Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

NMR Characterization of a Cu(I)-bound Peptide Model of Copper Metallochaperones: Insights on the Role of Methionine

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Experimental

Peptide Synthesis

2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and *t*Boc-amino acids were obtained from Novabiochem. Dimethylformamide (DMF), *N*,*N*-diisopropylethylamine (DIPEA), diethyl ether, acetonitrile and trifluoroacetic acid (TFA) were obtained from Biosolve. S-tritylmercaptopropionic acid was obtained from Peptides International. Triisopropylsilane (TIS) and *p*-cresol were obtained from Sigma-Aldrich. 4-methylbenzhydrylamine (MBHA) resin was obtained from Anaspec. Hydrogen fluoride (HF) and formic acid were obtained from Fluka.

Manual solid phase peptide synthesis (SPPS) for the H_2N-CSRPGMTCSG-MPAL peptide was employed on a 0.3 mmol scale using the *in situ* neutralization/HBTU activation procedure for Boc chemistry as previously described by Schnölzer *et al*.[1] To yield the C-terminal MPAL thioester the peptide was synthesized on a Trityl-Associated Mercaptopropionic Acid-Leucine (TAMPAL) resin as described by Hackeng *et al*.[2] The TAMPAL resin was prepared by coupling Boc-Leu to the 4-methylbenzhydrylamine (MBHA) resin by completing one synthetic cycle. This synthetic cycle consisted of a 1-3 minute activation of the *t*Boc amino acid (1.1 mmol) by HBTU (1.0 mmol, 0.5 M in DMF) in the presence of DIPEA (3 mmol). The amino acid was coupled for 10 minutes. All unbound amino acid was removed by a DMF flow wash and the *t*Boc group was removed by a two times 1 minute treatment with TFA followed by a DMF flow wash.

To continue the preparation of the TAMPAL resin 1.1 mmol of S-tritylmercaptopropionic acid was activated as mentioned above and coupled for 30 min to Leu-MBHA resin. The protecting trityl group was removed by the addition of TFA containing 2.5% (v/v) TIS and 2.5% (v/v) H_2O. The thioester bond was formed after coupling of the next C-terminal amino acid to the resin. After formation of the MPAL thioester on the resin, the remaining amino acids were coupled in the typical manner. All activated amino acids were coupled for 10 minutes except for serine and arginine, which were coupled for 20 minutes. The crude peptide on the resin was cleaved from the resin by treatment with anhydrous HF for 1h at 0°C with 4% (v/v) *p*-cresol as a scavenger. Subsequently the deprotected free peptide was precipitated in cold Et_2O, collected on a filter, dissolved in

50% (v/v) CH_3CN in H_2O (+ 0.1% TFA) and lyophilized yielding the crude peptide. To accomplish the cyclic peptide, native chemical ligation between the C-terminal thioester and the N-terminal cysteine (yielding a native peptide bound at the site of ligation) was performed. Therefore the crude peptide was dissolved in a buffer (10 mg/ml) containing 0.07 M Tris, 6 M guanidine hydrochloride (GuHCl), pH 8. 2% (v/v) Thiophenol was added and the reaction was kept at 37°C. After 1 hour cyclisation of the peptide was completed and the cyclic peptide was purified using reversed phase high pressure liquid chromatography (RP-HPLC). This semi-preparative RP-HPLC was performed on a Waters Deltaprep System using a Vydac RP C18 column (10 mm x 250 mm, 5 ml/min flow rate) by running a gradient of 5-25% CH_3CN in H_2O (+ 0.1% TFA) in 70 min. The cyclic peptide was characterized using liquid chromatography-mass spectrometry (LC-MS). LC-MS was performed on a Thermo LCQ Fleet system using a GraceSmart RP C18 column (50 mm x 2.1 mm, 0.2 ml/min flow rate) by running a gradient of 2-70% CH_3CN in H_2O (+ 0.1% HCOOH) in 9 min. The observed mass of 979.8 Da corresponded to the calculated molecular mass of 980.2 Da.

Sample Preparation

When the peptide was analyzed in water, the amide region of the spectrum underwent exchange with the water under the experimental conditions and the signals were broadened out. Therefore, the analysis was carried out in DMSO, which and has been shown to be a close mimic of the aqueous physiological environment.[3, 4]

Sample from acidic conditions: The peptide was dissolved in triple distilled water (TDW) and the pH was determined as 3.0. After lyophilization the apo peptide (2.0 mg, 2.04 μmol) was dissolved in 500 μL DMSO-d_6 and used as a control solution. In addition, 2.0 mg of the peptide (2.04 μmol) were dissolved in 450 μL DMSO-d_6 and 50 μL of a stock solution of CuCl (41.0 mM) in DMSO-d_6 were added. Dilution factor 10 – final concentration of CuCl was 4.1 mM.

