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Self-Assembling Cyclic Tetrapeptide from Alternating C-Linked Carbo-β-amino Acid [(*S*)-β-Caa] and α-Aminoxy Acid [(*R*)-Ama]: A Selective Chloride Ion Receptor

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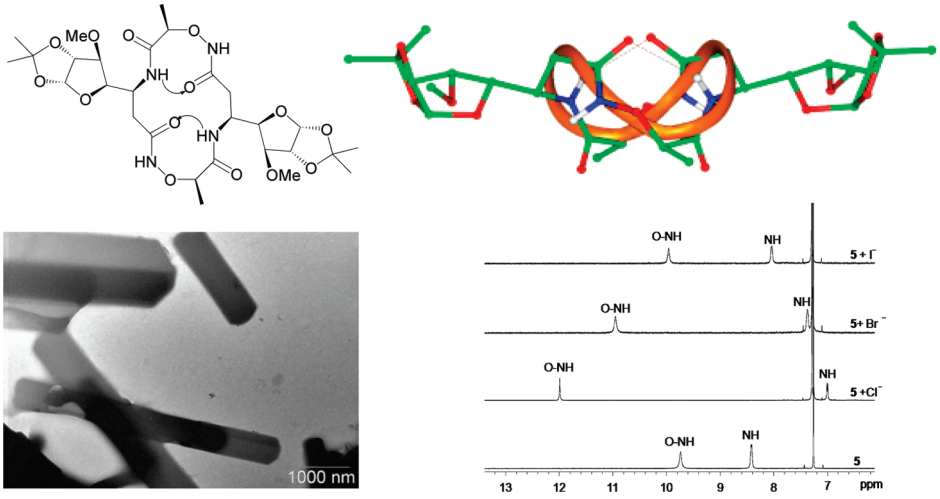

A cyclic tetrapeptide is prepared from alternating (*S*)-β-Caa (C-linked carbo-β-amino acid) and (*R*)-Ama (α-aminoxy acid). Extensive NMR (in $CDCl_3$ solution) and mass spectral (MS) studies show its halide binding capacity, with a special affinity to the chloride ion. At higher concentration it was found to form molecular aggregates as evidenced from transmission electron microscopic and atomic force microscopic analysis, confirming the formation of nanorods.

Introduction

Design and synthesis of β- and hybrid peptides from α- and homologous unnatural amino acids with interesting secondary and higher order structures has been a fascinating area of research in biopolymers.[1] For the first time in 1972, Hassal et al.[2] predicted the cyclic tetrapeptide, derived from 1:1 α- and β-amino acids, to form nanotubular structures. Since a vast number of naturally occurring cyclic peptides were shown to display biological activities,[3] research on the cyclic peptides from unnatural amino acids has drawn considerable attention. The observation from Seebach et al.[4] on stacking through intermolecular H-bonding in a cyclic β-tetrapeptide and the findings of Ghadiri et al.[5] on the transmembrane channels in β-peptides has been responsible for the surfeit of activity on cyclic β- and hybrid peptides. Further, Yang et al.[6] have reported a new class of cyclic

(1) (a) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893–4011. (b) Seebach, D.; Matthews, J. L. *Chem. Commun.* **1997**, 2015–2022. (c) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173–180. (d) Kirschenbaum, K.; Zuckerman, R. N.; Dill, D. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530–535. (e) Stigers, K. D.; Soth, M. J.; Nowick, J. S. *Curr. Opin. Chem. Biol.* **1999**, *3*, 714–723. (f) Smith, M. D.; Fleet, G. W. J. *J. Pept. Sci.* **1999**, *5*, 425–441. (g) Cheng, R. P.; Gellman, S. H.; De Grado, W. F. *Chem. Rev.* **2001**, *101*, 3219–3232. (h) Venkatraman, J.; Shankaramma, S. C.; Balaram, P. *Chem. Rev.* **2001**, *101*, 3131–3152. (i) Cubberley, M. S.; Iverson, B. L. *Curr. Opin. Chem. Biol.* **2001**, *5*, 650–653. (j) Martinek, T. A.; Fülöp, F. *Eur. J. Biochem.* **2003**, *270*, 3657–3666. (k) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodivers.* **2004**, *1*, 1111–1239. (l) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. *Nature Chem. Biol.* **2007**, *3*, 252–262. (m) Hecht, S., Huc, I., Eds. *Foldamers: Structure, Properties and Applications*; Wiley-VCH: Weinheim, Germany, 2007.

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peptides derived from α-aminoxy acids,[6a] α-aminoxy acids with α-amino acids,[6b] and small organic molecules[6c] as selective chloride ion receptors.[7] The advantage of the design in these peptides, unlike the α- and β-cyclic peptides, is that the aminoxy amide[8] NH has higher acidity over the normal amide NH and, hence, becomes a better binder of anions. Similarly, Le Grel et al.[9a] reported aza β^3-cyclopeptides, as a different class of cyclic peptides, from aza-β^3-amino acids.[9b,c] In continuation of our studies[10] on peptides from unnatural amino acids with carbohydrate side chains, herein we report the synthesis and structural investigations of linear peptide **4** and cyclic tetrapeptide **5** (Figure 1) from 1:1 alternating (*S*)-β-Caa **1** (C-linked carbo β-amino acid) and α-aminoxy acid **2** (*R*-Ama) using NMR, mass, MD, transmission electron microscopy (TEM), and atomic force microscopy (AFM).

Unlike the oligomers of α-aminoxy acid (Ama), which provided a novel class of very robust foldamers, stabilized by N−O turns,[8] the peptides reported[11] by our group prepared from (*R*)-Ama and (*R*)-β-Caa with 1:1 alternation, demonstrated a "new motif" for a 12/10-mixed helix. In these oligomers, the conformational preference of β-Caa governs that of (*R*)-Ama, driving it to behave more like a (*S*)-β^2-amino acid, thus resulting in pseudo β^2/β^3-peptides. This investigation is also pertinent, as we were intrigued by the fact that side chains play a special role in controlling the secondary structures.[11] To further explore the competing conformational preferences of these monomers, we present our studies on cyclic peptide derived from alternating (*S*)-β-Caa and

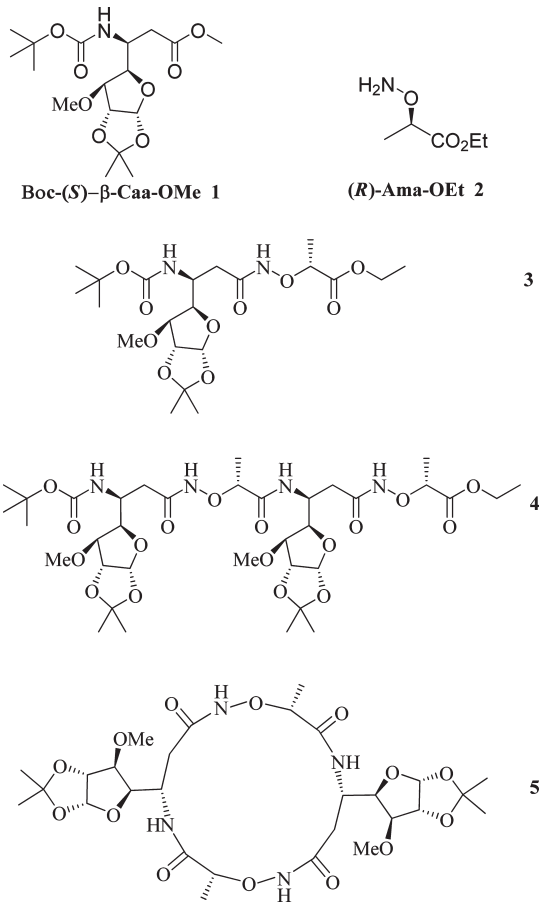


FIGURE 1. Structures of hybrid peptides **3**−**5**.

