Supplementary Information

Insights into the secondary structures of lactam *N*-substituted stapled peptides

Aldrin V. Vasco,[a] Celia González,[b] Stefan Gröger,[c] Wolfgang Brandt,[a] Jochen Balbach,[c] Carlos S. Pérez,[b] Ludger A. Wessjohann,[a,*] and Daniel G. Rivera,[a,b,*]

[a]Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120, Halle/Saale, Germany.

[b]Center for Natural Products Research, Faculty of Chemistry, University of Havana, Zapata y G, 10400, La Habana, Cuba.

[c]Institute of Physics/Biophysics and Center for Structural and Dynamics of Proteins, Martin Luther University Halle-Wittenberg, D-06120, Germany.

Corresponding Authors: *Emails: dgr@fq.uh.cu; wessjohann@ipb-halle.de

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Abbreviations

Alloc, allyloxycarbonyl; CD, circular dichroism; DCM, dichloromethane; DIC, diisopropylcarbodiimide; DIPEA, diisopropylethylamine; DMF, dimethylformamide; ESI-MS, electrospray ionization mass spectrometry; FA, formic acid; Fmoc, 9-fluorenylmethyloxycarbonyl; HOBt, 1-hydroxybenzotriazol, HR-MS, high resolution mass spectrometry; IR, infrared; MeOH, methanol; NMR, nuclear magnetic resonance; PyAOP (7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; RP-HPLC, reserved-phase high performance liquid chromatography; RT, room temperature; SPPS, solid phase peptide synthesis; tBu, tert-butyl; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; TG-S-RAM, Tentagel resin bounded Rink amide; THF, tetrahydrofuran; TIS, triisopropylsilane; TLC, thin layer chromatography; TMS, tetramethyl silane; Trt, triphenylmethyl.

S1. General

All starting materials were purchased from commercial sources and used without further purification.

S1.1. Spectroscopic characterization

NMR spectra were recorded at 298 K either on a Varian Mercury 400 NMR spectrometer at 399.94 MHz and 100.57 MHz for ^{1}H and ^{13}C, on an Agilent (Varian) VNMRS 600 NMR spectrometer at 599.83 MHz and 150.83 MHz for ^{1}H and ^{13}C, or in a Bruker Avance III spectrometer at 600.60 MHz and 151.02 MHz for ^{1}H and ^{13}C, respectively. Chemical shifts (δ) for intermediates characterized in common organics solvents are reported in ppm relative to the TMS (^{1}H NMR) and to the solvent signal (^{13}C NMR). For water-recorded NMR spectra, the chemical shifts are reported relative to TSP-d4 and ^{13}C chemical shifts were inferred from ^{1}H-1D spectra

according to IUPAC recommendations.[1] For cyclic pentapeptides, the *s-cis*/*s-trans* ratio was determined by integration of key signals in the unidimensional ^{1}H-PRESAT spectrum. The results of the 1D integration did not greatly differ (i.e. no more than 8 %) from those obtained when averaging the integration of the crosspeaks from the HSQC or NOESY spectra. Therefore, for cyclic dodecapeptides where 1D integration was not possible, HSQC integration[2] of the crosspeaks belonging to the exocyclic N^{α} methylene group was employed for estimating the *s-cis*/*s-trans* ratio and the results were averaged with those obtained by integration of the same signals in the NOESY spectrum. It should be point out that the integration in the NOESY spectrum relies on the assumption that the mobility of this portion of the molecule does not significantly changes from one to another conformer, not necessarily met, but apparently not greatly influencing the results. Nevertheless, in the case of experiments conducted in TFE/water, only estimation of the conformational ratio by NOESY integration could be achieved due to high noise from the solvent signal in the HSQC spectrum. It should be noted that, in any case, a quantitative determination of the conformational ratio was attempted, and these results should be interpreted as an estimation of the isomer population to infer which conformer is more populated in any case. Circular dichroism spectra were recorded on a Jasco J-815 spectropolarimeter equipped with a temperature controller at 20ºC. A total of 16 accumulations from 260 to185 nm at 50 nm/sec using 1 mm cuvettes were done.

S1.2. Chromatographic methods

Analytical and preparative RP-HPLC of crude peptides was carried out with an Agilent 1260 Infinity series system equipped with two preparative pumps and one analytical quaternary pump, coupled to a MWD detector and an Agilent 6120 Quadrupole LC/MS detector using API-ES as ion source. Reverse phase YMC-ODS-A column (150 × 4.6 mm I.D., 5 µm particle size) and YMC-ODS-A (150 × 20 mm I.D., 5 µm particle size) columns were used for analytical and preparative scale respectively. Analyses of pure peptides were performed on a Waters Acquity UHPLC BEH C18 column (1.7 µm, 2.1 mm × 50 mm) using a Waters Acquity UHPLC system coupled to an LCQ Deca XP MAX (Thermo Scientific) mass spectrometer. The ESI IT mass spectra were recorded with a 4.0 kV spray voltage; sheath gas nitrogen; capillary temperature, 275 °C; capillary voltage, 30 V. The column was maintained at 40 °C. Unless otherwise stated, a linear gradient from 5% to 90% of solvent B (0.1% (v/v) formic acid (FA) in acetonitrile) in solvent A (0.1% (v/v) formic acid (FA) in water) over 10 min at a flow rate of 0.15 mL min^{-1} was used. The mass spectra were evaluated by the Thermo software Xcalibur 2.0.7. Mass Spectrometry Characterization.

[1] Harris RK, Becker ED, Cabral De Menezes SM, Goodfellow R, Granger P. NMR nomenclature: Nuclear spin properties and conventions for chemical shifts (IUPAC recommendations 2001). *Concepts Magn Reson Part A Bridg Educ Res*. 2002;14(5):326-346. doi:10.1002/cmr.10035.

[2] Fardus-Reid F, Warren J, Le Gresley A. Validating heteronuclear 2D quantitative NMR. *Anal Methods*. 2016;8(9):2013-2019. doi:10.1039/c6ay00111d

S1.3. ESI-HRMS

A TripleToF 6600-1 mass spectrometer (Sciex) was used for high-resolution mass spectrometry, which was equipped with an ESI-DuoSpray-Ion-Source (it operated in positive ion mode) and was controlled by Analyst 1.7.1 TF software (Sciex). The ESI source operation parameters were as follows: ion spray voltage: 5,500 V, nebulizing gas: 60 p.s.i., source temperature: 450 °C, drying gas: 70 p.s.i., curtain gas: 35 p.s.i. Data acquisition was performed in the MS1-ToF mode, scanned from 100 to 1500 Da with an accumulation time of 50 ms.

S2. Experimental Methods

S2.1. General methods for solid-phase peptide synthesis and macrocyclization

Solid Phase Peptide Synthesis: Coupling reactions were carried out automatically on an INTAVIS ResPepSL automated peptide synthesizer by a stepwise Fmoc/tBu strategy using a 5-fold excess of amino acid and PyBop and a 10-fold excess of NMM at R.T. for 15 min. A 50 µmol scale on TG-S-RAM (Iris Biotech) resin (217 mg, 0.23 mmol/mg) was utilized.

Swelling: The resin is swelled for 20 min in dichloromethane before bring it into the synthesizer. After the coupling of all amino acids, the resin is manually washed with DCM (3×1 min) and DMF (2×1 min) and reacted with 10 eq. of Ac_2O/DIEA in 2 mL of DMF for 30 min. The completeness of the reaction is confirmed through the Kaiser test and the resin is manually washed with DCM (3×1 min).

Alloc/Allyl Removal: The resin is washed with dry dichloromethane (2×2 min) under a stream of nitrogen. A solution of phenylsilane (20 eq.) in dry dichloromethane and tetrakis(triphenylphosphine) palladium(0) (0.2 eq.) are added to the resin under a continuous stream of nitrogen. The mixture is stirred in the dark for 10 min, and the procedure is repeated once more. Finally, the resin is washed with 0.5% of sodium diethyldithiocarbamate trihydrate in DMF (5×2 min) and DCM (2×2 min).

Aminocatalysis-mediated Ugi-4C cyclization: The side chain deprotected resin-bound peptide is washed with THF (4×1 min) and treated with a suspension of paraformaldehyde (4 eq.) and pyrrolidine (4 eq.) in THF/MeOH (1:1) for 30 min. The excess of reagents is removed by washing the beads with THF (4×1min). The resin is then washed with DCM (4×1min) and DCM/TFE 1:1 solution (2×1min). A solution of the isocyanide (4 eq.) in 2 mL DCM/TFE 1:1 (or THF:MeOH 1:1 for relative polar isocyanides) is added to the resin and the suspension is stirred overnight (18hrs). Completion is evaluated by ESI-MS or RP-HPLC monitoring after mini-cleavages. Afterwards, the resin is washed with DCM (3×1 min) and DMF (2×1 min). Finally, the resin is washed with DCM (5×2 min) and Et_2O (3×1 min).

Minicleavage: A small amount of the dry resin (less than 10 mg) is transferred to an Eppendorf vial and 400 µL of the cleavage cocktail (TFA/TIS/H_2O 95:2.5:2.5) is added. The suspension is gently agitated during 45 min and afterwards the cleavage cocktail is concentrated under N_2 flow and the remaining oil is precipitated by addition of 500 µL diethyl ether. The suspension is centrifuged for 5 min after what the solid and liquid phases are separate by settling. The precipitated peptide is dissolved in a mixture AcN/H_2O in order to be analyzed by HPLC.

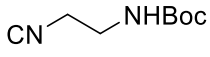
Cleavage: The resin is treated with the cocktail TFA/TIS/H_2O (95:2.5:2.5). The peptide is precipitated from frozen diethyl ether, then taken up in 1:2 AcN/H_2O and lyophilized.

S2.2. Synthesis of isocyanides

General protocol for the synthesis of isocyanides from amines

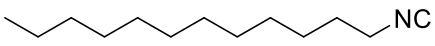
A solution of the amine (25 mmol) in ethyl formate (20 mL) is heated at reflux for 12 h to afford the formamide. The resulting solution is concentrated under vacuum and used without further purification. The formamide is then dissolved in 40 mL of dry THF, mixed with Et_3N (4 equiv.) and the system is filled with a N_2 atmosphere. The reaction is cooled to 0 °C and a solution of $POCl_3$ (1.3 equiv.) in 15 mL of THF is added dropwise during 30 minutes. The resulting mixture is stirred at 0 °C for 1 h, then allowed to reach room temperature and stirred for additional 2 h. The reaction mixture is quenched with 50 mL of 10 % aqueous $NaHCO_3$ and extracted with EtOAc (2×50 mL). The combined organic phases are combined, washed with brine (20 mL), dried over anh. Na_2SO_4, and concentrated under reduced pressure to dryness. The crude product is purified by column chromatography.

(*N*-tert-Butyloxycarbonyl)-aminoethyl isocyanide



The compound was synthesized according to the published protocol in Ref. 3 with a total yield of 67%.

n-dodecylisocyanide



The compound was synthesized according to the published protocol in Ref. 4.

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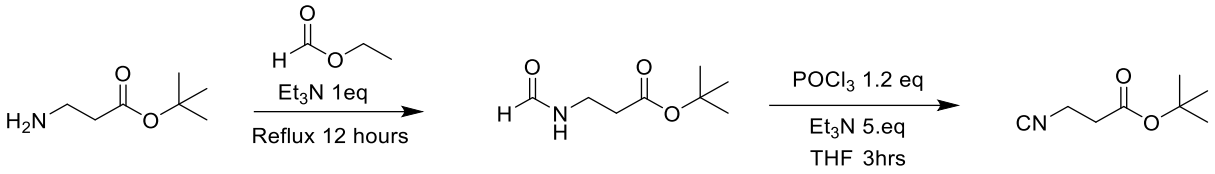
(3) Szymański, W.; Velema, W. A.; Feringa, B. L. *Angew. Chemie Int. Ed.* **2014**, *53* (33), 8682–8686.

(4) Pérez-Labrada, K.; Brouard, I.; Méndez, I.; Rivera, D. G. *J. Org. Chem.* **2012**, *77* , 4660–4670.

***t*-Butyl 3-isocyanopropanoate**



A solution of β-alanine t-butyl ester hydrochloride (27.5 mmol, 5.00 g) in ethyl formate (20 mL) was heated at reflux for 3 hours. The solvent was evaporated, yielding the formamide product as a clear colourless oil, which was not further purified. This latter product (4.8 g) and triethylamine (137.6 mmol, 19.2 mL) were dissolved in freshly destilled THF (20 mL) and the solution was cooled to -60 □C. Phosphorus oxychloride (33.0 mmol, 3.08 mL) was added dropwise, keeping the temperature below -55 □C. The cooling was removed and the reaction mixture was allowed to warm up to room temperature and stirred for additional 2 h. The reaction mixture was then poured into ice-cooled sat. aq. NaHCO3 (40 mL) and the organic phase was separated. The aqueous phase was washed with chloroform (2 × 25 mL). The collected organic phases were dried (MgSO4) and the solvent was evaporated. The product was purified by flash chromatography (n-hexane/AcOEt, 8:1, v/v; Rf = 0.68). Yield (3.8 g) 86.9%; yellow oil. 1H NMR (400 MHz, CDCl3) δ = 3.57 (tt, J = 6.8, 1.9 Hz, 2H), 2.56 (tt, J = 6.8, 2.1 Hz, 2H), 1.41 (d, s, 9H). 13C NMR (101 MHz, CDCl3) δ 167.59 (CO), 156.16 (t, J = 5.4 Hz), 80.86 (C(CH3)3), 36.40 (t, J = 7.1 Hz, CH2), 34.24(CH2), 27.00(C(CH3)3).

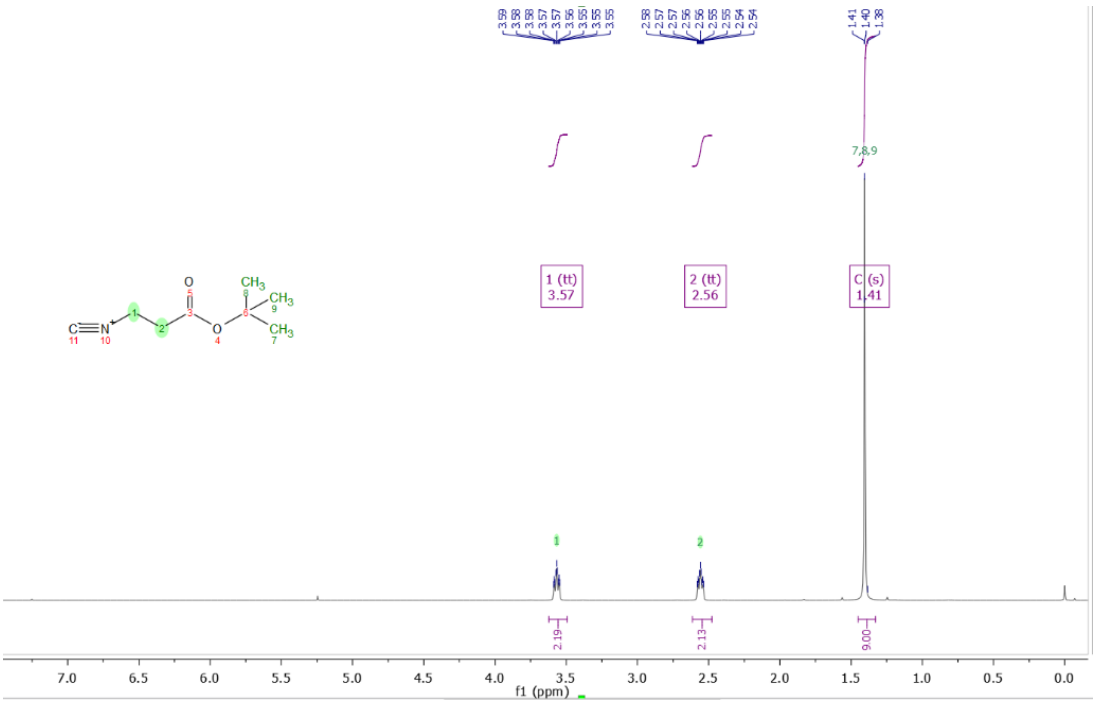


Figure S1. [1]H-NMR spectrum of *tert*-butyl 3-isocyanopropanoate

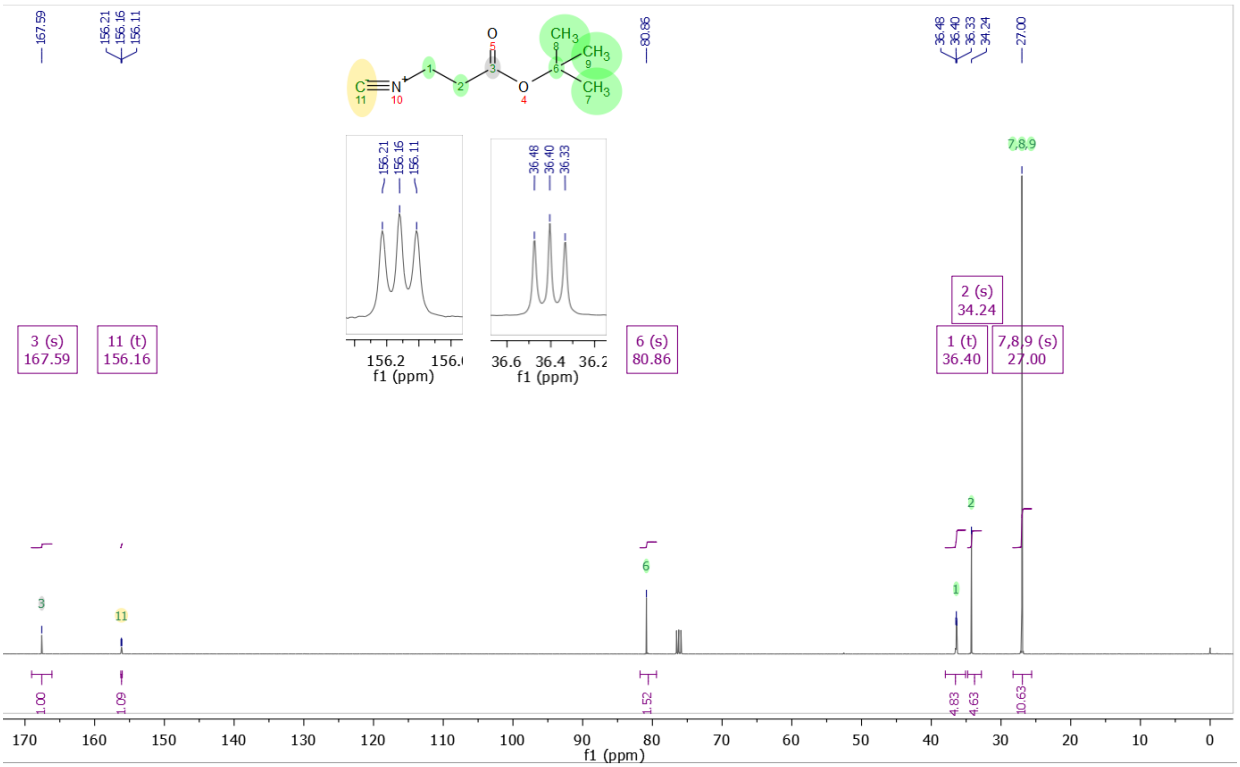
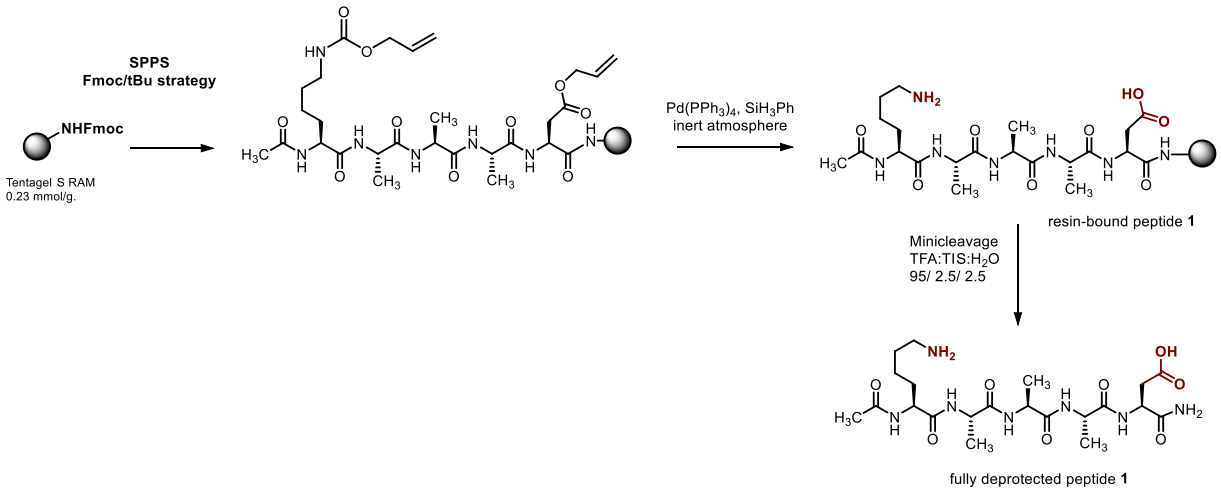


Figure S2. ^{13}C-NMR spectrum of *tert*-butyl 3-isocyanopropanoate

S2.3. Synthesis of peptides

Ac-Lys-Ala-Ala-Ala-Asp-NH$_2$ (1)



Resin-bound peptide **1** was synthesized in 50 µmol scale on TentaGel S RAM resin (217 mg, 0.23 mmol/mg) according to the protocols described in section S2.1. The purity of the resin-bound peptide was evaluated through minicleavages according to the protocol described in S2.1 (R_t 1.09 min), affording the fully deprotected peptide **1**, which was characterized by analytical UHPLC (R_t 1.07 min, > 95% purity (crude). ESI-HRMS, calcd for $C_{21}H_{38}N_7O_8$: 516.2782 $[M+H]^+$; Found: *m/z* 516.2774 $[M + H]^+$.

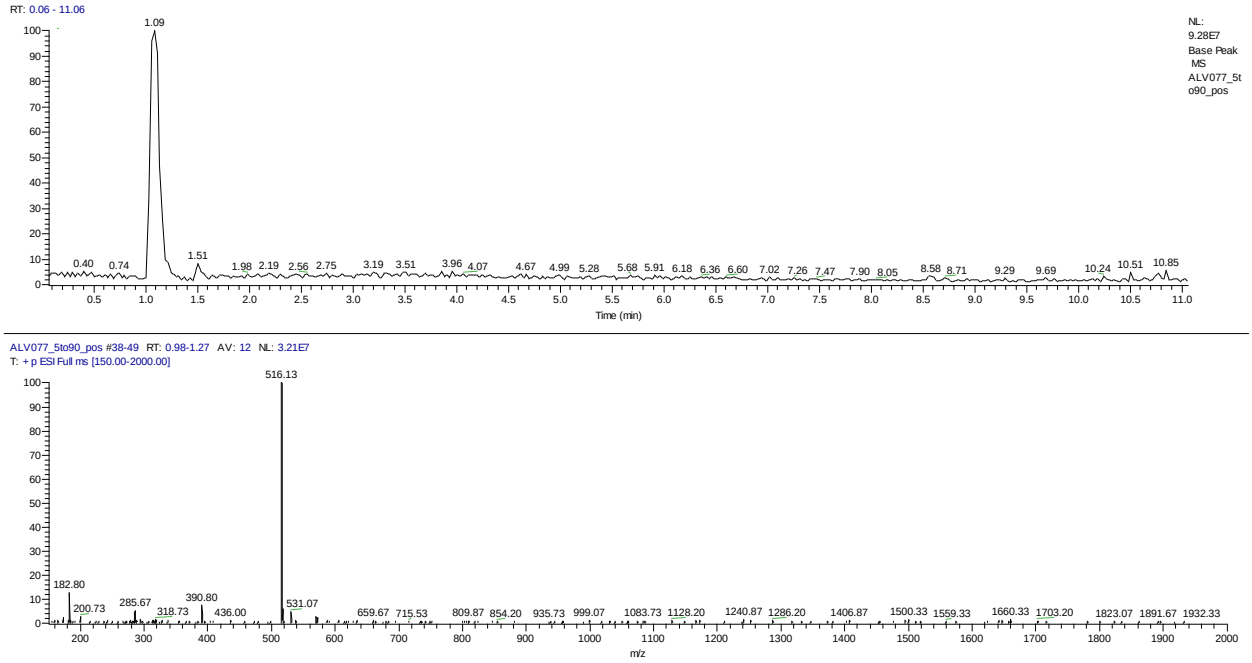
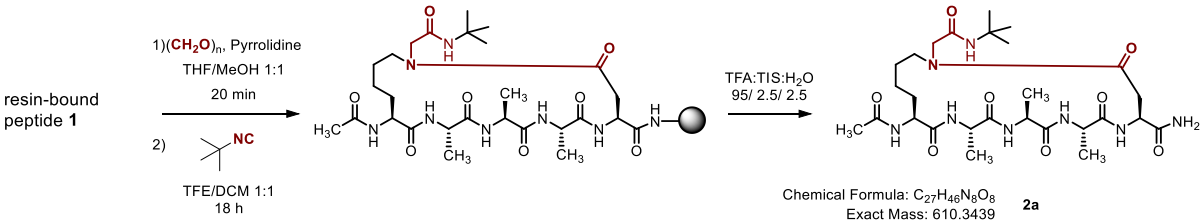


Figure S3. UHPLC-MS chromatogram of fully deprotected peptide **1**

Ac-(*N*-(2-(*tert*-butylamino)-2-oxoethyl)-(cyclo-1,5))-[KAAAD]-NH_2 (2a)



Cyclic peptide **2a** was produced by on-resin cyclization in a 50 µmol scale from resin-bound peptide **1** according to the aminocatalysis-mediated Ugi macrocyclization protocol described in S2.1, in the presence of *tert*-butylisocyanide. Cleavage with TFA/H_2O/TIS 95:2.5:2.5 for 2 h afforded peptide **2a** (88% cleavage yield), which was purified by semi-preparative RP-HPLC (17.1 mg, 56% isolated yield); R_t 4.55 min, > 95% purity. ESI-HRMS, calcd. for $C_{27}H_{47}N_8O_8$: 611.3517 $[M + H]^+$ Found: *m/z* 611.3502 $[M + H]^+$.