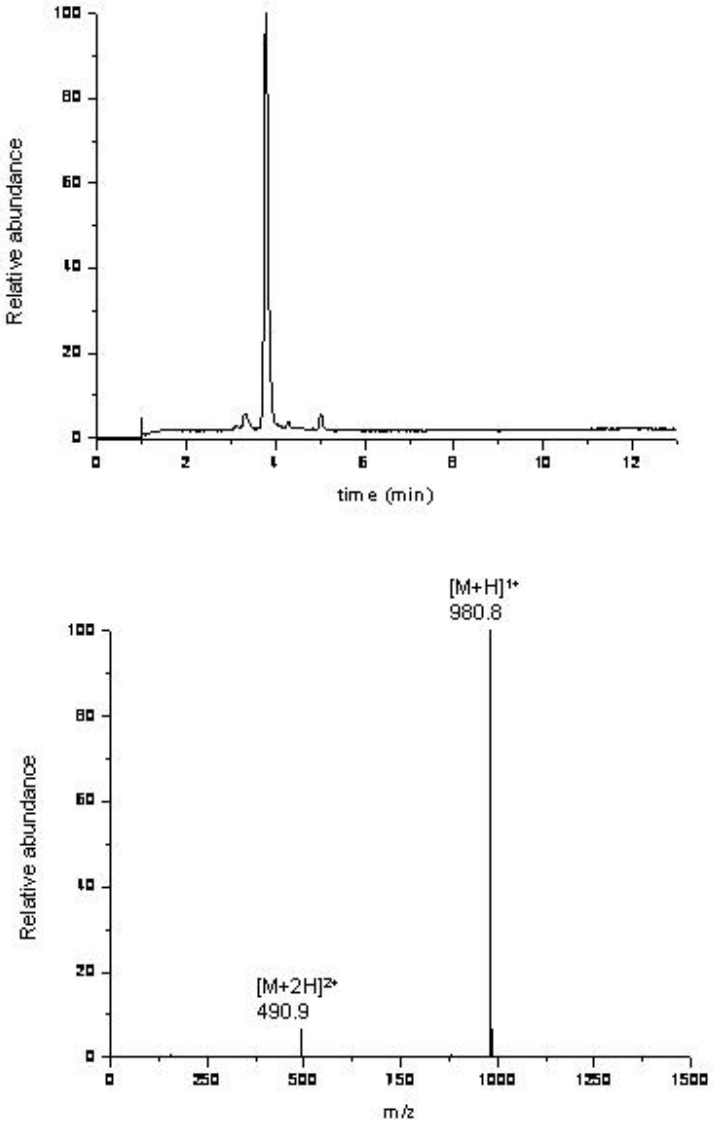
Sample from neutral conditions: The apo peptide (4.0 mg, 4.08 μmol) was dissolved in 1000 μL TDW and the pH was adjusted to 6.8 ±0.3 with a fresh solution of NaOH 0.1 N. The sample was lyophilized and 2.0 mg of the peptide was dissolved in 500 μL DMSO-d_6 and used as a control solution. In addition, 2.0 mg of the lyophilized peptide was dissolved in 450 μL DMSO-d_6, and 50 μL of a stock solution of CuCl (41.0 mM) in DMSO-d_6 was added. Dilution factor 10 – final concentration of CuCl was 4.10 mM.

Sample from basic conditions: The apo peptide (4.0 mg, 4.08 μmol) was dissolved in 1000 μL TDW and the pH was adjusted to 8.5±0.3 with a fresh solution of NaOH 0.1 N. The sample was lyophilized and 2.0 mg of the peptide was dissolved in 500 μL DMSO-d_6 and used as a control solution. In addition, 2.0 mg of the lyophilized peptide was dissolved in 450 μL DMSO-d_6, and 50 μL of a stock solution of CuCl (41.0 mM) in DMSO-d_6 was added. Dilution factor 10 – final concentration of CuCl was 4.10 mM.

LCMS of Cyclic peptide

Figure S1

LCMS analysis of the cyclic peptide with the sequence MTCSGCSRPG.



LCMS was performed on a Thermo LCQ Fleet system using a GraceSmart RP C18 column (50 mm x 2.1 mm, 0.2 ml/min flow rate) by running a gradient of 2-70% CH_3CN in H_2O (+ 0.1% HCOOH) in 9 min.

NMR structure determination

NMR experiments were performed on a Bruker Avance 600 MHz DMX spectrometer operating at the proton frequency of 600.13 MHz, using a 5-mm selective probe equipped with a self-shielded xyz-gradient coil at 20.0 ±0.1 °C. The transmitter frequency was set on the hydrogen-deuterium exchange in water signal, which was calibrated to 4.821 ppm. Correlation spectroscopy (COSY)[5], total correlation spectroscopy (TOCSY)[6] MLEV-17 based two-dimensional homonuclear magnetization transfer spectroscopy,[7] using the MLEV-17 pulse scheme for the 150 ms spin lock[6] and rotating frame nuclear Overhauser effect spectroscopy (ROESY)[6, 8, 9] experiments were acquired under identical conditions for all samples. The NOESY experiment gave a null spectrum under the conditions used. ROESY was performed using States-TPPI water suppression with a 3-9-19 pulse sequence with gradients for water saturation[10] and a mixing time of 150 ms.

