Supporting Information

Design, Synthesis, and Biological Evaluation of New Cyclic Melanotropin Peptide Analogues Highly Selective for the Human Melanocortin-4 Receptor

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NMR experiments:

NMR samples were prepared in D_2O with a peptide concentration of about 4.0 mM. All spectra were acquired on a Bruker DRX-600 spectrometer at 25 °C using a Nalorac triple-resonance single-axis gradient 5 mm probe, processed using the Bruker software XWINNMR. Chemical shifts were referenced to the residual water peak at 4.76 ppm.

Due to the presence of rotameric NMR resonance lines for peptides **1**, **3**, **5**, **6**, and **8**, ^{1}H chemical shifts and $^3J_{\text{HN-H}\alpha}$ are measurable for peptides **2**, **4**, and **7** only from the 1D ^{1}H NMR spectra. The ratio of rotamers for peptides **1**, **3**, **5**, **6**, and **8** was determined from the intensities of the most downfield peaks and is given below.

1: 44% (1:0.81)

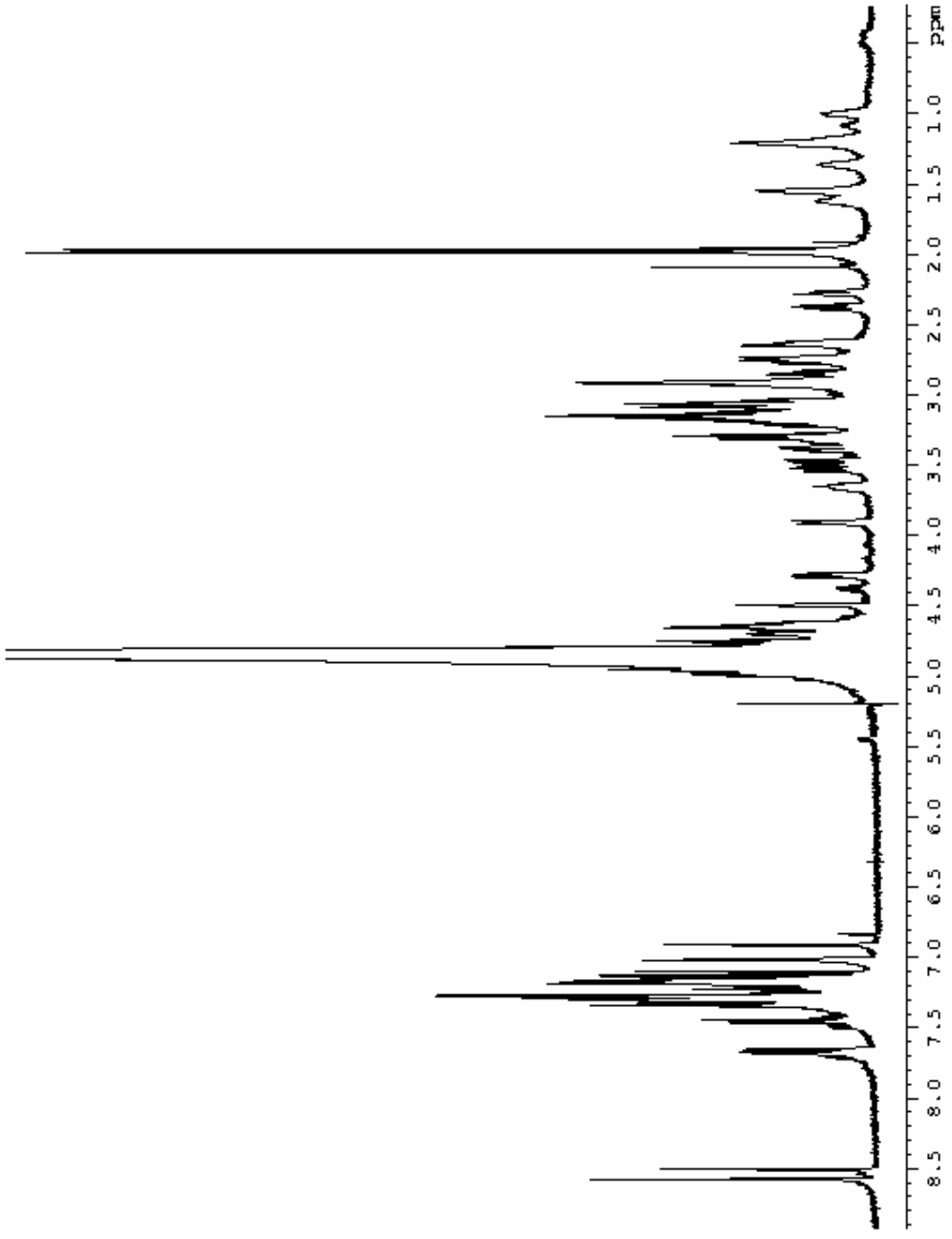
3: 44% (1:0.78)

5: 28%, 35%, 27% (1:0.91:0.69)

6: 22% (1:0.29)

8: 26% (1:0.36)

