SUPPLEMENTAL MATERIAL

Structural Variation of Glycolipids from *Meiothermus taiwanensis* ATCC BAA-400 under Different Growth Temperatures

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Experimental section

NMR. NMR spectra were recorded on 400 and 500 MHz spectrometers in $CDCl_3/CD_3OD$ = 10:1 for **1** and C_6D_6 for **1a-4a** at 300 K. 1D and 2D TOCSY spectra were observed with mixing times 80~120 ms. ROESY spectra were recorded with 400 ms mixing time. HSQC spectra were obtained with $^1J_{H\text{-}C}$ = 145 Hz and HMBC spectra were recorded with $^3J_{H\text{-}C}$ or $^2J_{H\text{-}C}$ = 8 Hz. $^1J_{H\text{-}C}$ coupling constant of anomeric protons were observed from HMQC without ^{13}C decoupling (PL12= 120 dB).

Mass. Molecular weight analysis of **1** was recorded on a desorption ionization on silicon time-of-flight (DIOS-TOF) mass spectrometry, and those of **2a-4a** were observed on MALDI-TOF mass Spectrometry. Tandem mass spectra of **1** were observed on ESI-LTQ mass spectrometry.

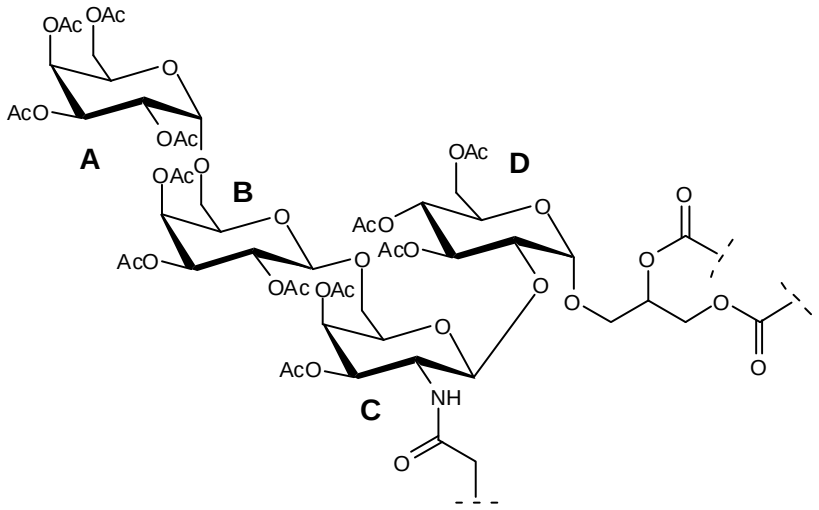
Material and purification of glycolipids 1 and 2a-4a. The incubational condition of *M. taiwanensis* was followed previous literature. The 55 ℃ cultural bacteria was extracted with ethanol then subjected to Si-gel chromatography eluted by a $CHCl_3/CH_3OH$ gradient from 10:1 to 3:1 to obtain GL1 and GL2 fractions. Both fractions were acetylated then purified by a Si-gel column eluted by $CHCl_3/CH_3OH$ = 100:1 to yield **2a** and a mixture of **3a** and **4a**. The 65 ℃ cultural bacteria was extracted and separated by the same method as above mention to obtain a mixture of glycolipid and phosphoglycolipid. The mixture was subjected to a Sephadex LH-20 column eluted by $CHCl_3/CH_3OH$ = 1:2 to give **1**.

Composition and linkage analysis. The fatty acid composition of the *O*-acyl groups in the glycolipid was determined by comparing the retention times of FAMEs (fatty acid methyl esters) from glycolipids to the standards in GC-MS analysis. The methyl esters were prepared by treatment of the glycolipids with 0.5 M HCl/CH_3OH at 80 ℃ for 1 h. Solvent was removed under a nitrogen stream, and the residue was partitioned between $CHCl_3$ and H_2O. FAMEs in organic phase were analyzed by GC-MS.

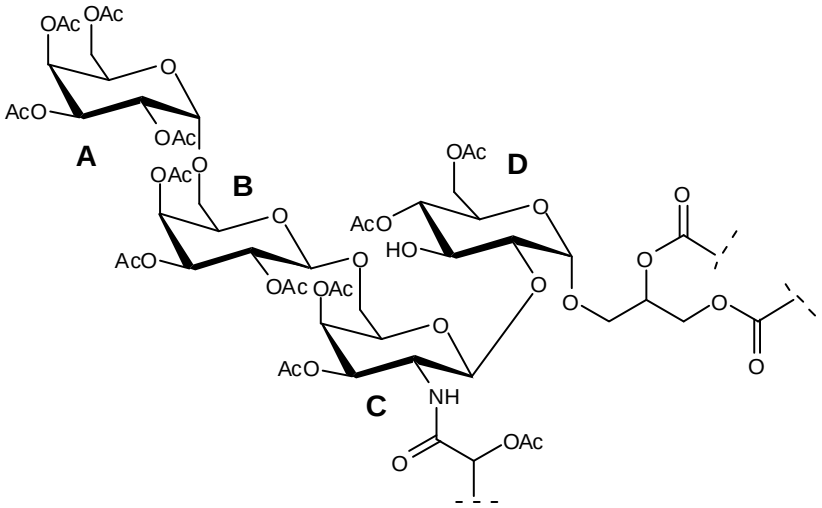
The sugar composition analysis was determined by GC-MS. The GC-MS analyses of glycolipid were performed by methanolysis with 0.5 M methanolic/HCl at 80 ℃ for 16 h, re-*N*-acetylation with pyridine/acetic anhydride (in low temperature with equivalent quantity of acetic anhydride), and trimethylsilylation with Sylon HTP (HMDS/TMCS/pyridine = 3:1:9) trimethylsilylation reagent. The final trimethylsilylated (TMS) derivatives were kept in *n*-hexane for GC-MS analysis.

For the carbohydrate linkage analysis, the Hakomori methylation analysis was carried out. The glycolipid was per-*O*-methylated with methyl iodide and dimethylsulfoxide anion in dimethylsulfoxide, and then hydrolyzed by 2 M trifluoroacetic acid at 100 ℃ for 5 h. The solvent was evaporated by compressed air, the residue was reduced with 0.25 M $NaBD_4$ in 1 M NH_4OH for 40 min. The reaction was quenched with 20% HOAc and coevaporated with CH_3OH. The residue was then per-acetylated with Ac_2O/pyridine (1:1, v/v) overnight, dried with toluene, and finally analyzed by GC-MS.

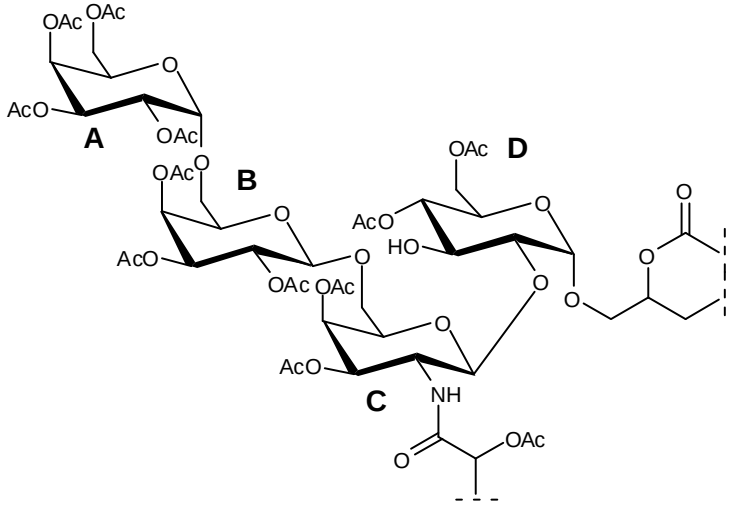
The GC-MS programs for analyses of TMS and FAMEs were set up at 60 ℃ for 1 min, increasing to 140 ℃ at 25 ℃ min^{-1}, to 200 ℃ at 5 ℃ min^{-1}, and finally to 300 ℃ at 10 ℃ min^{-1}. For partial methylated aditol acetates derivatives, the oven was programmed at 60 ℃ for 1 min before increasing to 290 ℃ at 8 ℃ min^{-1}, and finally to 300 ℃ at a rate of 10 ℃ min^{-1}. Peaks were analyzed by GC-MS and compared with the database. The arabitol derivative was used as an internal standard.



2a



3a



4a

Structures of compounds 2a-4a

Fatty acid compositions of 1-4

	Compositions (%)		
Fatty acids	**1**	**2**	**3** and **4**
Isobranched			
14:0	3.2	-	-
15:0	46.3	27.0	37.6
16:0	17.9	20.9	15.8
17:0	17.2	23.6	17.5
Anteisobranched			
15:0	5.0	8.9	6.8
17:0	2.8	19.6	12.0
Unsaturated			
18:1	4.5	-	-
others	3.1	-	10.3

NMR Data (500 MHz) of 1 in $CDCl_3/CD_3OD$ (10:1) and 1a in C_6D_6.[a]

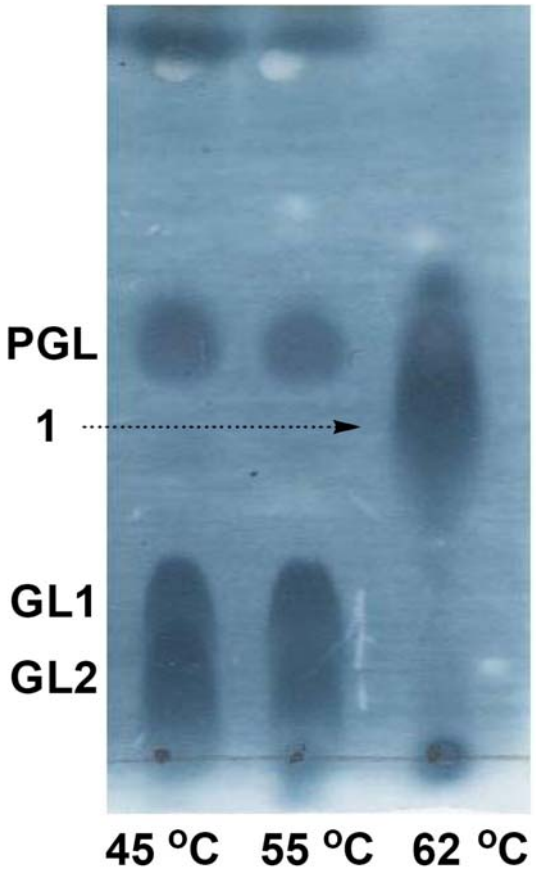
	1		**1a**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
A1	5.02 (s)	109.5	5.55 (s)	107.5
A2	3.91 (d, 2.4)	80.7	5.33 (d, 1.0)	81.7
A3	3.83 (dd, 4.8, 2.4)	77.5	5.39 (dd, 3.5, 1.0)	76.7
A4	3.90 (m)	84.6	4.87 (dd, 4.9, 3.5)	81
A5	3.57 (m)	71	5.87 (ddd, 6.0, 5.1, 4.9)	69.7
A6	3.46 (m)	62.9	4.61 (dd, 11.6, 5.1)	62.1
			4.53 (dd, 11.6, 6.0)	
B1	4.71 (d, 3.7)	98.5	5.63 (d, 3.9)	96.7
B2	3.71 (m)	67.8	5.57 (dd, 10.6, 3.9)	70.7
B3	3.61 (m)	77.4	4.58 (dd, 10.6, 3.4)	72
B4	3.90 (m)	69.3	5.82 (d, 3.4)	70.1
B5	3.65 (m)	70.4	4.56 (m)	62.4
B6	3.55 (m)	61	4.42 (m)	61.9
C1	4.52 (d, 8.4)	101.1	5.51 (d, 8.4)	99.7
C2	3.44 (m)	56.3	3.25 (ddd, 10.0, 8.4, 7.7)	57.2
C3	3.27 (m)	76.1	6.71 (t, 10.0)	71.2
C4	3.21 (m)	70.9	5.43 (m)	69.4
C5	3.32 (m)	74.4	3.55 (m)	73.1
C6	3.72 (m)	66.5	3.71 (dd, 11.9, 2.9)	65.4
	3.59 (m)		3.62 (m)	
NH			5.10 (d, 7.7)	
D1	4.68 (d, 3.7)	98.5	5.38 (d, 3.6)	99.1
D2	3.38 (m)	79.1	4.07 (dd, 9.5, 3.6)	76.1
D3	3.61 (m)	72	5.99 (t, 9.5)	72.1
D4	3.24 (m)	69.9	5.47 (m)	69.2
D5	3.35 (m)	71.1	4.47 (m)	67.7
D6	3.55 (m)	61.0	4.44 (m)	67.3
			4.30 (m)	
Diol				
1	3.44 (m)	69.3	4.09 (m)	70.1
	3.32 (m)		4.01 (dd, 10.7, 5.4)	
2	4.81 (m)	72.5	5.51 (m)	72.2

[a] The lipids signals are not listed.