Spectra were processed and analyzed with the TopSpin (Bruker Analytische Messtechnik GmbH) and SPARKY3 software.[11] Resonance assignment followed the sequential assignment methodology developed by Wüthrich.[12] Peak intensities were manually assigned from the Van der Waals radius until as strong (from the Van der Waals radius until 2.5 Å), medium (3.5 Å), weak (4.5 Å) and very weak (5.5 Å) with a ±0.5 Å error. The three-dimensional structures of the peptides were calculated using XPLOR (version 3.856)[13] by hybrid distance geometry-dynamical simulated annealing. The copper-sulfur bonds were introduced using patches within XPLOR. There were 50 initial structures, generated by simulated annealing. The NOE energy was introduced as a square-well potential. Molmol[14] was used to create the final ensemble of structures. Low energy structures chosen for further analysis had no NOE violations, deviations from ideal bond lengths of less than 0.05 Å, and bond angle deviations from ideality of less than 5º. Analysis and figures were made using Chimera.[15]

Figure S2

NMR TOCSY (green) and ROESY (purple) assignment spectra of cyclic MTCSGCSRPG from acidic conditions.

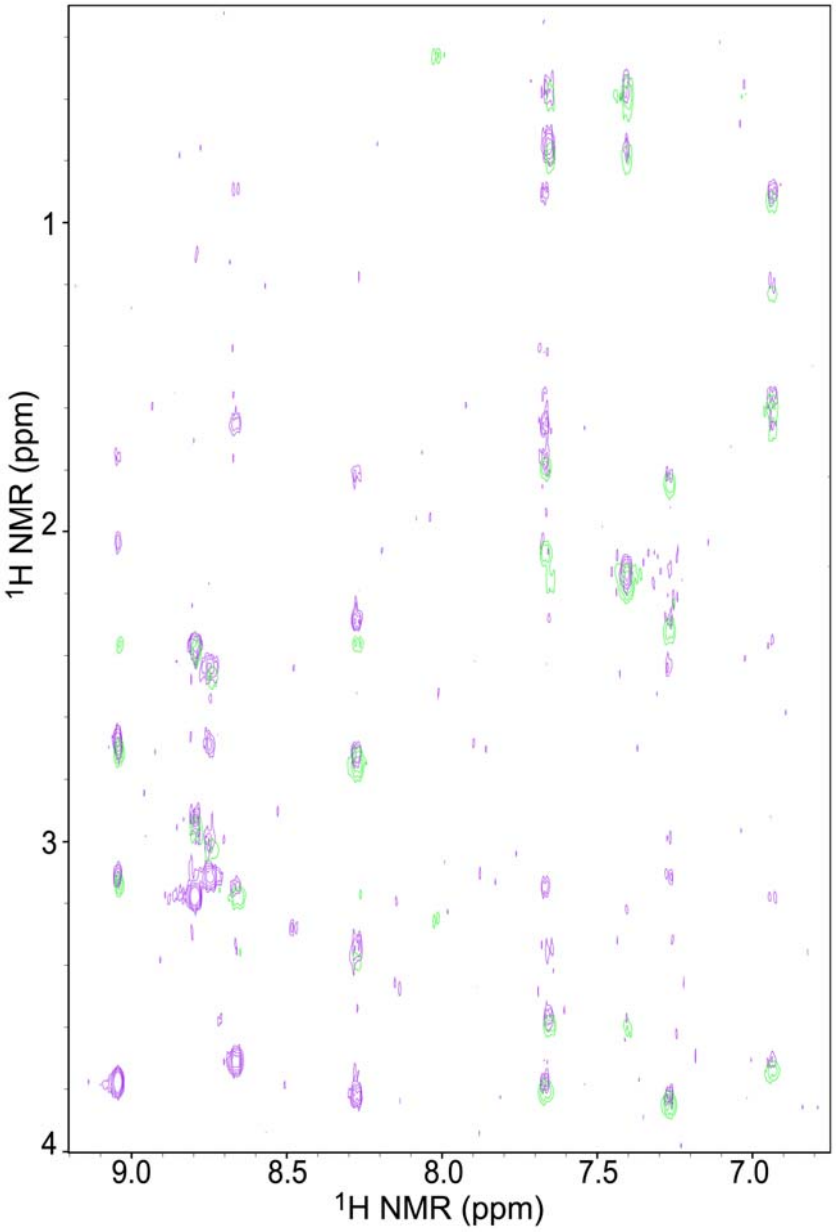


Figure S3

NMR TOCSY spectra of cyclic MTCSGCSRPG from acidic (green) and neutral (purple) conditions.

