Supporting information for

Assembly of Peptides Derived from β-Sheet Regions of β-Amyloid

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I. SUPPLEMENTAL FIGURES

II. MATERIALS AND METHODS

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I. SUPPLEMENTAL FIGURES

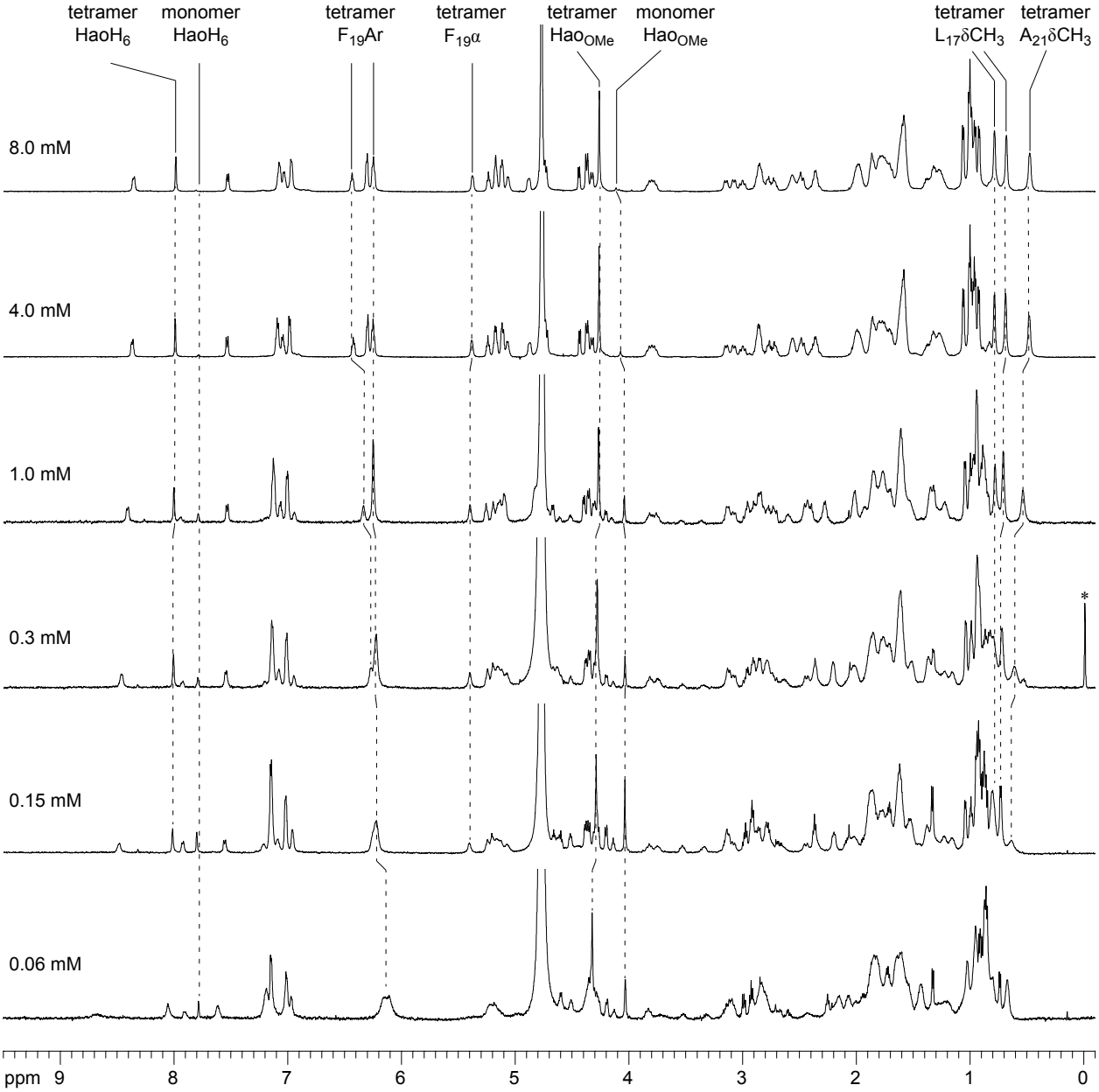


Figure S1. ^{1}H NMR spectra of peptide **1a** at various concentrations in D_2O at 600 MHz and 298 K. The 0.3 mM sample contains DSA as an internal standard, which is marked by an asterisk (*).

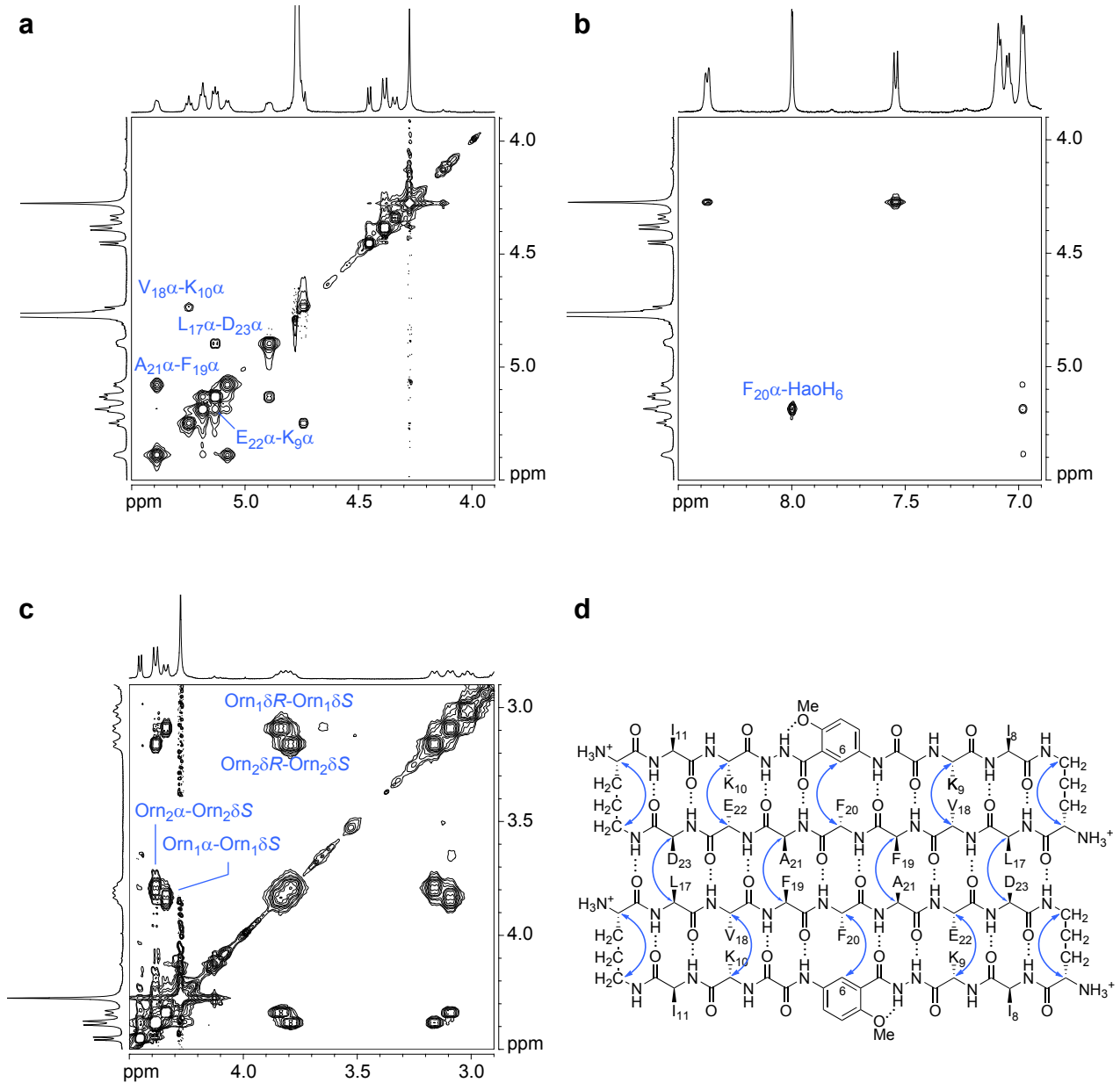


Figure S2. Expansions of the NOESY spectrum of peptide **1a** at 8.0 mM in D_2O at 600 MHz and 298 K. Key NOEs associated with β-sheet folding and dimerization are highlighed in blue. The $^{\delta}$Orn *pro-R* δ-protons are designated Ornδ*R*; the $^{\delta}$Orn *pro-R* δ-protons are designated Ornδ*R*.

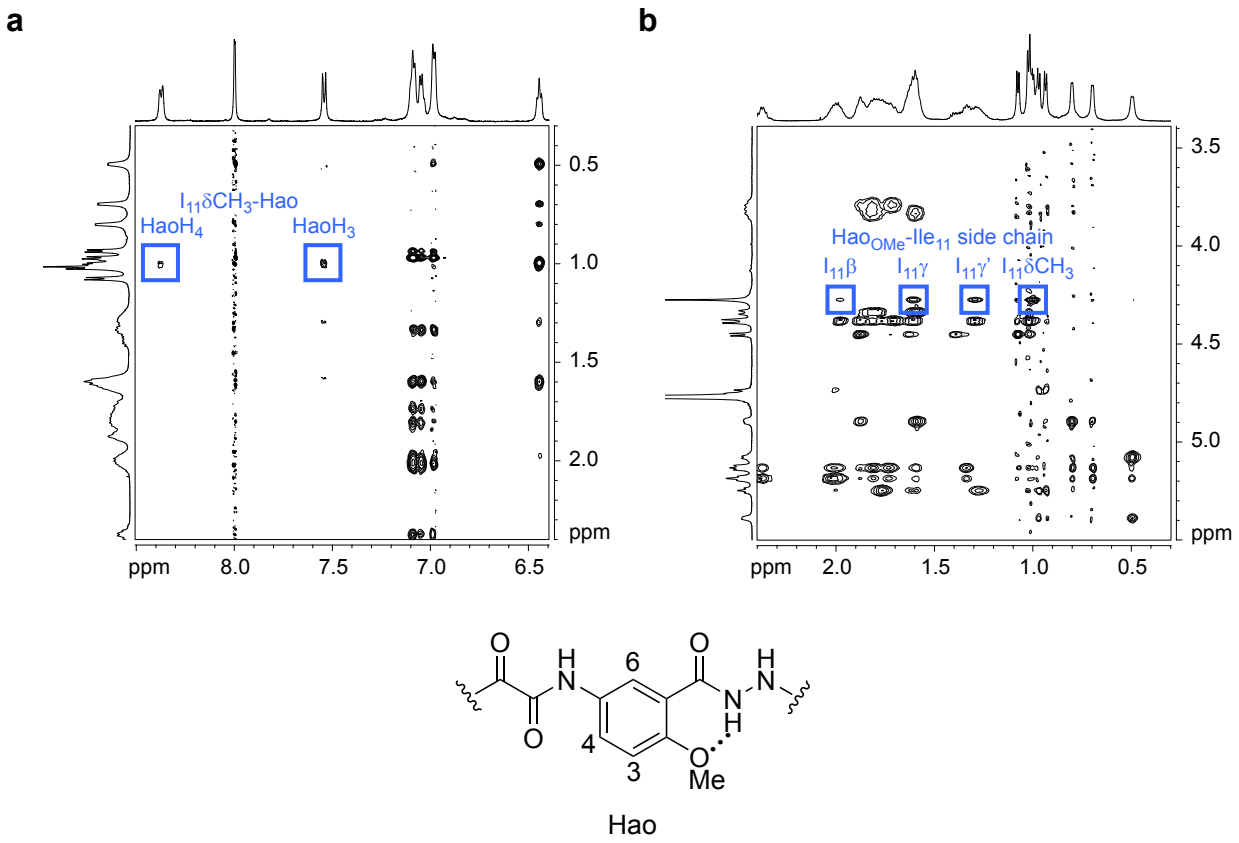


Figure S3. Expansions of the NOESY spectrum of peptide **1a** at 8.0 mM in D_2O at 600 MHz and 298 K. Key interlayer NOEs associated with tetramerization are highlighted in blue.

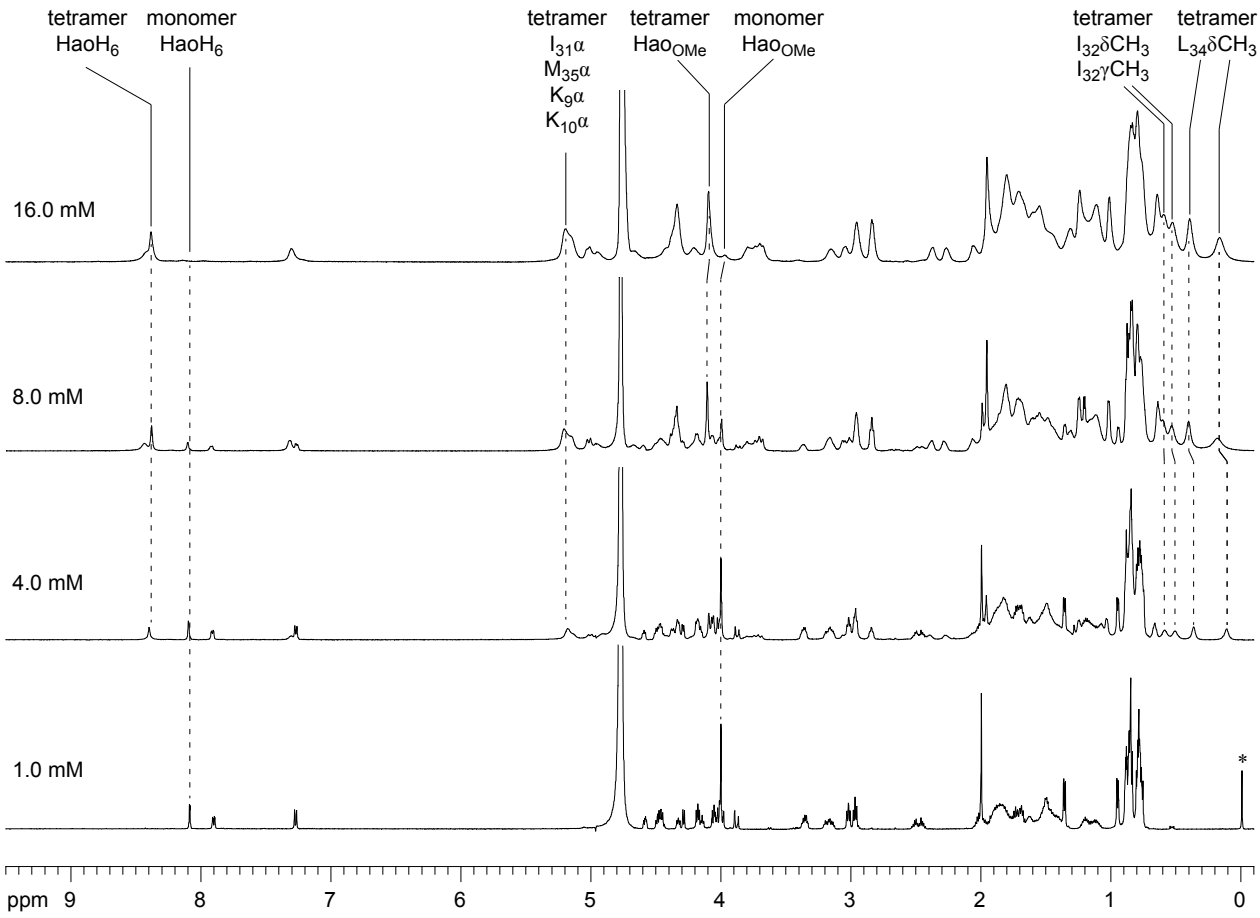


Figure S4. ^{1}H NMR spectra of peptide **1b** at various concentrations in D_2O at 600 MHz and 298 K. The 1.0 mM sample contains DSA as an internal standard, which is marked by an asterisk (*).

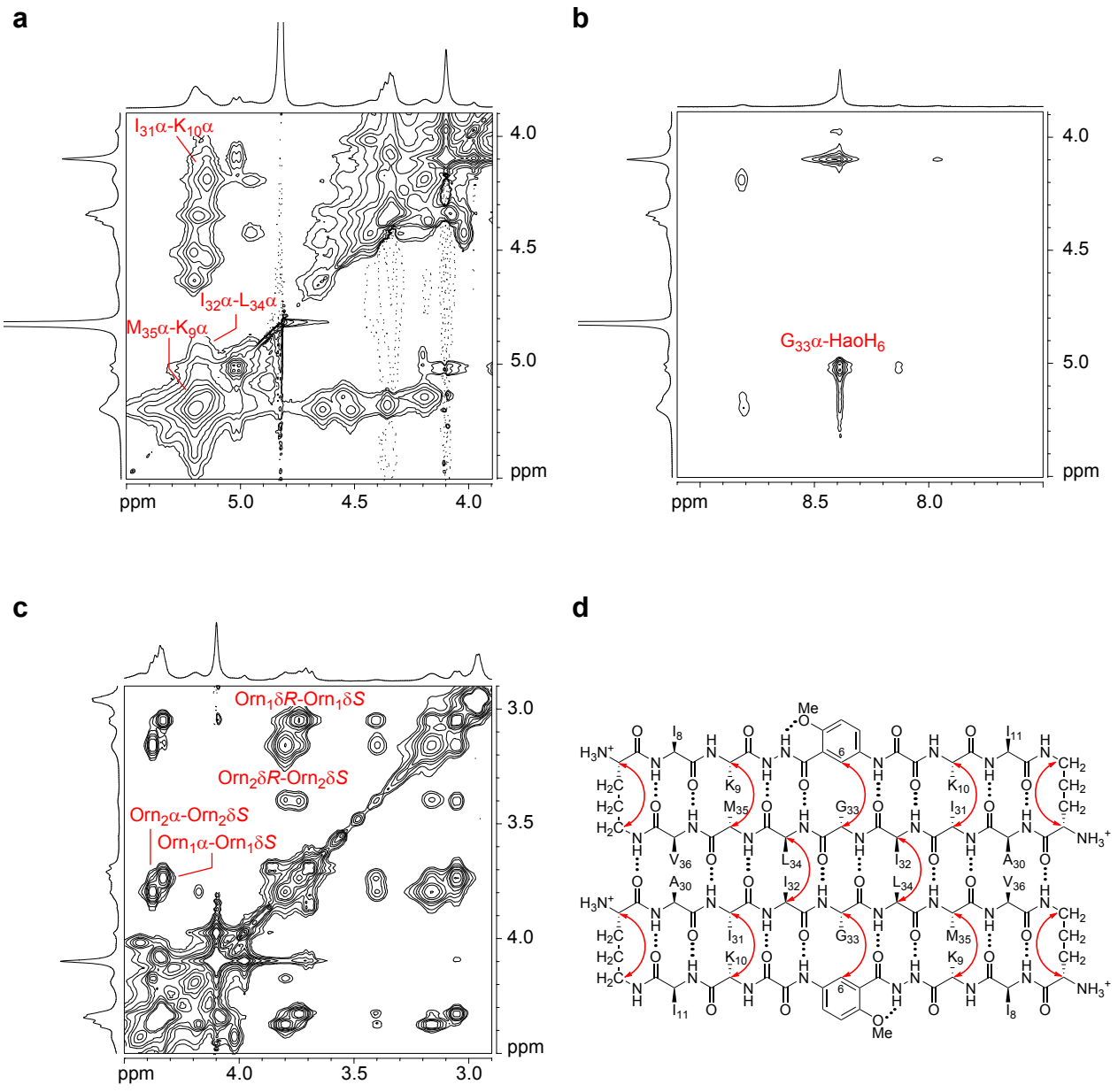


Figure S5. Expansions of the NOESY spectrum of peptide **1b** at 16.0 mM in D_2O at 600 MHz and 293 K. Key NOEs associated with β-sheet folding and dimerization are highlighed in red. The G_{33} *pro-R* α-proton is designated $G_{33}α$; the $^{\delta}$Orn *pro-R* δ-protons are designated Ornδ*R*; the $^{\delta}$Orn *pro-R* δ-protons are designated Ornδ*R*.

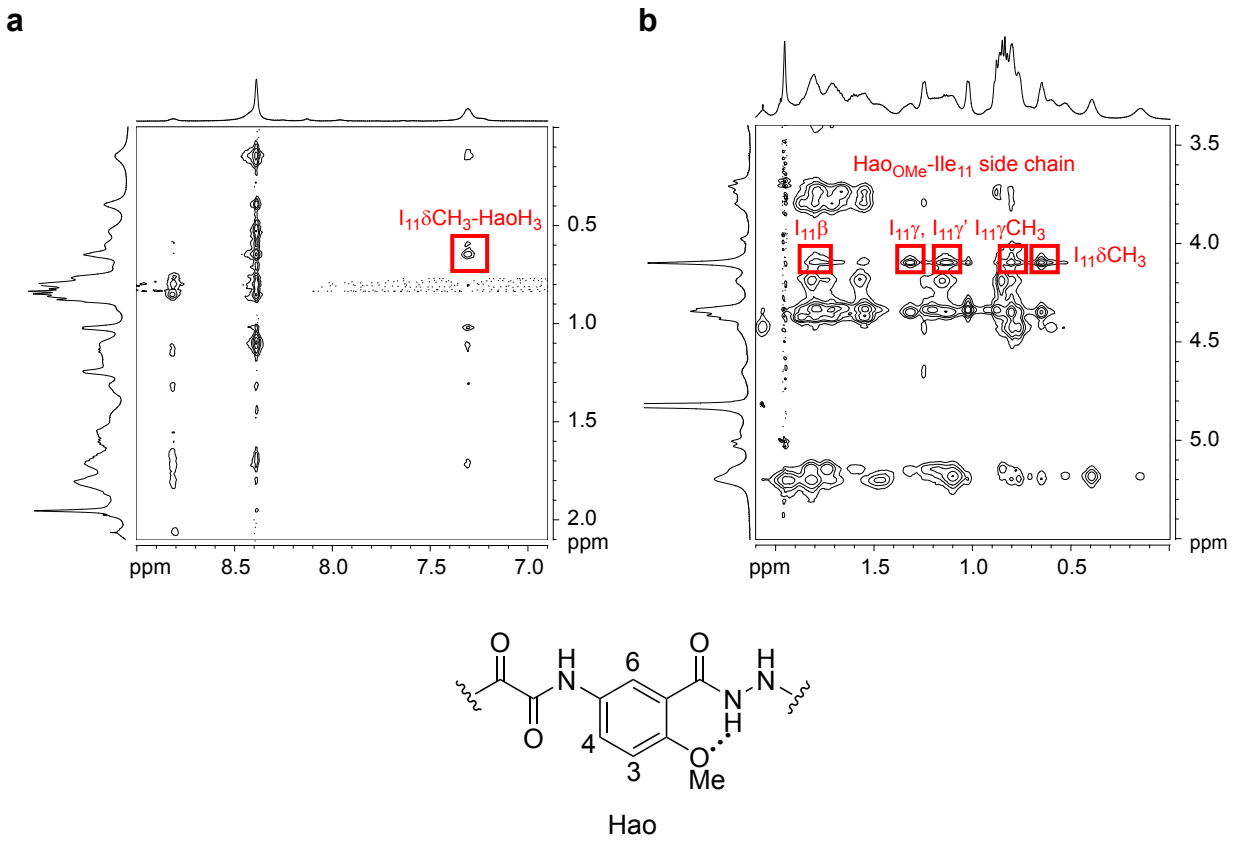


Figure S6. Expansions of the NOESY spectrum of peptide **1b** at 8.0 mM in D_2O at 600 MHz and 293 K. Key interlayer NOEs associated with tetramerization are highlighted in red.

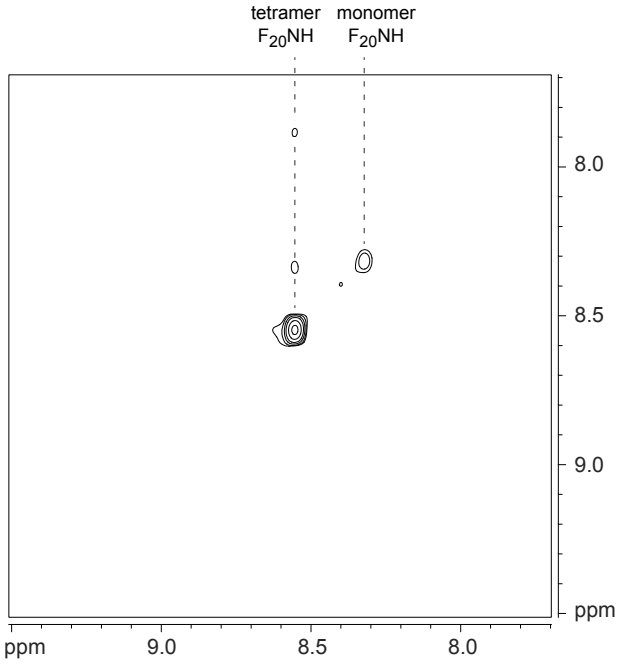


Figure S7. ^{15}N-Edited NOESY spectrum of peptide [^{15}N]**1a** at 8.0 mM in 9:1 H_2O/D_2O at 600 MHz and 293 K.

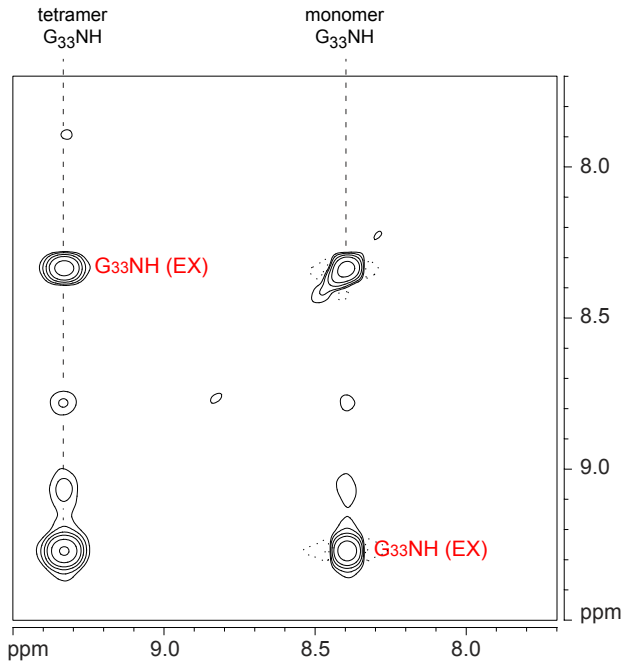


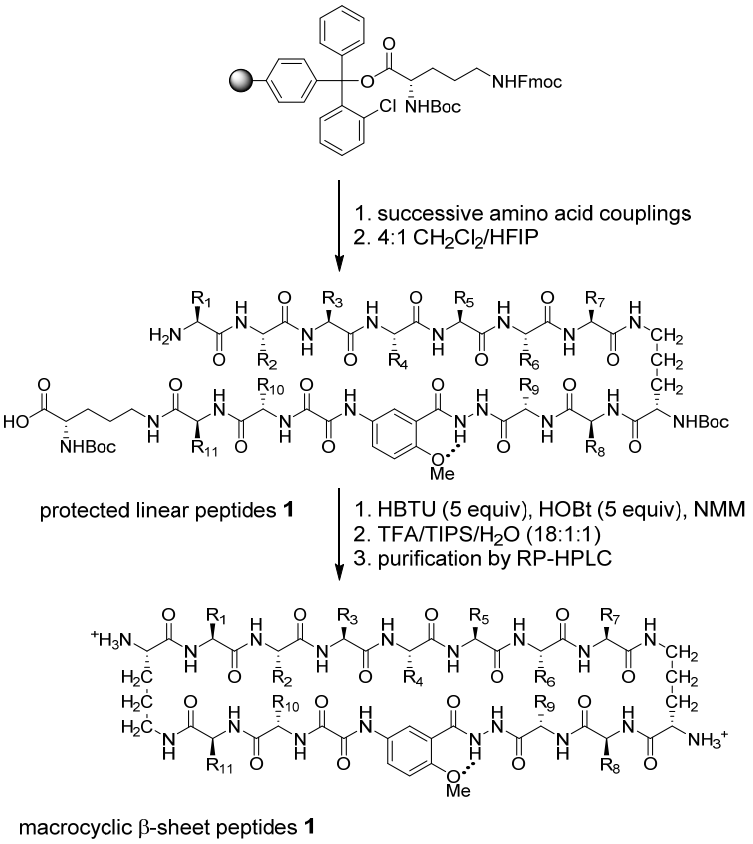
Figure S8. ^{15}N-Edited NOESY spectrum of peptide [^{15}N]**1b** at 8.0 mM in 9:1 H_2O/D_2O at 600 MHz and 293 K. Crosspeaks associated with chemical exchange between the monomers and tetramers are labeled EX.

II. MATERIALS AND METHODS

General

N,*N*-Dimethylformamide (DMF), 2,4,6-collidine, and piperidine were purchased from Alfa Aesar and used without further purification. HPLC grade acetonitrile (CH_3CN) was purchased from VWR International and used without further purification. Methylene chloride (CH_2Cl_2) was purchased from Fisher Scientific, stored under argon, and passed through a column of alumina before use.[1] Boc-Orn(Fmoc)-OH, HCTU, HBTU and HOBt were purchased from GL Biochem Ltd (Shanghai). 2-Chlorotrityl chloride resin and Fmoc protected amino acids were purchased from Chem-Impex International. *N*,*N*-Diisopropylethylamine (DIPEA), *N*-methylmorpholine (NMM), trifluoroacetic acid (TFA), and triisopropylsilane (TIPS) were purchased from Oakwood Chemical. Isotopically labeled glycine (^{15}N, 98%), phenylalanine (^{15}N, 98%), and deuterium oxide (D, 99.96%) were purchased from Cambridge Isotope Laboratories, Inc. Fmoc-Hao-OH was synthesized according to previously reported procedures.[2]

Synthesis of Peptides 1



Resin Loading. 2-Chlorotrityl chloride resin (300 mg, 1.1 meq/g, 100–200 mesh) was suspended in ca. 8 mL of CH_2Cl_2 in a 10-mL Bio-Rad Poly-Prep column and allowed to swell (15 min). The CH_2Cl_2 was drained and a solution of Boc-Orn(Fmoc)-OH (0.22 mmol, 100.0 mg) in CH_2Cl_2 (7.6 mL) and 2,4,6-collidine (0.4 mL), was added. The suspension was agitated gently overnight (10–12 h) and the solution was drained. The capping solution 17:2:1 CH_2Cl_2/MeOH/DIPEA (8 mL) was added. The mixture was agitated gently (1 h), and then the solution was drained.

Solid-Phase Peptide Synthesis. The loaded resin was transferred to a solid-phase peptide synthesis vessel with DMF (3 × 2 mL). Successive rounds of solid-phase peptide synthesis were performed on a PS3^{TM} Peptide Synthesizer (Protein Technologies) using the following conditions: The Fmoc deprotection steps (2 × 5 min) were performed with a 20% piperidine in DMF solution. The coupling steps (1 × 20 min) were performed for the amino acids (4 equiv) with HCTU (4 equiv) and a 20% 2,4,6-collidine in DMF solution. The unnatural amino acid Fmoc-Hao-OH (2 equiv) was coupled twice with 2 equiv of HCTU per coupling (60 min) to achieve complete coupling. DMF was used to rinse the resin after each deprotection (6 × 3 mL) and after each amino acid coupling (6 × 3 mL).

Cleavage from Resin. After the synthesis of each peptide was complete, the resin was transferred into the Poly-Prep column with CH_2Cl_2 (ca. 2 mL) and the solution was drained. The solid-phase peptide synthesis vessel was rinsed with ca. two additional portions of CH_2Cl_2 to ensure the complete transfer of the resin and the removal of DMF. A 1:4 HFIP/CH_2Cl_2 solution (8 mL) was added to the resin and the mixture was agitated gently. After 1 h, the solution was drained into a 250-mL round-bottom flask and the treatment with HFIP/CH_2Cl_2 solution was repeated. The combined solutions were evaporated under vacuum to give the protected linear peptides **1**.

Cyclization. The protected linear peptides **1** were cyclized with HBTU (5 equiv), HOBt (5 equiv), and NMM (8 equiv) in a solution of DMF (125 mL). The solution was stirred under N_2 overnight (12–24 h), and then the DMF was evaporated under vacuum. The peptides were placed under vacuum (ca. 0.1 mmHg) overnight to ensure complete removal of any residual DMF.

Deprotection. The protected cyclic peptides **1** were deprotected under acidic conditions with a solution of 18:1:1 TFA/triisopropylsilane/H_2O (10 mL). The solution was stirred for 2 h, then evaporated under vacuum. For peptides containing a methionine (**1b** and [^{15}N]**1b**), 50 mg of dithiothreitol (DTT) was added to the solution to prevent sulfur oxidation.

RP-HPLC Purification. The peptides were suspended in a solution of 20% aqueous CH_3CN (ca. 8 mL) and the suspensions were filtered through a 0.2 μm filter. The purity of each peptide was analyzed by analytical RP-HPLC on a Phenomenex Aeris 2.6μ XB-C18 column (150 mm x 4.6 mm) with a 5–100% gradient over 20 min of CH_3CN in H_2O with 0.1% TFA at 1.0 mL/min. The purification of each peptides was performed by preparative RP-HPLC on an Agilent Zorbax 7 μM SB-C18 Prep HT column (21.2 mm x 250 mm) with a 15–30% gradient over 10 min and 30–60% gradient over 45 min of CH_3CN in H_2O with 0.1% TFA at 15.0 mL/min. The pure fractions were combined and concentrated under vacuum. The peptides were re-suspended in a solution of H_2O with 0.1% TFA (ca. 10–15 mL), then lyophilized to give peptides **1** as a white powder in 8–22% yield (30–80 mg) based on the resin loading of the first amino acid Boc-Orn(Fmoc)-OH).

Fmoc-Protection of ^{15}N-Labeled Amino Acids[3]

Fmoc-[^{15}N]Phe-OH: A 100-mL one-neck round-bottom flask equipped with a magnetic stirring bar was charged with ^{15}N-labeled phenylalanine (1.0 g, 6 mmol) and a solution of 1:1 CH_3CN/H_2O (50 mL). Et_3N (0.6 g, 6 mmol) and Fmoc-OSu (1.9 g, 5.7 mmol) were added, then the reaction mixture was stirred until the solution turned clear (ca. 15 min). Additional Et_3N was added until the pH was roughly 8.5, then the mixture was stirred for 1 h. The mixture was poured into a solution of 1.0 M HCl (250 mL) in a 400-mL beaker while stirring vigorously. The Fmoc-[^{15}N]Phe-OH precipitated from the solution and the solid was isolated by filtering the mixture through a sintered glass filter funnel with a medium frit. The funnel was covered with a piece of filter paper and the solid was dried by aspirating air through the funnel. The solid was suspended in ca. 200 mL of EtOAc to form a turbid solution. The solution was stirred vigorously for 10 min, dried over $MgSO_4$, filtered, and then concentrated under vacuum to give a white solid. The isolated solid was ground into a fine powder to give ca. 1.94 g (92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77 (d, J = 7.5 Hz, 2H), 7.55 (t, J = 6.2 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 6.80 (m, 5H), 7.15 (d, J = 6.6 Hz, 2H), 5.19 (dd, J = 91.9, 8.2 Hz, 1H), 4.70 (m, 1H), 4.46 (dd, J = 10.4, 7.3 Hz, 1H), 4.37 (t, J = 8.7 Hz, 1H), 4.21 (t, J = 6.7 Hz, 1H), 3.18 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.2, 156.0 (d, $^1J_{CN}$ = 25 Hz), 144.0, 141.6, 135.6, 129.6, 129.0, 128. 0, 127.6, 127.3, 125.3, 120.3, 67.3, 54.7 (d, $^1J_{CN}$ = 13.8 Hz), 47.4, 37.9, 30.0.

Fmoc-[^{15}N]Gly-OH: A 100-mL one-neck round-bottom flask equipped with a magnetic stirring bar was charged with ^{15}N-labeled glycine (1.0 g, 13 mmol) and a solution of 1:1 CH_3CN/H_2O (50 mL). Et_3N (1.3 g, 13 mmol) and Fmoc-OSu (4.2 g, 12.5 mmol) were added, then the reaction mixture was stirred until the solution turned clear (ca. 15 min). Additional Et_3N was added until the pH was roughly 8.5, then the mixture was stirred for 1 h. The mixture was poured into a solution of 1.0 M HCl (250 mL) in a 400-mL beaker while stirring vigorously. The Fmoc-[^{15}N]Gly-OH precipitated from the solution and the solid was isolated by filtering the mixture through a sintered glass filter funnel with a medium frit. The funnel was covered with a piece of filter paper and the solid was dried by aspirating air through the funnel. The solid was suspended in ca. 200 mL of EtOAc to form a turbid solution. The solution was stirred vigorously for 10 min, dried over $MgSO_4$, filtered, and then concentrated under vacuum to give a white solid. The isolated solid was ground into a fine powder to give ca. 3.48 g (92%) isolated yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 7.4 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.5, 1 Hz, 2H), 5.28 (dt J = 92.6, 5.6 Hz), 4.43 (d, J = 7.0 Hz, 2H), 4.24 (t, J = 7.0 Hz, 1H), 4.04 (d, 5.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.6, 156.5, 144.0, 141.6, 128.0, 127.3, 125.3, 120.3, 67.6, 47.3, 42.6 (d, $^1J_{CN}$ = 13.8 Hz).

NMR Spectroscopy of Peptides 1

Sample Preparation. NMR spectroscopy of peptides **1a** and **1b** was performed in D_2O. The solutions were prepared by dissolving a weighed portion of the peptide in the appropriate volume of solvent. The molecular weights of the peptides were calculated as the TFA salts with all amino groups assumed to be protonated (**1a**, M.W. 2223.85 and **1b**, M.W. 2099.91). The solutions were allowed to stand for 24 h to allow complete hydrogen to deuterium exchange of the amide NH protons.

^{1}H NMR, TOCSY, ROESY, and NOESY Data Collection. NMR spectra were recorded on a Bruker 600 MHz spectrometer with a TBI probe. Presaturation water suppression was applied as needed. TOCSY spectra were recorded with 2048 points in the f_2 dimension and 512 increments in the f_1 dimension with a 150-ms spin-lock mixing time. ROESY spectra were recorded with 2048 points in the f_2 dimension and 512 increments in the f_1 dimension with a 200-ms spin-lock mixing time. NOESY spectra were recorded with 2048 points in the f_2 dimension and 512 increments in the f_1 dimension with a 150-ms mixing time.

^{1}H NMR, TOCSY, ROESY, and NOESY Data Processing. NMR spectra were processed with Bruker XwinNMR software. Automatic baseline correction was applied in both dimensions after phasing the spectra. TOCSY and ROESY spectra were Fourier transformed to a final matrix size of 2048 x 1024 real points using a Qsine weighting function and forward linear prediction. NOESY spectra were Fourier transformed to a final matrix size of 2048 x 2048 real points using a Qsine weighting function and forward linear prediction.

Diffusion-Ordered Spectroscopy (DOSY) Experiments. DOSY experiments were performed on a Bruker 500 MHz spectrometer equipped with a TCI cryoprobe, with a diffusion delay (Δ) of 75-ms and a diffusion gradient length (δ) of 2.5-ms. Sixteen sets of FIDs were recorded with the gradient strength incremented from 5%–95% using a linear ramp. The combined FIDs were Fourier transformed in Bruker's TopSpin^{TM} software to give a pseudo-2D spectrum. After phasing and performing baseline correction, each pseudo-2D spectrum was processed with logarithmic scaling on the Y-axis. The Y-axis was calibrated to the diffusion coefficient of the residual HOD peak in D_2O (1.9×10^{-9} m^2/s at 298 K).[4] The diffusion coefficients of the peptides were read and converted from logarithmic values to linear values.