(*R*)-Ama. In contrast to the linear oligomers with alternating (*R*)-β-Caa and an (*R*)-Ama, which could not be cyclized, the cyclization of tetrapeptide with alternating (*S*)-β-Caa and an (*R*)-Ama was rather facile. This observation is analogous to the inference drawn by Le Grel et al.[9] in aza-β^3-peptides.[12] Similar observations were made by Yang et al.[13] in the oligomers of α-aminoxy acids where the chirality switch from homo- to heterochiral resulted in helical scaffolds and cyclized systems, respectively. As the amide protons of the α-aminoxy acid are known to be better H-bond donors while interacting with anions,[6b,8] in the present study, the anion-binding properties of cyclic-[(*S*)-β-Caa-(*R*)-Ama-(*S*)-β-Caa-(*R*)-Ama] **5** (Figure 1) have been explored in detail. Further, the cyclic peptide **5** has favored the molecular assembly, and we have shown the formation of nanorods.

Results and Discussion

Synthesis of Hybrid Peptides 3−5. The peptides **3**−**5** (Figure 1) were synthesized in solution phase from the

(2) Hassal, C. H. In *Chemistry and Biology of Peptides: Proceedings of the Third American Peptide Symposium*; Meinhoffer, J., Ed.; Ann Arbor Sci.: Ann Arbor, MI, 1972; pp 153−157.

(3) Liskamp, R. M. J.; Rijkers, D. T. S.; Bakker, S. E. Bioactive Macrocyclic Peptides and Peptide Mimetics. In *Modern Supramolecular Chemistry: Strategies for Macrocycle Synthesis*; Diederich, F., Stang, P. J., Tykwinski, R. R., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, 2008.

(4) Seebach, D.; Matthews, J.; Meden, A.; Wessels, T.; Baerlocher, C.; McCusker, L. B. *Helv. Chim. Acta* **1997**, *80*, 173–182.

(5) (a) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993**, *366*, 324–327. (b) Clark, T. D.; Buehler, L. K.; Ghadiri, M. R. *J. Am. Che, Soc.* **1998**, *120*, 651–656. (c) Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 988–1011.

(6) (a) Yang, D.; Qu, J.; Li, W.; Zhang, Y.-H.; Ren, Y.; Wang, D.-P.; Wu, Y.-D. *J. Am. Chem. Soc.* **2002**, *124*, 12410–12411. (b) Yang, D.; Li, X.; Sha, Y.; Wu, Y.-D. *Chem.—Eur. J.* **2005**, *11*, 3005–3009. (c) Li, X.; Shen, B.; Yao, X.-Q.; Yang, D. *J. Am. Chem. Soc.* **2007**, *129*, 7264–7265.

(7) (a) Gerenscser, G. *Chloride Transport Coupling in Biological Membranes and Epithelia*; Elsevier: Amsterdam, 1984. (b) Aschroft, F. M. *Ion Channels and Disease*; Academic Press: New York, 2000.

(8) Li, X.; Yang, D. *Chem. Commun.* **2006**, 3367–3379.

(9) (a) Salaun, A.; Mocquer, C.; Perochon, R.; Lecorgne, A.; Le Grel, B.; Potel, M.; Le Grel, P. *J. Org. Chem.* **2008**, *73*, 8579–8582. (b) Cheguillaume, A.; Salaun, A.; Sinbandhit, S.; Potel, M.; Gall, P.; Baudy-Floc'h, M.; Le Grel, P. *J. Org. Chem.* **2001**, *66*, 4923–4929. (c) Cheguillaume, A.; Doubli-Bounoua, I.; Baudy-Floc'h, M.; Le Grel, P. *Synlett* **2000**, 331–334. (d) Le Grel, P.; Salaun, A.; Potel, M.; Le Grel, B.; Lassagne, F. *J. Org. Chem.* **2006**, *71*, 5638–5645.

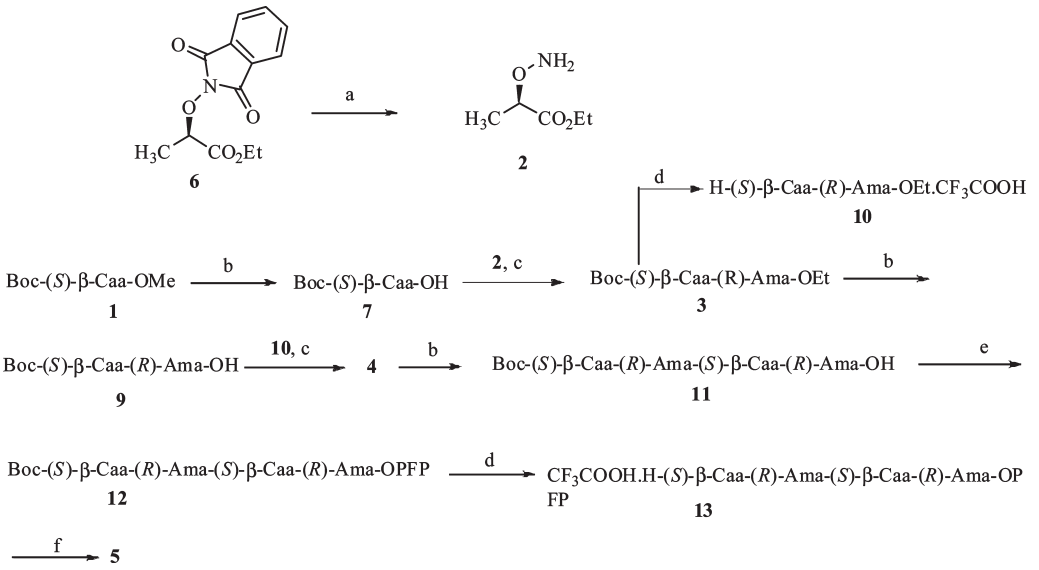
(10) (a) Sharma, G. V. M.; Ravinder Reddy, K.; Radha Krishna, P.; Ravi Sankar, A.; Narsimulu, K.; Kiran Kumar, S.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. *J. Am. Chem. Soc.* **2003**, *125*, 13670–13671. (b) Sharma, G. V. M.; Ravinder Reddy, K.; Radha Krishna, P.; Ravi Sankar, A.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. *Angew. Chem., Int. Ed.* **2004**, *43*, 3961–3965. (c) Sharma, G. V. M.; Nagender, P.; Radha Krishna, P.; Jayaprakash, P.; Ramakrishna, K. V. S.; Kunwar, A. C. *Angew. Chem., Int. Ed.* **2005**, *44*, 5878–5882. (d) Sharma, G. V. M.; Jadhav, V. B.; Ramakrishna, K. V. S.; Jayaprakash, P.; Narsimulu, K.; Subash, V.; Kunwar, A. C. *J. Am. Chem. Soc.* **2006**, *128*, 14657–14668. (e) Srinivasulu, G.; Kiran Kumar, S.; Sharma, G. V. M.; Kunwar, A. C. *J. Org. Chem.* **2006**, *71*, 8395–8400. (f) Sharma, G. V. M.; Babu, B. S.; Ramakrishna, K. V. S.; Nagendar, P.; Kunwar, A. C.; Schramm, P.; Baldauf, C.; Hofmann, H.-J. *Chem.—Eur. J.* **2009**, *15*, 5552–5566.

