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Synthesis of β-Peptides with β-Helices from New C-Linked Carbo-β-Amino Acids: Study on the Impact of Carbohydrate Side Chains

Gangavaram V. M. Sharma,*[a] Kota Sudhakar Rao,[a] Rapolu Ravi,[b] Kongari Narsimulu,[b] Pendem Nagendar,[a] Chirutha Chandramouli,[a] Singarapu Kiran Kumar,[b] and Ajit C. Kunwar*[b]

Abstract: The design and synthesis of β-peptides from new C-linked carbo-β-amino acids (β-Caa) presented here, provides an opportunity to understand the impact of carbohydrate side chains on the formation and stability of helical structures. The β-amino acids, Boc-(*S*)-β-$Caa_{(g)}$-OMe **1** and Boc-(*R*)-β-$Caa_{(g)}$-OMe **2**, having a D-galactopyranoside side chain were prepared from D-galactose. Similarly, the homo C-linked carbo-β-amino acids (β-hCaa); Boc-(*S*)-β-$hCaa_{(x)}$-OMe **3** and Boc-(*R*)-β-$hCaa_{(x)}$-OMe **4**, were prepared from D-glucose. The peptides derived from the above monomers were investigated by NMR, CD, and MD studies. The β-peptides, especially the shorter ones obtained from the epimeric (at the amine stereocenter C_β) **1** and **2** by the concept of alternating chirality, showed a much smaller propensity to form 10/12-helices. This substantial destabilization of the helix could be attributed to the bulkier D-galactopyranoside side chain. Our efforts to prepare peptides with alternating **3** and **4** were unsuccessful. However, the β-peptides derived from alternating geometrically heterochiral (at C_β) **4** and Boc-(*R*)-β-$Caa_{(x)}$-OMe **5** (D-xylose side chain) display robust right-handed 10/12-helices, while the mixed peptides with alternating **4** and Boc-β-hGly-OMe **6** (β-homoglycine), resulted in left-handed β-helices. These observations show a distinct influence of the side chains on helix formation as well as their stability.

Keywords: amino acids · carbohydrates · chirality · helical structures · peptides

Introduction

The protein functions are intimately related to their three-dimensional structures. Therefore, an understanding of the physical principles involved in the formation of their secondary and tertiary structural elements is very important. The research efforts in the last 20 years have led to the evolution of the principles that govern the folding in peptides derived from α-amino acids, besides de novo designs.[1] The hints for the possible formation of folded structures in the homo-oligomers of β-and γ-amino acids,[2] were realized, for the first time, by the independently discovered novel 14-helix in β-peptides by Gellman et al.[3a] and Seebach et al.[3b] These results subsequently prompted the identification of new folding patterns such as 12-, 10-, 8- and 10/12-helices and led to the emergence of 'foldamers',[4] with the creation of skeletal, secondary, and tertiary structural diversity.[5] Thus, the new designs and motifs to develop unprecedented folded patterns would provide an opportunity to understand the factors that govern the protein structure and function.[6] The theoretical studies performed on unsubstituted β-peptides by Wu and Wang[7a,b] and Hofmann et al.[7c] revealed that the mixed 10/12-helices are intrinsically the most stable secondary structures. The mixed 10/12-helices, also referred to as β-helices,[7] containing intertwined 10- and 12-membered (mr) H-bond rings, unique to β-peptides, were first realized by Seebach et al.[8] by using alternating β^2/β^3-amino acids. Subsequently, Kessler et al.[9] observed similar helices, in the peptides derived from furanoside β-amino acid (FAA) and β-hGly. In our search for novel foldamers, we have synthe-

[a] Dr. G. V. M. Sharma, K. S. Rao, P. Nagendar, C. Chandramouli
D-211, Discovery Laboratory
Organic Chemistry Division III
Indian Institute of Chemical Technology
Hyderabad 500 007 (India)
Fax: (+91) 40-27160387/27193108
E-mail: esmvee@iict.res.in

[b] R. Ravi, K. Narsimulu, S. K. Kumar, Dr. A. C. Kunwar
Center for Nuclear Magnetic Resonance
Indian Institute of Chemical Technology
Hyderabad 500 007 (India)
E-mail: kunwar@iict.res.in

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sized new β-amino acids, the C-linked carbo-β-amino acids (β-Caa$_{(x)}$),[10] from D-glucose, and used them in different designs to obtain β-helices. Thus, in our earlier studies,[11a] the peptides derived with 'alternating' epimeric (at the amine stereocenter C_β) (*S*)- and (*R*)-β-Caa$_{(x)}$ generated only right-handed 10/12-mixed helices, even though they are enantiomeric at the amine stereocenters. Likewise, in our other design on mixed peptides,[11b] from (*S*)-β-Caa$_{(x)}$/β-hGly and (*R*)-β-Caa$_{(x)}$/β-hGly, both the left- and right-handed 10/12-helices respectively were identified, wherein, the handedness of the helix is governed by the stereocenter of the β-Caa$_{(x)}$ used in the design. More recently, our designs using alternating α- and γ-amino acids (γ-Caa$_{(x)}$)[12a] and β-Caa$_{(x)}$ and α-aminoxy amino acid,[12b] resulted in new motifs to realize the 10/12-helix.

The mass spectral studies[13] on the dipeptides derived from alternating (*S*)- and (*R*)-β-Caa having different carbohydrate side chains, revealed that the fragmentation pattern for the peptides with (*S*)-β-Caa at the N-terminus is different from the dipeptide with the N-terminus (*R*)-β-Caa. The above difference in the behavior was attributed to the bulk of the side chain and stereochemistry at the amine center (at C_β). This observation has provided us with the impetus to investigate the impact of the side chains on helix formation and stability of the secondary structures in β-peptides containing new β-Caas. Hence, two different β-monomers were proposed: a) to have a bulky pyranoside side chain and b) to introduce flexibility through a methylene spacer between the backbone and the carbohydrate side chain.

In pursuit of the new designs on β-Caa monomers, a literature survey[14] revealed that peptidyl nucleosides, amipurimycins,[14,15] and miharamycins[14,16] having branched pyranose sugars, C-linked to glycine, show biological activity against rice blast disease. In view of this, new β-Caas, Boc-(*S*)-β-Caa$_{(g)}$-OMe **1** and Boc-(*R*)-β-Caa$_{(g)}$-OMe **2**, having a pyranosidic side chain were designed from D-galactose.

In a further study on mixed β-peptides,[11b] it was observed that the flexibility provided through the backbone (in β-hGly) permitted a switch in handedness of the helices. Thus, based on the mass spectral studies[13] and switch in handedness of the helix,[11b] a new β-Caa design, with flexibility in the side chain, was envisaged. Further, a literature[14] survey indicated that the higher-carbon sugar nucleosides[17] C-linked to lysine have an antifungal activity. In these systems, the sugar and amino acid components are separated by a methylene unit. Hence, in yet another novel design, homo C-linked carbo-β-amino acids (β-hCaa$_{(x)}$), Boc-(*S*)-β-hCaa$_{(x)}$-OMe **3** and Boc-(*R*)-β-hCaa$_{(x)}$-OMe **4** were synthesized from D-glucose, wherein, the carbohydrate side chain and the amino acid backbone are separated by a methylene group as a spacer. It was anticipated that the spacer group would provide additional flexibility and facilitate a relieving of the steric strain inherent in the β-Caa$_{(x)}$. Thus, the present work encompasses the synthesis of new β-Caas: Boc-(*S*)-β-Caa$_{(g)}$-OMe **1** and Boc-(*R*)-β-Caa$_{(g)}$-OMe **2**, with a bulkier galactopyranoside side chain, from D-galactose; β-hCaas: Boc-(*S*)-β-hCaa$_{(x)}$-OMe **3** and Boc-(*R*)-β-hCaa$_{(x)}$-OMe **4** (Figure 1), having a D-xylose side chain with a methylene spacer between the backbone and side chain. These β-Caas have been used to synthesize β-peptides **7**–**16** (Figure 2). Extensive NMR, MD, and CD studies have been carried out to understand the impact of the side chains and to explore the structures present in the above β-peptides.

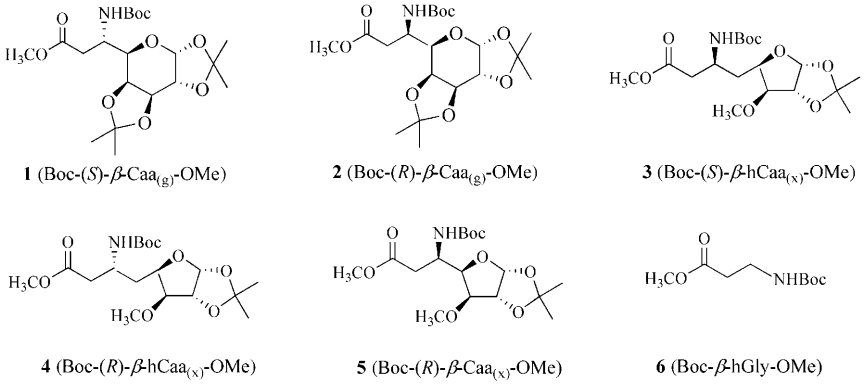


Figure 1. Structures of β-amino acids **1**–**6**.

Results and Discussion

Synthesis of Amino Acids 1 and 2

The monomers **1** and **2** (Scheme 1) were prepared from the ester **18**, synthesized from known aldehyde **17**[18] by aza-Michael addition with benzylamine.[19]

Synthesis of Amino Acids 3 and 4

Accordingly, treatment of the known[20] diol **21** with Ph_3P, imidazole, and iodine in CH_2Cl_2 at room temperature for 2 h afforded the 5,6-olefin **22** (78%). Wacker reaction of the olefin **22** (Scheme 2) with catalytic $PdCl_2$ and CuCl in the presence of O_2 in THF/H_2O (6:1) at room temperature for 6 h underwent the anti-Markonikov addition to afford the aldehyde **23** in 85% yield. Olefination of the aldehyde **23** by the Wittig reaction with (methoxycarbonylmethylene)-triphenyl phosphorane in benzene at reflux gave **24** (70%) as a *cis–trans* mixture, which on further reaction with benzylamine[16] gave a separable mixture of esters **25** (35%) and **26** (30%), epimeric at the amine stereocenter (C_β). The esters **25** and **26** were subjected to hydrogenolysis with 10% Pd–C in MeOH under hydrogen atmosphere to give the respective amines **25a** and **26a** (Scheme 2), which on subse-

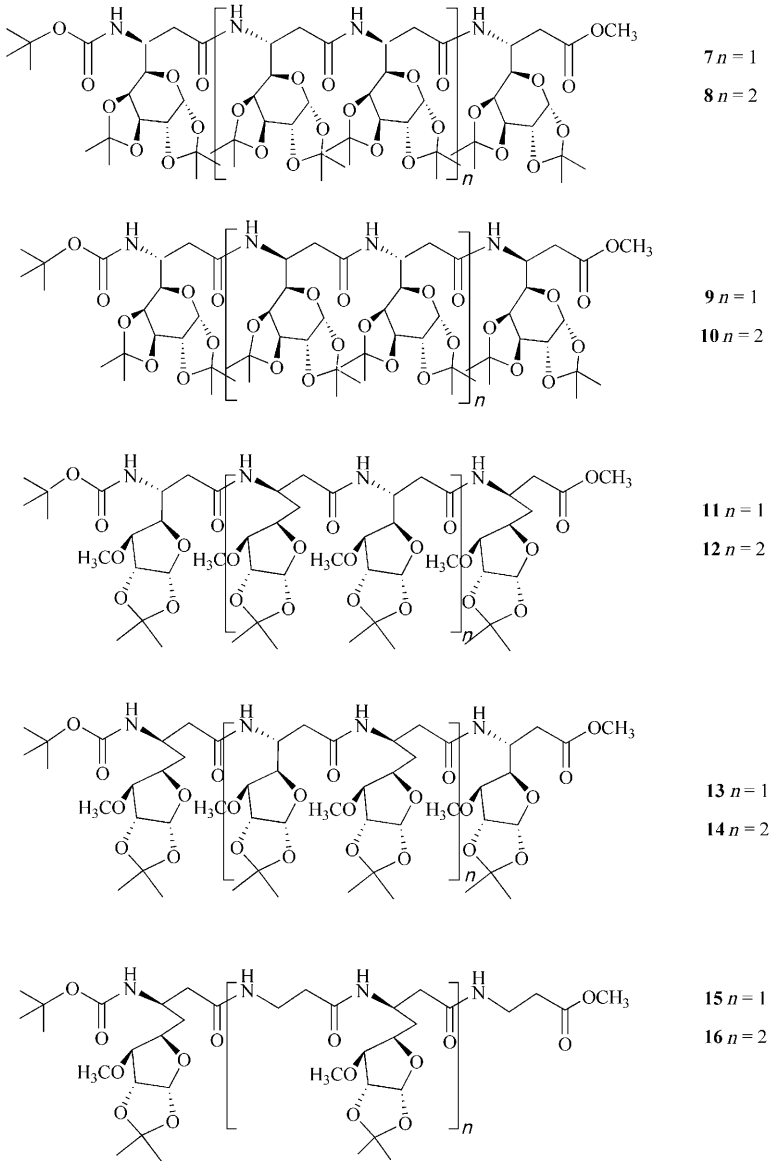


Figure 2. Structures of peptides **7**–**16**.

