Supplementary Information

Synthesis and structural studies of peptides containing a mannose-derived furanoid sugar amino acid

Tushar Kanti Chakraborty,* Dipankar Koley, Rapolu Ravi
and Ajit Chand Kunwar*

Indian Institute of Chemical Technology Hyderabad – 500 007, India
chakraborty@iict.res.in; kunwar@iict.res.in

Experimental procedures

General procedures. All reactions were carried out in oven or flame-dried glassware with magnetic stirring under nitrogen atmosphere using dry, freshly distilled solvents, unless otherwise noted. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm silica gel plates with UV light, I_2, 7% ethanolic phosphomolybdic acid-heat and 2.5% ethanolic anisaldehyde (with 1% AcOH and 3.3% conc. H_2SO_4)-heat as developing agents. Silica gel finer than 200 mesh was used for flash column chromatography. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated. Melting points are uncorrected. IR spectra were recorded as thin film or KBr pellets on FT-IR spectrometre. Mass spectra were obtained under liquid secondary ion mass spectrometric (LSIMS) technique. Optical rotations were measured in $CHCl_3$ solutions with a digital polarimeter.

Table 1. ^{1}H NMR chemical shifts δ in ppm with multiplicity and coupling constants J in Hz (in parentheses) of **1** (500 MHz, $CDCl_3$, 303 K).

Residue / Protons	Maa-1	Phe-2	Leu-3
NH	4.87 (dd) (5.0,7.2)	7.12 (d)	6.17 (d) (8.2)
CαH/C6H (pro-*S*)	3.37 (ddd) (5.0,6.3,14.0)	4.56 (ddd)	4.52 (m)
Cα'H/C6 H (pro-*R*)	3.27 (ddd) (5.8,7.2,14.0)		
CβH/C5H	4.03 (ddd) (2.5,5.8,6.3)	2.99 (dd) (6.3,14.0)	1.56 (m)
Cβ'H/C5'H		2.79 (dd) (7.3,14.0)	1.46 (m)
CγH/C4H	3.80 (dd) (1.6,2.5)		1.56/1.46 (m)
CδH/C3H	4.38 (bs)		0.90 (d) (6.3)
CδH/C3H	4.38 (bs)		0.90 (d) (6.3)
C2H	4.54 (bs)		
Others – aromatic region: 7.37-7.10 (15H), all -OCH_2s: 4.68,4.54,4.39,4.35, -OCH_3: 3.69 (s, 3H), Boc: 1.46			

Table 2. ^{1}H NMR chemical shifts δ in ppm with multiplicity and coupling constants J in Hz (in parentheses) of **2** (500 MHz, $CDCl_3$, 303 K).

Residue / Protons	Maa-1	Phe-2	Leu-3	Maa-4	Phe-5	Leu-6
NH	4.98 (dd) (6.7,8.2)	7.21 (d)	6.33 (d) (*J*=8.2Hz)	6.50 (d) (6.7,8.2)	7.32 (d)	6.14 (d) (8.5)
CαH/C6H (pro-*S*)	3.30 (ddd)	4.45 (ddd)	4.31 (m)	3.57 (ddd) (3.9,6.7,13.8)	4.56 (ddd)	4.48 (m)
Cα'H/C6H (pro-*R*)	3.24 (ddd) (5.1,8.2,13.8)			3.22 (ddd) (4.8,8.2,13.8)		
CβH/C5H	4.0 (m)	2.94 (dd) (6.5,13.6)	1.70 (m)	4.02 (m)	3.01 (dd)	1.55 (m)
Cβ'H/C5'H		2.81(dd) (7.2,13.6)	1.47 (m)		2.86 (dd) (7.4,13.6)	1.45 (m)
CγH/C4H	3.77 (m)		1.47 (m)	3.77 (m)		1.45 (m)
CδH/C3H	4.34 (bs)		0.86 (d) (6.1)	4.32 (bs)		0.87 (d) (6.1)
Cδ'H/C3'H			0.86 (d) (6.1)			0.87 (d) (6.1)
C2H	4.51 (bs)			4.51 (s)		
Others – aromatic region: 7.35-7.07 (30H), All -OCH_2s: 4.69, 4.63, 4.53, 4.47, -OCH_3: 3.65 (s, 3H), Boc: 1.45 (9H)						

Table 3. ^{1}H NMR chemical shifts δ in ppm with multiplicity and coupling constants J in Hz (in parentheses) of **3** (600 MHz, $CDCl_3$, 283 K).

Residue / Protons	Maa-1	Phe-2	Leu-3	Maa-4	Phe-5	Leu-6	Maa-7	Phe-8	Leu-9
NH	6.11 (dd) (5.2,7.7)	7.38 (d) (10.5)	8.16 (d) (9.4)	8.54 (d) (10.2)	8.25 (d) (11.0)	9.07 (d) (9.2)	8.37 (d) (10.2)	8.12 (d) (10.6)	8.48 (d) (9.8)
CαH/C6H (pro-*S*)	3.28 (ddd) (3.0,7.7,14.5)	5.19 (ddd) (5.0,9.8,10.5)	5.06 (ddd)	3.82 (m)	5.24 (ddd)	5.14 (ddd)	3.82 (m)	5.14 (ddd)	4.78 (ddd)
Cα'H/C6H (pro-*R*)	3.21 (ddd) (5.2,12.0,14.5)			3.14 (m)			3.11 (m)		
CβH/C5H	4.04 (m)	3.12 (dd) (5.0,14.1)	1.38 (m)	4.12 (m)	2.86 (m)	1.71 (m)	4.05 (m)	2.95 (dd)	1.37 (m)
Cβ'H/C5'H		2.62 (dd) (9.8,14.1)	1.29 (m)		2.86 (m)	1.71 (m)		2.78 (m)	1.37 (m)
CγH/C4H	3.73 (m)		1.27 (m)	3.70 (m)		1.64 (m)	3.72 (m)		1.31 (m)
CδH/C3H	4.44 (bs)		0.61 (d) (6.5)	4.63 (bs)		0.89 (d) (6.5)	4.58 (bs)		0.88 (d) (6.8)
Cδ'H/C3'H			0.59 (d) (6.5)			0.79 (d) (6.5)			0.66 (d) (6.5)
C2H	4.51 (bs)			4.92 (s)			5.04 (bs)		
Others – aromatic region: 7.45-6.87 (45H), All OCH_2: 4.91-4.88 (3H), 4.66-4.53 (5H), 4.33 (2H), 4.26 (2H), OCH_3: 3.46 (s, 3H), Boc: 1.44 (9H)									

Table 4. ^{1}H NMR chemical shifts δ in ppm with multiplicity and coupling constants J in Hz (in parentheses) of **4** (750 MHz, $CDCl_3$, 278 K).

Residue / Protons	Maa-1	Phe-2	Leu-3	Maa-4	Phe-5	Leu-6	Maa-7	Phe-8	Leu-9	Maa-10	Phe-11	Leu-12
NH	6.03 (dd) (5.2,7.7)	7.30 (d)	8.07 (d) (9.3)	8.47 (d) (10.1)	8.15 (d) (10.7)	8.90 (d) (8.8)	8.40 (d) (10.0)	8.17 (d) (10.7)	9.07 (d) (8.9)	8.27 (d) (10.2)	8.04 (d) (10.5)	8.42 (d) (10.5)
CαH/C6H (pro-*S*)	3.21 (m)	5.10 (ddd)	4.98 (ddd)	3.73 (m)	5.16 (ddd)	4.83 (ddd)	3.72 (m)	5.15 (ddd)	5.04 (ddd)	3.72 (m)	5.06 (ddd) (5.9,9.2, 10.5)	4.71 (ddd)
Cα'H/C6H (pro-*R*)	3.12 (m)			3.06 (m)			3.04 (m)			3.02 (m)		
CβH/C5H	3.96 (m)	3.04 (dd)	1.29 (m)	4.03 (m)	2.77 (m)	1.52 (m)	4.03	2.77 (m)	1.57 (m)	3.97 (m)	2.87 (dd) (9.2,14.3)	1.28 (m)
Cβ'H/C5'H		2.54 (dd) (6.2,14.3)	1.20 (m)		2.77 (m)	1.42 (m)		2.77 (m)	1.54 (m)		2.70 (dd) (5.9,14.3)	1.28 (m)
CγH/C4H	3.66 (m)		1.20 (m)	3.64 (m)		1.19 (m)	3.66 (m)		1.29 (m)	3.63 (m)		1.28 (m)
CδH/C3H	4.36 (bs)		0.90 (d) (6.7)	4.54 (bs)		0.62 (d) (6.7)	4.55 (bs)		0.80 (d) (6.7)	4.50 (bs)		0.66 (d) (6.7)
Cδ'H/C3'H			0.51 (d) (6.7)			0.44 (d) (6.7)			0.67 (d) (6.7)			0.58 (d) (6.7)
C2H	4.44 (bs)			4.81 (bs)			4.83 (bs)			4.96 (bs)		
Others – aromatic region: 7.41-6.80 (60H), All -0CH_2s: 4.87-4.80 (5H), 4.55-4.53 (3H), 4.51-4.49 (1H), 4.27-4.25 (2H), 4.19-4.15 (3H), 4.09 (2H), -OCH_3: 3.39 (s, 3H), Boc: 1.37 (9H)												

NMR Studies. NMR spectra for **1**-**4** were obtained in 4-6 mM solutions in $CDCl_3$ on 500, 600 and 750 MHz spectrometers at temperatures between 278-303 K (unless otherwise stated) using tetramethylsilane (TMS) as internal reference and the chemical shifts are shown in δ scales. Multiplicities of NMR signals are designated as s (singlet), d (doublet), t (triplet), q (quartet), br (broad), m (multiplet, for unresolved lines), etc. The proton resonance assignments were carried out by using two-dimensional total correlation spectroscopy (TOCSY) and rotating frame Overhauser effect spectroscopy (ROESY) experiments. To obtain the spatial proximity of the protons ROESY experiments were used for all the compounds. ROESY experiments were performed with mixing time of 0.3 s for compounds **1** and **2**, 0.22 s for **3** and 0.2 s for **4**. For ROESY experiments a spin-locking field of about 2.5 kHz was used. The TOCSY experiments were performed with the spin locking fields of about 10 kHz and a mixing time of 0.08 s. All the experiments were carried out in the phase sensitive mode. The spectra were acquired with 2×256 or 2×192 free induction decays (FID) containing 8-32 transients with relaxation delays of 1.5 s. The two-dimensional data were processed with Gaussian apodization in both the dimensions. Information on the H-bonding in $CDCl_3$ was carried by solvent titration studies by sequentially adding the polar solvent DMSO-d_6 up to 33% v/v.

The monomer-dimer inter-conversion rate was determined from a quantitative evaluation of the ROESY spectra. Cross peaks originating from exchange between two conformers have the same sign as diagonal peaks. For **3**, at 283 K, the ratio of the intensities of the dimer diagonal-peak and its cross correlation peak with the corresponding monomer, is about 84:16 (average of 8 distinct amide protons) (mixing time of 220 ms in the ROESY experiment). Using the procedure,[1,2] reported in the literature, this corresponds to an exchange rate of about 0.9 Hz. For **4** in the ROESY

experiments performed at 303 K with 200 ms mixing time, the corresponding ratio is 98:2 (average of 5 distinct amide protons), which gives the monomer to dimer exchange rate of about 0.1 Hz.

Dimerization constants for 3 and 4:

The dimerization constants (K_{dim}) have been obtained from standard kinetics equations (1) and (2):[3,4]

$$[A] + [A] \underset{}{\overset{K_{dim}}{\rightleftharpoons}} [B] \qquad (1)$$

$$K_{dim} = \frac{[B]}{[A]^2} \qquad (2)$$

We performed the experiments for **3** on a 4 mM solution in $CDCl_3$ at 283 K, where as for **4**, 6 mM solution at 278 K was studied.

Dimerization constant for 3. From the ^{1}H NMR spectrum of **3**, in a 4 mM solution in $CDCl_3$ at 283 K, the ratio of the minor (monomer) and major (dimer) species was found to be about 40:60.

Monomer concentration = 40% of 4 mM = 1.6 mM

Dimer concentration = (60% of 4 mM)/2 = 1.2 mM

$$K_{dim} = \frac{1.2X10^{-3}}{[1.6X10^{-3}]^2}$$

$$K_{dim} = 4.7X10^{2}\ M^{-1}$$

Dimerization constant for 4. In the ^{1}H NMR spectrum of **4**, in a 6 mM solution at 303 K, the ratio of the minor (monomer) and major (dimer) species is about 5:95.

Monomer concentration = 5% of 6 mM = 0.3 mM

Dimer concentration = [95% of 6 mM]/2 = 2.85 mM

$$K_{dim} = \frac{2.85x10^{-3}}{[0.3x10^{-3}]^2}$$

$$K_{dim} = 3.2x10^4\ M^{-1}$$

It is worth mentioning that we could only obtain the upper limit of 5% for the monomer concentration in **4**. Actually for **4**, K_{dim} will be $> 3.2x10^4\ M^{-1}$.

Molecular dynamics studies. Model building and molecular dynamics simulations were carried out using Insight II (97.0) / Discover1 program on a Silicon Graphics Octane and Fuel workstations using IRIX64 (6.5) operating system. The cvff force field with default parameters was used throughout the simulations. The initial minimizations were done with constraints, first with steepest decent, followed by conjugate gradient methods for a maximum of 3000 iterations each or RMS deviation of 0.001 kcal/mol, whichever was earlier. The energy-minimized structures were then subjected to MD simulations. The inter-atomic distances were obtained from the volume integrals of the ROESY spectra using two-spin approximation and the distance between the geminal protons of 1.80 Å. The upper and lower bounds of the constraints in the restraints in the MD runs were derived respectively by adding and subtracting 10% of the derived inter-atomic distances. For MD runs, a temperature of 300 K was used. The molecules were initially equilibrated for 50 ps and subsequently subjected to a 1 ns dynamics with a step size of 1 fs, sampling the trajectory at equal intervals of 10 ps. In this trajectory, 100 samples were generated and were again energy minimized without constraints, by using the above-mentioned protocol. The lowest energy structures for **3** and **4** are shown in Figure 3.

References:

[1] J. Cavanagh, W. J. Fairbrother, A. G. Palmer III, N. J. Skeleton, in *Protein NMR Spectroscopy*; Academic Press, New York, 1996.

[2] B. Reif, V. Wittmann, H. Schwalbe, C. Griesinger, K. Worner, K. Jahn-Hofmann, J. W. Engels, *Helv. Chim. Acta* **1997**, *80*, 1952-1971.

[3] C. S. Wilcox, In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H.-J. Durr, H., Eds.; VCH: New York, 1991; p 123.

[4] H.-D. Arndt, D. Bockelmann, A. Knoll, S. Lamberth, C. Griesinger, U. Koert, *Angew Chem. Int. Ed.* **2002**, *41*, 4062-4065.

Distance constraints used in MD simulation study

Table 5: Distance constraints used in MD calculations for peptide **3**, derived from ROESY experiment in $CDCl_3$ (600 MHz, 283 K).

