SUPPORTING INFORMATION

Structure Activity Relationship Study of the Cleistrioside/Cleistetroside natural products for Antibacterial/Anticancer Activity

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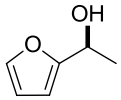
Section A: General Methods

Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon/nitrogen using oven-dried glassware and standard syringe/septa techniques. Ether, tetrahydrofuran, methylene chloride and methanol were dried by passing through activated alumina column with argon gas pressure. Hexanes refer to the petroleum fraction bp 40-60 ^{o}C. Commercial reagents were used without purification unless otherwise noted. Flash chromatography was performed using the indicated solvent system on silica gel standard grade 60 (230-400 mesh). Rf values are reported for analytical TLC using the specified solvents and 0.25 mm silica gel 60 F254 plates that were visualized by UV irradiation (254 nm) or by staining (465 mL of 95% EtOH, 17 mL conc. H_2SO_4, 5 mL acetic acid, and 13 mL anisaldehyde). Optical rotations were obtained using a digital polarimeter at sodium D line (589 nm) and were reported in concentration of g / 100 mL at 21 ^{o}C. ^{1}H and ^{13}C spectra were recorded on 600 M spectrometer, Chemical shifts are reported relative to $CHCl_3$ (δ 7.26 ppm) for ^{1}H and $CHCl_3$ (δ 77.0 ppm) for ^{13}C. IR was recorded on FT-IR Spectrometer; thin film was formed in $CHCl_3$ solution. Melting points are uncorrected.

Section B: Synthetic Procedures

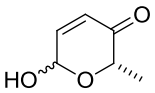
(*S*)-1-(furan-2-yl)ethanol (A)[1]



To a solution of acylfuran 11 (70 g, 636 mmol) in 90 ml CH_2Cl_2 was added the mixed solution of formic acid/triethylamine (1:1, 120 mL) and Noyori asymmetric transfer hydrogenation catalyst (*R*)-Ru(η^6-mesitylene)-(*S, S*)-TsDPEN (167 mg, 0.04 mol%). The resulting solution was stirred at room temperature for 72 h. The reaction mixture was diluted with water (1000 mL) and extracted with EtOAc (3 x 700 mL). The combined organic layers were washed with saturated $NaHCO_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography eluting with 30% EtOAc/hexane to give furan alcohol A (58.4 g, 521.5 mmol, 82%): colorless oil; Rf (30% EtOAc/hexane) = 0.41; $[\alpha]^{25}_{D}$ = + 21 (c = 1.0, CH_2Cl_2); IR (thin film, cm^{-1}) 3360, 2980, 2935, 1668, 1505, 1467, 1370, 1229, 1149, 1007, 877, 734; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30 (d, J = 1.8, 1H), 6.26 (dd, J = 3.0, 1.8 Hz, 1H), 6.15 (d, J = 3.0, 1H), 4.78 (dq, J =6.6, 6.6 Hz, 1H), 3.11 (s, 1H), 1.46 (d, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.7, 141.6, 109.9, 104.9, 63.3, 21.1.

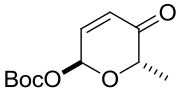
(2*S*, 6*R*)-6-hydroxy-2-methyl-2H-pyran-3(6H)-one (B):

[1] Spectral data of compounds 13, C, D, 14, E, F, 15, 16 and 17 were reported previously, see: Wu, B.; Li, M.; O'Doherty, G. A. *Org. Lett*. **2010**, *12*, 5466–5469.



Compound furan alcohol A (33.6 g, 300 mmol), 330 mL of THF, and 110 mL of H_2O were added to a 1000 ml round bottom flask and cooled to 0 ^oC. Solid $NaHCO_3$ (45 g, 549 mmol), $NaOAc•3H_2O$ (75 g, 551 mmol), and NBS (54 g, 305 mmol) were added to the solution and the mixture was stirred for 1 h at 0 ^oC. The reaction was quenched with saturated $NaHCO_3$ (450 mL), extracted (3 x 600 mL) with Et_2O, dried Na_2SO_4), concentrated under reduced pressure and purified by silica gel chromatography eluting with 25% EtOAc/hexane to give pyranone B (34.8 g, 272 mmol, 90%): Rf (60% EtOAc/hexane) = 0.29; $[\alpha]^{25}_D$ = + 44 (c = 1.0, CH_2Cl_2); IR (thin film, cm^{-1}) 3381, 2988, 2942, 1692, 1447, 1373, 1232, 1021, 937; ^1H NMR (600 MHz, $CDCl_3$) major isomer δ 6.82 (dd, J = 10.2, 3.0 Hz, 1H), 5.96 (d, J = 10.2, 1H), 5.48 (d, J = 3.0 Hz, 1H), 3.99 (q, J = 7.2 Hz, 1H), 1.23 (d, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) major isomer δ 197.6, 145.3, 126.6, 87.2, 74.8, 15.1.

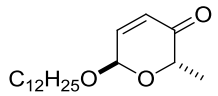
(2*S*, 6*S*)-tert-butyl -5,6-dihydro-6-methyl-5-oxo-2H-pyran-2-yl carbonate (12):



Pyronone alcohol B (30 g, 234.4 mmol) was dissolved in CH_2Cl_2 (250 mL) and the solution was cooled to -78 ^oC. A CH_2Cl_2 (50 mL) solution of $(Boc)_2O$ (70 g, 300 mmol) and a catalytic amount of DMAP (3 g, 23 mmol) was added to the reaction mixture. The reaction was stirred for 1 h at -78 ^oC, and quenched with 500 mL of

saturated $NaHCO_3$, extracted with Et_2O (3 x 700 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified using silica gel flash chromatography eluting with 6% EtOAc/hexane to give 40.5 g (177.6 mmol, 76%) of two diastereomers of Boc-protected pyranone 4α and 4β in 2.5:1: Rf (20% Et_2O/hexane) = 0.58; $[\alpha]^{25}_D$ = +98 (c = 1.0, CH_2Cl_2); IR (thin film, cm^{-1}) 2984, 2942, 1752, 1703, 1371, 1273, 1254, 1153, 938, 838; ^1H NMR (600 MHz, $CDCl_3$) δ 6.78 (dd, J = 10.2, 3.6 Hz, 1H), 6.22 (d, J = 3.6 Hz, 1H), 6.09 (d, J = 10.2 Hz, 1H), 4.53 (q, J = 6.6 Hz, 1H), 1.40 (s, 9H), 1.28 (d, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.5, 151.7, 140.9, 128.2, 89.1, 83.3, 72.0, 27.5, 15.1; ClHRMS Calculated for [$C_{11}H_{16}O_5Na^+$]: 251.0890, Found: 251.0883.

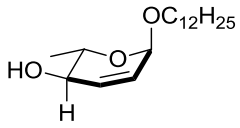
(1*R*, 5*S*)-1-dodecoxy-5-methyl-1H-pyran-4-one (13):



To a solution of Boc pyranone 12 (11.42 g, 50 mmol) and dodecan-1-ol (13.97 g, 75 mmol) in 50 mL CH_2Cl_2 at 0 ^oC was added 4Å molecular sieve (3.0 g) and PPh_3 (78.7 mg, 0.3 mmol). The mixture was stirred for 10 min and then $Pd_2(dba)_3 \cdot CHCl_3$ (77.6 mg, 0.075 mmol) was added. The reaction mixture was stirred and warmed to rt. After 2 h the reaction was quenched by adding 50 mL saturated $NaHCO_3$, followed by extraction with Et_2O (100 mL × 3). The organic layers were combined, washed by 50 mL saturated NaCl, dried over Na_2SO_4 and concentrated under reduced. The crude

product was purified using silica gel flash chromatography eluting with 5~10% EtOAc/hexane to give pyranone 13 (12.86 g, 87%): Rf (30% EtOAc/hexane) = 0.65; $[\alpha]^{25}_{D}$ = + 24.72 (c = 1.1, CH_2Cl_2); IR (thin film, $cm^{-1)}$ 2924, 2854, 1702, 1467, 1374, 1359, 1231, 1158, 1129, 1104, 1086, 1039, 843, 808, 723; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.82 (dd, J = 10.2, 3.6 Hz, 1H), 6.06 (d, J = 10.2 Hz, 1H), 5.16 (d, J = 3.0 Hz, 1H), 4.55 (q, J = 6.6 Hz, 1H), 3.82 (dt, J = 6.6, 9.6Hz, 1H), 3.57 (dt, J = 6.6, 9.6Hz, 2H), 1.61 (m, 2H) 1.38 (d, J = 6.6Hz, 3H), 1.26~1.37 (m, 20H), 0.87 (t, J = 6.6, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.1, 143.6, 127.2, 93.2, 70.3, 69.5, 31.9, 29.68, 29.63, 29.61, 29.59, 29.56, 29.37, 29.32, 26.1, 22.7, 15.2, 14.1; HRMS (ESI) calcd for $[C_{18}H_{32}O_3 + H]^+$: 297.2424, Found: 297.2425.

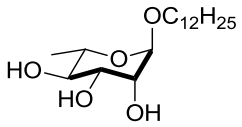
(1*R*, 4*R*, 5*S*)-1-dodecoxy-5-methyl-1,4-dihydro-5H-pyran-4-ol (C):



To a solution of compound 13 (12.0 g, 40.5 mmol) in CH_2Cl_2 (40 mL) at -78 ^{o}C was added $CeCl_3$/MeOH solution (0.4 M, 40 mL) and $NaBH_4$ (1.84 g, 48.6 mmol). The reaction mixture was stirred at -78 ^{o}C for 2 hours. The reaction mixture was quenched with 50 mL of saturated aqueous $NaHCO_3$, extracted with Et_2O (2 x 100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified using silica gel flash chromatography eluting with 5~20% EtOAc/hexane to give alcohol C (10.72 g, 89%): m.p. 39.1~40.2 ^{o}C; Rf (30% EtOAc/hexane) = 0.45; $[\alpha]^{25}_{D}$

= - 24.92 (c = 1.36, CH_2Cl_2); IR (thin film, cm^{-1}) 3383, 2922, 2854, 1458, 1378, 1325, 1297, 1190, 1129, 1147, 1132, 1102, 1046, 1002, 886, 845, 829, 721; ^1H NMR (600 MHz, $CDCl_3$) δ 5.87 (d, J = 10.2 Hz, 1H), 5.68 (d, J = 10.2, 2.2 Hz, 1H), 4.88 (s, 1H), 3.72 (m, 1H), 3.58 (q, J = 6.6 Hz, 1H), 3.46 (t, J = 6.6 Hz, 1H), 3.43 (t, J = 6.6 Hz, 1H), 2.54 (d, J = 8.4 Hz, 1H), 1.54 (d, J = 6.6 Hz, 3H), 1.26 (t, 20H), 0.85 (t, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.1, 143.6, 127.2, 93.2, 70.3, 69.5, 31.9, 29.7, 29.6, 29.6, 29.6, 29.6, 29.4, 29.3, 26.1, 22.7, 15.2, 14.1; HRMS (ESI) calcd for $[C_{18}H_{34}O_3 + Na]^+$: 321.2400, Found:321.2402.

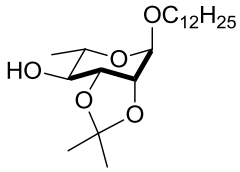
(3*S*,4*S*,5*R*)-2-(dodecyloxy)-6-methyltetrahydro-2H-pyran-3,4,5-triol (D):



To a solution of allylic alcohol C (10.0 g, 33.5 mmol) in t-butanol/acetone (1:1, 67 mL) at 0 °C was added a solution of N-methyl morpholine N-oxide/water (50% w/v) (20 mL) and OsO_4 (42.6 mg, 0.168 mmol). The reaction mixture was stirred at rt for 12 h and then concentrated. The residue was pipetted directly on to a silica gel column using a small amount of CH_3OH in three portions. Impurities were eluted with ether and the product was eluted with 90% EtOAc/Hexane. Pure fractions were combined and concentrated to afford triol D (10.22 g, 92%): Rf (100% EtOAc/hexane) = 0.28; $[\alpha]^{25}_D$ = - 45.80 (c = 1, CH_2Cl_2); IR (thin film, cm^{-1}) 3384, 2922, 2854, 1457, 1380, 1224, 1131, 1094, 1053, 983, 910, 882, 837, 809, 721, 677; ^1H NMR (600 MHz,

$CDCl_3$) δ 4.72 (s, 1H), 4.47 (s, 1H), 3.89 (s, 1H), 3.85 (s, 1H), 3.75 (dd, J = 9.0, 3.6 Hz, 1H), 3.62 (m, 1H), 3.46 (d, J = 9.0 Hz, 1H), 3.38 (t, J = 6.6 Hz, 1H), 3.35 (t, J = 6.6 Hz, 1H), 1.53 (d, J = 6.6 Hz, 3H), 1.26 (t, 20H), 0.85 (t, J = 6.6, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 99.8, 72.8, 71.7, 71.1, 68.2, 67.9, 32.0, 29.8, 29.6, 29.6, 29.6, 29.6, 29.4, 29.4, 26.2, 22.8, 17.6, 14.2; HRMS (ESI) calcd for $[C_{18}H_{36}O_5 + Na]^+$: 355.2455, Found: 355.2457.

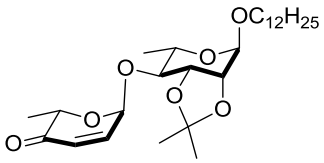
(14):



To a solution of triol D (10.0 g, 30.08 mmol) in acetone (80 mL) and 2,2-dimethoxypropane (74 mL, 600 mmol) at 0 ^oC was added p-Toluenesulfonic acid monohydrate (57 mg, 0.30 mmol). The reaction mixture was stirred for 4 h. The reaction mixture was neutralized with 2 mL of Et_3N and then was concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography eluting with 10~30% EtOAc/Hexane to give mono alcohol 14 (11.12 g, 99%): m.p. 56.0~57.2 ^oC; Rf (50% EtOAc/hexane) = 0.65; $[\alpha]^{25}_D$ = - 20.08 (c = 1.2, CH_2Cl_2); IR (thin film, cm^{-1}) 3461, 2924, 2855, 1457, 1382, 1244, 1220, 1170, 1140, 1094, 1088, 1055, 1022, 998, 922, 861, 818, 787, 724; ^1H NMR (600 MHz, $CDCl_3$) δ 4.93 (s, 1H), 4.13 (d, J = 6.0 Hz, 1H), 4.09 (dd, J = 7.2, 6.0 Hz, 1H), 3.68 (m, 2H), 3.42 (dq, J = 9.6, 6.6 Hz, 1H), 3.40 (dd, J = 9.6, 7.2 Hz, 1H), 2.34 (d, J = 4.2 Hz, 1H), 1.60 (s,

3H), 1.57 (m, 2H), 1.52 (s, 3H), 1.35 (s, 3H), 1.28 (m, 18H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 109.5, 97.2, 78.4, 76.0, 74.5, 67.9, 66.1, 32.0, 29.7, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 28.0, 26.2, 26.2, 22.8, 17.7, 14.2; HRMS (ESI) calcd for $[C_{21}H_{40}O_5 + H]^+$: 373.2948, Found: 373.2951.

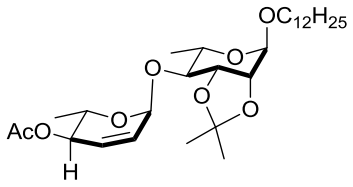
(E):



To a solution of Boc pyranone 12 (7.07 g, 30.98 mmol) and mono alcohol 14 (9.62 g, 25.82 mmol) in 30 mL CH_2Cl_2 at 0 ^oC was added 4Å molecular sieve (2.0 g) and PPh_3 (40.6 mg, 0.155 mmol). The mixture was stirred for 10 min and then $Pd_2(dba)_3 \cdot CHCl_3$ (40.4 mg, 0.039 mmol) was added. The reaction mixture was stirred and warmed to rt. After 2 h the reaction was quenched by adding 30 mL saturated $NaHCO_3$, followed by extraction with Et_2 (80 mL × 3). The organic layers were combined, washed by 50 mL saturated NaCl, dried over Na_2SO_4 and concentrated under reduced. The crude product was purified using silica gel flash chromatography eluting with 5~15% EtOAc/hexane to give pyranone E (11.08 g, 89%): Rf (30% EtOAc/hexane) = 0.62; $[\alpha]^{25}_D$ = - 11.70 (c = 1, CH_2Cl_2); IR (thin film, cm^{-1}) 2985, 2924, 2855, 1702, 1455, 1381, 1241, 1221, 1161, 1139, 1083, 1045, 1021, 990, 862, 788, 726; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.84 (dd, J = 10.2, 3.6 Hz, 1H), 6.06 (d, J = 10.2 Hz, 1H), 5.76 (d, J = 3.0 Hz, 1H), 4.95 (s, 1H), 4.52 (q, J = 6.6 Hz, 1H), 4.21 (dd, J = 6.6, 6.0 Hz, 1H), 4.11

(d, J = 6.0 Hz, 1H), 3.67 (m, 3H), 3.41 (dt, J = 9.6, 6.6 Hz, 1H), 1.66 (s, 3H), 1.56 (m, 2H), 1.33 (m, 9H), 1.26 (m, 18H), 0.87 (t, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.0, 143.7, 127.1, 127.1, 109.6, 97.0, 92.4, 79.0, 79.0, 76.5, 70.5, 67.8, 64.0, 32.0, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 28.1, 26.5, 26.2, 22.7, 17,5, 15.2, 14.2; HRMS (ESI) calcd for $[C_{27}H_{46}O_7 + Na]^+$: 505.3136, Found: 505.3140.

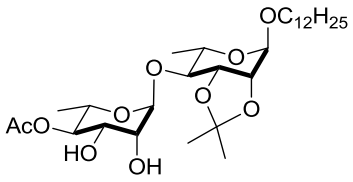
(F):



To a solution of disaccharide pyranone E (10.88 g, 22.54 mmol) in CH_2Cl_2 (25 mL) at -78 ^{o}C was added $CeCl_3$/MeOH solution (0.4 M, 25 mL) and $NaBH_4$ (1.02 g, 27.05 mmol). The reaction mixture was stirred at -78 ^{o}C for 2 hours. The reaction mixture was quenched with 30 mL of saturated aqueous $NaHCO_3$, extracted (2 x 100 mL) with Et_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified using silica gel flash chromatography eluting with 10~25% EtOAc/Hexane to give the allylic alcohol as a yellow oil. To a solution of the allylic alcohol in pyridine (40 mL) was added acetic anhydride (15 mL) and a catalytic amount of 4-dimethylaminopyridine (50 mg). After stirring for 3 h, the mixture was diluted in ether, washed with saturated $CuSO_4$ solution (3 x 100 mL) and the solvent was evaporated. The crude product was purified by silica gel flash chromatography eluting with 5~15% EtOAc/Hexane to give ester F (9.83g, 83% in two steps) as a

yellow oil: Rf (30% EtOAc/hexane) = 0.62; $[\alpha]^{25}_{D}$ = - 46.20 (c =1.50, CH_2Cl_2); IR (thin film, cm^{-1}) 2925, 2855, 1740, 1454, 1404, 1374, 1234, 1194, 1138, 1084, 1044, 1024, 988, 917, 861, 815, 723; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.83 (m,2H), 5.49 (s, 1H), 5.04 (d, J = 9.0 Hz, 1H), 4.94 (s, 1H), 4.19 (dd, J = 7.2, 6.0 Hz, 1H),4.09 (d, J = 5.4 Hz, 1H), 3.91 (dq, J = 9.0, 6.0 Hz, 1H), 3.66 (m, 2H), 3.60 (dd, J = 9.6, 7.2 Hz, 1H), 3.10 (dt, J = 9.6, 6.6 Hz, 1H), 2.08 (s, 3H), 1.61 (s, 3H), 1.56 (m, 2H), 1.34 (s, 3H), 1.27 (m, 21H), 1.20 (d, J = 6.0 Hz, 3H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.5, 132.7, 128.5, 128.0, 127.8, 127.3, 99.7, 99.6, 99.4, 95.2, 74.9, 69.9, 69.0, 68.9, 68.8, 68.0, 67.2, 48.0, 47.7, 18.0, 17.9, 17.8, 16.7; HRMS (ESI) calcd for $[C_{29}H_{50}O_8 + Na]^+$: 549.3398, Found: 549.3402.

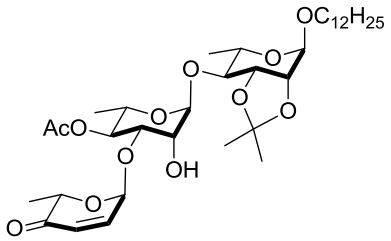
(15):



To a solution of allylic ester F (9.22 g, 17.5 mmol) in t-butanol/acetone (1:1, 35 mL) at 0 °C was added a solution of N-methyl morpholine N-oxide/water (50% w/v) (12 mL) and OsO_4 (22.4 mg, 0.168 mmol). The reaction mixture was stirred at rt for 12 h and then concentrated. The residue was pipetted directly on to a silica gel column using a small amount of CH_3OH in three portions. Impurities were eluted with ether and the product was eluted with 90% EtOAc/Hexane. Pure fractions were combined

and concentrated to afford diol 15 (9.02 g, 92%): Rf (50% EtOAc/hexane) = 0.32; $[\alpha]^{25}_{D}$ = - 76.20 (c = 0.80, CH_2Cl_2); IR (thin film, cm^{-1}) 3339, 2925, 2855, 1741, 1455, 1378, 1140, 1240, 1046, 1023, 994; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.36 (d, J = 1.2 Hz, 1H), 4.91 (s, 1H), 4.81 (dd, J = 9.6, 9.6 Hz, 1H), 4.14 (dd, J = 7.2, 5.4 Hz, 1H), 4.05 (d, J = 5.4 Hz, 1H), 3.93 (dd, J = 3.0, 1.8 Hz, 1H), 3.79-3.74 (m, 2H), 3.65-3.60 (m, 2H), 3.47 (dd, J = 10.2, 7.2 Hz, 1H), 3.38 (ddd, J = 9.6, 6.6, 6.6 Hz, 1H), 2.86 (s, br, 2H), 2.09 (s, 3H), 1.55-1.52 (m, 2H), 1.50 (s, 3H), 1.30 (s, 3H), 1.26-1.23 (m, 18H), 1.24 (d, J = 6.6 Hz, 3H), 1.17 (d, J = 6.0 Hz, 3H), 0.85 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.8, 109.4, 98.1, 96.8, 78.4, 77.5, 76.2, 75.1, 71.2, 70.0, 67.7, 66.3, 63.8, 31.9, 29.6, 29.6, 29.5, 29.4, 29.3, 27.9, 26.3, 26.1, 22.6, 21.0,18.0, 17.3, 14.1; HRMS (ESI) calcd for $[C_{29}H_{52}O_{10} + Na]^+$: 583.3453, Found: 583.3456.

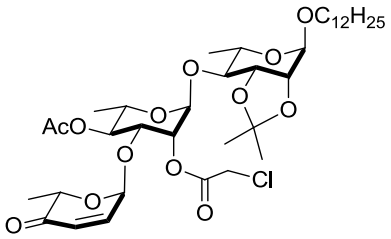
(16):



To a solution of diol 15 (7.80 g, 13.9 mmol) in methanol (14 mL) was added dibutyl tin oxide (3.81 g, 15.3 mmol). The solution was heated to reflux until the tin oxide dissolved. The solvent was then removed under vacuum. The residue was dissolved in dry CH_2Cl_2 (28 mL). To the solution was added Boc-pyranone (3.50 g, 15.3 mmol), $Pd_2(dba)_3$•$CHCl_3$ (70 mg, 67.6 μmol) and PPh_3 (70 mg, 270 μmol) at 0 ^oC under argon

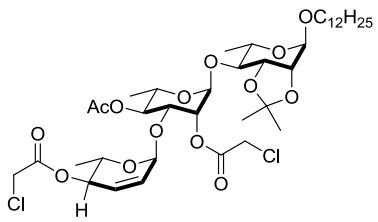
atmosphere. After stirring for 2 h from 0 ^{o}C to room temperature, the reaction mixture was quenched with 50 mL of saturated $NaHCO_3$, extracted (3 x 100 mL) with Et_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified using silica gel flash chromatography eluting with 5~20% EtOAc/Hexane to give pyranone 16 (7.08 g, 76%): Rf (50% EtOAc/hexane) = 0.54; $[\alpha]^{25}_{D}$ = - 54.80 (c = 0.60, CH_2Cl_2); IR (thin film, cm^{-1}) 3474, 2925, 2855, 1747, 1702, 1456, 1376, 1225, 1087, 1043, 993; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.67 (dd, J = 10.2, 3.6 Hz, 1H), 6.08 (d, J = 10.2 Hz, 1H), 5.39 (d, J = 1.8 Hz, 1H), 5.31 (d, J = 3.0 Hz, 1H), 5.08 (dd, J = 9.6, 9.6 Hz, 1H), 4.93 (s, 1H), 4.58 (dd, J = 13.8, 6.6 Hz, 1H), 4.17 (dd, J = 7.2, 5.4 Hz, 1H), 4.07 (m, 2H), 4.00 (dd, J = 9.6, 3.6 Hz, 1H), 3.83-3.79 (m, 1H), 3.70-3.63 (m, 2H), 3.51 (dd, J = 9.6, 7.2 Hz, 1H), 3.40 (ddd, J = 9.6, 6.6, 6.6 Hz, 1H), 2.07 (s, 3H), 1.57-1.54 (m, 2H), 1.51 (s, 3H), 1.37 (d, J = 6.6 Hz, 3H), 1.33-1.24 (m, 18H), 1.30 (s, 3H), 1.27 (d, J = 6.0 Hz, 3H), 1.17 (d, J = 6.6 Hz, 3H), 0.85 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.1, 169.8, 142.2, 127.6, 109.5, 98.1, 96.9, 94.7, 78.4, 77.9, 77.5, 76.3, 72.8, 71.2, 70.8, 67.8, 66.7, 63.8, 31.9, 29.63, 29.60, 29.5, 29.41, 29.39, 29.3, 27.9, 26.4, 26.1, 22.6, 20.9, 18.0, 17.4, 15.2, 14.1; HRMS (ESI) calcd for $[C_{35}H_{58}O_{12} + Na]^{+}$: 693.3820, Found: 693.3824.

(17):



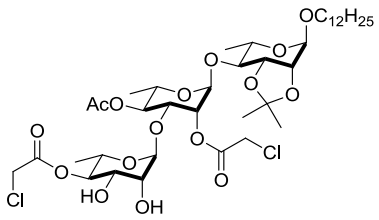
To a solution of the alcohol 16 (6.71 g, 10.0 mmol) in pyridine (30 mL) was added chloroacetic anhydride (5.13 g, 30 mmol) and a catalytic amount of 4-dimethylaminopyridine (30 mg). After stirring for 3 h, the mixture was diluted in ether, washed with saturated $CuSO_4$ solution (3 x 60 mL) and the solvent was evaporated. The crude product was purified by silica gel flash chromatography eluting with 5~15% EtOAc/Hexane to give chloro ester 17 (7.23 g, 97%) as a colorless oil: Rf (30% EtOAc/hexane) = 0.40; $[\alpha]^{25}_D$ = - 22.53 (c = 1.50, CH_2Cl_2); IR (thin film, cm^{-1}) 2928, 2855, 1751, 1702, 1376, 1225, 1138, 1086, 1044, 1004; ^1H NMR (600 MHz, $CDCl_3$) δ 6.58 (dd, J = 10.2, 3.6 Hz, 1H), 6.03 (d, J = 10.2 Hz, 1H), 5.38 (dd, J = 3.6, 1.8 Hz, 1H), 5.27 (d, J = 1.8 Hz, 1H), 5.25 (d, J = 3.6 Hz, 1H), 5.01 (dd, J = 9.6, 9.6 Hz, 1H), 4.92 (s, J = 1H), 4.51 (q, J = 6.6 Hz, 1H), 4.17 (dd, J = 7.2, 6.0 Hz, 1H), 4.13 (dd, J = 10.2, 3.6 Hz), 4.12 (s, 2H), 4.08 (d, J = 6.0 Hz, 1H), 3.84-3.79 (m, 1H), 3.72-3.67 (m,1H), 3.65 (ddd, J = 9.6, 6.6, 6.6 Hz, 1H), 3.45 (dd, J = 9.6, 7.2 Hz, 1H), 3.40 (ddd, J = 9.6, 6.6, 6.6 Hz), 2.08 (s, 3H), 1.58-1.53 (m, 2H), 1.49 (s, 3H), 1.35 (d, J = 7.2 Hz, 3H), 1.32-1.23 (m, 18H), 1.30 (s, 3H), 1.27 (d, J = 6.6 Hz, 3H), 1.18 (d, J = 6.6 Hz, 3H), 0.85 (t, J = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.2, 169.6, 166.7, 141.6, 127.6, 109.5, 96.9, 95.8, 95.2, 78.1, 77.9, 76.2, 75.1, 73.3, 72.8, 70.7, 67.8, 67.1, 63.6, 40.8, 31.9, 29.6, 29.6, 29.5, 29.4, 29.3, 27.9 , 26.3, 26.1, 22.6, 20.8, 18.0, 17.4, 14.9, 14.1; HRMS (ESI) calcd for $[C_{37}H_{59}ClO_{13} + Na]^+$: 769.3536, Found: 769.3541.

(G):



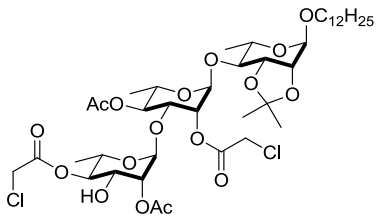
77% as a gummy solid: R_f (30% EtOAc/hexane) = 0.46; $[\alpha]^{25}_D$ = - 52.28 (*c* = 0.75, CH_2Cl_2); IR (thin film, cm^{-1}) 2925, 2855, 1750, 1379, 1224, 1085, 1047, 997; ^1H NMR (600 MHz, $CDCl_3$) δ 5.82 (d, *J* = 10.2 Hz, 1H), 5.64 (ddd, *J* = 10.8, 2.4, 2.4 Hz, 1H), 5.33 (dd, *J* = 3.0, 1.8 Hz, 1H), 5.27 (d, *J* = 1.8 Hz, 1H), 5.06-5.05 (m, 1H), 5.02 (brs, 1H), 5.00 (dd, *J* = 9.6, 9.6 Hz, 1H), 4.92 (s, 1H), 4.20 (d, *J* = 15.0 Hz, 1H), 4.17 (d, *J* = 15.0 Hz, 1H), 4.16 (m, 1H), 4.09 (d, *J* = 15.0 Hz, 1H), 4.08 (d, *J* = 5.4 Hz, 1H), 4.06 (d, *J* = 15.0 Hz, 1H), 4.05 (d, *J* = 15.0 Hz, 1H), 4.04 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.89-3.84 (m, 1H), 3.82-3.77 (m, 1H), 3.72-3.63 (m, 2H), 3.46 (dd, *J* = 9.6, 7.2 Hz, 1H), 3.40 (ddd, *J* = 9.6, 6.6, 6.6 Hz, 1H), 2.06 (s, 3H), 1.57-1.55 (m, 2H), 1.49 (s, 3H), 1.30 (s, 3H), 1.34-1.24 (m, 18H), 1.27 (d, *J* = 6.0 Hz, 3H), 1.22 (d, *J* = 6.6 Hz, 3H), 1.17 (d, *J* = 6.0 Hz, 3H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.6,167.0, 166.7, 129.5, 127.3, 109.5, 96.9, 96.6, 95.8, 78.0, 77.9, 76.2, 75.0, 73.9 , 72.7, 72.4, 67.8, 67.1, 65.0, 63.6, 41.0, 40.8, 31.9, 29.64, 29.61, 29.5, 29.4, 29.3, 27.9, 26.3, 26.1, 22.6, 20.8, 18.0, 17.7, 17.4, 14.1; HRMS (ESI) calcd for $[C_{39}H_{62}Cl_2O_{14} + Na]^+$: 847.3409, Found: 847.3418.

(18):



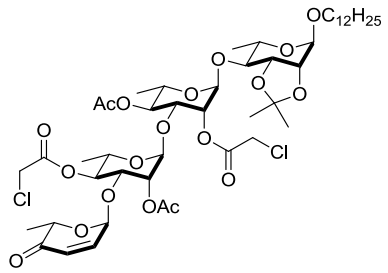
86% as a gummy solid: R_f (50% EtOAc/hexane) = 0.28; $[\alpha]^{25}_D$ = - 65.26 (*c* = 0.75, CH_2Cl_2); IR (thin film, cm^{-1}) 3481, 2925, 2855, 1765, 1736, 1379, 1240, 1052, 1033; ^1H NMR (600 MHz, $CDCl_3$) δ 5.28-5.27 (m, 2H), 5.02 (dd, *J* = 9.6, 9.6 Hz, 1H), 4.92 (s, 1H), 4.90 (s, 1H), 4.85 (dd, *J* = 9.6, 9.6 Hz, 1H), 4.18 (d, *J* = 15.0 Hz, 1H), 4.17 (m, 1H), 4.17 (d, *J* = 15.0 Hz, 1H), 4.14 (d, *J* = 15.0 Hz, 1H), 4.13 (d, *J* = 15.0 Hz, 1H), 4.10 (d, *J* = 15.0 Hz, 1H), 4.08 (d, *J* = 6.0 Hz, 1H), 4.06 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.86-3.82 (m, 1H), 3.80-3.76 (m, 1H), 3.70-3.63 (m, 2H), 3.45 (dd, *J* = 9.6, 7.2 Hz, 1H), 3.40 (ddd, *J* = 9.6, 6.6, 6.6 Hz, 1H), 2.75 (d, *J* = 6.6 Hz, 1H), 2.65 (d, *J* = 4.2 Hz, 1H), 2.07 (s, 3H), 1.58-1.54 (m, 2H), 1.50 (s, 3H), 1.30 (s, 3H), 1.27-1.24 (m, 18H), 1.26 (d, *J* = 6.6 Hz, 3H), 1.19 (d, *J* = 6.6 Hz, 3H), 1.17 (d, *J* = 6.0 Hz, 3H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.0, 168.1, 166.7, 109.6, 101.3, 96.8, 95.6, 78.1, 77.9, 76.5, 76.2, 74.4, 73.2, 72.8, 71.1, 69.4, 67.8, 67.0, 66.4, 63.6, 40.8, 40.7, 31.9, 29.62, 29.61, 29.5, 29.4, 29.3, 27.9, 26.3, 26.1, 22.6, 20.9, 18.0, 17.31, 17.29, 14.1; HRMS (ESI) calcd for $[C_{39}H_{64}Cl_2O_{16} + Na]^+$: 881.3464, Found: 881.3475.

(H):



91% as a gummy solid: R_f (50% EtOAc/hexane) = 0.52; $[\alpha]^{25}_D$ = - 40.36 (*c* = 1.10, CH_2Cl_2); IR (thin film, cm^{-1}) 3488, 2927, 2855, 1749, 1377, 1232, 1137, 1087, 1043, 989; ^1H NMR (600 MHz, $CDCl_3$) δ 5.26 (d, *J* = 1.8 Hz, 1H), 5.24 (dd, *J* = 3.6, 2.4 Hz, 1H), 5.04 (dd, *J* = 10.2, 9.6 Hz, 1H), 4.925 (s, 1H), 4.915 (s,1H), 4.88 (dd, *J* = 9.6, 9.6 Hz, 1H), 4.84 (dd, *J* = 3.6, 1.8 Hz, 1H), 4.18 (d, *J* = 15.0 Hz, 1H), 4.14 (d, *J* = 14.4 Hz, 1H), 4.13 (m, 2H), 4.07 (d, *J* = 5.4 Hz, 1H), 4.02 (dd, *J* = 10.2, 3.0 Hz, 1H), 3.90-3.84 (m, 2H), 3.79-3.74 (m, 1H), 3.68-3.62 (m, 2H), 3.44 (dd, *J* = 9.6, 7.2 Hz, 1H), 3.41 (ddd, *J* = 9.6, 6.6, 6.6 Hz, 1H), 2.13 (s, 3H), 2.12(s, 3H), 2.01 (d, *J* = 9.0 Hz, 1H), 1.58-1.53 (m, 2H), 1.49 (s, 3H), 1.30 (s, 3H), 1.28-1.24 (m, 18H), 1.25 (d, *J* = 6.0 Hz, 3H), 1.20 (d, *J* = 6.0 Hz, 3H), 1.17 (d, *J* = 6.6 Hz, 3H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.25, 170.20, 167.7, 166.8, 109.5, 99.0, 96.8, 95.7, 78.1, 77.9, 76.2, 76.0, 74.8, 73.2, 72.8, 72.2, 67.8, 67.7, 67.2, 66.5, 63.6, 40.8, 40.7, 31.9, 29.62, 29.60, 29.5, 29.4, 29.3, 27.9, 26.3, 26.1, 22.6, 20.9, 20.8, 18.0, 17.3, 17.2, 14.1; HRMS (ESI) calcd for $[C_{41}H_{66}Cl_2O_{17} + Na]^+$: 923.3569, Found: 923.3582.

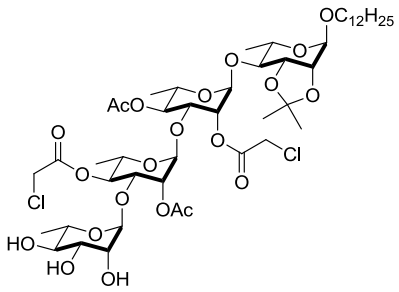
(I):



83% as a gummy solid: R_f (30% EtOAc/hexane) = 0.38; $[\alpha]^{25}_D$ = - 38.52 (*c* = 1.20, CH_2Cl_2); IR (thin film, cm^{-1}) 2927, 2856, 1747, 1703, 1376, 1229, 1137, 1083, 1041, 1008; ^1H NMR (600 MHz, $CDCl_3$) δ 6.68 (dd, J = 10.2, 3.6 Hz, 1H), 6.03 (d, *J* = 10.2 Hz, 1H), 5.30 (dd, *J* = 3.0, 1.8 Hz, 1H), 5.28 (d, *J* = 1.8 Hz, 1H), 5.25 (d, *J* = 3.0 Hz, 1H), 5.08-5.03 (m, 3H), 4.92 (s, 1H), 4.88 (d, *J* = 1.8 Hz, 1H), 4.35 (ddd, *J* = 7.2, 7.2, 6.6 Hz, 1H), 4.22 (d, *J* = 15.0 Hz, 1H), 4.17 (d, *J* = 15.0 Hz, 1H), 4.16 (dd, *J* = 6.0, 5.4 Hz, 1H), 4.07 (d, *J* = 5.4 Hz, 1H), 4.05-4.02 (m, 3H), 4.02 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.93-3.88 (m, 1H), 3.81-3.76 (m, 1H), 3.69-3.62 (m, 2H), 3.45 (dd, *J* = 9.6, 7.2 Hz, 1H), 3.42 (ddd, *J* = 9.6, 6.6, 6.6 Hz, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 1.58-1.53 (m, 2H), 1.50 (s, 3H), 1.32 (d, *J* = 7.2 Hz, 3H), 1.30 (s, 3H), 1.27-1.24 (m, 18H), 1.25 (d, *J* = 6.0 Hz, 3H), 1.20 (d, *J* = 6.0 Hz, 1H), 1.18 (d, *J* = 6.0 Hz, 6H), 0.85 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 196.3, 170.3, 170.1, 166.9, 166.4, 141.9, 127.6, 109.5, 99.0, 96.8, 77.9, 76.2, 75.1, 75.0, 74.0, 73.1, 72.2, 71.9, 70.6, 67.8, 67.2, 67.1, 63.6, 40.8, 40.5, 31.9, 29.62, 29.60, 29.5, 29.4, 29.3, 27.9, 26.3, 26.1, 22.6, 20.9, 20.7,

18.0, 17.4, 17.3, 14.7, 14.1; HRMS (ESI) calcd for $[C_{47}H_{72}Cl_2O_{19} + Na]^+$: 1033.3937, Found: 1033.3947.

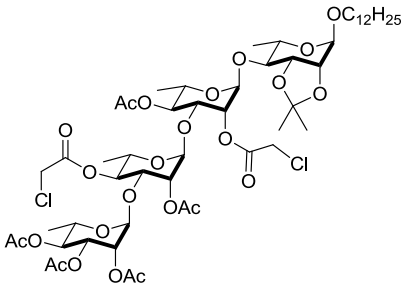
(20):



76% as a gummy solid: R_f (100% EtOAc/hexane) = 0.38; $[\alpha]^{25}_D$ = - 48.54 (*c* = 1.10, MeOH); IR (thin film, cm^{-1}) 3428, 2926, 2855, 1748, 1230, 1138, 1087, 1046, 988; ^1H NMR (600 MHz, $CDCl_3$) δ 5.274-5.267 (m, 2H), 5.05 (dd, *J* = 4.8, 4.2 Hz, 1H), 5.02 (dd, *J* = 4.8, 4.2 Hz, 1H), 4.93-4.92 (m, 2H), 4.88 (s, 1H), 4.83 (s, 1H), 4.23 (d, *J* = 15.0 Hz, 1H), 4.17 (d, *J* = 15.0 Hz, 1H), 4.15 (dd, *J* = 6.6, 6.0 Hz, 1H), 4.07 (d, *J* = 5.4 Hz, 1H), 4.06 (s, 2H), 4.01 (dd, *J* = 10.2, 3.0 Hz, 1H), 3.95 (dd, *J* = 10.2, 3.0 Hz, 1H), 3.88-3.84 (m, 1H), 3.79-3.75 (m, 2H), 3.68-3.62 (m, 2H), 3.61 (dd, *J* = 9.6, 3.0 Hz, 1H), 3.52-3.47 (m, 1H), 3.45 (dd, *J* = 9.6, 7.2 Hz, 1H), 3.42-3.38 (m, 2H), 2.12 (s, 3H), 2.11 (s, 3H), 1.58-1.53 (m, 2H), 1.49 (s, 3H), 1.30 (s, 3H), 1.32-1.24 (m, 18H), 1.25 (d, *J* = 6.6 Hz, 3H), 1.21 (d, *J* = 6.0 Hz, 3H), 1.18 (d, *J* = 6.0 Hz, 3H), 1.17 (d, *J* = 6.0 Hz, 3H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.4, 170.3, 166.9, 166.7, 109.6, 102.0, 98.9, 96.8, 95.6, 78.0, 77.9, 76.2, 75.2, 74.6, 74.2, 73.1, 72.9, 72.2, 71.9, 71.4, 70.8, 68.9, 67.8, 67.1, 67.0, 63.6, 40.8, 40.6, 31.9, 29.63, 29.61,

29.5, 29.4, 29.3, 27.9, 26.3, 26.1, 22.7, 20.9, 20.8, 18.0, 17.4, 17.3, 17.1, 14.1; HRMS (ESI) calcd for $[C_{47}H_{76}Cl_2O_{21} + Na]^+$: 1069.4148, Found: 1069.4164.

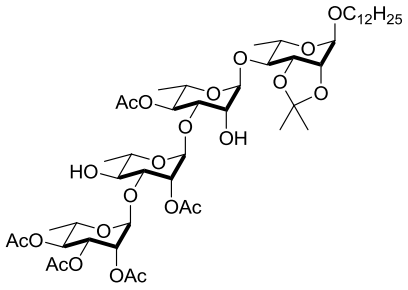
(21):



95.0 % as a colorless liquid: R_f (33% EtOAc/Hexane) = 0.29; $[\alpha]^{25}_D$ = - 35.73 (*c* = 1.75, $CHCl_3$); IR (thin film, cm^{-1}) 2926, 2855, 1748, 1371, 1223, 1139, 1087, 1046, 989; ^1H NMR (600 MHz, $CDCl_3$) δ 5.29 (s, 1H), 5.28 (dd, *J* = 4.8, 1.8 Hz, 1H), 5.12 (dd, *J* = 10.2, 3.6 Hz, 1H), 5.10 (dd, *J* = 10.2, 9.6 Hz, 1H), 5.05 (dd, *J* = 10.2, 9.6 Hz, 1H), 5.02 (dd, *J* = 10.2, 10.2 Hz, 1H), 5.00 (dd, *J* = 3.0, 1.8 Hz, 1H), 4.96 (dd, *J* = 3.6, 1.8 Hz, 1H), 4.93 (s, 1H), 4.88 (d, *J* = 1.8 Hz, 1H), 4.84 (d, *J* = 1.8 Hz, 1H), 4.26 (d, *J* = 14.4 Hz, 1H), 4.19 (d, *J* = 14.4 Hz, 1H), 4.17 (dd, *J* = 10.8, 6.0 Hz, 1H), 4.15 (d, *J* = 15 Hz, 1H), 4.14 (d, *J* = 15 Hz, 1H), 4.09 (d, *J* = 6.0 Hz, 1H), 4.03 (dd, *J* = 9.6, 3.0 Hz, 1H), 3.97 (dd, *J* = 9.6, 3.0 Hz, 1H), 3.87 (dq, *J* = 10.2, 6.6 Hz, 1H), 3.79 (dq, *J* = 9.6, 6.0 Hz, 1H), 3.78 (dq, *J* = 9.6, 6.0 Hz, 1H), 3.68 (dq, *J* = 8.6, 6.0 Hz, 1H), 3.66 (ddd, *J* = 9.6, 7.2, 6.6 Hz, 1H), 3.47 (dd, *J* = 10.2, 7.2 Hz, 1H), 3.41 (ddd, *J* = 9.6, 6.6, 6.6 Hz, 1H), 2.18 (s, 3H), 2.12 (s, 6H), 2.03 (s, 3H), 1.97 (s, 3H), 1.60 – 1.55 (m, 2H), 1.51 (s, 3H), 1.32 (s, 3H), 1.34 – 1.25 (m, 18H), 1.26 (d, *J* = 6.0 Hz, 3H), 1.21 (d, *J* =

6.0 Hz, 3H), 1.19 (d, J = 6.6 Hz, 3H), 1.17 (d, J = 6.6 Hz, 3H), 0.88 (dd, J = 7.2, 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.4, 170.2, 170.0 (2C), 169.7, 166.9, 166.8, 109.6, 99.1, 98.8, 96.8, 95.6, 78.0, 77.9, 76.2, 74.9, 74.3, 73.8, 73.1, 72.3, 71.4, 70.8, 70.1, 68.5, 67.8, 67.3 (2C), 67.1, 63.6, 40.7 (2C), 31.9, 29.6 (3C), 29.5, 29.4 (2C), 29.3, 27.9, 26.3, 26.1, 22.7, 20.9, 20.8 (2C), 20.7 (2C), 18.0, 17.3 (2C), 17.1, 14.1; HRMS (ESI): calcd. for $[C_{53}H_{82}Cl_2O_{24} + Na]^+$: 1195.44653, Found: 1195.44626.