NMR Spectroscopy of Peptides [^{15}N]1

Sample Preparation. NMR spectroscopy of peptides [^{15}N]**1a** and [^{15}N]**1b** was performed in 9:1 H_2O/D_2O. The solutions were prepared by dissolving a weighed portion of the peptide in the appropriate volume of solvent. The molecular weights of the peptides were calculated as the TFA salts with all amino groups assumed to be protonated ([^{15}N]**1a**, M.W. 2224.85 and [^{15}N]**1b**, M.W. 2100.91). 4,4-Dimethyl-4-silapentane-1-ammonium trifluoroacetate (DSA) was added as an internal standard for referencing chemical shifts.[5]

^{1}H NMR, ^{1}H,^{15}N HSQC, and ^{1}H,^{15}N NOESY-HSQC (^{15}N-edited NOESY) Data Collection. NMR spectra were recorded on a Bruker 600 MHz spectrometer with either a TBI probe or a BBFO cryoprobe. Gradient water suppression was applied as needed. ^{1}H,^{15}N HSQC spectra were recorded with 1024 points in the f_2 dimension and 512 increments in the f_1 dimension. ^{1}H,^{15}N NOESY-HSQC spectra were recorded with a 150-ms mixing time, and with 2048 points in the f_3 dimension (^{1}H), 1 increment in the f_2 dimension (^{15}N), and 1024 increments in the f_1 dimension (^{1}H).

^{1}H NMR, ^{1}H,^{15}N HSQC, and ^{1}H,^{15}N NOESY-HSQC (^{15}N-edited NOESY) Data Processing. NMR spectra were Fourier transformed in Bruker XwinNMR software with forward linear prediction and a Qsinc weighting function. Automatic baseline correction was applied in both dimensions after phasing the spectra. The ^{1}H,^{15}N HSQC spectra were processed to a final matrix size of 2048 x 1024 real points and with GB = 0.1 in the f_2 dimension. The ^{1}H,^{15}N NOESY-HSQC spectra were processed to a final 2D matrix size of 4096 x 2048 real points (f_3, f_1) and with GB = 0.05 in both dimensions.

Molecular Modeling of Peptides 1a and 1b.

Molecular models of the tetramers of peptides **1a** and **1b** were generated from the X-ray crystallographic structure of a similar macrocyclic β-sheet peptide (PDB 3T4G). This peptide contains AIIGLMV ($A\beta_{30–36}$) in the heptapeptide strand and $KFF^{Br}K$ in positions R_8-R_{11} in the template strand. The PDB coordinates were imported into PyMOL. Symmetry mates were generated to create two hydrogen-bonded dimers sandwiched on the surface displaying the side chains of A_{30}, I_{32}, L_{34}, and V_{36}. The alignment of each dimer was shifted by two residues to match the alignment of the dimers of peptides **1a** and **1b**. The residues of the dimers were mutated to match peptide **1a** or peptide **1b**, and the side chain torsion angles of χ_1 and χ_2 were adjusted for Ile (180^o and 60^o) and Phe (180^o). The dimers were then rotated manually to reflect the observed interlayer NOEs between Ile_{11} and the methoxy group of Hao.

The coordinates were exported from PyMOL. [Note that .pdb was used, but .mol2 file format is actually preferable and is recommended instead of .pdb.] The file was imported into MacroModel with the Maestro user interface. Atom types and bond orders were edited as needed to correct errors in bond type and charge. Distance constraints were applied to reflect the folding and dimerization of the macrocycles. Four interlayer distance constraints between the δ-methyl group of Ile_{11} and the methoxy group of Hao were applied to reflect the observed interlayer contacts. Minimization was performed with the MMFFs force field and GB/SA water solvation. All constraints were removed and minimization was repeated to generate a minimum-energy conformation (local minimum). The coordinates were exported in .pdb file format and imported into PyMOL.

III. REFERENCES

1. Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518–1520.

2. (a) Nowick, J. S.; Chung, D. M.; Maitra, K.; Maitra, S.; Stigers, K. D.; Sun, Y. *J. Am. Chem. Soc.* **2000**, *122*, 7654–7661; (b) Cheng, P.-N.; Nowick, J. S. *J. Org. Chem.* **2011**, *76*, 3166–3173.

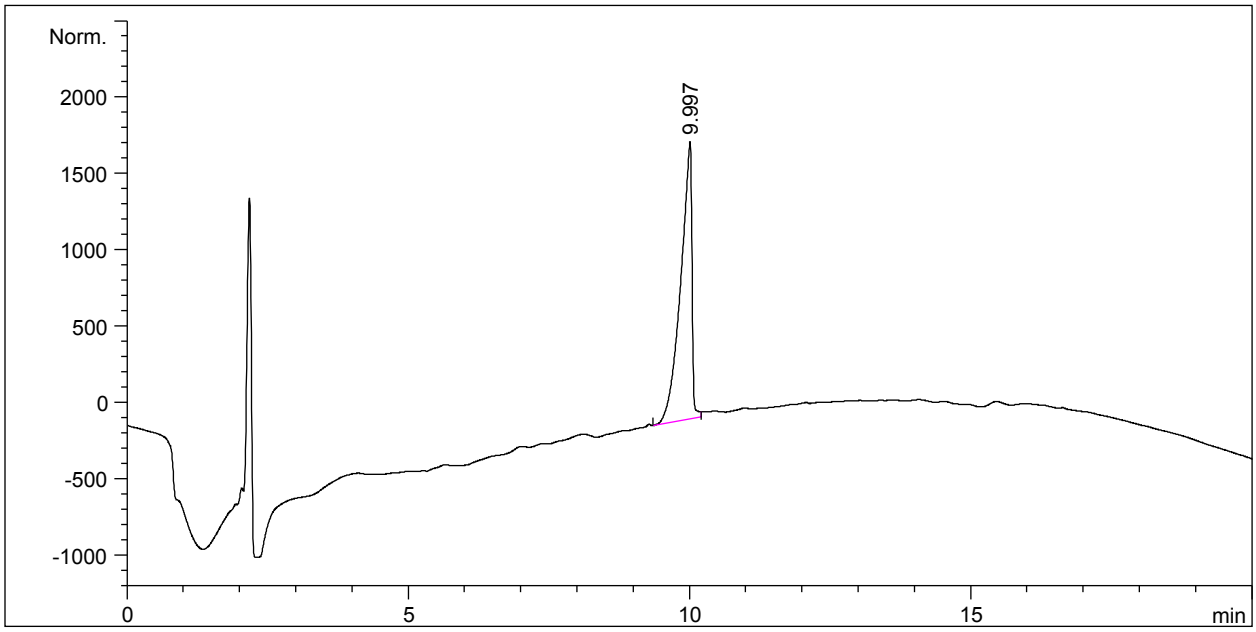
3. Podlech, J.; Gurrath, M.; Müller, G. 9-Fluorenylmethoxycarbonyl Group. In *Houben-Weyl Methods of Organic Chemistry*, Goodman, M., Ed. Thieme: Stuttgart, 2003; Vol. E22a, p 61.

4. Longsworth, L. G. *J. Phys. Chem.* **1960**, *64*, 1914–1917.

5. Nowick, J. S.; Khakshoor, O.; Hashemzadeh, M.; Brower, J. O. *Org. Lett.* **2003**, *5*, 3511–3513.

IV. CHARACTERIZATION DATA

RP-HPLC of peptide **1a**



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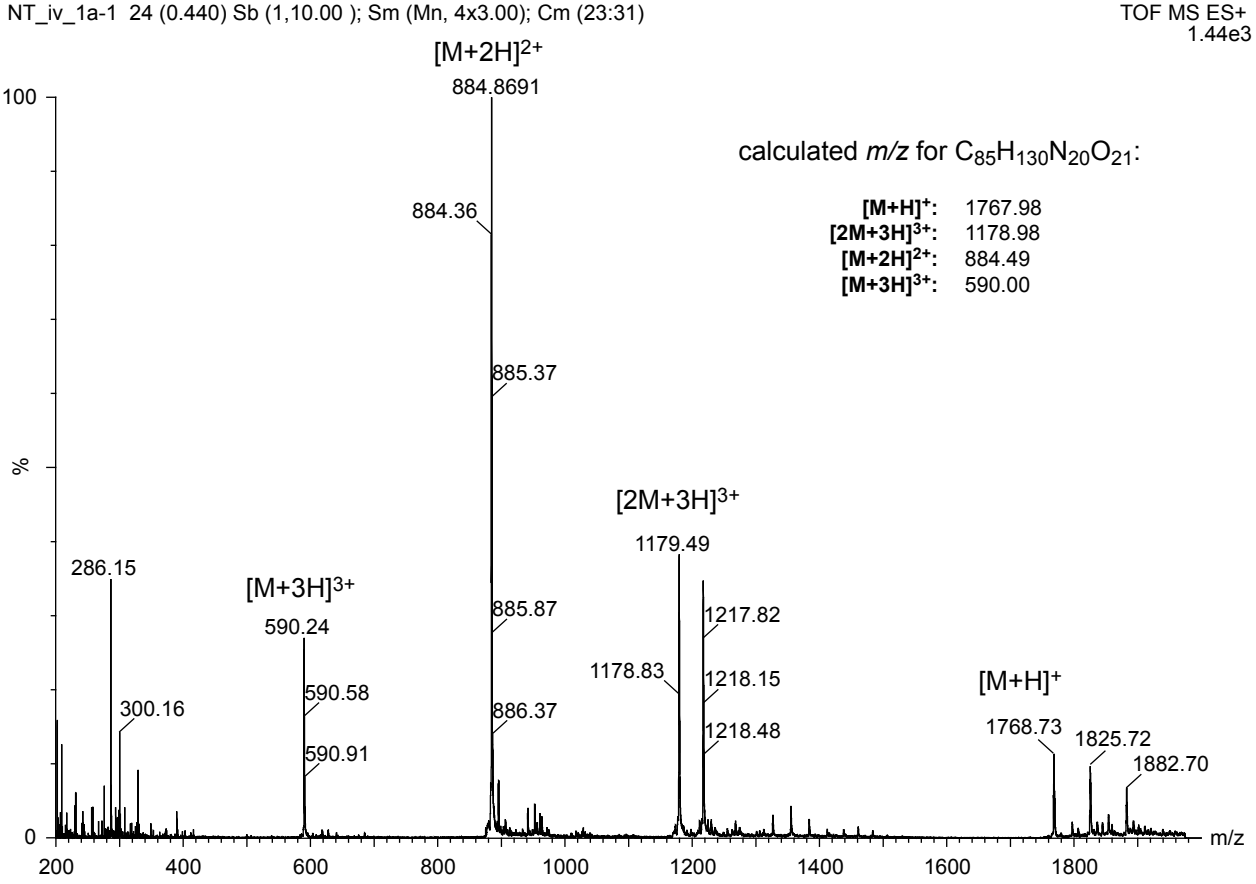
Totals :				2.50833e4	1820.18323	

column: Aeris XB-C18 2.6µ
dimensions: 150 mm x 4.6 mm
mobile phase: A: H_2O, 0.1% TFA
B: CH_3CN, 0.1% TFA
gradient: A/B (95:5) to (0:100) in 20 min
flow rate: 1.0 mL/min
detection: VWD, wavelength = 214 nm
temperature: 298 K

MS (ESI) of peptide **1a**



calculated *m/z* for $C_{85}H_{130}N_{20}O_{21}$:

$[M+H]^+$: 1767.98
$[2M+3H]^{3+}$: 1178.98
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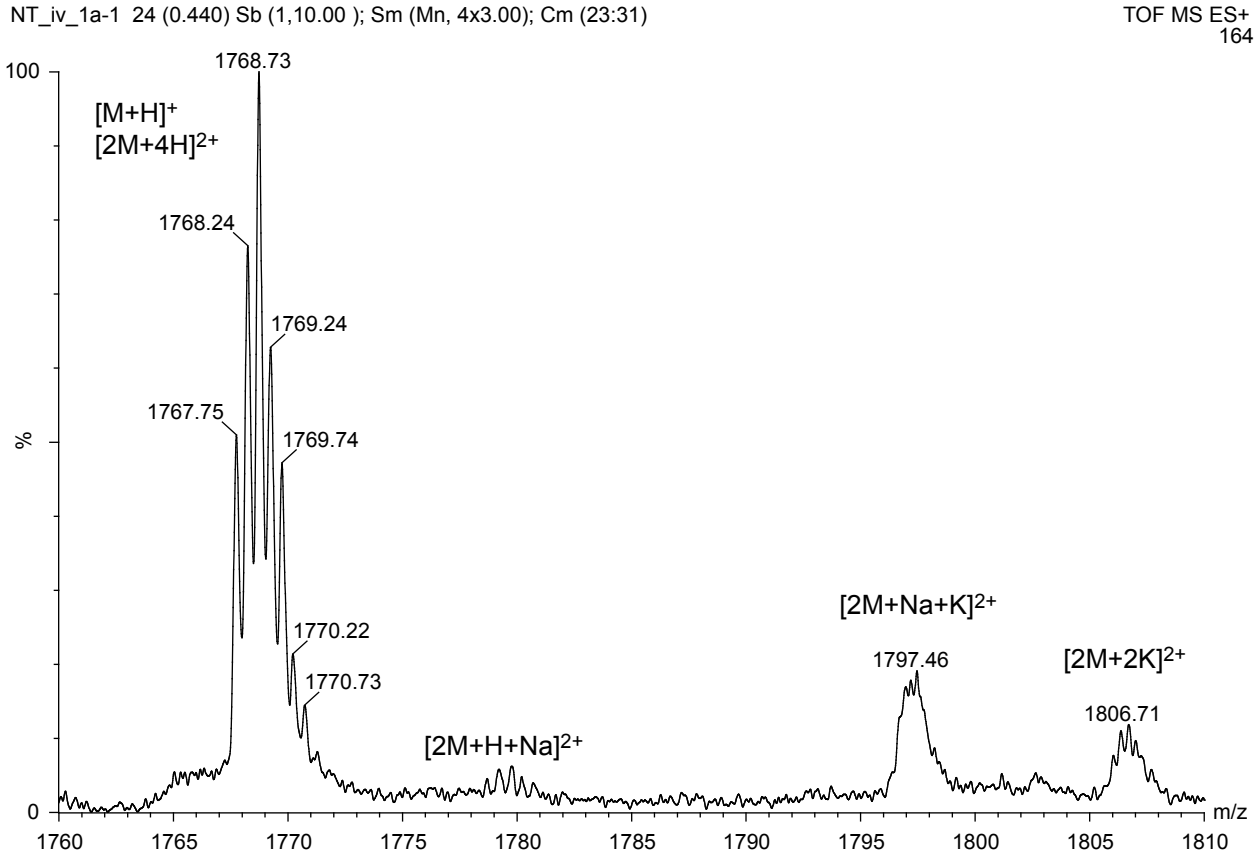
$[M+3H]^{3+}$: 590.00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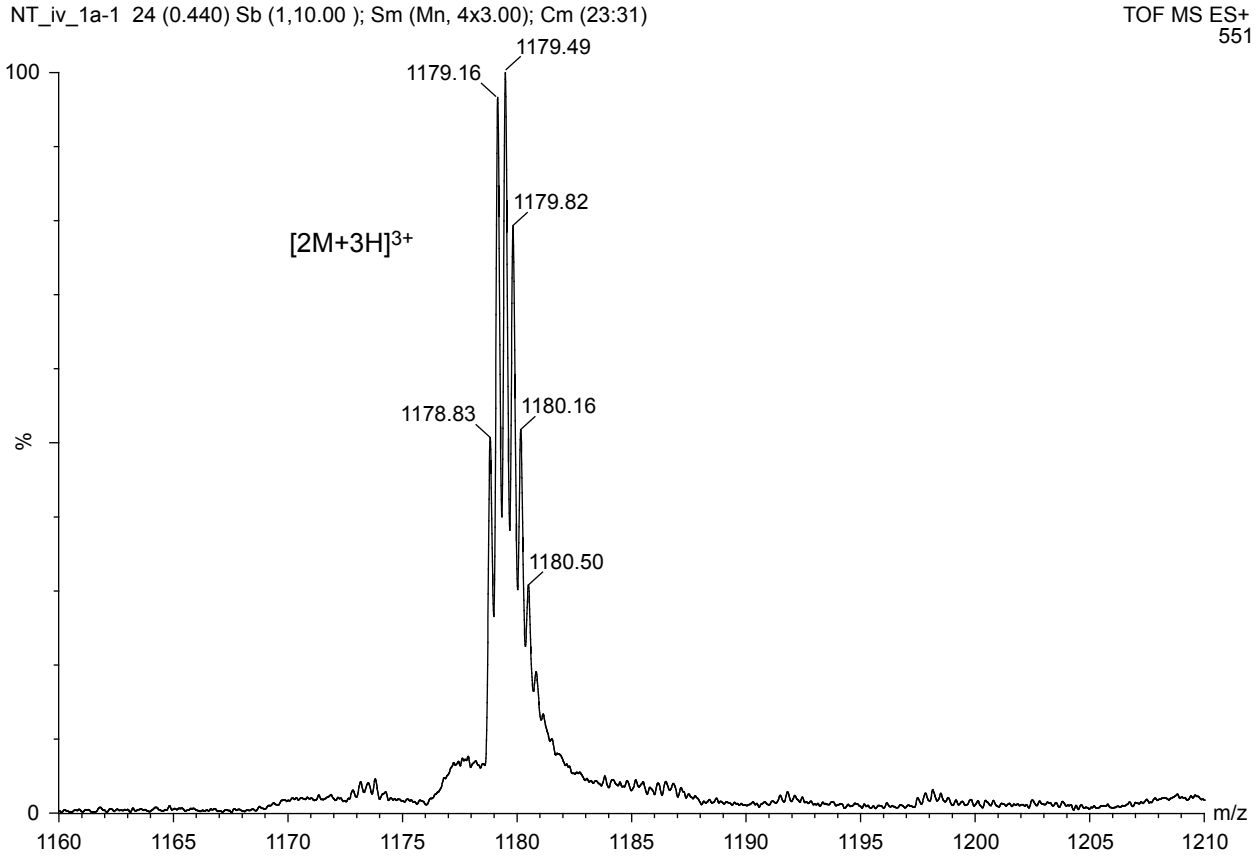
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%
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1179.16
1179.82
[2M+3H]3+
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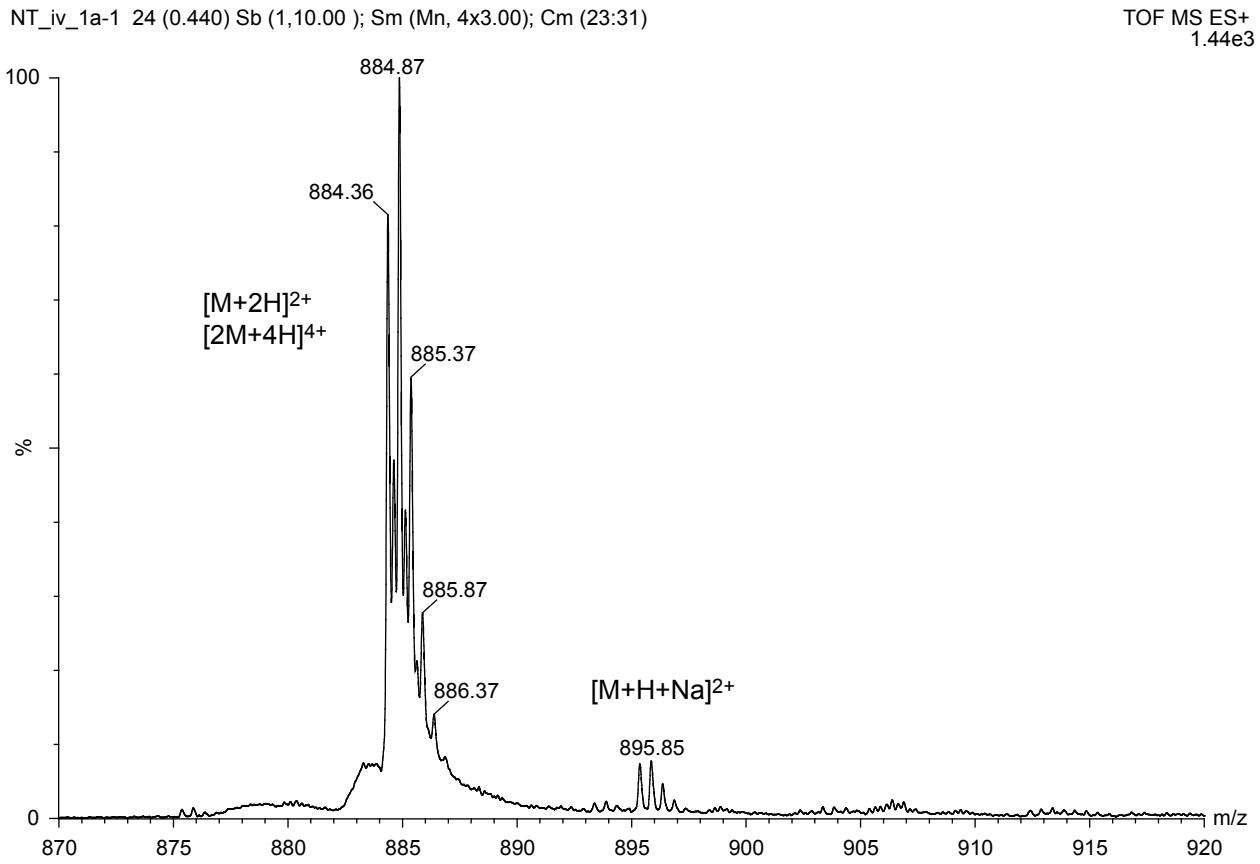
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100
%
0
884.87
884.36
[M+2H]2+
[2M+4H]4+
885.37
885.87
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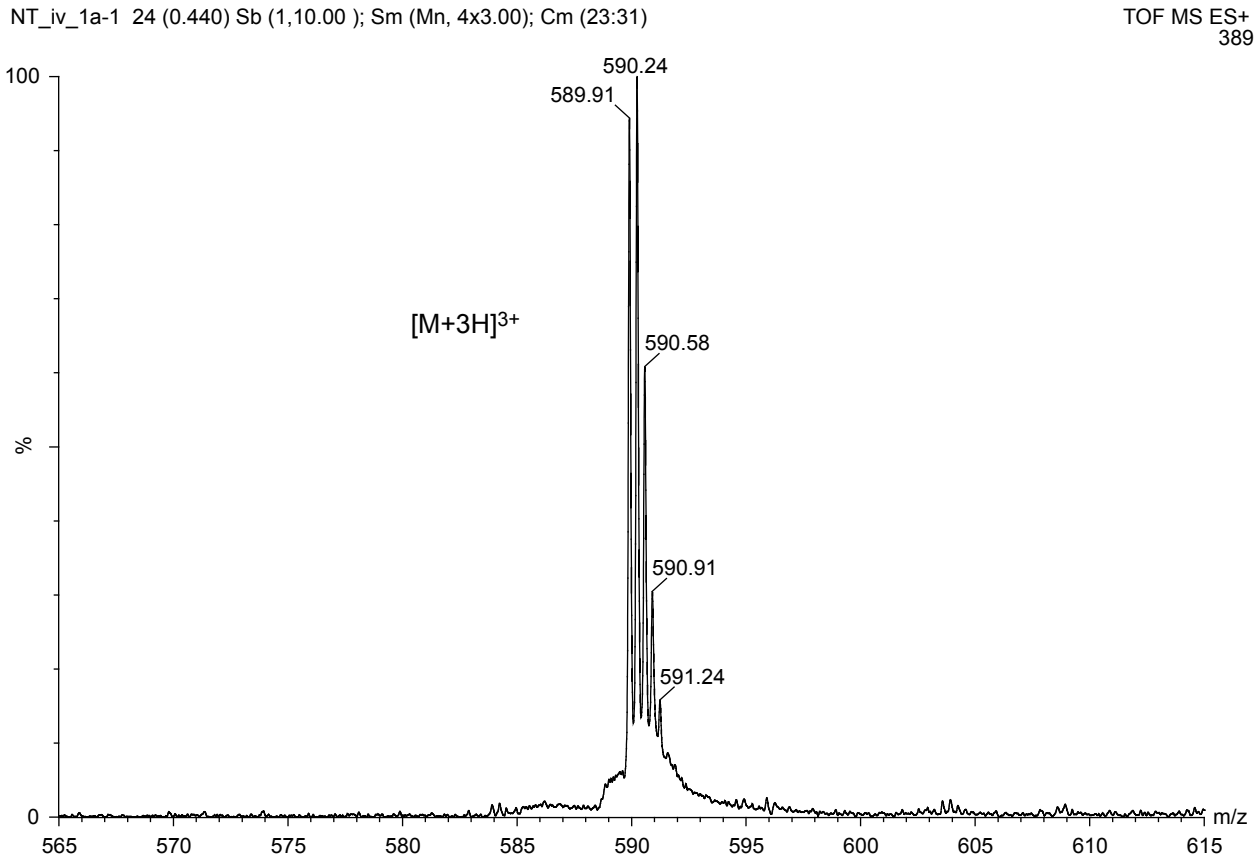
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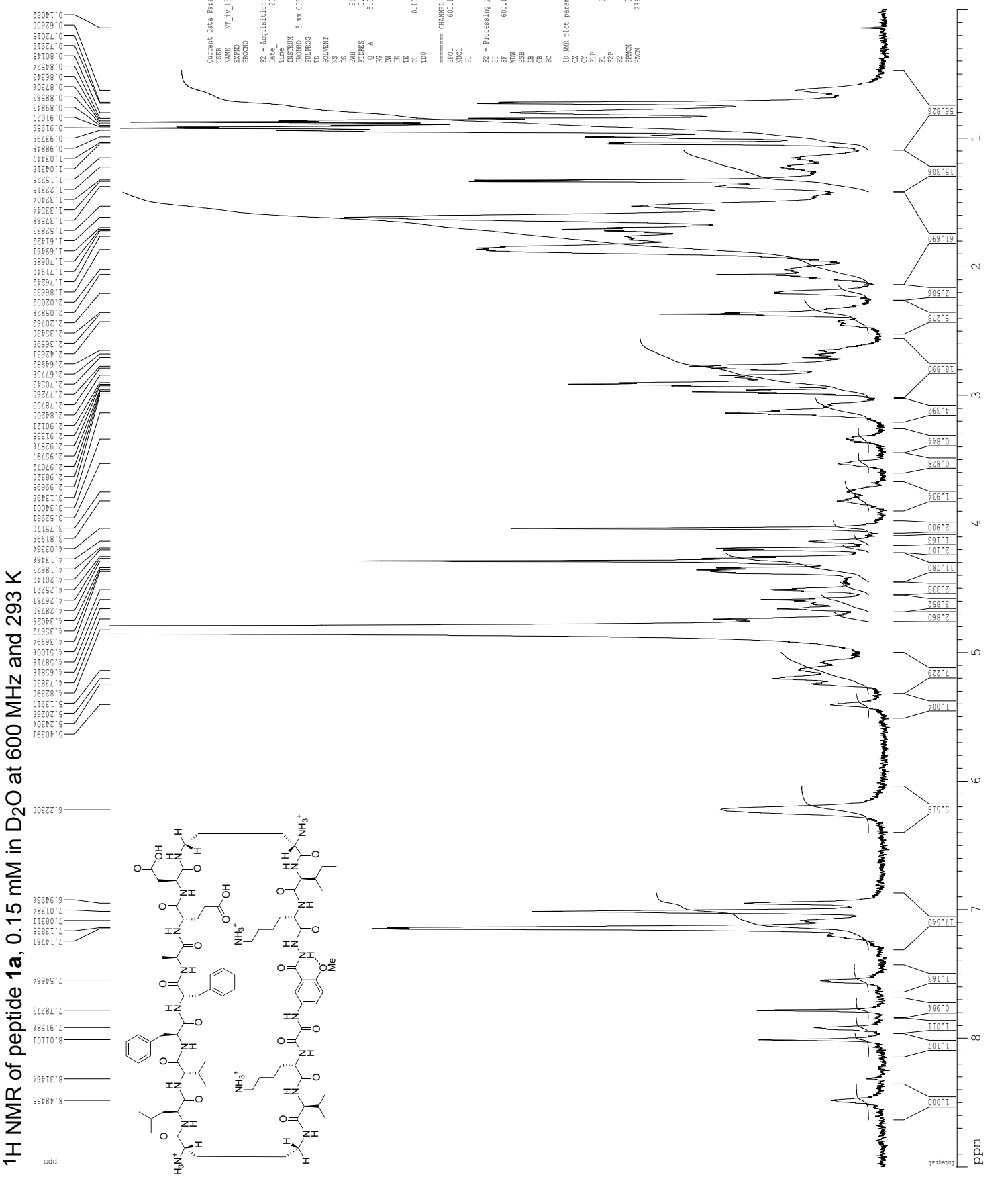
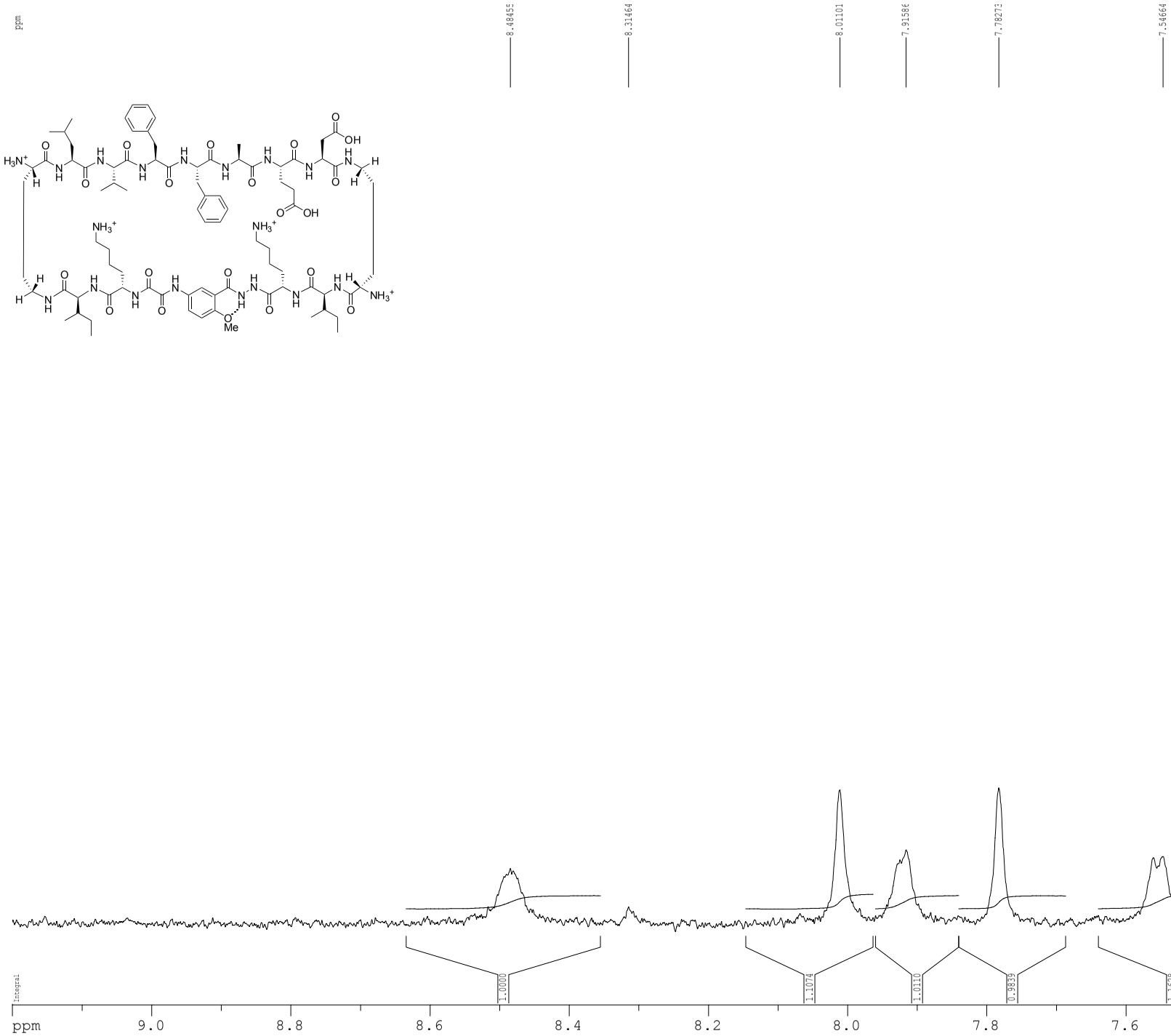
TOF MS ES+
389
100
%
0
590.24
589.91
[M+3H]3+
590.58
590.91
591.24
m/z
565
570
575
580
585
590
595
600
605
610
615

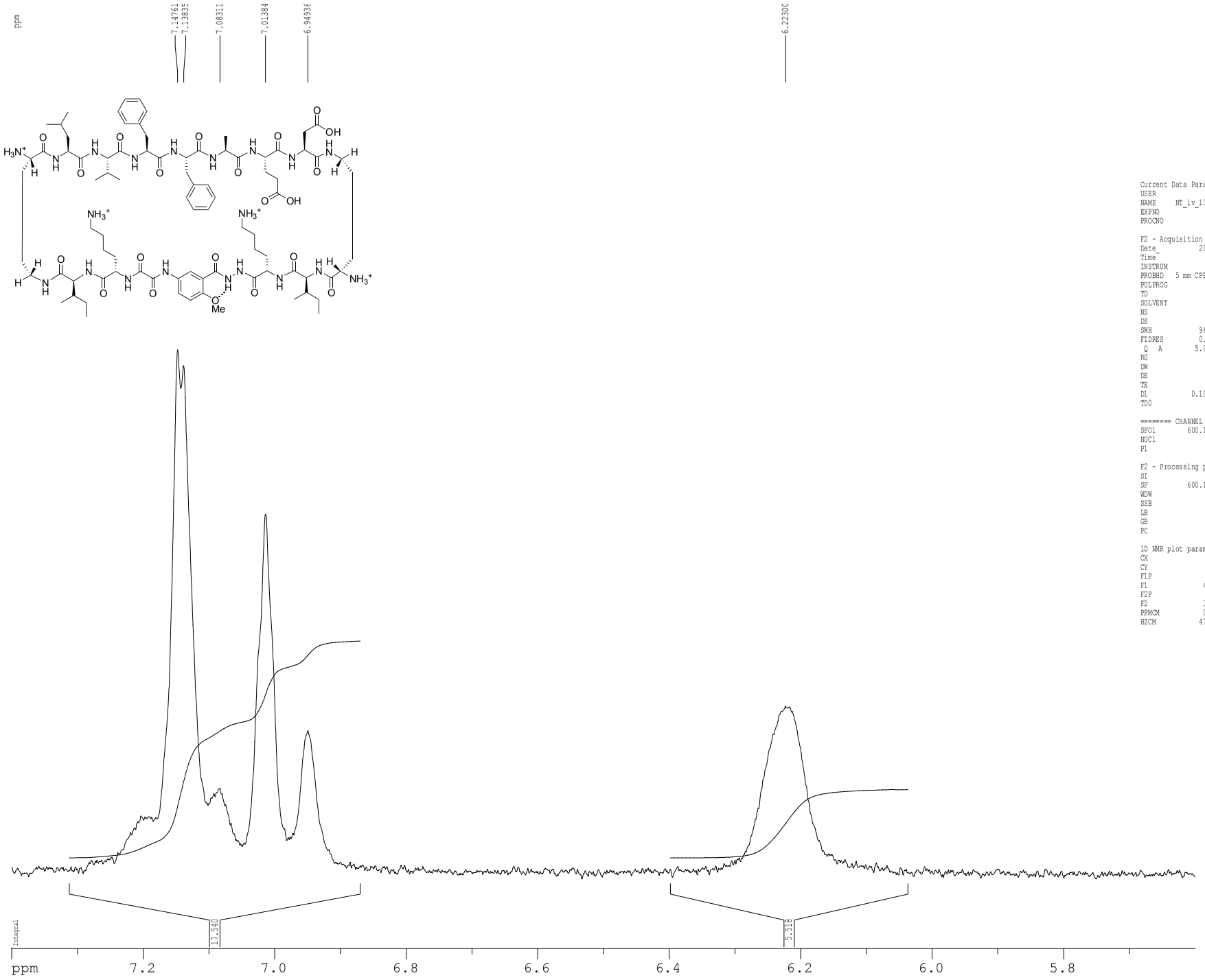
^{1}H NMR of peptide **1a**, 0.15 mM in D_2O at 600 MHz and 293 K



^{1}H NMR of peptide **1a**, 0.15 mM in D_2O at 600 MHz and 293 K



^{1}H NMR of peptide **1a**, 0.15 mM in D_2O at 600 MHz and 293 K

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DS                   2
SWH            9615.385 Hz
FIDRES         0.098194 Hz
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RG                12.7
DW              52.000 usec
DE              13.70 usec
TE              293.0 K
D1           0.10000000 sec
TD0                  1

======= CHANNEL f1 =======
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NUC1               1H
P1              12.00 usec

F2 - Processing parameters
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SF          600.1299881 MHz
WDW                EM
SSB                 0
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GB                  0
PC               1.00