S.No.	From	To	Intra/Inter	Lower bound (Å)	Upper bound (Å)
1	1 Maa NH	1 Maa 2H	Intra	3.632	4.437
2	2 Phe NH	1 Maa 2H	Intra	3.278	4.026
3	2 Phe NH	3 Leu NH	Intra	2.080	2.543
4	4 Maa NH	3 Leu αH	Intra	2.160	2.641
5	4 Maa NH	4 Maa 2H	Intra	3.958	4.838
6	4 Maa NH	4 Maa 5H	Intra	3.278	4.007
7	4 Maa NH	5 Phe NH	Intra	3.857	4.714
8	5 Phe NH	4 Maa 2H	Intra	3.034	3.708
9	5 Phe NH	4 Maa 5H	Intra	2.744	3.354
10	5 Phe NH	6 Leu NH	Intra	2.267	2.772
11	6 Leu NH	5 Phe αH	Intra	2.850	3.483
12	7 Maa NH	6 Leu αH	Intra	2.228	2.723
1 3	7 Maa NH	7 Maa 5H	Intra	3.327	4.067
14	7 Maa NH	8 Phe NH	Intra	3.906	4.775
15	8 Phe NH	7 Maa 2H	Intra	3.347	4.091
16	8 Phe NH	7 Maa 5H	Intra	2.906	3.551
17	8 Phe NH	9 Leu NH	Intra	2.294	2.803

18	9 Leu NH	8 Phe αH	Intra	3.644	4.453
19	Boc-	-OCH3	Inter	2.685	3.25
20	1 Maa NH	-OCH3	Inter	3.252	3.975
21	1 Phe NH	-OCH3	Inter	3.998	3.664
22	1 Maa NH	9 Leu αH	Inter	2.428	2.968
23	1 Maa 2H	8 Phe αH	Inter	3.405	4.161
24	2 Phe αH	7 Maa 2H	Inter	2.687	3.284
25	3 Leu NH	9 Leu NH	Inter	2.630	3.214
26	3 Leu NH	8 Phe NH	Inter	3.403	4.159
27	3 Leu αH	7 Maa NH	Inter	2.517	3.077
28	3 Leu αH	6 Leu αH	Inter	2.554	3.122
29	4 Maa NH	7 Maa NH	Inter	3.306	4.041
30	4 Maa NH	6 Leu αH	Inter	2.623	3.206
31	4 Maa 2H	5 Phe αH	Inter	3.050	3.728

Table 6: Distance constraints used in MD calculations for peptide **4**, derived from ROESY experiment in $CDCl_3$ (750 MHz, 278 K).

S.No.	From	To	Intra/Inter	Lower bound (Å)	Upper bound (Å)
1	1 Maa NH	1 Maa 5H	Intra	2.964	3.623
2	1 Maa NH	1 Maa 2H	Intra	3.244	3.965
3	2 Phe NH	1 Maa 2H	Intra	2.496	3.051
4	2 Phe NH	1 Maa 5H	Intra	2.412	2.948
5	2 Phe NH	3 Leu NH	Intra	2.051	2.507
6	6 Leu NH	2 PheαH	Intra	2.784	3.402
7	4 Maa NH	3 Leu αH	Intra	1.979	2.419
8	4 Maa NH	4 Maa 5H	Intra	2.920	3.569
9	4 Maa NH	4 Maa 2H	Intra	3.711	4.539
10	5 Phe NH	4 Maa 2H	Intra	2.516	3.075
11	5 Phe NH	4 Maa 5H	Intra	2.345	2.866
12	5 Phe NH	6 Leu NH	Intra	2.244	2.743
13	7 Maa NH	7 Maa 5H	Intra	2.750	3.361

14	8 Phe NH	7 Maa 2H	Intra	2.516	3.075
15	8 Phe NH	7 Maa 5H	Intra	2.345	2.866
16	8 Phe NH	9 Leu NH	Intra	2.256	2.757
17	10 Maa NH	9 Leu αH	Intra	1.976	2.415
18	10 Maa NH	10 Maa 5H	Intra	2.528	3.453
19	11 Phe NH	10 Maa 5H	Intra	2.510	3.068
20	11 Phe NH	12 Leu NH	Intra	2.850	3.484
21	12 Leu NH	11 PheαH	Intra	3.647	4.457
22	Boc	-OCH3	Inter	2.685	3.315
23	1 Maa NH	-OCH3	Inter	3.252	3.975
24	1 Phe NH	-OCH3	Inter	3.998	3.664
25	1 Maa NH	12 Leu αH	Inter	2.060	2.518
26	1 Maa 2H	11 Phe αH	Inter	2.221	2.715
27	2 Phe αH	10 Maa 2H	Inter	1.991	2.434
28	3 Leu NH	12 Leu NH	Inter	3.191	3.90
29	3 Leu αH	10 Maa NH	Inter	2.517	3.077
30	3 Leu αH	9 Leu αH	Inter	2.554	3.122
31	4 Maa NH	9 Leu αH	Inter	2.623	3.206
32	6 Leu NH	9 Leu NH	Inter	3.191	3.90

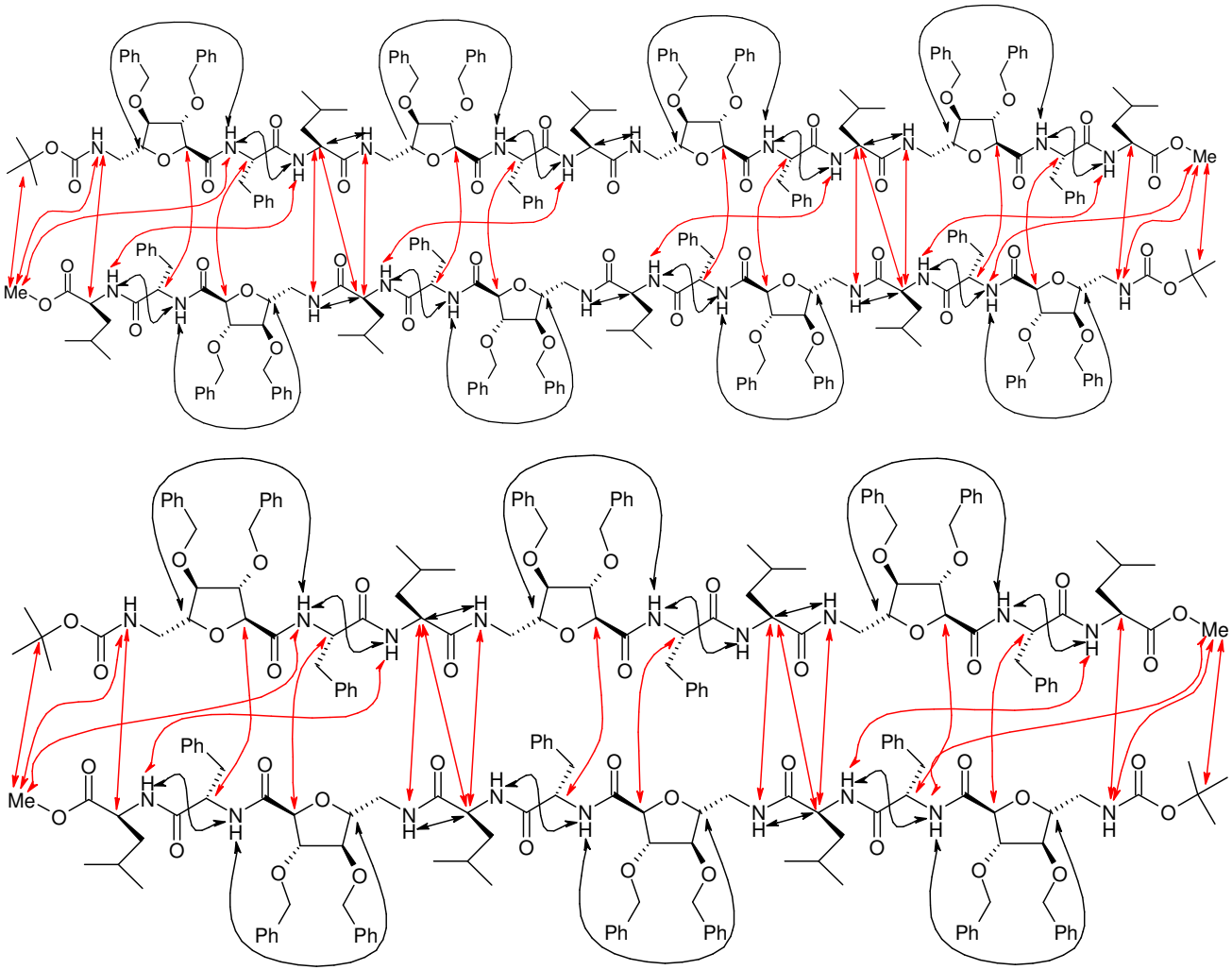


Figure 1: Characteristic inter residue nOes (red), intra residue nOes (black) are shown in the above figures for compounds **4** (top) and **3** (bottom).

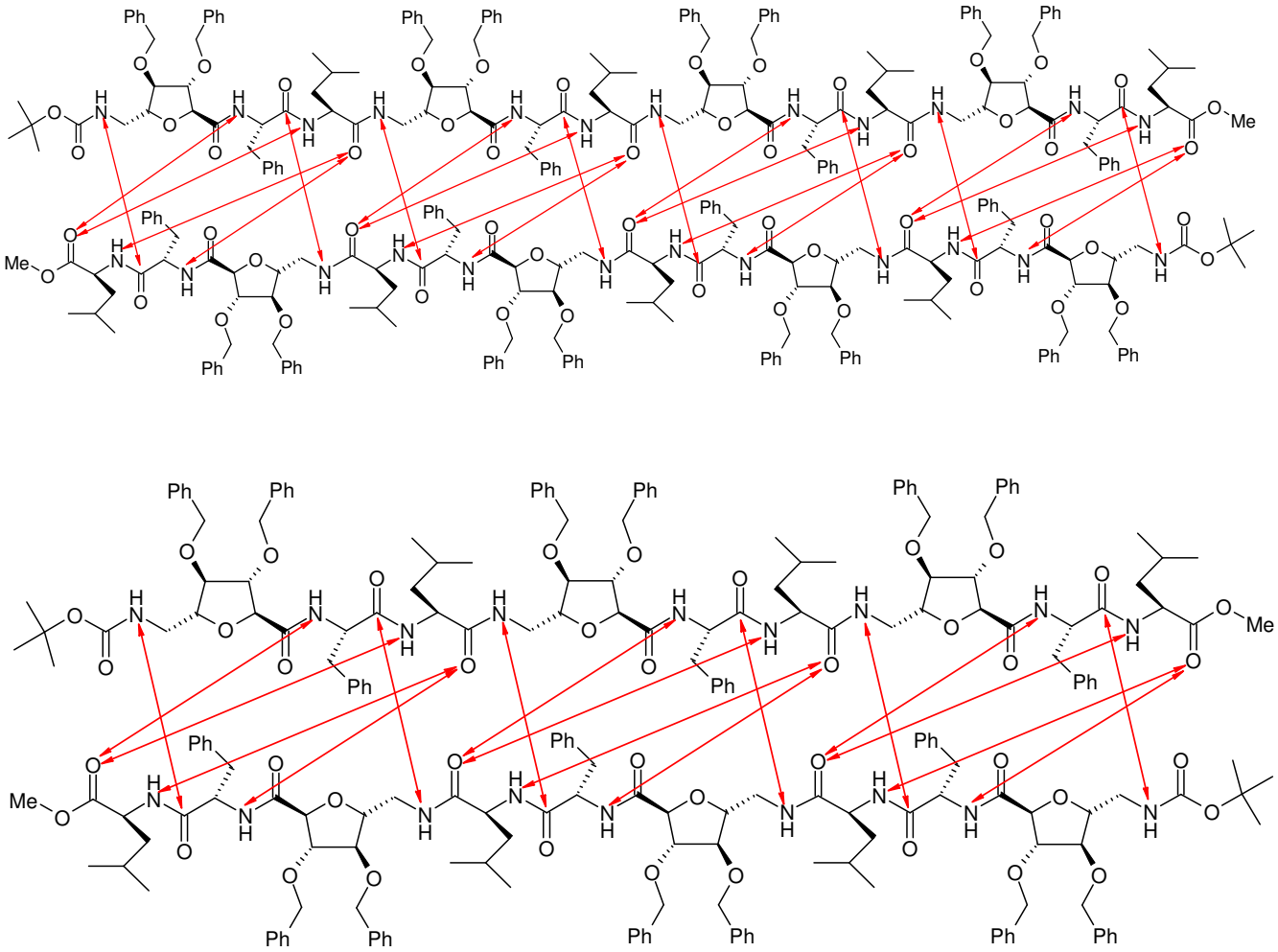


Figure 2: All H-bondings are shown in the above structure for compounds **4** (top) and **3** (bottom).

