Use of Disulfide "Staples" to Stabilize β-Sheet Quaternary Structure

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Supporting information

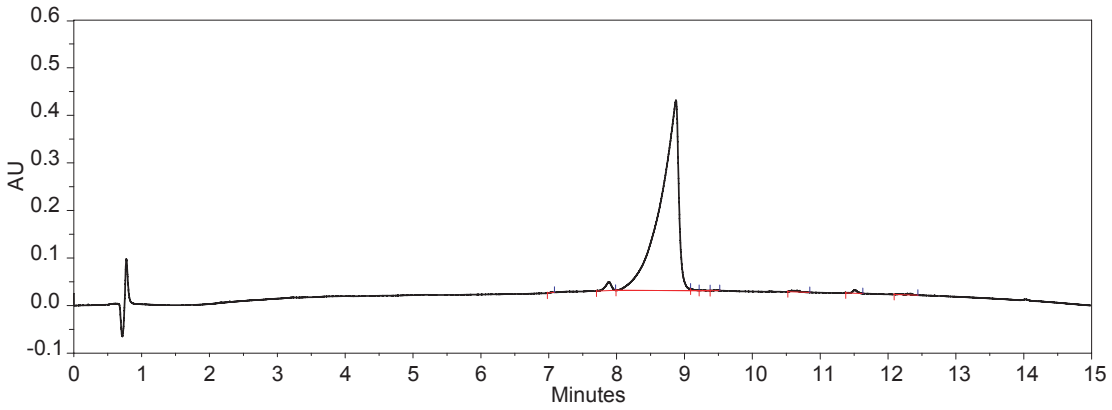
General procedures

Analytical reverse phase HPLC (RP-HPLC) was performed on a Beckman (Agilent Zorbax 80 SB C_{18} column, 50 x 4.6 mm; solvent A: H_2O/0.1% TFA, solvent B: CH_3CN/0.1% TFA), and preparative RP-HPLC was carried out on a Rainin machine (Agilent Zorbax 80 SB C_{18} column, 250 x 21.2 mm; solvent A: H_2O/0.1% TFA, solvent B: CH_3CN/0.1% TFA). ^1H NMR Spectra were acquired on a Varian INOVA800 (800 MHz) spectrometer. The 800 MHz 1D NMR spectra were processed by using MestreNova software, and the 800 MHz 2D NMR spectra were processed by using NmrPipe software. The NMR data were reported in ppm using either TMS (δ = 0.00 ppm) or HOD (δ = 4.80 ppm at 298 K and 4.60 ppm at 318 K) as internal standard.

Synthesis of cyclic peptides 1b and 1c

The syntheses of cyclic peptides **1b** and **1c** were carried out in a similar fashion to those of other 54-membered-ring cyclic peptides introduced in *J. Am. Chem. Soc.* **2007**, *129*, 5558–5569. As an exception, dithiothreitol was added to the deprotection cocktail to trap the carbocations released during the side-chain deprotection. Peptides **1b** and **1c** were respectively prepared in 13% and 4% overall yield with sufficient purity (>95%, based on HPLC and LRMS) to be used in the next step (oxidation-induced dimerization). NMR studies of these cyclic peptides were not performed, because they oxidize rapidly in aqueous solutions.

Analytical RP-HPLC of peptide **1b**

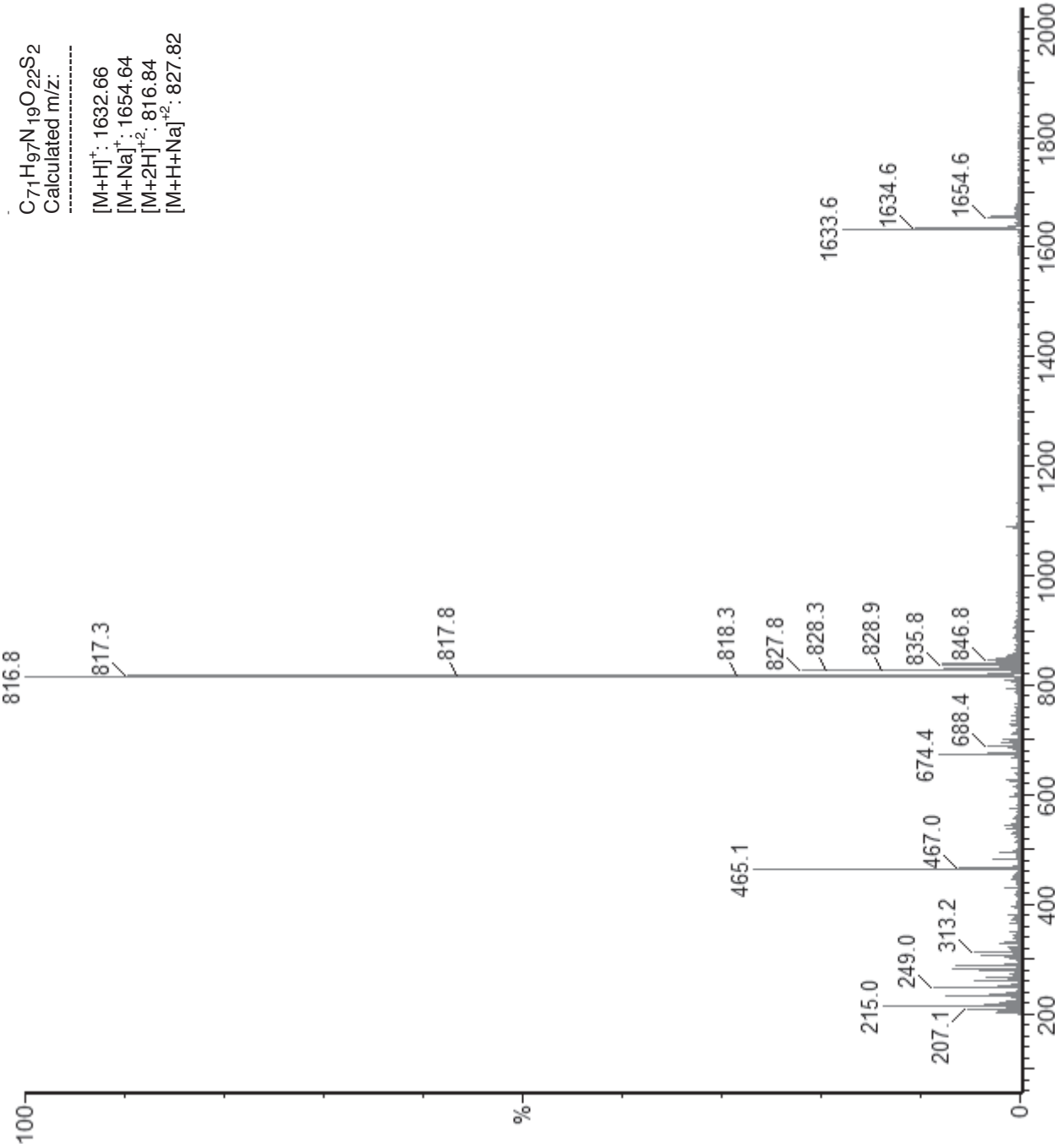


Time	Area	Area %	Height	Height %
7.055	1989	0.03	590	0.14
7.885	109497	1.45	18362	4.23
8.875	7330595	97.39	399747	92.06
9.090	11338	0.15	3320	0.76
9.263	3780	0.05	865	0.20
9.475	3001	0.04	732	0.17
10.608	20112	0.27	2432	0.56
11.512	32377	0.43	6793	1.56
12.183	14505	0.19	1376	0.32

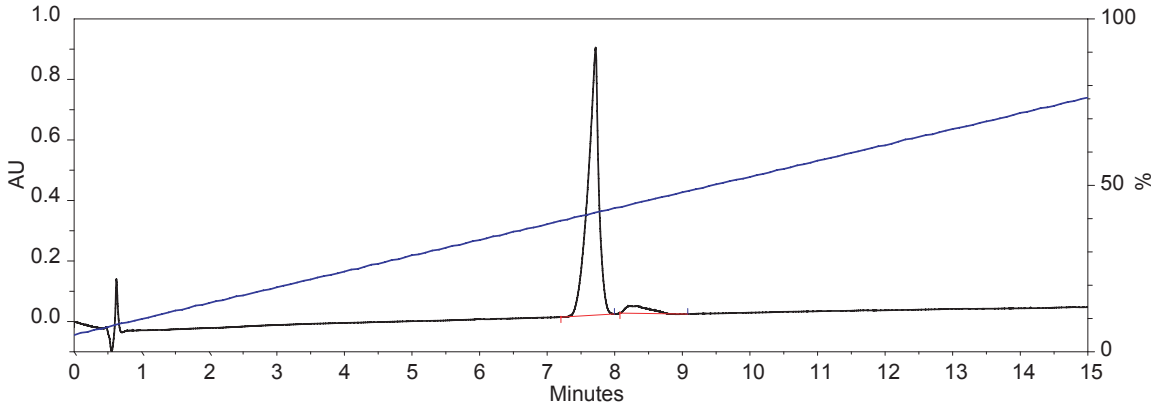
Totals	7527194	100.00	434217	100.00

λ = 214 nm. 80 SB Zorbax column
gradient 5%–100% solvent B over 20 min.
solvent A: Water (TFA 0.1%); solvent B: CH3CN (TFA 0.1%)
flow = 1 mL/min

LRMS(ESI) of peptide **1b**



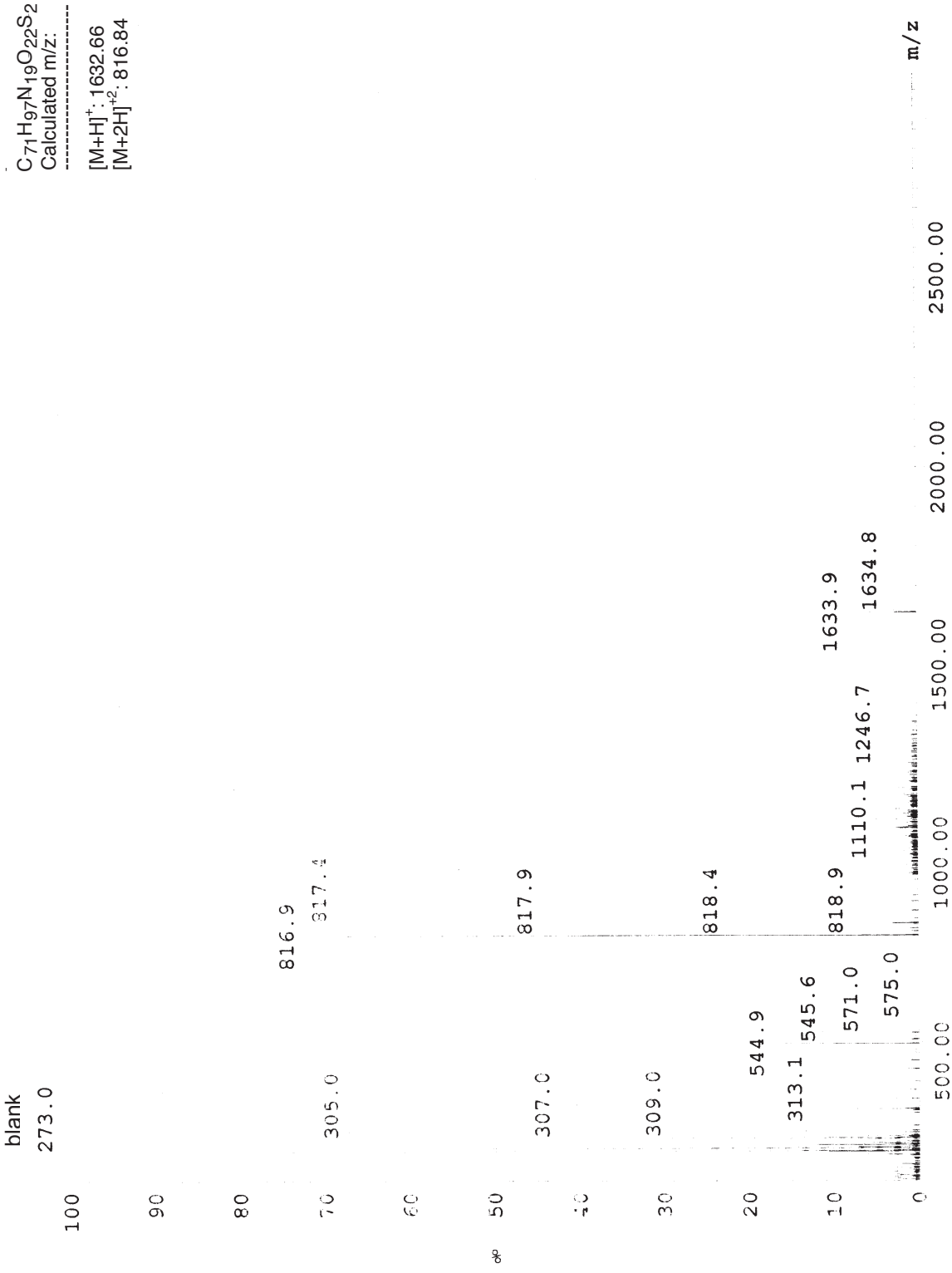
Analytical RP-HPLC of peptide **1c**



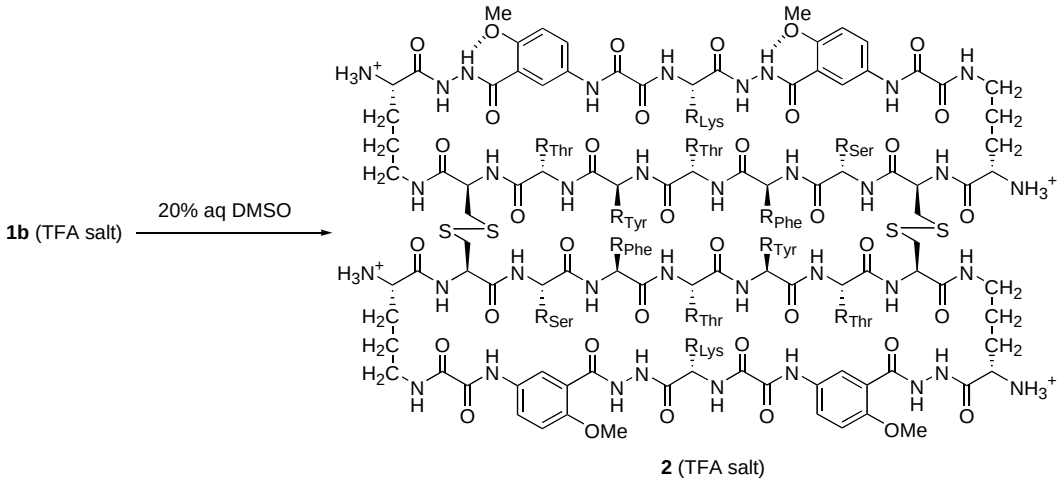
Time	Area	Area %	Height	Height %
7.717	9798347	94.44	884040	97.37
8.327	576361	5.56	23837	2.63
Totals	10374708	100.00	907877	100.00

