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PAPER

Design and synthesis of *trans*-3-aminopyran-2-carboxylic acid (APyC) and α/β-peptides with 9/11-helix†‡

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A new β-amino acid, *trans*-3-aminopyran-2-carboxylic acid (APyC), was designed and synthesized from (*R*)-glyceraldehyde derivative and used in the synthesis of α/β-peptides in a 1 : 1 alternating pattern with D-Ala. The presence of oxygen atom at the Cβ²-position in APyC was envisaged to provide opportunity for additional interaction. These hybrid peptides have shown the presence of 9/11-helix through extensive NMR and MD studies. The amide protons of D-Ala, in addition to participating in 9-mr H-bonding with CO of succeeding β-residue, were also involved in additional electrostatic interaction with pyran ring oxygen of preceding β-residue, which facilitated further stabilization to the 9/11-mixed helix. The study thus results in a new 'motif' for a 9/11-helix, and the first example from a cyclic β-amino acid.

Introduction

Designing and engineering of bio-molecules such as peptides and proteins,[1] either to understand their functions or to define new functions, has become an important area of research towards bio-medical and materials applications. The initial studies of Gellman *et al.* and Seebach *et al.* on β-peptides, resulting in new 12- and 14-helical patterns,[2] led to the emergence of a frontier field of 'foldamers'.[3] Further quest of the investigators in this active field resulted in a wealth of new peptide classes with heterogeneous backbones having more than one type of monomer residues. These efforts led to the synthesis of α/β-, α/γ-, α/δ-, α/ε-, β/γ- and other classes of hybrid peptides[4,5] with a variety of unidirectional and mixed helical patterns. More recently, 'hybrid helices' resulted from tethering together different helix types as shown by Gellman *et al.*[6] and Sharma *et al.*[7]

In the case of heterogeneous peptides, extensive studies have been carried out on α/β-peptides. The first results by Gellman *et al.*[4a] and Reiser *et al.*[4b] have shown 'split' 11, 14/15-helices and 13-helix respectively. Later designs by Sharma *et al.*[4c] with L-Ala and (*S*)-β-Caa (C-linked carbo β-amino acid) led to the emergence of another new 9/11(11/9)-mixed helix, while Chandrasekhar *et al.*[4d] reported a split pattern in α/β-peptides derived from sugar amino acid. Gellman *et al.*[8] have published several studies on α/β-peptides and arrived at different structural and biological features. The theoretical studies,[9] from the initial years of the work in this area, complemented the experimental results. Further, the studies on α/β-peptides by Hofmann *et al.*[10] predicted 9/11-helix ($i \rightarrow i+1/i \leftarrow i+3$) and 11/9-helix ($i \leftarrow i+3/i \rightarrow i+1$) as the most stable conformations.

In the earlier study on α/β-peptides, Gellman *et al.*[4a,11] noticed that the ACPC-containing peptides showed non-sequential nOes giving strong evidence for a folded conformation, while, the peptides made from ACHC showed no helical pattern. In view of the above observations, it was felt worthwhile to explore possibilities of additional interactions which may stabilize folded structures in six membered side chains like ACHC. The studies by Grierson *et al.*[12] on the oligomers of phenylisoserine, based on the literature findings[13] on taxol side chain, revealed stabilization of C6 stranded folds, by additional 5-mr H-bonding[14] between the N–H···O (hydroxyl). We have thus proposed to introduce an oxygen atom at β²-position in ACHC, replacing the '–CH_2–' group, to result in the design of a new pyran based β-amino acid, *trans*-3-aminopyran-2-carboxylic acid (APyC). A literature survey on peptides from sugar amino acids (SAA),[15a–f] though revealed no such H-bonding pattern, a recent report by Gervay-Hague *et al.*[15g] on sialic acid derived α/δ-hybrid peptides has shown such an interaction of the pyranose ring oxygen of Neu2en with L-Gluc HN, which appears to be unique to sialic acid moieties. Based on the above assumptions, the present study describes the design and synthesis of new β-amino acid, APyC **1**. Conversion of **1** into a series of α/β-peptides **3–10** (Fig. 1 and 2) with 1 : 1 alternation of D-Ala **2**, and the conformational analysis of the above peptides by extensive NMR spectroscopy supported by Molecular Dynamics (MD) and Infrared (IR) studies.

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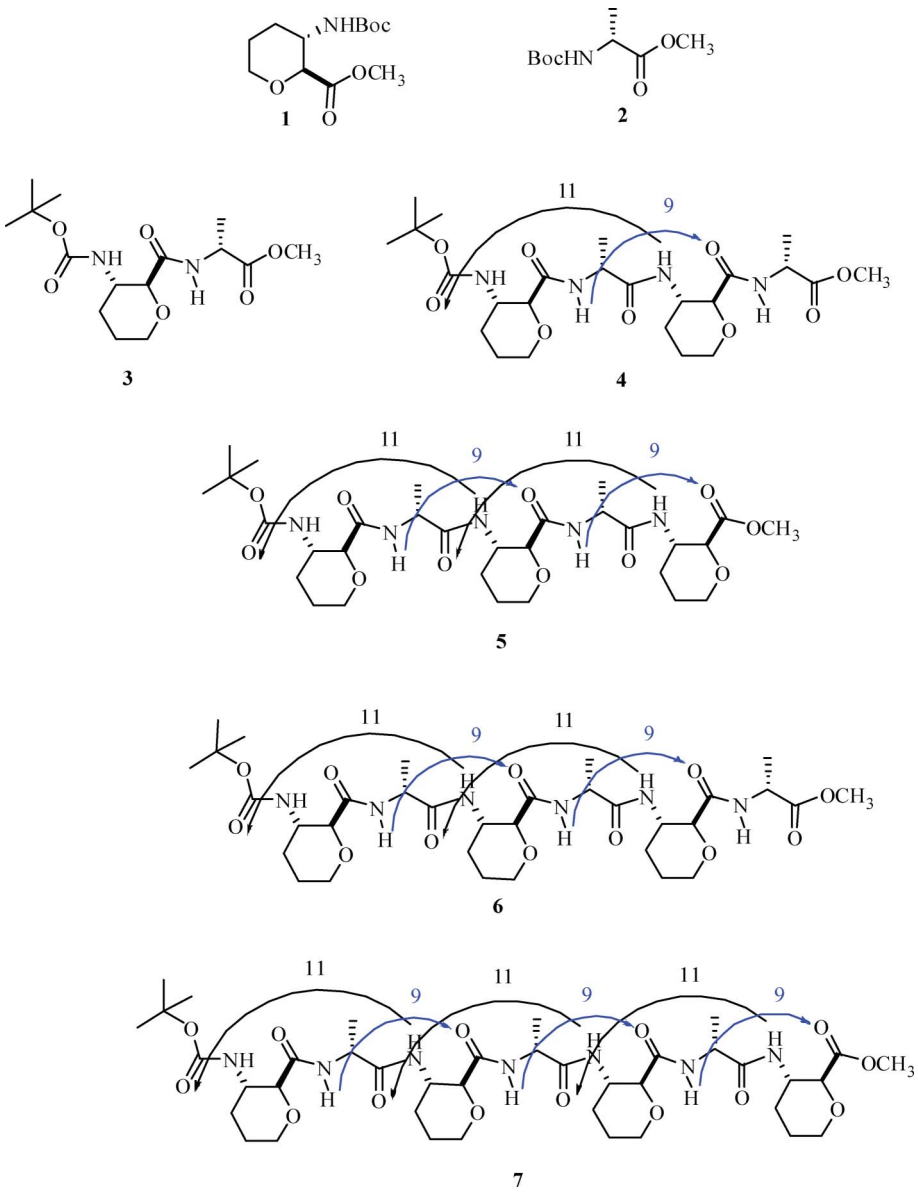


Fig. 1 Structures of the peptides **3–7** (arrows show hydrogen bonding).

Results and discussions

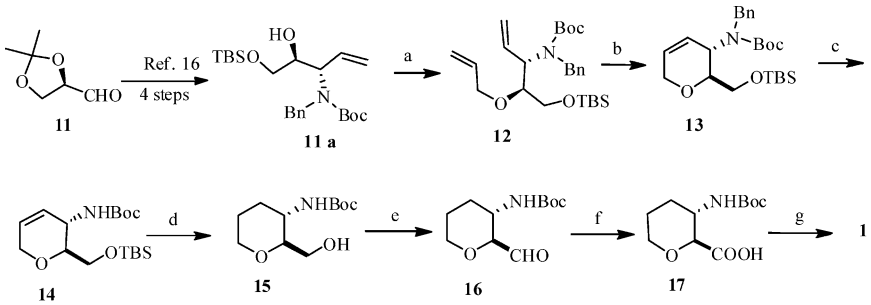
1. Synthesis of amino acid 1

The new pyran β-amino acid (**1**) was synthesized from the known compound **11a**, which was derived from (*R*)-2,3-*O*-isopropylidene-D-glyceraldehyde **11**[16] (Scheme 1). Accordingly, reaction of **11a** with allyl bromide (NaH, DMF) afforded the allyl ether **12** (79%). RCM reaction on diene **12** in toluene at reflux for 8 h in the presence of Grubb's catalyst I[17] (5 mol%) afforded **13** (80%).