NMR data (500 MHz) of 2a-4a in C_6D_6.[a]

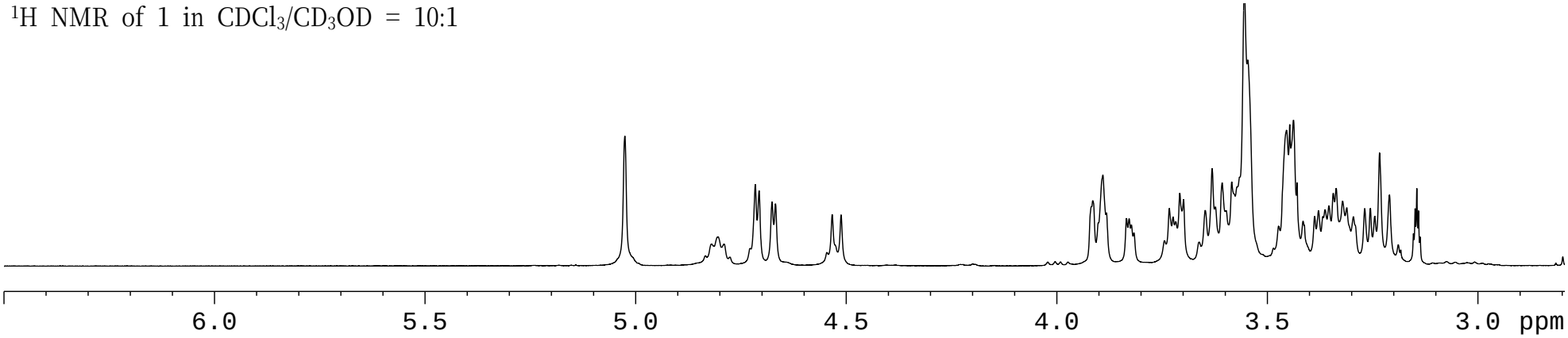
	2a		3a		4a	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
A 1	5.16 (d, 3.2)	96.8	5.13 (d, 3.2)	96.9	5.15 (d, 3.2)	96.8
A2	5.60 (dd, 10.8, 3.2)	68.1	5.64 (dd, 8.4, 3.2)	68.0	5.64 (dd, 8.4, 3.2)	68.0
A3	5.76 (dd, 10.8, 3.2)	68.0	5.74 (dd, 8.4, 3.0)	68.1	5.74 (dd, 8.4, 3.0)	68.1
A4	5.81 (d, 3.2)	68.4	5.86 (br s)	68.3	5.86 (br s)	68.3
A5	4.34 (dd, 6.4, 5.6)	67.3	4.48 (m)	67.4	4.48 (m)	67.4
A6	4.39 (dd, 10.0, 5.6)	61.6	4.46 (m)	61.6	4.46 (m)	61.6
	4.22 (dd, 10.0, 6.4)		4.31 (m)		4.31 (m)	
B1	4.46 (d, 8.0)	101.2	4.44 (d, 8.8)	102.3	4.48 (d, 8.8)	101.9
B2	5.61 (dd, 10.0, 8.0)	68.0	5.60 (dd, 8.8, 7.2)	69.0	5.6 (dd, 8.8, 7.2)	69.0
B3	5.23 (dd, 10.0, 3.2)	71.5	5.21 (m)	71.1	5.21 (m)	71.1
B4	5.54 (d, 3.2)	67.8	5.51 (br s)	68.0	5.51 (br s)	68.0
B5	3.54 (dd, 6.8, 6.0)	71.8	3.48 (m)	72.0	3.48 (m)	72.0
B6	3.85 (dd, 10.0, 6.0)	65.9	3.89 (m)	66.9	3.89 (m)	66.9
	3.37 (dd, 10.0, 6.8)		3.48 (m)		3.48 (m)	
C1	5.43 (d, 8.4)	100.4	4.72 (d, 8.4)	104.2	4.64 (8.4)	104.3
C2	3.66 (ddd, 11.2, 10.4, 8.4)	53.7	4.63 (dt, 12.0, 8.4)	51.2	4.63 (dt, 12.0, 8.4)	51.2
C3	6.06 (dd, 11.2, 2.8)	68.6	5.28 (dd, 12.0, 3.2)	70.8	5.28 (dd, 12.0, 3.2)	70.8
C4	5.63(br s)	67.8	5.51 (d, 3.2)	67.1	5.51 (d, 3.2)	67.1
C5	3.86(m)	72.4	4.05 (m)	72.3	4.05 (m)	72.3
C6	4.07(m)	67.3	4.30 (m)	69.7	4.06 (m)	69.5
	3.85(m)		3.65 (dd, 12.0, 3.2)		3.71 (m)	
NH	5.82 (d, 10.4)		6.85 (d, 8.4)		6.65 (d, 8.4)	
D1	5.24 (d, 3.2)	99.1	5.45 (d, 3.2)	98.8	5.42 (d, 3.2)	98.9
D2	4.07 (dd, 10.0, 3.2)	76.0	3.71 (m)	82.7	3.71 (m)	82.7
D3	5.81 (dd, 10.0, 9.6)	72.2	4.46 (m)	70.3	4.46 (m)	70.3
D4	5.31 (t, 9.6)	69.2	5.32 (dd, 10.0, 9.6)	70.1	5.32 (dd, 10.0, 9.6)	70.1
D5	4.19(m)	68.0	4.21 (m)	68.6	4.21 (m)	68.6
D6	4.46(m)	62.1	4.35 (dd, 12.0, 5.6)	62.6	4.44 (m)	62.7
	4.16(m)		4.23 (m)		4.27 (m)	
Diol 1					4.43 (m)	70.2
					3.90 (m)	
2					5.44 (m)	72.4
Glycerol 1	3.96 (dd, 10.8, 5.6)	66.9	4.17 (dd, 11.2, 5.4)	67.0		
	3.64 (m)		3.97 (dd, 11.2, 4.4)			
2	5.48 (m)	69.7	5.68 (m)	70.3		
3	4.70 (dd, 12.0, 2.4)	63.0	4.88 (dd, 12.0, 2.4)	63.3		
	4.57 (dd, 12.0, 6.4)		4.63 (dd, 12.0, 7.6)			
CO*CH*(OH)			4.96 (dd, 6.8, 5.6)	75.0	4.96 (dd, 6.8, 5.6)	75.0

[a] The lipids signals are not listed.

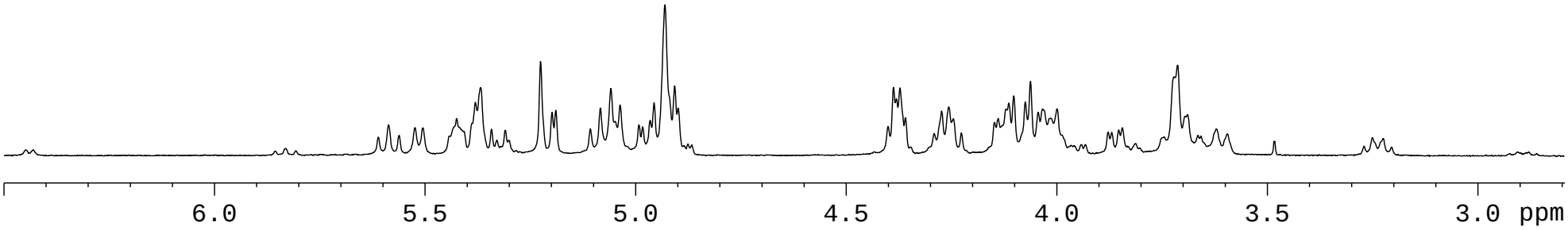


TLC profiles ($CHCl_3/CH_3OH$ = 3:1) of polar lipid extracts from *M. taiwanensis*. PGL: phosphoglycolipid; GL: glycolipid

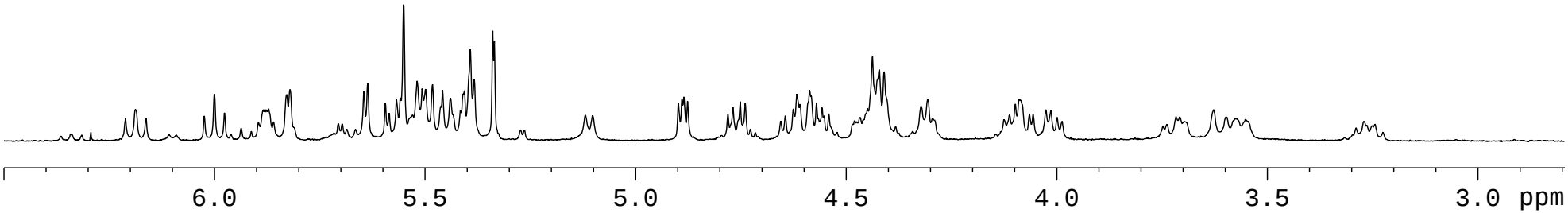
^{1}H NMR of 1 in $CDCl_3/CD_3OD$ = 10:1



