1. General information.

NMR experiments were acquired at 10 °C on a Varian Inova 600 spectrometer and processed with XWINNMR. LC-ESI-MS was carried out using an Agilent 1100 series binary pump together with a reversed-phase HPLC C18 column (Macherey-Nagel) and a Finnigan Thermoquest LCQ. Purification of β-peptides was performed with an Agilent 1100 Series with a Nucleodur C18 gravity column (Macherey-Nagel) at a flow rate of 25 mL/min. UV spectra were obtained in a Varian Cary 100 Bio spectrophotometer. Reactions under microwave irradiation were carried out in a CEM Explorer microwave, a monomodal synthesis reactor equipped with a 300 W (max) power source, an infrared sensor for temperature control and an automated synthesis workstation module.

Tenta Gel R PHB resin with a loading of 0.2 mmol/g was purchased from Rapp Polymere GmbH. D_2O was purchased from Deutero GmbH. PyBOP® was obtained from Novabiochem. Commercial α-*L*-amino acids were obtained from Aldrich-Sigma and Novabiochem. All other reagents were purchased from Aldrich-Sigma, Fluka and Acros.

2. b-Peptide synthesis.

A. β-Amino acid building blocks

Fmoc-protected β^3-*L*-amino acid derivatives were prepared by homologation from the corresponding commercial α-*L*-amino acids as described.[1,2] The Fmoc-protected disubstituted $\beta^{2,3}$-*L*-amino acid derivative *trans*-aminocyclohexyl carboxylic acid (ACHC), was synthesized as described.[3]

β-Peptide synthesis

Loading of the resin. Tenta Gel R PHB resin (1.0 g, 0.2 mmol/g resin initial loading) was swollen in dry CH_2Cl_2 (10.0 mL) for 30 min. A solution of Fmoc-β^3Tyr(*t*Bu)-OH (284.1 mg, 3 equiv) and DIC (46.7 µL, 1.5 equiv) in dry CH_2Cl_2 (1.5 mL) stirred for 20 min at room temperature and then added to the resin, followed by dimethylaminopyridine (DMAP) (36.7 mg, 1.5 equiv), and the suspension was stirred at room

[1] D. Seebach, M. Overhand, F. Kühnle, N. M. Florina, B. Martinoni, L. Oberer, U. Hommel, H. Widmer *Helv. Chim. Acta* **1996**, *79*, 913-941.
[2] E. Vaz, L. Brunsveld *Org. Lett.* **2006**, *8*, 4199-4202.
[3] M. Schinnerl, J. K. Murray, J. M. Langehan, S. H. Gellman *Eur. J. Org. Chem.* **2003**, 721-726.

temperature for 21 h. The resin was washed with CH_2Cl_2 (5 x 30 s), NMP (5 x 30 s), 90:10 NMP/MeOH (10 min), NMP (5 x 30 s), CH_2Cl_2 (5 x 30 s) and shrunk down with diethyl ether (5 x 30 s) and dried under vacuum. Fmoc-determination by UV-spectrophotometry at 301 nm with a small sample of the resin (ca. 2 mg) resulted in a calculated loading of 0.15 mmol/g resin. Unreacted sites of the resin were capped with a mixture of acetic acid anhydride (7.5 mL, 100 equiv), DMAP (107.5 mg, 1.1 equiv) and NMP (40 mL) by stirring for 70 min at room temperature. The resin was subsequently washed with NMP (5 x 30 s), CH_2Cl_2 (5 x 30 s) and Et_2O (5 x 30 s).

Synthesis of β*-peptides* **1-3**. β-Peptides were synthesized on a 27 μmol scale (150 mg of resin) using standard Fmoc-chemistry. One cycle of peptide elongation consisted of the following steps. The loaded resin was first swollen with *N*-methyl-2-pyrrolidone (NMP) (1 x 30 min) for initial resin swelling and the Fmoc protecting group was removed by treatment with 20% piperidine/DMF (3 mL, 1 x 15 min) followed by 2% *v/v* piperidine, 2% *v/v* 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) in NMP (3 mL, 1 x 15 min), then washed with NMP (5 x 30 s). The deprotected resin was treated for 2 h with a cocktail containing the appropriate β-amino acid (3 equiv), PyBOP® (39.3 mg, 0.076 mmol, 2.8 equiv), HOBt (11.6 mg, 0.076 mmol, 2.8 equiv) and diisopropylethylamine (DIEA) (38.8 μL, 0.227 mmol, 8.4 equiv) in NMP (1.0 mL) prepared immediately before addition to the resin. TNBS tests after each coupling were negative, nevertheless after washing with NMP (3 x 30 s), capping was performed by treatment with 7% *v/v* acetic anhydride and 7% *v/v* DIEA in NMP (5 mL, 5 min) and the capped resin was washed with NMP (5 x 30 s).

For the coupling and Fmoc deprotection of (*S,S*)-*trans*-aminocyclohexane carboxylic acid (ACHC) units, microwave irradiation was used. Coupling of the ACHC unit was achieved *via* treatment of the deprotected resin with a cocktail containing Fmoc-ACHC-OH (29.6 mg, 3 equiv), HBTU (30.7 mg, 3 equiv), HOBt (12.4 mg, 3 equiv) and DIEA (27.7 μL, 6 equiv) in 3 mL of 0.8 M LiCl in NMP under microwave irradiation (50 W maximum power, 45 ºC, ramp 2 min, hold 4 min). After filtration the process was repeated. The Fmoc deprotection was carried out with 20% *v/v* piperidine/NMP (3 mL, 50 W maximum power, 60 ºC, ramp 2 min, hold 2 min), the mixture was filtered and the deprotection protocol was repeated once, then the resin was washed with NMP (5 x 30 s).

The different steps were repeated until the complete β-peptide sequence was assembled.

For the cyclic β3-peptides **2**, the cyclization step was performed first removing the Allyl and Alloc protecting groups by treating the resin swollen in degassed CH_2Cl_2 in the presence of Ar, with PhSiH3 (24 equiv) and Pd(PPh3)4 (0.25 equiv) in CH_2Cl_2 for 30 min under Argon and continuous shaking. The peptide resin was washed with CH_2Cl_2 (3 x 2 min), NMP (3 x 2 min) and again with CH_2Cl_2 (4 x 2 min) and the process was repeated. Finally, the resin was washed with CH_2Cl_2 (3 x 2 min), NMP (3 x 2 min), a 0.02 M solution of Et2NCS2Na in NMP (3 x 2 min) and again with NMP (5 x 30 s). To the Alloc/Allyl deprotected β3-peptide resin was added HATU (1 equiv), HOBt (1 equiv) and DIEA (3 equiv) in 3 mL of NMP and shaken 12 h to undergo cyclization via the free carboxylic acid side-chain and the free amino group side chain. This process was repeated until a negative TNBS test resulted.

The final Fmoc protecting group was removed as described above, the resin was washed with NMP (5 x 30 s), CH_2Cl_2 (5 x 30 s) and Et_2O (5 x 30 s) and dried under vacuum for 3 h. The resin was treated for 3 h with a cleavage cocktail composed of 2% *v/v* water and 2% *v/v* triisopropylsilane (TIPS) in trifluoroacetic acid (TFA). The cleaved resin was washed with TFA (3 x 15 s) and the cleaved β-peptide was collected, concentrated by rotary evaporation into less than 1 mL solution and precipitated by addition of cold Et_2O (10 mL). The mixture was cooled in a liquid N_2 bath for 1 min, centrifuged (10000 rpm, 5 min, 4 °C) and the Et_2O was decanted from the pellet. Cold Et_2O was added again and the procedure was repeated twice. The crude peptide obtained was dissolved in H_2O/MeCN and lyophilized to dryness.

