

**Synthesis and antibacterial activity of novel neamine derivatives:
preponderant role of the substituent position on the neamine core.**

Nicolas Gernigon, Valérie Bordeau, Fabienne Berrée,
Brice Felden,* Bertrand Carboni *

Supporting Information

Synthesis of neamine derivatives 3 and 4	2
Biological assays for compounds 8a+8b and 8a	3
¹ H and ¹³ C NMR spectra of the neamine derivatives 1, 2 and 5-18	4

1,3,2',6'-Tetraazido-6,3',4'-tri-*O*-acetylneamine **3**¹

To a solution of 1,3,2',6'-tetraazidoneamine (3 g, 7 mmol) in a mixture of CH₂Cl₂/pyridine (45 mL, 1:1 v/v) at 0°C was added Ac₂O (45 mL). The reaction mixture was stirred at room temperature for 2h. It was then diluted with CH₂Cl₂ (50 mL) and slowly poured on an ice-cold aqueous NaHCO₃ saturated solution under stirring. The layers were separated, the organic layer was washed with H₂O (2 x 20 mL) and concentrated under reduced pressure. The residue was taken up in EtOAc (50 mL) and washed with aqueous NH₄Cl (2 x 50 mL) and brine (50 mL). The organic layer was dried (MgSO₄), concentrated *in vacuo*, and the residue was purified by column chromatography (EtOAc/cyclohexane 15% to 20%) to afford **3** (2.5 g, 4.6 mmol, 64%) (R_f 0.5 EtOAc/cyclohexane 2:3) and the tetracetylated neamine derivative (0.8 g, 1.4 mmol, 20%). (R_f 0.6 EtOAc/cyclohexane 2:3). ¹H NMR (300 MHz, CDCl₃) δ 5.50 (dd, *J* = 9.4, 10.5 Hz, 1H, H-3'), 5.36 (d, *J* = 3.6 Hz, 1H, H-1'), 5.06 (dd, *J* = 9.3, 10.6 Hz, 1H, H-4'), 4.94 (dd, *J* = 8.7, 9.8 Hz, 1H, H-6), 4.35 (ddd, *J* = 2.8, 4.7, 10.1 Hz, 1H, H-5'), 3.75-3.63 (m, 2H, H-5, H-2'), 3.62-3.50 (m, 2H, H-1, H-4), 3.50-3.28 (m, 4H, H-6'_{eq}, H-6'_{ax}, H-3, OH), 2.42 (ddd, *J* = 4.2, 4.2, 13.6 Hz, 1H, H-2'_{eq}), 2.19 (s, 3H, CH₃), 2.11 (s, 3H, CH₃), 2.07 (s, 3H, CH₃), 1.62 (ddd, *J* = 13.5, 13.5, 13.5 Hz, 1H, H-2'_{ax}); ¹³C NMR (75 MHz, CDCl₃) δ 169.9 (CH₃-C=O), 169.7 (CH₃-C=O), 169.2 (CH₃-C=O), 98.3 (C-1'), 83.4, 74.4, 73.9, 70.8, 69.0, 68.8, 61.3 (C-N₃), 57.8 (C-N₃), 57.4 (C-N₃), 50.4 (C-6'), 31.5 (C-2), 20.3 (CH₃), 20.2 (CH₃), 20.1 (CH₃).

3',4'-Di-*O*-acetyl-1,3,2',6'-tetraazidoneamine **4**²

To a solution of **3** (4 g, 7.2 mmol) in THF (80 mL) was added NaOH 0.1N (90 mL, 9 mmol). After stirring at room temperature for 1h, quantitative conversion of the starting material was observed by TLC (40% EtOAc in cyclohexane). The solution was neutralized by saturated NH₄Cl aqueous solution (20 mL). The remaining solvent was removed *in vacuo* and the aqueous layer was extracted with EtOAc (3 x 50 mL). The organic layers were combined, dried (MgSO₄), and concentrated under reduced pressure to give a yellow oil which was purified by column chromatography on silica gel using 15% to 20% EtOAc in cyclohexane to obtain **4** (2.8 g, 5.4 mmol, 75%) (R_f 0.4 EtOAc/cyclohexane 2:3). ¹H NMR (300 MHz, CDCl₃) δ 5.51 (dd, *J* = 9.3, 10.5 Hz, 1H, H-3'), 5.36 (d, *J* = 3.6 Hz, 1H, H-1'), 5.07 (dd, *J* = 10.1, 10.1 Hz, 1H, H-4'), 4.36 (ddd, *J* = 2.7, 4.8, 10.2 Hz, 1H, H-5'), 3.79 (d, *J* = 2.6 Hz, 1H), 3.70 (dd, *J* = 3.6, 10.5 Hz, 1H, H-2'), 3.60-3.25 (m, 7H), 3.00 (s, 1H), 2.37 (ddd, *J* = 4.1, 4.1, 13.2 Hz, 1H, H-2'_{eq}), 2.11 (s, 3H, CH₃), 2.07 (s, 3H, CH₃), 1.56 (ddd, *J* = 12.3, 12.3, 12.3 Hz, 1H, H-2'_{ax}); ¹³C NMR (75 MHz, CDCl₃) δ 170.2 (C=O), 169.8 (C=O), 98.6 (C-1'), 82.7, 76.0, 75.5, 71.0, 69.4, 69.3, 61.6 (C-N₃), 59.7 (C-N₃), 58.6 (C-N₃), 50.8 (C-6'), 31.8 (C-2), 20.7 (CH₃), 20.6 (CH₃); HRMS (electrospray) Calcd for C₁₆H₂₂N₁₂O₈Na [M+Na]⁺: 533.1581, found: 533.1565.

¹ W. K. C. Park, M. Auer, H. Jaksche, C-H. Wong, *J. Am. Chem. Soc.* 1996, **118**, 10150-10155.

² J. Li, J. Wang, P. G. Czyryca, H. Chang, T. W. Orsak, R. Evanson, C.-W. T. Chang, *Org. Lett.* 2004, **6**, 1381-1384.

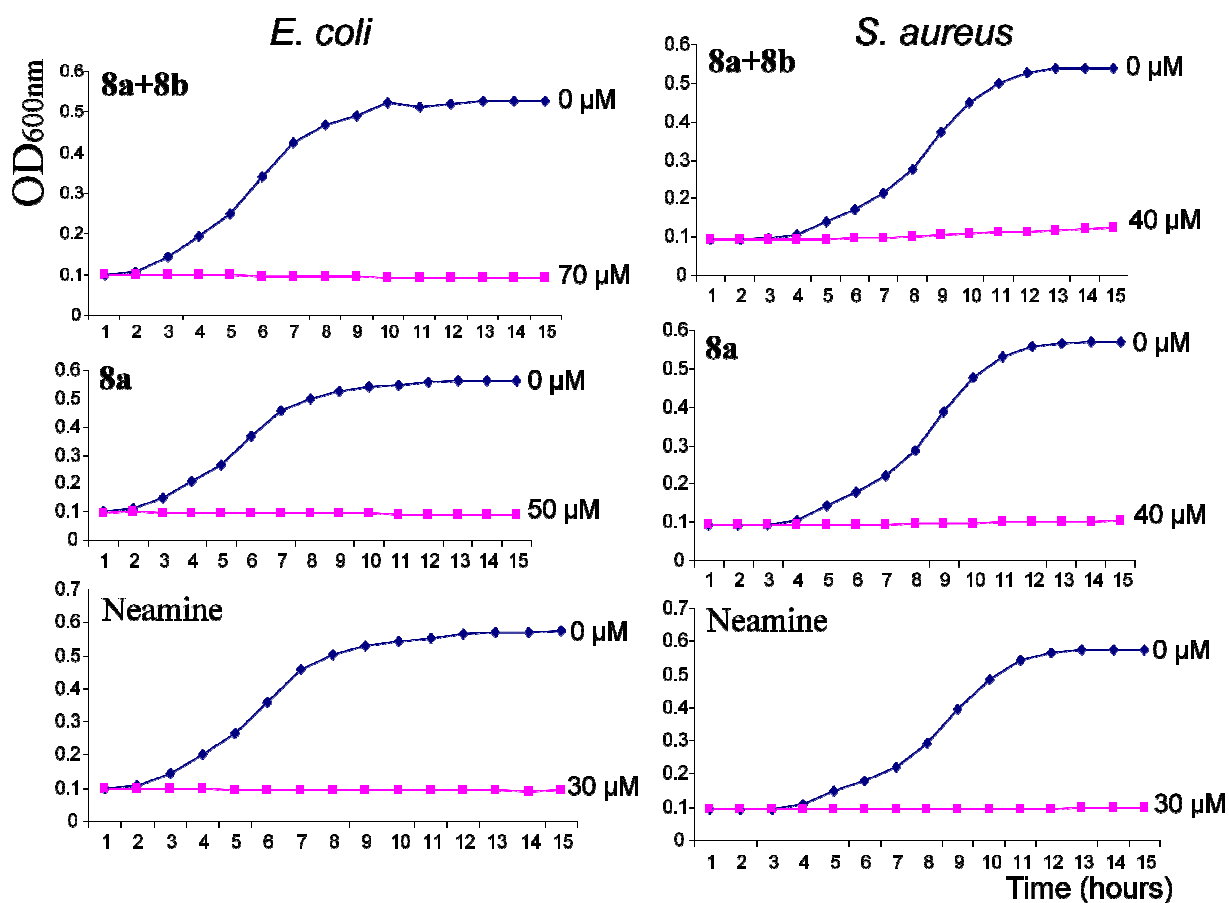
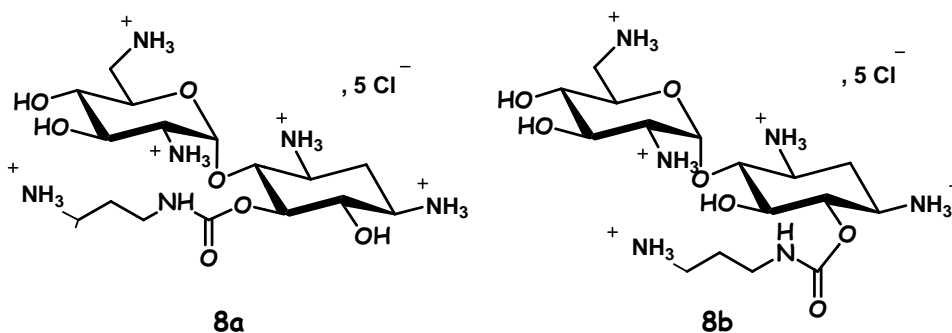
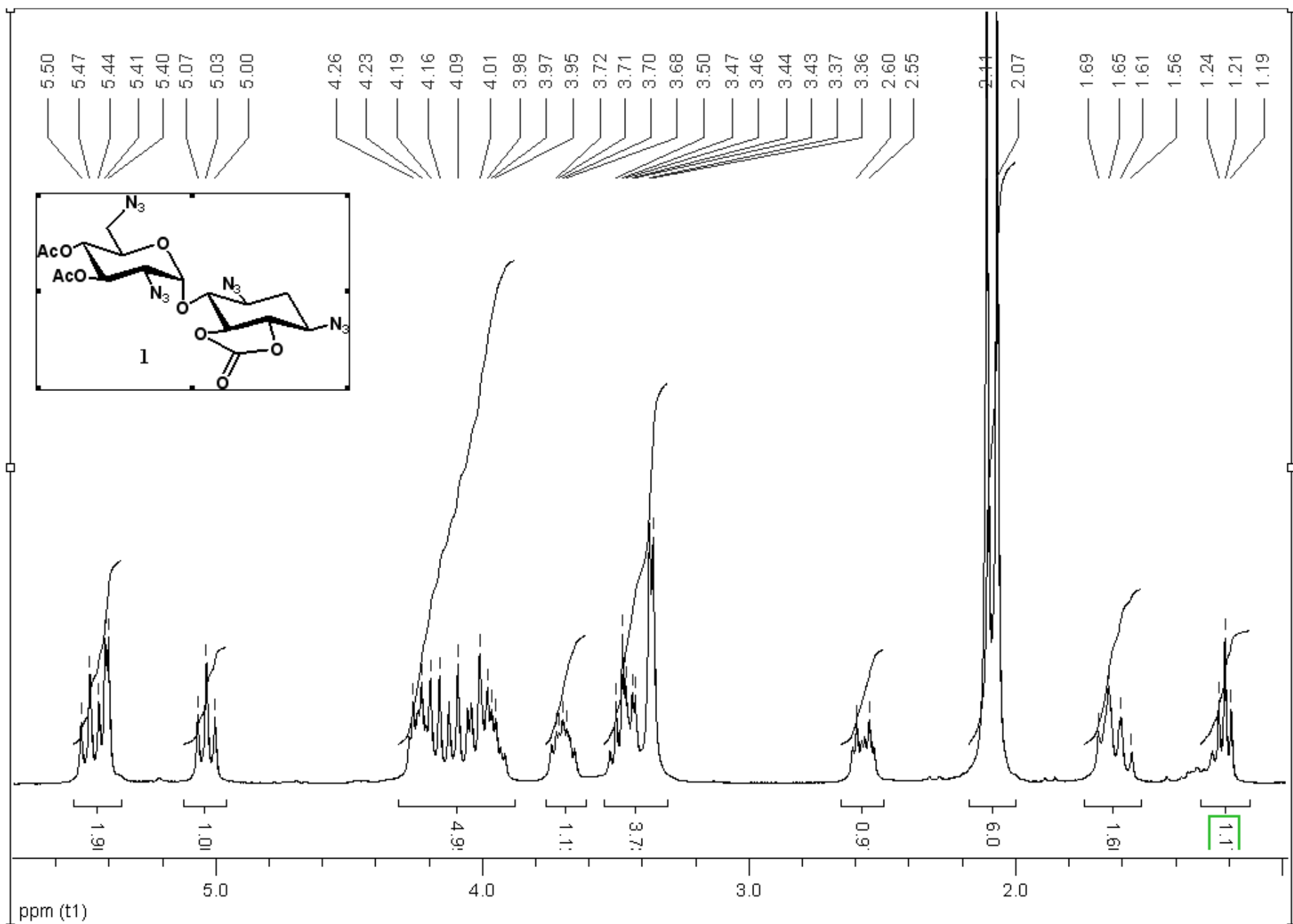
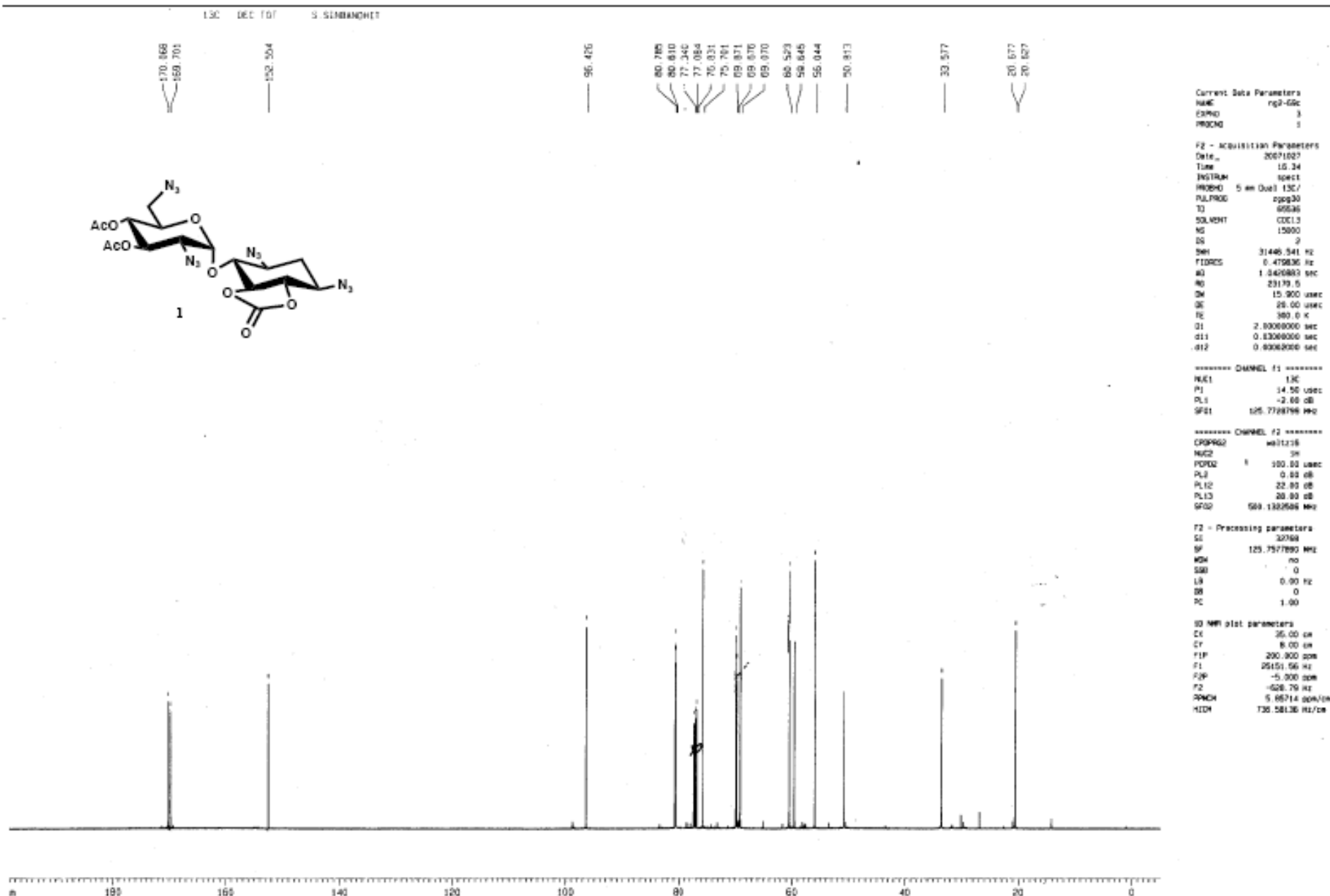


