SUPPORTING INFORMATION

Halogen bonding: a powerful tool for modulation of peptide conformation.

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Table of content

1. Nomenclature and peptide numbering

The peptides included in this paper are shown in Figure S1. The synthesis, full characterization and conformational analysis of peptides **1a-b** and **2a-b** are provided in reference 1 and 2. The synthesis, full characterization and conformational analysis of peptides **3a-b**, **4a-b**, **5** and **8** are given below. The following amino acid abbreviations have been used in this supporting information: D-P= D-Proline, G=Glycine, A=Alanine, S=Serine, V=Valine, N=Asparagine, Q=Glutamine, S(Me)=*O*-methyl-serine, hS(Me)=*O*-methyl-homoserine, X=2-Aminobuty acid, A(Cl)=3-Chloroalanine, A(Br)=3-Bromoalanine. For side chain positions the following notations have been used: alfa (α), beta (β), gamma (γ), delta (δ) and epsilon (ε).

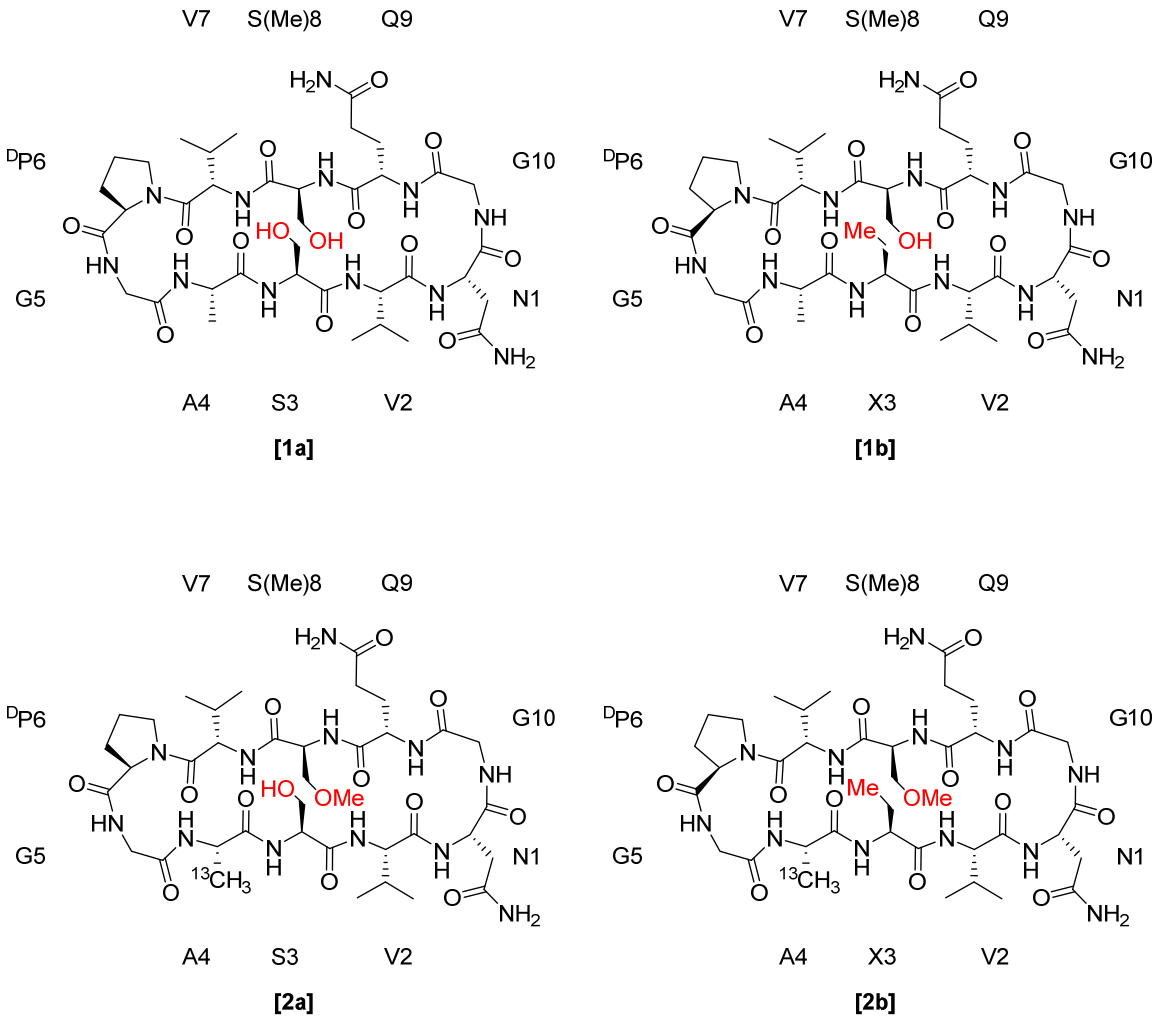
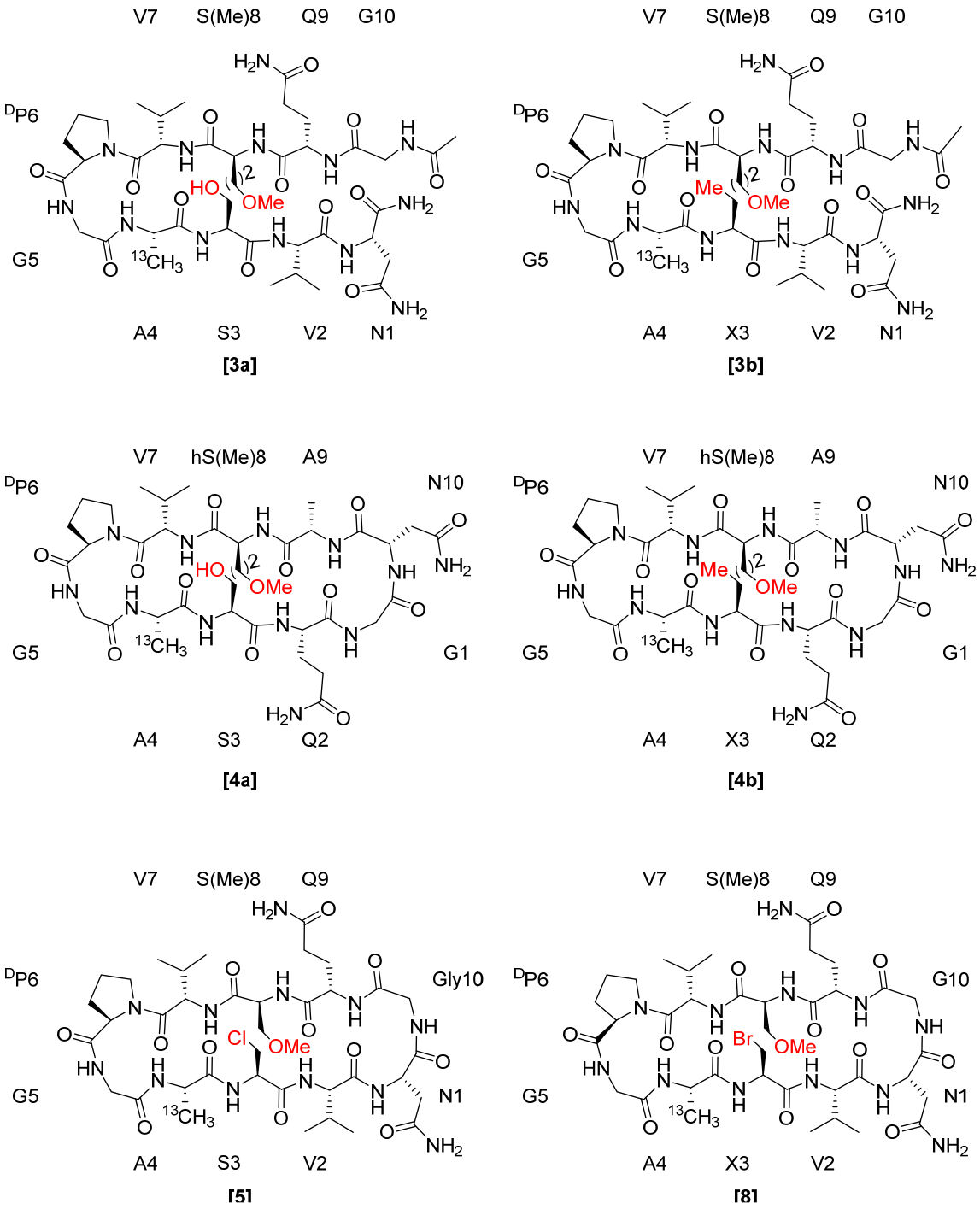


Figure S1. Structures of the included cyclic peptides, with the numbering used and the amino acids given as three letter acronyms. The synthesis and characterization of these peptides are previously reported.[1,2] *Figure S1 continued on p. S3.*



Continued Figure S1. Structures of the investigated cyclic peptides, with the numbering used and the amino acids given as three letter acronyms. The synthesis and characterization of these peptides are given below.

2. Peptide Synthesis

2.1 General information

The synthetic schemes for peptides **3a-b, 4a-b, 5 and 8** are outlined in scheme S1–4. Commercially available reagents and solvent were used without further purification. Asparagine substituted Rink MBHA resin (Fmoc-L-Asn(Trt)-Rink MBHA, loading 0.58 mmol/g), Fmoc-L-Ser(Me)-OH, Fmoc-L-Hse(Me)-OH and Fmoc-L-Ala(3-Cl)-OH were purchased from Iris Biotech. All other Fmoc-protected amino acids, glycine substituted 2-chlorotrityl resin (loading 0.46 mmol/g) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) were purchased from AAPPtec. Dimethyl formamide (DMF, ACS reagent grade), piperidine (99%), *N*,*N*-Diisopropylethylamine (DIPEA), triisopropyl silane (TIPS), Fmoc-[3-^{13}C]-Ala-OH, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), 2,4,6-trimethyl pyridine (TMP), and trifluoroacetic acid (TFA) were obtained from Sigma Aldrich. 3-(Trimethylammonium)propyl carbonate-functionalized silica gel (loading 0.46 mmol/g) was obtained from Sigma Aldrich/Silicycle inc. C_{18} – cartridges were obtained from Isolute. Solid phase peptide synthesis was carried out using an automated benchtop PS3 Peptide Synthesizer from Protein Technologies. Analytical reversed phase liquid chromatography (RP-HPLC)–mass spectrometry (MS) was performed on a API SCIEX 150 EX Perkin Elmer ESI-MS (30 eV) connected to a Perkin Elmer gradient pump system and a C8 column (120 Å, 4 μm, 4.6 × 50 mm) using gradients of acetonitrile/water (CH_3CN/H_2O) with 1% formic acid (HCOOH) as mobile phase at a flow rate of 1 ml/min. Preparative RP-HPLC was performed on a VWR LaPrep system with single wavelength detection (220 nm), using a Gemini Phenomenex C_{18} column (110 Å, 10 μm, 21.2 × 250 mm) and gradients of CH_3CN/H_2O (0.1% HCOOH) as mobile phase at a flow rate of 20 ml/min. High resolution LCMS analyses were performed on a Halo RP-Amide column (2.1 × 50 mm) with a flow of 250 μl/min and with a gradient of 5–95% ACN/MeOH 1:1 in H_2O. TOF-MS was used with 1 scan/s and extern calibration.

2.2 General procedure of peptide synthesis

The linear peptides were synthesized following the standard Nα-Fmoc protecting group strategy[3] on a 300 or 400 μmol scale. *Tert*-butyl (Ser) and Trityl (Gln, Asn) were used as side chain protecting groups. Before the first coupling, the resin was swollen in DMF for 3 × 10 min. Coupling reactions were performed using TBTU (2 equiv.) and DIPEA (4 equiv.) in DMF (3 ml/coupling). All solvents were stored over 3 Å molecular sieves activated at 220 °C before use. Double couplings were used for the peptide coupling steps (2 × 60 min, except for the first coupling in the synthesis and coupling to D-Pro or Val for which 2 × 90 min was used). Two equivalents were used of all amino acids except for Fmoc-[3-^{13}C]-Ala-OH of which 1.5 equivalents was used. Capping of unreacted sites was completed after each coupling with 20% acetic anhydride in DMF (5 ml) for 20 min. Fmoc-deprotection was accomplished by treatment with 20% piperidine in DMF (5 mL, 3 × 5 min).

2.3 Synthesis and purification protocols

Synthesis of linear peptides 3a and 3b. The linear peptides **3a** and **3b** were synthesized as described above using Fmoc-Asn(Trt)-Rink MBHA resin on 0.3 mmol scale (Scheme S1). After synthesis of the full peptide and removal of the final Fmoc-group, the resulted free amine was capped with 20% acetic anhydride in DMF (5 ml). The resin was transferred to a syringe fitted with a porous polypropylene frit and was washed several times with DCM. The peptide was then cleaved from the resin and the side chain protecting groups were removed by addition of TFA/TIPS/H_2O (95:2.5:2.5, 10 mL). The syringe was put on a shaker for 2 h and the cleavage was monitored with LCMS analysis. The solution containing the cleaved peptide was filtered and the resin was washed with TFA followed by DCM. The combined filtrates

were concentrated under a stream of nitrogen gas and the peptide was precipitated from ice cooled diethyl ether (20–30 ml DEE/1 ml DCM). The resulting precipitate was filtered and washed with cold ether and the isolated crude was dried under vacuum. The crude peptides were stored at −18 °C.

Purification of linear peptides 3a and 3b by RP-HPLC. 3a was purified using a gradient of 5–55% CH_3CN in H_2O (0.1% HCOOH) over 54 min at a flow rate of 20 ml/min and UV detection at λ = 220 nm. After evaporation **3a** was isolated as a white solid to a total yield of 34% over the full synthesis. MS m/z $[M+H]^+$ calculated for $C_{40}{}^{13}CH_{69}N_{13}O_{15}$: 985.51. Found: 985.5148. **3b** was purified using a gradient of 5–75% CH_3CN in H_2O (0.1% HCOOH) over 50 min at a flow rate of 20 ml/min and UV detection at λ = 220 nm. After evaporation **3b** was isolated as a white solid to a total yield of 45% over the full synthesis. MS m/z $[M+H]^+$ calculated for $C_{41}{}^{13}CH_{71}N_{13}O_{14}$: 983.53. Found: 983.5355.

Synthesis of linear peptides 11a and 11b. The linear peptides were synthesized as described above using H-Gly-2-chlorotrityl resin on 0.3 mmol scale (Scheme S2). After synthesis of the full peptide and removal of the final Fmoc-group, the resin was transferred to a fritted column with a teflon T-bore stopcock and was washed several times with DCM. The linear peptide was cleaved from the resin by addition of 1% TFA in DCM. Nitrogen was gently bubbled through the mixture for approximately 5 min and the solution containing the cleaved peptide was then filtered into a vial containing 10% pyridine/MeOH. The procedure was repeated 6 times and the resin was then washed with several portions of DCM and MeOH. The combined filtrates were concentrated under a stream of nitrogen gas and the peptide was precipitated from water (20–30 ml water/1 ml DCM/MeOH solution). The resulting precipitate was filtered off and washed with water. The isolated crude peptide was dried under vacuum and was then were stored at −18 °C.