β-peptide purification and analysis. The crude reaction mixture of each β-peptide synthesized was analyzed by LC-MS. β-Peptides were then purified by reversed-phase preparative HPLC on a Nucleodur C18 Gravity column (125 x 21 mm, Macherey-Nagel) with a linear gradient of A (0.1% HCOOH in H_2O) and B (0.1% HCOOH in CH_3CN) from 10% to 50% of B and a flow rate of 25 mL min^{-1} and were detected at 214 nm, 254 nm and 280 nm using a diode array UV/Vis detector. The identities and purities of the purified β-peptides were assessed by LC-MS (ESI mass spectrometry) (Table S1). Following purification, all β-peptides were lyophilized and kept at –20 °C.

Table S1. Analytical data of synthesized β-peptides.

β-peptide	Mass $[M+H]^+$		Purity	Yield
	calcd	found		
Lin(2,5)-LysGlu **1a**	936.58	936.52	> 99%	33%
Lin(2,5)-LysAsp **1b**	922.56	922.52	> 99%	35%
Cy(2,5)-LysGlu **2a**	918.57	918.52	> 99%	13%
Cy(2,5)-LysAsp **2b**	904.55	904.49	> 99%	9%
ACHC(2,5) **3**	901.58	901.51	> 99%	13%

3. Circular Dichroism Spectroscopy

CD spectra were recorded on a JASCO-815 spectrometer at 20 °C in MeOH or in a 10 mM sodium phosphate buffer (pH 7.4) using a 0.1 cm path length CD cell. For measurements at different pH, data were obtained from solutions in 100 mM AcOK/AcOH buffer (pH 1.75 and pH 3.6) or 50 mM $NaHCO_3$/NaOH buffer (pH 9.6). For the evaluation of the ionic strength on structural stability, increasing concentrations of NaCl ranging from 0 to 1.6 M in 10 mM sodium phosphate buffer (pH 7.4) were prepared. Each peptide was analyzed at a concentration of 150-200 μM. The final concentration of the β-peptide in each MeOH and aqueous buffer solutions was determined immediately before obtaining the CD spectrum by the UV absorbance of the β^3-Tyr residue using the extinction coefficient of 1420 cm^{-1} M^{-1} at 275 nm, the extinction coefficient of α-tyrosine.[4] Spectra represent the average of 10 scans (0.5 nm data pitch, continuous scanning mode, 20 nm min^{-1} scanning speed, 0.5 nm bandwidth) and were smoothed by Jasco software. Data were converted to mean-residue ellipticity MRE (in deg·cm^2·$dmol^{-1}$·$residue^{-1}$) according to the equation:

$$MRE = \text{CD effect} / C \cdot l \cdot n_{res}$$

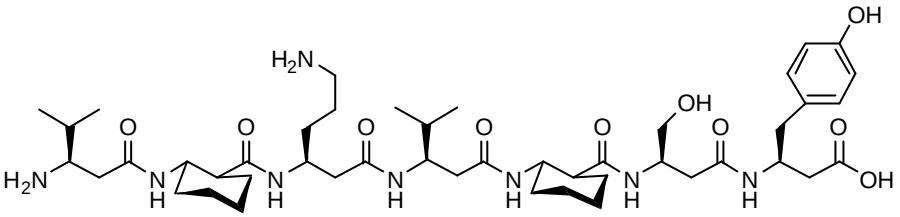
where the CD effect is in millidegrees, the concentration (C) is in moles per liter, the path length (l) is in millimeters and n_{res} is the number of residues.

[4] T. E. Creighton *Proteins: Structures and Molecular Principles*, 2nd ed.; W. H. Freeman and Company: New York, 1993; p14.

4. LC-MS

LC-MS spectra of the β-peptide **3** containing two ACHC units are given.

ACHC(2,5) **3**: calc. mass $[M+H]^+$ 901.58



ACHC(2,5) **3**

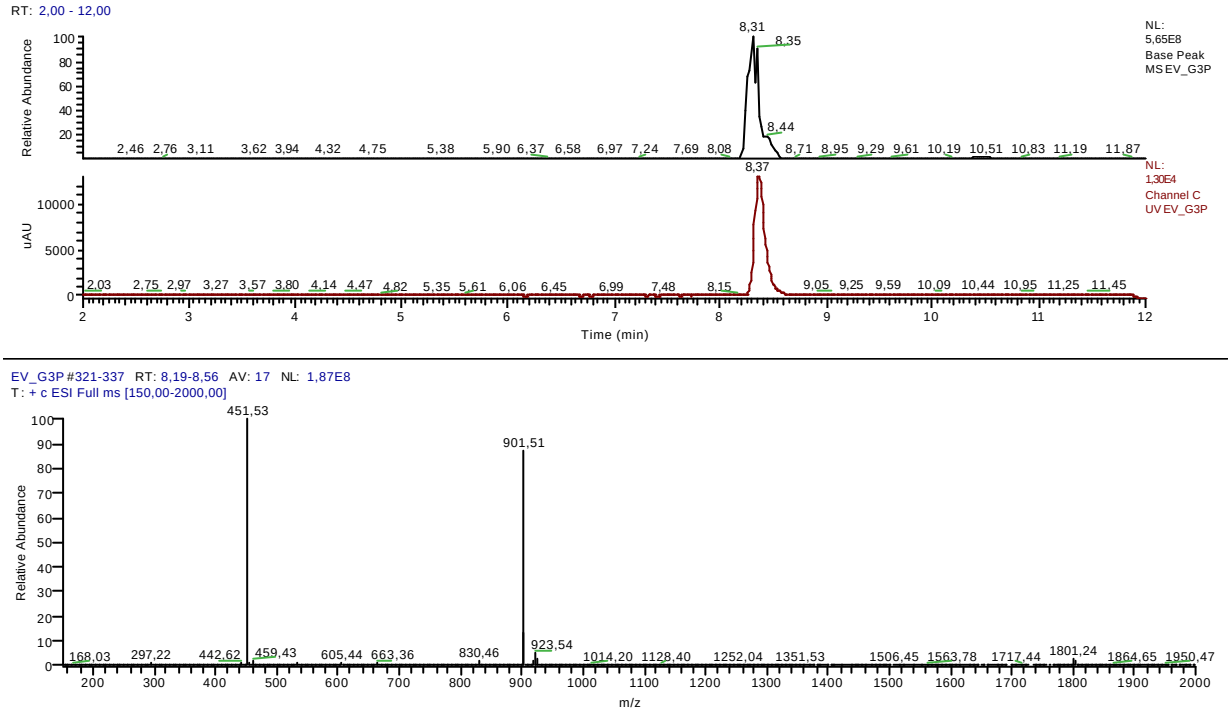


Figure S1. LC-MS spectrum of purified β-peptide **3** containing two ACHC units. Top, MS and UV (280 nm) traces. Bottom, mass spectrum of purified product.

