Supporting Information for:

Polymorphism of Oligomers of a Peptide from β-Amyloid

Authors: Johnny D. Pham,[a] Borries Demeler,[b] James S. Nowick*,[a]

Affiliations:

[a] Department of Chemistry, University of California, Irvine, Irvine, CA 92697-2025.

[b] Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78229-3900.

E-mail: jsnowick@uci.edu

This PDF file includes:

Materials and Methods

Supplemental Figures and Tables

Materials and Methods:

Peptides **1–5** were prepared and studied as the trifluoroacetate (TFA) salts.

*Synthesis of macrocyclic β-sheet peptides **1–4**.*

Macrocyclic peptide **1** was synthesized as described previously, by automated solid-phase peptide synthesis of the corresponding linear peptide on chlorotrityl resin, followed by solution-phase cyclization, deprotection, and purification.[1] Macrocyclic peptides **2a-2c, 3,** and **4** were synthesized in a similar fashion, using procedures previously reported for the synthesis of **1** and of other macrocyclic β-sheet peptides.[1,2,3] Boc-Orn(Fmoc)-OH was used to introduce the δ-linked ornithine turn units. Fmoc-Hao-OH[2] was used to introduce the unnatural amino acid Hao.[4] Standard Fmoc-protected amino acids were used to introduce the other residues: Fmoc-Ala-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-N(Me)-Phe-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Tyr(OtBu)-OH, Fmoc-Val-OH. In the synthesis of macrocyclic peptide **4**, Fmoc-N(Me)-Phe-OH was introduced using normal coupling times and conditions (20 min coupling with HCTU) and the subsequent amino acid, Fmoc-Phe-OH, was introduced by double coupling with HOAT (4 equiv.), HATU (4 equiv.), and 1 hour coupling times using automated solid phase peptide synthesis.

*Synthesis of linear peptide **5**.*

A 10 mL Bio-Rad Poly-Prep chromatography column was charged with Rink amide resin (300 mg, 0.73 mmol/g loading, 0.22 mmol) and ca. 6 mL of CH_2Cl_2. After 30 min, the solvent was drained and the resin was washed with ca. 3 mL of DMF. The resin was then submitted to cycles of standard Fmoc-based solid phase peptide synthesis on a PS3 automated peptide synthesizer (Protein Technologies, Inc.) using Fmoc-protected amino acid building blocks (4

equiv, with HCTU as coupling agent and 2,4,6-collidine as base). The final step of the synthesis of the protected peptide involved acetylating the amino-terminus by treating the resin with 2 mL of acetic anhydride on the PS3 synthesizer using 2,4,6-collidine as base. The resin was stirred with 10 mL of trifluoroacetic acid/triisopropylsilane/water (38:1:1 v/v) for 4 hours under nitrogen. The solution was concentrated under vacuum. The residue was dissolved in ca. 5 mL of water/acetonitrile (1:1), and centrifuged for 5 min at 14,000 rpm. The liquid portion was decanted from the supernatant, then filtered through a 0.45 micron filter, and purified by RP-HPLC on a C18 column (elution with acetonitrile and water containing 0.1% TFA, linear gradient from 20-35% acetonitrile over 38 min). The pure fractions were lyophilized to yield 3.1 mg of acyclic control peptide **5** (1% yield based on resin loading): ESI-MS m/z for $C_{64}H_{102}N_{16}O_{17}$ $[M + 2H]^{2+}$ calcd 683.37, found 683.34.

*NMR sample preparation, data collection, and data processing of peptides **1–5**.*

^{1}H NMR experiments of macrocyclic β-sheet peptides **1–5** were performed in D_2O at 500 MHz and 600 MHz at varying temperatures. Solutions of the peptides were prepared gravimetrically by dissolving an appropriate weight of each peptide in an appropriate volume of solvent. In calculating molecular weights, all amino groups were assumed to be protonated as the TFA salts. The HOD peak was used as a reference after the HOD peak was calibrated based on temperature.[5] All macrocyclic β-sheet peptides were allowed to stand for 6 hours to 36 hours to allow full hydrogen-deuterium exchange of the amide and ammonium hydrogens. 2D TOCSY, 2D COSY, 2D ROESY and 2D NOESY spectra were collected with 2048 data points in the f_2 domain and 512 data points in the f_1 domain. 2D TOCSY, 2D COSY and 2D ROESY data were processed to a 1024 x 1024 real matrix with a Qsine weighting function and with forward linear

prediction in the f_1 domain. 2D NOESY data were processed to a 1024 x 1024 real matrix with a Qsinc weighting function, a forward linear prediction in the f_1 domain, and with the parameter GB set at 0.05 in the f_2 and f_1 domain. The data were processed with the Bruker XwinNMR software.

In order to observe amide resonances for further resonance assignments, ^{1}H NMR studies of macrocyclic β-sheet peptide **2a** were also performed in a H_2O/D_2O mixture (9:1) at 800 MHz and 298 K using WATERGATE. 2D TOCSY and 2D NOESY data for **2a** in the H_2O/D_2O mixture (9:1) were collected with 4096 data points in the f_2 domain and 512 data points in the f_1 domain. The collected data were processed with zero filling to a final matrix of 4096 x 1024 real points with a Qsine weighting function and with a forward linear prediction in the f_1 domain. The data were processed with the Bruker XwinNMR software.

*Modeling of the solution-state tetramer of macrocyclic β-sheet peptide **2a**.*

We used the X-ray crystallographic structure of the tetramer of macrocyclic β-sheet **1** to create a model of the solution-state tetramer of macrocyclic β-sheet **2a**. We generated the initial coordinates for the model in PyMOL by (1) changing the *p*-bromophenylalanine of **1** to tyrosine, (2) shifting the crystallographic dimers out of alignment by two residues toward the C-termini, and (3) moving the dimers so that they packed through the LFA faces, instead of the VF faces. The rotamer of F_{20} with χ_1=180° was then selected to avoid inter-chain steric clashes, and the shifted dimer layers were oriented to approximately match the observed interlayer NOEs between the methoxy group of Hao_2 and the methyl group of threonine.

The resulting initial structure was then imported into Maestro and a minimum-energy structure was generated by applying distance constraints to match observed NOEs as follows:

Using MacroModel with the Maestro user interface, NOE constraints to match the observed intra- and intermolecular NOEs illustrated in Figures 3, 4, 6, and 7 were applied. Distance constraints of 2.2 to 2.8 Å were applied for the following intramolecular NOEs: $K_{16}\alpha$–Yα, $F_{20}\alpha$–Kα, $V_{18}\alpha$–Hao_1H_6, and $E_{22}\alpha$–Hao_2H_6 (16 constraints total within the tetramer). Distance constraints of 2.2 to 3.6 Å were applied for the following intermolecular intralayer NOEs: $L_{17}\alpha$–$D_{23}\alpha$ and $F_{19}\alpha$–$A_{21}\alpha$ (8 constraints total within the tetramer). Distance constraints of 2.2 to 5.0 Å were applied for the following intermolecular interlayer NOEs: Hao_2OMe–$ThrCH_3$, Hao_1H_4–Hao_2H_4, Hao_1H_4–Hao_2H_3, and Hao_1H_3–Hao_2-H_4 (16 constraints total between the layers of the tetramer). The structure was minimized with these constraints using the MMFFs force field with GB/SA water solvation. The NOE constraints were removed, and minimization was repeated using the MMFFs force field with GB/SA water solvation to generate a minimum-energy structure (local minimum).

This minimum-energy was then imported into PyMOL, and PyMOL was used to generate the images in Figures 10, 11B, and 12B.[6] A β-strand of nine glycine residues (G9) was used to generate a cartoon of the template strand Hao-K-Hao-Y-T. Specifically, the pdb coordinates from each unnatural amino acid Hao were used to generate tri-glycine segments, and the pdb coordinates of the threonine, tyrosine, and lysine residues were also used to generate three glycine residues of the G9 β-strand.

*Diffusion-ordered spectroscopy (DOSY) experiments of macrocyclic β-sheet peptides **1–4**.*

The diffusion coefficients of macrocyclic β-sheet peptides **1**-**4** were determined by DOSY experiments on a Bruker Avance 600 MHz spectrometer in D_2O at 298 K. The experiments comprised a series of 16 pulsed field gradient spin-echo experiments in which the

gradient strength was incremented to allow ca. 5–95% signal attenuation with a linear ramp. A 75-ms diffusion delay was used. Diffusion gradient lengths of 1.75 – 3.0 ms were selected to achieve appropriate attenuation of each macrocyclic β-sheet peptide. Data were processed to give a pseudo-2D spectrum. The residual HOD peak in D_2O was set as a reference (19.0×10^{-10} m^2/s at 298 K).[7]

*Analytical ultracentrifugation studies of macrocyclic β-sheet peptide **2b**.*

Analytical ultracentrifugation (AUC) sedimentation velocity (SV) studies were performed on macrocyclic β-sheet **2b** to further elucidate its self-association behavior. Solutions of **2b** were prepared gravimetrically as 0.10, 0.30, and 0.60 mM and determined spectrophotometrically to be 98.7, 304.0, and 657.6 μM based on a molar extinction coefficient of 2897 $M^{-1}cm^{-1}$ at 344 nm and a molar extinction coefficient of 22,260 $M^{-1}cm^{-1}$ at 280 nm,[a,8] Sedimentation experiments were performed with a Beckman Optima XL-I at the Center for Analytical Ultracentrifugation of Macromolecular Assemblies at the University of Texas Health Science Center at San Antonio. All measurements were made in intensity mode, measuring at 344 nm in 25 mM aqueous NaCl. The experimental data were collected at 20°C, and at 60,000 rpm, using 1.2 cm titanium 2-channel centerpieces for the 98.7 μM and 304.0 μM samples, and a 3 mm titanium 2-channel centerpiece for the 657.6 μM sample. Hydrodynamic corrections for buffer density, viscosity and partial specific volume (0.7179 ml/g for **2b**)[b] were made according to methods outlined in Laue et al.[9] and as implemented in UltraScan.[10]

The experimental data from SV experiments were analyzed with UltraScan v. 9.9 [10,11]

[a] The extinction coefficient of **2b** was calculated to be 22,260 $M^{-1}cm^{-1}$ from the extinction coefficient of Hao (9850 $M^{-1}cm^{-1}$) and Tyr (1280 $M^{-1}cm^{-1}$) at 280 nm.
[b] The partial specific volume of the Hao subunit was determined to be 0.65 cm^3/g as described previously.[8] The molar mass of the Hao subunit is 235.12 g.

and modeled with solutions of the Lamm equation.[12,13] Optimization was performed by 2-dimensional spectrum analysis (2DSA)[14] with simultaneous removal of time- and radially-invariant noise contributions.[15] 2DSA solutions are subjected to parsimonious regularization by genetic algorithm analysis,[16] and are further refined using Monte Carlo analysis to determine confidence limits for the determined parameters.[17] The calculations are computationally intensive and are carried out on high-performance computing platforms.[18] All calculations were performed on the Lonestar and Ranger clusters at the Texas Advanced Computing Center at the University of Texas at Austin, and on the Jacinto cluster at the Bioinformatics Core Facility at the University of Texas Health Science Center at San Antonio.

A comparison of sedimentation profiles measured at 344 nm from multiple loading concentrations (98.7 μM, 304.0 μM, and 657.6 μM) indicated a mass-action driven shift in the sedimentation profile, suggesting the presence of a reversible reaction (Figure S8). Genetic algorithm – Monte Carlo fitting of the individual concentrations suggested the presence of monomer and tetramer species at different ratios, depending on concentration. From these ratios, we estimated K_{assoc} = 1.93 x 10^{14}, 5.66 x 10^{15}, and 8.89 x 10^{14} M^{-3} for the respective experiments.[c] Detailed hydrodynamic fitting results for these fits are shown in Table S4. Based on these results, we fitted the lowest concentration — which displayed the largest signal for the monomer — to a reversible self-associating model for a monomer-tetramer equilibrium using a 200-iteration genetic algorithm-Monte Carlo analysis.[13,19] This treatment resulted in a very good fit to the monomer-tetramer model — with random residuals (Figure S9) — and gave K_{assoc} = 1.93 x 10^{14} M^{-3}.

[c] A K_{assoc} of 1.93 x 10^{14} M^{-3} corresponds to a 1:1 molar ratio of monomer and tetramer at 0.086 mM total concentration of **2b** and a 4:1 molar ratio of monomer and tetramer at 0.022 mM total concentration of **2b**.

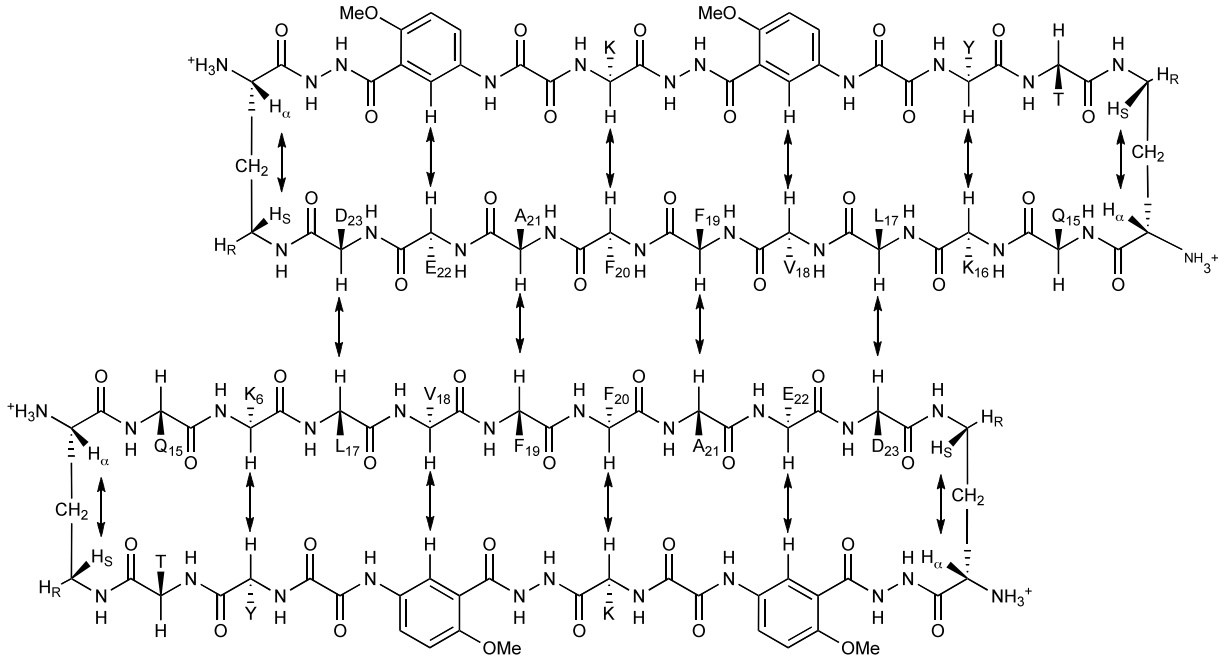


Figure S1a. Key NOEs associated with folding and dimerization of macrocyclic β-sheet peptide **2a**. Interstrand main chain-main chain NOEs were observed for **2a** in the NOESY 800 MHz spectrum with WATERGATE (8.0 mM in H_2O-D_2O (9:1) and 298 K).

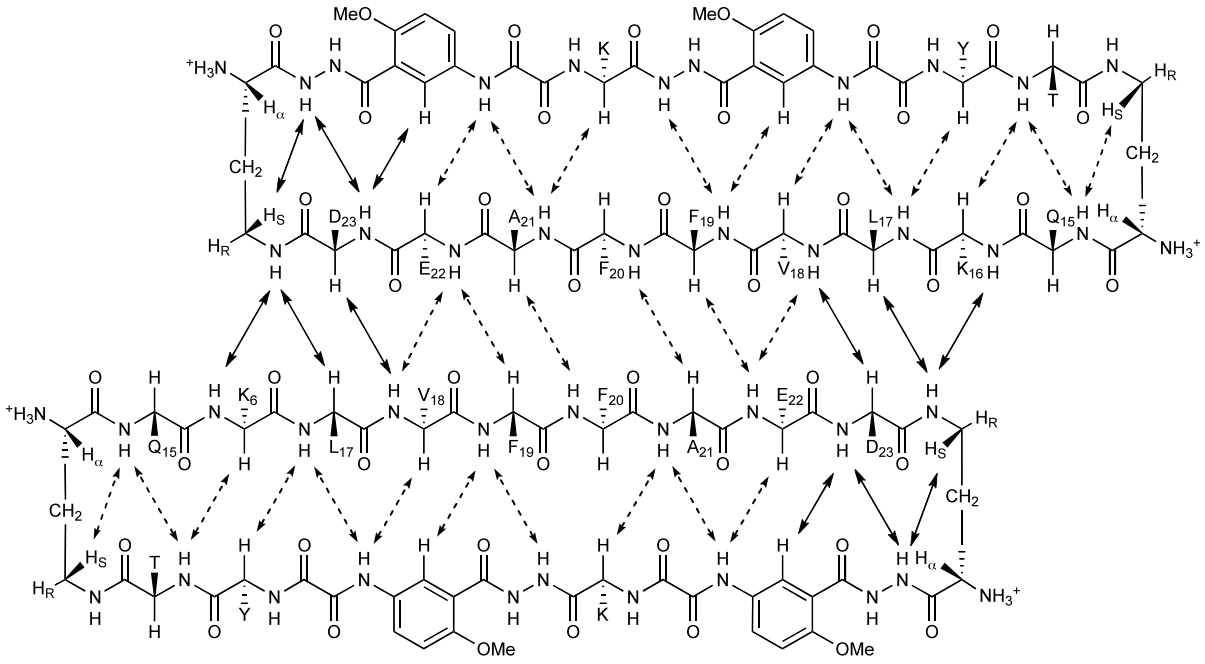


Figure S1b. Key NOEs associated with folding and dimerization of macrocyclic β-sheet peptide **2a**. Interstrand main chain-main chain NOEs were observed for **2a** in the NOESY 800 MHz spectrum with WATERGATE (8.0 mM in H_2O-D_2O (9:1) and 298 K). Dashed lines represent weak or ambiguous NOEs.

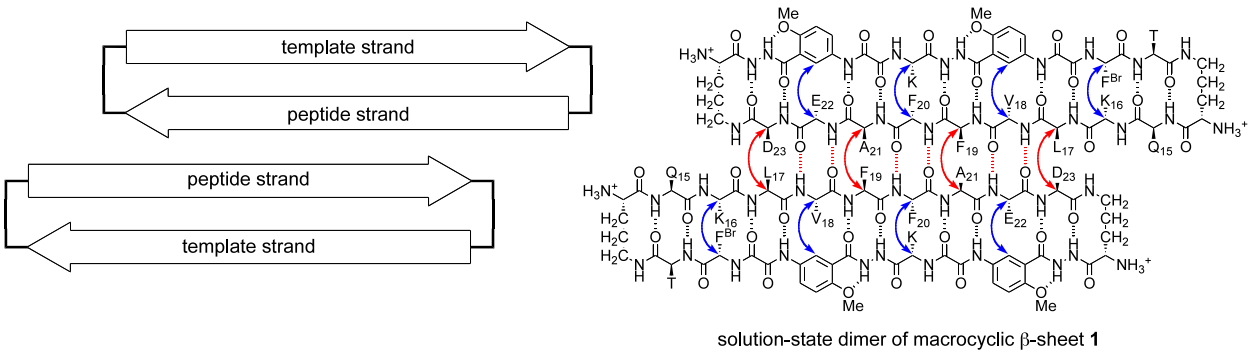


Figure S2a. Cartoon and chemical structure illustrating the hydrogen-bonded dimer formed by macrocyclic sheet β-peptide **1** in solution. Key NOEs associated with solution-state dimerization and folding of **1** are shown with red and blue arrows.

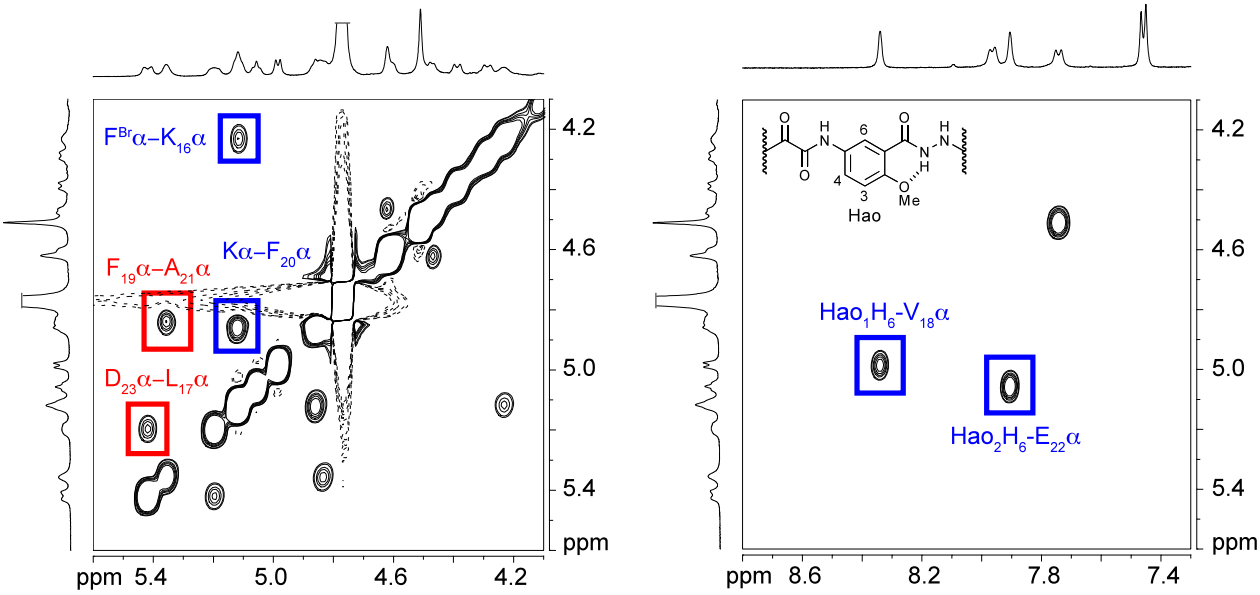


Figure S2b. Selected expansions of the NOESY spectrum of macrocyclic β-sheet peptide **1** at 2.0 mM in D_2O at 500 MHz and 298 K. Key intermolecular interstrand NOEs associated with dimerization are highlighted in red; key intramolecular interstrand NOEs associated with folding are highlighted in blue.

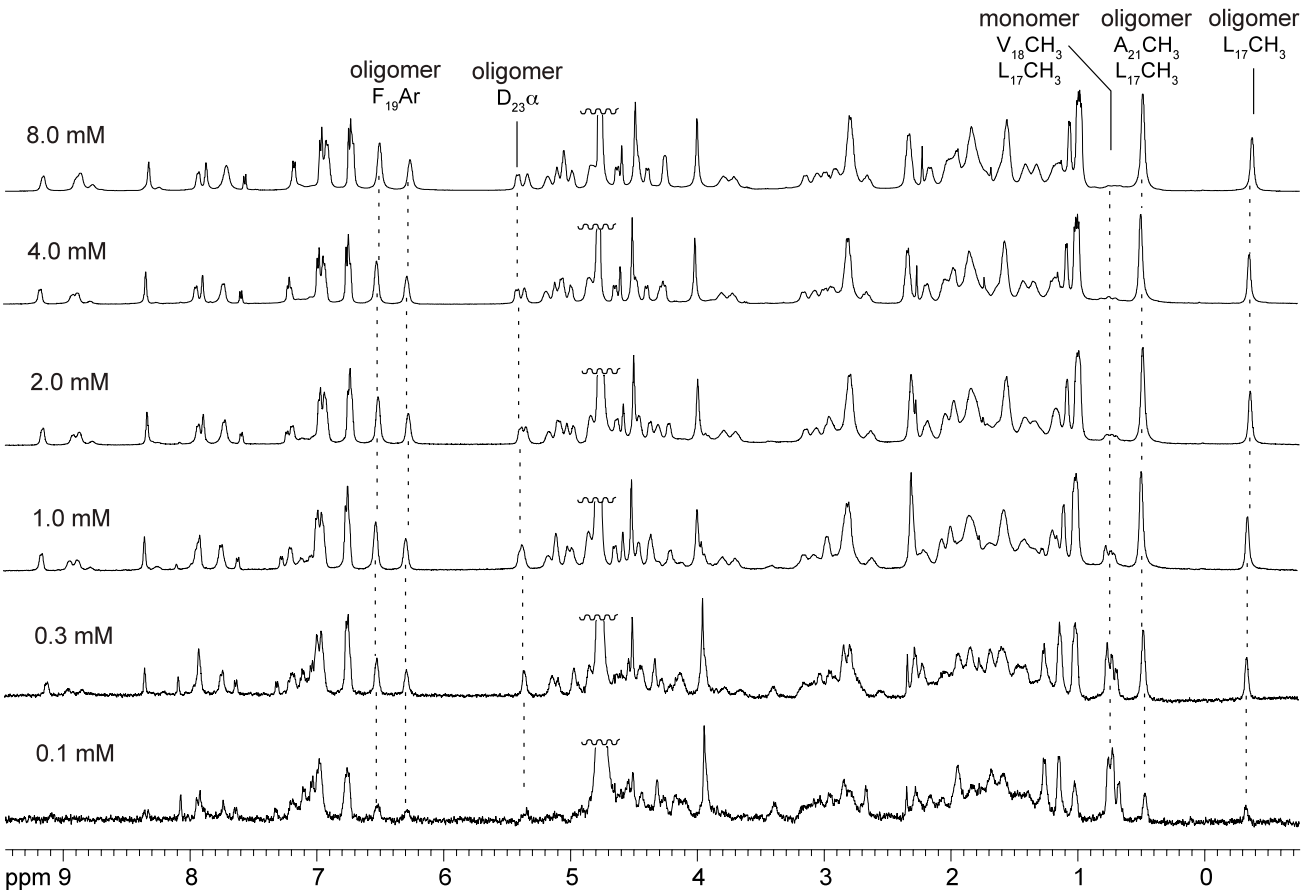


Figure S3. ^{1}H NMR spectra of macrocyclic β-sheet peptide **2a** at various concentrations in D_2O at 500 MHz and 298 K. Noteworthy characteristic resonances of the monomer and the oligomer are labeled and highlighted with dashed lines.

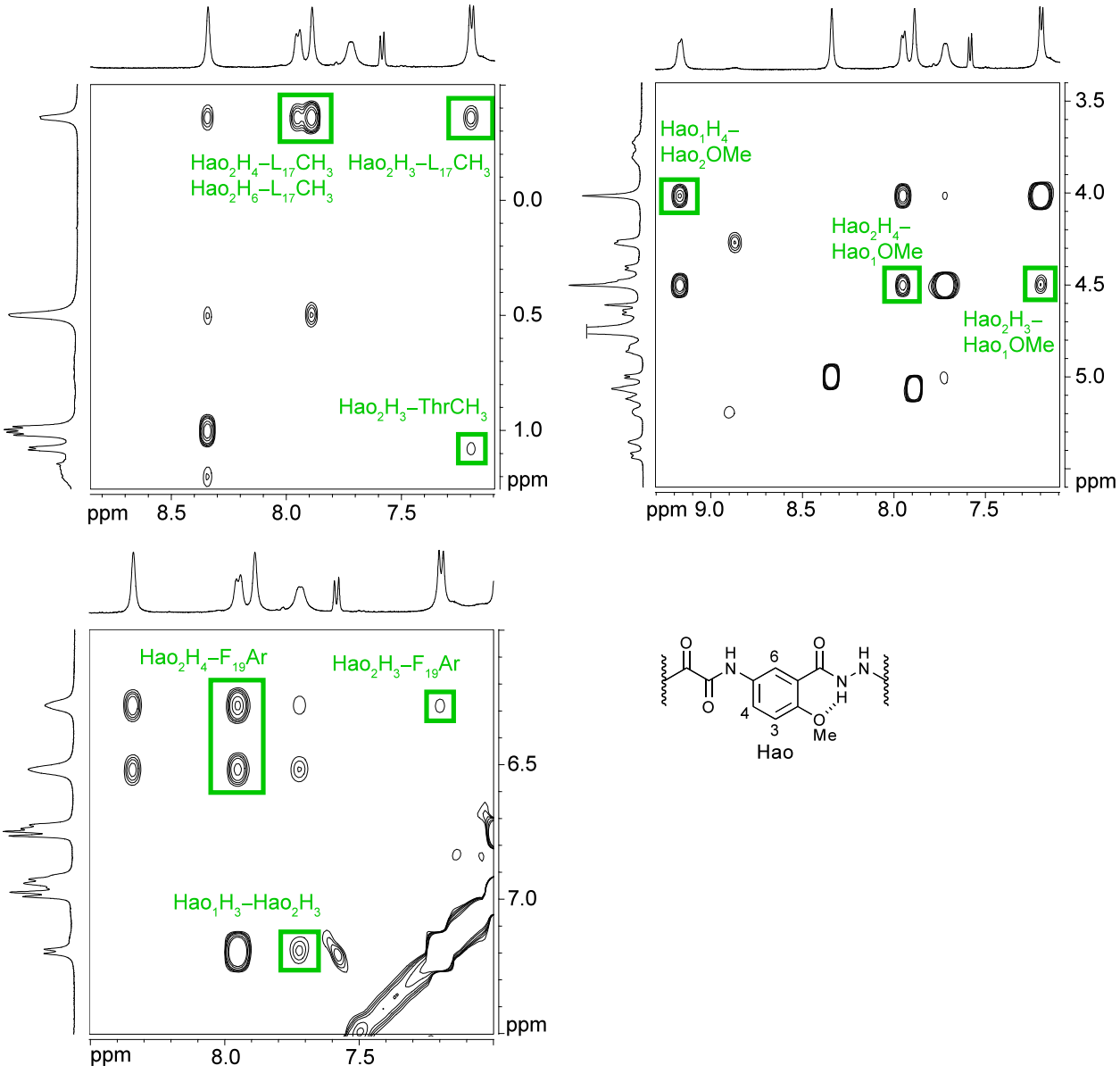


Figure S4. Selected expansions of the NOESY spectrum of macrocyclic β-sheet peptide **2a** at 8.0 mM in D_2O at 500 MHz and 300.5 K. Key interlayer NOEs associated with tetramerization are highlighted in green.

Table S1. Key NOEs associated with interlayer contacts in tetramer formation of 2a.[a]

	Hao_2H_3	Hao_2H_4	Hao_2H_6	Hao_2OMe
$F_{19}Ar$	weak	strong	-	-
$L_{17}\delta$	medium	medium	strong	-
T γ	weak	-	-	medium
Hao_1H_3	weak	medium	-	-
Hao_1H_4	strong	strong	-	strong
Hao_1OMe	weak	strong	-	-

[a]Interlayer contacts are observed in Figure 6 and Figure S4.

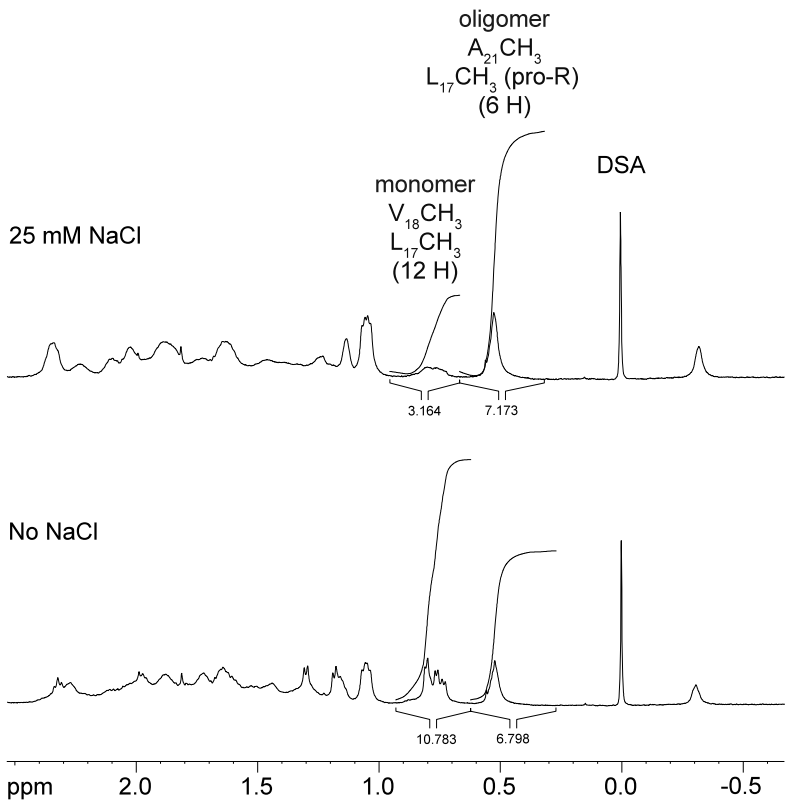


Figure S5. Expansions of the ^{1}H NMR spectra of macrocyclic β-sheet peptide **2a** at 0.3 mM in D_2O at 500 MHz and 298 K with 0 mM and 25 mM NaCl. (DSA = 4,4-dimethyl-4-silapentane-1-ammonium trifluoroacetate.)

Table S2. Percentage of monomer and tetramer of 2a at 0.3 mM with 0 and 25 mM NaCl (based on the relative integrals for selected ^{1}H NMR resonances).

[NaCl] (mM)	$L_{17}CH_3$, $V_{18}CH_3$ (as monomer)	$L_{17}CH_3$ (*pro*-R), $A_{21}CH_3$ (as oligomer)	% Monomer	% Tetramer
0	0.90/H	1.13/H	44.2	55.8
25	0.26/H	1.20/H	18.1	81.9

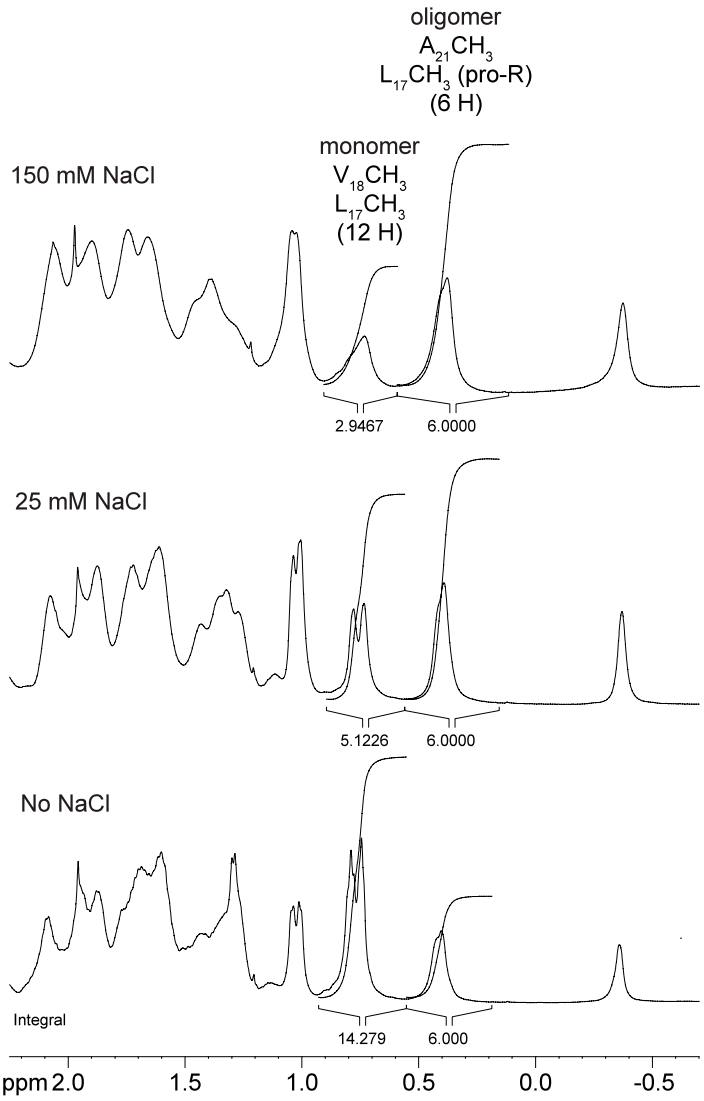


Figure S6. Expansions of the ^{1}H NMR spectra of macrocyclic β-sheet peptide **3** at 4.0 mM in D_2O at 500 MHz and 298 K with 0 mM, 25 mM NaCl, and 150 mM NaCl.

Table S3. Percentage of monomer and tetramer of 3 at 4.0 mM with 0, 25, and 150 mM NaCl (based on the relative integrals for selected ^{1}H NMR resonances).

[NaCl] (mM)	$L_{17}CH_3$, $V_{18}CH_3$ (as monomer)	$L_{17}CH_3$ (*pro*-R), $A_{21}CH_3$ (as oligomer)	% Monomer	% Tetramer
0	1.19/H	1.00/H	54.3	45.7
25	0.43/H	1.00/H	29.9	70.1
150	0.25/H	1.00/H	19.7	80.3

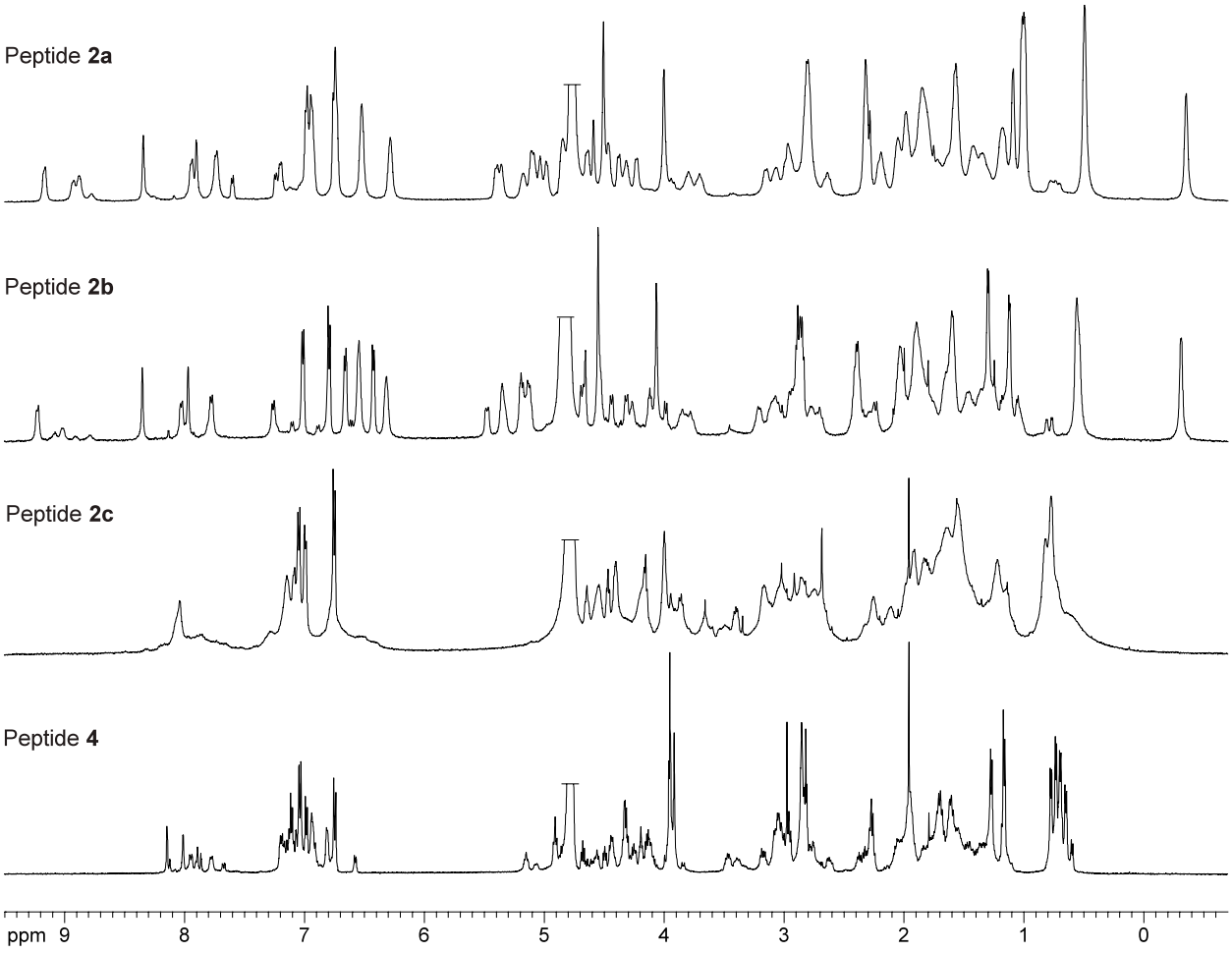


Figure S7. ^{1}H NMR spectra of macrocyclic β-sheet peptides at 2.0 mM at 298 K in D_2O at 500 MHz: **2a** (tetramer predominates), **2b** (tetramer predominates), **2c** (monomer predominates), and **4** (monomer predominates). The spectrum of **4** shows multiple sets of resonances, which are associated with amide-bond rotamers.

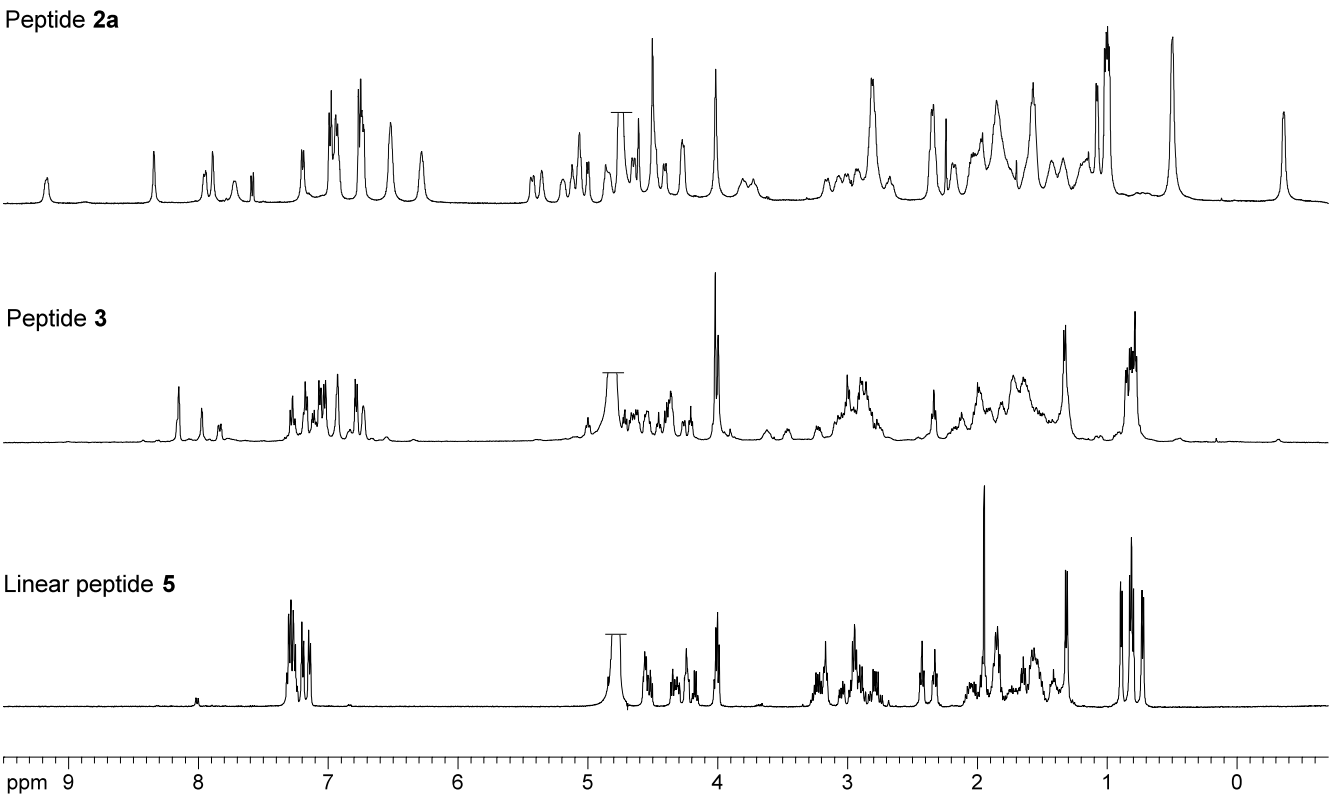


Figure S8. ^{1}H NMR spectra of macrocyclic β-sheet peptide **2a** at 8.0 mM at 300.5 K in D_2O at 500 MHz (tetramer predominates), macrocyclic β-sheet peptide **3** at 2.0 mM at 298 K in D_2O at 500 MHz (monomer predominates), and linear peptide **5** at 1.2 mM at 298 K in D_2O at 500 MHz (monomer).

Table S4. ^{1}H NMR chemical shifts of the α-protons of the 2a tetramer, the 3 monomer, and linear peptide 5.

Residue	Peptide **2a** δ (ppm)	Peptide **3** δ (ppm)	Peptide **5** δ (ppm)	Δδ (ppm) **2a - 5**	Δδ (ppm) **3 - 5**
Q_{15}	4.67	4.49	4.24	0.43	0.25
K_{16}	4.29	4.31	4.25	0.04	0.06
L_{17}	5.22	4.51	4.31	0.91	0.20
V_{18}	5.03	4.35	3.99	1.04	0.36
F_{19}	5.38	4.67	4.55[a]	0.85[a]	0.14[a]
F_{20}	4.88	4.57	4.51[a]	0.35[a]	0.04[a]
A_{21}	4.86	4.32	4.17	0.69	0.15
E_{22}	5.10	4.32	4.35	0.75	-0.03
D_{23}	5.45	4.59	4.59	0.86	0.00

[a] assignment of F_{19} and F_{20} of peptide **5** arbitrary. The average δ for F_{19} and F_{20} of peptide **5** was used in calculating Δδ.

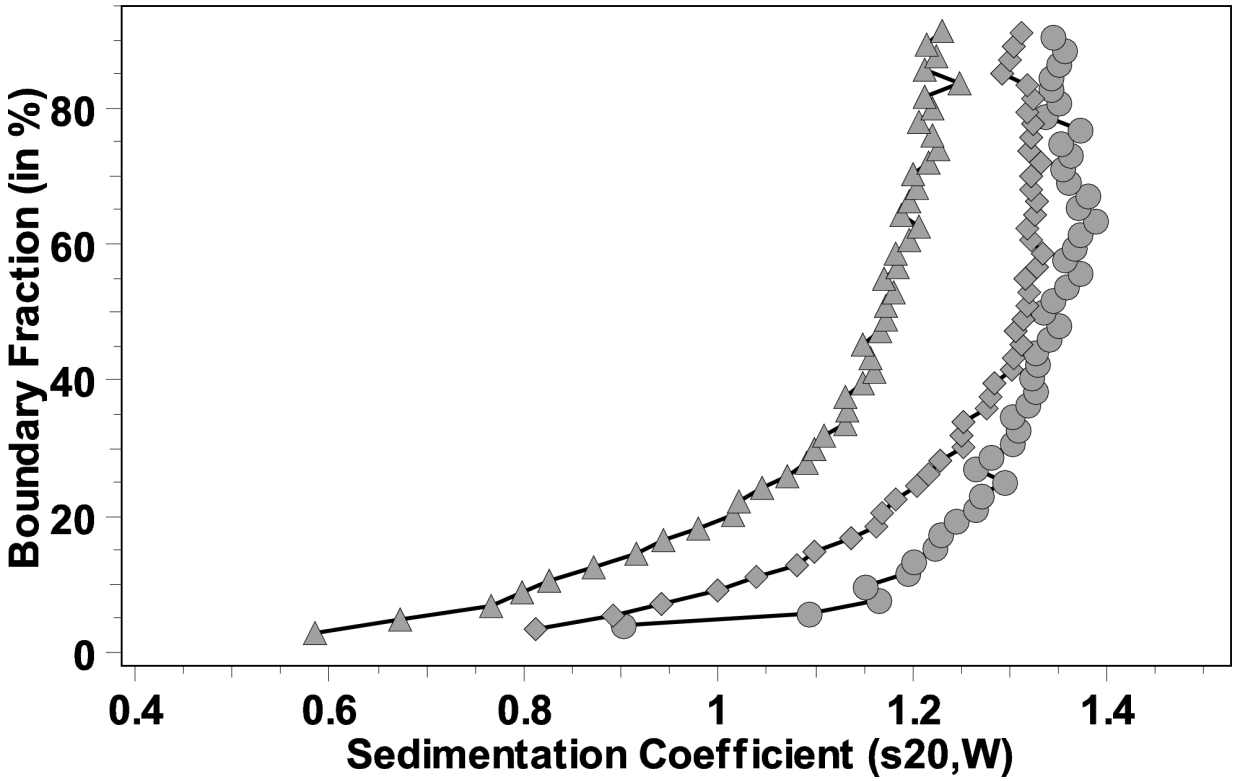


Figure S9. Sedimentation coefficient distributions of macrocyclic β-sheet peptide **2b** for loading concentrations at 98.7 μM (triangles), 304.0 μM (squares) and 657.6 μM (circles) obtained from sedimentation velocity experiments performed at 344 nm. The increase in sedimentation coefficient as a function of increase in loading concentration suggests the presence of a mass-action driven self-association reaction.

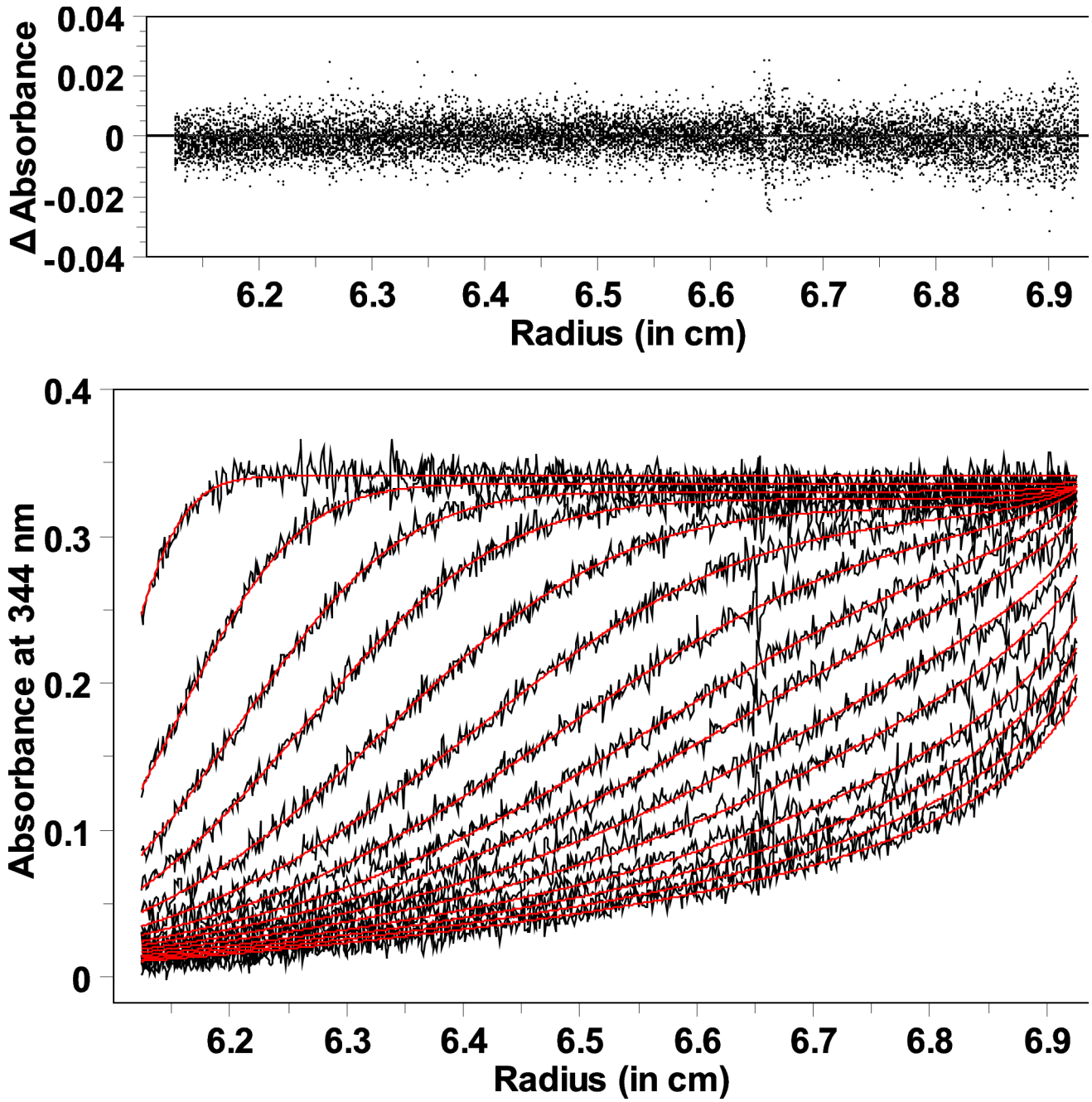


Figure S10. Sedimentation velocity data (black lines, lower plot) for macrocyclic β-sheet peptide **2b** (98.7 μM) when fitted with a reversible model for a monomer-tetramer equilibrium[13,19] using genetic algorithm-Monte Carlo analysis (red lines). Residuals are random, and shown in the upper portion of this plot. For clarity, only every fifth scan in this experiment is shown.

Table S5. Hydrodynamic measurements for macrocyclic β-sheet peptide **2b**.[a]

	Monomer (98.7 μM)	**Tetramer (98.7 μM)**	**Monomer (304.0 μM)**	**Tetramer (304.0 μM)**	**Monomer (657.6 μM)**	**Tetramer (657.6 μM)**
Partial concentration (% of total OD)	31.06%	68.94%	12.02%	87.98%	2.73%	97.27%
Molecular weight (Da)	2752.8	8946.4	3316.4	8947.7	2548.6	8507.2
Sedimentation Coefficient (x 10^{13} s)	0.60	1.30	0.59	1.27	0.65	1.25
Diffusion Coefficient (x 10^{7} cm^2/s)	18.75	12.50	15.22	14.33	21.94	15.60
Anisotropy (f/f_0)	1.24	1.26	1.44	1.24	1.09	1.21
K_{assoc} (M^{-3})	1.93 x 10^{14}		5.66 x 10^{15}		8.89 x 10^{14}	

[a]Hydrodynamic measurements for macrocyclic β-sheet **2b** from a genetic algorithm-Monte Carlo fit to a non-interacting model with 50 iterations, fitting each loading concentration individually.[12] Only two species were detected in each sample, corresponding in molecular weight to the monomer and tetramer of **2b**. The shift in partial concentration is consistent with a reversible self-association model for a monomer-tetramer equilibrium. K_{assoc} is estimated based on the relative amounts of monomer and tetramer determined in the genetic algorithm-Monte Carlo analysis, but due to the low amount of monomer in the two higher concentrations, the K_{assoc} from the lowest concentration is considered to be the most reliable.