1D NMR plot parameters
CX              22.80 cm
CY              15.00 cm
F1P             7.400 ppm
F1             4440.96 Hz
F2P             5.600 ppm
F2             3360.73 Hz
PPMCM          0.07895 ppm/cm
HZCM          47.37868 Hz/cm
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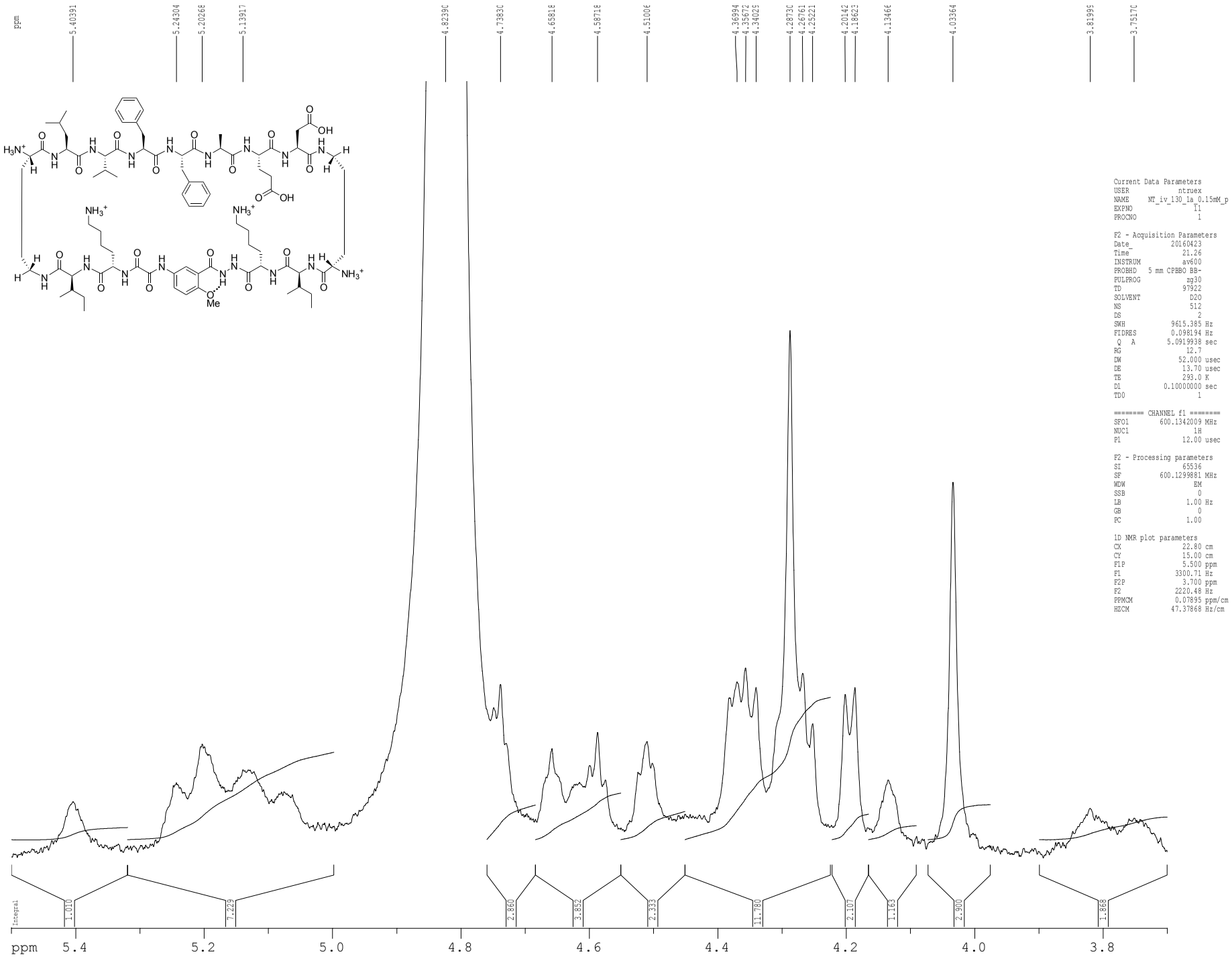
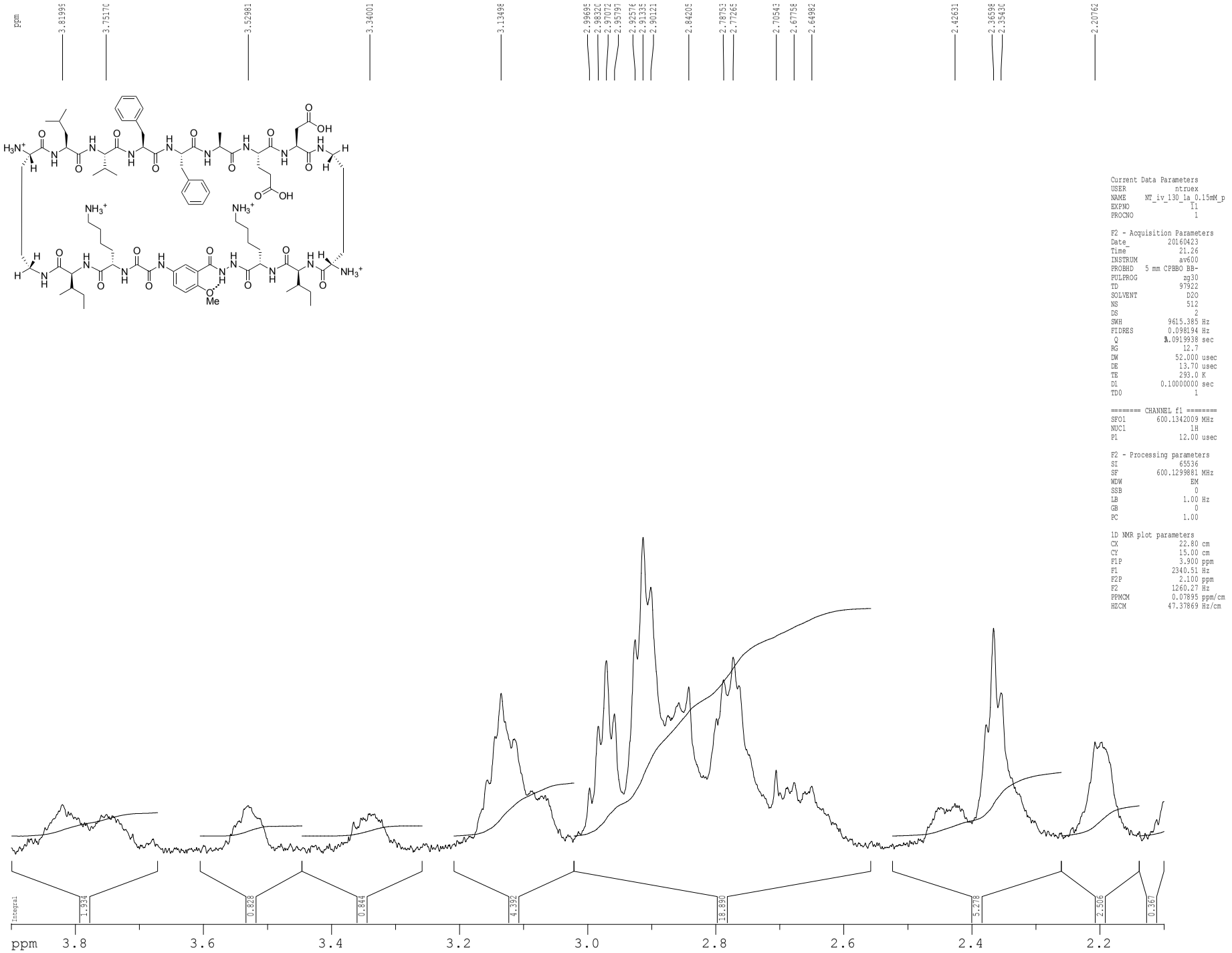
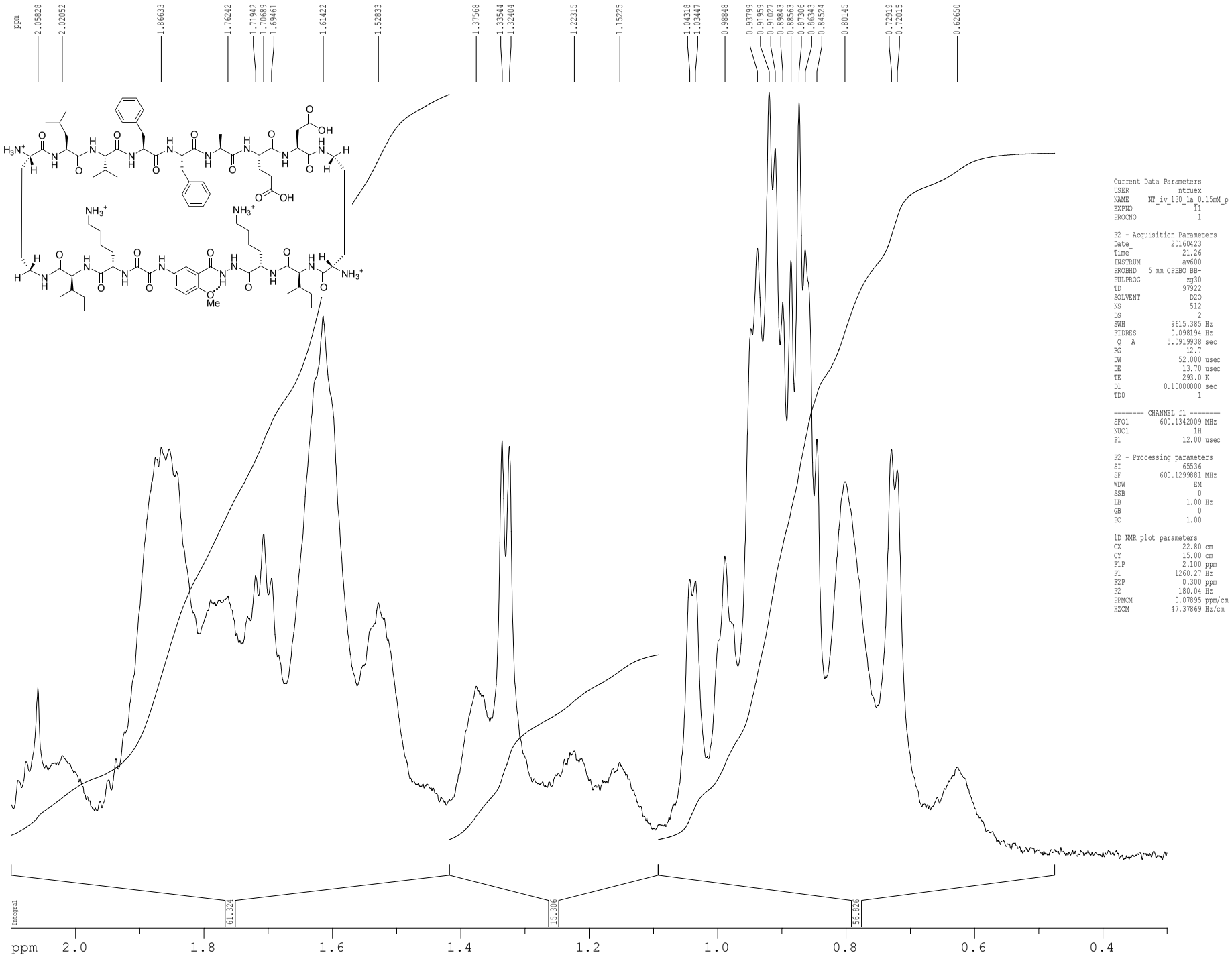
^{1}H NMR of peptide **1a**, 0.15 mM in D_2O at 600 MHz and 293 K



^{1}H NMR of peptide **1a**, 0.15 mM in D_2O at 600 MHz and 293 K



^{1}H NMR of peptide **1a**, 0.15 mM in D_2O at 600 MHz and 293 K



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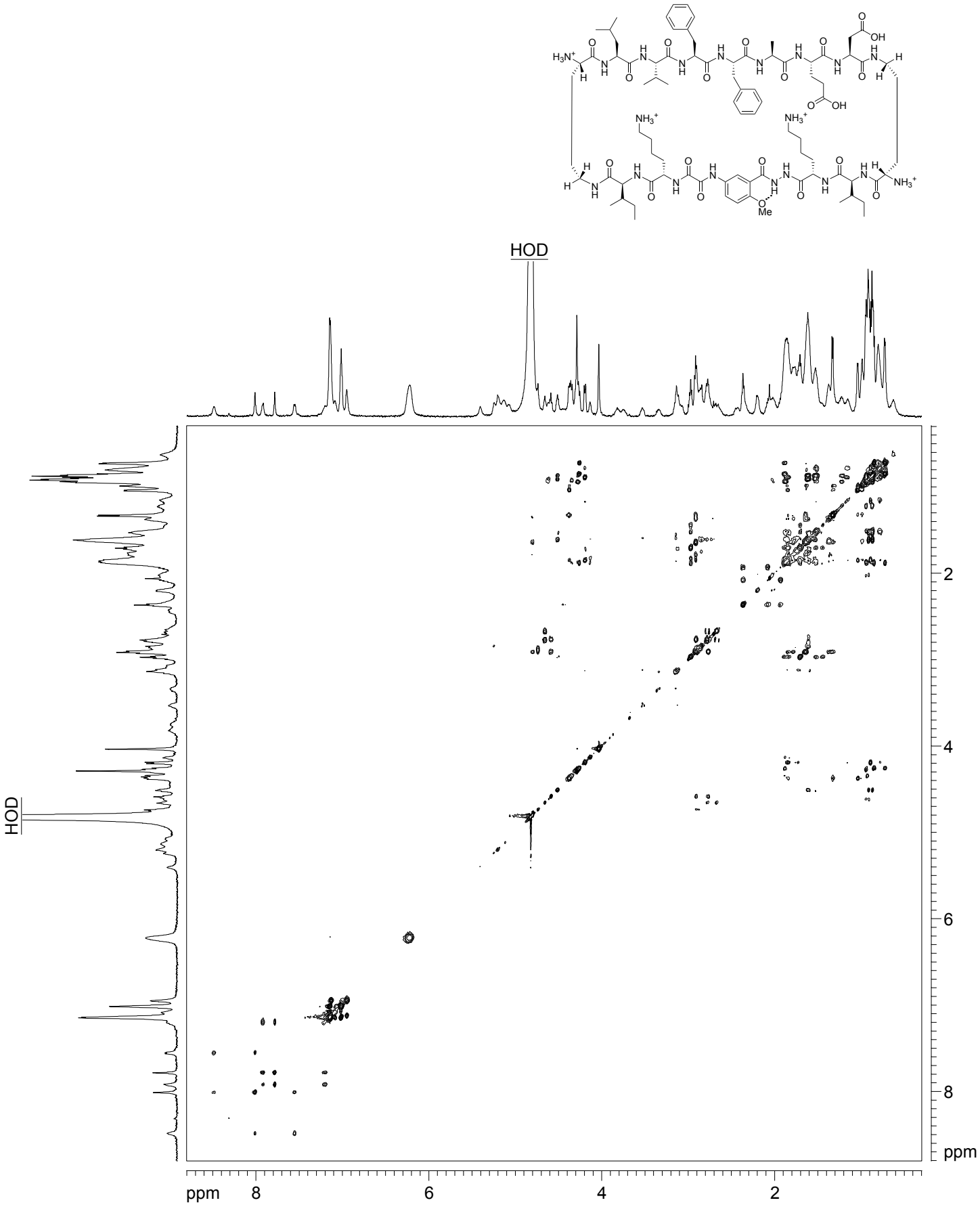
NS 512
DS 2
SWH 9615.385 Hz
FIDRES 0.098194 Hz
Q A 5.0919938 sec
RG 12.7
DW 52.000 usec
DE 13.70 usec
TE 293.0 K
D1 0.10000000 sec
TD0 1

======= CHANNEL f1 =======
SFO1 600.1342009 MHz
NUC1 1H
P1 12.00 usec

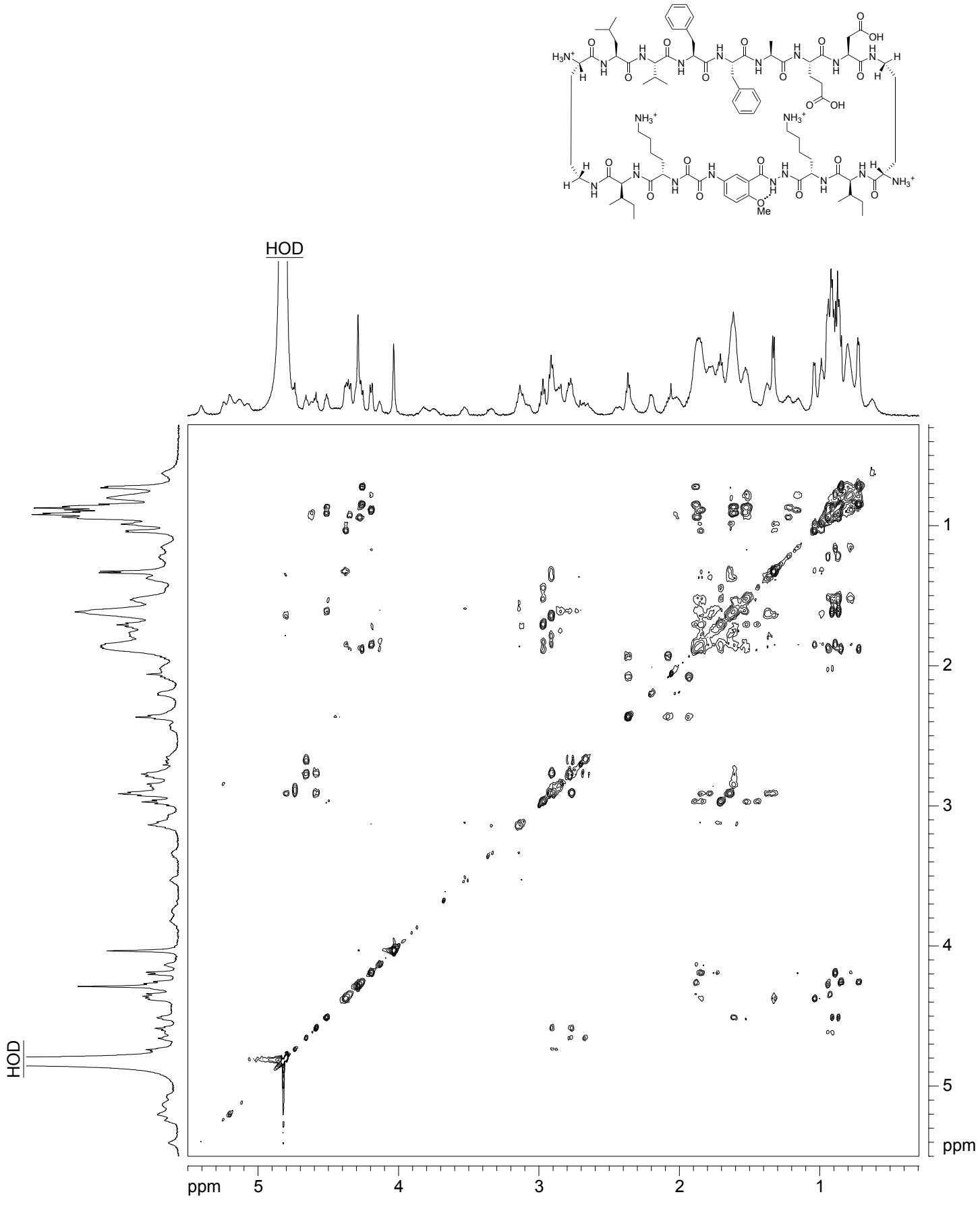
F2 - Processing parameters
SI 65536
SF 600.1299881 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 2.100 ppm
F1 1260.27 Hz
F2P 0.300 ppm
F2 180.04 Hz
PPMCM 0.07895 ppm/cm
HZCM 47.37869 Hz/cm

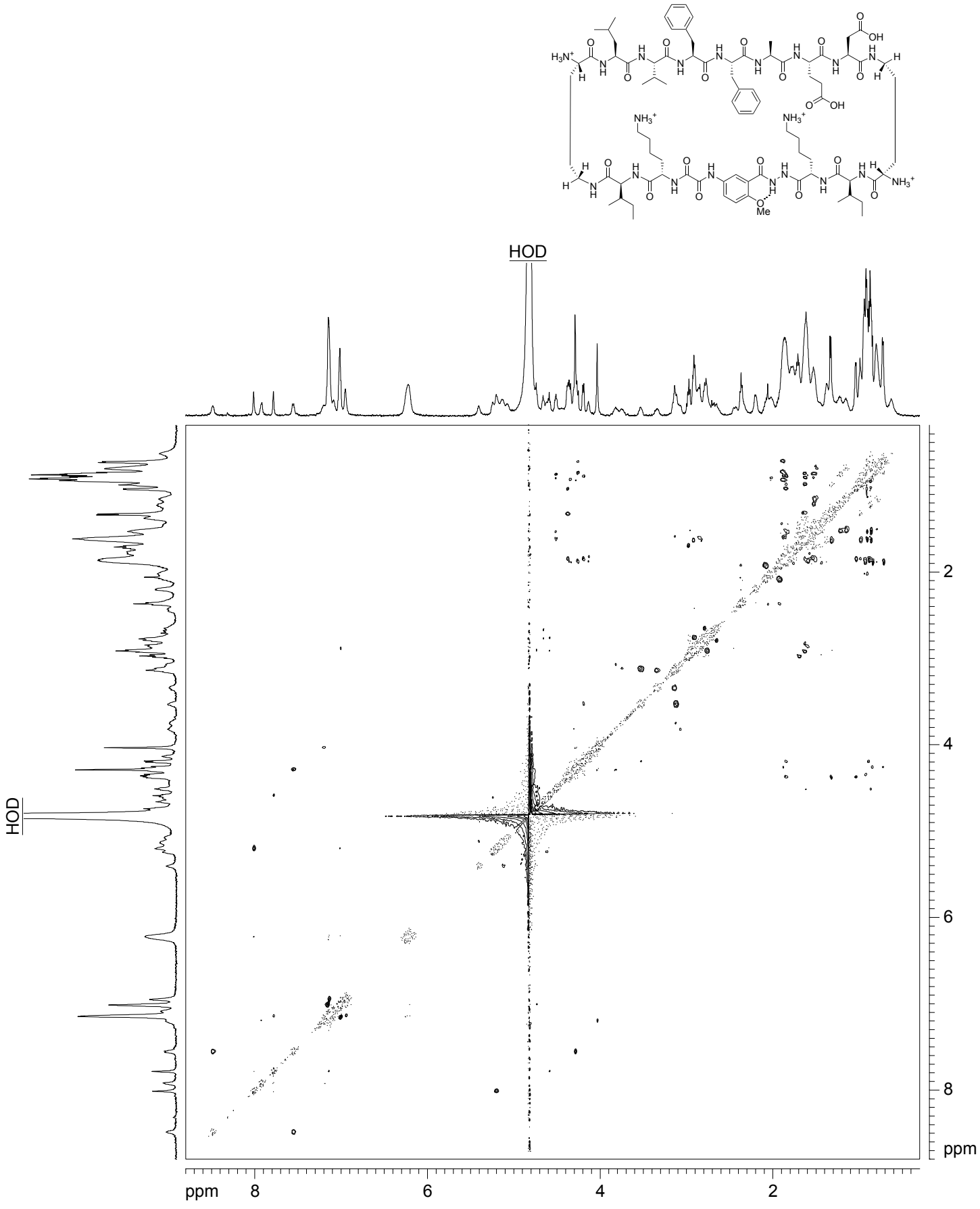
^{1}H NMR 2D TOCSY of peptide **1a** with presaturation suppression of the HOD peak
0.15 mM in D_2O at 600 MHz and 293 K with 150-ms spin-lock mixing time



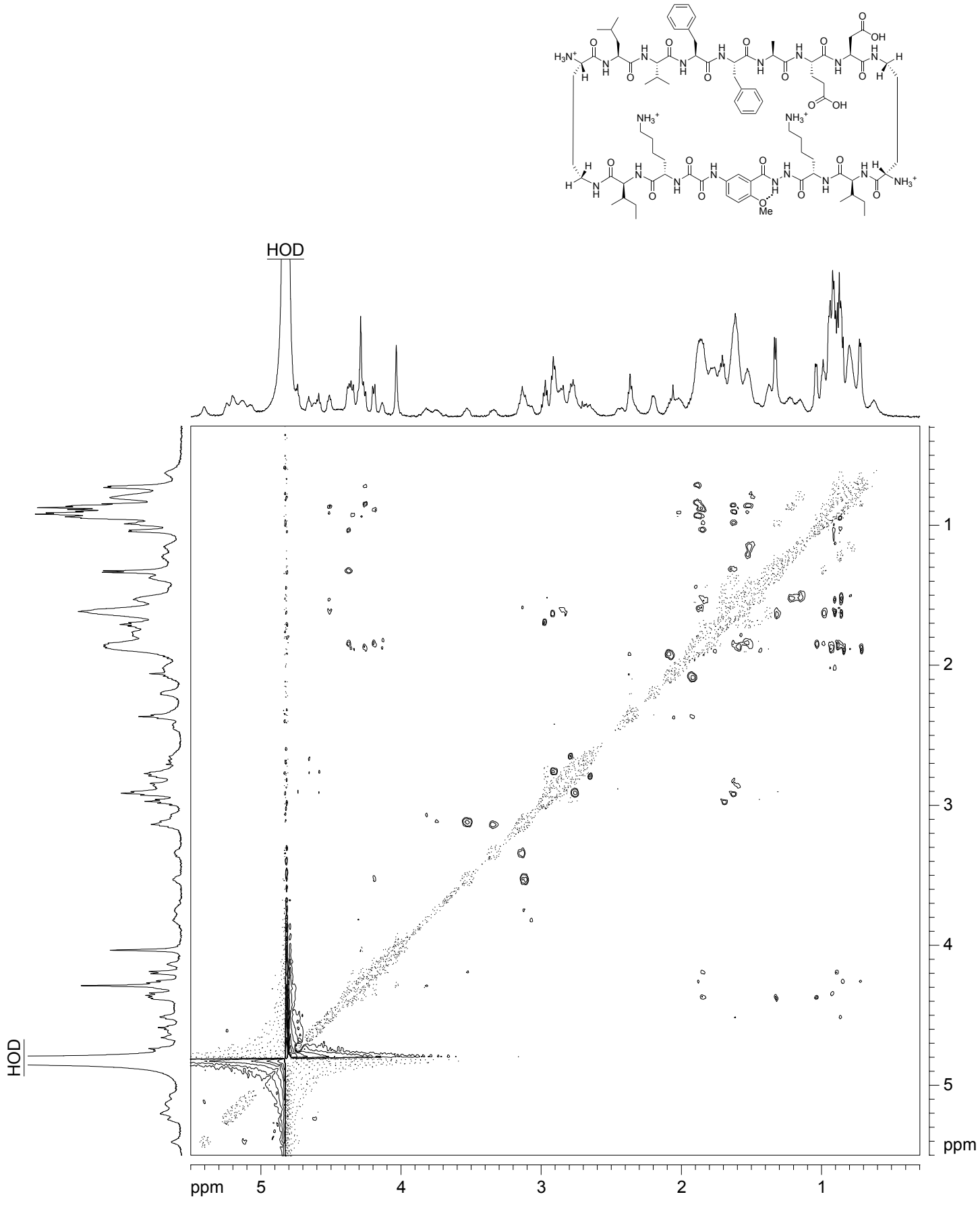
^{1}H NMR 2D TOCSY of peptide **1a** with presaturation suppression of the HOD peak
0.15 mM in D_2O at 600 MHz and 293 K with 150-ms spin-lock mixing time



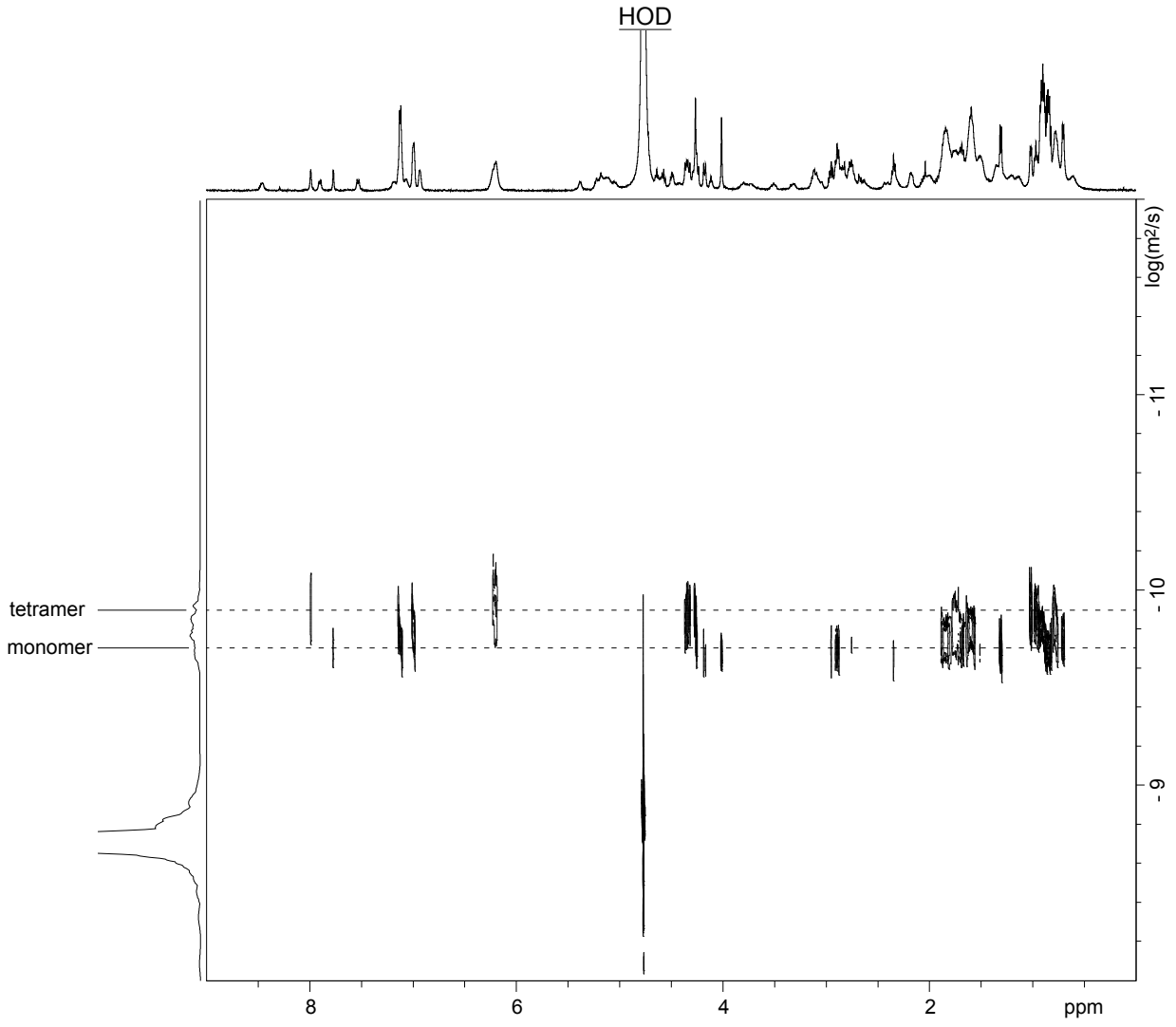
^{1}H NMR 2D ROESY of peptide **1a** with presaturation suppression of the HOD peak
0.15 mM in D_2O at 600 MHz and 293 K with 200-ms spin-lock mixing time



^{1}H NMR 2D ROESY of peptide **1a** with presaturation suppression of the HOD peak
0.15 mM in D_2O at 600 MHz and 293 K with 200-ms spin-lock mixing time



^{1}H NMR DOSY of peptide **1a**, 0.15 mM in D_2O at 500 MHz and 298 K



Calculations for peptide **1a** at 0.15 mM

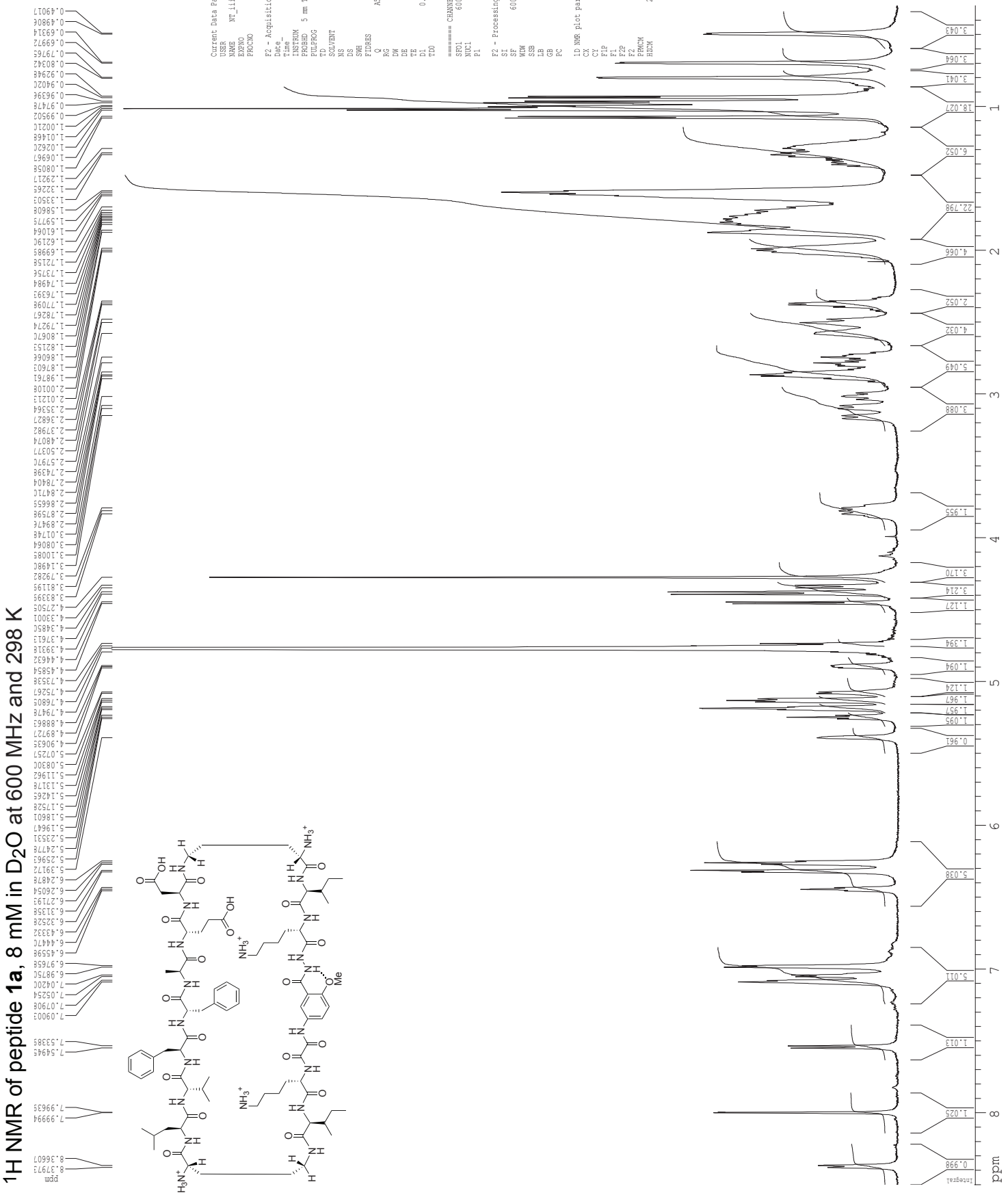
D_{HOD} = 19.0 x 10^{-10} m^2/s [a]
log (D_{HOD}) = -8.721

$D_{monomer}$: log(D) = -9.69; D = $10^{-9.69}$ = 20.4 ± 1.7 x 10^{-11} m^2/s

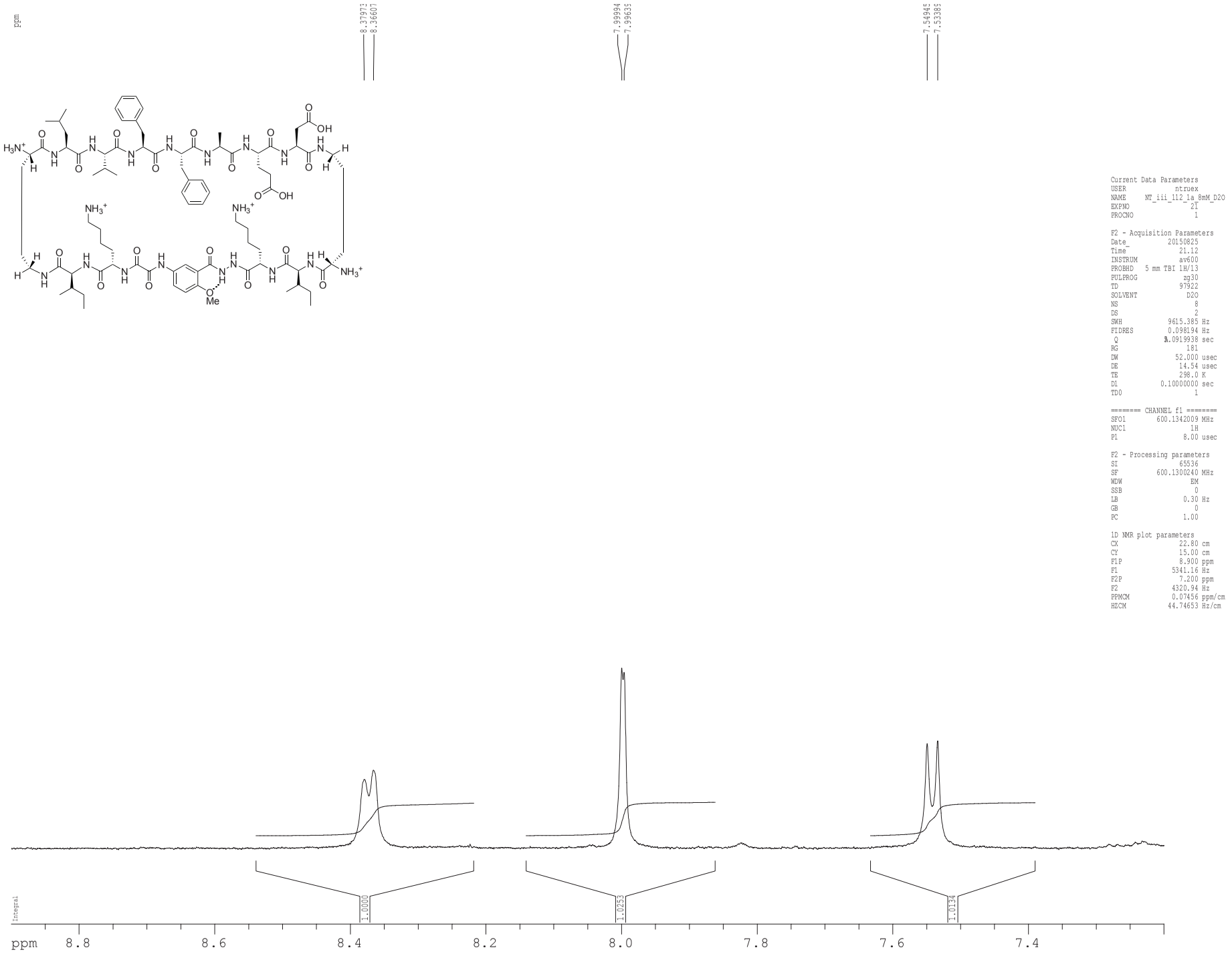
$D_{tetramer}$: log(D) = -9.90; D = $10^{-9.90}$ = 12.6 ± 1.6 x 10^{-11} m^2/s

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

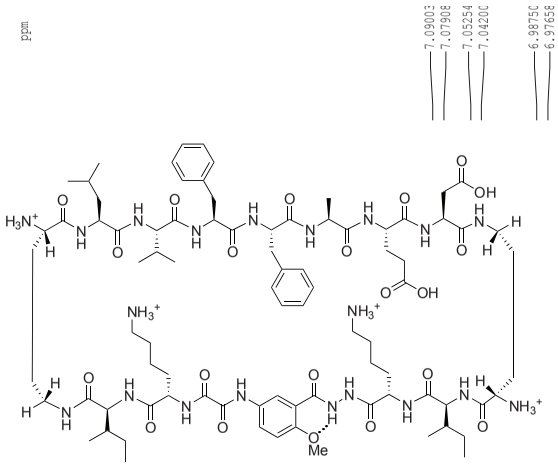
^{1}H NMR of peptide **1a**, 8 mM in D_2O at 600 MHz and 298 K

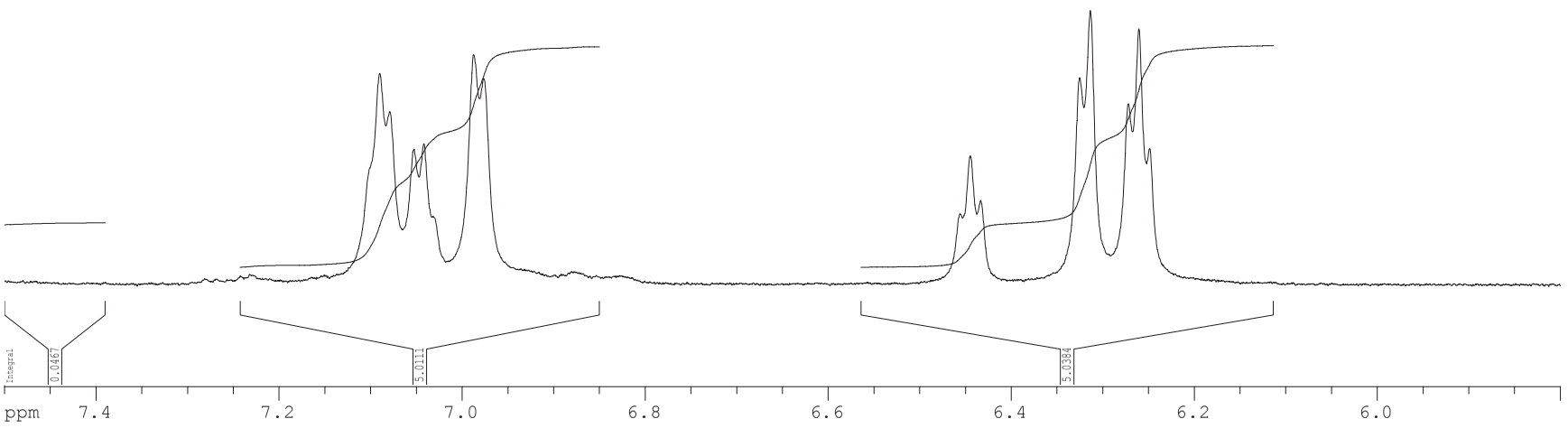


^{1}H NMR of peptide **1a**, 8 mM in D_2O at 600 MHz and 298 K



^{1}H NMR of peptide **1a**, 8 mM in D_2O at 600 MHz and 298 K



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DS                   2
SWH            9615.385 Hz
FIDRES         0.098194 Hz
AQ             2.0919938 sec
RG                 181
DW               52.000 usec
DE               14.54 usec
TE               298.0 K
D1            0.10000000 sec
TD0                  1