(11) Sharma, G. V. M.; Manohar, V; Dutta, S. K.; Subash, V; Kunwar, A. C. *J. Org. Chem.* **2008**, *73*, 3689–3698.

(12) (a) De Santis, P.; Morosetti, S.; Rizzo, R. *Macromolecules* **1974**, *7*, 52–58. (b) Khazanovich, N.; Granja, J. R.; McRee, D. E.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994**, *116*, 6011–6012. (c) Hartgerink, J. N.; Granja, J. R.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1996**, *118*, 43–50. (d) Clark, T. D.; Buriak, J. M.; Kobayashi, K.; Isler, M. P.; McRee, D. E.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 8949–8962. (e) Bong, D. T.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 2163–2166. (f) Rosenthal-Aizman, K.; Svensson, G.; Undén, A. *J. Am. Chem. Soc.* **2004**, *126*, 3372–3373. (g) Ashkenasy, N.; Horne, W. S.; Ghadiri, M. R. *Small* **2006**, *1*, 99–101.

(13) Li, X.; Wu, Y.-D.; Yang, D. *Acc. Chem. Res.* **2008**, *41*, 1428–1438.

SCHEME 1. Synthesis of Peptides 3−5[a]



[a]Key: (a) $N_2H_4 \cdot .H_2O$, CH_3OH, rt, 2 h; (b) aq 4 N NaOH, CH_3OH, 0 °C to rt, 2 h; (c) HOBt, EDCI, DIPEA, dry CH_2Cl_2, 0 °C−rt, 5 h; (d) CF_3COOH, dry CH_2Cl_2, 0 °C−rt, 2 h; (e) EDCI, pentafluorophenol (PFP), dry CH_2Cl_2, 0 °C−rt, 2 h; (f) DIPEA, dry CH_3CN, 70 °C, 3 h.

monomers **1** and **6**. Accordingly, ester **1** (Scheme 1) was subjected to hydrolysis with aq 4 N NaOH at room temperature to afford the acid **7** in 92% yield. Coupling of the acid **7** with the ester **2** (prepared from **6**[11] with hydrazine hydrate in methanol) in the presence of EDCI and HOBt in CH_2Cl_2 at room temperature for 5 h furnished the dipeptide **3** in 67% yield.

Base (aq 4 N NaOH) hydrolysis of dipeptide **3** gave the acid **9**, while the corresponding amine salt **10** was derived from **3** on exposure to CF_3COOH in CH_2Cl_2 for 2 h. The thus-derived acid **9** was then coupled (EDCI, HOBt, DIPEA) with amine **10** in CH_2Cl_2 for 5 h to furnish the pseudo β^2- and β^3-tetrapeptide **4** in 52% yield. Reaction of **4** with NaOH in CH_3OH gave the corresponding acid **11** (92%), which on reaction with pentafluorophenol[14] in the presence of EDCI in CH_2Cl_2 at room temperature for 2 h furnished the ester **12**. Further, ester **12** on exposure to CF_3COOH in CH_2Cl_2 for 2 h was converted into the corresponding amine salt **13**. Slow addition of a solution of **13** into acetonitrile containing DIPEA at 70 °C for 2 h furnished the cyclic tetrapeptide **5** in 55% yield.

Conformational Analysis. The NMR studies on peptides **4** (3.5 mM) and **5** (1.3 mM) were undertaken in $CDCl_3$ solution. For the linear tetrapeptide **4**, it was difficult to obtain detailed structural information due to the presence of several isomers and the resulting broadening of the spectral lines and exchange peaks in the ROESY experiments. For the major isomer, lack of dispersion in the ^{1}H NMR and non distinctive values of $^3J_{NH\text{-}C\beta H} \approx 8$ Hz for the β-Caa residues suggest either an averaging over several structures or absence of a well-defined structure. This was further confirmed from the solvent titration studies (adding 33% v/v of DMSO-d_6 in $CDCl_3$ solution), wherein only one amide proton (NH(3)) appeared to be H-bonded as is reflected in the small change in its chemical shift ($\Delta\delta$) of 0.41 ppm.[15] However, several medium range NOE correlations such as NH(1)/C4H(1), CβH(1)/NH(2), CαH(1)/NH(2), NH(3)/C4H(3), CβH(3)/NH(4), CαH(3)/NH(4), C4H(1)/NH(2), C4H(3)/NH(4), and NH(2)/NH(3) were observed.

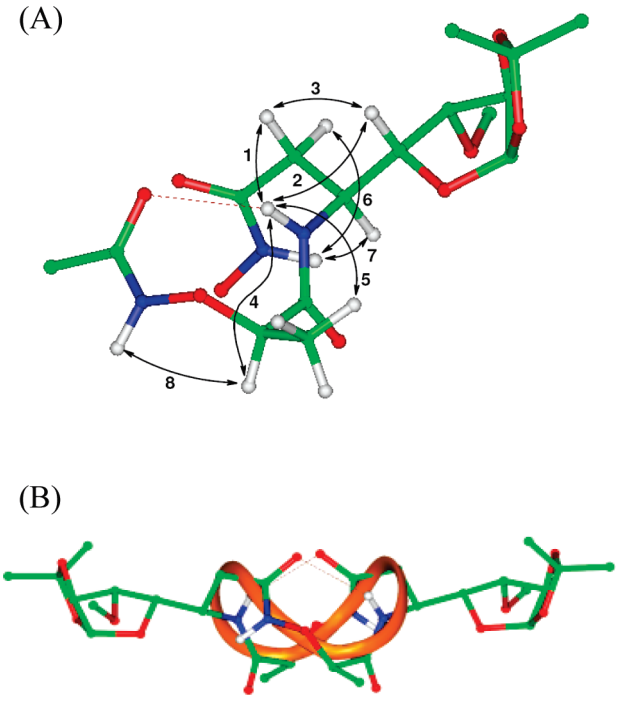


FIGURE 2. (A) Characteristic NOE correlations: β-CaaNH/β-CaaCαH(*pro-S*), β-CaaNH/β-CaaC4H, β-CaaC4H/β-CaaCαH-(*pro-S*), β-CaaNH/AmaCαH, β-CaaNH/Ama CβH, AmaNH/β-CaaCαH(*pro-R*), AmaNH/β-CaaCβH and AmaNH/AmaCαH are depicted as 1−8, respectively, in the partial structure of peptide **5**. (B) One of the lowest energy structures derived from the restrained MD studies, where the backbone is highlighted with a band.