Crude material of ACHC(2,5) **3**:

The β-peptide ACHC(2,5) **3** (*m/z* 901.51 at 11.55 min) was the major component in the obtained crude mixture after cleavage from the resin. Other unidentified minor products with lower mass were detected (shown in the lower mass chromatogram).

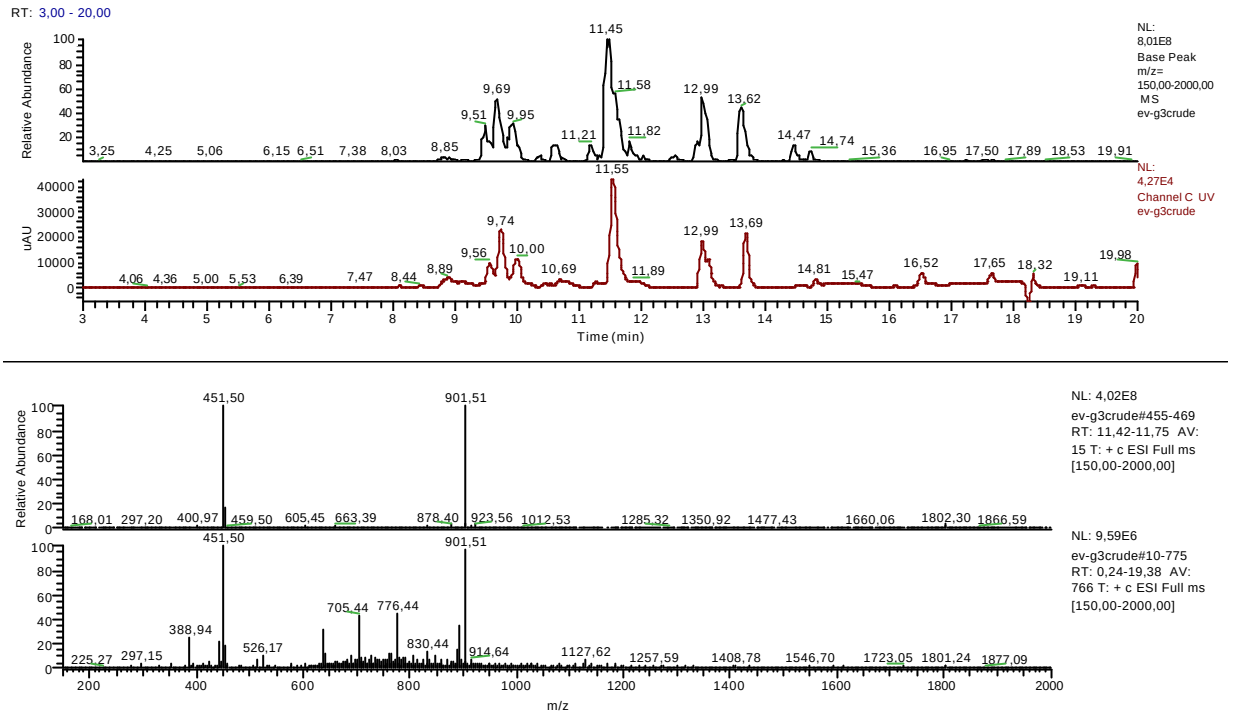


Figure S2. LC-MS spectrum of the crude of β-peptide **3**. Top, MS and UV (280 nm) traces. Bottom, mass spectrum of 11,42-11,75 min. retention time (desired product) and mass spectrum of 0,24.19,38 min. retention time (all signals).

5. NMR characterization

NMR samples contained 1.0-2.0 mM β-peptide in 10 mM sodium phosphate buffer (pH 7.4) with 9:1 H_2O:D_2O. The assignment strategy was based on the identification of individual resonance spin systems from TOCSY experiments following NOE correlations with ROESY experiments (300 ms of mixing time, optimized).

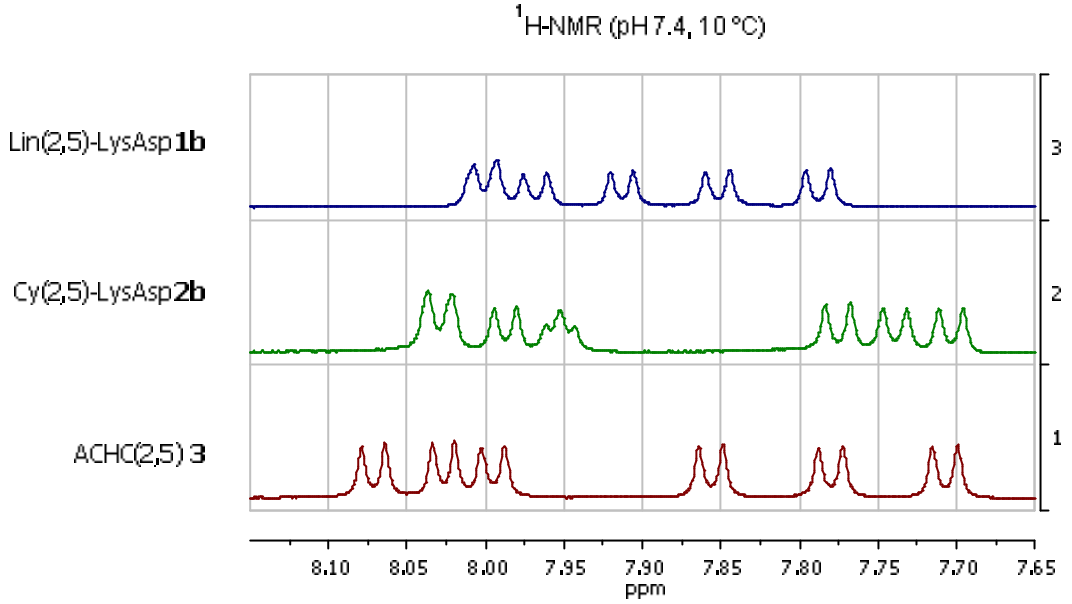
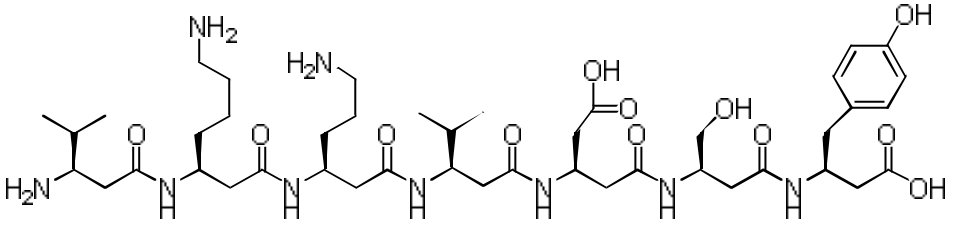


Figure S3. ^{1}H NMR of the amide proton dispersion for **1b**, **2b** and **3**.β-Peptides **1b** and **2b** showed overlapping amide protons corresponding to NH(Lys2) and NH(Asp5) at 8.00 ppm for **1b** and 8.03 ppm for **2b**. The triplet signal in the spectrum of **2b** results from the amide proton in the cyclized side chain to side chain linkage. The dispersion of **3** is most significant, followed by **2b**, and then **1b**.

