"Macrocyclic Design Strategies for Small, Stable Parallel β-Sheet Scaffolds"

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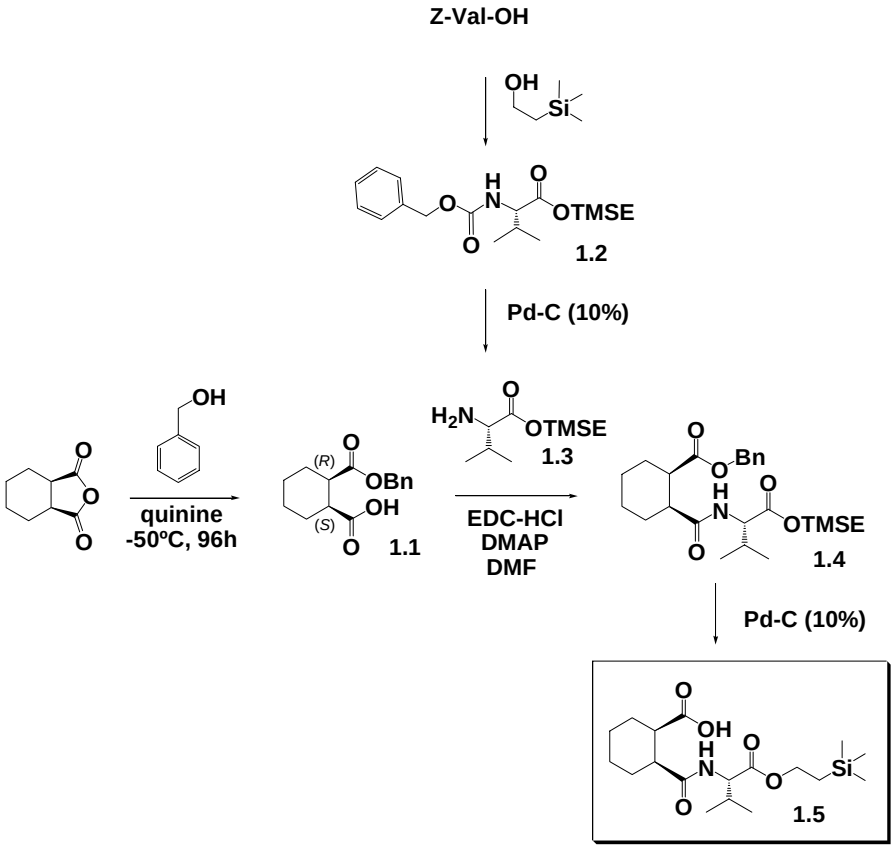
Supporting Information

Synthesis of cyclic peptides.

Materials. Novasyn TGR Resin, Novasyn TGA Resin, 2-Chlorotrityl Resin, hydroxybenzotriazole (HOBT), and all protected amino acids were obtained from Novabiochem. Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU), 1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC.HCl) were obtained from Chem. Impex. 7-azabenzotriazol-1-yloxy-tris-(pyrrolidino)phosphonium hexafluorophosphate (PyAOP), was obtained from Applied Biosystems. Solvents and all other chemicals were purchased from Aldrich or Fisher and used as received.

Synthesis of N-to-N linkers.

Scheme 1. Synthesis of the N-to-N linker 1.5.



(1S,2R)-2-((Benzyloxy)carbonyl)cyclohexanecarboxylic acid (1.1)

(ref. S1) Anhydride **1.1** (3.3 g, 21.4 mmol) and quinidine (7.6 g, 23.6 mmol) were dissolved in 250 mL of freshly distilled toluene under N_2. The mixture was cooled at -50ºC. Benzyl alcohol (6.7 mL , 64.7 mmol) was added as a single portion, and the reaction was allowed to stir for 5 days at -50ºC. The toluene was then removed under reduced pressure. The remaining oil was then dissolved in 125 mL of ether. The organic solution was washed with 2 N HCl (3 x 30 mL). The aqueous phase was then back-extracted with ether (5 x 50 mL). These five ether washes were combined and extracted with sat. $NaHCO_{3(aq)}$ (5 x 75 mL). The aqueous phase was washed with ether (100 mL) to remove any residual benzyl alcohol, acidified with 8 N HCl and extracted with CH_2Cl_2 (5 x 100 mL). The organic phase was then dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure to leave 3.36 g of clear oil (60 % yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23–1.44 (m, 4H), 1.72–1.86 (m, 2H), 1.98–2.10 (m, 2H), 2.82–2.90 (m, 2H), 5.10, 5.15 (AB_q, J = 12.6 Hz, 2H), 7.26–7.38 (m, 5H);^{13}C NMR (300 MHz, $CDCl_3$) δ 23.87, 23.95, 26.28, 26.48, 42.7, 66.5, 113.0, 128.3, 128.7, 136.2, 173.6, 179.8 ESI-MS calc. $[C_{15}H_{18}O_4Na]^+$= 285.1103 obs. $[C_{15}H_{18}O_4Na]^+$= 285.1093

Z-Val-OTMSE (1.2)

Z-Val-OH (3 g, 8.33 mmol) was dissolved in 85 mL of CH_2Cl_2. 2-Trimethylsilylethanol (1.25 g, 10.6 mmol), EDC (2.032 g, 10.6 mmol) and DMAP (cat.) were added to the solution, and the mixture was stirred at room temperature for 14 h. The reaction mixture was washed with 1 N HCl (3 x 100 mL), sat. $NaHCO_{3\,(aq)}$ (3 x 100 mL) and brine (1 x 100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to leave clear oil. Purification by chromatography on silica gel (EtOAc-Hexanes, 3:7), afforded 3.360 g of clear oil (87 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): 0.00 (s, 9H), 0.87 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H), 0.89-1.01 (m, 2H), 2.07-2.18 (m, 1H), 4.16-4.25 (m, 3H), 4.33-4.36 (m, 2H), 5.27 (d, J = 9.1 Hz, 3H), 7.23-7.36 (m, 4H), 7.55 (d, J = 7.3 Hz, 2H), 7.70 (d, J = 7.6 Hz, 2H) . ^{13}C NMR (300 MHz, $CDCl_3$) 0.0, 19.0, 19.1, 20.5, 32.9, 48.8, 60.6, 65.6, 68.5, 121.5, 126.6, 128.5, 129.2, 130.2, 142.8, 143.3, 145.5, 157.7, 168.7, 173.7 ESI-MS calc. $[C_{25}H_{33}NO_4SiNa]^+$= 462.2072 obs. $[C_{25}H_{33}NO_4SiNa]^+$= 462.2082

H-Val-OTMSE (1.3)

Protected amino acid **1.2** (1.0 g) was dissolved in 10 mL of 4.4 % formic acid-methanol. This solution was added to a round bottom flask containing 1.0 g of (Pd-C 10 %) and 50 mL of 4.4 % formic acid-MeOH. The mixture was allowed to stir overnight under an N_2 atmosphere. The suspension was filtered, and the solvent was removed under reduced pressure yielding **1.3** (643 mg, 80 %).

^{1}H NMR (300 MHz, $CDCl_3$): 0.00 (s, 9H), 0.85 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.6 Hz, 3H), 0.93-0.99 (m, 2H), 1.92-2.03 (m, 1H), 3.21 (d, J = 4.7 Hz, 1H), 4.13-4.19 (m, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) 0.0, 18.7, 20.8, 33.7, 51.5, 64.5, 177.1 ESI-MS calc. $[C_{25}H_{33}NO_4SiNa]^+$= 240.1498 obs. $[C_{25}H_{33}NO_4SiNa]^+$= 240.0568

BnO-(1*S*,2*R*)-CHDA-Val-OTMSE (1.4)

Compound **1.1** (1.0 g, 3.82 mmol) and **1.3** (0.994 g, 4.578 mmol) were dissolved in 46 mL CH_2Cl_2. PyBOP (2.38 g, 4.578 mmol), and DMAP (cat) were added to the solution, which was allowed to stir for 48 h. The solution was washed with 1 N HCl (3 x 100 mL), sat. $NaHCO_{3\,(aq)}$ (2 x 100 mL) and brine (1 x 100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to leave an orange oil. Purification by chromatography on silica gel (EtOAc-Hexanes, 3:7), afforded 1.02 g of white solid product (62 % yield).

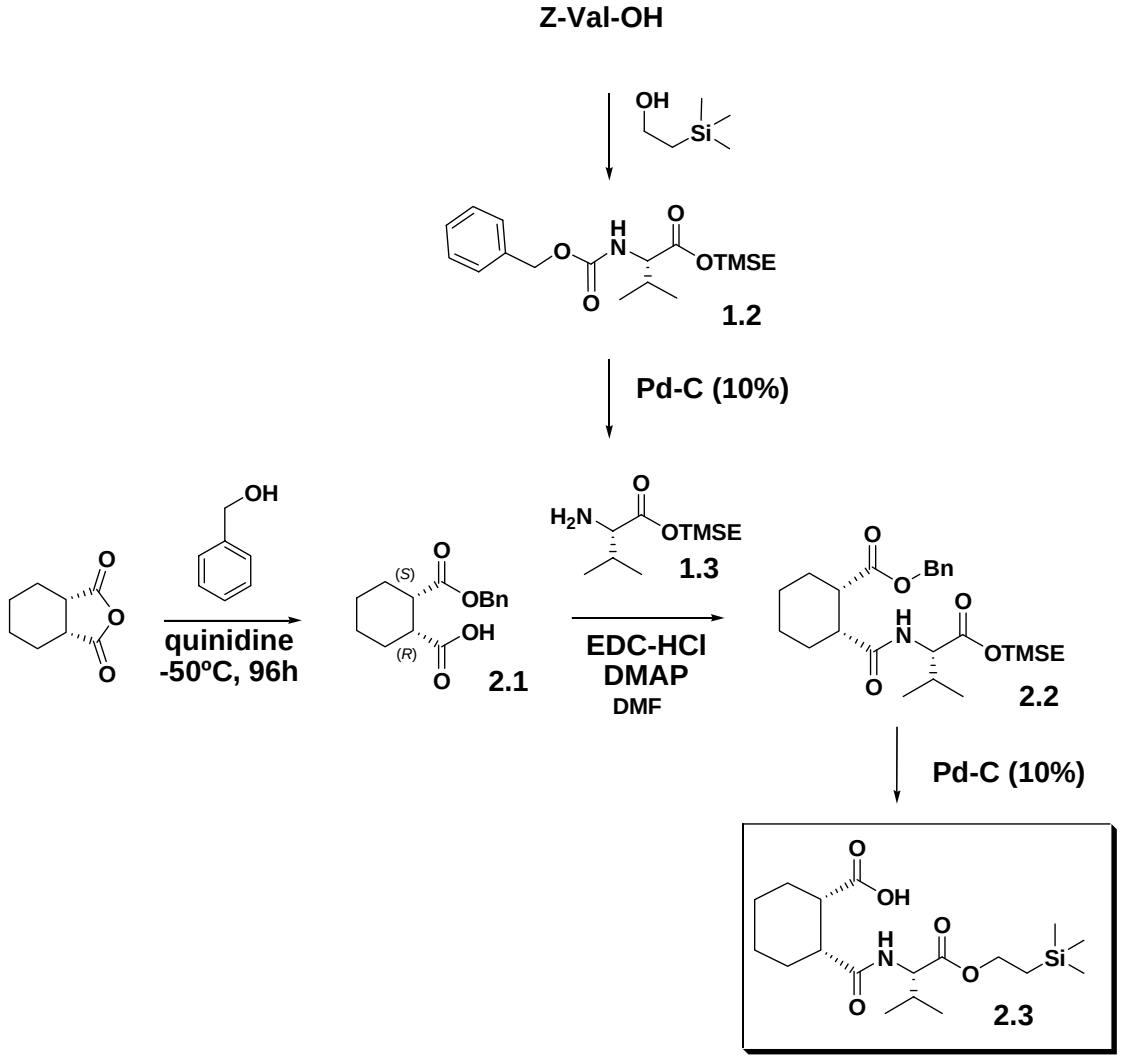
^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 9H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.93-0.99 (m, 2H), 1.31-1.55 (m, 3H), 1.57-1.78 (m, 3H), 2.01-2.13 (m, 3H), 2.66-2.72 (m, 1H), 2.85-2.91 (m, 1H), 4.13-4.19 (m, 2H), 4.48 (dd, *J* = 4.7 and 8.4 Hz, 1H), 5.04 (d, *J* = 12.4 Hz, 1H), 5.08 (d, *J* = 12.4 Hz, 1H), 6.20 (d, *J* = 8.8 Hz, 1H), 7.22-7.31 (m, 5H) ^{13}C NMR (300 MHz, $CDCl_3$) δ 0.0, 19.0, 19.3, 20.4, 25.0, 25.3, 28.2, 28.4, 33.0, 44.0, 45.4, 58.3, 65.0, 67.6, 129.5, 130.0, 137.8, 173.7, 174.7, 157.4 ESI-MS calc. = $[C_{25}H_{39}NO_5SiNa]^+$= 484.2490 obs. $[C_{21}H_{30}N_2O_4Na]^+$= 484.2501

HO-(1*S*,2*R*)-CHDA-Val-OTMSE (1.5)

Protected peptide **1.4** (1.0 g) was dissolved in 10 mL of 4.4 % formic acid-methanol. This solution was added to a round bottom flask containing 1.0 g of (Pd-C 10 %) and 50 mL of 4.4 % formic acid-MeOH. The mixture was allowed to stir overnight under an N_2 atmosphere. The suspension was filtered, and the solvent was removed under reduced pressure yielding pure compound **1.5** as a clear oil (765 mg, 95%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 9H), 0.91 (d, *J* = 7.3 Hz, 3H), 0.93 (d, *J* = 6.9 Hz, 3H) 0.99-1.04 (m, 2H), 1.38-1.84 (m, 6H), 1.88-1.97 (m, 1H), 2.07-2.15 (m, 2H), H), 2.63-2.69 (m, 1H), 2.88-2.92 (m, 1H), 4.15-4.21 (m, 2H), 4.48 (dd, *J* = 4.7 Hz and 8.4 Hz, 1H), 6.32 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 0.0, 19.1, 19.3, 20.4, 24.9, 35.4, 28.4, 29.0, 32.9, 44.4, 45.7, 58.7, 65.4, 173.7, 176.2, 179.0 ESI-MS calc. = $[C_{18}H_{33}NO_5SiNa]^+$= 394.2021 obs. $[C_{18}H_{33}NO_5SiNa]^+$= 394.2004

Scheme 2. Synthesis of the N-to-N linker 2.3.