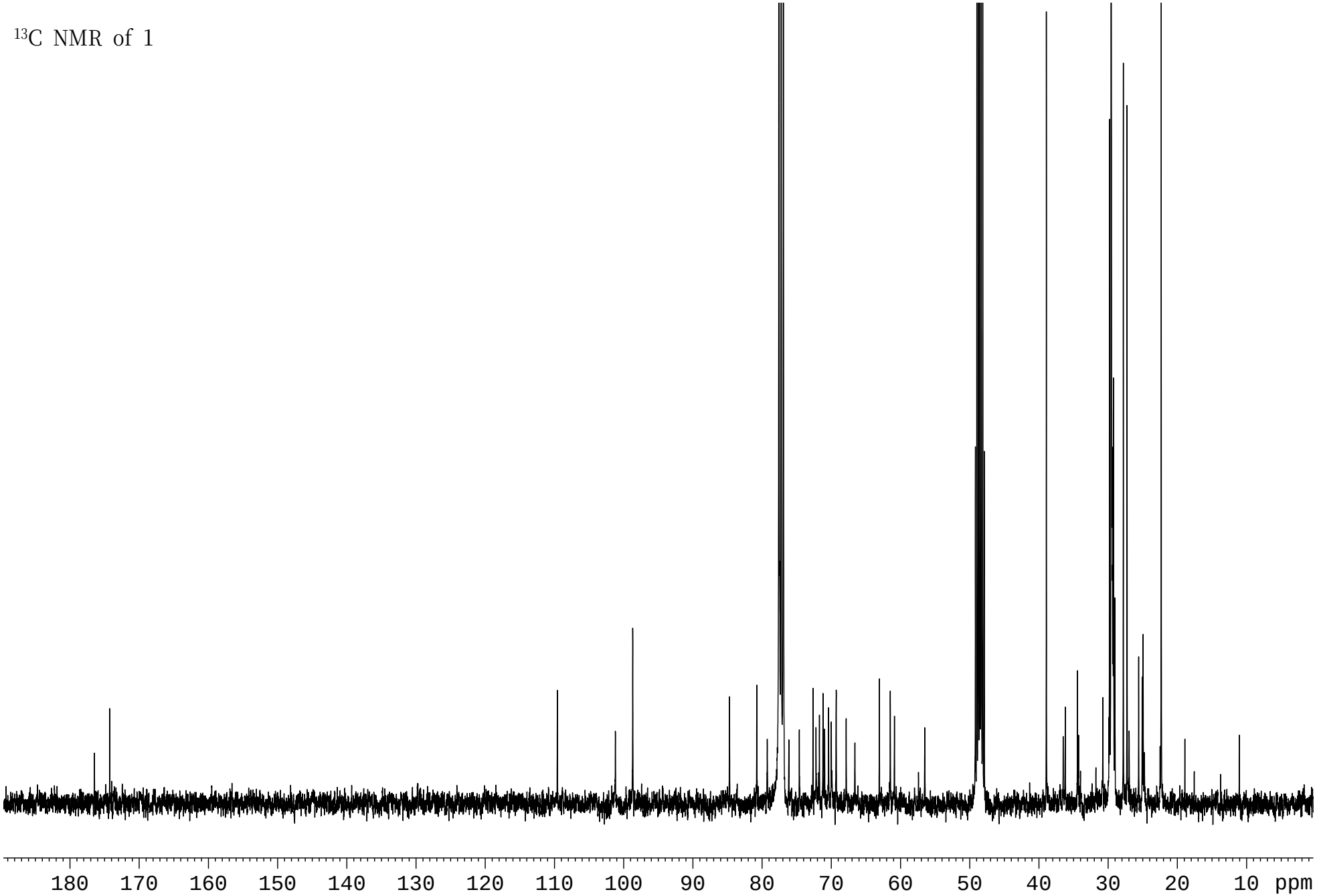
^{1}H NMR of 1a in $CDCl_3$



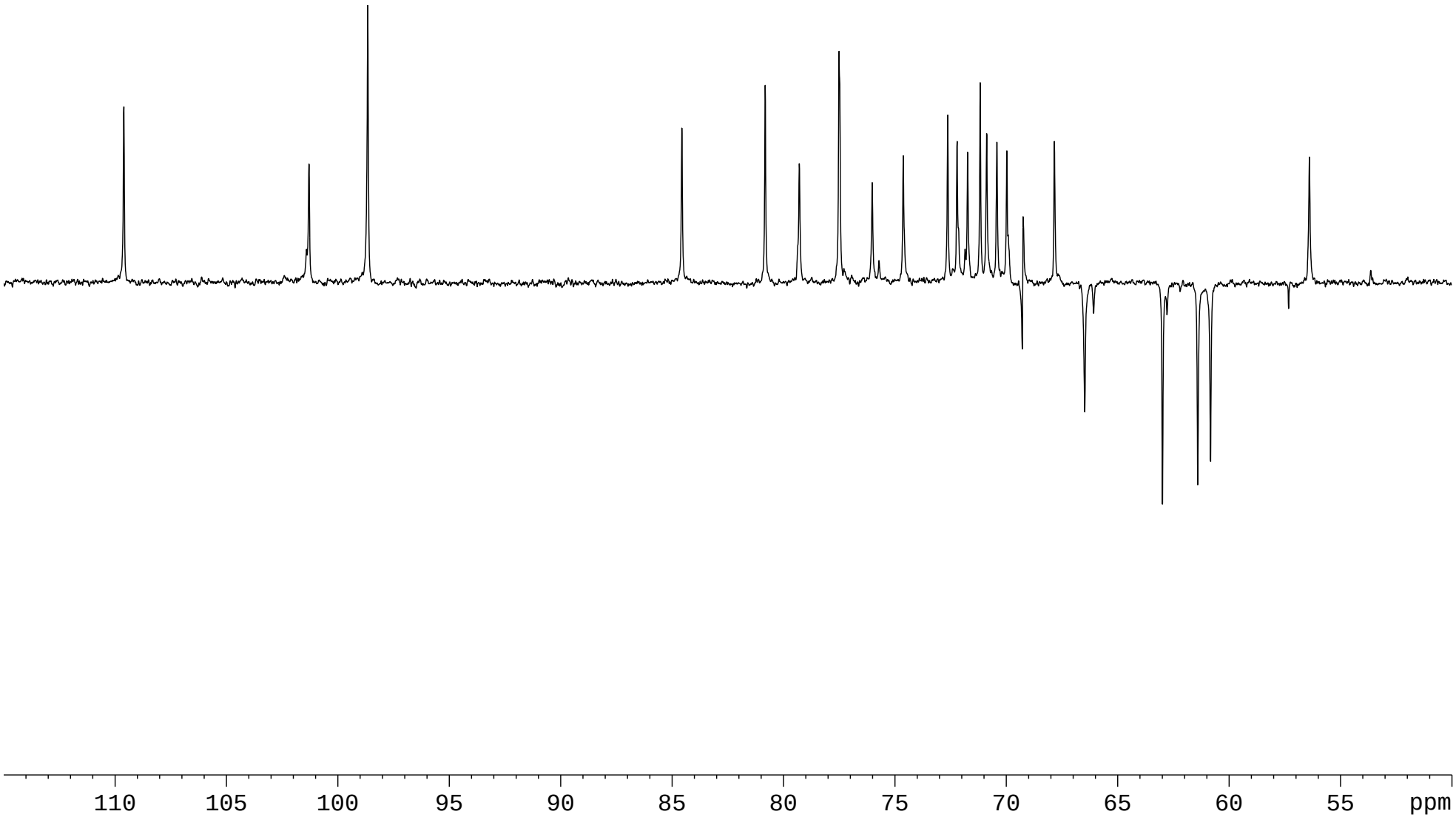
^{1}H NMR of 1a in C_6D_6

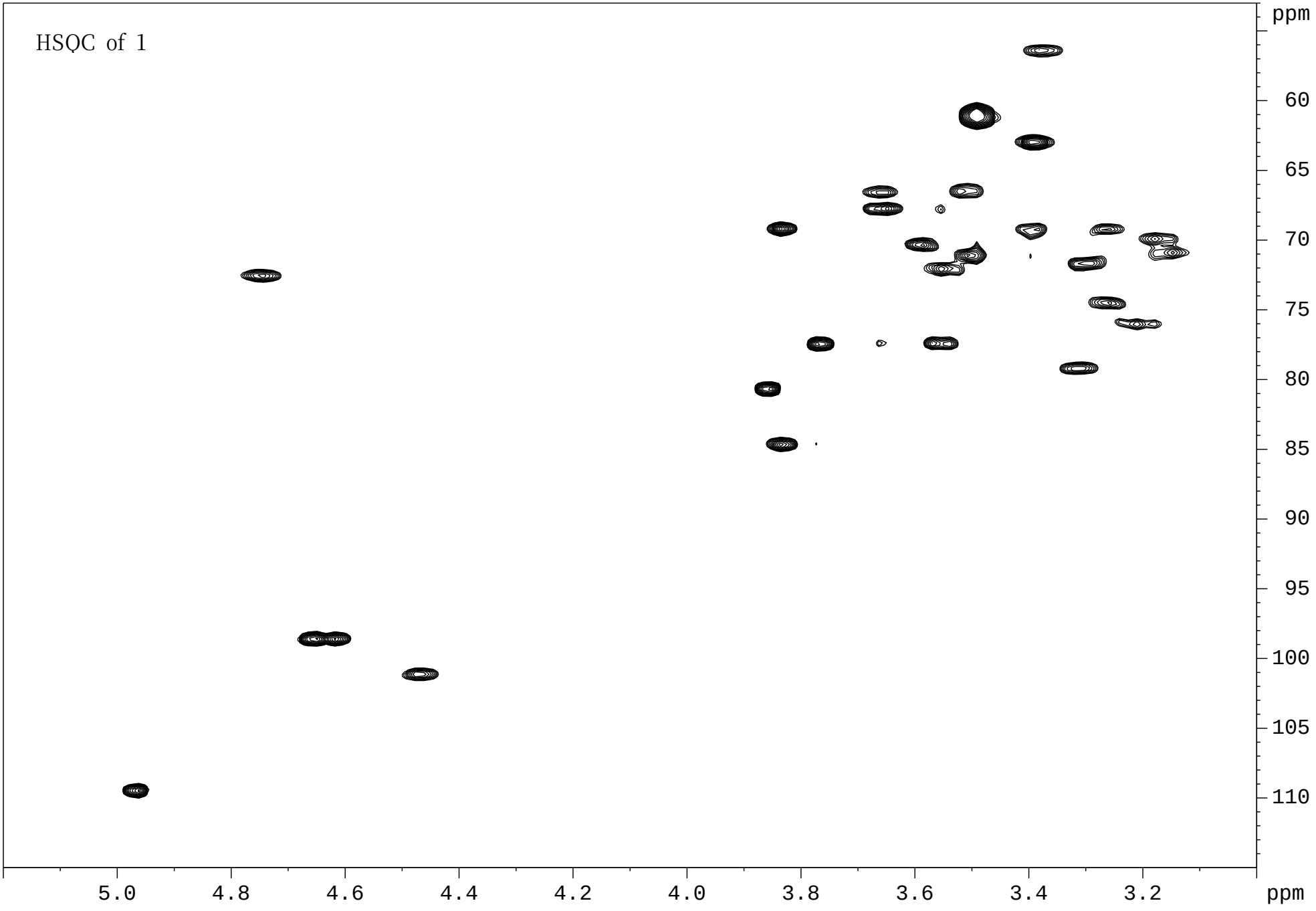


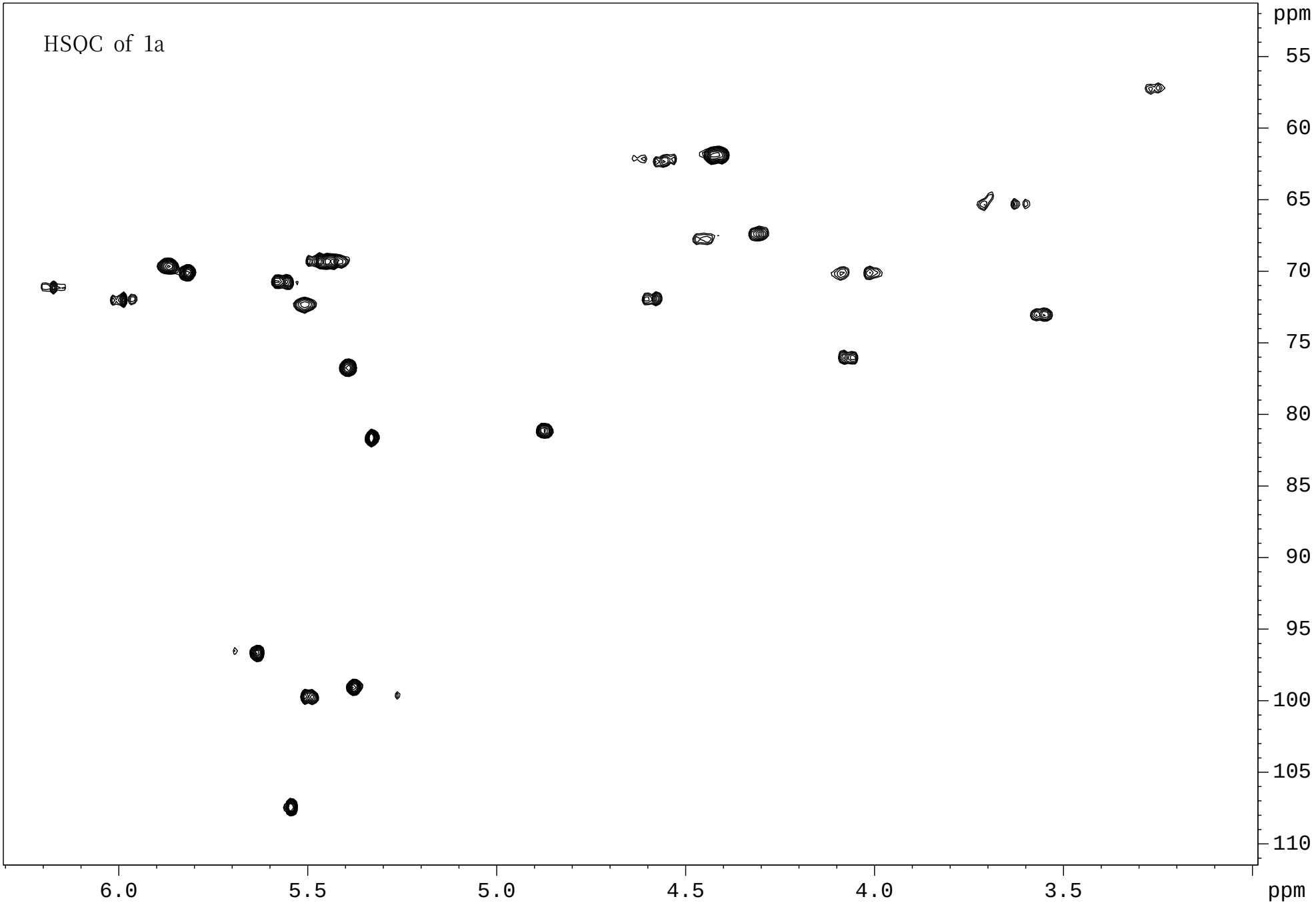
^{13}C NMR of 1

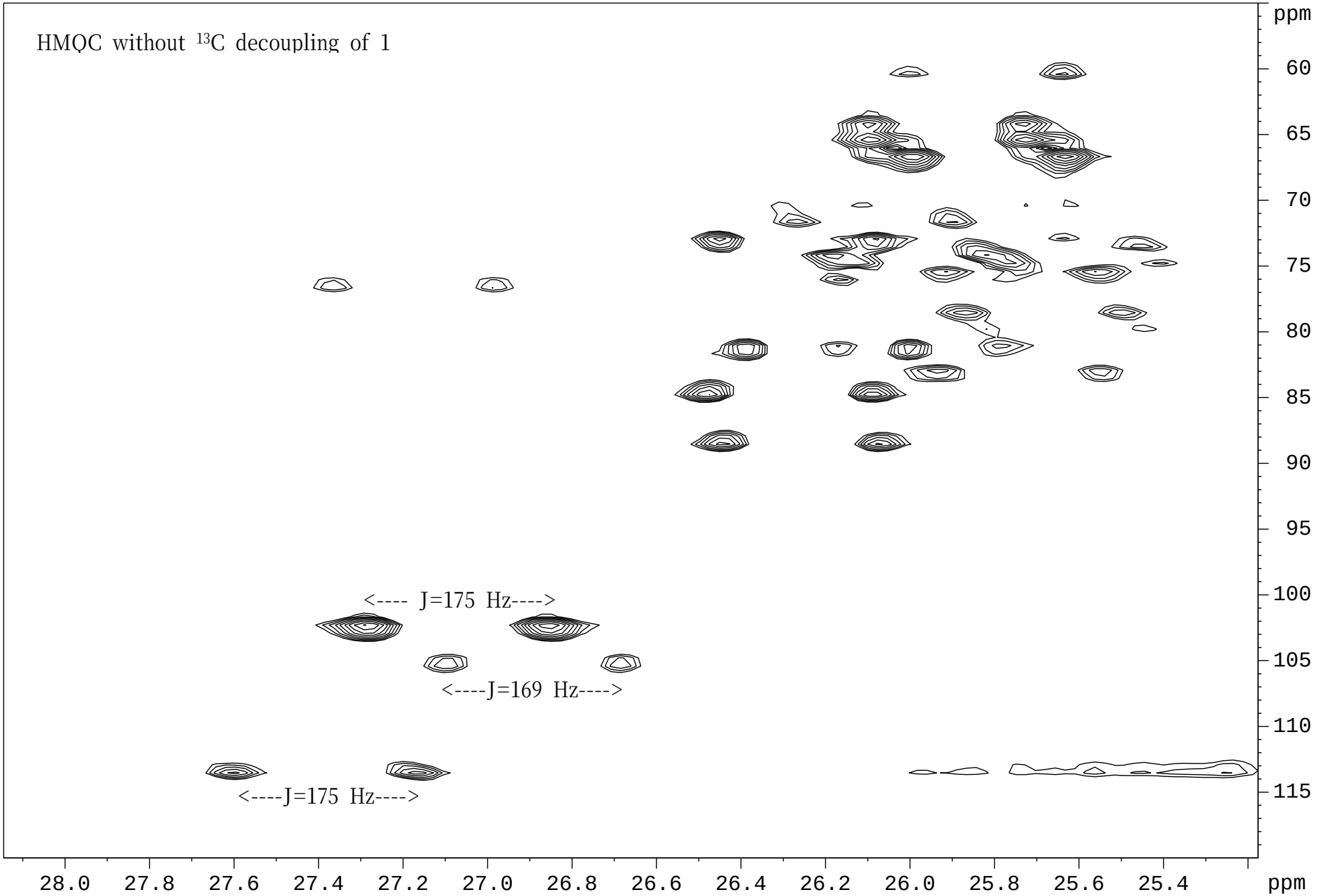


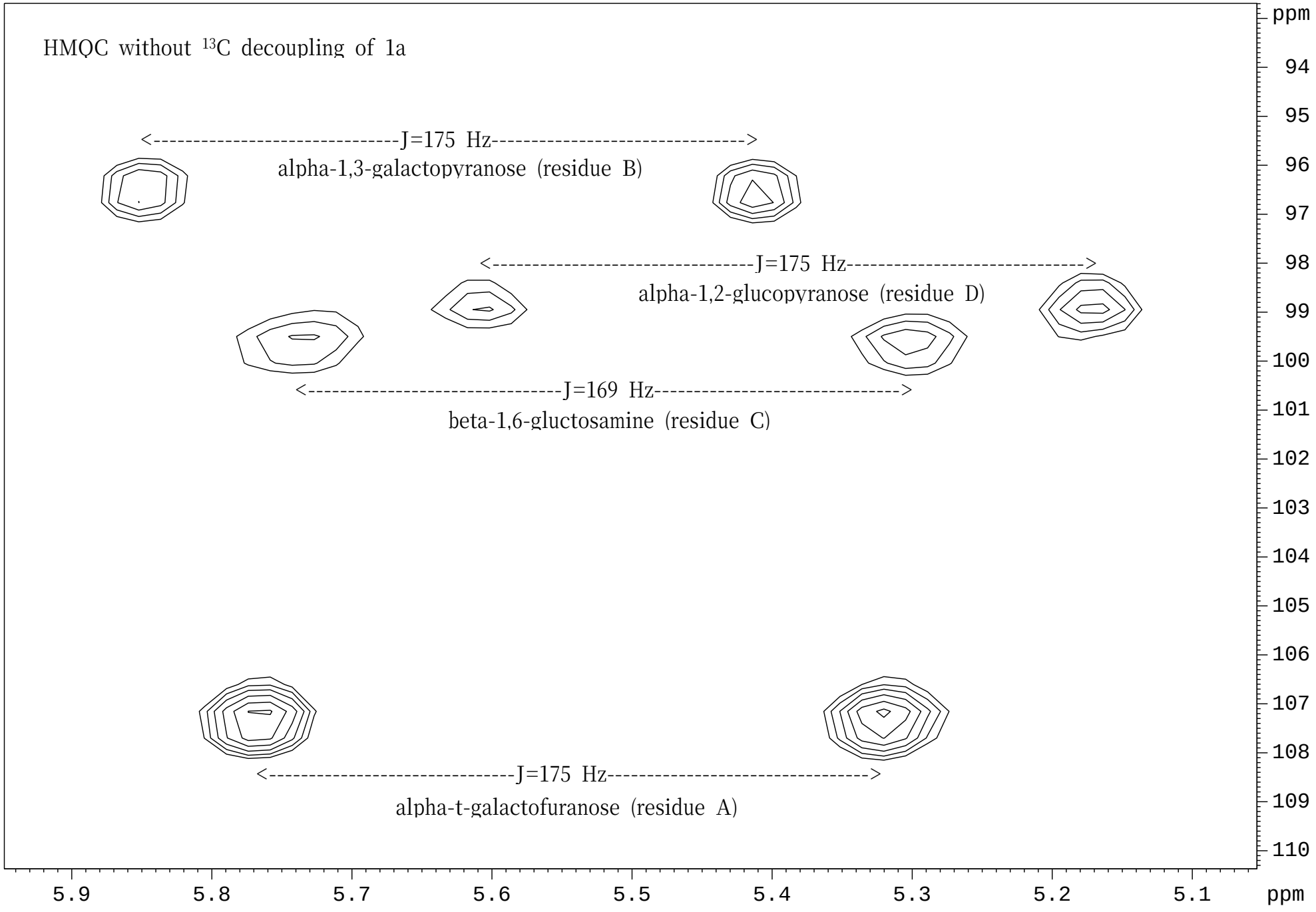