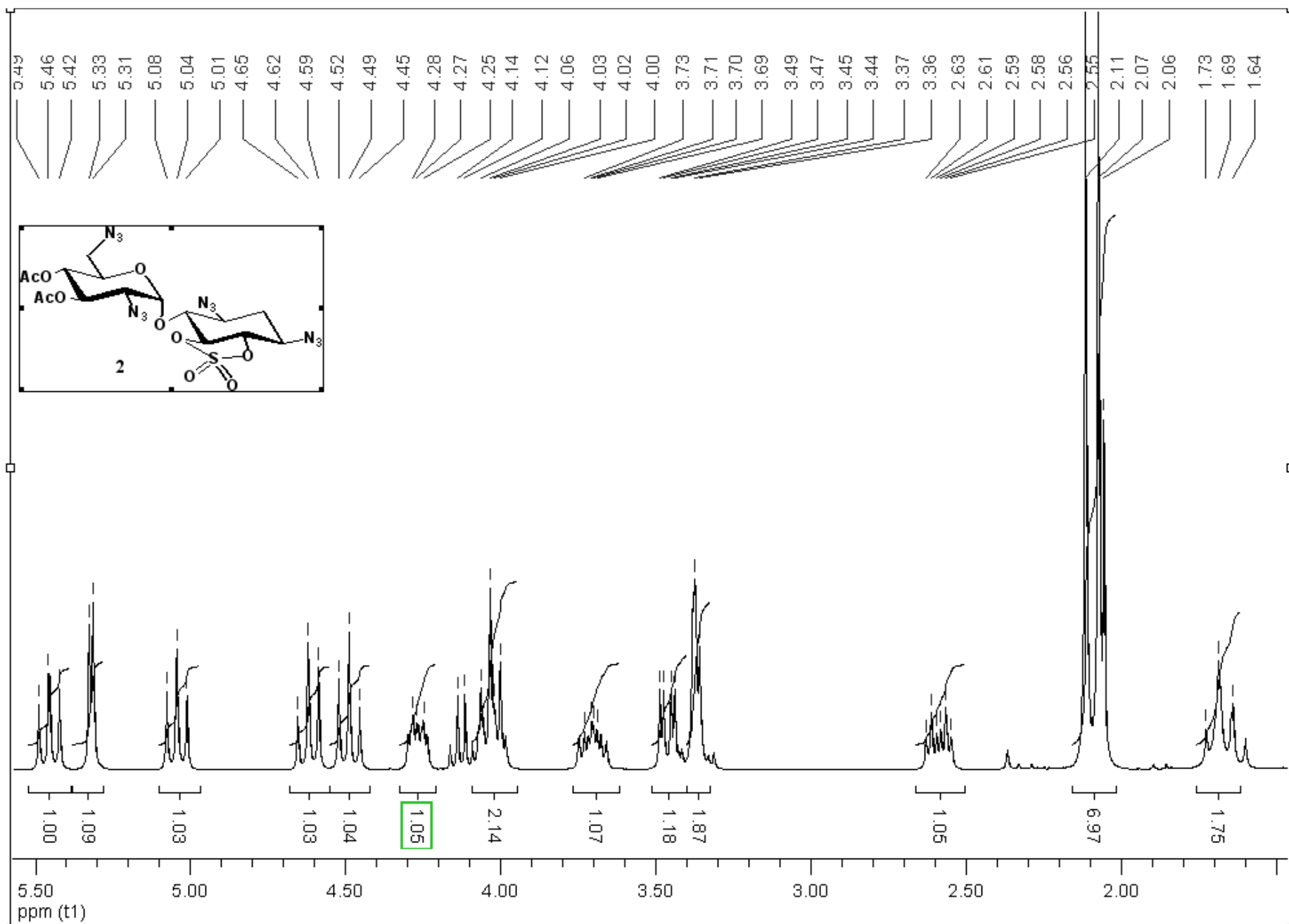
Figure 1

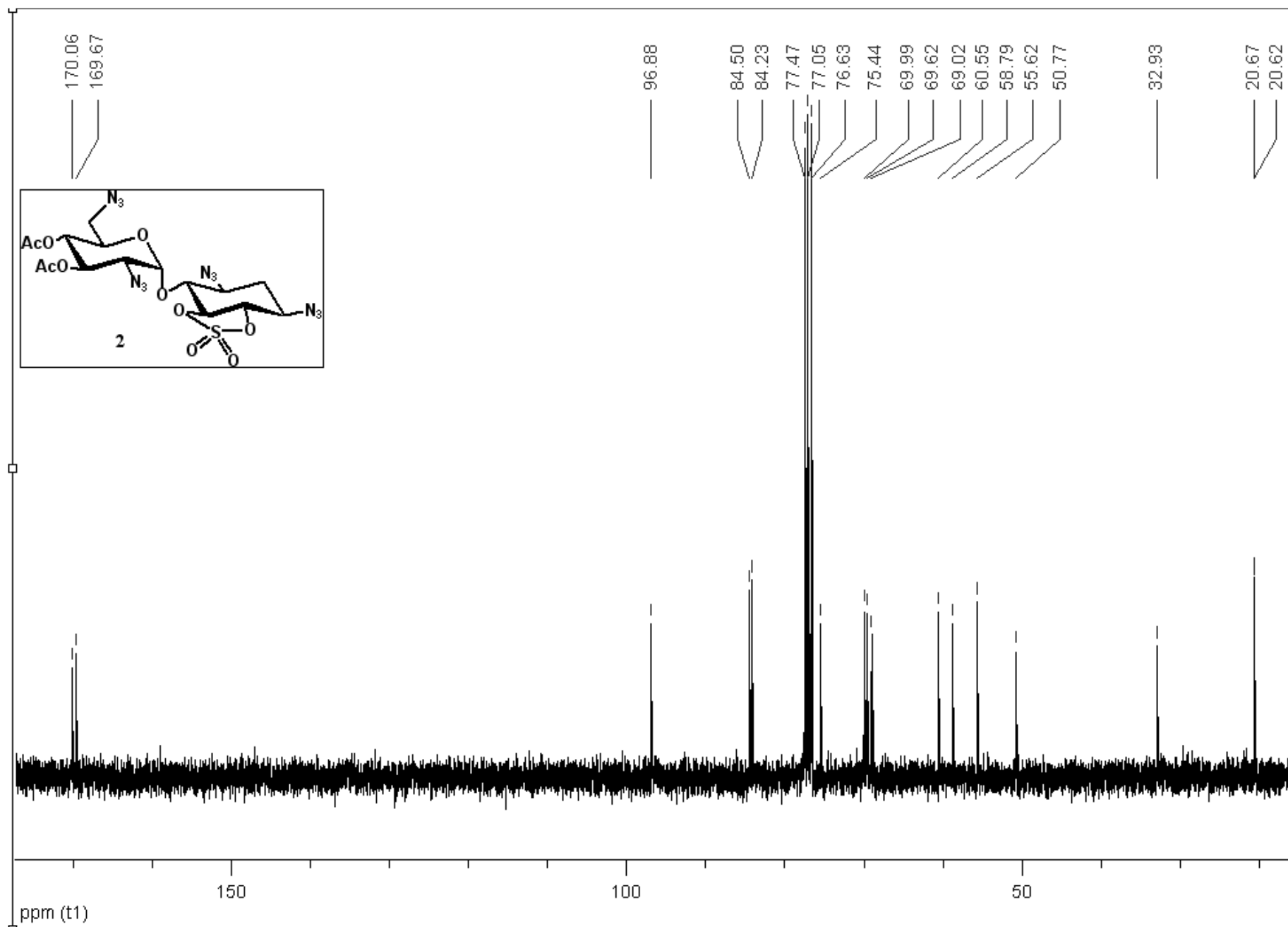
Figure 1 legend: Growth inhibition of the aminoglycoside derivatives on *E. coli* and *S. aureus* bacteria. Growth inhibition patterns of compounds **8a+8b** and **8a** compared to the effect of the neamine (internal control) during *E. coli* (left panels) and *S. aureus* (right panels) growths. For all the aminoside derivatives, only one concentration is indicated corresponding to the lowest that suppresses bacterial growth during a 15 hour incubation time (in red). The growth curves in the absence of the aminoglycosides are in blue.











170.06
169.67

96.88

84.50

84.23

77.47

77.05

76.63

75.44

69.99

69.62

69.02

60.55

58.79

55.62

50.77

32.93

20.67

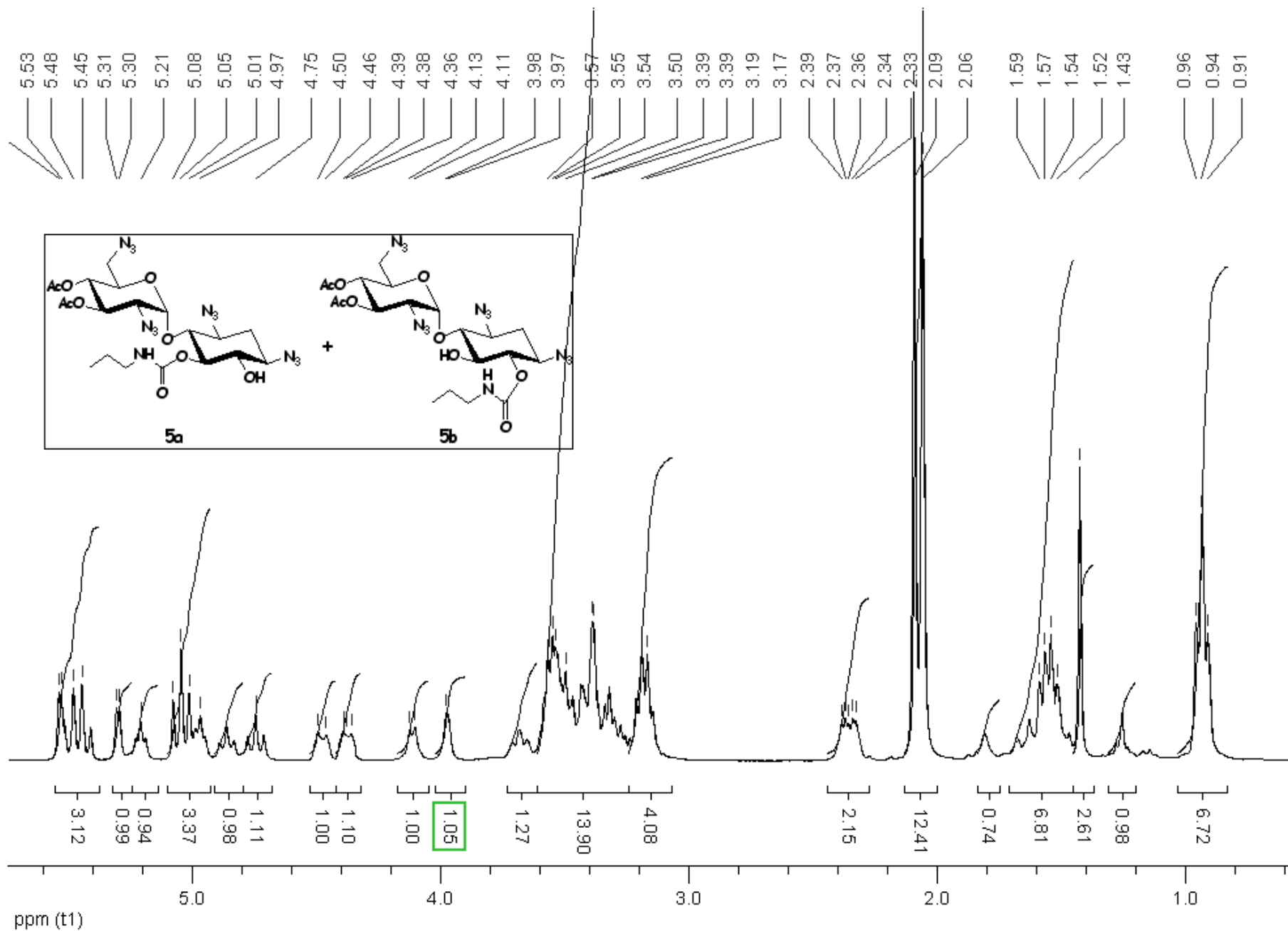
20.62

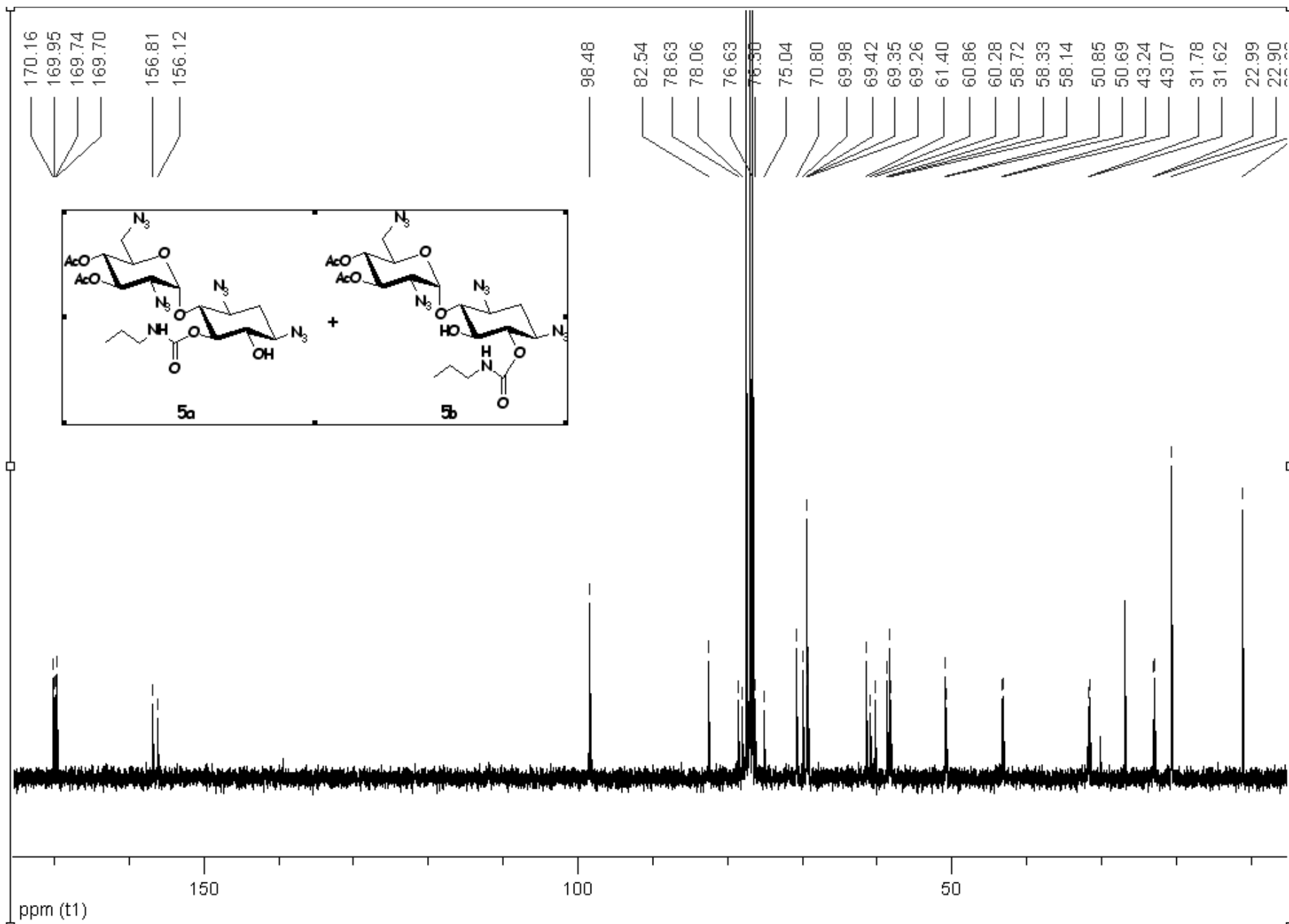
ppm (t1)

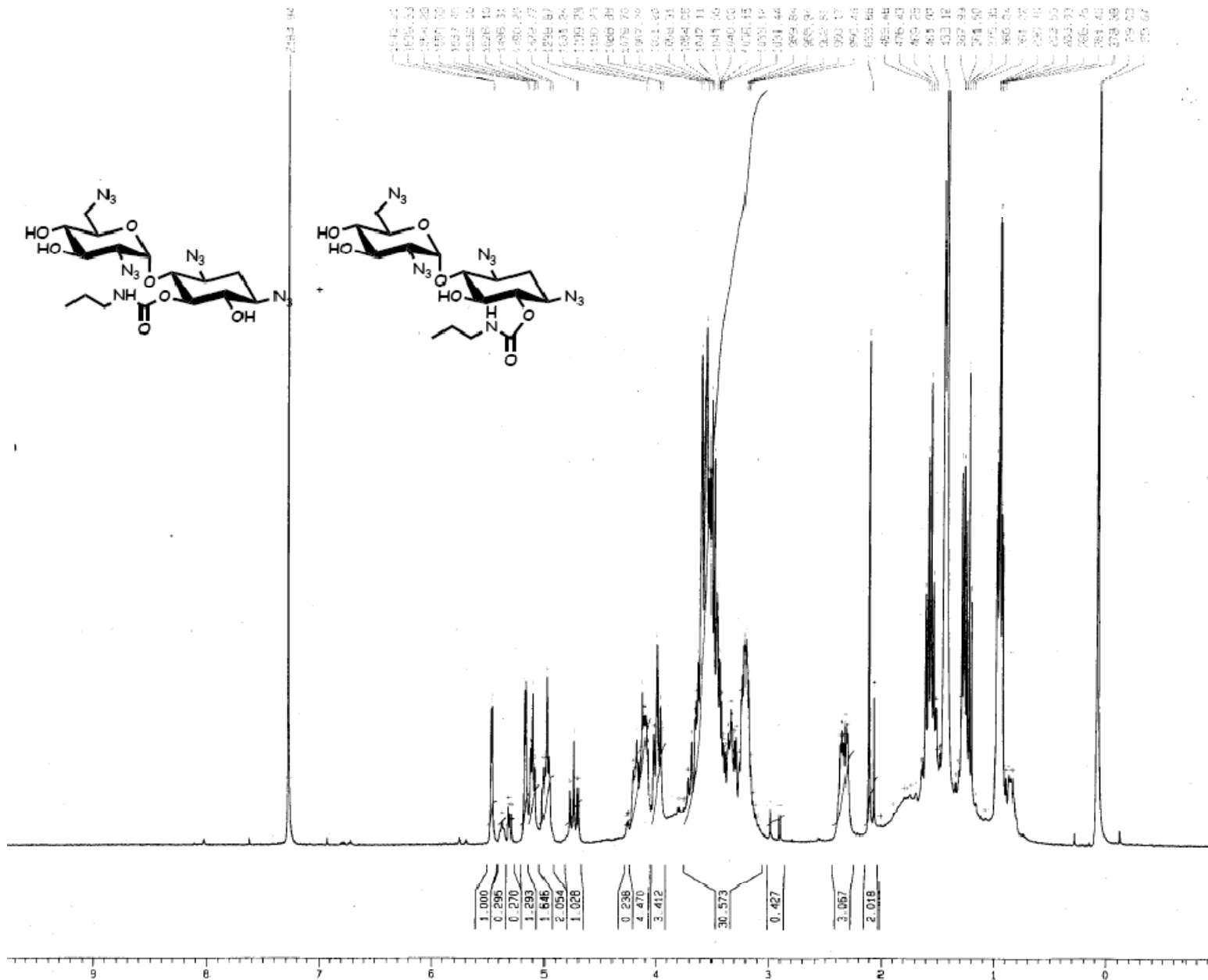
150

100

50







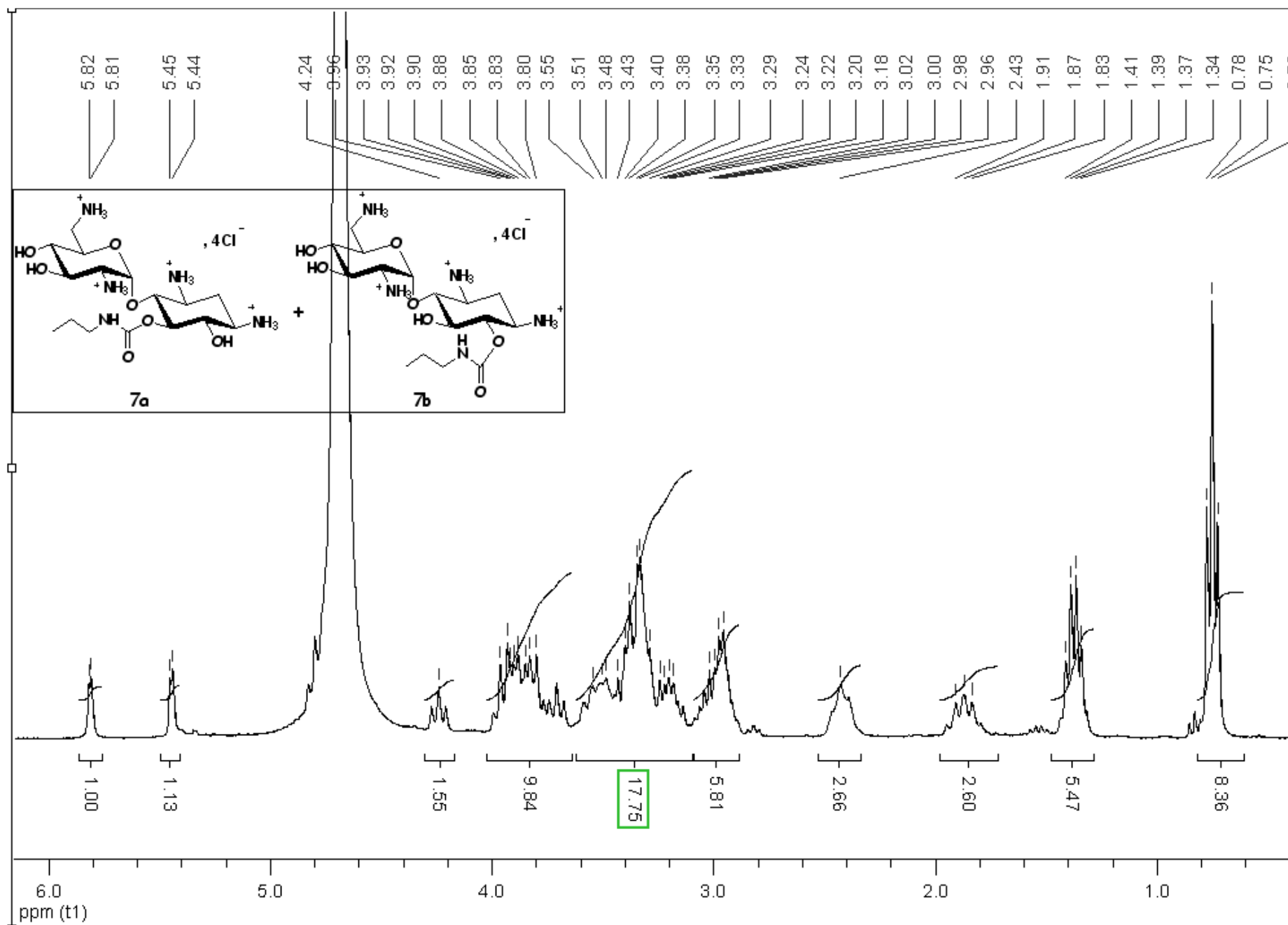
Current Data Parameters
 NAME ng2-83b
 EXPNO 1
 PROCNO 1