Synthesis of cyclic peptides 4a and 4b. Head-to-tail macrolactamization was performed in solution under pseudo-high dilution conditions.[4] The linear peptide precursor (**11a** or **11b**, 0.11 mmol) was dissolved in dry DMF (12 ml). Molecular sieves (3 Å) were added and the vial was flushed with nitrogen. HATU (3 equiv.) was dissolved in another portion of dry DMF (12 mL) under N_2. The two solutions were transferred to syringes and added dropwise, under N_2, into a round-bottomed flask containing HATU (0.1 equiv.), DMF (12 mL) and DIPEA (6 equiv.). The addition was performed a rate of 10 µL/min using a dual syringe-pump. After complete addition, the reaction mixture was stirred for another 15 min. LCMS analysis showed full conversion of the starting material. In order to remove 1-hydroxy-7-azabenzotriazole (HOAt) the reaction mixture was filtered through a plug of 3-(trimethylammonium)propyl carbonate-functionalized silica gel (1 g, 1.3 equiv. relative to HATU). The plug was rinsed with DMF until no product could be observed by LCMS in the filtrate. The DMF solution was then cooled on ice and slowly, while cooling, diluted with distilled water to a 1:1 mixture of DMF/water. The solution was then passed through a C18 cartridge (5 g, end-capped) conditioned with water. The column was run using a gradient of water in CH_3CN (50–0%) and the peptides were eluted with CH_3CN/water at 85:15. The solvent was removed under reduced pressure and the crude cyclic peptide was obtained as a white solid. For deprotection of amino acid side chains a mixture of TFA/H_2O/TIPS (95:2.5:2.5) was added to the crude peptide at 0 °C. The mixture was allowed to reach room temperature and was then stirred for two hours until LCMS showed full conversion of the starting material. The TFA was evaporated under nitrogen flush and cold ether was added to precipitate the product. The precipitate was filtered, washed with cold ether and dried under vaccum. The crude peptides were stored at −18 °C.

Purification of cyclic peptides 4a and 4b by RP-HPLC. 4a and **4b** were purified using a gradient of 5–55% CH_3CN in H_2O (0.1% HCOOH) over 54 min at a flow rate of 20 ml/min and UV detection at λ = 220 nm. After evaporation **4a** was isolated as a white solid to a total yield of 6% over the full synthesis. MS m/z [M+H]+ calculated for $C_{36}{}^{13}CH_{60}N_{12}O_{14}$: 898.44. Found: 898.4463. **4b** was isolated as a white solid to a total yield of 10% over the full synthesis. MS m/z [M+H]+ calculated for $C_{37}{}^{13}CH_{62}N_{12}O_{13}$: 896.46. Found: 896.4671.

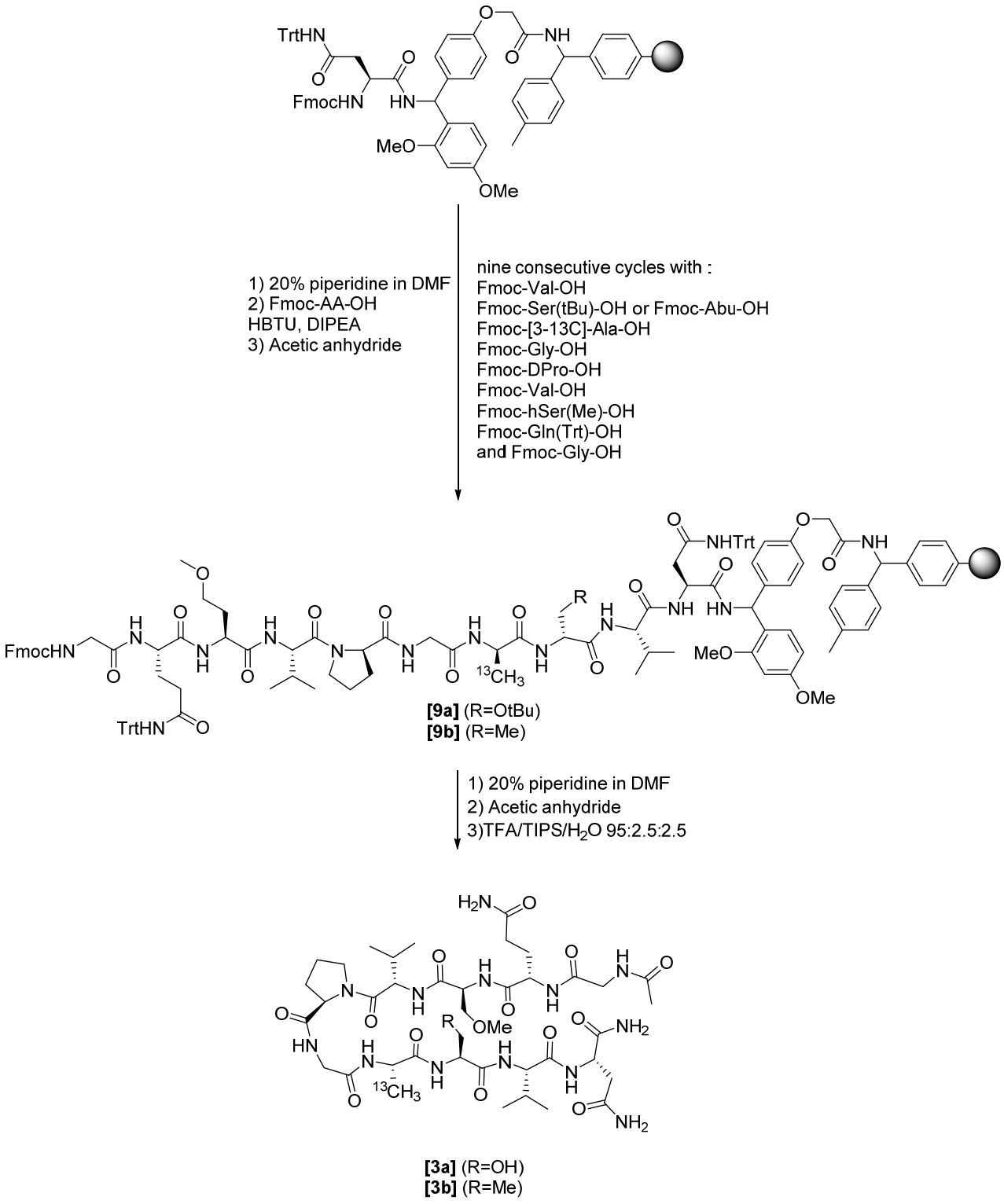
Synthesis of linear peptide 7. The linear peptide **6** was synthesized as described above using H-Gly-2-chlorotrityl resin on 0.4 mmol scale (Scheme S3). After removal of the final Fmoc-group, the resin was removed from the peptide synthesizer and transferred to a syringe fitted with a porous polypropylene frit and stopper, and several portions of DMF were added to wash the resin. The last two amino acids (Fmoc-Ala(3-Cl)-OH and Fmoc-[3-^{13}C]-Ala-OH) were coupled manually (2 × 1 h) in DMF with HATU (2 equiv.) as coupling reagent and TMP (2 equiv.) as a base. With these conditions no trace of the β-eliminated peptide was observed by LC-MS/UV. Fmoc-deprotection was carried out with fast addition (1 min) of 20% piperidine (3 mL), giving product and β-eliminated byproduct in a 2:1 ratio. The coupling reactions were followed by LCMS and the Kaiser test was carried out to ensure complete coupling. After the final Fmoc-deprotection, the resin was washed with DCM and dried under a steam of nitrogen gas and then under vacuum. The resin was stored at −18 °C. In order to cleave the peptide from the solid support, the resin was swollen in DCM for 30 min followed by addition of 1% TFA in DCM. The solution was mixed by rotation on a tube rotator for 5 min and was then filtered into a vial containing 10% pyridine/MeOH. The procedure was repeated 6 times and the resin was then washed with several portions of DCM and MeOH. The combined filtrates were concentrated under a stream of nitrogen gas and the peptide was precipitated from water (20–30 ml water/1 ml DCM/MeOH solution). The crude peptide was filtered off and dried under vacuum.

Synthesis of cyclic peptide 5. Head-to-tail macrolactamization was performed in solution under pseudo-high dilution conditions.[4] The linear peptide precursor 7 (0.12 mmol) was dissolved in dry DMF (14 ml). Molecular sieves (3 Å) were added and the vial was flushed with nitrogen. HATU (3 equiv.) was dissolved in another portion of dry DMF (14 mL) under N_2. The two solutions were transferred to syringes and added dropwise, under N_2, into a round-bottomed flask containing HATU (0.1 equiv.), DMF (14 mL) and TMP (6 equiv.). The addition was carried out a rate of 10 µL/min using a dual syringe-pump. After complete addition, the reaction mixture was stirred for another 15 min. LCMS analysis showed full conversion of the starting material. The reaction mixture was then cooled on ice and slowly, while cooling, diluted with MQ-H_2O to a 1:1 mixture of DMF/ H_2O. The solution was then passed through a C18 cartridge (5 g, end-capped) conditioned with water. The column was run using a gradient of water in acetonitrile (70–0%) and the peptides were eluted with CH_3CN/water at 85:15. The solvent was removed under reduced pressure and the crude cyclic peptide was obtained as a white solid. For deprotection of amino acid side chains a mixture of TFA/H_2O/TIPS (95:2.5:2.5) was added to the crude peptide at 0 °C. The mixture was allowed to reach room temperature and was then stirred for 2.5 hours until LCMS showed full conversion of the starting material. The TFA was evaporated under nitrogen flush and cold ether was added to precipitate the product. The precipitate was filtered, washed with cold ether and dried under vaccum. The crude peptide was stored at −18 °C.

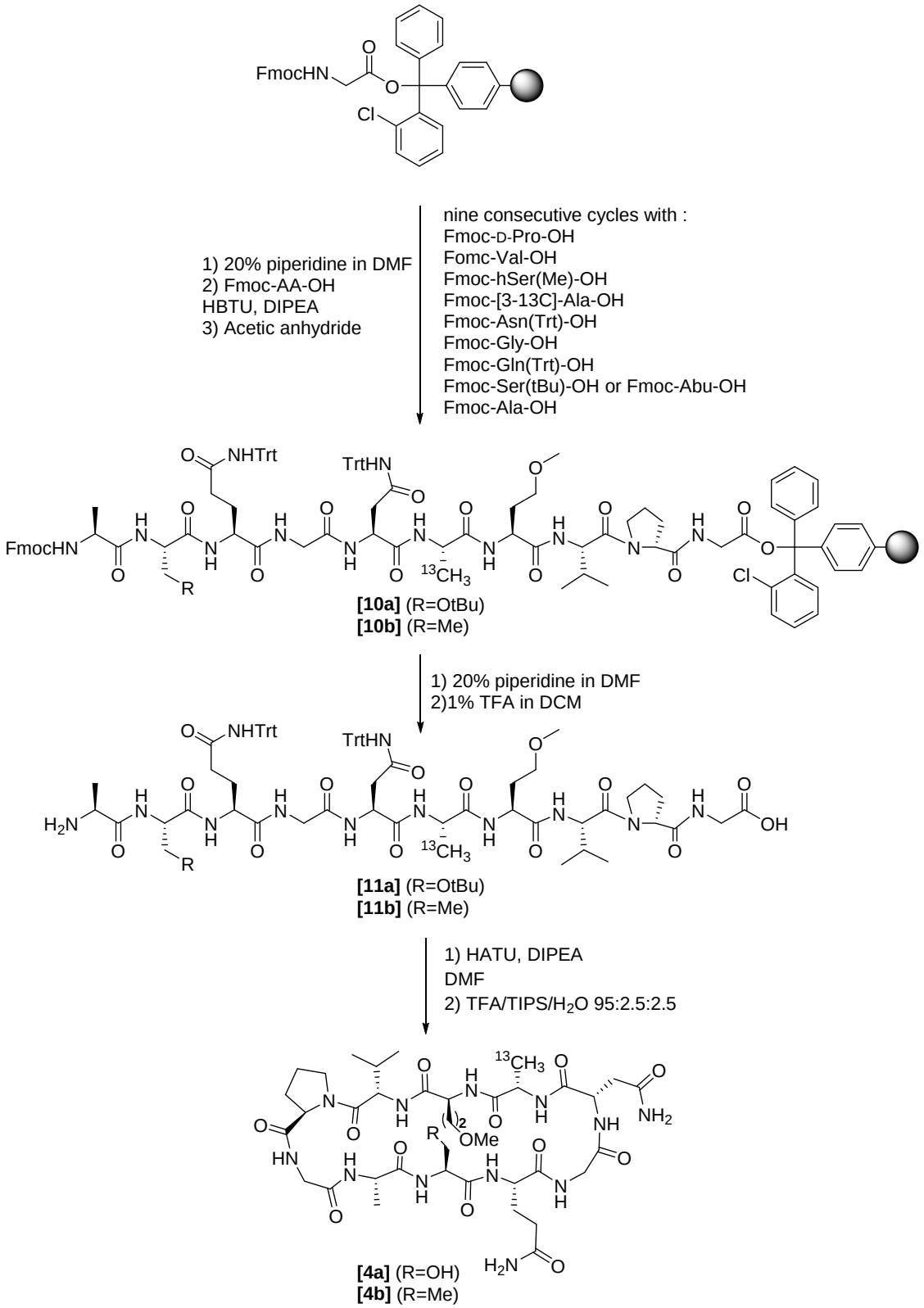
Purification of cyclic peptide 5 by RP-HPLC. Peptide **5** was purified using a gradient of 10–90% CH_3CN in H_2O (0.1% HCOOH) over 33 min at a flow rate of 20 ml/min and UV detection at λ = 220 nm. After evaporation **5** was isolated as a colorless solid to a total yield of 18% over the full synthesis. MS m/z $[M+H]^+$ calculated for $C_{37}{}^{13}CH_{61}ClN_{12}O_{13}$: 930.42. Found: 930.42755.

Synthesis of cyclic peptide 8. Peptide **2a** (28.7 mg, 31.5 μmol) and sodium bromide (32.4 mg, 314.7 μmol, dried at 150 °C overnight) were weighted into two separate vials. Molecular sieves (3 Å) were added to both vials followed by anhydrous DMF (0.4 mL). The vials were capped under nitrogen and the solutions were agitated for 1 h using an orbital shaker. The Vilsmeier reagent was prepared by adding PBr_3 to anhydrous DMF (0.5 mL) stirred in a 5 mL Biotage MW vial over molecular sieves (3 Å) and under nitrogen atmosphere at 0 °C.[5] After 30 min the DMF solution of **2a**, followed by the DMF solution of sodium bromide, were added dropwise against the walls of the MW vial. The reaction mixture was stirred under nitrogen atmosphere covered with aluminum foil with the temperature slowly increasing from 0 °C to room temperature. After 5.5 days the reaction mixture was filtered and purified.