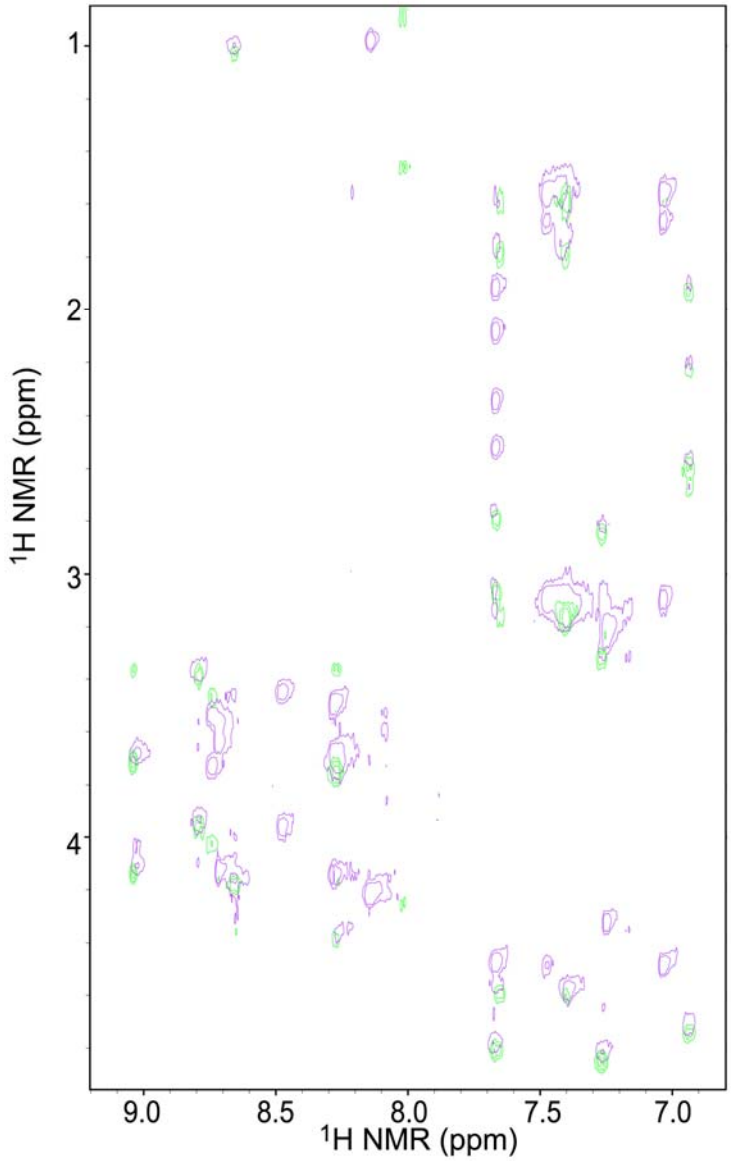
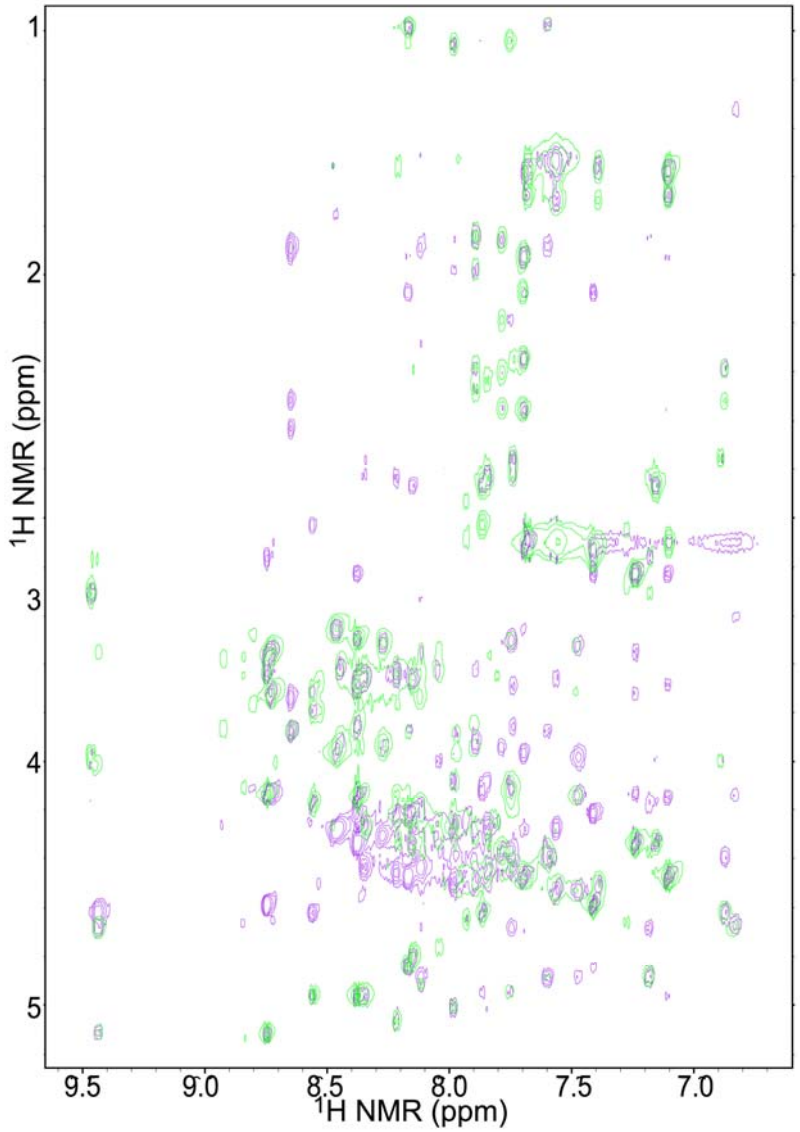


Figure S4

NMR TOCSY (green) and ROESY (purple) assignment spectra of cyclic MTCSGCSRPG from basic conditions.



