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Hybrid Helices: Motifs for Secondary Structure Scaffolds in Foldamers

Gangavaram V. M. Sharma,*,† Nagula Chandramouli,† Madavi Choudhary,‡ Pendem Nagendar,† Kallaganti V. S. Ramakrishna,‡ Ajit C. Kunwar,*,‡ Peter Schramm,§ and Hans-Jörg Hofmann*,§

Organic Chemistry Division III, Indian Institute of Chemical Technology (CSIR), Hyderabad 500 607, India, Centre for Nuclear Magnetic Resonance, Indian Institute of Chemical Technology (CSIR), Hyderabad 500 607, India, and Institute of Biochemistry, Faculty of Biosciences, University of Leipzig, Brüderstrasse 34, D-04103 Leipzig, Germany

Received August 24, 2009; E-mail: esmvee@iict.res.in; kunwar@iict.res.in; hofmann@uni-leipzig.de

Abstract: The concept of "hybrid helices" as a new motif for foldamers is presented. Hybrid helices can be realized by a combination of two or more different types of homologous and hybrid peptides, for example, β-peptides and α/β- and α/γ-hybrid peptides, within the same oligomer. The different helix types of the various peptide foldamer classes are maintained and form a regular helix along the sequence of the oligomer. The transition from one helix type to another was found to be rather smooth with high compatibility of the different helix types. Such hybrid helices represent novel motifs of secondary structure scaffolds. They open up the possibility to change the direction of helix propagation in a subtle manner. Hybrid helices enrich the arsenal of defined foldamer structures for a structural and functional mimicry of native peptides and proteins.

Introduction

The investigation of protein structures has been a very fascinating area of active research, since diverse protein functions are associated with their structures. The endless efforts and contributions resulted in several revelations about designing and engineering of proteins for achieving the required functional features for applications in the area of biology and material science.[1] This quest has prompted the design and generation of various well-defined backbones with unnatural amino acids, using higher homologues of α-amino acids, beginning with the synthesis of β-peptides as oligomers of β-amino acids, by Gellman et al.[2] and Seebach et al.[3] in the mid 1990s. These findings have been the precursor to a wealth of novel secondary structural elements comprising different helix types, strands, and turns in the families of β- and γ-peptides.[1−4] The new classes of "peptide foldamers" were found to be resistant against proteolytic enzymes, thus increasing their stability and bioavailability. Some of them displayed biological functions.[5] Our efforts in this direction with new types of β-amino acids, the C-linked carbo-β-amino acids (β-Caas),[6] led to peptides with right- and left-handed 12/10-helical patterns.[7] More recently, Gellman et al.[8] and Reiser et al.[9] suggested hybrid peptide designs with 1:1 alternation of α- and β-amino acids, which resulted in new helix types of such α/β-hybrid peptides and extended the foldamer portfolio considerably.[10] The α/β-hybrid peptides made from L-Ala/β-Caa and α/β-amino acids with proteinogenic side chains by us resulted in mixed 11/9-helices[11] and three residue turns[12] nucleated by an 11/9-helix. Extension of our activities to other hybrid peptide classes by combining

† Organic Chemistry Division III, Indian Institute of Chemical Technology.
‡ Centre for Nuclear Magnetic Resonance, Indian Institute of Chemical Technology.
§ University of Leipzig.

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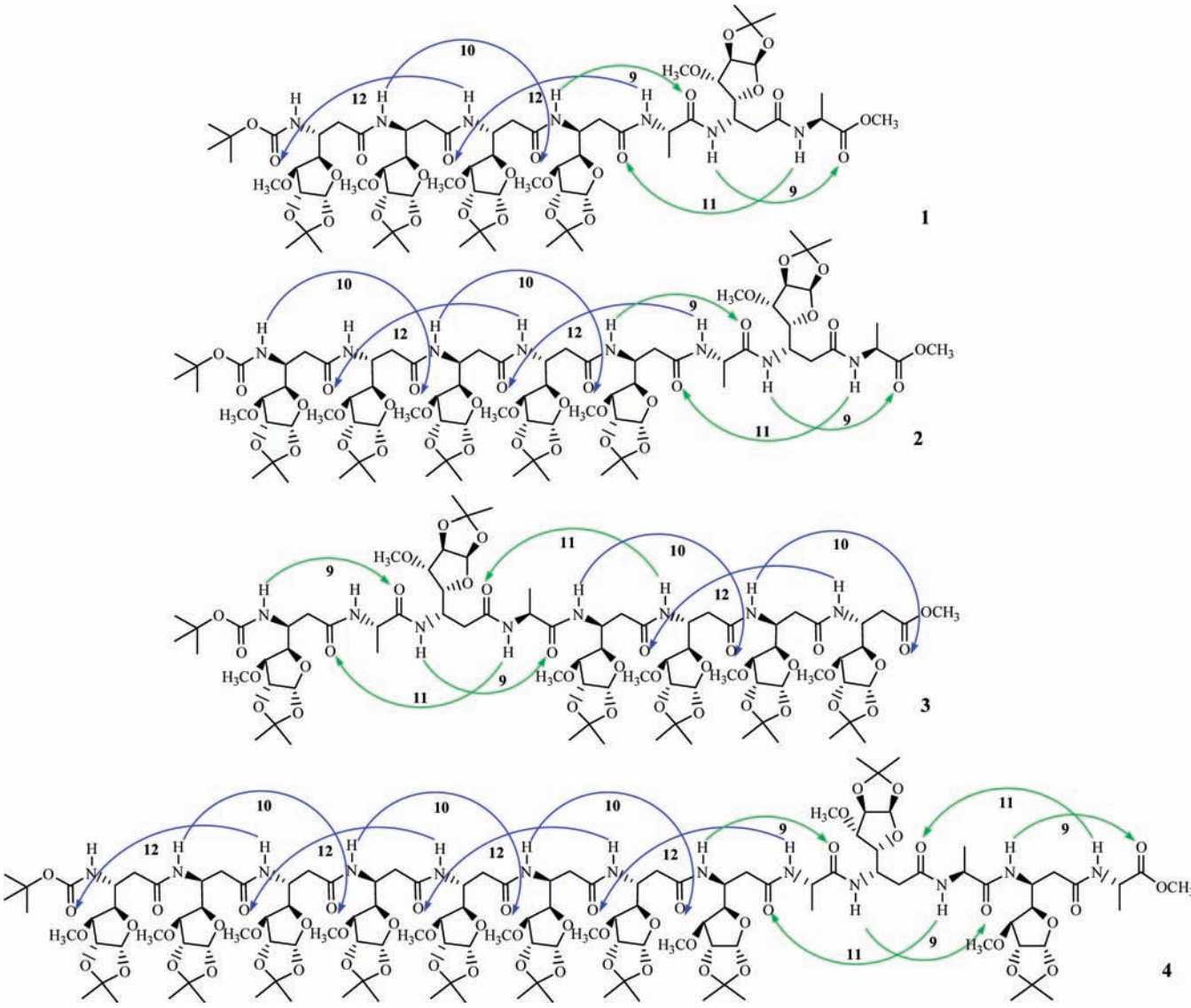

Figure 1. Structures of peptides **1**−**4** (arrows indicate the hydrogen bonds of the preferred mixed helix types).

two other homologous amino acids in 1:1 alternation provided 12/10- (in α/γ-hybrid peptides),[13a,b] 11/13- (in β/γ-hybrid peptides),[13a,b] 13/11- (in α/δ-hybrid peptides),[13c] and 14/12-helices (in α/ε-hybrid peptides).[13d]

In continuation of our interest in accessing new classes of scaffolds having homogeneous or heterogeneous backbones with characteristic folding patterns, similar to the designs on chimeric (α/β+α) peptides for protein surface recognition by Gellman et al.,[5b,g,h,10] we developed the concept of "hybrid helices", using a variety of helices obtained by us in different peptide foldamer families as building blocks. In this report, we present several examples of hybrid helices **1**−**7** (Figures 1 and 2), derived from

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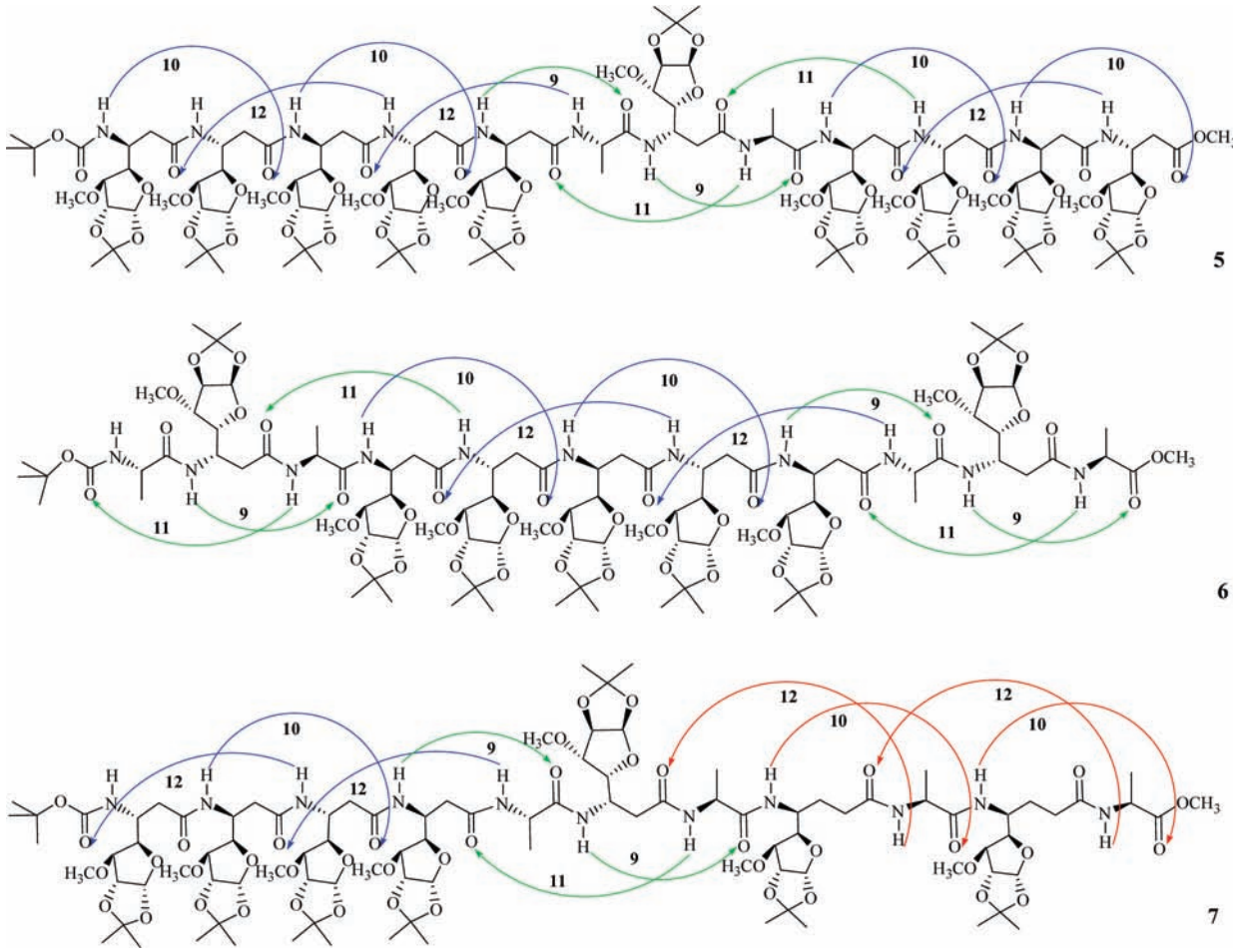