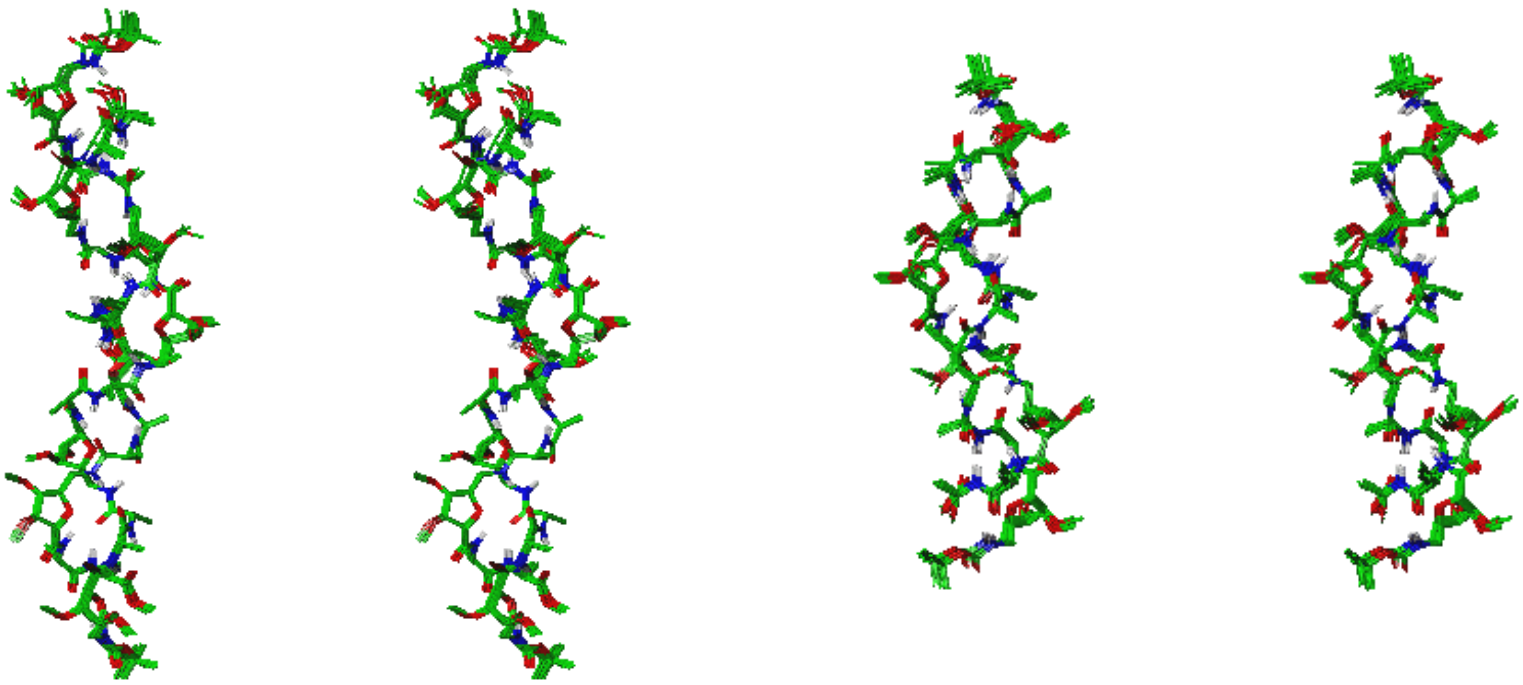
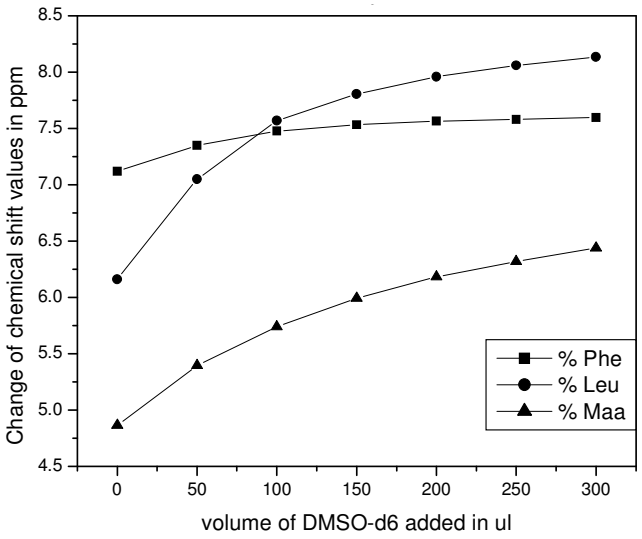
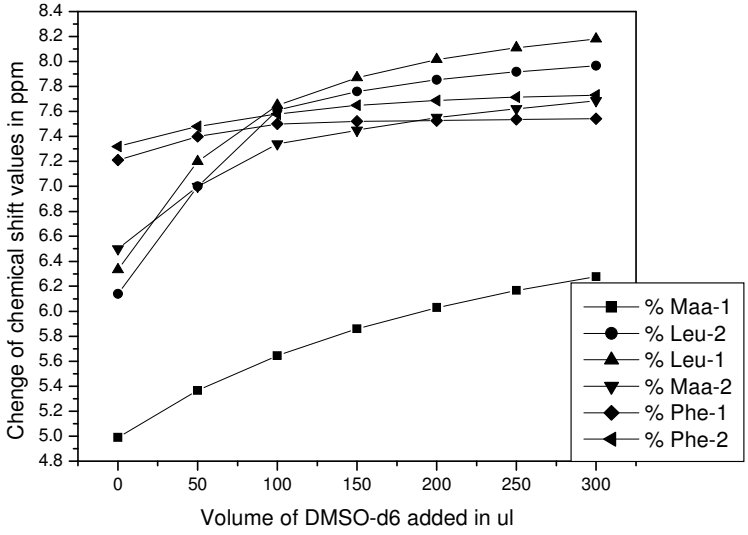


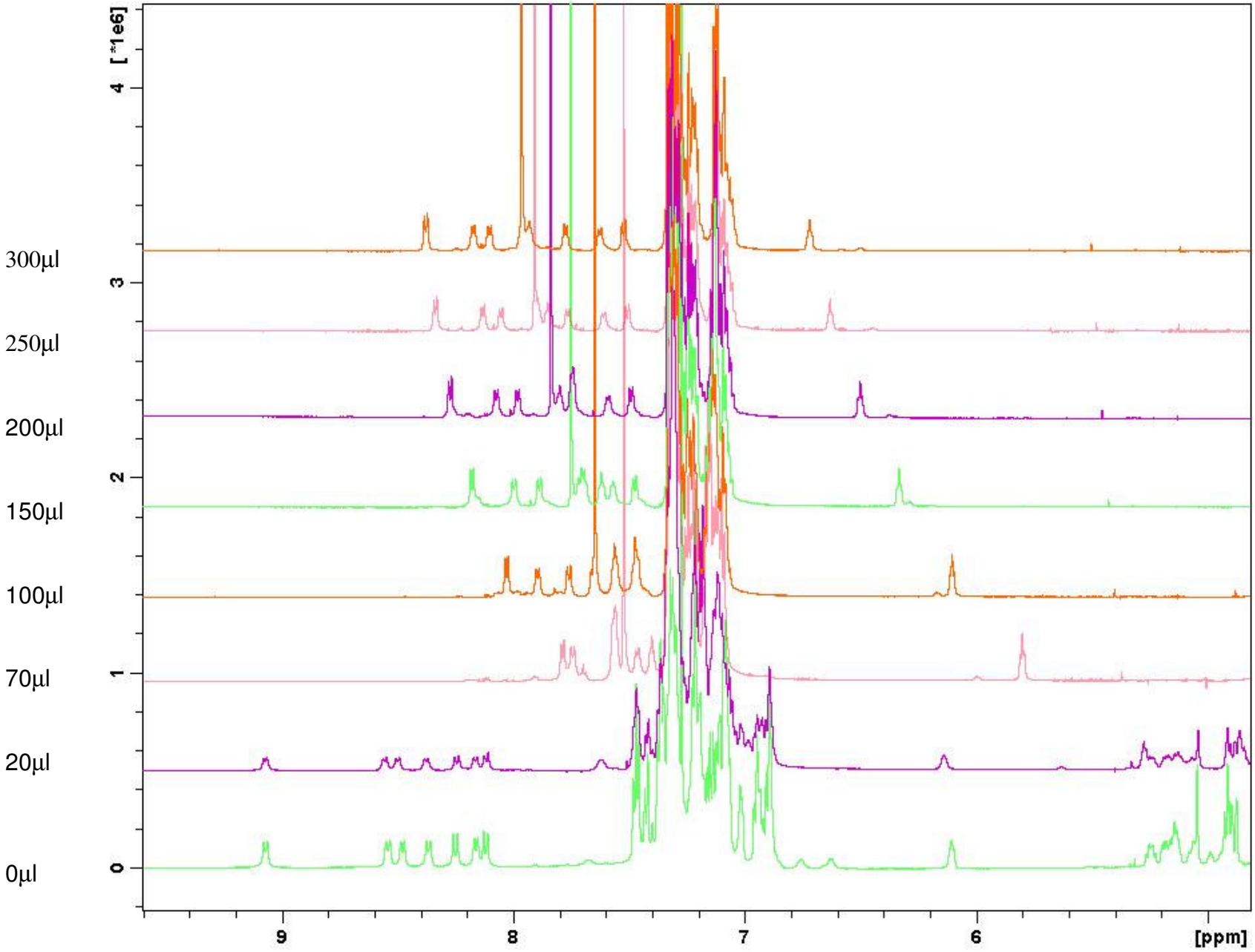
Figure 3: Stereo views of 10 lowest energy superimposed MD structures of compounds **4** (left) and **3** (right). -CH_3s are used here in place of –CH_2Phs and -$CH_2CH(CH_3)_2$s to enhance the clarities of the figures.