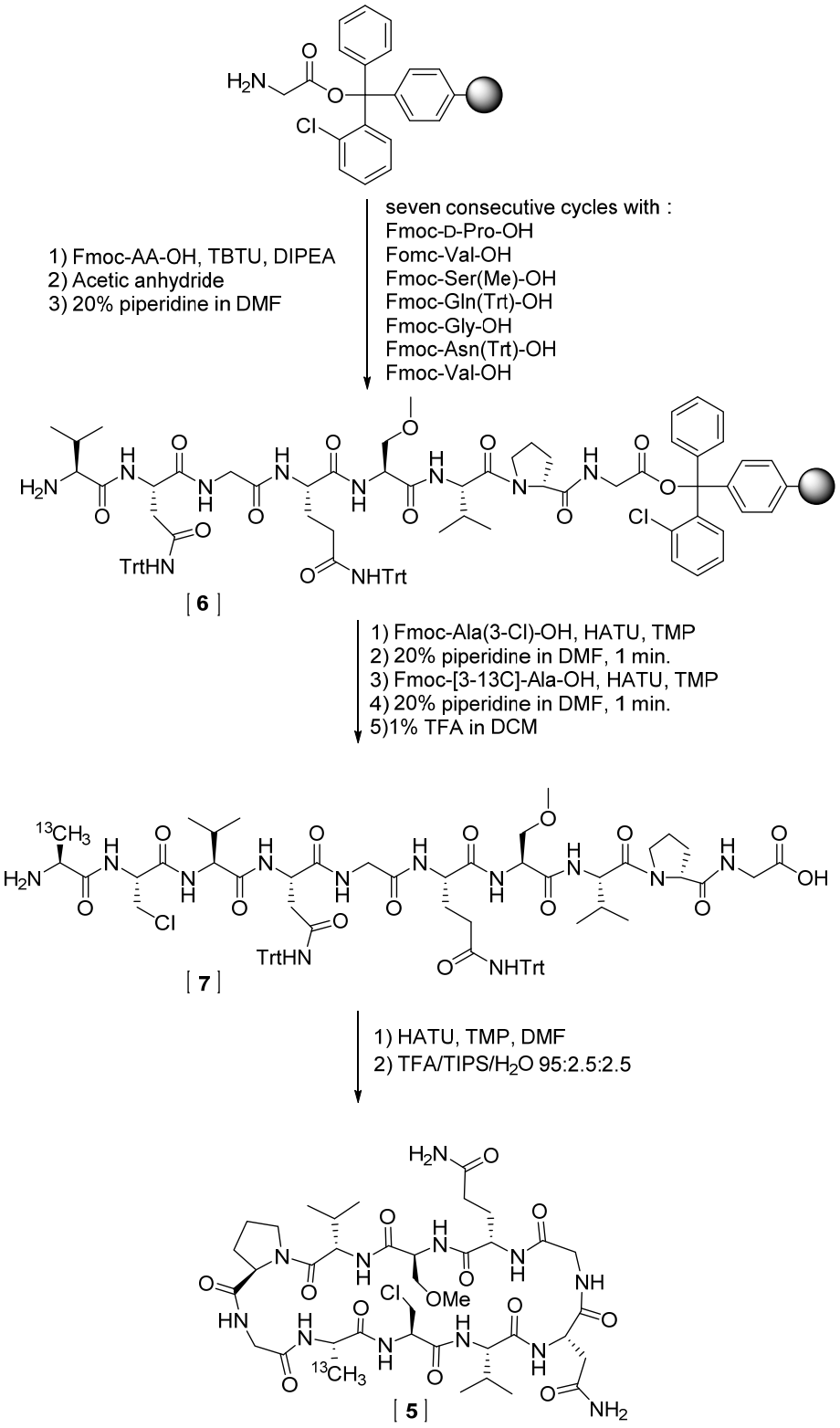
Purification of cyclic peptide 8 by RP-HPLC. Peptide **8** was purified using a gradient of 5–48% CH_3CN in H_2O (0.1% HCOOH) over 35 min at a flow rate of 20 ml/min and UV detection at λ = 220 nm. Selected fractions were analyzed using HPLC-MS/UV and those containing pure product were combined and lyophilized to obtain the product as a white solid (2.6 mg, 8.5%). MS (ESI^+) m/z: 975.9 $[M + H]^+$, 977.6 $[M + 2 + H]^+$.



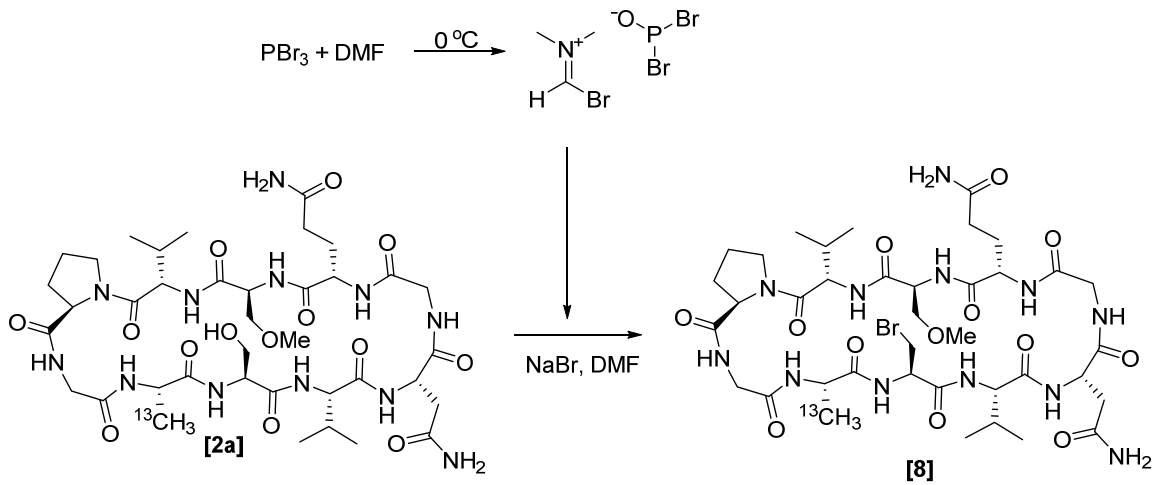
Scheme S1. Synthesis of the linear Peptides **3a** (R=OH) and **3b** (R=Me).



Scheme S2. Synthesis of the cyclic peptides **4a** (R=OH) and **4b** (R=Me).



Scheme S3. Synthesis of the chloro-substituted peptide **5**.



Scheme S4. Synthesis of the bromo-substituted peptide **8**.

3. NMR spectroscopy

Assignment of protons for all peptides except peptide **8** were derived from TOCSY and NOESY NMR spectra recorded at 25 °C on a 900 MHz BRUKER Avance III HD NMR spectrometer equipped with a TCI cryoprobe in DMSO-d_6. Assignment of protons for peptide **8** were derived from TOCSY and NOESY NMR spectra recorded at –10 °C on a 800 MHz BRUKER Avance III HD NMR spectrometer equipped with a TXO cryoprobe in DMF-d_7. The chemical shifts are shown in Tables S1–S6. $^3J_{CH\alpha\text{-}NH}$ coupling constants were determined from ^{1}H NMR spectra measured on a 400 MHz Varian spectrometer using DMSO-d_6 as solvent. The coupling constants are given in Table S8.

3.1 ^{1}H Chemical shift assignments

Table S1. ^{1}H NMR chemical shift assignment (δ, ppm) for **3a** at 25 °C in DMSO-d_6.

Residue	Hα	Hα1	Hα2	Hβ	Hβ1	Hβ2	Hγ	Hγ1	Hγ2	Hδ1	Hδ2	Hε1	Hε2	HN
N1	4.45				2.52	2.37				6.88	7.27			7.98
V2	4.11			2.02				0.86	0.82					7.78
S3	4.40				3.62	3.57								8.12
A4	4.38				1.29	1.14								7.88
G5		3.67	3.63											7.99
P6	4.28			2.04				1.86	1.75	3.71	3.54			
V7	4.32			1.95			0.85							7.95
hS8	4.38				1.72	1.89	3.28							8.09
Q9	4.24				1.86	1.70	2.07					7.23	6.74	8.09
G10		3.69	3.29											8.09

Table S2. ^{1}H NMR chemical shift assignment (δ, ppm) for **3b** at 25 °C in DMSO-d_6.

Residue	Hα	Hα1	Hα2	Hβ	Hβ1	Hβ2	Hγ	Hγ1	Hγ2	Hδ1	Hδ2	Hε1	Hε2	HN
N1	4.44			2.42	2.49					7.03	6.88			8.05
V2	4.13			1.97	0.82									7.78
X3	4.28			1.51			0.82							8.11
A4	4.38				1.28	1.14								7.84
G5		3.67	3.61											8.04
P6	4.28			2.04				1.85	1.77	3.68	3.55			
V7	4.33			1.94				0.85	0.84					8.02
hS8	4.44				1.89	1.72	3.28							8.15
Q9	4.28				1.85	1.69	2.06					7.23	6.74	8.09
G10	3.69													8.08

Table S3. ^{1}H NMR chemical shift assignment (δ, ppm) for **4a** at 25 °C in DMSO-d_6.

Residue	Hα	Hα1	Hα2	Hβ	Hβ1	Hβ2	Hγ	Hγ1	Hγ2	Hδ1	Hδ2	Hε1	Hε2	HN
G1		3.90	3.33											8.71
Q2	4.30				1.88	1.76	2.00					7.15	6.71	8.12
S3	4.59				3.62	3.55								8.29
A4	4.57			1.26										7.80
G5		3.77	3.52											8.44
P6	4.28			2.05			1.86			3.57				
V7	4.37			1.91				0.83	0.78					8.25
hS8	4.62				1.94	1.68	3.26							8.49
A9	4.42				1.27	1.14								7.70
N10	4.52				2.63	2.48				7.35	6.90			8.22

Table S4. ^{1}H NMR chemical shift assignment (δ, ppm) for **4b** at 25 °C in DMSO-d_6.

Residue	Hα	Hα1	Hα2	Hβ	Hβ1	Hβ2	Hγ	Hγ1	Hγ2	Hδ1	Hδ2	Hε1	Hε2	HN
G1		3.80	3.32											8.90
Q2	4.32				1.77	1.71	1.95					7.17	6.71	8.14
X3	4.65				1.63	1.48	0.80							8.38
A4	4.61			1.26										7.74
G5		3.78	3.45											8.47
P6	4.27			2.06			1.86			3.62	3.56			
V7	4.38			1.90				0.82	0.78					8.40
hS8	4.73				1.93	1.65	3.26							8.49
A9	4.52				1.29	1.15								7.73
N10	4.54				2.63	2.43				7.30	6.85			8.38

Table S5. ^{1}H NMR chemical shift assignment (δ, ppm) for **5** at 25 °C in DMSO-d_6.

Residue	Hα	Hα1	Hα2	Hβ	Hβ1	Hβ2	Hγ	Hγ1	Hγ2	Hδ1	Hδ2	Hε1	Hε2	HN
N1	4.07			2.91	2.43					7.38	6.82			9.32
V2	4.19			1.82				0.82	0.78					8.60
A(Cl)3	5.25				3.70	3.48								8.75
A4	4.66				1.31	1.15								7.67
G5		3.84	3.39											8.61
P6	4.29			2.03			1.84			3.56	3.48			
V7	4.30			1.89				0.81	0.76					8.51
S(Me)8	4.80				3.55	3.44								8.78
Q9	4.53				1.84	1.71	2.04					7.04	6.72	7.57
G10		3.84	3.25											8.18

Table S6. ^{1}H NMR chemical shift assignment (δ, ppm) for **8** at -10 °C in DMF-d_7.

Residue	Hα	Hα1	Hα2	Hβ	Hβ1	Hβ2	Hγ	Hγ1	Hγ2	Hδ1	Hδ2	Hε1	Hε2	HN
N1	4.55				3.24	3.16								9.73
V2	4.38			2.09			0.98							7.99
A(Br)3	4.67			3.92										8.33
A4	4.22				1.35	1.20								
G5		3.71	3.58											8.78
P6	4.67			2.50				2.10	2.03	3.91				
V7	4.50			1.98			0.89							7.72
hS8	4.69				3.72	3.58								8.76
Q9	4.69			2.05			2.50							7.58
G10		3.93	3.56											8.82

^{15}N Chemical shift assignments

Table S7. ^{15}N Chemical shift assignments (δ, ppm) at 25 °C in DMSO-d_6 .

	^{15}N chemical shift								
	N1	**V2**	**S/X3**	**A4**	**G5**	**V7**	**hS(Me)8**	**Q9**	**G10**
3a	120.1	117.2	114.6	120.6	106.1	119.4	119.7	118.4	111.3
3b	120.9	116.2	119.4	120.6	106.3	119.5	120.1	118.1	111.9
	G1	**Q2**	**S/X3**	**A4**	**G5**	**V7**	**hS(Me)8**	**A9**	**N10**
4a	112.9	120.9	116.6	120.6	109.0	120.7	121.1	119.5	120.6
4b	115.3	121.3	121.3	120.5	109.3	120.5	121.3	119.2	122.4

J-couplings

Table S8. $^{3}J_{CH\alpha,NH}$ for Peptides **3a-b, 4a-b and 5**.

	$^{3}J_{CH\alpha,NH}$				
Residue position	**3a**	**3b**	**4a**	**4b**	**5**
1	7.94				6.75
2	5.55	5.98	7.20	7.03	8.44
3				6.99	8.45
4	7.11	7.19		6.83	8.28
5					
6					
7	8.11	8.37	9.00	9.18	7.98
8		8.14	8.20		7.80
9	8.15		7.34		8.44
10			7.83		

NOE-buildup analysis

NOESY spectra were recorded on a 900 MHz BRUKER Avance III HD NMR spectrometer equipped with a TCI cryoprobe. NOE build-ups were recorded without solvent suppression with mixing times of 100, 200, 300, 400, 500, 600 and 700 ms. The relaxation delay was set to 2.5 s, and 16 scans were recorded with 16384 points in the direct dimension and 512 points in the indirect dimension. Distances were calculated using geminal methylene protons (1.78 Å) as reference. The NOE peak intensities were calculated using normalization of both cross peaks and diagonal peaks according to ([cross peak$_1$ × cross peak$_2$]/[diagonal peak$_1$ × diagonal peak$_2$])$^{0.5.}$ At least 5 mixing times giving a linear (r^2>0.98) initial NOE rate for every distance were used to determine σ_{ij} build-up rates (Figures S2–S6) according to the equation $r_{ij}=r_{ref}(\sigma_{ref}/\sigma_{ij})^{(1/6)}$, where r_{ij} is the distance between protons i and j in Ångström and σ_{ij} is the normalized intensity obtained from NOESY experiments. The calculated distances are given in Tables S9-S13.

Table S9. Interproton distances (Å) for Peptide **3a** derived from NOE build-up measurements.