Ac-c[Cys-His-*D*-Phe-N^{α}-guanidinylbutyl-Cys]-Trp-NH_2 **1**



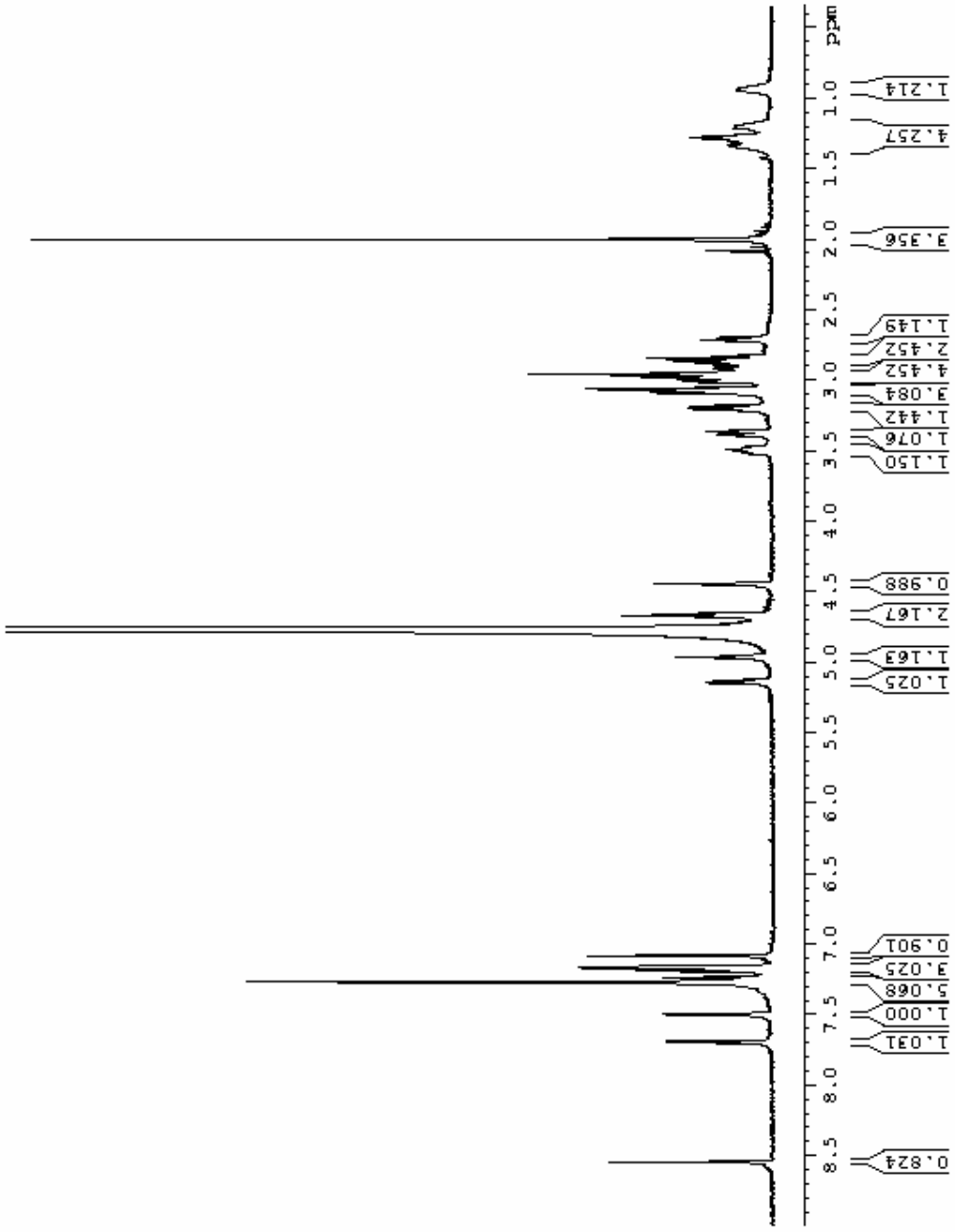
Ac-c[Cys-His-*D*-Phe-N^{α}-guanidinylbutyl-*D*-Cys]-Trp-NH_2 **2**
(Totally 38 observable hydrogens in D_2O):

0.88-0.98 (1H, m)
1.14-1.41 (4H, m)
2.00 (3H, s)
2.70 (1H, dd, *J* = 3.2, 13.3 Hz)
2.82-2.90 (2H, m)
2.93-3.02 (4H, m)
3.04-3.12 (3H, m)
3.19 (1H, dd, *J* = 8.7, 14.7 Hz)
3.37 (1H, dd, *J* = 5.5, 14.2 Hz)
3.45-3.55 (1H, m)

