SUPPORTING INFORMATION

Glucuronic acid as a helix-inducing linker in short peptides

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Contents

1. General

Fmoc-L-amino acids were purchased from Novabiochem (Melbourne, Australia) and Chemimpex (USA). O-Benzotriazole-N,N,N',N'-tetramethyl-uroniumhexafluorophosphate (HBTU) was obtained from IRIS Biotech GmbH (Marktredwitz, Germany) and Chemimpex (USA). (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexa-fluorophosphate) HATU was purchased from Chemimpex (USA). All other reagents were of peptide synthesis grade and obtained from Auspep (Melbourne, Australia). Peptides were synthesized manually by standard solid phase methods using HBTU or HATU/DIPEA activation of Fmoc protected amino acids on Rink amide resin (substitution 0.35 mmol/g).

2. Manual Synthesis

All peptides were assembled on Rink Amide resin at 0.1 mmol scale. Two equivalents of Fmoc-protected amino acid, 2.0 equivalents of HBTU, and 2.0 equivalents of DIPEA were employed in each coupling. For specialty protected amino acid **5** and Fmoc-Lys(Alloc)-OH, 2 equivalents of Fmoc-protected amino acid, 2 equivalents of HATU, and 4.5 equivalents of Collidine or 2 equivalents of DIPEA were employed in each coupling. Fmoc deprotections were achieved by 2 min treatments with excess 20% piperidine in DMF. Coupling progress was monitored by ninhydrin test.

3. Peptide Cyclization (Solid Phase)

The allyl protecting group of compound **5** and alloc group of lysine were removed from the peptide-resin with 20% $Pd(Ph_3)_4$ (Tetrakis(triphenylphosphine)palladium) in dichloromethane (DCM) in the presence of 20 equivalents phenylsilane (2 hours). Then the resin was washed with DMF, DCM, DMF, and DCM until the dark colour disappears. The cyclization of the resin bound peptide was performed with PyBOP (4.0 equiv) and Collidine (4.5 equiv) in DMF for 4 hours or overnight at room temperature.

4. Peptide Cleavage

The peptide was cleaved from the resin by treatment with a 5 mL solution containing 95% trifluoroacetic acid (TFA): 2.5% H_2O: 2.5% triisopropylsilane (TIPS) for 1.5 hours at room temperature. TFA solutions were filtered and evaporated under N_2. The

peptides were purified by semi-preparative rp-HPLC on a 22 × 250 mm column using a linear gradient ACN/H_2O (0% B to 40% B over 40 min).

5. Semi-preparative rp-HPLC purification

Semi-preparative rp-HPLC purification of the peptides was performed using a a Shimadzu Preparative HPLC system fitted with a Waters 486 tunable absorbance detector with detection at 214 nm or a Shimadzu PDA detector. Purification was performed by eluting with solvents A (0.1% TFA in water) and B (90:10 CH_3CN : H_2O, 0.1% TFA) on a C18 250 x 22 mm (300 Å) Phenomenex Lunar C18 columns.

6. Analylical rp-HPLC

Analytic rp-HPLC was performed using an Agilent 1200 Series with a diode-array detector on a Phenomenex Luna 5 μm, C18 or C8 250 × 4.60 mm column. The solvent gradient was A (0.1% TFA in water) and B (CH_3CN/H_2O = 90:10, 0.1% TFA).

7. CD Spectroscopy

CD measurements were performed using a Jasco model J-710 spectropolarimeter, which was routinely calibrated with (1S)-(+)-10-camphorsulfonic acid. A stock solution was prepared by dissolving 1-2mg of peptide in 630μL H_2O and 70μL D_2O. 500μL solutions of 100μM peptides were then prepared using an appropriate amount of stock solution and making up the difference with 10mM Phosphate Buffer (pH 7.4) and TFE (2,2,2-Trifluoroethanol). Spectra were recorded at room temperature (298K), with a 0.1 cm Jasco quartz cell over the wavelength range 260-185 nm at 50 nm/min, with a bandwidth of 1.0 nm, response time of 1 s, resolution step width of 1 nm and sensitivity of 20-50 Mdeg. Each spectrum represents the average of 5 scans. Spectra were analysed using the spectral analysis software and smoothed using 'adaptive smoothing' function.

Concentrations were determined using the PULCON method[1]. NMR solutions were prepared with 540 μL of stock solution and 60 μL of D_2O. 90° pulses were accurately determined and then 1D Spectra were acquired using the standard watergate sequence with a ns= 16, d1= 30s. Spectra were also acquired for a 4.76 mM solution of L-histidine as the reference standard. The fully resolved, most downfield amide resonance was integrated and used to calculate the concentration from the equation: where *c* is the concentration, *S* is the integral (in absolute units) number of protons, T

is the temperature in Kelvin, θ_{360} is the 360° rf pulse, *n* is the number of scans, and *rg* is the receiver gain used for measuring the reference (R) and unknown (U) samples.

$$C_u = C_R \frac{S_U T_U \vartheta_{360}^U n_R r g_R}{S_R T_R \vartheta_{360}^R n_U r g_U}$$

8. Percentage Helicity[2]

Percentage helicity of peptides were calculated from residue-molar eliticity at 220 nm using eq

$$f_{helix} = \frac{[\theta]_{220} - [\theta]_0}{[\theta]_{max} - [\theta]_0}$$

Where $[\theta]_{max}$ ($[\theta]_{max} = [\theta]_\infty(n - x)/n$) is the maximum theoretical mean residue ellipticity for a helix of n residues, $[\theta]_\infty$ is the mean residue ellipticity of an infinite helix, and x is an empirical constant that can be interpreted as the effective number of amides missing as a result of end effects, usually about 2.4-4 (we used x=2.5) and $[\theta]_\infty$ = (-44000 + 250T) (T is temperature of the peptide solution in °C). $[\theta]_0$ is the mean residue ellipticity of the peptide in random coil conformation and equals to (2220 – 53T) and $[\theta]_{220}$ ($[\theta]_{220} = 1/n \cdot [\theta_{obs}]/(10 \times l \times C)$) is the observed residue ellipticity of peptide at 220 nm. Where θ_{obs} = measured ellipticity in mdeg; n = number of peptide residues; C = sample concentration (mol/L); l = optical path length of the cell in cm.

9. NMR Spectroscopy

The samples for the NMR analyses of peptides were prepared by dissolving the peptide (1-2mg) in 540µL H_2O and 60µL D_2O at pH 5.0. 1D and 2D ^{1}H-NMR spectra were recorded on a Bruker Avance DRX-600 spectrometer. 2D ^{1}H-spectra were recorded in phase-sensitive mode using time-proportional phase incrementation for quadrature detection in the *t*1 dimension. The 2D experiments included wgTOCSY (standard Bruker mlevgpph pulse program), esROESY (standard Bruker roesyesgpph pulse program), edHSQC (standard Bruker hsqcedetgpsisp pulse program) and COSY (standard Bruker cosygpprqf pulse program). TOCSY spectra were acquired over 6887 Hz with 4096 complex data points in *F2*, 512 increments in *F*1 and 16 scans per increment. ROESY spectra were acquired over 6887 Hz with 4096 complex data points in *F2*, 512 increments in *F*1 and 16 scans per increment. TOCSY and ROESY

spectra were acquired with several isotropic mixing times of 80, 100 ms for TOCSY, 250-350 ms for ROESY. For all NMR experiments, water suppression was achieved using modified WATERGATE. For 1D ^{1}H NMR spectra acquired in H_2O/D_2O (9:1), the water resonance was suppressed by low power irradiation during the relaxation delay (1.5 to 3.0 s). The variable NMR experiments were performed over the range of 278-318K. Spectra were processed using Topspin (Bruker, Germany) software and ROE intensities were collected manually. The *t*1 dimensions of all 2D spectra were zero-filled to 1024 real data points with 90° phaseshifted QSINE bell window functions applied in both dimensions followed by Fourier transformation and fifth order polynomial baseline correction. ^{1}H chemical shifts were referenced to DSS (δ 0.00 ppm) in water. ^{3}JNHCHα coupling constants were measured from 1D ^{1}H NMR. ^{13}C spectra were obtained (where required) with a sweep width of 20840 Hz with 20000-10000 scans and 65K data points. ^{13}C NMR spectra were ^{1}H-decoupled. Phase sensitive HSQC spectra were obtained with 900 increments in *F*1, 2K data points in *F*2 and 32 scans per increment. These were a 2-D ^{1}H/^{13}C correlation via double inept transfer using sensitivity improvement with standard Bruker pulse programs of invietgssi or invietgpsi.

10. Structure Calculations[3]

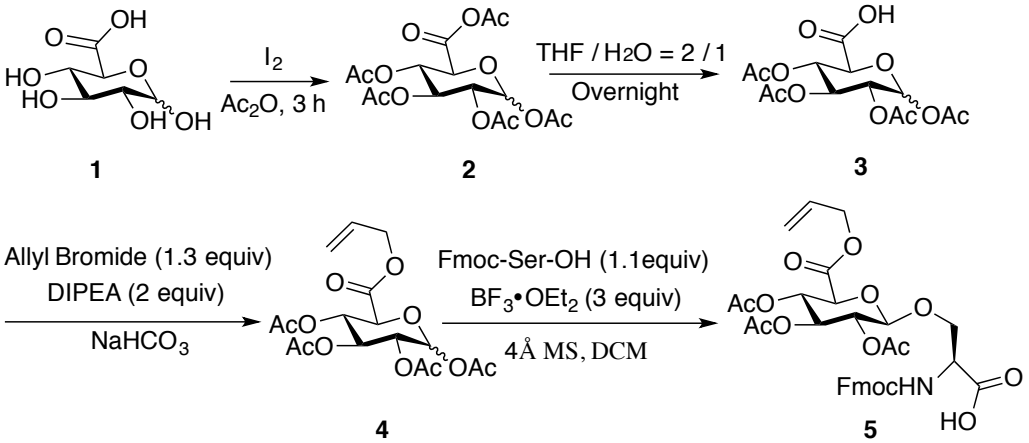
The distance restraints used in calculating the structure for peptides in water were derived from ROESY spectra (recorded at 298K, 293K for **9**) using mixing time of 300 ms (ROESY). ROE cross-peak volumes were classified manually as strong (upper distance constraint ≤ 2.7Å), medium (≤ 3.5Å), weak (≤ 5.0Å) and very weak (≤ 6.0Å). Standard pseudoatom distance corrections were applied for non-stereospecifically assigned protons. To address the possibility of conformational averaging, intensities were classified conservatively and only upper distance limits were included in the calculations to allow the largest possible number of conformers to fit the experimental data. Backbone dihedral angle restraints were inferred from ^{3}JNHCHα coupling constants in 1D spectra, φ was restrained to –65 ± 30° for ^{3}JNHCHα. There was clearly no evidence at all for *cis*-amides about peptide bonds (*i.e.* no CHα-CHα (*i*, *i*+1) ROEs) in the ROESY spectra so all ψ-angles were set to trans (ψ = 180°). Starting structures with randomised φ and ψ angles and extended side chains were generated using an *ab initio* simulated annealing protocol. The calculations were performed using the standard forcefield parameter set

(PARALLHDG5.2.PRO) and topology file (TOPALLHDG5.2.PRO) in XPLOR-NIH with in house modifications to generate lactam bridges between lysine and carboxylate acid residue of the sugar moiety. Structures were visualised with InsightII and analysed for distance (>0.2Å) and dihedral angle (>5^o) violations using noe.inp files. Final structures contained no distance violations (>0.2Å) or angle violations (>5^o).

11. Synthesis and analysis of 5

11.1 Synthesis

Compound **2** was prepared based on literature.[4] The NMR data was consistent with the reported data. Compound **4** was prepared as literature reported method,[5] with some modifications, the ^{1}H spectrum is consistent with data in literature.



Step 1. D-Glucuronic acid **1** (3.88 g, 20 mmol) was dissolved in 50 mL acetic anhydride and the mixture stirred in ice-water bath. Catalytic amount of Iodine (0.065 equiv, 330 mg) was added in portions and the red solution stirred at 0 ºC for 2 h. The ice-water bath was removed and the reaction mixture stirred for further 3 h. Acetic anhydride was removed under high vacuum. The residue was dissolved in DCM (60 mL). The solution was washed with 10% aqueous $Na_2S_2O_3$ solution (2 × 30 mL), brine and dried ($MgSO_4$). The solution was filtered and the filtrate concentrated under vacuum to dryness to give the crude mixed anhydride **2**.

Step 2. The above crude was dissolved in THF (20 mL) and water (10mL). The mixture was stirred at RT overnight. The most of THF was removed under vacuum and the residue extracted with EtOAc (2 × 30 mL). The combined EtOAc

phase was dried and evaporated under vacuum to provide the crude compound **3**, which was used without further purification.

Step 3. The above crude **3** (5.8 g, 16 mmol) was dissolved in DMF (40 mL), DIPEA (2 equiv, 32 mmol, 5.59 mL) was added. Allyl bromide (1.3 equiv, 20.8 mmol, 1.8 mL) was added dropwise into the reaction solution. The mixture was stirred ar RT overnight. The following day, TLC (PE/EA = 2/1) showed the complete disappearance of **3**. DMF was removed under high vacuum and the residue was dissolved in DCM. The solution was washed with brine, dried ($MgSO_4$). Flash column chromatography (silica gel 70-230 mesh, gradient elution with petroleum ether – EtOAc 3:1 to 1:1) provided **4** as a white powder (β/α = 4/1, 69% yield over 3 steps).

Step 4. A mixture of peracetylated allyl glucuronate **4** (4 g, 9.95 mmol) and Fmoc-Ser-OH (6.5 g, 20 mmol) were dried by co-evaporation with anhydrous toluene (3× 5 mL). The residue was dissolved in anhydrous DCM (40 mL) and the freshly activated 4Å molecular sieves (about 20 mg) were added. The mixture was stirred at RT under Ar gas for half an hour. The freshly distilled $BF_3 \cdot Et_2O$ (1.5 equiv, 15 mmol, 1.9 mL) was added dropwise. The mixture was stirred overnight; TLC (PE/EA = 2/1) indicated the presence of starting materials. Another 1.5 equiv of $BF_3 \cdot Et_2O$ was added and the mixture stirred for another 20 h. TLC showed some **4** and Fmoc-Ser-OH remained. The reaction was quenched by addition of 1 M HCl solution (20 mL), extracted with DCM (80 mL). The organic phase was washed by brine and dried ($MgSO_4$). After removing the solvent, the crude product was purified by flash column chromatography and HPLC (70% B (90% acetonitrile/ 10% water/ 0.1% TFA) in A (100% water/0.1% TFA)), obtained in 20.4% yield.

