Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 9191

www.rsc.org/obc

PAPER

Synthesis of C-linked carbo-β^2-amino acids and β^2-peptides: design of new motifs for left-handed 12/10- and 10/12-mixed helices†

Gangavaram V. M. Sharma,*[a] Nelli Yella Reddy,‡[a] Rapolu Ravi,‡[b] Bommagani Sreenivas,[a] Gattu Sridhar,[a] Deepak Chatterjee,[b] Ajit C. Kunwar*[b] and Hans-Jörg Hofmann*[c]

Received 15th August 2012, Accepted 2nd October 2012
DOI: 10.1039/c2ob26615f

C-linked carbo-β^2-amino acids (β^2-Caa), a new class of β-amino acid with a carbohydrate side chain having D-*xylo* configuration, were prepared from D-glucose. The main idea behind the design of the new β-amino acids was to move the steric strain of the bulky carbohydrate side chain from the Cβ- to the Cα-carbon atom and to explore its influence on the folding propensities in peptides with alternating (*R*)- and (*S*)-β^2-Caas. The tetra- and hexapeptides derived were studied employing NMR (in $CDCl_3$), CD, and molecular dynamics simulations. The β^2-peptides of the present study form left-handed 12/10- and 10/12-mixed helices independent of the order of the alternating chiral amino acids in the sequence and result in a new motif. These results differ from earlier findings on β^3-peptides of the same design, containing a carbohydrate side chain with D-*xylo* configuration, which form exclusively right-handed 12/10-mixed helices. Quantum chemical calculations employing *ab initio* MO theory suggest the side chain chirality as an important factor for the observed definite left- or right-handedness of the helices in the β^2- and β^3-peptides.

Introduction

The research activity on β-peptides has grown immensely since the appearance of the first reports by Gellman *et al.*[1] and Seebach *et al.*,[2] leading to the emergence of the area of foldamers.[3] Meanwhile, a large number of ω-amino acids and their oligomers have been designed to realize a wide variety of secondary structures.[3,4] In the β-peptide domain, oligomers of β^3-amino acids have been extensively studied,[3] whereas β^2-peptides derived from β^2-amino acids[5] have received less attention.[6] Interestingly, a few β^2-amino acids form partial structures of natural products[7] and are known to exhibit diverse biological activities. The natural occurrence of β^2-hAla[8a] as an exclusive component of rare natural peptides was established in 1951.[8b,c] Some unusual motifs[9] have been generated in β^2-peptides. One of the most interesting and unique helical structures, the 12/10-mixed helix,[10,11] was first observed in β-peptides having a backbone with alternating β^2- and β^3-residues. Similarly, a hairpin turn[12] was observed in peptides containing β^2-amino acids. Seebach *et al.*[6a] reported that homochiral β^2-peptides with proteinogenic side chains adopt a right-handed 14-helix, in contrast to the left-handed 14-helix observed in β^3-peptides.[2] Seebach's group have also used β^2-amino acids in mixed peptides to design 9- and 10-membered (mr) H-bonded turns.[13] Kaur *et al.*[14] synthesized β^2-peptides using 2,3-diamino propionic acid, whose CD spectra suggested the presence of solvent-dependent secondary structures, while the hydrazino peptides reported by Seebach and Lelais[15] did not show secondary structures. Recently, Mikata *et al.*[16a] reported the synthesis of and conformational studies on β^2-peptides from C-glycosyl β^2-amino acids.[16b]

Earlier we reported[11b] right-handed 12/10- (10/12-) helices in peptides from alternating (*R*)- and (*S*)-C-linked carbo β^3-amino acids (β^3-Caa; epimeric at Cβ),[17] while, both the right- and left-handed 12/10- (10/12-) helices[18] were observed in peptides from (*R*)- and (*S*)-β-Caas alternating with β-hGly (β-homo glycine), respectively. The backbone flexibility available in β-hGly was found to be responsible for the 'switch' in the handedness,[18] dictated by the epimeric β^3-Caas. Furthermore, the mass spectral studies[19] on the β^3-dipeptides, derived from epimeric β^3-Caas, revealed a steric strain at the Cβ-carbon due to the presence of the carbohydrate side chain. The results described above, in particular the switch in handedness of the 14-helix[2] in homo-oligomeric β^2-peptides compared to β^3-peptides,[6a] prompted us to

[a]*Organic and Biomolecular Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad-500 007, India*
[b]*Centre for Nuclear Magnetic Resonance, CSIR-Indian Institute of Chemical Technology, Hyderabad-500 007, India. E-mail: esmvee@iict.res.in, kunwar@iict.res.in, hofmann@uni-leipzig.de*
[c]*Institute of Biochemistry, Faculty of Biosciences, Pharmacy, and Psychology, University of Leipzig, Brüderstraße 34, D-04103 Leipzig, Germany*

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c2ob26615f

‡These authors contributed equally to this work.

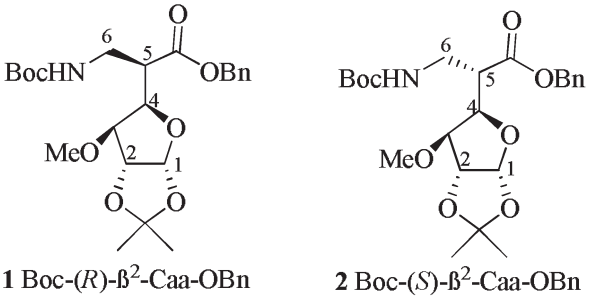


Fig. 1 Structures of β²-amino acids **1** and **2**.

investigate the folding propensities in β²-peptides with alternating chirality. We propose a new set of β-amino acids, which are C-linked carbo-β²-amino acids (β²-Caa), with a carbohydrate side chain at the Cα-carbon. The envisaged relief in the strain at the Cβ-carbon is expected to influence the conformational features of the β²-peptides. The β²-Caas **1** and **2** (Fig. 1), which are epimeric at the Cα-stereocenter, were synthesized to investigate new motifs.

The predictions of theoretical studies,[20,21] that the 'unlike' β²-peptides favor the formation of 10/12-mixed helices, prompted us to explore experimentally the design of 'alternating chirality' in β²-peptides from **1** and **2** for the first time. We report the synthesis of β²-peptides **3** and **4** with an (*S*)-β²-Caa at the N-terminus and **5** and **6** with an (*R*)-β²-Caa at the N-terminus (Fig. 2) and their conformational studies employing NMR, CD, MD, and quantum chemical methods.

Results and discussion

1. Synthesis of β²-amino acids 1 and 2

The main strategy in the synthesis[22] of the β²-Caas **1** and **2** from D-glucose would be the conversion of the C-6 hydroxy group into the amino group, while the requisite carboxylic group is introduced at the C-5 stereocentre. Accordingly, the known diol **7** was treated with TrCl and Et_3N in CH_2Cl_2 at room temperature to afford the trityl ether **8** in 89% yield (Scheme 1). The alcohol **8** was oxidized under Swern reaction conditions to give ketone **9** (98%), which on Wittig olefination with (methyl)triphenylphosphonium iodide and *t*-BuOK in THF at 0 °C gave **10** in 51% yield. Subsequently, the trityl group in **10** was deprotected using CF_3COOH in CH_2Cl_2 at room temperature to afford allylic alcohol **11** in 79% yield. The alcohol **11** under Mitsunobu[23] conditions was treated with phthalimide, Ph_3P, and DIAD in dry THF to furnish **12** in 71% yield (along with DIAD byproduct as an impurity). Compound **12** was treated with hydrazine hydrate in MeOH to give the amine, which on subsequent treatment with $(Boc)_2O$ at room temperature afforded **13** in 79% yield.

Hydroboration of **13** with BH_3·DMS in dry THF at room temperature furnished the alcohol **14** (Scheme 1) as an inseparable diastereomeric mixture (7 : 3). The terminal alcohol **14** on oxidation under Swern reaction conditions furnished aldehyde **15** (96%), which on further oxidation with $NaClO_2$ and H_2O_2 in *tert*-BuOH : H_2O (7 : 3) gave acid **16** (85%) as an inseparable diastereomeric mixture. To overcome the racemisation problem, the acid was converted into the benzyl ester with Bn–Br, DIPEA, and DMAP (cat.) in CH_2Cl_2 at room temperature to give a separable diastereomeric mixture of **1** (40%) and **2** (23%). The esters **1** and **2** on exposure to CF_3COOH afforded the TFA salts **17** and **18**, respectively.

To ascertain the absolute stereochemistry of the new stereocentre at C-5 in **1** and **2**, a cyclic ether was envisaged and synthesized from the known diol **19**.[24] Accordingly, **19** on reaction with dihydropyran in CH_2Cl_2 and PTSA (cat.) at room temperature gave THP ether **20** in 70% yield (Scheme 2). The carbinol **20** on Swern oxidation afforded the corresponding ketone **21**, which on Wittig olefination furnished **22** (56%). Reaction of **22** with PTSA in aq. MeOH at room temperature gave alcohol **23** (71%), which under Mitsunobu[23] conditions furnished **24** in 77% yield.

Treatment of **24** with hydrazine hydrate in MeOH at room temperature followed by reaction with $(Boc)_2O$ at room temperature gave **25** in 79% yield. Hydroboration of **25** with BH_3·DMS in dry THF at room temperature afforded the alcohols **26** (25%) and **27** (10%) as a separable diastereomeric mixture. The benzyl ether **26** was subjected to hydrogenolysis with Pd/C (10%) in EtOAc under a hydrogen atmosphere to give diol **28** in 92% yield. Reaction of diol **28** with *p*-TsCl and Et_3N in CH_2Cl_2 at room temperature furnished the corresponding tosylate, which underwent concomitant cyclization to afford the cyclic ether **29** in 80% yield. The structure and the stereochemistry at the C-5 stereocentre were unambiguously assigned by NMR studies.

The conformational analysis of **29** (Fig. 3) showed strong indicative nOes between C3H and $-CH_2NHBoc$ reflecting the presence of C3H and $-CH_2NHBoc$ at the same face, clearly giving evidence for the stereochemical assignment at the C-5 stereocentre.

Subsequently, the diol **28** was converted into the dimethyl ether using Ag_2O and MeI to afford **30** (42%), $[\alpha]_D^{25} = -107$ (*c* 0.14, $CHCl_3$). To further ascertain the absolute stereochemistry of the new β²-Caas **1** and **2**, they were converted into the dimethyl ethers **30** and **33**, respectively. Accordingly, **1** and **2** were independently treated with $LiAlH_4$ to afford the corresponding alcohols **31** (87%) and **32** (79%). Alkylation of the alcohols **31** and **32** with MeI in the presence of Ag_2O in DMF gave the respective ethers **30** (56%), $[\alpha]_D^{25} = -109.2$ (*c* 1.49, $CHCl_3$) and **33** (47%), $[\alpha]_D^{25} = -40.4$ (*c* 0.5, $CHCl_3$). From the spectral data and optical rotation values, it can be concluded that **30**, derived from **28** and **1**, is the same and thus has the same stereochemistry at C-5.

2. Synthesis of peptides

The synthesis of the peptides **3–6** is outlined in Scheme 3. Accordingly, Boc-(*S*)-β²-Caa-OBn **2** (Scheme 3) on hydrogenolysis with 10% Pd/C in EtOAc gave acid **34** in 92% yield, which on coupling with **17** in the presence of EDCI, HOBt, and NMM in CH_2Cl_2 afforded the dipeptide **35** in 69% yield. Hydrogenolysis (10% Pd/C in EtOAc) of **35** gave acid **36**, while **35** on exposure to CF_3COOH in CH_2Cl_2 was converted into the salt **37**. Condensation of the acid **36** with the salt **37** in the presence of EDCI, HOBt, and NMM in CH_2Cl_2 furnished the tetrapeptide **3** in 54% yield. Hydrogenolysis of peptide **3** afforded the

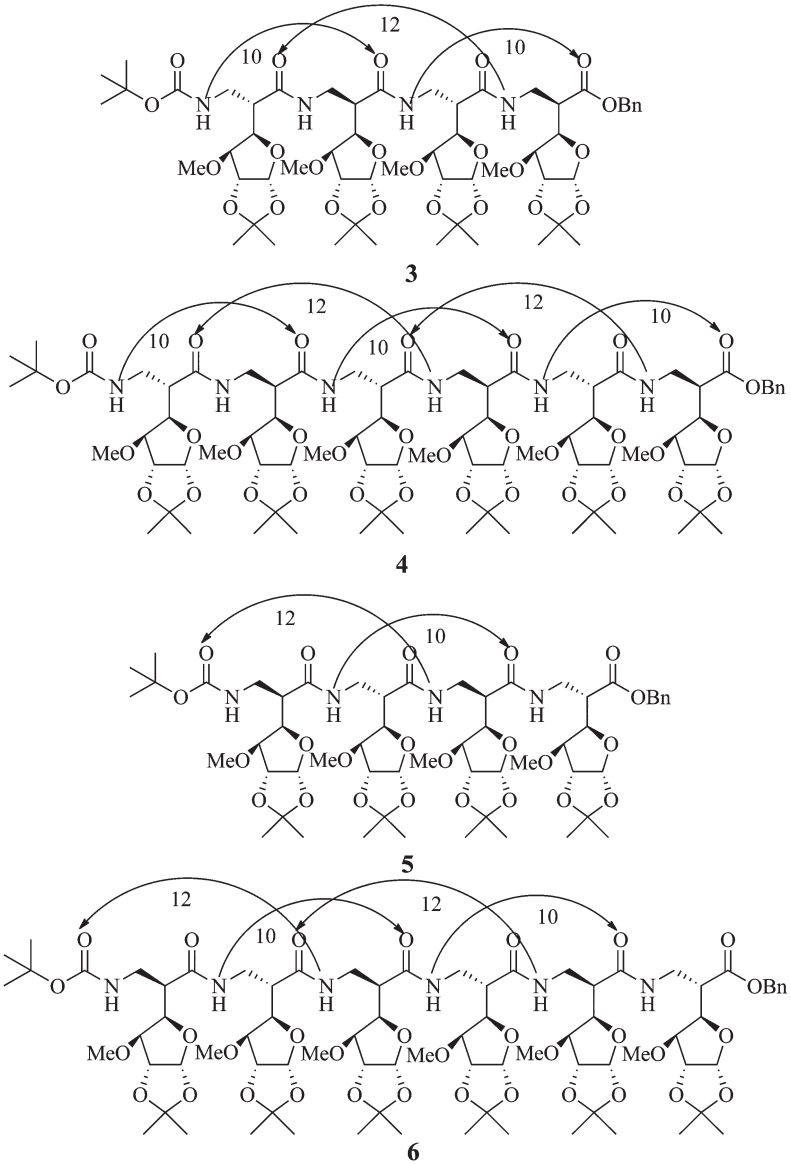


Fig. 2 Structures of β²-peptides **3** to **6** (arrows indicate the H-bonding pattern deduced from structural studies).

