipolar coupling between heteronuclear pairs of spins that is removed by magic-angle spinning [44]. REDOR experiments are always done in two parts, once with rotor-synchronized dephasing pulses (S) and once without (S_0). The dephasing pulses (*Fig. 19,a*) change the sign of the heteronuclear dipolar coupling, and this interferes with the spatial averaging resulting from the motion of the rotor. The difference in signal intensity ($\Delta S = S_0 - S$) for the observed spin in the two parts of the REDOR experiment is directly related to the corresponding distance to the dephasing spin [45].

In an $^{15}N \rightarrow {}^{13}C$ TEDOR experiment, nonobservable bilinear coherence is developed from ^{15}N magnetization (initially transferred from protons) by means of ^{13}C-REDOR pulses (*Fig. 7,b*). This coherence is transferred from the ^{15}N channel to the ^{13}C channel by coincident $\pi/2$ pulses and then developed into observable ^{13}C magnetization by ^{15}N REDOR pulses [46]. This process allows a selective observation of signals from ^{13}C labels that are directly bonded to ^{15}N labels. TEDOR was used as a filter for $^{13}C-{}^{19}F$ REDOR distance measurements with no interference from the natural abundance background [47].

REDOR and TEDOR-REDOR were performed with a 4-frequency transmission-line probe [48] having a 14-mm long, 9-mm inside-diameter anal. coil, and a *Chemagnetics/Varian* stator and spinner housing. Powdered samples were contained in *Chemagnetics/Varian* 7.5-mm outside-diameter zirconia rotors. The rotors were spun at 5000 Hz with the speed under active control to within ± 2 Hz. Experiments were done with a 4.7-Tesla magnet (200 MHz for protons) and a *Tecmag* pulse programmer. Radiofrequency pulses were produced by *Kalmus* and *American Microwave Technology* power amplifiers. The π-pulse lengths were 10 μs for ^{13}C, ^{15}N, and ^{19}F. Standard XY-8 phase cycling [49] was used for all dephasing pulses. C,H cross-polarization transfers were made in 2 ms at 50 kHz. Proton dipolar decoupling was 100 kHz during data acquisition. The ^{13}C chemical-shift scale was referenced relative to external TMS. The accuracy of distance measurements with ^{19}F dephasing pulses was confirmed by the two-bond coupling of [^{19}F]polycarbonate as described in [50]. Calculations of the dephasing by CF_3 groups took orientational effects into account [51].

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Received August 5, 2002