11. 2 Analytical data for compound 5

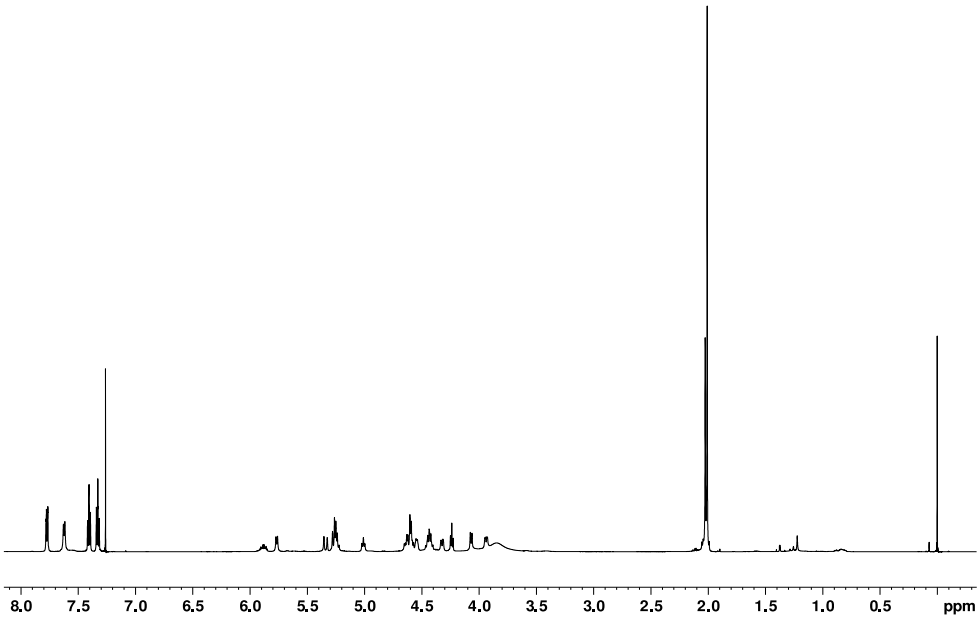
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, 2H, *J* = 7.52, Ar H), 7.62 (d, 2H, *J* = 6.99 Hz, Ar H), 7.41 (t, *J* = 7.48 Hz, 2H, Ar H), 7.33 (t, *J* = 7.46 Hz, 2H, Ar H), 5.89 (m, 1H, C*H*=CH_2), 5.76 (d, 1H, *J* = 7.80 Hz, NH), 5.34 (d, 1H, *J* = 16.98 Hz, CH=C*H*$_2$), 5.28 – 5.24 (m, 3H, CH=C*H*$_2$, H-3 and H-4 overlap), 5.01 (t, 1H, $J_{1,2}$ = 7.82 Hz, H-2), 4.65 – 4.57 (m, 3H, H-1 and OC*H*$_2$CH=CH_2 overlap), 4.55 (m, 1H, αH), 4.47 – 4.41 (m, 2H), 4.33 (dd, 1H, Ser-βHa), 4.24 (t, 1H, *J* = 6.81 Hz, Fmoc), 4.07 (d, 1H, $J_{4,5}$ = 8.96 Hz, H-5), 3.94 (dd, 1H, *J* = 10.73, 3.02 Hz, Ser-βHb), 2.03 (s, 3H, 1 COC*H*$_3$), 2.01 (s, 6H, 2 CO*CH*$_3$)

^{13}C NMR (150 MHz, $CDCl_3$) δ 172.0, 170.1, 169.6, 169.5, 166.7, 156.2, 130.9, 127.8, 127.1, 125.1, 120.0, 119.8, 100.8, 72.2, 71.9, 71.1, 69.7, 69.1, 67.3, 66.9, 54.0, 47.1, 20.6(0), 20.5(8), 20.5(5)

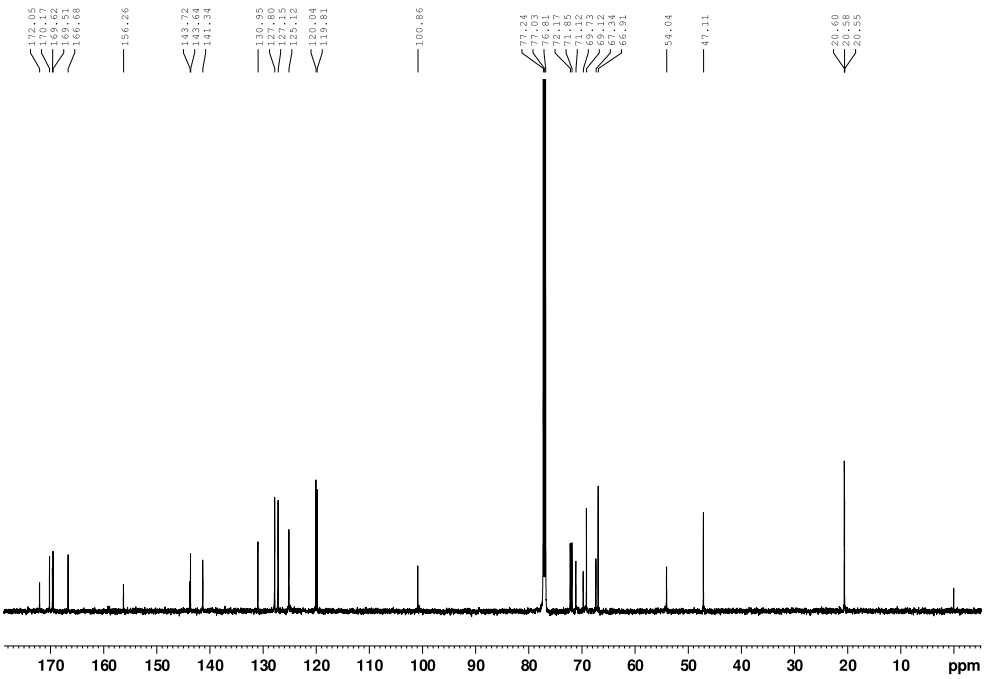
HRMS (ESI): Calcd for $C_{33}H_{36}N_7O_{14}$ [M + H^+]: 670.2130, found: 670.2131;

$[\alpha]_D^{20}$ = –53.6° (c 0.158, $CHCl_3$);

^{1}H NMR for compound 5



^{13}C NMR for compound 5



12. CD Spectra

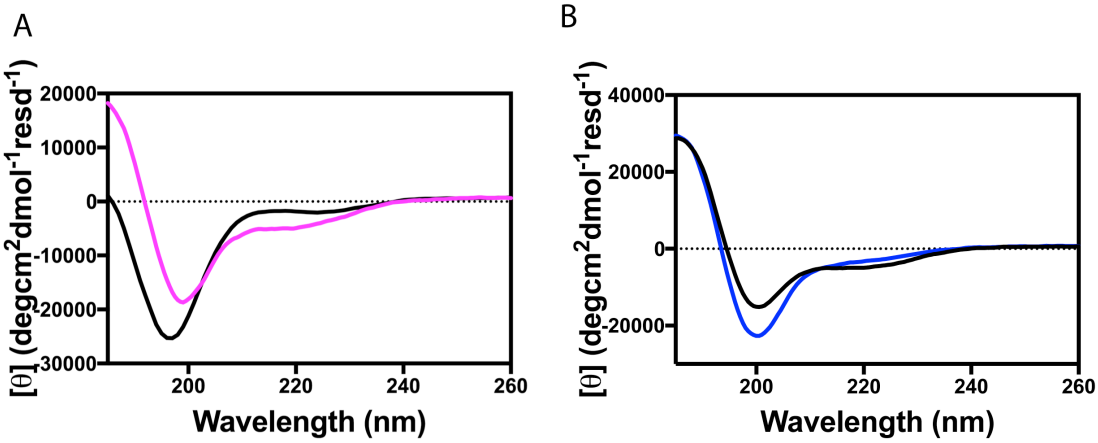


Figure S1. A) CD spectra of linear peptide **6** in 10 mM phosphate buffer (black) and 50% TFE/PBS (magenta) at 25 °C. B) CD spectra of cyclised peptide **7** in 10 mM phosphate buffer (black) and 50% TFE/PBS (blue) at 25°.

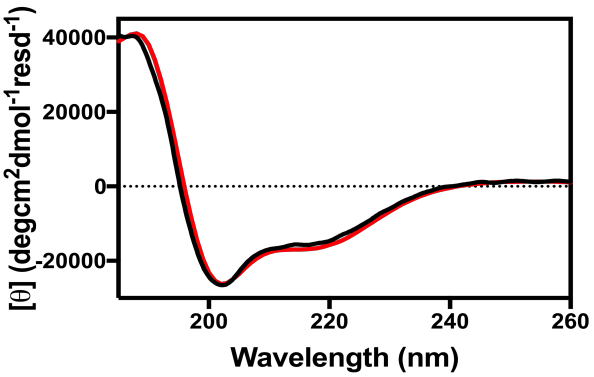


Figure S2. CD spectra of cyclised peptides **9** (black) and **10** (red) in 50% TFE/PBS at 25°.

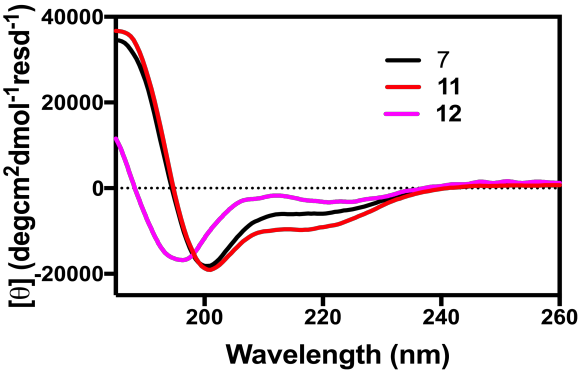


Figure S3. CD spectra of the cyclised peptides: **7** (blue), **11** (purple) and **12** (orange) in 10 mM phosphate solution (pH = 7.4) solution at 298K.

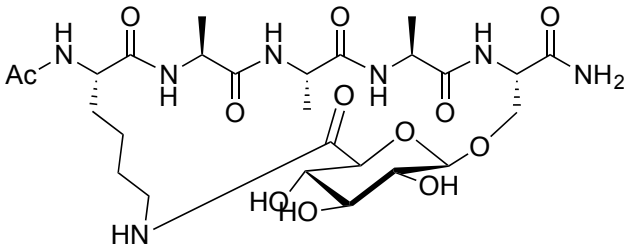
13. Analytical data for linear peptides

Ac-S*AAAK-NH_2, 6. HRMS (ESI): Calcd for $C_{26}H_{46}N_7O_{13}$ [M + H^+]: 664.3150, Found: 664.3148. Linear gradient from 0% to 40% B over 20 min, Rt =8.34 min.

Ac-KAAAS*-NH_2, 8. HRMS (ESI): Calcd for $C_{26}H_{45}N_7O_{13}Na$ [M + Na^+]: 686.2968, Found: 686.2968. Linear gradient from 0% to 40% B over 20 min, Rt =7.41 min.

14. Analysis of cyclised peptides

Peptide 9



HRMS (ESI): Calcd for $C_{26}H_{43}N_7O_{12}Na$ [M + Na^+]: 668.2862, Found: 668.2860. Linear gradient from 0% to 40% B over 20 min, Rt =11.37 min.

Table S1. Molar residue ellipticities at 220 nm in 10 mM phosphate buffer (pH 7.4, 298 K) and percentage helicity for peptide **9.**

	Peptide	$[\theta]_{220}$	Helicity (%)
1	Ac-[KAAAS*]-NH_2	-11681.9	64

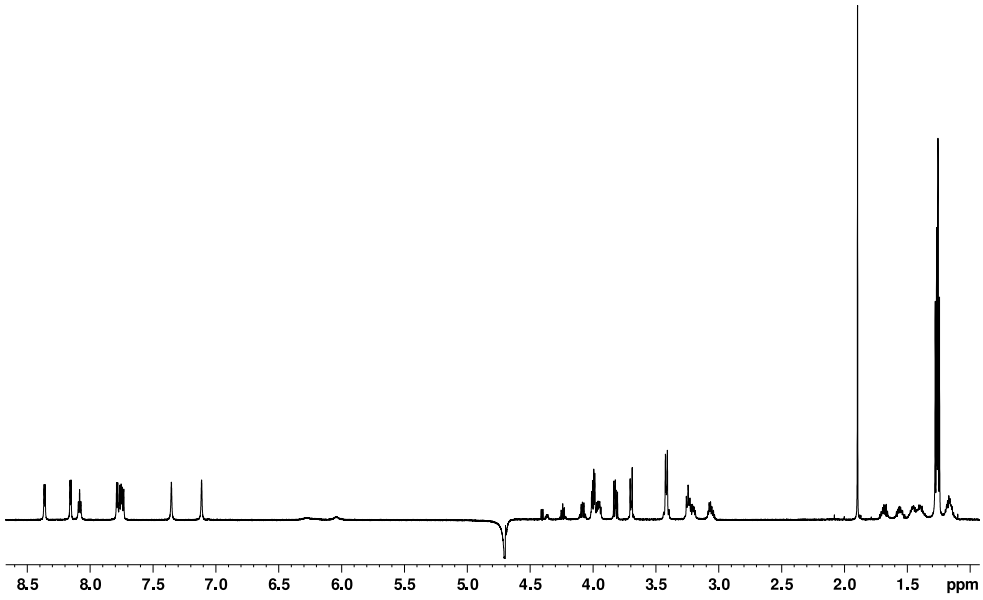


Figure S4. 600 MHz ^{1}H NMR spectrum of **9** in H_2O/D_2O (9:1) at 293K.

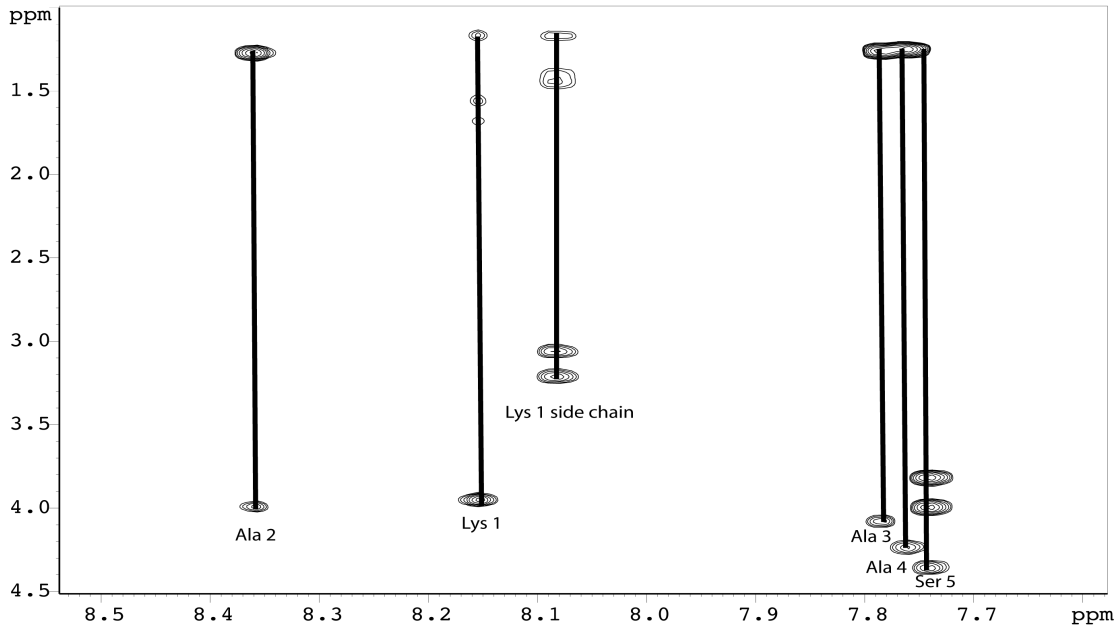


Figure S5. 600MHz TOCSY fingerprint region of **9** in H_2O/D_2O (9:1) at 293K. Connectivity of the NH, Cα, Cβ, Cγ protons for the peptide is shown by the solid line.