(1*R*,2*S*)-2-((Benzyloxy)carbonyl)cyclohexanecarboxylic acid (2.1)
Compound **1.1** (3.3 g, 21.4 mmol) and quinine (7.6 g, 23.6 mmol) were dissolved in 100 mL of freshly distilled toluene under N_2. The mixture was cooled at -50 ºC. Benzyl alcohol (6.7 mL) was added as a single portion and the reaction was allowed to stir for 5 days at -50ºC. The toluene was then removed under reduced pressure. The remaining oil was then dissolved in 125 mL of ether. The organic solution was washed with 2 N HCl (3 x 30 mL). The aqueous phase was then back extracted with ether (5 x 50 mL). The combined organic layers were extracted with sat. $NaHCO_{3(aq)}$ (5 x 75 mL). The aqueous phase was washed with ether (100 mL) to remove any residual benzyl alcohol, acidified with 8 N HCl and extracted with CH_2Cl_2 (5 x 100 mL). The organic phase was then dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure to leave 3.36 g of clear oil (60 % yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23–1.44 (m, 4H), 1.72–1.86 (m, 2H), 1.98–2.10 (m, 2H), 2.82–2.90 (m, 2H), 5.10, 5.15 (AB_q, *J* = 12.6 Hz, 2H), 7.26–7.38 (m, 5H) ^{13}C NMR (300 MHz, $CDCl_3$) δ 23.90, 23.94, 26.31, 26.48, 42.6, 42.7, 66.6, 128.3, 128.7, 136.3, 173.6, 178.9 ESI-MS calc. $[C_{15}H_{18}O_4Na]^+$= 285.1103 obs. $[C_{15}H_{18}O_4Na]^+$= 285.1095

BnO-(1*R*,2*S*)-CHDA-Val-OTMSE (2.2)

Compound **2.1** (1.0 g, 3.82 mmol) and **1.3** (0.994 g, 4.578 mmol) were dissolved in 46 mL CH_2Cl_2. PyBOP (2.38 g, 4.578 mmol), and DMAP (cat) were added to the solution, which was allowed to stir for 48 h. The solution was washed with 1 N HCl (3 x 100 mL), sat. $NaHCO_{3\,(aq)}$ (2 x 100 mL) and brine (1 x 100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to leave a orange oil. The product was purified by silica column chromatography (EtOAc-Hexanes, 3:7), yielding 1.00 g of white solid product (60 % yield).

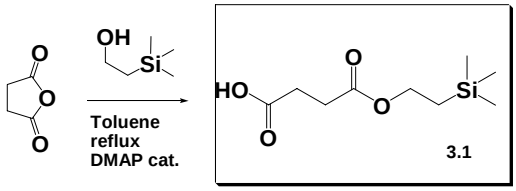
^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 9H), 0.85 (d, *J* = 6.9 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.93-0.99 (m, 2H), 1.31-1.77 (m, 6H), 2.06-2.15 (m, 3H), 2.67-2.72 (m, 1H), 2.85-2.90 (m, 1H), 4.13-4.18 (m, 2H), 4.48 (dd, *J* = 4.4 and 8.4 Hz, 1H), 5.04 (d, *J* = 12.1 Hz, 1H), 5.08 (d, *J* = 12.3 Hz, 1H), 6.21 (d, *J* = 8.8 Hz, 1H), 7.20-7.31 (m, 5H) ^{13}C NMR (300 MHz, $CDCl_3$) δ 0.0, 0.3, 19.0, 19.3, 20.4, 25.0, 25.3, 28.2, 28.4, 33.0, 44.0, 45.4, 58.3, 65.0, 67.6, 129.5, 129.9, 137.8, 173.7, 174.7, 157.4 ESI-MS calc. = $[C_{25}H_{39}NO_5SiNa]^+$= 484.2490 obs. $[C_{21}H_{30}N_2O_4Na]^+$= 484.2501

HO-(1*S*,2*R*)-CHDA-Val-OTMSE (2.3)

Protected peptide **2.2** (1 g) was dissolved in 10 mL of 4.4 % formic acid-methanol. This solution was added to a round bottom flask containing 1 g of (Pd-C 10 %) and 50 mL of 4.4 % formic acid-MeOH. The mixture was allowed to react overnight under an N_2 atmosphere. The suspension was filtered and the solvent was removed under reduced pressure yielding pure compound **2.3** (805 mg, 80 %).

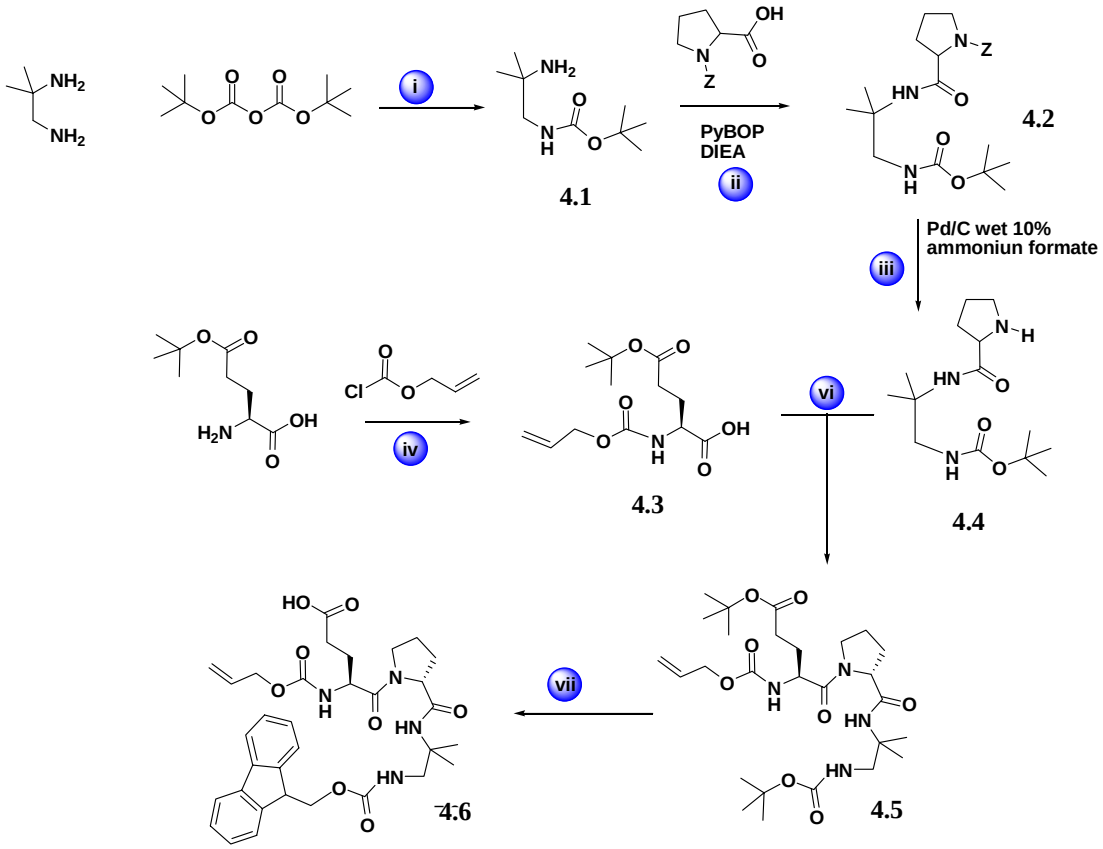
^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 9H), 0.91 (d, *J* = 6.9 Hz, 3H), 0.93 (d, *J* = 6.9 Hz, 3H) 0.99-1.05 (m, 2H), 1.38-1.52 (m, 2H), 1.59-1.81 (m, 4H), 1.93-2.03 (m, 1H), 2.12-2.24 (m, 1H), 2.73-2.78 (m, 1H), 2.82-2.87 (m, 1H), 4.22-4.26 (m, 2H), 4.52 (dd, *J* = 4.7 Hz and 8.4 Hz, 1H), 6.33 (d, *J* = 8.8 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 0.0, 19.1, 19.3, 20.4, 24.9, 35.4, 28.4, 29.0, 32.9, 44.4, 45.7, 58.7, 65.4, 173.7, 176.2, 179.0 ESI-MS calc. = $[C_{18}H_{33}NO_5SiNa]^+$= 394.2021 obs. $[C_{18}H_{33}NO_5SiNa]^+$= 394.2004

Scheme 3. Synthesis of the N-to-N linker 3.1.



3-((2-(Trimethylsilyl)ethoxy)carbonyl)propanoic acid (3.1)
Succinic anhydride (2 g, 20.0 mmol) and was dissolved in toluene (20 mL). 2-Trimethylsilylethanol (1.25 g, 10.6 mmol) and DMAP (cat.) were added to the solution, and the mixture was stirred at reflux for 14 h. The solution was washed with 1 N HCl (3 x 100 mL), and brine (1 x 100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to leave compound **3.1** as a clear oil (4.3 g, 99 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.01 (s, 9H), 0.95 (m, 2H), 2.55 (m, 2H), 2.63 (m, 2H), 4.14 (m, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 0.0, 18.82, 30.46, 30.84, 64.45, 64.58, 173.77, 179.48 ESI-MS calc. = $[C_{18}H_{33}NO_5SiNa]^+$= 241.0974 obs. $[C_{18}H_{33}NO_5SiNa]^+$= 241.0032.

Scheme 4. Synthesis of the C-to-C linker 4.6.

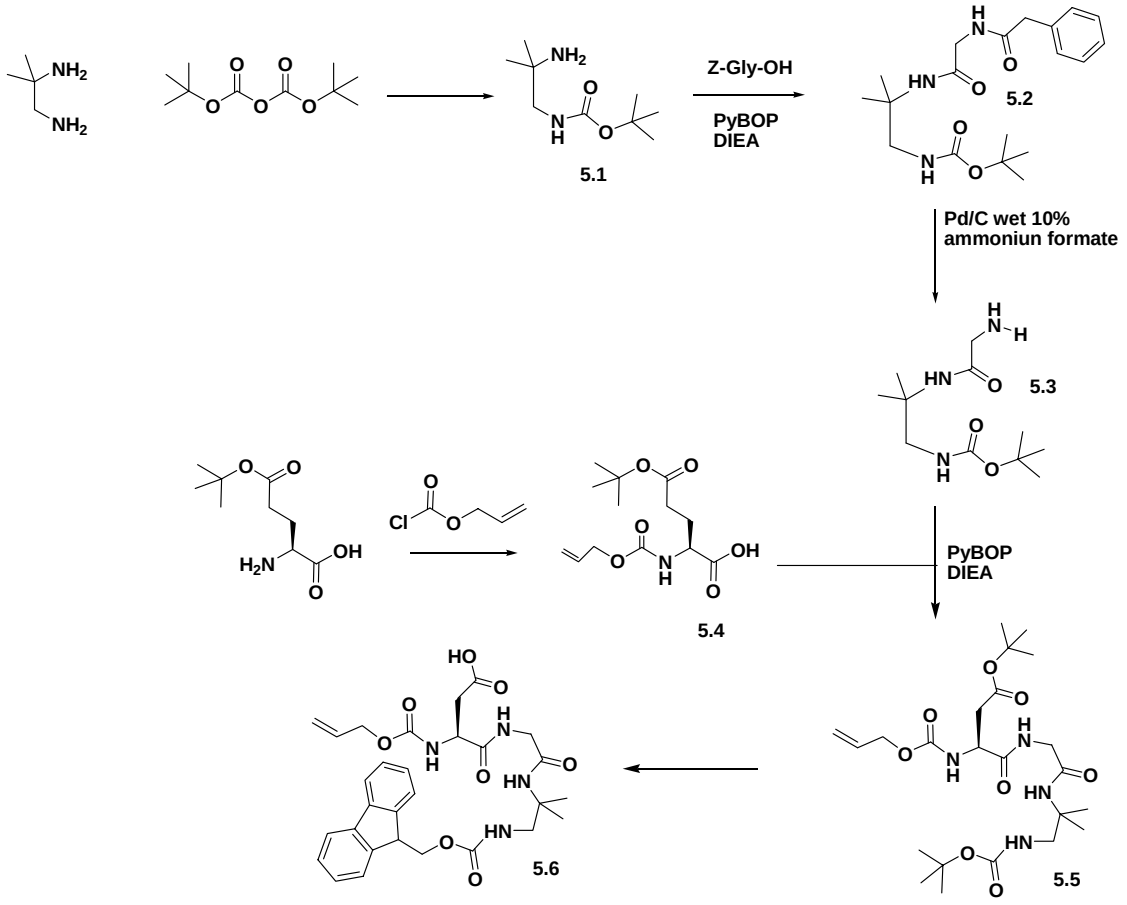


This material was prepared as described in the supporting information of Ref. The steps indicated in Scheme 4 are are summarized below:

i) 2-Methyl-1,2-diaminopropane (4 ml, 38.2 mmol), $(Boc)_2O$ (2.94 g), CH_2Cl_2 (150 mL). 70 % yield. ii) **4.1** (2.73 g, 15.4 mmol), Z-DPro-OH (4.6 g, 18.5 mmol), PyBOP (9.6 g, 18.5 mmol), DIEA (4.3 mL, 18.5 mmol), CH_2Cl_2 (100 ml); 72 % yield. iii) **4.2** (4.25 g, 10.3 mmol), ammonium formate (3.26 g, 51.7 mmol), Pd-C (wet 10 %) (3 g), MeOH (150 ml); 90 % yield. iv) H-Glu(O^{t}Bu)-OH (2.02 g, 11.8 mmol), dioxane (60 mL), sat.

aq. $NaHCO_3$ (20 mL), allyloxy chloroformate (1.3 mL, 11.8 mmol); 90 % yield. v) **4.3** (2.59 g, 9.08 mmol), **4.4** (3.13 g, 10.89 mmol), PyBOP (5.66 g, 10.89 mmol), DIEA (1.42 g, 10.89 mmol), CH_2Cl_2 (90 ml); 80 % yield. vi) a) **4.5** (4 g, 7.25 mmol), 4 N HCl in Dioxane (40 mL). 1.5h; remove solvent under reduced pressure; b) acetone (500 mL), 1 N $NaHCO_3$ (100 mL), Fmoc-OSu (2.55 g, **4.6** mmol), 4 h; 90 % yield.

Scheme 5. Synthesis of the C-to-C linker 5.6.



Tert-butyl-2-amino-2-methylpropylcarbamate (H-Dadme-Boc) 5.1
2-Methyl-1,2-diaminopropane (4 mL) was dissolved in 160 mL of CH_2Cl_2. $(Boc)_2O$ (2.95 g, 13.5 mmol) was added dropwise as a solution in 150 mL of CH_2Cl_2 (over 6 h). The mixture (white suspension) was stirred for 18 h and then transferred to a separatory funnel and extracted with aqueous $NaHCO_3$ (10%). The aqueous layer was then back-extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and evaporated, to leave compound **5.1** as clear oil (4 g, 56 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.09 (s, 6H), 1.46 (s, 9H), 3.01 (s, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 28.5, 50.3, 52.4, 79.2, 156.6 ESI-MS calc. = $[C_9H_{20}N_2O_2Na]^+$= 211.2673 obs. $[C_9H_{20}N_2O_2Na]^+$= 211.0561.