Chemical shifts and constraints data

Table S1

^{1}H chemical shift assignment (ppm) of the cyclic peptide from acidic conditions.

	HN	**Hα**	**Hβ**	**Others**
Met1	6.94	4.71	2.19, 1.90	CH_2 γ 2.65, 2.56
Thr2	8.66	4.15		CH_3 γ 0.99
Cys3	7.67	4.78	3.03, 2.77	
Ser4	9.04	4.11	3.68, 3.43	
Gly5	8.75	3.99, 3.44		
Cys6	7.27	4.82	3.29, 2.81	
Ser7	8.27	4.34	3.72, 3.33	
Arg8	7.66	4.57	1.76	Hγ 1.56, Hε 3.13, Hη 7.41
Pro9		4.18	2.12, 1.72	Hδ 3.71, Hγ1 1.98, Hγ2 1.85
Gly10	8.80	3.93, 3.36		

Table S2

NOE interaction statistics of the cyclic peptide from acidic conditions

Total number of restraints	65
Intra-residual restraints	32
i+1 restraints	27
i+2 restraints	5
i+3 restraints	1

Table S3

NOE constraints used to generate structures of the cyclic peptide from acidic conditions

```
!i.HN
!!Restraints i,i+1.
  assign (resid 3  and name HN) (resid 2  and name HN) 2.5  0.7  0.0
  assign (resid 6  and name HN) (resid 5  and name HN) 2.5  0.7  0.0
  assign (resid 6  and name HN) (resid 7  and name HN) 3.5  1.7  0.0
  assign (resid 8  and name HN) (resid 7  and name HN) 2.5  0.7  0.0
  assign (resid 1  and name HN) (resid 10 and name HN) 3.0  1.2  0.0
!i.FP
!!Intra-residual restraints.
  assign (resid 1  and name HA) (resid 1  and name HN) 3.5  1.7  0.0
  assign (resid 1  and name HB#) (resid 1  and name HN) 4.0  2.2  0.0
```

```
  assign (resid 1  and name HB#) (resid 1  and name HN) 3.0  1.2  0.0
  assign (resid 1  and name HG#) (resid 1  and name HN) 4.5  2.7  0.0
  assign (resid 1  and name HG#) (resid 1  and name HN) 3.5  1.7  0.0
  assign (resid 2  and name HA) (resid 2  and name HN) 3.5  1.7  0.0
  assign (resid 2  and name HG*) (resid 2  and name HN) 3.5  1.7  0.0
  assign (resid 3  and name HA) (resid 3  and name HN) 3.0  1.2  0.0
  assign (resid 3  and name HB#) (resid 3  and name HN) 4.5  2.7  0.0
  assign (resid 3  and name HB#) (resid 3  and name HN) 3.5  1.7  0.0
  assign (resid 4  and name HA) (resid 4  and name HN) 3.0  1.2  0.0
  assign (resid 4  and name HB#) (resid 4  and name HN) 3.0  1.2  0.0
  assign (resid 5  and name HA#) (resid 5  and name HN) 3.5  1.7  0.0
  assign (resid 5  and name HA#) (resid 5  and name HN) 2.5  0.7  0.0
  assign (resid 6  and name HA) (resid 6  and name HN) 3.0  1.2  0.0
  assign (resid 6  and name HB#) (resid 6  and name HN) 3.5  1.7  0.0
  assign (resid 6  and name HB#) (resid 6  and name HN) 4.0  2.2  0.0
  assign (resid 7  and name HA) (resid 7  and name HN) 3.0  1.2  0.0
  assign (resid 7  and name HB#) (resid 7  and name HN) 2.5  0.7  0.0
  assign (resid 8  and name HA) (resid 8  and name HN) 3.0  1.2  0.0
  assign (resid 8  and name HB*) (resid 8  and name HN) 2.5  0.7  0.0
  assign (resid 8  and name HG*) (resid 8  and name HN) 3.0  1.2  0.0
  assign (resid 10 and name HA#) (resid 10 and name HN) 3.0  1.2  0.0