λ = 214 nm. 80 SB Zorbax column
gradient 5%–100% solvent B over 20 min.
solvent A: Water (TFA 0.1%); solvent B: CH3CN (TFA 0.1%)
flow = 1 mL/min

LRMS(ESI) of peptide **1c**

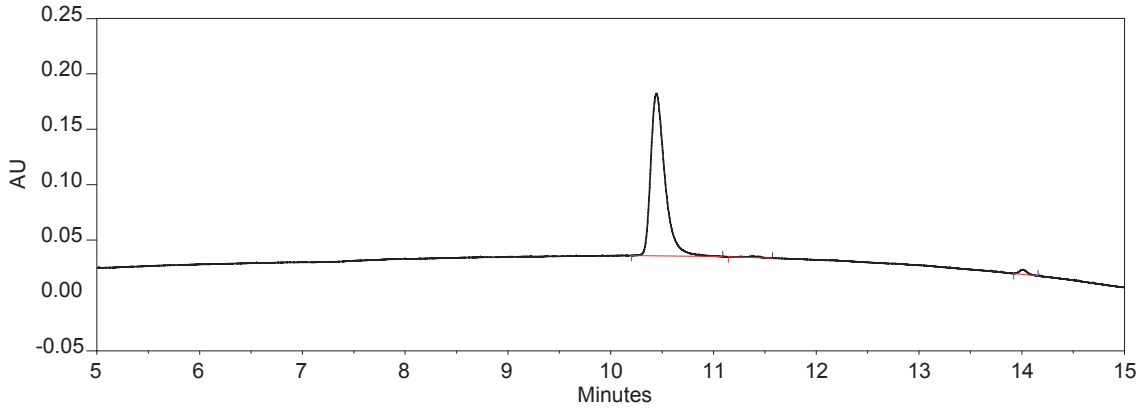


Synthesis of covalent dimer 2



Cyclic peptide **1b** (20 mg, 10.0 μmol) was treated with a 20% aqueous DMSO solution (5 mL). The oxidation reaction was monitored by analytical RP-HPLC. After 24 h, the reaction mixture was directly subjected to RP-HPLC purification (gradient elution with water/acetonitrile from 95:5 to 50:50, both solvents contained 0.1% TFA). Upon purification, 7 mg covalent dimer **2** (35% yield) was obtained. (Oxidation of 8.0 mg peptide **1b** in another batch afforded 6.0 mg dimer **2** in 75% yield). The purity of peptide **2** was verified by analytical RP-HPLC and LRMS (ESI).

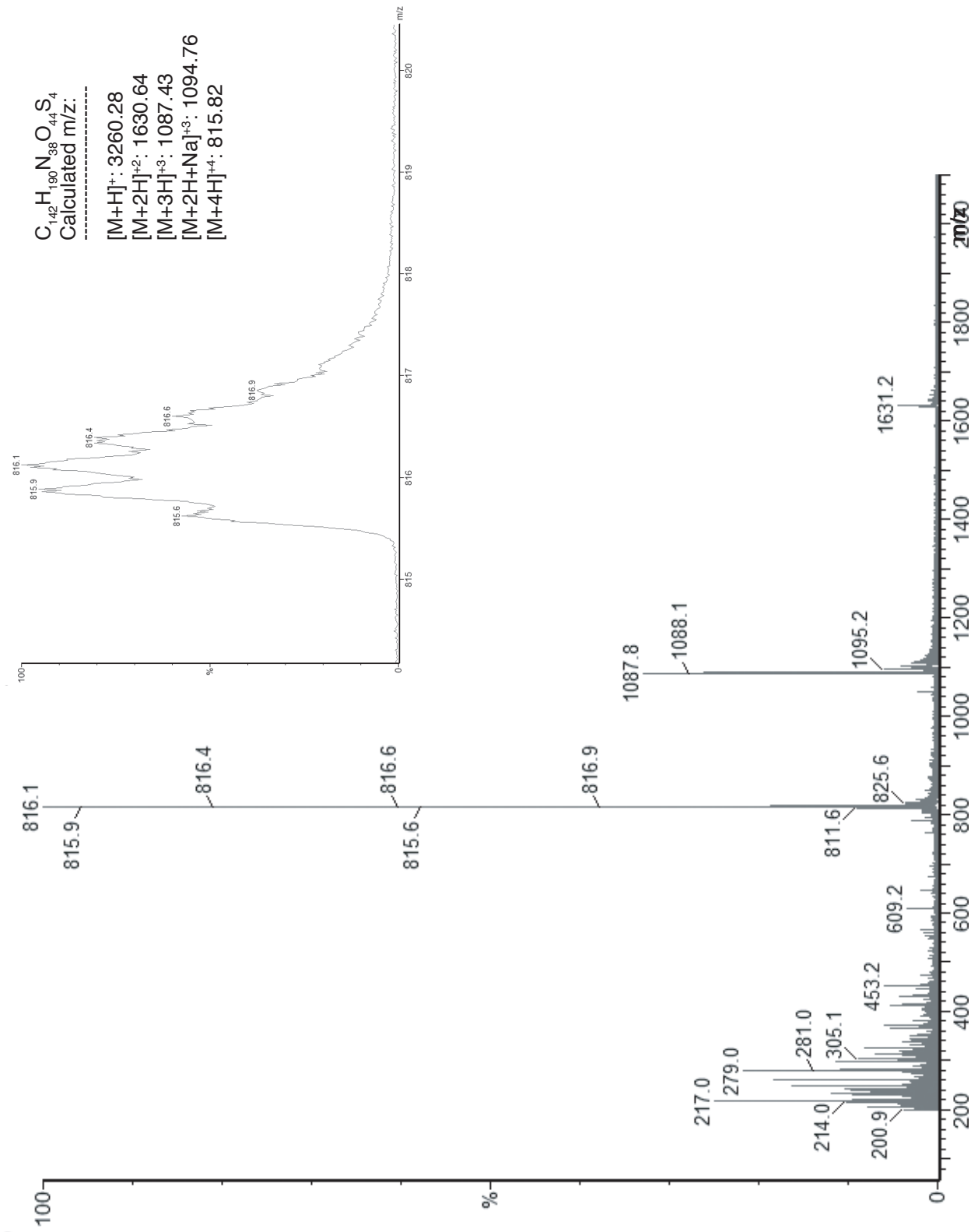
Analytical RP-HPLC of dimer **2**



Time	Area	Area %	Height	Height %
10.445	1398441	97.80	146454	96.52
11.388	8584	0.60	975	0.64
14.012	22930	1.60	4306	2.84
Totals	1429955	100.00	151735	100.00

λ = 214 nm. 80 SB Zorbax column
gradient 5%–100% solvent B over 20 min.
solvent A: Water (TFA 0.1%); solvent B: CH_3CN (TFA 0.1%)
flow = 1 mL/min

LRMS(ESI) of dimer **2**



^{1}H NMR studies of covalent dimer 2

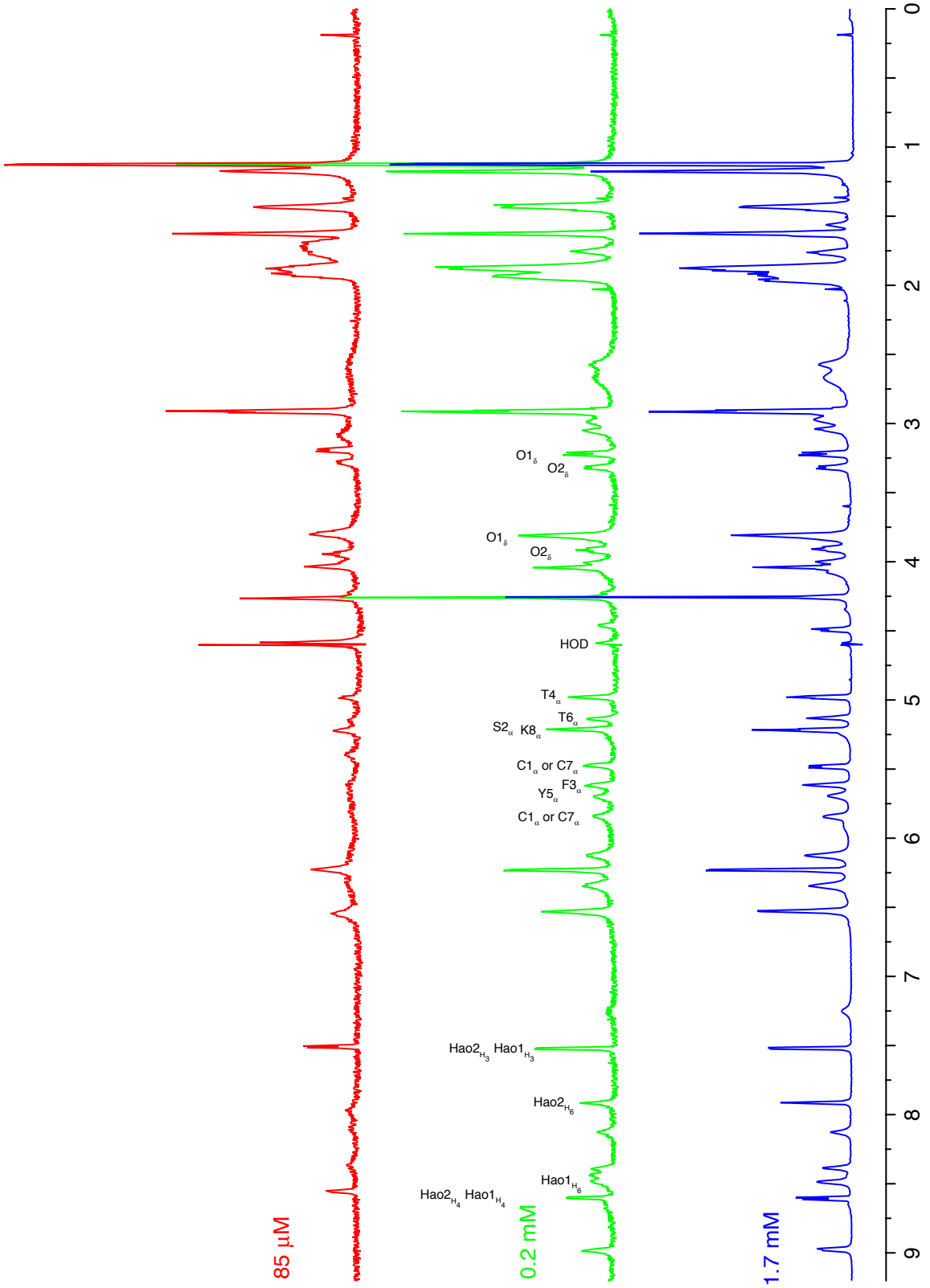
Peptide **2** was lyophilized with D_2O twice prior to NMR studies to attenuate resonances associated with water and exchangeable protons. Solutions of the peptide were prepared gravimetrically by dissolving an appropriate weight of the peptides in an appropriate volume of solvent. In calculating molecular weights, all amino groups were assumed to be protonated as TFA salts.

^{1}H NMR assignments. The ^{1}H NMR resonances of peptide **2** were assigned by an 800 MHz NOESY experiment at 318 K on a 0.2 mM D_2O solution of the peptide and by 800 MHz TOCSY and NOESY experiments at 298 K on a 0.7 mM DMSO-d6 solution of the peptide. Mixing times of 200 ms and 150 ms were used for the NOESY experiments in D_2O and DMSO-d6, respectively. A spin-lock time of 75 ms was used for the TOCSY experiment in DMSO-d6.