Debenzylation of **13** under Birch reaction conditions (Li, Liq. NH_3) at −78 °C in THF for 1 h afforded the amide **14** (68%), which on hydrogenation with 10% Pd–C in MeOH under hydrogen atmosphere gave **15** (93%). Sequential oxidation of alcohol **15** under Swern reaction conditions using $(COCl)_2$, DMSO and Et_3N in CH_2Cl_2, followed by further oxidation of aldehyde **16** using $NaClO_2$ and 30% H_2O_2 in *t*-BuOH–H_2O furnished the acid **17**. Treatment of **17** with diazomethane (CH_2N_2) generated *in situ* at 0 °C, for 1 h afforded ester **1** in 79% yield (over 3 steps).

2. Synthesis of peptides 3–10

The peptides **3–10** (Fig. 1 and 2) were prepared by alternating use of **1** and D-Ala **2**, under standard peptide coupling[18] conditions with EDCI, HOBt and DIPEA in CH_2Cl_2. Accordingly, APyC monomer **1** on hydrolysis with LiOH in THF : MeOH : H_2O (3 : 1 : 1) furnished the acid **17**, while, **1** on exposure to CF_3COOH in CH_2Cl_2 gave the salt **17a** (Scheme 2). Peptide coupling of acid **17** in the presence of EDCI, HOBt and DIPEA in CH_2Cl_2 with the HCl salt of D-Ala-OMe (**18b**) gave the dipeptide **3** (93%). Base (LiOH) hydrolysis of ester **3** afforded the acid **19a**, while, reaction of **3** with CF_3COOH in CH_2Cl_2 furnished the salt **19b**. Acid **19a** on coupling (EDCI, HOBt and DIPEA) with salt **17a** in CH_2Cl_2 gave the tripeptide **20** (75%). Hydrolysis of tripeptide **20** with base



Reagents and conditions: a) Allyl bromide, NaH, DMF, 0 °C - rt, 4 h; b) Grubb's-I catalyst, Toluene, Reflux, 8 h; c) Li, Liq NH_3, THF, -78 °C, 1 h; d) 10% Pd-C, MeOH, H_2, 6 h; e) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C; f) $NaClO_2$, 30% H_2O_2, t-BuOH: H_2O (7:3); g) CH_2N_2, ether, 1 h.

Scheme 1 Synthesis of *trans* APyC monomer **1**.

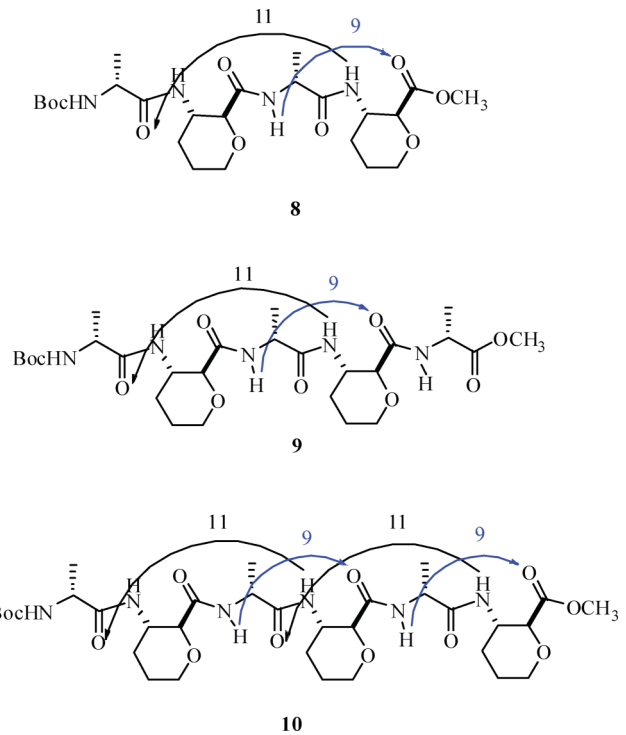


Fig. 2 Structures of the peptides **8–10** (arrows show hydrogen bonding).

(LiOH) gave the acid **20a**. Likewise, acid **18a** on coupling with the salt **17a** under standard peptide coupling conditions furnished the dipeptide **21** (86%). Hydrolysis of dipeptide **21** with LiOH gave the acid **21a**, while, reaction of **21** with CF_3COOH in CH_2Cl_2 afforded the salt **21b**. Peptide coupling of acid **19a** with the salt **19b** in the presence of EDCI, HOBt and DIPEA in CH_2Cl_2 afforded the tetrapeptide **4** (80%). Base (LiOH) hydrolysis of peptide **4** gave the acid **22a**, which on further coupling with **19b** in the presence of EDCI, HOBt and DIPEA afforded the hexapeptide **6** (64%). Similarly, peptide coupling of acid **20a** with the salt **21b** in the presence of EDCI, HOBt and DIPEA in CH_2Cl_2 afforded the pentapeptide **5** (75%).

Peptide **5** on hydrolysis of with LiOH gave the acid **23a**, which on further coupling with the salt **21b** under standard peptide coupling conditions afforded the heptapeptide **7** (43%). Likewise, acid **21a** on coupling with the salt **18b** in the presence of EDCI, HOBt and DIPEA in CH_2Cl_2 afforded the tripeptide **24** (83%). Base (LiOH) hydrolysis of peptide **24** gave the acid **24a**, which on further coupling with the salt **19b** under standard peptide coupling conditions furnished the pentapeptide **9** (67%). Acid **21a** on coupling with **21b** in the presence of EDCI, HOBt and DIPEA in CH_2Cl_2 gave the tetrapeptide **8** (75%). Similarly, base hydrolysis of **8** gave the acid **25a**, which on coupling with the salt **21b** in the presence of EDCI, HOBt and DIPEA in CH_2Cl_2 afforded the hexapeptide **10** (65%).

3. Conformational analysis of peptides 3–10

The peptides **3–10** (Fig. 1 and 2), having alternating **1** and **2** residues, were prepared under standard peptide coupling[18] conditions with EDCI, HOBt and DIPEA in CH_2Cl_2[19] and the ^{1}H NMR studies for all the peptides were undertaken as 3–5 mM solution in $CDCl_3$.[19]

The ^{1}H NMR spectrum[19] of **3** showed low field chemical shift (δ) > 7 ppm for NH(2), which along with small value of $\Delta\delta$ (0.56) in solvent titration study[20] confirmed its participation in possible interaction. $^3J_{C\alpha H\text{-}C\beta H}$ > 9 Hz corresponds to a *trans* orientation of CαH-CβH, implying a value of (θ) ≈ 60° for dihedral angle N-C(β)-C(α)-CO. A strong nOe correlation NH(1)/CαH(1) further supports this value. The nOe correlation NH(1)/Cγ′H, CβH(1)/CδH(1) together with large value of $^3J_{C\gamma' H\text{-}C\beta H}$ and four bond 'ω' coupling confirmed $^4C_1$ chair conformation of pyran ring as shown in the Fig. 3. Additionally, the presence of weak nOe correlation between CδH(1)/NH(2) and CεH(1)/NH(2) suggests the proximity of NH of D-Ala and ring oxygen in the pyran of APyC, providing evidence for a weak interaction between NH(2) and O(Cβ(1)). In order to understand such conformational constraints in more detail, the study was extended to higher homologues (Fig. 1 and 2).

The proton NMR spectrum[19] of tetrapeptide **4** in $CDCl_3$ showed a substantial dispersion in the amide region, indicating the presence of a secondary structure. The δ > 7 ppm for NH(2)–NH(4) along with small change in the chemical shift values in solvent titration studies[20] confirmed their participation in H-bonding. For β-amino acid residues β(1) and β(3), the value of $^3J_{NH\text{-}C\beta H}$ > 8.9 Hz was consistent with a ϕ_β (C(O)-*N*-Cβ-Cα) ≈ −120° and large $^3J_{C\alpha H\text{-}C\beta H}$ value of ~10.0 Hz corresponds to θ ≈ 60°. For Ala(2), $^3J_{NH\text{-}C\alpha H}$ value of 9.3 Hz correspond to a $\phi_\alpha \approx 120$