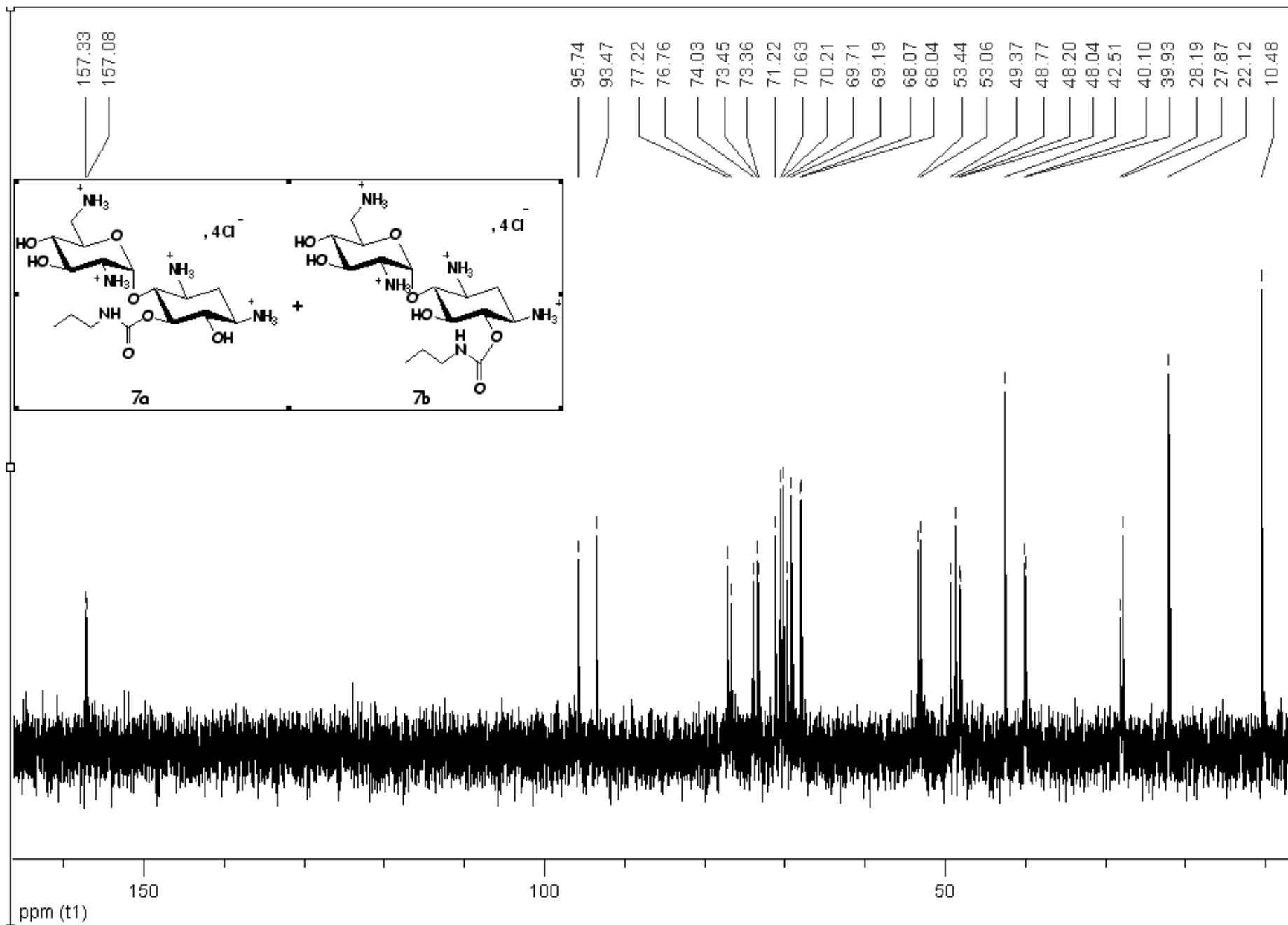
F2 - Acquisition Parameters
 Date_ 20071207
 Time 13.52
 INSTRUM spect
 PROBHD 5 mm BBO BB-1H
 PULPROG zg30
 TD 16384
 SOLVENT CDCl3
 NS 67
 DS 2
 SWH 6172.839 Hz
 FIDRES 0.376760 Hz
 AQ 1.3271540 sec
 RG 362
 DW 81.000 usec
 DE 5.00 usec
 TE 300.0 K
 D1 1.0000000 sec

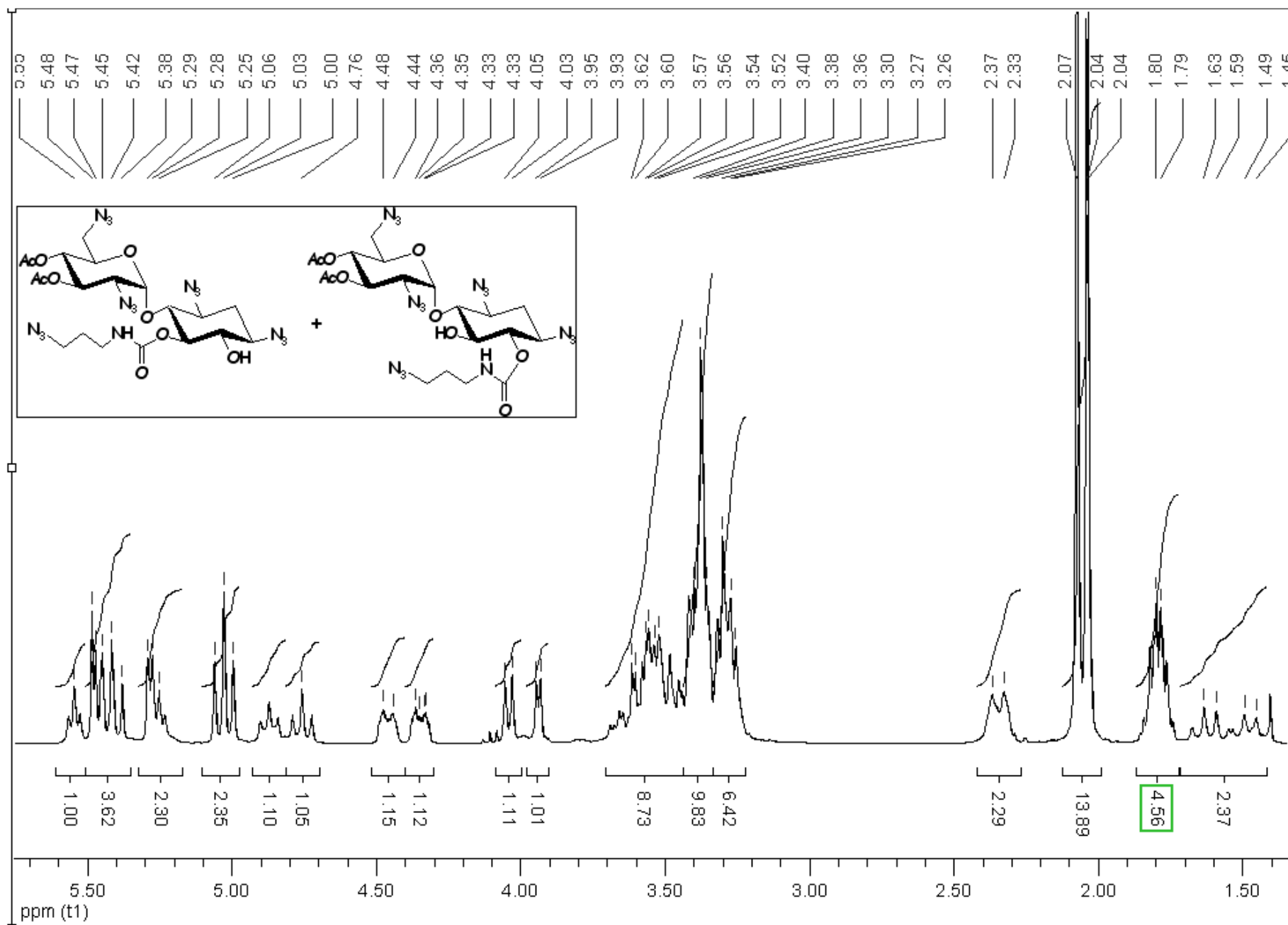
***** CHANNEL f1 *****
 NUC1 1H
 P1 8.50 usec
 PL1 0.00 dB
 SFO1 300.1310534 MHz

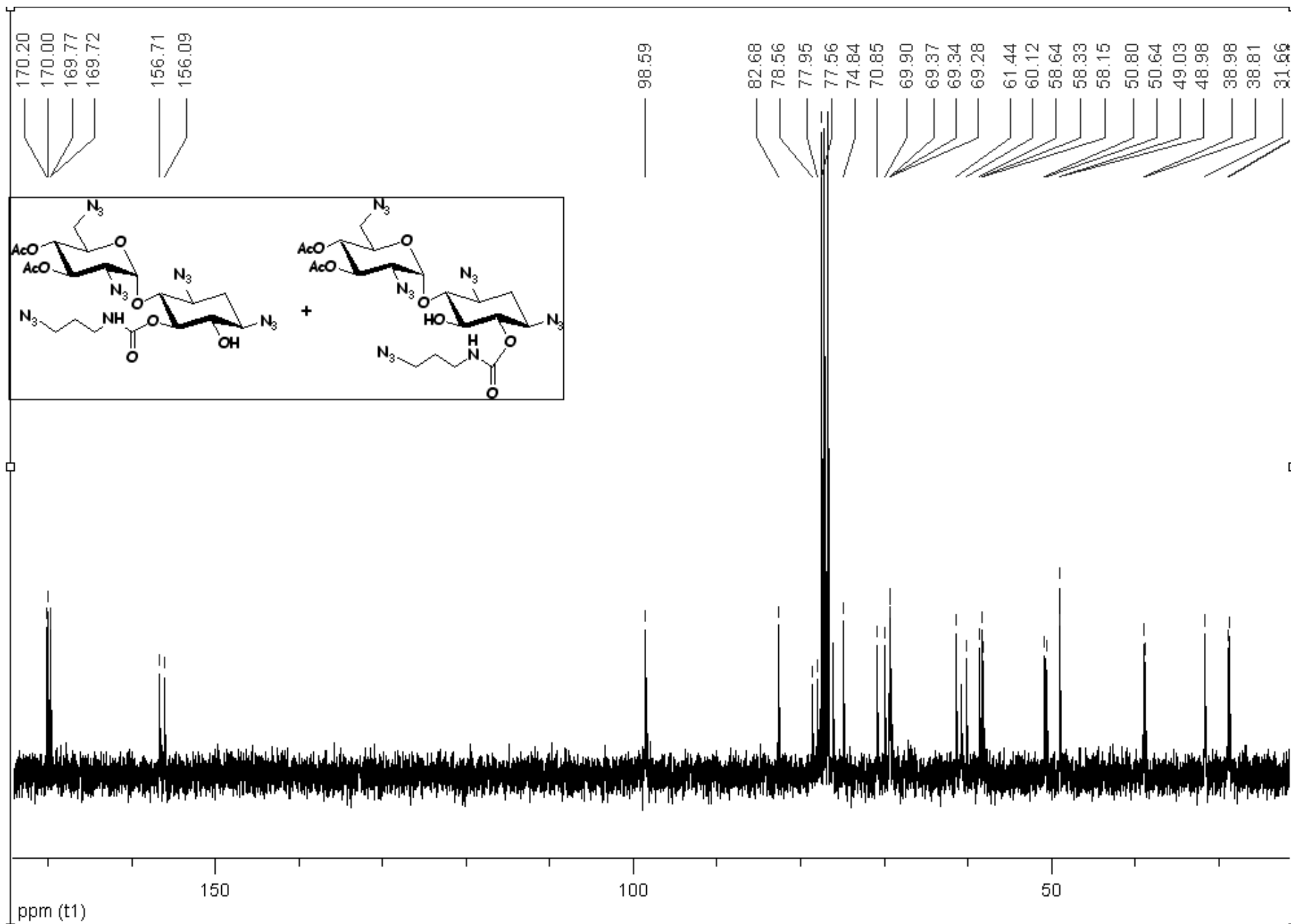
F2 - Processing parameters
 SI 16384
 SF 300.1300000 MHz
 MDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

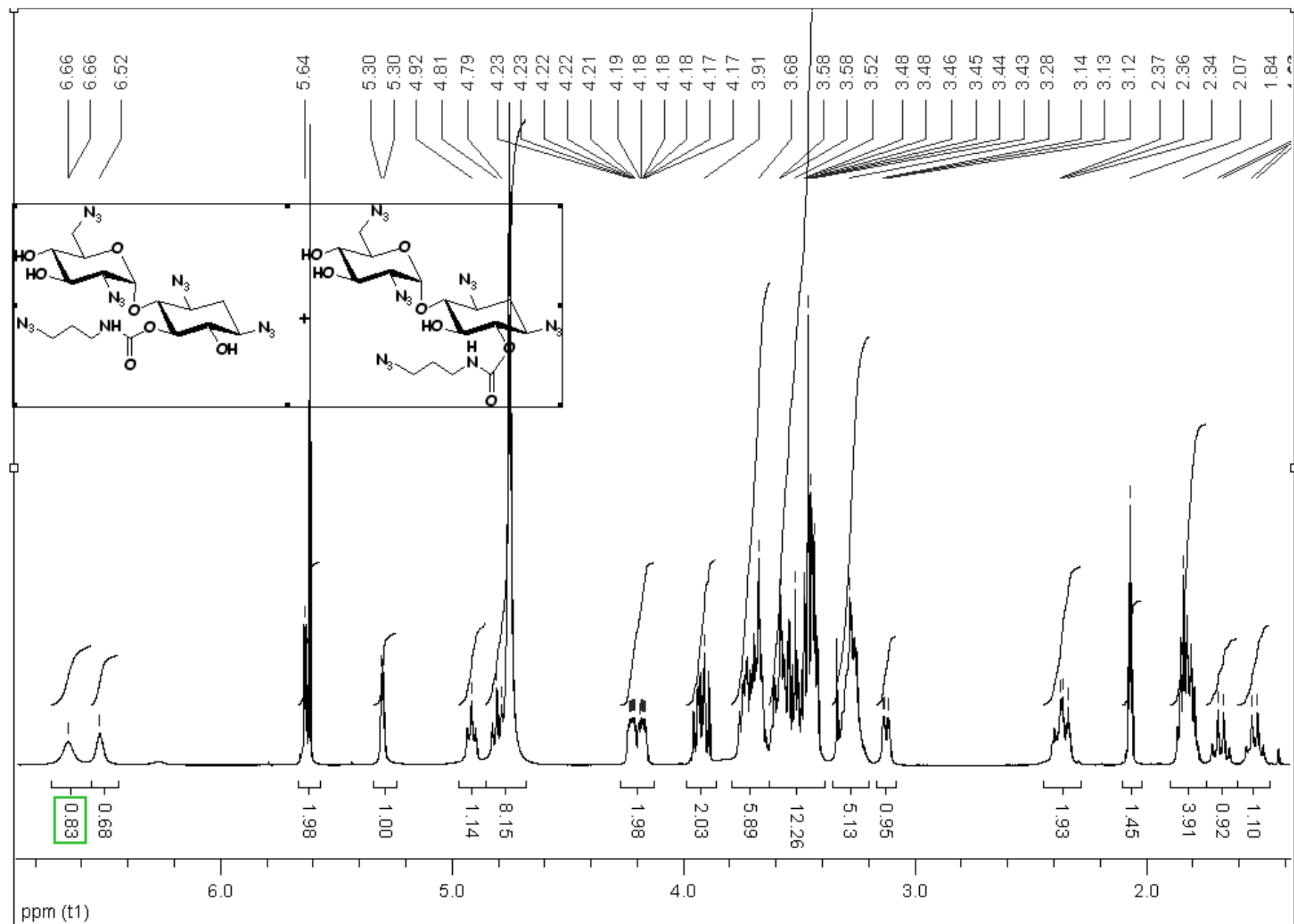
1D NMR plot parameters
 CX 35.50 cm
 CY 200.00 cm
 F1P 11.000 ppm
 F1 3301.43 Hz
 F2P -1.000 ppm
 F2 -300.13 Hz
 PPMCM 0.33803 ppm/cm
 HZCM 101.45241 Hz/cm

