Peptide Synthesis and Purification – Ac-HNPGYP-NH_2 was synthesized according to published methods using standard solid-phase synthesis techniques with a manual methodology.[1,2] Protected amino acids and other chemicals were purchased from Peptide International, Fluka, Sigma-Aldrich. The resin TentaGel R RAM (Rapp Polymers; capacity 0.18 mmol g^{-1}, 1 g) was treated with piperidin (20%) in dimethylformamide and all amino acids were linked with the use of DIPCI/HOBt methodology. The coupling reaction time was 1 h. Piperidine (20%) in dimethylformamide was used to remove the Fmoc group at all steps. After deprotection of the last N^{α}-Fmoc group, the peptide resin was washed and treated with *N*-acetylimidazol for 24 hours. After washing with methanol the protected peptide resin was dried *in vacuo*. Then resin was treated with trifluoroacetic acid/H_2O/phenol/triisopropylsilan (reagent B, 8.8/0.5/0.5/0.2) 10 ml per 1 g of resin at room temperature for 2 hours. After filtration of the exhausted resin, the solvent was concentrated *in vacuo* and the residue triturated with ether. The crude peptide was purified with HPLC using Vydac C_{18} semi-preparative column (10 × 250mm, 5 μm). The fraction containing the pure peptide was lyophilized twice and the purity assessed by a MALDI-TOF analysis using a Bruker Biflex III Maldi-Tof Mass Spectrometer (Bruker Daltonics).

Analytical data were as follows**:**

Ac-HNPGYP-NH_2 — R_t (HPLC) = 15.97 min, M = 724.7 M^+ + 1 (MALDI-TOF) = 725.1

NMR experiments: The peptide was dissolved in water containing deuterium oxide 10% or deuterium oxide. Solutions were carefully deoxygenated. The pH was adjusted at 7.8 with either DCl or NaOD. The desired concentrations of metal ions were obtained by adding aliquots of stock water solutions of $Cu(NO_3)_2$, and the pH was again checked and eventually re-adjusted.

NMR spectroscopy. NMR experiments were carried out at 14.1 T and at controlled temperature (± 0.1 K) on a Bruker Avance 600 spectrometer equipped with a Silicon Graphics workstation. Suppression of residual water signal was achieved by excitation sculpting, using a selective square