Table S6. Hydrodynamic measurements for macrocyclic β-sheet peptide **2b** at 98.7 μM from a genetic algorithm-Monte Carlo fit to a reversible monomer-tetramer model.[a]

	Monomer	**Tetramer**
Molecular weight (Da)	2137.0 (2116.5, 2157.6)	8548.0 (8466.0, 8630.4)
Sedimentation Coefficient (x 10^{13} s)	0.514 (0.511, 0.518)	1.223 (1.220, 1.225)
Diffusion Coefficient (x 10^{7} cm^2/s)	21.27 (21.17, 21.36)	12.64 (12.54, 12.74)
Anisotropy (f/f_0)	1.18 (1.17, 1.19)	1.25 (1.24, 1.26)
K_{assoc} (M^{-3})	1.93 (1.80, 2.05) x 10^{14}	

[a]Hydrodynamic measurements for macrocyclic β-sheet **2b** from a genetic algorithm-Monte Carlo fit to a reversible self-association model for a monomer-tetramer with 200 iterations.[19] The K_{assoc} observed in this fit matched well the K_{assoc} observed with the non-interacting fit. Values in parenthesis reflect the 95% confidence intervals for each parameter.

References and Notes:

1. Pham, J. D.; Chim, N.; Goulding, C. W.; Nowick, J. S. *J. Am. Chem. Soc.* **2013**, *135*, 12460−12467.
2. Cheng, P.-N.; Nowick, J. S. *J. Org. Chem.* **2011**, *76*, 3166–3173.
3. Cheng, P.-N.; Liu, C.; Zhao, M.; Eisenberg, D.; Nowick, J. S. *Nat. Chem.* **2012**, *4*, 927–933.
4. Nowick, J. S.; Chung, D. M.; Maitra, K.; Maitra, S.; Stigers, K. D.; Sun, Y. J. *J. Am. Chem. Soc.* **2000**, *122*, 7654–7661.
5. Gottlieb, H. E.; Kotllyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512–7515.
6. The PyMOL Molecular Graphics System, Schrödinger, LLC, available at www.pymol.org.
7. Longsworth, L. G. *J. Phys. Chem.* **1960**, *64*, 1914–1917.
8. Khakshoor, O.; Demeler, B.; Nowick, J. S. *J. Am. Chem. Soc.* **2007**, *129*, 5558–5569.
9. Laue, T. M.; Shah, B. D.; Ridgeway, T. M.; Pelletier, S. L. Computer-aided interpretation of analytical sedimentation data for proteins. In *Analytical Ultracentrifugation in Biochemistry and Polymer Science*; Harding, S. E., Rowe, A. J., Horton, J. C., Eds.; Cambridge, Royal Society of Chemistry: Cambridge, United Kingdom, 1992; 90–125.
10. Demeler, B. UltraScan: A Comprehensive Data Analysis Software Package for Analytical Ultracentrifugation Experiments. In *Modern Analytical Ultracentrifugation: Techniques and Methods;* Scott, D. J., Harding, S. E., Rowe, A. J., Eds.; Royal Society of Chemistry: Cambridge, United Kingdom, 2005; 210–229
11. Demeler B. UltraScan-II version 9.9, release 1528 - A comprehensive data analysis software package for analytical ultracentrifugation experiments, 2011, University of Texas Health Science Center at San Antonio, Dept. of Biochemistry. http://www.ultrascan.uthscsa.edu
12. Cao, W.; Demeler, B. *Biophys. J.* **2005**, *89*, 1589–1602.

13. Cao, W.; Demeler, B. *Biophys. J.* **2008**, *95,* 54–65.

14. Brookes, E.; Cao, W; Demeler, B. *Eur. Biophys. J.* **2010**, *39,* 405–414.

15. Schuck, P; Demeler, B. *Biophysical J.* **1999**, *76,* 2288–2296.

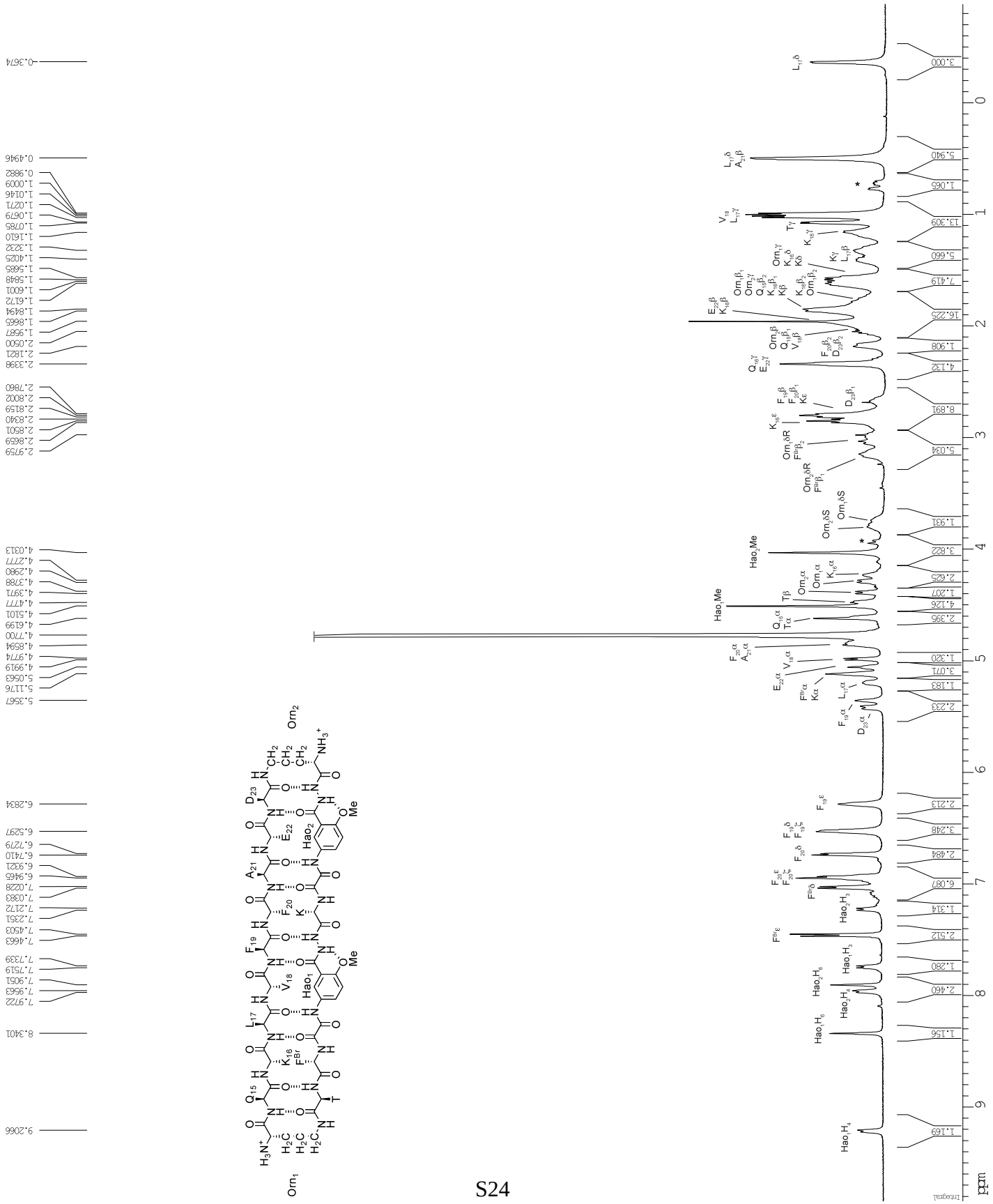
16. Brookes, E.; Demeler, B. Parsimonious Regularization using Genetic Algorithms Applied to the Analysis of Analytical Ultracentrifugation Experiments. In GECCO Proceedings, ACM 978-1-59593-697-4/07/0007, **2007**.

17. Demeler, B.; Brookes, E. *Colloid Polym. Sci.* **2008**, *286,*129–137.

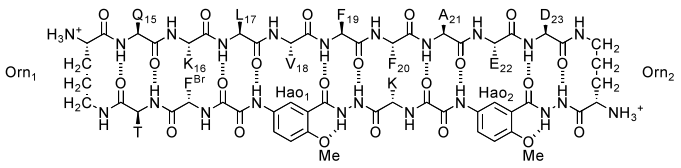
18. Brookes, E.; Demeler, B. *Colloid Polym. Sci.* **2008**, *286,* 138–148.

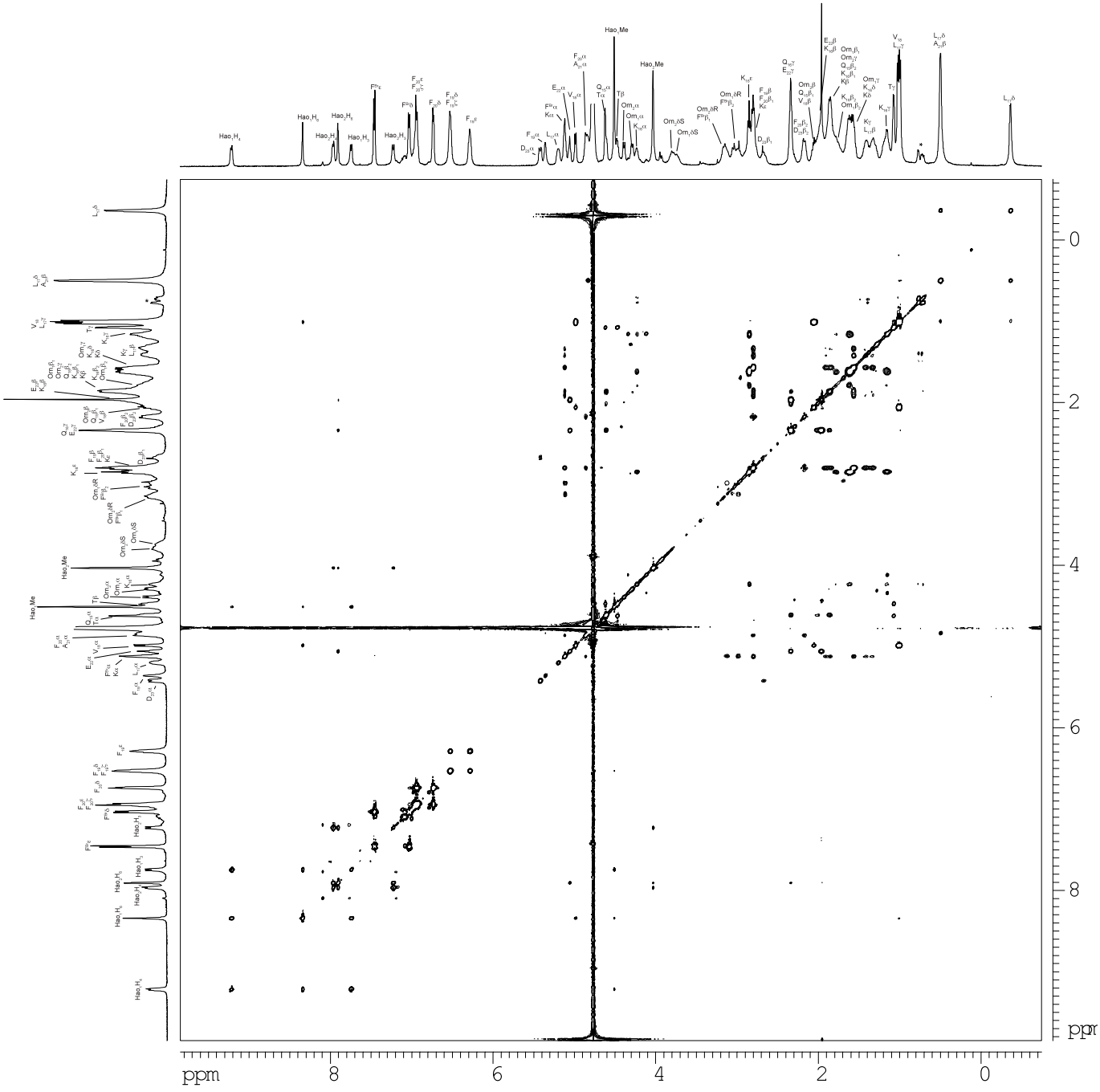
19. Demeler, B.; Brookes, E.; Wang, R.; Schirf, V.; Kim, C. A. *Macromol. Biosci.* **2010**, *10,* 775–782.

1D ^{1}H NMR spectrum of macrocyclic β-sheet **1**
2 mM in D_2O, 500 MHz, 298 K
tetramer predominates, monomer denoted with asterisk (*)

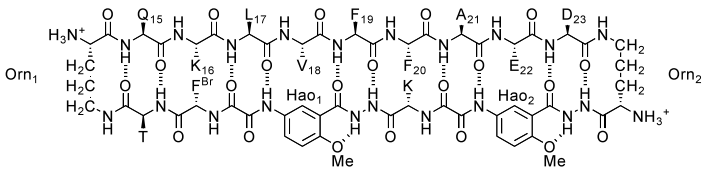


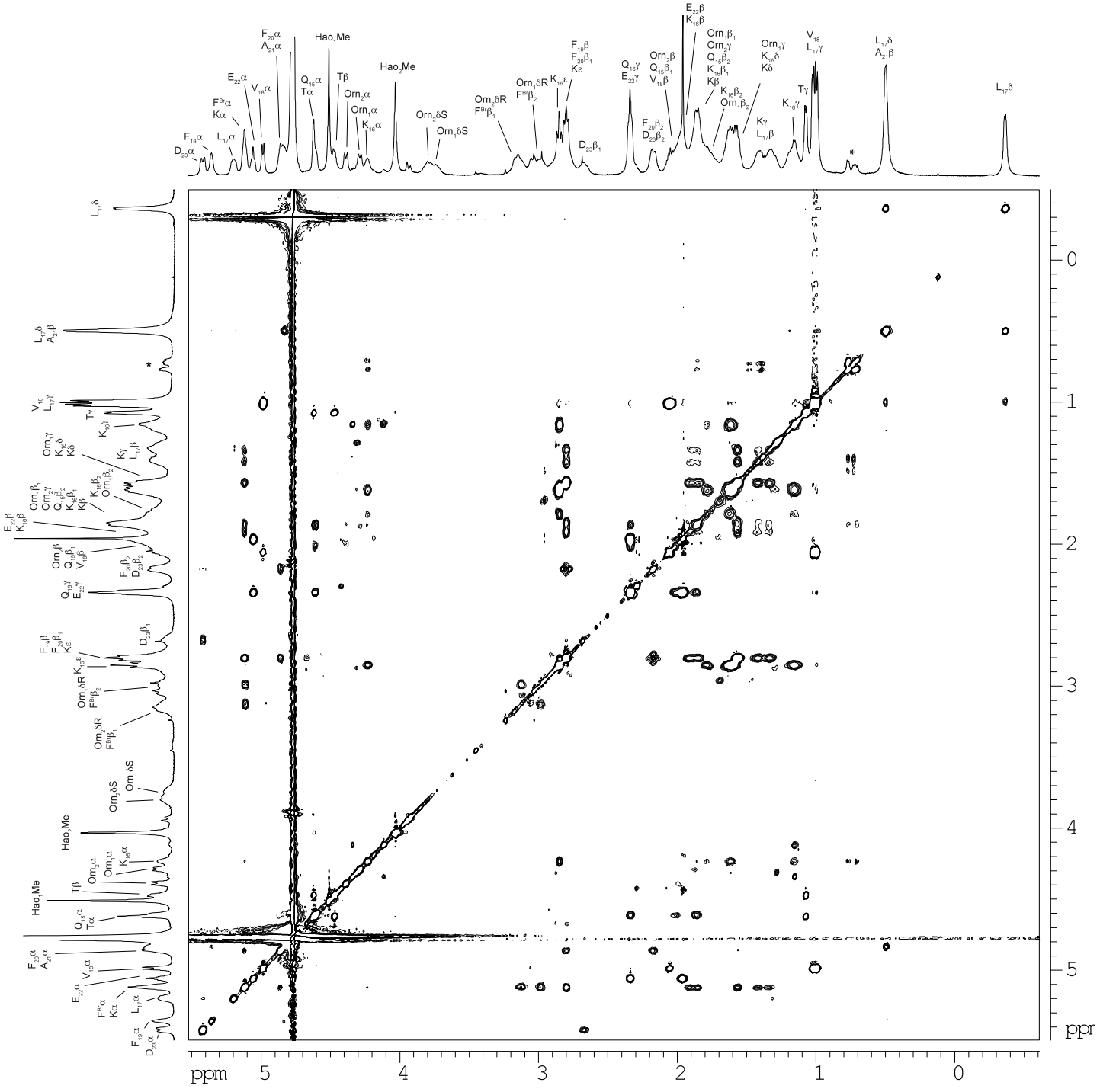
2D TOCSY spectrum of macrocyclic β-sheet **1** as tetramer
2 mM in D_2O, 500 MHz, 298 K, 150-ms spin-locking mixing time
tetramer predominates, monomer denoted by asterisk (*)



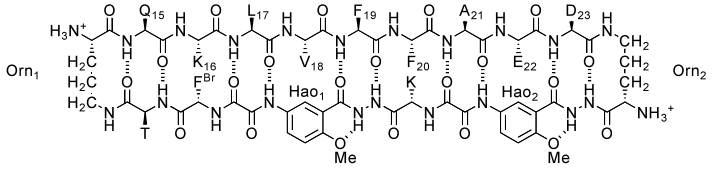


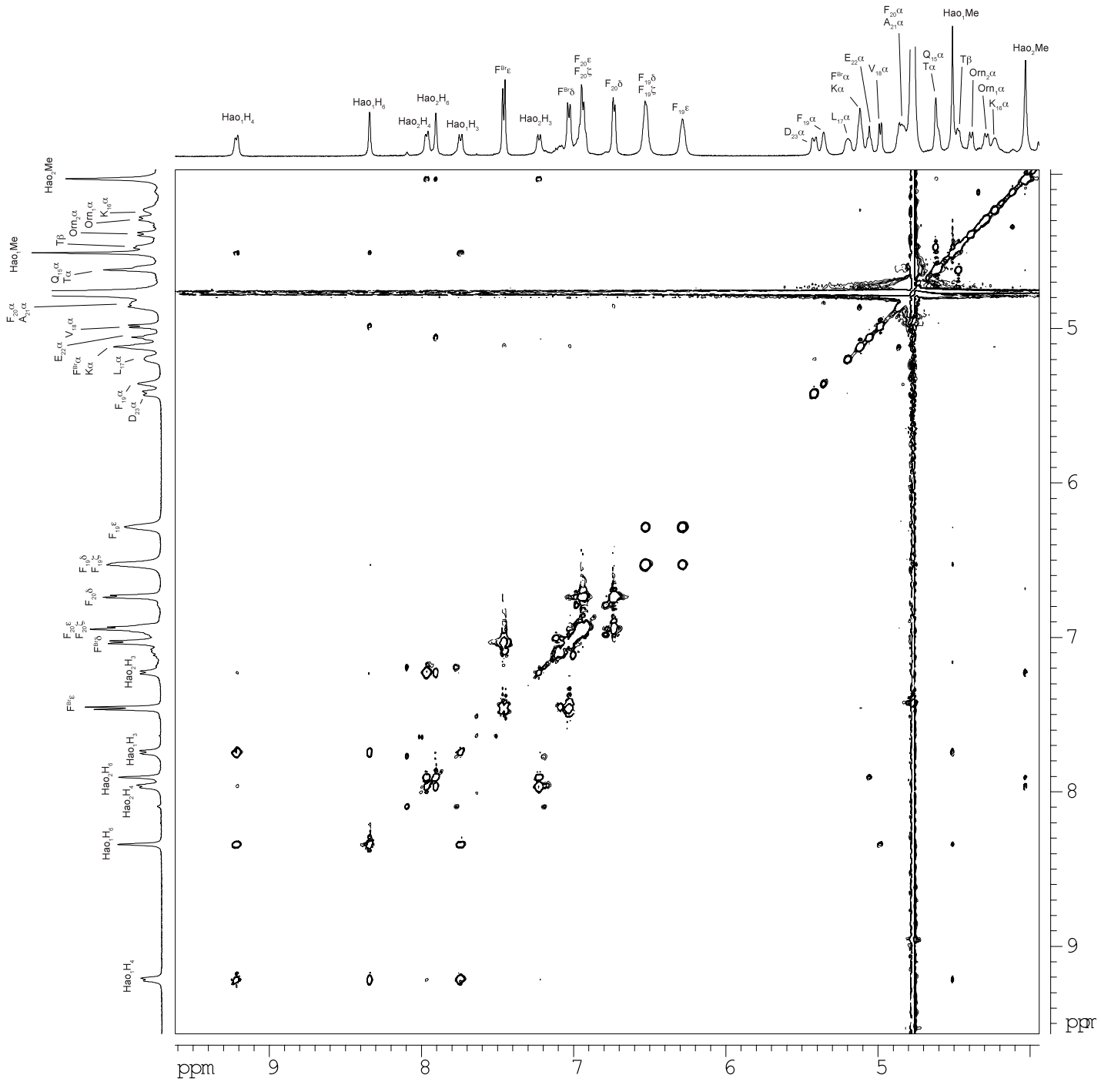
2D TOCSY spectrum of macrocyclic β-sheet **1** as tetramer
2 mM in D_2O, 500 MHz, 298 K, 150-ms spin-locking mixing time
tetramer predominates, monomer denoted by asterisk (*)



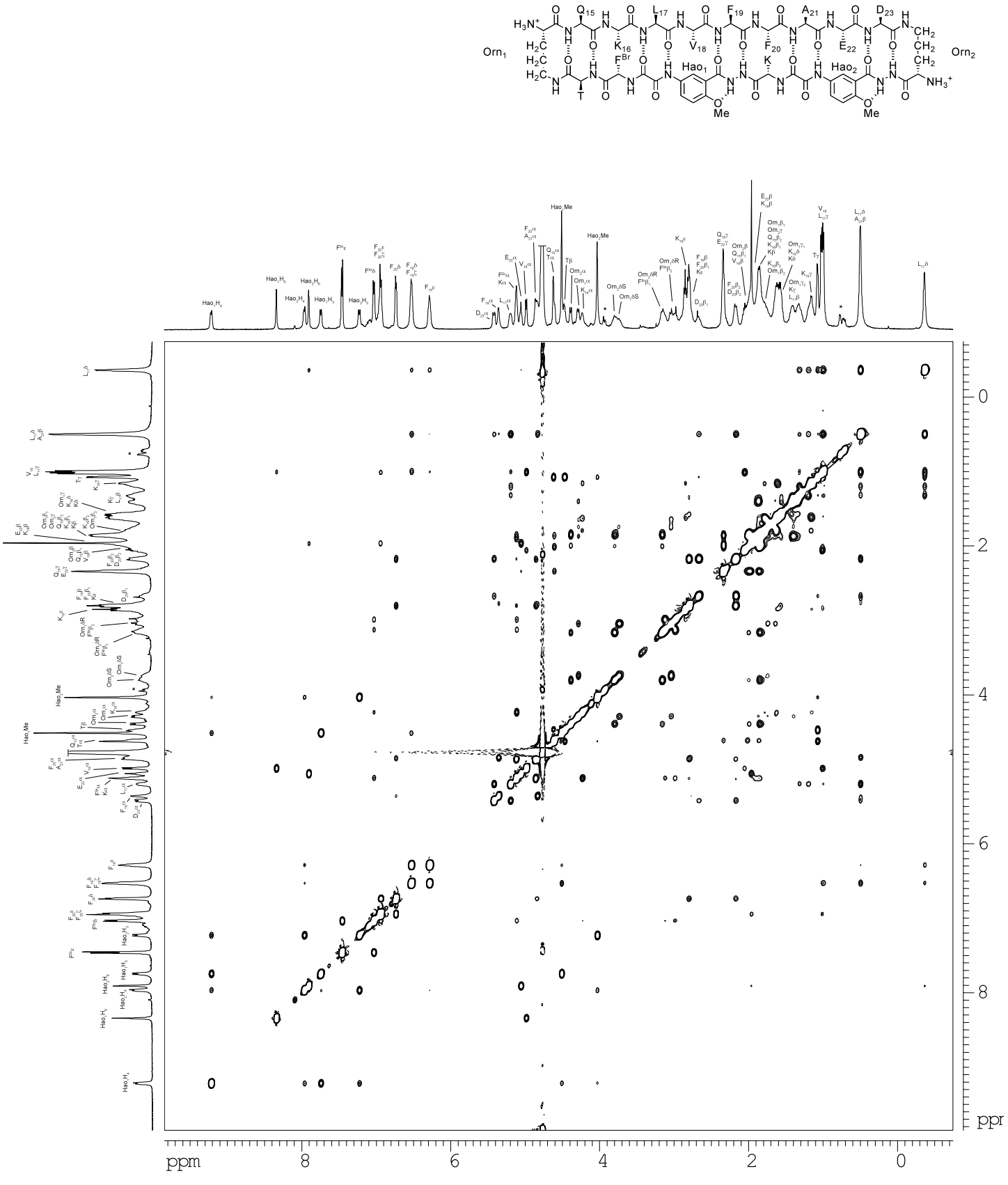


2D TOCSY spectrum of macrocyclic β-sheet **1** as tetramer
2 mM in D_2O, 500 MHz, 298 K, 150-ms spin-locking mixing time
tetramer predominates

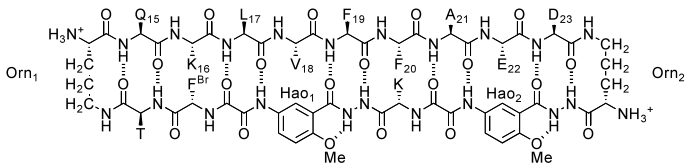


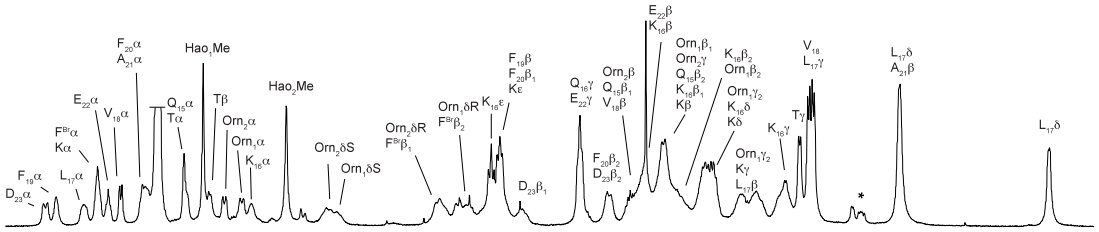


2D NOESY spectrum of macrocyclic β-sheet **1** as tetramer
2 mM in D_2O, 500 MHz, 298 K, 200-ms spin-locking mixing time
tetramer predominates, monomer denoted by asterisk (*)

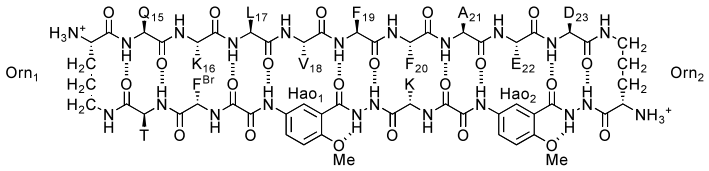


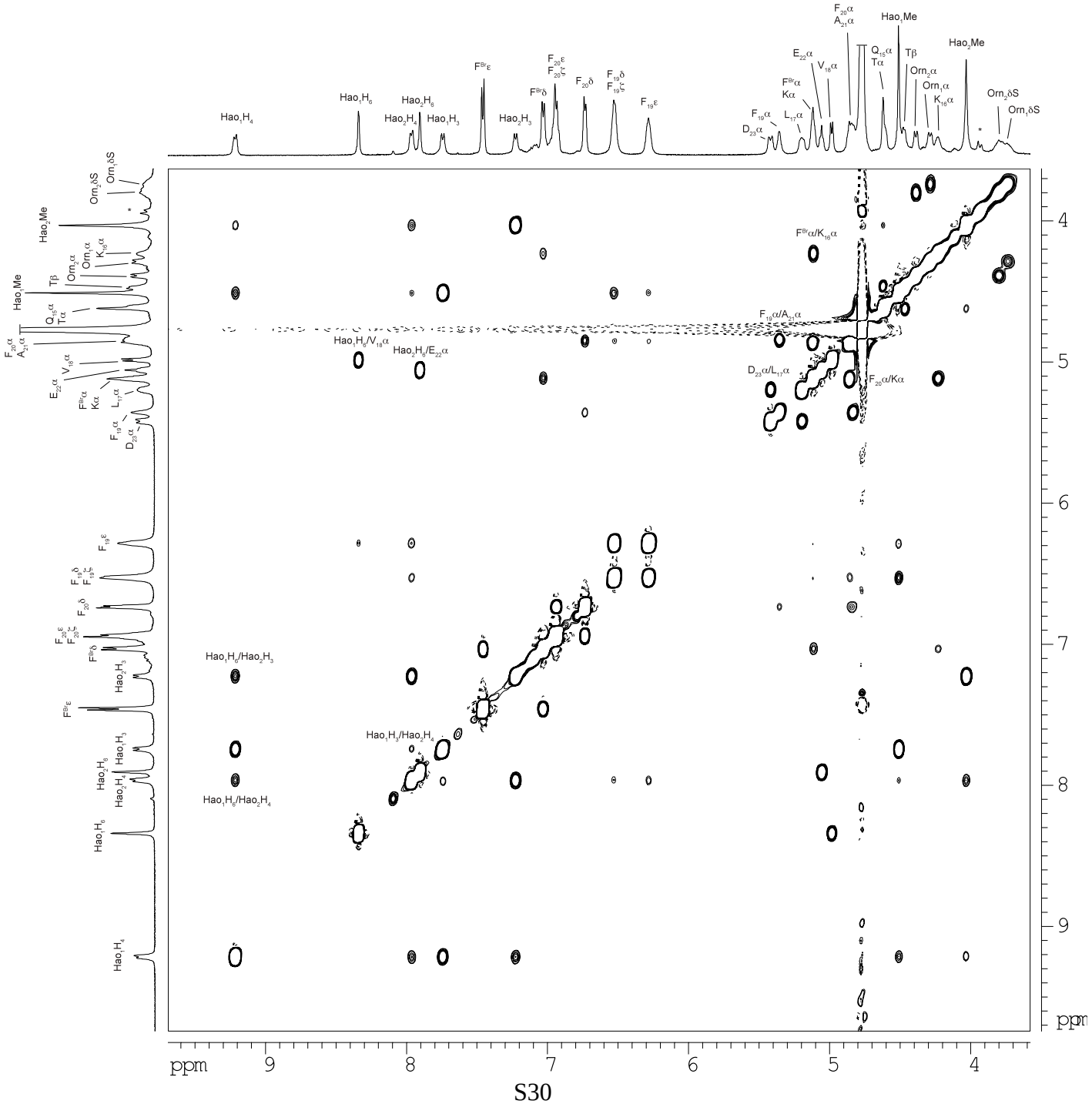
2D NOESY spectrum of macrocyclic β-sheet **1** as tetramer
2 mM in D_2O, 500 MHz, 298 K, 200-ms spin-locking mixing time
tetramer predominates, monomer denoted by asterisk (*)
#select NMR crosspeaks are labeled



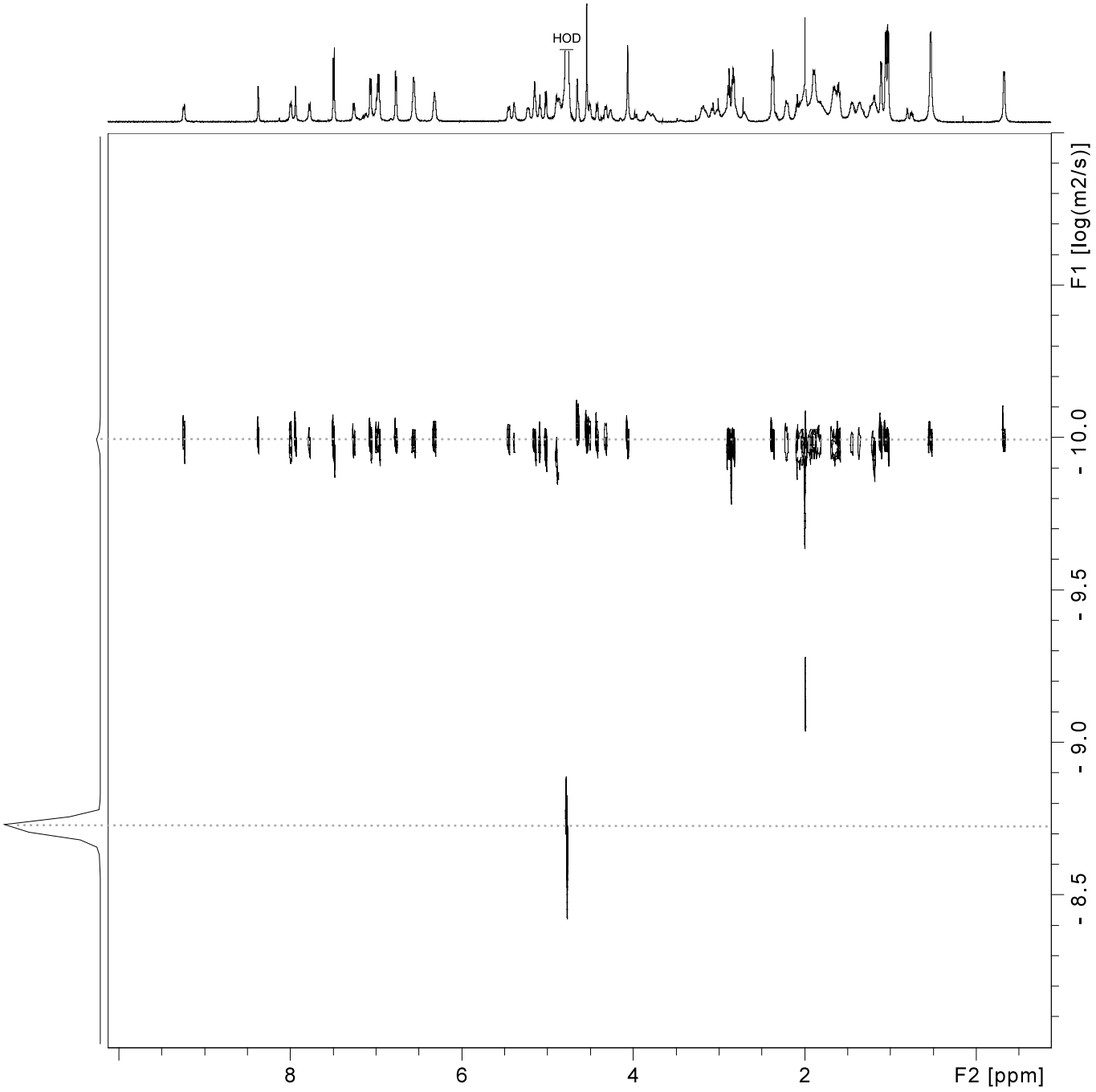


2D NOESY spectrum of macrocyclic β-sheet **1**
2 mM in D_2O, 500 MHz, 298 K, 200-ms spin-locking mixing time
tetramer predominates, monomer denoted by asterisk (*)
#select NMR crosspeaks are labeled





2D DOSY spectrum of macrocyclic β-sheet **1**
2 mM in D_2O, 600 MHz, 298 K
tetramer predominates

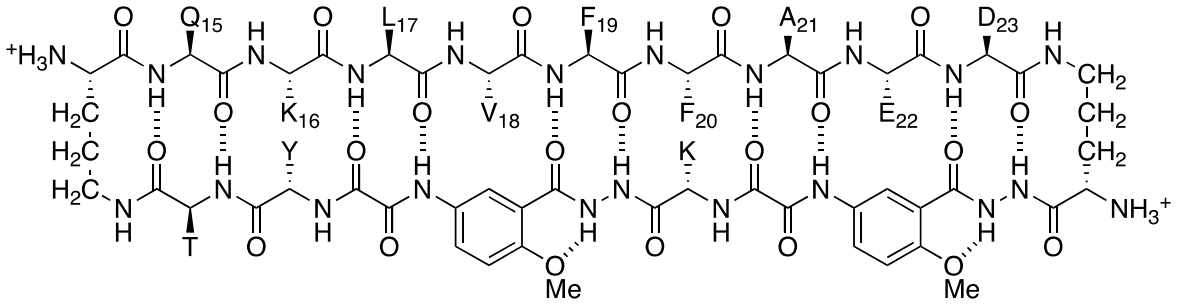


Calculation for 1 at 2.0 mM

DC_{HOD} = 19.0 x 10^{-10} m^2/s [a]
log DC_{HOD} = -8.721

For 1 tetramer, log DC (m^2/s) = -9.99(7), DC = $10^{-9.997}$ m^2/s = 10.1 x 10^{-11} m^2/s = 10.1 x 10^{-7} cm^2/s

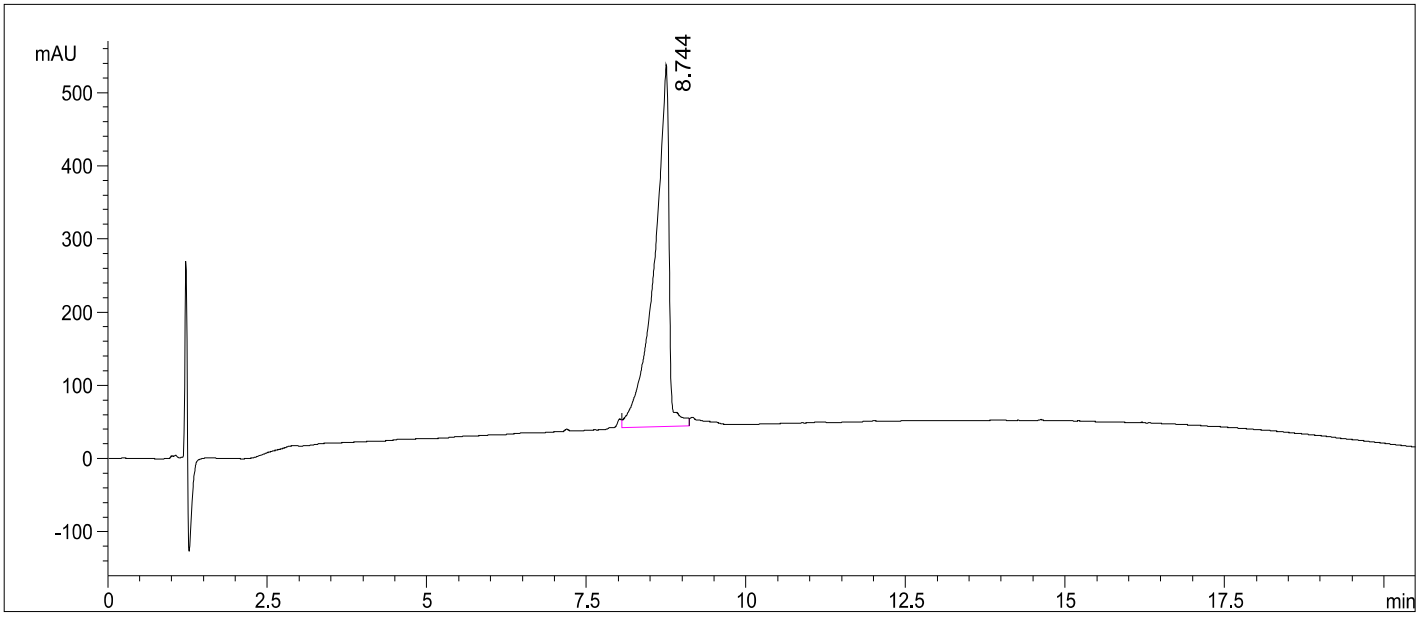
[a] Longsworth, L. G. J. Phys. Chem. 1960, 64, 1914–1917.



macrocyclic β-sheet peptide **2a** (as the TFA salt)

molecular weight calculated for $C_{101}H_{141}N_{25}O_{29} \cdot 4CF_3CO_2H$ (TFA salt of **2a**): 2625.44
molecular weight calculated for $C_{101}H_{141}N_{25}O_{29}$ (free base of **2a**): 2169.35
exact mass calculated for $C_{101}H_{141}N_{25}O_{29}$ (free base of **2a**): 2168.03

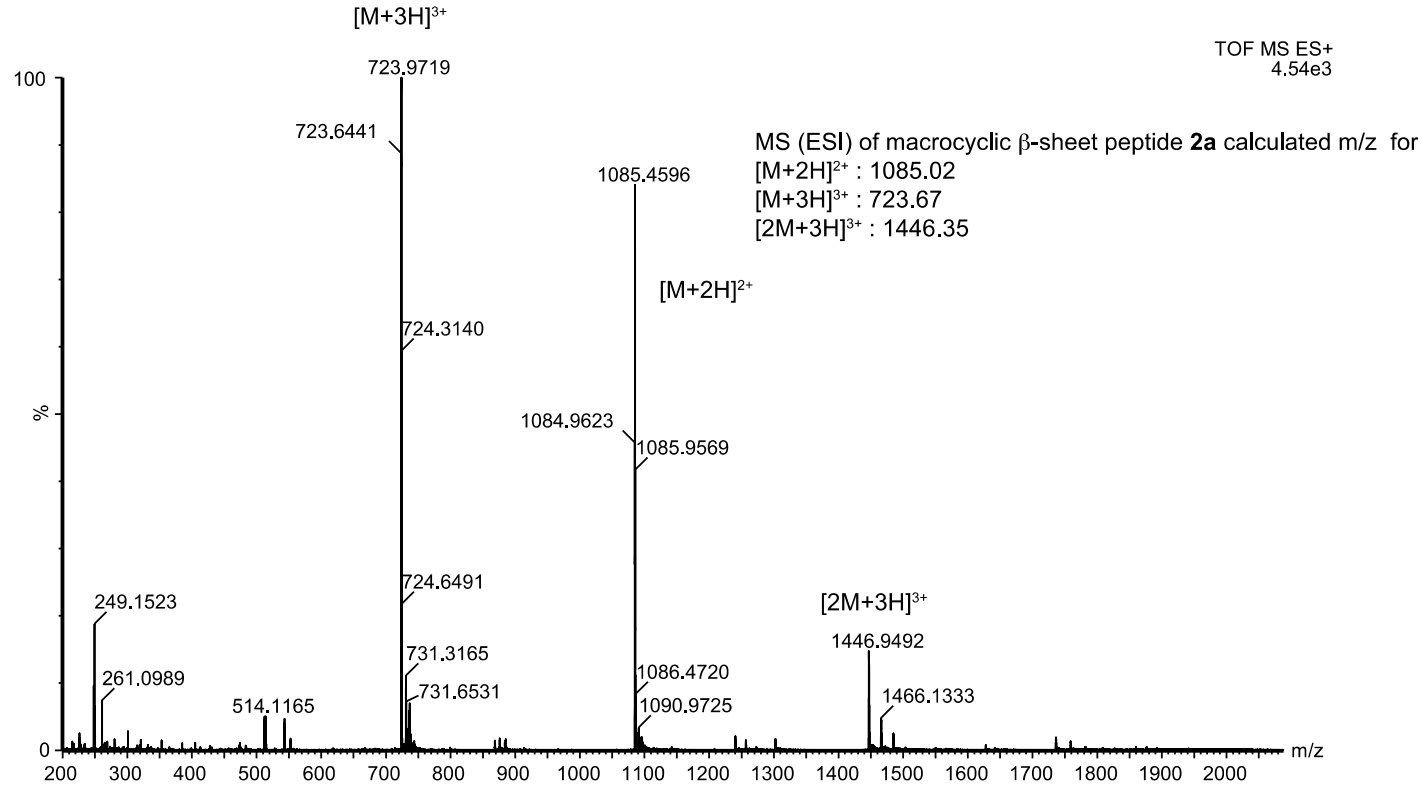
Analytical RP-HPLC of macrocyclic β-peptide **2a**

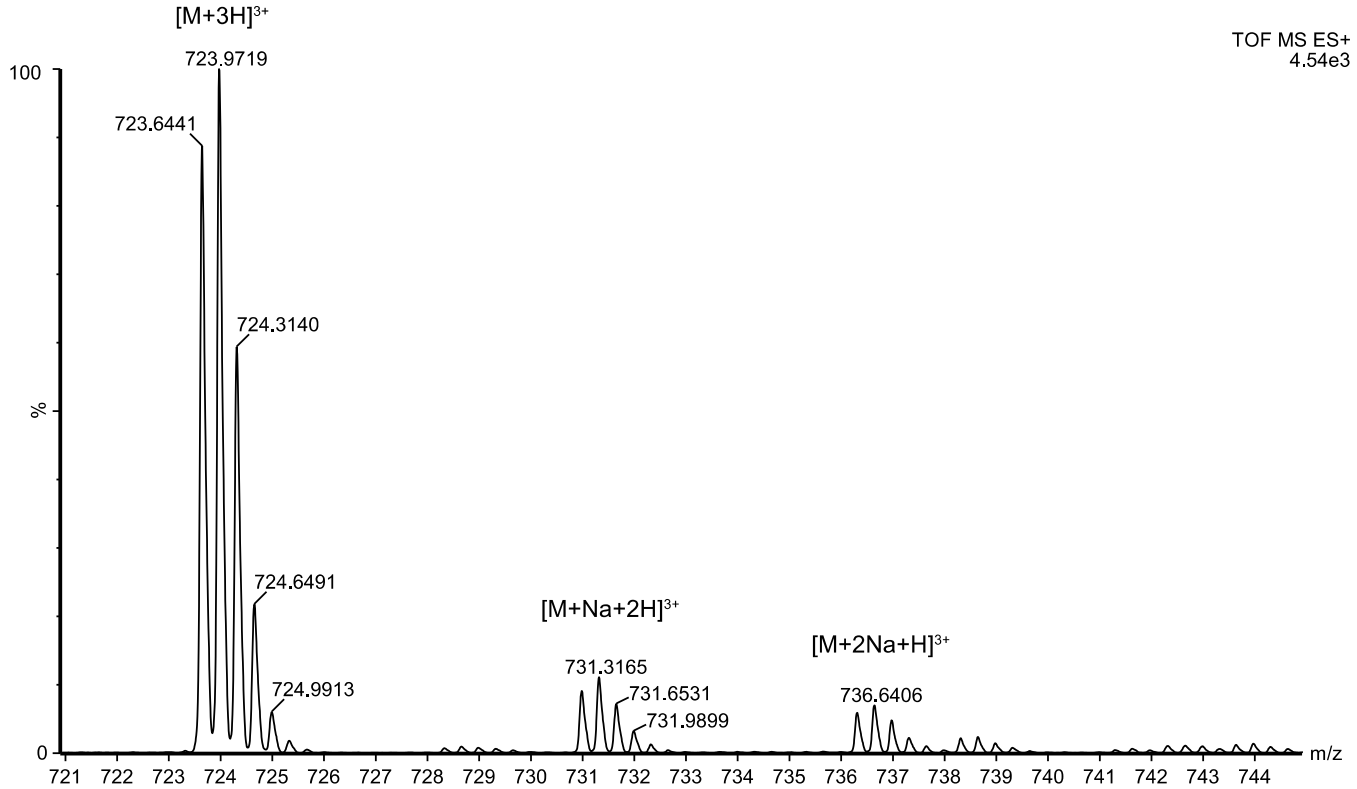


Signal 1: VWD1 A, Wavelength=214 nm

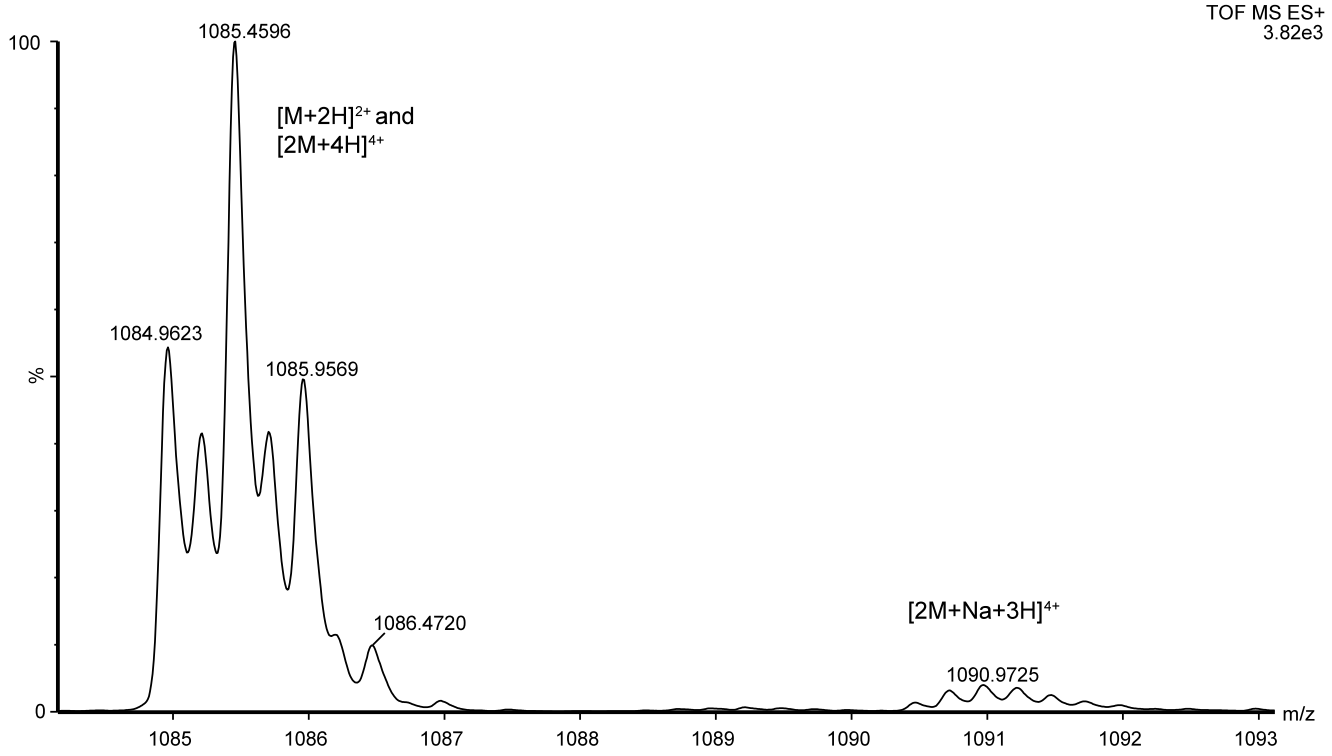
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	8.744	VV	0.2105	7939.88721	494.81061	100.0000
Totals :				7939.88721	494.81061	

