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Chirality and Template-Mediated Induction of Helical Preferences in Achiral β-Peptides

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Dedicated to Professor C. L. Khetrapal on the occasion of his 75th birthday

Abstract: This study describes chirality- or template-mediated helical induction in achiral β-peptides for the first time. A strategy of end capping β-peptides derived from β-hGly (the smallest achiral β-amino acid) with a chiral β-amino acid that possesses a carbohydrate side chain (β-Caa; C-linked carbo β-amino acid) or a small, robust helical template derived from β-Caas, was adopted to investigate folding propensity. A single chiral (*R*)-β-Caa residue at the C- or N-terminus in these oligomers led to a preponderance of right-handed 12/10-helical folds, which was reiterated more strongly in peptides capped at both the C- and N-terminus. Likewise, the presence of a template (a 12/10-helical trimer) at both the C- and N-terminus resulted in a very robust helix. The propagation of the helical fold and its sustenance was found in a homo-oligomeric sequence with as many as seven β-hGly residues. In both cases, the induction of helicity was stronger from the N terminus, whereas an anchor at the C terminus resulted in reduced helical propensity. Although these oligomers have been theoretically predicted to favor a 12/10-mixed helix in apolar solvents, this study provides the first experimental evidence for their existence. Diastereotopicity was found in both the methylene groups of the β-hGly moieties due to chirality. Additionally, the β-hGly units have shown split behavior in the conformational space to accommodate the 12/10-helix. Thus, end capping to assist chiralty- or template-mediated helical induction and stabilization in achiral β-peptides is a very attractive strategy.

Keywords: diastereotopicity · end capping · helical structures · peptides · template

Introduction

Template-nucleated helices in chiral α peptides, in which induction has been achieved by introduction of a few residues fixed in a helical conformation by covalent or metal-supported side-chain–side-chain bridges have been extensively studied.[1]Likewise, the concept of induction of helical screw sense in achiral peptides that contain rapidly interconverting left- and right-handed helices has evinced a great deal of interest.[2] The early work of Toniolo et al.[3] on achiral Aib (amino isobutyric acid) oligomers showed that the direction of the helical handedness is dependent on the position of the chiral guest residue. A further systematic study by Inai et al.[4] showed the influence of a chiral residue at either the C- or N-terminus, or both, on the induction of helical screw sense in achiral peptides with a propensity to form a 3_{10} helix. Though a single L-Leu residue is adequate to initiate left-handed helical folding, Ousaka and Inai[7] observed that oligomeric L-Leu (two or more residues) at the N terminus promotes a right-handed helical propensity. The phenomenon has been described as a domino effect[5,6] because a single chiral residue infers a profound influence to provide a distinct structure in the peptide. Interestingly, the generation of left-handed helices by a monomeric L-Leu terminal residue conforms with the observations of Shellman.[8] Chiral helical induction in aromatic foldamers instigated by a terminal chiral residue was also demonstrated by Huc et al.[9] Further research endeavors from Clayden et al.[10] resulted in the development of various NMR spectroscopic methods that utilized remote diastereotopic "reporter" groups to understand, both qualitatively and quantitatively, the handedness of the helical conformations that are rapidly interconverting. Such studies have provided evidence and rationale

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201201892. It contains NMR spectra, CD spectra, details of the MD calculations, and experimental details for compounds **4–6**, **11–12**, **18**, **21–23**, **26**, **28–33**, **36–37**, **39**, **42**, **45**, and **48**.

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for the role of the terminal/internal chiral residue, bound covalently or noncovalently, in the control of the helical screw sense.

Recent studies by Gellman et al.[11] in their chimeric design of the α/β+α-peptide family, reported that the α/β-peptide template (in this case, a 14/15-helical motif) facilitates the nucleation of a right-handed α-helical fold in the α-peptide region. More recently, we have demonstrated that a variety of compatible helical templates can be combined to generate a novel hybrid helical fold, which consists of up to three different helix types.[12]

Although all the above studies present different designs to induce helical screw sense in the achiral α peptides, such endeavors in the domain of achiral β-peptides are unexplored. Thus, it is imperative to investigate the induction of helicity in oligomers of achiral β-amino acids. The archetype of such β-peptidic homo-oligomers can be constructed from an achiral β-alanine, β-hGly (the simplest β-amino acid). β-hGly permits both *syn*- and *anti*-periplanar dihedral angles about Cα–Cβ.[13] Gellman et al.[14] predicted enhanced folding propensity in the oligomers of β-hGly very early from FTIR studies and attributed it to the repulsive interaction of the amide groups.[15,16] Interestingly, from NMR spectroscopic studies on poly(β-hGly), Glickson and Applequist[17] inferred that these peptides are disordered and possess little intermolecular hydrogen bonding. Further, Bode and Applequist have theoretically explored various types of helices in $(\beta\text{-hGly})_{12}$.[17c,18] Pavone et al.[19] have shown that the cyclic peptides that contain β-hGly adopt a well-defined 10-membered-ring hydrogen bond unit due to a *gauche* configuration. The ab initio quantum mechanical calculations by Hofmann et al.[20] and Wu and Wang[21] and coworkers predicted a variety of ordered secondary structures[22] in β-peptides. Though the β-hGly residue is known to destabilize the helices,[23] several groups[24–26] reported the presence of 12/10- and 14-helices in the peptides, derived from alternating chiral β-amino acids and β-hGly. The conformational preference to such foldings is attributed to the influence of the neighboring chiral[15,16] β-amino acid residues.

Based on the findings on the induction of helicity/screw sense[2,4–7,9–12] in achiral oligomers and the behavior of β-hGly, it was proposed that β-hGly oligomers, which do not reveal well-defined structures, could be persuaded to have helical folding. Thus, we planned to adopt the strategy of end capping the β-peptide oligomers formed from β-hGly, either with a chiral amino acid (β-Caa)[27] residue or with a robust template[28] derived from β-Caas (Figure 1), at the peptide termini to stimulate a secondary structure. This philosophy of anchoring the termini by two helical fragments

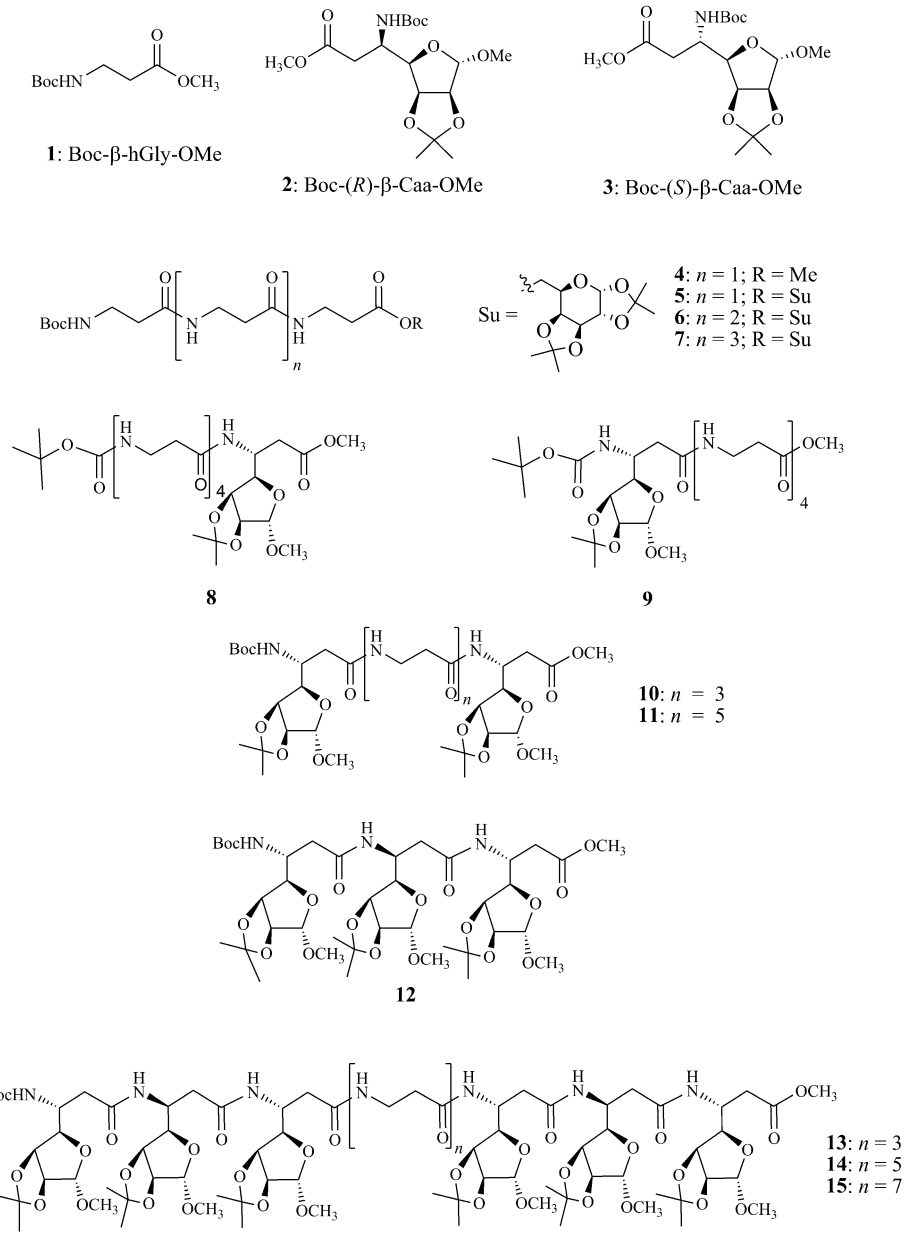

Figure 1. Structures of monomers **1**–**3** and peptides **4**–**15** (Boc = *tert*-butoxycarbonyl).

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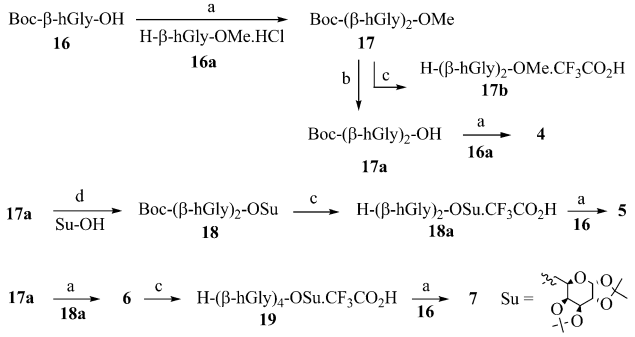
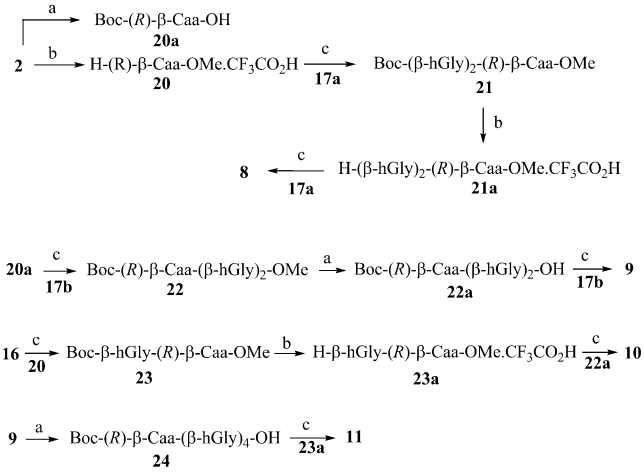
was also adopted by Huc et al.[29] in probing the helix-forming propensity of monomers. The present report more specifically describes our attempts towards the synthesis and investigation of the helix-forming potential of achiral β-peptides rich in β-hGly residues by NMR and circular dichroism (CD) spectroscopy and molecular dynamics (MD) studies.

Results and Discussion

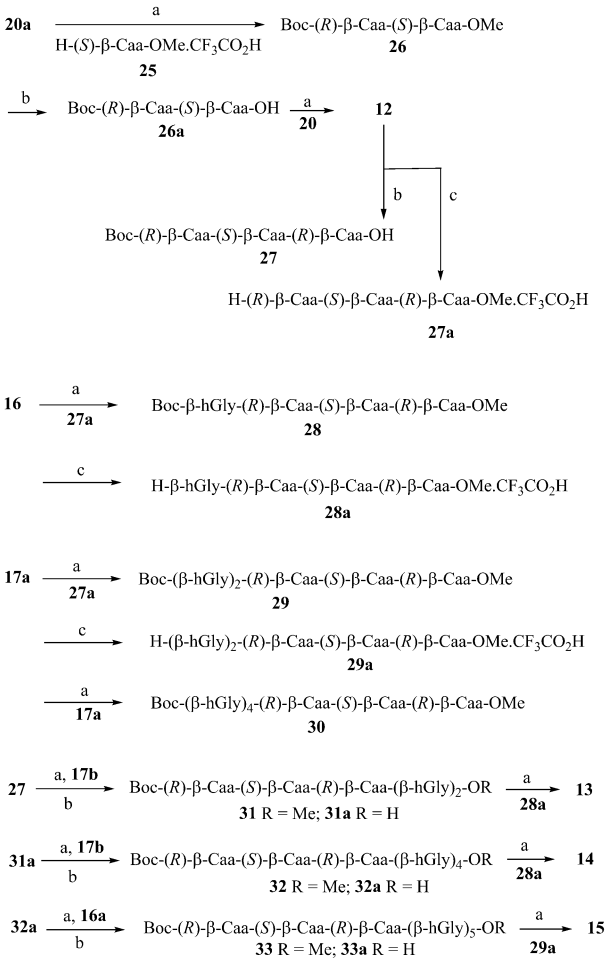
Peptide synthesis: The peptides **4–7** (Scheme 1), **8–11** (Scheme 2), **12–15**, and **29–32** (Scheme 3) were prepared from β-hGly **1**, β-Caas **2** and **3**, and standard peptide coupling reagents 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), 1-hydroxybenzotriazole (HOBt), and *N*,*N*-diisopropylethylamine (DIPEA)[30] in solution in CH_2Cl_2.



Scheme 1. Synthesis of peptides **4—7**. Reagents and conditions: a) 1-hydroxybenzotriazole (HOBt; 1.2 equiv), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI; 1.2 equiv), *N*,*N*-diisopropylethylamine (DIPEA; 2 equiv), dry CH_2Cl_2, 0 °C–RT; b) 4 N NaOH (aq), MeOH, 0 °C–RT, 2 h; c) CF_3COOH, dry CH_2Cl_2, 2 h; d) *N*,*N*-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), CH_2Cl_2, 0 °C–RT.