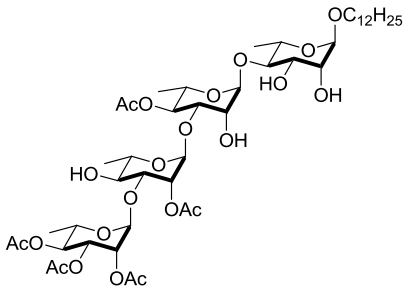
(J):



96% as a white gummy solid: R_f (50% EtOAc/hexane) = 0.43; $[\alpha]^{25}_D$ = - 48.07 (c = 1.68, $CHCl_3$); IR (thin film, cm^{-1}) 3447 (brs), 2926, 2855, 1745, 1372, 1222, 1138, 1082, 1045, 991; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.37 (d, J = 1.2 Hz, 1H), 5.32 (dd, J = 3.0, 1.2 Hz, 1H), 5.17 (dd, J = 10.2, 3.6 Hz, 1H), 5.08 (dd, J = 10.2, 9.6 Hz, 1H), 5.03 (dd, J = 10.2, 9.6 Hz, 1H), 4.97 (dd, J = 3.6, 1.8 Hz, 1H), 4.96 (d, J = 1.8 Hz, 1H), 4.93 (s, 1H), 4.87 (d, J = 1.8 Hz, 1H), 4.14 (dd, J = 7.2, 5.4 Hz, 1H), 4.08 (d, J = 5.4 Hz, 1H), 3.98 (dd, J = 2.4, 1.8 Hz, 1H), 3.91 (dd, J = 9.6, 3.6 Hz, 1H), 3.83 (dd, J = 9.6, 3.0 Hz, 1H), 3.81-3.75 (m, 3H), 3.66 (ddd, J = 9.6, 6.6, 6.0 Hz, 1H), 3.65 (dq, J = 9.6, 6.0 Hz, 1H), 3.62 (dd, J = 9.6, 9.0 Hz, 1H), 3.49 (dd, J = 10.2, 7.2 Hz, 1H),

3.41 (ddd, *J* = 9.6, 6.6, 6.6 Hz, 1H), 2.96 (s, 1H) 2.82 (s, 1H), 2.16 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.60 – 1.55 (m, 2H), 1.52 (s, 3H), 1.31 (s, 3H), 1.34 – 1.25 (m, 18H), 1.34 (d, *J* = 6.0 Hz, 3H), 1.26 (d, *J* = 6.0 Hz, 3H), 1.18 (d, *J* = 6.6 Hz, 3H), 1.17 (d, *J* = 6.0 Hz, 3H), 0.87 (dd, *J* = 7.2, 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.0, 170.6 (2C), 170.4, 170.1, 109.7, 99.7, 99.5, 98.3, 97.1, 78.7, 78.5, 77.9, 76.5, 72.4, 72.2, 72.1, 71.3, 70.9, 70.0, 69.4, 69.2, 68.0, 67.4, 67.1, 64.0, 33.1, 29.8 (3C), 29.7, 29.6 (2C), 29.5, 28.1, 26.6, 26.3, 22.9, 21.1(2C), 21.0 (3C), 18.2, 17.9, 17.6, 17.3, 14.3; HRMS (ESI): calcd. for $[C_{49}H_{80}O_{22} + Na]^+$: 1043.50334, Found: 1043.50367.

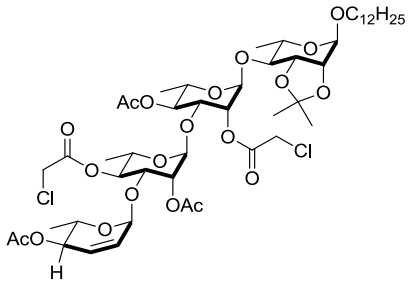
Cleistetroside-New2 (10):



97.0% as a gummy solid: R_f (20% MeOH/EtOAc) = 0.77; $[\alpha]^{25}_D$ = - 63.92 (*c* = 0.96, $CHCl_3$); IR (thin film, cm^{-1}) 3489 (brs), 2924, 2852, 1747, 1372, 1229, 1138, 1046, 990; ^{1}H NMR (600 MHz, CD_3OD) δ 5.36 (dd, *J* = 3.6, 1.8 Hz, 1H), 5.23 (d, *J* = 1.8 Hz, 1H), 5.14 (dd, *J* = 10.2, 3.6 Hz, 1H), 5.11 (dd, *J* = 9.6, 9.6 Hz, 1H), 5.08 (d, *J* = 1.8 Hz, 1H), 5.05 (dd, *J* = 3.6, 1.8 Hz, 1H), 4.99 (dd, *J* = 10.2, 9.6 Hz, 1H), 4.84 (d, *J* = 1.8 Hz, 1H), 4.64 (d, *J* = 1.8 Hz, 1H), 4.08 (dd, *J* = 3.0, 1.8 Hz, 1H), 4.05 (dd, *J* = 9.0, 3.0 Hz, 1H), 3.91-3.86 (m, 3H), 3.86 (dq, *J* = 10.2, 6.6 Hz, 1H), 3.76 (dd, *J* = 9.6,

3.6 Hz, 1H), 3.73 (dd, *J* = 3.6, 1.8 Hz, 1H), 3.67 (ddd, *J* = 9.6, 6.6, 6.6 Hz, 1H), 3.64 (dq, *J* = 9.6, 6.0 Hz, 1H), 3.52 (dd, *J* = 9.6, 8.4 Hz, 1H), 3.51 (dd, *J* = 9.6, 9.6 Hz, 1H), 3.40 (ddd, *J* = 10.2, 6.6, 6.0 Hz, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 1.95 (s, 3H), 1.64 – 1.54 (m, 2H), 1.33 – 1.28 (m, 21H), 1.28 (d, *J* = 6.0 Hz, 3H), 1.16 (d, *J* = 6.0 Hz, 3H), 1.15 (d, *J* = 6.0 Hz, 3H), 0.91 (dd, *J* = 7.2, 6.6 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 172.2 (2C), 171.9 (2C), 171.8, 103.2, 101.6, 101.1, 100.9, 81.4, 79.0, 77.5, 74.1, 73.6, 73.5, 73.4, 73.0, 72.3, 72.2, 71.1, 70.9, 70.5, 68.7 (2C), 68.4, 68.2, 33.2, 30.9 (3C), 30.8, 30.7, 30.6 (2C), 27.5, 23.9, 21.2, 21.0, 20.8 (3C), 18.9, 18.0, 17.8, 17.7, 14.6; HRMS (ESI): calcd. for $[C_{46}H_{76}O_{22} + Na]^+$: 1003.47204, Found: 1003.47190.

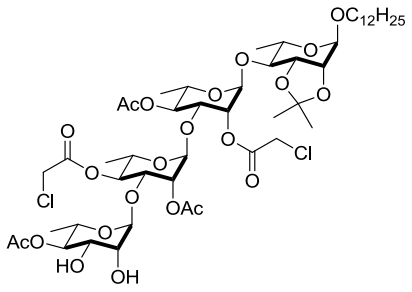
(K):



78% as a gummy solid: R_f (30% EtOAc/hexane) = 0.44; $[\alpha]^{25}_D$ = - 48.59 (*c* = 1.10, CH_2Cl_2); IR (thin film, cm^{-1}) 2926, 2856, 1745, 1375, 1230, 1137, 1085, 1039; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.80 (d, *J* = 10.2 Hz, 1H), 5.66 (ddd, *J* = 10.2, 2.4, 2.4 Hz, 1H), 5.30 (dd, *J* = 3.0, 1.8 Hz, 1H), 5.28 (d, *J* = 1.2 Hz, 1H), 5.08-5.01 (m, 4H), 4.99 (dd, *J* = 9.6, 1.2 Hz, 1H), 4.92 (s, 1H), 4.88 (d, *J* = 1.8 Hz, 1H), 4.22 (d, *J* = 15.0 Hz, 1H), 4.17 (d, *J* = 15.0 Hz, 1H), 4.16 (dd, *J* = 6.0, 5.4 Hz, 1H), 4.07 (d, *J* = 5.4 Hz, 1H),

4.03-4.01 (m, 3H), 3.98 (dd, *J* = 10.2, 3.0 Hz, 1H), 3.91-3.86 (m, 1H), 3.81-3.76 (m, 1H), 3.75-3.71 (m, 1H), 3.69-3.63 (m, 2H), 3.45 (dd, *J* = 9.6, 7.2 Hz, 1H), 3.42 (ddd, *J* = 10.2, 6.6, 6.6 Hz, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 1.58-1.54 (m, 2H), 1.50 (s, 3H), 1.30 (s, 3H), 1.29-1.25 (m, 18H), 1.26 (d, *J* = 6.0 Hz, 3H), 1.19 (d, *J* = 6.0 Hz, 3H), 1.18 (d, *J* = 6.0 Hz, 3H), 1.16 (d, *J* = 6.0 Hz, 6H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.43, 170.39, 170.0, 166.9, 166.5, 130.4, 126.7, 109.5, 98.9, 96.8, 95.6, 77.94, 77.89, 76.2, 74.8, 74.5, 74.2, 73.1, 72.5, 72.2, 70.6, 67.8, 67.2, 67.1, 65.1, 63.6, 31.9, 29.62, 29.60, 29.5, 29.4, 29.3, 27.9, 26.3, 26.1, 22.6, 21.0, 20.9, 20.7, 18.0, 17.52, 17.47, 17.3, 14.1; HRMS (ESI) calcd for $[C_{49}H_{76}Cl_2O_{20} + Na]^+$: 1077.4199, Found: 1077.4210.

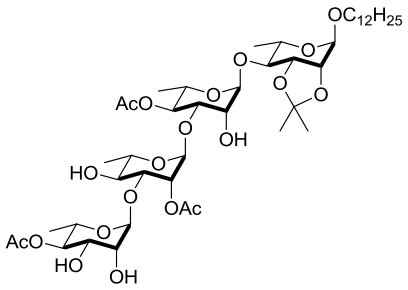
(19):



80% as a gummy solid: R_f (50% EtOAc/hexane) = 0.19; $[\alpha]^{25}_D$ = - 38.66 (*c* = 1.50, MeOH); IR (thin film, cm^{-1}) 3470, 2926, 2855, 1745, 1376, 1231, 1137, 1085, 1037, 989; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.27-5.26 (m, 2H), 5.05 (dd, *J* = 9.6, 3.0 Hz, 1H), 5.04 (dd, *J* = 10.2, 3.0 Hz, 1H), 4.92 (dd, *J* = 3.0, 1.8 Hz, 1H), 4.92 (s, 1H), 4.87 (d, *J* = 1.8 Hz, 1H), 4.75 (dd, *J* = 9.6, 9.6 Hz, 1H), 4.21 (d, *J* = 15.0 Hz, 1H), 4.16 (d, *J* =

15.0 Hz, 1H), 4.15 (dd, *J* = 7.2, 6.0 Hz, 1H), 4.07 (d, *J* = 5.4 Hz, 1H), 4.04-4.03 (m, 2H), 4.02 (dd, *J* = 10.2, 3.0 Hz, 1H), 3.96 (dd, *J* = 10.2, 3.0 Hz, 1H), 3.89-3.84 (m, 1H), 3.81-3.75 (m, 2H), 3.98 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.68-3.60 (m, 3H), 3.44 (dd, *J* = 9.6, 7.2 Hz, 1H), 3.41 (ddd, *J* = 10.2, 6.6, 6.6 Hz, 1H), 2.91 (brs, 1H), 2.65 (brs, 1H), 2.12 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 1.58-1.53 (m, 2H), 1.49 (s, 3H), 1.30 (s, 3H), 1.34-1.24 (m, 18H), 1.25 (d, *J* = 6.6 Hz, 3H), 1.18 (d, *J* = 6.0 Hz, 3H), 1.17 (d, *J* = 6.0 Hz, 3H), 1.13 (d, *J* = 6.0 Hz, 3H), 0.85 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.0, 170.2, 170.1, 166.9, 166.6, 109.6, 101.7, 98.8, 96.8, 95.6, 78.0, 77.9, 76.2, 75.0, 74.7, 74.0, 73.1, 72.2, 71.8, 70.8, 70.0, 67.8, 67.1, 67.0, 66.6, 63.6, 40.7, 40.5, 31.9, 29.62, 29.60, 29.5, 29.4, 29.3, 27.9, 26.3, 26.1, 22.6, 21.0, 20.9, 20.7, 18.0, 17.4, 17.3, 17.0, 14.1; HRMS (ESI) calcd for $[C_{49}H_{78}Cl_2O_{22} + Na]^+$: 1111.4254, Found: 1111.4264.

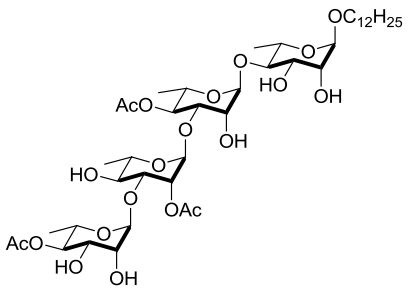
(L):



87.0 % as a white gummy solid: R_f (75% EtOAc/hexane) = 0.20; $[\alpha]^{25}_D$ = - 54.58 (*c* = 1.28, $CHCl_3$); IR (thin film, cm^{-1}) 3454 (brs), 2925, 2855, 1740, 1453, 1374, 1235, 1137, 1078, 1041, 992, 917, 861, 732; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.35 (d, *J* = 0.6

Hz, 1H), 5.08 (s, 1H), 5.05 (dd, *J* = 10.2, 9.6 Hz, 1H), 4.94 (dd, *J* = 3.0, 1.8 Hz, 1H), 4.93 (s, 1H), 4.85 (d, *J* = 1.2 Hz, 1H), 4.84 (dd, *J* = 9.6, 9.6 Hz, 1H), 4.15 (dd, *J* = 7.2, 6.0 Hz, 1H), 4.14-4.12 (m, 1H), 4.07 (d, *J* = 6.0 Hz, 1H), 4.04 (dd, *J* = 9.6, 3.0 Hz, 1H), 4.00 (dd, *J* = 2.4, 1.8 Hz, 1H), 3.86 (dq, *J* = 9.6, 6.0 Hz, 1H), 3.81 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.81 (dd, *J* = 9.6, 3.0 Hz, 1H), 3.79 (dq, *J* = 9.6, 6.0 Hz, 1H), 3.69 (dq, *J* = 9.6, 6.0 Hz, 1H), 3.66 (ddd, *J* = 9.6, 7.2, 6.6 Hz, 1H), 3.65 (dq, *J* = 10.2, 6.6 Hz, 1H), 3.57 (dd, *J* = 9.6, 9.0 Hz, 1H), 3.48 (dd, *J* = 10.2, 7.2 Hz, 1H), 3.41 (ddd, *J* = 9.6, 6.6, 6.6 Hz, 1H), 2.16 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 1.60 – 1.55 (m, 2H), 1.52 (s, 3H), 1.32 (s, 3H), 1.33 – 1.25 (m, 24H), 1.18 (d, *J* = 6.6 Hz, 3H), 1.15 (d, *J* = 6.0 Hz, 3H), 0.88 (dd, *J* = 7.2, 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.9, 171.3, 170.7, 109.6, 102.5, 99.5, 98.4, 97.1, 78.8, 78.7, 77.8 (2C), 76.5, 74.7, 72.5, 72.3, 72.1, 71.3, 70.9, 69.8, 69.3, 68.0, 67.2, 67.1, 64.0, 32.1, 29.8 (3C), 29.7.7, 29.6 (2C), 29.5, 28.1, 26.6, 26.3, 22.9, 21.3, 21.1, 21.0, 18.3, 17.9, 17.5, 17.3, 14.3; HRMS (ESI): calcd. for $[C_{45}H_{76}O_{20} + Na]^+$: 959.48222, Found: 959.48279.

Cleistetroside-New 1 (9):



95.0% as a gummy solid: R_f (17% MeOH/EtOAc) = 0.33; $[\alpha]^{25}_D$ = - 61.38 (*c* = 0.75, $CHCl_3$); IR (thin film, cm^{-1}) 3458 (brs), 2925, 2860, 1742, 1376, 1238, 1135, 1079, 1043, 990; ^1H NMR (600 MHz, CD_3OD) δ 5.23 (d, *J* = 1.8 Hz, 1H), 5.12 (dd, *J* = 10.2, 9.6 Hz, 1H), 5.01 (dd, *J* = 3.6, 1.8 Hz, 1H), 5.00 (d, *J* = 1.2 Hz, 1H), 4.90 (dd, *J* = 10.2, 9.6 Hz, 1H), 4.83 (d, *J* = 1.8 Hz, 1H), 4.65 (d, *J* = 1.2 Hz, 1H), 4.07 (dd, *J* = 3.0, 1.8 Hz, 1H), 4.01 (dd, *J* = 9.6, 3.0 Hz, 1H), 3.96 (dd, *J* = 3.6, 1.8 Hz, 1H), 3.88 (dq, *J* = 9.0, 6.0 Hz, 1H), 3.87 (dd, *J* = 10.2, 3.0 Hz, 1H), 3.86 (dq, *J* = 9.0, 6.0 Hz, 1H), 3.76 (dd, *J* = 9.0, 3.6 Hz, 1H), 3.73 (dd, *J* = 9.6, 3.0 Hz, 1H), 3.73 (dd, *J* = 3.6, 1.8 Hz, 1H), 3.68 (dq, *J* = 10.2, 6.6 Hz, 1H), 3.67 (ddd, *J* = 9.6, 6.6, 6.6 Hz, 1H), 3.64 (dq, *J* = 9.6, 6.0 Hz, 1H), 3.52 (dd, *J* = 9.0, 9.0 Hz, 1H), 3.46 (dd, *J* = 10.2, 9.6 Hz, 1H), 3.40 (ddd, *J* = 9.6, 6.6, 6.0 Hz, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 1.63 – 1.54 (m, 2H), 1.39 – 1.25 (m, 18H), 1.28 (d, *J* = 6.0 Hz, 6H), 1.14 (d, *J* = 6.0 Hz, 3H), 1.11 (d, *J* = 6.6 Hz, 3H), 0.91 (dd, *J* = 7.2, 6.6 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 172.7, 172.2, 172.1, 104.1, 103.1, 101.6, 100.9, 81.4, 78.8, 77.8, 75.7, 74.0, 73.9, 73.5, 73.4, 73.0, 72.3 (2C), 70.6, 70.5, 68.7 (2C), 68.4, 68.2, 33.2, 30.9 (3C), 30.8, 30.7, 30.6 (2C), 27.5, 23.9, 21.2 (2C), 21.0, 18.9, 18.1, 17.8 (2C), 14.3; HRMS (ESI): calcd. for $[C_{42}H_{72}O_{20} + Na]^+$: 919.45092, Found: 919.45128.

Determination of the linkage of the Ac group in Cleistetrosides

The regio- and stereo-chemical structural assignments of all eight oligosaccharides naturally occurring oligosaccharides were made by comparison of the spectral data to

that reported by Seidel and Hu. To establish the regio- and stereo-chemistry of the two new oligosaccharides (9 and 10) a detail NMR analysis was carried out for oligosaccharide 9 (*vide infra*) and the stereochemistry of 10 was determined by analogy. Finally structural confirmation for all ten oligosaccharides (1-10) was obtained by the detailed analysis of oligosaccharides 7 and 9 in combination with knowledge of the synthetic sequence for all ten oligosaccharides (i.e., proof of the routes regio- and stereoselectivity of each branching points).

Examination of the ^{1}H NMR spectra of cleistetrosides show similar splitting patterns for the protons of each of the A-D sugar moieties and also for the side chain aliphatic dodecyl groups. Each of sugar moieties contains eight-spin systems. It should be noted that the ^{1}H and ^{13}C NMR spectra does not reveal a greater separation between the carbonyl frequencies nor the methyl hydrogen and carbon frequencies for the acyl groups. Take cleistetroside-New-1 (9) as example:

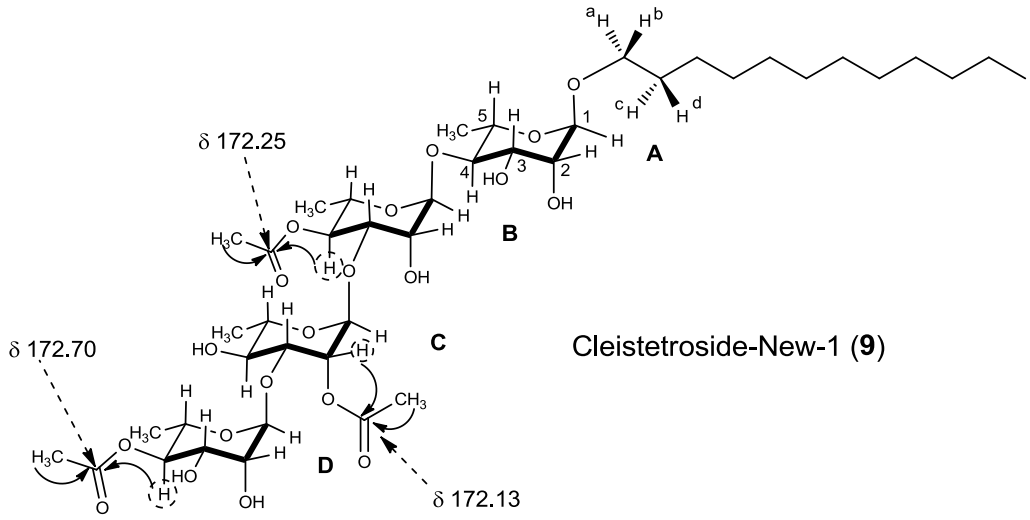


Figure S1. Structure of Cleistetroside-New-1 (9)

The ^{1}H NMR spectrum of cleistetroside-New1 (9) shows the presence of three singlets at 2.08, 2.09, and 2.12 ppm which are typical for the methyl of the Acyl groups, as well as the ^{13}C NMR spectrum displays three peaks at 172.13, 172.25, and 172.70 ppm, which are also characteristic for the carbonyl resonances for Acyl groups. The strategy for determination of the linkage positions of the three Acyl groups based on the following HMBC correlations: B-H4 → B-4-$CH_3\underline{COO}$ ($^{3}J_{HC}$) and B-4-$\underline{CH_3}COO$ → B-4-$CH_3\underline{COO}$ ($^{2}J_{HC}$); C-H2 → C-2-$CH_3\underline{COO}$ ($^{3}J_{HC}$) and C-2-$\underline{CH_3}COO$ → C-2-$CH_3\underline{COO}$ ($^{2}J_{HC}$); D-H4 → D-4-$CH_3\underline{COO}$ ($^{3}J_{HC}$) and D-4-$\underline{CH_3}COO$ → D-4-$CH_3\underline{COO}$ ($^{2}J_{HC}$).

In HMBC, observed three ($^{3}J_{HC}$) and two bond ($^{2}J_{HC}$) correlations, confirm the positions of the three Ac groups in cleistetroside-new-1. (Figure S2)

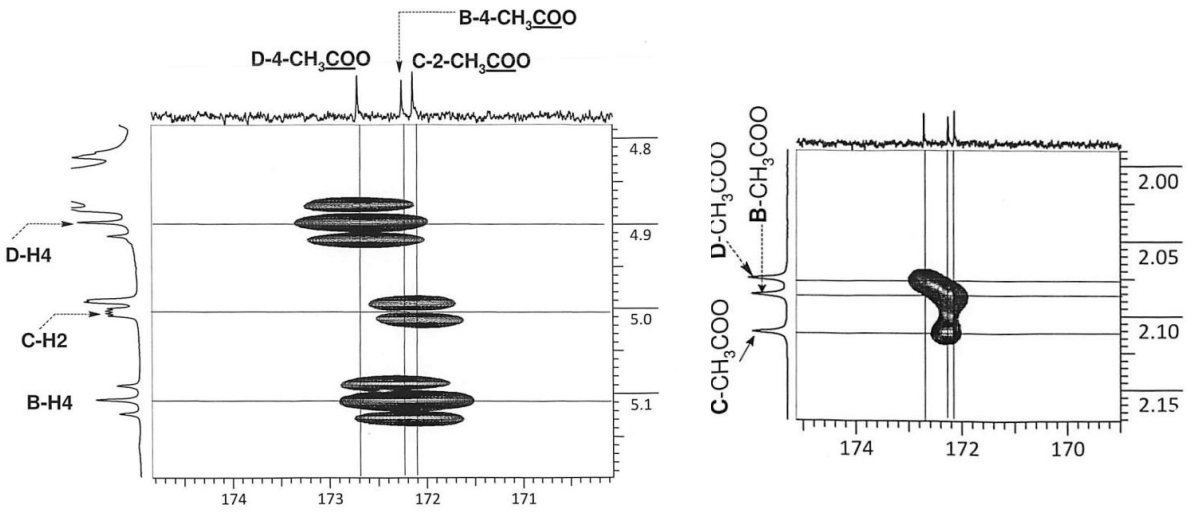


Figure S2. Chemical shifts of the carbonyls in cleistetroside-new-1 (9)

In the ^{1}H NMR spectrum of cleistetroside-3 the four acyl methyl's appear at 2.09, 2.13, 2.14, 2.15 ppm, the ^{13}C NMR spectrum of cleistetroside-3 shows four carbonyl resonances at 172.08, 172.29, 172.35, and 172.39 ppm and four acyl group methyl resonances at 20.95, 20.96, 21.06, and 21.19.

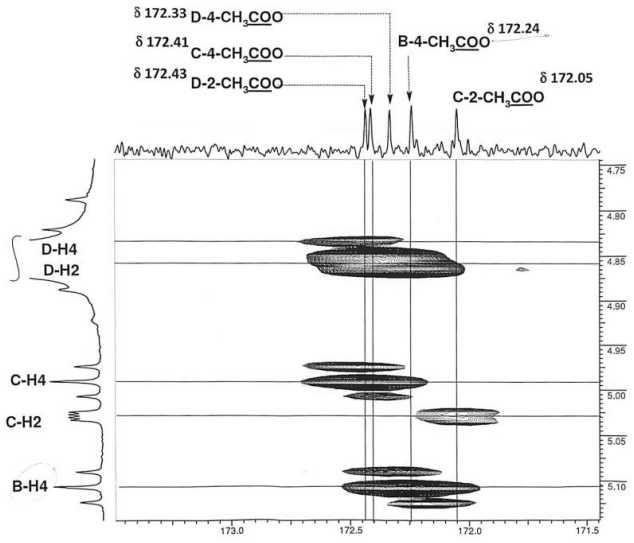


Figure S3. Chemical shifts of the carbonyls in cleistetroside-6 (7)

Whereas the ^{1}H NMR spectrum of cleistetroside-6 shows five acyl group methyl resonances at 2.09, 2.12, 2.12, 2.14, and 2.15 ppm. Similarly, whereas the ^{13}C NMR spectrum of cleistetroside-6 shows five carbonyl resonances at 172.05, 172.24, 172.33, 172.41 and 172.43 and also five acyl methyl resonances at 20.93, 20.94, 21.07, 21.08, and 21.18.

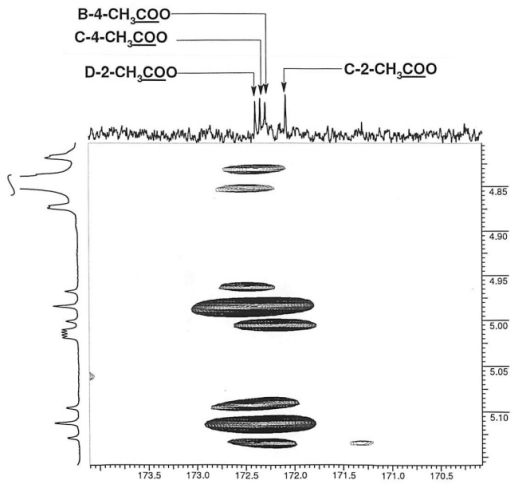


Figure S4. Chemical shifts of the carbonyls in cleistetroside-6 (7)

Section C: Antibacterial Activity MIC Assays[2]

The two strains of *E. coli* (MG1655 and BAS901 *imp*-4213, *zab*4292::Tn5)[3] bacterium were obtained from Prof. Kim Lewis, Department of Biology, Northeastern University. The *B. subtilis* strain (JH642, *trpC2 pheA1*)[4] was obtained from Prof. Alan D. Grossman, Department of Biology, Massachusetts Institute of Technology.

Preparation of inoculum: The Gram-(–) wild-type *E. coli* and Gram-(+) *B. subtilis* were cultured in liquid Luria Broth (LB) and Gram-(+)-like *imp* bacteria was cultured in liquid LB containing Kanamycin at 50 μg/mL. Strains were cultured overnight in liquid LB medium and further diluted 10-fold into 5 mL fresh LB medium. Cultures were incubated with shaking at 37 ^{o}C for 45 min to 1 h to obtain the cell population (approx. 10^8 CFU/mL) with desired optimal optical density (OD_{600}). The cultures were further diluted approximately 1000-fold (10^5 CFU/mL) into fresh liquid LB medium and kept at room temperature until needed.

Preparation of Broth Macrodilution: The ten target oligosaccharide stock solutions were prepared at 20 mM in dimethyl sulfoxide (DMSO). Working solutions were prepared in liquid LB with the tested compounds at 4X, where X is the final concentration desired. To each well of a 96- well microtiter plate was dispensed 100 μL of fresh liquid LB. A 100 μL aliquot of each tested compound was added to the first well of a row, which was then serially diluted 1:2 to give a series of seven concentrations. Then 100 μL of the already prepared culture solution was added to each well. Optical densities were recorded on Biotek synergy HT plate reader at 600

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[2] The assay was performed by the broth dilution method described by the National Committee for Clinical Laboratory Standard Methods (M7-A6, 2003)

[3] Sampson, B. A.; Misr, R.; Benson, S. A. *Genetics,* **1989**, *122*, 491–501.

[4] Perego, M.; Spiegelman, G. B.; Hoch, J. A. *Molecular Microbiology*, **1988**, *2*, 689-699

nm absorbance with constant shaking. After 16 h, minimum inhibitory concentration (MIC) values were recorded as the lowest concentration at which no visible growth of bacteria was observed.

Section D: MTT Colorimetric Assays[5]

The human lung epithelial cell line NCI-H460 was obtained from the American Type Culture Collection (ATCC, Manassas,VA). The cells were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum and 2 mM L-glutamine and 100 units/mL penicillin/streptomycin. Cell cultures were maintained in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were passaged at preconfluent densities using a solution containing 0.25% trypsin and 0.5 mM EDTA (Invitrogen). Cells were seeded at a density of 10,000 cell/well in a 96 well plate for 12 hours with 10% FBS, 1% penicillin and streptomycin, and 1% L-glutamine resulting in 80% confluency. Each dose was prepared in 1% FBS medium by 1000X dilution of the drug which was prepared in dimethyl sulfoxide (DMSO) solution to ensure DMSO concentration less than 0.1%. Control experiments showed that 0.1% DMSO had no effect on cytotoxicity. The cell viability was measured by incubating the treated cell with 10 μL of 5mg/mL MTT solution in deionized water per well for 4 h, followed by solublizing the resulting formazan salt with DMSO for 45min. Absorbance was detected by a Gen5 Reader at 562 nm. The experiment was performed in 3 replicate wells of each compound or concentration with at least three experimental runs (N = 9). Data were analyzed by using GraphPad Prism version 5.03 for Windows, GraphPad Software, San Diego California USA. The cell survival at 100 μM was < 1 %.

[5] (a)Mosmann, T. *J. Immunological Methods,* **1983**, *65*, 55-63. (b) Chanvorachote, P.; Nimmannit, U.; Stehlik, C.; Wang, L.; Jiang, B.-H.; Ongpipatanakul, B.; Rojanasakul, Y. *Cancer Res.,* **2006**, *66*, 6353-6360

Table S1. Nonlinear-regression analysis of MTT dose-dependent experiment for the ten oligosaccharides (SE = Standard Error).

Compd	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
IC_{50} (μM)	90.9	12.5	9.1	12.8	12.2	15.4	7.5	16.4	16.5	9.8
SE (μM)	1.791	1.078	1.033	1.103	1.073	1.294	1.052	1.222	1.182	1.213
R^2	0.9719	0.9819	0.9653	0.9728	0.9619	0.9651	0.9414	0.9445	0.9490	0.9608

Section E: NCI Growth Inhibition Assays[6]

The human tumor cell lines were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. Cells (100 μL) were inoculated into 96 well microtiter plates at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold higher concentration than the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional four or 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different drug dilutions were added to the appropriate microtiter wells already containing 100

[6] Screening Services - NCI-60 DTP Human Tumor Cell Line Screen Home Page. http://dtp.nci.nih.gov/branches/btb/ivclsp.html (accessed October 15, 2010)

μL of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For cells in suspension, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$[(Ti-Tz)/(C-Tz)] \times 100$ for concentrations for which $Ti \geq Tz$

$[(Ti-Tz)/Tz] \times 100$ for concentrations for which $Ti<Tz$.

Growth inhibition of 50% (GI_{50}) was calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation.[7]

[7] NCI-60 DTP Human Tumor Cell Line Screen Program in this assay only reported 59 cell lines.

Table S2. GI_{50} (μM) values for seven oligosaccharides against 60 cancer cell lines.

		Compound						
Cell Type	**Cell line**	**1**	**2**	**3**	**4**	**7**	**8**	**10**
Leukemia	CCRF-CEM	3.88	1.19	2.25	4.5	1.07	1.25	1.18
	HL-60(TB)	8.23	1.39	2.12	6.42	1.11	1.09	1.07
	K-562	7.49	1.85	2.41	5.36	1.33	1.51	1.58
	MOLT-4	5.95	1.57	2.20	5.76	1.17	1.42	1.18
	RPMI-8226	4.12	1.00	1.86	2.84	0.96	1.04	0.92
	SR	3.20	1.20	1.45	3.83	0.86	0.92	0.85
Non-Small Cell Lung Cancer	A549/ATCC	6.69	2.08	9.47	8.18	1.18	0.80	0.97
	EKVX	6.40	1.60	7.55	8.12	0.77	0.78	0.64
	HOP-62	7.61	1.78	6.02	7.36	0.82	0.81	0.88
	HOP-92	3.20	0.97	0.86	1.63	NA	0.61	0.60
	NCI-H23	7.53	1.80	6.36	7.48	0.94	0.86	0.86
	NCI-H322M	7.83	NA	6.81	6.24	0.96	0.81	0.76
	NCI-H460	6.32	1.54	7.58	8.43	0.94	0.93	0.96
	NCI-H522	5.78	1.07	2.26	5.25	0.84	0.80	0.78
Colon Cancer	COLO-205	6.87	0.88	5.17	5.25	0.84	0.81	0.86
	HCC-2998	6.99	0.93	6.94	9.08	0.86	0.88	0.92
	HCT-116	3.17	1.66	2.86	7.40	0.84	0.79	0.83
	HCT-15	8.39	7.38	7.54	8.33	1.01	0.82	1.52
	HT-29	4.35	1.21	8.02	8.13	0.97	0.91	0.91
	KM12	5.64	1.33	6.02	7.32	0.89	0.90	0.87
	SW-620	7.66	1.89	6.04	7.31	0.85	0.85	0.84
CNS Cancer	SF-268	5.54	1.61	4.43	6.18	0.78	0.74	0.69
	SF-295	6.96	1.14	7.13	8.97	0.94	0.88	0.58
	SF-539	7.11	3.10	4.70	7.17	0.83	0.80	0.91
	SNB-19	7.44	2.36	5.59	7.15	0.81	0.78	0.81
	SNB-75	3.42	0.90	1.36	2.18	0.50	0.54	0.62
	U251	5.86	1.73	4.44	7.40	0.81	0.81	0.81
Melanoma	LOX IMVI	4.23	0.85	1.15	5.14	0.82	0.82	0.82
	MALME-3M	5.34	NA	5.27	6.11	0.53	0.59	0.70
	M14	6.78	1.48	6.11	7.23	0.83	0.82	0.85
	MDA-MB-435	7.68	1.49	6.00	7.25	0.83	0.88	0.84

	SK-MEL-2	7.11	1.61	5.89	7.16	0.89	0.83	0.88
	SK-MEL-28	7.01	1.75	1.96	6.04	0.84	0.83	0.86
	SK-MEL-5	6.21	1.23	1.76	6.27	0.74	0.76	0.82
	UACC-257	7.80	2.53	7.70	8.05	0.93	0.83	1.05
	UACC-62	6.15	1.98	4.36	6.54	0.78	0.74	0.77
Ovarian Cancer	IGROV1	2.48	NA	2.03	6.14	0.83	0.81	0.82
	OVCAR-3	5.91	1.49	4.84	6.07	0.77	0.84	0.80
	OVCAR-4	6.83	1.94	8.71	7.47	0.77	0.82	0.88
	OVCAR-5	6.55	1.75	6.39	6.70	0.79	0.77	0.80
	OVCAR-8	9.10	3.09	9.12	9.12	1.54	0.90	0.89
	NCI/ADR-RES	9.29	7.92	9.15	8.90	3.38	0.92	2.24
	SK-OV-3	8.44	4.04	8.36	7.59	0.93	0.79	0.80
Renal Cancer	786-0	8.34	3.42	6.71	8.65	0.96	0.90	0.93
	A498	6.73	5.13	7.57	5.64	0.73	0.67	0.02
	ACHN	8.23	4.11	7.93	7.58	0.96	0.87	0.89
	CAKI-1	8.78	6.57	8.45	9.24	1.42	0.86	0.60
	RXF-393	5.24	0.97	2.10	6.65	0.76	0.64	0.72
	SN12C	7.65	1.72	3.65	6.66	0.85	0.82	0.82
	TK-10	7.87	4.21	8.08	8.22	1.12	0.98	0.94
	UO-31	6.61	NA	7.43	5.70	0.87	0.72	0.69
Prostate Cancer	PC-3	2.37	0.89	5.30	6.55	0.82	0.71	0.75
	DU-145	7.94	2.48	7.92	7.47	0.80	0.80	0.79
Breast Cancer	MCF7	4.84	1.62	1.74	5.29	0.87	0.89	0.94
	MDA-MB-231/ATCC	6.86	1.13	2.56	6.10	0.87	0.80	0.88
	HS 578T	5.41	1.13	2.37	6.32	0.92	0.78	0.78
	BT-549	7.90	4.54	8.46	8.39	0.94	1.05	0.88
	T-47D	5.62	1.65	1.46	3.56	0.79	0.75	0.74
	MDA-MB-468	3.32	0.93	1.47	2.65	0.78	0.75	0.79

Section F: ^{1}H NMR and ^{13}C NMR Spectra

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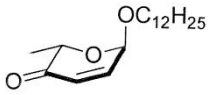

^{1}H NMR of (**13**)
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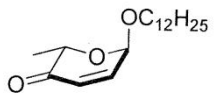

^{13}C NMR of (**13**)
150 MHz, $CDCl_3$

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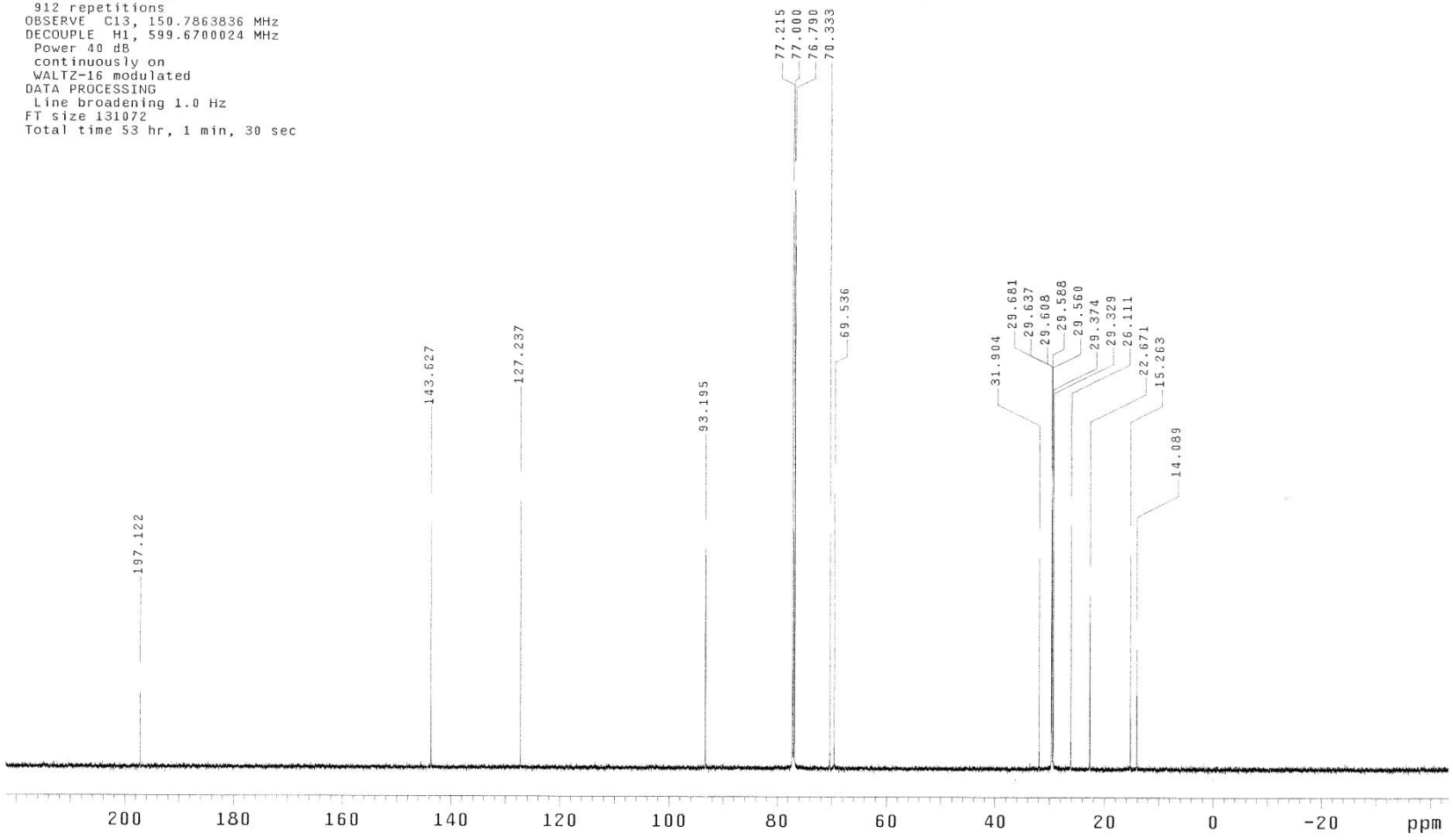