Macrocyclic β-sheet peptide **2a**

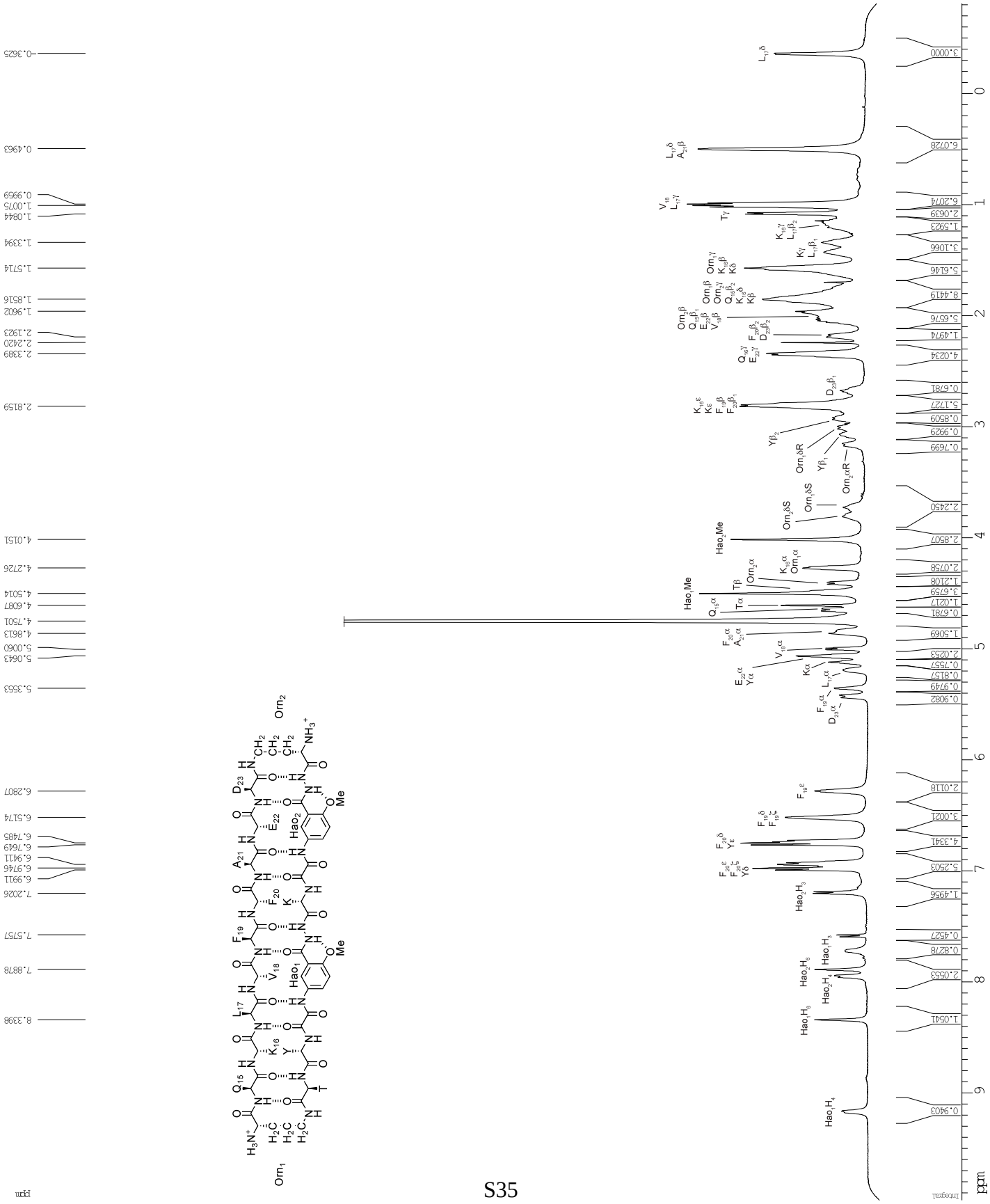




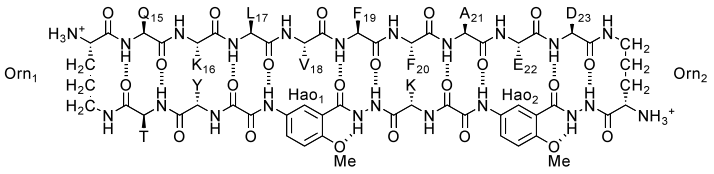
Macrocyclic β-sheet peptide **2a**

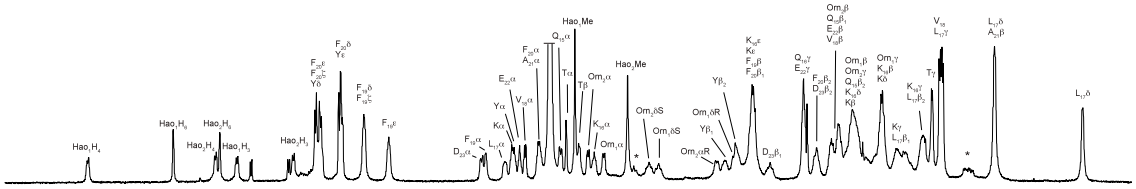


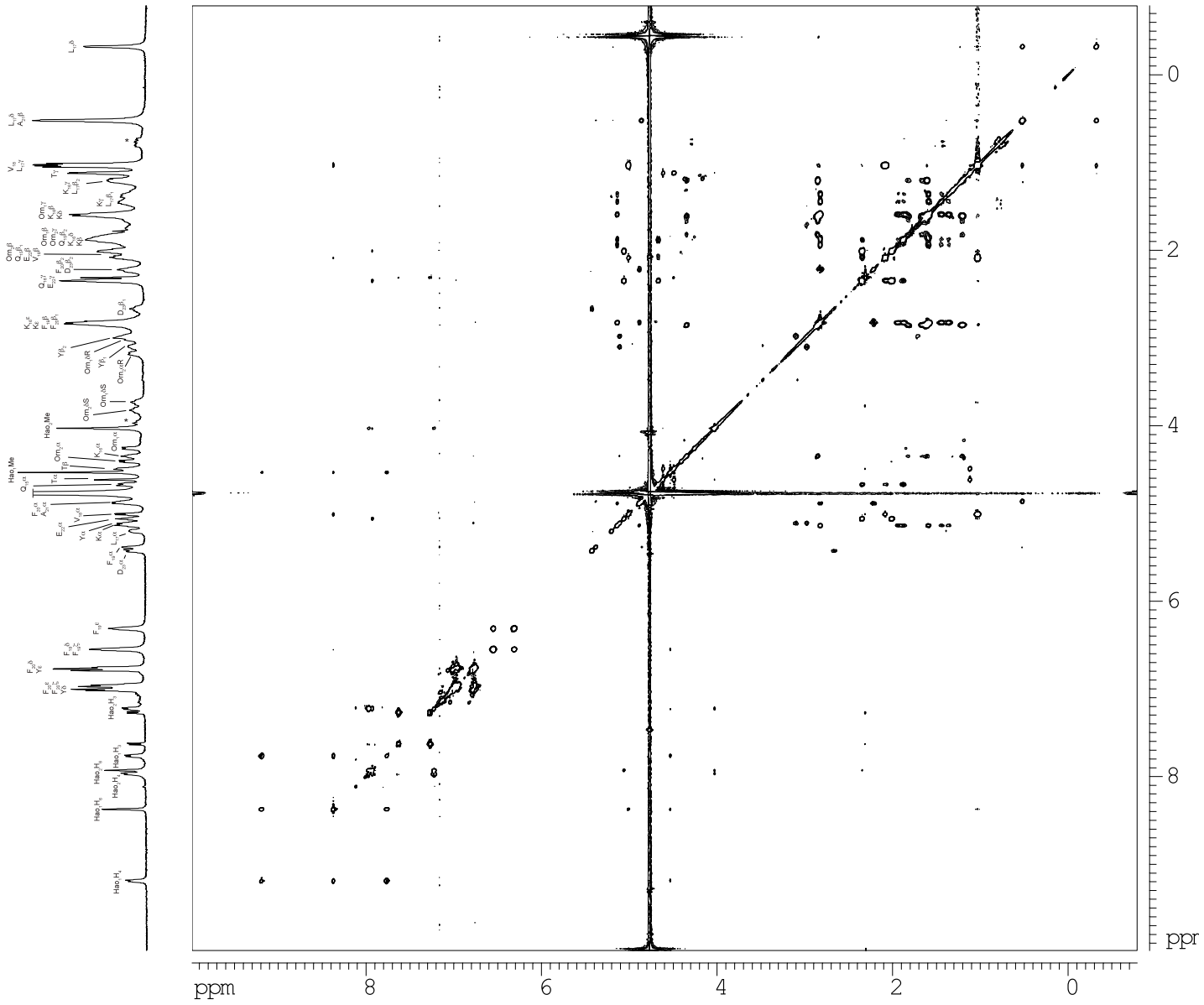
1D ^{1}H NMR spectrum of macrocyclic β-sheet **2a**
8 mM in D_2O, 500 MHz, 300.5 K
tetramer predominates



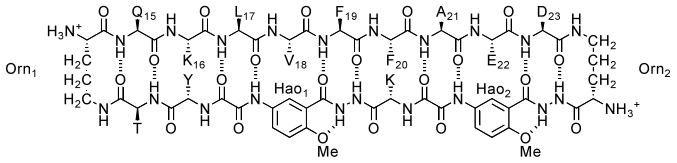
2D TOCSY spectrum of macrocyclic β-sheet **2a**
2 mM in D_2O, 500 MHz, 300.5 K, 150-ms spin-locking mixing time
tetramer predominates, monomer denoted with an asterisk (*)

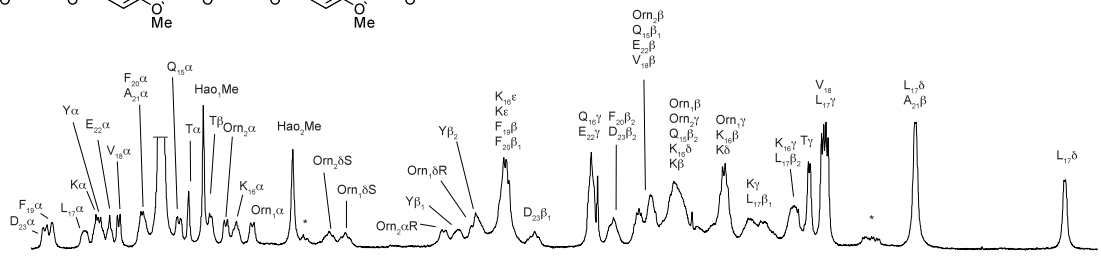


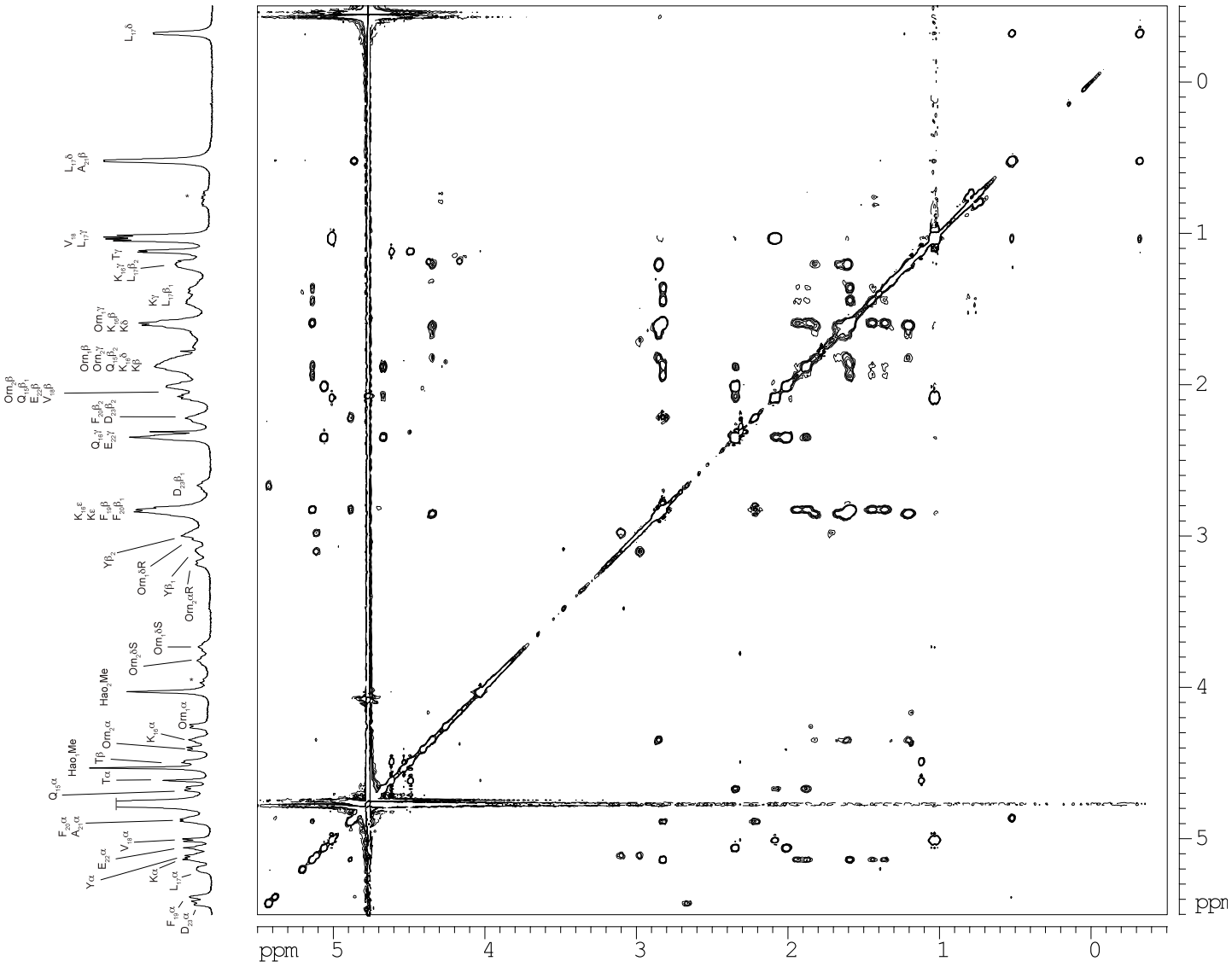




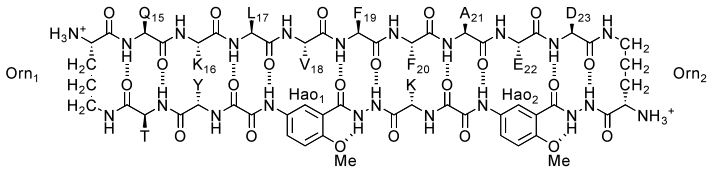
2D TOCSY spectrum of macrocyclic β-sheet **2a**
2 mM in D_2O, 500 MHz, 300.5 K, 150-ms spin-locking mixing time
tetramer predominates, monomer denoted with an asterisk (*)

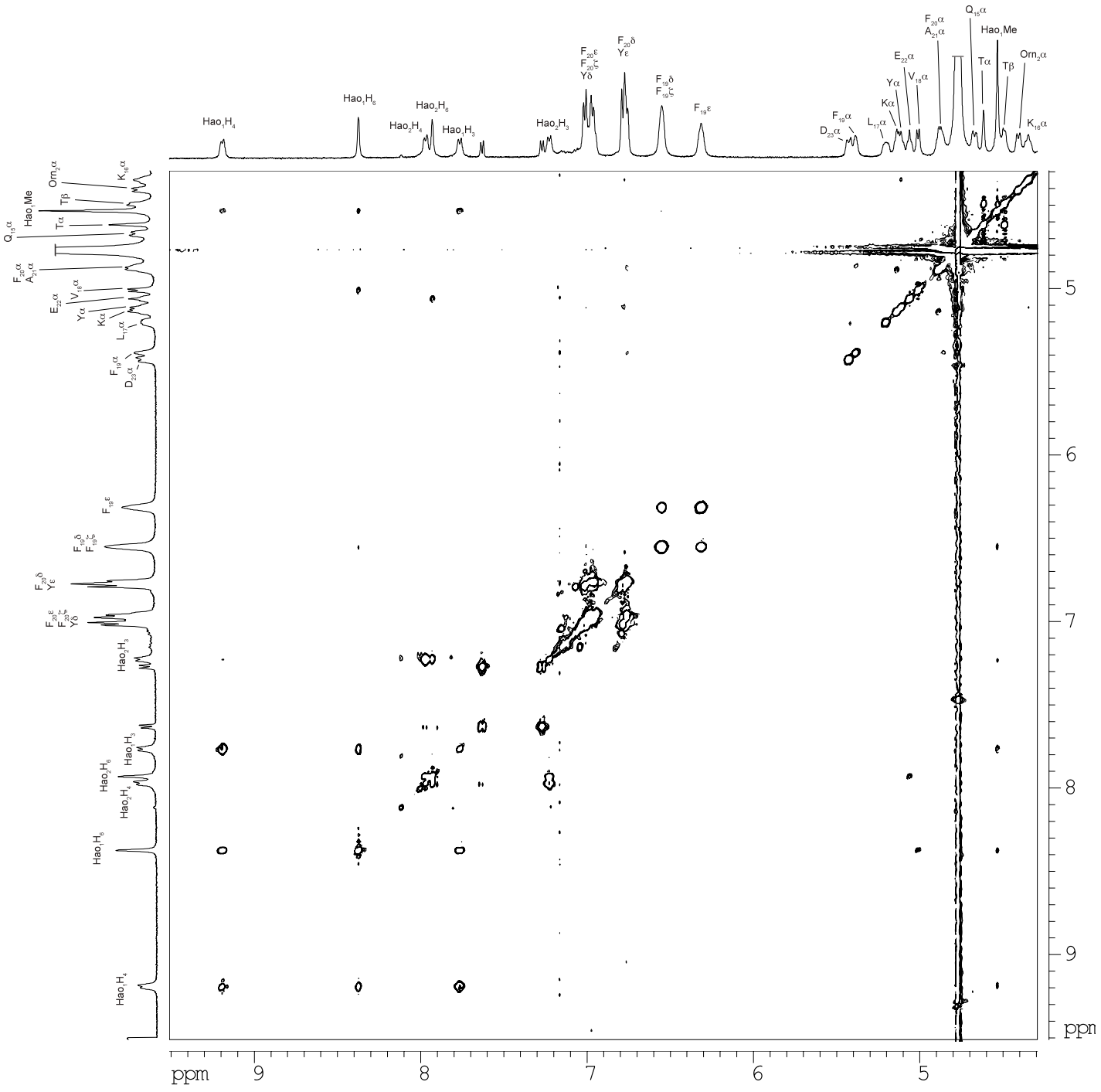




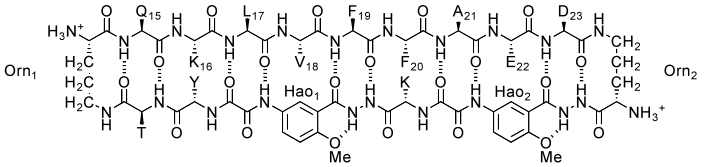


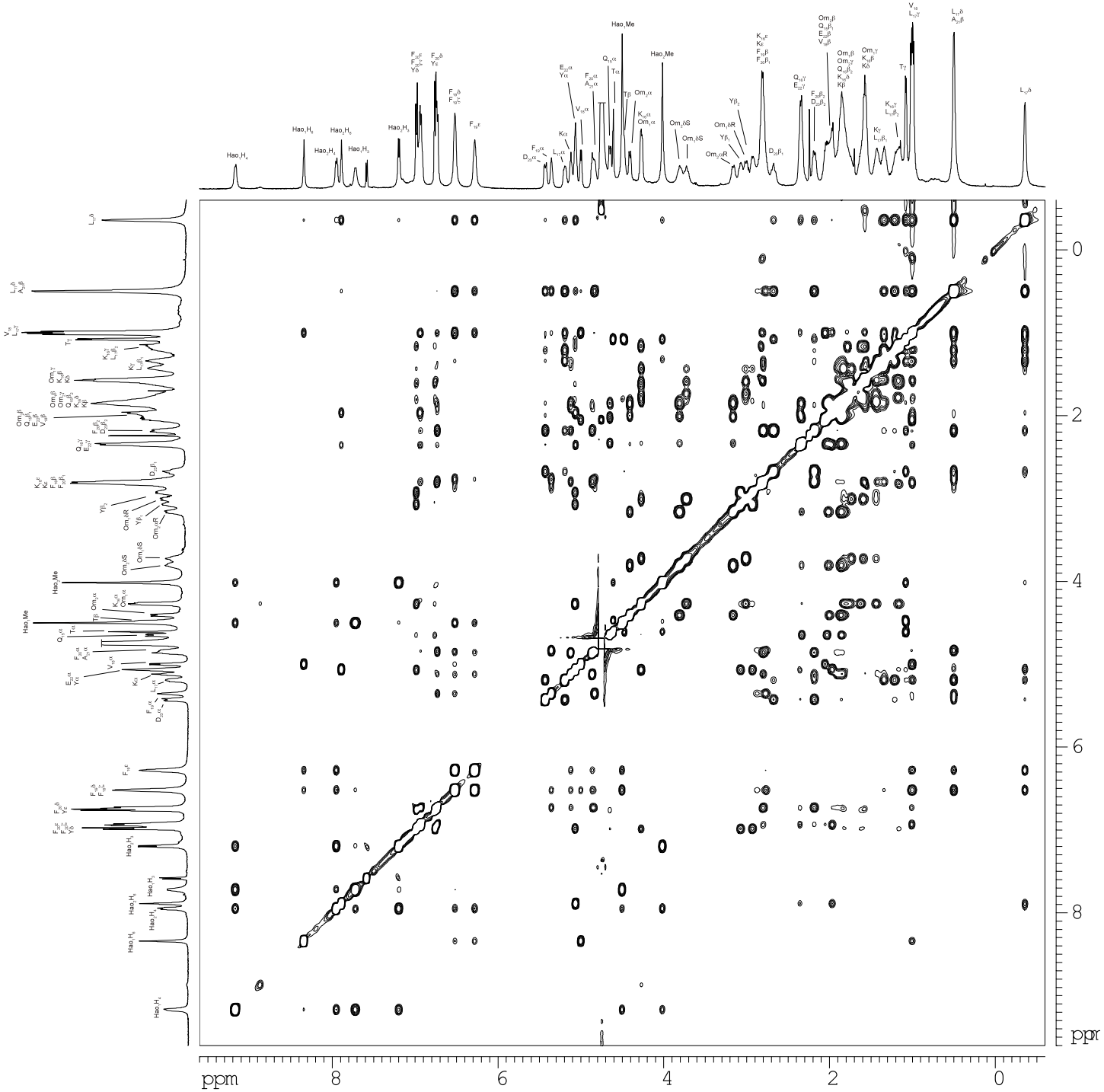
2D TOCSY spectrum of macrocyclic β-sheet **2a**
2 mM in D_2O, 500 MHz, 300.5 K, 150-ms spin-locking mixing time
tetramer predominates, monomer denoted with an asterisk (*)



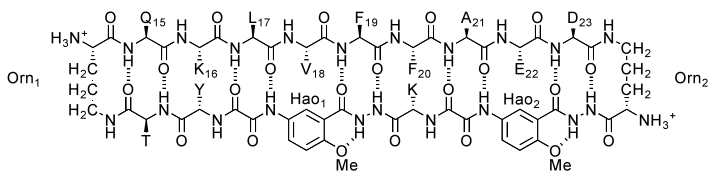


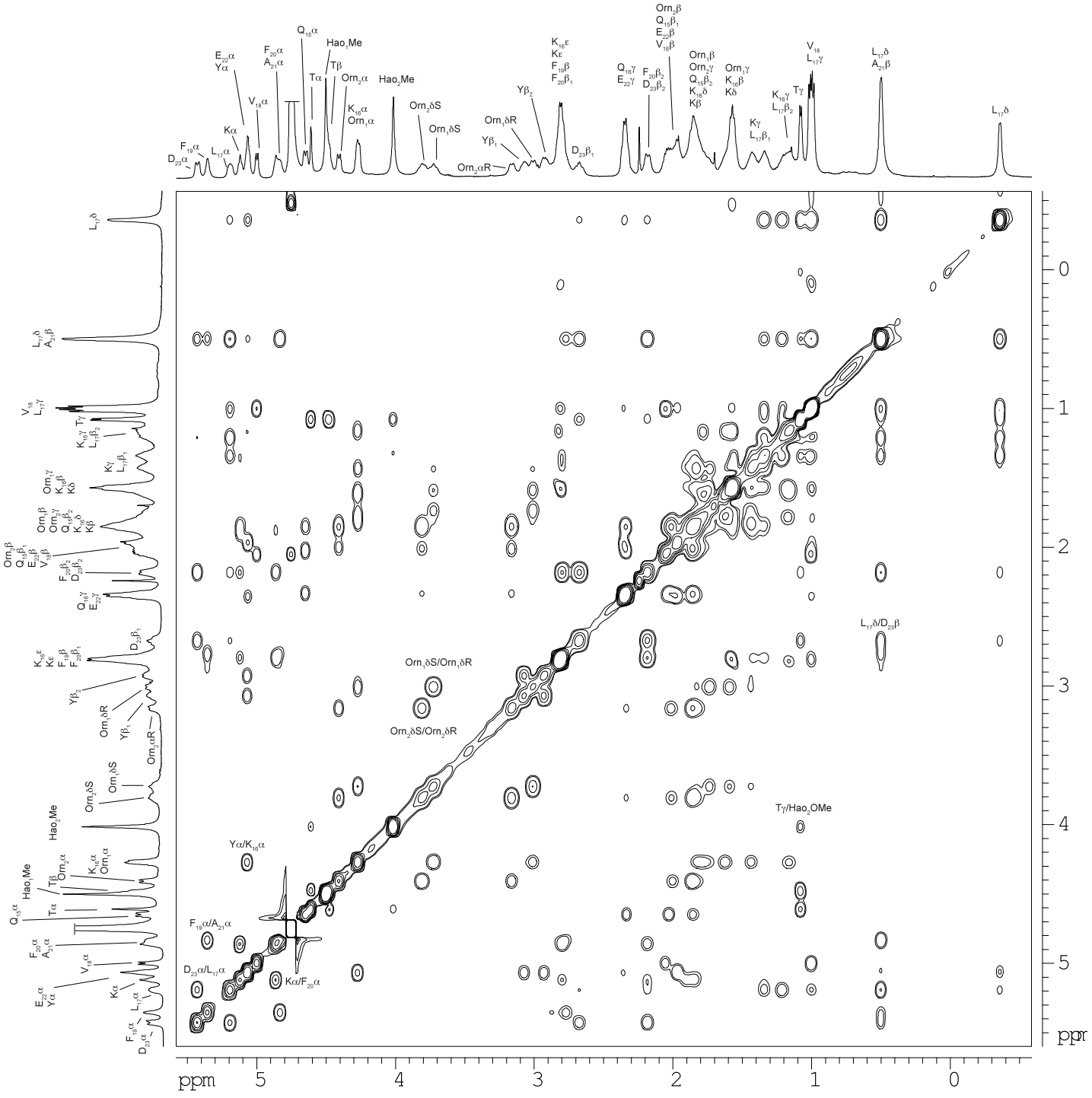
2D NOESY spectrum of macrocyclic β-sheet **2a**
8 mM in D_2O, 500 MHz, 300.5 K, 150-ms spin-locking mixing time
tetramer predominates



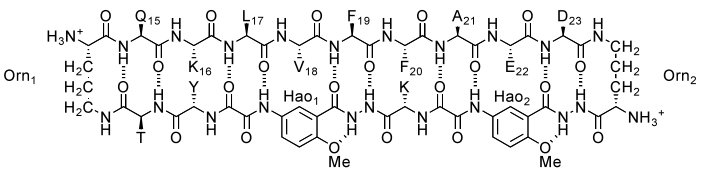


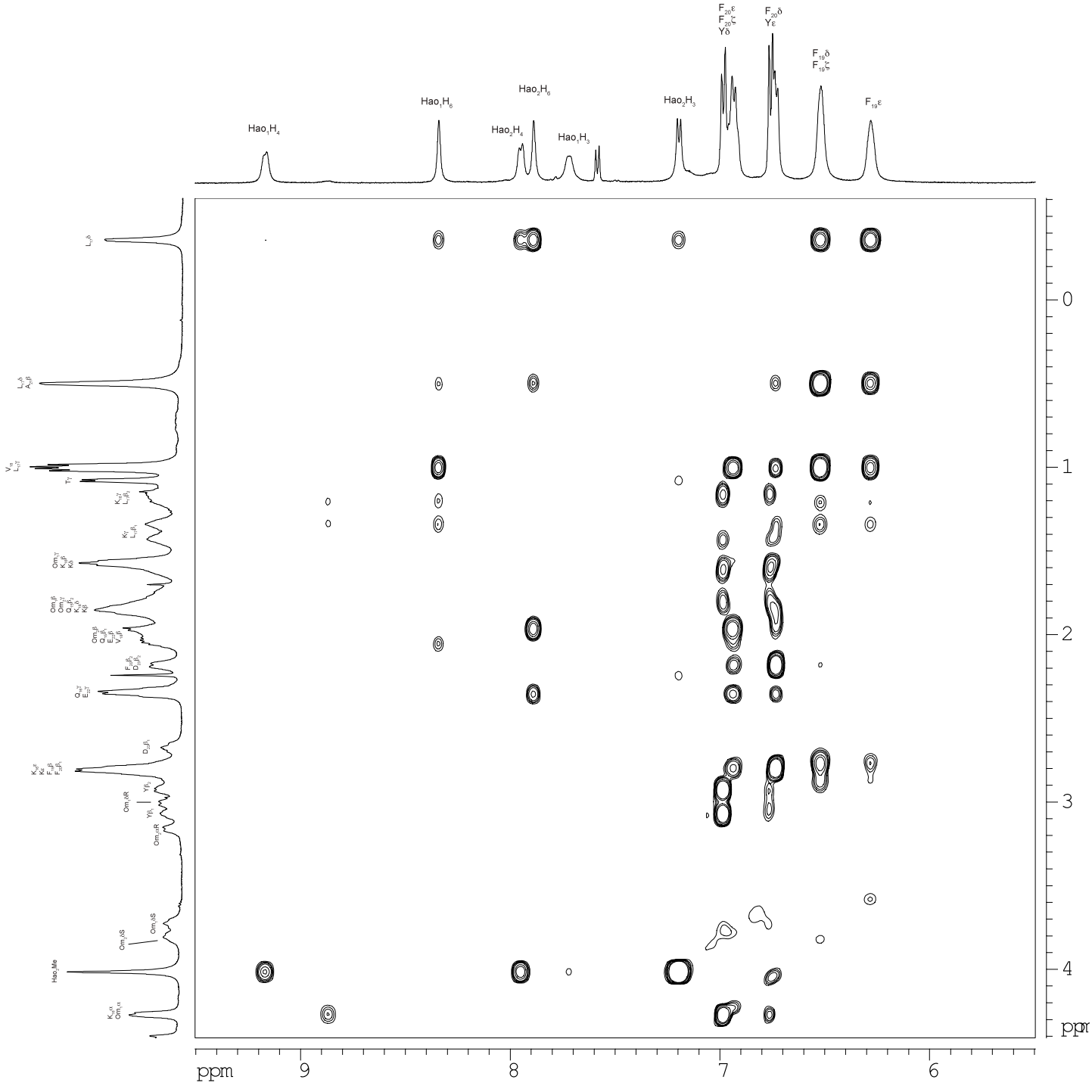
2D NOESY spectrum of macrocyclic β-sheet **2a**
8 mM in D_2O, 500 MHz, 300.5 K, 150-ms spin-locking mixing time
tetramer predominates
#select NMR crosspeaks are labeled



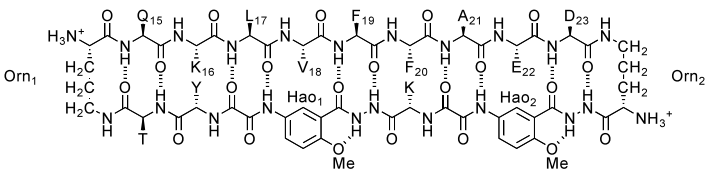


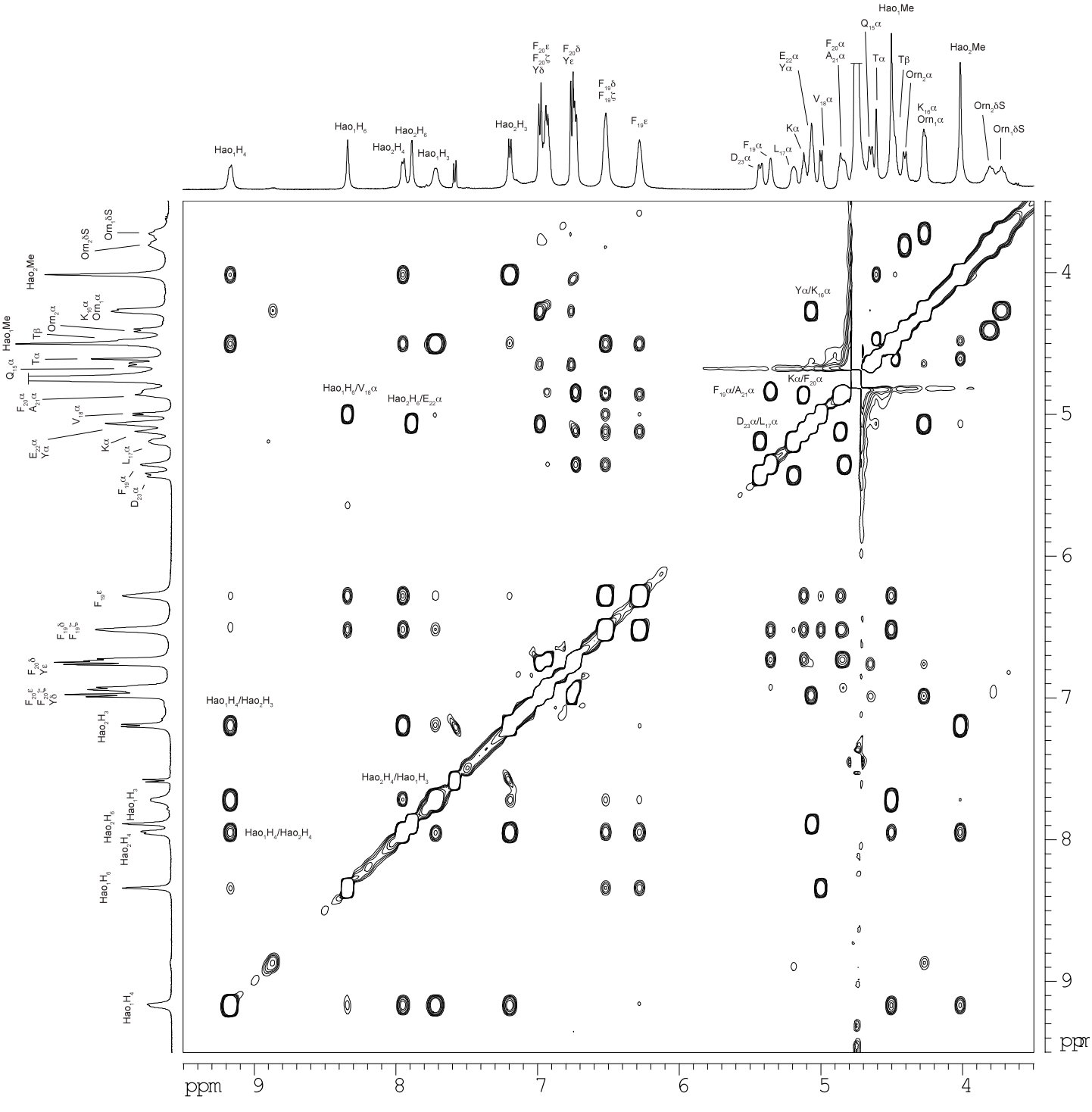
2D NOESY spectrum of macrocyclic β-sheet **2a**
8 mM in D_2O, 500 MHz, 300.5 K, 150-ms spin-locking mixing time
tetramer predominates



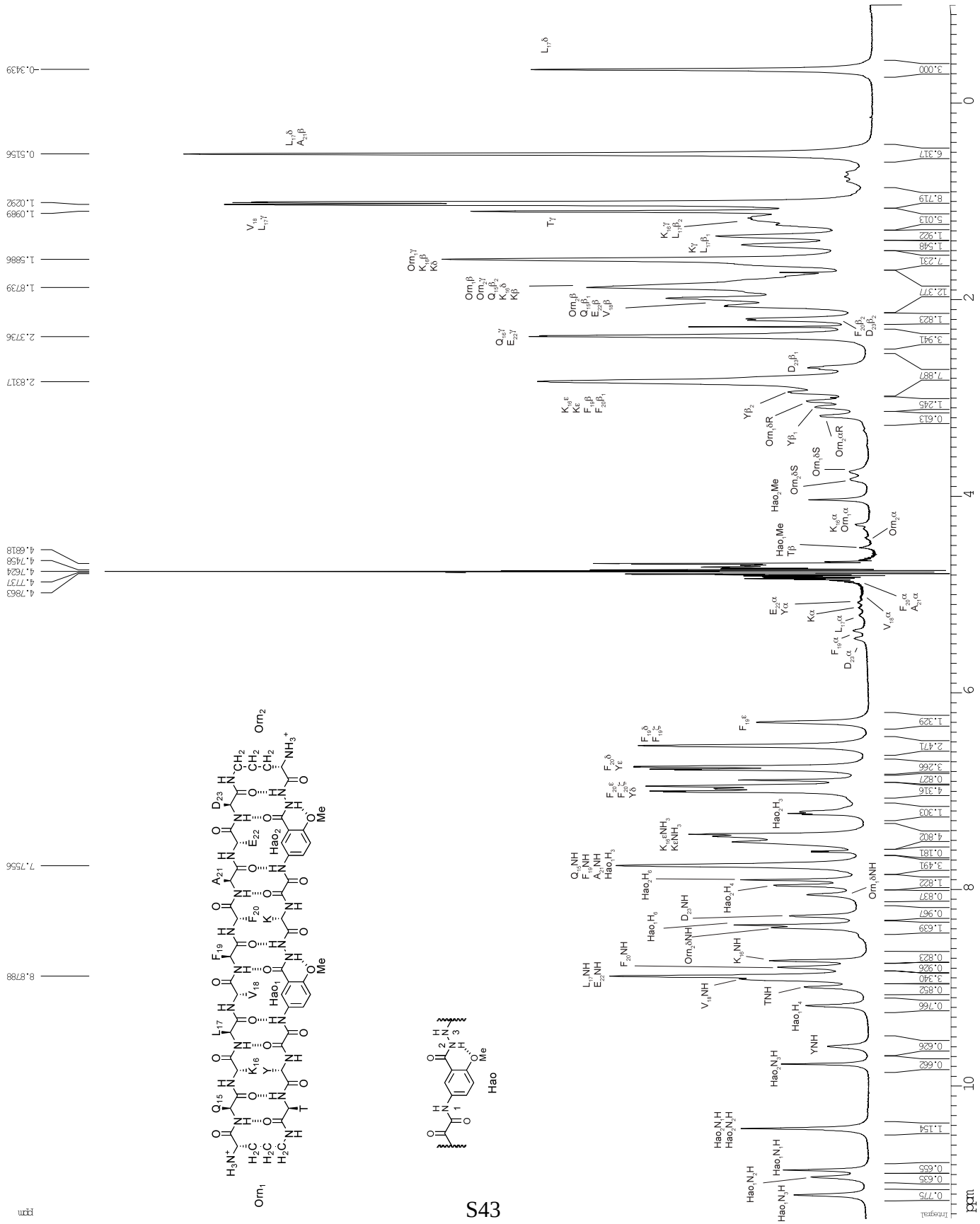


2D NOESY spectrum of macrocyclic β-sheet **2a**
8 mM in D_2O, 500 MHz, 300.5 K, 150-ms spin-locking mixing time
tetramer predominates
#select NMR crosspeaks are labeled

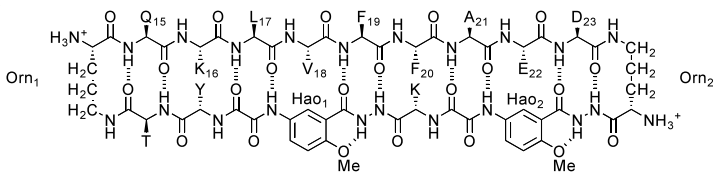


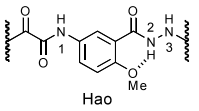


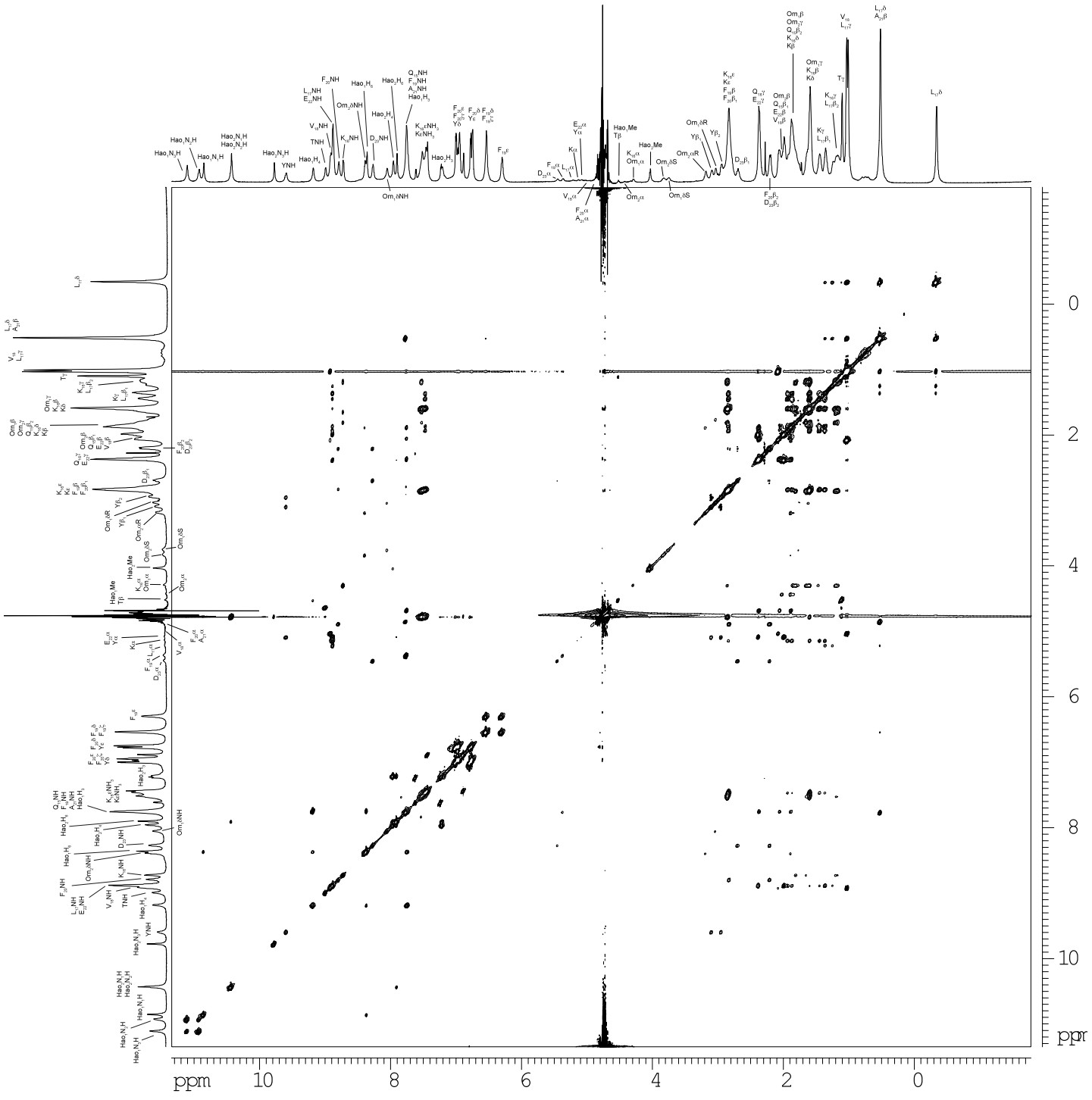
1D ^{1}H NMR spectrum of macrocyclic β-sheet **2a**
with WATERGATE
8 mM in 9:1 H_2O/D_2O, 800 MHz, 298 K
tetramer predominates



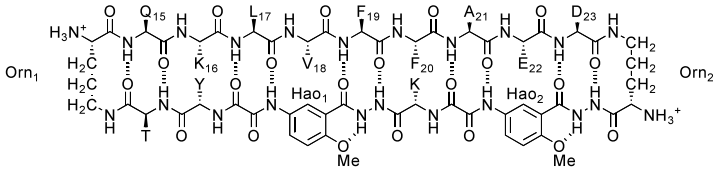
2D TOCSY spectrum of macrocyclic β-sheet **2a** with WATERGATE
8 mM in 9:1 H_2O/D_2O, 800 MHz, 298 K
75-ms spin-locking mixing time
tetramer predominates

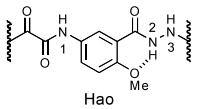


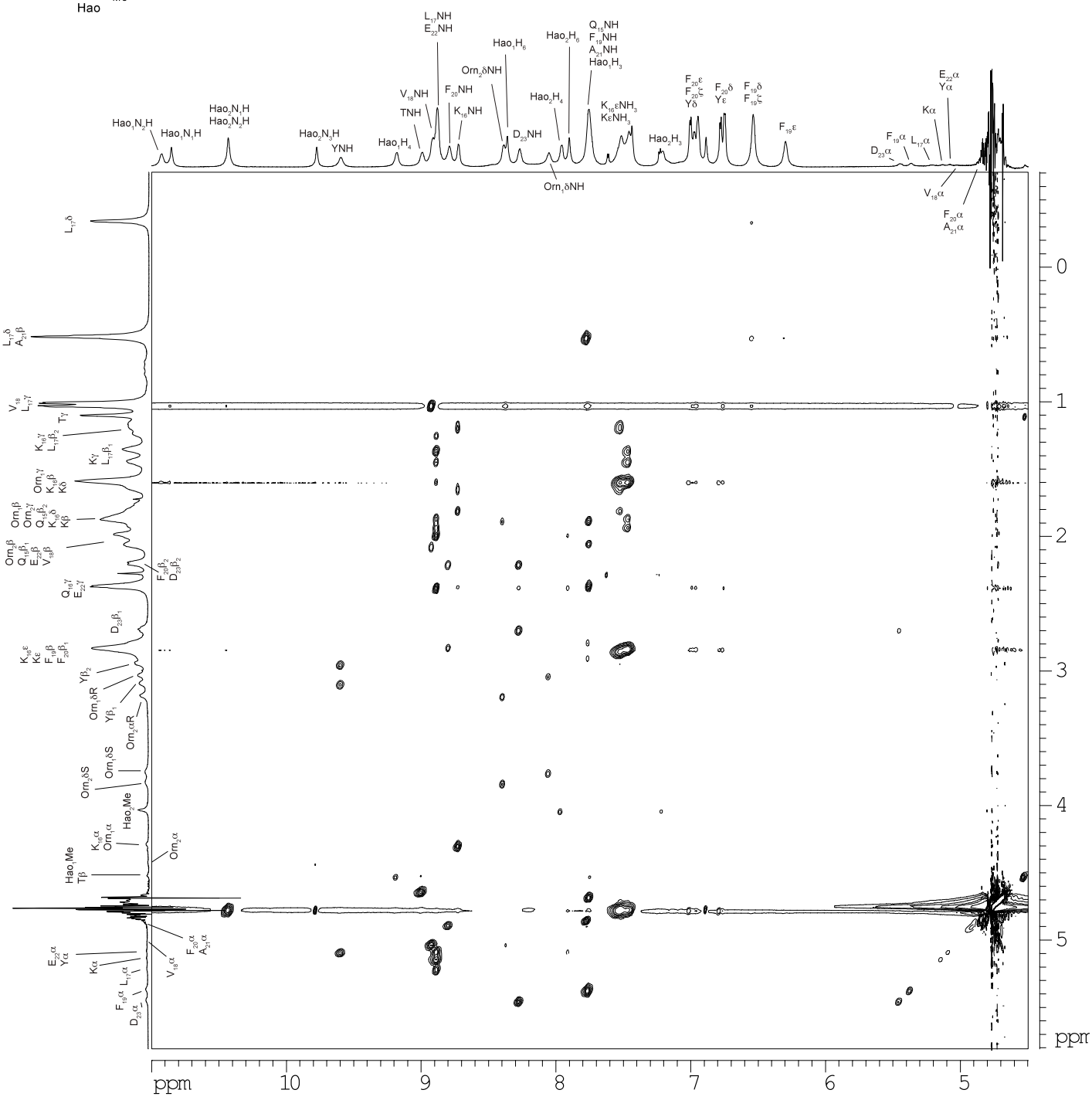




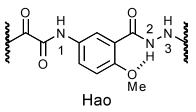
2D TOCSY spectrum of macrocyclic β-sheet **2a** with WATERGATE
8 mM in 9:1 H_2O/D_2O, 800 MHz, 298 K
75-ms spin-locking mixing time
tetramer predominates

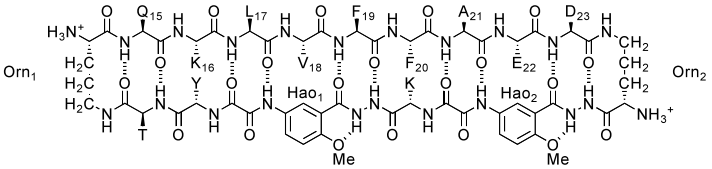


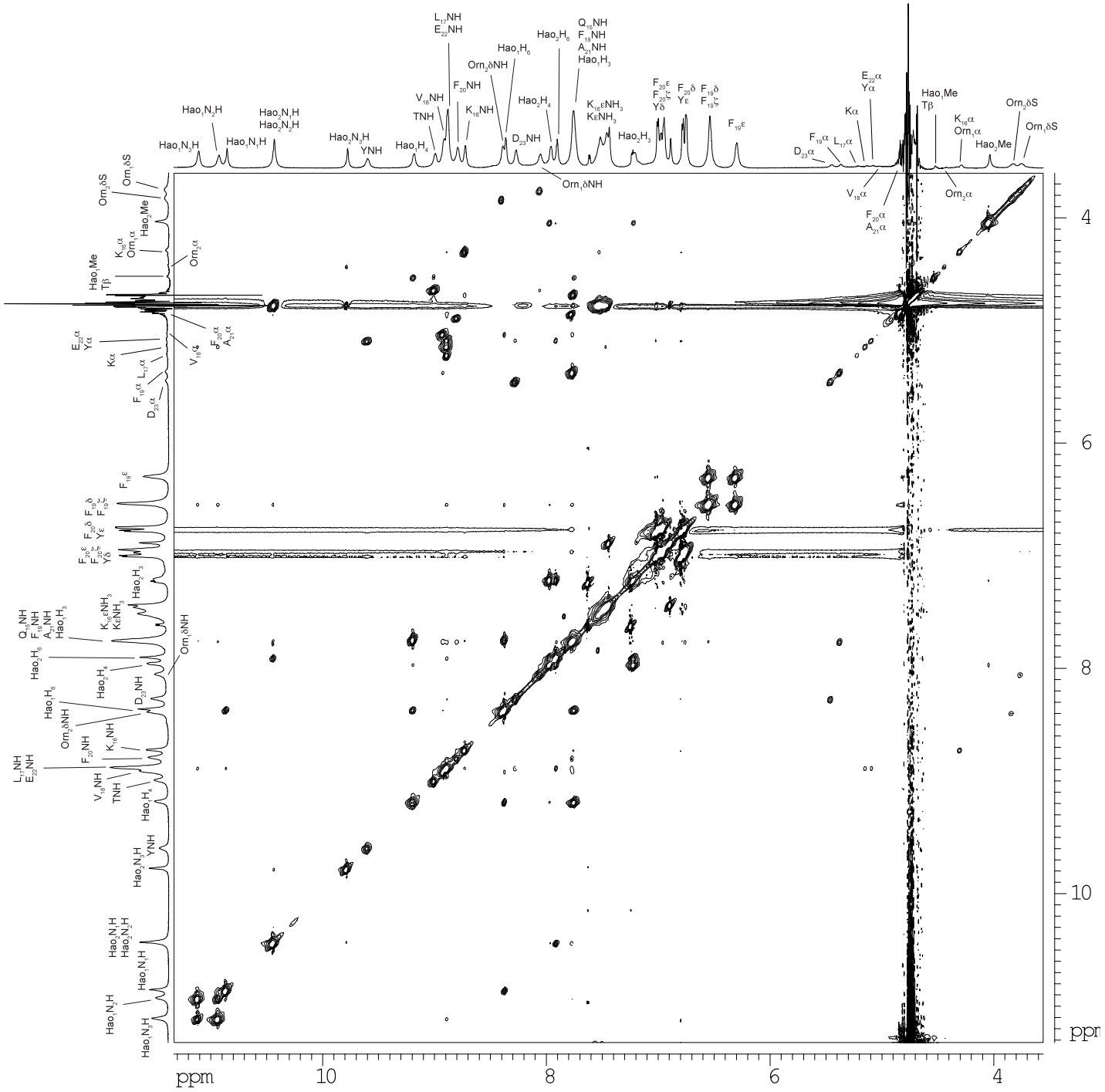




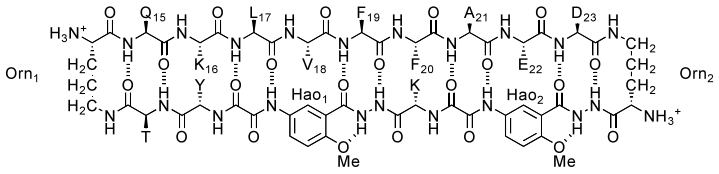
2D TOCSY spectrum of macrocyclic β-sheet **2a** with WATERGATE
8 mM in 9:1 H_2O/D_2O, 800 MHz, 298 K
75-ms spin-locking mixing time
tetramer predominates

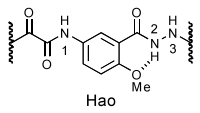


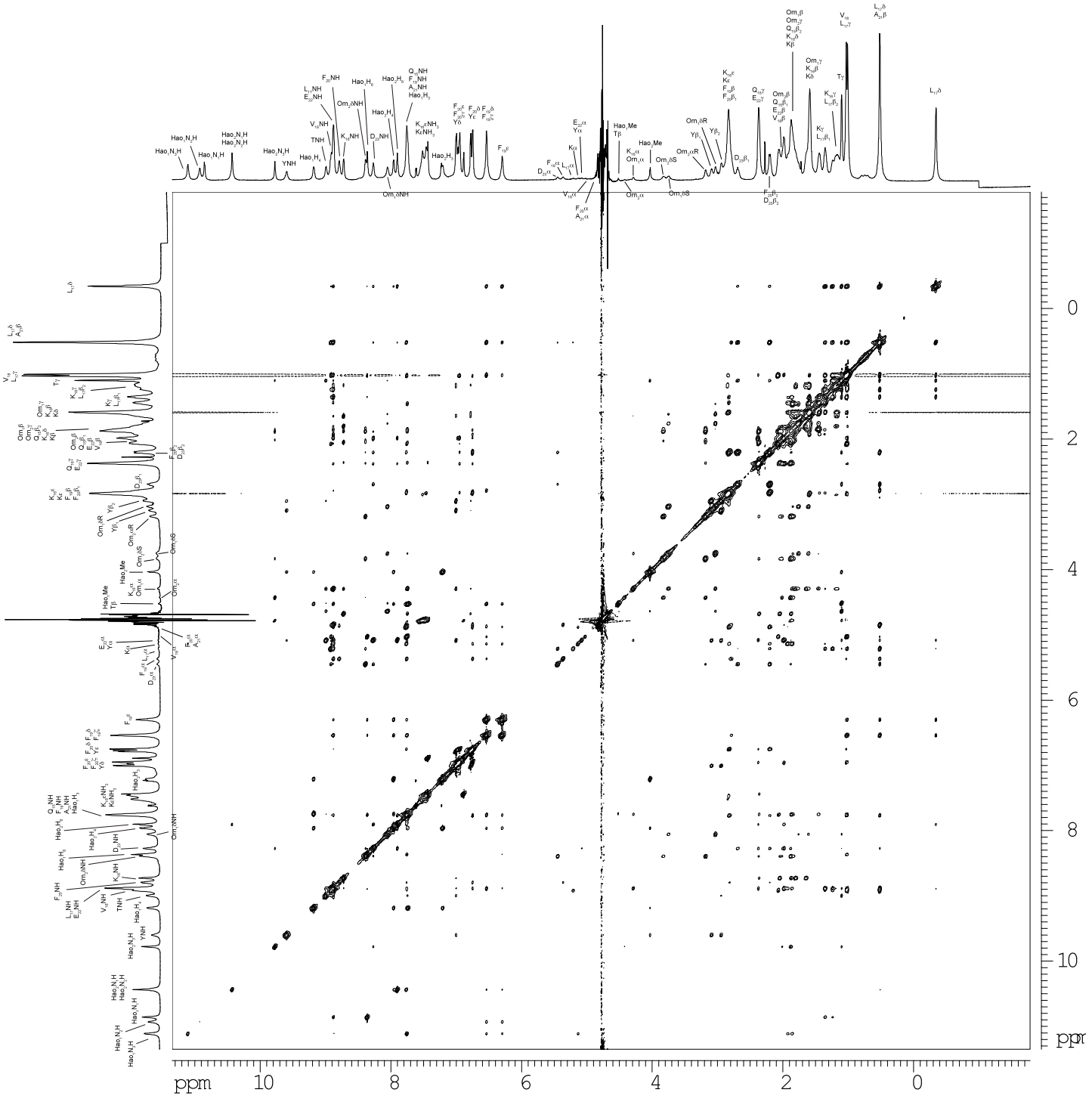




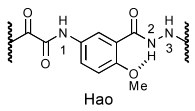
2D NOESY spectrum of macrocyclic β-sheet **2a** with WATERGATE
8 mM in 9:1 H_2O/D_2O, 800 MHz, 298 K
225-ms spin-locking mixing time
tetramer predominates