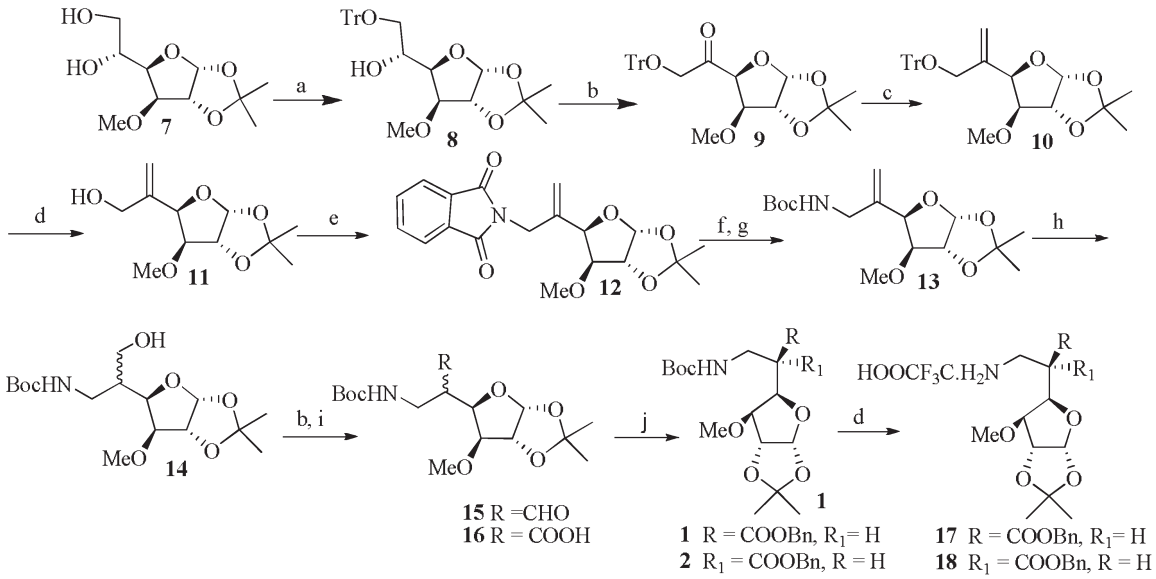
corresponding acid **38**, which on coupling (EDCI, HOBt and NMM) with **37** in CH_2Cl_2 gave the hexapeptide **4** in 32% yield.

Likewise, coupling (EDCI, HOBt and NMM) of acid **39** (obtained from **1**) with salt **17** in CH_2Cl_2 furnished the dipeptide **40** in 61% yield. Ester **40** on hydrogenolysis gave the corresponding acid **41**, which on coupling with the salt **42** (obtained from **40** by exposure to CF_3COOH) gave tetrapeptide **5** in 55% yield. Hydrogenolysis and coupling of the corresponding acid **43** with **42** in the presence of EDCI, HOBt and NMM in CH_2Cl_2 afforded the hexapeptide **6** in 36% yield.

3. Conformational analysis

The ^{1}H NMR studies[25] on peptides **3–6** were performed in ~5 mM solutions in $CDCl_3$ on a 600 MHz spectrometer. The spectrum of peptide **3** with an (*S*)-β²-Caa residue at the N-terminus showed wide dispersion in the chemical shifts (δ) of amide as well as CβH protons. The NH-3 and NH-4 resonances appeared at low field (δ >7 ppm), indicating their participation in hydrogen bonding. The solvent titration studies[26] showed that all amide protons, except NH-2, display modest changes of their chemical shifts ($\Delta\delta$NH <0.66 ppm) upon addition of up to 33% v/v DMSO-d_6 in $CDCl_3$ solution, suggesting their inaccessibility to DMSO-d_6, and confirming their involvement in intramolecular H-bonding. For the residues in the middle of the sequence, the coupling constants $^3J_{NH-C\beta H}$ ~9.0 Hz and ~4.0 Hz imply that the torsion angles C(O)–N–Cβ–Cα (ϕ) are constrained with (*R*)-β²-Caa and (*S*)-β²-Caa having values of ~120° and ~−120°, respectively (Fig. 4).

Additionally, $^3J_{C\alpha H-C\beta H}$ couplings of about ~4.0 Hz for (*R*)-β²-Caa imply that the torsion angles N–Cβ–Cα–C(O) (θ) are ~−60°, whereas, the $^3J_{C\alpha H-C\beta H}$ values of ~10.0 Hz and ~3.0 Hz for (*S*)-β²-Caa correspond to θ values of ~−60° or ~180°. The



Scheme 1 *Reagents and conditions*: (a) Et_3N, TrCl, CH_2Cl_2, 0 °C–rt, 8 h; (b) $(COCl)_2$, dry DMSO, Et_3N, CH_2Cl_2, −78 °C, 3 h; (c) Ph_3P + CH_3I, *t*-BuOK, dry THF, 0 °C, 17 h; (d) CF_3COOH, CH_2Cl_2, 0 °C–rt, 2 h; (e) phthalimide, DIAD, Ph_3P, dry THF, 0 °C–rt, 6 h; (f) $NH_2NH_2{\cdot}H_2O$, MeOH, rt, 5 h; (g) $(Boc)_2O$, MeOH, 0 °C–rt, 6 h; (h) $BH_3{\cdot}DMS$, 30% H_2O_2, NaOH, dry THF, 10 h; (i) $NaClO_2$, H_2O_2, *t*-BuOH : H_2O 10 h; (j) DIPEA, BnBr, DMAP, CH_2Cl_2, rt, 8 h.

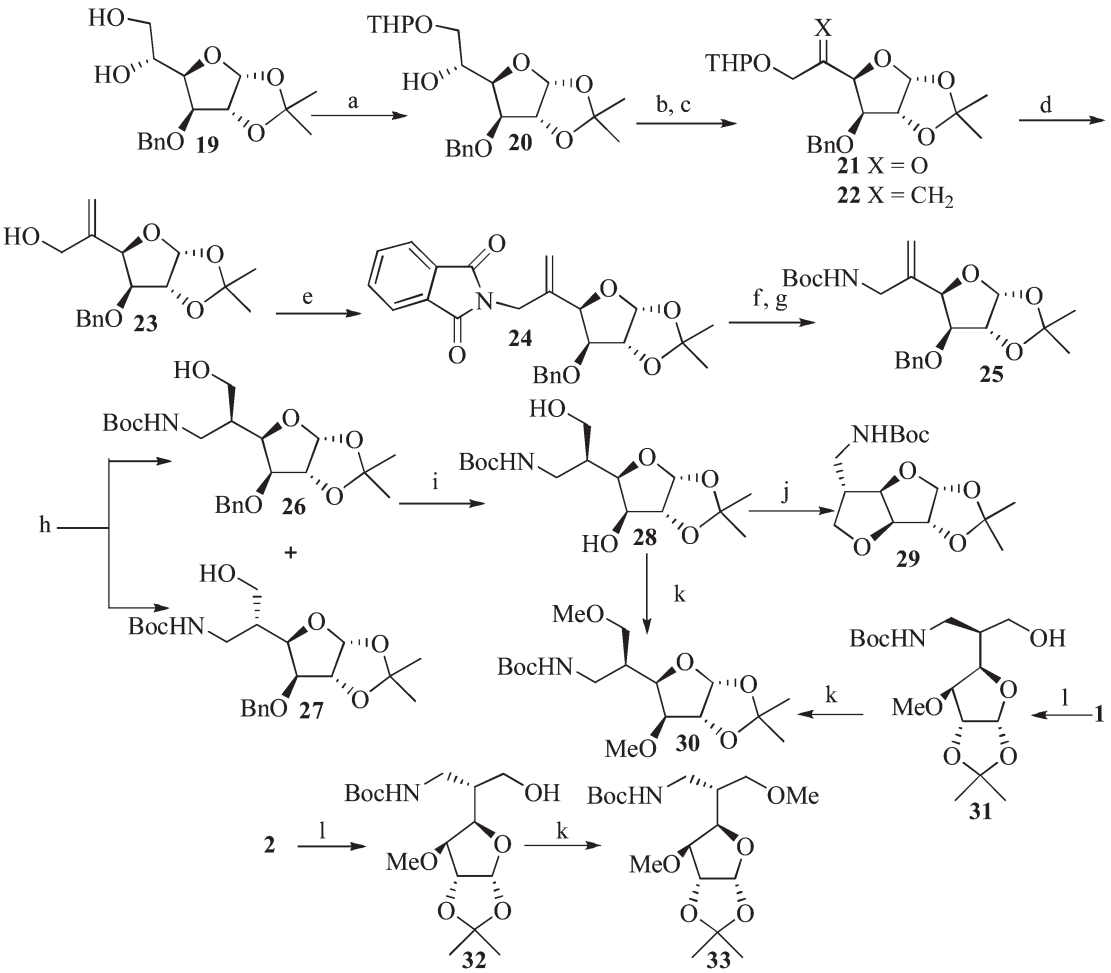
sequential nOe correlation (Fig. 5) $C\beta H_{(pro\text{-}S)}(2)$/NH(3) and intra-residue nOe correlations NH(1)/CαH(1) and NH(3)/CαH(3) along with almost extremum values for $^3J_{C\alpha H\text{–}C\beta H}$ (discussed above) imply a predominant conformation with $\theta \sim -60°$ and Cβ–Cα–C(O)–N(ψ) of ~±120° (Fig. 4) for the second and the third residue. For the two terminal residues, the lack of the extremum values of the couplings between backbone protons suggests averaging over several conformers. In addition to these conclusions on the backbone dihedral angles, the nOes $C\beta H_{(pro\text{-}S)}(2)$/NH(4) and $C\beta H_{(pro\text{-}S)}(2)$/C4H(4) provide support for a well defined structure with H-bonding between NH(4) and CO(1), corresponding to a 12-mr pseudo ring, while the nOe correlations NH(1)/NH(2) and NH(3)/NH(4) imply that NH(1) and NH(3) are H-bonded with CO(2) and CO(4), respectively, to form 10-mr pseudo rings.

Thus, peptide **3** showed a propensity for a left-handed 10/12-mixed helix (10/12/10…hydrogen bond pattern), unlike the observed right-handed 10/12-helix in β^3-peptides.[11b] A similar switch in the handedness of the 14-helix in the homo-oligomeric β^3- and β^2-peptides was earlier reported by Seebach *et al.*[2,6a]

The ^{1}H NMR spectrum of **4** showed that several amide protons have δ >7 ppm (except NH-1 and NH-2) and $\Delta\delta$NH <0.53 (except NH-2) in solvent titration studies, suggesting their involvement in H-bonding.[26] Like **3**, for all central residues (excluding the residues at the termini), $^3J_{NH\text{–}C\beta H}$ >9.5 Hz and <3.0 Hz imply ϕ ~120° and ~−120° for (*R*)-β^2-Caa and (*S*)-β^2-Caa, respectively. Similarly, $^3J_{C\alpha H\text{–}C\beta H}$ <4.0 Hz and >10.0 Hz suggest the preponderance of a single rotamer about Cα–Cβ. This information along with the nOes NH(3)/$C\beta H_{(pro\text{-}S)}(2)$, NH(5)/$C\beta H_{(pro\text{-}S)}(4)$, NH(1)/C$\alpha$H(1), NH(3)/C$\alpha$H(3), and NH(5)/C$\alpha$H(5) indicate that the angles θ and ψ for these residues are ~−60° and ~±120°, respectively. Additionally, the nOe correlations $C\beta H_{(pro\text{-}S)}(2)$/NH(4), $C\beta H_{(pro\text{-}S)}(2)$/C4H(4), $C\beta H_{(pro\text{-}S)}(4)$/NH(6), $C\beta H_{(pro\text{-}S)}(4)$/C4H(6), NH(1)/NH(2), NH(3)/NH(4), and NH(5)/NH(6) support the preponderance of structures having NH(1)–CO(2), NH(3)–CO(4), NH(5)–CO(6), NH(4)–CO(1), and NH(6)–CO(3) hydrogen bonds. The above data show the presence of a left-handed mixed helix with a 10/12/10/12/10-hydrogen bonding pattern.