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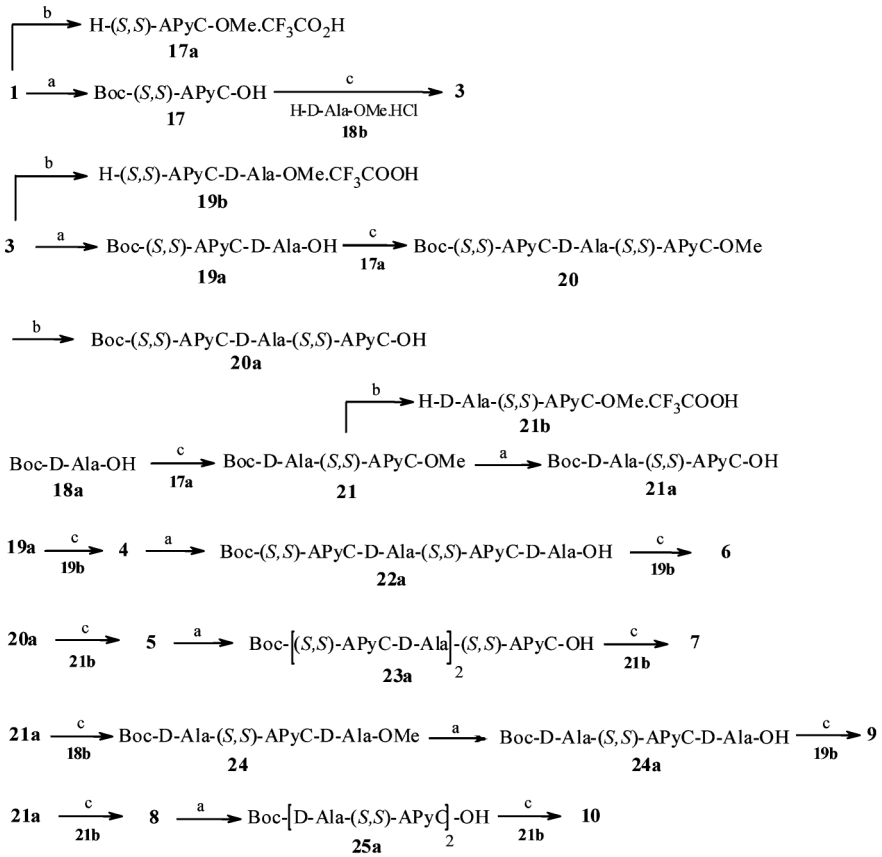

Reagents and conditions: a) LiOH, THF:MeOH:H_2O (3:1:1), 0 °C - rt, 2 h; b) CF_3COOH, dry CH_2Cl_2, 0 °C - rt, 2 h; c) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (2 equiv), dry CH_2Cl_2, 0 °C - rt, 8 h.

Scheme 2 Synthesis of peptides **3–10**.

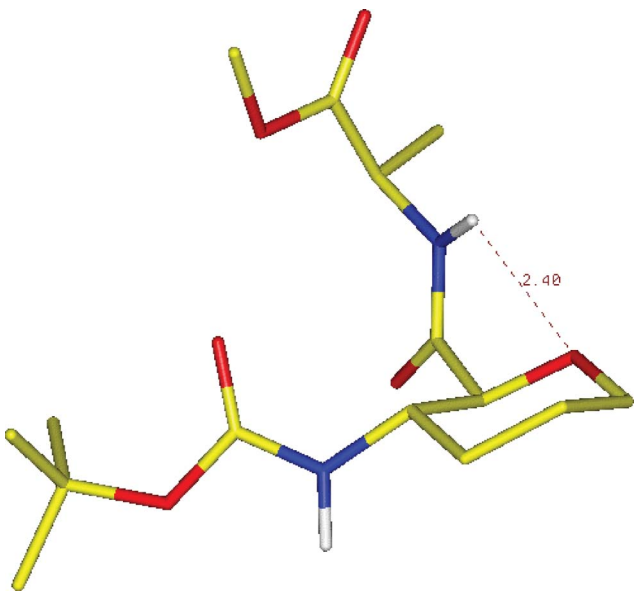

Fig. 3 Representation of electrostatic interaction of (D-Ala)-NH—O-(pyran) in peptide **3**.

whereas for Ala (4), $^3J_{NH\text{-}C\alpha H}$ value of 7.2 Hz is not very distinct and may reflect fraying in the termini.

The nOe correlations CβH(1)/NH(3), CαH(2)/NH(3) in **4** support an 11-membered H-bond between Boc CO and NH(3). Further, the nOe correlation NH(2)/NH(3) supports the presence of a 9-mr H-bonding between NH(2) and CO(3). The above characteristic nOes confirm the presence of a 9/11-helix[21] in peptide **4**. Similar to the observation made for peptide **3**, the MD structures of **4** revealed the presence of proximity for NH(2) and NH(4) to the pyran ring oxygen[15g] of preceding β(1) and β(3) residues. The distance of 2.4 Å provides an evidence for weak electrostatic interactions in peptide **4**.[22] This observation was further supported by weak nOe correlations CδH(1)/NH(2), CεH(1)/NH(2), CδH(3)/NH(4), CεH(3)/NH(4).

A systematic propagation of H-bonding pattern was observed in peptides **5** and **6**. All the nOe correlations and coupling constants defined the presence of 9/11-helix, with additional stabilization from the weak electrostatic interactions between the amide protons α(i) and pyran oxygen of β(i-1). The characteristic nOes have been labeled in the ROESY spectrum of peptide **6** (Fig. 4).

^{1}H NMR spectrum[19] of peptide **7** showed a very well resolved amide region. Their low field δ and small Δδ values in solvent titration studies,[20] imply that many of the amide protons participated in H-bonding. Large values of $^3J_{NH\text{-}C\beta H} > 9$ Hz and $^3J_{C\beta H\text{-}C\alpha H} > 9$ Hz are consistent with $\phi \approx -120°$ and $\theta \approx 60°$ for β-amino acid residues β(1), β(3) and β(5). For the second and the fourth D-Ala residues, large $^3J_{NH\text{-}C\alpha H} > 9$ Hz are consistent with ϕ_α (C(O)-*N*-Cα-C(O))

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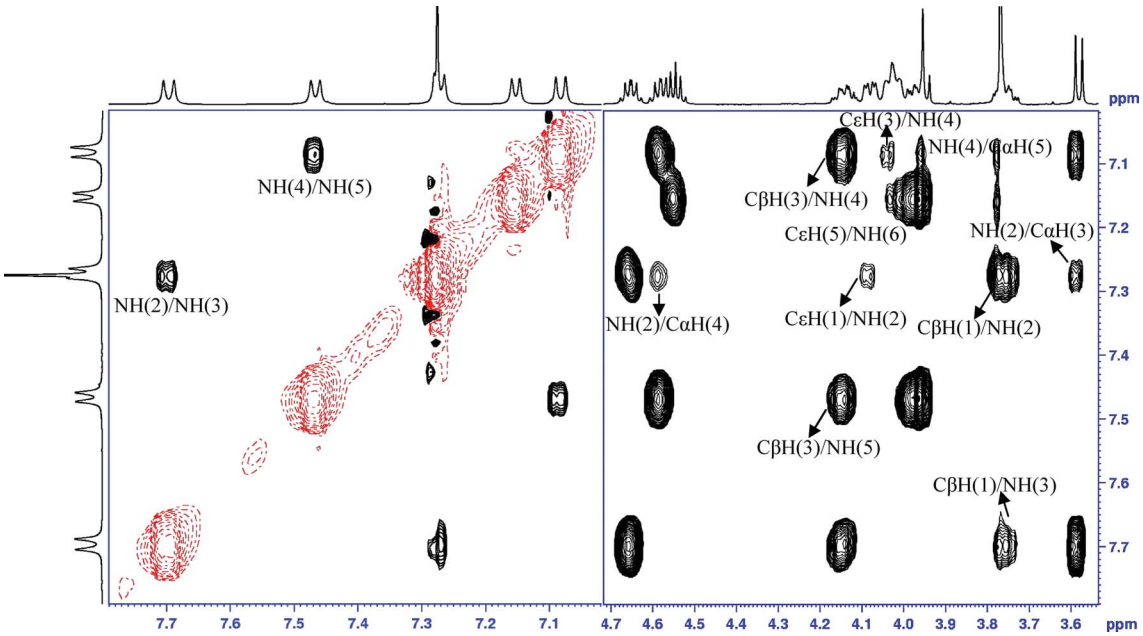

Fig. 4 ROESY spectrum of peptide **6**.

≈ 120°. The characteristic nOe correlation CβH(i)/NH(i+2) was observed for the first and third β-residues, while, due to the spectral overlap, several characteristic nOe correlations were ambiguous, though NH(i)/NH(i+1) (i = 2 and 4) were distinctly observed.

For the restrained molecular dynamics (MD) studies,[19] the constraints were derived from the intensities of the nOe cross peaks in the ROESY spectra using two spin approximation. Fig. 5 shows 20 superimposed minimum energy structures of peptides **4** and **6**, in which the RMSD of the backbone and heavy atoms are 0.35 and 0.53 Å for **4** and 0.40 and 0.65 Å for **6** respectively. The average backbone dihedral angles are derived by excluding the first and last residues. For **4**, ϕ, θ and ψ values for APyC are –92 ± 2°, 65 ± 2°, 74 ± 3° respectively, whereas, ϕ = 140 ± 3° and ψ = –75 ± 2° for D-Ala. Similarly, corresponding values for **6** are –95 ± 2°, 66 ± 1°, 77 ± 3°, 136 ± 3° and –75 ± 2°. The ϕ, θ and ψ in α/β-peptides for 9/11-helix predicted theoretically by Hofmann *et al.*[10] are ~–90°, ~60°, ~90° respectively for β-residue, and ϕ ~ 130° and ψ ~ –60° for α-residue.