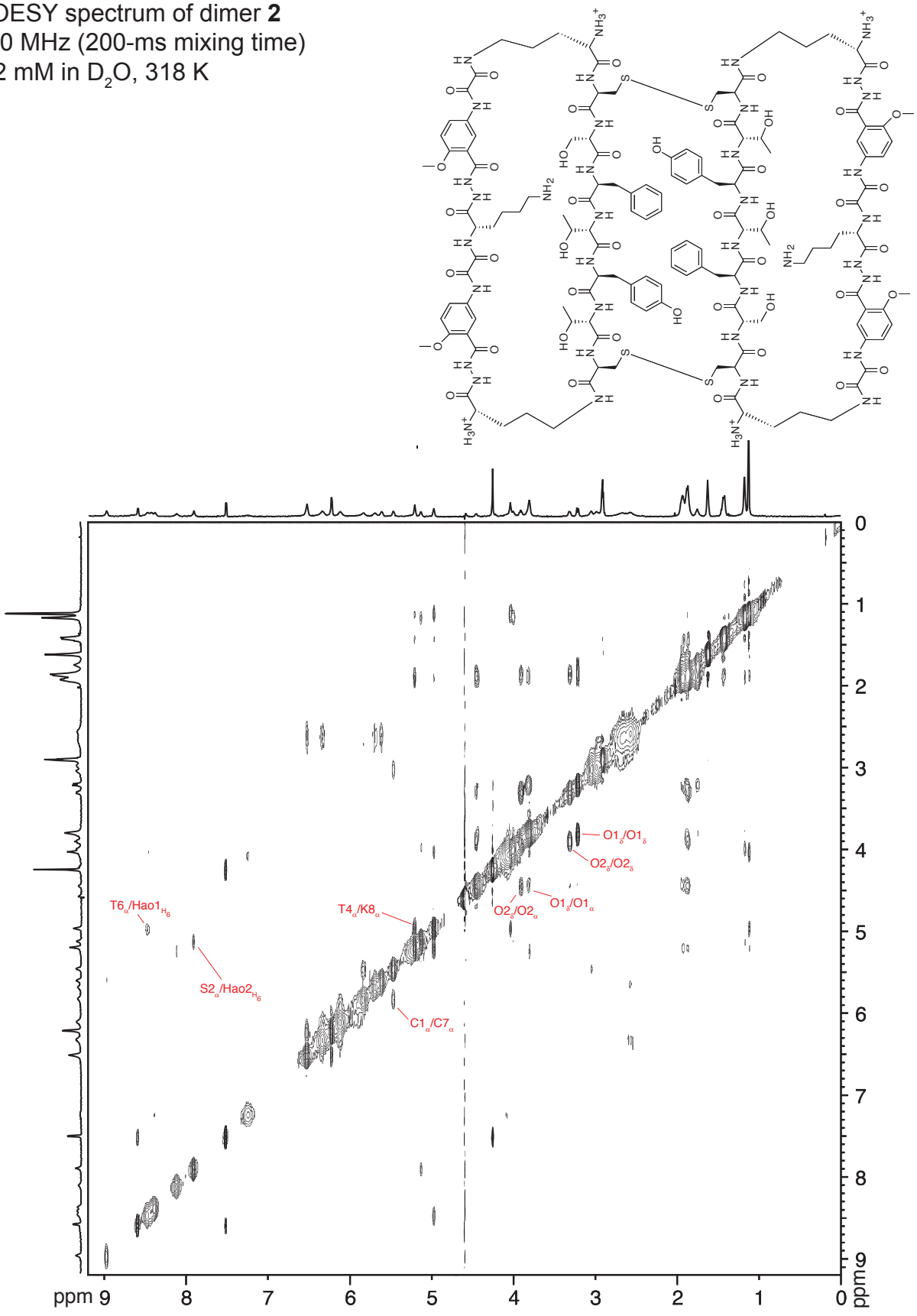
The 2D NOESY spectra in DMSO-d6 were acquired with 6000 data points in the f_2 domain and 300 data points in the f_1 domain and were processed by zero-filling to a final matrix of 4096 x 1048 real points. The 2D TOCSY spectra in DMSO-d6 were acquired with 2048 data points in the f_2 domain and 300 data points in the f_1 domain and were processed by zero-filling to a final matrix of 4096 x 1048 real points. The 2D NOESY spectrum in D_2O was acquired with 3640 data points in the f_2 domain and 128 data points in the f_1 domain and was processed by zero-filling to a final matrix of 4096 x 1048 real points.

Self-association studies. The self-association properties of peptide **2** in D_2O were studied by comparing the ^{1}H NMR spectra at various concentrations (17 μM–1.7 mM) and various temperatures (280–318 K).

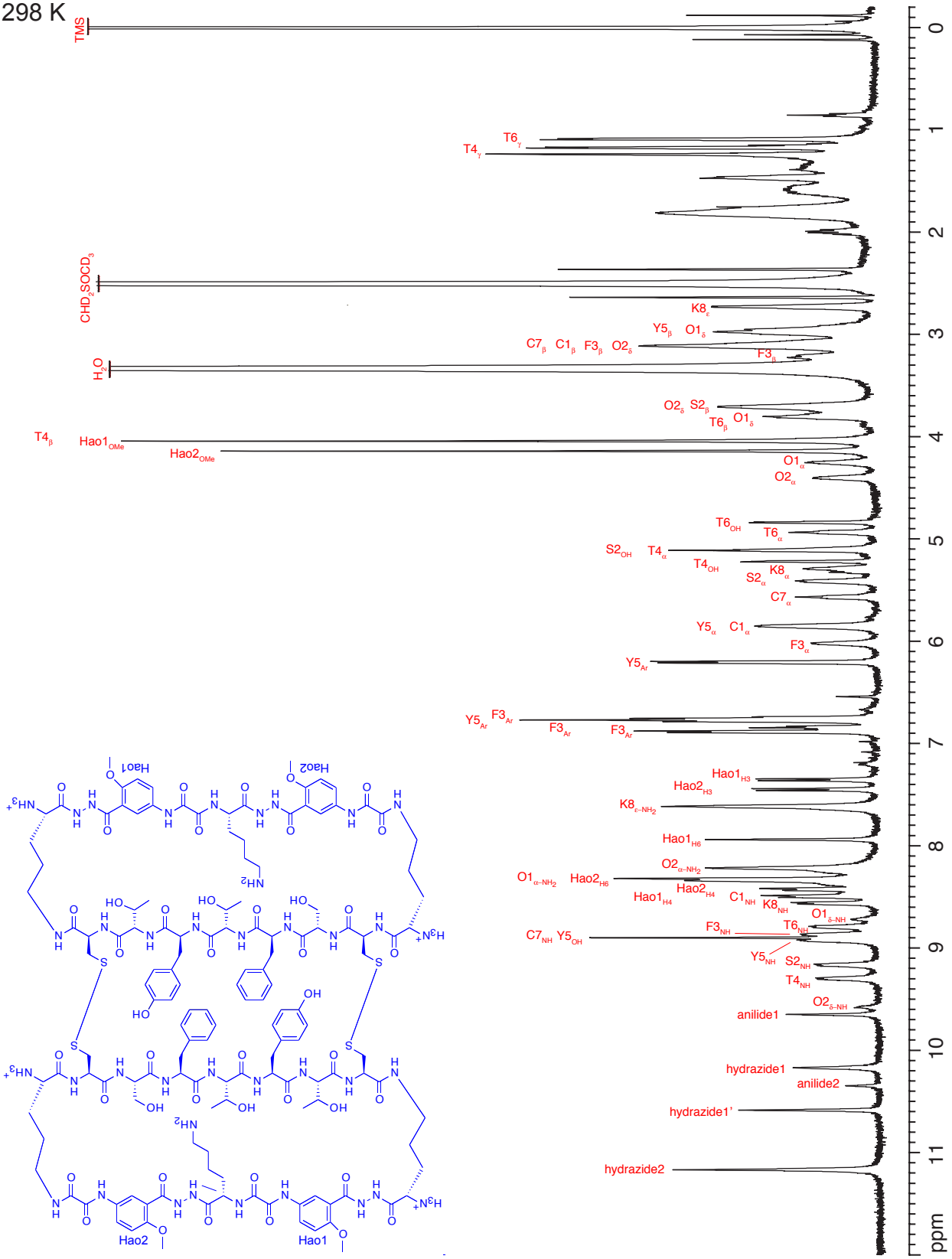
800 MHz ^{1}H NMR spectra of dimer **2**
D_2O, 318 K



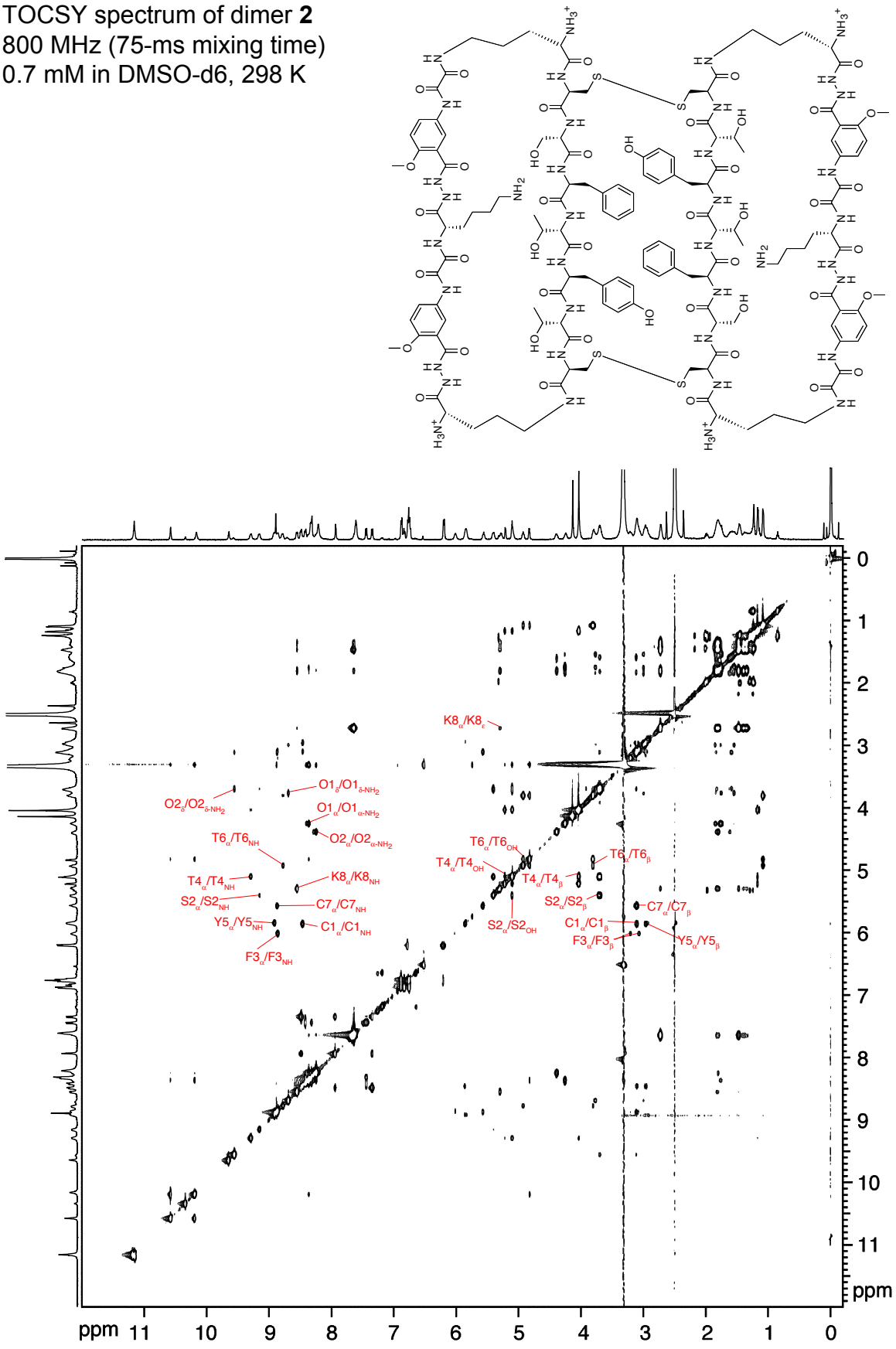
NOESY spectrum of dimer **2**
800 MHz (200-ms mixing time)
0.2 mM in D_2O, 318 K



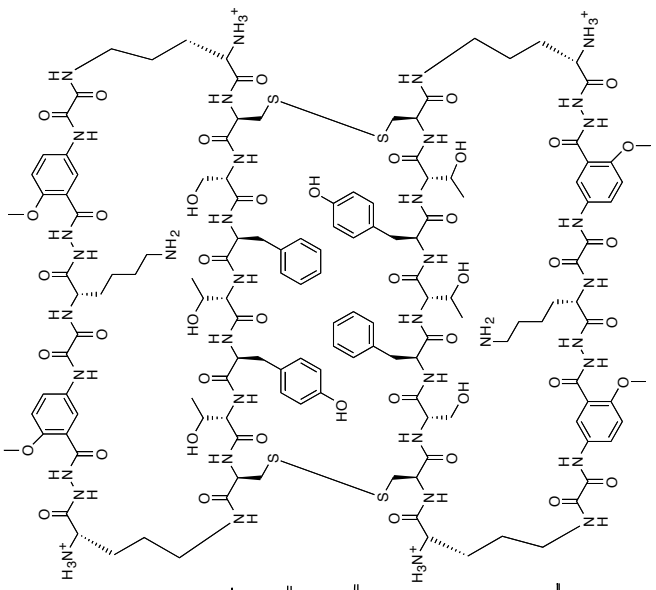
800 MHz ^{1}H NMR spectrum of dimer **2**
0.7 mM in DMSO-d6
298 K