(14) Seebach, D.; Overhand, M.; Kuhnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913–941.

(15) See the Supporting Information.

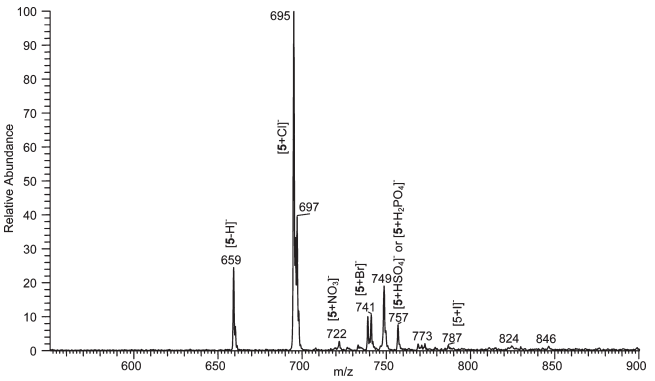


FIGURE 3. Negative ion ESI mass spectrum of cyclic peptide **5**.

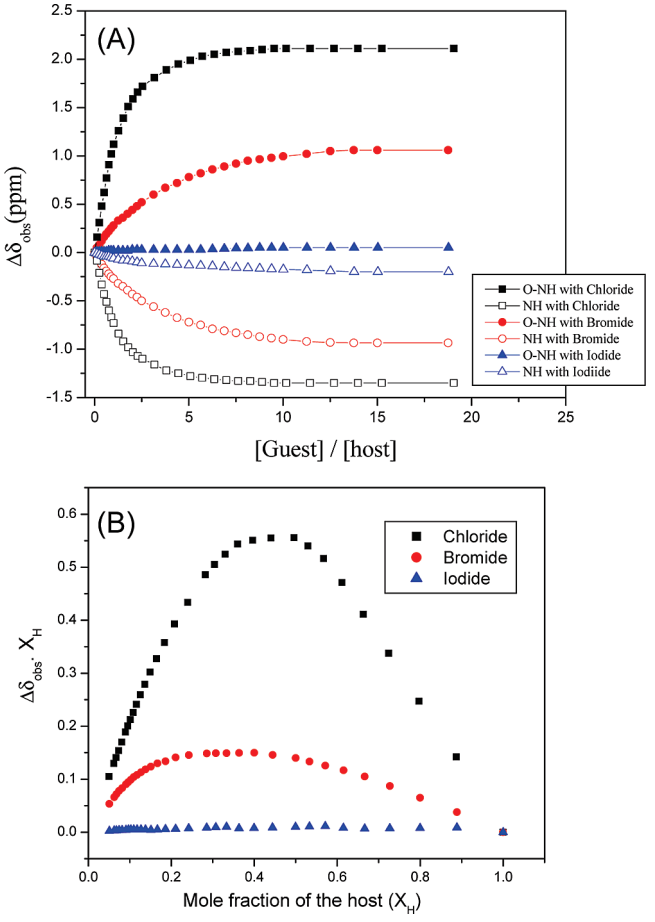


FIGURE 4. (A) Change in chemical shift of amide protons and (B) Job plot of peptide **5** upon addition of anions (Cl^-, Br^-, and I^-).

As discussed above, a facile macrocyclization of **4** is suggestive of the proximity of two termini. The presence of only one set of peaks from (*R*)-Ama and (*S*)-*β*-Caa in **5** is consistent with 2-fold molecular symmetry in the NMR time scale. The amide protons for the (*S*)-*β*-Caa residue appeared at 8.35 ppm, suggesting their participation in H-bonding. This was further confirmed from the solvent titration studies, wherein a very small change in its chemical shift ($\Delta\delta$) of 0.09 ppm was observed.[15] For (*S*)-*β*-Caa, the large $^3J_{\text{NH-C}\beta\text{H}}$ (~10 Hz) and NOE correlations *β*-Caa NH/*β*-CaaCαH(*pro-S*) and *β*-CaaNH/*β*-CaaC4H suggest the dihedral angles around

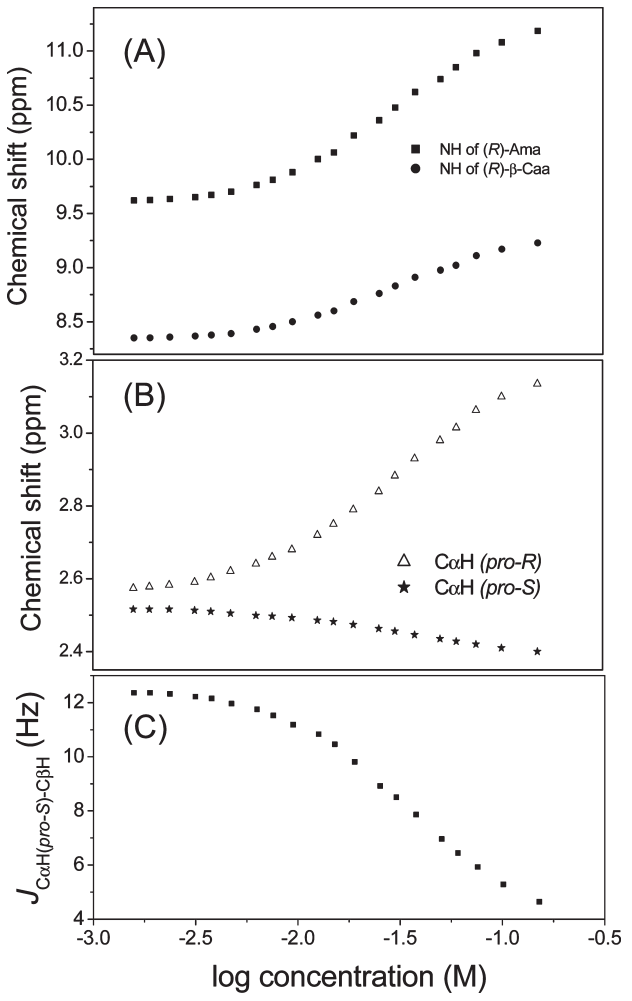


FIGURE 5. (A) Concentration dependence of amide protons and (B) CαHs of *β*-Caa of peptide **5** and (C) change of $^3J_{\text{C}\alpha\text{H}(pro\text{-}S)\text{-C}\beta\text{H}}$ with concentration in peptide **5**.