DEPT135 of 1

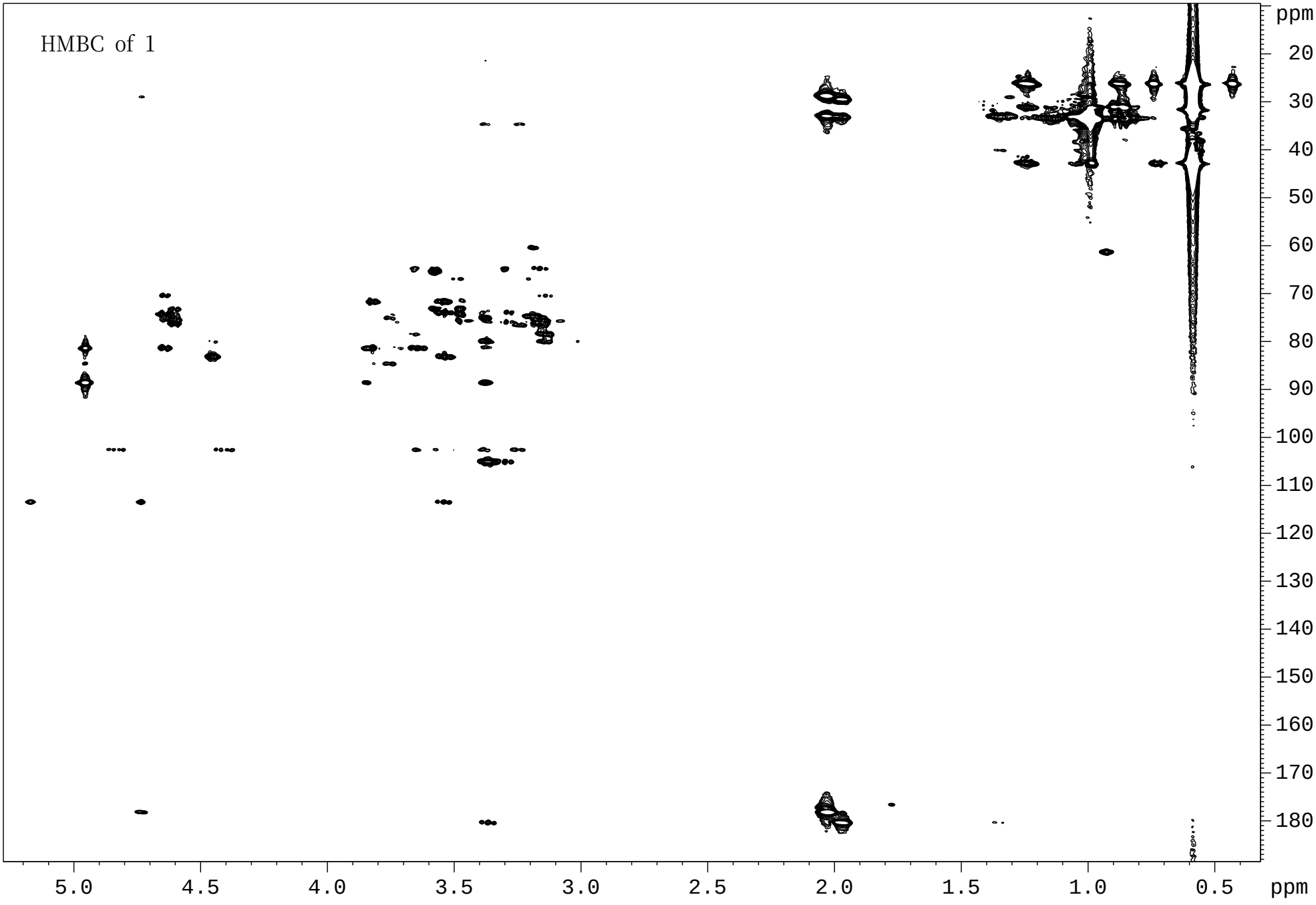


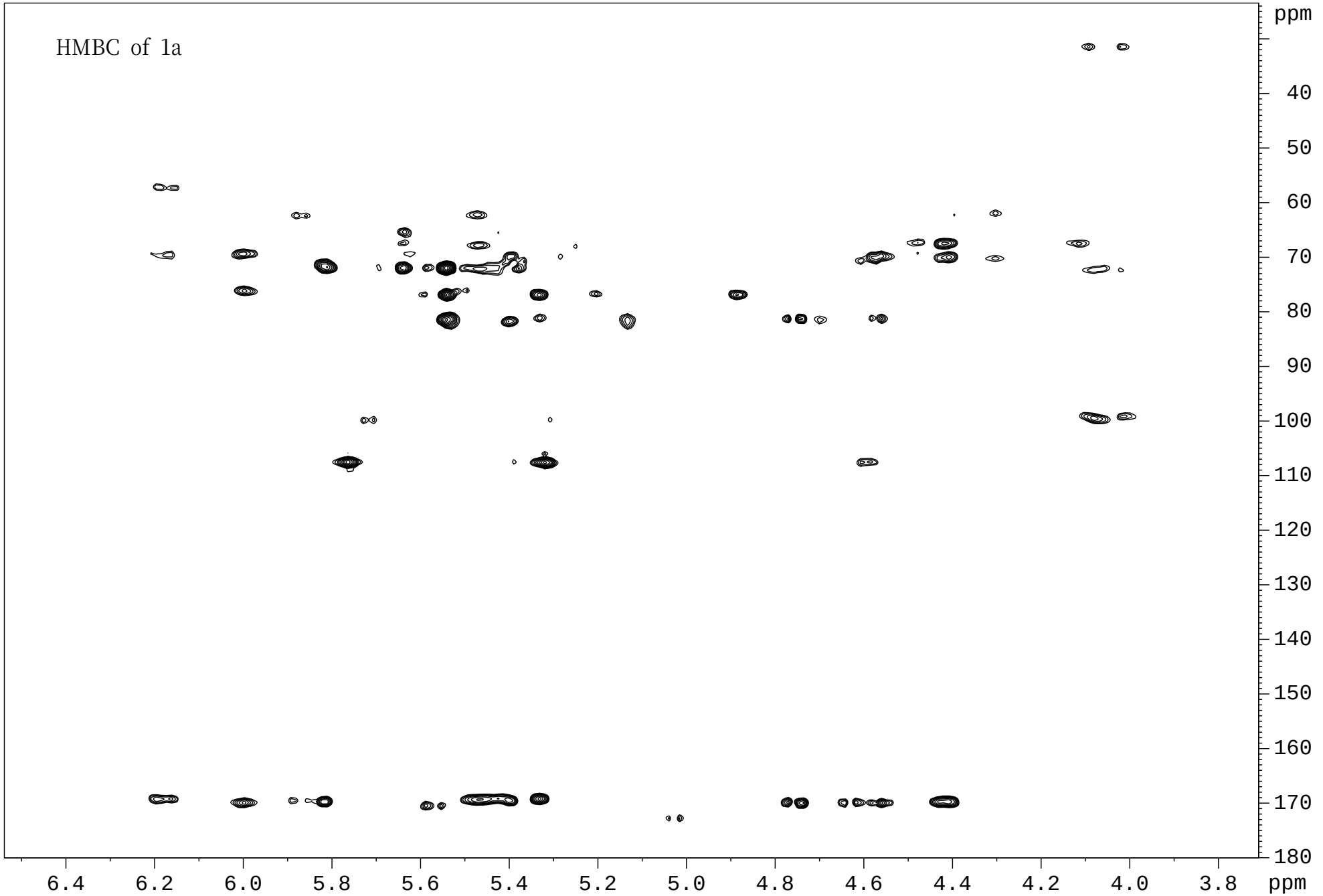


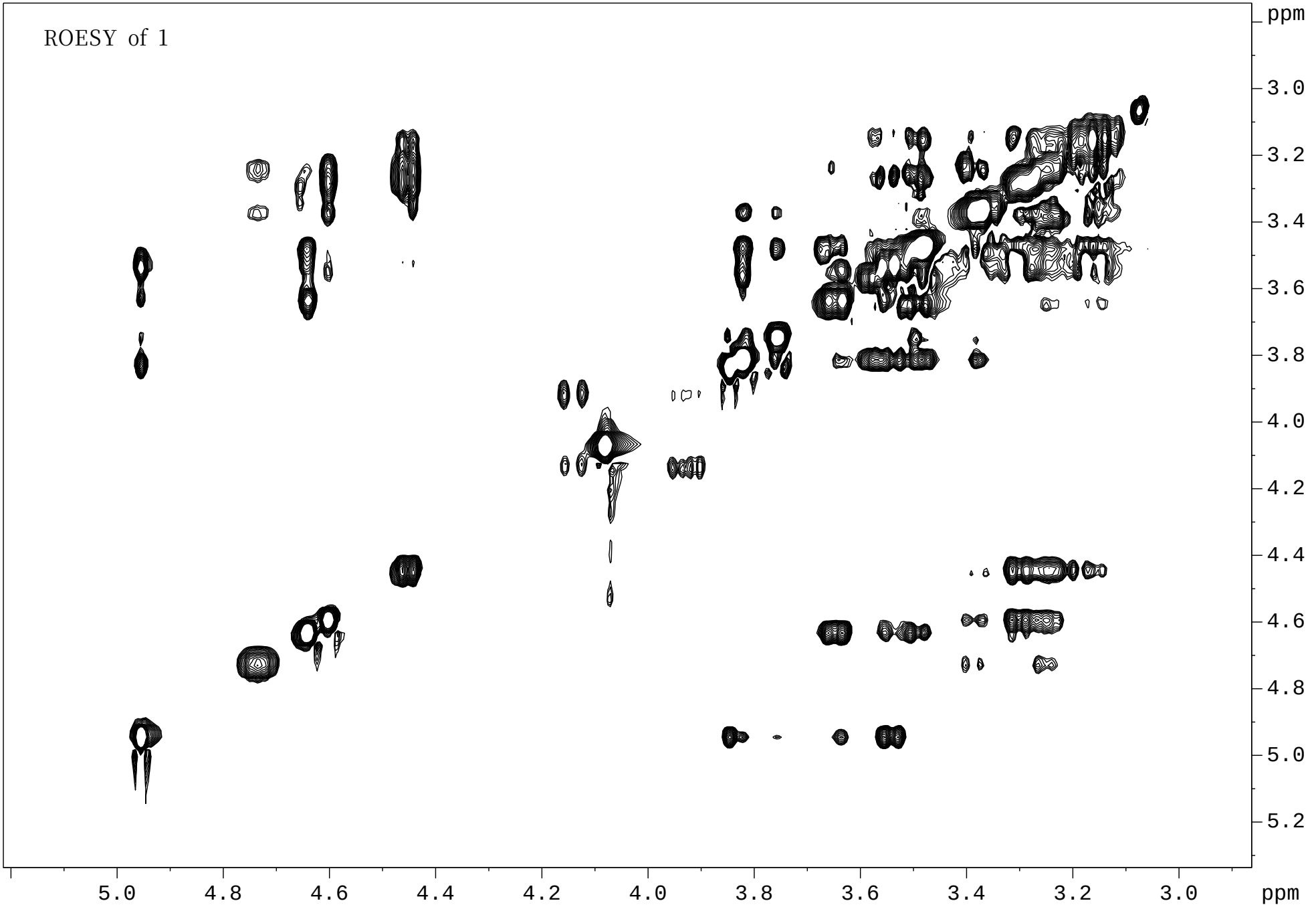


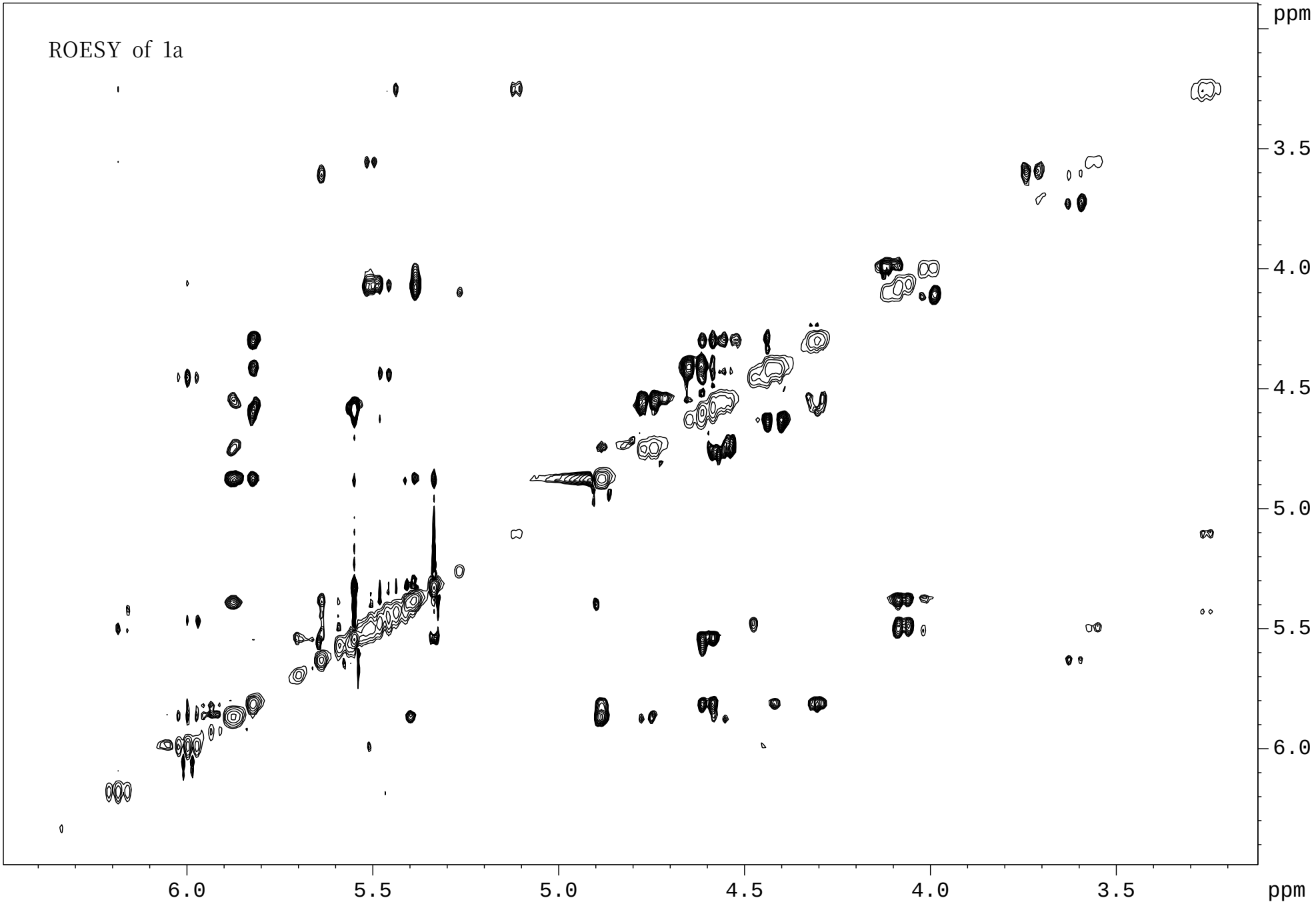


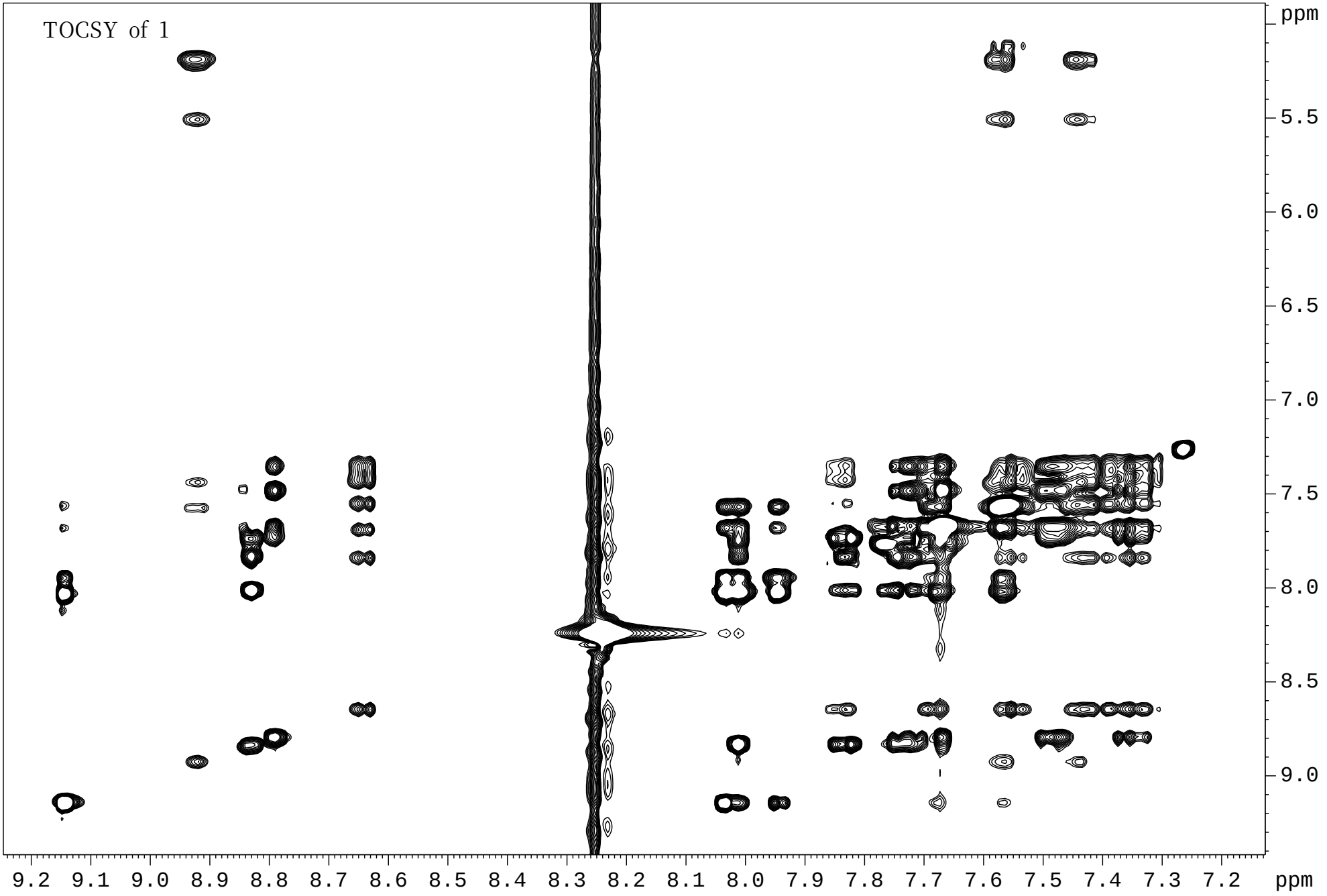


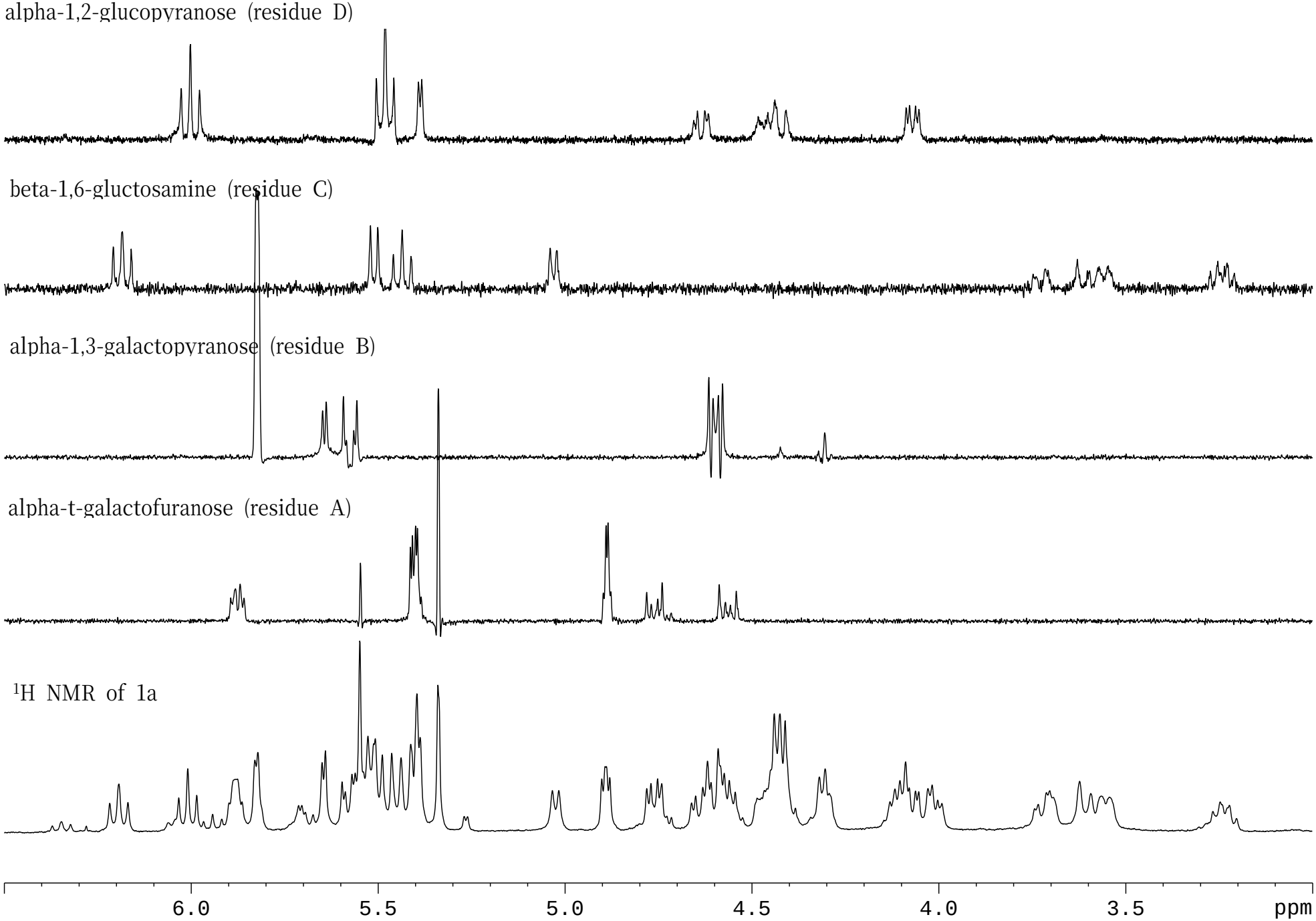


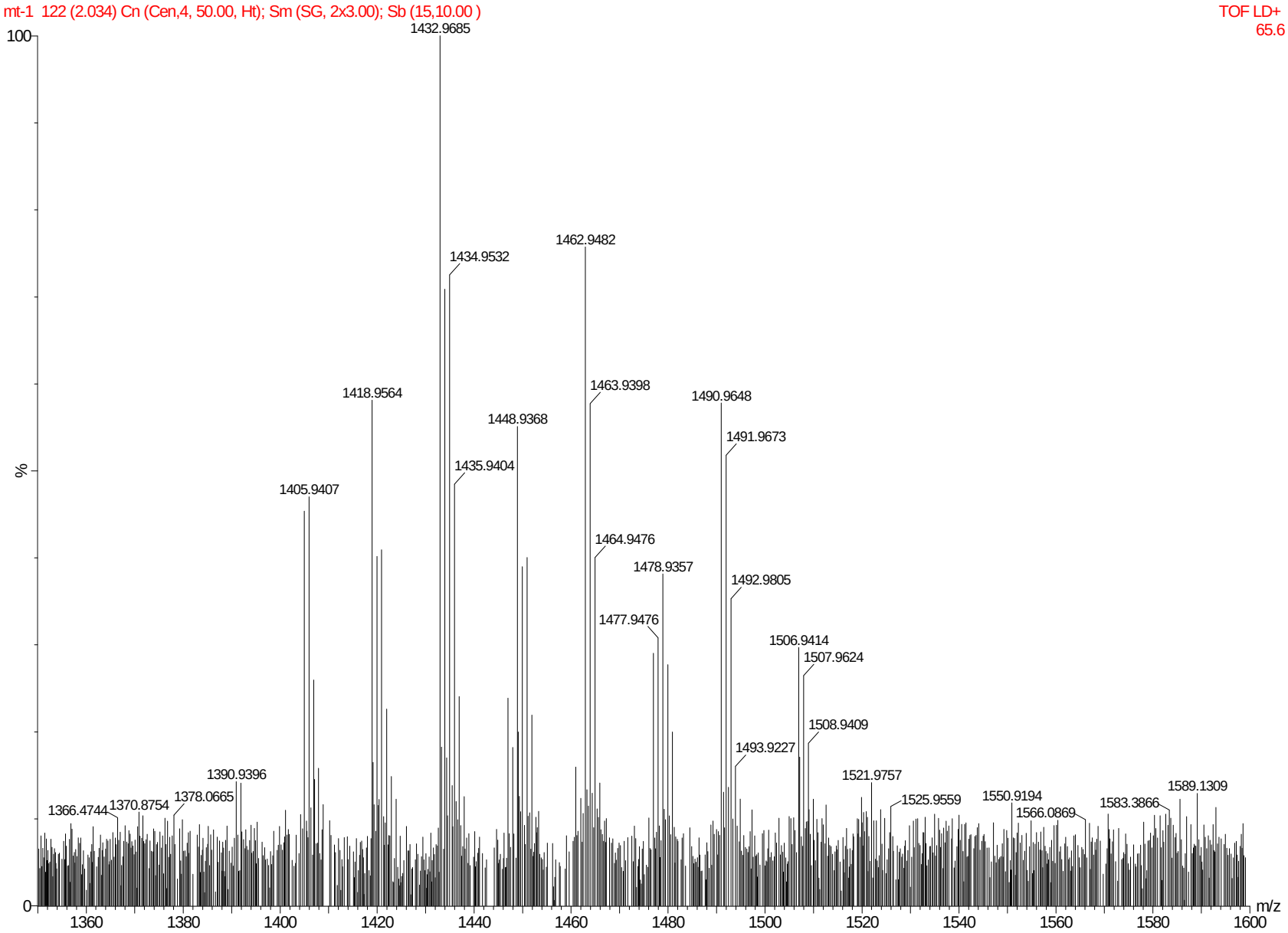


