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Design of a "New Motif" with β-Amino Acids and α-Aminoxy Acids: Synthesis of Hybrid Peptides with 12/10-Helix

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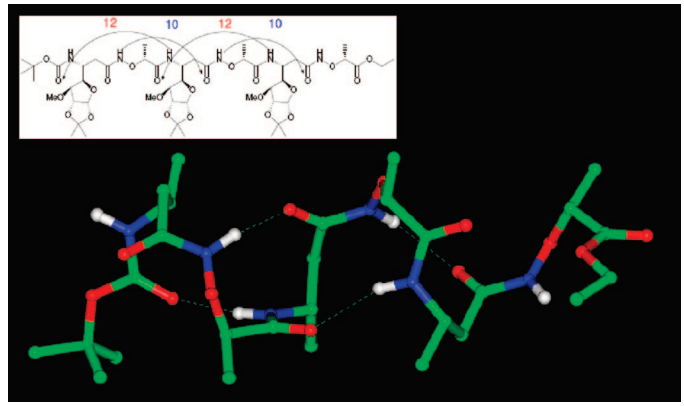

Hybrid peptides are prepared from a C-linked carbo-β-amino acid ester (*R*-β-Caa) and an α-aminoxy acid (*R*-Ama) derived from *S*-lactic acid. Extensive NMR (in $CDCl_3$ solution), CD, and MD studies on the tetra- and hexapeptides led to identification of robust 12/10-mixed helices. The dipeptide repeat having an *R*-β-Caa and an *R*-Ama thus provides a "new motif" to realize a 12/10-mixed helix, for the first time, in oligomers containing *R*-Ama. To understand the impact of side chains in the mixed helix formation, *R*-β-Caa/Ama (with no substitution in Ama) and *S*-β-hAla/*R*-Ama oligomers were investigated. NMR studies revealed the existence of 12/10-helices in these hybrid peptides, and the side chains of monomers were found to have a profound influence on their stabilities. These observations imply that the propensity of β-amino acid to prefer a mixed 12/10-helix governs the structural behavior in these peptides. The structural consequences of the lone-pair repulsion between nitrogen and oxygen atoms result in a new and interesting structural motif which behaves like "pseudo" β^3,β^2-peptides in generating 12/10-mixed helices.

Introduction

The functions of proteins are associated with their compact three-dimensional structures, which in turn are derived from the secondary and tertiary structures. Rapid progress has been made to understand the protein structures and their functions, thus resulting in many synthetic peptides with conformations similar to the natural ones.[1] The recent past witnessed intense activity for the design and synthesis of non-natural peptides[2] with well-defined secondary structures, from β-, γ- and δ- amino acids.[2–4] Gellman et al.[5] and Seebach et al.[6] first reported the

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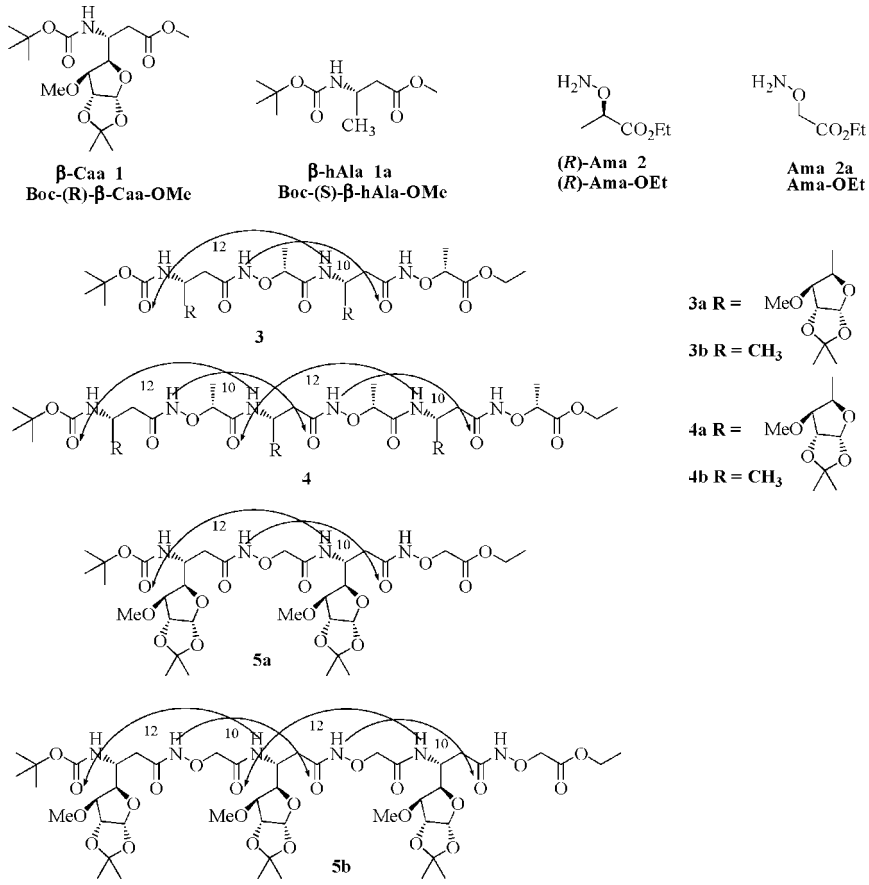

FIGURE 1. Structures of hybrid peptides **3** to **5** (arrows indicate the H-bonds).

presence of 14-helix in β-peptides. This area of research, coined as "foldamers"[7] by Gellman, received very significant attention, and β-peptides obtained from β-amino acids have resulted in a variety of helices, sheets, and turns.[8] To create skeletal diversity for attaining a variety of secondary structures leading to new classes of foldamers, modification of the backbone or the amino acid side chain are interesting protocols. Thus, the replacement of the Cα or Cβ atom in β-amino acids by heteroatoms such as an oxygen or nitrogen is an attractive extension to the β-peptides. Extensive studies by Yang et al.[9] have shown that the oligomers of α-aminoxy acids (Ama), which are oxa analogues of β-amino acids, wherein the β-carbon is replaced with an oxygen atom, generate a very robust 8-helix, referred to as an N−O turn. Further, a variety of aminoxy acids were prepared and converted into yet another class of foldamers by their group.[10] In similar studies on the backbone replacement with heteroatoms, oligomers of α-hydrazino acids, which are aza analogues of β-amino acids, wherein the β-carbon is replaced with a nitrogen atom, have been reported by Grel et al.[11] and Seebach et al.[12] Theoretical calculations by Hofmann et al.[13] described the potential of α-hydrazino acids in the extension of the domain of β-peptides to derive a variety of secondary structures. In our search for new scaffolds, we found that oligomers derived from C-linked carbo-β-amino acid esters (β-Caa) result in very robust helices.[14] The wide variety of helices observed for α/β- as well as α/γ- and β/γ-mixed peptides containing β-Caa suggests that there is considerable scope to enhance the conformational space; especially fascinating has been their propensity to generate mixed helices.[15,16] The oligomers with alternating D-α-amino acid and L-Ama, which resemble α/β-peptides in the backbone space, resulted in a 7/8-helix.[17] Based on the above disclosures about Ama and β-Caa, with their respective preferences for the N−O turns and mixed helices, respectively, a new design, inspired by the report of Seebach et al.[18] on β^2,β^3-peptides, led

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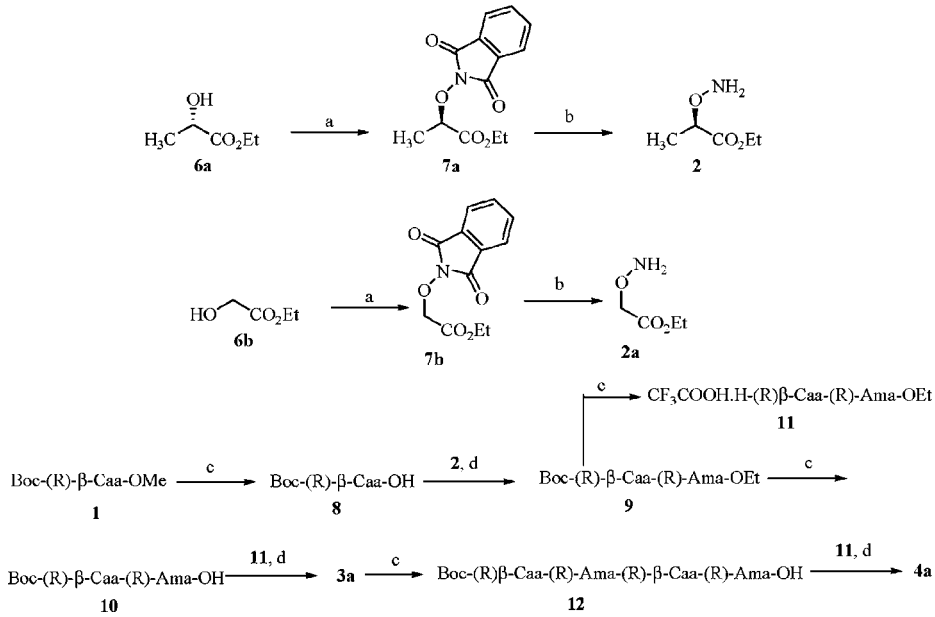
SCHEME 1. Synthesis of α-Aminoxy Amino Acids 2 and 2a and Hybrid Peptides 3a and 4a[a]