Scheme 2. Synthesis of peptides **8—11**. Reagents and conditions: a) 4 N NaOH (aq), MeOH, 0 °C–RT; b) CF_3COOH, dry CH_2Cl_2, 0 °C–RT; c) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (2 equiv), dry CH_2Cl_2, 0 °C–RT.



Scheme 3. Synthesis of peptides **13–15** and **29—32**. Reagents and conditions: a) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (2 equiv), dry CH_2Cl_2, 0 °C–RT; b) 4 N NaOH (aq), MeOH, 0 °C–RT; c) CF_3COOH, dry CH_2Cl_2, 0 °C–RT.

*Synthesis of peptides **4–7**:* The synthesis of peptides **4–7** is outlined in Scheme 1. Accordingly, peptide coupling of acid **16** with the HCl salt of β-hGly-OMe (**16a**) gave dipeptide **17** (82 %). Hydrolysis of **17** with 4 N NaOH (aq) furnished acid **17a**, whereas reaction of **17** with CF_3COOH in CH_2Cl_2 afforded salt **17b**. Coupling of acid **17a** with **16a** gave tripeptide **4** (65 %). Esterification of acid **17a** with galactose diacetonide, [(3a*R*,5*R*,5a*S*,8a*S*,8b*R*)-2,2,7,7-tetramethylperhydrodi[1,3]dio-xolo[4,5-b:4,5-d]pyran-5-yl]methanol, in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 gave ester **18** (83 %).

Exposure of dipeptide **18** to CF_3COOH in CH_2Cl_2 gave salt **18a**, which, on further reaction with acid **16** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2, afforded tripeptide **5** (62 %). Similarly, acid **17a** furnished tetrapeptide **6** (70 %) on peptide coupling with salt **18a**. Treatment of **6** with CF_3COOH in CH_2Cl_2 gave salt **19**, which on further condensation (EDCI, HOBt, and DIPEA) with **16** in CH_2Cl_2 furnished pentapeptide **7** (56 %) [Scheme 1].

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*Synthesis of peptides **8–11***: The synthesis of peptides **8–11** is outlined in Scheme 2. Accordingly, hydrolysis of ester **2** with 4 N NaOH (aq) afforded acid **20a**, whereas exposure of **2** to CF_3COOH in CH_2Cl_2 gave salt **20**. Peptide coupling of acid **17a** with salt **20** furnished tripeptide **21** (82%). Reaction of peptide **21** with CF_3COOH in CH_2Cl_2 gave salt **21a**, which afforded pentapeptide **8** (76%) on further coupling with acid **17a** under standard peptide coupling conditions.

Reaction of acid **20a** with salt **17b** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 afforded tripeptide **22** (82%). Base hydrolysis of peptide **22** gave acid **22a**, which furnished peptide **9** (79%) on further coupling with salt **17b** under standard conditions.

On reaction with salt **20** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2, acid **16** gave peptide **23** (87%). Treatment of peptide **23** with CF_3COOH in CH_2Cl_2 furnished salt **23a**, which afforded peptide **10** (79%) on further coupling with acid **22a** under standard conditions. Similarly, hydrolysis of **9** with 4 N NaOH (aq) gave acid **24**, which gave peptide **11** (66%) after coupling with salt **23a** in CH_2Cl_2.

*Synthesis of peptides **12–15** and **29–32***: Peptide coupling of acid **20a** with salt **25** (prepared by *tert*-butoxycarbonyl (Boc) deprotection of **3**) afforded dipeptide **26** (88%). Base hydrolysis [4 N NaOH (aq)] of ester **26** gave acid **26a**, which furnished tripeptide **12** (87%) on peptide coupling with salt **20**.[28] Further peptide coupling of acid **16** with salt **27a** gave tetrapeptide **28** (71%), which afforded salt **28a** after treatment with CF_3COOH in CH_2Cl_2. Peptide coupling of acid **17a** with salt **27a** furnished pentapeptide **29** (70%). Treatment of **29** with CF_3COOH in CH_2Cl_2 gave salt **29a**. Peptide coupling of acid **17a** with **29a** under standard coupling conditions afforded heptapeptide **30** (Scheme 3).

Similarly, acid **27** reacted with **17b** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 and gave pentapeptide **31** (93%), which subsequently afforded acid **31a** after base hydrolysis. Peptide coupling of acid **31a** with salt **28a** furnished peptide **13** (70%). Likewise, reaction of acid **31a** with **17b** in the presence of EDCI, HOBt, and DIPEA gave **32** (89%). Hydrolysis of peptide **32** with 4 N NaOH (aq) afforded acid **32a**, which furnished peptides **14** (70%) and **33** (63%) after coupling with either salt **28a** or **16a**, respectively, in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2. Peptide **33** gave acid **33a** after hydrolysis with 4 N NaOH (aq), which reacted with **29a** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 to afford peptide **15** (68%) [Scheme 3].

Conformational analysis: ^{1}H NMR spectroscopic studies on peptides **4–15** and **29–32** were carried out in $CDCl_3$ ($c = 3$–7 mM). The ^{1}H NMR spectrum of **5**, unlike achiral peptide **4**, revealed the signatures of chiral induction in the form of distinctly different chemical shifts for both the Cα protons ($\delta = 2.53$ and 2.63 ppm) and Cβ protons ($\delta = 3.39$ and 3.68 ppm) for the third β-hGly residue, whereas the protons of other β-hGly residues did not display diastereotopicity. Despite the observation of diastereotopic protons in **5**, in general, in both **4** and **5**, the amide protons were found to not be hydrogen bonded (deduced from the chemical shifts $\delta < 7$ ppm and very large changes in their chemical shifts of $\Delta\delta > 1.00$ ppm detected by solvent titration studies).[31,32] Peptide **6** behaved similarly to **5** and no structural features could be deciphered from its ^{1}H NMR spectrum, which may be due to its small size and fraying in the terminal residues. However, the ^{1}H NMR spectrum of **7**[32] supported the presence of two isomeric species in a ratio of 70:30, inferred from an extensive array of exchange peaks in the ROESY spectrum. Although both isomers showed the presence of hydrogen bonding, suggested by distinctive δ and $\Delta\delta$ values,[31,32] the broadening of resonances that arises due to exchange between the isomers prevented deduction of more meaningful information on their structures. Diastereotopicity was also observed for the methylene protons of the C-terminal β-hGly residue linked to D-galactose in peptides **6** and **7**. This finding is unlike the report of Clayden et al.,[33] who have shown diastereotopicity due to a single chiral center propagating as far as 60 bonds away.

The above results indicate the role of chiral induction by a carbohydrate residue, but also open up several possibilities. To investigate further, peptide **8** with a C-terminal (*R*)-β-Caa residue[26,28] (a β-amino acid with a carbohydrate side chain) was synthesized and investigated. The ^{1}H NMR spectrum of **8** displayed diastereotopicity for the methylene protons of all of the β-hGly residues. The spectrum showed the presence of two isomers in a 1:2 ratio. A careful study revealed the involvement of the last four amide protons ($\delta >$ 7.19 ppm and $\Delta\delta < 0.60$ ppm) in hydrogen bonding for the major isomer. The $^3J(H_N,H_{C\beta})$ couplings are not very distinctive for the last three residues (for the third and fourth residues the values are $J = 5.2$ and 7.1 Hz, respectively, whereas for the fifth residue $J = 8.2$ Hz), although their values seem to suggest some degree of constraint about ϕ (C(O)-N-Cβ-Cα)[34] with preponderance of $|\phi| \approx 120°$ (Figure 2).

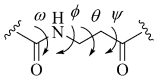

Figure 2. Definition of the dihedral angles in β-amino acids.

The above facts, as well as several discrete medium-range nOe correlations [for example, CβH(1)(*pro-R*)/CαH(3)(*pro-R*), CβH(1)(*pro-R*)/NH(3), CβH(3)(*pro-R*)/NH(5), CβH(3)(*pro-R*)/CαH(5)(*pro-R*)], along with sequential correlations CβH(1)(*pro-R*)/NH(2), CαH(1)(*pro-S*)/NH(2), NH(2)/CαH(3)(*pro-R*), CαH(2)(*pro-R*)/NH(3), CβH(3)(*pro-R*)/NH(4), CαH(3)(*pro-S*)/NH(4), NH(4)/CαH(5)(*pro-R*), CαH(4)(*pro-R*)/NH(5), and NH(4)/NH(5), provide emphatic support for the presence of a 12/10-helix.[26,28] These findings are very important; they not only provide unmistakable evidence for the induction of a helical preference in achiral peptides, but also provide the first unequivocal experimental proof for the theoretically predicted preference for the 12/10-helix in β-peptides in apolar solvents.[20,21]

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MD calculations were undertaken by using the distance constraints obtained from the ROESY data, and two spin approximations were employed. Dihedral angle constraints were not used in these calculations, although starting geometries were consistent with the data. Twenty superimposed low-energy structures[32] very clearly showed a right-handed 12/10-helix with average root-mean-square deviation (RMSD) values for the backbone and heavy atoms of 0.70 and 0.98 Å, respectively. Significant fraying at the peptide termini can be noticed. This result clearly demonstrates that the chirality end capping by (*R*)-β-Caa with a furanoside side chain induced right-handed helicity in the achiral β-hGly oligomer **8**.

Like the major isomer of **8**, the minor isomer displayed diastereotopicity for each of the methylene protons for all of the β-hGly residues. The minor isomer of **8** showed very distinct signatures of hydrogen bonding of the amide protons [$\delta = 6.99$, 7.99, 7.62, and 8.13 ppm for the second to fifth amide protons, respectively, and $\Delta\delta < 0.71$ ppm]. In addition, the $^3J(H_N,H_{C\beta})$ values for (*R*)-β-Caa ($J = 8.5$ Hz) and β-hGly ($J > 8.2$ Hz or $J < 4.6$ Hz) residues were consistent with $|\phi| \approx 120°$. For the minor isomer of **8**, nOe correlations NH(1)/NH(2) and NH(1)/NH(3) were also observed in the ROESY spectrum. However, due to lack of sufficient nOe constraints, deduction of the structure was not possible. In addition, though theoretically a 14-helix is an energetically favored conformation, the above data does not support the presence of such a structure for the minor isomer.

Having observed remarkable induction of a right-handed helical structure prompted by the chirality of a carbohydrate side chain in peptide **8**, peptide **9**, with an N-terminal (*R*)-β-Caa residue, was investigated. The inference from NMR spectroscopic studies of **9** in $CDCl_3$ was found to be very similar to **8**. A 12/10-helical pattern, as discussed below, was observed with a slightly larger population (≈70%) for the major isomer. The involvement of the amide protons of last four residues in hydrogen bonding is supported ($\delta >$ 7.19 ppm and $\Delta\delta_{NH} < 0.70$ ppm). A large number of observed couplings in **9** provided emphatic support for the constrained values for ϕ and θ. For β-hGly, $^3J(H_N,H_{C\alpha}) <$ 4.7 Hz or $^3J(H_N,H_{C\alpha}) > 7.8$ Hz and $^3J(H_{C\alpha},H_{C\beta}) > 11$ Hz or $^3J(H_{C\alpha},H_{C\beta}) < 4.5$ Hz are consistent with $|\phi| \approx 120°$ and $\theta \approx 60°$. The nOe correlations CβH(1)/NH(3), CβH(1)/CαH(3)(*pro-R*), NH(2)/CαH(3)(*pro-R*), NH(2)/NH(3), CβH(3)(*pro-R*)/NH(5), CβH(3)(*pro-R*)/CαH(5)(*pro-R*), NH(4)/CαH(5) (*pro-R*), and NH(4)/NH(5), besides the observations mentioned above, strongly support a 12/10-helix in **9**. Twenty superimposed structures from the MD calculations (Figure 3) with backbone and heavy atom RMSD values of 0.65 and 0.83 Å, respectively, also support the above findings for **9**.

The results for the minor isomers are very similar to those for **7** and **8**.[32] All of the amide protons, except NH(1), appeared at $\delta > 6.92$ ppm, which, along with $\Delta\delta < 0.75$ ppm determined by solvent titration studies, confirms their involvement in hydrogen bonding. The ^{1}H NMR spectrum was well dispersed; all of the protons could be assigned properly and

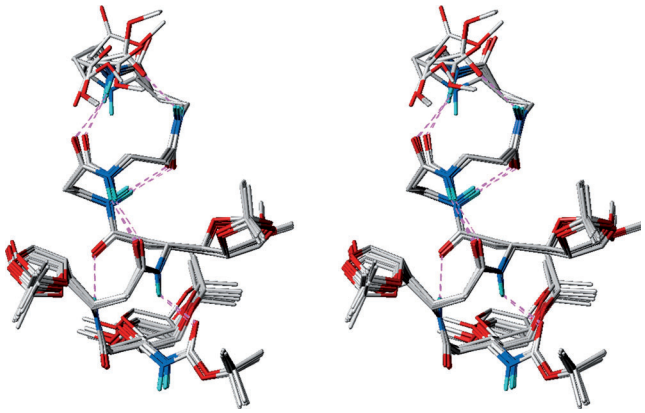

Figure 3. Stereoviews of the superimposition of the 20 lowest-energy structures of peptide **9** determined on the basis of NMR spectroscopic measurements in solution in $CDCl_3$ (hydrogen bonds are shown as dotted lines).

coupling information could be obtained for most of them. Prochirality of the methylene groups was assigned with help of the exchange peaks between the analogous protons of the major isomer.

As in the case of the minor isomer of peptide **8**, $^3J(H_N,H_{C\beta}) \leq 4.5$ Hz and $^3J(H_N,H_{C\beta}) \geq 7.7$ Hz for β-hGly and $^3J(H_N,H_{C\beta}) = 9.9$ Hz for (*R*)-β-Caa indicate $|\phi| \approx 120°$; one large and one small value for $^3J(H_{C\beta},H_{C\alpha})$ is consistent with $\theta \approx 60°$. Also, the nOe correlations NH(1)/NH(2) and NH(1)/NH(3) were present, as shown in the expansion of the amide region in the ROESY spectrum (Figure 4). Though the above information and the absence of an NH(2)/NH(3) nOe correlation in the minor isomer indicate some departure from a 12/10-helical pattern at the N terminus, we still failed to decipher the structure.

The findings for achiral β-peptide **9**, which show the presence of a higher population of the 12/10-helix, further support stronger induction from the N terminus, as was observed for achiral α-peptides.[4,10] Though the revelation of major isomers with right-handed 12/10 helices in **8** and **9** is quite an encouraging result and reflects the important role played by the chiral side chain in helical induction in β-hGly oligomers, the presence of a sizeable population of the other isomer reflects the compromised robustness of the helix. Thus, it was felt worthwhile to adopt the strategy of end capping the β-hGly oligomers at both the C- and N-terminus with an (*R*)-β-Caa residue.

