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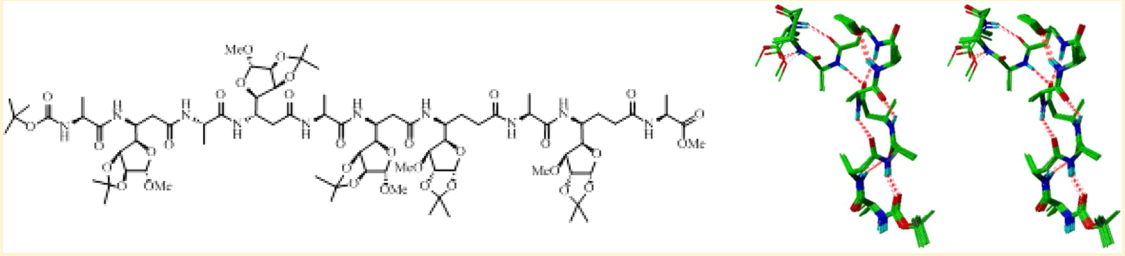
Design and Synthesis of Peptides with Hybrid Helix-Turn-Helix (HTH) Motif and Their Conformational Study

Gangavaram V. M. Sharma,*[,†] Prashanth Thodupunuri,[†,§] Katukuri Sirisha,[‡,§] Shaik Jeelani Basha,[‡] Pottireddygari Gurava Reddy,[‡,∥] and Akella V. S. Sarma*[,‡]

[†]Organic and Bimolecular Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India

[‡]Centre for Nuclear Magnetic Resonance and Structural Chemistry, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India

Supporting Information



ABSTRACT: The present study is aimed at the design and synthesis of peptides with hybrid helix-turn-helix (HTH) motif and their conformational analysis (NMR, MD, and CD studies). The requisite peptides with heterogeneous backbones were prepared from β-, γ-, and δ-amino acids with carbohydrate side chains and α-amino acid, L-Ala. The α/β-peptides were prepared from (*S*)-β-Caa$_{(l)}$ (C-linked carbo-β-amino acid with D-*lyxo* furanoside side chain) and L-Ala with a 1:1 alternation. The α/β-peptides with "helix-turn" motif displayed a 11/9-helix nucleating a 13-atom H-bonding turn. The α/β-octapeptides showed the presence of HTH structures with bifurcated 11/15-H-bonded turn. Further, the α/β-hexapeptide with HT motif, independently on coupling with $\gamma/\alpha/\gamma/\alpha$- and $\delta/\alpha/\delta/\alpha$-tetrapeptides at the C-terminus provided access to the decapeptides with "hybrid HTH" motifs. The decapeptide ("$\alpha-\beta-\alpha-\beta-\alpha-\beta-\gamma-\alpha-\gamma-\alpha$") showed a hybrid HTH with "11/9/11/9/11/16/9/12/10" H-bonding, while the decapeptide ("$\alpha-\beta-\alpha-\beta-\alpha-\beta-\delta-\alpha-\delta-\alpha$") revealed the presence of a "11/9/11/9/11/17/9/13/11" helical pattern. The above peptides thus have shown compatibility between different types of helices and serendipitous bifurcated 11/16- and 11/17-turns. The present study thus provided the first opportunity for the design and study of "hybrid HTH" motifs with more than one kind of helical structures in them.

INTRODUCTION

The primary sequence of proteins and peptides made from α-amino acids fold into complex structures that are responsible for their diverse functions.[1] The interaction of stable and fundamental secondary structures,[2] by their assembly, generate tertiary and quaternary structures of higher order. Such a relationship between the structure and function afforded an opportunity for the development of peptides from non-natural amino acids, leading to "foldamers".[3] Extensive and sustained efforts resulted in the development of peptide foldamers with homogeneous and heterogeneous backbones constituting folding propensities of several types.[3] Though the intensified efforts resulted in the identification of tertiary and quaternary structures in peptides designed from non-natural amino acids,[4] the progress has been limited.[5] However, efforts by different research groups led to the identification of quaternary structures such as helix bundles in β- and α/β-peptides.[6]

The helix-turn-helix (HTH) structural motif is one of the simplest functional assemblies and has been implicated in various important functions in DNA-binding proteins.[7] The HTH motif is a tertiary structure constructed from two helices that are connected through a tight turn that resembles the seven membered H-bonded ring of the classical γ-turn. The turn of the HTH motif is distinct from the more common β-turn.[8] The HTH turn lacks the H-bond of the γ-turn, and consequently the backbone angles differ from the classical γ-turn angles. The type II and II′ β-turns have sequence chiralities that make them particularly facile targets for de novo design.[9] Etzkorn et al.[10] reported a peptidomimetic[11] of the turn in the HTH motif of DNA-binding proteins. The conformational constraint in this system was achieved by an unusual linking of two amino acids with a side chain C−C bond and determined the structure. Durani et al.[12] reported a de novo design of a potential turn-helix motif. In further investigations, Koksch et al.[13] reported a study to identify extended sequences of β- and γ-amino acids in β/γ-hybrid peptides that appear to be well suited to mimic an α-helical coiled coil to produce artificial chimeric folding motifs. The β/γ-hybrid peptides[14] with an alternating backbone pattern were utilized as structural mimics of an α-helix.[15]

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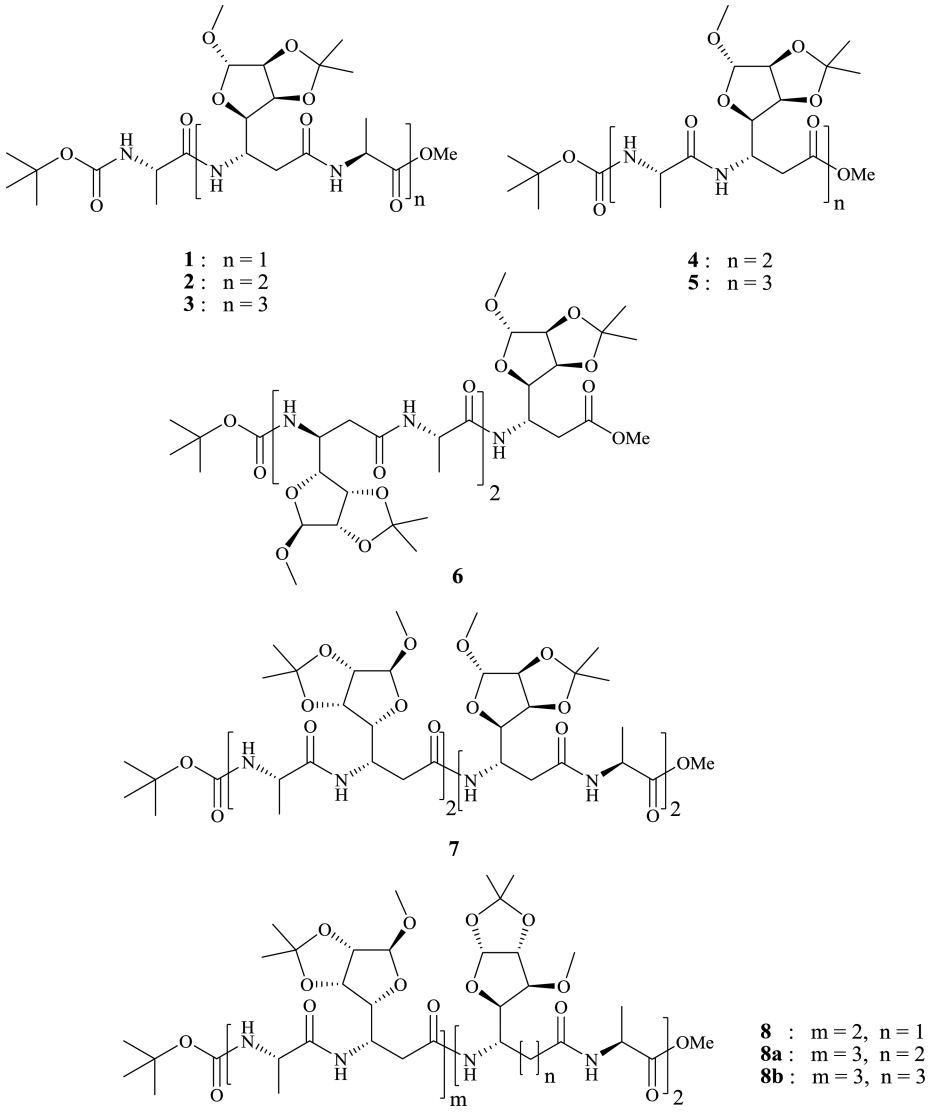

Figure 1. Structures of peptides **1**−**8**, **8a**, and **8b**.

Our group has earlier reported[16] designs on HTH motif, with two helices connected by a turn motif. A de novo design from *β*-peptides with 12/10-helix[17,18] derived from C-linked carbo-*β*-amino acid, (*S*)-*β*-Caa$_{(l)}$[19] with a D-*lyxo* furanoside side chain and a 7-membered turn motif, was reported[16] to result in HTH motif. Likewise, a study on *α*/*β*-peptides[20] revealed that a 11/9-helix[21,22] [a 11/9-helix as defined by Hofmann, has 9-atom H-bond between the *β*-residue NH(*i*)···O═C(*i*+1) of *α*-residue (β_i/α_{i+1}) and 11-atom H-bond between the *α*-residue NH(*i* +3)···O═C(*i*) of *β*-residue (α_i/β_{i+3}) interaction][21b] nucleated by a serendipitous 7/13-atom turn to give a helix-turn (HT), which in turn was converted into HTH motif with a 11/15-atom bifurcated H-bonding.[23a] Further studies from our group generated HT peptides with 12-, 14-, and 15-atom turns [between NH(i)/CO(i+2)], supported by a 7-atom turn [between CO(i)/NH(i+2)] in *α*/*β*-peptides[23b] having *β*−*α*−*α*, *β*−*α*−*γ* and *β*−*α*−*δ* sequences at the C-terminus respectively, derived from (*S*)-*β*-Caa$_{(x)}$. However, replacement of L-Ala in *β*−*α*−*β* sequence with Gly, generated a turn with 13-atom H-bonding.[23b] Our recent results on the *α*/*β*-peptides[24] derived from (*S*)-*β*-Caa$_{(r)}$ with D-*ribo* furanoside side chain and $\beta^{2,2}$-peptides from $\beta^{2,2}$-Caa$_{(x)}$[25] have shown the presence of electrostatic interactions between the NH of amide and -OMe at C-3 of furanoside side chain. Having found interesting information from the above studies on the difference in the behavior of carbohydrate side chains (D-*xylo* and D-*ribo*), it was proposed to utilize *β*-Caas with D-*lyxo* furanoside side chain[16,26] in the design of *α*/*β*-peptides. Herein, we report the synthesis of *α*/*β*-peptides from (*S*)-*β*-Caa$_{(l)}$ with D-*lyxo* furanoside side chain and L-Ala alternatingly, to generate HT peptides and use them in the design of peptides with "hybrid HTH", with more than one kind of helical patterns in them (Figure 1). The conformational analysis (NMR, MD, and CD) of the new peptides **1**−**8**, **8a**, and **8b**, would give us an opportunity to understand the effect of carbohydrate side chain on the formation of helix and turn, as well as their stabilities, besides the successful evaluation on the concept of hybrid HTH.

RESULTS AND DISCUSSION

Synthesis of Peptides 1−8. Peptides **1**−**8** were prepared by standard peptide coupling methods[27] using EDCI, HOBT, and DIPEA in solution phase. Accordingly, condensation of acid **9** with the known[16] salt **10a** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 afforded the dipeptide **11** in 85% yield

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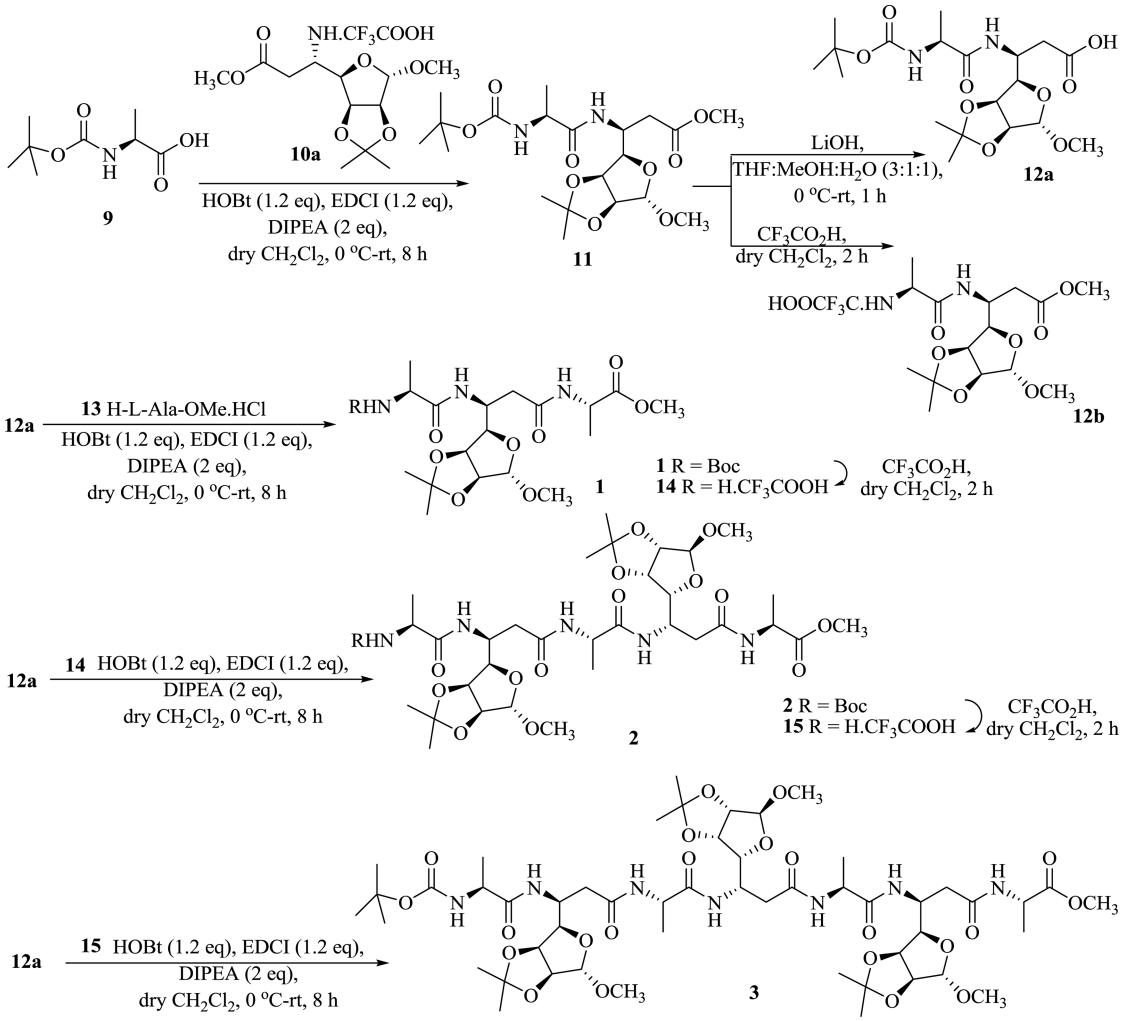
Scheme 1. Synthesis of Peptides 1−3



(Scheme 1). Ester **11** on hydrolysis with LiOH furnished the acid **12a**, while **11** on reaction with CF_3COOH in CH_2Cl_2 gave **12b**. Coupling (EDCI, HOBt, and DIPEA) of acid **12a** with salt **13** in CH_2Cl_2 furnished the tripeptide **1** (62%), which on acid (CF_3COOH) mediated reaction in CH_2Cl_2 gave the salt **14**.