[a] Key: (a) Ph_3P, *N*-hydroxyphthalimide, DIAD, THF, 0 °C to rt, 5 h; (b) $N_2H_4 \cdot H_2O$, CH_3OH, rt, 2 h; (c) aq 4 N NaOH, CH_3OH, 0 °C to rt, 2 h; (d) HOBt, EDCl, DIPEA, dry CH_2Cl_2, 0 °C to rt, 5 h; (e) CF_3COOH, dry CH_2Cl_2, 0 °C to rt, 2 h.

us to use the dipeptide repeats of alternating "*R*-*β*-Caa and *R*-Ama" as a "new motif". The amino acids **1/1a** and **2/2a** were utilized for the synthesis of hybrid peptides **3** to **5** (Figure 1) under standard peptide-coupling protocols.[19] Detailed structural studies were undertaken using NMR, CD, and molecular dynamics (MD) studies.

In the α-aminoxy acids, the lone-pair electron repulsion between the nitrogen and oxygen atoms makes their backbone more rigid compared to the *β*-amino acids. The theoretical and experimental studies[9,20] revealed that the N−O turn H-bonding is most favorable in these peptides. It has been further shown by theoretical calculations[20] that the 12/10-helix is less stable in the oligomers of aminoxy acids and each structure has a *gauche* N−O−C−C dihedral angle, similar to those observed in *β*-peptides, where the oxygen atom is replaced with a carbon atom.[21] In oxa-peptides, the electron-withdrawing ability of the N−O group, besides the rigidity about the N−O bond, promotes the H-bonding and favors the C8 conformation in these systems. In contrast, such C8-helices are quite high in energy[21,22] in *β*-peptides and therefore are less stable and can be ruled out, while these peptides have intrinsic preference for 12/10-helices and thus are the most favored structures. Further, theoretical studies predicted that the β^2,β^3-peptides have higher propensity for 12/10-helices.[21,22] Thus, peptides of the present design, obtained from *R*-β^3-Caa (**1**)/*R*-Ama (**2**; oxa-analogue of β^2-amino acid) can be viewed as "pseudo" β^3,β^2-peptides containing β^3-amino acids and an oxa analogue of β^2-amino acid. Since the constituent monomers, *R*-β^3-Caa (**1**) and *R*-Ama (**2**), in the new design of hybrid peptides have high propensity for different secondary structures, it is of special interest to see which of these propensities govern the structure of these oligomers.

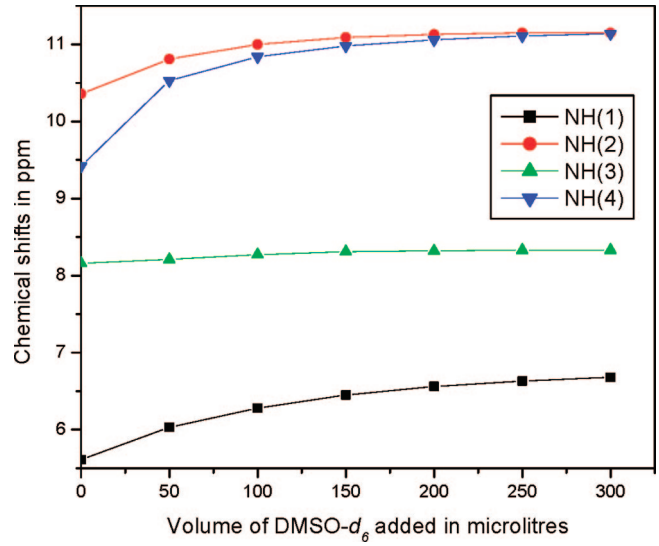


FIGURE 2. Solvent titration studies for peptide **3a**.

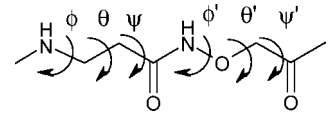


FIGURE 3. Definition of various dihedral angles in *β*-amino acid and α-aminoxy acid.

Results and Discussion

1. Synthesis of Hybrid Peptides 3a and 4a. The *R*-Ama (**2**) and Ama (**2a**) were prepared from *S*-ethyl lactate (**6a**) and

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TABLE 1. ^{1}H NMR (600 MHz, $CDCl_3$, 278 K) of **3a**: Chemical Shifts (δ in ppm), Multiplicity, and Coupling Constants (J in Hz)

	residue			
protons	β-Caa(1)	*R*-Ama(2)	β-Caa(3)	*R*-Ama(4)
NH	5.61 (d, J = 10.0)	10.33 (s)	8.16 (d, J = 9.0)	9.42 (s)
CαH	2.32 (*pro-R*)(dd, J = 9.6, 13.8)	4.25 (m)	2.52 (*pro-R*)(dd, J = 9.0, 14.2)	4.58 (m)
Cα′H	2.61 (*pro-S*)(dd, J = 3.5, 13.8)		2.62 (*pro-S*)(dd, J = 3.7, 14.2)	
CβH	4.43 (dddd, J = 3.5, 7.7, 9.6, 10.0)	1.43 (d, J = 6.6)	4.54 (dq, J = 3.7, 9.0)	1.50 (d, J = 7.1)
C4H	4.10 (dd, J = 3.0, 7.7)		4.25 (m)	
C3H	3.74 (d, J = 3.0)		3.70 (d, J = 3.0)	
C2H	4.56 (d, J =3.9)		4.57 (d, J = 3.9)	
C1H	5.9 (d, J = 3.9)		5.91 (d, J = 3.9)	
	acetonides	1.47, 1.46, 1.45, 1.44 (4s, 12H, acetonide-CH_3)		
	OMe	3.41 (s, 3H, OCH_3-1), 3.36 (s, 3H, OCH_3-3)		
	ethyl ester	4.26 (m, 2H, -CH_2), 1.32 (t, J = 7.2 Hz, 3H, -CH_3)		
	Boc	1.43 (s, 9H, Boc)		

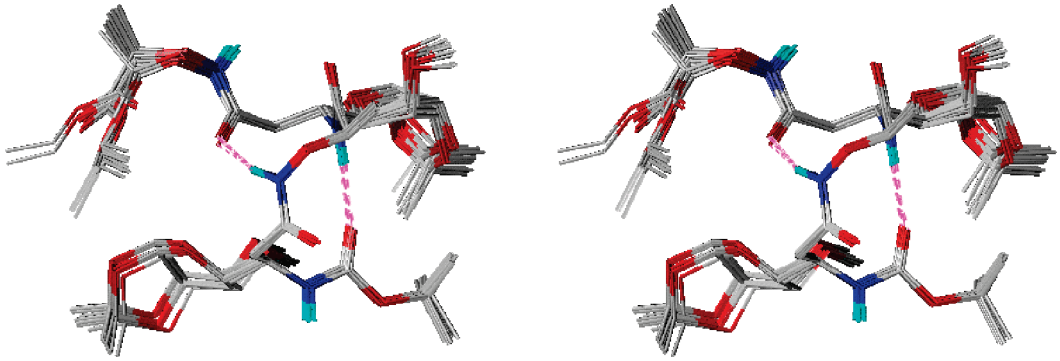


FIGURE 4. Stereoview of 20 superimposed structures of **3a** (H-bonds are shown as dotted lines).

ethyl glycolate (**6b**), respectively (Scheme 1). Thus, reaction of **6a** and **6b** with Ph_3P, DIAD, and *N*-hydroxyphthalimide in THF under Mitsunobu conditions at 0 °C for 5 h afforded the respective *N*-oxyimides **7a** (72%) and **7b** (75.9%). Further, treatment of **7a** and **7b** with hydrazine in methanol gave the respective *N*-aminoxy derivatives **2** and **2a** (Scheme 1). The peptides **3a** and **4a** were synthesized in the solution phase from the monomers **1** and **2** (Scheme 1). Accordingly, ester **1** was subjected to hydrolysis with aq 4 N NaOH at room temperature to afford the acid **8** in 92% yield. Coupling of acid **8** with the *N*-aminoxy derivative **2** in the presence of EDCI and HOBt in CH_2Cl_2 at room temperature for 5 h furnished the dipeptide **9** in 67% yield, $[\alpha]_D = +116.9$ (c 0.25, $CHCl_3$). Base (aq 4 N NaOH) hydrolysis of dipeptide **9** gave the acid **10**, while **9** was converted into the corresponding amine salt **11** on exposure to CF_3COOH in CH_2Cl_2 for 2 h. The thus-derived acid **10** was then coupled (EDCI, HOBt, DIPEA) with amine fuction of **11** in CH_2Cl_2 for 5 h to furnish the tetrapeptide **3a** in 52% yield, $[\alpha]_D = +143.6$ (c 0.3, $CHCl_3$). Reaction of **3a** with NaOH in CH_3OH gave the corresponding acid **12** (92%), which on coupling with the salt **11** under the above reaction conditions afforded the hexapeptide **4a** in 44% yield, $[\alpha]_D = +318.59$ (c 0.13, $CHCl_3$).