As envisaged, the NMR spectrum of **10** showed enhanced population of the major isomer (95%) with all the signatures of a robust 12/10-helix.[32] The involvement of the amide protons of the last four residues in hydrogen bonding was supported ($\delta > 7.54$ ppm and $\Delta\delta = 0.12$ to -0.60 ppm). All of the derived couplings that involved the backbone support a fairly robust helical pattern. A large number of the observed couplings provided emphatic support for constrained dihedral angles (β-hGly: $^3J(H_N,H_{C\alpha}) > 9.5$ Hz or $^3J(H_N,H_{C\alpha}) < 4.0$ Hz and $^3J(H_{C\alpha H},H_{C\beta}) > 9.3$ Hz or $^3J(H_{C\alpha H},H_{C\beta}) < 4.3$ Hz).

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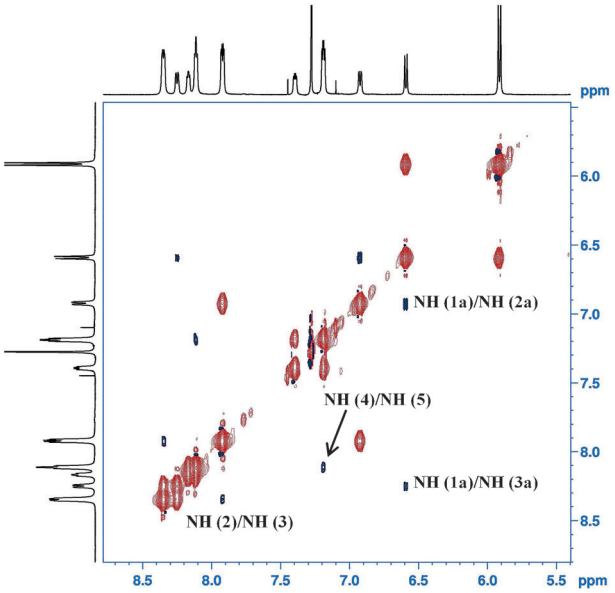

Figure 4. Expansion of the amide region in the ROESY spectrum of peptide **9** to show NH–NH nOe correlations (black) and exchange peaks (red) between the two isomers. 1a, 2a, and 3a refer to the peaks of the minor isomer for the amide protons of residues 1, 2, and 3, respectively.

The nOe correlations,[32] coupling constants, and MD data, support a very robust 12/10-helix compared to oligomers **8** and **9** capped at only the C- or N-terminus, respectively. Twenty superimposed structures of **10** with backbone and heavy atom RMSD values of 0.64 and 0.81 Å, respectively, are shown in Figure 5. However, the structure of the minor isomer was unresolved.

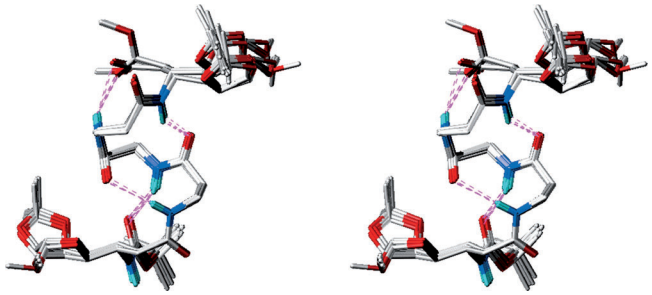

Figure 5. Stereoviews of the superimposition of the 20 lowest-energy structures of peptide **10** determined on the basis of NMR spectroscopic measurements in solution in $CDCl_3$ (hydrogen bonds are shown as dotted lines).

Heptapeptide **11** showed a trend similar to that displayed by **10**, with a population of about 92% for the major isomer. The last six amide protons appear to be hydrogen bonded ($\delta > 7.63$ ppm and $\Delta\delta_{NH} = 0.09$ to -0.82 ppm). The derived couplings that involve the backbone protons imply constrained dihedral angles (β-hGly: $^3J(H_N,H_{C\alpha}) < 4.2$ Hz or $^3J(H_N,H_{C\alpha}) > 8.5$ Hz and $^3J(H_{C\alpha},H_{C\beta}) > 9.3$ Hz or $^3J(H_{C\alpha},H_{C\beta}) < 4.3$ Hz). The nOe correlations contain distinct signatures of the 12/10-helix and the robustness of the helix is further supported by the MD data.[32]

It was reassuring to be able to induce a helical structure with a preference to exist as right-handed 12/10-helix in achiral β-peptides **8**–**11** by end capping with (*R*)-β-Caa. Although the populations of the major isomers increased substantially when both the peptide termini were anchored with (*R*)-β-Caa, all of the peptides studied above showed the presence of sizeable populations of other structures. Consequently, we decided to explore helix motif **12**,[28] based on the concept of hybrid helices, for the induction of enhanced helical propensities in β-hGly oligomers and prepared peptides **13**–**15**.

For peptide **13**, a highly dispersed ^{1}H NMR spectrum[32] infers the existence of a well-defined structure. The ROESY spectrum displayed several exchange peaks that implied the presence of two isomers. However, the ^{1}H NMR spectrum did not reveal significant signatures of the minor isomer, which suggested a minuscule population (possibly $<1\%$). All of the amide protons, except NH(1), had $\delta > 7.94$ ppm and only a very small change in their chemical shifts ($\Delta\delta = 0.01$ to -0.32 ppm) was detected by solvent titration studies (Figure 6),[32] which provided adequate support for their involvement in intramolecular hydrogen-bonded structures. This indicates that the helical structures are retained in the terminal tripeptide fragments, which also ensures induction and continuity of the helical fold in the β-hGly segment. It was observed that the backbone dihedral angles of β-Caa as well as β-hGly residues are constrained, reflected by the coupling constants.[32] All six of the β-Caa residues at the termini have $^3J(H_N,H_{C\beta}) > 9.0$ Hz, which corresponds to dihedral angles $\phi \approx -120°$ and 120° for (*R*)-β-Caa and (*S*)-β-Caa, respectively. This implies an *anti*-periplanar arrangement of NH and CβH. One small and one large coupling constant ($^3J(H_{C\alpha},H_{C\beta}) \approx 3$ and 12 Hz) for (*R*)-β-Caa support a value for θ of about 60° or 180°. These couplings, as well as one

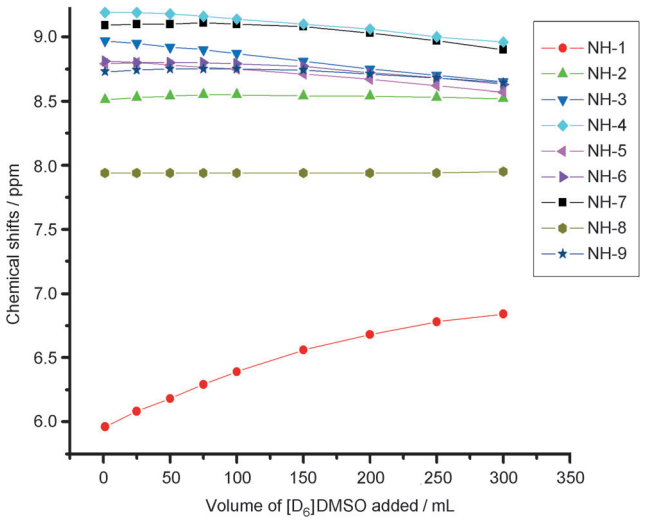

Figure 6. Solvent dependence of NH chemical shifts at varying concentration of [D_6]-DMSO in $CDCl_3$ (600 μL) from titration studies of peptide **13**.

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strong and another medium intensity inter-residue nOe correlation that involves CαH and NH supported $\theta \approx 60°$. Similarly, for (*S*)-β-Caa, two small coupling constants ($^3J(H_{C\alpha},H_{C\beta}) \approx 5$ and 3 Hz) are consistent with $\theta \approx 60°$. This also enabled us to deduce from the very strong nOe correlations NH(2)/CαH(1)(*pro-S*), NH(4)/CαH(3)(*pro-S*), and NH(8)/CαH(7)(*pro-S*) that the dihedral angle $\psi \approx 100°$ for (*R*)-β-Caa. Similarly, the presence of NH(3)/CαH(2)(*pro-R*) and NH(9)/CαH(8)(*pro-R*) specifies $\psi \approx -100°$ for (*S*)-β-Caa. The terminal capping sequences also display medium range nOe correlations [CβH(1)/NH(3), CβH(1)/CαH(3)-(*pro-R*), NH(2)/CαH(3)(*pro-R*), NH(2)/NH(3), CβH(7)/NH(9), CβH(7)/CαH(9)(*pro-R*), NH(8)/CαH(9)(*pro-R*), and NH(8)/NH(9)], which additionally qualifies a mixed 12/10-helix for the tripeptide motifs at the peptide termini.

The β-hGly fragment in **13** also displayed signatures of a structure that corresponds to a mixed 12/10-helix.[32] Due to spectral overlap, only a few of the $^3J(H_{C\alpha},H_{C\beta})$ coupling constants could be obtained, however, all of them had extreme values ($^3J(H_{C\alpha},H_{C\beta}) > 12.5$ Hz or $^3J(H_{C\alpha},H_{C\beta}) < 4.5$ Hz ; $^3J(H_N,H_{C\beta}) > 8.4$ Hz or $^3J(H_N,H_{C\beta}) < 4.1$ Hz). The nOe correlations [CβH(3)/NH(4), CβH(3)/NH(5), CβH(3)/CαH(5)(*pro-R*), NH(4)/CαH(3)(*pro-S*), NH(4)/CαH(5)(*pro-R*), NH(4)/NH(5), CβH(5)(*pro-R*)/NH(6), CβH(5)(*pro-R*)/NH(7), NH(6)/CαH(5)(*pro-S*), and NH(6)/CαH(7)(*pro-R*)], along with the structural features already elaborated, confirm the continuity of a mixed helical structure. Thus, the peptide **13** has a 12/10-helix with a $(12/10)_4$ hydrogen-bonded arrangement. Terminal helical motifs nucleate these folds, which effectively regulate and propagate the helix throughout the length of the oligomer. In this helical organization, it was remarkable to note that the β-hGly residues in the fourth and sixth positions behave differently compared to that in the fifth position. For β-hGly(4) and β-hGly(6), the observed sequential nOe correlations NH(*i*)/CαH(*pro-S*)(*i*−1) and NH(*i*)/CαH(*pro-R*)(*i*+1) [*i* = 4, 6] are noticed for the amide protons of (*S*)-β-Caa, whereas for β-hGly(5), the nOe correlation NH(5)/CαH(*pro-R*)(4) is similar to that for the amide protons of (*R*)-β-Caa. This interesting split behavior of the β-hGly residues, which span two different conformational spaces, is a consequence of the alternating chirality in the helix design of peptide **13** and is an elegant demonstration of the accommodative nature of β-hGly in the mixed-helical folds. Similar split behavior is observed in all of the peptides studied above.

Twenty superimposed low-energy structures of **13** obtained from the MD calculations are shown in the Figure 7. The average RMSD values of the backbone and heavy atoms are 0.52 and 0.68 Å, respectively.

Close scrutiny of the above nOe correlations reveals that in β-peptides with an alternation of (*R*)- and (*S*)-β-Caa residues the nOe correlations required to establish a right-handed 12/10-helical pattern can be generalized (Figure 8). The nOe correlations NH(*i*)/CαH(*i*−1)(*pro-S*), NH(*i*)/CαH-(*i*+1)(*pro-R*), and NH(*i*)/NH(*i*+1) support formation of 10-membered-ring hydrogen bonding of NH(*i*) with CO(*i*+1), whereas NH(*i*+1)/CαH(*i*+1)(*pro-R*), NH(*i*+1)/CαH(*i*)(*pro-R*), CβH(*i*−1)/NH(*i*), CβH(*i*−1)/NH(*i*+1), and CβH(*i*−1)/CαH(*i*+1)(*pro-R*) confirm 12-membered-ring hydrogen bonding of NH(*i*+1) with CO (*i*−2) [Figure 8]. In the region rich in β-hGly, the relations modify nominally. Specifically, we need to replace CβH(*i*−1) with CβH(*i*−1)(*pro-R*) for β-hGly residues.

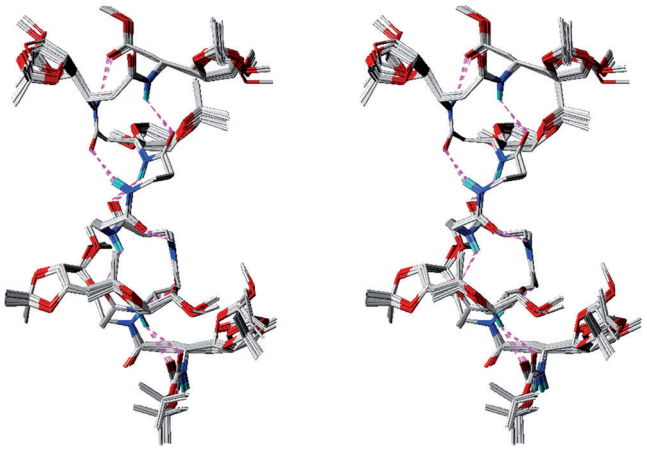

Figure 7. Stereoviews of the superimposition of the 20 lowest-energy structures of peptide **13** determined on the basis of NMR spectroscopic measurements in solution in $CDCl_3$ (hydrogen bonds are shown as dotted lines).

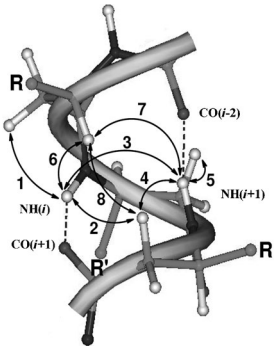

Figure 8. The nOe correlations required to establish a right-handed 10/12-helical pattern in β-peptides (*R*)-β-Caa (R = sugar, R' = H), (*S*)-β-Caa (R = H, R' = sugar), and β-hGly (R = R' = H). 1) NH(*i*)/CαH(*i*−1)(*pro-S*); 2) NH(*i*)/CαH(*i*+1)(*pro-R*); 3) NH(*i*)/NH(*i*+1); 4) NH(*i*+1)/CαH(*i*+1)-(*pro-R*); 5) NH(*i*+1)/CαH(*i*)(*pro-R*); 6) CβH(*i*−1)*/NH(*i*); 7) CβH-(*i*−1)*/NH(*i*+1); 8) CβH(*i*−1)*/CαH(*i*+1)(*pro-R*). NH(*i*) participates in 10-membered-ring hydrogen bonding with CO(*i*+1) and NH(*i*+1) forms a 12-membered-ring hydrogen bonding system with CO(*i*−2). *For β-hGly, CβH(*i*−1) is replaced by CβH(*i*−1)(*pro-R*).