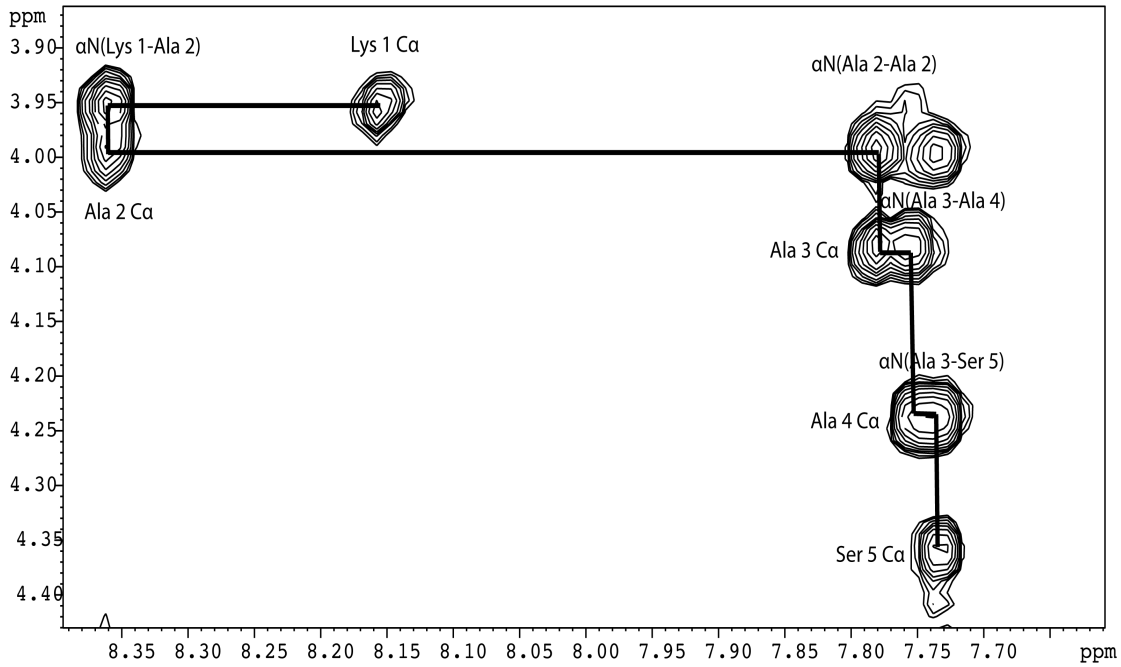


Figure S6. Magnified NH-CHα region from the 600 MHz ROESY spectrum of **9** in H_2O/D_2O (9:1) at 293K. Sequential connectivity for the peptide is shown by the solid line.

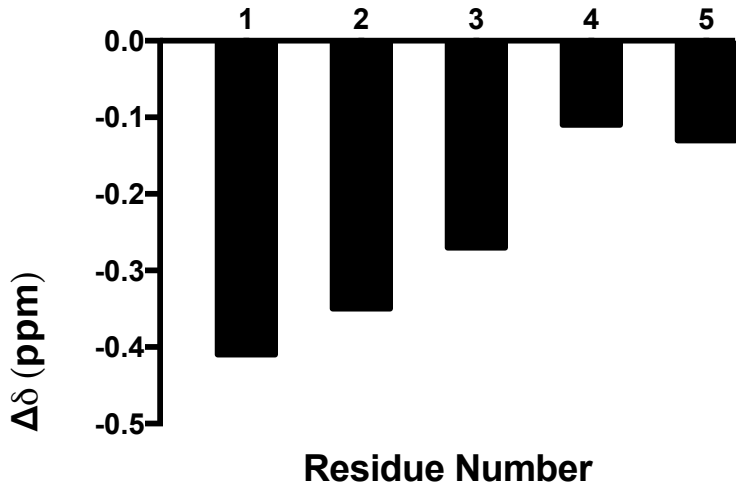


Figure S7. Δδ Hα versus the corresponding amino acids in random coil[6] for **9** in water at 293K

Table S2. ^{1}H NMR resonance assignments and chemical shifts (δ ppm) for **9** in H_2O/D_2O (9:1) at 293K.

Residue	NH	H α	H β	Other
Ac-K1	8.15	3.95	1.68, 1.55	1.35-1.49 (2δH); 1.11-1.21(2γH); 3.17-3.23(1εH), 3.02-3.09(1εH);
A2	8.36	4.00		1.23-1.28(3 CH3)
A3	7.78	4.08		1.89 OAc cap
A4	7.76	4.24		
S5-NH_2	7.74	4.37	4.00, 3.82	T: 7.35, 7.11
Sugar H1 4.40	H2 3.24	H3 3.41	H4 3.41 overlap	H5 3.69

Table S3. Amide NH coupling constants and Δδ/T for **9** in H_2O/D_2O (9:1) at 293K.

Residue	K1	A2	A3	A4	S5	NT1	NT2	NH-side
^{3}JNH--CHα (Hz)	4.76	4.59	5.33	7.02	7.27	N/A	N/A	N/A
Δδ/K (--ppb)	7.4	6.7	6.4	1.3	1.9	3.0	5.9	7.8

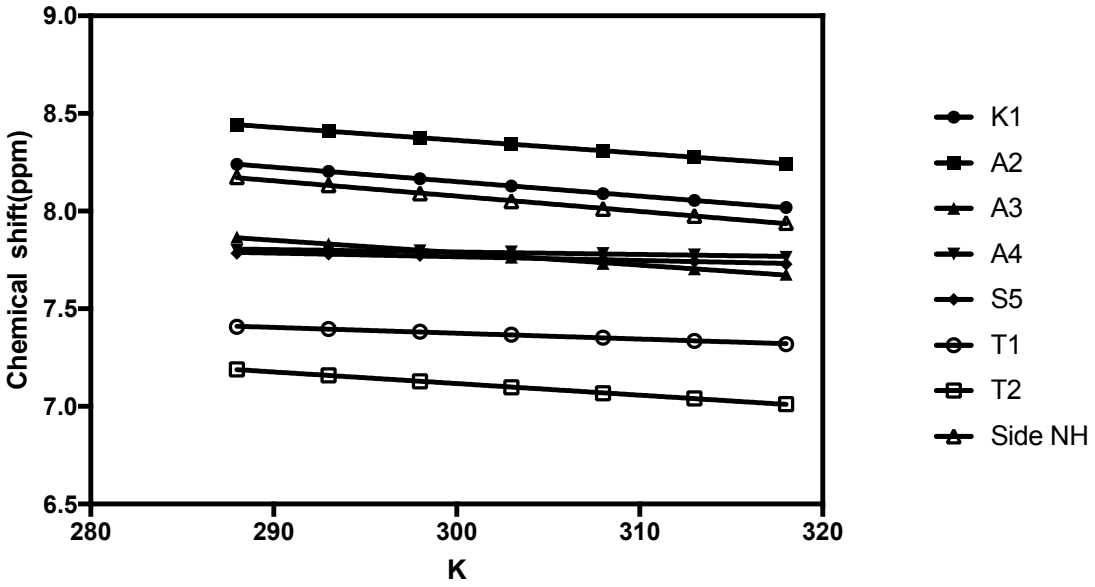


Figure S8. Temperature dependence of the amide NH chemical shifts for **9** in H_2O/D_2O (9:1). Line slopes indicating temperature coefficients ($\Delta\delta/T$) for each residue are shown in brackets. Lys1 [7.4 ppb/K], Ala2 [6.7 ppb/K], Ala3 [6.4 ppb/K], Ala4 [1.3 ppb/K], Ser5 [1.9 ppb/K], Ser5 H1 [3.0 ppb/K], Ser5 H2 [5.9 ppb/K], Lys1 side NH [7.8 ppb/K]. Values of less than 4 ppb/K indicate hydrogen-bonds.[6]

Table S4. ROE-derived distances, ^3JNH-CHα derived ϕ-angle restraints and hydrogen bond restraints used for calculating the solution structure of **9** in H_2O/D_2O (9:1) at 293K.

1	Acetyl 1 Hα*	Lys 2 HN	4.2 Å; Strong + 1.5Å correction
2	Acetyl 1 Hα*	Ala 3 HN	7.5 Å; Very Weak + 1.5Å correction
3	Acetyl 1 Hα*	Ala 4 HN	7.5 Å; Very Weak + 1.5Å correction
4	Lys 2 Hα	Ala 3 HN	2.7 Å; Strong
5	Lys 2 Hα	Ala 5 HN	6.0 Å; Very Weak
6	Lys 2 HN	Ala 3 HN	6.0 Å; Very Weak
7	Lys 2 HB1	Ala 3 HN	5.0 Å; Weak
8	Lys 2 HZ1	Ala 3 Hα	6.0 Å; Very Weak
9	Lys 2 HZ1	Ala 5 Hα	6.0 Å; Very Weak
10	Lys 2 Hα	Ala 5 Hβ*	6.5 Å; Weak + 1.5Å correction
11	Lys 2 Hγ*	Ala 3 Hα	7.0 Å; Very Weak + 1.0Å correction
12	Lys 2 Hε2	Ala 5 Hα	6.0 Å; Very Weak
13	Ala 3 Hα	Ala 3 Hα	3.5 Å; Medium
14	Ala 3 Hα	Ser 6 HN	6.0 Å; Very Weak
15	Ala 3 Hα	Ser 6 H1	5.0 Å; Weak
16	Ala 3 Hα	Ser 6 H2	6.0 Å; Very Weak
17	Ala 3 Hα	Ala 4 Hβ*	6.5 Å; Weak + 1.5Å correction
18	Ala 3 HN	Ala 4 HN	5.0 Å; Weak
19	Ala 4 HN	Ala 5 Hβ*	6.5 Å; Weak + 1.5Å correction
20	Ala 4 Hα	Ala 5 HN	3.5 Å; Medium
21	Ala 4 Hα	Ser 6 H1	6.0 Å; Very Weak
22	Ala 5 Hα	Ser 6 HN	3.5 Å; Medium
23	Ser 6 HN	Ser 6 H1	6.0 Å; Very Weak

24	Ser 6 HN	Ser 6 H2	6.0 Å; Very Weak
25	Ser 6 Hα	Ser 6 H1	3.5 Å; Medium
26	Ser 6 Hα	Ser 6 H2	6.0 Å; Very Weak
27	Ser 6 Hβ1	Ser 6 H1	6.0 Å; Very Weak
28	Ser 6 Hβ2	Ser 6 H1	5.0 Å; Weak
29	Ser 6 HN	Ala 5 Hβ*	6.5 Å; Weak + 1.5Å correction
30	Sugar H5	Lys 2 HZ	3.5 Å; Medium
31	Sugar H1	Ser 6 HN	6.0 Å; Vey Weak
32	Sugar H5	Ala 4 Hα	6. 0 Å; Vey Weak
33	Sugar H1	Ser 6 Hβa	2.7 Å; Strong
34	Sugar H1	Ser 6 Hβb	2.7 Å; Strong
35	Sugar H2	Ala 4 Hβ	6.0 Å; Very Weak + 1.5Å correction

φ-angle restraints

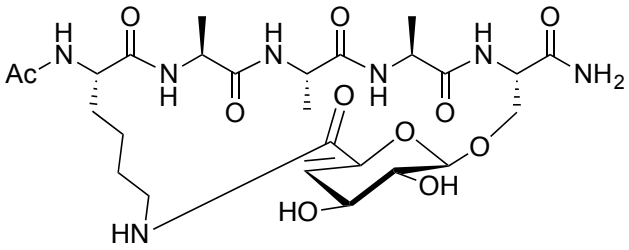
	Residue	^{3}JNH-CHα	φ-dihedral angle restraint
1	Lys 1	4.76	-65 ± 30°
2	Ala 2	4.59	-65 ± 30°
3	Ala 3	5.33	-65 ± 30°

Hydrogen-bond restraints

	Donor	Acceptor	H-O Distance	N-O Distance
1	Ser6 H1	Ala3 O	1.88[-.3 Å,+.42 Å]	2.88 ± 0.3 Å
2	Ser6 NH	Lys2 O	1.88[-.3 Å,+.42 Å]	2.88 ± 0.3 Å
3	Ala5 NH	Acetly1 O	1.88[-.3 Å,+.42 Å]	2.88 ± 0.3 Å

* Represents protons that were not stereospecifically assigned and whose distance restraints were adjusted with standard pseudoatom corrections.[7]

Peptide 10



HRMS (ESI): Calcd for $C_{26}H_{41}N_7NaO_{11}$ [M + Na^+]: 650.2756, found: 650.2756. Linear gradient from 0% to 40% B over 20 min, Rt =12.50 min

Table S5. Molar residue ellipticities at 220 nm in 10 mM phosphate buffer (pH 7.4, 298 K) and percentage helicity for peptides **10**.

	Peptide	[θ]220	Helicity (%)
1	Ac-[KAAAS*]-NH_2	-7403.5	42

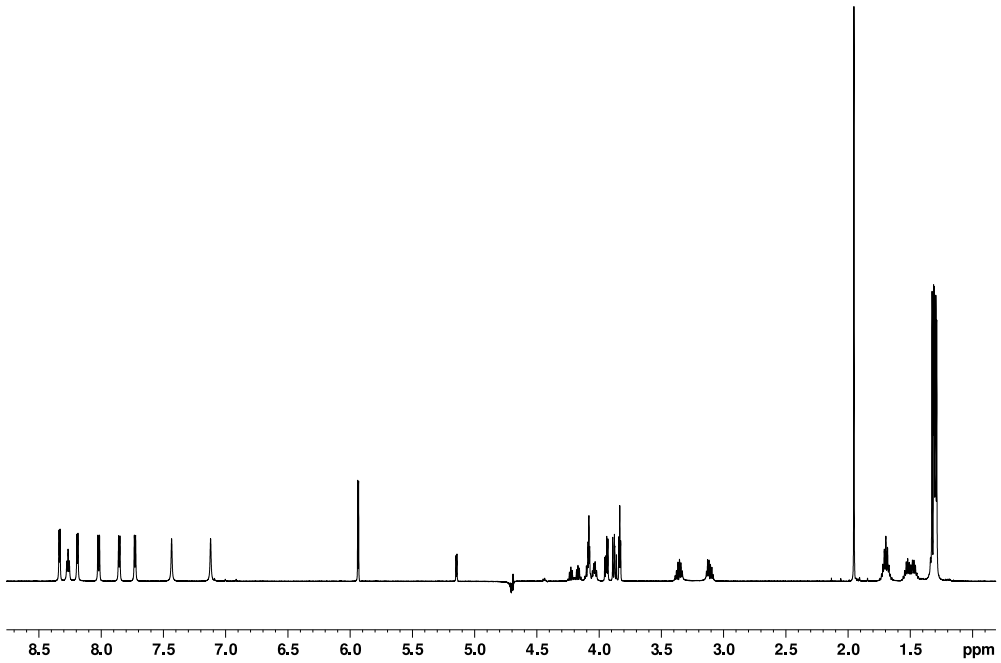


Figure S9. 600MHz ^{1}H NMR spectrum of **10** in H_2O/D_2O (9:1) at 298K.