======= CHANNEL f1 =======
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NUC1               1H
P1                8.00 usec

F2 - Processing parameters
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GB                  0
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1D NMR plot parameters
CX                22.80 cm
CY                15.00 cm
F1P               7.500 ppm
F1              4500.98 Hz
F2P               5.800 ppm
F2              3480.75 Hz
PPMCM          0.07456 ppm/cm
HZCM           44.74653 Hz/cm
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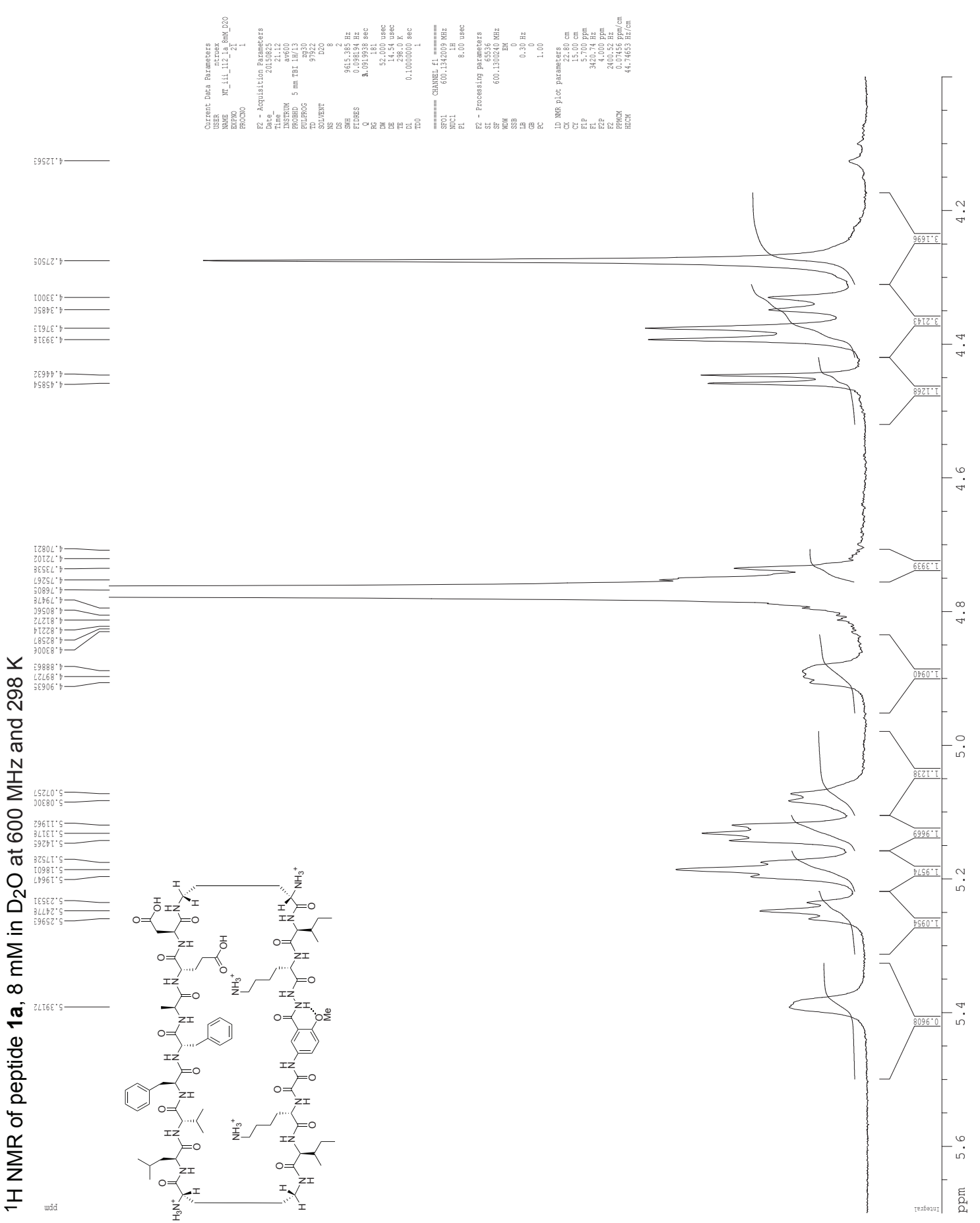
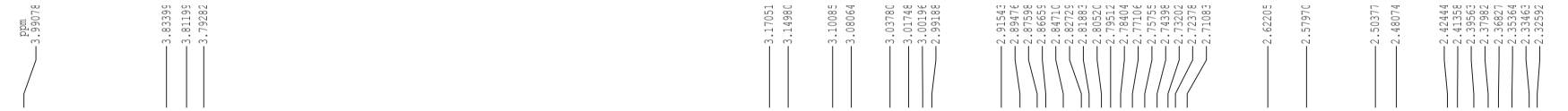
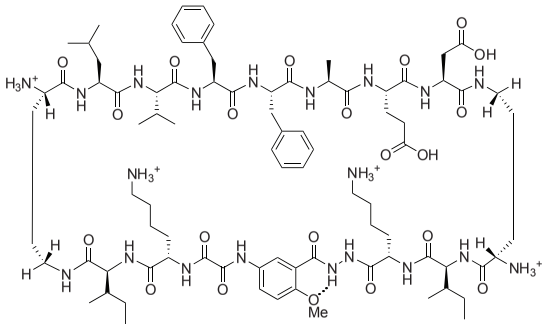
^{1}H NMR of peptide **1a**, 8 mM in D_2O at 600 MHz and 298 K



^{1}H NMR of peptide **1a**, 8 mM in D_2O at 600 MHz and 298 K



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PULPROG           zg30
TD               97922
SOLVENT            D2O
NS                   8
DS                   2
SWH            9615.385 Hz
FIDRES         0.098194 Hz
AQ             3.0919938 sec
RG                 181
DW              52.000 usec
DE              14.54 usec
TE              298.0 K
D1            0.10000000 sec
TD0                  1

======= CHANNEL f1 =======
SFO1        600.1342009 MHz
NUC1               1H
P1               8.00 usec

F2 - Processing parameters
SI                65536
SF          600.1300240 MHz
WDW                EM
SSB                 0
LB               0.30 Hz
GB                  0
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1D NMR plot parameters
CX              22.80 cm
CY              15.00 cm
F1P             4.000 ppm
F1             2400.52 Hz
F2P             2.300 ppm
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HZCM           44.74654 Hz/cm
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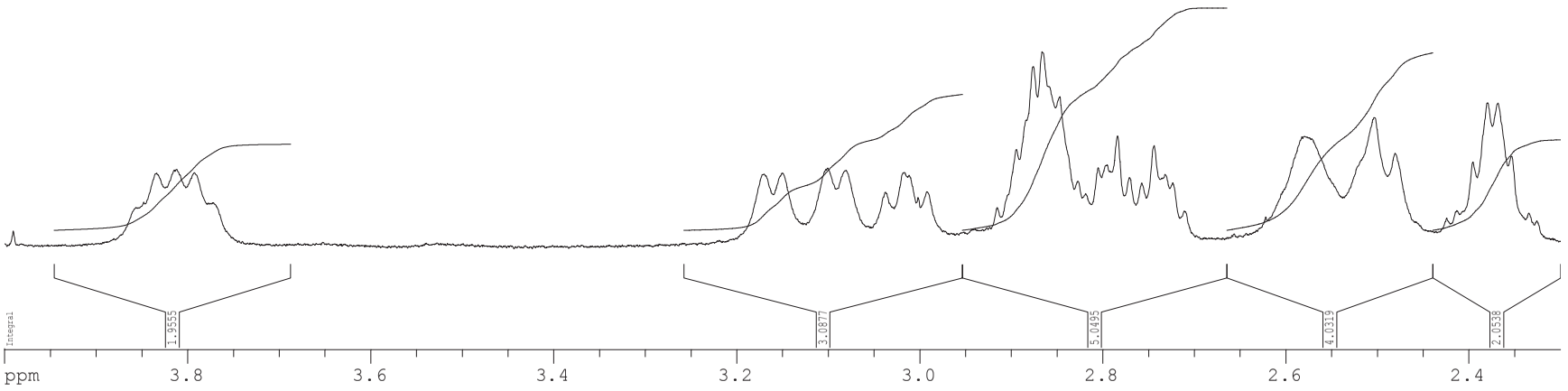
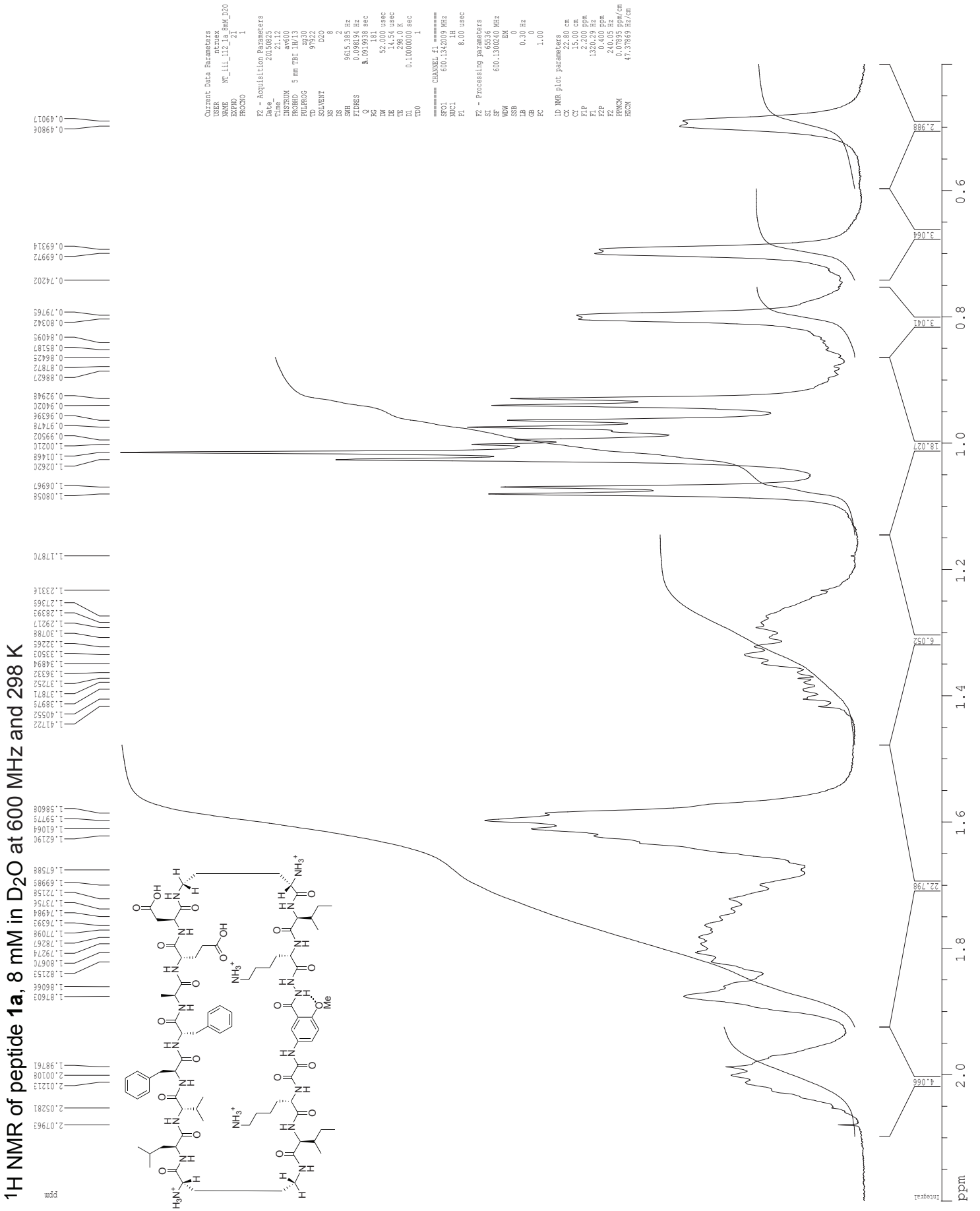
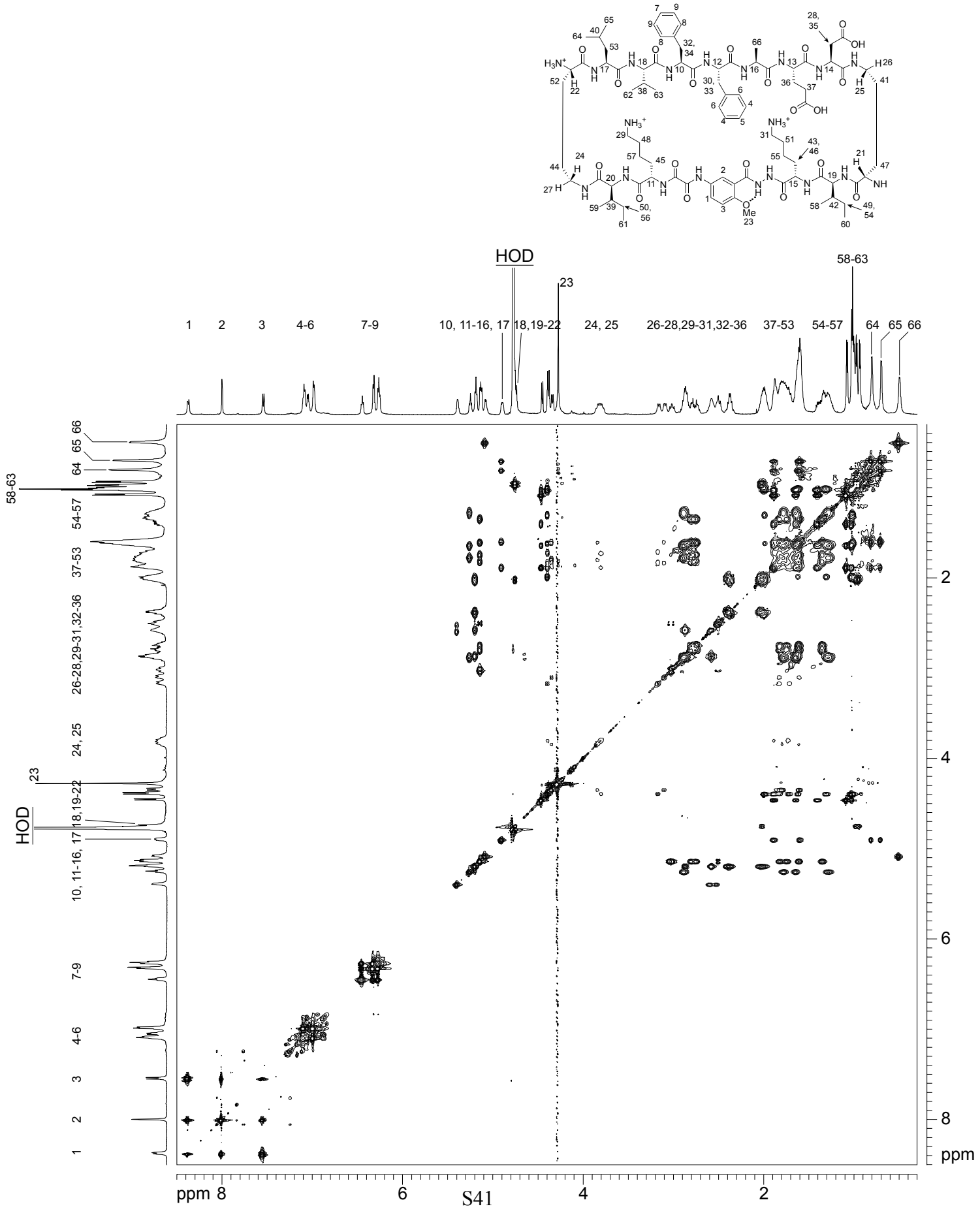


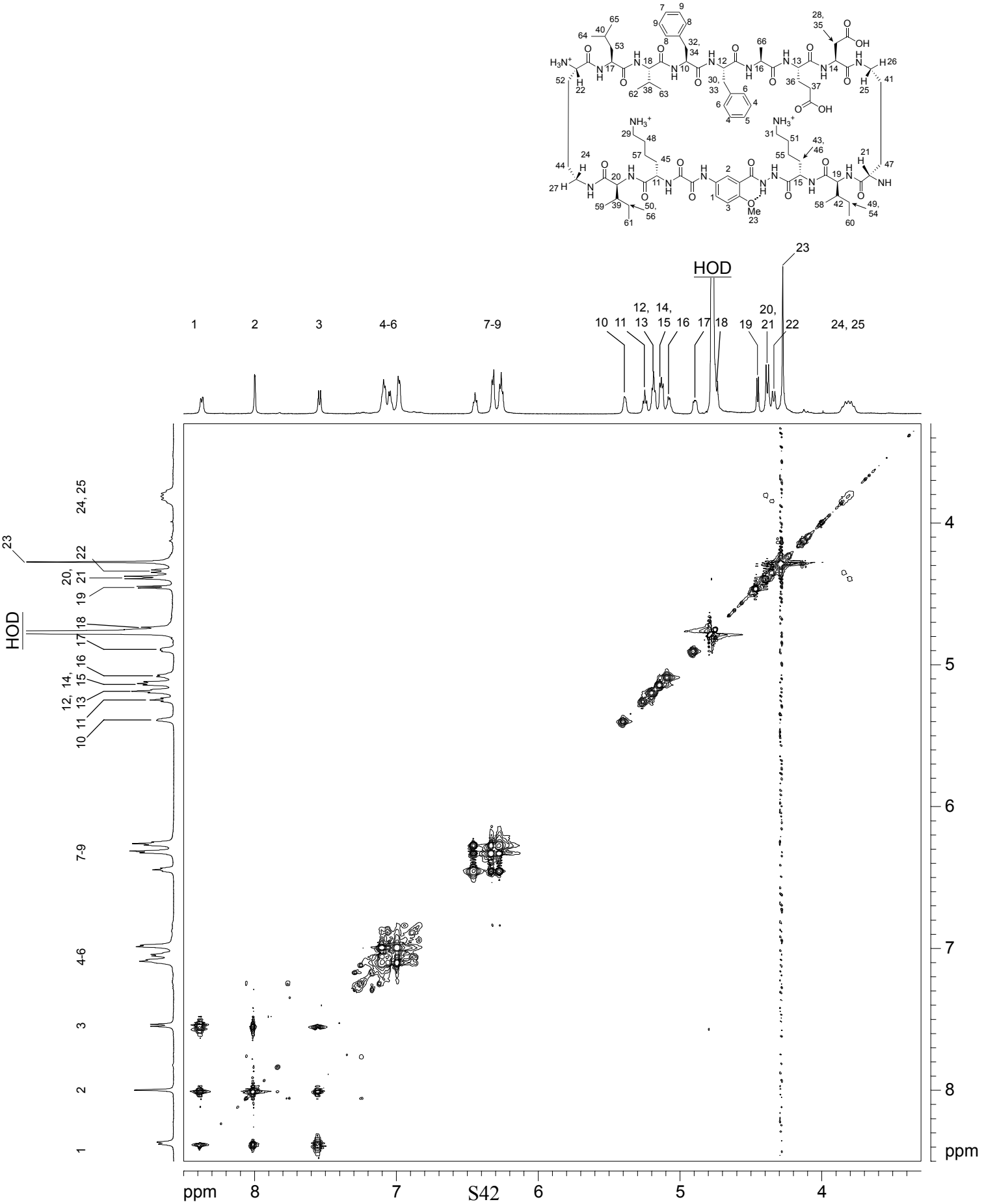
^{1}H NMR of peptide **1a**, 8 mM in D_2O at 600 MHz and 298 K



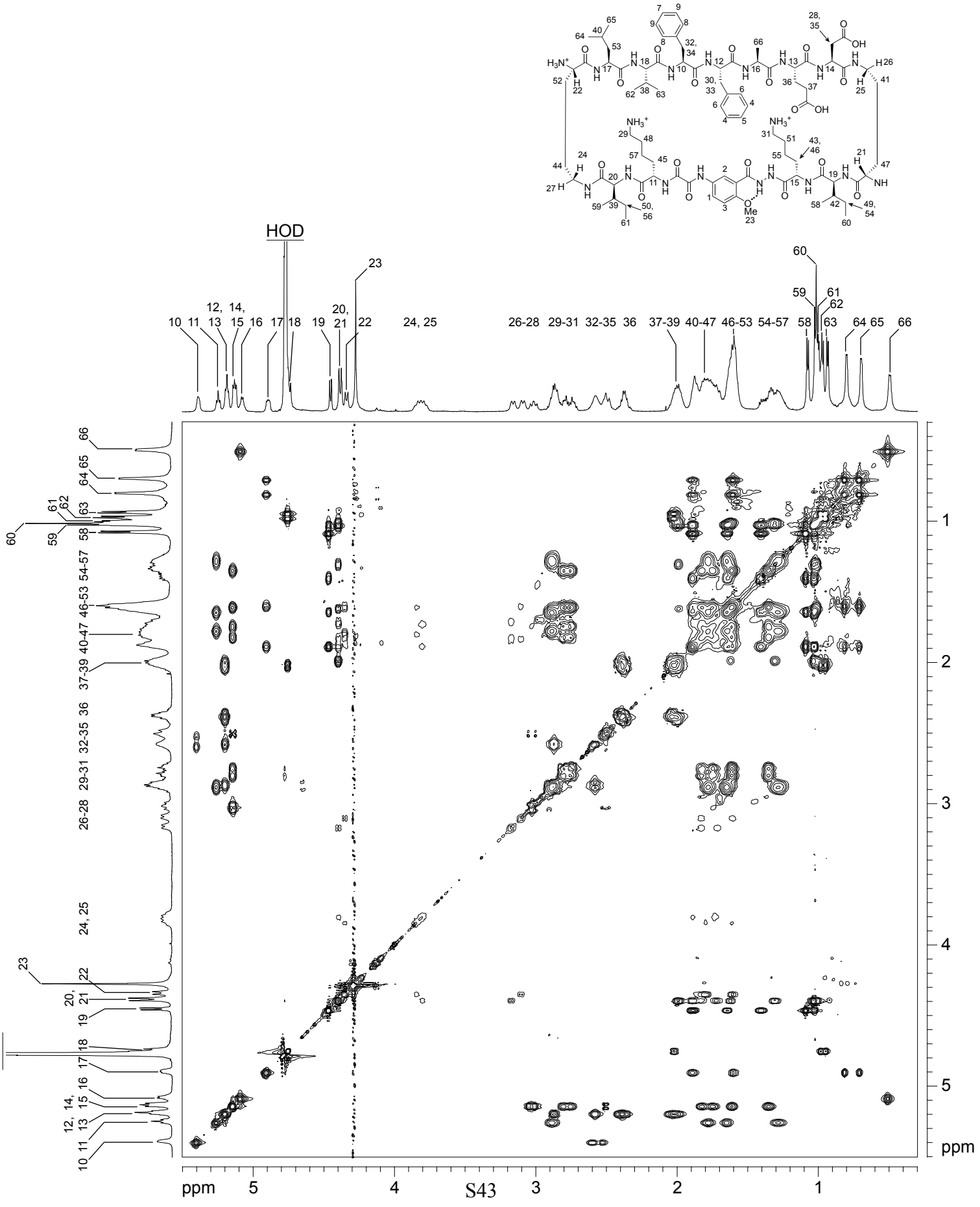
^{1}H NMR 2D TOCSY of peptide **1a** with presaturation suppression of the HOD peak
8 mM in D_2O at 600 MHz and 298 K with 150-ms spin-lock mixing time



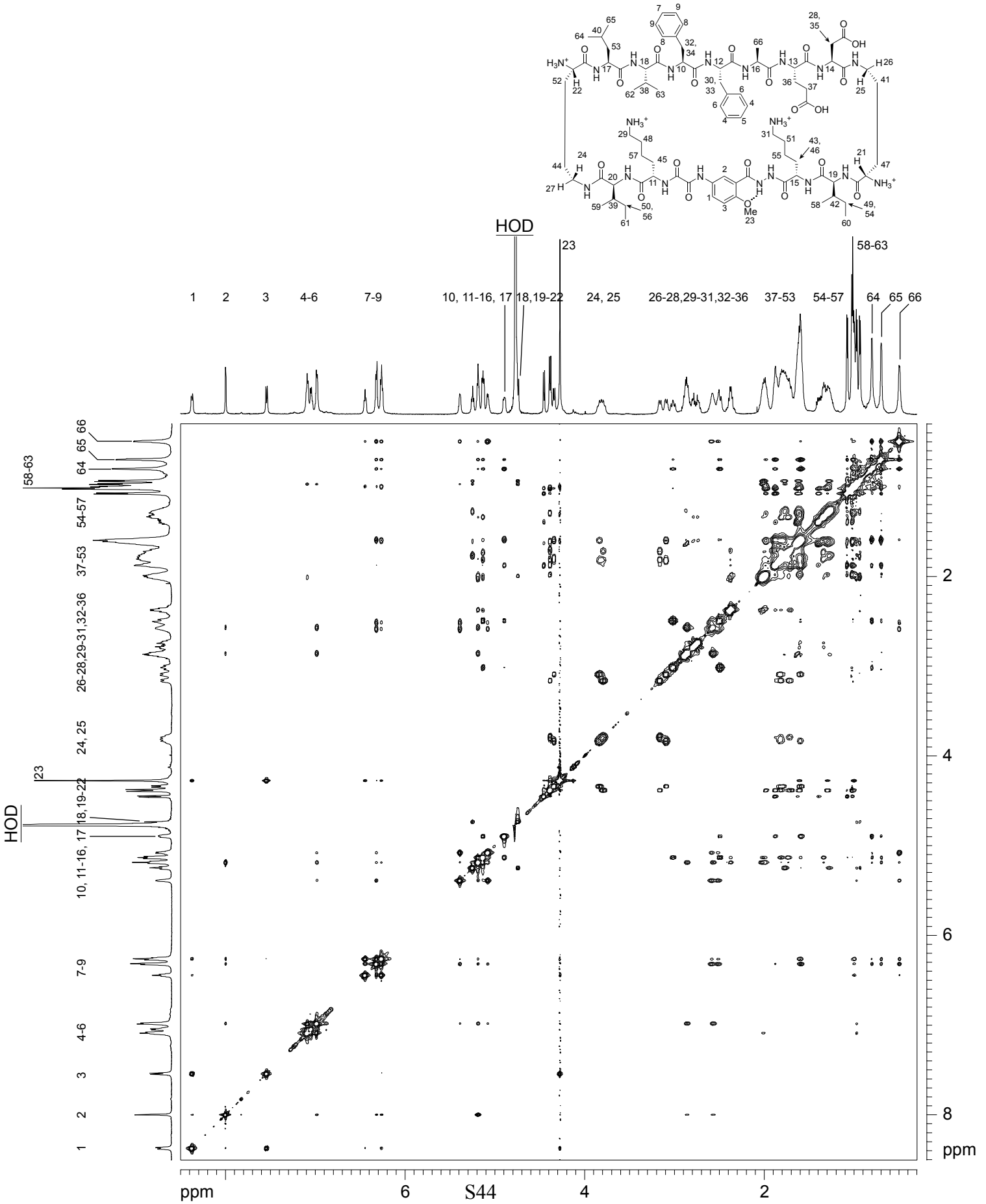
^{1}H NMR 2D TOCSY of peptide **1a** with presaturation suppression of the HOD peak
8 mM in D_2O at 600 MHz and 298 K with 150-ms spin-lock mixing time



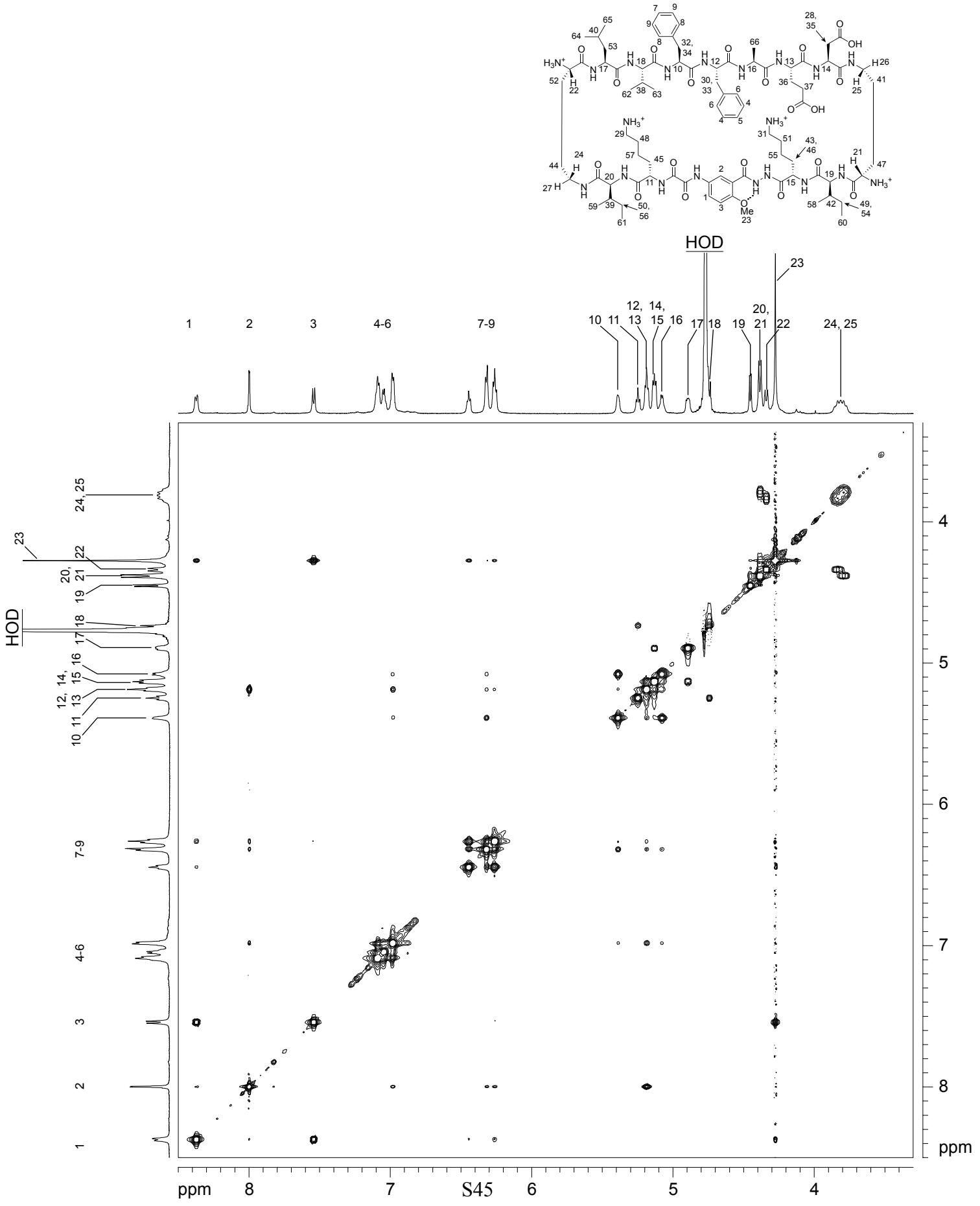
^{1}H NMR 2D TOCSY of peptide **1a** with presaturation suppression of the HOD peak
8 mM in D_2O at 600 MHz and 298 K with 150-ms spin-lock mixing time



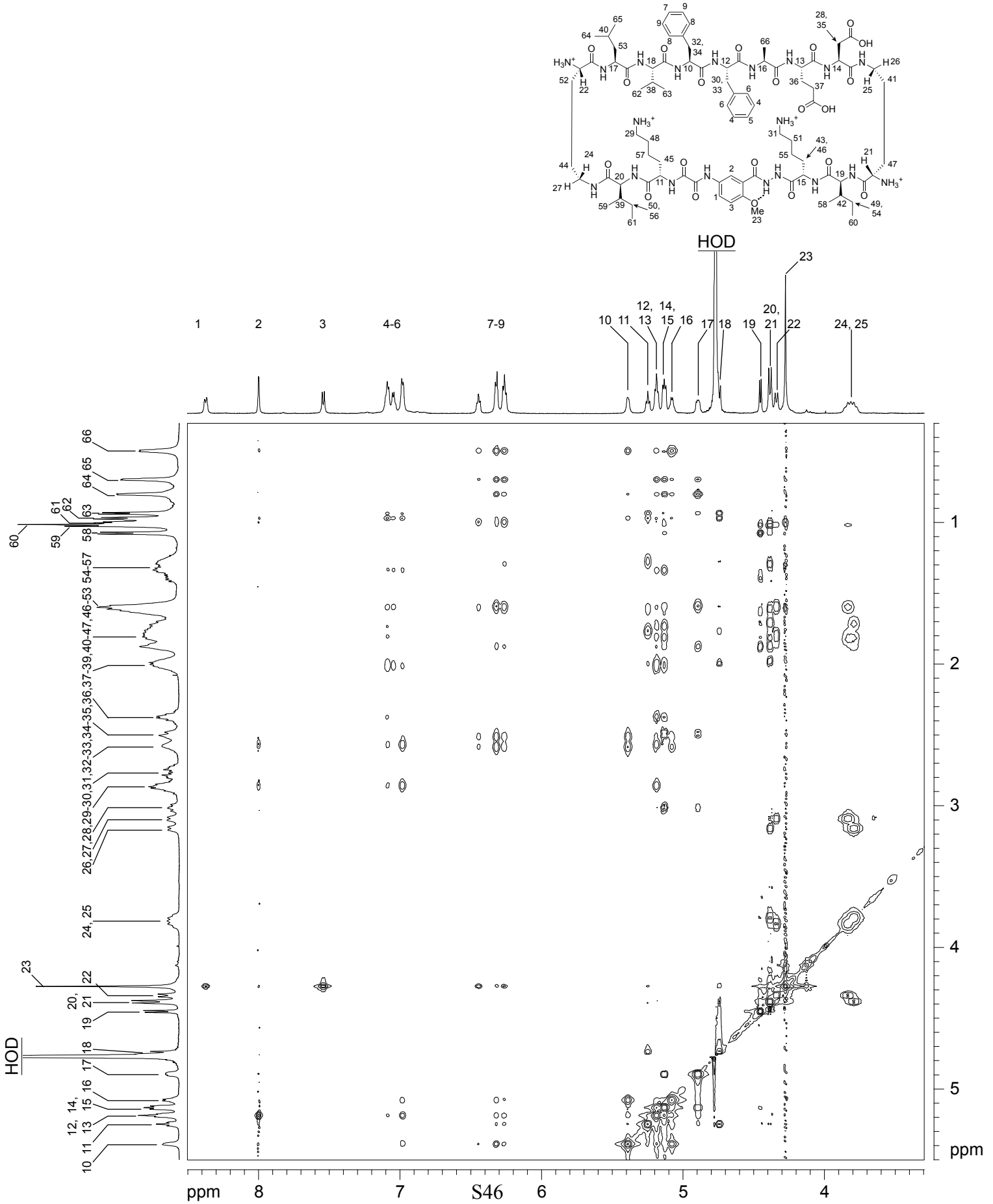
^{1}H NMR 2D NOESY of peptide **1a** with presaturation suppression of the HOD peak
8 mM in D_2O at 600 MHz and 298 K with 150-ms mixing time



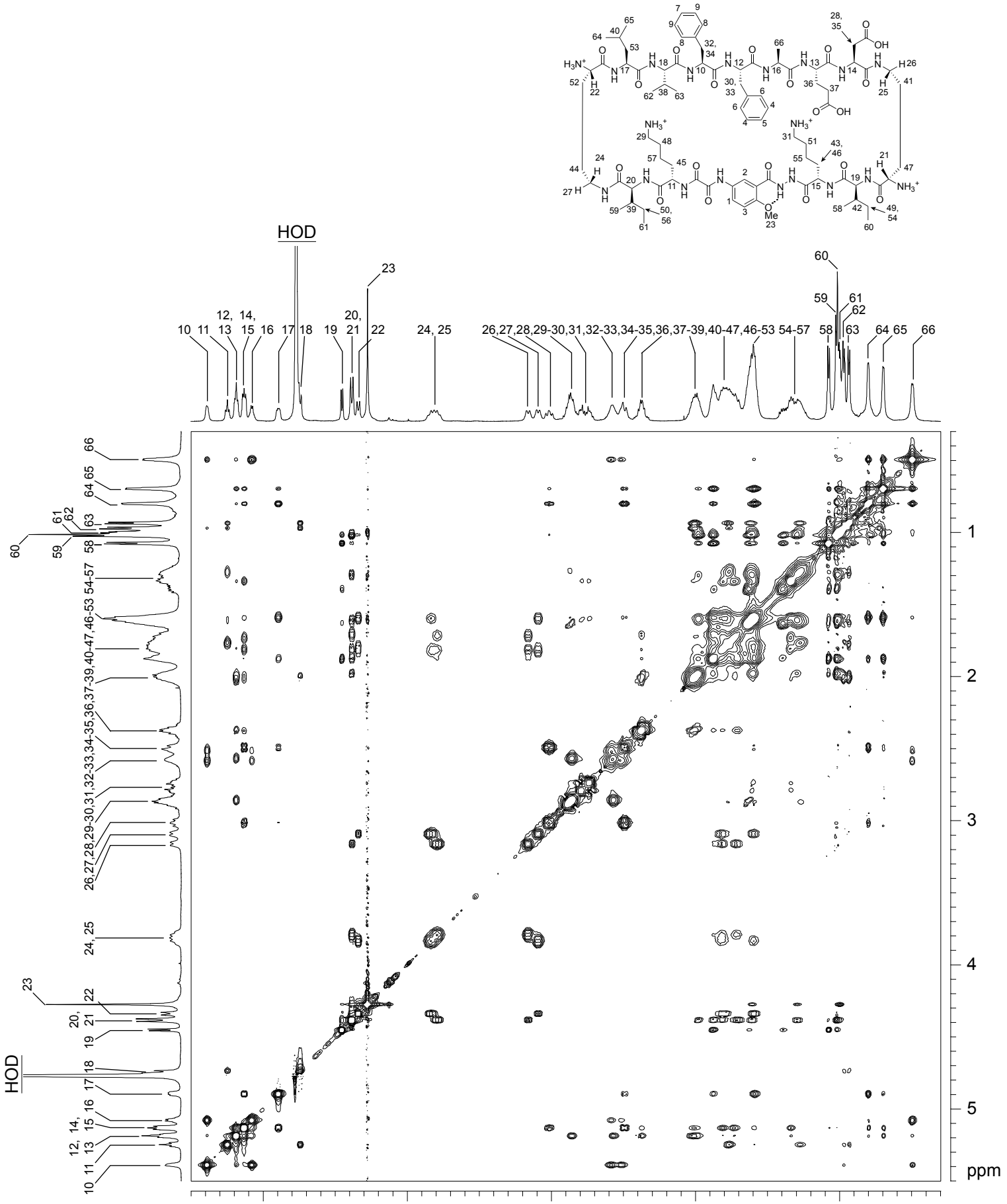
^{1}H NMR 2D NOESY of peptide **1a** with presaturation suppression of the HOD peak
8 mM in D_2O at 600 MHz and 298 K with 150-ms mixing time