The above peptides consistently showed absorption maxima in the NH- stretching region around 3310–3330, 3400 and 3440 cm^{-1} (Fig. 6). The small NH stretch at 3440 cm^{-1} corresponds to non-hydrogen bonded NH. The major band at 3310–3330 cm^{-1} corresponds to the conventional H-bonding and increases with length of the peptide chain. Another small NH stretch at 3400 cm^{-1} may be attributed to weak interaction between NH of α(i) and pyran oxygen of β(i-1), which is in accordance with the observation made by MD studies.[19] From the IR studies it was concluded that the folding of these peptides is dominated by 9/11-H-bonding and further stabilized by weak electrostatic interaction.[22] The distance between the amide proton and pyran oxygen of preceding residue was measured as 2.4 Å and an angle of 102°.[15g] Though the distance is higher than for a normal H-bond, similar observation in the form of bifurcated H-bonds were reported by Gellman *et al.*[14c] Further, they compared the relative stabilities of these H-bonds using IR data. The 5-mr H-bonding is

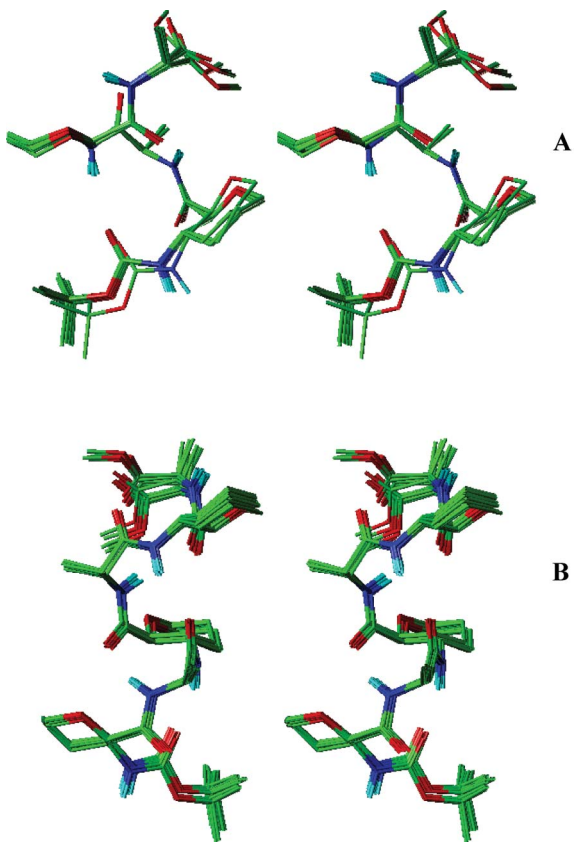

Fig. 5 MD structures of peptides: (A) **4** and (B) **6** (stereoview of 20 superimposed minimum energy structures; hydrogens are removed after the calculations for clarity).

characterized by N–H···O angle of 101° and H···O distance of 2.34 Å. In another study, inter (5-mr)/intra (6-mr) bifurcated H-bonding interactions were reported in a homo β-peptide.[13] The

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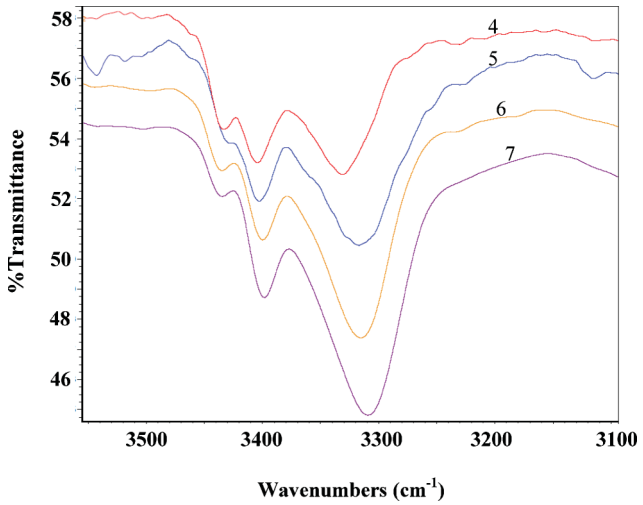

Fig. 6 IR spectra of peptides **4–7**.

5-mr H-bond shows a mean distance O···HN ~ 2.26 Å and mean angle O···H–N ~ 109°, whereas, the 6-mr H-bond shows a mean distance NH···O ~ 2.35 Å and mean angle formed by N–H···O=C ~ 118°, which spans the length of the peptide. Though the 5-mr and 6-mr H-bonding[14b] is commonly observed in natural products,[14a] their overall contribution to the conformations in the oligomers is small.

Based on the above results, the study was extended to peptides **8–10** (Fig. 2) with an α-residue at the N-terminus. As was observed in peptides **4–7**, these peptides also showed a 9/11-mixed helical pattern with α-residue participating in 9-mr H-bonding and β-residue in 11-mr H-bonding. Though several amide protons showed their involvement in H-bonding as deduced from δNH and the solvent titration studies,[20] the first and second amide protons do not seem to participate in H-bonding. The amide protons of terminal α-residues show significantly large value of Δδ (~ 1 ppm) in solvent titration studies. The value of $^{3}J_{NH\text{-}C\alpha H} > 9.0$ Hz for D-Ala(3) was consistent with a $\phi \approx 120°$. Large values of $^{3}J_{NH\text{-}C\beta H} > 8.9$ Hz and $^{3}J_{C\beta H\text{-}C\alpha H} > 9.0$ Hz are consistent with $\phi \approx -120°$ and $\theta \approx 60°$ for the second and the fourth β residues. Similar to peptides **4–7**, the peptides **8–10** exhibited the characteristics of a 9/11-mixed helix. Further proof of structural confirmation was obtained from the MD studies.[19]

NMR studies on peptide **9** were carried out in CD_3OH to understand the stability of the secondary structure in a polar solvent. The presence of characteristic nOes such as Cβ(2)/NH(4), Cε(4)/NH(5), NH(1)/NH(2) and NH(3)/NH(4) with low intensities support small population of 9/11-helical structures. The exchange studies in CD_3OD do not seem to suggest very robust folds in the pentapeptide **9**.[19] These observations are consistent with the conclusions derived from theoretical studies by Hofmann *et al.*[10]

4. Conclusion

In summary, the peptides prepared with 1 : 1 alternating APyC, the new β-amino acid (*trans*-3-aminopyran-2-carboxylic acid) and D-Ala, have shown the participation of the amide proton of α-residue in a 9-mr H-bonding and that of β-residue in 11-mr H-bonding, giving rise to a right-handed 9/11-mixed helix. In addition to the mixed helix, the amide proton of α(i) showed a weak electrostatic interaction with the oxygen atom present in the Cβ²-position of the β(i-1), which may strengthen the 9/11-folds realized. The present design generated a new motif for 9/11-helix, for the first time from a cyclic β-amino acid (APyC). The participation of oxygen substituent in weak electrostatic interaction in APyC, opens up new fronts for the use of such monomers in different designs. Further studies on the use of new β-amino acid are in progress.

Experimental

NMR spectra (1D and 2D experiments) for peptides **3–10** were obtained at 500 and 600 MHz (^{1}H), and at 75 MHz, 100 MHz, and 150 MHz (^{13}C). Chemical shifts are reported in δ scale with respect to internal TMS reference. IR spectra were recorded with FT-IR spectrometer. Melting points were determined in open capillaries and were not corrected.

Restrained molecular dynamics (MD) studies were carried out using the INSIGHT-II Discover[13] module employing an SGI workstation. 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-(*S*,*S*)-APyC-D-Ala -OMe (3)

To a solution of ester **1** (0.6 g, 2.31 mmol) in THF : MeOH : H_2O (3 : 1 : 1), LiOH (0.13 g, 5.41 mmol) was added at 0 °C and stirred at room temperature for 2 h. The reaction mixture was adjusted to pH 2–3 with aq. 1 N HCl and extracted with EtOAc (2 × 15 mL). The organic layer was dried (Na_2SO_4) and evaporated to give acid **17**.

