Supporting Information

Sugar furanoid trans-vicinal diacid as γ-turn inducer: Synthesis and Conformational study

Madhuri Vangala,[a] Snehal A. Dhokale,[b] Rupesh L. Gawade,[c] Rajamohanan R. Pattuparambil,[b] Vedavati G Puranik[c] and Dilip D. Dhavale[a]*

[a] *Department of Chemistry, Garware Research Centre, University of Pune, Pune, 411 007, India. E-mail: ddd@chem.unipune.ac.in*
[b] *Central NMR Facility, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411 008, India.*
[c] *Centre for Materials Characterization, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411 008, India.*

Experimental Section

General Methods. Reactions were carried out with distilled and dried solvents using oven-dried glassware. All reagents, protected amino acids, 2-Chloro Trityl chloride resin, were purchased from commercial sources. ^{1}H NMR and ^{13}C NMR were recorded in $CDCl_3$ and DMSO-d_6 using TMS as internal standard. Melting points are uncorrected. Optical rotations were measured on a polarimeter. Kaiser test was used for detection of primary amines on the solid phase. HPLC analyses was carried out using analytical (RP Luna 5µC18(2) 100Å Column 250 x 4.6 mm) with gradient elution: Mass samples were analyzed by High-resolution mass spectrometry using ESI TOF and MALDI TOF/TOF. IR spectra were obtained using FT-IR spectrophotometer using KBr pellets and NaCl plates and recorded in cm^{-1}. Circular dichroism (CD) was performed on spectrophotometer using a cell of 2mm path length. Spectra were recorded as an accumulation of 3 scans using a scan speed of 100nm/min, with resolution of 1.0 nm, band-width 1.0 nm and a response of 1 sec. Spectra were smoothened(5) and plotted using OriginPro 6.1.

1,2:5,6-di-*O*-isopropylidene-3-*C*-trichloromethyl-α-D-allofuranose (2)
To a solution of ketone (5.0 g, 19.3 mmol) in dry THF (50 mL) and $CHCl_3$ (12.0 mL, 144.2 mmol) under nitrogen atmosphere, cooled at -78 ºC in julabo was added 1M LHMDS soln (38.7 mL, 38.7 mmol) dropwise over a period of 20 min. Reaction mixture was stirred at this temperature for 3h and then brought to 0 ºC. Reaction mixture was quenched with saturated solution of $NaHCO_3$ at 0 ºC to neutralize. The resulting mixture was concentrated at rotary evaporation and extracted with $CHCl_3$ (75 mL X 3). The combined organic layer was washed with water, brine and dried over Na_2SO_4 and concentrated. Purification of the residue by column chromatography over silica with 9:1 hexane/EtOAc as eluent afforded trichloromethyl alcohol **2** as a white crystalline solid: (3.5g, 48%) R_f = 0.5 (hexane/EtOAc, 85:15); $[\alpha]_D^{26}$ = +31.8 (*c* 1.0, $CHCl_3$) [lit.[ref-15]: +30.1(*c* 1.0, $CHCl_3$)]; mp- 136-138 ºC; IR (KBr, *v*, cm^{-1}) 3435 (OH), 2991, 1452, 1375, 1267, 1159, 850, 642; ^{1}HNMR (300 MHz, $CDCl_3$) δ (ppm) 1.36 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 3.86 (s, exchangeable, 1H, OH), 3.91 (dd, *J* = 8.4, 7.1 Hz, 6CHa), 4.20-4.10 (m, 2H, 4CH, 6CHa'), 4.73-4.67 (m, 1H, 5CH), 4.80 (d, *J* = 4.4 Hz, 1H, 2CH), 5.91 (d, *J* = 4.4 Hz, 1H, 1CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 25.5 (CH_3), 26.3 (CH_3), 26.6 (CH_3), 26.9 (CH_3), 67.8 (C6), 71.9 (C5), 82.0 (C2), 85.0 (C4), 87.5 (C3), 100.8 (CCl_3), 103.9 (C1), 109.8 ($\underline{C}(CH_3)_2$), 113.3 ($\underline{C}(CH_3)_2$). HRMS (TOF ES$^+$, CH_3CN) calcd for $C_{13}H_{19}Cl_3O_6$ $[M+Na]^+$ = 399.0144, found 399.0145.

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*C*-benzylcarbamoyl-α-D-glucofuranose(3)
To a solution of trichloromethyl alcohol **2** (1.40 g, 3.69 mmol) in dioxane (20 mL), cooled to 10 ºC, was added NaOH (591 mg, 14.79 mmol) dissolved in 40 mL of H_2O followed, by immediate addition of NaN_3 (480 mg, 7.39 mmol) and TBAI (136 mg, 0.36 mmol). Reaction mixture was warmed to rt and stirred for 1h. Dioxane removed on rotary evaporatory and reaction mixture acidified to pH 3 using solid NH_4Cl. The reaction mixture was extracted with EtOAc (75 mL X 3), combined organic layers dried over Na_2SO_4 and concentrated to obtain azido acid. To crude azido acid compound (1.1 g, 3.34 mmol) in dry DCM (12 mL) was added EDCI (832 mg, 4.34 mmol), HOBt (586 mg, 4.34 mmol), mol.seives under nitrogen atmosphere. Reaction mixture was stirred at rt for 15 min. $BnNH_2$ (1.1 mL, 10.02 mmol) was added dropwise and stirred at rt for overnight. Water was added to the reaction mixture and extracted into DCM (50 mL X 2). Combined organic layer washed with water, brine, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography over silica with 8.5:1.5 hexane/EtOAc as eluent to give compound **3** as a white solid: R_f = 0.5 (hexane/EtOAc, 80:20); (1.08 g, 78%); $[\alpha]_D^{26}$ = +58.32 (*c* 1.0, $CHCl_3$); mp- 146-148 ºC; IR ($CHCl_3$, *v*, cm^{-1}) 3290, 2923, 2127, 1669, 1547, 1373, 850; ^{1}HNMR (300 MHz, $CDCl_3$) δ (ppm) 1.22 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 3.95 (dd, *J* = 8.6, 5.4 Hz, 1H, 6CHa), 4.25-4.10 (m, 2H, 5CH, 6CHa'), 4.31-4.26 (m, 1H, 4CH), 4.49 (d, *J* = 5.0 Hz, 2H, CH_2Ph), 4.89 (d, *J* = 3.2 Hz, 1H, 2CH), 5.82 (d, *J* =3.2 Hz, 1H, 1CH), 7.40-7.20 (m, 5H, Ph), 7.69 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 24.8 (CH_3), 25.6 (CH_3), 26.4 (CH_3), 26.8 (CH_3), 43.9 (CH_2Ph), 67.9 (C6), 71.7 (C5), 75.3 (C3), 79.9 (C4), 85.1 (C2), 103.2 (C1), 110.4 ($\underline{C}(CH_3)_2$), 113.3 ($\underline{C}(CH_3)_2$), 127.6 (Ph), 128.0 (Ph), 128.6 (Ph), 137.1 (Ph), 164.6 (CONH). HRMS (TOF ES$^+$, CH_3CN) calcd for $C_{20}H_{26}N_4O_6$ $[M+Na]^+$ = 441.1749, found 441.1750.

3-Azido-3-deoxy 1,2-*O*-isopropylidene 3-*C*-benzyl carbamoyl α-D-glucofuranose (4)
A soln of **3** (750 mg) in AcOH (8 mL, 85%) was heated to 50 ºC for 3h. Reaction mixture neutralized with solid $NaHCO_3$ and extracted into EtOAC (25 mL X 3). Combined organic layer washed with sat. $NaHCO_3$ (5 mL X 3), brine, dried over Na_2SO_4 and concentrated. Residue after purification by chromatography over silica 4:6 hexane/EtOAc as eluent gave product **4** as a sticky compound: R_f = 0.3 (hexane/EtOAc, 40:60) (603.0 mg, 90%); $[\alpha]_D^{24}$ = +43.48 (*c* 0.51, $CHCl_3$); IR (neat, *v*, cm^{-1}) 3345 (br), 2115, 1660, 1376, 1030, 874; ^{1}HNMR (300 MHz, $CDCl_3$) δ (ppm) 1.35 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 2.80-3.60 (br, exchangeable, 2H, OH), 3.66 (dd, *J* = 11.9, 5.1 Hz, 1H, 6CHa), 3.80 (dd, *J* = 11.9, 3.2 Hz, 1H, 6CHa'), 4.10- 3.90 (m, 1H, 5CH), 4.41 (d, *J* = 9.2 Hz, 1H, 4CH), 4.46 (dd, *J* = 15, 5.5 Hz, 1H, CH_2Ph), 4.54 (dd, *J* = 15, 5.9 Hz, 1H, CH_2Ph), 4.74 (d, *J* = 3.6 Hz, 1H, 2CH), 5.87 (d, *J* = 3.6 Hz, 1H, 1CH), 7.40-7.20 (m, 5H, Ph), 7.65 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 26.5 (CH_3), 26.6 (CH_3), 43.8 (CH_2Ph), 64.0 (C6), 69.0 (C5), 75.5 (C3), 79.6 (C4), 84.7 (C2),

103.8 (C1), 113.6 (C(CH$_3$)$_2$), 127.4 (Ph), 127.5 (Ph), 128.6 (Ph), 137.1 (Ph), 166.1 (CONH). HRMS (TOF ES$^+$, CH$_3$CN) calcd for $C_{17}H_{22}N_4O_6$ [M+Na]$^+$ = 401.1436, found 401.1436.

3-Azido-3-deoxy 1,2-*O*-isopropylidene 3-*C*-benzyl carbamoyl α- D-glucofuranose 5-carboxylic acid (5)

To the diol **4** (590 mg, 1.56 mmol) in THF (10 mL) and H_2O (2.5 mL) was added $NaIO_4$ (433 mg, 2.02 mmol) in two portions and reaction mixture stirred at 0 ºC for 30min. Ethylene glycol (0.15 mL) was added to quench the reaction mixture and extracted with EtOAc (25 mL X 2). Combined organic layer washed with water, brine, dried over Na_2SO_4 and concentrated to give aldehyde as white foam. To the crude aldehyde (480 mg, 1.38 mmol) and oxone (420 mg, 1.38 mmol) taken in a RB was added dry DMF (8 mL) under N_2 atmosphere. Reaction mixture stirred at RT for 3h and then quenched by adding sat. NH_4Cl. DMF removed on rota under reduced pressure and residue extracted with EtOAc (75 mL X 3) and dried over Na_2SO_4. Crude compound was purified by chromatography over silica using 9.5:0.5 EtOAc/MeOH as eluent to give product **5** as a white solid: R_f = 0.1 (EtOAc/MeOH, 90:10); (426 mg, 85%); $[\alpha]_D^{24}$ = +106.81 (*c* 0.57, MeOH); mp- 153-155 ºC; IR ($CHCl_3$, *v*, cm^{-1}) 3392 (br), 2121, 1739, 1662, 1383, 1247, 1041, 873; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 1.26 (s, 3H, CH$_3$), 1.40 (s, 3H, CH$_3$), 4.50-4.30 (m, 2H, CH$_2$ Ph), 4.58 (s, 1H, 4CH), 4.82 (br s, 1H, 2CH), 5.86 (br s, 1H, 1CH), 7.40-7.15 (m, 5H, Ph), 11.4-11.2 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 26.3 (CH$_3$), 26.5 (CH$_3$), 42.1 (CH$_2$NH), 75.4 (C4), 78.0 (C3), 84.2 (C2), 102.0 (C1), 111.6 (C(CH$_3$)$_2$), 126.7 (Ph), 126.8 (Ph), 128.2 (Ph), 138.7 (Ph), 165.0 (CONH), 169.0 (COOH). HRMS (TOF ES$^+$, MeOH) calcd for $C_{16}H_{18}N_4O_6$ [M+Na]$^+$ = 385.1123, found 385.1125.

Synthesis and Analysis of peptides

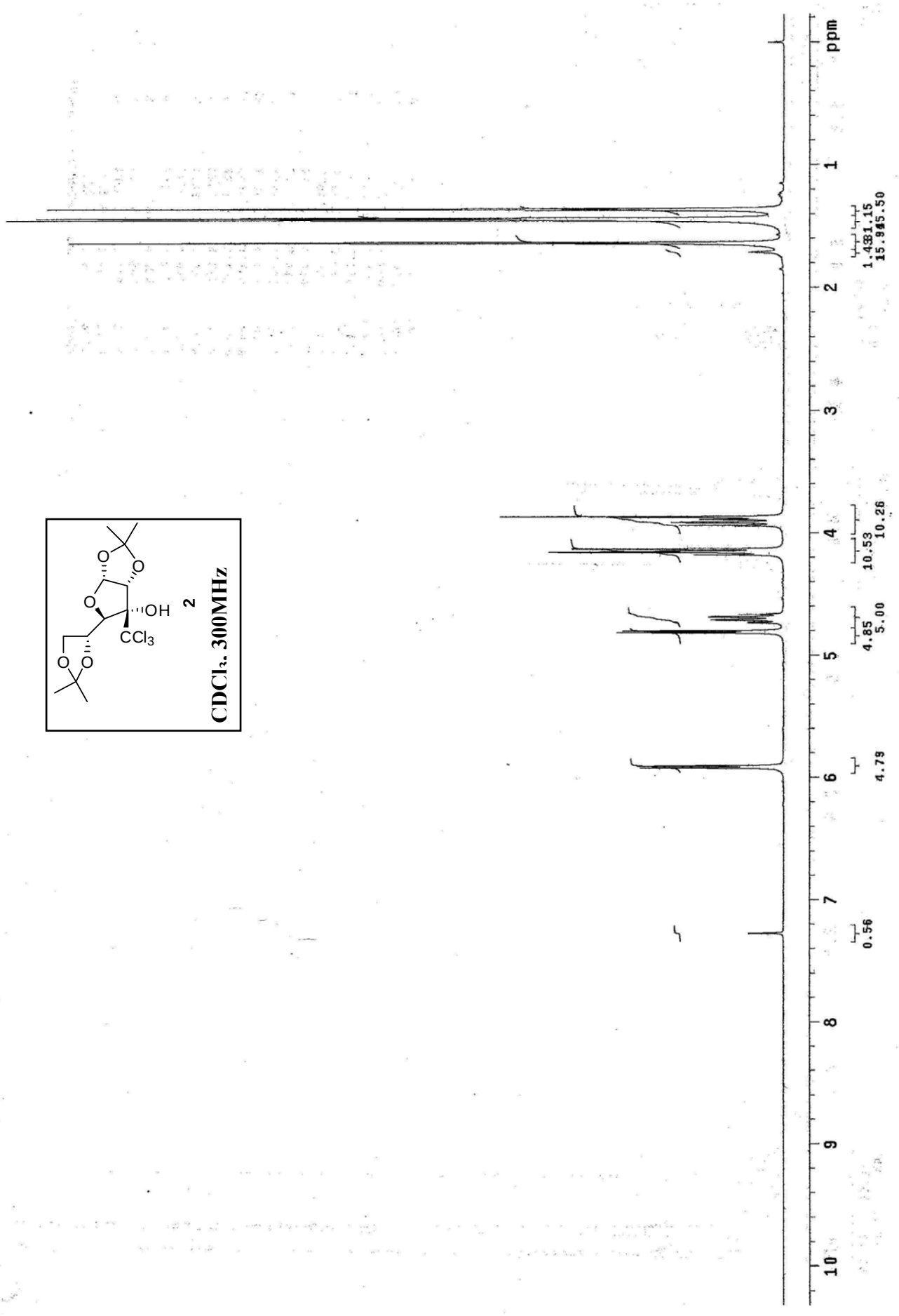
2-chlorotrityl chloride resin (0.8 mmol/g) was used. FmocAA/SAA-**5** (3 eq each), HBTU (3 eq), HOBt (3 eq) was dissolved in 200µL of DMF, followed by addition of DIPEA (7 eq). The solution was agitated at rt for 2 min and transferred to the resin. The coupling reaction was performed for 4 h, after which the resin was drained and washed with several times with DMF/DCM/DMF. The *N*-terminal Fmoc group was removed by addition of 20% piperidine in DMF (2 x 1.5 mL) and monitored by Kaiser's test. Finally, after the last coupling with SAA-**5**, resin is washed with DMF (4 × 1 mL), DCM (4 × 1 mL), MeOH (1 × 1 mL), DCM (4 × 1 mL) and dried under vacuum.