Figure 2. Structures of peptides **5**−**7** (arrows indicate the hydrogen bonds of the preferred mixed helix types).

two or more different backbone types of β-peptides and α/β- and α/γ-hybrid peptide sequences[7,11−13] within a single oligomer.

Results and Discussion

The peptides **1**−**6** combine two different helical types, the 10/12- and 11/9-helices of β-peptides and α/β-hybrid peptides. There are two such helices in the peptides **1**-**4** and three in the peptides **5** and **6**. Peptide **7** also consists of three helices representing three different foldamer classes now, the 12/10-helices of β-peptides and α/γ-hybrid peptides and the 11/9-helix of α/β-hybrid peptides. The short helices were in turn prepared from L-Ala, the β-Caas **8** and **9**, and the γ-Caa **18**, by standard peptide coupling methods.[14] The syntheses (Schemes 1 and 2) are described in detail in Supporting Information[15] and in the Experimental Section. The syntheses of the peptides **1**−**4** (Scheme 1) start from β-Caas **8**, **9**, and peptide **13**.[12] For the syntheses of the peptides **5**−**7** (Scheme 2), besides peptide **3**, peptide **17**[12] and the γ-Caa **18** are necessary.

Structural Studies. The structures of the peptides **1**-**7** were investigated by extensive NMR studies in $CDCl_3$.[15] In peptide **1**, the backbone resonances were well resolved. Low field chemical shifts of the amide protons ($\delta > 7$ ppm), except NH(1), suggested their participation in hydrogen bonding. This was further confirmed by solvent titration studies,[16] where only small changes in their chemical shifts were noticed ($\Delta\delta < 0.42$ ppm) after adding up to 33% DMSO-d_6(v/v). From our earlier studies[7a] the peptides containing β-Caas, with alternating chirality, have been known to take a right-handed 12/10-helical structure. Thus, the first four residues in peptide **1** at the N-terminus are expected to form a 12/10-helix, if the structural perturbation from the following residues is small. The coupling values $^3J_{\mathrm{NH\text{-}C\beta H}} > 9.7$ Hz, $^3J_{\mathrm{C\beta H-C\alpha H}(pro\text{-}R)}$ and $^3J_{\mathrm{C\beta H\text{-}C\alpha H}(pro\text{-}S)} \approx$ 12 and 3 Hz, respectively, for the *R*-residues and about 5 and 3 Hz respectively for the *S*-residues as well as the medium range backbone NOE correlations, like CβH(1)/NH(3) and CβH(1)/CαH *(pro-R)*(3), support the presence of a 12-membered (mr) H-bond between NH(3)-CO(Boc) as the sequential CβH(1)/NH(2) and NH(2)/NH(3) nOes support a 10-mr H-bond between NH(2)−CO(3). All these observations are fully consistent with a right-handed mixed 12/10-helix at the N-terminus in peptide **1**.

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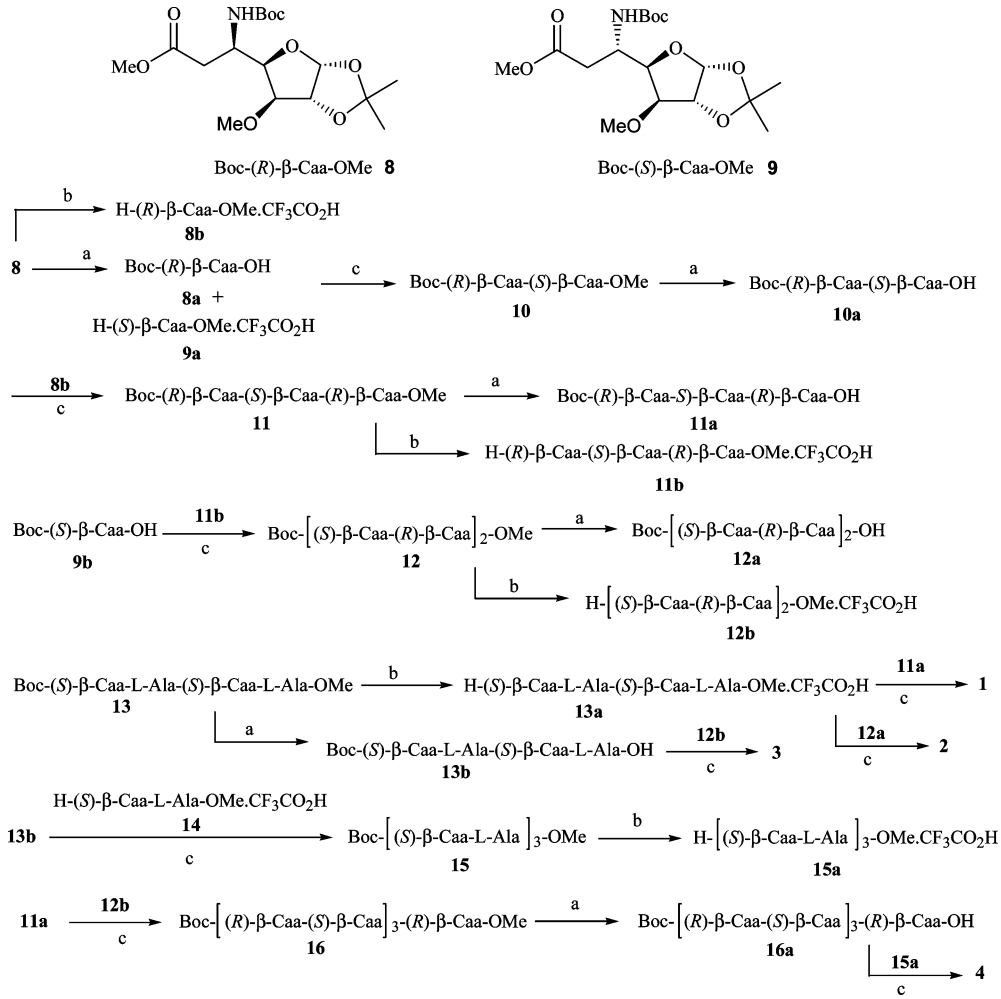
(15) See Supporting information.

(16) Solvent titration studies were carried out by sequentially adding up to 33% of DMSO-d_6 (v/v) to 600 uL $CDCl_3$ solutions of the peptides.

Scheme 1. Synthesis of Peptides **1**−**4**[a]



[a] Reagents and conditions: (a) aq. 4N NaOH, MeOH, 0 °C - rt; (b) CF_3CO_2H, dry CH_2Cl_2; (c) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (1.5 equiv), dry CH_2Cl_2, 0 °C − rt.

As already discussed, peptides with alternating *S*-β-Caa and L-Ala residues generate a right-handed 11/9-helix.[7a] In peptide **1**, we found tell tale signatures of a very well-defined 11/9-helical structure involving the last four residues. The coupling constants $^3J_{NH\text{-}C\beta H} \approx 8.5$ Hz, $^3J_{NH\text{-}C\alpha H}$ ($\alpha-5$ residue) = 4.5 Hz, and $^3J_{C\alpha H-C\beta H} < 5.6$ Hz along with CβH(3)/NH(4), CαH(5)/NH(7) and NH(4)/NH(5), NH(6)/NH(7) nOes are consistent with the presence of an 11-mr hydrogen bond between NH(7)−CO(4) and a 9-mr hydrogen bond between NH(4)−CO(5) and NH(6)−CO(7) in **1**.

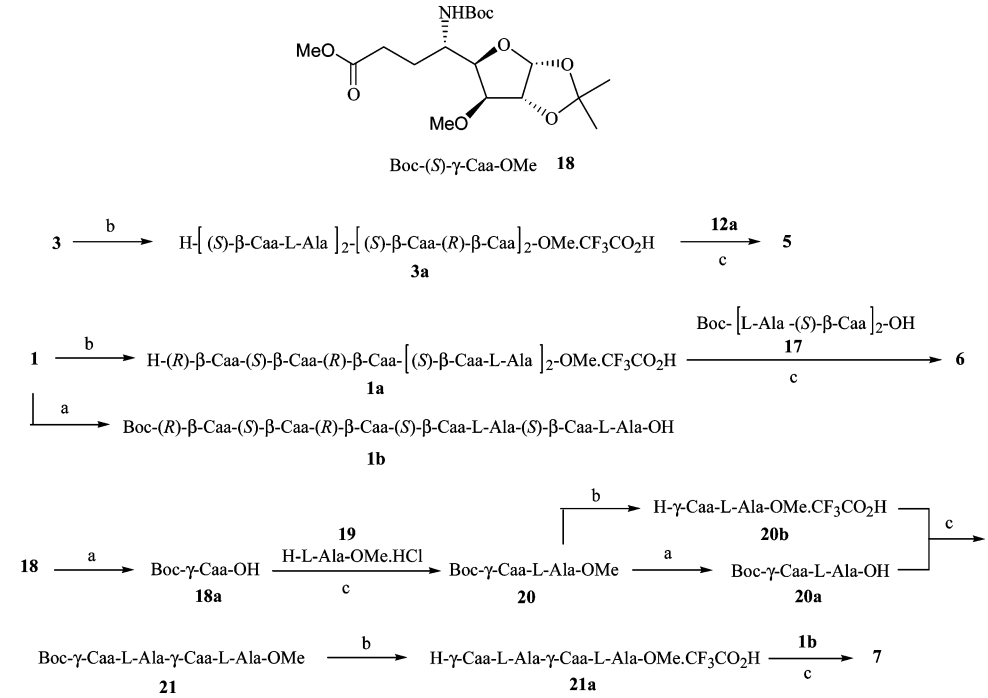
A detailed look at the structure of the peptide **1** shows that the fourth residue is in the "hinge" region of the peptide. It is part of both different helix types, since it can be accommodated in a 12/10-helix at the N-terminus as well as in an 11/9-helix at the C-terminus. A very distinct NOE [CβH(3)/NH(5)] connecting the two helices and the presence of a NH(5)-CO(2) H-bond show that NH(5) is also part of the N-terminus 12/10-helix. Thus, the hybrid helix has a 12/10/12/9/11/9-hydrogen bond sequence (alternate NH(i+3)−CO(i) and NH(i+2)−CO(i+3) hydrogen bonds). Obviously, the two helix types are well accommodated within the oligomer with negligible deviations in the hinge region.