Lin(2,5)-β^{3}-hLys,β^{3}-hAsp **1b**



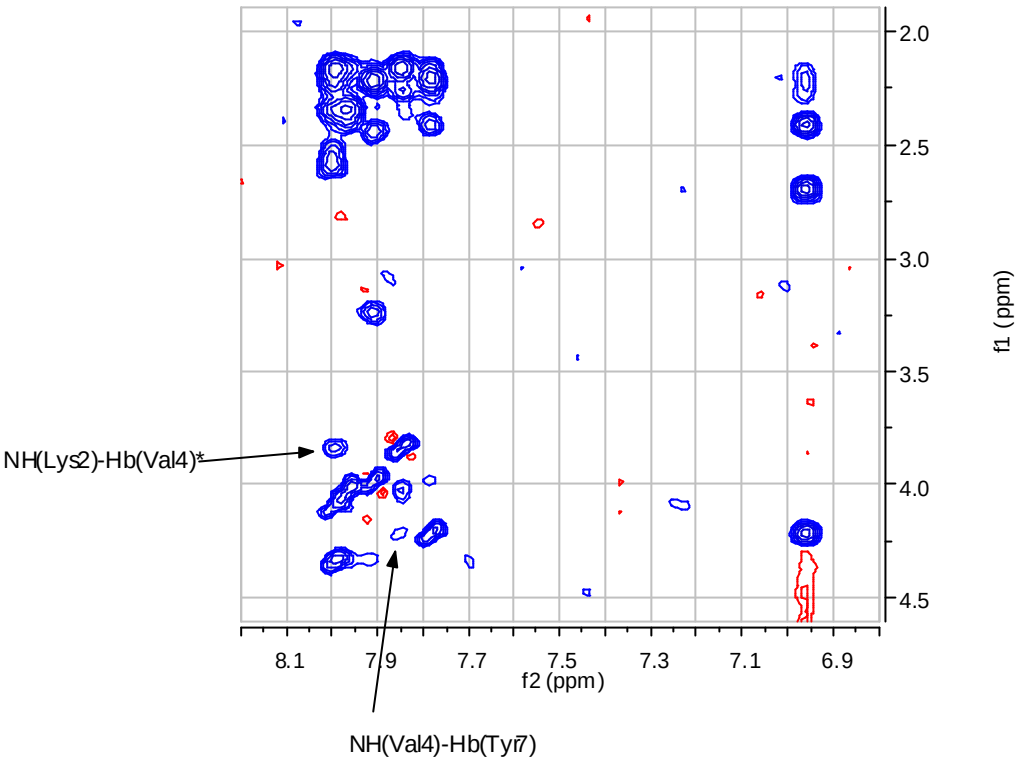


Figure S4. 2D NMR ROESY of **1b** (pH 7.4, 10 ºC, 300 ms mixing time) of the amide proton region. * ambiguously assigned.

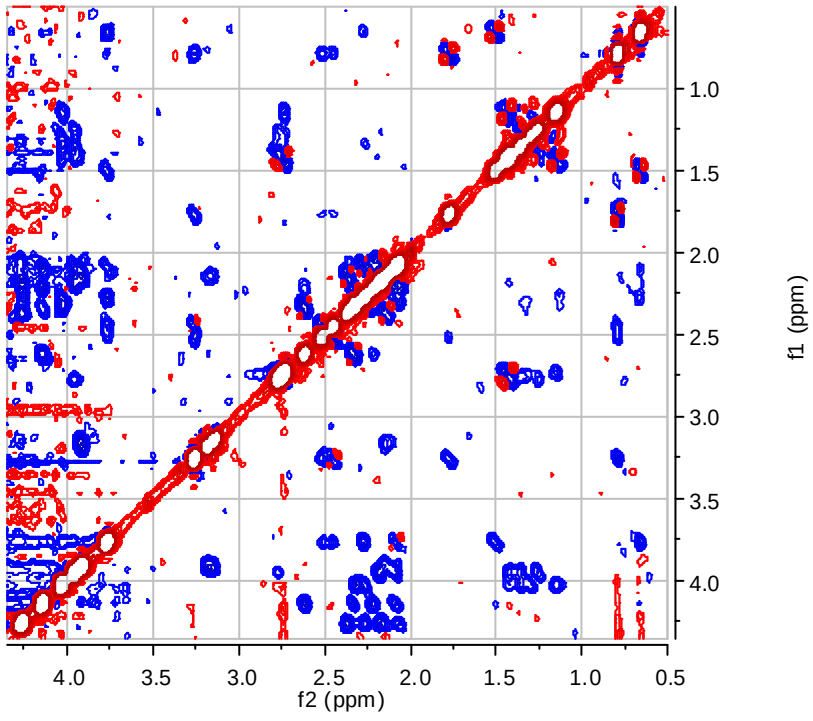


Figure S5. 2D NMR ROESY of **1b** (pH 7.4, 10 ºC, 300 ms mixing time) of the aliphatic proton region. None of the NOE signals corresponded to long-range correlations between residues (*i*, *i*+2) or (*i*, *i*+3).

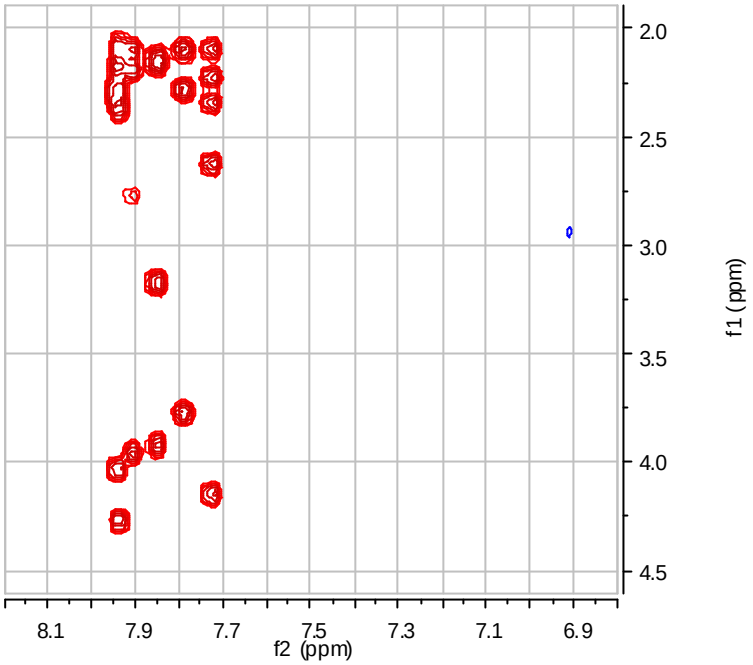


Figure S6. 2D NMR TOCSY of **1b** (pH 7.4, 10 ºC) of the amide proton region.