2. Conformational Analysis of Hybrid Peptides 3a and 4a. ^{1}H NMR studies were carried out in 8−11 mM solutions in $CDCl_3$. Both **3a** and **4a** show the presence of three isomers, which display exchange peaks in the ROESY spectra. The major isomers, with a population of about 90%, was studied in detail. Resonance assignments were obtained from a combination of TOCSY, ROESY, HSQC, and HMBC experiments. For **3a**, a highly dispersed NMR spectrum suggests the existence of a well-defined structure. Apart from NH(1), all of the amide proton chemical shifts (δ) are >7 ppm. A small change in the chemical shifts ($\Delta\delta$) of NH(2) and NH(3) during the solvent titration studies (Figure 2) provides sufficient support for the propensity of intramolecular H-bonded structures. The dihedral angles defining the conformations of these oligomers are described in Figure 3. For β-amino acid and α-aminoxy acid, the dihedral angles about the amide bond C(O)−N(H) are ∼180°, corresponding to a *trans*-amide bond, while other dihedral angles for β-amino acid, C(O)−N−Cβ−Cα(ϕ), N−Cβ−Cα−C(O) (θ), Cβ−Cα−C(O)−N (ψ), and for α-minoxy acid, C(O)−N−O−Cα(ϕ'), N−O−Cα−C(O) (θ'), and O−Cα−C(O)−N (ψ'), are expected to vary. It was observed that the backbone dihedral angles of β-Caa residues are constrained, as reflected by the coupling constants (Table 1). An *anti*periplanar arrangement of NH and CβH is indicated by $^3J_{\text{NH-C}\beta\text{H}} > 9.0$ Hz, corresponding to the dihedral angle ϕ ∼120°. The $^3J_{\text{C}\alpha\text{H}-\text{C}\beta\text{H}} > 9.6$ Hz or <3.7 Hz support a value of 60° or 180° for the dihedral angle θ. The high-field CαH was assigned as CαH(*pro-R*), based on these couplings as well as a strong NOE correlation between it and NH, permitting us to choose between the two options for the θ and confirm a value of $\theta \sim$ 60°. This also enabled us to deduce from the very strong NOE correlations, NH(2)/CαH(1)(*pro-S*) and NH(4)/CαH(3)(*pro-S*), that the dihedral angle ψ is ∼100°. In the absence of a very few protons in the *R*-Ama residue, information can be gleaned for only dihedral angles ψ' from the NOE data. A strong correlation NH(3)/CαH(2) implies $\psi' \sim -100°$ for the *R*-Ama(2) at the second residue. In addition to above information, the medium-range NOEs, CβH(1)/NH(3), CβH(1)/CαH(3)(*pro-R*), and NH(2)/NH(3), provide the tell tale signatures of a 12-membered H-bond (NH(3)-BocCO) and a 10-membered H-bond (NH(2)-CO(3)) corresponding to a right handed 12/10-mixed helix.[14a]

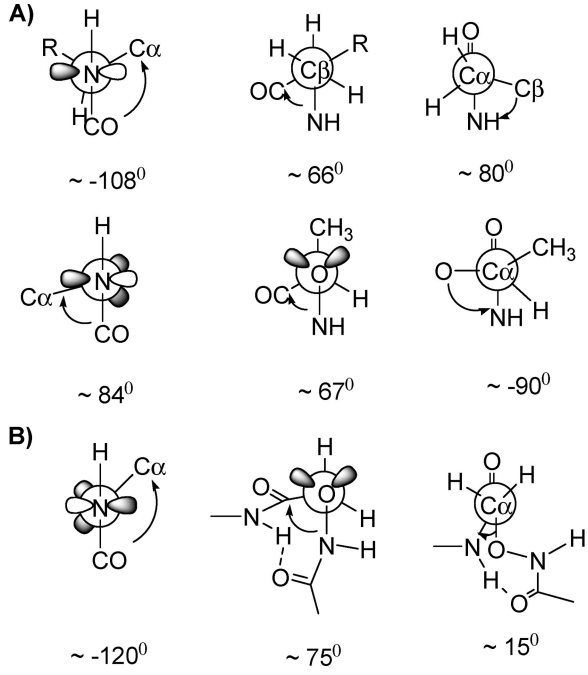


FIGURE 5. (A) Dihedral angles for hybrid peptide **3a** (from NMR data). (B) Dihedral angles of *R*-Ama forming right handed 8-helix.[9b,20]

For restrained MD calculations (Figure 4), the distance constraints were obtained from ROESY data of **3a**, using two spin approximation.[23] Dihedral angle constraints were not used in these calculations, though starting geometries were consistent with the data.[19]

Twenty superimposed low energy structures are shown in the Figure 4. The average rmsd of the backbone and heavy atoms are 0.31 and 0.51 Å, respectively. The fraying at the termini is seen in these structures.

The dramatic difference in conformation of *R*-Ama between the 12/10-helix and 8-helix can be attributed to change in ϕ' and ψ' (Figure 5). The values of ϕ' have a magnitude of about 100°, though they differ in the sign. On the other hand, in 8-helix, ψ' is required to be closer to 0° making the conformation eclipsed, while in 12/10-helix it takes more sterically favorable *gauche* conformation. As β-Caa is energetically reluctant to take part in 8-helix conformation, it can be envisioned that the steric relaxation favors formation of a 12/10-helix.

For the hexamer **4a**, the resonance assignments were made as for **3a**, using the couplings and the NOEs. The data support the involvement of NH(2) to NH(5) in H-bonding.[19] For β-Caa residues, *anti*periplanar arrangement of the NH and the CβH is reflected in $^3J_{\text{NH-C}\beta\text{H}} > 9.4$ Hz (Table 2). Similarly the value of $\theta \sim 60°$ is inferred from $^3J_{\text{C}\alpha\text{H}-\text{C}\beta\text{H}} > 11.2$ Hz or <2.9 Hz and the NOEs similar to those discussed for **3a**, which also permitted us to assign the CαH(*pro-R*) and CαH(*pro-S*) protons. Presence of expected medium range NOEs, CβH(1)/NH(3), CβH(1)/CαH(3)(*pro-R*), CβH(3)/NH(5), CβH(3)/CαH(5)(*pro-R*), NH(2)/NH(3), and NH(4)/NH(5) as well as NH(2)/CαH(4) and NH(4)/CαH(6) in the ROESY spectrum (Figure 6) along with the above data provide compelling evidence of a 12/10/12/10 H-bonded arrangement.