Table S1. Full assignment of the NMR resonances for cyclic peptide **2a**

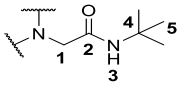
Major conformer(*s-cis*) 63%

Residue	HN(^3J)	HA/CA	HB/CB	CO	others
Ace	-	1.99/21.57	-	176.8	-
Lys[1]	8.19(5.9)	4.19/56.4	1.68, 1.78/ 33.7	177.5	1.49/ 24.9 γ; 1.60, 1.71/ 31.1 δ; 3.28, 3.60/ 52.8ε
Ala[2]	8.64 (4.6)	4.29/52.6	1.47/ 19.2	178.7	-
Ala[3]	8.50 (3.5)	4.14/54.5	1.45/ 18.4	179.3	-
Ala[4]	8.10 (5.0)	4.25/53.1	1.40/ 18.3	174.7	-
Asp[5]	7.84 (7.2)	4.60/53.8	2.79, 3.32/ 36.8	179.0	174.7 γ; 7.30, 7.16 (*terminal NH2*)

Minor conformer(*s-trans)* 37 %

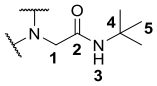
Residue	HN(^3J)	HA/CA	HB/CB	CO	others
Ace	-	2.07/24.4	-	177.6	-
Lys[1]	8.28 (3.6)	4.12/ 57.8	1.77, 1.80/ 31.8	n.d	1.13, 1.57/ 24.8 γ; 1.84, 1.36/ 28.1 δ; 2.69, 3.97/ 52.0 ε
Ala[2]	8.42 (3.7)	4.07/ 54.9	1.42/ 17.9	180.2	-
Ala[3]	7.82 (5.9)	4.18/ 53.7	1.41/ 18.6	179.5	-
Ala[4]	7.96 (5.3)	4.15/ 54.1	1.43/ 18.5	179.3	-
Asp[5]	8.14 (6.5)	4.59/ 53.7	2.78, 2.93/ 37.4	n.d	7.28, 7.04 (*terminal NH2*)

N -substitution



Atom group	δ_H/δ_C
1	3.95/ 52.8
2	/172.3
3	7.37/ -
4	/ 54.3
5	1.32/ 28.6

N -substitution



Atom group	δ_H/δ_C
1	3.89, 4.16/ 56.9
2	/172.1
3	7.65/ -
4	/ 54.6
5	1.32/ 30.5

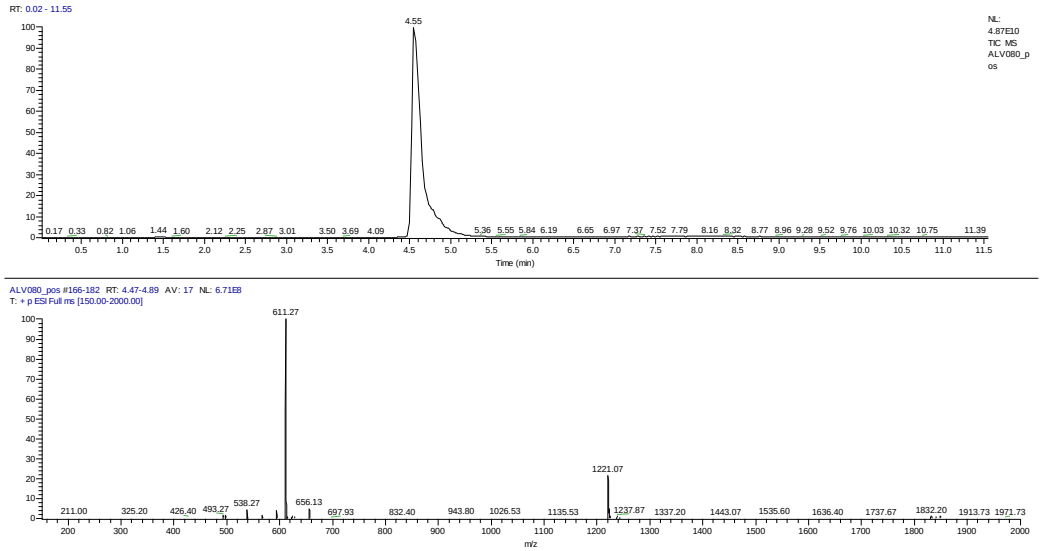


Figure S4. UHPLC-MS chromatogram of pure peptide **2a**

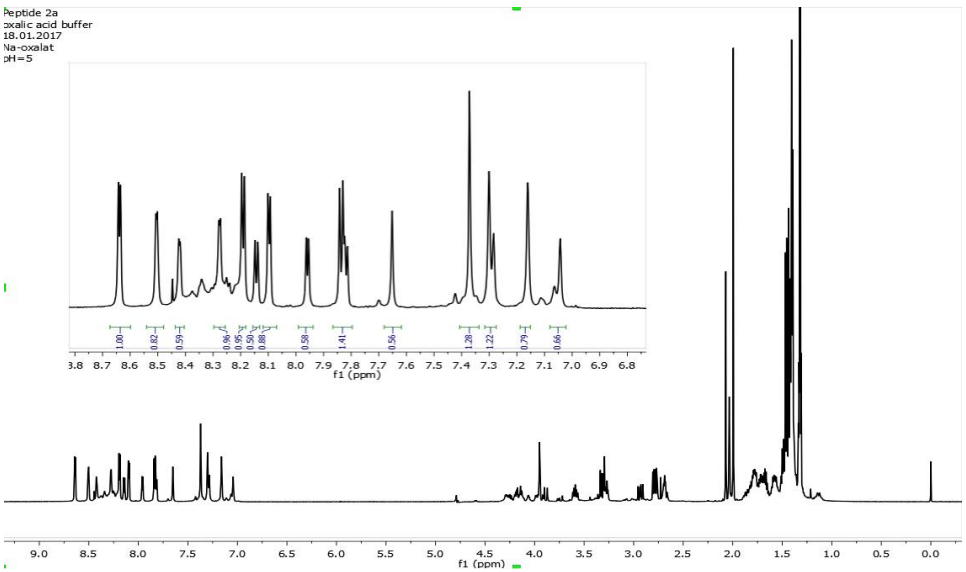


Figure S5. ^{1}H-NMR spectrum of peptide **2a** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1 obtained using the WATERGATE pulse sequence for water suppression.

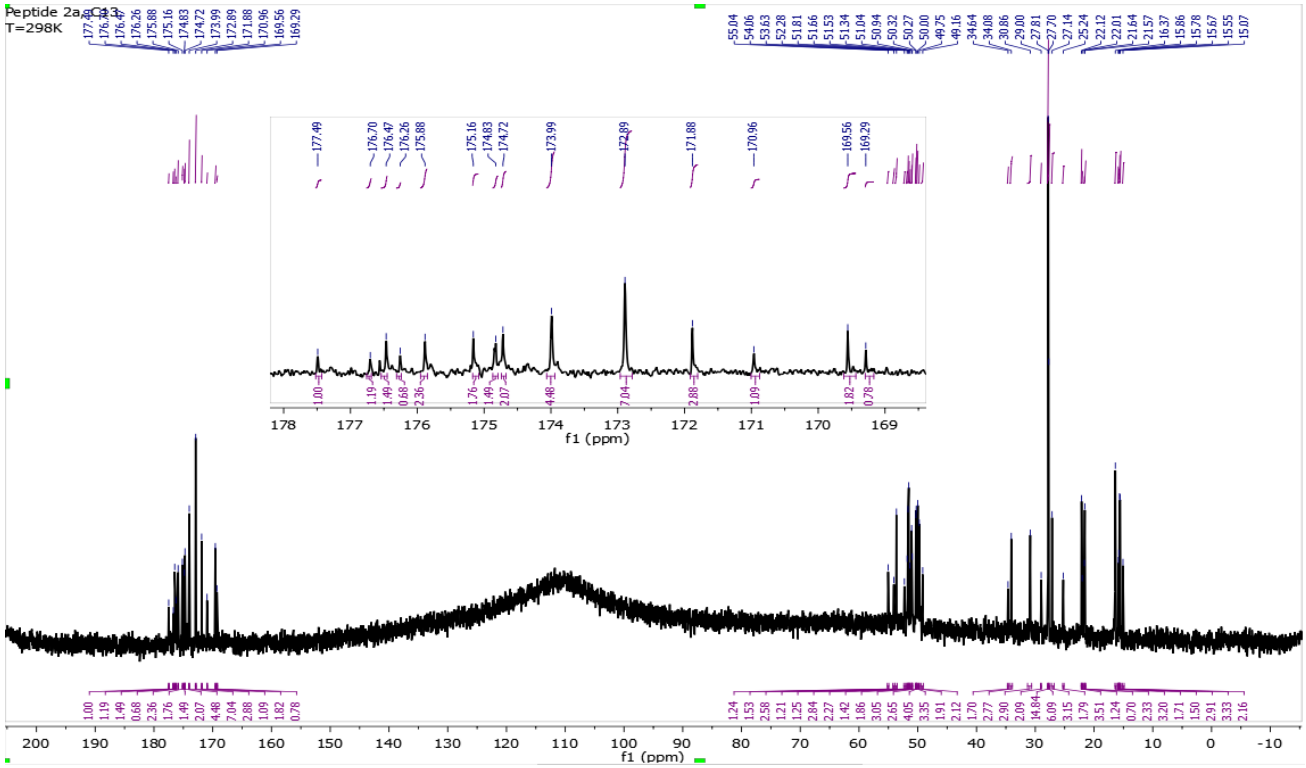


Figure S6. ^{13}C-NMR spectrum of peptide **2a** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1

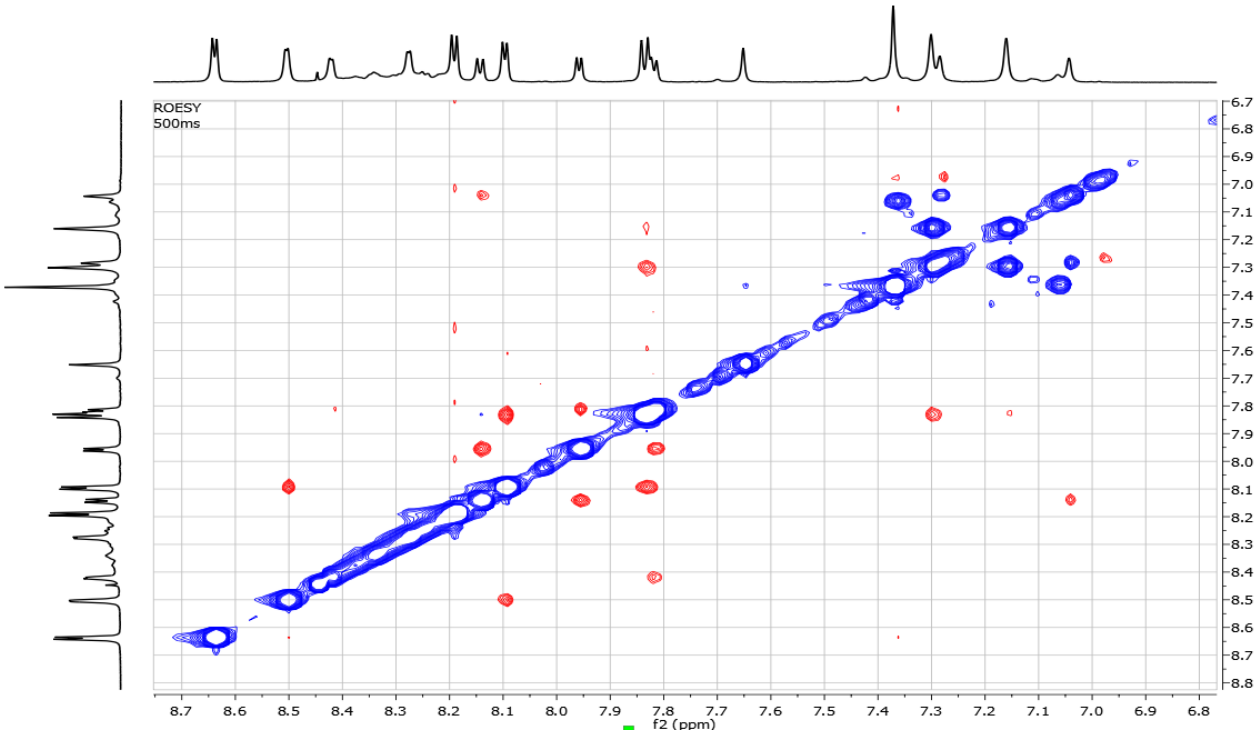


Figure S7. H^N-H^N section of the ROESY spectrum of peptide **2a** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1

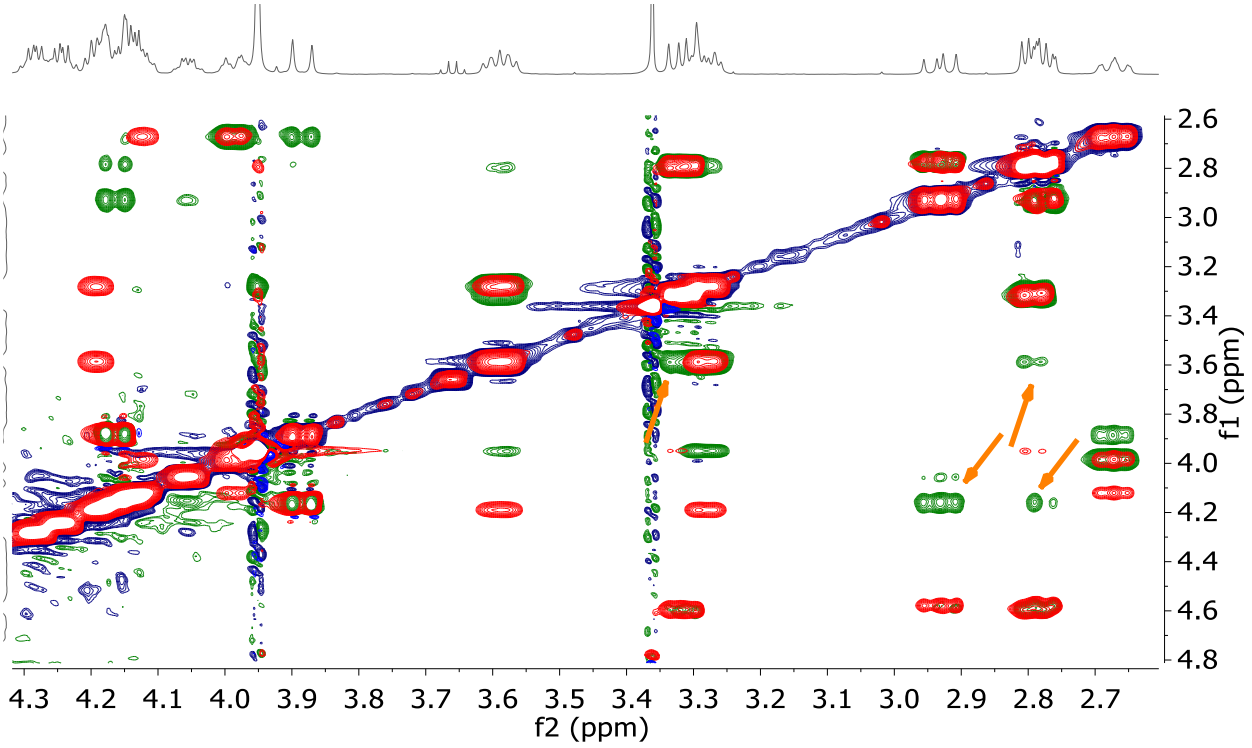


Figure S8. Superimposition of the zTOCSY(red) and ROESY(green-blue) spectra showing in orange arrows important cross peaks defining the *s- cis* and *s-trans* configuration around the exocyclic tertiary amide.

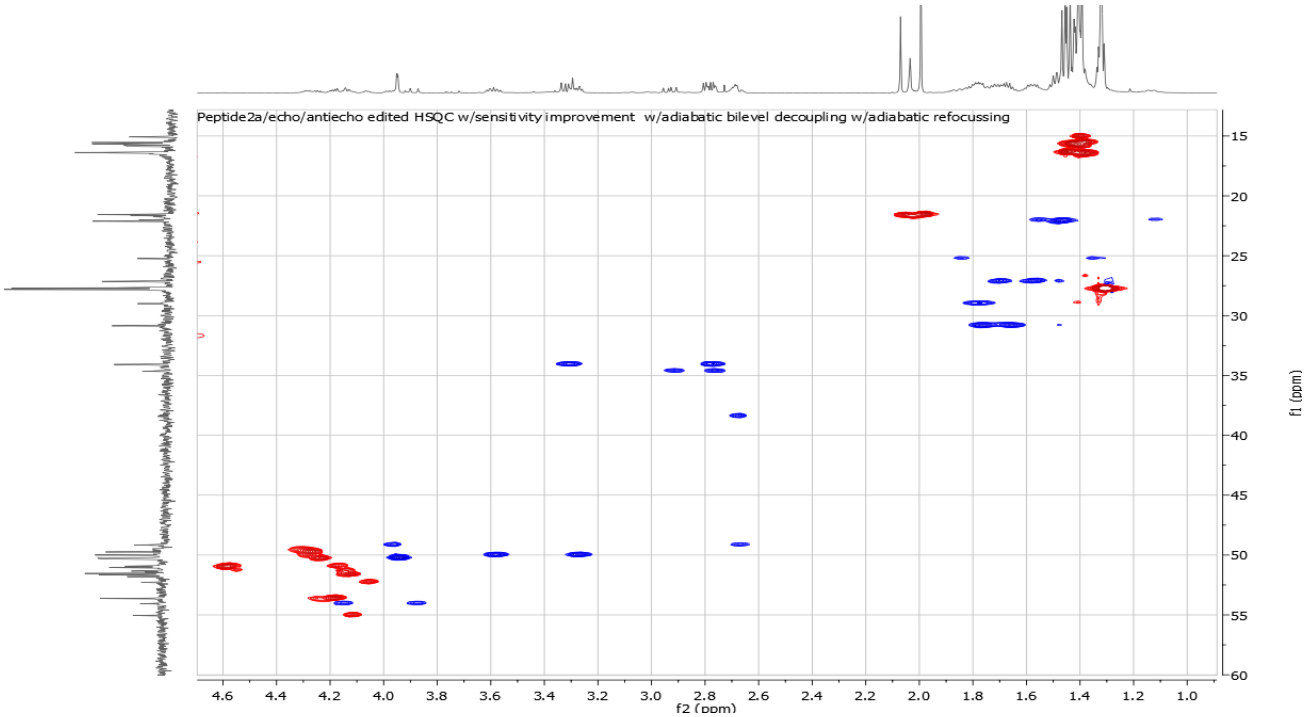


Figure S9. HSQC-edited spectrum of peptide **2a** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1.

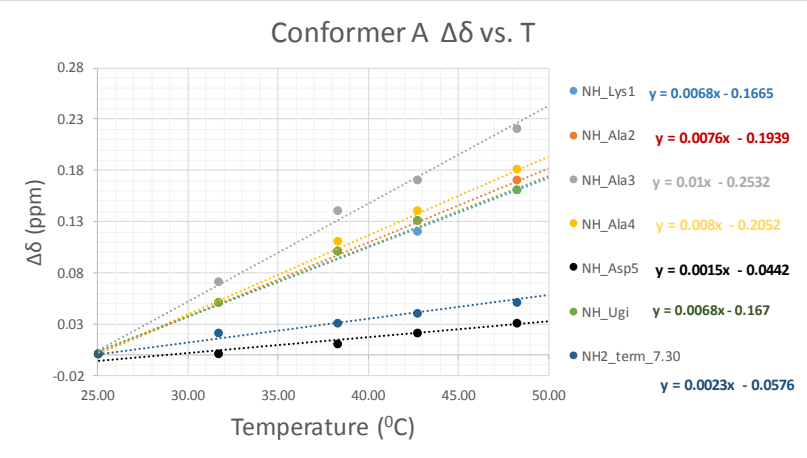


Figure S10. Temperature dependence of amide chemical shifts for the Conformer A of Peptide **2a**

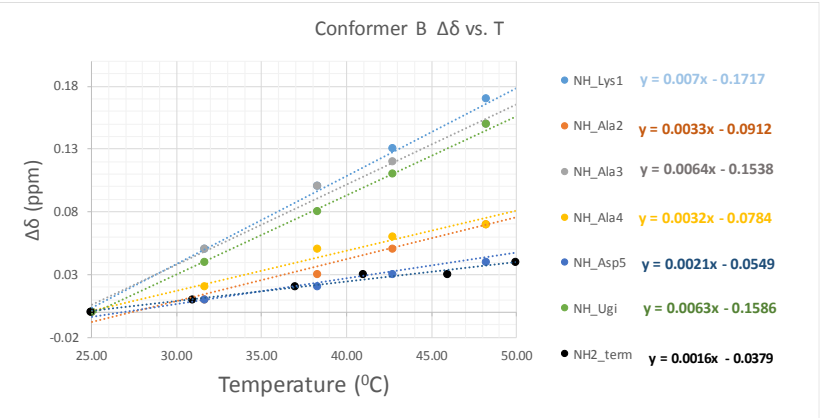
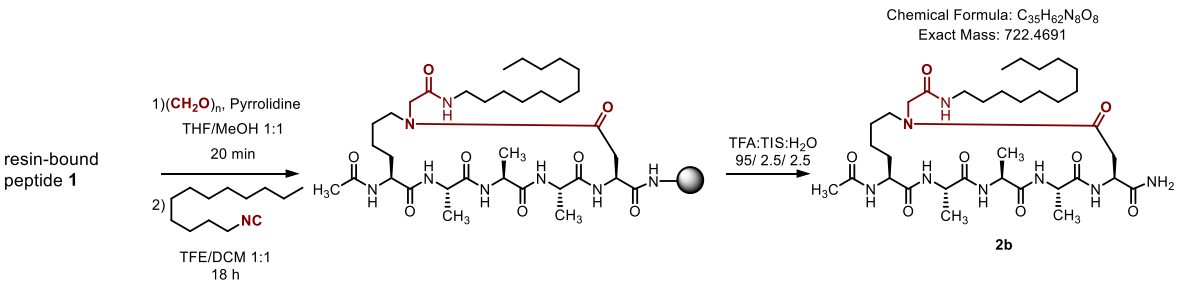


Figure S11. Temperature dependence of amide chemical shifts for the Conformer B of Peptide **2a**

Ac-(*N*-(2-(*n*-dodecylamino)-2-oxoethyl)-(cyclo-1,5))-[KAAAD]-NH_2 (2b)



Cyclic peptide **2b** was produced by on-resin cyclization in a 50 µmol scale from resin-bound peptide **1** according to the aminocatalysis-mediated Ugi macrocyclization protocol described in S2.1, in the presence of *n*-dodecylisocyanide. Cleavage with TFA/H_2O/TIS 95:2.5:2.5 for 2 h afforded peptide **2b** (78% cleavage yield), which was purified by semi-preparative RP-HPLC (12.4 mg, 34% isolated yield); R_t 9.03 min, > 95% purity. ESI-HRMS, calcd. for $C_{35}H_{63}N_8O_8$: 723.4769 $[M + H]^+$ Found: *m/z* 723.4744 $[M + H]^+$.

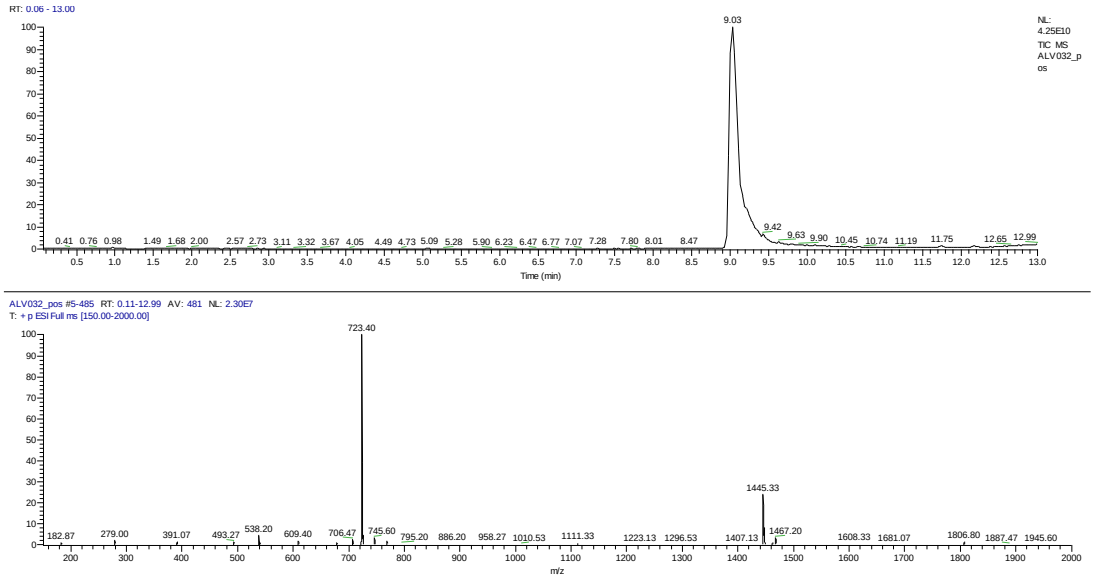


Figure S12. UHPLC-MS chromatogram of pure peptide **2b**

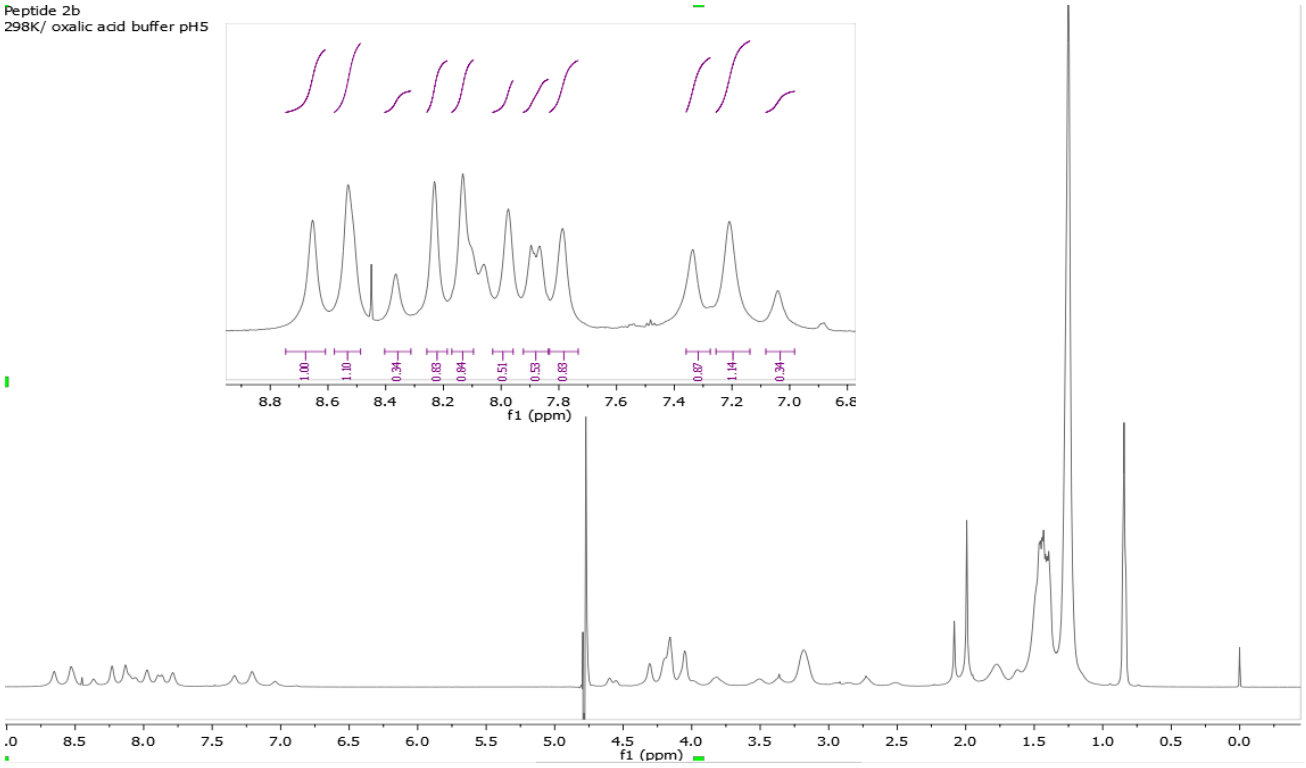
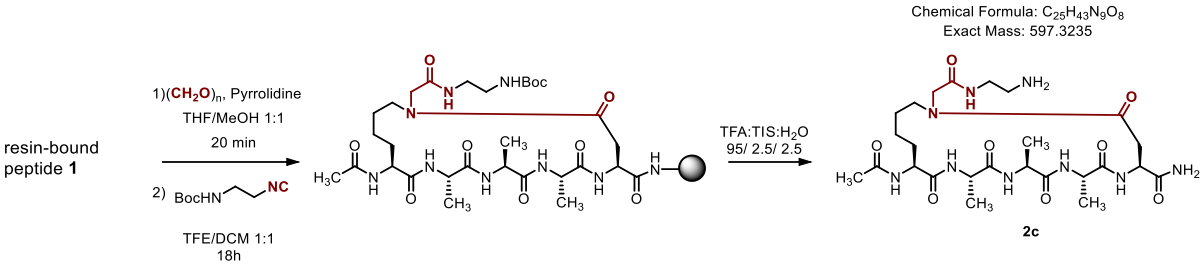


Figure S13. ^{1}H-NMR spectrum of peptide **2b** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1 obtained using the PRESAT pulse sequence for water suppression.