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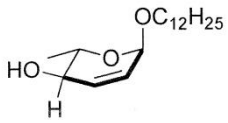
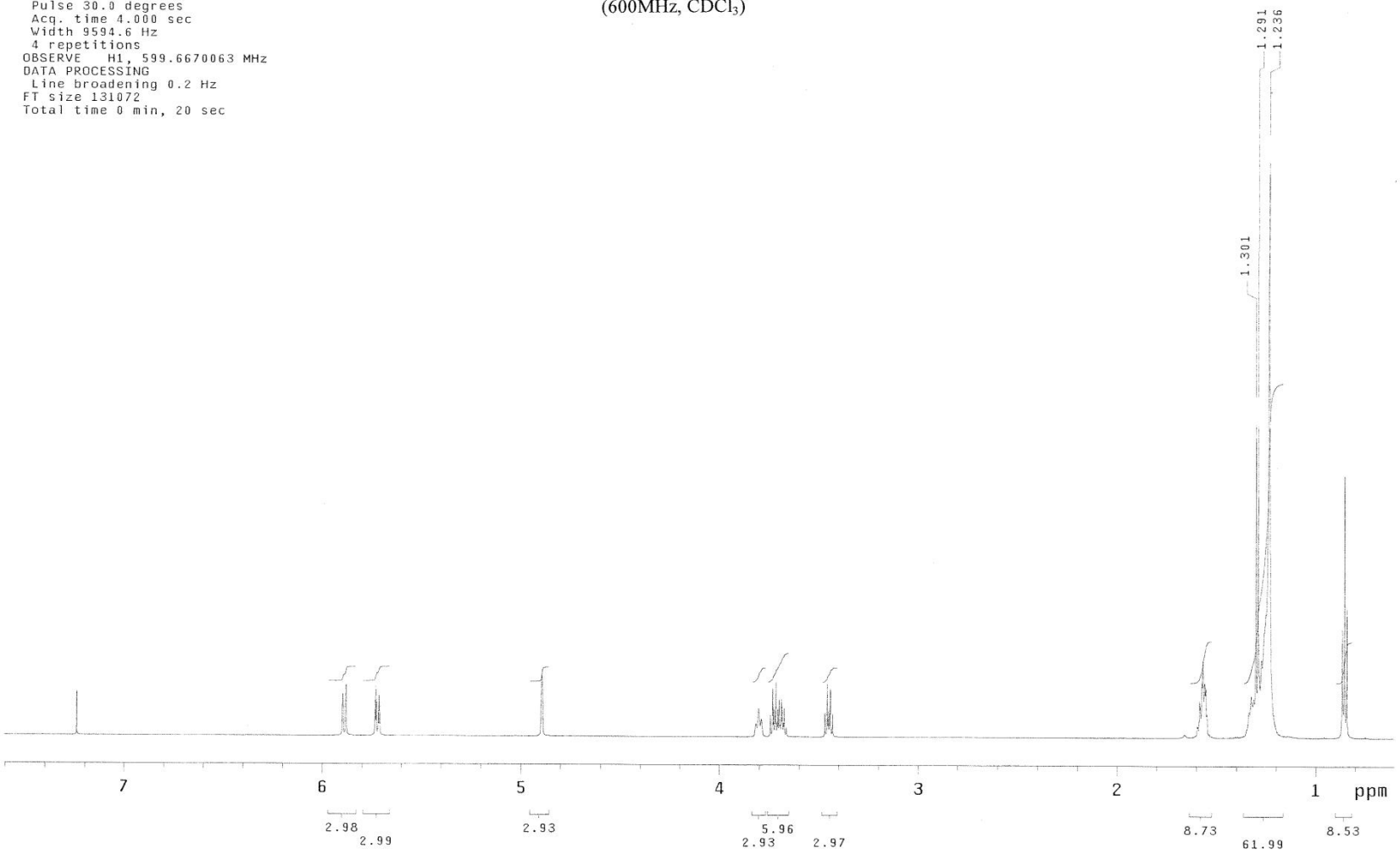


^{1}H NMR of **(C)**
(600MHz, $CDCl_3$)



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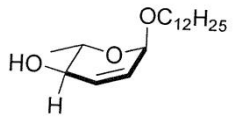
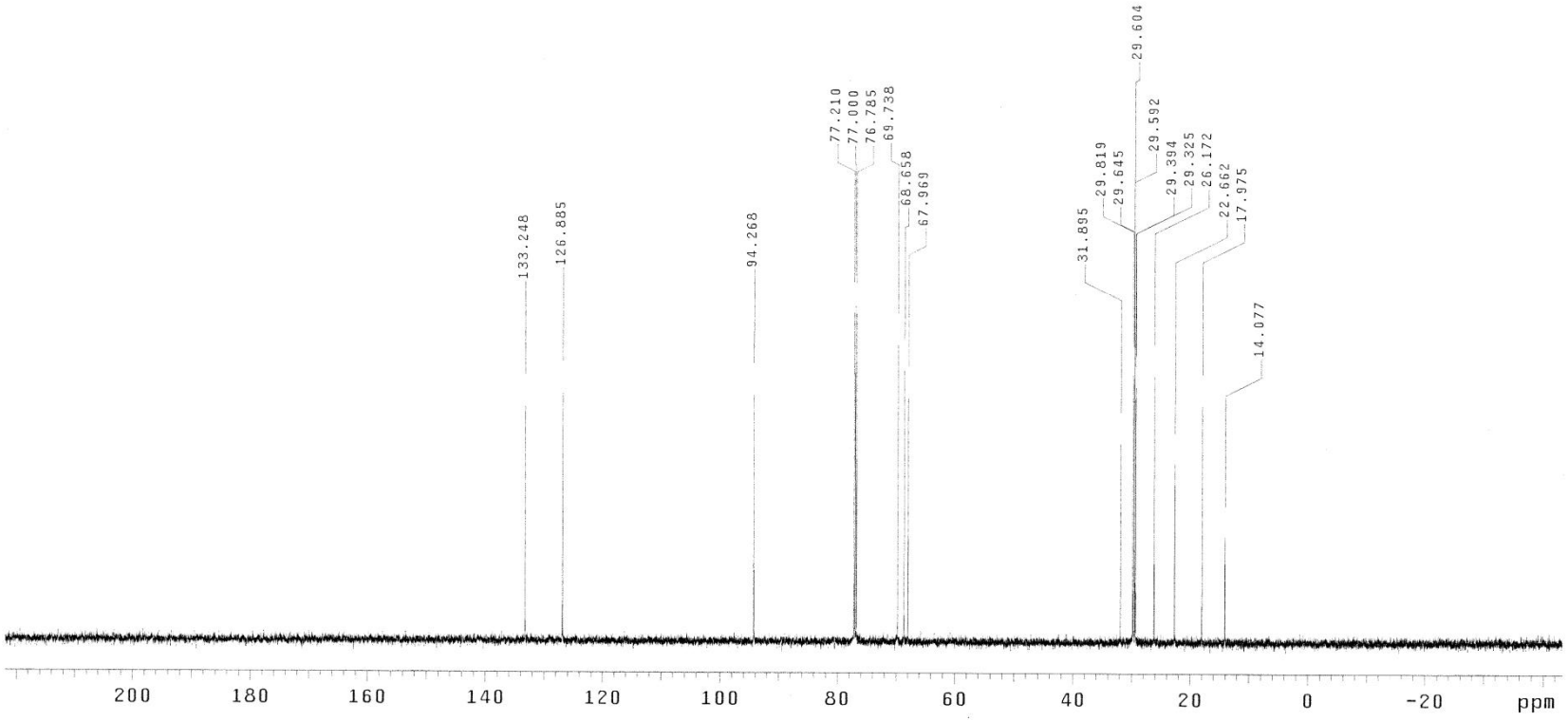


^{13}C NMR of **(C)**
(150 MHz, $CDCl_3$)



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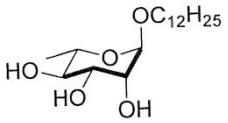
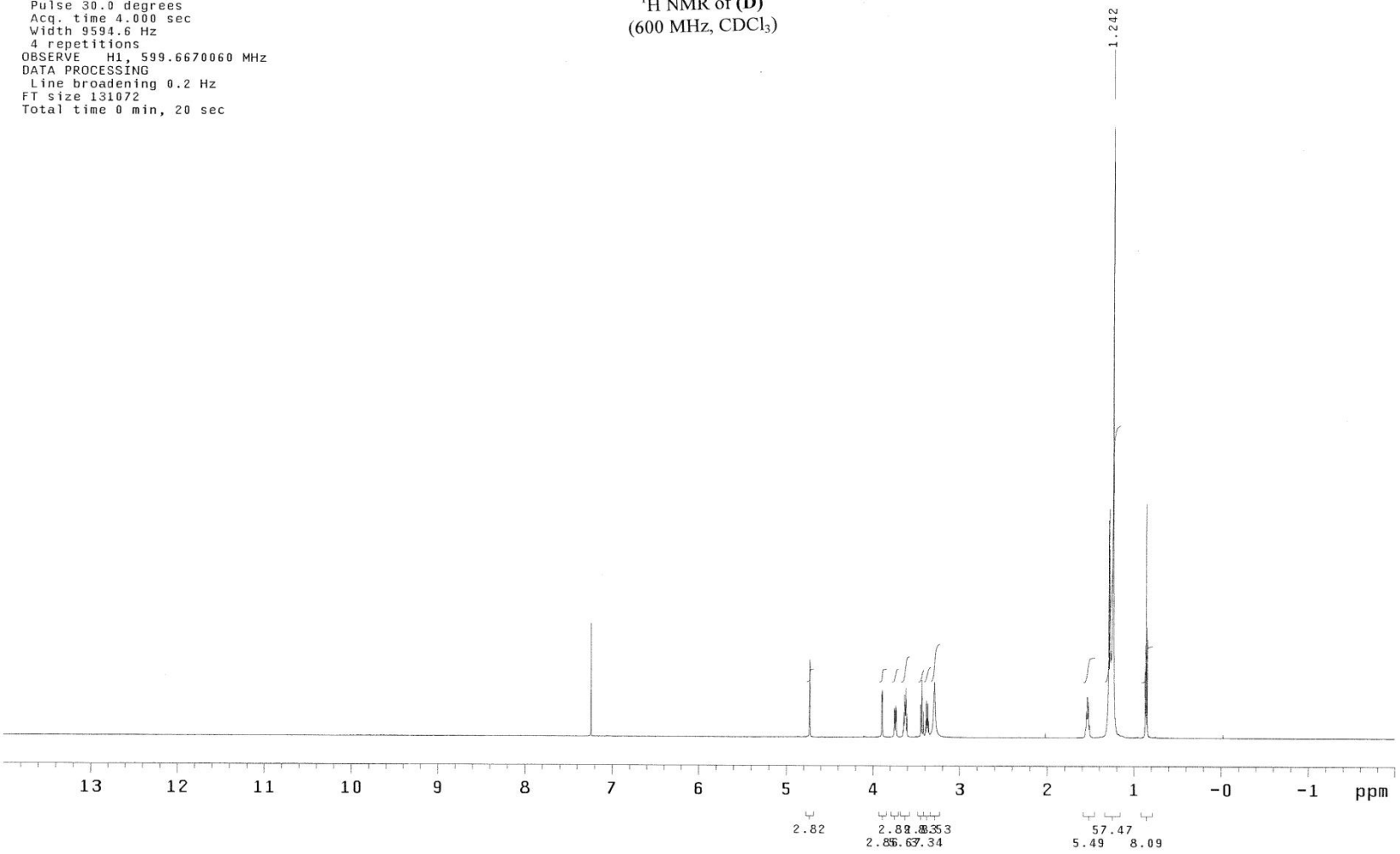
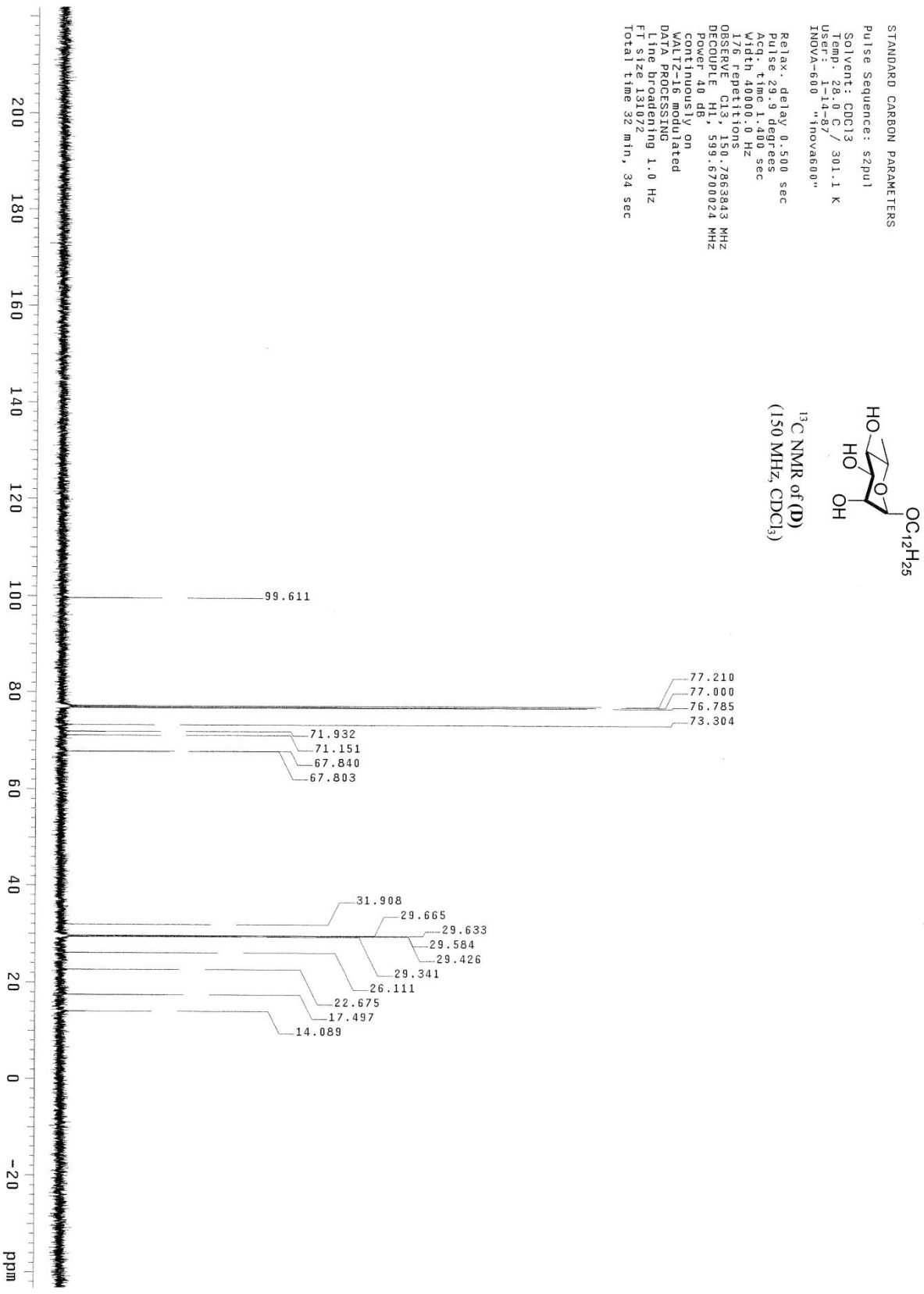


^{1}H NMR of **(D)**
(600 MHz, $CDCl_3$)

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HO
O
OH
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(150 MHz, CDCl3)
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STANDARD PROTON PARAMETERS

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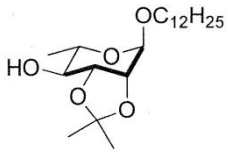
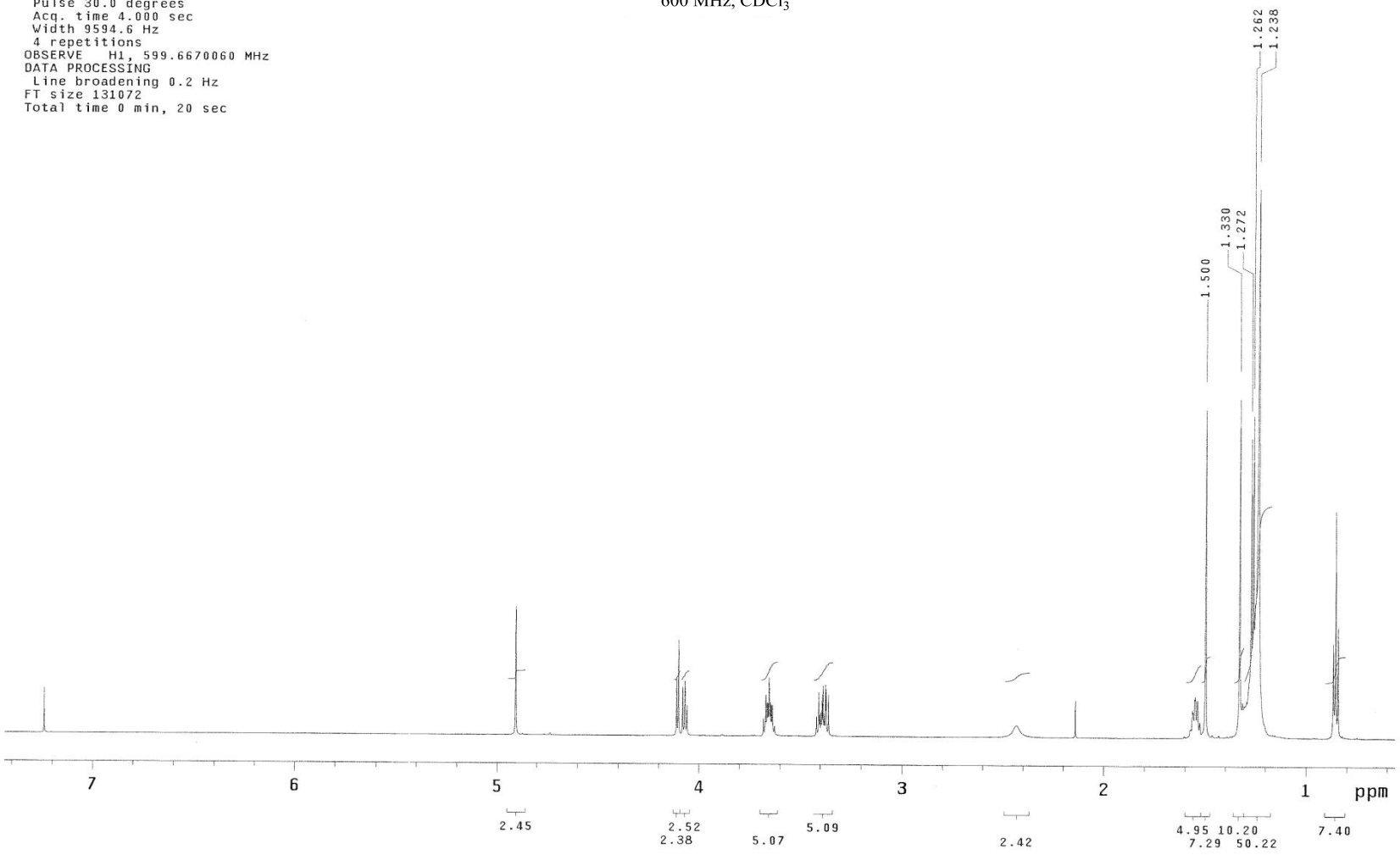
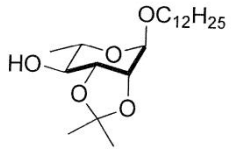
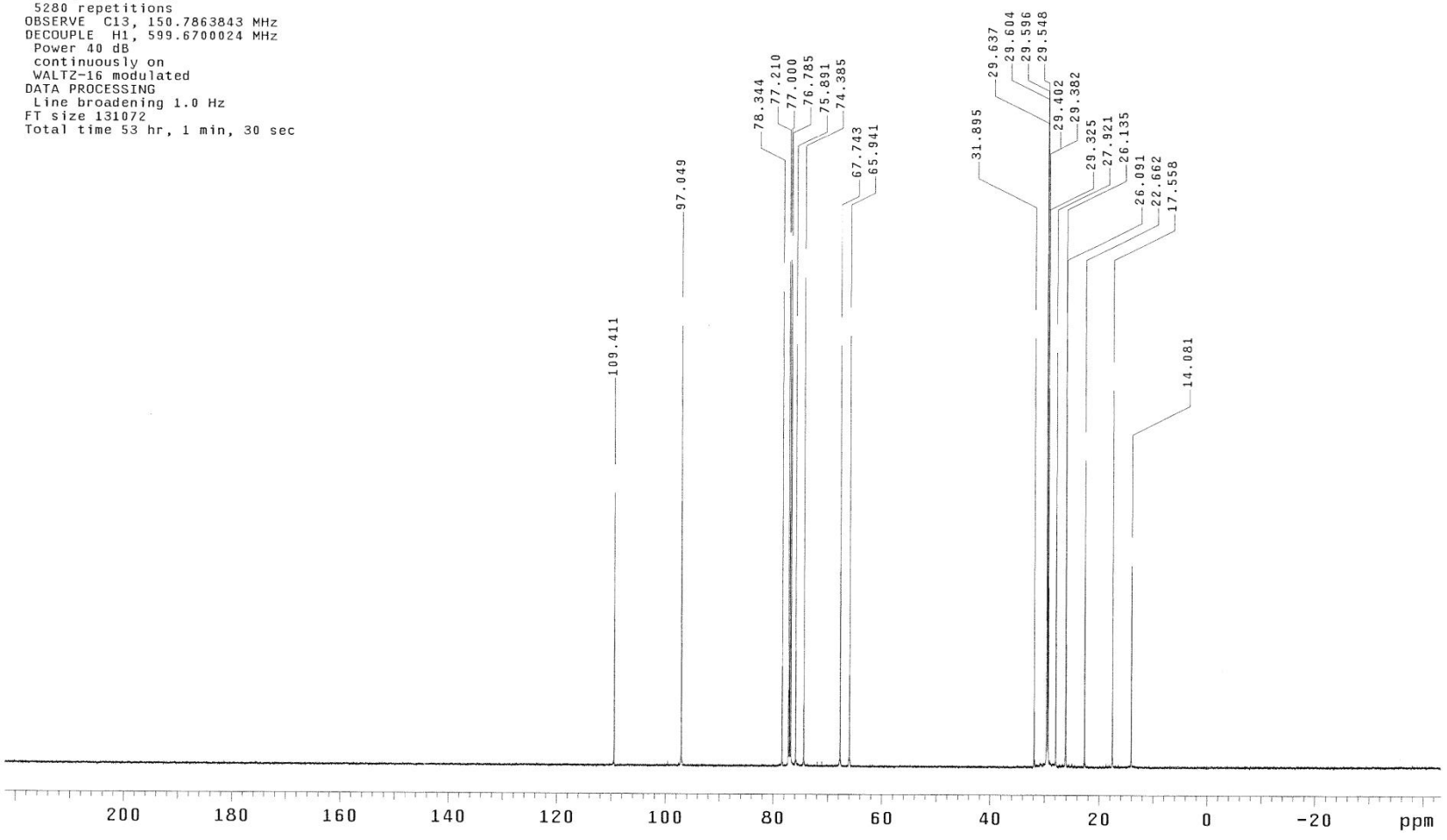


^{1}H NMR of (**14**)
600 MHz, $CDCl_3$





^{13}C NMR of (**14**)
150 MHz, $CDCl_3$



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4 repetitions
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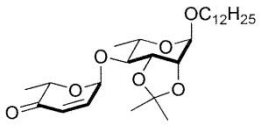
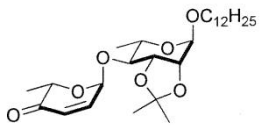


^{1}H NMR of **(E)**
(600 MHz, $CDCl_3$)



^{13}C NMR of **(E)**
(150 MHz, $CDCl_3$)