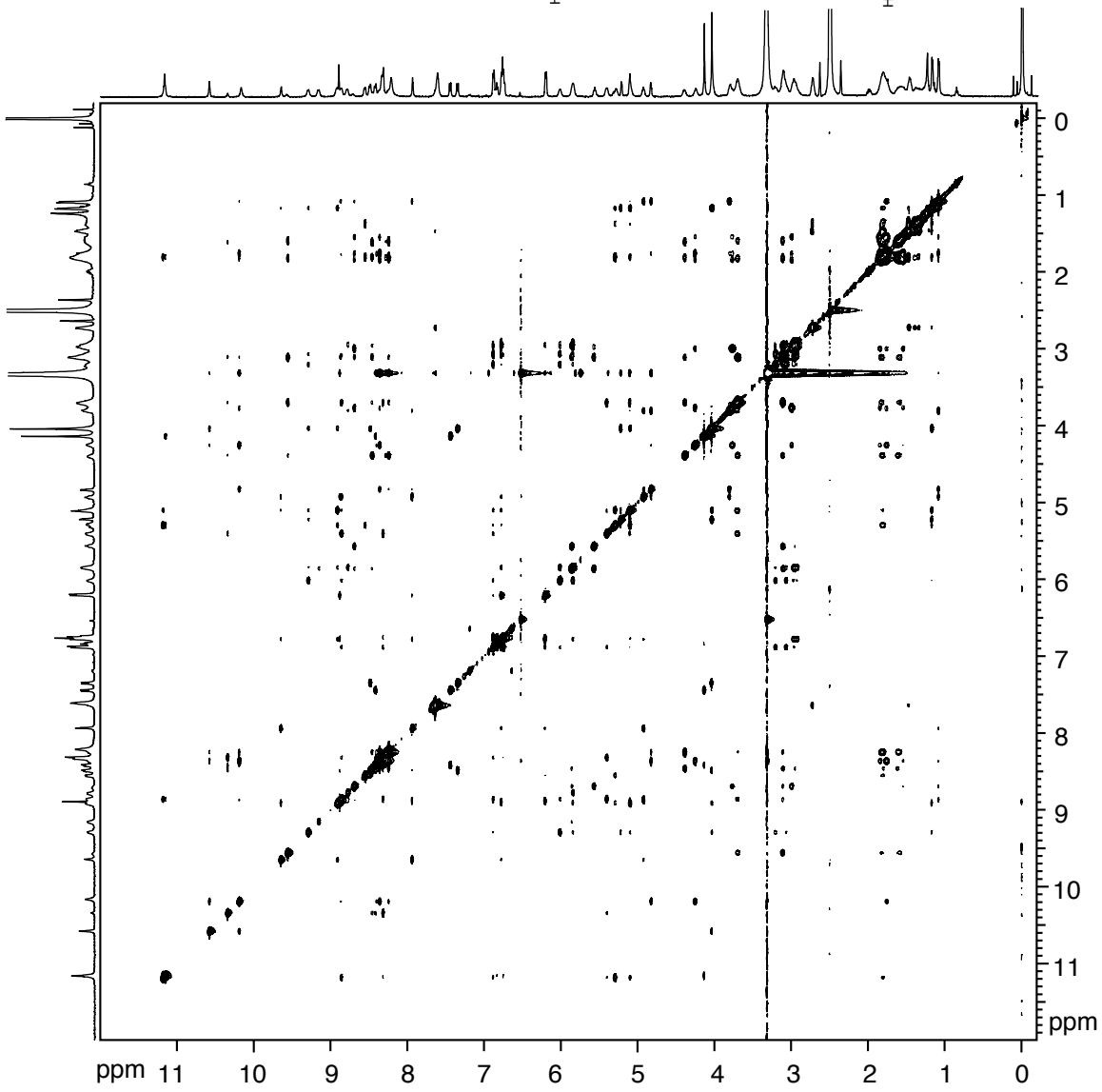


TOCSY spectrum of dimer **2**
800 MHz (75-ms mixing time)
0.7 mM in DMSO-d6, 298 K

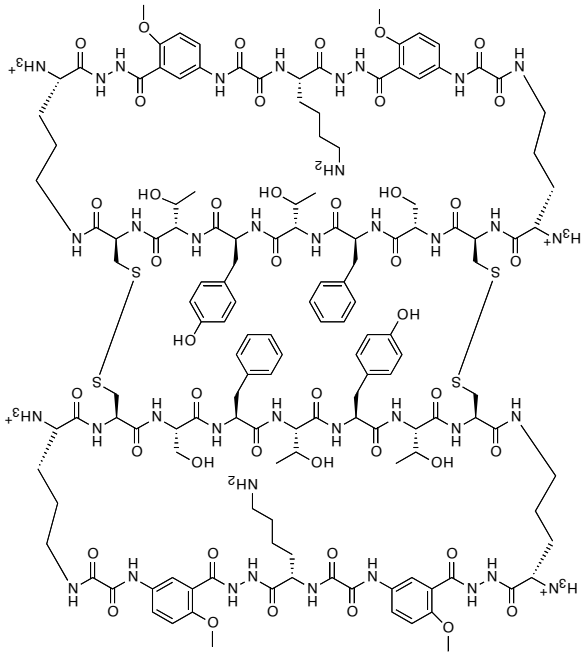


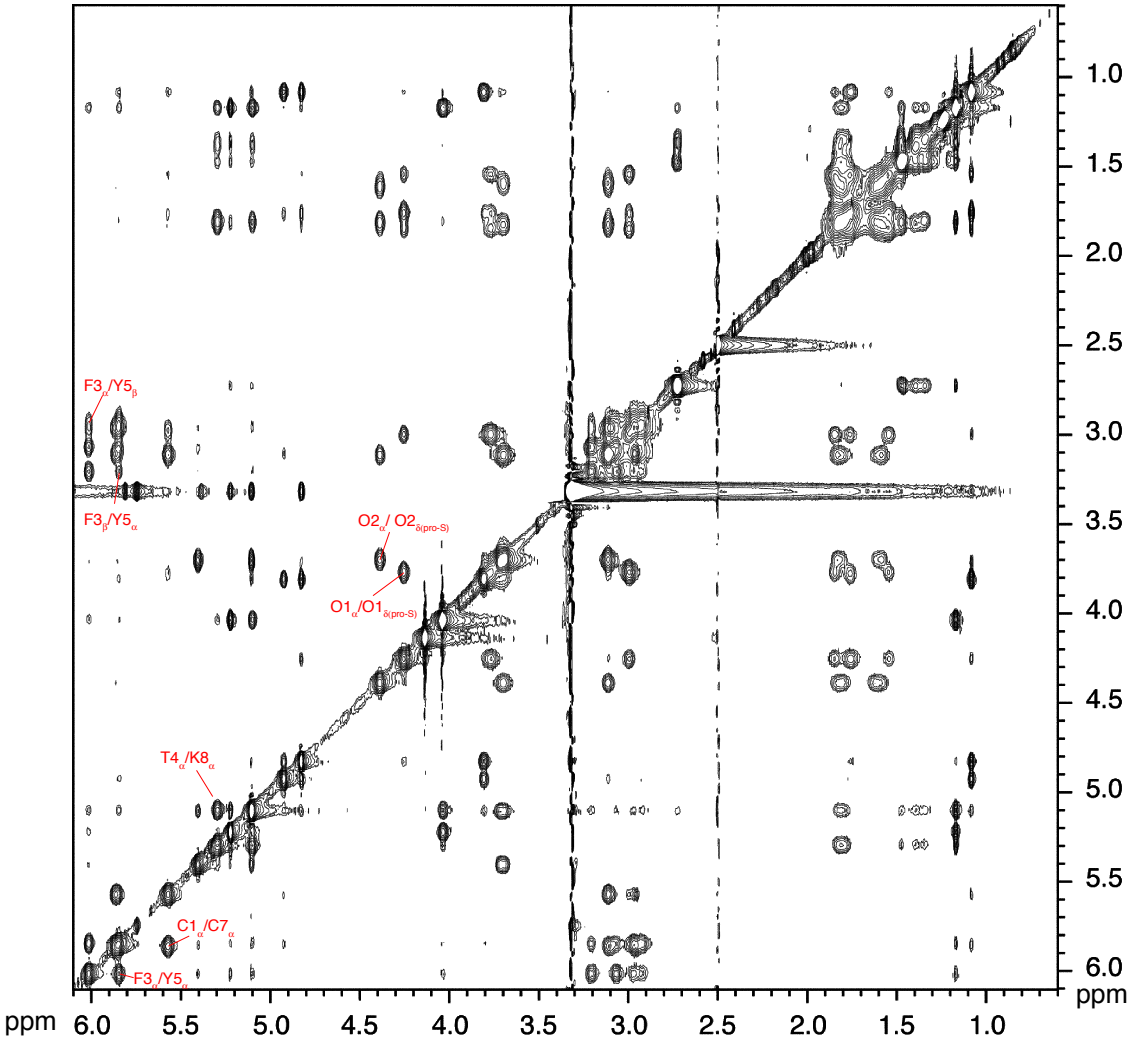
NOESY spectrum of dimer **2**
800 MHz (150-ms mixing time)
0.7 mM in DMSO-d6, 298 K



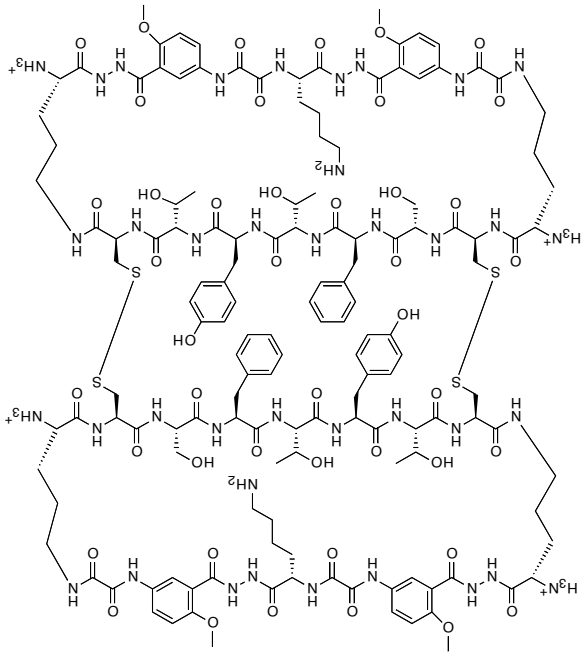


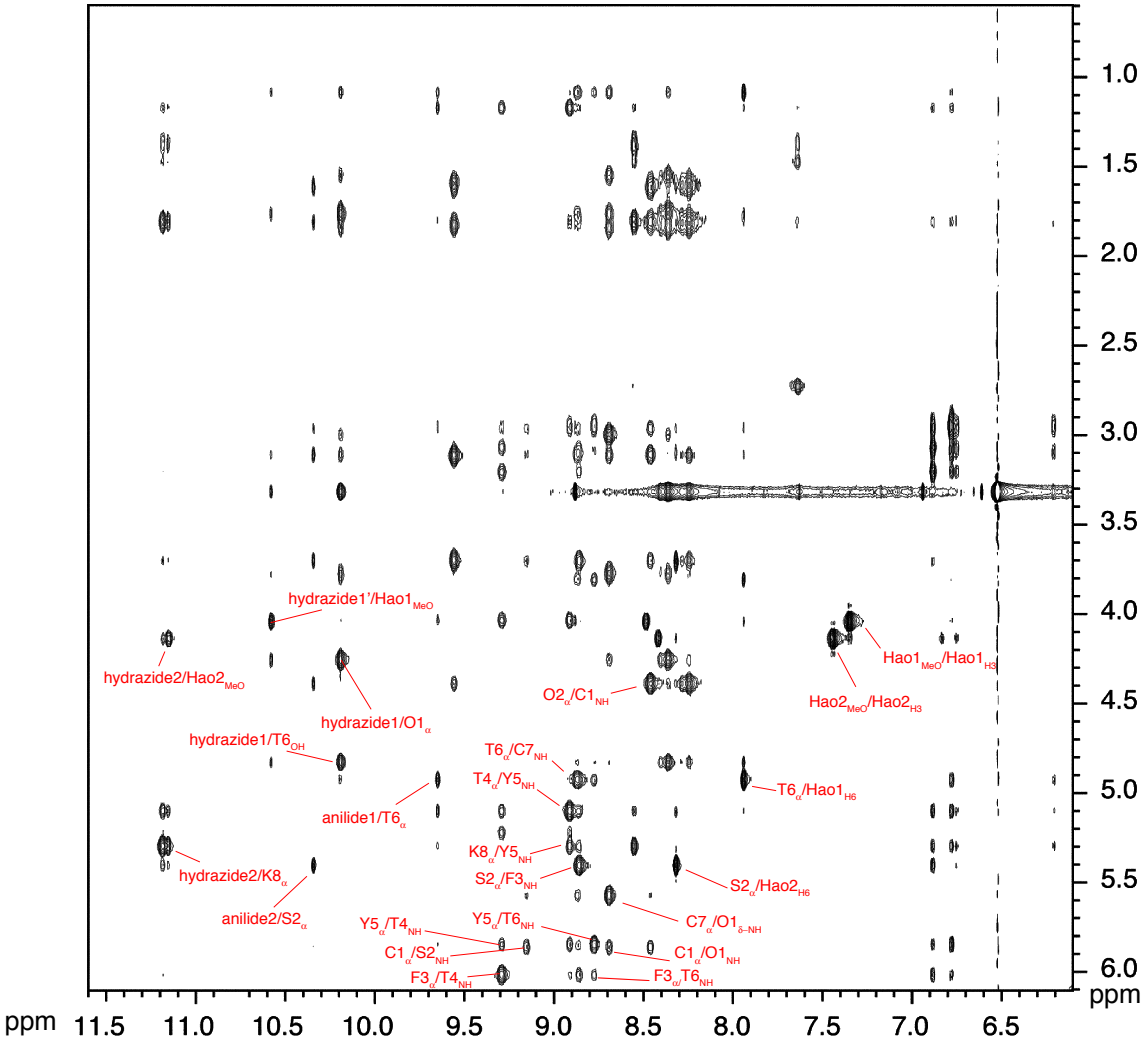
NOESY spectrum of dimer **2**
800 MHz (150-ms mixing time)
0.7 mM in DMSO-d6, 298 K