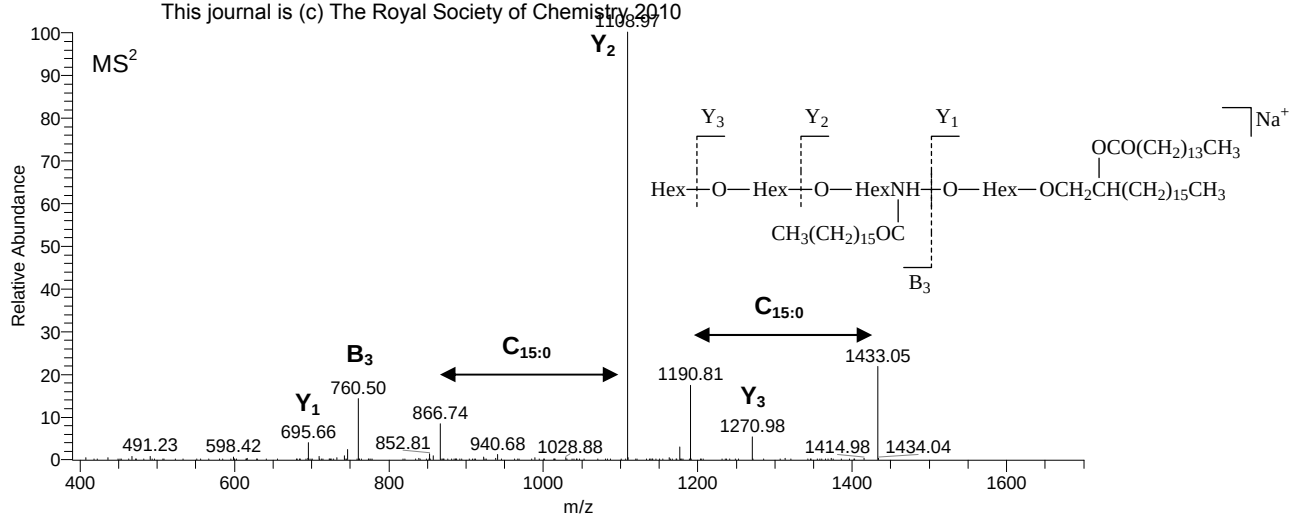


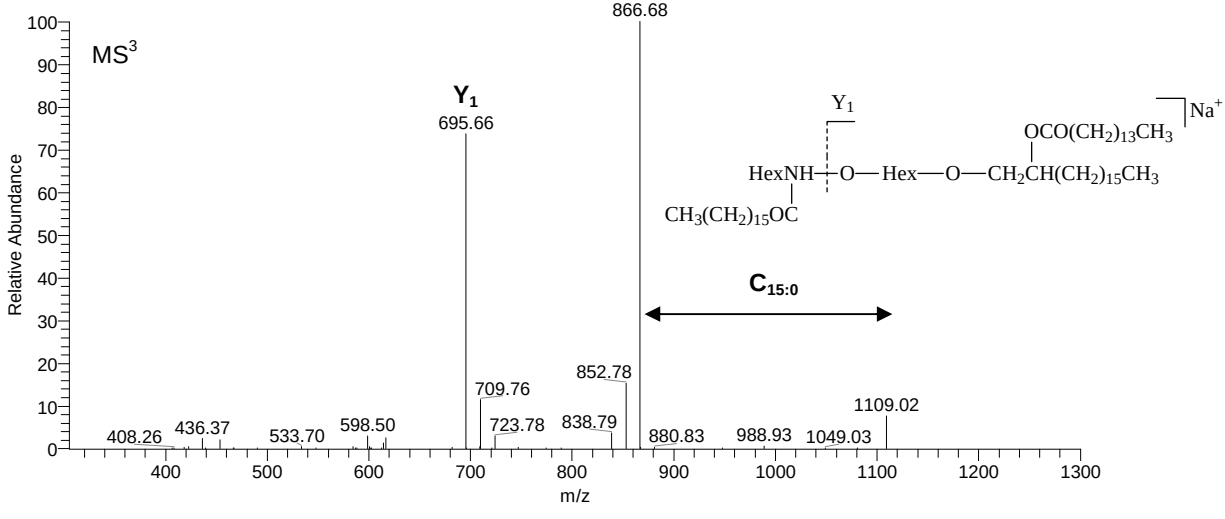


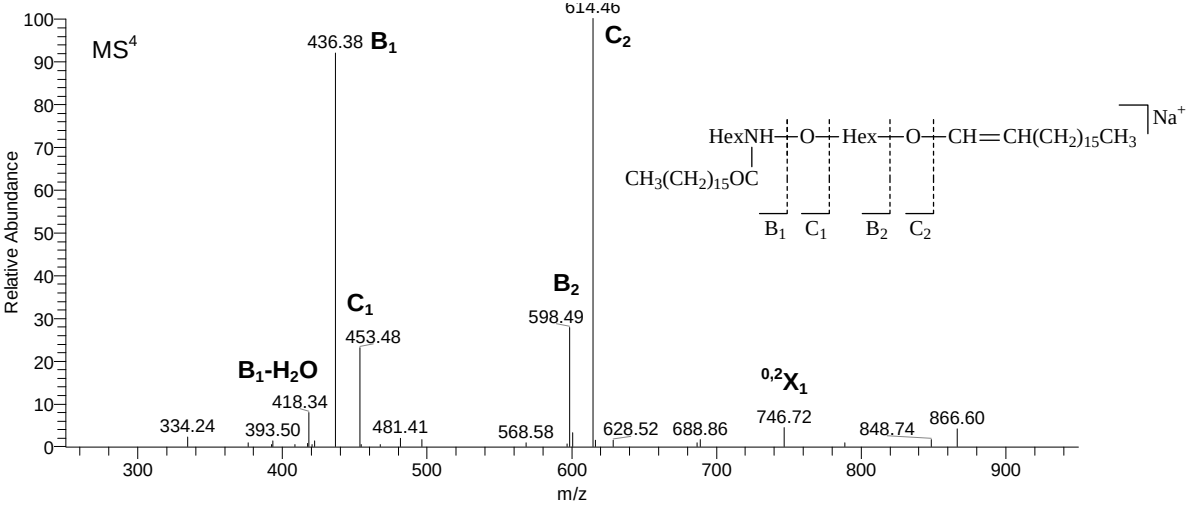




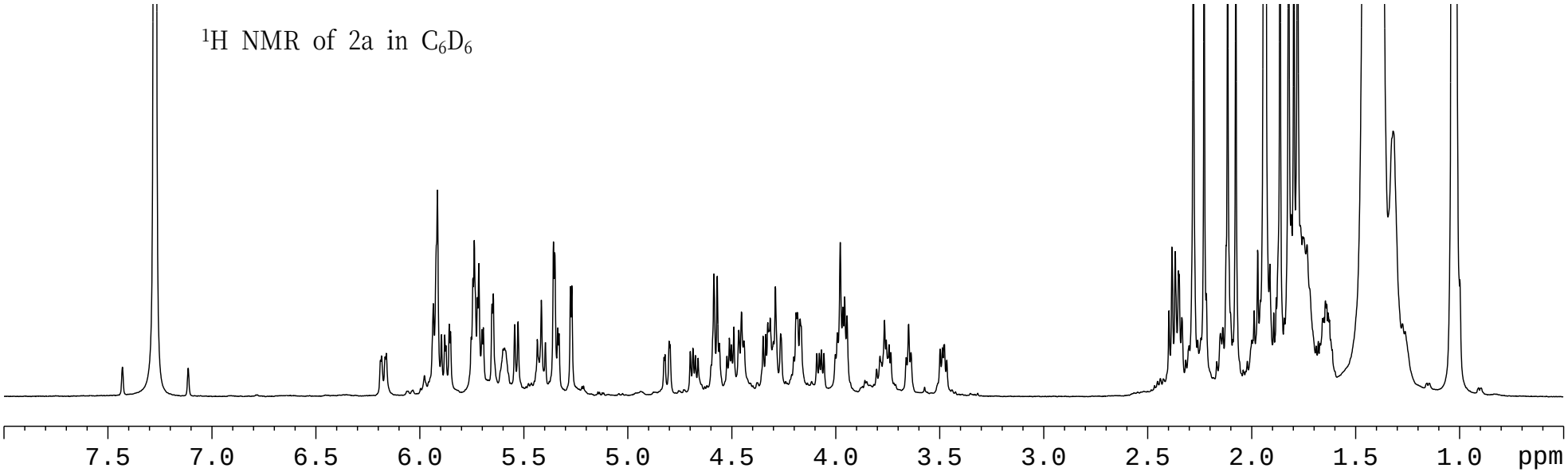



MS2
Relative Abundance
m/z
1108.97
Y2
B3
760.50
C15:0
Y1
695.66
866.74
852.81
940.68
1028.88
491.23
598.42
1190.81
Y3
1270.98
C15:0
1433.05
1414.98
1434.04