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STANDARD CARBON PARAMETERS

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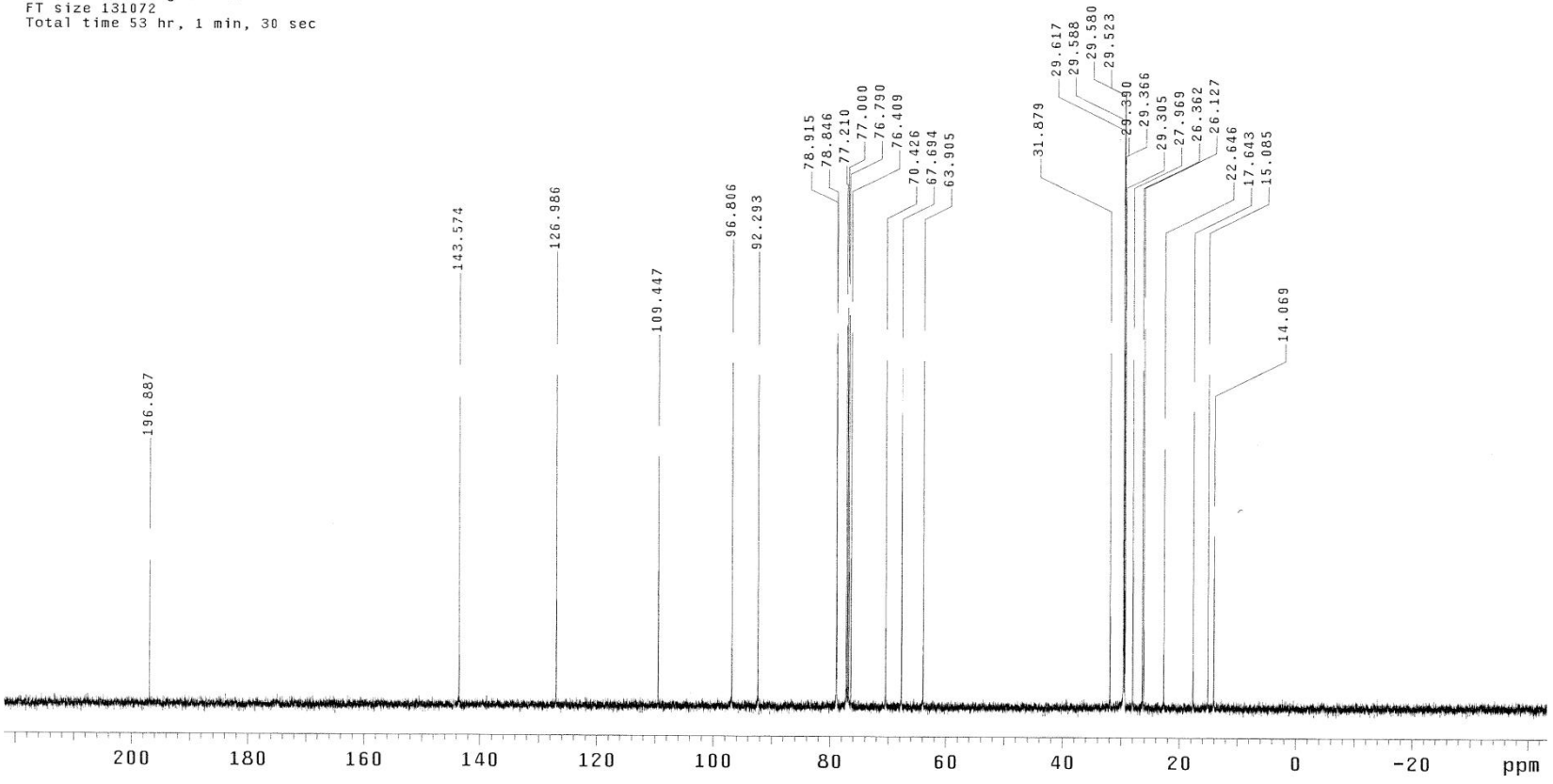
 Pulse 29.9 degrees
 Acq. time 1.400 sec
 Width 40000.0 Hz
 144 repetitions
OBSERVE  C13, 150.7863855 MHz
DECOUPLE  H1, 599.6700024 MHz
 Power 40 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 1.0 Hz
FT size 131072
Total time 53 hr, 1 min, 30 sec
```



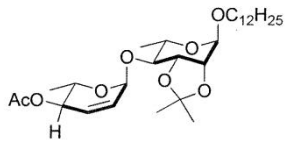
STANDARD PROTON PARAMETERS

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:
File: PROTON

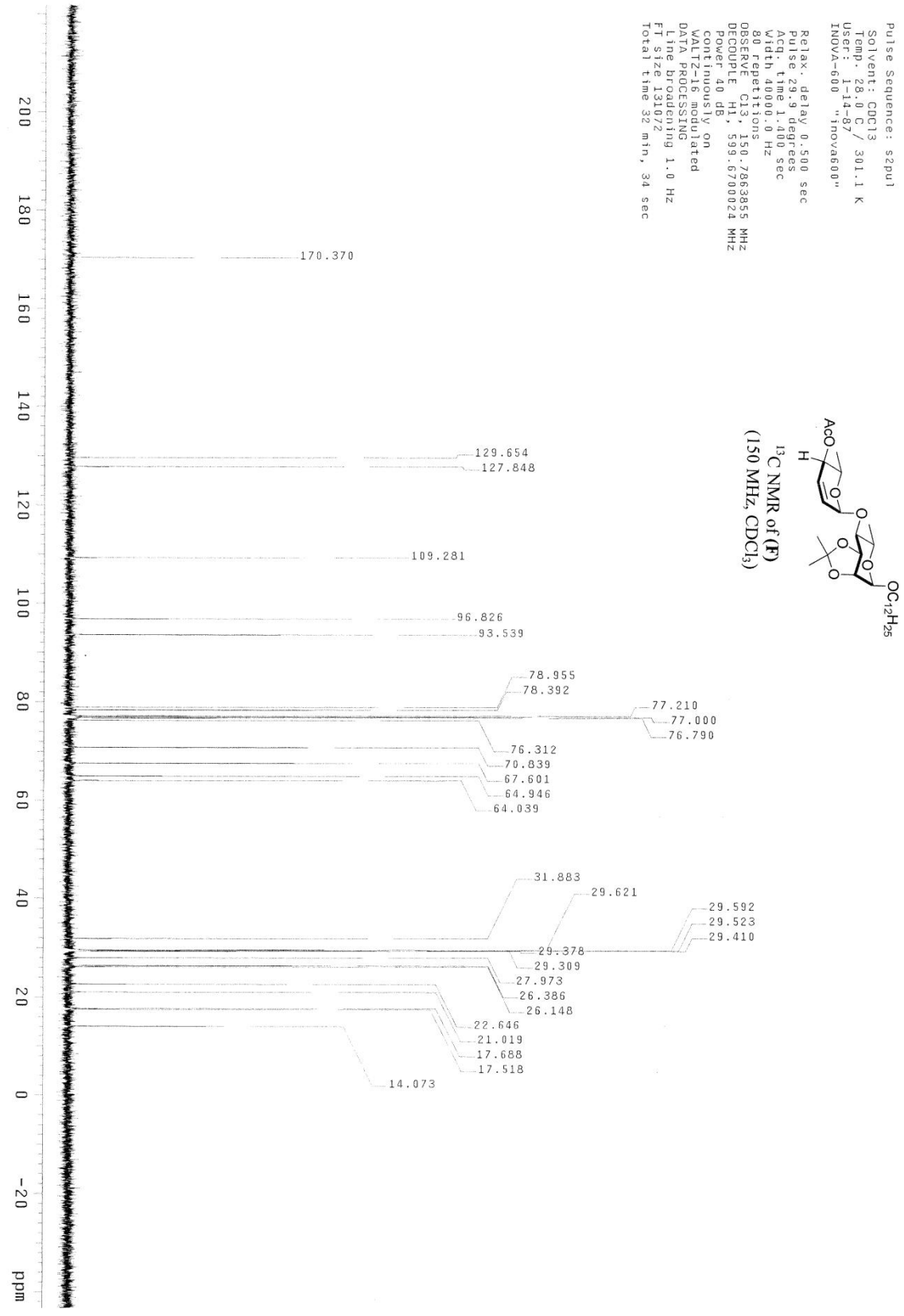
Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 28.0 C / 301.1 K
INOVA-600 "inova600"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 4.000 sec
Width 9594.6 Hz
4 repetitions
OBSERVE H1, 599.6670063 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 131072
Total time 0 min, 20 sec



^{1}H NMR of **(F)**
(600 MHz, $CDCl_3$)


STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
User: l-14-87
INOVA-600 "inova600"
Relax. delay 0.500 sec
Pulse 29.9 degrees
Acq. time 1.400 sec
Width 40000.0 Hz
80 repetitions
OBSERVE C13, 150.7863855 MHz
DECOUPLE H1, 599.6700024 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 32 min, 34 sec
170.370
129.654
127.848
109.281
96.826
93.539
78.955
78.392
77.210
77.000
76.790
76.312
70.839
67.601
64.946
64.039
31.883
29.621
29.592
29.523
29.410
29.378
29.309
27.973
26.386
26.148
22.646
21.019
17.688
17.518
14.073
200
180
160
140
120
100
80
60
40
20
0
-20
ppm
AcO
H
O
O
O
O
O
O
OC12H25
13C NMR of (F)
(150 MHz, CDCl3)

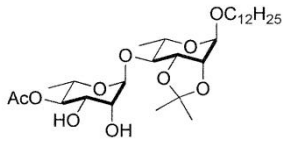
STANDARD PROTON PARAMETERS

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:
File: PROTON

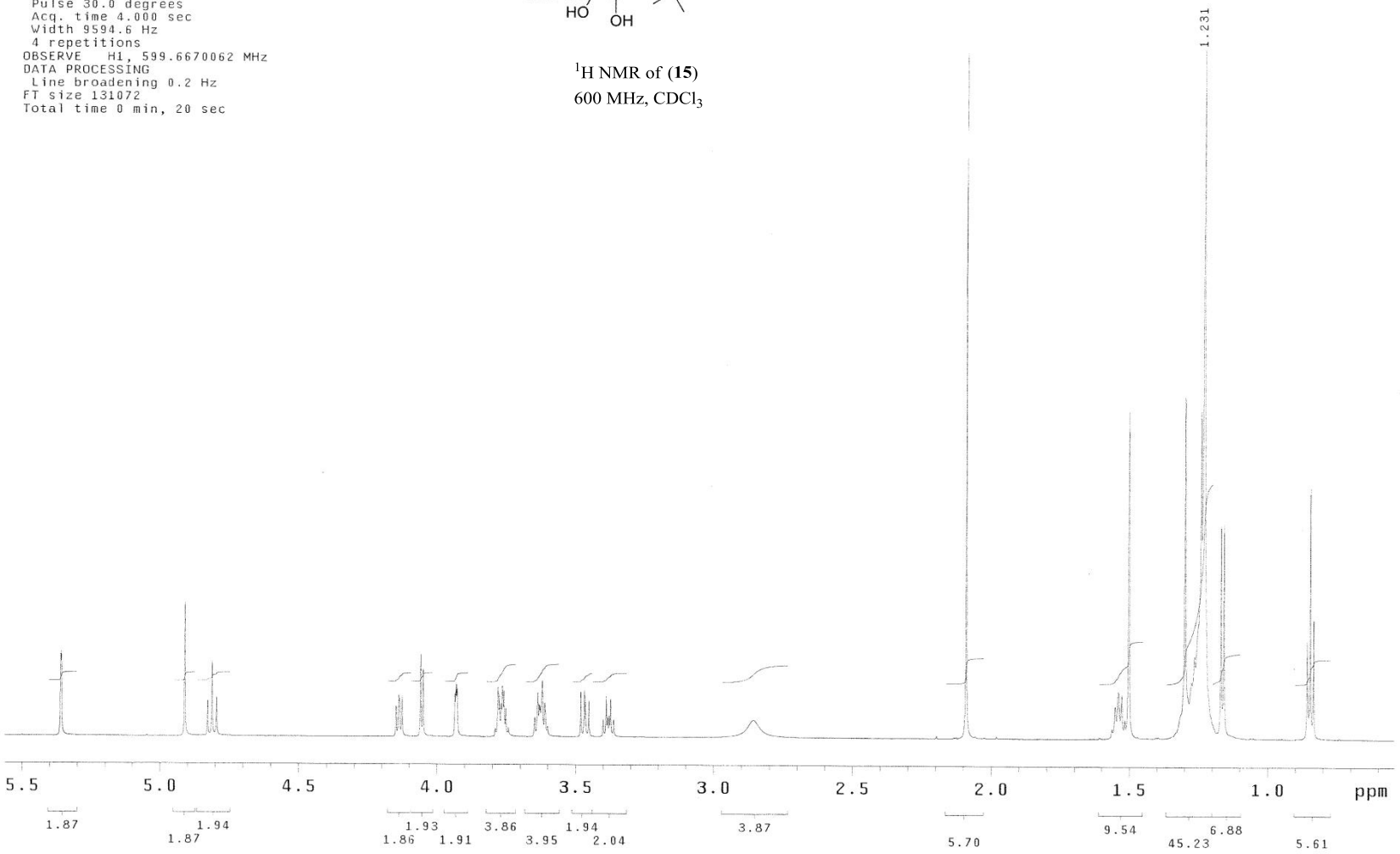
Pulse Sequence: s2pul

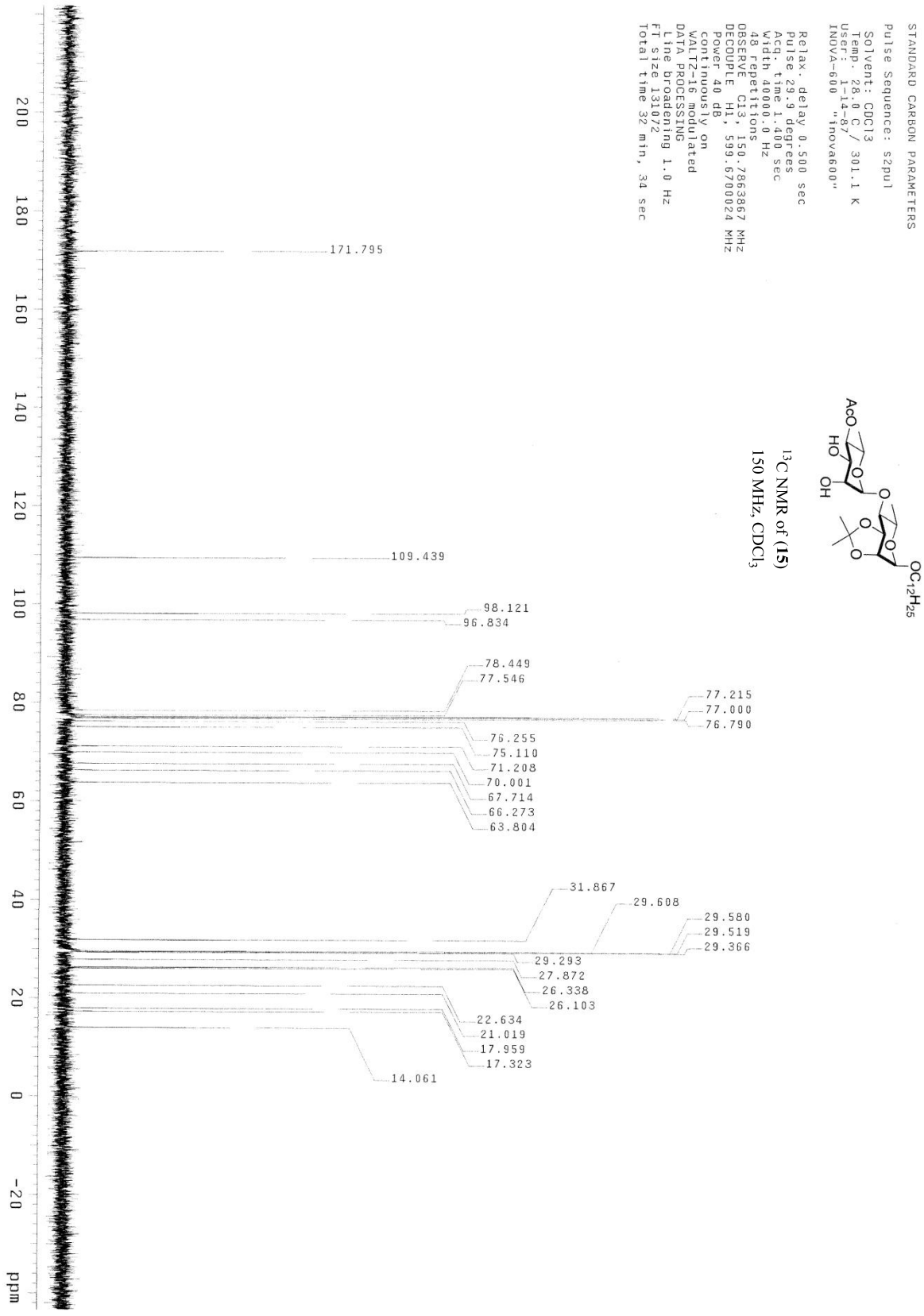
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
INOVA-600 "inova600"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 4.000 sec
Width 9594.6 Hz
4 repetitions
OBSERVE H1, 599.6670062 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 131072
Total time 0 min, 20 sec



^{1}H NMR of (**15**)
600 MHz, $CDCl_3$




STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
User: 1-14-87
INOVA-600 "inova600"
Relax. delay 0.500 sec
Pulse 29.9 degrees
Acq. time 1.400 sec
Width 40000.0 Hz
48 repetitions
OBSERVE C13, 150.7863867 MHz
DECOUPLE H1, 599.6700024 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 32 min, 34 sec
AcO
HO
OH
O
OC12H25
13C NMR of (15)
150 MHz, CDCl3
171.795
109.439
98.121
96.834
78.449
77.546
77.215
77.000
76.790
76.255
75.110
71.208
70.001
67.714
66.273
63.804
31.867
29.608
29.580
29.519
29.366
29.293
27.872
26.338
26.103
22.634
21.019
17.959
17.323
14.061
200
180
160
140
120
100
80
60
40
20
0
-20
ppm

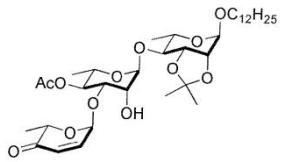
STANDARD PROTON PARAMETERS

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:

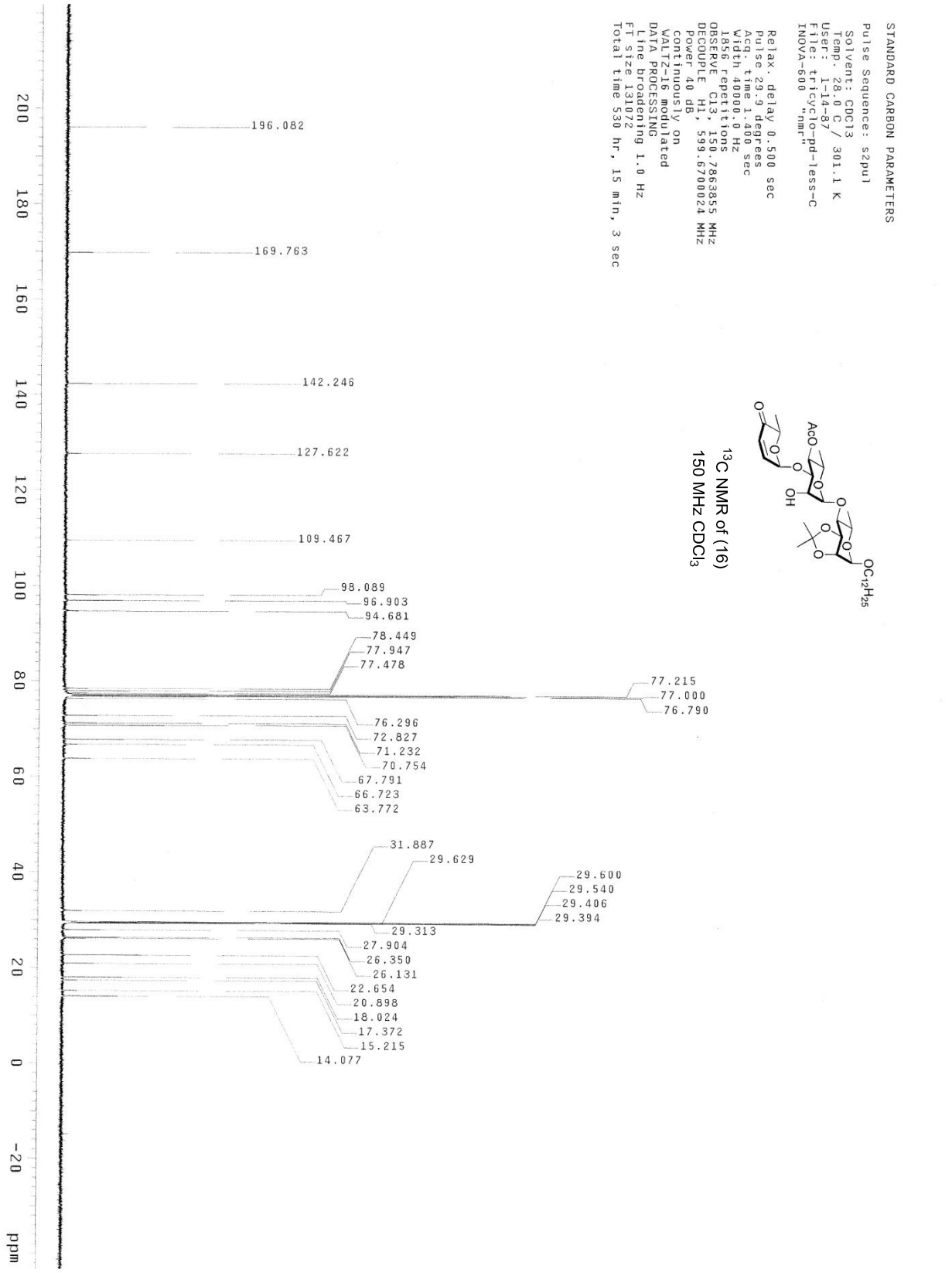
Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 28.0 C / 301.1 K
File: tricyclo-pd-top
INOVA-600 "nmr"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 4.000 sec
Width 9594.6 Hz
4 repetitions
OBSERVE H1, 599.6670084 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 131072
Total time 0 min, 20 sec



^{1}H NMR of (16)
600 MHz $CDCl_3$


STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
User: I_14-87
File: tricyclo-pd-less-C
INOVA-600 "nmr"
Relax. delay 0.500 sec
Pulse 29.9 degrees
Acq. time 1.400 sec
Width 40000.0 Hz
1856 repetitions
OBSERVE C13, 150.7863855 MHz
DECOUPLE H1, 599.6700024 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 530 hr, 15 min, 3 sec
13C NMR of (16)
150 MHz CDCl3
AcO
OH
OC12H25
196.082
169.763
142.246
127.622
109.467
98.089
96.903
94.681
78.449
77.947
77.478
77.215
77.000
76.790
76.296
72.827
71.232
70.754
67.791
66.723
63.772
31.887
29.629
29.600
29.540
29.406
29.394
29.313
27.904
26.350
26.131
22.654
20.898
18.024
17.372
15.215
14.077
200
180
160
140
120
100
80
60
40
20
0
-20
ppm

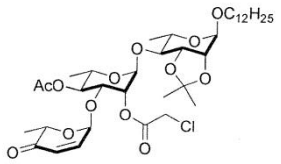
STANDARD PROTON PARAMETERS

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:
File: PROTON