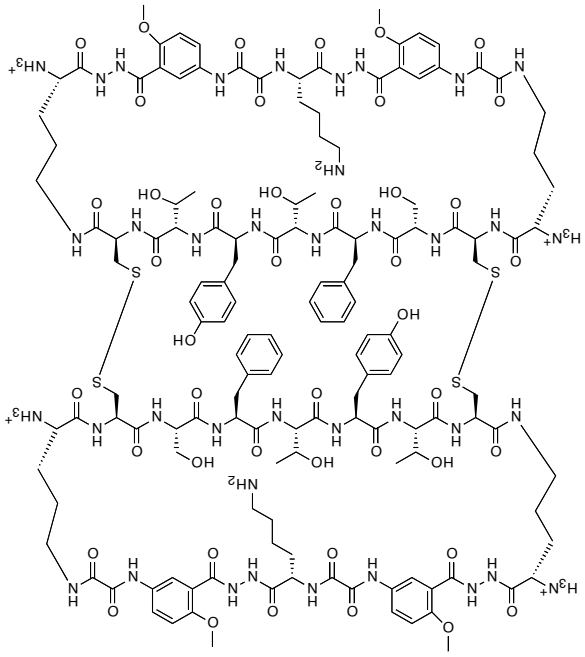


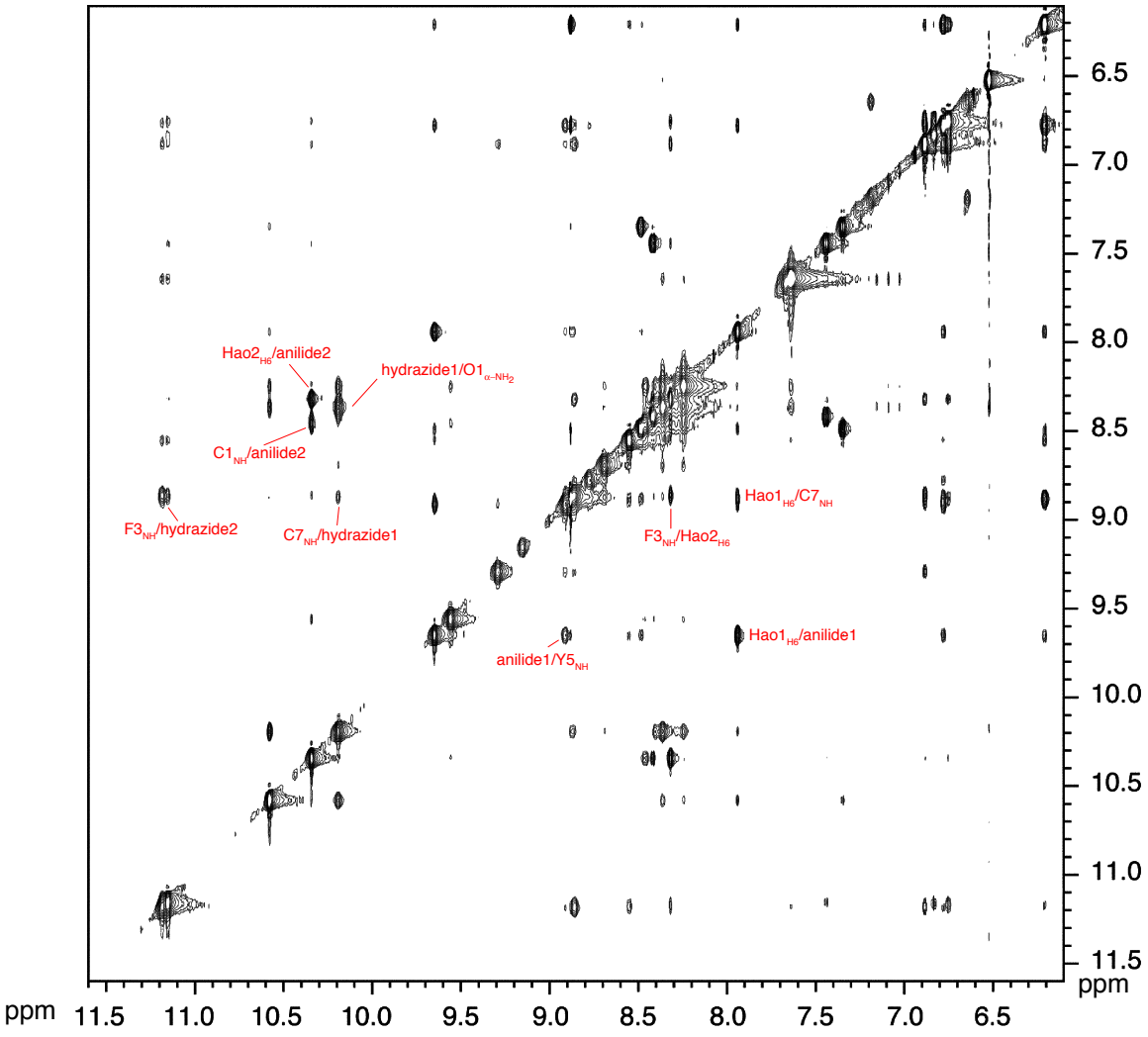
NOESY spectrum of dimer **2**
800 MHz (150-ms mixing time)
0.7 mM in DMSO-d6, 298 K



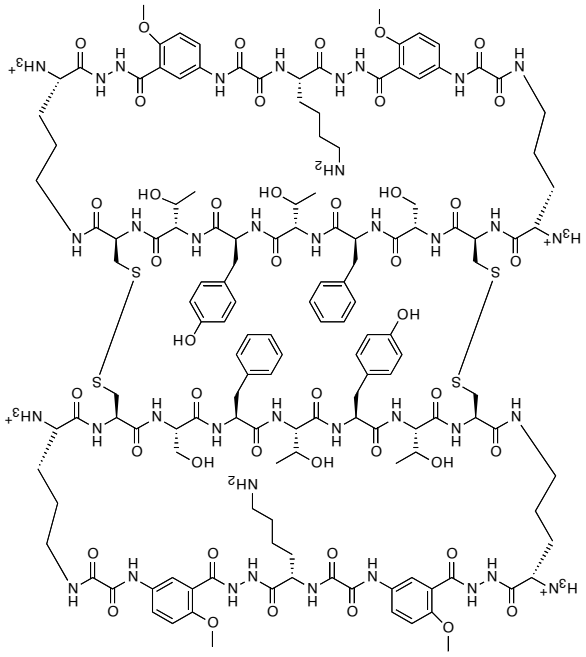


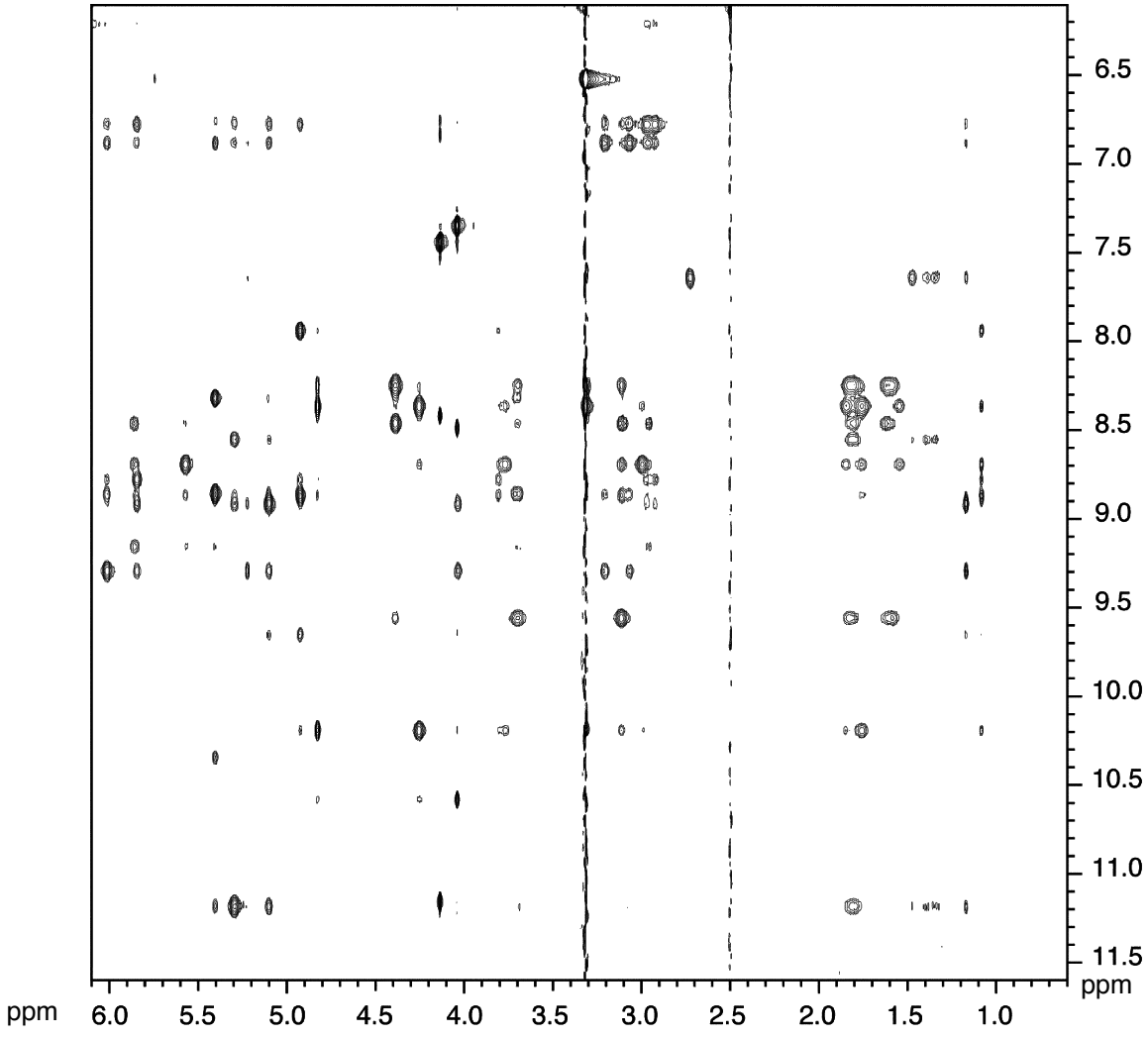
NOESY spectrum of dimer **2**
800 MHz (150-ms mixing time)
0.7 mM in DMSO-d6, 298 K





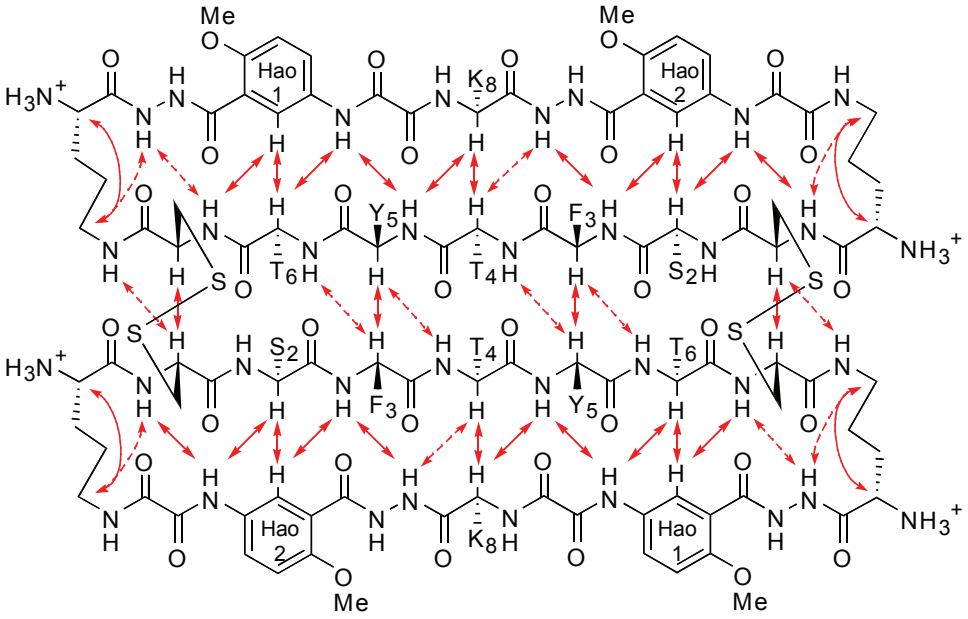
NOESY spectrum of dimer **2**
800 MHz (150-ms mixing time)
0.7 mM in DMSO-d6, 298 K