As already mentioned, Gellman et al.[5b,g,h,10] have elegantly designed similar classes of peptide ($\alpha/\beta+\alpha$) families in the development of very potent and novel foldamer-based inhibitors of protein−protein interactions. In such peptides, the 14/15-helical fold was observed to nucleate[5b] a helix in the short α-peptide moiety.

The details on the conformational analyses for peptides **2** and **3** are discussed in the Supporting Information. Similar to peptide **1**, both peptides form stable hybrid helices. In peptide **2**, the first four residues form a 12/10-helix, while the fifth residue is part of both the 12/10-helix at the N-terminus and the 11/9-helix formed by the residues at the C-terminus. As expected, peptide **3** begins with an 11/9-helix of the first four residues, which is connected by the 12/10-helix of the remaining residues at the C-terminus.

The solvent titration studies[16] of the tridecamer **4**, which is the largest peptide in the present study, indicate the participation of all the amide protons, except NH(1), with $\Delta\delta < 0.57$ ppm, in H-bonding. The NOE correlations CβH(1)/NH(3), CβH(1)/CαH(*pro-R*)(3), NH(2)/NH(3), CβH(3)/NH(5), CβH(3)/CαH(*pro-R*)(5), NH(4)/NH(5), CβH(5)/NH(7), CβH(5)/CαH(*pro-R*)(7), NH(6)/NH(7) (Figure 3) and the coupling constant $^3J_{NH\text{-}C\beta H} > 8.3$ Hz (for all β-residues) support a mixed 12/10-helix involving the first seven residues. Though we could obtain all but one $^3J_{C\beta H\text{-}C\alpha H(pro\text{-}S)}$ (<3 Hz), only a single $^3J_{C\beta H\text{-}C\alpha H(pro\text{-}R)}$ with a value of 12.8 Hz for the fifth residue could be derived due to spectral overlap. The couplings involving the last six residues, $^3J_{NH\text{-}C\beta H} = 9.2$ Hz [for β-Caa(8)], $^3J_{NH\text{-}C\beta H} = 9.8$ Hz [for β-Caa(10)], $^3J_{NH\text{-}C\beta H} = 9.2$ Hz [for β-Caa(12)], $^3J_{NH\text{-}C\alpha H} = 4.3$ Hz

Scheme 2. Synthesis of Peptides **5**−**7**[a]



[a] Reagents and conditions: (a) aq. 4N NaOH, MeOH, 0 °C - rt; (b) CF_3CO_2H, dry CH_2Cl_2; (c) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (1.5 equiv), dry CH_2Cl_2, 0 °C − rt.

[L-Ala(9)], $^3J_{NH\text{-}C\alpha H} = 5.2$ Hz [L-Ala(11)], $^3J_{NH\text{-}C\alpha H} = 7.9$ Hz [L-Ala(13)] and the small values of $^3J_{C\alpha H-C\beta H}$ (due to overlaps not all the couplings could be obtained) and the nOes NH(8)/NH(9), CαH(9)/NH(11), NH(10)/NH(11), CαH(11)/NH(13), and NH(12)/NH(13) confirm an 11/9-helix at the C-terminus. In the hinge region of the two helix types, the presence of the NOE correlation CβH(7)/NH(9) supports the continuity of the helical pattern as observed for peptides **1**-**3**.

Fifteen superimposed structures of **4** obtained from restrained MD studies are shown in Figure 4a. The backbone and heavy atom root-mean-square deviations are 0.57 Å and 1.13 Å, respectively. In Figure 5a, the helical structure of peptide **4** is illustrated as result of a quantum chemical geometry optimization at the HF/6-31G* level of ab initio MO theory. The backbone torsion angles are given in the Supporting Information. The high degree of regularity of the hybrid helix along the sequence of the oligomer accommodating the two different helix types without problems can well be seen.

The ^{1}H NMR spectrum of the dodecamer **5** in $CDCl_3$ showed very well dispersed signals from the backbone protons. All amide protons, except NH(2), participate in hydrogen bonding as suggested by their low field δ and small Δδ in solvent titration studies (Δδ < 0.43 ppm).[16] For the first eight residues the structure is very similar to that derived for peptide **2**. The first five residues show all the nOes expected for a 12/10-helix and the residues 5 to 9 display those defining the 11/9-helix, such as CβH(2)/NH(4), CβH(2)/CαH(*pro-R*)(4), NH(3)/NH(4), NH(5)/NH(6), CαH(6)/NH(8), and NH(7)/NH(8), including the NOE, CβH(4)/NH(6) in the hinge region (Figure 6). The nOes expected for a 12/10-helix involving the last four residues, like NH(9)/NH(10), CβH(10)/NH(12), CβH(10)/CαH(*pro-R*)(12), and NH(11)/NH(12), were also obtained (Figure 6). The presence of the nOes CαH(8)/NH(10) and CαH(8)/CαH(*pro-R*)(10) shows again that the transition from the 11/9- to the 12/10-helix is rather smooth. Only few $^3J_{C\alpha H-C\beta H}$ couplings could be obtained due to extensive overlaps, but all the couplings $^3J_{NH\text{-}C\beta H}$ (>9.2 Hz) and $^3J_{NH\text{-}C\alpha H}$ (~5 Hz) were observed.

In Figure 5b, the optimized structure of peptide **5** can be seen with the 10/12-helices of the terminal β-peptide parts and the central 9/11-helix of the α/β-hybrid peptide. It is interesting to compare this helix with a regular mixed β-peptide helix with alternating 10- and 12-membered hydrogen-bonded rings. Figure 7a shows a superimposition of the hybrid helix of peptide **5** with an optimized mixed 10/12-helix of a β-peptide sequence with the same number of amino acid constituents. Most interesting is that both helices have practically the same length.

Obviously, the poly proline II conformation of the alanine residue in the 9/11-helix of the α/β-hybrid peptides with average backbone torsion angles of $\varphi \approx -59°$ and $\psi \approx 146°$ replaces rather perfectly the second β-amino acid constituent in the mixed 10/12-helix of β-peptides in peptide **5**. The backbone torsion angles of the second β-amino acid constituents in β-peptides and the β-amino acid constituent in α/β-hybrid peptides have anyway nearly the same values. The average backbone torsion angle values for the two β-amino acid residues in the 10/12-helical parts of peptide **5** are $\varphi = 87.7°$, $\theta = 66.1°$, and $\psi = -106.2°$ and $\varphi = -97.5°$, $\theta = 61.8°$, and $\psi = 87.8°$, respectively. The average values for the β-amino acid constituent of the 9/11-helix in the α/β-hybrid peptide part are $\varphi = 85.2°$, $\theta = 62.2°$, and $\psi = -101.2°$.

The NMR data on peptide **6**[15] in $CDCl_3$ show again the smooth switch between both helix types. They are discussed in detail in the Supporting Information. The optimized structure is given in Figure 5c and shows the regularity of the hybrid helix. As in the case of peptide **5**, an overlay of the hybrid helix of **6** with a 12/10-helix of the β-peptides demonstrates the similarity of the mixed and hybrid helices (Figure 7b). This time a β-peptide 12/10-helix has to be selected for the superimposition since the hybrid helix of **6** has the 11/9-helix parts at the termini and not in the central part of the sequence as in **5**.