This study establishes β^2-peptides with alternating chirality as a new motif for the realization of 10/12-left-handed mixed helices. Interestingly, left-handed helices are very rare in natural proteins, as reflected in the protein data bank (PDB).[27,28] An occasional incidence of left-handed helices has been noticed at ligand binding sites and other functional sites, which confirms their significance in protein functions.[29]

For further investigations on the impact of alternating chirality, the peptides **5** and **6** were synthesized having an (*R*)-β^2-Caa residue at the N-terminus. The ^{1}H NMR spectrum of **5** displayed two sets of resonance peaks in the ratio of 73 : 27. Based on the presence of exchange peaks in the ROESY spectrum, the existence of two isomers in **5** is implied, unlike in the case of the corresponding β^3-peptides.[11b] Only the isomer with the larger population was investigated in detail, since it was not possible to deduce specific structural information for the minor one. The δ >7.19 ppm and $\Delta\delta$NH <0.45 ppm for NH-2 and NH-3 imply their participation in H-bonding.[25] The $^3J_{NH\text{–}C\beta H}$ values of ~7.5 Hz and ~5.0 Hz reflect contributions from several conformers, though the predominance of structures with $\phi \sim \pm 120°$ can be inferred. The $^3J_{C\alpha H\text{–}C\beta H}$ values of ~5.0 Hz and ~5.0 Hz for (*R*)-β^2-Caa and ~8.5 Hz and ~4.0 Hz for (*S*)-β^2-Caa along with the strong nOes NH(2)/$C\beta H_{(pro\text{-}S)}(1)$, NH(4)/$C\beta H_{(pro\text{-}S)}(3)$, NH(2)/C$\alpha$H(2), and NH(4)/C$\alpha$H(4) indicate the presence of a substantial population of conformers with $\theta \sim -60°$ and $\psi \sim \pm 120°$. The nOe correlations $C\beta H_{(pro\text{-}S)}(1)$/NH(3), $C\beta H_{(pro\text{-}S)}(1)$/C4H(3), and NH(2)/NH(3) along with the above information suggest that a significant population of structures has a left-handed mixed helix with a 12/10-hydrogen bonding pattern.



Scheme 2 *Reagents and conditions*: (a) 3,4-dihydropyran, PTSA, CH_2Cl_2, 0 °C–rt, 3 h; (b) $(COCl)_2$, dry DMSO, Et_3N, CH_2Cl_2, −78 °C, 3 h; (c) $Ph_3P + CH_3I$, *t*-BuOK, dry THF, 0 °C, 17 h; (d) PTSA, aq. MeOH, rt, 2 h; (e) phthalimide, DIAD, Ph_3P, dry THF, 0 °C–rt, 6 h; (f) $NH_2NH_2{\cdot}H_2O$, MeOH, rt, 5 h; (g) MeOH, $(Boc)_2O$, 0 °C–rt, 6 h; (h) $BH_3{\cdot}DMS$, 30% H_2O_2, NaOH, dry THF, 12 h; (i) H_2, 10% Pd/C, EtOAc, rt, 12 h; (j) *p*-TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C–rt, 12 h; (k) Ag_2O, DMF, MeI, rt, 12 h; (l) $LiAlH_4$, dry THF, 0.5 h, 0 °C.

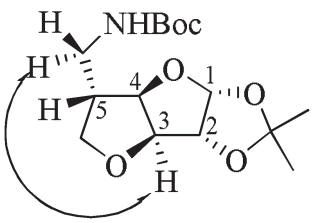
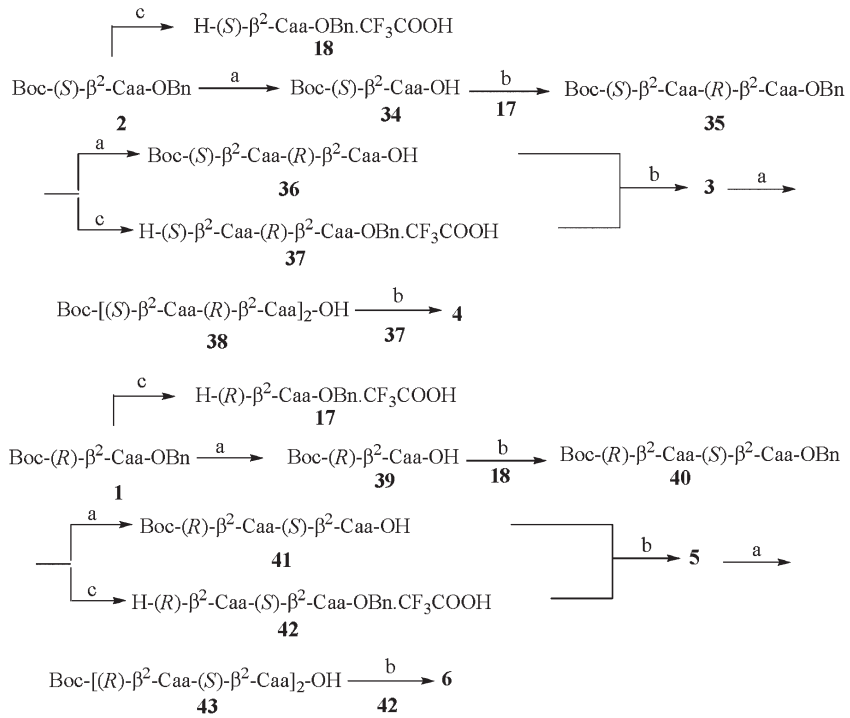


Fig. 3 Characteristic nOes (shown by double arrow) observed in **29**.

Peptide **6** showed a very wide dispersion in chemical shifts of amide proton (3.30 ppm) and CβH (1.58 ppm), while participation of several amide protons in H-bonding[25] was indicated by δ >7 ppm and $\Delta\delta$NH <0.45 ppm.[26] The values of $^3J_{NH-C\beta H}$ ~9 Hz and ~4 Hz imply that ϕ is ~±120°. Also $^3J_{C\alpha H-C\beta H}$ couplings of about ~4.0 Hz for the (*R*)-β^2-Caa-residues and ~9.5 Hz and ~4.0 Hz for the (*S*)-β^2-Caa-residues along with the nOes (Fig. 6) NH(2)/CβH$_{(pro\text{-}S)}$(1), NH(4)/CβH$_{(pro\text{-}S)}$(3), NH(6)/CβH$_{(pro\text{-}S)}$(5), NH(2)/CαH(2), NH(4)/CαH(4), and NH(6)/CαH(6), CβH$_{(pro\text{-}S)}$(1)/NH(3), CβH$_{(pro\text{-}S)}$(1)/C4H(3), CβH$_{(pro\text{-}S)}$(3)/NH(5), CβH$_{(pro\text{-}S)}$(3)/C4H(5), NH(2)/NH(3), and NH(4)/NH(5) provide sufficient evidence for the presence of a left-handed mixed helix with a 12/10/12/10 H-bonding pattern.

The couplings and nOes involving the side chains in peptides **3–6** have been used to obtain information on the structure of the furanoside sugar ring as well as on the dihedral angle Cβ–Cα–C4–O (χ1). For all residues, values of $^3J_{C\alpha H-C4H}$ ≥9.0 imply χ1 ~180° for the (*S*)-residues and ~60° for the (*R*)-residues (Fig. 7). This was further confirmed by the intra-residue nOe correlations CβH$_{(pro\text{-}S)}$/C4H and CβH$_{(pro\text{-}R)}$/C4H in all (*S*)-β-Caa residues and NH/C4H in all (*R*)-β^2-Caa residues. The couplings $^3J_{C1H-C2H}$ ~4 Hz, $^3J_{C2H-C3H}$ ~0 Hz, and $^3J_{C3H-C4H}$ ~3.0 Hz for the sugar rings correspond to a sugar pucker of $^3T_2$.[11b] Strong nOes Me$_{(pro\text{-}S)}$/C1H, Me$_{(pro\text{-}S)}$/C2H as well as a weak Me$_{(pro\text{-}R)}$/C4H nOe correlation support an envelope conformation of the isopropylidine ring.

For the restrained molecular dynamics (MD) studies[25] (Fig. 8), the constraints were derived from the volume integrals obtained from the ROESY spectra using ISPA (Isolated Spin-Pair Approximation).[30] Fig. 8A and 8B show the superimposition of the 10 lowest energy structures of **3** and **4**, respectively.



Scheme 3 *Reagents and conditions*: (a) H_2, 10% Pd/C, EtOAc, rt, 2 h; (b) HOBt (1.2 equiv.), EDCI (1.2 equiv.), NMM (4 equiv.), dry CH_2Cl_2, 0 °C–rt; (c) CF_3COOH, dry CH_2Cl_2, 0 °C–rt, 2 h.

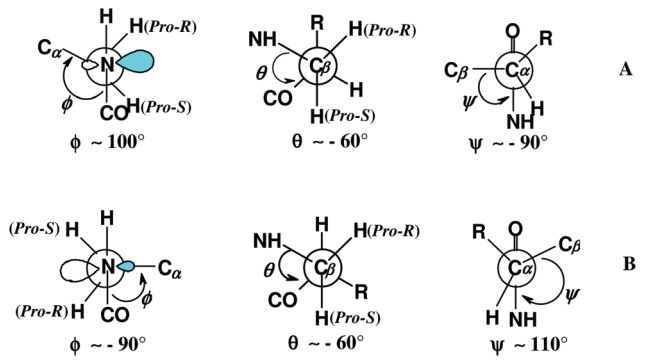


Fig. 4 Schematic Newman projections showing the orientation of the substituents in a left-handed 10/12-helix for the ideal values of torsional angles ϕ, θ, and ψ.[20,21] (A) depicts the view along the backbone atoms in (*R*)-β^2-Caa and (B) that in (*S*)-β^2-Caa.

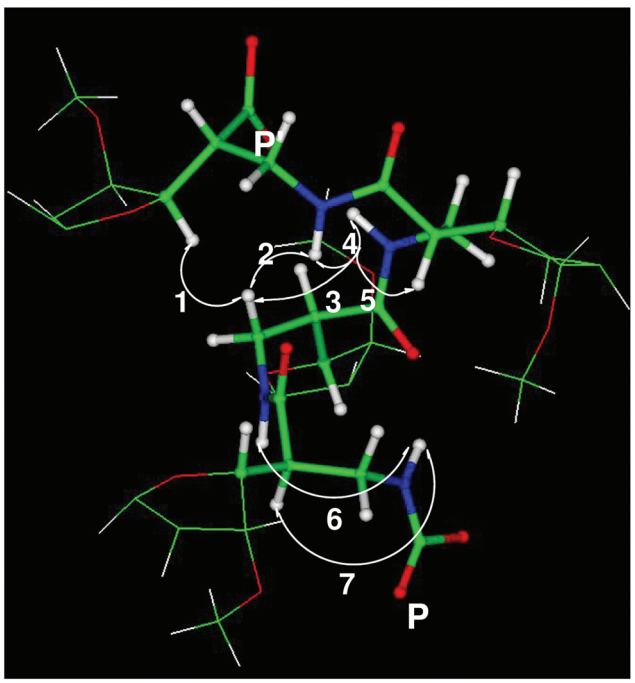


Fig. 5 Characteristic nOes of peptide **3** are shown in 3D model: (1) $C\beta H_{(pro\text{-}S)}(2)$/C4H(4); (2) $C\beta H_{(pro\text{-}S)}(2)$/NH(4); (3) $C\beta H_{(pro\text{-}S)}(2)$/NH(3); (4) NH(3)/NH(4); (5) NH(3)/CαH(3); (6) NH(1)/NH(2); (7) NH(1)/CαH (1). P = Boc; P′ = OBn.

The MD structures depict the features already alluded to with backbone and heavy atom RMSDs of 0.58 Å and 1.21 Å for **3** and 0.85 Å and 1.64 Å for **4**, respectively. Similar structural details were found for **5** and **6** from the MD analysis.[25] In order to highlight the contributions of the peptidic backbone atoms, while taking RMSD values, the *tert*-butyl group in Boc and the benzyl group in the –OBn moieties were replaced by hydrogen atoms for the peptides **3–6**. The NMR data suggest that the fraying of the termini is more conspicuous in the β^2-peptides compared to β^3-peptides, suggesting that the robustness of the helices in the β^2-peptides is compromised. The MD data confirm these observations with somewhat larger RMSD deviations for the heavy atoms and the backbone atoms.[11b] This observation is also in agreement with the reduced robustness of the 14-helix in β^2-peptides compared to the β^3-peptides.[2,6a]

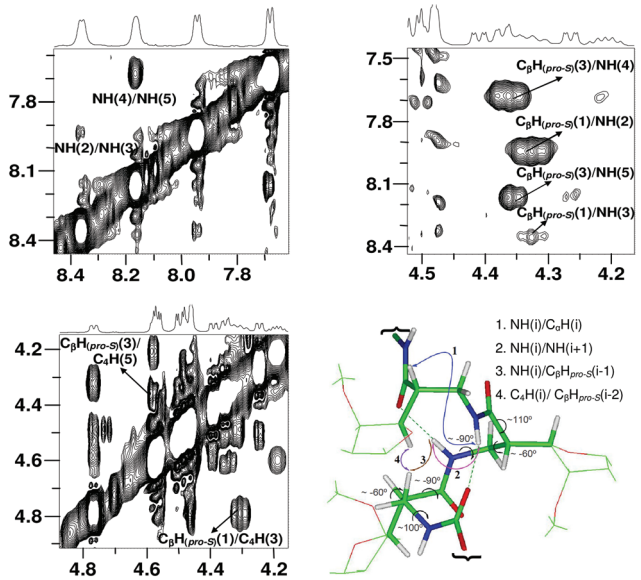


Fig. 6 ROESY spectrum of peptide **6** with the characteristic nOe correlations supporting a 12/10 helix (dotted lines represent H-bonds).