	Residue A	**Residue B**	**δA (ppm)**	**δB (ppm)**	**σ**	**R2**	**Distance rAB (Å)**
1	A4NH	P6α	7.88	4.28	6.9E-06	0.99	3.55
2	S3NH	A4NH	8.12	7.88	0.000034	0.99	2.72
3	S3NH	V2NH	8.12	7.78	6.14E-05	0.99	2.47
4	N1NH	V2NH	7.98	7.78	4.79E-05	0.98	2.57
5	N1NH	V2α	7.98	4.11	0.000128	0.99	2.18
6	A4NH	V7β	7.88	1.95	4.3E-06	0.99	3.84
7	A4NH	G5α	7.88	3.67	0.000237	0.99	1.97
8	hS8α	S3β1	4.38	3.62	5.68E-05	0.99	2.50
9	V2NH	S3β1	7.78	3.62	2.7E-05	0.99	2.83
10	P6δ1	V7α	3.71	4.32	0.000199	0.99	2.03
Ref.	P6δ1	P6δ2	3.71	3.54	0.000436	0.99	1.78

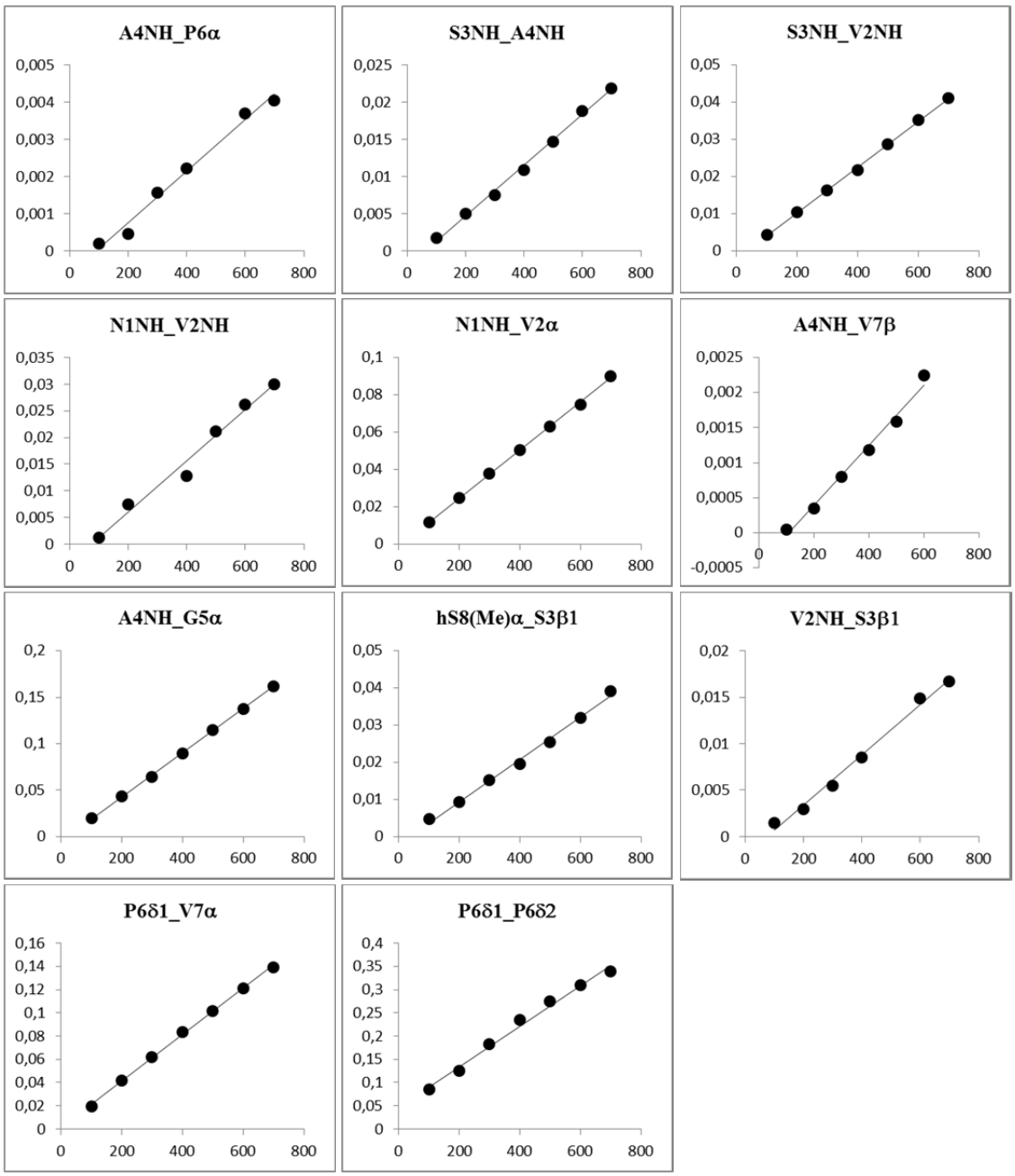


Figure S2. Build-up curves for interproton distances of Peptide **3a**.

Table S10. Interproton distances (Å) for Peptide **3b** derived from NOE build-up measurements.

	Residue A	Residue B	δA (ppm)	δB (ppm)	σ	R2	Distance rAB (Å)
1	hS8NH	V7NH	8.15	8.02	0.0000769	0.99	2.42
2	X3NH	A4NH	8.11	7.84	0.0000299	0.99	2.83
3	X3NH	V2NH	8.11	7.78	0.0000501	0.99	2.59
4	X3NH	A4α	8.11	4.38	0.000292	0.99	1.93
5	V2NH	X3β	7.78	1.51	0.0000161	0.99	3.14
6	V7α	P6δ2	4.33	3.55	0.0002994	0.99	1.93
7	A4NH	G5α1	7.84	3.67	0.0001495	0.99	2.16
8	A4NH	G5α2	7.84	3.61	0.0001714	0.99	2.11
Ref.	P6δ1	P6δ2	3.68	3.55	0.0004809	0.99	1.78

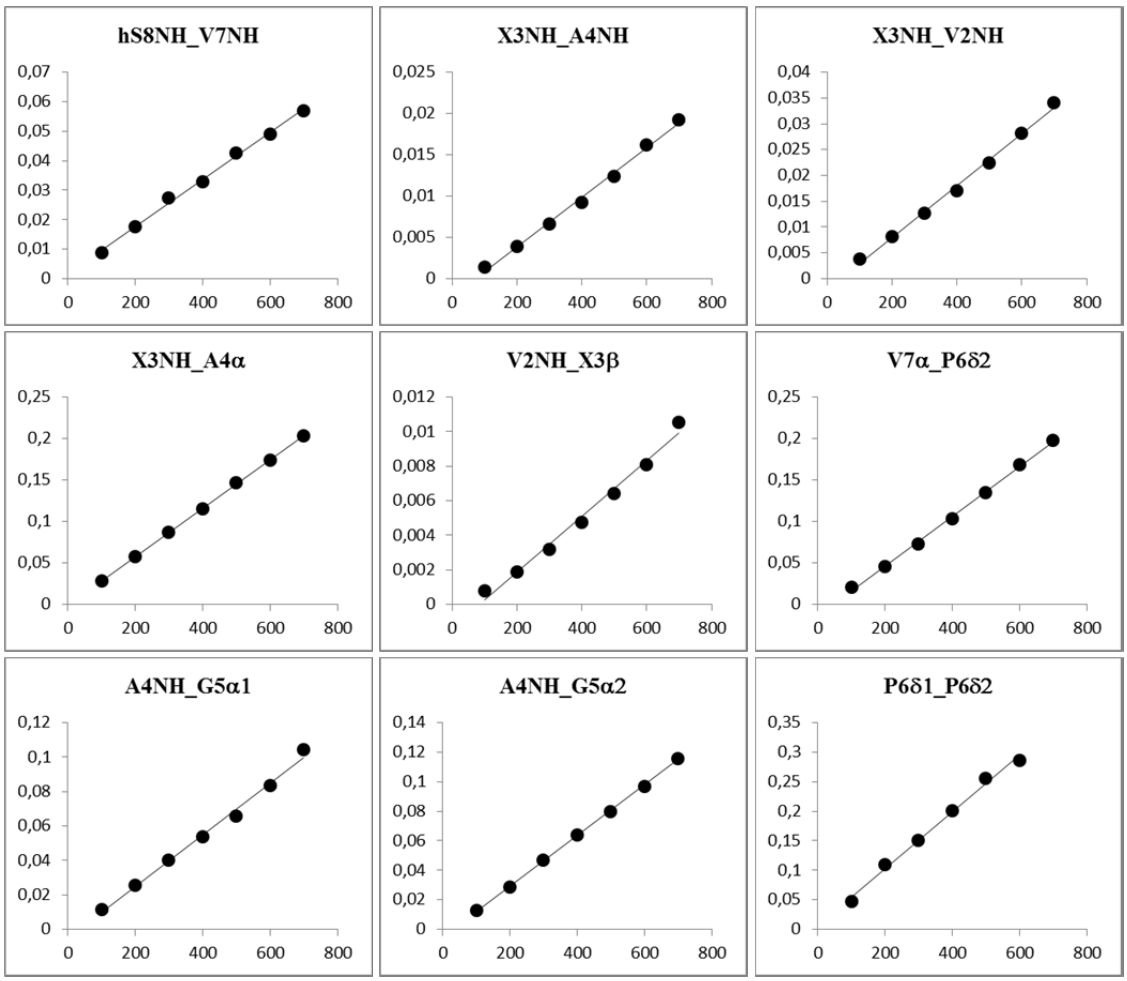


Figure S3. Build-up curves for interproton distances of Peptide **3b**.

Table S11. Interproton distances (Å) for Peptide **4a** derived from NOE build-up measurements.

	Residue A	Residue B	δA (ppm)	δB (ppm)	σ	R2	Distance rAB (Å)
1	G1NH	Q2NH	8.71	8.12	0.0000672	**0.99**	2.73
2	G1NH	N10NH	8.71	8.22	0.0000445	**0.99**	2.92
3	G1NH	Q2α	8.71	4.30	0.0002559	**0.99**	2.18
4	G1NH	A9NH	8.71	7.70	0.0000093	**0.99**	3.79
5	hS8NH	A9α	8.49	4.42	0.0001050	**0.99**	2.53
6	hS8NH	A9NH	8.49	7.70	0.0001164	**0.99**	2.49
7	hS8NH	V7NH	8.49	8.25	0.0000405	**0.99**	2.97
8	G5NH	A4NH	8.44	7.80	0.0001222	**0.99**	2.47
9	G5NH	P6α	8.44	4.28	0.0002312	**0.99**	2.22
10	S3NH	Q2NH	8.29	8.12	0.0000366	**0.99**	3.02
11	S3NH	A4NH	8.29	7.80	0.0000481	**0.99**	2.88
12	V7NH	hS8α	8.25	4.62	0.0003674	**0.99**	2.06
13	V7NH	A4NH	8.25	7.80	0.0000275	**0.99**	3.17
14	N10NH	A9NH	8.22	7.70	0.0001477	**0.99**	2.39
15	Q2NH	S3α	8.12	4.59	0.0003236	**0.99**	2.10
16	Q2NH	A9NH	8.12	7.70	0.0000311	**0.99**	3.10
17	A4NH	P6α	7.80	4.28	0.0000207	**0.98**	3.32
18	A9NH	N10α	7.70	4.52	0.0000948	**0.99**	2.58
19	S3α	Q2α	4.59	4.30	0.0000069	**0.99**	3.99
Ref.	G5α1	G5α2	3.77	3.52	0.0008702	**0.99**	1.78

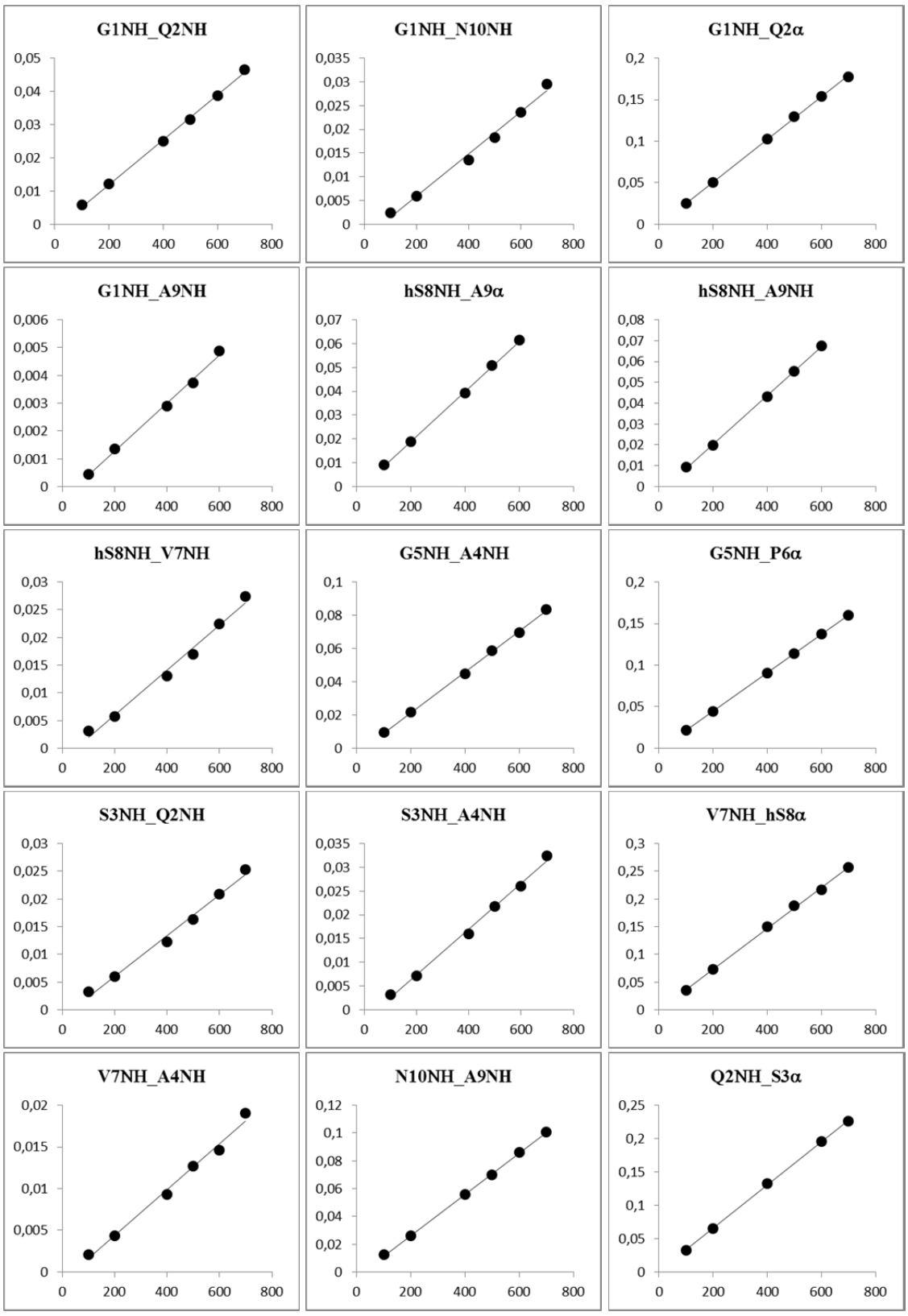


Figure S4. Build-up curves for interproton distances of Peptide **4a**. *Continued on next page.*

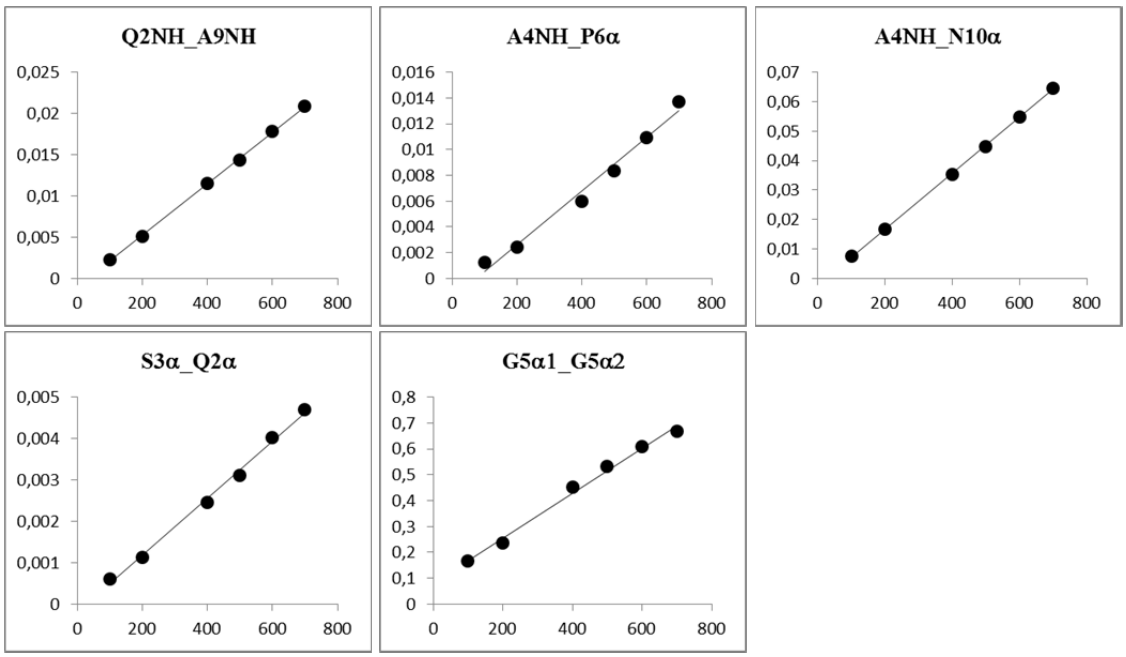


Figure S4 *continued*. Build-up curves for interproton distances of Peptide **4a**.