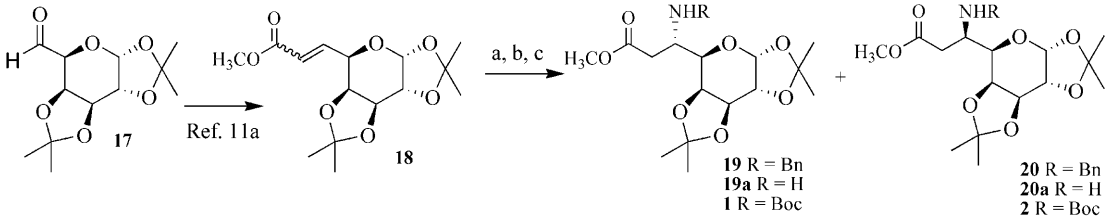
quent treatment with $(Boc)_2O$ and Et_3N in THF resulted in **3** (93%) and **4** (85%). The absolute stereochemistry of the newly created amine centers in β-$hCaa_{(x)}$ **3** and **4**, was established by the method of Riguera et al.[21]

Synthesis of Peptides 7–10 Containing (*S*)-β-$Caa_{(g)}$ 1 and (*R*)-β-$Caa_{(g)}$ 2

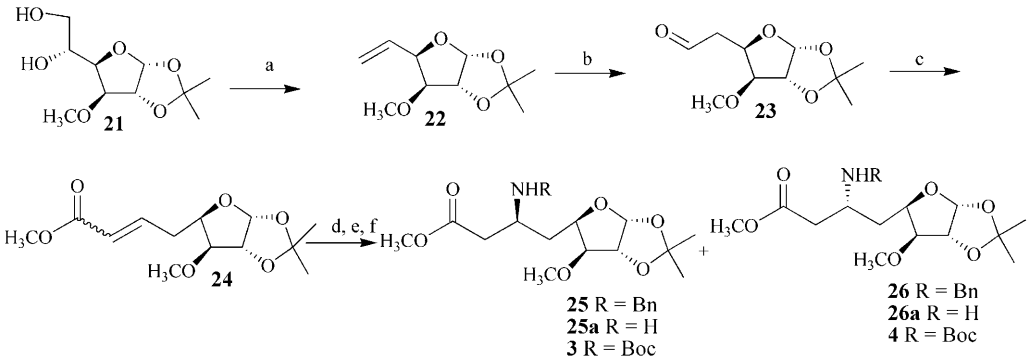
In our earlier studies,[11a] the β-peptides were synthesized based on the concept of alternating chirality,[7a,b] from (*R*)-β-$Caa_{(x)}$ and (*S*)-β-$Caa_{(x)}$. Under the influence of identical carbohydrate side chains, these peptides, although enantiomeric at the amine stereocenters in the backbone, have been identified with right-handed 12/10-helices. In the present study, the same design principle is adopted for the synthesis of β-peptides from the new β-amino acids, to understand the impact of the side chains.

Accordingly, (*S*)-β-$Caa_{(g)}$ (Boc-(*S*)-β-$Caa_{(g)}$-OMe) **1** (Scheme 3) on reaction with aqueous 4N NaOH gave the corresponding acid **27** (92%). Peptide coupling of **27**, with the salt **28** (prepared by the exposure of **2** to CF_3COOH) in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 afforded the dipeptide **29** (88%).

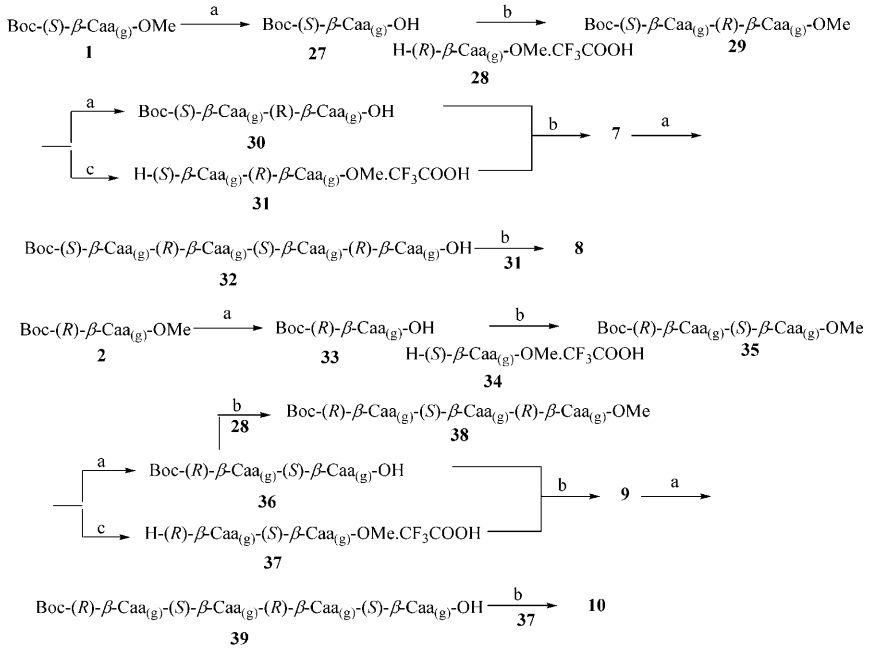
Hydrolysis of the ester **29** with base (4N NaOH) furnished the acid **30**, while treatment of the ester **29** with CF_3COOH in CH_2Cl_2 resulted in the salt **31**. Further, the condensation of the acid **30** and salt **31** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 gave the tetrapeptide **7** in 75% yield. Compound **7** on base hydrolysis and coupling of the resultant acid **32**, with the dipeptide salt **31** under standard reaction conditions (EDCI, HOBt, and DIPEA in CH_2Cl_2) afforded the hexapeptide **8** (47%).



Scheme 1. Synthesis of amino acids **1** and **2**. a) BnNH2, RT, 12h; b) H2, 10% Pd–C, CH_3OH, RT, 12h; c) $(Boc)_2O$, Et_3N, THF, 0°C–RT, 2h.



Scheme 2. Synthesis of amino acids **3** and **4**. a) Ph_3P, imidazole, I_2, CH_2Cl_2, 0°C–RT, 2 h; b) $PdCl_2$, CuCl, O_2, THF/H_2O (6:1), RT, 6 h; c) Ph_3P=$CHCO_2Me$, benzene, reflux, 6 h; d) $BnNH_2$, RT, 12 h; e) H_2, 10%, Pd–C, RT, 12 h; f) $(Boc)_2$, Et_3N, THF, 0°–RT, 2 h.



Scheme 3. Synthesis of peptides **7**–**10** containing **1** and **2**. a) 4 N NaOH, CH_3OH, 0°C–RT; b) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (1.5 equiv), dry CH_2Cl_2, 0°C–RT; c) CF_3COOH, dry CH_2Cl_2, 0°C–RT.

Likewise, Boc-(*R*)-β-$Caa_{(g)}$-OMe **2** (Scheme 3) was saponified with aqueous 4 N NaOH to give acid **33**, which on coupling with **34** (obtained by the Boc deprotection of **1** with CF_3COOH), in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 afforded the dipeptide **35** (91%). Base hydrolysis of **35** furnished the acid **36**, which on reaction with the salt **28** gave tripeptide **38** in 62% yield, while, coupling of the acid **36** with salt **37** (obtained from **35** by the exposure to CF_3COOH) gave the tetrapeptide **9** (64%). Ester **9** on hydrolysis with base (4 N NaOH) gave the corresponding acid **39**, which on peptide coupling with the salt **37** afforded the hexapeptide **10** (49%).

Synthesis of Peptides 11–14 with Alternating 4 and 5

In the first design, it was proposed to use the new monomers (*S*)-β-$hCaa_{(x)}$ **3** and (*R*)-β-$hCaa_{(x)}$ **4** with the concept of alternating chirality. However, the attempted synthesis failed to give the expected peptides. This may be attributed to the larger conformational space and steric interactions experienced by the carbohydrate side chains arising from the additional flexibility in the C_4–C_5 (methylene spacer) between the backbone and side chain in the amino acids **3** and **4**.

In yet another design, it was envisaged to use the new amino acid (*R*)-β-$hCaa_{(x)}$ **4**, alternating with (*R*)-β-$Caa_{(x)}$ **5**. The observations made earlier by us from the mass spectral studies[13] as well as the mixed peptides,[11b] prompted us to use monomer **4**, which is geometrically homochiral with (*S*)-β-$Caa_{(x)}$, in the present design. Accordingly, reaction of acid **40** with **41** (prepared from **4** on treatment with CF_3COOH in CH_2Cl_2) in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 afforded the dipeptide **42** in 76% yield (Scheme 4). Ester **42** on base hydrolysis gave the acid **43**, while, on exposure to CF_3COOH, it gave the amine salt **44**. Peptide coupling of **43** and **44** afforded the tetrapeptide **11** (43%). Further, **11** was hydrolysed using 4 N NaOH to give the acid **45**, which on condensation with the salt **44** furnished hexapeptide **12** (27%).

In a further study, ester **4** on hydrolysis with base gave the acid **46**, which on coupling with the salt **47** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 afforded the dipeptide **48** in 78% yield (Scheme 4). The acid **49**, obtained by base hydrolysis of ester **48**, on condensation with the salt **50** (prepared by Boc deprotection with CF_3COOH in CH_2Cl_2), gave tetrapeptide **13** (55%). Further, hydrolysis of compound **13** with 4 N NaOH gave the acid **51**, which on

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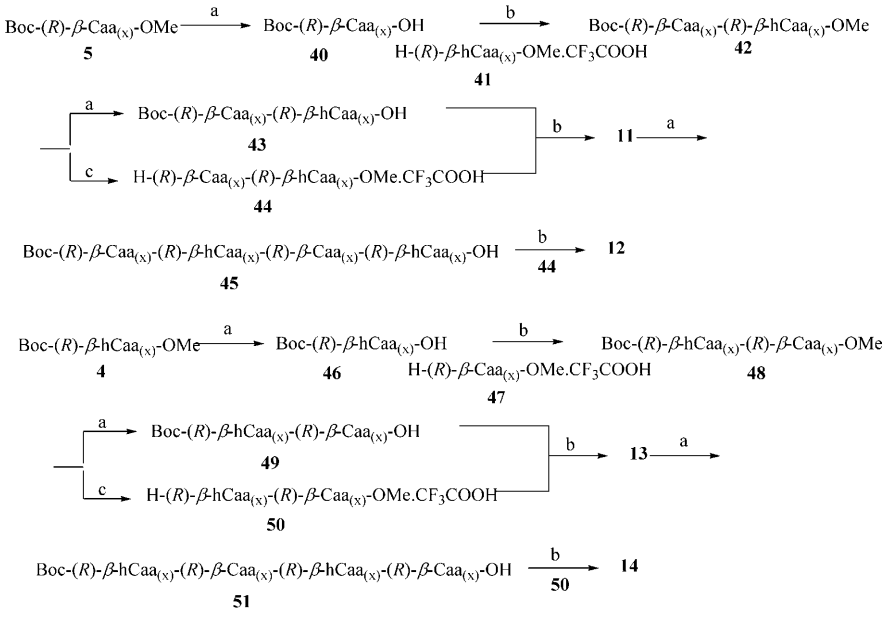


Scheme 4. Synthesis of peptides **11–14** having alternating **4** and **5**. a) 4 N NaOH, CH_3OH, 0°C–RT; b) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (1.5 equiv), dry CH_2Cl_2, 0°C–RT; c) CF_3COOH, dry CH_2Cl_2, 0°C–RT.

coupling with the salt **50** furnished the hexapeptide **14** (28%).

Synthesis of Peptides 15 and 16 Containing 4 and 6

In our earlier studies,[11b] the (*S*)-β-$Caa_{(x)}$/β-hGly mixed peptides switched the helical handedness compared to (*S*)-β-$Caa_{(x)}$/(*R*)-β-$Caa_{(x)}$ and (*R*)-β-$Caa_{(x)}$/β-hGly peptides. Since (*R*)-β-$hCaa_{(x)}$ **4**, is geometrically homochiral with (*S*)-β-$Caa_{(x)}$, it was felt worthwhile to investigate the influence of the conformational freedom around the C_4–C_5 in the side chain on helical stability, by synthesizing peptides with alternating **4** and **6**. Accordingly, condensation of acid **46** with the salt **52** (obtained by the deprotection of **6** on reaction with CF_3COOH) in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 afforded the dipeptide **53** in 85% yield (Scheme 5). The dipeptide **53** on Boc deprotection with CF_3COOH gave corresponding amine salt **55**, which, on peptide coupling with the acid **54** (prepared by the base hydrolysis of ester **53**) afforded the tetrapeptide **15** (68%). Base hydrolysis of **15** gave acid **56**, which on further coupling with the salt **55** afforded the hexapeptide **16** (48%).

Conformational Analysis of Peptides 7–16

Most of the NMR studies were carried out in 5–10 mM solutions at 303 K, while peptides **7–10** and **13–16** were studied in $CDCl_3$, peptides **11** and **12** were investigated in [D_5]pyridine.[22] The resonance assignments were made with the help of total correlation spectroscopy (TOCSY) and rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments.[23] Usually the N-terminal Boc-amide proton appears at higher field, permitting an easy assignment for the resonance of the first residue. For sequential assignments, strong $C_\alpha H(i)/NH(i+1)$ and weak $C_\alpha H(i)/NH(i)$ NOE correlations provided the clue. Additionally, for most of the molecules, the first and the last residue display NH/Boc(CH_3)$_3$ and $C_\alpha H$/C(O)OMe NOE correlations, respectively. Another distinguishing feature for the first and the last residue was the presence of only one NOE correlation between the NH and the $C_\alpha H$ protons, unlike other residues which had two such peaks. The conformation in β-amino acids is defined by four dihedral angles (Figure 3). We have derived the structural information using the coupling constants (*J*), the NOE, and the H-bonding information.

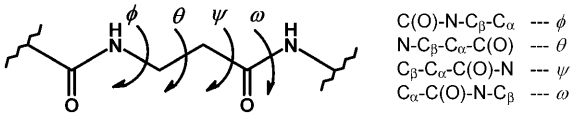
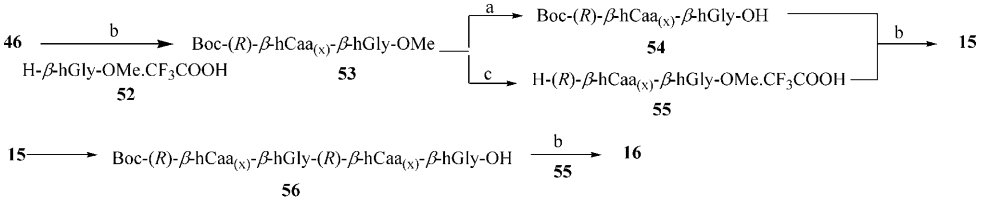


Figure 3. Schematic representation of backbone dihedral angles in β-peptides.