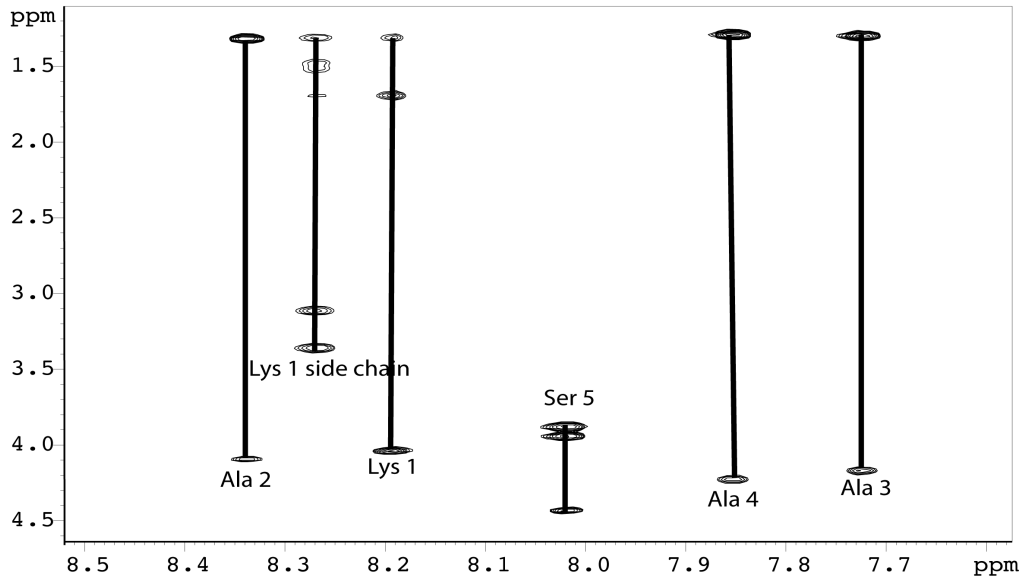


Figure S10. 600MHz TOCSY fingerprint region of **10** in H_2O/D_2O (9:1) at 298K. Connectivity of the NH, Cα, Cβ, Cγ protons for the peptide is shown by the solid line.

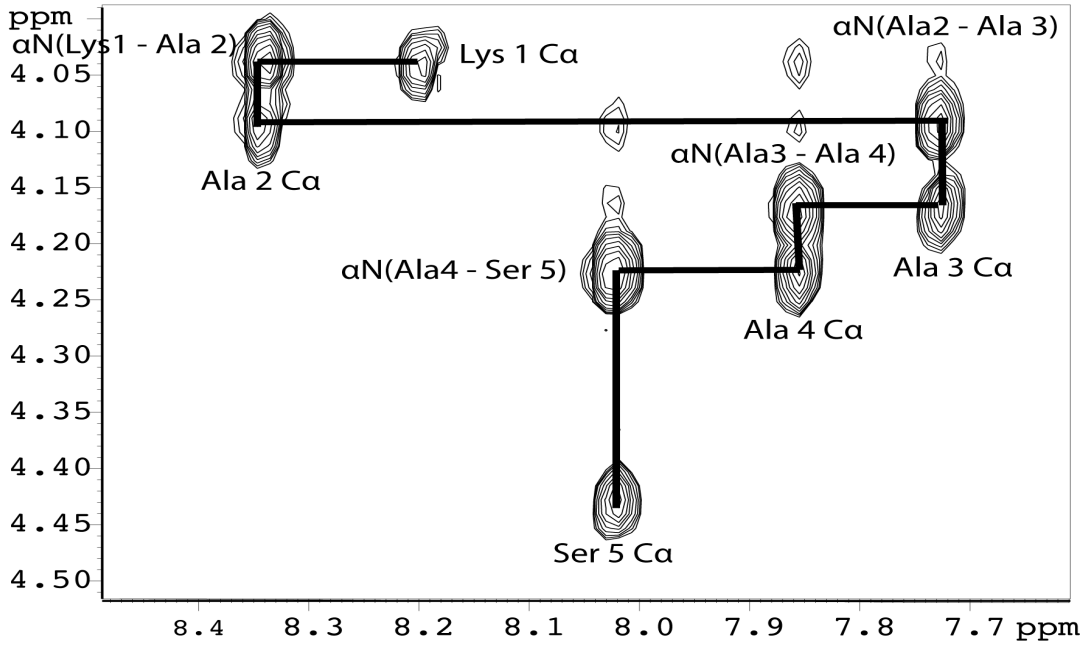


Figure S11. Magnified NH-CHα region from the 600MHz ROESY spectrum of **10** in H_2O/D_2O (9:1) at 298K. Sequential connectivity for the peptide is shown by the solid line.

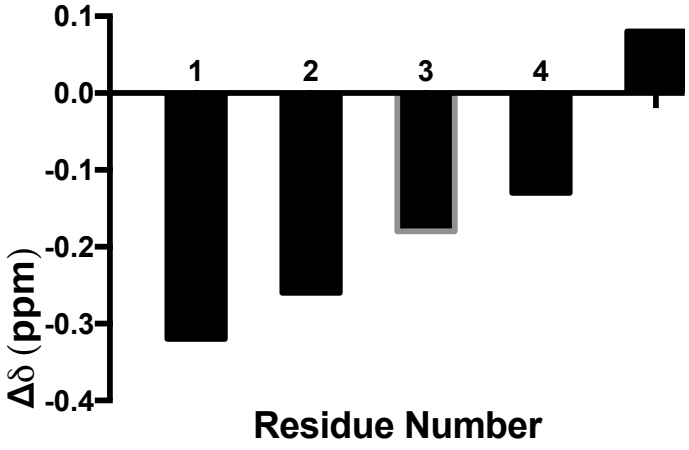


Figure S12. Δδ Hα versus the corresponding amino acids in random coil for **10** in water at 298K.

Table S6. ^{1}H NMR resonance assignments and chemical shifts (δ ppm) for **10** in H_2O/D_2O (9:1) at 298K.

Residue	NH	H α	H β	Other
Ac-K1	8.19	4.04	2β H 1.70	2εH 3.36, 3.11; 2δ H 1.47-1.53; 2 γH 1.30-1.32;

A2	8.34	4.09	1.32	
A3	7.73	4.17	1.30	
A4	7.86	4.22	1.29	
S5-NH_2	8.05	4.43	3.94, 3.88	
Sugar H1 5.15	H2 3.83	H3 4.08	H4 5.94	

Table S7. Amide NH coupling constants and Δδ/T for **10.**

Residue	K1	A2	A3	A4	S5	NT1	NT2	NH-side
^{3}JNH--CHα (Hz)	5.22	4.92	6.00	6.36	7.44	N/A	N/A	N/A
Δδ/K (--ppb)	7.4	6.6	4.9	3.3	4.6	4	5.5	8.3

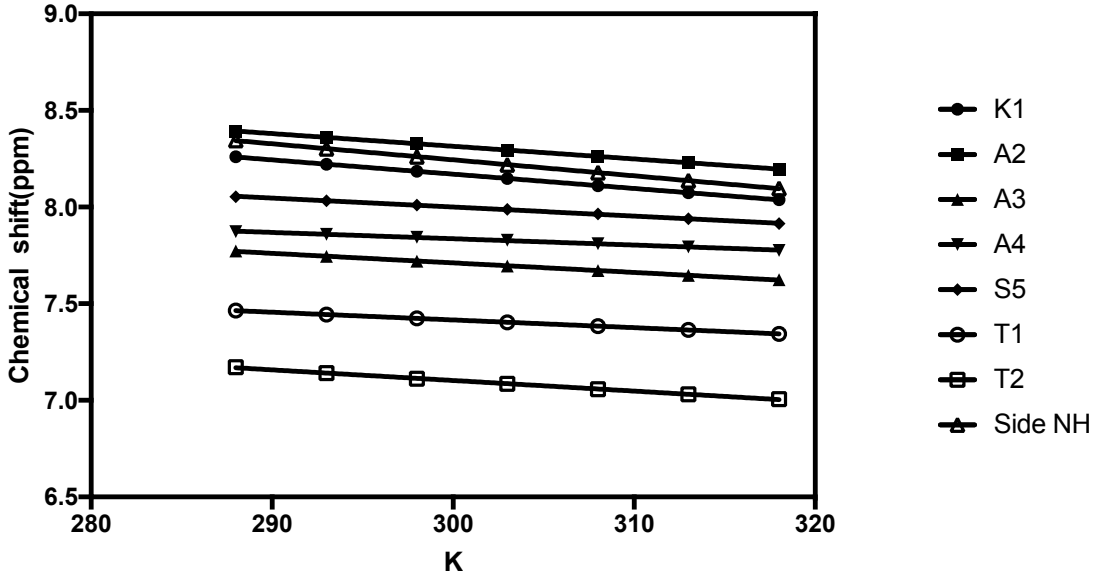


Figure S13. Temperature dependence of the amide NH chemical shifts for **10** in H_2O/D_2O (9:1). Line slopes indicating temperature coefficients (Δδ/T) for each residue are shown in brackets. Lys1 [7.4 ppb/K], Ala2 [6.6 ppb/K], Ala3 [4.9 ppb/K], Ala4 [3.3 ppb/K], Ser5 [4.6 ppb/K], Ser5 H1 [4.0 ppb/K], Ser5 H2 [5.5 ppb/K], Lys1 side NH [8.3 ppb/K].

Table S8. ROE-derived distances, ^{3}JNH-CHα derived **φ**-angle restraints and hydrogen bond restraints used for calculating the solution structure of **10** in H_2O/D_2O (9:1) at 298K.

1	Acetyl 1 Hα*	Lys 2 HN	4.2 Å; Strong + 1.5Å correction
2	Acetyl 1 Hα*	Ala 3 HN	7.5 Å; Very Weak + 1.5Å correction
3	Acetyl 1 Hα*	Ala 4 HN	7.5 Å; Very Weak + 1.5Å correction
4	Lys 2 Hα	Ala 3 HN	2.7 Å; Strong
5	Lys 2 Hα	Ala 4 HN	6.0 Å; Very Weak
6	Lys 2 Hα	Ala 5 HN	5.0 Å; Weak
7	Lys 2 HN	Ala 3 HN	5.0 Å; Weak

8	Lys 2 Hβ*	Ala 3 HN	5.0 Å; Weak
9	Lys 2 Hβ*	Ala 4 HN	6.0 Å; Very Weak
10	Ala 3 Hα	Ala 4 HN	2.7 Å; Strong
11	Ala 3 Hα	Ala 5 HN	6.0 Å; Very Weak
12	Ala 3 Hα	Ser 6 HN	6.0 Å; Very Weak
13	Ala 3 HN	Ala 4 HN	5.0 Å; Weak
14	Ala 4 HN	Ala 5 HN	5.0 Å; Weak
15	Ala 4 Hα	Ala 5 HN	2.7 Å; Strong
16	Ala 4 Hα	Ser 6 HN	6.0 Å; Very Weak
17	Ala 4 Hα	Ser 6 H2	6.0 Å; Very Weak
18	Ala 5 HN	Ser 6 HN	5.0 Å; Weak
19	Ala 5 Hα	Ser 6 HN	2.7 Å; Strong
20	Ala 5 Hα	Ser 6 H2	6.0 Å; Very Weak
21	Ser 6 HN	Ser 6 H1	6.0 Å; Very Weak
22	Ser 6 HN	Ser 6 H2	5.0 Å; Weak
23	Ser 6 Hα	Ser 6 H1	5.0 Å; Weak
24	Ser 6 Hα	Ser 6 H2	3.5 Å; Medium
25	Ser 6 Hβ1	Ser 6 H2	5.0 Å; Weak
26	Ser 6 Hβ2	Ser 6 H2	5.0 Å; Weak
27	Ser 6 Hβ1	Ser 6 H1	6.0 Å; Very Weak
28	Ser 6 Hβ2	Ser 6 H1	6.0 Å; Very Weak

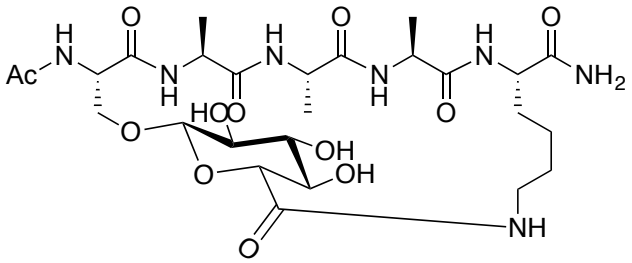
φ-angle restraints

	Residue	^{3}JNH-CHα	φ-dihedral angle restraint
1	Lys 1	5.22	-65 ± 30°
2	Ala 2	4.92	-65 ± 30°
3	Ala 3	6.00	-65 ± 30°

Hydrogen-bond restraints

	Donor	Acceptor	H-O Distance	N-O Distance
1	Ser6 H1	Ala3 O	1.88[-.3 Å,+.42 Å]	2.88 ± 0.3 Å
2	Ser6 NH	Lys2 O	1.88[-.3 Å,+.42 Å]	2.88 ± 0.3 Å
3	Ala5 NH	Acetly1 O	1.88[-.3 Å,+.42 Å]	2.88 ± 0.3 Å

Peptide 7



HRMS (ESI): Calcd for $C_{26}H_{44}N_7O_{12}$+H [M + H^+]: 646.3042, Found: 646.3037; Linear gradient from 0% to 40% B over 20 min, Rt =11.21 min

Table S9. Molar residue ellipticities at 220 nm in 10 mM phosphate buffer (pH 7.4, 298 K) and percentage helicity for peptides **7**.

	Peptide	[θ]220	Helicity (%)
1	Ac-[S*AAAK]-NH_2	-5795.8	34

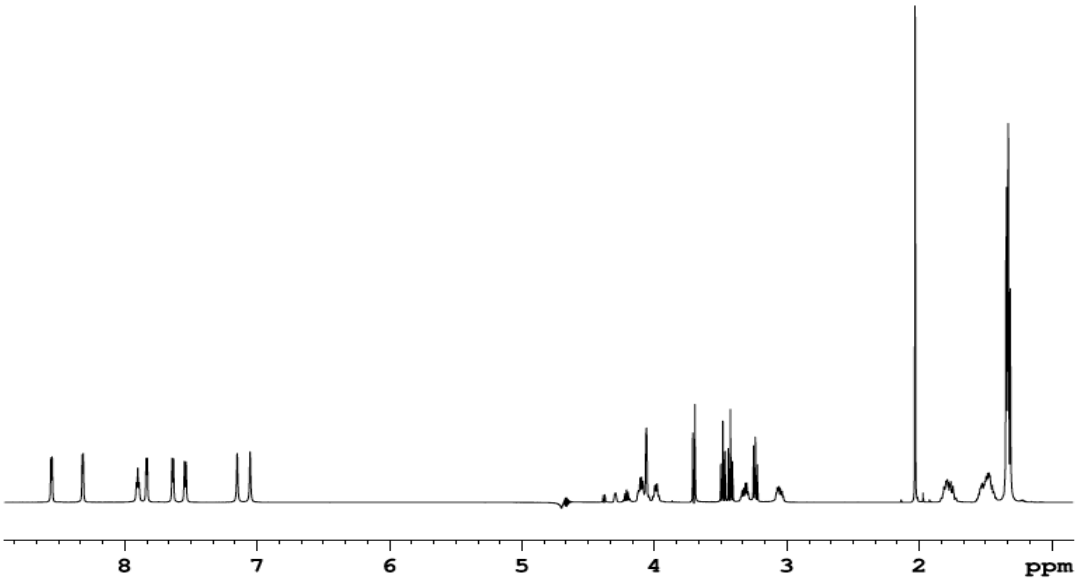


Figure S14. 600MHz ^{1}H NMR spectrum of **7** in H_2O/D_2O (9:1) at 298K.