A mixture of acid **17** (0.6 g, 2.44 mmol), HOBt (0.39 g, 2.93 mmol) and EDCI (0.56 g, 2.93 mmol) in CH_2Cl_2 (25 mL) was stirred at 0 °C for 15 min and treated with salt **18b** (0.39 g, 2.93 mmol) under N_2 atmosphere and continued stirring at room temperature for 8 h. The reaction mixture was quenched at 0 °C with sat. NH_4Cl (15 mL) solution. After 10 min, reaction mixture was diluted with $CHCl_3$ (50 mL), washed with 1 N HCl (25 mL), water (25 mL), aq. sat. $NaHCO_3$ (15 mL) and brine (25 mL). The organic layers were dried (Na_2SO_4), evaporated and the residue was purified by column chromatography (60–120 mesh Silica gel, 50% ethyl acetate in pet. ether) to afford **3** (0.72 g, 90%) as a white solid; m.p. 155 °C; $[\alpha]_D = +45.8$ (*c* 0.5, $CHCl_3$); IR ($CHCl_3$): 3410, 3009, 2981, 2863, 1741, 1710, 1673, 1510, 1502, 1452, 1367, 1343, 1307 cm^{-1};^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.03 (d, 1H, $J = 7.5$ Hz, NH-2), 5.38 (br, 1H, NH-1), 4.58 (p, 1H, $J = 7.5$ Hz, CαH-2), 4.01 (dddd, 1H, $J = 1.4, 3.0, 4.3, 11.3$ Hz, CεH-1), 3.76 (s, 3H, $COOCH_3$), 3.63 (d, 1H, $J = 9.1$ Hz, CαH-1), 3.51 (m, 1H, CβH-1), 3.48 (dt, 1H, $J = 2.7, 11.3$ Hz, Cε′H-1), 2.42 (m, 1H, CγH-1), 1.72 (m, 1H, CδH-1), 1.67 (m, 1H, Cδ′H-1), 1.42 (m, 1H, Cγ′H-1), 1.42 (d, 1H, $J = 7.5$ Hz, CH_3-2) 1.42 (s, 9H, Boc);^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.0, 169.4, 155.5, 79.3, 78.9, 67.6, 52.5, 50.1, 47.5, 30.1, 28.3 (3C), 24.2, 18.3; HRMS (ESI): *m/z* calcd for $C_{15}H_{26}N_2O_6Na$: 353.1688 $[M+Na]^+$; found: 353.1686.

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Boc-(*S*,*S*)-APyC-D-Ala-(*S*,*S*)-APyC-OMe (20)

A solution of ester **3** (0.5 g, 1.51 mmol) in THF: MeOH: H_2O (3 : 1 : 1) was treated with LiOH (0.09 g, 3.78 mmol) at 0 °C and continued stirring at room temperature for 2 h. Work up as described for **17** gave the acid **19a**.

A mixture of acid **19a** (0.45 g, 1.42 mmol), HOBt (0.23 g, 1.70 mmol), EDCI (0.32 g, 1.70 mmol) in CH_2Cl_2 (15 mL) was stirred at 0 °C for 15 min and treated with the salt **17a** (0.36 g, 1.42 mmol) under N_2 atmosphere for 8 h. Workup as described for **3** and purification by column chromatography (60–120 mesh silica gel, 1.5% methanol in $CHCl_3$) afforded **20** (0.49 g, 75%) as a white solid; m.p. 230 °C; $[\alpha]_D$ = +32.5 (*c* 0.25, $CHCl_3$); IR ($CHCl_3$): 3400, 3325, 3008, 2930, 2858, 1735, 1676, 1514, 1238, 1167, 1090, 1053 cm^{-1}; ^1H NMR ($CDCl_3$, 298 K, 600 MHz): δ 7.38 (d, 1H, *J* = 9.1 Hz, NH-3), 6.63 (d, 1H, *J* = 9.6 Hz, NH-2), 4.75 (d, 1H, *J* = 9.6 Hz, NH-1), 4.63 (dq, 1H, *J* = 9.6, 7.0 Hz, CαH-2), 4.11 (dq, 1H, *J* = 3.9, 9.6 Hz, CβH-3), 4.06 (m, 2H, CεH-1, CεH-3), 3.94 (d, 1H, *J* = 9.7 Hz, CαH-3), 3.77 (s, 3H, $COOCH_3$), 3.70 (m, 1H, CβH-1), 3.47 (d, 1H, *J* = 9.6 Hz, CαH-1), 3.43 (dt, 1H, *J* = 2.4, 11.9 Hz, Cε′H-3), 3.41 (dt, 1H, *J* = 2.4, 12.0 Hz, Cε′H-1), 2.14 (m, 1H, CγH-1), 2.08 (m, 1H, CγH-3), 1.82 (m, 1H, CδH-3), 1.81 (m, 1H, CδH-1), 1.74 (m, 1H, Cδ′H-1), 1.73 (m, 1H, Cδ′H-3), 1.68 (m, 1H, Cγ′H-3), 1.43 (m, 1H, Cγ′H-1), 1.43 (s, 9H, Boc), 1.30 (d, 3H, *J* = 7.0 Hz, CH_3-2);^{13}C NMR ($CDCl_3$, 150 MHz): δ 171.5, 170.2, 169.9, 155.8, 82.1, 80.2, 80.1, 67.5 (2C), 52.6, 50.1, 47.6, 46.7, 30.7, 29.3, 28.2 (3C), 25.4, 24.8, 16.9; HRMS (ESI): *m*/*z* calcd for $C_{21}H_{35}N_5O_8Na$: 480.2473 $[M+Na]^+$; found: 480.2488.

Boc-D-Ala-(*S*,*S*)-APyC-OMe (21)

A solution of acid **18a** (0.13 g, 0.67 mmol), HOBt (0.11 g, 0.80 mmol) and EDCI (0.16 g, 0.80 mmol) in CH_2Cl_2 (15 mL) was stirred at 0 °C for 15 min and treated with the salt **17a** [prepared from **1** (0.18 g, 0.67 mmol) and CF_3COOH (0.2 mL) in CH_2Cl_2 (0.5 mL)] and DIPEA (0.3 mL, 1.34 mmol) under nitrogen atmosphere at room temperature for 8 h. Workup as described for **3** and purification by column chromatography (60–120 mesh Silica gel, 60% ethyl acetate in pet. ether) gave **21** (0.19 g, 86%) as a white solid; m.p. 143 °C; $[\alpha]_D$ = +107.6 (*c* 0.25, $CHCl_3$); IR ($CHCl_3$): 3431, 3013, 2980, 2932, 2860, 1690, 1494, 1448, 1369, 1286, 1231, 1165, 1122, 1094 cm^{-1};^1H NMR (600 MHz, $CDCl_3$, 278 K): δ 6.54 (d, 1H, *J* = 8.6 Hz, NH-2), 4.92 (d, 1H, *J* = 7.3 Hz, NH-1), 4.17 (p, 1H, *J* = 7.3 Hz, CαH-2), 4.12 (m, 1H, CβH-2), 4.02 (m, 2H, CεH-2), 3.82 (d, 1H, *J* = 8.1 Hz, CαH-2), 3.75 (s, 3H, $COOCH_3$), 3.51 (m, 1H, Cε′H-2), 2.03 (m, 1H, CγH-2), 1.74 (m, 2H, CδH-2, Cδ′H-2), 1.56 (m, 1H, Cγ′H-2), 1.46 (s, 9H, Boc), 1.34 (d, 3H, *J* = 7.2 Hz, CH_3-1);^{13}C NMR ($CDCl_3$, 150 MHz): δ 172.0, 169.8, 155.7, 80.4, 79.2, 66.7, 53.4, 52.5, 50.1, 46.7, 28.6 (3C), 23.5, 17.6; HRMS (ESI): *m*/*z* calcd for $C_{15}H_{26}N_2O_6Na$: 353.1688 $[M+Na]^+$; found: 353.1701.

Boc-(*S*,*S*)-APyC-D-Ala-(*S*,*S*)-APyC-D-Ala-OMe (4)

A mixture of acid **19a** (0.15 g, 0.46 mmol), HOBt (0.08 g, 0.56 mmol) and EDCI (0.11 g, 0.56 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 15 min and treated with **19b** [prepared from **3** (0.16 g, 0.46 mmol) and CF_3COOH (0.2 mL) in CH_2Cl_2 (2 mL)] and DIPEA (0.16 mL, 0.92 mmol) under N_2 atmosphere for 8 h. Workup as described for **3** and purification by column chromatography (60-120 mesh Silica gel, 2.5% methanol in $CHCl_3$) afforded **4** (0.2 g, 80%) as a white solid; m.p. 250 °C; $[\alpha]_D$ = 157.3 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$): 3432, 3404, 3331, 3006, 2934, 2860, 1741, 1672, 1513, 1452, 1371, 1310, 1231, 1163, 1090 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, 278 K): δ 7.60 (d, 1H, *J* = 8.9 Hz, NH-3), 7.22 (d, 1H, *J* = 9.3 Hz, NH-2), 7.02 (d, 1H, *J* = 7.2 Hz, NH-4), 4.75 (d, 1H, *J* = 9.8 Hz, NH-1), 4.63 (dq, 1H, *J* = 9.3, 7.0 Hz, CαH-2), 4.51 (d, 1H, *J* = 7.2 Hz, CαH-4), 4.04 (m, 1H, CεH-3), 4.03 (m, 1H, CεH-1), 3.96 (dq, 1H, *J* = 3.9, 9.6 Hz, CβH-3), 3.84 (dq, 1H, *J* = 4.1, 9.8 Hz, CβH-1), 3.79 (d, 1H, *J* = 9.6 Hz, CαH-3), 3.77 (s, 3H, $COOCH_3$), 3.47 (d, 1H, *J* = 9.8 Hz, CαH-1), 3.44 (dt, 1H, *J* = 2.4, 11.9 Hz, Cε′H-3), 3.38 (dt, 1H, *J* = 2.4, 12.0 Hz, Cε′H-1), 2.13 (m, 1H, CγH-3), 2.11 (m, 1H, CγH-1), 1.82 (m, 1H, CδH-1), 1.80 (m, 1H, CδH-3), 1.69 (m, 1H, Cδ′H-1), 1.68 (m, 1H, Cδ′H-3), 1.64 (d, 1H, *J* = 3.9, 12.6 Hz, Cγ′H-3) 1.43 (s, 9H, Boc), 1.42 (d, 1H, *J* = 7.2 Hz, CH_3-4), 1.39 (dq, 1H, *J* = 3.8, 12.5 Hz, Cγ′H-1), 1.30 (d, 1H, *J* = 7.0 Hz, CH_3-2); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 173.1, 171.5, 169.4, 169.3, 155.7, 82.4, 80.0, 79.9, 67.8, 67.4, 52.4, 49.7, 48.6, 47.8, 47.0, 30.8, 30.0, 28.2 (3C), 25.2, 25.1, 18.1, 16.9; HRMS (ESI): *m*/*z* calcd for $C_{24}H_{40}N_4O_9Na$: 551.2692 $[M+Na]^+$; found: 551.2702.