  assign (resid 10 and name HA#) (resid 10 and name HN) 2.5  0.7  0.0
!i+1.FP
!!Restraints i,i+1.
  assign (resid 1  and name HA) (resid 2  and name HN) 2.5  0.7  0.0
  assign (resid 1  and name HB#) (resid 2  and name HN) 4.5  2.7  0.0
  assign (resid 1  and name HG#) (resid 2  and name HN) 3.5  1.7  0.0
  assign (resid 2  and name HA) (resid 3  and name HN) 3.5  1.7  0.0
  assign (resid 3  and name HA) (resid 4  and name HN) 2.5  0.7  0.0
  assign (resid 3  and name HB#) (resid 4  and name HN) 3.5  1.7  0.0
  assign (resid 3  and name HB#) (resid 4  and name HN) 4.5  2.7  0.0
  assign (resid 4  and name HA) (resid 5  and name HN) 2.5  0.7  0.0
  assign (resid 4  and name HB#) (resid 5  and name HN) 3.0  1.2  0.0
  assign (resid 5  and name HA#) (resid 6  and name HN) 3.5  1.7  0.0
  assign (resid 5  and name HA#) (resid 6  and name HN) 5.5  3.7  0.0
  assign (resid 6  and name HA) (resid 7  and name HN) 2.5  0.7  0.0
  assign (resid 6  and name HB#) (resid 7  and name HN) 2.5  0.7  0.0
  assign (resid 6  and name HB#) (resid 7  and name HN) 3.5  1.7  0.0
  assign (resid 7  and name HA) (resid 8  and name HN) 4.5  2.7  0.0
  assign (resid 8  and name HH#) (resid 7  and name HN) 5.5  3.7  0.0
  assign (resid 9  and name HA) (resid 10 and name HN) 2.5  0.7  0.0
  assign (resid 9  and name HB#) (resid 10 and name HN) 5.5  3.7  0.0
  assign (resid 10 and name HA#) (resid 1  and name HN) 4.5  2.7  0.0
  assign (resid 10 and name HA#) (resid 1  and name HN) 5.5  3.7  0.0


!i+2.FP
!!Restraints i,i+2.
  assign (resid 1  and name HB#) (resid 3  and name HN) 3.5  1.7  0.0
  assign (resid 1  and name HE*) (resid 3  and name HN) 5.5  3.7  0.0
  assign (resid 1  and name HG#) (resid 3  and name HN) 3.0  1.2  0.0
  assign (resid 4  and name HA) (resid 6  and name HN) 4.0  2.2  0.0
  assign (resid 9  and name HA) (resid 1  and name HN) 4.5  2.7  0.0
!i.AL
!!Intra-residual restraints.
  assign (resid 1  and name HA) (resid 1  and name HE*) 5.5  3.7  0.0
  assign (resid 8  and name HB*) (resid 8  and name HH#) 3.5  1.7  0.0
```

```
  assign (resid 8  and name HE*) (resid 8  and name HH#) 2.5  0.7  0.0
  assign (resid 8  and name HG*) (resid 8  and name HH#) 3.0  1.2  0.0
  assign (resid 9  and name HB#) (resid 9  and name HA) 2.5  0.7  0.0
  assign (resid 9  and name HB#) (resid 9  and name HA) 3.0  1.2  0.0
  assign (resid 9  and name HG#) (resid 9  and name HD#) 3.0  1.2  0.0
  assign (resid 9  and name HG#) (resid 9  and name HD#) 3.5  1.7  0.0
!i+1.AL
!!Restraints i,i+1.
  assign (resid 9  and name HD#) (resid 8  and name HA) 2.5  0.7  0.0
  assign (resid 9  and name HD#) (resid 8  and name HA) 2.5  0.7  0.0
!i+3.AL
!!Restraints i,i+3.
  assign (resid 8  and name HB*) (resid 1  and name HG#) 4.5  2.7  0.0
```