C(O)−N−C*β*−Cα (ϕ) ≈ 120° and N−C*β*−Cα−C(O) (θ) ≈ −60°. Unlike for the linear analogue from (*R*)-Ama/(*R*)-*β*-Caa, where the N−O turns are conspicuous by their absence, these dihedral angles along with the NOE correlations, *β*-CaaNH/Ama CαH, *β*-Caa NH/Ama C*β*H, Ama NH/*β*-Caa C*β*H, Ama NH/*β*-Caa CαH(*pro-R*), and Ama NH/Ama CαH (Figure 2A), support an 8-membered N−O turn between carbonyls and amides of the two *β*-Caa residues [*β*-Caa(CO) -*β*-Caa′(NH) and *β*-Caa′(CO)-*β*-Caa (NH)]. One of the lowest energy structures derived from the restrained molecular dynamics (MD) studies is shown in Figure 2, where the backbone bracelet-like structure is highlighted with a band. Very similar structures have been observed by Yang et al.[13] for 16- and 24-membered macrocycles from cyclic-$(Ama)_4$ and cyclic-$(Ama)_6$, respectively, and a 21-membered macrocycle of cyclic-(Ama-α amino acid)$_3$.

Anion-Binding Studies. Anion-binding properties were investigated with the help of NMR and mass spectrometry. Negative-ion electrospray ionization of a methanolic solution of the cyclic peptide **5** mixed with NH_4F, NH_4Cl, NH_4Br, NH_4I, $NaNO_2$, $NaNO_3$, NaN_3, Na_2SO_4, $NaHSO_4$, Na_3PO_4, Na_2HPO_4, NaH_2PO_4, $KHCO_3$, and K_2CO_3 solutions gave rise to the spectrum shown in Figure 3. The spectrum shows

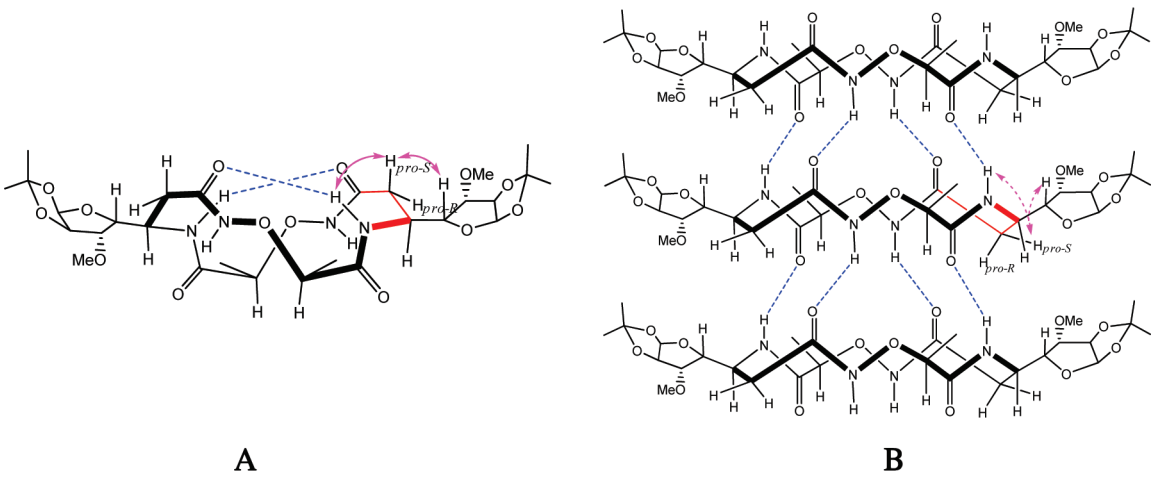


FIGURE 6. Dihedral angle N−Cβ−Cα−C(O) (θ) of (*S*)-β-Caa (shown in red) in (A) dilute solution (1.3 mM), $\theta \approx -60°$ and (B) concentrated solutions (~100 mM), $\theta \approx 60°$ forming nanorod like structure in $CDCl_3$ (the H-bonds arerepresented by blue dashed lines; the solid and dashed arrows in magenta represent strong and weak intensity NOE correlations, respectively).

the peaks corresponding to $[\mathbf{5} + Cl]^-$ (*m*/*z* 695), $[\mathbf{5} + Br]^-$ (*m*/*z* 739), $[\mathbf{5} + I]^-$ (*m*/*z* 787), $[\mathbf{5} + NO_3]^-$ (*m*/*z* 722), and $[\mathbf{5} + HSO_4]^-$ or $[\mathbf{5} + H_2PO_4]^-$ (*m*/*z* 757).

It is noteworthy that the abundance of $[\mathbf{5} + Cl]^-$ is higher as compared to other adduct ions, suggesting stronger affinity of Cl^- toward the cyclic peptide **5**. To investigate the nature of the adduct ion complex, the MS/MS spectrum of $[M + Cl]^-$ (*m*/*z* 695) was examined. The spectrum[15] shows mainly $[M - H]^-$ (*m*/*z* 659) at various collision energies.

This suggests that a loosely bound noncovalent ion−molecule complex is formed between Cl^- and the peptide under the given experimental conditions. NMR titration studies provide further evidence for the anion binding (Figure 4), wherein sequential addition of the halogen ions (Cl^-, Br^-, I^-) reveals significant changes in the spectra with the signals of the aminoxy amides shifting downfield, while those of the β-Caa amide protons show upfield shift (Figure 4).[16] These results imply that the aminoxy amides in the **5**−anion complex are H-bonded, unlike in the free peptide **5**. The NMR studies of the halogen binding[15] are comparable with the data reported by Yang et al.,[6b] implying identical mode of binding. Thus, the 8-membered N−O turn gets disrupted (β-CaaNH shifts upfield) and the acidic aminoxy NH forms strong H-bond with the Cl^- (large downfield shift observed upon complexation). A Job plot of the amide proton chemical shift against the mole fraction of the ions provided emphatic support for a 1:1 complex of the Cl^- ion with **5** (Figure 4).[16b,17] In conformity with mass spectrometry data, the complex with I^- was rather weak, and no definitive information on it could be obtained. However, the complex with the Br^- ion appears to be a 1:1 complex. The association constant (K_a) was determined by a titration method reported by Kelly et al.[18] The complex is very selective for Cl^-, with an association constant (K_a) of about 513 ± 70 M^{-1}. The K_a value for the $[\mathbf{5} + Br]^-$ complex is about 111 ± 11 M^{-1}. These observations are in line with the mass spectrometric data, where the halogen ion−**5** complex intensities follow the $Cl^- > Br^- > I^-$ pattern. These values of the K_a are much smaller than those found in cyclic hexapeptides by Yang et al.[6b] presumably due to smaller ring size in **5**. The linear peptide **4** displayed very poor binding with these halide anions.[15]

Studies on Molecular Association and Nanorod Assembly. Changes in the chemical shifts as a function of the concentration provide a handy tool for studying molecular association. We have observed very substantial changes in chemical shifts of both the amide and Cα protons of (*S*)-β-Caa (Figure 5), supporting molecular association. Amide protons display large downfield shifts (1.58 ppm and 0.88 ppm for (*R*)-Ama and (*S*)-β-Caa, respectively) implying their involvement in H-bonding.