Table S12. Interproton distances (Å) for Peptide **4b** derived from NOE build-up measurements.

	Residue A	Residue B	δA (ppm)	δB (ppm)	σ	R2	Distance rAB (Å)
1	G1NH	Q2NH	8.90	8.14	0.0000207	0.99	3.35
2	G1NH	Q2α	8.90	4.32	0.0002400	0.99	2.22
3	G1NH	A9NH	8.90	7.73	0.0000036	0.99	4.48
4	hS8NH	A9α	8.49	4.52	0.0001990	0.99	2.30
5	G5NH	P6α	8.47	4.27	0.0001786	0.99	2.34
6	G5NH	A4NH	8.47	7.74	0.0000506	0.99	2.88
7	V7NH	hS8α	8.40	4.73	0.0002186	0.99	2.26
8	Q2NH	X3α	8.14	4.65	0.0002692	0.99	2.18
9	Q2NH	A9NH	8.14	7.73	0.0000159	0.99	3.50
Ref.	G5α1	G5α2	3.78	3.45	0.0009145	0.99	1.78

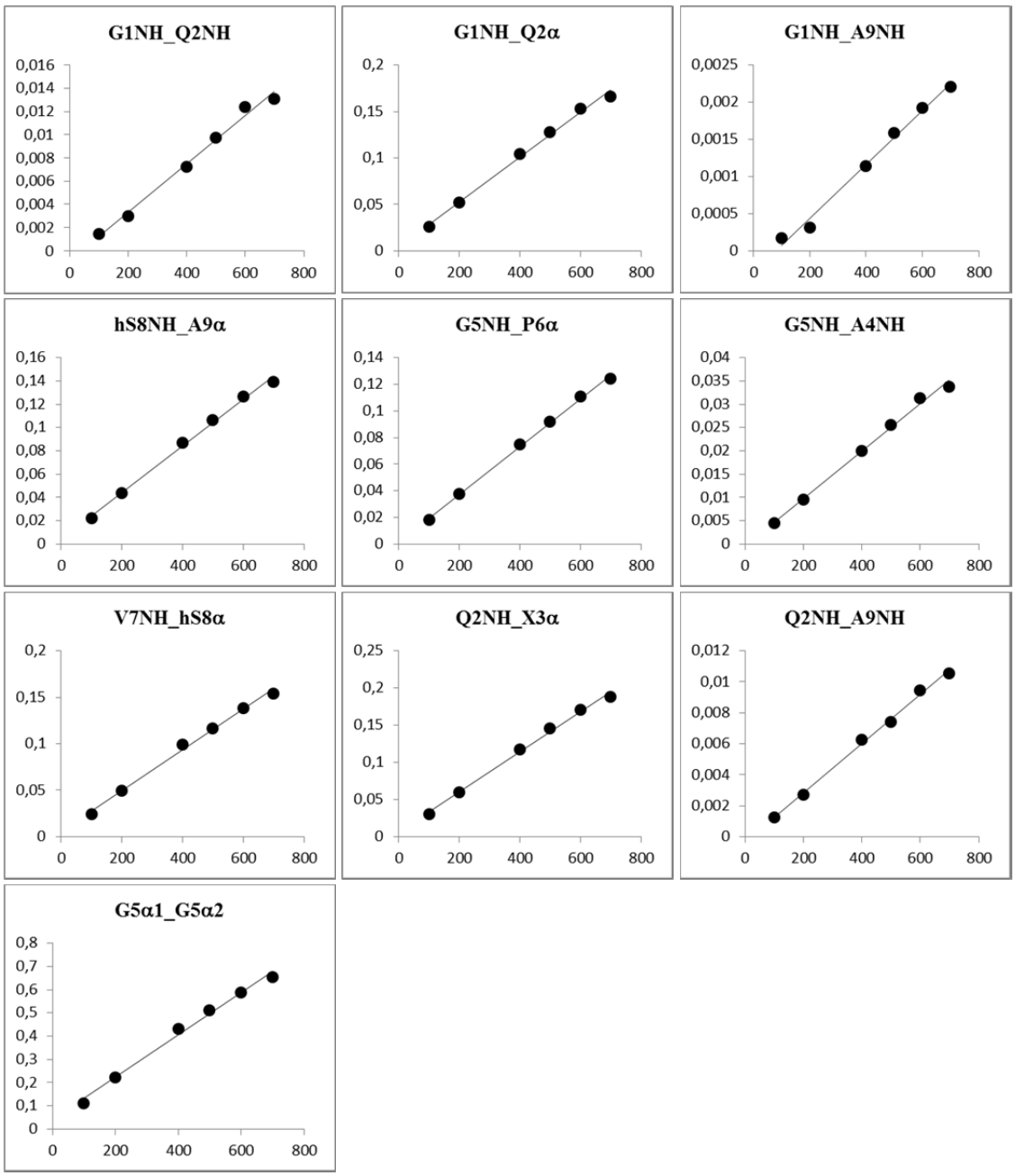


Figure S5. Build-up curves for interproton distances of Peptide **4b**.

Table S13. Interproton distances (Å) for Peptide **5** derived from NOE build-up measurements.

	Residue A	Residue B	δA (ppm)	δB (ppm)	σ	R2	Distance rAB (Å)
1	N1NH	Q9NH	9.32	7.57	1.22E-05	0.99	3.25
2	N1NH	G10NH	9.32	8.18	9.86E-05	0.99	2.30
3	N1NH	V2α	9.32	4.19	0.000422	0.99	1.80
4	S(Me)8NH	Q9α	8.78	4.53	0.000358	0.99	1.85
5	A(Cl)3NH	A4NH	8.75	7.67	1.93E-05	0.98	3.01
6	G5NH	A4NH	8.61	7.67	0.000133	0.99	2.18
7	V2NH	G10NH	8.60	8.18	1.93E-05	0.99	3.01
8	V2NH	A(Cl)3α	8.60	5.25	0.000373	0.99	1.84
9	V2NH	Q9NH	8.60	7.57	8.11E-05	0.99	2.37
10	V7NH	A4NH	8.51	7.67	5.10E-05	0.99	2.56
11	V7NH	A(Cl)3α	8.51	5.25	8.48E-05	0.99	2.35
12	G10NH	N1α	8.18	4.07	0.000119	0.99	2.22
13	G10NH	V2α	8.18	4.19	2.74E-05	0.99	2.84
14	G10NH	Q9NH	8.18	7.57	0.00013	0.99	2.19
15	Q9NH	S(Me)8α	7.57	4.80	5.56E-06	0.98	3.71
16	Q9NH	N1α	7.57	4.07	1.32E-05	0.99	3.21
17	A(Cl)3α	A4α	5.25	4.66	1.60E-05	0.98	3.11
18	A(Cl)3α	Q9α	5.25	4.53	4.35E-06	0.99	3.86
19	A(Cl)3α	S(Me)8α	5.25	4.80	0.000168	0.99	2.10
20	S(Me)8α	Q9α	4.80	4.53	2.09E-05	0.99	2.97
Ref.	N1β1	N1β2	2.91	2.43	0.000453	0.99	1.78

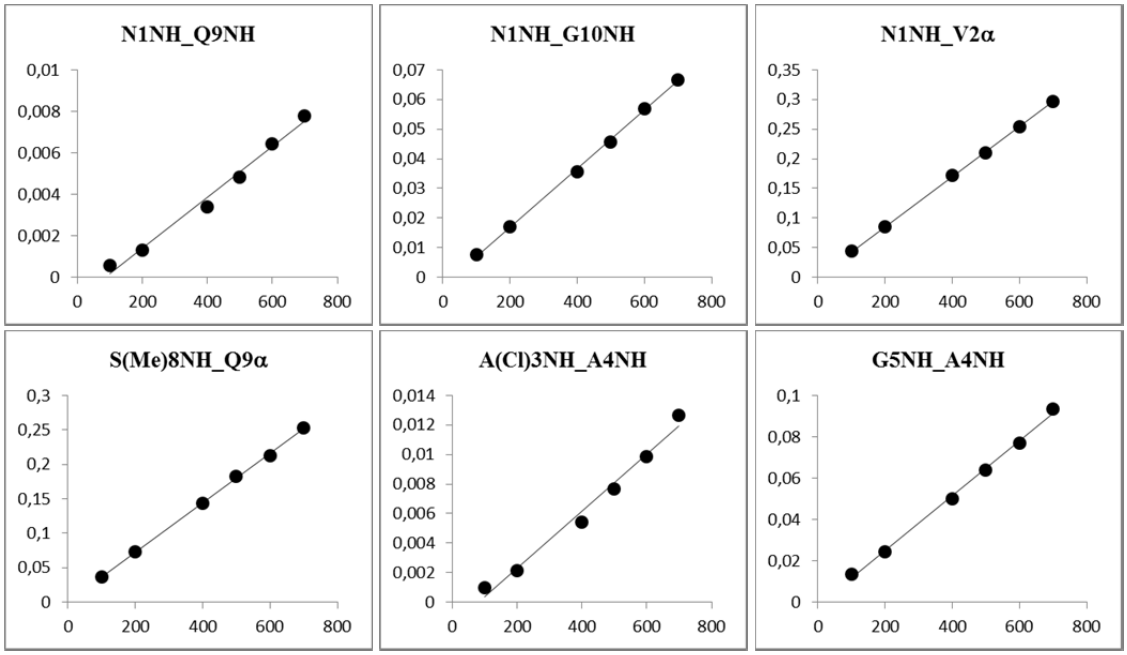


Figure S6. Build-up curves for interproton distances of Peptide **5.** *Continued on next page.*

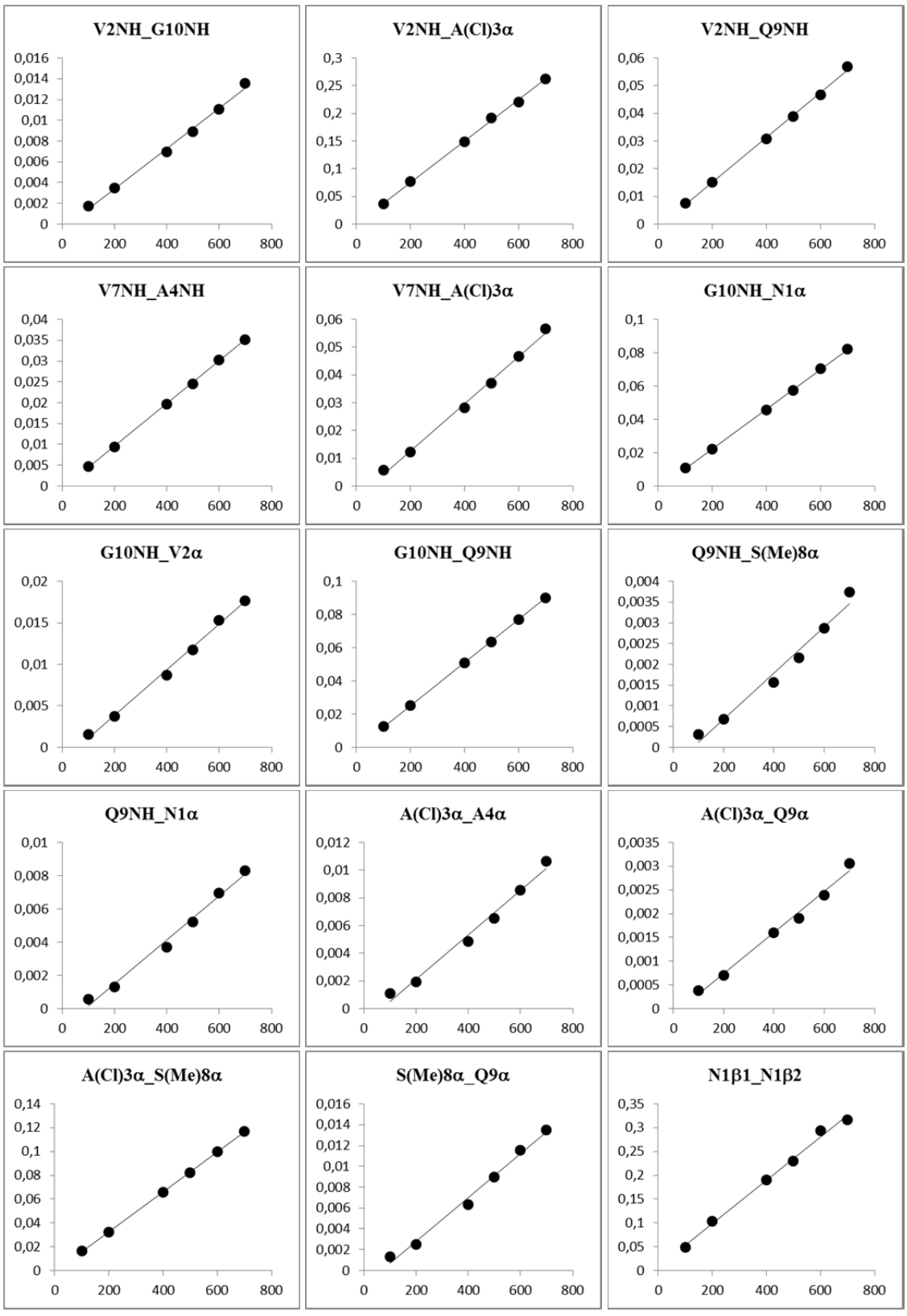


Figure S6 *continued*. Build-up curves for interproton distances of Peptide **5.**

VT-NMR (temperature coefficients)

Amide temperature coefficients, $\Delta\delta_{NH}/\Delta T$ (ppb K^{-1}), were obtained by running ^{1}H and TOCSY NMR spectra at 25–65 °C on a 500 MHz Varian spectrometer in DMSO-d_6. The

amide temperature coefficient ($\Delta\delta_{NH}/\Delta T$) were obtained from $(\delta_{T,high}-\delta_{T,low})/(T_{high}-T_{low})$, which provides negative values but are reported as positive values. $\Delta\delta_{NH}/\Delta T < 3$ indicates a strong intramolecular hydrogen bond, $\Delta\delta_{NH}/\Delta T = 3–5$ indicates that the amide proton is in equilibrium between a solvent exposed and an intramolecular hydrogen bond and $\Delta\delta_{NH}/\Delta T >5$ that the amide proton is solvent exposed.[6] Plotting δNH against temperature gave $r^2 > 0.99$ for all amide protons. The DMSO-d_6 residual solvent signal was used as chemical shift reference, with its temperature dependence taken in consideration following published calibration data.[7] The results are summarized in Tables S14–S18.