Further NMR spectroscopic studies of **14** and **15**, with five and seven β-hGly residues, respectively, helix capped by the (*R*,*S*,*R*) template at both the C- and N-terminus,[28] indicated the propagation of the 12/10-mixed-helical pattern. In **14**, it very clearly emerged that the $(\beta\text{-hGly})_5$ segment end capped between the two 12/10-helices at the termini is accommodated in an elongated helix with a $(12/10)_5$ hydrogen-bonded configuration. However, for peptide **15** although there was a severe overlap, most of the characteristic cou-

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plings and nOe correlations, along with the derived hydrogen-bonding information, provided unmistakable signatures of the 12/10-helix. Thus, similar propagation of the helix in peptide **15** further shows that as many as seven consecutive β-hGly residues are held in a 12/10-helical fold by end capping with two robust helices.

Twenty superimposed low-energy structures for **14** deduced from MD studies[32] have shown the average RMSD of the backbone and heavy atoms are 0.63 and 0.96 Å, respectively. However, for **15**, due to severe overlap of the nOe cross peaks in the ROESY spectrum,[32] it was difficult to deduce the volume integrals and, hence, the distance constraints, which restricted the use of MD studies.

The above observations on induction and propagation of helicity in achiral β-peptides (*R*,*S*,*R*) helix-capped at both the C- and N-terminus, along with the findings of Gellman et al.,[11a] naturally led us to study single-capped peptides **29** and **30** (capped at the C terminus) and **31** and **32** (capped at the N terminus).

From the ^{1}H NMR spectrum of **29** and **30**, two isomers were found in equilibrium, in ratios of 70:30 and 75:25, respectively. The results show that the major isomers contain all the signatures of a 12/10-helix.[32] For the minor isomers, although several hydrogen-bonded amide protons were observed, it was not possible to get many structural details. The MD structure for the major isomer of **29** confirmed the 12/10-helix,[32] whereas for **30** such calculations could not be undertaken due to spectral overlap and broadening of the resonances.

Similarly, both peptides **31** and **32**, end capped with a single (*R*,*S*,*R*) helix at the N terminus, displayed all the characteristic signatures for the presence and propagation of a mixed 12/10-helix. The exchange peaks in the ROESY spectra of **31** and **32** imply an equilibrium between two isomers, in which the populations of the minor isomers was too small to be determined (probably $<1\,\%$), unlike in **29** and **30**.

It was gratifying to learn that 12/10-helical folds were induced in oligomers rich in β-hGly residues (**13**–**15** and **29**–**32**) by the robust terminal (*R*,*S*,*R*)-helical segments. The observation that a helix at either the C- or N-terminus is adequate to induce the desired helical fold in the achiral β-hGly oligomer is notable. All four peptides **29**–**32** generated a mixed 12/10-helix as the major isomer, although the peptides capped at the N terminus have shown one isomer almost excusively and, thus, appear to have more robust helices.

To evaluate the effectiveness of helical induction by (*R*)-β-Caa in polar solvents, NMR spectroscopic studies were also carried out on **13** in deuterated methanol ($c=3$–5 mM); a 98:2 ratio of major/minor isomer was detected. H/D exchange studies of the amide protons are typically used to obtain information on hydrogen bonding in CD_3OD. For peptide **13** it was observed that the amide protons of the fourth to sixth β-hGly residues exchange rapidly (within a minute), whereas all the protons of the (*R*,*S*,*R*)-helical motifs, except NH(1), were present for about an hour.[32] This implies that the template has a weak tendency to retain its helical fold and induce helicity, and suggests that **13** contains a substantial population of disordered structures in solution in methanol. This conclusion was further supported by the ROESY data obtained in CD_3OH,[32] which, despite severe overlap, showed the presence of weak nOe cross peaks [NH(2)/NH(3), NH(8)/NH(9), and CβH(1)/NH(3)] that corresponded to the 12/10-helical folds of the templates. Coupling constants of $^3J(H_N,H_{C\beta})>8.4$ Hz for all of the β-Caa residues (except for the seventh residue) provided additional evidence for the structure. Although the Cα and Cβ protons of all the β-hGly residues displayed diastereotopicity, only the sixth residue had two distinct coupling constants ($^3J(H_N,H_{C\beta}=5.2$ and 6.5 Hz). These observations show that helical induction in methanol is significantly weaker than that observed in $CDCl_3$.

The CD spectra[32] of peptides **8**–**11** (Figure 9a), **13**–**15** (Figure 9b), and **29**–**32** (Figure 9c) were obtained from solutions in methanol ($c=0.2$ mM). Peptides **13**–**15** and **29**–**32** showed distinct maxima at about 202 nm, with positive molar ellipticity (θ) values above 195 nm, which is consistent with a 12/10-helix.[26,28,35] These results, as indicated from the NMR spectroscopic studies, show that as the β-hGly sequence becomes longer, the value of θ decreases, which implies that integrity of the helical folds is being challenged and, thus, the robustness of the 12/10-helix is compromised.

However, the observed lower molar ellipticity values[32] for peptides **8**–**11**, with maxima less-than-half as intense compared to **13**–**15**, may be attributed to weak helical induction by a single chiral amino acid residue relative to template-mediated induction by **12** (see reference [28] for the CD spectrum). In addition, the CD data is consistent with the NMR spectroscopic observations for stronger helical induction from the N terminus.[32]

The CD spectra of all of the peptides, except for **8**, showed similar θ maxima that corresponded to a 12/10-helix. As already discussed, a comparison of the NMR spectroscopic data of **8** and **9** in $CDCl_3$ indicated the presence of a 12/10-helix in peptide **8**, although the induction was shown to be stronger in **9**. Consistent with NMR spectroscopic observations, the CD spectrum of **9** in methanol reflects a better definition of the 12/10-helix, with a maxima at $\theta=$ 204 nm. However, for **8**, the CD spectrum looked different, which suggested very weak 12/10-helical folding (Figure 9a).

Having observed a rather distinctive helical induction by end capping β-hGly oligomers with (*R*)-β-Caa, we thought it was meaningful to explore the generality of this induction with other β-amino acids. To extend the study, cyclic and acylic β-amino acids *trans*-3-aminopyran-2-carboxylic acid (APyC) **34**[36] and β-hPhe **35** were utilized for the synthesis of end-capped pentapeptides **36** and **37** (Figure 10), respectively, to further understand the helical induction.

Synthesis of peptides 36 and 37: The synthesis of the peptides **36** and **37** is outlined in Scheme 4. Accordingly, coupling of acid **16b** with salt **38** (prepared from **34**[36] in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2) furnished dipeptide **39** (88 %), which on reaction with CF_3COOH in CH_2Cl_2

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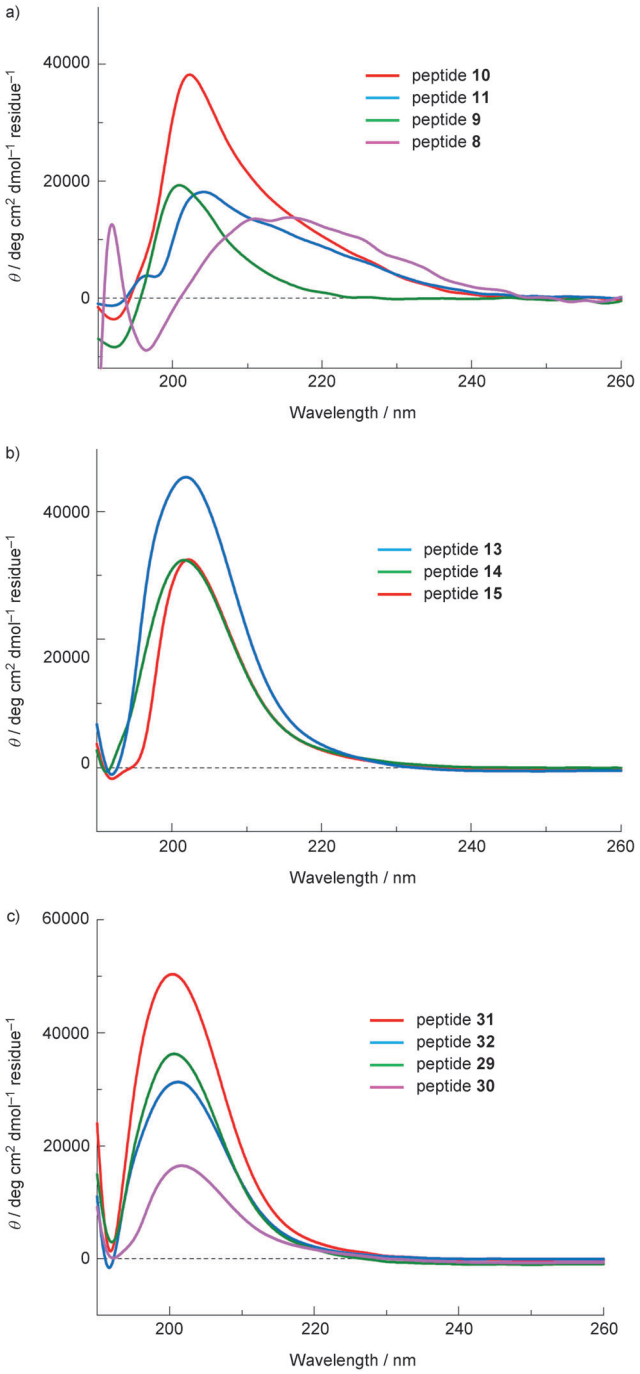


Figure 9. a) CD spectra of peptides **8**, **9**, **10**, and **11** in MeOH; b) CD spectra of peptides **13**, **14**, and **15** in MeOH; c) CD spectra of peptides **29**, **30**, **31**, and **32** in MeOH.

gave salt **40**. Coupling of acid **41** (prepared from **34**)[36] with salt **17b** under standard conditions afforded tripeptide **42** (79%). Hydrolysis of **42** with 4N NaOH (aq) afforded acid **43**. Finally, treatment of **43** with salt **40**, in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 furnished pentapeptide **36** (60%).

Similarly, synthesis of peptide **37** was achieved from **35** as shown in Scheme 4. Thus, acid **17a** was coupled (EDCI,

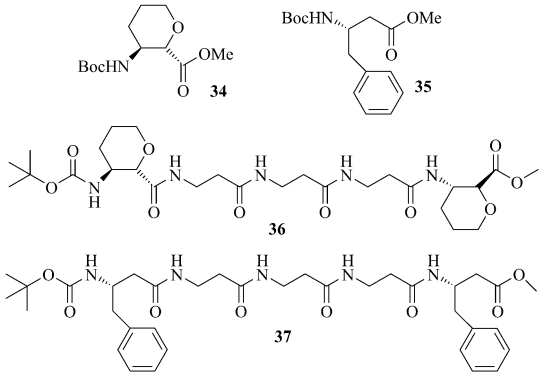
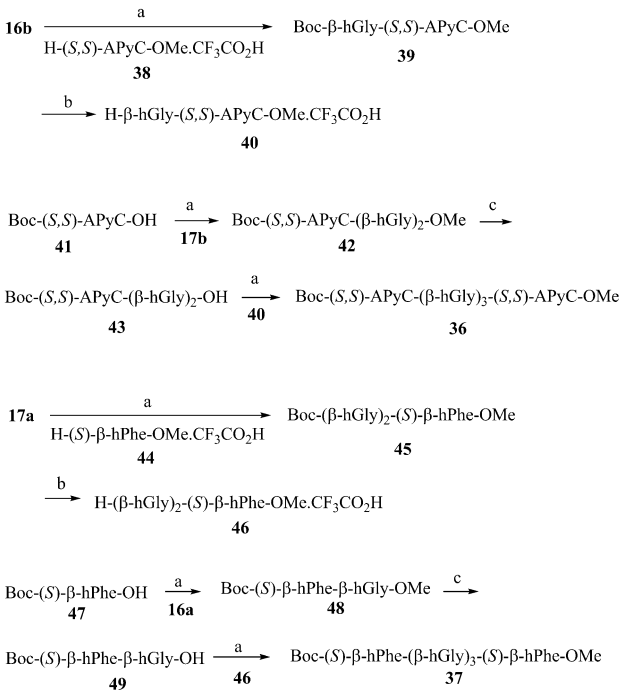


Figure 10. Structures of monomers **34** and **35** and peptides **36** and **37**.



Scheme 4. Synthesis of peptides **36** and **37**. Reagents and conditions: a) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (2 equiv), dry CH_2Cl_2, 0°C–RT; b) CF_3COOH, dry CH_2Cl_2, 2 h; c) 4N NaOH (aq), MeOH, 0°C–RT, 2 h.

HOBt, DIPEA) with salt **44** (prepared from **35**) in CH_2Cl_2 to give tripeptide **45** (75%), which afforded salt **46** on exposure to CF_3COOH in CH_2Cl_2. Likewise, coupling of acid **47** (prepared from **35**) with salt **16a** under standard peptide coupling conditions furnished dipeptide **48** (71%), which gave acid **49** after hydrolysis with 4N NaOH (aq). Finally, treatment of **49** with salt **46** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 furnished pentapeptide **37** (29%).