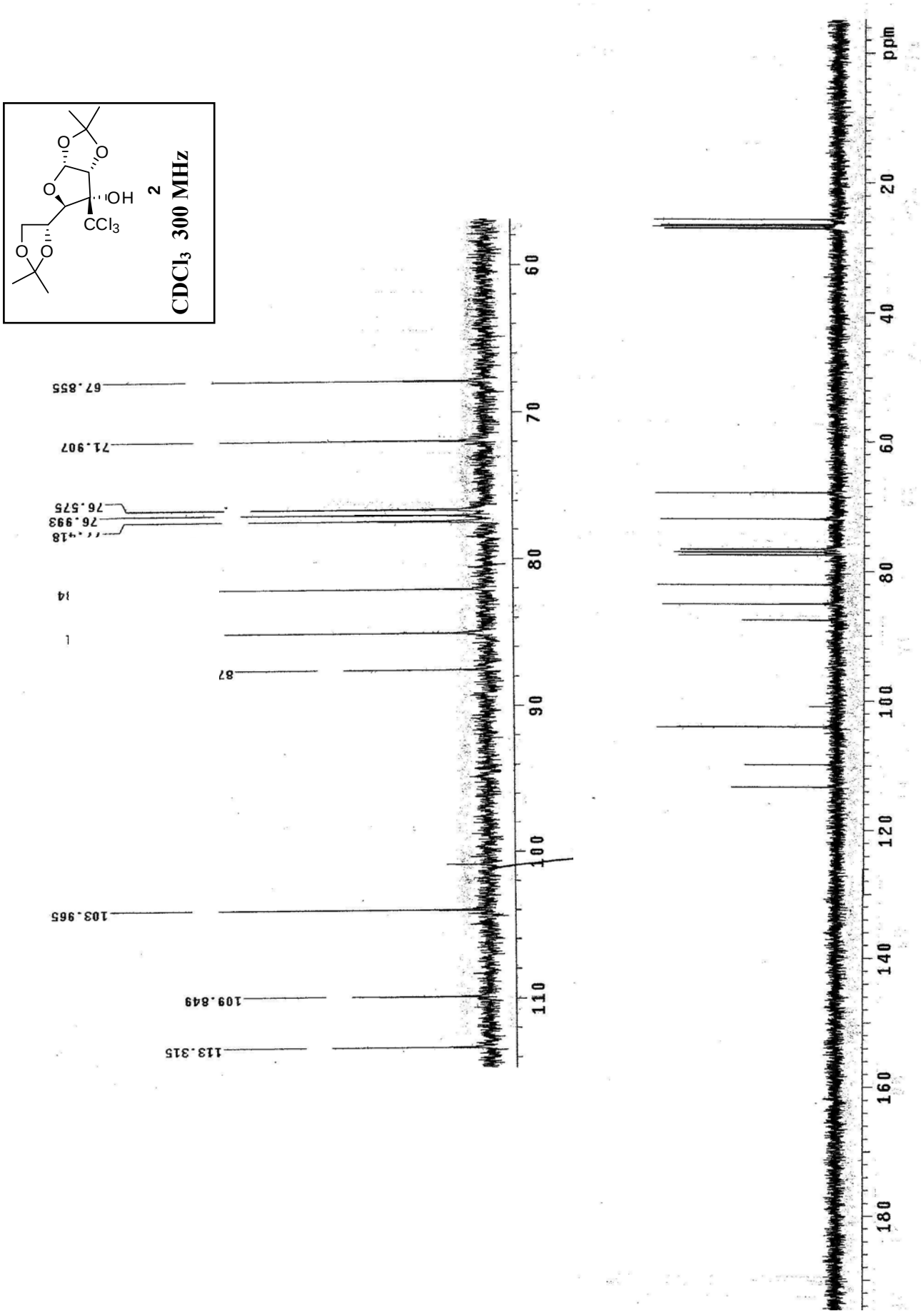
Cleavage of 8 from resin

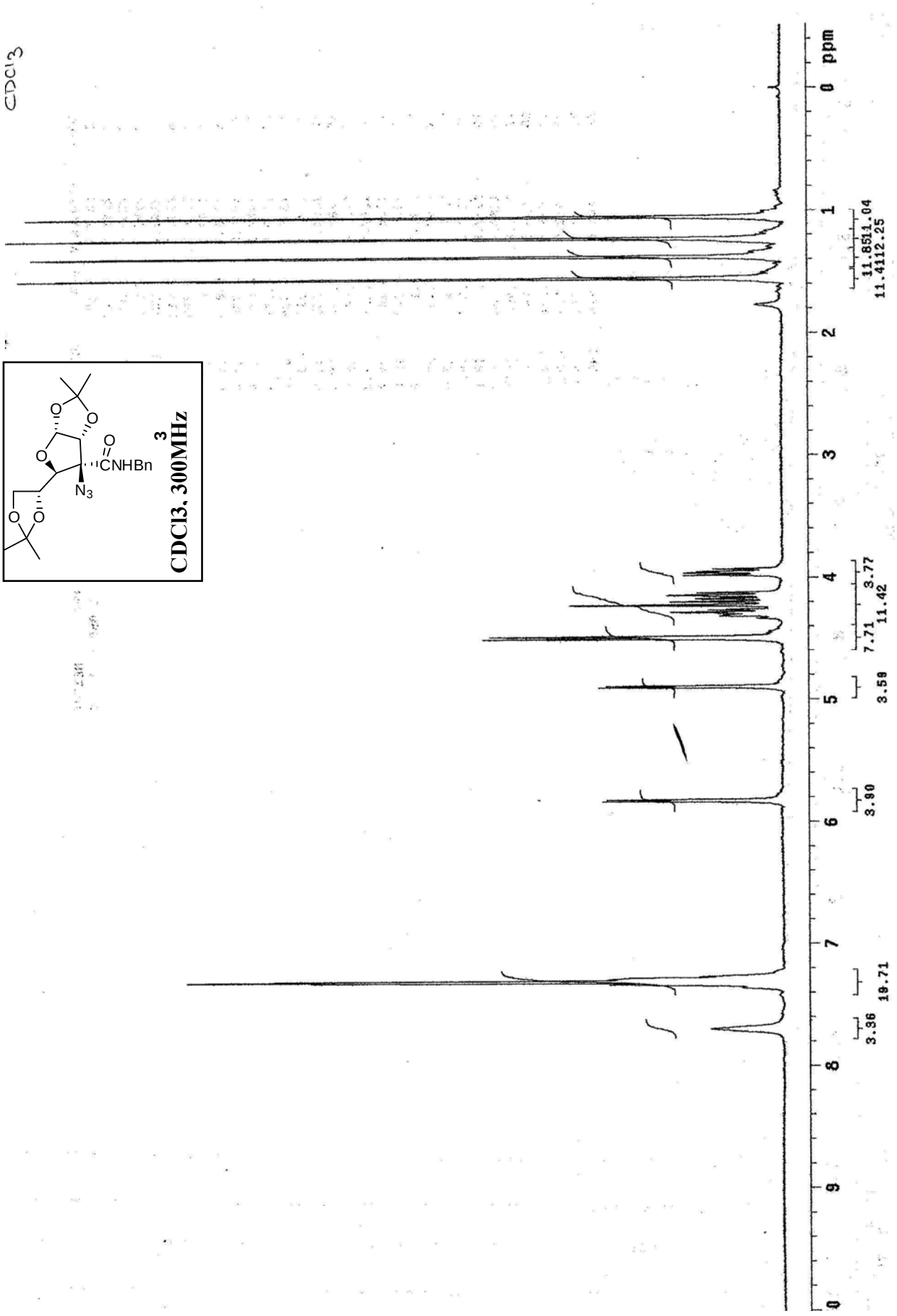
Resin was cleaved under very mild conditions using the following protocol: To 50 mg resin in a vial was added 400 µL of cooled 0.2% TFA in DCM for 15 min. The reaction mixture was filtered through a sintered funnel, the residue washed with DCM and the combined filtrate and washings were evaporated under vacuum to get residue which was redissolved in 200 µL of HPLC grade CH$_3$CN for rp-HPLC analysis and purification to give colourless amorphous solid. (t_R=25.7 min,[A = 0.05% TFA in H_2O (100%), B = 0.05% TFA in CH$_3$CN (100%) with flow rate 1.0 mL min^{-1} (Linear gradient from A to 100% B in 30 min) UV detection: 220, 260 nm]. IR ($CHCl_3$, *v*, cm^{-1}) 3450-3410 (br), 2922, 2115, 1637-1630 (br); ^{1}H NMR (500MHz ,CD$_3$CN) δ (ppm) 1.11 (d, *J* = 6.3 Hz, 3H), 1.16 (s, 9H), 1.20 (t, *J* = 7.6 Hz, 2H), 1.33 (s, 3H), 1.36 (br s, 2H), 1.39 (s, 9H), 1.50 (s, 3H), 1.54 (d, *J* = 7.0 Hz, 1H), 1.77 - 1.69 (m, 1H), 2.85 (dd, *J* = 14.1, 8.7 Hz, 1 H), 2.95 (q, *J* = 6.6 Hz, 2H), 3.06 (dd, *J* = 14.1, 4.9 Hz, 2H), 3.14 - 3.09 (m, 1H), 3.22 (dd, *J* = 14.8, 5.3 Hz, 1H), 4.21 (dq, *J* = 6.2, 2.8Hz, 1H), 4.37 - 4.33 (m, 1H), 4.39 (dd, *J* = 8.3, 2.6 Hz, 1H), 4.43 (d, *J* = 5.8 Hz, 2H), 4.56 - 4.50 (m, 1H), 4.66 - 4.60 (m, 1H), 4.85 (m, 2H), 4.86 (br s, 1H), 5.33(br s, 1H), 5.92 (d, *J* = 3.2 Hz, 1H), 6.93 (br s, 1H), 6.95 (br s, 1H), 7.03 (s, 1H), 7.06 - 7.04 (m, 1H), 7.09 (d, *J* = 2.3 Hz, 2H), 7.11 (s, 1H), 7.16 (br s, 1H), 7.19 (s, 1H), 7.20 (s, 1H), 7.22 (s, 1H), 7.23 (s, 2H), 7.29 (s, 2H), 7.31 (s, 1H), 7.33 (s, 2 H), 7.37 (s, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 8.75 (t, *J* = 5.3 Hz, 1H), 9.21 (br s, 1H); ^{13}C NMR (500MHz, CD$_3$CN) δ (ppm) 20.2, 23.2, 26.6, 27.1, 28.4, 28.5, 30.2, 32.2, 37.9, 40.8, 43.7, 53.9, 54.9, 55.2, 58.3, 67.7, 75.4, 76.5, 78.7, 79.0, 85.3, 104.6, 110.8, 112.2, 114.6, 118.2, 119.3, 119.8, 122.4, 124.8, 127.6, 128.0, 128.4, 129.7, 129.4, 130.3, 137.3, 137.5, 139.3, 157.0, 165.3, 169.0, 170.9, 172.0, 172.7. HRMS (TOF ES$^+$, CH$_3$CN) calcd for $C_{55}H_{72}N_{10}O_{13}$ [M+Na]$^+$ = 1103.5178, found 1103.5216.

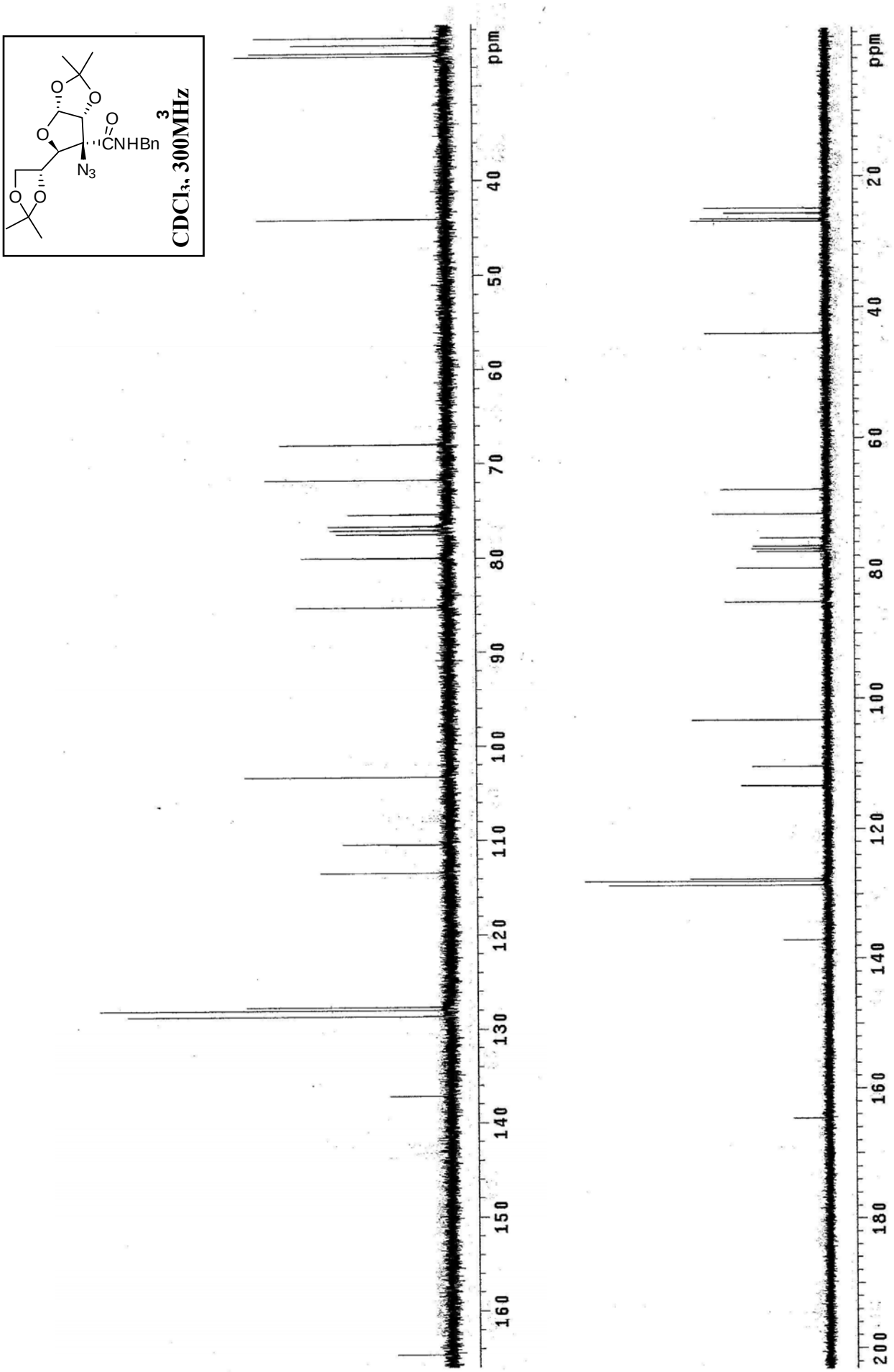
NMR and MD Simulations

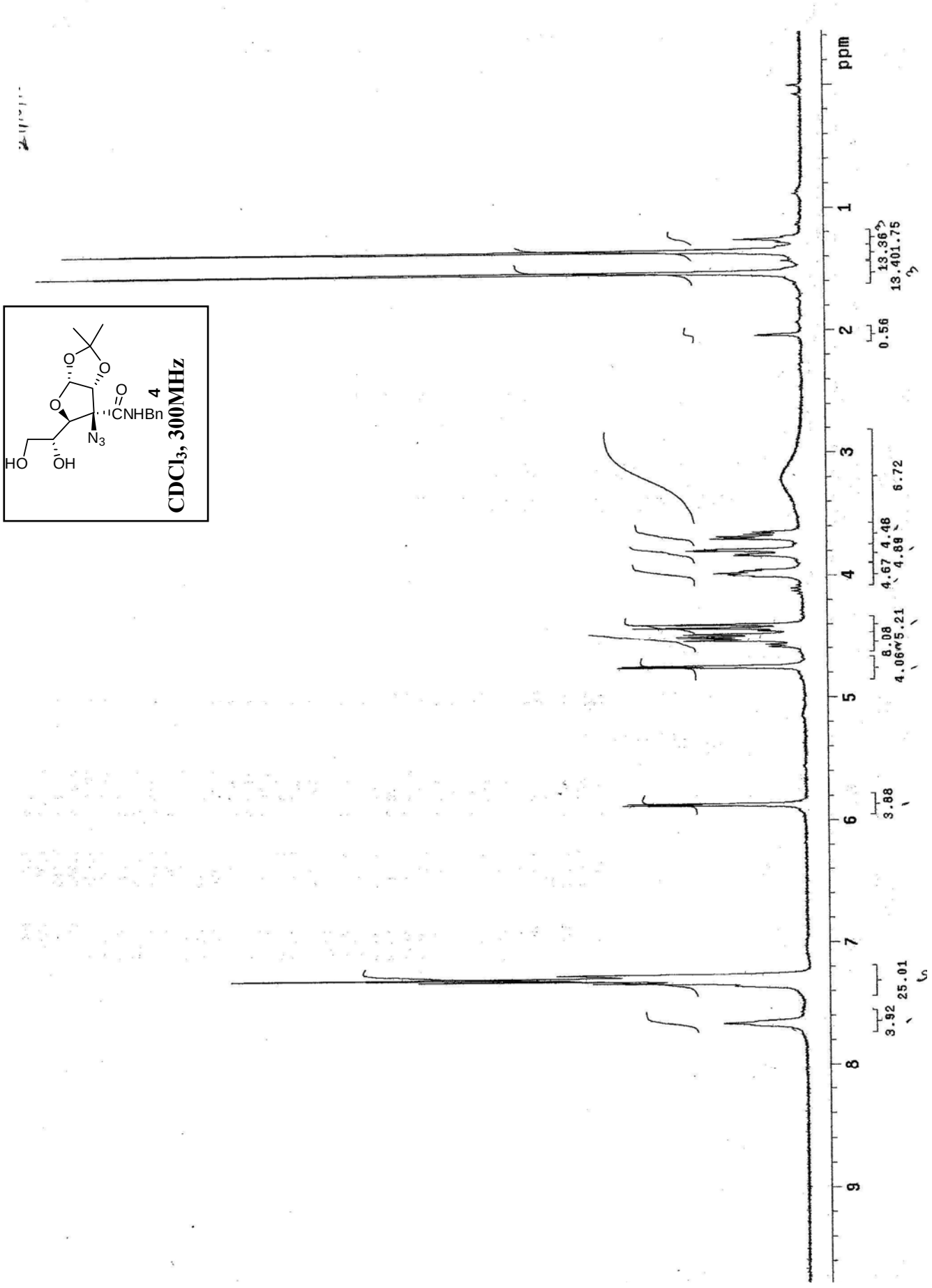
All NMR studies were carried out on 500 MHz spectrometer at a probe temperature of 295 K. For NOE experiment 12 scans were done and mixing time was (D_8)1S. MD simulations were carried out using Schrödinger software. Minimization was done with steepest decent, using conjugate gradient methods. For 500 iterations each, the energy-minimized structures were then subjected to MD simulations at 295K with dielectric constant 37.5 for CD$_3$CN. Distance constraints obtained from NOESY experiment were used in MD calculations in Schrödinger software by using macromodule application.