Z-Gly-Dadme-Boc 5.2

H-Dadme-Boc (2 g, 11.29 mmol) and Z-Gly-OH (2.83, 13.5 mmol) were dissolved in 120 mL of CH_2Cl_2. PyBOP (7.04 g, 13.54 mmol) and DIEA (1.76 g, 13.54 mmol) were added to the solution, which was allowed to stir for 4 h. The solution was washed with 1 N HCl (3 x 100 mL), sat. $NaHCO_{3\,(aq)}$ (2 x 100 mL) and brine (1 x 100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to leave an orange oil. This material was purified by silica column chromatography (EtOAc-Hexanes, 2:8),, to yield 4.10 g of white solid product (100 % yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 6H), 1.40 (s, 9H), 3.21 (d, *J* = 6.2 Hz, 2H), 3.73 (d, *J* = 5.5 Hz, 2H), 5.09 (s, 2H), 5.32 (br s, 1H), 5.61 (br s, 1H), 6.73 (s, 1H), 7.25-7.34 (m, 5H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 24.4, 28.4, 45.0, 49.6, 55.5, 67.1, 79.8, 128.1, 128.6, 136.3, 156.7, 157.4, 168.8. ESI-MS calc. = $[C_{19}H_{23}N_3O_4Na]^+$= 386,2518, obs. $[C_{19}H_{23}N_3O_4Na]^+$= 386,0853.

H-Gly-Dadme-Boc 5.3

Protected molecule **5.2** (3 g) was dissolved in 20 mL of 4.4 % formic acid-methanol. This solution was added to a round bottom flask containing 2 g of (Pd-C 10 %) and 100 mL of 4.4 % formic acid-MeOH. The mixture was allowed to stir overnight under an N_2 atmosphere. The suspension was filtered, and the solvent was removed from the filtrate under reduced pressure yielding pure compound **5.3** (2.3 g, 80 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (s, 6H), 1.44 (s, 9H), 2.71-2.83 (m, 2H), 3.22-3.38 (m, 2H), 5.44-5.48 (m, 1H), 7.33 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 25.0, 28.5, 45.0, 49.5, 54.4, 79.3, 156.9, 172.2. ESI-MS calc. = $[C_{11}H_{23}N_3O_3Na]^+$= 267.1661, obs. $[C_{11}H_{23}N_3O_3Na]^+$= 266.9985.

Alloc-Glu(O^t Bu)-OH 5.4

H-Glu(O^{t}Bu)-OH (2.02 g, 9.84 mmol) was dissolved in 60 mL of dioxane and 20 mL of sat. aqueous $NaHCO_3$. Allyloxy chloroformate was added as a single portion (1.3 mL**,** 12.2 mmol). The opaque mixture was stirred for 24 h and then transferred to a separatory funnel and acidified with 1 N HCl. The solution was extracted with 3 x 100 CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and evaporated, to leave compound **5.4** as a clear oil (3.1 g, 100%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.94-2.06 (m, 1H), 2.14-2.26 (m, 1H), 2.30-2.50 (m, 2H), 4.34-4.41 (m, 1H), 4.58 (d, *J* = 5.1 Hz, 2H), 5.20-5.30 (m, 2H), 5.53 (d, *J* = 7.3 Hz, 1H), 5.84-5.97 (m, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 27.3, 28.0, 31.5, 65.9, 66.9, 81.0, 117.8, 132.4, 156.0, 172.3, 175.2. ESI-MS calc. = $[C_{13}H_{21}NO_6Na]^+$= 310.1369, obs. $[C_{13}H_{21}NO_6Na]^+$= 310.1195.

Alloc-Glu(O^t Bu)- Gly-Dadme-Boc 5.5

Alloc-Glu(O^t Bu)-OH **5.4** (3.23 g, 11.25 mmol) and H-Gly-Dadme-Boc **5.3** (2.3 g, 9.38 mmol), were dissolved in 100 mL of CH_2Cl_2. PyBOP (5.85 g, 11.25 mmol), and DIEA (3.37 g, 11.25 mmol) were added to the solution, which was allowed to stir for 12 h. The solution was washed with 1 N HCl (3 x 100 mL), sat. $NaHCO_{3\ (aq)}$ (2 x 100 mL) and brine (1 x 100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to leave an orange oil. Purification by chromatography on silica gel (EtOAc-Hexanes, 3:7), afforded 3 g of white solid product (65 % yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (s, 6H), 1.44 (s, 9H), 1.90-2.03 (m, 1H), 2.04-2.17 (m, 1H), 2.25-2.46 (m, 2H), 3.27 (d, *J* = 6.2 Hz, 1H), 3.75-3.92 (m, 1H), 4.17-4.23 (m, 1H), 4.50-4.62 (m, 2H), 5.19-5.32 (m, 2H), 5.37-5.40 (m, 1H), 5.83-5.96 (m, 1H), 6.02 (d, *J* = 6.2 Hz, 1H), 6.71 (s, 1H), 7.12-7.14 (m, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.4, 21.2, 24.6, 28.3, 28.6, 43.8, 49.4, 55.2, 60.6, 66.2, 81.3, 118.2, 132.6, 157.4, 168.6, 171.3, 172.3. ESI-MS calc. = $[C_{23}H_{40}N_4O_8Na]^+$= 523.2846, obs. $[C_{23}H_{40}N_4O_8Na]^+$= 523.1956.

Alloc-Glu(OH)- Gly-Dadme-Fmoc 5.6

Alloc-Glu(O^t Bu)-Gly-Dadme-Boc **5.5** (3 g**,** 5.99 mmol) was dissolved in 40 mL of 4 N HCl in dioxane, and the solution was stirred for 1.5 h. HCl-dioxane was removed with a high vacuum rotavapor. The resulting solid was resuspended in aAcetone (200 mL), and 100 mL of 1 N aqueous $NaHCO_3$ was added. Fmoc-OSu (1.05 eq) was added, and the mixture was stirred for 4 h. The acetone was removed by using a rotavapor. The remaining liquid was transferred to a separatory funnel, acidified and extracted with EtOAc (3 x 100 mL), The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to leave 2 g of clear oil **(**compound **5.6).**

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (s, 3H), 1.29 (s, 3H), 1.83-2.16 (m, 2H), 2.43-2.46 (m, 2H), 3.36 (d, *J* = 6.2 Hz, 1H), 3.64-3.93 (m, 1H), 4.17-4.22 (m, 1H), 4.30-4.55 (m, 4H), 5.10-5.32 (m, 2H), 5.70-5.80 (m, 1H), 6.06 (d, *J* = 6.2 Hz, 1H), 6.36 (d, *J* = 6.9 Hz, 1H), 6.45 (s, 1H), 6.75 (s, 1H), 7.26-7.40 (m, 4H), 7.73-7.75 (m, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 24.7, 25.6, 26.2, 47.4, 58.3, 66.3, 67.0, 109.0, 111.2, 118.2, 120.2, 125.3, 127.3, 127.9, 128.0, 132.5, 141.5, 141.6, 144.0, 166.0, 169.5, 172.7, 172.9. ESI-MS calc. = $[C_{29}H_{34}N_4O_8Na]^+$= 589.6023, obs. $[C_{29}H_{34}N_4O_8Na]^+$= 589.3556.

Structures of macrocycles 10 and 11, which are diastereomers of macrocycles 1 and 2 (see Fig 1 in the main text), respectively.

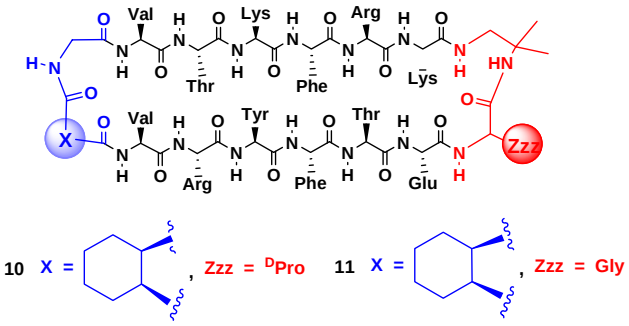
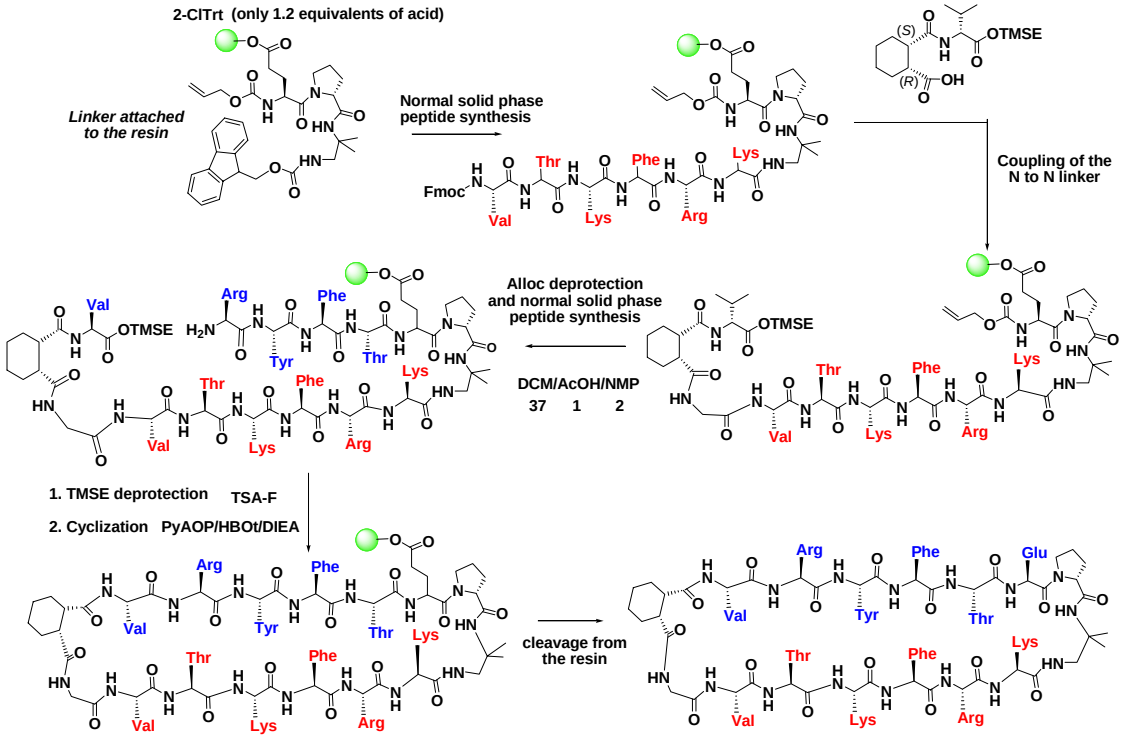


Figure 1S. Macrocycles **10-11**.

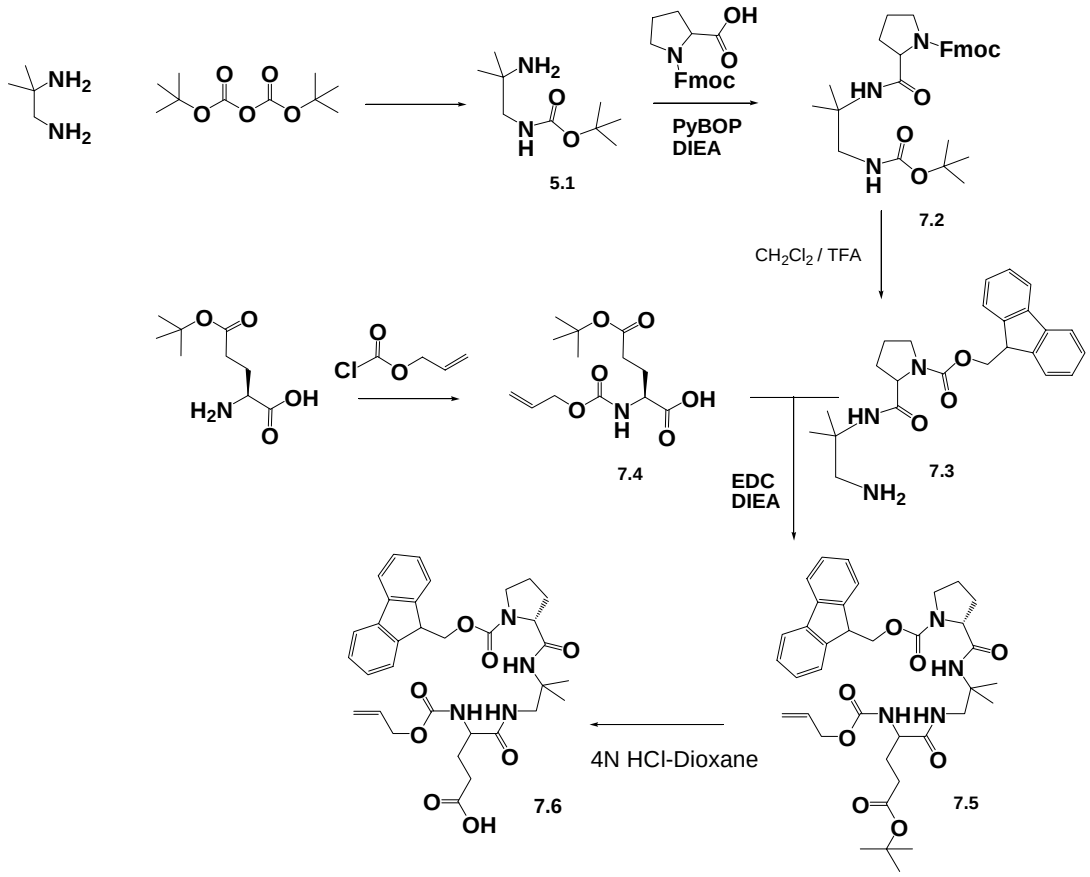
Scheme 6. Synthesis of the cyclic peptides



Synthesis of cyclic peptides **1, 3,** and **10** began with the preparation in solution of the fragment Alloc-Glu-Val-D-Pro-DADME-Fmoc (**4.6**), summarized in Scheme 4. Synthesis of cyclic peptides **2**, **4**, and **11** began with the preparation in solution of the fragment Alloc-Glu-Val-Gly-DADME-Fmoc (**5.6**), summarized in Scheme 5. This

fragment was attached to Nova Syn TGA resin via the Glu side chain carboxyl group, and residues corresponding to Lys(1) to Gly(7) were appended via Fmoc-based solid phase synthesis, using standard Fmoc procedures. Fmoc amino acids were activated as HOBT esters with HBTU and DIEA. All amino acids with reactive side chains were protected. The N-to-N linker unit, **2.3** (Scheme 2) or **1.5** (Scheme 1) or **3.1** (Scheme 3)) was treated as an amino acid and coupled to Gly(7) using the same protocol employed for other couplings. The alloc protecting group was removed with Pd^0 catalyst, and the amino acid residues corresponding to Thr(13) through Arg(10) in peptides **1-2** and **10-11**, or Thr(13) through Val (9) in peptides **3** and **4**, were introduced via standard methods. The Fmoc was removed from Arg(10) or Val(9), TSAF (4 eq., 4 h) was used to remove the TMSE protecting group, and cylization was achieved on resin with standard coupling reagents (PyAOP, HOBT, DIEA in DMF).