Table S14. Amide proton temperature coefficients $\Delta\delta_{NH}/\Delta T$ (ppb K^{-1}) in DMSO-d_6 for **3a**.

Temp. (°C)	N1-NH	V2-NH	S3-NH	A4-NH	G5-NH	V7-NH	hS8-NH	Q9-NH	G10-NH
25	7.970	7.773	8.117	7.870	7.978	7.931	8.089	8.074	8.096
33	7.939	7.734	8.066	7.838	7.954	7.881	8.048	8.038	8.052
40	7.912	7.703	8.026	7.811	7.935	7.841	8.010	8.004	8.014
47	7.887	7.672	7.985	7.785	7.914	7.801	7.972	7.985	7.972
55	7.856	7.636	7.939	7.754	7.887	7.771	7.939	7.940	7.939
65	7.822	7.593	7.875	7.717	7.853	7.702	7.884	7.900	7.867
$\Delta\delta_{NH}$	-0.148	-0.180	-0.242	-0.153	-0.125	-0.229	-0.205	-0.174	-0.229
$\Delta\delta_{NH}/\Delta T$	-3.6	-4.4	-6.0	-3.8	-3.1	-5.6	-5.1	-4.3	-5.6

Table S15. Amide proton temperature coefficients $\Delta\delta_{NH}/\Delta T$ (ppb K^{-1}) in DMSO-d_6 for **3b.**

Temp. (°C)	N1-NH	V2-NH	X3-NH	A4-NH	G5-NH	V7-NH	hS8-NH	Q9-NH	G10-NH
25	8.034	7.773	8.107	7.837	8.039	8.013	8.146	8.083	8.077
33	7.996	7.735	8.057	7.809	8.017	7.956	8.101	8.048	8.042
40	7.962	7.699	8.015	7.782	7.989	7.910	8.059	8.017	8.017
47	7.937	7.668	7.984	7.759	7.964	7.882	8.023	7.993	7.988
55	7.889	7.627	7.931	7.728	7.924	7.827	7.976	7.954	7.938
65	7.846	7.581	7.878	7.695	7.883	7.762	7.919	7.910	7.890
$\Delta\delta_{NH}$	-0.188	-0.192	-0.229	-0.142	-0.156	-0.251	-0.227	-0.173	-0.187
$\Delta\delta_{NH}/\Delta T$	-4.6	-4.7	-5.6	-3.5	-3.8	-6.2	-5.6	-4.3	-4.6

Table S16. Amide proton temperature coefficients $\Delta\delta_{NH}/\Delta T$ (ppb K^{-1}) in DMSO-d_6 for **4a.**

Temp. (°C)	G1-NH	Q2-NH	S3-NH	A4-NH	G5-NH	V7-NH	hS8-NH	A9-NH	N10-NH
25	8.714	8.130	8.308	7.812	8.444	8.244	8.499	7.714	8.219
33	8.657	8.114	8.273	7.807	8.393	8.21	8.459	7.693	8.171
47	8.563	8.086	8.21	7.795	8.308	8.151	8.389	7.662	8.094
55	8.513	8.069	8.173	7.789	8.261	8.117	8.349	7.646	8.054
65	8.451	8.045	8.126	7.777	8.203	8.074	8.297	7.627	8.008
$\Delta\delta_{NH}$	-0.263	-0.085	-0.182	-0.035	-0.241	-0.17	-0.202	-0.087	-0.211
$\Delta\delta_{NH}/\Delta T$	-6.6	-2.1	-4.6	-0.9	-6.0	-4.2	-5.1	-2.2	-5.3

Table S17. Amide proton temperature coefficients $\Delta\delta_{NH}/\Delta T$ (ppb K^{-1}) in DMSO-d_6 for **4b**.

Temp. (°C)	G1-NH	Q2-NH	X3-NH	A4-NH	G5-NH	V7-NH	hS8-NH	A9-NH	N10-NH
25	8.825	8.166	8.351	7.802	8.383	8.387	8.412	7.763	8.368
33	8.769	8.147	8.311	7.797	8.332	8.358	8.371	7.747	8.307
40	8.716	8.127	8.284	7.79	8.297	8.332	8.332	7.73	8.273
55	8.606	8.081	8.218	7.774	8.203	8.27	8.244	7.696	8.189
65	8.534	8.052	8.182	7.764	8.138	8.218	8.2	7.676	8.121
$\Delta\delta_{NH}$	-0.291	-0.114	-0.169	-0.038	-0.245	-0.169	-0.212	-0.087	-0.247
$\Delta\delta_{NH}/\Delta T$	-7.3	-2.9	-4.2	-0.9	-6.1	-4.2	-5.3	-2.2	-6.2

Table S18. Amide proton temperature coefficients $\Delta\delta_{NH}/\Delta T$ (ppb K^{-1}) in DMSO-d_6 for **5.**

Temp. (°C)	N1-NH	V2-NH	A(Cl)3-NH	A4-NH	G5-NH	V7-NH	S(Me)8-NH	Q9-NH	G10-NH
25	9.316	8.582	8.754	7.671	8.599	8.514	8.779	7.576	8.173
30	9.287	8.566	8.730	7.665	8.583	8.499	8.755	7.568	8.142
35	9.255	8.550	8.703	7.658	8.567	8.482	8.728	7.559	8.108
40	9.223	8.533	8.678	7.652	8.551	8.465	8.702	7.552	8.075
45	9.189	8.515	8.651	7.646	8.533	8.447	8.674	7.543	8.041
50	9.153	8.496	8.624	7.639	8.514	8.427	8.645	7.534	8.006
$\Delta\delta_{NH}$	-0.163	-0.086	-0.130	-0.032	-0.085	-0.087	-0.134	-0.042	-0.167
$\Delta\delta_{NH}/\Delta T$	-6.5	-3.4	-5.2	-1.3	-3.4	-3.5	-5.4	-1.7	-6.7

Methoxy temperature coefficients

The hS8 methoxy temperature dependence, for peptides **2a-b** and **5**, were measured and calculated the same way as described above for the amide protons.

Table S19. hS8 Methoxy methyl proton temperature coefficients $\Delta\delta/\Delta T$ (ppb K^{-1}) in DMSO-d_6, for the temperature interval 25–100 °C.

	2a	2b	5
$\Delta\delta$	– 0.014	– 0.005	– 0.024
ΔT	75	75	75
$\Delta\delta/\Delta T$	– 0.19	– 0.07	– 0.32

4. Computational conformational analysis

Conformational searches were performed using the Monte Carlo algorithm with intermediate torsion sampling, 50000 Monte Carlo steps, and RMSD cut-off set to 2.0 Å, followed by Molecular Mechanics energy minimization with the software Macromodel (v.9.1) as implemented in the Schrödinger package. For **3a-b** and **4 a-b**, two independent searches were performed using OPLS-2005 or Amber* as force field, and with water solvation model. The conformational search for peptide **5** and **8** was also performed with the force field OPLS-3[8], a new version of OPLS with parameters for aromatic halogen bonding included. Energy minimization was performed using the Polak-Ribiere type conjugate gradient (PRCG) with maximum iteration steps set to 5000. All conformations within 42 kJ/mol from the global minimum were saved. The ensembles from the conformational searches using the different force fields were combined and elimination of redundant conformtions was preformed by the comparsion of heavy atom coordinates applying a RMSD cutoff of 2.5 Å for the cyclic peptides and 3.0 Å for the linear ones, giving the ensembles used in NAMFIS. The results are summarized in Table S20. Since the force fields used do not include parameters for aliphatic halogen bonds, no conformations with halogen bonding interaction were obtained from the conformational analyses of peptide **5** and **8**. Therefore, a hairpin conformer was minimized with constrained Cl/Br-OMe distances and angles and these conformers were then added to the corresponding ensemble. The constrains used to generate these conformers were: Cl-OMe

distance 2.94 Å +/– 10% (corresponding to 90% of the sum of the van der Waals radie), Br-OMe distance 3.03 Å +/– 10% (corresponding to 90% of the sum of the van der Waals radie), C-Cl-O(Me) angle (180° +/–10%) and C-Br-O(Me) angle (180° +/–10%).

Table S20. Results of the conformational analysis.

		Number of conformations		
		Total[a]	Within 12.6 kJ/mol[b]	[c]Redundant conformer elimination
3a	OPLS	1502	32	427
	Amber*	520	16	
3b	OPLS	1404	23	396
	Amber*	746	20	
4a	OPLS	173	13	52
	Amber*	201	21	
4b	OPLS	184	31	34
	Amber*	361	9	
5	OPLS	204	23	
	Amber*	135	10	138
	OPLS-3	35	4	
8	OPLS	289	17	
	Amber*	216	11	97
	OPLS-3	22	3	

[a]Total number of unique conformations found. [b]Conformations found within 12.6 kJ/mol (3.0 kcal/mol) of the global minimum. [c]Conformations obtained after redundant conformation elimination with the root-mean-square deviation cutoff 2.5 Å (cyclic peptides) and 3.0 Å (linear peptides) for heavy atoms. This conformation ensemble is used as input in the NAMFIS analysis. To the ensembles of **5** and **8** a halogen bond constrained peptide was added, see text for details.

5. Identification of solution ensemble using the NAMFIS algorithm

Solution ensembles were determined by fitting the experimentally measured distances and coupling constants to those back-calculated for computationally predicted conformations following previously described protocols.[9] Dihedral angles were calculated from experimental coupling constants using a Karplus equation specifically developed for peptides.[10,11] NOE-derived distances are presented in Tables S9–S13, whereas coupling constants are presented in Table S8. The results of the NAMFIS-analysis are given in Table S21. Since the side chain orientations are poorly predicted by the conformational searches only back bone interactions were included in the NAMFIS analyses, with the exception of the highly flexible linear peptides where some side chain positions had to be included in order to get enough data to perform the analysis. The ensemble analyses were validated using standard methods, that is,

through evaluation of the reliability of the conformational restraints by the addition of 10% random noise to the experimental data, by the random removal of individual restraints, and by comparison of the experimentally observed and back-calculated distances and scalar coupling constants.

Table S21. Result of the NAMFIS-analyses for peptides **3a-b**, **4a-b** and **5**.

3a		**3b**		**4a**		**4b**		**5**	
Conf. No.[a]	%[b]	Conf. No.[a]	%[b]	Conf. No.[a]	%[b]	Conf. No.[a]	%[b]	Conf. No.[a]	%[b]
1	2	1	12	1	14	***1***	***13***	***1***	***9***
2	23	2	5	2	4	***2***	***14***	2	2
3	***11***	3	19	3	2	3	26	***3***	***16***
4	18	4	13	4	15	4	7	4	4
5	2	5	21	***5***	***36***	***5***	***4***	5	12
6	18	6	6	6	10	6	5	6	7
7	13	7	20	7	9	7	2	***7***	***26***
8	12	8	3	8	5	8	28	***8***	***19***
				9	4			***9***	***4***

[a]The structures of the most populated conformations are shown in Figures S7–S11. [b] The hairpin population in solution. Hairpin conformations are indicated by numbers in *italic*. The hairpin content was calculated to 11% for **3a**, 0% for **3b**, 36% for **4a**, 31% for **4b** and 74% for **5.**

Table S22. Experimentally determined and back-calculated (NAMFIS) interproton distances (Å) and coupling constants (Hz) for the solution ensemble of **3a**.

Interproton distances		**Coupling constants**	
Exp.	Calc.	Exp.	Calc.
3.55	4.14	7.9	7.9
2.72	2.68	8.1	8.0
2.47	2.51	8.2	7.9
2.57	2.70	5.5	5.6
2.18	2.42	7.1	7.3
3.84	4.56		
1.97	2.59		
2.50	2.91		
2.83	2.89		
2.03	2.44		
RMSD distances = 0.41		*RMSD coupling constants = 0.17*	

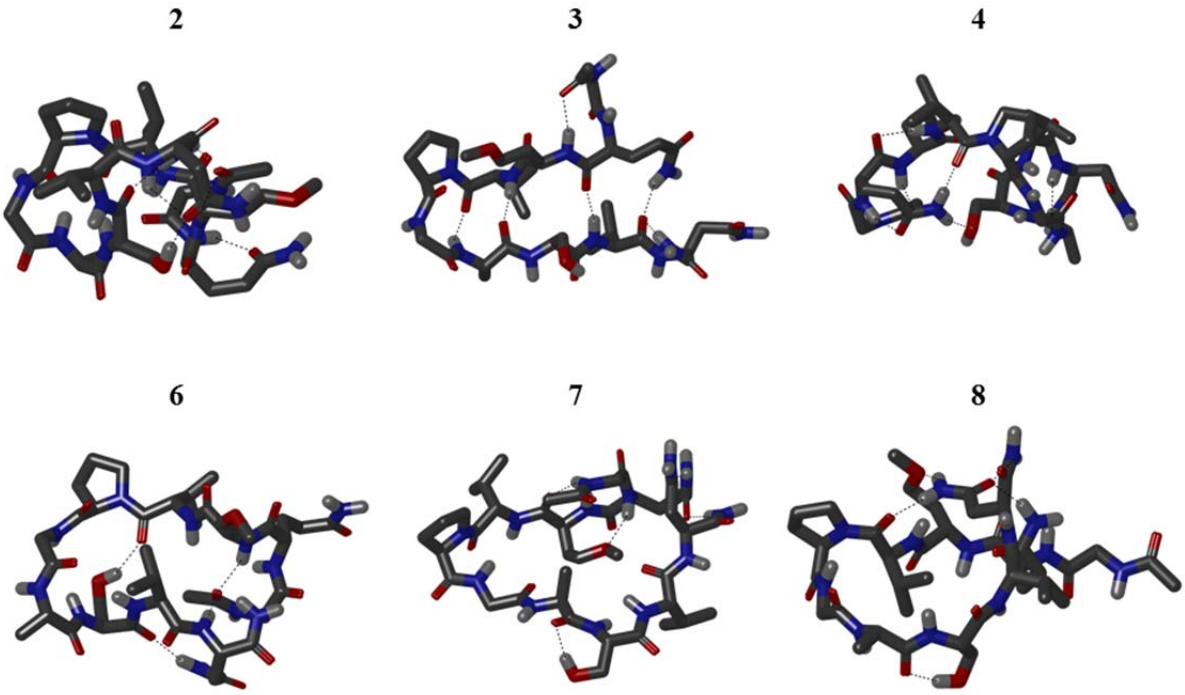


Figure S7. The most populated solution conformations of **3a**, as selected by the NAMFIS-analysis. Populations in % are given in Table S21. Hydrogen bonds are indicated by black dotted lines. Non-polar hydrogens are omitted for clarity.