Twenty lowest energy superimposed structures from the MD calculations are shown in Figure 7, with a heavy atom and backbone rmsd of 0.69 and 0.80 Å, respectively. Excluding the

TABLE 2. ^{1}H NMR (600 MHz, $CDCl_3$, 278 K) of **4a**: Chemical Shifts (δ in ppm), Multiplicity, and Coupling Constants (J in Hz)

	residue					
protons	β-Caa(1)	*R*-Ama(2)	β-Caa(3)	*R*-Ama(4)	β-Caa(5)	*R*-Ama(6)
NH	5.55 (d, J = 10.6)	10.83 (s)	8.98 (d, J = 10.0)	11.12 (s)	8.74 (d, J = 9.4)	9.48 (s)
CαH	2.15 (*pro-R*) (t, J = 13.2)	4.19 (q, J = 6.6)	2.36 (*pro-R*) (t, J = 13.2)	4.28 (q, J = 6.6)	2.49 (*pro-R*) (dd, J = 11.2, 14.3)	4.68 (q, J = 7.0)
CαH	2.74 (*pro-S*) (dd, J = 2.9, 13.2)		2.88 (*pro-S*) (dd, J = 2.9, 13.2)		2.58 (*pro-S*) (dd, J = 2.9, 14.3)	
CβH	4.59 (m)	1.37 (d, J = 6.6)	4.61 (m)	1.40 (d, J = 6.6)	4.68 (m)	1.51 (d, J = 7.0)
C4H	4.07 (m)		4.18 (dd, J = 3.1, 9.6)		4.08 (m)	
C3H	3.71 (d, J = 3.4)		3.59 (d, J = 3.1)		3.70 (d, J = 3.2)	
C2H	4.54 (d, J = 3.8)		4.56 (d, J = 3.8)		4.55 (d, J =3.8)	
C1H	5.91 (d, J = 3.8)		5.88 (d, J = 3.8)		5.89 (d, J = 3.8)	
acetonides	1.45, 1.38 1.37, 1.30, 1.29, 1.28 (6s, 18H, acetonide-CH_3)					
OMe	3.40 (s, 3H, OCH_3-1), 3.34 (s, 3H, OCH_3-5), 3.30 (s, 3H, OCH_3-3)					
ethyl ester	4.26 (q, J = 7.0 Hz, 2H, -CH_2), 1.32 (d, J = 7.0 Hz, 3H, -CH_3)					
Boc	1.43 (s, 9H, Boc)					

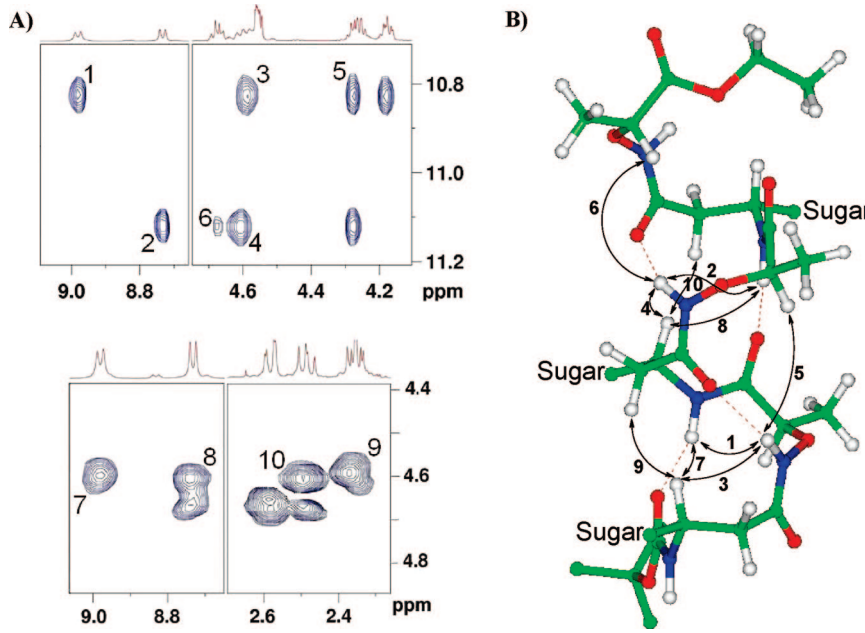


FIGURE 6. ROESY expansions for **4a**: (A) The NOE correlations, NH(2)/NH(3), NH(4)/NH(5), CβH(1)/NH(2), CβH(3)/NH(4), NH(2)/CαH(4), NH(4)/CαH(6), CβH(1)/ NH(3), CβH(3)/NH(5), CβH(1)/CαH(3)(*pro-R*), and CβH(3)/CαH(5)(*pro-R*) are marked as 1−10, respectively. (B) The characteristic NOEs have been depicted in the energy-minimized structure of **4a**.

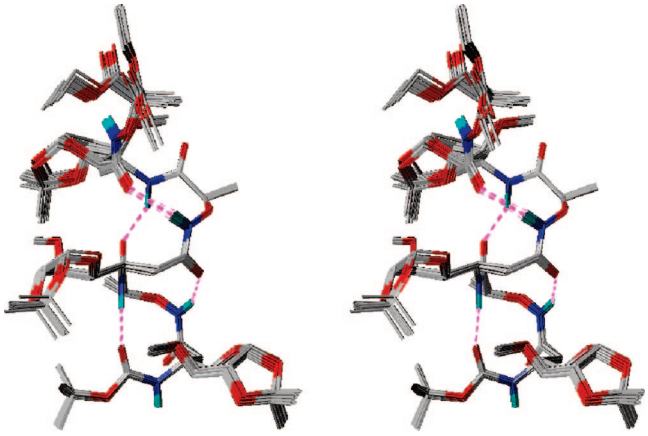


FIGURE 7. Stereoview of 20 superimposed structures of **4a** (H-bonds are shown as dotted lines).

first and last residues, the average backbone dihedral angles for the *R*-Ama residues are $\phi' = 84 \pm 2°$, $\theta' = 67 \pm 2°$, and $\psi' = -92 \pm 1°$, while for the β-Caa residues they are $\phi = -108 \pm 2°$, $\theta = 66 \pm 1°$ and $\psi = 80 \pm 1°$, respectively. The pitch for the helix is ~5.5 Å, number of residues per turn ~2.7, and the rise per turn ~2.1 Å.

The values observed above are very similar to those obtained for other 12/10-helices reported[9b,c,10,16] previously (Figure 8).

The CD spectra of 0.2 mM solution of **3a** and **4a**, like the oligomers of *R*-Ama, were studied in trifluoroethanol (TFE) and methanol.[9] The presence of a 12/10-mixed helix[14] is very well supported by a maximum at ~199−202 nm.[19]

3. Synthesis of Hybrid Peptides 3b/4b and 5a/5b. Thus, the structural analysis of the hybrid peptides **3a** and **4a** amply revealed that the conformational preference of *R*-β-Caa influenced *R*-Ama to behave like β^2-amino acid resulting in the formation of 12/10-mixed helix as was the case with β^2,β^3-peptides reported by Seebach et al.[18] Earlier studies by Yang et al.[10] suggested that the formation of the N−O turn is independent of the side-chain variation and is solely controlled by the nature of the backbone. Since the peptides with the new design having "*R*-β-Caa/*R*-Ama" exist in a 12/10-helical structure, where *R*-Ama deviates from its conformational preference in the aminoxy peptides, the study was further extended to understand the side chain effects in these "pseudo" β^3,β^2-peptides. Hence, new peptides with alternating *R*-β-Caa and Ama (with no substitution), and *S*-β-hAla and *R*-Ama, were studied to explore the influence of the side chain in helix formation.

The *R*-β-Caa/Ama peptides **5a** and **5b** and *S*-β-hAla/*R*-Ama peptides **3b** and **4b** were prepared (Scheme 2) following the protocol described for the synthesis of peptides **3a** and **4a**.[19]

4. Conformational Analysis of Hybrid Peptides 3b/4b and 5a/5b. The NMR data for the tetramers **3b** and **5a** show the presence of four isomers in ratios of 84:10:4:2 (**3b**) and 75:11:9:5 (**5a**). For both peptides, structural information could be obtained only for the major isomers. Characteristic NMR signatures on H-bonding as well as on couplings and NOE correlations confirm the presence of a 12/10-helix. However, weaker NOE cross peaks in these peptides, compared to those for **3a**, suggest the reduced stability of the helical structures.[19] MD calculations further support these observations. Twenty superimposed low energy structures for these peptides, showed that the average rmsd of the backbone and the heavy atoms are 0.61 and 0.96 Å, respectively, for **3b** and 0.79 and 1.03 Å, respectively, for **5a**.[19]

For peptides **4b** and **5b**, three isomers were observed in the NMR spectra. For **4b**, the isomeric ratio was 91:6:3, and only the major isomer was investigated in detail. The distinctive signatures for the propagation of 12/10-helix though were visible in the NMR spectra of **4b**,[19] weaker NOE correlations suggest the presence of larger population of disordered structures. From the MD calculations of **4b**, 20 superimposed low energy structures showed that the average rmsd of the backbone and

(23) Neuhaus, D. Williamson, M. P. The Nuclear Overhauser Effect in Structural and Conformational Analysis; VCH Publishers: New York, 1989.