Scheme 7. Synthesis of the C-to-C linker 7.6.

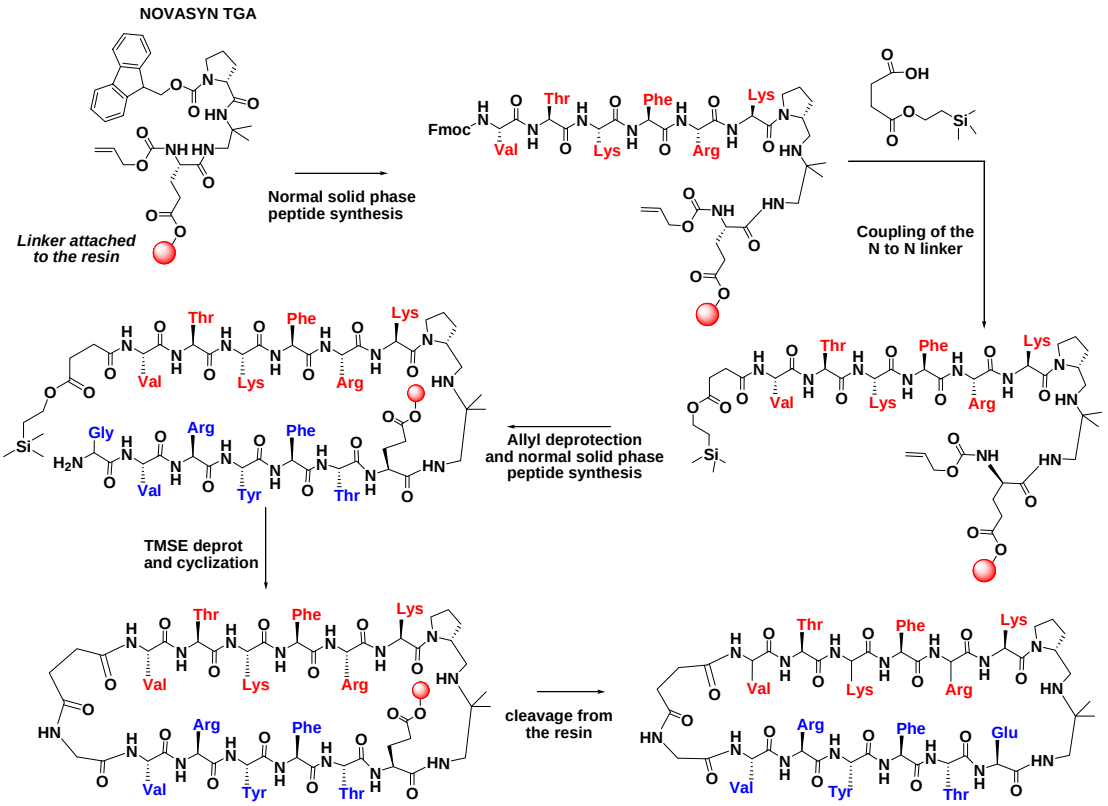


i) 2-Methyl-1,2-diaminopropane (4 ml, 38.2 mmol), $(Boc)_2O$ (2.94 g), CH_2Cl_2 (150 ml). 70 % yield. ii) **7.1** (2.72 g, 15.4 mmol), Fmoc-DPro-OH (6.2 g, 18.48), PyBOP (9.6 g, 18.5 mmol), DIEA (4.3 mL, 18.5 mmol) CH_2Cl_2 (100 ml). 72 % yield. iii) CH_2Cl_2:TFA (1:1) iv) H-Glu(O^{t}Bu)-OH (2.02 g, 11.8 mmol), Dioxane (60mL), $NaHCO_3$ sat (20mL), Allyloxy chloroformiate (1.3 mL, 11.8 mmol). 90 % yield. v) **7.3** (2.00 g, 4.93 mmol), **7.4** (1.84 g, 6.42 mmol), EDC (1.23 g, 6.42 mmol), DIEA (3.0 mL, 19.72 mmol) CH_2Cl_2 (60 ml). 60 % yield. vi) a) **7.5** (1.5 g, 2.12 mmol), 4N HCl in Dioxane (50 mL). 1.5h. remove solvent under reduced pressure. 90% yield.

Fmoc-^{D}Pro-Dadme-Glu(OH)-Alloc (7.6).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (s, 3H), 1.30 (s, 3H), 1.82-2.08 (m, 6H), 2.31-2.46 (m, 2H), 3.44-3.75 (m, 6H), 4.04-4.47(m, 6H), 5.12-5.26 (m, 2H), 5.82-5.92 (m, 2H), 6.36 (s, 1H), 7.31-7.37 (m, 4H), 7.59 (d, *J* = 6.6 Hz, 2H). 7.74 (d, *J* = 7.6 Hz, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 24.7, 24.8, 24.9, 29.9, 30.0, 30.4, 42.8, 43.0, 47.3, 58.3, 61.9, 66.0, 68.0, 71.3, 72.5, 111.2, 117.9, 120.1, 124.5, 125.3, 127.3, 127.4, 141.5, 143.8, 172.4, 175.9, 179.4 ESI-MS calc. = $[C_{33}H_{40}N_4O_8Na]^+$= 643.2846, obs. $[C_{29}H_{34}N_4O_8Na]^+$= 643.0964.

Scheme 8. Synthesis of the cyclic peptide 9



Synthesis of cyclic peptide **(9)** began with the preparation in solution of the fragment Fmoc-^{D}Pro-Dadme-Glu(OH)-Alloc (**7.6**), described in Scheme 7. This fragment was attached to Nova Syn TGA resin via the Glu side chain carboxyl group, and residues corresponding to Lys(14) to Val(9) were appended via Fmoc-based solid phase synthesis, using standard Fmoc procedures. Fmoc amino acids were activated as HOBT esters with HBTU and DIEA. All amino acid with reactive side chains were protected. The N-to-N linker unit (**4.6**) was treated as an amino acid and coupled to the Val(9) using the protocol for normal couplings. The alloc protecting group was removed with Pd^{0} catalyst, and the amino acids residues corresponding to Thr(2) through Gly(7) were introduced via standard methods. The Fmoc was removed from Gly(7). TSAF (4 eq., 4 h) was used to remove the TMSE protecting group, and cylization was achieved on resin with standard coupling reagents (PyAOP, HOBT, DIEA in DMF).

NMR Protocols.

NMR sample preparation and experiments

NMR samples were prepared by dissolving lyophilized peptides in 9:1 H_2O/D_2O, 100 mM pH 3.80 sodium deuterioacetate buffer (buffer pH was not corrected for isotope effects). Peptide concentrations were generally 2.5 mM. Samples were prepared with total volumes of approximately 300 μL for 3 mm tubes and 600 μL for 5 mm NMR tubes. Trace amounts of 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were added to samples as internal reference. The NMR samples were stable in solution for weeks to months showing no apparent precipitation of peptide or decrease in NMR signal strength over the entire period of study. In all cases sharp lines were observed in 1D spectra suggesting that the peptides were not aggregated in solution.

NMR experiments were performed on Varian INOVA 600 MHz spectrometers at 4ºC using 3 mm or 5 mm probes.

Inova 600 probes
Varian 3 mm $^{1}H/^{13}C/^{15}N$ with 3 axis PFG
Varian 5 mm $^{1}H/^{13}C/^{15}N$ with 3 axis PFG

The probe thermocouple temperatures were calibrated with a methanol chemical shift thermometer. Chemical shifts measurements were performed at 5 K intervals set via the probe thermocouple calibration. The reported temperatures are presumed to be accurate to ≈ 1 K. Solvent suppression was achieved by 0.6-1.5 second solvent presaturation during the relaxation delay. Watergate solvent suppression as implemented in Varian´s Protein Pack suite of experiments was used instead of presaturation in some ROESY and TOCSY experiments. Spectral windows of 6600 Hz were used. Standard Varina pulse sequences were used, and data were processed using Varian VNMR 6.1 software and analyzed with the sparky (ref S3) program. Shifted sine bell window functions were generally applied before Fourier transformation. For all samples GCOSY spectra were obtained in absolute mode with gradient echo coherence selection; TOCSY (ref S4) and ROESY (ref S4, S6) spectra were acquired in the sensitive mode with hypercomplex phase cycling (States-Haberkorn method). All experiments were performed by collecting 2048 points in f2 and 300-600 points in f1. TOCSY experiments employed a standard MLEV-17 spin lock sequence with a spin lock field of 7-8 KHz and mixing time of 80 ms. ROESY experiments used spinlocking fields of 3 kHzs and mixing times of 200-250 ms. The ^{1}H chemical shift assignment of the peptides was achieved by the sequential assignment procedures (ref. S7) ROEs were characterized as strong, medium or weak based on visual comparison of well resolved geminal proton pairs.

NMR Structure Ensemble Generation.

Distance constrains were derived from the intensity of NOE cross-peaks in 600 MHz ROESY spectra with 200 ms mixing time at 277 K. Using the $I = c.r^{-6}$ approximation (ref S7) (where I is the NOE intensity, r the distance between protons, and c a constant) and the NOE between well resolved diastereotopic CH_2 protons as an internal standard, the interactions were grouped into very strong (≤2.5 Å), strong (≤3.0Å), medium (≤3.5Å), and weak (≤4.5Å) categories. NMR structure ensembles were generated using v1.1 of CNS (ref. S9). The constraints employed for the ensembles are summarized in Table 7S-11S. The default CNS simulated annealing script, which includes high-temperature dynamics and then a cooling cycle in torsion space (with K_{NOE} = 150) followed by further cooling in Cartesian space (K_{NOE} = 50) and minimization (K_{NOE} = 75), was modified in the following ways: (a) the high-temperature torsion dynamics and cooling phases were each doubled in length (2000 15-fs steps each, initially 50 000 K with cooling during the last 2000 steps); (b) the Cartesian cooling cycle (starting at 2000 K, K_{NOE} = 75 or 50 Kcal/Å^2) was also doubled in length (6000 5-fs steps) which allowed us to ramp the E_{repel} scaled factor from 0.2 to 1.0 during the cycle; (c) chemical shift and H-bond constrains were not employed, and the dihedral angle energy function was disabled. In present study, 500 random structures were used, producing 20 accepted structures with no NOE distance restraint violations in excess of 0.15 Å.

Use of NMR to determine whether peptides self-associate

Diffusion experiments (ref. S9) for the extraction of the hydrodynamic radius R_h were performed by using a water-sLED pulse sequence (ref S10) through acquisition of spectra with varying gradient strength and plots of variations in signal as a function of gradient strength, according to the equation:

$$\ln I/I_0 = -\gamma^2 G^2 \delta^2 D(\Delta - \delta/3)$$

$$\ln \frac{I}{I_0} = -b.\ D \qquad b = \gamma^2\, G^2\, \delta^2\, (\Delta - \delta/3)$$

$$D = \frac{K_b T}{6\pi\eta r_s}$$

where I refers to the peak intensity for a given gradient strength G; I_0 to the peak intensity in the absence of gradients; Δ denotes the separation between leading edges of the gradients; δ is the duration of the gradient pulse of strength G, and γ the magnetogyric ratio of ^1H. A non-interacting compound, dioxane (R_h=2.12 Å), was added to the peptide solutions to serve as a reference, and $R_h^{peptide}$ was obtained from the slopes, S_{pept} and S_{ref} of the semilogarithmic fits of data for the peptide and the reference compound to the equation above according to the next equation.

$$R_h^{peptide} = \frac{slope_{ref}}{slope_{pep}} R_h^{ref}$$

The diffusion experiments were performed for peptides **3** and **10** in 90 % H_2O/10 % D_2O containing 20 µl of 1 % dioxane in D_2O, with gradient strengths between 2 and 46 G/cm at 277 K. The delays Δ and δ where held constant in all the experiments at 70 and 5 ms. Peak intensities for several peaks across the spectrum were measured using MestreNova 5.1.0.(ref S11). All data fitting was performed in GraphPad Prism 4 (ref S 12).

These measurements were performed for peptides **10** and **3** in a range of concentrations between 300 µM and 9 mM, but the deduced hydrodynamic radius did not change over this range, indicating that these peptides do not form aggregates in this range of concentrations. For peptide **10**, R_h = 8.30 ± 0.20 (0.3 mM, 2.5 mM and 8 mM). For peptide **3**, the R_h = 8.19 ± 0.25 (0.3 mM, 2.5 mM and 5 mM). 1D proton NMR spectra displayed sharp peaks in this concentration range, which supports our conclusion that there is no peptide aggregation in this range of concentrations.

Table 1S. Proton resonances (ppm relatice to DSS) for **1**:
c(VYFTE^{D}P-dadme-KRFKTVG-(1*S*,2*R*)-CHDA)
(2.5mM peptide in 9:1 H_2O:D_2O pH 3.80, 100 mM acetate buffer, 277 K)

Residue	HN	Hα	Hβ, Hβ´	Others
Lys1	8.862	4.936	1.830	γ 1.479, 1.587, δ 1.742, ε 3.017 τ 7.667,
Arg2	9.051	4.750	1.788	γ 1.510 δ 2.894, ε 6.914
Phe3	8.864	5.557	2.938, 3.085	δ 7.142, ε 7.305, τ 7.256
Lys4	8.805	4.469	1.697	γ 1.112, 1.209, δ 1.493, 1.606, ε 2.890, τ 7.728
Thr5	8.730	4.737	3.771	γ 0.705
Val6	7.998	4.157	2.201	γ 0.862, 0.975
Gly7	8.280	3.773, 4.100		
CHDA8				3.175, 2.528, 2.107, 1.936, 1.846, 1.753, 1.546, 1.332
Val9	8.072	4.534	1.757	γ 0.472, 0.672
Arg10	8.266	4.515	1.661	γ 1.407, δ 3.063, ε 7.153.
Tyr11	8.745	5.212	2.568, 2.642	δ 6.873 , ε 6.662
Phe12	8.840	4.826	2.637, 3.032	δ 7.148, ε 7.304, τ 7.264
Thr13	8.794	4.890	3.888	γ 0.988
Glu14	8.761	4.979	2.039, 2.083	γ 2.433
^{D}Pro15		4.232	1.901, 2.272	γ 1.995, 2.153, δ 3.783, 3.874l
Dadme16	8.092			3.456, 3.585, 1.117, 1.307

Table 2S. Proton resonances (ppm relatice to DSS) for **10**:
c(VYFTE^{D}P-dadme-KRFKTVG-(1*R*,2*S*)-CHDA)
(2.5 mM peptide in 9:1 H_2O:D_2O pH 3.80, 100 mM acetate buffer, 277 K)