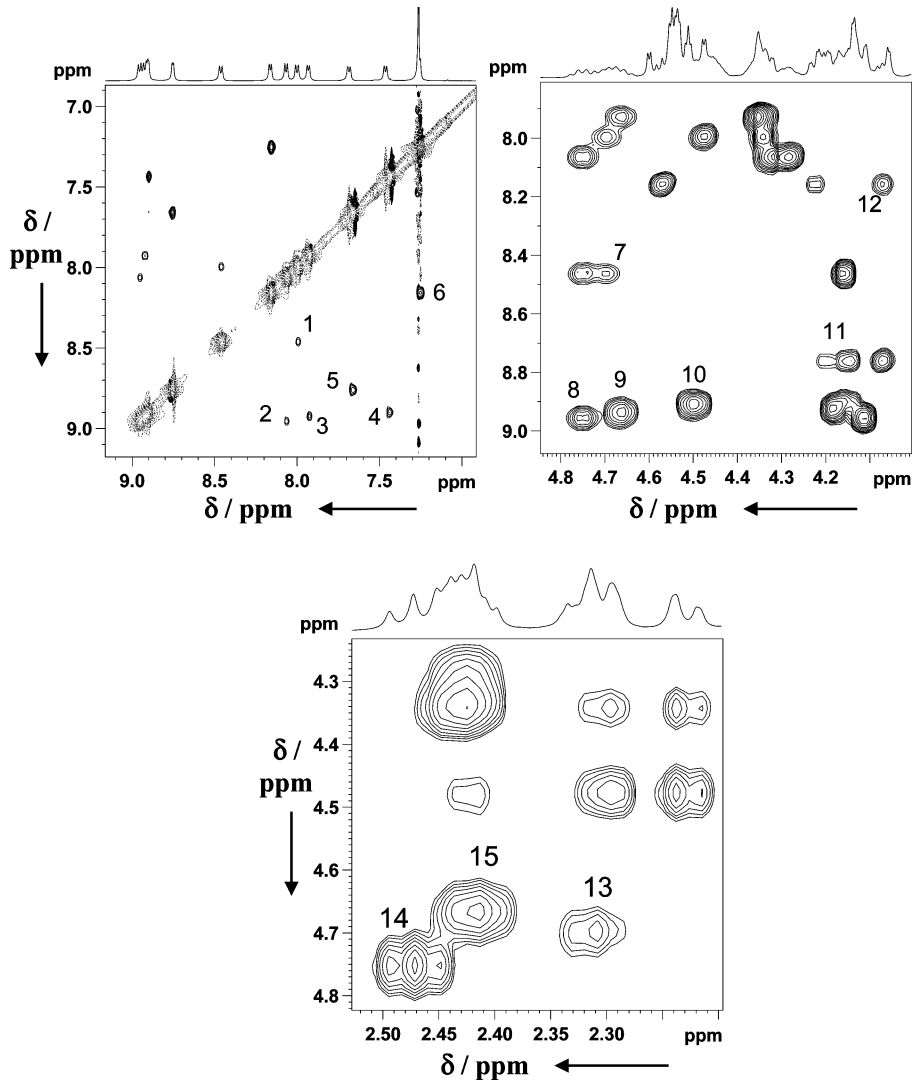


Figure 3. ROESY spectrum (in $CDCl_3$) of **4** (The nOes NH(2)/NH(3), NH(4)/NH(5), NH(6)/NH(7), NH(8)/NH(9), NH(10)/NH(11), NH(12)/NH(13), CβH(1)/NH(3), CβH(3)/NH(5), CβH(5)/NH(7), CβH(7)/NH(9), CαH(9)/NH(11), CαH(11)/NH(13), CβH(1)/CαH(*pro-R*)(3), CβH(3)/CαH(*pro-R*)(5), and CβH(5)/CαH(*pro-R*)(7) are marked as 1−15.).

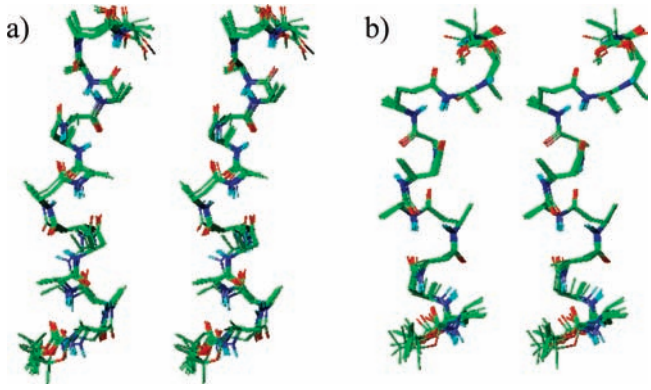


Figure 4. Stereoview of 15 superimposed minimum energy structures for (a) peptide **4** and (b) peptide **7** from restrained MD studies (The sugar moieties were replaced by methyl groups after the calculations for clarity.).

Peptide **7** combines three different peptide backbones, namely β-peptide, α/β-, and α/γ-hybrid peptide sequences. The conformational analysis demonstrates that the three different helix types can be realized in a hybrid helix with a continuous propagation along the sequence of the oligomer. All amide protons, except NH(1) and NH(10), participate in hydrogen bonding, as suggested by the low $\Delta\delta$ values (<0.50 ppm) in the solvent titration studies[16] and also by their low field δ values. The first seven residues in **7** are identical to those in peptide **1**. All the nOes required for a compound helix, as noticed in **1**, with 12/10- and 11/9-helical pattern, such as CβH(1)/NH(3), CβH(1)/CαH(*pro-R*)(3), NH(2)/NH(3), CβH(3)/NH(5), NH(4)/NH(5), CαH(5)/NH(7), and NH(6)/NH(7), were observed again. For the last four residues of the γ/α-peptide fragment, the corresponding 12/10-helical pattern is adequately characterized by CαH(7)/NH(9), NH(8)/NH(9), CαH(9)/NH(11), and NH(10)/NH(11) NOE correlations. Additional support for the helix comes from the nOes C_4H(6)/CαH(*pro-R*)(8) and C_4H(8)/CαH(*pro-R*)(10), involving the sugar protons. Because of spectral overlap, the only couplings that could be obtained involving the backbone protons were those with the amide protons [$^3J_{\text{NH-C}\beta\text{H}}$ (>8.8 Hz), $^3J_{\text{NH-C}\gamma\text{H}}$ (>9.0), and $^3J_{\text{NH-C}\alpha\text{H}} = 4.5$ and 7.2 Hz; only two such couplings could be obtained]. The 11/9-helix again smoothly changes into the 12/10-helix of the alternating γ- and α-residues. The NH(10), which resonated at $\delta = 6.85$ ppm having a $\Delta\delta$ of 1.07 ppm, does not appear to be

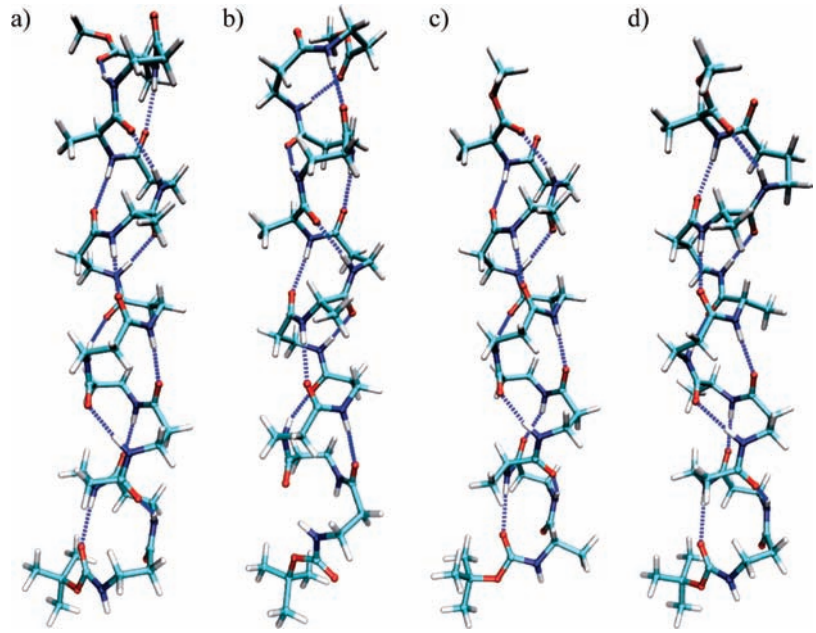


Figure 5. Hybrid helices for the peptides (a) **4**, (b) **5**, (c) **6**, and (d) **7** according to complete geometry optimization at the HF/6-31G* level of ab initio MO theory (Sugar side chains were omitted for clarity.).

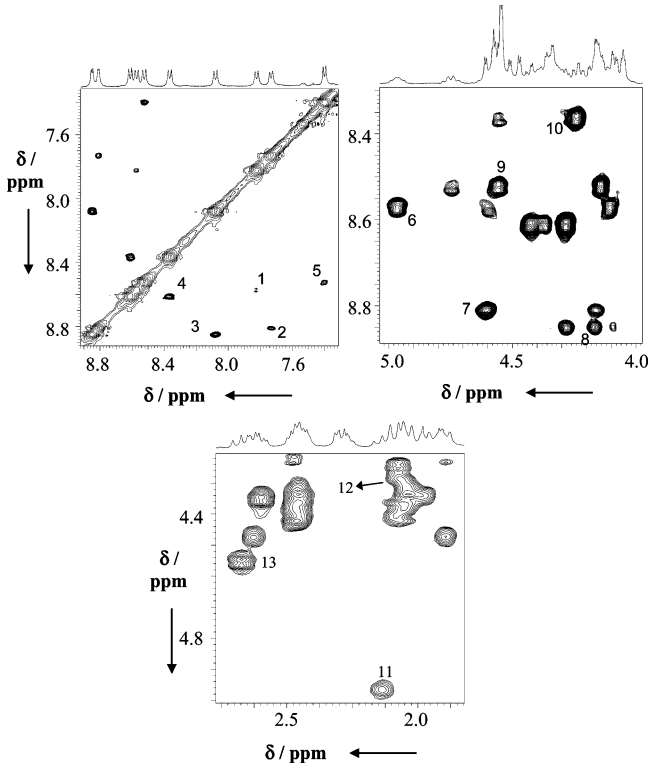


Figure 6. ROESY spectrum of **5** in $CDCl_3$ (The nOes NH(3)/NH(4), NH(5)/NH(6), NH(7)/NH(8), NH(9)/NH(10), NH(11)/NH(12), CβH(2)/NH(4), CβH(4)/NH(6), CαH(6)/NH(8), CαH(8)/NH(10), and CβH(10)/NH(12), CβH(2)/CαH(*pro-R*)(4), CβH(8)/CαH(*pro-R*)(10), and CβH(10)/CαH(*pro-R*)(12) are marked as 1−13).