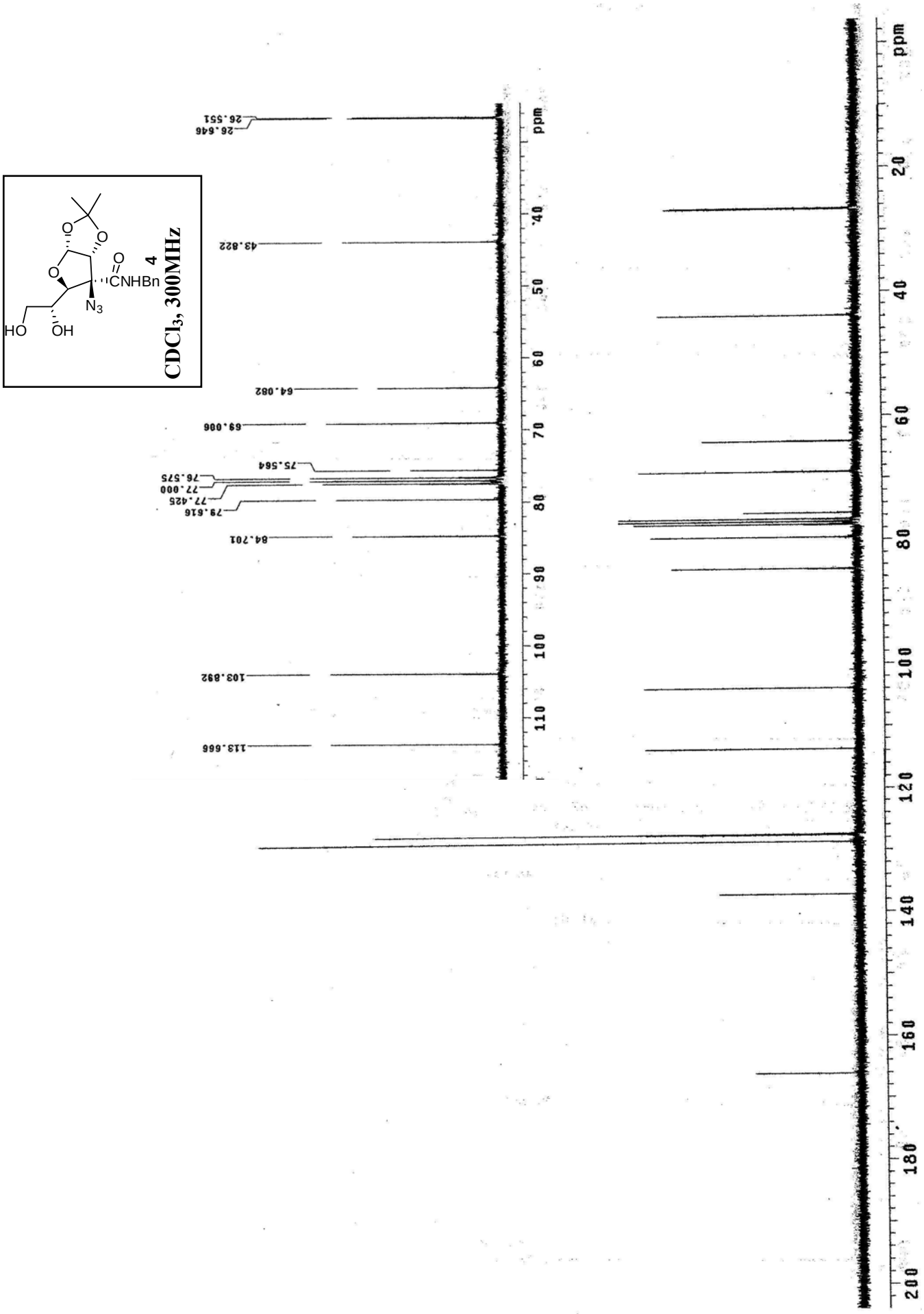


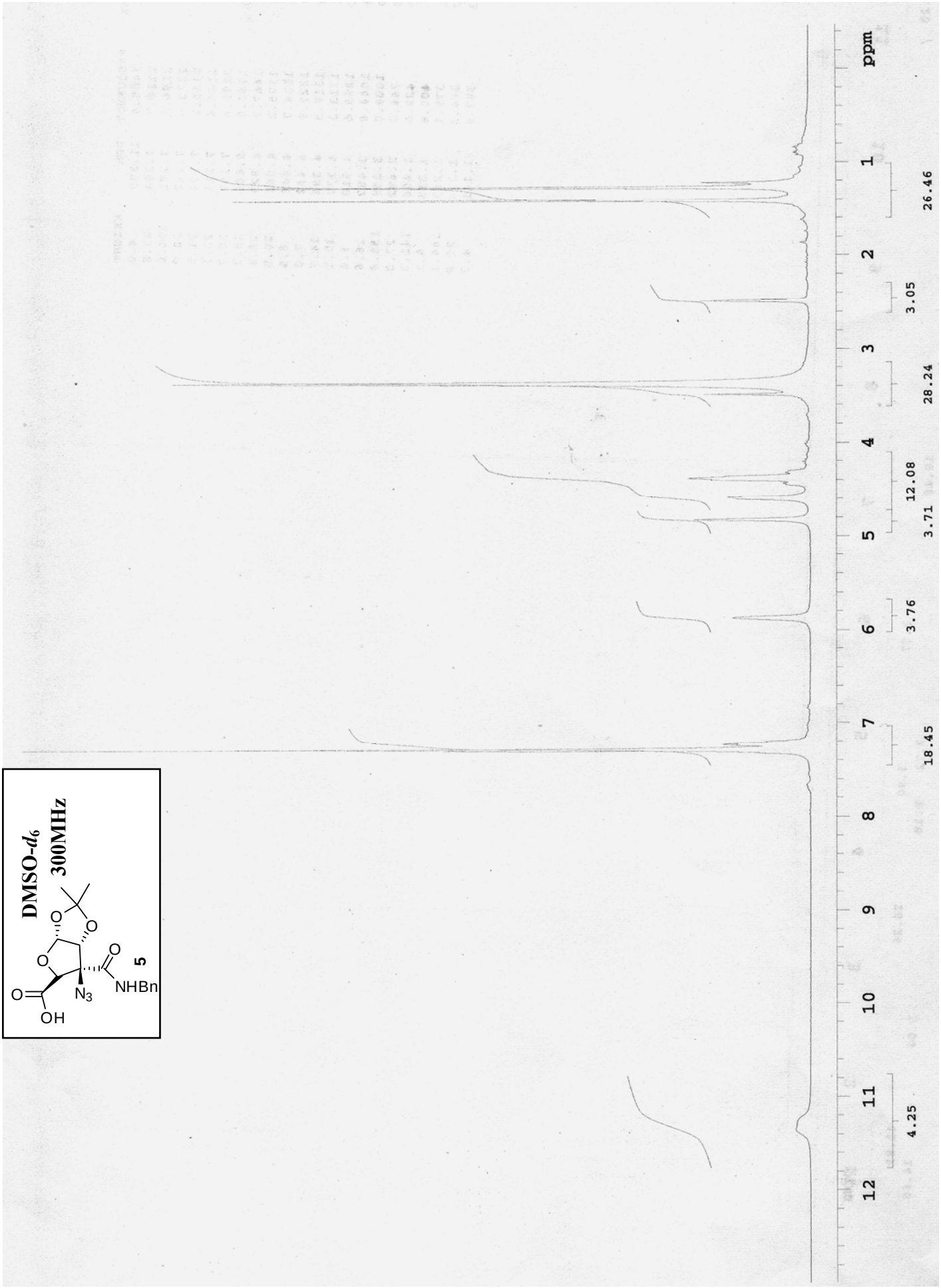


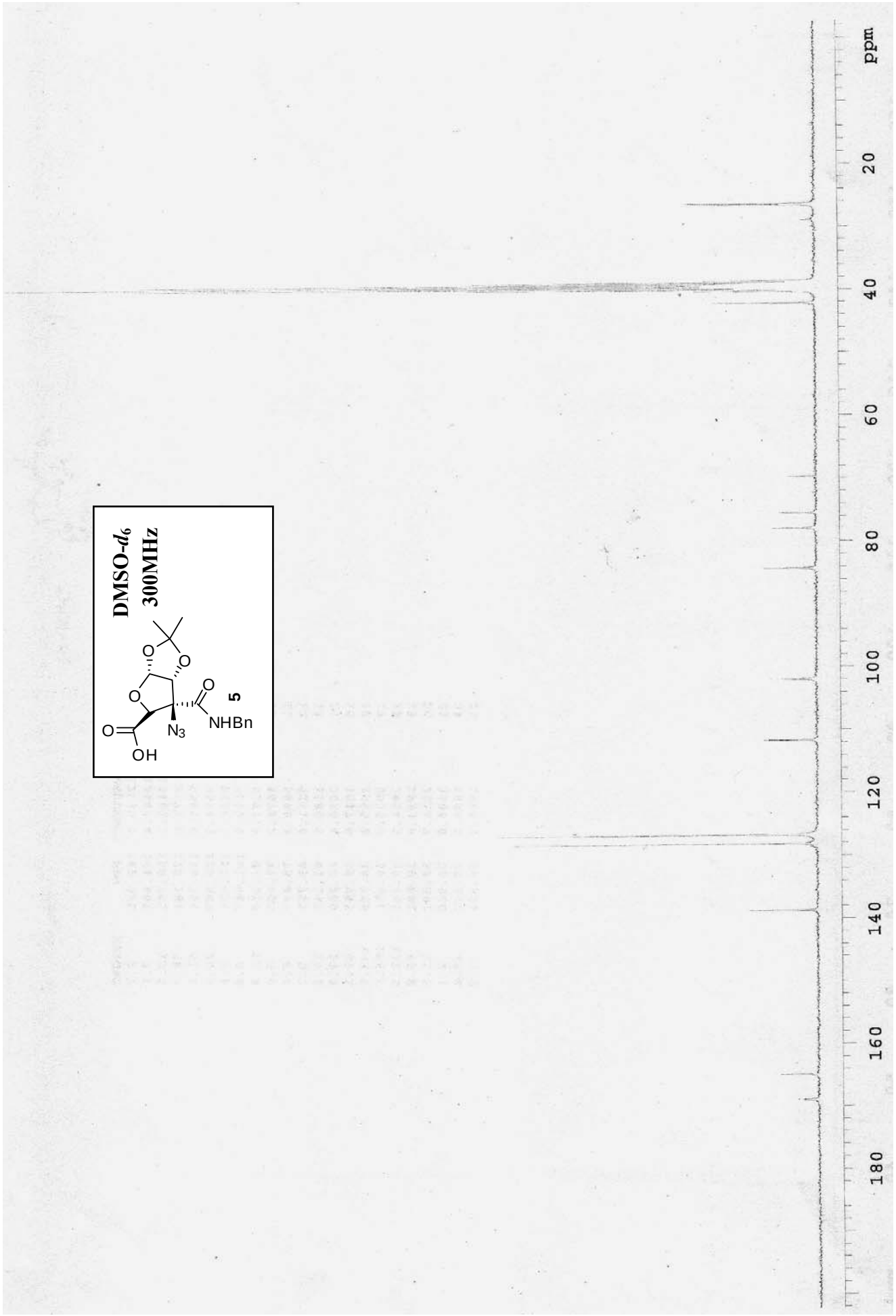


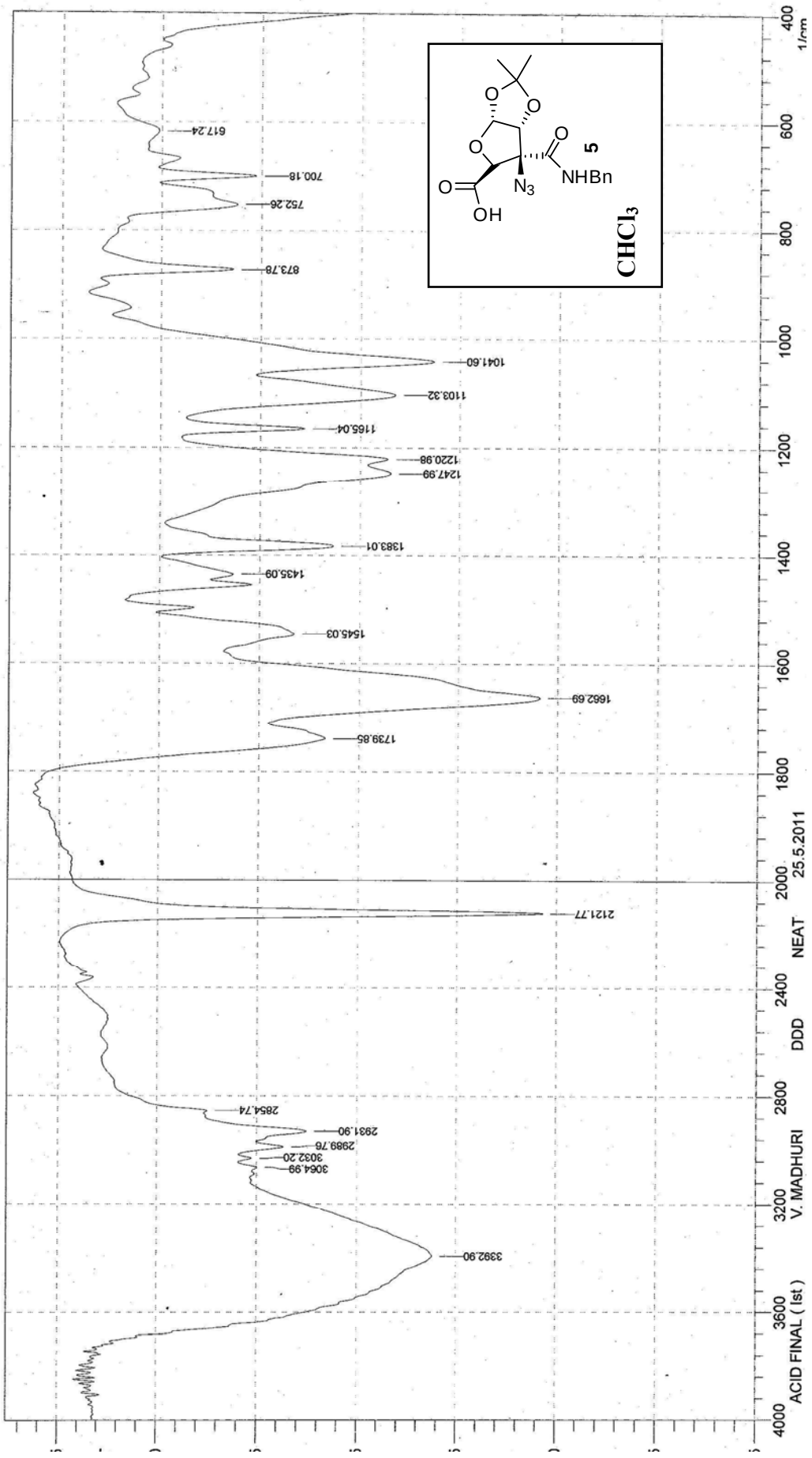


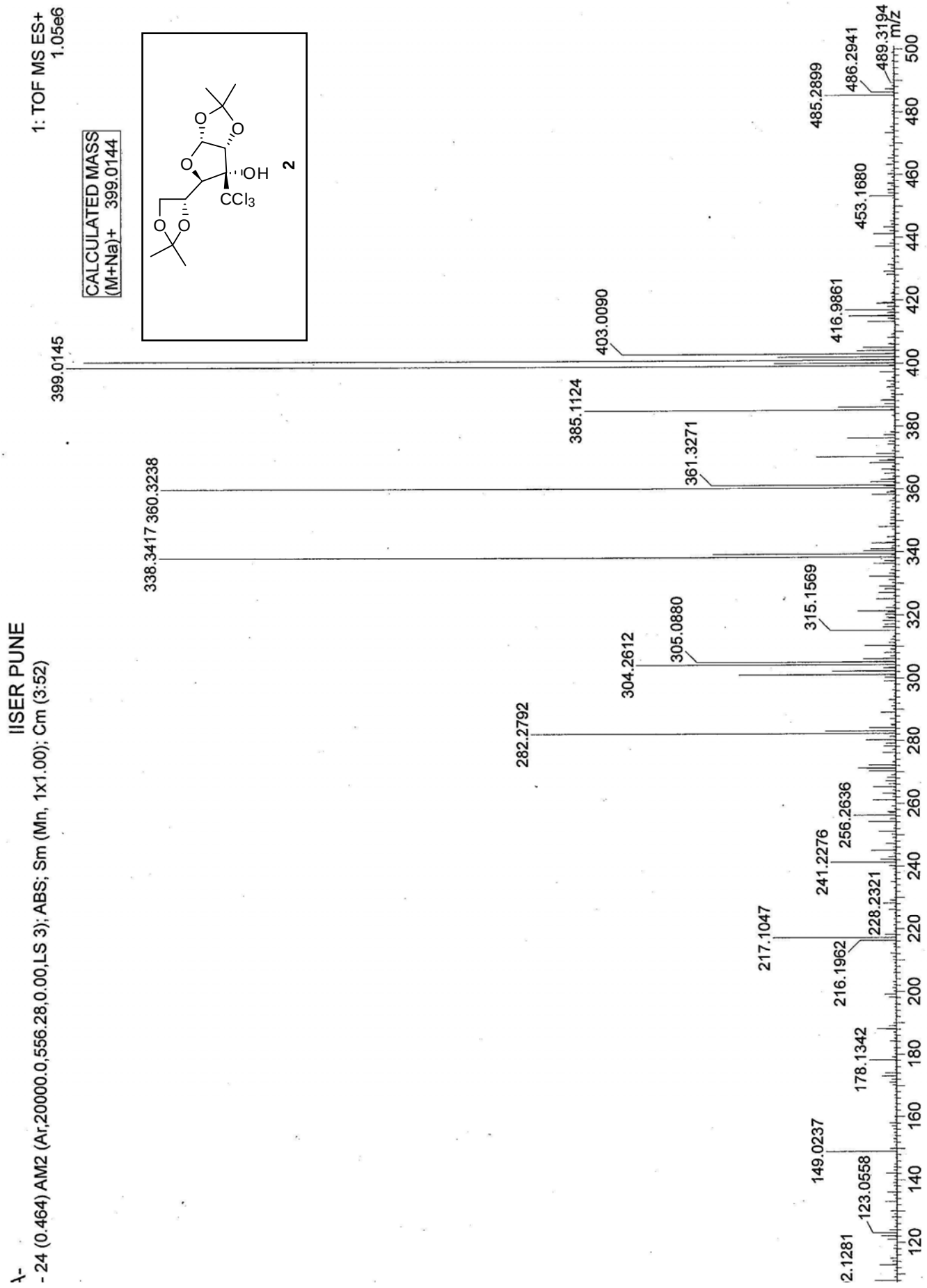


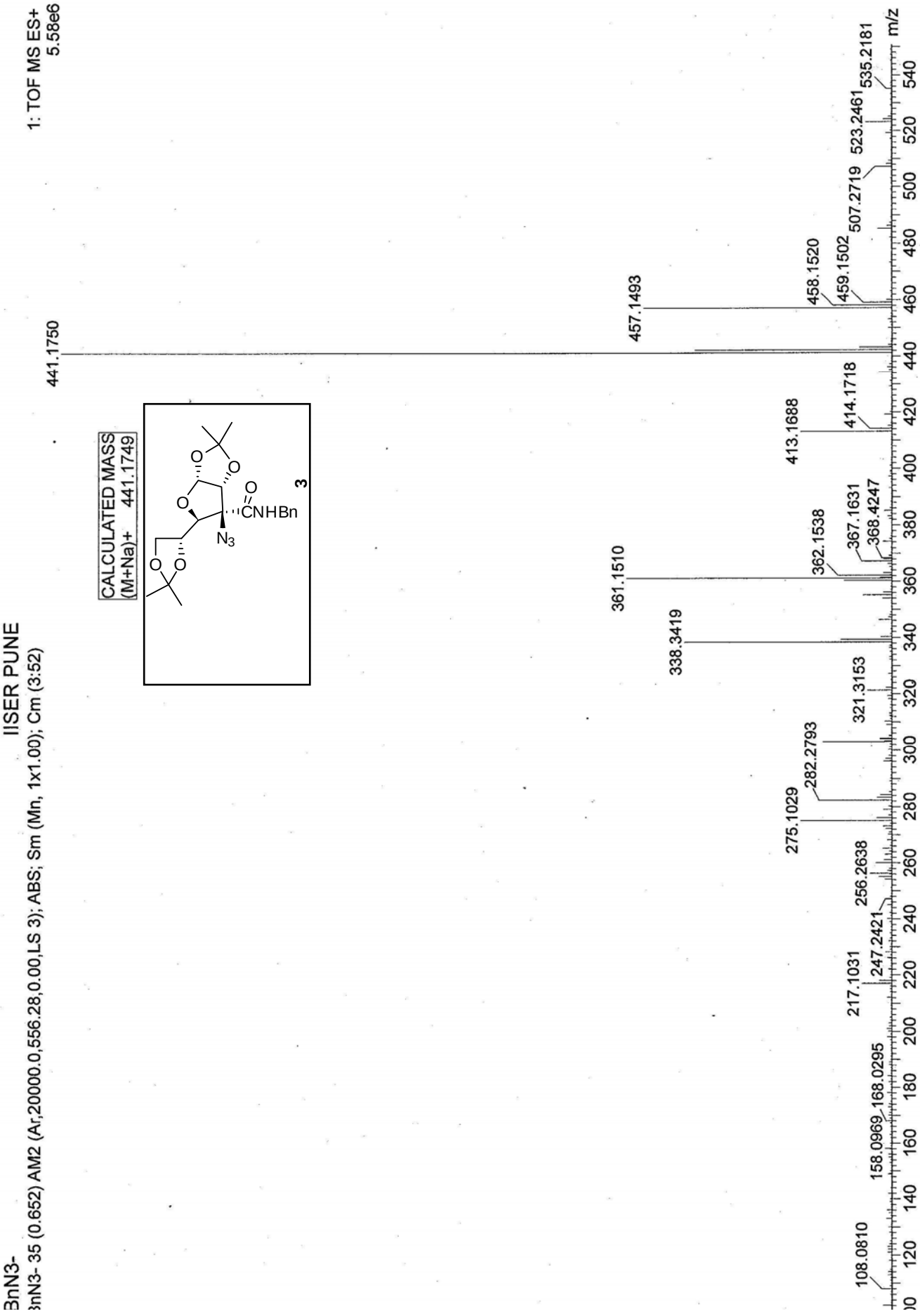


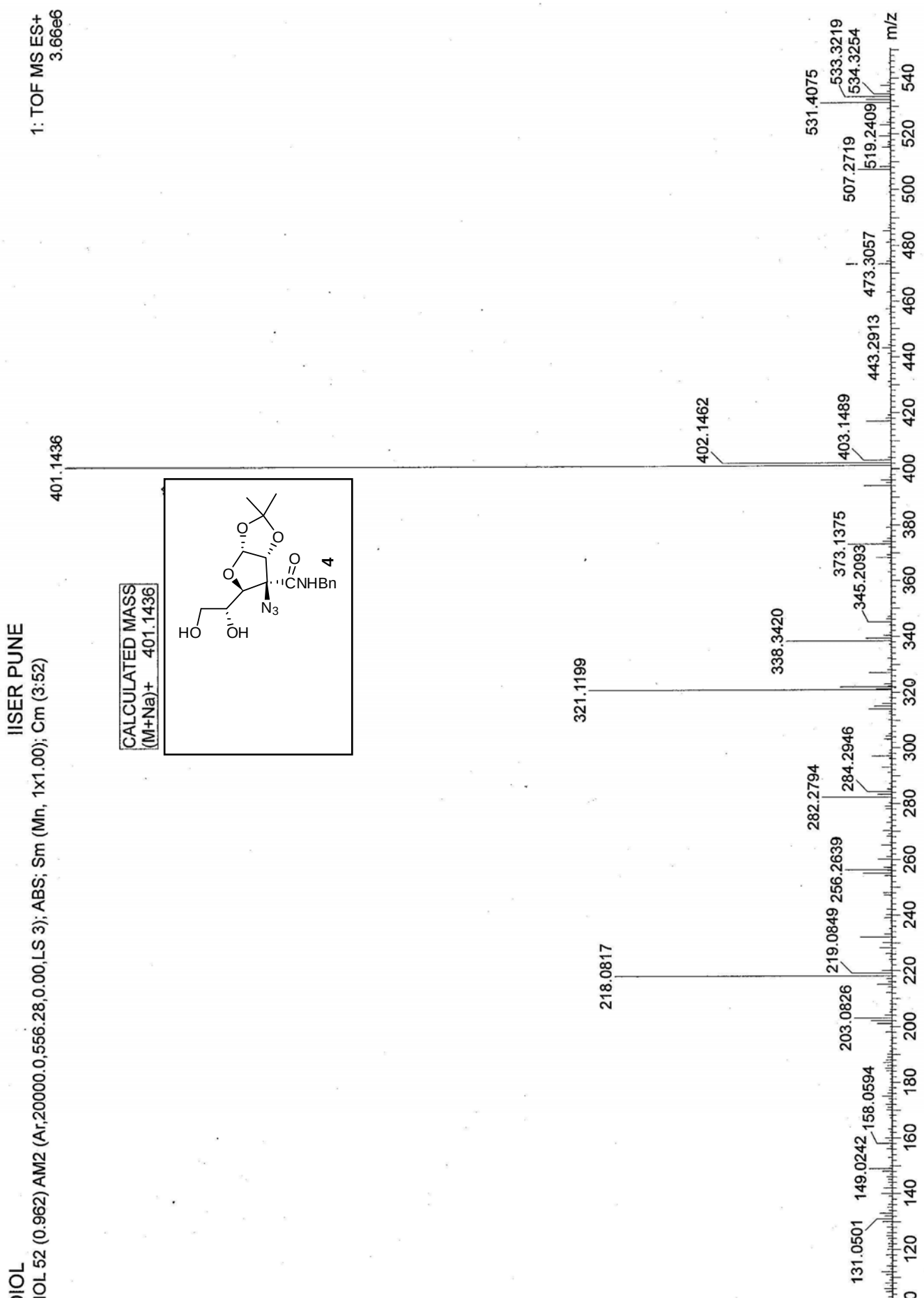


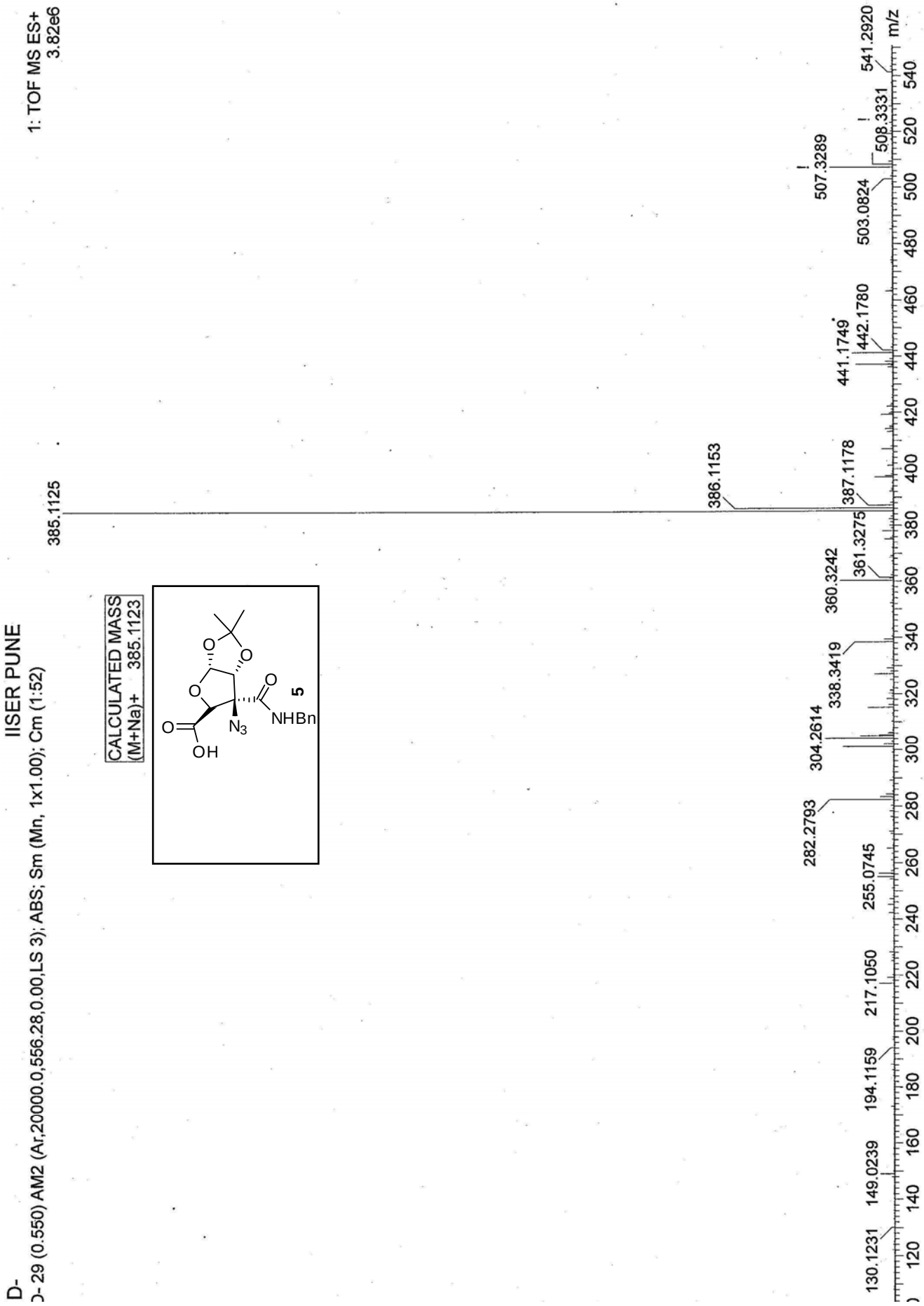

DMSO-d_6
300MHz
5
NHBn
N_3
OH
O
O
O
O
O
ppm
20
40
60
80
100
120
140
160
180

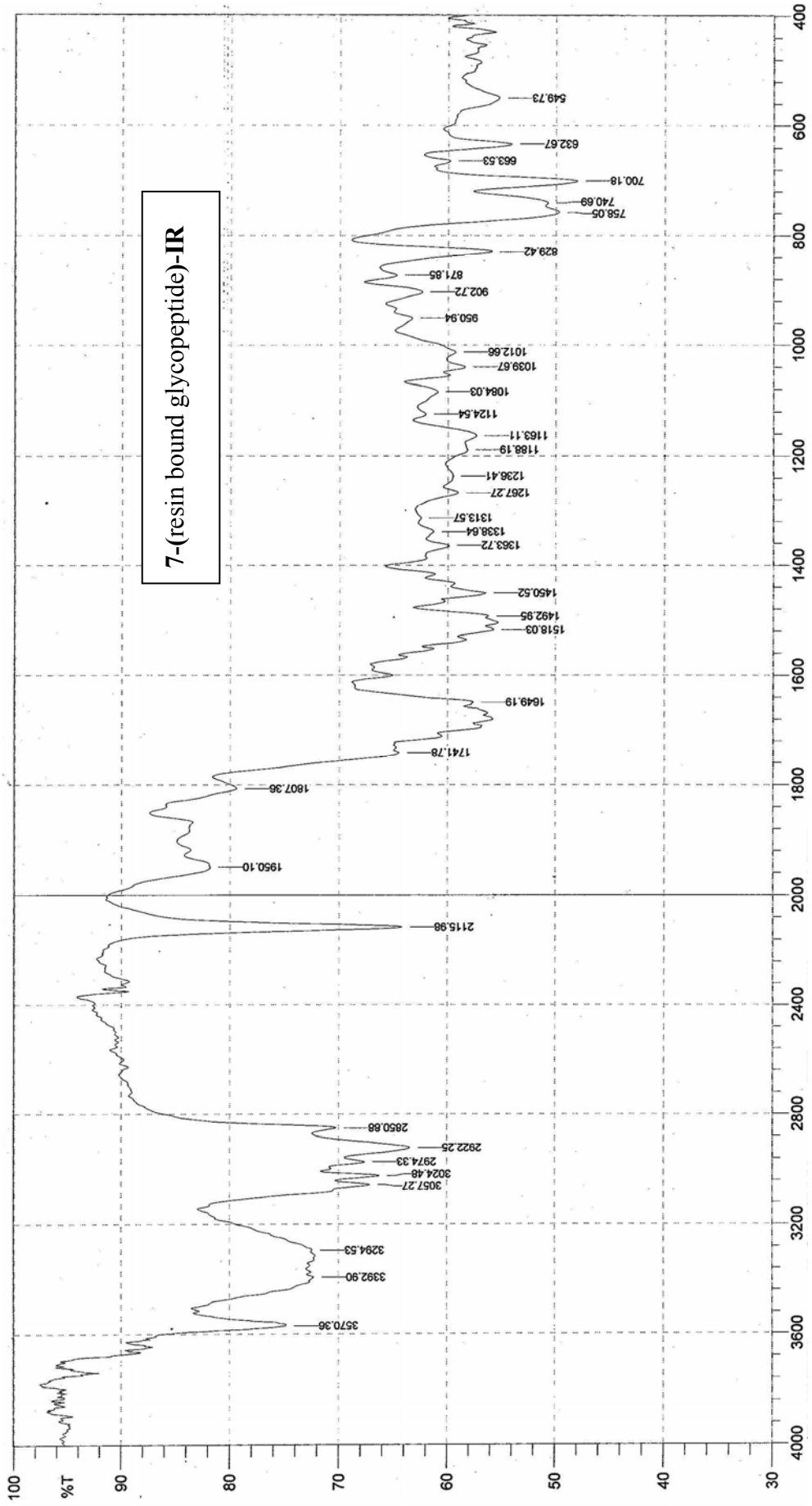



IISER PUNE
- 24 (0.464) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (Mn, 1x1.00); Cm (3:52)
1: TOF MS ES+
1.05e6
CALCULATED MASS
(M+Na)+ 399.0144
OH
CCl3
2
m/z
399.0145
403.0090
416.9861
485.2899
486.2941
489.3194
453.1680
385.1124
361.3271
338.3417
360.3238
315.1569
305.0880
304.2612
282.2792
256.2636
241.2276
228.2321
217.1047
216.1962
178.1342
149.0237
123.0558
2.1281