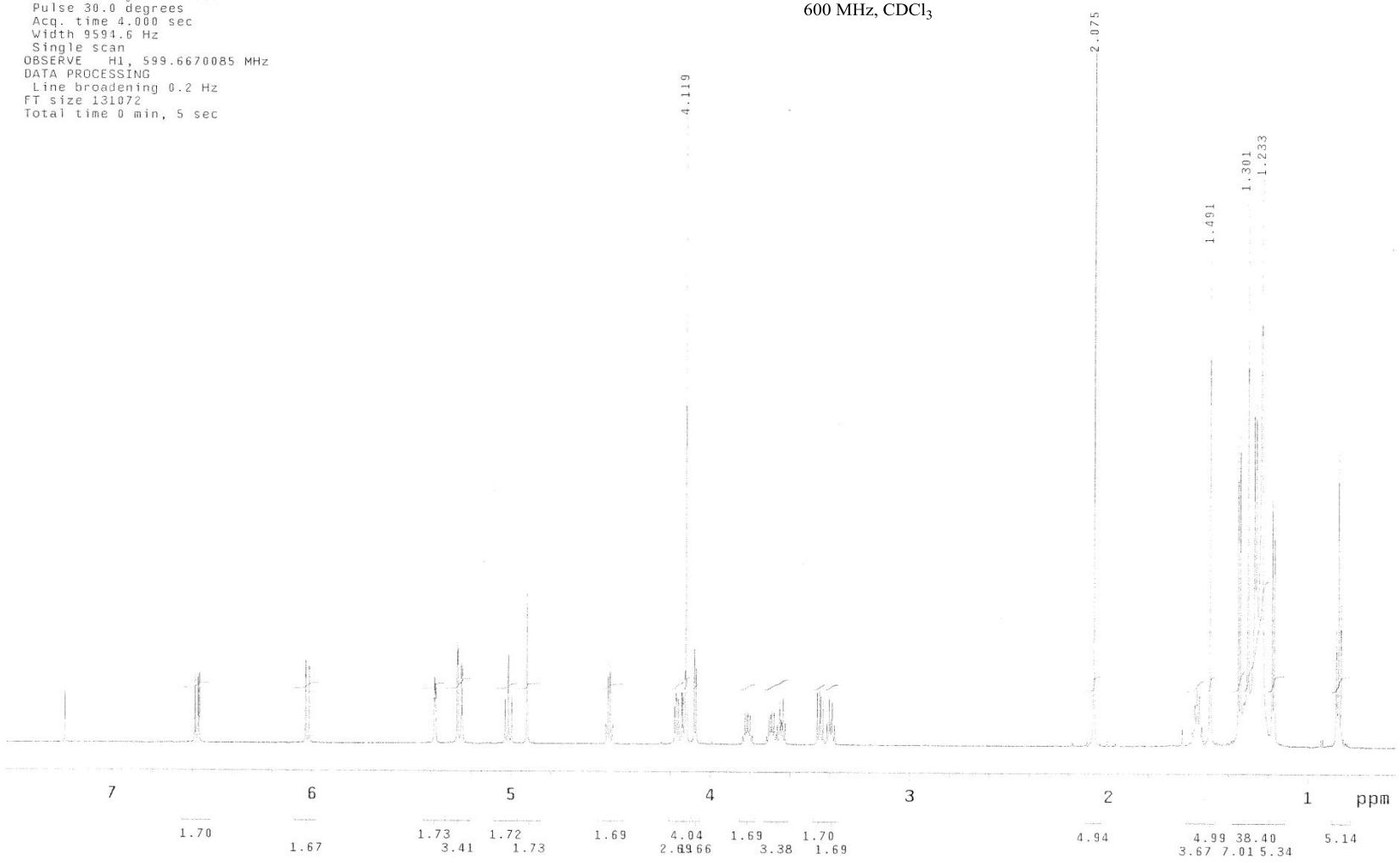
Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 28.0 C / 301.1 K
INOVA-600 "inova600"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 4.000 sec
Width 9594.6 Hz
Single scan
OBSERVE H1, 599.6670085 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 131072
Total time 0 min, 5 sec



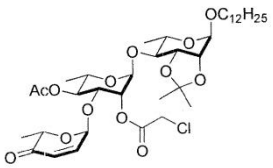
^{1}H NMR of (**17**)
600 MHz, $CDCl_3$



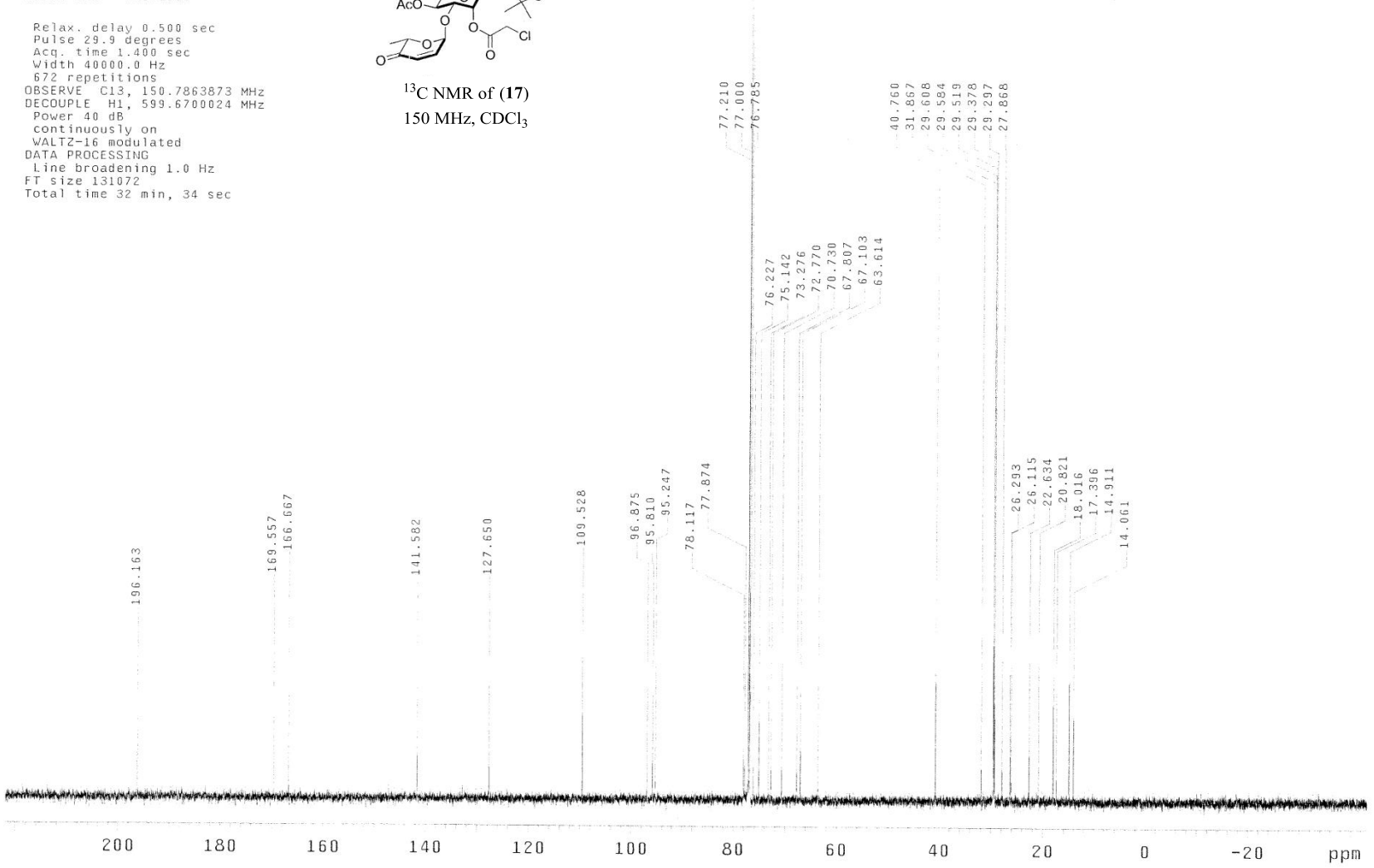
STANDARD CARBON PARAMETERS

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
User: 1-14-87
INOVA-600 "inova600"

Relax. delay 0.500 sec
Pulse 29.9 degrees
Acq. time 1.400 sec
Width 40000.0 Hz
672 repetitions
OBSERVE C13, 150.7863873 MHz
DECOUPLE H1, 599.6700024 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 32 min, 34 sec



^{13}C NMR of (**17**)
150 MHz, $CDCl_3$

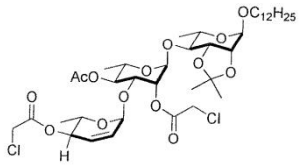


STANDARD PROTON PARAMETERS

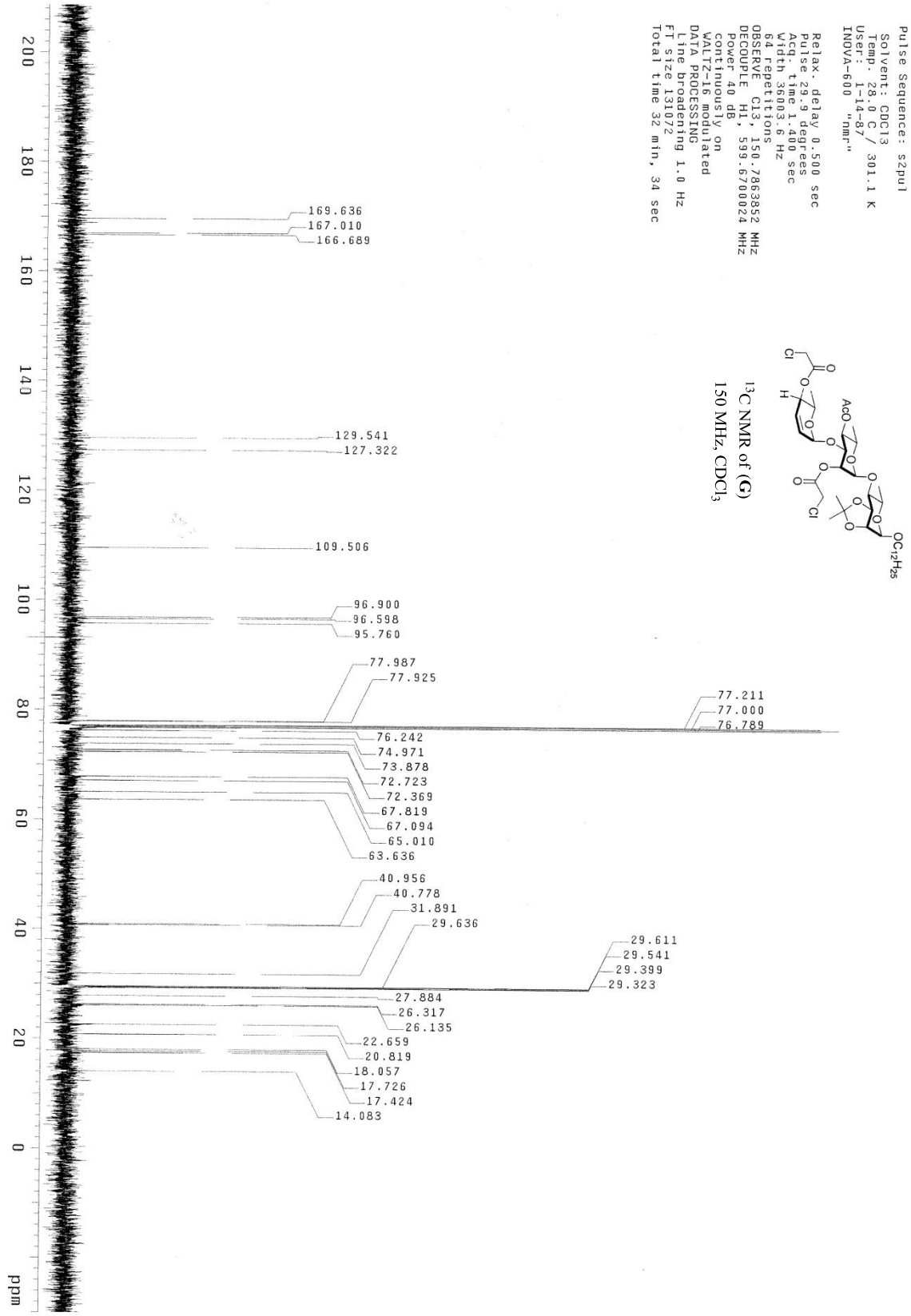
Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
File: 020409-1H
INOVA-600 "nmr"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 4.000 sec
Width 9594.6 Hz
Single scan
OBSERVE H1, 599.6670079 MHz
DATA PROCESSING
FT size 131072
Total time 0 min, 5 sec



^{1}H NMR of (**G**)
600 MHz, $CDCl_3$



^{13}C NMR of (**G**)
150 MHz, $CDCl_3$

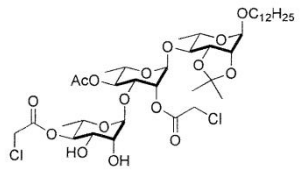
STANDARD PROTON PARAMETERS

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:

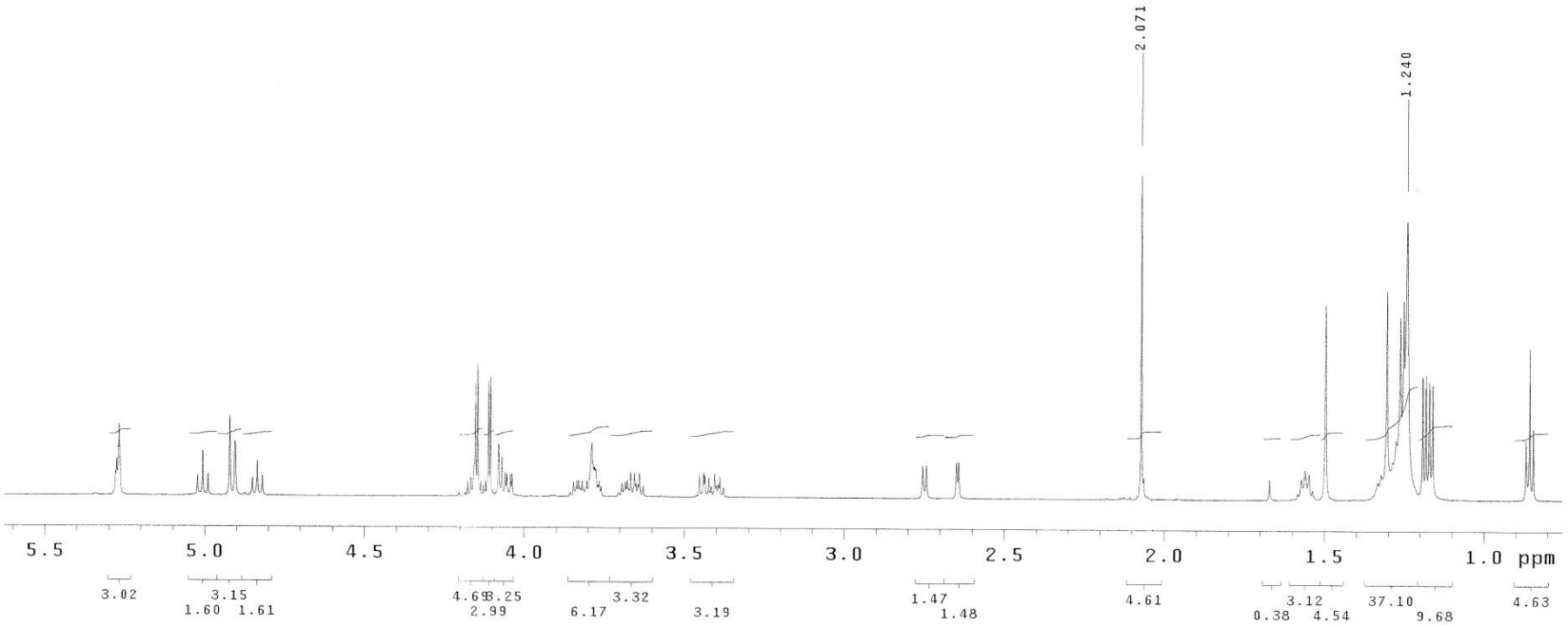
Pulse Sequence: s2pul

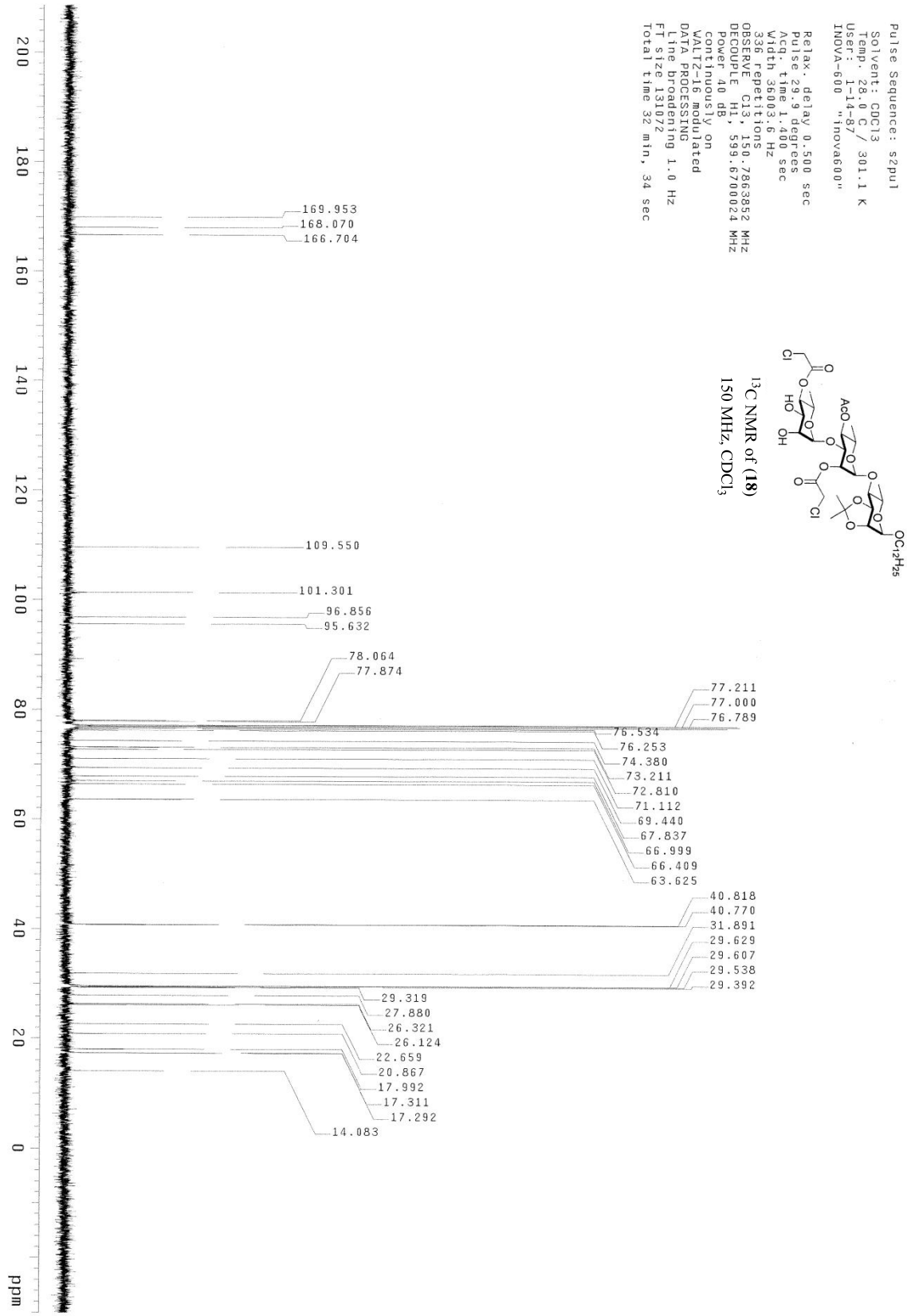
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
File: 020609-1H
INOVA-600 "nmr"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 4.000 sec
Width 9594.6 Hz
Single scan
OBSERVE H1, 599.6670071 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 131072
Total time 0 min, 5 sec



^{1}H NMR of (**18**)
600 MHz, $CDCl_3$





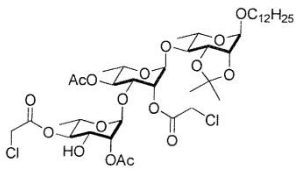
^{13}C NMR of (**18**)
150 MHz, $CDCl_3$

STANDARD PROTON PARAMETERS

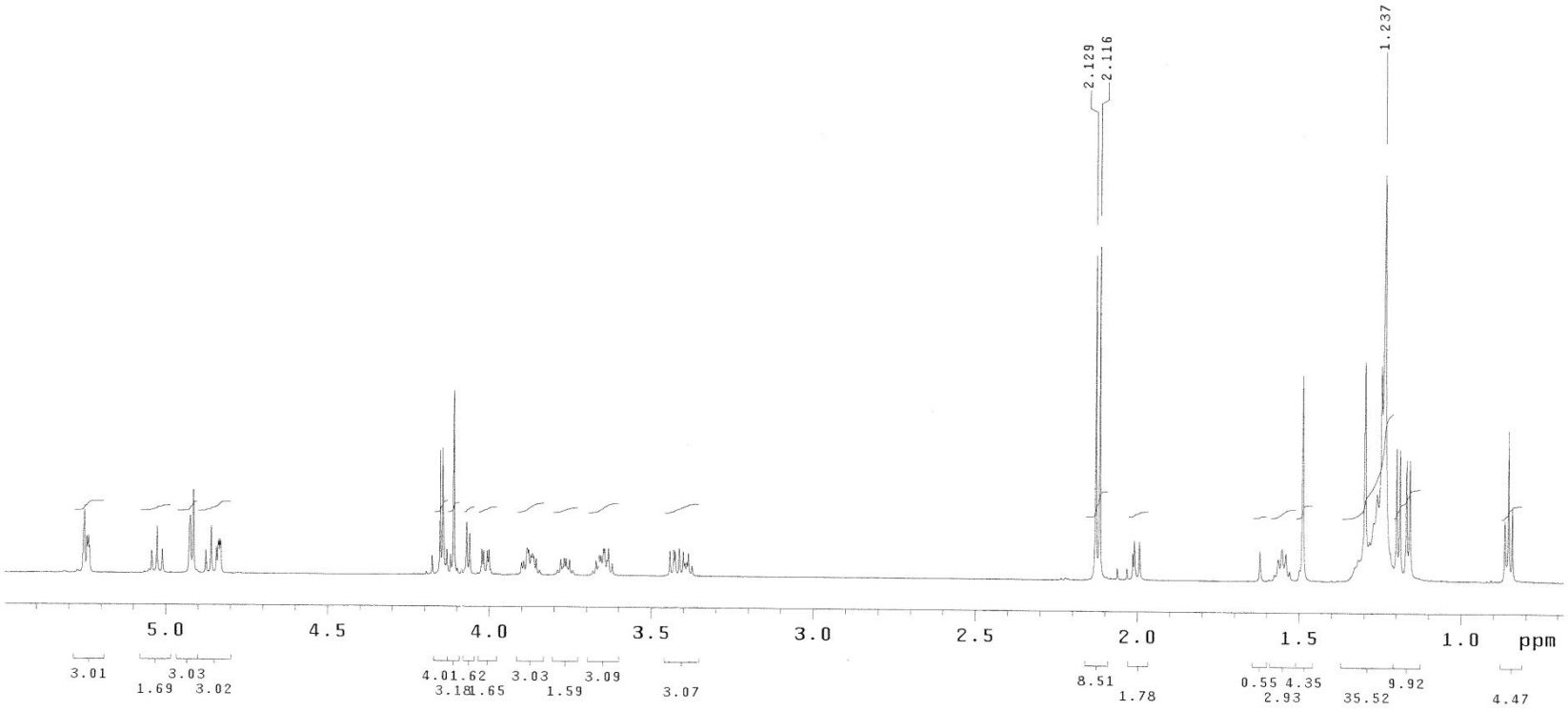
Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
File: 020909-1H
INOVA-600 "nmr"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 4.000 sec
Width 9594.6 Hz
4 repetitions
OBSERVE H1, 599.6670079 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 131072
Total time 0 min, 20 sec



^{1}H NMR of (**H**)
600 MHz, $CDCl_3$

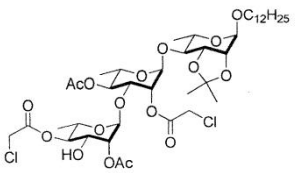


STANDARD CARBON PARAMETERS

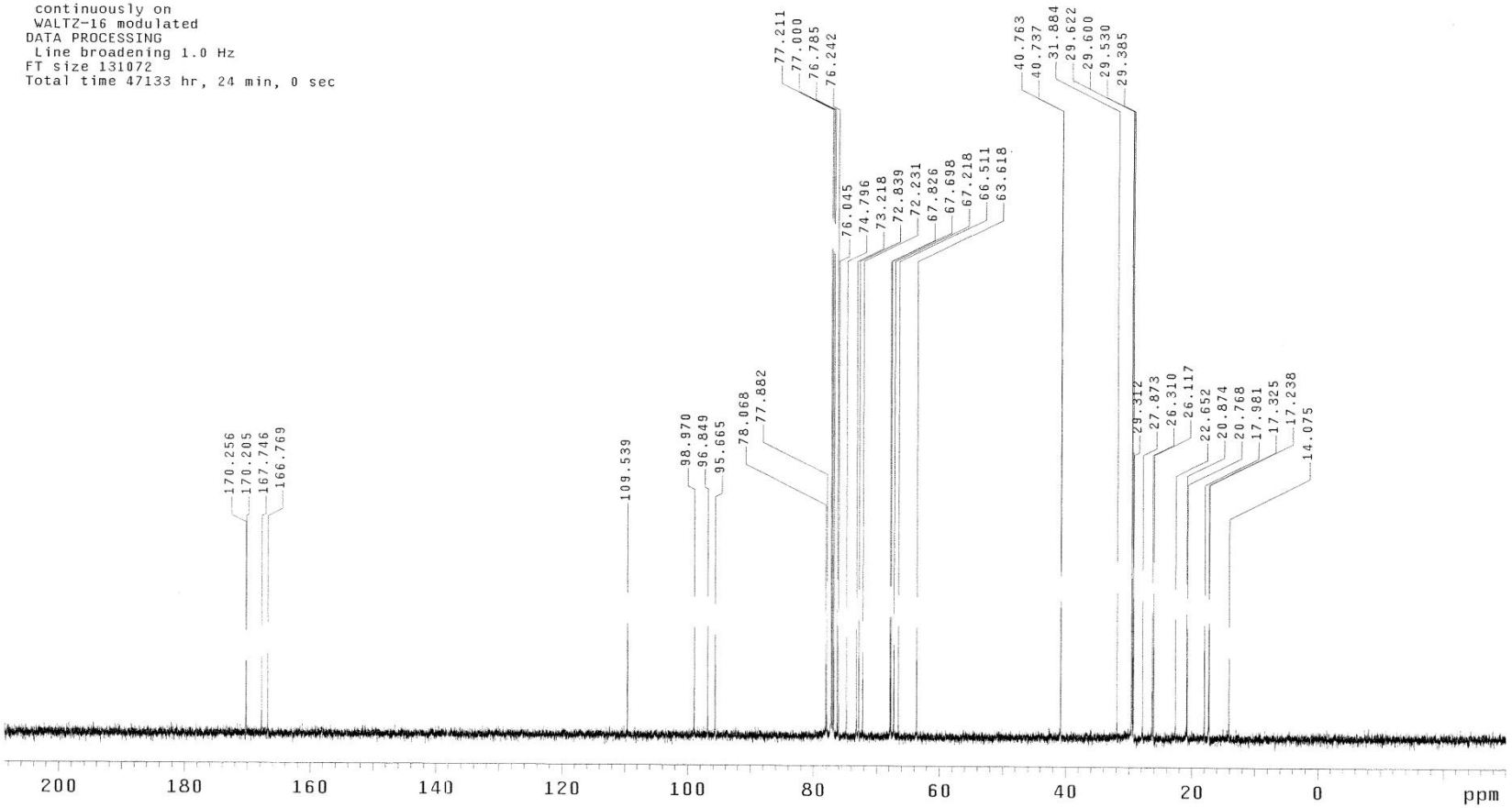
Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 28.0 C / 301.1 K
User: 1-14-87
File: 020909-1C
INOVA-600 "nmr"

Relax. delay 0.500 sec
Pulse 29.9 degrees
Acq. time 1.400 sec
Width 36003.6 Hz
240 repetitions
OBSERVE C13, 150.7863857 MHz
DECOUPLE H1, 599.6700024 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 47133 hr, 24 min, 0 sec



^{13}C NMR of (**H**)
150 MHz, $CDCl_3$



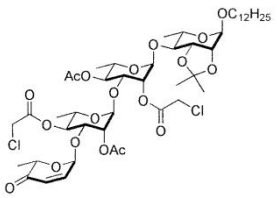
STANDARD PROTON PARAMETERS

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:
File: PROTON

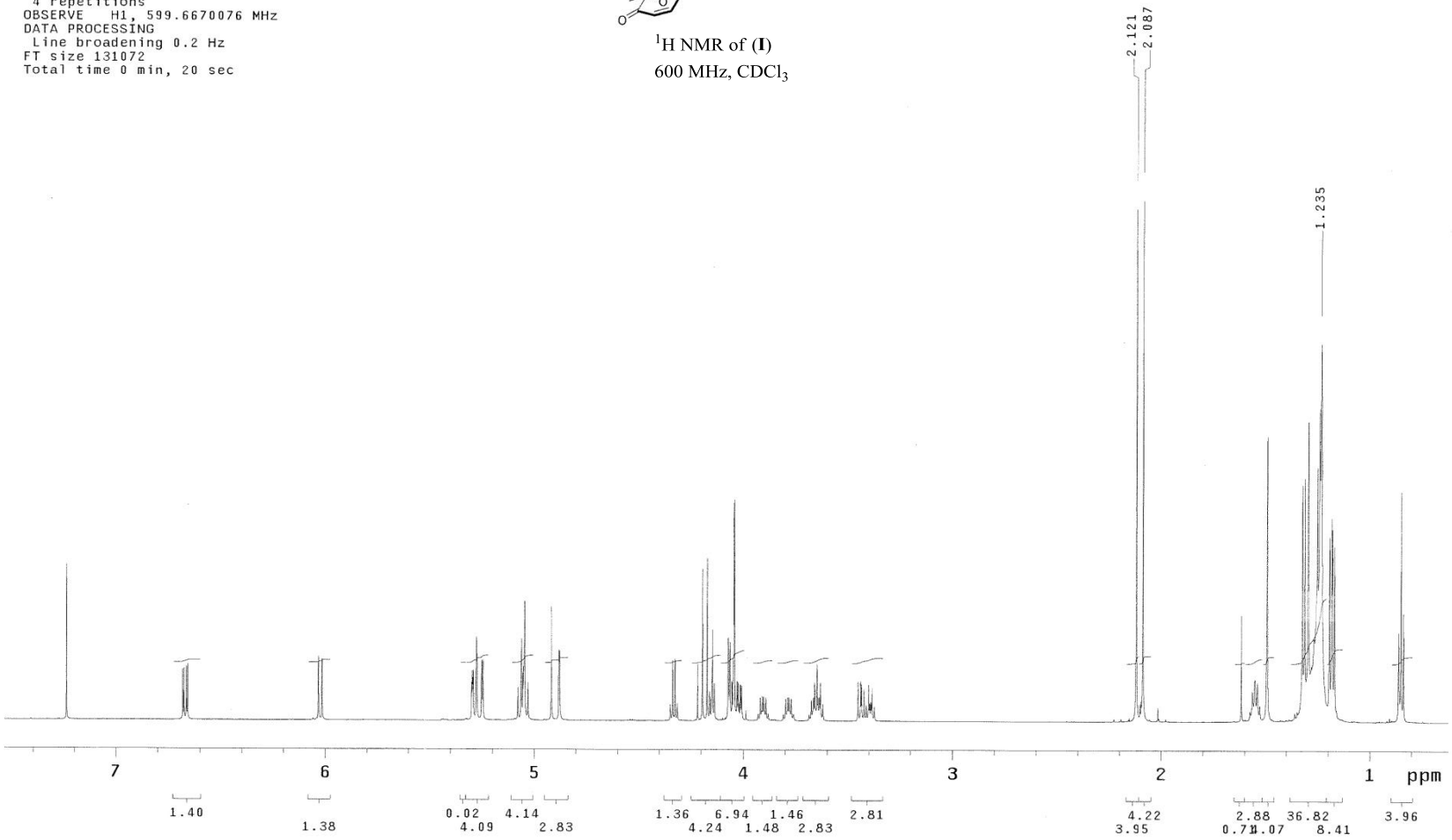
Pulse Sequence: s2pul

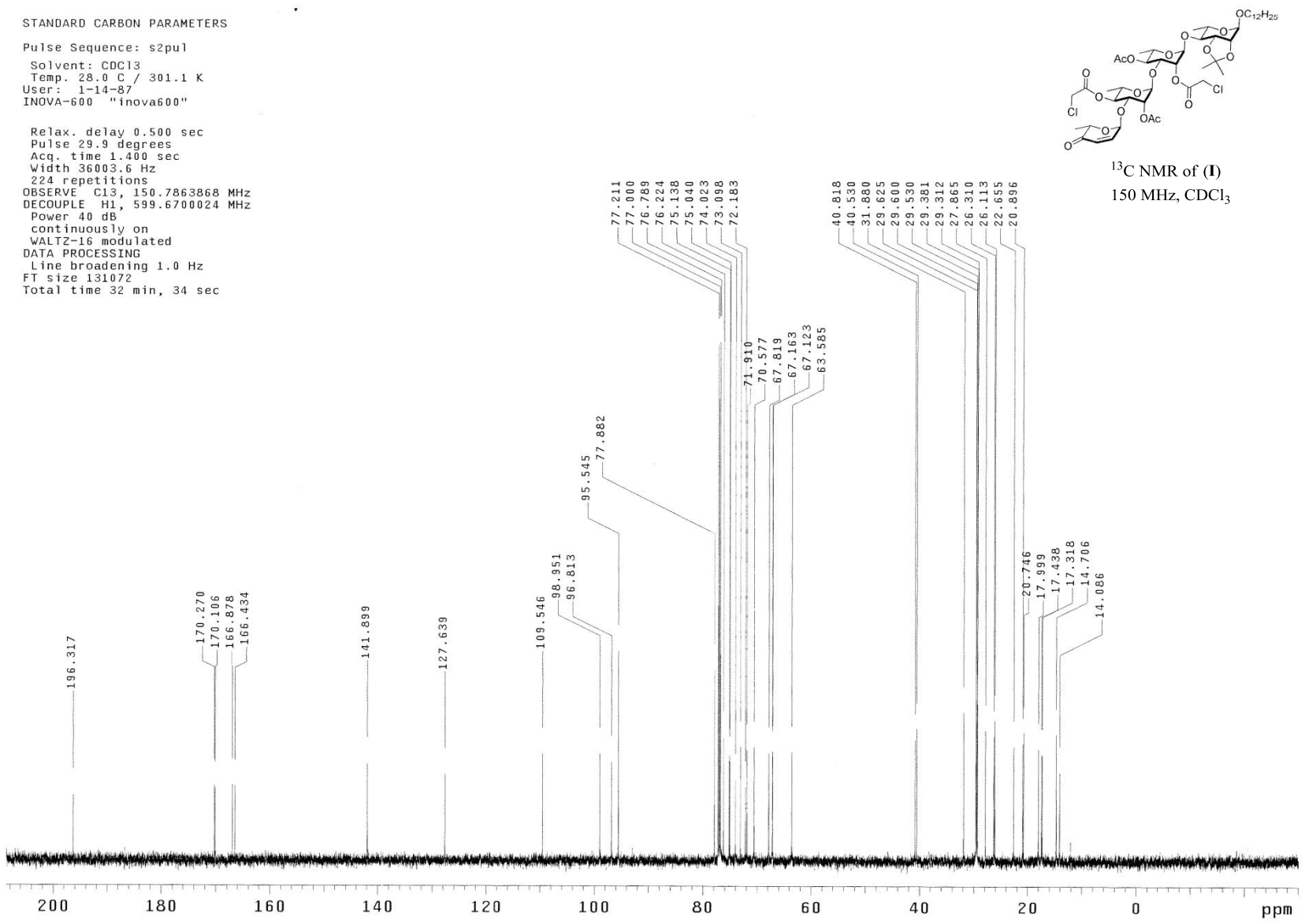
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
INOVA-600 "inova600"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 4.000 sec
Width 9594.6 Hz
4 repetitions
OBSERVE H1, 599.6670076 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 131072
Total time 0 min, 20 sec



^{1}H NMR of (**I**)
600 MHz, $CDCl_3$





^{13}C NMR of (**I**)
150 MHz, $CDCl_3$

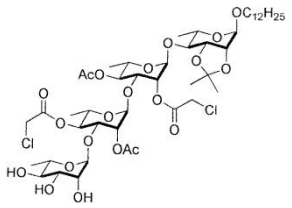
STANDARD PROTON PARAMETERS

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:

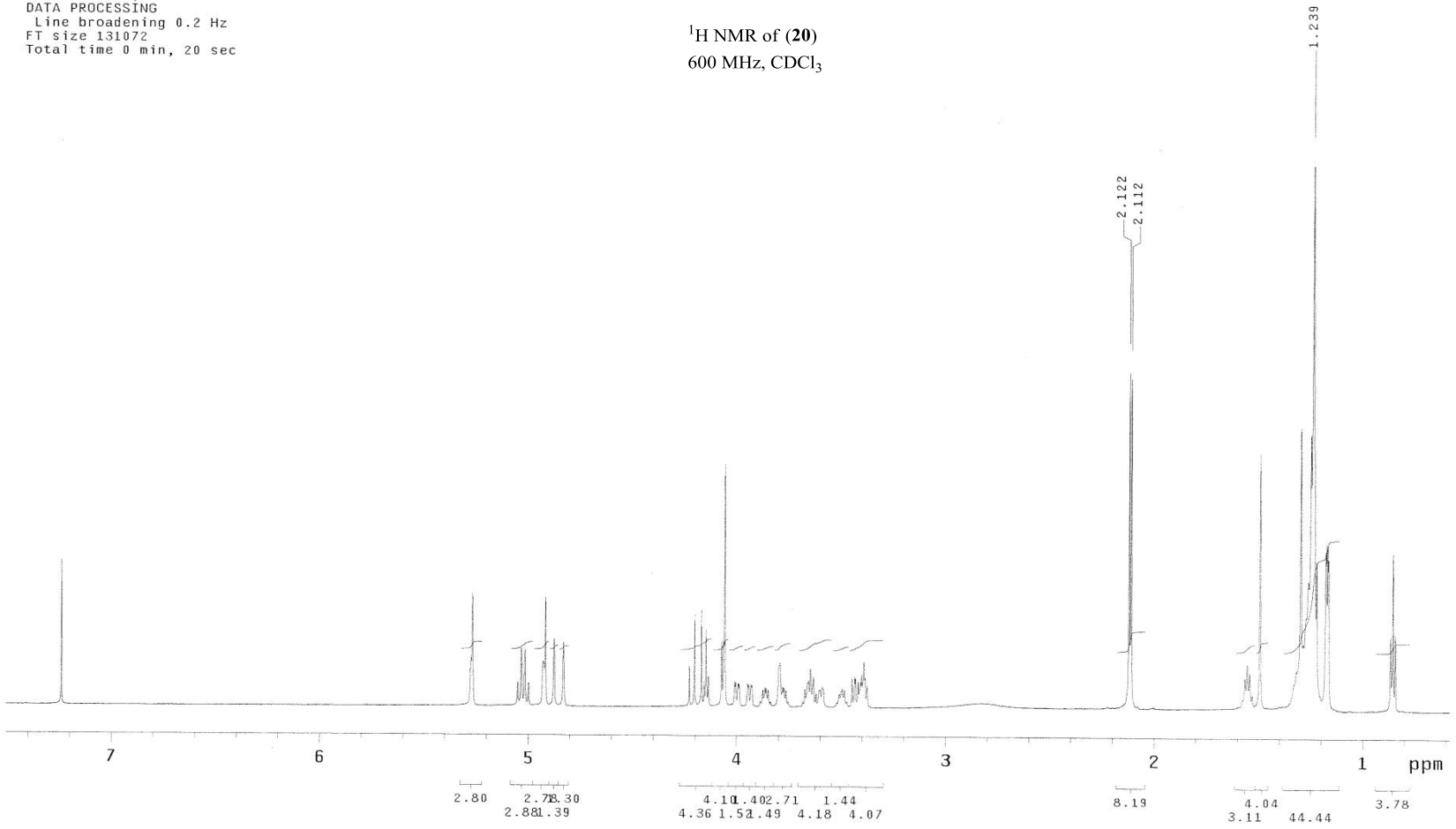
Pulse Sequence: s2pul

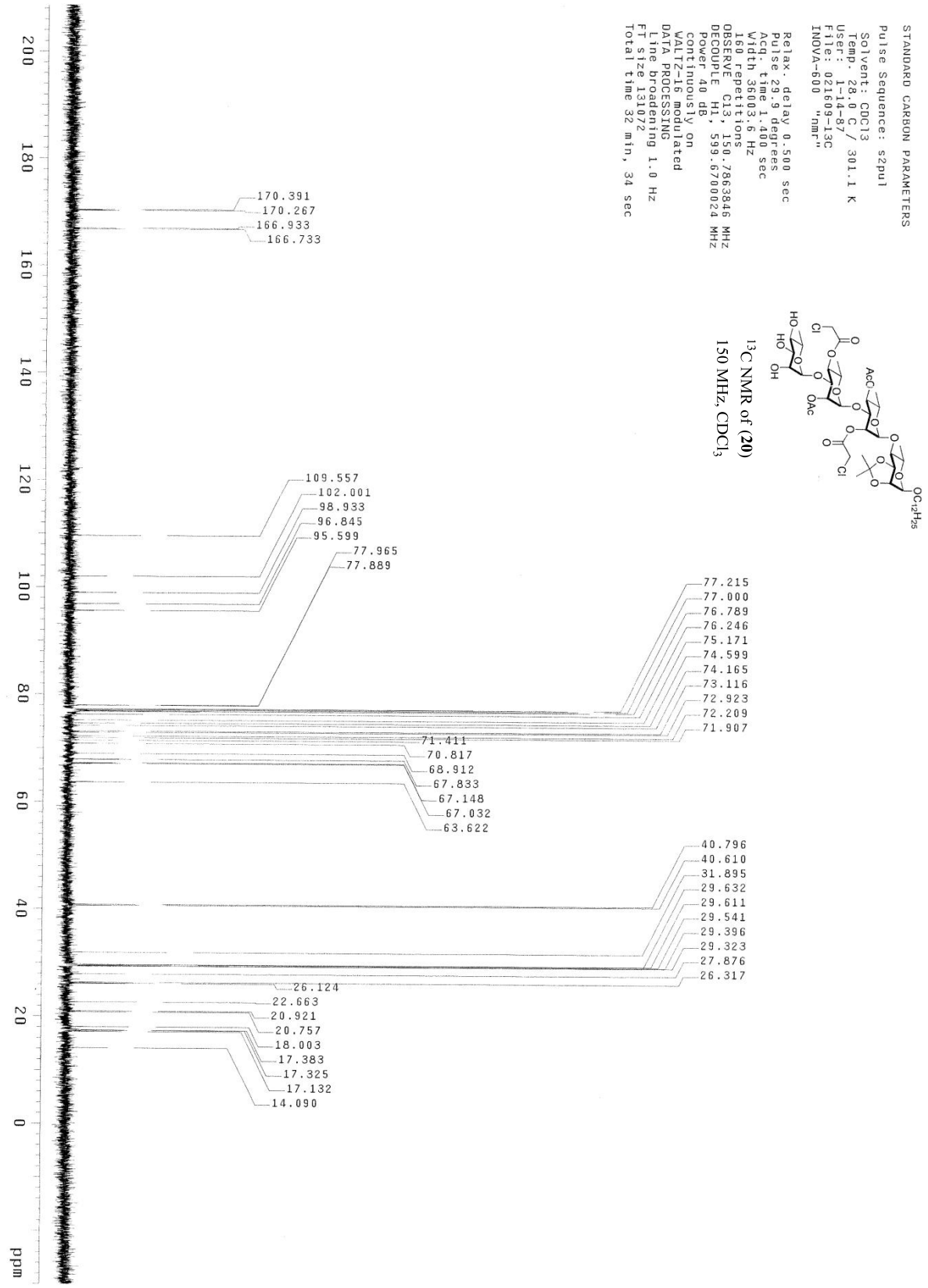
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
File: 021609-13H
INOVA-600 "nmr"

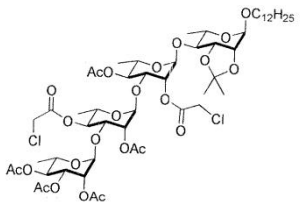
Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 4.000 sec
Width 9594.6 Hz
4 repetitions
OBSERVE H1, 599.6670081 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 131072
Total time 0 min, 20 sec



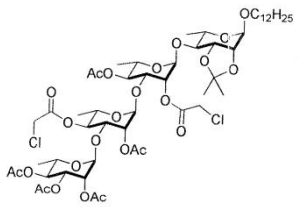
^{1}H NMR of (**20**)
600 MHz, $CDCl_3$



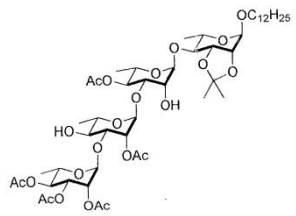