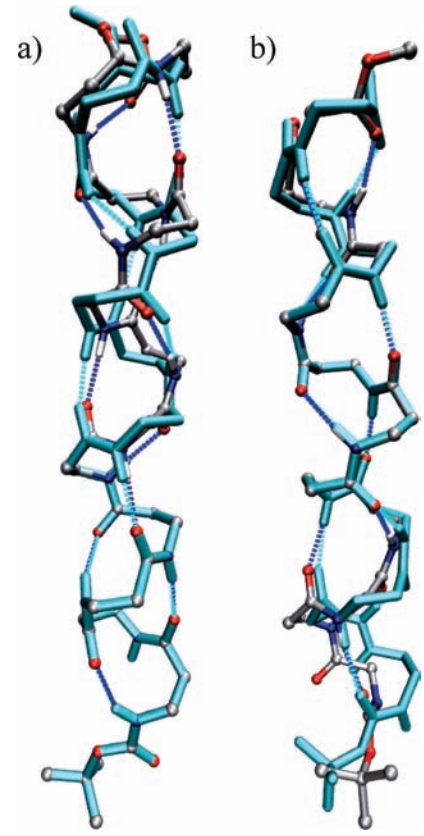


Figure 7. Superimpositions of (a) the hybrid helix of peptide **5** and a mixed 10/12-helix of a β-peptide with the same number of amino acid constituents; (b) the hybrid helix of peptide **6** with a 12/10-helix of a β-peptide with the same number of amino acid constituents (β-peptide in cyan; side-chains and hydrogen atoms were omitted for clarity).

hydrogen-bonded due to fraying at the C-terminus, a situation also observed in our study on the tetramers of α/γ-hybrid peptides.[13a]

In Figure 4b, fifteen superimposed structures of **7** obtained from restrained MD studies are shown. The backbone and heavy atom root-mean-square deviations are 0.77 Å and 1.03 Å, respectively. In Figure 5d, the structure of **7** is shown as obtained from geometry optimization at the HF/6-31G* level of ab initio MO theory. It can well be seen that a regular helix extends throughout the oligomer and connects the three different mixed

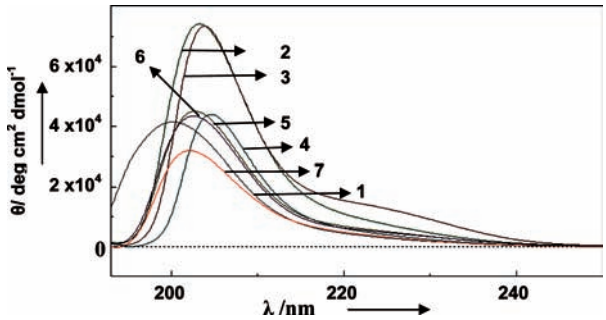


Figure 8. CD spectra of the peptides **1**−**7** in MeOH.

helix types. In comparison to the other hybrid helices, there is a more distinct kink in the hinge region between the α/β- and α/γ-parts (∼15−20°). Such "kinks" impart subtle variations in the direction of helix propagation.[17]

The CD spectra (in MeOH) for **1**−**7** are presented in the Figure 8. They display a maximum around 200−207 nm, a feature common to all the individual helical components, that is, a 10/12-helix[7] from β-, an 11/9-helix[11a] from α/β-, and a 12/10-helix[13a] from α/γ-hybrid peptides. All these molecules have a 10/12-helix from the β-peptide family. The other feature expected for an 11/9-helix, a broad shoulder at around 220−225 nm, is rather subdued and visible only in **3**. A look at the published literature[7,11a] brings out the fact that the molecular ellipticities of 11/9-fragments are much smaller (by about a factor of 5) than those of the 10/12-helix from the β-peptide family, which might be responsible for the mild presence of 11/9-helical features in the CD spectra. Obviously, the features of 12/10-helix seem to dominate in all the peptides.

Conclusions

In this work, we successfully presented the concept of "hybrid helices" as novel motifs of secondary structural scaffolds. Such motifs can be obtained by a combination of two or more different types of homologous and hybrid peptides with structural and conformational diversity, resulting in well-defined helical patterns. The transition from one helix type to another was found to be rather smooth with high compatibility of the various helix types due to the availability of very stable and robust mixed helices. Hybrid helices open up the possibility to change the direction of the helix propagation in a subtle manner enabling to control the orientation of the side chains. The availability of such new motifs, in combination with other peptide foldamers, may help in attaining a functional mimicry of natural proteins.

Experimental Section

General. NMR spectra (1D and 2D experiments) for peptides **1**−**7** were obtained in $CDCl_3$ at 500 and 600 MHz (^1H), and at 100 and 150 MHz (^{13}C). Chemical shifts are reported in δ scale with respect to internal TMS reference. IR spectra were recorded with a FT-IR spectrometer in between 400−4000 cm^{-1} in KBr pellets. Melting points were determined in open capillaries and were not corrected. The CD spectra were obtained with a spectropolarimeter using rectangular fused quartz cells of 0.2 cm path length in 200 μM methanol solutions. The binomial method was used to smooth the spectra. The values are expressed in terms of $[\theta]$, the total molar ellipticity (deg·cm^2·$dmol^{-1}$) per residue. Restraint molecular dynamics (MD) studies were carried out using the INSIGHT-II Discover module. The constraints were derived from the volume integrals obtained from the ROESY spectra using a two-spin approximation and a 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%.

Boc-(*R*)-β-Caa-(*S*)-β-Caa-OCH$_3$ (10). A cooled (0 °C) solution of **8** (0.21 g, 0.56 mmol) in methanol (2.5 mL) was treated with aq. 4 N NaOH solution (2.5 mL) and stirred at room temperature. After 2 h, methanol was removed and adjusted at a pH of 2−3 with aq. 1 N HCl solution at 0 °C and extracted with ethyl acetate (2 × 10 mL). The organic layers were dried (Na_2SO_4) and concentrated to give **8a** (0.2 g, 99%) as a white solid, which was used as such for the next step.

A solution of **8a** (0.2 g, 0.55 mmol), HOBt (0.09 g, 0.66 mmol) and EDCI (0.13 g, 0.66 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C for 15 min and treated with **9a** prepared by Boc deprotection of **9**[12] and DIPEA (0.14 mL, 0.83 mmol) under N_2 atmosphere for 8 h. The reaction mixture was quenched at 0 °C with sat. NH_4Cl solution (10 mL). After 10 min, the reaction mixture was diluted with $CHCl_3$ (10 mL), washed with 1N HCl (10 mL), aq. sat. $NaHCO_3$ solution (10 mL), and brine (10 mL). The organic layer was dried (Na_2SO_4), evaporated, and the residue purified by column chromatography (Silica gel, 55% ethyl acetate in pet. ether) to afford **10** (0.31 g) in 88% yield as a white solid; mp 157−160 °C; $[\alpha]_D$ = −40.8 (*c* 0.49, $CHCl_3$); IR (KBr): ν 3335, 3269, 2991, 2938, 1730, 1700, 1650, 1526, 1167, 1074, 1013 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.48 (d, 1H, *J* = 8.3 Hz, NH-2), 5.91 (d, 1H, *J* = 3.9 Hz, C_1H), 5.87 (d, 1H, *J* = 3.9 Hz, C_1H), 5.68 (d, 1H, *J* = 8.7 Hz, NH-1), 4.59 (m, 1H, $C_\beta H$), 4.55 (d, 1H, *J* = 3.9 Hz, C_2H), 4.53 (d, 1H, *J* = 3.9 Hz, C_2H), 4.37 (m, 1H, C_4H), 4.26 (m, 1H, $C_\beta H$), 4.25 (m, 1H, C_4H), 3.74 (d, 1H, *J* = 3.3 Hz, C_3H), 3.73 (d, 1H, *J* = 3.3 Hz, C_3H), 3.67 (s, 3H, COOMe), 3.40 (s, 3H, OMe), 3.38 (s, 3H, OMe), 2.71 (dd, 1H, *J* = 6.4, 14.6 Hz, $C_\alpha H'$), 2.62 (dd, 1H, *J* = 5.9, 14.6, $C_\alpha H$), 2.57 (dd, 1H, *J* = 5.2, 15.3 Hz, 1H, $C_\alpha H'$), 2.50 (dd, 1H, *J* = 4.2, 15.3 Hz, $C_\alpha H$), 1.48 (s, 3H, Me), 1.46 (s, 3H, Me), 1.43 (s, 9H, Boc), 1.31 (s, 3H, Me), 1.30 (s, 3H, Me); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 171.7, 170.4, 155.3, 111.7 (2C), 104.9, 104.7, 84.5, 83.9 (2C), 81.4 (2C), 80.0, 79.0, 57.8, 57.6, 51.7, 46.1, 45.6, 37.9, 36.2, 28.3 (3C), 26.8, 26.7, 26.3, 26.2; HRMS (ESI): *m/z* calculated for $C_{28}H_{46}N_2O_{13}$ $(M + H)^+$ 619.3101, found 619.3120.

Boc-(*R*)-β-Caa-(*S*)-β-Caa-(*R*)-β-Caa-OCH$_3$ (11). As described for the synthesis of **8a**, a solution of **10** (0.2 g, 0.31 mmol) gave **10a** (0.19 g, 96%) as a white solid, which was used as such for the next step.

A solution of **8** (0.11 g, 0.30 mmol) and TFA (0.2 mL) in CH_2Cl_2 (1 mL) was stirred at room temperature for 1 h. After completion of the reaction, the solvent was evaporated under reduced pressure and the resulting **8b** was dried under high vacuum and used as such for the next step.

As described for the synthesis of **10**, a mixture of **10a** (0.19 g, 0.30 mmol), HOBt (0.05 g, 0.36 mmol), and EDCI (0.07 g, 0.36 mmol) in CH_2Cl_2 (3 mL) was stirred at 0 °C for 15 min and treated with the above amine and DIPEA (0.08 mL, 0.45 mmol) under N_2 atmosphere for 8 h. Workup and purification by column chromatography (Silica gel, 90% ethyl acetate in pet. ether) afforded **11** (0.2 g) in 75% yield as a white solid; mp 108−111 °C; $[\alpha]_D$ = −5.1 (*c* 0.45, $CHCl_3$); IR (KBr): ν 3364, 3308, 2996, 2940, 1717, 1670, 1542, 1371, 1164, 1077, 1010 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.08 (d, 1H, *J* = 9.5 Hz, NH-3), 7.19 (d, 1H, *J* = 9.6 Hz, NH-2), 5.89 (d, 1H, *J* = 3.9 Hz, C_1H-3), 5.89 (d, 1H, *J* = 3.9 Hz, C_1H-2), 5.85 (d, 1H, *J* = 4.0 Hz, C_1H-1), 5.26 (d, 1H, *J* = 10.5 Hz, NH-1), 4.69 (m, 1H, $C_\beta H$-3), 4.57 (d, 1H, *J* = 3.9 Hz, C_2H-2), 4.53 (d, 1H, *J* = 3.9 Hz, C_2H-3), 4.53 (m, 1H, $C_\beta H$-1), 4.52 (d, 1H, *J* = 4.0 Hz, C_2H-1), 4.50 (m, 1H, $C_\beta H$-2), 4.30 (dd, 1H, *J* = 3.2, 9.8 Hz, C_4H-2), 4.17 (dd, 1H, *J* = 3.1, 9.7 Hz, C_4H-3), 4.13 (d, 1H, *J* = 3.0 Hz, C_3H-2), 3.99 (dd, 1H, *J* = 3.1, 7.3 Hz, C_4H-1),

(17) Brown, J. H.; Kim, K.-H.; Jun, G.; Greenfield, N. J.; Dominguez, R.; Volkmann, N.; Hitchcock-DeGregori, S. E.; Cohen, C. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8496–8501.