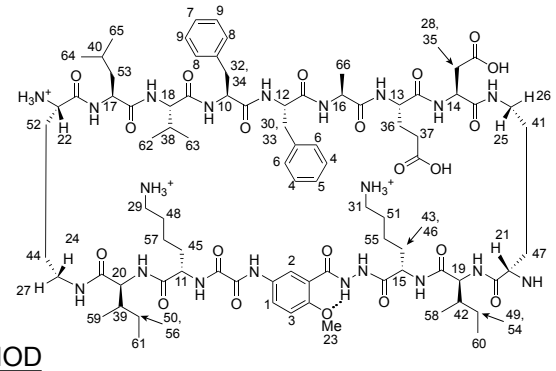
^{1}H NMR 2D NOESY of peptide **1a** with presaturation suppression of the HOD peak
8 mM in D_2O at 600 MHz and 298 K with 150-ms mixing time

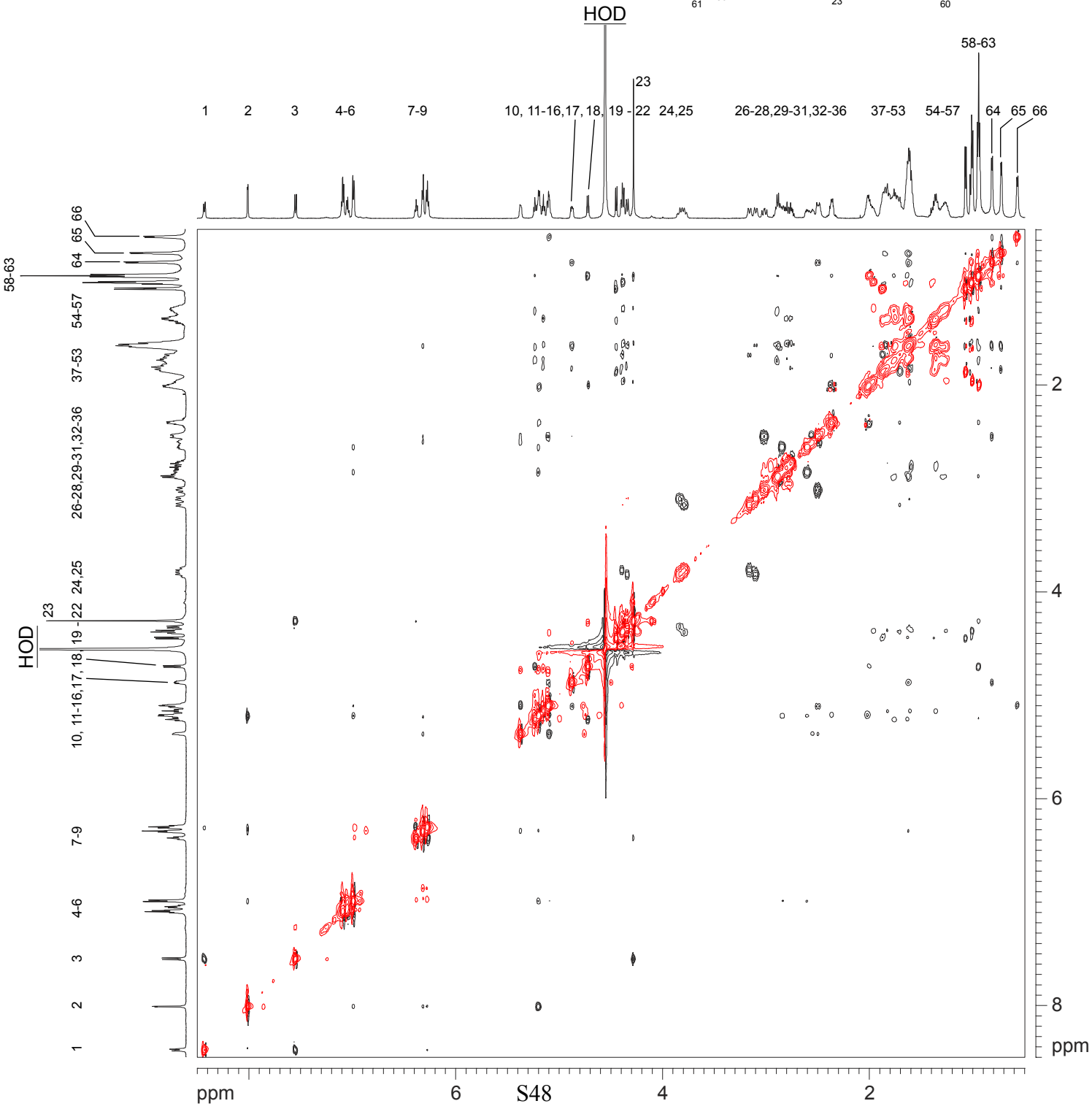


^{1}H NMR 2D NOESY of peptide **1a** with presaturation suppression of the HOD peak
8 mM in D_2O at 600 MHz and 298 K with 150-ms mixing time

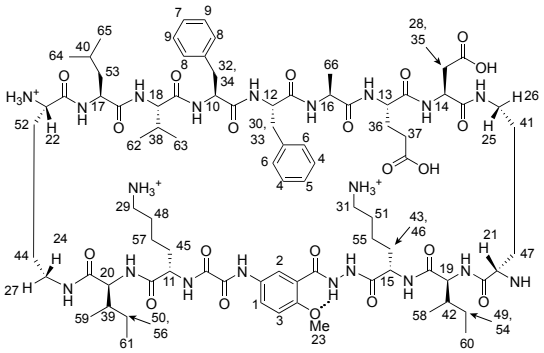


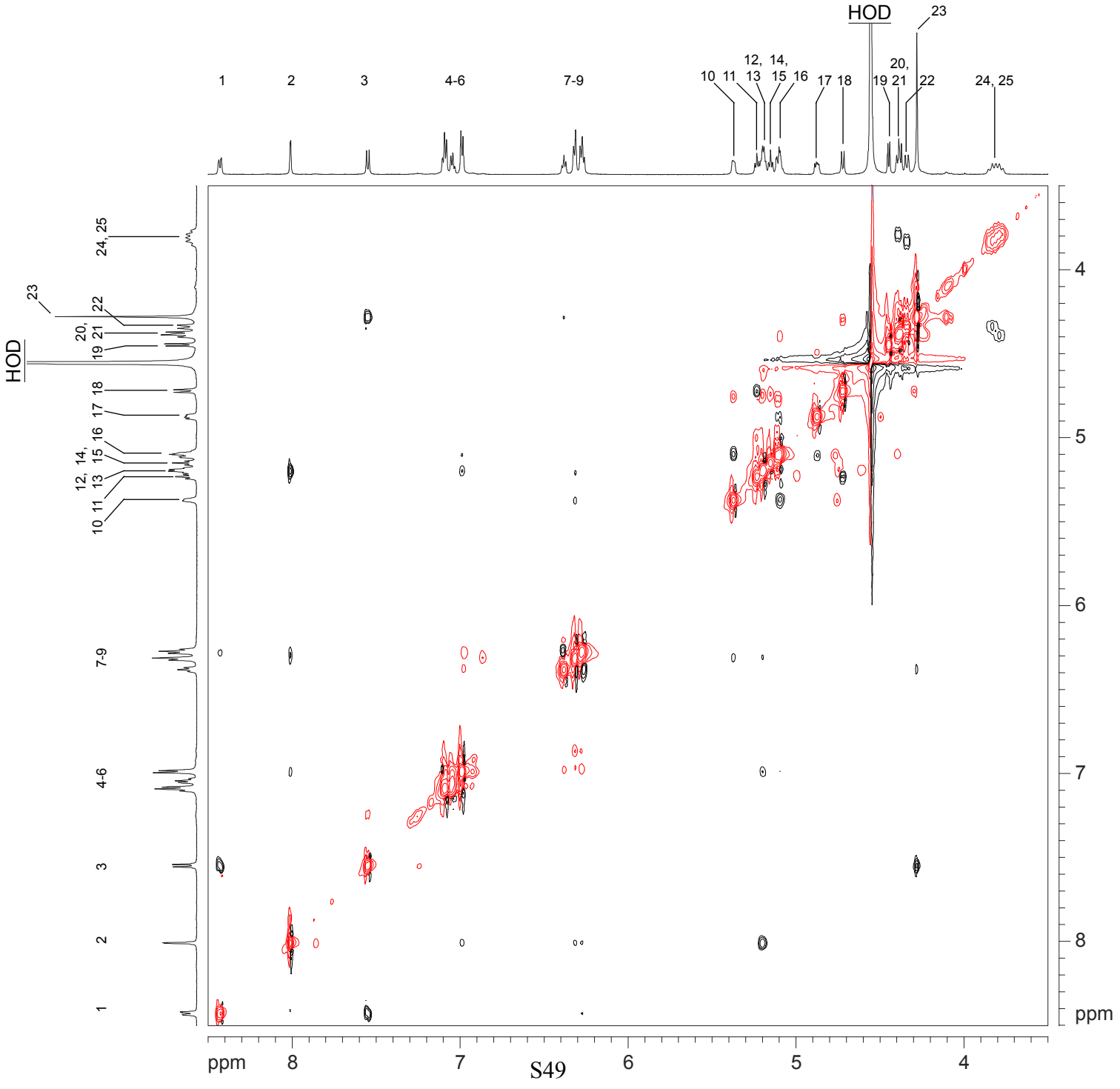
^{1}H NMR 2D EXSY of peptide **1a** with presaturation suppression of the HOD peak
8 mM in D_2O at 600 MHz and 318 K with 200-ms spin-lock mixing time
Exchange crosspeaks are shown in red;
ROE crosspeaks are shown in black.



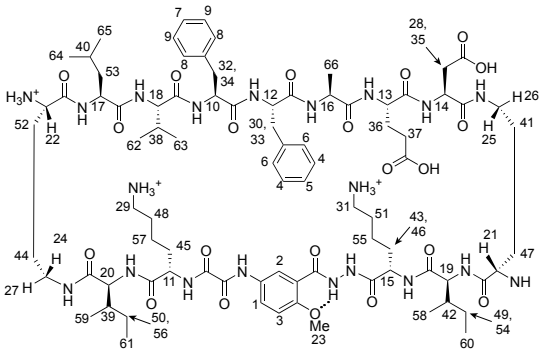


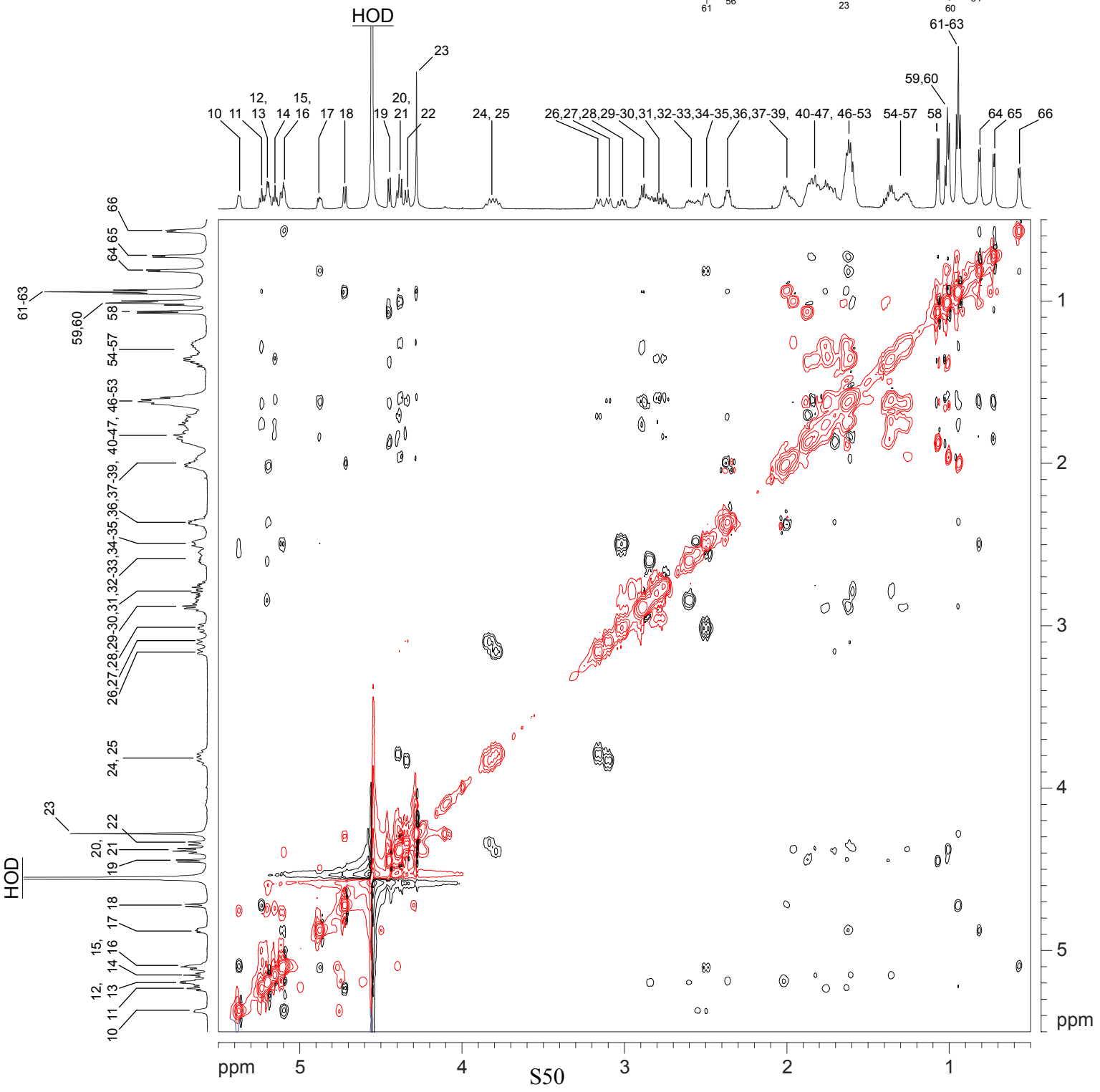
^{1}H NMR 2D EXSY of peptide **1a** with presaturation suppression of the HOD peak
8 mM in D_2O at 600 MHz and 318 K with 200-ms spin-lock mixing time
Exchange crosspeaks are shown in red;
ROE crosspeaks are shown in black.



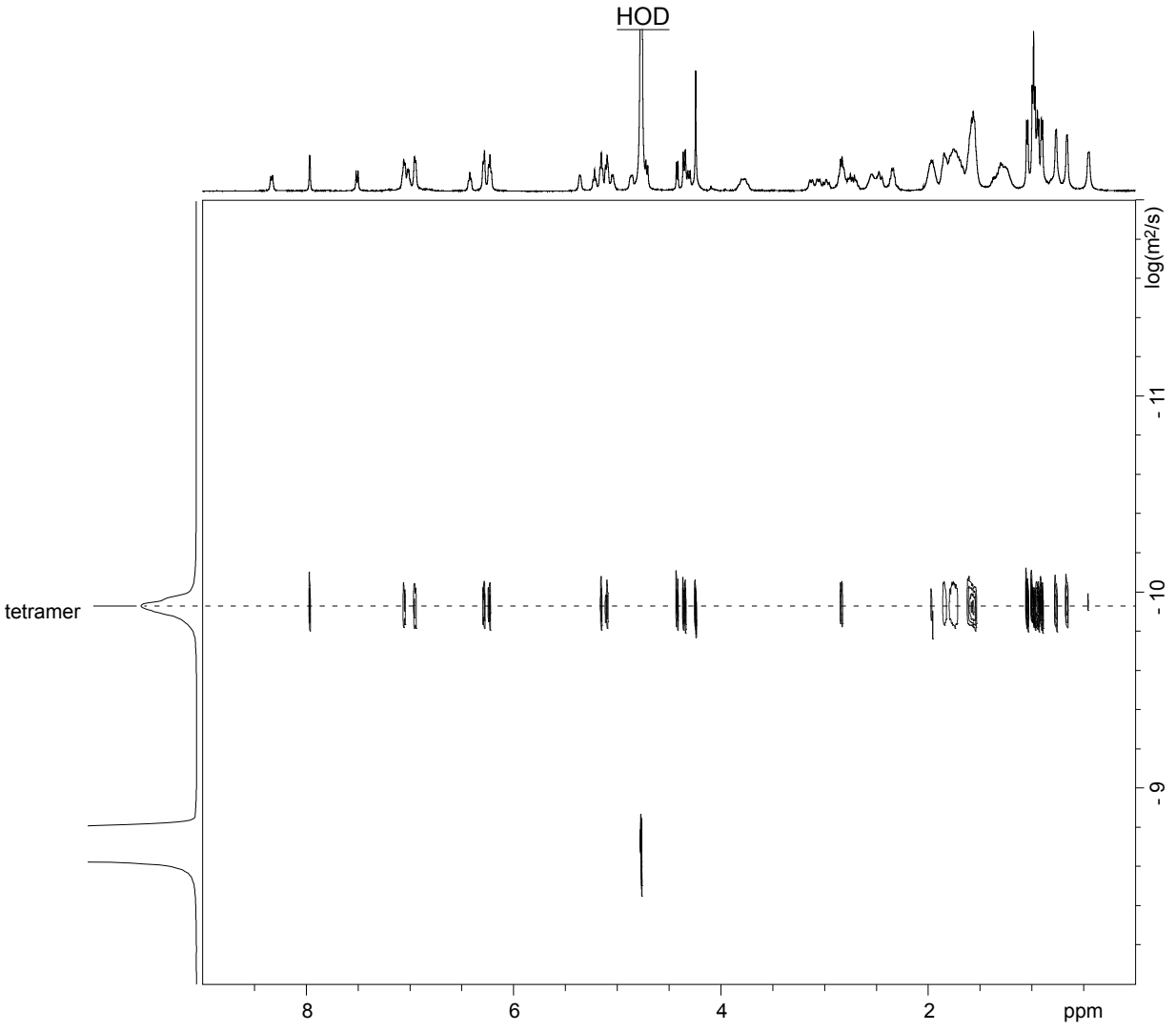


^{1}H NMR 2D EXSY of peptide **1a** with presaturation suppression of the HOD peak
8 mM in D_2O at 600 MHz and 318 K with 200-ms spin-lock mixing time
Exchange crosspeaks are shown in red;
ROE crosspeaks are shown in black.





^{1}H NMR DOSY of peptide **1a**, 8 mM in D_2O at 500 MHz and 298 K
tetramer predominates



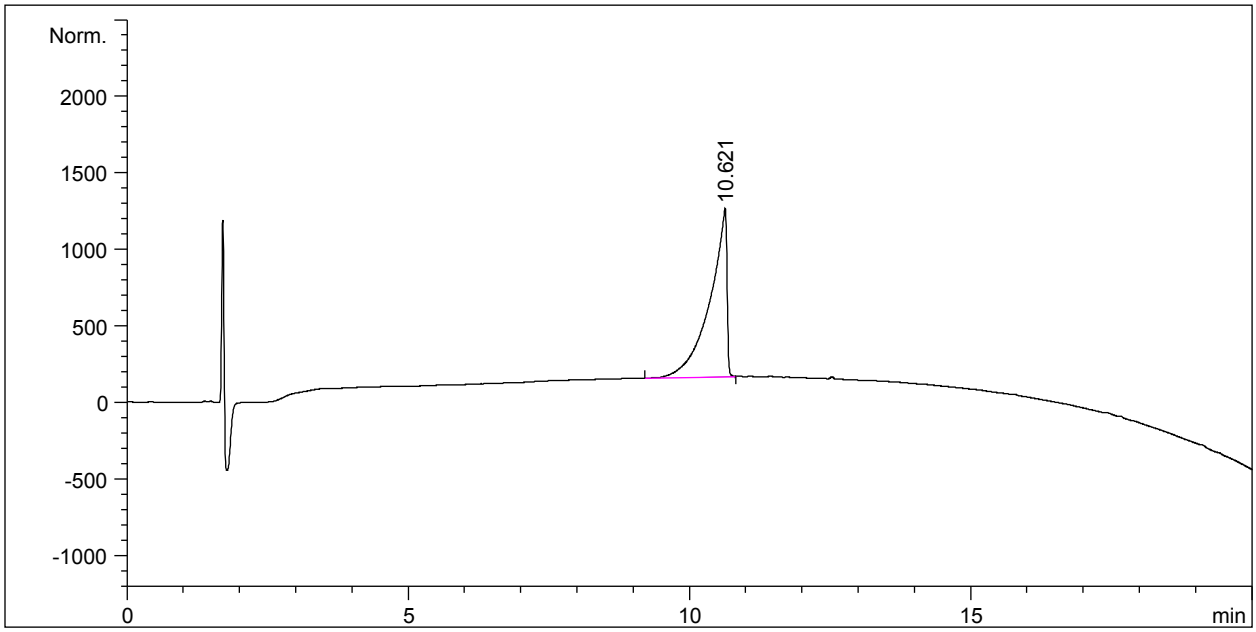
Calculations for peptide **1a** at 8.0 mM

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

$D_{tetramer}$: log(D) = -9.928; D = $10^{-9.928}$ = 11.8 ± 1.0 x 10^{-11} m^2/s

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

RP-HPLC of peptide **1b**



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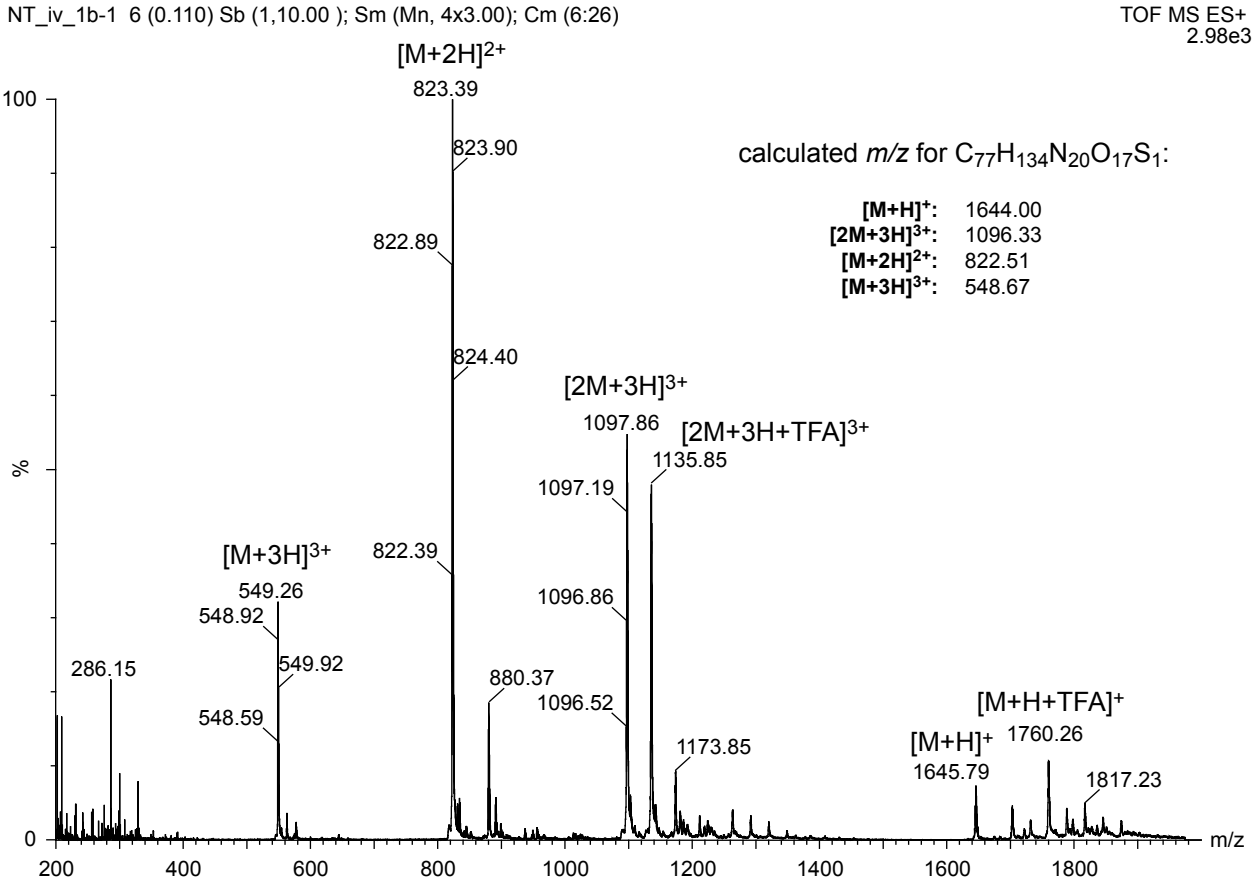
Totals :				2.29549e4	1098.58997	

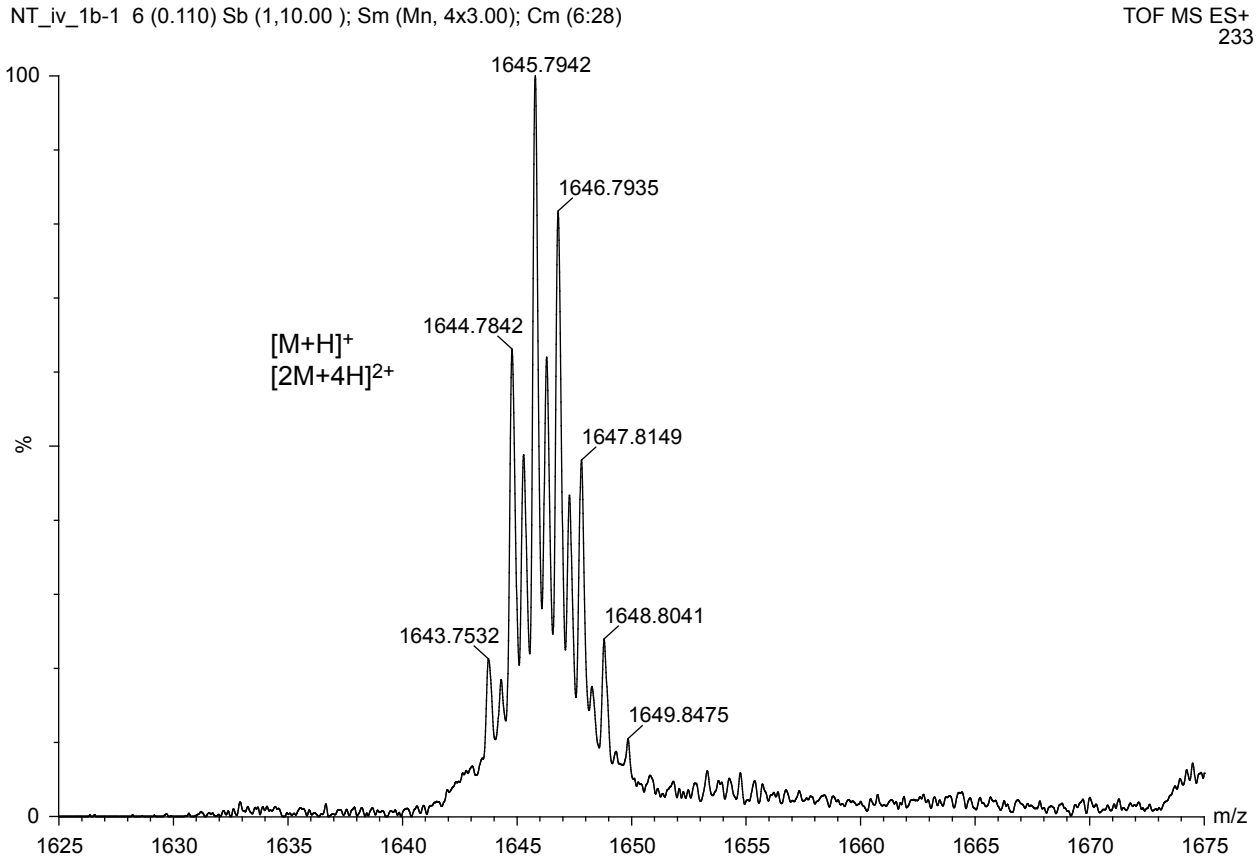
column:	Aeris XB-C18 2.6µ
dimensions:	150 mm x 4.6 mm
mobile phase:	A: H_2O, 0.1% TFA B: CH_3CN, 0.1% TFA
gradient:	A/B (95:5) to (0:100) in 20 min
flow rate:	1.0 mL/min
detection:	VWD, wavelength = 214 nm
temperature:	298 K

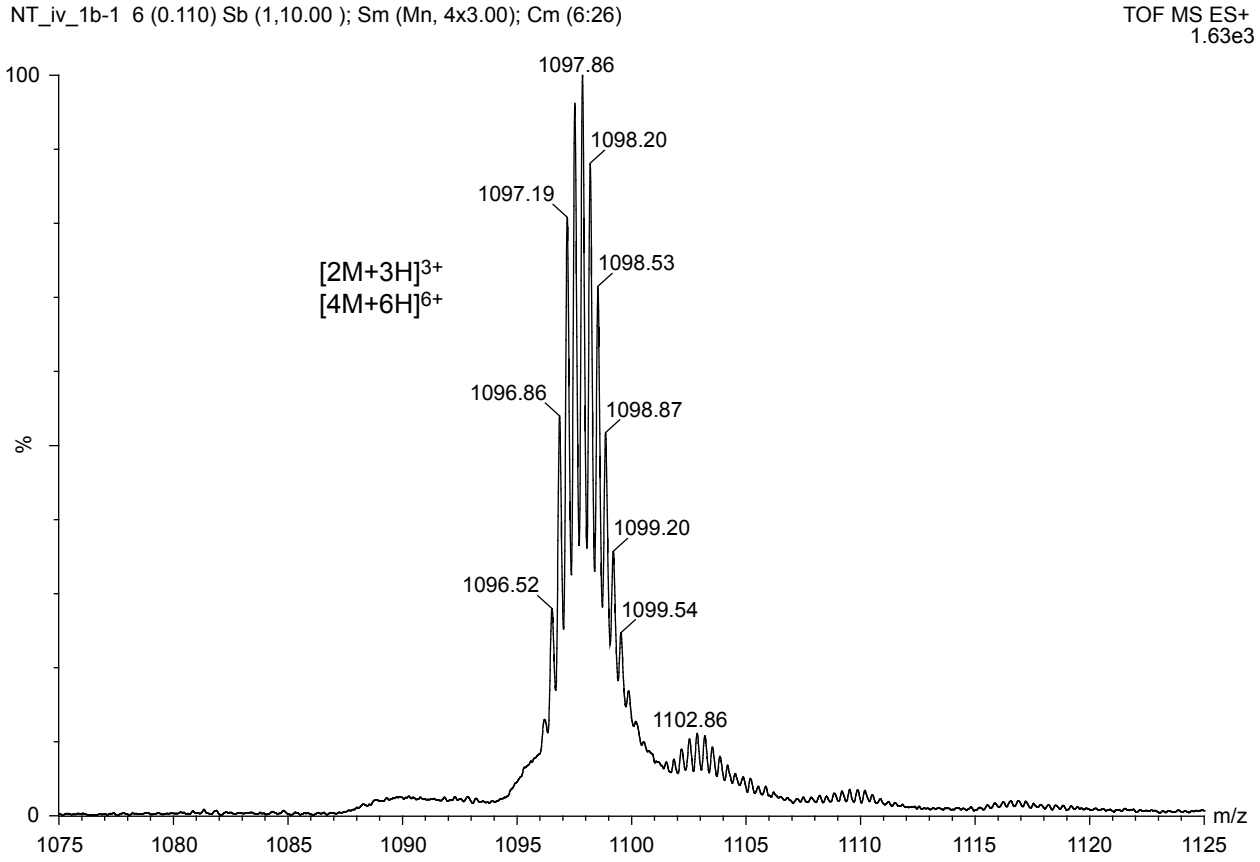
MS (ESI) of peptide **1b**

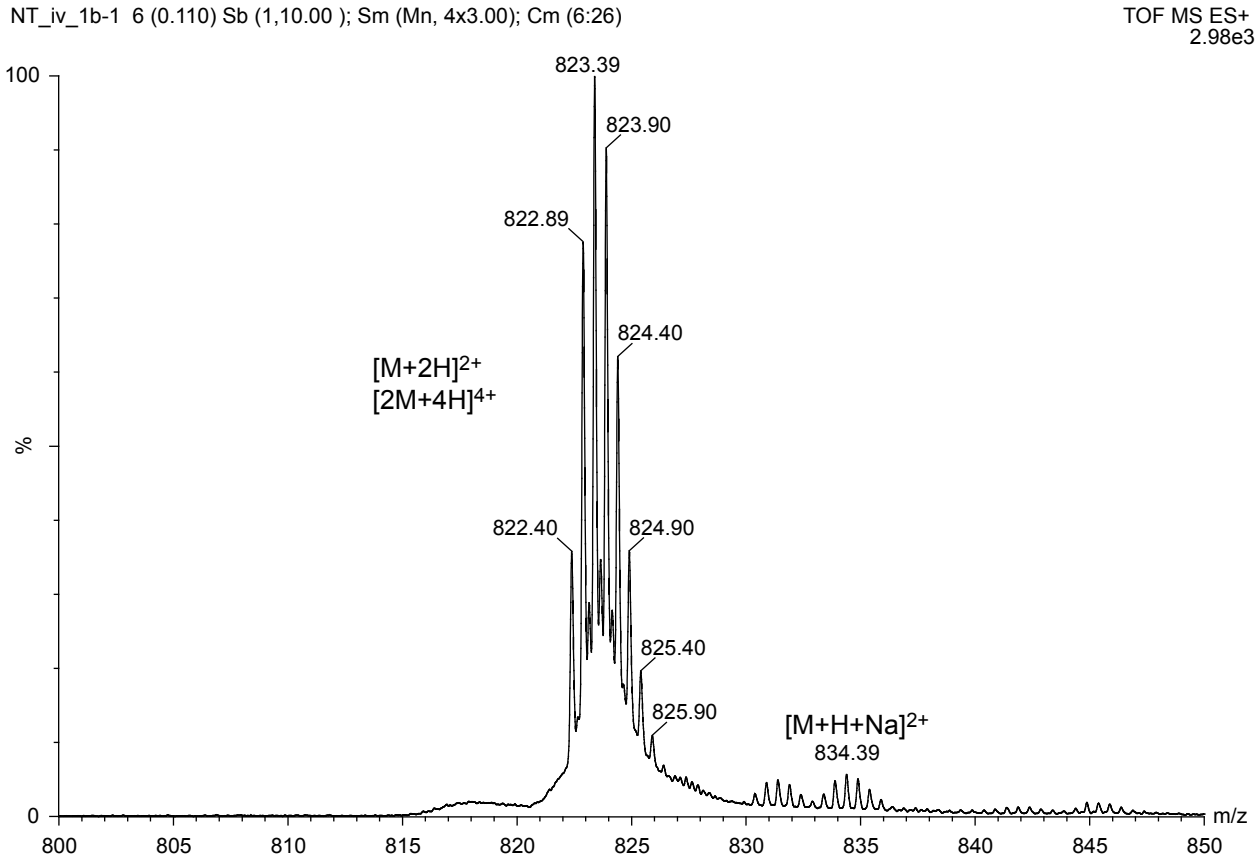


calculated *m/z* for $C_{77}H_{134}N_{20}O_{17}S_1$:

$[M+H]^+$:	1644.00
$[2M+3H]^{3+}$:	1096.33
$[M+2H]^{2+}$:	822.51
$[M+3H]^{3+}$:	548.67


NT_iv_1b-1 6 (0.110) Sb (1,10.00); Sm (Mn, 4x3.00); Cm (6:28)
TOF MS ES+
233
1645.7942
1646.7935
1644.7842
[M+H]+
[2M+4H]2+
1647.8149
1648.8041
1643.7532
1649.8475
100
%
0
m/z
1625
1630
1635
1640
1645
1650
1655
1660
1665
1670
1675


NT_iv_1b-1 6 (0.110) Sb (1,10.00); Sm (Mn, 4x3.00); Cm (6:26)
TOF MS ES+
1.63e3
1097.86
1098.20
1097.19
[2M+3H]3+
[4M+6H]6+
1098.53
1096.86
1098.87
1099.20
1096.52
1099.54
1102.86
100
%
0
m/z
1075
1080
1085
1090
1095
1100
1105
1110
1115
1120
1125