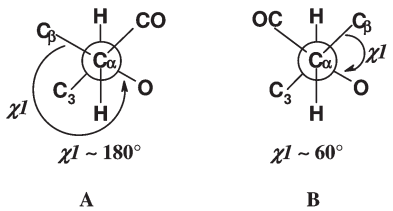


Fig. 7 Newman projections showing the orientations of the substituents for the predominant conformers around the Cα–C4 (χ1) bond, appending the sugar side chains to the peptide backbone. (A) (*S*)-β^2-Caa and (B) (*R*)-β^2-Caa.

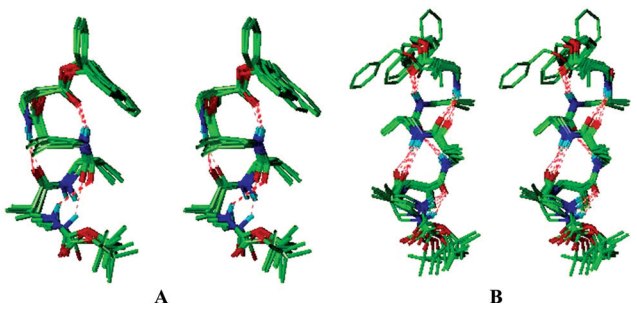


Fig. 8 Stereoview of the MD structures of peptide **3** (A) and peptide **4** (B); sugars are replaced by methyl groups after the calculations for clarity.

CD spectroscopy, unlike for natural proteins and peptides,[31] has been less extensively used for the understanding of folding patterns of β- and α/β-peptides,[32] which can be attributed to the scarce availability of high resolution structural information.[32,33]

The CD studies (Fig. 9) on peptides **3–6** have been undertaken in 0.2 mM solution in methanol, since chloroform absorptions interfere with the requisite CD absorptions.[34] The CD spectra very clearly depict a negative maximum at about 203 nM,

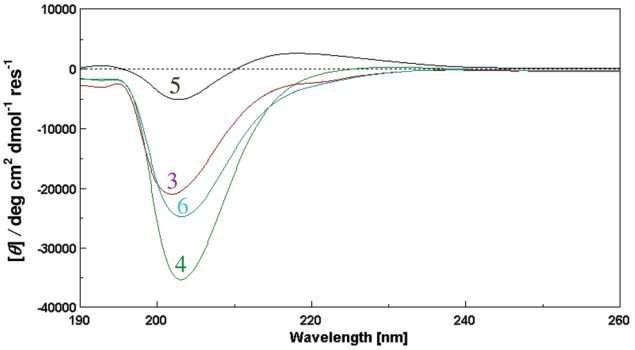


Fig. 9 CD spectra of peptides **3–6** in MeOH.

corresponding to the signatures of a left-handed 10/12-helix in these β-peptides. Interestingly, like the results from NMR in $CDCl_3$ solution, the helices in β^2-peptides appear less robust, as reflected from the smaller molecular ellipticities observed for the peptides **3–6**, compared to those obtained for β^3-peptides.[11b] Peptide **5**, however, showed a very small molecular ellipticity and a loss of structure, as was exhibited by the two populations from the NMR study.

4. Theoretical studies

The most striking result of the present work on β^2-peptides with alternating chirality design employing (*R*)- and (*S*)-β^2-amino acids, which are epimeric at the amine stereocentre, is the occurrence of exclusively left-handed mixed 12/10- and 10/12-helical conformations, independent of the order of the chiral amino acid constituents. The left-handed 10/12/10-hydrogen bonding pattern is favored in sequences with an (*S*)-β^2-Caa at the N-terminus, while the left-handed 12/10/12-hydrogen bonding pattern appears in sequences with an (*R*)-β^2-Caa at the N-terminus. A similarly surprising result was already observed in β^3-peptides of the same class, where right-handed mixed helices are exclusively formed.[10b,17]

According to general ideas on the side chain influence on the structure of mixed helices,[20a,c,21a,b] a switch in handedness can be expected with a change in the chirality of the amino acids. Referring to the β-peptide design of 12/10- and 10/12-mixed helices, four helix possibilities can formally be expected, *viz.* left-handed or right-handed helices with 12/10- or, alternatively, 10/12-hydrogen bonded pseudo rings. Moreover, it can be assumed that, if a left-handed 12/10-helix is preferred in β^2-peptides having a (*R*,*S*,*R*…)-amino acid sequence, the corresponding right-handed helix with the same 12/10-hydrogen bonded ring order is the preferred one in β^2-peptides with a β^2-(*S*,*R*,*S*…)-amino acid sequence. Likewise, the corresponding conclusions are valid for 10/12-ring sequences. This is confirmed by the stabilities of all mixed 12/10- and 10/12-helices of blocked β^2- and β^3-hexapeptides consisting of mono-substituted β^2- and β^3-amino acid constituents with achiral methyl side chains, obtained at the HF/6-31G* level of *ab initio* MO theory. These data are given in Table 5 of the ESI† (see also ref. 20c). In Table 5 (ESI†), both the cases with alternating chirality, which is the subject of this manuscript, and also the cases of non-alternating chirality were considered for completeness. The study indicates

Table 1 Relative energies[a] of all right-handed and left-handed 12/10- and 10/12-mixed helices of blocked β^2- and β^3-hexapeptides with alternating chirality consisting of mono-methyl-substituted β-amino acids obtained at the HF/6-31G* level of *ab initio* MO theory[b]

Helix[b]	Relative energy
β^2-peptides	
β(*2R,2S*)rh10/12 ≡ β(*2S,2R*)lh10/12	42.2
β(*2S,2R*)rh10/12 ≡ β(*2R,2S*)lh10/12	1.3
β(*2S,2R*)rh12/10 ≡ β(*2R,2S*)lh12/10	47.7
β(*2R,2S*)rh12/10 ≡ β(*2S,2R*)lh12/10	**0.0**[c]
β^3-peptides	
β(*3R,3S*)rh10/12 ≡ β(*3S,3R*)lh10/12	1.3
β(*3S,3R*)rh10/12 ≡ β(*3R,3S*)lh10/12	93.9
β(*3S,3R*)rh12/10 ≡ β(*3R,3S*)lh12/10	**0.0**[d]
β(*3R,3S*)rh12/10 ≡ β(*3S,3R*)lh12/10	96.6

[a] In kJ mol^{-1}. [b] The first substitution and configuration symbols in the parentheses correspond to the first mono-substituted amino acid constituent in the sequence, the second symbols to the second constituent; rh = right-handed, lh = left-handed. [c] Total energy E_T = −1956.369187 a.u. [d] Total energy E_T = −1956.378003 a.u.

Table 2 Relative energies[a] of the right-handed and left-handed 12/10- and 10/12-mixed helices obtained for the β^2- and β^3-peptides with alternating chirality consisting of β^2- and β^3-Caas at the HF/6-31G* level of *ab initio* MO theory[b]

Helix[c]	Relative energy
β^2-Caa peptides	
β(*2R,2S*)rh10/12	28.8
β(*2R,2S*)lh12/10	41.0
β(*2S,2R*)lh10/12	**0.0**[d]
β(*2S,2R*)rh12/10	137.9
β^3-Caa-peptides	
β(*3R,3S*)rh12/10	21.7
β(*3R,3S*)lh10/12	112.8
β(*3S,3R*)rh10/12	**0.0**[e]
β(*3S,3R*)lh12/10	72.2

[a] In kJ mol^{-1}. [b] The β^2-peptide structures correspond to peptide **6**, in the β^3-peptides only the side chains are shifted to Cβ; for details of the calculations, see ESI.† [c] The first substitution and configuration symbols in the parentheses correspond to the first mono-substituted amino acid constituent in the sequence, the second symbols to the second one; rh = right-handed, lh = left-handed. [d] Total energy E_T = −5590.195546 a.u. [e] Total energy E_T = −5590.200755 a.u.

that all formally possible mixed 12/10- and 10/12-helices can be localized as minimum conformations for the blocked model hexapeptides. The theoretical data are impressively in agreement with the experimental data reported by Seebach and co-workers,[10d] where the formation of right-handed 10/12-helices is particularly favored in alternate sequences beginning with an (*S*)-β^2-amino acid constituent followed by an (*S*)-β^3-amino acid constituent, while right-handed 12/10-helices are favored in sequences beginning with an (*S*)-β^3-amino acid residue followed by an (*S*)-β^2-amino acid residue.

Since the focus of the present work involves the concept of alternating chirality, we selected only the data for these cases from Table 5 (ESI†) and present them in Table 1. As expected, right-handed mixed helices of β^2- and β^3-peptides of the same hydrogen bonding type (10/12- or 12/10-) with an (*S*,*R*,*S*…)-sequence are equivalent to the left-handed mixed helices of the same hydrogen bonding type in the corresponding (*R*,*S*,*R*…)-sequences and *vice versa*. Moreover, the stability of the right-handed 10/12-helix in β^2-peptides with an (*S*,*R*,*S*…)-sequence of β^2-amino acids is comparable with that of the left-handed 12/10-helices with the same chirality sequence. The same conclusions can be drawn for β^2-peptides with an (*R*,*S*,*R*…)-sequence of β^2-amino acids and for the corresponding situations in β^3-peptides.

In order to explain the preference for the same handedness in β^2- (left-handed) and β^3-peptides (right-handed) independent of the order of the alternate chiral β-Caas, we have to look for another explanation. Unlike the model hexapeptides with achiral methyl side chains in Table 1, the epimeric β^2- and β^3-Caa constituents in our study with their chiral carbohydrate side chains, are diastereomeric. Obviously, the preference for the same handedness independent of the order of the chiral constituents in the peptides from alternating (*S*)- and (*R*)-β-Caas is governed by the different steric situation arising from the chiral carbohydrate side chains.

A systematic theoretical conformational analysis for β^3-peptides with the carbohydrate (D-*xylo* configuration) side chains, absolutely confirms the preference for right-handedness both in β^3-(*R*,*S*,*R*…)- and β^3-(*S*,*R*,*S*…)-amino acid sequences (Table 2, for details of the calculations, see ESI†). In the case of a β^3-(*R*,*S*,*R*...)-amino acid sequence, the right-handed 12/10-hydrogen-bonded ring sequence is favored, whereas, a right-handed 10/12-ring order is preferred in the β^3-(*S*,*R*,*S*...)-amino acid sequence. Likewise, the theoretical and experimental data are in good agreement for the (*S*,*R*,*S*...)-amino acid sequences of β^2-peptides, which favor the formation of left-handed mixed 10/12-helices. In the case of the β^2-(*R*,*S*,*R*...)-amino acid sequences, two conformers were indicated in the NMR experiments, wherein the left-handed 12/10-helix was the most stable conformer, while the structure of the other could not be analyzed. However, according to the theoretical data (Table 2), a right-handed 10/12-helix is slightly favored over the experimentally determined left-handed 12/10-helix. As long as the nature of the second conformer is unknown, a final evaluation of the theoretical data is difficult.

In comparison to the model peptides with achiral methyl side chains of Table 1, the space-filling properties of the chiral side chains in the β^2- and β^3-Caas exclude the existence of most mixed helix types. These findings further reiterate the role of the chiral side chains in defining the handedness of the observed helices in β^2- and β^3-peptides.

5. Conclusions

In this study, β^2-Caas with carbohydrate side chain at the Cα stereocentre have been prepared for the first time, inspired by our observations on the mass spectral studies on β^3-peptides derived from β^3-Caas. The study provided experimental proof of the theoretical predictions on the concept of unlike β^2-peptides resulting in 12/10- or 10/12-mixed helices. The design of peptides with alternating chirality, employing the epimeric (*R*)- and (*S*)-β^2-Caas resulted in a new motif for the generation of left-handed 12/10- or 10/12-mixed helices. The preference for left-handedness in the helices of β^2-peptides, independent of the order of the alternate epimeric (*R*)- and (*S*)-β^2-Caas in the sequence, suggests a determining influence of the side chain

chirality. Obviously, the same reasons determine the formation of exclusively right-handed 12/10- and 10/12-mixed helices in the corresponding β^3-peptides. Furthermore, this study shows that the shift of the carbohydrate side chain from the Cβ to the Cα positions, reduces the steric strain at Cβ, and may support the switch in the handedness. Interestingly, the relief in steric strain seems to decrease the helix stability in the β^2-peptides compared to the β^3-peptides. The suggested new motifs for a predictable rational design of left-handed secondary structures could stimulate further studies towards skeletal and conformational diversity in foldamers.