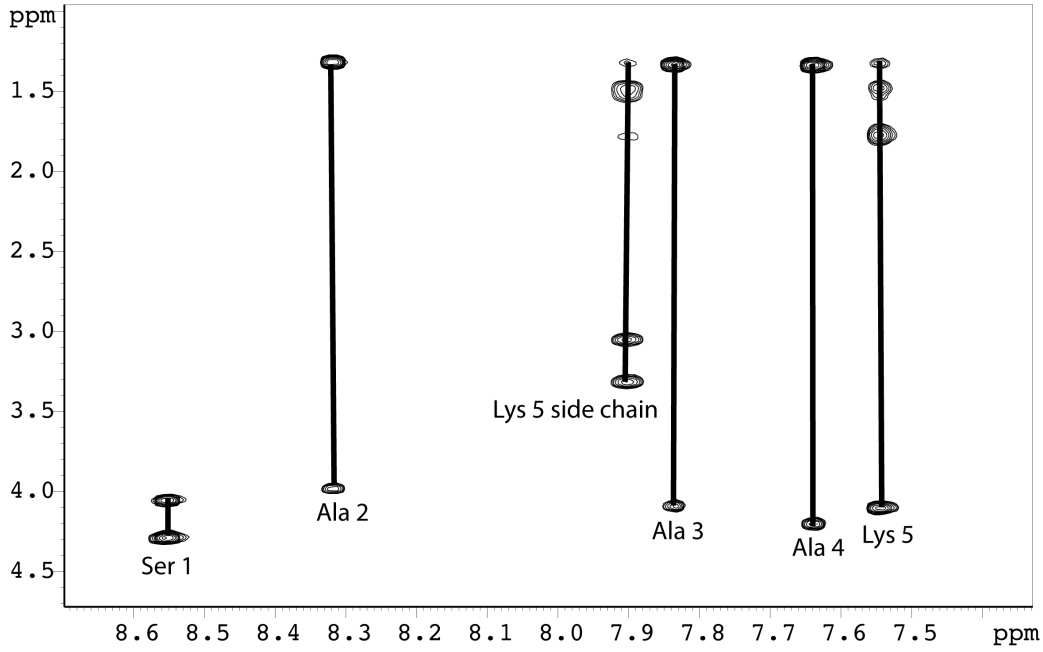


Figure S15. 600MHz TOCSY fingerprint region of 7 in H_2O/D_2O (9:1) at 298K. Connectivity of the NH, C**α**, C**β**, C**γ** protons for the peptide is shown by the solid line.

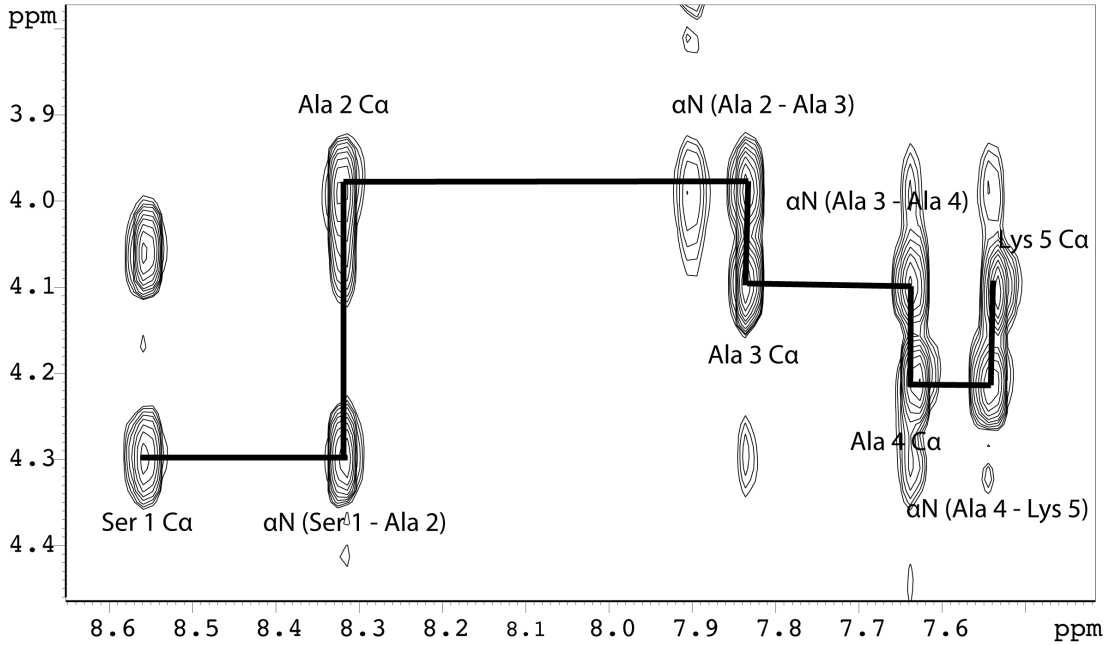


Figure S16. Magnified NH-CHα region from the 600MHz ROESY spectrum of **7** in H_2O/D_2O (9:1) at 298K. Sequential connectivity for the peptide is shown by the solid line.

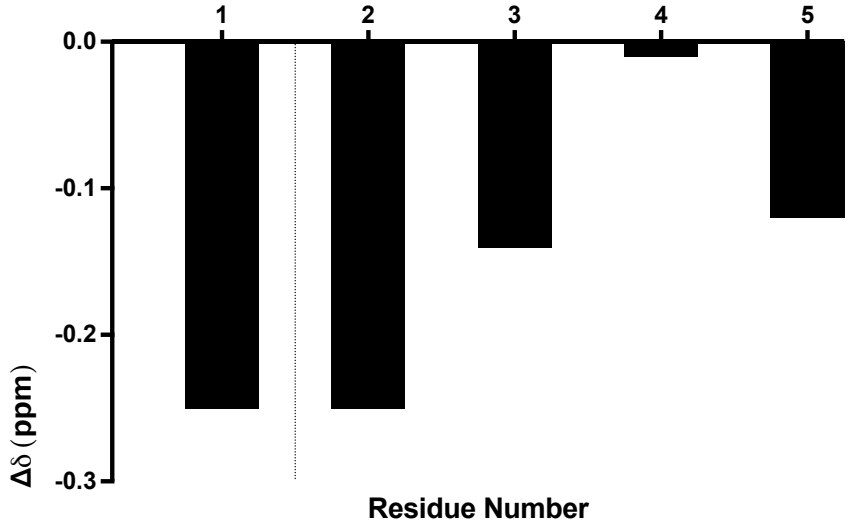


Figure S17. Δδ Hα versus the corresponding amino acids in random coil for **7** in water at 298K.

Table S9. 1H NMR resonance assignments and chemical shifts (δ ppm) for **7** in H_2O/D_2O (9:1) at 298K.

Residue	NH	H α	H β	Other
Ac-Ser1	8.55	4.29	2H 4.06	OAc 2.03
A2	8.32	3.99	1.32	
A3	7.83	4.10	1.34	
A4	7.64	4.21	1.34	
K5-NH_2	7.54	4.11	2H 1.78	εH_2 3.32, 3.06; 2δ H and 1

T 7.15 7.05				γH 1.44-1.54; 1γH 1.33
Sugar H1 4.38	H2 3.24	H3 3.43	H4 3.48	H5 3.70

Table S10. Amide NH coupling constants and Δδ/T for **7.**

Residue	S1	A2	A3	A4	K5	NT1	NT2	NH-side
^{3}JNH--CHα (Hz)	5.48	4.55	5.06	6.18	7.16	N/A	N/A	N/A
Δδ/K (--ppb)	7.7	6.6	5.5	1.9	1.0	1.8	7.4	7.1

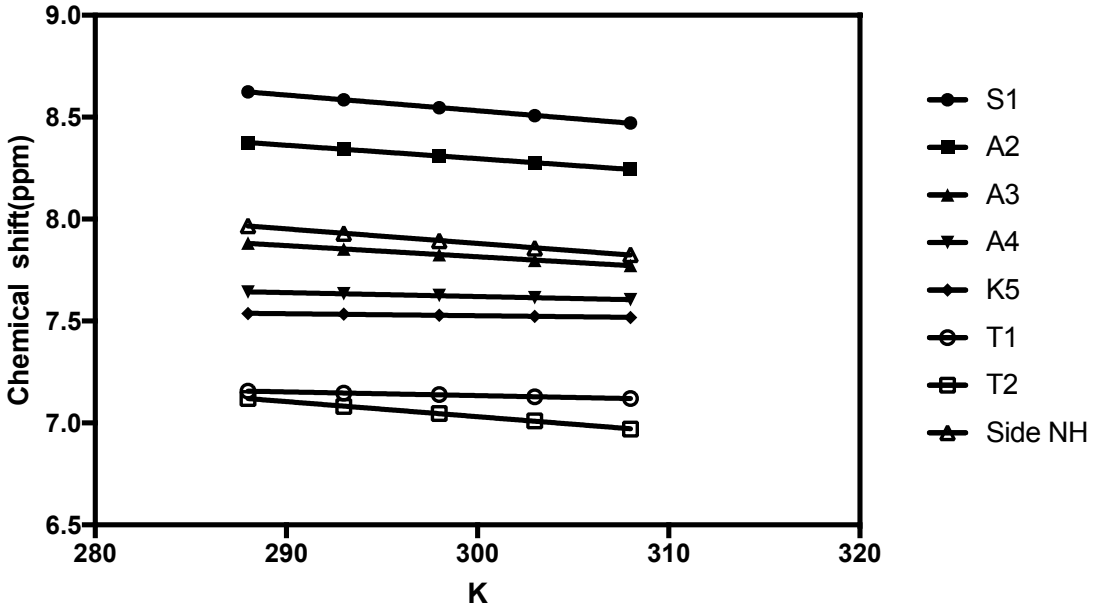
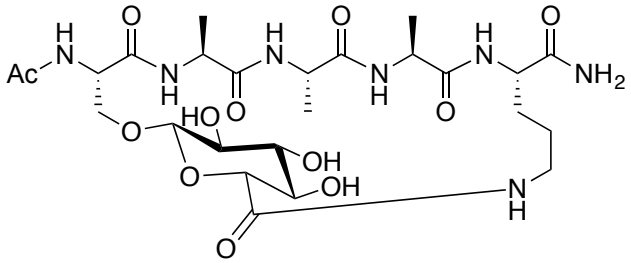


Figure S18. Temperature dependence of the amide NH chemical shifts for **7** (9:1). Line slopes indicating temperature coefficients (Δδ/T) for each residue. Ser1 [7.7 ppb/K], Ala2 [6.6 ppb/K], Ala3 [5.5 ppb/K], Ala4 [1.9 ppb/K], Lys5 [1.0 ppb/K], Lys5 H1 [1.8 ppb/K], Lys5 H2 [7.4 ppb/K], Lys5 side NH [7.1 ppb/K].

Peptide 11



HRMS (ESI): Calcd for $C_{25}H_{41}N_7O_{12}$ [M + Na^+]: 654.2705, Found: 654.2707; Linear gradient from 0% to 40% B over 20 min, Rt =16.42 min

Table S11. Molar residue ellipticities at 220 nm in 10 mM phosphate buffer (pH 7.4, 298 K) and percentage helicity for peptides **11**.

	Peptide	$[\theta]_{220}$	Helicity (%)
1	Ac-[S*AAAO]-NH_2	-8304.0	46.5

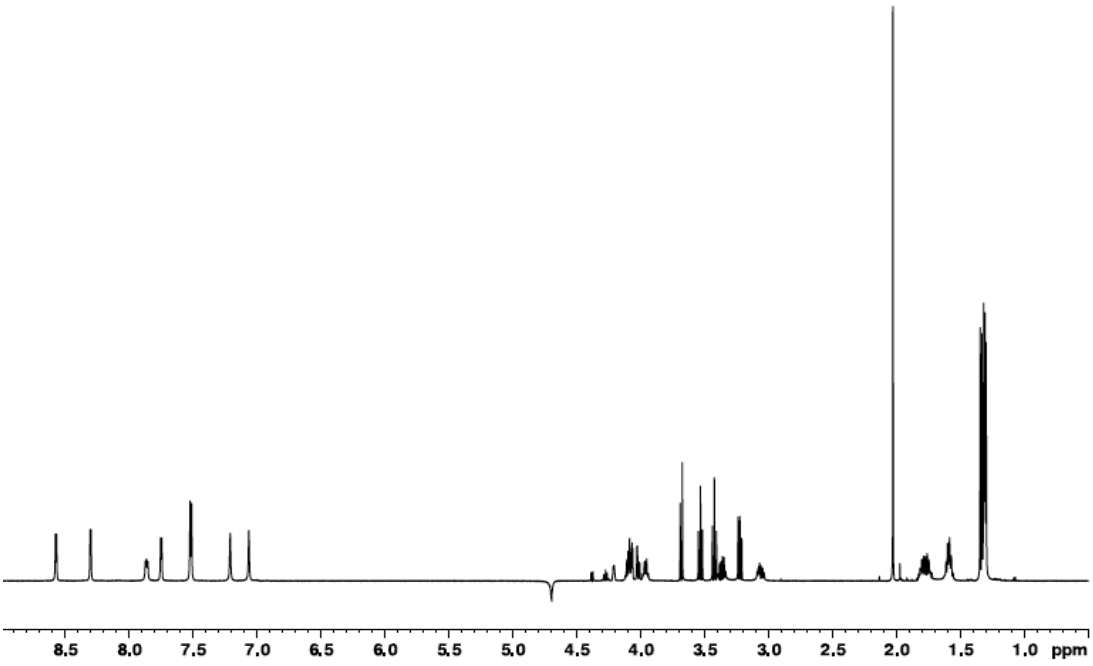


Figure S19. 600MHz ^{1}H NMR spectrum of **11** in H_2O/D_2O (9:1) at 298K.

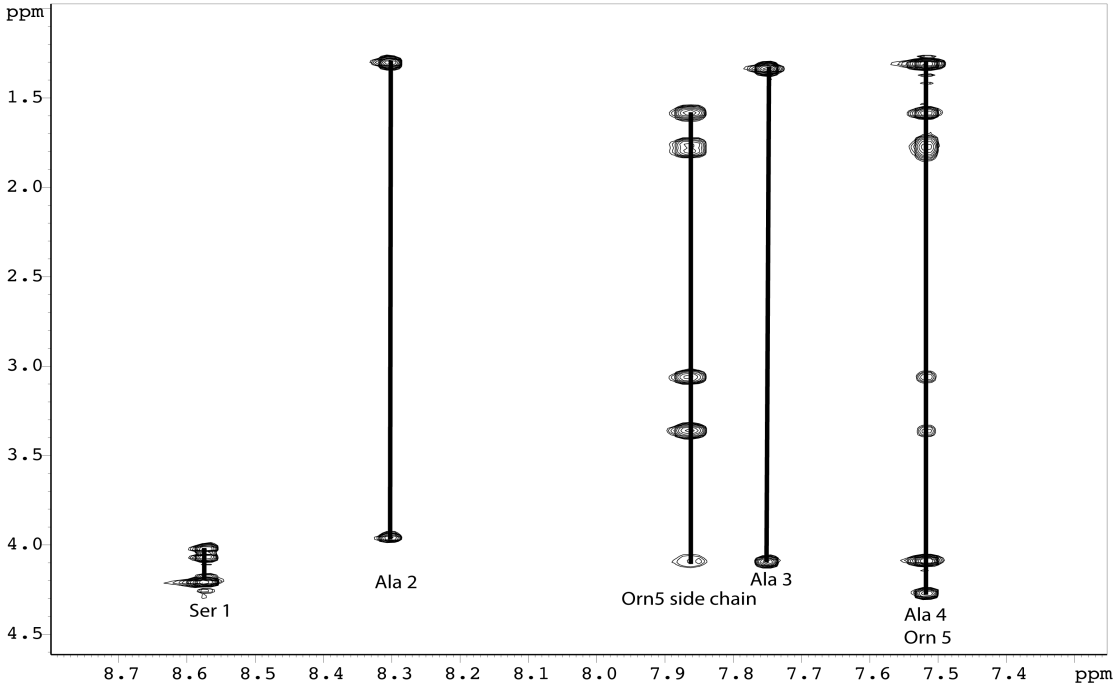


Figure S20. 600MHz TOCSY fingerprint region of **11** in H_2O/D_2O (9:1) at 298K. Connectivity of the NH, C**α**, C**β**, C**γ** protons for the peptide is shown by the solid line.