3.71 (d, 1H, J = 3.1 Hz, C_3H-1), 3.70 (d, 1H, J = 3.1 Hz, C_3H-3), 3.66 (s, 3H, COOMe), 3.39 (s, 3H, OMe), 3.38 (s, 3H, OMe), 3.36 (s, 3H, OMe), 2.85 (dd, 1H, J = 3.0, 13.0 Hz, $C_\alpha H_{(pro\text{-}S)}$-3), 2.78 (dd, 1H, J = 2.7, 12.9 Hz, $C_\alpha H_{(pro\text{-}S)}$-1), 2.53 (dd, 1H, J = 10.7, 13.0 Hz, $C_\alpha H_{(pro\text{-}R)}$-3), 2.34 (dd, 1H, J = 4.9, 13.1 Hz, $C_\alpha H_{(pro\text{-}R)}$-2), 2.22 (dd, 1H, J = 3.3, 13.1 Hz, $C_\alpha H_{(pro\text{-}S)}$-2), 2.16 (dd, 1H, J = 12.4, 12.9 Hz, $C_\alpha H_{(pro\text{-}R)}$-1), 1.44 (s, 6H, 2× Me), 1.43 (s, 9H, Boc), 1.39 (s, 3H, Me), 1.31 (s, 3H, Me), 1.30 (s, 3H, Me), 1.29 (s, 3H, Me); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 173.5, 169.6, 169.0, 156.1, 111.4, 111.3, 111.2, 104.9 (2C), 104.8, 84.2, 83.4, 83.3, 81.7, 81.5, 81.3, 81.0, 80.8, 80.4, 79.7, 58.2, 57.6, 57.2, 52.1, 48.4, 46.2, 45.5, 40.8, 38.3, 37.5, 28.4 (3C), 26.8, 26.7, 26.6, 26.3, 26.2, 26.1; HRMS (ESI): m/z calculated for $C_{39}H_{63}N_3O_{18}$ $(M + H)^+$ 862.4221, found 862.4219.

Boc-(*S*)-β-Caa-(*R*)-β-Caa-(*S*)-β-Caa-(*R*)-β-Caa-OCH$_3$ (12). As described for the synthesis of **10**, a mixture of **9b** (prepared by base hydrolysis of ester **9**[12] (0.1 g, 0.27 mmol), HOBt (0.04 g, 0.33 mmol), EDCI (0.06 g, 0.33 mmol) in CH_2Cl_2 (3 mL) was stirred at 0 °C for 15 min and treated with **11b** [prepared from **11** (0.24 g, 0.27 mmol) and TFA (0.3 mL) in CH_2Cl_2 (2 mL)] and DIPEA (0.07 mL, 0.41 mmol) under N_2 atmosphere for 8 h. Workup and purification by column chromatography (Silica gel, 1.8% methanol in $CHCl_3$) afforded **12** (0.57 g) in 76% yield as a white solid; mp 124−127 °C; $[\alpha]_D$ = −15.2 (c 0.3, $CHCl_3$); IR (KBr): ν 3366, 3286, 2987, 2936, 1718, 1655, 1541, 1374, 1211, 1166, 1081, 1021 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.23 (d, 1H, J = 8.2 Hz, NH-4), 7.12 (d, 1H, J = 9.1 Hz, NH-3), 6.83 (d, 1H, J = 10.0 Hz, NH-2), 5.99 (d, 1H, J = 8.9 Hz, NH-1), 5.89 (d, 1H, J = 4.0 Hz, C_1H-1), 5.88 (d, 1H, J = 3.7 Hz, C_1H-2), 5.87 (d, 1H, J = 3.7 Hz, C_1H-3), 5.87 (d, 1H, J = 4.0 Hz, C_1H-4), 4.82 (m, 1H, C_βH-2), 4.64 (m, 1H, C_βH-4), 4.57 (d, 1H, J = 4.0 Hz, C_2H-1), 4.55 (d, 1H, J = 3.7 Hz, C_2H-2), 4.53 (d, 1H, J = 3.7 Hz, C_2H-3), 4.51 (d, 1H, J = 4.0 Hz, C_2H-4), 4.41 (m, 1H, C_βH-3), 4.35 (dd, 1H, J = 3.4, 8.8 Hz, C_4H-1), 4.28 (dd, 1H, J = 3.1, 9.9 Hz, C_4H-3), 4.14 (m, 1H, C_βH-1), 4.12 (dd, 1H, J = 2.8, 9.4 Hz, C_4H-4), 4.10 (dd, 1H, J = 3.4, 6.0 Hz, C_4H-2), 4.09 (d, 1H, J = 3.1 Hz, C_3H-3), 3.80 (d, 1H, J = 2.9 Hz, C_3H-4), 3.78 (d, 1H, J = 3.4 Hz, C_3H-1), 3.73 (d, 1H, J = 3.4 Hz, C_3H-2), 3.66 (s, 3H, COOMe), 3.41 (s, 3H, OMe), 3.37 (s, 3H, OMe), 3.37 (s, 3H, OMe), 3.36 (s, 3H, OMe), 2.83 (dd, 1H, J = 3.2, 12.9 Hz, $C_\alpha H_{(pro\text{-}S)}$-4), 2.67 (dd, 1H, J = 2.3, 12.7 Hz, $C_\alpha H_{(pro\text{-}S)}$-2), 2.50 (dd, 1H, J = 9.7, 12.9 Hz, $C_\alpha H_{(pro\text{-}R)}$-4), 2.44 (dd, 1H, J = 12.8, 13.0 Hz, $C_\alpha H_{(pro\text{-}S)}$-3), 2.43 (dd, 1H, J = 4.1, 12.8 Hz, $C_\alpha H_{(pro\text{-}S)}$-1), 2.41 (dd, 1H, J = 5.2, 12.8 Hz, $C_\alpha H_{(pro\text{-}R)}$-1), 2.18 (dd, 1H, J = 3.6, 13.0 Hz, $C_\alpha H_{(pro\text{-}R)}$-3), 2.13 (dd, 1H, J = 10.9, 12.7 Hz, $C_\alpha H_{(pro\text{-}R)}$-2), 1.49 (s, 3H, Me), 1.44 (s, 3H, Me), 1.44 (s, 9H, Boc), 1.43 (s, 3H, Me), 1.30 (s, 3H, Me), 1.30 (s, 3H, Me), 1.30 (s, 3H, Me), 1.29 (s, 3H, Me), 1.26 (s, 3H, Me); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 173.5, 170.1, 169.5, 169.0, 156.6, 111.4, 111.3 (2C), 111.1, 104.9, 104.8, 104.7 (2C), 84.2, 83.4, 83.3, 83.1, 81.5, 81.4, 81.3, 81.0 (2C), 80.8, 80.5, 80.4, 79.7, 79.6, 58.2, 57.6, 57.2, 57.1, 52.1, 48.4, 46.2, 45.5, 40.8, 38.3, 38.2, 37.5, 28.4 (3C), 26.8, 26.7, 26.6, 26.5, 26.3, 26.2 (2C), 26.1; HRMS (ESI): m/z calculated for $C_{50}H_{80}N_4O_{23}$ (M^+ + H) 1104.5372, found 1104.5351.

Boc-(*R*)-β-Caa-(*S*)-β-Caa-(*R*)-β-Caa-(*S*)-β-Caa-L-Ala-(*S*)-β-Caa-L-Ala-OCH$_3$ (1). As described for the synthesis of **8a**, a solution of **11** (0.4 g, 0.45 mmol) gave **11a** (0.25 g, 63%) as a white solid, which was used as such in the next step.