Ac-(*N*-((2-(aminoethyl)amino)-2-oxoethyl)-(cyclo-1,5))-[KAAAD]-NH_2 (2c)



Cyclic peptide **2c** was produced by on-resin cyclization in a 50 µmol scale from resin-bound peptide **1** according to the aminocatalysis-mediated Ugi macrocyclization protocol described in S2.1, in the presence of (*N*-Boc)-aminoethyl isocyanide. Cleavage with TFA/H_2O/TIS 95:2.5:2.5 for 2 h afforded peptide **2c** (78% cleavage yield), which was purified by semi-preparative RP-HPLC (11.7 mg, 39% isolated yield); R_t 1.92 min, > 95% purity. ESI-HRMS, calcd. for $C_{25}H_{44}N_9O_8$: 598.3313 $[M + H]^+$ Found: *m/z* 598.3299 $[M + H]^+$.

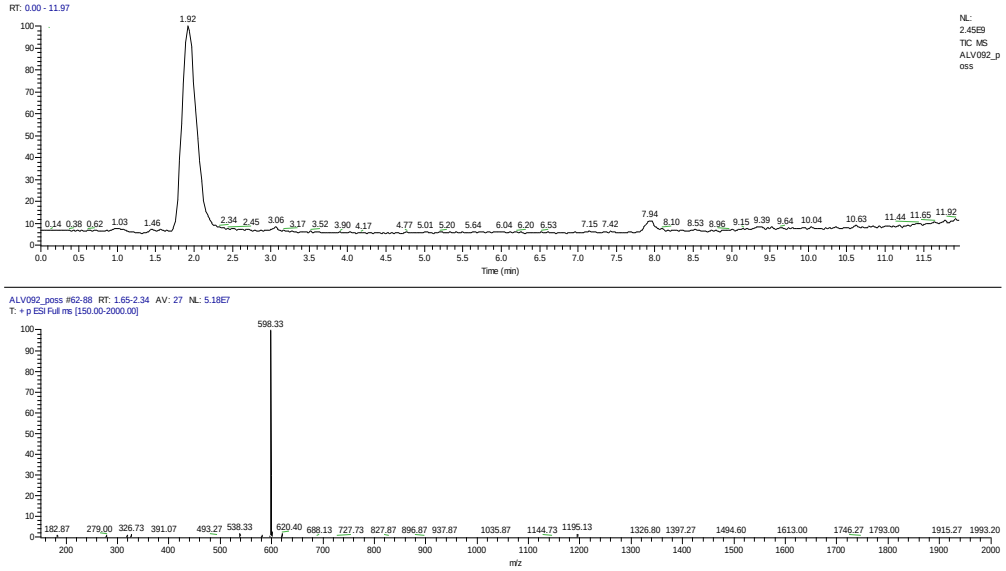


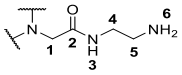
Figure S14. UHPLC-MS chromatogram of pure peptide **2c**

Table S2. Full assignment of the NMR resonances for cyclic peptide **2c**

Major conformer(*s-cis*) 78%

Residue	HN(^3J)	HA/CA	HB/CB	CO	others
Ace	-	2.00/24.4	-	176.7	-
Lys[1]	8.21 (5.7)	4.20/56.5	1.70-1.78/33.7	176.6	1.48/24.8 γ; 1.56/29.7 δ; ε 3.35/52.8
Ala[2]	8.61 (4.7)	4.30/52.5	1.48/19.2	178.8	-
Ala[3]	8.56 (n/d)	4.15/54.6	1.45/18.4	179.4	-
Ala[4]	8.17 (4.8)	4.25/53.1	1.41/18.3	177.5	-
Asp[5]	7.88 (7.3)	4.61/53.9	2.80-3.36/36.8	178.1	175.2 γ; 7.32, 7.29 (*terminal NH2*)

N-substitution

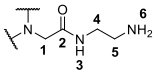


Atom group	δ_H/δ_C
1	4.18-3.92/52.5
2	/174.4
3	8.14
4	3.58-3.52/39.9
5	3.18-3.57/42.1
6	n.d

Minor conformer(*s-trans*) 22%

Residue	HN(^3J)	HA/CA	HB/CB	CO	others
Ace	-	2.08/24.5	-	177.7	-
Lys[1]	8.31 (n/d)	4.14/57.8	.79-1.84/31.'	(n/d)	1.36,1.90/27.8 γ; (n/d) δ; 2.72, 3.97/51.5 ε
Ala[2]	8.45 (n/d)	4.05/55.2	1.43/17.9	(n/d)	-
Ala[3]	7.87 (5.5)	4.17/53.9	1.47/(n/d)	179.6	-
Ala[4]	7.96 (5.3)	4.16/54.2	1.43/18.6	179.4	-
Asp[5]	8.12 (n/d)	4.59/53.6	.83-2.94/37.'	178.2	7.04, 7.29 (*terminal NH2*)

N-substitution



Atom group	δ_H/δ_C
1	4.34-4.01/56.1
2	174.40
3	(n/d)
4	(n/d)
5	(n/d)
6	(n/d)

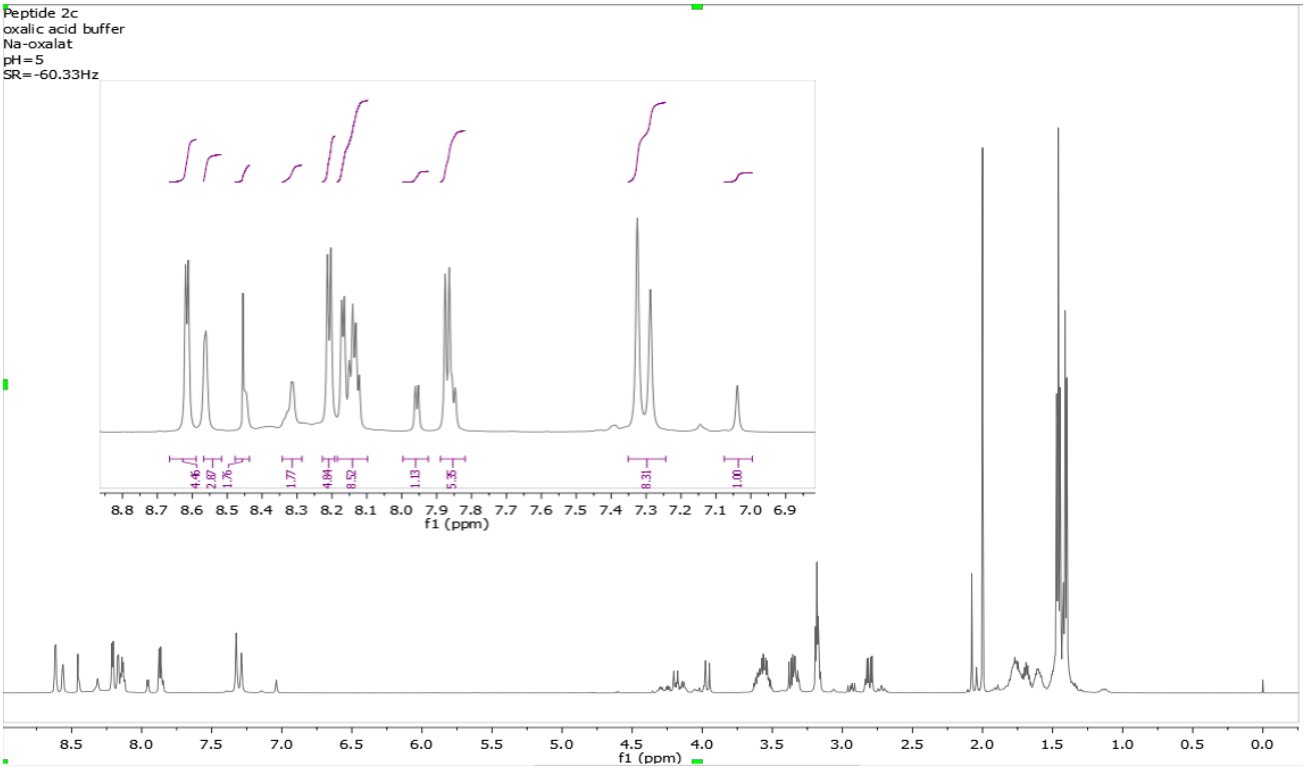


Figure S15. ^{1}H-NMR spectrum of peptide **2c** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1 obtained using the WATERGATE pulse sequence for water suppression.

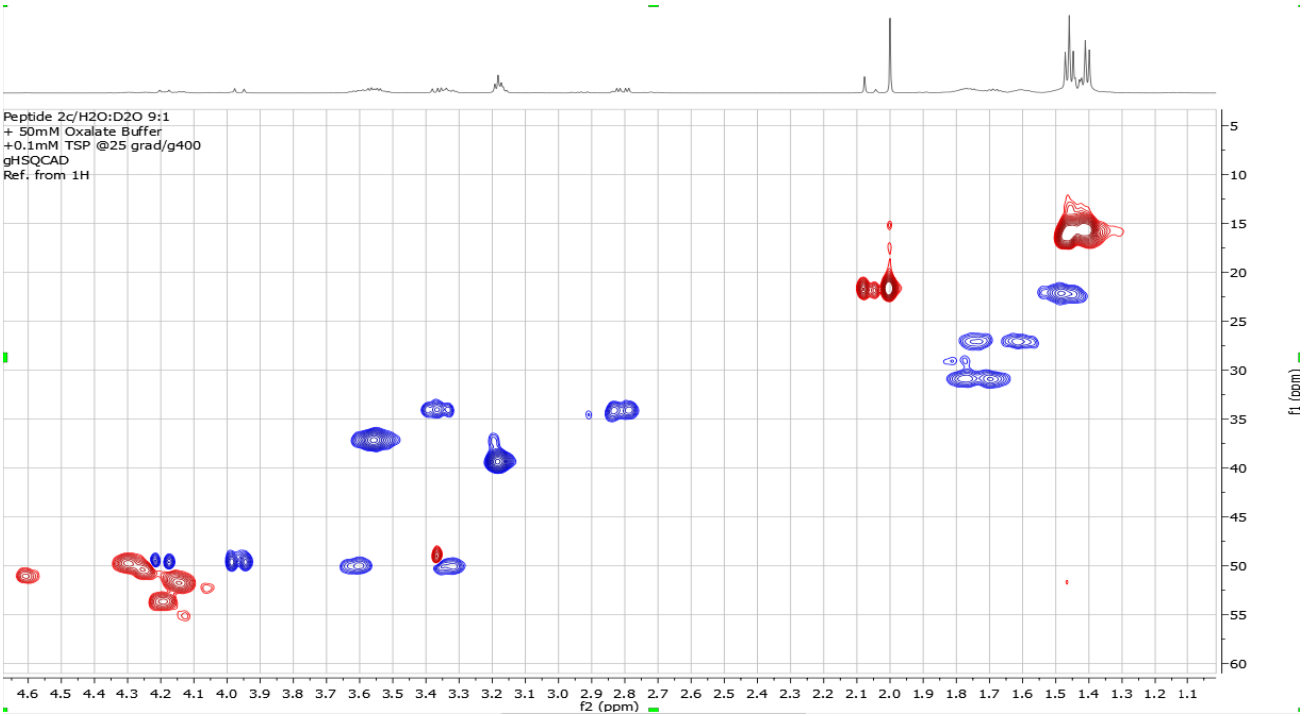
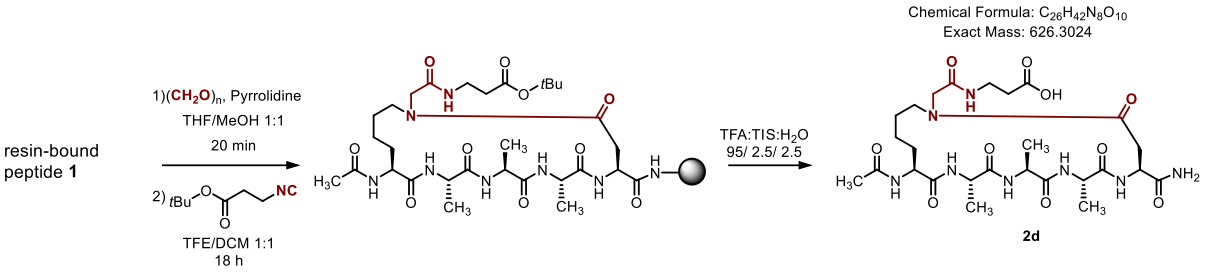


Figure S16. HSQC-edited spectrum of peptide **2c** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1.

Ac-(*N*-((2-(carboxyethyl)amino)-2-oxoethyl)-(cyclo-1,5))-[KAAAD]-NH_2 (2d)



Cyclic peptide **2d** was produced by on-resin cyclization in a 50 µmol scale from resin-bound peptide **1** according to the aminocatalysis-mediated Ugi macrocyclization protocol described in S2.1, in the presence of *t*ert-butyl-3-isocyanopropanoate. Cleavage with TFA/H_2O/TIS 95:2.5:2.5 for 2 h afforded peptide **2d** (83% cleavage yield), which was purified by semi-preparative RP-HPLC (9.8 mg, 31% isolated yield); R_t 3.28 min, > 93% purity. ESI-HRMS, calcd. for $C_{26}H_{43}N_8O_{10}$: 626.3102 $[M + H]^+$ Found: *m/z* 627.3091 $[M + H]^+$.

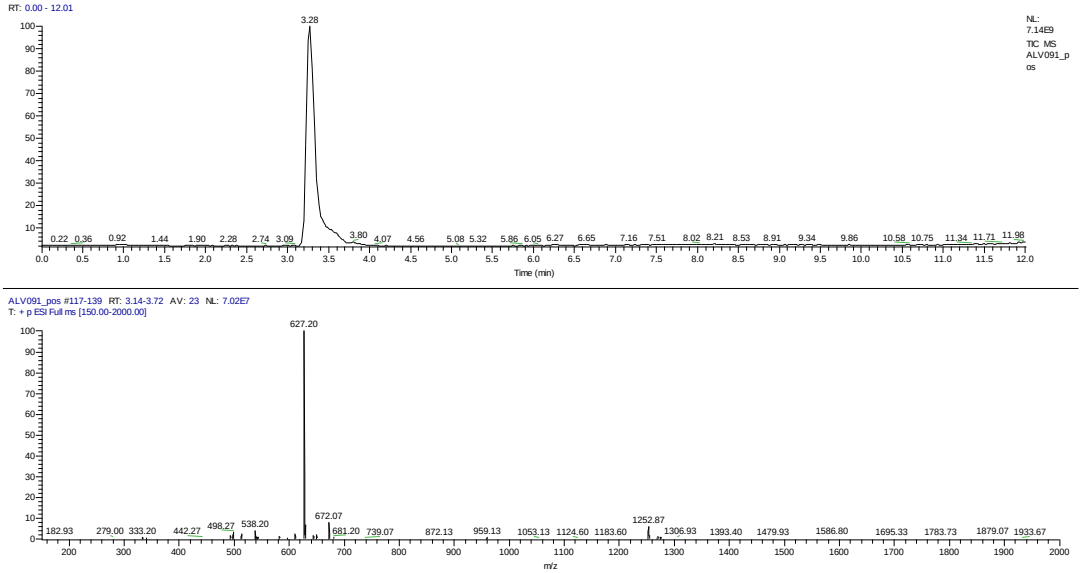


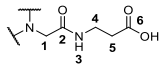
Figure S17. UHPLC-MS chromatogram of pure peptide **2d**

Table S3. Full assignment of the NMR resonances for cyclic peptide **2d**

Major conformer (~71.5%, *s*-cis)

Residue	HN(^3J)	HA/CA	HB/CB	CO	others
Ace[1]	-	1.99/24.5	-	176,9	-
Lys[2]	8.19 (5.8)	4.18/56.5	1.68-1.77/33.7	176,8	1.48/30.0 γ; 1.74-1.59/29.9 δ; 3.57-3.29/52.9 ε
Ala[3]	8.60 (4.7)	4.28/52.6	1.46/19.2	178,8	-
Ala[4]	8.51 (3.5)	4.14/54.6	1.44/18.6	179,4	-
Ala[5]	8.12 (4.8)	4.24/53.3	1.40/18.4	177,6	-
Asp[6]	7.84 (7.1)	4.58/53.9	2.80-3.32/36.9	178,2	174.9 γ; 7.30-7.20 (*terminal NH2*)

N-substitution

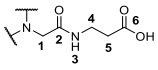


Atom group	δ_H/δ_C
1	4.10-3.96/52.4
2	173,50
3	7.92 (6.0)
4	3.45/39.0
5	2.51/37.9
6	181,00

Minor conformer (~28.5%, *s*-trans)

Residue	HN(^3J)	HA/CA	HB/CB	CO	others
Ace[1]	-	2.06/24.6	-	177,7	-
Lys[2]	8.29 (3.6)	4.12/57.9	1.80/37.9	n/d	1.87-1.36/28.0 δ; 3.95-2.70/51.6 ε
Ala[3]	8.43 (3.7)	4.06/55.2	1.40/19.3	180,2	-
Ala[4]	7.82 (5.7)	4.18/53.9	1.41/18.0	179,5	-
Ala[5]	7.95 (6.4)	4.15/54.2	1.43/18.0	179,3	-
Asp[6]	8.15 (6.5)	4.59/53.9	2.80-2.93/37.5	179,1	173.8 γ; 7.05-7.28 (*terminal NH2*)

N-substitution



Atom group	δ_H/δ_C
1	4.27-3.96/56.2
2	173,20
3	8.10 (5.9)
4	3.46/39.2
5	2.50/38.0
6	181,10

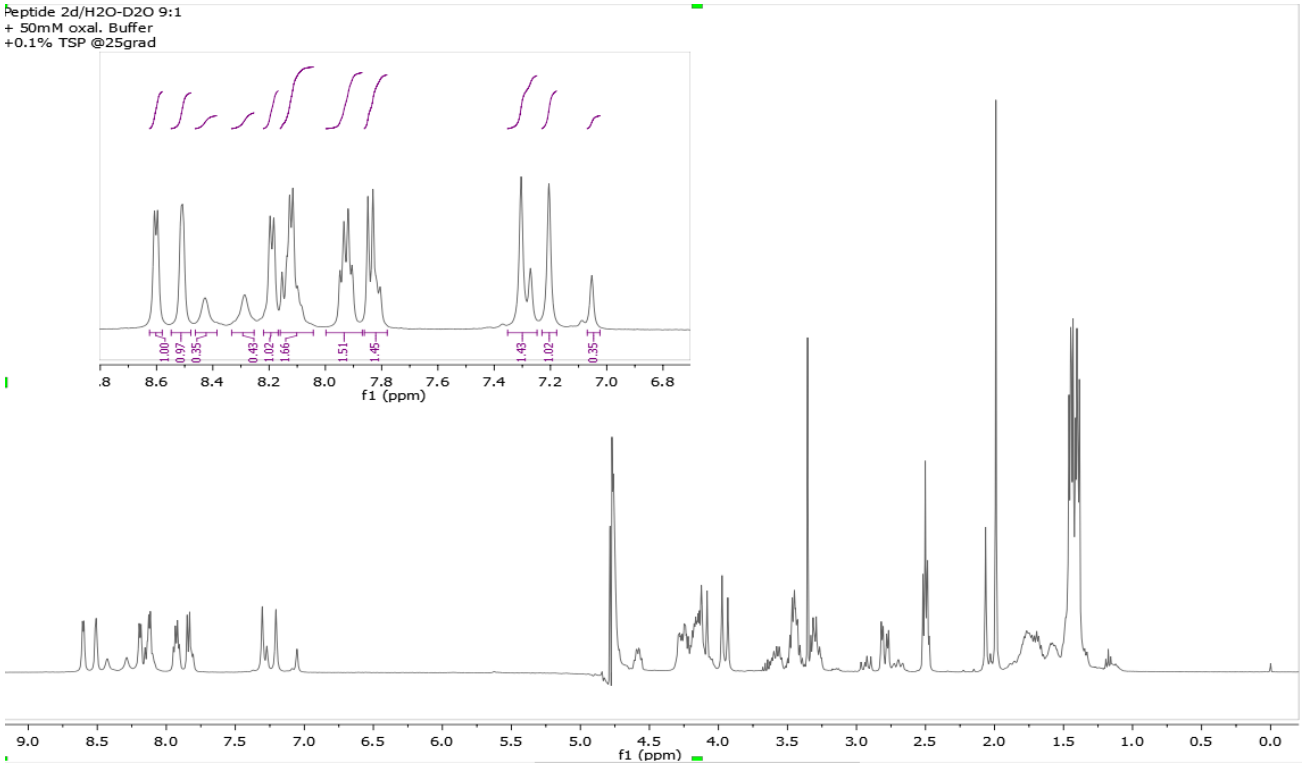


Figure S18. ^{1}H-NMR spectrum of peptide **2d** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1 obtained using the PRESAT pulse sequence for water suppression.

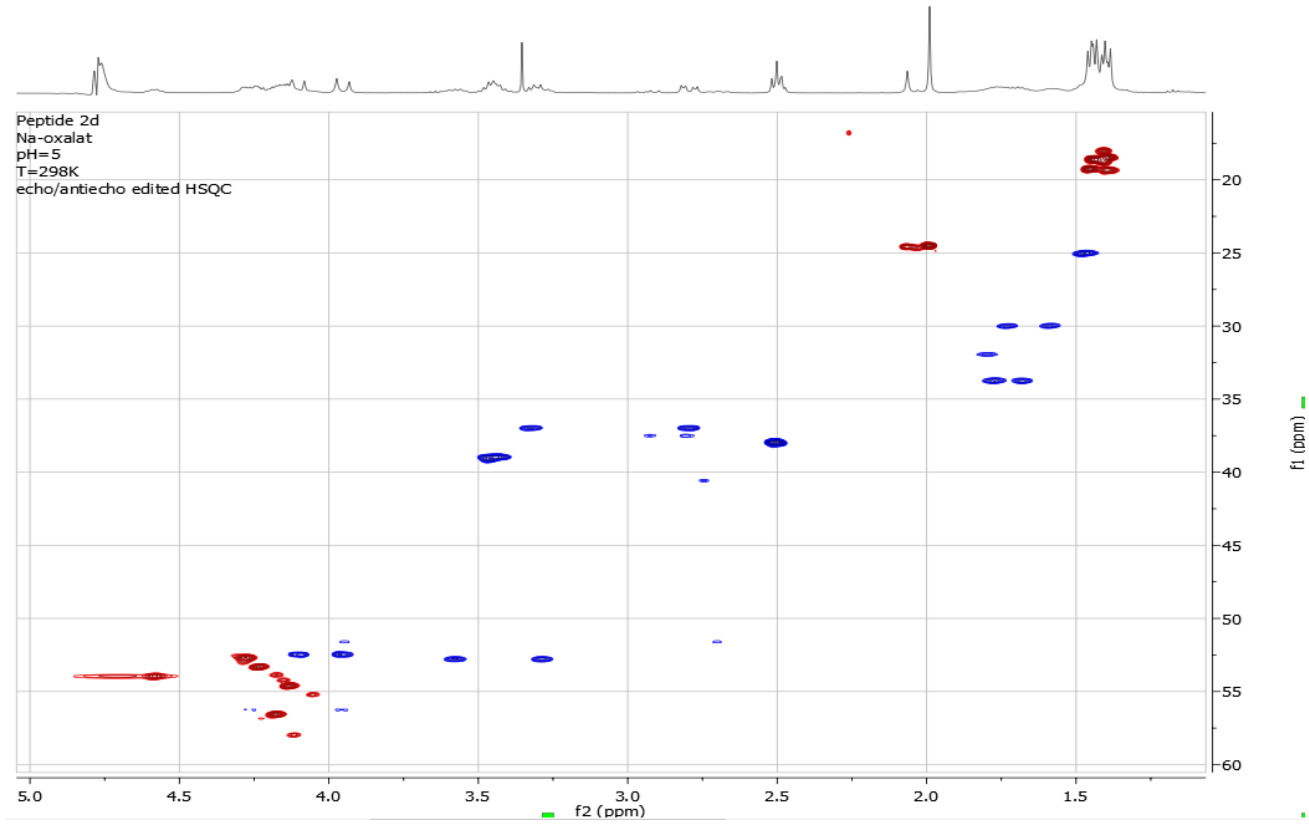


Figure S19. HSQC-edited spectrum of peptide **2d** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1.

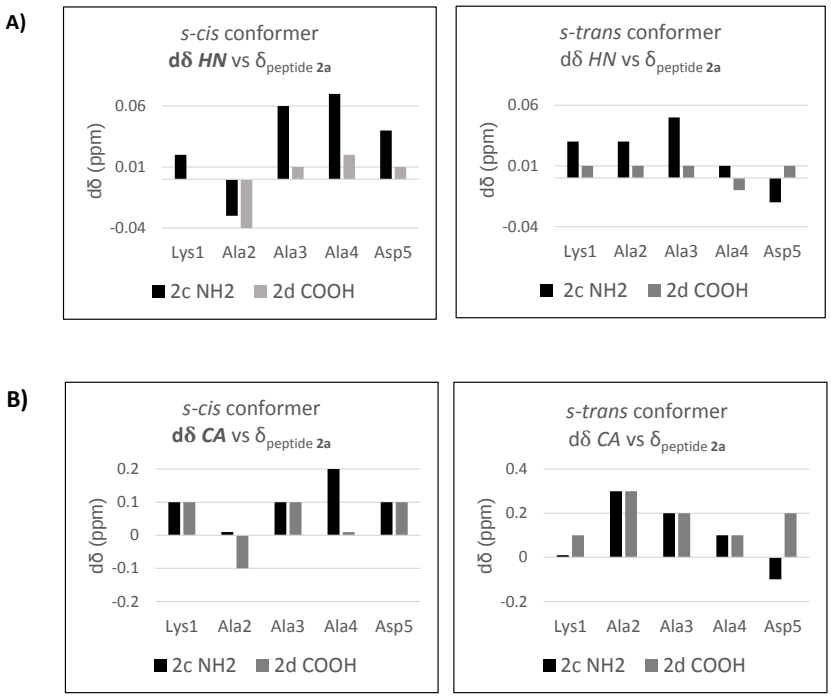
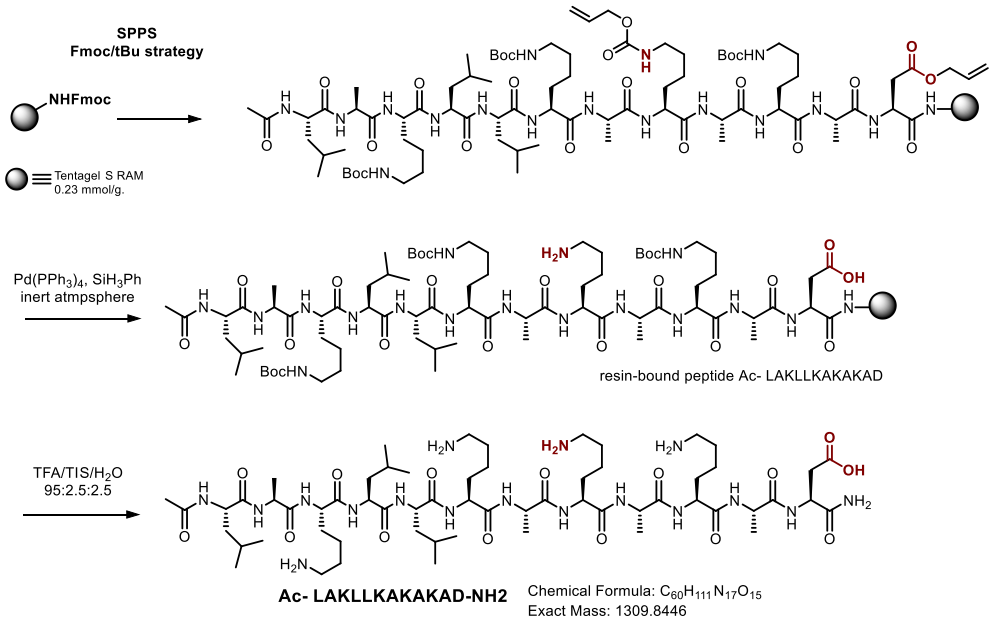


Figure S20. Chemical shift deviations of peptides **2c** and **2d** against peptide **2a** for a) amide protons and b) alpha carbons.