NT_iv_1b-1 6 (0.110) Sb (1,10.00); Sm (Mn, 4x3.00); Cm (6:26)
TOF MS ES+
2.98e3
100
%
0
823.39
823.90
822.89
824.40
[M+2H]2+
[2M+4H]4+
822.40
824.90
825.40
825.90
[M+H+Na]2+
834.39
m/z
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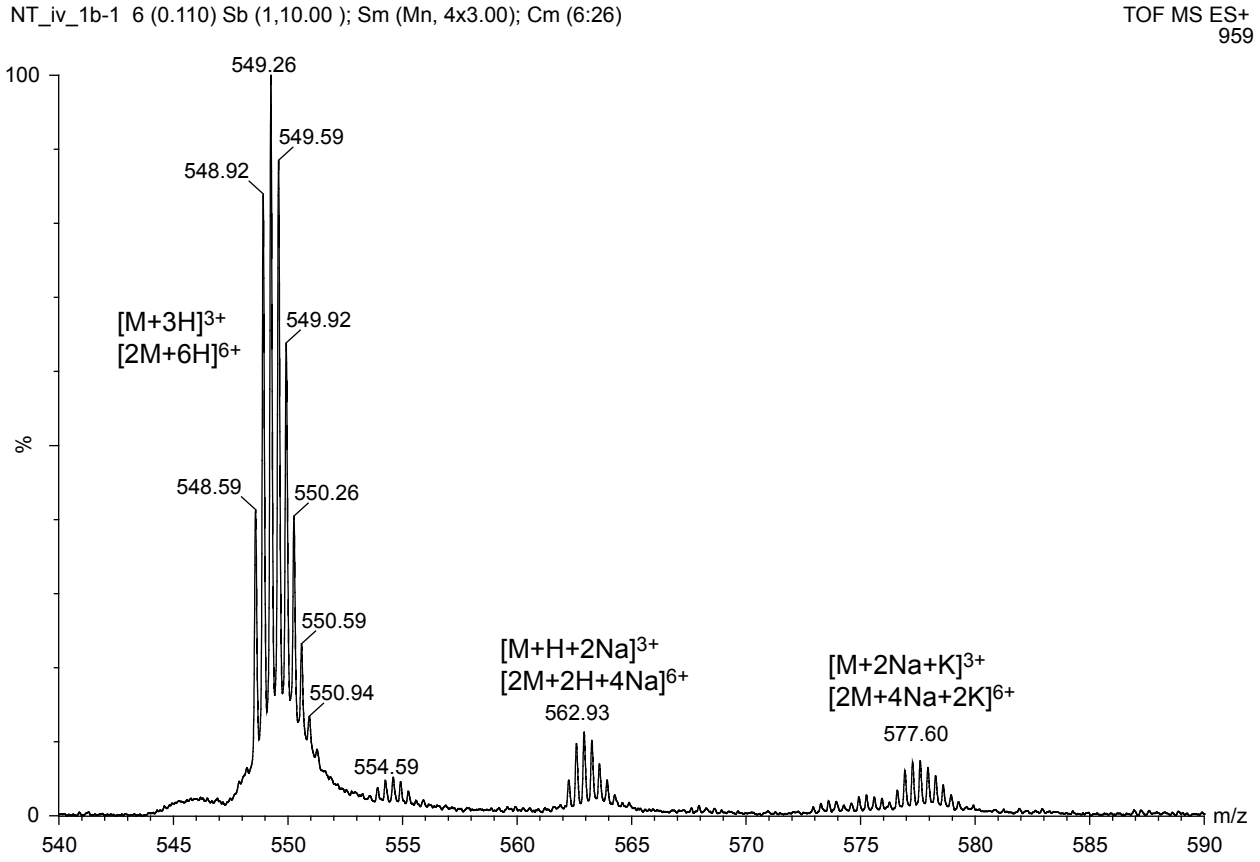
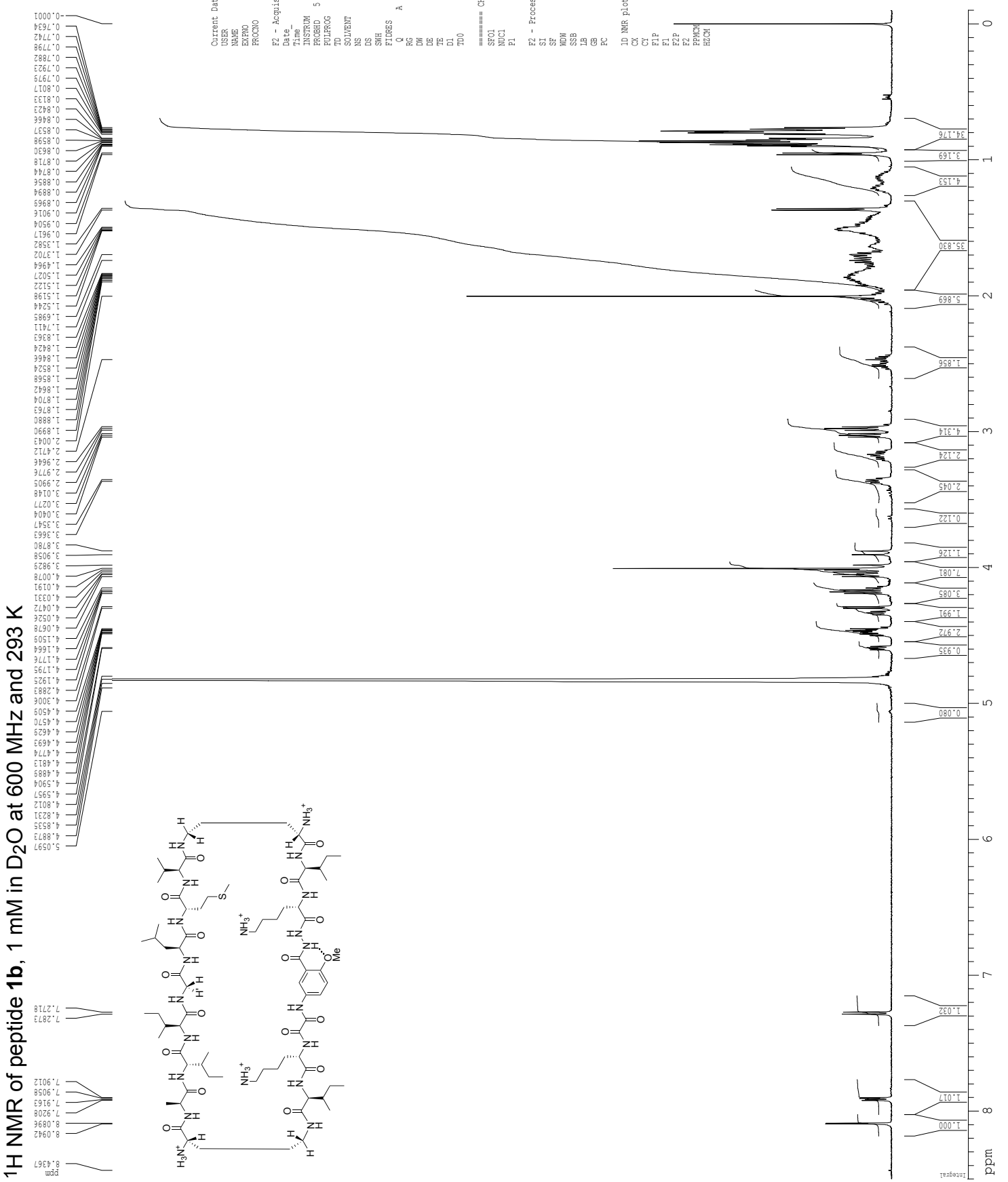
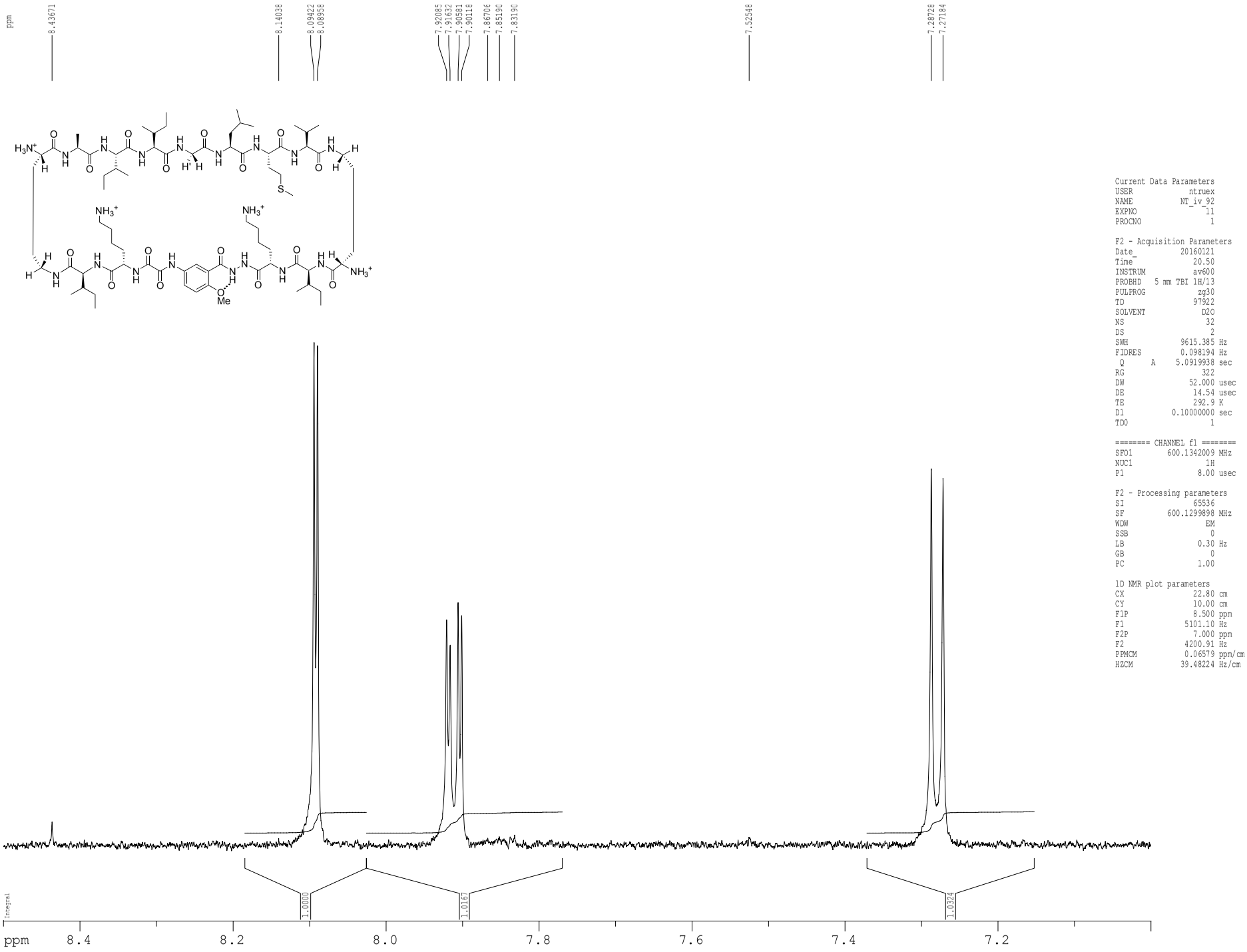
805
810
815
820
825
830
835
840
845
850


NT_iv_1b-1 6 (0.110) Sb (1,10.00); Sm (Mn, 4x3.00); Cm (6:26)
TOF MS ES+
959
100
%
0
549.26
549.59
548.92
[M+3H]3+
[2M+6H]6+
549.92
548.59
550.26
550.59
550.94
554.59
[M+H+2Na]3+
[2M+2H+4Na]6+
562.93
[M+2Na+K]3+
[2M+4Na+2K]6+
577.60
m/z
540
545
550
555
560
565
570
575
580
585
590

1H NMR of peptide **1b**, 1 mM in D_2O at 600 MHz and 293 K



^{1}H NMR of peptide **1b**, 1 mM in D_2O at 600 MHz and 293 K



Current Data Parameters
USER ntruex
NAME NT_iv_92
EXPNO 11
PROCNO 1

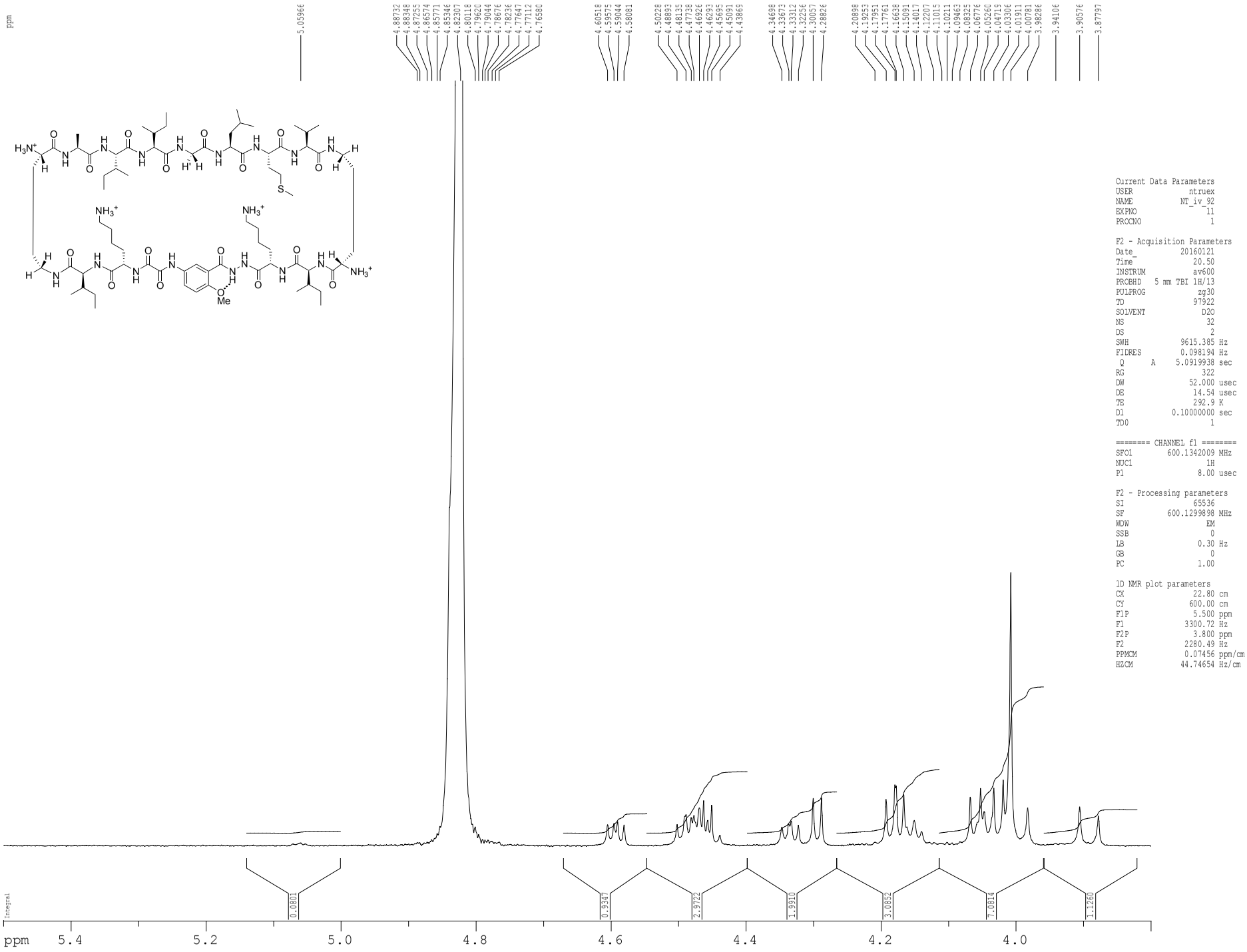
F2 - Acquisition Parameters
Date_ 20160121
Time 20.50
INSTRUM av600
PROBHD 5 mm TBI 1H/13
PULPROG zg30
TD 97922
SOLVENT D2O
NS 32
DS 2
SWH 9615.385 Hz
FIDRES 0.098194 Hz
Q A 5.0919938 sec
RG 322
DW 52.000 usec
DE 14.54 usec
TE 292.9 K
D1 0.10000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 600.1342009 MHz
NUC1 1H
P1 8.00 usec

F2 - Processing parameters
SI 65536
SF 600.1299898 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 22.80 cm
CY 10.00 cm
F1P 8.500 ppm
F1 5101.10 Hz
F2P 7.000 ppm
F2 4200.91 Hz
PPMCM 0.06579 ppm/cm
HZCM 39.48224 Hz/cm

^{1}H NMR of peptide **1b**, 1 mM in D_2O at 600 MHz and 293 K

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Current Data Parameters
USER              ntruex
NAME           NT_iv_92
EXPNO                11
PROCNO                1

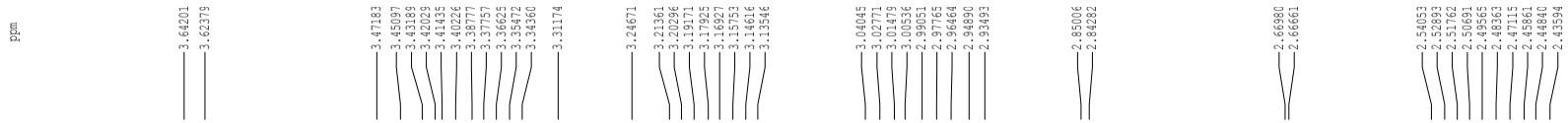
F2 - Acquisition Parameters
Date_          20160121
Time              20.50
INSTRUM           av600
PROBHD   5 mm TBI 1H/13
PULPROG             zg30
TD                97922
SOLVENT             D2O
NS                   32
DS                    2
SWH            9615.385 Hz
FIDRES        0.098194 Hz
 Q      A     5.0919938 sec
RG                  322
DW               52.000 usec
DE               14.54 usec
TE               292.9 K
D1            0.10000000 sec
TD0                   1

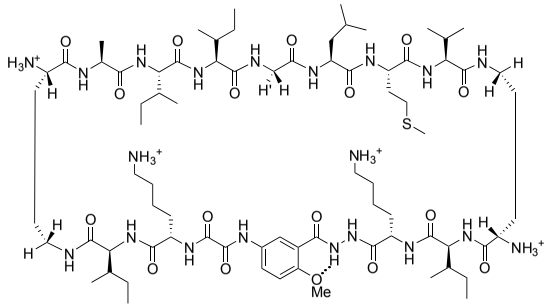
======== CHANNEL f1 ========
SFO1         600.1342009 MHz
NUC1                1H
P1                8.00 usec

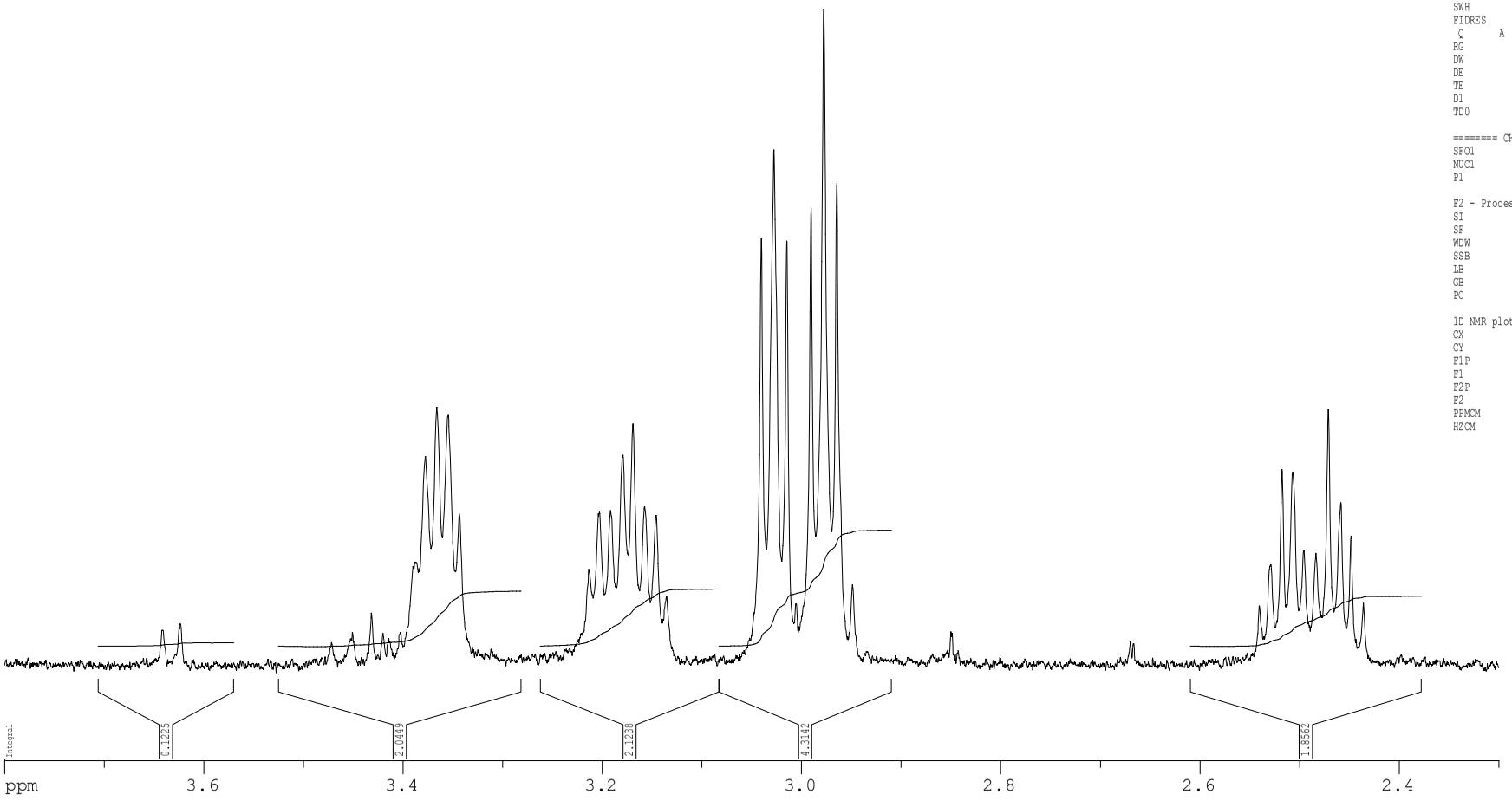
F2 - Processing parameters
SI                65536
SF           600.1299898 MHz
WDW                  EM
SSB                   0
LB                0.30 Hz
GB                    0
PC                1.00

1D NMR plot parameters
CX               22.80 cm
CY              600.00 cm
F1P               5.500 ppm
F1              3300.72 Hz
F2P               3.800 ppm
F2              2280.49 Hz
PPMCM          0.07456 ppm/cm
HZCM          44.74654 Hz/cm
```

^{1}H NMR of peptide **1b**, 1 mM in D_2O at 600 MHz and 293 K





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Current Data Parameters
USER                ntruex
NAME             NT_iv_92
EXPNO                  11
PROCNO                  1

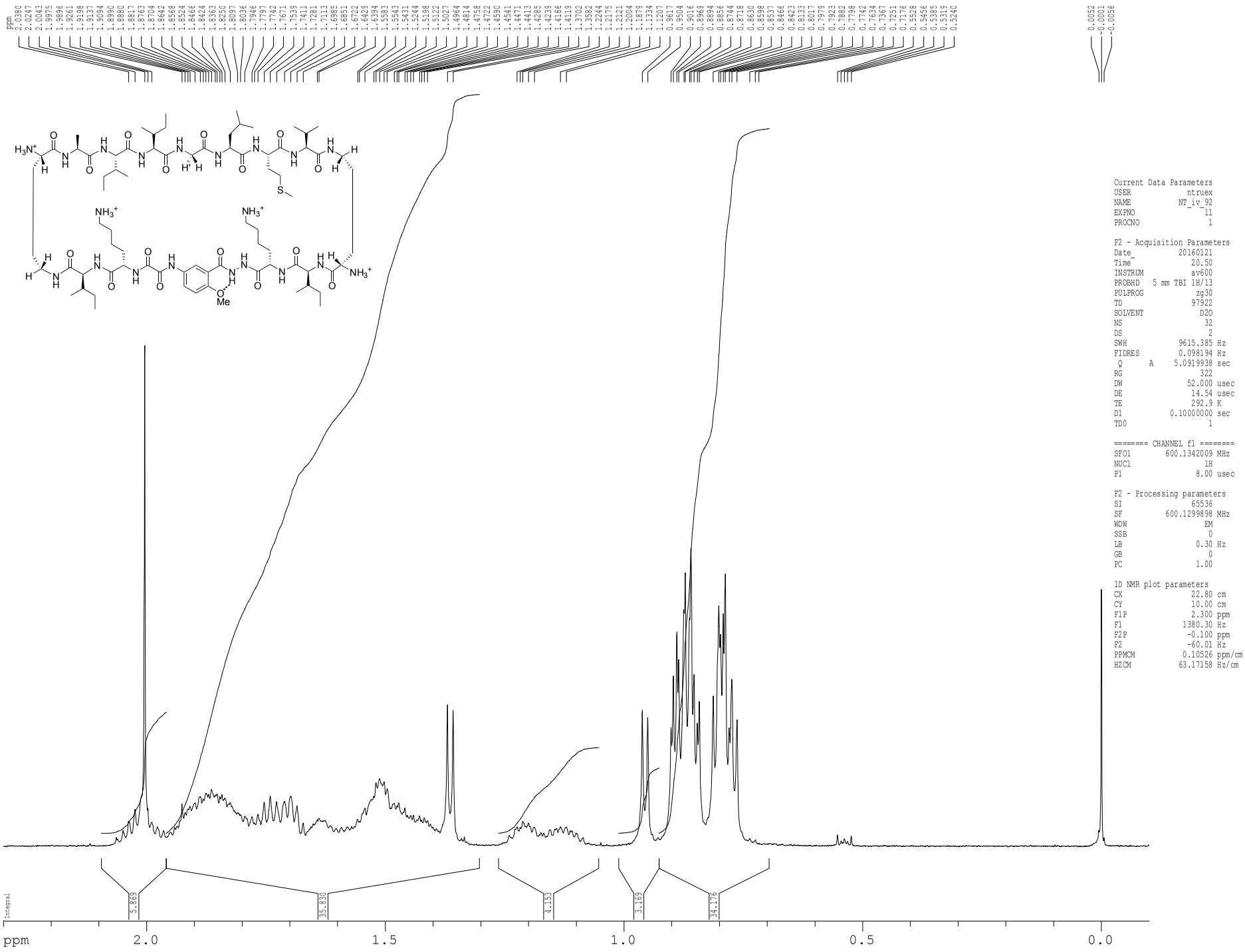
F2 - Acquisition Parameters
Date_            20160121
Time                20.50
INSTRUM             av600
PROBHD    5 mm TBI 1H/13
PULPROG              zg30
TD                  97922
SOLVENT               D2O
NS                     32
DS                      2
SWH              9615.385 Hz
FIDRES           0.098194 Hz
 Q       A     5.0919938 sec
RG                    322
DW                 52.000 usec
DE                 14.54 usec
TE                 292.9 K
D1              0.10000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          600.1342009 MHz
NUC1                   1H
P1                  8.00 usec

F2 - Processing parameters
SI                  65536
SF            600.1299898 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00

1D NMR plot parameters
CX                  22.80 cm
CY                  10.00 cm
F1P                 3.800 ppm
F1                2280.49 Hz
F2P                 2.300 ppm
F2                1380.30 Hz
PPMCM            0.06579 ppm/cm
HZCM            39.48224 Hz/cm
```

^{1}H NMR of peptide **1b**, 1 mM in D_2O at 600 MHz and 293 K



Current Data Parameters
USER ntruex
NAME NT_iv_92
EXPNO 11
PROCNO 1

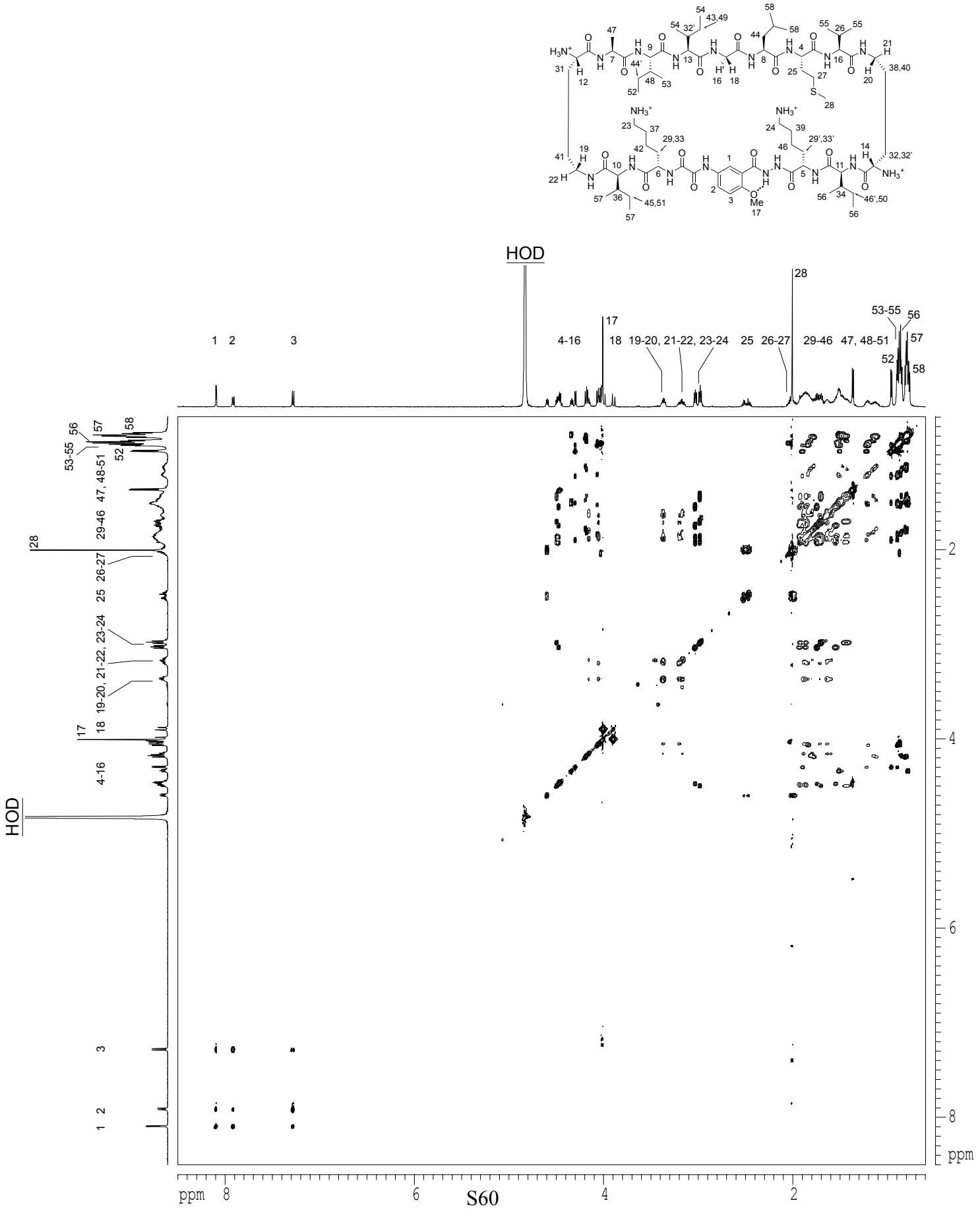
F2 - Acquisition Parameters
Date_ 20160121
Time 20.50
INSTRUM av600
PROBHD 5 mm TBI 1H/13
PULPROG zg30
TD 97922
SOLVENT D2O
NS 32
DS 2
SWH 9615.385 Hz
FIDRES 0.098194 Hz
Q A 5.0919938 sec
RG 322
DW 52.000 usec
DE 14.54 usec
TE 292.9 K
D1 0.10000000 sec
TD0 1

======= CHANNEL f1 =======
SFO1 600.1342009 MHz
NUC1 1H
P1 8.00 usec

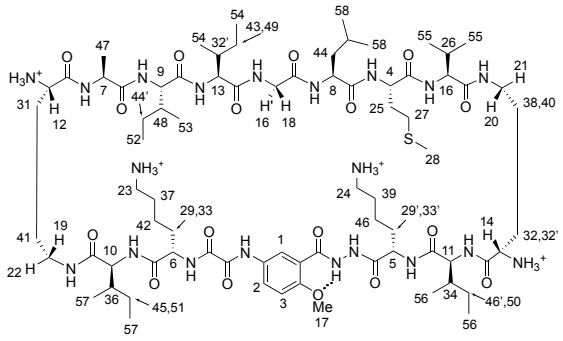
F2 - Processing parameters
SI 65536
SF 600.1299898 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

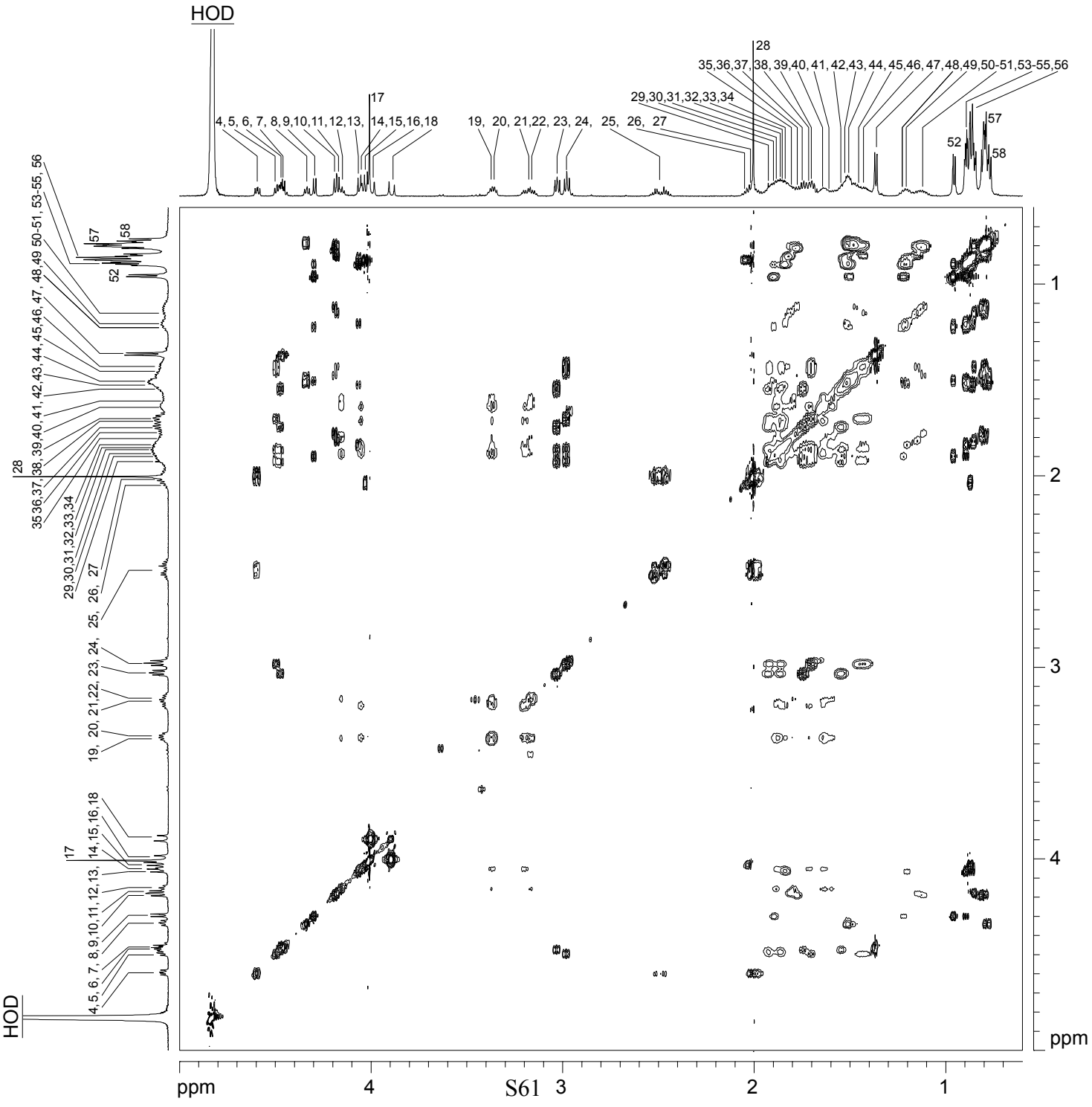
1D NMR plot parameters
CX 22.80 cm
CY 10.00 cm
F1P 2.300 ppm
F1 1380.30 Hz
F2P -0.100 ppm
F2 -60.01 Hz
PPMCM 0.10526 ppm/cm
HZCM 63.17158 Hz/cm

^{1}H NMR 2D TOCSY of macrocycle **1b** with presaturation suppression of the HOD peak
1 mM in D_2O at 600 MHz and 293 K with 150-ms spin-lock mixing time

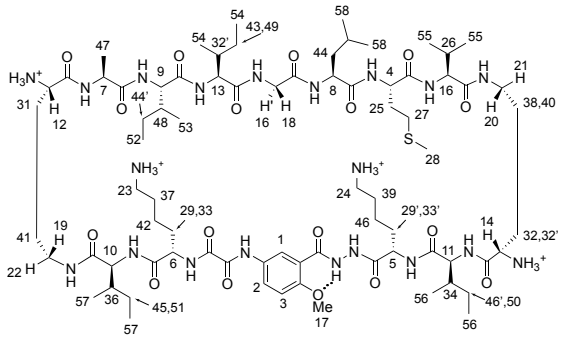


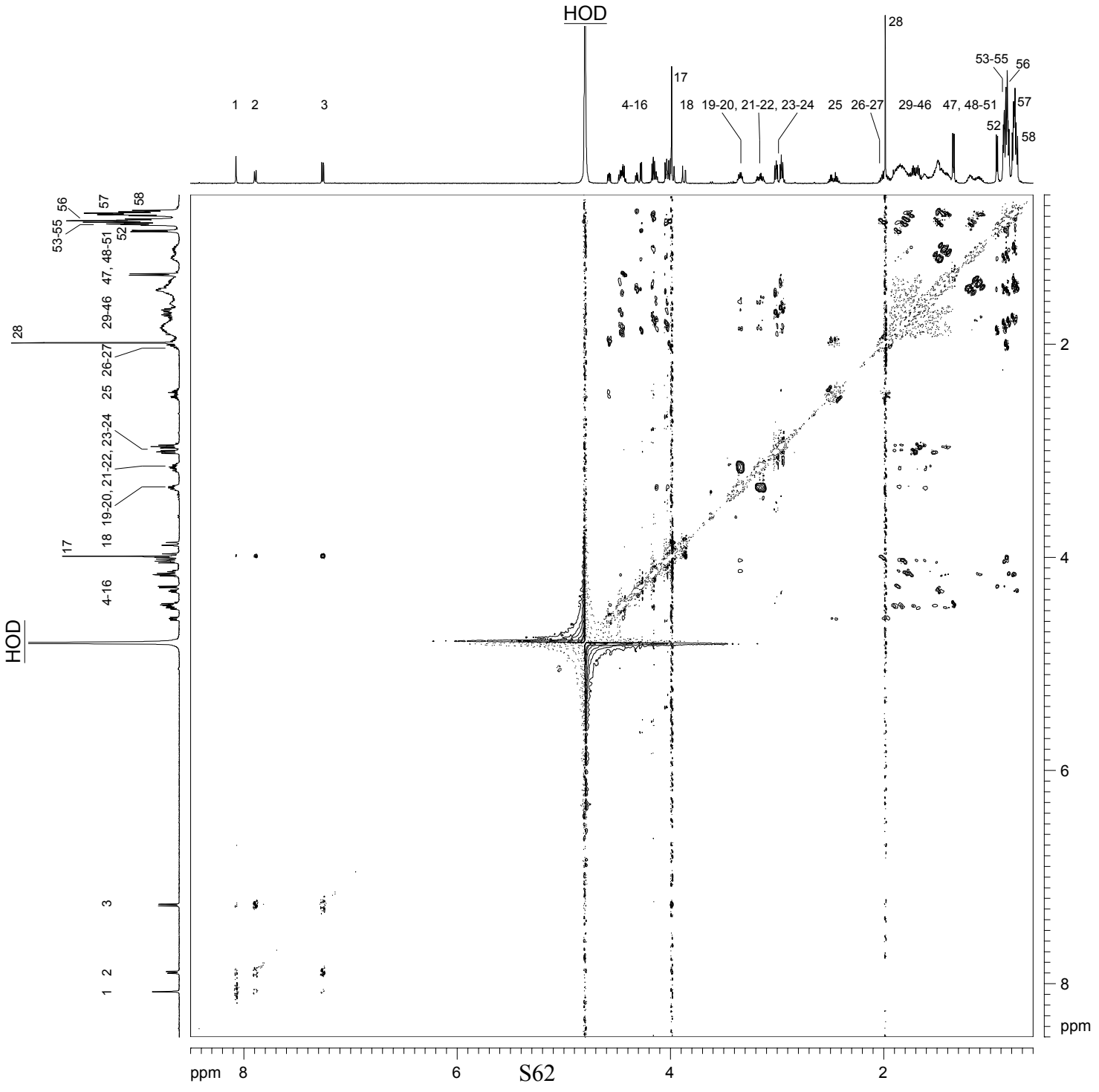
^{1}H NMR 2D TOCSY of macrocycle **1b** with presaturation suppression of the HOD peak
1 mM in D_2O at 600 MHz and 293 K with 150-ms spin-lock mixing time