Likewise, coupling of acid **12a** with the salt **14** in CH_2Cl_2 afforded the pentapeptide **2** in 40% yield, which on hydrolysis with CF_3COOH gave the salt **15**. Similarly, acid **12a** on coupling (EDCI, HOBt and DIPEA) with the salt **15** in CH_2Cl_2 furnished the heptapeptide **3** (32%).

In a further study, acid **12a** on coupling with the salt **12b** in CH_2Cl_2 gave the tetrapeptide **4** in 61% yield (Scheme 2). Peptide **4** on base (LiOH) hydrolysis furnished **16a**, while **4** on treatment with CF_3COOH afforded the salt **16b**. Acid **12a** on coupling (EDCI, HOBt, and DIPEA) with the salt **16b** in CH_2Cl_2 afforded the hexapeptide **5** (60%), which on base hydrolysis furnished acid **5a**. Similarly, coupling of acid **10b** with the salt **16b** in CH_2Cl_2 gave the pentapeptide **6** in 55% yield.

Likewise, acid **10b** on coupling (EDCI, HOBt, and DIPEA) with the salt **14** in CH_2Cl_2 furnished the tetrapeptide **17** in 55% yield, which on reaction with CF_3COOH in CH_2Cl_2 gave the salt **17a**. Acid **16a** on coupling with the salt **17a** in CH_2Cl_2 afforded the octapeptide **7** in 27% yield, while reaction of **16a** with the known salt **18**[21a] gave the octapeptide **8** in 29% yield (Scheme 3).

CONFORMATIONAL ANALYSIS

The NMR spectra of the peptides **1**−**8** (Figure 1) were recorded as 3−5 mM solution in $CDCl_3$.[28] The initial studies on the monomer (*S*)-*β*-Caa$_{(l)}$ **10c** with D-*lyxo* furanoside side chain and (*S*)-*β*-Caa$_{(x)}$ **10d** with D-*xylo* furanoside side chain revealed that both of them have comparable stability. The side chains in **10c** and **10d** have puckered conformations, while in D-*lyxo* furanoside side chain of **10c**, C4 is in plane, whereas it is out of plane in D-*xylo* furanoside side chain of **10d**. Though the acetonide protection is at C1/C2 in **10d**, while on C2/C3 in **10c**, no significant structural difference was found in both these *β*-residues, except for the furanoside conformation. For **10c**, small $^3J_{C\beta H-C\alpha H}$ values and medium NOE's with NH reveal $\theta_\beta \approx 60°$. One of the *α*-protons with a relatively strong NOE with NH and relatively large $^3J_{C\beta H-C\alpha H}$, is assigned as *pro-R*, while the other proton is assigned as *pro-S*. Likewise, C*α*H$_{(pro\text{-}S)}$, showed relatively strong NOE with C3H than C*α*H$_{(pro\text{-}R)}$. The $^3J_{C\beta H-C4H}$ = 7.3 Hz indicates that averaging over several conformations and medium NOE between NH/C4H shows the C*β*H and C4H are antiperiplanar to each other. The $^3J_{C4H-C3H}$ = 3.5 Hz, $^3J_{C3H-C2H}$ = 5.8 Hz, and $^3J_{C2H-C1H}$ = 0 Hz are consistent with a sugar pucker as shown in Figure 2. In addition, the OMe is closer to C*α*H$_{(pro\text{-}S)}$ in **10d**, whereas it is proximal to C*β* proton in **10c**.

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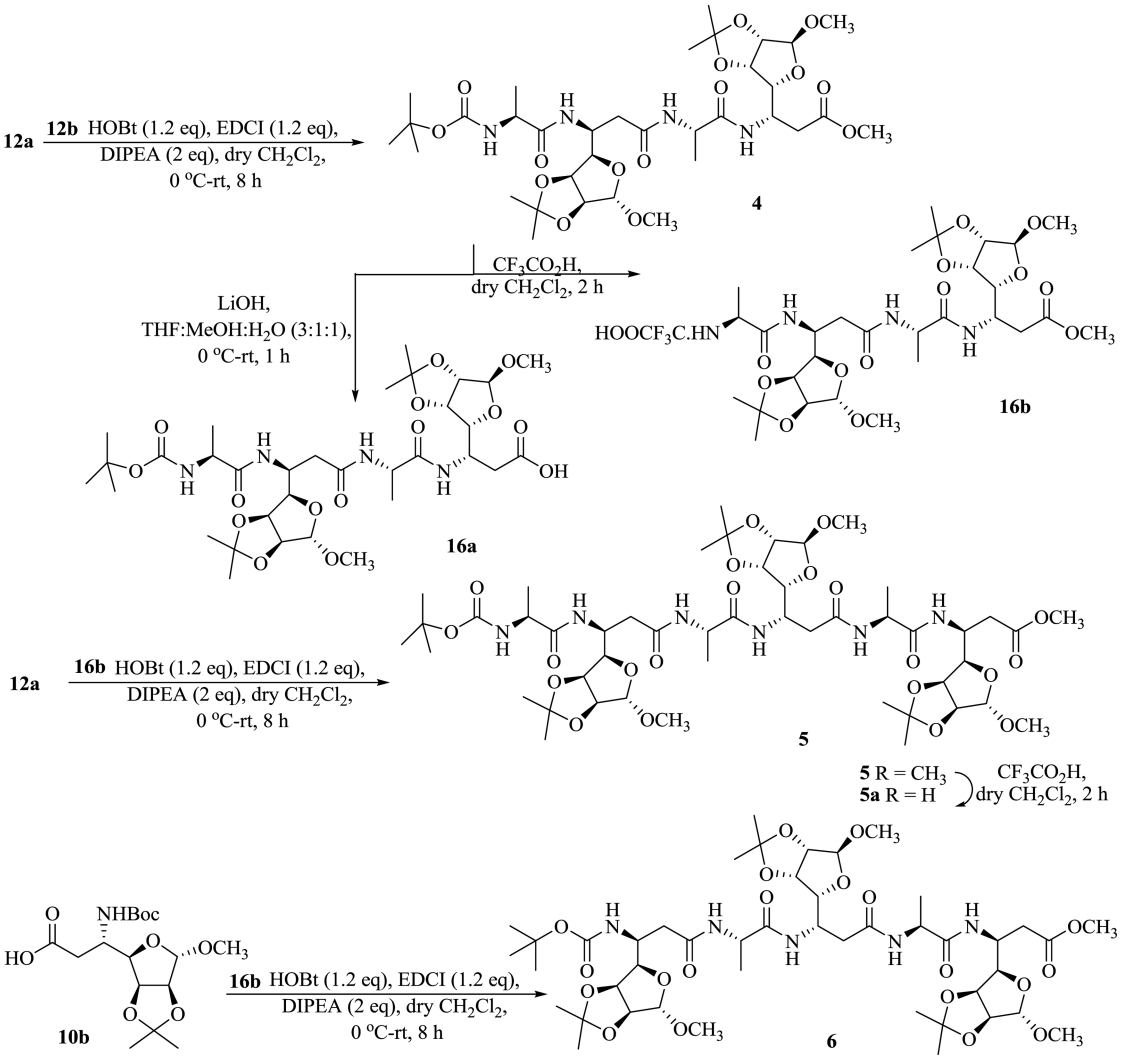
Scheme 2. Synthesis of Peptides 4−6



Based on the above findings, oligomers from (*S*)-*β*-Caa$_{(l)}$ and L-Ala in 1:1 ratio alternating were studied.[28] Peptides **1**−**3** with L-Ala at the C-terminus displayed the characteristics of 11/9-mixed helix. The proton NMR spectrum of peptide **1**[28] showed a downfield shift of amide protons and small chemical shift change in the solvent titration study,[29] indicating their participation in H-bonding, except for NH(1). Together with characteristic NOE's NH(2)/NH(3) and C*α*H(1)/NH(3) information, some population of NH(2) showed 9-membered and NH(3) showed 11-atom hydrogen bonding. Even though the presence of downfield chemical shifts and small $\Delta\delta$ values from solvent titration were observed in **1**, it showed only the presence of a nascent structure, despite the presence of few medium range NOEs. This observation in **1** shows a difference in the structural stability with respect to the known[21a] tripeptide prepared from (*S*)-*β*-Caa$_{(x)}$, having a D-*xylo* furanoside side chain.

Detailed analysis of heptamer **3** showed a well resolved proton NMR spectrum[28] with five of the seven amide protons resonating above 8 ppm and NH(6) resonating at 7.65 ppm, indicating their participation in H-bonding. The quantitative information about H-bonding was obtained from solvent titration study[29] with DMSO-d_6, where a very small change in chemical shifts indicated the strength of their H-bonding interaction. For Boc NH, the large value of $\Delta\delta$ indicated the absence of H-bonding. Small values of $^3J_{C\alpha H/C\beta H}$ (<5.1 Hz) and large values of $^3J_{NH\text{-}C\beta H}$ (~9 Hz) for *β*-Caa$_{(l)}$ residues are consistent with $\theta \approx 60°$ and $\phi \approx 100°$, respectively. The $^3J_{NH\text{-}C\alpha H}$ values of ~6 Hz for the first three *α*-residues indicated the possibility of averaging of several conformations with a $\phi_\alpha \approx -60$ or +180, whereas, for the residue *α*(4), large value of $^3J_{NH\text{-}C\alpha H}$ indicates ϕ_α may be around −120°. As described for the monomer, the protons of C*α*H were stereospecifically assigned based on the couplings with *β*-protons and proximity from the amide protons. C$\alpha H_{(pro\text{-}R)}$ is assigned based on its relatively large coupling with C*β*H and strong NOE with succeeding amide proton. The large value of $^3J_{C\beta H-C4H} \approx 10$ Hz may be the result of a single conformation about χ_1(H−C$_\beta$-C$_4$−H) ≈ 180°. From the detailed analysis of **3** from the ROESY spectrum (Figure 3), it was realized that the characteristic NOE correlations C*α*H(1)/NH(3), C*α*H(3)/NH(5), C*α*H(5)/NH(7), NH(2)/NH(3), NH(4)/NH(5), and NH(6)/NH(7) together with coupling information correspond to the predominance of a right-handed 11/9-mixed helix. The presence of 11/9-helix received support from CD profile of **3** (Figure 4) with a maximum at 202 and a shoulder at 225 nm.

Overlay of 20 superimposed minimum energy structures of peptide **3**, with average pairwise heavy atom and backbone RMSD value of 0.39 and 0.18 Å, respectively, as is shown in

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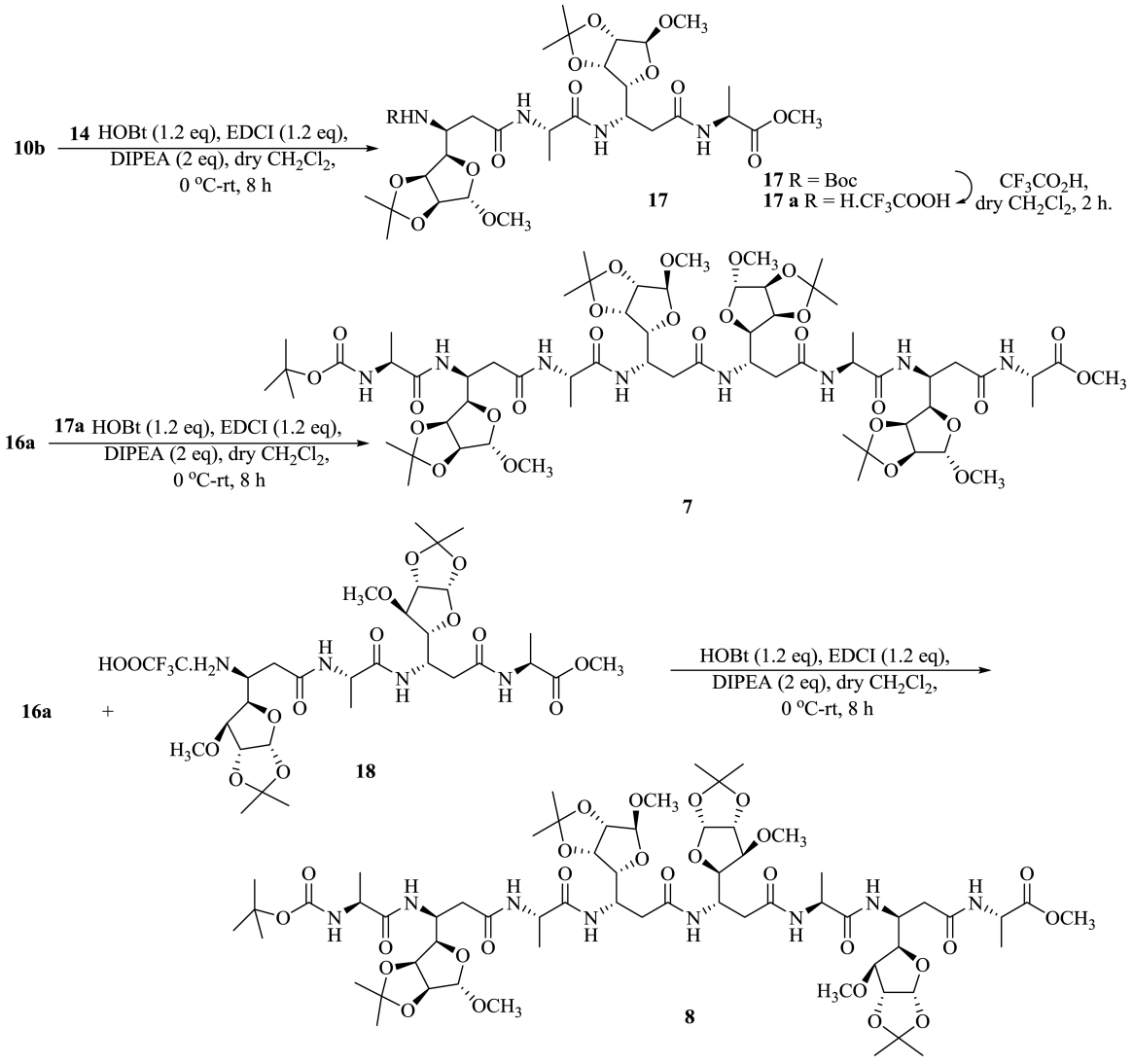
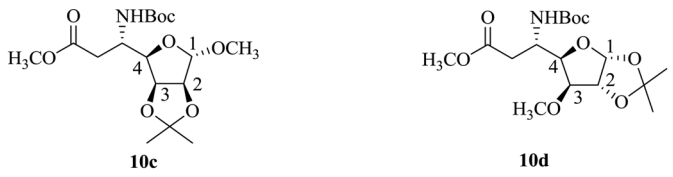
Scheme 3. Synthesis of Peptide 7 and 8





Structures of monomers: (a) (S)-β-Caa$_{(l)}$ **10c**; (b) (S)-β-Caa$_{(x)}$ **10d**.

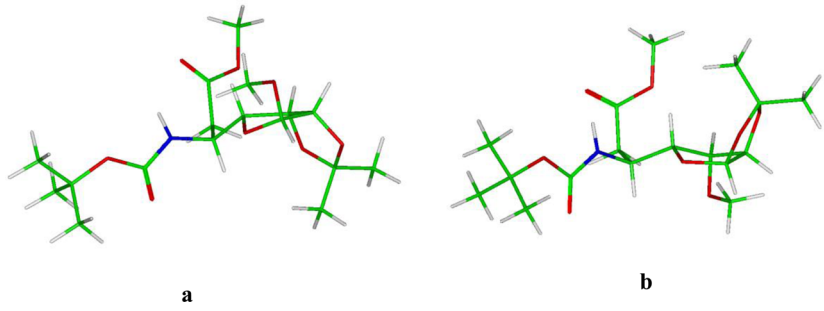


Figure 2. Energy minimized structures of monomers. (a) (S)-*β*-Caa$_{(l)}$ **10c**; (b) (S)-*β*-Caa$_{(x)}$**10d**.