Reagents and conditions: a) 4 N NaOH, CH_3OH, 0 °C-RT; b) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (1.5 equiv), dry CH_2Cl_2, 0 °C-RT; c) CF_3COOH, dry CH_2Cl_2, 0 °C-RT

Scheme 5. Synthesis of peptides **15–16** with alternating **4** and **6**. a) 4 N NaOH, CH_3OH, 0°C–RT; b) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (1.5 equiv), dry CH_2Cl_2, 0°C–RT; c) CF_3COOH, dry CH_2Cl_2, 0°C–RT.

In the ^{1}H NMR spectrum of **7** in $CDCl_3$, NH(1)–NH(4) have chemical shift (δ) values of 5.65, 7.03, 6.96, and 8.16 ppm, respectively, suggesting participation of some of them in H-bonding. In order to confirm their involvement in H-bonding, solvent titration studies[24] were carried out, by adding up to 33% v/v [D_6]DMSO, in these solutions. Amide protons, which are already intramolecularly H-bonded, do not get exposed to the [D_6]DMSO solution, resulting in minimal changes in their chemical shifts ($\Delta\delta_{NH}$).[25] Exposed NH protons, which do not participate in intramolecular H-bonding, show large downfield shifts in these experiments. The observation of a small variation in the chemical shifts ($\Delta\delta_{NH}$) of <0.16 ppm for all but the NH(2), confirm their involvement in H-bonding. For the second and third residue, $^3J_{NH-C\beta H}$ are 9.6 and 9.0 Hz respectively, which permits us to infer an *anti-periplanar* (*ap*) disposition of the NH and C_βH protons, corresponding to C(O)–*N*–C_β–C_α (ϕ) $\sim\pm120°$ (Figure 4). On the other hand, $^3J_{NH-C\beta H}$ values of 7.6 and

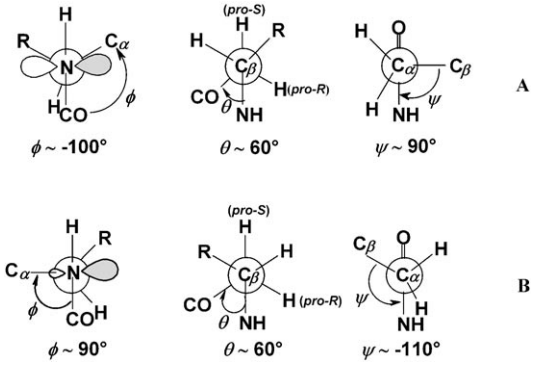


Figure 4. Schematic Newman projections showing the orientation of substituents in 10/12-right-handed helix for the ideal values of torsional angles ϕ, θ and ψ.[5b] view along the backbone atoms in: (A) (*R*)-β-$Caa_{(g)}$ and (B) (*S*)-β-$Caa_{(g)}$.

8.2 Hz for the two residues at the termini, show a definite influence of fraying and lack of robustness of the helix, which results in the reduction of the couplings arising from averaging over several conformations. Most of the $^3J_{C\alpha H-C\beta H}$ values are either >10 Hz or <5 Hz, thus supporting the predominance of a single conformation around C_α–C_β. Sequential NOEs, NH(2)/$C_\alpha H_{(pro-R)}$(1), NH(3)/$C_\alpha H_{(pro-S)}$(2), NH(4)/$C_\alpha H_{(pro-R)}$(3), and individual $^3J_{C\alpha H-C\beta H}$ confirm a substantial population of a single conformation with N–C_β–C_α–C(O) (θ) of ~60° and C_β–C_α–C(O)–N (ψ) of $\sim\pm120°$ (Figure 4). This information along with very weak NOE correlations, C_βH(2)/NH(4), C_βH(2)/$C_\alpha H_{(pro-R)}$(4) and NH(3)/C_βH(2), indicate the presence of a right-handed 10/12-mixed helix, which is in variance with the finding of very robust helices in our earlier studies on tetrapeptides, derived from alternating (*R*)- and (*S*)-β-$Caa_{(x)}$.[11a] The destabilization of the helical structure in peptide **7**, reflects the influence of the bulky pyranoside side chain in the new β-amino acids **1** and **2**.

^{1}H NMR spectrum of **8**, which is well dispersed (both amide and C_αH region) along with several amide proton resonances at $\delta>7$ ppm, suggested the presence of a stable secondary structure. A solvent titration study confirmed that apart from NH(2), all the amide protons, with a small $\Delta\delta_{NH}$ (<0.13 ppm), are involved in H-bonding. The distinctive $^3J_{NH-C\beta H}$ and $^3J_{C\alpha H-C\beta H}$ couplings and the characteristic NOE correlations like: C_βH(2)/NH(4), C_βH(2)/$C_\alpha H_{(pro-R)}$(4), C_βH(4)/NH(6), C_βH(4)/$C_\alpha H_{(pro-R)}$(6), NH(3)/NH(4), NH(5)/NH(6), NH(3)/C_βH(2), and NH(5)/C_βH(4), along with information collated above provide compelling evidence for a right-handed 10/12-helix with a 10/12/10/12/10 H-bonded arrangement in peptide **8** (Figure 5).

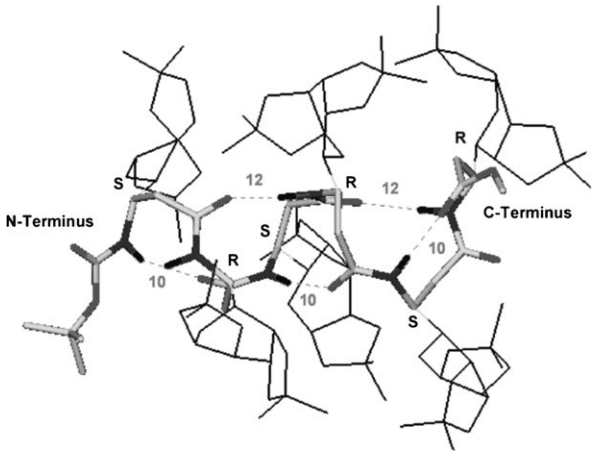


Figure 5. Schematic model of peptide **8** showing the H-bonds. The numbers reflect the number of atoms involved in the H-bonded pseudo ring.

The tripeptide **38** derived from (*R*)- and (*S*)-β-$Caa_{(g)}$, with an (*R*)-residue at the N-terminus, unlike the tripeptide obtained from alternating (*R*)- and (*S*)-β-$Caa_{(x)}$,[11a] has shown no secondary structure. However, the NMR spectrum of tetrapeptide **9**, displays the signatures of the nucleation of a nascent helix. The participation of NH(2) and NH(3) in H-bonding is implied by their low field shifts ($\delta>7$ ppm) as well as the small $\Delta\delta_{NH}$ in the solvent titration studies. Further support for the nascent mixed 10/12-helix comes from the diagnostic coupling constants and rather weak NOEs, C_βH(1)/NH(3) and C_βH(1)/$C_\alpha H_{(pro-R)}$(3).[22]

For **10**, a large dispersion of the amide chemical shifts (about 2.96 ppm) and C_αH chemical shifts (about 0.72 ppm) suggested a well-defined secondary structure. The amide protons, NH(2)–NH(5), resonate at 7.44, 8.82, 7.91, and 8.20 ppm respectively, indicating their participation in H-bonding, which was further confirmed from solvent titration studies, which display $\Delta\delta_{NH}<0.14$ ppm. ROESY spectrum (Figure 6) shows medium-range NOEs, C_βH(1)/NH(3), C_βH(1)/$C_\alpha H_{(pro-R)}$(3), C_βH(3)/NH(5), and C_βH(3)/$C_\alpha H_{(pro-R)}$(5), characteristic of 12-mr H-bonding involving NH(3)–CO(Boc) and NH(5)–CO(2). Further, NOE correlations, NH(2)/NH(3), NH(4)/NH(5), NH(2)/C_βH(1), NH(4)/C_βH(3), and NH(6)/C_βH(5) characterize 10-mr H-bonding

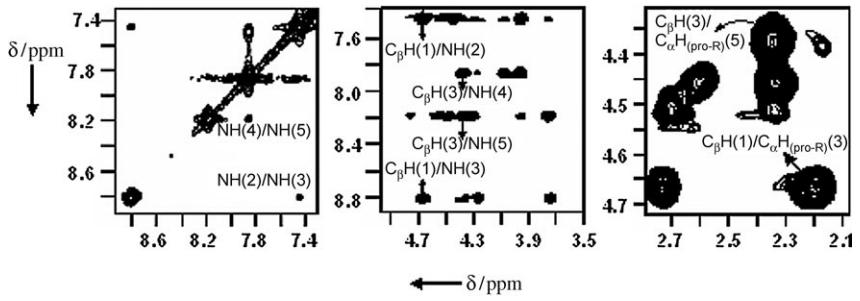


Figure 6. The characteristic NOE correlations in **10**.

involving NH(2)–CO(3)and NH(4)–CO(5). These characteristic NOEs in conjunction with the $^3J_{NH-C\beta H}$ ~9.4 Hz and $^3J_{C\alpha H-C\beta H}$ values of >10 Hz and <5 Hz for most of the residues, clearly suggest that **10** is forming a stable 10/12-helix with 12/10/12/10 H-bonded arrangement. These findings very clearly bring out the fact that while the shorter peptides **7** and **9**, barely exhibit an incipient helix, the right-handed 12/10-helices are more stable for larger peptides **8** and **10**. Thus the impact of the larger pyranoside side chain in destabilizing the helix is very pronounced for the shorter peptides compared to that for the longer ones. Thus, the peptide backbone made from epimeric (at C_β) (*R*)- and (*S*)-β-$Caa_{(g)}$, although enantiomeric at the amine center, the identical chiral carbohydrate side chains govern the handedness of the secondary structure to give right-handed helices.

The tetrapeptide, **11**, contains alternating **5** ((*R*)-β-$Caa_{(x)}$) and **4** ((*R*)-β-$hCaa_{(x)}$). Arising from the broadening of spectral lines in $CDCl_3$ solution, ^{1}H NMR studies on **11** were carried out in [D_5]pyridine. Wide dispersion of all the resonances, especially in the amide and C_αH region, indicates a possibility for a periodic secondary structure. The amide protons NH(1)–NH(4) resonate at 7.86, 8.81, 8.59, and 8.98 ppm, respectively. To gain insight on intramolecular hydrogen bonding patterns in a pyridine solution, the temperature dependence of amide proton chemical shifts is studied. The internal hydrogen bonding is disrupted when the temperature is raised, and the chemical shifts of the amides change. However, if the amide protons participate in intramolecular H-bonding, upon increasing the temperature, the propensity for breaking these bonds is small and the change in chemical shift or temperature coefficient ($\Delta\delta/\Delta T$) will be rather small. The NH temperature coefficients were measured in the temperature range from 303 K to 333 K. It is observed that $\Delta\delta/\Delta T$ are smaller for NH(2) (15.1 ppb K^{-1}) and NH(3) (12.2 ppb K^{-1}) compared to NH(1) (21.2 ppb K^{-1}) and NH(4) (17.7 ppb K^{-1}),[26] which point to their participation in intramolecular H-bonding. $^3J_{C\alpha H-C\beta H}$ >9.4 Hz and <5 Hz clearly demonstrate the presence of predominantly a single conformation around C_α–C_β. Considering individual $^3J_{C\alpha H-C\beta H}$ and various sequential C_αH/NH and intra-residue C_αH/C_βH NOE correlations, a value of θ ~60° for all residues was derived. Similarly, $^3J_{NH-C\beta H}$ ~9 Hz, points to a preponderance of an *ap* arrangement of the NH and C_βH protons. These observations along with the presence of medium-range NOEs, C_βH(1)/NH(2), C_βH(1)/NH(3), C_βH(1)/$C_\alpha H_{(pro-R)}$(3), and C_βH(3)/NH(4) confirm the 12/10 H-bonded arrangement in tetrapeptide **11**. The structure is stabilized by one 12-mr H-bond [NH(3)–CO(Boc)] and one 10-mr H-bond [NH(2)–CO(3)].

Hexapeptide **12**, displayed all the signatures of a well-defined 10/12-mixed helix. All the amide protons, excluding NH(1) and NH(6), participate in intramolecular H-bonding, which was confirmed by the variable temperature studies. Coupling constants (very similar to that for **11**) and the distinctive NOEs (Figure 7), C_βH(1)/NH(2), C_βH(1)/NH(3), C_βH(1)/$C_\alpha H_{(pro-R)}$(3), NH(2)/NH(3), C_βH(3)/NH(4), C_βH(3)/NH(5), C_βH(3)/$C_\alpha H_{(pro-R)}$(5), NH(4)/NH(5), and C_βH(5)/NH(6), along with the information on H-bonding, provide compelling evidence for the presence of a 12/10/12/10 H-bonded arrangements for the peptide **12**.[22]

For peptide **13**, apart from NH(2), all other amide protons participate in intramolecular H-bonding,[22] while $^3J_{NH-C\beta H}$ >9.6 Hz corresponds to ϕ ~±120°, $^3J_{C\alpha H-C\beta H}$ >8.2 Hz and <5 Hz support the presence of predominantly a single conformation around C_α–C_β. These observations along with medium-range NOEs, C_βH(2)/NH(4), C_βH(2)/$C_\alpha H_{(pro-R)}$(4) C_βH(2)/NH(3), NH(1)/NH(2), and NH(3)/NH(4), confirm the right-handed 10/12-helix with 10/12/10 H-bonded arrangement. For hexapeptide **14**, NMR data provide all the evidence for a 10/12-helix with 10/12/10/12/10 H-bonded configuration.[22]

From the ^{1}H NMR spectrum and the solvent titration studies of mixed tetrapeptide **15**, with alternating **4** and **6** residues, despite broadening of the spectral lines, resulting in a concomitant loss of information, NH(2) and NH(3) were found to participate in the H-bonding. The observation of medium-range NOE correlations, C_βH(1)/NH(3) and

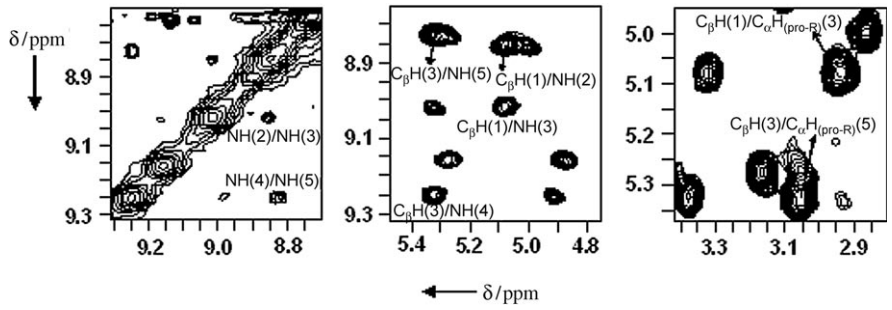


Figure 7. The characteristic NOE correlations in **12**.