STANDARD CARBON PARAMETERS
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
User: l-14-87
File: 021609-13C
INOVA-600 "nmr"
Relax. delay 0.500 sec
Pulse 29.9 degrees
Acq. time 1.400 sec
Width 36003.6 Hz
160 repetitions
OBSERVE C13, 150.7863846 MHz
DECOUPLE H1, 599.6700024 MHz
Power 40 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 32 min, 34 sec
13C NMR of (20)
150 MHz, CDCl3
170.391
170.267
166.933
166.733
109.557
102.001
98.933
96.845
95.599
77.965
77.889
77.215
77.000
76.789
76.246
75.171
74.599
74.165
73.116
72.923
72.209
71.907
71.411
70.817
68.912
67.833
67.148
67.032
63.622
40.796
40.610
31.895
29.632
29.611
29.541
29.396
29.323
27.876
26.317
26.124
22.663
20.921
20.757
18.003
17.383
17.325
17.132
14.090
ppm



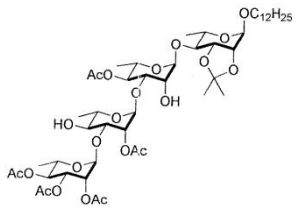
^{1}H NMR of (**21**)
600 MHz, $CDCl_3$



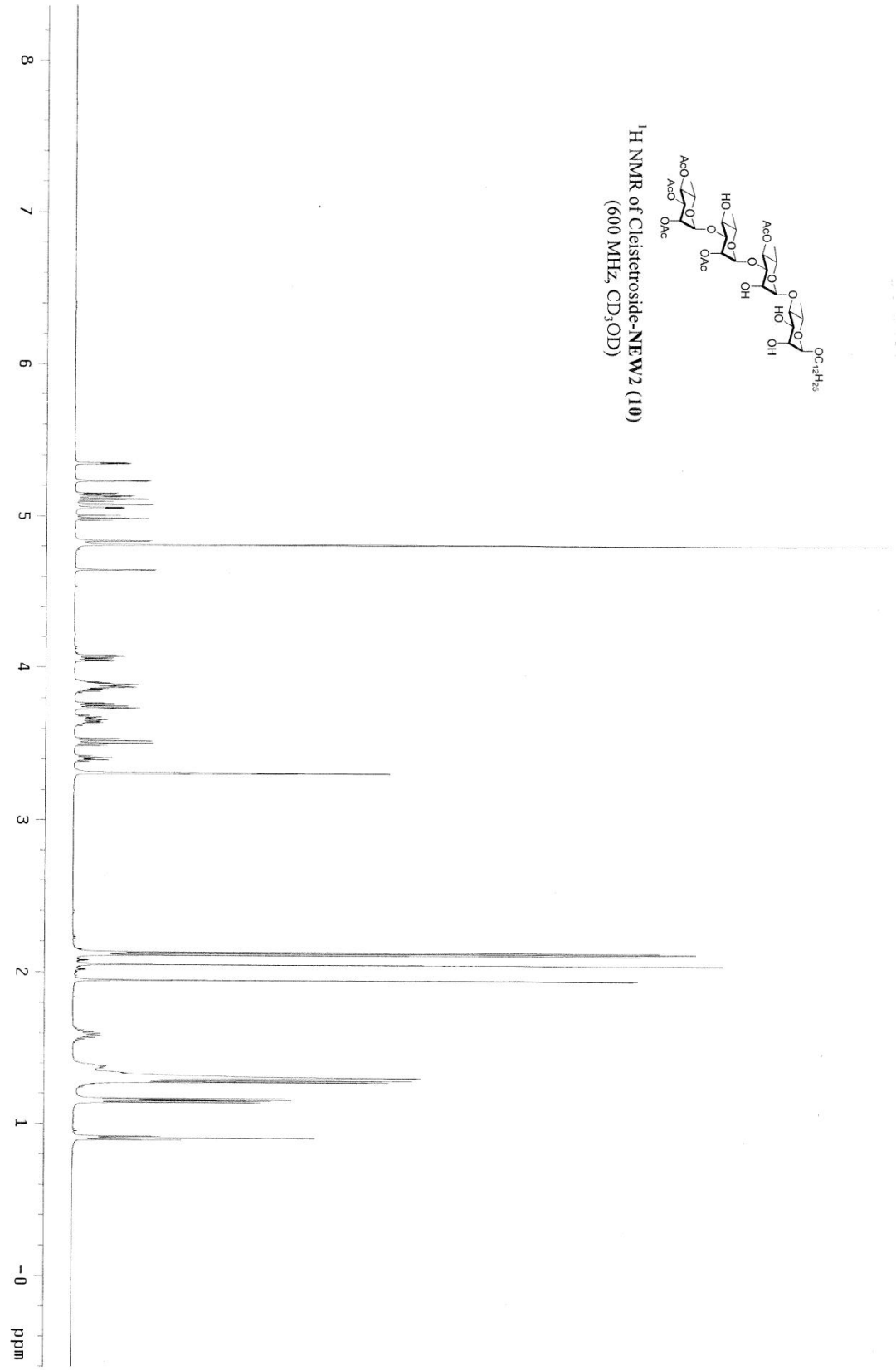
^{13}C NMR of (**21**)
150 MHz, $CDCl_3$



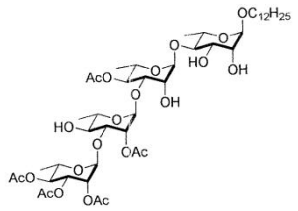
^{1}H NMR of (**J**)
600 MHz, $CDCl_3$



^{13}C NMR of (**J**)
150 MHz, $CDCl_3$



^{1}H NMR of Cleistetroside-**NEW2 (10)**
(600 MHz, CD_3OD)



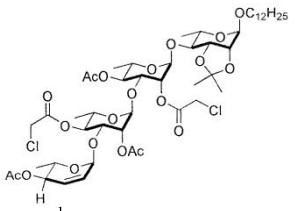
^{13}C NMR of Cleistetroside-**NEW2 (10)**
(150 MHz, CD_3OD)

STANDARD PROTON PARAMETERS

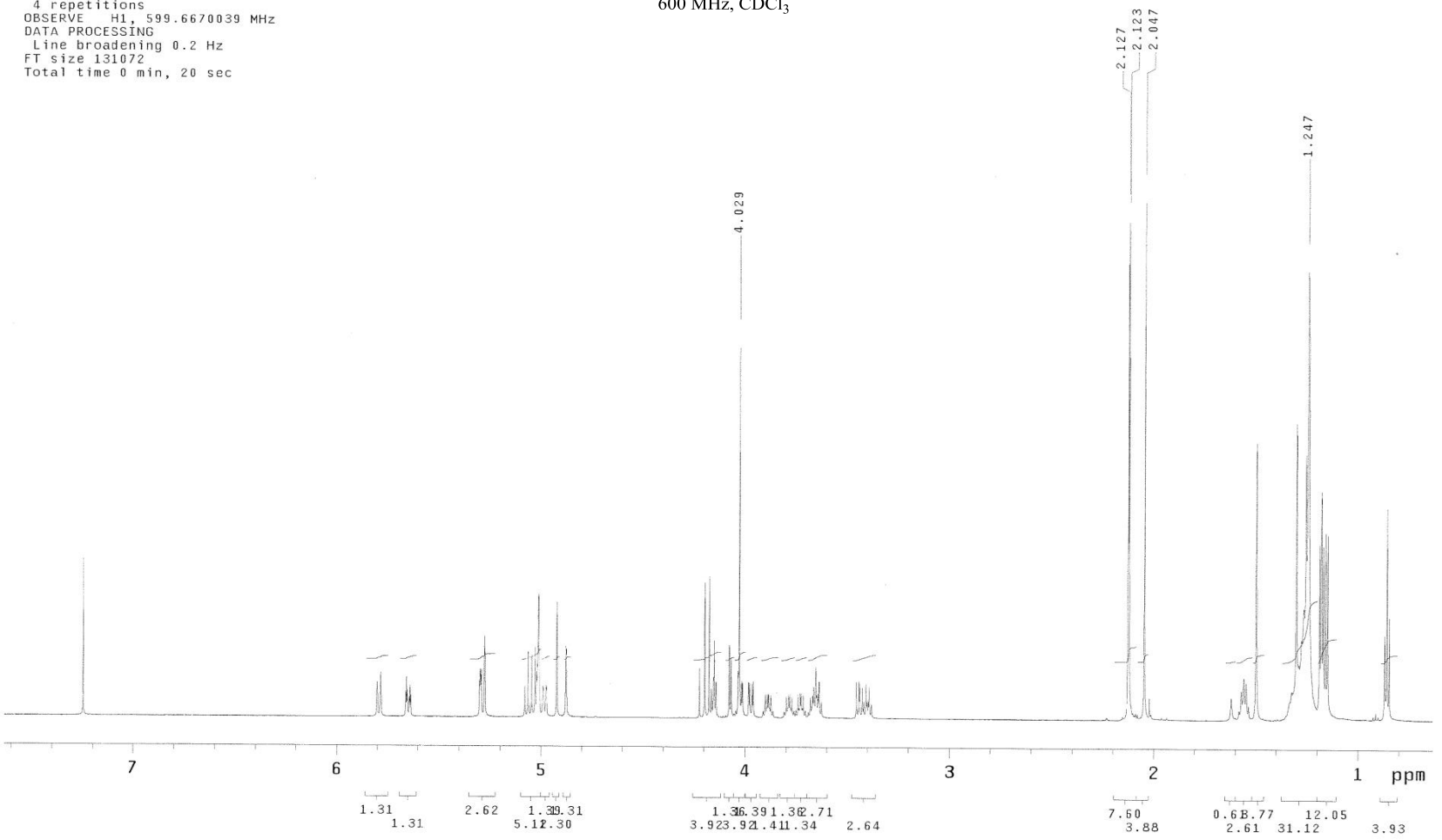
Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 28.0 C / 301.1 K
File: 021309-1H
INOVA-600 "inova600"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 4.000 sec
Width 9594.6 Hz
4 repetitions
OBSERVE H1, 599.6670039 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 131072
Total time 0 min, 20 sec



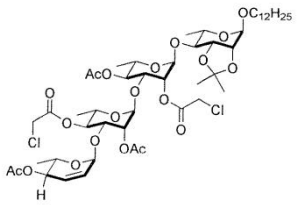
^{1}H NMR of (**K**)
600 MHz, $CDCl_3$



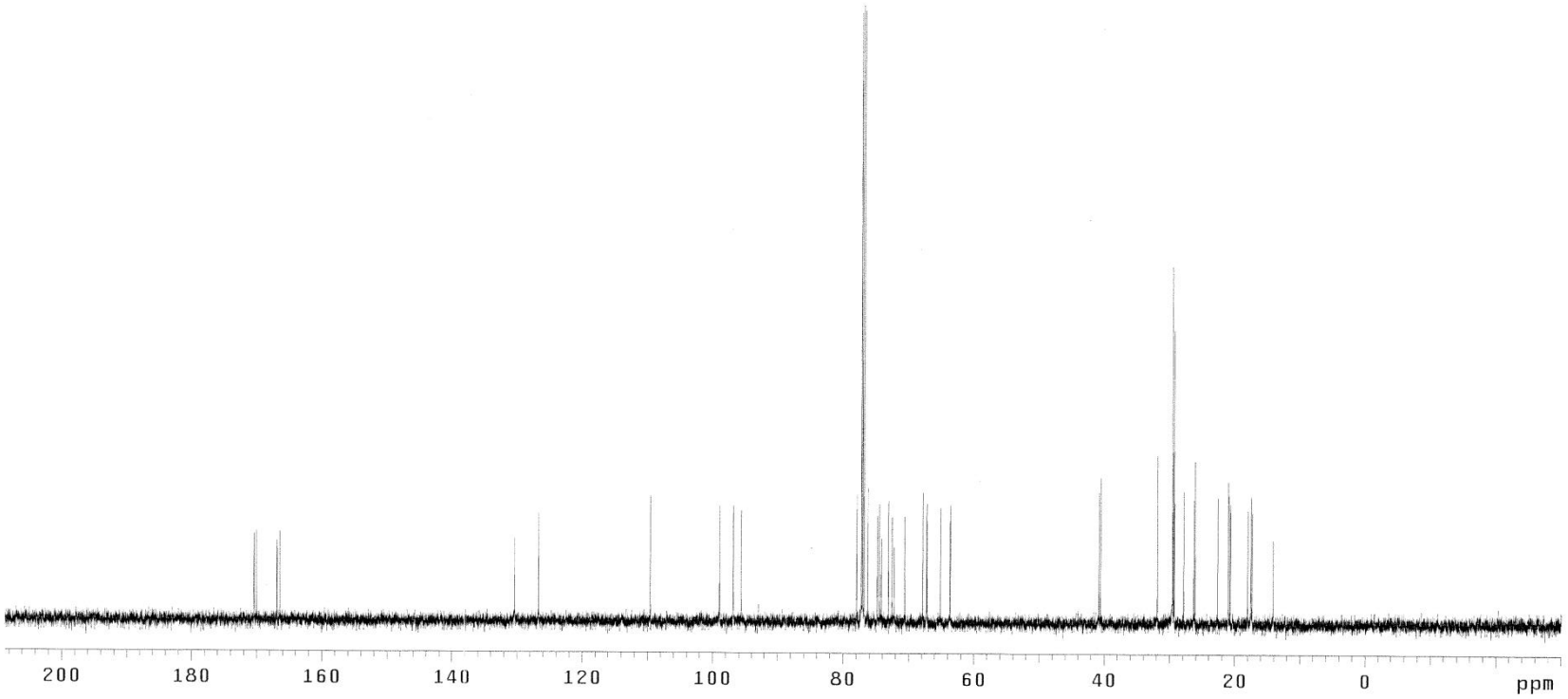
STANDARD CARBON PARAMETERS

Pulse Sequence: s2pul
 Solvent: CDCl3
 Temp. 28.0 C / 301.1 K
User: 1-14-87
File: 021309-1C
INOVA-600 "inova600"

 Relax. delay 0.500 sec
 Pulse 29.9 degrees
 Acq. time 1.400 sec
 Width 36003.6 Hz
 240 repetitions
OBSERVE C13, 150.7863857 MHz
DECOUPLE H1, 599.6700024 MHz
 Power 40 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 1.0 Hz
FT size 131072
Total time 32 min, 34 sec



^{13}C NMR of (**K**)
150 MHz, $CDCl_3$



S15

STANDARD PROTON PARAMETERS

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:

Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 28.0 C / 301.1 K
File: 021609-7H
INOVA-600 "inova600"

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Pulse 30.0 degrees
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4 repetitions
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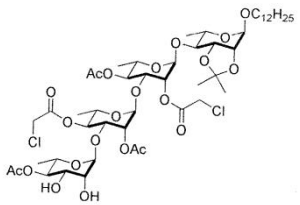
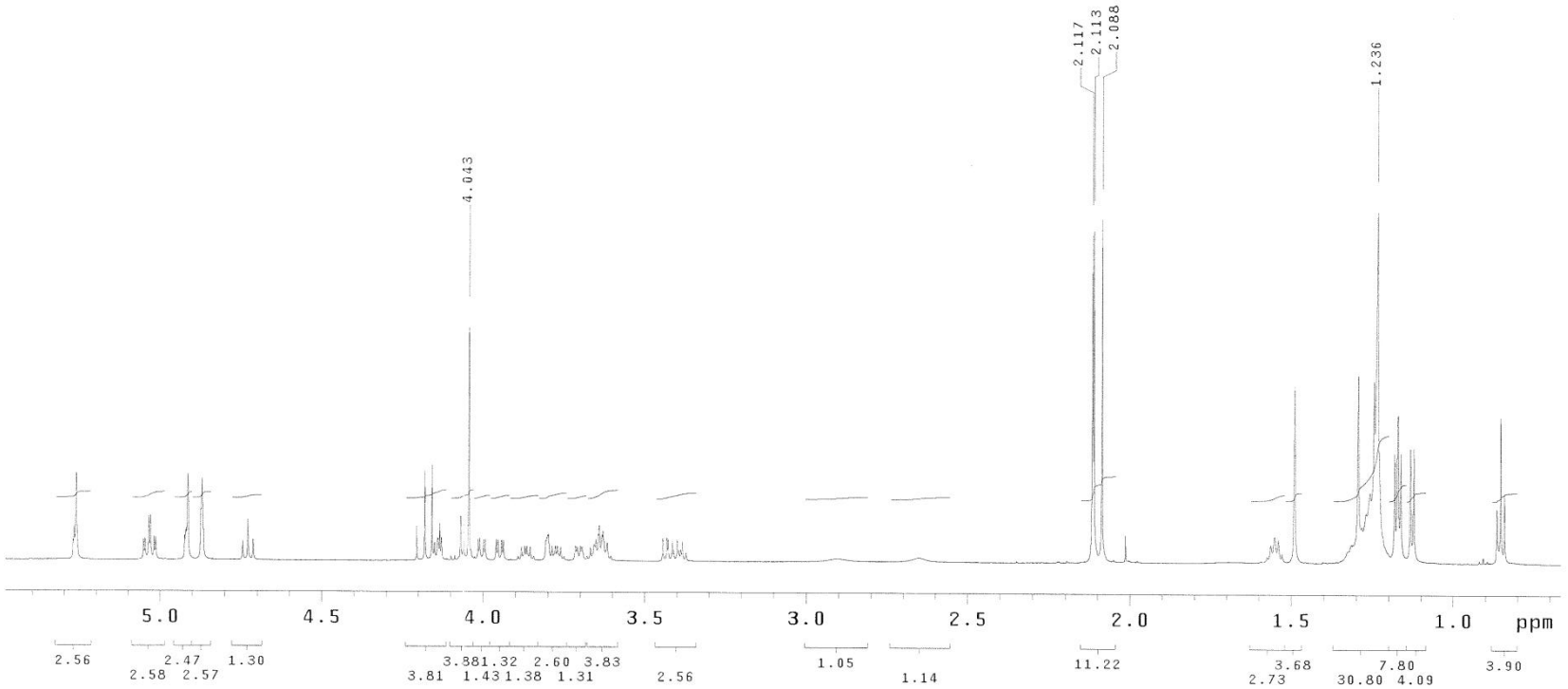
DATA PROCESSING
Line broadening 0.2 Hz
FT size 131072
Total time 0 min, 20 sec



^{1}H NMR of (**19**)
600 MHz, $CDCl_3$



STANDARD CARBON PARAMETERS

Pulse Sequence: s2pul
 Solvent: CDCl3
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User: 1-14-87
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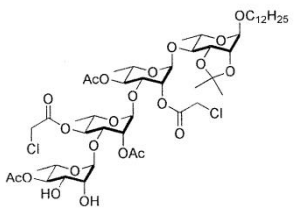
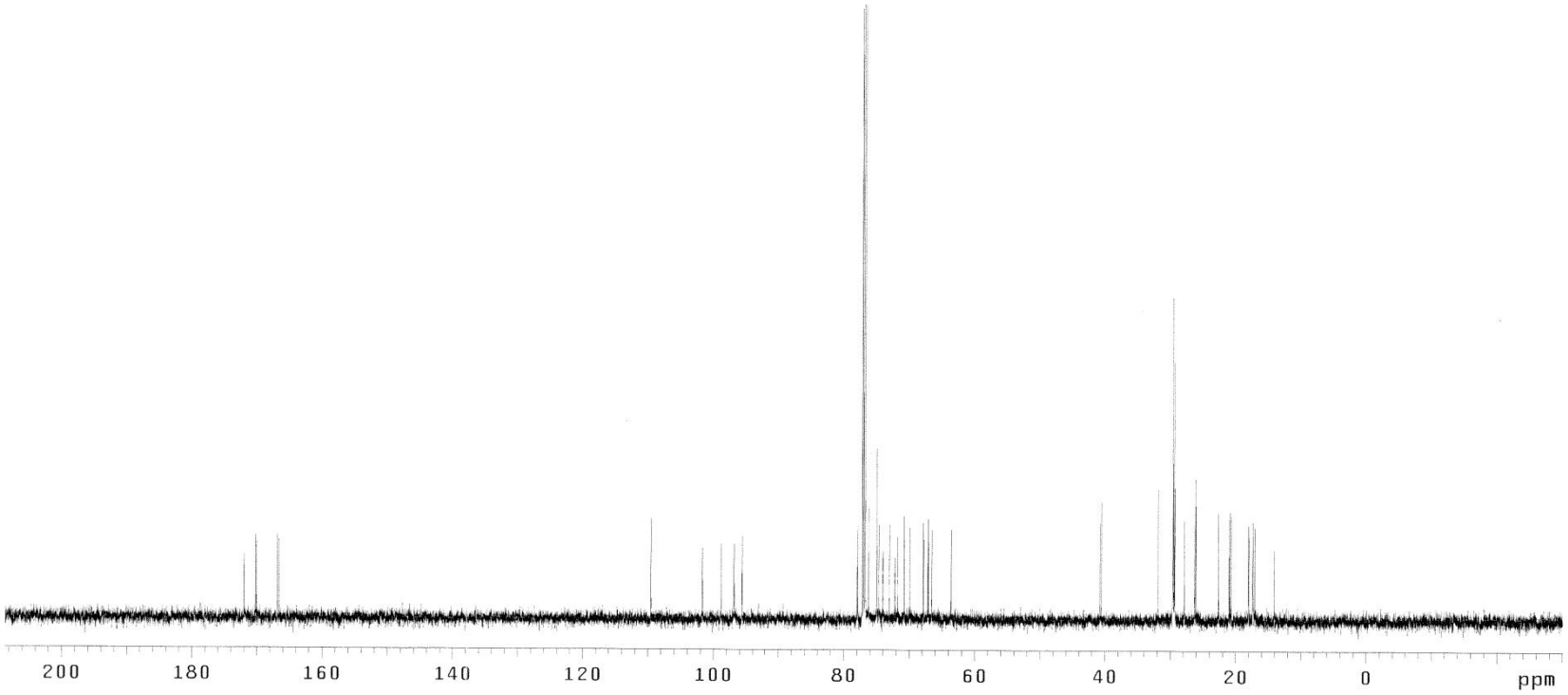
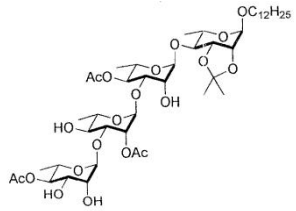
 Pulse 29.9 degrees
 Acq. time 1.400 sec
 Width 36003.6 Hz
 224 repetitions
OBSERVE C13, 150.7863857 MHz
DECOUPLE H1, 599.6700024 MHz
 Power 40 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 1.0 Hz
FT size 131072
Total time 32 min, 34 sec

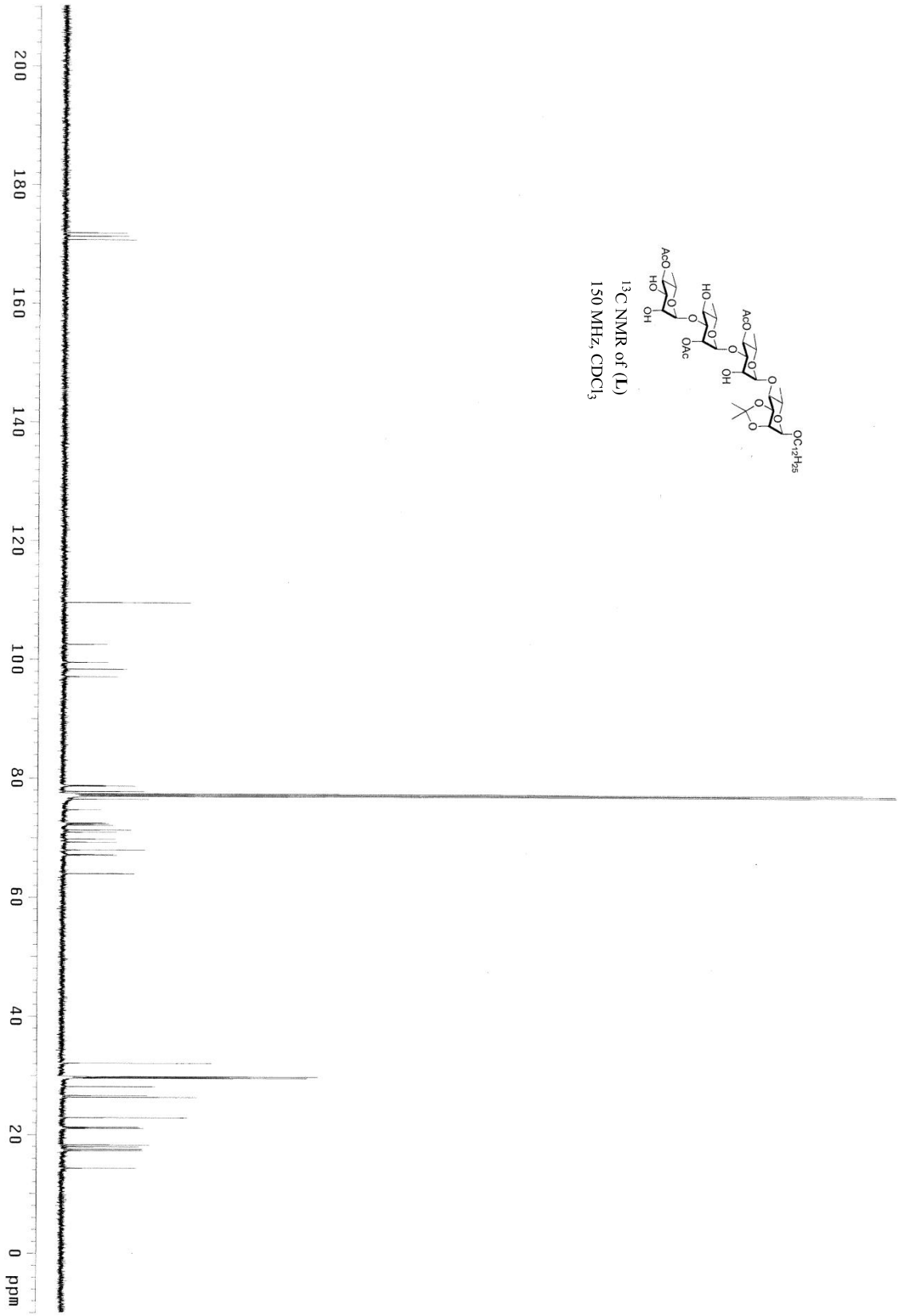


^{13}C NMR of (**19**)
150 MHz, $CDCl_3$

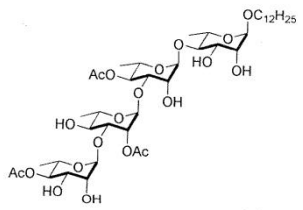




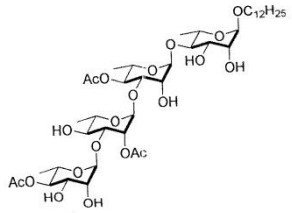
^{1}H NMR of (**L**)
600 MHz, $CDCl_3$



^{13}C NMR of (**L**)
150 MHz, $CDCl_3$



^{1}H NMR of Cleistetroside-**NEW1 (9)**
(600 MHz, CD_3OD)



^{13}C NMR of Cleistetroside-**NEW1 (9)**
(150 MHz, CD_3OD)

Table 1. ^{1}H NMR chemical shifts (δ/ppm) of Cleistetrosides (**3**, **6**, and **new**)[a]

Compd	Ring	H1	H2	H3	H4	H5	5-CH_3	Others
Cleistetro-	A	4.6382	3.7261	3.7413	3.5143	3.6350	1.2773	2.0877, 2.1339, 2.1369, 2.1451 (**CH_3**COO)
side-3	B	5.2236	4.0560	3.8774	5.1112	3.8869	1.1455	3.3967 (dt, $^{2}J_{ab}$= 9.6 Hz, $^{3}J_{Ha,Hc}$ = $^{3}J_{Ha,Hd}$ = 6.2 Hz, H^a),
	C	4.8724	5.0139	4.2317	4.9836	4.0415	1.1635	3.6629 (dt, H^b); 1.53 - 1.64 (m, H^c/H^d);