Titration study of compound **1**

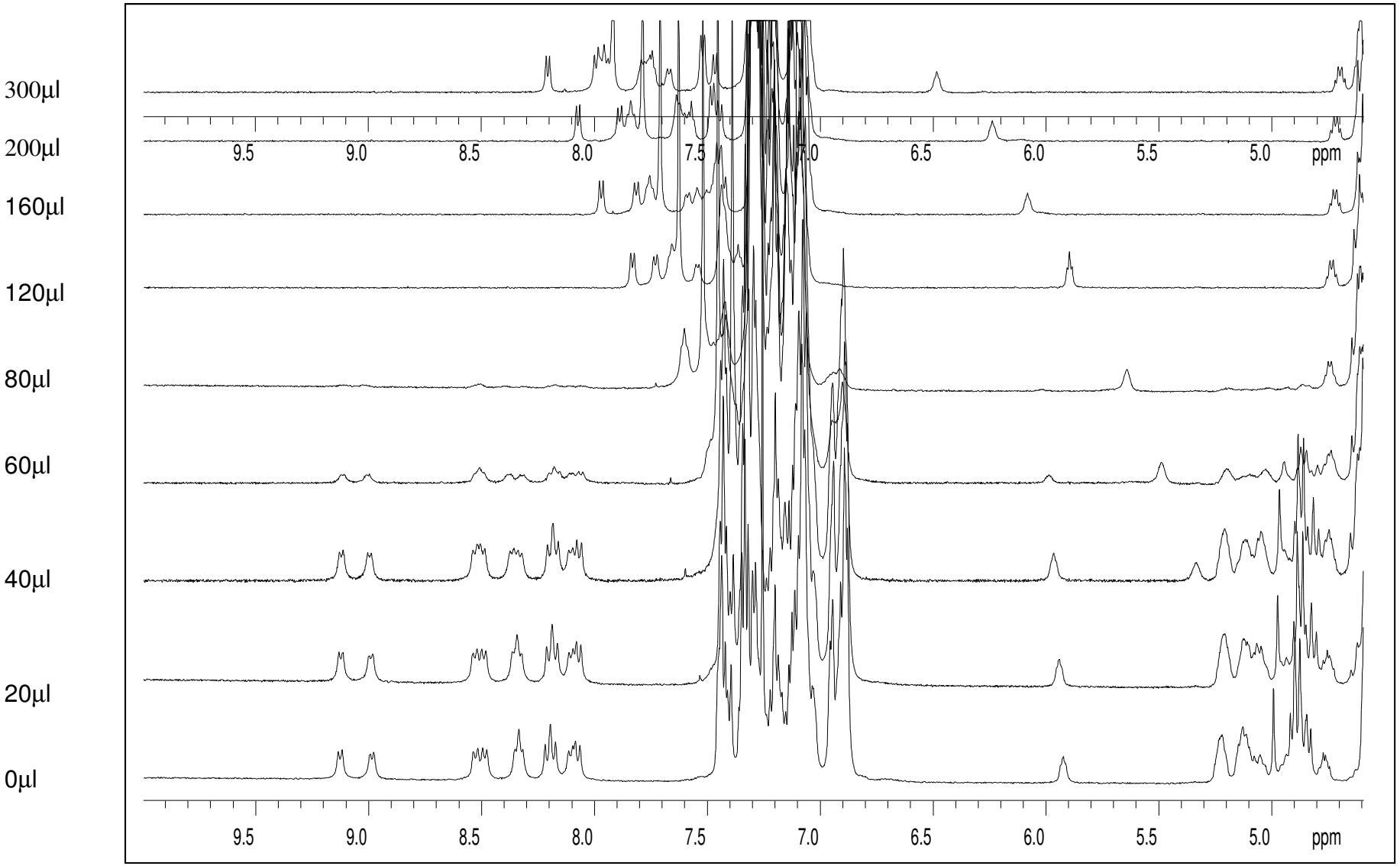


Titration study of compound **2**

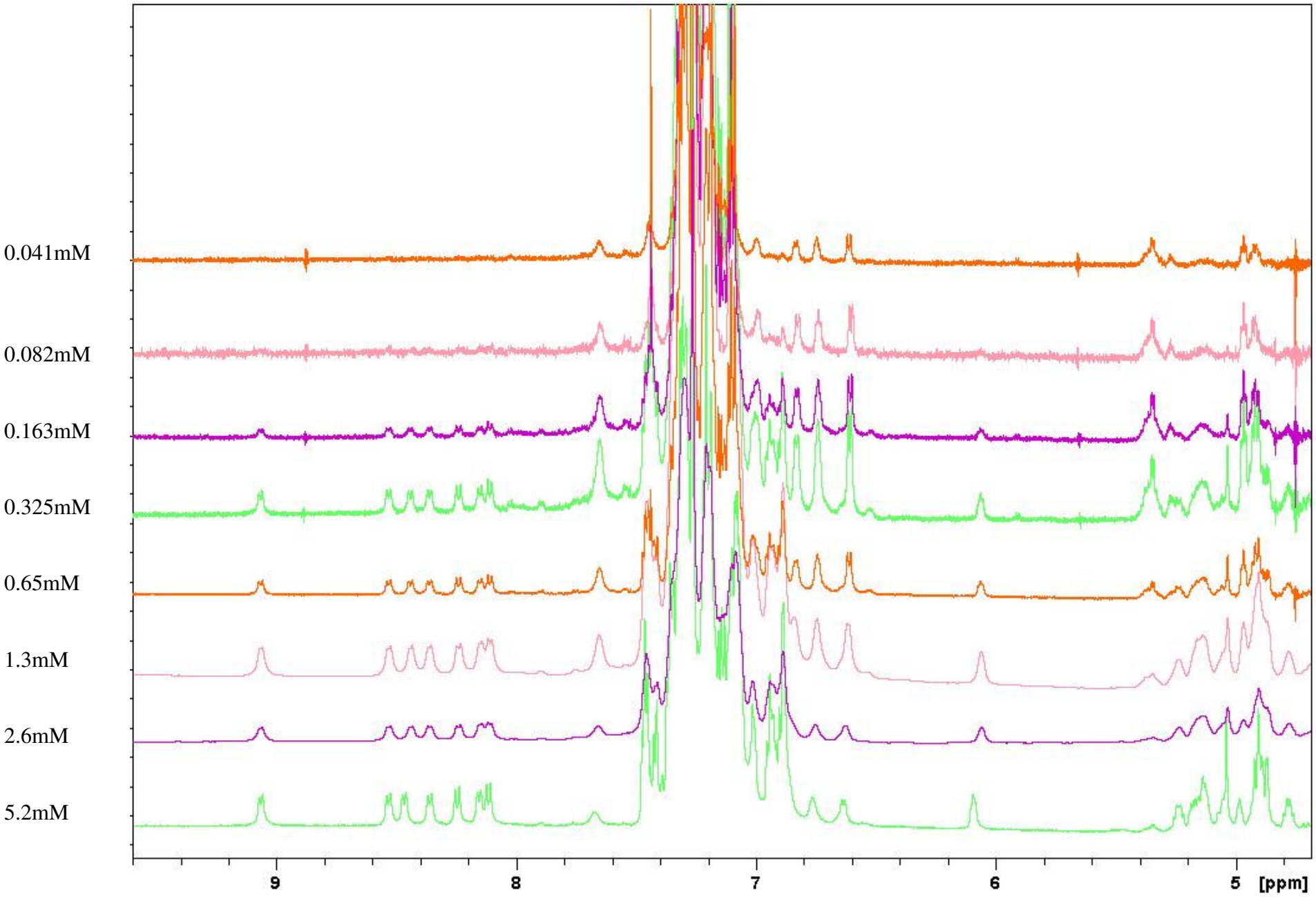




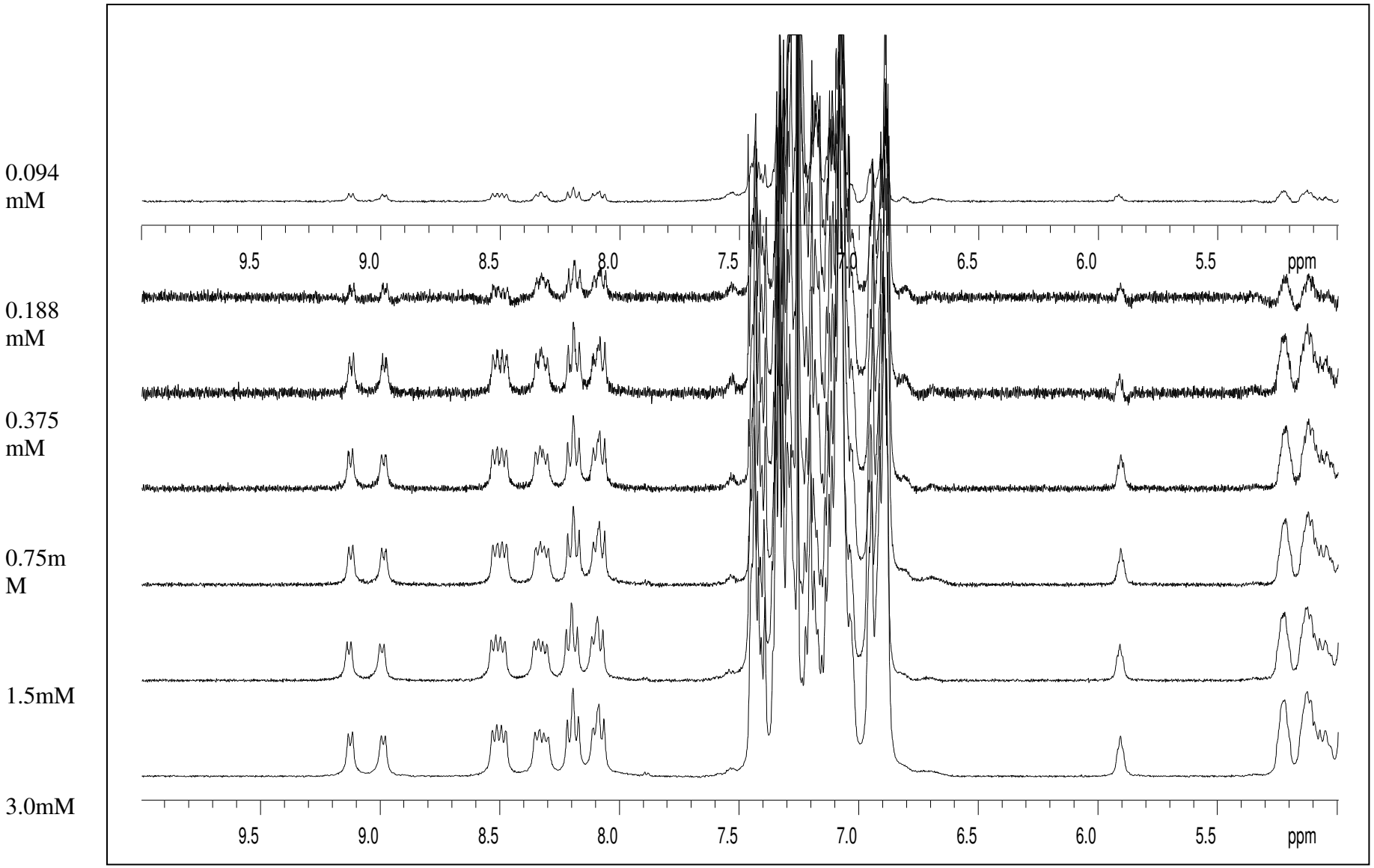
Titration plot of compound **3** (600 MHz, 283 K, $CDCl_3$ vs DMSO-d_6)



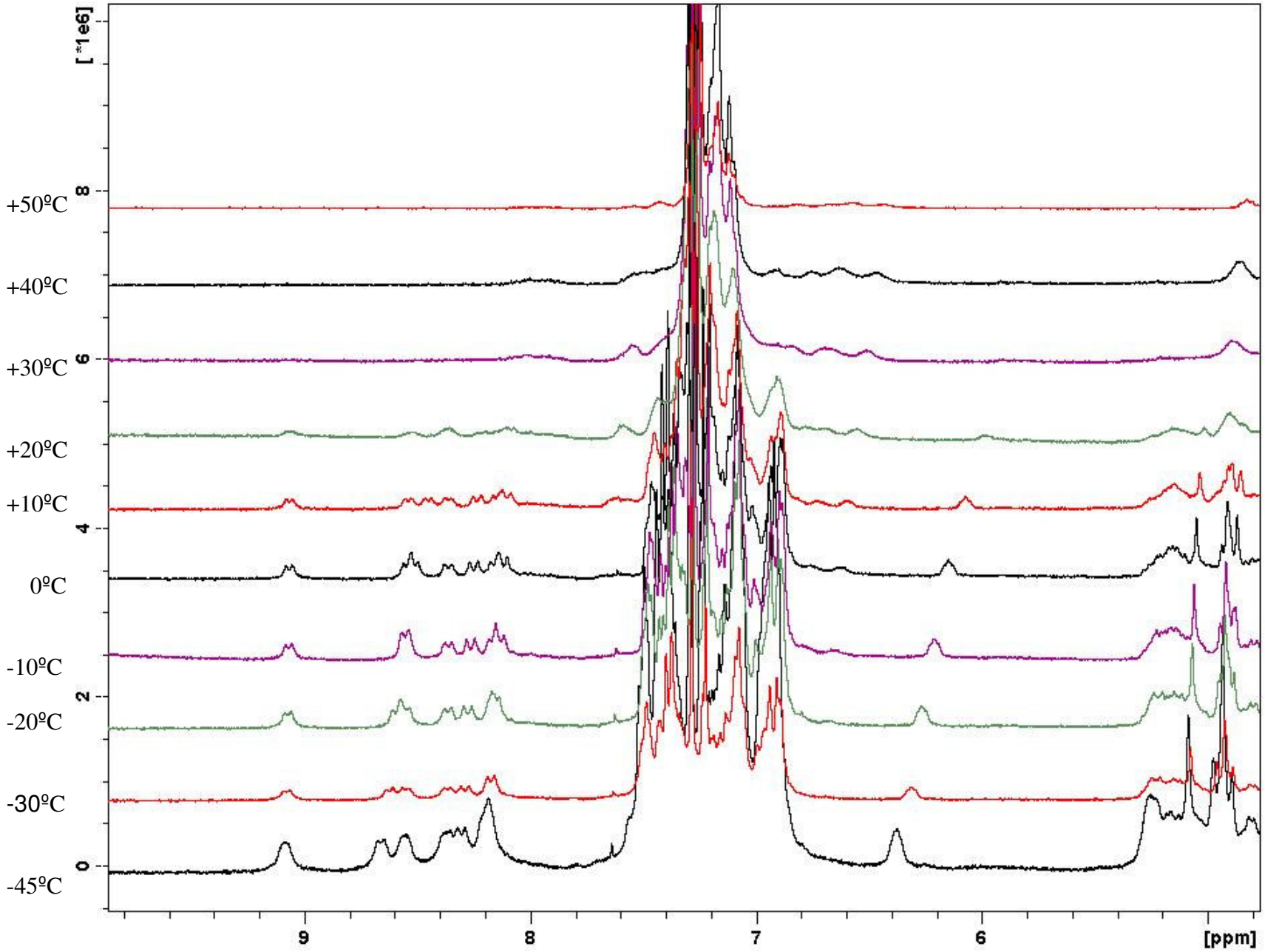
Titration plot of compound **4** (500 MHz, 303 K, $CDCl_3$ vs DMSO-d_6)



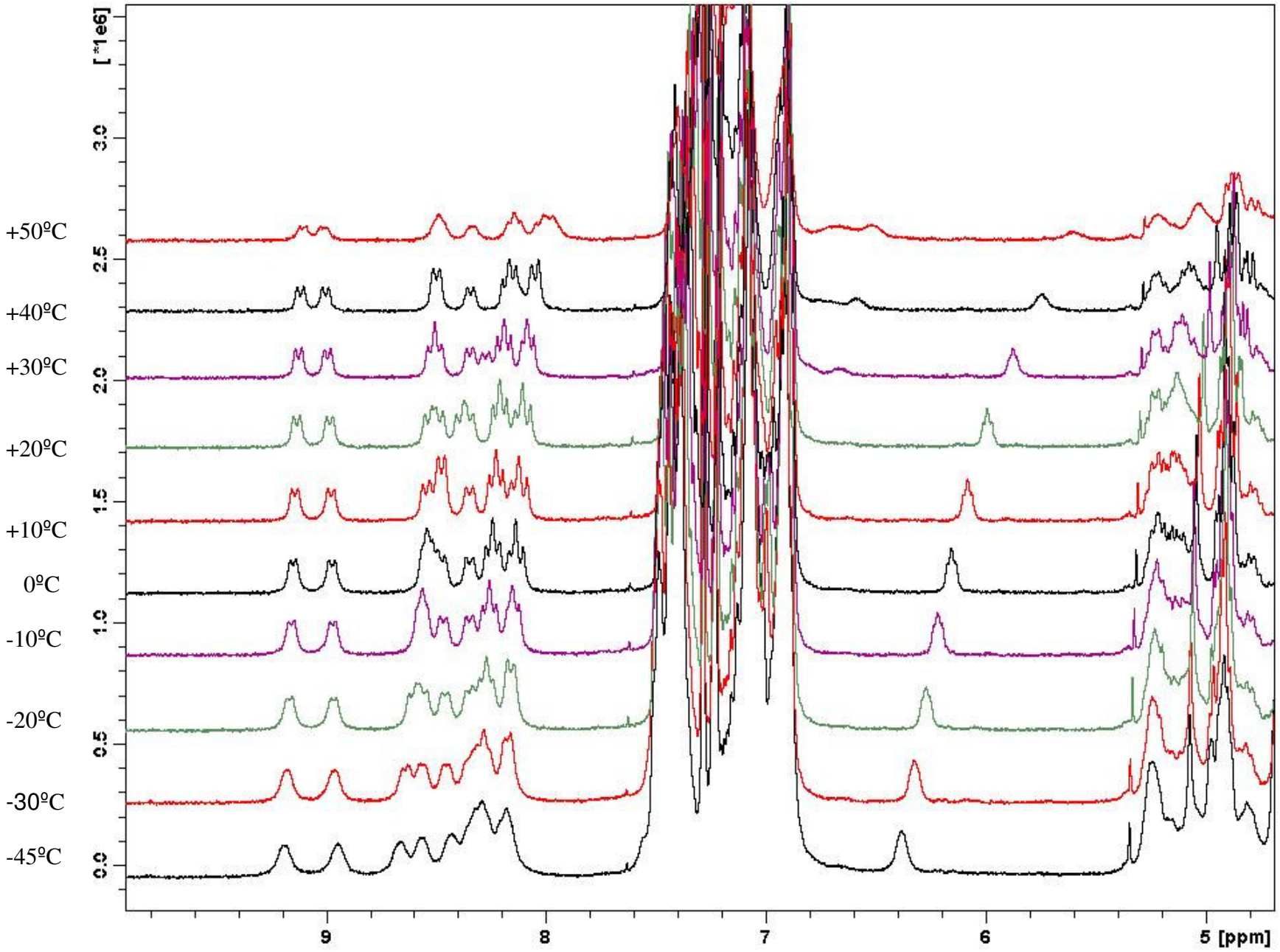
Dilution study of compound **3** from 5.2 mM to 0.041mM (600 MHz, 288 K, $CDCl_3$)



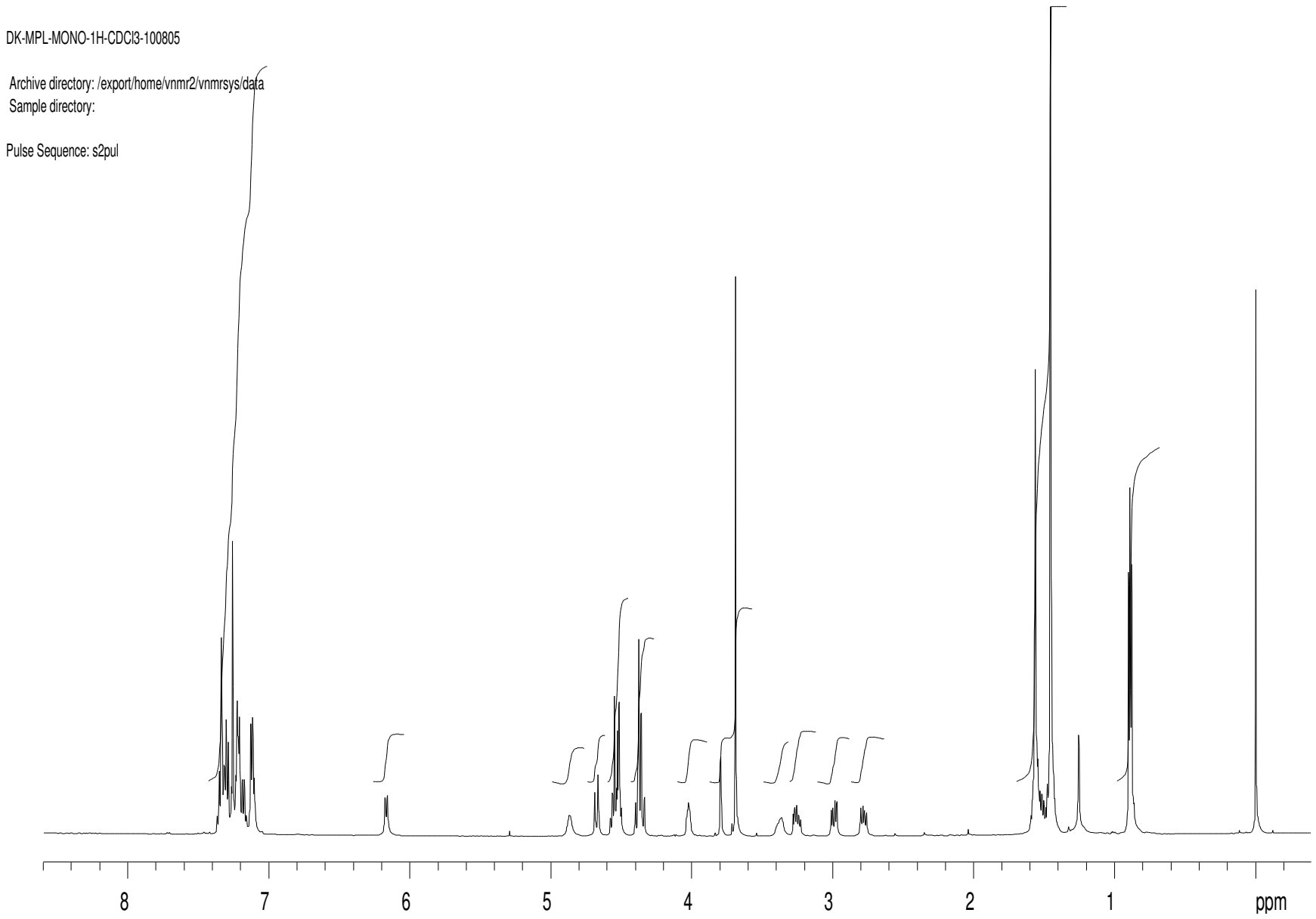
Dilution study of compound **4** from 6.0 mM to 0.094mM (500 MHz, 30 ºC, $CDCl_3$)