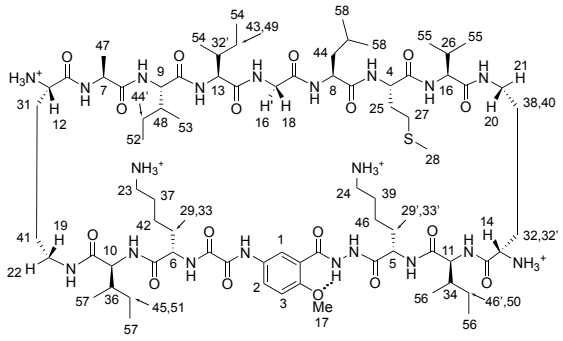


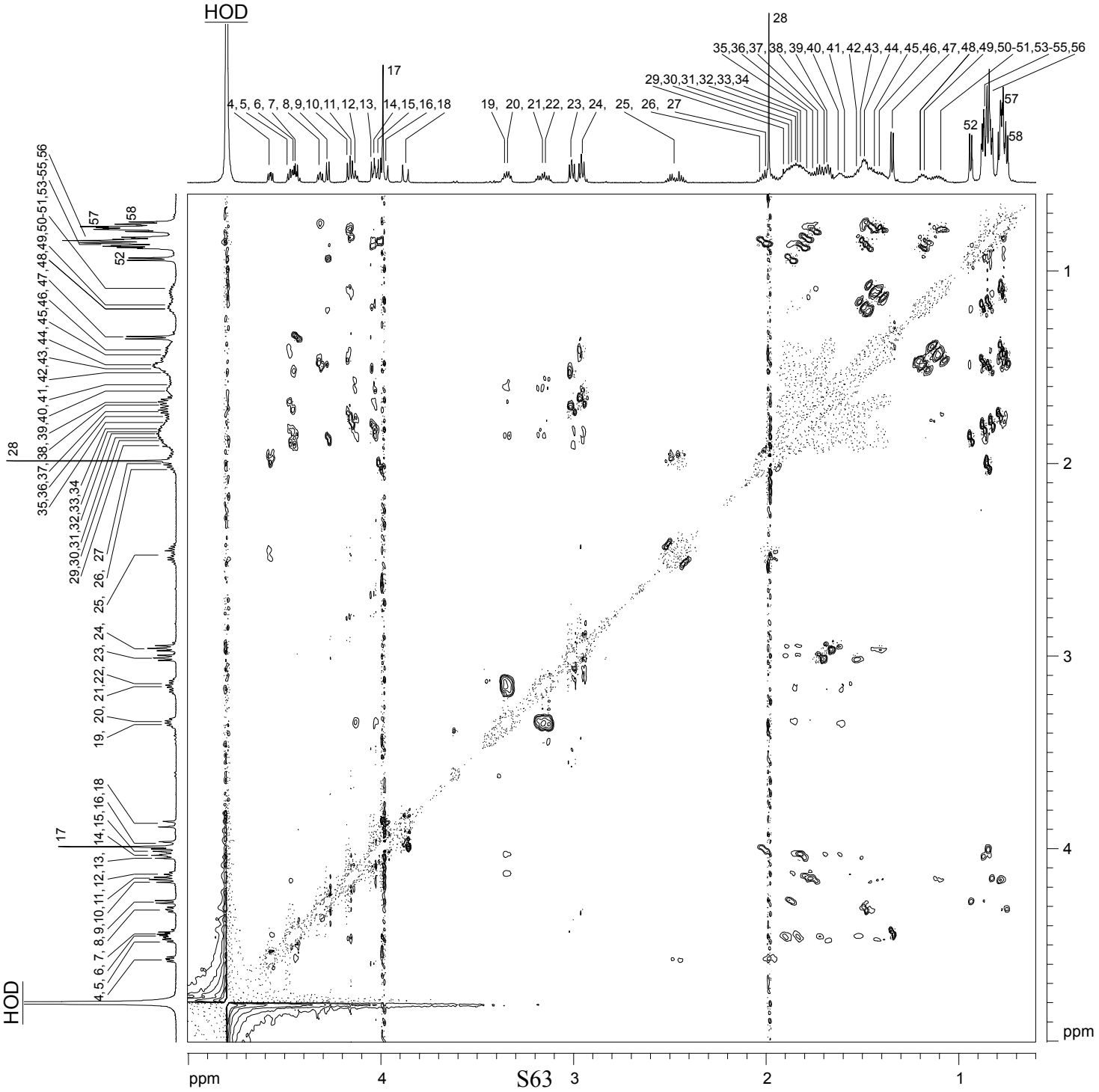
^{1}H NMR 2D ROESY of macrocycle **1b** with presaturation suppression of the HOD peak
1 mM in D_2O at 600 MHz and 293 K with 200-ms spin-lock mixing time



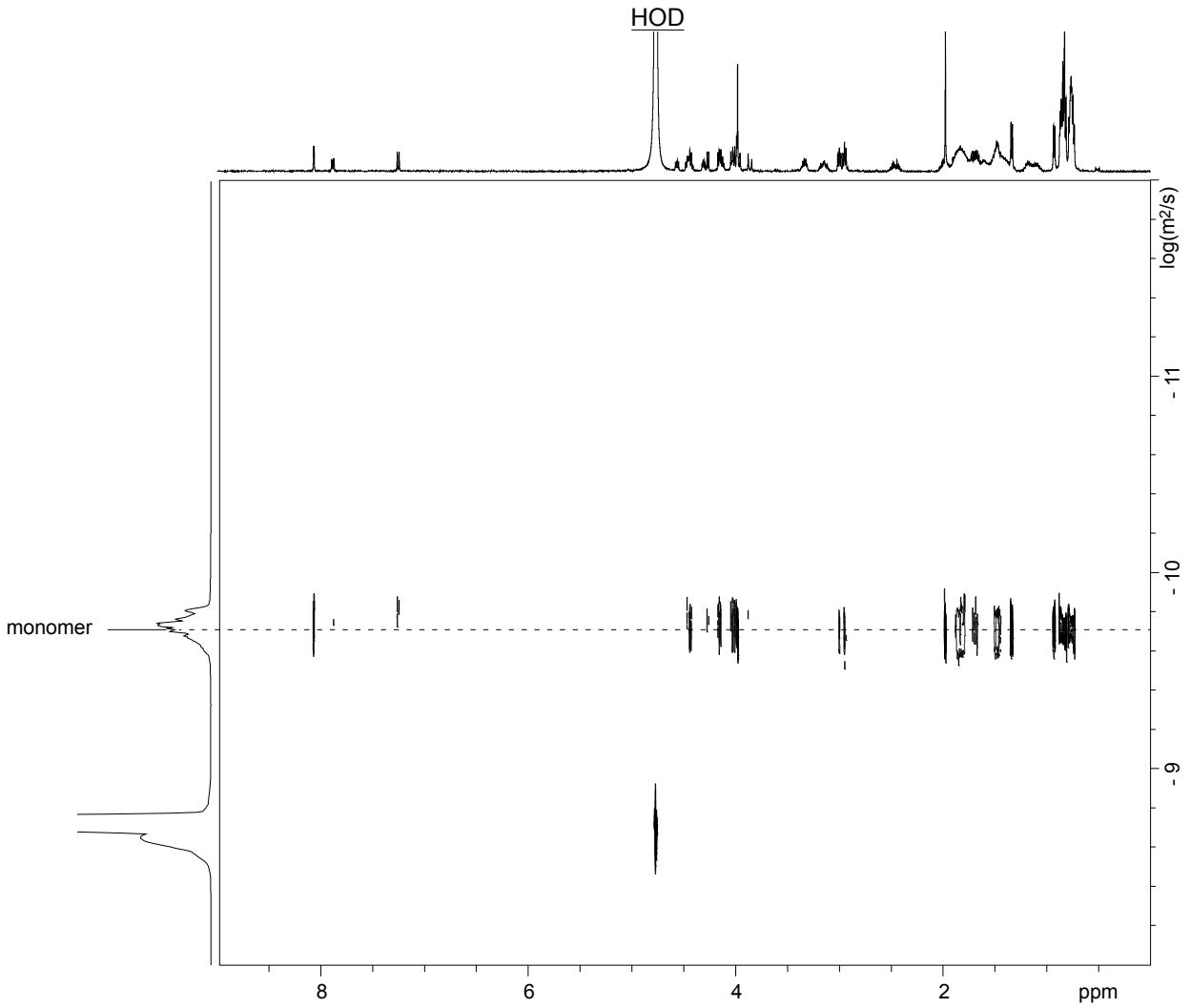


^{1}H NMR 2D ROESY of macrocycle **1b** with presaturation suppression of the HOD peak
1 mM in D_2O at 600 MHz and 293 K with 200-ms spin-lock mixing time





^{1}H NMR DOSY of peptide **1b**, 1 mM in D_2O at 500 MHz and 298 K
monomer predominates



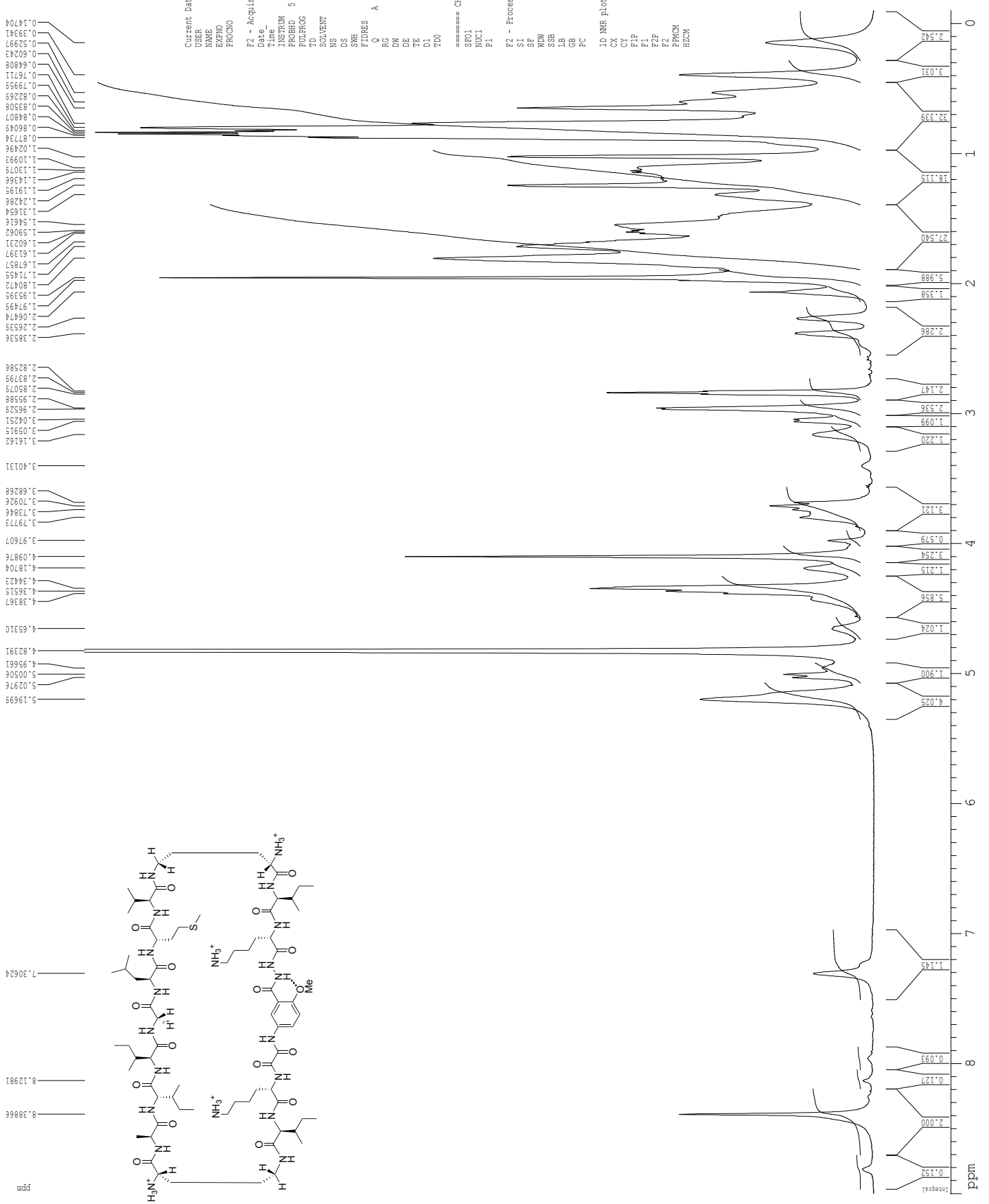
Calculations for peptide **1b** at 1.0 mM

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

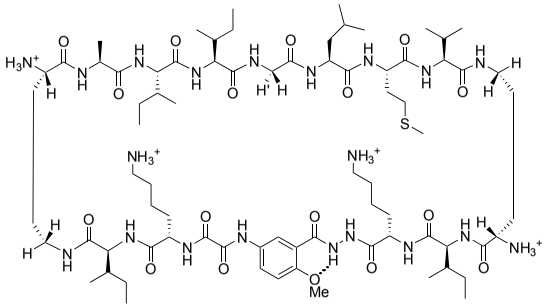
$D_{monomer}$: log(D) = -9.712; D = $10^{-9.712}$ = 19.4 ± 1.7 x 10^{-11} m^2/s

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

^{1}H NMR of peptide **1b**, 16 mM in D_2O at 600 MHz and 293 K



^{1}H NMR of peptide **1b**, 16 mM in D_2O at 600 MHz and 293 K

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NAME           NT_iv_122
EXPNO                 12
PROCNO                 1

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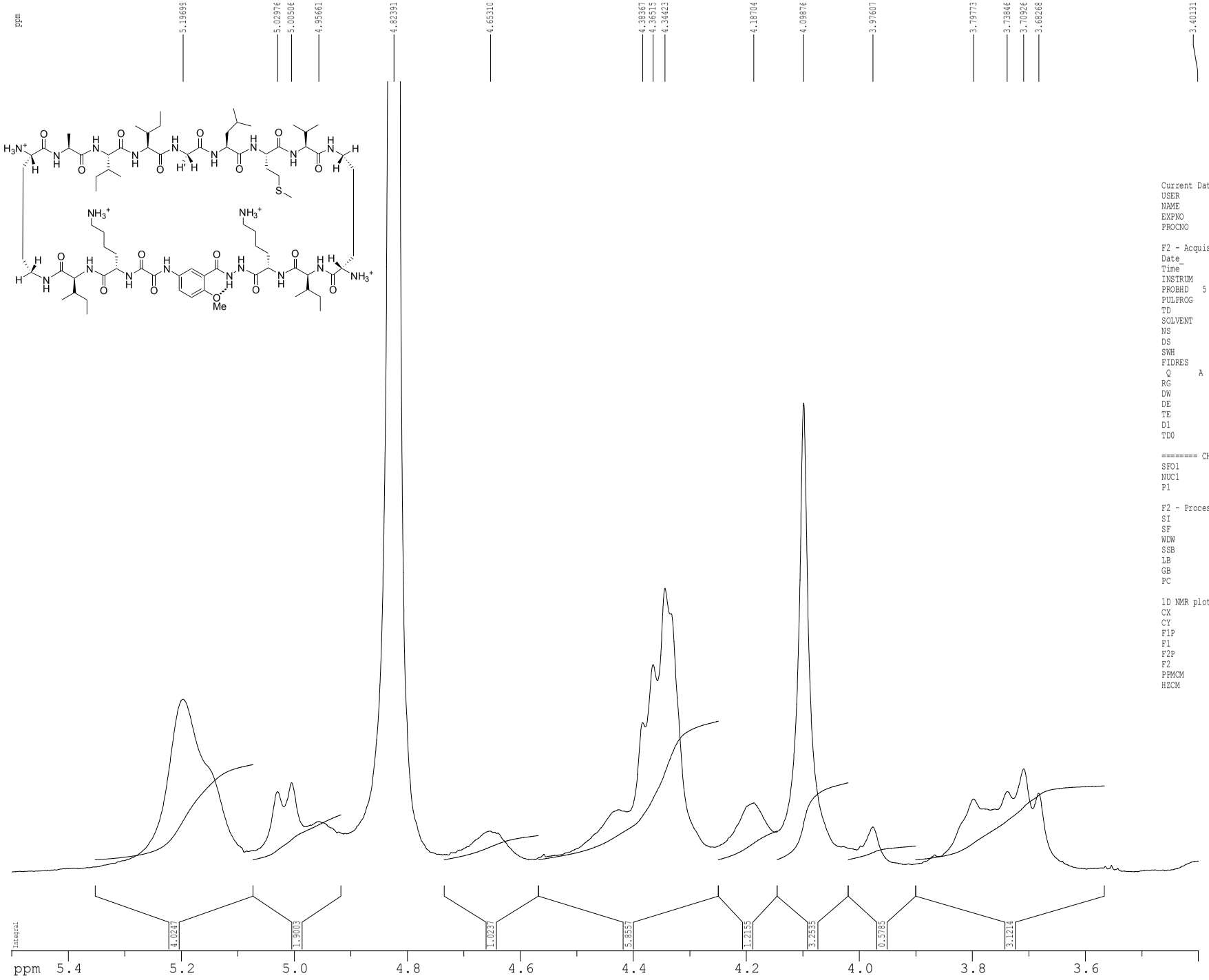
PROBHD   5 mm CPBBO BB-
PULPROG            zg30
TD                97922
SOLVENT             D2O
NS                   32
DS                    2
SWH            9615.385 Hz
FIDRES        0.098194 Hz
 Q       A    5.0919938 sec
RG                    9
DW               52.000 usec
DE               13.70 usec
TE               293.0 K
D1           0.10000000 sec
TD0                   1

======== CHANNEL f1 =======
SFO1         600.1342009 MHz
NUC1                 1H
P1               12.00 usec

F2 - Processing parameters
SI                65536
SF           600.1299883 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.00

1D NMR plot parameters
CX                22.80 cm
CY                15.00 cm
F1P               9.000 ppm
F1              5401.17 Hz
F2P               6.900 ppm
F2              4140.90 Hz
PPMCM          0.09211 ppm/cm
HZCM          55.27513 Hz/cm
```

^{1}H NMR of peptide **1b**, 16 mM in D_2O at 600 MHz and 293 K



Current Data Parameters
USER ntruex
NAME NT_iv_122
EXPNO 12
PROCNO 1

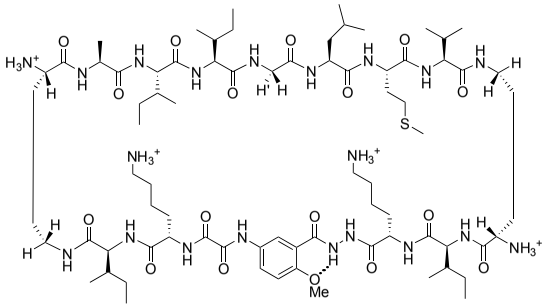
F2 - Acquisition Parameters
Date_ 20160331
Time 20.34
INSTRUM av600
PROBHD 5 mm CPBBO BB-
PULPROG zg30
TD 97922
SOLVENT D2O
NS 32
DS 2
SWH 9615.385 Hz
FIDRES 0.098194 Hz
Q A 5.0919938 sec
RG 9
DW 52.000 usec
DE 13.70 usec
TE 293.0 K
D1 0.10000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 600.1342009 MHz
NUC1 1H
P1 12.00 usec

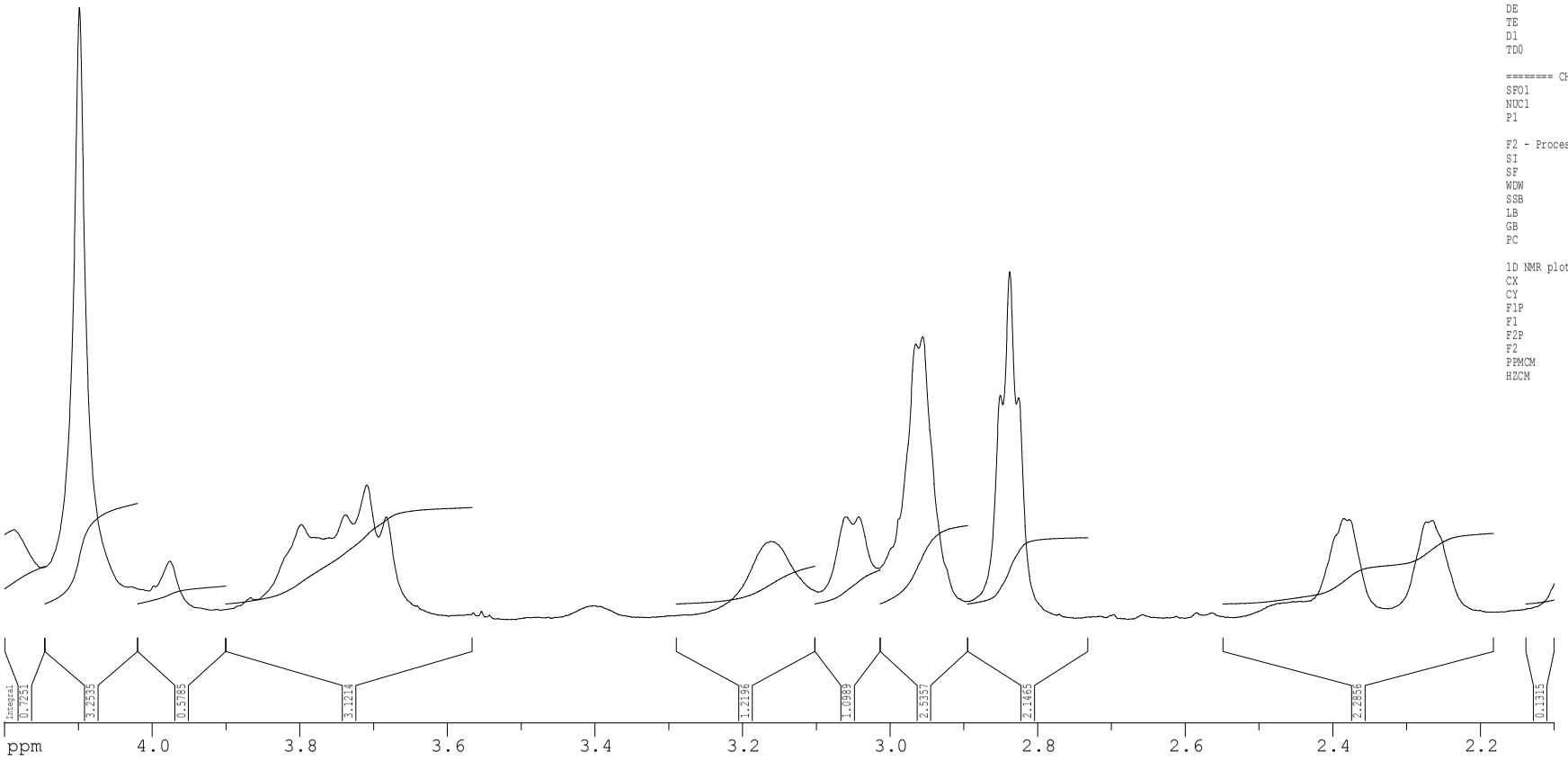
F2 - Processing parameters
SI 65536
SF 600.1299883 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 5.500 ppm
F1 3300.72 Hz
F2P 3.400 ppm
F2 2040.44 Hz
PPMCM 0.09211 ppm/cm
HZCM 55.27513 Hz/cm

^{1}H NMR of peptide **1b**, 16 mM in D_2O at 600 MHz and 293 K



89S

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Current Data Parameters
USER              ntruex
NAME           NT_iv_122
EXPNO                 12
PROCNO                 1

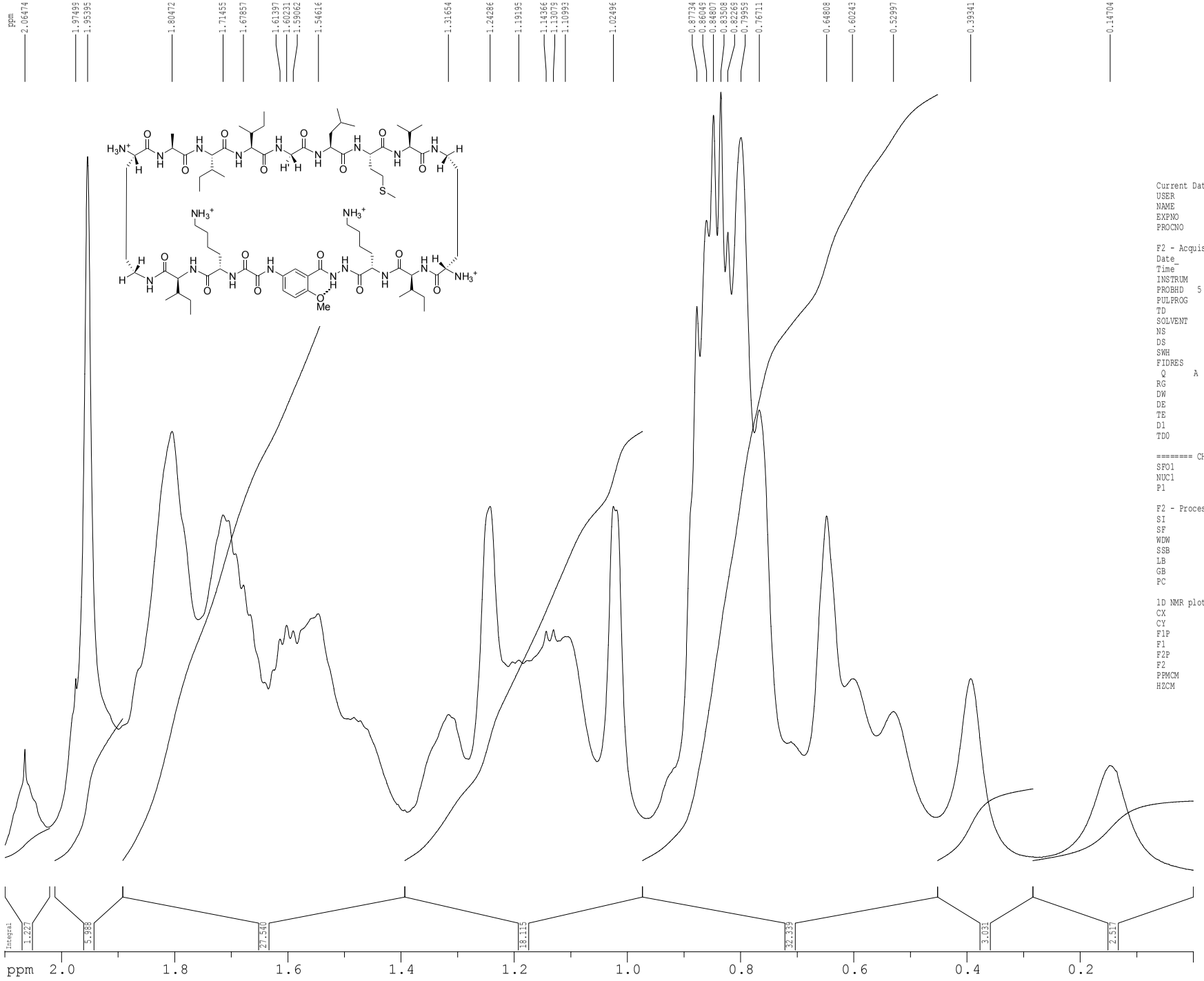
F2 - Acquisition Parameters
Date_           20160331
Time              20.34
INSTRUM           av600
PROBHD   5 mm CPBBO BB-
PULPROG            zg30
TD                97922
SOLVENT             D2O
NS                   32
DS                    2
SWH            9615.385 Hz
FIDRES        0.098194 Hz
 Q       A    5.0919938 sec
RG                    9
DW               52.000 usec
DE               13.70 usec
TE                293.0 K
D1           0.10000000 sec
TD0                   1

======== CHANNEL f1 =======
SFO1         600.1342009 MHz
NUC1                 1H
P1                12.00 usec

F2 - Processing parameters
SI                65536
SF           600.1299883 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.00

1D NMR plot parameters
CX                22.80 cm
CY                15.00 cm
F1P               4.200 ppm
F1              2520.55 Hz
F2P               2.100 ppm
F2              1260.27 Hz
PPMCM          0.09211 ppm/cm
HZCM          55.27513 Hz/cm
```

^{1}H NMR of peptide **1b**, 16 mM in D_2O at 600 MHz and 293 K



Current Data Parameters
USER ntruex
NAME NT_iv_122
EXPNO 12
PROCNO 1

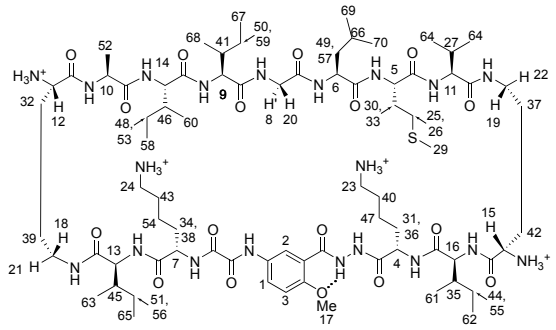
F2 - Acquisition Parameters
Date_ 20160331
Time 20.34
INSTRUM av600
PROBHD 5 mm CPBBO BB-
PULPROG zg30
TD 97922
SOLVENT D2O
NS 32
DS 2
SWH 9615.385 Hz
FIDRES 0.098194 Hz
Q A 5.0919938 sec
RG 9
DW 52.000 usec
DE 13.70 usec
TE 293.0 K
D1 0.10000000 sec
TD0 1

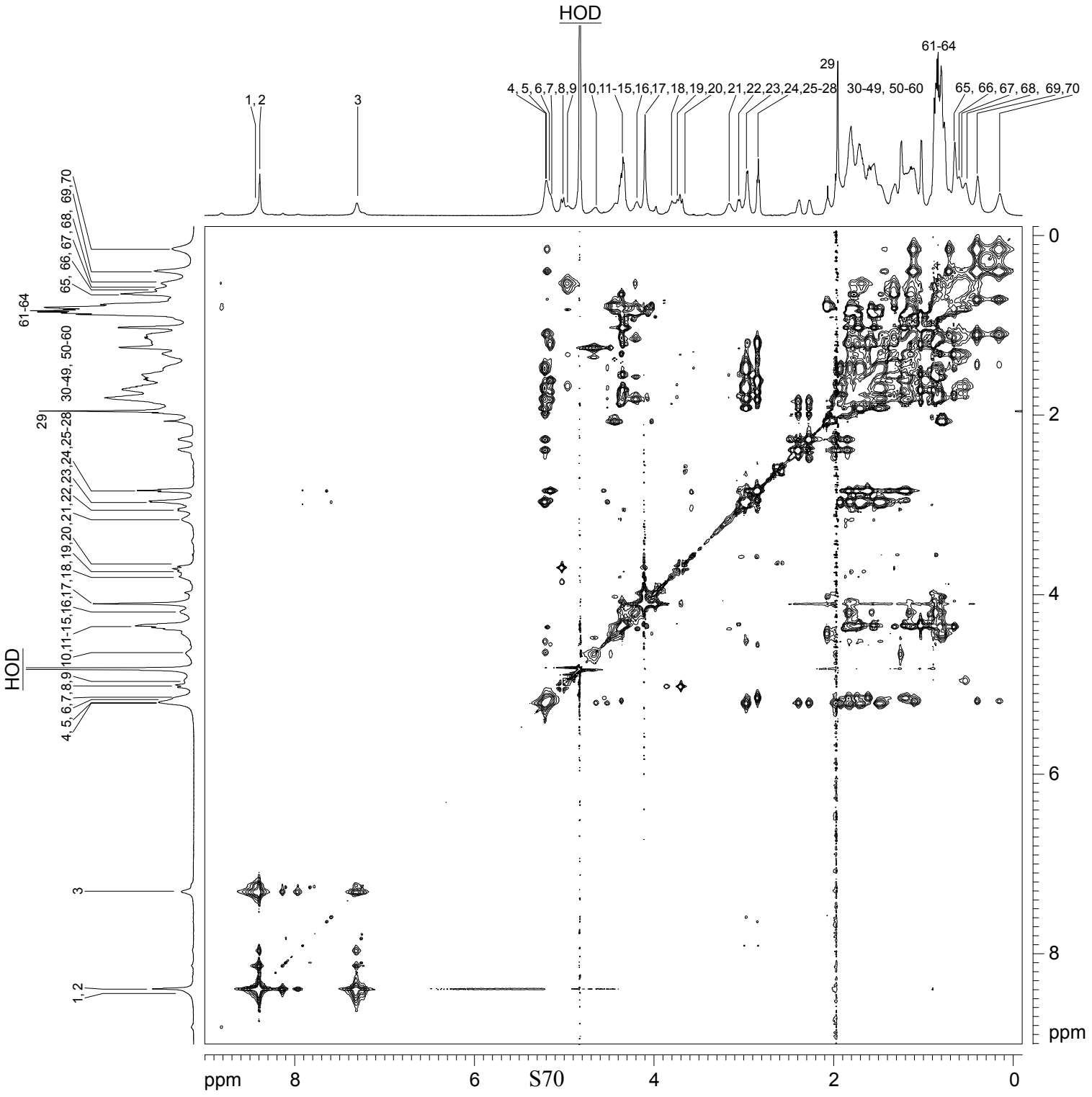
======== CHANNEL f1 ========
SFO1 600.1342009 MHz
NUC1 1H
P1 12.00 usec

F2 - Processing parameters
SI 65536
SF 600.1299883 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

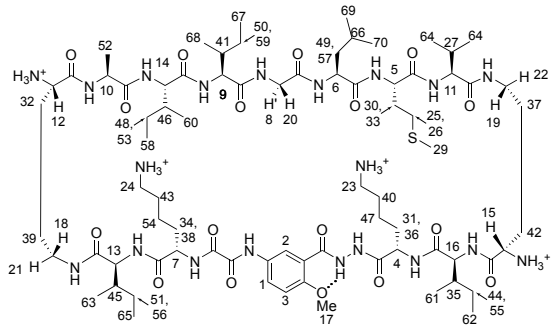
1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 2.100 ppm
F1 1260.27 Hz
F2P 0.000 ppm
F2 0.00 Hz
PPMCM 0.09211 ppm/cm
HZCM 55.27513 Hz/cm

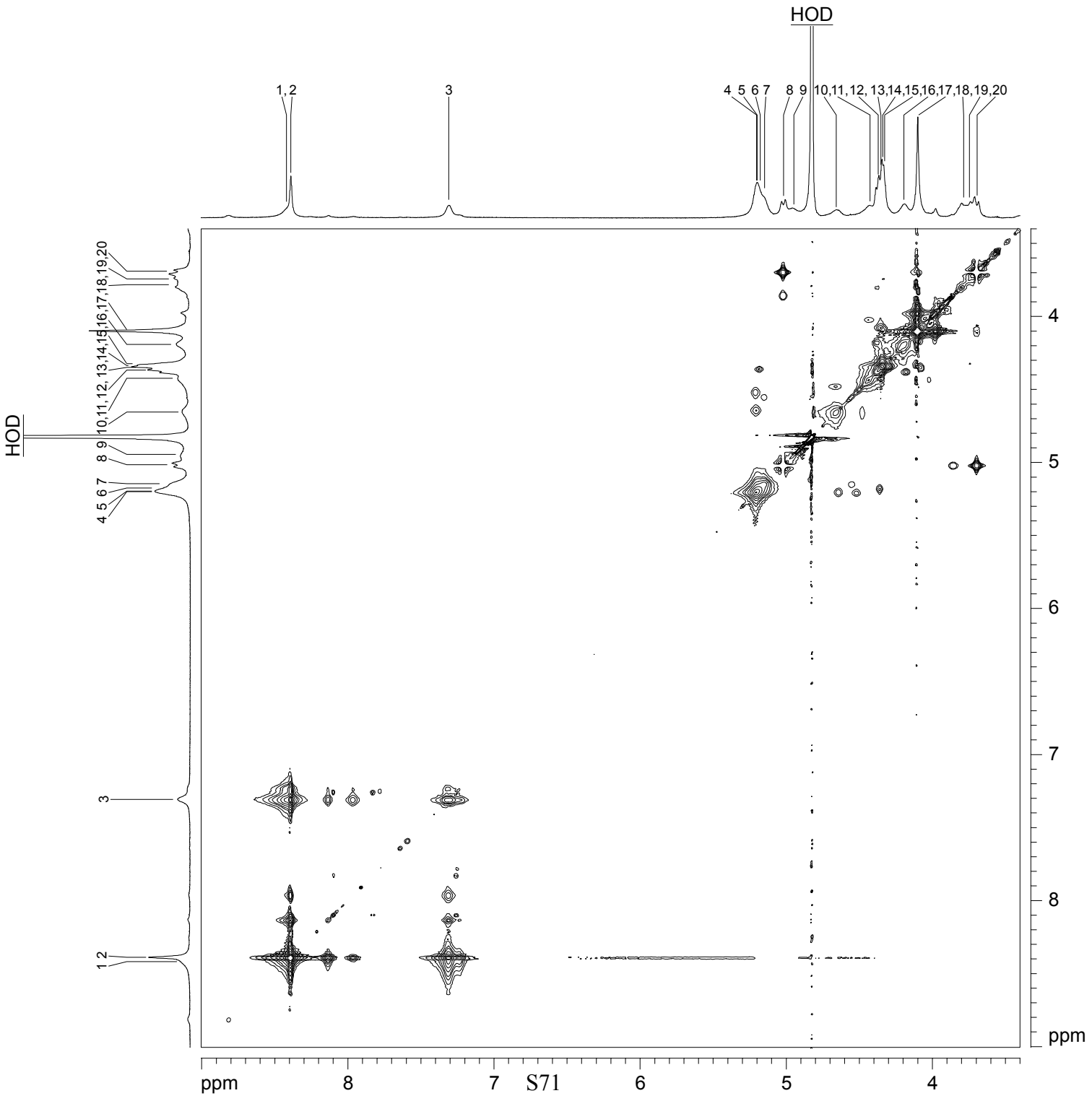
^{1}H NMR 2D TOCSY of macrocycle **1b** with presaturation suppression of the HOD peak
16 mM in D_2O at 600 MHz and 293 K with 150-ms spin-lock mixing time



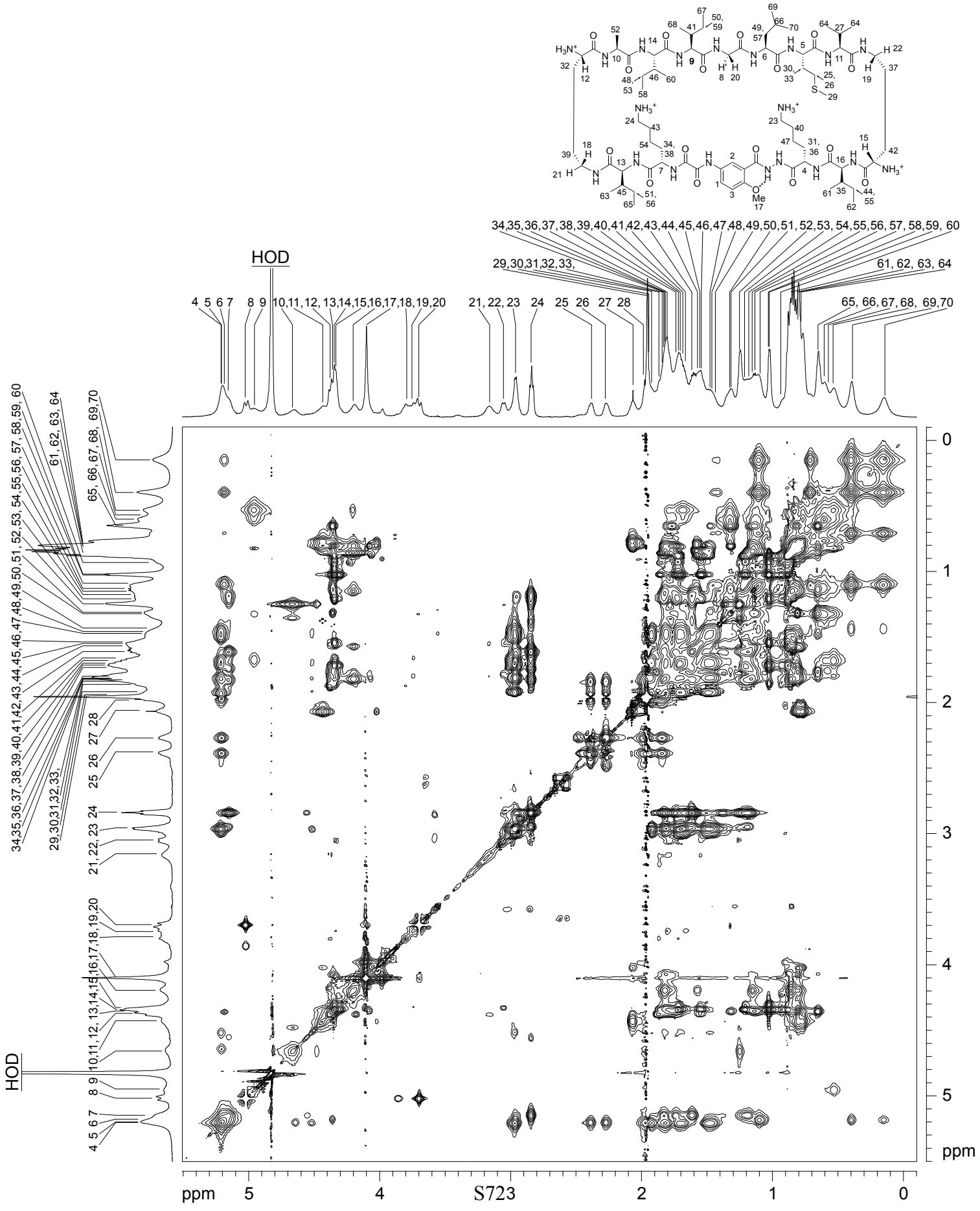


^{1}H NMR 2D TOCSY of macrocycle **1b** with presaturation suppression of the HOD peak
16 mM in D_2O at 600 MHz and 293 K with 150-ms spin-lock mixing time