Resin bounded Peptide Ac- LAKLLKAKAKAD



Resin-bound peptide Ac-Leu-Ala-Lys(Boc)-Leu-Leu-Lys(Boc)-Ala-Lys-Ala-Lys(Boc)-Ala-Asp was synthesized in 50 µmol scale on TentaGel S RAM resin (217 mg, 0.23 mmol/mg) according to the protocols described in section S2.1. The purity of the resin-bound peptide was evaluated through minicleavages according

to the protocol described in S2.1. Analytical UHPLC: R_t 4.54 min, > 87% purity (crude); ESI-HRMS, calcd for $C_{60}H_{112}N_{17}O_{15}^{2+}$: 655.9301 $[M+2H]^{2+}$; Found: *m/z* 655.9333 $[M + 2H]^{2+}$.

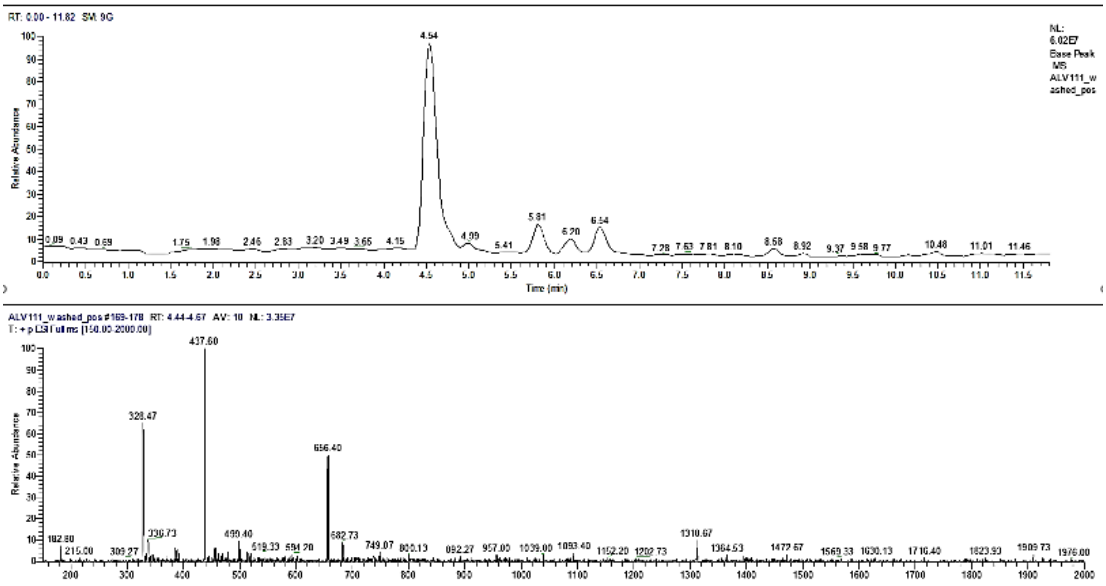
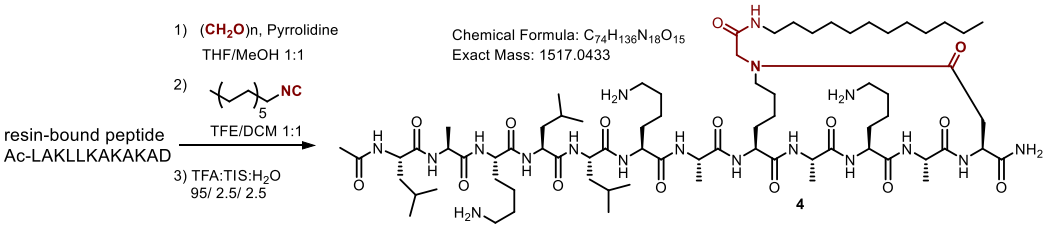


Figure S21. UHPLC-MS chromatogram of crude peptide Ac-LAKLLKAKAKAD-NH_2

Ac-(*N*-(2-(*n*-dodecylamino)-2-oxoethyl)-(cyclo-8,12))-[LAKLLKAKAKAD]-NH_2 (4)



Cyclic peptide **4** was produced by on-resin cyclization in a 50 µmol scale from resin-bound peptide Ac-LAKLLKAKAKAD according to the aminocatalysis-mediated Ugi macrocyclization protocol described in S2.1, in the presence of *n*-dodecylisocyanide. Cleavage with TFA/H_2O/TIS 95:2.5:2.5 for 2 h afforded peptide **4** (87% cleavage yield), which was purified by semi-preparative RP-HPLC (32.3 mg, 43% isolated yield); R_t 8.17 min, > 91% purity. ESI-HRMS, calcd. for $C_{74}H_{138}N_{18}O_{15}^{2+}$: 759.5295 $[M + 2H]^{2+}$ Found: *m/z* 759.5273 $[M + 2H]^{2+}$.

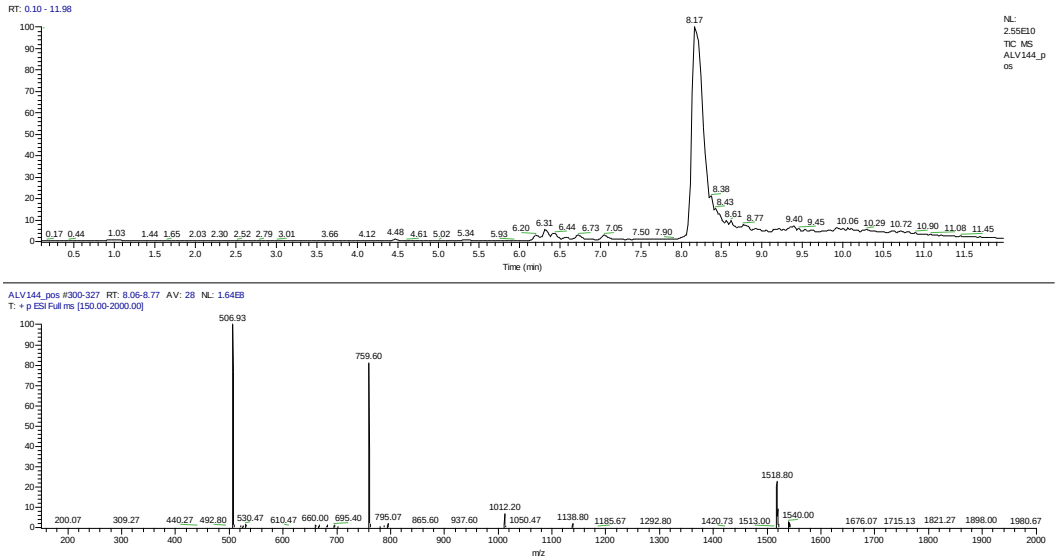


Figure S22. UHPLC-MS chromatogram of pure peptide **4**

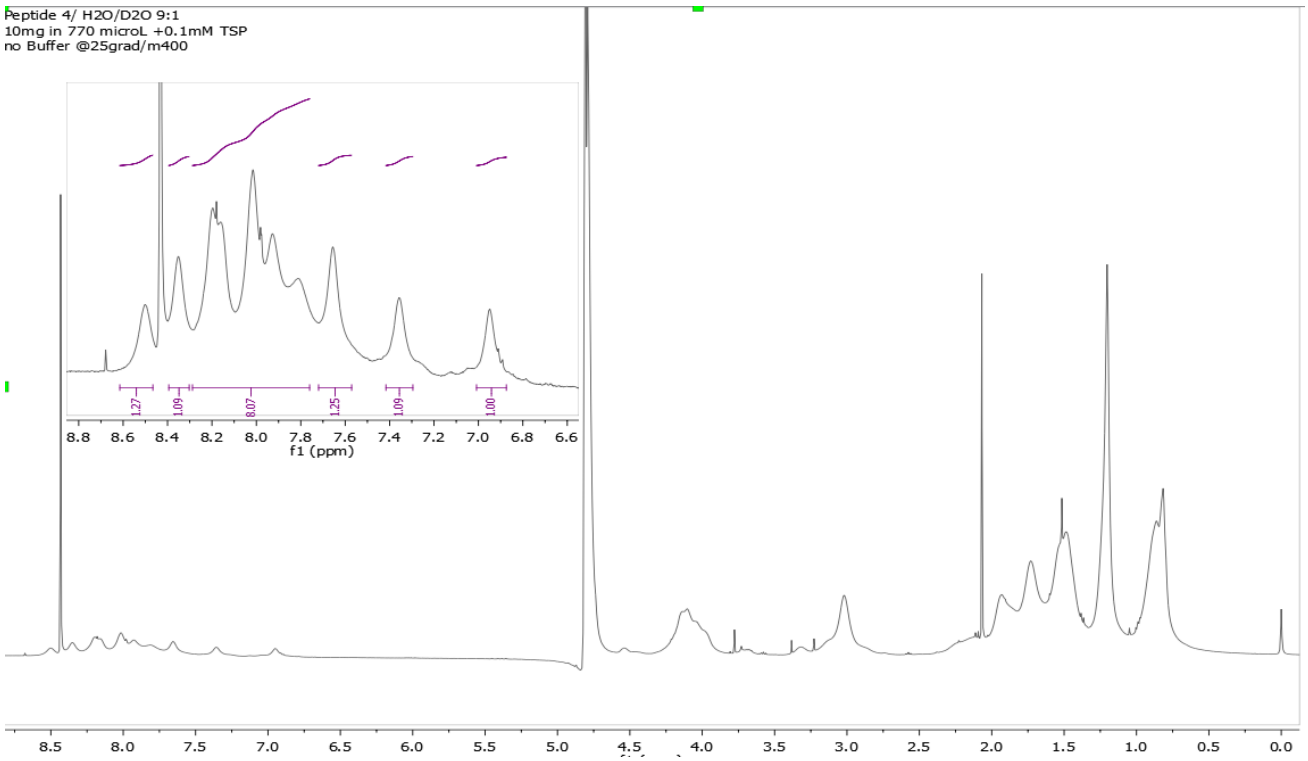


Figure S23. ^{1}H NMR spectrum of peptide **4** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1 obtained using the PRESAT pulse sequence for water suppression.

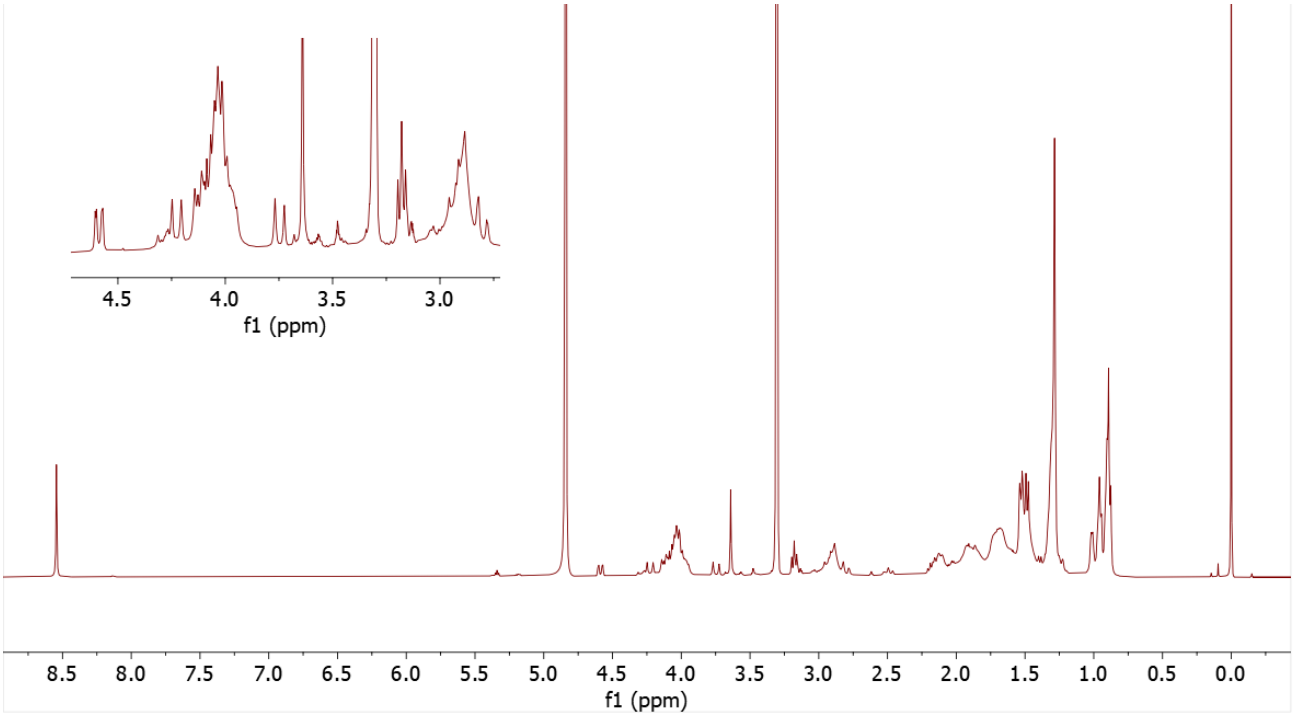


Figure S24. ^{1}H NMR spectrum of peptide **4** in Methanol-d4.

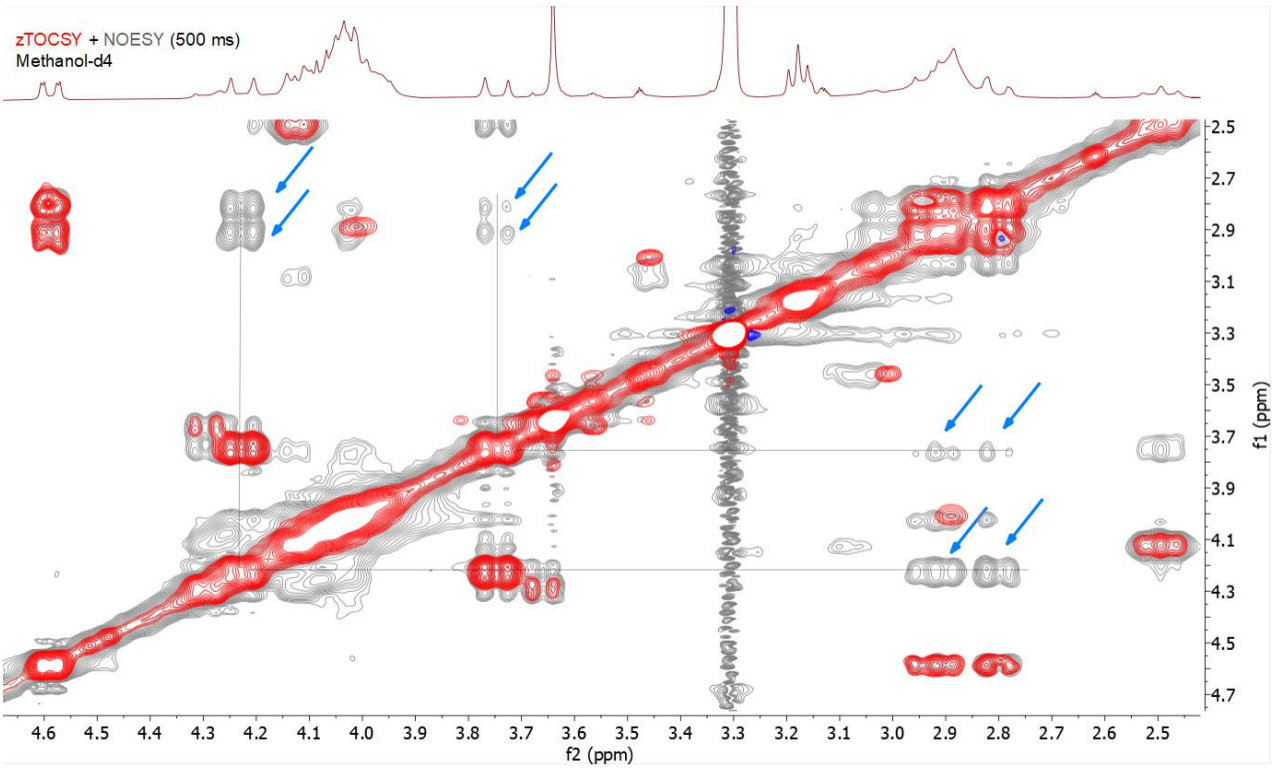


Figure S25. Section of the superimposed zTOCSY(red) and NOESY(grey) spectra for peptide **4** in Methanol-d4. With blue arrows the crosspeaks indicating spatial proximity between the exocyclic glycine residue and the beta protons of the aspartic acid for the major conformer (*s-cis*/*s-trans* ratio 10:90).

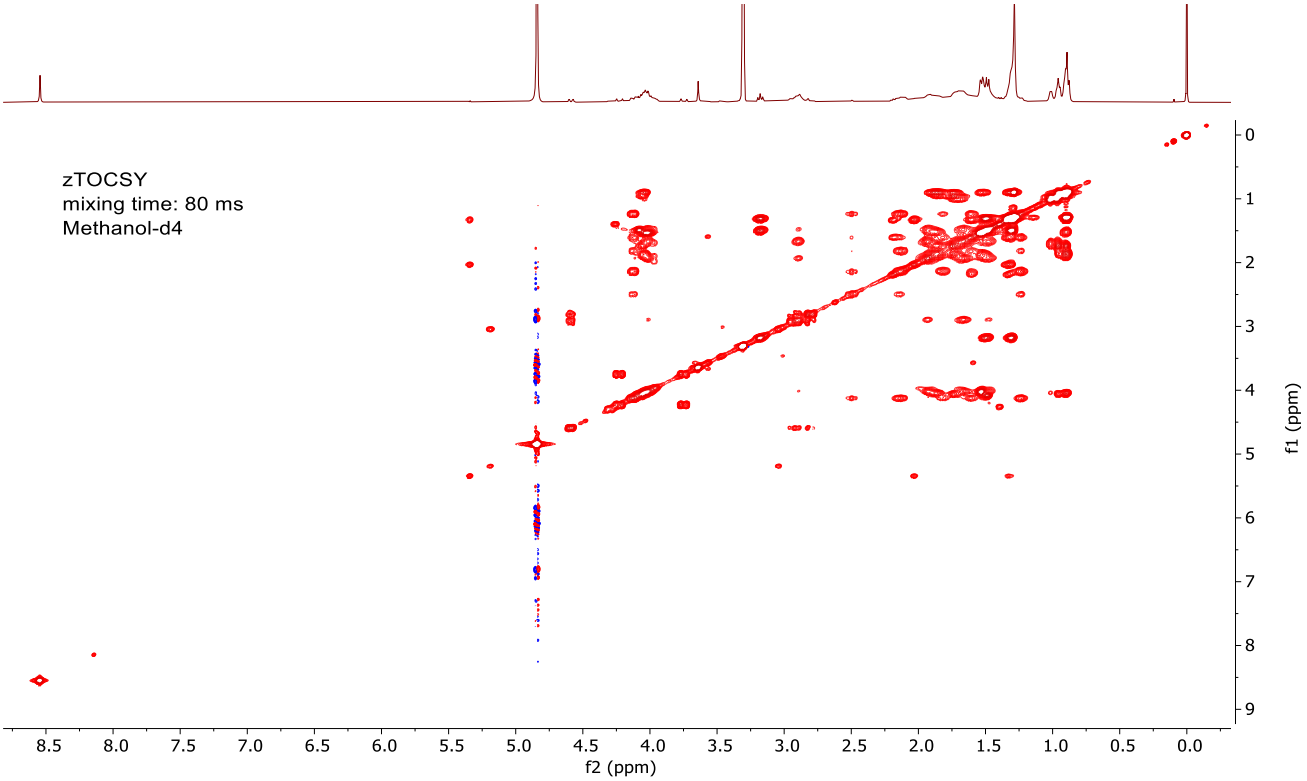


Figure S26. zTOCSY spectrum of peptide **4** in Methanol-d4.

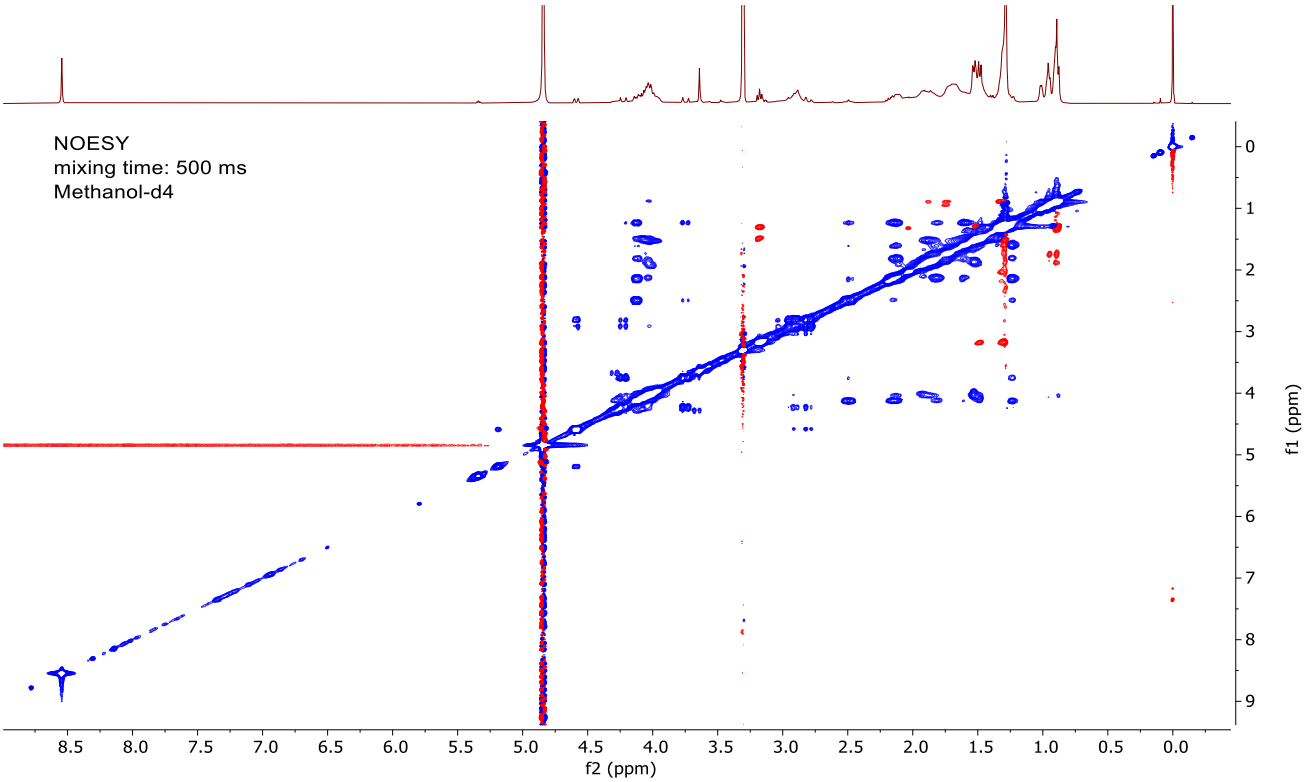


Figure S27. NOESY spectrum of peptide **4** in Methanol-d4.

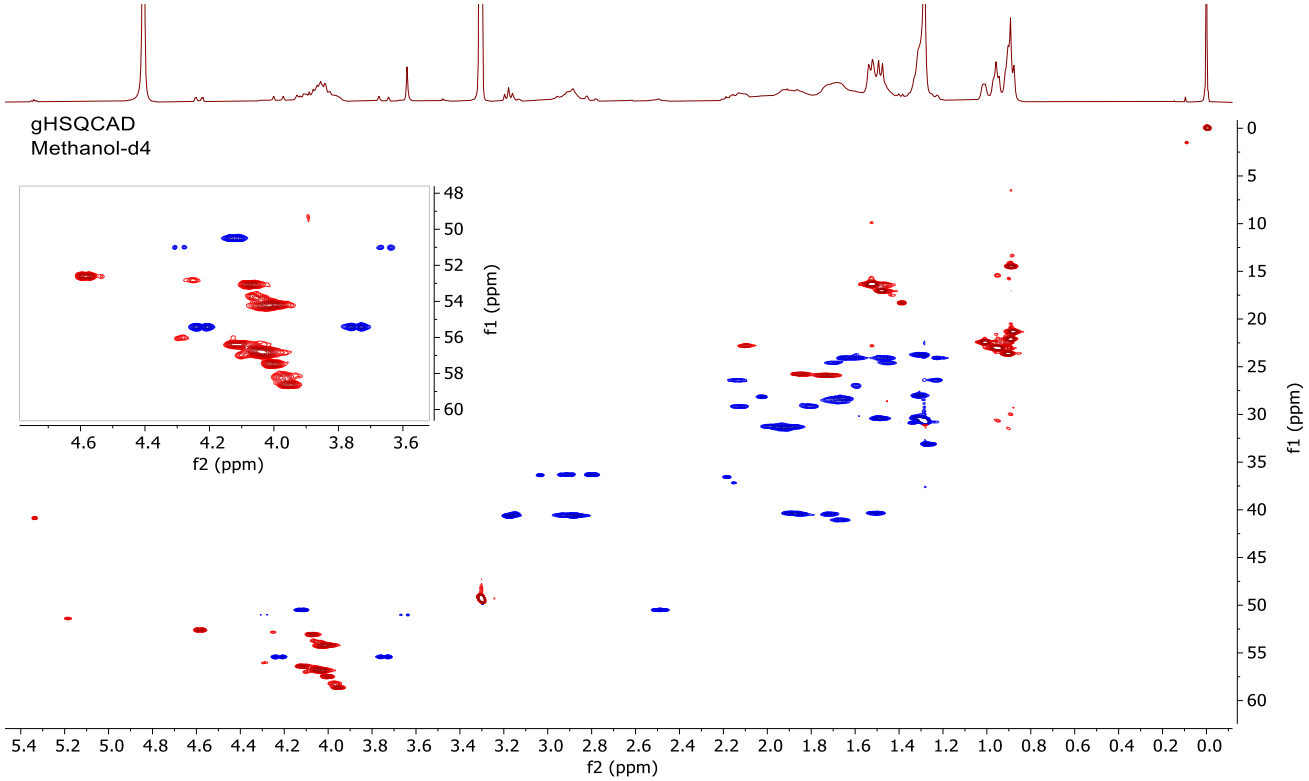


Figure S28. HSQC-edited spectrum of peptide **4** in Methanol-d4.