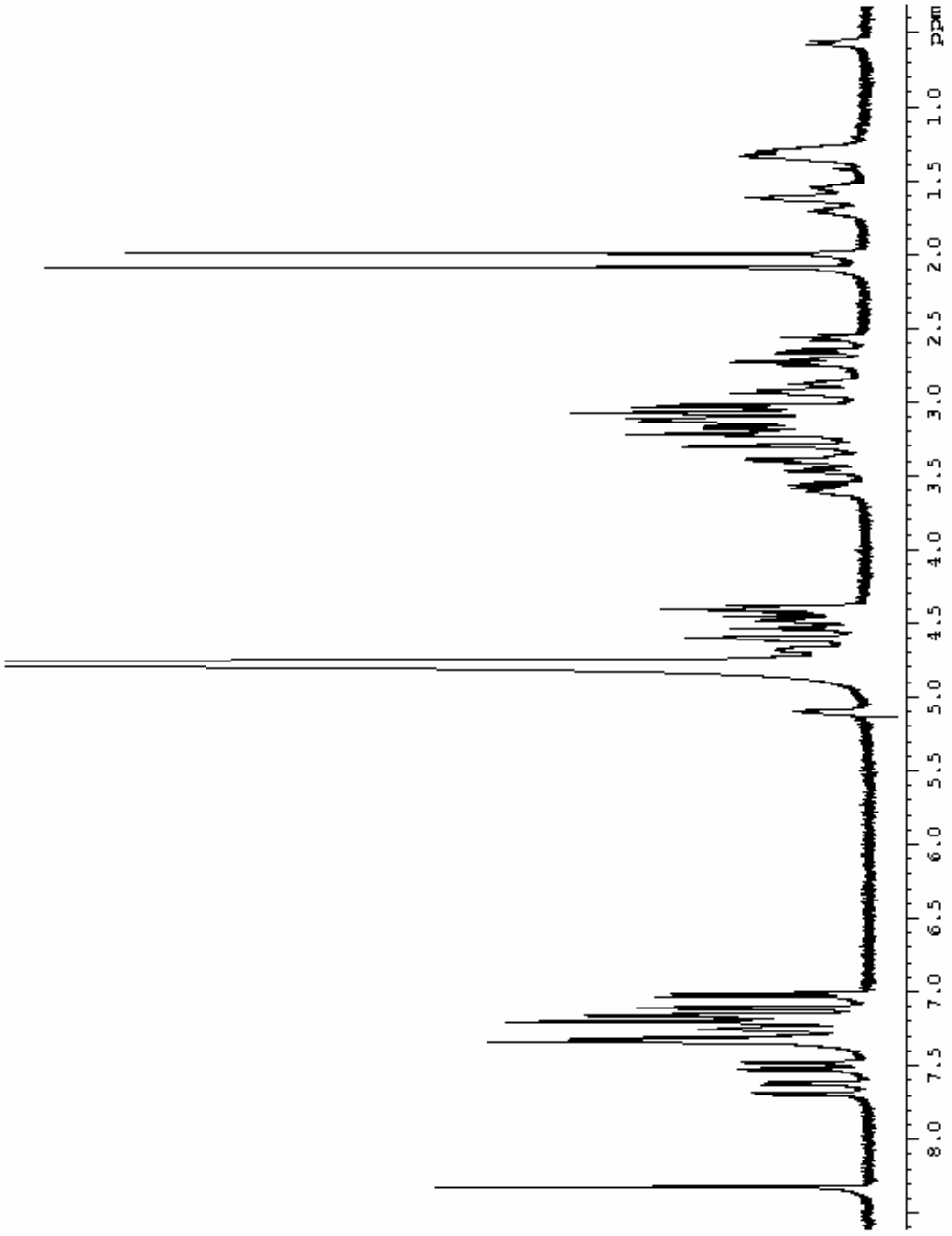
4.45 (1H, t, *J* = 4.1 Hz)
4.63-4.71 (2H, m)
4.96 (1H, t, *J* = 7.3 Hz)
5.14 (1H, dd, *J* = 3.7, 11.5 Hz)

7.08 (1H, s)
7.13-7.21 (3H, m)
7.22-7.32 (5H, m)
7.50 (1H, d, *J* = 8.3 Hz)
7.69 (1H, d, *J* = 7.8 Hz)
8.54 (1H, s)