	D	4.8166	4.8443	3.6845	3.3325	3.5104	1.2190	1.26-1.40 (m, 18H), 0.91 (t, ^{3}J = 7.1 Hz, 3H, H^{α})
Cleistetro-	A	4.6398	3.7292	3.7425	3.5153	3.6349	1.2773	2.0943, 2.1164, 2.1237, 2.1410, 2.1514 (**CH_3**COO)
side-6	B	5.2233	4.0597	3.8790	3.8882	5.1058	1.1450	3.3977 (dt, $^{2}J_{ab}$= 9.6 Hz, $^{3}J_{Ha,Hc}$ = $^{3}J_{Ha,Hd}$ = 6.2 Hz, H^a),
	C	4.8746	5.0233	4.2695	4.9901	4.0528	1.1674	3.6489 (dt, H^b); 1.53-1.64 (m, H^c/H^d);
	D	4.8545	4.8645	3.8483	4.8545	3.7030	1.1158	1.26-1.40 (m, 18H), 0.91 (t, ^{3}J =7.1 Hz, 3H, H^{α})
Cleistetro-	A	4.6421	3.7312	3.7525	3.5198	3.6357	1.2796	2.0837, 2.0945, 2.1197 (**CH_3**COO)
side-new	B	5.2302	3.8702	3.8815	5.1120	3.8815	1.1437	3.3999 (dt, $^{2}J_{ab}$= 9.6 Hz, $^{3}J_{Ha,Hc}$ = $^{3}J_{Ha,Hd}$ = 6.2 Hz, H^a),
	C	4.8258	5.0082	4.0088	3.4564	3.8574	1.2825	3.6586 (dt, H^b); 1.53 - 1.64 (m, H^c/H^d);
	D	4.9943	3.9585	3.7291	4.9021	3.6769	1.1041	1.26-1.40 (m, 18H), 0.90 (t, ^{3}J =7.1 Hz, 3H, H^{α})

(a) Chemical shifts and coupling constants were determined by simulation

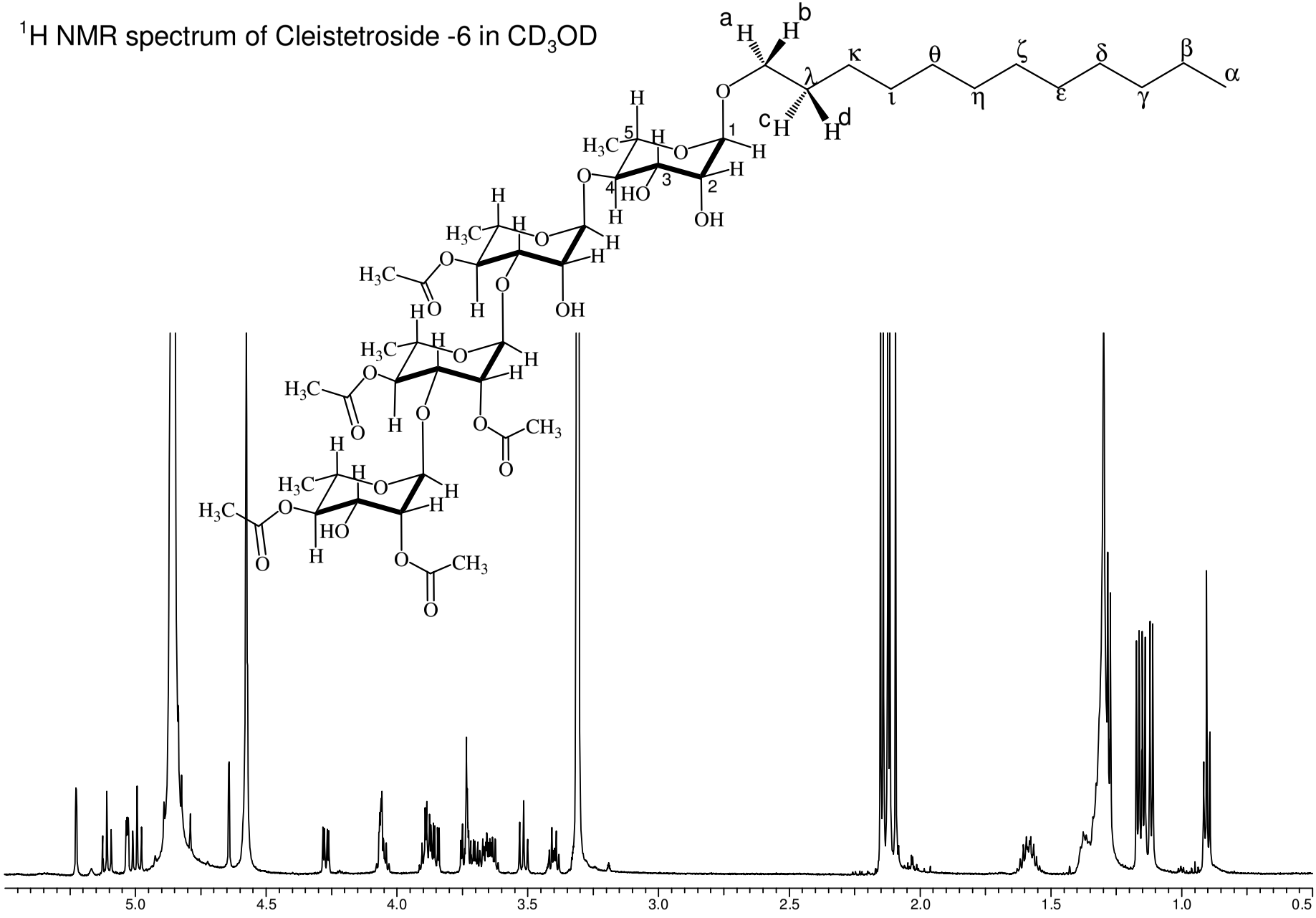
Table 2. $^{3}J_{HH}$ coupling constants (J/Hz) in Cleistetrosides (**3**, **6**, and **new**)

Compd	$^{3}J_{HH}$	Ring A	Ring B	Ring C	Ring D
Cleistetroside-3	H1 – H2	1.74	1.71	1.64	1.72
	H2 – H3	3.45	3.32	3.50	3.42
	H3 – H4	9.42	9.83	9.95	9.77
	H4 – H5	9.42	9.83	9.95	9.77
	H5- 5-CH_3	6.22	6.28	6.28	6.28
Cleistetroside-6	H1 – H2	1.71	1.75	1.70	1.71
	H2 – H3	3.36	3.40	3.49	3.55
	H3 –H4	9.51	9.88	9.94	9.91
	H4 – H5	9.51	9.88	9.94	9.91
	H5- 5-CH_3	6.22	6.28	6.31	6.28
Cleistetroside-new	H1 – H2	1.70	1.98	1.73	1.68
	H2 – H3	3.36	3.31	3.44	3.48
	H3 – H4	9.22	9.85	9.67	9.82
	H4 – H5	9.22	9.85	9.67	9.82
	H5- 5-CH_3	6.22	6.28	6.28	6.28

Table 3. ^{13}C NMR chemical shifts (δ/ppm) of Cleistetrosides (**3**, **6**, and **new**)

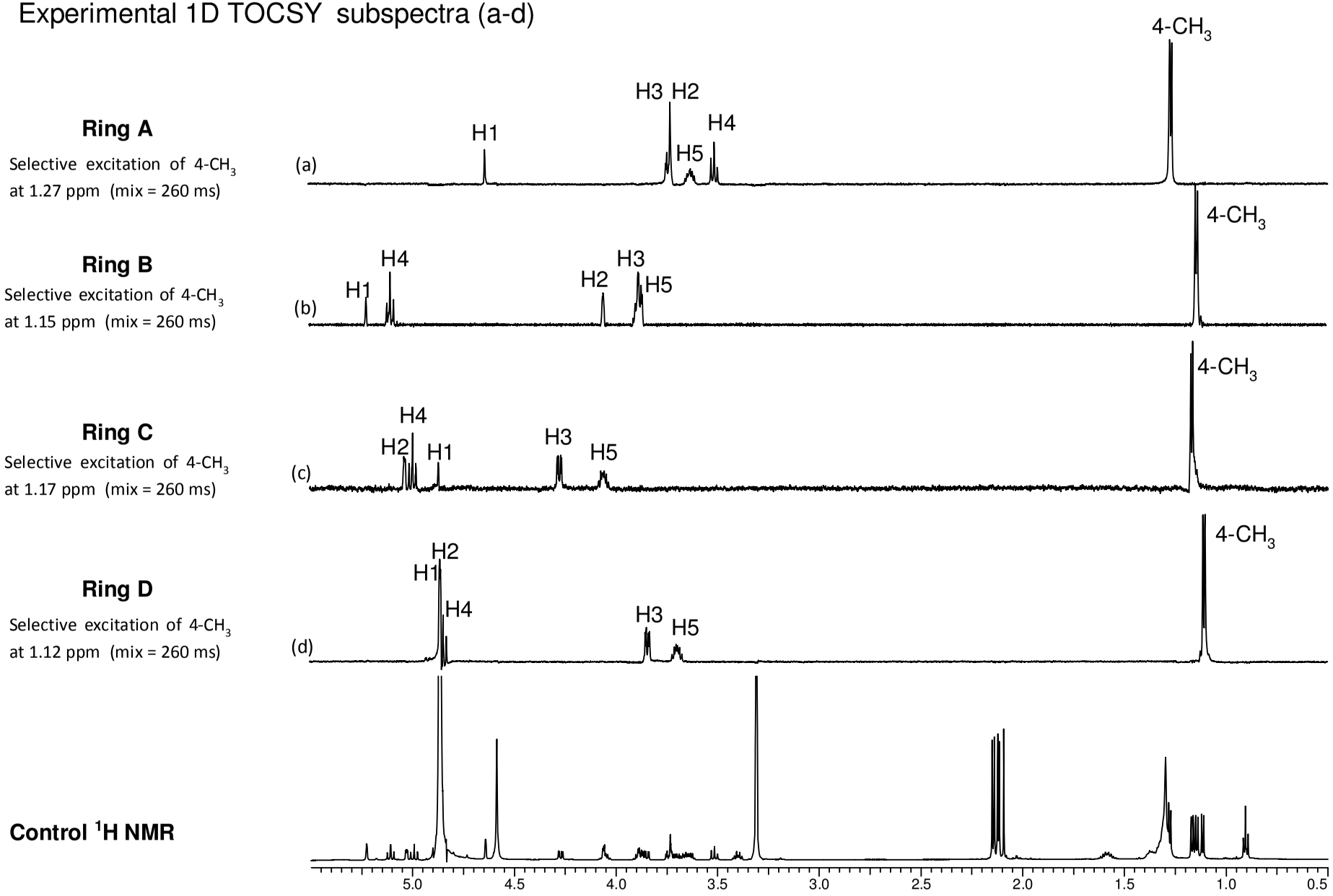
Compd	Ring	C1	C2	C3	C4	C5	Others
Cleistetro-	A	101.58	73.00	73.39	81.45	68.19	17.77, 17.84, 17.87, 18.86 (A-5-CH_3, B-5-CH_3, C-5-CH_3, D-5-CH_3)
side-3	B	103.11	72.23	73.98	79.09	68.71	20.95,20.96, 21.06,21.19 (**CH_3**COO); 172.08, 172.29, 172.35, 172.39
	C	100.81	73.65	73.94	76.56	68.27	(CH_3**CO**O); 14.60 (C_α), 23.89 (C_β), 33.23 (C_γ), 27.46 (C_k), 30.71 (C_λ),
	D	101.32	74.24	70.37	73.97	70.74	68.64 (C), 30,57, 30,61, 30,84, 30,87, 30,88, 30.89 (C_ε, C_ξ, C_ν, C_θ, C_ι)
Cleistetro-	A	101.58	72.99	73.43	81.46	68.19	17.67, 17.76, 17.81, 18.86 (A-5-CH_3, B-5-CH_3, C-5-CH_3, D-5-CH_3)
side-6	B	103.11	72.22	68.29	73.97	68.68	20.93,20.94, 21.07,21.08, 21.18 (**CH_3**COO); 172.05, 172.24, 172.33,
	C	100.92	73.43	76.27	74.04	68.29	172.41, 172.43 (CH_3**CO**O); 14.61 (C_α), 23.89 (C_β), 33.22 (C_γ), 27.46 (C_k),
	D	100.96	74.14	68.66	75.20	68.45	30.70 (C_λ), 68.66 (C), 30,56, 30,61, 30,84, 30,87, 30,88, 30.89 (C_ε, C_ξ, C_ν, C_θ, C_ι)
Cleistetro-	A	101.57	73.09	73.39	81.34	68.19	17.77 (B-5-CH_3, D-5-CH_3), 18.10, 18.87 (A-5-CH_3, C-5-CH_3)
side-new	B	103.12	72.25	78.82	74.02	68.74	20.99, 21.17, 21.19 (**CH_3**COO); 172.13, 172.25, 172.70 (CH_3**CO**O);
	C	100.94	73.89	77.86	73.50	70.58	14.60 (C_α), 23.88 (C_β), 33.22 (C_γ), 27.45 (C_k), 30.70 (C_λ), 68.65 (C),
	D	104.15	72.25	70.50	75.63	68.36	30,55, 30,60, 30,83, 30,86, 30,88 (C_ε, C_ξ, C_ν, C_θ, C_ι)

^{1}H NMR spectrum of Cleistetroside -6 in CD_3OD

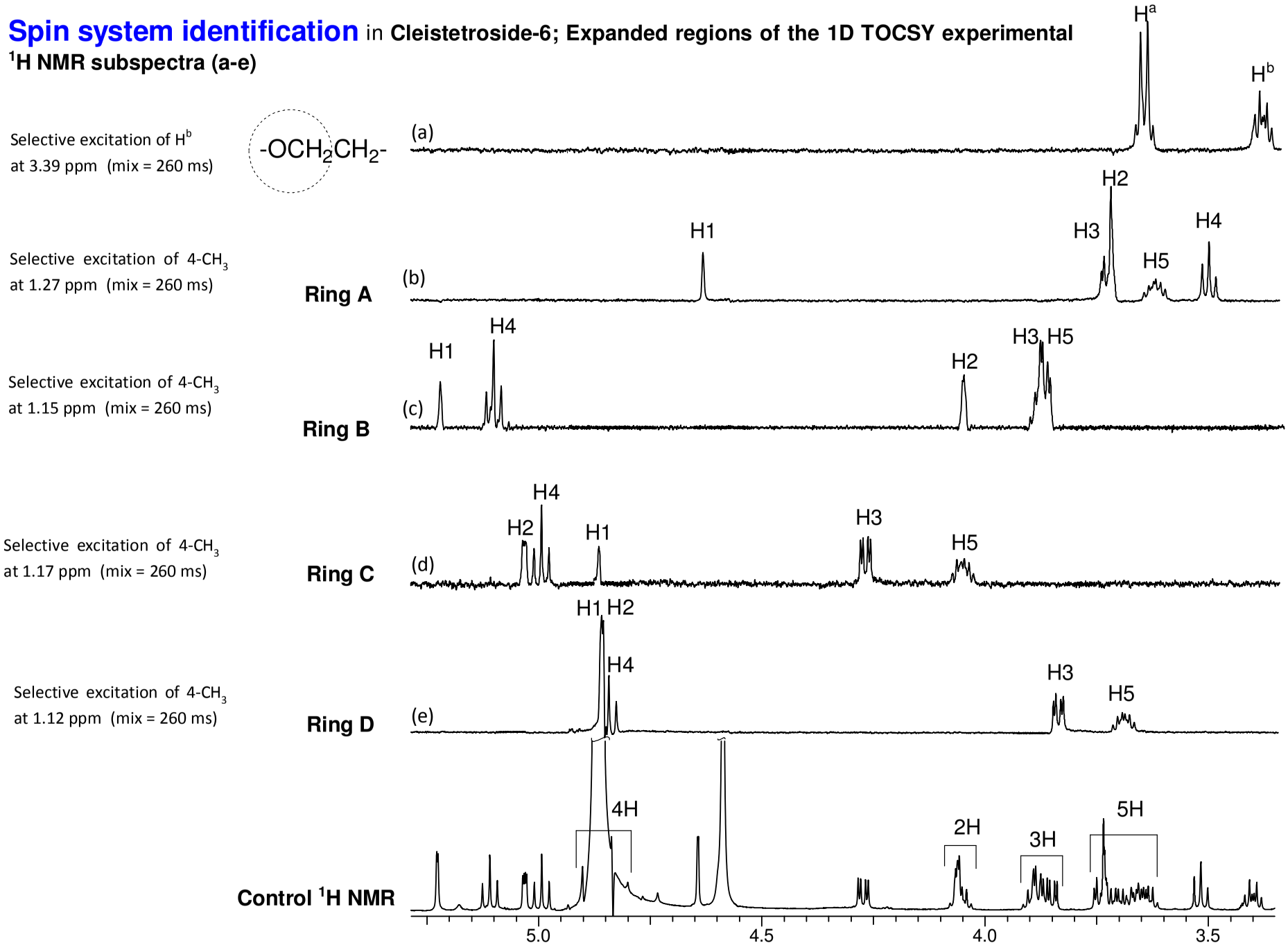


Magnetization transfer from methyl's

Experimental 1D TOCSY subspectra (a-d)



Spin system identification in **Cleistetroside-6; Expanded regions of the 1D TOCSY experimental ^{1}H NMR subspectra (a-e)**

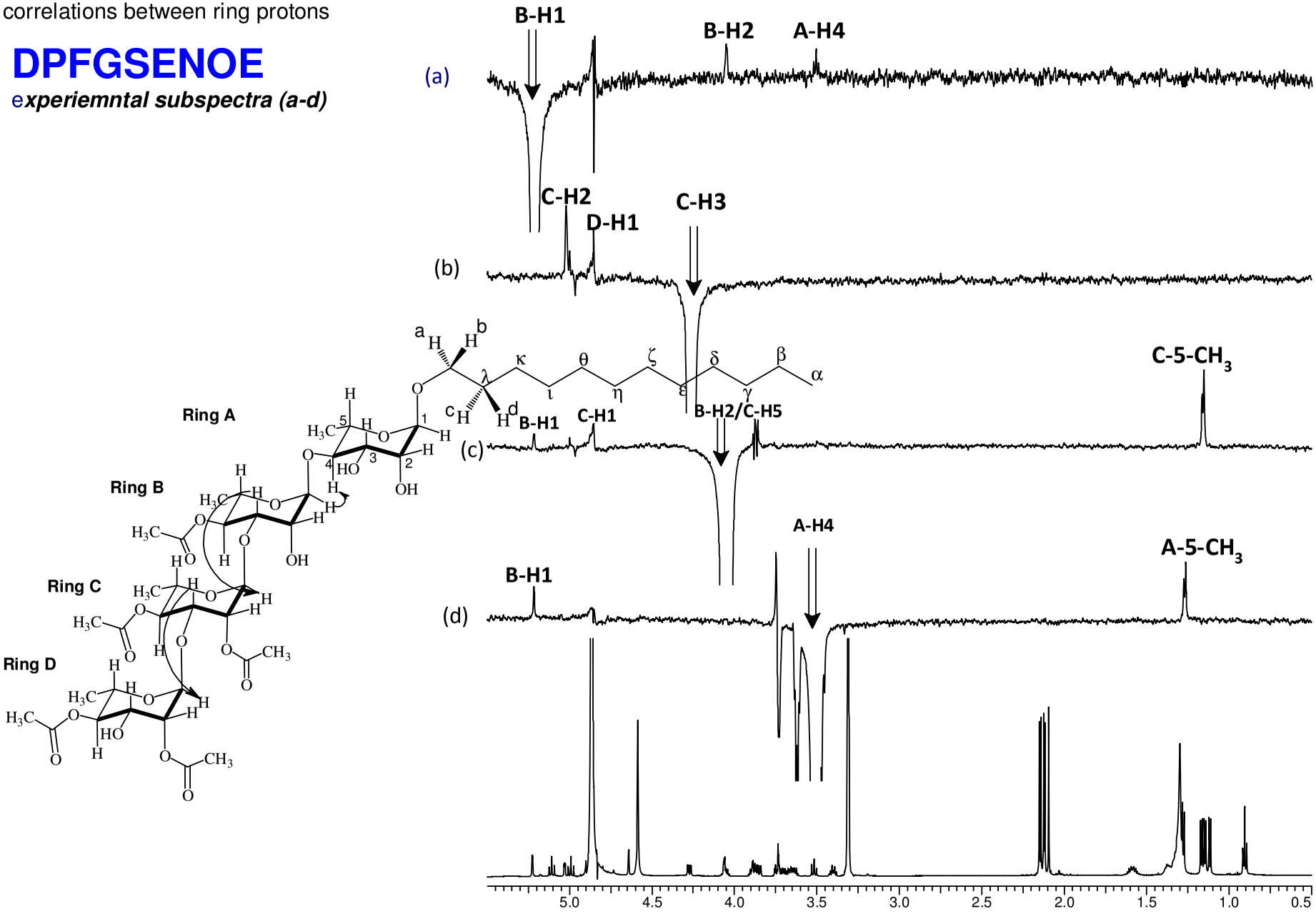


Observed **NOESY**

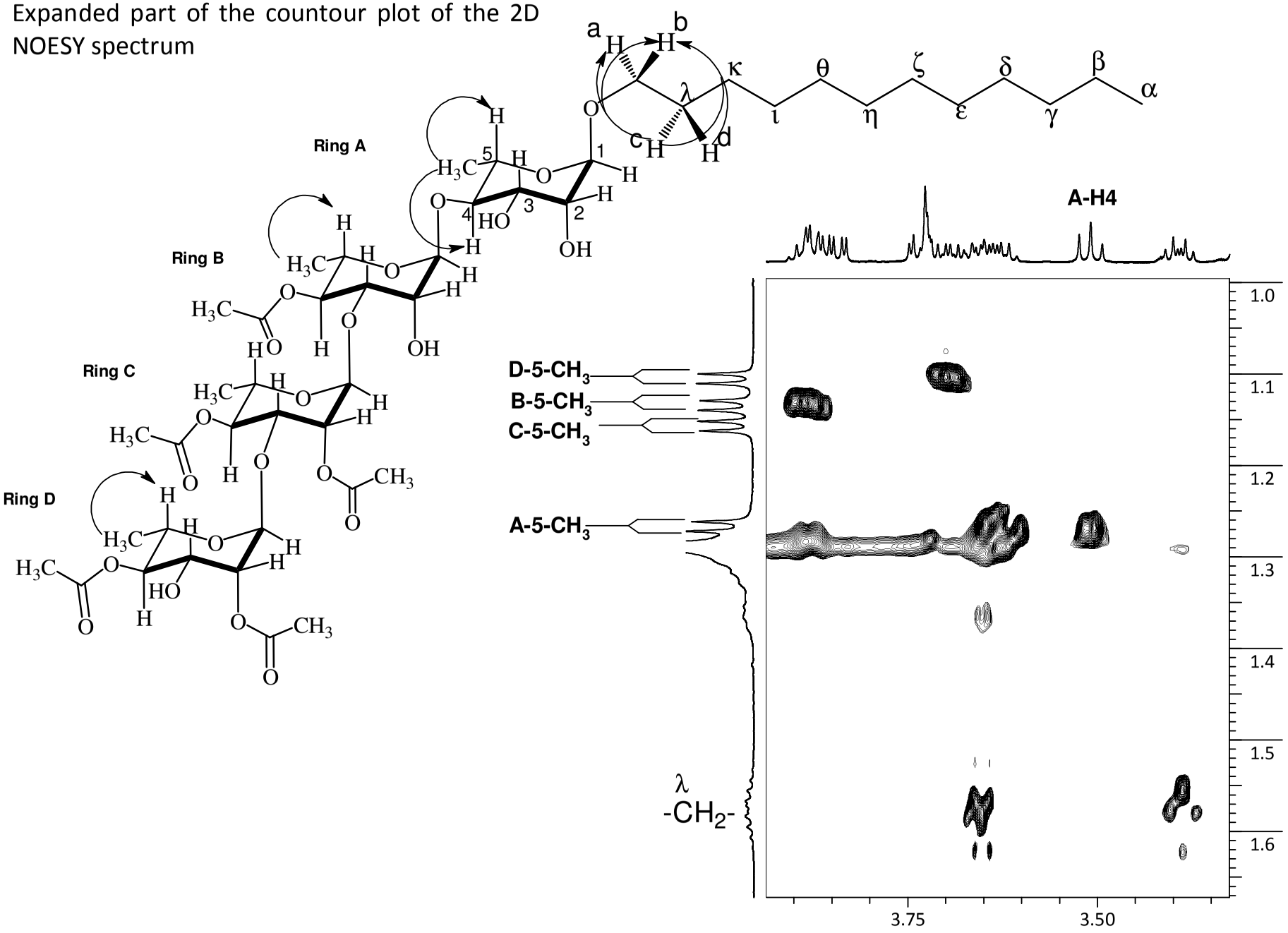
correlations between ring protons

DPFGSENOE

experiemntal subspectra (a-d)



Expanded part of the countour plot of the 2D NOESY spectrum

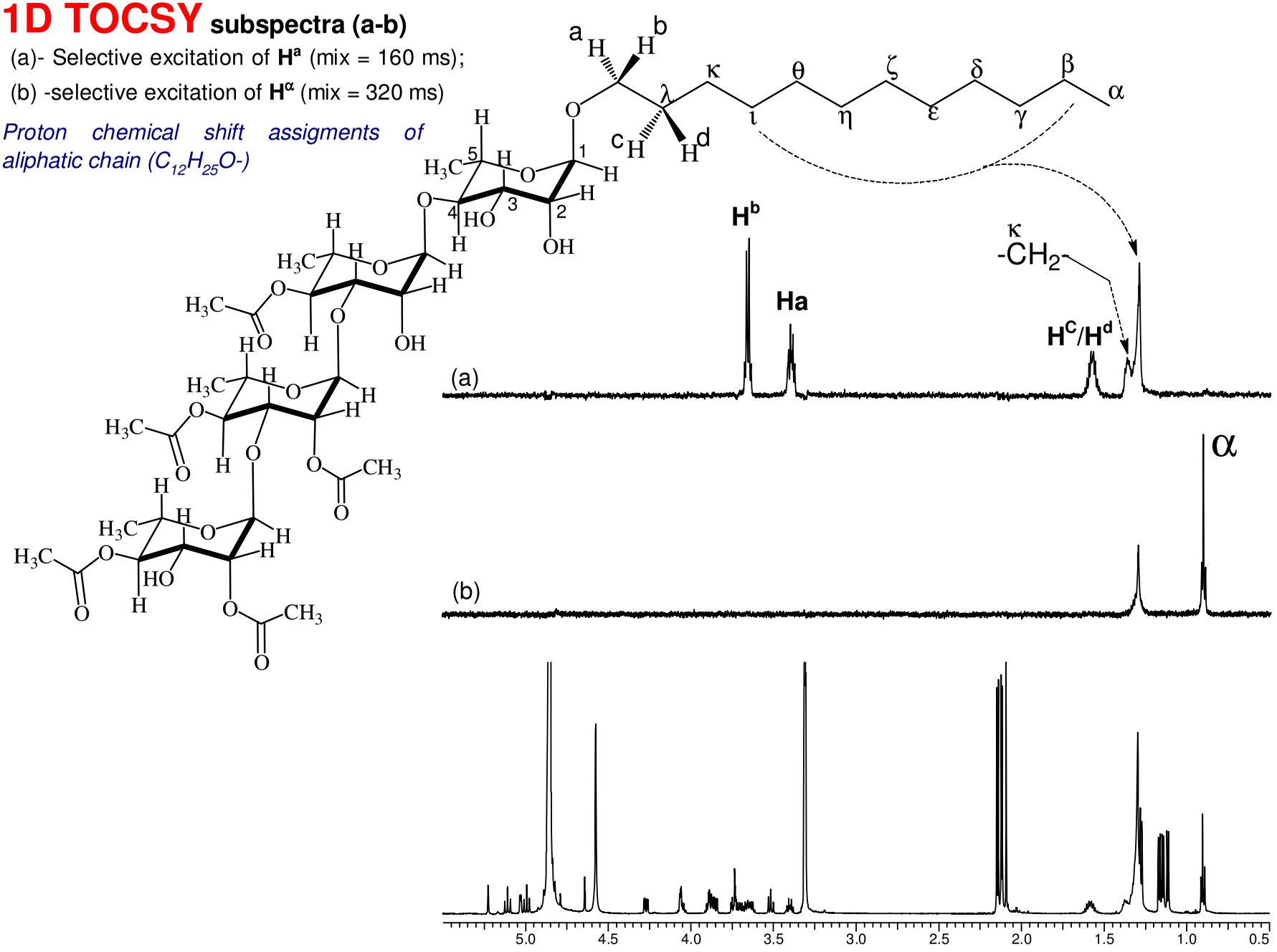


1D TOCSY subspectra (a-b)

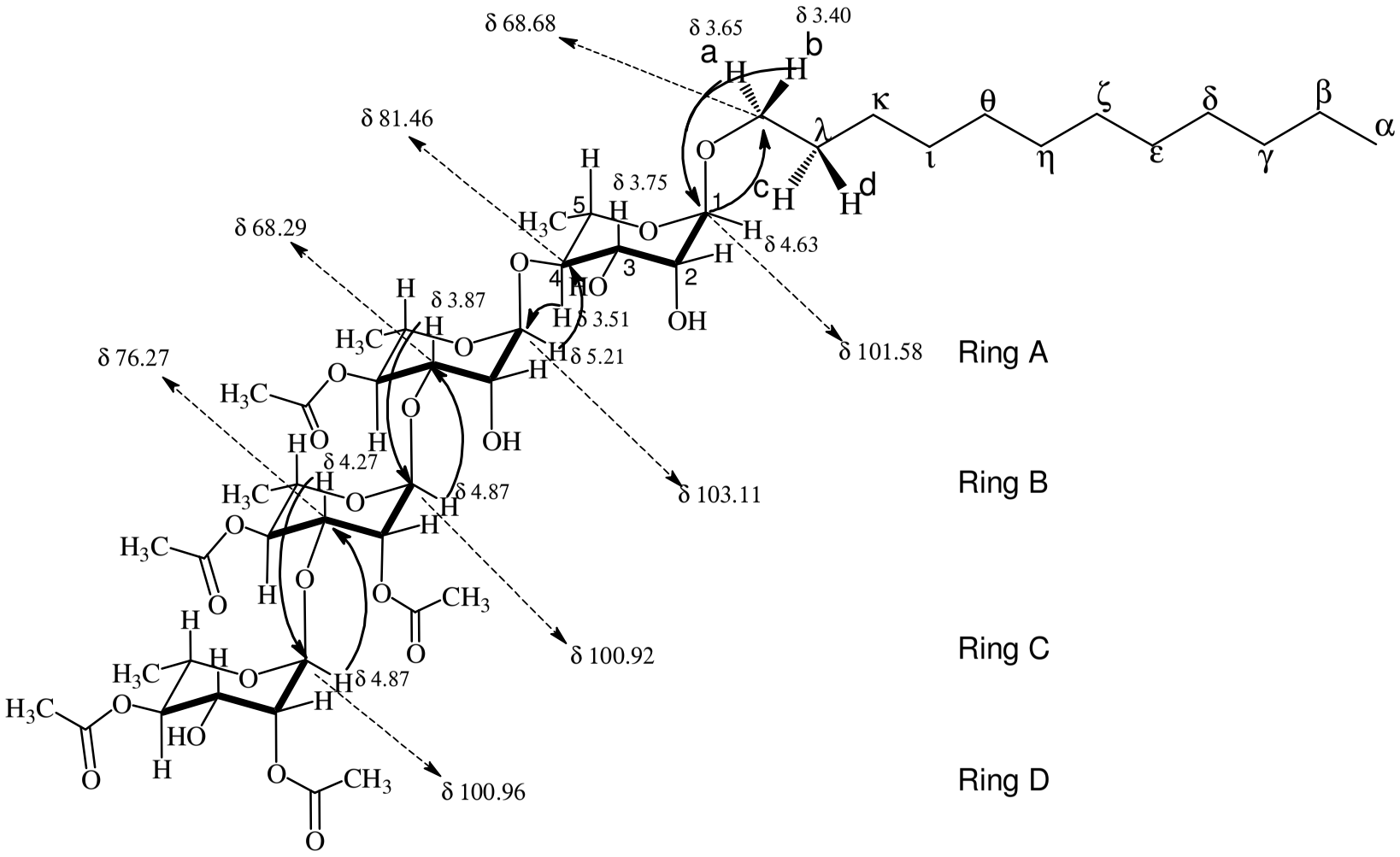
(a)- Selective excitation of H^a (mix = 160 ms);

(b) -selective excitation of H^α (mix = 320 ms)

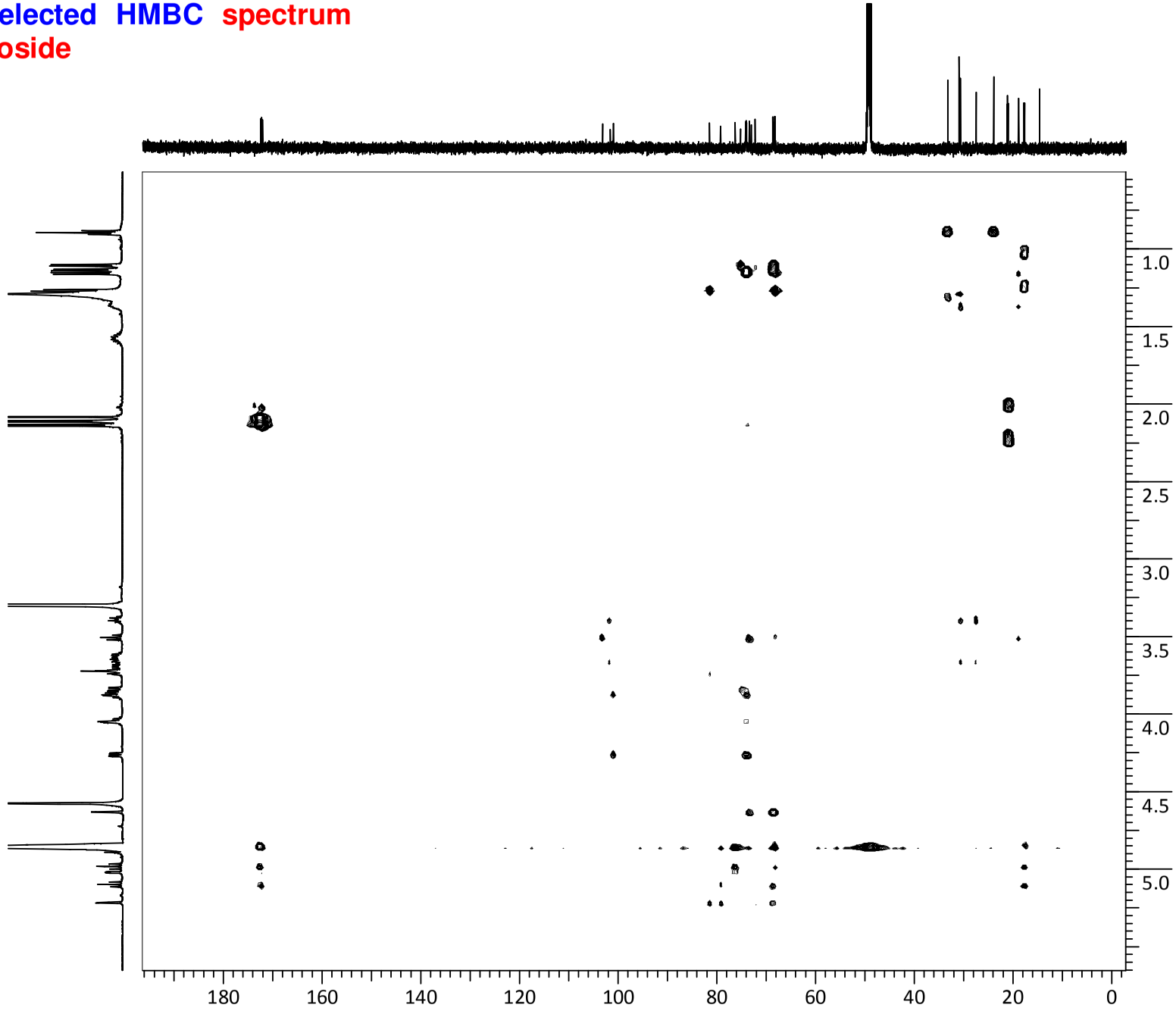
Proton chemical shift assigments of aliphatic chain ($C_{12}H_{25}O$-)



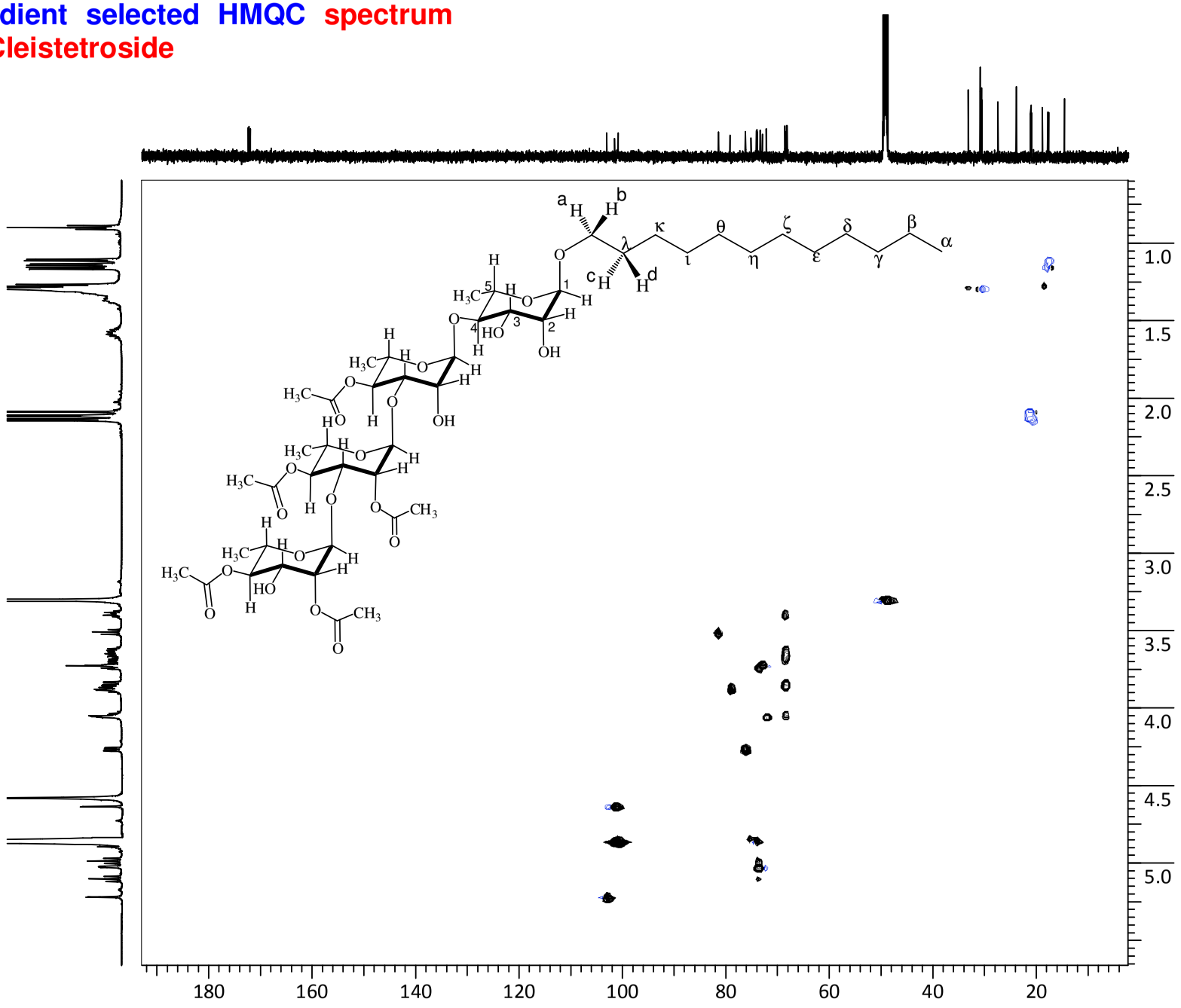