Conformational analysis of 36 and 37: The ^{1}H NMR spectrum of **36** in $CDCl_3$ (293 K)[32] showed a well-dispersed spectrum, which implied the presence of secondary structure

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(>99% population). Except NH(1), all of the amide protons resonated at $\delta > 7$ ppm, which indicated their participation in hydrogen bonding. This was further confirmed by the small $\Delta\delta$ value detected from solvent titration studies[31] ($\Delta\delta < 0.38$ ppm). For the (*S*,*S*)-APyC residues, coupling constants $^3J(H_N,H_{C\beta}) > 9.1$ Hz and $^3J(H_{C\alpha},H_{C\beta}) > 9.5$ Hz are consistent with $\phi \approx -120°$ and $\theta \approx 60°$. Strong nOe correlations for NH(*i*)/CαH(*i*) support the deduced value of θ. For the β-hGly residues coupling constants of $^3J(H_N,H_{C\beta}) > 9.4$ Hz or $^3J(H_N,H_{C\beta}) < 2.7$ Hz and $^3J(H_{C\alpha},H_{C\beta}) > 12.5$ Hz or $^3J(H_{C\alpha},H_{C\beta}) < 4.3$ Hz reflect constrained values of ϕ and θ. A careful examination of the coupling constants suggests that they are consistent with angles of $\phi \approx -120°$ for β-hGly(2) and β-hGly(4) and $\phi \approx 120°$ for β-hGly(3). The data further indicates that $\theta \approx 60°$ for all the β-hGly residues. Additionally, the nOe correlations CβH(1)/NH(3), CβH(1)/CαH(3)(*pro-R*), NH(2)/CαH(3)(*pro-R*), NH(2)/NH(3), CβH(3)(*pro-R*)/NH(5), CβH(3)(*pro-R*)/CαH(5), NH(4)/CαH(5), and NH(4)/NH(5), along with the values of the coupling constants, emphatically support the propagation of a 12/10-helix along the length of the oligomer. These results are comparable with those derived for the analogous peptide **10**, end capped with (*R*)-β-Caa. Twenty superimposed low-energy structures of **36** obtained from the MD calculations[32] are shown in Figure 11. The average RMSD values of the backbone and heavy atoms are 0.31 and 0.56 Å, respectively. The CD spectrum in methanol supports the presence of a secondary structure.[32]

NMR spectroscopic studies on peptide **36** were also carried out in deuterated methanol;[32] a ratio of 94:6 for the major/minor populated isomers was detected in CD_3OH. H/D exchange studies in CD_3OD showed that all of the amide proton resonances disappeared within 1 h, suggestive of weak helical induction. Observation of only a few nOe correlations [NH(1)/NH(2), NH(2)/NH(3), and CβH(1)/NH(3)] further supported the presence of a weak helical structure and the substantial population of disordered structures.

The ^{1}H NMR spectra of peptide **37** in $CDCl_3$ (273 K)[32] showed the presence of two isomers in equilibrium in a ratio of 63:37. For the major isomer all the amide protons, except NH(1), resonated at $\delta > 7.2$ ppm, which indicated their participation in hydrogen bonding. The small $\Delta\delta$ value detected from solvent titration studies[31] ($\Delta\delta < 0.39$ ppm) further confirmed the presence of hydrogen bonding. In the major isomer, the large coupling constant ($^3J(H_N,H_{C\beta}) > 8.5$ Hz) for β-hPhe shows an *anti*-periplanar arrangement of NH and CβH. Due to severe spectral overlap, many of the coupling constants could not be obtained, especially for the β-hGly residues. However, the presence of characteristic nOe correlations [CβH(1)/NH(3), CβH(3)/NH(5), CβH(1)/Cα'H(3), CβH(3)/Cα'H(5), NH(4)/NH(5), NH(2)/Cα'H(3), and NH(4)/Cα'H(5)] provides sufficient evidence for the presence of a 12/10-helix.

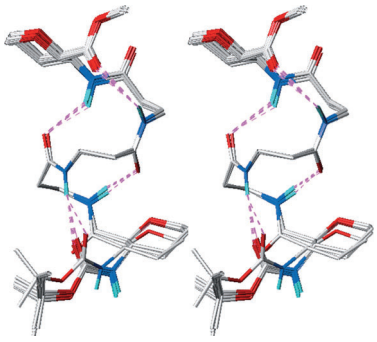

Figure 11. Stereoviews of the superimposition of the 20 lowest-energy structures of peptide **36** determined on the basis of NMR spectroscopic measurements in solution in $CDCl_3$ (hydrogen bonds are shown as dotted lines).

In the case of the minor isomer,[32] the amide protons NH(3) and NH(5) resonate at $\delta > 7.8$ ppm and, additionally, a small $\Delta\delta$ value supports their involvement in hydrogen bonding ($\Delta\delta < 0.23$ ppm). For β-hPhe, the coupling constant value of $^3J(H_N,H_{C\beta}) > 8.5$ Hz suggests the *anti*-periplanar arrangement of NH and CβH. Lack of sufficient information for the coupling constants and nOe correlations prevented the elucidation of the structure of the minor isomer, as was the case with other peptides.

The above analyses on peptides **36** and **37** amply demonstrates that chiral induction of a 12/10-helix in achiral β-peptides can be achieved by β-amino acids other than β-Caa. The preorganized cyclic β-amino acid **34** almost exclusively generated a 12/10-helix, whereas induction was much weaker for the unconstrained β-amino acid **35** with a proteinogenic side chain.

We performed variable temperature (VT) NMR studies on **37** because of the very good quality of the ^{1}H NMR spectrum, with sharp resonances, as well as the largest population of the minor isomer among the peptides investigated. A study between $T = 223$ K and 298 K showed that the population of the minor isomer is practically unchanged (33 ± 2% at 223 K and 37 ± 2% at 298 K). From the populations of the two isomers, it was inferred that the major isomer is energetically more favored by ≈ 0.3 kcal mol^{-1} relative to the minor isomer.[32] The energy differences between the major and minor isomers of all the peptides are provided in the Supporting Information.

Finally, it is pertinent to mention that all of the peptides, whether end capped with chiral residues or templated, have shown the induction of helicity and distereotopicity in the β-hGly residues. Diastereotopicity was observed for the Cα and Cβ protons of all the β-hGly residues in the peptides end capped with (*R*)-β-Caa or the tripeptide helical template, which enables them to be used as reporter groups to indicate chiral induction in an achiral β-peptide.[10,33] Interestingly, the peptides with a D-galactose protecting group do not induce such universal diastereotopicity, which highlights that the chemical environment in the helical backbone results in very significant asymmetry.

Yet another important finding of the study, as already discussed for peptide **13**, emanates from the presence of a 12/10-helical structure in most of the peptides. This implies that β-hGly residues do not occupy the same conformational

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space, which infers the split behavior. Thus, the amide protons of the even-numbered β-hGly residues participated in hydrogen bonding with the CO moiety of the subsequent residue [NH(*i*)···CO(*i*+1)], whereas the amide protons of the odd-numbered residues [except NH(1)] participated in hydrogen bonding with the carbonyl group located three residues behind [NH(*i*)···CO(*i*−3)]. Thus, it is important to note that to accommodate a mixed 12/10-helix, β-hGly residues participate in alternate 10- and 12-membered-ring hydrogen bonding, which implicates split behavior.

Conclusion

The present account delineates that chirality from a single chiral β-amino acid [(*R*)-β-Caa] can efficiently induce helicity (12/10-mixed helix) in achiral β-peptides derived from β-hGly residues. It was also demonstrated that the nucleation of helicity mediated by template capping of peptides with short and robust preorganized motifs is an elegant strategy. In addition, it was evident that induction from the N terminus led to more robust helical patterns relative to those capped at the C terminus. Chiral induction was inferred by the observed diastereotopicity of the Cα and Cβ protons of the β-hGly residues in all of the peptides studied. The presence of a minor folded structure was noticed for all of the peptides, in which several amide protons were hydrogen-bonded, yet it was not possible to exactly decipher the folding pattern. The generality of the chiral induction was further reiterated by the studies on peptides end capped with (*S*,*S*)-APyC and β-hPhe. Stronger helical induction was observed by end capping with a preorganized β-amino acid, relative to the β-amino acids with less-constrained side chains. This study also exemplifies the split behavior of β-hGly; alternating β-hGly units participate in 12- and 10-membered-ring hydrogen bonding to form robust 12/10 helices. These results provide, for the first time, the experimental evidence for the reported theoretical predictions of the preference for a 12/10-helical fold in β-peptides in apolar solvents. It establishes that proper nucleation plays a pivotal role in defining the helix formation and stability in oligomers with a flexible backbone. This work envisages that chirality- or template-assisted induction in achiral β-peptides might facilitate further novel conformations.

Experimental Section

General: NMR spectra (1D and 2D experiments) for all the peptides were obtained in $CDCl_3$ at 500, 600, and 700 MHz (^1H), and at 100, 150, and 175 MHz (^{13}C). Chemical shifts (δ) are reported in ppm with respect to an internal tetramethylsilane reference. IR spectra were recorded with a FTIR spectrometer as KBr pellets in the range $\tilde{\nu}$ = 400–4000 cm^{-1}. Melting points were determined in open capillaries and were not corrected. The CD spectra were obtained with a spectropolarimeter by using rectangular fused quartz cells of 0.2 cm path length as solutions in methanol (200 μM). The binomial method was used to smooth the spectra. The values are expressed in terms of the total molar ellipticity (θ) [deg cm^2 $dmol^{-1}$ $residue^{-1}$]. Restraint molecular dynamics (MD) studies were carried out by using the INSIGHT-II Discover module. The constraints were derived from the volume integrals obtained from the ROESY spectra by using a two-spin approximation and a reference distance of 1.8 Å for the geminal protons. The upper and lower bounds of the distance constraints were obtained by enhancement and reduction of the derived distance by 10%.

Peptide 17: A solution of **16** (1.2 g, 6.34 mmol), HOBt (1.02 g, 7.61 mmol), and EDCI (1.46 g, 7.61 mmol) in CH_2Cl_2 (25 mL) was stirred at 0 °C under N_2 atmosphere for 15 min, then treated sequentially with amine salt **16a** (0.88 g, 6.34 mmol) and DIPEA (2.18 mL, 12.6 mmol) and stirred at RT for 8 h. The reaction mixture was quenched at 0 °C with a saturated aqueous solution of NH_4Cl (15 mL). After 10 min, the reaction mixture was diluted with $CHCl_3$ (20 mL), washed with 1 N HCl (15 mL), water (15 mL), a saturated aqueous solution of $NaHCO_3$ (15 mL), and brine (15 mL). The organic layer was dried (Na_2SO_4), evaporated, and the residue was purified by column chromatography (60–120 mesh silica gel, 55% EtOAc/petroleum ether) to give **17** (1.42 g, 82%) as a white solid. M.p. 143–145 °C; ^1H NMR (500 MHz, $CDCl_3$): δ = 6.18 (m, 1H; NH-2), 5.16 (m, 1H; NH-1), 3.70 (s, 3H; $COOCH_3$), 3.53 (m, 2H; CβH-2), 3.38 (m, 2H; CβH-1), 2.54 (m, 2H; CαH-2), 2.37 (m, 2H; CαH-1), 1.43 ppm (s, 9H; Boc); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.5, 171.4, 156.0, 78.9, 51.3, 36.5, 36.2, 34.9, 33.9, 28.3 ppm (3C); IR (KBr): $\tilde{\nu}$ = 3290, 3080, 2927, 2854, 2355, 1734, 1638, 1536, 1293, 1174, 1107 cm^{-1}; MS (FAB): *m*/*z* calcd (%) for $C_{12}H_{22}N_2O_5$: 275 (52) $[M+H]^+$, 175 (100) $[M+H-Boc]^+$.

Peptide 7: A mixture of **16** (0.2 g, 0.76 mmol), HOBt (0.12 g, 0.92 mmol), and EDCI (0.17 g, 0.92 mmol) in CH_2Cl_2 (15 mL) was stirred at 0 °C for 15 min then treated with **19** [prepared from **6** (0.44 g, 0.76 mmol) and CF_3COOH (0.5 mL) in CH_2Cl_2 (4 mL)] and DIPEA (0.26 mL, 1.53 mmol)] under N_2 atmosphere for 8 h. Workup as described for **17** and purification by column chromatography (60–120 mesh silica gel, 4.2% methanol/$CHCl_3$) gave **7** (0.31 g, 56%) as a white solid. M.p. 228–230 °C; $[\alpha]_D^{25} = -60.0$ (c = 0.1 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ = 7.29 (m, 1H; NH-5), 7.21 (m, 1H; NH-3), 7.19 (m, 1H; NH-2), 6.98 (m, 1H; NH-4), 5.57 (d, J = 5.0 Hz, 1H; C1H), 5.44 (m, 1H; NH-1), 4.64 (dd, J = 2.5, 8.1 Hz, 1H; C3H), 4.37 (dd, J = 2.5, 5.0 Hz, 1H; C2H), 4.34 (dd, J = 8.7, 11.8 Hz, 1H; C6H), 4.25 (m, 1H; C4H), 4.25 (m, 1H; C6H), 4.08 (ddd, J = 1.9, 3.1, 8.7 Hz, 1H; C5H), 3.66 (m, 1H; CβH-5), 3.58 (m, 2H; CβH-3), 3.54 (m, 2H; CβH-2), 3.52 (m, 2H; CβH-4), 3.48 (m, 2H; CβH-1), 3.44 (m, 1H; CβH-5), 2.63 (m, 1H; CαH-5), 2.58 (m, 1H; CαH-5), 2.42 (m, 2H; CαH-1), 2.41 (m, 2H; CαH-3), 2.36 (m, 2H; CαH-4), 2.34 (m, 2H; CαH-2), 1.52 (s, 3H; CH_3), 1.46 (s, 6H; 2×CH_3), 1.34 (s, 3H; CH_3), 1.43 ppm (s, 9H; Boc); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 172.3, 172.1, 172.0, 171.9, 171.6, 156.2, 109.8, 108.9 (2C), 96.2 (2C), 79.3, 70.8, 70.4, 66.0, 63.8, 37.1, 36.6, 36.3, 36.0, 35.9, 35.8, 35.5, 34.5, 29.7, 28.3 (3C), 25.8 (2C), 24.8, 24.3 ppm; IR (KBr): $\tilde{\nu}$ = 3301, 3080, 2929, 1743, 1638, 1544, 1544, 1257, 1071, 1006, 800, 699 cm^{-1}; HRMS (ESI): *m*/*z* calcd for $C_{32}H_{53}N_5O_{13}Na$: 738.3537 $[M+Na]^+$; found: 738.3515.

Peptide 8: A mixture of **17a** (0.1 g, 0.38 mmol), HOBt (0.06 g, 0.46 mmol), and EDCI (0.09 g, 0.46 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C for 15 min then treated with **21a** [prepared from **21** (0.2 g, 0.38 mmol) and CF_3COOH (0.2 mL) in CH_2Cl_2 (2 mL)] and DIPEA (0.13 mL, 0.76 mmol) under N_2 atmosphere at RT for 8 h. Workup was as described for **17** and purification by column chromatography (60–120 mesh silica gel, 2.5% methanol/$CHCl_3$) gave **8** (0.19 g, 76%) as a white solid. M.p. 185 °C; $[\alpha]_D^{25} = +207.33$ (c = 0.1 in $CHCl_3$); IR (KBr): $\tilde{\nu}$ = 3310, 3017, 1658, 1514, 1440, 1216, 759, 670 cm^{-1}.