Hex—O—Hex—O—HexNH—O—Hex—OCH2CH(CH2)15CH3
OCO(CH2)13CH3
CH3(CH2)15OC
Y3
Y2
Y1
B3
Na+

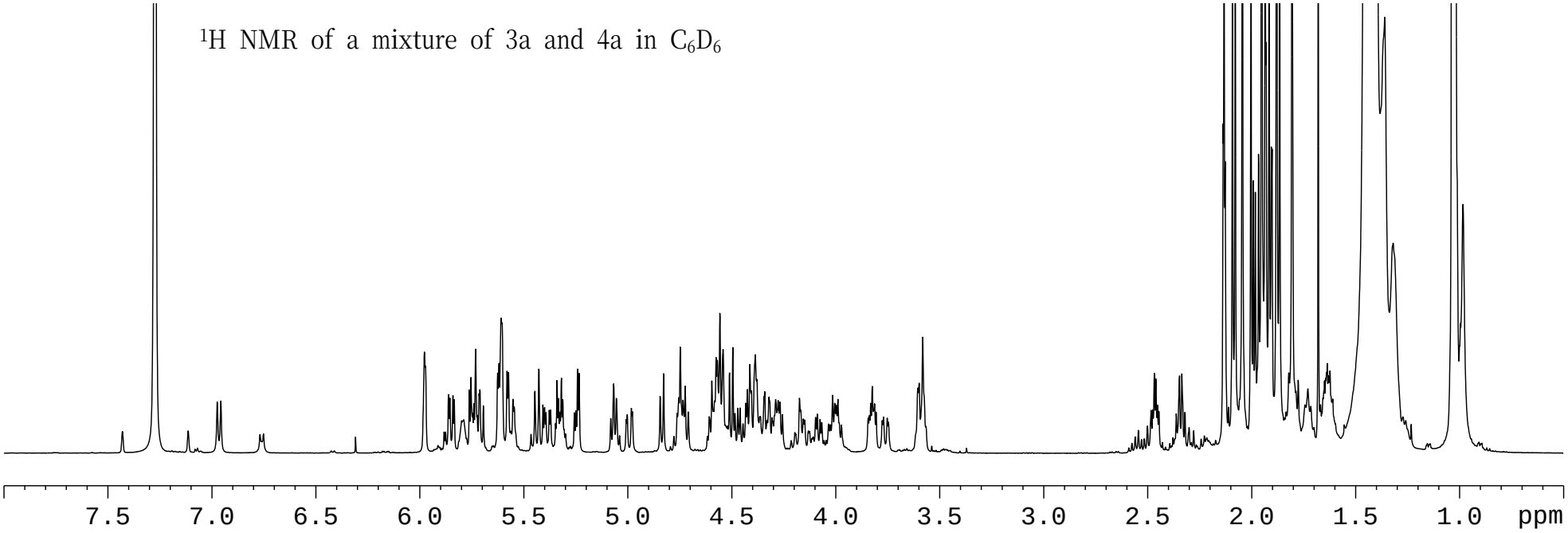

MS3
Relative Abundance
m/z
866.68
Y1
695.66
C15:0
852.78
709.76
723.78
838.79
880.83
988.93
1049.03
1109.02
408.26
436.37
533.70
598.50
HexNH—O—Hex—O—CH2CH(CH2)15CH3
OCO(CH2)13CH3
CH3(CH2)15OC
Y1
Na+