Boc-(*S*,*S*)-APyC-[D-Ala-(*S*,*S*)-APyC]$_2$-D-Ala-OMe (6)

To a solution of ester **4** (0.06 g, 0.11 mmol) in THF: MeOH: H_2O (3 : 1 : 1), LiOH (0.005 g, 0.22 mmol) was added at 0 °C and continued stirring at room temperature for 2 h. Work up as described for **17** gave the acid **22a**.

A mixture of acid **22a** (0.05 g, 0.09 mmol), HOBt (0.01 g, 0.1 mmol) and EDCI (0.02 g, 0.1 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C for 15 min and treated with **19b** [prepared from **3** (0.03 g, 0.09 mmol) and CF_3COOH (0.1 mL) in CH_2Cl_2 (1 mL)] and DIPEA (0.1 mL, 0.18 mmol) under nitrogen atmosphere at room temperature for 8 h. Workup as described for **3** and purification by column chromatography (60–120 mesh Silica gel, 3.5% methanol in $CHCl_3$) gave **6** (0.05 g, 64%) as a white solid; m.p. 280 °C; $[\alpha]_D$ = +262.8 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$): 3428, 3402, 3317, 3005, 2932, 2858, 1740, 1686, 1672, 1666, 1514, 1451, 1372, 1311, 1237, 1212, 1161, 1091 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, 278 K): δ 7.69 (d, 1H, *J* = 9.8 Hz, NH-3), 7.47 (d, 1H, *J* = 8.3 Hz, NH-5), 7.29 (d, 1H, *J* = 9.4 Hz, NH-2), 7.15 (d, 1H, *J* = 7.3 Hz, NH-6), 7.11 (d, 1H, *J* = 9.1 Hz, NH-4), 4.79 (d, 1H, *J* = 9.9 Hz, NH-1), 4.65 (dq, 1H, *J* = 9.4, 7.0 Hz, CαH-2), 4.58 (dq, 1H, *J* = 9.1, 7.0 Hz, CαH-4), 4.54 (p, 1H, *J* = 7.3 Hz, CαH-6), 4.14 (dq, 1H, *J* = 4.0, 9.8 Hz, CβH-3), 4.08 (m, 1H, CεH-1), 4.03 (m, 1H, CεH-3), 4.01 (m, 1H, CεH-5), 3.98 (m, 1H, CβH-5), 3.94 (d, 1H, *J* = 9.7 Hz, CαH-5), 3.78 (s, 3H, $COOCH_3$), 3.75 (m, 1H, CβH-1), 3.58 (d, 1H, *J* = 9.8 Hz, CαH-3), 3.49 (d, 1H, *J* = 9.7 Hz, CαH-1), 3.48 (m, 1H, Cε′H-5), 3.44 (m, 1H, Cε′H-3), 3.39 (m, 1H, Cε′H-1), 2.10 (m, 2H, CγH-5, CγH-1), 2.03 (m, 1H, CγH-3), 1.86 (m, 1H, CδH-1), 1.79 (m, 1H, CδH-3), 1.77 (m, 1H, CδH-5), 1.76 (m, 1H, Cγ′H-3), 1.75 (m, 1H, Cγ′H-5), 1.72 (m, 1H, Cδ′H-1), 1.69 (m, 1H, Cδ′H-3), 1.67 (m, 1H, Cδ′H-5), 1.47 (s, 9H, Boc), 1.43 (d, 3H, *J* = 7.3 Hz, CH_3-6), 1.43 (m, 1H, Cγ′H-1), 1.33 (d, 3H, *J* = 7.1 Hz, CH_3-4), 1.26 (d, 3H, *J* = 7.0 Hz, CH_3-2); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 173.2, 172.7, 171.3, 169.9, 169.5, 169.4, 155.8, 82.7, 81.7, 80.0, 79.6, 67.6, 67.4 (2C), 52.4, 50.0, 48.7, 48.4, 47.7, 47.2, 46.6, 30.6, 29.5, 29.3, 28.1 (3C), 25.4, 25.2, 25.0, 18.1, 17.1, 17.0; HRMS (ESI): *m*/*z* calcd for $C_{33}H_{54}N_6O_{12}Na$: 749.3697 $[M+Na]^+$; found: 749.3724.

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Boc-(*S*,*S*)-APyC-D-Ala-(*S*,*S*)-APyC-D-Ala-(*S*,*S*)-APyC-OMe (5)

To a solution of ester **20** (0.2 g, 0.17 mmol) in THF: MeOH: H_2O (3 : 1 : 1), LiOH (0.015 g, 0.34 mmol) was added at 0 °C and continued stirring at room temperature for 2 h. Work up as described for **17** gave the acid **20a**.

A mixture of acid **20a** (0.1 g, 0.31 mmol), HOBt (0.05 g, 0.37 mmol) and EDCI (0.07 g, 0.37 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 15 min and treated with **21b** [prepared from **21** (0.14 g, 0.31 mmol) and CF_3COOH (0.2 mL) in CH_2Cl_2 (2 mL)] and DIPEA (0.11 mL, 0.63 mmol) under N_2 atmosphere for 8 h. Workup as described for **3** and purification by column chromatography (60–120 mesh Silica gel, 3.0% methanol in $CHCl_3$) afforded **5** (0.15 g, 75%) as a white solid; m.p. 255 °C; $[\alpha]_D$ = +220.1 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$): 3432, 3400, 3315, 3008, 2942, 2861, 1734, 1665, 1516, 1447, 1370, 1238, 1160, 1089 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, 288 K): δ 7.71 (d, 1H, *J* = 9.7 Hz, NH-3), 7.38 (d, 1H, *J* = 8.7 Hz, NH-5), 7.30 (d, 1H, *J* = 9.4 Hz, NH-2), 6.65 (d, 1H, *J* = 9.6 Hz, NH-4), 4.80 (d, 1H, *J* = 10.1 Hz, NH-1), 4.66 (dq, 1H, *J* = 9.4, 6.9 Hz, CαH-2), 4.62 (dq, 1H, *J* = 9.6, 7.0 Hz, CαH-4), 4.10 (m, 1H, CβH-5), 4.08 (m, 1H, CεH-1), 4.07 (m, 3H, CβH-3, CεH-3, CεH-5), 3.78 (s, 3H, $COOCH_3$), 3.73 (m, 1H, CβH-1), 3.58 (d, 1H, *J* = 9.7 Hz, CαH-3), 3.49 (d, 1H, *J* = 9.7 Hz, CαH-1), 3.47 (dt, 1H, *J* = 2.5, 11.9 Hz, Cε′H-3), 3.45 (dt, 1H, *J* = 2.4, 11.6 Hz, Cε′H-5), 3.39 (dt, 1H, *J* = 2.4, 11.9 Hz, Cε′H-1), 2.11 (m, 1H, CγH-1), 2.05 (m, 1H, CγH-3), 2.04 (m, 1H, CγH-5), 1.88 (m, 1H, CδH-5), 1.84 (m, 2H, CδH-1, Cδ′H-5), 1.82 (m, 2H, CδH-3, Cδ′H-3), 1.75 (m, 1H, Cγ′H-3), 1.72 (m, 1H, CδH-1), 1.69 (m, 1H, Cγ′H-5), 1.44 (m, 1H, Cγ′H-1), 1.43 (s, 9H, Boc), 1.33 (d, *J* = 7.0 Hz, 3H, CH_3-4), 1.25 (d, 3H, *J* = 6.9 Hz, CH_3-2); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 172.9, 171.3, 170.3, 169.8, 169.4, 155.9, 82.7, 81.5, 80.1, 79.9, 67.5, 67.4 (2C), 52.4, 50.1, 49.0, 47.7, 46.9, 46.6, 30.7, 29.2, 28.9, 28.2 (3C), 25.4 (2C), 24.8, 17.0, 16.9; HRMS (ESI): *m*/*z* calcd for $C_{30}H_{49}N_5O_{11}Na$: 678.3326 $[M+Na]^+$; found: 678.3349.