Major isomer: ^1H NMR (600 MHz, $CDCl_3$): δ = 7.58 (d, J = 8.1 Hz, 1H; NH-5), 7.30 (t, J = 6.1 Hz, 1H; NH-2), 7.24 (dd, J = 5.2, 7.1 Hz, 1H; NH-3), 7.19 (dd, J = 5.2, 7.1 Hz, 1H; NH-4), 5.39 (t, J = 6.3 Hz, 1H; NH-1), 4.89 (s, 1H; C1H-5), 4.77 (dd, J = 3.5, 6.0 Hz, 1H; C3H-5), 4.70 (ddt, J = 4.6, 7.2, 8.1 Hz, 1H; CβH-5), 4.54 (d, J = 6.0 Hz, 1H; C2H-5), 4.02 (dd, J = 3.5, 7.2 Hz, 1H; C4H-5), 3.81 (m, 1H; $CβH_{(pro\text{-}R)}$-3), 3.72 (m, 1H; $CβH_{(pro\text{-}S)}$-2), 3.70 (m, 1H; $CβH_{(pro\text{-}S)}$-4), 3.70 (s, 3H; $COOCH_3$), 3.58 (dddd, J = 4.2, 6.3, 8.6, 13.5 Hz, 1H; $CβH_{(pro\text{-}R)}$-1), 3.39 (m, 1H; $CβH_{(pro\text{-}S)}$-3), 3.39 (m, 1H; $CβH_{(pro\text{-}S)}$-1), 3.33 (m, 1H; $CβH_{(pro\text{-}R)}$-4), 3.33 (m, 1H; $CβH_{(pro\text{-}R)}$-2), 3.30 (s, 3H; OCH_3), 2.86 (dd, J = 4.6, 14.2 Hz, 1H; $CαH_{(pro\text{-}$

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$_{R)}$-5), 2.63 (dd, J=8.1, 14.2 Hz, 1H; CαH$_{(pro\text{-}S)}$-5), 2.46 (m, 1H; CαH$_{(pro\text{-}S)}$-1), 2.46 (m, 1H; CαH$_{(pro\text{-}R)}$-3), 2.37 (m, 1H; CαH$_{(pro\text{-}S)}$-3), 2.37 (m, 1H; CαH$_{(pro\text{-}R)}$-1), 2.30 (m, 1H; CαH$_{(pro\text{-}S)}$-4), 2.30 (m, 1H; CαH$_{(pro\text{-}S)}$-2), 2.30 (m, 1H; CαH$_{(pro\text{-}R)}$-4), 2.30 (m, 1H; CαH$_{(pro\text{-}R)}$-2), 1.49 (s, 3H; CH$_3$), 1.43 (s, 9H; Boc), 1.31 ppm (s, 3H; CH$_3$); ^{13}C NMR (150 MHz, CDCl$_3$): δ= 173.9, 173.0, 172.2, 172.0, 171.6, 156.2, 112.7, 106.7, 84.7, 79.5, 79.4, 78.8, 54.7, 52.2, 46.1, 39.2, 37.6, 37.3, 37.2, 36.9, 36.7, 36.3, 36.2, 35.9, 28.3 (3C), 25.9, 24.4 ppm.

Minor isomer: ^{1}H NMR (600 MHz, CDCl$_3$): δ=8.13 (d, J=8.5 Hz, 1H; NH-5), 7.99 (dd, J=3.5, 9.0 Hz, 1H; NH-3), 7.62 (dd, J=4.6, 8.2 Hz, 1H; NH-4), 6.99 (dd, J=3.6, 9.1 Hz, 1H; NH-2), 6.38 (dd, J=1.9, 8.4 Hz, 1H; NH-1), 4.89 (s, 1H; C1H-5), 4.77 (dd, J=3.5, 6.0 Hz, 1H; C3H-5), 4.70 (m, 1H; CβH-5), 4.54 (d, J=6.0 Hz, 1H; C2H-5), 4.03 (m, 1H; CβH-3), 3.98 (dd, J=3.5, 7.8 Hz, 1H; C4H-5), 3.91 (m, 1H; Cβ'H-2), 3.81 (m, 1H; Cβ'H-4), 3.74 (m, 1H; Cβ'H-1), 3.70 (s, 3H; COOCH$_3$), 3.32 (m, 1H; Cβ'H-3), 3.30 (s, 3H; OCH$_3$), 3.28 (m, 1H; CβH-1), 3.27 (m, 1H; CβH-2), 3.23 (m, 1H; CβH-4), 3.00 (dd, J=3.5, 13.3 Hz, 1H; CαH-5), 2.54 (dd, J=9.3, 13.3 Hz, 1H; Cα'H-5), 2.51 (m, 1H; CαH-3), 2.49 (m, 1H; CαH-1), 2.46 (m, 1H; Cα'H-3), 2.36 (m, 1H; Cα'H-1), 2.23 (m, 1H; Cα'H-4), 2.23 (m, 1H; CαH-4), 2.22 (m, 1H; CαH-2), 2.10 (ddd, J=3.0, 10.5, 13.5 Hz, 1H; Cα'H-2), 1.49 (s, 3H; CH$_3$), 1.31 (s, 3H; CH$_3$), 1.43 ppm (s, 9H; Boc); HRMS (ESI): m/z calcd for $C_{29}H_{49}N_5O_{12}Na$: 682.3275 $[M+Na]^+$; found: 682.3286.

Peptide 9: A solution of ester **22** (0.2 g, 0.38 mmol) in methanol (1.5 mL) was treated with 4 N NaOH (1.5 mL) at 0 °C–RT for 2 h. Workup as described for **17a** gave acid **22a**.

A mixture of acid **22a** (0.15 g, 0.29 mmol), HOBt (0.05 g, 0.35 mmol), and EDCI (0.06 g, 0.35 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 15 min then treated with **17b** [prepared from **17** (0.08 g, 0.29 mmol) and CF_3COOH (0.1 mL) in CH_2Cl_2 (1 mL)] and DIPEA (0.1 mL, 0.59 mmol) under N_2 atmosphere at RT for 8 h. Workup as described for **17** and purification by column chromatography (60–120 mesh silica gel, 2.5% methanol/$CHCl_3$) gave **9** (0.15 g, 79%) as a white solid. M.p. 188–190 °C; $[\alpha]_D^{25}$=+167.50 (c=0.25 in $CHCl_3$); IR (KBr): $\tilde{\nu}$=3312, 3017, 2360, 1653, 1440, 1215, 1099, 759, 669 cm^{-1}; HRMS (ESI): m/z calcd for $C_{29}H_{50}N_5O_{12}$: 660.3455 $[M+H]^+$; found: 660.3473.

Major isomer: ^{1}H NMR (500 MHz, CDCl$_3$): δ=8.35 (dd, J=3.7, 9.2 Hz, 1H; NH-3), 8.11 (dd, J=4.5, 7.8 Hz, 1H; NH-5), 7.92 (dd, J=4.0, 9.0 Hz, 1H; NH-2), 7.19 (dd, J=4.7, 8.5 Hz, 1H; NH-4), 5.91 (d, J=6.6 Hz, 1H; NH-1), 4.92 (s, 1H; C1H-1), 4.75 (dd, J=3.6, 6.0 Hz, 1H; C3H-1), 4.68 (m, 1H; CβH-1), 4.54 (d, J=6.0 Hz, 1H; C2H-1), 4.02 (m, 1H; CβH$_{(pro\text{-}R)}$-3), 3.97 (dd, J=3.6, 5.0 Hz, 1H; C4H-1), 3.94 (m, 1H; CβH$_{(pro\text{-}S)}$-2), 3.89 (dddd, J=3.8, 7.8, 8.7, 13.3 Hz, 1H; CβH$_{(pro\text{-}R)}$-5), 3.82 (m, 1H; CβH$_{(pro\text{-}S)}$-4), 3.70 (s, 3H; COOCH$_3$), 3.33 (ddt, J=6.2, 13.2, 4.5 Hz, 1H; CβH$_{(pro\text{-}S)}$-5), 3.28 (s, 3H; OCH$_3$), 3.27 (ddt, J=3.7, 13.1, 4.0 Hz, 1H; CβH$_{(pro\text{-}S)}$-3), 3.20 (m, 1H; CβH$_{(pro\text{-}R)}$-4), 3.07 (dddd, J=3.0, 4.0, 11.6, 13.2 Hz, 1H; CβH$_{(pro\text{-}R)}$-2), 2.69 (ddd, J=3.8, 6.2, 14.3 Hz, 1H; CαH$_{(pro\text{-}S)}$-5), 2.66 (dd, J=2.8, 12.6 Hz, 1H; CαH$_{(pro\text{-}S)}$-1), 2.46 (ddd, J=4.5, 8.7, 14.3 Hz, 1H; CαH$_{(pro\text{-}R)}$-5), 2.43 (m, 1H; CαH$_{(pro\text{-}S)}$-3), 2.40 (ddd, J=4.0, 11.8, 13.2 Hz, 1H; CαH$_{(pro\text{-}R)}$-3), 2.40 (dd, J=11.5, 12.6 Hz, 1H; CαH$_{(pro\text{-}R)}$-1), 2.34 (ddd, J=3.8, 11.1, 13.2 Hz, 1H; CαH$_{(pro\text{-}R)}$-4), 2.28 (ddd, J=3.8, 11.6, 12.6 Hz, 1H; CαH$_{(pro\text{-}R)}$-2), 2.20 (ddd, J=2.8, 4.3, 13.2 Hz, 1H; CαH$_{(pro\text{-}S)}$-4), 2.19 (ddd, J=3.0, 4.2, 12.6 Hz, 1H; CαH$_{(pro\text{-}S)}$-2), 1.51 (s, 3H; CH$_3$), 1.49 (s, 3H; CH$_3$), 1.43 ppm (s, 9H; Boc); ^{13}C NMR (150 MHz, CDCl$_3$): δ=173.6, 172.9, 172.4, 172.2, 170.5, 156.6, 112.7, 106.5, 84.7, 80.2, 79.9, 79.6, 54.5, 52.0, 49.4, 39.8, 37.4, 37.3, 36.7, 36.4, 36.0, 35.7, 35.5, 34.4, 28.3 (3C), 25.5, 24.3 ppm.

Minor isomer: ^{1}H NMR (500 MHz, CDCl$_3$): δ=8.25 (dd, J=2.5, 9.7 Hz, 1H; NH-3), 8.17 (dd, J=4.5, 7.7 Hz, 1H; NH-5), 7.40 (dd, J=4.3, 9.0 Hz, 1H; NH-4), 6.92 (dd, J=2.6, 10.3 Hz, 1H; NH-2), 6.59 (d, J=9.9 Hz, 1H; NH-1), 4.85 (s, 1H; C1H-1), 4.70 (dd, J=3.1, 5.7 Hz, 1H; C3H-1), 4.56 (m, 1H; CβH-1), 4.51 (d, J=5.7 Hz, 1H; C2H-1), 4.17 (dddd, J=3.5, 9.7, 13.3, 13.5 Hz, 1H; CβH$_{(pro\text{-}S)}$-3), 4.05 (m, 1H; CβH$_{(pro\text{-}R)}$-2), 3.99 (m, 1H; CβH$_{(pro\text{-}S)}$-5), 3.89 (dddd, J=3.2, 4.3, 9.0, 12.5 Hz, 1H; CβH$_{(pro\text{-}R)}$-4), 3.70 (s, 3H; COOCH$_3$), 3.69 (dd, J=3.1, 8.3 Hz, 1H; C4H-1), 3.28 (s, 3H; OCH$_3$), 3.26 (m, 1H; CβH$_{(pro\text{-}R)}$-5), 3.17 (m, 1H; CβH$_{(pro\text{-}R)}$-3), 3.15 (m, 1H; CβH$_{(pro\text{-}S)}$-4), 3.13 (m, 1H; CβH$_{(pro\text{-}S)}$-2), 2.73 (dd, J=2.8, 12.6 Hz, 1H; CαH-1), 2.70 (ddd, J=4.0, 6.7, 13.8 Hz, 1H; CαH-5), 2.52 (dt, J=13.3, 3.3 Hz, 1H; CαH$_{(pro\text{-}R)}$-3), 2.48 (ddd, J=4.5, 9.7, 13.8, 1H; CαH$_{(pro\text{-}S)}$-5), 2.44 (dt, J=5.0, 13.3, 1H; CαH$_{(pro\text{-}S)}$-3), 2.40 (t, J=12.5 Hz, 1H; CαH$_{(pro\text{-}R)}$-1), 2.25 (ddd, J=3.1, 3.9, 13.5 Hz, 1H; CαH$_{(pro\text{-}R)}$-2), 2.21 (m, 1H; CαH$_{(pro\text{-}S)}$-4), 2.19 (m, 1H; CαH$_{(pro\text{-}R)}$-4), 2.06 (ddd, J=3.1, 11.8, 13.5 Hz, 1H; CαH$_{(pro\text{-}S)}$-2), 1.51 (s, 3H; CH$_3$), 1.49 (s, 3H; CH$_3$), 1.43 ppm (s, 9H; Boc).

Peptide 10: A solution of ester **22** (0.2 g, 0.38 mmol) in methanol (1.5 mL) was treated with 4 N NaOH (1.5 mL) at 0 °C–RT for 2 h. Workup as described for **17a** gave the acid **22a**.