$C_\beta H(1)/C_\alpha H_{(pro-S)}(3)$, support the presence of a 10/12-helix in the tetramer. Higher mixed peptide **16**, with better spectral resolution, was found to have a fairly robust helical structure. The low-field amide δ values and the solvent titration studies confirmed that H bonding occurs for NH(2)–NH(5). The $^3J_{NH-C\beta H}$ values ($^3J_{NH-C\beta H}$ ~9.0 Hz for ((*R*)-β-hCaa$_{(x)}$); $^3J_{NH-C\beta H}$ ~9.0 Hz and ~2.5 Hz for β-hGly) correspond to ϕ ~±120°. The predominance of a single conformer about θ was deduced from the $^3J_{C\alpha H-C\beta H}$ values (~9 Hz or ~5 Hz). The sequential NOE correlations, NH(2)/$C_\alpha H_{(pro-R)}(1)$, NH(3)/$C_\alpha H_{(pro-S)}(2)$, NH(4)/$C_\alpha H_{(pro-R)}(3)$, NH(5)/$C_\alpha H_{(pro-S)}(4)$, NH(6)/$C_\alpha H_{(pro-R)}(5)$, and intraresidue correlations, NH(i)/$C_\beta H_{(pro-S)}$(i), along with well-defined $^3J_{C\alpha H-C\beta H}$ values imply a predominant conformation with θ ~ −60°, for all these residues. This unusual change of sign of θ compared to other peptides, suggests the likely presence of a left-handed 10/12-helix. Above details, along with the NOEs, $C_\beta H(1)$/NH(3), $C_\beta H(1)/C_\alpha H_{(pro-S)}(3)$, $C_\beta H(3)$/NH(5), and $C_\beta H(3)/C_\alpha H_{(pro-S)}(5)$, provide ample support for the left-handed 10/12-helix with 12/10/12/10 H-bonded pattern in **16**. Thus, monomer **4**, geometrically homochiral with (*S*)-β-Caa$_{(x)}$,[10] behaved in a similar way[11b] in mixed peptide **16** and effected a switch in handedness to result in the left-handed 12/10-helix.

The couplings involving the side chains in peptides **7**–**10** have been used to obtain the information on the structure of the pyranose sugar ring as well as on the dihedral angle N–C_β–C_5–O ($\chi'1$). Except for the second residue in **7** and **8** and the first residue in **9** and **10**, for most of the β-Caa$_{(g)}$, $^3J_{C\beta H-C5H}$ ~9.0 Hz, implies $\chi'1$ ~180° for (*R*)-β-Caa$_{(g)}$, and ~60° for (*S*)-β-Caa$_{(g)}$. However as noticed for (*S*)-β-Caa$_{(x)}$/(*R*)-β-Caa$_{(x)}$ peptides,[11a] for the second residue in **7** and **8** and for the first residue in **9** and **10**, which incidentally is (*R*)-β-Caa$_{(g)}$, $^3J_{C\beta H-C5H}$ has a value of ~6 Hz, corresponding to $\chi'1$ ~60°. The galactose ring like moiety observed earlier,[27] exists in a slightly distorted boat form as supported by the couplings, $^3J_{C1H-C2H}$ ~5.0 Hz, $^3J_{C2H-C3H}$ ~2.5 Hz, $^3J_{C3H-C4H}$ ~7.8 Hz, $^3J_{C4H-C5H}$ ~1.7 Hz, and a C_3H/C_5H NOE correlation. The lower intensity of the $Me_{(pro-S)}/C_1H$ NOE compared to $Me_{(pro-S)}/C_2H$ and $Me_{(pro-R)}/C_5H$, further show the envelope conformation of the isopropylidine ring containing the C_1–C_2 bond. Similarly, envelope conformation of the isopropylidine ring at the C_3–C_4 bond was confirmed by the NOEs between $Me_{(pro-R)}/C_3H$ and $Me_{(pro-R)}/C_4H$.

In peptides **11**–**16**, for monomer (*R*)-β-hCaa$_{(x)}$, $J_{C\beta H-C5H(pro-R)}$ ~9 Hz, $^3J_{C\beta H-C5H(pro-S)}$ ~5 Hz, $^3J_{C4H-C5H(pro-S)}$ ~9 Hz and $^3J_{C4H-C5H(pro-R)}$ ~5 Hz suggest restricted rotations about C_β–C_5 and C_5–C_4 bonds. These couplings along with intra-residue NOE correlations, NH/$C_5H_{(pro-R)}$ and NH/C_4H, imply preponderance of conformers with N–C_β–C_5–C_4 (χl) ~60° and C_β–C_5–C_4–O (χ2) ~60° (Figure 8). For (*R*)-β-Caa$_{(x)}$, $^3J_{C\beta H-C4H}$ ~9.0 Hz and the intra-residue NOEs, NH/C_4H, $C_4H/C_\alpha H_{(pro-R)}$ and $C_4H/C_\alpha H_{(pro-S)}$, are consistent with N–$C_\beta$–$C_4$–O ($\chi''1$) ~180°. The couplings, $^3J_{C1H-C2H}$ ~4 Hz, $^3J_{C2H-C3H}$ ~0 Hz, and $^3J_{C3H-C4H}$ ~3 Hz, for all the sugar rings in both β-Caa$_{(x)}$ and β-hCaa$_{(x)}$, correspond to a sugar pucker of $^3T_2$. Strong NOEs $Me_{(pro-S)}/C_1H$ and $Me_{(pro-S)}/C_2H$ as well as weak $Me_{(pro-R)}/C_4H$ NOE correlation further show the envelope conformation of the isopropylidine ring.

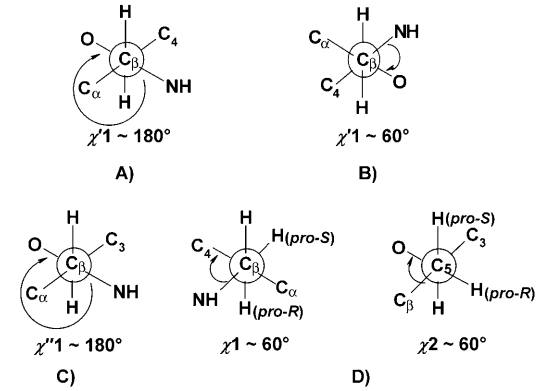


Figure 8. Newman projections showing the orientations of various substituents for the predominant conformers about the C–C bonds, appending the sugar side chains to the peptide backbone. About C_β-C_5 ($\chi'1$) (A) for (*R*)-β-Caa$_{(g)}$ and (B) for (*S*)-β-Caa$_{(g)}$; (C) About C_β-C_4 ($\chi''1$) for (*R*)-β-Caa$_{(x)}$; (D) About C_β-C_5 (χ1) and C_5-C_4 (χ2) for (*R*)-β-hCaa$_{(x)}$.

The CD spectra (200 μM solution in methanol) of the peptides **7**–**10** (Figure 9) and **11**–**14**[22] show diagnostic signatures of a right-handed 10/12-mixed helix, with maxima at about 203 nm. On the other hand the peptides **15**–**16**,[22] as anticipated, gave characteristic signatures for a left-handed 10/12-mixed helix, with a minima at ~202 nm. The small values of molar ellipticities ($[\theta]_{max} < 35\,000$ deg cm^2 dmol^{-1}) in the CD spectra of **7** and **9** (Figure 9) clearly show that the helical secondary structure has just begun to nucleate in these smaller oligomers. The $[\theta]_{max}$ for the peptides **8** and **10** (Figure 9) ~300 000 deg cm^2 dmol^{-1} and ~469 000 deg cm^2 dmol^{-1} respectively, are pronounced, indicating the stability of the mixed helices. The difference in their molar ellipticities indicate that the peptide **10**, with (*R*)-β-Caa$_{(g)}$ at the N-terminus, is more robust than **8**, where the N-terminus residue is (*S*)-β-Caa$_{(g)}$. Similarly, the $[\theta]_{max}$ for **12** and **14** ~160 000 deg cm^2 dmol^{-1} and ~69 700 deg cm^2 dmol^{-1}, respectively, indicate that peptide **12** has more robust helical pattern compared to **14**.

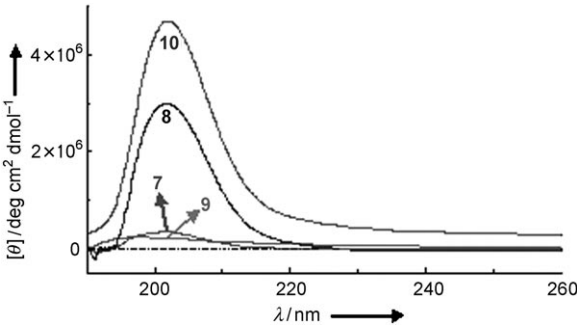


Figure 9. CD spectra of **7**–**10**.

Thus, from the above CD spectral details, it was observed that peptides (**8** and **14**) with 10/12-helical pattern have

lower molar ellipticities than the peptides (**10** and **12**) with 12/10-helices. The theoretical predictions made by Wu and Wang,[7a] where the 12/10-helices, (nucleating the mixed helix at the N-termini with a 12-mr H-bonding) are energetically more favorable over the 10/12-helices (where the 10-mr H-bonding initiates the helix at the N-termini) by 1.5 kcal, support these findings.

For the restraint molecular dynamics (MD) studies (Figure 10 and 11), constraints were derived from the volume integrals obtained from the ROESY spectra using a

Figure 10. Stereoview of MD structures of (A) Peptide **10** and (B) Peptide **12** (sugars are replaced with methyl groups, for clarity, after calculations).

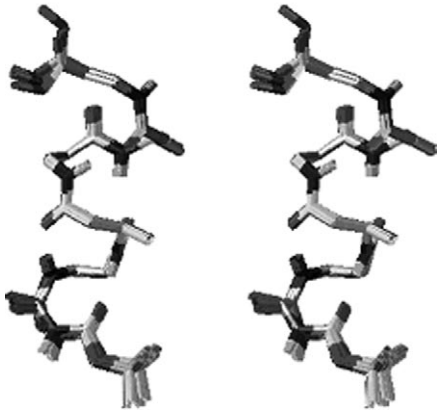


Figure 11. Stereoview of MD structures of **16** (sugars are replaced with methyl groups, for clarity, after calculations).

two-spin approximation. Figure 10 A and 10 B show 20 lowest energy superimposed structures of hexapeptides **10** and **12**, respectively. The MD structures depict the features that have already been suggested, with the backbone and heavy atom RMSDs of 0.78 Å and 1.23 Å for **10** and 0.63 Å and 1.02 Å for **12**, respectively. These values are significantly larger than those observed for the corresponding peptides studied earlier, which resulted in very robust mixed helices.[11a] Figure 11 shows the 20 lowest energy superimposed structures for **16**. The backbone and heavy atom RMSDs for the left-handed 10/12-helix are 0.58 Å and 0.79 Å.

Conclusions

In conclusion, new amino acids (*R*)- and (*S*)-β-Caa$_{(g)}$, having a bulky D-galactopyranoside side chain, were synthesized from D-galactose, while the (*R*)- and (*S*)-β-hCaa$_{(x)}$, with a methylene spacer between the backbone and side chain, were prepared from D-glucose. As anticipated, there is a significant impact of the side chains on the helix formation and its stability in the β-peptides derived from these β-amino acids. Unlike in our earlier studies,[11a] the tripeptide made from alternating **1** and **2**, has shown no secondary structure. Similarly, the tetrapeptides synthesized from **1** and **2**, barely show nucleation of an incipient 10/12-mixed helix. Further, unlike in the earlier studies with (*R*)- and (*S*)-β-Caa$_{(x)}$, attempted synthesis of β-peptides using alternating (*S*)-β-hCaa$_{(x)}$ **3** and (*R*)-β-hCaa$_{(x)}$ **4** failed, which again reflects a subtle impact of the respective side chains. However, synthesis using alternating geometrically heterochiral (*R*)-β-Caa$_{(x)}$ and (*R*)-β-hCaa$_{(x)}$ **4** was successful and yielded the peptides with robust right-handed 10/12-mixed helices. Similarly, the mixed β-peptides from (*R*)-β-hCaa$_{(x)}$ **4** and β-hGly have shown left-handed 10/12-helical structures. Thus, this study with the new monomers amply demonstrates that the side chains do have a substantial influence on the stability of the secondary structures besides controlling the design and synthesis. The addition of new β-amino acid monomers, with their larger conformational requirements, might help in designing novel secondary structures in the arena of synthetic peptides and proteins research.

Experimental

General

CH_2Cl_2 was distilled over CaH_2 and stored over 4 Å molecular sieves. Et_3N was dried on KOH and distilled from ninhydrin. Amino acids and peptide coupling reagents were purchased from standard catalogues. HOBT (1-Hydroxy-1H benzotriazole) and EDCI (1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride) were used for peptide synthesis. All coupling and Boc deprotection reactions were carried out under nitrogen atmosphere. TLC was performed on Silica gel 60 F_{254} plates and detection with UV or dipping into the solution of ninhydrin followed by heating. Melting points were determined in open capillaries and were not corrected.