Ac-c[Cys-His-*D*-Phe-N^{α}-guanidinylbutyl-*D*-Cys]-Trp-NH_2 **2**



Ac-c[Cys-His-*D*-Phe-Cys]-N^{α}-guanidinylbutyl-Trp-NH_2 **3**



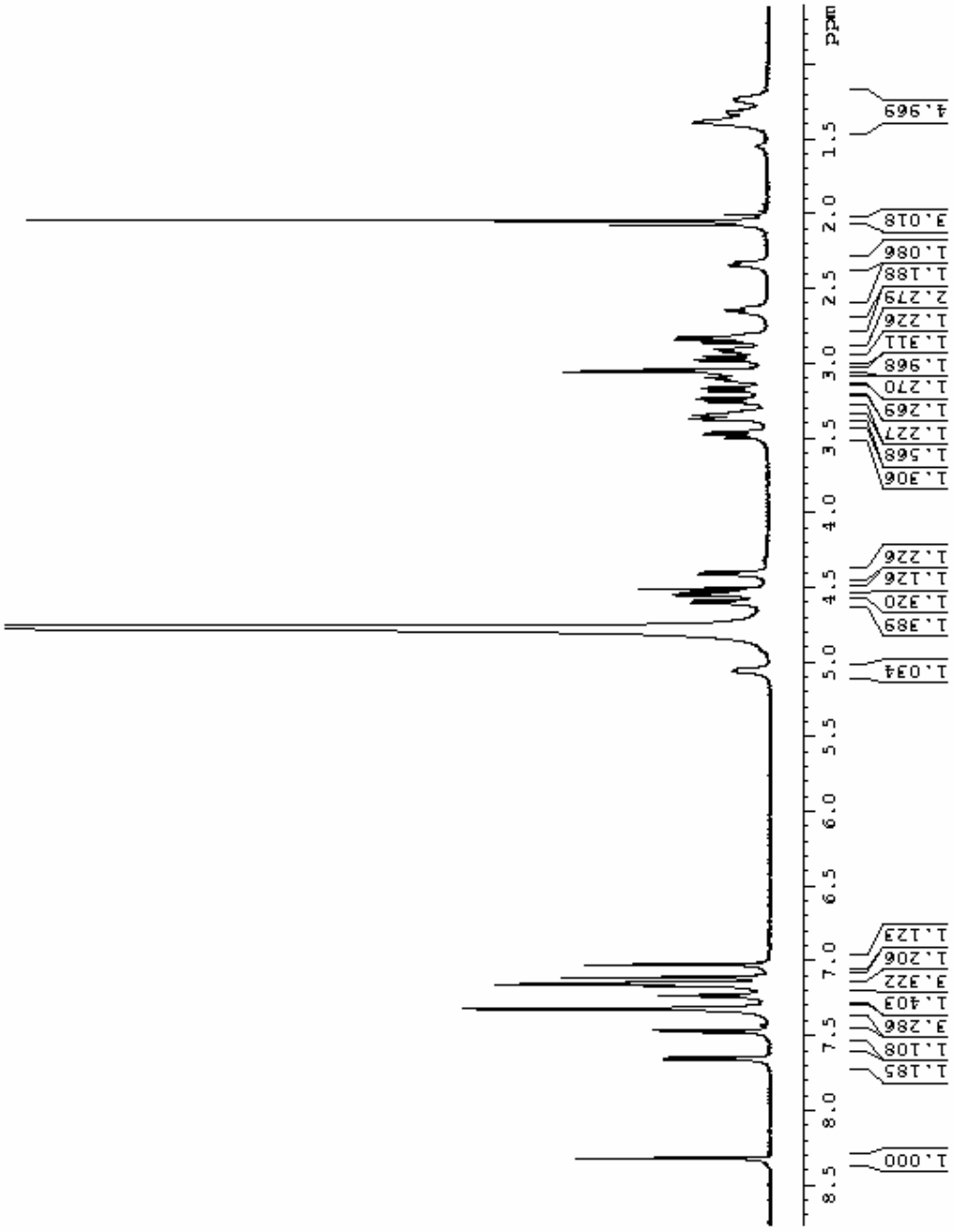
Ac-c[Cys-His-*D*-Phe-Cys]-N^{α}-guanidinylbutyl-*D*-Trp-NH_2 **4**
(Totally 38 observable hydrogens)

1.16-1.47 (5H, m)
2.04 (3H, s)
2.33 (1H, d, *J* = 12.4 Hz)
2.57-2.71 (1H, m)
2.78-2.87 (2H, m)
2.88-2.94 (1H, m)
2.97 (1H, dd, *J* = 1.8, 14.7 Hz)
3.05 (2H, d, *J* = 7.8 Hz)
3.07-3.14 (1H, m)
3.17 (1H, dd, *J* = 4.6, 14.2 Hz)
3.25 (1H, dd, *J* = 7.8, 14.7 Hz)
3.36 (1H, dd, *J* = 5.0, 14.7 Hz)
3.48 (1H, dd, *J* = 11.9, 15.1 Hz)