A close look at ^1H NMR spectra with varying concentration revealed that in addition to changes in the chemical shifts, the coupling constant $^3J_{C\alpha H(pro\text{-}S)\text{-}C\beta H}$ also changes as a function of concentration (from a value of 12.4 to 4.6 Hz in 1.3 and 151.5 mM solution in $CDCl_3$, respectively), indicating a definite conformational change in backbone conformation.

Two small $^3J_{C\alpha H-C\beta H}$ values (4.1 and 4.6 Hz in 151.5 mM solution in $CDCl_3$) and the weakening the NOEs of β-CaaCαH-(*pro*-*S*) with both β-CaaNH and β-CaaC4H, compared to that in dilute solutions, gives strong evidence of change in the dihedral angle θ from ~−60° (Figure 6A) to ~60° (Figure 6B). This in turn disrupts the 8-membered N−O turn and leaves the amide and carbonyl groups in axial disposition, facilitating the intermolecular H-bonding (Figure 6). A plausible model has been presented in Figure 6B. The side chains thus occupy equatorial position on the exterior of the flat, disklike peptide ring, as observed for other β-peptides.[5]

Additional evidence for the molecular association is garnered from the presence of *m*/*z* 1319 ($[2M - H]^-$), a dimeric peak of **5** in the MS/MS.[15] Very large intensity of the $[M - H]^-$ (*m*/*z* 659) peak in the MS/MS suggests that the dimeric complex of the peptide is loosely bound.[15]

Transmission electron microscopy (TEM) was used to obtain further insight of the molecular association and self-assembly of the peptide **5** into nanorods (Figure 7). Aliquots of the sample were applied to previously cleaved mica and left to evaporate. The samples were subsequently floated on a water−methanol mixture and collected over 400 mesh

(16) (a) Macomber, R. S. *J. Chem. Educ.* **1992**, *5*, 375–378. (b) Blanda, M. T.; horner, J. H.; Newcomb, M. *J. Org. Chem.* **1989**, *54*, 4626–4636.
(17) Gil, V. M. S.; Oliveira, N. C. *J. Chem. Educ.* **1990**, *67*, 473–478.
(18) Kelly, T. R.; Kim, M. H. *J. Am. Chem. Soc.* **1994**, *116*, 7072–7080.

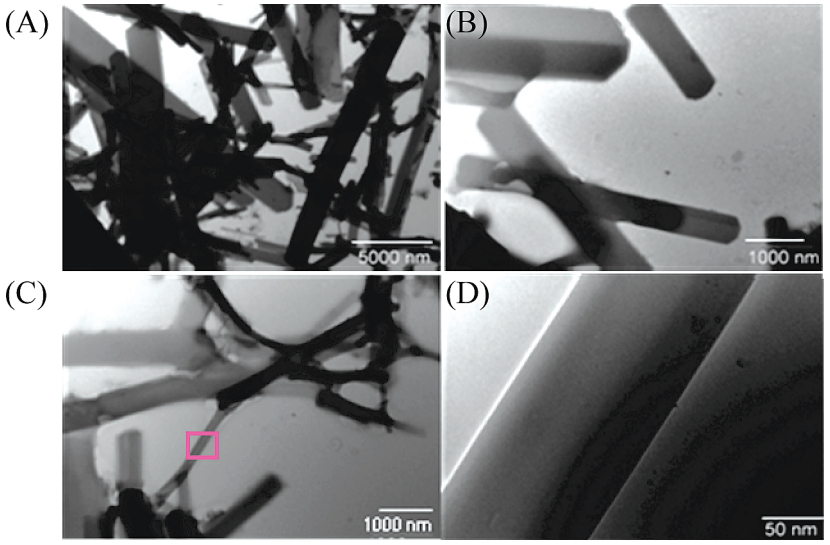


FIGURE 7. (A) TEM image of the nanorods formed by **5** from a 1 mM solution in $H_2O-MeOH$. (B) Magnified image of the micrograph. (C) TEM image of nanorods formed by **5** from diluted solution (1 μM). (D) Magnified portion from image C showing single nanorod of width ~120 nm.

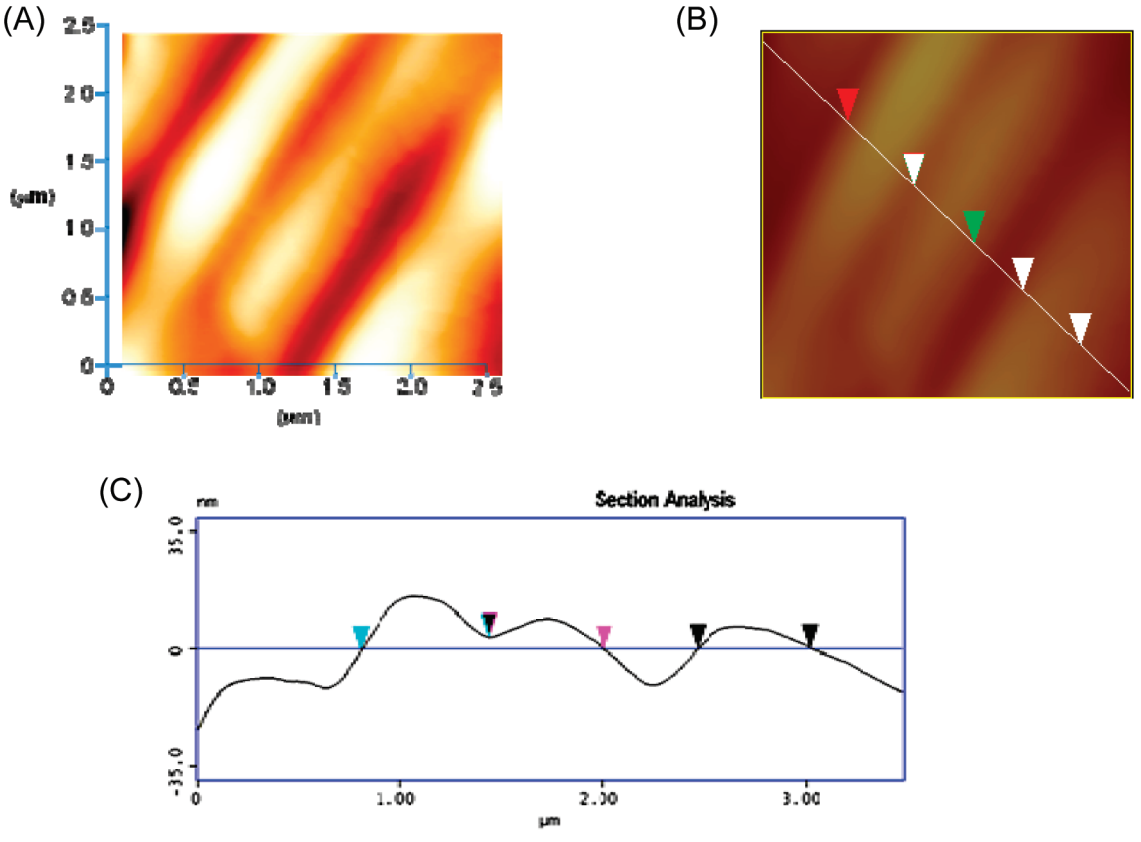


FIGURE 8. (A) AFM image of peptide **5** from 50 μM solution in MeOH. (B) A topographic profile of (A). (C) Section analysis of (B).