IR spectra were recorded on KBr pallets in the 400–4000 cm^{-1} range. NMR spectra were recorded in 5–10 mM solutions in $CDCl_3$ and [D_5]pyridine on 500 (^{1}H: 500 MHz, ^{13}C: 125 MHz), 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), 600 (^{1}H: 600 MHz, ^{13}C: 150 MHz) and 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz) MHz spectrometers. The spectra were invariably obtained at 303 K. Chemical shifts (δ) are reported in ppm downfield with respect to internal TMS ($SiMe_4$) ($\delta = 0$ ppm). CD spectra were recorded on 0.2 mM solutions in MeOH at room temperature using a 2 mm pathlength cell and a spectral range of 260–190 nm, with a scanning speed of 50 nm min^{-1}, bandwidth of 2 nm and data pitch of 0.1 nm with 2 accumulations. The values are expressed in terms of [θ], the total molar ellipticity (deg cm^2 $dmol^{-1}$). The binomial method is used for smoothing of the spectra.

Restraint molecular dynamics (MD) studies were carried out using INSIGHT-II. The constraints were derived from the volume integrals obtained from the ROESY spectra using a two-spin approximation and the reference distance of 1.8 Å for the geminal protons. The upper and lower

bound of the distance constraints have been obtained by enhancing and reducing the derived distance by 10%.

Synthesis

Boc-(*S*)-β-Caa$_{(g)}$-OCH$_3$ (1). A solution of **19** (1.10 g, 3.01 mmol) in methanol (5.0 mL) was treated with 10% Pd–C (0.10 g) and stirred for 12 h at room temperature. The reaction mixture was filtered through celite and the organic layer was dried (Na_2SO_4). Solvent was evaporated under reduced pressure to give **19a** (1.32 g, 93%) as a pale yellow liquid.

A solution of **19a** (1.32 g, 3.98 mmol) and Et_3N (1.38 mL, 9.95 mmol) in THF (13 mL) at 0°C was treated with $(Boc)_2O$ (0.92 mL, 3.98 mmol) and stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc (2×10 mL) and washed with water (5 mL). Organic layer was dried (Na_2SO_4), evaporated under reduced pressure, and the residue was purified by column chromatography (Silica gel, 15% EtOAc in petroleum ether) to give **1** (1.61 g, 93%) as a white solid: m.p. 105–108°C; $\alpha_D^{27} = -136.3$ ($c = 0.8$, $CHCl_3$); IR (KBr): $\tilde{\nu} = 3404$, 2984, 2931, 2827, 1744, 1709, 1516, 1440, 1368, 1174, 1072, 1022 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 5.53$ (d, 1H, $J = 5.0$ Hz, C_1H), 5.07 (d, 1H, $J = 7.9$ Hz, NH), 4.58 (dd, 1H, $J = 2.4$, 7.9 Hz, C_3H), 4.29 (dd, 1H, $J = 2.4$, 5.0 Hz, C_2H), 4.26 (dd, 1H, $J = 1.7$, 7.9 Hz, C_4H), 4.15 (m, 1H, C_βH), 4.00 (dd, 1H, $J = 1.7$, 6.5 Hz, C_5H), 3.66 (s, 3H, COOMe), 2.79 (dd, 1H, $J = 6.5$, 16.5 Hz, C_αH), 2.73 (dd, 1H, $J = 5.5$, 16.5 Hz, $C_{\alpha'}$H), 1.50 (s, 3H, Me), 1.44 (s, 3H, Me), 1.43 (s, 9H, Boc), 1.32 (s, 3H, Me), 1.31 ppm (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 172.0$, 155.5, 109.4, 108.7, 96.5, 71.7, 71.0, 70.6, 66.8, 51.5, 48.6, 35.8, 28.3, 25.9, 25.8, 24.9, 24.3 ppm; HRMS (ESI): *m/z* calculated for $C_{20}H_{34}NO_9$ $[M+H]^+$ 432.2203, found 432.2202.

Boc-(*R*)-β-Caa$_{(g)}$-OCH$_3$ (2). A solution of **20** (0.90 g, 2.13 mmol) in methanol (2.7 mL) was treated with 10% Pd-C (0.09 g) as described for **19a** to give **20a** (0.652 g, 92%) as a yellow liquid.

As described for **1**, a solution of **20a** (0.652 g, 1.97 mmol) and Et_3N (0.68 mL, 4.93 mmol) in THF (7 mL) was treated with $(Boc)_2O$ (0.45 g, 1.97 mmol) to give **2** (0.773 g, 91%) as a syrup after chromatographic purification (Silica gel, 14% EtOAc in petroleum ether): $\alpha_D^{27} = -81.13$ ($c = 0.46$, $CHCl_3$); IR (Neat): $\tilde{\nu} = 3410$, 3390, 2925, 1723, 1460, 1306, 1230, 1150, 980, 847 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 5.48$ (d, 1H, $J = 5.0$ Hz, C_1H), 5.33 (d, 1H, $J = 9.3$ Hz, NH), 4.58 (dd, 1H, $J = 2.5$, 8.0 Hz, C_3H), 4.32 (dd, 1H, $J = 1.7$, 8.0 Hz, C_4H), 4.26 (dd, 1H, $J = 2.5$, 5.0 Hz, C_2H), 4.17 (m, 1H, C_βH), 3.97 (dd, 1H, $J = 1.7$, 7.0 Hz, C_5H), 3.67 (s, 3H, COOMe), 2.78 (dd, 1H, $J = 6.1$, 16.4 Hz, C_αH), 2.72 (dd, 1H, $J = 5.2$, 16.4 Hz, $C_{\alpha'}$H), 1.49 (s, 3H, Me), 1.45 (s, 3H, Me), 1.43 (s, 9H, Boc), 1.33 (s, 3H, Me), 1.31 ppm (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 166.7$, 150.4, 131.4, 130.3, 128.7, 127.2, 112.6, 105.6, 85.2, 82.7, 80.3, 46.2, 29.6, 28.5, 26.9, 26.3, 20.0 ppm; HRMS (ESI): *m/z* calculated for $C_{20}H_{34}NO_9$ $[M+H]^+$ 432.2204, found 432.2201.

Boc-(*S*)-β-Caa$_{(g)}$-(*R*)-β-Caa$_{(g)}$-OCH$_3$ (29). A solution of ester **1** (1.1 g, 2.55 mmol) in methanol (4.4 mL) was treated with aq. 4N NaOH solution (4.4 mL) at 0°C to room temperature. After 2 h, methanol was removed and the pH adjusted to 2–3 with aq. 1N HCl solution at 0°C and extracted with EtOAc (2×10 mL). The organic layer was dried (Na_2SO_4) and concentrated to give **27** (0.98 g, 92%) as a white solid, which was used as obtained for the next step.

A solution of **2** (0.853 g, 1.98 mmol) and TFA (0.8 mL) in CH_2Cl_2 (5 mL) was stirred at room temperature for 1 h. Solvent was evaporated under reduced pressure and resulting amine salt **28** was dried under high vacuum and used as obtained for the next step.

A mixture of **27** (0.825 g, 1.98 mmol), HOBt (0.320 g, 2.37 mmol), and EDCI (0.454 g, 2.37 mmol) in CH_2Cl_2 was stirred at 0°C for 15 min and the reaction mixture was treated with **28**, DIPEA (0.51 mL, 2.97 mmol) and stirred at room temperature for 8 h. It was quenched at 0°C with sat. NH_4Cl (5 mL) solution. After 10 min, the reaction mixture was diluted with $CHCl_3$ (10 mL), washed with 1N HCl (5 mL), water (5 mL), aq. sat $NaHCO_3$ (5 mL) solution, and brine (5 mL). The organic layer was dried (Na_2SO_4) and evaporated to give the residue, which was purified by column chromatography (Silica gel, 40% EtOAc in petroleum ether) to give **29** (1.28 g, 88%) as a white solid: m.p. 105–107°C; $\alpha_D^{27} = -119.89$ ($c = 0.9$, $CHCl_3$); IR (KBr): $\tilde{\nu} = 3431.9$, 3043.9, 2927.5, 1734.5, 1689.4, 1508.2, 1375.8, 1170.3, 1021.6, 996.3, 872.6 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 6.52$ (d, 1H, $J = 8.7$ Hz, NH-2), 5.52 (d, 1H, $J = 5.0$ Hz, C_1H-1), 5.48 (d, 1H, $J = 5.0$ Hz, C_1H-2), 5.14 (d, 1H, $J = 6.8$ Hz, NH-1), 4.58 (dd, 1H, $J = 2.4$, 8.0 Hz, C_3H-2), 4.57 (dd, 1H, $J = 2.5$, 7.9 Hz, C_3H-1), 4.45 (m, 1H, C_βH-2), 4.32 (dd, 1H, $J = 1.7$, 7.9 Hz, C_4H-1), 4.30 (dd, 1H, $J = 1.8$, 8.0 Hz, C_4H-2), 4.29 (dd, 1H, $J = 2.5$, 5.0 Hz, C_2H-1), 4.27 (dd, 1H, $J = 2.4$, 5.0 Hz, C_2H-2), 4.06 (dd, 1H, $J = 1.8$, 7.7 Hz, C_5H-2), 4.02 (dd, 1H, $J = 1.7$, 7.6 Hz, C_5H-1), 4.00 (m, 1H, C_βH-1), 3.66 (s, 3H, COOMe), 2.80 (dd, 1H, $J = 6.1$, 16.8 Hz, C_αH-2), 2.70–2.56 (m, 3H, C_αH-1, $C_{\alpha'}$H-1, $C_{\alpha'}$H-2), 1.53 (s, 3H, Me), 1.49 (s, 3H, Me), 1.45 (s, 3H, Me), 1.44 (s, 12H, Boc, Me), 1.33 (s, 3H, Me),1.31 (s, 6H, Me), 1.30 ppm (s, 6H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 172.3$, 170.2, 156.0, 109.3, 108.8, 108.7, 96.5, 71.5, 71.0, 70.9, 70.8, 70.7, 70.5, 67.3, 66.5, 51.5, 49.4, 46.5, 37.4, 34.6, 28.4, 26.0, 25.9, 25.9, 25.8, 25.0, 24.9, 24.4, 24.2 ppm; HRMS (ESI): *m/z* calculated for $C_{34}H_{55}N_2O_{15}Na$ $[M+Na]^+$ 753.3421, found 753.3413.

Boc-(*S*)-β-Caa$_{(g)}$-(*R*)-β-Caa$_{(g)}$-(*S*)-β-Caa$_{(g)}$-(*R*)-β-Caa$_{(g)}$-OCH$_3$ (7). As described for **27**, a solution of **29** (0.65, 0.89 mmol), on reaction with 4N NaOH in MeOH gave **30** (0.276 g, 94%) as a white solid, which was used as such for further reaction.

As described for the synthesis of **29**, a mixture of **30** (0.35 g, 0.48 mmol), HOBt (0.079 g, 0.58 mmol), and EDCI (0.111 g, 0.58 mmol) in CH_2Cl_2 (3 mL) was stirred at 0°C for 15 min and treated with **31** [prepared from **29** (0.357 g, 0.488 mmol), TFA (0.35 mL) in CH_2Cl_2 (2.0 mL), and DIPEA (0.12 mL, 0.72 mmol)] under N_2 atmosphere for 8 h. Workup and purification by column chromatography (Silica gel, 2.0% methanol in $CHCl_3$) afforded **7** (0.48 g, 75%) as a white solid: m.p. 152–155°C; $\alpha_D^{27} = -101.84$ ($c = 0.15$, $CHCl_3$); IR (KBr): $\tilde{\nu} = 3331$, 2927, 1744, 1708, 1605, 1547, 1389, 1121, 916, 802 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.16$ (d, 1H, $J = 8.2$ Hz, NH-4), 7.03 (d, 1H, $J = 9.6$ Hz, NH-2), 6.96 (d, 1H, $J = 9.0$ Hz, NH-3), 5.65 (d, 1H, $J = 7.6$ Hz, NH-1), 5.54 (d, 1H, $J = 5.1$ Hz, C_1H-3), 5.53 (d, 1H, $J = 4.9$ Hz, C_1H-1), 5.52 (d, 1H, $J = 5.0$ Hz, C_1H-4), 5.41 (d, 1H, $J = 5.0$ Hz, C_1H-2), 4.76 (m, 1H, C_βH-2), 4.75 (dd, 1H, $J = 1.7$, 7.9 Hz, C_4H-3), 4.64 (dd, 1H, $J = 2.5$, 7.8 Hz, C_3H-4), 4.59 (dd, 2H, $J = 2.4$, 7.9 C_3H-1 and 3), 4.58 (dd, 1H, $J = 2.3$, 7.9 Hz, C_3H-2), 4.46 (m, 1H, C_βH-4), 4.39 (dd, 1H, $J = 1.6$, 7.8 Hz, C_4H-4), 4.38 (m, 1H, C_βH-3), 4.35 (dd, 1H, $J = 1.7$, 8.1 Hz, C_4H-1), 4.33 (dd, 1H, $J = 1.5$, 7.9 Hz, C_4H-2), 4.31 (dd, 1H, $J = 2.5$, 5.0 Hz, C_2H-4), 4.29 (dd, 1H, $J = 2.4$, 4.9 Hz, C_2H-1), 4.27 (dd, 1H, $J = 2.3$, 5.0 Hz, C_2H-2), 4.26 (dd, 1H, $J = 2.4$, 5.1 Hz, C_2H-3), 4.23 (m, 1H, C_βH-1), 3.93 (dd, 1H, $J = 1.7$, 9.0 Hz, C_5H-3), 3.85 (dd, 1H, $J = 1.7$, 8.7 Hz, C_5H-1), 3.84 (dd, 1H, $J = 1.6$, 8.8 Hz, C_5H-4), 3.77 (dd, 1H, $J = 1.5$, 5.0 Hz, C_5H-2), 3.63 (s, 3H, COOMe), 2.81 (dd, 1H, $J = 3.8$, 13.8 Hz, $C_\alpha H_{(pro-S)}$-4), 2.71 (dd, 1H, $J = 6.5$, 14.8 Hz, $C_\alpha H_{(pro-R)}$-1), 2.60 (dd, 1H, $J = 3.1$, 12.8 Hz, $C_\alpha H_{(pro-S)}$-2), 2.57 (dd, 1H, $J = 4.7$, 14.8 Hz, $C_\alpha H_{(pro-S)}$-1), 2.52 (dd, 1H, $J = 9.8$, 13.8 Hz, $C_\alpha H_{(pro-R)}$-4), 2.51 (dd, 1H, $J = 3.1$, 13.6 Hz, $C_\alpha H_{(pro-R)}$-3), 2.41 (dd, 1H, $J = 5.0$, 13.6 Hz, $C_\alpha H_{(pro-S)}$-3), 2.35 (dd, 1H, $J = 11.3$, 12.8 Hz, $C_\alpha H_{(pro-R)}$-2), 1.55 (s, 3H, Me), 1.50 (s, 3H, Me), 1.48 (s, 6H, Me), 1.45 (s, 3H, Me), 1.44 (s, 9H, Boc), 1.43 (s, 3H, Me), 1.41 (s, 3H, Me), 1.38 (s, 3H, Me), 1.34 (s, 3H, Me), 1.33 (s, 3H, Me), 1.31 (s, 3H, Me), 1.30 (s, 9H, Me), 1.29 ppm (s, 6H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 173.8$, 171.3, 169.8, 155.8, 109.5, 109.3, 109.0, 108.9, 108.7, 108.4, 108.2, 96.6, 96.3, 78.7, 71.4, 71.1, 70.7, 70.6, 70.4, 70.3(2), 70.1, 68.0, 67.8, 67.7, 66.2, 52.1, 50.1, 49.7, 47.7, 46.5, 39.4, 37.4, 36.7, 36.0, 29.6, 28.4, 26.1, 26.0 (2), 25.9, 25.5, 25.2, 25.0, 24.9, 24.8, 24.7, 24.4, 24.3, 24.1 ppm; HRMS (ESI): *m/z* calculated for $C_{62}H_{96}N_4O_{27}Na$ $[M+Na]^+$ 1351.6128, found 1351.6116.