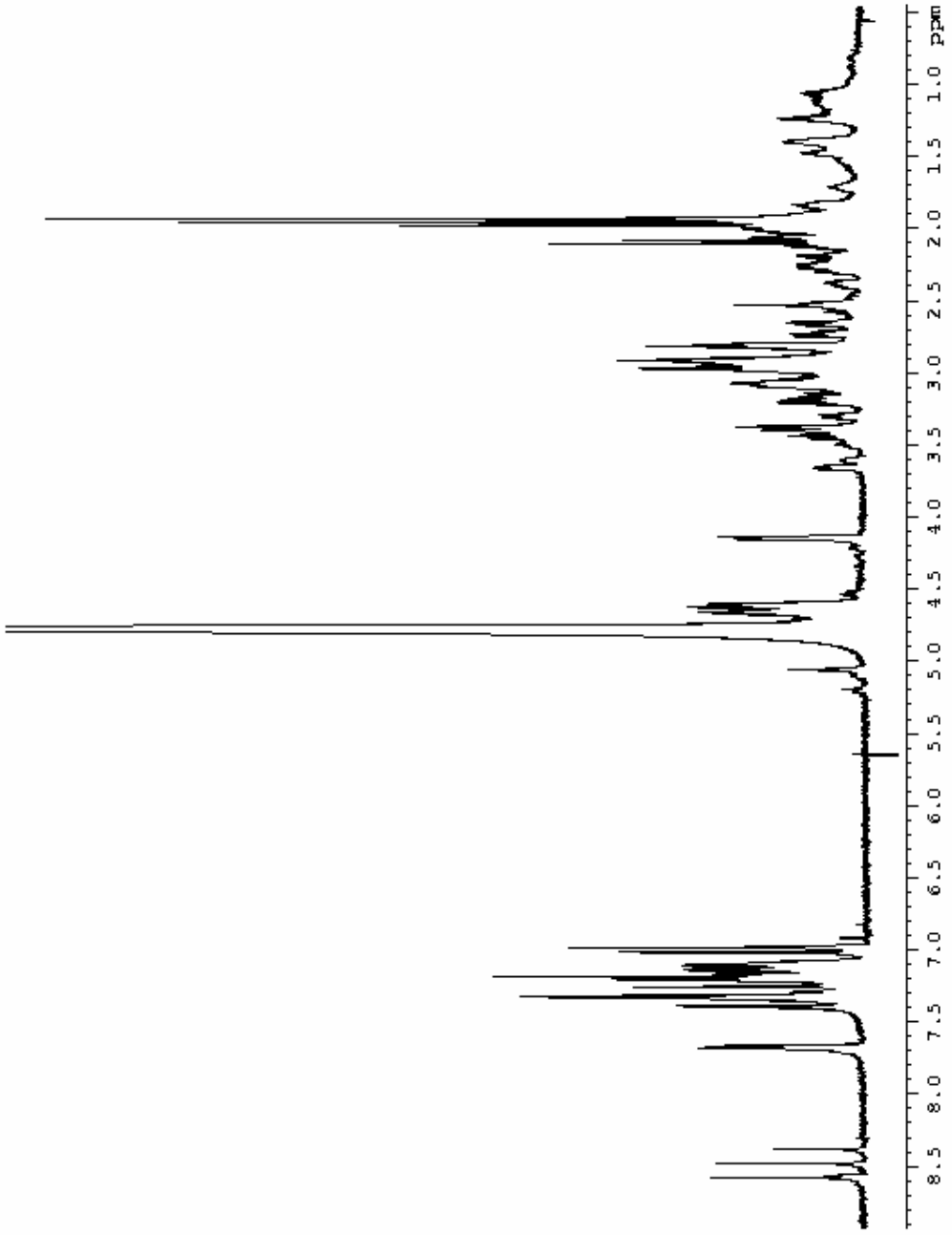
4.40 (1H, dd, *J* = 5.0, 11.0 Hz)
4.51 (1H, t, *J* = 8.25 Hz)
4.55 (1H, dd, *J* = 2.8, 7.3Hz)
4.60 (1H, dd, *J* = 1.8, 10.5 Hz)
5.06 (1H, bs)

7.02 (1H, s)
7.11 (1H, s)
7.13-7.20 (3H, m)
7.23 (1H, t, *J* = 7.3 Hz)
7.28-7.38 (3H, m)
7.47 (1H, d, *J* = 8.3 Hz)
7.65 (1H, d, *J* = 7.8 Hz)
8.32 (1H, s)

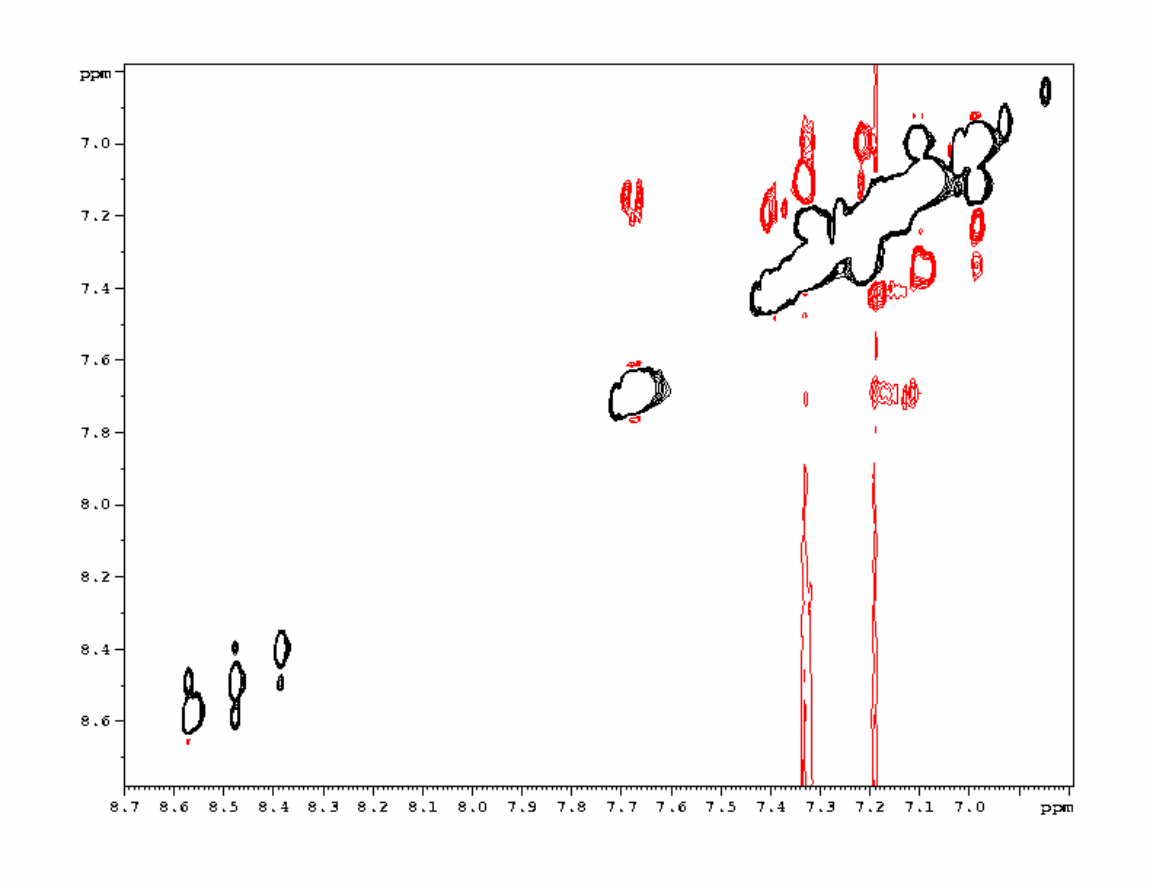
Ac-c[Cys-His-*D*-Phe-Cys]-N^{α}-guanidinylbutyl-*D*-Trp-NH_2 **4**