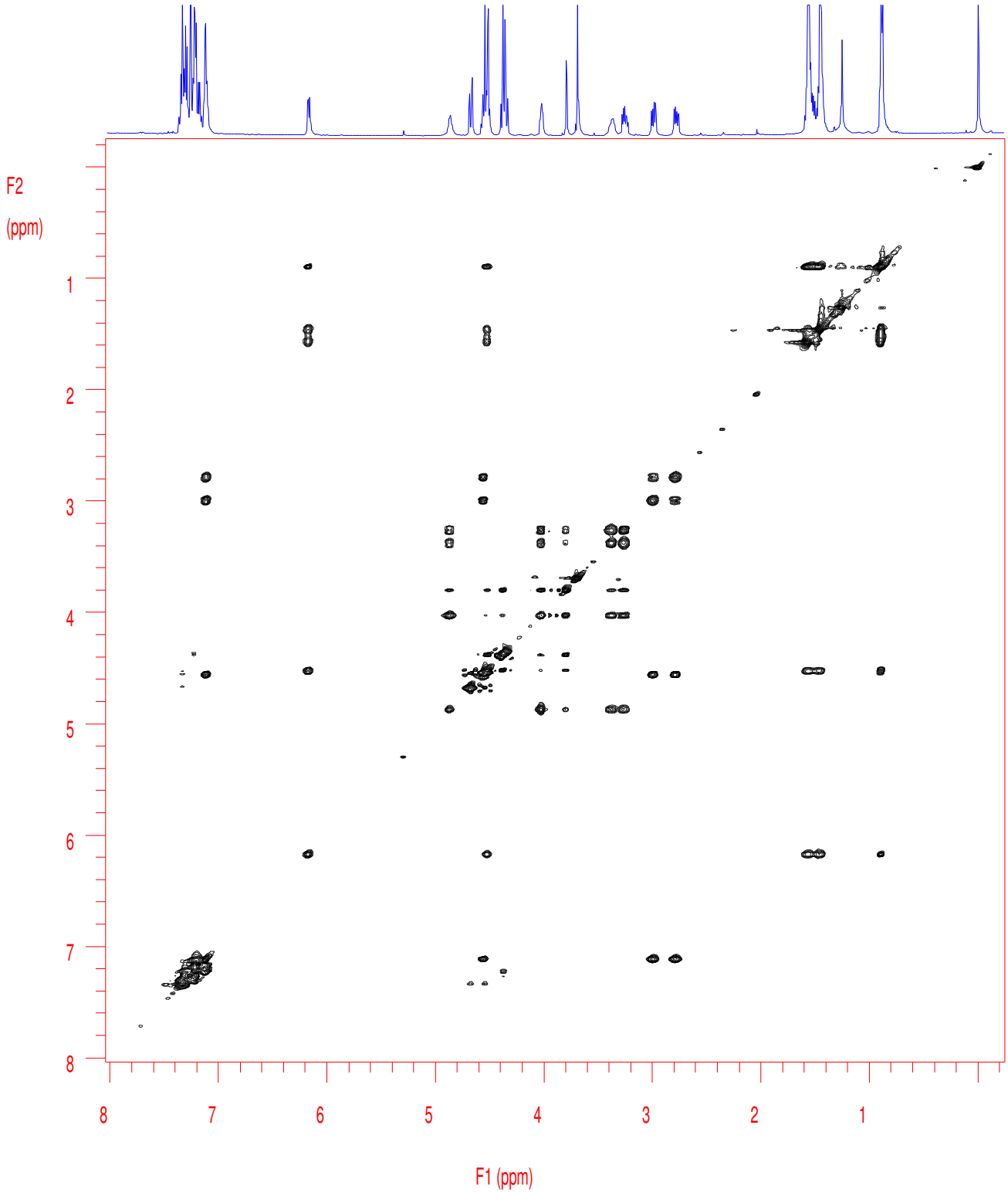
Variable Temperature study of compound **3** from 228 K to 323 K (300 MHz, $CDCl_3$)



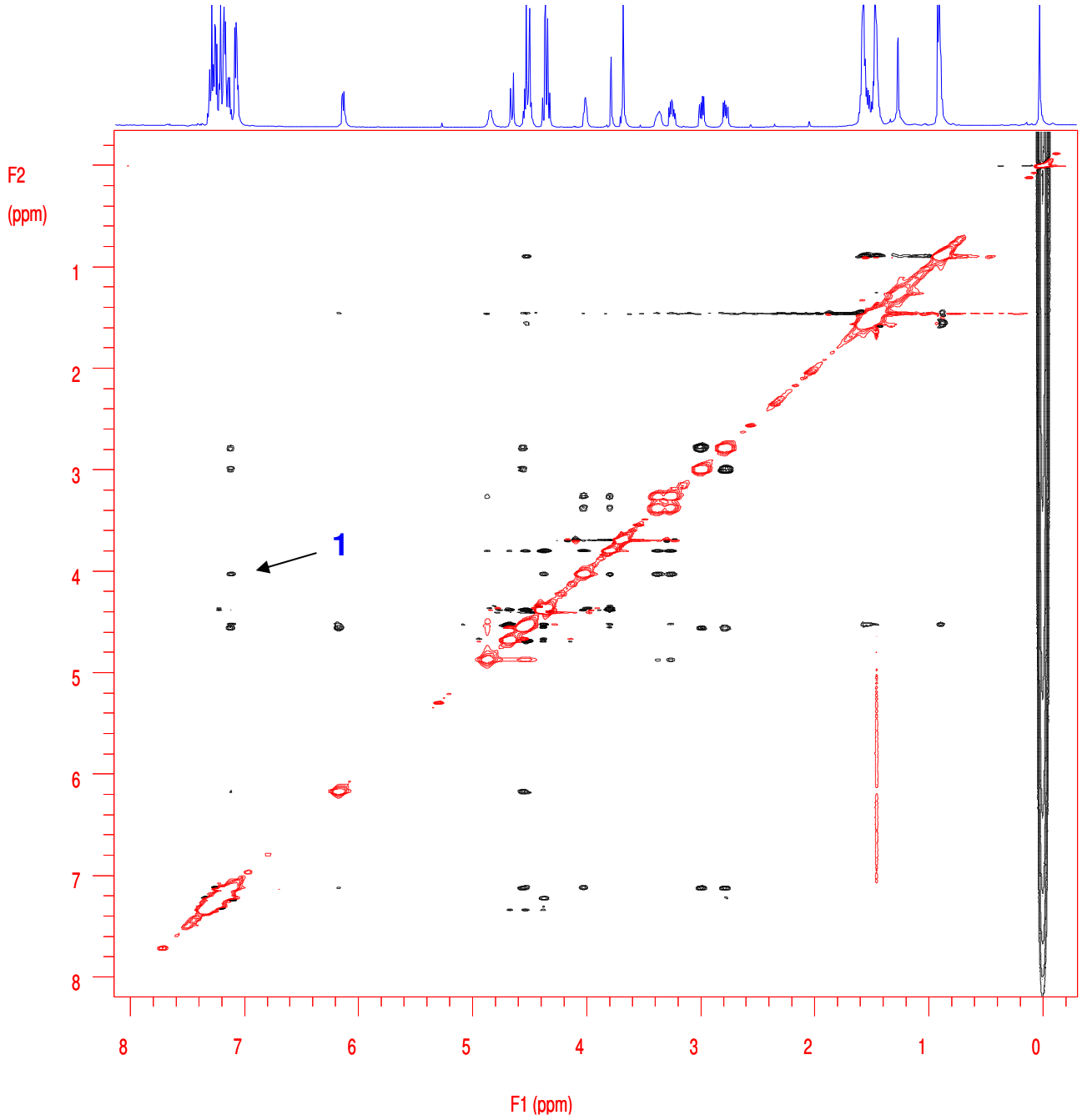
Variable Temperature study of compound **4** from 228 K to 323 K (300 MHz, 4.81 mM, $CDCl_3$)



^{1}H NMR spectrum of compound **1** (500 MHz, 303 K, $CDCl_3$)

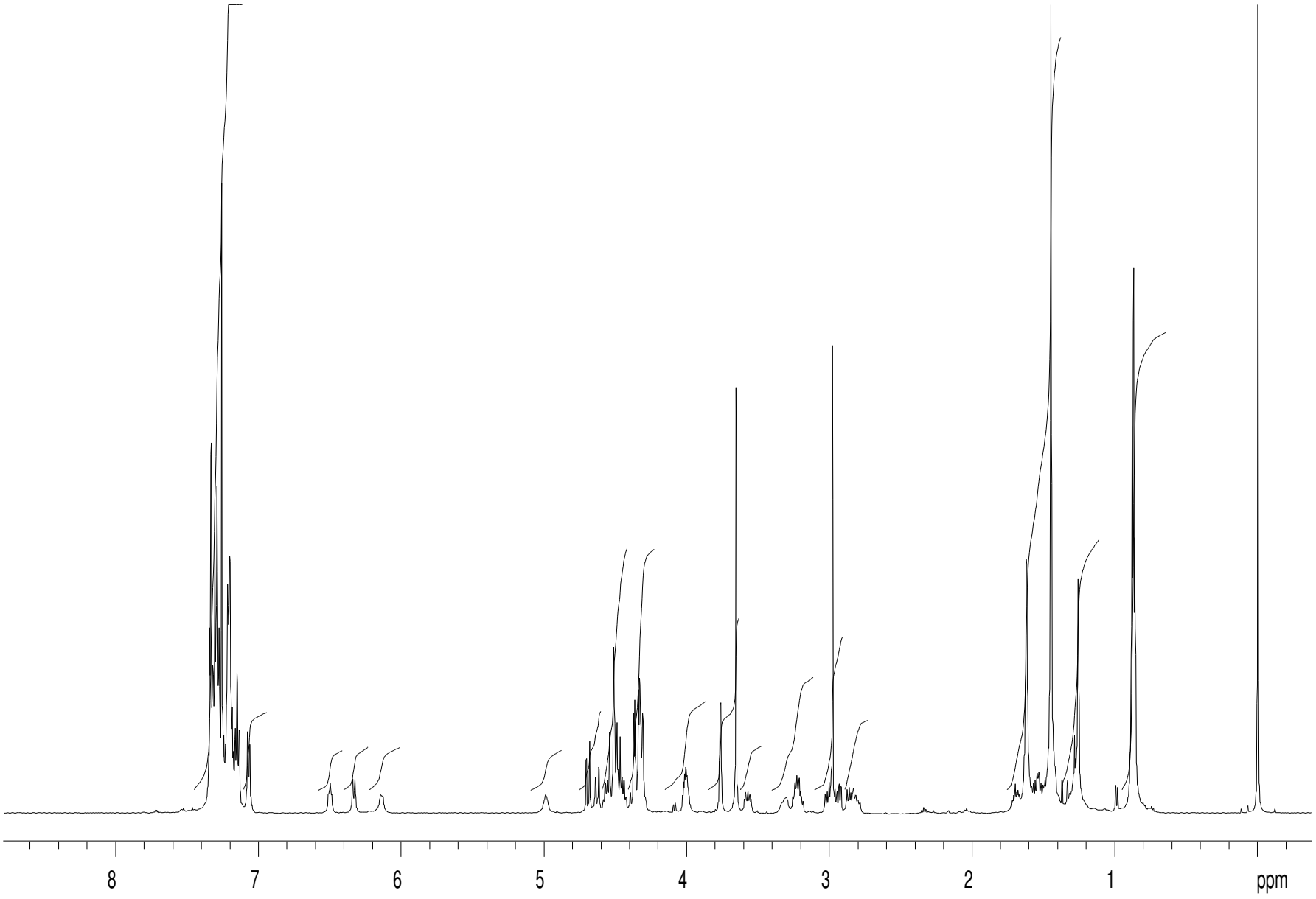


TOCSY spectrum of compound **1** (500 MHz, $CDCl_3$, 303 K)

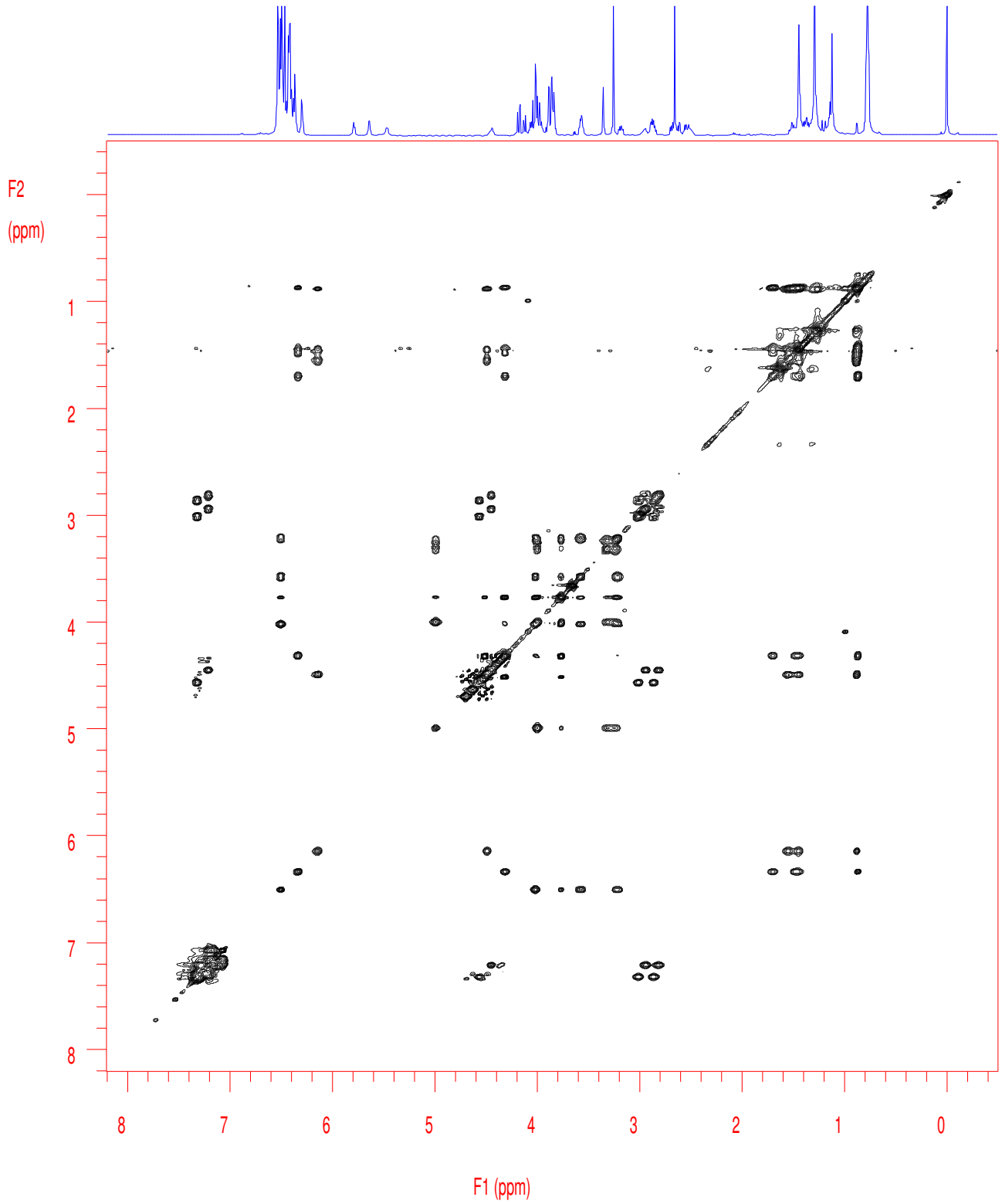


ROESY spectrum of compound **1** (500 MHz, $CDCl_3$, 303 K)