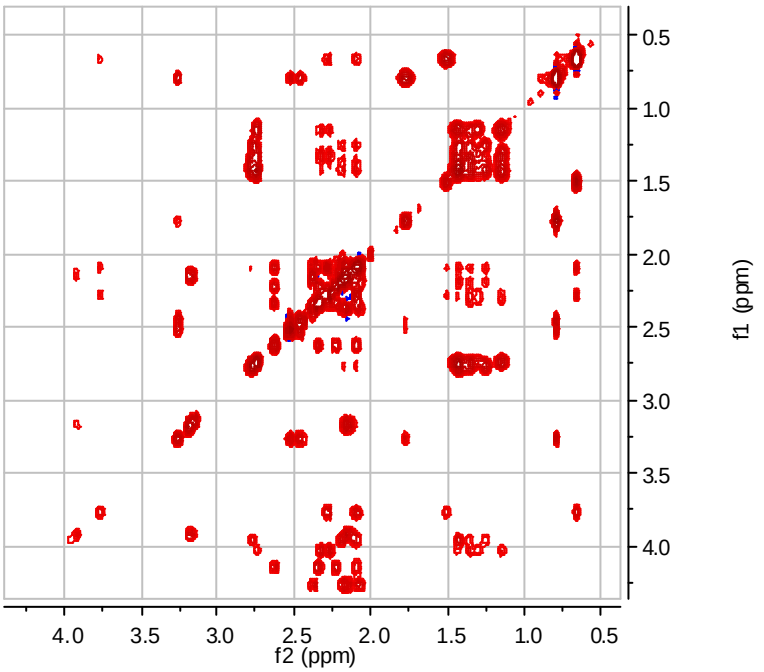


Figure S7. 2D NMR TOCSY of **1b** (pH 7.4, 10 ºC) of the aliphatic proton region.

Cy(2,5)-β^3-hLys,β^3-hAsp **2b**

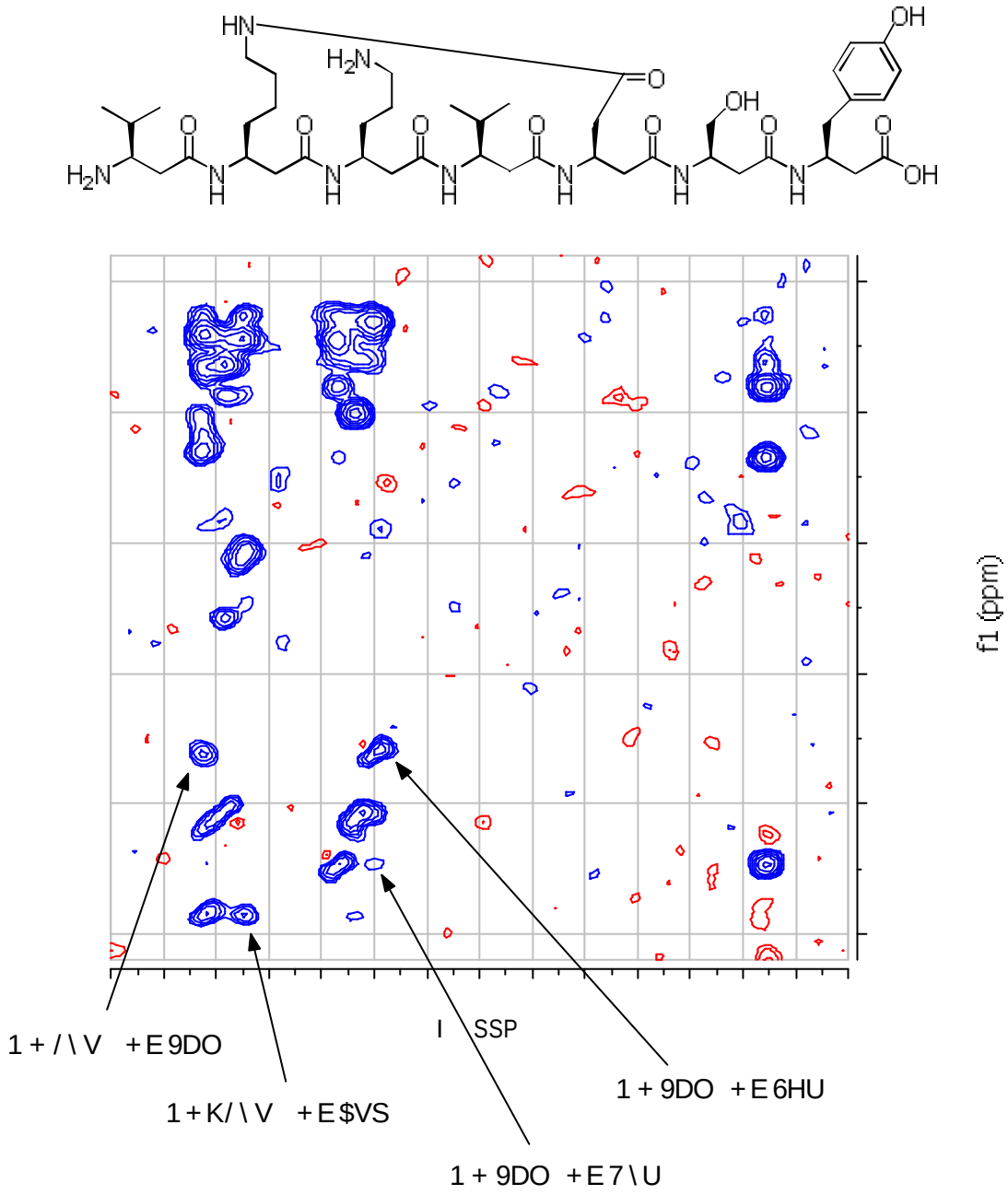


Figure S8. 2D NMR ROESY of **2b** (pH 7.4, 10 ºC, 300 ms mixing time) of the amide proton region.