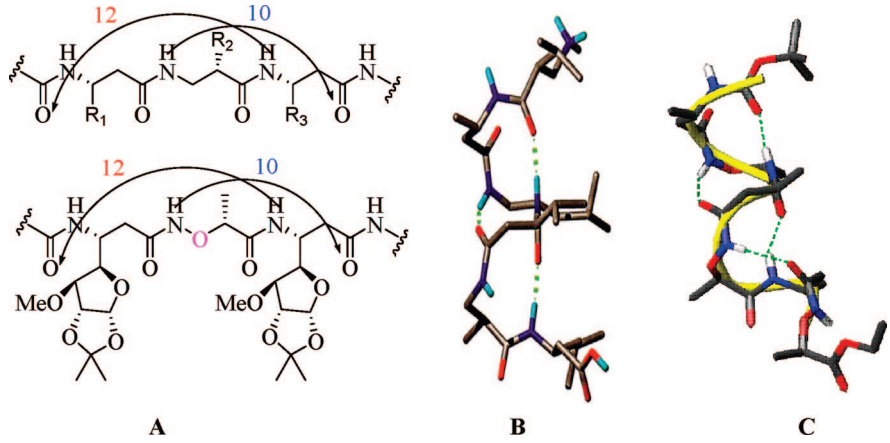
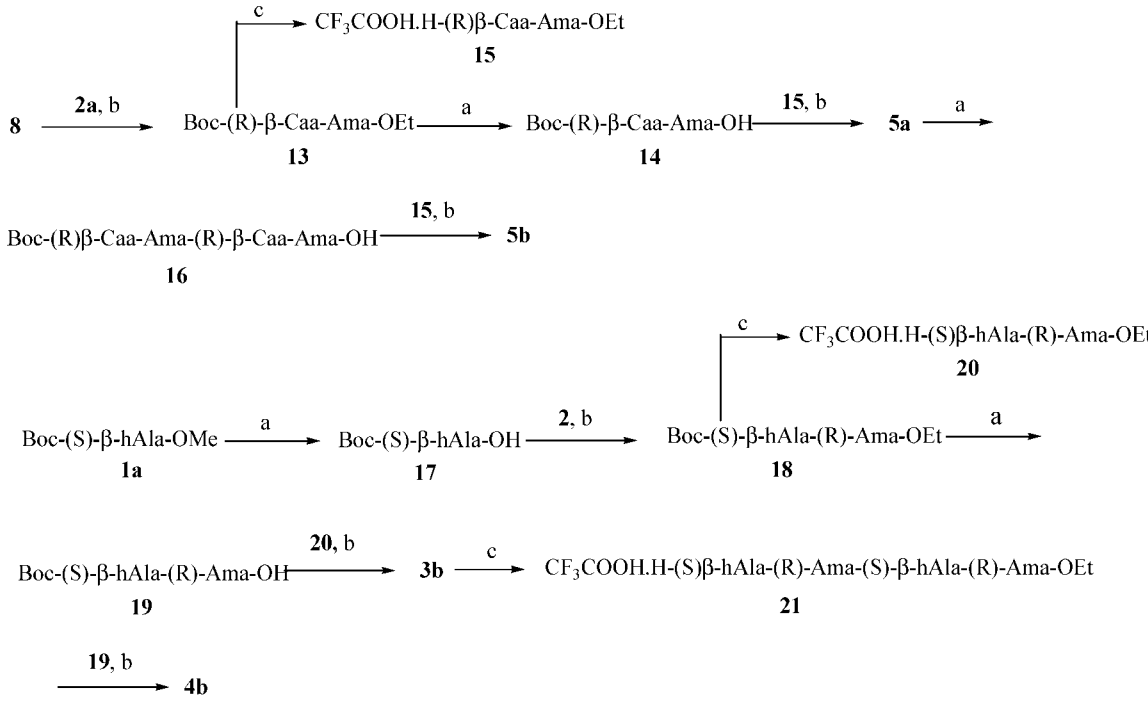


FIGURE 8. (A) Chemical structures of β^3,β^2- and "pseudo" β^3,β^2-peptides. (B) 12/10-helix (β^3,β^2-peptide);[18] (C) 12/10-helix ("pseudo" β^3,β^2-peptide) (backbone is highlighted).

SCHEME 2. Synthesis of Hybrid Peptides 3b/4b and 5a/5b[a]



[a] Key: aq 4 N NaOH, CH_3OH, 0 °C to rt, 2 h; (b) HOBt, EDCl, DIPEA, dry CH_2Cl_2, 0 °C to rt, 5 h; (c) CF_3COOH, dry CH_2Cl_2, 0 °C to rt, 2 h.

the heavy atoms are 1.24 and 1.96 Å, respectively.[19] The spectral overlap of signals in **5b**, forbids the identification of the individual isomer populations and precludes obtaining the structural information.

The CD spectra for peptides **3b/4b** and **5a/5b** were studied in 0.2 mM solution of both TFE and methanol. The maxima of about 200 nm reveal the presence of a 12/10-mixed helix for **3b** and **4b**, however, reduced molar ellipticities at the maxima reflect the lack of stability to these structures. The CD spectra for **5a** and **5b** in TFE show further destabilization of the secondary structures as indicated from very small molar ellipticities at the maxima. On the other hand, in methanol solution, complete loss of structure was observed.[19]

The above structural elucidation very clearly demonstrates the influence of side chains in the helix forming capabilities of these peptides. Thus, the peptides **3a** and **4a** display a very robust 12/10-helix, while, **3b** and **4b**, though reveal the presence of 12/10-helical structures, they are not found to be as robust as in **3a** and **4a**. Further destabilization of helices was observed in peptides **5a** and **5b**. These observations suggest that the substituents and their size play an important role in deciding the stability of the conformations in this class of foldamers.

5. Conclusions

To conclude, the study emphatically demonstrates that the "*R*-β-Caa/*R*-Ama" oligomers really behave like peptides with alternating β^3- and β^2-residues and generate 12/10-helices in these "pseudo" β^3,β^2-peptides. It appears to be an outcome of the dominance of *R*-β-Caa to provide robust mixed helices over that of *R*-Ama to form an 8-helix. The difference in the robustness of the 12/10-helices in these peptides further suggests that the stability of mixed helices is sensitive to the variation of the substituents at β^3- and pseudo β^2-amino acids. This study demonstrates that further expansion of the foldamer domain is possible by exploiting the oligomers derived from dipeptide

repeats containing the versatile building blocks, Ama, and β-Caa or β-amino acids with different side chains. In view of the recent developments on tertiary and quaternary structures derived from β- and α/β-peptides,[24] these results create additional opportunities for the design and synthesis of several such hybrid foldamers.

Experimental Section

^{1}H NMR were recorded on 600 MHz spectrometers at 278 K, with 8−11 mM solution in $CDCl_3$ using tetramethylsilane (TMS) as internal standard. The chemical shifts are shown in δ scales. The proton resonance assignments were carried out by using two-dimensional total correlation spectroscopy (TOCSY), rotating frame Overhauser effect spectroscopy (ROESY), heteronuclear single quantum correlation (HSQC), and heteronuclear multiple bond correlation (HMBC) experiments. ROESY experiments, which provide the spatial proximity of the protons, were performed with a mixing time of 0.2 s with a spin-locking field of about 2.5 kHz. The TOCSY experiments were performed with the spin-locking fields of about 10 kHz and a mixing time of 0.08 s. TOCSY and ROESY spectra were acquired with 2 × 256 or 2 × 192 free induction decays (FID) containing 16−24 transients with relaxation delays of 1.5 s. HSQC and HMBC experiments, optimized for J_{C-H} = 145 and 10 Hz, respectively, were carried out with 256 × 2048 and 128 × 4096 free induction decays (FID), respectively, containing 4−8 transients. All of the experiments were carried out in the phase-sensitive mode. Information on the H-bonding in $CDCl_3$ was obtained from solvent titration studies at 278 K by sequentially adding up to 300 μL of DMSO-d_6 in 600 μL of $CDCl_3$ solution of peptides. The two-dimensional data were processed with shifted sine bell or Gaussian apodization in both the dimensions. The CD spectra were recorded in quartz cells of 2 mm path length at room temperature with scan range of 190−260 nm and scanning speed of 20 nm/min, using peptide concentration of 0.2 mM in MeOH and trifluoroethanol (TFE). All spectra represent 1 scan, each of 100 ms time constant and are background corrected and smoothened over 2−5 data points using a binomial method. The Insight-II program was used for moleculer model construction and for structural analysis of the different obtained conformations. The DISCOVER software was used for molecular modeling calculations, including energy minimization. The cvff MSI version with default parameter was used as force field throughout the calculation using a distance dependent dielectric constant with ε = 4.8 (dielectric constant of deuterated chloroform). The interproton distances used in restrained molecular dynamics simulation were obtained from the volume integrals of the ROESY spectra using two-spin approximation and a reference distance of 1.80 Å between the geminal protons at CαH.[25] Dihedral angle constraints were not used in these calculations. The upper and lower bounds of the constraints were derived, respectively, by subtracting and adding 10% to thus obtain interatomic distances. The complete set of NOE distance constrains used for structure calculation is shown in the Supporting Information.[19] As a first step, a mild minimization with constrains was performed in order to remove bad steric contacts and improve the stability of the calculations. The following general protocol was used for minimizing energy. Each structure was energy minimized by steepest descent method, followed by conjugate gradient method for a maximum of 10000 iterations each or rms deviation of 0.001 kcal/mol, whichever was earlier. For MD runs, a temperature of 300 K was used. The molecules were initially equilibrated for 50 ps and subsequently subjected to a 1 ns dynamics with a step size of 1 fs, sampling the trajectory at equal intervals of 10 ps. In this trajectory, 105 samples were generated and were again energy minimized.