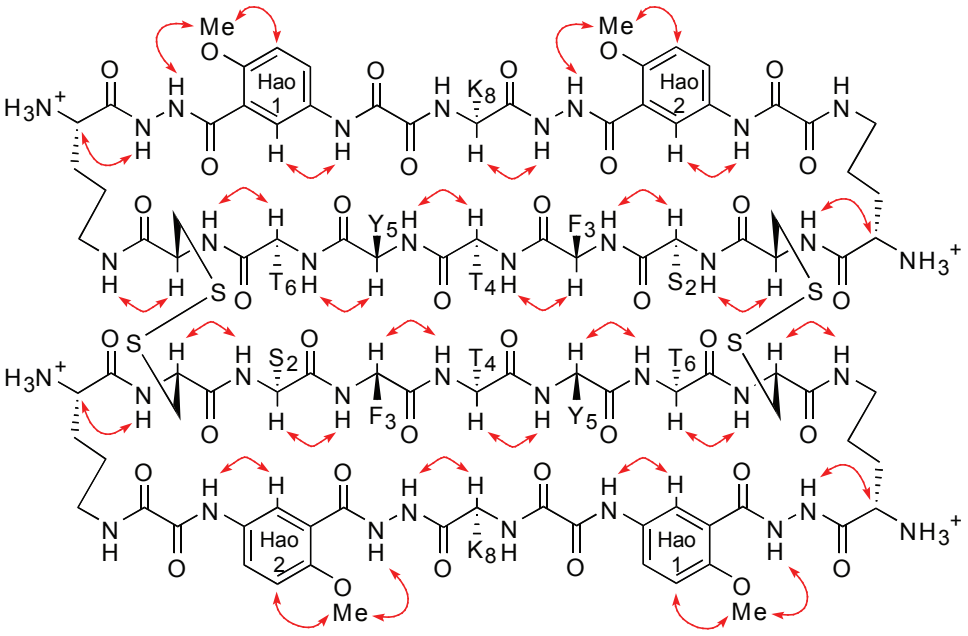


Key NOEs in dimer **2**
(800 MHz, DMSO-d6, 298 K)

a. Interstrand main chain–main chain NOEs



b.d. Intrastrand NOEs



dashed arrows represent the weak or ambiguous NOEs

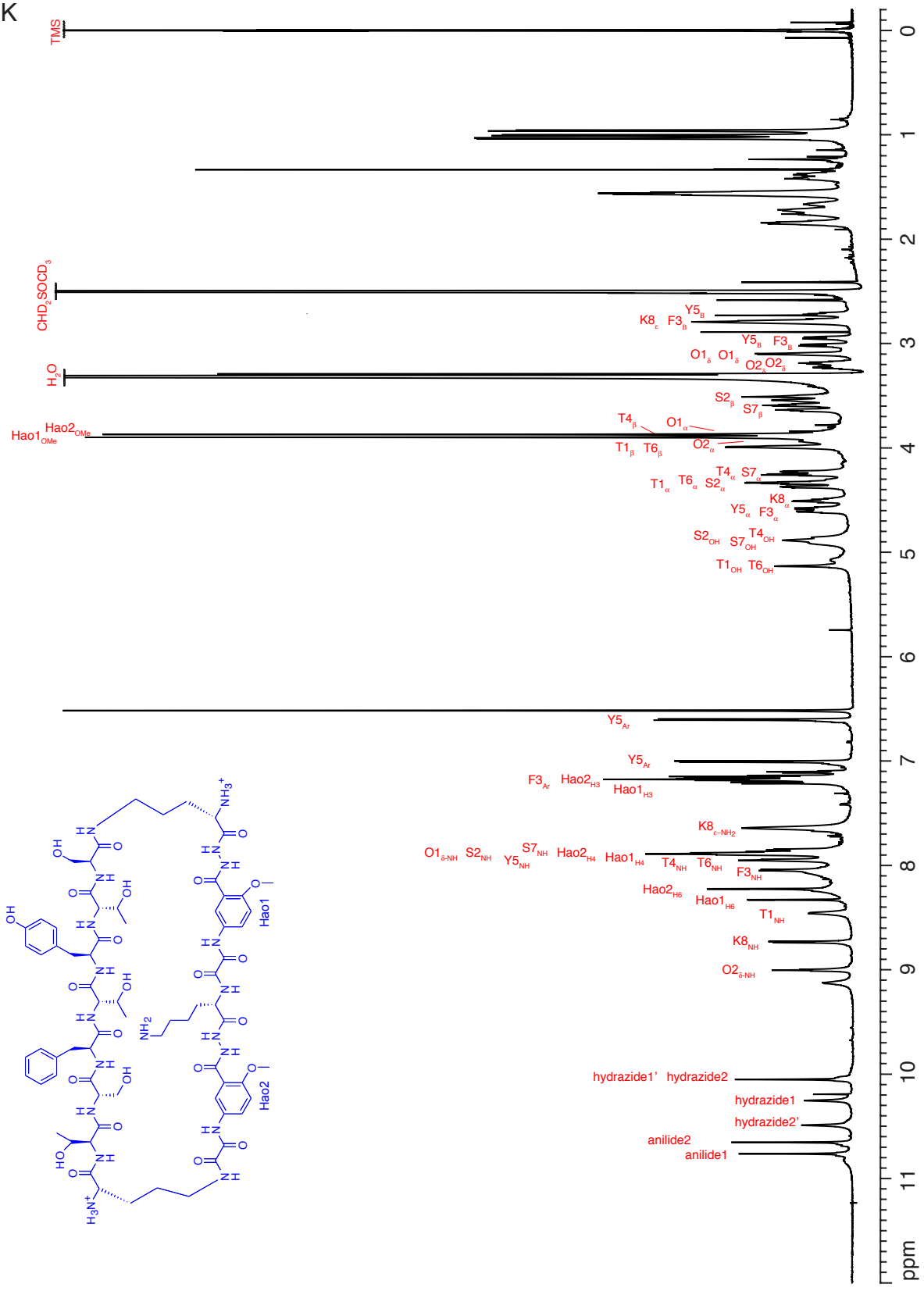
^{1}H NMR studies of cyclic peptide 1a

Solutions of peptide **1a** [cyclic peptide **3a** in *J. Am. Chem. Soc.* **2007**, *129*, 5558–5569] were prepared gravimetrically by dissolving an appropriate weight of the peptides in an appropriate volume of solvent. In calculating molecular weights, all amino groups were assumed to be protonated as TFA salts.

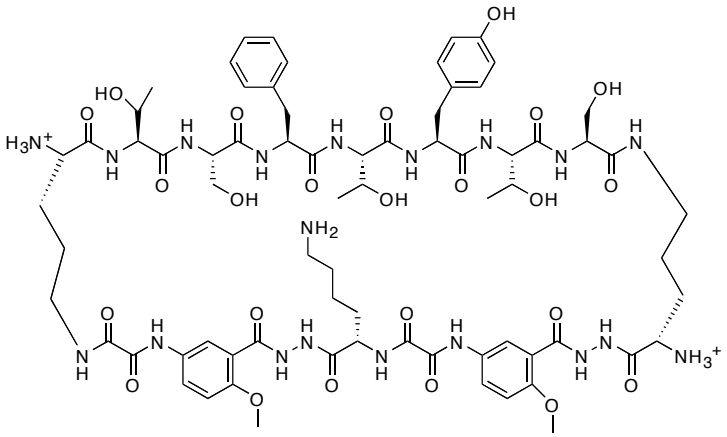
^{1}H NMR assignments. The ^{1}H NMR studies of the monomer and the tetramer of peptide **1a** in D_2O were previously reported (reference above). The ^{1}H NMR resonances of peptide **1a** in DMSO-d6 were assigned by 800 MHz TOCSY and NOESY experiments at 298 K on a 0.7 mM solution of the peptide. A spin-lock time of 75 ms was used for the TOCSY experiment and a mixing time of 150 ms was used for the NOESY experiment.

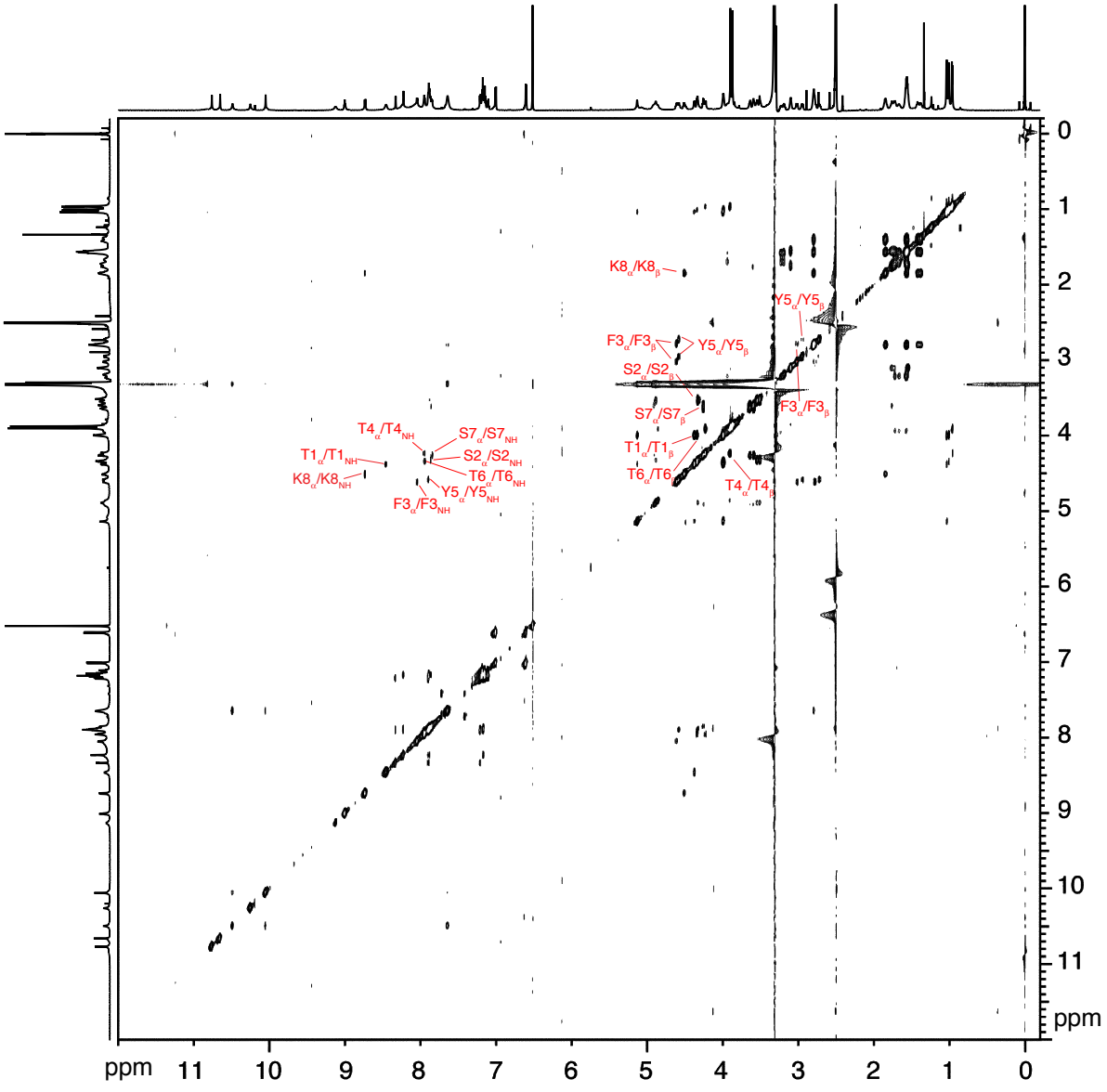
The 2D NOESY spectra were acquired with 6000 data points in the f_2 domain and 300 data points in the f_1 domain and were processed by zero-filling to a final matrix of 4096 x 1048 real points. The 2D TOCSY spectra were acquired with 2048 data points in the f_2 domain and 300 data points in the f_1 domain and were processed by zero-filling to a final matrix of 4096 x 1048 real points.