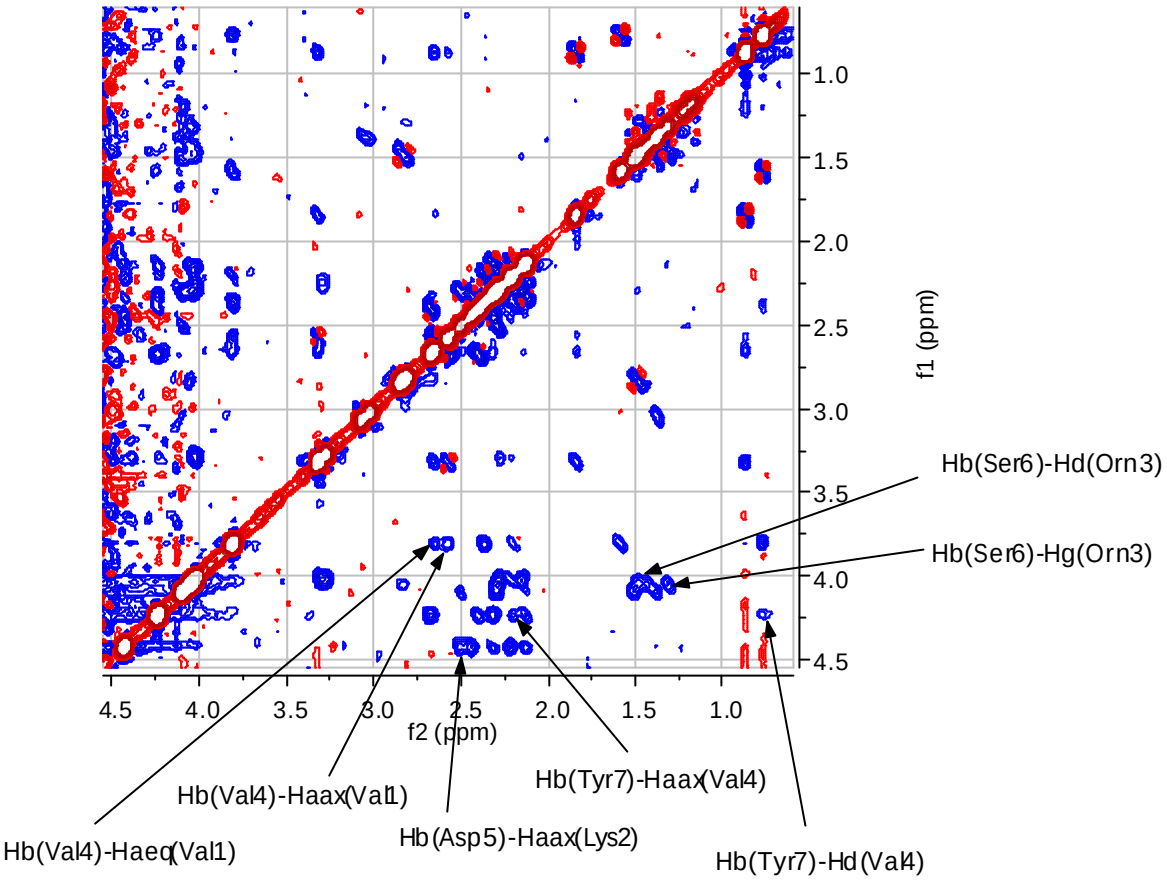


Figure S9. 2D NMR ROESY of **2b** (pH 7.4, 10 ºC, 300 ms mixing time) of the aliphatic proton region, showing the NOE signals corresponding to long-range backbone and side chain to side chain correlations between residues (*i*, *i*+2) or (*i*, *i*+3).

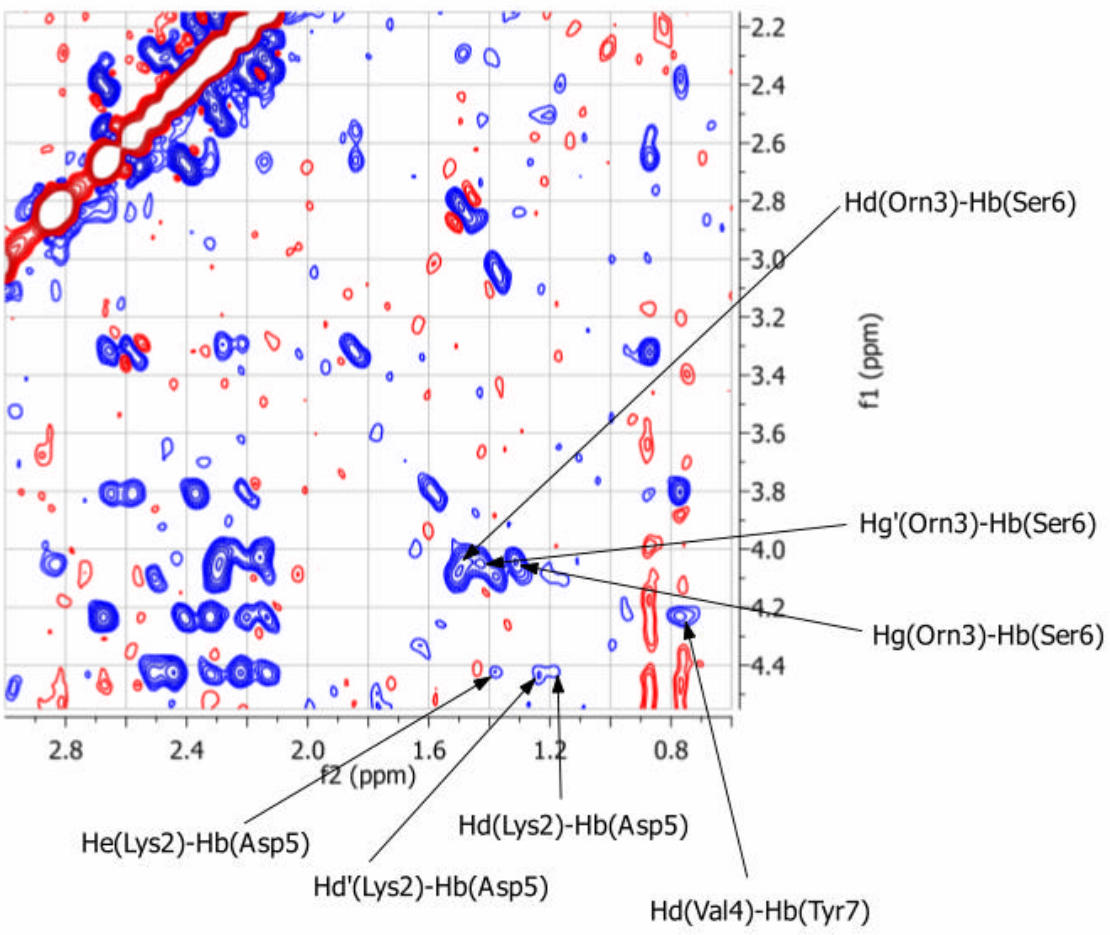


Figure S10. 2D NMR ROESY of **2b** (pH 7.4, 10 ºC, 300 ms mixing time) of the aliphatic proton region, showing the NOE signals corresponding to long-range side-chain correlations between residues (*i*, *i*+2) or (*i*, *i*+3).

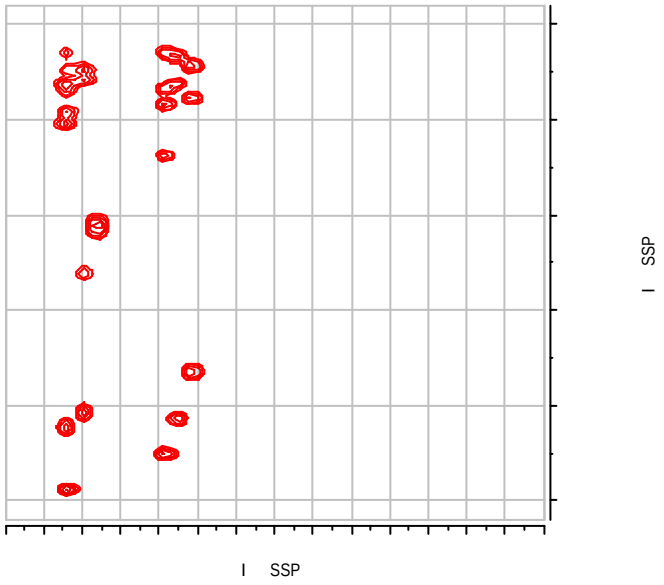


Figure S11. 2D NMR TOCSY of **2b** (pH 7.4, 10 ºC) of the amide proton region.

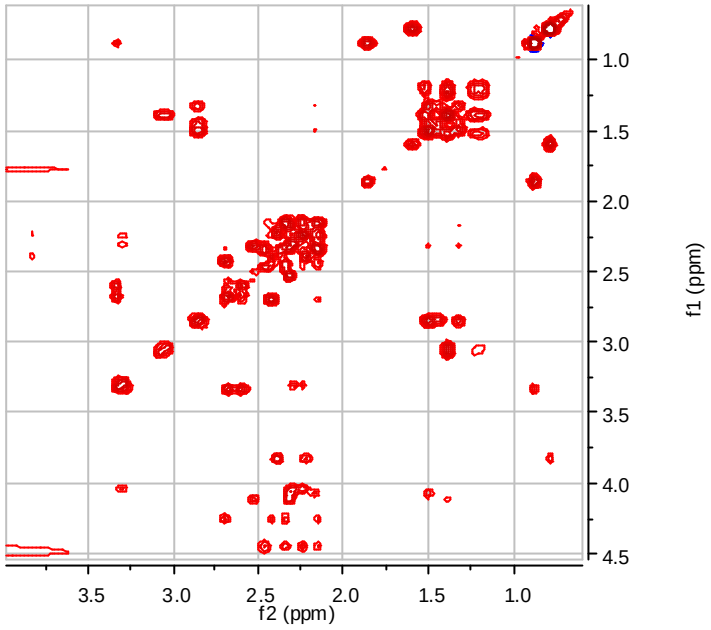


Figure S12. 2D NMR TOCSY of **1b** (pH 7.4, 10 ºC) of the aliphatic proton region.