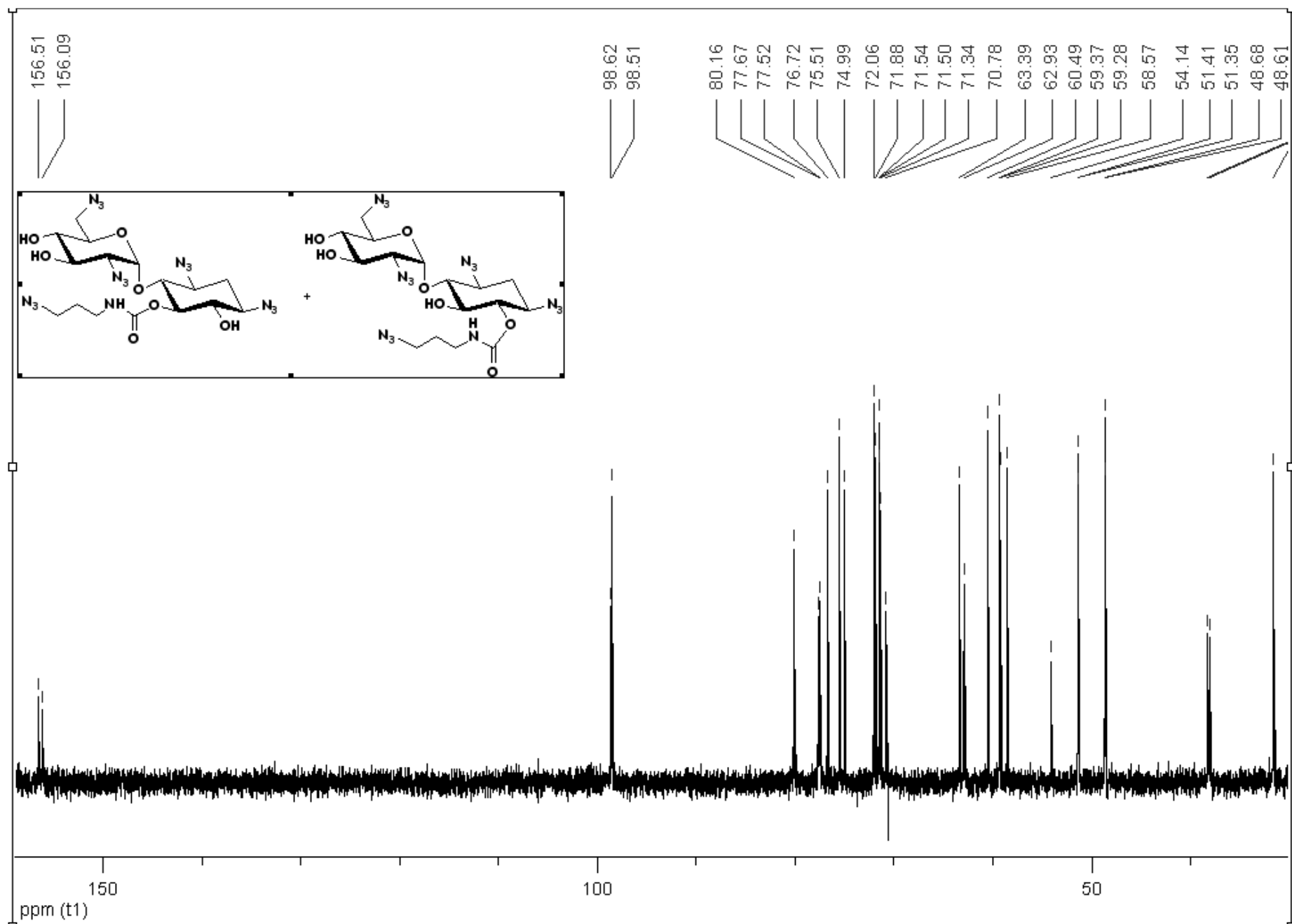


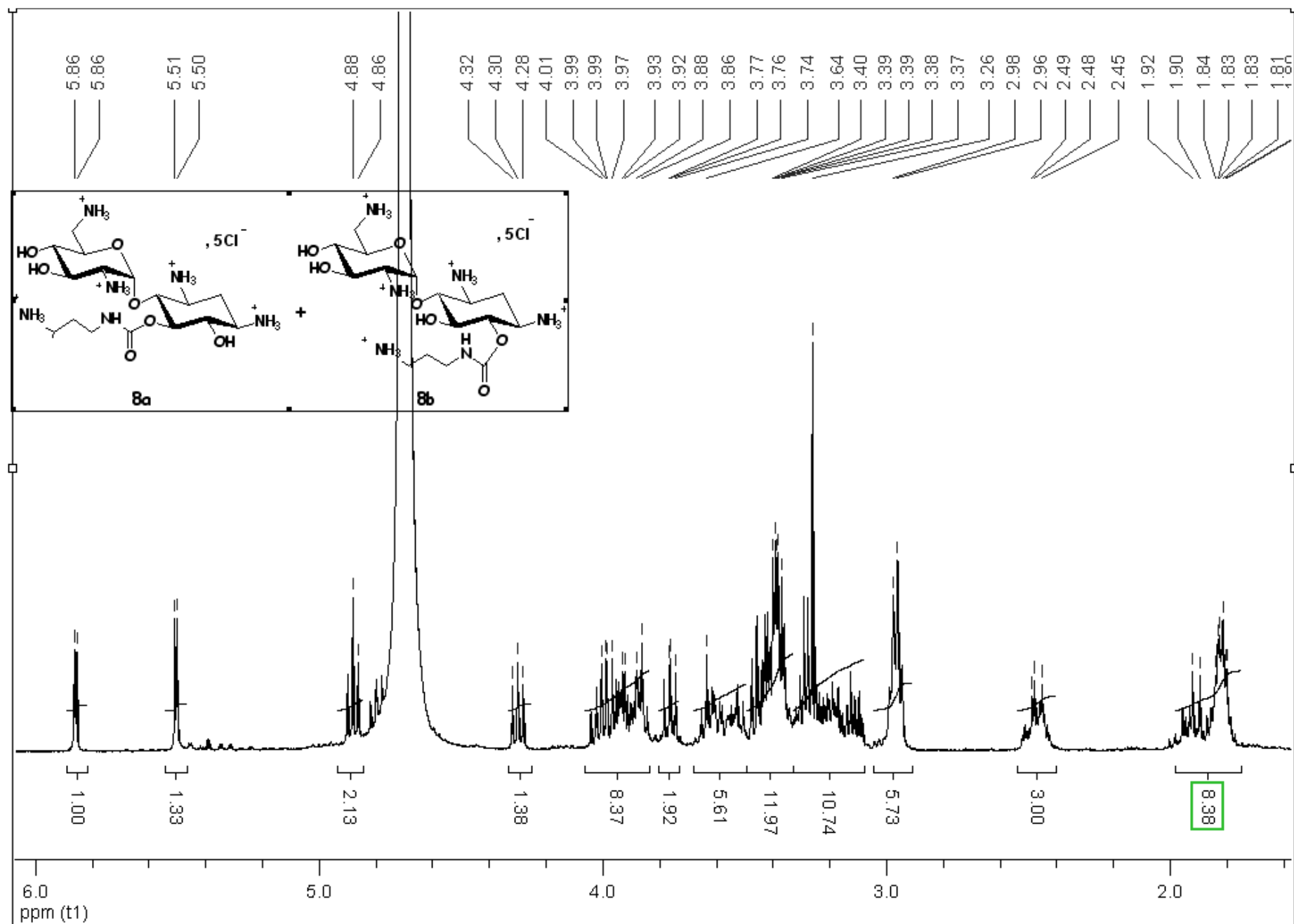


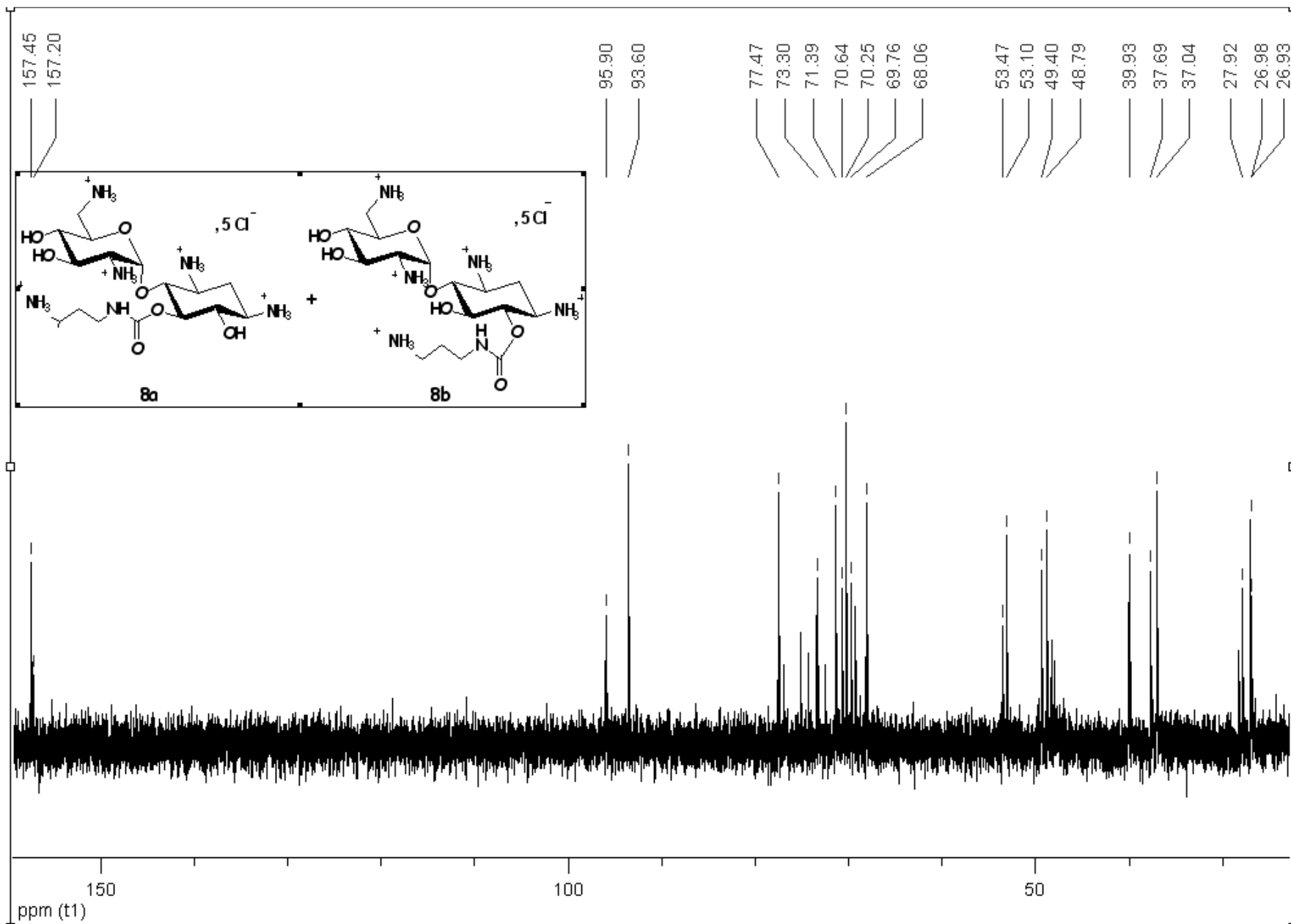


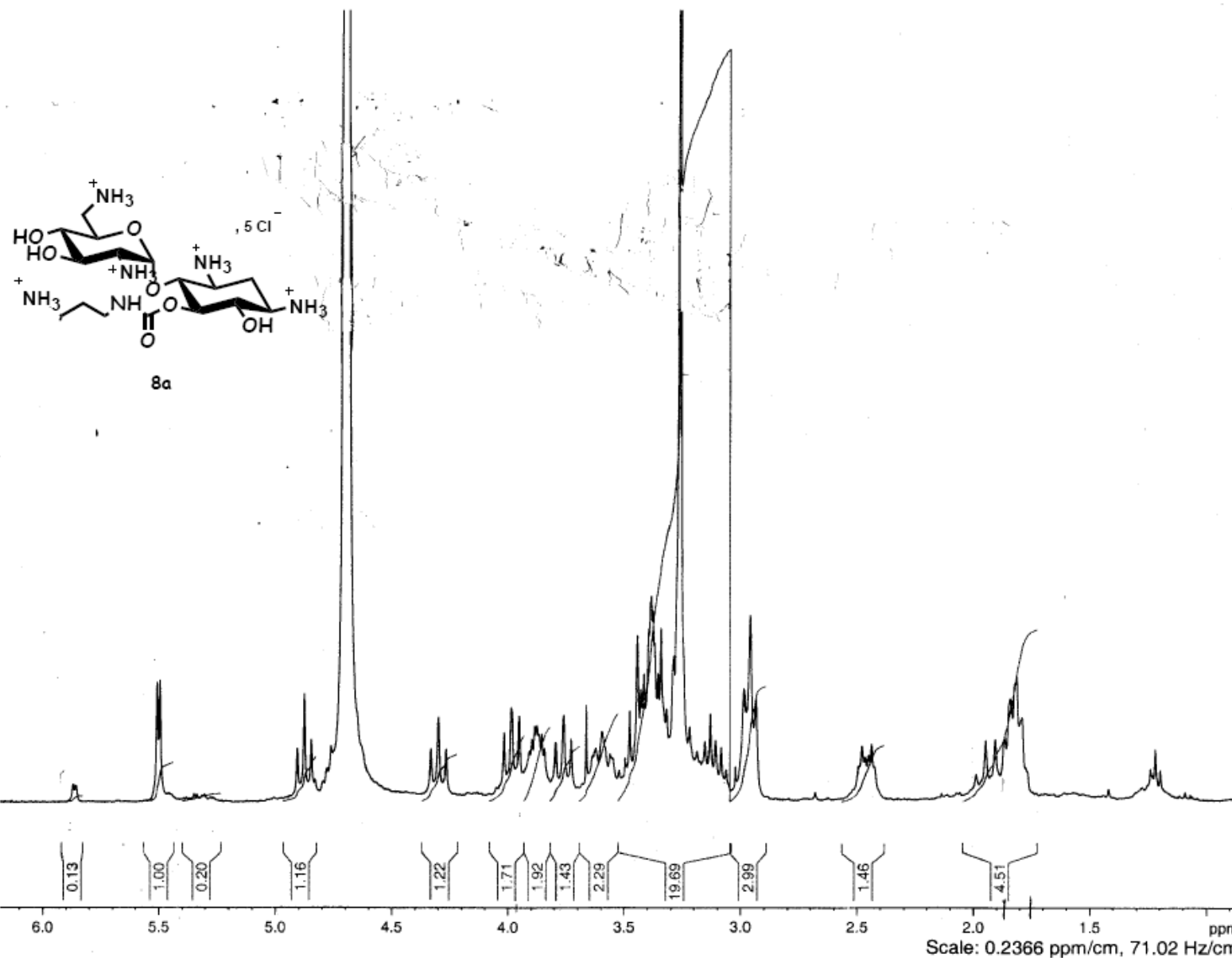












NAME FB3-46a
EXPNO 1
PROCNO 1
Date_ 20100219
Time 11.08
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 32768
SOLVENT D2O
NS 64
DS 2
SWH 6172.839 Hz
FIDRES 0.188380 Hz
AQ 2.6542580 sec
RG 512
DW 81.000 usec
DE 6.50 usec
TE 296.8 K
D1 1.00000000 sec
TD0 1

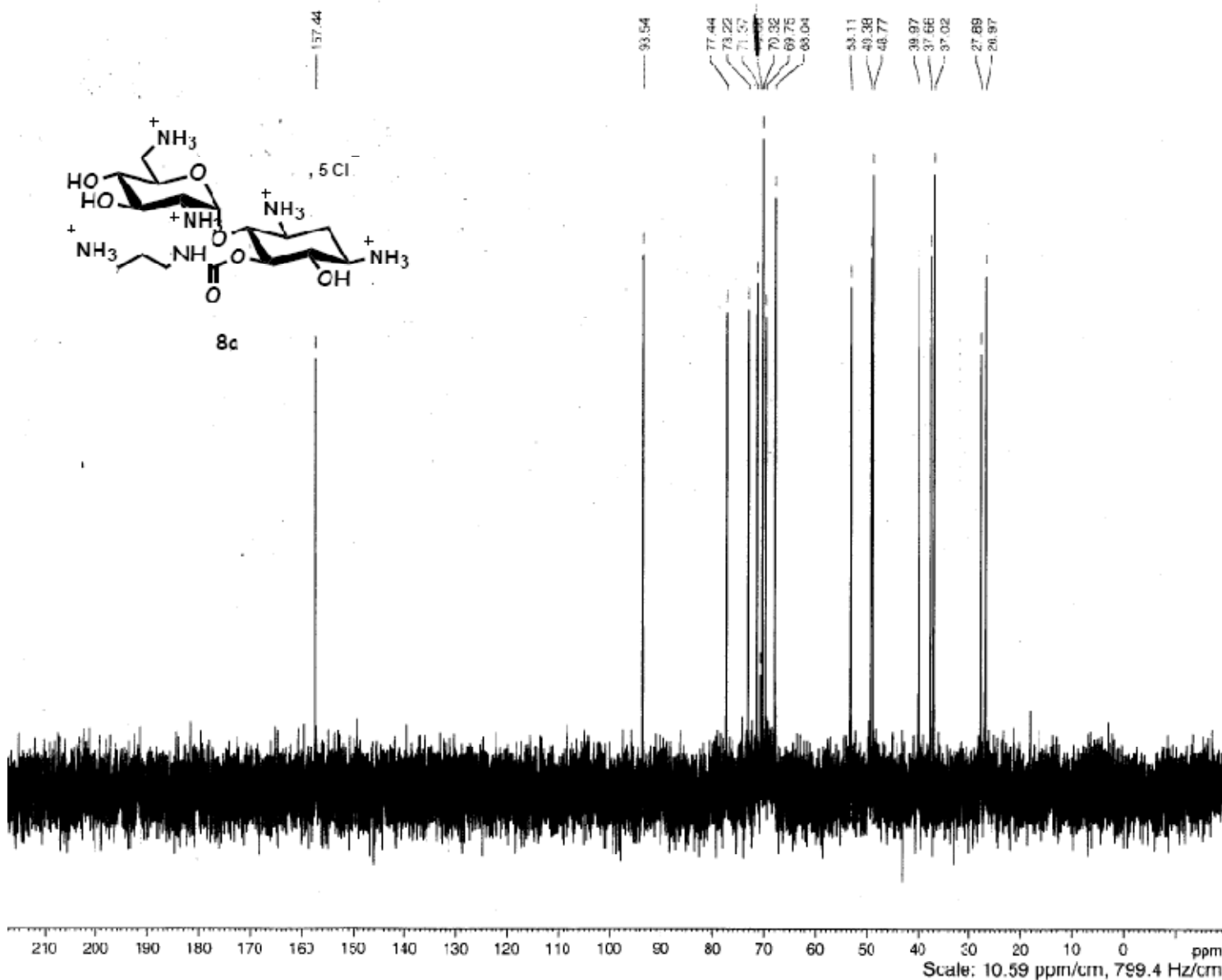
===== CHANNEL f1 =====
NUC1 1H
P1 8.20 usec
PL1 -2.50 dB
PL1W 23.22754669 W
SFO1 300.1318534 MHz
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
FC 1.00



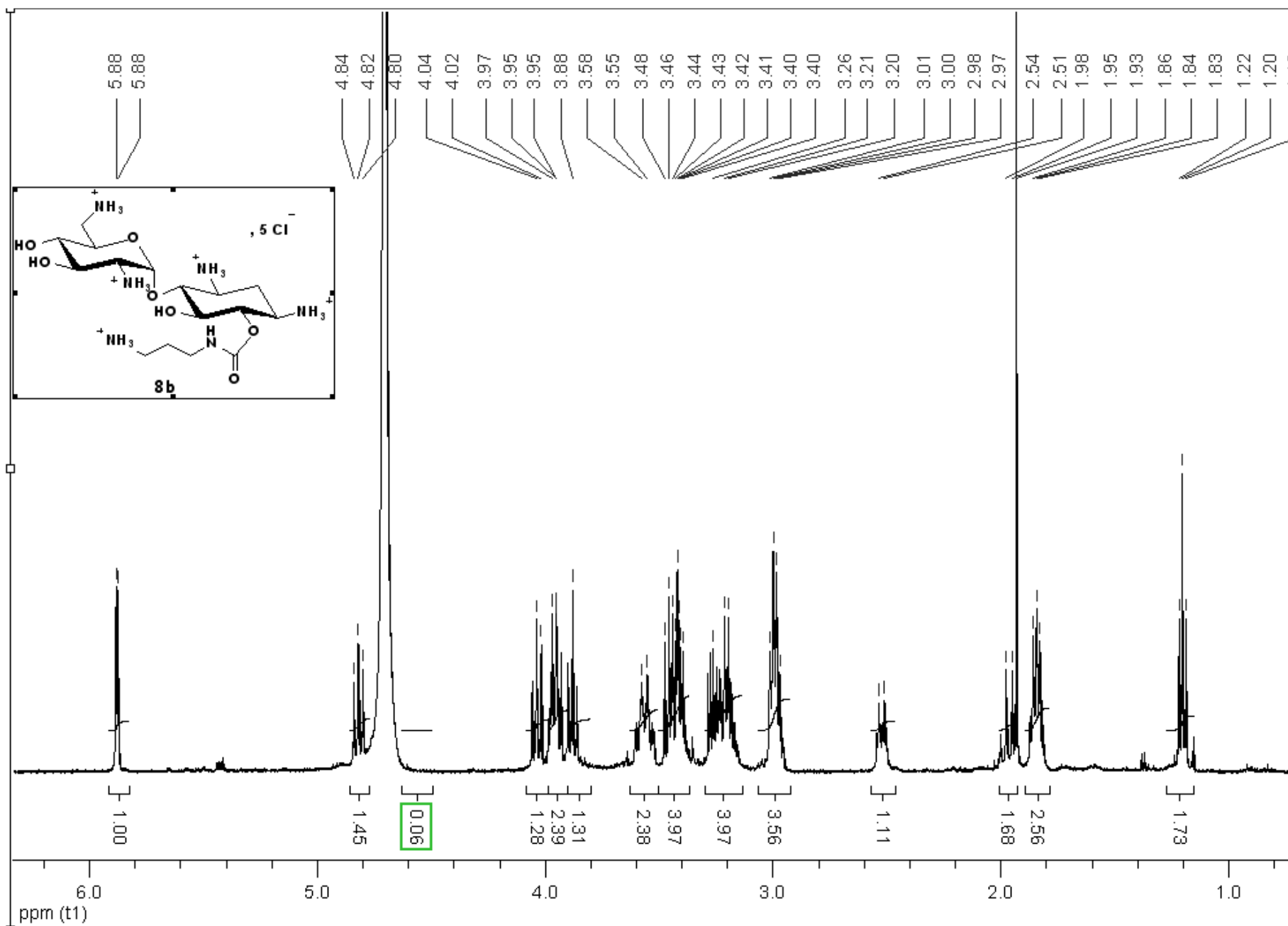
NAME 783-46e
EXPNO 10
PROCNO 1
Date_ 20100223
Time 1.28
INSTRUM spect
PROBHD 5 mm PABBO B5-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 650
DS 2
SWH 17985.611 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 sec
RG 23170.5
DW 27.800 usec
DE 6.50 usec
TE 297.2 K
D1 2.0000000 sec
D11 0.0300000 sec
CNO 1