A mixture of **22a** (0.15 g, 0.29 mmol), HOBt (0.04 g, 0.35 mmol), and EDCI (0.06 g, 0.35 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C for 15 min then treated with **23a** [prepared from **23** (0.13 g, 0.29 mmol) and CF_3COOH (0.15 mL) in CH_2Cl_2 (1 mL)] and DIPEA (0.1 mL, 0.59 mmol) under N_2 atmosphere at RT for 8 h. Workup as described for **17** and purification by column chromatography (60–120 mesh silica gel, 2% methanol/$CHCl_3$) gave **10** (0.19 g, 79%) as a white solid. M.p. 140 °C; $[\alpha]_D^{25}$=−174.66 (c=0.1 in $CHCl_3$); ^{1}H NMR (600 MHz, CDCl$_3$): δ=8.51 (dd, J=3.3, 9.8 Hz, 1H; NH-3), 8.47 (d, J=8.8 Hz, 1H; NH-5), 8.06 (dd, J=3.6, 9.5 Hz, 1H; NH-2), 7.54 (dd, J=4.0, 9.5 Hz, 1H; NH-4), 5.90 (d, J=10.3 Hz, 1H; NH-1), 4.93 (s, 1H; C1H-1), 4.89 (s, 1H; C1H-5), 4.78 (dd, J=3.4, 5.8 Hz, 1H; C3H-5), 4.74 (dd, J=3.5, 5.8 Hz, 1H; C3H-1), 4.70 (m, 1H; CβH-5), 4.70 (m, 1H; CβH-1), 4.54 (d, J=5.8 Hz, 1H; C2H-5), 4.54 (d, J=5.8 Hz, 1H; C2H-1), 4.15 (dddd, J=3.3, 9.8, 12.8, 13.1 Hz, 1H; CβH$_{(pro\text{-}R)}$-3), 4.00 (ddt, J=9.5, 13.7, 4.0 Hz, 1H; CβH$_{(pro\text{-}S)}$-2), 3.96 (dd, J=3.5, 5.1 Hz, 1H; C4H-1), 3.91 (m, 1H; CβH$_{(pro\text{-}S)}$-4), 3.90 (dd, J=3.4, 8.1 Hz, 1H; C4H-5), 3.70 (s, 3H; COOCH$_3$), 3.30 (s, 3H; OCH$_3$), 3.29 (s, 3H; OCH$_3$), 3.20 (ddt, J=4.3, 13.1, 3.3 Hz, 1H; CβH$_{(pro\text{-}S)}$-3), 3.10 (dddd, J=2.4, 3.6, 4.0, 12.0 Hz, 1H; CβH$_{(pro\text{-}R)}$-4), 3.06 (dd, J=3.7, 13.1 Hz, 1H; CαH$_{(pro\text{-}S)}$-5), 2.99 (dddd, J=2.6, 3.6, 12.1, 13.7 Hz, 1H; CβH$_{(pro\text{-}R)}$-2), 2.67 (dd, J=2.6, 12.5 Hz, 1H; CαH$_{(pro\text{-}S)}$-1), 2.51 (dt, J=13.0, 3.3 Hz, 1H; CαH$_{(pro\text{-}S)}$-3), 2.46 (dd, J=9.3, 13.1 Hz, 1H; CαH$_{(pro\text{-}R)}$-5), 2.40 (ddd, J=4.3, 12.8, 13.0 Hz, 1H; CαH$_{(pro\text{-}R)}$-3), 2.35 (dd, J=12.1, 12.5 Hz, 1H; CαH$_{(pro\text{-}R)}$-1), 2.31 (ddd, J=3.6, 12.1, 12.5 Hz, 1H; CαH$_{(pro\text{-}R)}$-4), 2.27 (ddd, J=4.0, 12.1, 12.4 Hz, 1H; CαH$_{(pro\text{-}R)}$-2), 2.16 (ddd, J=2.4, 4.3, 12.5 Hz, 1H; CαH$_{(pro\text{-}S)}$-4), 2.13 (ddd, J=2.6, 4.0, 12.4 Hz, 1H; CαH$_{(pro\text{-}S)}$-2), 1.52 (s, 3H; CH$_3$), 1.49 (s, 3H; CH$_3$), 1.31 (s, 3H; CH$_3$), 1.30 (s, 3H; CH$_3$), 1.43 ppm (s, 9H; Boc); ^{13}C NMR (150 MHz, CDCl$_3$): δ=174.3, 173.6, 173.0, 171.8, 170.7, 157.0, 112.8, 112.7, 107.0, 106.5, 84.9, 84.7, 80.2 (2C), 54.6, 52.4, 51.2, 51.0, 50.2, 46.3, 40.4, 39.2, 38.8, 37.8, 37.6, 37.0, 36.8, 36.7, 36.3, 28.4, 28.2 (3C), 26.0, 25.6, 24.8, 24.3 ppm; IR (KBr): $\tilde{\nu}$=3302, 3088, 2984, 2938, 1740, 1646, 1550, 1441, 1372, 1240, 1169, 1103, 1017, 857, 590 cm^{-1}; HRMS (ESI): m/z calcd for $C_{37}H_{61}N_5O_{16}Na$: 854.4113 $[M+Na]^+$; found: 854.4142.

Peptide 13: A solution of ester **31** (0.4 g, 0.39 mmol) in methanol (1.6 mL) was treated with 4 N NaOH (1.6 mL) at 0 °C–RT for 2 h. Workup as described for **17a** gave the acid **31a**.

A mixture of **31a** (0.07 g, 0.07 mmol), HOBt (0.01 g, 0.09 mmol), and EDCI (0.02 g, 0.09 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 15 min then treated with **28a** [prepared from **28** (0.07 g, 0.07 mmol) and CF_3COOH (0.07 mL) in CH_2Cl_2 (0.5 mL)] and DIPEA (0.02 mL, 0.14 mmol) under N_2 atmosphere at RT for 8 h. Workup as described for **17** and purification by column chromatography (60–120 mesh silica gel, 2.5% methanol/$CHCl_3$) gave **13** (0.09 g, 70%) as a white solid. M.p. 150 °C; $[\alpha]_D^{25}$=+386.1 (c=0.5 in $CHCl_3$); ^{1}H NMR (600 MHz, CDCl$_3$): δ=9.19 (dd, J=3.4, 9.1 Hz, 1H; NH-4), 9.09 (J=9.5, Hz 1H; NH-7), 8.97 (d, J=9.4 Hz, 1H; NH-3), 8.81 (dd, J=4.1, 9.0 Hz, 1H; NH-6), 8.79 (dd, J=3.3, 8.4 Hz, 1H; NH-5), 8.72 (d, J=9.5 Hz, 1H; NH-9), 8.51 (d, J=9.6 Hz, 1H; NH-2), 7.94 (d, J=9.4 Hz, 1H; NH-8), 5.96 (d, J=9.4 Hz, 1H; NH-8), 5.96 (d, J=10.3 Hz, 1H; NH-1), 5.10 (dd, J=3.3, 5.9 Hz, 1H; C3H-8), 5.01 (dd, J=3.3, 5.9 Hz, 1H; C3H-2), 4.93 (s, 1H; C1H-2), 4.90 (s, 1H; C1H-1), 4.89 (s, 1H; C1H-9), 4.88 (s, 1H; C1H-8), 4.86 (s, 1H; C1H-7), 4.83 (s, 1H; C1H-3), 4.79 (dd, J=3.4, 5.8 Hz, 1H; C3H-9), 4.75 (m, 1H; CβH-9), 4.74 (dd, J=3.0, 5.7 Hz; C3H-7), 4.72 (m, 1H; CβH-1), 4.71 (dd, J=3.4, 5.9 Hz, 1H; C3H-1), 4.69 (dd, J=3.2, 5.9 Hz, 1H; C3H-3), 4.66 (m, 1H; CβH-7), 4.65 (m, 1H; CβH-3), 4.61 (m, 1H; CβH-2), 4.58 (d, J=5.9 Hz, 1H; C2H-2), 4.56 (d, J=5.7 Hz; C2H-9), 4.53 (d, J=5.7 Hz; C2H-7), 4.53 (d, J=5.9 Hz, 1H; C2H-1), 4.52 (d, J=5.9 Hz, 1H; C2H-3), 4.50 (m, 1H; CβH-8), 4.45 (d, J=5.9 Hz, 1H; C2H-

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8), 4.30 (ddt, J=3.3, 13.9, 8.4 Hz, 1H; CβH$_{(pro\text{-}R)}$-5), 4.08 (dd, J=3.3, 10.5 Hz, 1H; C4H-8), 4.06 (dd, J=3.3, 10.5 Hz, 1H; C4H-2), 4.04 (dd, J=3.2, 9.7 Hz, 1H; C4H-3), 3.98 (m, 1H; CβH$_{(pro\text{-}S)}$-4), 3.96 (dd, J=3.4, 9.2 Hz, 1H; C4H-9), 3.94 (dd, J=3.4, 9.5 Hz, 1H; C4H-1), 3.94 (m, 1H; CβH$_{(pro\text{-}S)}$-6), 3.70 (s, 3H; COOCH$_3$), 3.34 (s, 3H; OCH$_3$), 3.32 (s, 6H; 2×OCH$_3$), 3.31 (s, 3H; OCH$_3$), 3.23 (s, 3H; OCH$_3$), 3.22 (s, 3H; OCH$_3$), 3.13 (dt, J=13.9, 3.3 Hz, 1H; CβH$_{(pro\text{-}S)}$-5), 3.05 (dd, J=2.9, 12.3 Hz, 1H; CαH-3), 3.02 (dd, J=3.5 Hz, 12 Hz, 1H; CαH$_{(pro\text{-}S)}$-7), 2.96 (dd, J=2.6, 12.4 Hz, 1H; CαH$_{(pro\text{-}S)}$-9), 2.96 (m, 1H; CβH$_{(pro\text{-}R)}$-5), 2.94 (m, 1H; CβH$_{(pro\text{-}R)}$-6), 2.74 (dd, J=2.0, 12.8 Hz, 1H; CαH-1), 2.65 (dt, J=12.5, 3.3 Hz, 1H; CαH-3), 2.56 (dd, J=3.0, 12.9 Hz, 1H; CαH-2), 2.47 (m, 1H; CαH$_{(pro\text{-}R)}$-7), 2.46 (m, 1H; CαH$_{(pro\text{-}R)}$-8), 2.45 (m, 1H; CαH$_{(pro\text{-}R)}$-6), 2.44 (m, 1H; CαH$_{(pro\text{-}S)}$-5), 2.43 (t, J=12.4 Hz, CαH$_{(pro\text{-}R)}$-9), 2.40 (m, 1H; CαH$_{(pro\text{-}S)}$-8), 2.37 (m, 1H; CαH$_{(pro\text{-}R)}$-1), 2.27 (dt, J=4.5, 12.5 Hz; CαH$_{(pro\text{-}R)}$-4), 2.21 (t, J=12.3 Hz, 1H; CαH-3), 2.17 (t, J=12.5 Hz; CαH$_{(pro\text{-}R)}$-7), 2.10 (dt, J=12.6, 3.6 Hz, 1H; CαH$_{(pro\text{-}S)}$-7), 2.08 (m, 1H; CαH$_{(pro\text{-}S)}$-4), 1.52 (s, 3H; CH$_3$), 1.49 (s, 3H; CH$_3$), 1.48 (s, 6H; 2×CH$_3$), 1.43 (s, 3H; CH$_3$), 1.40 (s, 3H; CH$_3$), 1.31 (s, 3H; CH$_3$), 1.30 (s, 6H; 2×CH$_3$), 1.28 (s, 6H; 2×CH$_3$), 1.27 (s, 3H; CH$_3$), 1.42 ppm (s, 9H; Boc); ^{13}C NMR (150 MHz, CDCl$_3$): δ=174.2, 173.1 (2C), 172.4, 171.4, 170.8 (2C), 169.7, 169.3, 157.0, 112.9, 112.8 (2C), 112.4, 112.3 (2C), 112.2 (2C), 108.0, 107.7, 107.3, 106.9, 106.6, 106.5, 85.1 (2C), 84.9, 84.8 (2C), 81.6, 81.2, 80.4, 79.8, 79.6 (2C), 79.5, 79.2 (2C), 78.9, 55.2 (2C), 54.9, 54.3 (2C), 54.2, 54.1, 52.3 (2C), 50.2, 47.7, 46.7, 46.3, 46.2, 45.9, 42.4, 41.8, 40.6, 39.5 (2C), 38.5, 37.3, 37.2, 36.9, 36.8, 36.5, 36.4, 29.7, 28.3 (3C), 26.4, 26.2, 26.1 (2C), 26.0, 25.7, 25.1, 25.0, 24.8, 24.6, 24.3, 24.1 ppm; IR (KBr): $\tilde{\nu}$=3300, 3090, 2987, 2939, 1650, 1554, 1445, 1377, 1272, 1099, 880, 593 cm^{-1}; HRMS (ESI): m/z calcd for $C_{81}H_{131}N_9O_{36}$: 902.9343 $[M+2H]^{2+}$; found: 902.9343.

Peptide 14: A solution of ester **32** (0.17 g, 0.14 mmol) in methanol (0.6 mL) was treated with 4 N NaOH (0.6 mL) at 0 °C–RT for 2 h. Workup as described for **17a** gave the acid **32a**.