Cu/Rh grids, which were then negatively stained with 2% uranyl acetate solution. Under these conditions, as can be seen from Figure 7, both 2D and 3D structures are formed probably due to piling of individual rods at 1 mM concentration. The nanorods are mainly observed as single entities or grouped into rods. However, when dilute (1 μM) samples were taken, the peptides self-assembled into rods of length in the micrometers range with a width of about 150 nm (Figure 7C).

The self-assembly of molecules into rodlike structures was also observed by atomic force microscopy (AFM) (Figure 8). At 50 μM concentration and longer incubation time, rodlike structures with a shape similar to those observed by TEM are formed. The height of the nanorods was obtained by doing section analysis of the observed image. The aggregates heights for the peptide deposited onto a mica substrate from a fresh 50 μM solution of **5** in MeOH (Figure 8) is found to be

in the range of 10−20 nm. Different heights were observed probably due to piling of individual rods as aggregates at 1 mM concentration.

Both TEM and AFM techniques, in spite of the use of two different substrates (Cu/Rh grid and mica respectively), provide equivalent nanorod structures. As depicted in Figure 6, intermolecular association of macrocyles give rise to nanostructured rods at 1 mM concentration. The surfaces of these assemblies appear smooth and uniform throughout the length, which suggests that the rods are made up of tens of tightly packed peptide nanorods aligned parallel to each other. Based on a "hierarchical" process proposed by Dory and co-workers,[20] it is believed that it involves an assembly of individual peptide nanorods over several generations to end up in the formation of nano- to micro- structured rods, which most probably happens due to noncovalent interactions among the nanorods.

Conclusions

The present study demonstrates that, unlike the peptides from (*R*)-*β*-Caa/(*R*)-Ama, (*S*)-*β*-Caa/(*R*)-Ama can easily be cyclized. NMR and mass spectral studies reveal that the symmetric cyclic peptide **5**, with two N−O turns, shows selective and appreciable binding affinity with the chloride ion, while the linear tetrapeptide **4** does not display this capability. NMR studies as a function of solute concentration provide ample support of the intermolecular H-bonding, which results in the nanomolecular assembly of these cyclic peptides explored in detail by TEM and the AFM studies. This new class of pseudo β^2- and β^3-peptides from (*S*)-*β*-Caa and (*R*)-Ama may aid in the design of diverse peptide scaffolds with desired functional features.

Experimental Section

The peptides were synthesized following standard solution-phase peptide coupling methods[19] using 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxy-benzotriazole (HOBt) as coupling agents and dry CH_2Cl_2 as solvent. Saponification of tetrapeptide using NaOH in MeOH gave free acid, and treatment with pentafluorophenol (PFP) in CH_2Cl_2 using EDCI as esterification agent gave activated ester. This activated ester was subjected to Boc deprotection using TFA in CH_2Cl_2. The TFA salt of activated ester was taken in CH_3CN (0.01 M) and slow addition into *N*,*N*-diisopropylethylamine (DIPEA; 1.5 equiv) in CH_3CN (0.003 M) at 70 °C, after aqueous workup and chromatographic purification furnished the product **5** in 55% yield.

Boc-(*S*)-*β*-Caa-(*R*)-Ama-OEt (3). A solution of ester **1**[21] (0.80 g, 2.13 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 (3 × 10 mL). The organic layer was dried (Na_2SO_4) and concentrated to give **7** (0.71 g, 92%) as a white solid, which was used for further reaction without any purification.

A solution of acid **7** (0.70 g, 1.93 mmol), HOBt (0.31 g, 2.32 mmol), and EDCI (0.44 g, 2.32 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** 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 (60−120 mesh silica gel, 50% ethyl acetate and petroleum ether) to afford **3** (0.62 g, 67%) as a white solid: mp 95−96 °C; $[\alpha]_D$ = +86.6 (*c* 0.26, $CHCl_3$); IR (KBr) 3267, 2988, 2930, 1751, 1736, 1670, 1660, 1550, 1168, 1055, 1021, 889, 855 cm^{-1} ; ^1H NMR (500 MHz, $CDCl_3$) *δ* 9.34 (s, 1H, NH-2), 5.90 (d, *J* = 3.9 Hz, 1H, C1H-1), 5.11 (br, 1H, NH-1), 4.58 (d, *J* = 3.9 Hz, 1H, C2H-1), 4.51 (q, *J* = 7.2 Hz, 1H, CαH-2), 4.31 (m, 1H, C*β*H-1), 4.23 (m, 2H, $-CH_2$), 4.10 (br, 1H, C4H-1), 3.76 (br, 1H, C3H-1), 3.41 (s, 3H, OCH_3-1), 2.54 (dd, *J* = 5.8, 15.0 Hz, 1H, CαH(*pro-S*)-1), 2.39 (dd, *J* = 5.0, 15.0 Hz, 1H, CαH(*pro-R*)-1), 1.66 (d, *J* = 7.2 Hz, 3H, C*β*H-2), 1.50, 1.48 (2s, 6H, Acetonide-CH_3), 1.43 (s, 9H, BOC), 1.31 (t, *J* = 7.2 Hz, 3H, $-CH_3$); ^{13}C NMR (150 MHz, $CDCl_3$, 278 K): *δ* 172.0, 168.1, 156.0, 111.8, 104.6, 83.8, 81.2, 80.0, 79.6, 79.2, 61.3, 57.3, 47.6, 36.0, 28.2, 26.7, 26.2, 16.2, 14.1; HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{36}N_2O_{10}$ (M^+ + Na) 499.2267, found 499.2256.

Boc-(*S*)-*β*-Caa-(*R*)-Ama-(*S*)-*β*-Caa-(*R*)-Ama-OEt (4). A solution of **3** (0.2 g, 0.42 mmol) as described for **7** gave Boc-(*S*)-*β*-Caa-(*R*)-Ama-OH (**9**; 0.17 g, 90%) as a white solid, which was used for further reaction without any purification.

A solution of **3** (0.18 g, 0.38 mmol) and CF_3COOH (0.4 mL) in CH_2Cl_2 (2 mL) was stirred at 0 °C to room temperature for 2 h. Solvent was evaporated under reduced pressure, and the resulting salt **10** was dried under high vacuum and used as such without any further purification.