Experimental section

For peptides **3–6**, NMR spectra (1D and 2D experiments) were acquired at 300 MHz, 400 MHz, 500 MHz, and 600 MHz (^{1}H), and at 75 MHz, 100 MHz, and 150 MHz (^{13}C). TMS was used as an internal reference for both ^{1}H and ^{13}C spectra and the chemical shifts are reported in the δ scale. The low field amide proton chemical shifts (>7 ppm) were used as an indicator of their involvement in H-bonding. The presence of H-bonds was further validated by solvent titration studies by sequentially adding up to 300 μL of DMSO-d_6 in 600 μL of $CDCl_3$ solution of peptides. The NMR spectra were obtained by using the standard programs provided by the manufacturer of the instruments. The 2-D spectra were run in phase sensitive mode by using the States-TPPI procedure,[35] while the TOCSY experiments were run with a spin locking field of about 10 KHz and a mixing time of 80 ms, the ROESY experiments were usually performed with mixing times of 300 ms using a continuous spin-locking field of about 3 KHz. The 2D data were processed with Gaussian apodization in both dimensions. The distance constraints for the MD calculation were obtained from the volume integrals of the cross peaks in the ROESY spectra using two spin approximation and a reference distance of 1.8 Å for the geminal protons at Cβ.

The CD spectra were run at room temperature (~298 K) on 0.2 mM peptide in MeOH using rectangular fused quartz cells of 0.2 cm path length with scan range of 190–260 nm and scanning speed of 50 nm min^{-1}. The spectra were smoothened using binomial method. The values are expressed in terms of [θ], the total molar ellipticity (deg cm^2 dmol^{-1} res^{-1}).

The structural analysis of different conformations, including molecular modeling calculations and energy minimization, were carried out with the help of the Insight-II(97.0)/Discover program. The CVFF force field with default parameters was employed for all the calculations using a distance dependent dielectric constant with ε = 4.7 (dielectric constant of $CDCl_3$), which allows the inclusion of the solvent implicitly. A large number of restraints were used quantitatively in the restraint MD calculations. The upper and lower bound of the distance constraints have been obtained by diminishing and enhancing the derived distance by 10%. These constraints with a force constant of 15 kcal mol^{-1} Å^{-2} were applied in the form of a flat-bottom potential. No restraints for dihedral angles and hydrogen bonds were included in the structure determination. The complete set of distance constraints used for peptides **3–6** have been presented in tabular form in the ESI.† These restraints were used throughout the MD simulations as well as the minimization. For the initiation of the dynamics the molecular model was built using the values of the dihedral angles deduced from the coupling constants as well as the nOes. As a first step, the steric contacts were removed by a mild minimization with the constraints. Subsequently energy minimizations were performed using steepest descent methods, followed by conjugate gradient methods for a maximum of 5000 iterations each or RMS deviation of 0.001 kcal mol^{-1}, whichever was achieved earlier. The 100 structures generated so were energy minimized with the above protocol and ten of the best possible structures were superimposed for display.

The HRMS spectra were recorded on Q-TOF mass spectrometer.

The quantum chemical conformational analysis of the peptides was performed at the HF/6-31G* level of *ab initio* MO theory. The details are described in the ESI.†

tert-Butyl *N*-2-[(3a*R*,5*R*,6*S*,6a*R*)-6-methoxy-2,2-dimethylperhydrofuro[2,3-*d*][1,3]-dioxol-5-yl]allylcarbamate (13)

To a stirred solution of alcohol **11** (4.0 g, 17.39 mmol), Ph_3P (6.83 g, 26.0 mmol) and phthalimide (3.78 g, 26.08 mmol) in dry THF (80 mL), DIAD (5.16 mL, 26.08 mmol) was added dropwise at 0 °C and the reaction stirred at room temperature for 6 h. Solvent was evaporated and the residue purified by column chromatography (silica gel 60–120 mesh, 25% EtOAc in petroleum ether) to furnish *2-2-[(3aR,5R,6S,6aR)-6-methoxy-2,2-dimethylperhydrofuro[2,3-d][1,3]dioxol-5-yl]allyl-1,3-isoindolinedione* (**12**; 4.45 g, 71%) as a pale yellow syrup (along with DIAD byproduct as impurity).

A solution of the above crude **12** (4.41 g, 12.32 mmol) and hydrazine hydrate (1.20 mL, 24.64 mmol) in MeOH (40 mL) was stirred at room temperature for 5 h. The reaction mixture was filtered and solvent evaporated. The above free amine dissolved in MeOH (50 mL) was treated with $(Boc)_2O$ (4.24 mL, 18.48 mmol) at 0 °C and stirred at room temperature for 6 h. Solvent was evaporated and the residue purified by column chromatography (silica gel 60–120 mesh, 14% EtOAc in petroleum ether) to furnish **13** (3.19 g, 79%) as a pale yellow syrup; $[\alpha]_D^{25}$ = −69.4 (*c* 1.1, $CHCl_3$); IR (neat): 3331, 2979, 1705, 1395, 1260, 1125, 1052 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.88 (d, 1H, *J* = 3.7 Hz, C_1H), 5.24 (s, 1H, olefinic), 5.14 (s, 1H, olefinic), 4.67–4.63 (m, 2H, NH, C_4H), 4.53 (d, 1H, *J* = 3.7 Hz, C_2H), 3.87 (dd, 1H, *J* = 7.5, 15.1 Hz, CHN) 3.77 (d, 1H, *J* = 3.2 Hz, C_3H), 3.66 (dd, 1H, *J* = 7.5, 15.1 Hz, CHN), 3.39 (s, 3H, OMe), 1.47 (s, 9H, Boc), 1.43 (s, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 155.9, 140.7, 113.6, 111.49, 104.4, 84.9, 81.4, 92.0, 80.7, 79.2, 57.7, 43.1, 28.3, 28.1, 26.6, 26.1; ESIMS (*m*/*z*, %): 352 (M^+ + Na, 100); HRMS (ESI): *m*/*z* calculated for $C_{16}H_{27}NO_6$ (M^+ + Na) 352.1736, found 352.1742.

tert-Butyl *N*-2-[(3a*R*,5*R*,6*S*,6a*R*)-6-methoxy-2,2-dimethylperhydrofuro [2,3-*d*][1,3]-dioxol-5-yl]-3-hydroxypropylcarbamate (14)

To a cooled (0 °C) solution of **13** (4.6 g, 13.98 mmol) in dry THF (30 mL) under N_2 atmosphere, BH_3·DMS (1.32 mL, 13.98 mmol) was added dropwise and the reaction stirred at

room temperature for 10 h. Subsequently, the above cold (0 °C) reaction mixture was treated with 2N aq. NaOH solution (13.8 mL, 3 mL g^{-1}) followed by 30% H_2O_2 (4.6 mL, 1 mL g^{-1}). It was warmed to room temperature and stirred for 3 h. Water (20 mL) was added and the mixture extracted with EtOAc (3 × 40 mL). Combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (silica gel 60–120 mesh, 25% EtOAc in petroleum ether) to furnish **14** as a 7 : 3 diastereomeric mixture (2.93 g, 60%) as a pale yellow syrup; IR (neat): 3415, 2980, 2934, 1690, 1518, 1370, 1167, 1079, 1021 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.84–5.80 (m, 1H, C_1H), 4.92 (m, 1H, NH), 4.54–4.51 (m, 1H, C_2H), 4.08 (dd, 0.3H, J = 3.0, 9.8 Hz, C_4H), 3.97 (dd, 0.7H, J = 3.0, 9.8 Hz, C_4H), 3.78–3.71 (m, 0.6H, C_6H), 3.66 (d, 0.3H, J = 3.0 Hz, C_2H), 3.63 (d, 0.7H, J = 3.0 Hz, C_2′H), 3.55–3.07 (m, 3.4H, C_6H, CHN), 3.43 (s, 0.9H, OMe), 3.41 (s, 2.1H, OMe′), 2.07–1.97 (m, 1H, C_5H), 1.50 (s, 3H, CH_3), 1.47 (s, 9H, Boc), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 158.0, 111.3, 104.9, 104.3, 86.1, 83.5, 81.8, 81.2, 80.0, 78.8, 59.6, 57.5, 57.4, 40.8, 38.3, 28.4, 28.3, 26.6, 26.5, 26.2, 26.1; ESIMS (m/z, %): 370 (M^+ + Na, 100); HRMS (ESI): m/z calculated for $C_{16}H_{29}NO_7$ (M^+ + Na) 370.1841, found 370.1837.

2-[(3a*R*,5*R*,6*S*,6a*R*)-6-Methoxy-2,2-dimethylperhydrofuro[2,3-*d*]-[1,3]dioxol-5-yl]-3-[(*tert*-butoxycarbonyl)amino]propanoic acid (16)

To a stirred solution of oxaloyl chloride (2.04 mL, 23.4 mmol), in dry CH_2Cl_2 (25 mL), DMSO (3.32 mL, 46.8 mmol) was added at −78 °C and the reaction stirred at the same temperature for 15 min. A solution of alcohol **14** (5.41 g, 15.6 mmol) in CH_2Cl_2 (25 mL) was added at −78 °C and the reaction stirred at the same temperature for 2.5 h. The reaction mixture was treated with Et_3N (13.02 mL, 93.6 mmol) at −78 °C. Work up as described for **9** afforded **15** (5.21 g, 96%) as a yellow syrup, which was used as such for the next reaction.

To a stirred solution of **15** (2.80 g, 8.11 mmol) in *t*-butanol : water (10 mL; 7 : 3), sodium chlorite (1.10 g, 12.17 mmol) and H_2O_2 (4.5 mL, 40.57 mmol, 30% aqueous solution) were added and the reaction stirred at room temperature for 10 h. The reaction mixture was concentrated and the residue dissolved in ethyl acetate (20 mL). It was washed with water (5 mL), brine (5 mL), dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography (Silica gel, 60–120 mesh, 60% EtOAc in petroleum ether) to give **16** as a diastereomeric mixture (2.50 g, 85%) as a colorless liquid; IR (neat): 3419, 2992, 2925, 1708, 1671, 1412, 1163 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.90 (d, 1H, J = 3.9 Hz, C_1H), 4.69–4.66 (m, 1H, C_4H), 4.55 (d, 1H, J = 3.7 Hz, C_2H), 3.84 (d, 1H, J = 3.5 Hz, C_3H), 3.50 (s, 3H, OMe), 3.68–3.37 (m, 2H, $C_\beta H$), 3.06–2.91 (m, 1H, $C_\alpha H$), 1.47 (s, 3H, CH_3) 1.43 (s, 9H, Boc), 1.32 (s, 3H, CH_3); ESIMS (m/z, %): 384 (M^+ + Na, 100); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 177.1, 177.0, 155.9, 155.8, 111.5, 111.4, 104.6, 84.4, 83.6, 81.4, 81.0, 80.2, 79.1, 78.9, 65.5, 65.4, 57.7, 57.5, 45.5, 45.2, 41.3, 39.1, 36.9, 29.6, 28.3, 27.4, 26.7, 26.1; HRMS (ESI): m/z calculated for $C_{16}H_{27}NO_8$ (M^+ + Na) 384.1634, found 384.1646.

Benzyl (2*R*)-2-[(3a*R*,5*R*,6*S*,6a*R*)-6-methoxy-2,2-dimethylperhydrofuro[2,3-*d*][1,3]dioxol-5-yl]-3-[(*tert*-butoxycarbonyl)amino]propanoate (1) and benzyl (2*S*)-2-[(3a*R*,5*R*,6*S*,6a*R*)-6-methoxy-2,2-dimethylperhydrofuro[2,3-*d*]-[1,3]dioxol-5-yl]-3-[(*tert*-butoxycarbonyl)amino]propanoate (2)

To a stirred solution of **16** (2.50 g, 6.92 mmol) and DIPEA (2.41 mL, 13.84 mmol) in CH_2Cl_2 (35 mL), BnBr (0.82 mL, 6.92 mmol) and DMAP (cat.) were added at 0 °C and the reaction stirred at room temperature for 8 h. The reaction mixture was washed with water (20 mL) and brine (20 mL). Solvent was evaporated and the residue purified by column chromatography. First eluted (silica gel, 60–120 mesh, 12% EtOAc in petroleum ether) was **1** (1.25 g, 40%) as a pale yellow syrup; $[\alpha]_D^{25}$ = +6.07 (c 0.73, $CHCl_3$); IR (neat): 3432, 2928, 1713, 1675, 1249, 1152, 1041 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.43–7.31 (m, 5H, Ar-H), 5.86 (d, 1H, J = 3.7 Hz, C_1H), 5.21 (d, 1H, J = 12.2 Hz, benzylic CH), 5.08 (d, 1H, J = 12.2 Hz, benzylic CH), 4.98 (t, 1H, J = 5.2 Hz, NH), 4.51 (d, 1H, J = 3.7 Hz, C_2H), 4.36 (dd, 1H, J = 3.2, 9.8 Hz, C_4H), 3.71 (d, 1H, J = 3.2 Hz, C_3H), 3.64–3.54 (m, 2H, $C_\beta H$), 3.14 (s, 3H, OMe), 3.01–2.92 (m, 1H, $C_\alpha H$), 1.5 (s, 3H, CH_3), 1.42 (s, 9H, Boc), 1.31 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.6, 155.6, 135.7, 128.2, 126.8, 111.5, 105.8, 104.7, 86.0, 84.1, 81.3, 79.2, 66.5, 57.4, 45.0, 40.7, 28.2, 26.7, 26.1; HRMS (ESI): m/z calculated for $C_{23}H_{33}NO_8Na$ (M^+ + Na) 474.2103, found 474.2127.