Boc-(*S*)-β-Caa$_{(g)}$-(*R*)-β-Caa$_{(g)}$-(*S*)-β-Caa$_{(g)}$-(*R*)-β-Caa$_{(g)}$-(*S*)-β-Caa$_{(g)}$-(*R*)-β-Caa$_{(g)}$-OCH$_3$ (8). As described for **27**, a solution of **7** (0.136, 0.10 mmol), on reaction with 4N NaOH in MeOH gave **32** (0.276 g, 94%) as a white solid, which was used as such for further reaction.

As described for the synthesis of **29**, a mixture of **32** (0.1 g, 0.076 mmol), HOBt (0.012 g, 0.091 mmol) and EDCI (0.018 g, 0.091 mmol) in CH_2Cl_2 (2 mL) was stirred at 0°C for 15 min and treated with **31** [prepared from **29** (0.056 g, 0.076 mmol) and TFA (0.06 mL) in CH_2Cl_2 (0.5 mL)] and DIPEA (0.02 mL, 0.114 mmol) under N_2 atmosphere for 8 h. Workup and purification by column chromatography (Silica gel, 2.3% methanol in $CHCl_3$) afforded **8** (0.07 g, 47%) as a white solid: m.p. 183–186°C; $\alpha_D^{27} = -112.4$ ($c = 0.21$, $CHCl_3$); IR (KBr): $\tilde{\nu} = 3314$, 2950, 2908, 2335, 1725, 1658, 1492, 1219, 1165, 911, 784 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$):

δ = 9.09 (d, 1H, J = 9.5 Hz, NH-4), 8.26 (d, 1H, J = 9.8 Hz, NH-6), 7.33 (d, 1H, J = 10.4 Hz, NH-3), 7.11 (d, 1H, J = 10.1 Hz, NH-2), 6.79 (d, 1H, J = 7.8 Hz, NH-5), 5.54 (d, 1H, J = 5.1 Hz, C_1H-1), 5.53 (d, 1H, J = 5.0 Hz, C_1H-3), 5.49 (d, 1H, J = 4.9, C_1H-6), 5.48 (brs, 1H, NH-1), 5.47 (d, 1H, J = 4.9 Hz, C_1H-4), 5.41 (d, 1H, J = 5.1 Hz, C_1H-5), 5.38 (d, 1H, J = 5.0 Hz, C_1H-2), 4.91 (m, 1H, C_βH-2), 4.86 (dd, 1H, J = 1.8, 8.0 Hz, C_4H-5), 4.77 (dd, 1H, J = 1.6, 8.4 Hz, C_4H-3), 4.63 (m, 1H, C_βH-6), 4.60 (dd, 1H, J = 2.4, 8.0 Hz, C_3H-1), 4.58 (m, 3H, C_3H-2, 4 and 5), 4.57 (m, 1H, C_3H-6), 4.54 (dd, 1H, J = 2.1, 8.4 Hz, C_3H-3), 4.44 (dd, 1H, J = 1.8, 8.0, C_4H-1), 4.39 (m, 1H, C_βH-3), 4.32 (dd, 1H, J = 1.7, 7.8 Hz, C_4H-4), 4.30 (m, 2H, C_5H-1, C_4H-6), 4.29 (dd, 2H, J = 2.3, 5.0 Hz, C_2H-1, 6), 4.28 (m, 1H, C_4H-2), 4.28 (dd, 1H, J = 2.2, 5.0 Hz, C_2H-4), 4.26 (dd, 1H, J = 2.2, 5.1 Hz, C_2H-5), 4.25 (dd, 1H, J = 2.2, 5.0 Hz, C_2H-2), 4.24 (dd, 1H, J = 2.1, 5.0 Hz, C_2H-3), 4.23 (m, 1H, C_βH-4), 4.05 (m, 1H, C_βH-5), 3.96 (dd, 1H, J = 1.8, 10.0 Hz, C_5H-5), 3.89 (dd, 1H, J = 1.6, 9.1 Hz, C_5H-3), 3.85 (m, 1H, C_βH-1), 3.76 (dd, 1H, J = 1.7, 4.9 Hz, C_5H-2), 3.67 (dd, 1H, J = 1.6, 9.6 Hz, C_5H-6), 3.64 (m, 1H, C_5H-4), 3.63 (s, 3H, COOMe), 2.90 (dd, 1H, J = 2.6, 15.7 Hz, $C_\alpha H_{(pro-S)}$-6), 2.75 (dd, 1H, J = 2.6, 13.5 Hz, $C_\alpha H_{(pro-S)}$-4), 2.64 (dd, 1H, J = 2.1, 11.9 Hz, $C_\alpha H_{(pro-S)}$-2), 2.58 (m, 2H, C_αH-1), 2.55 (dd, 1H, J = 12.2, 15.7 Hz, $C_\alpha H_{(pro-R)}$-6), 2.49 (dd, 1H, J = 2.3, 11.8 Hz, $C_\alpha H_{(pro-R)}$-3), 2.46 (dd, 1H, J = 4.3, 13.2 Hz, $C_\alpha H_{(pro-R)}$-5), 2.39 (dd, 1H, J = 2.7, 13.2 Hz, $C_\alpha H_{(pro-S)}$-5), 2.38 (dd, 1H, J = 3.9, 11.8 Hz, $C_\alpha H_{(pro-S)}$-3), 2.22 (t, 1H, J = 11.9 Hz, $C_\alpha H_{(pro-R)}$-2), 2.16 (dd, 1H, J = 12.5, 13.5, $C_\alpha H_{(pro-R)}$-4), 1.57 (s, 15H, Me), 1.53 (s, 3H, Me), 1.50 (s, 3H, Me), 1.47 (s, 3H, Me), 1.46 (s, 3H, Me), 1.45 (s, 3H, Me), 1.44 (s, 9H, Boc), 1.41 (s, 3H, Me), 1.39 (s, 3H, Me), 1.33 (s, 9H, Me), 1.31 (s, 6H, Me), 1.29 (s, 6H, Me), 1.28 (s, 6H, Me), 1.26 ppm (s, 9H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6, 172.0, 171.7, 171.0, 170.5, 169.1, 155.7, 109.5, 109.4, 109.2, 109.1, 109.0(2), 108.7, 108.6, 108.5, 108.2, 96.8, 96.6, 96.5, 96.4, 96.3, 96.1, 95.6, 78.6, 71.9, 71.3, 71.0, 70.9, 70.8, 70.7 (2), 70.6, 70.5, 70.4, 70.2, 70.0, 69.9, 69.8, 69.6, 69.4, 69.3, 67.9, 67.5, 65.9, 51.6, 51.5, 50.1, 48.4, 48.3, 47.7, 44.0, 40.2, 39.6, 38.0, 37.9, 37.6, 36.3, 29.6, 28.4, 27.4, 27.0, 26.9, 26.3, 26.2, 26.1, 26.0, 25.9, 25.6, 25.4, 25.2, 25.1, 24.9, 24.6, 24.5, 24.4(2), 24.1 ppm; HRMS (ESI): *m/z* calculated for $C_{90}H_{138}N_6O_{39}Na$ $[M+Na]^+$ 1949.9049, found 1949.9056.

Boc-(*R*)-β-hCaa$_{(x)}$-OCH$_3$ (4). As described for the synthesis of **19a**, a solution of **26** (1.2 g, 3.2 mmol) in methanol (4.0 mL) was treated with 10% Pd–C (0.12 g) for 12 h to give **26a** (0.85 g, 93%) as a pale yellow liquid.

As described for the synthesis of **1**, a solution of **26a** (0.85 g, 2.98 mmol) and Et_3N (0.829 mL, 5.96 mmol) in THF (10 mL) at 0 °C was treated with $(Boc)_2O$ (0.8 mL, 3.5 mmol) and stirred at room temperature for 3 h. Workup and purification by column chromatography (Silica gel, 15% EtOAc in petroleum ether) gave **4** (0.98 g, 85%) as a syrup: $\alpha_D^{27} = -18.3$ ($c = 0.6$, $CHCl_3$); IR (Neat): $\tilde{\nu}$ = 3404, 2984, 2931, 2827, 1744, 1709, 1516, 1440, 1368, 1174, 1072, 1022 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.85 (d, 1H, J = 3.9 Hz, C_1H), 5.22 (d, 1H, J = 8.8 Hz, NH), 4.56 (d, 1H, J = 3.9 Hz, C_2H), 4.24 (dt, 1H, J = 3.3, 7.1 Hz, C_4H), 4.08 (m, 1H, C_βH), 3.67 (s, 3H, COOMe), 3.56 (d, 1H, J = 3.3 Hz, C_3H), 3.41 (s, 3H, OMe), 2.65 (m, 1H, J = 5.9, 15.9 Hz, C_αH), 2.58 (m, 1H, J = 6.3, 15.9 Hz, C_αH), 1.93 (m, 2H, C_5H, C_5H), 1.43 (s, 9H, Boc), 1.49 (s, 3H, Me)1.32 ppm (s, 3H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 166, 149, 105, 98.7, 98.0, 75.6, 78.4, 71.8, 51.7, 45.0, 39.0, 33.7, 22.5, 20.7, 20.3 ppm; HRMS (ESI): *m/z* calculated for $C_{18}H_{32}NO_8$ $[M+H]^+$ 390.2192, found 390.2201.

Boc-(*S*)-β-hCaa$_{(x)}$-OCH$_3$ (3). As described for the synthesis of **19a**, a solution of **25** (1.0 g, 2.6 mmol) in methanol (3.0 mL) was treated with 10% Pd–C (0.1 g) for 12 h to give **25a** (0.7 g, 92%) as a pale yellow liquid.

As described for the synthesis of **1**, a solution of **25a** (0.7 g, 2.4 mmol) and Et_3N (0.67 mL, 4.8 mmol) in THF (8 mL) at 0 °C was treated with $(Boc)_2O$ (0.67 mL, 2.94 mmol) and stirred at room temperature for 2 h. Workup and purification by column chromatography (Silica gel, 15% EtOAc in petroleum ether) gave **3** (0.88 g, 93%) as a white solid: m.p. 125–128 °C; $\alpha_D^{27} = -48.5$ ($c = 0.54$, $CHCl_3$); IR (KBr): $\tilde{\nu}$ = 3383, 2980, 2935, 1721, 1705, 1499, 1308, 1160, 1071, 1011 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.85 (d, 1H, J = 3.9 Hz, C_1H), 5.14 (d, 1H, J = 9.4 Hz, NH), 4.55 (d, 1H, J = 3.9 Hz, C_2H), 4.21 (ddd, 1H, J = 3.7, 6.2, 7.9 Hz, C_4H), 4.01 (m, 1H, C_βH), 3.77 (d, 1H, J = 3.7 Hz, C_3H), 3.68 (s, 3H, COOMe), 3.43 (s, 3H, OMe), 2.57 (m, 2H, C_αH, C_αH), 1.92 (ddd, 1H, J = 4.4, 7.9, 14.1 Hz, C_5H), 1.87 (ddd, 1H, J = 6.2, 9.7, 14.1 Hz, C_5H), 1.43 (s, 9H, Boc), 1.47 (s, 3H, Me), 1.31 ppm (s, 3H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 166.0, 149.2, 105.6, 98.6, 79, 75.4, 71.9, 51.5, 45.7, 39.9, 33.3, 26.1, 22.5, 20.5, 20.3 ppm; HRMS (ESI): *m/z* calculated for $C_{18}H_{32}NO_8$ $[M+H]^+$ 390.2163, found 390.2275.