As described for the synthesis of **10**, a mixture of **11a** (0.21 g, 0.25 mmol), HOBt (0.05 g, 0.30 mmol), EDCI (0.06 g, 0.30 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C for 15 min and treated with **13a** [prepared from **13**[12] (0.19 g, 0.25 mmol) and TFA (0.3 mL) in CH_2Cl_2 (0.2 mL)] and DIPEA (0.07 mL, 0.37 mmol) under N_2 atmosphere for 8 h. Workup and purification by column chromatography (Silica gel, 3.0% methanol in $CHCl_3$) afforded **1** (0.12 g, 31%) as a white solid; mp 150−152 °C; $[\alpha]_D$ = +49.8 (c 0.1, $CHCl_3$); IR (KBr): ν 3428, 3076, 2986, 2937, 2831, 1720, 1649, 1546, 1451, 1375, 1299, 1218, 1165, 1119, 1081, 1022, 888, 854, 640, 550 cm^{-1}; ^{1}H NMR ($CDCl_3$, 288 K, 600 MHz): δ 8.98 (d, 1H, J = 4.8 Hz, NH-5), 8.42 (d, 1H, J = 9.9 Hz, NH-3), 8.26 (d, 1H, J = 7.9 Hz, NH-7), 8.03 (d, 1H, J = 9.8 Hz, NH-2), 7.46 (d, 1H, J = 8.8 Hz, NH-4), 7.01 (d, 1H, J = 8.5 Hz, NH-6), 5.95 (d, 1H, J = 3.7 Hz, C_1H) 5.89−5.86 (m, 3H, C_1H), 5.81 (d, 1H, J = 3.7 Hz, C_1H), 5.32 (d, 1H, J = 10.5 Hz, NH-1), 4.66 (m, 1H, C_βH-1), 4.58−4.53 (m, 3H, C_2H), 4.58 (m, 1H, C_βH-3), 4.57 (m, 1H, C_αH-7) 4.51 (m, 1H, C_βH-2), 4.50 (d, 1H, J = 4.0 Hz, C_2H), 4.48 (d, 1H, J = 3.8 Hz, C_2H), 4.39 (m, 1H, C_βH-6), 4.37 (d, 1H, J = 3.3, 9.6 Hz, C_4H-2), 4.35 (m, 1H, C_βH-4), 4.23 (m, 1H, C_4H-6), 4.22 (m, 1H, C_4H-4), 4.19 (m, 1H, C_4H-4), 4.07 (d, 1H, J = 2.8 Hz, C_3H-2), 4.05 (d, 1H, J = 3.4 Hz, C_3H-6), 4.03 (m, 1H, C_αH-5), 4.03 (dd, 1H, J = 3.9, 8.0 Hz, C_4H-1), 4.03 (d, 1H, J = 3.4 Hz, C_3H-4), 3.72 (s, 3H, COOMe), 3.67 (m, 2H, C_3H-1, 3), 3.38 (s, 3H, OMe), 3.37 (s, 3H, OMe), 3.35 (s, 3H, OMe), 3.32 (s, 3H, OMe), 3.23 (s, 3H, OMe), 2.89 (dd, 1H, J = 2.7, 12.9 Hz, $C_\alpha H_{(pro\text{-}S)}$-3), 2.83 (dd, 1H, J = 2.9, 12.6 Hz, $C_\alpha H_{(pro\text{-}S)}$-1), 2.64 (dd, 1H, J = 5.5, 12.9 Hz, $C_\alpha H_{(pro\text{-}R)}$-6), 2.62 (dd, 1H, J = 4.8, 12.6 Hz, $C_\alpha H_{(pro\text{-}R)}$-4), 2.36 (dd, 1H, J = 5.7, 13.0 Hz, $C_\alpha H_{(pro\text{-}R)}$-2), 2.30 (t, 1H, J = 12.7 Hz $C_\alpha H_{(pro\text{-}R)}$-3), 2.23 (dd, 1H, J = 2.7, 13.0 Hz, $C_\alpha H_{(pro\text{-}S)}$-2), 2.19 (dd, 1H, J = 2.8, 12.9 Hz, $C_\alpha H_{(pro\text{-}S)}$-6), 2.10 (t, 1H, J = 12.4 Hz, $C_\alpha H_{(pro\text{-}R)}$-1), 2.06 (dd, 1H, J = 3.1, 12.6 Hz, $C_\alpha H_{(pro\text{-}S)}$-4), 1.49 (s, 3H, Me), 1.47 (s, 6H, Me, CH_3-5), 1.45 (m, 6H, CH_3-7, Me), 1.43 (s, 12H, Boc, Me), 1.36 (s, 3H, Me), 1.31 (s, 3H, Me), 1.30 (s, 6H, 2× Me), 1.28 (s, 6H, 2× Me); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 175.3, 174.0, 171.3 (2C), 170.9, 170.1, 170.0, 156.3, 111.7, 111.5, 111.4 (2C), 111.0, 105.0, 104.9 (2C), 104.8, 104.7, 84.0, 83.5, 83.3, 82.9 (2C), 81.4, 81.1 (2C), 81.0 (3C), 80.4 (2C), 80.3, 79.8, 79.7, 57.4, 57.3, 57.2 (2C), 57.1, 52.5, 52.0, 48.5, 48.4, 47.4, 46.8, 46.7, 46.5, 41.0, 40.8, 38.2, 36.6 (2C), 28.3 (3C), 27.1, 26.6 (3C), 26.3 (2C), 26.2 (3C), 25.9, 16.1, 16.0; HRMS (ESI): m/z calculated for $C_{67}H_{107}N_7O_{30}$ $(M + Na)^+$ 1512.6955, found 1512.7027.

Boc-(*R*)-β-Caa-(*S*)-β-Caa-(*R*)-β-Caa-(*S*)-β-Caa-(*R*)-β-Caa-(*S*)-β-Caa-(*R*)-β-Caa-(*S*)-β-Caa-L-Ala-(*S*)-β-Caa-L-Ala-(*S*)-β-Caa-L-Ala-OCH$_3$ (4). As described for the synthesis of **8a**, a solution of **16** (0.05 g, 0.03 mmol) gave **16a** (0.05 g, 95%) as a white solid, which was used as such in the next step.

As described for the synthesis of **10**, a mixture of **16a** (0.05 g, 0.027 mmol), HOBt (0.005 g, 0.03 mmol), EDCI (0.007 g, 0.03 mmol) in CH_2Cl_2 (3 mL) was stirred at 0 °C for 15 min and treated with **15a** [prepared from **15** (0.03 g, 0.03 mmol) and TFA (0.06 mL) in CH_2Cl_2 (0.5 mL)] and DIPEA (0.007 mL, 0.04 mmol) under N_2 atmosphere for 8 h. Workup and purification by column chromatography (Silica gel, 4.5% methanol in $CHCl_3$) afforded **4** (0.04 g, 52%) as a white solid; mp 238−241 °C; $[\alpha]_D$ = +60.8 (c 0.05, $CHCl_3$); IR (KBr): ν 3427, 3086, 2986, 2930, 2851, 1736, 1647, 1560, 1546, 1535, 1460, 1452, 1375, 1216, 1165, 1120, 1081, 1023, 889, 854, 637, 553 cm^{-1}; ^{1}H NMR ($CDCl_3$, 288 K, 600 MHz): δ 8.96 (d, 1H, J = 9.5 Hz, NH-5), 8.92 (d, 1H, J = 9.5 Hz, NH-7), 8.90 (d, 1H, J = 4.3 Hz, NH-9), 8.77 (d, 1H, J = 5.2 Hz, NH-11), 8.46 (d, 1H, J = 9.9 Hz, NH-3), 8.15 (d, 1H, J = 7.9 Hz, NH-13), 8.06 (d, 1H, J = 9.2 Hz, NH-4), 7.99 (d, 1H, J = 10.0 Hz, NH-2), 7.92 (d, 1H, J = 8.3 Hz, NH-6), 7.64 (d, 1H, J = 9.8 Hz, NH-10), 7.41 (d, 1H, J = 9.2 Hz, NH-8), 7.25 (d, 1H, J = 9.2 Hz, NH-12), 5.91−5.88 (m, 6H, C_1H), 5.86 (d, 1H, J = 3.1 Hz, C_1H), 5.84 (d, 1H, J = 3.8 Hz, C_1H), 5.83 (d, 1H, J = 3.5 Hz, C_1H), 5.81 (d, 1H, J = 3.4 Hz, C_1H), 5.22 (d, 1H, J = 10.6 Hz, NH-1), 4.75 (m, 1H, C_βH-3), 4.70 (m, 1H, C_βH-1), 4.67 (m, 1H, C_βH-5), 4.59 (d, 1H, J = 3.8 Hz, C_2H), 4.56 (m, 1H, C_αH-13), 4.55−4.50 (m, 9H, C_2H), 4.50 (m, 1H, C_βH-7), 4.48 (m, 1H, C_βH-2), 4.46 (m, 1H, C_βH-10), 4.45 (m, 1H, C_βH-12), 4.38 (m, 1H, C_4H-6), 4.36 (m, 1H, C_βH-8), 4.34 (m, 2H, C_4H-2, C_βH-6), 4.33 (m, 1H, C_4H-4), 4.28 (m, 1H, C_βH-4), 4.23 (m, 1H, C_4H-10), 4.21 (m, 1H, C_4H-12), 4.20 (m, 1H, C_4H-8), 4.18 (m, 1H, C_4H-7), 4.17 (m, 1H, C_αH-9), 4.16 (m, 1H, C_4H-3), 4.14 (m, 1H, C_3H-8), 4.13 (m, 2H, C_3H-4, C_3H-6), 4.11 (m, 1H, C_4H-5), 4.10 (m, 1H, C_3H-2), 4.07 (m, 1H, C_αH-11), 4.05 (m, 1H, C_3H-12), 3.99 (m, 1H, C_3H-10), 3.98 (m, 1H, C_4H-1), 3.74 (d, 1H, J = 3.5 Hz, C_3H-7), 3.73 (s, 3H, COOMe), 3.65 (d, 2H, J = 3.7, C_3H-1,5), 3.59 (d,

1H, $J = 3.0$, C_3H-3), 3.40 (s, 6H, 2×OMe), 3.36 (s, 3H, OMe), 3.35 (s, 6H, 2×OMe), 3.34 (s, 3H, OMe), 3.33 (s, 3H, OMe), 3.32 (s, 3H, OMe), 3.29 (s, 3H, OMe), 3.26 (s, 3H, OMe), 2.97 (dd, 1H, $J = 2.2$, 12.5 Hz, $C_\alpha H_{(pro\text{-}S)}$-3), 2.89 (dd, 1H, $J = 2.8$, 12.9 Hz, $C_\alpha H_{(pro\text{-}S)}$-1), 2.82 (dd, 1H, $J = 2.5$, 12.9 Hz, $C_\alpha H_{(pro\text{-}S)}$-5), 2.77 (dd, 1H, $J = 2.9$, 12.8 Hz, $C_\alpha H_{(pro\text{-}S)}$-7), 2.65 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-10), 2.64 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-12), 2.62 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-8), 2.48 (t, 1H, $J = 12.8$ Hz, $C_\alpha H_{(pro\text{-}R)}$-5), 2.43 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-6), 2.42 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-4), 2.41 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-7), 2.31 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-3), 2.29 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-2), 2.23 (dd, 1H, $J = 2.8$, 12.9 Hz, $C_\alpha H_{(pro\text{-}S)}$-2), 2.16 (dd, 1H, $J = 2.4$, 10.6 Hz, $C_\alpha H_{(pro\text{-}S)}$-4), 2.14 (m, 1H, $C_\alpha H_{(pro\text{-}S)}$-12), 2.12 (m, 1H, $C_\alpha H_{(pro\text{-}S)}$-6), 2.08 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-1), 2.07 (m, 1H, $C_\alpha H_{(pro\text{-}S)}$-8), 2.05 (m, 1H, $C_\alpha H_{(pro\text{-}S)}$-10), 1.51 (s, 3H, Me), 1.48 (s, 6H, Me), 1.47 (s, 3H, Me), 1.46 (m, 6H, Me, CH_3-9), 1.45 (s, 6H, 2×Me), 1.44 (m, 3H, CH_3-13), 1.44 (s, 3H, Me), 1.42 (m, 15H, Boc, CH_3-11, Me), 1.35 (s, 3H, Me), 1.34 (s, 6H, 2×Me), 1.31 (s, 6H, 2×Me), 1.30 (s, 6H, 2×Me), 1.28 (s, 3H, Me), 1.27 (s, 6H, 2×Me), 1.25 (s, 3H, Me); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 175.6, 174.8, 174.5, 172.2, 171.8 (2C), 171.2, 170.8, 170.7, 170.4, 170.2 (2C), 169.8, 156.5, 112.0, 111.7, 111.6 (2C), 111.5 (3C), 110.8, 110.4 (2C), 105.5, 105.2 (2C), 105.0 (5C), 104.8 (2C), 84.3 (2C), 83.5 (3C), 83.4 (5C), 82.8, 82.0 (2C), 81.5 (3C), 81.4 (3C), 81.3 (4C), 80.8 (4C), 80.6, 80.4, 79.8, 79.7, 57.3 (2C), 57.2 (3C), 57.1 (2C), 57.0 (3C), 52.4 (3C), 52.2, 48.6 (2C), 47.6, 47.3 (3C), 47.1 (2C), 46.7, 46.5, 41.2, 40.8, 40.6, 40.2, 38.3, 38.1 (3C), 37.9, 36.7, 28.5 (3C), 27.6 (2C), 27.4, 26.8 (2C), 26.7 (2C), 26.6 (2C), 26.5 (2C), 26.4 (3C), 26.3, 26.2 (2C), 26.1 (2C), 26.0, 16.5, 16.1, 14.1; HRMS (ESI): *m/z* calculated for $C_{125}H_{199}N_{13}O_{56}$ $(M + 2H)^+$ 1389.1556, found 1389.1552.