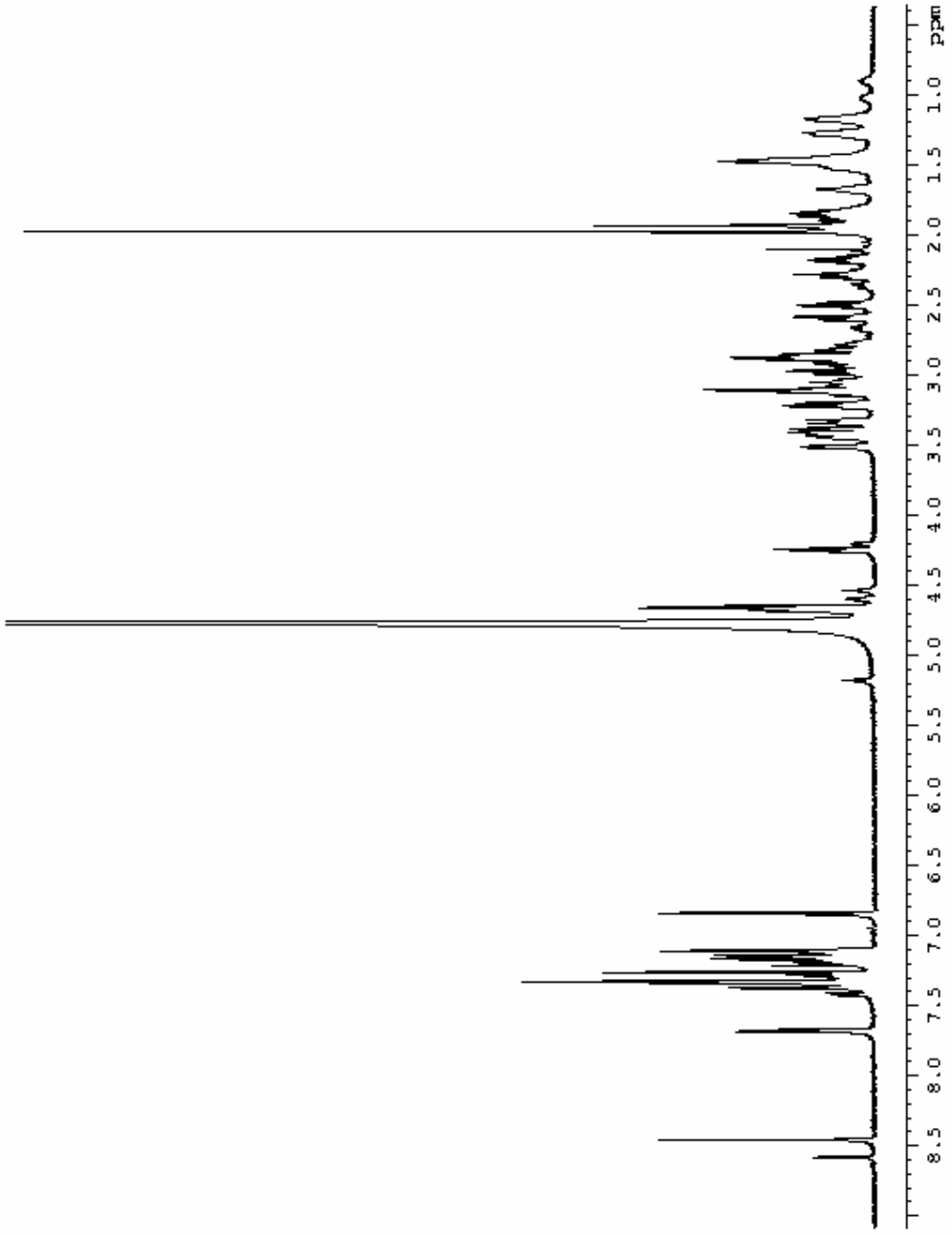
Ac-c[Glu-His-*D*-Phe-N^{α}-guanidinylbutyl-Dab]-Trp-NH_2 **5**