Residue	HN	Hα	Hβ, Hβ´	Others
Lys1	8.852	4.934	1.829	γ 1.485, 1.589, δ 1.760, ε 3.018
Arg2	9.047	4.761	1.716	γ 1.512, 1.795, δ 2.905, ε 6.914
Phe3	8.833	5.592	2.917, 3.075	δ 7.148, ε 7.305, τ 7.236
Lys4	8.741	4.537	1.658	γ 1.058, 1.189, δ 1.448, 1.594, ε 2.873
Thr5	8.695	4.782	3.785	γ 0.748
Val6	8.097	4.261	1.902	γ 0.828, 0.880
Gly7	8.152	3.923, 3.463		
CHDA8				3.041, 2.414, 2.070, 1.775, 1.576, 1.461, 1.333
Val9	7.853	4.294	1.805	γ 0.394, 0.787
Arg10	8.378	4.514	2.639, 2.569	γ 1.498, 1.675, δ 3.127, ε 7.202
Tyr11	8.661	5.170	2.557, 2.632	δ 6.883 , ε 6.665
Phe12	8.878	4.748	2.660, 3.056	δ 7.153, ε 7.306, τ 7.229
Thr13	8.827	4.909	3.911	γ 1.022
Glu14	8.768	4.985	2.037, 2.104	γ 2.425
^{D}Pro15		4.247	1.907, 2.274	γ 2.054, 2.108, δ 3.810, 3.842
Dadme16	8.094			3.454, 3.592, 1.124, 1.315

Table 3S. Proton resonances (ppm relatice to DSS) for **2**:
c(VYFTEG-dadme-KRFKTVG-(1*S*,2*R*)-CHDA)
(2.5 mM peptide in 9:1 H_2O:D_2O pH 3.80, 100 mM acetate buffer, 277 K)

Residue	HN	Hα	Hβ, Hβ´	Others
Lys1	8.871	4.916	1.842	γ 1.496, 1.594, δ 1.764, ε 3.014 τ 7.682,
Arg2	9.020	4.749	1.783	γ 1.516, δ 2.924, ε 6.925
Phe3	8.855	5.536	2.957, 3.056	δ 7.159, ε 7.295, τ 7.302
Lys4	8.800	4.473	1.699	γ 1.109, 1.203, δ 1.495, 1.624, ε 2.894, τ 7.645
Thr5	8.742	4.736	3.782	γ 0.711
Val6	8.012	4.157	2.201	γ 0.863, 0.966
Gly7	8.510	3.818		
CHDA8				3.168, 2.528, 2.096, 1.960, 1.855, 1.742, 1.531, 1.346
Val9	8.096	4.540	1.748	γ 0.478, 0.676
Arg10	8.264	4.509	1.646	γ 1.415, δ 3.066, ε 7.153.
Tyr11	8.752	5.223	2.580, 2.656	δ 6.884 , ε 6.705
Phe12	8.836	4.844	2.657, 3.039	δ 7.147, ε 7.227, τ 7.303
Thr13	8.836	4.880	3.922	γ 1.015
Glu14	8.705	4.646	2.001	γ 2.407
Gly15	9.186	3.613, 3.818		
Dadme16	8.097			3.482, 3.532, 1.158, 1.320

Table 4S. Proton resonances (ppm relatice to DSS) for **11**:
c(VYFTEG-dadme-KRFKTVG-(1*R*,2*S*)-CHDA)
(2.5 mM peptide in 9:1 H_2O:D_2O pH 3.80, 100 acetate buffer, 277 K)

Residue	HN	Hα	Hβ, Hβ´	Others
Lys1	8.871	4.898	1.840	γ 1.494, 1.595, δ 1.770, ε 3.021 τ 7.699,
Arg2	9.009	4.748	1.788	γ 1.511, 1.782 δ 2.910, ε 6.921
Phe3	8.858	5.575	2.683, 2.918	δ 7.162, ε 7.295, τ 7.295
Lys4	8.737	4.472	1.667	γ 1.044, 1.192, δ 1.510, 1.678, ε 2.869, τ 7.736
Thr5	8.708	4.782	3.779	γ 0.743
Val6	7.998	4.268	1.908	γ 0.844
Gly7	8.510	3.788		
CHDA8				3.129, 2.928, 1.846, 1.833, 1.755, 1.592, 1.484, 1.444
Val9	7.876	4.286	1.791	γ 0.390, 0.773
Arg10	8.382	4.510	1.686	γ 1.480, δ 3.127, ε 7.208.
Tyr11	8.675	5.165	2.539, 2.638	δ 6.856 , ε 6.669
Phe12	8.867	4.814	2.669, 3.061	δ 7.152, ε 7.287, τ 7.239
Thr13	8.832	4.890	3.932	γ 1.023
Glu14	8.722	4.659	2.057	γ 2.398
Gly15	9.188	3.620, 3.802		
Dadme16	8.092			3.462, 3.539, 1.158, 1.321

Table 5S. Proton resonances (ppm relatice to DSS) for **3**:
c(VYFTE^{D}P-dadme-KRFKTVG-succ)
(2.5 mM peptide in 9:1 H_2O:D_2O pH 3.80, 100 mM acetate buffer, 277 K)

Residue	HN	Hα	Hβ, Hβ´	Others
Lys1	8.867	4.939	1.840	γ 1.480, 1.603, δ 1.755, ε 3.025
Arg2	9.049	4.757	1.801	γ 1.511, δ 2.898, ε 6.915
Phe3	8.843	5.559	2.931, 3.076	δ 7.292, ε 7.306, τ 7.240
Lys4	8.781	4.478	1.692	γ 1.092, 1.216, δ 1.485, ε 2.884
Thr5	8.699	4.767	3.773	γ 0.726
Val6	8.058	4.206	2.158	γ 0.863, 0.963
Gly7	8.645	3.905, 4.059		
Succ8				2.507, 2.697, 2.764
Val9	8.357	4.398	1.768	γ 0.417, 0.725
Arg10	8.303	4.530	1.674	γ 1.437 δ 3.085, ε 7.149
Tyr11	8.737	5.210	2.562, 2.636	δ 6.871 , ε 6.669
Phe12	8.842	4.829	2.645, 3.042	δ 7.147, ε 7.236, τ 7.307
Thr13	8.798	4.897	3.893	γ 1.002
Glu14	8.761	5.019	2.061	γ 2.431
^{D}Pro15		4.243	1.894, 2.278	γ 2.007, 2.169, δ 3.783, 3.873
Dadme16	8.100			3.459, 3.586, 1.123, 1.314

Table 6S. Proton resonances (ppm relatice to DSS) for **4**:
c(VYFTEG-dadme-KRFKTVG-succ)
(2.5 mM peptide in 9:1 H_2O:D_2O pH 3.80, 100 mM acetate buffer, 277 K)

Residue	HN	Hα	Hβ, Hβ´	Others
Lys1	8.541	4.260	1.787	γ 1.423, 1.498, δ 1.724, ε 3.003
Arg2	8.364	4.314	1.751	γ 1.537, 1.676, δ 3.098, ε 7.168
Phe3	8.375	4.644	2.983, 3.088	δ 7.215, ε 7.310, τ 7.215
Lys4	8.443	4.249	1.685	γ 1.282, δ 1.593, ε 2.932
Thr5	8.464	4.307	4.102	γ 1.115
Val6	8.793	4.168	2.086	γ 0.957
Gly7	8.561	3.934, 3.792		
Succ8				2.584, 2.688
Val9	8.645	3.701	2.047	γ 0.822, 0.900
Arg10	8.652	4.302	1.635	γ 1.374, 1.503 δ 3.105, ε 7.158
Tyr11	8.565	4.614	2.821, 2.960	δ 7.055 , ε 6.746
Phe12	8.546	4.329	2.942, 3.091	δ 7.215, ε 7.310, τ 7.215
Thr13	8.311	4.329	4.137	γ 1.164
Glu14	8.596	4.335	1.972, 2.101	γ 2.420
Gly15	8.561	3.934, 3.792		
Dadme16	8.287			3.486, 1.130, 1.125

Table 7S. Proton resonances (ppm relatice to DSS) for **9**:
c(VTKFRK^{D}P-dadme-ETFYRVG-succ)
(2.5m M in 9:1 H_2O:D_2O pH 3.80, 100 mM acetate buffer, 277 K)

Residue	HN	Hα	Hβ, Hβ´	Others
Glu1	8.794	4.922	2.074	γ 2.466
Thr2	8.639	4.573	4.135	γ 1.156
Phe3	8.797	5.437	2.851	δ 7.053, ε 7.214, τ 7.053
Tyr4	8.629	4.854	2.705, 3.016	δ 7.000 , ε 6.743
Arg5	8.708	5.025	1.680	γ 1.480, δ 3.104, ε 7.216
Val6	8.104	4.060	2.171	γ 0.744, 0.951
Gly7	8.804	0.744, 0.965		
Succ8				2.587, 2.780
Val9	8.466	4.427	1.932	γ 0.806, 0.905
Thr10	8.385	4.429	3.880	γ 0.982
Lys11	8.665	4.850	1.642	γ 1.253, 1.134, δ 1.557, ε 2.784, τ 7.505
Phe12	8.700	4.719	2.666, 3.032	δ 7.053, ε 7.214, τ 7.053
Arg13	8.734	4.918	1.620	γ 1.356, δ 3.104, ε 7.216
Lys14	8.637	4.851	1.824	γ 1.416, δ 1.702, ε 2.645, τ 7.511
^{D}Pro15		4.260	2.102	γ 2.069, δ 3.793
Dadme16	8.244			1.165, 1.343, 3.513, 3.623

Table 8S. Selected long distance NOEs observed for **1** in aqueous solution.
(100 mM acetate buffer, pH 3.8, 277 K)

Residue	H-atom	Residue	H-atom	NOE intensity
Lys(1)	HG#	Pro(15)	H##	Very strong
Lys(1)	HA	Glu(14)	HN	Very strong
Lys(1)	HG#	Glu(14)	HB#	Very strong
Lys(1)	HD#	Glu(14)	HB#	Very strong
Lys(4)	HD#	Tyr(11)	HD#	Very strong
Lys(4)	HD#	Tyr(11)	HE#	Very strong
Lys(4)	HE#	Val(9)	HG#	Very strong
Lys(4)	HE#	Val(9)	HG#	Very strong
Lys(4)	HG#	Val(9)	HG#	Very strong
Lys(4)	HG#	Tyr(11)	HD#	Very strong
Lys(4)	HD#	Val(9)	HG#	Very strong
Lys(4)	HG#	Val(9)	HG#	Very strong
Thr(5)	HA	Arg(10)	HN	Very strong
Thr(5)	HB	Phe(3)	HE#	Very strong
Thr(5)	HG2#	Phe(12)	HD#	Very strong
Thr(5)	HG2#	Phe(12)	HB#	Very strong
Val(6)	HB	Val(9)	HG##	Very strong
Val(9)	HA	Val(6)	HB	Very strong
Val(9)	HG##	Val(6)	HB	Very strong
Val(9)	HG##	Tyr(11)	HE	Very strong
Arg(10)	HG#	Thr(5)	HG2#	Very strong
Phe(12)	HB#	Phe(3)	HZ	Very strong
Phe(12)	HB#	Thr(5)	HG2#	Very strong
Thr(13)	HA	Arg(2)	HN	Very strong
Glu(14)	HG#	Phe(3)	HE	Very strong
Phe(3)	HE#	Phe(12)	HN	Strong
Phe(3)	HA	Phe(12)	HN	Strong
Lys(4)	HG#	Val(6)	HG##	Strong
Lys(4)	HG#	Arg(2)	HG#	Strong
Thr(5)	HA	Arg(10)	HG#	Strong
Thr(5)	HB	Phe(12)	HD#	Strong
Thr(5)	HG2#	Phe(3)	HE#	Strong
Val(9)	HA	Val(6)	HN	Strong
Val(9)	HB	Val(6)	HB	Strong
Tyr(11)	HD#	Val(9)	HG##	Strong
Tyr(11)	HA	Lys(4)	HG##	Strong
Phe(12)	HB#	Phe(3)	HE#	Strong
Phe(12)	HB#	Phe(3)	HZ	Strong
Thr(13)	HA	Arg(2)	HG#	Strong
Glu(14)	HG#	Phe(12)	HD#	Strong
Lys(4)	HG#	Tyr(11)	HD#	Medium
Arg(10)	HG#	Phe(3)	HE#	Medium
Val(9)	HB	CHDA(8)	H21	Medium
Pro(15)	HG##	Pro(15)	HD#	Weak
Thr(5)	HG2#	Phe(12)	HN	Weak
Thr(13)	HG2#	Lys(1)	HB#	Weak

Table 9S. Selected long distance NOEs observed for **10** in aqueous solution.
(100 mM acetate buffer, pH 3.8, 277 K)

Residue	H-atom	Residue	H-atom	NOE intensity
Arg(2)	HA	Lys(1)	HN	Very strong
Arg(2)	HG#	Tyr(11)	HD#	Very strong
Phe(3)	HA	Arg(2)	HN	Very strong
Lys(4)	HA	Lys(4)	HG#	Very strong
Lys(4)	HG#	Val(6)	HG##	Very strong
Lys(4)	HE#	Val(6)	HG##	Very strong
Lys(4)	HE#	Val(9)	HG##	Very strong
Lys(4)	HE#	Val(9)	HG##	Very strong
Val(6)	HG##	Lys(4)	HE#	Very strong
Val(9)	HA	Val(6)	HN	Very strong
Val(9)	HG##	Lys(4)	HG#	Very strong
Val(9)	HG##	Tyr(11)	HE#	Very strong
Arg(10)	HN	Thr(5)	HA	Very strong
Phe(12)	HB#	Phe(3)	HZ	Very strong
Thr(13)	HA	Arg(2)	HG#	Very strong
Thr(13)	HA	Arg(2)	HG#	Very strong
Thr(13)	HA	Arg(2)	HN	Very strong
Thr(13)	HG2#	Arg(2)	HG#	Very strong
Glu(14)	HG#	Phe(3)	HE#	Very strong
Dadme(16)	H4#	Lys(1)	HB#	Very strong
Arg(2)	HD#	Tyr(11)	HE#	Strong
Lys(4)	HD#	Tyr(11)	HE#	Strong
Lys(4)	HN	Tyr(11)	HA	Strong
Lys(4)	HG#	Tyr(11)	HD#	Strong
Lys(4)	HB#	Tyr(11)	HD#	Strong
Thr(5)	HA	Arg(10)	HN	Strong
Val(9)	HG##	Val(6)	HB	Strong
Arg(10)	HD#	Phe(12)	HE#	Strong
Phe(12)	HB#	Phe(3)	HD#	Strong
Thr(13)	HG2#	Tyr811)	HD#	Strong
Lys(1)	HA	Glu(14)	HN	Medium
Lys(1)	HA	Dadme(16)	H11	Medium
Phe(3)	HA	Phe(12)	HN	Medium
Thr(5)	HG2#	Phe(3)	HE#	Medium
Thr(5)	HG2#	Arg(10)	HG#	Medium
Thr(5)	HG2#	Phe(12)	HD#	Medium
Thr(5)	HG2#	Phe(12)	HZ	Medium
Lys(1)	HD#	Glu(14)	HB#	Medium
Arg(2)	HD#	Lys(4)	HD#	Weak
Thr(5)	HG2#	Arg(10)	HG#	Weak
Phe(12)	HA	Phe(3)	HE#	Weak