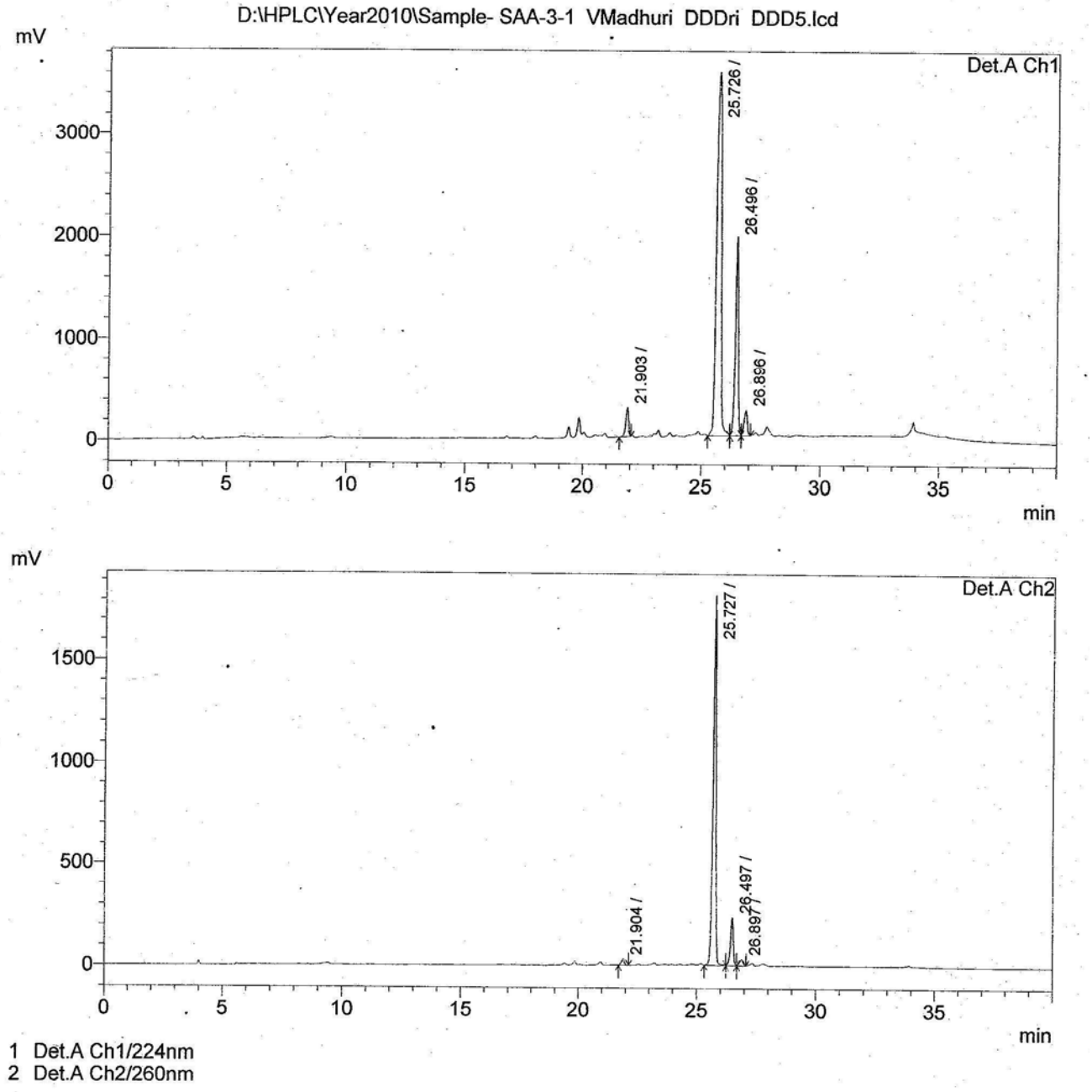

D-
D- 29 (0.550) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS; Sm (Mn, 1x1.00); Cm (1:52)
IISER PUNE
1: TOF MS ES+
3.82e6
CALCULATED MASS
(M+Na)+ 385.1123
5
NHBn
N3
OH
385.1125
386.1153
387.1178
441.1749
442.1780
503.0824
507.3289
508.3331
541.2920
361.3275
360.3242
338.3419
304.2614
282.2793
255.0745
217.1050
194.1159
149.0239
130.1231
m/z