Pth-(*R*)-Ama-OEt (7a). To a solution of **6a** (1.5 g, 12.7 mmol), Ph_3P (4.9 g, 19.06 mmol), and *N*-hydroxyphthalimide (3.06 g, 19.06 mmol) in dry THF (15 mL) was added DIAD (3.7 mL, 19.06 mmol) at 0 °C and the mixture stirred under N_2 atmosphere for 5 h. Solvent was evaporated under reduced pressure, and the residue obtained was purified by column chromatography (silica gel, 10% ethyl acetate and petroleum ether) to afford **7a** (2.4 g, 72%) as a colorless syrup: $[\alpha]_D$ = +84.6 (*c* 0.3, $CHCl_3$); IR (KBr) 2986, 1737, 1373, 1188, 1082, 703 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$, 288K): δ 7.86 (dd, *J* = 2.9, 5.5 Hz, 2H, Ar-H), 7.78 (dd, *J* = 2.9, 5.5 Hz, 2H, Ar-H), 4.9 (q, *J* = 7.0 Hz, 1H, CαH), 4.25 (m, 2H, $-CH_2$), 1.66 (d, *J* = 7.0 Hz, 1H, Cβ*H*), 1.30 (t, *J* = 7.0 Hz, 3H, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170, 163, 134, 128.5, 124.5, 81, 62, 16, 14; HRMS (ESI+) *m*/*z* calcd for $C_{13}H_{13}NO_5$ (M^+ + Na) 286.0691, found 286.0688.

H-(*R*)-Ama-OEt (2)]. A solution of **7a** (0.7 g, 2.66 mmol) and $N_2H_4 \cdot H_2O$ (0.39 mL, 7.98 mmol) in CH_3OH (10 mL) was stirred at room temperature for 2 h. The solvent was removed under reduced pressure, and the residue treated with 5% aq $NaHCO_3$ (10 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layer was dried (Na_2SO_4) and evaporated under high vacuum to afford **2**, which was used as such for further reaction.

Pth-Ama-OEt (7b). To a solution of **6b** (1.0 g, 9.60 mmol), Ph_3P (3.77 g, 14.41 mmol), and *N*-hydroxyphthalimide (2.32 g, 14.41 mmol) in dry THF (15 mL) was added DIAD (2.65 mL, 14.41 mmol) at 0 °C and the mixture stirred under N_2 atmosphere for 5 h. Workup as described for **7a** and purification by column chromatography (silica gel, 12% ethyl acetate and petroeum ether) afforded **7b** (1.8 g, 75.9%) as a white solid: mp 90−91 °C; IR (KBr) 2986, 1737, 1373, 1188, 1082, 703 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$, 288K): δ 7.88 (dd, *J* = 2.9, 5.5 Hz, 2H, Ar−H), 7.86 (dd, *J* = 2.9, 5.5 Hz, 2H, Ar-H), 4.84 (*s*, 2H, CαH), 4.28 (*q*, *J* = 7.4, 2H, $-CH_2$), 1.32 (d, *J* = 7.4 Hz, 3H, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ168, 162.8,156.4, 134.6, 128.5, 123.6, 72.9, 21.8, 13.9.; HRMS (ESI+) *m*/*z* calcd for $C_{12}H_{12}NO_5$ (M^+ + Na) 250.0715, found 250.0715.

H-Ama-OEt (2a). A solution of **7b** (0.7 g, 2.83 mmol) and $N_2H_4 \cdot H_2O$ (0.41 mL, 8.50 mmol) in CH_3OH (10 mL) was stirred at room temperature for 2 h. Workup as described for **2** afforded **2a**, which was used as such for further reaction.

Boc-(*R*)-β-Caa-OH (8). A solution of ester **1** (0.85 g, 2.26 mmol) in methanol (4 mL) was treated with aq 4 N NaOH solution (4 mL) at 0 °C to room temperature. After 2 h, methanol was removed and adjusted pH to 2−3 with aq 1 N HCl solution at 0 °C and extracted with EtOAc (2 × 10 mL). The organic layer was dried (Na_2SO_4) and concentrated to give **8** (0.71 g, 86.8%) as a white solid: mp 124−127 °C; $[\alpha]_D$ = −22.5 (*c* 0.55, $CHCl_3$); IR (KBr) 3324, 2983, 2929, 1708, 1643, 1410, 1262, 1160, 1076, 1018 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.88 (d, *J* = 3.8 Hz, 1H, C1H), 5.33 (d, *J* = 9.4 Hz, 1H, NH), 4.55 (d, *J* = 3.8 Hz, 1H, C2H), 4.37 (dddd, *J* = 4.9, 5.6, 7.6, 9.4 Hz, 1H, Cβ*H*), 4.32 (dd, *J* = 2.6, 7.6 Hz, 1H, C4H), 3.73 (d, *J* = 2.6 Hz, 1H, C3H), 3.41 (s, 3H, OCH_3), 2.77 (dd, *J* = 5.6, 16.2 Hz, 1H, CαH), 2.72 (dd, *J* = 4.9, 16.2 Hz, 1H, CαH′), 1.48 (s, 3H, CH_3), 1.44 (s, 9H, Boc), 1.32 (s, 3H, CH_3); FABMS 362 [$(M + H)^+$], 262 [$(M + H - Boc)^+$].

Boc-(*R*)-β-Caa-(*R*)-Ama-OEt (9). A solution of acid **8** (0.70 g, 1.93 mmol), HOBt (0.315 g, 2.32 mmol), and EDCI (0.446 g, 2.32 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere

(24) (a) Raguse, T. L.; Lai, J. R.; LePlae, P. R.; Gellman, S. H. *Org. Lett.* **2001**, *3*, 3963–3966. (b) Cheng, R. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **2002**, *124*, 11564–11565. (c) Qiu, J. X.; Petersson, E. J.; Matthews, E. E.; Schepartz, A. *J. Am. Chem. Soc.* **2006**, *128*, 11338–11339. (d) Sharma, G. V. M.; Subash, V.; Narsimulu, K.; Ravi Sankar, A.; Kunwar, A. C. *Angew. Chem., Int. Ed.* **2006**, *45*, 8207–8210. (e) Horne, W. S.; Price, J. L.; Keck, J. L.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 4178–4180. (f) Daniels, D. S.; Petersson, E. J.; Qiu, J. X.; Schepartz, A. *J. Am. Chem. Soc.* **2007**, *129*, 1532–1533. (g) Price, J. L.; Horne, W. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 6376–6377. (h) Petersson, E. J.; Craig, C. J.; Daniels, D. S.; Qiu, J. X.; Schepartz, A. *J. Am. Chem. Soc.* **2007**, *129*, 5344–5345. (i) Petersson, E. J.; Schepartz, A. *J. Am. Chem. Soc.* **2008**, *130*, 821–823.

(25) For **3b** and **5a**, because of the very small difference in the chemical shifts between the geminal CαH protons and the presence of exchange peaks between the different isomers, the NH−Cβ*H* distance of 3.08 Å (*anti*periplanar) was used.

for 15 min, treated with the above crude amine **2** and DIPEA (0.50 mL, 2.90 mmol), and stirred at room temperature for 5 h. The reaction mixture was quenched with satd aq NH_4Cl (10 mL) at 0 °C and diluted with $CHCl_3$ (10 mL). It was sequentially washed with 1 N HCl (10 mL), water (10 mL), and aq NaCl solution (10 mL). The organic layer was dried (Na_2SO_4) and evaporated to give the residue, which was purified by column chromatography (silica gel, 50% ethyl acetate in petroleum ether) to afford **9** (0.62 g, 67%) as a white solid: mp 116−119 °C; $[\alpha]_D = +116.9$ (*c* 0.25, $CHCl_3$); IR (KBr) 3269, 2987, 2937, 1754, 1739, 1688, 1661, 1554, 1373, 1168, 1058, 1021, 889, 858 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.20 (s, 1H, NH-2), 5.87 (d, $J = 3.9$ Hz, 1H, C1H-1), 5.49 (d, $J = 9.7$ Hz, 1H, NH-1), 4.55 (d, $J = 3.9$ Hz, 1H, C2H-1), 4.51 (q, $J = 7.2$ Hz, 1H, CαH-2), 4.24 (m, 1H, CβH-1), 4.23 (dd, $J = 3.9$, 7.2 Hz, 1H, C4H-1), 4.22 (m, 2H, $-CH_2$), 3.72 (d, $J = 3.2$ Hz, 1H, C3H-1), 3.41 (s, 3H, OCH_3-1), 2.51 (dd, $J = 5.7$, 14.5 Hz, 1H, CαH(*pro-S*)-1), 2.43 (dd, $J = 4.8$, 14.5 Hz, 1H, CαH(*pro-R*)-1), 1.48 (d, $J = 7.2$ Hz, 3H, CβH-2), 1.46, 1.31 (2s, 6H, acetonide-CH_3), 1.43 (s, 9H, BOC), 1.30 (t, $J = 7.2$ Hz, 3H, $-CH_3$); ^{13}C NMR (150 MHz, $CDCl_3$, 278 K) δ 172.0, 169.2, 155.1, 111.8, 104.8, 83.4, 81.4, 80.1, 79.3, 79.1, 61.2, 57.8, 45.8, 34.9, 28.2, 26.6, 26.2, 16.1, 14.0; HRMS (ESI+) *m/z* calcd for $C_{21}H_{36}N_2O_{10}$ (M^+ + Na) 499.2267, found 499.2257.