Boc-(*S*,*S*)-APyC-[D-Ala-(*S*,*S*)-APyC]$_2$-D-Ala-(*S*,*S*)-APyC-OMe (7)

To a solution of ester **5** (0.12 g, 0.11 mmol) in THF: MeOH: H_2O (3 : 1 : 1), LiOH (0.016 g, 0.22 mmol) was added at 0 °C and continued stirring at room temperature for 2 h. Work up as described for **17** gave the acid **23a**.

A mixture of acid **23a** (0.1 g, 0.31 mmol), HOBt (0.05 g, 0.37 mmol) and EDCI (0.07 g, 0.37 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 15 min and treated with **21b** [prepared from **21** (0.20 g, 0.31 mmol) and CF_3COOH (0.2 mL) in CH_2Cl_2 (2 mL)] and DIPEA (0.11 mL, 0.63 mmol) under N_2 atmosphere for 8 h. Workup as described for **3** and purification by column chromatography (60–120 mesh Silica gel, 4.0% methanol in $CHCl_3$) afforded **7** (0.12 g, 43%) as a white solid; m.p. 295 °C; $[\alpha]_D$ = +358.7 (*c* 0.25, $CHCl_3$); IR ($CHCl_3$): 3430, 3339, 3309, 3007, 2944, 2862, 1661, 1543, 1447, 1372, 1237, 1154, 1089 cm^{-1};^1H NMR (600 MHz, $CDCl_3$, 278 K): δ 7.64 (d, 1H, *J* = 9.7 Hz, NH-3), 7.55 (d, 1H, *J* = 9.6 Hz, NH-5), 7.35 (d, 1H, *J* = 8.4 Hz, NH-7), 7.20 (d, 1H, *J* = 9.2 Hz, NH-2), 7.12 (d, 1H, *J* = 9.4 Hz, NH-4), 6.60 (d, 1H, *J* = 9.4 Hz, NH-6), 4.73 (d, 1H, *J* = 9.8 Hz, NH-1), 4.65 (m, 1H, CαH-2), 4.63 (m, 1H, CαH-4), 4.60 (m, 1H, CαH-6), 4.14 (dq, 1H, *J* = 4.1, 9.7 Hz, CβH-3), 4.09 (m, 1H, CβH-7), 4.07 (m, 4H, CεH-1, CβH-5, CεH-5, CαH-7), 4.06 (m, 1H, CεH-3, CεH-7), 3.77 (s, 3H, -$COOCH_3$), 3.74 (dq, 1H, *J* = 4.1, 9.8 Hz, CβH-1), 3.68 (d, 1H, *J* = 9.7 Hz, CαH-5), 3.57 (d, 1H, *J* = 9.7 Hz, CαH-3), 3.47 (m, 1H, Cε′H-5), 3.45 (m, 1H, Cε′H-7), 3.43 (dt, 1H, *J* = 2.5, 11.7 Hz, Cε′H-3), 3.38 (dt, 1H, *J* = 2.4, 11.8 Hz, Cε′H-1), 2.11 (m, 1H, CγH-1), 2.04 (m, 1H, CγH-7), 2.01 (m, 1H, CγH-3), 2.00 (m, 1H, CγH-5), 1.96 (m, 1H, CδH-5), 1.89 (m, 1H, CδH-7), 1.87 (m, 1H, CδH-1), 1.86 (m, 1H, CδH-3), 1.82 (m, 2H, Cδ′H-5, Cδ′H-7), 1.81 (m, 1H, Cδ′H-3), 1.74 (m, 1H, Cγ′H-5), 1.72 (m, 1H, Cδ′H-1), 1.71 (m, 1H, Cγ′H-3), 1.68 (m, 1H, Cγ′H-7), 1.47 (s, 9H, Boc), 1.43 (m, 1H, Cγ′H-1), 1.32 (d, 3H, *J* = 7.1 Hz, CH_3-6), 1.27 (d, 3H, *J* = 7.0 Hz, CH_3-4), 1.25 (d, 3H, *J* = 7.0 Hz, CH_3-2); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 173.1, 172.5, 171.4, 170.3, 170.0, 169.9, 169.4, 155.8, 82.7, 82.1, 81.2, 80.0, 79.8, 67.5 (2C), 67.4 (2C), 52.3, 50.1, 49.0 (2C), 47.7, 46.9, 46.6, 46.5, 30.6, 29.1, 28.9 (2C), 28.2 (3C), 25.4 (2C), 25.3, 24.8, 17.2, 17.1, 17.0; HRMS (ESI): *m*/*z* calcd for $C_{39}H_{63}N_7O_{14}Na$: 876.4413 $[M+Na]^+$; found: 876.4434.

Boc-D-Ala-(*S*,*S*)-APyC-D-Ala-OMe (24)

To a solution of ester **21** (0.5 g, 1.51 mmol) in THF: MeOH: H_2O (3 : 1 : 1) LiOH (0.09 g, 3.78 mmol) was added at 0 °C continue stirring at room temperature for 2 h. Work up as described for **17** gave the acid **21a**.

A mixture of acid **21a** (0.3 g, 0.94 mmol), HOBt (0.15 g, 1.13 mmol) and EDCI (0.21 g, 1.13 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 15 min and treated with the salt **18b** (0.15 g, 1.13 mmol) under N_2 atmosphere for 8 h. Workup as described for **3** and purification by column chromatography (60–120 mesh Silica gel, 2% methanol in $CHCl_3$) afforded **24** (0.31 g, 83%) as a white solid; m.p. 140 °C; $[\alpha]_D$ = +41.46 (*c* 0.5, $CHCl_3$); IR (KBr): 3317, 3296, 2979, 2849, 2361, 1748, 1666, 1535, 1452, 1369, 1330, 1216, 1164, 1097, 1047, 963, 640 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, 298 K): δ 7.02 (d, 1H, *J* = 7.5 Hz, NH-2), 6.78 (d, 1H, *J* = 7.1 Hz, NH-3), 5.11 (br, 1H, NH-1), 4.52 (p, 1H, *J* = 7.1 Hz, CαH-3), 4.17 (p, 1H, *J* = 7.2 Hz, CαH-1), 4.04 (m, 1H, CεH-2), 3.80 (m, 1H, CβH-2), 3.75 (s, 3H, $COOCH_3$), 3.65 (d, 1H, *J* = 9.4 Hz, CαH-2), 2.38 (m, 1H, CγH-2), 1.85 (m, 1H, CδH-2), 1.76 (m, 1H, Cδ′H-2), 1.68 (m, 1H, Cγ′H-2), 1.45 (s, 9H, Boc), 1.42 (d, 3H, *J* = 7.1 Hz, CH_3-1), 1.32 (d, 3H, *J* = 7.1 Hz, CH_3-3); ^{13}C NMR ($CDCl_3$, 150 MHz): 172.9, 172.5, 169.3, 155.5, 79.9, 78.8, 67.8, 52.6, 50.3, 49.1, 47.7, 29.8, 28.3 (3C), 24.3, 18.6, 18.2; HRMS (ESI): *m*/*z* calcd for $C_{18}H_{31}N_3O_7Na$: 401.2241 $[M+Na]^+$; found: 401.2258.

Boc-D-Ala-(*S*,*S*)-APyC-D-Ala-(*S*,*S*)-APyC-D-Ala-OMe (9)

To a solution of ester **24** (0.08 g, 1.51 mmol) in THF: MeOH: H_2O (3 : 1 : 1) LiOH (0.02 g, 3.78 mmol) was added at 0 °C and continued stirring at room temperature for 2 h. Work up as described for **17** gave the acid **24a**.

A solution of acid **24a** (0.02 g, 0.06 mmol), HOBt (0.01 g, 0.07 mmol) and EDCI (0.01 g, 0.07 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 15 min and treated with **19b** [prepared from **3** (0.03 g, 0.06 mmol) and CF_3COOH (0.02 mL) in CH_2Cl_2 (0.5 mL)] and DIPEA (0.1 mL, 0.59 mmol) under nitrogen atmosphere at room temperature for 8 h. Workup as described for **3** and purification by column chromatography (60–120 mesh Silica gel,