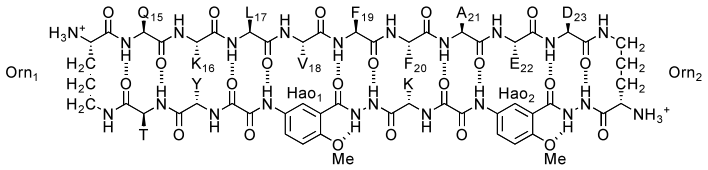


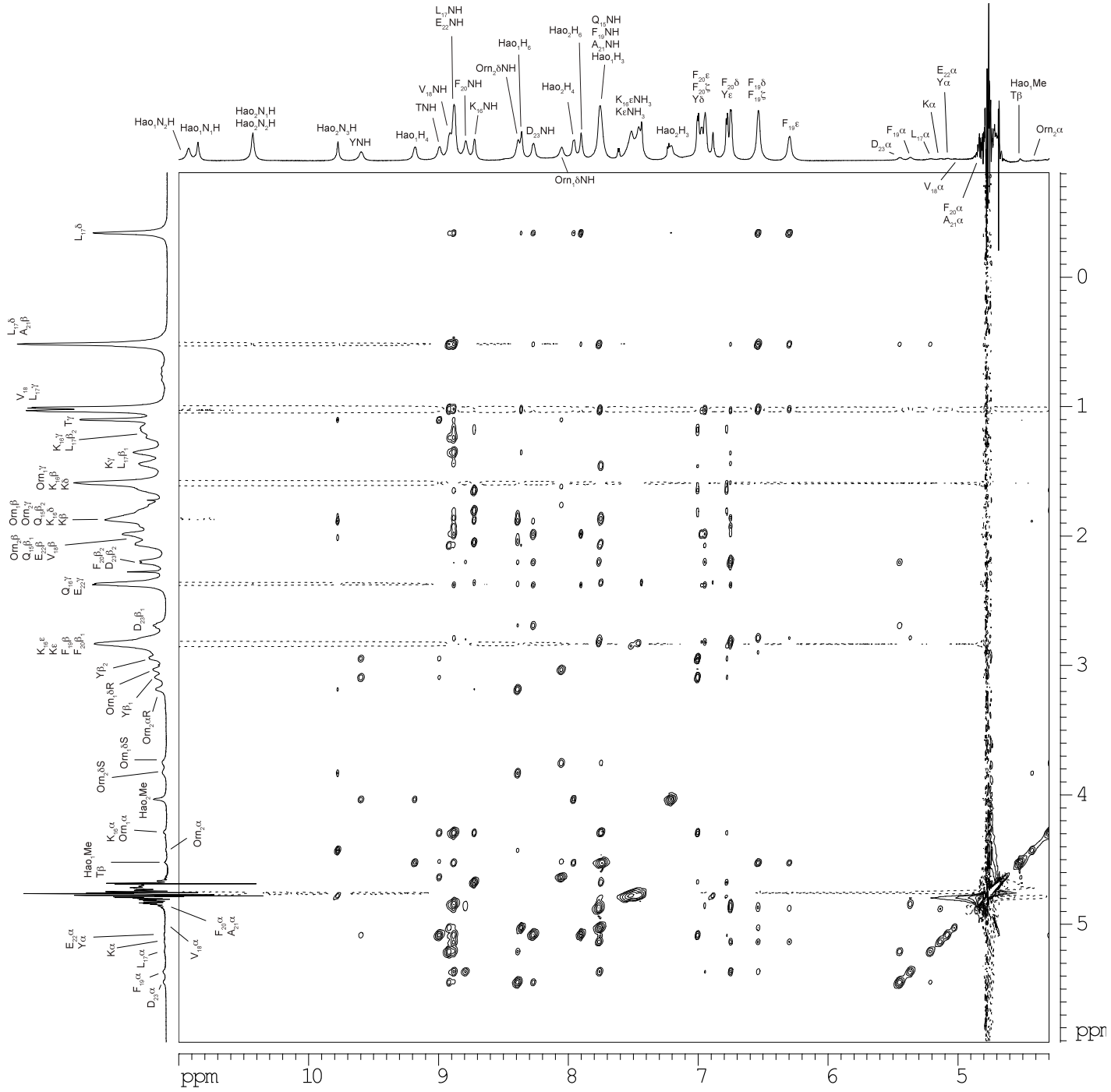




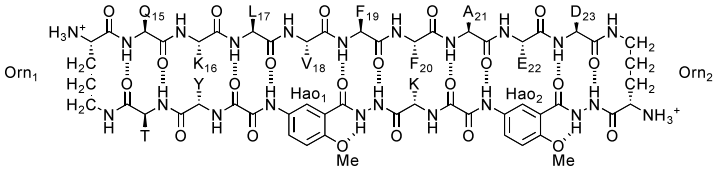
2D NOESY spectrum of macrocyclic β-sheet **2a** with WATERGATE
8 mM in 9:1 H_2O/D_2O, 800 MHz, 298 K
225-ms spin-locking mixing time
tetramer predominates

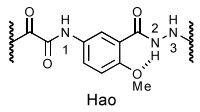


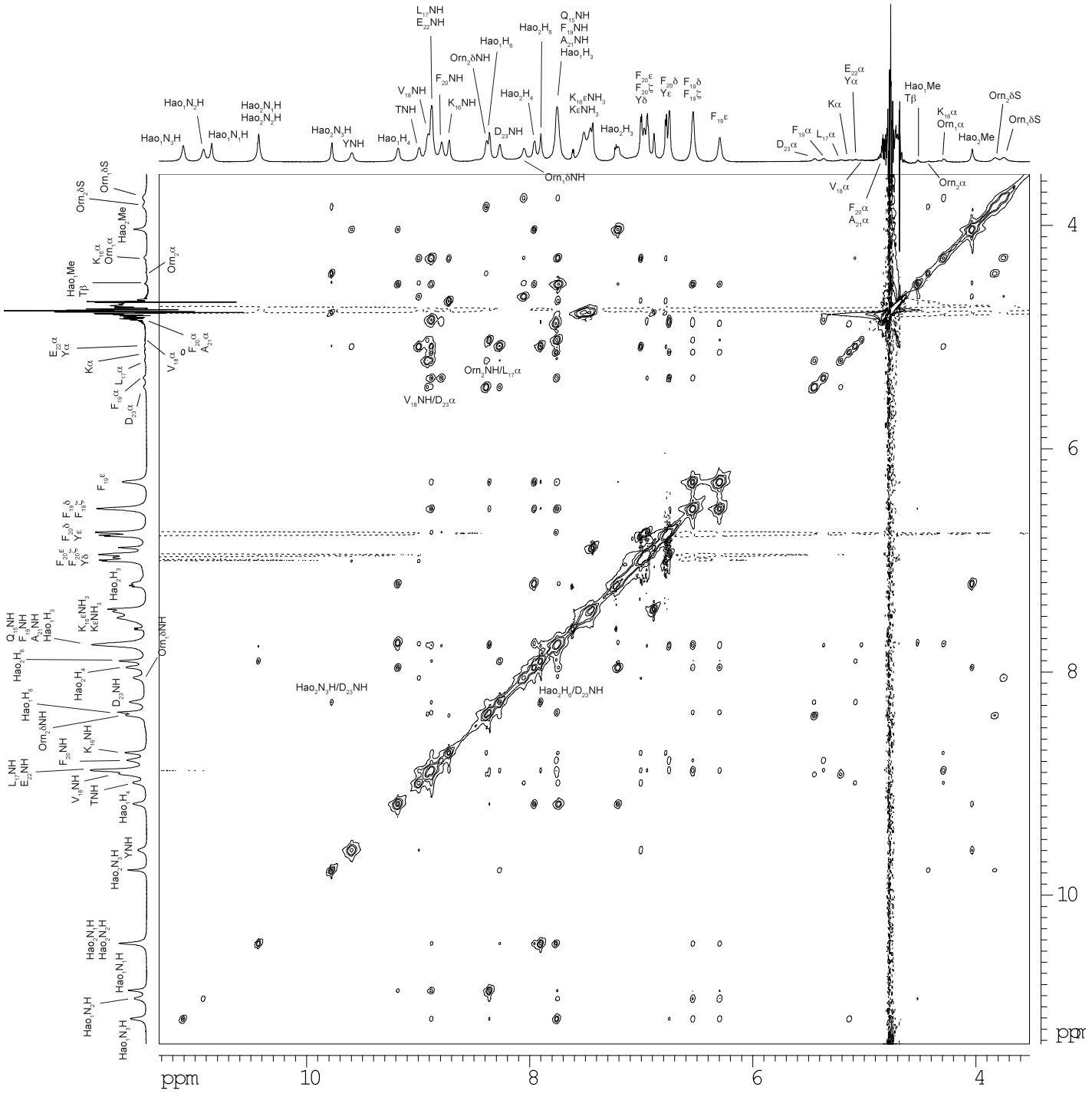




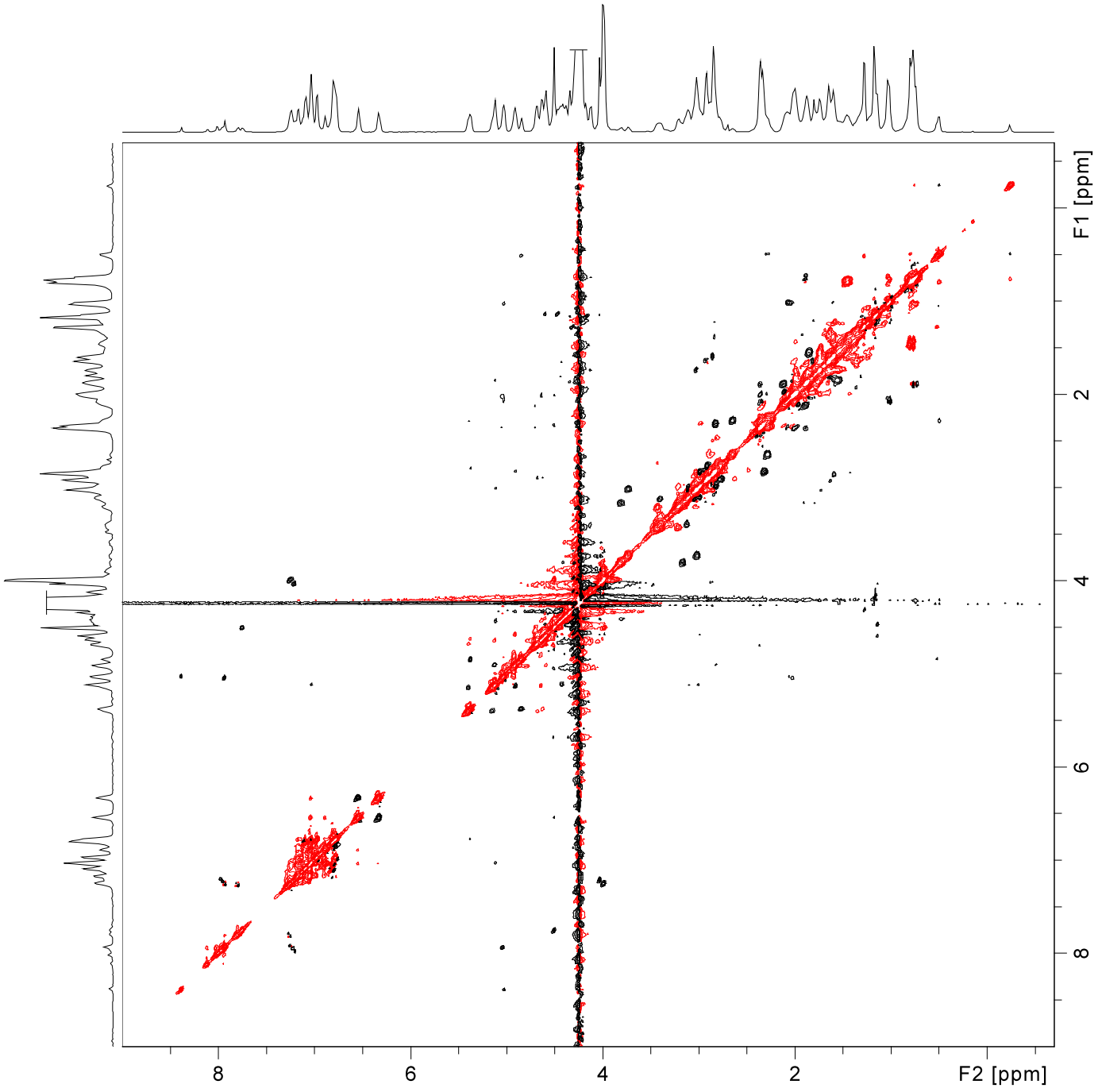
2D NOESY spectrum of macrocyclic β-sheet **2a** with WATERGATE
8 mM in 9:1 H_2O/D_2O, 800 MHz, 298 K
225-ms spin-locking mixing time
tetramer predominates
#select NMR crosspeaks are labeled



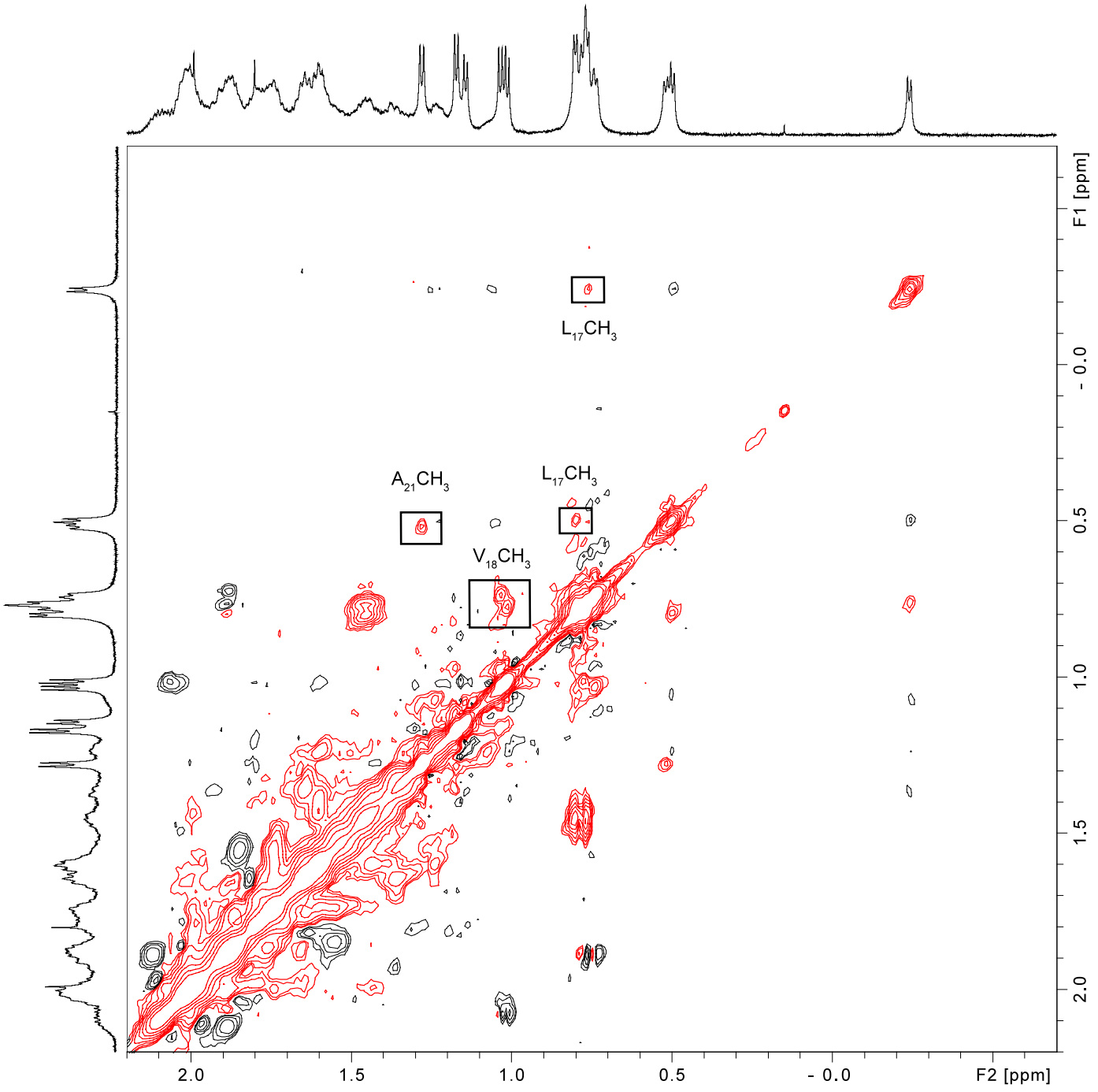




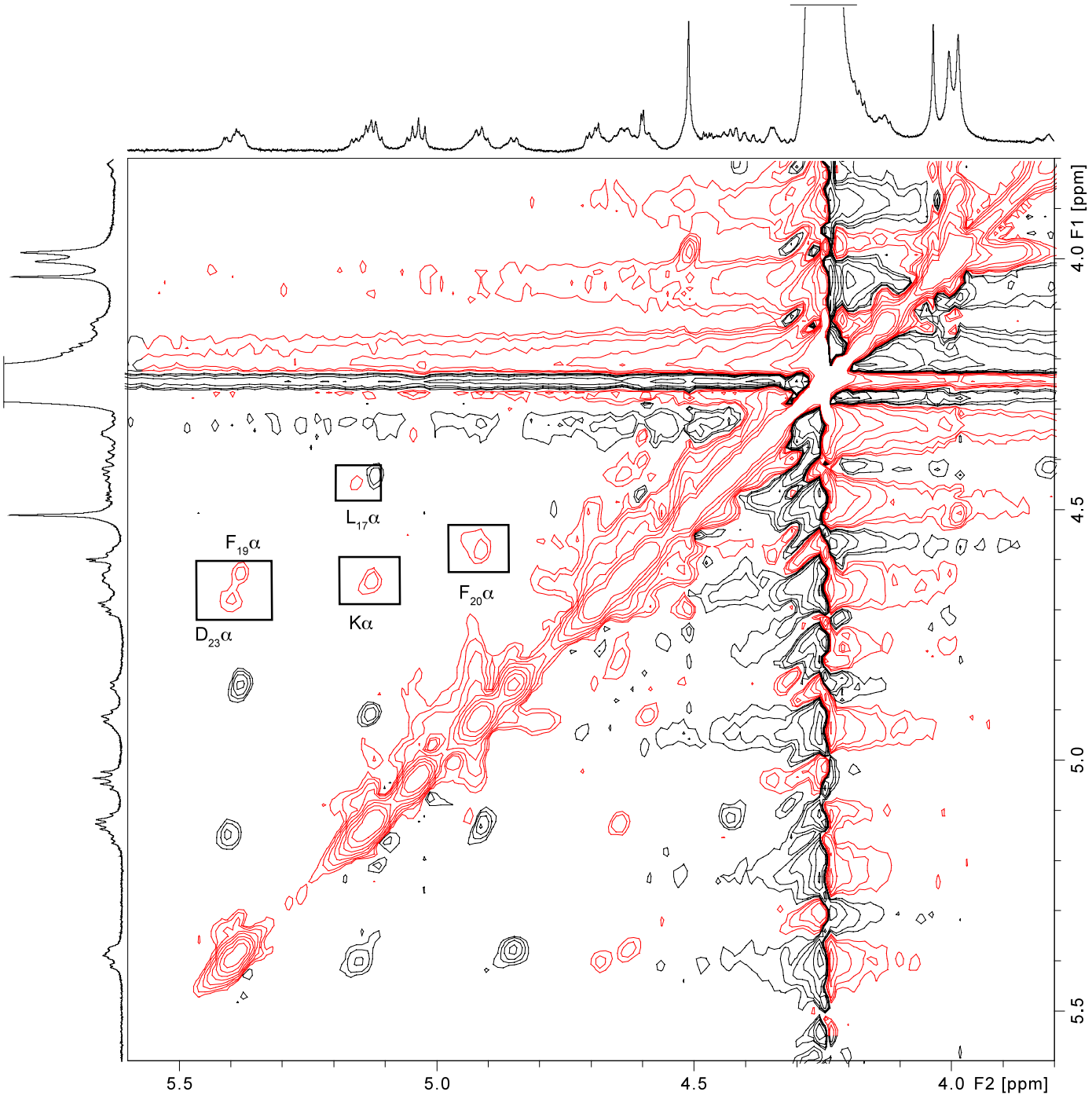
^{1}H NMR 2D EXSY (ROESY spectra showing exchange crosspeaks)
Peptide **2a**, 2 mM in D_2O, 600 MHz, 350 K
200 ms spin-locking time
Exchange crosspeaks shown in red; ROE crosspeaks shown in black



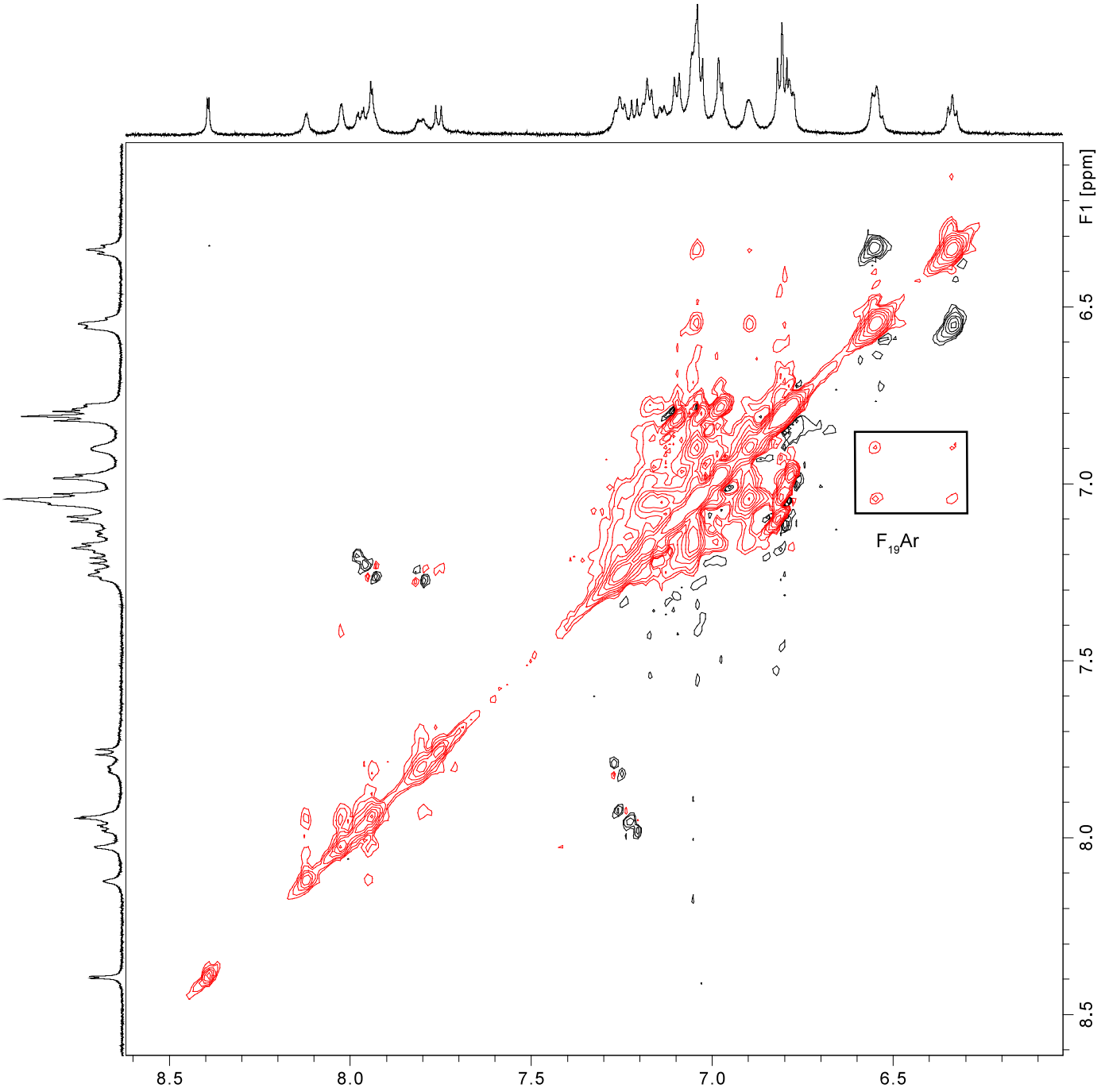
^{1}H NMR 2D EXSY (ROESY spectra showing exchange crosspeaks)
Peptide **2a**, 2 mM in D_2O, 600 MHz, 350 K
200 ms spin-locking time
Exchange crosspeaks shown in red; ROE crosspeaks shown in black



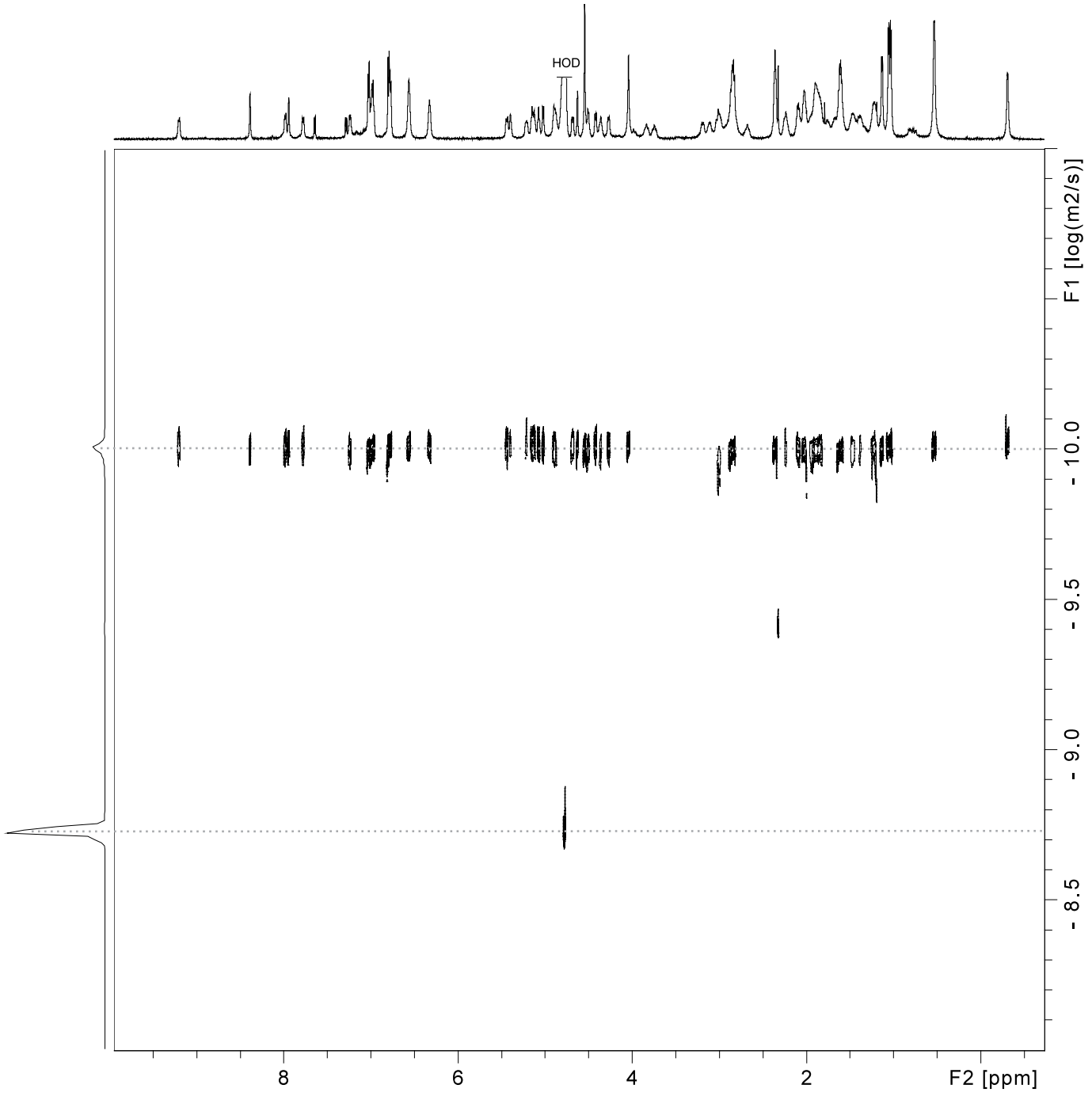
^{1}H NMR 2D EXSY (ROESY spectra showing exchange crosspeaks)
Peptide **2a**, 2 mM in D_2O, 600 MHz, 350 K
200 ms spin-locking time
Exchange crosspeaks shown in red; ROE crosspeaks shown in black



^{1}H NMR 2D EXSY (ROESY spectra showing exchange crosspeaks)
Peptide **2a**, 2 mM in D_2O, 600 MHz, 350 K
200 ms spin-locking time
Exchange crosspeaks shown in red; ROE crosspeaks shown in black



2D DOSY spectrum of macrocyclic β-sheet **2a**
2 mM in D_2O, 600 MHz, 298 K
tetramer predominates



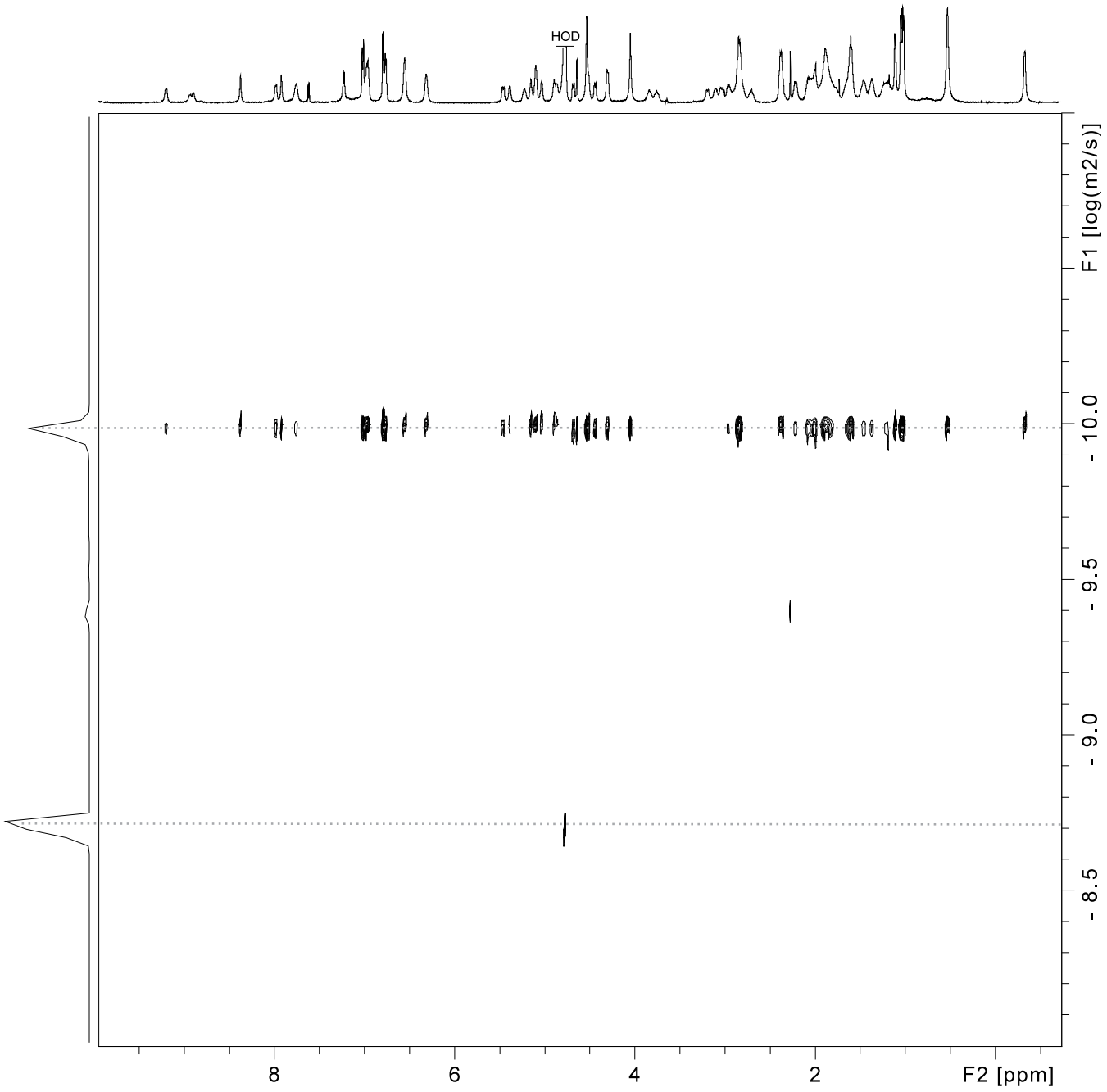
Calculation for 2a at 2.0 mM

DC_{HOD} = 19.0 x 10^{-10} m^2/s [a]
log DC_{HOD} = -8.721

For 2a tetramer, log DC (m^2/s) = -10.00(1), DC = $10^{-10.001}$ m^2/s = 10.0 x 10^{-11} m^2/s = 10.0 x 10^{-7} cm^2/s

[a] Longsworth, L. G. J. Phys. Chem. 1960, 64, 1914–1917.

2D DOSY spectrum of macrocyclic β-sheet **2a**
8 mM in D_2O, 600 MHz, 298 K
tetramer predominates

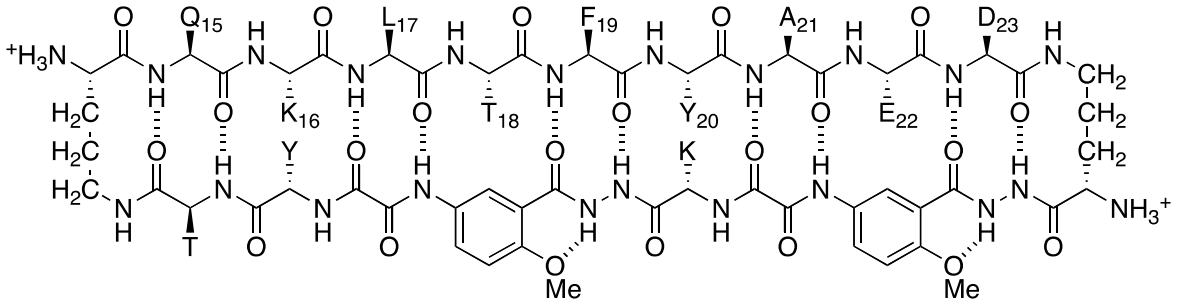


Calculation for 2a at 8.0 mM

DC_{HOD} = 19.0 x 10^{-10} m^2/s [a]
log DC_{HOD} = -8.721

For 2a tetramer, log DC (m^2/s) = -9.99(4), DC = $10^{-9.994}$ m^2/s = 10.1 x 10^{-11} m^2/s = 10.1 x 10^{-7} cm^2/s

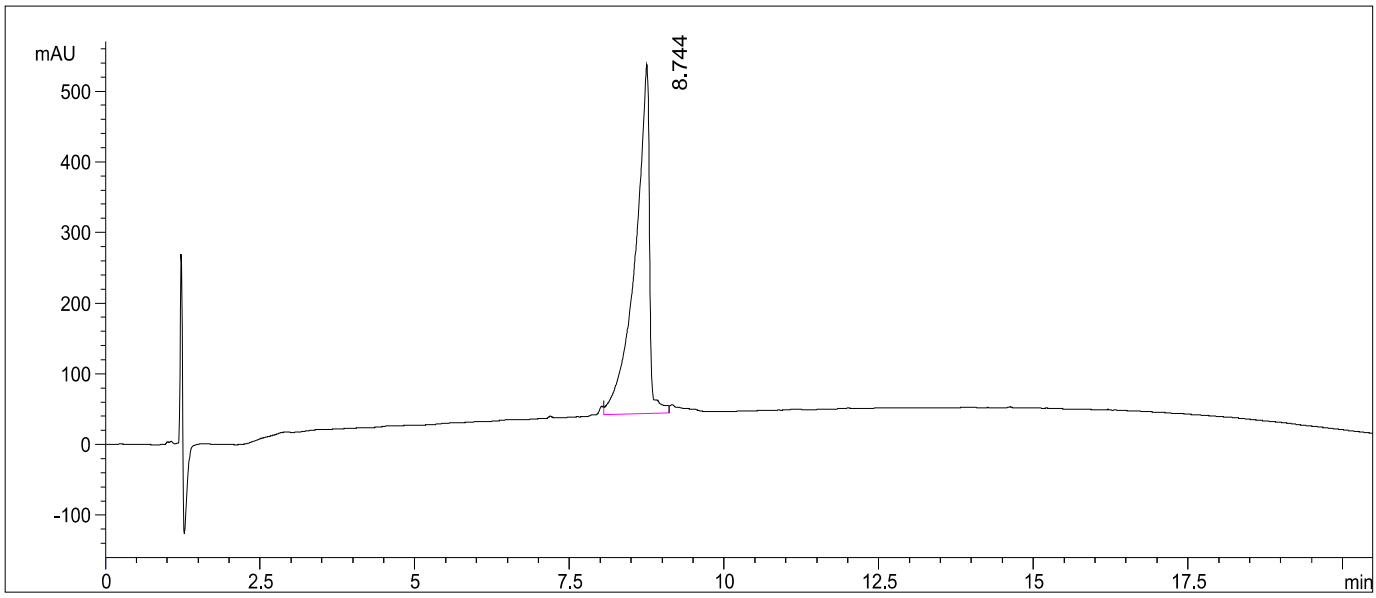
[a] Longsworth, L. G. J. Phys. Chem. 1960, 64, 1914–1917.



macrocyclic β-sheet peptide **2b** (as the TFA salt)

molecular weight calculated for $C_{100}H_{139}N_{25}O_{31} \cdot 4CF_3CO_2H$ (TFA salt of **2b**): 2643.42
molecular weight calculated for $C_{100}H_{139}N_{25}O_{31}$ (free base of **2b**): 2187.32
exact mass calculated for $C_{100}H_{139}N_{25}O_{31}$ (free base of **2b**): 2186.01

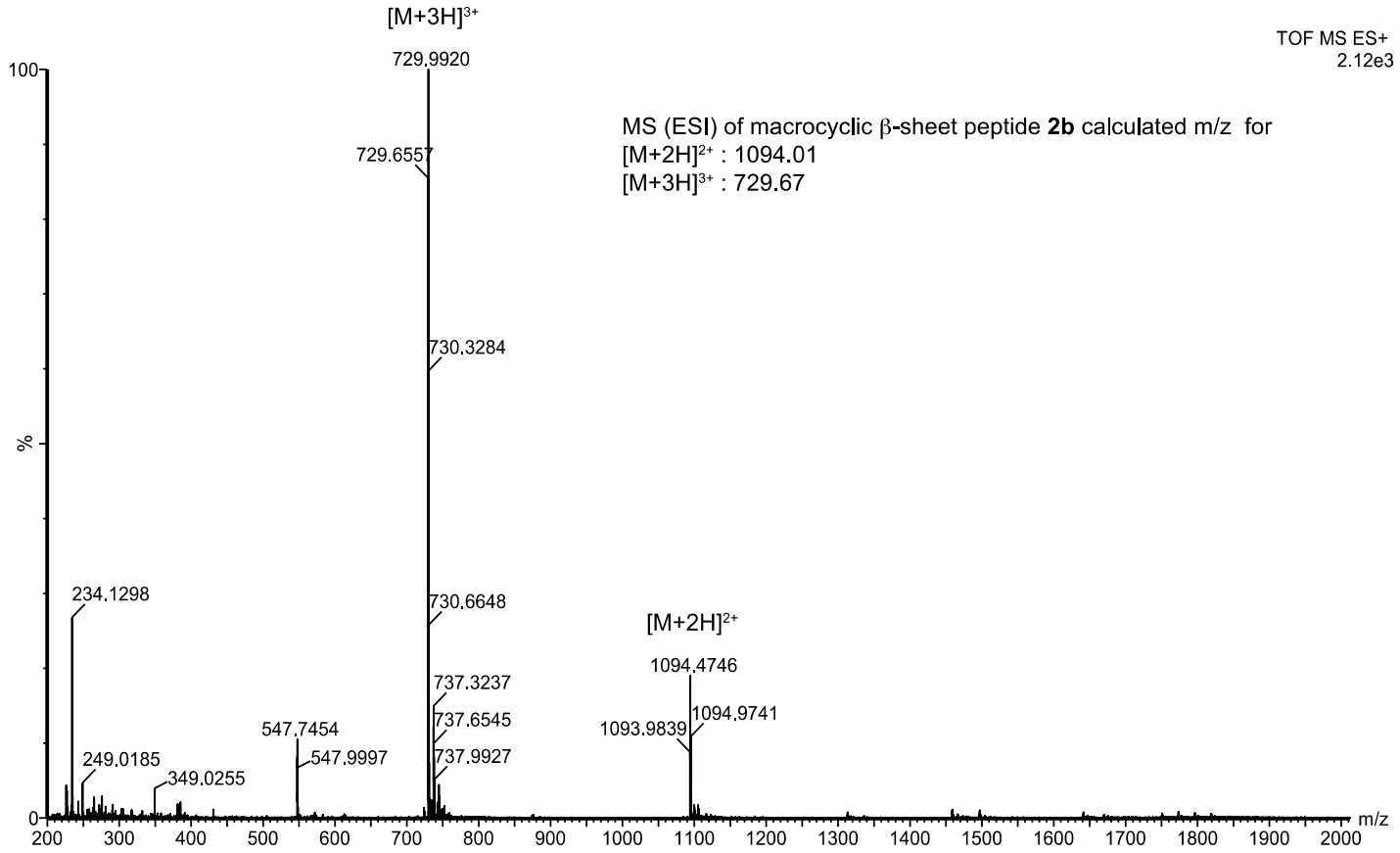
Analytical RP-HPLC of macrocyclic β-peptide **2b**

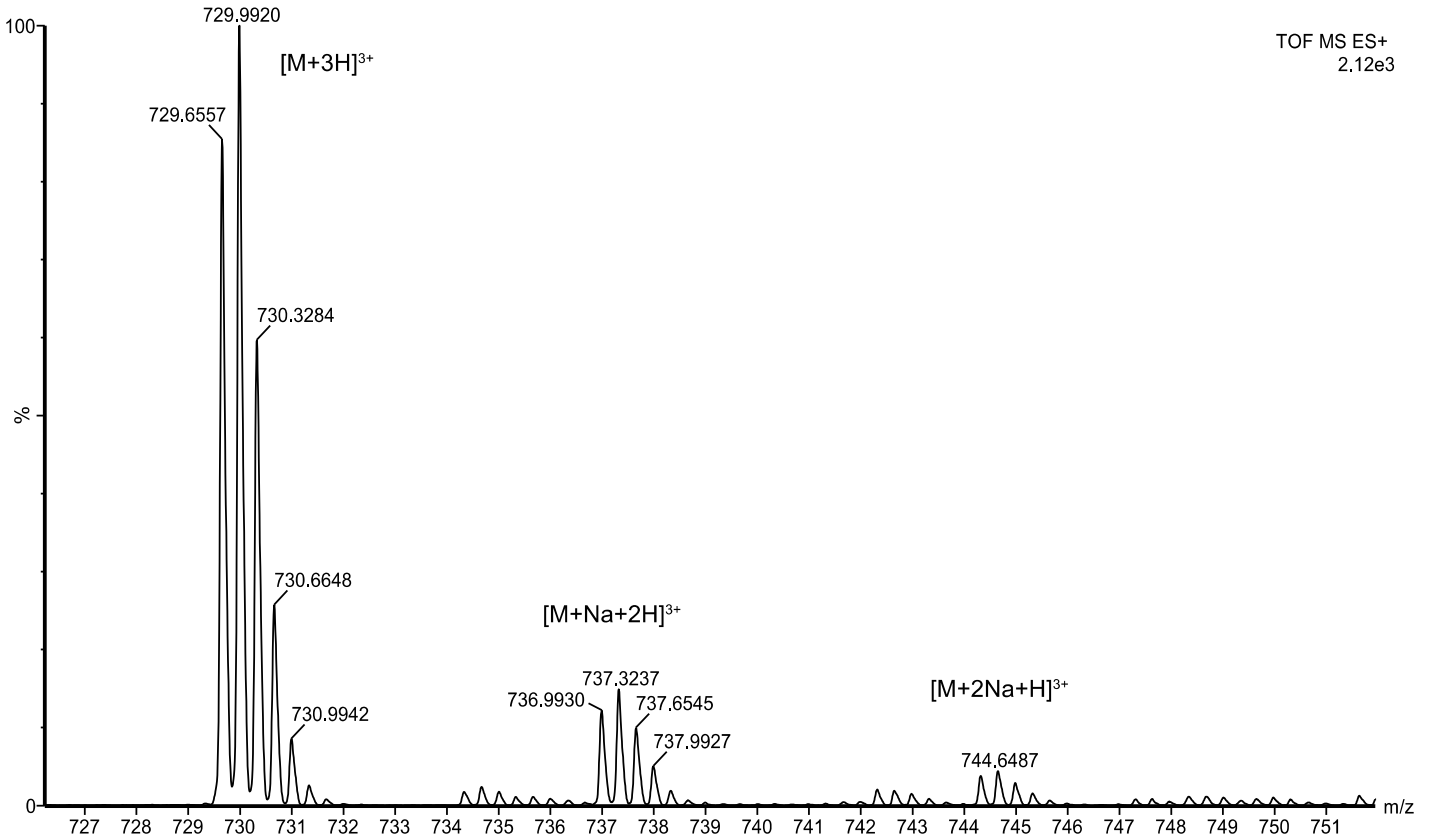


Signal 1: VWD1 A, Wavelength=214 nm

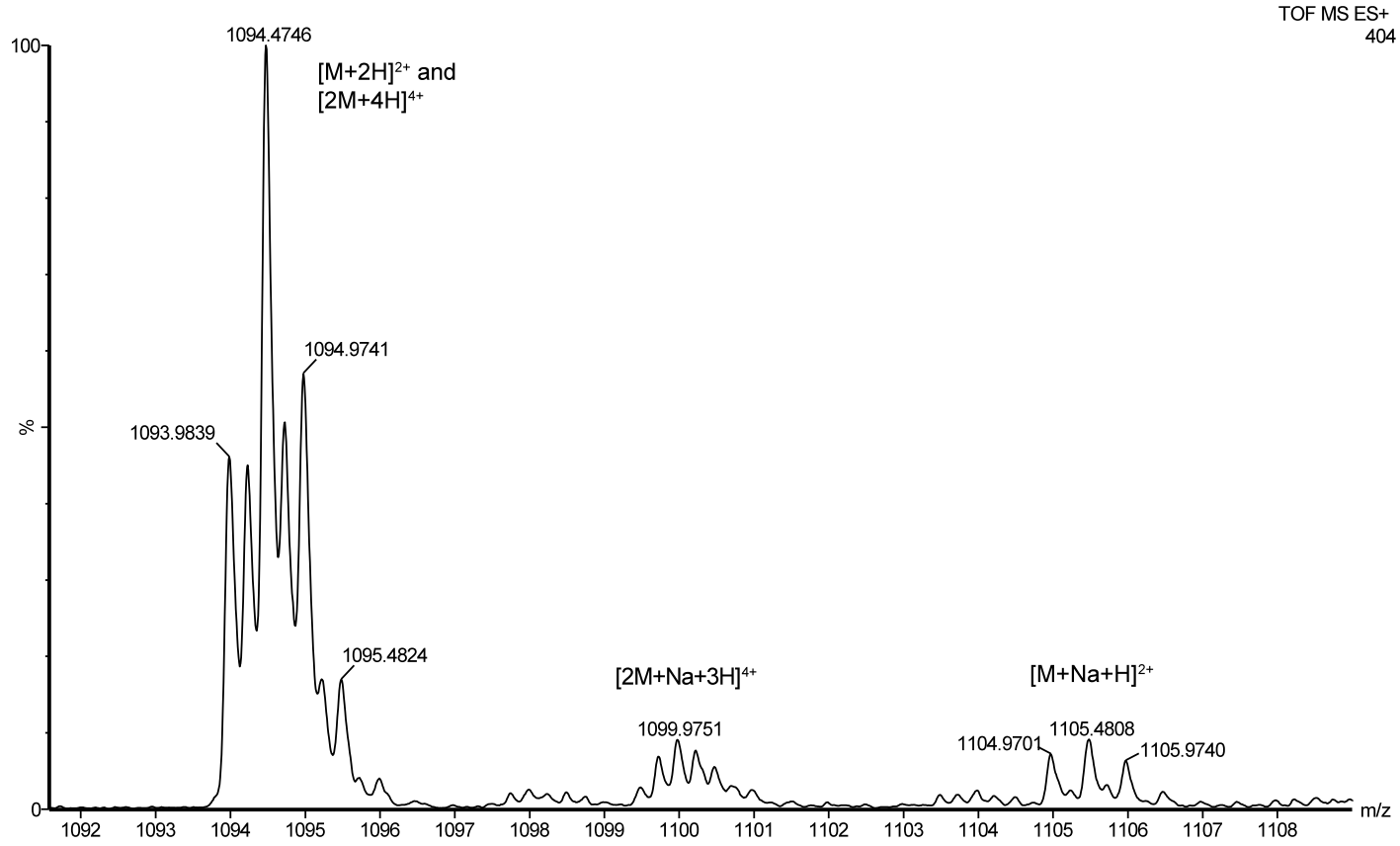
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	8.744	VV	0.2105	7939.88721	494.81061	100.0000
Totals :				7939.88721	494.81061	

Macrocyclic β-sheet peptide **2b**

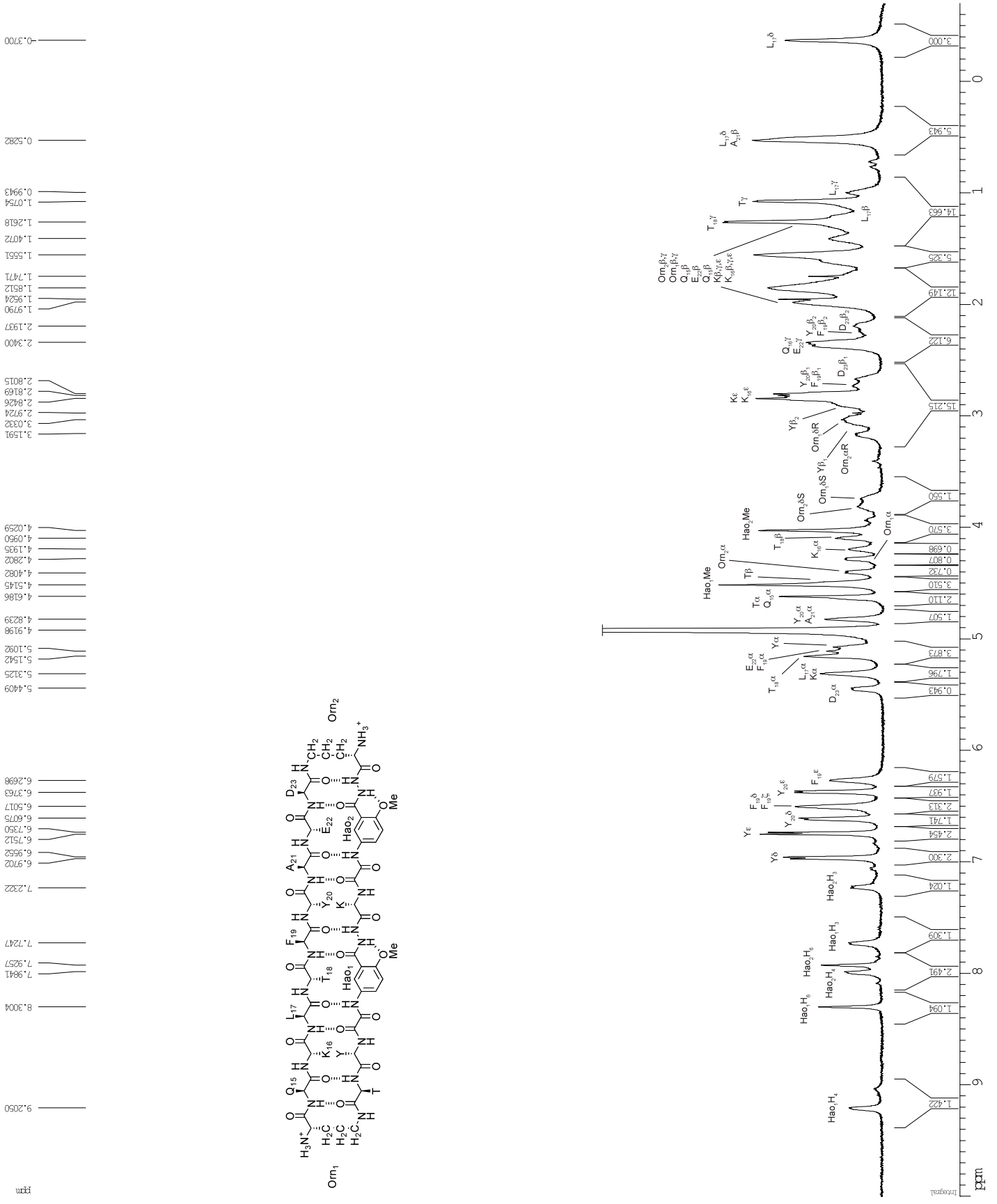




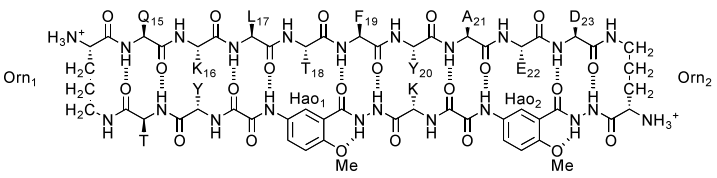
Macrocyclic β-sheet peptide **2b**

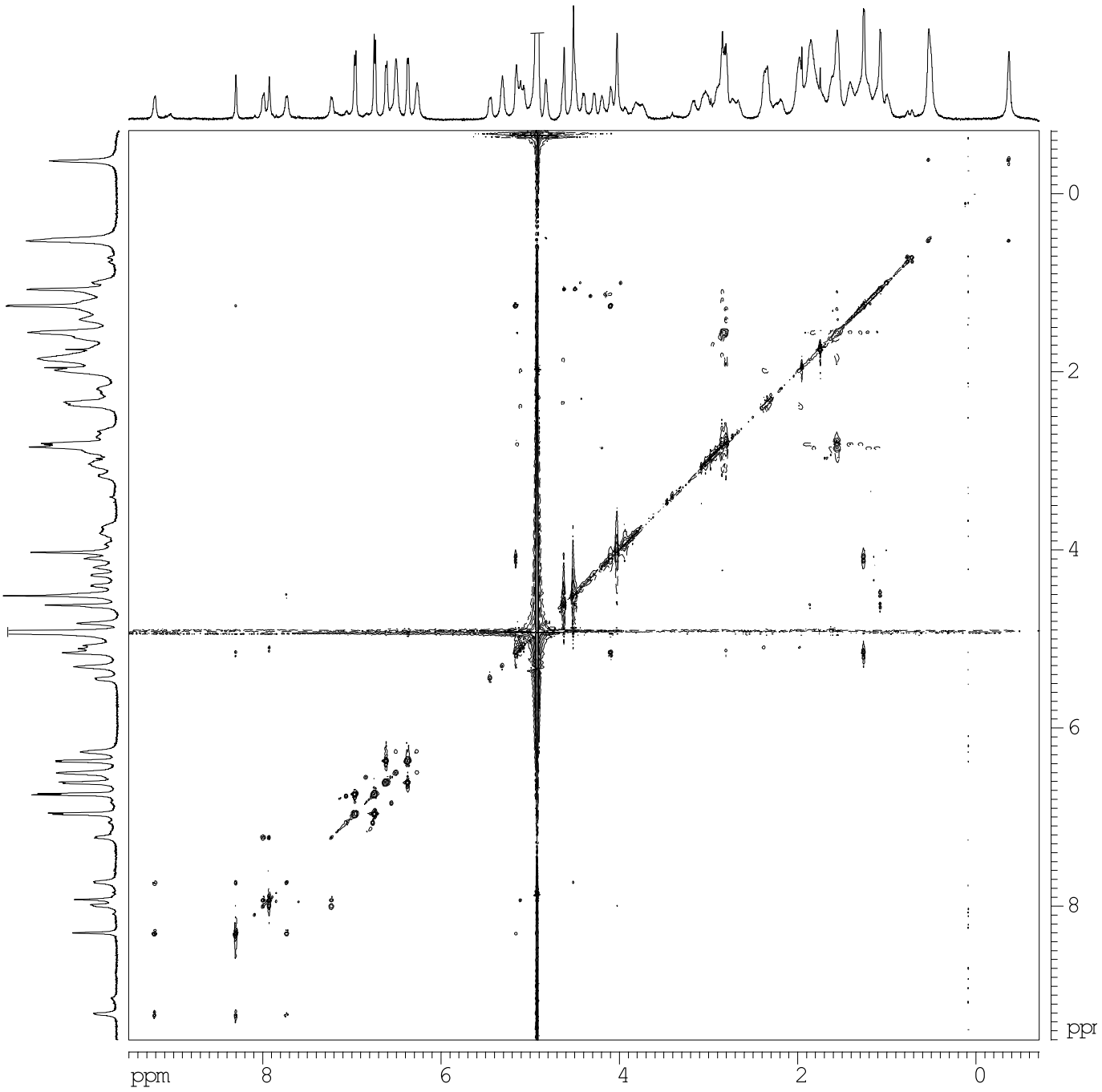


1D ^{1}H NMR spectrum of macrocyclic β-sheet **2b**
2 mM in D_2O, 500 MHz, 285 K
tetramer predominates