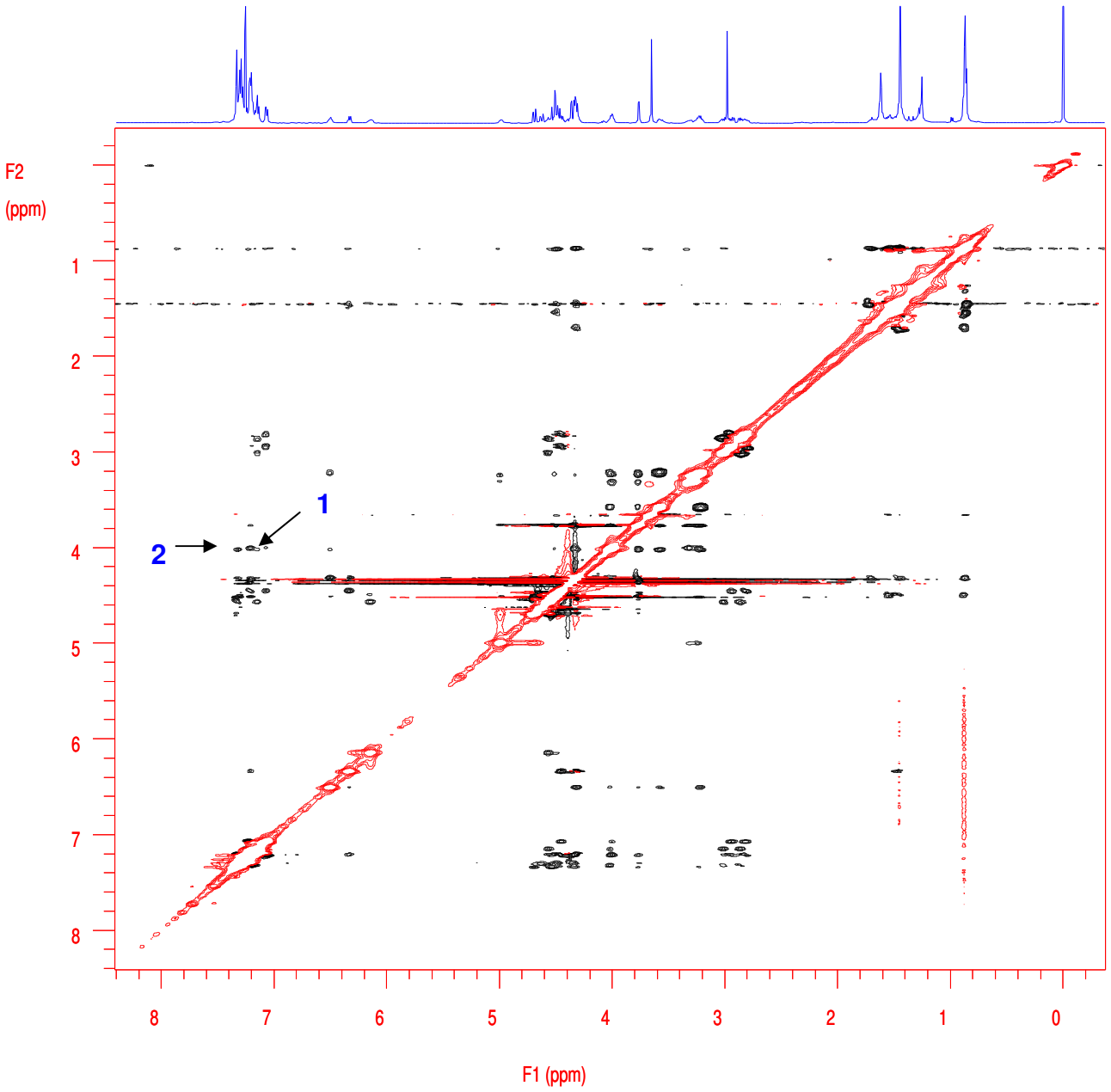
PheNH/MaaC5H nOe has been marked as **1**



^{1}H NMR spectrum of compound **2** (500 MHz, 303 K, $CDCl_3$)

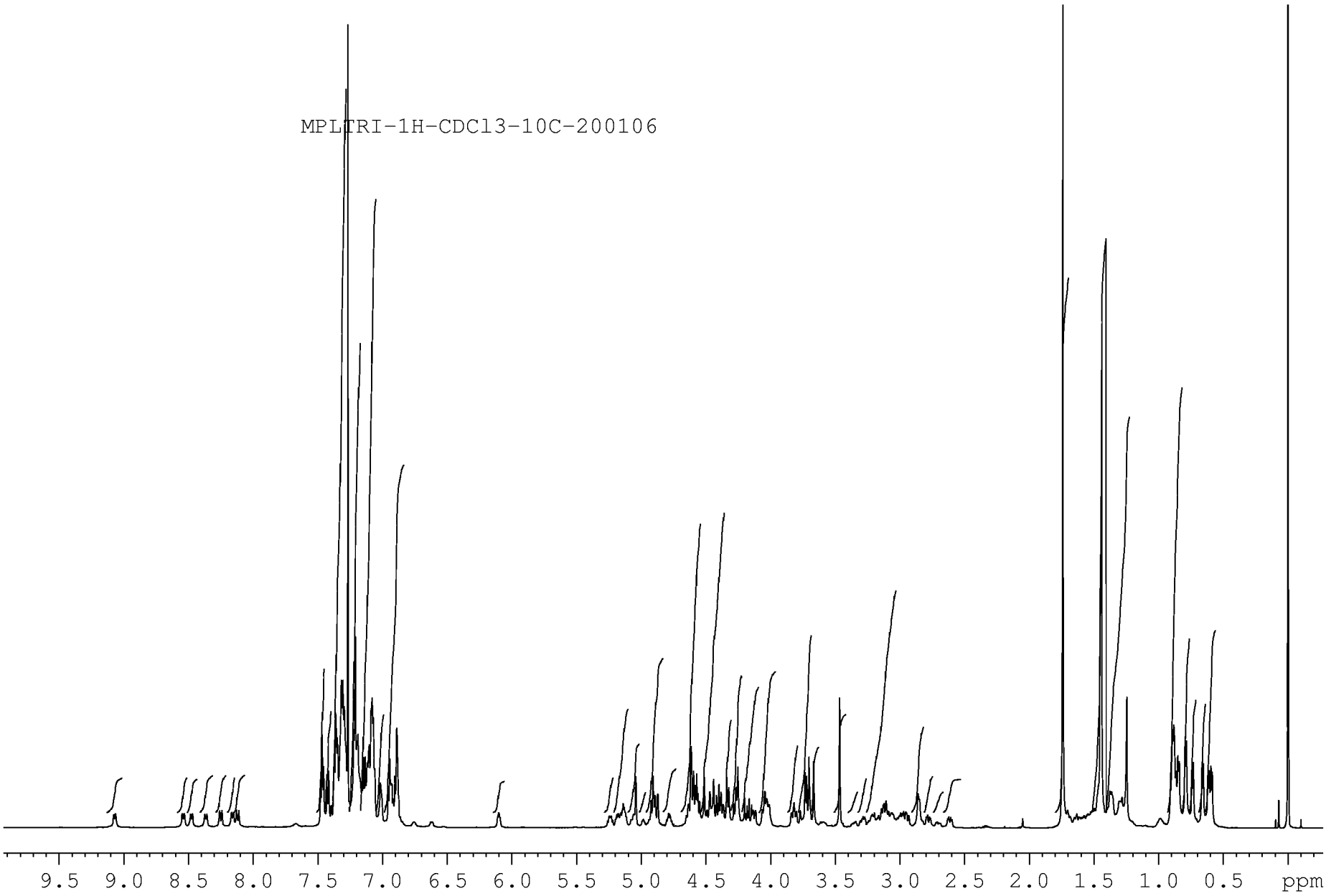


TOCSY spectrum of compound **2** (500 MHz, $CDCl_3$, 303 K)

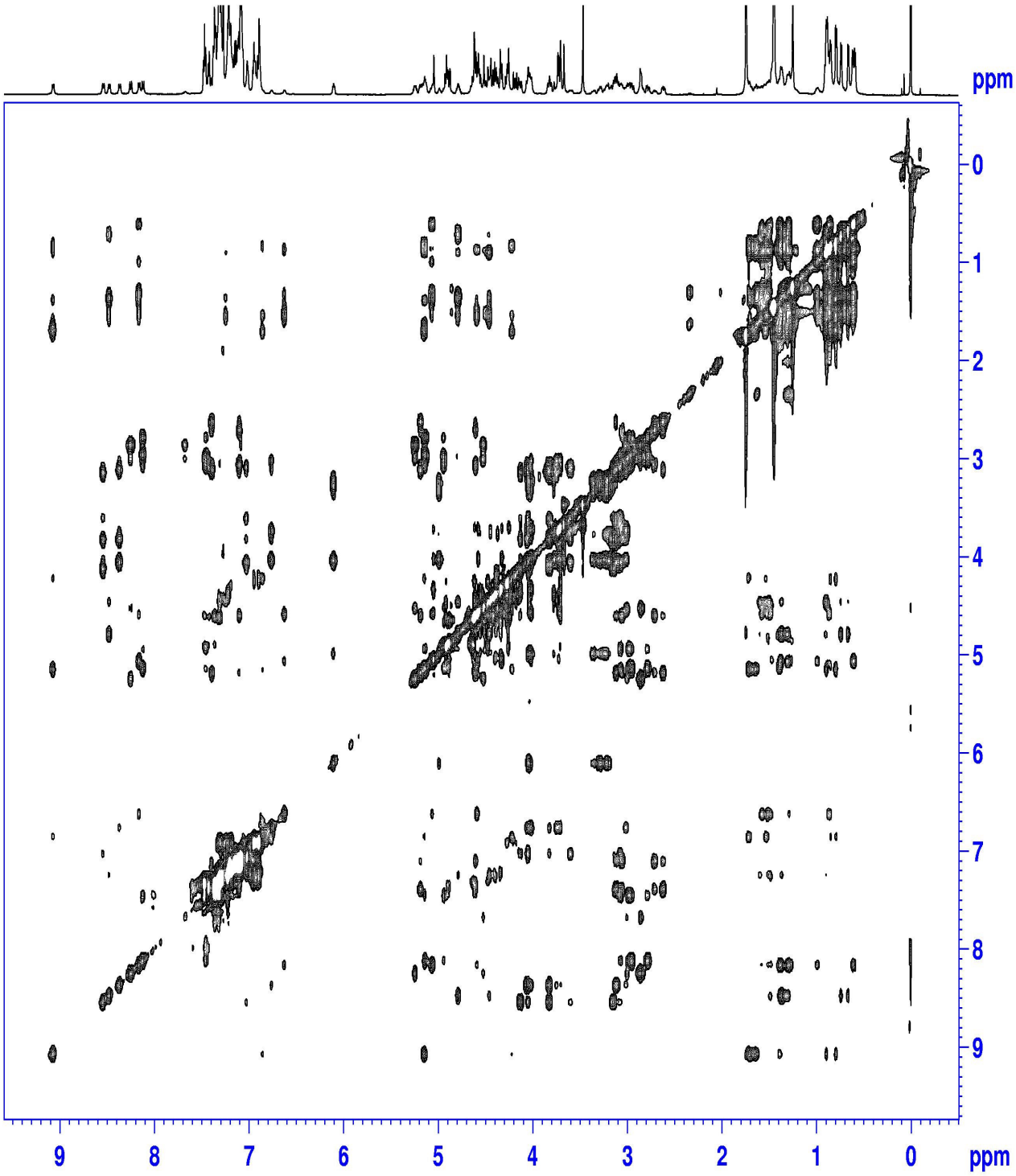


ROESY spectrum of compound **2** (500 MHz, $CDCl_3$, 303 K)

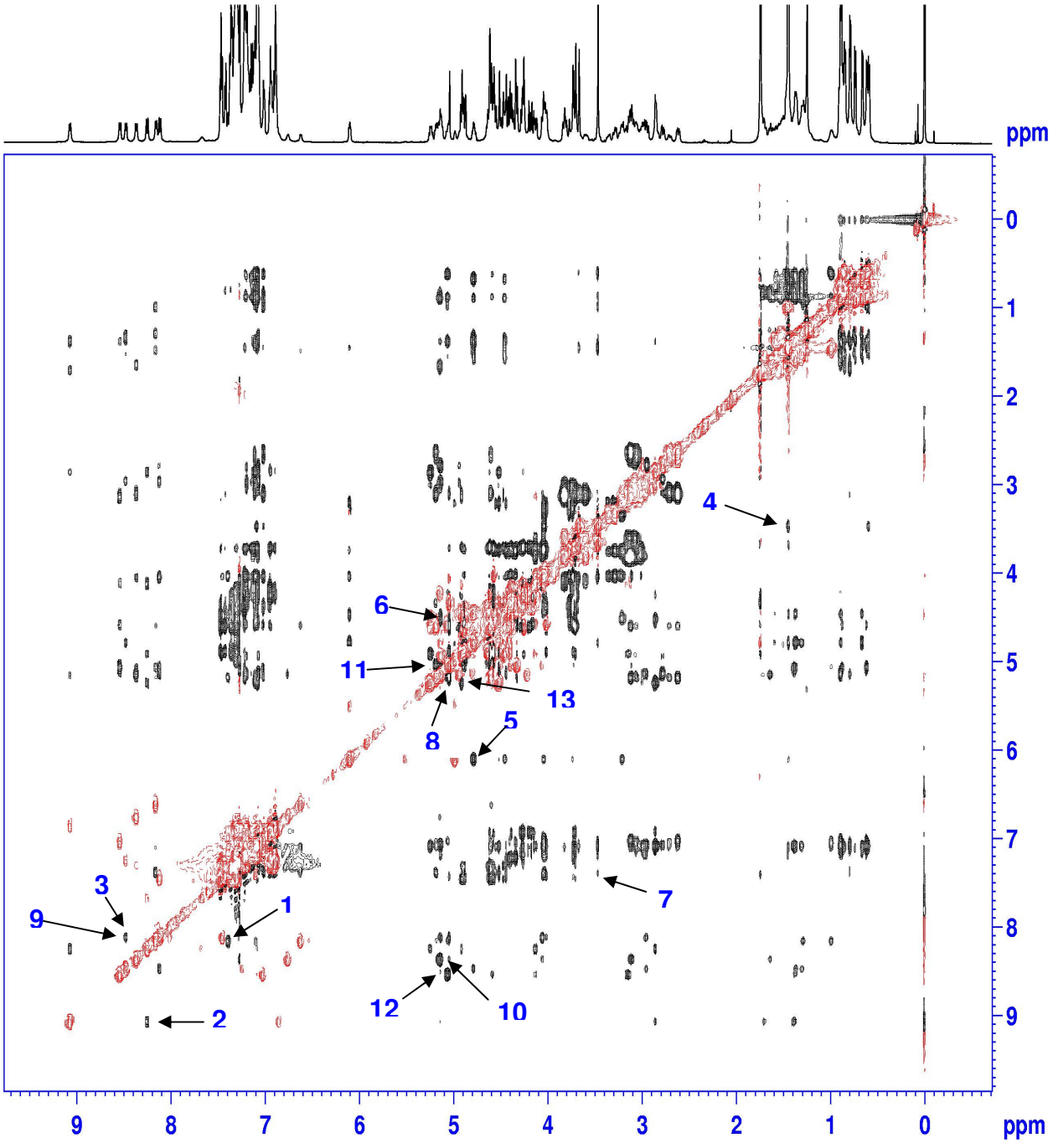
Phe(2)NH/Maa(1)C5H and Phe(5)NH/Maa(4)C5H nOe correlations are shown as **1** and **2,** respectively.