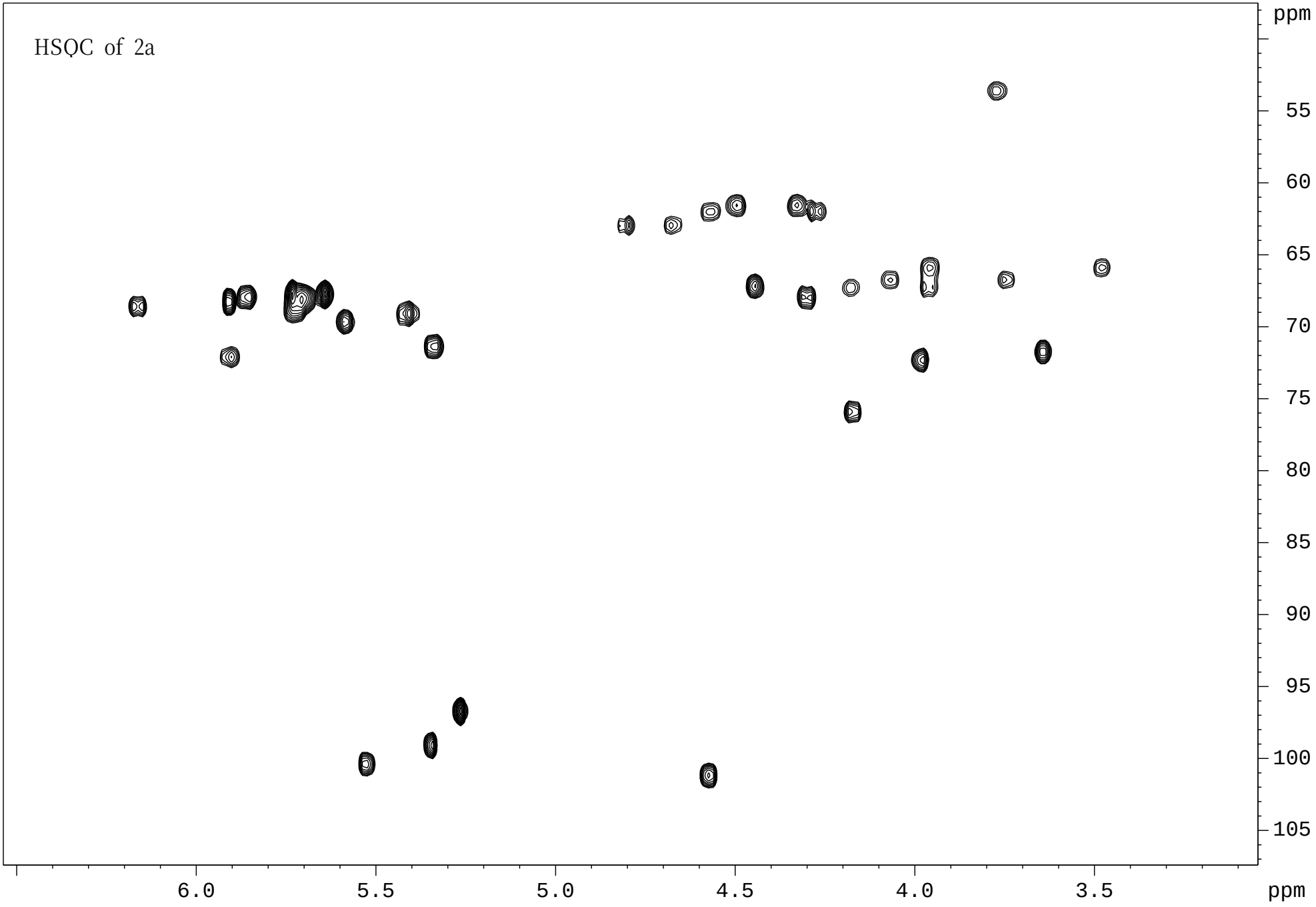

MS4
Relative Abundance
m/z
614.46
C2
436.38 B1
B2
598.49
C1
453.48
B1-H2O
418.34
0,2X1
746.72
866.60
334.24
393.50
481.41
568.58
628.52
688.86
848.74
HexNH—O—Hex—O—CH=CH(CH2)15CH3
CH3(CH2)15OC
B1 C1 B2 C2
Na+

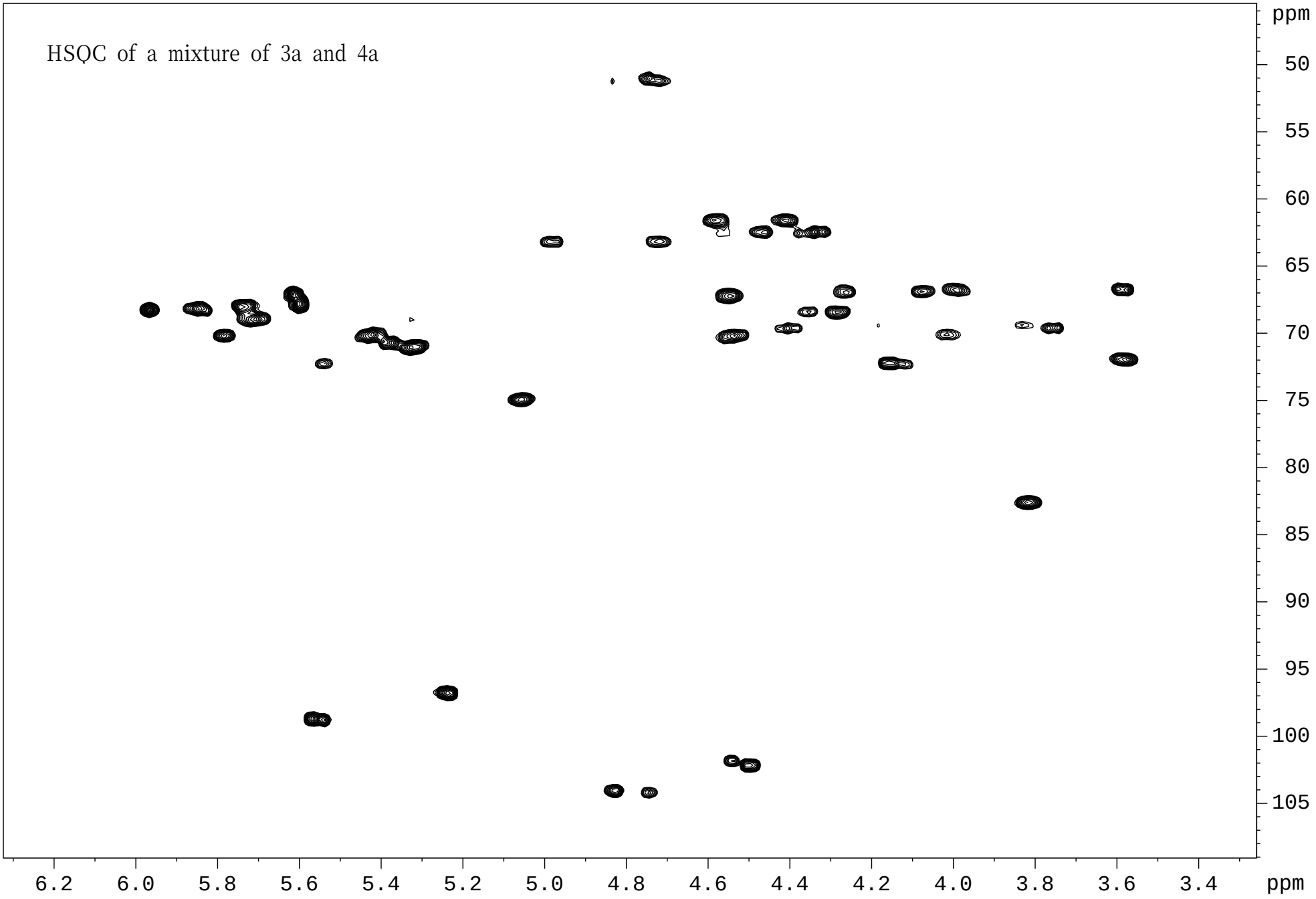
^{1}H NMR of 2a in C_6D_6

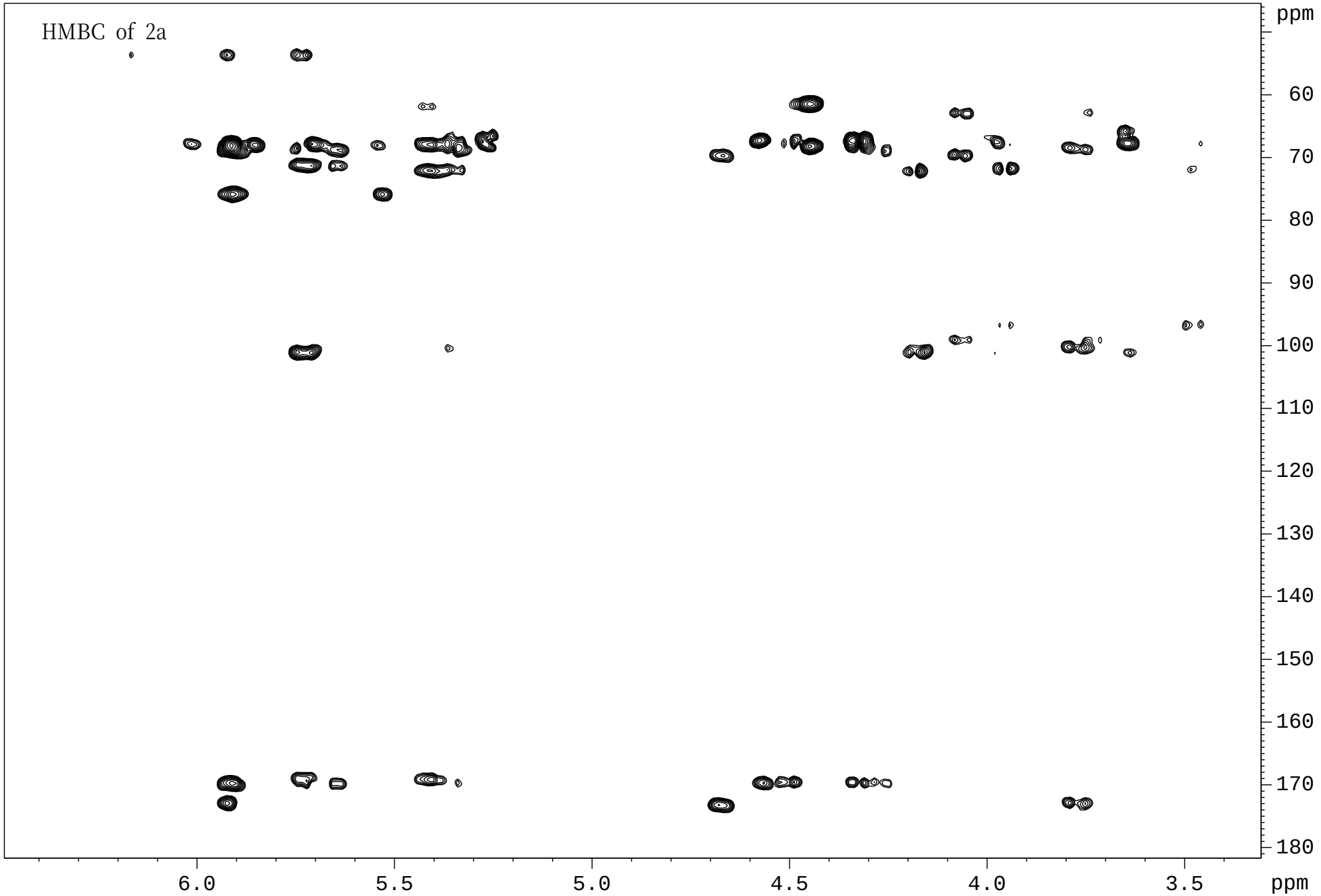


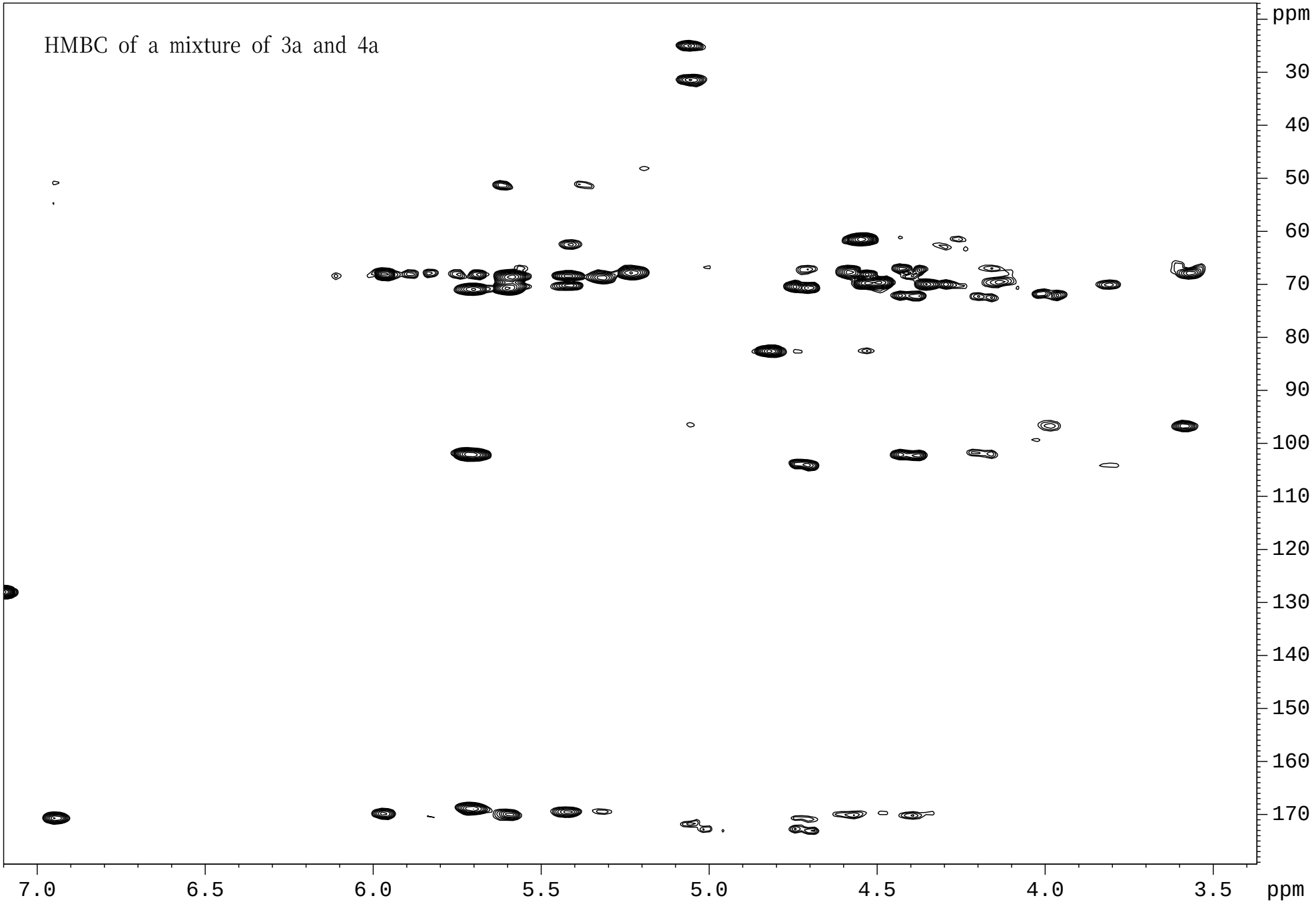
^{1}H NMR of a mixture of 3a and 4a in C_6D_6