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3.0% methanol in $CHCl_3$) gave **9** (0.03, 67%) as a white solid; m.p. 265 °C; $[\alpha]_D$ = +41.0 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$): 3409, 3331, 3005, 2933, 2861, 2300, 1670, 1525, 1451, 1371, 1242, 1162, 1093, 1670, 1525, 1450, 1371, 1242, 1162, 1093 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, 278 K): δ 7.46 (d, 1H, *J* = 8.6 Hz, NH-4), 7.19 (d, 1H, *J* = 8.9 Hz, NH-3), 7.07 (d, 1H, *J* = 7.3 Hz, NH-5), 6.63 (d, 1H, *J* = 8.9 Hz, NH-2), 5.32 (d, 1H, *J* = 8.1 Hz, NH-1), 4.52 (d, 1H, *J* = 7.3 Hz, CαH-5), 4.50 (dq, 1H, *J* = 8.9, 7.0 Hz, CαH-3), 4.21 (p, 1H, *J* = 7.4 Hz, CαH-1), 4.09 (m, 1H, CβH-2), 4.05 (m, 1H, CεH-2), 4.03 (m, 1H, CεH-4), 3.94 (m, 1H, CβH-4), 3.87 (d, 1H, *J* = 9.9 Hz, CαH-4), 3.77 (s, 3H, -$COOCH_3$), 3.48 (d, 1H, *J* = 9.5 Hz, CαH-2), 3.47 (dt, 1H, *J* = 2.1, 11.9 Hz, Cε′H-4), 3.41 (dt, 1H, *J* = 2.3, 12.1 Hz, Cε′H-2), 2.09 (m, 1H, CγH-4), 2.08 (m, 1H, CγH-2), 1.83 (m, 1H, CδH-2), 1.79 (m, 1H, CδH-4), 1.72 (m, 2H, Cδ′H-4, Cδ′H-2), 1.68 (m, 1H, Cγ′H-4), 1.51 (dq, 1H, *J* = 4.0, 12.5 Hz, Cγ′H-2), 1.43 (s, 9H, Boc), 1.43 (d, 3H, *J* = 7.3 Hz, CH_3-5), 1.33 (d, 3H, *J* = 7.4 Hz, CH_3-1), 1.31 (d, 3H, *J* = 7.0 Hz, CH_3-3); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 173.0, 172.9, 171.2, 169.6, 169.4, 155.8, 128.4, 96.0, 81.5, 80.2, 79.5, 67.7, 67.5, 52.4, 50.1, 48.5, 48.2, 47.7, 47.4, 30.2, 29.6, 28.2 (3C), 25.0, 18.1, 17.0; HRMS (ESI): *m/z* calcd for $C_{27}H_{45}N_5O_{11}Na$: 622.3064 $[M+Na]^+$; found: 622.3079.

Boc-D-Ala-(*S*,*S*)-APyC-D-Ala-(*S*,*S*)-APyC-OMe (8)

A solution of ester **21** (0.06 g, 0.18 mmol) in THF: MeOH: H_2O (3 : 1 : 1) LiOH (0.01 g, 0.45 mmol) was added at 0 °C and continued stirring at room temperature for 2 h. Work up as described for **17** gave the acid **21a**.

A solution of acid **21a** (0.05 g, 0.17 mmol), HOBt (0.03 g, 0.20 mmol) and EDCI (0.04 g, 0.20 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 15 min and treated with the salt **21b** [prepared from **21** (0.06 g, 0.17 mmol) and CF_3COOH (0.1 mL) DIPEA (0.05 mL, 0.34 mmol) in CH_2Cl_2 under N_2 atmosphere for 8 h. Workup as described for **3** and purification by column chromatography (60–120 mesh Silica gel, 2.8% methanol in $CHCl_3$) gave **8** (0.07, 75%) as a white solid; m.p. 198 °C; $[\alpha]_D$ = +103.0 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$): 3406, 3334, 3010, 2934, 2860, 1670, 1530, 1448, 1371, 1238, 1226, 1163, 1091 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, 278 K): δ 7.38 (d, *J* = 8.5 Hz, 1H, NH-4), 6.71 (d, 1H, *J* = 8.9 Hz, NH-2), 6.65 (d, 1H, *J* = 9.1 Hz, NH-3), 5.37 (d, 1H, *J* = 8.3 Hz, NH-1), 4.52 (dq, 1H, *J* = 9.1, 7.1 Hz, CαH-3), 4.21 (d, 1H, *J* = 7.2 Hz, CαH-1), 4.08 (m, 1H, CεH-2), 4.06 (m, 1H, CαH-4), 4.06 (m, 1H, CβH-4), 4.02 (m, 1H, CεH-4), 3.99 (m, 1H, CβH-2), 3.78 (s, 3H, -$COOCH_3$), 3.46 (m, 2H, Cε′H-2, CαH-2), 3.45 (m, 1H, Cε′H-4), 2.10 (m, 1H, CγH-2), 2.02 (m, 1H, CγH-4), 1.88 (m, 1H, CδH-4), 1.83 (m, 2H, CδH-2, Cδ′H-4), 1.76 (m, 1H, Cδ′H-2), 1.71 (m, 1H, Cγ′H-4), 1.52 (m, 1H, Cγ′H-2), 1.43 (s, 9H, Boc), 1.33 (m, 3H, CH_3-1), 1.32 (m, 3H, CH_3-2); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 173.1, 171.3, 170.4, 169.5, 156.0, 81.3, 80.3, 79.7, 67.6, 67.4, 52.5, 50.0, 48.4, 47.9, 47.3, 30.2, 29.7, 28.3 (3C), 25.2, 24.7, 17.7, 17.0; HRMS (ESI): *m/z* calcd for $C_{24}H_{40}N_4O_9Na$: 551.2692 $[M+Na]^+$; found: 551.2682.

Boc-D-Ala-[(*S*,*S*)-APyC-D-Ala]$_2$-(*S*,*S*)-APyC-OMe (10)

A solution of ester **8** (0.04 g, 0.75 mmol) in THF: MeOH: H_2O (3 : 1 : 1) LiOH (0.01 g, 0.45 mmol) was added at 0 °C and continued stirring at room temperature for 2 h. Work up as described for **17** gave the acid **25a**.

A solution of acid **25a** (0.04 g, 0.07 mmol), HOBt (0.01 g, 0.08 mmol), and EDCI (0.02 g, 0.08 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C for 15 min and treated with **21b** [prepared from **21** (0.02 g, 0.07 mmol) and CF_3COOH (0.02 mL) in CH_2Cl_2 (0.5 mL)] and DIPEA (0.12 mL, 0.14 mmol) in CH_2Cl_2 under N_2 atmosphere for 8 h. Workup as described for **3** and purification by column chromatography (60–120 mesh Silica gel, 4.0% methanol in $CHCl_3$) gave **10** (0.03 g, 65%) as a white solid; m.p. 270 °C; $[\alpha]_D$ = +142.4 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$): 3402, 3310, 3008, 2935, 2861, 1665, 1533, 1449, 1373, 1236, 1205, 1158, 1092 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, 278 K): δ 7.62 (d, 1H, *J* = 9.6 Hz, NH-4), 7.37 (d, 1H, *J* = 8.6 Hz, NH-6), 7.31 (d, 1H, *J* = 9.1 Hz, NH-3), 6.66 (d, 1H, *J* = 9.5 Hz, NH-5), 6.60 (d, 1H, *J* = 9.6 Hz, NH-2), 5.33 (d, 1H, *J* = 8.1 Hz, NH-1), 4.60 (dq, 1H, *J* = 9.6, 7.1 Hz, CαH-5), 4.55 (dq, 1H, *J* = 9.1, 7.0 Hz, CαH-3), 4.22 (p, 1H, *J* = 7.2 Hz, CαH-1), 4.07 (m, 1H, CεH-4), 4.06 (m, 6H, CβH-2, CεH-2, CβH-4, CαH-6, CβH-6, CεH-6), 3.78 (s, 3H, -$COOCH_3$), 3.63 (d, 1H, *J* = 9.8 Hz, CαH-4), 3.48 (d, 1H, *J* = 9.9 Hz, CαH-2), 3.47 (m, 1H, Cε′H-4), 3.45 (m, 1H, Cε′H-6), 3.41 (m, 1H, Cε′H-2), 2.08 (m, 1H, CγH-2), 2.03 (m, 1H, CγH-6), 2.00 (m, 1H, CγH-4), 1.92 (m, 1H, CδH-4), 1.88 (m, 1H, CδH-6), 1.85 (m, 1H, CδH-1), 1.83 (m, 1H, Cδ′H-6), 1.81 (m, 1H, Cδ′H-4), 1.75 (m, 1H, Cγ′H-4), 1.74 (m, 1H, Cδ′H-2), 1.68 (m, 1H, Cγ′H-6), 1.52 (dq, 1H, *J* = 4.0, 12.5 Hz, Cγ′H-2), 1.47 (s, 9H, Boc), 1.33 (d, 3H, *J* = 7.1 Hz, CH_3-1), 1.33 (d, 3H, *J* = 7.1 Hz, CH_3-5), 1.26 (d, 3H, *J* = 7.0 Hz, CH_3-3); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 173.2, 172.4, 171.4, 170.3, 169.9, 169.6, 155.8, 82.0, 81.2, 80.4, 79.8, 67.5 (2C), 67.4, 52.4, 49.0, 48.4, 47.7, 47.0 (2C), 46.9, 30.2, 29.7, 29.0, 28.9 (3C), 28.4, 25.4, 25.1, 17.1 (2C), 17.0; HRMS (ESI): *m/z* calcd for $C_{33}H_{54}N_6O_{12}Na$: 749.3697 $[M+Na]^+$; found: 749.3737.

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20 Solvent titration studies were carried out by sequentially adding up to 33% (v/v) of DMSO-d_6 to 600 μL $CDCl_3$ solutions of the peptides.
21 (*a*) As defined by Hofmann *et al.*,[10] a 9/11-helix is one in which the amide proton of α-residue participates in 9-mr H-bonding, whereas that of the β-residue participates in 11-mr H-bonding; (*b*) In our earlier studies,[4c] we have defined, based on the convention of Wu and Wong,[9b] a 9/11-helix as one in which the first H-bonding is a 9-membered one.
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