Figure 5, gave evidence for the presence of the NMR derived proposed conformation with 11/9-helical pattern.

Pentapeptide **2**, from its corresponding NMR, CD, and MD data,[28] revealed the presence of a right-handed 11/9-helix. Thus,

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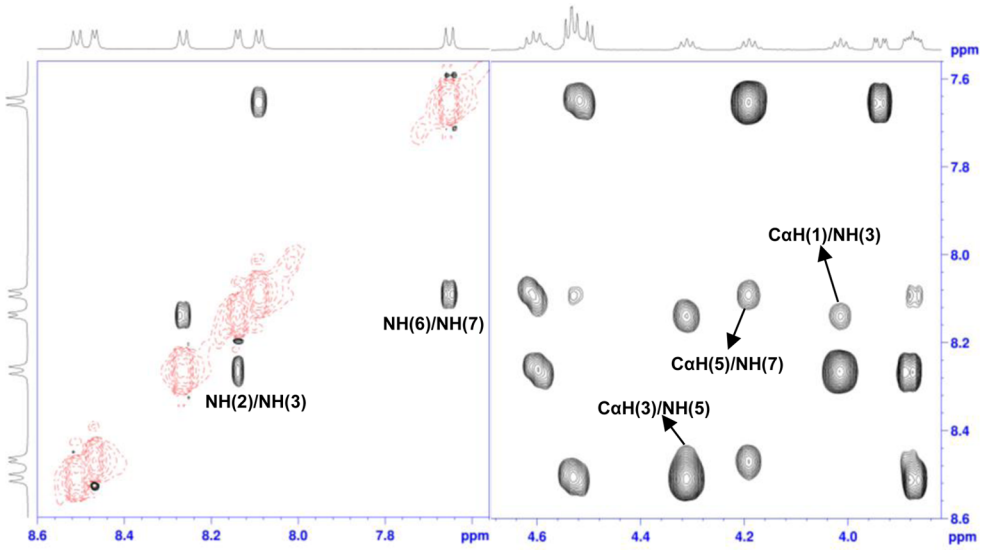

Figure 3. ROESY spectrum of peptide **3** depicting the characteristic NOE correlations.

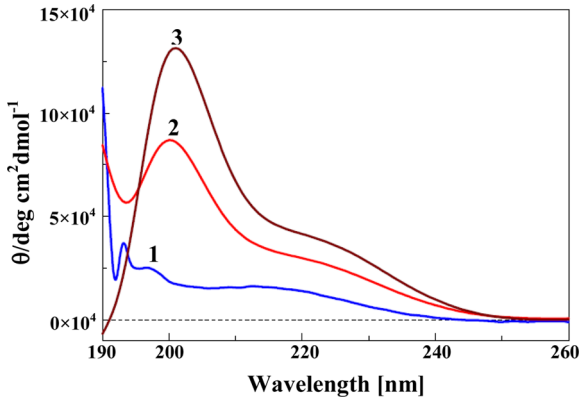

Figure 4. CD spectra of peptides **1**−**3** in MeOH.

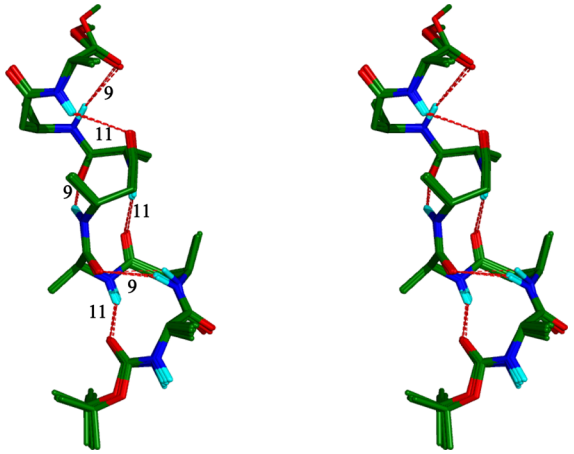

Figure 5. Stereoview of superimposed 20 minimum energy structures of **3** (sugars are replaced with methyls for clarity).

the above study on peptides **1**−**3** with "*α*−*β*−*α*" sequence at the C-terminus though inferred the presence of a right-handed 11/9-helix, the stability of the secondary structures are found to be relatively weak when compared to the oligomers reported[21a] with D-*xylo* furanoside side chain.

Further, studies were undertaken on the peptides **4**−**6** with a *β*−*α*−*β* sequence at the C-terminus. Detailed study of hexapeptide **5** showed all the amide protons except NH(1) resonating at ≥7 ppm, indicating the probability of their participation in H-bonding.[28] Quantitative H-bonding information was obtained from solvent titration study,[29] which clearly indicated that except for NH(1) and NH(6) all the amide protons were involved in H-bonding. The standard characteristic NOE correlations encompassing residues 1−4 at the N-terminus end of the peptide were observed in peptide **5** that correspond to a 11/9-helix, which are supported by $^{3}J_{\text{NH-C}\alpha\text{H}}$ = 6.0 Hz for *α*-residue, $^{3}J_{\text{NH-C}\beta\text{H}}$ > 9.1 Hz and $^{3}J_{\text{C}\alpha\text{H-C}\beta\text{H}}$ < 5.0 Hz for the *β*-residues as well as C*α*H(1)/NH(3), C*α*H(3)/NH(5), and NH(2)/NH(3) NOE signatures. Further, the distinguishing NOE correlations NH(4)/NH(5), NH(5)/NH(6), NH(4)/NH(6), and C4H(4)/NH(6), as shown in Figure 6, involving the C-terminal residues, confirmed a variation in the structure compared to peptides **1**−**3**.

The proximity among the amide protons at C-terminus and NOE between C4H(4) with NH(6) support the presence of an unusual three residue turn stabilized by a 13-atom H-bonding between NH(4) and CO(6). However, peptide **5** differs from the reported[23a] peptide with D-*xylo* furanoside side chain, which displayed a 7/13-turn. Such a difference may be attributed to the presence of a bulky 2,3-acetonide group in the D-*lyxo* furanoside ring from the *β*-face, while, the 1,2-acetonide group is in *α*-face in D-*xylo* furanoside.

Restrained MD studies on peptide **5** revealed the presence of a helix-turn (HT) motif (Figure 7). The stereoview of 20 superimposed minimum energy structures of peptide **5** with average pairwise heavy atom and backbone RMSD values of 0.57 and 0.23 Å, confirm the presence of a 13-atom H-bonding at C-terminus, nucleated by a 11/9-helix.

Similar secondary structural propensity was observed in the other oligomers **4** and **6**, as revealed from the standard characteristic NOE correlations and H-bonding information obtained from solvent titration.[28] It is noticed that the extent of stability in the helix-turn peptides **4**−**6** is less, in comparison to the reported peptides[23a] with D-*xylo* furanoside side chain. Such a lesser stability in the conformational behavior may be attributed

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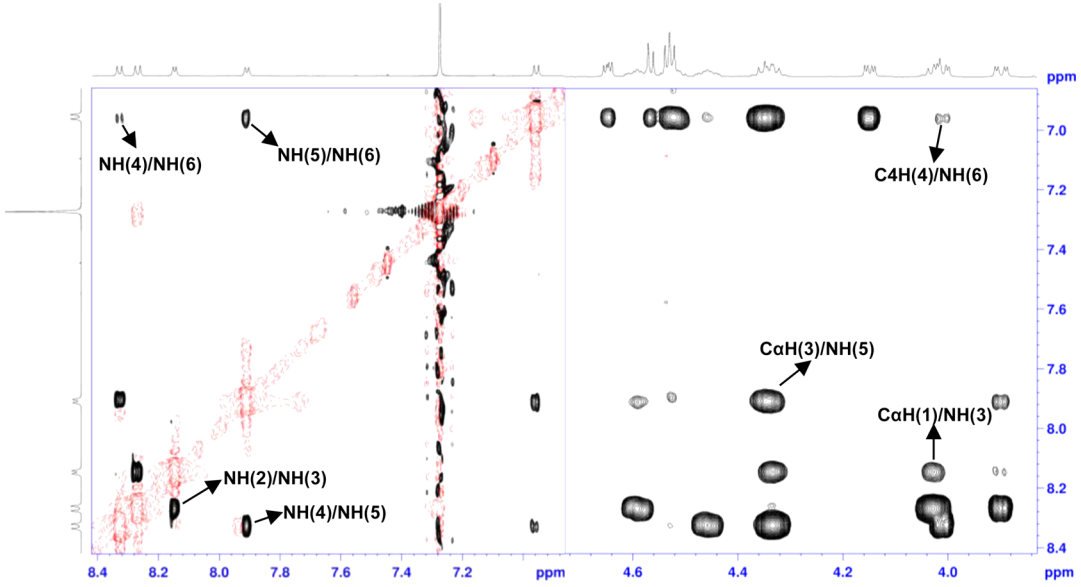

Figure 6. ROESY spectrum of peptide **5** depicting the characteristic NOE correlations.

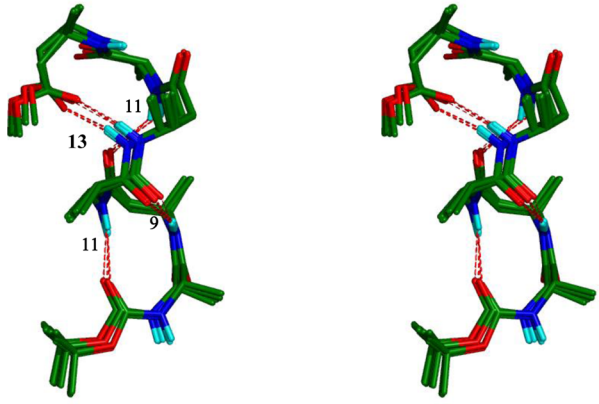

Figure 7. Stereoview of superimposed 20 minimum energy structures of **5** (sugars are replaced with methyls for clarity).

to the absence of additional stabilization from the γ-turn (7-atom H-bonding) in the present series of peptides.

Further, the studies were extended to understand the secondary structures in octapeptides **7** and **8** with HTH motif. The downfield chemical shift of several amide protons in proton NMR spectrum of **7** indicated their H-bonding nature.[28] Except NH(1), all the amide protons displayed small $\Delta\delta$ values (<0.72 ppm) in solvent titration[29] with DMSO-d_6 indicating their participation in H-bonding. The NOE correlations, couplings and MD studies confirmed that the peptide showed some difference in the folding pattern, when compared to the helix-turn structure observed in peptides **4**-**6**. Based on the usual NOE correlations of CαH(1)/NH(3), NH(2)/NH(3), CαH(6)/NH(8), and NH(7)/NH(8) together with the coupling constants $^3J_{\text{NH-C}\beta\text{H}}$ = 8.5 Hz for β(2), $^3J_{\text{NH-C}\alpha\text{H}}$ = 6.2 Hz for α(1), $^3J_{\text{NH-C}\alpha\text{H}}$ = 7.0 Hz for α(3), $^3J_{\text{NH-C}\beta\text{H}}$ = 9.4 Hz for β(7), $^3J_{\text{NH-C}\alpha\text{H}}$ = 4.6 Hz for α(6), and small values of $^3J_{\text{C}\alpha\text{H-C}\beta\text{H}}$, the N-terminal part of the peptide encompassing residues α(1)-β(2)-

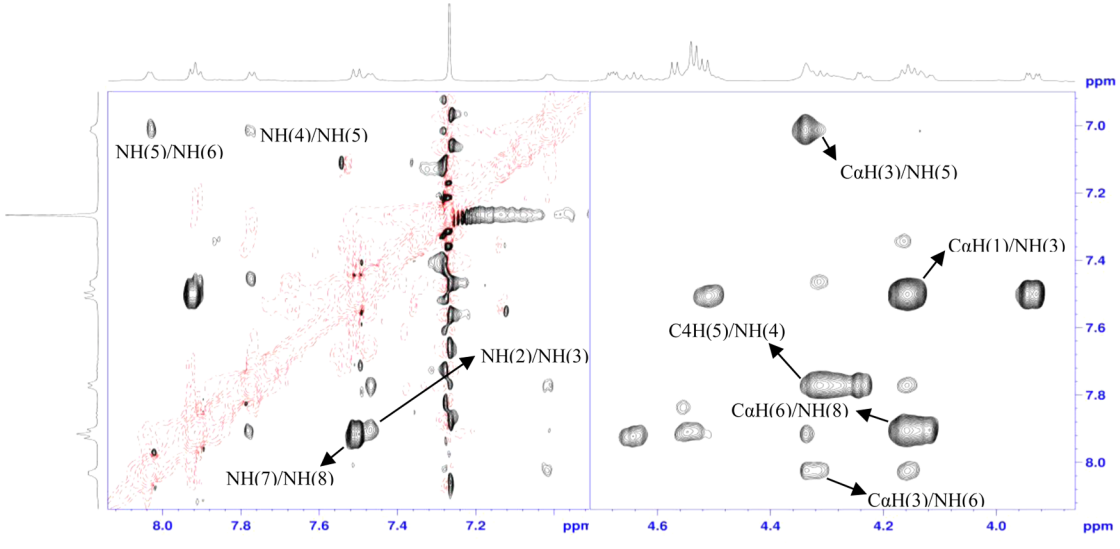

Figure 8. ROESY spectrum of peptide **7** depicting the characteristic NOE correlations.

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α(3) and C-terminal part of the peptide encompassing the residues α(6)-β(7)-α(8) showed the propensity of 11/9-helix. The turn region encompassing the residues α(3)-β(4)-β(5)-α(6) showed new characteristic NOE correlations (Figure 8).

The presence of NH(4)/NH(5), NH(5)/NH(6), CαH(3)/NH(5), and CαH(3)/NH(6) NOE's indicated a bifurcated three center H-bond shared between CO(2) with NH(5) and NH(6) forming 11/15-membered pseudo rings, respectively. Likewise, the NOE correlation NH(4)/C4H(5) supports a 9-atom H-bonding between NH(4) and oxygen on sugar ring of β(5) residue imparting further stabilization to the overall structure observed in HTH motif.

The restrained MD calculations, as displayed in Figure 9, further confirmed a HTH structure in peptide **7**. The overlay of

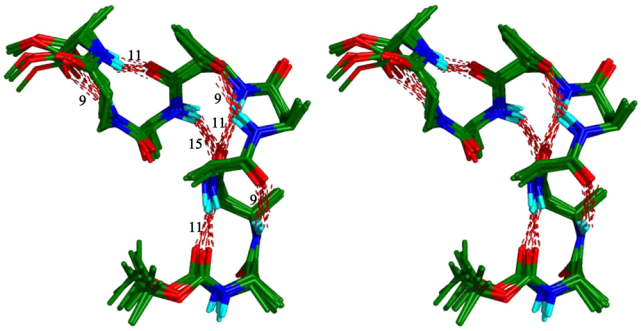

Figure 9. Stereoview of superimposed 20 minimum energy structures of **7** (sugars are replaced with methyls for clarity).

20 minimum energy structures with an average pairwise heavy atom and backbone RMSD values of 0.90 and 0.85 Å, respectively, clearly showed the HTH pattern in peptide **7**.

Conformational analysis of peptide **7** was also carried out in a polar solvent like methanol.[28] Though a majority of the NOE correlations characteristic of a HTH motif were observed in methanol, they were however found to be weak when compared to the NOE's observed in $CDCl_3$.