Boc-(*R*)-β-Caa-(*R*)-Ama-(*R*)-β-Caa-(*R*)-Ama-OEt (3a). A solution of **9** (0.2 g, 0.42 mmol) as described for **8** gave Boc-(*R*)-β-Caa-(*R*)-Ama-OH (**10**; 0.17 g, 90.4%) as a white solid, which was used for the further reaction.

A solution of **9** (0.18 g, 0.38 mmol) and CF_3COOH (0.4 mL) in CH_2Cl_2 (2 mL) was stirred at 0 °C to rt for 2 h. Solvent was evaporated under reduced pressure, and the resulting salt **11** was dried under high vacuum and used as such for further reaction.

A solution of **10** (0.17 g,0.38 mmol), HOBt (0.062 g, 0.456 mmol), and EDCI (0.087 g, 0.456 mmol) in dry CH_2Cl_2 (2 mL) was stirred at 0 °C for 15 min and treated with the above-obtained amine TFA salt **11** and DIPEA (0.098 mL, 0.57 mmol) under nitrogen atmosphere for 5 h. Workup as described for **9** and purification by column chromatography (silica gel, 1.8% CH_3OH in $CHCl_3$) afforded **3a** (0.16 g, 52.3%) as a white solid: mp 84−85 °C; $[\alpha]^{20}_D = +143.66$ (*c* 0.3 in $CHCl_3$); IR (KBr) 3431, 2985, 1683, 1552, 1165, 1081, 1021, 855 cm^{-1}; ^{13}C NMR (150 MHz, $CDCl_3$, 278 K) δ 172.3, 170.7, 168.8, 168.5, 156.1, 111.8, 111.7, 104.9, 104.8, 83.9, 83.4, 81.4, 81.1, 81.0, 80.3, 80.0, 79.9, 79.8, 61.4, 58.1, 57.8, 47.2, 45.7, 36.4, 35.6, 29.7, 29.4, 28.3, 26.6, 26.1, 16.3, 15.7, 14.1; HRMS (ESI+) *m/z* calcd for $C_{35}H_{58}N_4O_{17}$ (M^+ + Na) 829.3694, found 829.3688.

Boc-(*R*)-β-Caa-(*R*)-Ama-(*R*)-β-Caa-(*R*)-Ama-(*R*)-β-Caa-(*R*)-Ama-OEt (4a). A solution of **3a** (0.14 g, 0.173 mmol) as described for **8** gave Boc-(*R*)-β-Caa-(*R*)-Ama-(*R*)-β-Caa-(*R*)-Ama-OH (**12**; 0.125 g, 92.6%) as a white solid, mp 96−97 °C.

To a solution of **12** (0.125 g, 0.16 mmol), HOBt (0.026 g, 0.193 mmol), and EDCI (0.037 g, 0.193 mmol) in dry CH_2Cl_2 (5 mL) was added amine salt **11** [prepared from **9** (0.077 g, 0.16 mmol) and CF_3COOH (0.2 mL) in CH_2Cl_2 (2 mL)] and the mixture stirred at room temperature for 5 h. Workup as described for **9** and purification by column chromatography (silica gel, 2.4% CH_3OH in $CHCl_3$) furnished **4a** (0.082 g, 44.3%) as a white solid: mp 97−98 °C; $[\alpha]_D = +318.59$ (*c* 0.13, $CHCl_3$); IR (KBr) 3423, 2986, 2925, 2852, 1669, 1537, 1456, 1374, 1253, 1218, 1165, 1117, 1081, 888, 854 cm^{-1}; ^{13}C NMR (150 MHz, $CDCl_3$, 278 K) δ 172.7, 170.3, 169.6,168.7, 168.6, 167.1, 156.7, 111.6, 111.3, 105.2, 105.0, 104.9, 84.3, 83.7, 83.1, 81.7, 81.0, 80.7, 80.6, 80.1, 80.0, 79.4, 78.9, 61.5, 58.2,57.8, 57.5, 48.6, 47.3, 45.1, 37.3, 36.0, 33.8, 29.7, 29.4, 28.3, 26.7, 26.4, 26.1, 26.0, 25.9, 24.8, 16.3, 15.4, 15.0, 14.2, 14.0; HRMS (ESI+) *m/z* calcd for $C_{49}H_{80}N_6O_{24}$ (M^+ + Na) 1159.5121, found 1159.5092.

Boc-(*R*)-β-Caa-Ama-OEt (13). A solution of acid **8** (0.80 g, 2.21 mmol), HOBt (0.360 g, 2.65 mmol), and EDCI (0.509 g, 2.65 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere for 15 min, treated with the above crude amine **2a** and DIPEA (0.57 mL,3.32 mmol), and stirred at room temperature for 5 h. Workup as described for **9** and purification by column chromatography (silica gel, 50% ethyl acetate and petroleum ether) afforded **13** (0.675 g, 66.29%) as a white solid: mp 153−155 °C; $[\alpha]_D = -69.54$ (*c*1.07, $CHCl_3$); IR (KBr) 3269, 2982, 2930, 1732, 1695, 1673, 1551, 1365, 1282, 1254, 1021, 858 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.30 (s, 1H, NH-2), 5.85 (d, $J = 3.6$ Hz, 1H, C1H-1), 5.50 (d, $J = 9.7$ Hz, 1H, NH-1), 4.54 (d, $J = 3.6$ Hz, 1H, C2H-1), 4.44 (s, 2H, CαH-2), 4.32 (m, 1H, CβH-1), 4.25 (dd, $J = 2.2$, 4.7 Hz, 1H, C4H-1), 4.22 (m, 2H, $-CH_2$), 3.71 (d, $J = 2.2$ Hz, 1H, C3H-1), 3.41 (s, 3H, OCH_3-1), 2.51 (dd, $J = 5.7$, 14.5 Hz, 1H, CαH(*pro-S*)-1), 2.43 (dd, $J = 4.8$, 14.5 Hz, 1H, CαH(*pro-R*)-1), 1.46, 1.31 (2s, 6H, acetonide-CH_3), 1.43 (s, 9H, BOC), 1.30 (t, $J = 6.2$ Hz, 3H, $-CH_3$); ^{13}C NMR (150 MHz, $CDCl_3$, 278 K) δ 169.5, 169.0, 155.2, 111.7, 104.7, 83.4, 81.3, 80.0, 79.4, 72.1, 61.3, 57.8, 45.6, 39.4, 28.2, 26.6, 26.2, 14.0; HRMS (ESI+) *m/z* calcd for $C_{20}H_{35}N_2O_{10}$ (M^+ + H) 463.2291, found 463.2270.

Boc-(*R*)-β-Caa-Ama-(*R*)-β-Caa-Ama-OEt (5a). A solution of **13** (0.2 g, 0.43 mmol) as described for **9** gave Boc-(*R*)-β-Caa-Ama-OH (**14**; 0.18 g, 96.2%) as a white solid, which was used for the further reaction.