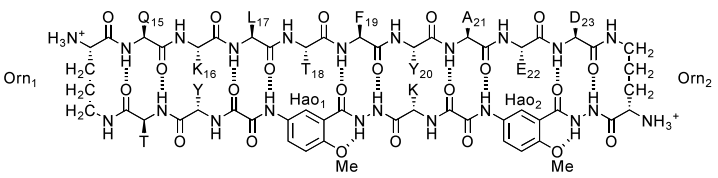


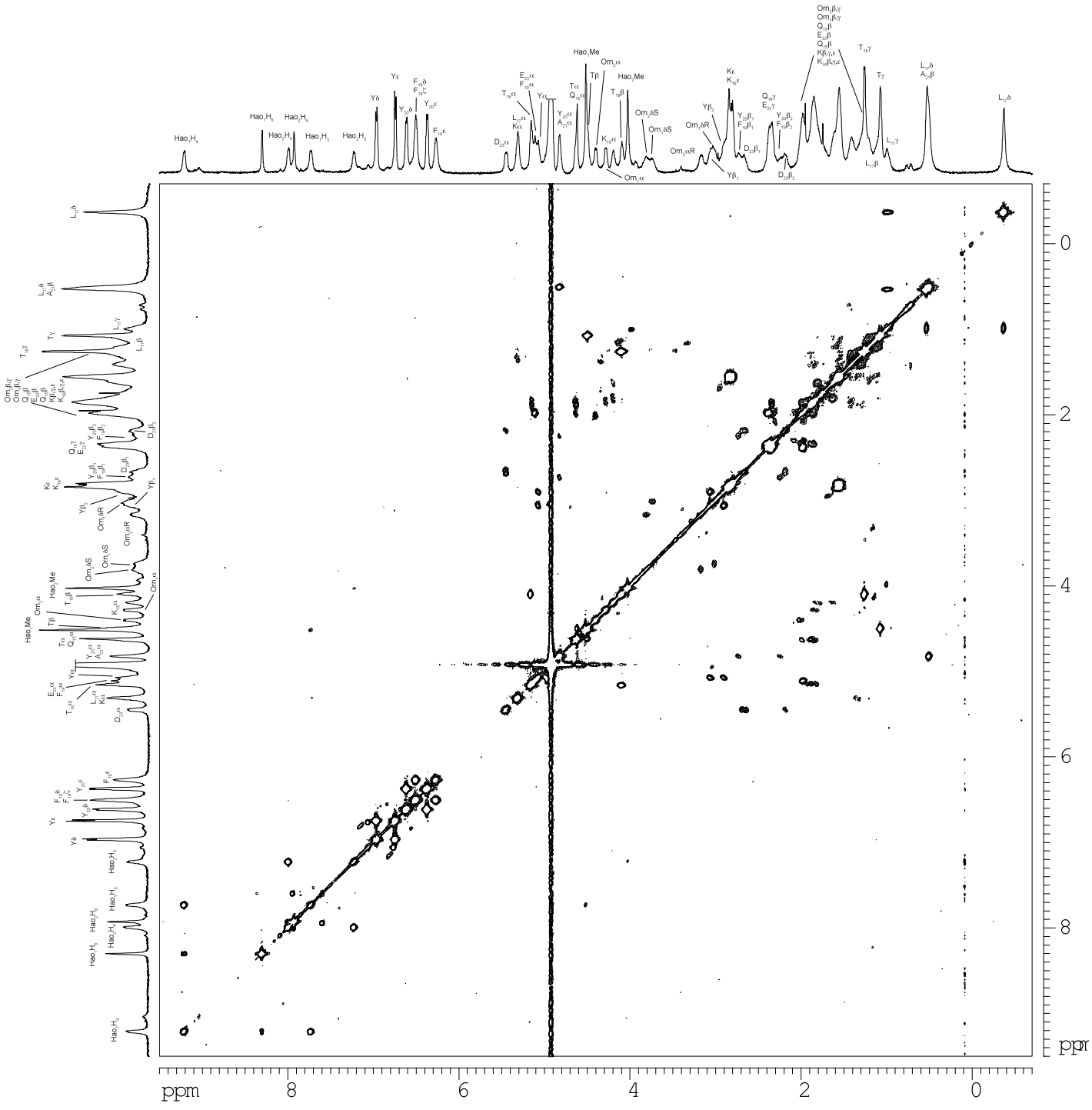
2D TOCSY spectrum of macrocyclic β-sheet **2b** as tetramer
2 mM in D_2O, 500 MHz, 285 K
150-ms spin-locking mixing time
tetramer predominates





2D COSY spectrum of macrocyclic β-sheet **2b** as tetramer
2 mM in D_2O, 500 MHz, 285 K
tetramer predominates



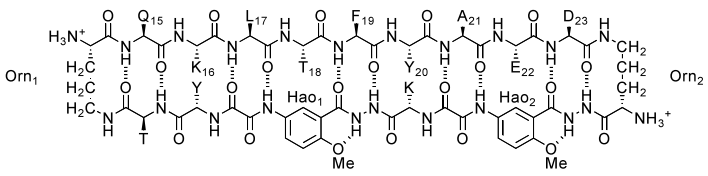


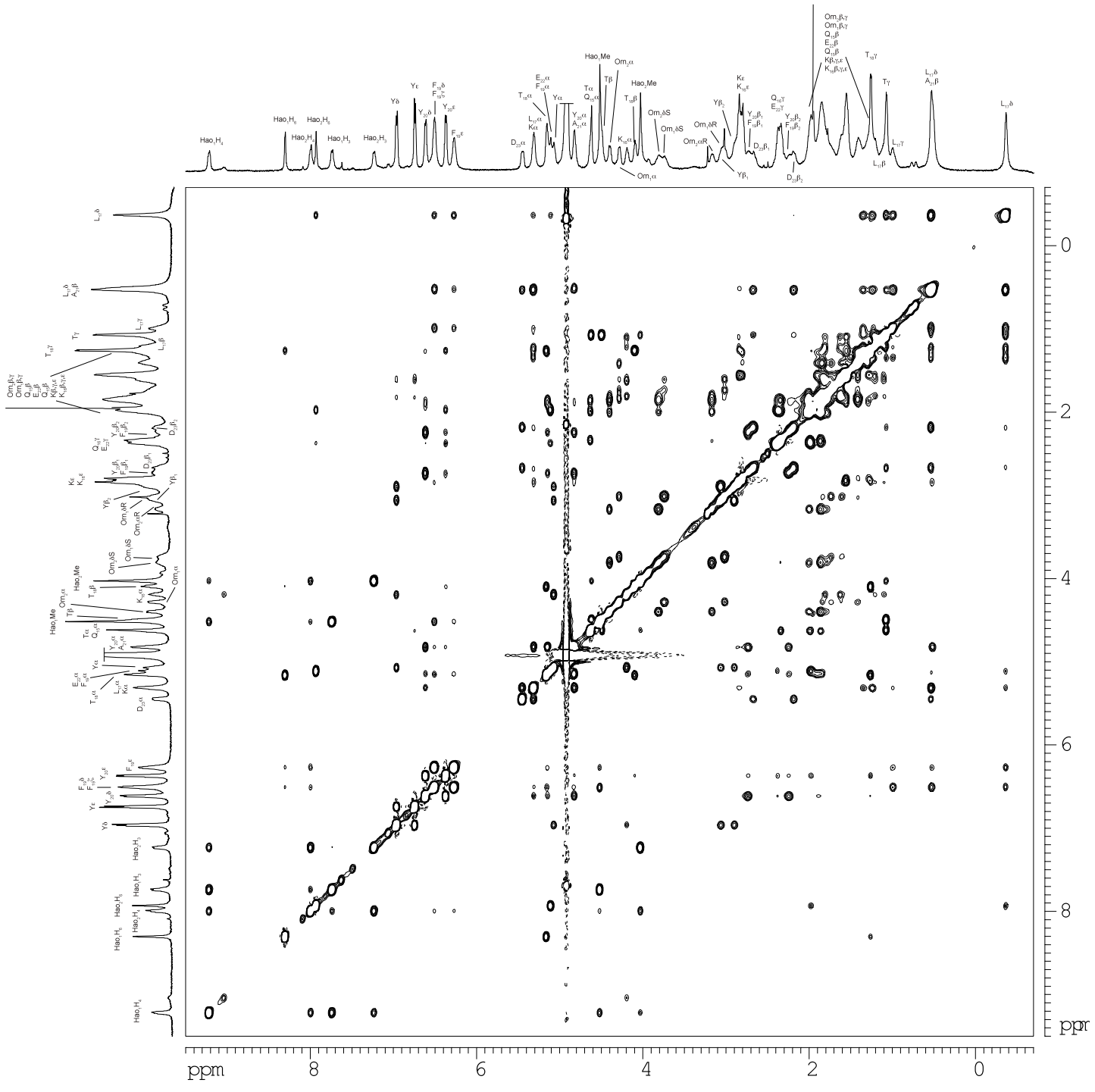
2D NOESY spectrum of macrocyclic β-sheet **2b** as tetramer

2 mM in D_2O, 500 MHz, 285 K

150-ms spin-locking mixing time

tetramer predominates





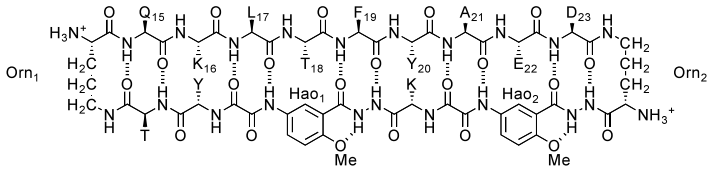
2D NOESY spectrum of macrocyclic β-sheet **2b** as tetramer

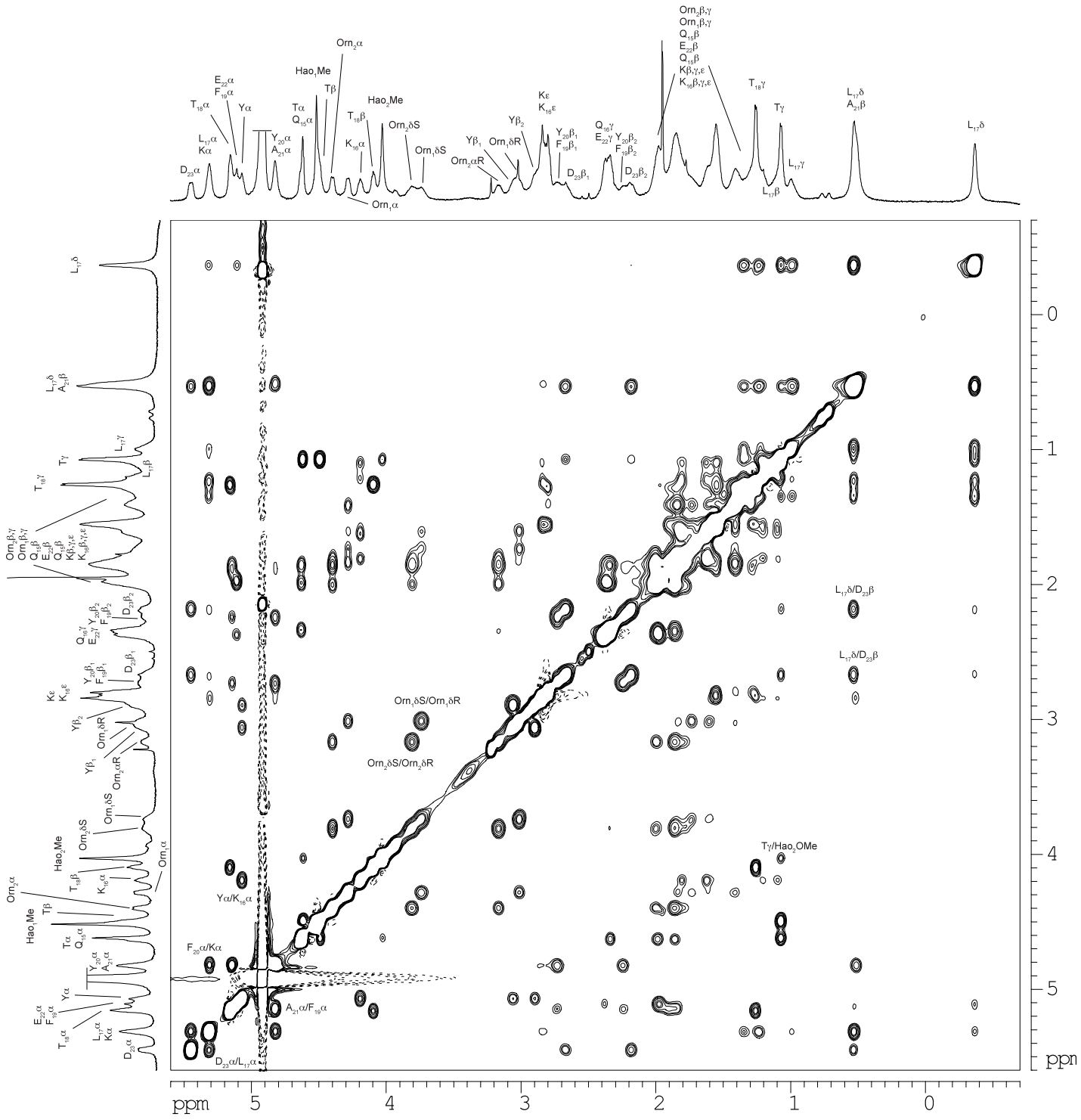
2 mM in D_2O, 500 MHz, 285 K

150-ms spin-locking mixing time

tetramer predominates

#select NMR crosspeaks are labeled





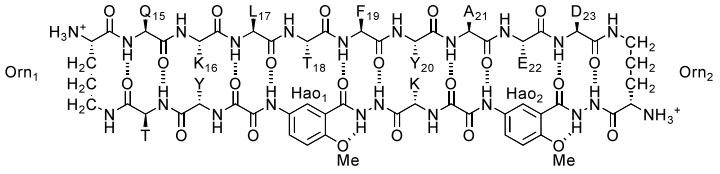
2D NOESY spectrum of macrocyclic β-sheet **2b** as tetramer

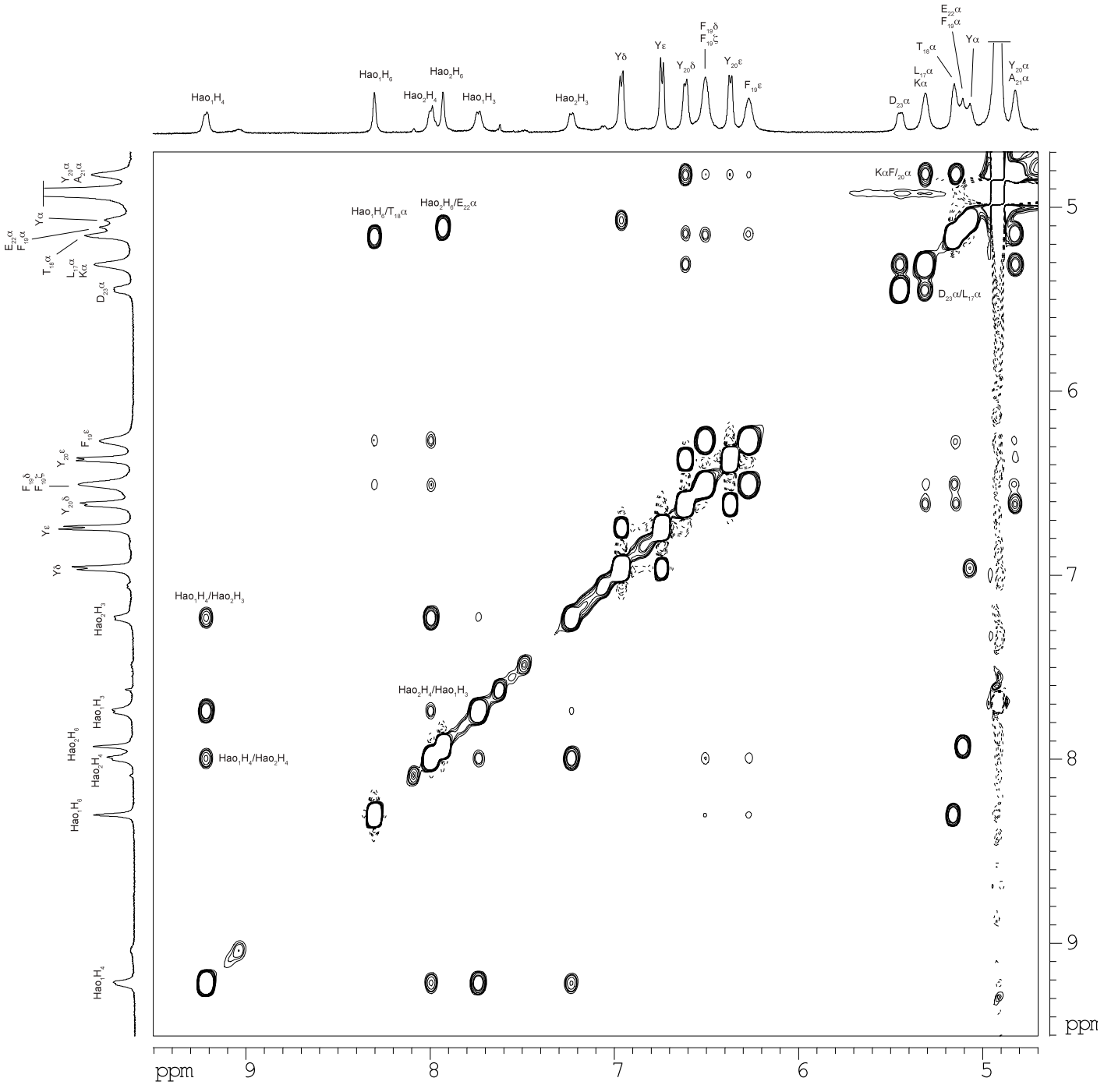
2 mM in D_2O, 500 MHz, 285 K

150-ms spin-locking mixing time

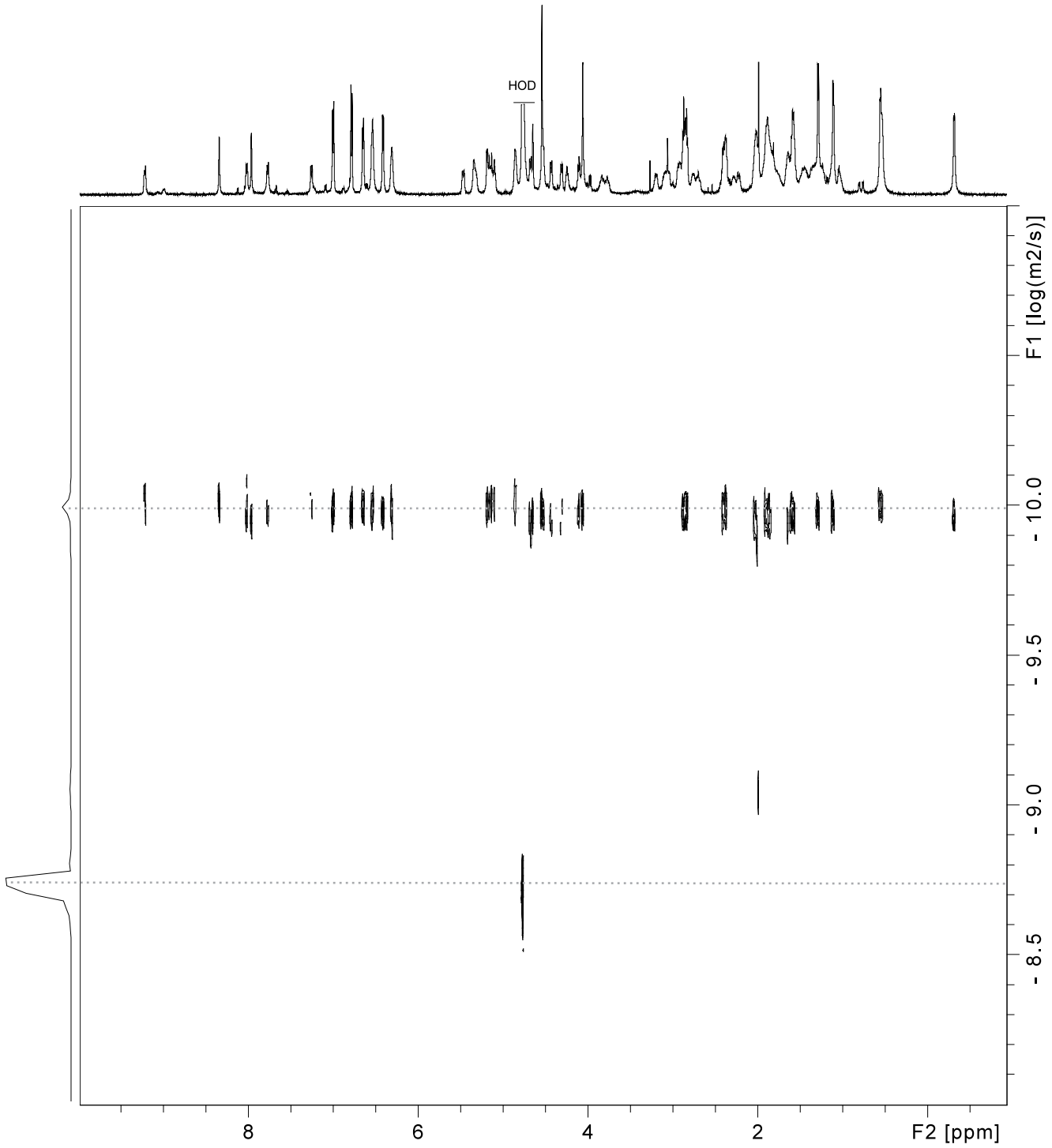
tetramer predominates

#select NMR crosspeaks are labeled





2D DOSY spectrum of macrocyclic β-sheet **2b**
2 mM in D_2O, 600 MHz, 298 K
tetramer predominates



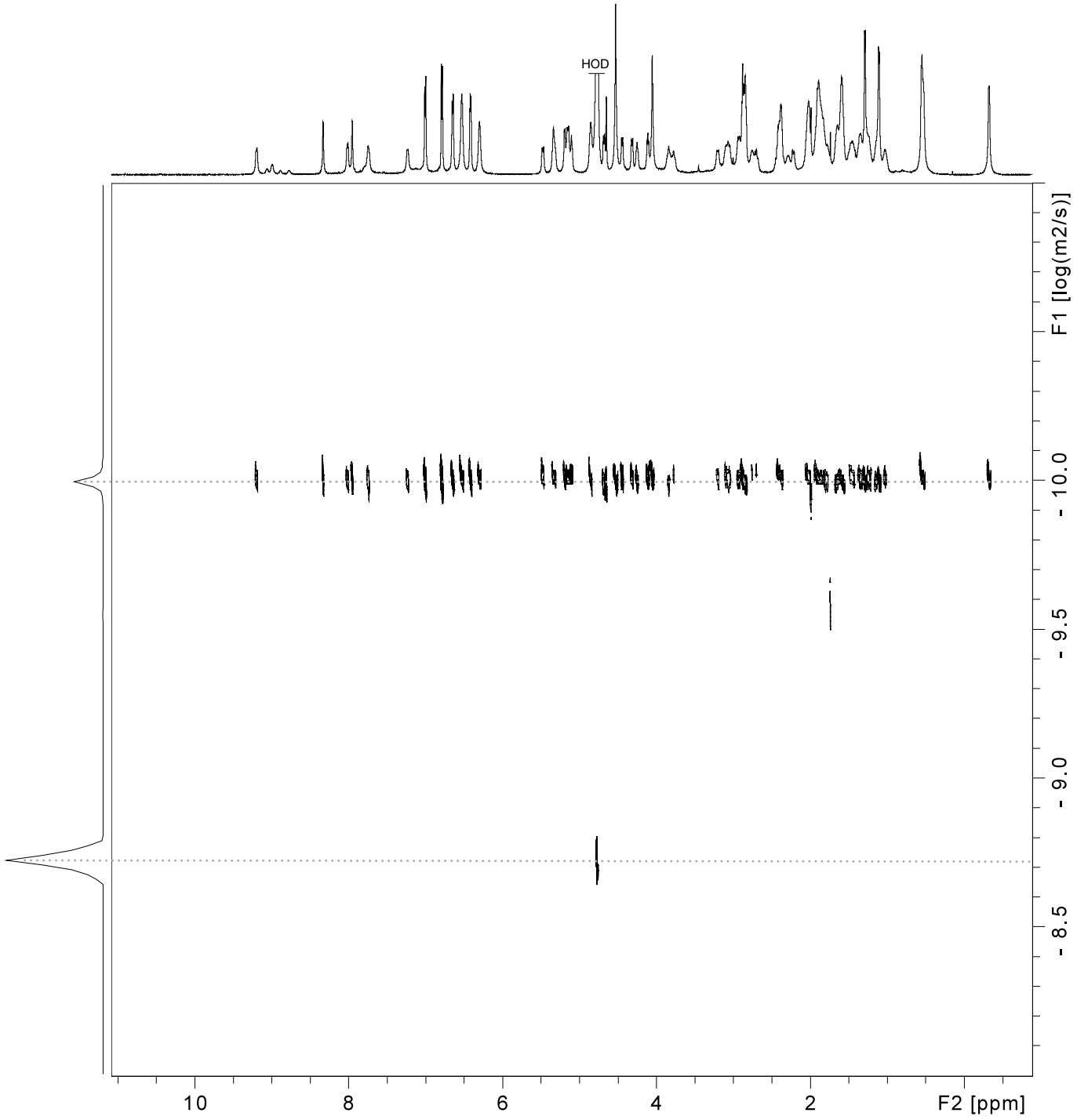
Calculation for 2b at 2.0 mM

DC_{HOD} = 19.0 x 10^{-10} m^2/s [a]
log DC_{HOD} = -8.721

For 2b tetramer, log DC (m^2/s) = -9.98(6), DC = $10^{-9.986}$ m^2/s = 10.3 x 10^{-11} m^2/s = 10.3 x 10^{-7} cm^2/s

[a] Longsworth, L. G. J. Phys. Chem. 1960, 64, 1914–1917.

2D DOSY spectrum of macrocyclic β-sheet **2b**
8 mM in D_2O, 600 MHz, 298 K
tetramer predominates

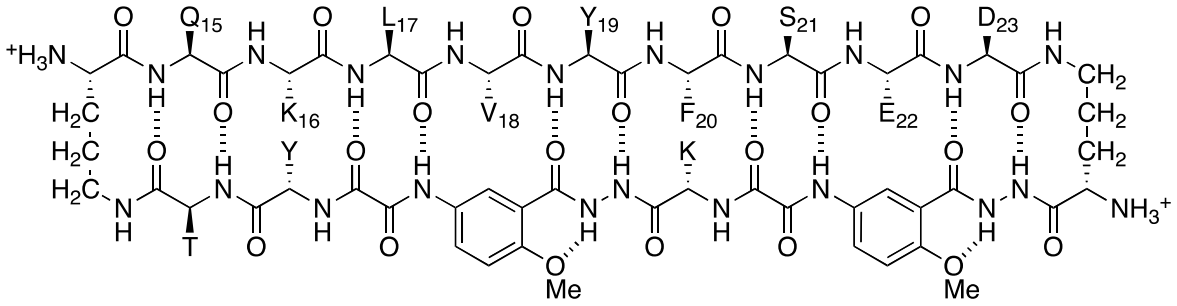


Calculation for 2b at 8.0 mM

DC_{HOD} = 19.0 x 10^{-10} m^2/s [a]
log DC_{HOD} = -8.721

For 2b tetramer, log DC (m^2/s) = -9.99(4), DC = $10^{-9.994}$ m^2/s = 10.1 x 10^{-11} m^2/s = 10.1 x 10^{-7} cm^2/s

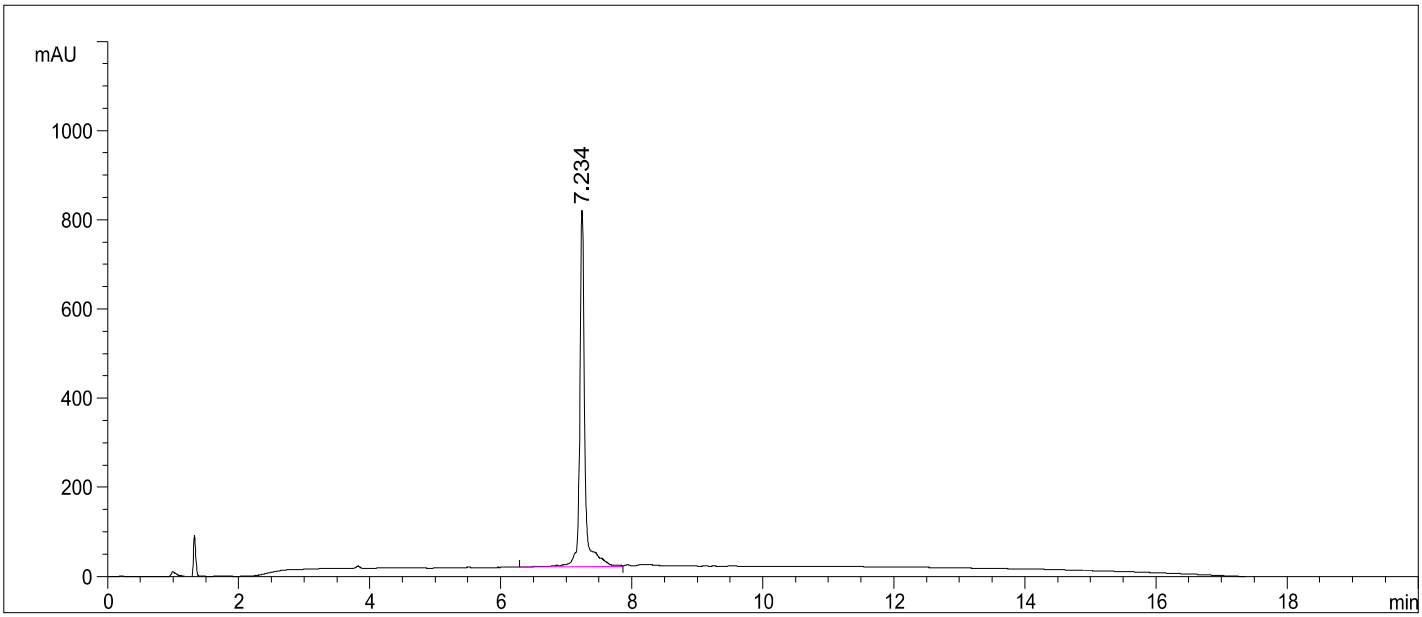
[a] Longsworth, L. G. J. Phys. Chem. 1960, 64, 1914–1917.



macrocyclic β-sheet peptide **2c** (as the TFA salt)

molecular weight calculated for $C_{101}H_{141}N_{25}O_{31} \cdot 4CF_3CO_2H$ (TFA salt of **2c**): 2657.44
molecular weight calculated for $C_{101}H_{141}N_{25}O_{31}$ (free base of **2c**): 2201.35
exact mass calculated for $C_{101}H_{141}N_{25}O_{31}$ (free base of **2c**): 2200.02

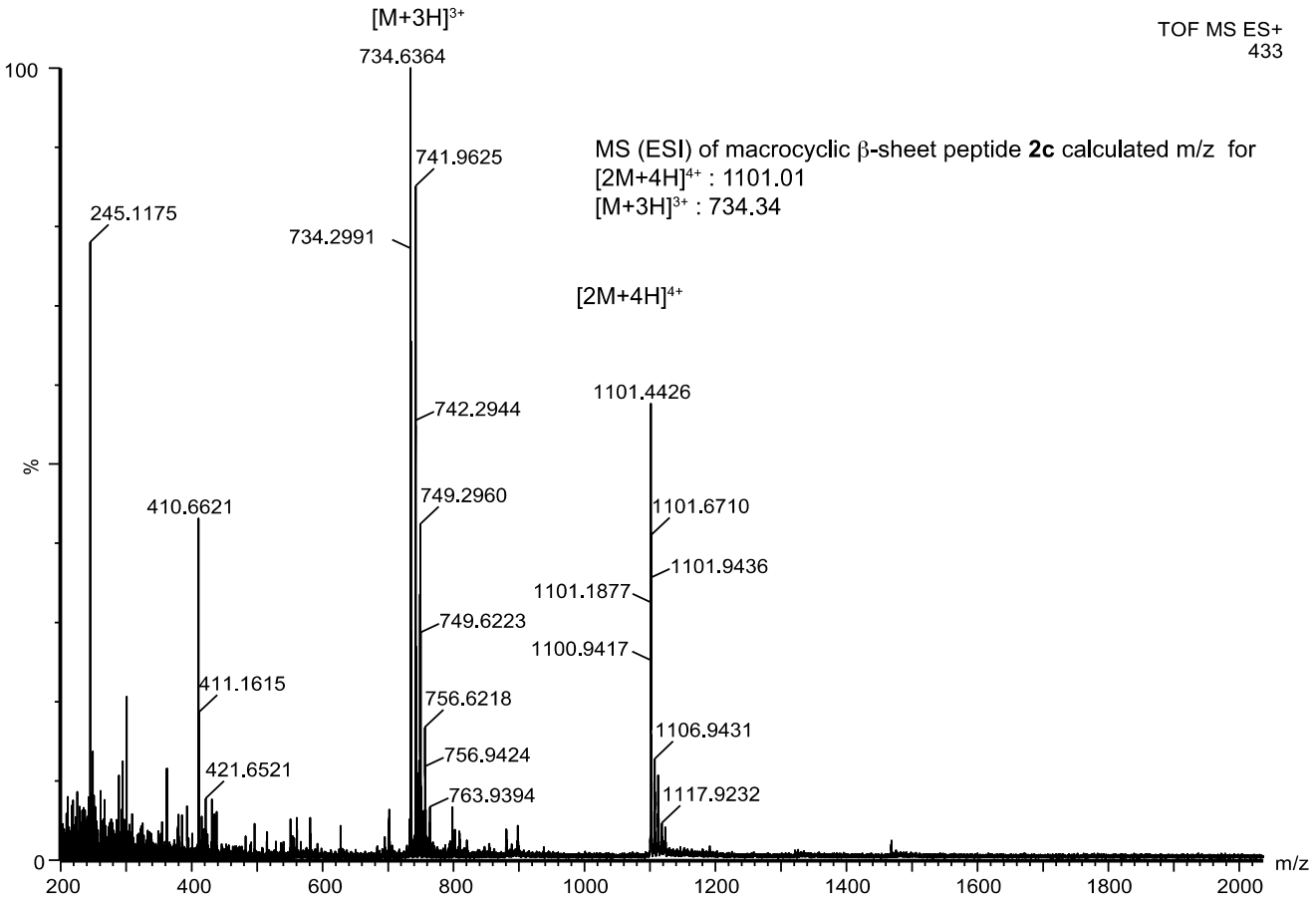
Analytical RP-HPLC of macrocyclic β-sheet peptide **2c**

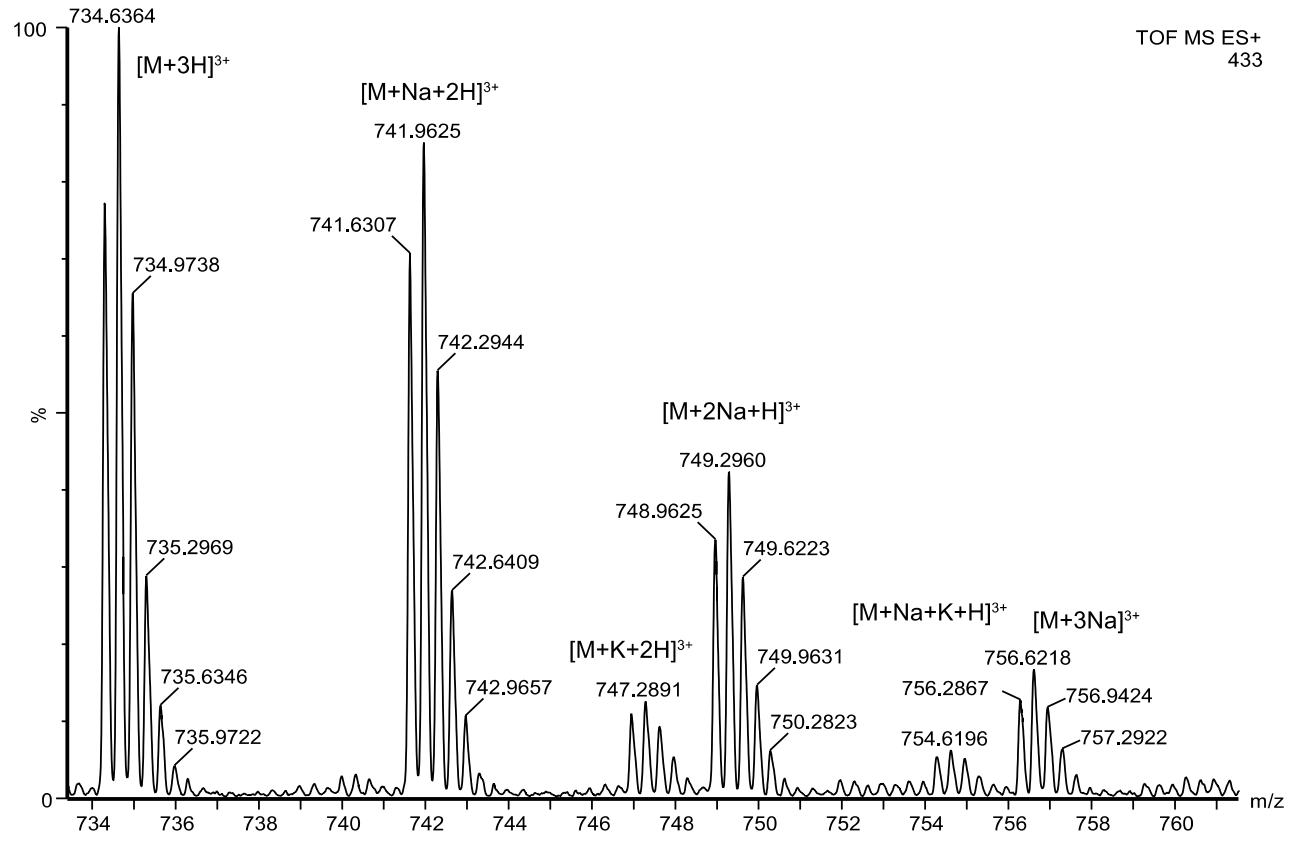


Signal 1: VWD1 A, Wavelength=214 nm

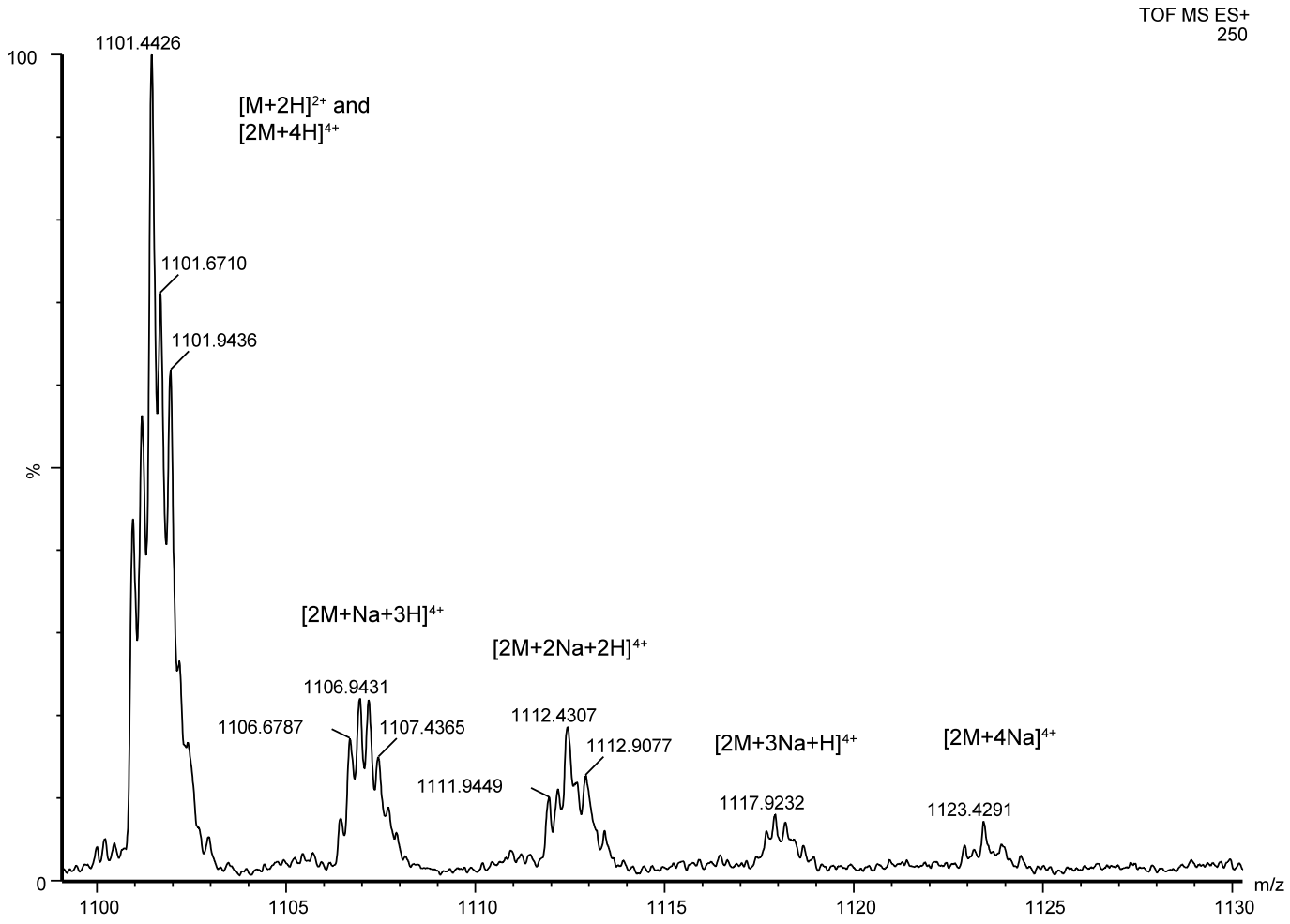
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.234	BV	0.0780	4340.98779	798.37006	100.0000
Totals :				4340.98779	798.37006	

Macrocyclic β-sheet peptide **2c**

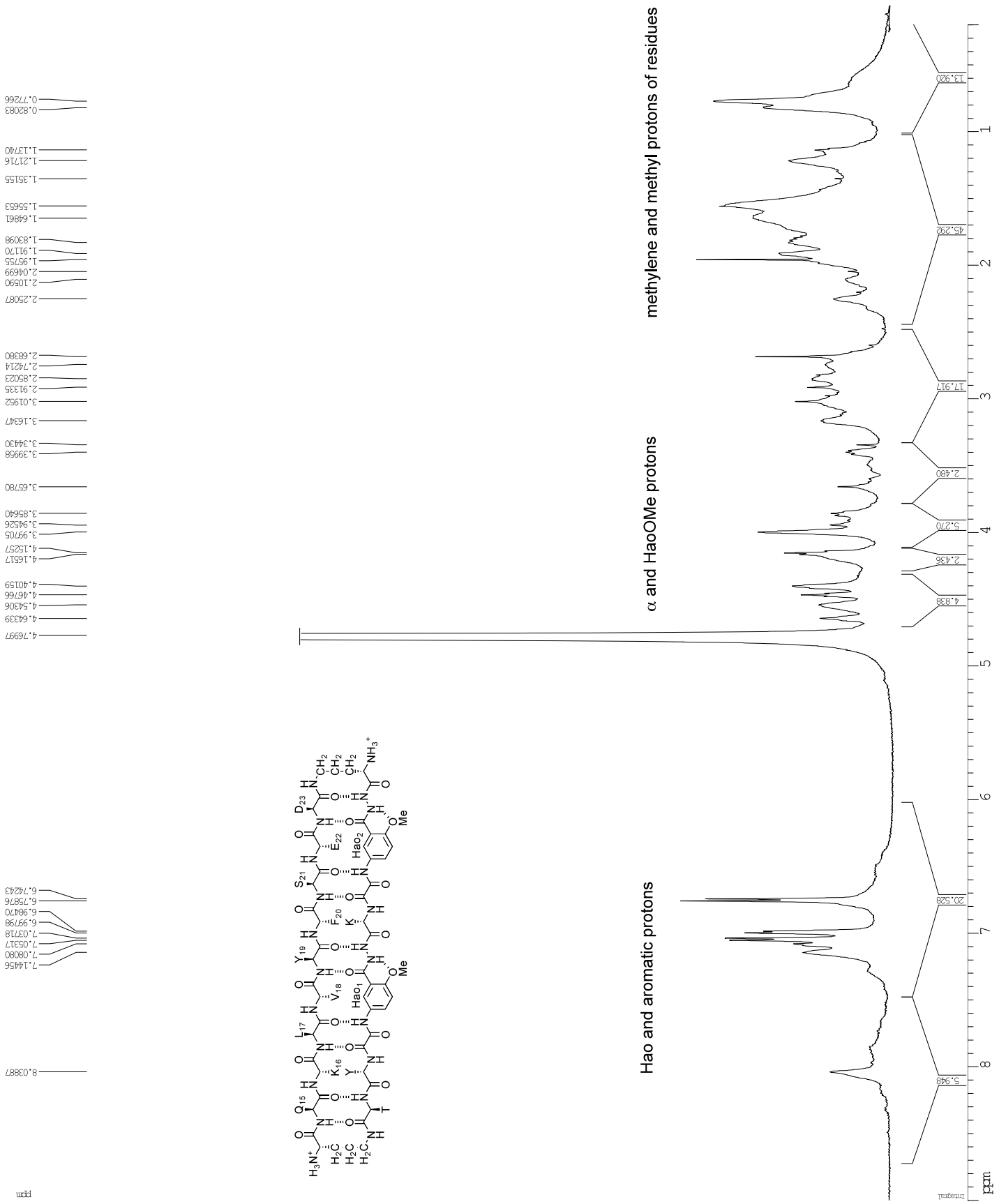




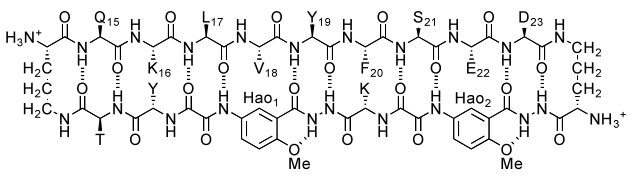
Macrocyclic β-sheet peptide **2c**

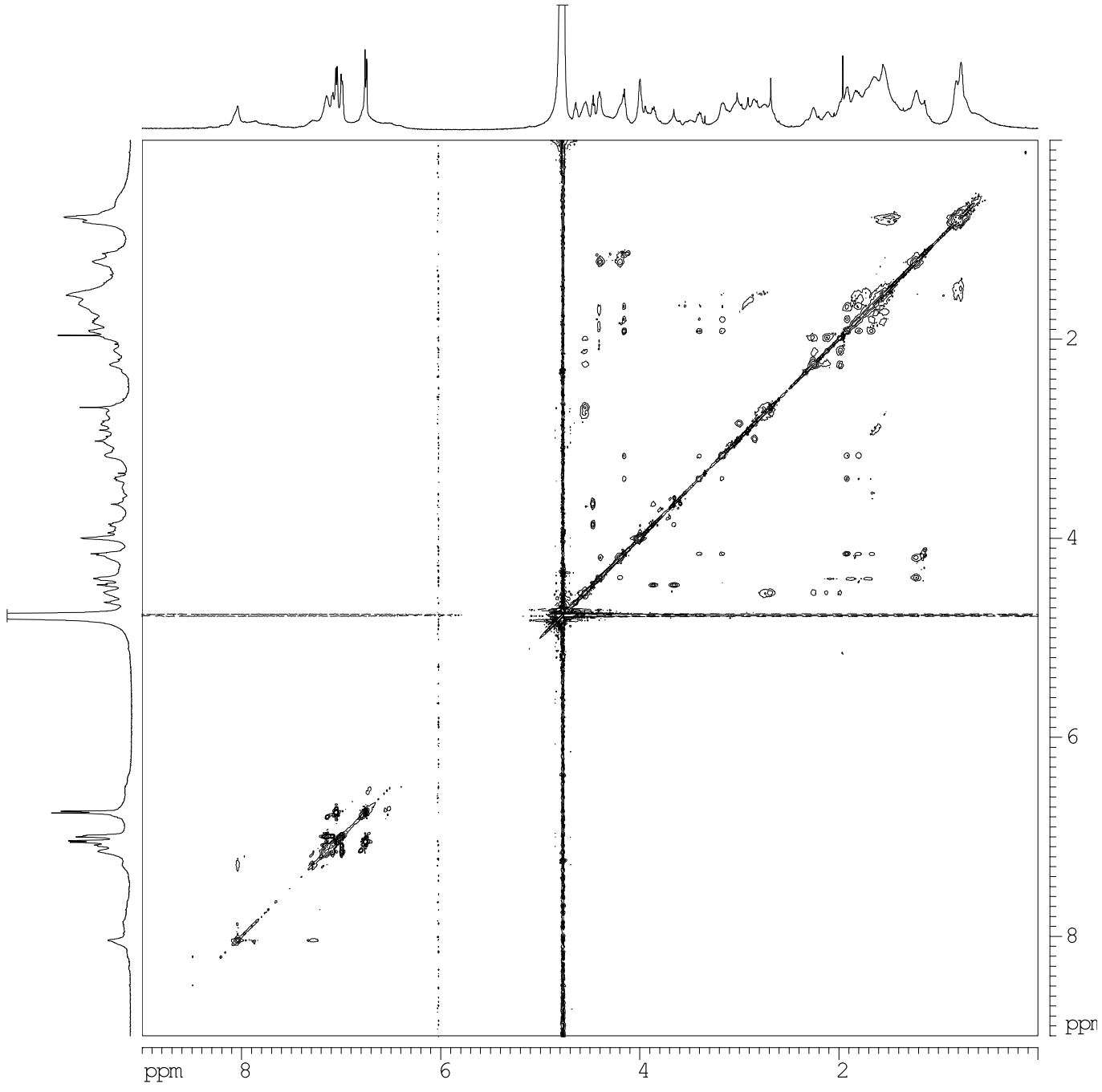


1D ^{1}H NMR spectrum of macrocyclic β-sheet **2c**
2 mM in D_2O, 500 MHz, 298 K

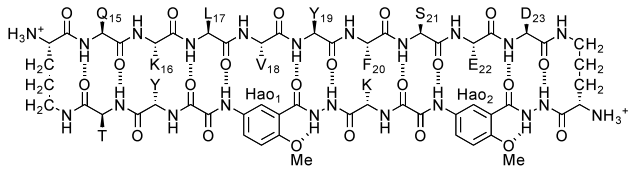


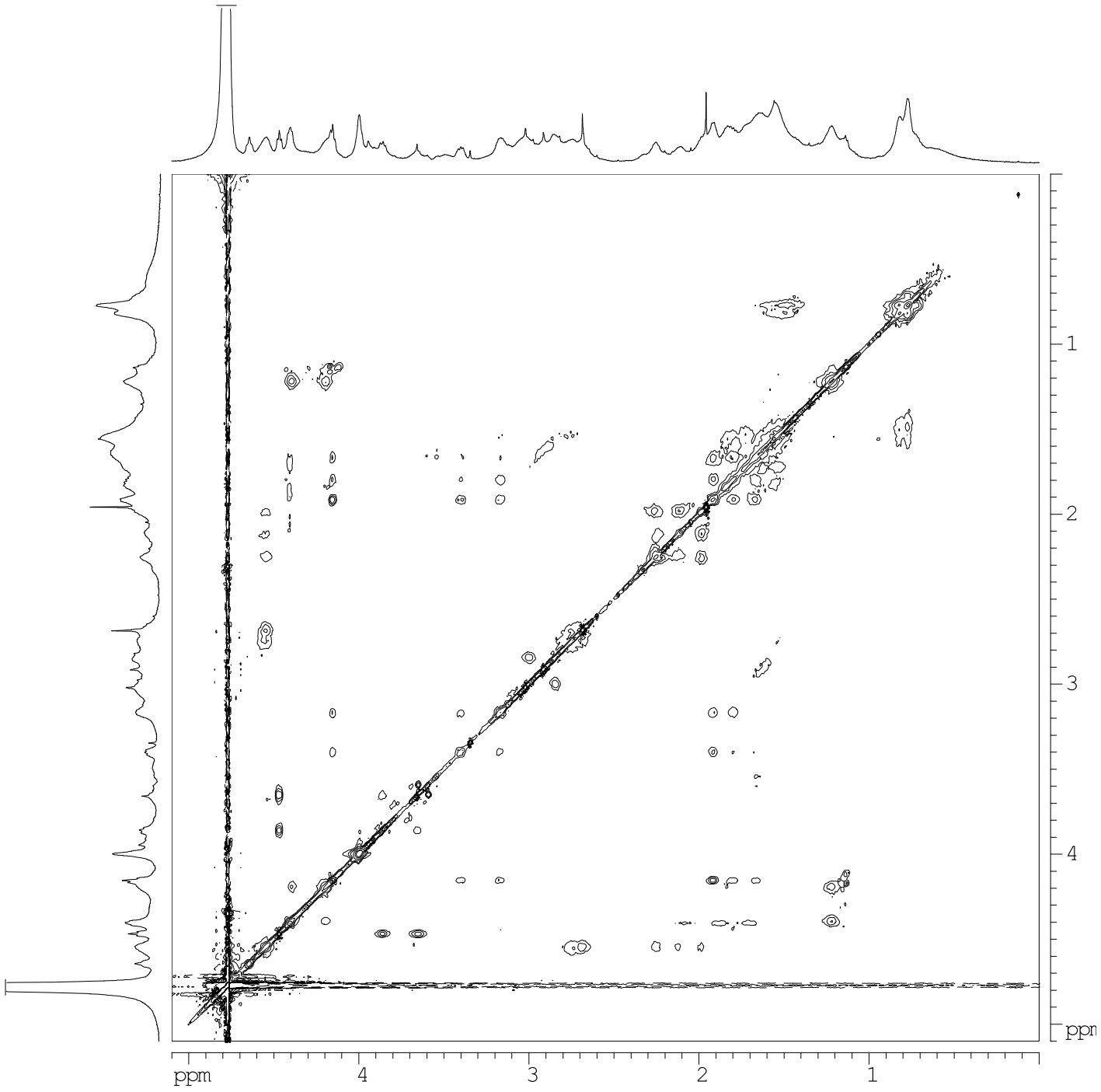
2D TOCSY spectrum of macrocyclic β-sheet **2c**
2 mM in D_2O, 500 MHz, 298 K
150-ms spin-locking mixing time