Table 10S. Selected long distance NOEs observed for **3** in aqueous solution.
(100 mM acetate buffer, pH 3.8, 277 K)

Residue	H-atom	Residue	H-atom	NOE intensity
Lys(1)	HA	Glu(14)	HN	Very strong
Lys(4)	HE#	Val(9)	HG##	Very strong
Thr(5)	HA	Arg(10)	HN	Very strong
Thr(5)	HB	Phe(3)	HZ	Very strong
Val(9)	HA	Val(6)	HB	Very strong
Val(9)	HG##	Val(6)	HB	Very strong
Tyr(11)	HA	Lys(4)	HN	Very strong
Phe(12)	HB#	Phe(3)	HE#	Very strong
Thr(13)	HA	Arg(2)	HN	Very strong
Thr(5)	HG2#	Arg(10)	HH##	Very strong
Val(9)	HG##	Tyr(11)	HE#	Very strong
Val(9)	HA	Val(6)	HN#	Strong
Tyr(11)	HD#	Lys(4)	HE#	Strong
Tyr(11)	HE#	Lys(4)	HE#	Strong
Tyr(11)	HD#	Lys(4)	HD#	Strong
Tyr(11)	HE#	Lys(4)	HD#	Strong
Tyr(11)	HD#	Lys(4)	HD#	Strong
Thr(13)	HA	Arg(2)	HB#	Strong
Thr(13)	HA	Arg(2)	HB#	Strong
Glu(14)	HG#	Phe(12)	HE#	Strong
Glu(14)	HG#	Phe(12)	HZ	Strong
Lys(1)	HA	Dadme(16)	H11	Medium
Thr(5)	HG2#	Phe(12)	HB#	Medium
Thr(5)	HG2#	Arg(10)	HG#	Medium
Glu(14)	HG#	Phe(12)	HB#	Medium
Phe(12)	HB#	Phe(3)	HZ	Medium
Thr(13)	HG2#	Arg(2)	HG#	Medium
Arg(2)	HG#	Tyr(11)	HD#	Weak
Thr(13)	HG2#	Dadme(16)	H3#	Weak
Dadme(16)	H4#	Arg(2)	HG#	Weak

Table 11S. Selected long distance NOEs observed for **11** in aqueous solution.
(100 mM acetate buffer, pH 3.8, 277 K)

Residue	H-atom	Residue	H-atom	NOE intensity
Lys(1)	HA	Glu(14)	HN	Very Strong
Lys(1)	HB#	Glu(14)	HB#	Very Strong
Arg(2)	HG#	Tyr(11)	HD#	Very Strong
Phe(3)	HE#	Glu(14)	HG2#	Very Strong
Phe(3)	HA	Phe(12)	HN	Very Strong
Lys(4)	HE#	Val(6)	HG#	Very Strong
Lys(4)	HE#	Val(9)	HG#	Very Strong
Lys(4)	HG2#	Val(9)	HG#	Very Strong
Val(9)	HA	Val(6)	HN	Very Strong
Val(9)	HG#	Tyr(11)	HE#	Very Strong
Arg(10)	HN	Thr(5)	HA	Very Strong
Tyr(11)	HE#	Val(6)	HG#	Very Strong
Phe(12)	HD#	Thr(5)	HG2#	Very Strong
Phe(12)	HH#	Thr(5)	HG2#	Very Strong
Thr(13)	HA	Arg(2)	HN	Very Strong
Phe(12)	HE#	Thr(5)	HG2#	Strong
Lys(1)	HA	Dadme(16)	H11	Strong
Arg(10)	HG#	Phe(3)	HE#	Medium
Thr(13)	HG2#	Dadme(16)	H1#	Medium
Tyr(11)	HA	Lys(4)	HN	Medium
Lys(4)	HE#	Val(9)	HG#	Medium
Phe(12)	HB#	Thr(5)	HG2#	Medium
Arg(2)	HD#	Tyr(11)	HE#	Medium
Arg(2)	HD#	Tyr(11)	HD#	Medium
Lys(4)	HG#	Tyr(11)	HD#	Medium

Table 12S. Selected long distance NOEs observed for **2** in aqueous solution.
(100 mM acetate buffer, pH 3.8, 277 K)

Residue	H-atom	Residue	H-atom	NOE intensity
Lys(1)	HA	Glu(14)	HN	Very strong
Lys(1)	HB#	Glu(14)	HB#	Very strong
Phe(3)	HA	Phe(12)	HN	Very strong
Lys(4)	HE#	Val(6)	HG#	Very strong
Lys(4)	HG#	Tyr(11)	HE#	Very strong
Thr(5)	HA	Arg(10)	HN	Very strong
Thr(5)	HG2#	Phe(3)	HE#	Very strong
Thr(5)	HG2#	Phe(12)	HD#	Very strong
Val(6)	HB	Val(9)	HG##	Very strong
Val(6)	HB	Val(9)	HG##	Very strong
Val(9)	HA	Val(6)	HN	Very strong
Arg(10)	HN	Thr(5)	HA	Very strong
Tyr(11)	HE#	Lys(4)	HN	Very strong
Tyr(11)	HA	Lys(4)	HN	Very strong
Thr(13)	HA	Arg(2)	HN	Very strong
Glu(14)	HG#	Phe(3)	HE#	Very strong
Dadme(16)	H5#	Arg(2)	HD#	Very strong
Arg(2)	HG#	Tyr(11)	HD#	Strong
Lys(4)	HE#	Val(9)	HG#	Strong
Lys(4)	HE#	Tyr(11)	HE#	Strong
Lys(4)	HG#	Tyr(11)	HD#	Strong
Val(6)	HA	Lys(4)	HG#	Strong
Val(9)	HA	Val(6)	HB	Strong
Val(9)	HG2#	Tyr(11)	HE#	Strong
Phe(12)	HB#	Thr(5)	HG2#	Strong
Thr(13)	HA	Arg(2)	HG#	Strong
Glu(14)	HB#	Phe(3)	HD#	Strong
Glu(14)	HG#	Phe(12)	HD#	Strong
CHDA(8)	H6#	Val(9)	HN	Medium
Phe(3)	HA	Phe(12)	HN	Weak
Thr(5)	HG2#	Phe(12)	HZ	Weak
Arg(2)	HG#	Thr(13)	HE#	Weak
Phe(12)	HB#	Phe(3)	HE#	Weak

Table 13S. Selected long distance NOEs observed for **9** in aqueous solution.
(100 mM acetate buffer, pH 3.8, 277 K)

Residue	H-atom	Residue	H-atom	NOE intensity
Val(6)	HB	Val(9)	HG##	Very Strong
Val(6)	HB	Val(9)	HG##	Very Strong
Succ(8)	H3#	Val(9)	HN	Very Strong
Succ(8)	H3#	Val(9)	HG##	Very Strong
Val(9)	HA	Val(6)	HB	Very Strong
Val(9)	HG##	Tyr(4)	HE#	Very Strong
Val(9)	HG##	Succ(8)	H3#	Very Strong
Thr(10)	HB	Phe(3)	HD#	Very Strong
Lys(11)	HG#	Tyr(4)	HD#	Very Strong
Lys(11)	HG#	Tyr(4)	HE#	Very Strong
Lys(11)	HG#	Val(9)	HG##	Very Strong
Arg(13)	HA	Thr(2)	HN	Very Strong
Lys(14)	HN	Glu(1)	HA	Very Strong
Thr(2)	HG2#	Arg(13)	HB#	Strong
Tyr(4)	HE#	Val(6)	HG2#	Strong
Arg(5)	HB#	Phe(3)	HB#	Strong
Val(6)	HG##	Lys(11)	HG#	Strong
Val(9)	HB	Val(6)	HG##	Strong
Lys(11)	HD#	Val(6)	HG##	Strong
Phe(12)	HB#	Phe(3)	HD#	Strong
Dadme(16)	H5#	Glu(1)	HG#	Strong
Phe(3)	HA	Phe(12)	HN	Strong
Phe(3)	HD	Thr(10)	HG2#	Medium
Val(9)	HG##	Tyr(4)	HD#	Medium
Tyr(4)	HD#	Val(6)	HG##	Medium
Lys(11)	HA	Tyr(4)	HN	Ambiguous-strong
Arg(5)	HA	Thr(10)	HN	Ambiguous-strong

Chemical shift deviation studies for macrocyclic peptides 10 and 11.

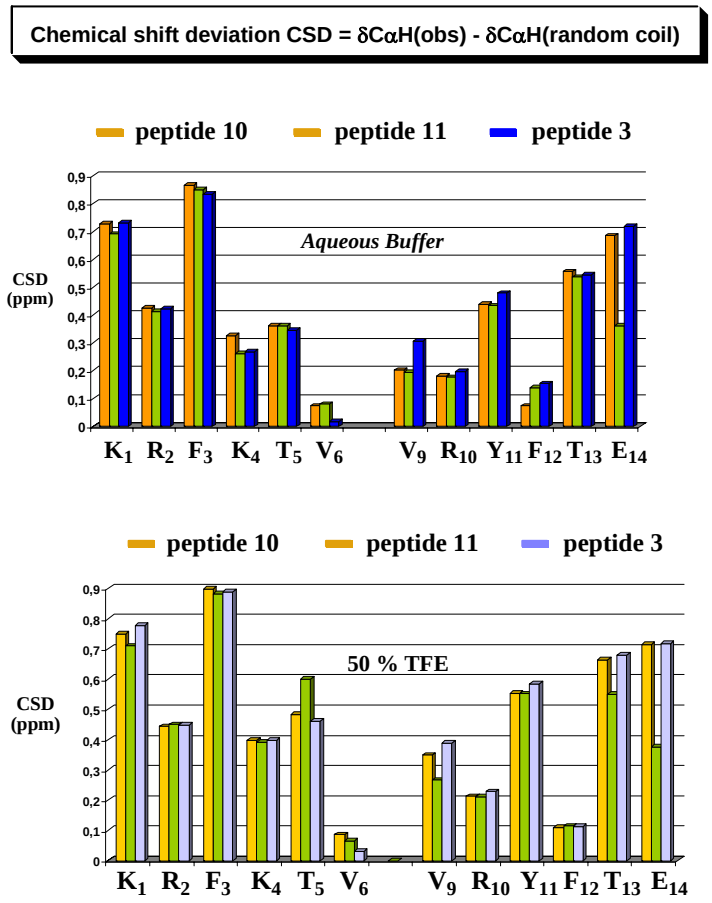


Figure 2S. $\Delta\delta C_\alpha H$ = $\delta C_\alpha H$(observed) - $\delta C_\alpha H$(random coil), or CSD, for α-amino acid residues of macrocycles **10-11** dissolved in aqueous buffer

The CSD data and the NMR-derived structures reveal very similar conformational behavior for peptides **1-3** (see Fig 2 in the main text), **10** and **11.** These similarities indicate that the presence of just one preorganized linker (along with one flexible linker) promotes parallel β-sheet secondary structure, i.e., that it is not necessary to use preorganized linkers for both N-to-N and C-to-C connections of the peptide strands.

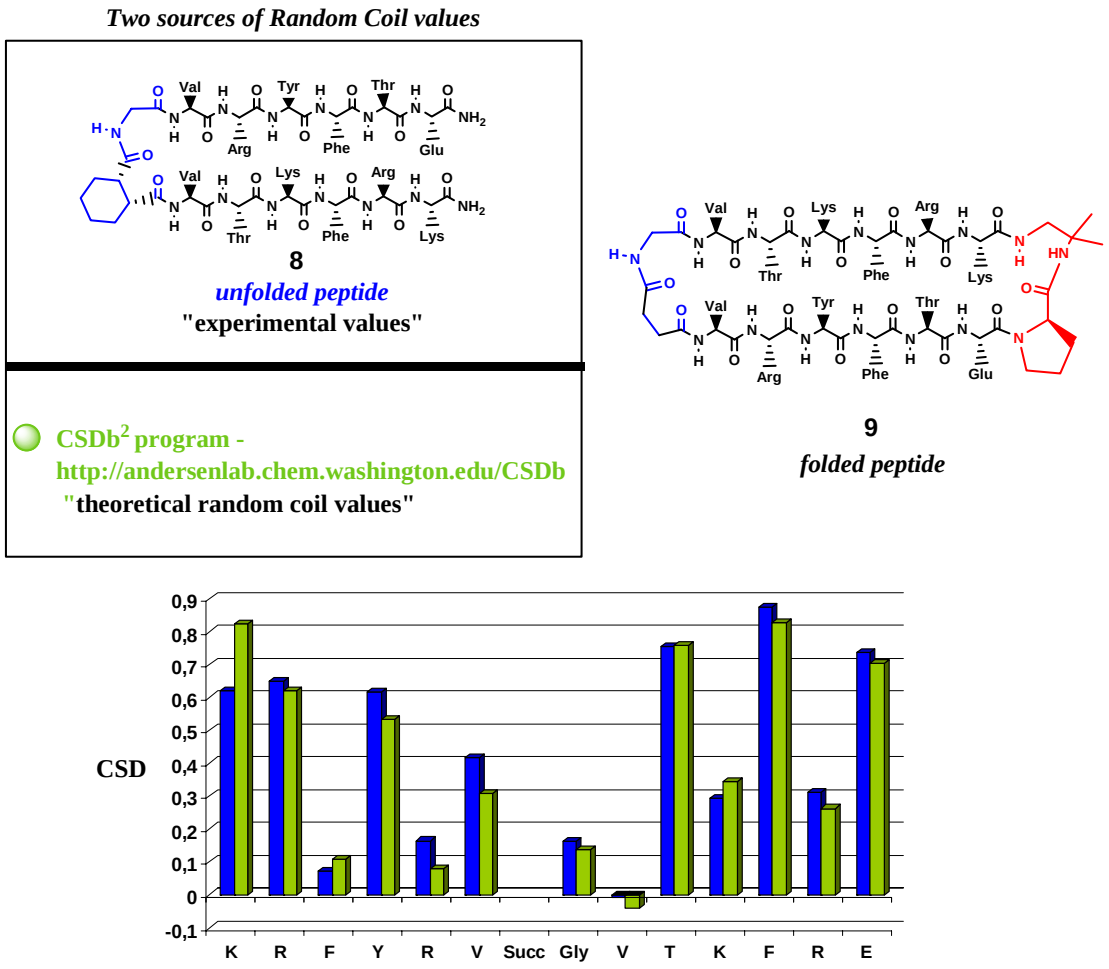


Figure 3S. $\Delta\delta C_\alpha H = \delta C_\alpha H$(observed) - $\delta C_\alpha H$(random coil), or CSD, for α-amino acid residues of macrocycle **9** dissolved in aqueous buffer using peptide **8 (blue bars)** or CSDb[2] (green bars) as the source of random coil values.

Is noteworthy that the CSD values obtained by using the CH_α values of unfolded peptide **8**, are very similar to those obtained by using the CSDb[2] program, a bioinformatics tool developed by Niels H. Andersen *et al.*, which highlights the value of CSDb[2] for generating random coil values.