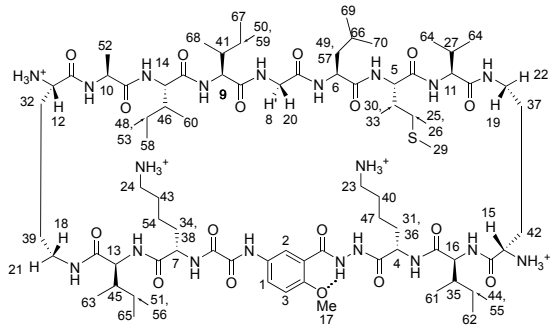


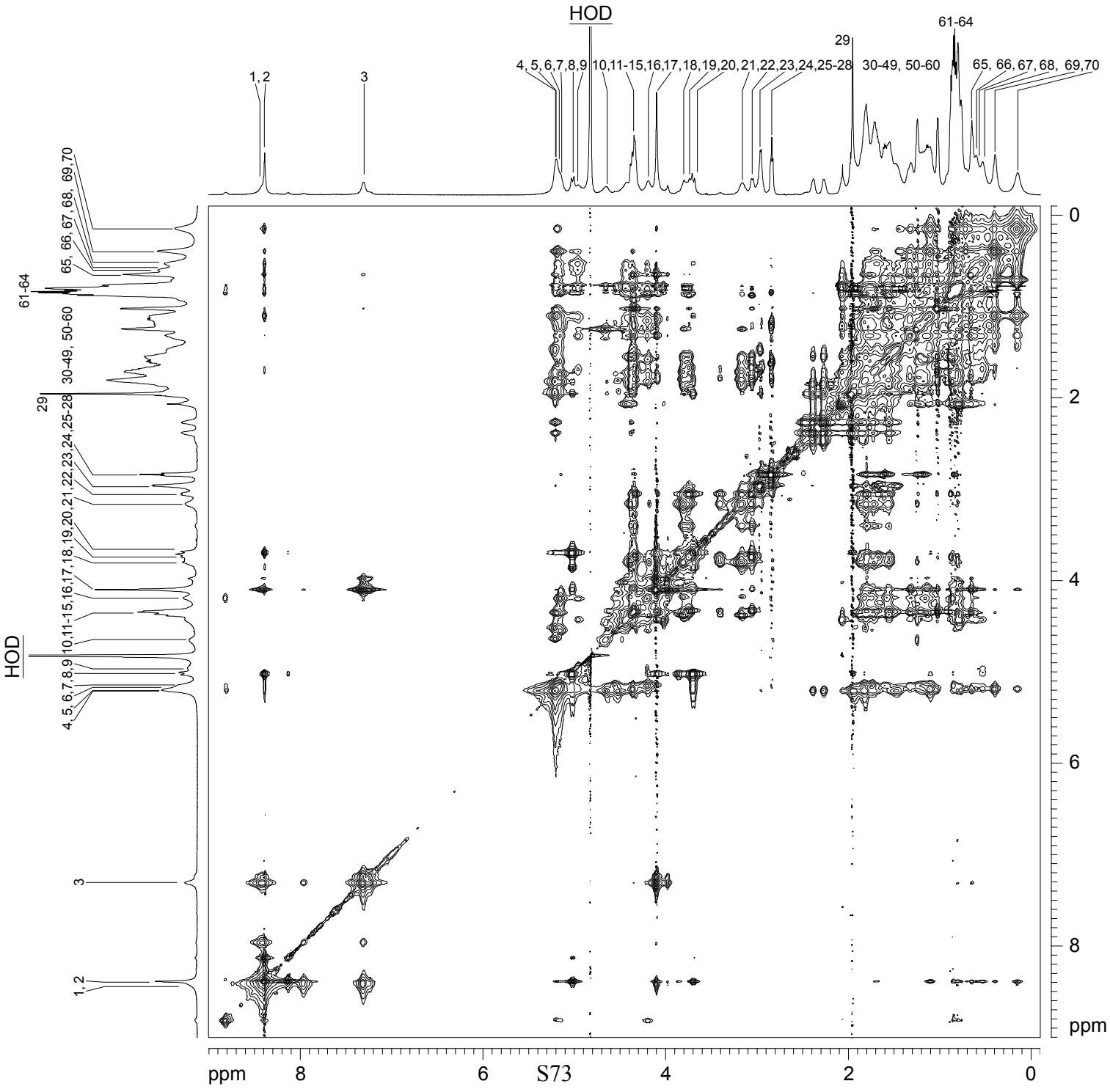


^{1}H NMR 2D TOCSY of macrocycle **1b** with presaturation suppression of the HOD peak
16 mM in D_2O at 600 MHz and 293 K with 150-ms spin-lock mixing time

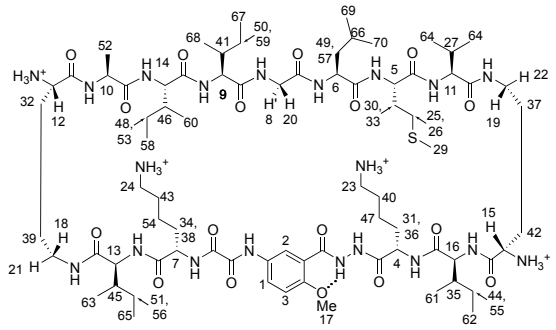


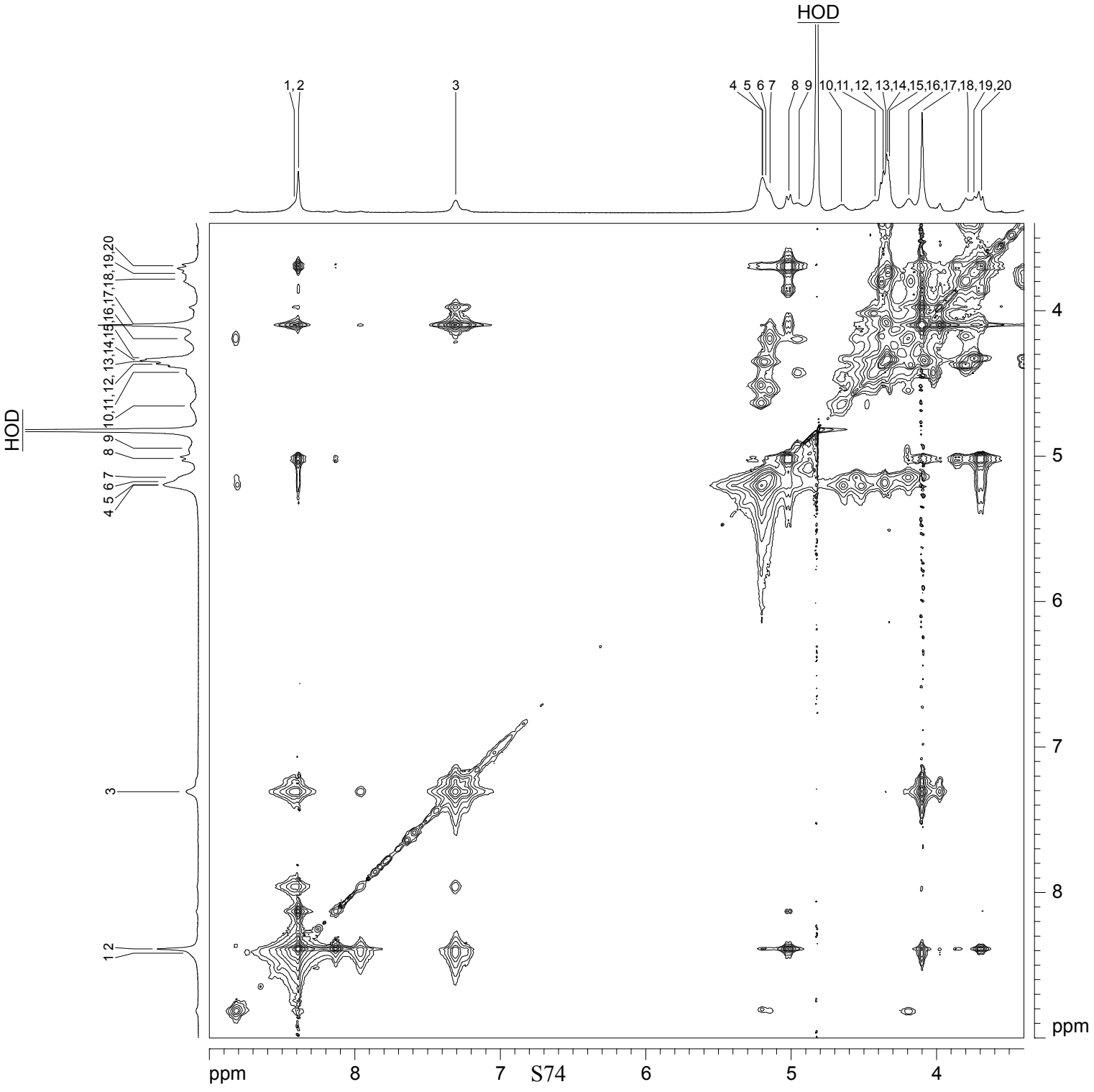
^{1}H NMR 2D NOESY of macrocycle **1b** with presaturation suppression of the HOD peak
16 mM in D_2O at 600 MHz and 293 K with 150-ms mixing time



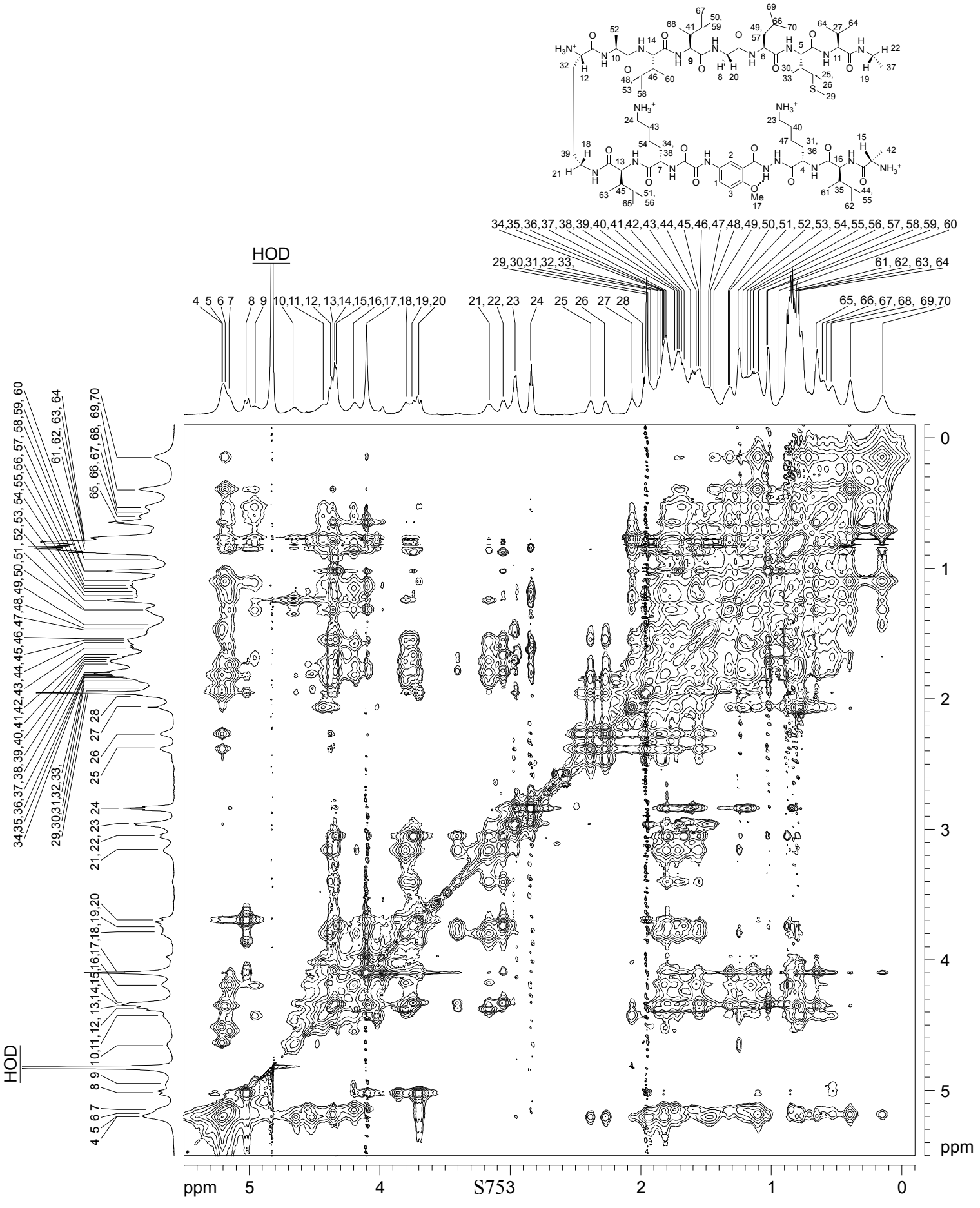


^{1}H NMR 2D NOESY of macrocycle **1b** with presaturation suppression of the HOD peak
16 mM in D_2O at 600 MHz and 293 K with 150-ms mixing time

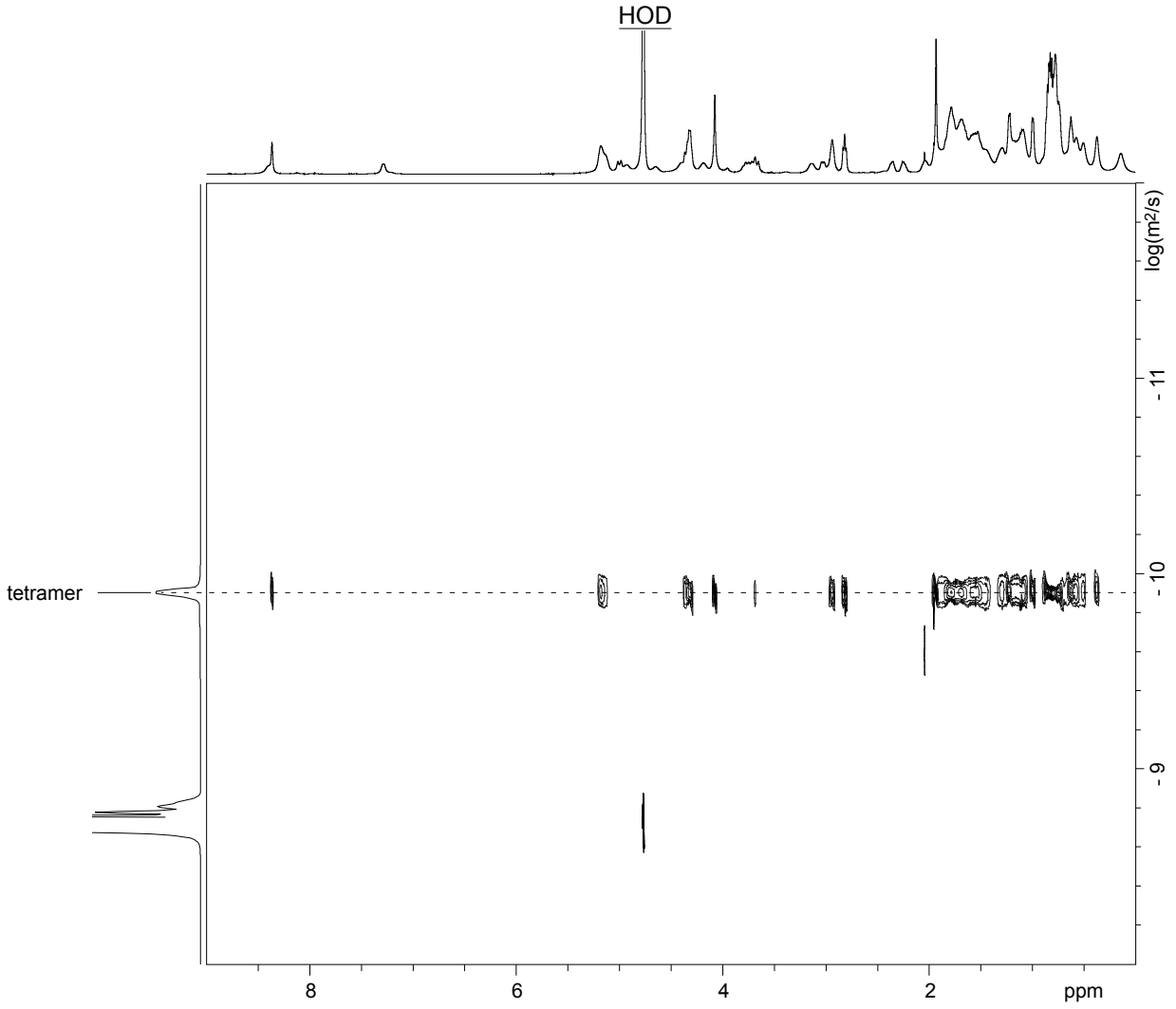




^{1}H NMR 2D NOESY of macrocycle **1b** with presaturation suppression of the HOD peak
16 mM in D_2O at 600 MHz and 293 K with 150-ms mixing time



^{1}H NMR DOSY of peptide **1b**, 16 mM in D_2O at 500 MHz and 298 K
tetramer predominates



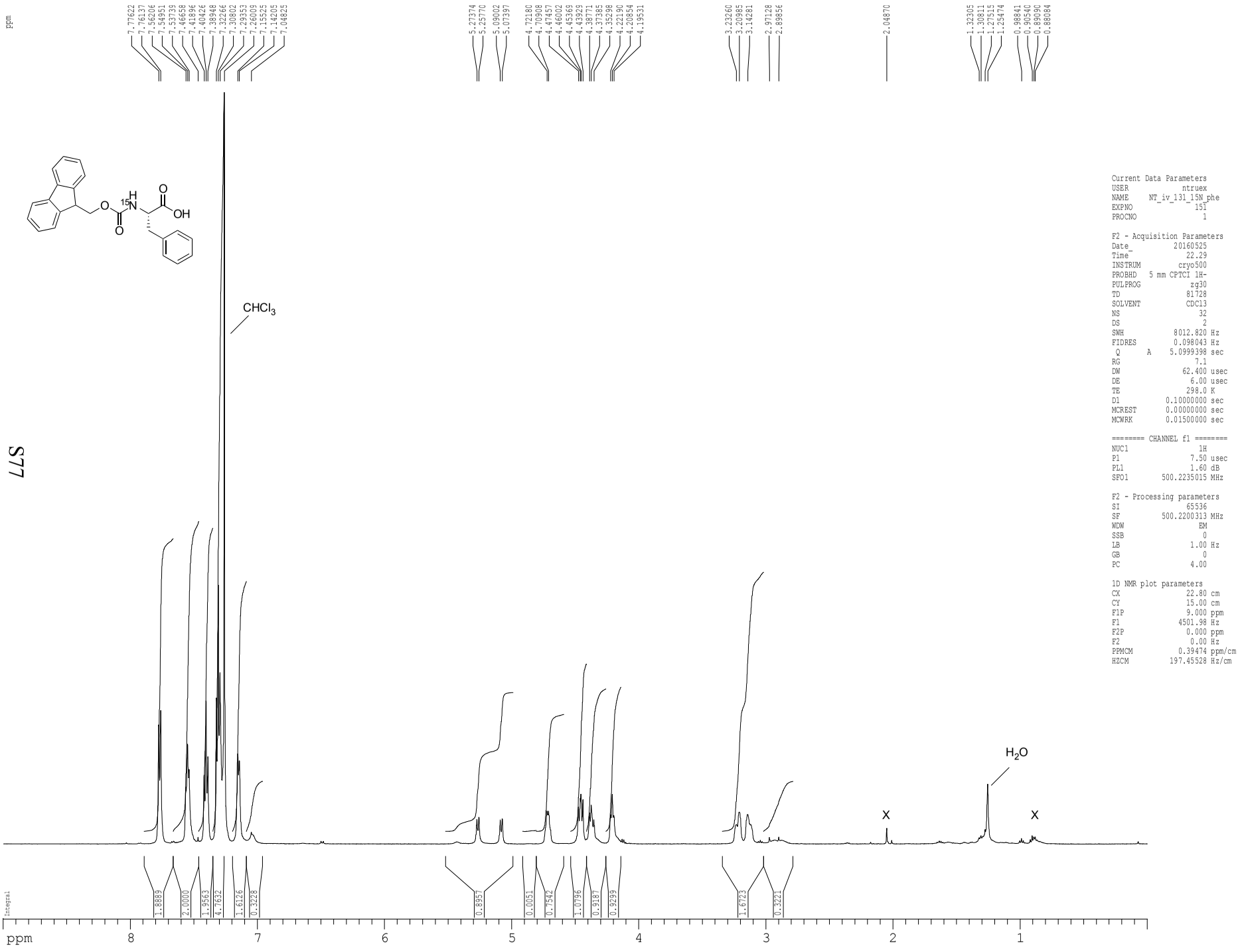
Calculations for peptide **1b** at 16.0 mM

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

$D_{tetramer}$: log(D) = -9.924; D = $10^{-9.924}$ = 11.9 ± 1.1 x 10^{-11} m^2/s

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

^{1}H NMR of Fmoc-[^{15}N]Phe-OH in $CDCl_3$ at 500 MHz and 298 K

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PROCNO                  1

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TD               81728
SOLVENT           CDCl3
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DS                    2
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FIDRES         0.098043 Hz
 Q       A     5.0999398 sec
RG                 7.1
DW              62.400 usec
DE               6.00 usec
TE              298.0 K
D1          0.10000000 sec
MCREST      0.00000000 sec
MCWRK       0.01500000 sec

======== CHANNEL f1 ========
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PL1              1.60 dB
SFO1       500.2235015 MHz

F2 - Processing parameters
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SF          500.2200313 MHz
WDW                EM
SSB                 0
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GB                  0
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1D NMR plot parameters
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CY              15.00 cm
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F1            4501.98 Hz
F2P             0.000 ppm
F2              0.00 Hz
PPMCM         0.39474 ppm/cm
HZCM        197.45528 Hz/cm
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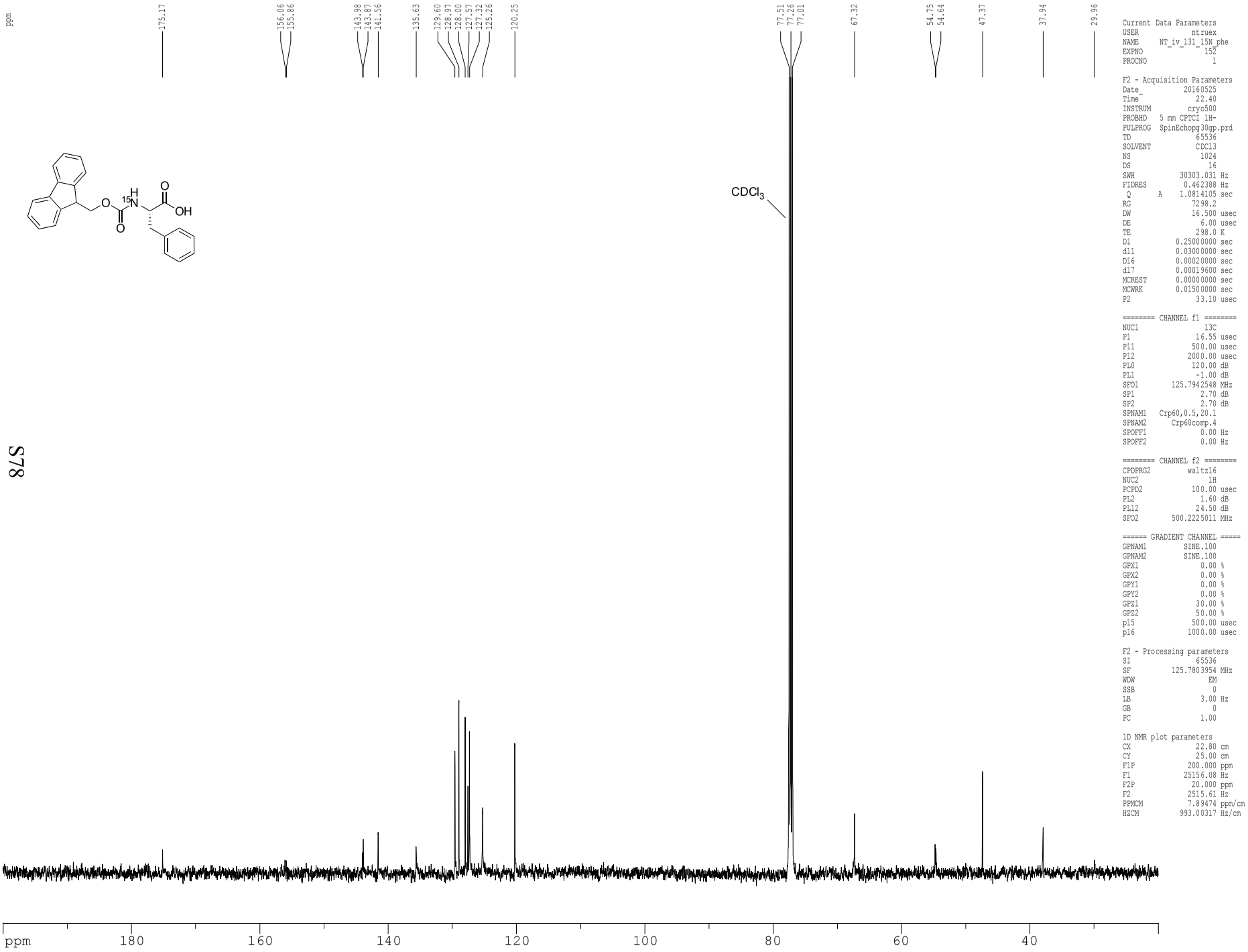
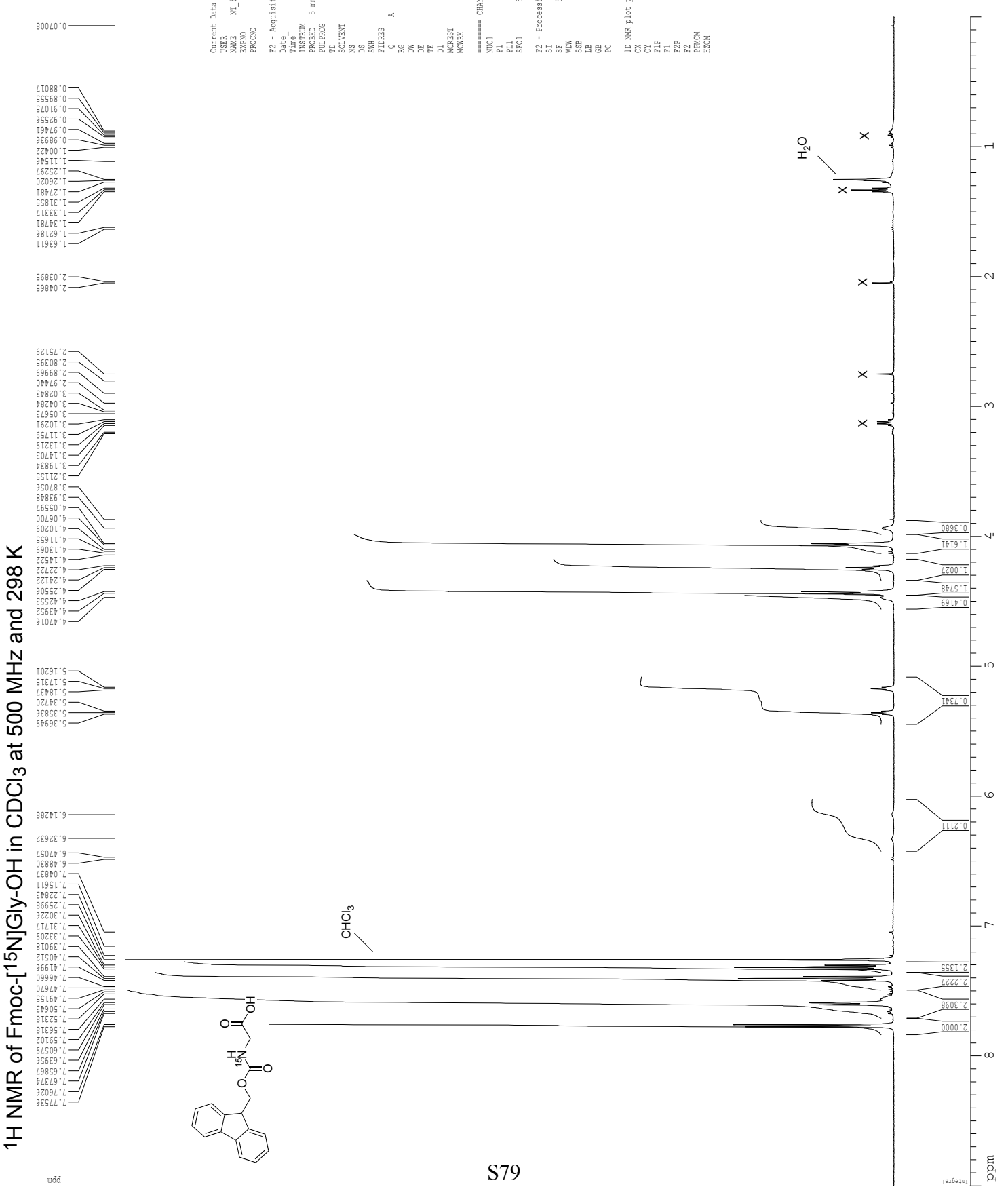
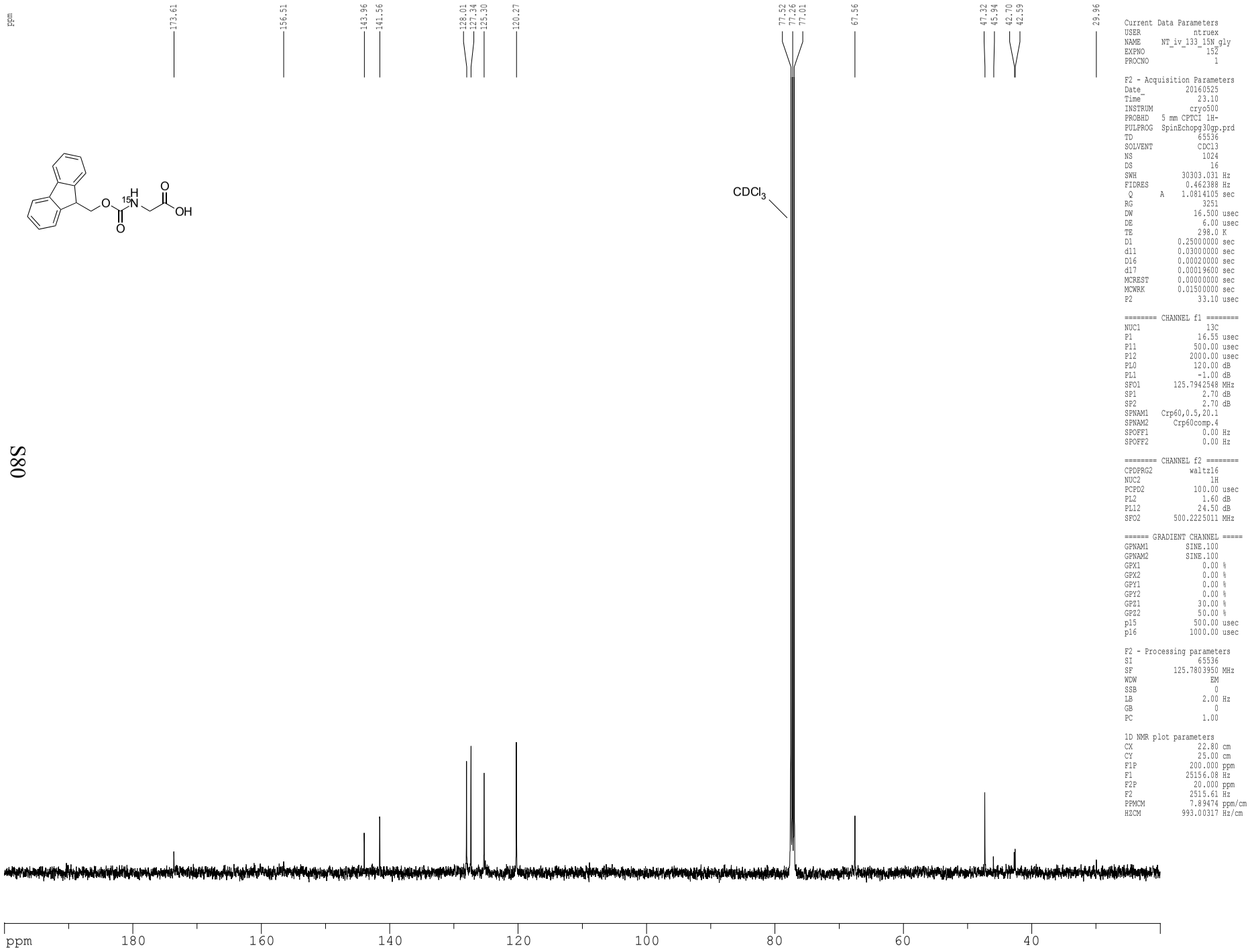
^{1}H NMR of Fmoc-[^{15}N]Phe-OH in $CDCl_3$ at 500 MHz and 298 K



^{1}H NMR of Fmoc-[^{15}N]Gly-OH in $CDCl_3$ at 500 MHz and 298 K



^{13}C NMR of Fmoc-[^{15}N]Gly-OH in $CDCl_3$ at 500 MHz and 298 K

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d17          0.00019600 sec
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P2                33.10 usec

======== CHANNEL f1 ========
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P1                16.55 usec
P11              500.00 usec
P12             2000.00 usec
PL0              120.00 dB
PL1               -1.00 dB
SFO1        125.7942548 MHz
SP1               2.70 dB
SP2               2.70 dB
SPNAM1   Crp60,0.5,20.1
SPNAM2      Crp60comp.4
SPOFF1               0.00 Hz
SPOFF2               0.00 Hz

======== CHANNEL f2 ========
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NUC2                1H
PCPD2            100.00 usec
PL2               1.60 dB
PL12             24.50 dB
SFO2        500.2225011 MHz

====== GRADIENT CHANNEL =====
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GPNAM2          SINE.100
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GPX2                0.00 %
GPY1                0.00 %
GPY2                0.00 %
GPZ1               30.00 %
GPZ2               50.00 %
p15              500.00 usec
p16             1000.00 usec

F2 - Processing parameters
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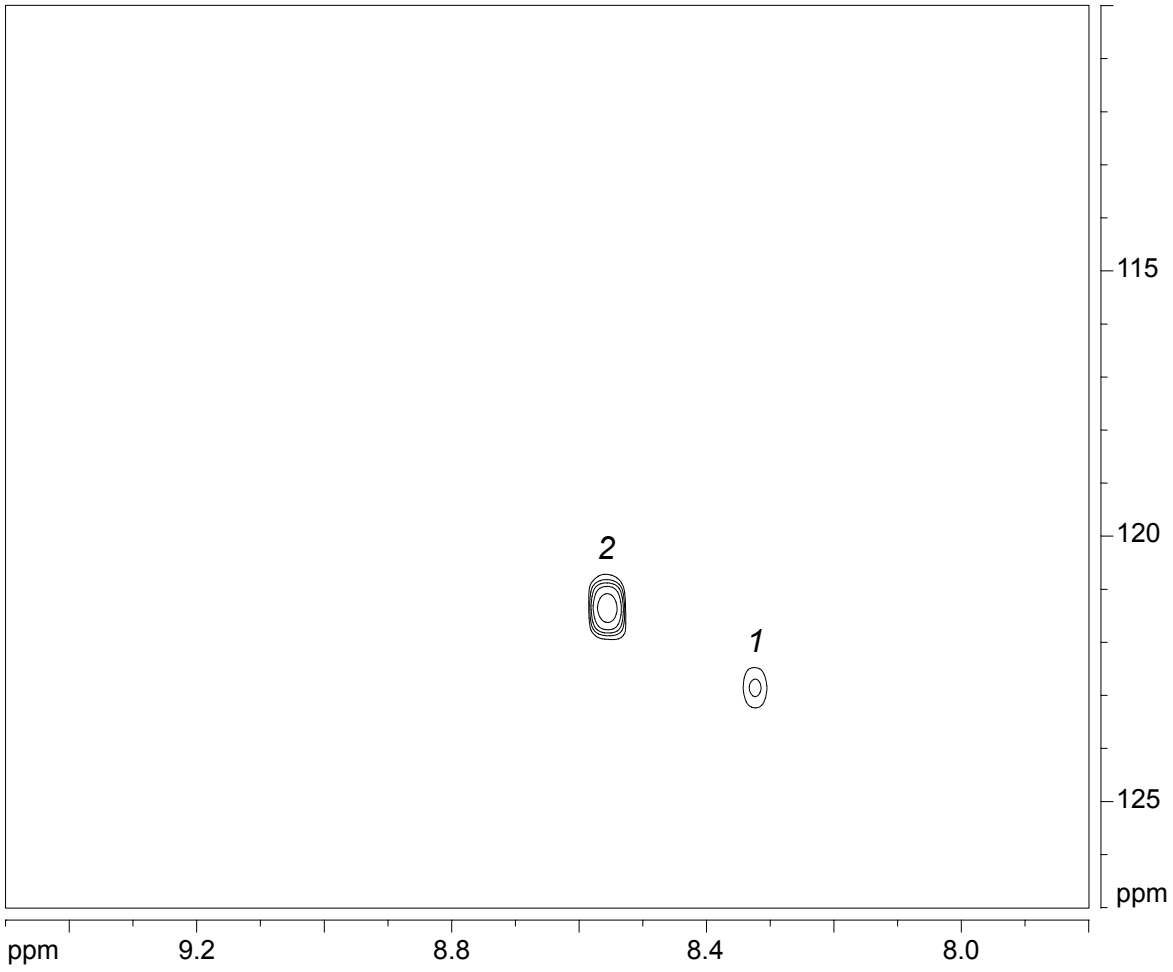
WDW                   EM
SSB                    0
LB                 2.00 Hz
GB                     0
PC                 1.00

1D NMR plot parameters
CX                22.80 cm
CY                25.00 cm
F1P             200.000 ppm
F1             25156.08 Hz
F2P              20.000 ppm
F2              2515.61 Hz
PPMCM           7.89474 ppm/cm
HZCM          993.00317 Hz/cm
```

$^{1}H,^{15}N$ HSQC of peptide [^{15}N]**1a** in 9:1 H_2O/D_2O at 600 MHz and 293 K
8.0 mM total concentration



$^{1}H,^{15}N$ HSQC of peptide [^{15}N]**1b** in 9:1 H_2O/D_2O at 600 MHz and 293 K
8.0 mM total concentration