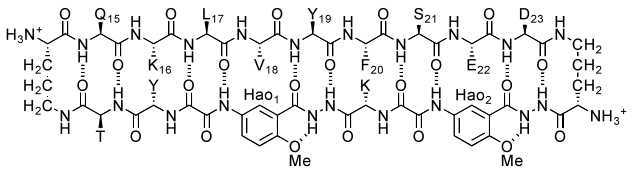


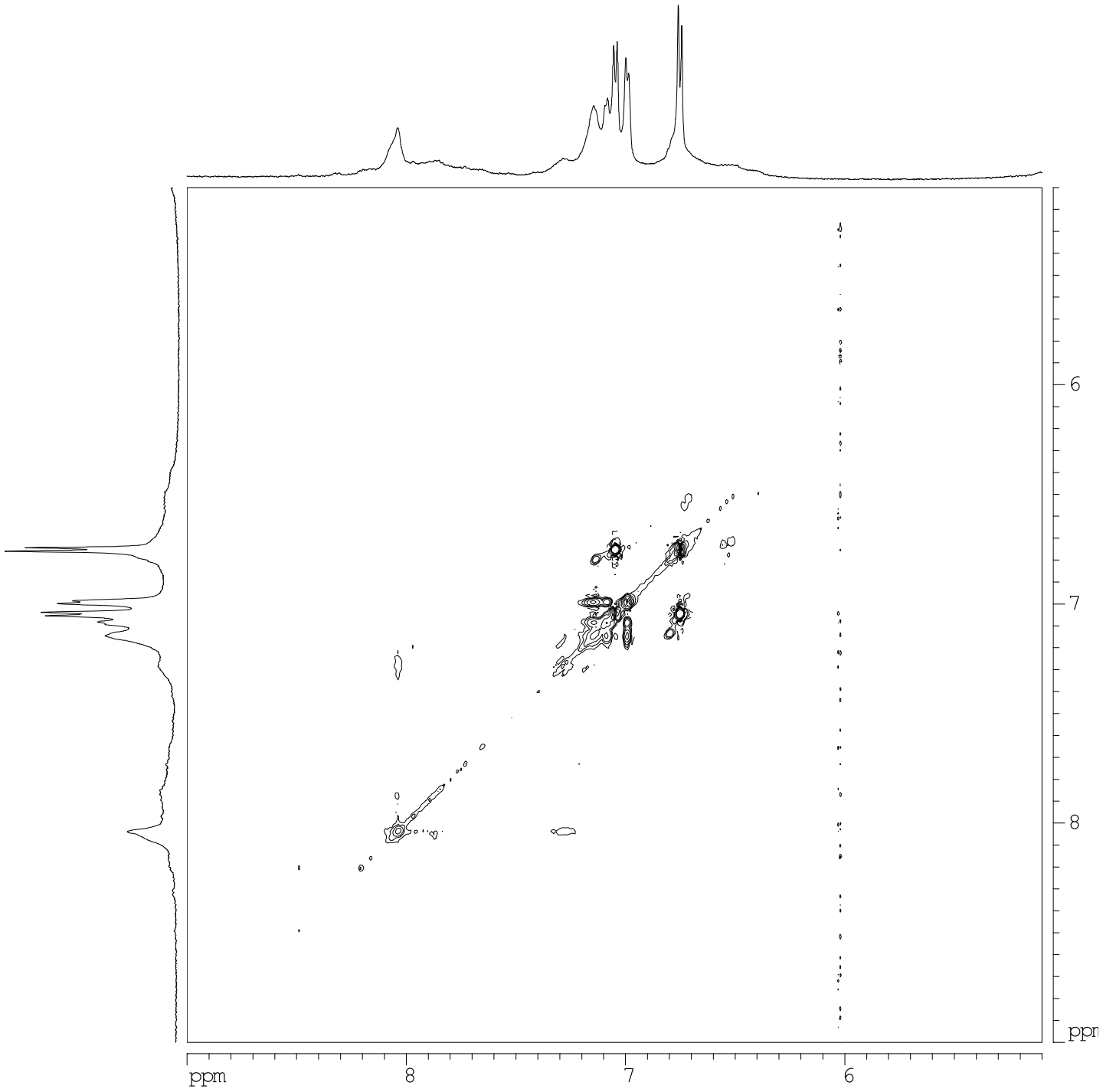
2D TOCSY spectrum of macrocyclic β-sheet **2c**
2 mM in D_2O, 500 MHz, 298 K
150-ms spin-locking mixing time



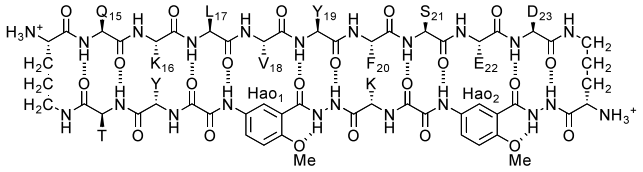


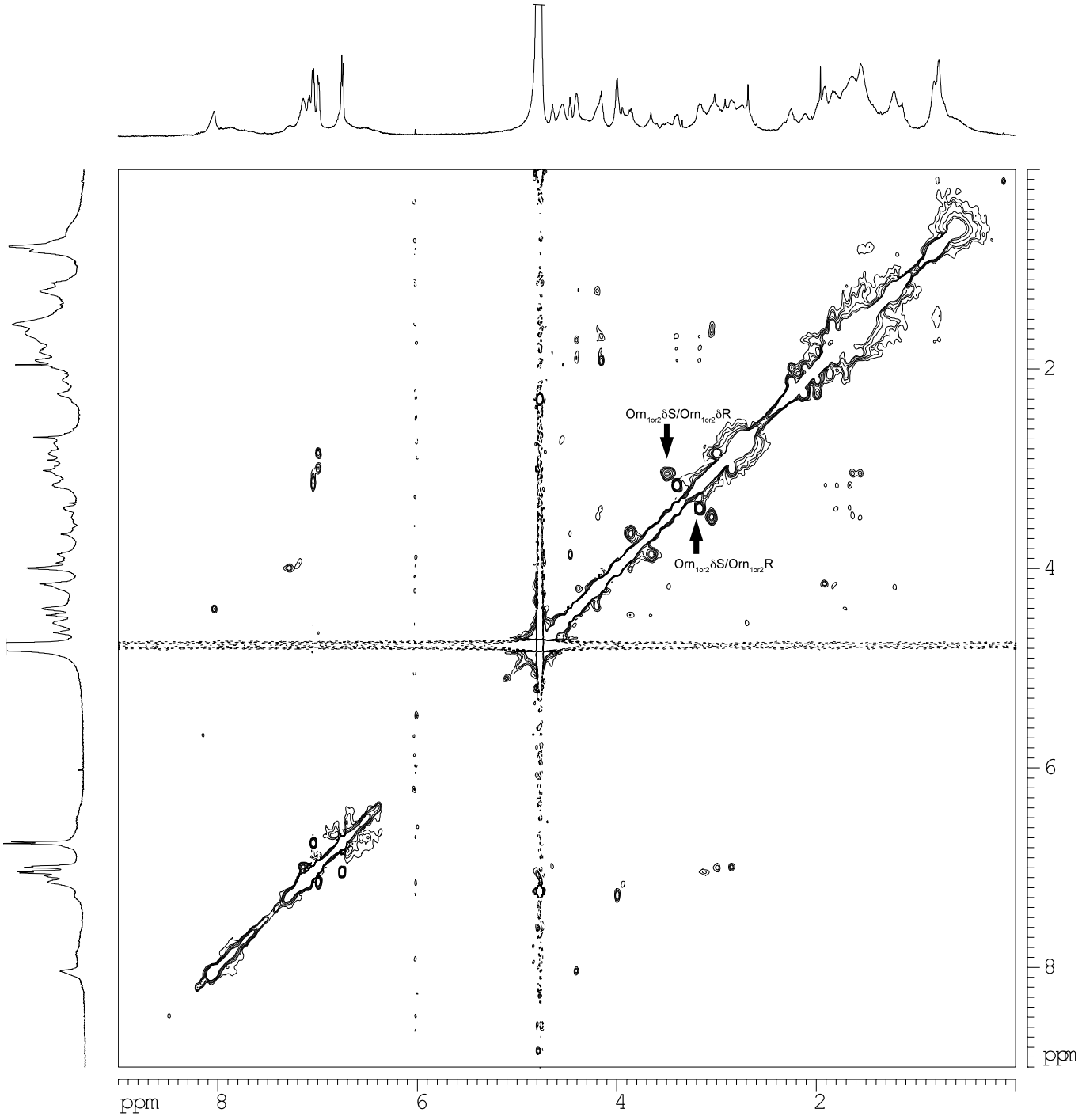
2D TOCSY spectrum of macrocyclic β-sheet **2c**
2 mM in D_2O, 500 MHz, 298 K
150-ms spin-locking mixing time



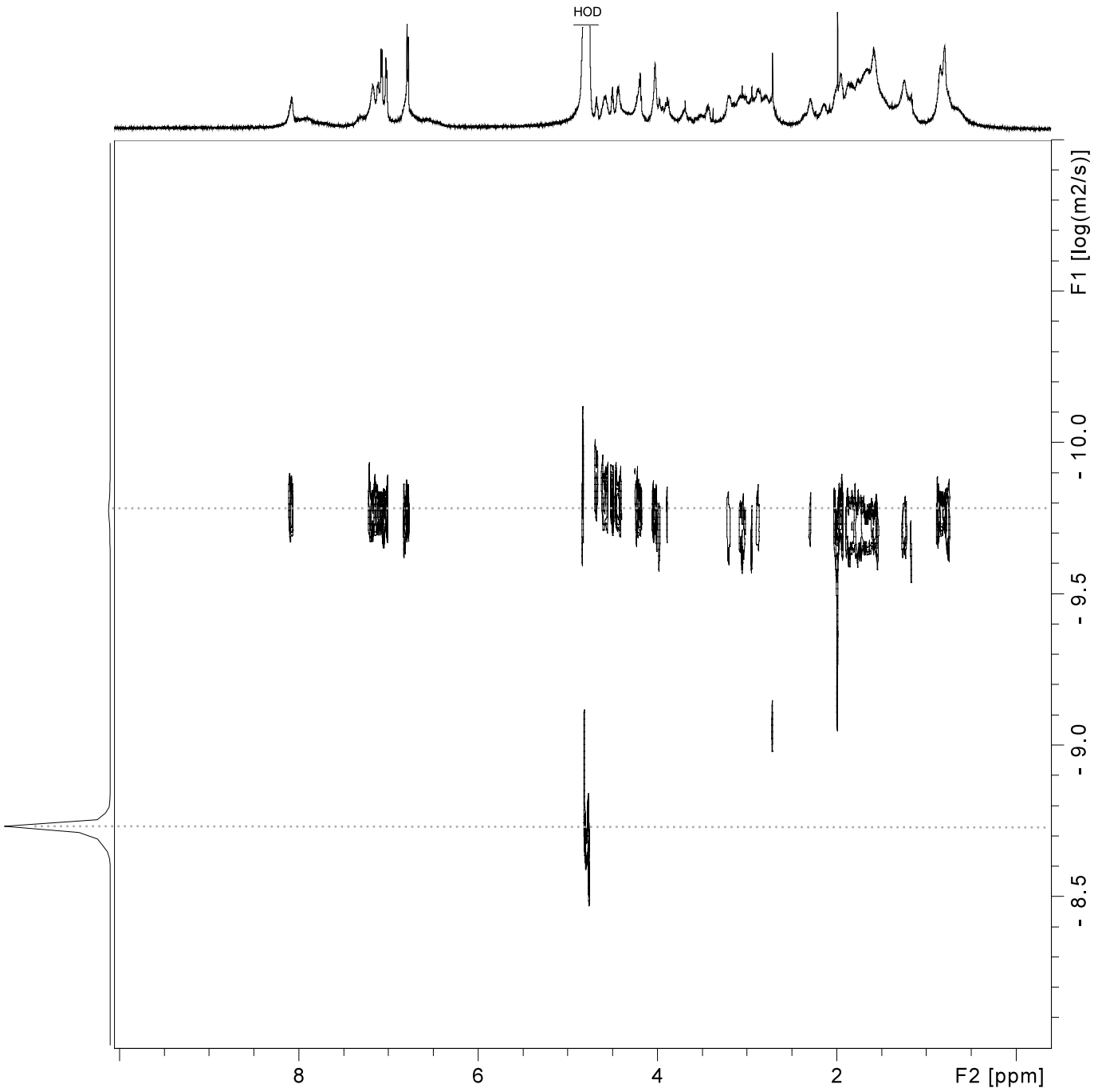


2D NOESY spectrum of macrocyclic β-sheet **2c**
2 mM in D_2O, 500 MHz, 298 K
250 ms spin-locking time





2D DOSY spectrum of macrocyclic β-sheet **2c**
2 mM in D_2O, 600 MHz, 298 K
monomer predominates

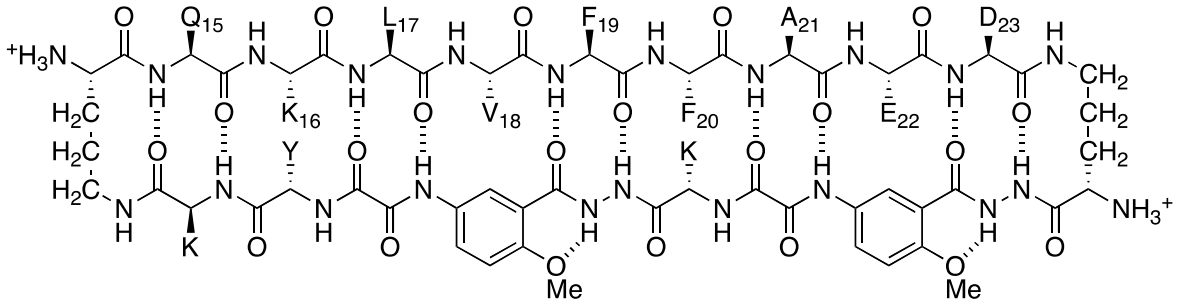


Calculation for 2c at 2.0 mM

DC_{HOD} = 19.0 x 10^{-10} m^2/s [a]
log DC_{HOD} = -8.721

For 2c tetramer, log DC (m^2/s) = -9.78(3), DC = $10^{-9.783}$ m^2/s = 16.5 x 10^{-11} m^2/s = 16.5 x 10^{-7} cm^2/s

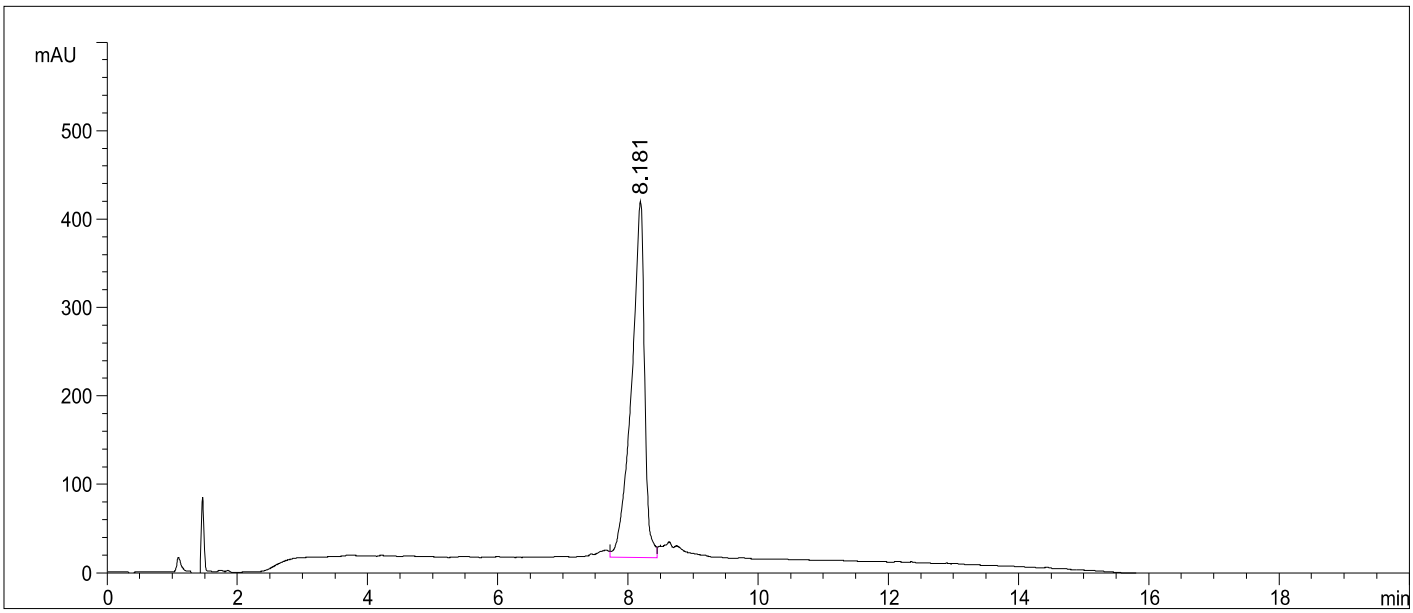
[a] Longsworth, L. G. J. Phys. Chem. 1960, 64, 1914–1917.



macrocyclic β-sheet peptide **3** (as the TFA salt)

molecular weight calculated for $C_{103}H_{146}N_{26}O_{28} \cdot 5CF_3CO_2H$ (TFA salt of **3**): 2766.54
molecular weight calculated for $C_{103}H_{146}N_{26}O_{28}$ (free base of **3**): 2196.42
exact mass calculated for $C_{103}H_{146}N_{26}O_{28}$ (free base of **3**): 2195.08

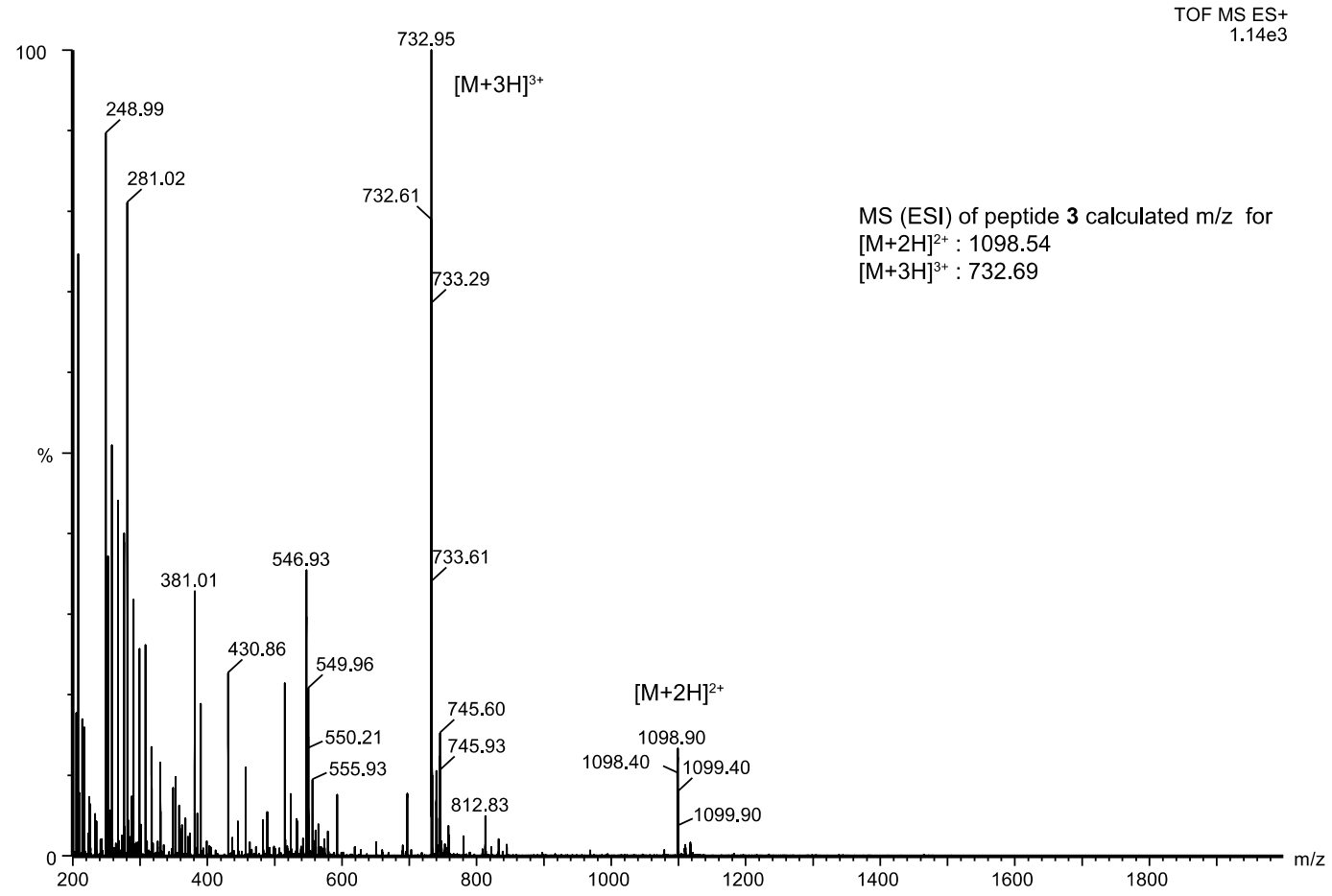
Analytical RP-HPLC of macrocyclic β-sheet peptide **3**



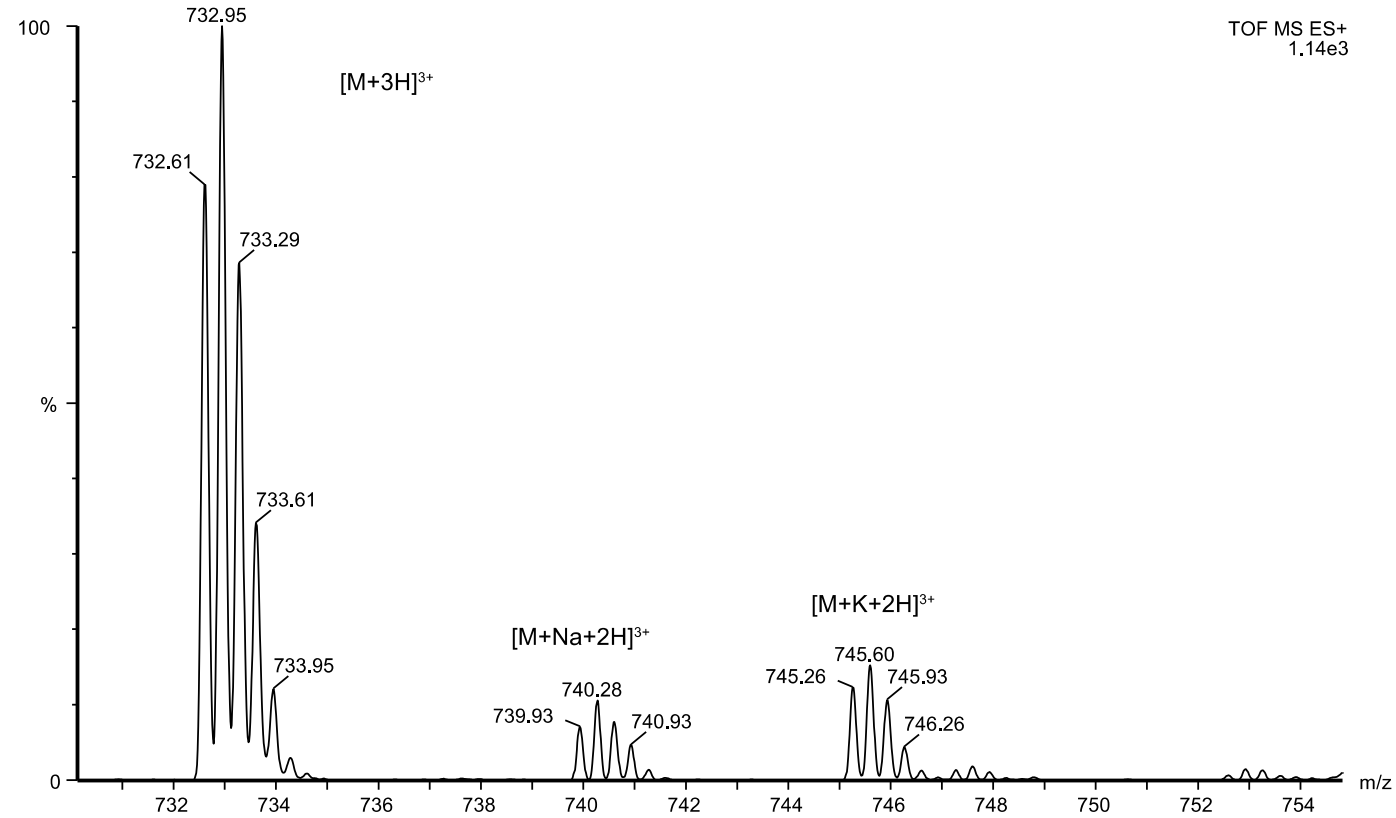
Signal 1: VWD1 A, Wavelength=214 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	8.181	VV	0.1970	5600.01514	403.11060	100.0000
Totals :				5600.01514	403.11060	

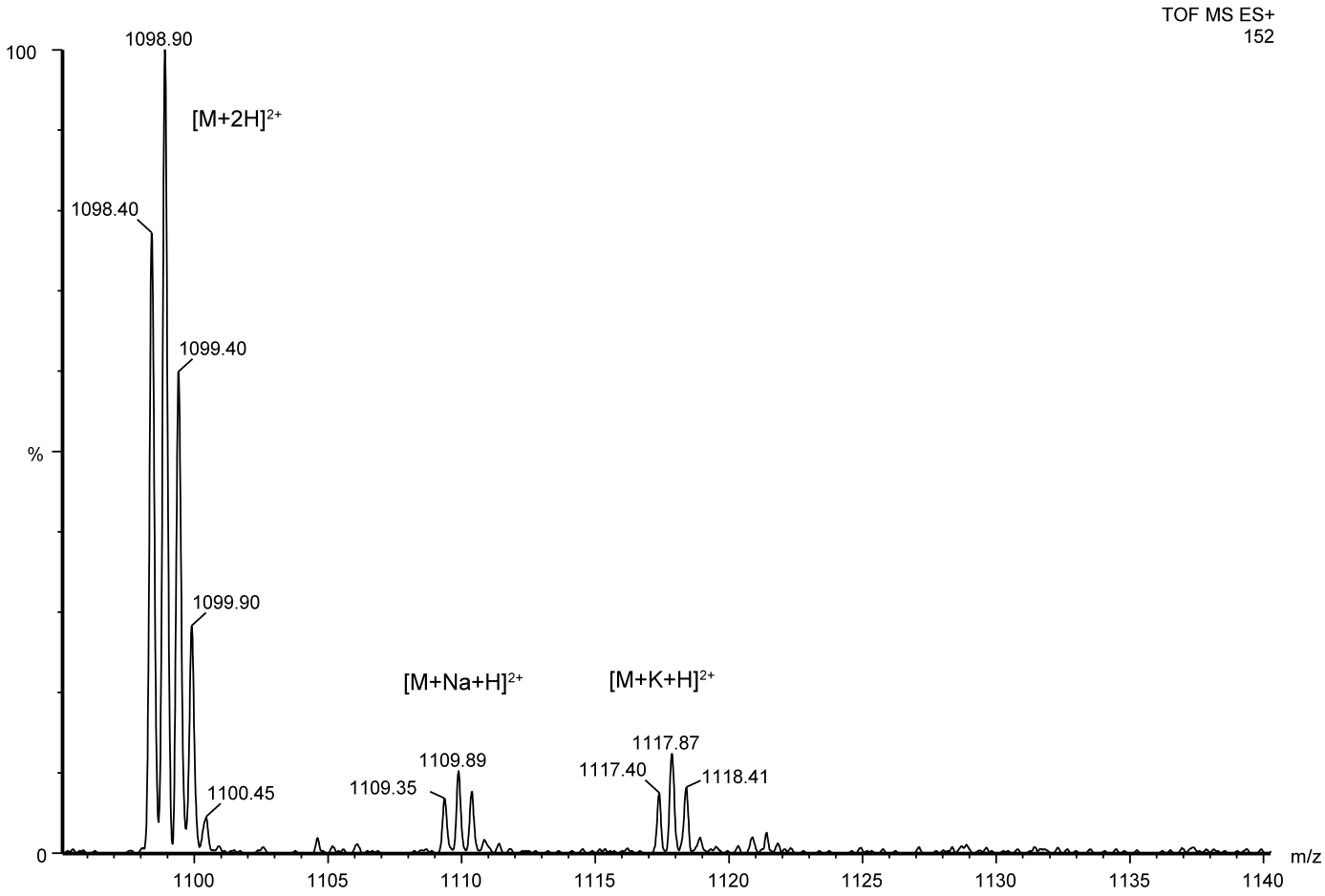
Macrocyclic β-sheet peptide **3**



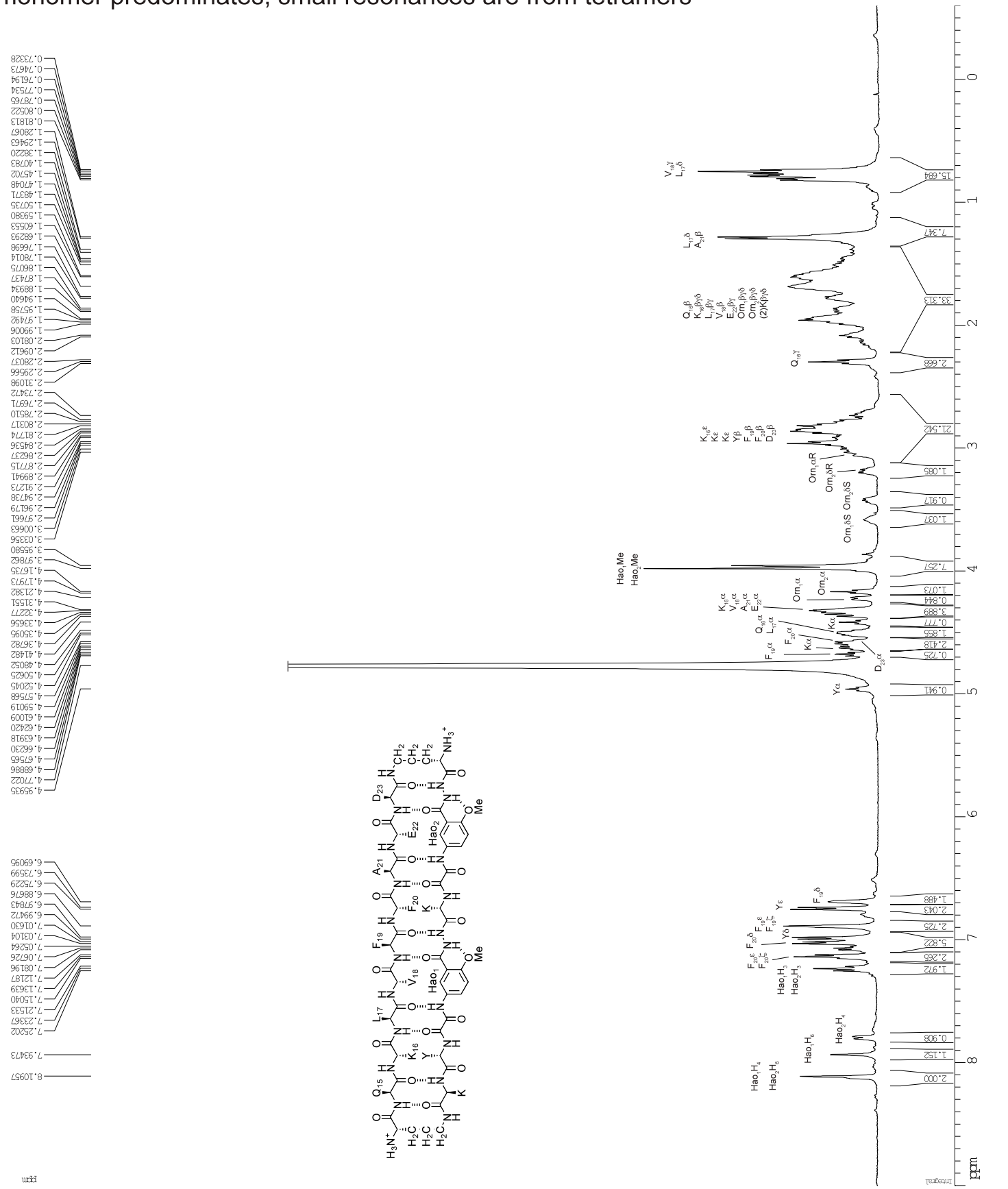
MS (ESI) of peptide **3** calculated m/z for
$[M+2H]^{2+}$: 1098.54
$[M+3H]^{3+}$: 732.69



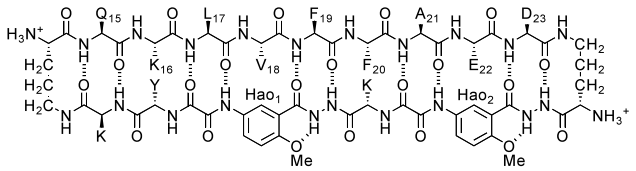
Macrocyclic β-sheet peptide **3**

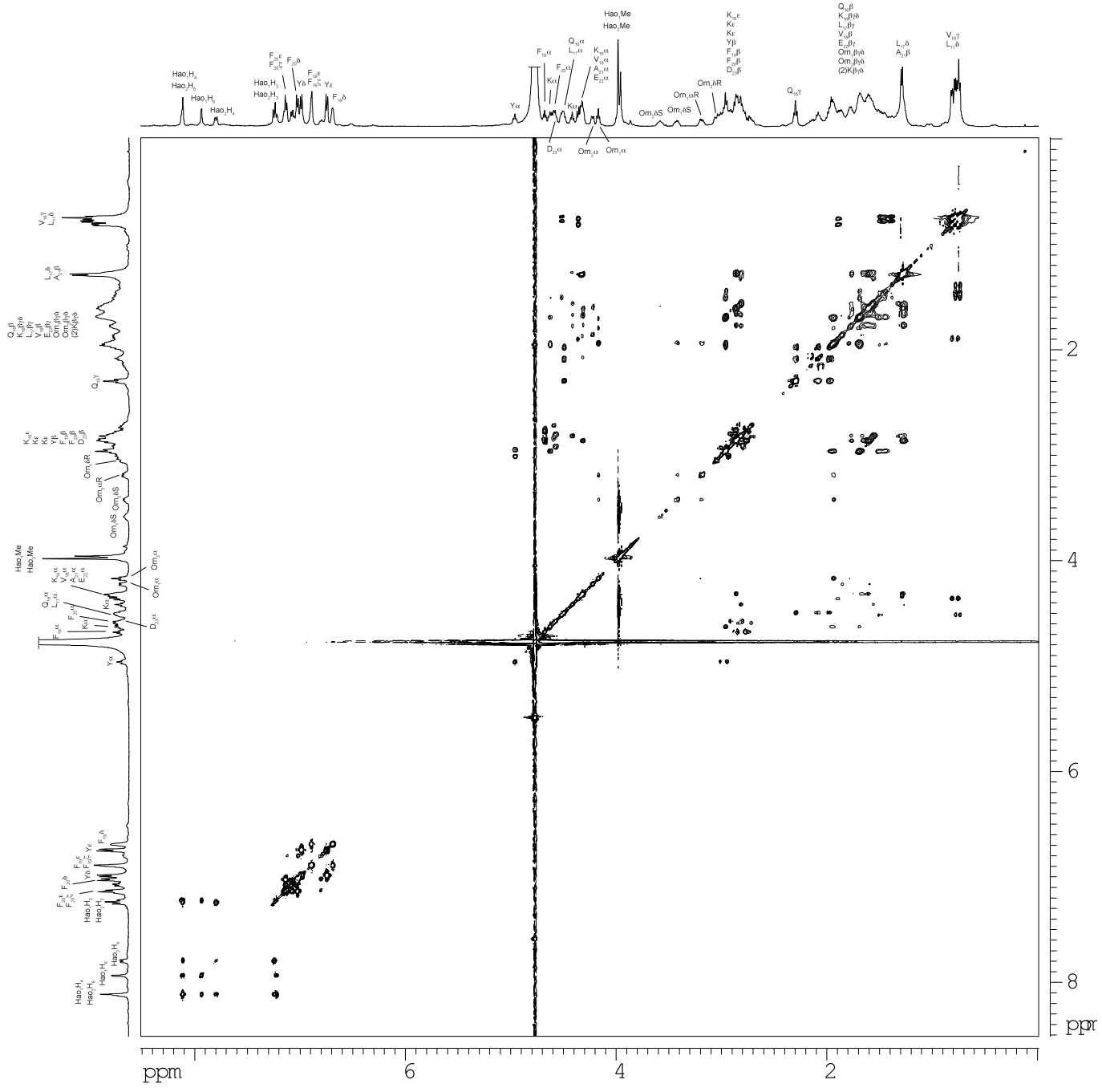


1D ^{1}H NMR spectrum of macrocyclic β-sheet **3**
2 mM in D_2O, 500 MHz, 298 K
monomer predominates, small resonances are from tetramers

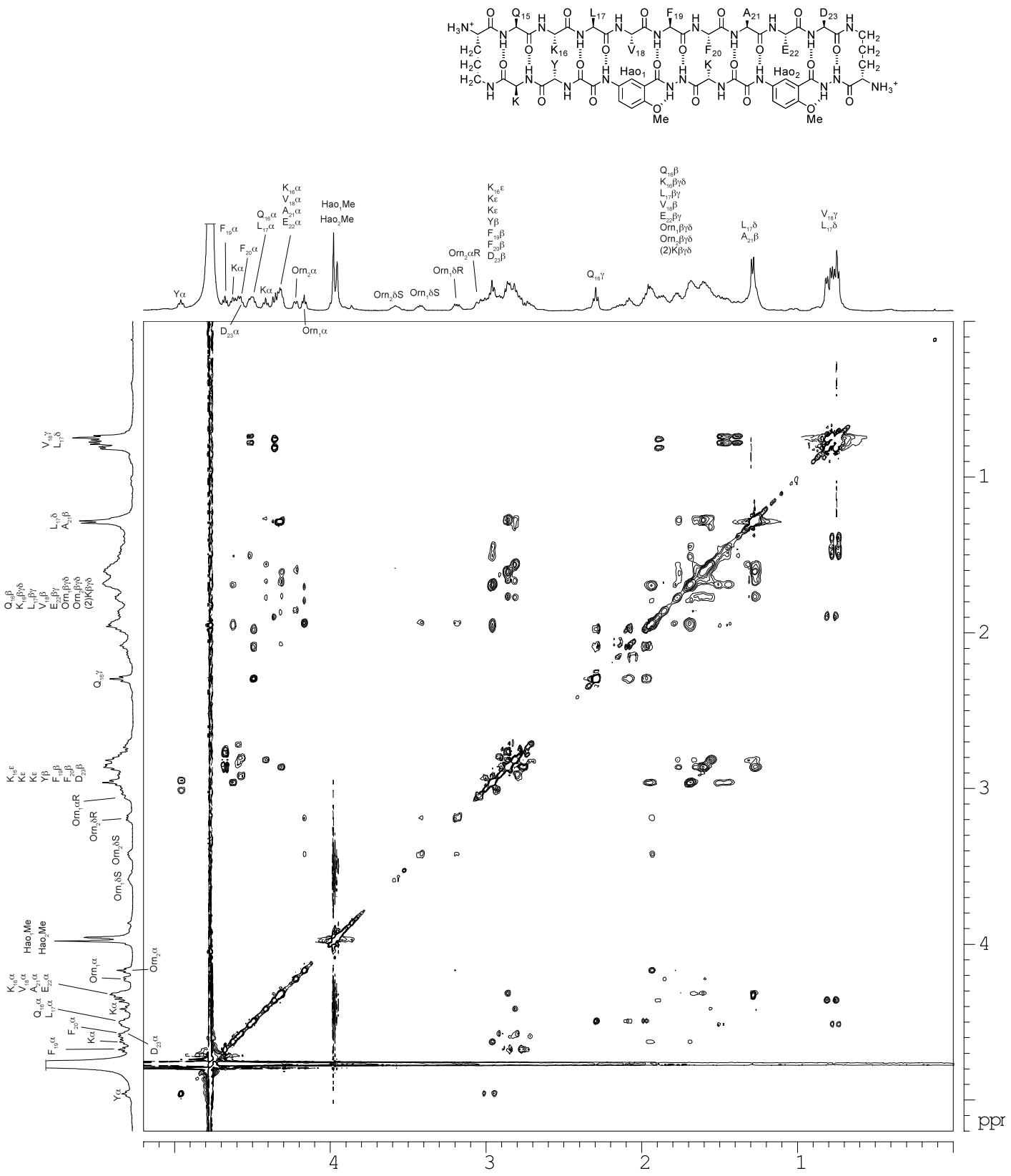


2D TOCSY spectrum of macrocyclic β-sheet **3**, 2 mM in D_2O, 500 MHz, 298 K
150-ms spin-locking mixing time
monomer predominates, small resonances from tetramer

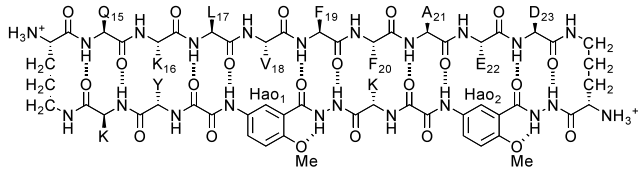


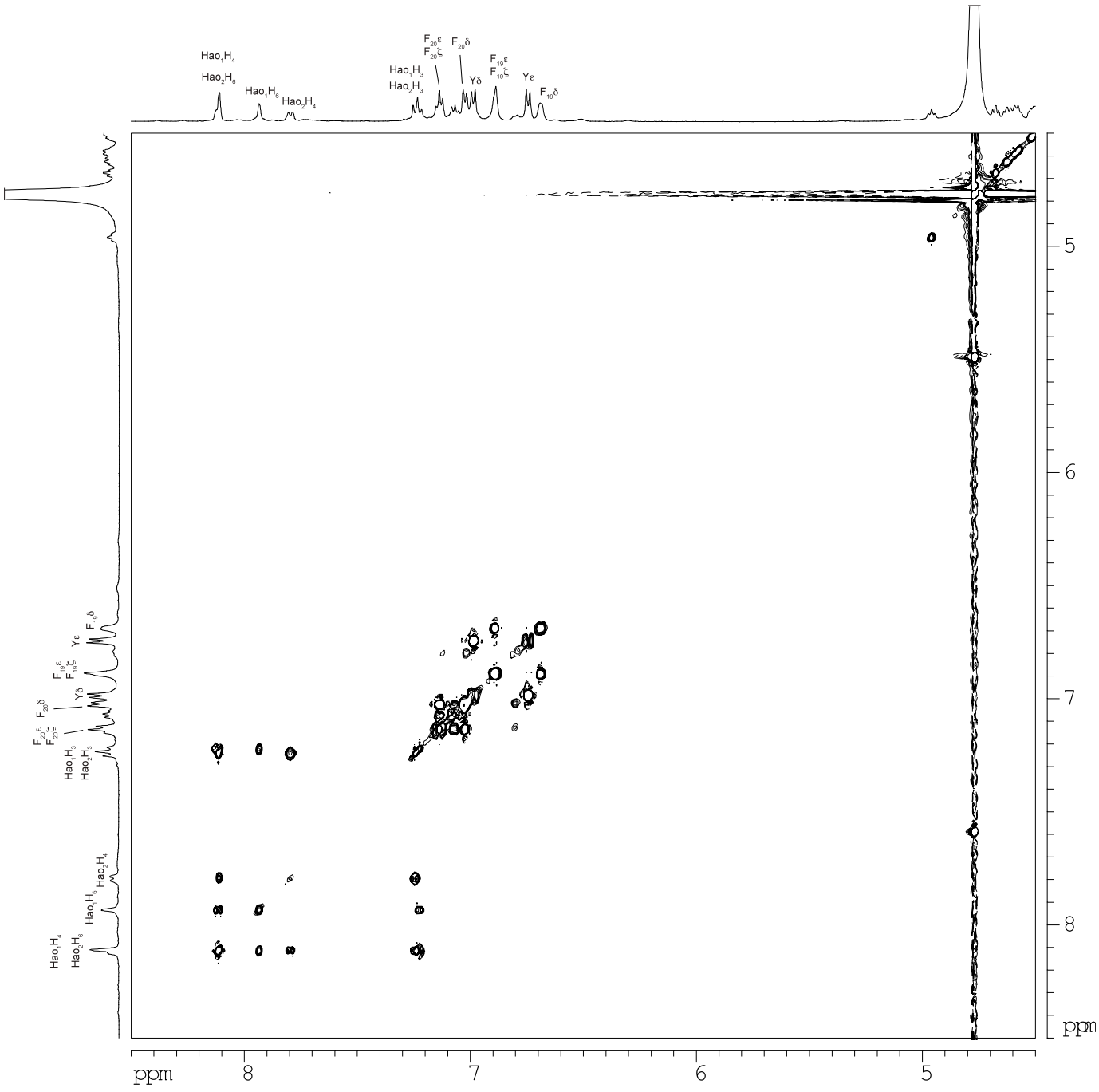


2D TOCSY spectrum of macrocyclic β-sheet **3**, 2 mM in D_2O, 500 MHz, 298 K
150-ms spin-locking mixing time
monomer predominates, small resonances from tetramer

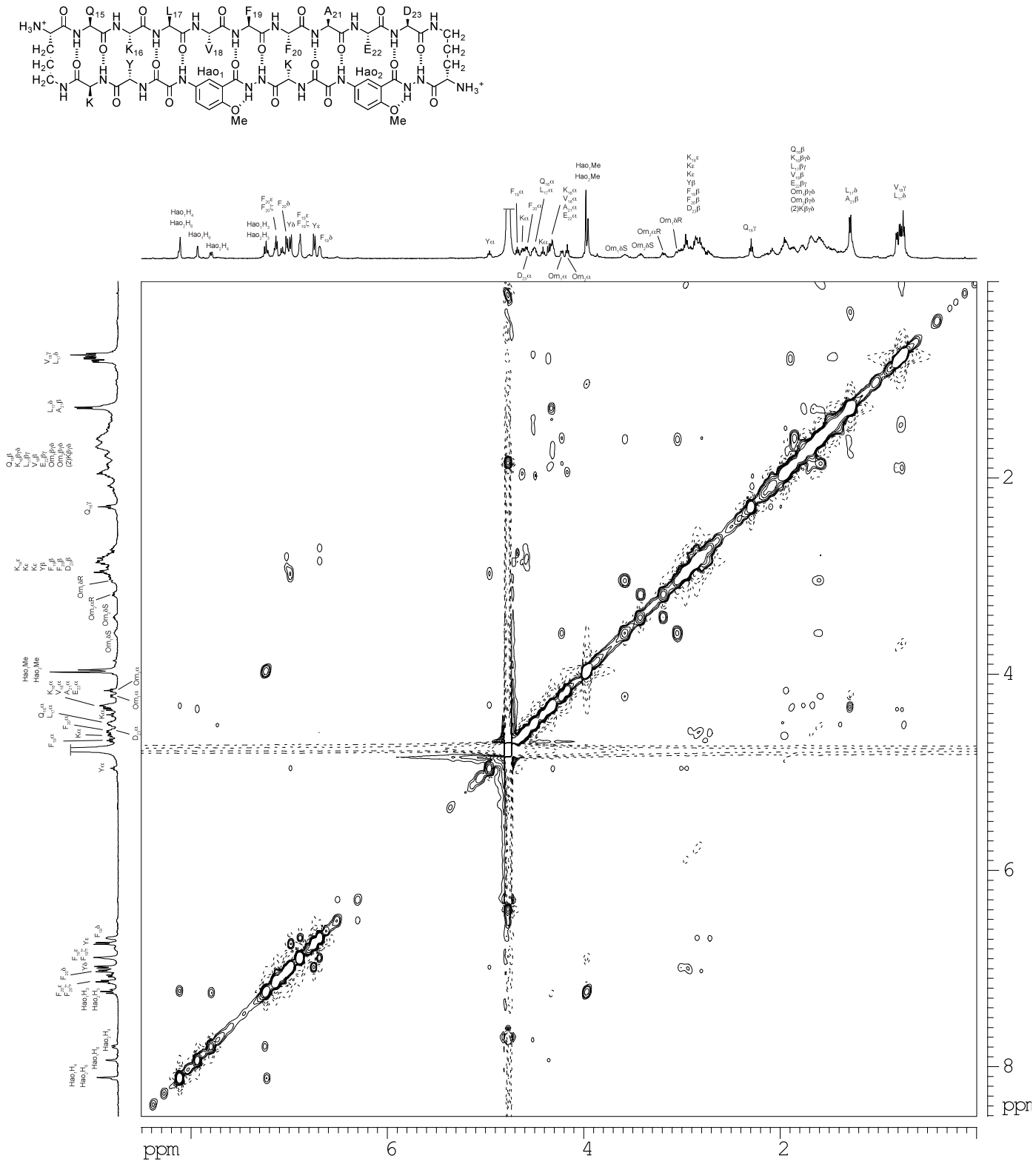


2D TOCSY spectrum of macrocyclic β-sheet **3**, 2 mM in D_2O, 500 MHz, 298 K
150-ms spin-locking mixing time
monomer predominates, small resonances from tetramer

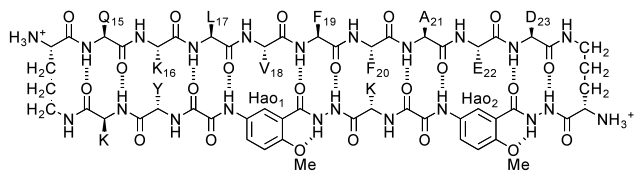


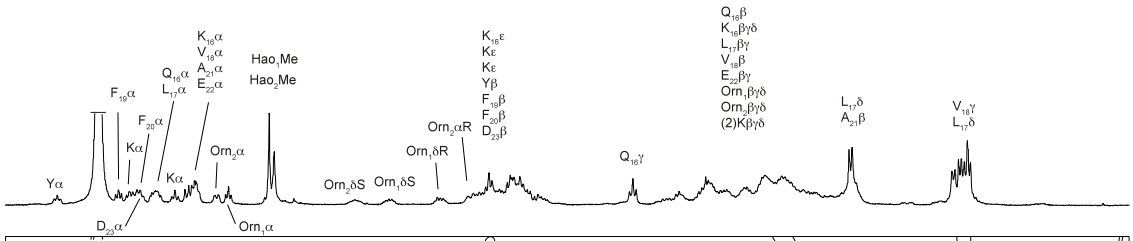


2D NOESY spectrum of macrocyclic β-sheet **3**
2 mM in D_2O, 500 MHz, 298 K
200-ms spin-locking mixing time
monomer predominates, small resonances are from tetramers