A solution of **9** (0.17 g, 0.38 mmol), HOBt (0.06 g, 0.45 mmol), and EDCI (0.08 g, 0.45 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 **10** and DIPEA (0.10 mL, 0.57 mmol) under nitrogen atmosphere for 5 h. Workup as described for **3** and purification by column chromatography (60−120 mesh silica gel, 1.8% CH_3OH in $CHCl_3$) afforded **4** (0.16 g, 52%) as a white solid: mp 94−96 °C; $[\alpha]^{20}_D$ = +137.4 (*c* 0.25 in $CHCl_3$); IR (KBr) 3431, 2985, 2934, 1682, 1552, 1456, 1374, 1166, 1082, 1021, 888, 856 cm^{-1} ; ^1H NMR (600 MHz, $CDCl_3$, 278 K) *δ* 10.32 (s, 1H, NH-2), 9.89 (s, 1H, NH-4), 7.81 (d, *J* = 8.2 Hz, 1H, NH-3), 5.91 (d, *J* = 3.9 Hz, 1H, C1H-3), 5.91 (d, *J* = 3.9 Hz, 1H, C1H-1), 5.38 (d, *J* = 7.7 Hz, 1H, NH-1), 4.59 (d, *J* = 3.9 Hz, 1H, C2H-3), 4.59 (d, *J* = 3.9 Hz, 1H, C2H-1), 4.54 (m, 1H, CαH-4), 4.48 (m, 1H, C*β*H-3), 4.30 (m, 1H, C4H-3), 4.28 (m, 1H, CαH-2), 4.26 (m, 1H, C4H-1), 4.21(m, 2H, ethyl ester ($-CH_2CH_3$) 4.17 (m, 1H, C*β*H-1), 3.74 (d, *J* = 3.3 Hz, 1H, C3H-1), 3.74 (d, *J* = 3.3 Hz, 1H, C3H-3), 3.37 (s, 6H, OCH_3-1, OCH_3-3), 2.50 (m, 1H, CαH-1), 2.50 (m, 1H, CàH-1), 2.48 (m,1H, CαH-3), 2.48 (m, 1H, CàH-3), 1.50 (s, 6H, acetonide-CH_3), 1.48 (m, 3H, C*β*H-4), 1.45 (m, 3H, C*β*H-2), 1.43 (s, 9H, BOC), 1.32 (s, 6H, acetonide-CH_3) 1.30(m, 3H, ethyl ester ($-CH_2CH_3$); ^{13}C (150 MHz, $CDCl_3$, 278 K): *δ* 171.1, 171.7, 169.1, 168.1, 156.5, 111.8, 111.7, 104.8, 104.7, 84.2, 83.9, 82.9, 81.3, 81.2, 80.6, 80.2, 80.0, 79.1, 61.3, 57.5, 57.4, 47.4, 45.9, 36.7, 36.4, 29.6, 29.3, 28.3, 26.7, 26.2, 16.8, 16.2, 14.0; HRMS (ESI+) *m*/*z* calcd for $C_{35}H_{58}N_4O_{17}$ (M^+ + Na) 829.3694, found 829.3669.

Cyclic-[(*S*)-*β*-Caa-(*R*)-Ama-(*S*)-*β*-Caa-(*R*)-Ama] (5). A solution of **4** (0.14 g, 0.17 mmol) as described for **7** gave Boc-(*S*)-*β*-Caa-(*R*)-Ama-(*S*)-*β*-Caa-(*R*)-Ama−OH (**11**; 0.12 g, 92%) as a white solid, mp 96−97 0 °C.

A solution of **11** (0.12 g, 0.16 mmol) was dissolved in CH_2Cl_2 (4 mL) at 0 °C and treated with pentafluorophenol (0.06 g, 0.32 mmol) and EDCI (0.06 g, 0.32 mmol) sequentially under nitrogen atmosphere. The resulting solution was stirred at room

(19) (a) Bodanszky, M.; Bodanszky, A. *The Practices of Peptide Synthesis*; Springer-Verlag: New York, 1984. (b) Grant, G. A. *Synthetic Peptides: A User's Guide*; W. H. Freeman: New York, 1992. (c) Bodanszky, M. *Peptide Chemistry: A Practical Textbook*; Springer-Verlag: Berlin, 1993.

(20) Leclair, S.; Baillargeon, P.; Skouta, R.; Gauthier, D.; Zhao, Y.; Dory, Y. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 349–353.

(21) Sharma, G. V. M.; Reddy, V. G.; Reddy, K. R. *Tetrahedron: Asymmetry* **2002**, *13*, 21–24.

temperature for 2 h. The reaction mixture was diluted with $CHCl_3$ (20 mL) and washed with 1 N HCl (20 mL) and saturated NaCl solution (20 mL). The solvent was removed under reduced pressure and residue washed with hexane to give **12** (0.14 g) in 93% yield. Crude **12** (0.14 g, 0.15 mmol) was deprotected as described for **10** to furnish amine salt **13**, which was dissolved in CH_3CN (0.01 M, 14.5 mL) and added slowly to a solution of DIPEA (0.04 mL, 0.22 mmol) in CH_3CN (73 mL, 0.003 M) at 70 °C for 2 h. Workup as described for **3** and purification by column chromatography (60−120 mesh silica gel, 2.0% CH_3OH in $CHCl_3$) furnished **5** (0.05 g, 55%) as a white solid: mp 255−257 °C; $[\alpha]_D = -50.4$ (*c* 0.25, $CHCl_3$); IR (KBr) 3416, 3281, 2988, 2928, 2854, 1704, 1659, 1567, 1380, 1260, 1232, 1164, 1079, 1025, 956, 860 cm^{-1}; ^1H NMR (600 MHz, 0.5 mg in 600 μL, concn 1.3 mM, $CDCl_3$, 303 K) δ 9.62 (s, 1H, NH-Ama), 8.35 (d, $J = 10.2$ Hz, 1H, NH-Caa), 5.88 (d, $J = 3.9$ Hz, 1H, C1H), 4.58 (d, $J = 3.9$ Hz, 1H, C2H), 4.57 (m, 1H, CβH-Caa), 4.39 (q, $J = 7.0$ Hz, 1H, CαH-Ama), 4.08 (dd, $J = 3.5, 8.4$ Hz, 1H, C4H), 3.61 (d, $J = 3.5$ Hz, 1H, C3H), 3.39 (s, 6H, OCH_3), 2.58 (dd, $J = 3.7, 16.7$ Hz, 1H, CαH (*pro-R*)-Caa), 2.52 (dd, $J = 12.3, 16.7$ Hz, 1H, CαH (*pro-S*)-Caa), 1.52 (s, 3H, acetonide-CH_3), 1.44 (d, $J = 7.0$ Hz, 3H, CβH-Ama), 1.31 (s, 6H, acetonide-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$, 278 K) δ 172.8, 167.5, 112.0, 104.8, 83.4, 82.5, 80.9, 80.6, 57.5, 29.3, 26.7, 17.4, 14.2; HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{44}N_4O_{14}$ ($M^+ + H$) 661.2932, found 661.2920.

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Supporting Information Available: General experimental procedures, protocols for NMR, mass, TEM, AFM, and MD studies, detailed mass spectral studies, copies of ^1H NMR and ^{13}C NMR data for peptides **3**−**5**, copies of TOCSY and ROESY spectra of peptides **4** and **5**, graph showing solvent dependence of NH chemical shifts at varying concentration of DMSO-d_6 in $CDCl_3$, distance constraints used in MD calculations, copies of mass spectra for peptide **5**, structural studies of peptide **5**, and chemical shift of amide protons of **5** at different temperatures in DMSO-d_6 solvent. This material is available free of charge via the Internet at http://pubs.acs.org.