Glycopeptide 8-CRUDE

Flow Rate : 1 ml / min

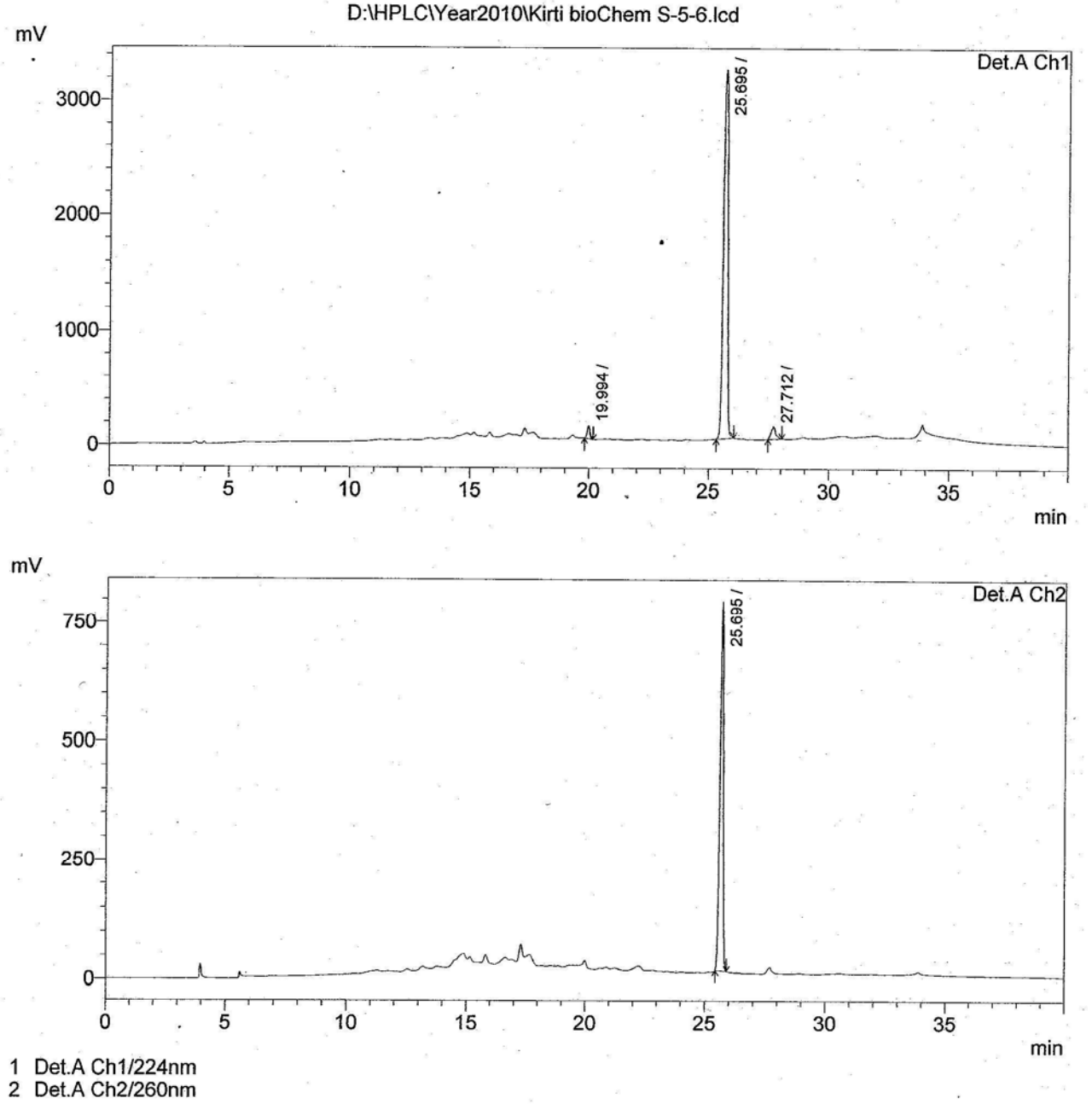
<Chromatogram>

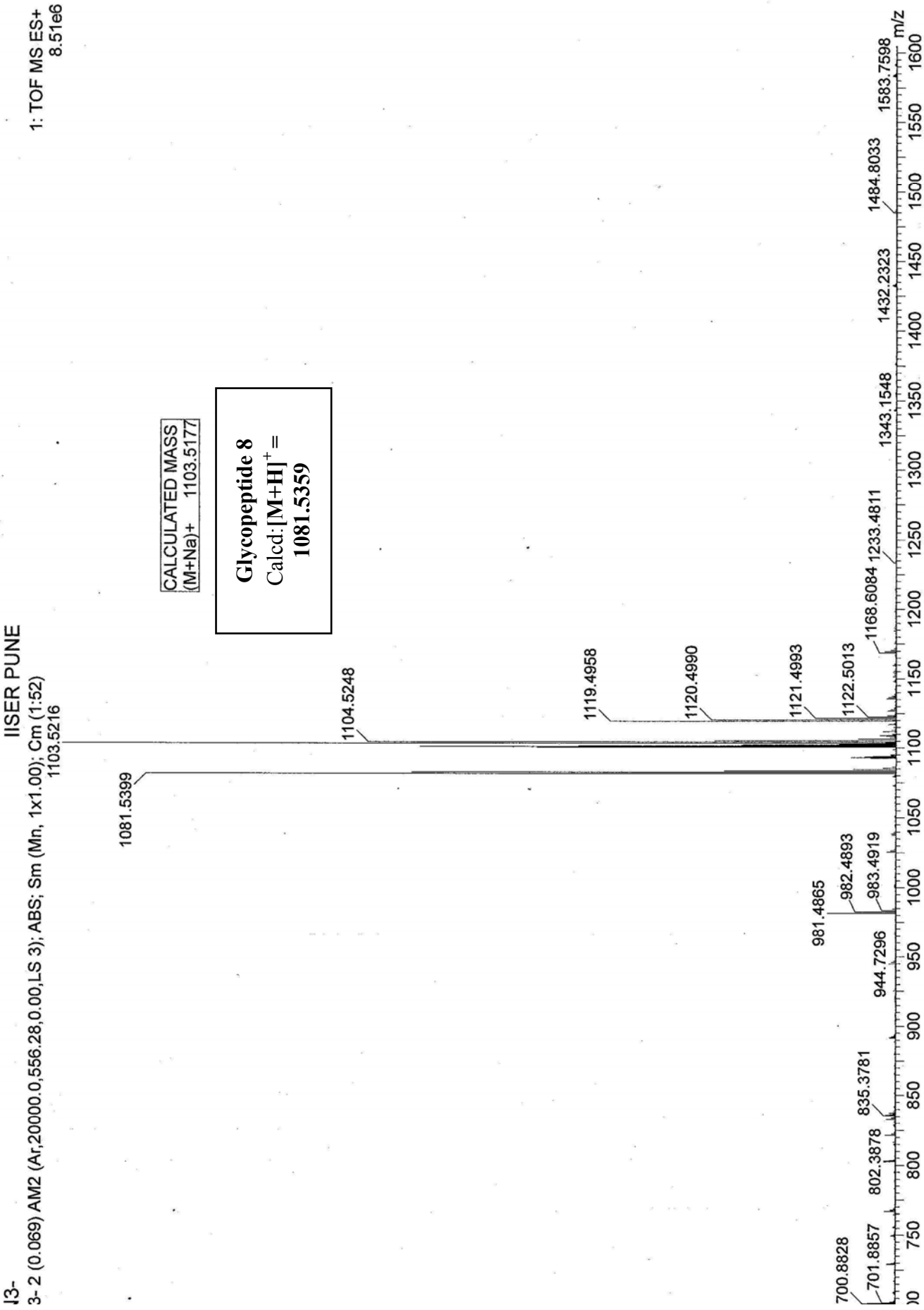


Glycopeptide 8- PURE

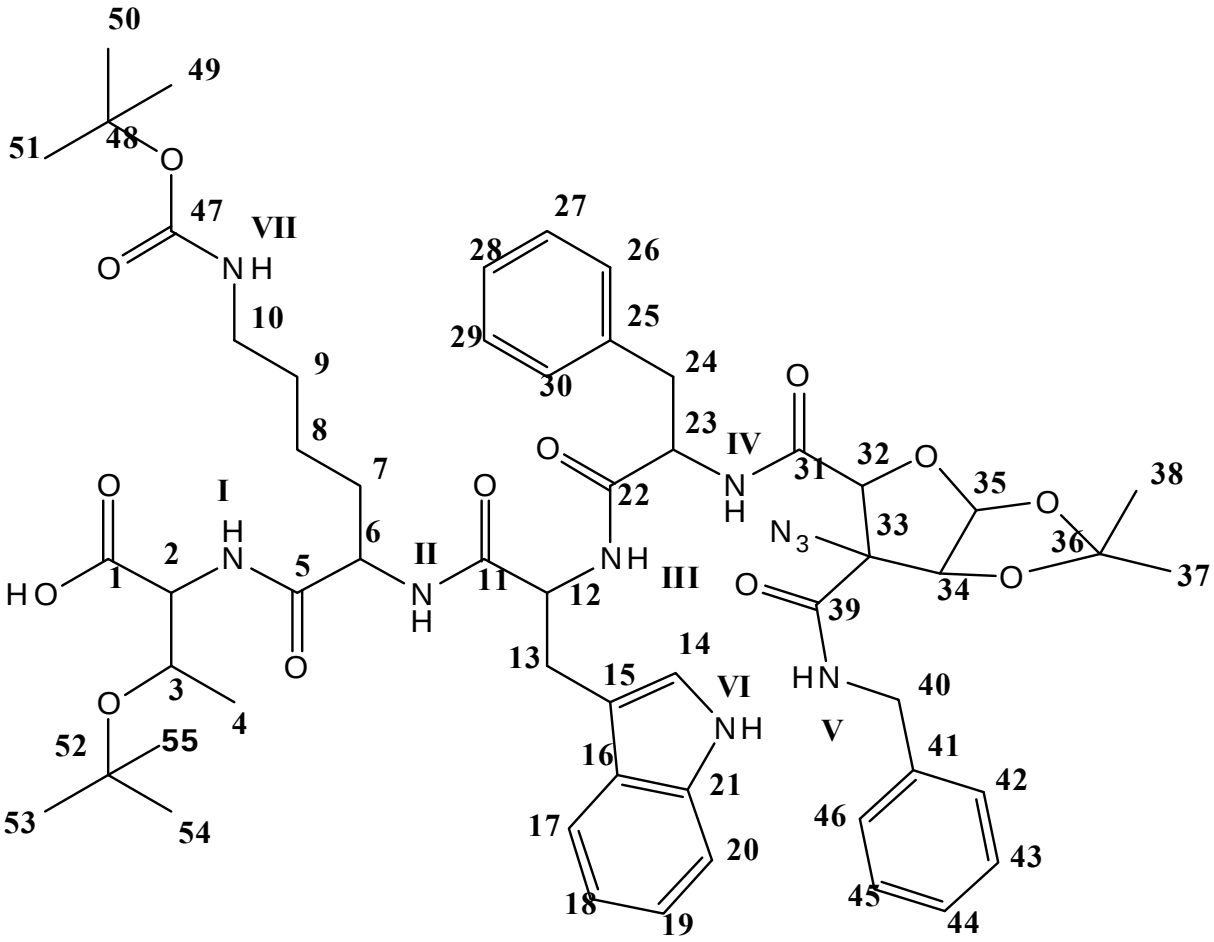
Flow Rate : 1 ml / min

<Chromatogram>



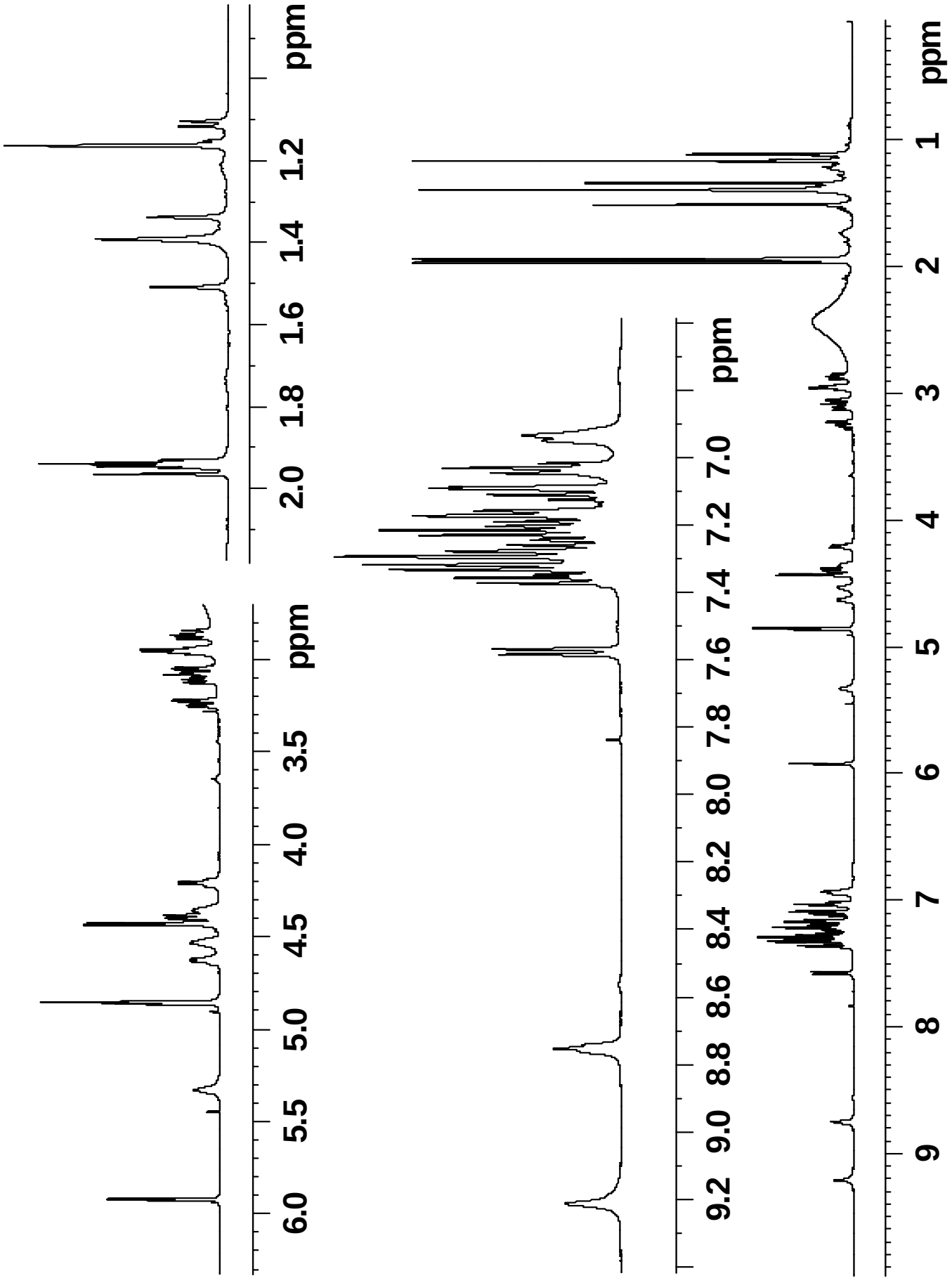


2D NMR Studies of Glycopeptide 8 in CD_3CN on 500MHz at 295K

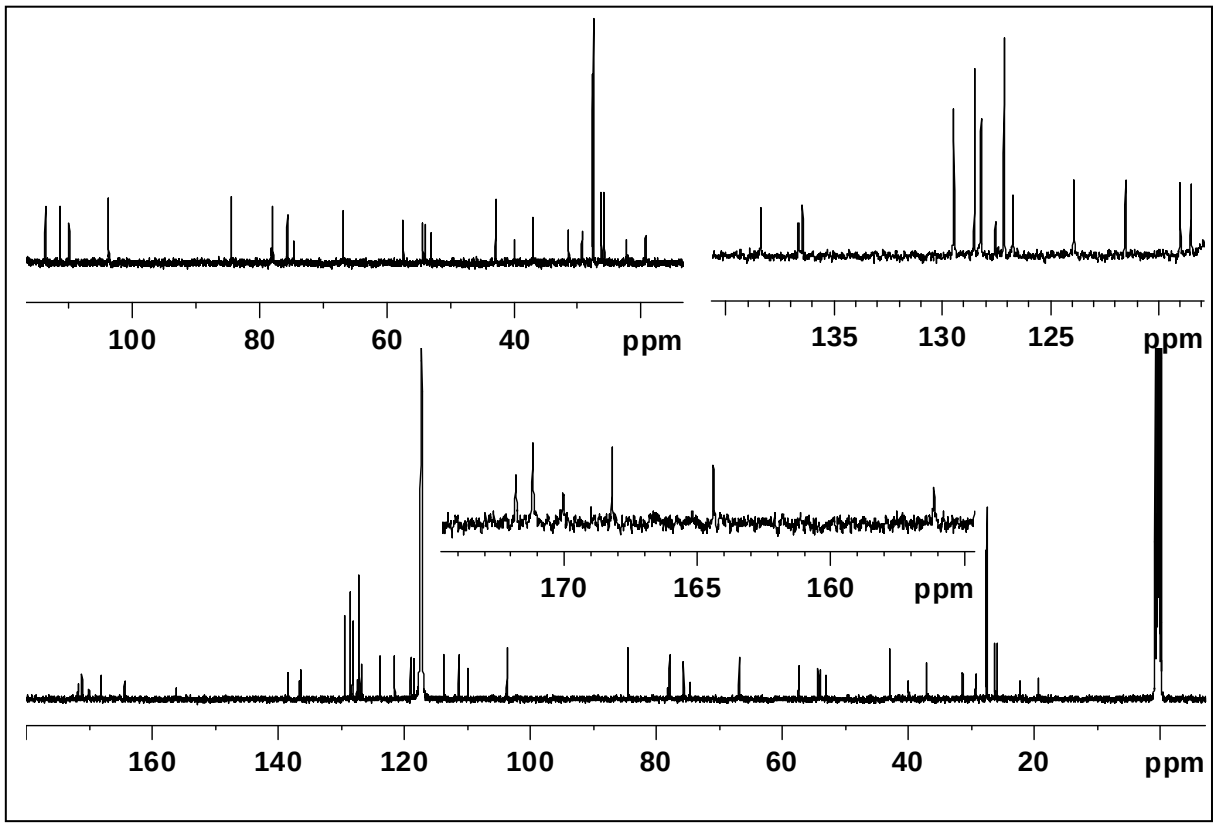


Glycopeptide 8 chemical structure with numbering

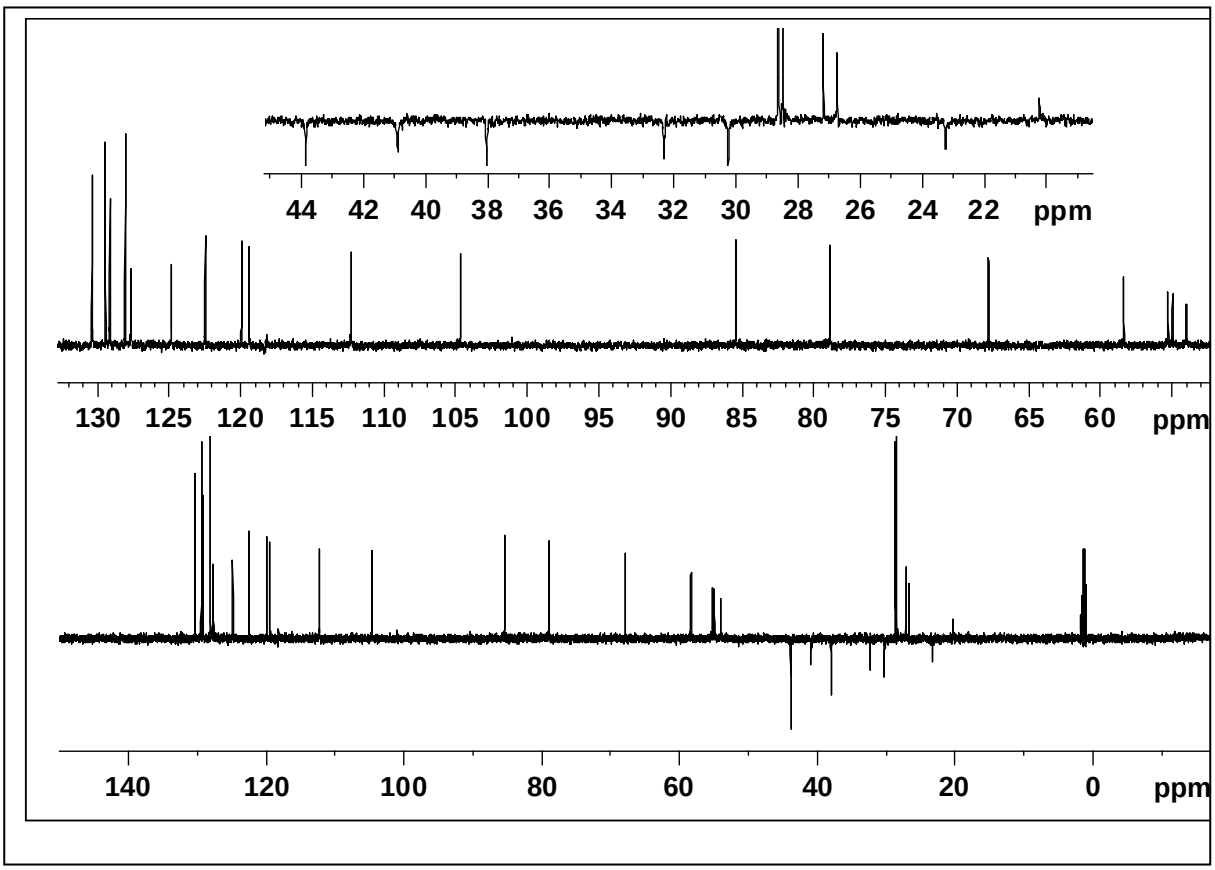
Glycopeptide 8 - ^{1}H,^{13}C HSQC Assignment	
^{1}H (δ/ppm)	^{13}C (δ/ppm)
1.16(C53H,C54H,C55H)	28.4
1.39(C49H,C50H,C51H)	28.5
4.39(C2H)	58.3
4.21(C3H)	67.7
1.11(C4H)	20.2
4.35(C6H)	53.9
1.73,1.55(C7H)	32.3
1.20(C8H)	23.2
1.36(C9H)	30.2
2.95(C10H)	40.9
4.64(C12H)	54.9
3.22,3.10(C13H)	28.5
7.09(C14H)	124.8
7.58(C17H)	119.4
7.04(C18H)	119.9
7.11(C19H)	122.4
7.37(C20H)	112.2
4.53(C23H)	55.2
3.04/3.05/3.08/3.07(C24H)	38.0
7.19(C26H),7.16(C30H)	130.3
7.23(C27H,C29H)	129.1
7.20(C28H)	127.6
4.85(C32H)	78.8
4.86(C34H)	85.3
5.92(C35H)	104.6
4.43(C40H)	43.8
7.29(C42H,C46H)	128.0
7.27(C44H)	128.0
7.33(C43H,C45H)	129.4
1.50(C37H)	26.7
1.33(C38H)	27.1