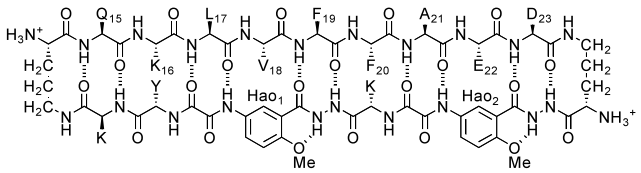


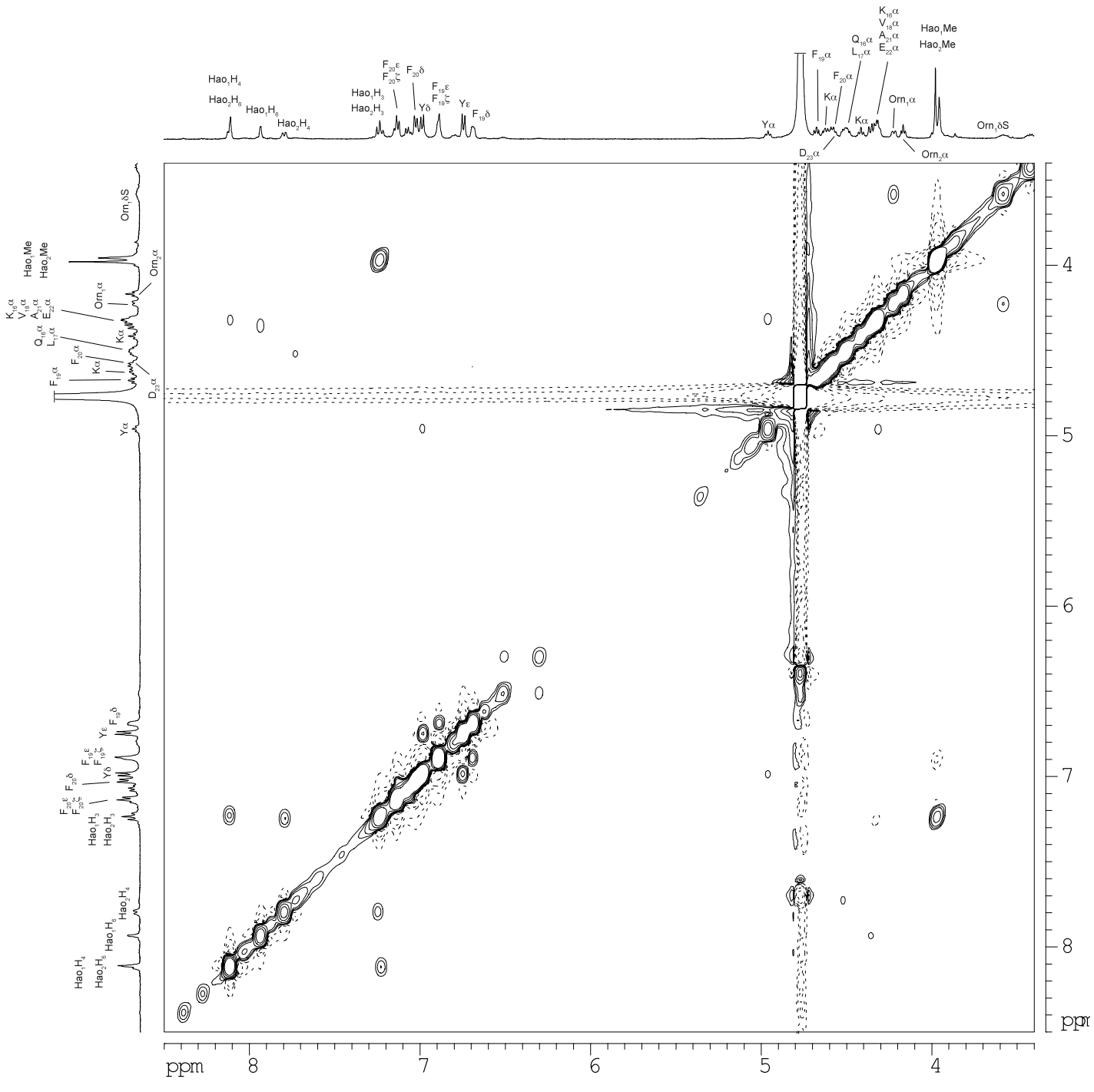
2D NOESY spectrum of macrocyclic β-sheet **3**, 2 mM in D_2O, 500 MHz, 298 K
200-ms spin-locking mixing time
monomer predominates, small resonances are from tetramers



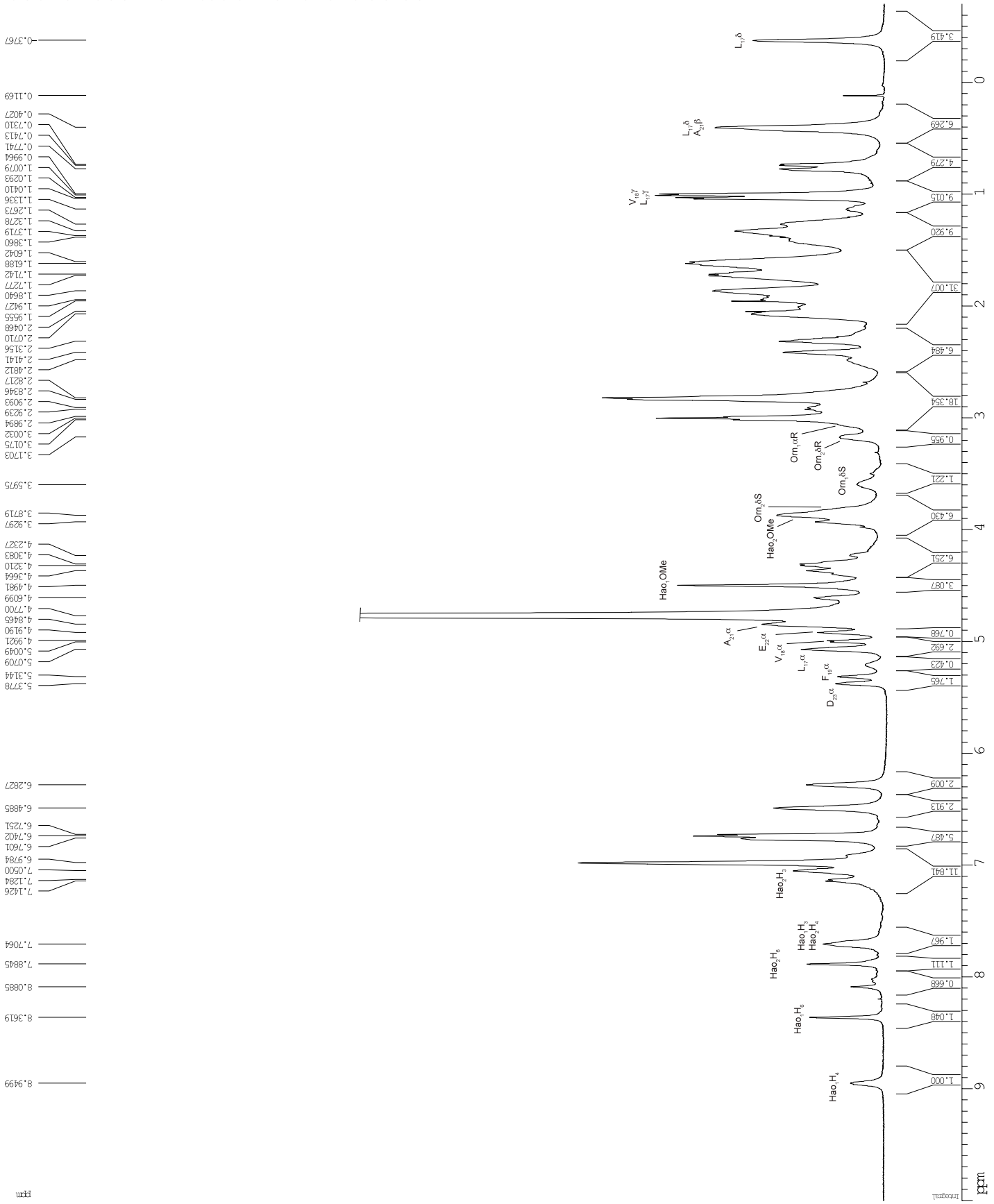


2D NOESY spectrum of macrocyclic β-sheet **3**, 2 mM in D_2O, 500 MHz, 298 K
200 ms spin-locking time
monomer predominates, small resonances are from tetramer

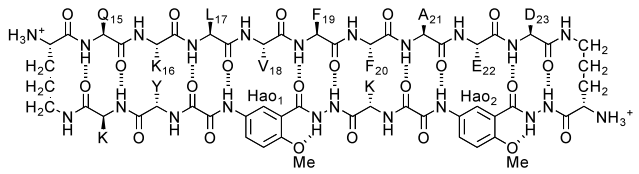


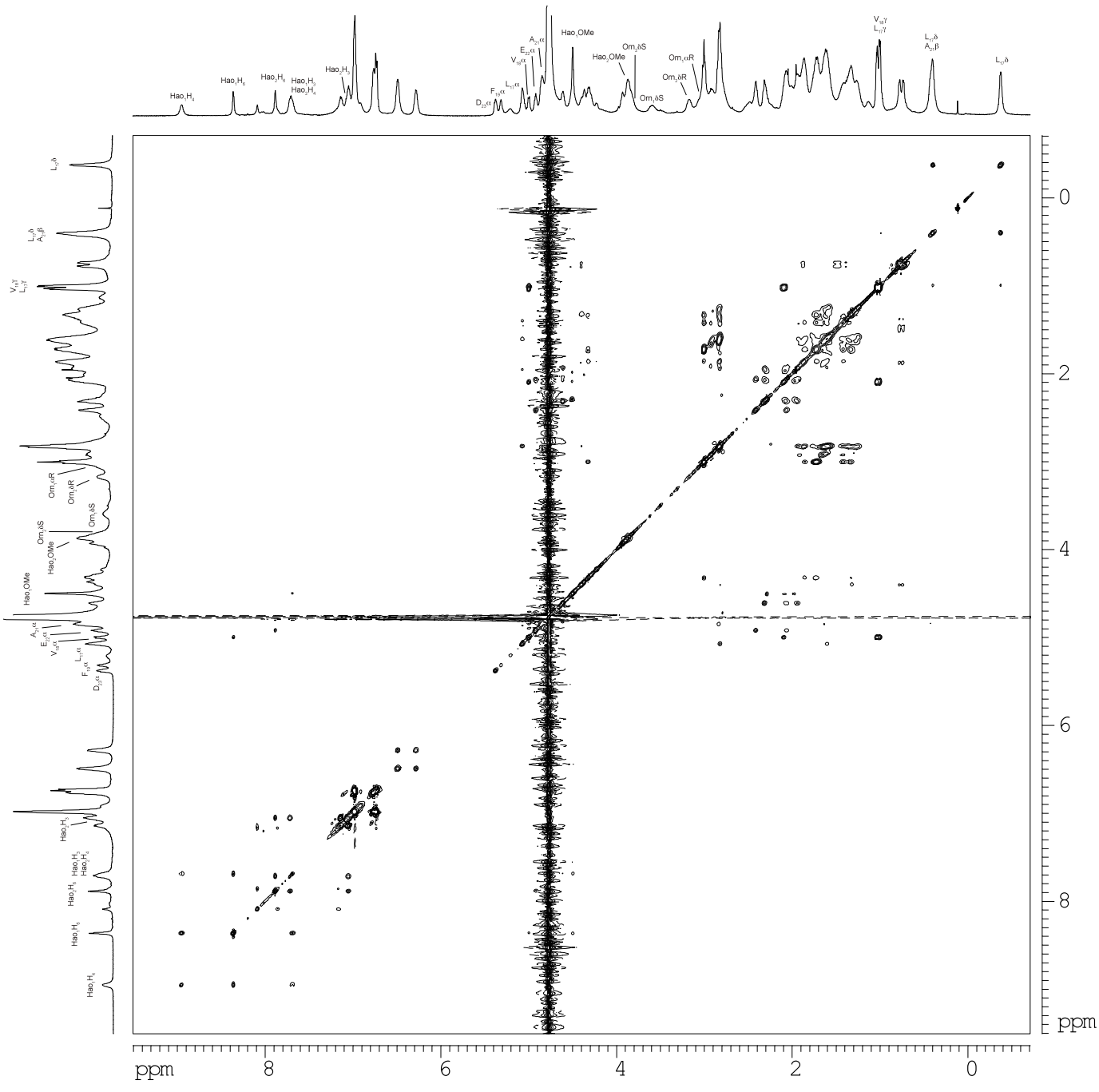


1D ^{1}H NMR spectrum of macrocyclic β-sheet **3**, 8 mM in D_2O, 500 MHz, 298 K
40/60 ratio of monomer to tetramer
resonances from tetramer are labeled

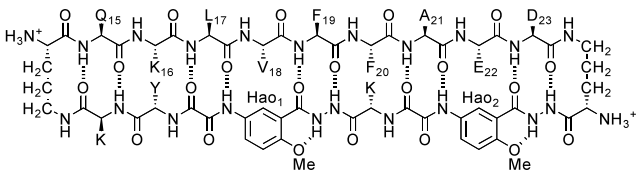


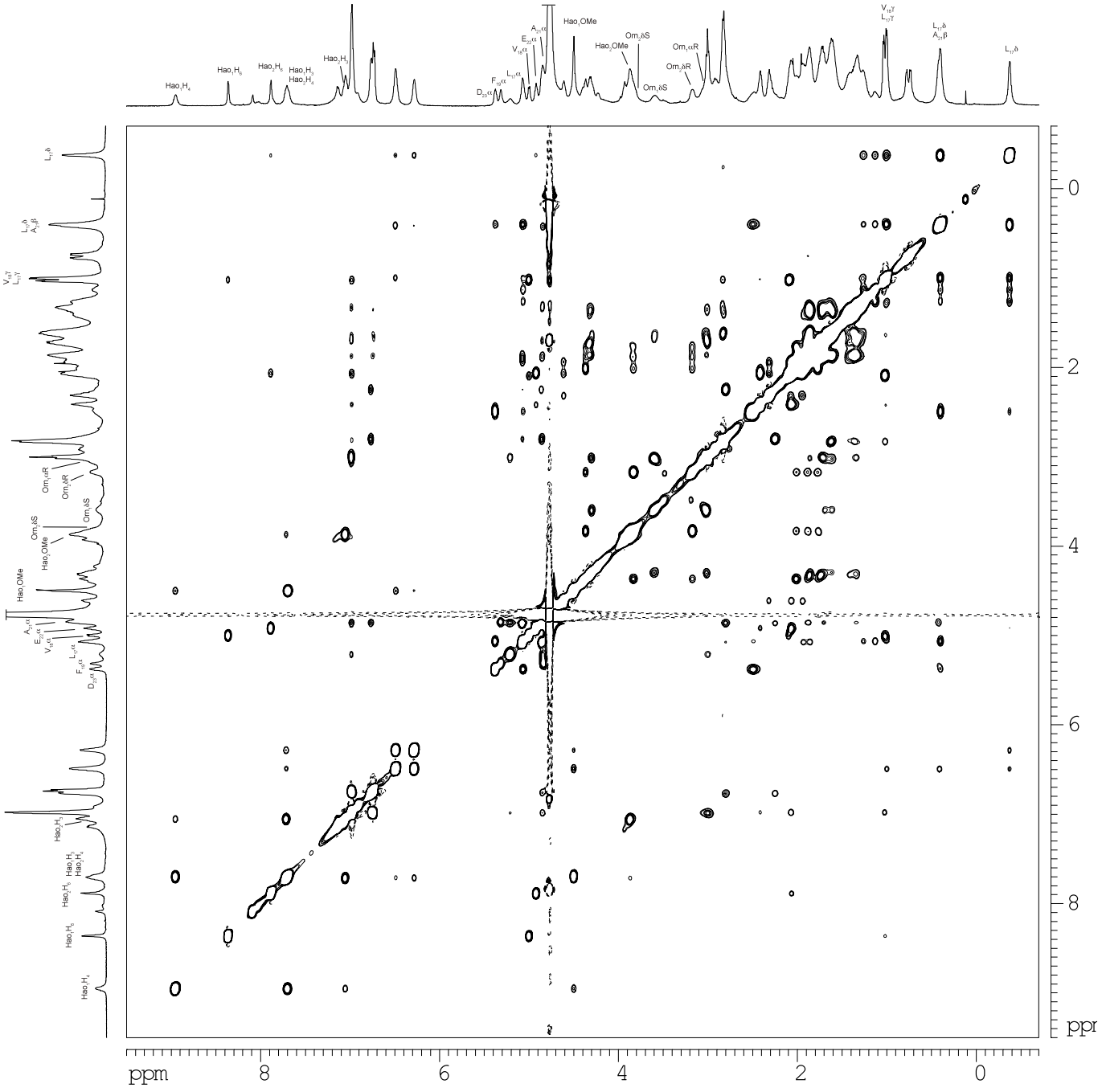
2D TOCSY spectrum of macrocyclic β-sheet **3**, 8 mM in D_2O, 500 MHz, 298 K
150-ms spin-locking mixing time
40/60 ratio of monomer to tetramer
resonances from tetramer are labeled



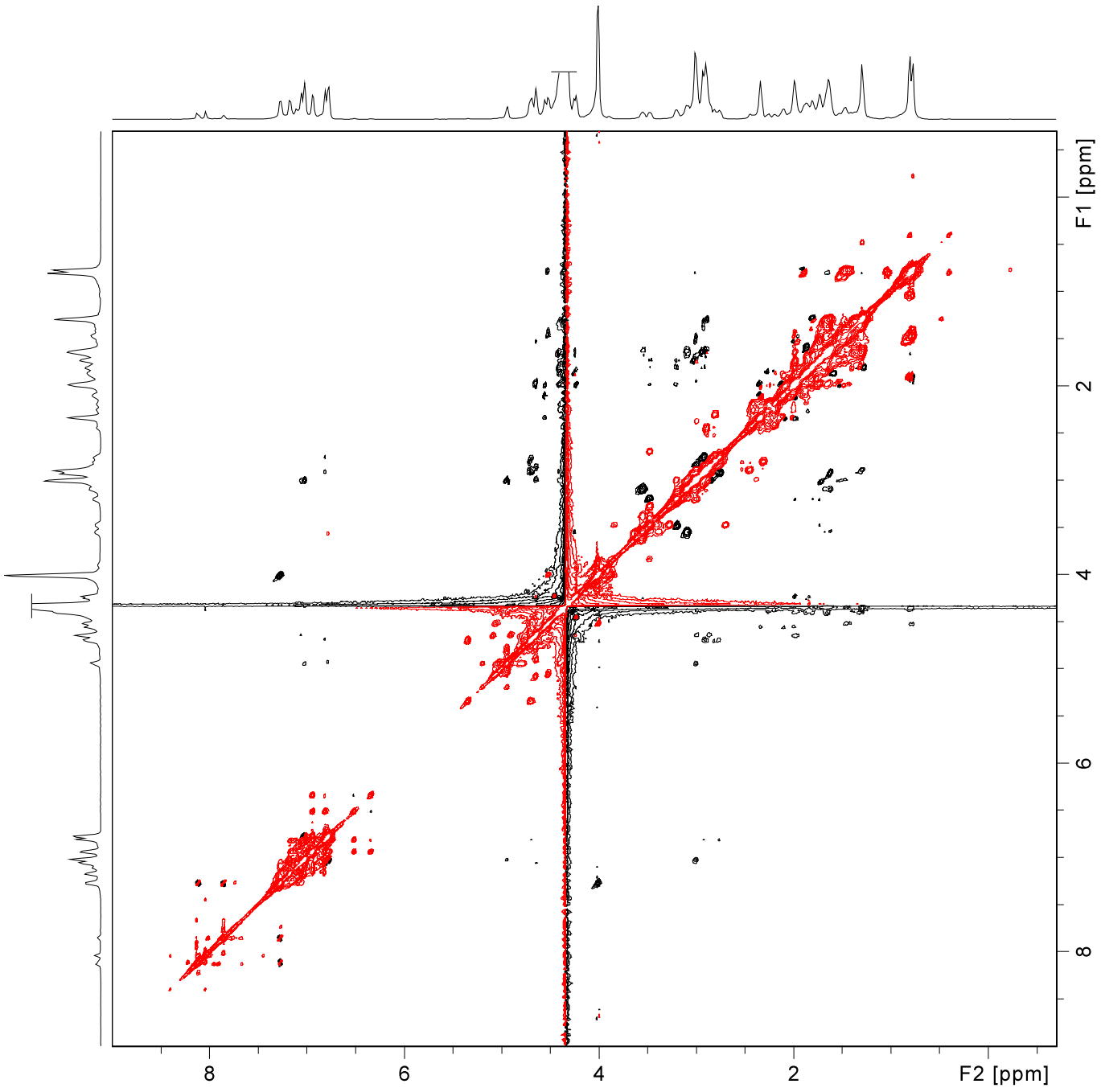


2D NOESY spectrum of macrocyclic β-sheet **3**, 8 mM in D_2O, 500 MHz, 298 K
200-ms spin-locking mixing time
40/60 ratio of monomer to tetramer
resonances from tetramer are labeled

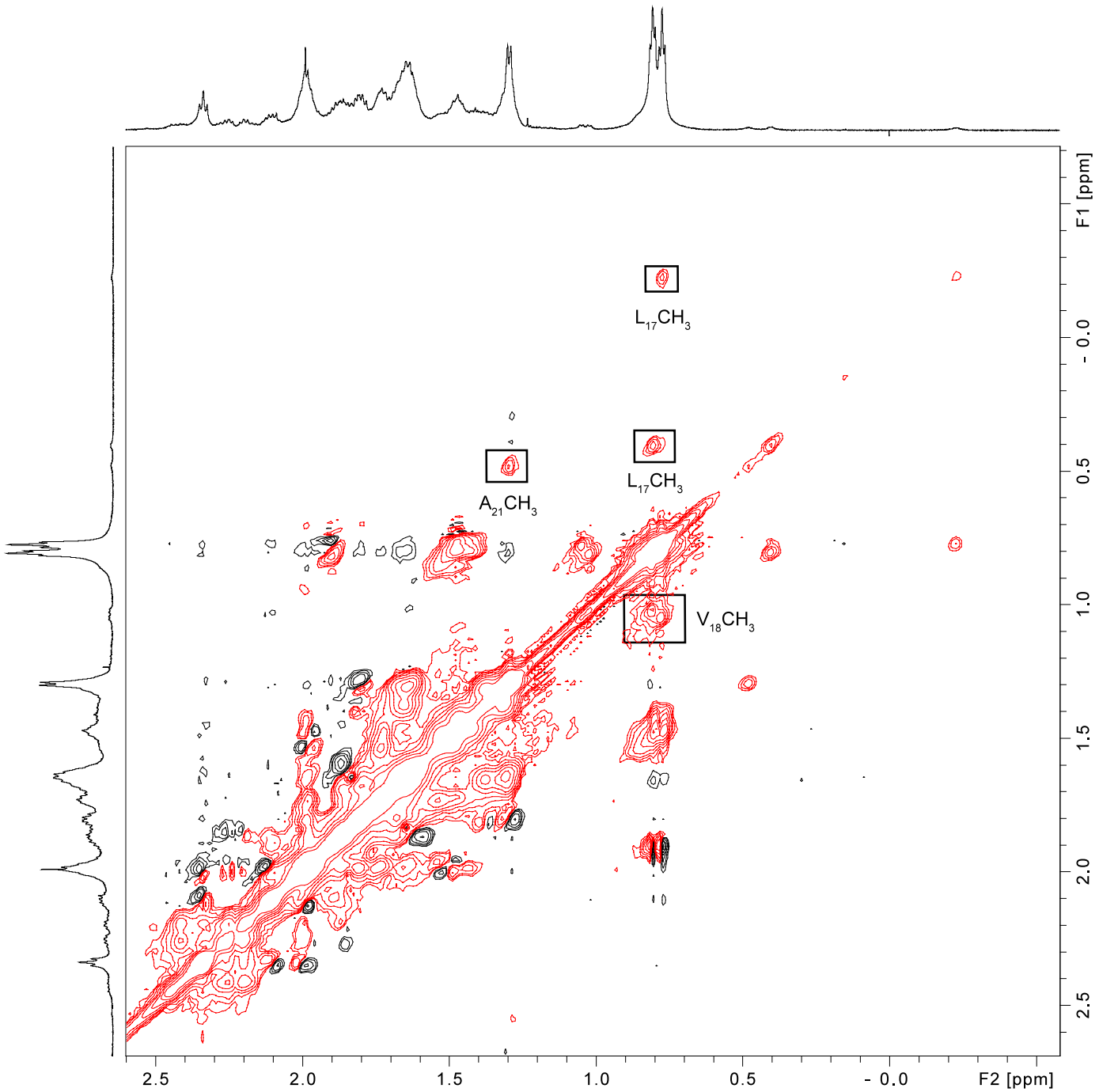




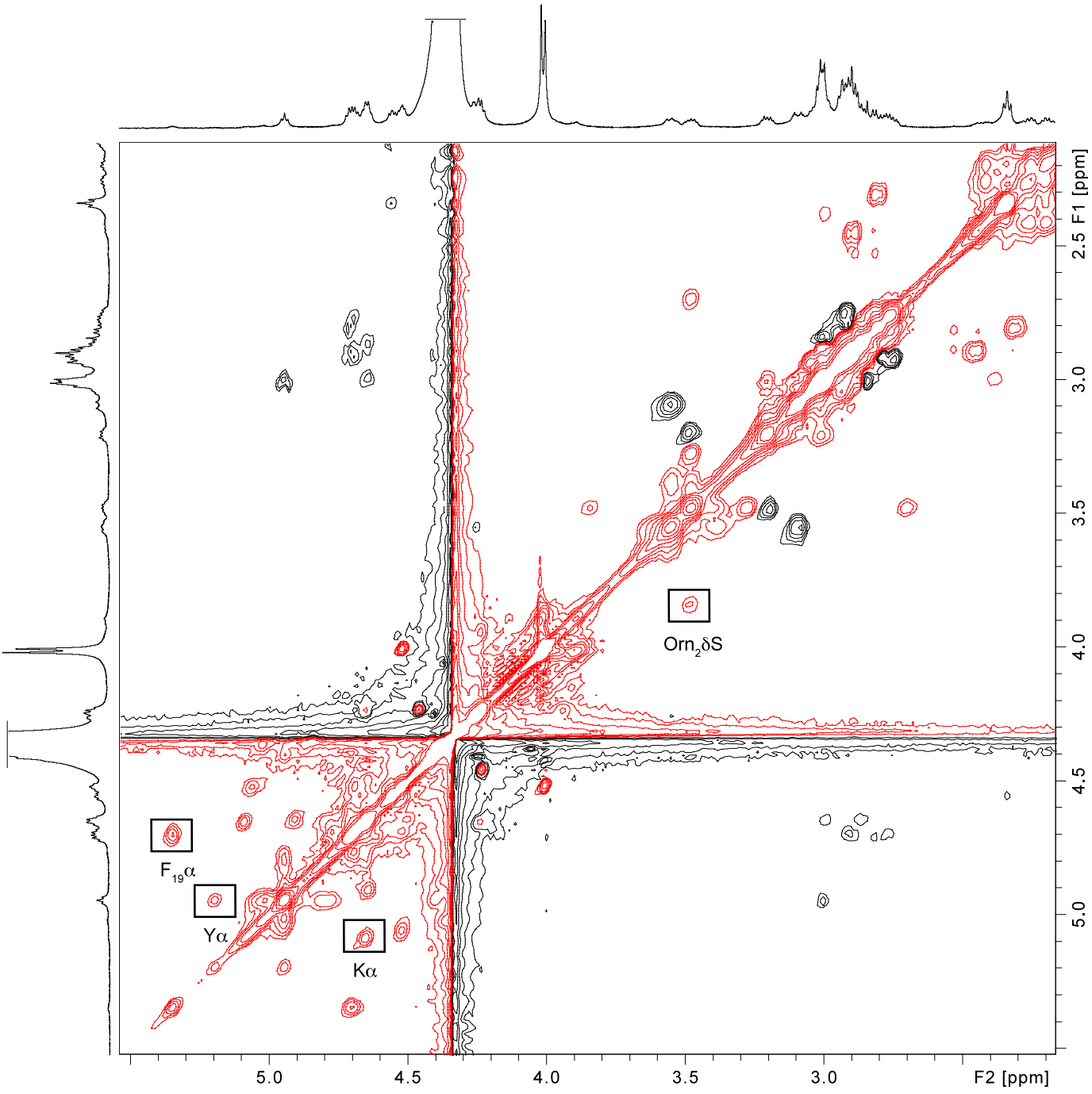
^{1}H NMR 2D EXSY (ROESY spectra showing exchange crosspeaks)
Peptide **3**, 4 mM in D_2O, 600 MHz, 340 K
200 ms spin-locking time
Exchange crosspeaks shown in red; ROE crosspeaks shown in black



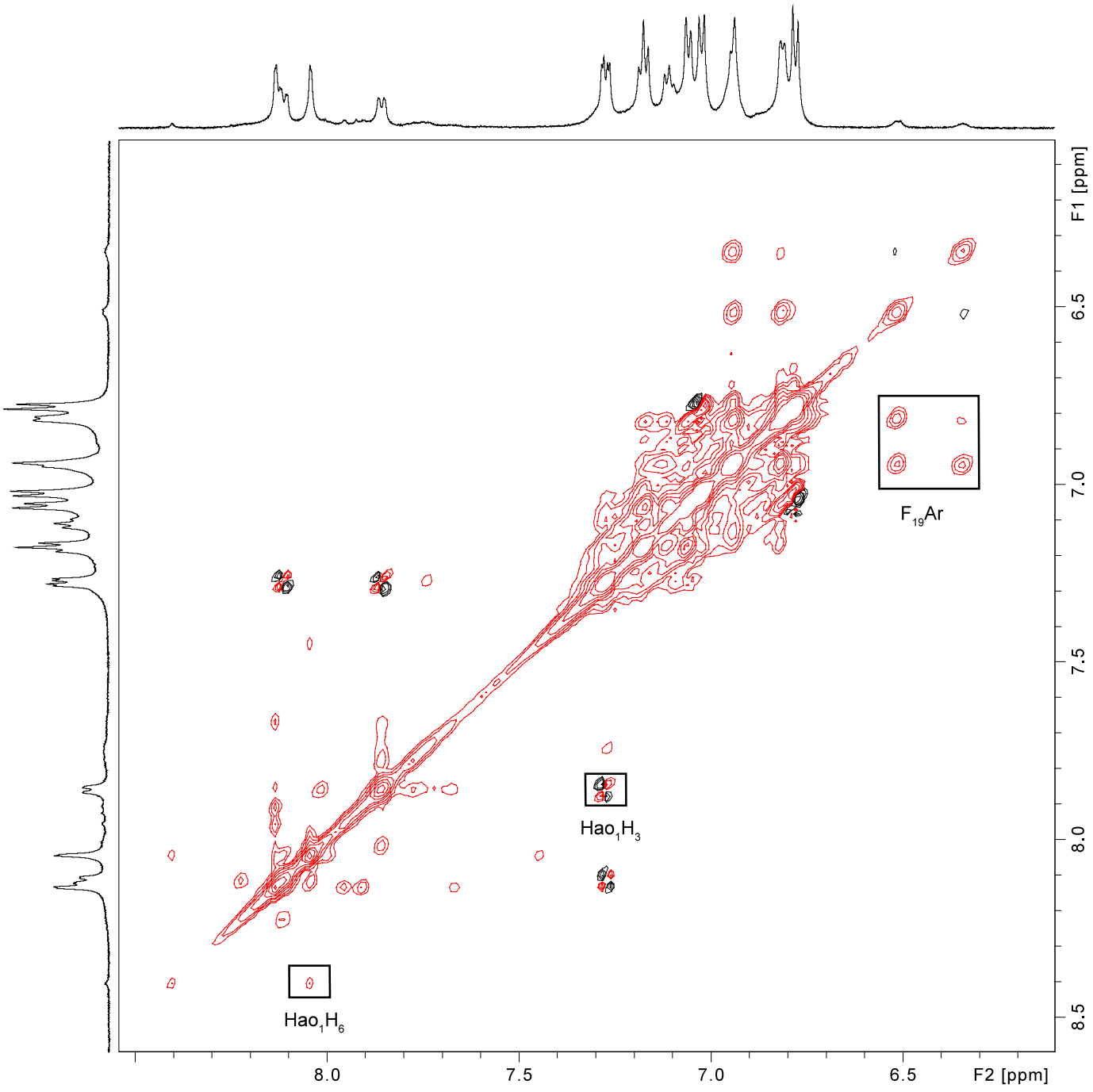
^{1}H NMR 2D EXSY (ROESY spectra showing exchange crosspeaks)
Peptide **3**, 4 mM in D_2O, 600 MHz, 340 K
200 ms spin-locking time
Exchange crosspeaks shown in red; ROE crosspeaks shown in black



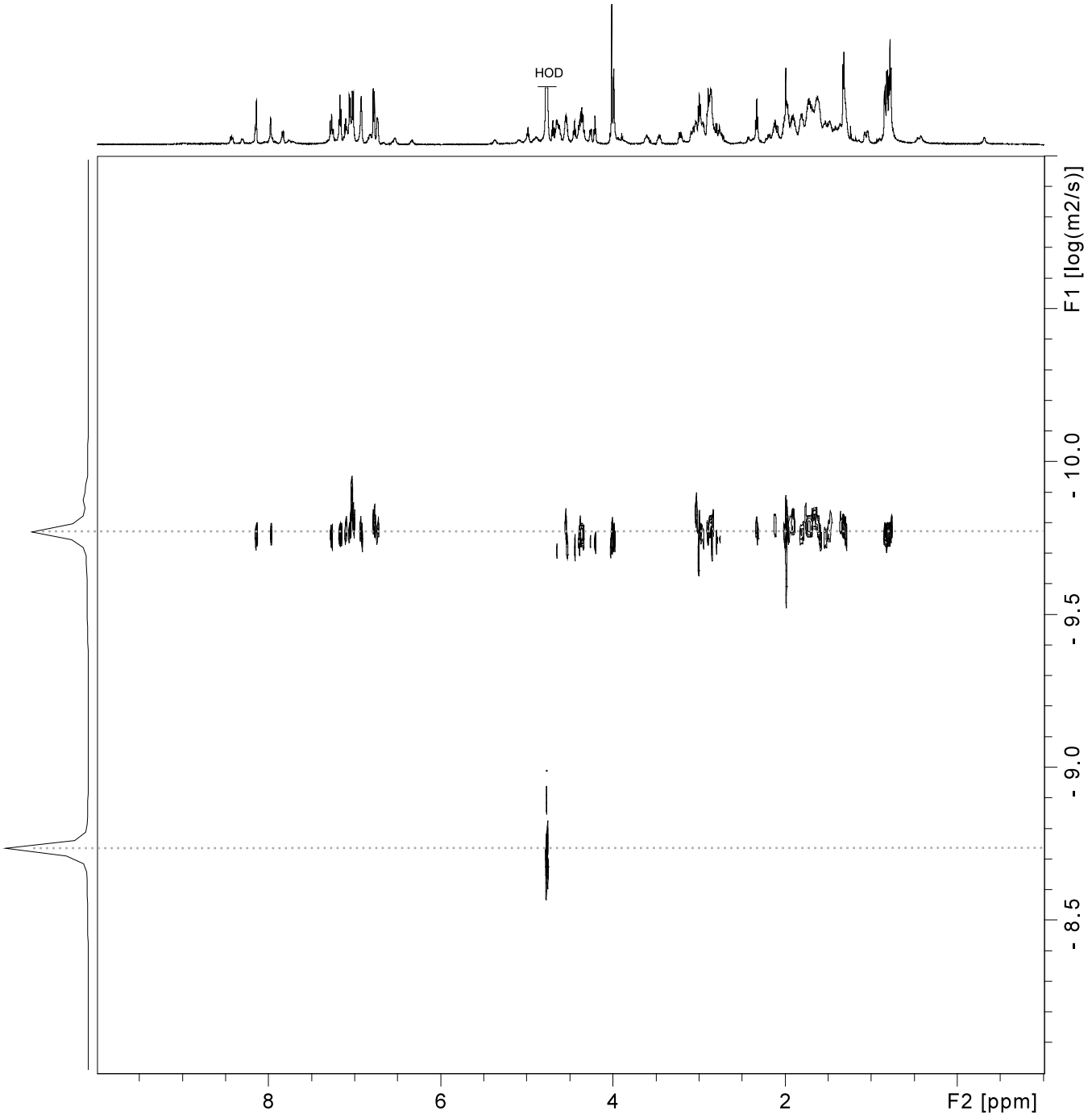
^{1}H NMR 2D EXSY (ROESY spectra showing exchange crosspeaks)
Peptide **3**, 4 mM in D_2O, 600 MHz, 340 K
200 ms spin-locking time
Exchange crosspeaks shown in red; ROE crosspeaks shown in black



^{1}H NMR 2D EXSY (ROESY spectra showing exchange crosspeaks)
Peptide **3**, 4 mM in D_2O, 600 MHz, 340 K
200 ms spin-locking time
Exchange crosspeaks shown in red; ROE crosspeaks shown in black



2D DOSY spectrum of macrocyclic β-sheet **3**
2 mM in D_2O, 600 MHz, 298 K
monomer predominates,
small resonances from tetramer

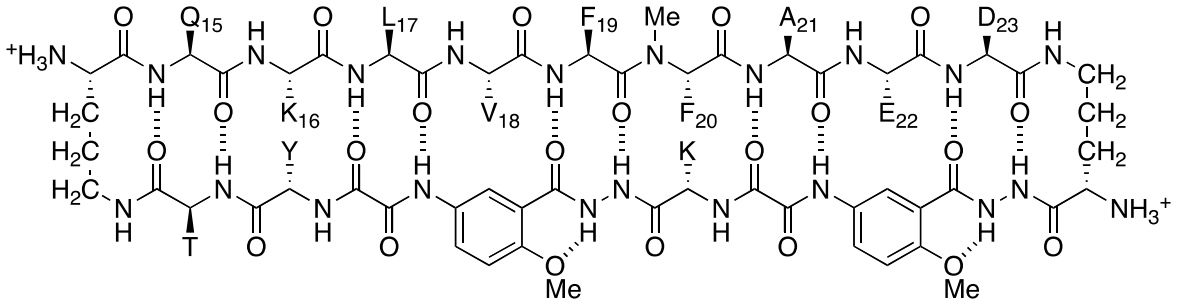


Calculation for 3 at 2.0 mM

DC_{HOD} = 19.0 x 10^{-10} m^2/s [a]
log DC_{HOD} = -8.721

For 3 tetramer, log DC (m^2/s) = -9.78(5), DC = $10^{-9.785}$ m^2/s = 16.4 x 10^{-11} m^2/s = 16.4 x 10^{-7} cm^2/s

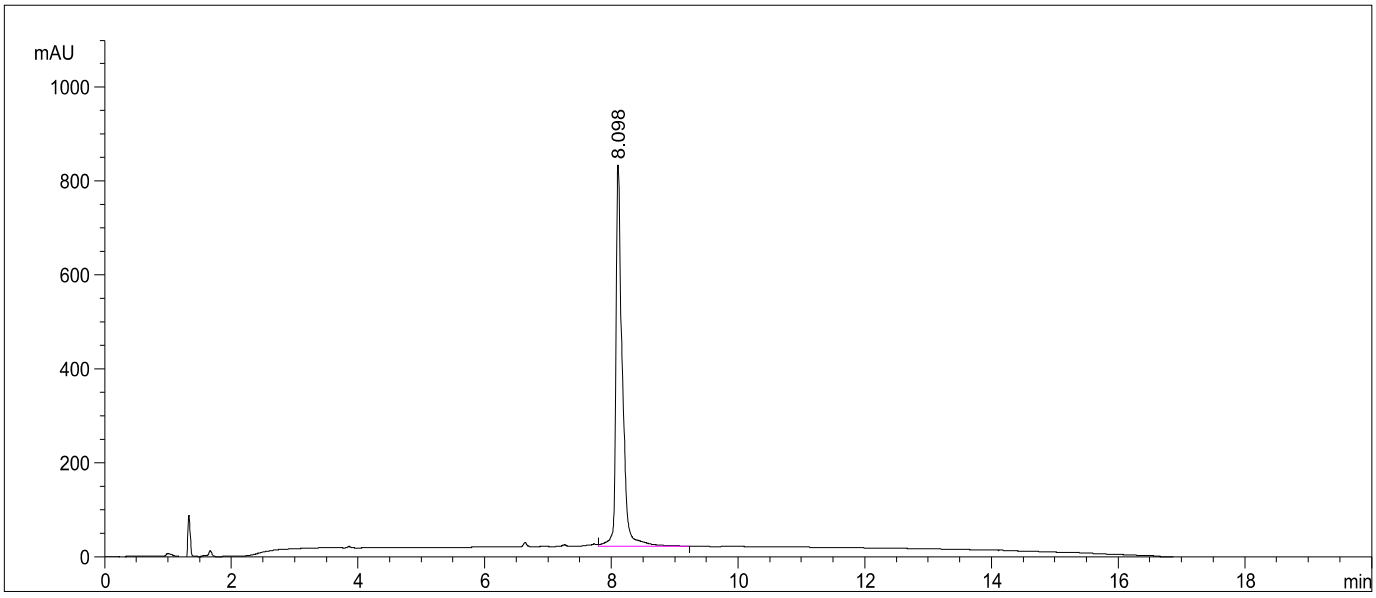
[a] Longsworth, L. G. J. Phys. Chem. 1960, 64, 1914–1917.



macrocyclic β-sheet peptide **4** (as the TFA salt)

molecular weight calculated for $C_{102}H_{143}N_{25}O_{29} \cdot 4CF_3CO_2H$ (TFA salt of **4**): 2639.47
molecular weight calculated for $C_{102}H_{143}N_{25}O_{29}$ (free base of **4**): 2183.38
exact mass calculated for $C_{102}H_{143}N_{25}O_{29}$ (free base of **4**): 2182.04

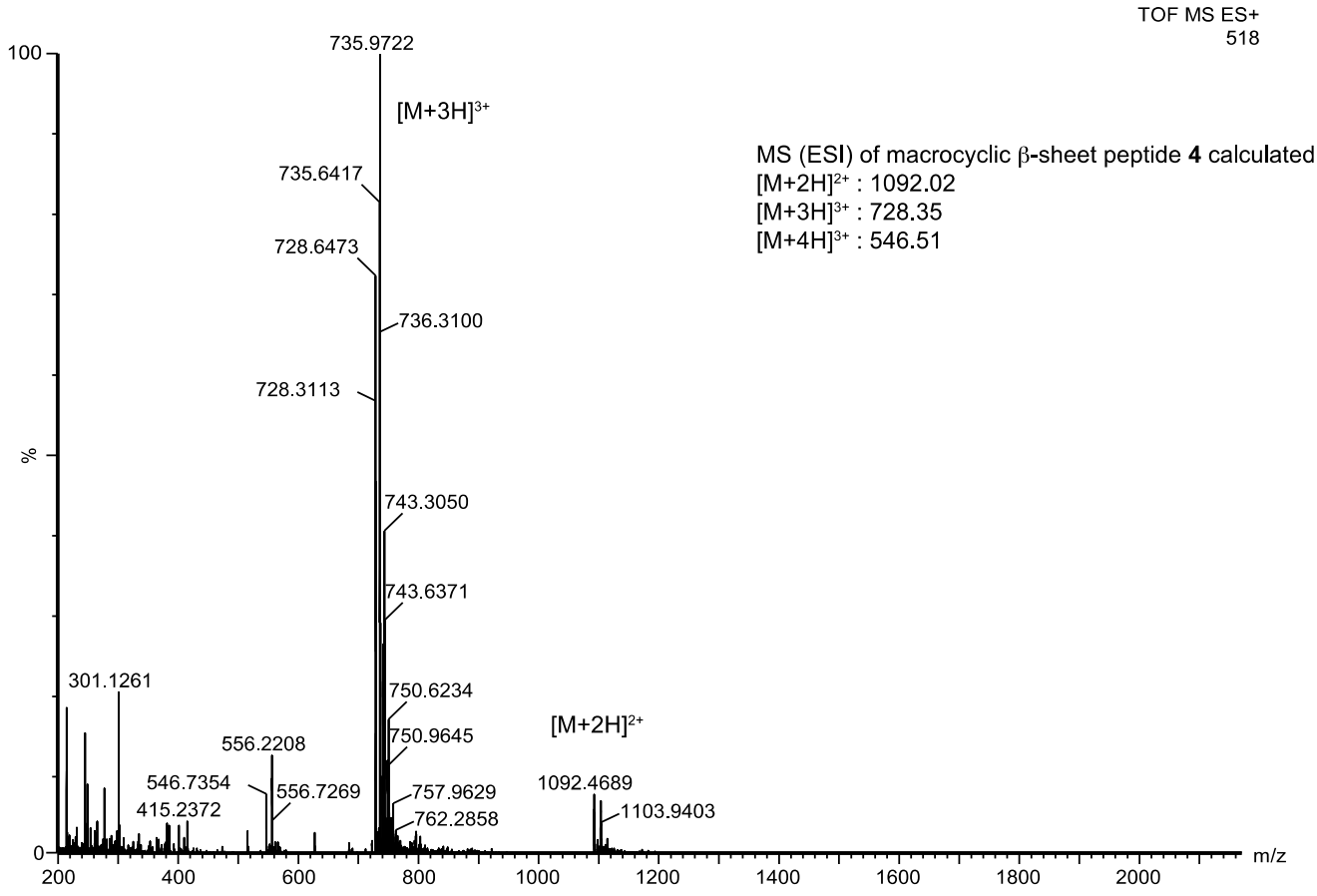
Analytical RP-HPLC of macrocyclic β-peptide **4**



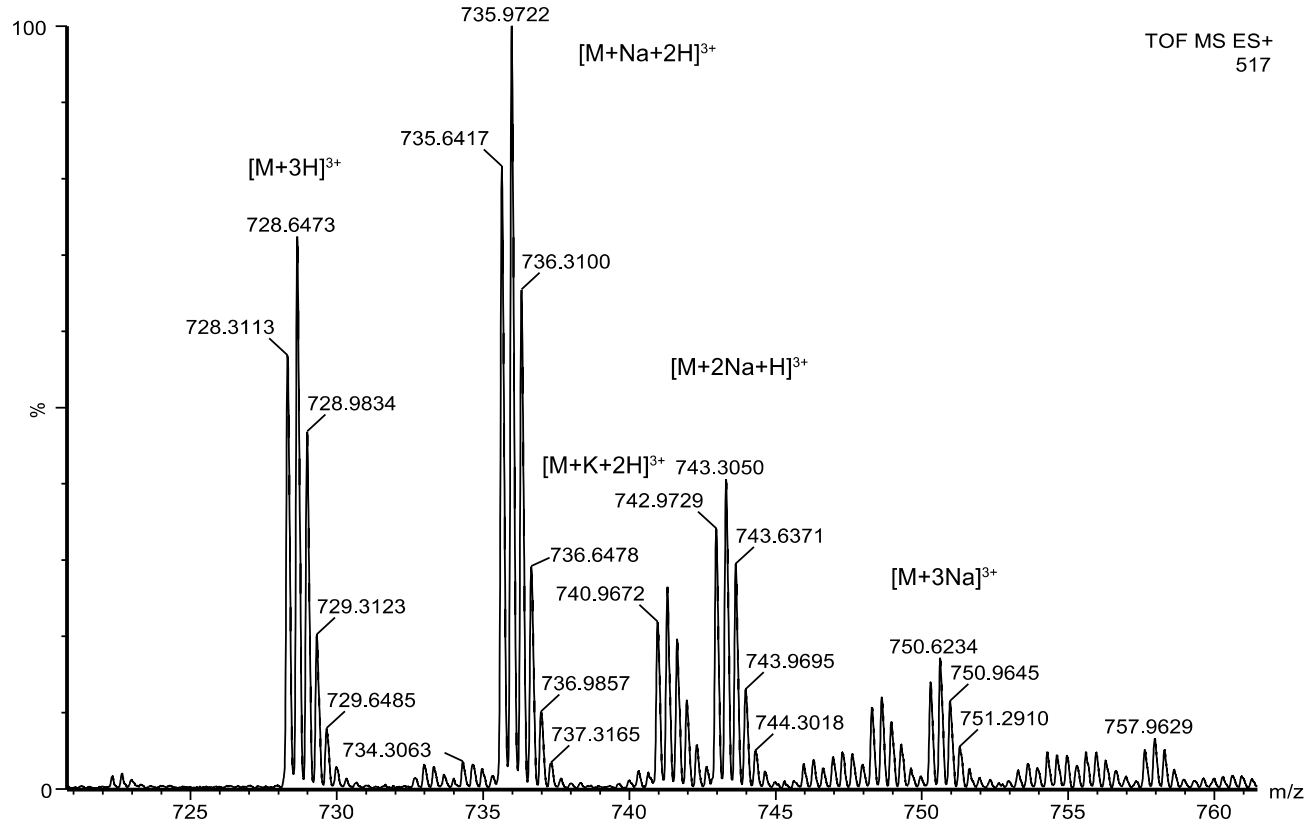
Signal 1: VWD1 A, Wavelength=214 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	8.098	VV	0.1003	5771.48926	812.59326	100.0000
Totals :				5771.48926	812.59326	

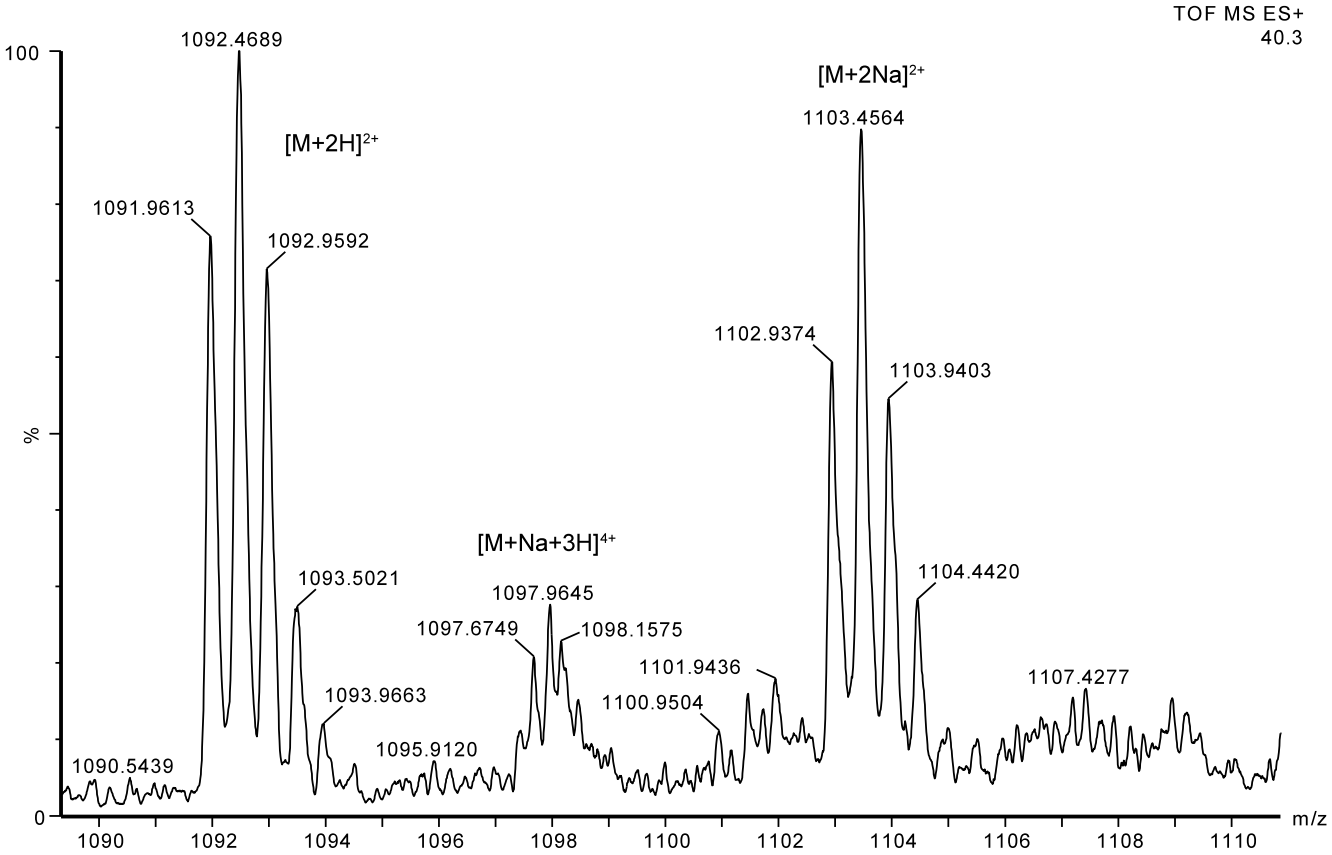
Macrocyclic β-sheet peptide **4**

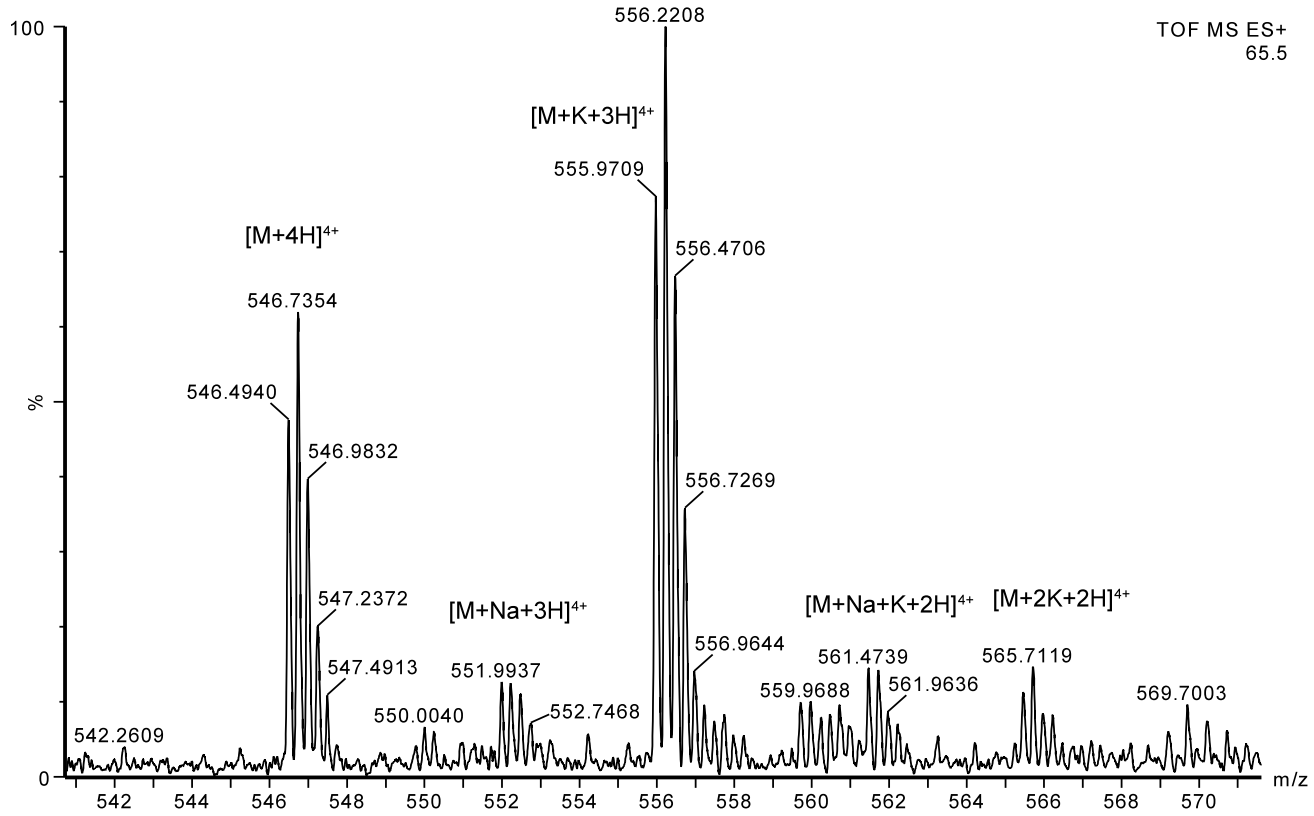


MS (ESI) of macrocyclic β-sheet peptide **4** calculated m/z for
$[M+2H]^{2+}$: 1092.02
$[M+3H]^{3+}$: 728.35
$[M+4H]^{3+}$: 546.51