Boc-(*S*)-β-Caa-(*R*)-β-Caa-(*S*)-β-Caa-(*R*)-β-Caa-(*S*)-β-Caa-L-Ala-(*S*)-β-Caa-L-Ala-(*S*)-β-Caa-(*R*)-β-Caa-(*S*)-β-Caa-(*R*)-β-Caa-OCH$_3$ (5). As described for the synthesis of **8a**, a solution of **12** (0.05 g, 0.04 mmol) gave **12a** (0.05 g, 99%) as a white solid, which was used as such in the next step.

As described for the synthesis of **10**, a mixture of **12a** (0.03 g, 0.03 mmol), HOBt (0.004 g, 0.03 mmol), EDCI (0.006 g, 0.03 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C for 15 min and treated with **3a** [prepared from **3** (0.045 g, 0.03 mmol) and TFA (0.06 mL) in CH_2Cl_2 (0.5 mL)] and DIPEA (0.006 mL, 0.03 mmol) under N_2 atmosphere for 8 h. Workup and purification by column chromatography (Silica gel, 4.5% methanol in $CHCl_3$) afforded **5** (0.03 g, 37%) as a white solid; mp 215−217 °C; $[\alpha]_D = +69.3$ (*c* 0.05, $CHCl_3$); IR (KBr): ν 3421, 3081, 2987, 2852, 1726, 1647, 1559, 1534, 1459, 1446, 1376, 1215, 1160, 1081, 1021, 888, 856, 638, 521 cm^{-1}; ^{1}H NMR ($CDCl_3$, 288 K, 600 MHz): δ 8.85 (d, 1H, $J = 5.5$ Hz, NH-8), 8.81 (d, 1H, $J = 4.6$ Hz, NH-6), 8.61 (d, 1H, $J = 9.2$ Hz, NH-9), 8.57 (d, 1H, $J = 9.6$ Hz, NH-4), 8.53 (d, 1H, $J = 9.6$ Hz, NH-12), 8.37 (d, 1H, $J = 9.9$ Hz, NH-10), 8.08 (d, 1H, $J = 9.7$ Hz, NH-7), 7.83 (d, 1H, $J = 9.7$ Hz, NH-3), 7.74 (d, 1H, $J = 9.2$ Hz, NH-5), 7.40 (d, 1H, $J = 8.5$ Hz, NH-11), 6.69 (d, 1H, $J = 10.5$ Hz, NH-2), 6.27 (d, 1H, $J = 9.7$ Hz, NH-1), 5.91−5.86 (m, 7H, C_1H), 5.85 (d, 1H, $J = 3.4$ Hz, C_1H), 5.80 (d, 1H, $J = 3.8$ Hz, C_1H), 5.76 (d, 1H, $J = 3.8$ Hz, C_1H), 4.97 (m, 1H, C_βH-2), 4.75 (m, 1H, C_βH-12), 4.61 (m, 1H, C_βH-4), 4.61 (d, 1H, $J = 3.6$ Hz, C_2H), 4.59−4.53 (m, 7H, C_2H), 4.56 (m, 1H, C_βH-10), 4.51 (d, 1H, $J = 3.4$ Hz, C_2H), 4.47 (d, 1H, $J = 3.4$ Hz, C_2H), 4.47 (m, 1H, C_βH-7), 4.41 (m, 1H, C_4H-12, C_βH-9), 4.40 (m, 1H, C_4H-3), 4.39 (m, 1H, C_βH-9), 4.38 (m, 1H, C_βH-3), 4.36 (m, 1H, C_4H-11), 4.34 (m, 2H, C_4H-1, C_βH-5), 4.33 (m, 1H, C_βH-11), 4.28 (m, 1H, C_αH-8), 4.24 (m, 1H, C_4H-10), 4.23 (m, 1H, C_4H-7), 4.18 (m, 1H, C_4H-5), 4.17 (m, 1H, C_3H-11), 4.16 (m, 1H, C_αH-6), 4.15 (m, 2H, C_4H-2, C_βH-1), 4.11 (m, 1H, C_4H-4), 4.09 (m, 1H, C_3H-5), 4.08 (d, 1H, $J = 3.2$ Hz, C_3H-9), 4.06 (d, 1H, $J = 3.9$ Hz, C_3H-3), 4.05 (d, 1H, $J = 3.9$ Hz, C_3H-7), 3.86 (d, 1H, $J = 3.6$ Hz, C_3H-1), 3.81 (d, 1H, $J = 3.3$ Hz, C_3H-10), 3.71 (m, 1H, C_3H-12), 3.71 (m, 1H, C_3H-4), 3.70 (m, 1H, C_3H-2), 3.65 (s, 3H, COOMe), 3.40 (s, 9H, 3×OMe), 3.39 (s, 3H, OMe), 3.37 (s, 6H, 2×OMe), 3.35 (s, 9H, 3×OMe), 3.25 (s, 3H, OMe), 2.91 (dd, 1H, $J = 2.3$, 12.7 Hz, $C_\alpha H_{(pro\text{-}S)}$-10), 2.85 (dd, 1H, $J = 3.5$, 13.4, Hz, $C_\alpha H_{(pro\text{-}S)}$-4), 2.78 (dd, 1H, $J = 3.5$, 13.4 Hz, $C_\alpha H_{(pro\text{-}S)}$-2), 2.77 (m, 1H, $C_\alpha H_{(pro\text{-}S)}$-12), 2.65 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-12), 2.60 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-7), 2.58 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-5), 2.48 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-9), 2.47 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-11), 2.46 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-3), 2.44 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-1), 2.35 (dd, 1H, $J = 7.5$, 14.8 Hz, $C_\alpha H_{(pro\text{-}S)}$-1), 2.21 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-4), 2.17 (m, 1H, $C_\alpha H_{(pro\text{-}S)}$-3), 2.16 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-10), 2.14 (m, 1H, $C_\alpha H_{(pro\text{-}S)}$-9), 2.13 (m, 1H, $C_\alpha H_{(pro\text{-}S)}$-11), 2.08 (m, 1H, $C_\alpha H_{(pro\text{-}S)}$-5), 2.04 (m, 1H, $C_\alpha H_{(pro\text{-}R)}$-2), 2.01 (m, 1H, $C_\alpha H_{(pro\text{-}S)}$-7), 1.53 (s, 3H, Me), 1.49 (s, 3H, Me), 1.47 (s, 3H, Me), 1.46 (m, 3H, CH_3-6), 1.45 (s, 3H, Me), 1.45 (s, 9H, Boc), 1.43 (s, 6H, 2×Me), 1.42 (s, 6H, 2×Me), 1.41 (m, 9H, CH_3-8, 2×Me), 1.36 (s, 3H, Me), 1.32 (s, 3H, Me), 1.30 (s, 6H, 2×Me), 1.29 (s, 6H, 2×Me), 1.28 (s, 6H, 2×Me), 1.27 (s, 6H, 2×Me); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 175.6, 175.0, 173.5, 172.3, 171.6, 171.4, 171.2, 171.1, 170.2 (2C), 170.1, 169.1, 156.0, 111.9, 111.7, 111.6 (2C), 111.5, 111.4, 111.3 (2C), 111.1, 110.7, 105.1, 105.0, 104.9 (5C), 104.8, 104.7, 104.6, 83.6 (2C), 83.3, 83.2, 83.1 (3C), 83.0, 82.9, 82.7, 81.6, 81.3 (4C), 81.2 (4C), 81.1 (2C), 80.9 (2C), 80.8, 80.7, 80.5, 80.4, 80.3 (2C), 79.0, 78.8, 58.2, 57.5, 57.4 (2C), 57.3, 57.2 (2C), 57.1, 57.0, 56.9, 52.2 (2C), 52.0, 48.5, 48.3, 48.0, 47.5, 47.2, 47.1, 46.8 (3C), 46.6, 41.4, 41.2, 40.0, 38.7 (2C), 38.1, 38.0, 37.8, 36.9, 36.6, 28.4 (3C), 27.3, 27.1, 26.9 (2C), 26.8 (2C), 26.7 (2C), 26.5, 26.4 (2C), 26.3 (5C), 26.2 (3C), 26.1, 16.6, 16.0; HRMS (ESI): *m/z* calculated for $C_{122}H_{194}N_{12}O_{55}$ $(M + 2H)^{2+}$ 1353.6371, found 1353.6368.

Theoretical Calculations. The quantum chemical calculations were performed employing the Gaussian03 program package.[18] Details of the calculations and the backbone torsion angles of the hybrid helices are given in the Supporting Information.

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Supporting Information Available: Complete ref 18, NMR/CD spectra, details of the MD calculations, and ab initio MO data for stabilities and torsion angles of the higher helix conformers at various approximation levels. Experimental details for the compounds **15**, **16**, **20**, **21**, **2**, **3**, **6**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Frisch, M. J.; et al. *Gaussian 03*, Revision C.02; Gaussian Inc.: Wallingford, CT, 2004.