The peptide **8**, designed with D-*lyxo* furanoside side chain in HT region and a helix at the C-terminus with a D-*xylo* furanoside side chain, was studied to understand the compatibility between two different carbohydrate side chains in a single peptide. The NMR analysis of **8** revealed all the NOE correlations, couplings and similar structural features that were observed for **7**, inferring the presence of similar HTH structure. MD studies[19] on **8** supported a 11/9-helix at the N- and C-termini with a 11/15-bifurcated H-bonding in the hinge region, wherein the conformation was stabilized by a 9-atom H-bonded turn structure between backbone amide proton of residue β(4) and side chain oxygen atom on sugar ring of β(5). The observations from the NMR studies, besides the comparable molar ellipticities in the CD profile (Figure 10), indicated their comparable stabilities.

This study revealed that the α/β-peptides with β-residues containing D-*lyxo* furanoside side chain displayed similar 11/9-helix structure but a small variation in the turn structure, whereas, HTH super secondary structure is relatively less stable than the peptides with D-*xylo* furanoside side chain.

Synthesis of Peptides with Hybrid HTH. Having synthesized the α/β-peptides with HT and HTH motifs from (*S*)-β-Caa$_{(l)}$ and L-Ala, the study was then extended to the synthesis of hybrid HTH peptides **8a** and **8b** (Scheme 4), by

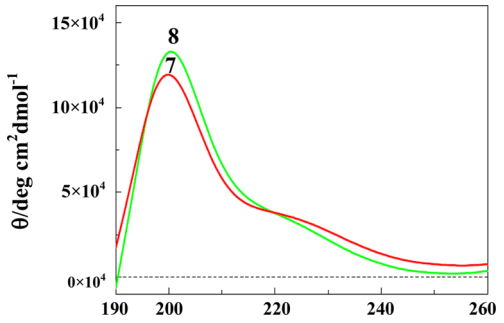

Figure 10. CD spectra of peptides **7** and **8**.

making use of HT peptide **5** and reported α/γ-tetrapeptide **19** with a 12/10-helix[30] and α/δ-tetrapeptide **20** with 11/13-helix.[31]

Accordingly, acid **5a** on condensation with the known[30] salt **19a** prepared from **19** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 gave the decapeptide **8a** in 37% yield (Scheme 4). Likewise, acid **5a** on coupling (EDCI, HOBt, and DIPEA) with the known[31] salt **20a** in CH_2Cl_2 afforded the decapeptide **8b** in 20% yield (Scheme 4).

The ^{1}H NMR spectrum of peptide **8a** showed well resolved downfield chemical shift of all the amide protons indicating a well-defined structure.[28] Except NH(1), all the amide protons displayed small $\Delta\delta$ values in solvent titration[29] with DMSO-d_6 indicating the participation of the amide protons in H-bonding. The NOE correlations, couplings, and MD studies confirmed that peptide **8a** showed similar folding pattern, when compared to helix-turn-helix structure observed above in peptides **7** and **8**. Based on the characteristic NOE correlations of CαH(1)/NH(3), NH(2)/NH(3), CαH(3)/NH(5), and NH(4)/NH(5) together with coupling constants $^3J_{\text{NH-C}\beta\text{H}}$ = 9.6 Hz, small values of $^3J_{\text{C}\alpha\text{H-C}\beta\text{H}} \leq 4.9$ for β-residues and $^3J_{\text{NH-C}\alpha\text{H}}$ = ~5.5 Hz for α-residues at the N-terminal part of the peptide encompassing residues α(1)-β(2)-α(3)-β(4)-α(5) showed the propensity of 11/9-helix. The C-terminal part of the peptide encompassing the residues α(8)-γ(9)-α(10) showed the characteristic signatures as CαH(8)/NH(10), CβH$_{(pro\text{-}R)}$(9)/NH(9), and NH(9)/NH(10) along with couplings $^3J_{\text{NH-C}\alpha\text{H}}$ = ~6.5 Hz for α-residues and $^3J_{\text{NH-C}\gamma\text{H}}$ = 8.9 Hz for γ-residues which correspond to a 12/10-mixed helix. The turn region encompassing the residues α(5)-β(6)-γ(7)-α(8) showed characteristic NOE correlations NH(6)/NH(7), CαH(5)/NH(7), and CαH(5)/NH(8) along with couplings $^3J_{\text{NH-C}\alpha\text{H}}$ = ~5.5 Hz for α-residues, $^3J_{\text{NH-C}\beta\text{H}}$ = 8.9, small values of $^3J_{\text{C}\alpha\text{H-C}\beta\text{H}} \leq 4.2$ for β-residues, and $^3J_{\text{NH-C}\gamma\text{H}}$ = 8.6 Hz for γ-residues. These characteristic features indicated the presence of a bifurcated three center H-bond shared between CO(4) with NH(7) and NH(8) forming a 11/16-atom H-bonding. Likewise, the NOE correlations NH(6)/C4H(7) and NH(6)/C1H(7) indicate a 9-atom H-bonding between NH(6) and oxygen on sugar ring of β(7) residue imparting further stabilization to the overall structure observed. The restrained MD calculations confirmed the above observation.[28]

Peptide **8b** also showed similar characteristics as observed for peptide **8a**, corresponding to a 11/9-helix at the N-terminus, 13/11-helix at the C-terminus, and 11/17- atom bifurcated pseudo ring in the turn region along with 9-atom interaction between backbone and side chain. The ROESY spectrum showed characteristic NOE correlations as shown in Figure 11.

The restrained MD calculations for **8b** further confirmed the above predictions, and the overlay of 20 minimum energy structures as shown in Figure 12 revealed the presence of a hybrid

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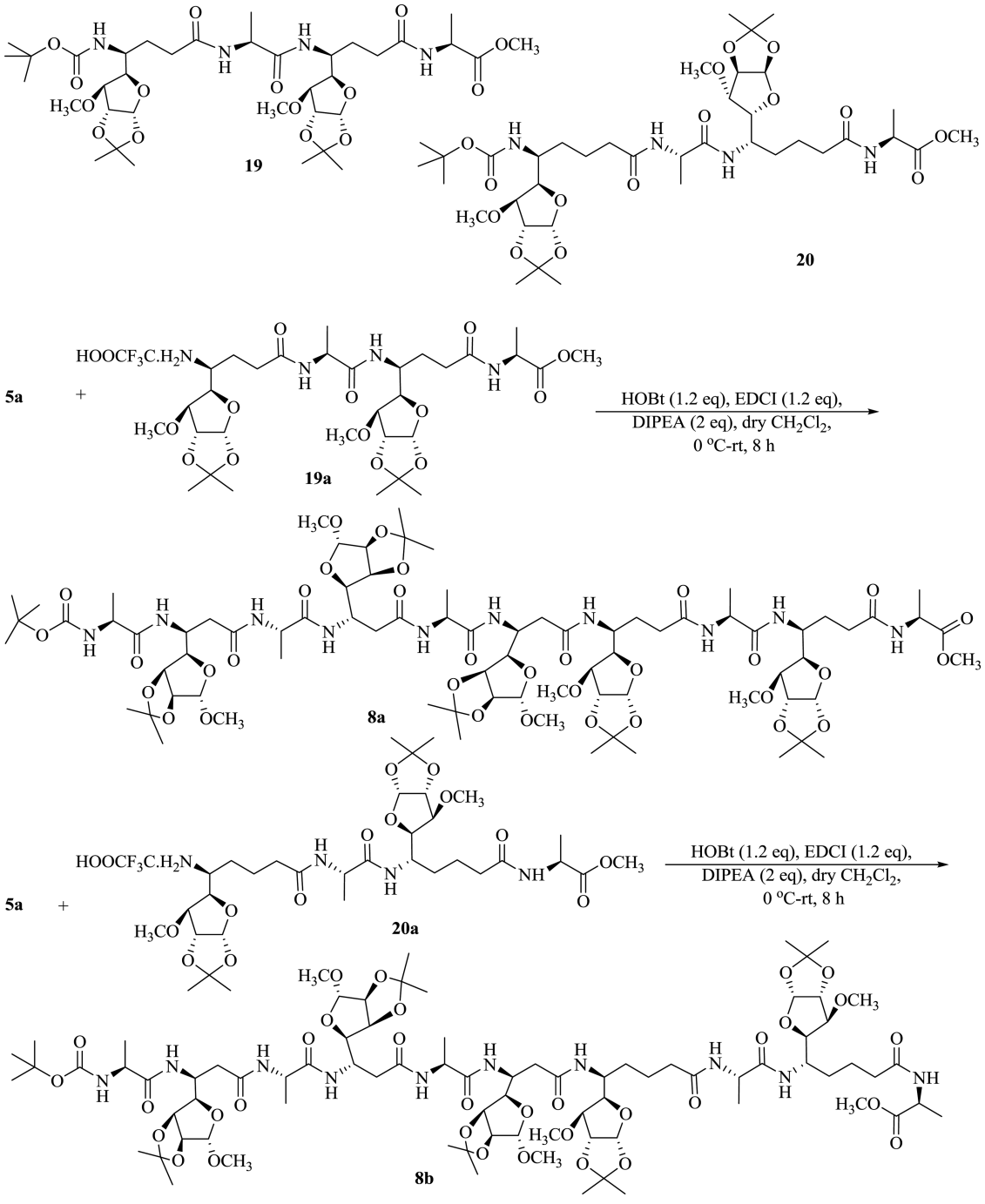
Scheme 4. Synthesis of Peptides 8a and 8b



HTH with 11/9/11/9/11/17/9/13/11-hydrogen bonding in peptide **8b**.

The structural information for the HTH super secondary structures observed above in peptides **8a** and **8b** are further supported by CD profiles (Figure 13).

CONCLUSION

In conclusion, the present study resulted in the design and synthesis of peptides with a new concept on "hybrid HTH", besides, showing the impact of D-*lyxo* furanoside side chain on the formation and stability of secondary structures and compatibility with (*S*)-β-Caa$_{(x)}$ with D-*xylo* furanoside side chain. Unlike the earlier reports, the new α/β-peptides from (*S*)-β-Caa$_{(l)}$, with D-*lyxo* furanoside side chain and L-Ala have shown (a) nascent population of 11/9-mixed helix in α/β-tripeptide, (b) 13-atom H-bonding turn in contrast to a reported 7/13-turn, in the $\beta-\alpha-\beta$ sequence at the C-terminus, and (c) bifurcated 11/15-turn supported by a 9-atom H-bonding with furan oxygen atom, with relatively less stability in HTH motifs. Such deviations may be attributed to the carbohydrate side chain and steric factors resulting from the acetonide group. Further, the present study evidently proved the concept of "hybrid HTH" motif in peptides prepared from the α/β-peptides on coupling with γ/α- or δ/α-tetrapeptides. These peptides displayed 11/9/11/9/11/16/9/12/10- and 11/9/11/9/11/17/9/13/11-helices, which include two new turns with 11/16- and 11/7-atom H-bonding,

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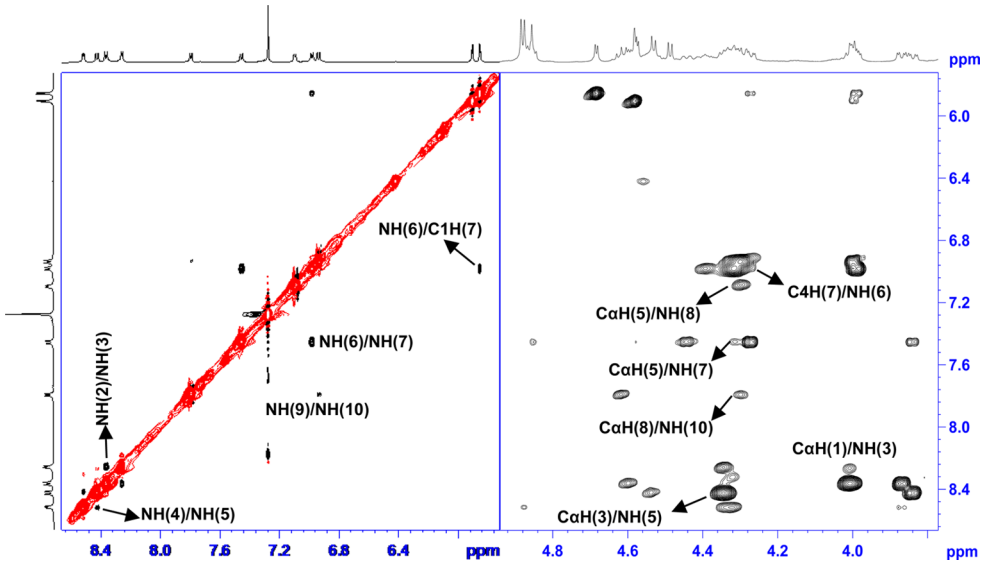

Figure 11. ROESY spectrum of peptide **8b** depicting the characteristic NOE correlations.

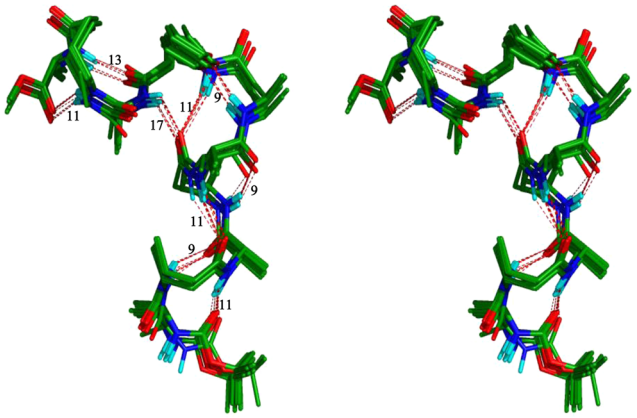

Figure 12. Stereoview of a superimposition of the 20 lowest energy structures for peptide **8b**.

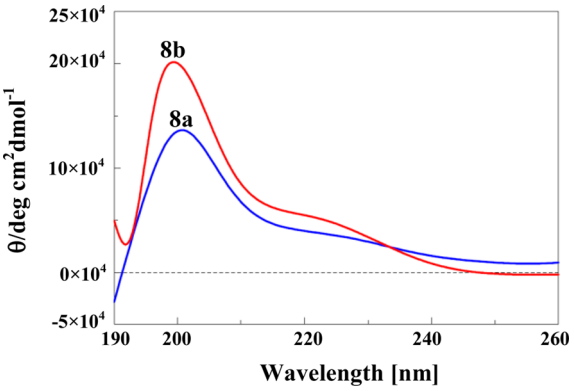

Figure 13. CD spectra of peptides **8a** and **8b** in MeOH.

in $\alpha-\beta-\gamma$ and $\alpha-\beta-\delta$ sequences. The study in the new class of peptides has shown good compatibility between the turn structure and more than one kind of helical patterns at the N- and C-termini. Further, it discloses that the carbohydrate side chains have definite influence on the helix formation and stability. In addition, the concept of "hybrid HTH" and understanding the impact of side chain would be of great use to generate peptides with super secondary structures and desirable functions in the area of foldamers.

EXPERIMENTAL SECTION

NMR spectra (1D and 2D experiments) were obtained on 400, 500, 600, and 700 MHz spectrometers at 278−303° K in chloroform solvent with tetramethyl silane (TMS) as an internal reference. All the chemical shifts were measured in a 1D NMR spectrum and coupling constants were measured with resolution enhanced 1D spectrum. Chemical shift correlations were done using two-dimensional NMR experiments such as total correlation spectroscopy (TOCSY) and rotating frame Overhauser effect spectroscopy (ROESY) in phase sensitive mode[32] with mixing times of 80 and 250−350 ms, respectively. The spectra were acquired with a 2 × 192 or 2 × 256 experiments containing 8−16 transients with a relaxation delay of 1.5−2 s. The 2D spectra were processed with a Gaussian apodization in both the dimensions. Solvent titration studies were done using sequential addition of 33% v/v DMSO-d_6 (up to 300 μL) to peptides dissolved in 600 μL of chloroform solutions.