Table S4

^{1}H chemical shift assignment (ppm) of the cyclic peptide from basic conditions

	HN	**Hα**	**Hβ**	**Others**
Met1	7.70	4.48	2.08, 1.93	CH_2 γ 2.56, 2.36
Thr2	8.17	4.22	4.85	CH_3 γ 0.99
Cys3	7.41	4.59	3.14	
Ser4	7.24	4.34	3.23	
Gly5	8.72	3.72, 3.55		
Cys6	8.75	4.13	3.64, 3.57	
Ser7	8.38	4.14	3.69, 3.50	
Arg8	7.11	4.47	1.68	Hγ 1.57, Hε 3.10, Hη 7.69
Pro9		4.28	2.13, 1.76	Hδ1 3.81, Hδ2 3.53, Hγ1 1.98, Hγ2 1.88
Gly10	8.46	3.96, 3.46		

Table S5

NOE interaction statistics of the cyclic peptide from basic conditions

Total number of restraints	111
Intra-residual restraints	67
***i*+1 restraints**	25
***i*+2 restraints**	10
***i*+3 restraints**	7
Long range restraints	2

Table S6

NOE constraints of the cyclic peptide from basic conditions

```
!i+1.HN
!!Restraints i,i+1.
  assign (resid 3  and name HN) (resid 2  and name HN) 2.5  0.5  0.35
  assign (resid 4  and name HN) (resid 5  and name HN) 3.0  1.0  0.35
  assign (resid 8  and name HN) (resid 7  and name HN) 2.5  0.5  0.35
  assign (resid 1  and name HN) (resid 10 and name HN) 4.0  2.0  0.35
!i.FP
!!Intra-residual restraints.
  assign (resid 1  and name HA) (resid 1  and name HN) 3.5  1.5  0.35
  assign (resid 1  and name HB#) (resid 1  and name HN) 4.5  2.5  0.35
  assign (resid 1  and name HB#) (resid 1  and name HN) 3.0  1.0  0.35
  assign (resid 1  and name HG#) (resid 1  and name HN) 4.0  2.0  0.35
  assign (resid 1  and name HG#) (resid 1  and name HN) 3.0  1.0  0.35
  assign (resid 2  and name HA) (resid 2  and name HN) 2.5  0.5  0.35
  assign (resid 2  and name HB*) (resid 2  and name HN) 3.0  1.0  0.35
  assign (resid 2  and name HG*) (resid 2  and name HN) 3.0  1.0  0.35
  assign (resid 3  and name HA) (resid 3  and name HN) 2.5  0.5  0.35
  assign (resid 3  and name HB*) (resid 3  and name HN) 3.0  1.0  0.35
  assign (resid 4  and name HA) (resid 4  and name HN) 3.0  1.0  0.35
  assign (resid 4  and name HB*) (resid 4  and name HN) 2.5  0.5  0.35
  assign (resid 5  and name HA#) (resid 5  and name HN) 3.0  1.0  0.35
  assign (resid 5  and name HA#) (resid 5  and name HN) 3.5  1.5  0.35
  assign (resid 6  and name HA) (resid 6  and name HN) 3.0  1.0  0.35
  assign (resid 6  and name HB#) (resid 6  and name HN) 3.0  1.0  0.35
  assign (resid 6  and name HB#) (resid 6  and name HN) 3.0  1.0  0.35
  assign (resid 7  and name HA) (resid 7  and name HN) 3.0  1.0  0.35
  assign (resid 7  and name HB#) (resid 7  and name HN) 2.5  0.5  0.35
  assign (resid 7  and name HB#) (resid 7  and name HN) 3.5  1.5  0.35
  assign (resid 8  and name HA) (resid 8  and name HN) 3.0  1.0  0.35
  assign (resid 8  and name HB*) (resid 8  and name HN) 3.0  1.0  0.35
  assign (resid 8  and name HD*) (resid 8  and name HN) 4.5  2.5  0.35
  assign (resid 8  and name HG*) (resid 8  and name HN) 2.5  0.5  0.35
  assign (resid 10 and name HA#) (resid 10 and name HN) 3.5  1.5  0.35
  assign (resid 10 and name HA#) (resid 10 and name HN)  2.5  0.5  0.35
!i+1.FP
!!Restraints i,i+1.
  assign (resid 1  and name HA) (resid 2  and name HN) 2.5  0.5  0.35
  assign (resid 1  and name HB#) (resid 2  and name HN) 3.0  1.0  0.35
  assign (resid 1  and name HB#) (resid 2  and name HN) 5.0  3.0  0.35
  assign (resid 2  and name HA) (resid 3  and name HN) 2.5  0.5  0.35
  assign (resid 2  and name HB*) (resid 3  and name HN) 4.0  2.0  0.35
  assign (resid 2  and name HG*) (resid 3  and name HN) 5.5  3.5  0.35
  assign (resid 3  and name HA) (resid 4  and name HN) 5.5  3.5  0.35
  assign (resid 4  and name HB*) (resid 3  and name HN) 3.5  1.5  0.35
  assign (resid 5  and name HA#) (resid 4  and name HN) 3.5  1.5  0.35
  assign (resid 5  and name HA#) (resid 4  and name HN) 4.0  2.0  0.35
  assign (resid 6  and name HA) (resid 5  and name HN) 4.5  2.5  0.35
  assign (resid 7  and name HA) (resid 8  and name HN) 3.0  1.0  0.35
  assign (resid 7  and name HB#) (resid 8  and name HN) 4.0  2.0  0.35
  assign (resid 7  and name HB#) (resid 8  and name HN) 5.0  3.0  0.35
  assign (resid 9  and name HA) (resid 10 and name HN) 2.5  0.5  0.35
  assign (resid 9  and name HB#) (resid 10 and name HN) 4.5  2.5  0.35
  assign (resid 10 and name HA#) (resid 1  and name HN) 3.0  1.0  0.35
```

```
  assign (resid 10 and name HA#) (resid 1  and name HN) 4.5  2.5  0.35
!i+2.FP
!!Restraints i,i+2.
  assign (resid 1  and name HB#) (resid 3  and name HN) 3.0  1.0  0.35
  assign (resid 3  and name HA) (resid 5  and name HN) 4.5  2.5  0.35
  assign (resid 3  and name HB#) (resid 1  and name HN) 4.0  2.0  0.35
  assign (resid 9  and name HA) (resid 1  and name HN) 3.5  1.5  0.35