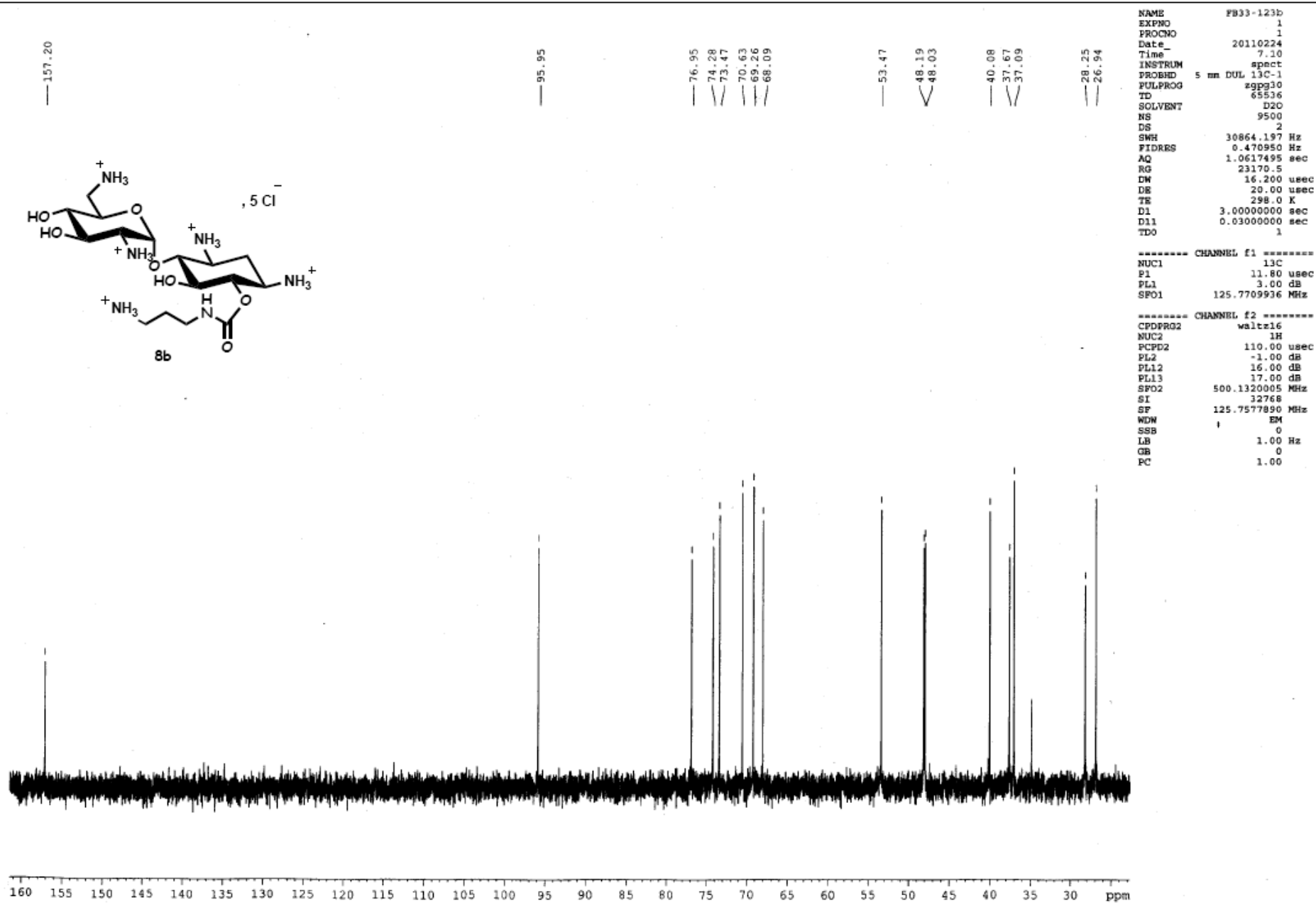
===== CHANNEL f1 =====
NUC1 13C
P1 6.35 usec
PL1 6.00 dB
PL1W 95.19999655 W
SFO1 75.4752948 MHz

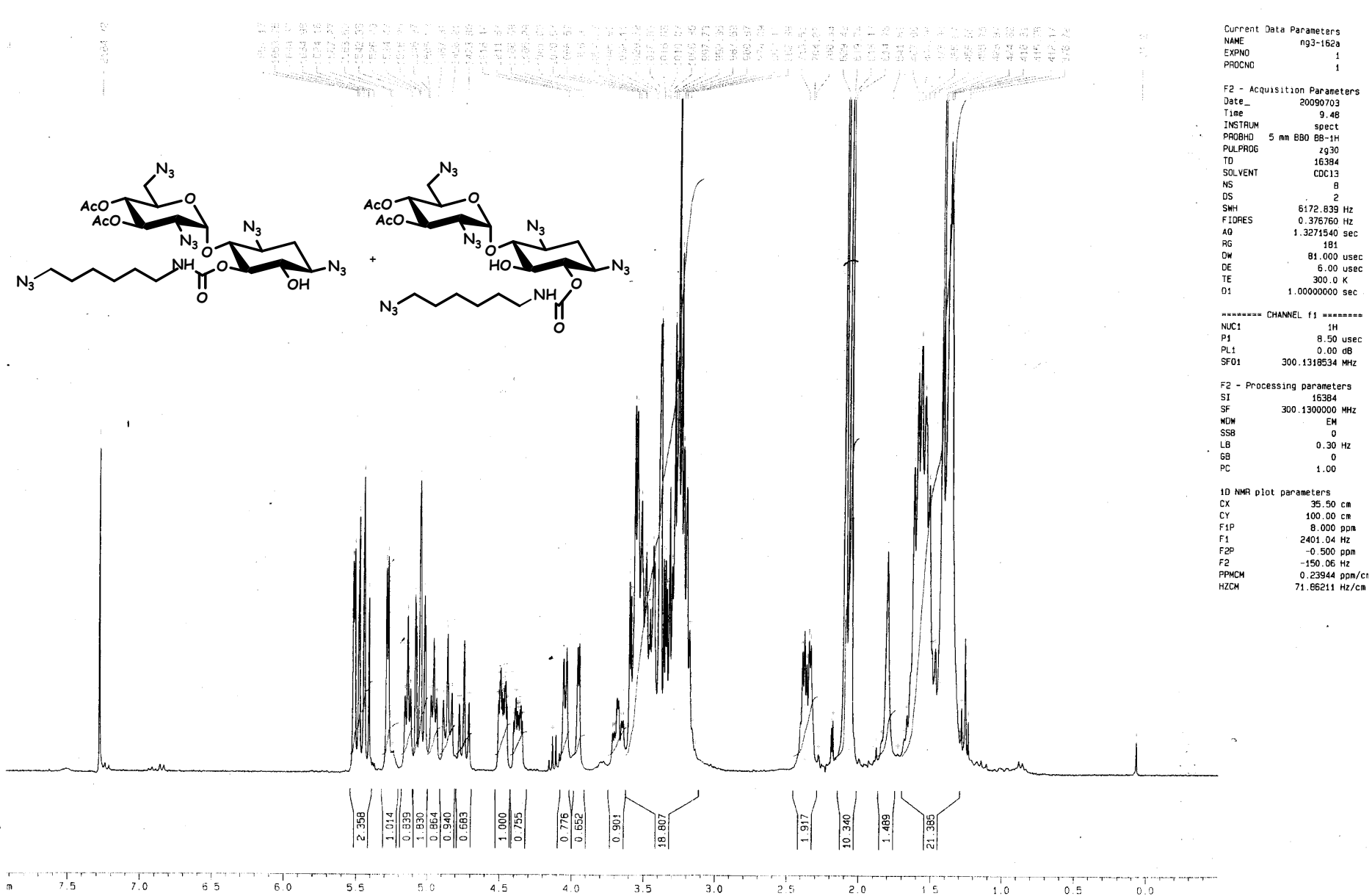
===== CHANNEL f2 =====
CDEPRO2 waltz16
NUC2 1H
PULP2 100.00 usec
PL2 -2.50 dB
PL12 19.50 dB
PL13 19.50 dB
PL2W 23.22754669 W
PL12W 0.14655592 W
PL13W 0.14655592 W
SFO2 300.1312005 MHz
SI 32768
SF 75.4677490 MHz
WDW EM
SGB 0
LB 0.60 Hz
GB 0
PC 1.40

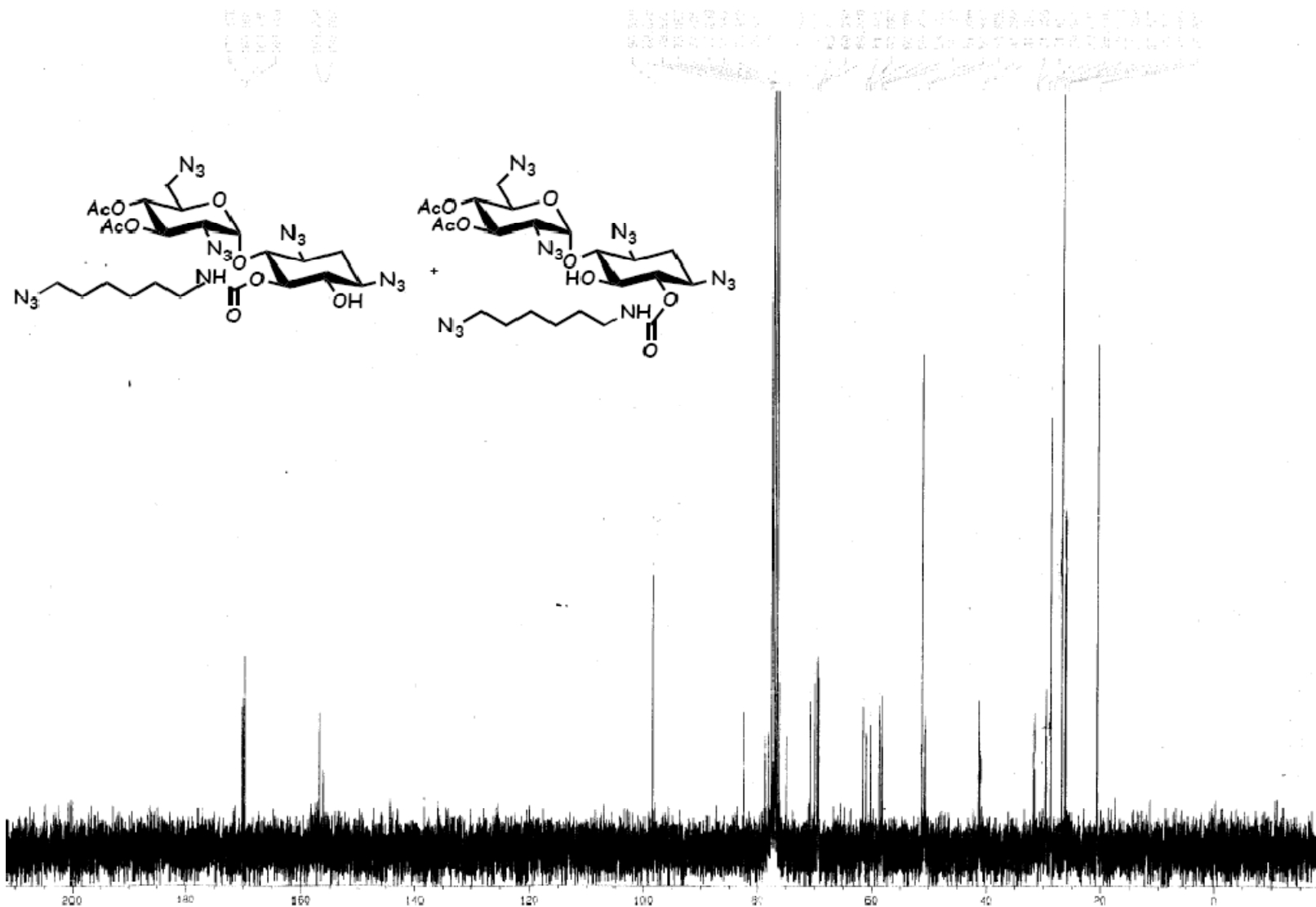


Scale: 10.59 ppm/cm, 799.4 Hz/cm









Current Data Parameters
NAME ng3-162a
EXPNO 2
PROCNO 1

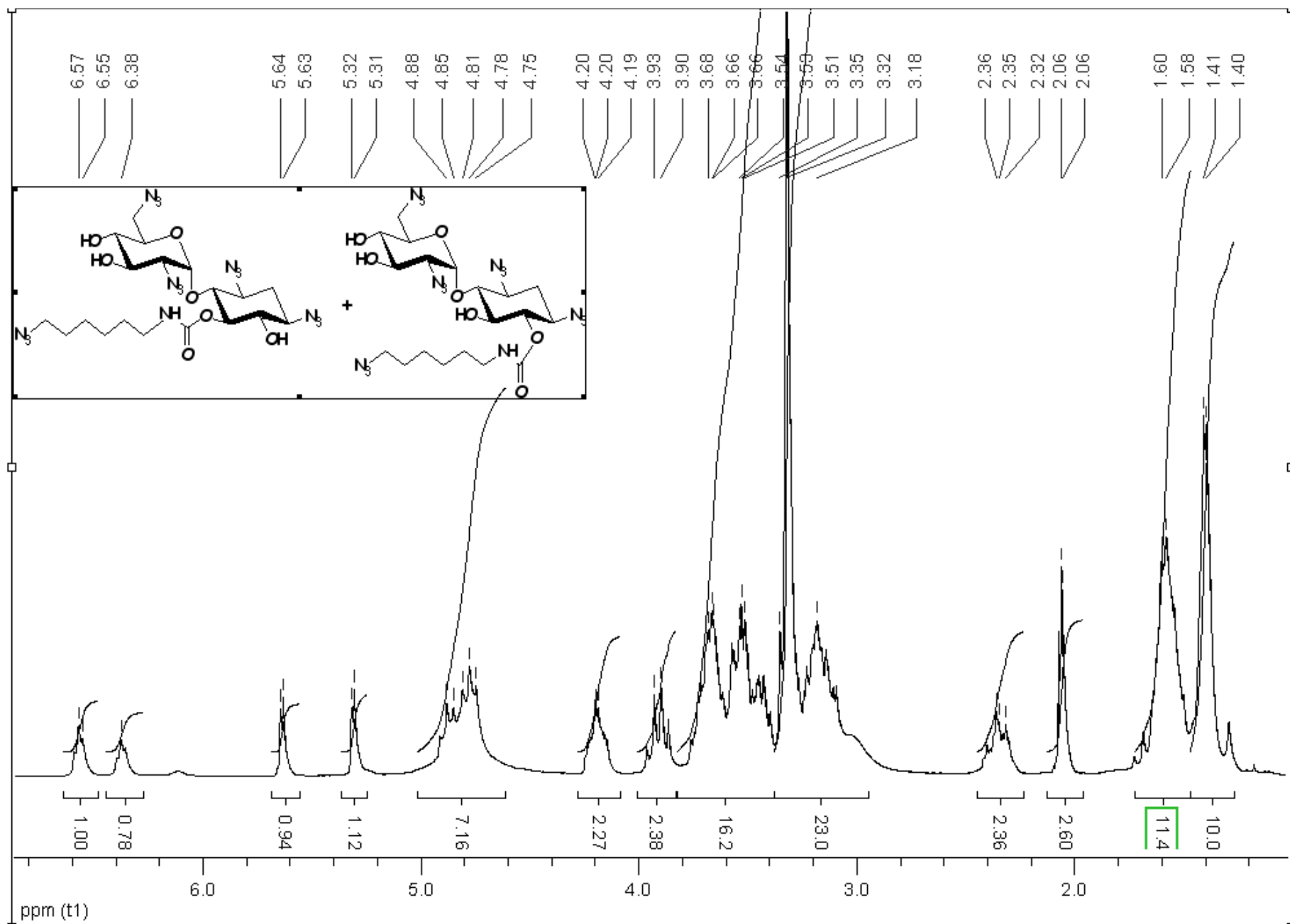
F2 - Acquisition Parameters
Date_ 20090703
Time 9.55
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 170
DS 2
SWH 17965.611 Hz
FIDRES 0.548877 Hz
AQ 0.9110004 sec
RG 11985.2
Dw 27.800 usec
DE 6.00 usec
TE 300.0 K
D1 2.0000000 sec
d11 0.0300000 sec
d12 0.0002000 sec

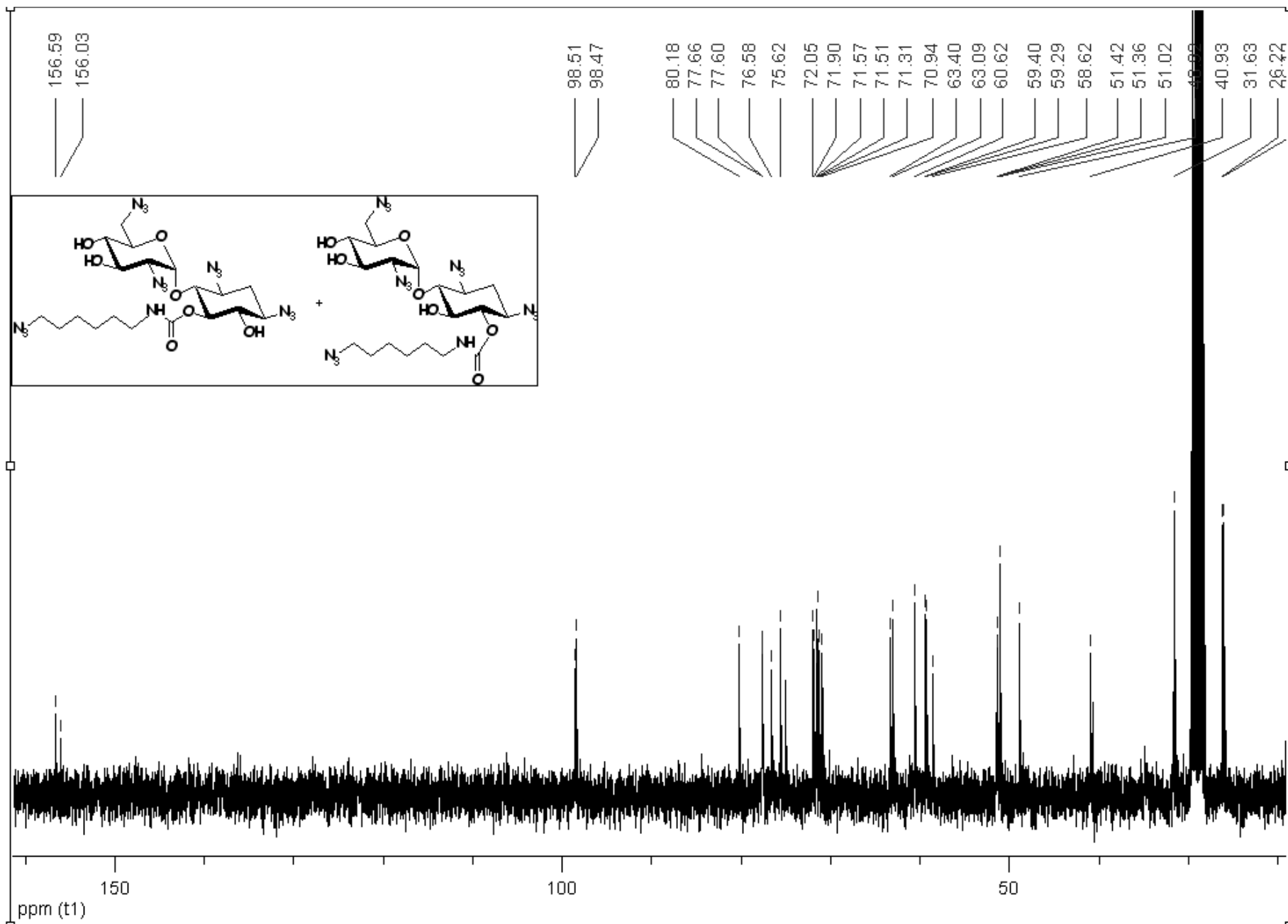
***** CHANNEL f1 *****
NUC1 13C
P1 5.20 usec
PL1 -6.00 dB
SFO1 75.4752953 MHz