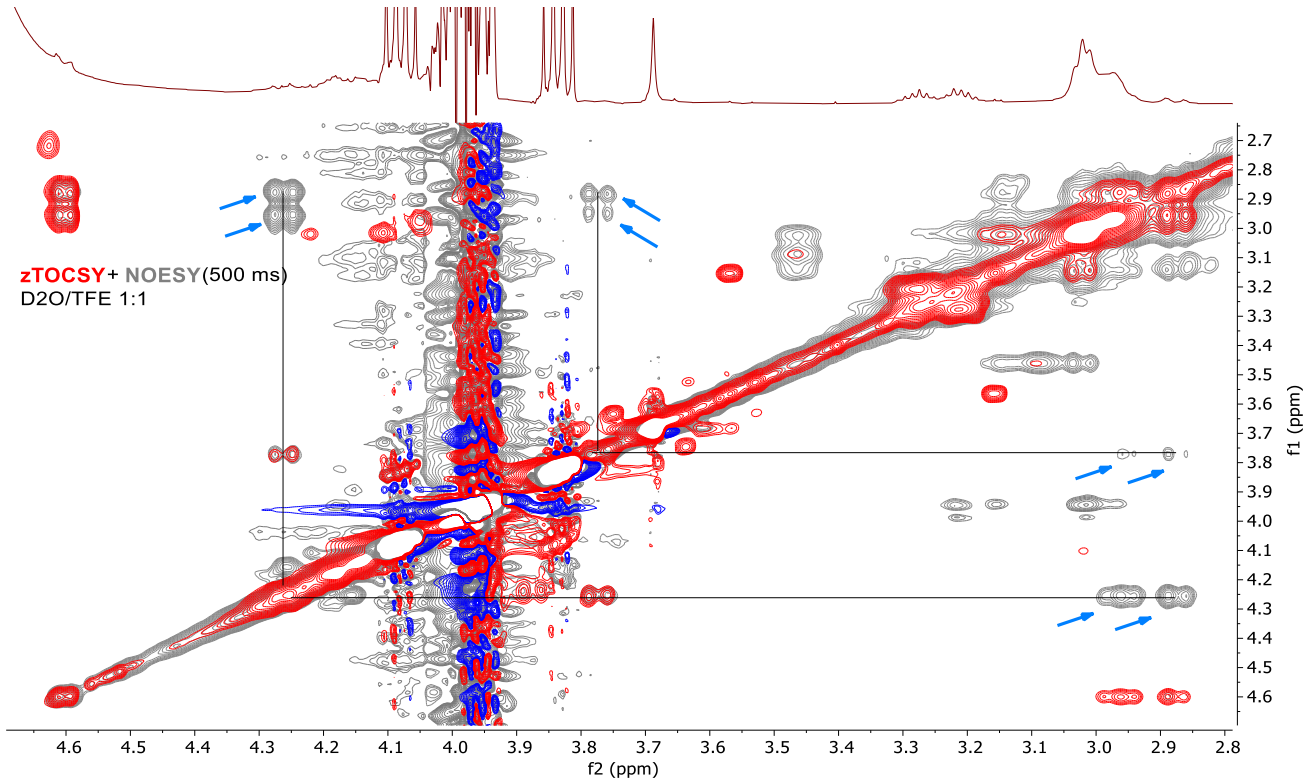


Figure S29. Section of the superimposed zTOCSY(red) and NOESY(grey) spectra for peptide **4** in D_2O/TFE 1:1. With blue arrows the crosspeaks indicating spatial proximity between the exocyclic glycine residue and the beta protons of the aspartic acid for the major conformer (*s-cis*/*s-trans* ratio 19:81).

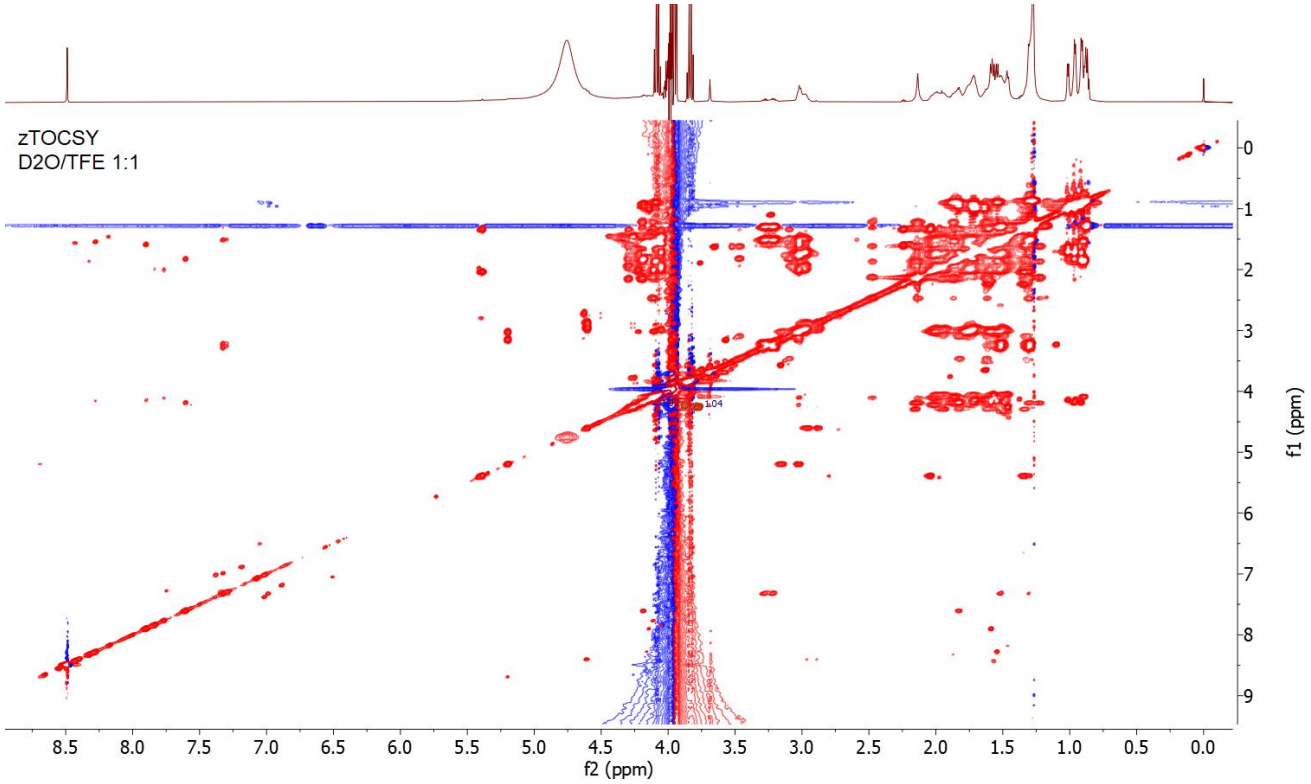


Figure S30. zTOCSY spectrum of peptide **4** in D_2O/TFE 1:1 (mixing time: 80 ms).

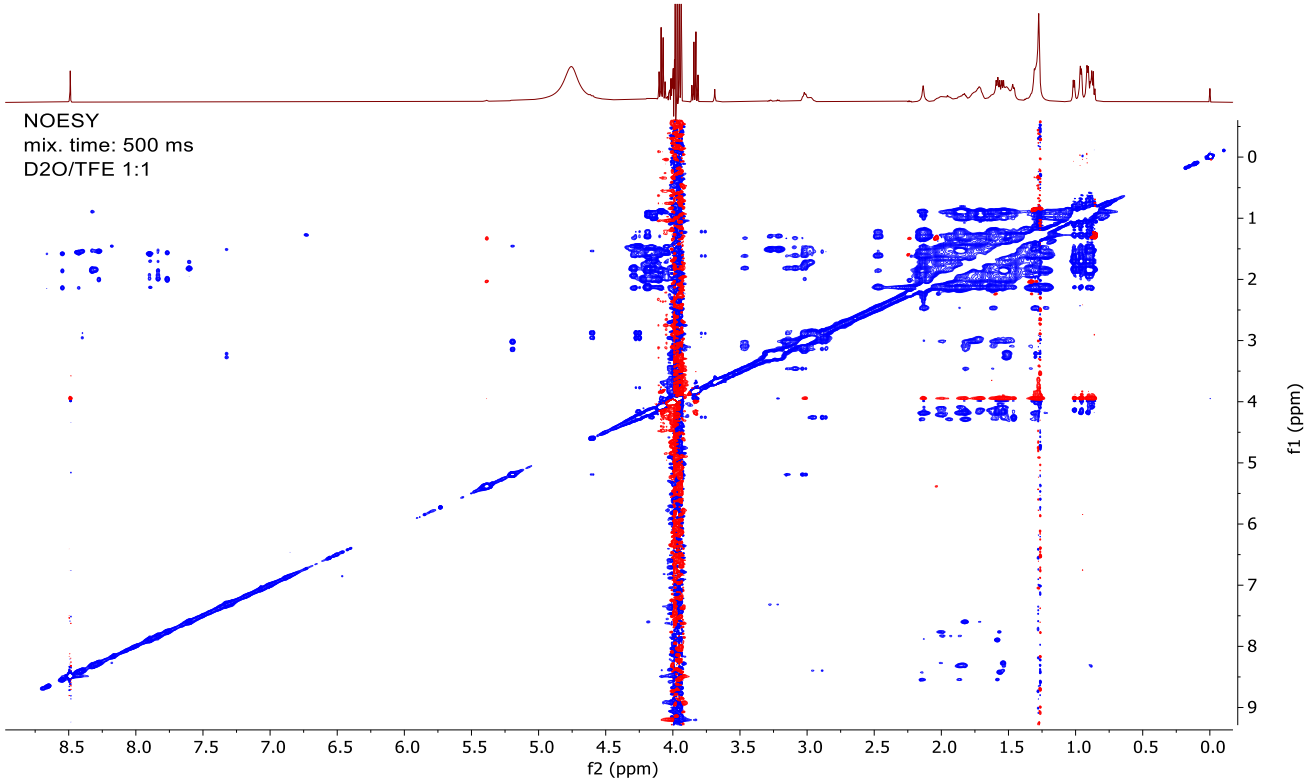


Figure S31. NOESY spectrum of peptide **4** in D_2O/TFE 1:1 (mixing time: 500 ms).

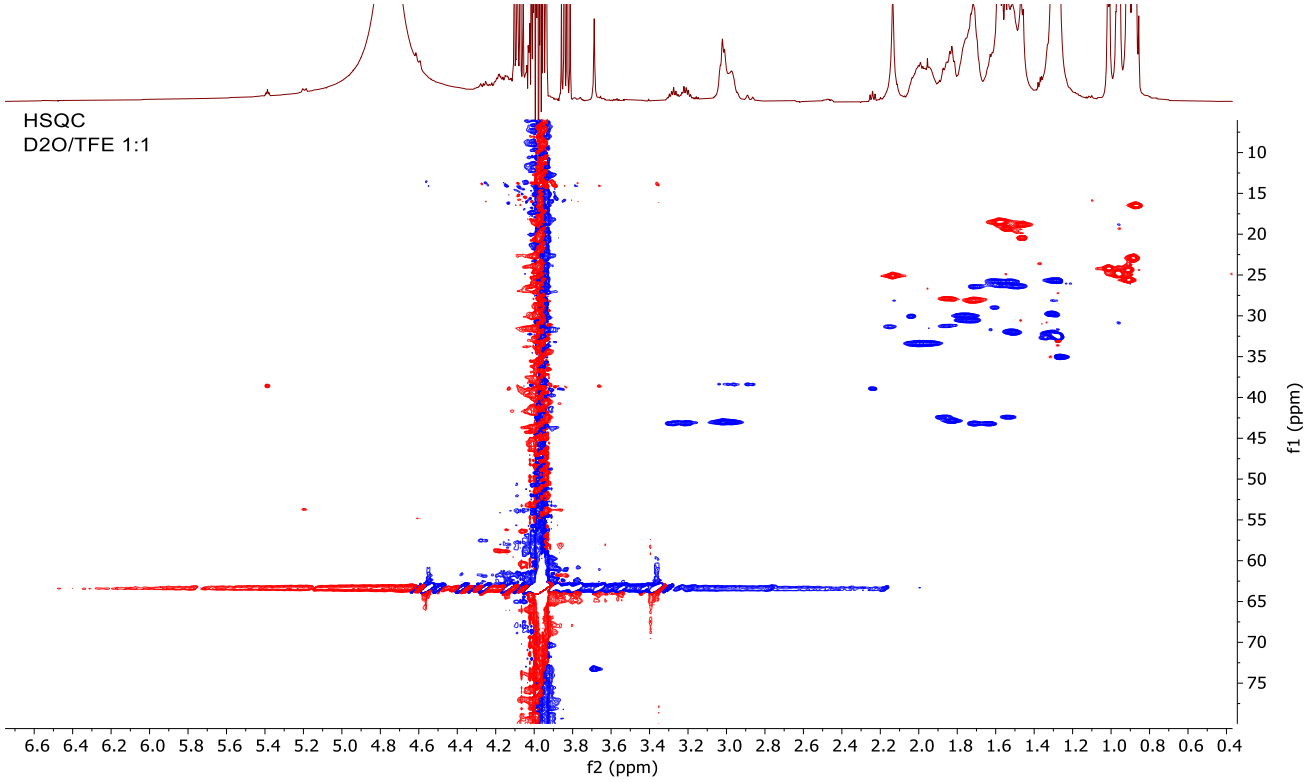
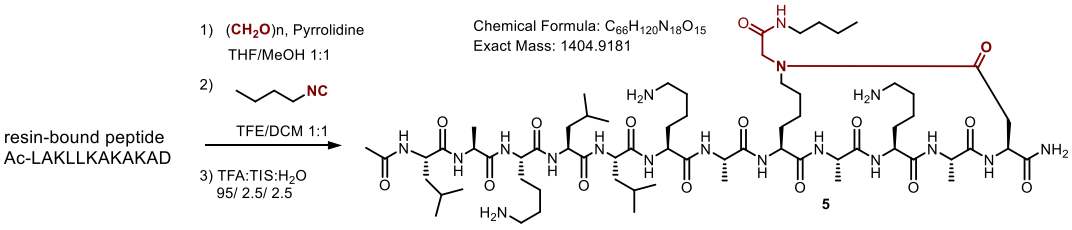


Figure S32. HSQC-edited spectrum of peptide **4** in D_2O/TFE 1:1.

Ac-(*N*-(2-(*n*-butylamino)-2-oxoethyl)-(cyclo-8,12))-[LAKLLKAKAKAD]-NH_2 (5)



Cyclic peptide **5** was produced by on-resin cyclization in a 50 µmol scale from resin-bound peptide Ac-LAKLLKAKAKAD according to the aminocatalysis-mediated Ugi macrocyclization protocol described in S2.1.4, in the presence of *n*-butylisocyanide. Cleavage with TFA/H_2O/TIS 95:2.5:2.5 for 2 h afforded peptide **5** (83% cleavage yield), which was purified by semi-preparative RP-HPLC (27.9 mg, 40% isolated yield); R_t 6.19 min, > 94% purity. ESI-HRMS, calcd. for $C_{66}H_{122}N_{18}O_{15}^{2+}$: 703.4669 $[M + 2H]^{2+}$ Found: *m/z* 703.4676 $[M + 2H]^{2+}$.

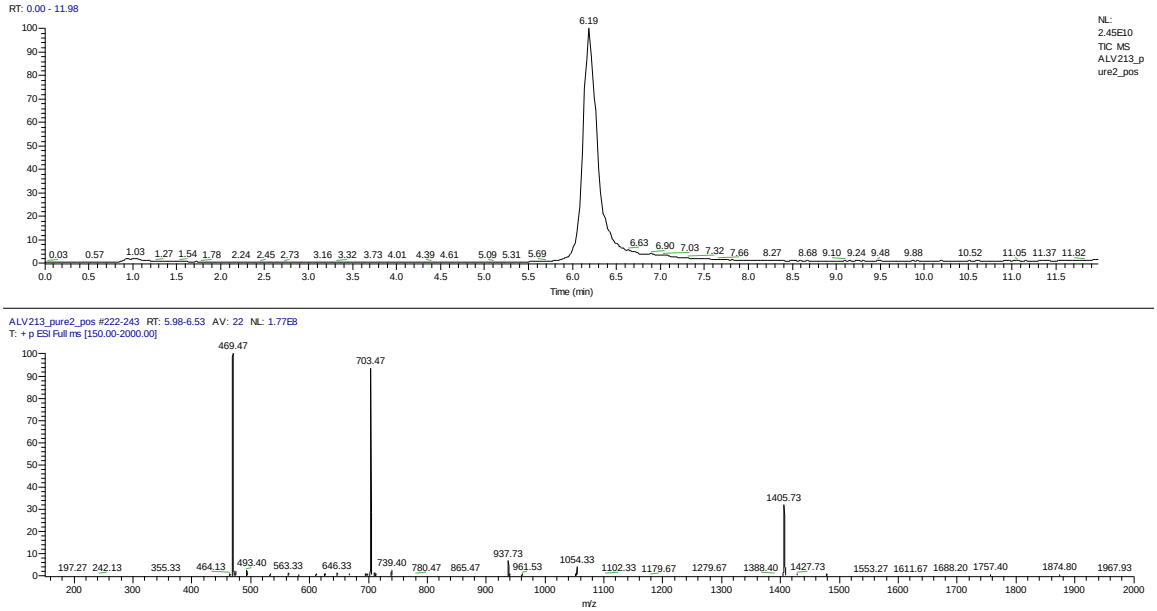


Figure S33. UHPLC-MS chromatogram of pure peptide **5**

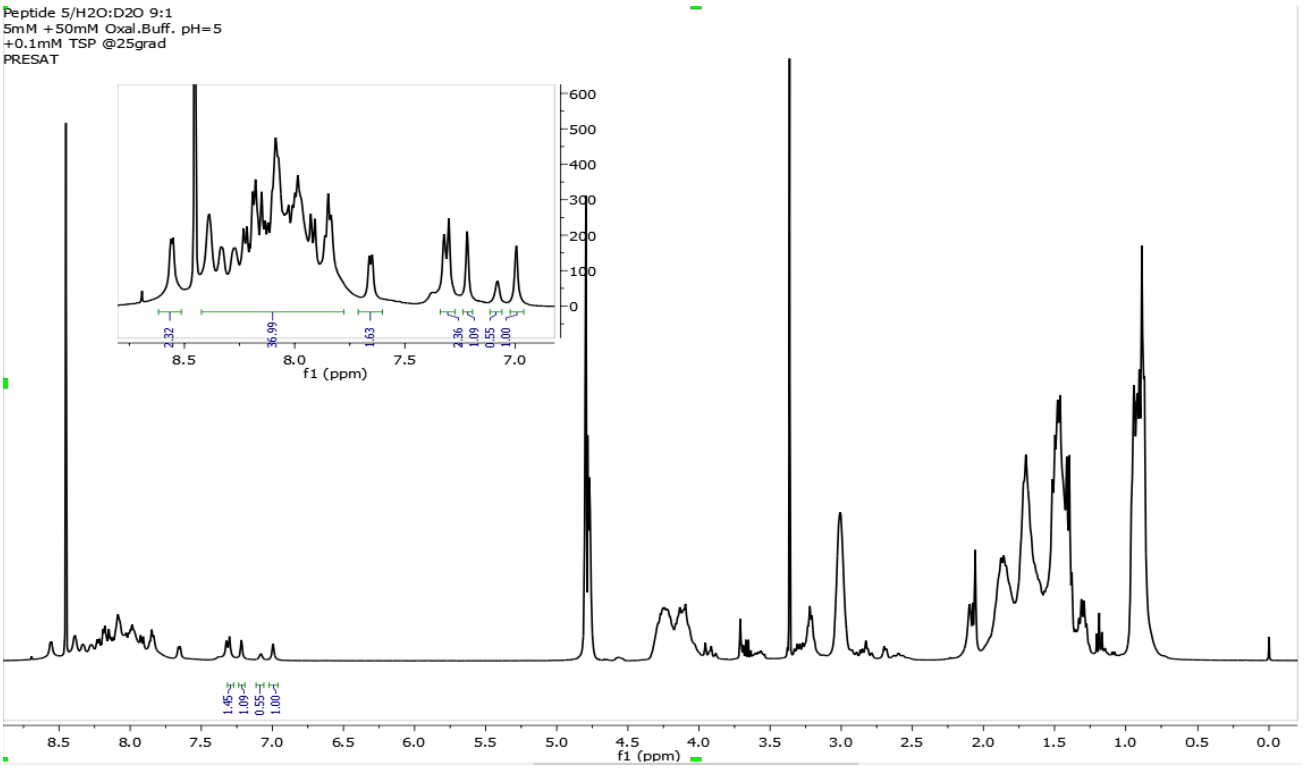


Figure S34. ^{1}H NMR spectrum of peptide **5** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1 obtained using the PRESAT pulse sequence for water suppression.

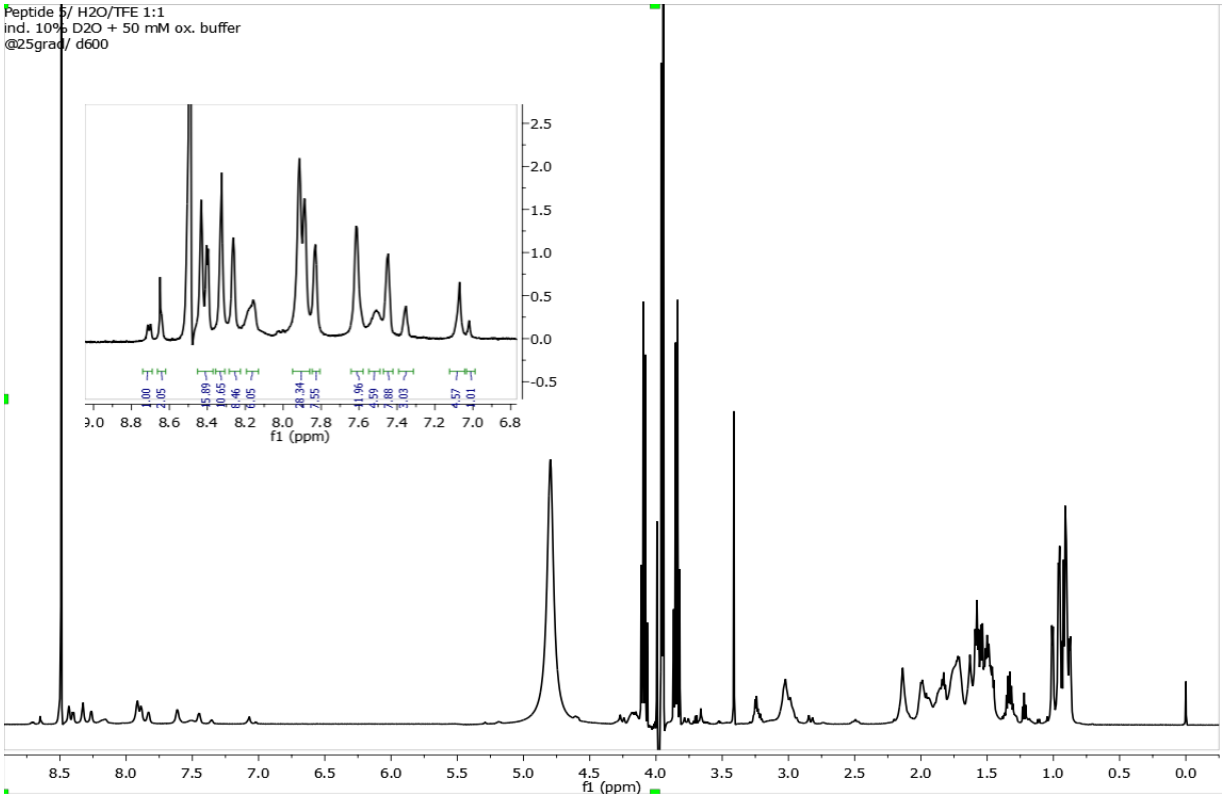


Figure S35. ^{1}H-NMR spectrum of peptide **5** in pH5 oxalic acid/oxalate buffer in TFE/Water 1:1 solution obtained using a multi-frequency PRESAT pulse sequence for water suppression.

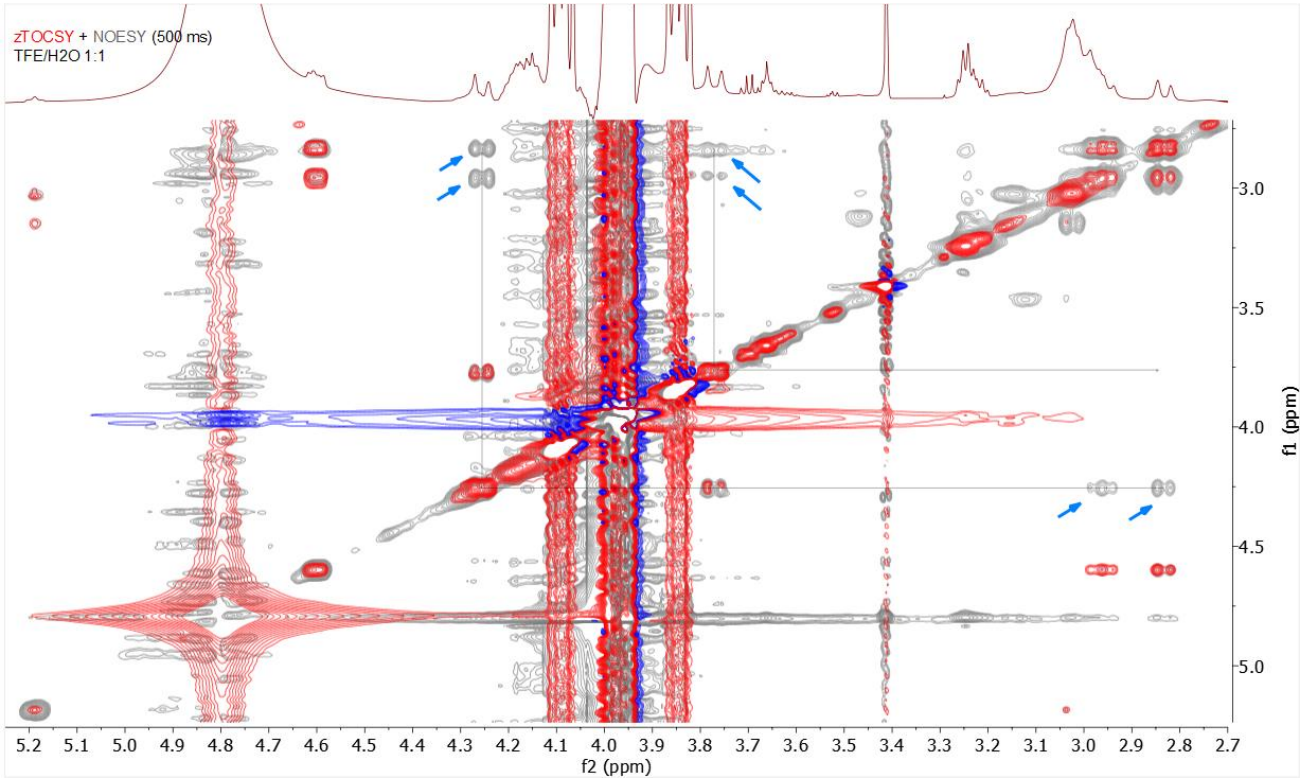


Figure S36. Section of the superimposed zTOCSY(red) and NOESY(grey) spectra for peptide **5** in TFE/H_2O 1:1. With blue arrows the crosspeaks indicating spatial proximity between the exocyclic glycine residue and the beta protons of the aspartic acid for the major conformer (*s-cis*/*s-trans* ratio 10:90).