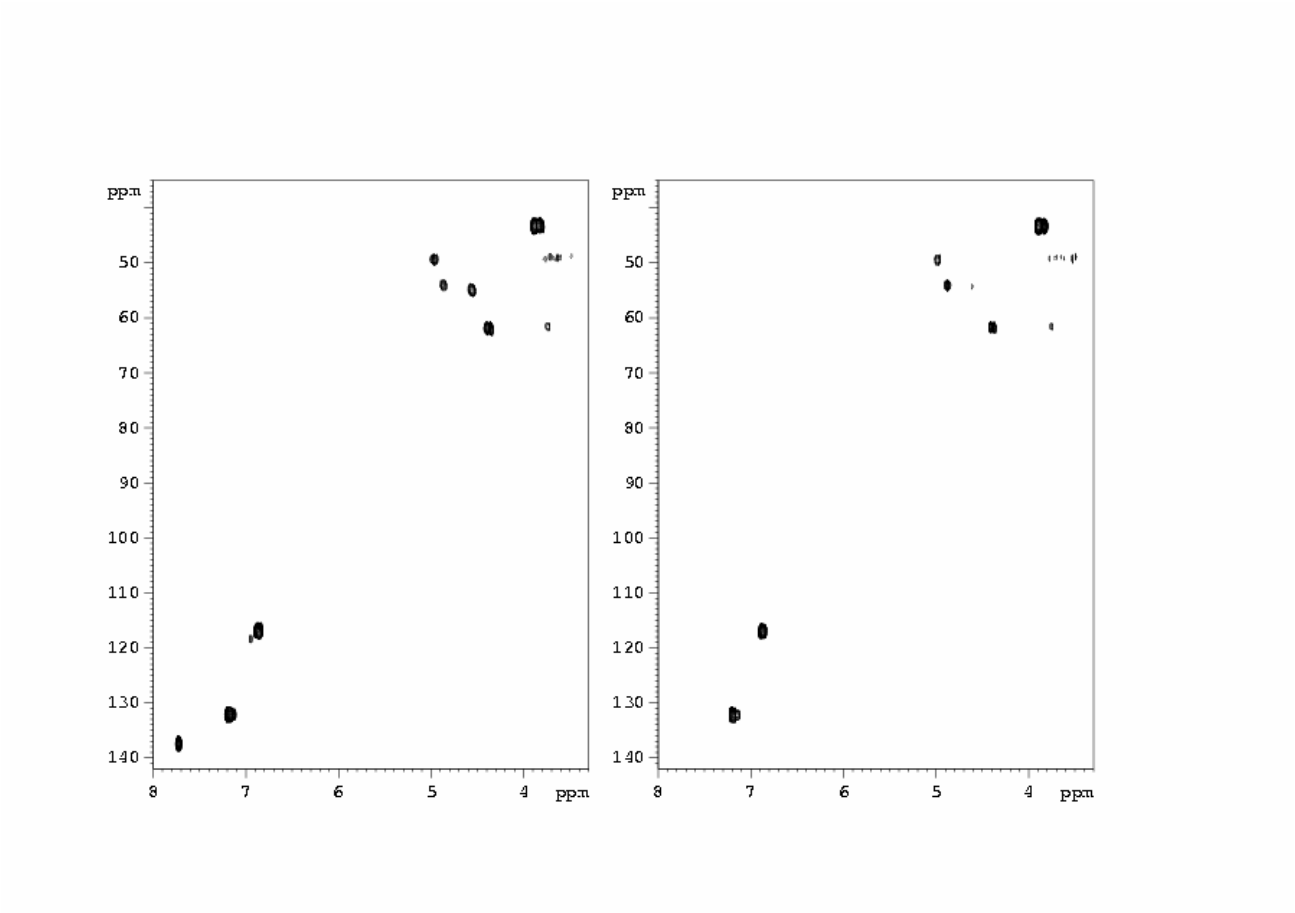
pulse on water 2 ms long. Proton resonance assignment for was obtained by standard COSY, TOCSY, NOESY and ROESY experiments. Proton spin-lattice relaxation rates ($R_1 = 1/T_1$) were measured with the standard inversion recovery pulse sequence; relaxation rates were calculated with regression analysis of the initial recovery curves of longitudinal magnetization components, leading to errors in the range ± 3%. Single- selective excitations where achieved by means of suitably shaped π-pulses generated by the SHAPE TOOL module of the Bruker program XWINNMR.

The IR-TOCSY sequence was used to calculate the relaxation rates of the overlapping ^{1}H NMR signal. This was obtained by introducing a ^{1}H 180° pulse followed by a variable delay in front of the TOCSY sequence. The obtained results were compared with those obtained from normal IR sequence. The agreement was found in the errors limit of both experiments.

References:

1. Atherton, E., and Sheppard, R. C., (1989) *Solid Phase Peptide Synthesis*, Rickwood, D., Hames, B. D., eds., IRL Press, Oxford
2. Fields, G. B., and Noble, R. L. (1990) *Int. J. Peptide Protein Res*., **35**, 161-214

^{1}H-^{13}C HSQC spectra of ChPrP(54-59) 5 mM, pD = 7.8, T = 298 before (left), after the addition of 0.01 equivalent of Cu(II) (right).



Supplementary Material (ESI) for Chemical Communications

Aromatic region of ^{1}H NMR spectrum of ChPrP(53-58) (upper trace) and ChPrP(54-59) (lower trace); 5mM, T 298, pD 7.8