Boc-(*R*)-β-Caa$_{(x)}$-(*R*)-β-hCaa$_{(x)}$-OCH$_3$ (42). As described for the synthesis of **27**, a solution of **5** (0.98 g, 2.61 mmol) on reaction with 4N NaOH in MeOH gave **40** (0.9 g, 96%) as a white solid and which was used as such for further reaction.

As described for the synthesis of **29**, a solution of **40** (0.9 g, 2.8 mmol), HOBt (0.46 g, 3.41 mmol), and EDCI (0.65 g, 3.41 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C under N_2 atmosphere for 15 min and treated with **41** [prepared from **4** (1.07 g, 2.8 mmol) and TFA (1.0 mL) in CH_2Cl_2 (10 mL)] and DIPEA (0.74 mL, 4.27 mmol) and stirred for 8 h. Workup and purification by column chromatography (Silica gel, 50% EtOAc in petroleum ether) gave **42** (1.20 g, 76%) as a white solid: m.p. 73–75 °C; $\alpha_D^{27} = -23.5$ ($c = 0.40$, $CHCl_3$); IR (KBr): $\tilde{\nu}$ = 3431.9, 3343.9, 2927.5, 1744.5, 1689.4, 1508.2, 1375.8, 1170.3, 1093.6, 1021.6, 996.3, 872.6 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.54 (d, 1H, J = 8.2 Hz, NH-2), 5.70 (d, 1H, J = 8.8 Hz, NH-1), 5.86 (d, 1H, J = 3.7 Hz, C_1H-1), 5.84 (d, 1H, J = 3.7 Hz, C_1H-2), 4.57 (d, 1H, J = 3.7 Hz, C_2H-2), 4.53 (d, 1H, J = 3.7 Hz, C_2H-1), 4.34 (m, 1H, C_4H-2), 4.24 (m, 3H, C_4H-1, C_βH-1, C_βH-2), 3.71 (d, 1H, J = 3.0 Hz, C_3H-1), 3.67 (s, 3H, COOMe), 3.63 (d, 1H, J = 3.3 Hz, C_3H-2), 3.41 (s, 3H, OMe), 3.40 (s, 3H, OMe), 2.70 (dd, 1H, J = 5.6, 15.8 Hz, C_αH-2), 2.58 (dd, 1H, J = 6.6, 15.8 Hz, C_αH-2), 2.52 (dd, 1H, J = 5.7, 15.2 Hz, C_αH-1), 2.47 (dd, 1H, J = 4.7, 15.2 Hz, C_αH-1), 1.96 (m, 2H, C_5H-2, C_5H-2), 1.43 (s, 9H, Boc), 1.48 (s, 3H, Me), 1.46 (s, 3H, Me), 1.31 (s, 3H, Me), 1.30 ppm (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.3, 170.4, 155.4, 111.7, 111.6, 105.0, 104.8, 84.7, 84.0, 81.6, 80.2, 78.1, 60.6, 57.8, 57.7, 46.2, 45.6, 38.0, 36.5, 29.6, 28.4, 26.8, 26.7, 26.4, 26.3 ppm; HRMS (ESI): *m/z* calculated for $C_{29}H_{48}N_2O_{13}Na$ $[M+Na]^+$ 655.3079, found 655.3052.

Boc-(*R*)-β-Caa$_{(x)}$-(*R*)-β-hCaa$_{(x)}$-(*R*)-β-Caa$_{(x)}$-(*R*)-β-hCaa$_{(x)}$-OCH$_3$ (11). As described for **27**, a solution of **42** (0.6 g, 0.94 mmol) on reaction with 4N NaOH in MeOH gave **43** (0.55 g, 95%) as a white solid, which was used as such for further reaction.

A mixture of acid **43** (0.50 g, 0.80 mmol), HOBt (0.13 g, 0.97 mmol), and EDCI (0.18 g, 0.97 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C under N_2 atmosphere for 15 min and treated with **44** [prepared from **42** (0.51 g, 0.8 mmol) and TFA (0.5 mL) in CH_2Cl_2 (3.0 mL)] and DIPEA (0.20 mL, 1.21 mmol) and stirred for 8 h. Workup as described for **29** and purification by column chromatography (Silica gel, 2% MeOH in $CHCl_3$) gave **11** (0.4 g, 43%) as a white solid: m.p. 90–93 °C; $\alpha_D^{27} = +1.9$ ($c = 0.30$, $CHCl_3$); IR (KBr): 3370, 2986, 2942, 1717, 1666, 1520, 1337, 1100, 972, 864 cm^{-1}; ^{1}H NMR (500 MHz, Pyridine-d_5): δ = 8.98 (d, 1H, J = 8.4 Hz, NH-4), 8.81 (d, 1H, J = 9.1 Hz, NH-2), 8.59 (d, 1H, J = 9.4 Hz, NH-3), 7.86 (d, 1H, J = 9.7 Hz, NH-1), 6.11 (d, 1H, J = 3.9 Hz, C_1H-1), 6.06 (d, 1H, J = 3.9 Hz, C_1H-2), 6.03 (m, 2H, C_1H-3, C_1H-4), 5.22 (m, 1H, C_βH-3), 5.03 (m, 1H, C_βH-1), 4.97 (m, 1H, C_βH-2), 4.85 (m, 1H, C_βH-4), 4.73 (d, 1H, J = 3.9 Hz, C_2H-2), 4.72 (m, 1H, C_4H-2), 4.71 (d, 1H, J = 3.9 Hz, C_2H-1), 4.71 (m, 1H, C_4H-3), 4.70 (m, 2H, C_2H-3, C_2H-4), 4.66 (m, 1H, C_4H-4), 4.62 (dd, 1H, J = 3.2, 8.7 Hz, C_4H-1), 4.01 (d, 1H, J = 3.2 Hz, C_3H-3), 4.00 (d, 1H, J = 3.2 Hz, C_3H-1), 3.85 (d, 1H, J = 3.2 Hz, C_3H-2), 3.77 (d, 1H, J = 3.2 Hz, C_3H-4), 3.63 (s, 3H, COOMe), 3.43 (s, 3H, OMe), 3.41 (s, 3H, OMe), 3.37 (s, 3H, OMe), 3.33 (s, 3H, OMe), 3.21 (dd, 1H, J = 3.9, 13.2 Hz, $C_\alpha H_{(pro-S)}$-1), 3.15 (dd, 1H, J = 6.1, 15.5 Hz, $C_\alpha H_{(pro-R)}$-4), 3.09 (dd, 1H, J = 4.5, 13.6 Hz, $C_\alpha H_{(pro-S)}$-3), 2.96 (dd, 1H, J 9.4,13.6 Hz, $C_\alpha H_{(pro-R)}$-3), 2.93 (dd, 1H, J = 7.3, 15.5 Hz, $C_\alpha H_{(pro-S)}$-4), 2.86 (dd, 1H, J = 5.2, 12.9 Hz, $C_\alpha H_{(pro-R)}$-2), 2.70 (dd, 1H, J = 9.7, 13.2 Hz, $C_\alpha H_{(pro-R)}$-4), 2.63 (dd, 1H, J = 4.5, 12.9 Hz, $C_\alpha H_{(pro-S)}$-2), 2.46 (m, 2H, $C_5H_{(pro-S)}$-2, $C_5H_{(pro-R)}$-2), 2.36 (ddd, 1H, J = 4.5, 8.4, 14.1 Hz, $C_5H_{(pro-R)}$-4), 2.27 (ddd, 1H, J = 5.5, 8.4, 14.1 Hz, $C_5H_{(pro-S)}$-4), 1.53 (s, 9H, Boc), 1.52–1.49 (s, 12H, Me), 1.33–1.31 ppm (s, 12H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 172.3, 170.6, 170.1, 16.8, 150.9, 111.6, 111.5, 111.4, 111.3, 105, 104.5, 84.8, 84.1, 83.5, 81.8, 81.4, 81.3, 80.8, 80.7, 79.4, 78.2, 77.7, 58.1, 57.7, 57.5, 51.7, 48.8, 45.8, 44.9, 45.0, 40.1,39.4, 39.3, 38.2, 31.7, 28.4, 26.7, 26.3, 26.2 ppm; HRMS (ESI): *m/z* calculated for $C_{52}H_{84}N_4O_{23}Na$ $[M+Na]^+$ 1155.5446, found 1155.5421.

Boc-(*R*)-β-Caa$_{(x)}$-(*R*)-β-hCaa$_{(x)}$-(*R*)-β-Caa$_{(x)}$-(*R*)-β-hCaa$_{(x)}$-(*R*)-β-Caa$_{(x)}$-(*R*)-β-hCaa$_{(x)}$-OCH$_3$ (12). As described for **27**, a solution of **11** (0.40 g, 0.34 mmol) on reaction with 4 N NaOH in MeOH gave **45** (0.35 g, 89%) as a white solid, which was used as obtained for further reaction.

A mixture of **45** (0.20 g, 0.17 mmol), HOBt (0.03 g, 0.21 mmol), and EDCI (0.04 g, 0.21 mmol) in CH_2Cl_2 (3 mL) was stirred at 0 °C for 15 min and treated with **44** [prepared from **42** (0.112 g, 0.178 mmol) and TFA (0.1 mL) in CH_2Cl_2 (3.0 mL)] and DIPEA (0.07 mL, 0.4 mmol) under nitrogen atmosphere for 8 h. Workup as described for **29** and purification by column chromatography (Silica gel, 2.5% methanol in $CHCl_3$) afforded **12** (0.08 g, 27%) as a white solid: m.p. 120–123 °C; $\alpha_D^{27} = +27.5$ ($c = 0.25$, $CHCl_3$); IR (KBr): $\tilde{\nu} = 3412$, 2939, 1720, 1657, 1546, 1378, 1209, 1101, 1026, 966, 879 cm^{-1}; ^{1}H NMR (500 MHz, Pyridine-d_5): $\delta = 9.25$ (d, 1H, $J = 9.1$ Hz, NH-4), 9.15 (d, 1H, $J = 8.5$ Hz, NH-6), 9.02 (d, 1H, $J = 10.1$ Hz, NH-3), 8.85 (d, 1H, $J = 9.5$ Hz, NH-2), 8.82 (d, 1H, $J = 9.9$ Hz, NH-5), 8.17(d, 1H, $J = 10.1$ Hz, NH-1), 6.16 (d, 1H, $J = 3.9$ Hz, C_1H-1), 6.14 (d, 1H, $J = 3.9$ Hz, C_1H-3), 6.05 (d, 1H, $J = 3.9$ Hz, C_1H-2), 6.02 (d, 1H, $J = 3.9$ Hz, C_1H-6), 5.99 (m, 2H, C_1H-4, C_1H-5), 5.32 (m, 1H, C_βH-3), 5.27 (m, 1H, C_βH-5), 5.08 (m, 1H, C_βH-1), 5.00 (m, 1H, C_βH-2), 4.91 (m, 1H, C_βH-4), 4.87 (m, 1H, C_βH-6), 4.77 (d, 1H, $J = 3.9$ Hz, C_2H-1), 4.73 (d, 1H, $J = 3.9$ Hz, C_2H-2), 4.71 (m, 3H, C_2H-4, C_2H-6, C_4H-4), 4.70 (d, 1H, $J = 3.9$ Hz, C_2H-3), 4.70 (m, 2H, C_4H-3, C_4H-6), 4.69 (m, 2H, C_4H-2, C_4H-5), 4.67 (d, 1H, $J = 3.9$ Hz, C_2H-5), 4.51 (dd, 1H, $J = 3.2$, 8.8 Hz, C_4H-1), 4.03 (d, 1H, $J = 3.2$ Hz, C_3H-3), 4.02 (d, 1H, $J = 3.2$ Hz, C_3H-5), 4.01 (d, 1H, $J = 3.2$ Hz, C_3H-1), 3.90 (d, 1H, $J = 3.2$ Hz, C_3H-4), 3.83 (d, 1H, $J = 3.2$ Hz, C_3H-2), 3.82 (d, 1H, $J = 3.2$ Hz, C_3H-6), 3.65 (s, 3H, COOMe), 3.51 (s, 3H, OMe), 3.49 (s, 3H, OMe), 3.44 (s, 3H, OMe), 3.41 (s, 3H, OMe), 3.39 (s, 3H, OMe), 3.37 (m, 1H, $C_\alpha H_{(pro-S)}$-3), 3.33 (s, 3H, C_3OMe-6), 3.32 (m, 1H, $C_\alpha H_{(pro-S)}$-1), 3.30 (m, 1H, $C_\alpha H_{(pro-R)}$-6), 3.16 (dd, 1H, $J = 3.3$, 13.1 Hz, $C_\alpha H_{(pro-S)}$-5), 3.10 (dd, 1H, $J = 5.2$, 12.1 Hz, $C_\alpha H_{(pro-R)}$-4), 3.05 (dd, 1H, $J = 10.7$, 13.1 Hz, $C_\alpha H_{(pro-R)}$-5), 2.94 (t, 1H, $J = 11.9$ Hz, $C_\alpha H_{(pro-R)}$-3), 2.91 (dd, 1H, $J = 7.8$, 15.5 Hz, $C_\alpha H_{(pro-S)}$-6), 2.86 (dd, 1H, $J = 4.9$, 12.7 Hz, $C_\alpha H_{(pro-R)}$-2), 2.59 (dd, 1H, $J = 3.6$, 12.1 Hz, $C_\alpha H_{(pro-S)}$-4), 2.54 (t, 1H, $J = 11.9$ Hz, $C_\alpha H_{(pro-R)}$-1), 2.46 (dd, 1H, $J = 4.3$, 12.7 Hz, $C_\alpha H_{(pro-S)}$-2), 2.51 (m, 2H, $C_5H_{(pro-S)}$-4, $C_5H_{(pro-R)}$-4), 2.38 (m, 2H, $C_5H_{(pro-S)}$-2, $C_5H_{(pro-R)}$-2), 2.37 (m, 1H, $C_5H_{(pro-S)}$-6), 2.28 (ddd, $J = 5.2$, 8.4, 14.0, Hz 1H, $C_5H_{(pro-R)}$-6), 1.52 (s, 9H, Boc), 1.52–1.50 (s, 18H, Me), 1.34–1.31 ppm (s, 18H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 174.8$, 174.7, 174.2, 173.2, 171.1, 169.8, 156.4, 111.7, 111.6, 114.4, 111.0, 110.9, 105.0, 104.9, 104.8, 104.7, 104.6, 85.7, 84.4, 84.3, 84.1, 83.9, 83.8, 81.8, 81.6, 81.5, 81.2, 81.1, 80.6, 80.4, 80.1, 78.8, 78.6, 78.2, 58.1, 57.8, 57.7, 57.4, 57.3, 52.0, 51.4, 50.1, 49.1, 48.5, 45.9, 45.8, 45.7, 42.1, 41.9, 39.0, 37.8, 29.9, 28.7, 27.3, 27.1, 27.0, 26.9, 26.8, 26.7, 26.6, 26.5, 26.5 ppm; HRMS (ESI): m/z calculated for $C_{75}H_{120}N_6O_{33}Na$ $[M+Na]^+$ 1655.6391, found 1655.6379.