Table S23. Experimentally determined and back-calculated (NAMFIS) interproton distances (Å) and coupling constants (Hz) for the solution ensemble of **3b**.

Interproton distances		**Coupling constants**	
Exp.	Calc.	Exp.	Calc.
2.42	2.43	8.1	7.9
2.83	2.86	7.2	7.2
2.59	2.56	8.4	8.2
1.93	2.27	6.0	6.1
3.14	3.40		
1.93	2.46		
2.16	2.52		
2.11	2.51		
RMSD distances = 0.31		*RMSD coupling constants = 0.18*	

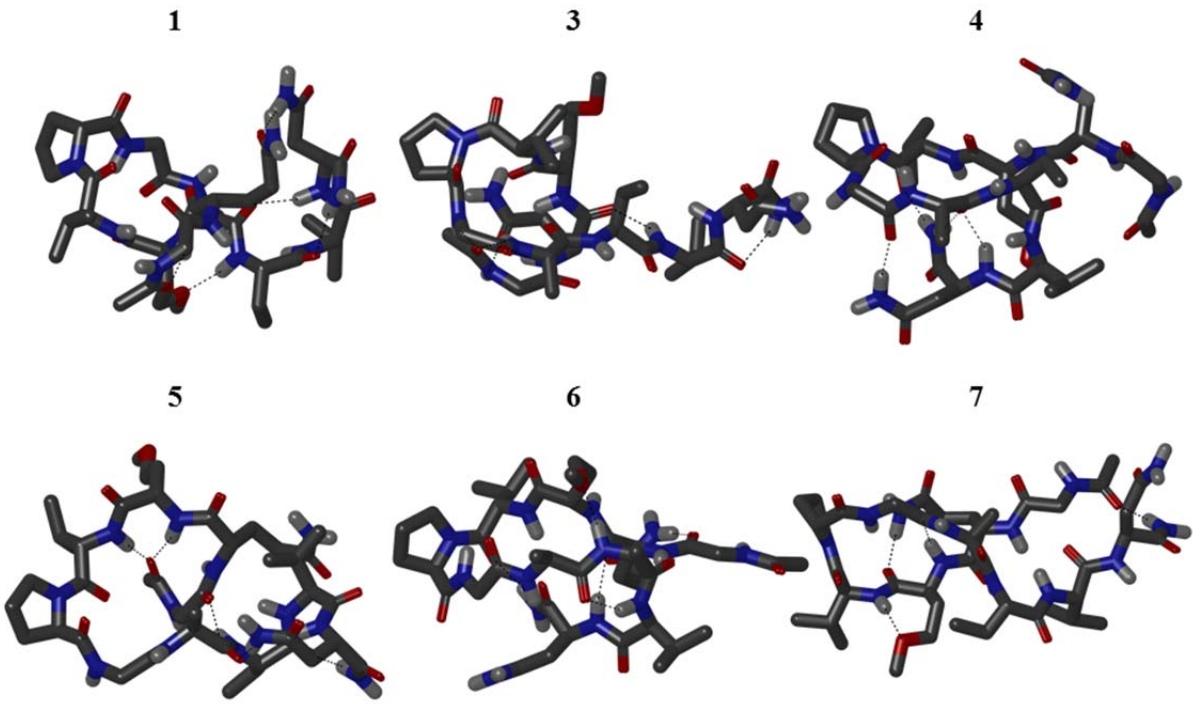


Figure S8. The most populated solution conformations of **3b**, as selected by the NAMFIS-analysis. Populations in % are given in Table S21. Hydrogen bonds are indicated by black dotted lines. Non-polar hydrogens are omitted for clarity.

Table S24. Experimentally determined and back-calculated (NAMFIS) interproton distances (Å) and coupling constants (Hz) for the solution ensemble of **4a**.

Interproton distances		**Coupling constants**	
Exp.	Calc.	Exp.	Calc.
2.73	3.13	8.2	8.0
2.92	2.98	7.8	7.2
2.18	2.33	9.0	8.7
3.79	3.51	7.2	7.2
2.53	3.34	7.3	7.7
2.49	2.37		
2.97	3.25		
2.47	2.48		
2.22	2.32		
3.02	3.08		
2.88	2.78		
2.06	2.30		
3.17	3.46		
2.39	2.55		
2.10	2.39		
3.10	3.38		
3.32	3.66		
2.58	2.58		
3.99	4.58		
RMSD distances = 0.31		*RMSD coupling constants = 0.34*	

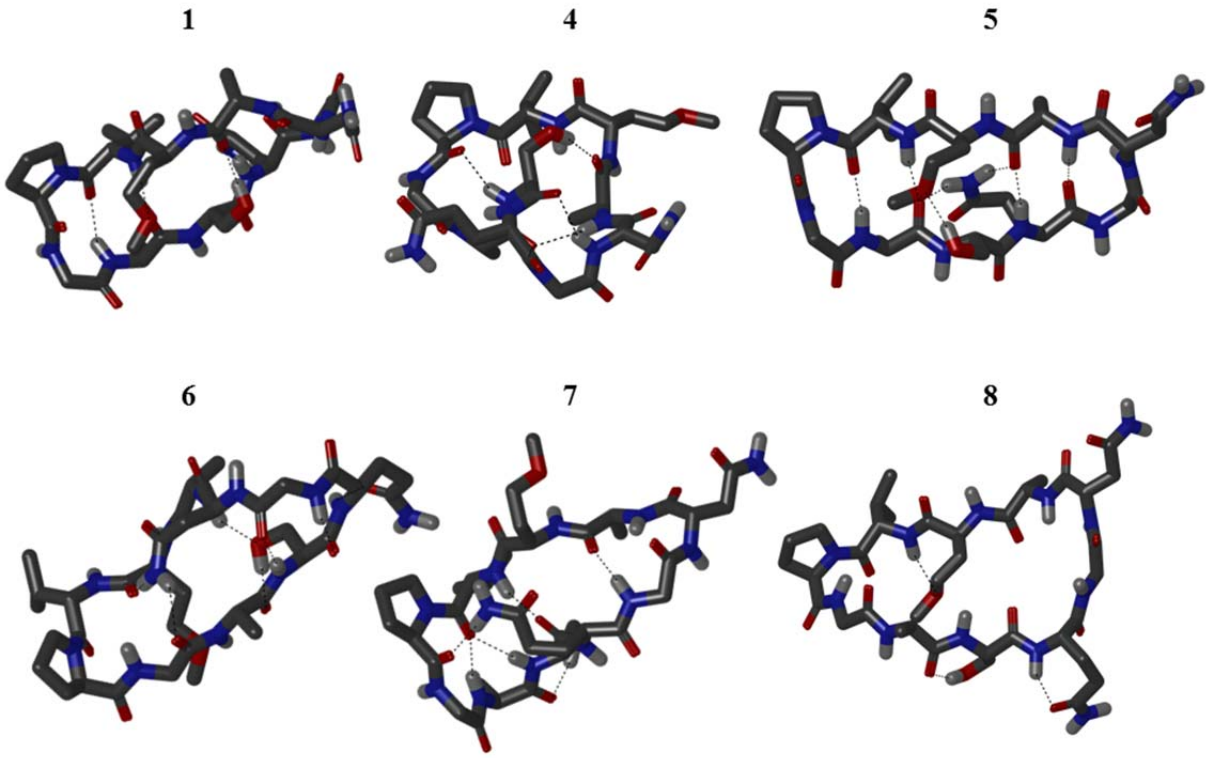


Figure S9. The most populated solution conformations of **4a**, as selected by the NAMFIS-analysis. Populations in % are given in Table S21. Hydrogen bonds are indicated by black dotted lines. Non-polar hydrogens are omitted for clarity.

Table S25. Experimentally determined and back-calculated (NAMFIS) interproton distances (Å) and coupling constants (Hz) for the solution ensemble of **4b**.

Interproton distances		Coupling constants	
Exp.	Calc.	Exp.	Calc.
3.35	3.29	7.2	7.2
2.22	2.26	7.0	7.2
4.48	4.41	6.8	7.2
2.30	2.35	9.2	8.8
2.34	2.36	7.0	7.1
2.88	2.95		
2.26	2.34		
2.18	2.40		
3.50	3.72		
RMSD distances = 0.12		*RMSD coupling constants = 0.25*	

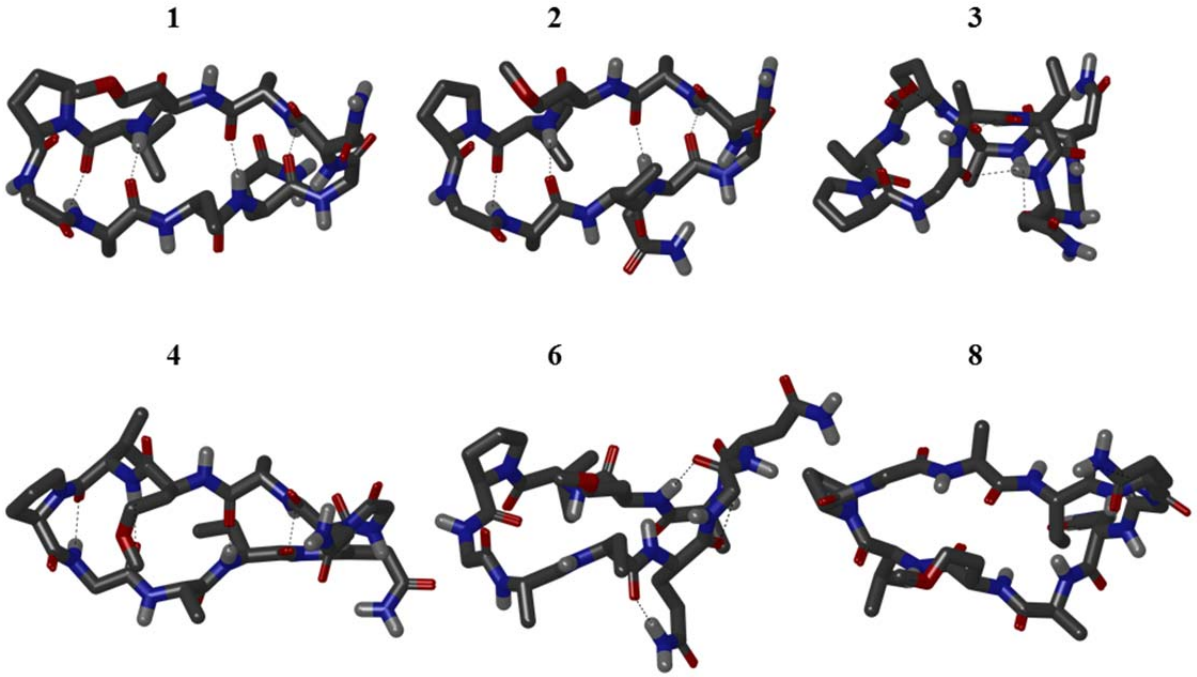


Figure S10. The most populated solution conformations of **4b**, as selected by the NAMFIS-analysis. Populations in % are given in Table S21. Hydrogen bonds are indicated by black dotted lines. Non-polar hydrogens are omitted for clarity.

Table S26. Experimentally determined and back-calculated (NAMFIS) interproton distances (Å) and coupling constants (Hz) for the solution ensemble of **5**.

Interproton distances		**Coupling constants**	
Exp.	Calc.	Exp.	Calc.
3.25	3.51	6.8	7.7
2.30	2.74	8.4	7.7
1.80	2.25	8.4	8.2
1.85	2.29	8.3	9.2
3.01	3.00	8.0	8.5
2.18	2.48	7.8	6.8
3.01	3.93	8.4	9.2
1.84	2.28		
2.37	3.12		
2.56	3.39		
2.35	2.98		
2.22	2.74		
2.84	4.03		
2.19	2.36		
3.71	4.70		
3.21	4.12		
3.11	4.61		
3.86	4.43		
2.10	2.47		
2.97	4.49		
RMSD distances = 0.77		*RMSD coupling constants = 0.78*	

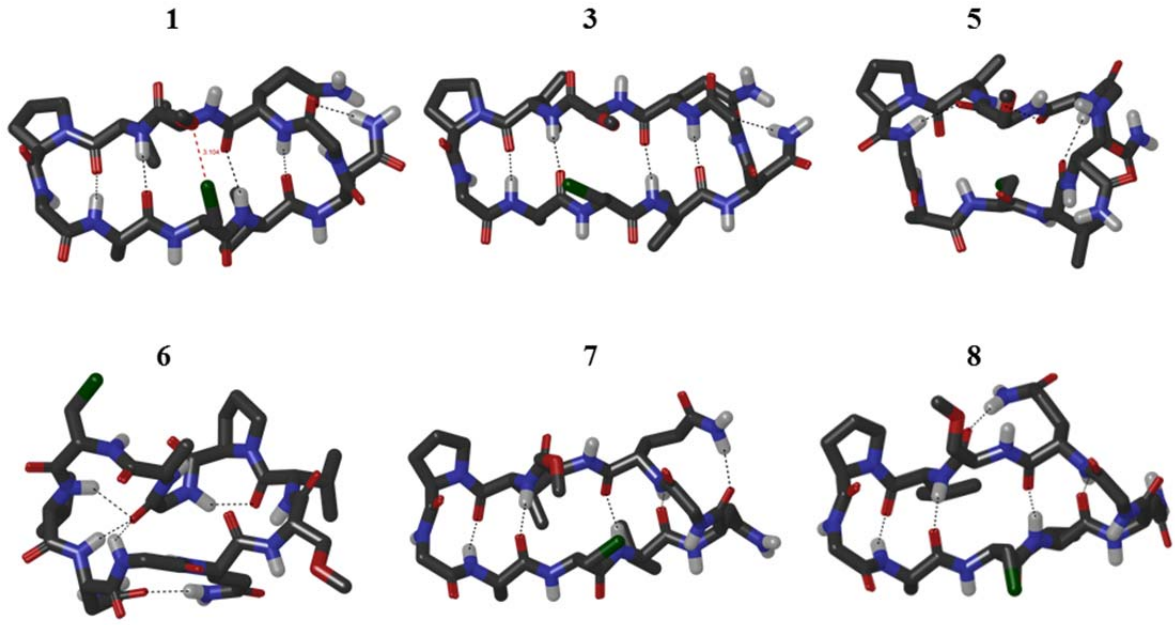


Figure S11. The most populated solution conformations of **5**, as selected by the NAMFIS-analysis. Populations in % are given in Table S21. Hydrogen bonds are indicated by black dotted lines. Non-polar hydrogens are omitted for clarity. Conformer 1 has a halogen bond interaction indicated by red dotted line, with a Cl···OMe distance of 3.104 Å and a C–Cl–O angle of 175.7º.

NAMFIS ensemble analysis at the interaction-site. In order to examine if the NMR-data could show the spatial orientation of the A(Cl) and S(Me) side chains, i.e. if a halogen bond interaction between these two residues occur, a NAMFIS analysis was run with constrains only concerning these residues. A hairpin conformation was selected and the Cα—Cβ bond was rotated, giving 9 different conformers (figure S12) which were used as input ensemble. The input distances are given in table S27.