Model building and restrained molecular dynamics simulations[33] on **2**−**8**, **8a**, and **8b** were carried out using the Insight II (97.0)/Discover program[34] on a Silicon Graphics O2 workstation. The CVFF force field with default parameters was used throughout the simulations using distance dependent dielectric constant with e = 4.8 for chloroform. The constraints were derived from the volume integrals obtained from ROESY spectra[35] using a two-spin approximation and a reference distance of 1.8 Å of geminal protons. The complete set of NOE distance constraints used for structure calculation is given in the Supporting Information. The following general protocol was used for minimizing energy. Minimizations were first done with steepest descent, followed by conjugate gradient method for a maximum of 1000 iterations each or RMS deviation of 0.001 kcal/mol, whichever was earlier. The energy-minimized structures were subjected to MD simulations. A number of interatomic distance constraints at a corresponding temperature were used for the MD run. The molecules were initially equilibrated for 50 ps and subsequently subjected to a simulated annealing protocol. Starting from 300 K, they were heated to 1500 K in four steps with increasing the temperature by 300 K and simulating for 2.5 ps at each step, and then subsequently cooled back to 300 K in 4 steps with decreasing the temperature by 300 K in each step again simulating for 2.5 ps at each step. After this a structure was saved and the above process repeated 100 times, each time saving a structure. The 100 structures generated were energy minimized again. From these 100 energy minimized structures, 20 of the lowest energy structures were superimposed for display. For peptide **2**, the backbone RMSD was 0.30 Å and the heavy atoms RMSD

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was 0.53 Å. The corresponding values for peptide **3**−**8**, **8a**, and **8b** backbone RMSD are 0.18, 0.28, 0.23, 0.81, 0.85, 0.29, 0.75, and 0.59 Å and heavy atom RMSD are 0.39, 0.50, 0.57, 0.83, 0.90, 0.40, 0.98, and 0.70 Å, respectively. For better visualization, in all the figures some atoms/groups have been removed after calculation.

CD spectra were acquired at room temperature in methanol using a 2 mm path length CD cell. Spectra were acquired with an average of 2 scans (100 ms time constant, 2 nm bandwidth), background corrected, and smoothed over 2−5 data points. The scans are carried out from 250 to 193 nM, at 200 μM concentration. Binomial method was used for smoothing the spectra. The molar ellipticities (θ) have been normalized and the data has been presented as deg cm^2 dmol^{-1}.

High-resolution mass spectra (HRMS) were recorded on a Q-TOF mass spectrometer using atmospheric pressure ionization (API), at a capillary voltage of 4.0 kV using a solution MeOH (80%) and water (20%) containing 0.1% of formic acid.

Boc-L-Ala-(*S*)-β-Caa$_{(I)}$-OMe (11). A solution of acid **9** (0.4 g, 2.11 mmol), HOBt (0.34 g, 2.53 mmol), and EDCI (0.48 g, 2.53 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min. It was treated sequentially with salt **10a** [prepared from **10c** (0.79 g, 2.11 mmol) in dry CH_2Cl_2 (1 mL) at 0 °C on treatment with CF_3COOH (0.5 mL)] and DIPEA (0.73 mL, 4.23 mmol) and stirred for 8 h. The reaction mixture was quenched with aq. satd. NH_4Cl solution (10 mL). After 10 min, it was diluted with $CHCl_3$ (2 × 10 mL) and washed with water (10 mL), $NaHCO_3$ solution (10 mL), and brine (10 mL). The organic layers were dried (Na_2SO_4) and evaporated, and the residue was purified by column chromatography (60−120 mesh silica gel, 55% ethyl acetate in pet. ether) to afford **11** (0.80 g, 85%) as a colorless syrup; $[\alpha]^{20}_D$ = +95.0 (*c* 0.1, $CHCl_3$); IR (KBr): ν 3333, 2979, 2932, 1715, 1674, 1514, 1449, 1368, 1216, 1165, 1094, 1024, 857, 771, 667 cm^{-1}; ^{1}H NMR (600 MHz, 298 K, $CDCl_3$): δ 6.63 (d, 1H, *J* = 6.45 Hz, NH-2), 5.04 (bs, 1H, NH-1), 4.88 (s, 1H, C_1H-2), 4.64 (qd, 1H, *J* = 7.5, 8.0 Hz, C_3H-2), 4.54 (m, 1H, C_βH-2), 4.53 (d, 1H, *J* = 5.7 Hz, C_2H-2), 4.18 (dd, 1H, *J* = 2.3, 7.4 Hz, C_4H-2), 3.68 (s, 3H, OMe), 3.29 (s, 3H, OMe), 2.82 (qd, 2H, *J* = 16.6, 22.6 Hz, C_αH-2, $C_{\alpha'}$H-2), 1.46 (s, 3H, Ac), 1.44 (s, 9H, Boc), 1.35 (d, 3H, *J* = 7.1 Hz, CH3−1), 1.28 (s, 3H, Ac); ^{13}C NMR (150 MHz, 298 K, $CDCl_3$): δ 172.1, 171.9, 155.3, 112.8, 106.8, 85.2, 79.8, 79.5, 78.9, 54.5, 51.7, 50.1, 46.2, 35.2, 28.2, 26.0, 24.7, 18.5; HRMS (ESI): *m*/*z* calculated for $C_{20}H_{34}N_2O_9Na$ (M^++ Na) 469.2162, found 469.2147.

Boc-L-Ala-(*S*)-β-Caa$_{(I)}$-L-Ala-OMe (1). A cooled (0 °C) solution of **11** (0.40 g, 0.89 mmol) in THF:MeOH:H_2O (3:1:1) (2 mL) was treated with LiOH (0.03 g, 1.34 mmol) and stirred at room temperature. After 1 h, pH was adjusted to 2−3 with 1 N HCl solution at 0 °C and extracted with ethyl acetate (2 × 50 mL). The organic layers were dried (Na_2SO_4) and evaporated to give **12a** (0.35 g, 88%) as a colorless solid, which was used as such for the next reaction.

A solution of **12a** (0.35 g, 0.81 mmol), HOBt (0.13 g, 0.97 mmol), and EDCI (0.18 g, 0.97 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min and treated sequentially with the salt **13** (0.11 g, 0.81 mmol) and DIPEA (0.28 mL, 1.62 mmol) and stirred for 8 h. Work up as described for **11** and purification of the residue by column chromatography (60−120 mesh silica gel, 1.4% MeOH in $CHCl_3$) afforded **1** (0.3 g, 62%) as a colorless solid; mp 161 °C; $[\alpha]^{20}_D$ = +243.3 (*c* 0.05, $CHCl_3$); IR (KBr): ν 3325, 2981, 2927, 1724, 1664, 1530, 1454, 1368, 1213, 1165, 1090, 1025, 970, 857, 751, 667 cm^{-1}; ^{1}H NMR (600 MHz, 298 K, $CDCl_3$): δ 7.53 (d, 1H, *J* = 8.0 Hz, NH-3), 7.29 (d, 1H, *J* = 9.5 Hz, NH-2), 5.04 (d, 1H, *J* = 6.8 Hz, NH-1), 4.94 (dd, 1H, *J* = 3.3, 5.4 Hz, C_3H-2), 4.87 (s, 1H, C_1H-2), 4.62 (qd, 1H, *J* = 7.5, 8.0 Hz, C_αH-2), 4.56 (m, 1H, C_βH-2), 4.53 (d, 1H, *J* = 5.4 Hz, C_2H-2), 3.93 (dd, 1H, *J* = 3.3, 10.1 Hz, C_4H-2), 3.93 (p, 1H, *J* = 6.8 Hz, C_αH-1), 3.76 (s, 3H, OMe), 3.27 (s, 3H, OMe), 2.57 (d, 1H, *J* = 3.8 Hz, C_αH-2), 2.57 (d, 1H, *J* = 3.8 Hz, $C_{\alpha'}$H-2), 1.45 (d, 1H, *J* = 7.5 Hz, CH_3-3), 1.45 (s, 3H, Ac), 1.42 (s, 9H, Boc), 1.38 (d, 1H, *J* = 6.8 Hz, CH_3-1), 1.29 (s, 3H, Ac); ^{13}C NMR (150 MHz, 298 K, $CDCl_3$): δ 175.6, 172.9, 171.1, 156.0, 112.4, 106.8, 85.1, 80.2, 79.5, 77.5, 54.0, 52.6, 51.3, 48.4, 46.8, 38.2, 29.7, 28.2, 26.1, 24.9, 17.5, 16.4; HRMS (ESI): *m*/*z* calculated for $C_{23}H_{39}N_3O_{10}Na$(M^++Na) 540.2533, found 540.2509.

Boc-L-Ala-(*S*)-β-Caa$_{(I)}$-L-Ala-(*S*)-β-Caa$_{(I)}$-L-Ala-OMe (2). A solution of acid **12a** (0.25 g, 0.58 mmol), HOBt (0.09 g, 0.69 mmol), and EDCI (0.13 g, 0.69 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min. It was treated sequentially with **14** [prepared from **1** (0.3 g, 0.58 mmol) and CF_3COOH (0.15 mL) in dry CH_2Cl_2 (1 mL) at 0 °C] and DIPEA (0.2 mL, 1.1 mmol) and stirred for 8 h. Work up as described for **11** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.1% MeOH in $CHCl_3$) afforded **2** (0.19 g, 40%) as a colorless solid; mp: 143−145 °C; $[\alpha]^{20}_D$ = +230.3 (*c* 0.1, $CHCl_3$); IR (KBr): ν 3311, 3018, 2924, 2852, 1648, 1534, 1455, 1371, 1214, 1165, 1087, 972, 750, 667 cm^{-1}; ^{1}H NMR (600 MHz, 278 K, $CDCl_3$): δ 8.24 (d, 1H, *J* = 6.0 Hz, NH-3), 8.15 (d, 1H, *J* = 9.7 Hz, NH-2), 8.10 (d, 1H, *J* = 8.0 Hz, NH-5), 7.68 (d, 1H, *J* = 10.0 Hz, NH-4), 5.05 (d, 1H, *J* = 6.0 Hz, NH-1), 4.97 (dd, 1H, *J* = 3.5, 6.0 Hz, C_3H-4), 4.88 (s, 1H, C_1H-2), 4.87 (dd, 1H, *J* = 3.3, 6.0 Hz, C_3H-2), 4.87 (s, 1H, C_1H-4), 4.60 (qd, 1H, *J* = 7.5, 8.0 Hz, C_αH-5), 4.58 (dddd, 1H, *J* = 10.5, 9.7, 4.8, 3.0 Hz, C_βH-2), 4.54 (d, 1H, *J* = 6.0 Hz, C_2H-4), 4.52 (d, 1H, *J* = 6.0 Hz, C_2H-2), 4.51 (dddd, 1H, *J* = 10.0, 7.8, 5.0, 2.8 Hz, C_βH-4), 4.18 (qd, 1H, *J* = 7.5, 6.0 Hz, C_αH-3), 4.02 (qd, 1H, *J* = 7.0, 6.0 Hz, C_αH-1), 3.93 (dd, 1H, *J* = 3.5, 10.0 Hz, C_4H-4), 3.85 (dd, 1H, *J* = 3.3, 10.5 Hz, C_4H-2), 3.77 (s, 3H, OMe), 3.31 (s, 3H, OMe), 3.28 (s, 3H, OMe), 2.58 (dd, 1H, *J* = 4.8, 12.6 Hz, C_αH-2), 2.53 (dd, 1H, *J* = 2.8, 13.0 Hz, C_αH-4), 2.47 (dd, 1H, *J* = 3.0, 12.6 Hz, $C_{\alpha'}$H-2), 2.41 (dd, 1H, *J* = 5.0, 13.0 Hz, $C_{\alpha'}$H-4), 1.46 (d, 1H, *J* = 7.5 Hz, CH_3-5), 1.46 (s, 3H, Ac), 1.44 (d, 1H, *J* = 7.5 Hz, CH_3-3), 1.43 (s, 3H, Ac), 1.42 (s, 9H, Boc), 1.39 (d, 1H, *J* = 7.0 Hz, CH_3-1), 1.31 (s, 3H, Ac), 1.30 (s, 3H, Ac); ^{13}C NMR (150 MHz, 298 K, $CDCl_3$): δ 176.2, 175.3, 173.1, 172.6, 171.2, 155.9, 112.4, 112.2, 106.6, 106.0, 84.9, 84.6, 80.2, 79.3, 79.2, 79.0, 78.8, 54.0, 53.7, 52.8, 52.2, 51.6, 51.7, 48.6, 47.3, 46.1, 38.0, 37.9, 28.1, 26.0, 25.9, 24.8, 24.5, 17.4, 16.4, 16.1; HRMS (ESI): *m*/*z* calculated for $C_{37}H_{61}N_5O_{16}Na$ (M^++ Na) 854.4011, found 854.3990.

Boc-L-Ala-(*S*)-β-Caa$_{(I)}$-L-Ala-(*S*)-β-Caa$_{(I)}$-L-Ala-(*S*)-β-Caa$_{(I)}$-L-Ala-OMe (3). A solution of **12a** (0.06 g, 0.13 mmol), HOBt (0.02 g, 0.16 mmol), and EDCI (0.03 g, 0.16 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min. It was treated sequentially with **15** [prepared from **2** (0.11 g, 0.14 mmol) and CF_3COOH (0.1 mL) in dry CH_2Cl_2 (0.5 mL) at 0 °C] and DIPEA (0.05 mL, 0.27 mmol) and stirred for 8 h. Work up as described for **11** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.6% MeOH in $CHCl_3$) afforded **3** (0.05 g, 32%) as a colorless solid; mp: 156−157 °C; $[\alpha]^{20}_D$ = +180.0 (*c* 0.1, $CHCl_3$); IR (KBr): ν 3747, 3298, 2924, 2853, 2312, 1726, 1645, 1538, 1455, 1372, 1271, 1206, 1110, 1029, 975, 876, 856, 768, 667 cm^{-1}; ^{1}H NMR (600 MHz, 293 K, $CDCl_3$): δ 8.51 (d, 1H, *J* = 9.7 Hz, NH-4), 8.46 (d, 1H, *J* = 5.9 Hz, NH-5), 8.26 (d, 1H, *J* = 9.7 Hz, NH-2), 8.14 (d, 1H, *J* = 6.0 Hz, NH-3), 8.09 (d, 1H, *J* = 8.0 Hz, NH-7), 7.65 (d, 1H, *J* = 8.6 Hz, NH-6), 5.01 (d, 1H, *J* = 6.0 Hz, NH-1), 4.98 (dd, 1H, *J* = 3.5, 5.8 Hz, C_3H-6), 4.88 (dd, 1H, *J* = 3.4, 5.9 Hz, C_3H-2), 4.88 (s, 1H, C_1H-2), 4.87 (s, 1H, C_1H-4), 4.86 (s, 1H, C_1H-6), 4.87 (m, 1H, C_3H-4), 4.60 (dq, 1H, *J* = 8.0, 7.5 Hz, C_αH-7), 4.60 (dddd, 1H, *J* = 10.0, 9.7, 4.7, 3.0 Hz, C_βH-2), 4.54 (d, 1H, *J* = 5.8 Hz, C_2H-4), 4.52 (d, 1H, *J* = 5.8 Hz, C_2H-6), 4.52 (dddd, 1H, *J* = 10.0, 9.7, 4.9, 2.7 Hz, C_βH-4), 4.50 (dddd, 1H, *J* = 10.0, 9.8, 5.1, 2.8 Hz, C_βH-6), 4.50 (d, 1H, *J* = 5.9 Hz, C_2H-2), 4.31 (qd, 1H, *J* = 7.2, 6.0 Hz, C_αH-3), 4.19 (qd, 1H, *J* = 7.2, 5.9 Hz, C_αH-5), 4.01 (qd, 1H, *J* = 7.0, 6.0 Hz, C_αH-1), 3.93 (dd, 1H, *J* = 3.5, 10.0 Hz, C_4H-6), 3.88 (dd, 1H, *J* = 3.4, 10.0 Hz, C_4H-2), 3.87 (dd, 1H, *J* = 3.3, 10.0 Hz, C_4H-4), 3.76 (s, 3H, OMe), 3.31 (s, 3H, OMe), 3.30 (s, 3H, OMe), 3.28 (s, 3H, OMe), 2.57 (dd, 1H, *J* = 4.7, 12.6 Hz, C_αH-2), 2.53 (dd, 1H, *J* = 2.8, 13.0 Hz, C_αH-6), 2.47 (dd, 1H, *J* = 3.0, 12.6 Hz, $C_{\alpha'}$H-2), 2.44 (dd, 1H, *J* = 2.7, 12.5 Hz, C_αH-4), 2.41 (dd, 1H, *J* = 5.1, 13.0 Hz, $C_{\alpha'}$H-6), 2.38 (dd, 1H, *J* = 4.9, 12.5 Hz, $C_{\alpha'}$H-4), 1.46 (d, 1H, *J* = 7.5 Hz, CH_3-7), 1.46 (d, 1H, *J* = 7.2 Hz, CH_3-5), 1.46 (s, 3H, Ac), 1.44 (d, 1H, *J* = 7.2 Hz, C_3H-3), 1.43 (s, 3H, Ac), 1.43 (s, 9H, Boc), 1.38 (d, *J* = 7.0 Hz, CH_3-1), 1.30 (s, 3H, Ac), 1.29 (s, 3H, Ac), 1.25 (s, 3H, Ac); ^{13}C NMR (150 MHz, 298 K, $CDCl_3$): δ 176.3, 175.7, 175.4, 173.2, 172.8, 172.7, 171.2, 156.0, 112.5, 112.3, 112.2, 106.8, 106.1, 106.0, 100.0, 85.0, 84.9, 80.3, 79.5, 79.3, 79.2, 79.1, 78.9, 78.7, 54.1, 53.7, 53.6, 52.8, 52.6, 51.7, 48.7, 47.5, 46.9, 46.2, 38.2, 38.1, 38.0, 29.7, 28.3, 26.1, 25.9, 25.8, 24.9, 24.7, 24.5, 17.4, 16.4, 16.1; HRMS (ESI): *m*/*z* calculated for $C_{51}H_{83}N_7O_{22}Na$(M^++Na) 1168.5488, found 1168.5431.