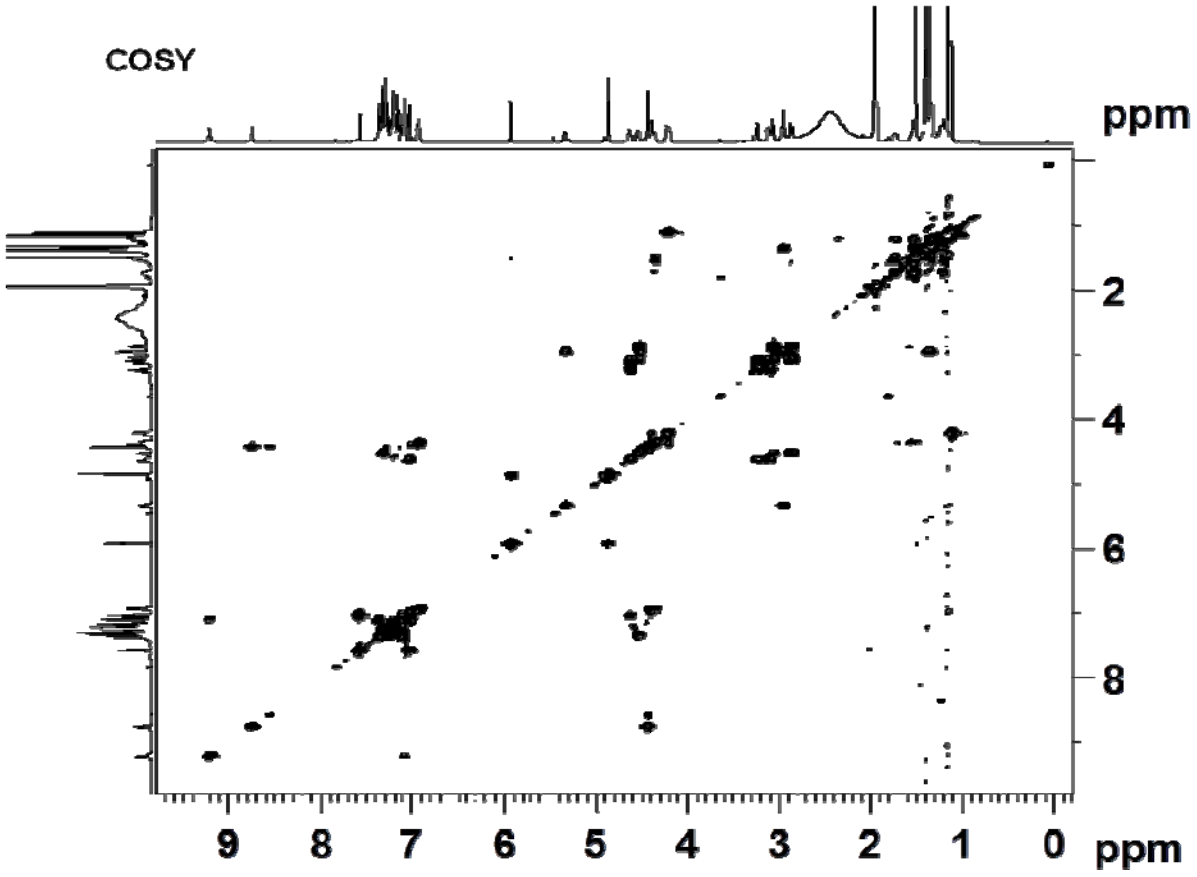


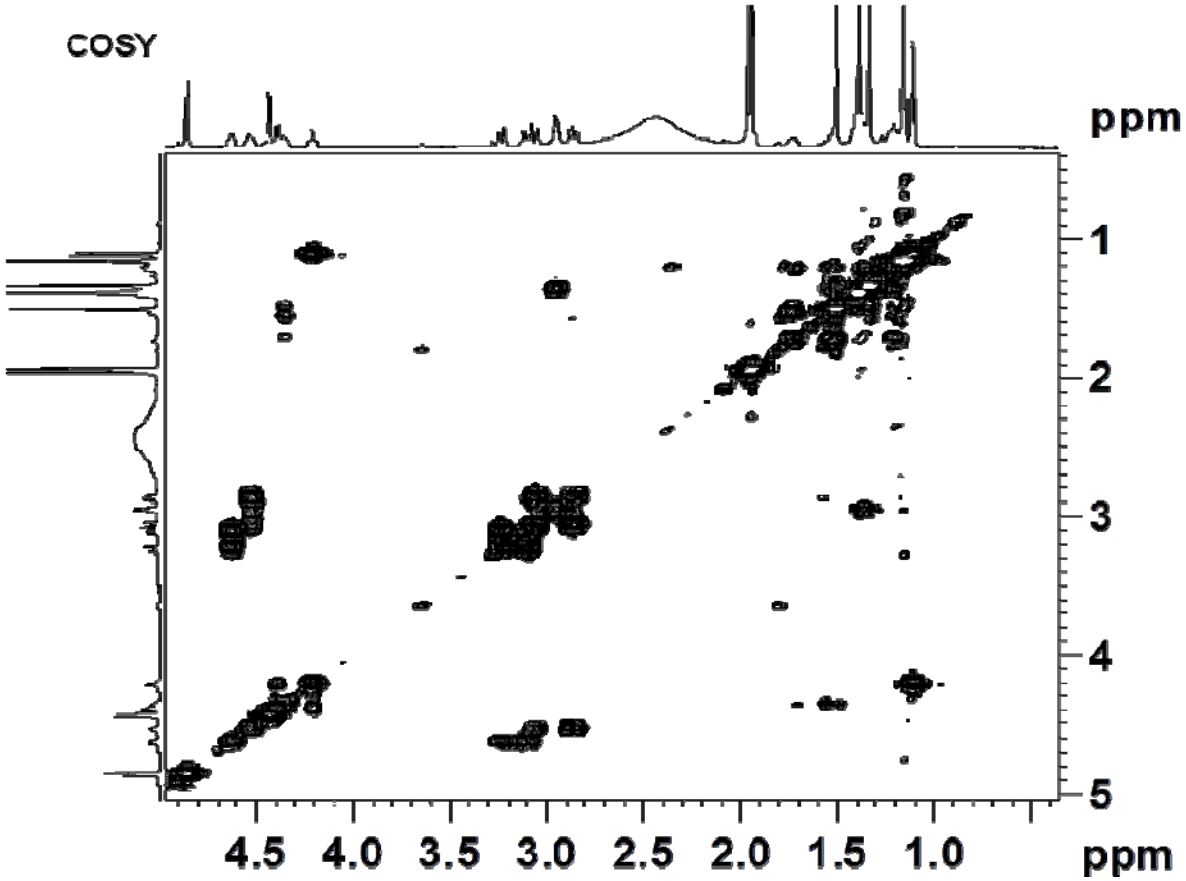
^{13}C NMR of **Glycopeptide 8**

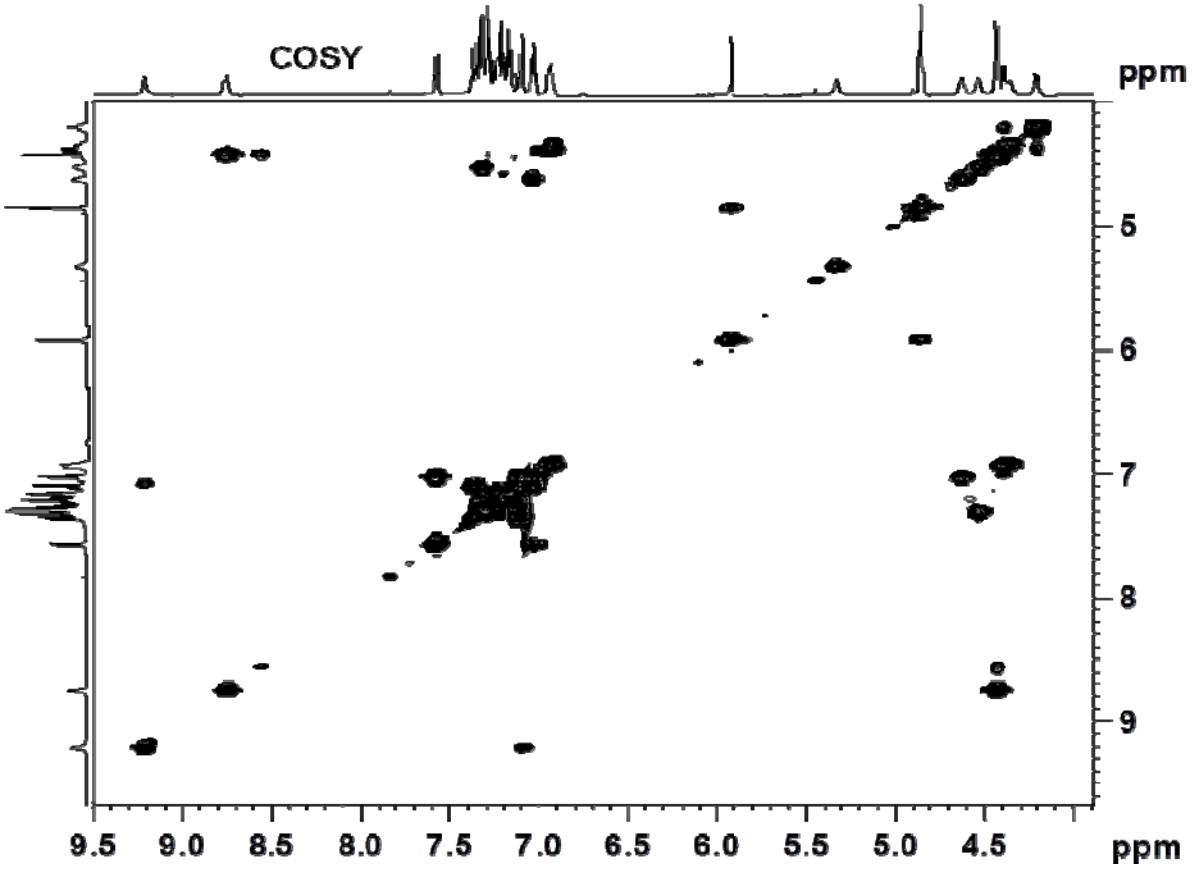


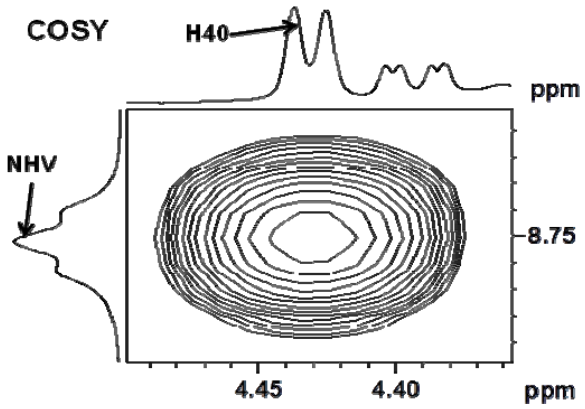
DEPT

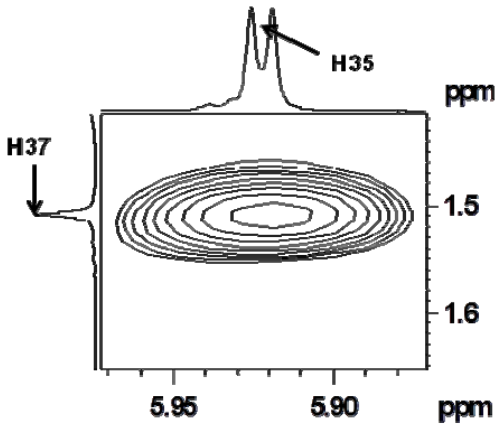


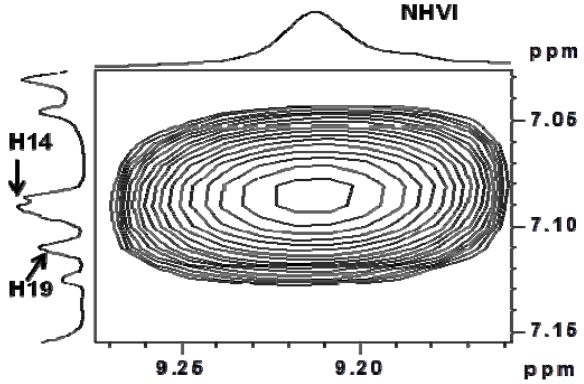


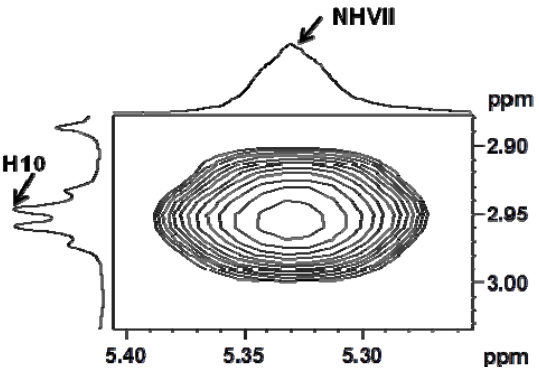


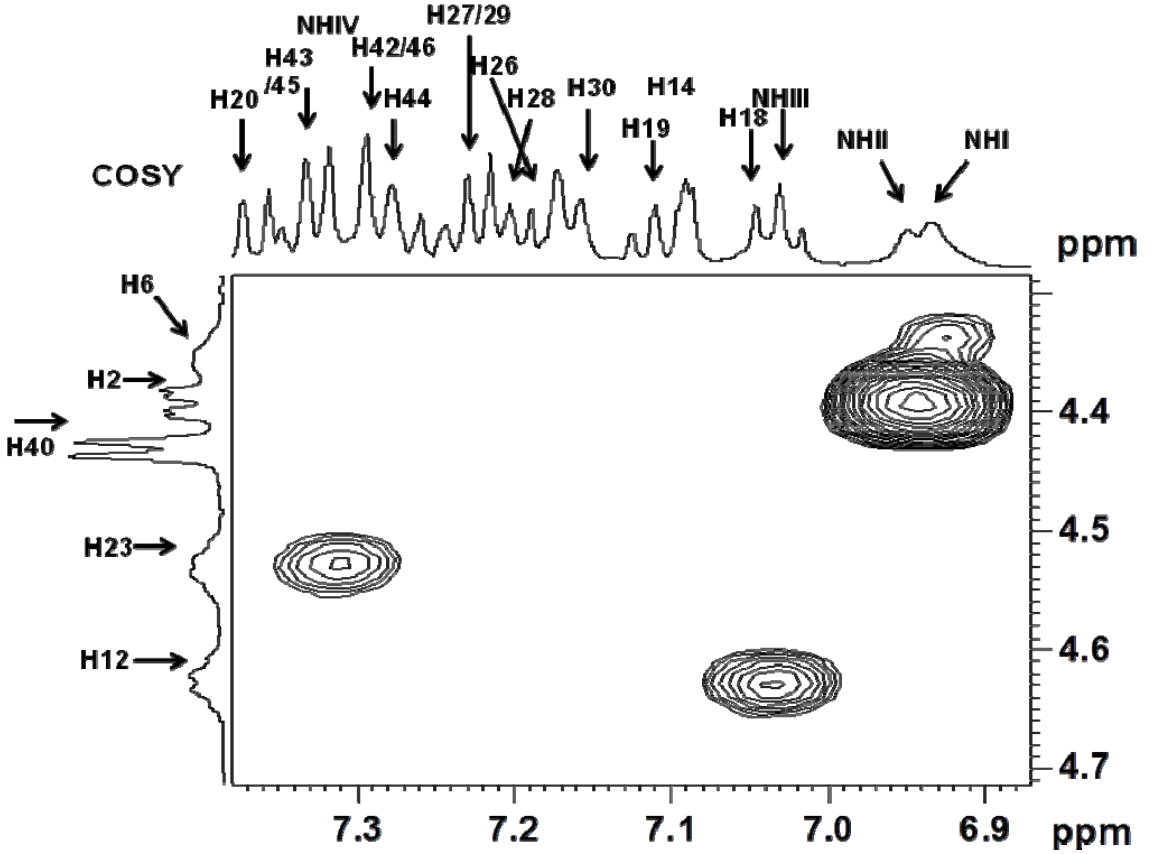


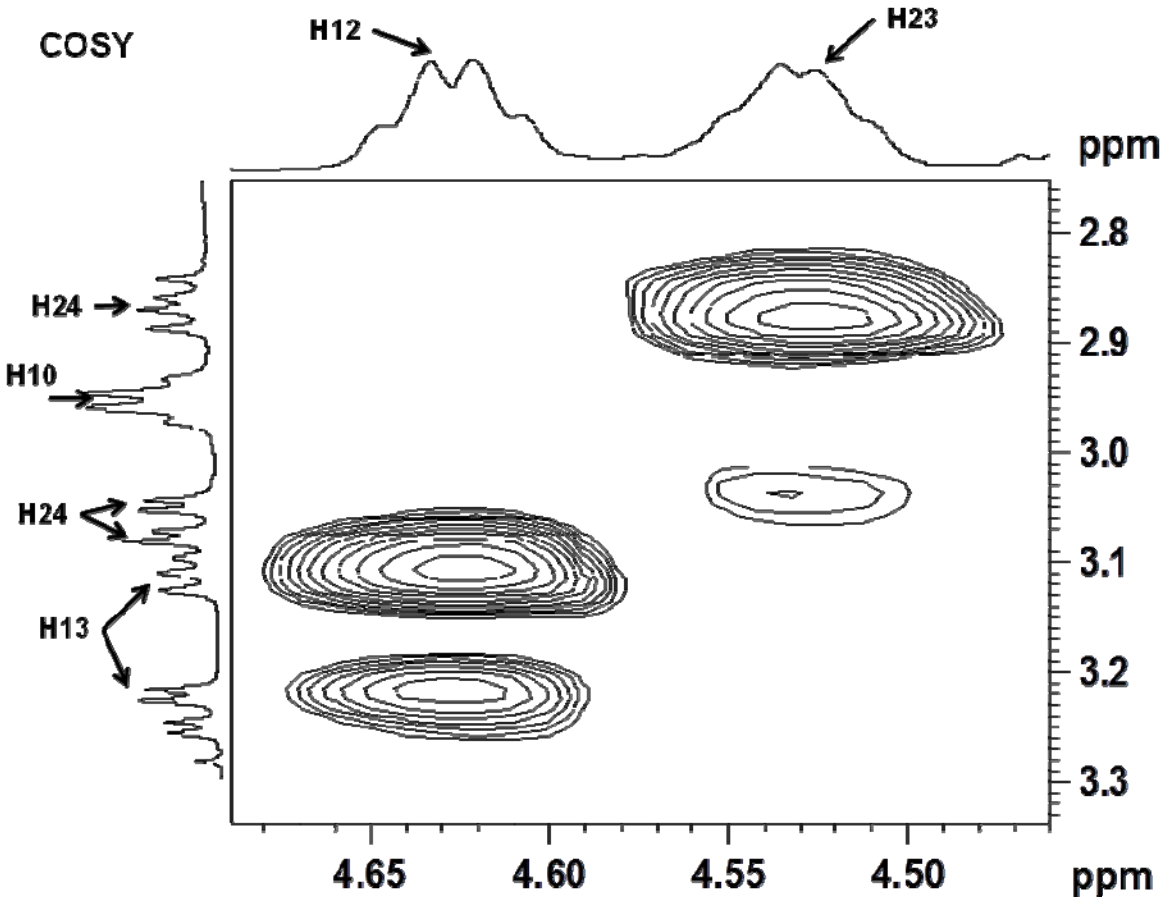


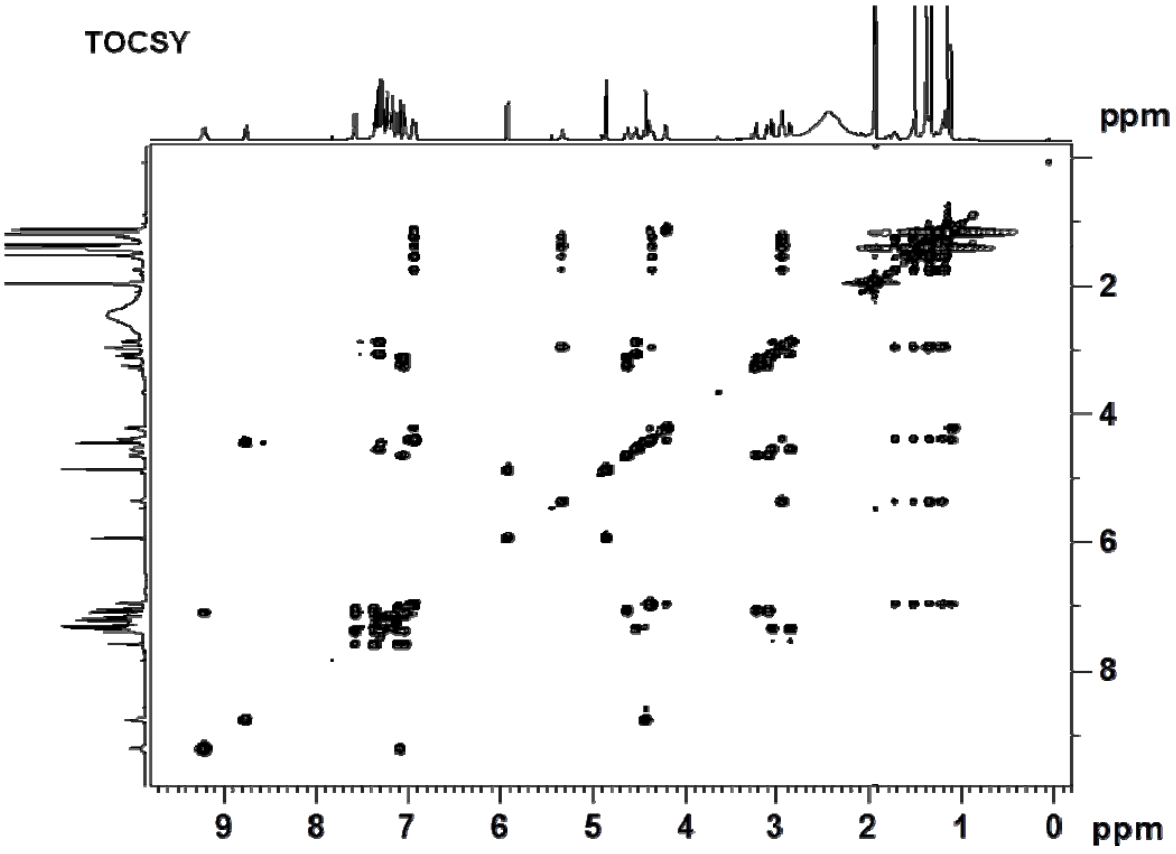


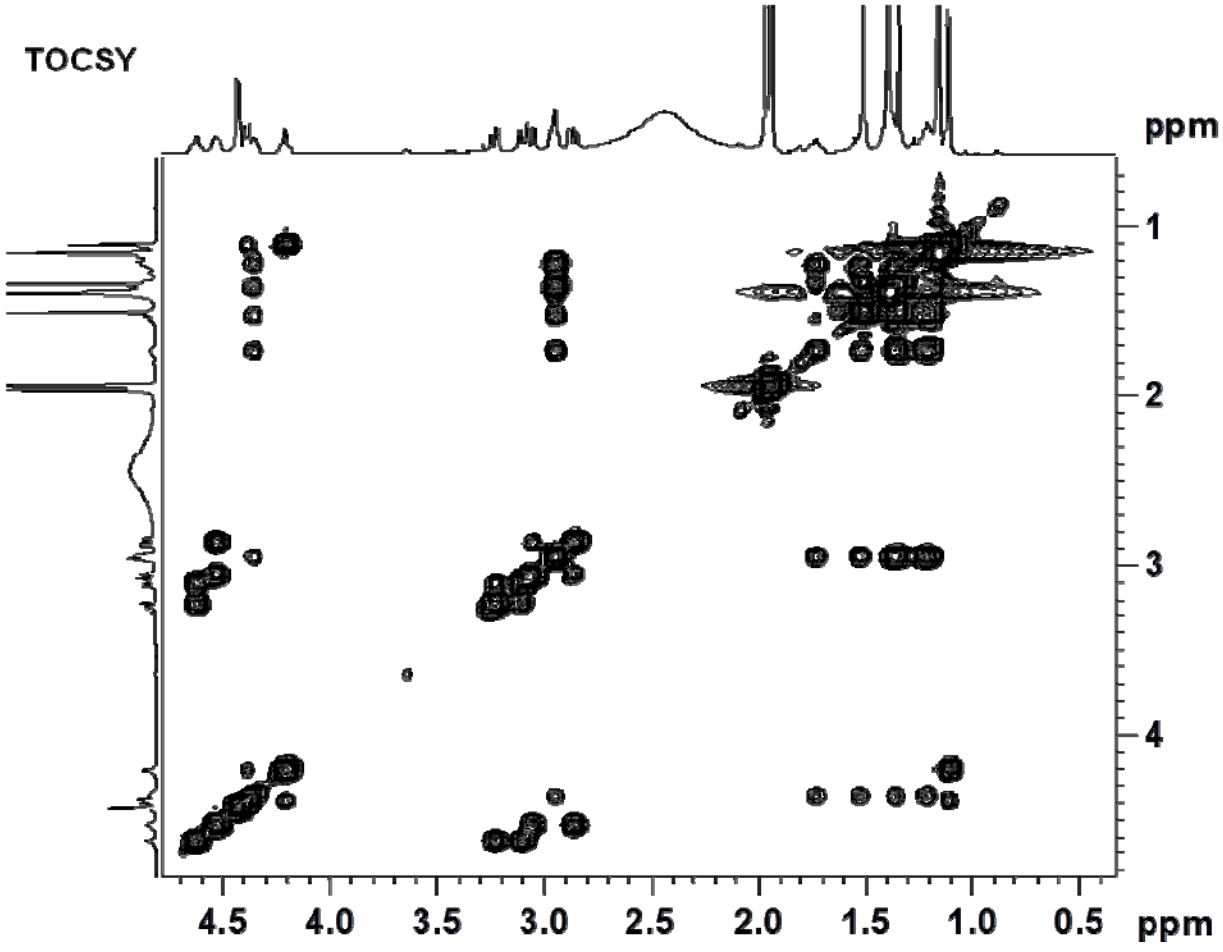


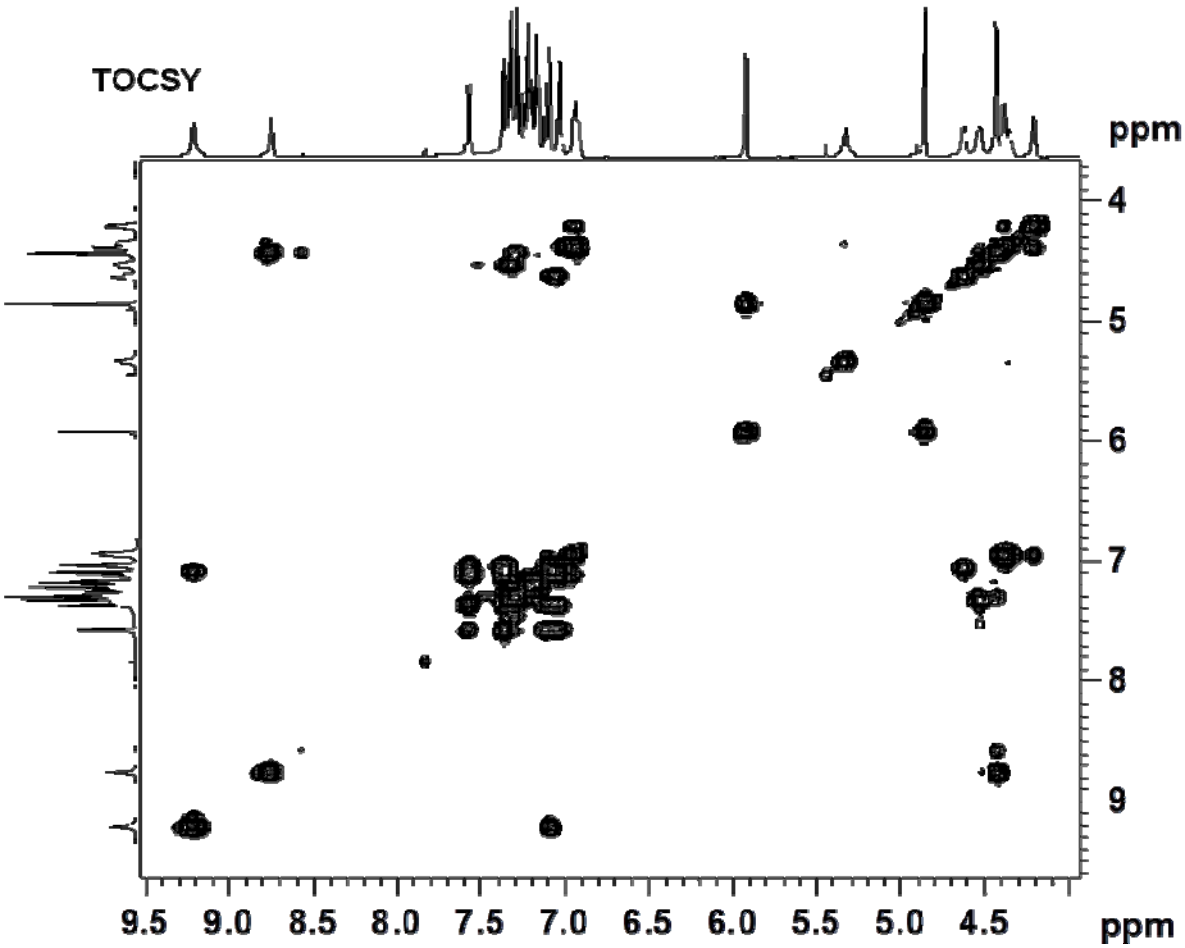


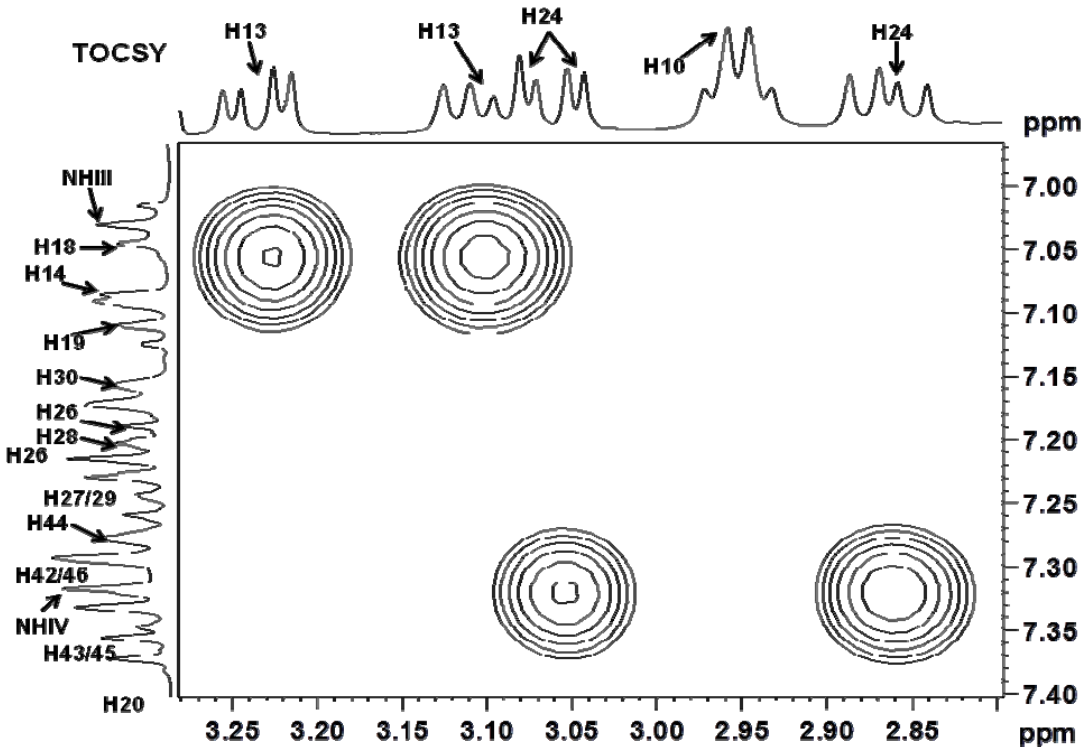


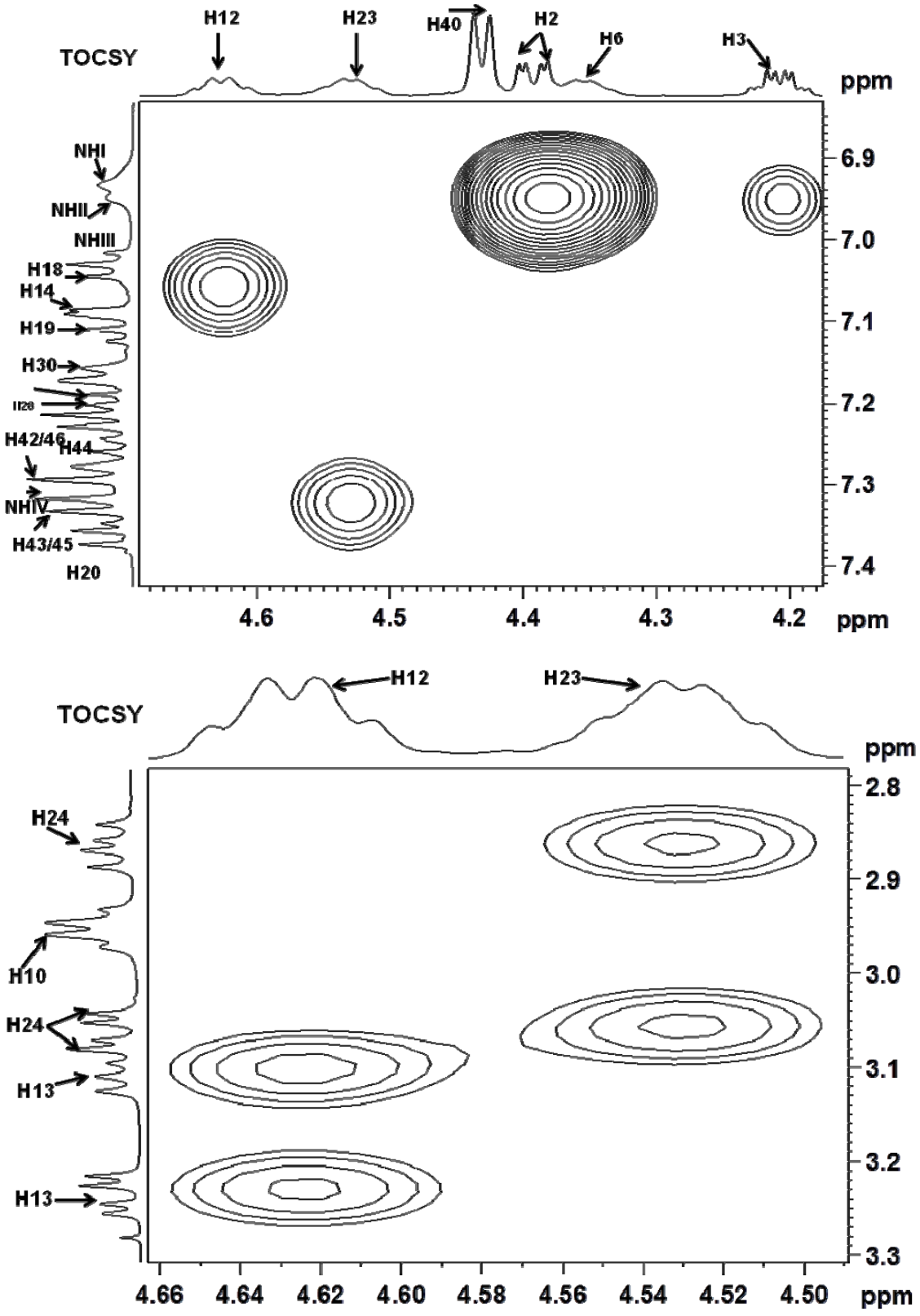


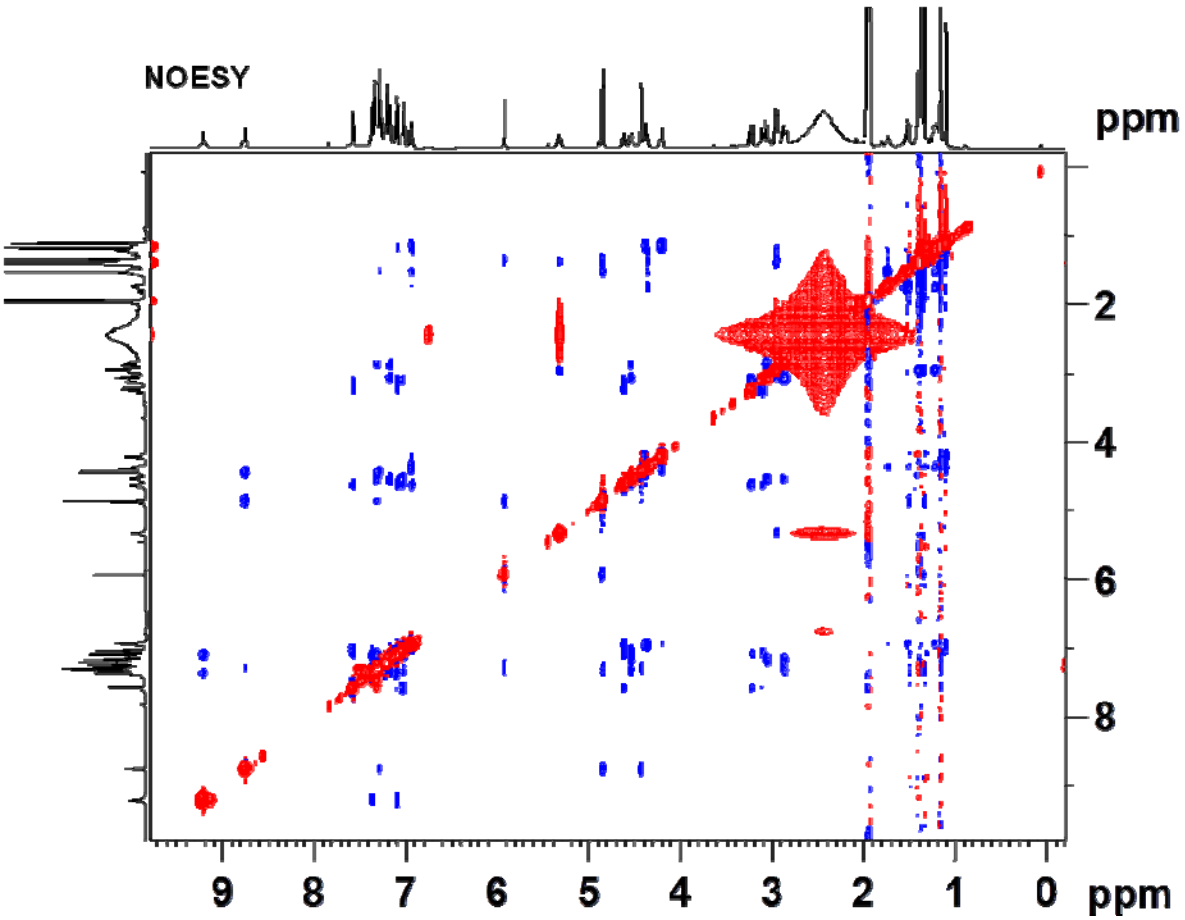


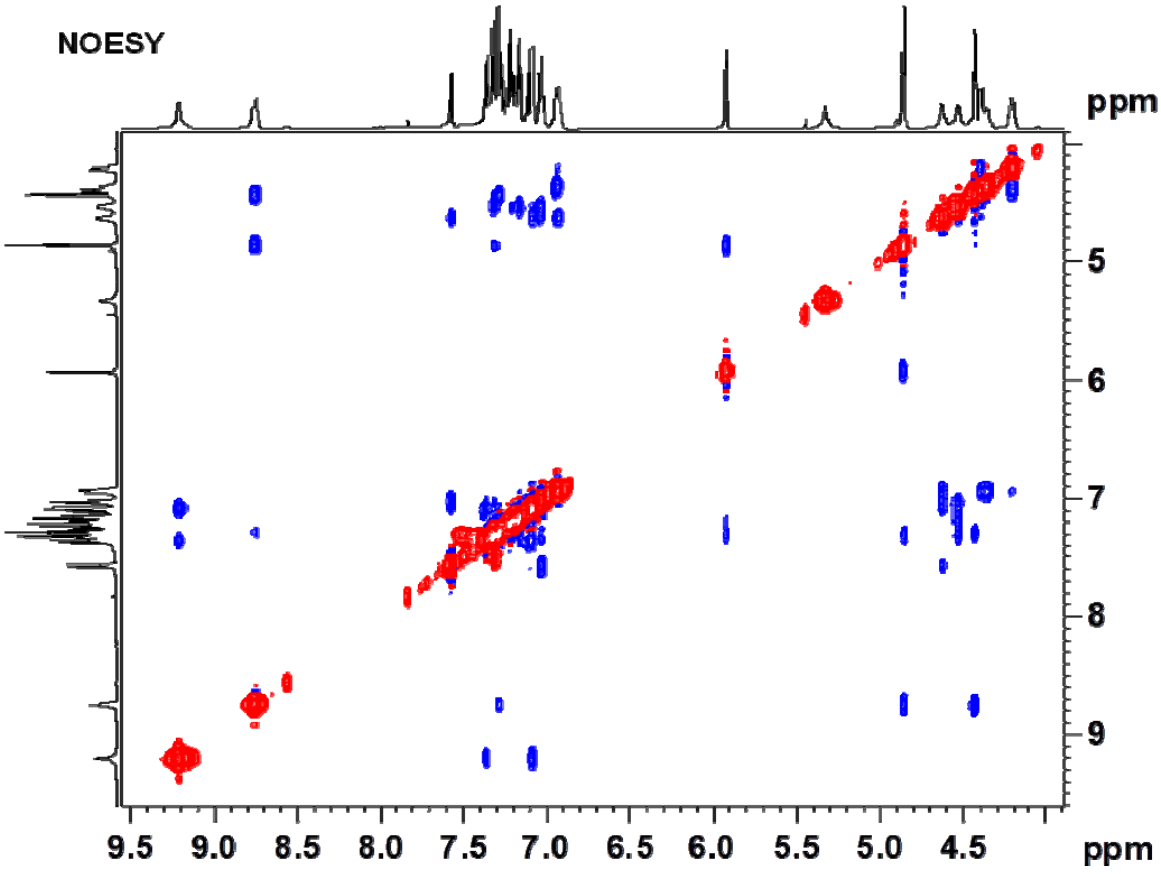


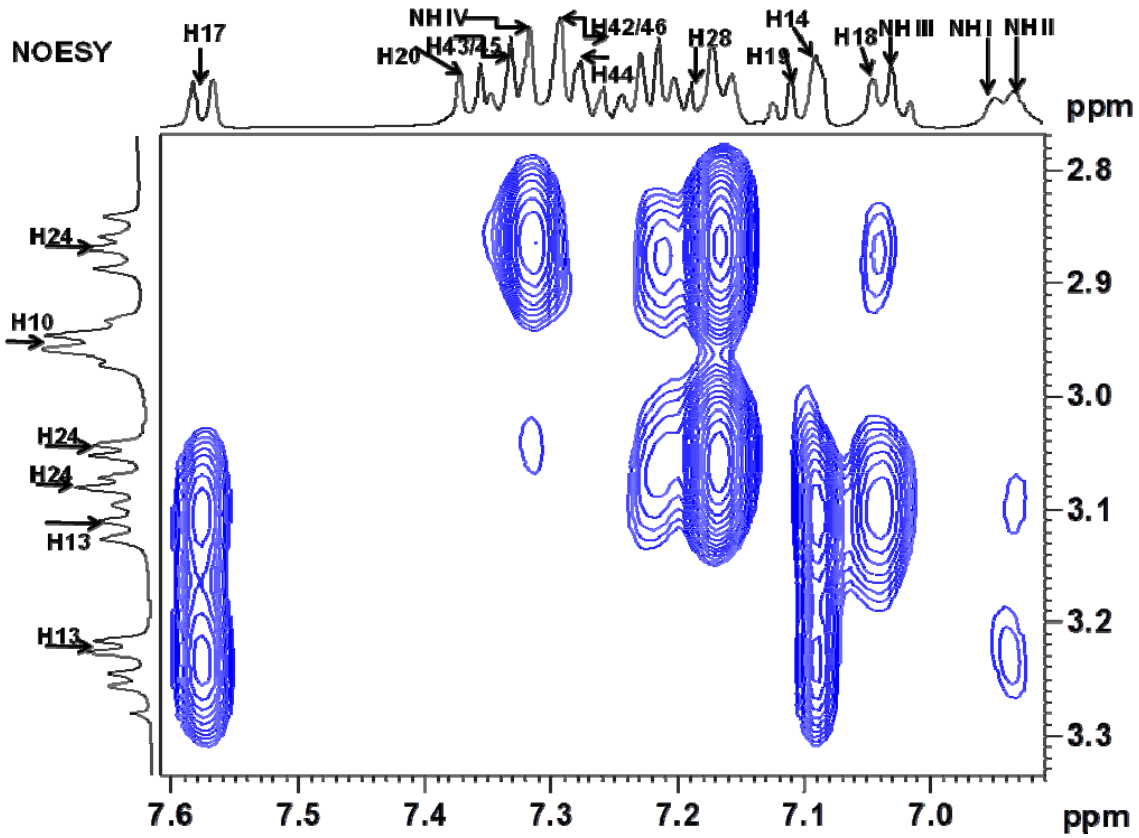


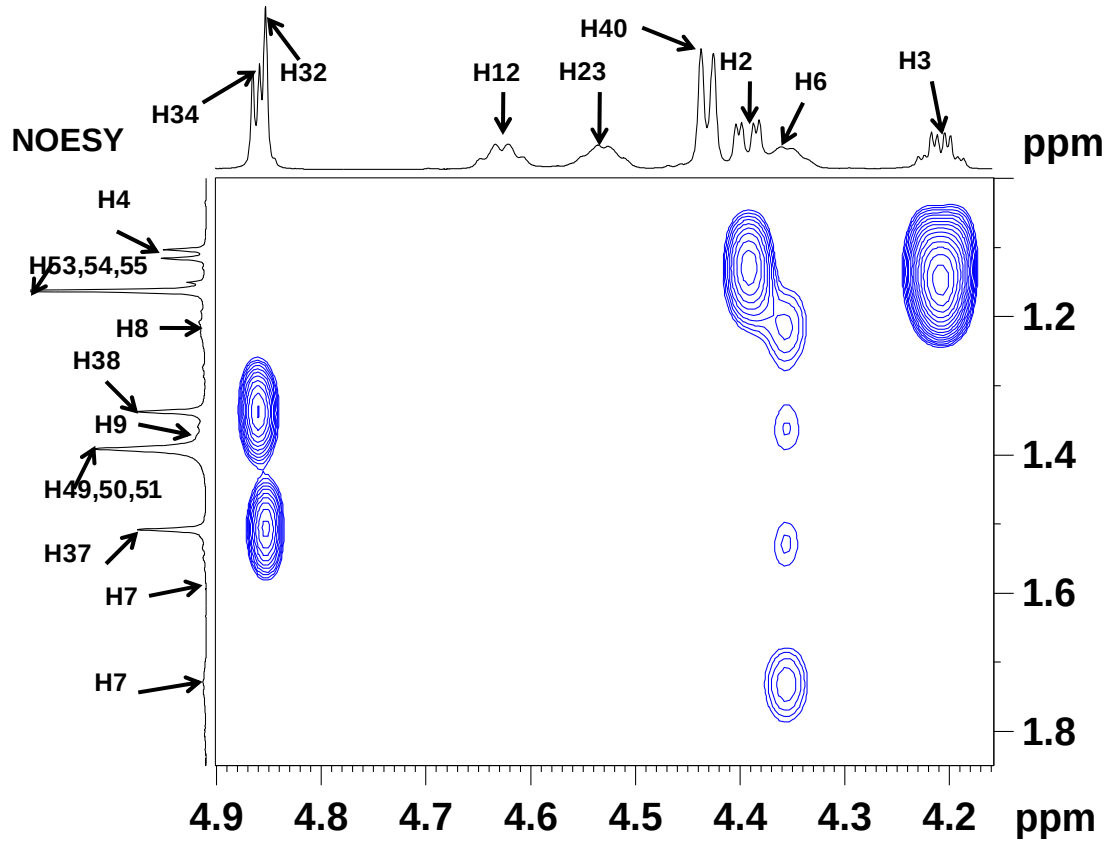