Effect of TFE on the chemical shifts of Hα protons

Table 14S. Chemical shifts of Hα protons observed for **1** in aqueous solution with different proportions of TFE (100 mM acetate buffer/ X % TFE, pH 3.80, 278 K).

	Aqueous buffer	10 % TFE	20 % TFE	30 %TFE	50 %TFE
Lys(1)	4,934	4,93	4,923	4,945	4,981
Arg(2)	4,75	4,751	4,741	4,75	4,789
Phe(3)	5,557	5,557	5,57	5,585	5,616
Lys(4)	4,469	4,484	4,526	4,571	4,603
Thr(5)	4,737	4,758	4,789	4,848	4,902
Val(6)	4,157	4,16	4,173	4,180	4,196
Val(9)	4,534	4,538	4,544	4,533	4,551
Arg(10)	4,515	4,523	4,522	4,547	4,562
Tyr(11)	5,212	5,234	5,243	5,284	5,324
Phe(12)	4,826	4,824	4,818	4,811	4,811
Thr(13)	4,89	4,913	4,946	4,982	5,03
Glu(14)	4,979	4,973	4,981	4,994	5,024

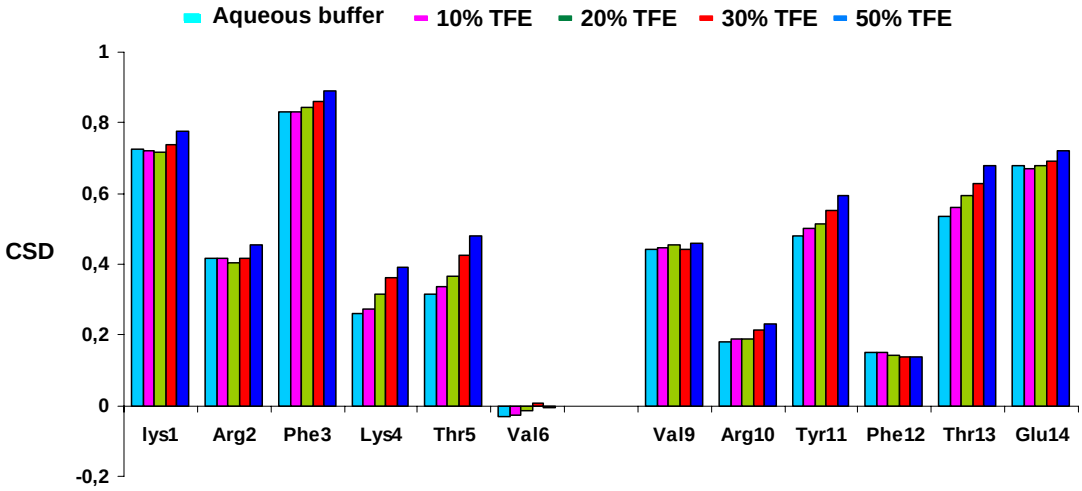


Figure 4S. Effect of TFE cosolvent proportion on Hα chemical shifts in cyclic reference peptide **1** (100 mM acetate buffer/ X % TFE, pH 3.8, 278 K).

Table 15S. Chemical shifts of Hα protons observed for **10** in aqueous solution with different proportions of TFE (100 mM acetate buffer/ X % TFE, pH 3.80, 278 K).

	Aqueous buffer	10 % TFE	20 % TFE	30 %TFE	50 %TFE
lys1	4,934	4,922	4,919	4,934	4,956
Arg2	4,761	4,759	4,739	4,748	4,779
Phe3	5,592	5,585	5,601	5,61	5,623
Lys4	4,537	4,488	4,519	4,559	4,607
Thr5	4,782	4,789	4,807	4,854	4,905
Val6	4,261	4,274	4,26	4,272	4,273
Val9	4,294	4,307	4,324	4,375	4,44
Arg10	4,514	4,517	4,516	4,53	4,546
Tyr11	5,17	5,181	5,198	5,236	5,284
Phe12	4,748	4,796	4,786	4,788	4,784
Thr13	4,909	4,911	4,94	4,981	5,016
Glu14	4,985	4,98	4,986	4,992	5,016

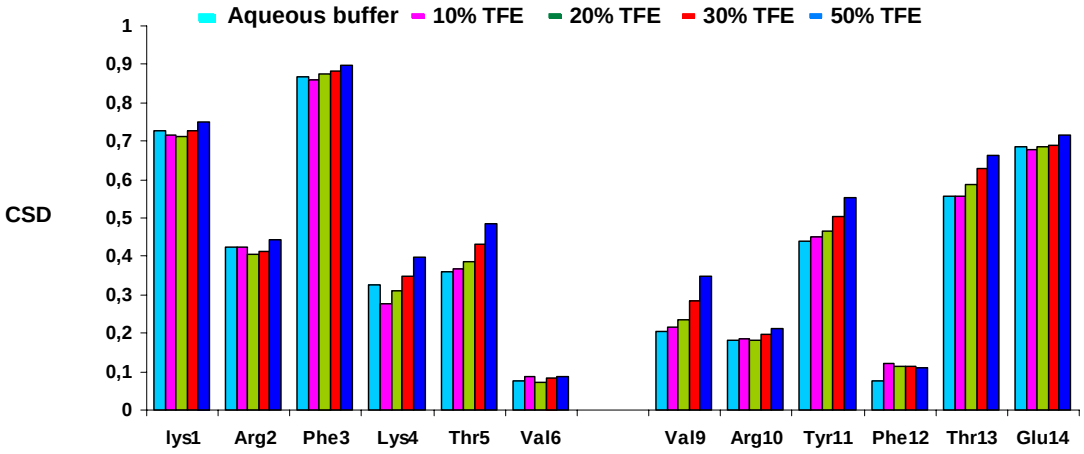


Figure 5S. Effect of TFE cosolvent proportion on Hα chemical shifts in cyclic reference peptide **10** (100 mM acetate buffer/ X % TFE, pH 3.8, 278 K).

Table 16S. Chemical shifts of Hα protons observed for **3** in aqueous solution with different proportions of TFE (100 mM acetate buffer/ X % TFE, pH 3.80, 278 K).

	Aqueous buffer	10 % TFE	20 % TFE	30 %TFE	50 %TFE
Lys(1)	4,939	4,929	4,919	4,917	4,96
Arg(2)	4,757	4,754	4,741	4,74	4,766
Phe(3)	5,559	5,57	5,575	5,582	5,609
Lys(4)	4,478	4,495	4,521	4,543	4,597
Thr(5)	4,767	4,785	4,805	4,822	4,875
Val(6)	4,206	4,218	4,214	4,206	4,223
Val(9)	4,398	4,403	4,407	4,422	4,466
Arg(10)	4,53	4,54	4,535	4,537	4,562
Tyr(11)	5,21	5,226	5,23	5,251	5,299
Phe(12)	4,829	4,823	4,811	4,799	4,794
Thr(13)	4,897	4,915	4,942	4,96	5,006

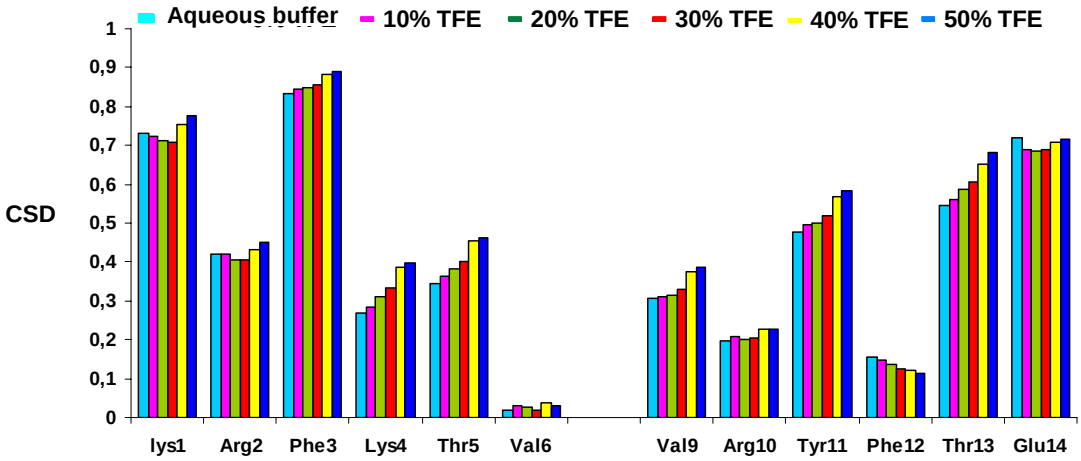


Figure 6S. Effect of TFE cosolvent proportion on Hα chemical shifts in cyclic reference peptide **3** (100 mM acetate buffer/ X % TFE, pH 3.8, 278 K).

Table 17S. Chemical shifts of Hα protons **2** observed for in aqueous solution with different proportions of TFE (100 mM acetate buffer/ X % TFE, pH 3.80, 278 K).

	Aqueous buffer	10 % TFE	20 % TFE	30 %TFE	50 %TFE
Lys(1)	4,916	4,881	4,886	4,917	4,906
Arg(2)	4,749	4,734	4,718	4,759	4,76
Phe(3)	5,536	5,537	5,55	5,588	5,584
Lys(4)	4,473	4,489	4,518	4,598	4,594
Thr(5)	4,736	4,756	4,786	4,889	4,877
Val(6)	4,157	4,168	4,156	4,334	4,325
Val(9)	4,54	4,538	4,517	4,581	4,577
Arg(10)	4,509	4,517	4,522	4,56	4,561
Tyr(11)	5,233	5,224	5,24	5,305	5,303
Phe(12)	4,844	4,892	4,814	4,793	4,796
Thr(13)	4,88	4,9	4,948	5,012	5,016

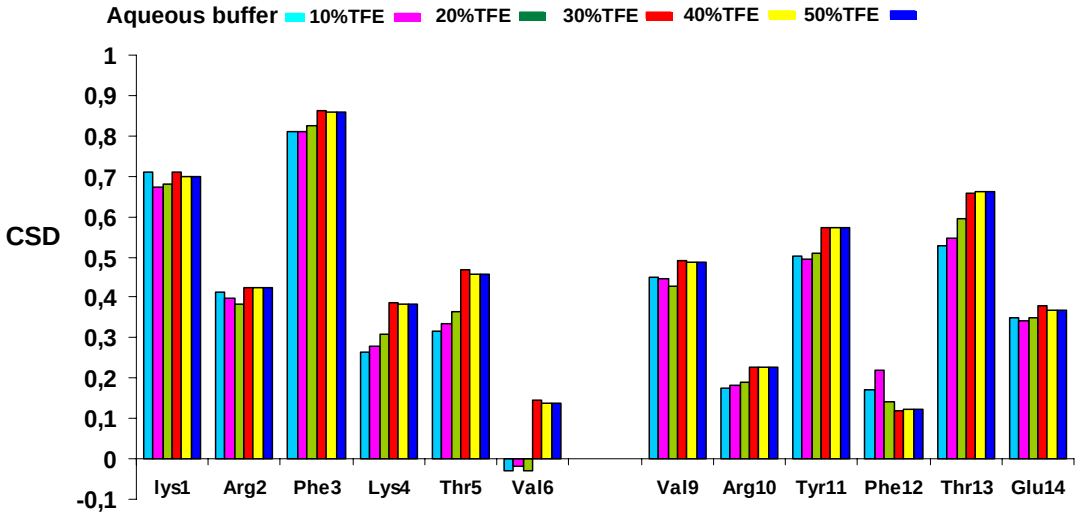


Figure 7S. Effect of TFE cosolvent proportion on Hα chemical shifts in cyclic reference peptide **2** (100 mM acetate buffer/ X % TFE, pH 3.8, 278 K).

Table 18S. Chemical shifts of Hα protons observed for **11** in aqueous solution with different proportions of TFE (100 mM acetate buffer/ X % TFE, pH 3.80, 278 K).

	Aqueous buffer	10 % TFE	20 % TFE	30 %TFE	50 %TFE
Lys(1)	4,898	4,889	4,88	4,902	4,924
Arg(2)	4,748	4,729	4,726	4,745	4,767
Phe(3)	5,575	5,583	5,587	5,607	5,607
Lys(4)	4,472	4,507	4,509	4,575	4,606
Thr(5)	4,782	4,808	4,815	4,885	5,013
Val(6)	4,268	4,274	4,266	4,276	4,267
Val(9)	4,286	4,322	4,331	4,347	4,358
Arg(10)	4,51	4,52	4,513	4,541	4,547
Tyr(11)	5,165	5,194	5,198	5,256	5,279
Phe(12)	4,814	4,809	4,803	4,799	4,799
Thr(13)	4,89	4,926	4,938	4,986	4,91

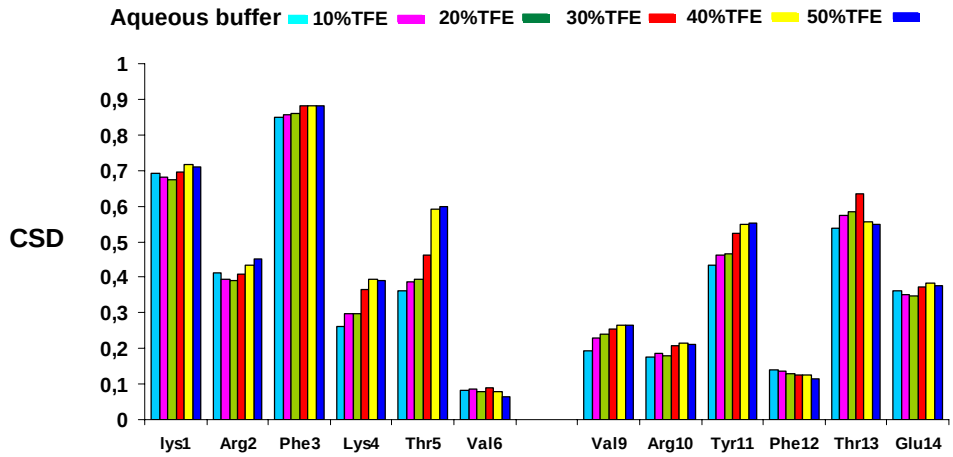


Figure 8S. Effect of TFE cosolvent proportion on Hα chemical shifts in cyclic reference peptide **11** (100 mM acetate buffer/ X % TFE, pH 3.8, 278 K).

Table 19S. Chemical shifts of Hα protons observed for **9** in aqueous solution with different proportions of TFE (100 mM acetate buffer/ X % TFE, pH 3.80, 278 K).