Expansion of the ROESY spectrum for
Ac-c[Glu-His-*D*-Phe-N^{α}-guanidinylbutyl-Dab]-Trp-NH_2 **5**



Ac-c[Glu-His-*D*-Phe-N^{α}-guanidinylbutyl-Orn]-Trp-NH_2 **6**



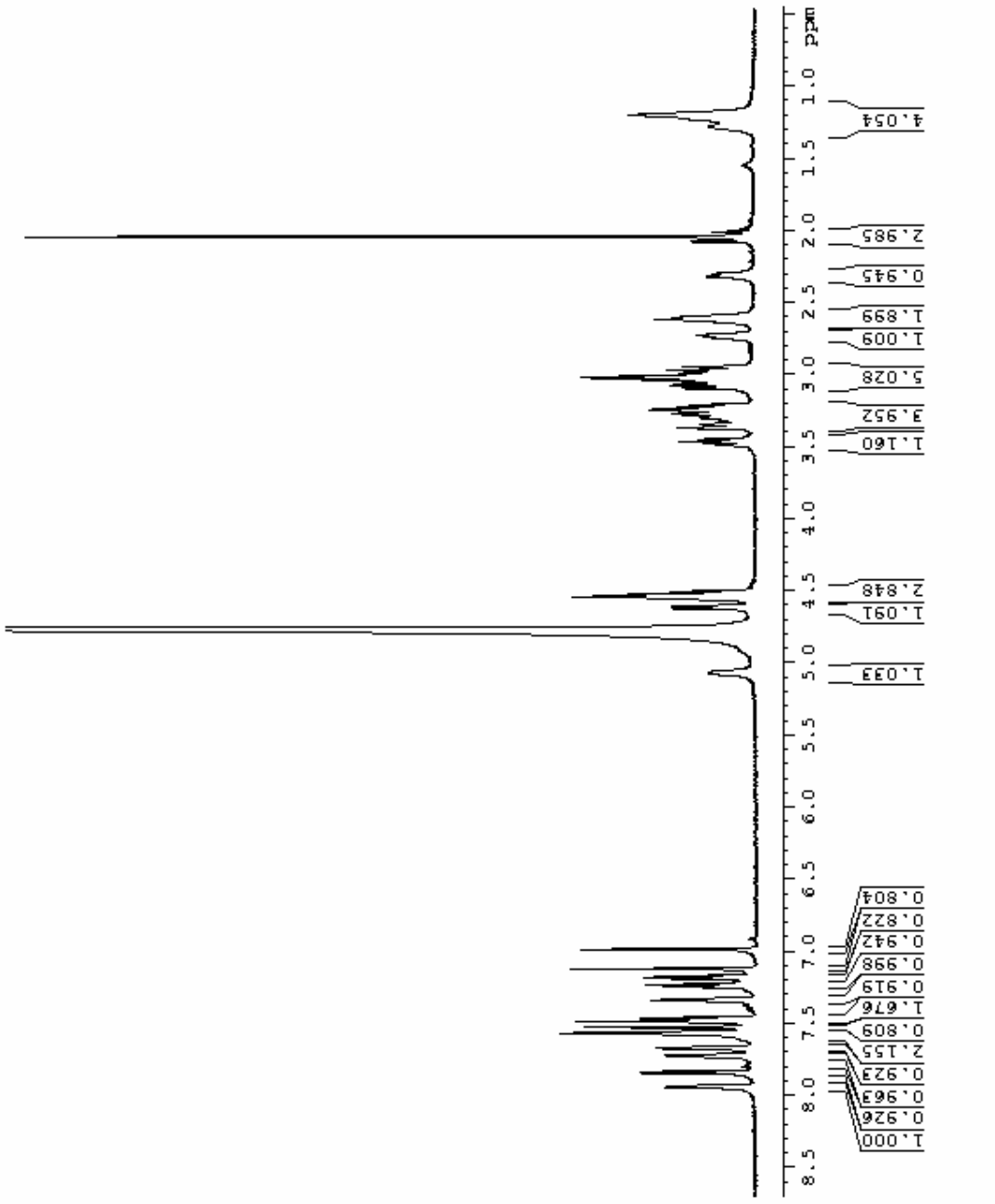
Ac-c[Cys-His-*D*-Nal(2′)-Cys]-N^{α}-guanidinylbutyl-*D*-Trp-NH_2 **7**
(Totally 40 observable hydrogens)

1.09-1.35 (4H, m)
2.04 (3H, s)
2.31 (1H, d, *J* = 12.8 Hz)
2.55-2.67 (2H, m)
2.69-2.80 (1H, m)
2.90-3.14 (5H, m)
3.18-3.40 (4H, m)
3.47 (1H, t, *J* = 11.9 Hz)