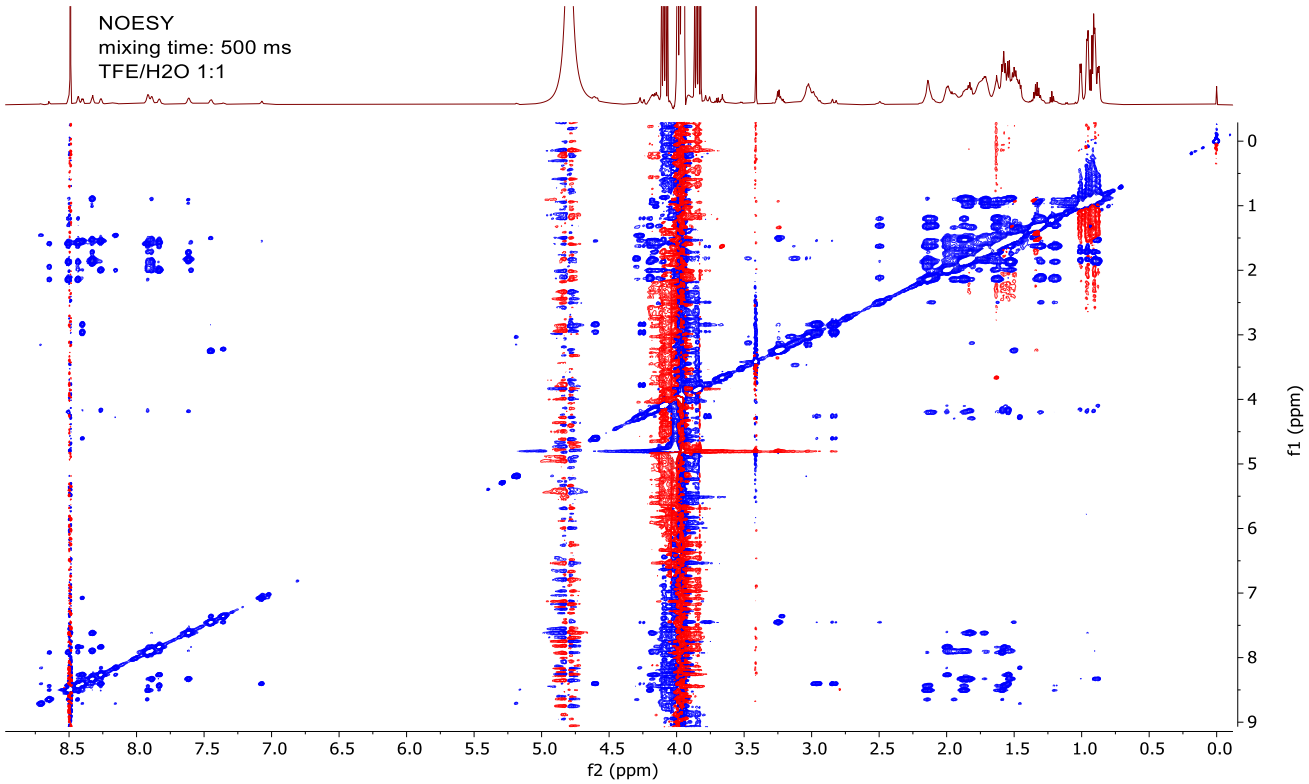


Figure S37. NOESY spectrum of peptide **5** in TFE/H_2O 1:1 (mixing time: 500 ms).

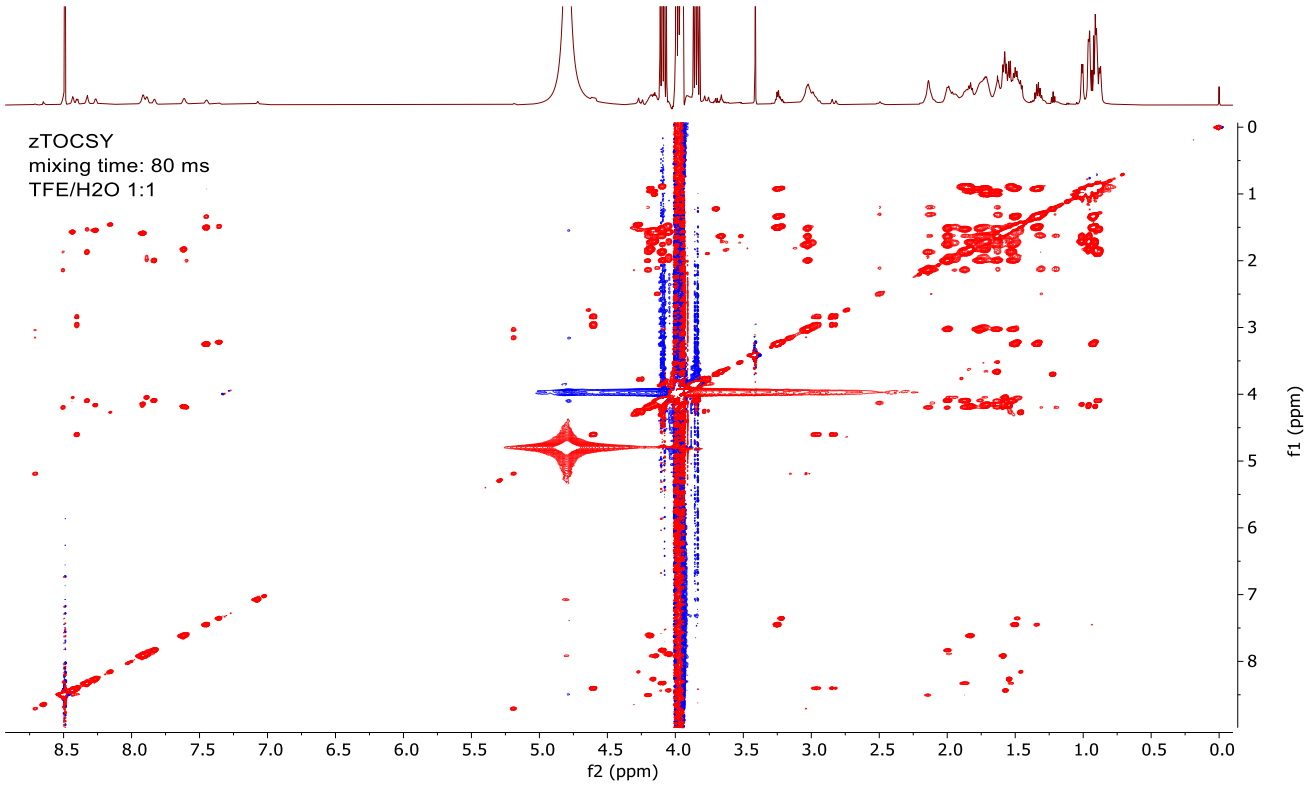


Figure S38. zTOCSY spectrum of peptide **5** in TFE/H_2O 1:1 (mixing time: 80 ms).

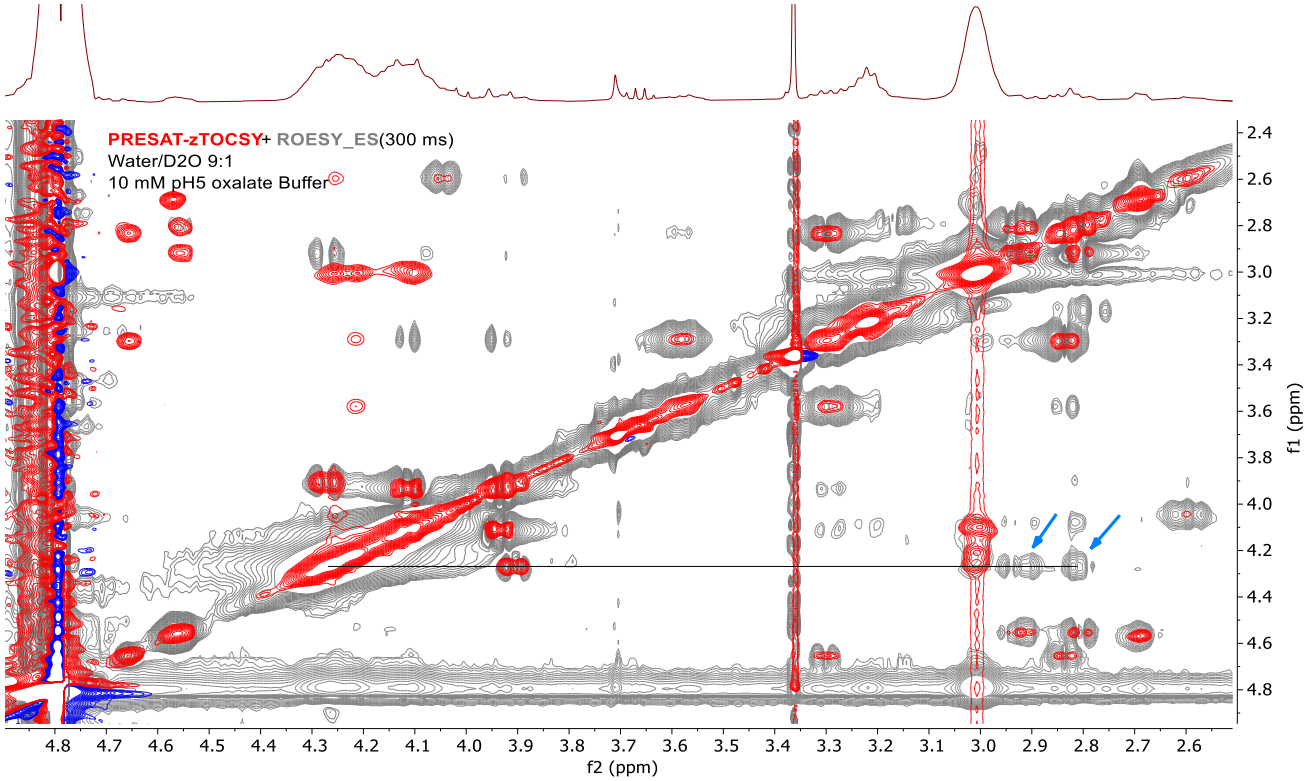


Figure S39. Section of the superimposed zTOCSY (red) and ROESY_ES (grey) spectra for peptide **5** in H_2O/D_2O 9:1 with pH 5 oxalate buffer. With blue arrows the crosspeaks indicating spatial proximity between the exocyclic glycine residue and the beta protons of the aspartic acid for the minor conformer (*s-cis*/*s-trans* ratio 57:43).

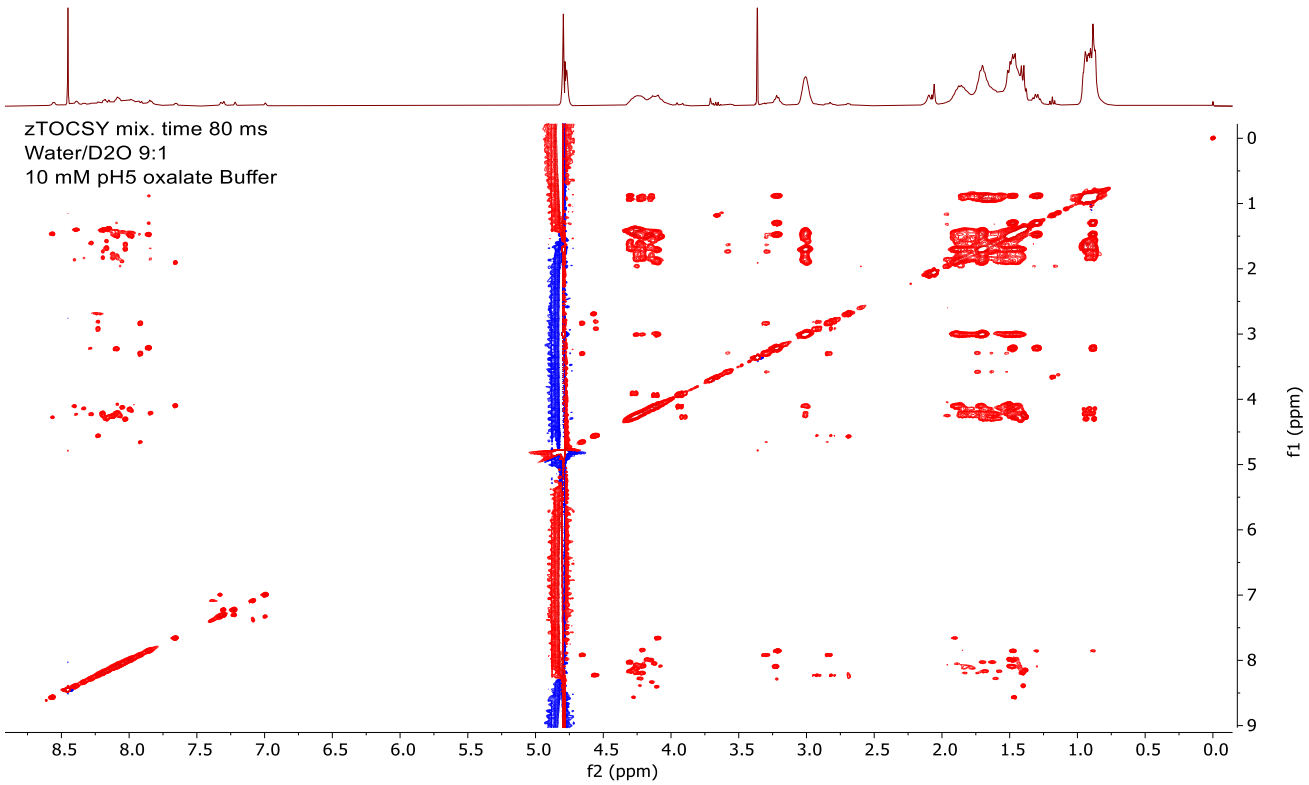


Figure S40. zTOCSY spectrum of peptide **5** in H_2O/D_2O 9:1 with pH 5 oxalate buffer (mixing time: 80 ms).

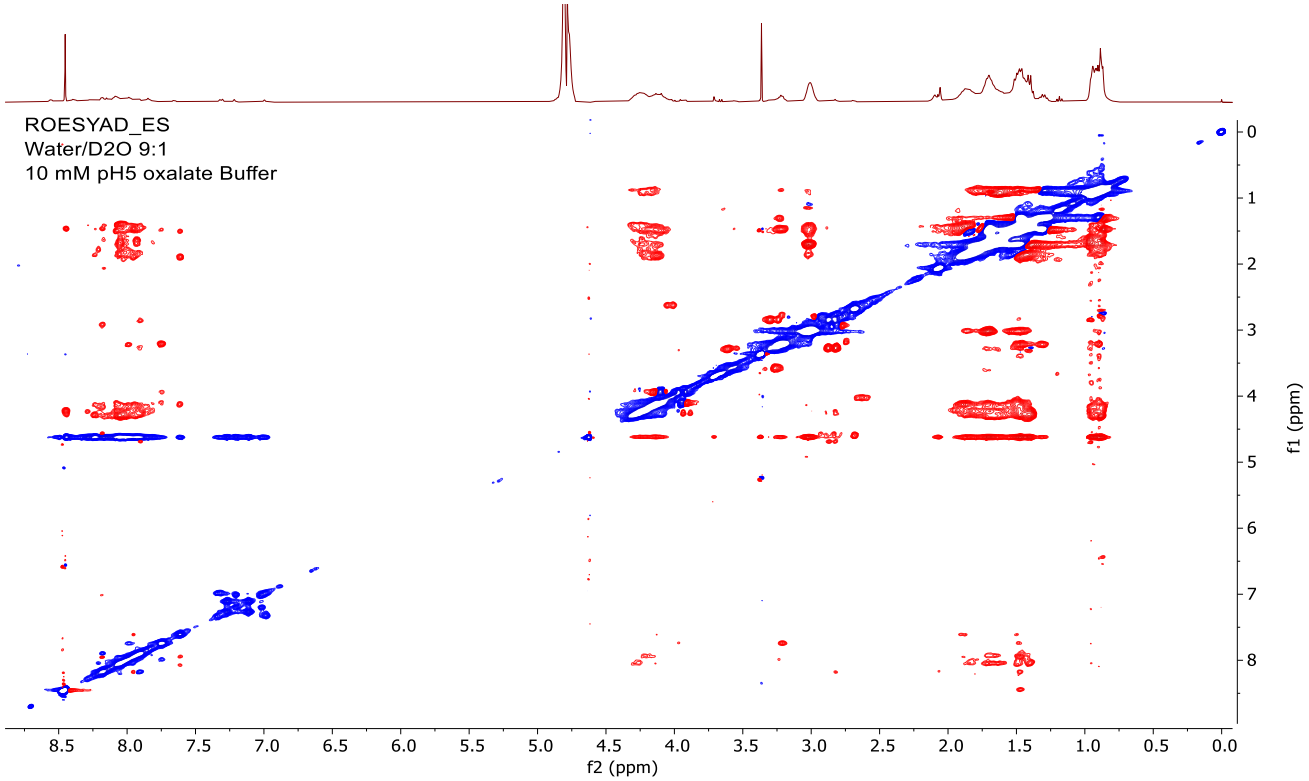


Figure S41. ROESY_ES spectrum of peptide **5** in H_2O/D_2O 9:1 with pH 5 oxalate buffer (mixing time: 300 ms).

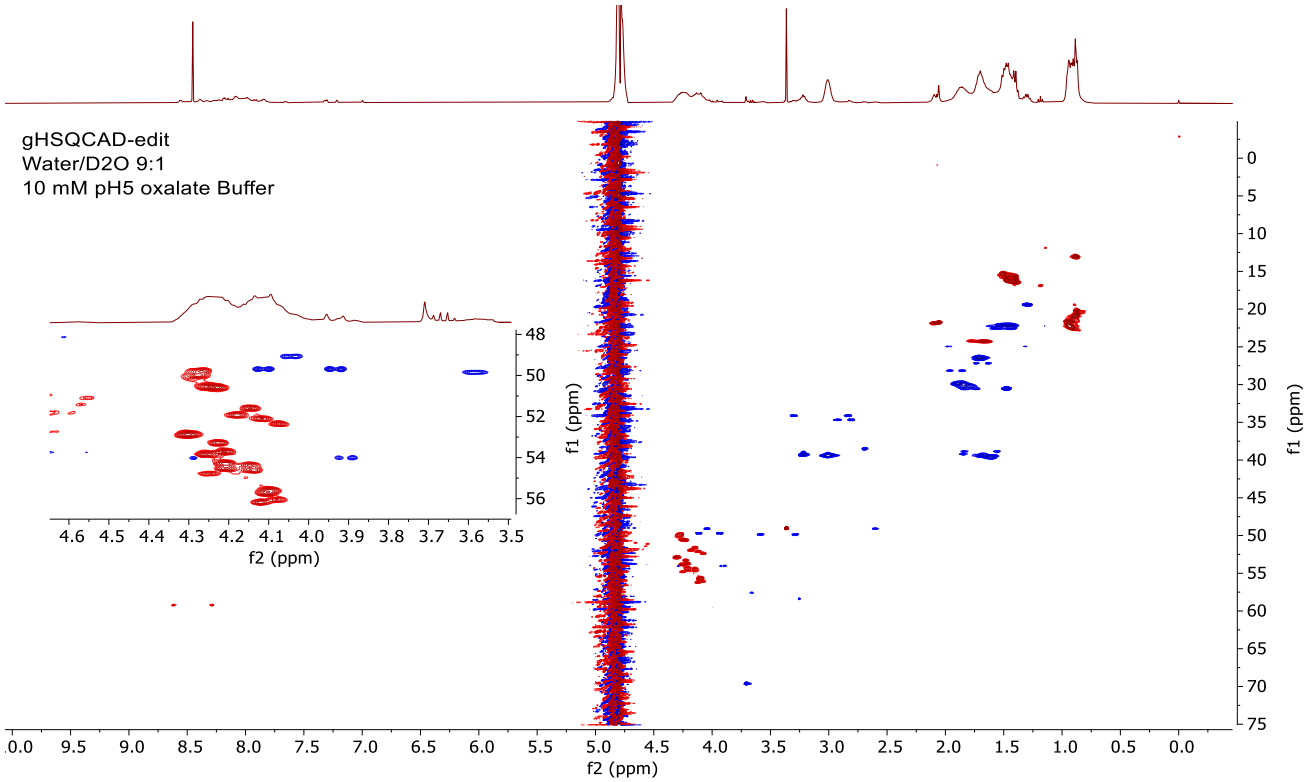


Figure S42. HSQC-edited spectrum of peptide **5** in H_2O/D_2O 9:1 with pH 5 oxalate buffer.

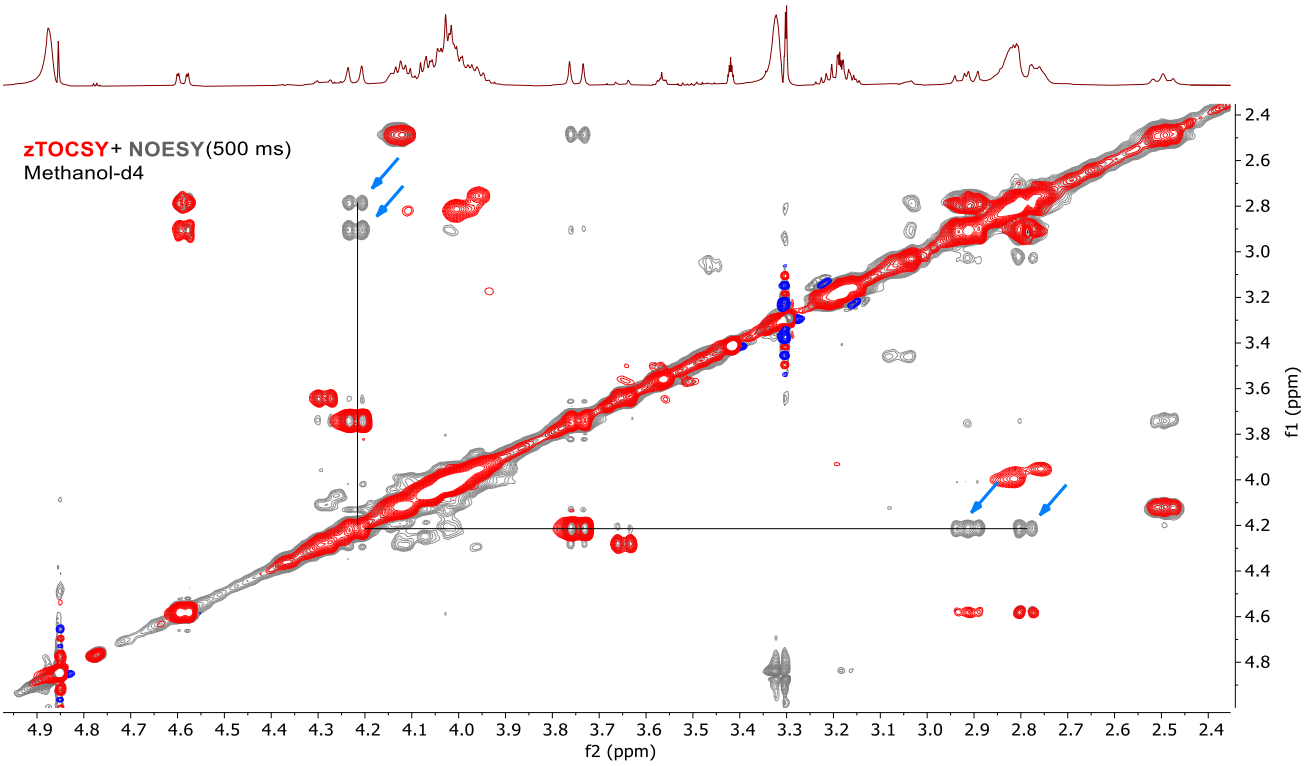


Figure S43. Section of the superimposed zTOCSY (red) and NOESY (grey) spectra for peptide **5** in Methanol-d4. With blue arrows the crosspeaks indicating spatial proximity between the exocyclic glycine residue and the beta protons of the aspartic acid for the major conformer (*s-cis*/*s-trans* ratio 13:87).

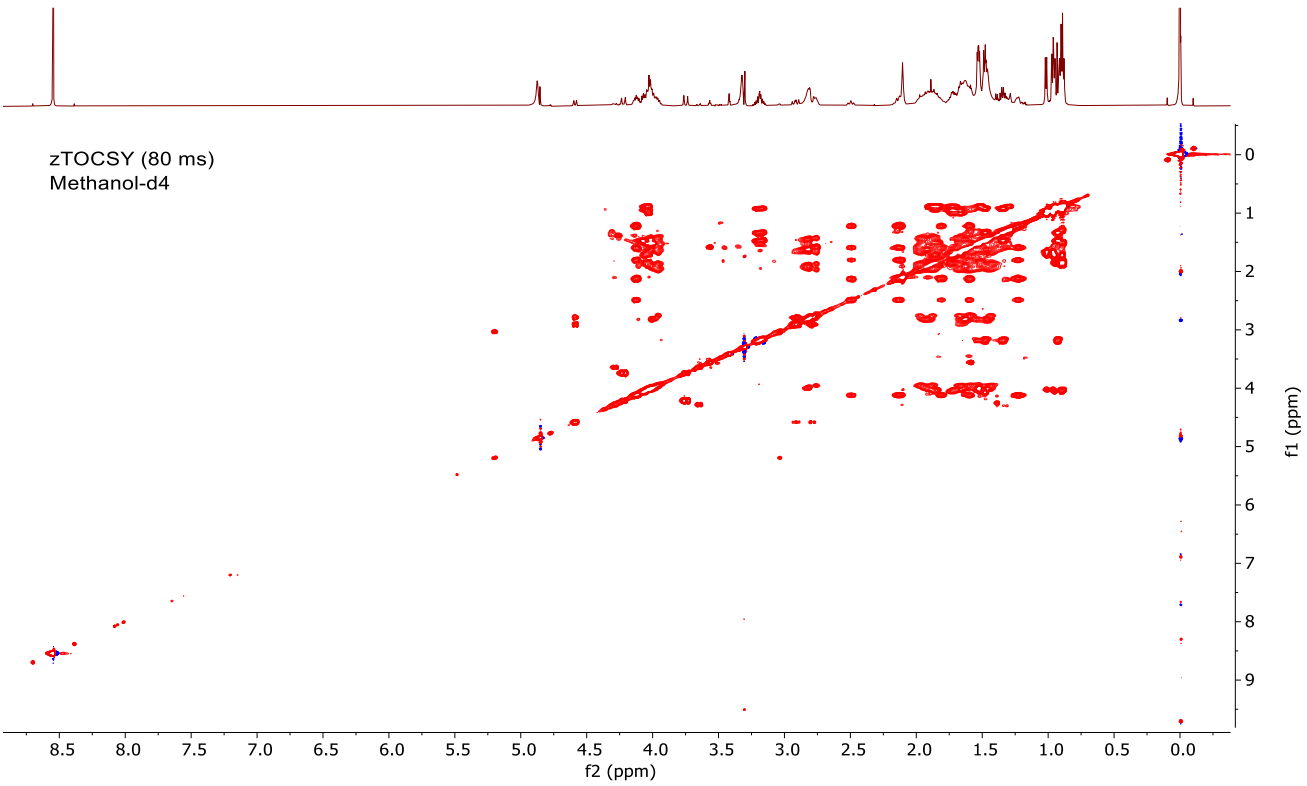


Figure S44. zTOCSY spectrum of peptide **5** in Methanol-d4 (mixing time: 80 ms).