Boc-(*R*)-β-hCaa$_{(x)}$-β-hGly-OCH$_3$ (53). As described for **27**, a solution of **4** (0.55 g, 1.41 mmol) on reaction with 4 N NaOH in MeOH gave **46** (0.51 g, 96%) as a white solid, which was used as obtained for further reaction.

A solution of **46** (0.5 g, 1.33 mmol), HOBt (0.216 g, 1.6 mmol), and EDCI (0.305 g, 1.6 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere for 15 min and treated sequentially with the amine **52** [prepared from **6** (0.269 g, 1.33 mmol), TFA (0.3 mL) in CH_2Cl_2 (3.0 mL) and DIPEA (0.344 mL, 1.99 mol)] and stirred for 8 h. Workup as described for **29** and purification by column chromatography (Silica gel, 50% EtOAc in petroleum ether) gave **53** (0.52 g, 85%) as a white solid: m.p. 90–94 °C; $\alpha_D^{27} = -23.5$ ($c = 0.40$, $CHCl_3$); IR (KBr): $\tilde{\nu} = 3385$, 2920, 2836, 1744, 1695, 1542, 1379, 1162, 1090, 1016, 879.6 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 6.67$ (d, 1H, $J = 7.4$ Hz, NH-2), 5.68 (d, 1H, $J = 8.2$ Hz, NH-1), 4.83 (s, 1H, C_1H-1), 4.82 (s, 1H, C_1H-2), 4.73 (m, 1H, C_δH-2), 4.62 (m, 1H, C_γH-2), 4.47 (m, 2H, C_2H-1, 2), 4.39 (m, 1H, C_βH-2), 4.14 (d, 1H, $J = 3.1$, 7.4 Hz, C_βH-1), 4.07 (m, 1H, C_γH-1), 3.67 (s, 3H, COOMe), 3.62 (m, 1H, C_δH-1), 3.30 (s, 3H, OMe), 3.29 (s, 3H, OMe), 2.72 (m, 1H, $C_\alpha H_{(pro-R)}$-1), 2.70 (m, 1H, $C_\alpha H_{(pro-S)}$-1), 2.67 (dd, 1H, $J = 5.4$, 14.0 Hz, $C_\alpha H_{(pro-R)}$-2), 2.49 (d, 1H, $J = 5.4$, 14.0 Hz, $C_\alpha H_{(pro-S)}$-2), 1.47 (s, 3H, Me), 1.43 (s, 9H, Boc), 1.42 (s, 3H, Me), 1.29 (s, 3H, Me), 1.28 ppm (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.7$, 170.4, 155.5, 111.7, 111.1, 104.7, 104.6, 84.6, 84.3, 81.6, 81.4, 79.2, 77.8, 57.2, 57.6, 51.8, 45.6, 45.4, 41.2, 36.3, 32.4, 28.4, 26.8, 26.7, 26.2 ppm; HRMS (ESI): m/z calculated for $C_{21}H_{36}N_2O_9Na$ $[M+Na]^+$ 488.2326, found 483.2309.

Boc-(*R*)-β-hCaa$_{(x)}$-β-hGly-(*R*)-β-hCaa$_{(x)}$-β-hGly-OCH$_3$ (15). A solution of **53** (0.21 g, 0.45 mmol), as described for **27**, on reaction with 4 N NaOH in MeOH gave **54** (0.20 g, 98%) as a white solid, which was used as obtained for further reaction.

A mixture of acid **54** (0.2 g, 0.45 mmol), HOBt (0.072 g, 0.537 mmol) and EDCI (0.102 g, 0.537 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere for 15 min and treated with the amine salt **55** [prepared from **53** (0.206 g, 0.45 mmol) and TFA (0.2 mL) in CH_2Cl_2 (2.0 mL), DIPEA (0.117 mL, 0.67 mmol)] under nitrogen and stirred for 8 h. Workup as described for **29** and purification by column chromatography (Silica gel, 2.1% MeOH in $CHCl_3$) gave **15** (0.240 g, 68%) as a white solid: m.p. 196–198 °C; $\alpha_D^{27} = +150.9$ ($c = 0.10$, $CHCl_3$); IR (KBr): $\tilde{\nu} = 3343$, 2988, 2936, 1685, 1531, 1520, 1373, 1167, 1076, 888 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.47$ (m, 1H, NH-2), 7.44 (d, 1H, $J = 8.4$ Hz, NH-3), 6.68 (t, 1H, $J = 6.3$ Hz, NH-4), 5.89 (d, 1H, $J = 4.3$ Hz, C_1H-3), 5.85 (d, 1H, $J = 4.3$ Hz, C_1H-1), 5.37 (d, 1H, $J = 9.5$ Hz, NH-1), 4.54 (d, 1H, $J = 4.3$ Hz, C_2H-1), 4.53 (d, 1H, $J = 4.3$ Hz, C_2H-3), 4.32 (m, 1H, C_βH-3), 4.19 (m, 1H, C_βH-1), 4.25 (m, 1H, C_4H-3), 4.23 (m, 1H, C_4H-1), 3.76 (m, 1H, $C_\beta H_{(pro-R)}$-2), 3.70 (s, 3H, COOMe), 3.54 (d, 1H, $J = 3.3$ Hz, C_3H-1), 3.54 (d, 1H, $J = 3.3$ Hz, C_3H-3), 3.22 (m, 1H, $C_\beta H_{(pro-S)}$-2), 3.49 (m, 2H, $C_\beta H_{(pro-R)}$-4, $C_\beta H_{(pro-R)}$-4), 3.39 (s, 3H, OMe), 3.38 (s, 3H, OMe), 2.54 (m, 2H, $C_\alpha H_{(pro-S)}$-4, $C_\alpha H_{(pro-R)}$-4), 2.53 (m, 1H, $C_\alpha H_{(pro-R)}$-1), 2.51 (m, 2H, $C_\alpha H_{(pro-S)}$-3, $C_\alpha H_{(pro-R)}$-3), 2.35 (dd, 1H, $J = 8.4$, 13.7 Hz, $C_\alpha H_{(pro-S)}$-1), 2.24 (m, 2H, $C_\alpha H_{(pro-S)}$-2, $C_\alpha H_{(pro-R)}$-2), 1.89 (m, 2H, $C_5H_{(pro-S)}$-3, $C_5H_{(pro-R)}$-3), 1.87 (m, 1H, $C_5H_{(pro-S)}$-1), 1.82 (ddd, 1H, $J = 4.1$, 8.5, 13.0 Hz, $C_5H_{(pro-R)}$-1), 1.41 (s, 9H, Boc), 1.46–1.31 ppm (s, 12H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 172.9$, 171.6, 171.4, 170.8, 155.8, 111.5, 111.4, 104.7, 404., 104.5, 85.4, 85.1, 81.4, 81.3, 79.3, 77.8, 77.6, 57.7, 57.5, 51.7, 47.2, 46.2, 42.2, 40.8, 36.8, 35.9, 34.9, 33.7, 33.4, 32.3, 28.4, 26.7, 26.3, 26.2 ppm; HRMS (ESI): m/z calculated for $C_{36}H_{60}N_4O_{15}Na$ $[M+Na]^+$ 811.3952, found 811.3934.

Boc-(*R*)-β-hCaa$_{(x)}$-β-hGly-(*R*)-β-hCaa$_{(x)}$-β-hGly-(*R*)-β-hCaa$_{(x)}$-β-hGly-OCH$_3$ (16). A solution of **15** (0.18 g, 0.23 mmol), as described for **27**, on reaction with 4 N NaOH in MeOH gave **56** (0.16 g, 93%) as a white solid, which was used as obtained for further reaction.

A mixture of **56** (0.16 g, 0.206 mmol), HOBt (0.034 g, 0.247 mmol) and EDCI (0.047 g, 0.247 mmol) in CH_2Cl_2 (2 mL)was stirred at 0 °C for 15 min and treated with amine salt **55** [prepared from **53** (0.095 g, 0.206 mmol), TFA (0.1 mL) in CH_2Cl_2 (1.0 mL)] and DIPEA (0.053 mL, 0.31 mmol)] under nitrogen atmosphere for 8 h. Workup as described for **29** and purification by column chromatography (Silica gel, 2.7% methanol in $CHCl_3$) afforded **16** (0.11 g, 48%) as a white solid: m.p. 231–233 °C; $\alpha_D^{27} = +62.5$ ($c = 0.05$, $CHCl_3$ IR (KBr): $\tilde{\nu} = 3322$, 2925, 2855, 1648, 1540, 1375, 1260, 1080, 1024, 884 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.41$ (dd, 1H, $J = 2.9$, 9.9 Hz, NH-2), 8.37 (d, 1H, $J = 8.2$ Hz, NH-5), 8.29 (dd, 1H, $J = 2.8$, 9.6 Hz, NH-4), 8.08 (d, 1H, $J = 9.7$ Hz, NH-3), 6.59 (m, 1H, NH-6), 5.05 (d, 1H, $J = 10.5$ Hz, NH-1), 5.86 (d, 1H, $J = 3.7$ Hz, C_1H-5), 5.81 (d, 1H, $J = 3.7$ Hz, C_1H-3), 5.77 (d, 1H, $J = 3.7$ Hz, C_1H-1), 4.54 (d, 1H, $J = 3.7$ Hz, C_2H-3), 4.52 (d, 1H, $J = 3.7$ Hz, C_2H-5), 4.52 (m, 1H, C_βH-3), 4.49 (d, 1H, $J = 3.7$ Hz, C_2H-1), 4.45 (m, 1H, C_βH-1), 4.40 (m, 1H, C_βH-5), 4.27 (m, 1H, C_4H-5), 4.25 (m, 1H, C_4H-1), 4.24 (m, 1H, C_4H-3), 3.56 (d, 1H, $J = 3.1$ Hz, C_3H-5), 3.54 (d, 1H, $J = 3.1$ Hz, C_3H-1), 3.44 (d, 1H, $J = 3.1$ Hz, C_3H-3), 3.99 (m, 1H, $C_\beta H_{(pro-R)}$-2), 3.91 (m, 1H, $C_\beta H_{(pro-R)}$-4), 3.71 (s, 3H, COOMe), 3.39–3.35 (s, 9H, OMe), 3.56 (m, 1H, $C_\beta H_{(pro-R)}$-6), 3.41 (m, 1H, $C_\beta H_{(pro-S)}$-6), 3.00 (m, 1H, $C_\beta H_{(pro-S)}$-4), 2.89 (m, 1H, $C_\beta H_{(pro-S)}$-2), 2.67 (dd, 1H, $J = 3.0$, 12.5 Hz, $C_\alpha H_{(pro-R)}$-1), 2.63 (dd, 1H, $J = 3.2$, 12.2 Hz, $C_\alpha H_{(pro-R)}$-3), 2.55 (m, 2H, $C_\alpha H_{(pro-S)}$-6, $C_\alpha H_{(pro-R)}$-6), 2.54 (m, 2H, $C_\alpha H_{(pro-S)}$-5, $C_\alpha H_{(pro-R)}$-5), 2.39 (t, 1H, $J = 12.5$ Hz, $C_\alpha H_{(pro-S)}$-3), 2.30 (m, 1H, $C_\alpha H_{(pro-S)}$-4), 2.19 (t, 1H, $J = 12.5$ Hz, $C_\alpha H_{(pro-S)}$-1), 2.16 (m, 1H, $C_\alpha H_{(pro-S)}$-2), 2.09 (m, 1H, $C_\alpha H_{(pro-R)}$-2), 2.08 (m, 1H, $C_\alpha H_{(pro-R)}$-4), 1.94 (m, 1H, $C_5H_{(pro-S)}$-1), 1.91 (m, 1H, $C_5H_{(pro-S)}$-3), 1.90 (m, 2H, $C_5H_{(pro-S)}$-5, $C_5H_{(pro-R)}$-5), 1.82 (m, 1H, $C_5H_{(pro-R)}$-3), 1.64 (ddd, 1H, $J = 2.9$, 9.5, 15.0 Hz, $C_5H_{(pro-R)}$-1), 1.41 (s, 9H, Boc), 1.47 (s, 3H, Me), 1.38 (s, 3H, Me), 1.30 (s, 3H, Me), 1.29 (s, 3H, Me), 1.27 (s, 3H, Me), 1.26 ppm (s, 3H, Me); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 173.3$, 172.1, 171.9, 171.5, 170.6, 156.2, 111.3, 111.2, 104.6, 104.5, 104.5, 84.6,

84.4, 81.4, 81.3, 79.7, 77.6, 57.6, 57.5, 51.8, 46.0, 46.0, 45.0, 43.6, 43.4, 43.1, 38.1, 36.7, 36.6, 36.2, 35.1, 34.6, 34.2, 33.3, 32.9, 31.9, 31.4, 30.2, 29.7, 28.4, 26.6, 26.5, 26.4, 26.2 ppm; HRMS (ESI): *m*/*z* calculated for $C_{51}H_{84}N_6O_{21}Na$ $[M+Na]^+$ 1139.5599, found 1139.5592.

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