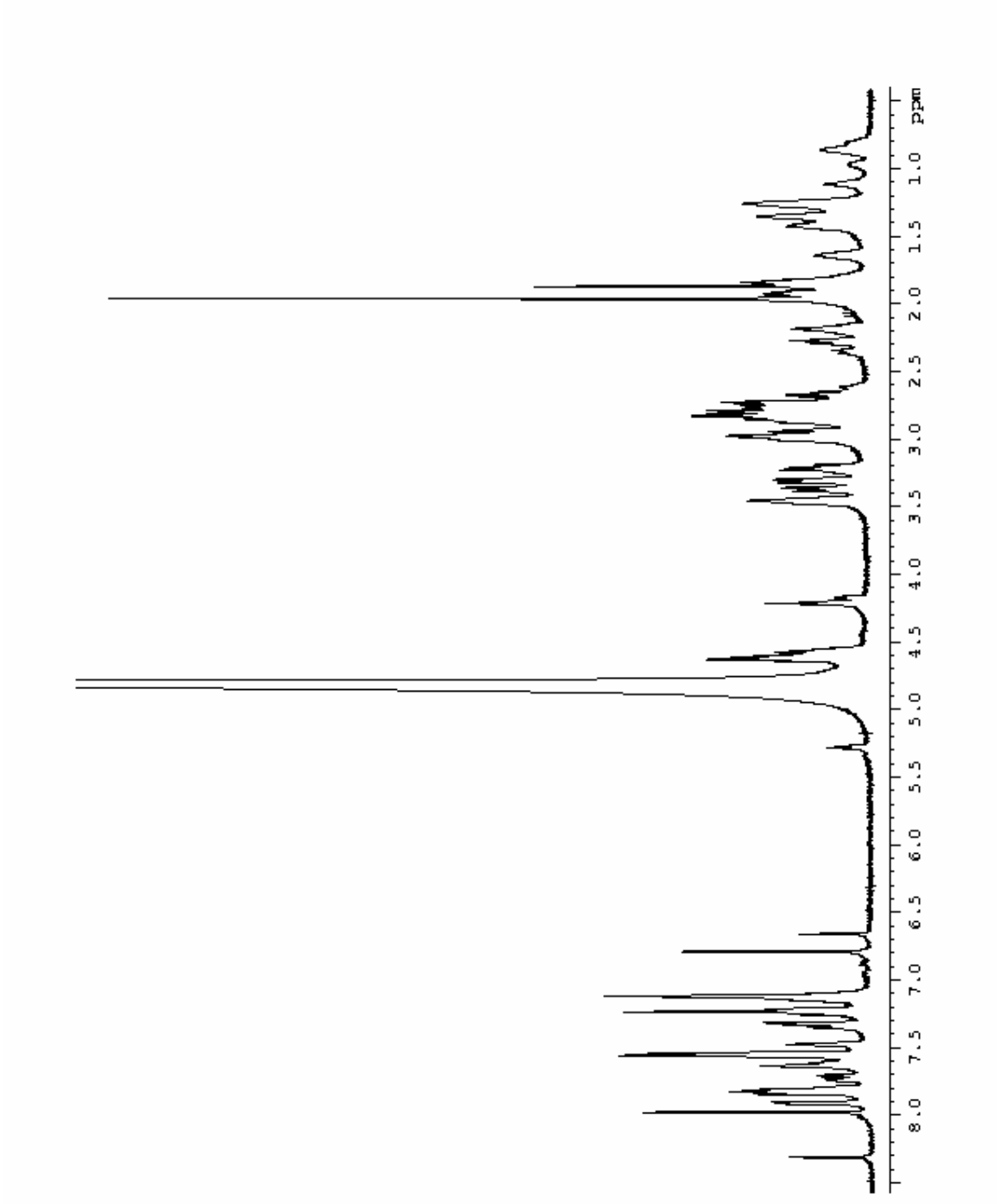
4.48-4.58 (3H, m)
4.61 (1H, dd, *J* = 2.3, 10.1 Hz)
5.07 (1H, bs)

6.99 (1H, s)
7.12 (1H, s)
7.18 (1H, t, *J* = 7.3 Hz)
7.24 (1H, t, *J* = 8.3 Hz)
7.33 (1H, d, *J* = 8.3 Hz)
7.44-7.51 (2H, m)
7.53 (1H, s)
7.57 (2H, dd, *J* = 3.2, 6.4 Hz)
7.64-7.69 (1H, m)
7.70-7.75 (1H, m)
7.84 (1H, d, *J* = 8.7 Hz)
7.91-7.98 (1H, m)

Ac-c[Cys-His-*D*-Nal(2′)-Cys]-N^{α}-guanidinylbutyl-*D*-Trp-NH_2 **7**



Ac-c[Glu-His-*D*-Nal(2′)-N^{α}-guanidinylbutyl-Orn]-Trp-NH_2 **8**



Dose-response curves of MTII in the presence of the antagonists developed in this study.