^{1}H NMR spectrum of compound **3** (600 MHz, 283 K, $CDCl_3$)

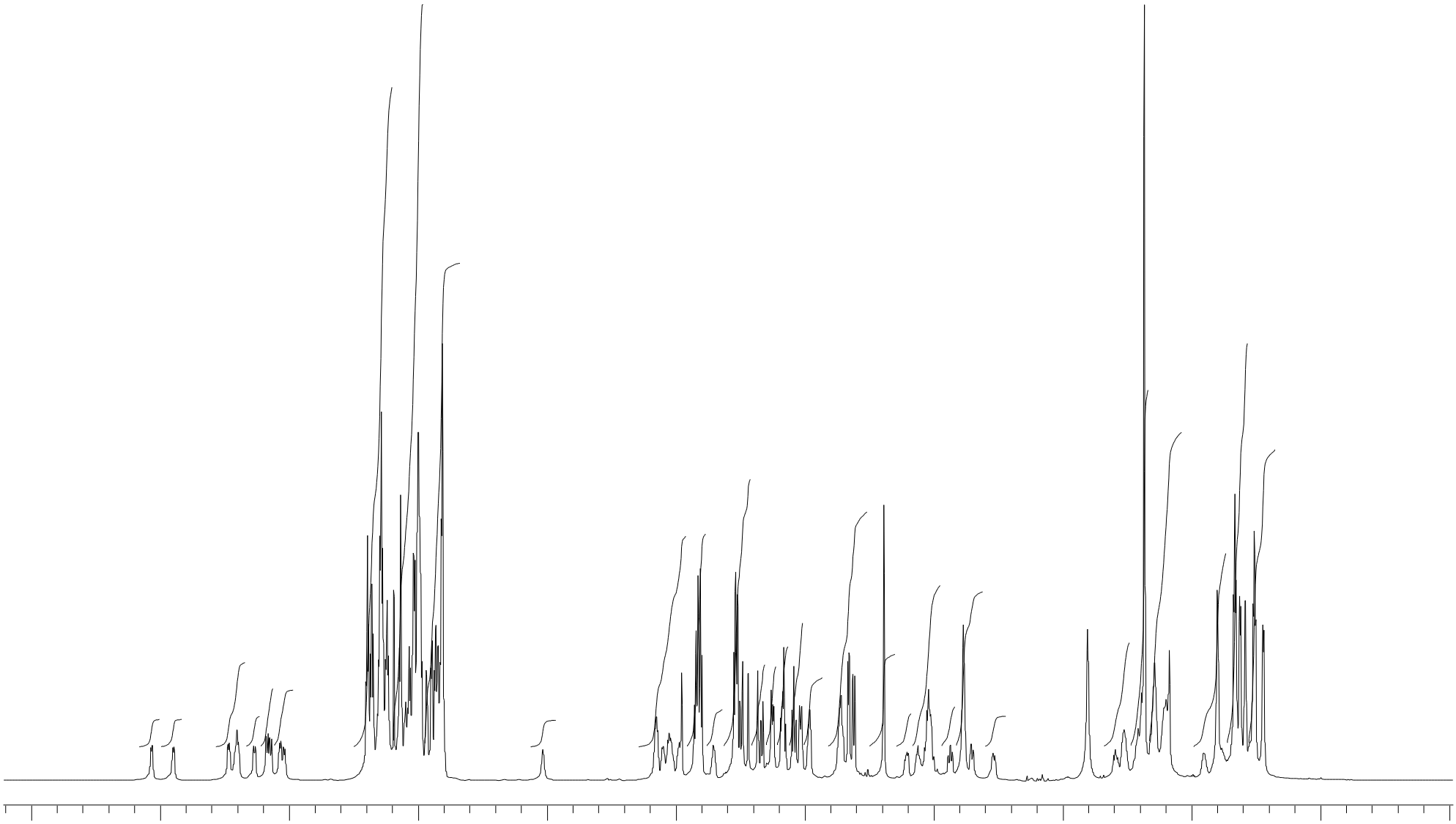


TOCSY spectrum of compound **3** (600 MHz, $CDCl_3$, 283 K)

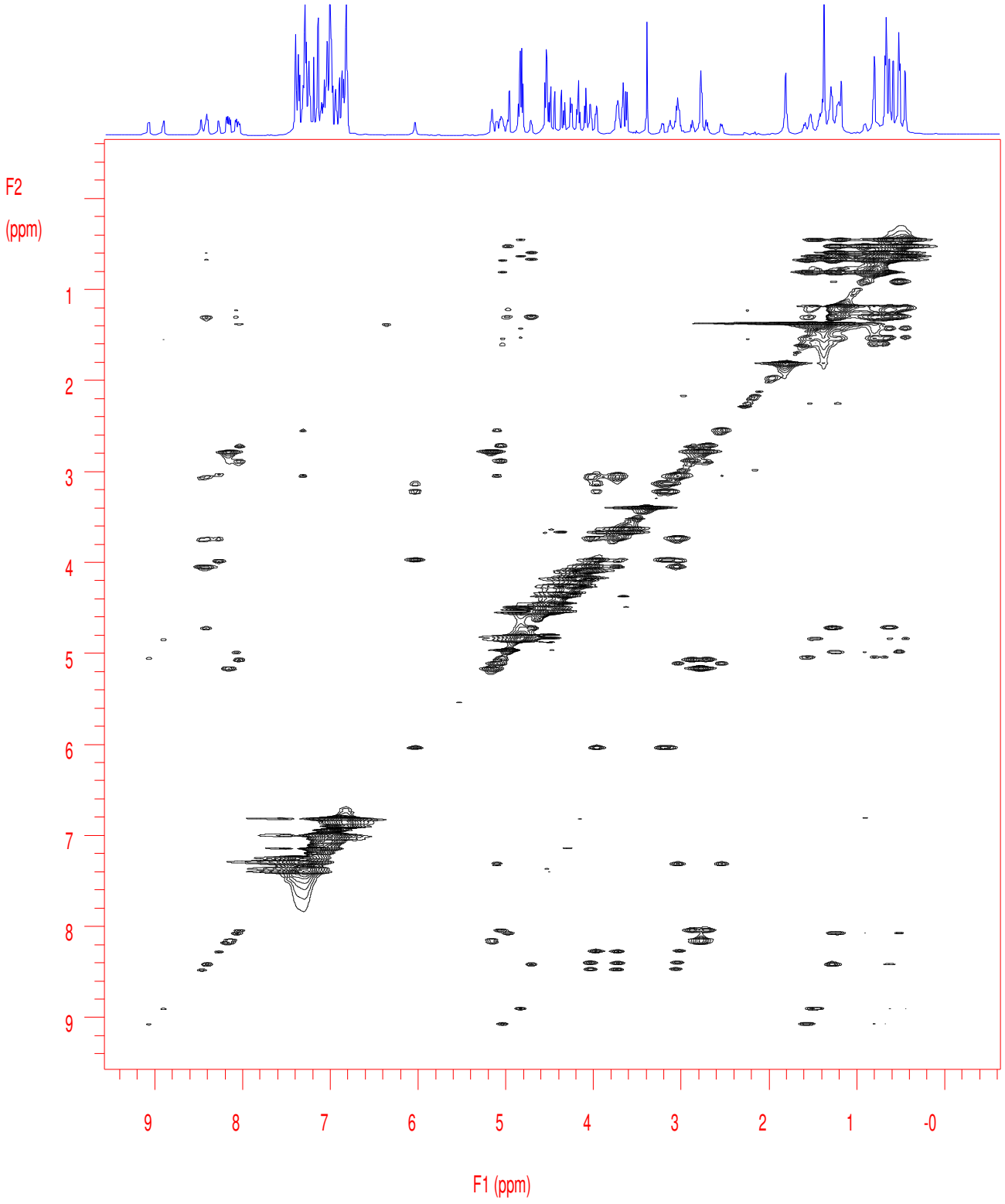


ROESY spectrum of compound **3** (600 MHz, $CDCl_3$, 283 K)

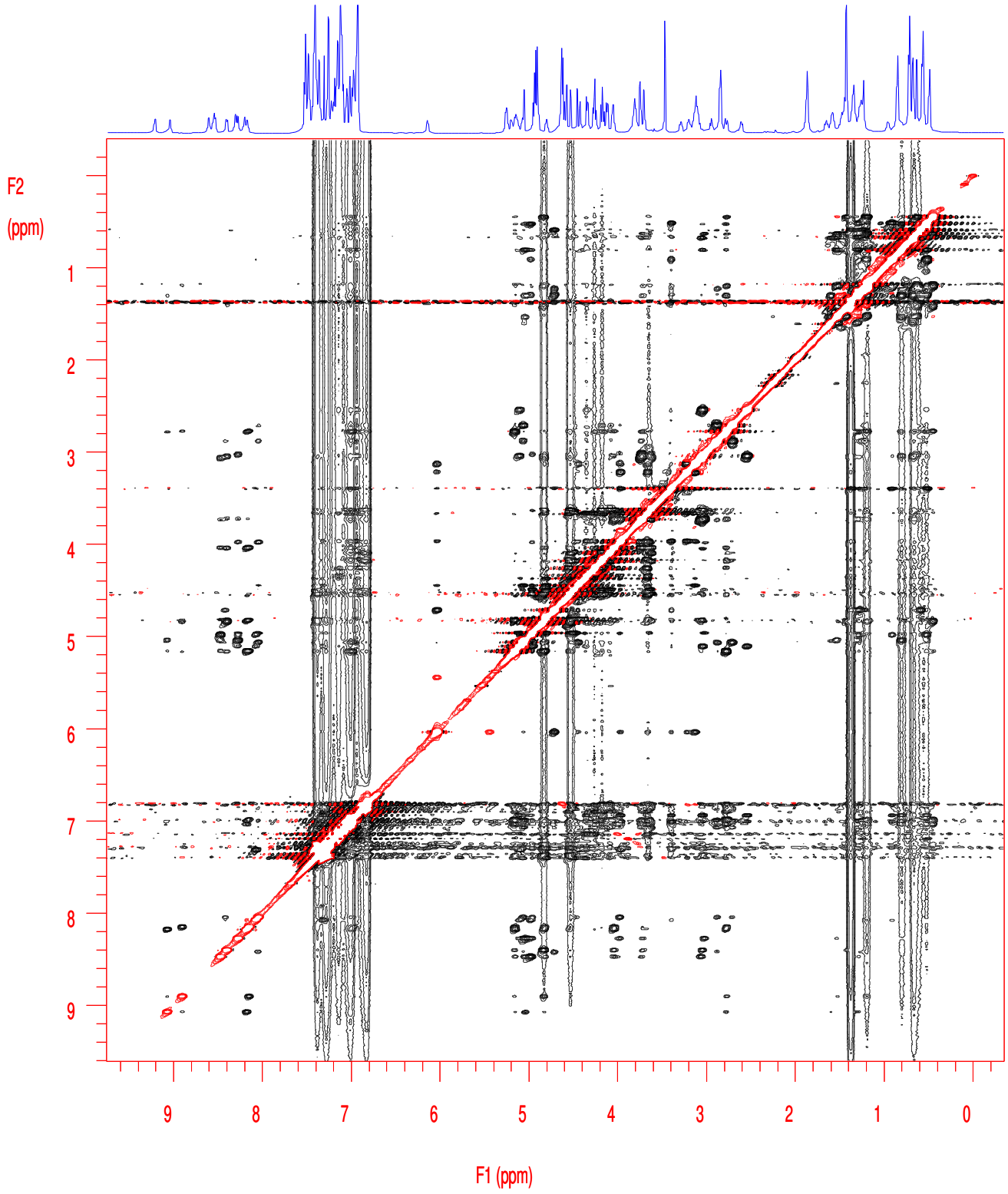
Diagonostic nOes are marked in the figure.
1. Phe(2)NH/Leu(3)NH, **2.** Phe(5)NH/Leu(6)NH, **3.** Phe(8)NH/Leu(9)NH, **4.** Boc/OMe, **5.** Maa(1)NH/Leu(9)CαH, **6.** Maa(1)C2H/Phe(8)CαH, **7.** Phe(2)NH/OMe, **8.** Phe(2)CαH/Maa(7)C2H, **9.** Leu(3)NH/Leu(9)NH, **10.** Leu(3)CαH/Maa(7)NH, **11.** Leu(3)CαH/Leu(6)CαH, **12.** Maa(4)NH/Leu(6)CαH, **13.** Maa(4)C2H/Phe(5)CαH