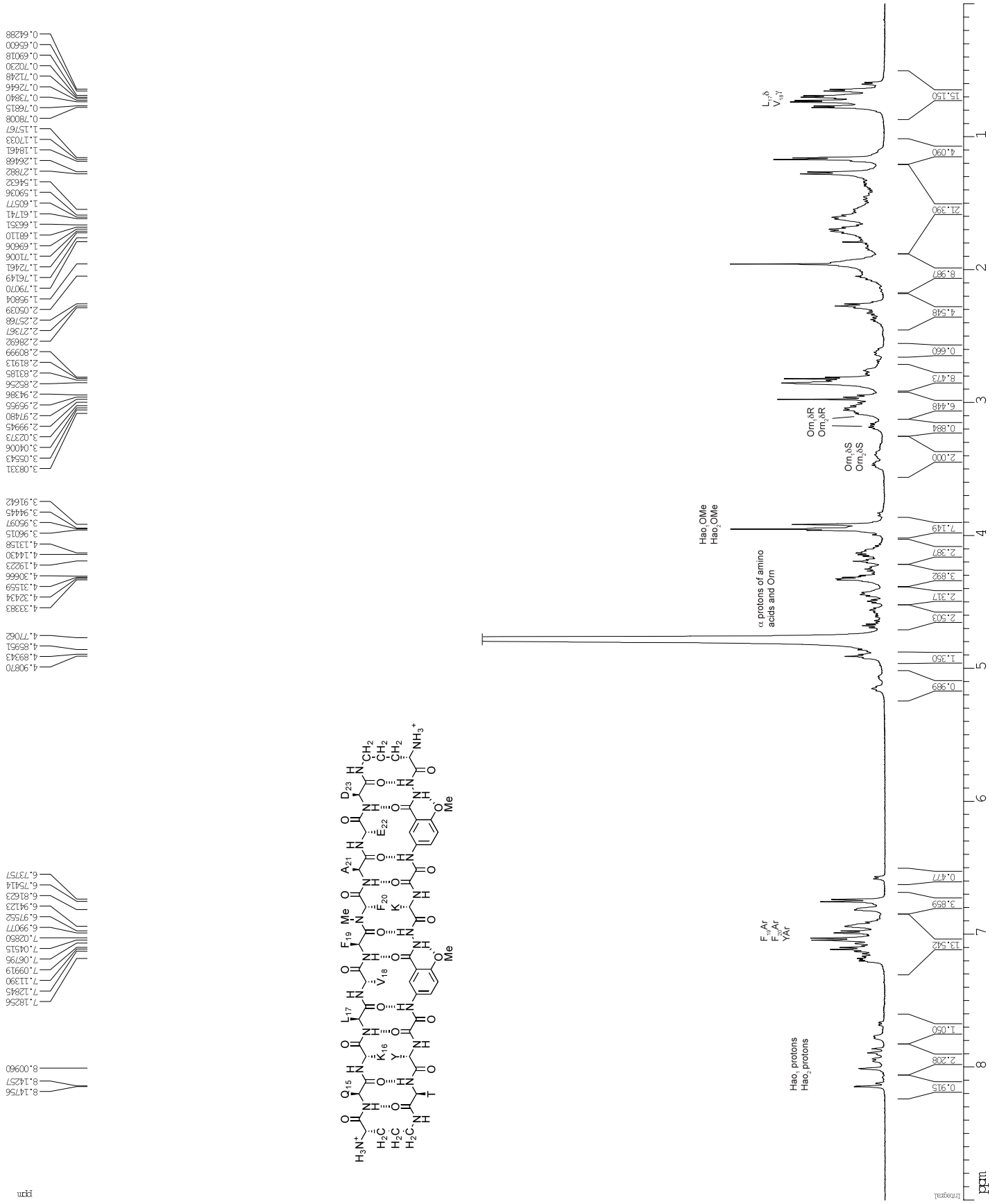


Macrocyclic β-sheet peptide **4**

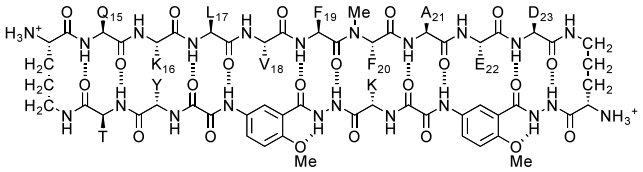


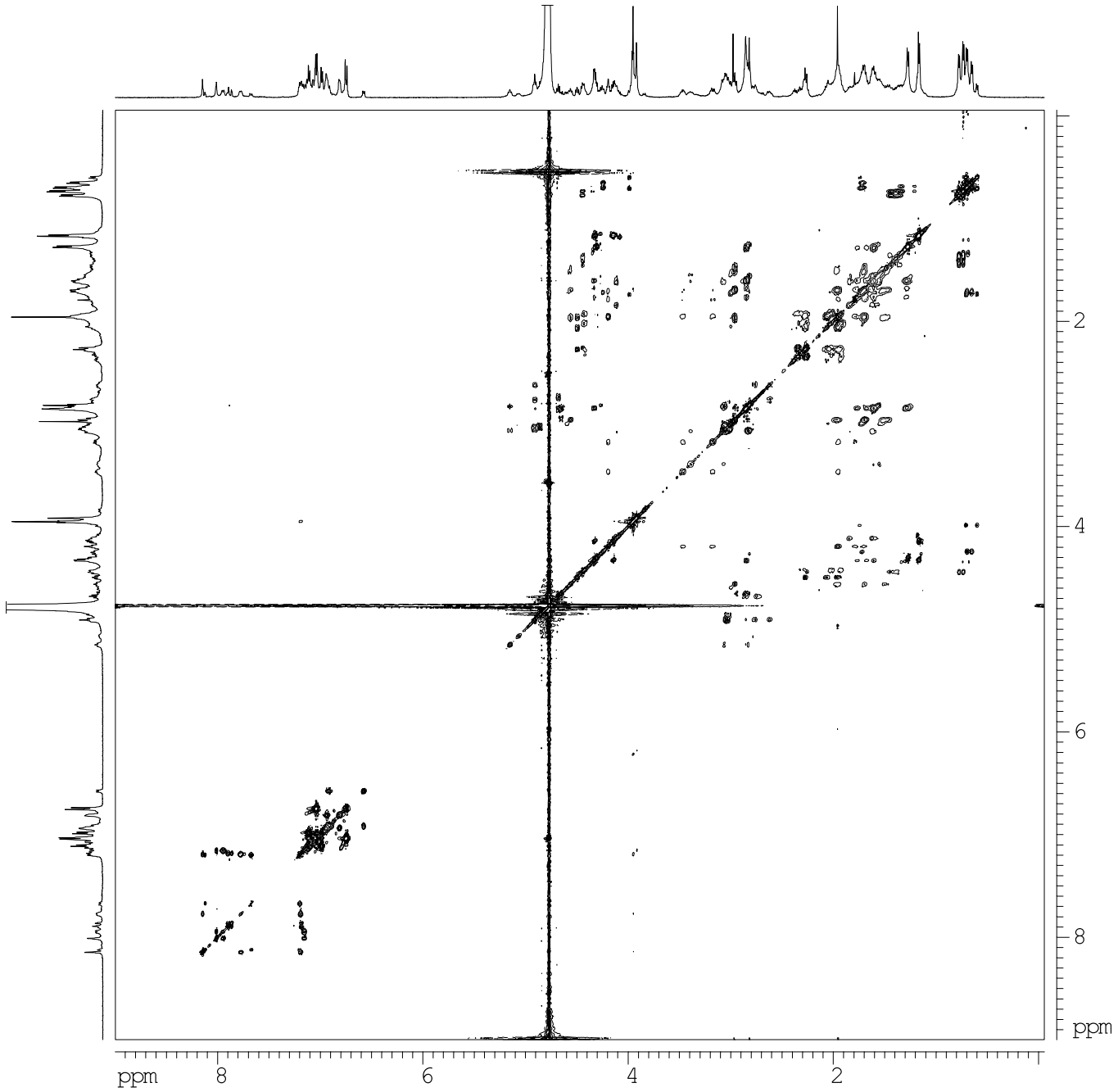


1D ^{1}H NMR spectrum of macrocyclic β-sheet peptide **4**
2 mM in D_2O, 500 MHz, 298 K

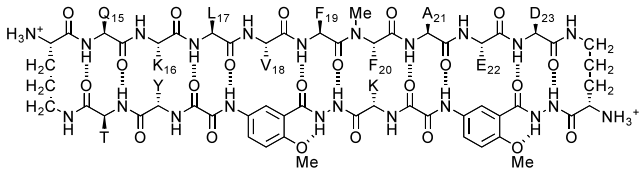


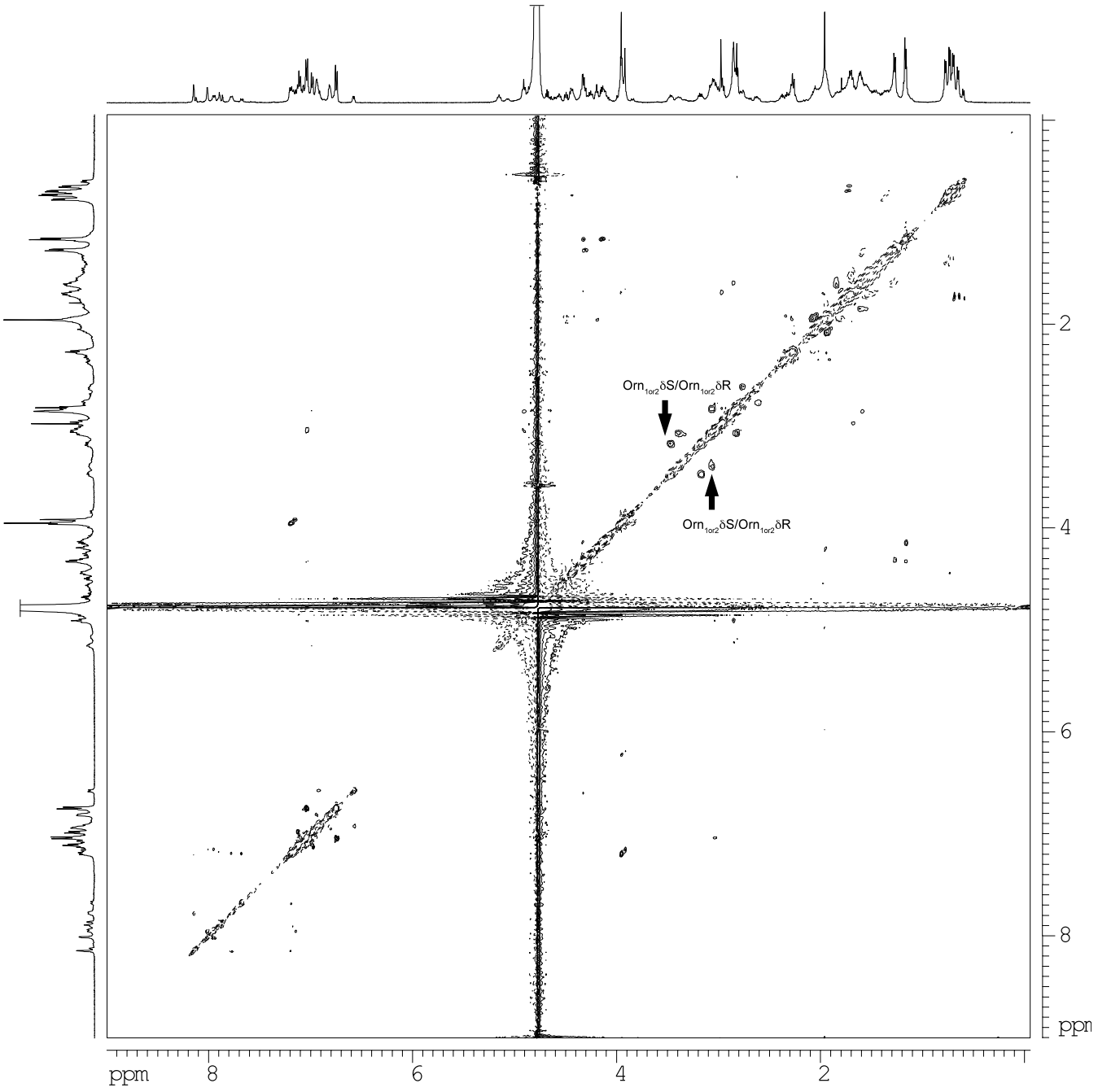
2D TOCSY spectrum of macrocyclic β-sheet peptide **4** as monomer
2 mM in D_2O, 500 MHz, 298 K
150 ms spin-locking time



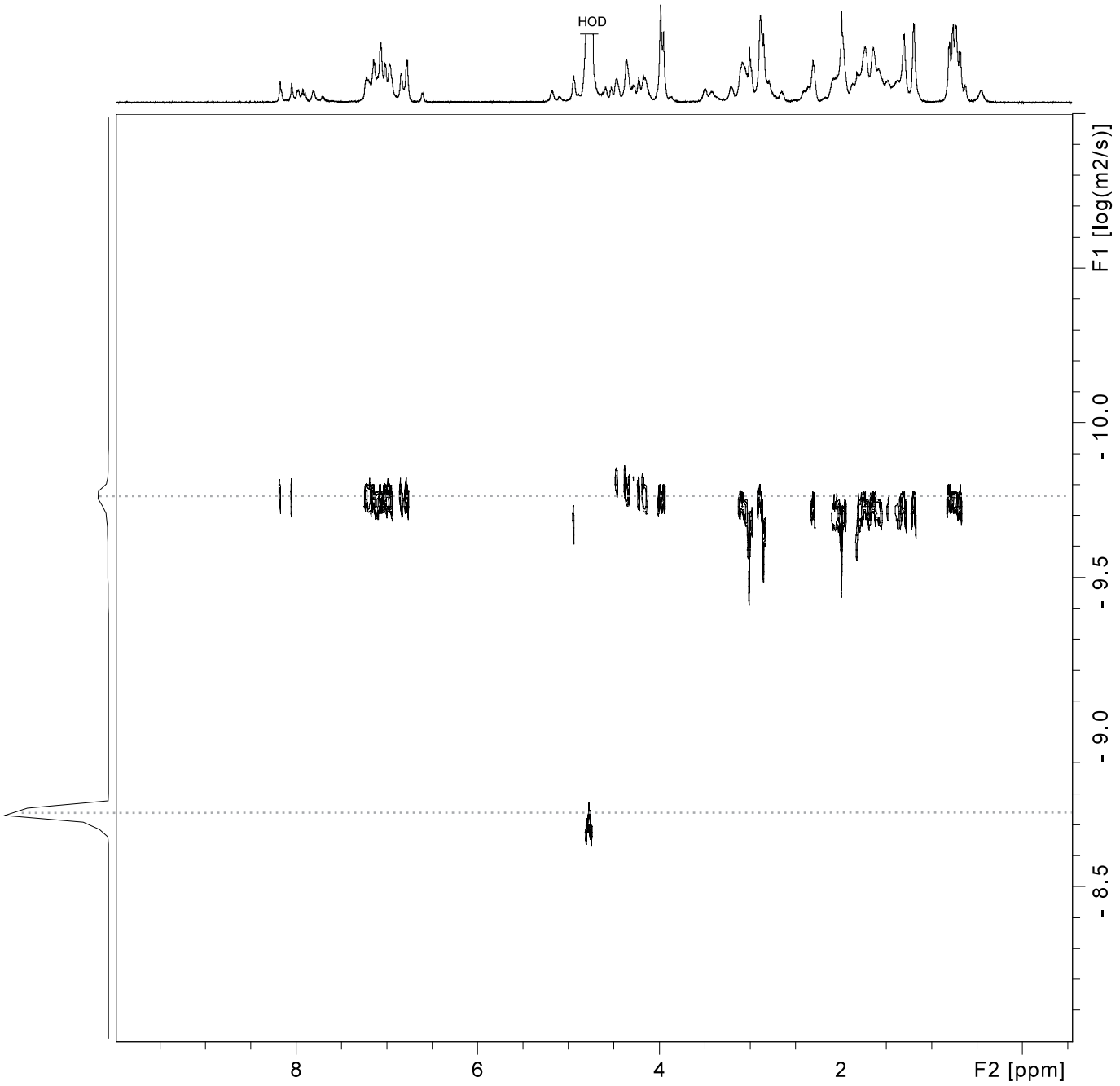


2D ROESY spectrum of macrocyclic β-sheet peptide **4**
2 mM in D_2O, 500 MHz, 298 K
200 ms spin-locking time





2D DOSY spectrum of macrocyclic β-sheet **4**
2 mM in D_2O, 600 MHz, 298 K
monomer predominates

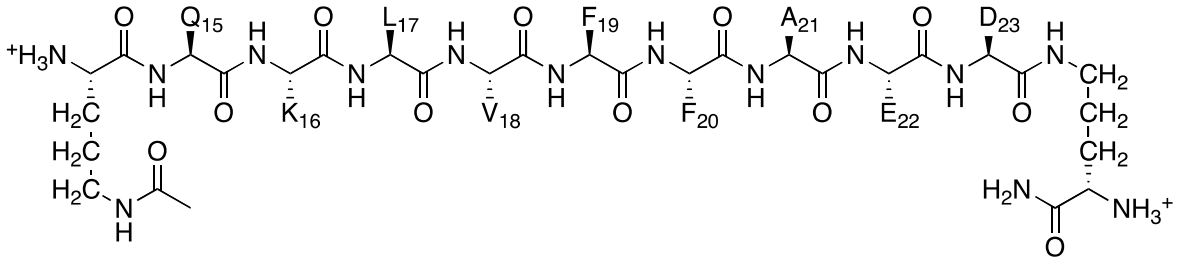


Calculation for 4 at 2.0 mM

$DC_{HOD} = 19.0 \times 10^{-10}$ m^2/s [a]
$\log DC_{HOD} = -8.721$

For 4 tetramer, log DC (m^2/s) = -9.75(4), DC = $10^{-9.754}$ m^2/s = 17.6×10^{-11} m^2/s = 17.6×10^{-7} cm^2/s

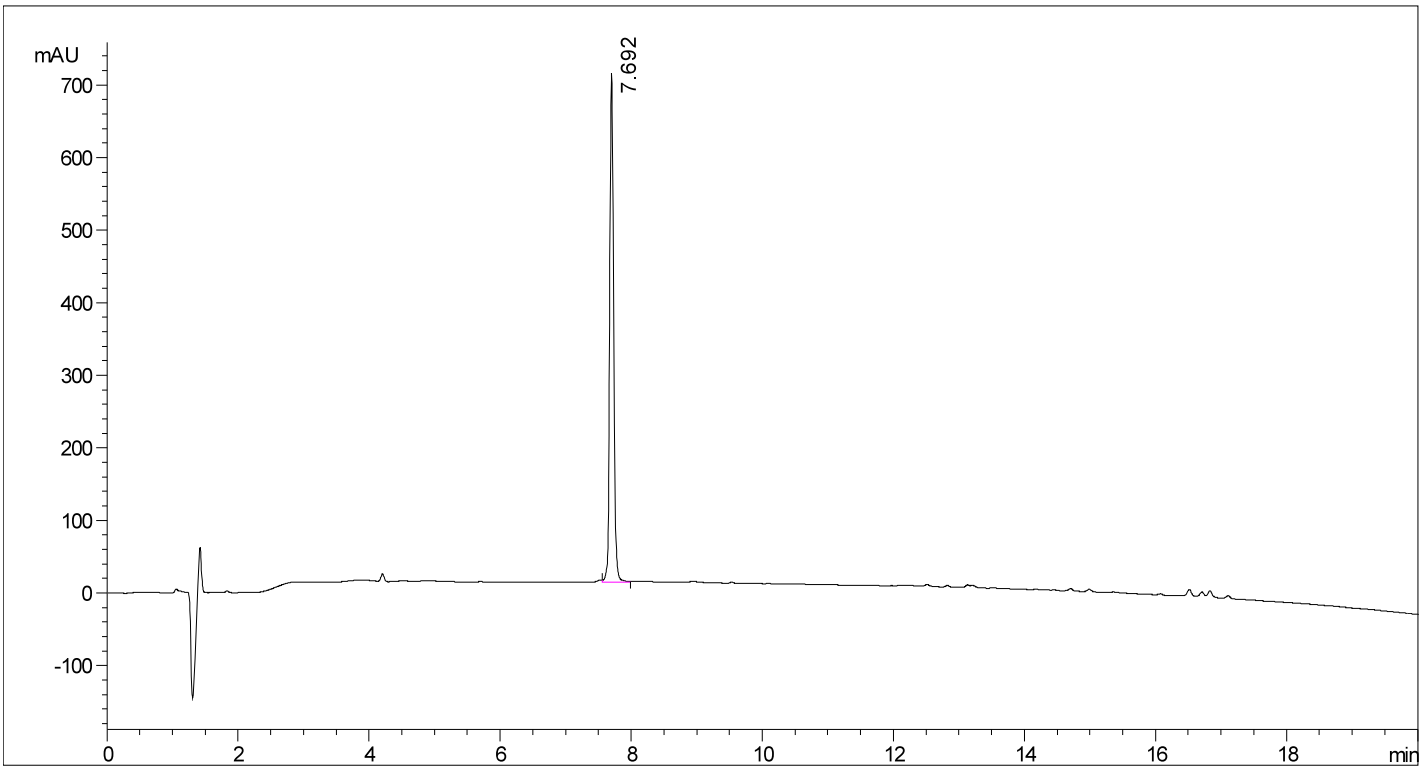
[a] Longsworth, L. G. J. Phys. Chem. 1960, 64, 1914–1917.



linear peptide **5** (as the TFA salt)

molecular weight calculated for $C_{64}H_{100}N_{16}O_{17} \cdot 3CF_3CO_2H$ (TFA salt of **5**): 1707.65
molecular weight calculated for $C_{64}H_{100}N_{16}O_{17}$ (free base of **5**): 1365.58
exact mass calculated for $C_{64}H_{100}N_{16}O_{17}$ (free base of **5**): 1364.75

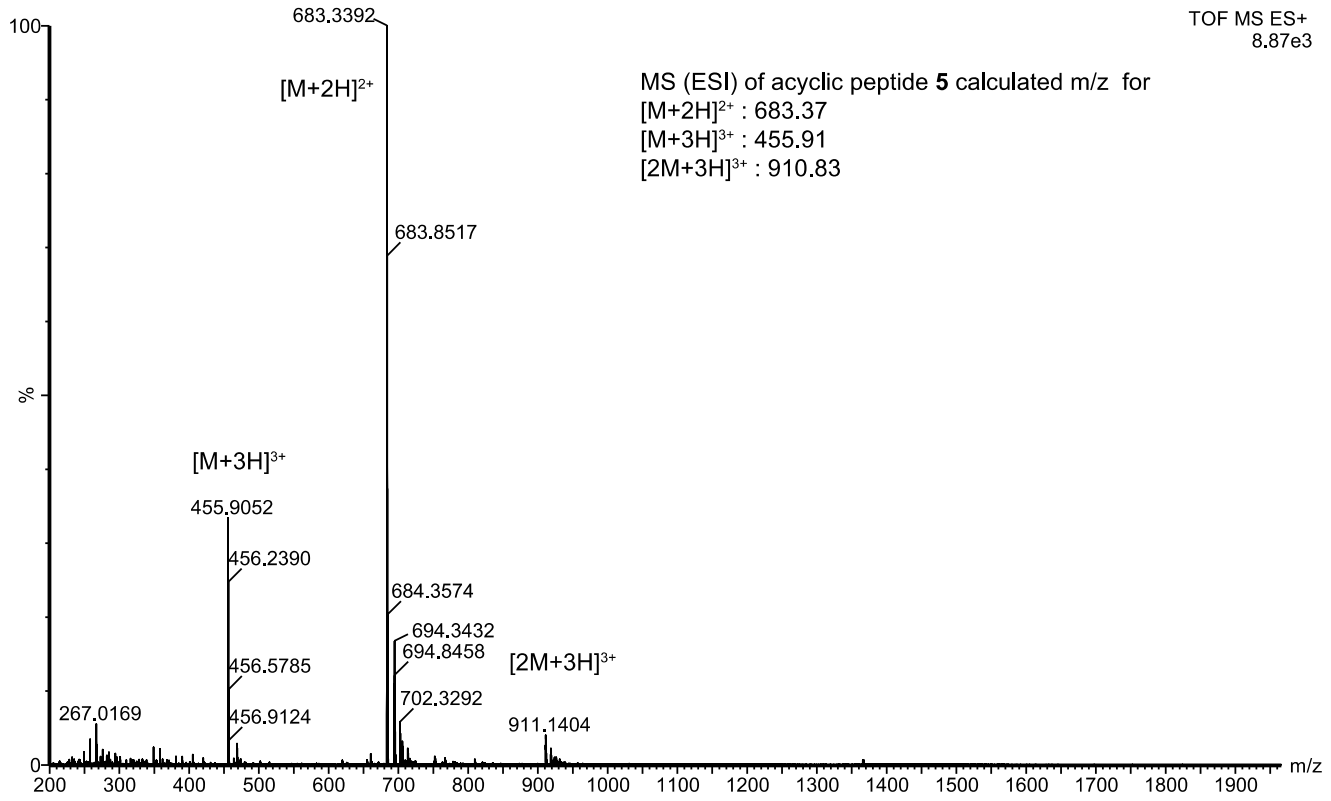
Analytical RP-HPLC of linear peptide **5**

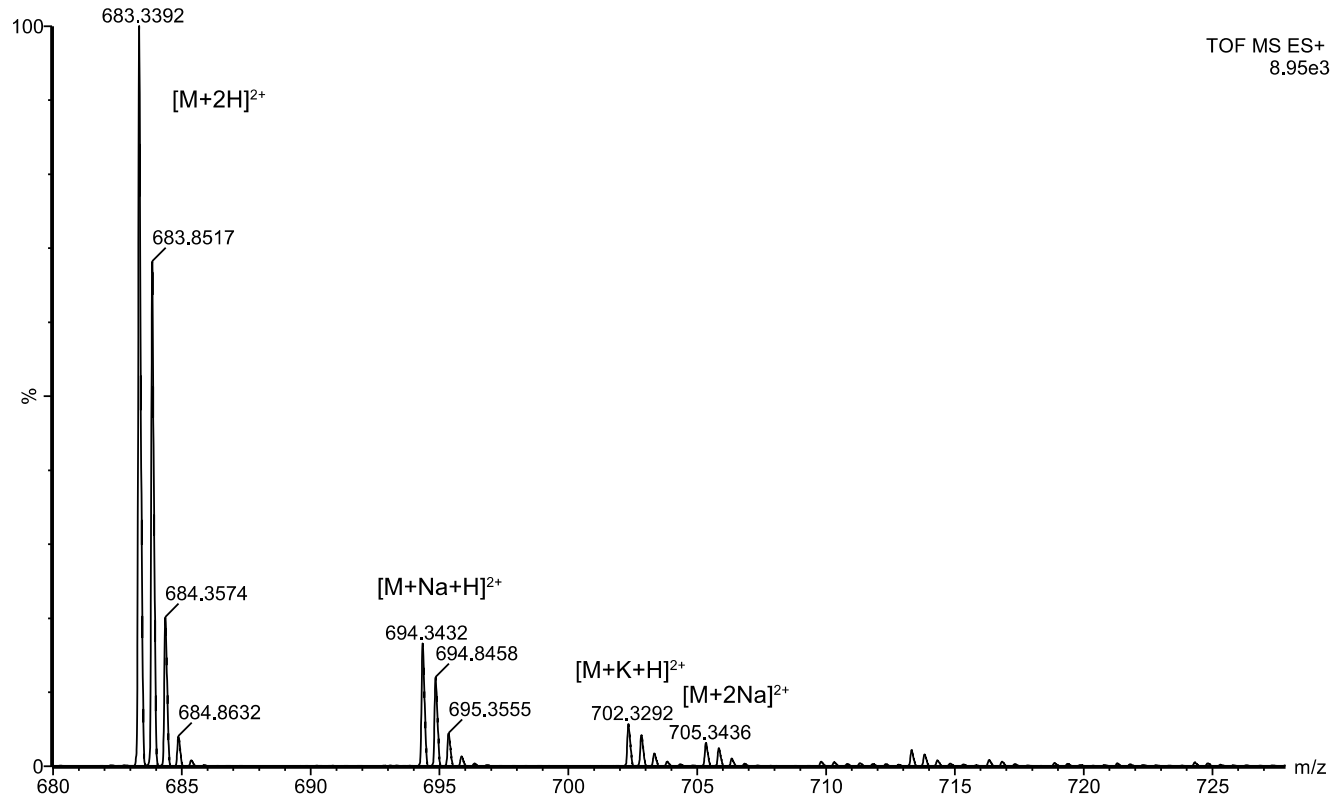


Signal 1: VWD1 A, Wavelength=214 nm

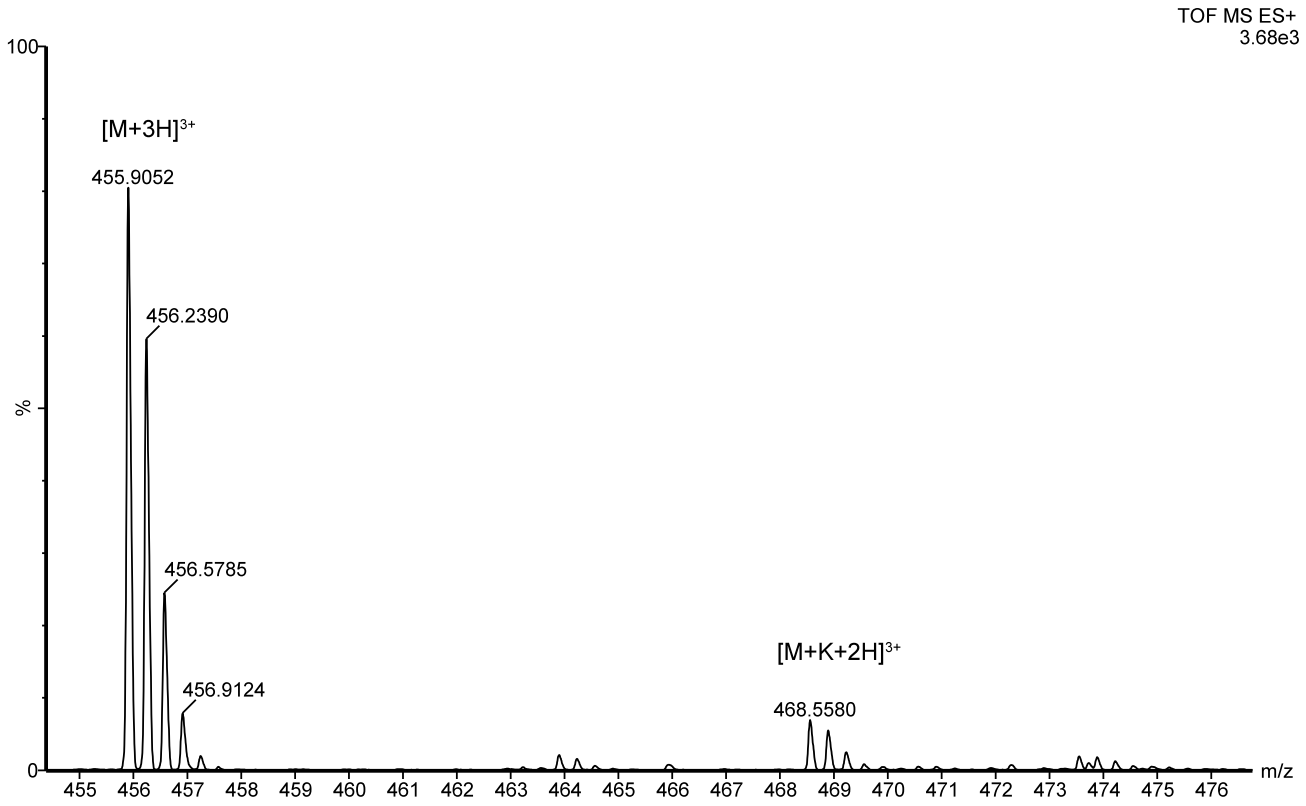
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.692	VV	0.0657	2891.74707	698.99927	100.0000
Totals :				2891.74707	698.99927	

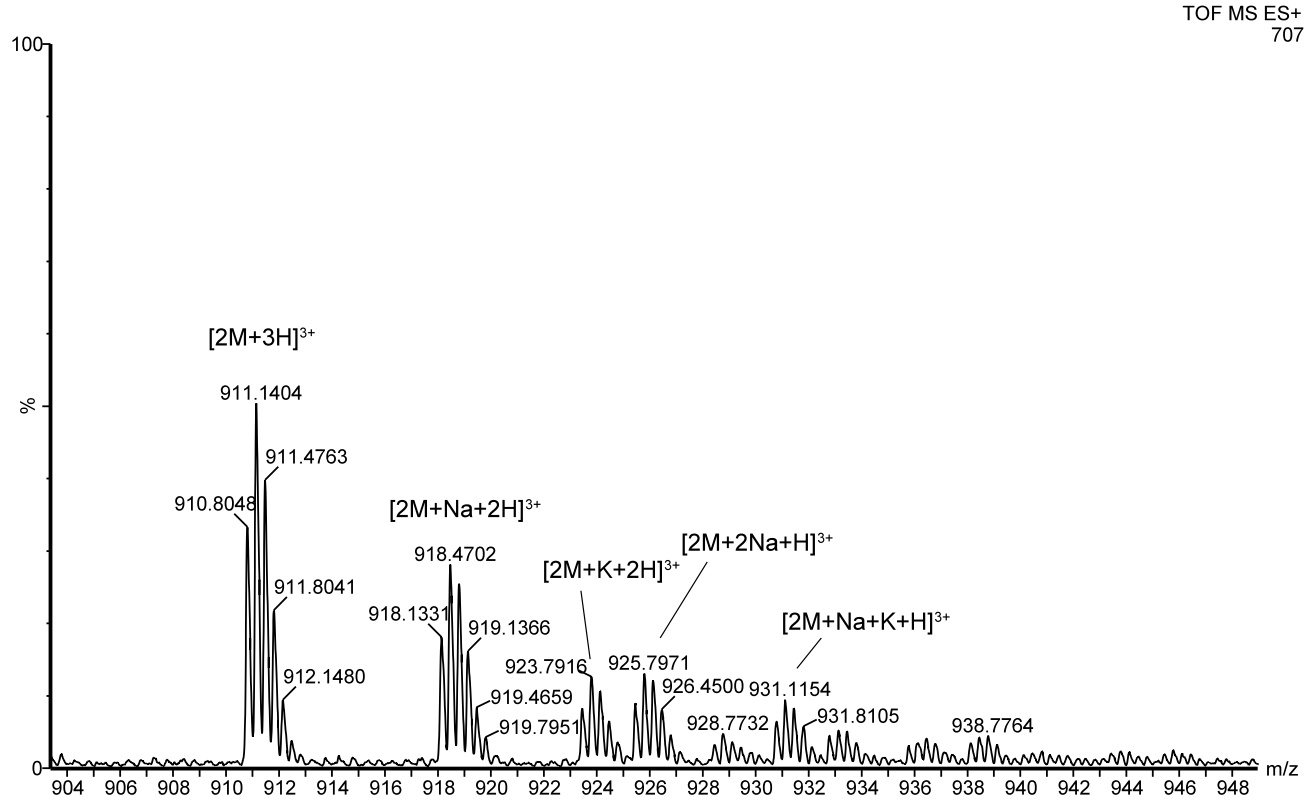
Linear peptide **5**



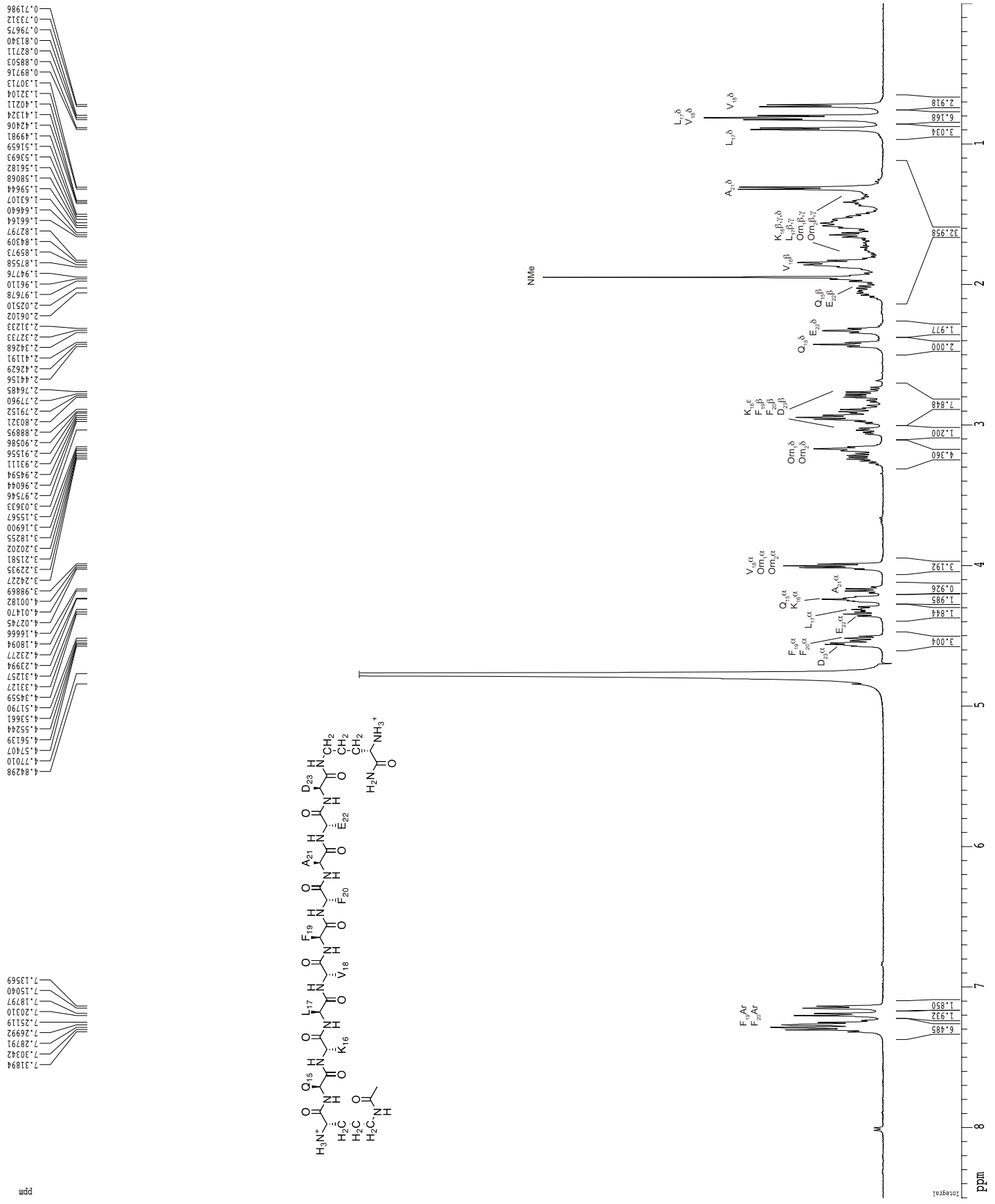


Linear peptide **5**

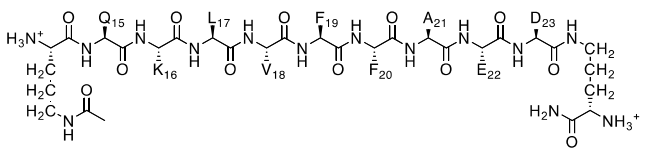


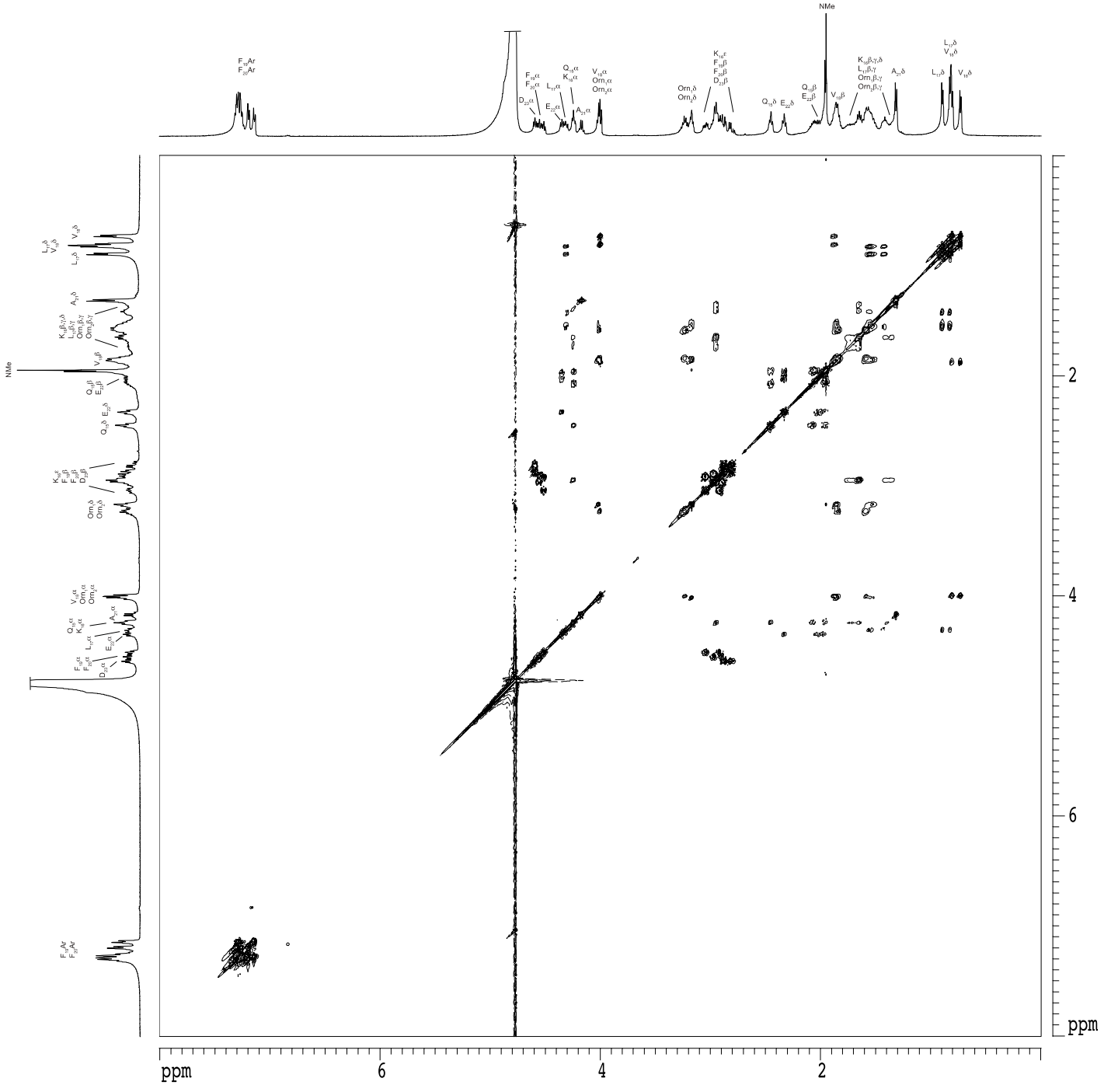


1D ^{1}H NMR spectrum of linear peptide **5** as control
1.2 mM in D_2O, 500 MHz, 298 K

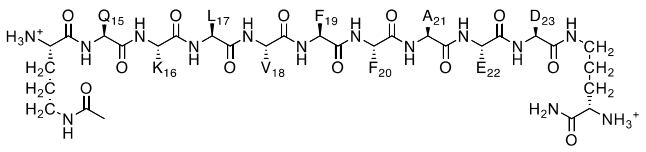


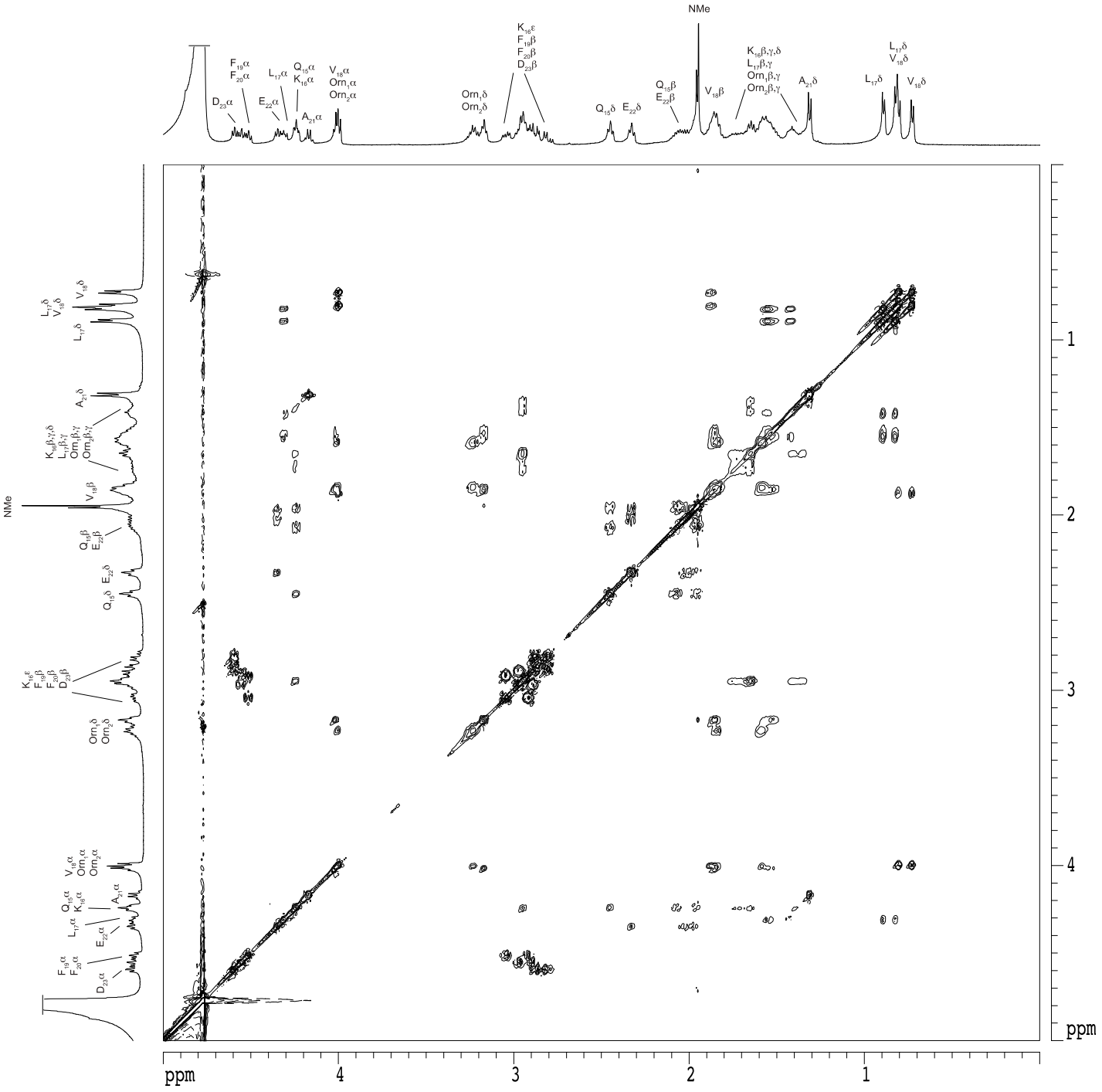
2D TOCSY NMR spectrum of linear peptide **5** as control
4.95 mM in D_2O, 500 MHz, 298 K
150 ms spin-locking time



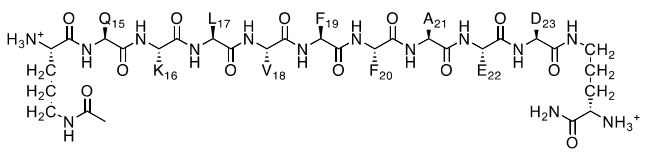


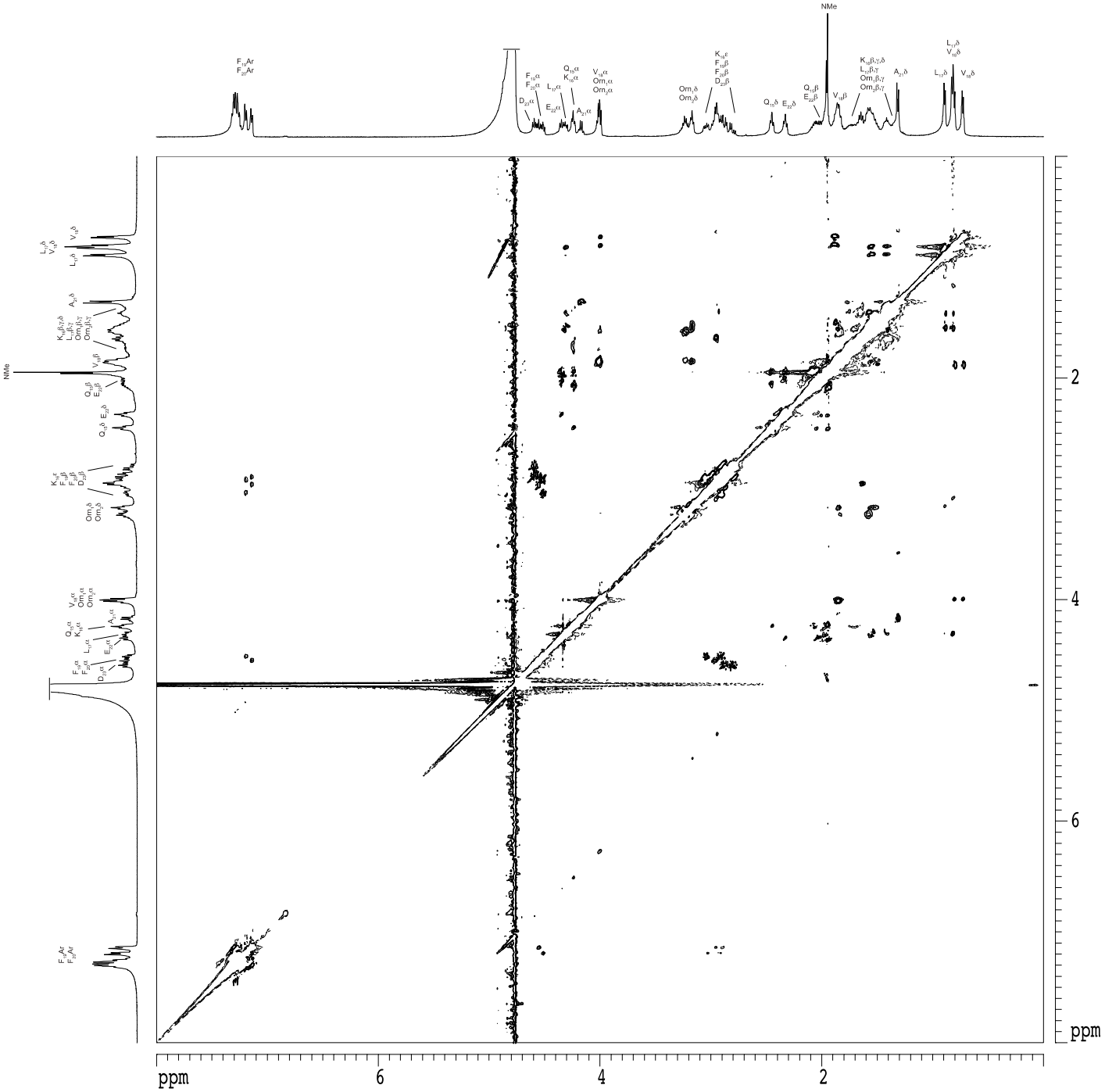
2D TOCSY NMR spectrum of linear peptide **5** as control
4.95 mM in D_2O, 500 MHz, 298 K
150 ms spin-locking time





2D ROESY NMR spectrum of linear peptide **5** as control
4.95 mM in D_2O, 500 MHz, 298 K
200 ms spin-locking time





2D ROESY NMR spectrum of linear peptide **5** as control
4.95 mM in D_2O, 500 MHz, 298 K
200 ms spin-locking time