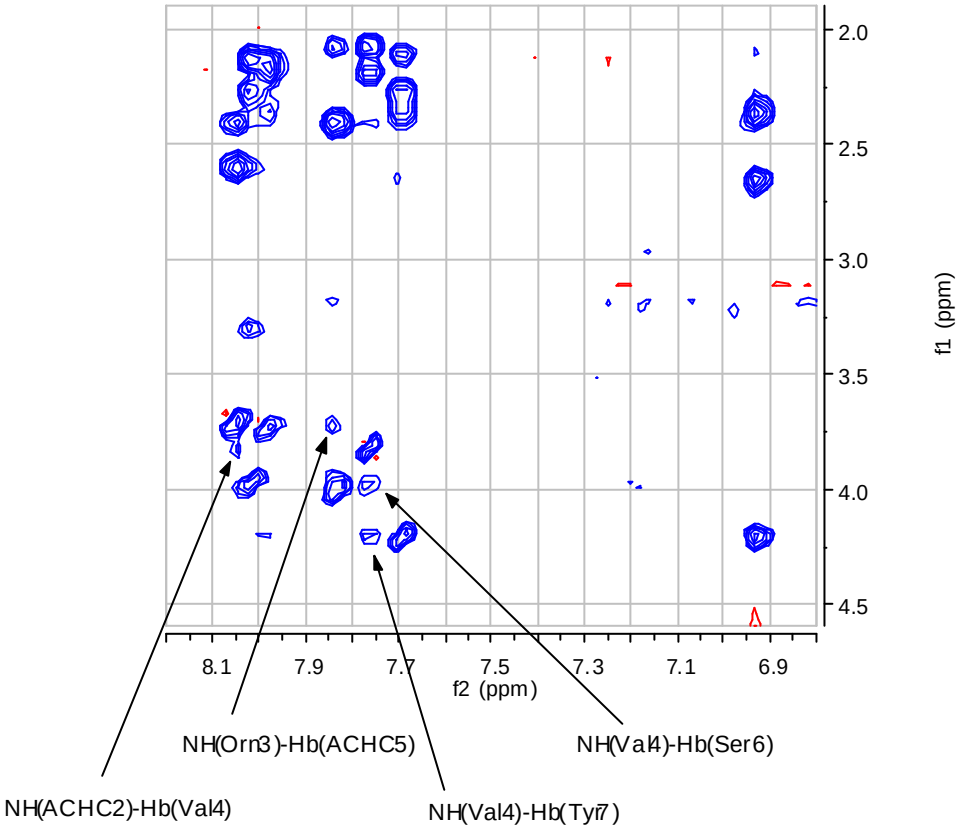


Figure S13. 2D NMR ROESY of **3** (pH 7.4, 10 ºC, 300 ms mixing time) of the amide proton region.

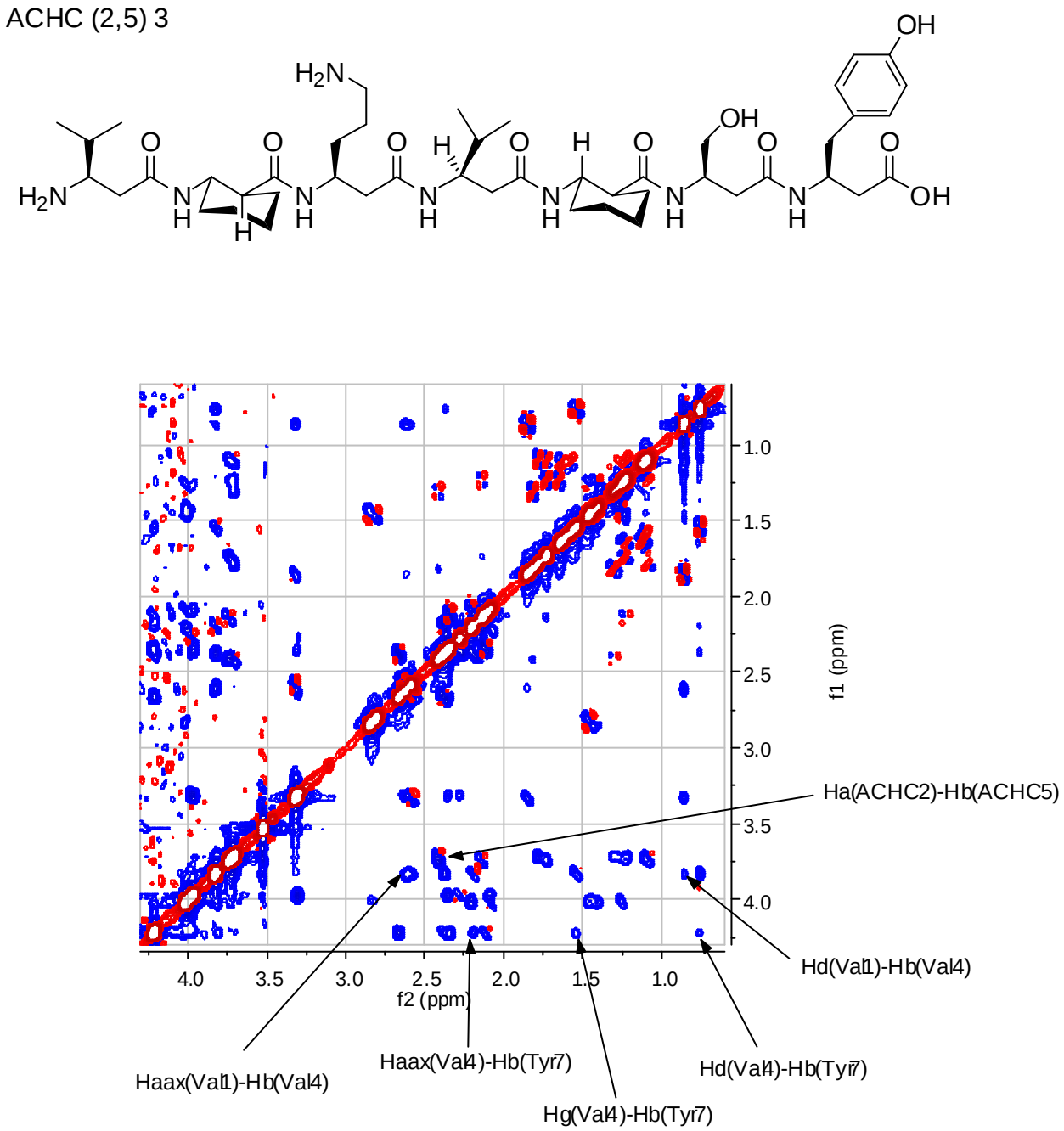


Figure S14. 2D NMR ROESY of **3** (pH 7.4, 10 ºC, 300 ms mixing time) of the aliphatic proton region, showing the NOE signals corresponding to long-range backbone and side-chain to side-chain correlations between residues (*i*, *i*+2) or (*i*, *i*+3).