Second eluted (silica gel, 15% EtOAc in petroleum ether) was **2** (0.72 g, 23%) as a colourless syrup; $[\alpha]_D^{25}$ = −36.4 (c 0.48, $CHCl_3$); IR (neat): 3435, 2931, 1715, 1370, 1166, 1079 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.38–7.30 (m, 5H, Ar-H), 5.87 (d, 1H, J = 3.8 Hz, C_1H), 5.26 (d, 1H, J = 12.4 Hz, benzylic CH), 5.13 (d, 1H, J = 12.4 Hz, benzylic CH), 4.96–4.78 (m, 1H, NH), 4.56 (d, 1H, J = 3.8 Hz, C_2H), 4.37 (dd, 1H, J = 2.6, 9.9 Hz, C_4H), 3.79 (d, 1H, J = 2.6 Hz, C_3H) 3.56–3.43 (m, 1H, $C_\beta H$), 3. 43 (s, 3H, OMe), 3.35–3.26 (m, 1H, $C_\beta H$), 3.12–3.06 (m, 1H, $C_\alpha H$), 1.49 (s, 3H, CH_3), 1.43 (s, 9H, Boc), 1.33 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.5, 135.6, 135.7, 128.4, 128.0, 127.8, 111.5, 104.5, 99.8, 83.3, 80.9, 78.6, 66.5, 57.3, 44.8, 38.9, 28.2, 26.5, 26.1; HRMS (ESI): m/z calculated for $C_{23}H_{33}NO_8Na$ (M^+ + Na) 474.2103, found 474.2125.

Boc-[(*S*)-β²-Caa-(*R*)-β²-Caa]$_2$-OBn (3)

A solution of **35** (0.37 g, 0.53 mmol) in EtOAc (3 mL) was treated with 10% Pd/C (0.03 g) and stirred at room temperature for 6 h. Work up as described for **34** gave **36** (0.3 g, 96%) as a white solid, which was used as such for further reaction.

A solution of **36** (0.28 g, 0.46 mmol) was treated with HOBt (0.075 g, 0.55 mmol), EDCI (0.106 g, 0.55 mmol) in CH_2Cl_2 (10 mL) and stirred at 0 °C under N_2 atmosphere for 15 min and treated with **37** (0.30 g, 0.50 mmol) [obtained from **35** on exposure to CF_3COOH (0.5 mL)] and NMM (0.20 mL, 1.84 mmol) under nitrogen atmosphere for 24 h. Work up as described for **35** and purification of the residue by column chromatography (silica gel 60–120 mesh, 1.3% methanol in $CHCl_3$) gave **3** (0.3 g, 54%) as a white solid; m.p. 109–110 °C; $[\alpha]_D^{25}$ = −54.5 (c 0.36, $CHCl_3$); IR (KBr): 3379, 2986, 1714, 1666, 1539, 1378, 1167, 1080, 1012 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 7.64 (dd, 1H, J = 5.0, 8.1 Hz, NH-4), 7.42–7.32 (m,

5H, Ar-H), 7.08 (dd, 1H, J = 4.0, 8.9 Hz, NH-3), 6.70 (dd, 1H, J = 4.0, 9.2 Hz, NH-2), 5.90 (bd, 1H, NH-1), 5.88 (d, 1H, J = 3.9 Hz, C_1H-2), 5.86 (d, 1H, J = 3.9 Hz, C_1H-1), 5.84 (d, 1H, J = 3.9 Hz, C_1H-4), 5.83 (d, 1H, J = 3.9 Hz, C_1H-3), 5.18 (d, 1H, J = 12.1 Hz, benzylic CH), 5.05 (d, 1H, J = 12.1 Hz, benzylic CH), 4.63 (dd, 1H, J = 3.4, 9.3 Hz, C_4H-4), 4.56 (d, 1H, J = 3.9 Hz, C_2H-2), 4.55 (d, 1H, J = 3.9 Hz, C_2H-1), 4.54 (d, 1H, J = 3.9 Hz, C_2H-3), 4.49 (d, 1H, J = 3.9 Hz, C_2H-4), 4.44 (dd, 1H, J = 3.4, 10.0 Hz, C_4H-2), 4.24 (dd, 1H, J = 3.1, 9.9 Hz, C_4H-1), 4.24 (dd, 1H, J = 3.1, 9.9 Hz, C_4H-3), 3.71 (m, 1H, CβH (pro-*R*)-4), 4.22 (m, 1H, CβH (pro-*R*)-2), 3.92 (d, 1H, J = 3.1 Hz, C_3H-1), 3.76 (d, 1H, J = 3.4 Hz, C_3H-2), 3.74 (ddd, 1H, J = 3.2, 8.9, 11.7 Hz, CβH (pro-*R*)-3), 3.71 (d, 1H, J = 3.4 Hz, C_3H-4), 3.70 (d, 1H, J = 3.1 Hz, C_3H-3), 3.48 (s, 3H, OMe), 3.44 (s. 3H, OMe), 3.39 (ddd, 1H, J = 3.0, 5.5, 10.8 Hz, CβH (pro-*S*)-1), 3.39 (m, 1H, CβH (pro-*R*)-1), 3.35 (m, 1H, CβH (pro-*S*)-4), .48 (s, 3H, OMe), 3.34 (s. 3H, OMe), 3.31 (m, CβH (pro-*S*)-2), 3.06 (ddd, 1H, J = 4.0, 9.9, 11.7 Hz, CβH (pro-*S*)-3), 3.05 (s, 3H, OMe), 2.96 (ddd, 1H, J = 4.6, 6.3, 9.3 Hz, CαH-4), 2.87 (td, 1H, J = 4.9, 10.0 Hz, CαH-2), 2.73 (dt, 1H, J = 3.2, 9.9 Hz, CαH-3), 2.67 (ddd, 1H, J = 3.0, 5.9, 9.9 Hz, CαH-1), 1.47 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.43 (s, 9H, Boc), 1.33 (s, 3H, CH_3), 1.30 (s, 9H, 3 × CH_3), 1.27 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9 (2C), 172.5, 171.1, 156.2, 135.8, 128.6 (2C), 128.5 (2C), 128.3, 111.9, 111.7, 111.6, 104.9, 104.7 (2C), 104.6, 84.3, 84.0, 83.8, 83.4, 81.7, 81.2, 81.1, 81.0, 79.3, 78.9, 78.8, 78.4, 77.2, 66.7, 57.8, 57.7, 57.6, 57.3, 45.9, 45.7, 45.6, 45.3, 39.8, 39.3, 38.8, 37.5, 29.7, 28.4 (3C), 26.9, 26.8 (2C), 26.7, 26.3 (2C), 26.2 (2C); HRMS (ESI): *m*/*z* calculated for $C_{56}H_{84}N_4O_{23}$ (M^+ + Na) 1203.5424, found 1203.5391.

Boc-[(*S*)-β^2-Caa-(*R*)-β^2-Caa]$_3$-OBn (4)

A solution of **3** (0.26 g, 0.22 mmol) in EtOAc (2 mL) was treated with 10% Pd/C (0.03 g) and stirred at room temperature for 6 h. Work up as described for **34** gave **38** (0.22 g, 94%) as a white solid, which was used as such for further reaction.

A solution of **38** (0.19 g, 0.17 mmol) was treated with HOBt (0.028 g, 0.20 mmol), EDCI (0.040 g, 0.20 mmol) in CH_2Cl_2 (5 mL) and stirred at 0 °C under N_2 atmosphere for 15 min and treated with **37** (0.10 g, 0.18 mmol) [obtained from **35** on exposure to CF_3COOH (0.3 mL)] and NMM (0.076 mL, 0.69 mmol) in CH_2Cl_2 (3 mL) under nitrogen atmosphere for 24 h. Work up as described for **35** and purification of the residue by column chromatography (silica gel 60–120 mesh, 1.7% methanol in $CHCl_3$) afforded **4** (0.09 g, 32%) as a white solid; m.p. 130–131 °C; $[\alpha]_D^{25}$ = −107.3 (*c* 0.395, $CHCl_3$); IR (KBr): 3325, 2987, 1715, 1656, 1456, 1380, 1219, 1080 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.46 (dd, 1H, J = 4.8, 8.3 Hz, NH-6), 8.31 (dd, 1H, J = 2.8, 10.0 Hz, NH-4), 8.10 (dd, J = 2.8, 9.6 Hz, NH-3), 7.54 (bd, 1H, NH-5), 7.45–7.32 (m, 5H, Ar-H), 6.78 (bd, 1H, NH-2), 5.88 (d, 1H, J = 3.9 Hz, C_1H-2), 5.86 (d, 1H, J = 3.9 Hz, C_1H-5), 5.84 (d, 1H, J = 3.8 Hz, C_1H-6), 5.82 (d, 1H, J = 3.9 Hz, C_1H-1), 5.78 (bd, 1H, NH-1), 5.75 (d, 1H, J = 3.8 Hz, C_1H-3), 5.20 (d, 1H, J = 11.9 Hz, benzylic CH), 5.07 (d, 1H, J = 11.9 Hz, benzylic CH), 4.83 (dd, 1H, J = 3.3, 9.9 Hz, C_4H-4), 4.70 (dd, 1H, J = 3.3, 9.9 Hz, C_4H-2), 4.68 (m, 1H, CβH (pro-*R*)-2), 4.61 (d, 1H, J = 3.9 Hz, C_2H-2), 4.54 (d, 1H, J = 3.9 Hz, C_2H-1), 4.52 (d, 1H, J = 3.9 Hz, C_2H-5), 4.50 (d, 1H, J = 3.8 Hz, C_2H-6), 4.39 (ddd, 1H, J = 4.5, 10.0, 14.1 Hz, CβH (pro-*R*)-4), 4.34 (dd, 1H, C_4H-5), 4.33 (m, 1H, CβH (pro-*R*)-6), 4.28 (dd, 1H, J = 3.1, 10.1 Hz, C_4H-3), 4.18 (dd, 1H, J = 3.1, 10.2 Hz, C_4H-1), 3.99 (d, 1H, J = 3.1 Hz, C_3H-1), 3.92 (d, 1H, J = 3.3 Hz, C_3H-2), 3.82 (m, 1H, CβH (pro-*R*)-5), 3.80 (m, 1H, CβH (pro-*R*)-3), 3.76 (d, 1H, J = 3.3 Hz, C_3H-4), 3.69 (d, 1H, J = 3.5 Hz, C_3H-6), 3.64 (d, 1H, J = 3.1 Hz, C_3H-3), 3.56 (d, 1H, J = 3.2 Hz, C_3H-5), 3.47 (s, 3H, OMe), 3.41 (m, 1H, CβH (pro-*R*)-4, 3.41 (m, 1H, CβH (pro-*S*)-1), 3.40 (s, 3H, OMe), 3.35 (ddd, 1H, J = 2.6, 9.7, 12.1 Hz, CβH (pro-*S*)-4), 3.34 (s, 3H, OMe), 3.31 (m, 1H, CβH (pro-*S*)-2), 3.29 (m, 1H, CβH (pro-*S*)-6), 3.29 (s, 3H, OMe), 3.22 (s, 3H, OMe), 3.01 (ddd, 1H, J = 4.7, 6.4, 9.0 Hz, CαH-6), 3.00 (s, 3H, OMe), 2.97 (td, 1H, J = 3.2, 9.0 Hz, CαH-2), 2.85 (m, 1H, CαH-4), 2.81 (m, 1H, CαH-5), 2.81 (m, 1H, CβH (pro-*S*)-5), 2.62 (ddd, 1H, J = 3.9, 3.9, 10.2 Hz, CαH-1), 2.53 (ddd, 1H, J = 2.6, 10.1, 10.6 Hz, CαH-3), 1.57 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.44 (s, 9H, Boc), 1.40 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.29 (s, 6H, CH_3), 1.27 (s, 9H, CH_3), 1.25 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.6, 173.4, 173.1, 173.0, 171.8, 170.4, 156.3, 135.4, 128.8 (2C), 128.5 (2C), 128.2, 112.1, 112.0, 111.6 (2C), 111.5 (2C), 104.8 (2C), 104.7, 104.5, 104.2, 84.3, 84.0, 83.8, 83.7, 83.5, 83.2, 81.8, 81.5, 81.2 (2C), 81.1, 81.0, 79.2, 78.8 (2C), 78.6, 78.3, 77.6, 77.2, 66.8, 57.9, 57.7, 57.6, 57.5, 57.2, 57.1, 47.0, 46.6, 46.3, 45.9, 45.1, 44.9, 39.9, 38.9 (2C), 38.4, 37.2, 36.9, 29.7, 28.4 (3C), 26.9 (2C), 26.8 (2C), 26.7 (2C), 26.4 (2C), 26.3 (2C), 26.2 (2C); HRMS (ESI): *m*/*z* calculated for $C_{78}H_{118}N_6O_{33}$ (M^+ + Na) 1689.7632, found 1689.7606.

Boc-[(*R*)-β^2-Caa-(*S*)-β^2-Caa]$_2$-OBn (5)

A solution of ester **40** (0.31 g, 0.44 mmol) in EtOAc (3 mL) was treated with 10% Pd/C (0.06 g) and stirred at room temperature for 6 h. Work up as described for **34** gave **41** (0.26 g, 98%) as a white solid, which was used as such for further reaction.