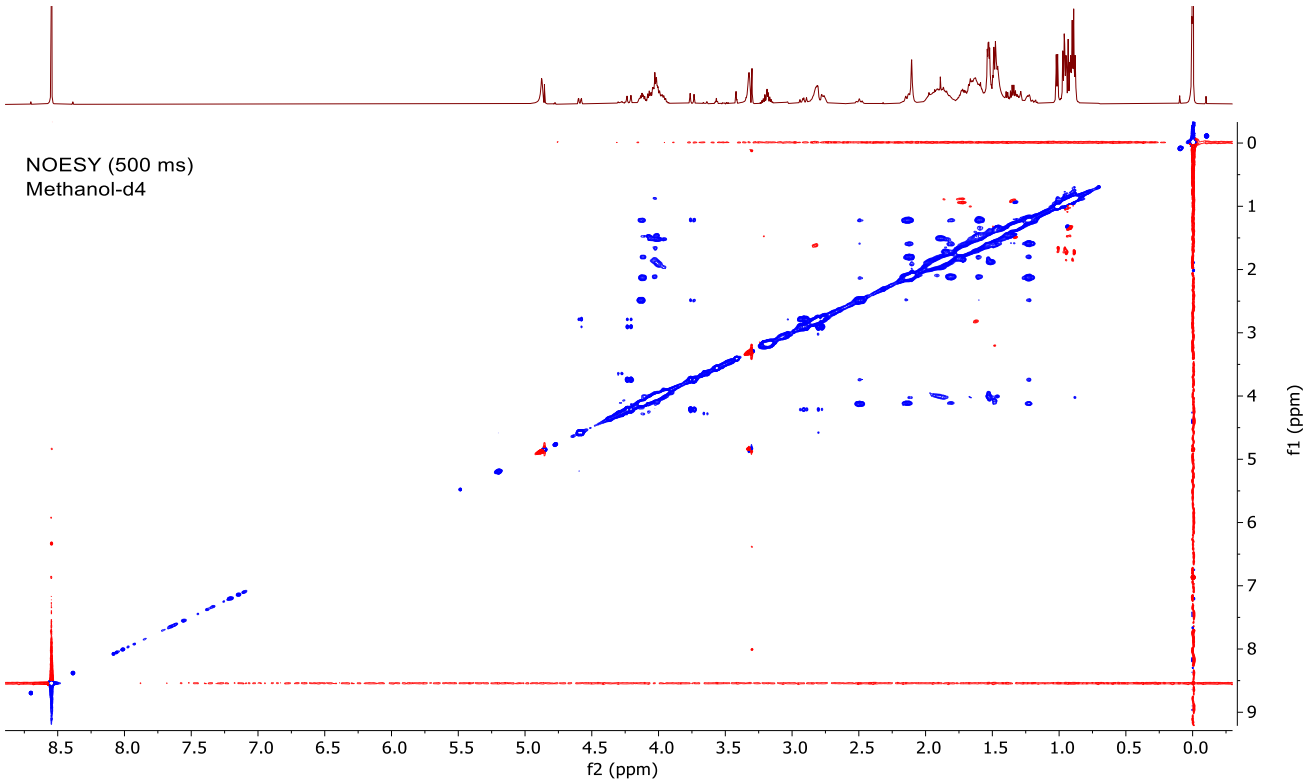


Figure S45. NOESY spectrum of peptide **5** in Methanol-d4 (mixing time: 500 ms).

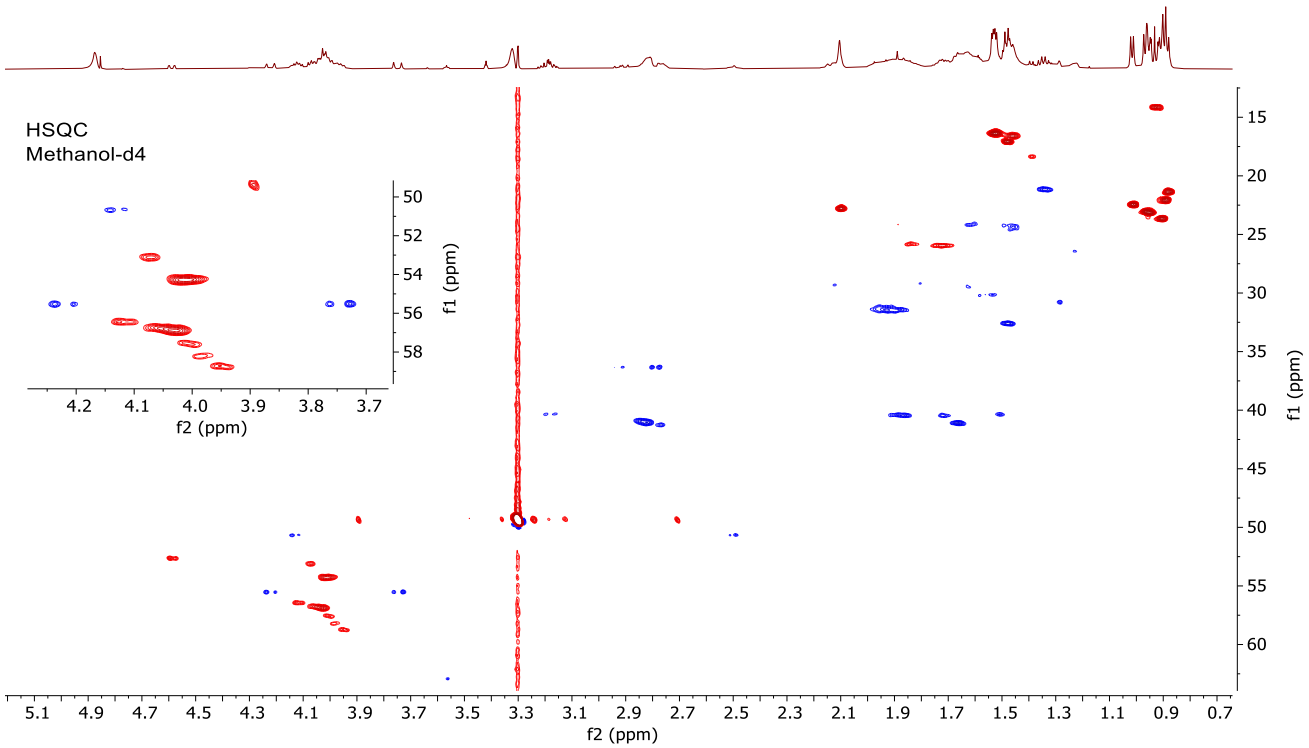
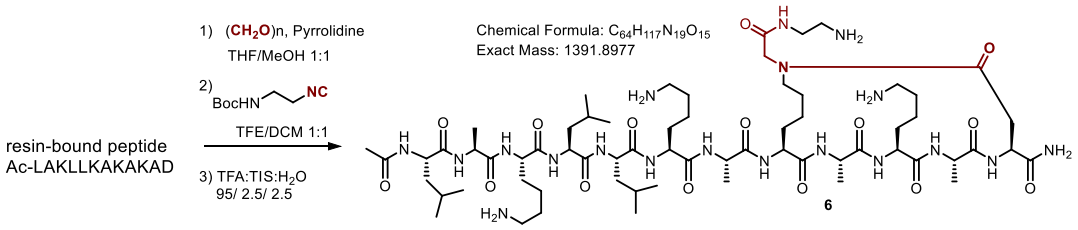


Figure S46. HSQC-edited spectrum of peptide **5** in Methanol-d4.

Ac-(*N*-((2-(aminoethyl)amino)-2-oxoethyl)-(cyclo-1,5))-[LAKLLKAKAKAD]-NH_2 (6)



Cyclic peptide **6** was produced by on-resin cyclization in a 50 µmol scale from resin-bound peptide Ac-LAKLLKAKAKAD according to the aminocatalysis-mediated Ugi macrocyclization protocol described in S2.1, in the presence of *n*-butylisocyanide. Cleavage with TFA/H_2O/TIS 95:2.5:2.5 for 2 h afforded peptide **6** (82% cleavage yield), which was purified by semi-preparative RP-HPLC (27.9 mg, 40% isolated yield); R_t 4.90 min, > 86% purity. ESI-HRMS, calcd. for $C_{64}H_{119}N_{19}O_{15}^{2+}$: 696.9567 $[M + 2H]^{2+}$ Found: *m/z* 696.9561 $[M + 2H]^{2+}$.

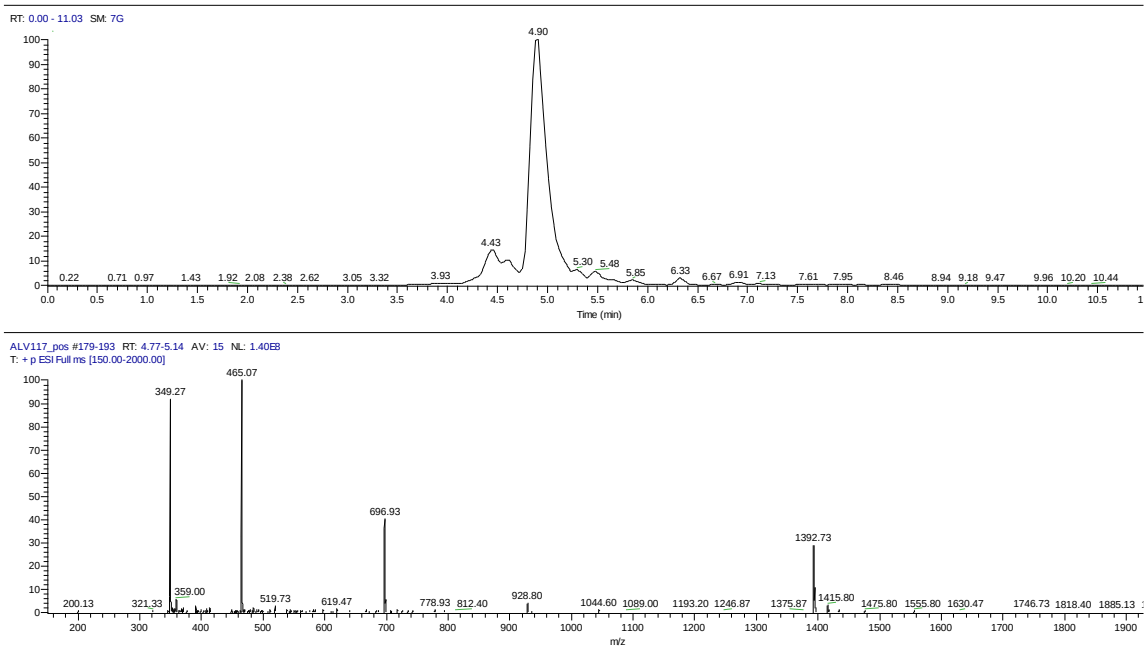


Figure S47. UHPLC-MS chromatogram of pure peptide **6**

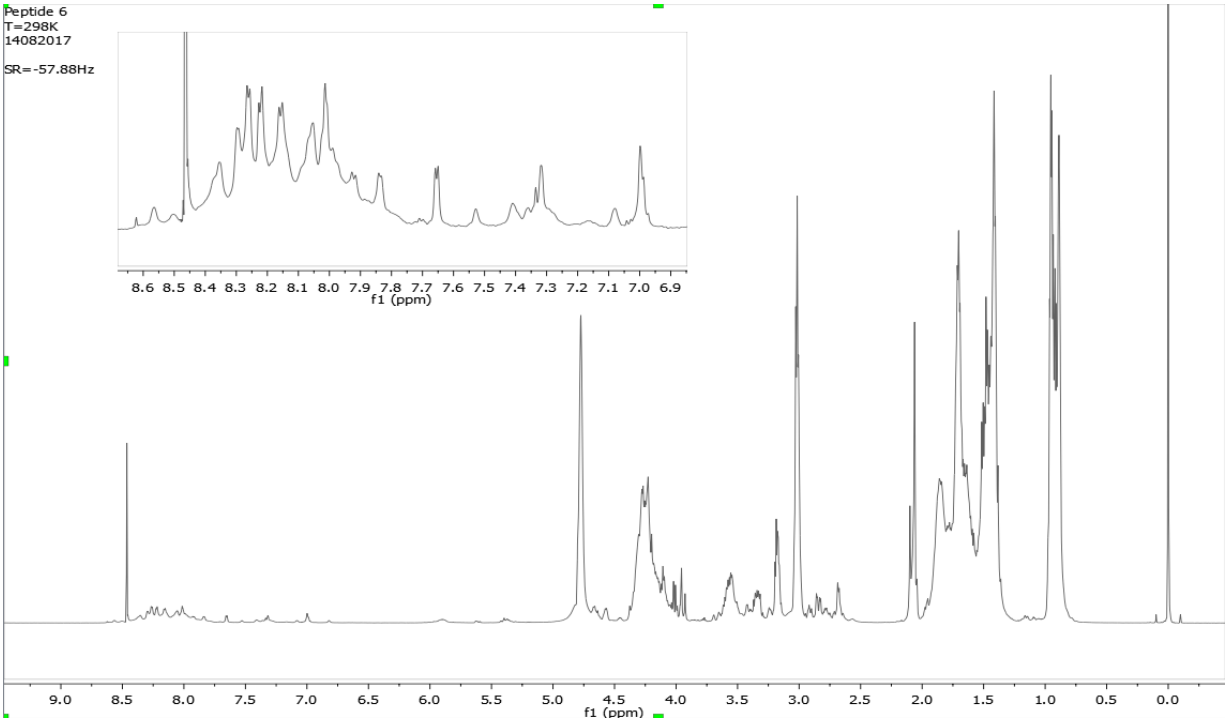
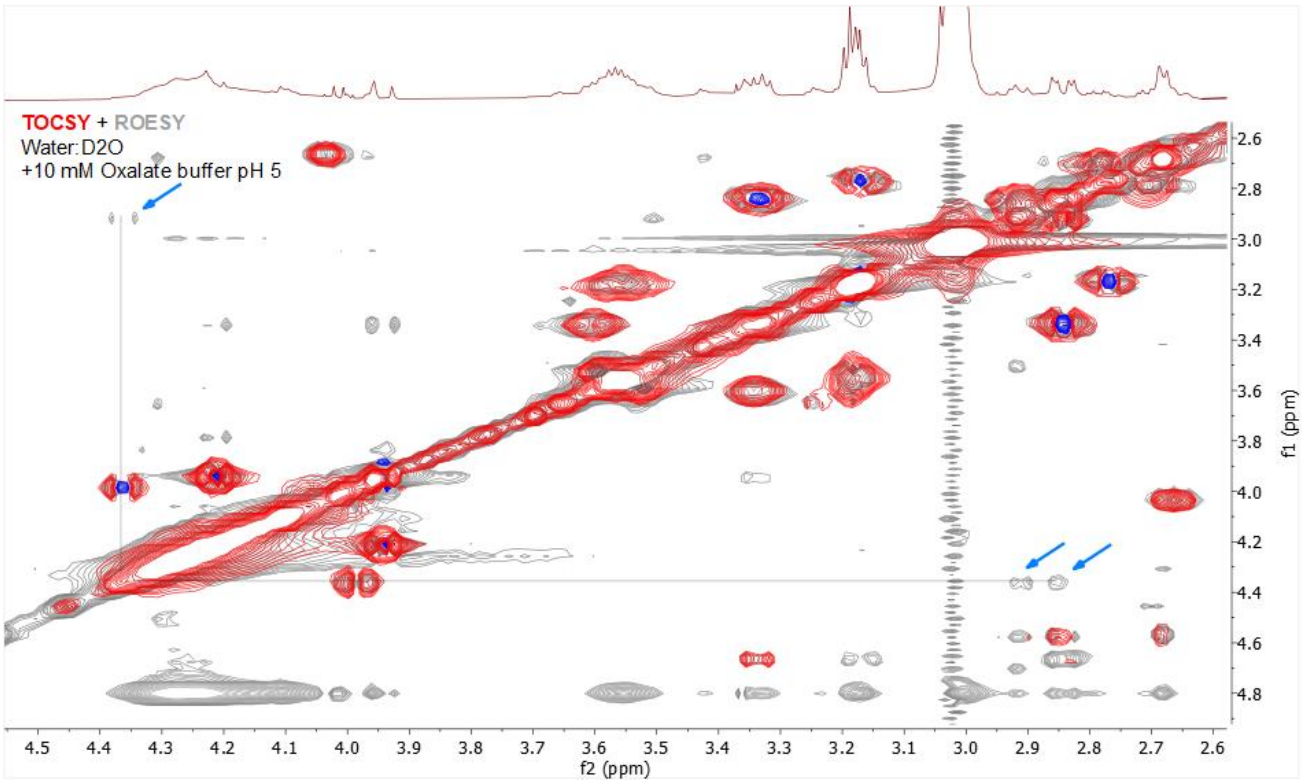


Figure S48. ^{1}H NMR spectrum of peptide **6** in a pH 5 oxalic acid/oxalate buffer in Water/D_2O 9:1 obtained using the PRESAT pulse sequence for water suppression.



Section of the superimposed zTOCSY (red) and ROESY (grey) spectra for peptide **6** in H_2O/D_2O 9:1 with pH 5 oxalate buffer. With blue arrows the crosspeaks indicating spatial proximity between the exocyclic glycine residue and the beta protons of the aspartic acid for the minor conformer (*s-cis*/*s-trans* ratio 79:21).

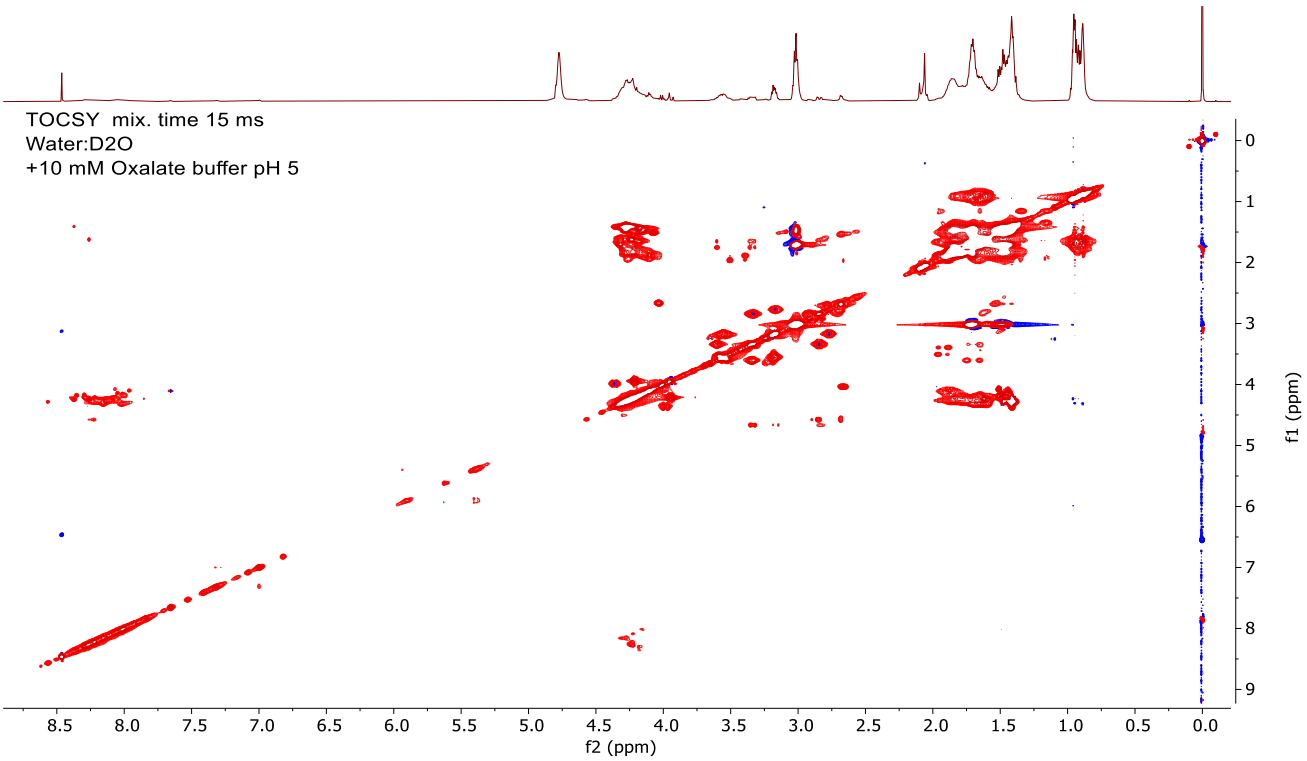


Figure S49. zTOCSY spectrum of peptide **5** in in H_2O/D_2O 9:1 with pH 5 oxalate buffer (mixing time: 15 ms).

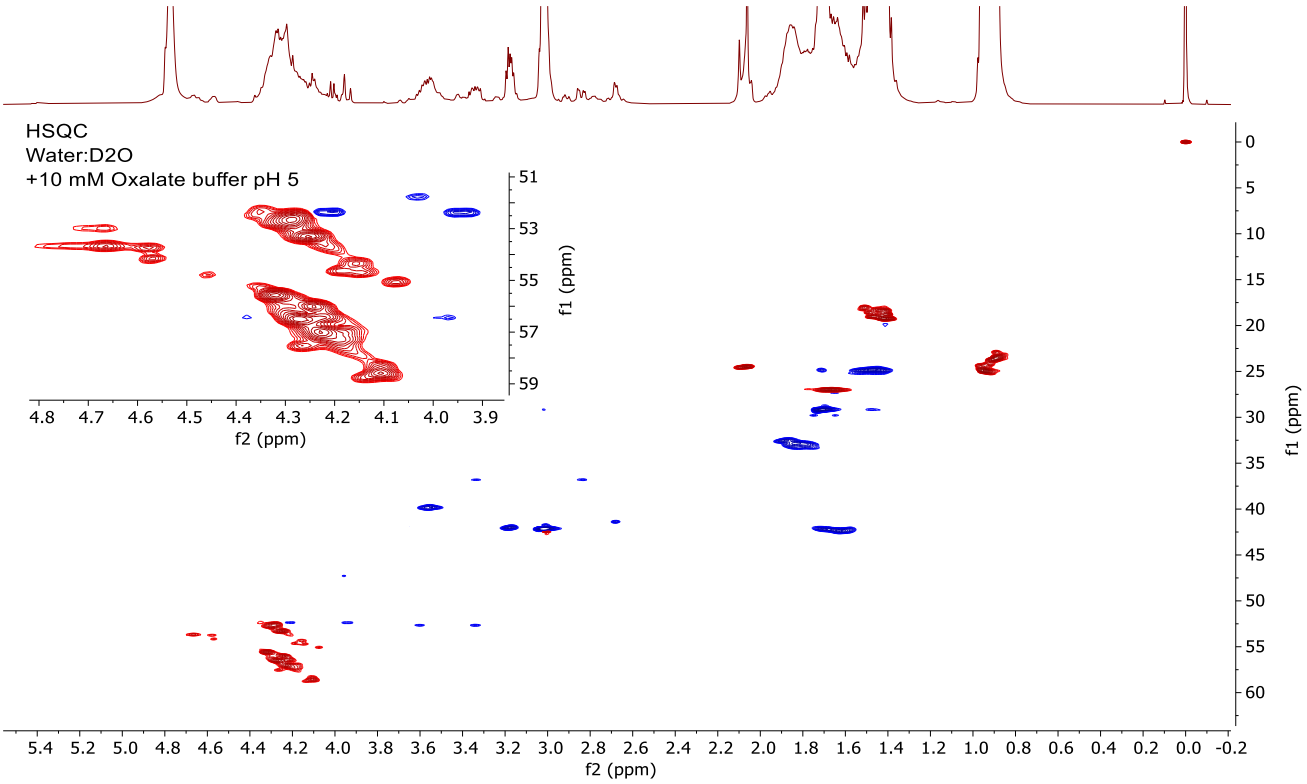


Figure S50. HSQC-edited spectrum of peptide **5** in in H_2O/D_2O 9:1 with pH 5 oxalate buffer.

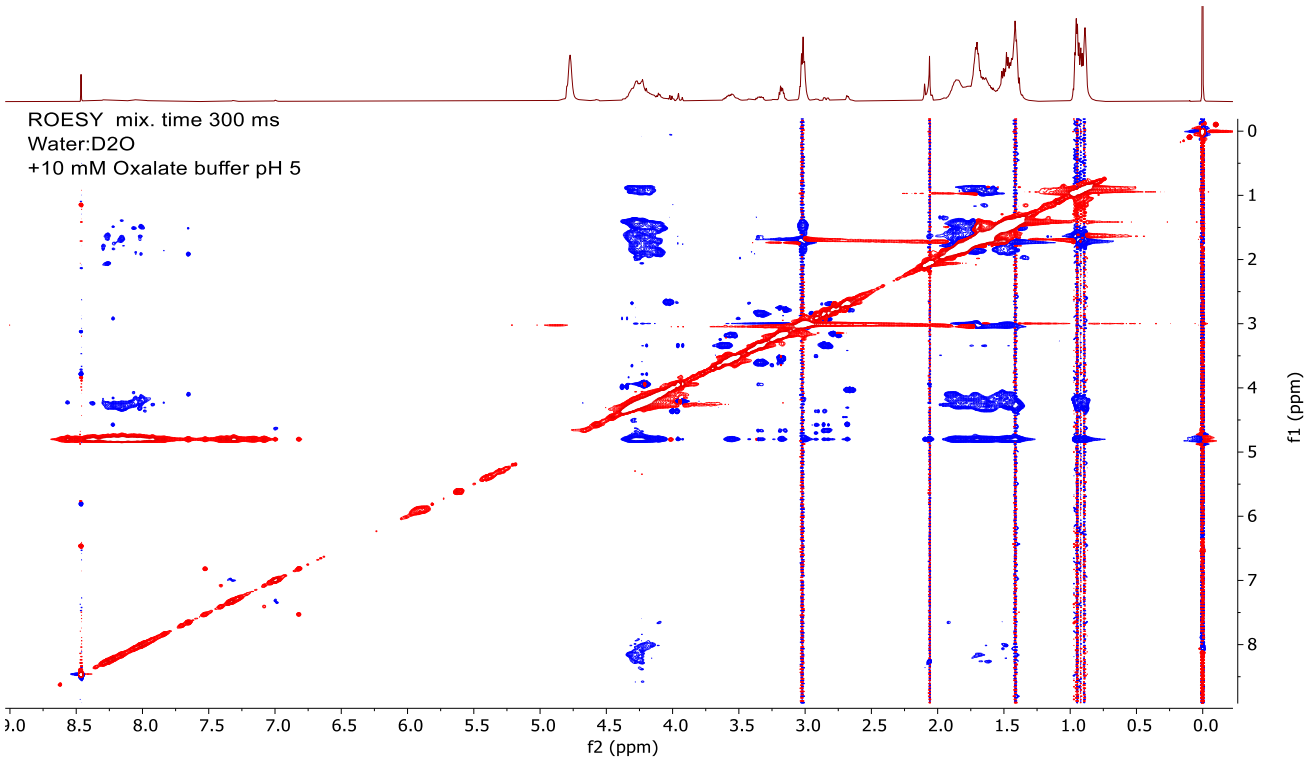


Figure S51. ROESY spectrum of peptide **5** in H_2O/D_2O 9:1 with pH 5 oxalate buffer (mixing time: 300ms).

Table S4. Ratio of conformers in different solvents

	cyclic peptide **4**			cyclic peptide **5**		
	s-cis	*s-trans*	Inferred by	*s-cis*	*s-trans*	Inferred by
Water	*not determined*			57	43	HSQC, NOESY
TFE:Water	19	81	NOESY	10	90	NOESY
MeOH	10	90	HSQC, NOESY	13	87	HSQC, NOESY

Table S5. ^{13}C Chemical shift for the exocyclic methylene group in different conformers for cyclic pentapeptides

	δ *vs.* TMSP (in ppm)	
Peptide	***s-cis***	***s-trans***
2a	52.8	56.9
2c	52.5	56.1
2d	52.4	56.2

Table S6. ^{13}C Chemical shift for the exocyclic methylene group in different conformers for cyclic dodecapepides

	cyclic peptide **4**		cyclic peptide **5**		cyclic peptide **6**	
	s-cis	*s-trans*	*s-cis*	*s-trans*	*s-cis*	*s-trans*
Water	*not determined*		52.5	56.8	52.4	56.5
TFE:Water	not determinec	57.5	*not determined*		*not determined*	
MeOH	51.0	55.5	50.7	55.3		

S3. Molecular dynamics simulations, structure determination and conformer distribution studies

S3.1. NMR structure determination

Cross-peaks in ROESY spectra were assigned and integrated in NMRFAM-SPARKY.[22] Distance constraints from ROE intensities were generated using pseudo-atoms corrections where needed, and placed into three groups: strong (2.8 Å upper limit), medium (3.5 Å upper limit) and weak (5.0 Å upper limit). The lower limit for distance restraints was always maintained at 1.8 Å. Backbone dihedral angle restraints were inferred from $^{3}J_{NHCHa}$ coupling constants in 1D spectrum at 300 K, φ was restrained to -120 ± 30° for $^{3}J_{NHCHa} \geq 8$ Hz as reported by Fairlie and co-workers.[19] Peptide backbone bond ω angles were all set *trans*. Structure calculations were carried out using *Xplor-NIH 2.43* package.[23]The calculations were performed using the standard force field parameter set and topology file within *Xplor-NIH* with in-house modifications to allow peptide cyclization and amide *N*-substitution. Structures were visualized and analyzed using *VMD-Xplor*. The *s-cis* or *s-trans* conformation around the tertiary amide was unequivocally inferred from the NMR data and fixed during the MD simulations.