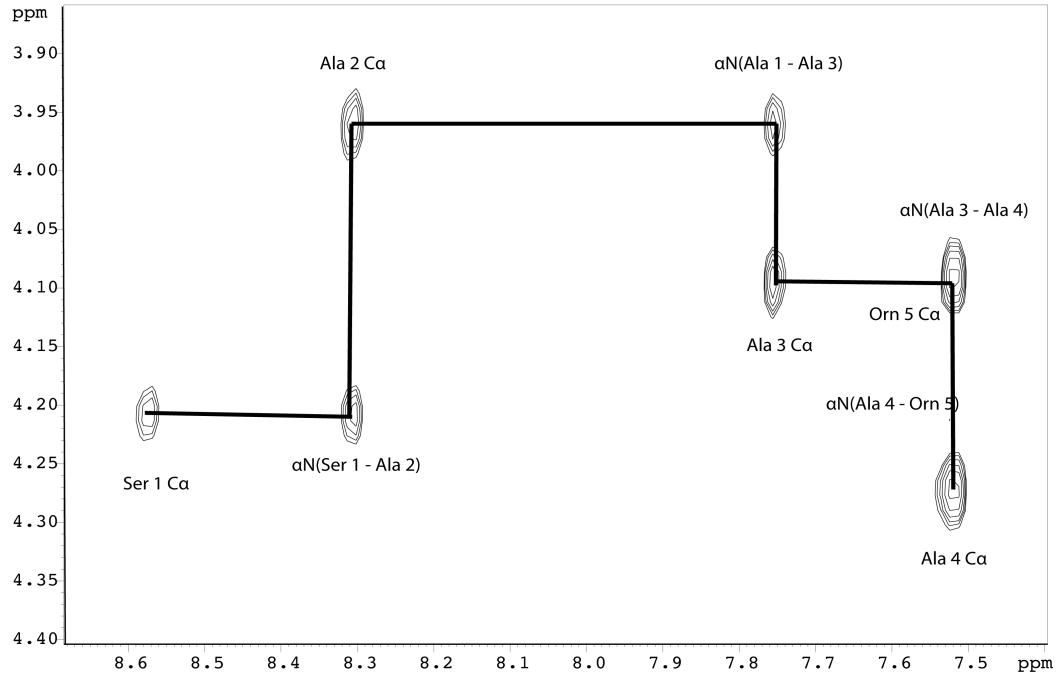


Figure S21. Magnified NH-CHα region from the 600MHz ROESY spectrum of **11** in H_2O/D_2O (9:1) at 298K. Sequential connectivity for the peptide is shown by the solid line.

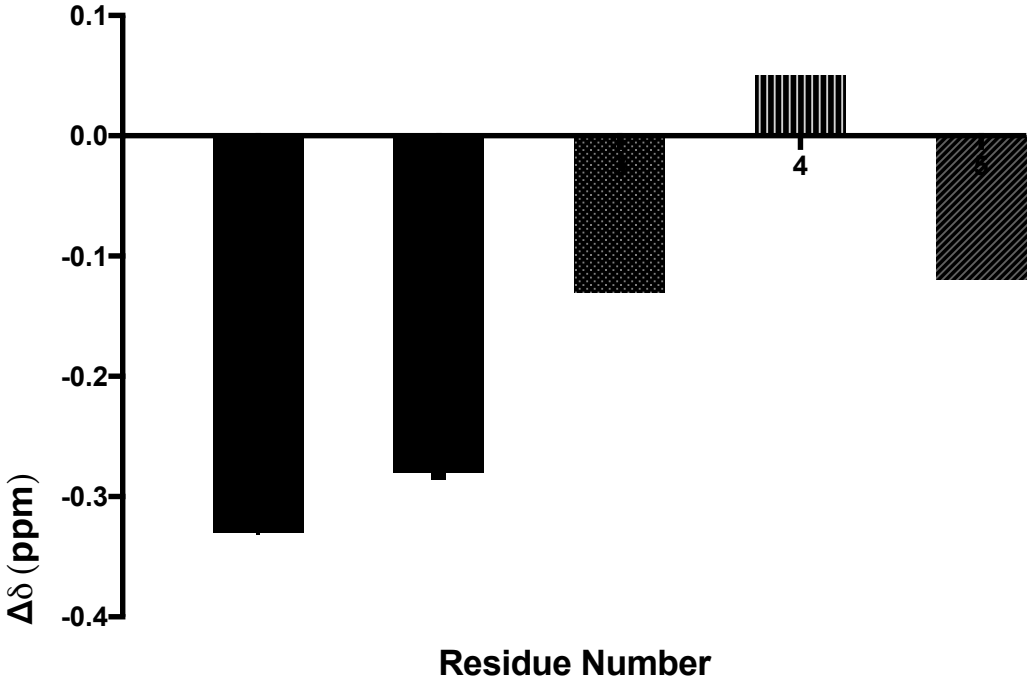


Figure S22. Δδ Hα versus corresponding amino acids in random coil for **11** in water at 298K.

Table S12. ^{1}H NMR resonance assignments and chemical shifts (δ ppm) for **11** in H_2O/D_2O (9:1) at 298K.

Residue	NH	H α	H β	Other
Ac-Ser1	8.57	4.21	4.07, 4.02	OAc: 2.03
A2	8.30	3.96	1.30	T1: 7.21
A3	7.75	4.10	1.34	T2: 7.06
A4	7.52	4.28	1.32	
O5-NH_2	7.52	4.09	1.72-1.83(2H)	1.55-1.62(2rH); 3.36 εH1, 3.06 εH2;
H1 4.38	H2 3.22	H3 3.42	H4 3.53	H5 3.68

Table S13. Amide NH coupling constants and Δδ/T for **11.**

Residue	S1	A2	A3	A4	O5	NT1	NT2	Side NH
^3JNH--CHα (Hz)	5.22	4.08	5.10	6.78	6.78	N/A	N/A	N/A
Δδ/K (--ppb)	7.9	5.9	4.8	1.3	1.3	1.8	6.5	6.9

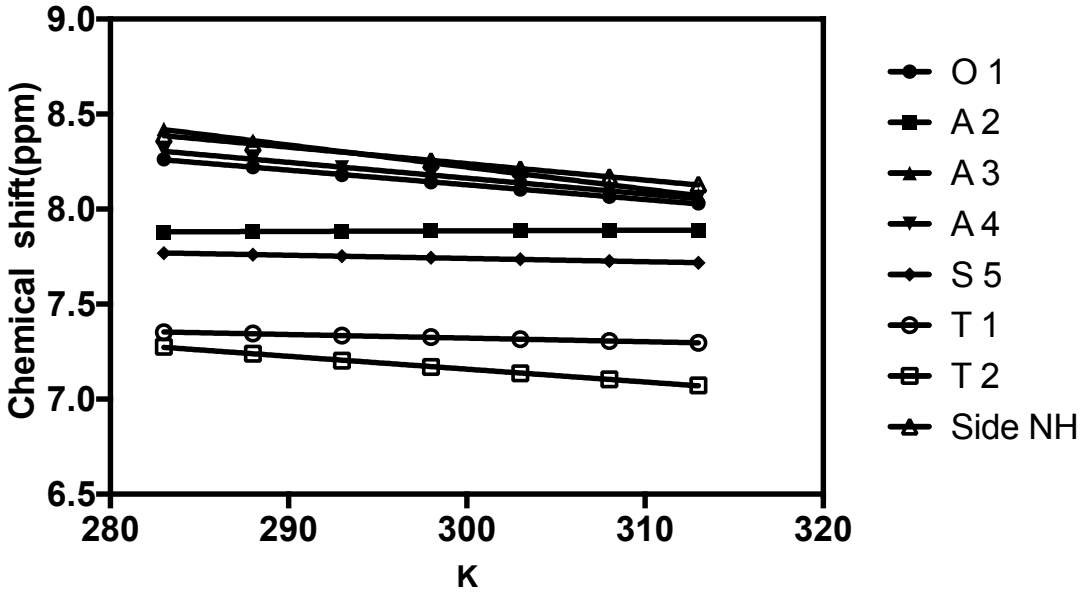
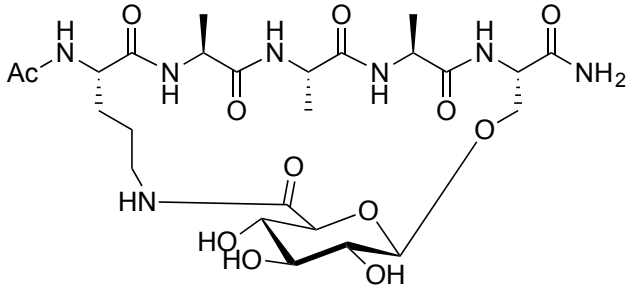


Figure S23. Temperature dependence of the amide NH chemical shifts for **11** in H2O/D2O (9:1). Line slopes indicating temperature coefficients (Δδ/T) for each residue are shown in brackets. Orn1 [7.9 ppb/K], Ala2 [5.9 ppb/K], Ala3 [4.8 ppb/K], Ala4 [1.3 ppb/K], Ser5 [1.3 ppb/K], Ser5 H1 [1.8 ppb/K], Ser5 H2 [6.5 ppb/K], Orn1 side NH [6.9 ppb/K].

Peptide 12



HRMS (ESI): Calcd for $C_{25}H_{41}N_7O_{12}$ [M + Na^+]: 654.2705, Found: 654.2709; Linear gradient from 0% to 40% B over 20 min, Rt =9.97 min

Table S14. Molar residue ellipticities at 220 nm in 10 mM phosphate buffer (pH 7.4, 298 K) and percentage helicity for peptides **12**.

	Peptide	[θ]220	Helicity (%)
1	Ac-[OAAAS*]-NH_2	-3536.5	22.4

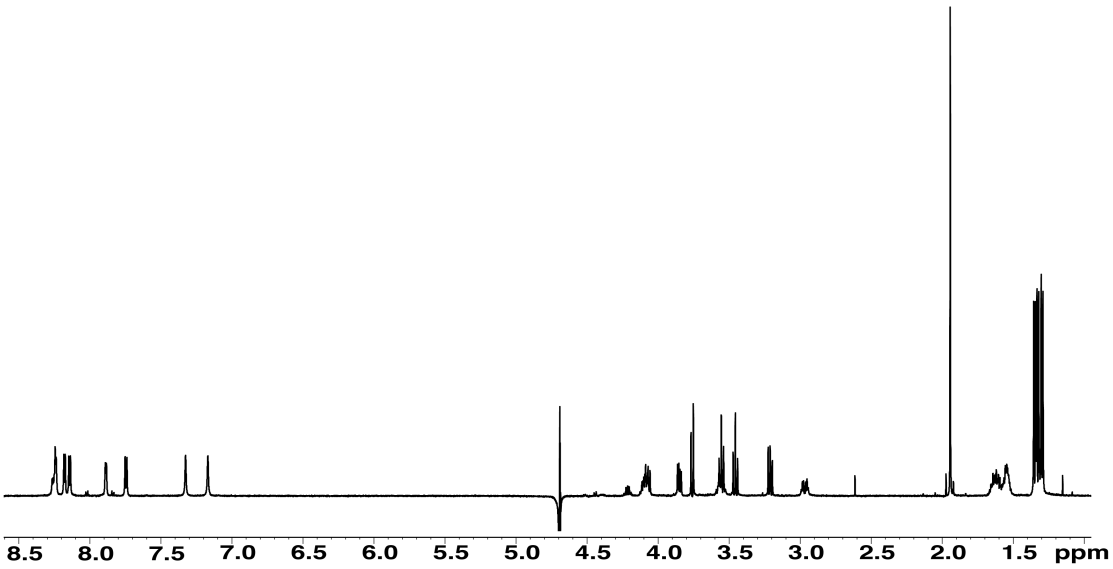


Figure S24. 600MHz ^{1}H NMR spectrum of **12** in H_2O/D_2O (9:1) at 298K.

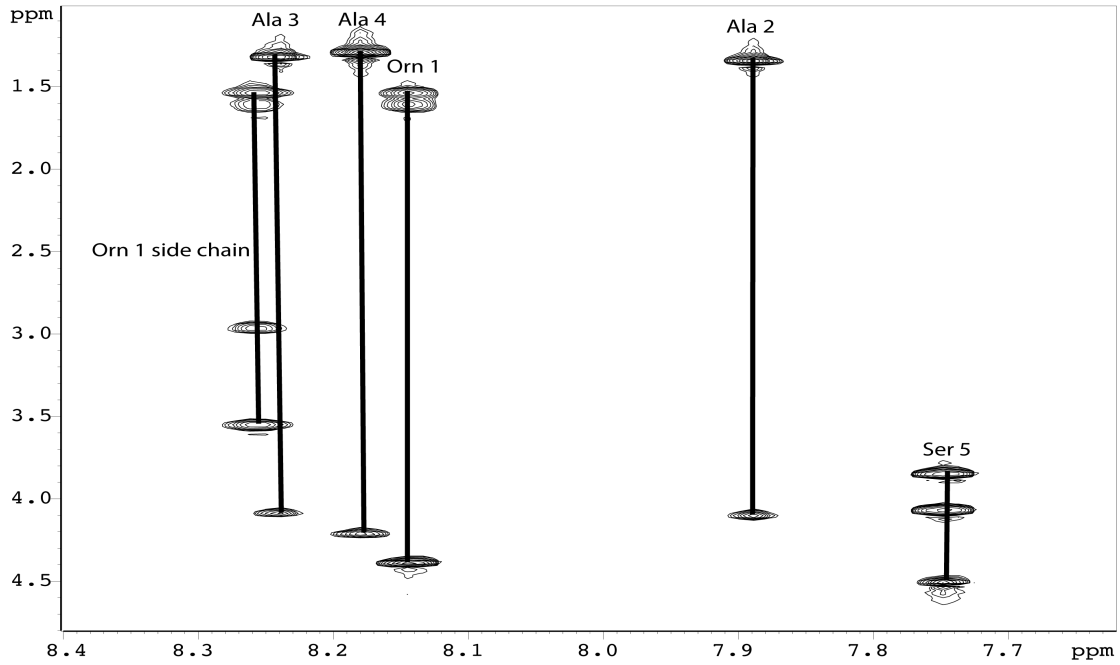


Figure S25. 600MHz TOCSY fingerprint region of **12** in H_2O/D_2O (9:1) at 298K. Connectivity of the NH, C**α**, C**β**, C**γ** protons for the peptide is shown by the solid line.