A mixture of **32a** (0.06 g, 0.05 mmol), HOBt (0.01 g, 0.06 mmol), and EDCI (0.01 g, 0.06 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C for 15 min then treated with **28a** [prepared from **28** (0.05 g, 0.05 mmol) and CF_3COOH (0.05 mL) in CH_2Cl_2 (0.5 mL)] and DIPEA (0.02 mL, 0.1 mmol) under N_2 atmosphere at RT for 8 h. Workup as described for **17** and purification by column chromatography (60–120 mesh silica gel, 3% methanol/$CHCl_3$) gave **14** (0.07 g, 70%) as a white solid. M.p. 155 °C; $[\alpha]_D^{25}$=+477.2 (c=0.5 in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ=9.05 (d, J=9.6 Hz, 1H; NH-9), 9.02 (dd, J=3.9, 9.6 Hz, 1H; NH-4), 8.96 (dd, J=3.2, 10.1 Hz, 1H; NH-7), 8.93 (d, J=9.4 Hz, 1H; NH-3), 8.86 (dd, J=3.5, 9.6 Hz, 1H; NH-5), 8.84 (dd, J=3.9, 9.4 Hz, 1H; NH-8), 8.69 (d, J=9.4 Hz, 1H; NH-11), 8.65 (dd, J=4.0, 9.1 Hz, 1H; NH-6), 8.44 (d, J=9.8 Hz, 1H; NH-2), 7.91 (d, J=9.3 Hz, 1H; NH-10), 5.97 (d, J=10.3 Hz, 1H; NH-1), 5.10 (dd, J=3.3, 5.7 Hz, 1H; C3H-10), 4.97 (dd, J=3.2, 5.7 Hz, 1H; C3H-2), 4.90 (s, 1H; C1H-1), 4.90 (s, 1H; C1H-2), 4.89 (s, 1H; C1H-11), 4.88 (s, 1H; C1H-10), 4.85 (s, 1H; C1H-9), 4.84 (s, 1H; C1H-3), 4.79 (dd, J=3.2, 5.7 Hz; C3H-11), 4.77 (m, 1H; CβH-11), 4.73 (dd, J=3.3, 5.7 Hz, 1H; C3H-1), 4.72 (dd, J=3.0, 5.7 Hz, 1H; C3H-3), 4.71 (m, 1H; CβH-1), 4.69 (m, 1H; CβH-3), 4.69 (dd, J=3.0, 5.7 Hz, 1H; C3H-9), 4.66 (m, 1H; CαH-9), 4.59 (ddt, J=3.2, 4.5, 9.8 Hz, 1H; CβH-2), 4.55 (d, J=5.7 Hz; C2H-11), 4.53 (d, J=5.7 Hz, 1H; C2H-1), 4.52 (d, J=5.7 Hz, 1H; C2H-3), 4.51 (d, J=5.7 Hz, 1H; C3H-9), 4.50 (m, 1H; CβH-10), 4.45 (d, J=5.7 Hz, 1H; C2H-10), 4.37 (m, 1H; CβH$_{(pro\text{-}R)}$-7), 4.36 (m, 1H; CβH$_{(pro\text{-}R)}$-5), 4.08 (dd, J=3.3, 10.3 Hz, 1H; C4H-10), 4.04 (dd, J=3.0, 9.4 Hz, 1H; C4H-3), 4.03 (dd, J=3.2, 8.4 Hz, 1H; C4H-2), 3.98 (m, 1H; CβH$_{(pro\text{-}S)}$-6), 3.97 (m, 1H; CβH$_{(pro\text{-}S)}$-4), 3.97 (dd, J=3.3, 9.2 Hz, 1H; C4H-1), 3.95 (dd, J=3.2, 9.2 Hz, 1H; CβH-11), 3.95 (m, 1H; CβH$_{(pro\text{-}S)}$-8), 3.86 (dd, J=3.0, 8.8 Hz, 1H; C4H-9), 3.70 (s, 3H; COOCH$_3$), 3.29 (s, 6H; 2×OCH$_3$), 3.24 (s, 6H; 2×OCH$_3$), 3.22 (s, 6H; 2×OCH$_3$), 3.20 (m, 1H; CβH$_{(pro\text{-}S)}$-5), 3.14 (m, 1H; CβH$_{(pro\text{-}S)}$-7), 2.96 (m, 1H; CαH$_{(pro\text{-}S)}$-11), 2.96 (m, 1H; CβH$_{(pro\text{-}R)}$-4), 2.95 (m, 1H; CβH$_{(pro\text{-}R)}$-8), 2.92 (m, 1H; CβH$_{(pro\text{-}R)}$-6), 2.73 (m, 1H; CαH$_{(pro\text{-}R)}$-1), 2.72 (m, 1H; CαH$_{(pro\text{-}S)}$-7), 2.62 (dt, J=12.8, 3.0 Hz, 1H; CαH$_{(pro\text{-}S)}$-5), 2.57 (dd, J=3.0, 12.9 Hz, 1H; CαH$_{(pro\text{-}S)}$-2), 2.47 (m, 1H; CαH$_{(pro\text{-}R)}$-2), 2.45 (m, 1H; CαH$_{(pro\text{-}R)}$-5), 2.45 (m, 1H; CαH-10), 2.44 (m, 1H; CαH$_{(pro\text{-}R)}$-11), 2.42 (m, 1H; CαH$_{(pro\text{-}R)}$-6), 2.42 (m, 1H; CαH-10), 2.40 (m, 1H; CαH$_{(pro\text{-}S)}$-1), 2.33 (m, 1H; CαH$_{(pro\text{-}R)}$-8), 2.29 (m, 1H; CαH$_{(pro\text{-}R)}$-4), 2.27 (m, 1H; CαH$_{(pro\text{-}R)}$-7), 2.23 (m, 1H; CαH$_{(pro\text{-}R)}$-3), 2.22 (m, 1H; CαH$_{(pro\text{-}R)}$-9), 2.12 (m, 1H; CαH$_{(pro\text{-}S)}$-8), 2.10 (m, 1H; CαH$_{(pro\text{-}S)}$-4), 2.08 (m, 1H; CαH$_{(pro\text{-}S)}$-6), 1.52 (s, 3H; CH$_3$), 1.49 (s, 3H; CH$_3$), 1.48 (s, 3H; CH$_3$), 1.47 (s, 3H; CH$_3$), 1.42 (s, 3H; CH$_3$), 1.40 (s, 3H; CH$_3$), 1.31 (s, 3H; CH$_3$), 1.29 (s, 6H; 2×CH$_3$), 1.28 (s, 6H; 2×CH$_3$), 1.27 (s, 3H; CH$_3$), 1.42 ppm (s, 9H; Boc); ^{13}C NMR (150 MHz, CDCl$_3$): δ=174.1, 173.6, 173.1, 173.0, 172.8, 172.3, 172.0, 171.5, 170.9 (2C), 169.7, 169.3, 156.9, 112.8 (2C), 112.7 (2C), 112.4, 112.3 (2C), 112.2, 107.9, 107.3, 107.2, 106.9, 106.5 (2C), 106.4, 85.0, 84.9, 84.8 (2C), 81.1 (2C), 80.3, 79.8, 79.6, 79.5, 79.4, 79.3, 79.2, 79.1, 79.0, 78.9, 55.1, 54.7, 54.3, 54.2 (2C), 54.1, 52.4, 50.2, 47.5, 46.7, 46.3, 46.2, 45.8, 42.0, 41.7, 40.5, 39.5, 39.3, 39.0, 38.8, 38.5, 37.3, 37.2, 37.1, 36.8, 36.7, 36.4, 36.3, 28.3 (3C), 26.3 (2C), 26.1 (2C), 25.6, 25.0 (2C), 24.8 (2C), 24.5, 24.2 ppm (2C); IR (KBr): $\tilde{\nu}$=3292, 3089, 2986, 2940, 1650, 1533, 1444, 1377, 1206, 1100, 1026, 966, 516 cm^{-1}; HRMS (ESI): m/z calcd for $C_{87}H_{141}N_{11}O_{38}$: 973.9714 $[M+2H]^{2+}$; found: 973.9717.

Peptide 15: A solution of ester **33** (0.07 g, 0.04 mmol) in methanol (0.3 mL) was treated with 4 N NaOH (0.3 mL) at 0 °C–RT for 2 h. Workup as described for **17a** gave the acid **33a**.

A mixture of **33a** (0.06 g, 0.04 mmol), HOBt (0.01 g, 0.05 mmol), and EDCI (0.01 g, 0.05 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C for 15 min then treated with **29a** [prepared from **29** (0.05 g, 0.04 mmol) and CF_3COOH (0.05 mL) in CH_2Cl_2 (0.4 mL)] and DIPEA (0.01 mL, 0.09 mmol) under nitrogen atmosphere at RT for 8 h. Workup as described for **17** and purification by column chromatography (60–120 mesh silica gel, 5% methanol/$CHCl_3$) gave **15** (0.07 g, 68%) as a white solid. M.p. 210 °C; $[\alpha]_D^{25}$=+191.9 (c=0.25 in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ=9.07 (dd, J=2.7, 9.8 Hz, 1H; NH-7), 9.04 (dd, J=4.1, 9.5 Hz, 1H; NH-4), 9.04 (d, J=9.5 Hz, 1H; NH-11), 8.97 (d, J=9.5 Hz, 1H; NH-3), 8.91 (dd, J=3.0, 9.8 Hz, 1H; NH-9), 8.89 (dd, J=3.5, 9.8 Hz, 1H; NH-5), 8.87 (dd, J=3.8, 9.4 Hz, 1H; NH-10), 8.77 (dd, J=4.0, 9.1 Hz, 1H; NH-8), 8.71 (d, J=9.8 Hz, 1H; NH-13), 8.59 (dd, J=4.1, 9.2 Hz, 1H; NH-6), 8.46 (d, J=9.7 Hz, 1H; NH-2), 7.93 (d, J=9.2 Hz, 1H; NH-12), 5.99 (d, J=10.2 Hz, 1H; NH-1), 5.10 (dd, J=3.3, 5.9 Hz, 1H; C3H-12), 4.97 (dd, J=3.3, 5.8 Hz, 1H; C3H-2), 4.90 (s, 1H; C1H-1), 4.90 (s, 1H; C1H-2), 4.89 (s, 1H; C1H-13), 4.88 (s, 1H; C1H-12), 4.85 (s, 1H; C1H-11), 4.84 (s, 1H; C1H-3), 4.79 (dd, J=3.6, 5.9 Hz, 1H; C4H-13), 4.78 (m, 1H; CβH-13), 4.74 (dd, J=3.0, 6.0 Hz, 1H; C4H-11), 4.72 (dd, J=3.0, 6.3 Hz, 1H; C3H-3), 4.72 (m, 1H; CβH-1), 4.70 (m, 1H; CβH-3), 4.69 (dd, J=3.0, 5.9 Hz, 1H; C3H-1), 4.69 (m, 1H; CβH-11), 4.60 (dddd, J=3.2, 4.5, 9.7, 10.0 Hz, 1H; CβH-2), 4.56 (d, J=5.9 Hz, 1H; C3H-13), 4.53 (d, J=5.9 Hz, 1H; C2H-1), 4.52 (d, J=3.9 Hz, 1H; C3H-12), 4.51 (d, J=6.0 Hz, 1H; C3H-11), 4.50 (m, 1H; CβH-12), 4.48 (d, J=5.8 Hz, 1H; C2H-2), 4.45 (d, J=6.3 Hz, 1H; C2H-3), 4.42 (m, 1H; CβH$_{(pro\text{-}R)}$-7), 4.41 (m, 1H; CβH$_{(pro\text{-}R)}$-5), 4.39 (m, 1H; CβH$_{(pro\text{-}R)}$-9), 4.08 (dd, J=3.3, 10.2 Hz, 1H; C4H-12), 4.04 (dd, J=3.3, 10.3 Hz, 1H; C4H-2), 4.03 (dd, J=3.0, 9.3 Hz, 1H; C4H-3), 4.00 (m, 1H; CβH$_{(pro\text{-}S)}$-4), 3.99 (m, 1H; CβH$_{(pro\text{-}S)}$-6), 3.96 (m, 1H; CβH$_{(pro\text{-}S)}$-10), 3.96 (dd, J=3.6, 9.0 Hz, 1H; CβH-13), 3.96 (dd, J=3.0, 9.0 Hz, 1H; C4H-1), 3.86 (dd, J=3.1, 8.9 Hz, 1H; CβH-11), 3.70 (s, 3H; COOCH$_3$), 3.33 (s, 3H; OCH$_3$), 3.31 (s, 6H; 2×OCH$_3$), 3.30 (s, 3H; OCH$_3$), 3.24 (s, 3H; OCH$_3$), 3.22 (s, 3H; OCH$_3$), 3.22 (m, 1H; CβH$_{(pro\text{-}S)}$-7), 3.20 (m, 1H; CβH$_{(pro\text{-}S)}$-9), 3.03 (m, 1H; CαH$_{(pro\text{-}S)}$-3), 3.00 (t, J=12.6 Hz, 1H; CαH-11), 2.98 (m, 1H; CβH$_{(pro\text{-}R)}$-10), 2.98 (m, 1H; CβH$_{(pro\text{-}R)}$-8), 2.91 (m, 1H; CβH$_{(pro\text{-}R)}$-4), 2.96 (m, 1H; CαH$_{(pro\text{-}S)}$-13), 2.95 (m, 1H; CβH$_{(pro\text{-}R)}$-6), 2.74 (m, 1H; CαH$_{(pro\text{-}S)}$-1), 2.72 (ddd, J=2.8, 5.8, 13.0 Hz, 1H; CαH-7), 2.72 (m, 1H; CαH$_{(pro\text{-}S)}$-9), 2.63 (dt, J=12.8, 2.8 Hz, 1H; CαH-5), 2.57 (dd, J=3.0, 13.0 Hz, 1H; CαH-2), 2.48 (m, 1H; CαH$_{(pro\text{-}R)}$-2), 2.45 (m, 1H; CαH$_{(pro\text{-}R)}$-12), 2.45 (m, 1H; CαH$_{(pro\text{-}R)}$-6), 2.45 (m, 1H; CαH$_{(pro\text{-}R)}$-5), 2.45 (m, 1H; CαH$_{(pro\text{-}S)}$-12), 2.43 (m, 1H; CαH$_{(pro\text{-}R)}$-13), 2.40 (m, 1H; CαH$_{(pro\text{-}R)}$-1), 2.34 (m, 1H; CαH$_{(pro\text{-}R)}$-10), 2.33 (m, 1H; CαH$_{(pro\text{-}R)}$-6), 2.30 (m, 1H; CαH$_{(pro\text{-}R)}$-9), 2.30 (m, 1H; CαH$_{(pro\text{-}S)}$-11), 2.29 (m, 1H; CαH$_{(pro\text{-}R)}$-8), 2.23 (m, 1H; CαH$_{(pro\text{-}R)}$-3), 2.22 (dt, J=6.0, 12.6 Hz, 1H; CαH$_{(pro\text{-}R)}$-4), 2.12 (m, 1H; CαH$_{(pro\text{-}S)}$-4), 2.12 (m, 1H; CαH$_{(pro\text{-}S)}$-10), 2.11 (m, 1H; CαH$_{(pro\text{-}S)}$-6), 2.11 (m, 1H; CαH$_{(pro\text{-}S)}$-8), 1.52 (s, 3H; CH$_3$), 1.50 (s, 3H; CH$_3$), 1.49 (s, 3H; CH$_3$), 1.48 (s, 3H; CH$_3$), 1.40 (s, 3H; CH$_3$), 1.31 (s, 3H; CH$_3$), 1.30 (s, 3H; CH$_3$), 1.29 (s, 3H; CH$_3$), 1.28 (s, 3H; CH$_3$), 1.27 (s, 6H; 2×CH$_3$), 1.42 ppm (s, 9H; Boc); ^{13}C NMR (150 MHz, CDCl$_3$): δ=174.1, 173.6, 173.0, 172.9, 179.8 (2C), 172.3, 172.1, 171.5, 170.9, 170.8, 169.7, 169.3, 156.9, 112.8, 112.7, 112.4, 112.3, 112.2, 107.9, 107.3, 107.2, 106.9, 106.5,

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106.4, 85.0 (2C), 84.9, 84.8 (2C), 81.1, 80.3, 79.8, 79.5 (2C), 79.4, 79.3, 79.2, 78.8, 55.1 (2C), 54.7 (2C), 54.3 (2C), 54.2 (2C), 54.1 (2C), 52.4 (2C), 50.2, 47.5, 46.7, 46.3, 46.2, 45.8, 42.0, 41.7, 40.5, 39.5, 39.3, 39.0, 38.8, 38.5, 37.3 (2C), 37.2, 37.0, 36.9, 36.8, 36.7, 36.4, 28.2 (3C), 26.3 (2C), 26.1 (2C), 26.0, 25.6, 25.0, 24.9, 24.8, 24.5, 24.2 ppm (2C); IR (KBr): $\tilde{\nu}$ = 3294, 3090, 2986, 2938, 1649, 1553, 1443, 1376, 1272, 1208, 1099, 1026, 965, 878, 593 cm^{-1}; HRMS (ESI): m/z calcd for $C_{93}H_{151}N_{13}O_{40}$: 1045.5101 $[M+2H]^{2+}$; found: 1045.5118.

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