	Aqueous buffer	10 % TFE	20 % TFE	30 %TFE	50 %TFE
Lys(14)	4,851	4,888	4,932	4,968	5,065
Arg(13)	4,918	4,906	4,929	4,949	4,949
Phe(12)	4,719	4,712	4,695	4,701	4,697
Lys(11)	4,85	4,87	4,848	4,853	4,843
Thr(10)	4,429	4,437	4,428	4,441	4,426
Val(9)	4,427	4,418	4,426	4,122	4,12
Val(6)	4,06	4,062	4,094	4,124	4,101
Arg(5)	5,025	4,957	5,14	5,162	5,173
Tyr(4)	4,854	4,833	5,005	4,949	4,969
Phe(3)	5,437	5,459	5,477	5,472	5,455
Thr(2)	4,573	4,583	4,631	4,631	4,652
Glu(1)	4,922	4,918	4,96	4,96	5,026

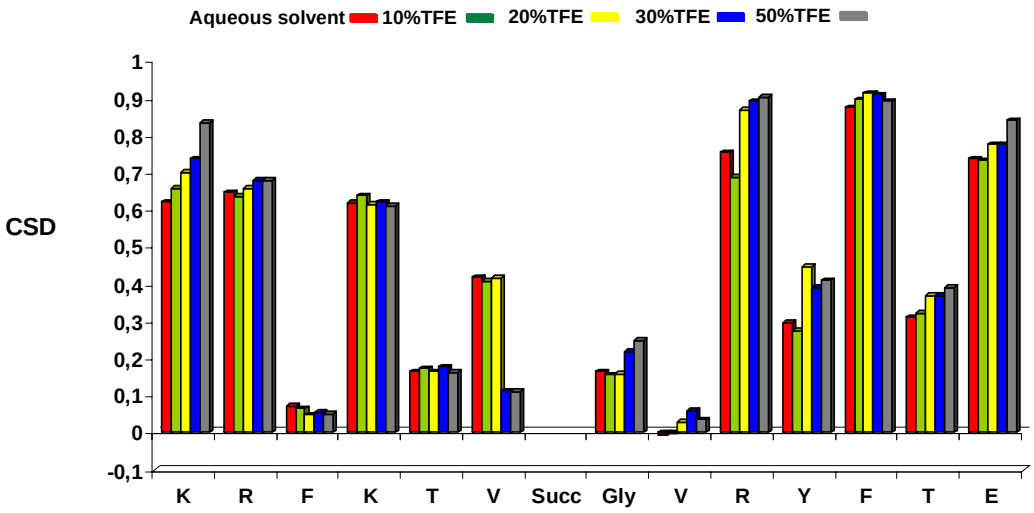


Figure 9S. Effect of TFE cosolvent proportion on Hα chemical shifts in cyclic reference peptide **9** (100 mM acetate buffer/ X % TFE, pH 3.8, 278 K).

NMR structures for macrocycles 10 and 11

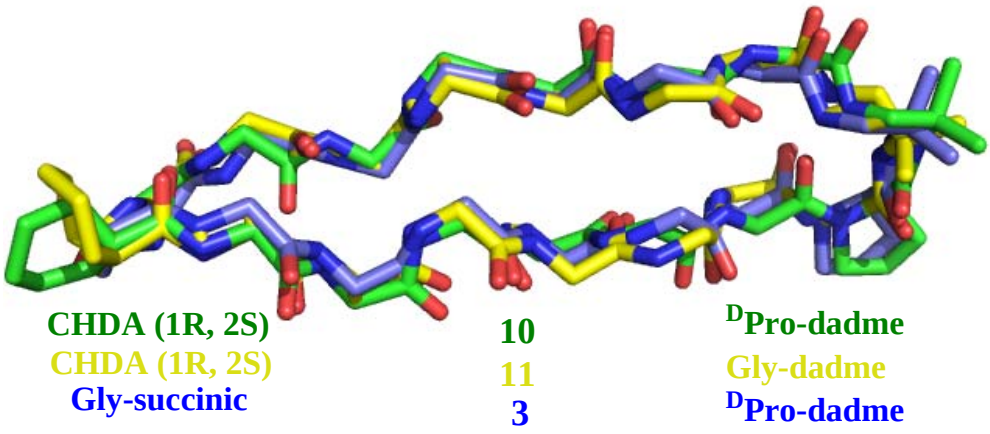


Figure 10S. Overlays of the lowest energy conformations identified by NOE-restrained dynamics analysis for macrocycles **3**, **10** and **11**. RMSD between the backbones of peptides **10** and **3** is 0.587 Å, and the RMSD between the backbones of peptides and **11** and **3** is 0.532 Å (linker regions not included). The image was generated with Pymol (ref S13)

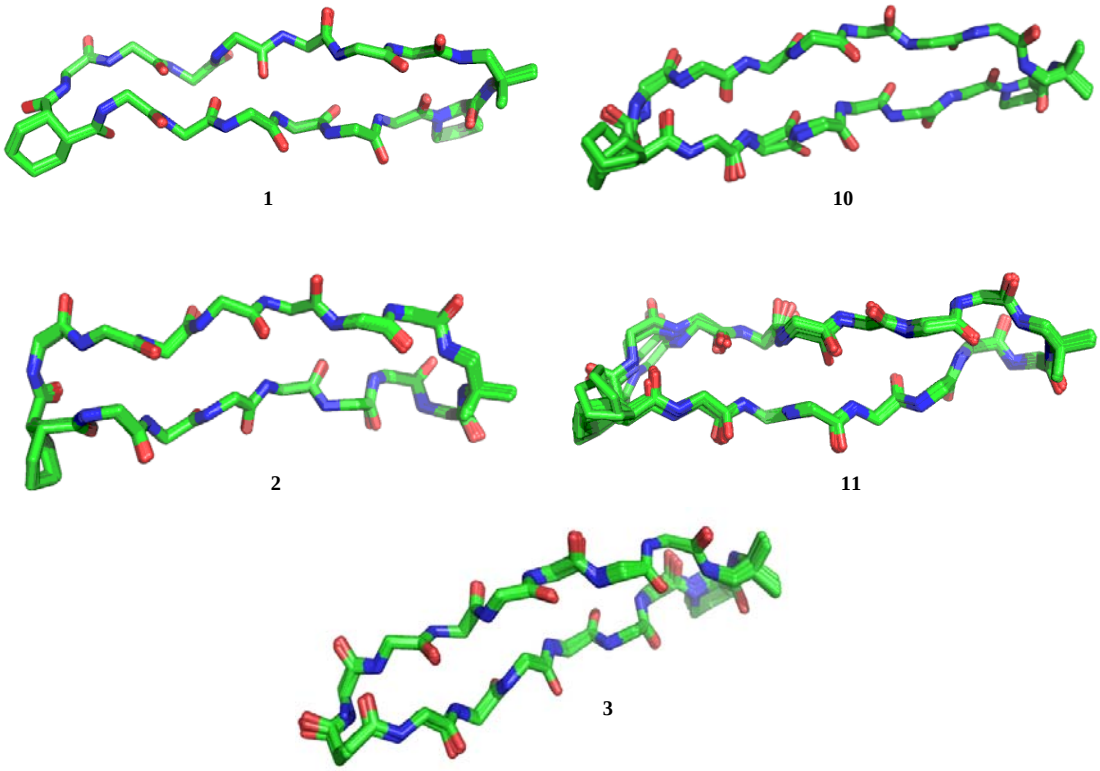


Figure 11S. Ten best NMR structures **1-3, 10-11** obtained from NOE-restrained dynamics calculations using CNS. The RMSD among backbone heavy atoms for the best structures of **1** is 0.035 ± 0.017 Å, of **10** is 0.119 ± 0.088 Å, of **2** is 0.284 ± 0.179 Å, of **11** is 0.149 ± 0.064 Å and of **3** is 0.300 ± 0.186 Å. The image was generated with Pymol (ref S13)

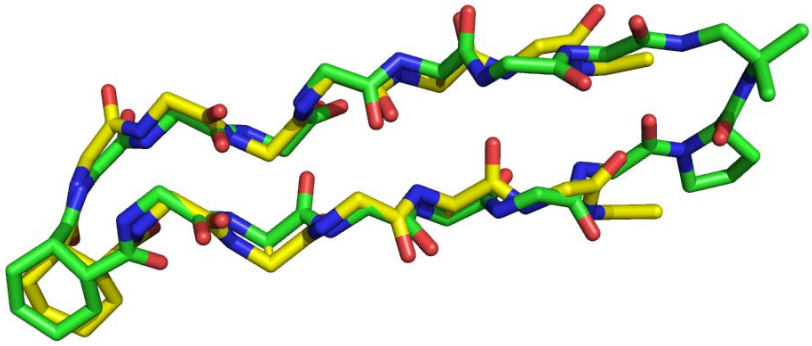


Figure 12S. Overlay of NMR structures for peptides **6** (yellow) and **1** (green, cyclic). The two peptides have very similar backbone conformations in these structures, which suggest that both adopt a β-hairpin conformation in solution. RMSD = 0.720 Å between the sets of Cα backbone atoms of the two peptides (residues 3 to 12). The image was generated with Pymol. The image was generated with Pymol (ref S13)

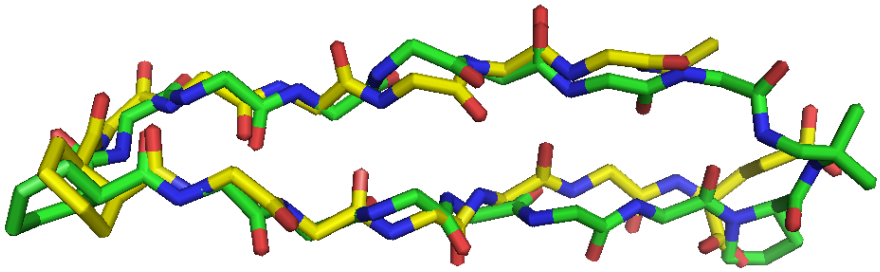


Figure 13S. Overlay of NMR structures for peptides **7** (yellow) and **10** (green, cyclic). The two peptides have very similar backbone conformations in these structures, which suggest that both adopt a β-hairpin conformation in solution. RMSD = 0.845 Å between the sets of Cα backbone atoms of the two peptides (residues 3 to 12). The image was generated with Pymol. The image was generated with Pymol (ref S13)

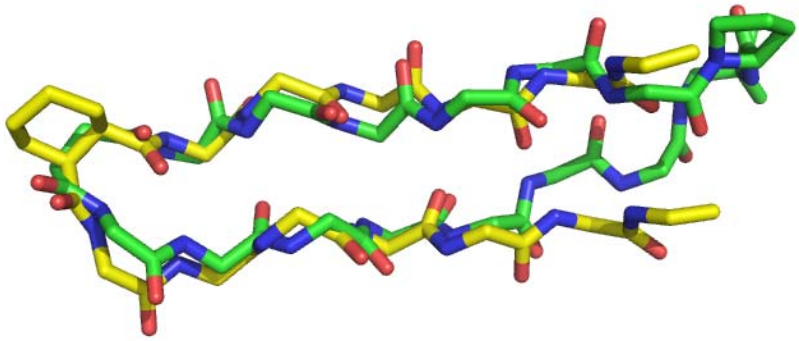


Figure 14S. Overlay of NMR structures for peptides **6** (yellow) and **3** (green, cyclic; the diacid portion is derived from succinic acid). The two peptides have very similar backbone conformations in these structures, which suggest that both adopt a β-hairpin conformation in solution. RMSD = 1.117 Å between the sets of Cα backbone atoms of the two peptides (residues 3 to 12). The image was generated with Pymol (ref S13).

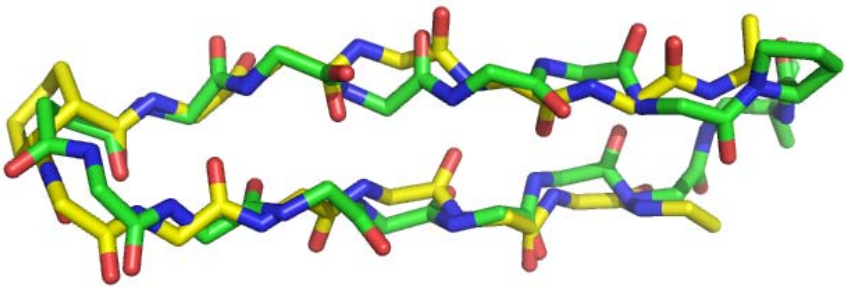


Figure 15S. Overlay of NMR structures for peptides **7** (yellow) and **3** (green, cyclic; the diacid portion is derived from succinic acid). The two peptides have very similar backbone conformations in these structures, which suggest that both adopt a β-hairpin conformation in solution. RMSD = 0.888 Å between the sets of Cα backbone atoms of the two peptides (residues 3 to 12). The image was generated with Pymol (ref S13)

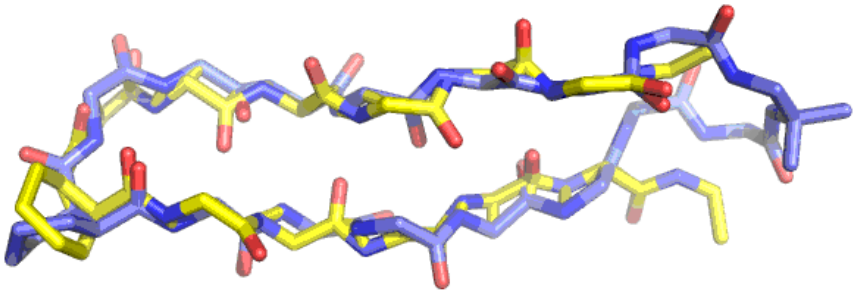


Figure 16S. Overlay of NMR structures for peptides **2** (yellow) and **11** (blue, cyclic; Gly instead of D-Pro). The two peptides have very similar backbone conformations in these structures, which suggest that both adopt a β-hairpin conformation in solution. RMSD = 1.143 Å between the sets of Cα backbone atoms of the two peptides (residues 3 to 12). The image was generated with Pymol (ref S13)

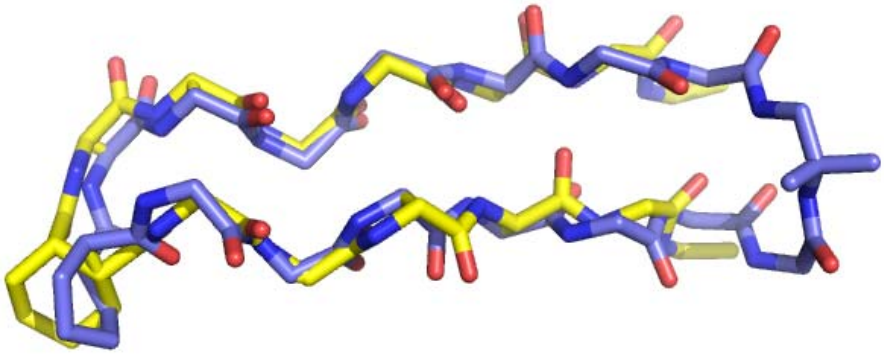


Figure 17S. Overlay of NMR structures for peptides **1** (yellow) and **10** (blue, cyclic; Gly instead of D-Pro). The two peptides have very similar backbone conformations in these structures, which suggest that both adopt a β-hairpin conformation in solution. RMSD = 0.801 Å between the sets of Cα backbone atoms of the two peptides (residues 3 to 12). The image was generated with Pymol (ref S13)

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(S13) Delano Scientific LLC.