Boc-L-Ala-(*S*)-β-Caa$_{(I)}$-L-Ala-(*S*)-β-Caa$_{(I)}$-OMe (4). A solution of **12a** (0.15 g, 0.34 mmol), HOBt (0.05 g, 0.41 mmol), and EDCI (0.08 g,

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0.41 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min. It was treated sequentially with **12b** [prepared from **11** (0.15 g, 0.34 mmol) and CF_3COOH (0.1 mL) in dry CH_2Cl_2 (0.5 mL) at 0 °C] and DIPEA (0.12 mL, 0.69 mmol) and stirred for 8 h. Work up as described for **11** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.0% MeOH in $CHCl_3$) afforded **4** (0.16 g, 61%) as a colorless solid; mp: 97−99 °C; $[\alpha]^{20}_D$ = +202.8 (*c* 0.05, $CHCl_3$); IR (KBr): ν 3310, 2925, 2853, 1654, 1531, 1452, 1370, 1216, 1165, 1090, 1025, 968, 878, 857, 771, 667 cm^{-1}; ^1H NMR (600 MHz, 283 K, $CDCl_3$): δ 7.90 (d, 1H, *J* = 9.0 Hz, NH-2), 7.53 (d, 1H, *J* = 7.2 Hz, NH-3), 7.01 (d, 1H, *J* = 8.1 Hz, NH-4), 5.09 (d, 1H, *J* = 6.5 Hz, NH-1), 4.97 (dd, 1H, *J* = 3.3, 5.8 Hz, C_3H-2), 4.87 (s, 1H, C_1H-2), 4.87 (s, 1H, C_1H-4), 4.65 (dd, 1H, *J* = 3.2, 5.6 Hz, C_3H-4), 4.56 (d, 1H, *J* = 5.6 Hz, C_2H-4), 4.53 (d, 1H, *J* = 5.8 Hz, C_2H-2), 4.52 (dddd, 1H, *J* = 10.2, 9.0, 4.6, 3.5 Hz, $C_\beta H$-2), 4.47 (dddd, 1H, *J* = 8.2, 8.1, 6.6, 4.8 Hz, $C_\beta H$-4), 4.37 (p, 1H, *J* = 7.2 Hz, $C_\alpha H$-3), 4.17 (dd, 1H, *J* = 3.2, 8.2 Hz, C_4H-4), 4.05 (qd, 1H, *J* = 7.2, 6.5 Hz, $C_\alpha H$-1), 4.02 (dd, 1H, *J* = 3.3, 10.2 Hz, C_4H-2), 3.71 (s, 3H, OMe), 3.28 (s, 3H, OMe), 3.26 (s, 3H, OMe), 2.85 (dd, 1H, *J* = 6.6, 16.2 Hz, $C_\alpha H$-4), 2.76 (dd, 1H, *J* = 4.8, 16.2 Hz, $C_{\alpha'}H$-4), 2.62 (dd, 1H, *J* = 4.6, 13.2 Hz, $C_\alpha H$-2), 2.55 (dd, 1H, *J* = 3.5, 13.2 Hz, $C_{\alpha'}H$-2), 1.46 (s, 2H, Ac), 1.42 (s, 9H, Boc), 1.40 (d, 1H, *J* = 7.2 Hz, CH_3-3), 1.37 (d, 1H, *J* = 7.2 Hz, CH_3-1), 1.30 (s, 3H, Ac), 1.29 (s, 3H, Ac); ^{13}C NMR (150 MHz, 298 K, $CDCl_3$): δ 173.3, 173.0, 172.5, 171.3, 156.0, 112.8, 112.4, 107.0, 106.7, 99.9, 85.2, 85.0, 80.0, 79.5, 79.5, 79.4, 78.9, 54.5, 54.2, 51.9, 51.1, 50.1, 47.0, 46.6, 38.0, 35.2,29.7, 28.4, 28.2, 26.1, 26.0, 24.8, 17.5, 16.7; HRMS (ESI): *m*/*z* calculated for $C_{34}H_{56}N_4O_{15}Na(M^+{+}Na)$ 783.3639, found 783.3633.

Boc-L-Ala-(*S*)-β-Caa$_{(I)}$-L-Ala-(*S*)-β-Caa$_{(I)}$-L-Ala-(*S*)-β-Caa$_{(I)}$-OMe (5). A solution of **12a** (0.04 g, 0.09 mmol), HOBt (0.01 g, 0.11 mmol), and EDCI (0.02 g, 0.11 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min. It was treated sequentially with **16b** [prepared from **4** (0.07 g, 0.09 mmol) and CF_3COOH (0.1 mL) in dry CH_2Cl_2 (0.5 mL) at 0 °C] and DIPEA (0.03 mL, 0.18 mmol) and stirred for 8 h. Work up as described for **11** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.9% MeOH in $CHCl_3$) afforded **5** (0.06 g, 60%) as a colorless solid; mp: 135−137 °C; $[\alpha]^{20}_D$ = +346.8 (*c* 0.05, $CHCl_3$); IR (KBr): ν 3304, 2983, 2931, 1727, 1646, 1536, 1453, 1370, 1210, 1087, 1026, 968, 876, 856, 753, 666 cm^{-1}; ^1H NMR (600 MHz, 278 K, $CDCl_3$): δ 8.33 (d, 1H, *J* = 9.1 Hz, NH-4), 8.27 (d, 1H, *J* = 9.7 Hz, NH-2), 8.15 (d, 1H, *J* = 6.0 Hz, NH-3), 7.91 (d, 1H, *J* = 7.1 Hz, NH-5), 6.96 (d, 1H, *J* = 8.6 Hz, NH-6), 5.05 (d, 1H, *J* = 5.9 Hz, NH-1), 4.98 (dd, 1H, *J* = 3.4, 5.9 Hz, C_3H-4), 4.90 (dd, 1H, *J* = 3.4, 5.8 Hz, C_3H-2), 4.89 (s, 1H, C_1H-6), 4.88 (s, 1H, C_1H-2), 4.86 (s, 1H, C_1H-4), 4.65 (dd, 1H, *J* = 3.5, 5.9 Hz, C_3H-6), 4.59 (dddd, 1H, *J* = 10.4, 9.7, 4.8, 3.0 Hz, $C_\beta H$-2), 4.56 (d, 1H, *J* = 5.9 Hz, C_2H-6), 4.53 (d, 1H, *J* = 5.9 Hz, C_2H-4), 4.53 (d, 1H, *J* = 5.8 Hz, C_2H-2), 4.52 (dddd, 1H, *J* = 8.6, 8.1, 6.2, 5.1 Hz, $C_\beta H$-6), 4.46 (dddd, 1H, *J* = 10.0, 9.1, 4.8, 3.1 Hz, $C_\beta H$-4), 4.35 (qd, 1H, *J* = 7.3, 7.1 Hz, $C_\alpha H$-5), 4.33 (qd, 1H, *J* = 7.2, 6.0 Hz, $C_\alpha H$-3), 4.14 (dd, 1H, *J* = 3.5, 8.1 Hz, C_4H-6), 4.02 (qd, 1H, *J* = 7.0, 5.9 Hz, $C_\alpha H$-1), 4.00 (dd, 1H, *J* = 3.4, 10.0 Hz, C_4H-4), 3.89 (dd, 1H, *J* = 3.4, 10.4 Hz, C_4H-2), 3.72 (s, 3H, OMe), 3.32 (s, 3H, OMe), 3.29 (s, 3H, OMe), 3.27 (s, 3H, OMe), 2.80 (dd, 1H, *J* = 6.27, 16.1 Hz, $C_\alpha H$-6), 2.76 (dd, 1H, *J* = 5.1, 16.1 Hz, $C_{\alpha'}H$-6), 2.56 (dd, 1H, *J* = 4.8, 12.6 Hz, $C_\alpha H$-2), 2.52 (dd, 1H, *J* = 3.1, 13.2 Hz, $C_\alpha H$-4), 2.47 (dd, 1H, *J* = 4.8, 13.2 Hz, $C_{\alpha'}H$-4), 2.46 (dd, 1H, *J* = 3.0, 12.6 Hz, $C_{\alpha'}H$-2), 1.46 (s, 6H, Ac), 1.44 (s, 3H, Ac), 1.42 (d, 1H, *J* = 7.2 Hz, CH_3-3), 1.42 (s, 9H, Boc), 1.41 (d, 1H, *J* = 7.3 Hz, CH_3-5), 1.39 (d, 1H, *J* = 7.0 Hz, CH_3-1), 1.30 (s, 9H, Ac); ^{13}C NMR (150 MHz, 298 K, $CDCl_3$): δ 175.3, 173.7, 173.2, 172.6, 172.5, 171.4, 155.8, 112.8, 112.3, 112.2, 106.6, 106.0, 100.0, 85.0, 84.8, 84.6, 80.1, 79.3, 79.1, 79.0, 78.8, 54.5, 54.1, 53.8, 52.0, 51.9, 51.5, 50.3, 47.3, 46.3, 46.1, 38.0, 37.7, 35.3, 28.1, 26.0, 25.8, 24.8, 24.7, 24.5, 17.5, 16.4, 16.3; HRMS (ESI): *m*/*z* calculated for $C_{48}H_{78}N_6O_{21}Na$-$(M^+{+}Na)$ 1097.5112, found 1097.5036.

Boc- β-Caa$_{(I)}$-L-Ala-(*S*)-β-Caa$_{(I)}$-L-Ala-(*S*)-β-Caa$_{(I)}$-OMe (6). A cooled (0 °C) solution of **10c** (0.06 g, 0.17 mmol) in THF:MeOH:H_2O (3:1:1) (1 mL) was treated with LiOH (0.01 g, 0.35 mmol) and stirred at room temperature. Work up as described for **12a** gave **10b** (0.6 g, 96%) as a colorless solid, which was used as such for the next reaction.

A solution of **10b** (0.06 g, 0.17 mmol), HOBt (0.05 g, 0.25 mmol) and EDCI (0.03 g, 0.25 mmol) in CH_2Cl_2 (3 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min. It was treated sequentially with **16b** [prepared from **4** (0.12 g, 0.17 mmol) and CF_3COOH (0.1 mL) in dry CH_2Cl_2 (1 mL) at 0 °C] and DIPEA (0.43 mL, 0.34 mmol) and stirred for 8 h. Work up as described for **11** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.1% CH_3OH in $CHCl_3$) afforded **6** (0.08 g, 48%) as a colorless solid; mp: 160 °C; $[\alpha]^{20}_D$ = +150 (*c* 0.1, $CHCl_3$); IR (KBr): ν 3745, 3394, 3291, 2922, 2852, 2313, 1646, 1550, 1521, 1457, 1376, 1337, 1213, 1164, 1106, 1049, 968, 752, 667 cm^{-1}; ^1H NMR (600 MHz, 278 K, $CDCl_3$): δ 7.94 (d, 1H, *J* = 9.0 Hz, NH-3), 7.62 (d, 1H, *J* = 7.3 Hz, NH-4), 6.91 (d, 1H, *J* = 8.4 Hz, NH-5), 6.47 (d, 1H, *J* = 5.7 Hz, NH-2), 5.81 (d, 1H, *J* = 8.5 Hz, NH-1), 4.94 (dd, 1H, *J* = 3.3, 5.8 Hz, C_3H-3), 4.91 (s, 1H, C_1H-1), 4.89 (s, 1H, C_1H-5), 4.87 (s, 1H, C_1H-3), 4.71 (dd, 1H, *J* = 3.5, 5.8 Hz, C_3H-1), 4.65 (dd, 1H, *J* = 3.5, 5.7 Hz, C_3H-5), 4.56 (d, 1H, *J* = 5.8 Hz, C_2H-5), 4.53 (d, 1H, *J* = 5.8 Hz, C_2H-3), 4.53 (d, 1H, *J* = 5.8 Hz, C_2H-1), 4.49 (dddd, 1H, *J* = 8.4, 8.1, 6.0, 5.4 Hz, $C_\beta H$-5), 4.48 (dddd, 1H, *J* = 10.0, 9.0, 4.6, 3.7 Hz, $C_\beta H$-3), 4.36 (p, 1H, *J* = 7.3 Hz, $C_\alpha H$-4), 4.24 (qd, 1H, *J* = 7.0, 5.7 Hz, $C_\alpha H$-2), 4.21 (ddt, 1H, *J* = 8.5, 8.0, 5.4 Hz, $C_\beta H$-1), 4.16 (dd, 1H, *J* = 3.5, 8.1 Hz, C_4H-5), 4.05 (dd, 1H, *J* = 3.5, 8.0 Hz, C_4H-1), 4.01 (dd, 1H, *J* = 3.3, 10.0 Hz, C_4H-3), 3.71 (s, 3H, OMe), 3.31 (s, 3H, OMe), 3.29 (s, 3H, OMe), 3.26 (s, 3H, OMe), 2.81 (dd, 1H, *J* = 6.0, 16.2 Hz, $C_\alpha H$-5), 2.77 (dd, 1H, *J* = 5.4, 16.2 Hz, $C_{\alpha'}H$-5), 2.64 (dd, 2H, *J* = 5.4 Hz, $C_\alpha H$-1), 2.59 (dd, 1H, *J* = 4.6, 13.6 Hz, $C_\alpha H$-3), 2.53 (dd, 1H, *J* = 3.7, 13.6 Hz, $C_{\alpha'}H$-3), 1.46 (s, 9H, Boc), 1.42 (d, 1H, *J* = 7.0 Hz, CH_3-1), 1.41 (s, 6H, Ac), 1.30 (s, 12H, Ac); ^{13}C NMR (150 MHz, 298 K, $CDCl_3$): δ 173.5, 172.7, 172.5, 171.5, 171.3, 155.9, 112.7, 112.5, 112.3, 106.8, 106.5, 106.3, 101.9, 85.0, 84.8, 79.5, 79.3, 79.2, 79.1, 78.8, 54.5, 54.3, 54.2, 52.0, 50.8, 50.1, 47.6, 47.0, 46.4, 38.4, 37.7, 35.2, 28.4, 26.0, 25.9, 24.7, 24.6, 24.5, 17.3; HRMS (ESI): *m*/*z* calculated for $C_{45}H_{73}N_5O_{20}Na(M^+{+}Na)$ 1026.4746, found 1026.4699.