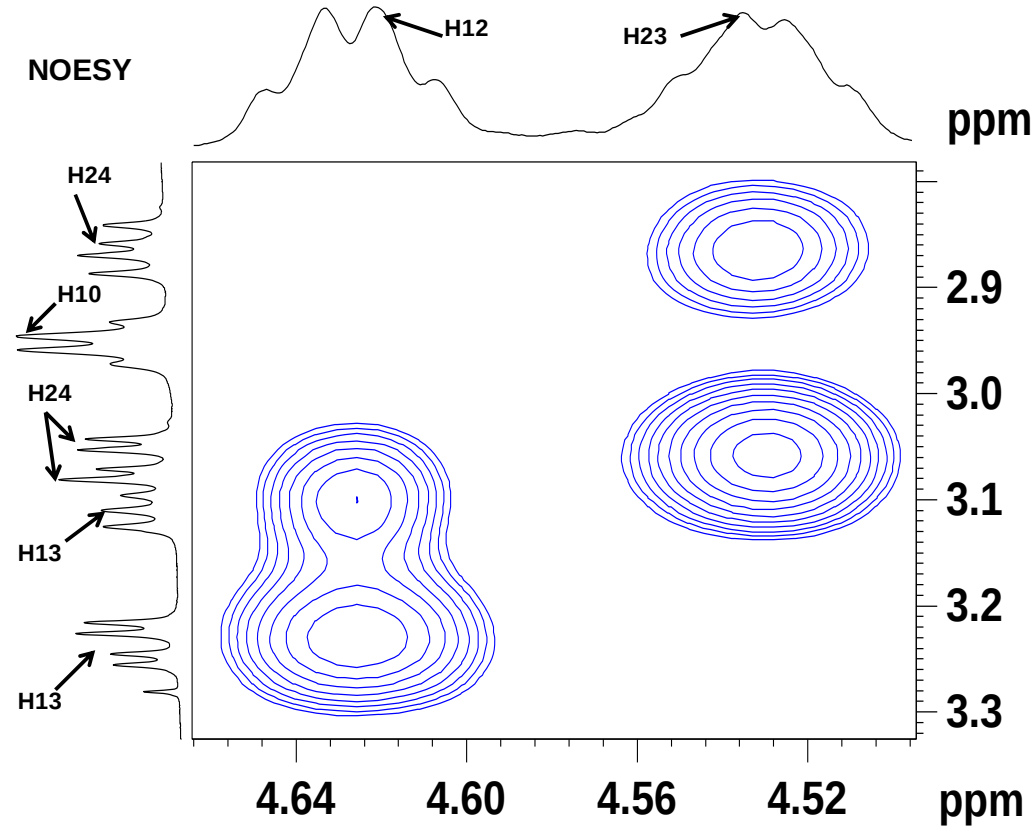


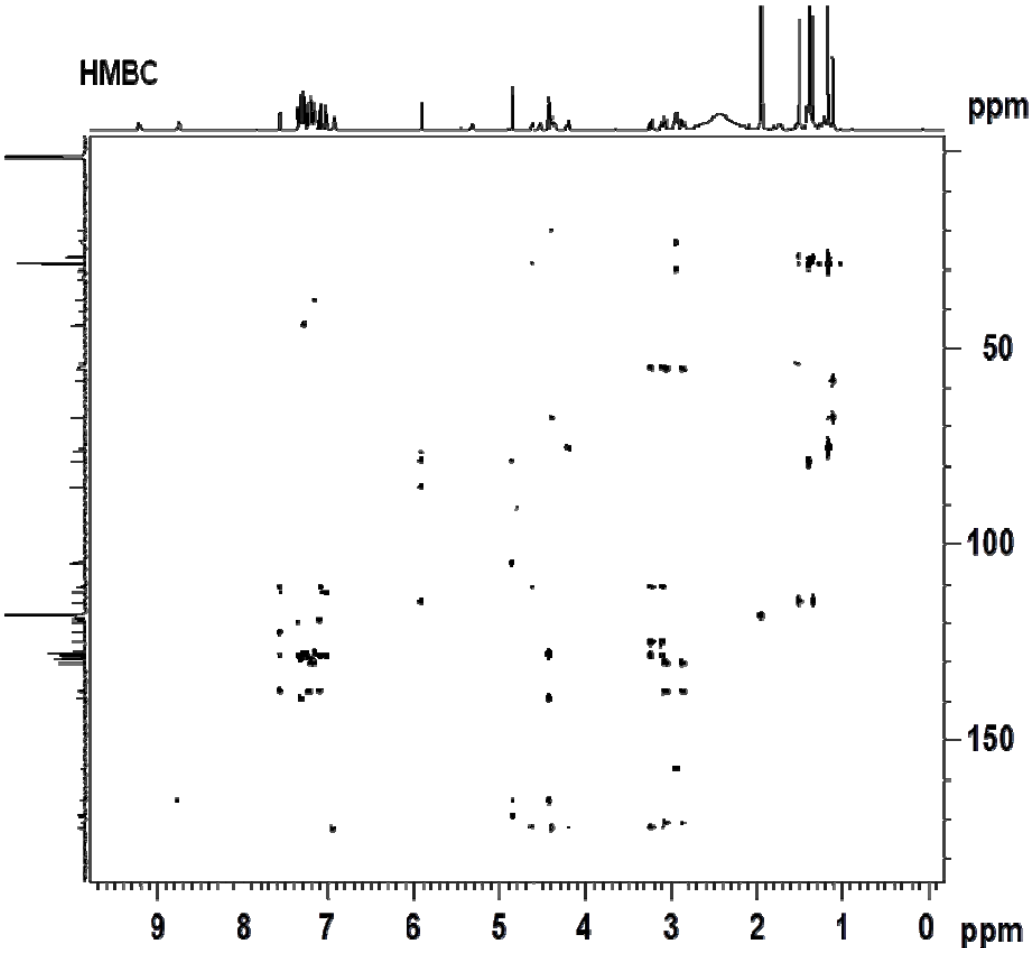


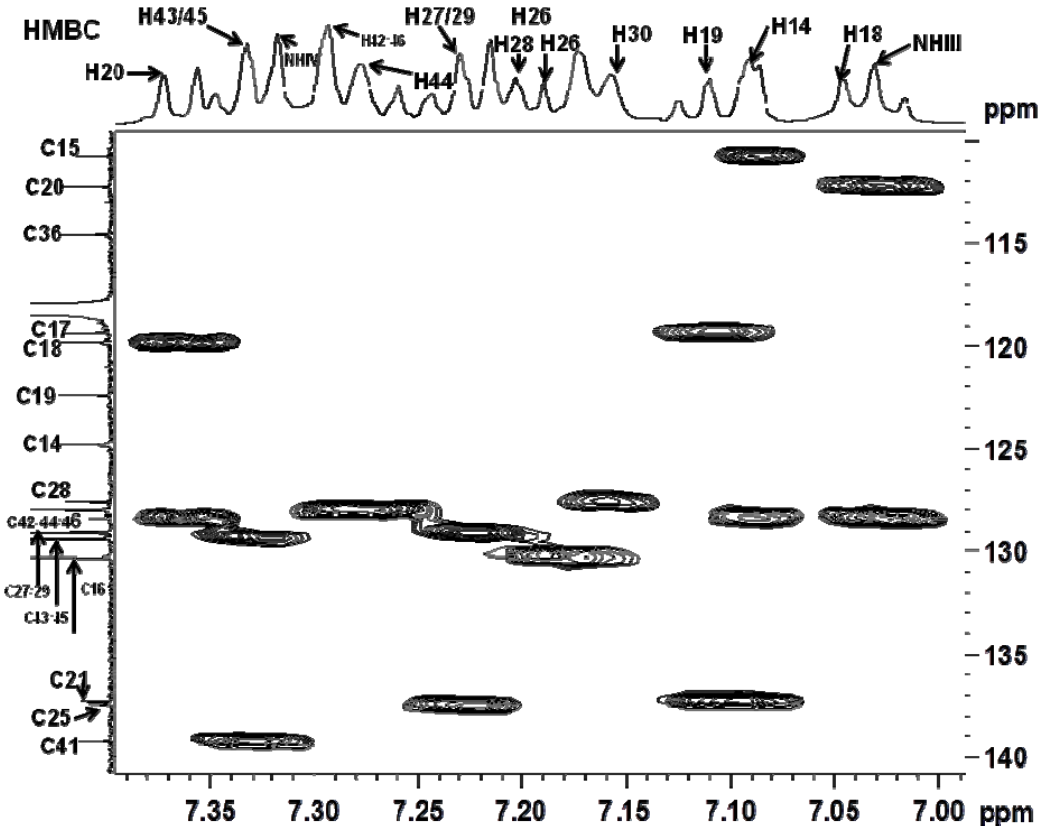


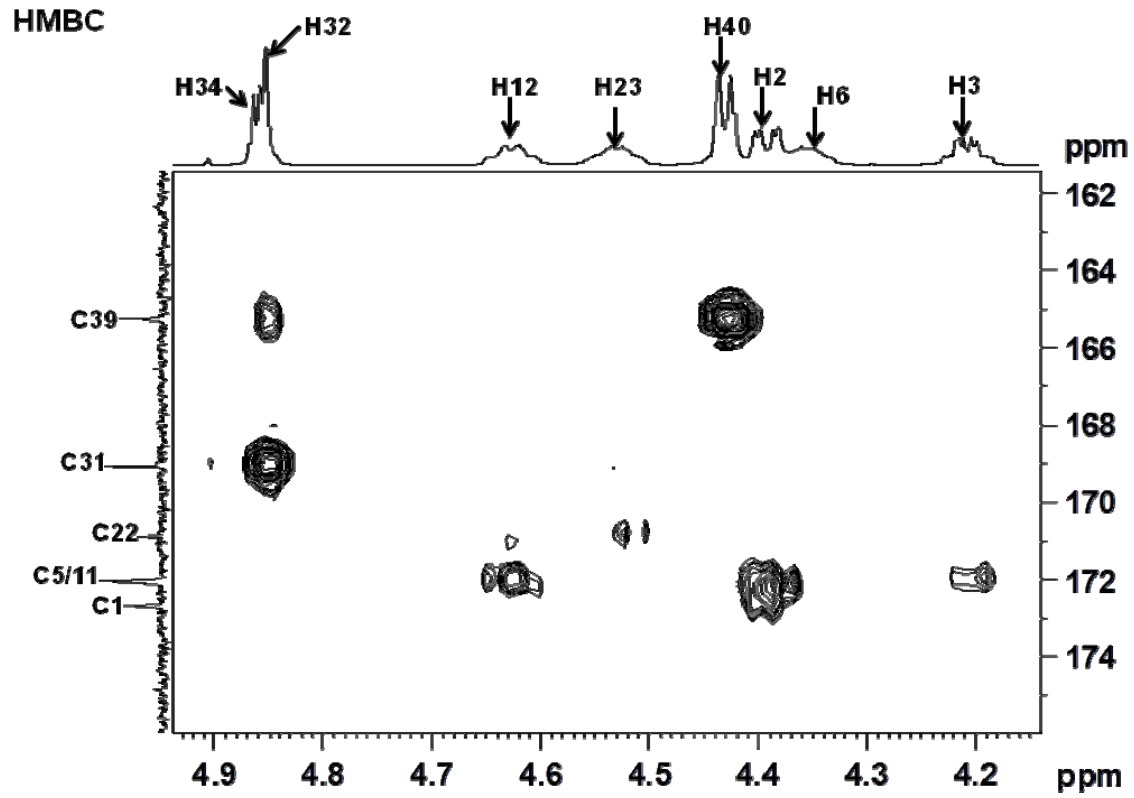


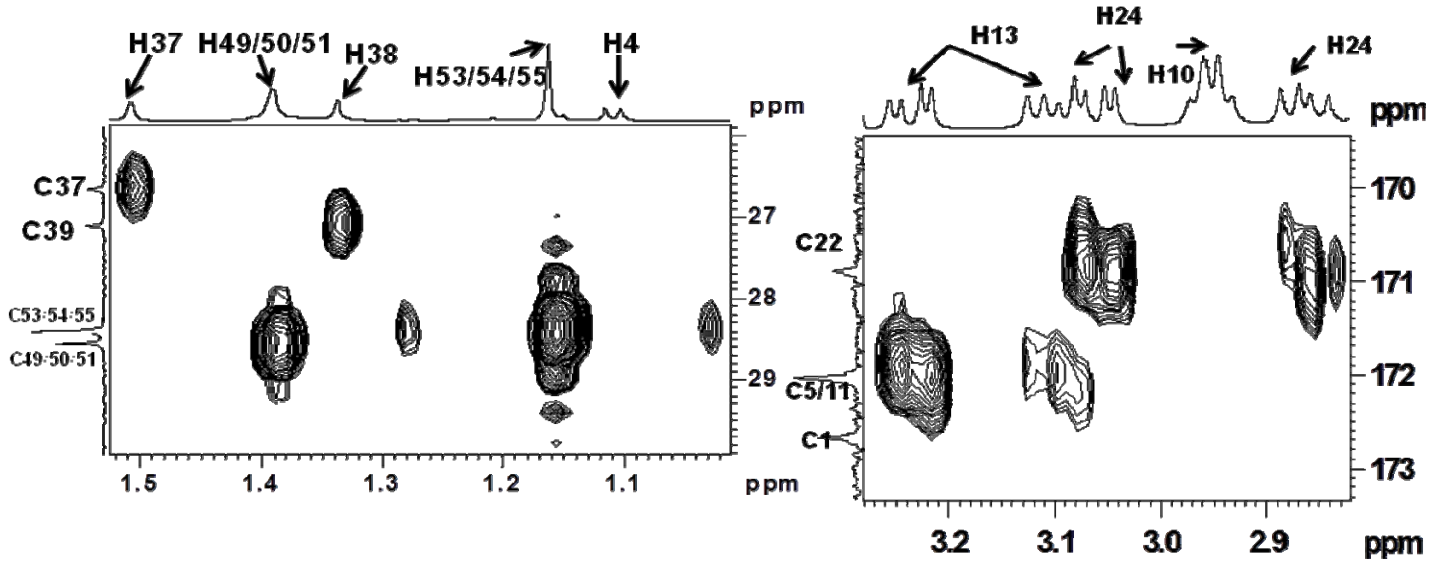


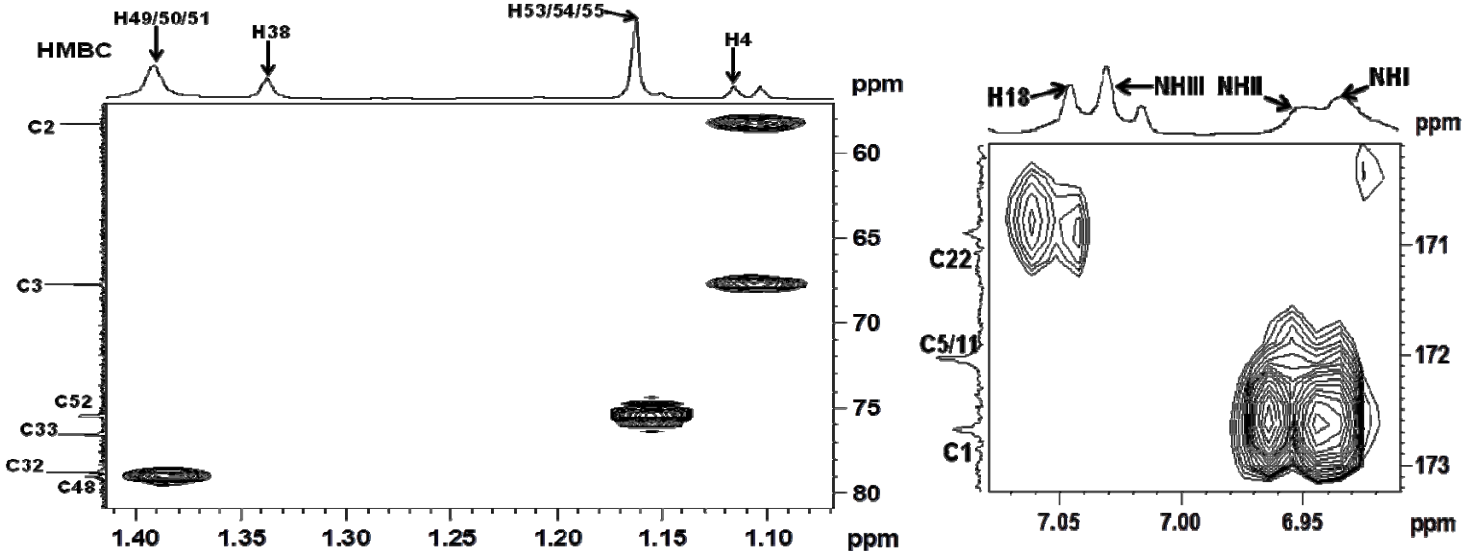


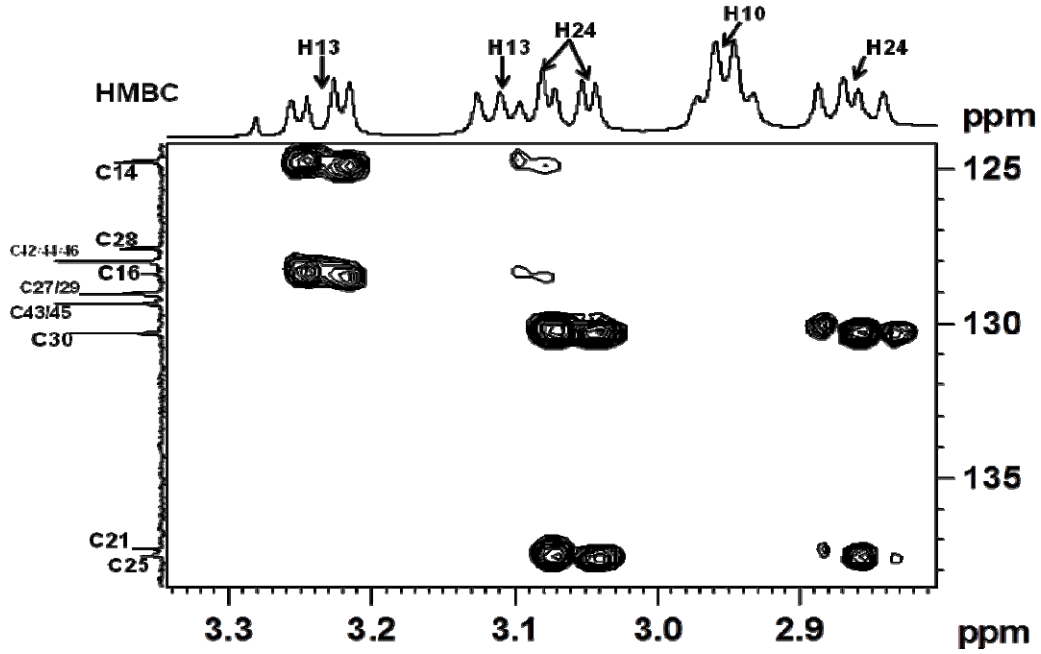


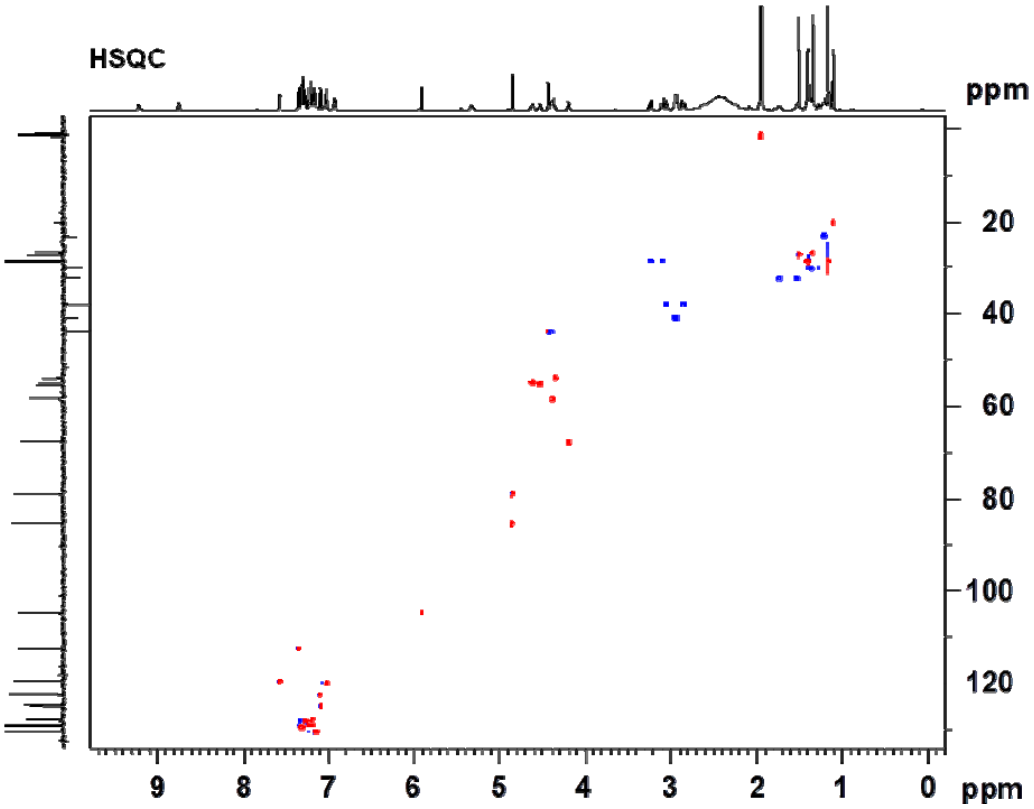


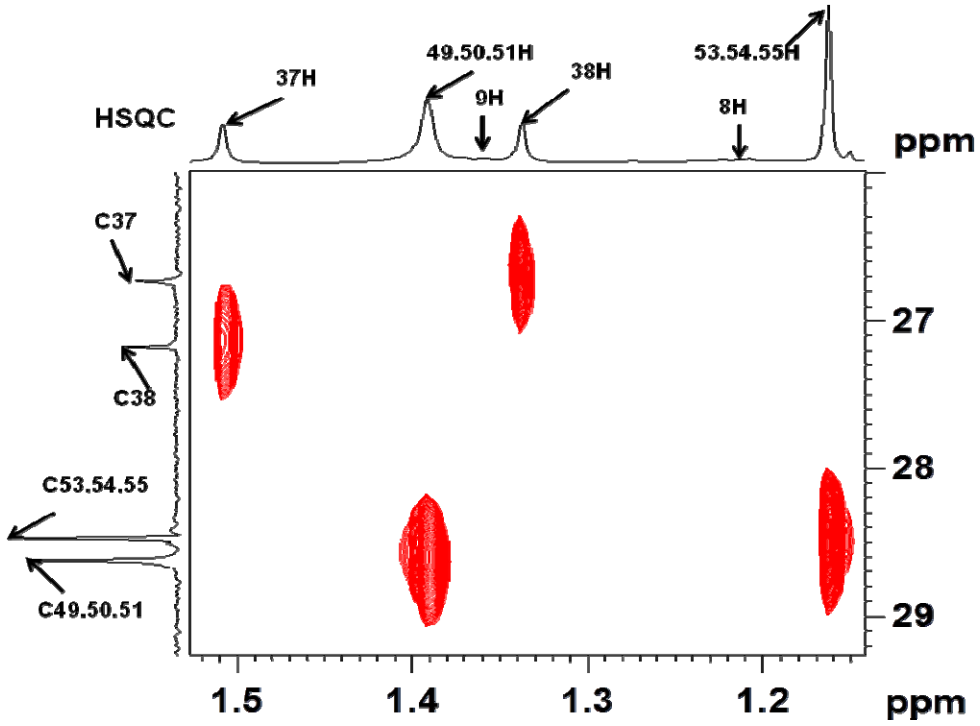


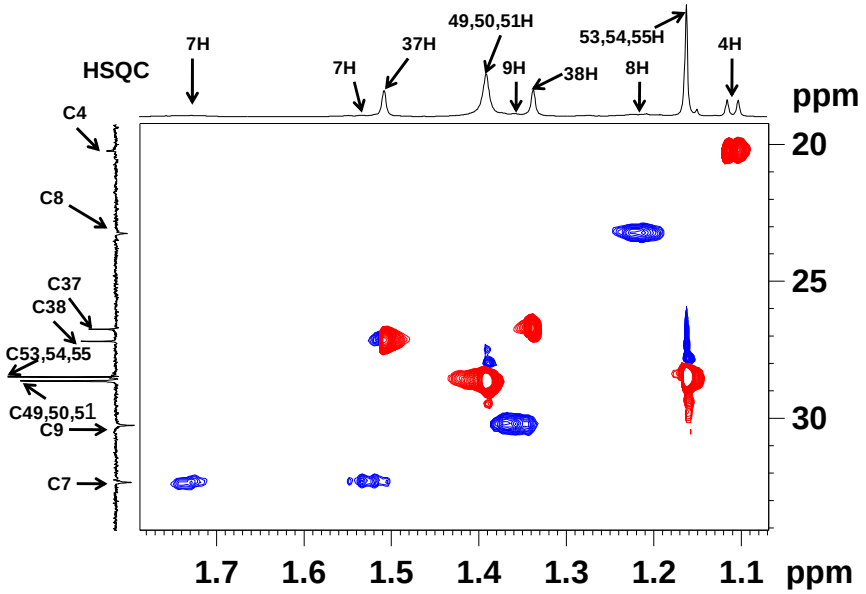


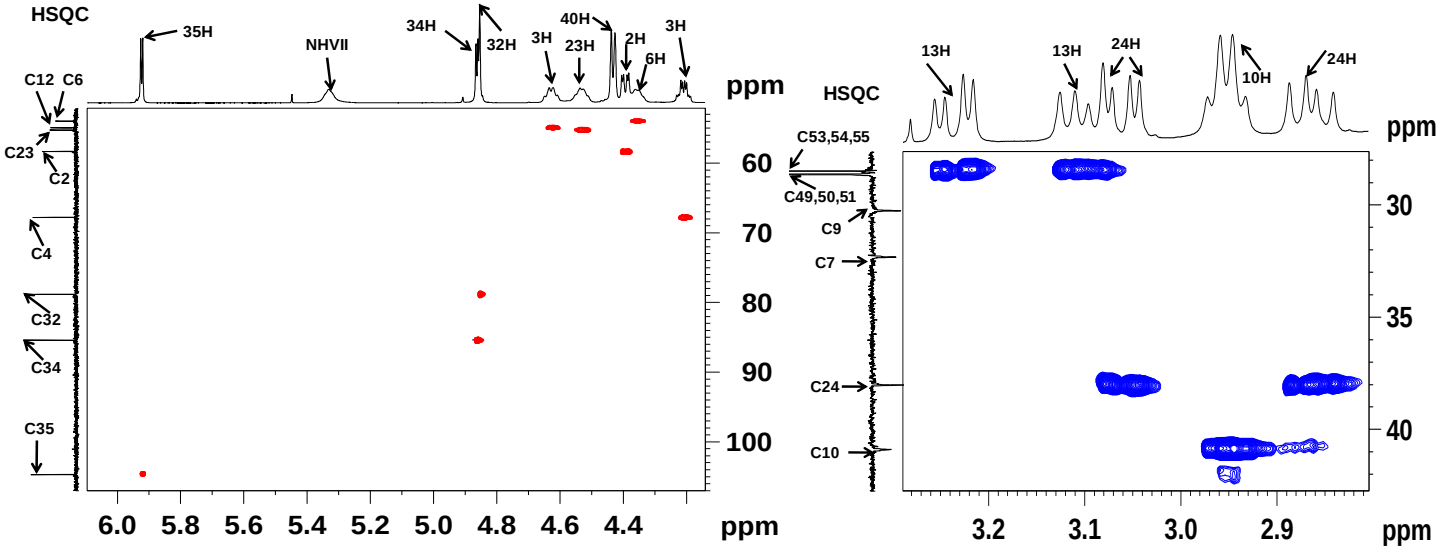


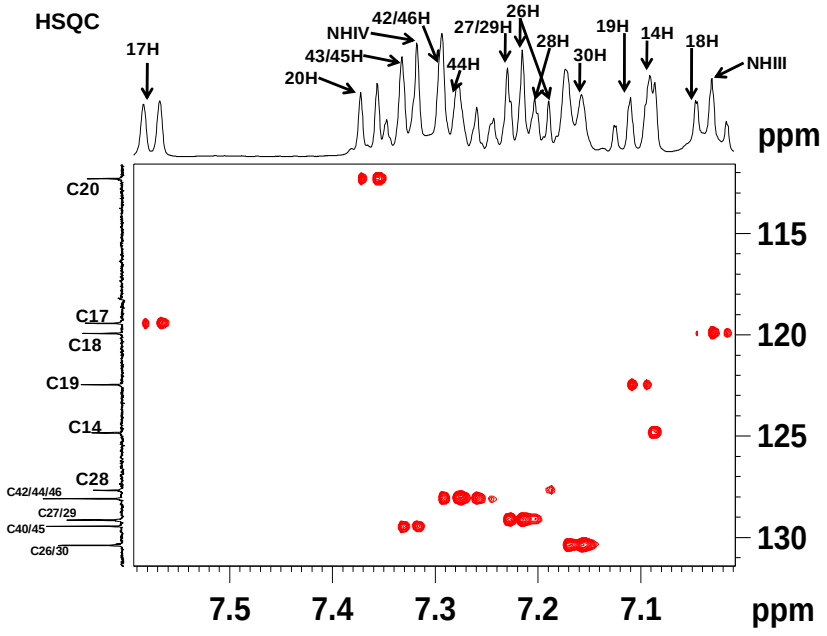


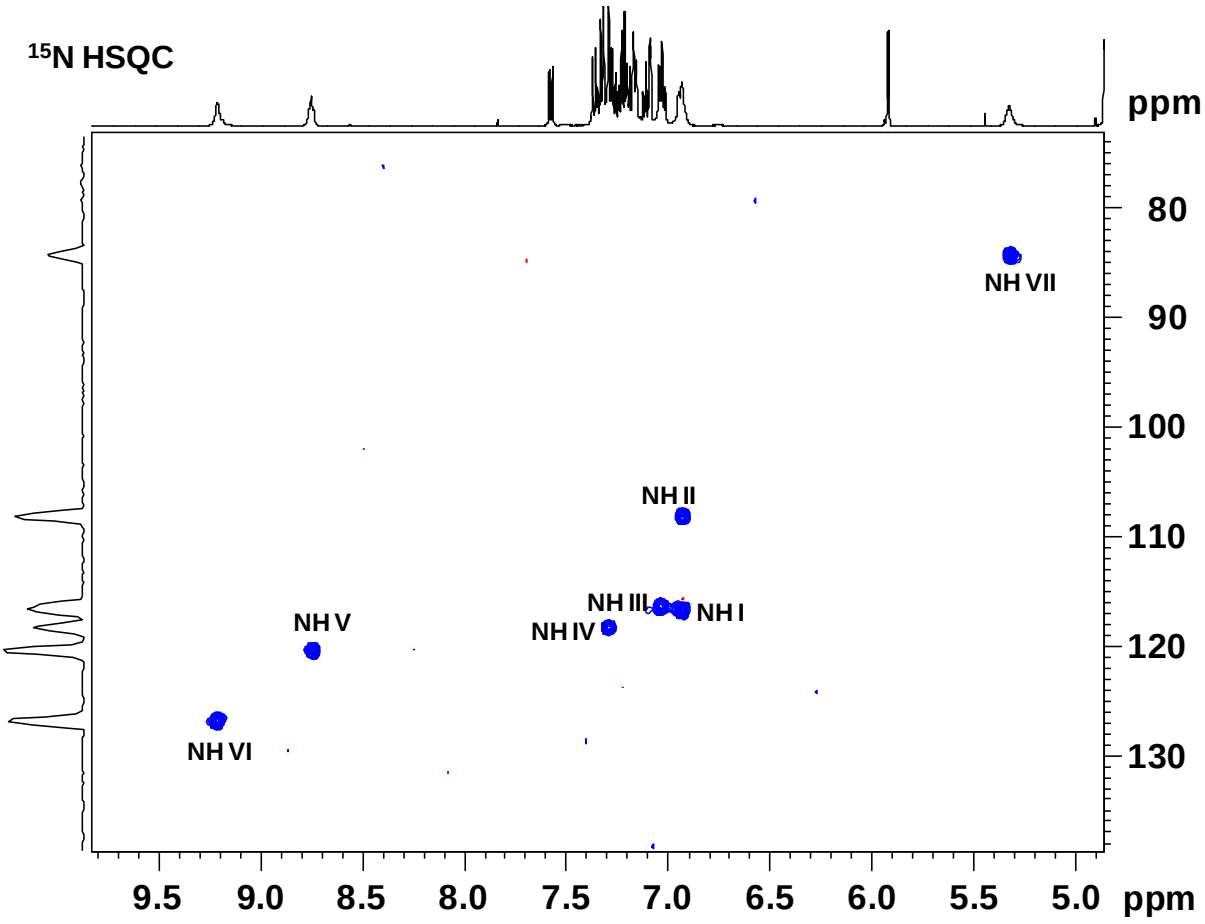




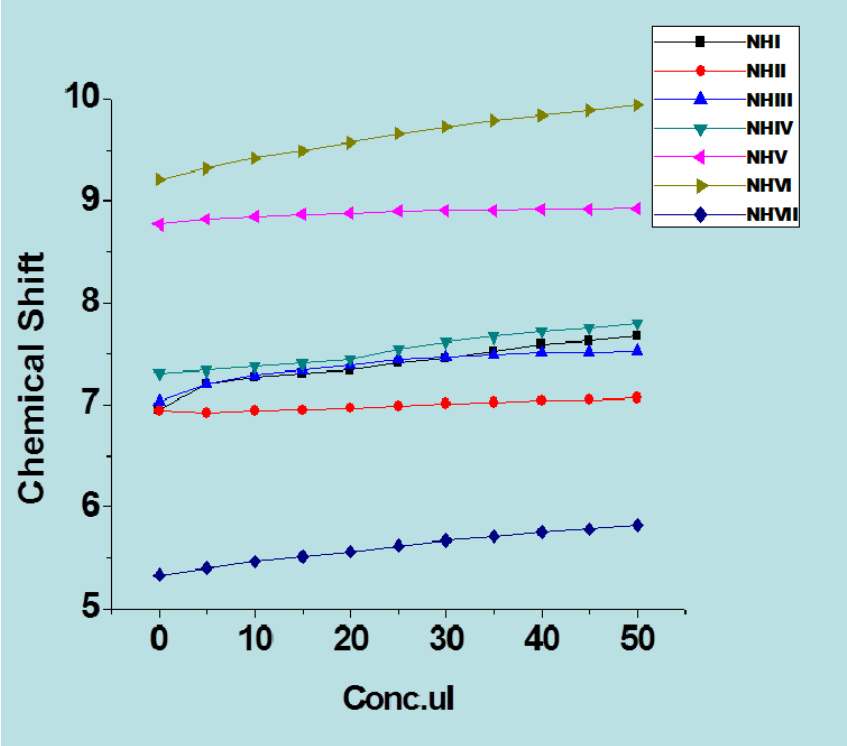








DMSO Titration Studies



DMSO Titration Studies –Change in Chemical Shifts

CONC. (ul)	Chemical Shift (ppm)						
	NHI	NHII	NHIII	NHIV	NHV	NHVI	NHVII
0	6.95	6.94	7.03	7.31	8.77	9.21	5.33