A solution of **41** (0.26 g, 0.43 mmol) was treated with HOBt (0.07 g, 0.52 mmol), EDCI (0.10 g, 0.52 mmol) in CH_2Cl_2 (5 mL) and stirred at 0 °C under N_2 atmosphere for 15 min and then treated with **42** (0.32 g, 0.47 mmol) [obtained from **40** on exposure to CF_3COOH] and NMM (0.19 mL, 1.72 mmol) in CH_2Cl_2 (3 mL) under nitrogen atmosphere for 24 h. Workup as described for **35** and purification of the residue by column chromatography (silica gel 60–120, 1.2% methanol in $CHCl_3$) gave **5** (0.27 g, 55%) as a white solid; m.p. 110–111 °C; $[\alpha]_D^{25}$ = −71.0 (*c* 0.27, $CHCl_3$); IR (KBr): 3379, 2986, 1714, 1666, 1539, 1378, 1167, 1080, 1012 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.49 (dd, 1H, J = 4.8, 7.5 Hz, NH-3), 7.45–7.29 (m, 5H, Ar-H), 7.19 (dd, 1H, J = 4.8, 7.6 Hz, NH-2), 6.65 (dd, 1H, J = 4.8, 7.3 Hz, NH-4), 5.89 (d, 1H, J = 3.8 Hz, C_1H-4), 5.87 (d, 1H, J = 3.8 Hz, C_1H-1), 5.86 (d, 1H, J = 3.8 Hz, C_1H-3), 5.83 (d, 1H, J = 3.8 Hz, C_1H-2), 5.37 (dd, 1H, J = 5.3, 7.4 Hz, NH-1), 5.22 (d, 1H, J = 12.5 Hz, benzylic CH), 5.17 (d, 1H, J = 12.5 Hz, benzylic CH), 4.58 (d, 1H, J = 3.8 Hz, C_2H-4), 4.54 (d, 1H, J = 3.8 Hz, C_2H-1), 4.54 (d, 1H, J = 3.8 Hz, C_2H-2), 4.53 (d, 1H, J = 3.8 Hz, C_2H-3), 4.48 (dd, 1H, J = 3.3, 9.1 Hz,

C_4H-3), 4.43 (dd, 1H, J = 3.3, 9.7 Hz, C_4H-4), 4.40 (dd, 1H, J = 3.3, 10.0 Hz, C_4H-1), 4.25 (dd, 1H, J = 3.3, 9.0 Hz, C_4H-2), 3.91 (ddd, 1H, J = 5.0, 7.5, 13.5 Hz, CβH (pro-*R*)-3), 3.79 (ddd, 1H, J = 4.9, 7.4, 13.9 Hz, CβH (pro-*R*)-1), 3.78 (d, 1H, J = 3.3 Hz, C_3H-4), 3.77 (d, 1H, J = 3.3 Hz, C_3H-1), 3.74 (d, 1H, J = 3.3 Hz, C_3H-2), 3.70 (d, 1H, J = 3.3 Hz, C_3H-3), 3.69 (ddd, 1H, J = 4.0, 7.6, 13.5 Hz, CβH (pro-*R*)-2), 3.63 (ddd, 1H, J = 4.0, 7.6, 13.5 Hz, CβH (pro-*R*)-4), 3.45 (ddd, 1H, J = 5.0, 5.3, 13.9 Hz, CβH (pro-*S*)-1), 3.45 (s. 3H, OMe), 3.40 (s. 3H, OMe), 3.39 (td, 1H, J = 4.8, 13.5 Hz, CβH (pro-*S*)-3), 3.37 (ddd, 1H, J = 4.8, 6.5, 13.5 Hz, CβH (pro-*S*)-4), 3.32 (s, 3H, OMe), 3.31 (s. 3H, OMe), 3.16 (ddd, 1H, J = 4.8, 8.7, 13.5 Hz, CβH (pro-*S*)-2), 3.07 (ddd, 1H, J = 4.1, 6.5, 9.7 Hz, CαH-4), 2.82 (ddd, 1H, J = 4.9, 5.0,, 10.0 Hz, CαH-1), 2.74 (ddd, 1H, J = 4.0,. 8.7, 9.0 Hz, CαH-2), 2.72 (ddd, 1H, J = 4.8, 5.0, 9.1 Hz, CαH-3), 1.51 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.41 (s, 9H, Boc), 1.31 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.24 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.6, 172.4, 172.1, 171.4, 155.9, 135.7, 128.5 (2C), 128.1, 128.0 (2C), 111.6 (2C), 111.5, 104.7, 104.6 (2C), 83.9, 83.8, 83.5, 83.4, 81.3, 81.2, 81.1, 81.0, 79.4 (2C), 79.0, 78.9, 77.2, 66.7, 57.7, 57.6 (2C), 57.4, 45.9, 45.6 (2C), 44.7, 40.7, 39.3, 37.8, 37.7 (2C), 28.3 (4C), 26.8 (2C), 26.7 (2C), 26.6 (2C), 26.1 (2C); HRMS (ESI): *m*/*z* calculated for $C_{56}H_{84}N_4O_{23}$ (M^+ + Na) 1203.5424, found 1203.5390.

Boc-[(*R*)-β^2-Caa-(*S*)-β^2-Caa]$_3$-OBn (6)

A solution of **5** (0.23 g, 0.19 mmol) in EtOAc (3 mL) was treated with 10% Pd/C (0.03 g) and stirred at room temperature for 6 h. Work up as described for **34** gave **43** (0.2 g, 97%) as a white solid, which was used as such for further reaction.

A solution of **43** (0.18 g, 0.16 mmol) was treated with HOBt (0.026 g, 0.19 mmol), EDCI (0.038 g, 0.19 mmol) in CH_2Cl_2 (5 mL) and stirred at 0 °C under N_2 atmosphere for 15 min and then treated with **42** (0.11 g, 0.166 mmol) [obtained from **40** on exposure to CF_3COOH] and NMM (0.072 mL, 0.664 mmol) in CH_2Cl_2 (4 mL) under nitrogen atmosphere for 24 h. Work up as described for **35** and purification of the residue by column chromatography (silica gel 60–120 mesh, 1.7% methanol in $CHCl_3$) furnished **6** (0.10 g, 36%) as a white solid; m.p. 127–129 °C; $[\alpha]_D^{25}$ = −115.2 (*c* 0.35, $CHCl_3$); IR (KBr): 3325, 2987, 1715, 1656, 1456, 1380, 1219, 1080 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 (dd, 1H, J = 2.9, 10.6 Hz, NH-3), 8.16 (dd, 1H, J = 4.1, 8.5 Hz, NH-5), 7.94 (dd, J = 2.5, 10.6 Hz, NH-2), 7.68 (dd, J = 2.6, 10.0 Hz, NH-3), 7.39–7.30 (m, 5H, Ar-H), 6.96 (t, 1H, J = 6.2 Hz, NH-6), 5.89 (d, 1H, J = 3.9 Hz, C_1H-6), 5.87 (d, 1H, J = 3.9 Hz, C_1H-1), 5.86 (d, 1H, J = 3.9 Hz, C_1H-4), 5.86 (d, 1H, J = 3.9 Hz, C_1H-5), 5.80 (d, 1H, J = 3.9 Hz, C_1H-3), 5.72 (d, 1H, J = 3.9 Hz, C_1H-2), 5.21 (d, 1H, J = 12.3 Hz, benzylic CH), 5.15 (d, 1H, J = 12.3 Hz, benzylic CH), 5.06 (dd, J = 2.8, 10.0 Hz, NH-1),4.79 (dd, 1H, J = 3.4, 10.0 Hz, C_4H-3), 4.60 (dd, 1H, J = 3.4, 9.4 Hz, C_4H-5), 4.60 (d, 1H, J = 3.9 Hz, C_2H-1), 4.59 (d, 1H, J = 3.9 Hz, C_2H-6), 4.53 (d, 1H, J = 3.9 Hz, C_2H-4), 4.51 (d, 1H, J = 3.9 Hz, C_2H-2), 4.51 (dd, 1H, J = 3.4, 10.4 Hz, C_4H-1), 4.49 (d, 1H, J = 3.9 Hz, C_2H-3), 4.48 (d, 1H, J = 3.9 Hz, C_2H-5), 4.41 (dd, 1H, J = 3.4, 9.8 Hz, C_4H-6), 4.37 (dd, 1H, J = 3.2, 9.8 Hz, C_4H-4), 4.37 (ddd, 1H, CβH (pro-*R*)-3), 4.33 (m, 1H, CβH (pro-*R*)-1), 4.26 (dd, 1H, J = 3.2, 9.7 Hz, C_4H-2), 4.21 (ddd, 1H, J = 6.9, 8.5, 14.0 Hz, CβH (pro-*R*)-5), 3.93 (d, J = 3.4 Hz, C_3H-1), 3.88 (ddd, 1H, J = 2.7, 10.1, 13.1 Hz, CβH (pro-*R*)-2), 3.82 (ddd, 1H, J = 3.7, 10.0, 12.1 Hz, CβH (pro-*R*)-4), 3.79 (d, 1H, J = 3.4 Hz, C_3H-6), 3.77 (d, 1H, J = 3.4 Hz, C_3H-5), 3.73 (d, 1H, J = 3.4 Hz, C_3H-3), 3.69 (d, 1H, J = 3.2 Hz, C_3H-2), 3.59 (m, 1H, CβH (pro-*R*)-6), 3.57 (d, 1H, J = 3.2 Hz, C_3H-4), 3.45 (s, 3H, OMe), 3.41 (m, 1H, CβH (pro-*S*)-6, 3.40 (m, 1H, CβH (pro-*S*)-1), 3.40 (s, 3H, OMe), 3.35 (s, 3H, OMe), 3.30 (m, 1H, CβH (pro-*S*)-3), 3.29 (s, 3H, OMe), 3.26 (s, 3H, OMe), 3.23 (s, 3H, OMe), 3.19 (td, 1H, J = 4.1, 14.0, CβH (pro-*S*)-5), 3.06 (ddd, 1H, J = 4.3, 7.1, 9.8 Hz, CαH-6), 2.94 (td, 1H, J = 3.2, 10.4 Hz, CαH-1), 2.85 (ddd, 1H, J = 4.1, 6.9, 9.4 Hz, CαH-5), 2.85 (m, 1H, CβH (pro-*S*)-2), 2.79 (ddd, 1H, J = 2.6, 9.7, 12.1 Hz, CβH (pro-*S*)-4), 2.79 (ddd, 1H, CαH-3), 2.73 (ddd, 1H, J = 3.7, 9.7, 9.8 Hz, CαH-4), 2.64 (ddd, 1H, J = 2.7, 9.7, 10.3 Hz, CαH-2), 1.57 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.47 (s, 6H, CH_3), 1.46 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.39 (s, 9H, Boc), 1.34 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.29 (s, 6H, CH_3), 1.27 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.6, 172.4, 172.3 (2C), 171.8, 170.4, 156.3, 135.8, 128.5 (2C), 128.3, 128.1 (2C), 111.9, 111.7, 111.6 (2C), 111.5, 111.4, 104.8, 104.7, 104.6, 104.5 (2C), 104.2, 84.0, 83.9 (2C), 83.4, 83.3 (2C), 81.8, 81.5, 81.1, 81.0, 79.9 (2C), 79.1, 79.0, 78.9, 78.7, 77.9, 77.3, 66.7, 57.9, 57.8, 57.5 (3C), 57.1, 47.0, 46.9 (2C), 45.1, 45.0, 44.9, 40.3, 40.1, 38.8, 37.9, 37.1, 37.0, 29.7, 28.4 (3C), 26.9 (2C), 26.8 (2C), 26.7 (2C), 26.3 (5C), 26.0; HRMS (ESI): *m*/*z* calculated for $C_{78}H_{118}N_6O_{33}$ (M^+ + Na) 1689.7632, found 1689.7647.

Acknowledgements

NYR, RR, GS, DC, BS are thankful to UGC and CSIR, New Delhi for financial support in the form of fellowship. H.-J. H. is obliged to Deutsche Forschungsgemeinschaft for financial support (Sonderforschungsbereich 610 and project HO/2346/1–3).