Boc-[(*S*)-β-Caa$_{(I)}$-L-Ala]$_2$-OMe (17). A cooled (0 °C) solution of **10c** (0.07 g, 0.18 mmol) in THF:MeOH:H_2O (3:1:1) (2 mL) was treated with LiOH (0.01 g, 0.28 mmol) and stirred at room temperature. Work up as described for **12a** gave **10b** (0.06 g, 89%) as a colorless solid, which was used as such for the next reaction.

A solution of **10b** (0.06 g, 0.17 mmol), HOBt (0.02 g, 0.19 mmol) and EDCI (0.04 g, 0.19 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min. It was treated sequentially with **14** [prepared from **1** (0.08 g, 0.17 mmol) and CF_3COOH (0.1 mL) in dry CH_2Cl_2 (0.5 mL) at 0 °C] and DIPEA (0.06 mL, 0.33 mmol) and stirred for 8 h. Work up as described for **11** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.1% MeOH in $CHCl_3$) afforded **17** (0.07 g, 55%) as a colorless solid; mp: 103−104 °C; $[\alpha]^{20}_D$ = +235.1 (*c* 0.1, $CHCl_3$); IR (KBr): ν 3325, 2980, 2926, 2853, 1742, 1705, 1656, 1502, 1453, 1368, 1210, 1161, 1083, 967, 858, 753, 665 cm^{-1}; ^1H NMR (500 MHz, 298 K, $CDCl_3$): δ 7.64 (d, 1H, *J* = 7.8 Hz, NH-3), 7.36 (d, 1H, *J* = 8.1 Hz, NH-4), 6.50 (b, 1H, NH-2), 5.54 (d, 1H, *J* = 5.7 Hz, NH-1), 4.91 (m, 1H, C_3H-3), 4.89 (s, 1H, C_1H-1), 4.86 (s, 1H, C_1H-3), 4.68 (dd, 1H, *J* = 3.7, 5.4 Hz, C_3H-1), 4.59 (t, 1H, *J* = 7.4, 15.2 Hz, C_3H-3), 4.53 (d, 3H, *J* = 5.1 Hz, C_2H-1, C_2H-3, $C_\beta H$-3), 4.21 (dt, 1H, *J* = 5.7, 11.5, 13.2 Hz, $C_\alpha H$-4), 4.13 (t, 1H, *J* = 7.0, 5.7 Hz, $C_\beta H$-1), 4.01 (dd, 1H, *J* = 3.0, 7.1 Hz, C_4H-1), 3.95 (dd, 1H, *J* = 2.7, 9.8 Hz, C_4H-3), 3.75 (s, 3H, OMe), 3.31 (s, 3H, OMe), 3.27 (s, 3H, OMe), 2.62 (d, 2H, *J* = 4.0 Hz, $C_{\alpha'}H$-1, $C_{\alpha'}H$-3), 2.55 (d, 3H, *J* = 3.7 Hz, $C_\alpha H$-1, $C_\alpha H$-3, $C_\alpha H$-2), 1.68 (s, 6H, Ac), 1.45 (s, 9H, Boc), 1.43 (d, 3H, *J* = 7.8 Hz, CH_3-2), 1.41 (d, 3H, *J* = 7.8 Hz, CH_3-4), 1.29 (s, 6H, Ac); ^{13}C NMR (150 MHz, 298 K, $CDCl_3$): δ 175.7, 172.5, 171.5, 171.0, 155.9, 112.4, 112.6, 106.7, 106.4, 85.0, 79.6, 79.5, 79.4, 79.2, 54.4, 54.1, 52.6, 50.8, 48.5, 47.7, 46.8, 38.6, 38.0, 28.4, 26.0, 25.9, 24.8, 24.6, 17.2, 16.4; HRMS (ESI): *m*/*z* calculated for $C_{34}H_{56}N_4O_{15}Na(M^+{+}Na)$ 783.3639, found 783.3633.

Boc-[L-Ala-(*S*)-β-Caa$_{(I)}$]$_2$-[(*S*)-β-Caa$_{(I)}$-L-Ala]$_2$-OMe (7). A cooled (0 °C) solution of **4** (0.07 g, 0.09 mmol) in THF:MeOH:H_2O (3:1:1) (2 mL) was treated with LiOH (0.004 g, 0.14 mmol) and stirred at room temperature. Work up as described for **12a** gave **16a** (0.06 g, 88%) as a colorless solid, which was used as such for the next reaction.

A solution of **16a** (0.06 g, 0.08 mmol), HOBt (0.01 g, 0.09 mmol), and EDCI (0.02 g, 0.09 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min. It was treated sequentially with **17a** [prepared from **17** (0.61 g, 0.08 mmol) and CF_3COOH (0.1 mL) in dry

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CH_2Cl_2 (0.5 mL) at 0 °C] and DIPEA (0.03 mL, 0.16 mmol) and stirred for 8 h. Work up as described for **11** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.8% MeOH in $CHCl_3$) afforded **7** (0.03 g, 27%) as a colorless solid; mp: 142−144 °C; $[\alpha]^{20}_D$ = +140 (*c* 0.1, $CHCl_3$); IR (KBr): ν 3312, 2925, 2853, 1647, 1537, 1454, 1374, 1213, 1164, 1096, 970, 748, 667 cm^{-1}; ^1H NMR (600 MHz, 293 K, $CDCl_3$): δ 8.03 (d, 1H, *J* = 4.6 Hz, NH-6), 7.92 (d, 1H, *J* = 8.0 Hz, NH-8), 7.91 (d, 1H, *J* = 8.5 Hz, NH-2), 7.77 (d, 1H, *J* = 7.4 Hz, NH-4), 7.50 (d, 1H, *J* = 9.4 Hz, NH-7), 7.47 (d, 1H, *J* = 7.0 Hz, NH-3), 7.01 (d, 1H, *J* = 6.2 Hz, NH-5), 5.06 (d, 1H, *J* = 6.2 Hz, NH-1), 4.97 (dd, 1H, *J* = 3.4, 5.8 Hz, C_3H-7), 4.90 (dd, 1H, *J* = 3.4, 5.8 Hz, C_3H-2), 4.87 (s, 1H, C_1H-2), 4.85 (s, 1H, C_1H-7), 4.84 (s, 1H, C_1H-5), 4.81 (s, 1H, C_1H-4), 4.80 (m, 1H, C_3H-5), 4.68 (dd, 1H, *J* = 3.4, 5.8 Hz, C_3H-4), 4.64 (qd, 1H, *J* = 7.5, 8.0 Hz, C_αH-8), 4.57 (d, 1H, *J* = 5.8 Hz, C_2H-2), 4.55 (m, 1H, C_βH-2), 4.53 (d, 2H, *J* = 5.8 Hz, C_2H-4, C_2H-7), 4.52 (d, 1H, *J* = 5.8 Hz, C_2H-5), 4.50 (m, 1H, C_βH-7), 4.33 (m, 1H, C_4H-5), 4.32 (m, 1H, C_βH-5), 4.31 (p, 1H, *J* = 7.0 Hz, C_αH-3), 4.27 (m, 1H, C_βH-4), 4.23 (dd, 1H, *J* = 3.4, 9.4 Hz, C_4H-4), 4.16 (qd, 1H, *J* = 7.0, 6.2 Hz, C_αH-1), 4.15 (qd, 1H, *J* = 7.3, 4.6 Hz, C_αH-6), 4.12 (dd, 1H, *J* = 3.4, 10.0 Hz, C_4H-2), 3.94 (dd, 1H, *J* = 3.4, 9.9 Hz, C_4H-7), 3.76 (s, 3H, OMe), 3.37 (s, 3H, OMe), 3.28 (s, 6H, OMe), 3.23 (s, 3H, OMe), 2.85 (dd, 1H, *J* = 8.9, 14.7 Hz, C_αH-4), 2.76 (dd, 1H, *J* = 4.1, 14.7 Hz, C_αH-5), 2.76 (dd, 1H, *J* = 4.1, 14.7 Hz, $C_{\alpha'}$H-4), 2.66 (dd, 1H, *J* = 5.0, 13.5 Hz, C_αH-2), 2.58 (m, 1H, C_αH-7), 2.58 (m, 1H, $C_{\alpha'}$H-2), 2.56 (m, 1H, $C_{\alpha'}$H-5), 2.53 (dd, 1H, *J* = 3.3, 13.1 Hz, $C_{\alpha'}$H-7), 1.49 (d, 3H, *J* = 7.3 Hz, CH_3-6), 1.46 (d, 3H, *J* = 7.5 Hz, CH_3-8), 1.45 (s, 3H, Ac), 1.44 (s, 9H, Boc), 1.42 (d, *J* = 7.0 Hz, CH_3-3), 1.39 (d, *J* = 7.0 Hz, CH_3-1), 1.30 (s, 3H, Ac), 1.29 (s, 3H, Ac), 1.28 (s, 3H, Ac), 1.25 (s, 3H, Ac) ; ^{13}C NMR (100 MHz, 293 K, $CDCl_3$): δ 175.8, 174.0, 173.6, 173.4, 171.9, 171.8, 171.2, 170.0, 156.3, 112.6, 112.5, 112.4, 106.9, 106.8, 106.7, 106.6, 85.3, 85.1, 80.0, 79.7, 79.6, 79.5, 79.4, 79.3, 54.4, 54.2, 54.1, 54.0, 52.6, 51.5, 51.2, 48.5, 47.8, 47.2, 47.0, 38.1, 37.9, 37.4, 36.6, 29.6, 28.3, 26.1, 26.1, 26.0, 26.0, 25.1, 25.0, 24.9, 24.7, 17.5, 17.3, 16.7, 16.2; HRMS (ESI): *m*/*z* calculated for $C_{62}H_{100}N_8O_{27}Na(M^+{+}Na)$ 1411.6590, found 1411.6512.

Boc-[L-Ala-(*S*)-β-Caa$_{(l)}$]$_2$-[(*S*)-β-Caa$_{(x)}$-L-Ala]$_2$-OMe (8). A cooled (0 °C) solution of **4** (0.07 g, 0.09 mmol) in THF:MeOH:H_2O (3:1:1) (2 mL) was treated with LiOH (0.004 g, 0.14 mmol) and stirred at room temperature. Work up as described for **12a** gave **16a** (0.06 g, 88%) as a colorless solid, which was used as such for the next reaction.

A solution of **16a** (0.06 g, 0.08 mmol), HOBt (0.01 g, 0.09 mmol), and EDCI (0.02 g, 0.09 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min. It was treated sequentially with **18** [prepared from **18a** (0.61 g, 0.08 mmol) and CF_3COOH (0.1 mL) in dry CH_2Cl_2 (0.5 mL) at 0 °C] and DIPEA (0.03 mL, 0.16 mmol) and stirred for 8 h. Work up as described for **11** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.8% MeOH in $CHCl_3$) afforded **8** (0.03 g, 29%) as a colorless solid; mp: 122−125 °C; $[\alpha]^{20}_D$ = +100.5 (*c* 0.1, $CHCl_3$); IR (KBr): ν 3326, 2986, 28935, 1656, 1542, 1454, 1377, 1211, 1166, 1084, 860, 520 cm^{-1}; ^1H NMR (600 MHz, 288 K, $CDCl_3$): δ 7.95 (d, 1H, *J* = 8.0 Hz, NH-8), 7.86 (d, 1H, *J* = 9.1 Hz, NH-2), 7.56 (d, 1H, *J* = 5.3 Hz, NH-6), 7.44 (d, 1H, *J* = 6.7 Hz, NH-4), 7.38 (br, 1H, *J* = 6.2 Hz, NH-3), 7.33 (d, 1H, *J* = 9.5 Hz, NH-5), 7.16 (d, 1H, *J* = 9.2 Hz, NH-7), 5.88 (d, 1H, *J* = 3.8 Hz, C_1H-7), 5.83 (d, 1H, *J* = 3.8 Hz, C_1H-5), 5.06 (d, 1H, *J* = 6.3 Hz, NH-1), 4.88 (dd, 1H, *J* = 3.4, 6.0 Hz, C_3H-2), 4.86 (s, 1H, C_1H-2), 4.79 (s, 1H, C_1H-4), 4.78 (dd, 1H, *J* = 3.4, 6.0 Hz, C_3H-4), 4.60 (d, 1H, *J* = 3.8 Hz, C_2H-5), 4.58 (d, 1H, *J* = 6.0 Hz, C_2H-2), 4.58 (d, 1H, *J* = 3.8 Hz, C_2H-7), 4.56 (dq, 1H, *J* = 8.0, 7.5 Hz, C_αH-8), 4.53 (m, 1H, C_βH-2), 4.52 (d, 1H, *J* = 6.0 Hz, C_2H-4), 4.47 (m, 1H, C_βH-5), 4.46 (m, 1H, C_βH-7), 4.39 (dd, 1H, *J* = 3.4, 10.0 Hz, C_4H-5), 4.25 (qd, 1H, *J* = 7.5, 6.2 Hz, C_αH-3), 4.21 (m, 1H, C_βH-4), 4.18 (dd, 1H, *J* = 3.3, 10.0 Hz, C_4H-7), 4.15 (dd, 1H, *J* = 3.4, 10.0 Hz, C_4H-4), 4.07 (qd, 1H, *J* = 7.0, 6.3 Hz, C_αH-4, 7), 4.06 (m, 1H, C_4H-2), 4.05 (qd, 1H, *J* = 7.5, 5.3 Hz, C_αH-6), 4.02 (d, 1H, *J* = 3.3 Hz, C_3H-7), 3.83 (d, 1H, *J* = 3.5 Hz, C_3H-5), 3.70 (s, 3H, OMe), 3.39 (s, 3H, OMe), 3.38 (s, 3H, OMe), 3.32 (s, 3H, OMe), 3.22 (s, 3H, OMe), 2.86 (dd, 1H, *J* = 6.6, 14.3 Hz, C_αH-4), 2.65 (dd, 1H, *J* = 3.7, 14.3 Hz, $C_{\alpha'}$H-4), 2.63 (dd, 1H, *J* = 4.4, 13.4 Hz, C_αH-2), 2.63 (dd, 1H, *J* = 5.0, 13.0 Hz, C_αH-7), 2.61 (dd, 1H, *J* = 5.0, 14.3 Hz, C_αH-5), 2.57 (dd, 1H, *J* = 3.3, 13.4 Hz, $C_{\alpha'}$H-2), 2.32 (dd, 1H, *J* = 6.4, 14.3 Hz, $C_{\alpha'}$H-5), 2.20 (dd, 1H, *J* = 3.5, 1.0 Hz, $C_{\alpha'}$H-7), 1.48 (d, 3H, *J* = 7.5 Hz, CH_3-6), 1.47 (s, 3H, Ac), 1.44 (d, 1H, *J* = 7.5 Hz, C_3H-8), 2.53 (dd, 1H, *J* = 3.3, 13.1 Hz, $C_{\alpha'}$H-7), 1.49 (d, 3H, *J* = 7.3 Hz, CH_3-6), 1.46 (d, 3H, *J* = 7.5 Hz, CH_3-8), 1.44 (s, 9H, Boc), 1.42 (s, 3H, Ac), 1.39 (d, *J* = 7.5 Hz, CH_3-3), 1.38 (d, *J* = 7.0 Hz, CH_3-1), 1.31 (s, 3H, Ac), 1.29 (s, 3H, Ac), 1.28 (s, 3H, Ac), 1.26 (s, 3H, Ac); ^{13}C NMR (100 MHz, 298 K, $CDCl_3$): δ 175.4, 174.2, 173.2, 173.0, 172.0, 171.2, 170.9, 170.5, 156.2, 112.4, 111.5, 106.7, 104.9, 104.6, 85.3, 85.1, 83.4, 83.2, 81.5, 81.4, 80.0, 79.9, 79.5, 79.4, 79.3, 79.1, 57.2, 57.3, 54.1, 54.0, 52.4, 51.6, 51.3, 48.6, 48.0, 47.2, 46.6, 46.7, 38.1, 38.0, 37.9, 36.8, 31.9, 29.6, 29.3, 28.3, 26.7, 26.4, 26.4, 26.1, 26.0, 26.0, 24.9, 22.6,17.5, 17.0, 16.7, 16.2, 14.1; HRMS (ESI): *m*/*z* calculated for $C_{62}H_{100}N_8O_{27}Na(M^+{+}Na)$ 1411.6590, found 1411.6496.