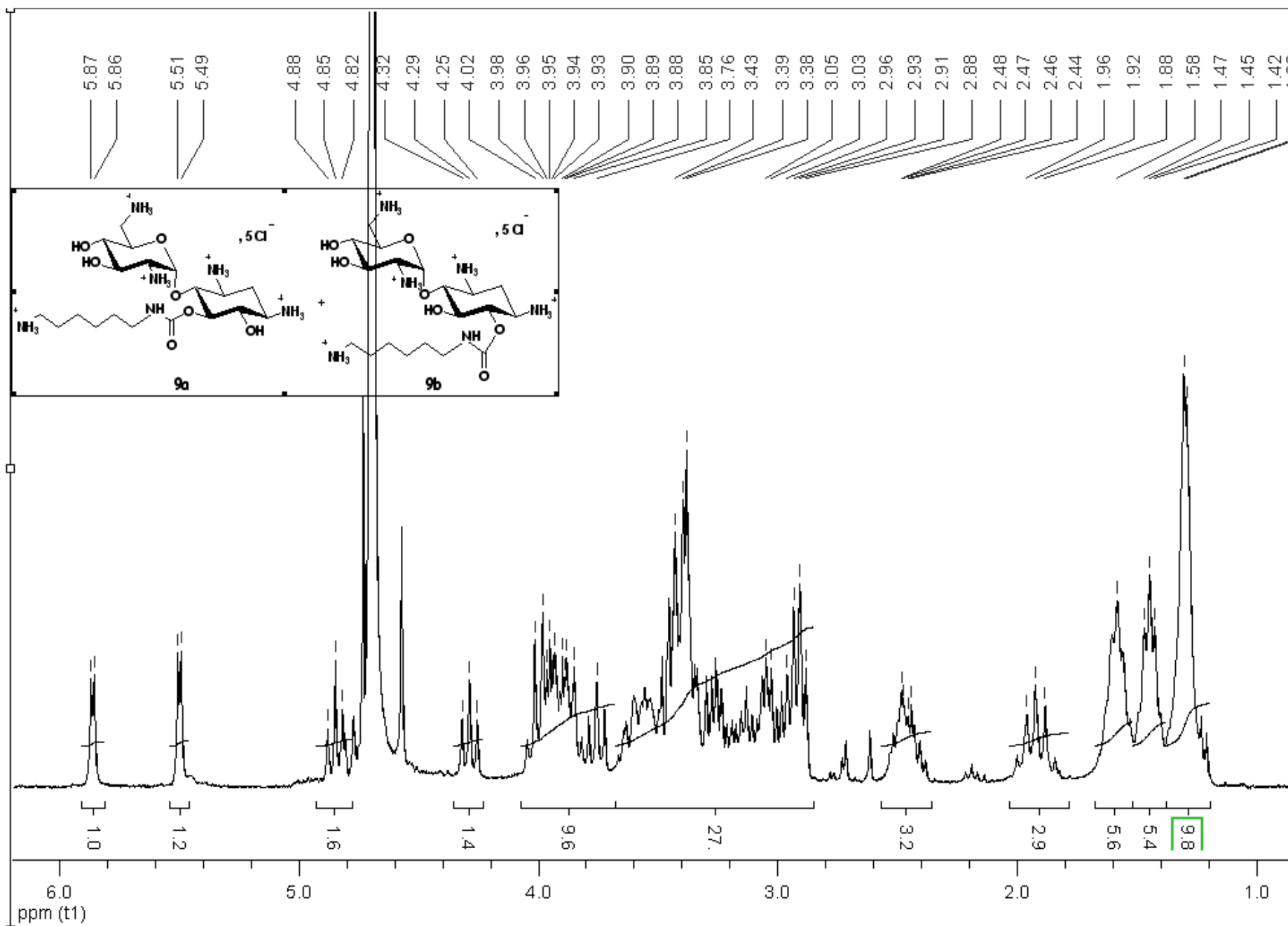
***** CHANNEL f2 *****
CROPRG2 waltz16
NUC2 1H
PCPRG2 80.00 usec
PL2 -6.00 dB
PL12 20.00 dB
PL13 20.00 dB
SFO2 300.1312005 MHz

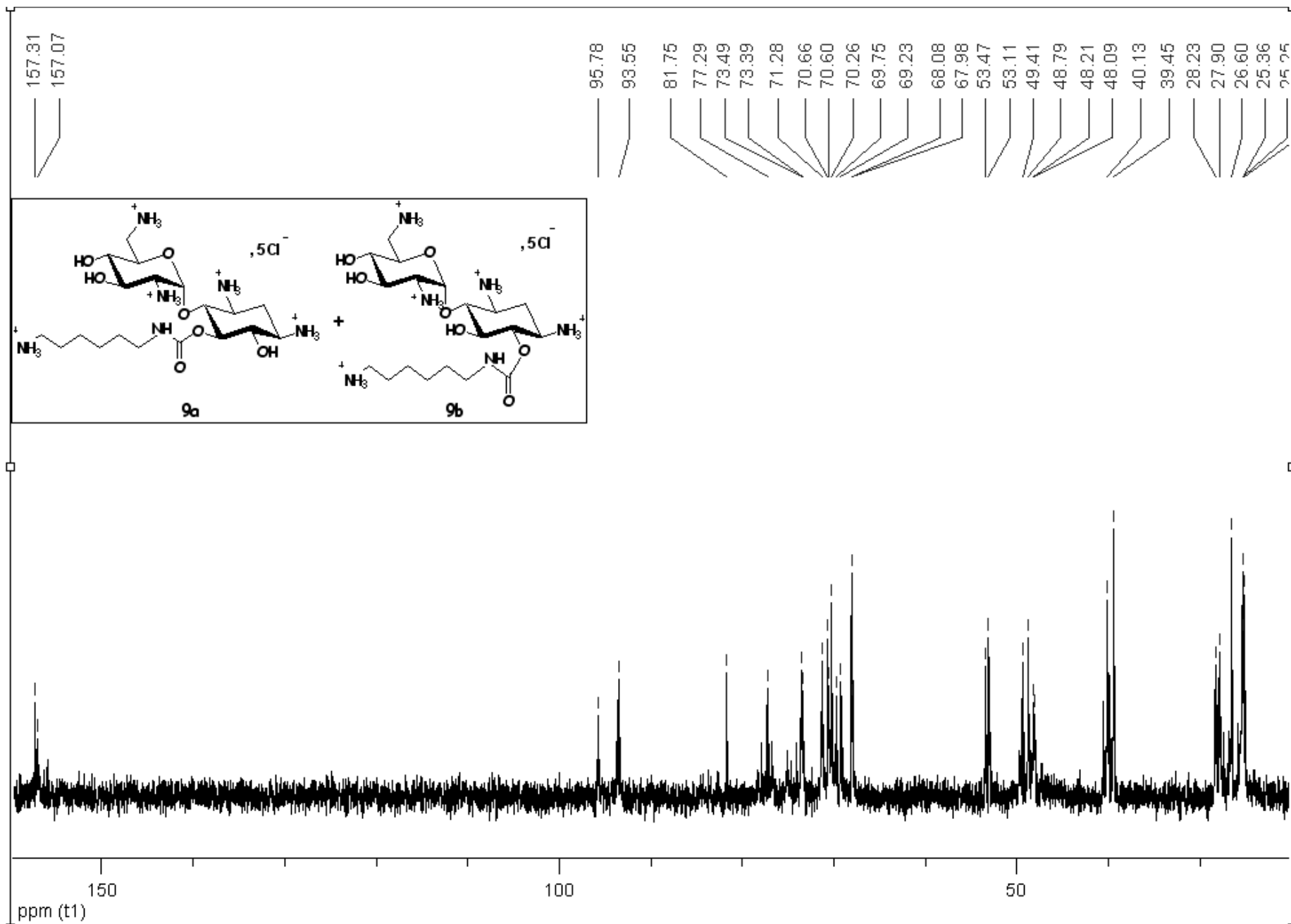
F2 - Processing parameters
SI 32768
SF 75.4677490 MHz
WDW EM
SSB 0
LB 0.55 Hz
GB 0
PC 1.40

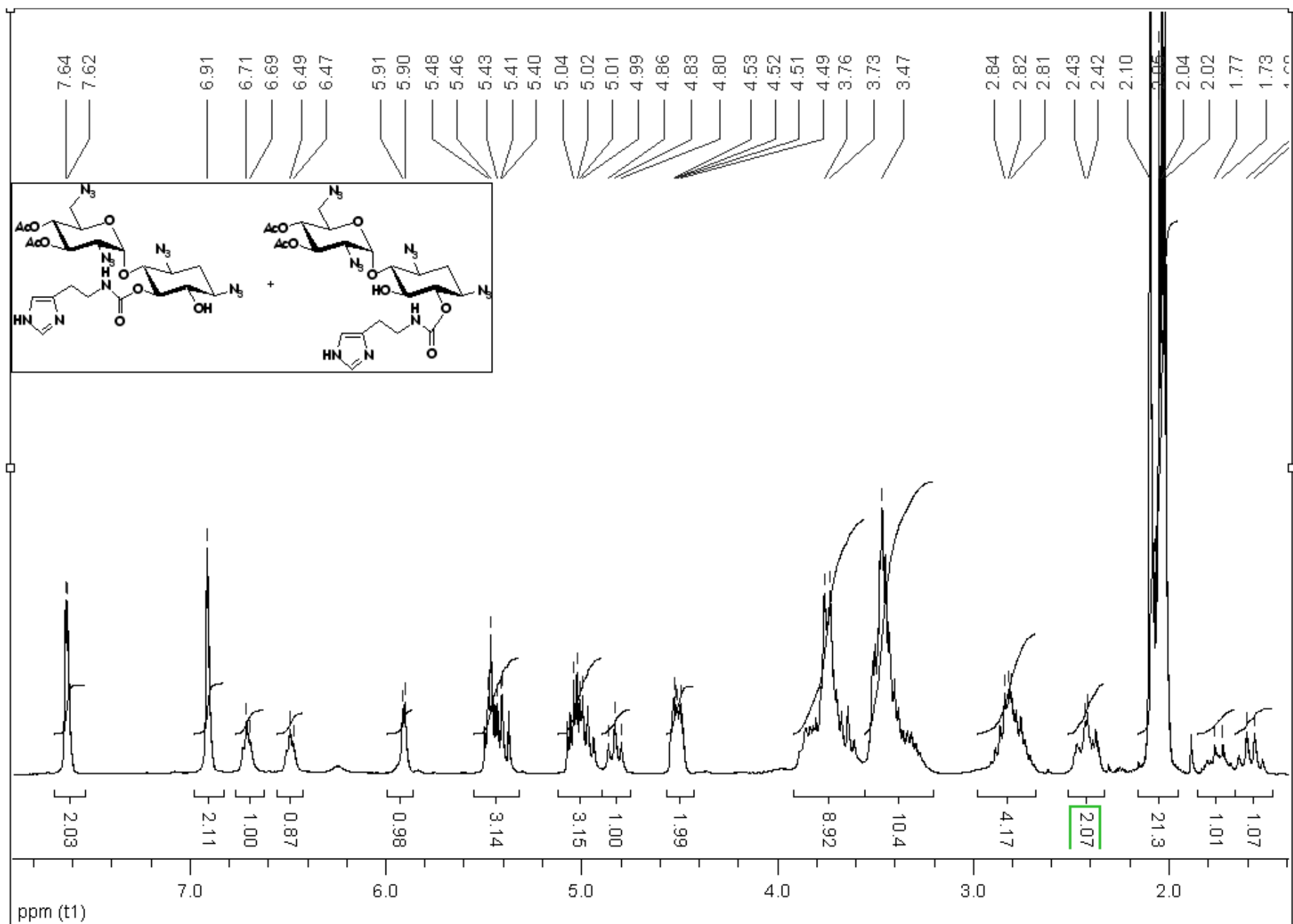
1D MFT D10t parameters
CX 35.50 cm
CY 20.00 cm
F1P 215.200 ppm
F1 16240.66 Hz
F2P -20.000 ppm
F2 -1509.35 Hz
PPMCM 6.62535 ppm/cm
HZCM 500.00040 Hz/cm