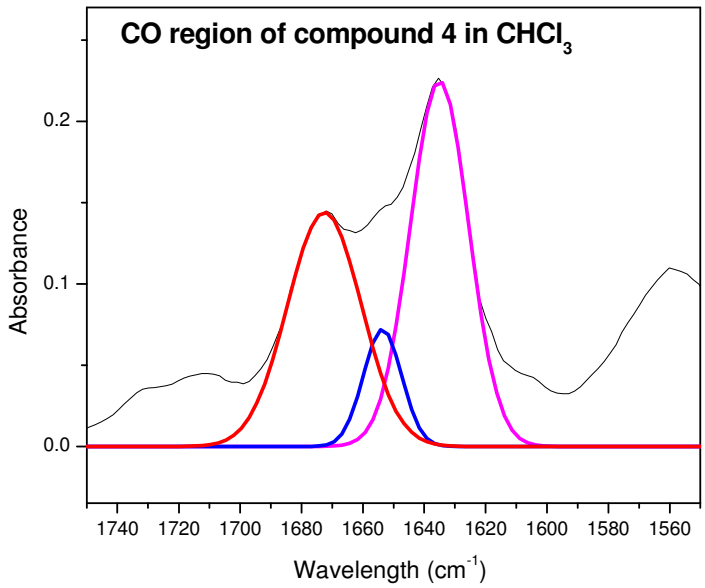
^{1}H NMR spectrum of compound **4** (750 MHz, 278 K, $CDCl_3$)

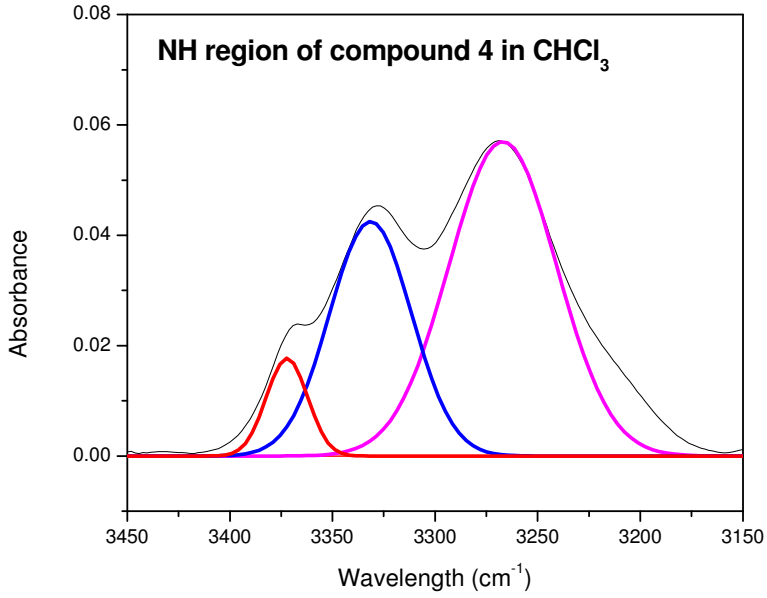


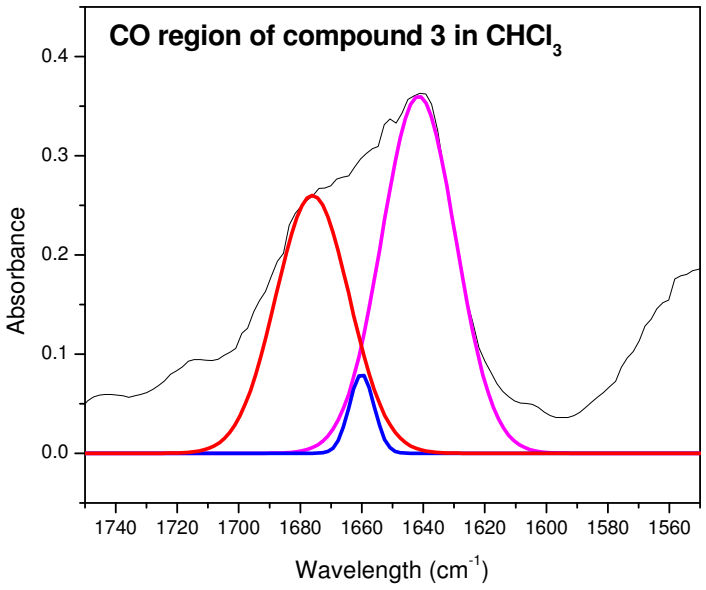
TOCSY spectrum of compound **4** (750 MHz, $CDCl_3$, 278 K)

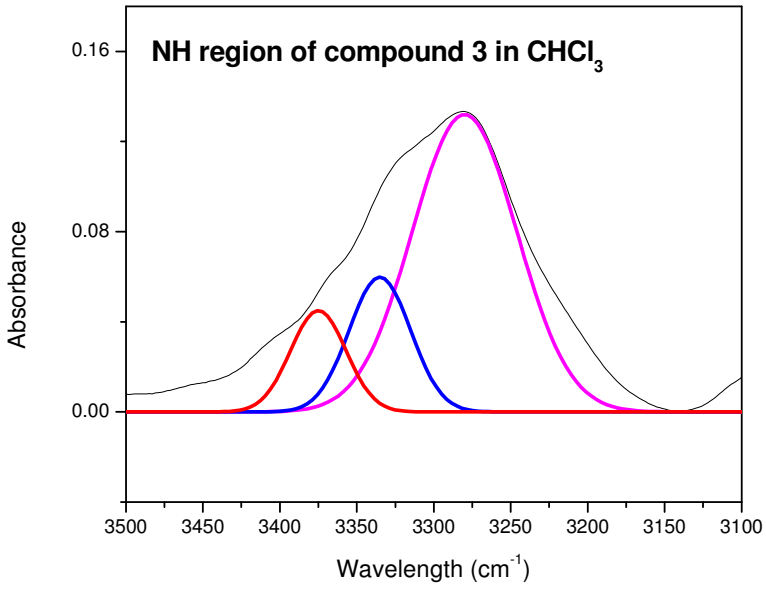


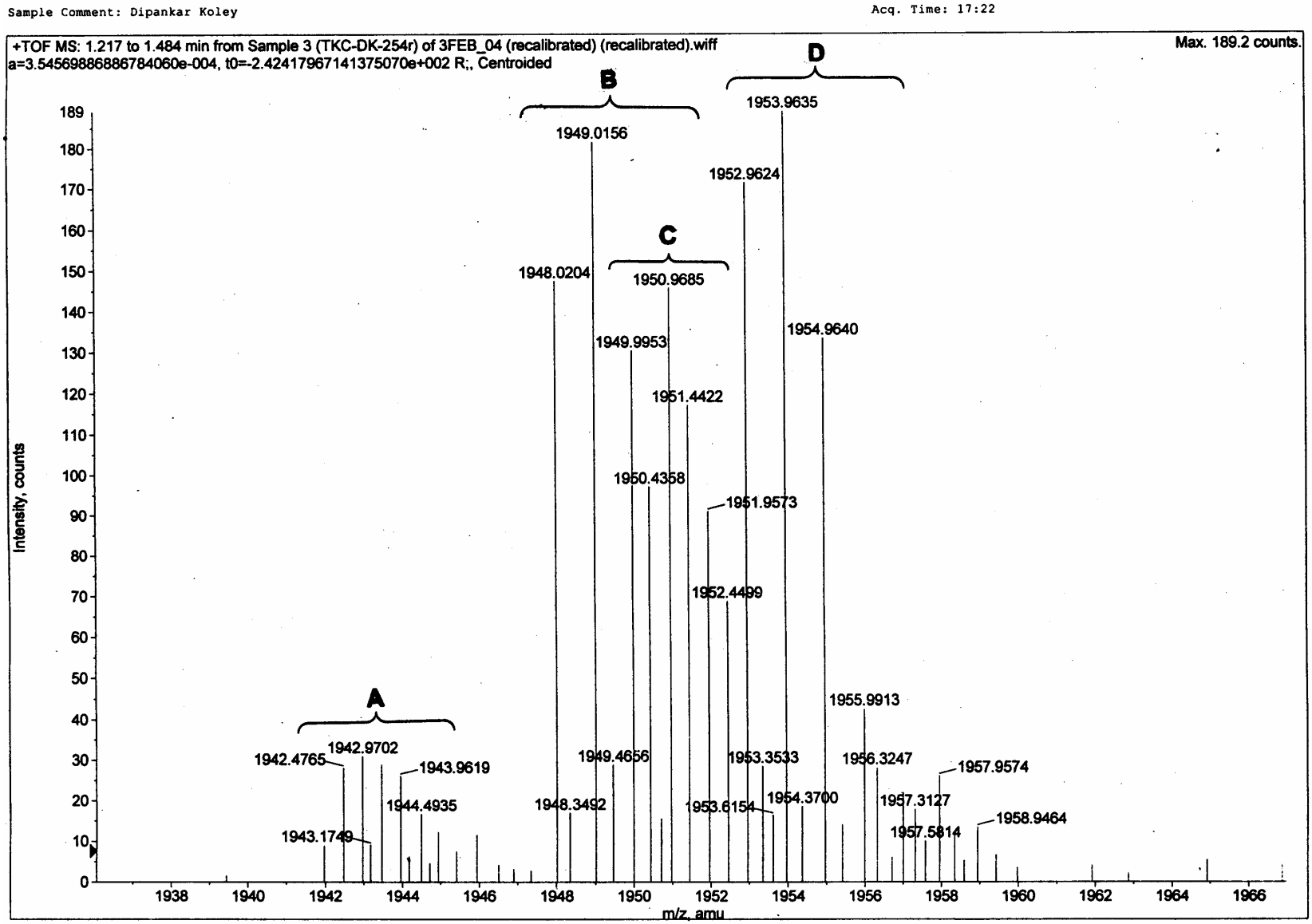
ROESY spectrum of compound **4** (750 MHz, $CDCl_3$, 278 K)


CO region of compound 4 in CHCl3
Absorbance
0.0
0.1
0.2
1740
1720
1700
1680
1660
1640
1620
1600
1580
1560
Wavelength (cm-1)

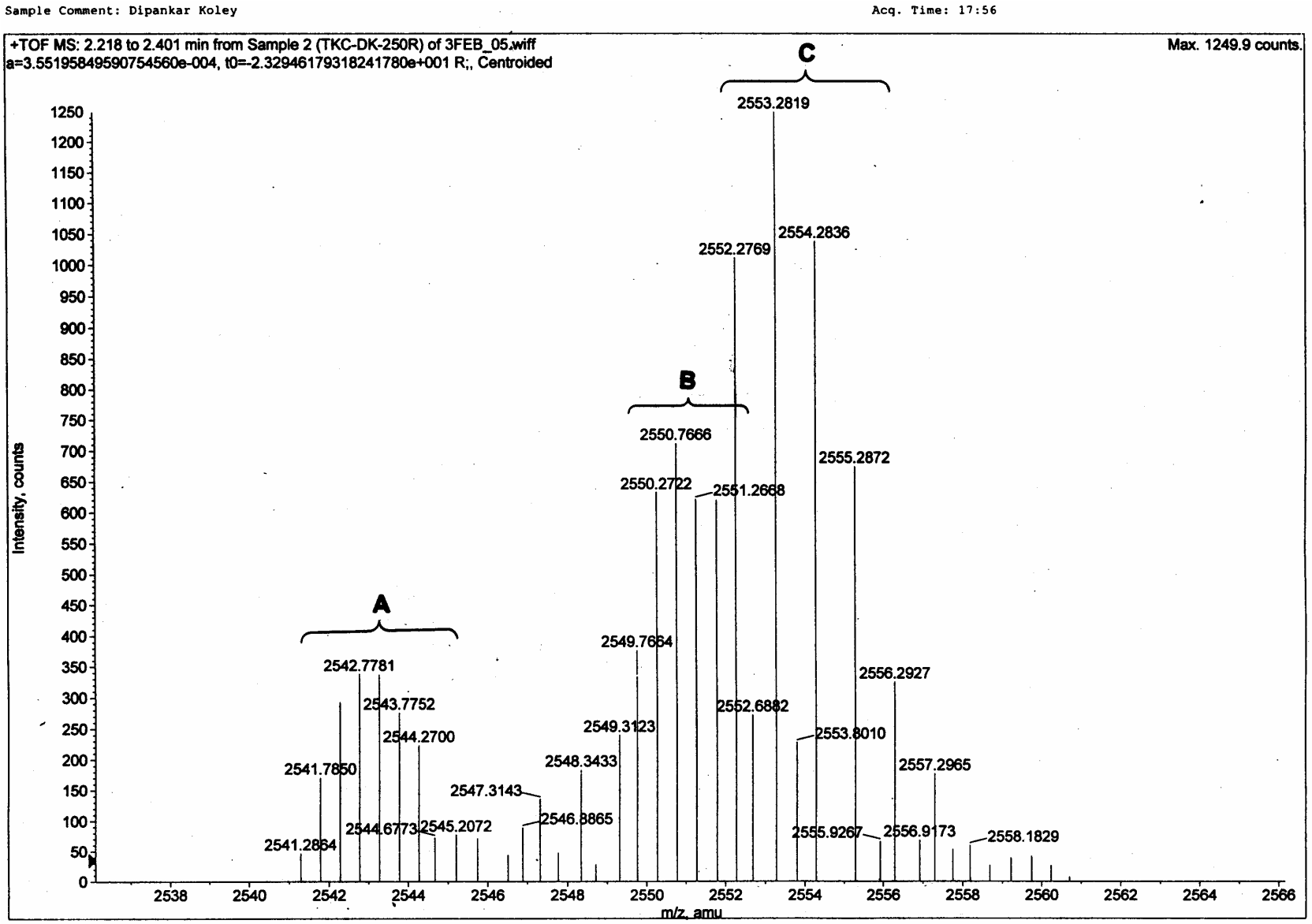

NH region of compound 4 in CHCl3
Absorbance
0.00
0.02
0.04
0.06
0.08
3450
3400
3350
3300
3250
3200
3150
Wavelength (cm-1)


CO region of compound 3 in CHCl3
Absorbance
0.4
0.3
0.2
0.1
0.0
1740 1720 1700 1680 1660 1640 1620 1600 1580 1560
Wavelength (cm-1)


NH region of compound 3 in CHCl3
Absorbance
0.16
0.08
0.00
3500 3450 3400 3350 3300 3250 3200 3150 3100
Wavelength (cm-1)



HRMS (ESI) of compound **3**: *m/z* calculated for $[2M + H + Na]^{2+}$ ($C_{222}H_{271}N_{18}O_{42}Na$)/2 :1941.9755, found 1941.9699 (**A**); *m/z* calculated for $[M + NH_4]^+$ ($C_{111}H_{139}N_{10}O_{21}$) :1948.0110, found 1948.0204 (**B**); *m/z* calculated for $[2M + NH_4 + Na]^{2+}$ ($C_{222}H_{274}N_{19}O_{42}Na$)/2 : 1950.4893 found 1950.4358 (**C**); *m/z* calculated for $[M + Na]^+$ ($C_{111}H_{135}N_9O_{21}Na$) :1952.9664, found 1952.9624 (**D**).



HRMS (ESI) of compound **4**: *m/z* calculated for $[2M + H + Na]^{2+}$ ($C_{292}H_{353}N_{24}O_{54}Na$)/2 : 2541.2750, found 2541.2864 (**A**); *m/z* calculated for $[2M + NH_4 + Na]^{2+}$ ($C_{292}H_{356}N_{25}O_{54}Na$)/2 : 2549.7883, found 2549.7664 (**B**); *m/z* calculated for $[M + Na]^{+}$ ($C_{146}H_{176}N_{12}O_{27}Na$) : 2552.2660, found 2552.2769 (**C**).