Boc-[L-Ala-(*S*)-β-Caa$_{(l)}$]$_3$-[(*S*)-γ-Caa$_{(x)}$-L-Ala]$_2$-OMe (8a). A cooled (0 °C) solution of **5** (0.06 g, 0.06 mmol) in THF:MeOH:H_2O (3:1:1) (2 mL) was treated with LiOH (0.002 g, 0.09 mmol) and stirred at room temperature. Work up as described for **12a** gave **5a** (0.05 g, 96%) as a colorless solid, which was used as such for the next reaction.

A solution of **5a** (0.05 g, 0.05 mmol), HOBt (0.008 g, 0.06 mmol), and EDCI (0.01 g, 0.06 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min, treated sequentially with **19a** [prepared from **19**[30] (0.04 g, 0.04 mmol) and CF_3COOH (0.1 mL) in dry CH_2Cl_2 (0.5 mL) at 0 °C] and DIPEA (0.02 mL, 0.07 mmol) and stirred for 8 h. Work up as described for **11** and purification of the residue by column chromatography (60−120 mesh silica gel, 3.2% MeOH in $CHCl_3$) afforded **8a** (0.03 g, 37%) as a colorless solid; mp: 148−151 °C; $[\alpha]^{20}_D$ = +129.1 (*c* 0.05, $CHCl_3$); IR (KBr): ν 3743, 3395, 2922, 2852, 2378, 2312, 1838, 1741, 1692, 1645, 1532, 1499, 1465, 1382, 1217, 1164, 1081, 1027, 971, 857, 772, 627, 607, 564 cm^{-1}; ^1H NMR (600 MHz, 298 K, $CDCl_3$): δ 8.41 (d, 1H, *J* = 5.3 Hz, NH-5), 8.26 (d, 1H, *J* = 9.6 Hz, NH-4), 8.20 (d, 1H, *J* = 9.6 Hz, NH-2), 8.10 (d, 1H, *J* = 5.7 Hz, NH-3), 7.64 (d, 1H, *J* = 6.9 Hz, NH-10), 7.43 (d, 1H, *J* = 9.1 Hz, NH-7), 7.21 (d, 1H, *J* = 6.1 Hz, NH-8), 7.00 (d, 1H, *J* = 8.2 Hz, NH-6), 6.57 (d, 1H, *J* = 8.9 Hz, NH-9), 5.87 (d, 1H, *J* = 3.8 Hz, C_1H-9), 5.83 (d, 1H, *J* = 3.9 Hz, C_1H-7), 5.07 (d, 1H, *J* = 6.0 Hz, NH-1), 4.98 (dd, 1H, *J* = 3.4, 5.7 Hz, C_3H-6), 4.86 (s, 2H, C_3H-2, C_3H-4), 4.85 (s, 2H, C_1H-2, C_1H-4), 4.84 (s, 1H, C_1H-6), 4.66 (d, 1H, *J* = 3.9 Hz, C_2H-7), 4.59 (m, 1H, C_βH-7), 4.55 (m, 1H, C_2H-6, C_αH-10), 4.54 (d, 1H, *J* = 3.8 Hz, C_2H-9), 4.52 (m, 1H, C_βH-4), 4.38 (m, 1H, C_βH-6), 4.37 (m, 1H, C_γH-7), 4.37 (m, 1H, C_αH-3), 4.35 (s, 1H, C_2H-2), 4.35 (s, 1H, C_2H-4), 4.34 (s, 1H, C_4H-7), 4.34 (s, 1H, C_αH-5), 4.31 (m, 1H, C_αH-8), 4.30 (m, 1H, C_γH-9), 4.08 (dd, 1H, *J* = 3.1, 7.1 Hz, C_4H-9), 4.03 (m, 1H, C_αH-1), 3.92 (dd, 1H, *J* = 3.2, 10.2 Hz, C_4H-6), 3.89 (m, 1H, C_4H-4), 3.87 (dd, 1H, *J* = 3.1, 7.1 Hz, C_4H-2), 3.73 (s, 3H, COOMe), 3.67 (m, 1H, C_3H-9), 3.65 (m, 1H, C_3H-7), 3.41 (s, 3H, OMe), 3.34 (s, 3H, OMe), 3.30 (s, 6H, OMe), 3.29 (s, 3H, OMe), 2.58 (dd, 1H, *J* = 4.6, 12.9 Hz, C_αH-2), 2.50 (dd, 1H, *J* = 2.8, 13.3 Hz, C_αH-4) 2.48 (m, 1H, C_αH-6, $C_{\alpha'}$H-6), 2.46 (dd, 1H, *J* = 3.2, 12.9 Hz, $C_{\alpha'}$H-2), 2.41 (dd, 1H, *J* = 4.9, 13.3 Hz, $C_{\alpha'}$H-4), 2.31 (m, 2H, C_αH-7, $C_{\alpha'}$H-7), 2.24 (m, 2H, C_αH-9, $C_{\alpha'}$H-9), 1.96 (m, 2H, C_βH-9), 1.75 (m, 2H, C_βH-7, $C_{\beta'}$H-7), 1.74 (m, 1H, C_βH-9), 1.67 (s, 21H, 7 x Ac), 1.44 (m, 3H, CH_3-3),1.42 (s, 6H, CH_3-8, CH_3-10),1.42 (s, 9H, Boc), 1.39 (m, 6H, CH_3-1, CH_3-5), 1.29 (s, 9H, 3 x Ac); ^{13}C NMR (150 MHz, 298 K, $CDCl_3$): δ 175.3, 173.9, 173.0 (3C), 172.9, 172.8, 172.6, 172.5, 171.1, 155.9, 112.5, 112.4, 112.3, 111.6, 111.5, 107.1, 106.5, 106.1, 104.8, 104.5, 85.1, 85.0, 84.8, 84.3, 82.9, 82.3, 81.7, 81.4, 81.2, 80.1, 79.3, 79.2, 79.2, 79.1, 79.0, 77.5, 57.8, 57.7, 54.3, 54.1, 53.6, 52.3, 52.2, 51.9, 51.6, 49.9, 48.1, 48.0, 47.9, 47.5, 47.2, 46.2, 38.2, 37.8, 37.7, 32.4, 32.1, 31.9, 30.0, 29.7, 29.3, 29.2, 28.6, 28.2, 26.7, 26.2, 26.0, 25.9, 25.0, 24.7, 24.6, 22.6, 17.6, 16.6, 16.5, 14.1; HRMS (ESI): *m*/*z* calculated for $C_{78}H_{126}N_{10}NaO_{33}$ $(M^+{+}Na)$ 1753.8381, found 1753.8278.

Boc-[L-Ala-(*S*)-β-Caa$_{(l)}$]$_3$-[(*S*)-δ-Caa$_{(x)}$-L-Ala]$_2$-OMe (8b). A cooled (0 °C) solution of **5** (0.06 g, 0.06 mmol) in THF:MeOH:H_2O (3:1:1) (2 mL) was treated with LiOH (0.002 g, 0.09 mmol) and stirred at room temperature. Work up as described for **12a** gave **5a** (0.05 g, 96%) as a colorless solid, which was used as such for the next reaction.

A solution of **5a** (0.09 g, 0.09 mmol), HOBt (0.01 g, 0.10 mmol), and EDCI (0.02 g, 0.10 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min, treated sequentially with **20a** [prepared from **20**[31] (0.07 g, 0.09 mmol) and CF_3COOH (0.1 mL) in dry CH_2Cl_2 (0.5 mL) at 0 °C] and DIPEA (0.22 mL, 0.12 mmol) and stirred for 8 h. Work up as described for **11** and purification of the residue by column

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chromatography (60−120 mesh silica gel, 3.1% MeOH in $CHCl_3$) afforded **8b** (0.03 g, 20%) as a colorless solid; mp: 149−151 °C; $[\alpha]^{20}_D$ = +109.60 (*c* 0.1, $CHCl_3$); IR (KBr): ν 3744, 2922, 2852, 1645, 1550, 1514, 1457, 1374, 1217, 1054, 1032, 1013, 771, 668 cm^{-1}; ^1H NMR (600 MHz, 298 K, $CDCl_3$): δ 8.44 (d, 1H, *J* = 5.8 Hz, NH-5), 8.33 (d, 1H, *J* = 9.8 Hz, NH-4), 8.27 (d, 1H, *J* = 9.7 Hz, NH-2), 8.18 (d, 1H, *J* = 5.9 Hz, NH-3), 7.65 (d, 1H, *J* = 7.8 Hz, NH-10), 7.35 (d, 1H, *J* = 9.9 Hz, NH-7), 6.91 (d, 1H, *J* = 8.9 Hz, NH-6), 6.86 (d, 1H, *J* = 7.3 Hz, NH-8), 6.79 (d, 1H, *J* = 9.9 Hz, NH-9), 5.84 (d, 1H, *J* = 3.9 Hz, C_1H-9), 5.78 (d, 1H, *J* = 3.9 Hz, C_1H-7), 4.99 (d, 1H, *J* = 5.8 Hz, NH-1), 4.97 (dd, 1H, *J* = 3.3, 5.8 Hz, C_3H-6), 4.81 (s, 1H, C_3H-4), 4.78 (s, 1H, C_3H-2), 4.79 (s, 1H, C_1H-4), 4.77 (s, 1H, C_1H-2), 4.61 (d, 1H, *J* = 3.9 Hz, C_2H-7), 4.52 (m, 1H,C_βH-2), 4.55 (m, 1H, C_αH-10), 4.54 (m, 1H, C_1H-6), 4.50 (d, 1H, *J* = 4.6 Hz, C_2H-4), 4.50 (d, 1H, *J* = 3.9 Hz, C_2H-9), 4.46 (m, 1H, C_βH-4), 4.45 (d, 1H, *J* = 5.8 Hz, C_2H-6), 4.41 (d, 1H, *J* = 6.0 Hz, C_2H-2), 4.37 (m, 1H, C_δH-7), 4.31 (m, 1H, C_βH-6), 4.27 (m, 1H, C_αH-3), 4.26 (m, 1H, C_δH-9), 4.24 (m, 1H, C_αH-8), 4.23 (m, 1H, C_αH-5), 4.21 (dd, 1H, *J* = 3.1, 10.1 Hz, C_4H-7), 3.93 (m, 1H, C_4H-6), 3.92 (m, 1H, C_4H-9), 3.92 (m, 1H, C_αH-1), 3.79 (dd, 1H, *J* = 3.1, 10.3 Hz, C_4H-2), 3.76 (dd, 1H, *J* = 3.2, 10.2 Hz, C_4H-4), 3.74 (s, 3H, COOMe), 3.55 (d, 1H, *J* = 3.1 Hz, C_3H-7), 3.53 (d, 1H, *J* = 3.3 Hz, C_3H-9), 3.39 (s, 3H, OMe), 3.35 (s, 3H, OMe), 3.31 (s, 3H, OMe), 3.29 (s, 3H, OMe), 3.28 (s, 3H, OMe), 2.50 (dd, 1H, *J* = 4.7, 12.8 Hz, C_αH-2), 2.44 (dd, 1H, *J* = 2.9, 13.2 Hz, C_αH-6), 2.40 (m, 2H, C_αH-4, C_αH-9), 2.38 (m, 1H, $C_{\alpha'}$H-6), 2.37 (m, 1H, $C_{\alpha'}$H-2), 2.28 (m, 1H, $C_{\alpha'}$H-4), 2.27 (m, 1H, C_αH-7), 2.14 (m, 1H, $C_{\alpha'}$H-7), 2.07 (m, 1H, $C_{\alpha'}$H-9), 1.74 (m, 1H, C_βH-7), 1.62 (m, 1H, C_βH-9), 1.59 (m, 1H, $C_{\beta'}$H-7), 1.58 (m, 1H, $C_{\beta'}$H-9), 1.44 (m, 1H, C_γH-7), 1.43 (m, 1H, C_γH-9), 1.41 (s, 3H, Ac), 1.39 (s, 3H, Ac), 1.37 (m, 3H, CH_3-3), 1.36 (m, 6H, 2 x Ac), 1.35 (s, 12H, Ac, Boc), 1.34 (m, 1H, C_γH-7), 1.32 (m, 3H, CH_3-10), 1.32 (m, 3H, Ac), 1.31 (m, 1H, C_γH-9), 1.31 (d, 6H, *J* = 7.0 Hz, CH_3-1, CH_3-5), 1.28 (d, 3H, *J* = 3.9 Hz, CH_3-8) 1.22 (s, 6H, 2 x Ac), 1.18 (s, 6H, 2 x Ac); ^{13}C NMR (150 MHz, 298 K, $CDCl_3$): δ 175.5, 175.4, 175.0, 173.4, 173.3, 173.1 (2C), 172.8, 172.7, 170.6, 156.0, 112.5, 112.3, 112.2, 111.5, 111.3, 107.1, 106.4, 106.1, 104.8, 104.3, 85.0, 84.9, 84.8, 84.2, 82.9, 82.8, 81.7, 81.5, 81.3, 80.2, 79.5, 79.4, 79.3, 78.9, 57.5, 54.1, 54.0, 53.6, 52.5, 52.4, 52.0, 51.7, 49.2, 48.0, 47.6, 47.5, 47.1, 46.9, 46.2, 38.2, 37.9, 35.0, 34.9, 31.9, 30.1, 29.8, 29.7, 29.6, 29.4, 29.3, 29.2, 28.2, 26.7, 26.2, 26.1, 26.0, 25.9, 25.8, 25.0, 24.8, 24.6, 22.6, 22.0, 21.3, 17.6, 17.4, 17.2, 16.5, 16.4, 14.1; HRMS (ESI): *m*/*z* calculated for $C_{80}H_{130}N_{10}NaO_{33}$ (M^++Na) 1781.8694, found 1781.8589.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra, solvent titration plots, distance constraints used in MD calculations, MD and CD structures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: esmvee@iict.res.in.

*E-mail: ssav@iict.res.in.

Notes

The authors declare no competing financial interest.

[§](P.T. and K.S.) Part of the thesis work.

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