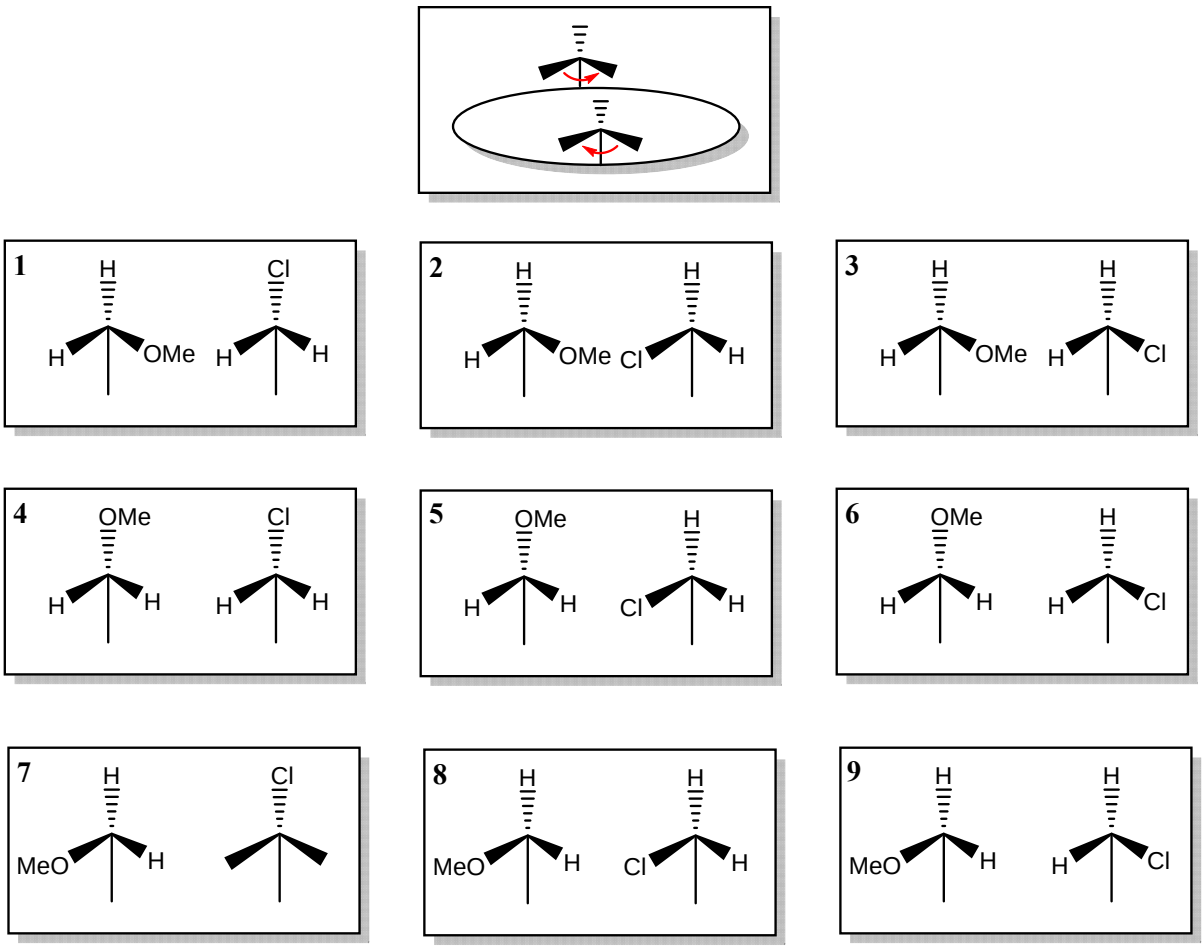


Figure S12. The input ensemble for the NAMFIS analyses for peptide **5** with the A(Cl)3 and S(Me)8 interactions as constrains. The ensemble was generated by rotating the Cα—Cβ bond, as indicated by the red arrows.

Table S27. Interproton distances (Å) for Peptide **5** concerning the A(Cl)3 and S(Me)8 residues derived from NOE build-up measurements.

No.	AA1	AA2	f2 ppm	f1 ppm	R2	Dist. (Å)
1	G10NH	A(Cl)3β1	8.18	3.70	0.99	3.36
2	Q9NH	A(Cl)3β1	7.57	3.70	0.99	3.56
3	S(Me)8NH	S(Me)8β2	7.57	3.44	0.99	2.13
4	S(Me)8NH	S(Me)8β1	7.57	3.55	0.99	2.37
5	A(Cl)3NH	A(Cl)3β1	8.75	3.70	0.99	2.48
6	A(Cl)3NH	A(Cl)3β2	8.75	3.48	0.99	2.32
7	V2NH	A(Cl)3β1	8.60	3.70	0.99	2.63
8	V7NH	S(Me)8β1	8.51	3.55	0.99	2.60
9	A(Cl)3α	A(Cl)3β1	5.25	3.70	0.99	2.19
10	A(Cl)3α	A(Cl)3β2	5.25	3.48	0.99	2.36
11	S(Me)8α	A(Cl)3β1	4.80	3.70	0.99	2.93
12	S(Me)8α	S(Me)8β2	4.80	3.44	0.98	2.16
13	S(Me)8α	S(Me)8β1	4.80	3.55	0.99	2.25
14	S(Me)8NH	S(Me)8δ	7.57	3.19	0.99	3.62
15	V7NH	S(Me)8δ	8.51	3.19	0.99	3.30
16	S(Me)8α	S(Me)8δ	4.80	3.19	0.99	2.95
ref	***N1β1***	***N1β2***	***2.91***	***2.43***	***0.99***	***1.78***

Since there are two possible β-protons on each residue (A(Cl)β1/β2 and S(Me)β1/β2), there are four possible ways to assign the NMR-data. NAMFIS analyses were run for all four combinations and the results are given in table S28 and S29.

Table S28. Result of the NAMFIS-analyses for peptide **5** with the A(Cl)3 and S(Me)8 interactions as constrains.

	Main conformer	Minor conformer	Msigma%	RMSD distances
NAMFIS 1	1 (92%)	4 (8%)	0.163D+11	1.56
NAMFIS 2	1 (92%)	4 (8%)	0.408D+08	1.07
NAMFIS 3	1 (92%)	4 (8%)	0.284D+10	1.51
NAMFIS 4	1 (92%)	4 (8%)	0.709D+07	1.01

Table S29. Experimentally determined and back-calculated (NAMFIS) interproton distances (Å) for the solution ensemble peptide **5** using the A(Cl)3 and S(Me)8 interactions as constrains.

	Interproton distances			
	NAMFIS 1	NAMFIS 2	NAMFIS 3	NAMFIS 4
Exp.	Calc.	Calc.	Calc.	Calc.
3.36	7.66	5.97	7.66	5.97
3.56	6.09	4.55	6.09	4.55
2.13	3.62	3.62	2.55	2.55
2.37	2.55	2.55	3.62	3.62
2.48	2.74	3.66	2.74	3.66
2.32	3.66	2.74	3.66	2.74
2.63	4.37	3.26	4.37	3.26
2.60	4.16	4.16	3.33	3.33
2.19	3.01	2.74	3.01	2.74
2.36	2.74	3.01	2.74	3.01
2.93	4.46	4.39	4.46	4.39
2.16	2.40	2.40	3.06	3.06
2.25	3.06	3.06	2.40	2.40
3.62	3.98	3.98	3.98	3.98
3.30	3.15	3.15	3.14	3.14
2.95	3.50	3.50	3.49	3.49
RMSD distances	*1.56*	*1.07*	*1.51*	*1.01*

6. DFT-calculations

The geometry optimization of **5** was performed by a two-layer ONIOM[12] calculation where DFT employing Truhlar's M06 exchange and correlation functional[13] was used for the high-level part and the PM6[14] method for the low-level part. The high-level part was chosen to comprise the residues S(Me)8 and A(Cl)3, incorporating all atoms of the peptide bonds to V2, A4, V7, and Q9, respectively, as well as the α C atoms of V2 and V2 and the H atoms bonded to them. The atoms at the cut between the two ONIOM layers were replaced by H in the high-level layer. For the treatment of the S(Me)8···A(Cl)3 dimer and its two fragments, the vacant bonds were saturated by H atoms, whose positions were reoptimized with the remaining atoms kept fixed. To get the geometry of the S(Me)8···A(H)3 dimer, the Cl atom in A(Cl)3 was replaced by an H atom, whose position was optimized again with the rest of the dimer kept rigid. For the DFT calculation, the triple-zeta version (pc-2) of Jensen's polarization consistent basis sets[15-17] was used. Diffuse functions[18] were added for the O and Cl atoms in the O···Cl bond as well as the nearest neighbors of these atoms and the H atom replacing the Cl in A(H)3. The basis-set superposition error was corrected by the Counterpoise method.[19] To avoid numerical problems that may arise when the M06 functional is applied for weakly bound complexes[20] a high-resolution integration grid was used for the DFT integrations (Gaussian keyword Grid=Ultrafine). Solvent effects were simulated by the Polarizable Continuum Model (PCM)[21] with dimethyl sulfoxide as solvent. For the optimization of the saturating H atoms in the S(Me)8···A(H)3 dimer, the diffuse basis functions as well as the PCM were omitted for cost reasons. Also, the Counterpoise corrections had to be calculated for the gas phase. All calculations were done with the Gaussian09 program package.[22]

In the following, the equilibrium geometries and energies for the structure investigated in this work are given. For peptide 5, the specification of the ONIOM layers is given in the Gaussian

input format, i.e. High and Low for the high-level and low-level layers, respectively, atoms that are replaced by a valence-saturating H atom in the high-level layer are marked by a H at the end of the line. For the two dimers, it is specified at the end of each line to which of the monomers they belong, the format corresponding to a Gaussian Counterpoise calculation. The geometries for the monomers are obtained by extracting the respective fragment from the dimer geometry (since no reoptimization is performed for the monomers).

Graphical representations av molecules and molecular surfaces were prepared with GaussView.[23]

```
---------------------------------------------------------------
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    H        ... pc-2

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E(RM06) =  -655.097260318

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Peptide 5 ONIOM(M06:PM6)

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 H        6.661732    4.447430    1.146507   Low
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 H        7.017729   -1.407805    0.441925   Low

 Small system: E(RPM6)  =    -0.359438869264
 Small system: E(RM06)  = -1487.031484837557
 Big system:   E(RPM6)  =    -1.055915343918
 Total:        E(ONIOM) = -1487.727961312211

------------------------------------------------------------
S(Me)8···A(Cl)3 extracted from peptide 5  M06
Fragment 1: A(Cl)3
Fragment 2: S(Me)8

C         -1.335016   -0.343293    2.704164 1
O         -0.214059    0.006762    2.395286 1
N         -2.274641   -0.748966    1.839248 1
C         -2.136662   -0.575464    0.411394 1
C         -3.420834    0.059812   -0.123128 1
O         -4.500625   -0.242193    0.349287 1
C         -1.964752   -1.890641   -0.323222 1
Cl        -0.381198   -2.644644    0.044317 1
H         -3.206148   -0.964493    2.167578 1
H         -1.276994    0.065169    0.213137 1
H         -2.735264   -2.602110   -0.034162 1
H         -2.004571   -1.740332   -1.404085 1
N         -3.230882    0.899634   -1.156061 1
```

```
C         -4.325179    1.456151   -1.925348 1
H         -2.276739    1.067918   -1.462865 1
H         -5.248655    1.008163   -1.517157 1
C          0.680823    0.477885   -2.352005 2
O         -0.270741    0.881991   -1.708831 2
N          1.873747    0.206979   -1.820861 2
C          2.083529    0.304503   -0.391263 2
C          2.122703    1.765005    0.068124 2
O          2.773311    2.596709   -0.532779 2
C          3.410188   -0.327315   -0.031190 2
O          3.393845   -1.672040   -0.413050 2
C          4.617113   -2.308831   -0.137030 2
H          2.614423   -0.159385   -2.397402 2
H          1.278446   -0.234723    0.120042 2
H          4.225426    0.211342   -0.538119 2
H          3.571424   -0.226964    1.050775 2
H          4.536134   -3.343151   -0.462710 2
H          5.443399   -1.829229   -0.672724 2
H          4.841489   -2.288961    0.934776 2
N          1.454009    1.986972    1.217062 2
C          1.430209    3.265378    1.890376 2
H          0.847356    1.253013    1.571979 2
H          2.234657    3.878958    1.440014 2
H         -1.668369   -0.393454    3.754318 1
H         -4.230494    1.200559   -2.979701 1
H         -4.378193    2.539450   -1.822236 1
H          0.635183    0.313158   -3.441432 2
H          1.616569    3.141102    2.955851 2
H          0.479082    3.781592    1.750478 2

 E(dimer)                   =  -1487.04008164
 E(fragment 1)              =   -916.063500025
 Counterpoise: BSSE energy =      0.001204181534

-------------------------------------------------------------
S(Me)8···A(H)3 extracted from peptide 5  M06
(Cl in A(Cl)3 replaced by H)
Fragment 1: S(Me)8
Fragment 2: A(H)3

C     -1.356886   -0.094657    2.783674 1
O     -0.243035    0.208546    2.406987 1
N     -2.288843   -0.680931    2.019943 1
C     -2.154829   -0.790472    0.585387 1
C     -3.451265   -0.297725   -0.058693 1
O     -4.524820   -0.520737    0.468637 1
C     -1.957815   -2.221409    0.124401 1
H     -3.215873   -0.844863    2.388316 1
H     -1.307793   -0.185545    0.260828 1
H     -2.714304   -2.876133    0.551593 1
H     -2.000975   -2.288460   -0.964674 1
N     -3.278014    0.324822   -1.237995 1
C     -4.383183    0.697564   -2.097450 1
H     -2.327429    0.447133   -1.576028 1
H     -5.297655    0.321733   -1.604892 1
C      0.640628   -0.251046   -2.343535 2
O     -0.318311    0.254166   -1.788876 2
N      1.838785   -0.389066   -1.774376 2
C      2.047226   -0.006984   -0.393132 2
C      2.058333    1.515975   -0.231552 2
O      2.692488    2.224646   -0.987687 2
C      3.386003   -0.530017    0.079078 2
```

```
O      3.395515   -1.923752   -0.029477 2
C      4.630983   -2.470222    0.361757 2
H      2.586172   -0.848099   -2.270283 2
H      1.252931   -0.449623    0.218033 2
H      4.190464   -0.086880   -0.527728 2
H      3.545710   -0.214786    1.119192 2
H      4.569890   -3.549847    0.247214 2
H      5.447619   -2.090440   -0.261625 2
H      4.855370   -2.234685    1.407549 2
N      1.385941    1.947970    0.853689 2
C      1.337695    3.333549    1.261222 2
H      0.793741    1.287313    1.349190 2
H      2.129942    3.861094    0.695109 2
H     -1.688778    0.057434    3.824433 1
H     -4.284004    0.240471   -3.080904 1
H     -4.457097    1.778677   -2.210197 1
H      0.597737   -0.628664   -3.378732 2
H      1.526859    3.425840    2.329450 2
H      0.376705    3.793883    1.026101 2
H     -0.980340   -2.585411    0.445625 1

 E(dimer)                  = -1027.43061020
 E(fragment 1)             =  -456.456445015
 E(fragment 2)             =  -570.959669452
 Counterpoise: BSSE energy =     0.001107246925

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