References

1 D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 1996, **118**, 13071–13072.
2 D. Seebach, M. Overhand, F. N. M. Kuhnle, B. Martinoni, L. Oberer, U. Hommel and H. Widmer, *Helv. Chim. Acta*, 1996, **79**, 913–941.
3 (*a*) D. Seebach and J. L. Matthews, *Chem. Commun.*, 1997, 2015–2022; (*b*) S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173–180; (*c*) K. Kirschenbaum, R. N. Zuckerman and D. A. Dill, *Curr. Opin. Struct. Biol.*, 1999, **9**, 530–535; (*d*) K. D. Stigers, M. J. Soth and J. S. Nowick, *Curr. Opin. Chem. Biol.*, 1999, **3**, 714–723; (*e*) M. D. Smith and G. W. J. Fleet, *J. Pept. Sci.*, 1999, **5**, 425–441; (*f*) R. P. Cheng, S. H. Gellman and W. F. DeGrado, *Chem. Rev.*, 2001, **101**, 3219–3232; (*g*) J. Venkatraman, S. C. Shankaramma and P. Balaram, *Chem. Rev.*, 2001, **101**, 3131–3152; (*h*) M. S. Cubberley and B. L. Iverson, *Curr. Opin. Chem. Biol.*, 2001, **5**, 650–653; (*i*) F. Fülöp, *Chem. Rev.*, 2001, **101**, 2181–2204; (*j*) D. Seebach, D. F. Hook and A. Glättli, *Biopolymers*, 2005, **84**, 23–37; (*k*) T. A. Martinek and F. Fülöp, *Eur. J. Biochem.*, 2003, **270**, 3657–3666; (*l*) D. Seebach, A. K. Beck and D. J. Bierbaum, *Chem. Biodiversity*, 2004, **1**, 1111–1239; (*m*) N. Rathore, S. H. Gellman and J. J. de Pablo, *Biophys. J.*, 2006, **91**, 3425–3435; (*n*) C. M. Goodman, S. Choi, S. Shandler and W. F. DeGrado, *Nat. Chem. Biol.*, 2007, **3**, 252–262; (*o*) *Foldamers: Structure, Properties, and Applications*, ed. S. Hecht and I. Huc,

Wiley-VCH, Weinheim, Germany, 2007; (*p*) I. Saraogi and A. D. Hamilton, *Chem. Soc. Rev.*, 2009, **38**, 1726–1743; (*q*) G. Guichard and I. Huc, *Chem. Commun.*, 2011, **47**, 5933–5941; (*r*) P. G. Vasudev, S. Chatterjee, N. Shamala and P. Balaram, *Chem. Rev.*, 2011, **111**, 657–687; (*s*) F. Bouillère, S. Thètiot-Laurent, C. Kouklovsky and V. Alezra, *Amino Acids*, 2011, **41**, 687–707; (*t*) L. K. A. Pilsl and O. Reiser, *Amino Acids*, 2011, **41**, 709–718; (*u*) T. A. Martinek and F. Fülöp, *Chem. Soc. Rev.*, 2012, **41**, 687–702.

4 (*a*) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes and J. S. Moore, *Chem. Rev.*, 2001, **101**, 3893–4011; (*b*) T. Hintermann, K. Gademann, B. Jaun and D. Seebach, *Helv. Chim. Acta*, 1998, **81**, 983–1002; (*c*) S. Hanessian, X. Luo, R. Schaum and S. Michnick, *J. Am. Chem. Soc.*, 1998, **120**, 8569–8570; (*d*) D. D. Long, N. L. Hungerford, M. D. Smith, D. E. A. Brittain, D. G. Marquess, T. D. W. Claridge and G. W. J. Fleet, *Tetrahedron Lett.*, 1999, **40**, 2195–2198.

5 (*a*) G. Lelais and D. Seebach, *Biopolymers*, 2004, **76**, 206–243; (*b*) H. Huang, X. Liu, J. Deng, M. Qiu and Z. Zheng, *Org. Lett.*, 2006, **8**, 3359–3362; (*c*) R. Hoen, T. Tiemersma-Wegman, B. Procuranti, L. Lefort, J. G. de Vries, A. J. Minnaard and B. L. Feringa, *Org. Biomol. Chem.*, 2007, **5**, 267–275; (*d*) M. K. Edmonds, F. H. M. Graichen, J. Gardiner and A. D. Abell, *Org. Lett.*, 2008, **10**, 885–887; (*e*) N. J. A. Martin, X. Cheng and B. List, *J. Am. Chem. Soc.*, 2008, **130**, 13862–13863.

6 (*a*) T. Hintermann and D. Seebach, *Synlett*, 1997, 437–438; (*b*) G. Guichard and D. Seebach, *Chimia*, 1997, **51**, 315–318; (*c*) G. Guichard, S. Abele and D. Seebach, *Helv. Chim. Acta*, 1998, **81**, 187–206.

7 (*a*) F. V. Nussbaum and P. Spiteller, β-Amino acids in nature, in *Highlights in Bioorganic Chemistry*, C. Schmuck and H. Wennemers, Wiley-VCH, Weinheim, Germany, 2003, p. 63; (*b*) P. Spitelle and F. V. Nussbaum, Biosynthesis of α-amino acids, in *Highlights in Bioorganic Chemistry*, C. Schmuck and H. Wennemers, Wiley-VCH, Weinheim, Germany, 2003, p. 90.

8 (*a*) W. F. DeGrado, J. P. Schneider and Y. Hamuro, *J. Pept. Res.*, 1999, **54**, 206–217; (*b*) H. R. Crumpler, C. E. Dent, H. Harris and R. G. Wetall, *Nature*, 1951, **167**, 307–308; (*c*) K. Fink, R. B. Henderson and R. M. Fink, *Proc. Soc. Exp. Biol. Med.*, 1951, **78**, 135–141.

9 (*a*) F. Gessier, C. Noti, M. Rueping and D. Seebach, *Helv. Chim. Acta*, 2003, **86**, 1862–1870; (*b*) Y. Takeuchi, M. Nabetani, K. Takagi, T. Hagi and T. Koizumi, *J. Chem. Soc., Perkin Trans. 1*, 1991, 49–53.

10 (*a*) D. Seebach, K. Gademann, J. V. Schreiber, J. L. Matthews, T. Hintermann, B. Jaun, L. Oberer, U. Hommel and H. Widmer, *Helv. Chim. Acta*, 1997, **80**, 2033–2038; (*b*) D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Jaun, J. L. Matthews, J. V. Schreiber, L. Oberer, U. Hommel and H. Widmer, *Helv. Chim. Acta*, 1998, **81**, 932–982; (*c*) D. Seebach, J. V. Schreiber, X. Daura and W. F. van Gunsteren, *Helv. Chim. Acta*, 2000, **83**, 34–57; (*d*) M. Rueping, J. V. Schreiber, G. Lelais, B. Jaun and D. Seebach, *Helv. Chim. Acta*, 2002, **85**, 2577–2593.

11 (*a*) H. Kessler, S. A. W. Gruner and V. Truffault, *Chem.–Eur. J.*, 2002, **8**, 4365–4376; (*b*) G. V. M. Sharma, K. R. Reddy, P. R. Krishna, A. R. Sankar, K. Narsimulu, S. K. Kumar, P. Jayaprakash, B. Jagannadh and A. C. Kunwar, *J. Am. Chem. Soc.*, 2003, **125**, 13670–13671; (*c*) G. V. M. Sharma, V. B. Jadhav, C. Madavi, K. V. S. Ramakrishna, P. Jayaprakash, K. Narsimulu, V. Subash and A. C. Kunwar, *J. Am. Chem. Soc.*, 2006, **128**, 14657–14668; (*d*) G. V. M. Sharma, V. Manohar, S. K. Dutta, V. Subash and A. C. Kunwar, *J. Org. Chem.*, 2008, **73**, 3689–3698.

12 (*a*) K. Gademann, T. Kimmerlin, D. Hoyer and D. Seebach, *J. Med. Chem.*, 2001, **44**, 2460–2468; (*b*) D. Seebach, S. Abele, K. Gademann and B. Jaun, *Angew. Chem., Int. Ed.*, 1999, **38**, 932–982; (*c*) X. Daura, K. Gademann, H. Schäfer, B. Jaun, D. Seebach and W. F. van Gunsteren, *J. Am. Chem. Soc.*, 2001, **123**, 2393–2404; (*d*) C. Peter, M. Rueping, H. J. Wörner, B. Jaun, D. Seebach and W. F. van Gunsteren, *Chem.–Eur. J.*, 2003, **9**, 5838–5849.

13 D. Seebach, B. Jaun, R. Sebesta, R. I. Mathad, O. Flögel, M. Limbach, H. Sellner and S. Cottens, *Helv. Chim. Acta*, 2006, **89**, 1801–1825.

14 S. Ahmed, R. Beleid, T. Sprules and K. Kaur, *Org. Lett.*, 2007, **9**, 25–28.

15 G. Lelais and D. Seebach, *Helv. Chim. Acta*, 2003, **86**, 4152–4168.

16 (*a*) Y. Inaba, T. Kawakami, S. Aimoto, T. Ikegami, T. Takeuchi, T. Nakazawa, S. Yano and Y. Mikata, *Carbohydr. Res.*, 2009, **344**, 613–626; (*b*) Y. Inaba, S. Yano and Y. Mikata, *Tetrahedron Lett.*, 2007, **48**, 993–997.

17 G. V. M. Sharma, V. G. Reddy, A. S. Chander and K. R. Reddy, *Tetrahedron: Asymmetry*, 2002, **13**, 21–24.

18 G. V. M. Sharma, K. R. Reddy, P. R. Krishna, A. R. Sankar, P. Jayaprakash, B. Jagannadh and A. C. Kunwar, *Angew. Chem., Int. Ed.*, 2004, **43**, 3961–3965.

19 R. Srikanth, P. Nagi Reddy, R. Narsimha, R. Srinivas, G. V. M. Sharma, K. R. Reddy and P. R. Krishna, *J. Mass Spectrom.*, 2004, **39**, 1068–1074.

20 (*a*) K. Möhle, R. Günther, M. Thormann, N. Sewald and H.-J. Hofmann, *Biopolymers*, 1999, **50**, 167–184; (*b*) C. Baldauf, R. Günther and H.-J. Hofmann, *Angew. Chem., Int. Ed.*, 2004, **43**, 1594–1597; (*c*) C. Baldauf, R. Günther and H.-J. Hofmann, *Biopolymers*, 2005, **80**, 675–687; (*d*) C. Baldauf, R. Günther and H.-J. Hofmann, *J. Org. Chem.*, 2004, **69**, 6214–6220; (*e*) P. Schramm, G. V. M. Sharma and H.-J. Hofmann, *Biopolymers*, 2010, **94**, 279–291.

21 (*a*) Y.-D. Wu and D.-P. Wang, *J. Am. Chem. Soc.*, 1998, **120**, 13485–13493; (*b*) Y.-D. Wu and D.-P. Wang, *J. Am. Chem. Soc.*, 1999, **121**, 9352–9362; (*c*) Y.-D. Wu, J.-Q. Lin and Y.-L. Zhao, *Helv. Chim. Acta*, 2002, **85**, 3144–3161; (*d*) Y.-D. Wu, W. Han, D.-P. Wang, Y. Gao and Y.-L. Zhao, *Acc. Chem. Res.*, 2008, **41**, 1418–1427.

22 G. V. M. Sharma, K. L. Reddy, P. S. Laxmi, R. Ravi and A. C. Kunwar, *J. Org. Chem.*, 2006, **71**, 3967–3399.

23 O. Mitsunobu, M. Wada and T. Sano, *J. Am. Chem. Soc.*, 1972, **102**, 679–680.

24 (*a*) K. Tadano, Y. Idogaki, H. Yamada and T. Suami, *J. Org. Chem.*, 1987, **52**, 1201–1210; (*b*) A. S. Meyer and T. Reichstein, *Helv. Chim. Acta*, 1946, **29**, 152–162.

25 See ESI.†

26 Solvent titration studies were carried out by sequentially adding up to 50% of DMSO-d_6 to 600 μL $CDCl_3$ solutions of the peptides. Small changes in amide proton chemical shift ($\Delta\delta$NH) have been used as an indicator for H-bonding.

27 (*a*) A. A. Adzhubei and M. J. E. Sternberg, *J. Mol. Biol.*, 1993, **229**, 472–493; (*b*) P. K. Vlasov, G. T. Kilosanidze, D. L. Ukrainskii, A. V. V. G. Tumanyan and N. G. Esipova, *Biophysics*, 2001, **3**, 546–550; (*c*) F. Jourdan, S. Lazzaroni, B. L. Mendez, P. Lo Cantore, M. de Julio, P. Amodeo, N. S. Iacobellis, A. Evidente and A. Motta, *Proteins: Struct., Funct., Genet.*, 2003, **52**, 534–543.

28 M. Novotny and G. J. Kleywegt, *J. Mol. Biol.*, 2005, **347**, 231–241.

29 (*a*) O. Hertzberg and J. Moult, *Proteins: Struct., Funct., Genet.*, 1991, **11**, 223–229; (*b*) J. M. Petock, I. Y. Torshin, I. T. Weber and R. W. Harrison, *Proteins: Struct., Funct., Genet.*, 2003, **53**, 872–879.

30 (*a*) D. Neuhaus and M. Williamson, *The Nuclear Overhauser Effect in Structural and Conformational Analysis*, VCH Publishers, New York, 1989; (*b*) P. D. Thomas, V. J. Basus and T. L. James, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 1237–1241.

31 T. E. Creighton, *Proteins: Structures and Molecular Properties*, W. H. Freeman and Company, New York, 2nd edn, 1993.

32 M. A. Schmitt, B. Weisblum and S. H. Gellman, *J. Am. Chem. Soc.*, 2007, **129**, 417–428.

33 A. Glättli, X. Daura, D. Seebach and W. F. van Gunsteren, *J. Am. Chem. Soc.*, 2002, **124**, 12972–12978.

34 R. Mahalakshmi, G. Shanmugam, L. P. Prasad and P. Balaram, *ChemBioChem*, 2005, **6**, 2152–2158.

35 (*a*) D. J. States, R. A. Haberkorn and D. J. Ruben, *J. Magn. Reson.*, 1982, **48**, 286–292; (*b*) D. Marion, M. Ikura, R. Tschudin and A. D. Bax, *J. Magn. Reson.*, 1989, **85**, 393–399.