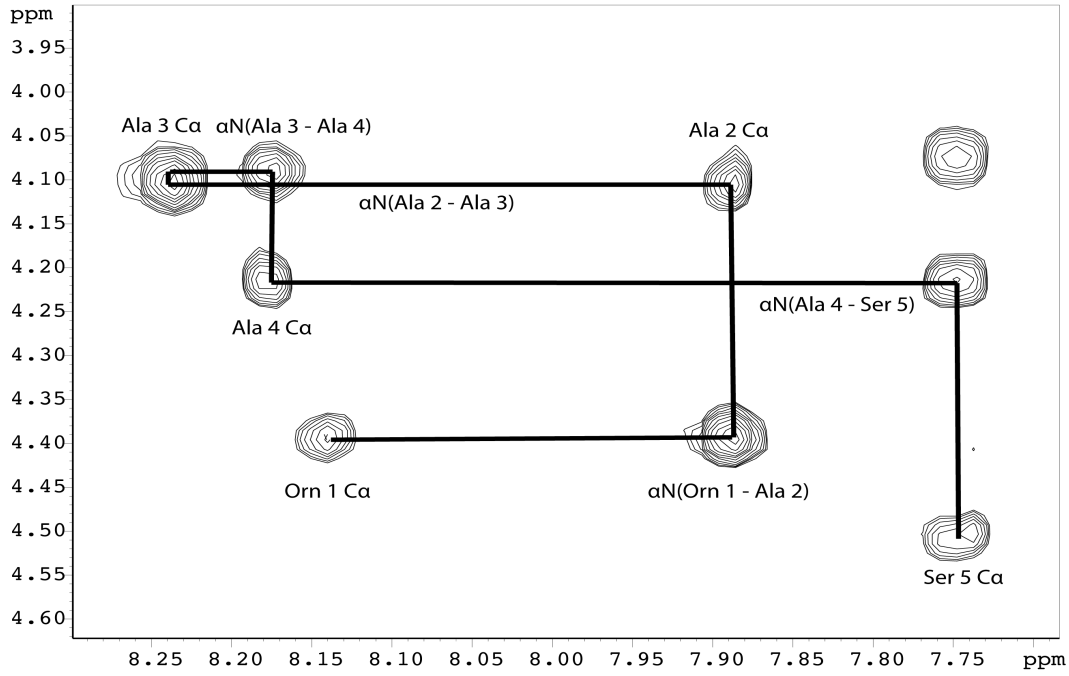


Figure S26. Magnified NH-CHα region from the 600MHz ROESY spectrum of **12** in H_2O/D_2O (9:1) at 298K. Sequential connectivity for the peptide is shown by the solid line.

Table S15. ^{1}H NMR resonance assignments and chemical shifts (δ ppm) for **12** in H_2O/D_2O (9:1) at 298K.

Residue	NH	H α	H β	Other

Ac-O1	8.14 side NH 8.389	4.21	1.58-1.67 (2H)	OAc: 1.94; 1.52-1.58(Y 2H); 2.94-3.00(εHb), 3.52-3.59 (εHa);
A2	7.89	4.10	1.35	
A3	8.24	4.09	1.33	
A4	8.18	4.21	1.30	
S5-NH_2	7.75	4.50	3.85 4.07	T1: 7.33; T2: 7.17;
H1 4.44	H2 3.21	H3 3.46	H4 3.55	H5 3.76

Table S16. Amide NH coupling constants and Δδ/T for **12**.

Residue	O1	A2	A3	A4	S5	NT1	NT2	Side NH
^3JNH--CHα (Hz)	6.66	4.50	4.58	5.64	7.62	N/A	N/A	N/A
Δδ/K (--ppb)	7.74	3.07	11.6	8.32	1.7	1.89	6.76	8.65

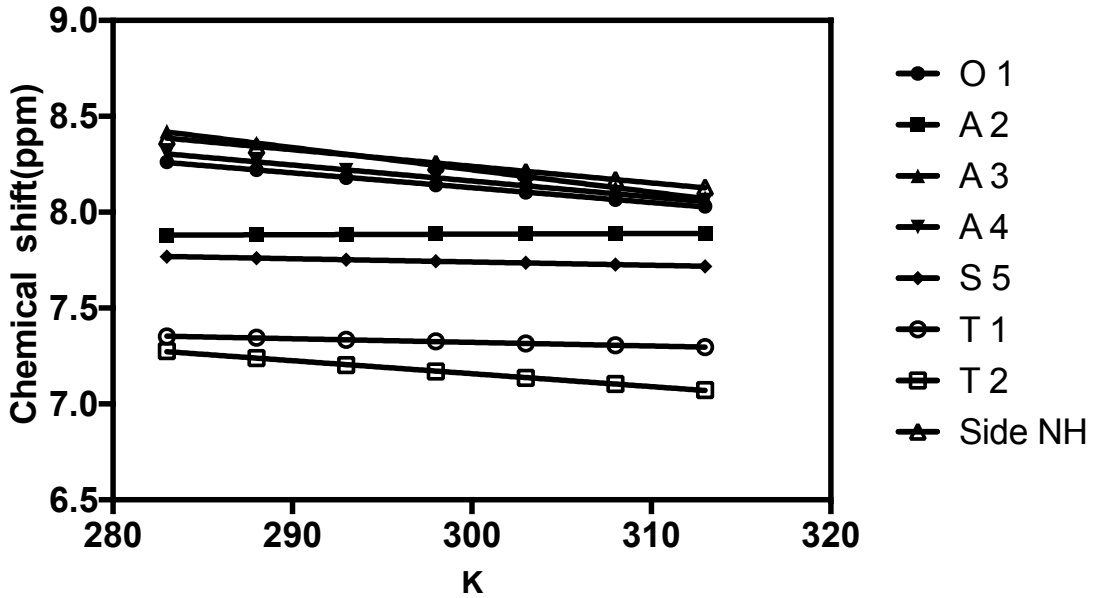
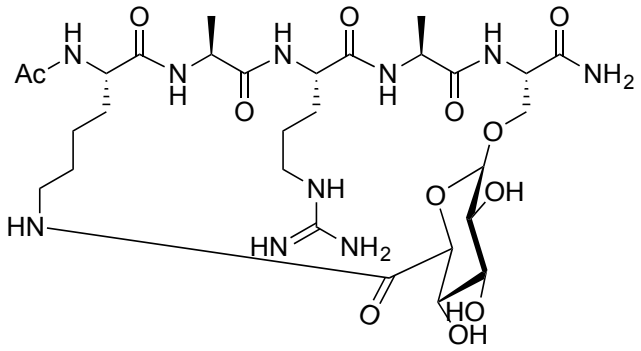


Figure S27. Temperature dependence of the amide NH chemical shifts for **12** in H_2O/D_2O (9:1). Line slopes indicating temperature coefficients (Δδ/T) for each residue are shown in brackets. Orn1 [7.7 ppb/K], Ala2 [0.3 ppb/K], Ala3 [11.6 ppb/K], Ala4 [8.3 ppb/K], Ser5 [1.7 ppb/K], Ser5 H1 [1.9 ppb/K], Ser5 H2 [6.8 ppb/K], Orn1 side NH [8.7 ppb/K].

Peptide 13



HRMS (ESI): Calcd for $C_{29}H_{50}N_{10}O_{12}$ [M + H^+]: 731.3682, Found: 731.3681; Linear gradient from 0% to 40% B over 20 min, Rt =9.92 min

Table S17. Molar residue ellipticities at 220 nm in 10 mM phosphate buffer (pH 7.4, 298 K) and percentage helicity for peptides **13**.

	Peptide	[θ]220	Helicity (%)
1	Ac-[KARAS*]-NH_2	-7040.2	40

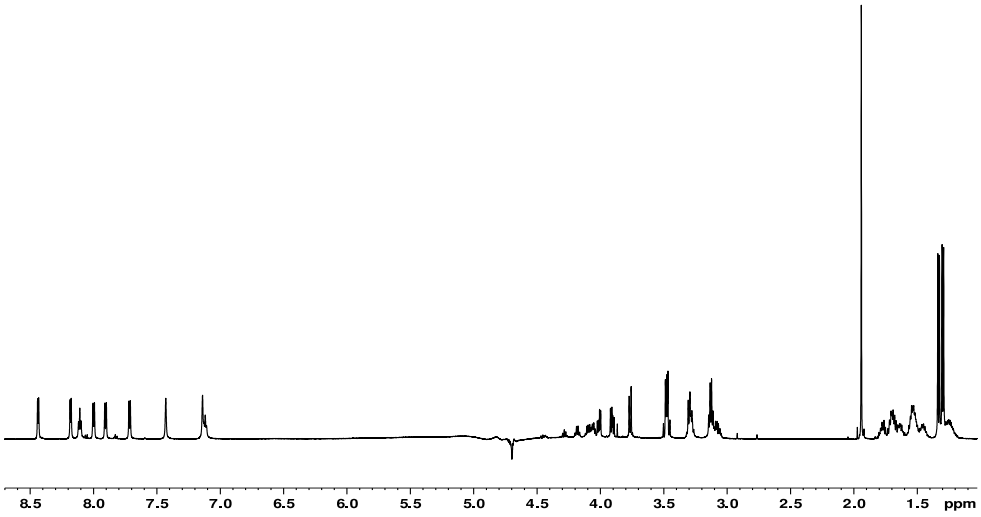


Table S18. ^{1}H NMR resonance assignments and chemical shifts (δ ppm) for **13** in H_2O/D_2O (9:1) at 298K.

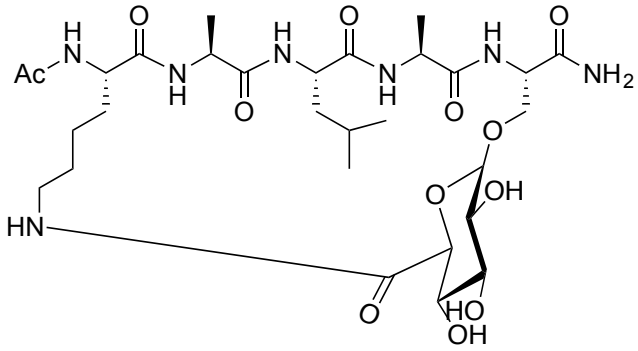
Residue	NH	H α	H β	Other
Ac-K1	8.18 side NH 8.11	4.06	βa 1.69, βb 1.63;	OAc: 1.94; 1.24(ϒ 2H); δa 1.45, δb 1.51; εa 3.29, εb 3.07;
A2	8.44	4.09	1.33	
R3	7.71	4.19	βa 1.77, βb 1.68;	1.54 (ϒ 2H); 3.13 (δ 2H);

A4	8.00	4.29	1.30	
S5-NH_2	7.91	4.43	βa 4.01, βb 3.91;	T1 7.43; T2 7.14;
H1 4.46	H2 3.30	H3 3.47	H4 3.48	H5 3.77

Table S19. Amide NH coupling constants for **13**.

Residue	K1	A2	R3	A4	S5	NT1	NT2	Side NH
^3JNH--CHα(Hz)	5.10	5.20	6.44	6.64	7.17	N/A	N/A	N/A

Peptide 14



HRMS (ESI): Calcd for $C_{29}H_{49}N_7O_{12}$ [M + Na^+]: 710.3331, Found: 710.3333; Linear gradient from 0% to 40% B over 20 min, Rt =16.21 min

Table S20. Molar residue ellipticities at 220 nm in 10 mM phosphate buffer (pH 7.4, 298 K) and percentage helicity for peptides **14**.

	Peptide	[θ]220	Helicity (%)
1	Ac-[KALAS*]-NH_2	-10032.4	55

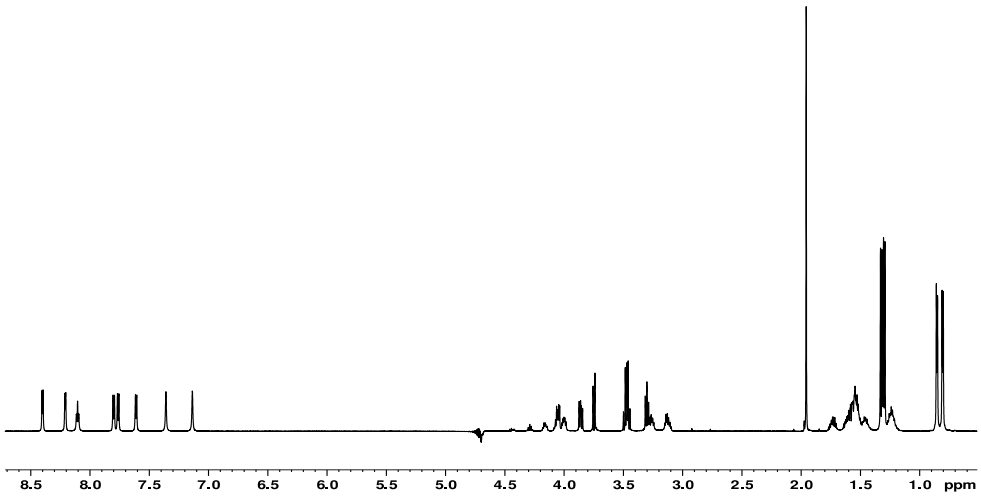


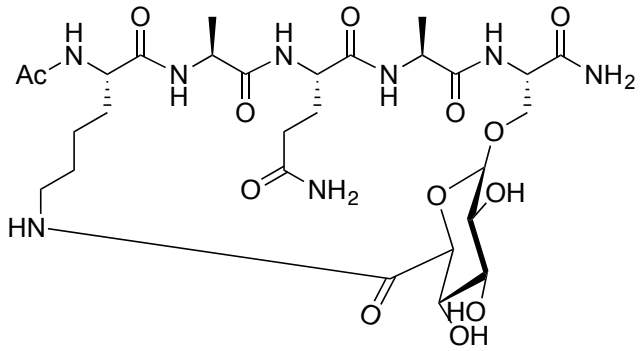
Table S21. ^{1}H NMR resonance assignments and chemical shifts (δ ppm) for **14** in H_2O/D_2O (9:1) at 298K.

Residue	**NH**	**H α**	**H β**	**Other**
Ac-K1	8.21 side NH 8.10	4.00	βa 1.73, βb 1.60;	OAc: 1.96; 1.24(ϒ 2H); δa 1.51, δb 1.45; εa 3.26, εb 3.12;
A2	8.40	4.06	1.32	
L3	7.61	4.16	1. 56 (β 2H)	ϒ 1.56
A4	7.80	4.42	1.30	
S5-NH_2	7.76	4.43	β a 4.05, βb 3.86;	T1 7.36; T2 7.14;
H1 4.45	H2 3.30	H3 3.46	H4 3.47	H5 3.75

Table S22. Amide NH coupling constants for **14**.

Residue	K1	A2	L3	A4	S5	NT1	NT2	Side NH
^{3}JNH---CHα(Hz)	4.55	5.03	6.52	6.85	7.14	N/A	N/A	N/A

Peptide 15



HRMS (ESI): Calcd for $C_{28}H_{46}N_8O_{13}$ [M + H^+]: 703.3257, Found: 703.3254; Linear gradient from 0% to 40% B over 20 min, Rt =10.73 min

Table S23**.** Molar residue ellipticities at 220 nm in 10 mM phosphate buffer (pH 7.4, 298 K) and percentage helicity for peptides **15**.