800 MHz ^{1}H NMR spectrum of dimer **1a**
0.7 mM in DMSO-d6
298 K

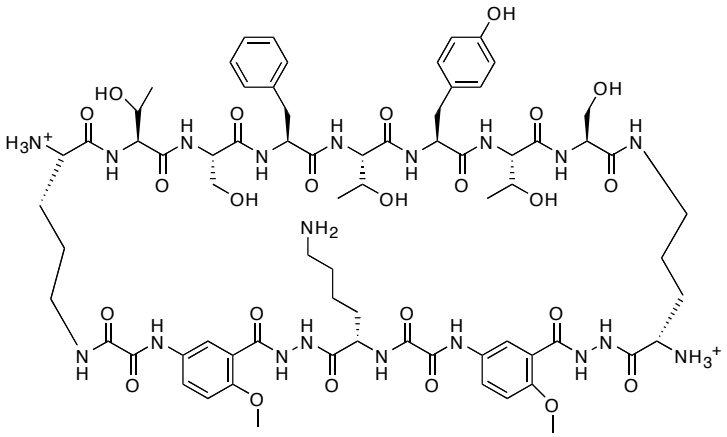


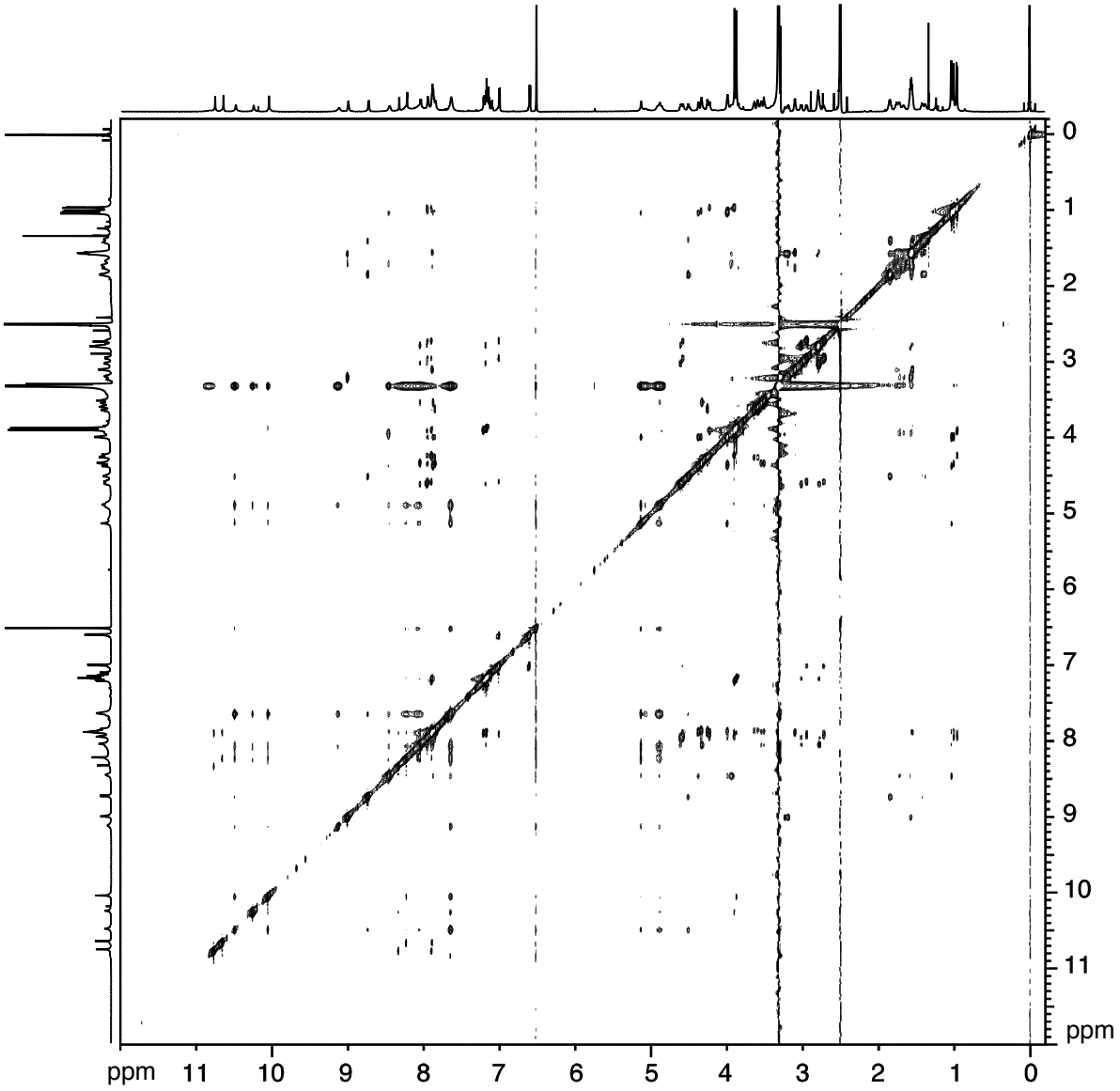
TOCSY spectrum of peptide **1a**
800 MHz (75-ms mixing time)
0.7 mM in DMSO-d6, 298 K



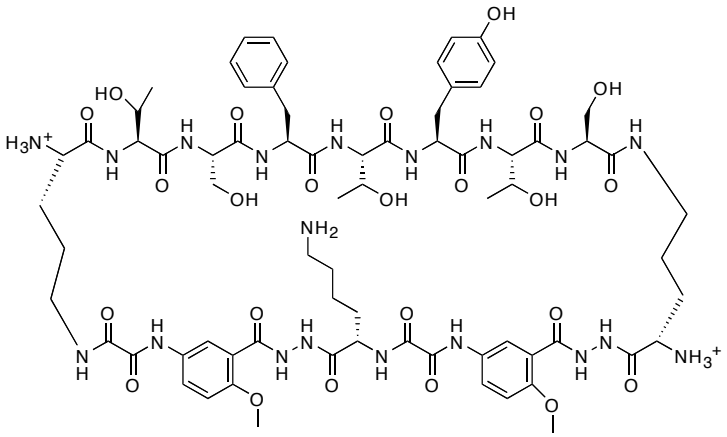


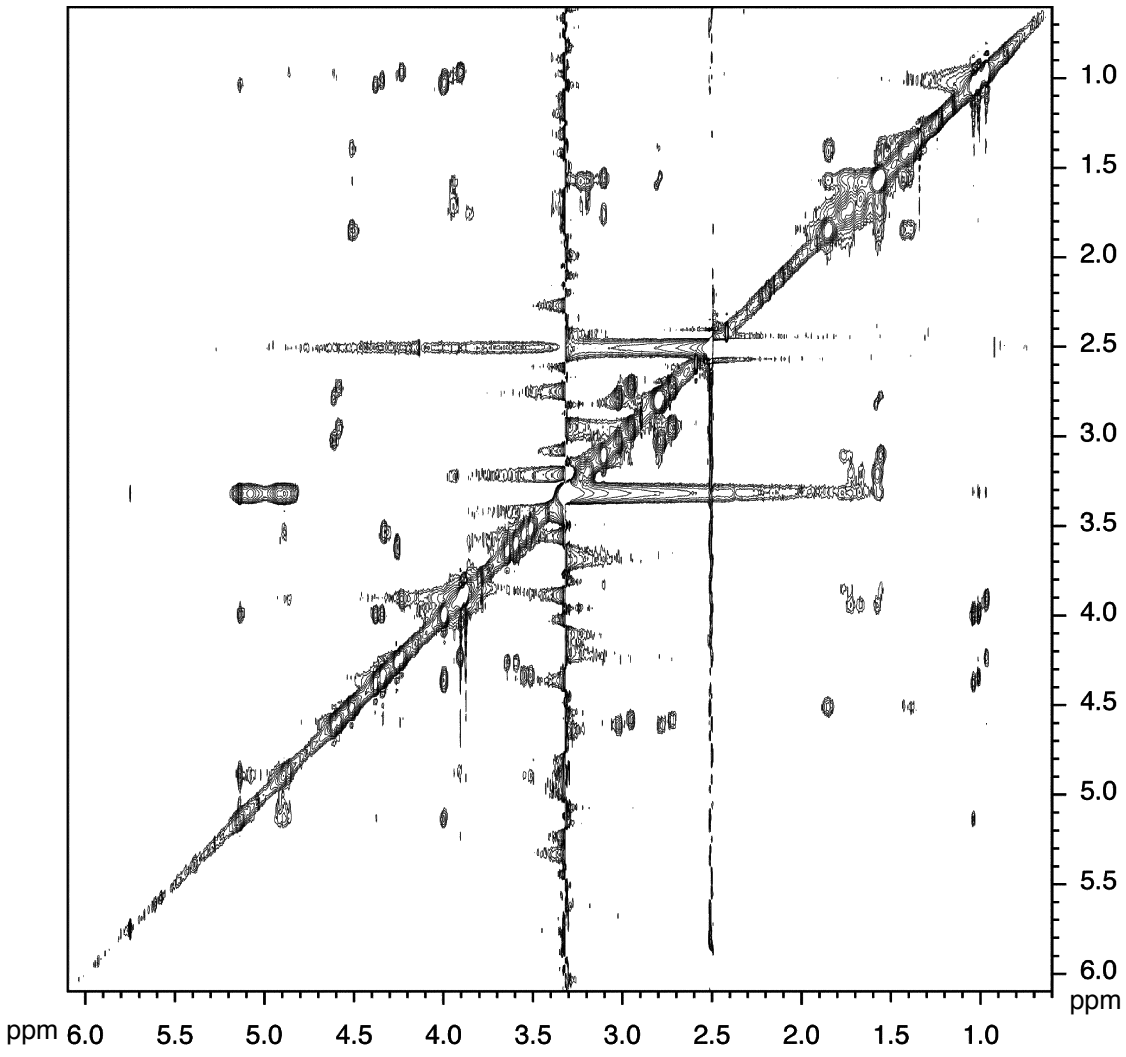
NOESY spectrum of peptide **1a**
800 MHz (150-ms mixing time)
0.7 mM in DMSO-d6, 298 K



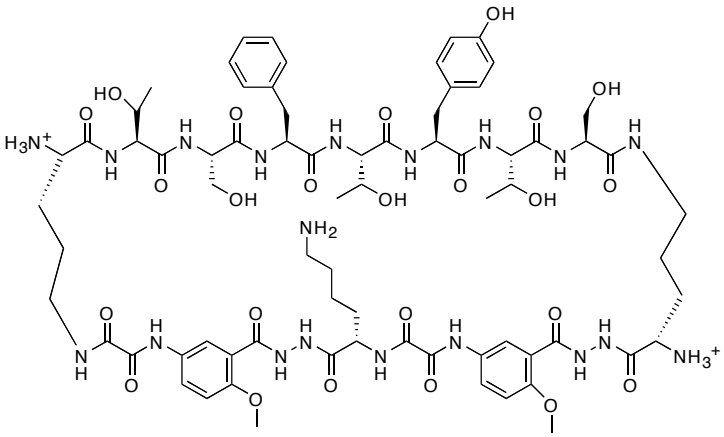


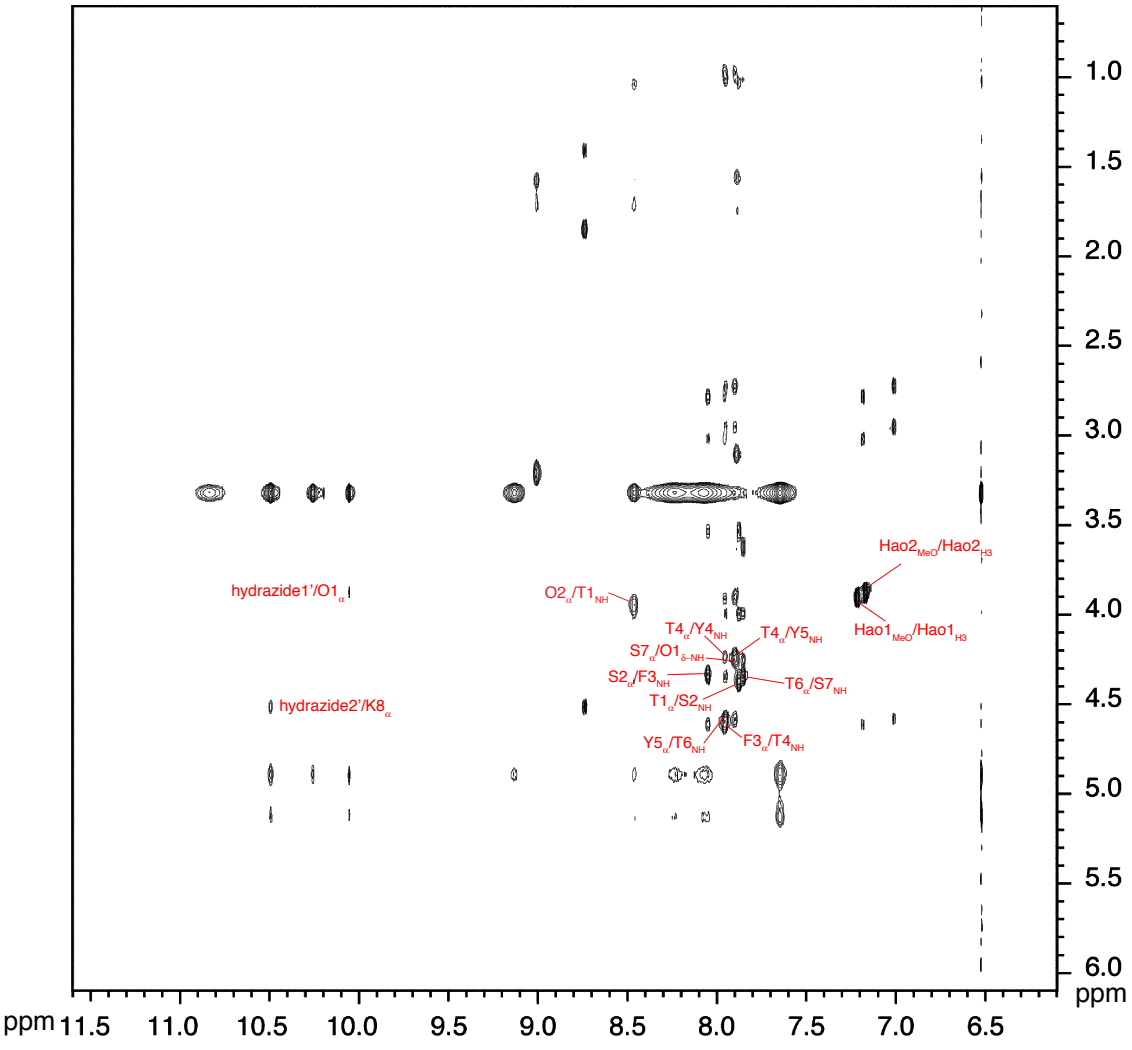
NOESY spectrum of peptide **1a**
800 MHz (150-ms mixing time)
0.7 mM in DMSO-d6, 298 K