MALDI-TOF of 2a

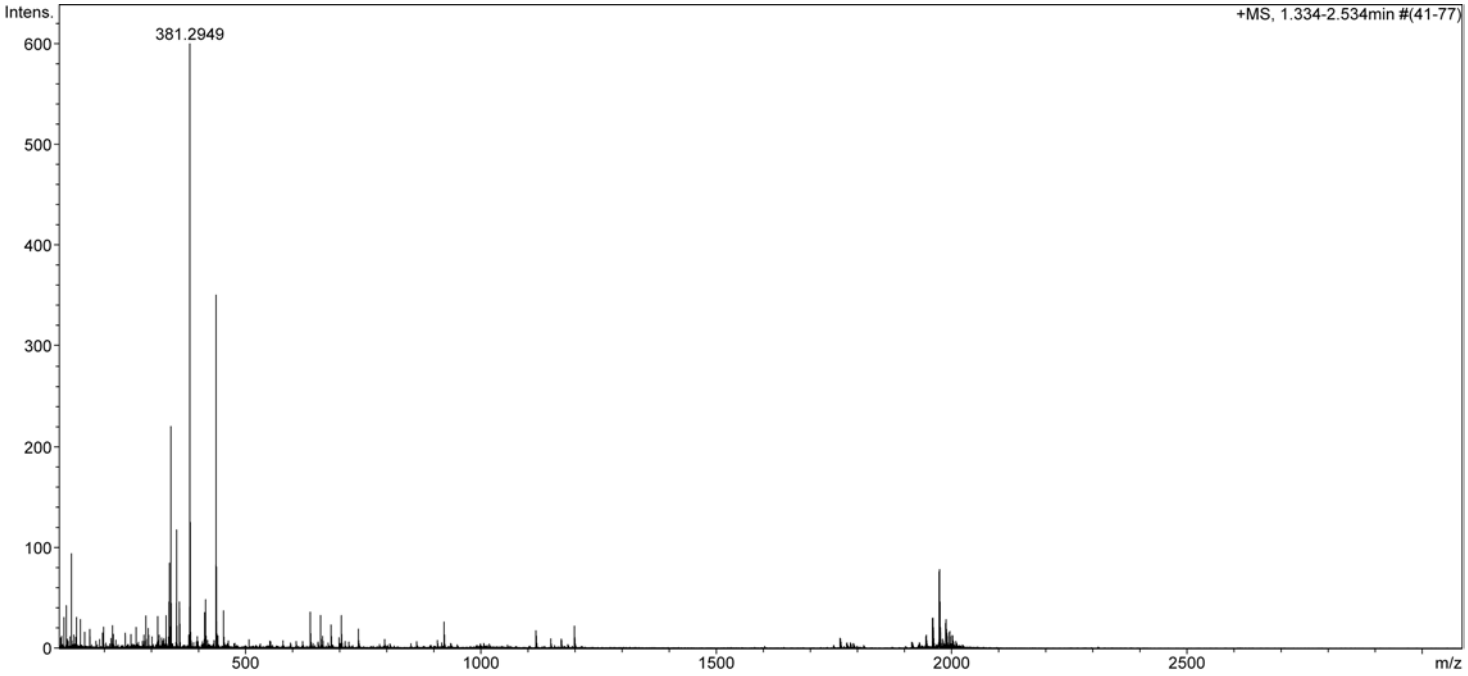
Display Report

Analysis Info

Analysis Name	D:\service_data\Bio-TOF\2007_0830\GL1OAc_34_01_8789.d\GL1OAc_34_01_8789.ser		
Method	methodname	Operator	operator name
Sample Name	samplename	Instrument	BioTOF II
Comment	comment		
Acquisition Date	2007/9/3 下午 06:55:29		

Acquisition Parameter

Capilary End Plate	n/a	Capilary Exit	n/a	detbias	1950 V
EndP	3500 V	Collision energy	n/a	Number of Averages	n/a



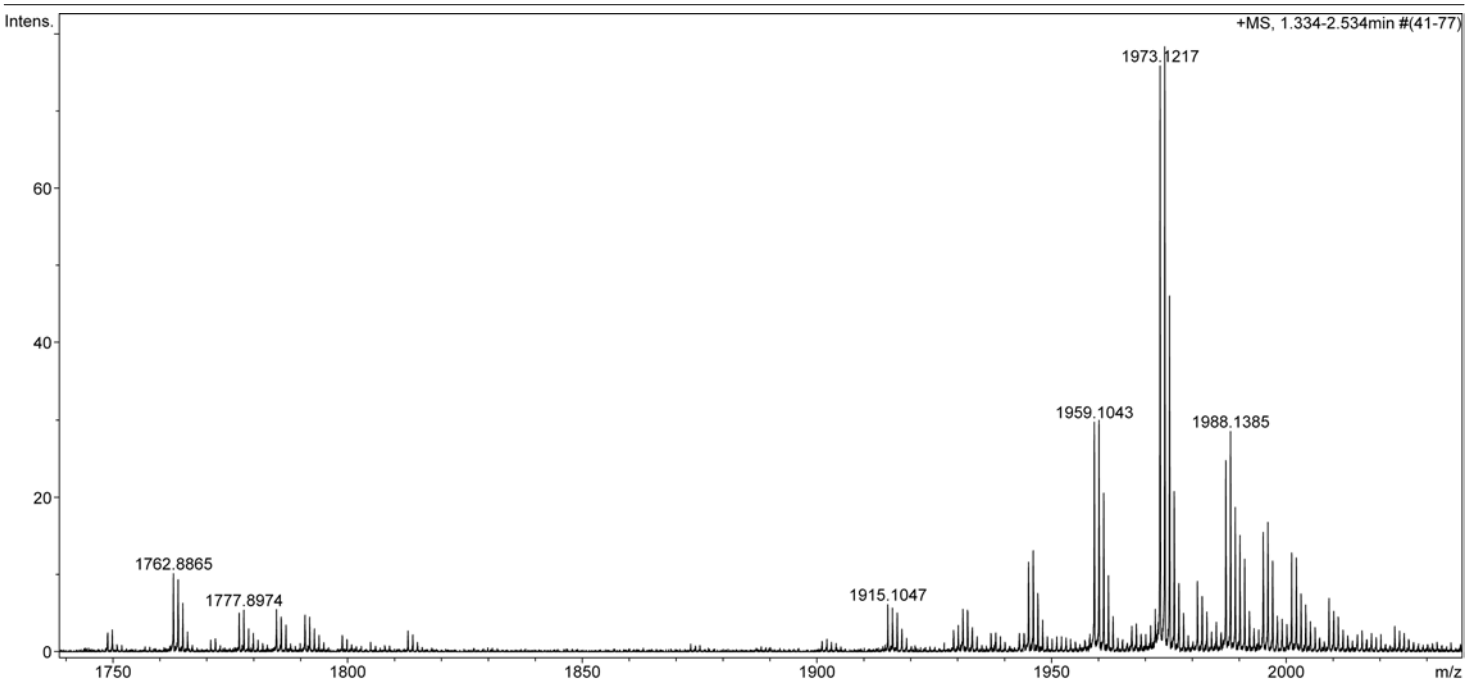
Display Report

Analysis Info

Analysis Name	D:\service_data\Bio-TOF\2007_0830\GL1OAc_34_01_8789.d\GL1OAc_34_01_8789.ser		
Method	methodname	Operator	operator name
Sample Name	samplename	Instrument	BioTOF II
Comment	comment		
Acquisition Date	2007/9/3 下午 06:55:29		

Acquisition Parameter

Capilary End Plate	n/a	Capilary Exit	n/a	detbias	1950 V
EndP	3500 V	Collision energy	n/a	Number of Averages	n/a



MALDI-TOF of a mixture of 3a and 4a

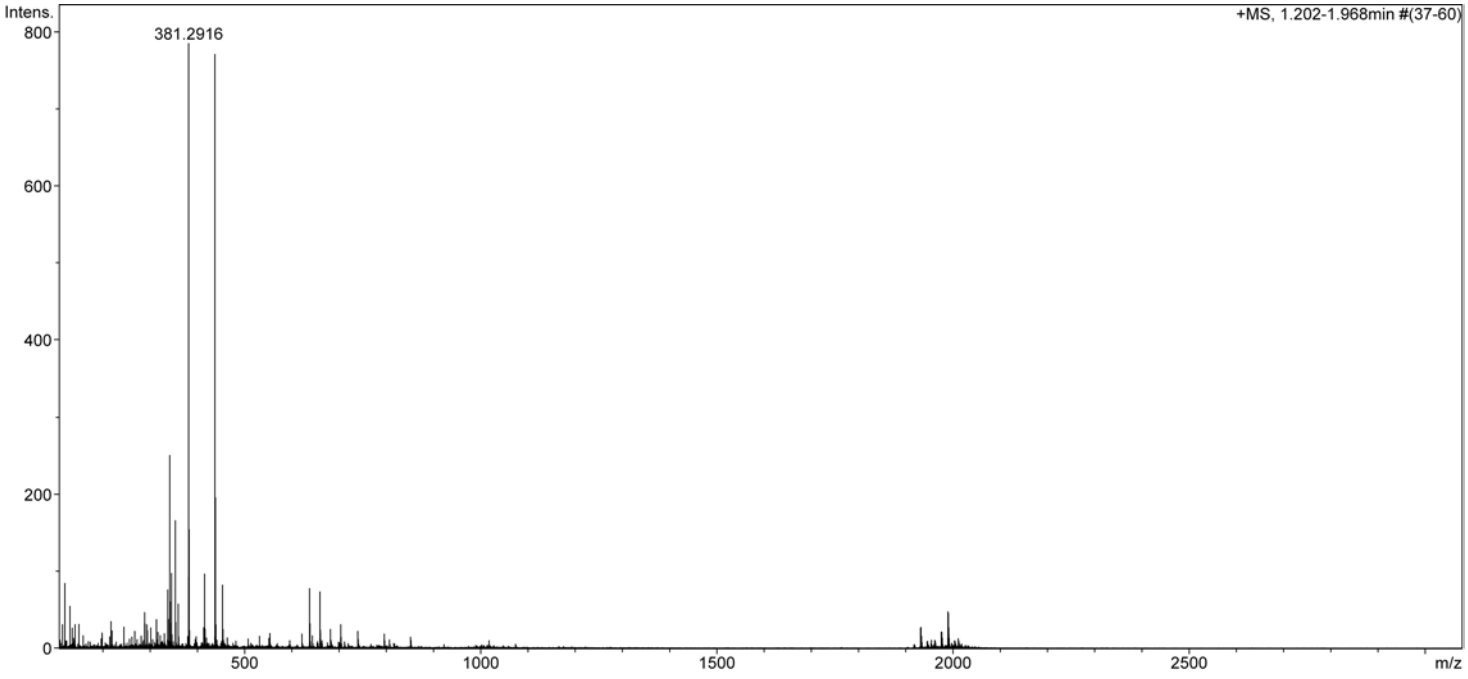
Display Report

Analysis Info

Analysis Name	D:\service_data\Bio-TOF\2007_0830\GLOAc5_33_01_8788.d\GLOAc5_33_01_8788.ser		
Method	methodname	Operator	operator name
Sample Name	samplename	Instrument	BioTOF II
Comment	comment		
Acquisition Date	2007/9/3 下午 06:48:47		

Acquisition Parameter

Capilary End Plate	n/a	Capilary Exit	n/a	detbias	1950 V
EndP	3500 V	Collision energy	n/a	Number of Averages	n/a



Display Report

Analysis Info

Analysis Name	D:\service_data\Bio-TOF\2007_0830\GLOAc5_33_01_8788.d\GLOAc5_33_01_8788.ser		
Method	methodname	Operator	operator name
Sample Name	samplename	Instrument	BioTOF II
Comment	comment		
Acquisition Date	2007/9/3 下午 06:48:47		

Acquisition Parameter

Capilary End Plate	n/a	Capilary Exit	n/a	detbias	1950 V
EndP	3500 V	Collision energy	n/a	Number of Averages	n/a