	Peptide	**[θ]220**	**Helicity (%)**
1	Ac-[KAQAS*]-NH_2	-9931.6	55

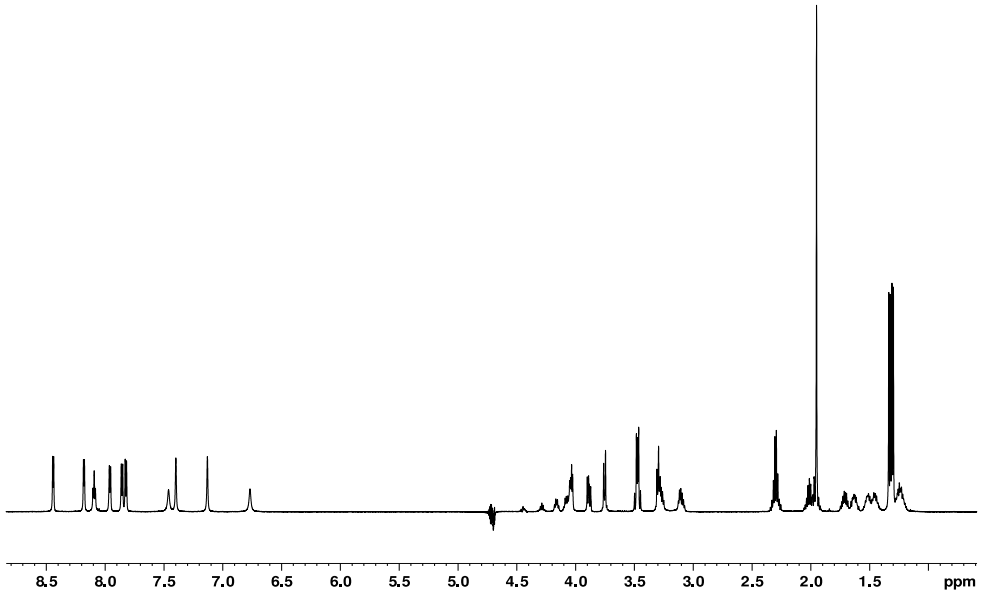


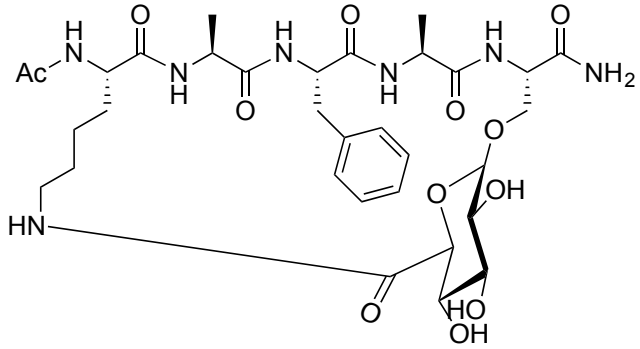
Table S24**.** ^{1}H NMR resonance assignments and chemical shifts (δ ppm) for **15** in H_2O/D_2O (9:1) at 298K.

Residue	NH	H α	H β	Other
Ac-K1	8.18 side NH 8.09	4.04	βa 1.71, βb 1.63;	OAc: 1.95; 1.24(Y 2H); δa 1.52, δb 1.46; εa 3.28, εb 3.10;
A2	8.44	4.08	1.33	
Q3	7.82	4.16	βa 2.01, βb 1.96	2.30 (Y 2H) ; side NHa 7.46, NHb 6.77;
A4	7.96	4.28	1.30	
S5-NH_2	7.86	4.43	βa 4.04, βb 3.89;	T1 7.40; T2 7.13;
H1 4.45	H2 3.29	H3 3.47	H4 3.48	H5 3.75

Table S25. Amide NH coupling constants for **15**.

Residue	K1	A2	Q3	A4	S5	NT1	NT2	Side NH
^{3}JNH--CHα(Hz)	5.09	5.01	6.66	6.61	7.16	N/A	N/A	N/A

Peptide 16



HRMS (ESI): Calcd for $C_{32}H_{47}N_7O_{12}$ [M + H^+]: 722.3355, Found: 722.3353; Linear gradient from 0% to 40% B over 20 min, Rt =16.85 min

Table S26**.** Molar residue ellipticities at 220 nm in 10 mM phosphate buffer (pH 7.4, 298 K) and percentage helicity for peptides **16**.

	Peptide	[θ]220	Helicity (%)
1	Ac-[KAFAS*]-NH_2	-4902.7	29

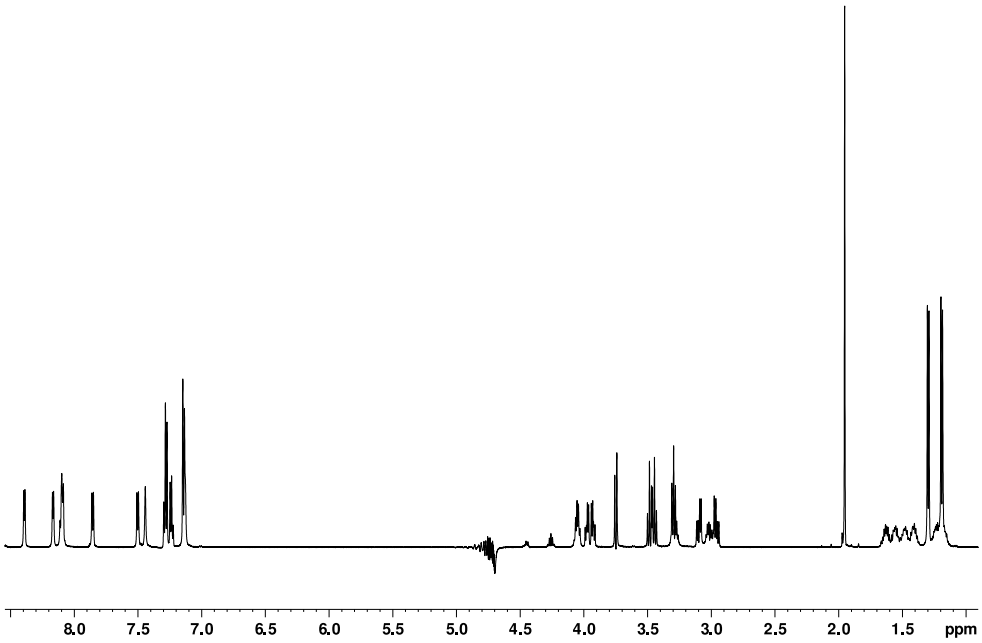


Table S27**.** ^{1}H NMR resonance assignments and chemical shifts (δ ppm) for **16** in H_2O/D_2O (9:1) at 298K.

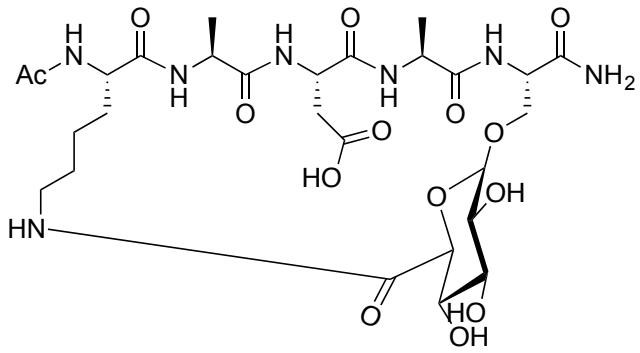
Residue	NH	H α	H β	Other
Ac-K1	8.17 side NH 8.10	4.05	βa 1.48, βb 1.41;	OAc: 1.95; Y 1a 1.22, Y 1b 1.17; δa 1.62, δb 1.55; εa 3.29, εb 3.01;

A2	8.39	4.05	1.19	
F3	7.50	4.47	βa 3.10, βb 2.96	7.14 (*O* 2H) ; 7.28 (*m* 2H); 7.24 (*p* 2H);
A4	8.09	4.25	1.30	
S5-NH_2	7.85	4.44	βa 3.98, βb 3.93;	T1 7.44; T2 7.13;
H1 4.45	H2 3.30	H3 3.45	H4 3.49	H5 3.75

Table S28**.** Amide NH coupling constants for **16**

Residue	K1	A2	F3	A4	S5	NT1	NT2	Side NH
^3JNH--CHα(Hz)	5.40	5.40	7.08	N/A	7.08	N/A	N/A	N/A

Peptide 17



HRMS (ESI): Calcd for $C_{27}H_{43}N_7O_{14}$ [M + H^+]: 690.2941, Found: 690.2939; Linear gradient from 0% to 40% B over 20 min, Rt =10.60 min

Table S29**.** Molar residue ellipticities at 220 nm in 10 mM phosphate buffer (pH 7.4, 298 K) and percentage helicity for peptides **17**.

	Peptide	[θ]220	Helicity (%)
1	Ac-[KADAS*]-NH_2	-8862.64	49

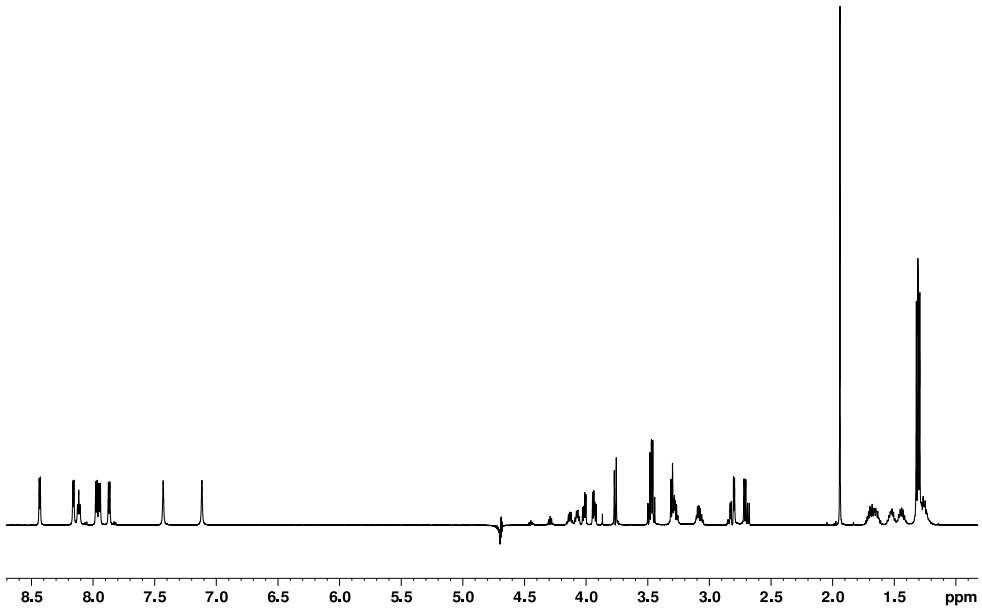


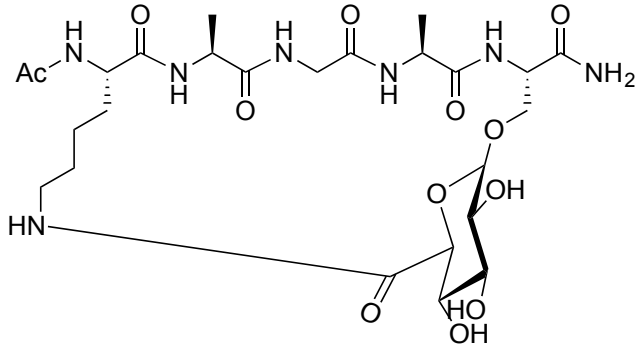
Table S30. ^{1}H NMR resonance assignments and chemical shifts (δ ppm) for **17** in H_2O/D_2O (9:1) at 298K.

Residue	NH	H α	H β	Other
Ac-K1	8.16 side NH 8.12	4.07	1.66 2H	OAc: 1.94; 1.25 (Y 2H); δa 1.52, δb 1.44; εa 3.28, εb 3.08;
A2	8.43	4.13	1.31	
D3	7.97	4.47	βa 2.81, βb 2.70	
A4	7.95	4.29	1.30	
S5-NH_2	7.87	4.43	βa 4.02, βb 3.93;	T1 7.43; T2 7.12;
H1 4.45	H2 3.29	H3 3.46	H4 3.48	H5 3.76

Table S31. Amide NH coupling constants for **17**

Residue	K1	A2	D3	A4	S5	NT1	NT2	Side NH
^{3}JNH--CHα(Hz)	5.34	5.40	6.78	6.90	7.14	N/A	N/A	N/A

Peptide 18



HRMS (ESI): Calcd for $C_{25}H_{42}N_7O_{12}$ [M + H^+]: 632.2886, Found: 632.2891; Linear gradient from 0% to 40% B over 20 min, Rt =9.71 min

Table S32**.** Molar residue ellipticities at 220 nm in 10 mM phosphate buffer (pH 7.4, 298 K) and percentage helicity for peptides **18**.

	Peptide	[θ]220	Helicity (%)
1	Ac-[KAGAS*]-NH_2	-2699.1	18

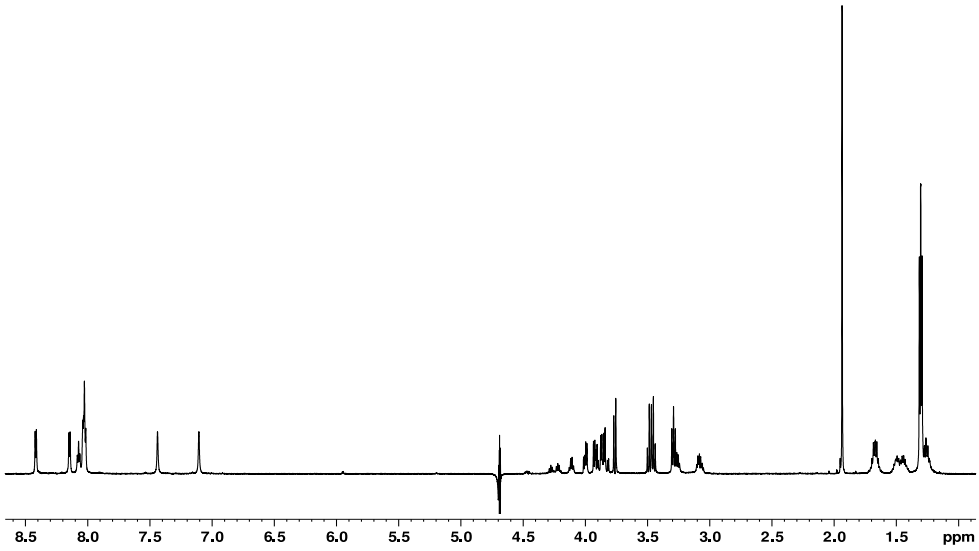


Table S33**.** ^{1}H NMR resonance assignments and chemical shifts (δ ppm) for **18** in H_2O/D_2O (9:1) at 298K.

Residue	NH	H α	H β	Other
Ac-K1	8.15 side NH 8.07	4.11	1.67 2H	OAc: 1.93; 1.26 (Y 2H); δa 1.50, δb 1.45; εa 3.27, εb 3.08;
A2	8.42	4.22	1.31	
G3	8.03	αa 3.88, αb 3.83		

A4	8.02	4.28	1.30	
S5-NH_2	8.03	4.47	βa 4.00, βb 3.92;	T1 7.44; T2 7.11;
H1 4.46	H2 3.29	H3 3.45	H4 3.49	H5 3.76

Table 34. Amide NH coupling constants for **18**

Residue	K1	A2	G3	A4	S5	NT1	NT2	Side NH
^{3}JNH--CHα(Hz)	5.85	5.99	N/A	N/A	N/A	N/A	N/A	N/A

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