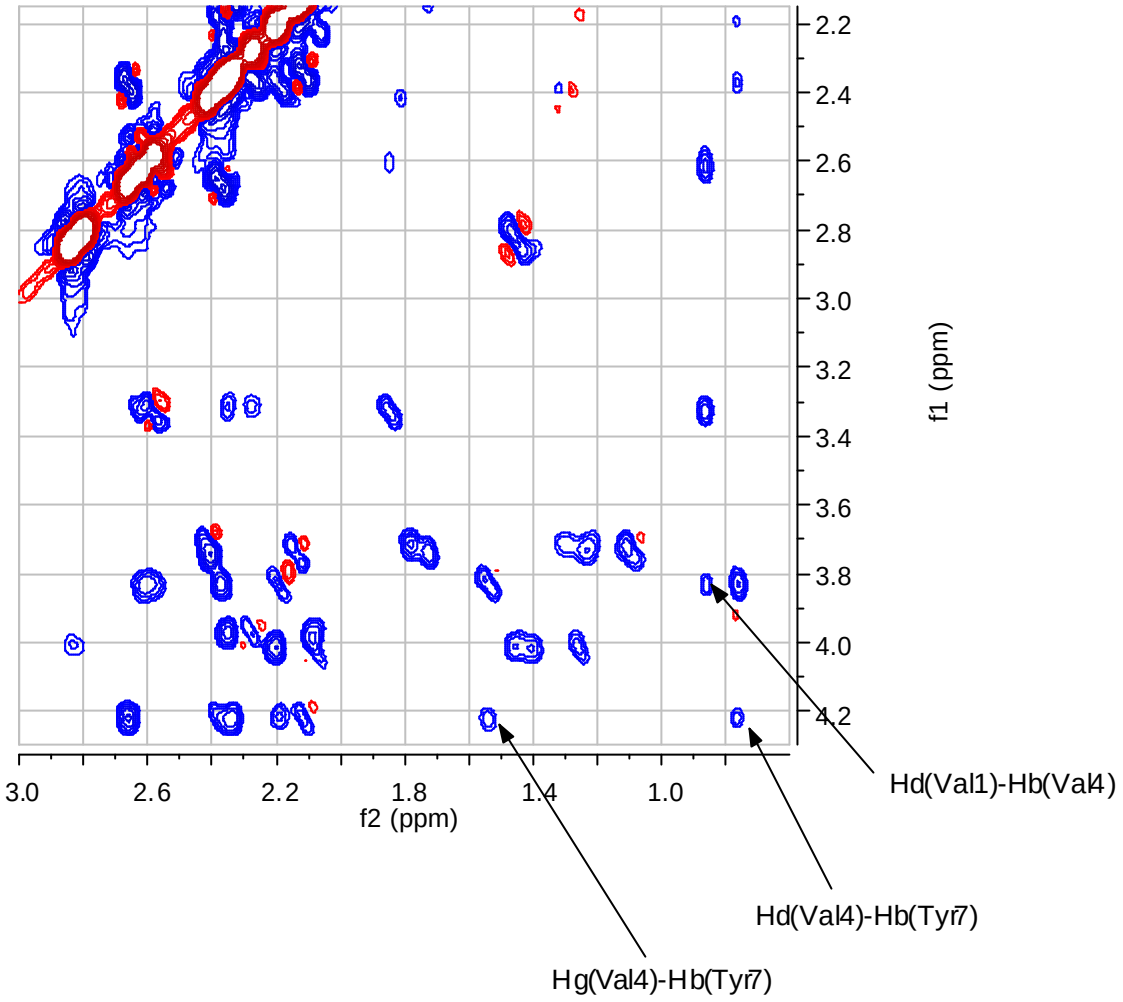


Figure S15. 2D NMR ROESY of **3** (pH 7.4, 10 ºC, 300 ms mixing time) of the aliphatic proton region, showing the NOE signals corresponding to long-range side-chain correlations between residues (*i*, *i*+2) or (*i*, *i*+3).

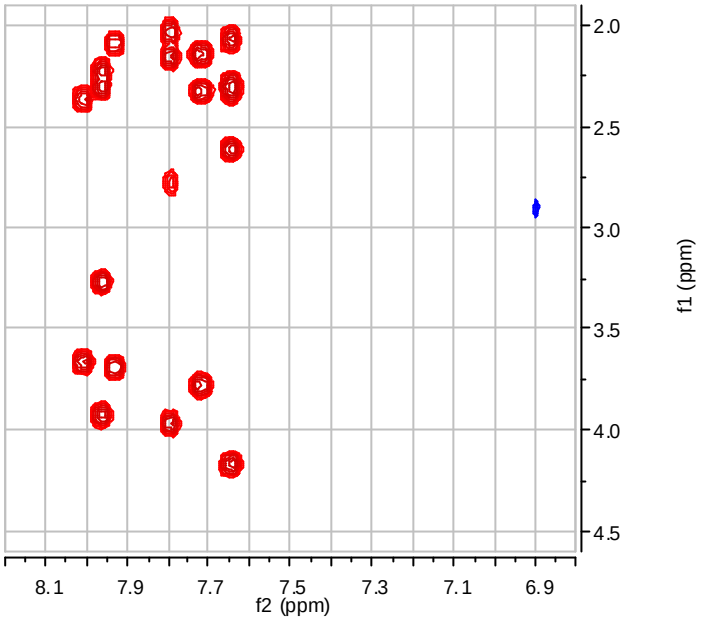


Figure S16. 2D NMR TOCSY of **3** (pH 7.4, 10 ºC) of the amide proton region.

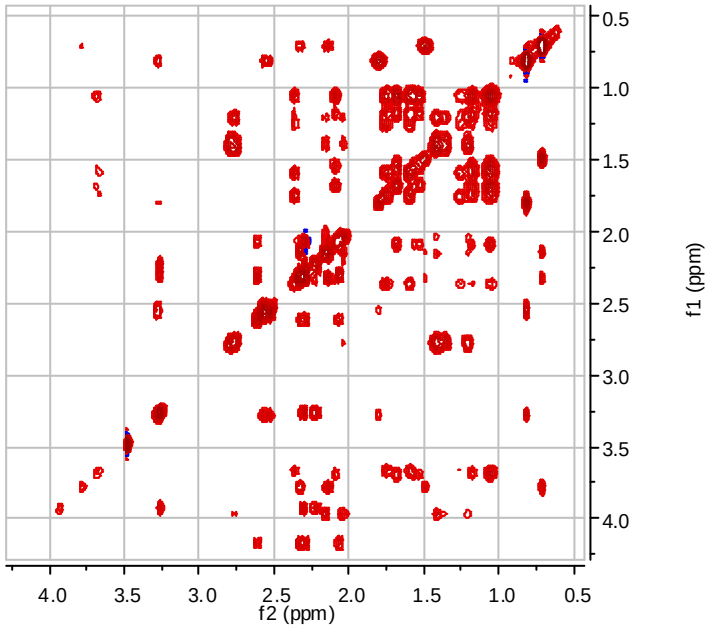


Figure S17. 2D NMR TOCSY of **1b** (pH 7.4, 10 ºC) of the aliphatic proton region.