A solution of **14** (0.18 g,0.41 mmol), HOBt (0.067 g, 0.49 mmol), and EDCI (0.095 g, 0.49 mmol) in dry CH_2Cl_2 (2 mL) was stirred at 0 °C for 15 min and treated with the above-obtained amine TFA salt **15** [prepared from the ester **13**] and DIPEA (0.102 mL, 0.62 mmol) under nitrogen atmosphere for 5 h. Workup as described for **9** and purification by column chromatography (silica gel, 1.8% CH_3OH in $CHCl_3$) afforded **5a** (0.17 g, 53.12%) as a white solid: mp 80−82 °C; $[\alpha]^{20}_D = -19.89$ (*c* 1.25, $CHCl_3$); IR (KBr) 3431, 2923, 2853, 1686, 1463, 1376, 1261, 1021, 855; ^{13}C (150 MHz, $CDCl_3$, 278 K) δ 183.2, 169.7, 168.8, 168.2, 155.9, 111.8, 104.8, 83.7, 83.6, 81.3, 81.2, 80.2, 80.0, 79.9, 75.6, 72.1, 61.3, 58.1, 57.8, 46.2, 45.1, 35.8, 35.5, 31.9, 29.7, 28.3, 27.0, 26.7, 26.2, 16.3, 14.1; HRMS (ESI+) *m/z* calcd for $C_{33}H_{55}N_4O_{17}$ (M^+ + H) 779.3562, found 779.3561.

Boc-(*R*)-β-Caa-Ama-(*R*)-β-Caa-Ama-(*R*)-β-Caa-Ama-OEt (5b). A solution of **5a** (0.15 g, 0.19 mmol) as described for **8** gave Boc-(*R*)-β-Caa)-Ama-(*R*)-β-Caa)-Ama-OH (**16**; 0.13 g, 90.27%) as a white solid, which was used for further reaction without any purification.

To a solution of **16** (0.13 g, 0.17 mmol), HOBt (0.028 g, 0.207 mmol), and EDCI (0.040 g, 0.207 mmol) in dry CH_2Cl_2 (5 mL) were added amine salt **15** [prepared from **13** (0.080 g, 0.17mmol) and CF_3COOH (0.2 mL) in CH_2Cl_2 (2 mL)] and DIPEA (0.045 mL, 0.25mmol) and the mixture stirred at room temperature for 5 h. Workup as described for **9** and purification by column chromatography (silica gel, 2.5% CH_3OH in $CHCl_3$) furnished **5b** (0.067 g, 35.4%) as a white solid: mp 86−90 °C; $[\alpha]_D = -1.85$ (*c* 0.09, $CHCl_3$); IR (KBr) 3423, 2925, 2853, 1670, 1545, 1456, 1379, 1261, 1117, 1081, 888 cm^{-1}; ^{13}C NMR (150 MHz, $CDCl_3$, 278 K) δ 183.3, 169.5, 169.3, 169.2, 168.8, 168.4, 155.8,139.4, 114.2, 111.9, 104.9, 83.7, 83.6, 83.5, 81.3, 81.2, 80.3, 80.2, 80.0, 75.5, 75.3, 72.0, 61.5, 57.9, 57.9, 57.8, 46.5, 45.2, 45.0, 38.4, 36.1, 35.9, 35.1, 33.9, 31.9, 29.7, 29.4, 28.4, 27.0, 26.7, 26.1, 22.7, 14.2, 14.1; HRMS (ESI+) *m/z* calcd for $C_{46}H_{74}N_6O_{24}$ (M^+ + Na) 1117.4652, found 1117.4644.

Boc-(*R*)-β-hAla-(*R*)-Ama-OEt (18). A solution of **1a** (0.9 g, 0.4.14 mmol) as described for **8** gave Boc-(*R*)-β-hAla-(*R*)-Ama-OH **17**; (0.83 g, 98.5%) as a white solid which was used for further reaction without any purification.

A solution of acid **17** (0.82 g, 4.03 mmol), HOBt (0.65 g, 4.84 mmol), and EDCI (0.92 g, 4.84 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere for 15 min, treated with the above crude amine **2** [prepared from **7a** (1.4 g, 2.665.32 mmol) and $N_2H_4 \cdot H_2O$ (0.78 mL, 15.96 mmol)] and DIPEA (0.50 mL,2.90 mmol), and stirred at room temperature for 5 h. Workup as described for **9** and purification by column chromatography (silica gel, 50% etthyl acetate and petroleum ether) gave **18** (0.75 g, 58.59%) as a white solid: mp 110−112 °C; $[\alpha]_D = +38.15$ (*c* 1.065,

$CHCl_3$); IR (KBr) 3269, 2986, 2937, 1736, 1672, 1536, 1456, 1365, 1273, 1058, 1021, 889, 858 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.95 (s, 1H, NH-2), 5.08 (br, 1H, NH-1), 4.57 (q, $J = 7.2$ Hz, 1H, CαH-2), 4.23(q, $J = 6.9$ Hz, 2H, $-CH_2$), 3.95 (m, 1H, CβH-1), 2.31 (m, $J = 2$H, CαH-1), 1.50 (d, $J = 7.2$, Hz, 3H, CβH-2), 1.43 (s, 9H, BOC), 1.30 (t, $J = 6.9$ Hz, 3H, $-CH_3$), 1.24 (d, $J = 6.5$ Hz, 2H, CγH-1); ^{13}C NMR (150 MHz, $CDCl_3$, 278 K) δ 172.4, 169.2, 155.3, 80.1, 78.8, 61.2, 45.8, 39.4, 28.5, 20.1, 16.3, 14.1; HRMS (ESI+) *m*/*z* calcd for $C_{14}H_{26}N_2O_6$ ($M^+ + Na$) 341.1688, found 341.1681.

Boc-(*R*)-*β*-hAa-(*R*)-Ama-(*R*)-*β*-hAa-(*R*)-Ama-OEt (3b). A solution of **18** (0.22 g, 0.69 mmol) as described for **8** gave Boc-(*R*)-*β*-hAa-(*R*)-Ama-OH **19**; 0.17 g, 94.5%) as a white solid, which was used for the further reaction.

A solution of **19** (0.17 g,0.58 mmol), HOBt (0.095 g, 0.70 mmol), and EDCI (0.134 g, 0.70 mmol) in dry CH_2Cl_2 (2 mL) was stirred at 0 °C for 15 min and treated with the above obtained amine TFA salt **20** [prepared from **18** (0.22 g, 0.69 mmol) and CF_3COOH (0.4 mL) in CH_2Cl_2 (2 mL)] and DIPEA (0.15 mL, 0.87 mmol) under nitrogen atmosphere for 5 h. Workup as described for **9** and purification by column chromatography (silica gel, 1.8% CH_3OH in $CHCl_3$) afforded **3b** (0.135 g, 48.21%) as a white solid: mp 158−160 °C; $[\alpha]^{20}_D = +246.7$ (*c* 1.04, $CHCl_3$); IR (KBr) 3340, 3269, 2980, 1748, 1685, 1560, 1525, 1265, 1141, 1091, 1061, 855 cm^{-1}; ^{13}C (150 MHz, $CDCl_3$, 278 K) δ 172.2, 171.1, 170.0, 168.7, 155.7, 81.6, 79.4, 79.2, 61.4, 44.2, 42.8, 40.0, 29.5, 28.6, 27.1, 21.1, 20.2, 16.3, 14.1; HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{39}N_4O_9$ ($M^+ + H$) 491.2717, found 491.2706.

Boc-(*R*)-*β*-hAa-(*R*)-Ama-(*R*)-*β*-hAa-(*R*)-Ama-(*R*)-*β*-hAa-(*R*)-Ama-OEt (4b). To a solution of **19** (0.075 g, 0.25 mmol), HOBt (0.042 g, 0.31 mmol), and EDCI (0.059 g, 0.31 mmol) in dry CH_2Cl_2 (5 mL) were added amine salt **21** [prepared from **3b** (0.12 g, 0.24 mmol) and CF_3COOH (0.4 mL) in CH_2Cl_2 (2 mL)] and DIPEA (0.067 mL, 0.38 mmol) and the mixture stirred at room temperature for 5 h. Workup as described for **9** and purification by column chromatography (silica gel, 2.6% CH_3OH in $CHCl_3$) furnished **4b** (0.041 g, 24.1%) as a white solid: mp 161−165 °C; $[\alpha]_D = +249.55$ (*c* 0.52, $CHCl_3$); IR (KBr) 3330, 3272, 2923, 2852, 1750, 1666, 1559, 1529, 1452, 1372, 1270, 1208, 1164, 1092, 888, 854 cm^{-1}; ^{13}C NMR (150 MHz, $CDCl_3$, 278 K) δ 184.2, 179.2, 172.6, 170.7, 169.2, 169.0, 168.8, 80.6, 80.4, 80.3, 78.9, 61.6, 53.4, 42.2, 41.6, 40.3, 38.5, 37.1, 22.2, 22.1, 20.8, 20.6, 16.3, 15.4, 15.4, 14.1; HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{51}N_6O_{12}$ ($M^+ + H$) 663.3564, found 663.3549.

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

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