  assign (resid 3  and name HB#) (resid 5  and name HN) 5.0  3.0  0.35
  assign (resid 3  and name HB#) (resid 5  and name HN) 5.0  3.0  0.35
  assign (resid 6  and name HA) (resid 4  and name HN) 3.5  1.5  0.35
!i+3.FP
!!Restraints i,i+3.
  assign (resid 3  and name HA) (resid 6  and name HN) 2.5  0.5  0.35
  assign (resid 3  and name HB#) (resid 6  and name HN) 3.5  1.5  0.35
  assign (resid 4  and name HA) (resid 7  and name HN) 2.5  0.5  0.35
  assign (resid 4  and name HB*) (resid 7  and name HN) 3.0  1.0  0.35
  assign (resid 1  and name HB#) (resid 8  and name HN) 4.5  2.5  0.35
  assign (resid 1  and name HG#) (resid 8  and name HN) 5.0  3.0  0.35
!i-lr.FP
!!Long range (further than i+3).
  assign (resid 2  and name HA) (resid 7  and name HN) 4.5  2.7  1.5
  assign (resid 4  and name HB*) (resid 8  and name HN) 3.0  1.0  0.35
!i.AL
!!Intra-residual restraints.
  assign (resid 1  and name HB#) (resid 1  and name HA) 2.5  0.5  0.35
  assign (resid 1  and name HB1) (resid 1  and name HB2) 2.5  0.5  0.35
  assign (resid 1  and name HB#) (resid 1  and name HG#) 3.5  1.5  0.35
  assign (resid 1  and name HB#) (resid 1  and name HG#) 4.5  2.5  0.35
  assign (resid 1  and name HB#) (resid 1  and name HA) 3.0  1.0  0.35
  assign (resid 1  and name HB#) (resid 1  and name HG#) 4.0  2.0  0.35
  assign (resid 1  and name HG#) (resid 1  and name HA) 3.5  1.5  0.35
  assign (resid 1  and name HG#) (resid 1  and name HB#) 3.5  1.5  0.35
  assign (resid 1  and name HG1) (resid 1  and name HG2) 2.5  0.5  0.35
  assign (resid 1  and name HG#) (resid 1  and name HA) 2.5  0.5  0.35
  assign (resid 2  and name HA) (resid 2  and name HB*) 2.5  0.5  0.35
  assign (resid 2  and name HG*) (resid 2  and name HA) 2.5  0.5  0.35
  assign (resid 2  and name HG*) (resid 2  and name HB*) 2.5  0.5  0.35
  assign (resid 3  and name HA) (resid 3  and name HB#) 3.0  1.0  0.35
  assign (resid 3  and name HA) (resid 3  and name HB#) 3.0  1.0  0.35
  assign (resid 4  and name HB*) (resid 4  and name HA) 2.5  0.5  0.35
  assign (resid 6  and name HB#) (resid 6  and name HA) 3.5  1.5  0.35
  assign (resid 6  and name HB1) (resid 6  and name HB2) 2.5  0.5  0.35
  assign (resid 7  and name HB#) (resid 7  and name HA) 2.5  0.5  0.35
  assign (resid 7  and name HB1) (resid 7  and name HB2) 2.5  0.5  0.35
  assign (resid 8  and name HA) (resid 8  and name HE) 3.0  1.0  0.35
  assign (resid 8  and name HB*) (resid 8  and name HA) 2.5  0.5  0.35
  assign (resid 8  and name HB*) (resid 8  and name HD*) 3.0  1.0  0.35
  assign (resid 8  and name HB*) (resid 8  and name HE) 4.0  2.0  0.35
  assign (resid 8  and name HD*) (resid 8  and name HA) 2.5  0.5  0.35
  assign (resid 8  and name HD*) (resid 8  and name HE) 3.0  1.0  0.35
  assign (resid 8  and name HG*) (resid 8  and name HA) 2.5  0.5  0.35
  assign (resid 8  and name HG*) (resid 8  and name HB*) 3.0  1.0  0.35
  assign (resid 8  and name HG*) (resid 8  and name HD*) 2.5  0.5  0.35
  assign (resid 8  and name HG*) (resid 8  and name HE) 3.0  1.0  0.35
  assign (resid 9  and name HB#) (resid 9  and name HA) 3.0  1.0  0.35
  assign (resid 9  and name HB#) (resid 9  and name HD#) 4.5  2.5  0.35
  assign (resid 9  and name HB#) (resid 9  and name HA) 3.5  1.5  0.35
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  assign (resid 9  and name HB1) (resid 9  and name HB2) 2.5  0.5  0.35
  assign (resid 9  and name HB#) (resid 9  and name HD#) 4.0  2.0  0.35
  assign (resid 9  and name HD1) (resid 9  and name HD2) 2.5  0.5  0.35
  assign (resid 9  and name HG#) (resid 9  and name HD#) 2.5  0.5  0.35
  assign (resid 9  and name HG#) (resid 9  and name HD#) 3.0  1.0  0.35
  assign (resid 9  and name HG#) (resid 9  and name HD#) 3.5  1.5  0.35
  assign (resid 9  and name HG#) (resid 9  and name HD#) 2.5  0.5  0.35
  assign (resid 10 and name HA1) (resid 10 and name HA2) 2.5  0.5  0.35
!i+1.AL
!!Restraints i,i+1.
  assign (resid 1  and name HB#) (resid 2  and name HA) 3.5  1.5  0.35
  assign (resid 9  and name HD#) (resid 8  and name HB*) 3.5  1.5  0.35
  assign (resid 9  and name HD#) (resid 8  and name HG*) 5.0  3.0  0.35
  assign (resid 9  and name HD#) (resid 8  and name HE) 5.0  3.0  0.35
!i+2.AL
!!Restraints i,i+2.
  assign (resid 1  and name HG#) (resid 3  and name HB#) 4.0  2.0  0.35
  assign (resid 1  and name HG#) (resid 3  and name HB#) 4.0  2.0  0.35
  assign (resid 9  and name HG#) (resid 7  and name HB#) 4.5  2.5  0.35
!i+3.AL
!!Restraints i,i+3.
  assign (resid 9  and name HG#) (resid 6  and name HB#) 4.0  2.0  0.35
```

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