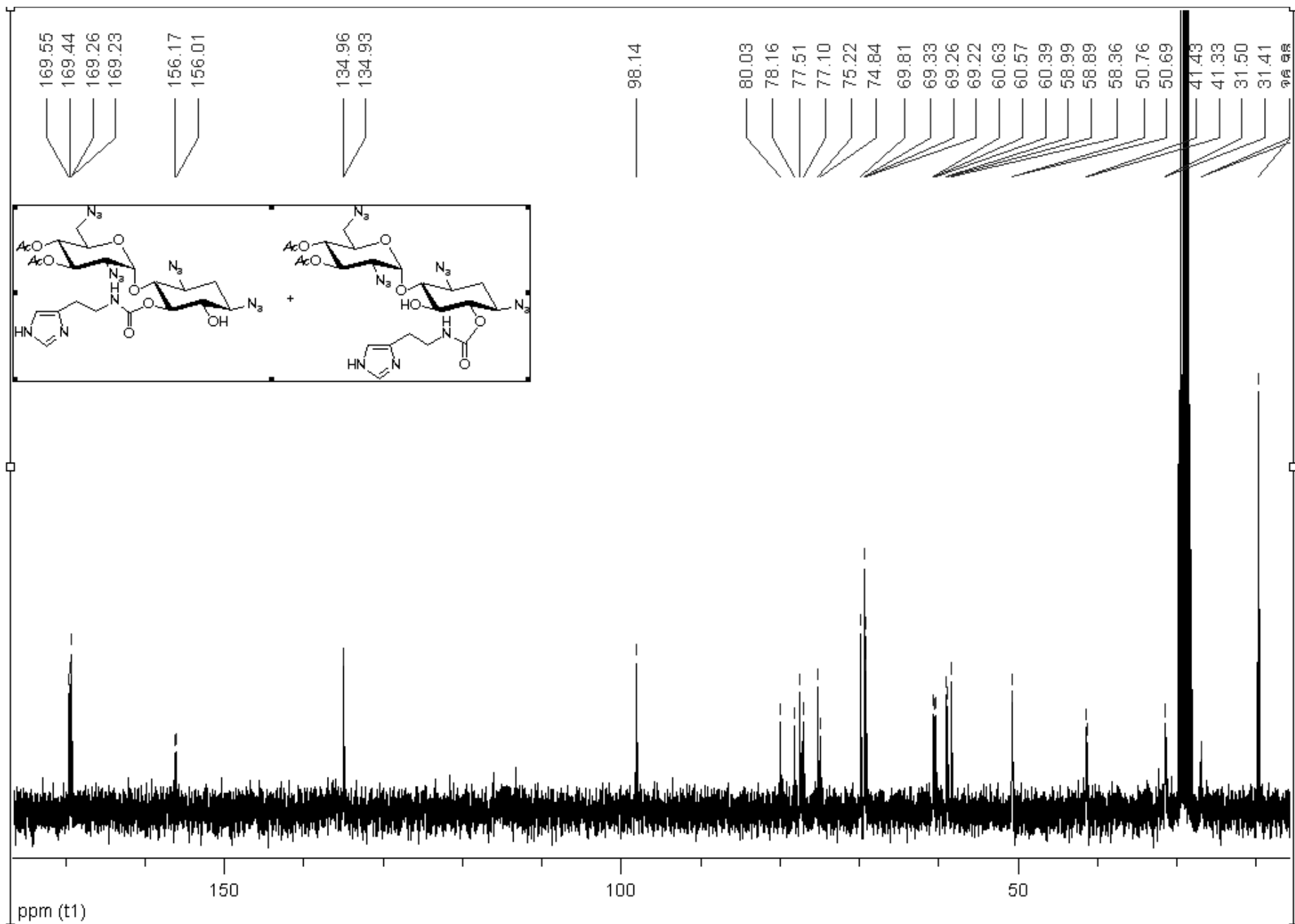


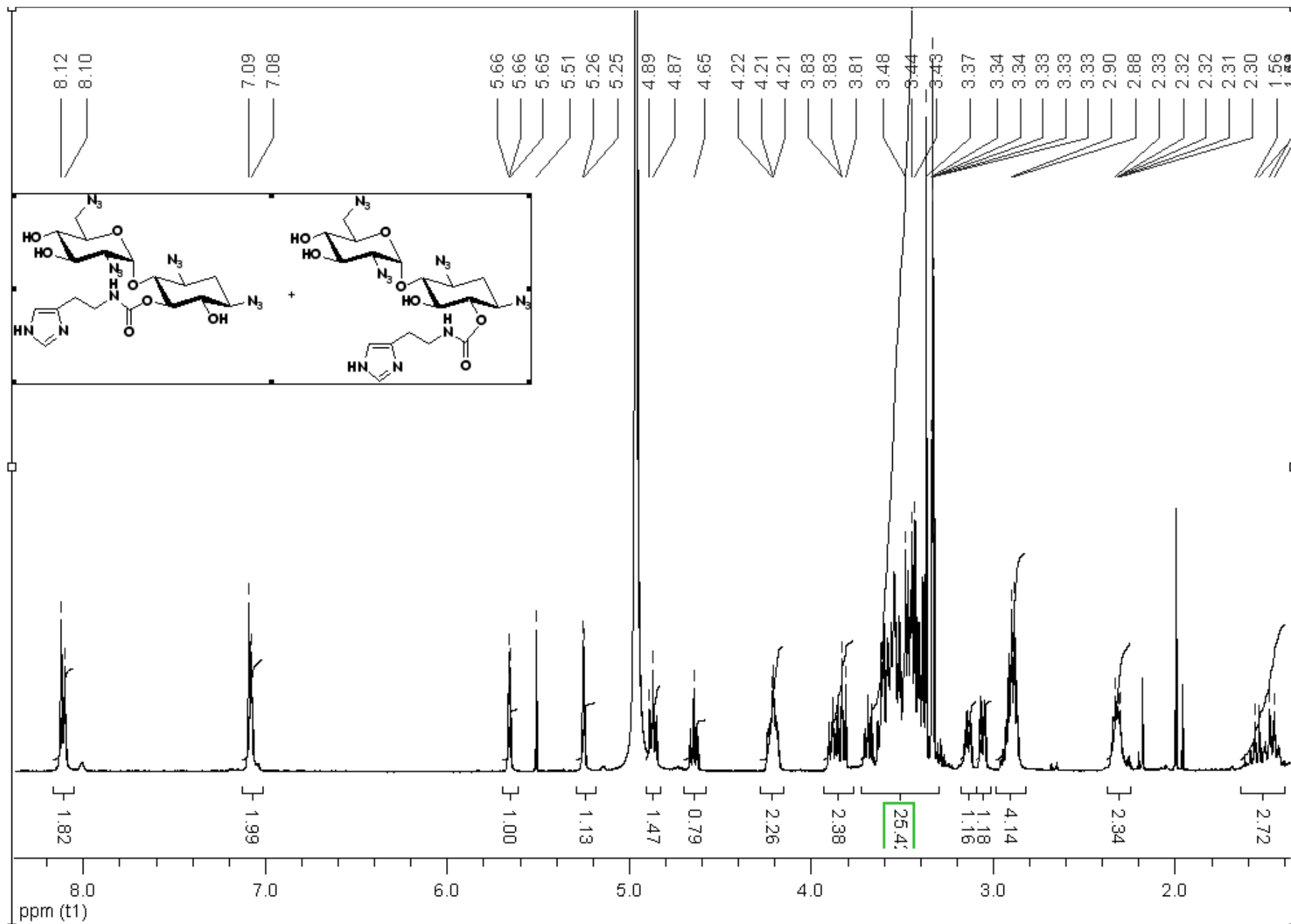


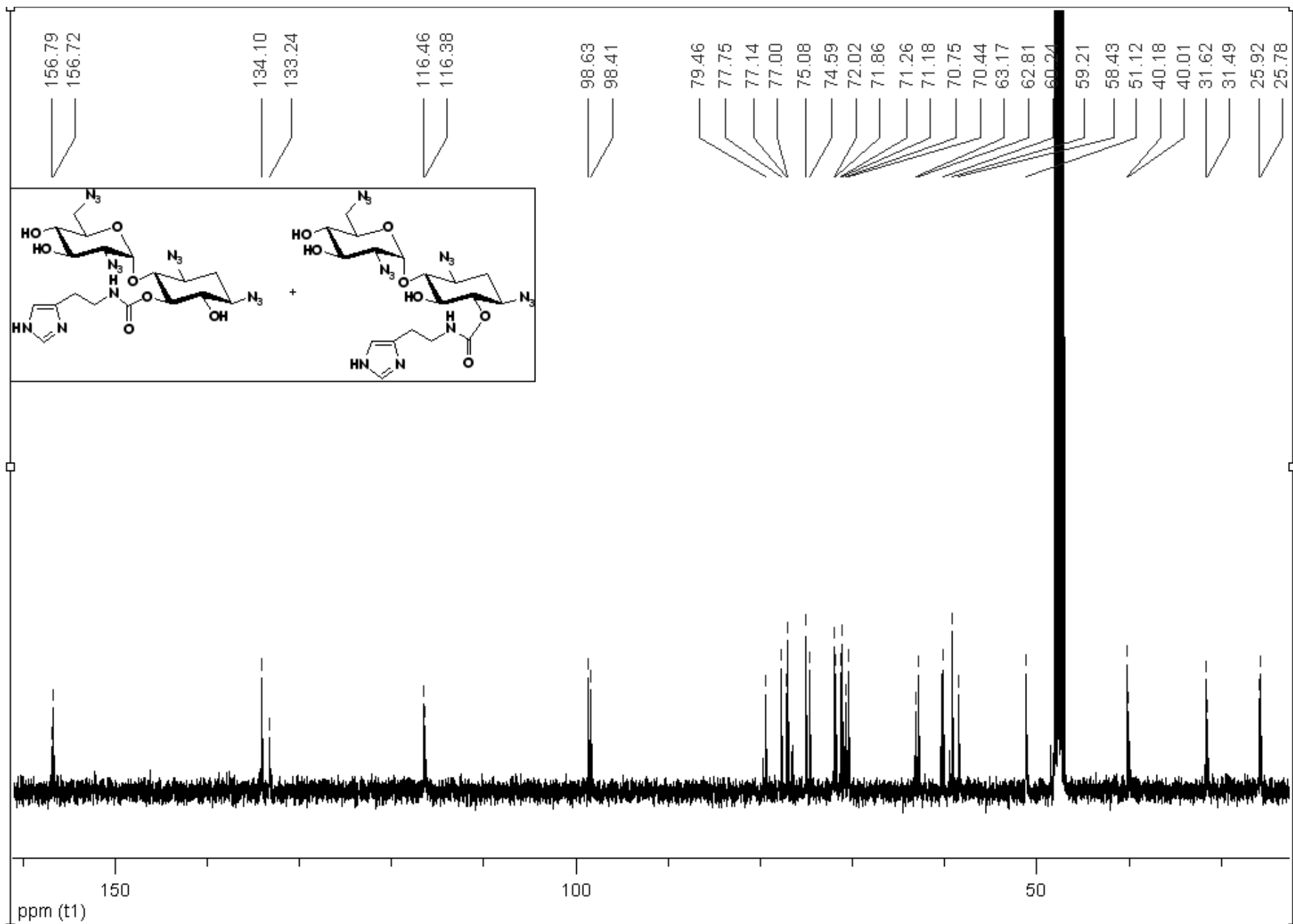


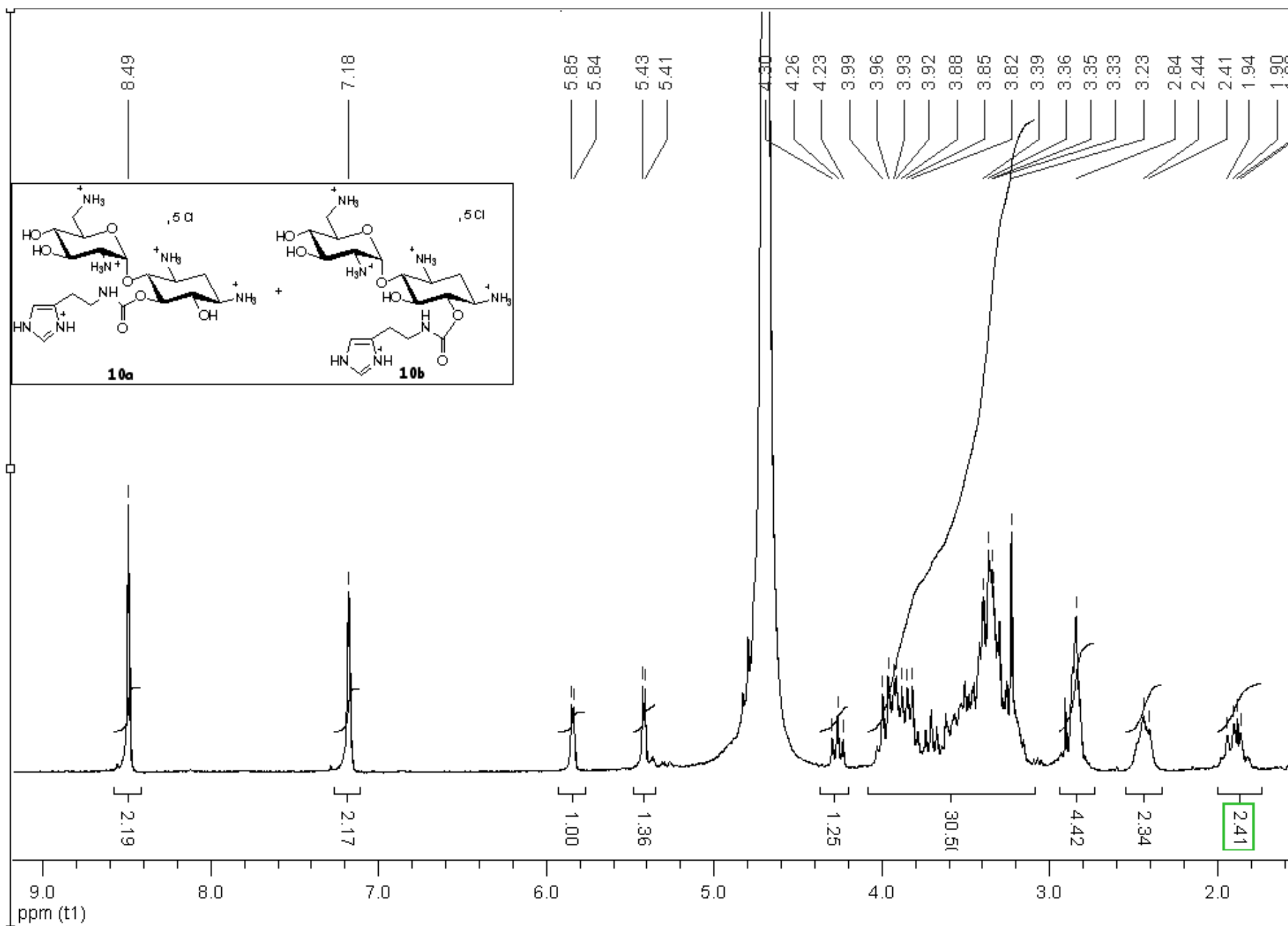


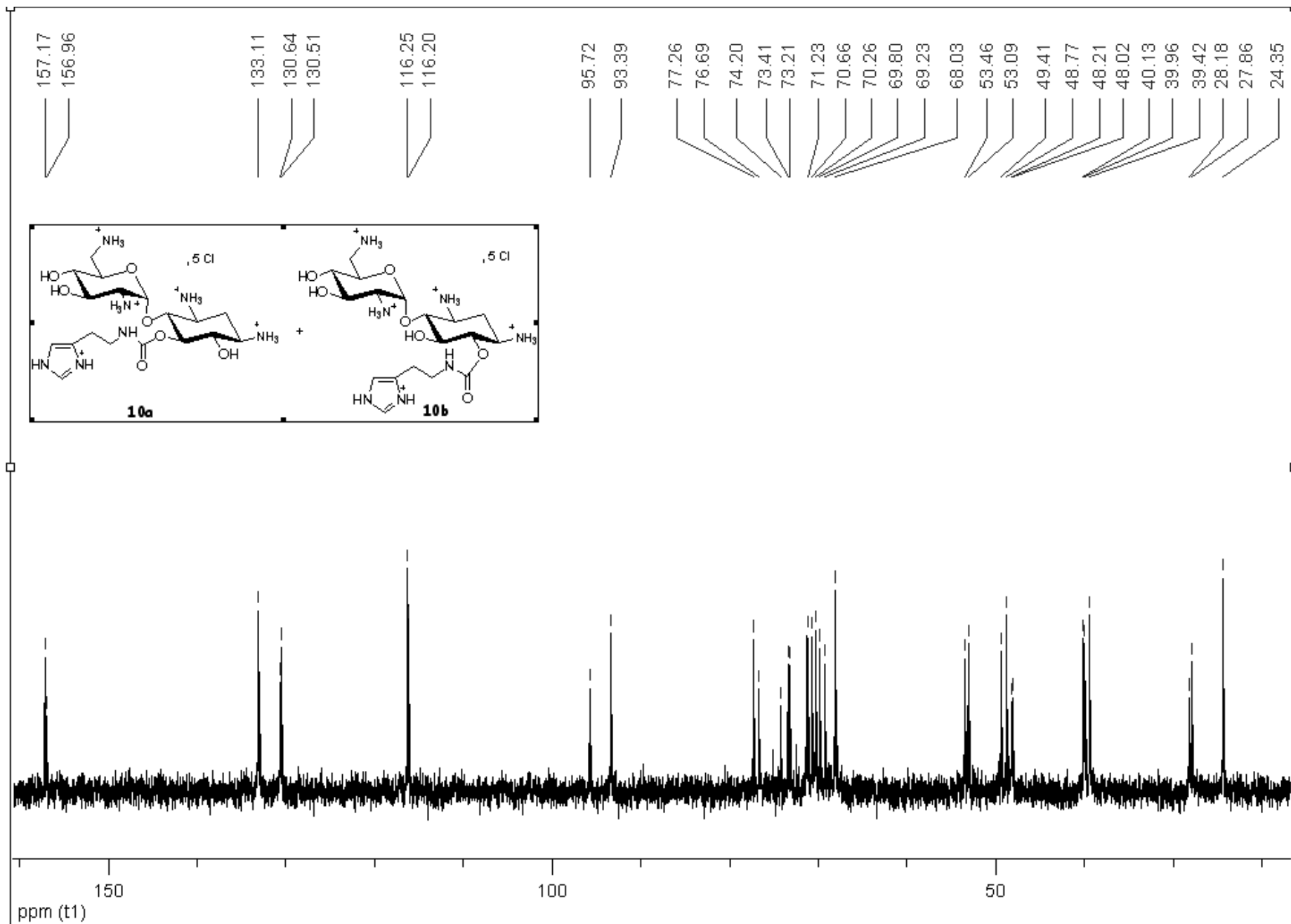


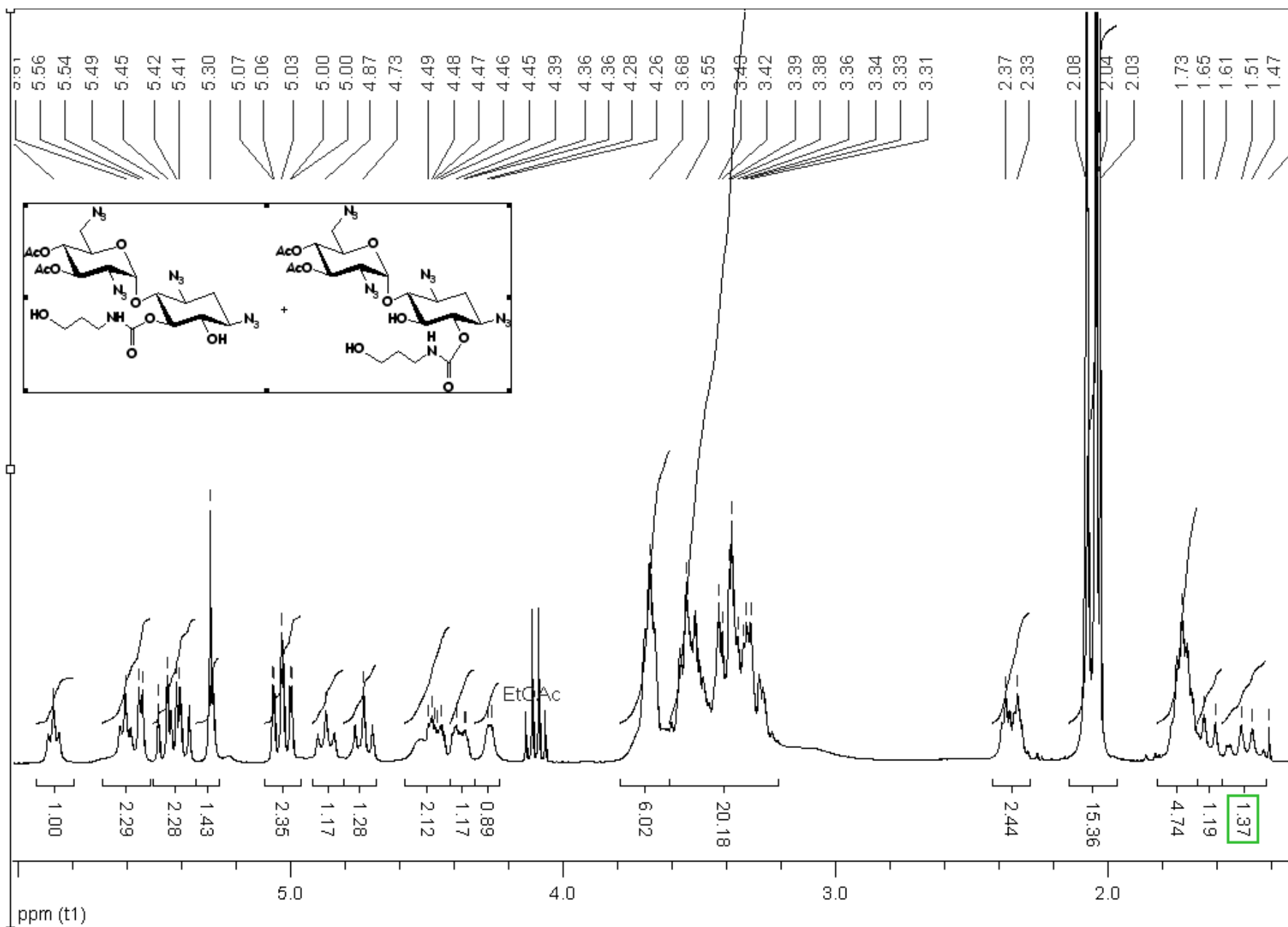


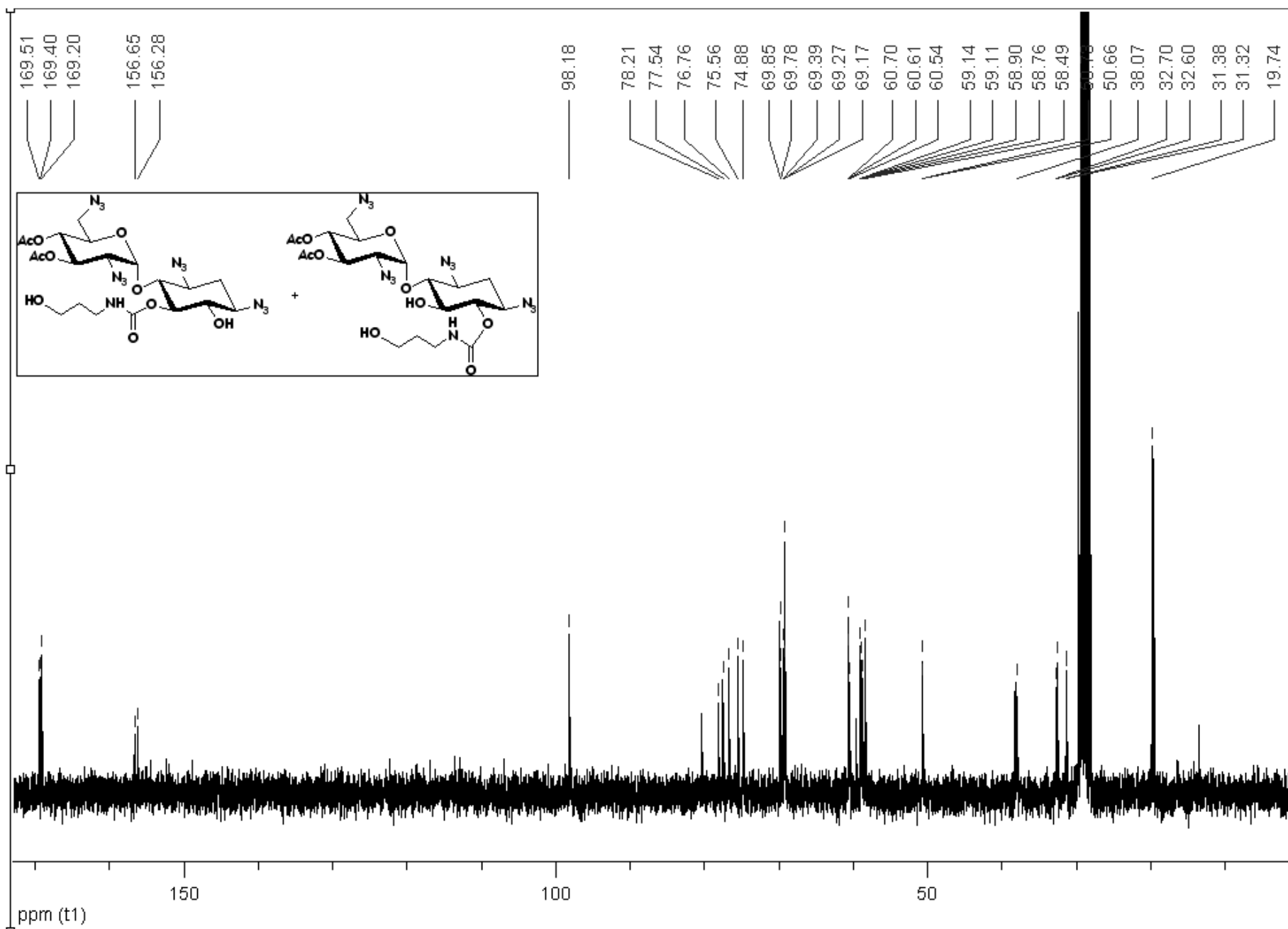


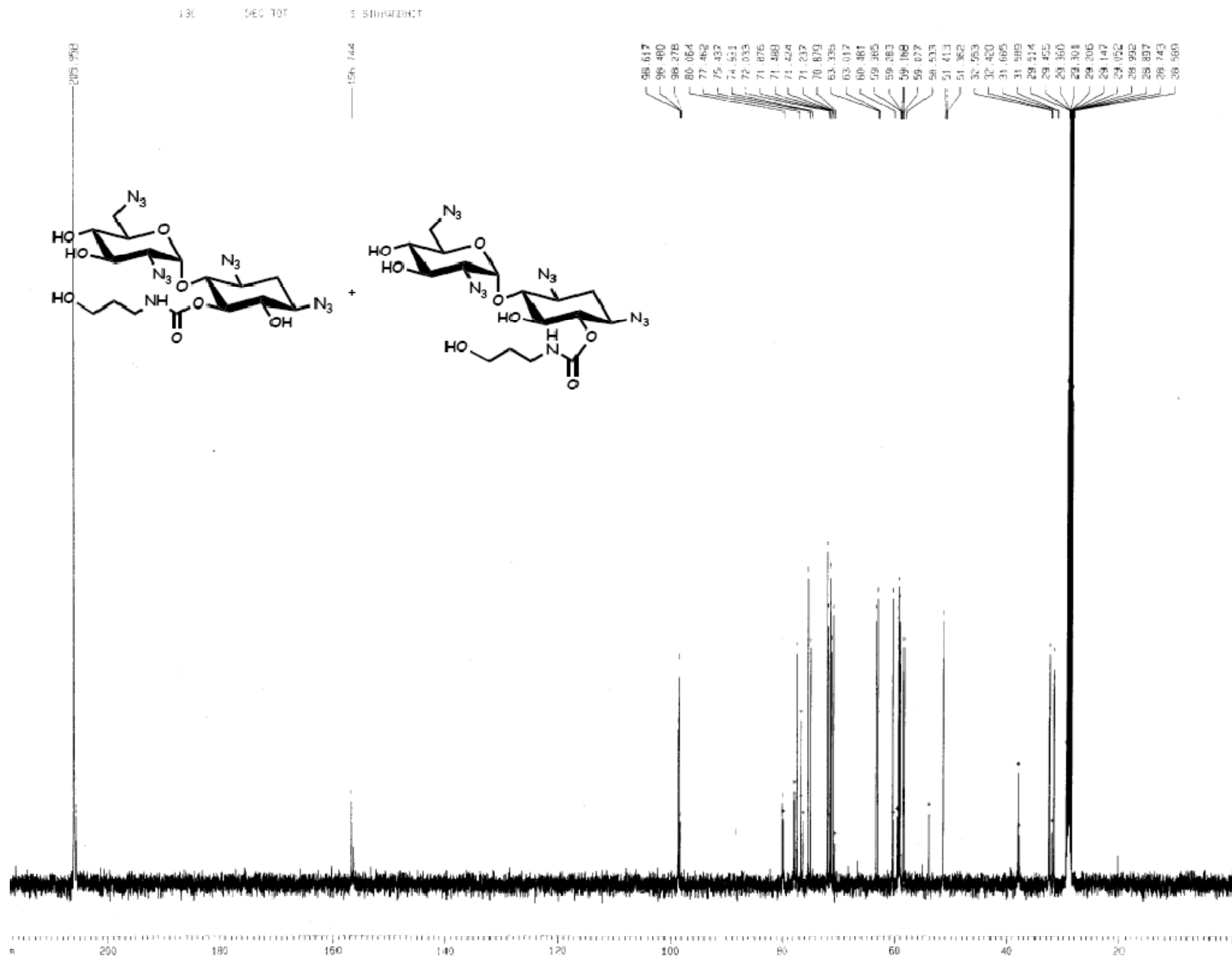












Current Data Parameters
 NAME ng3-92a
 EXPNO 2
 PROCNO 1

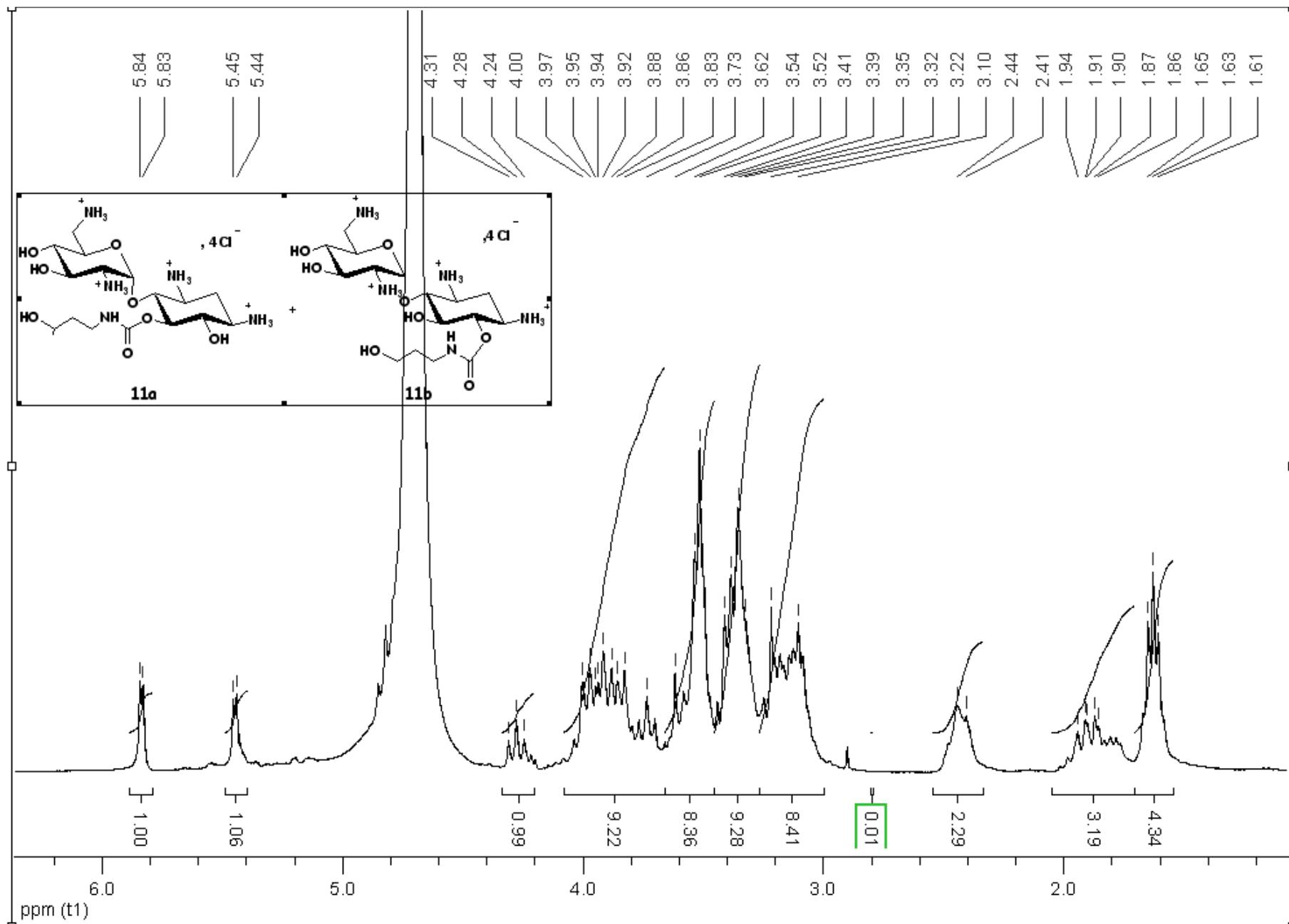
F2 - Acquisition Parameters
 Date_ 20090408
 Time 10.52
 INSTRUM spect
 PROBHD 5 mm QNP 13C/1
 PULPROG zgpg30
 TO 69.35
 SOLVENT CDCl3
 NS 95
 DS 4
 SWH 30103.030 Hz
 FIDRES 0.462368 Hz
 AQ 1.0914105 sec
 RG 18390.4
 DW 16.500 usec
 DE 20.00 usec
 TE 300.0 K
 D1 3.00000000 sec
 d11 0.03000000 sec
 d12 0.00002000 sec

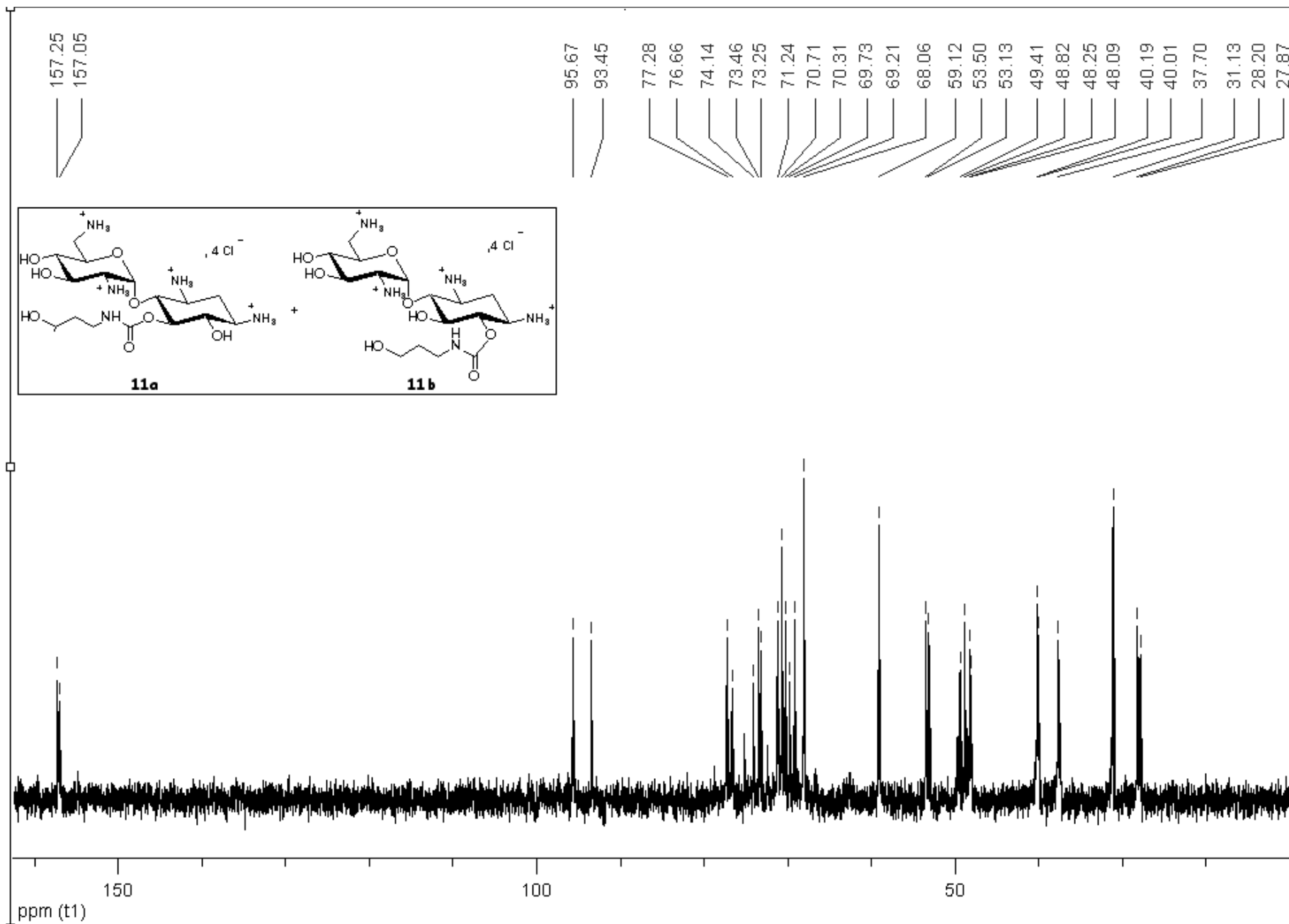
----- CHANNEL f1 -----
 NU1 13C
 P1 9.40 usec
 PL1 4.00 dB
 SF01 125.7716224 MHz

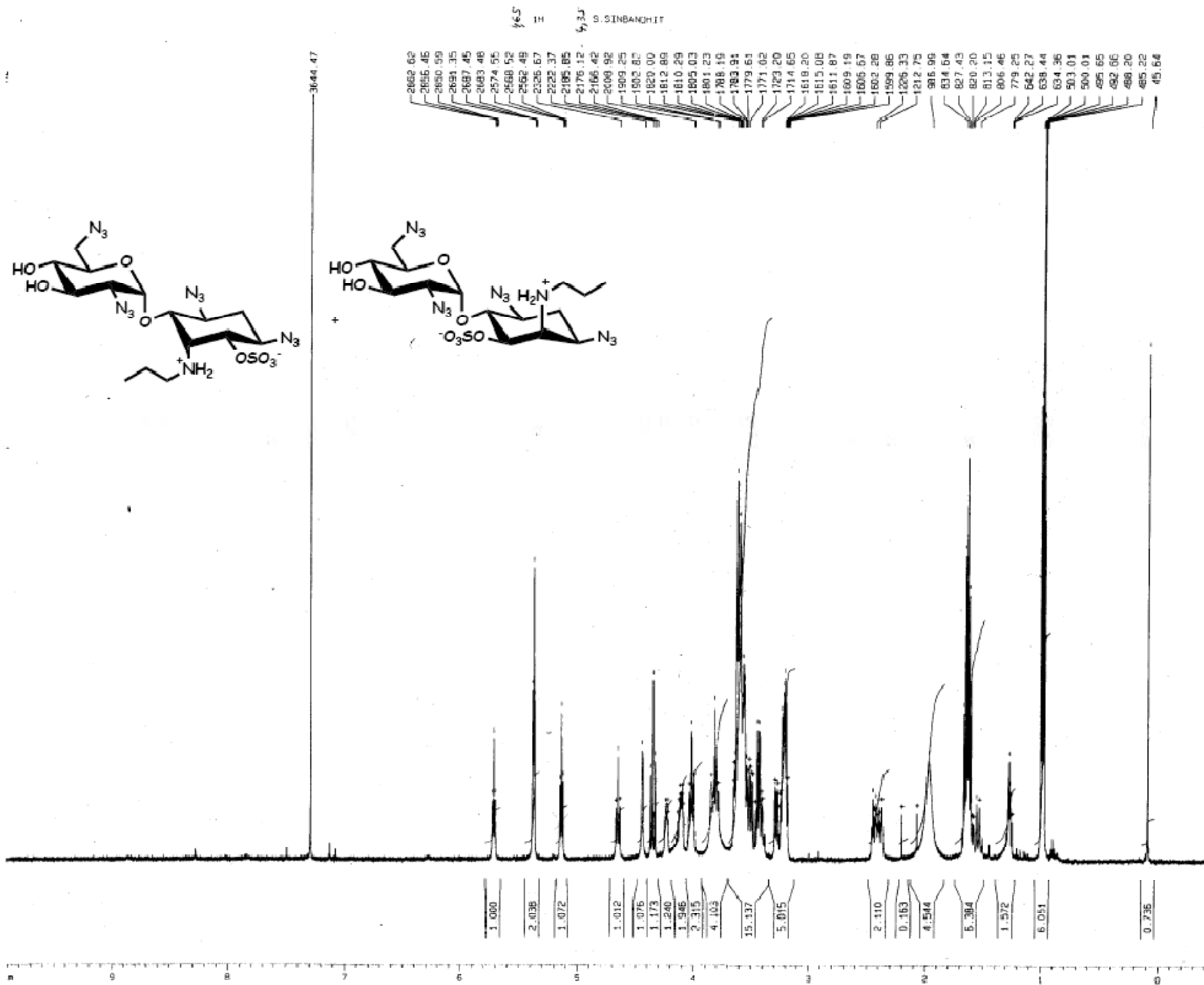
----- CHANNEL f2 -----
 CPDPRG2 waltz16
 NU2 1H
 P2 100.00 usec
 PL2 0.00 dB
 PL12 17.00 dB
 PL13 17.00 dB
 SF02 500.1320005 MHz

F2 - Processing parameters
 SI 32768
 SF 125.7578956 MHz
 MDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.00

3D NMR plot parameters
 CX 35.00 cm
 CY 100.00 cm
 F1P 820.000 ppm
 F1 27866.71 Hz
 F2P -0.500 ppm
 F2 -62.88 Hz
 PPMX 6.30000 ppm/cm
 XZCM 792.27405 Hz/cm







Current Data Parameters
 NAME: mg2-40c
 EXNO: 1
 PROCNO: 1

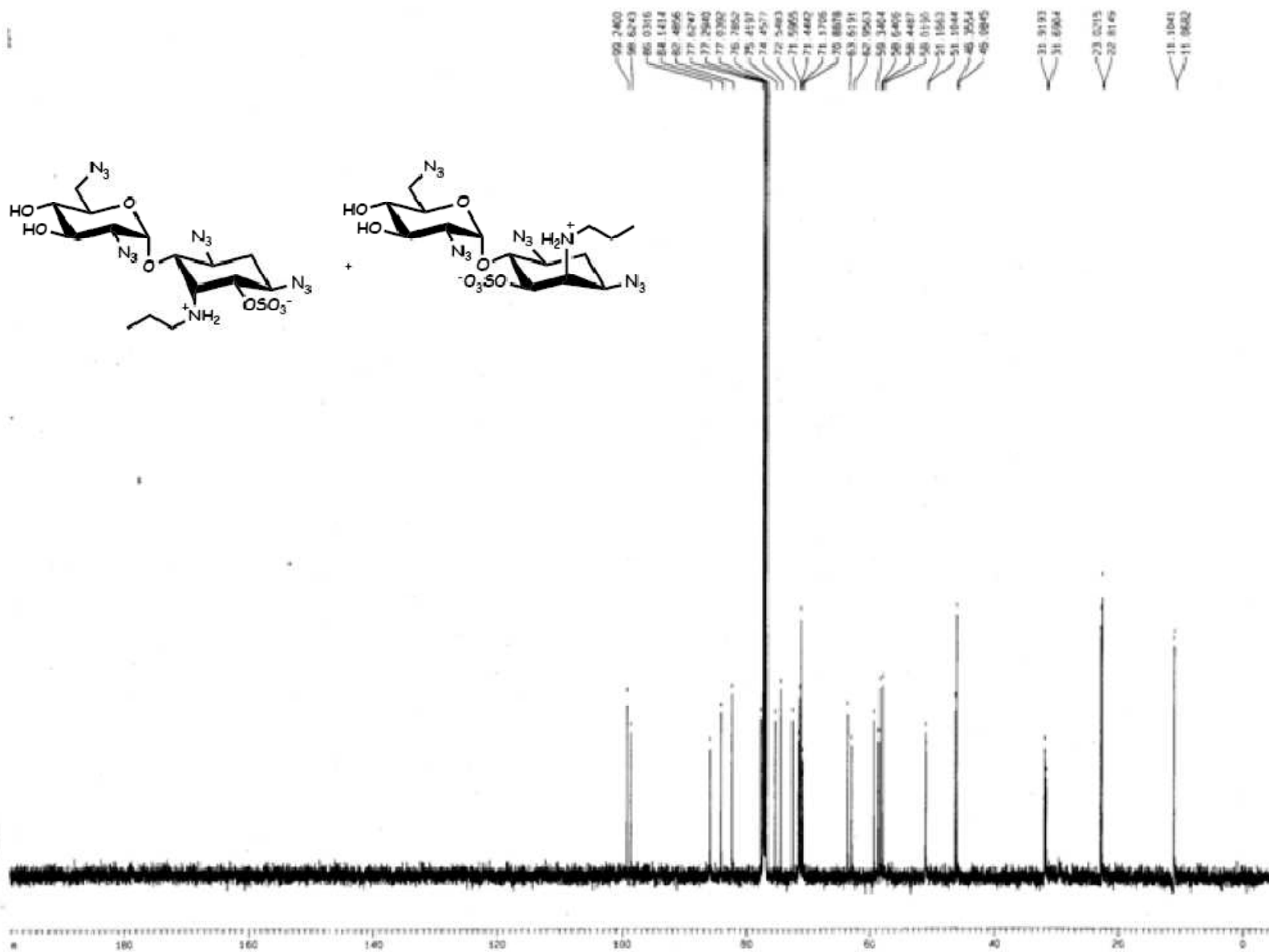
F2 - Acquisition Parameters
 Date_: 20070722
 Time: 10.35