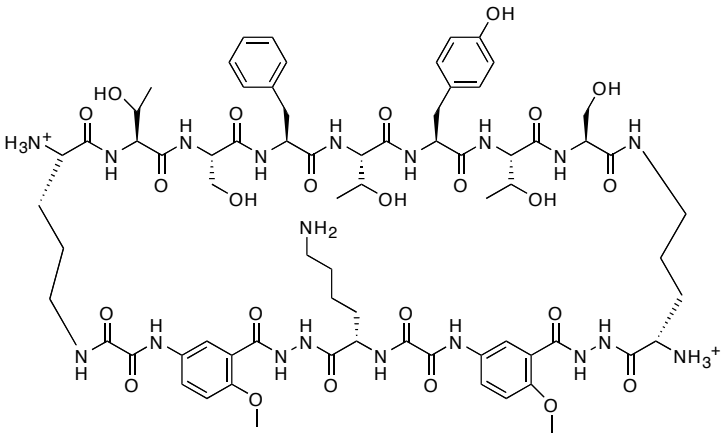


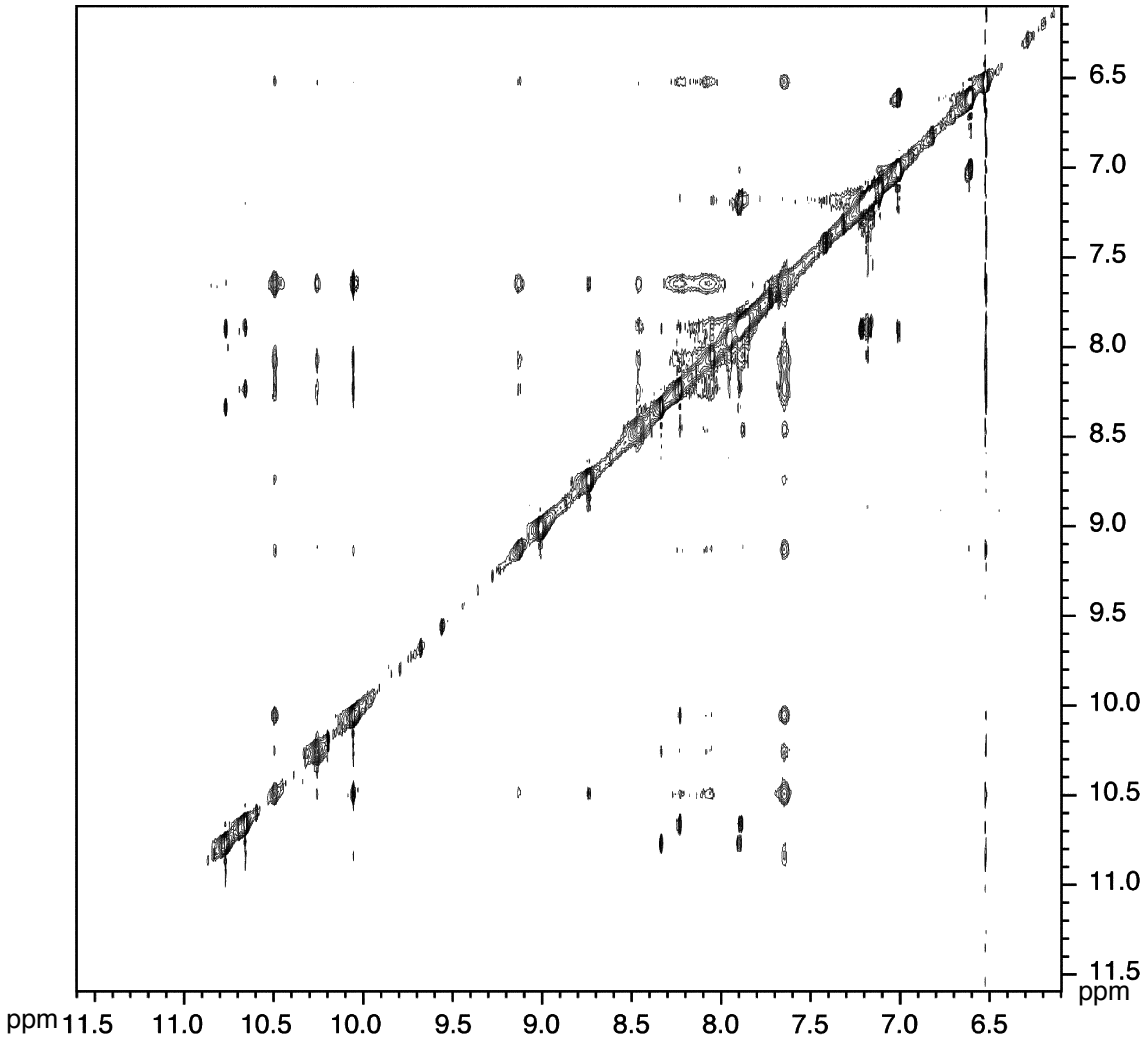
NOESY spectrum of peptide **1a**
800 MHz (150-ms mixing time)
0.7 mM in DMSO-d6, 298 K



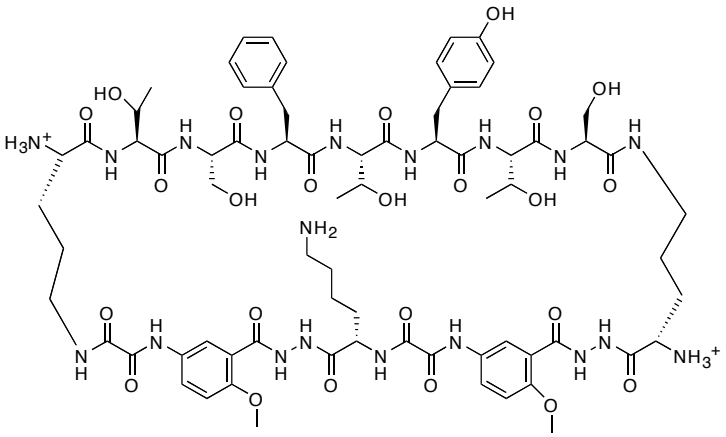


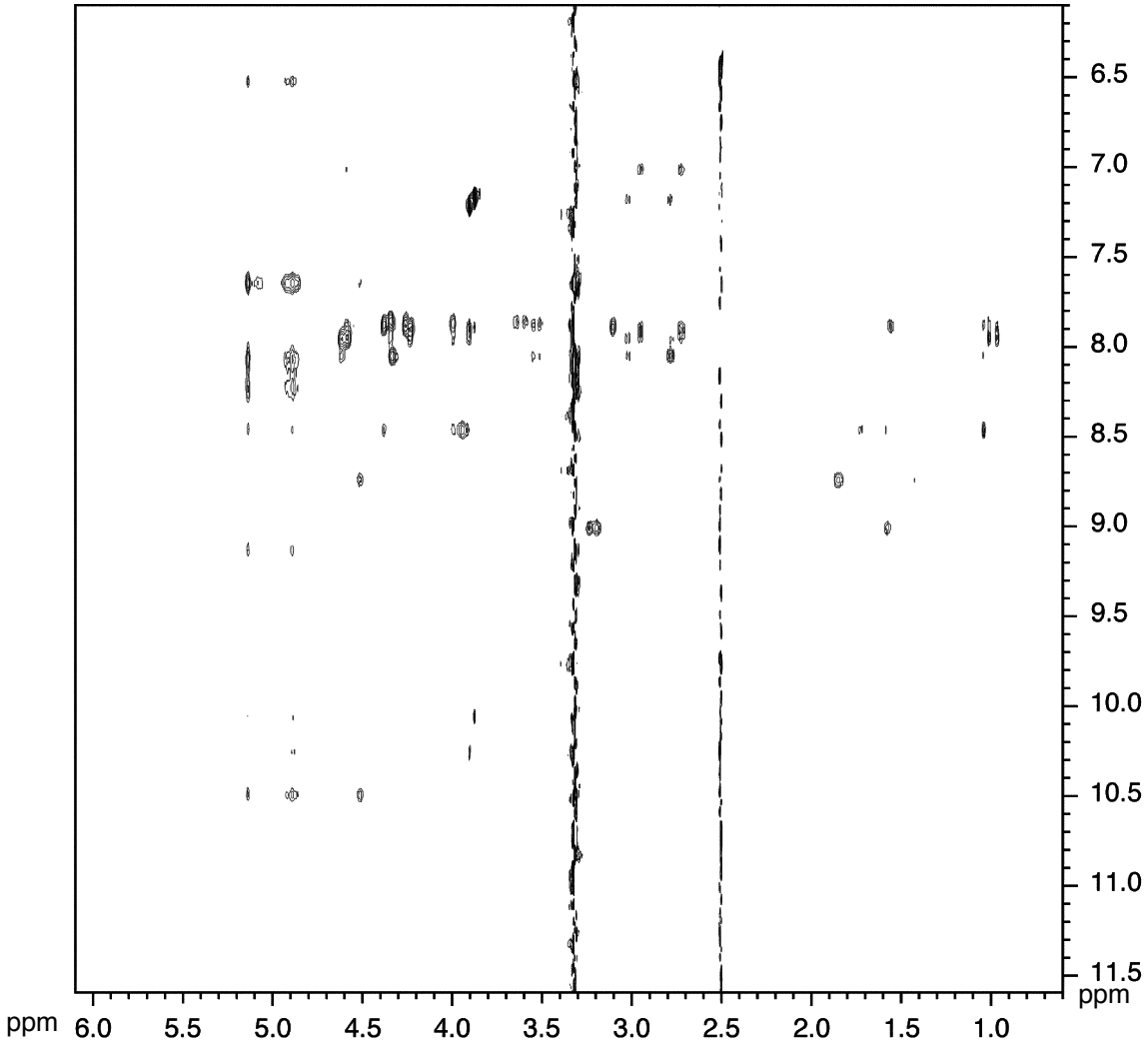
NOESY spectrum of peptide **1a**
800 MHz (150-ms mixing time)
0.7 mM in DMSO-d6, 298 K





NOESY spectrum of peptide **1a**
800 MHz (150-ms mixing time)
0.7 mM in DMSO-d6, 298 K

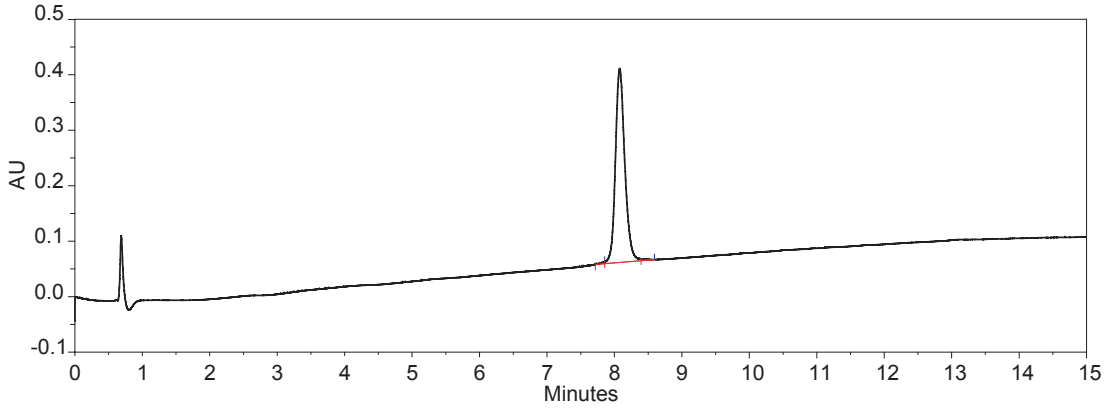




Synthesis of covalent dimer of 1c

Cyclic peptide **1c** (8 mg, 4.0 μmol) was dissolved in 0.4 mL of water and was stirred under air. The oxidation reaction was monitored by analytical RP-HPLC. (The viscosity of the solution increased over time.) After one week, the reaction mixture was diluted in 10 mL of water and lyophilized. Purification of the remaining material by RP-HPLC (gradient elution with water/acetonitrile from 95:5 to 50:50, both solvents contained 0.1% TFA) afforded 3 mg covalent dimer of **1c** (38% yield). The purity of the dimer was determined by analytical RP-HPLC and LRMS (ESI).

Analytical RP-HPLC of dimer of **1c**



Time	Area	Area %	Height	Height %
7.717	10347	0.30	0	0.00
8.078	3445284	99.33	349454	99.38
8.395	13037	0.38	2168	0.62
Totals	3468668	100.00	351622	100.00

λ = 214 nm. 80 SB Zorbax column
gradient 5%–100% solvent B over 20 min.
solvent A: Water (TFA 0.1%); solvent B: CH_3CN (TFA 0.1%)
flow = 1 mL/min

LRMS(ESI) of dimer of **1c**