NMR structure determination was performed through simulated annealing regularization and refinement in torsion angle space, using experimental data as inter-proton distances and dihedral angles restraints. For simulated annealing regularization 200 starting structures were randomly generated. A 100 ps molecular dynamics simulation at 3500 K was performed with a time-step of 3 fs. The system was cooled from 3500 to 25 K, with a temperature step of 12.5 K. At each temperature step, 0.2 ps of molecular dynamics simulation was performed. A 500 steps torsion angle minimization was performed and finally the system was optimized by means of 500 steps conjugated gradient Powell Cartesian minimization.

The refinement protocol consisted in a slow cooling simulated annealing from the regularized structures. A 10 ps molecular dynamics simulation at 1500 K was achieved with a time-step of 3 fs. The system was cooled with a temperature step of 12.5 K and a simulation time of 0.2 ps at each temperature. A 500 torsion angle minimization was performed afterwards a second 500 steps minimization was achieved in Cartesian coordinates. A finally 1000 steps Powell minimization with an energy function non-dependent of experimental restraints was executed.

S3.2. Molecular Dynamics Simulations

MD simulations were conducted within YASARA (www.yasara.org). For dodecapeptide **4**, a structure was created within MOE (www.chemcomp.com) and further imported into YASARA. For the modelling of compound **4** including two cyclolipopeptides, two molecules in an ideal helical conformation were built in MOE

and a protein-protein docking using Rigid Body refinement was assessed in order to determine the best intermolecular fitting. In all cases, the structures imported into YASARA were firstly optimized and the obtained coordinates were employed as starting point for a 100 ns MD simulation using AMBER 12 force field. The starting model was placed in a water box with a physiological NaCl concentration of 0.9%. Periodic boundary conditions with pressure control at 298 K were utilized. Force field parametrizations for the novel residues based on semi-empirical calculations were automatically calculated. The final trajectory was analyzed within YASARA, as well as VMD.[24] An initial 600ps regularization was conducted with increased weighting of the planar energy terms in order to keep the configuration close to helical. For the production phase, the weighting of all functions were reset to their standard values.

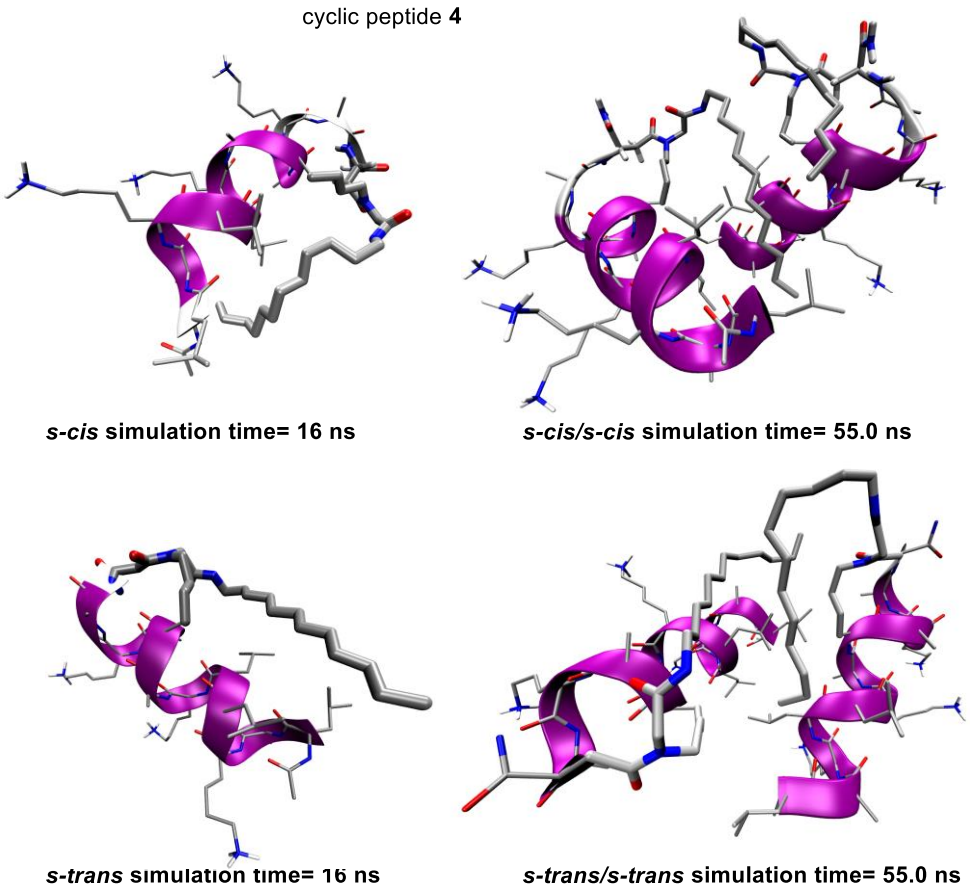


Figure S52. Snapshots of the MD simulations in explicit water solvent at pH 7.4 from ideal helical structures for cyclic peptide **4** starting from s-cis or s-trans configuration and including one or two molecules.

A) B)

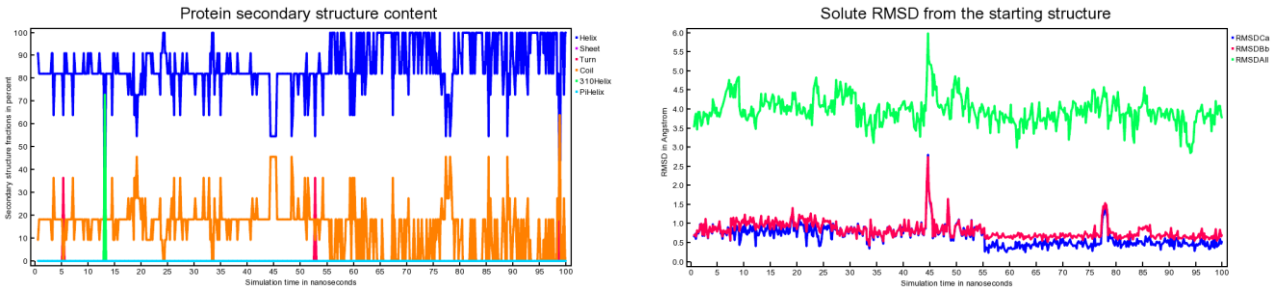


Figure S53. A) Secondary structure content (blue: helix) and B) RMSD from starting structure during 100 ns of MD simulations from a single molecule of **4** from ***s-cis*** configuration at the lactam bridge.

A) B)

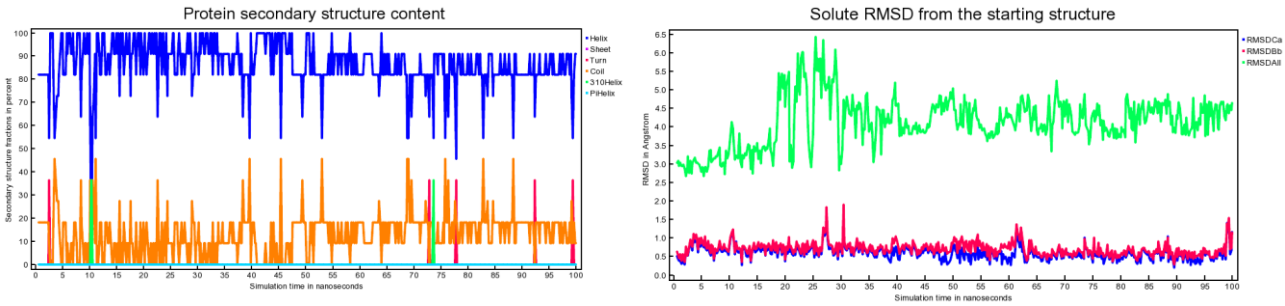


Figure S54. A) Secondary structure content (blue: helix) and B) RMSD from starting structure during 100 ns of MD simulations from a single molecule of **4** from ***s-trans*** configuration at the lactam bridge.

A) B)

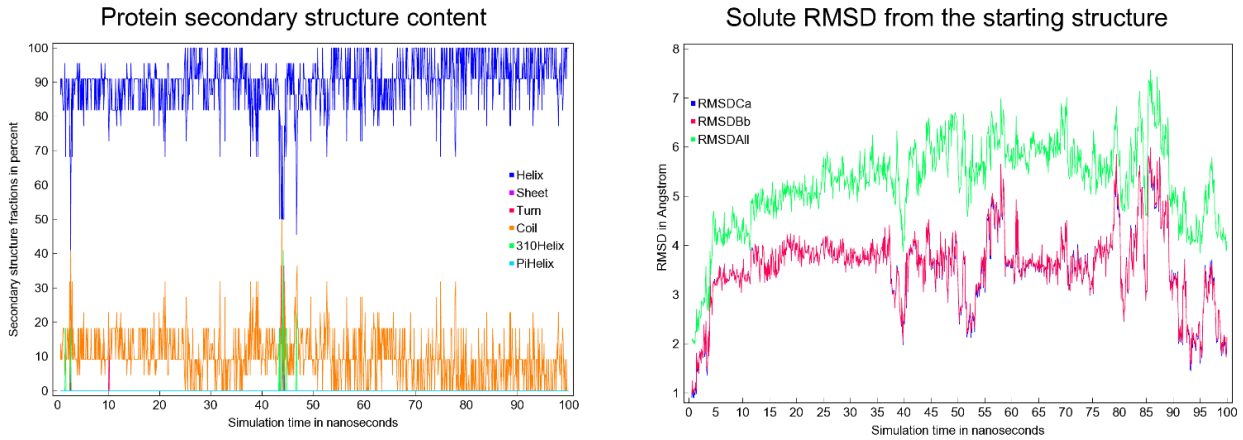


Figure S55. A) Secondary structure content (blue: helix) and B) RMSD from starting structure during 100 ns of MD simulations comprising two molecules of **4** with ***s-cis*** configuration at the lactam bridge.

A) B)

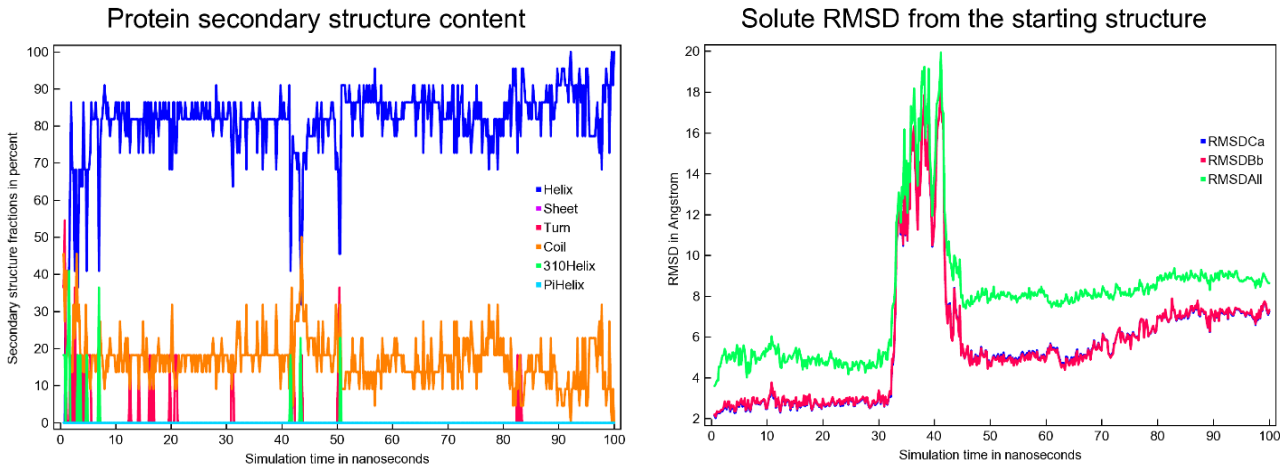


Figure S56. A) Secondary structure content (blue: helix) and B) RMSD from starting structure during 100 ns of MD simulations comprising two molecules of **4** with s-trans configuration at the lactam bridge.

S3.3. Conformer search distribution studies by Molecular Mechanics

To get further insights into the tendency of *s-cis* and *s-trans* conformers to occur in an alpha helical structure we attempted to perform a conformational search analysis within MOE (www.chemcomp.com), by restricting the peptide backbone to exist in a helical arrangement and explore different conformations by allowing rotation of side chains, including the isomerization at the lactam bridge. We utilize the LowMode conformational search method implemented in MOE and Amber10:EHT was utilized as the forcefield. Generalized Born solvation model was employed. A window of 10 kcal/mol was employed as threshold from the best structure was set to show the final conformers. Both, cyclic peptide **4** and **5** were studied resulting in 87 and 156 final structures respectively. Starting from both, *s-cis* and *s-trans* configurations at the exocyclic amide (in both cases allowing the *s-cis*/*s-trans* isomerization), all the final conformers presented *s-trans* configuration (See Fig. S59 and S60). This latter suggests that, despite the *N*-substituent, the *s-trans* conformation is thermodynamically more stable when the peptide skeleton is in the ideal helical conformation also in correspondence with the lack of helicity of the peptides when *s-cis* conformations are populated.

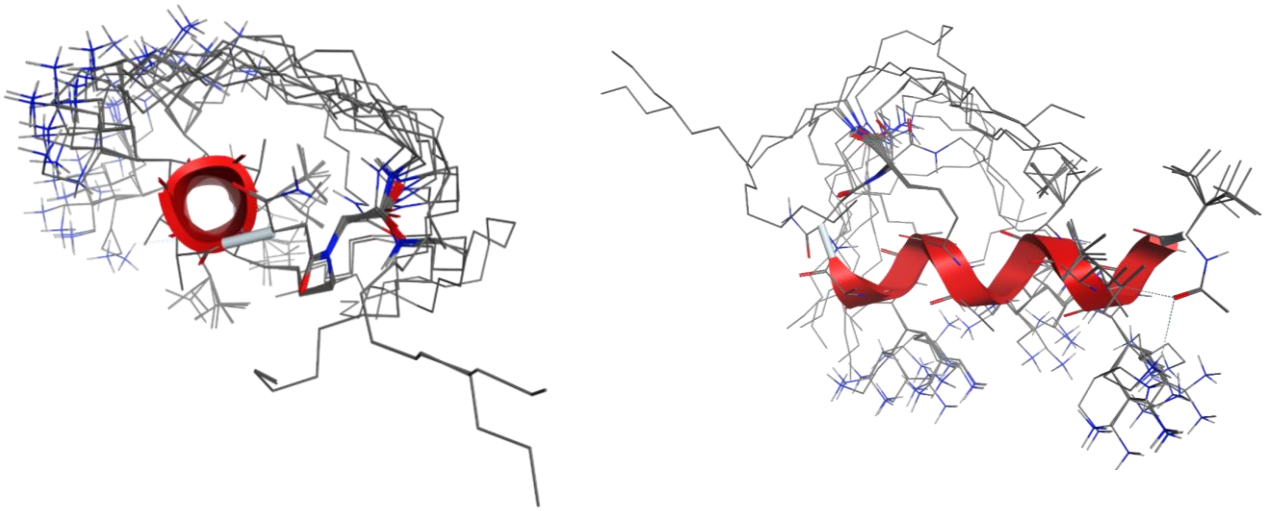


Figure S57. Superimposition of the 20 lowest energy structures resulting from the conformational search for cyclic peptide **4**, a) view from top of the helix and b) side view.

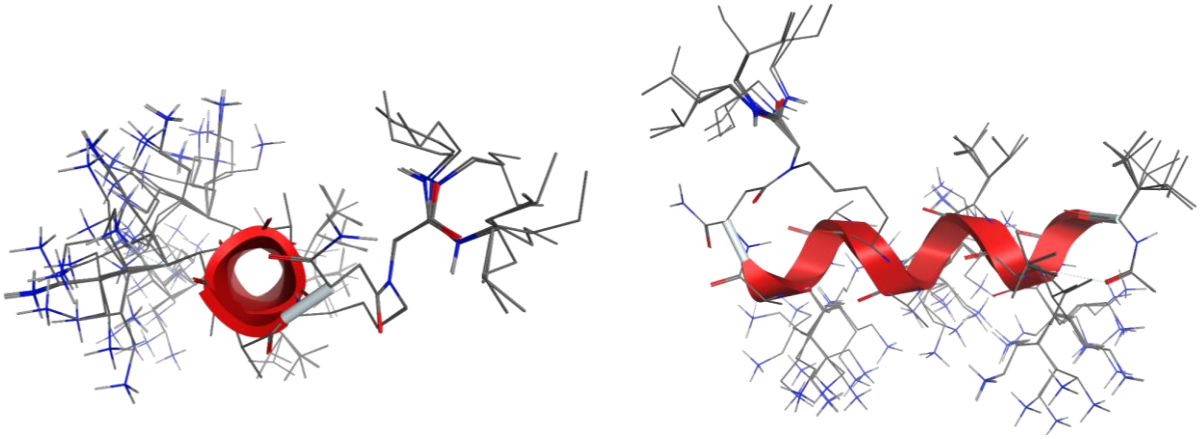


Figure S58. Superimposition of the 20 lowest energy structures resulting from the conformational search for cyclic peptide **5**, a) view from top of the helix and b) side view.

Table S7. Energy and dihedral angle at the exocyclic amide for the 87 generated conformers for cyclic peptide **4**

Conformer	E(kcal/mol)	dE	dihedral at tertiary amide (CE-N-C-CB)	Conformer	E(kcal/mol)	dE	dihedral at tertiary amide (CE-N-C-CB)
1	-219.36	0.00	175.49	51	-211.71	7.65	178.71
2	-219.17	0.19	177.13	52	-211.63	7.73	174.99
3	-219.13	0.23	176.39	53	-211.60	7.77	178.49
4	-218.65	0.72	176.15	54	-211.45	7.91	178.75
5	-218.40	0.96	176.21	55	-211.43	7.93	173.53
6	-217.46	1.90	175.47	56	-211.35	8.01	178.01
7	-217.36	2.00	175.36	57	-211.28	8.08	175.68
8	-217.33	2.03	177.62	58	-211.26	8.11	175.50
9	-216.90	2.46	176.99	59	-211.24	8.12	175.91
10	-216.64	2.73	175.66	60	-211.18	8.18	174.86
11	-216.23	3.14	178.21	61	-211.13	8.23	176.29
12	-216.11	3.25	179.80	62	-211.11	8.25	175.46
13	-215.73	3.64	177.08	63	-211.01	8.35	175.44
14	-215.64	3.72	175.90	64	-210.95	8.42	176.34
15	-215.11	4.25	174.63	65	-210.72	8.64	175.67
16	-215.08	4.28	176.63	66	-210.66	8.70	178.46
17	-214.79	4.57	177.84	67	-210.50	8.86	-179.31
18	-214.78	4.58	176.19	68	-210.46	8.90	179.83
19	-214.73	4.63	175.91	69	-210.43	8.93	176.85
20	-214.70	4.67	177.49	70	-210.37	8.99	175.55
21	-214.66	4.70	174.56	71	-210.34	9.03	175.29
22	-214.66	4.70	176.50	72	-210.19	9.17	177.69
23	-214.62	4.74	175.76	73	-210.16	9.20	175.88
24	-214.36	5.00	175.30	74	-210.00	9.37	176.66
25	-214.21	5.16	177.92	75	-210.00	9.37	-179.35
26	-214.05	5.31	175.95	76	-209.99	9.37	174.34
27	-213.84	5.52	178.56	77	-209.93	9.44	178.80
28	-213.82	5.54	175.81	78	-209.91	9.46	176.83
29	-213.81	5.56	179.15	79	-209.79	9.57	176.01
30	-213.72	5.64	175.45	80	-209.79	9.58	-166.35
31	-213.70	5.67	177.84	81	-209.79	9.58	-164.60
32	-213.52	5.84	175.43	82	-209.73	9.63	177.37
33	-213.45	5.91	175.64	83	-209.73	9.63	176.25
34	-213.38	5.98	178.40	84	-209.72	9.64	179.98
35	-213.34	6.02	176.52	85	-209.71	9.66	175.51
36	-213.29	6.07	175.73	86	-209.58	9.78	175.75
37	-213.22	6.14	175.34	87	-209.49	9.87	176.47
38	-213.04	6.32	179.37				
39	-212.89	6.47	178.00				
40	-212.82	6.54	176.94				
41	-212.78	6.58	177.95				
42	-212.69	6.67	176.32				
43	-212.67	6.69	175.08				
44	-212.41	6.96	177.58				
45	-212.25	7.12	176.83				
46	-212.19	7.17	175.31				
47	-212.11	7.25	179.29				
48	-212.09	7.27	-178.71				
49	-211.89	7.48	174.96				
50	-211.80	7.56	174.90				

Table S8. Energy and dihedral angle at the exocyclic amide for the 87 generated conformers for cyclic peptide **4**

Conformer	E(kcal/mol)	dE	dihedral at tertiary amide (CE-N-C-CB)	Conformer	E(kcal/mol)	dE	dihedral at tertiary amide (CE-N-C-CB)	Conformer	E(kcal/mol)	dE	dihedral at tertiary amide (CE-N-C-CB)
1	-229.09	0.00	175.89	61	-222.56	6.53	175.63	121	-220.22	8.87	175.29
2	-229.04	0.05	176.06	62	-222.55	6.54	175.58	122	-220.15	8.93	175.80
3	-227.34	1.75	176.19	63	-222.54	6.55	175.90	123	-220.15	8.94	175.67
4	-227.33	1.76	175.50	64	-222.54	6.55	175.24	124	-220.14	8.95	175.38
5	-227.12	1.97	175.76	65	-222.50	6.59	178.80	125	-220.03	9.06	176.19
6	-226.90	2.19	175.94	66	-222.45	6.64	175.46	126	-220.02	9.07	175.81
7	-226.34	2.75	175.93	67	-222.45	6.64	176.06	127	-219.97	9.12	175.85
8	-226.27	2.82	176.06	68	-222.34	6.75	175.89	128	-219.93	9.16	175.80
9	-226.21	2.88	175.54	69	-222.34	6.75	175.33	129	-219.88	9.21	175.81
10	-226.09	3.00	176.06	70	-222.32	6.77	175.72	130	-219.87	9.21	175.82
11	-225.78	3.30	175.63	71	-222.30	6.79	175.63	131	-219.87	9.22	175.42
12	-225.61	3.48	175.41	72	-222.21	6.88	175.67	132	-219.76	9.33	175.41
13	-225.50	3.58	175.33	73	-222.19	6.90	178.59	133	-219.68	9.40	175.81
14	-225.27	3.82	175.28	74	-222.18	6.91	175.76	134	-219.67	9.42	175.63
15	-225.26	3.83	176.08	75	-222.09	6.99	175.46	135	-219.63	9.46	-165.87
16	-224.86	4.22	176.06	76	-221.93	7.16	175.38	136	-219.59	9.50	175.28
17	-224.77	4.32	175.20	77	-221.85	7.24	175.49	137	-219.58	9.51	175.85
18	-224.73	4.36	175.73	78	-221.77	7.32	176.21	138	-219.49	9.60	175.33
19	-224.70	4.39	175.80	79	-221.73	7.36	174.73	139	-219.48	9.61	-165.44
20	-224.60	4.48	175.85	80	-221.67	7.42	175.63	140	-219.46	9.63	175.97
21	-224.59	4.50	175.63	81	-221.61	7.48	175.50	141	-219.44	9.64	175.33
22	-224.58	4.51	175.89	82	-221.57	7.51	175.80	142	-219.41	9.68	-165.94
23	-224.47	4.62	176.15	83	-221.50	7.59	177.92	143	-219.40	9.69	175.84
24	-224.46	4.62	175.93	84	-221.49	7.60	176.31	144	-219.40	9.69	175.33
25	-224.46	4.63	176.01	85	-221.36	7.73	175.59	145	-219.39	9.70	175.68
26	-224.43	4.66	175.72	86	-221.35	7.74	175.89	146	-219.38	9.71	175.54
27	-224.36	4.73	175.38	87	-221.33	7.76	175.45	147	-219.37	9.72	175.24
28	-224.34	4.75	175.59	88	-221.31	7.78	175.38	148	-219.37	9.72	175.37
29	-224.34	4.75	175.72	89	-221.23	7.86	176.19	149	-219.27	9.82	175.94
30	-224.22	4.87	175.59	90	-221.23	7.86	175.29	150	-219.23	9.85	176.53
31	-224.21	4.88	175.79	91	-221.23	7.86	175.85	151	-219.23	9.86	175.63
32	-224.13	4.96	175.49	92	-221.20	7.89	175.58	152	-219.23	9.86	175.37
33	-224.13	4.96	174.39	93	-221.18	7.91	176.19	153	-219.21	9.88	175.80
34	-224.08	5.01	175.89	94	-221.15	7.94	175.71	154	-219.19	9.89	175.73
35	-223.98	5.10	175.19	95	-221.11	7.98	175.85	155	-219.19	9.90	-165.39
36	-223.92	5.17	175.89	96	-221.11	7.98	175.84	156	-219.18	9.91	175.63
37	-223.91	5.18	175.42	97	-221.07	8.02	175.64				
38	-223.84	5.25	175.50	98	-221.06	8.03	175.58				
39	-223.80	5.29	175.41	99	-221.01	8.07	175.81				
40	-223.71	5.38	175.76	100	-220.98	8.11	175.60				
41	-223.61	5.48	175.80	101	-220.96	8.13	175.68				
42	-223.56	5.52	175.63	102	-220.93	8.16	175.41				
43	-223.49	5.60	175.84	103	-220.92	8.17	175.85				
44	-223.46	5.63	175.37	104	-220.88	8.21	175.72				
45	-223.45	5.64	175.25	105	-220.85	8.23	176.06				
46	-223.37	5.72	175.77	106	-220.85	8.24	175.64				
47	-223.28	5.81	175.63	107	-220.80	8.29	175.63				
48	-223.27	5.82	175.68	108	-220.79	8.30	175.29				
49	-223.22	5.87	175.46	109	-220.74	8.35	175.73				
50	-223.21	5.88	176.02	110	-220.72	8.37	175.42				
51	-223.20	5.89	175.81	111	-220.69	8.40	175.41				
52	-223.17	5.92	175.85	112	-220.64	8.45	176.10				
53	-223.10	5.99	175.45	113	-220.63	8.45	175.37				
54	-223.03	6.05	175.38	114	-220.59	8.50	175.34				
55	-223.01	6.07	175.41	115	-220.55	8.54	175.89				
56	-222.94	6.15	175.80	116	-220.47	8.61	175.54				
57	-222.89	6.19	175.67	117	-220.37	8.72	177.01				
58	-222.71	6.38	175.71	118	-220.36	8.73	176.27				
59	-222.67	6.41	175.80	119	-220.30	8.79	175.80				
60	-222.57	6.52	176.10	120	-220.23	8.86	175.47				