 INSTRUM: spect
 PROBHD: 5 mm Dual 13C/
 PULPROG: zg30
 TD: 65536
 SOLVENT: CDCl3
 VS: 16
 OS: 2
 SSB: 7002.001 Hz
 FIDRES: 0.106854 Hz
 AQ: 4.6793910 sec
 RG: 406.4
 DM: 71.400 usec
 DE: 12.00 usec
 TE: 300.0 K
 D9: 0.5000000 sec

***** CHANNEL f1 *****
 NUC1: 1H
 P1: 8.65 usec
 PL1: 0.05 dB
 SFO1: 500.1330008 MHz

F2 - Processing parameters
 SI: 32768
 SF: 500.1330000 MHz
 WDW: no
 SSB: 0
 LB: 0.00 Hz
 GB: 0
 PC: 1.00

F2 NMR plot parameters
 CX: 35.00 cm
 CY: 60.00 cm
 FSP: 10.000 ppm
 F1: 500.130 MHz
 F2: -250.06 Hz
 PRNCH: 0.30000 ppm/cm
 ROCK: 150.03899 Hz/cm

13C DEC T01 5 SINBANDH1



===== DATA PARAMETERS =====
 NAME: AG2-H0c
 CONNO: 3
 PROCD: 1

F2 - Acquisition Parameters
 Date_: 20070720
 Time: 14.56
 INSTRM: spect
 PROBHD: 5 mm QNP 13C/
 PULPROG: zgpg30
 TD: 65536
 SOLVENT: CDCl3
 NS: 2548
 DS: 2
 SWH: 30446.540 Hz
 FIDRES: 0.478028 Hz
 AQ: 1.0420883 sec
 RG: 23170.5
 DW: 15.960 usec
 DE: 29.00 usec
 TE: 300.0 K
 D1: 2.00000000 sec
 d11: 0.00000000 sec
 d12: 0.00000000 sec

===== CHANNEL F1 =====
 NUC1: 13C
 P1: 24.50 usec
 PL1: -2.00 dB
 SFO1: 100.628160 MHz

===== CHANNEL F2 =====
 CPDPRG2: waltz16
 NUC2: 1H
 PCPD2: 100.00 usec
 PL2: 0.00 dB
 PL12: 22.00 dB
 PL13: 20.00 dB
 SFO2: 500.137800 MHz

F2 - Processing parameters
 SI: 32768
 SF: 100.628160 MHz
 WDW: EM
 SSB: 0
 GB: 1.00 Hz
 SC: 0
 PC: 1.00

===== 13C NMR plot parameters =====
 CH: 35.00 um
 CR: 8.00 um
 FIDP: 200.000 um
 FI: 25151.56 Hz
 FIDP: -5.000 um
 F2: -628.78 Hz
 RMWD: 5.85714 um/s
 SCA: 736.58136 Hz/cm

