Supplementary Figure 1. Sedimentation equilibrium analysis of the interaction of CCK_8PO_4 with ferric ions.

Samples of 100 µL containing 25 µM CCK_8PO_4 and 25 µM EDTA (A) or 25 µM $FeCl_3$ (B) in 10 mM Na PIPES, pH 6.5, 100 mM NaCl and 0.005% Tween 20 were centrifuged in a ProteomeLabXL1 analytical ultracentrifuge at a final rotor speed of 60,000 rpm at 20 °C as described in Supplementary Experimental. The experimental data (○) were fitted directly to equation 1 given by Bailey and coworkers [1], assuming a single ideal solute at sedimentation equilibrium. The molecular masses determined for CCK_8PO_4 in the presence of EDTA (1326 Da) or Fe^{3+} ions (1257 Da) were similar to each other and to the theoretical value of 1142.7.

Supplementary Table 1. ^{1}H Chemical shifts of CCK_8SO_4 at pH 6.5, 298 K [a]

One dimensional, TOCSY, DQF-COSY and NOESY experiments at 500 and 600 MHz allowed complete assignment of the spectrum of 3.5 mM CCK_8SO_4 in $H_2O/^2H_2O$ 9:1, pH 6.5 and 298 K. The results are in agreement with the assignments of Fournié-Zaluski et al. [2] from 1D spectra of 5 mM CCK_8SO_4 in $H_2O/^2H_2O$, pH 6, recorded at 270 and 400 MHz. However, we assign the lower field methionine NH resonance to Met3 rather than Met6. The TOCSY, DQF-COSY spectra and higher fields used in our study also allow assignment of overlapping resonances in the aromatic region, and the NOESY allows assignment of the lower field S-CH_3 resonance to Met3.

	NH	CHα	CHβ	other
Asp 1	*b*	4.11	2.74, 2.63	
Tyr 2	*b,c*	4.60	3.08, 3.01	H2,6 and H3,5 7.24
Met 3	8.40	4.35	1.92, 1.84	CHγ 2.47, 2.39 S-CH_3 2.04
Gly 4	7.93	3.86		
Trp 5	8.08	4.50	3.20, 2.95	H2 7.22, H4 7.56 H5 7.12, H6 7.20 H7 7.47, HN 10.12
Met 6	7.82	4.14	1.69, 1.63	CHγ 2.09, 2.06 S-CH_3 1.95
Asp 7	7.91	4.40	2.58, 2.41	
Phe 8	7.92	4.65	3.27, 3.23	H2,6 7.25, H3,5 7.32 H4 7.27

a Chemical shifts relative to DSS with H_2O at 4.77 ppm.
b Not observed at this pH.
c 8.72 ppm at pH 4.0

Supplementary Table 2. ^{1}H Chemical shifts of CCK_8PO_4 at pH 6.5, 298 K

	NH	CHα	CHβ	other
Asp 1	*b*	4.12	2.77, 2.67	
Tyr 2	*b,c*	4.66	3.14, 2.94	H2,6 7.14 H3,5 7.19
Met 3	8.46	4.15	1.97, 1.93	CHγ 2.55, 2.47 S-CH_3 2.09
Gly 4	8.17	3.95 3.91		
Trp 5	8.11	4.55	3.23, 2.99	H2 7.27, H4 7.61 H5 7.25, H6 7.16 H7 7.51, HN 10.17
Met 6	7.82	4.14	1.69, 1.63	CHγ 2.09, 2.06 S-CH_3 1.95
Asp 7	7.94	4.40	2.62, 2.46	
Phe 8	8.01	4.61	3.31, 3.27	H2,6, 7.27 H3,5 7.36 H4 7.31

a Chemical shifts relative to DSS with H_2O at 4.77 ppm.
b Not observed at this pH.
c 8.69 ppm at pH 4.0

EXPERIMENTAL

Analytical ultracentrifugation.

Analytical ultracentrifugation experiments were conducted in a ProteomeLabXL1 analytical ultracentrifuge (Beckman-Coulter) using an An-Ti60 rotor and double-sector 12 mm path length cells containing quartz windows and charcoal-filled Epon centerpieces. Samples of 100 µL containing 25 µM CCK_8PO_4 and 25 µM EDTA or $FeCl_3$ in 10 mM Na acetate (pH 4.0) or 10 mM PIPES (pH 6.5) containing 100 mM NaCl and 0.005% Tween 20 were centrifuged at a final rotor speed of 60,000 rpm at 20 °C. At 1 h intervals, the 270 nm absorbance of each sample was measured at 0.001 cm increments along the centrifuge cell. The time for the attainment of equilibrium was generally 24 h, the criterion being that consecutive scans were superimposable. At equilibrium, the cells were scanned 10 times as described above, the data averaged, and Mr values calculated as described previously [1]. Because it was impossible to spin the sample fast enough to deplete the peptide from the meniscus for baseline measurement, the baseline was fitted as an additional parameter.

REFERENCES.

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2. Fournie-Zaluski, M.C., Belleney, J., Lux, B., Durieux, C., Gerard, D., Gacel, G., Maigret, B., and Roques, B.P. (1986). Conformational analysis of cholecystokinin CCK26-33 and related fragments by 1H NMR spectroscopy, fluorescence-transfer measurements, and calculations. Biochemistry **25**, 3778-3787.

Suppl.
Figure 1