5	7.21	6.92	7.20	7.34	8.82	9.32	5.40
10	7.28	6.94	7.29	7.38	8.85	9.42	5.46
15	7.31	6.95	7.34	7.41	8.87	9.49	5.51
20	7.34	6.97	7.39	7.45	8.88	9.57	5.56
25	7.41	6.99	7.45	7.55	8.90	9.66	5.62
30	7.46	7.01	7.47	7.62	8.91	9.73	5.67
35	7.53	7.02	7.49	7.67	8.91	9.79	5.71
40	7.59	7.04	7.51	7.72	8.92	9.84	5.75
45	7.63	7.05	7.52	7.76	8.92	9.89	5.78
50	7.67	7.07	7.53	7.80	8.93	9.94	5.82
Δδ	*0.72*	*0.13*	*0.5*	*0.49*	*0.16*	*0.73*	*0.49*

Distance constraints used in MD calculations for Glycopeptide 8 from NOESY experiment

Sr.No.	From	To	Distance(Å)	Sr.No.	From	To	Distance(Å)
1.	NHV	32H	2.76-2.26	7.	53/54/55H	17H	4.60-3.76
2.	NHV	19H	5.44-4.45	8.	35H	38H	3.46-2.83
3.	17H	6H	4.86-3.98	9.	34H	38H	2.94-2.41
4.	53/54/55H	18H	4.50-3.68	10.	32H	37H	3.04-2.49
5.	53/54/55H	14H	3.96-3.24				
6.	42H	37H	4.00-3.27