Observed key three-bond HMBC correlations confirms connectivity between four sugar untis (A-D)



Gradient selected HMBC spectrum of Cleistetroside

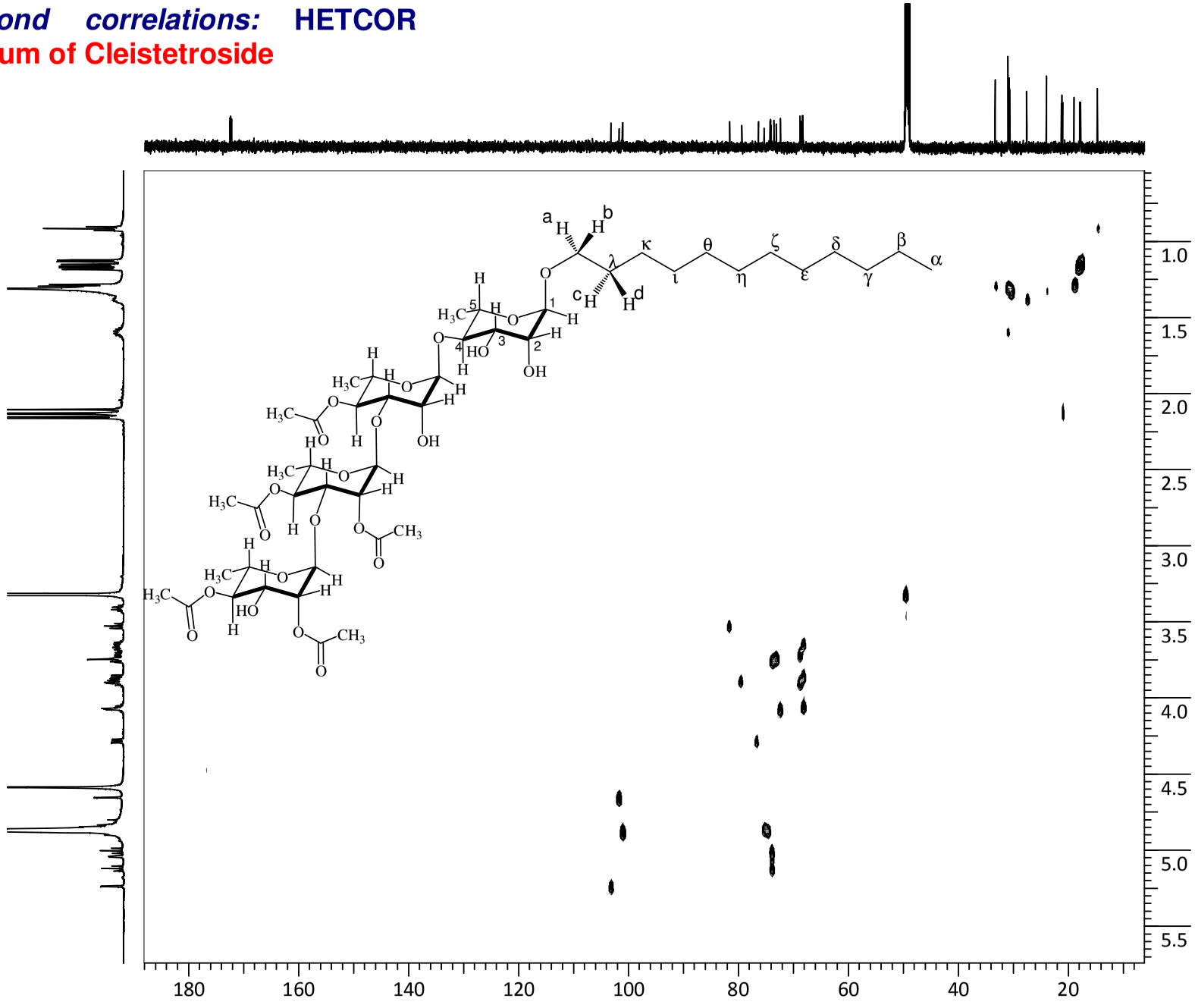


Gradient selected HMQC spectrum of Cleistetroside

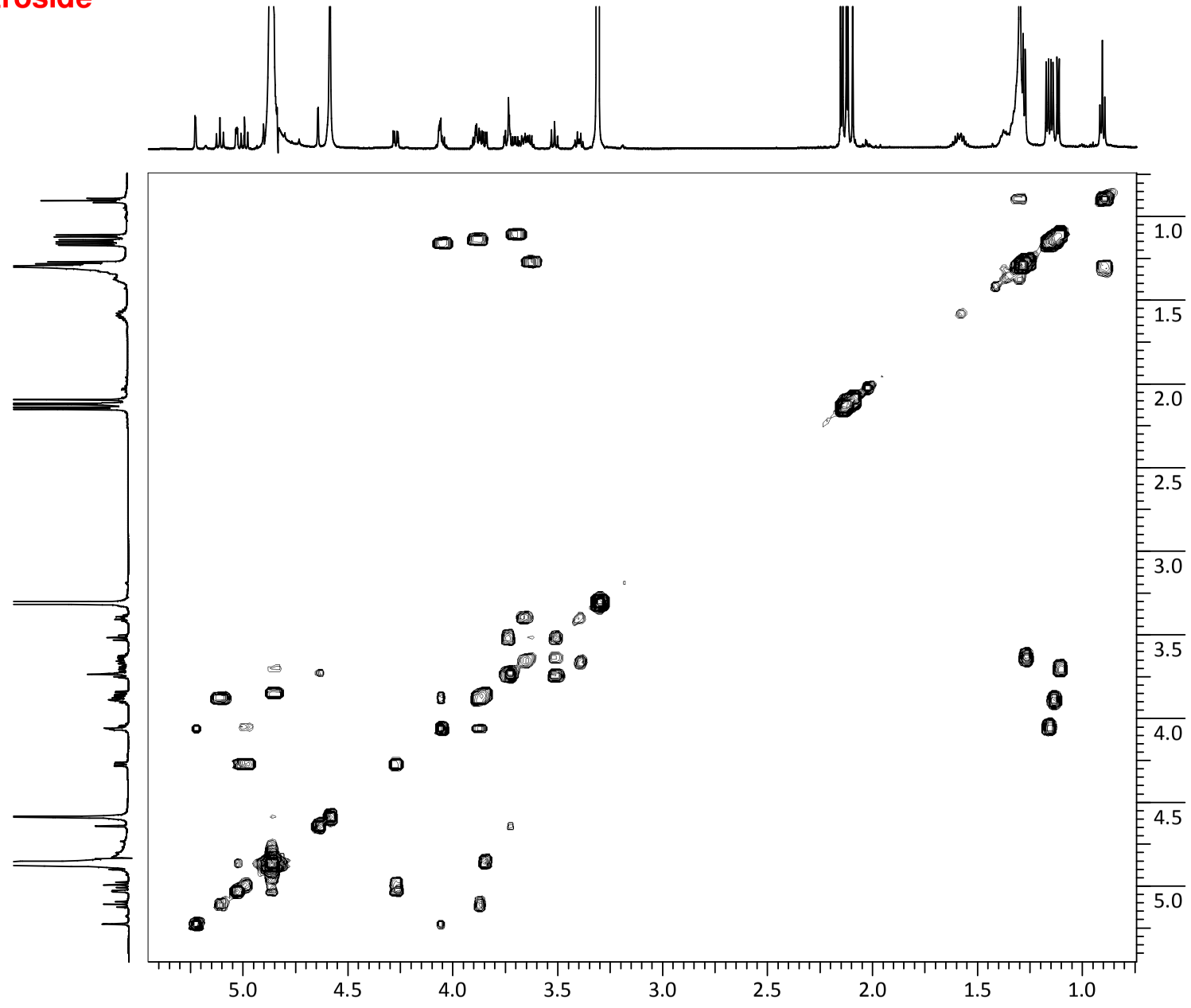


One-bond correlations: **HETCOR**

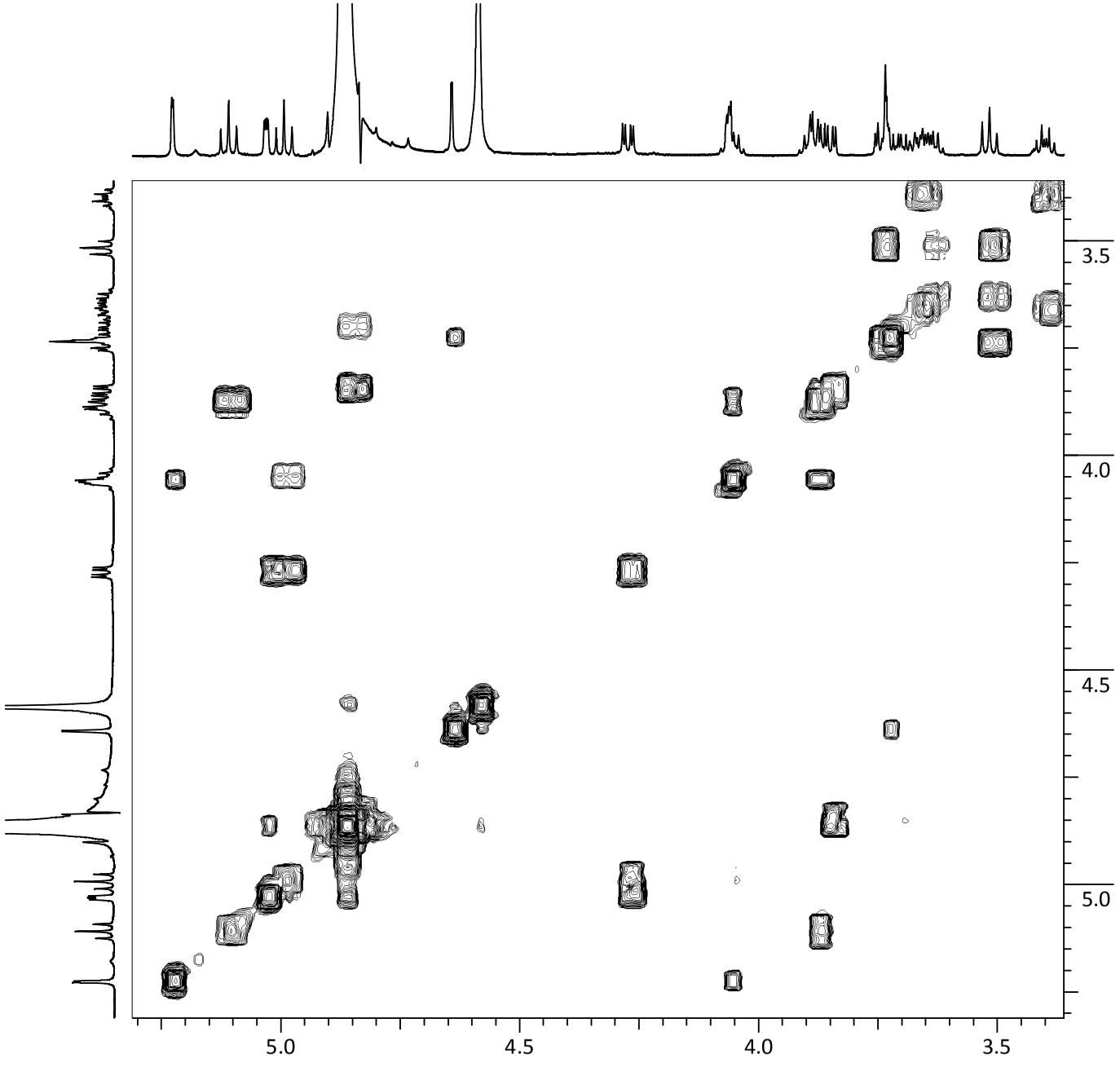
spectrum of Cleistetroside



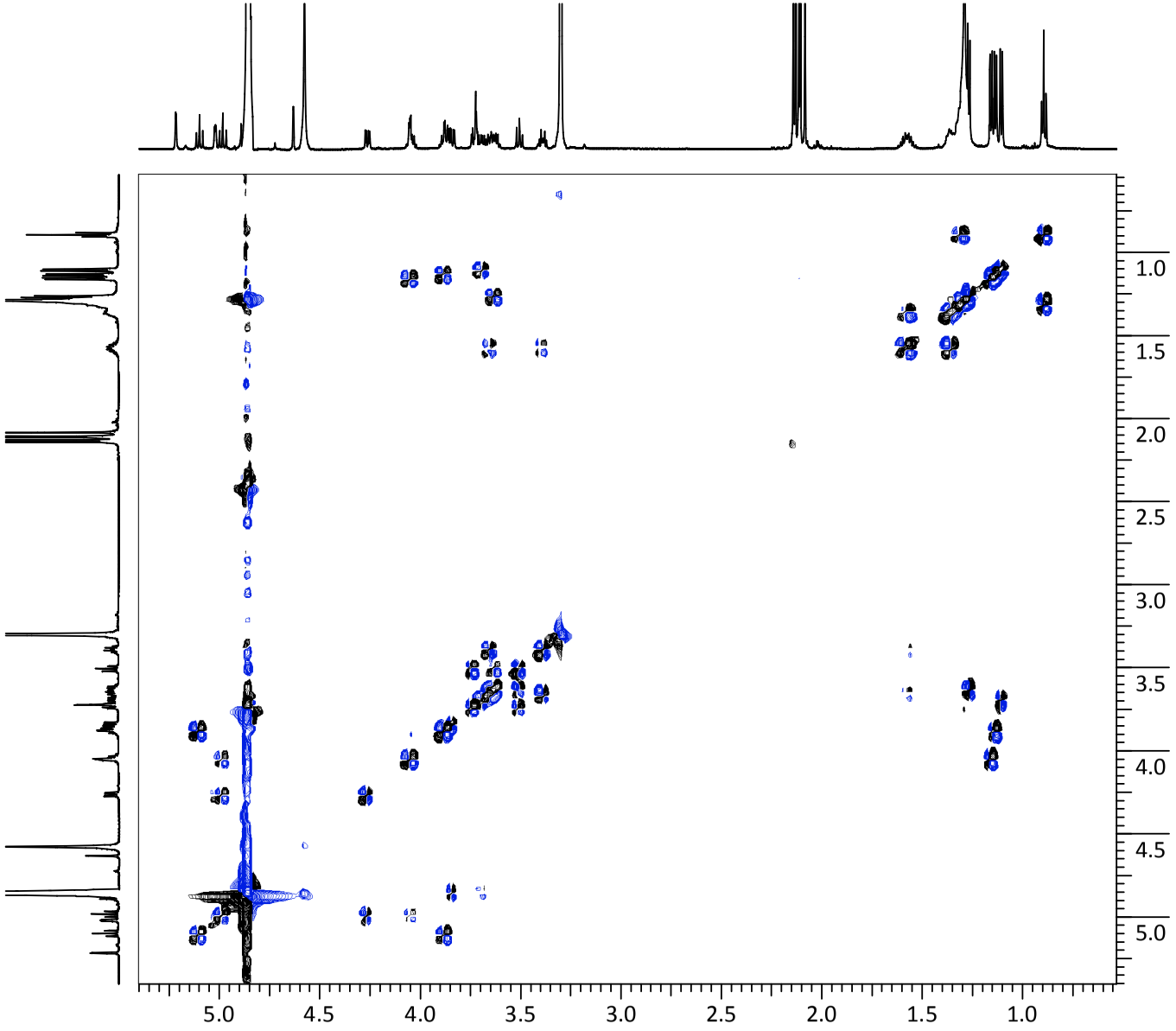
Gradient selected COSY spectrum of Cleistetroside



Exapnded portion of the gCOSY spectrum of Cleistetroside

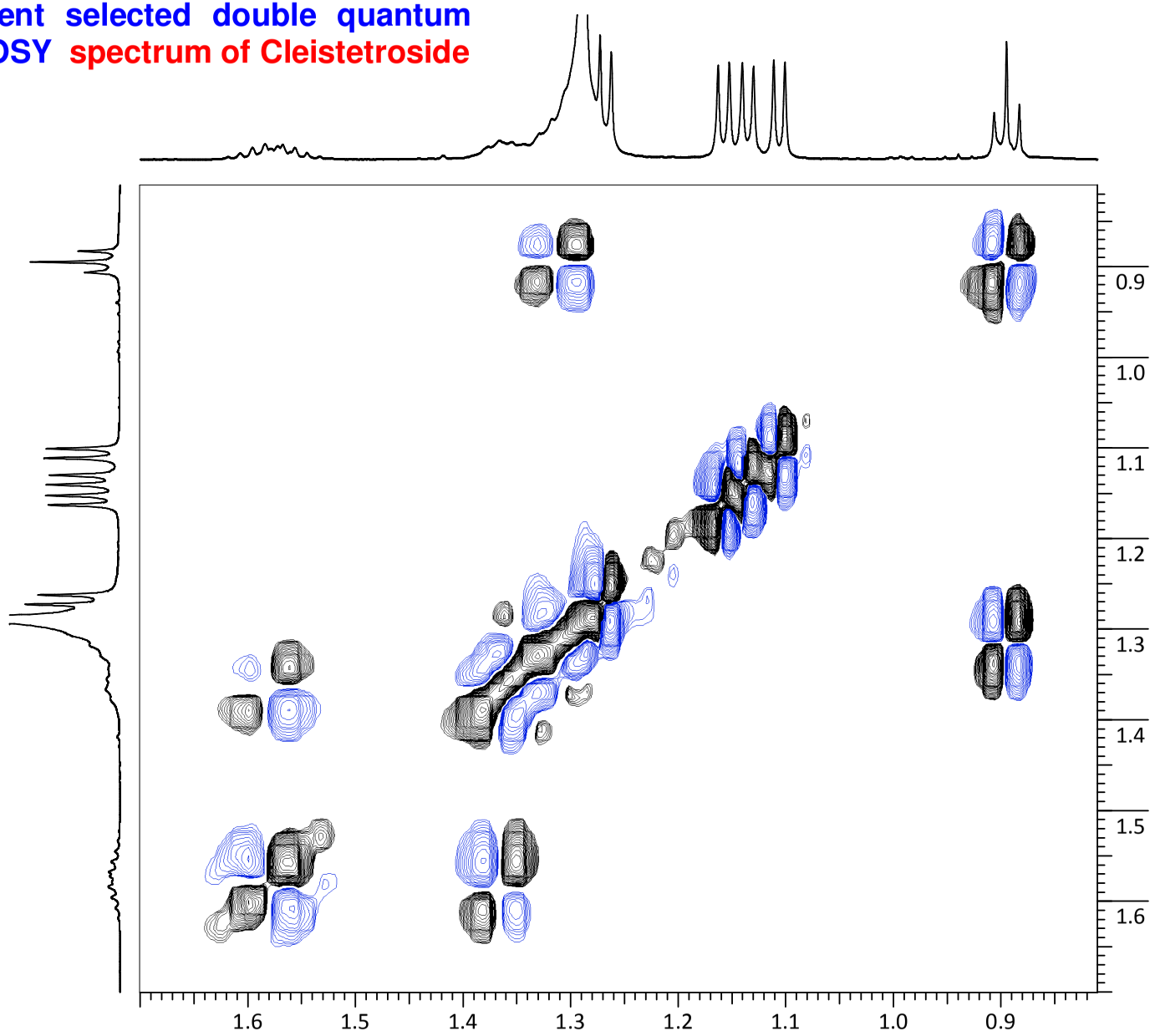


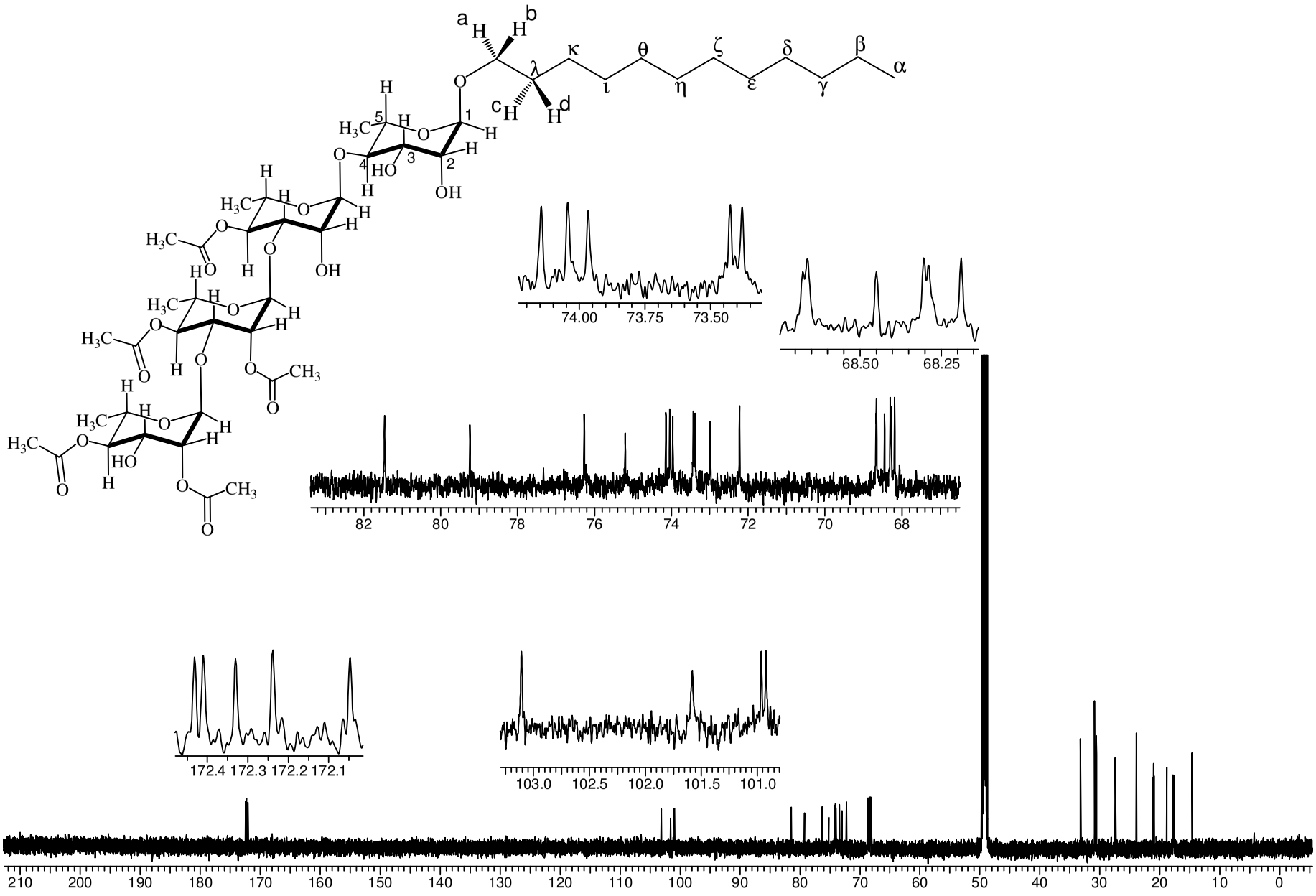
Gradient selected double quantum DQCOSY spectrum of Cleistetroside



Expansion of the countour plot of the DQCOSY spectrum

Gradient selected double quantum DQCOSY spectrum of Cleistetroside

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68.50 68.25
82 80 78 76 74 72 70 68
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103.0 102.5 102.0 101.5 101.0
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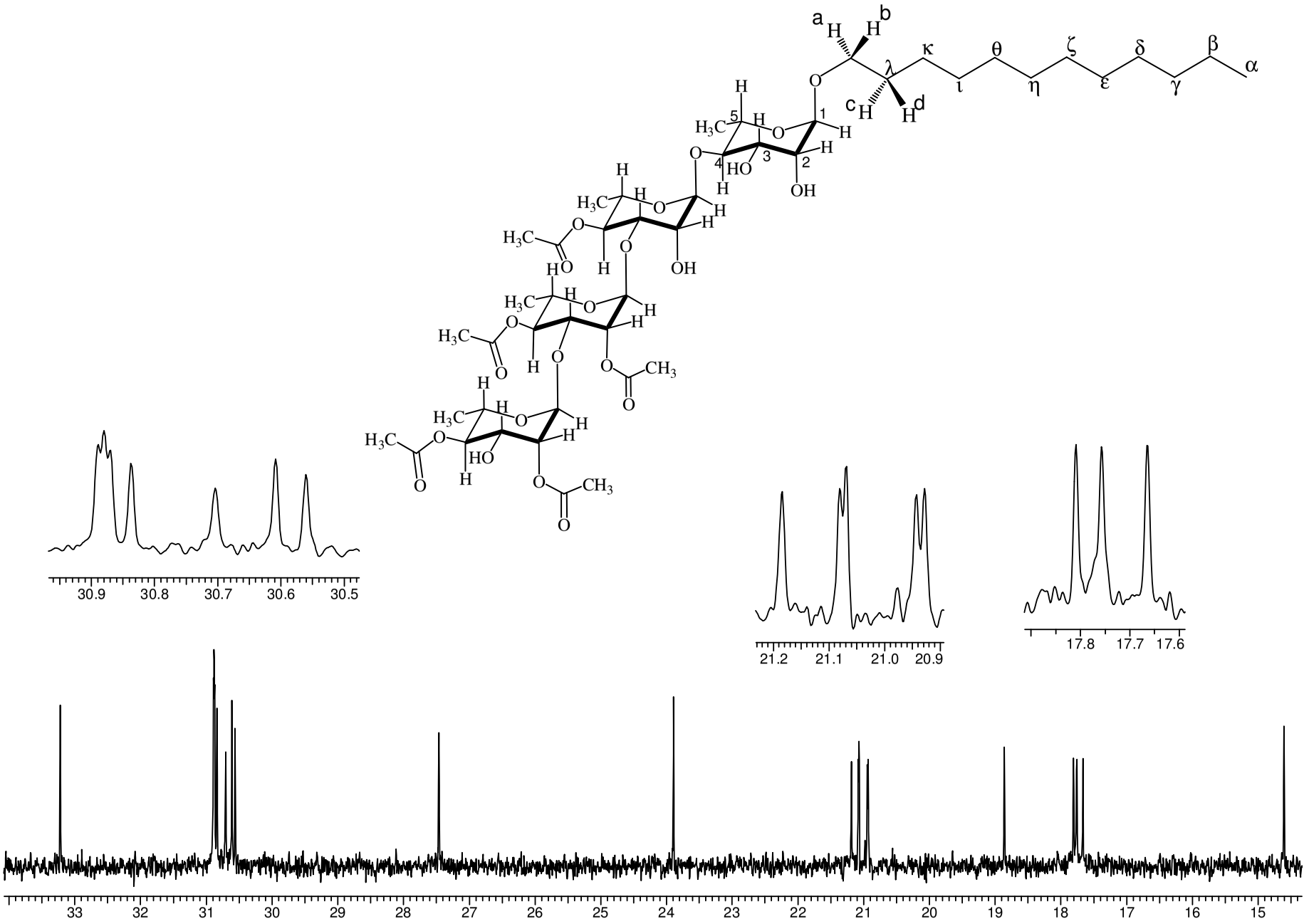
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21.1
21.0
20.9
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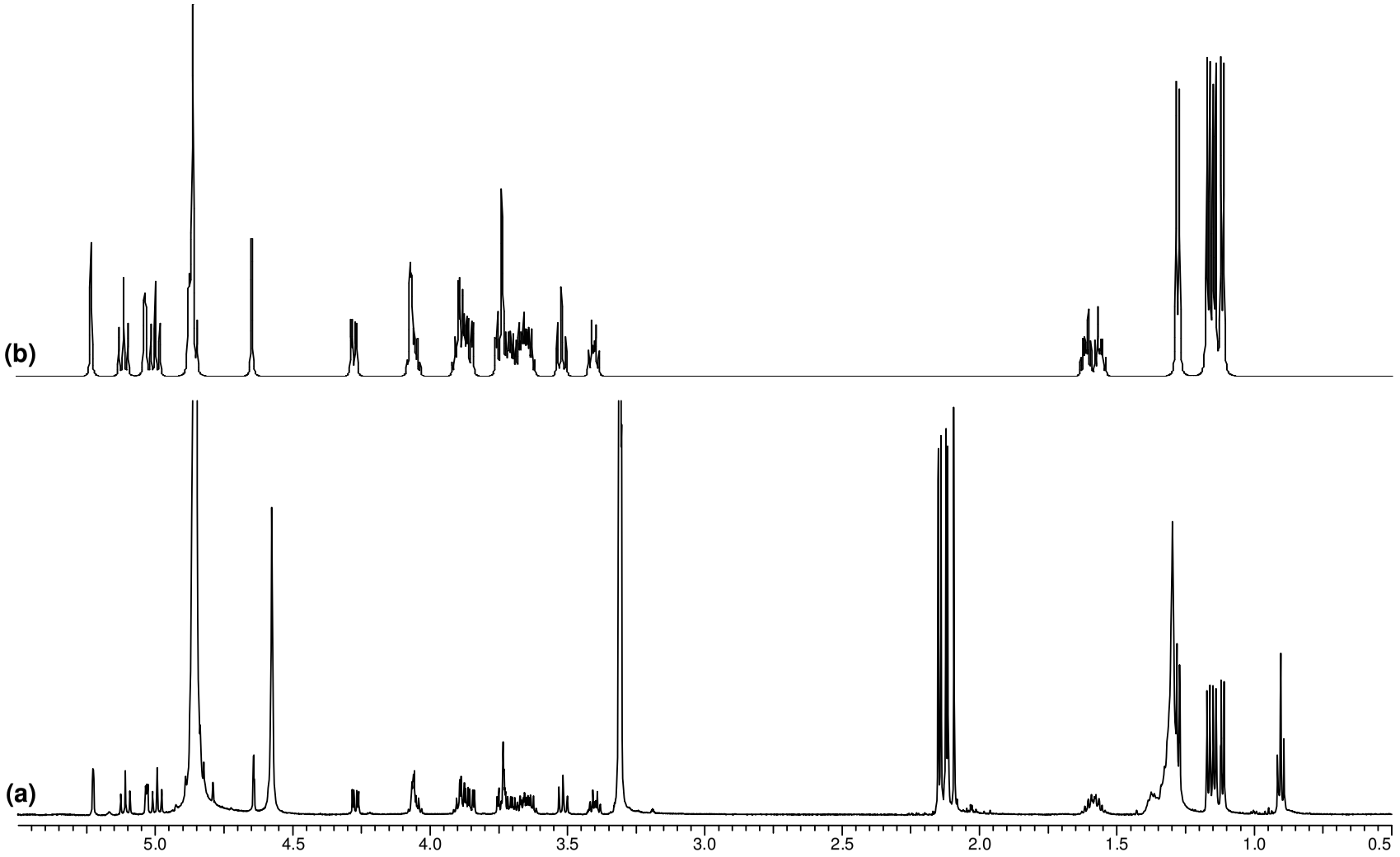
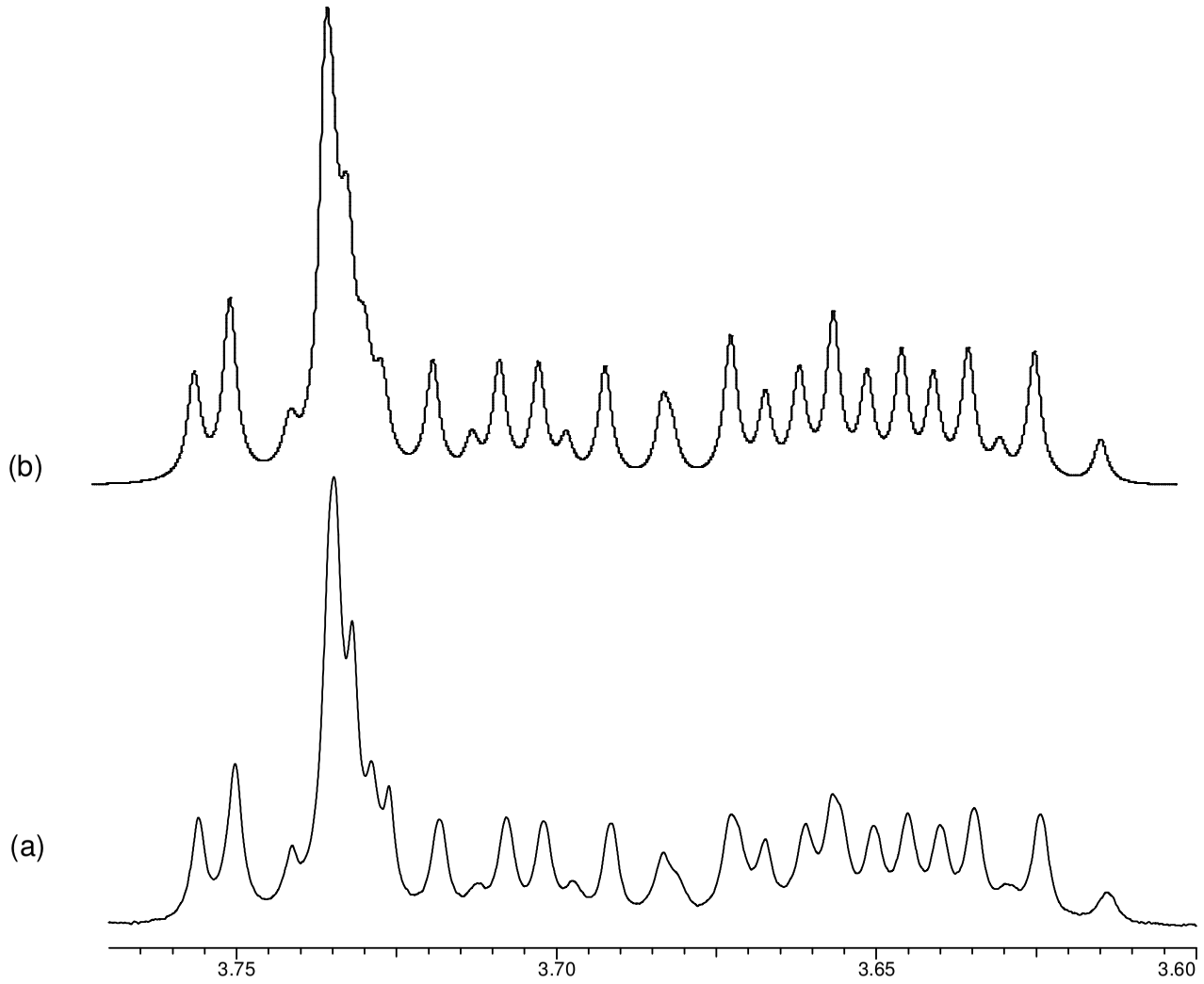
15

Experimental (a) and calculated (b) ^{1}H NMR spectrum



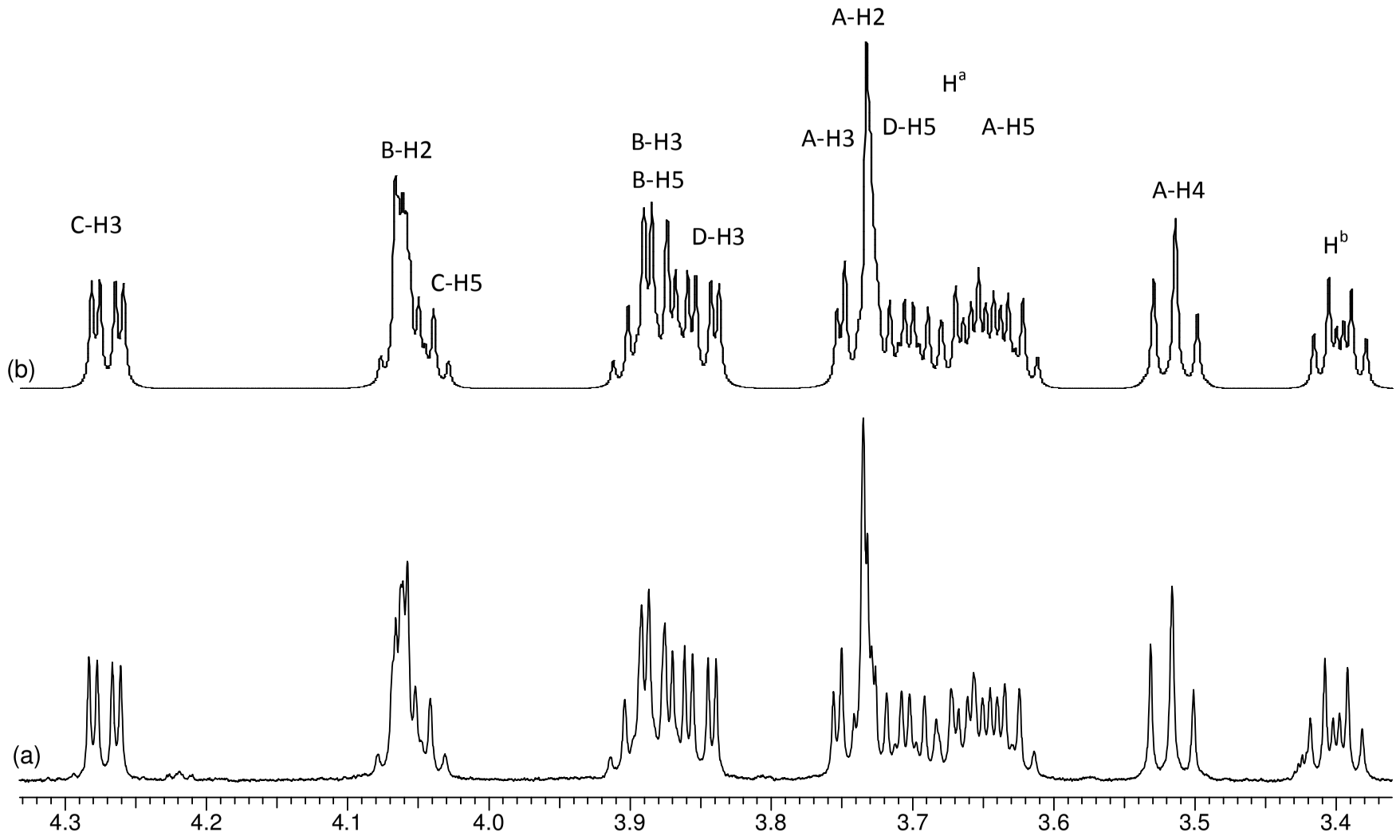
Experimental (a) and calculated (b) ^{1}H NMR spectrum

Expanded portion of the ^{1}H NMR spectrum; spectral region (3.60 - 3.80 ppm)



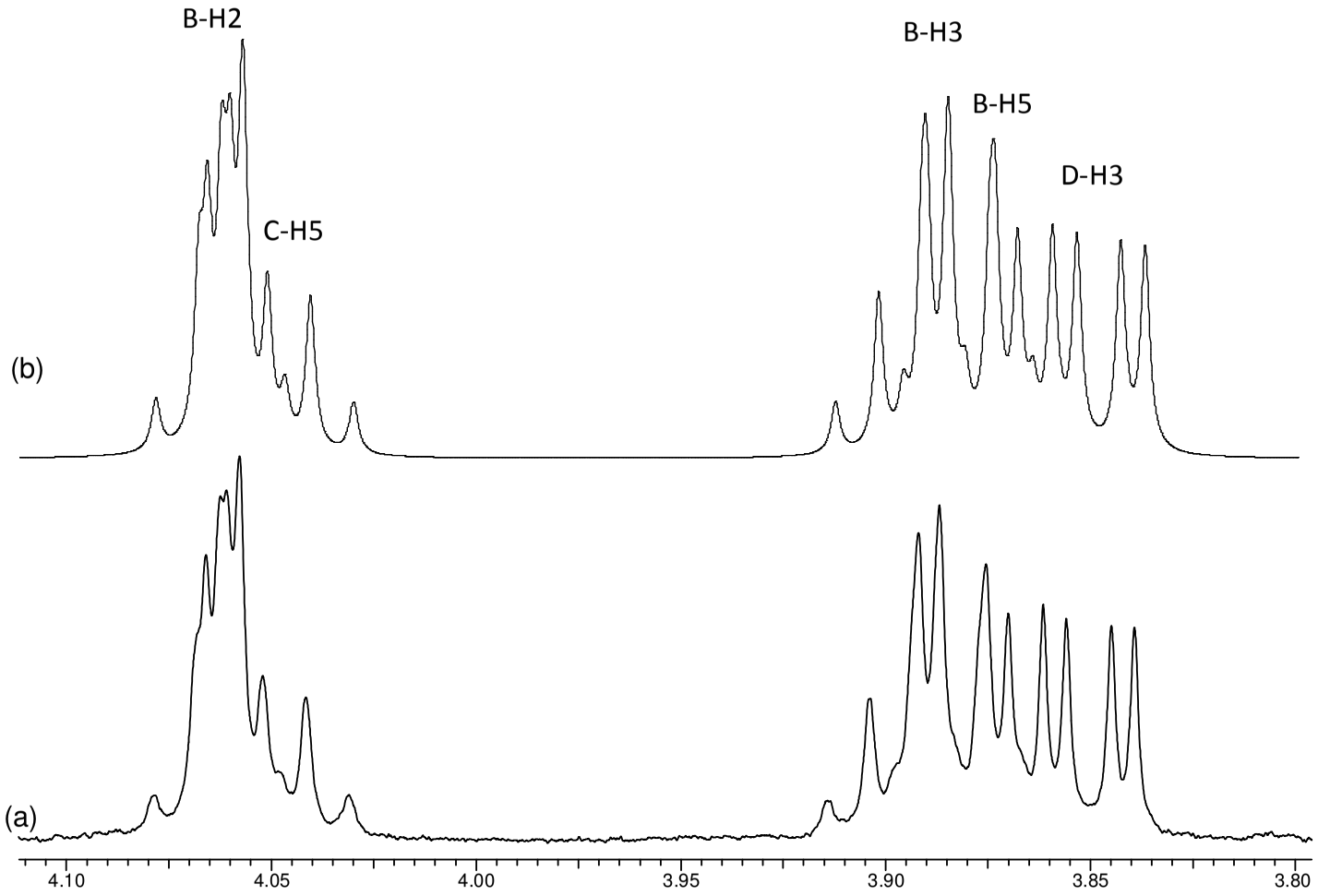
Experimental (a) and calculated (b) ^{1}H NMR spectrum

Expanded portion of the ^{1}H NMR spectrum; spectral region (3.361 - 4.332 ppm)



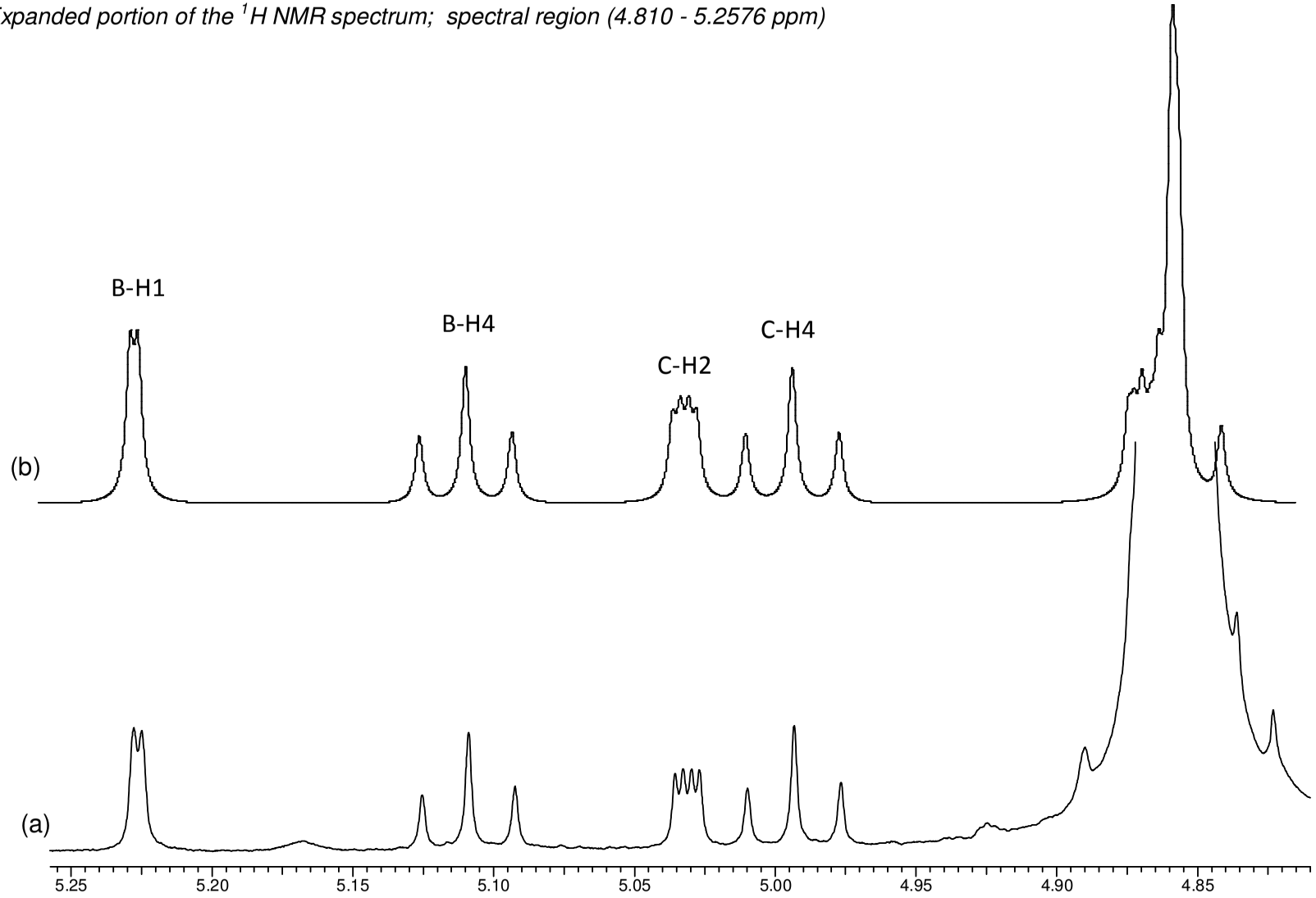
Experimental (a) and calculated (b) ^{1}H NMR spectrum

Expanded portion of the ^{1}H NMR spectrum; spectral region (3.8 - 4.1 ppm)

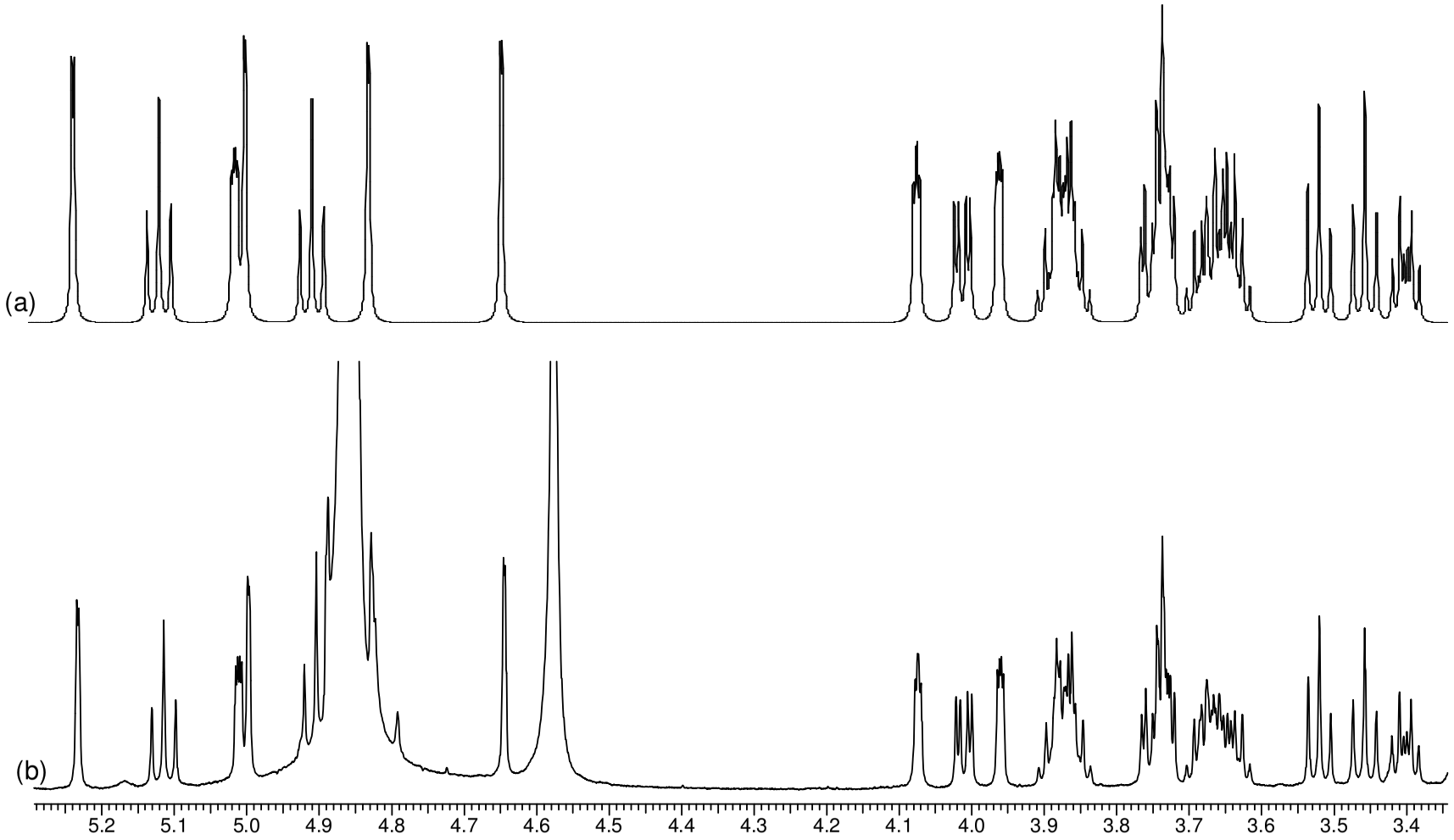


Experimental (a) and calculated (b) ^{1}H NMR spectrum

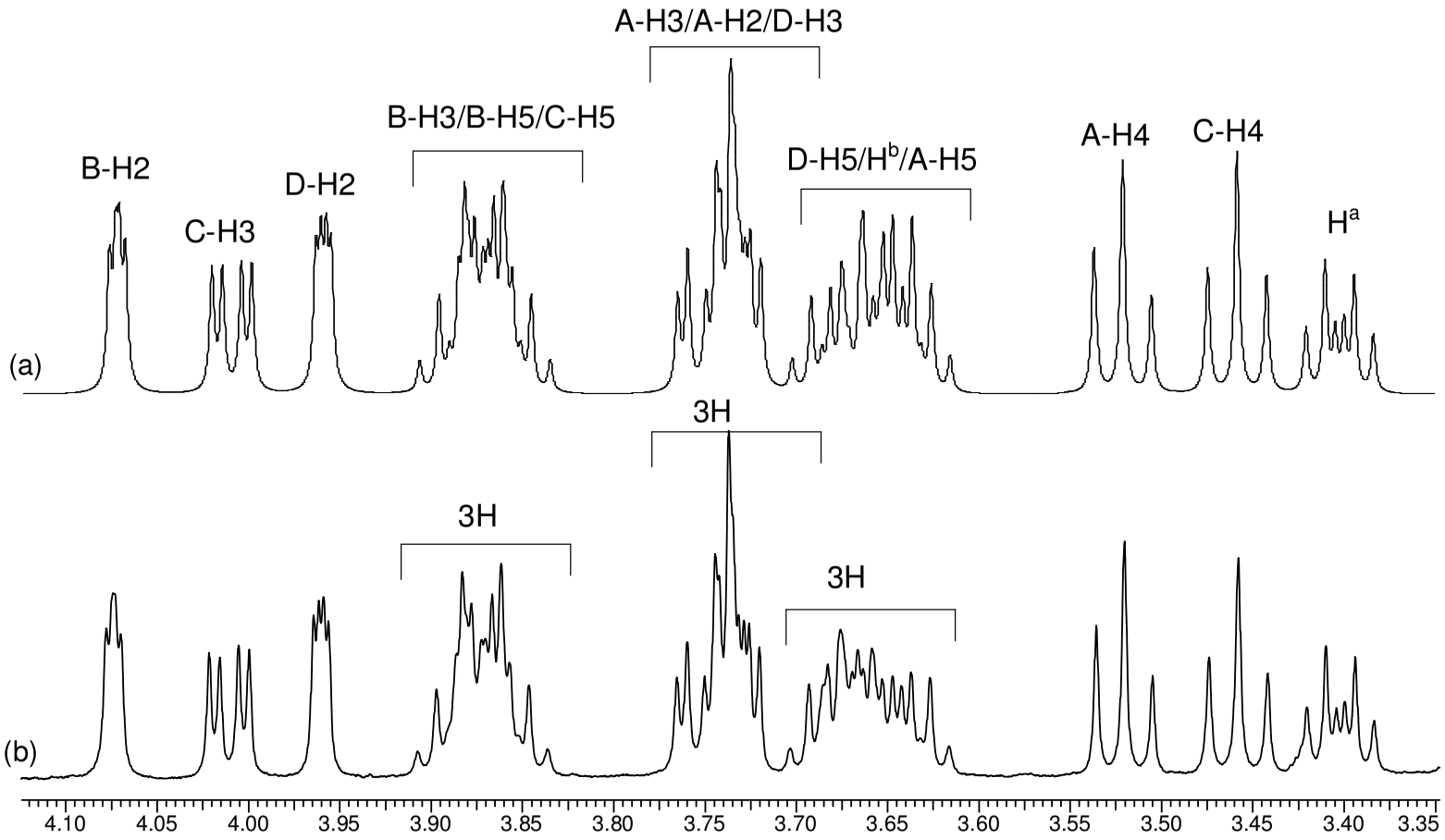
Expanded portion of the ^{1}H NMR spectrum; spectral region (4.810 - 5.2576 ppm)



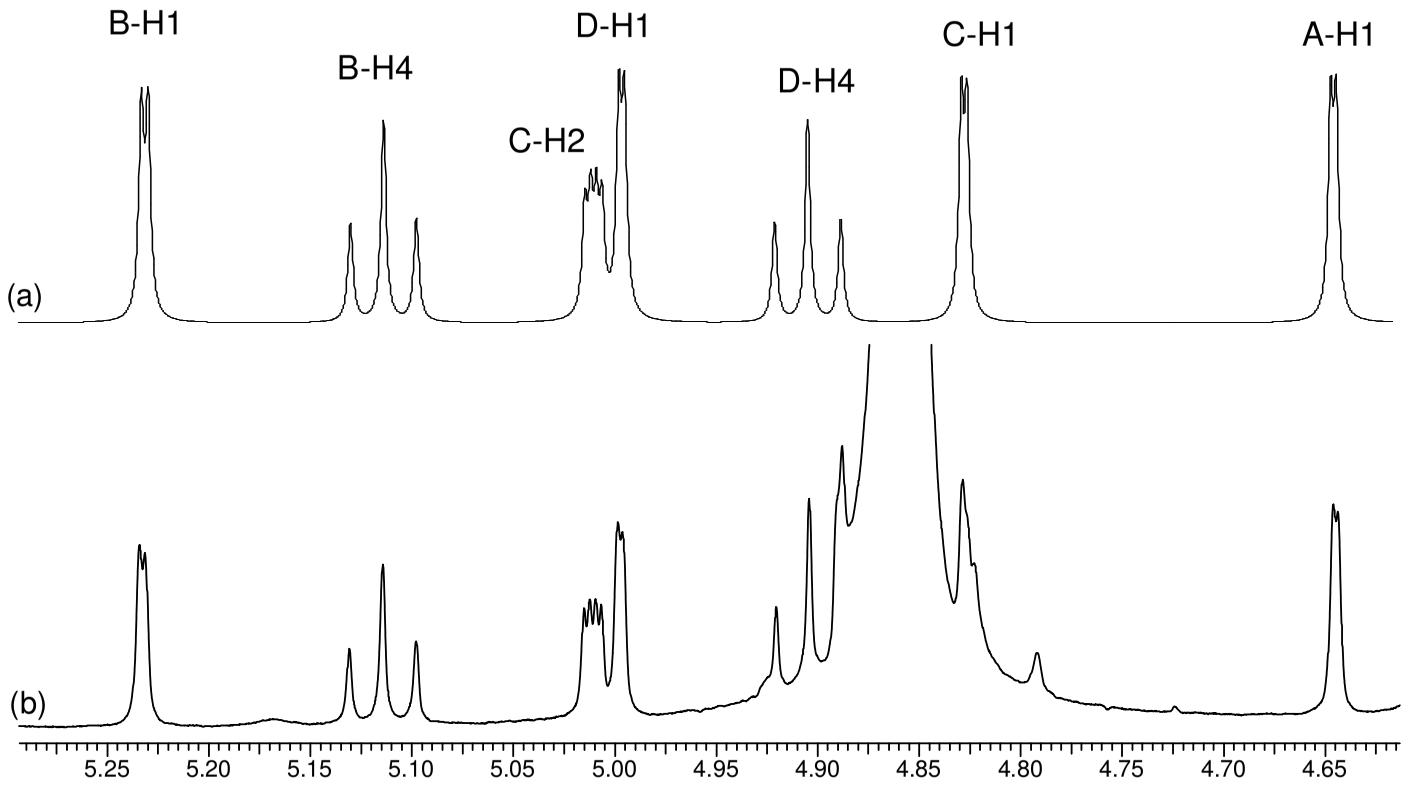
Calculated (a) and experimental (b) ^{1}H NMR spectrum *(spectral region: 3.344 - 5.293 ppm)*



Calculated (a) and experimental (b) ^{1}H NMR spectrum *(spectral region: 3.348 - 4.124 ppm)*

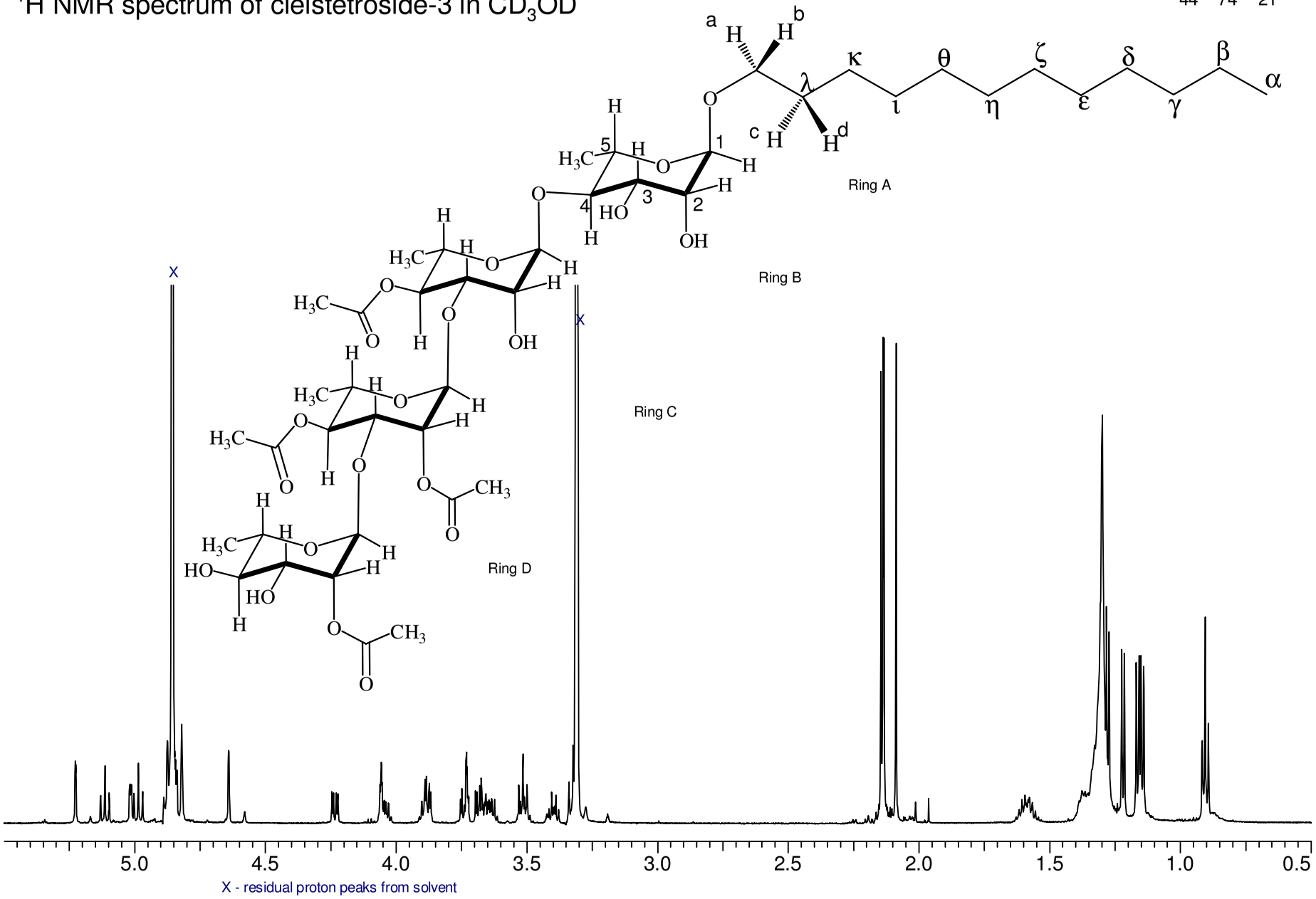


Calculated (a) and experimental (b) ^{1}H NMR spectrum *(spectral region: 4.6133 - 5.2933 ppm)*

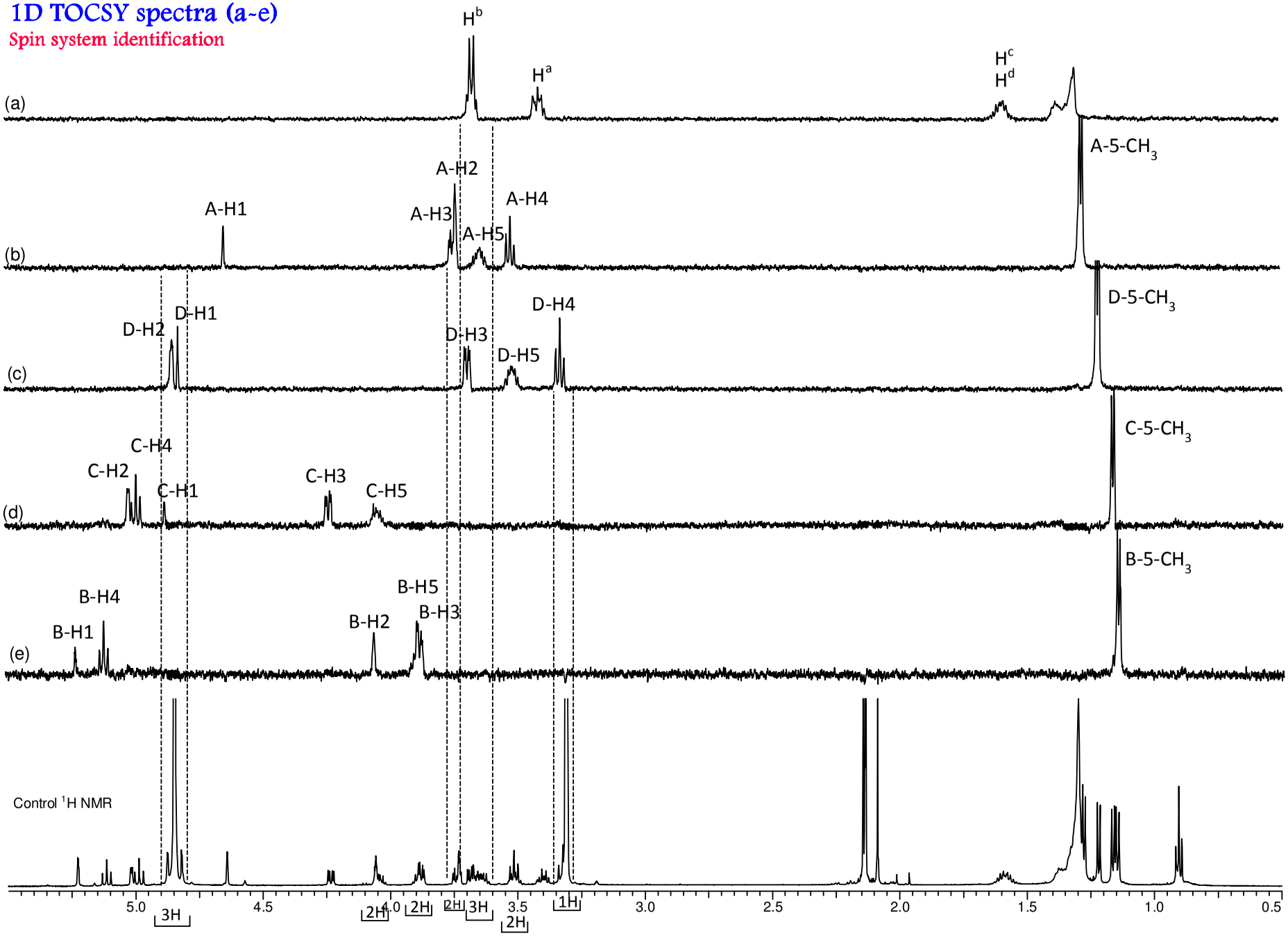


^{1}H NMR spectrum of cleistetroside-3 in CD_3OD

Formula: $C_{44}H_{74}O_{21}$

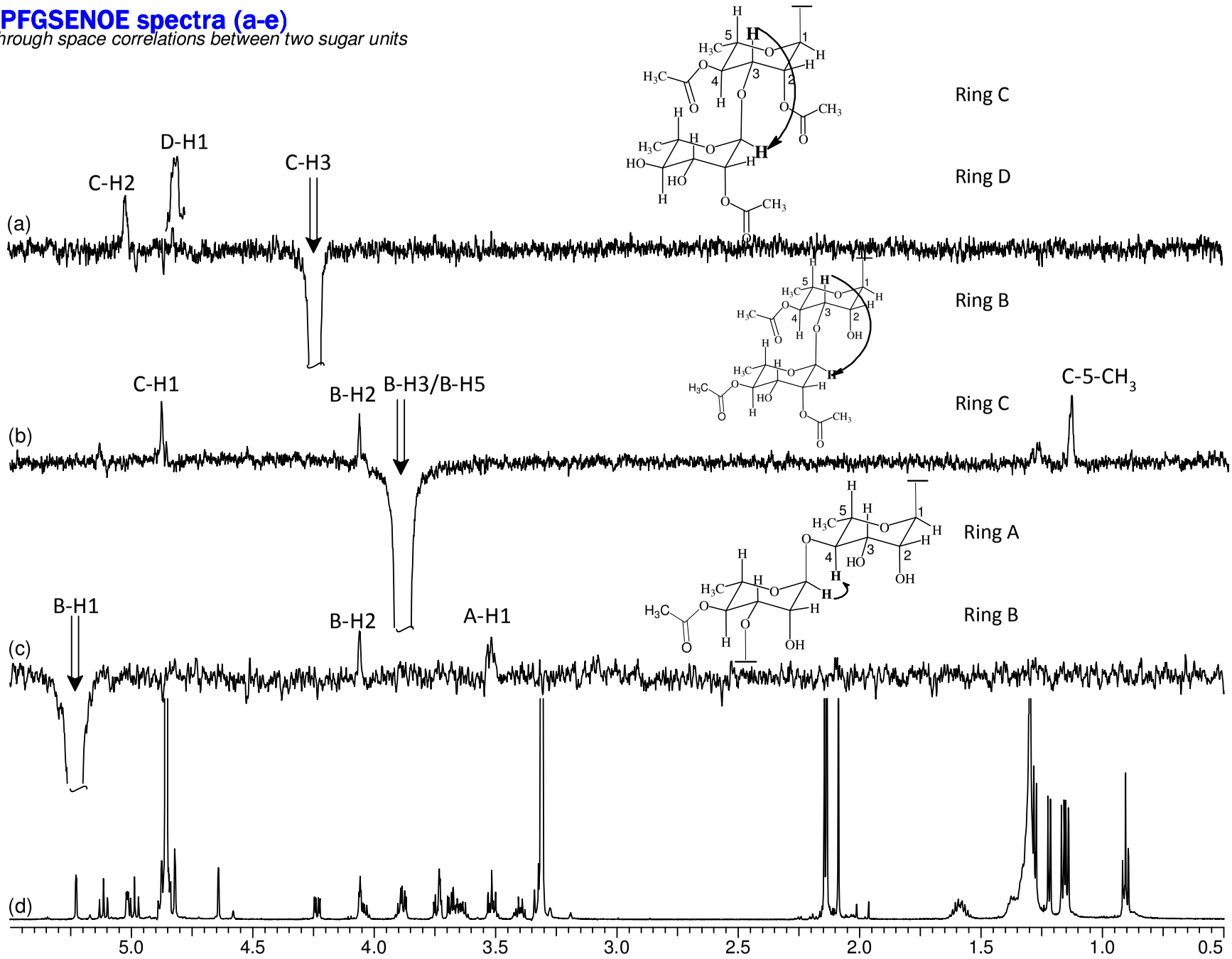


X - residual proton peaks from solvent

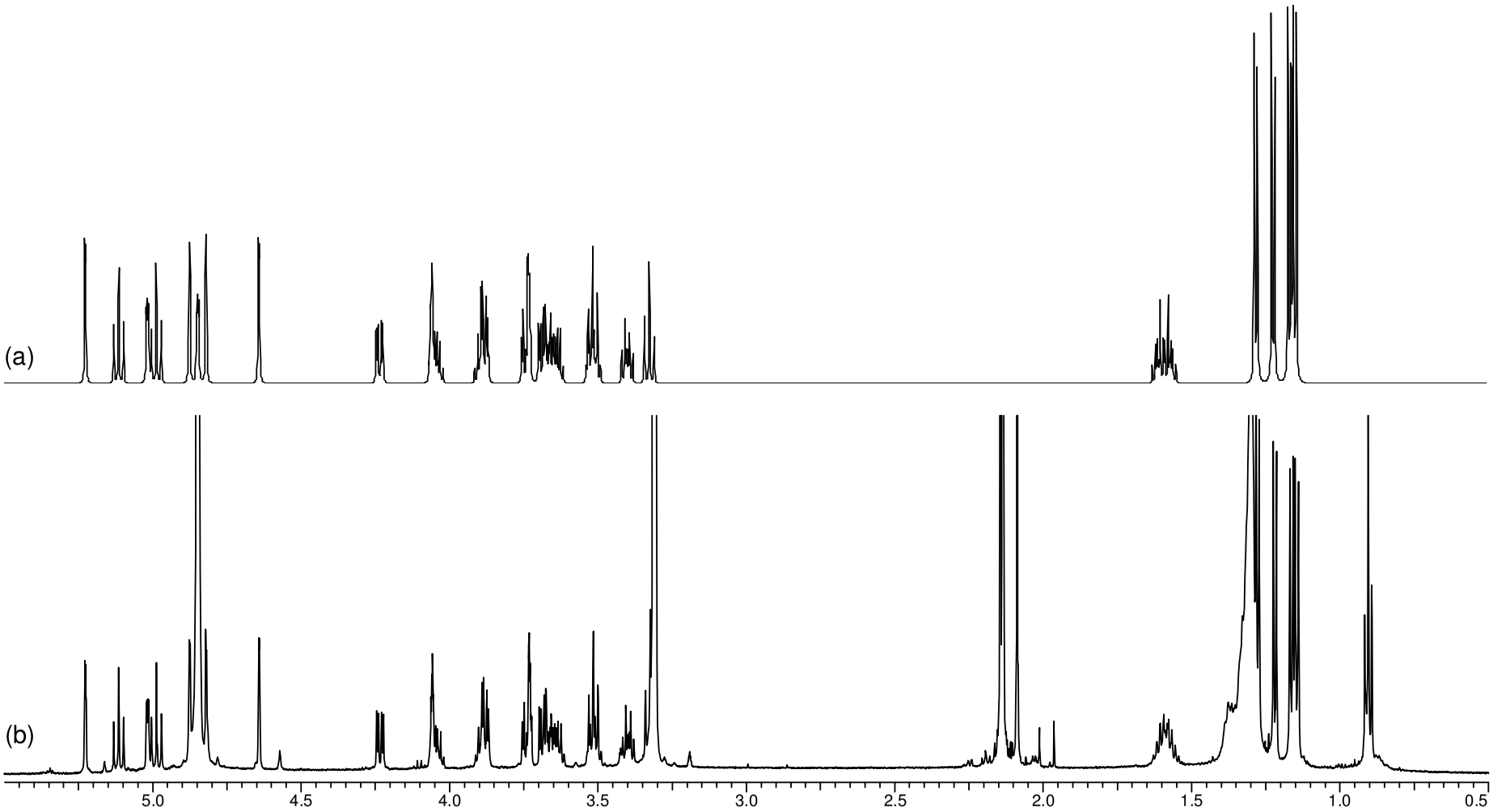

1D TOCSY spectra (a~e)
Spin system identification
(a)
(b)
(c)
(d)
(e)
Control ^{1}H NMR
H^b
H^a
H^c
H^d
A-5-CH$_3$
A-H2
A-H1
A-H3
A-H4
A-H5
D-5-CH$_3$
D-H2
D-H1
D-H3
D-H4
D-H5
C-5-CH$_3$
C-H4
C-H2
C-H1
C-H3
C-H5
B-5-CH$_3$
B-H4
B-H5
B-H1
B-H2
B-H3
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
3H
2H
2H
2H
3H
2H
1H

DPFGSENOE spectra (a-e)

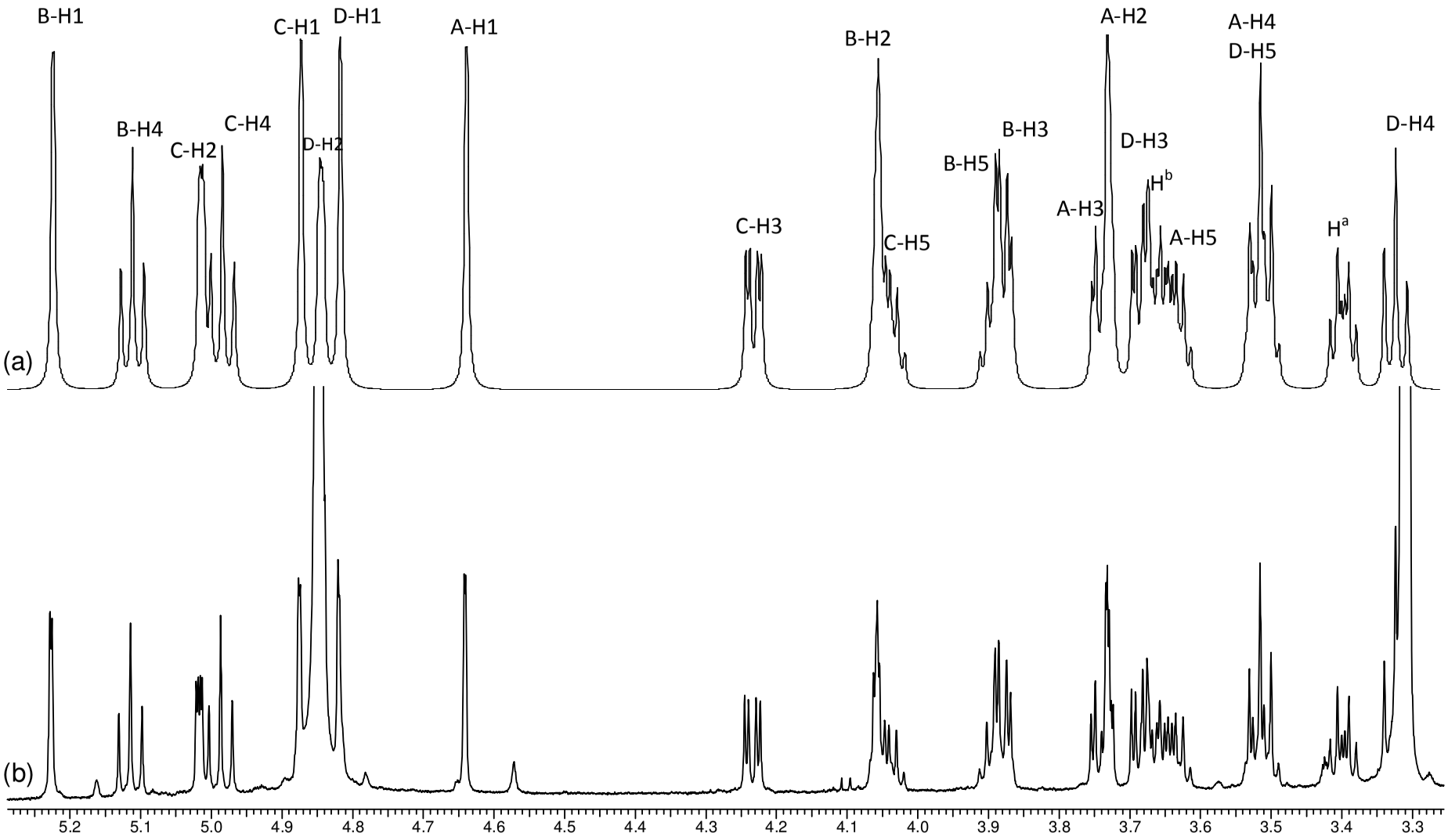
through space correlations between two sugar units



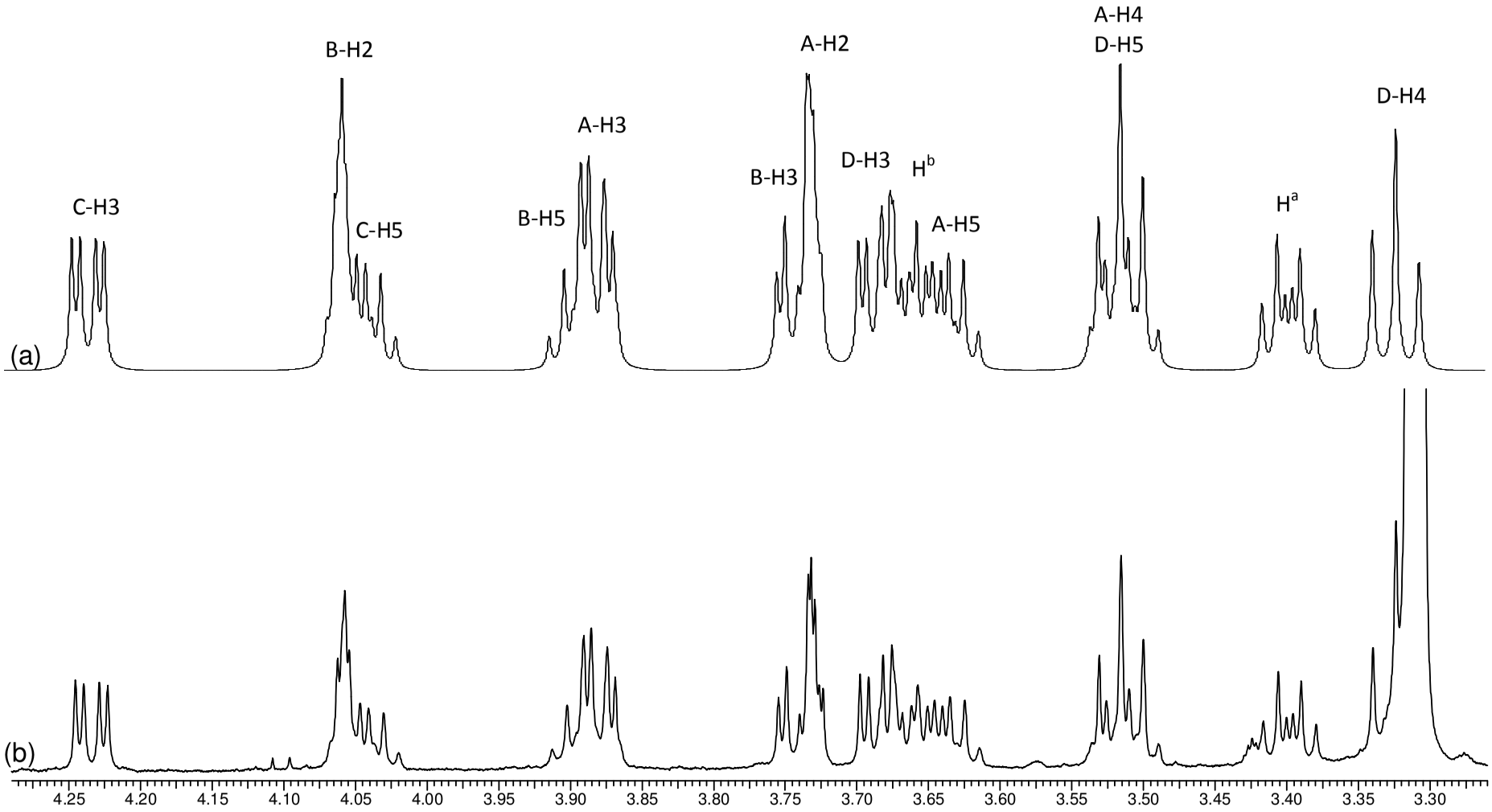
Calculated (a) and experimental (b) ^{1}H NMR spectrum



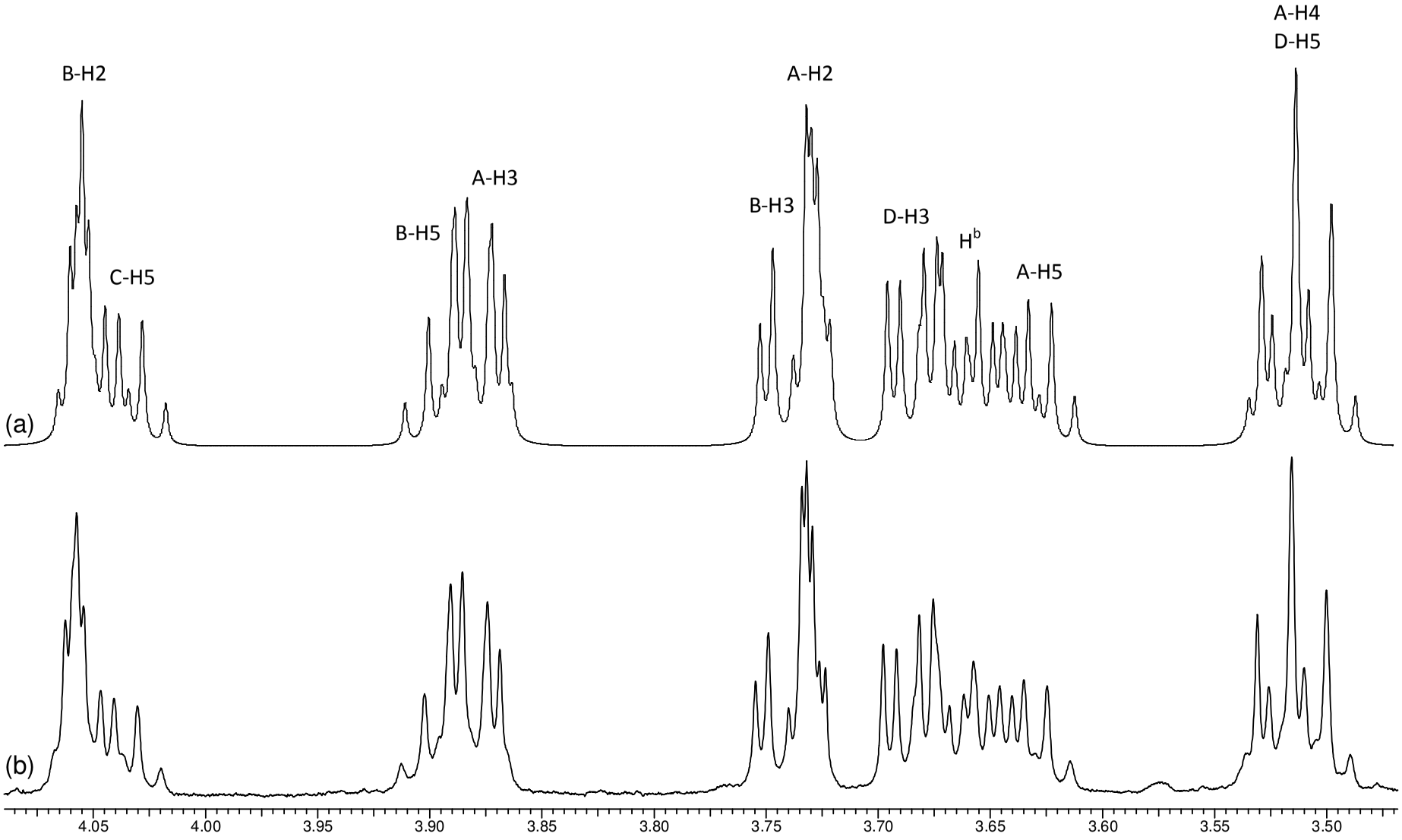
Expanded portion of calculated (a) and experimental (b) ^{1}H NMR spectrum



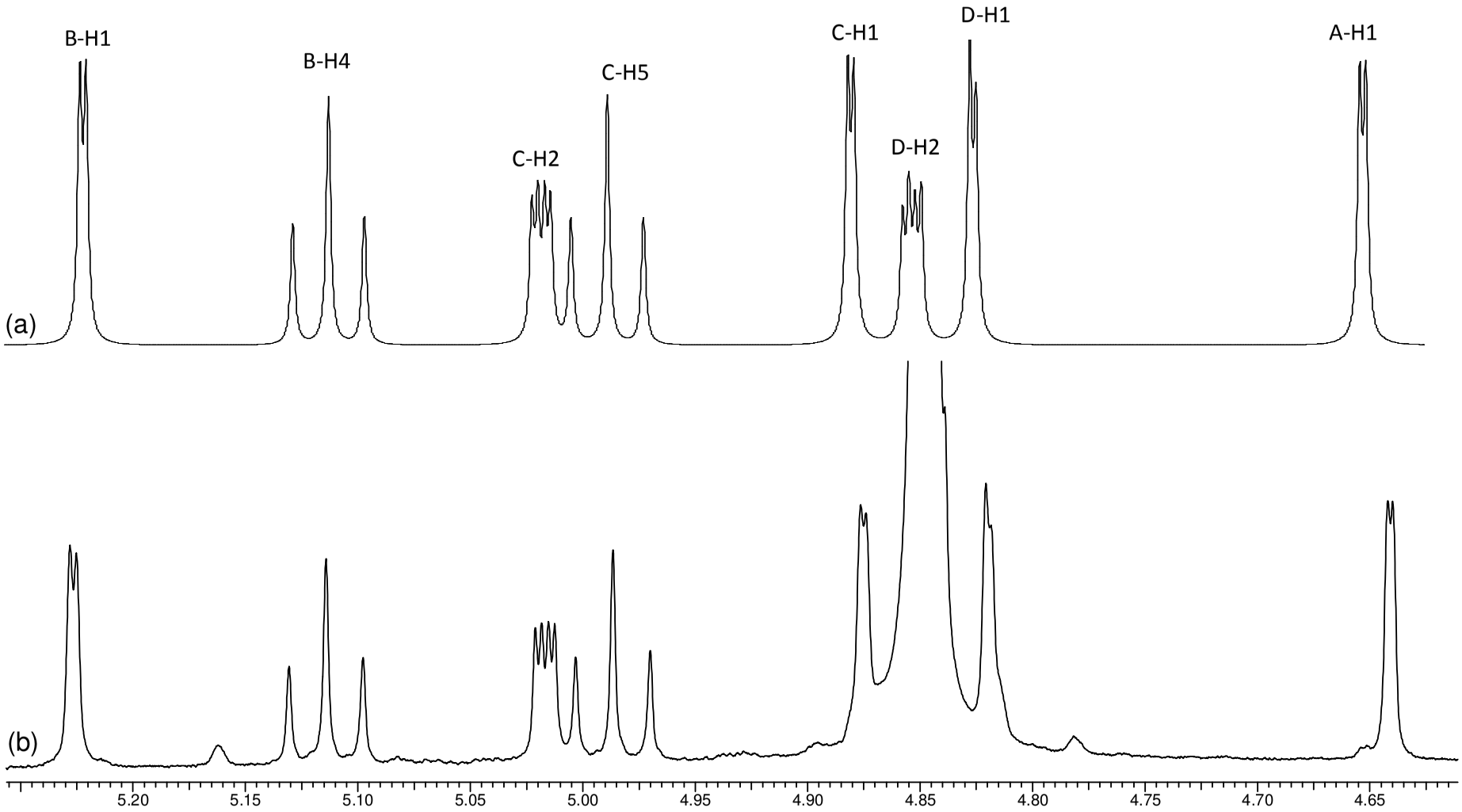
Expanded portion of calculated (a) and experimental (b) ^{1}H NMR spectrum



Expanded portion of calculated (a) and experimental (b) ^{1}H NMR spectrum

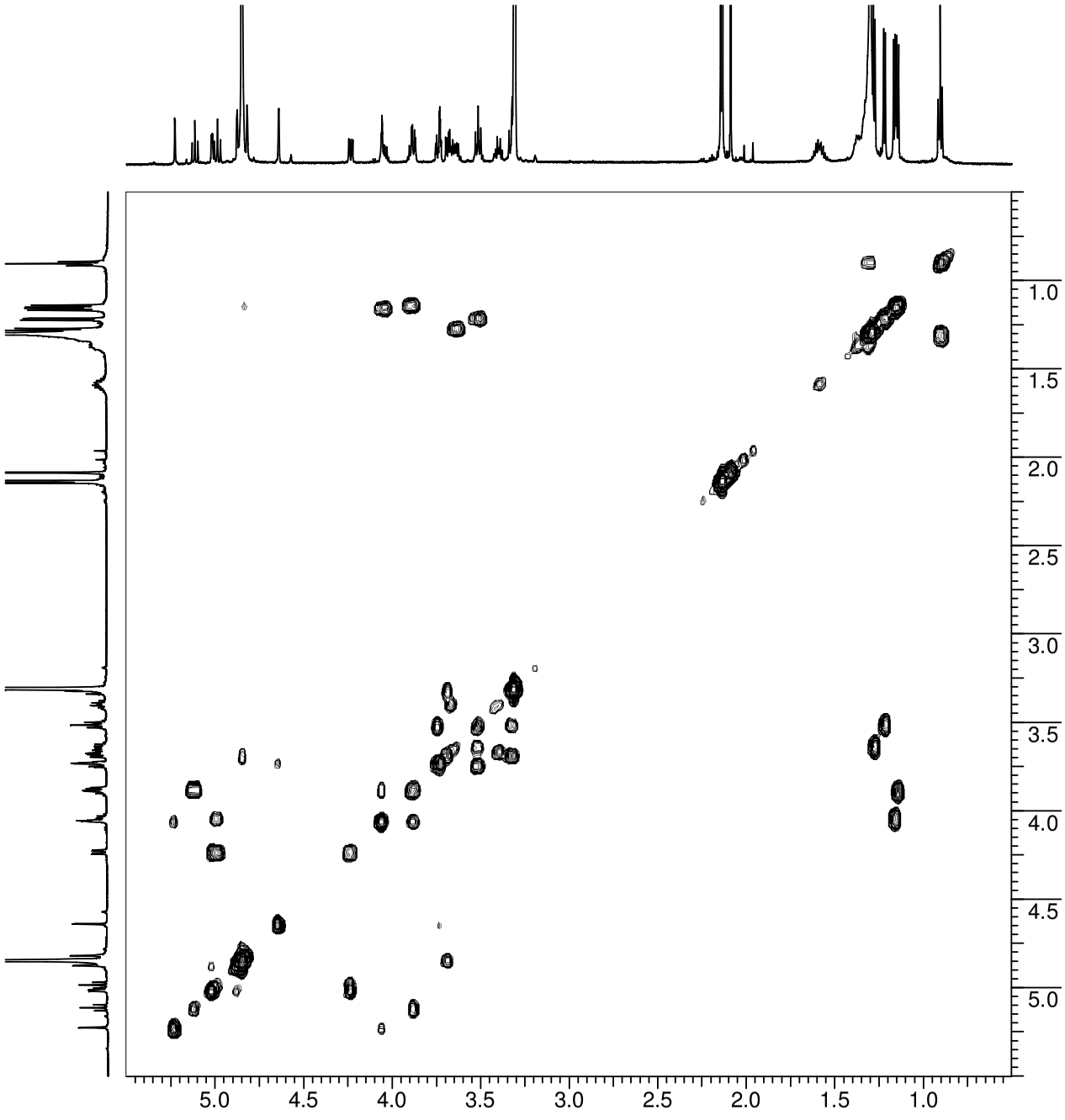


Expanded portion of calculated (a) and experimental (b) ^{1}H NMR spectrum

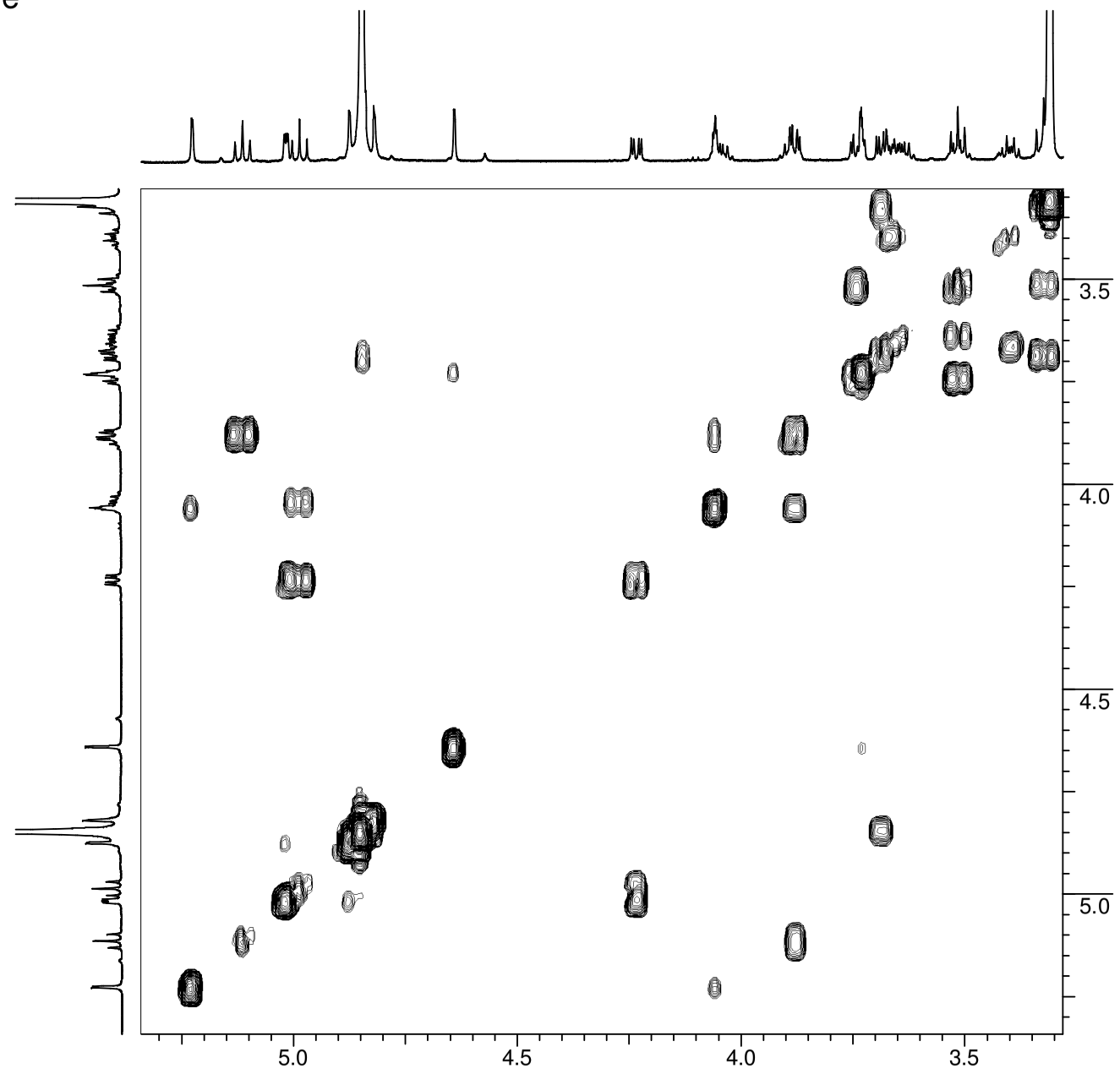


gCOSY

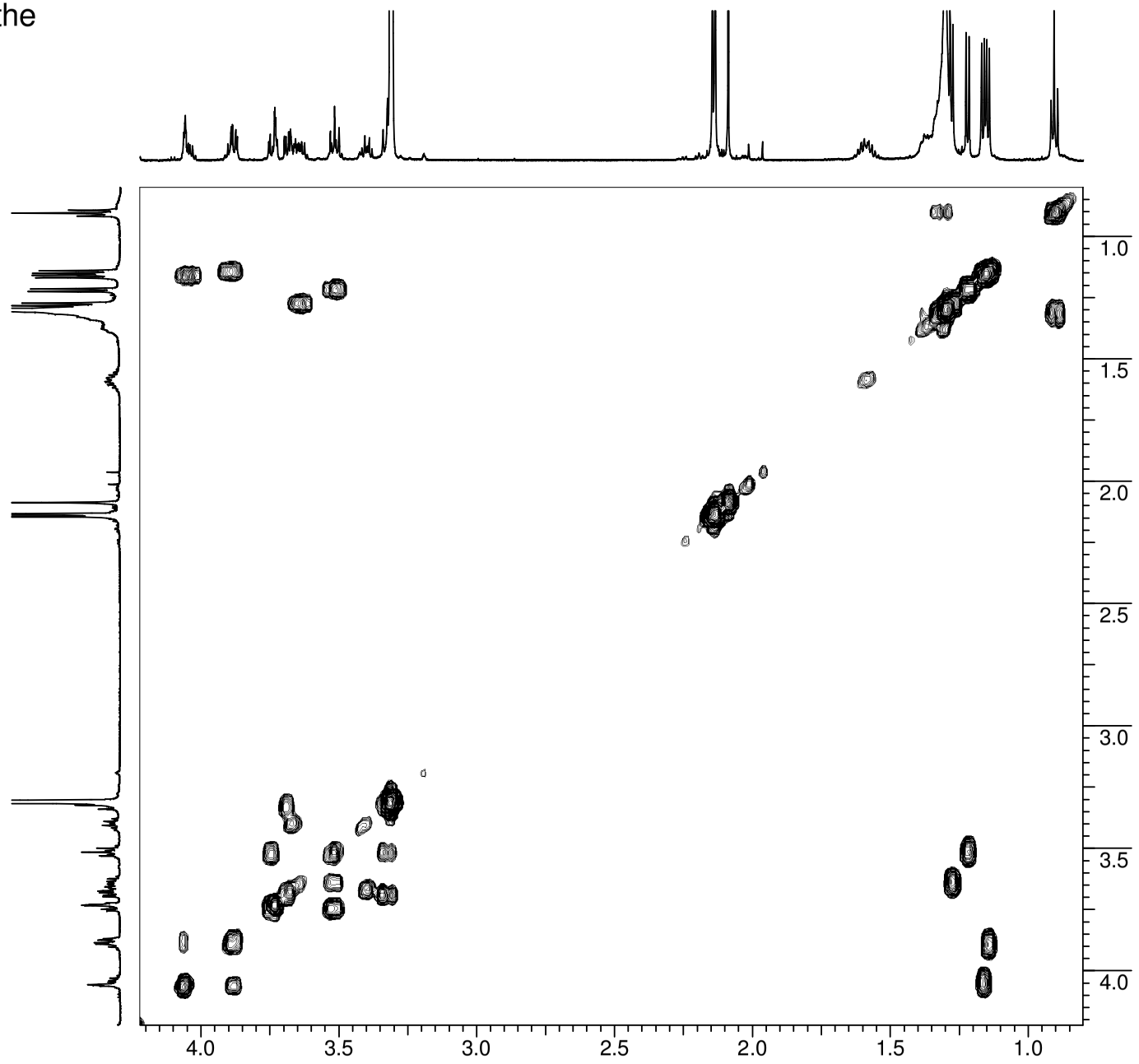
spectrum



Expanded portion of the
gCOSY spectrum

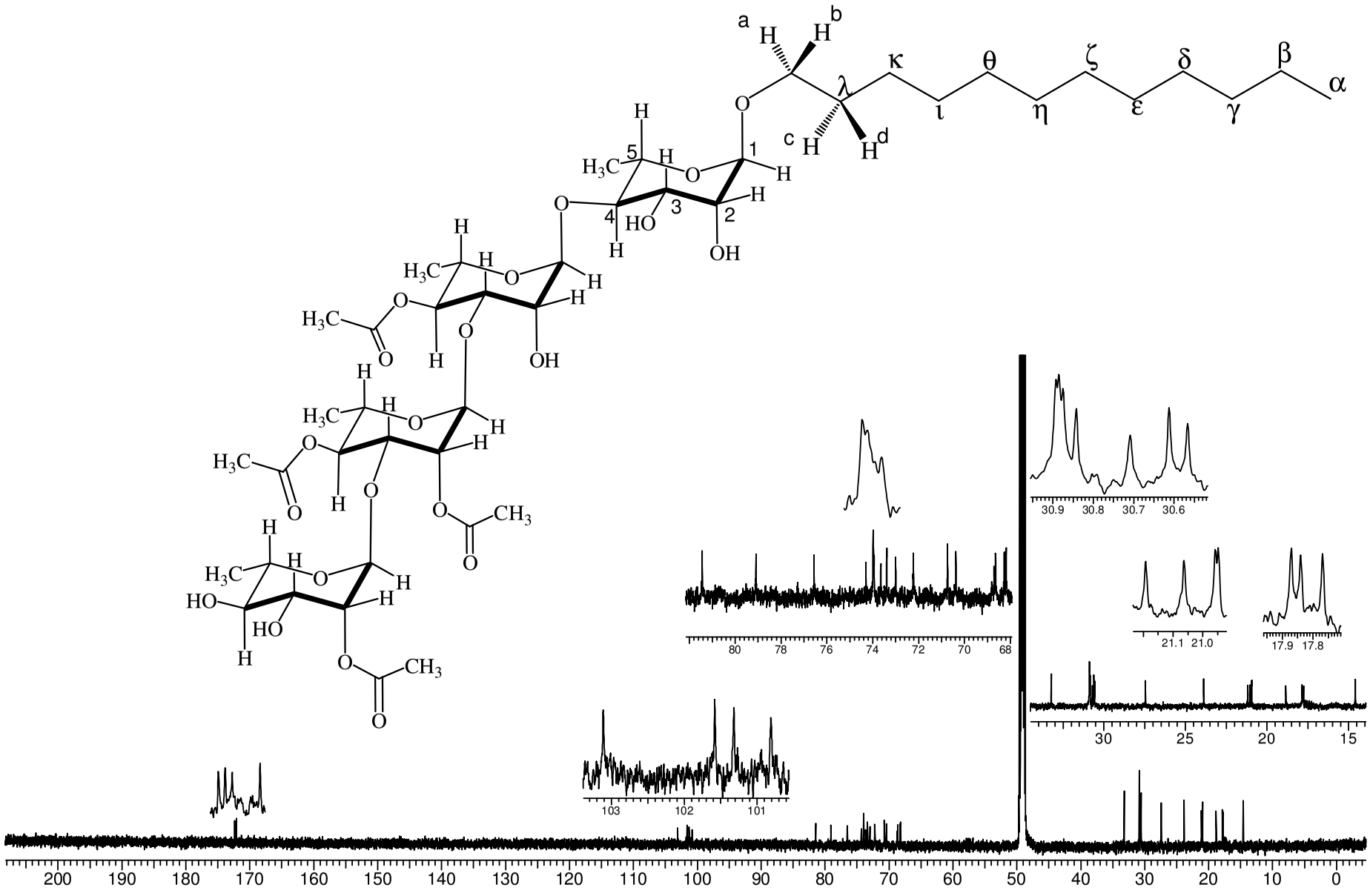


Expanded portion of the
gCOSY spectrum

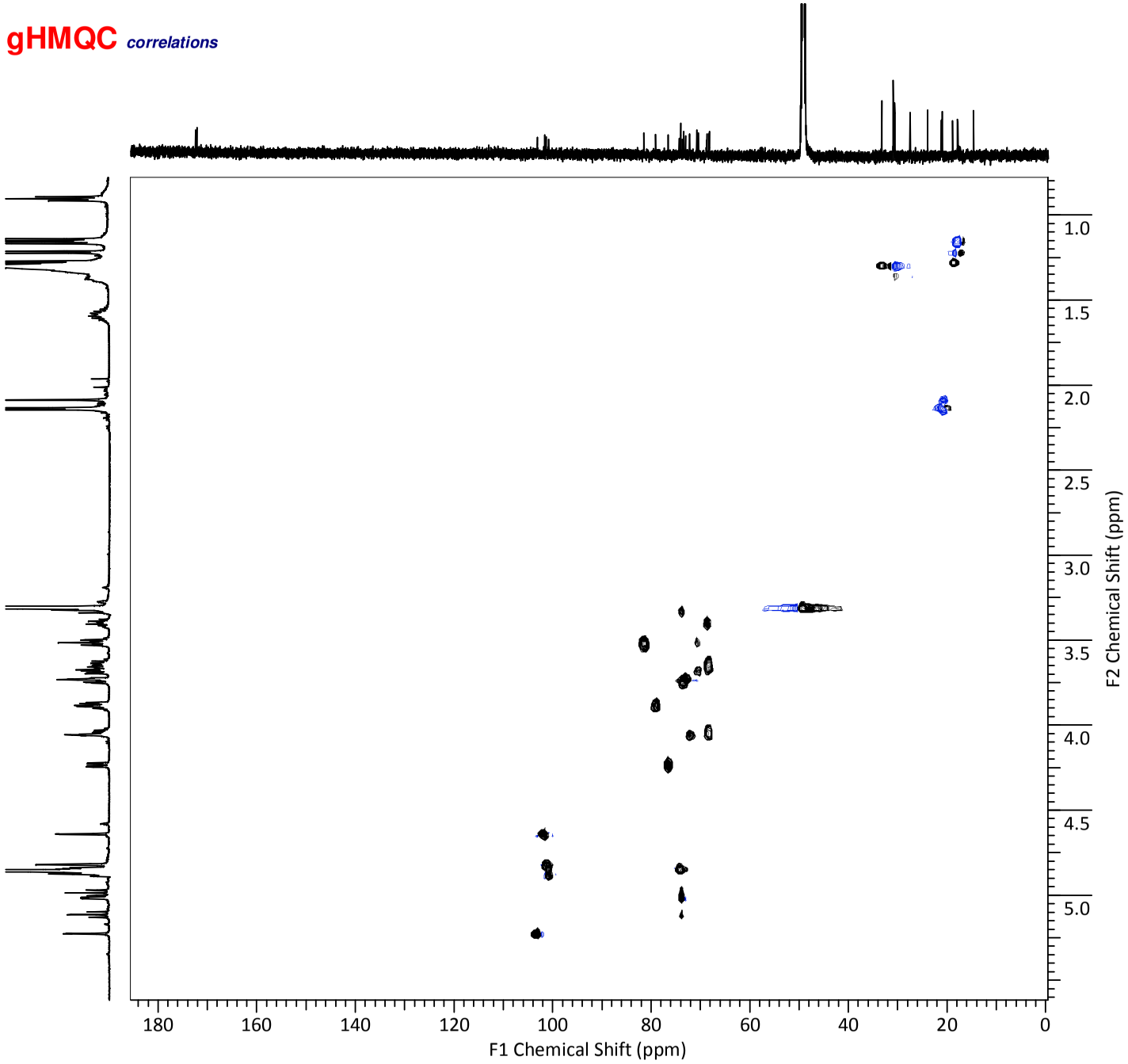


^{13}C NMR spectrum of cleistetroside-3 in CD_3OD

Formula: $C_{44}H_{74}O_{21}$

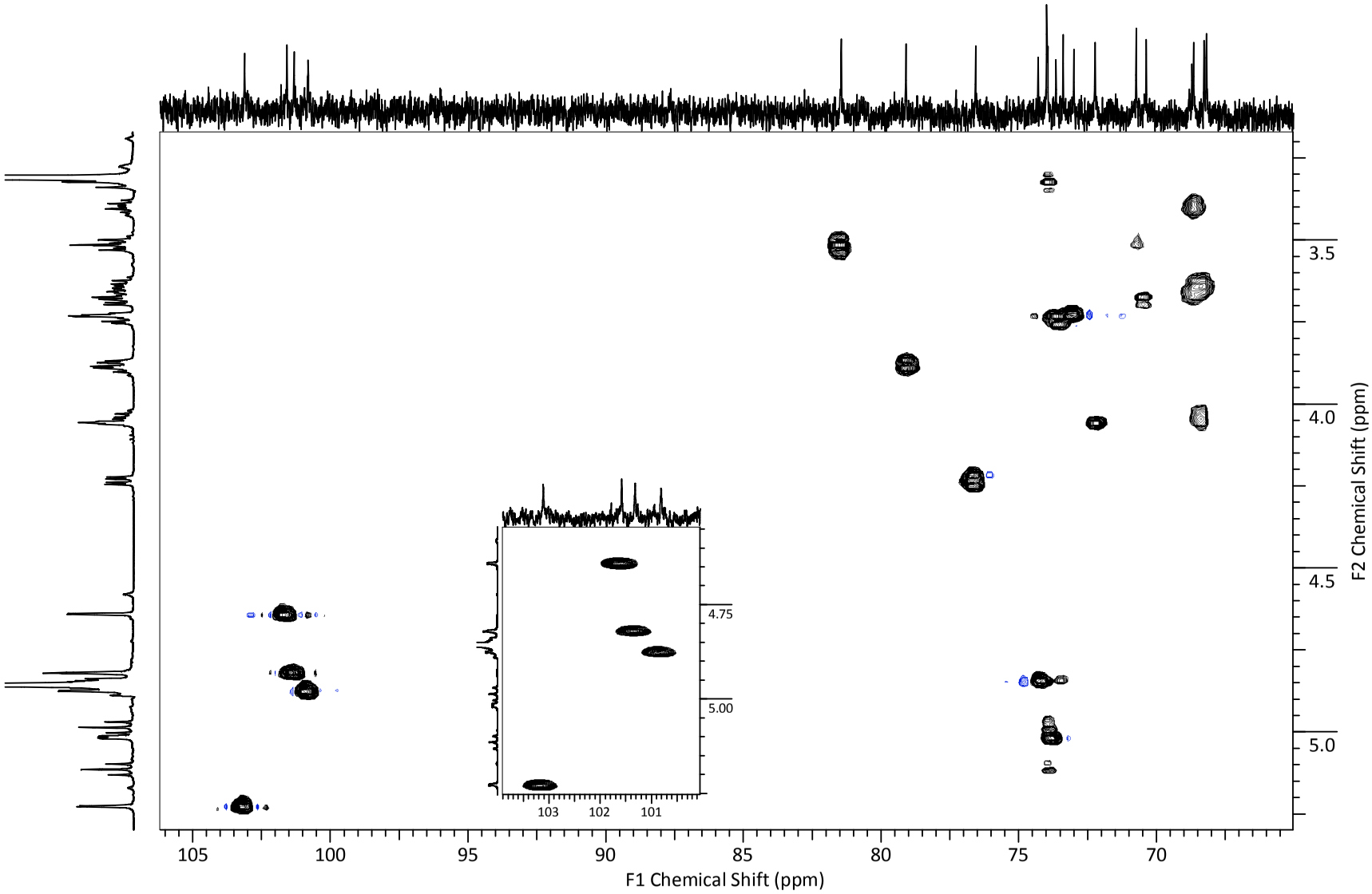


One-bond gHMQC *correlations*

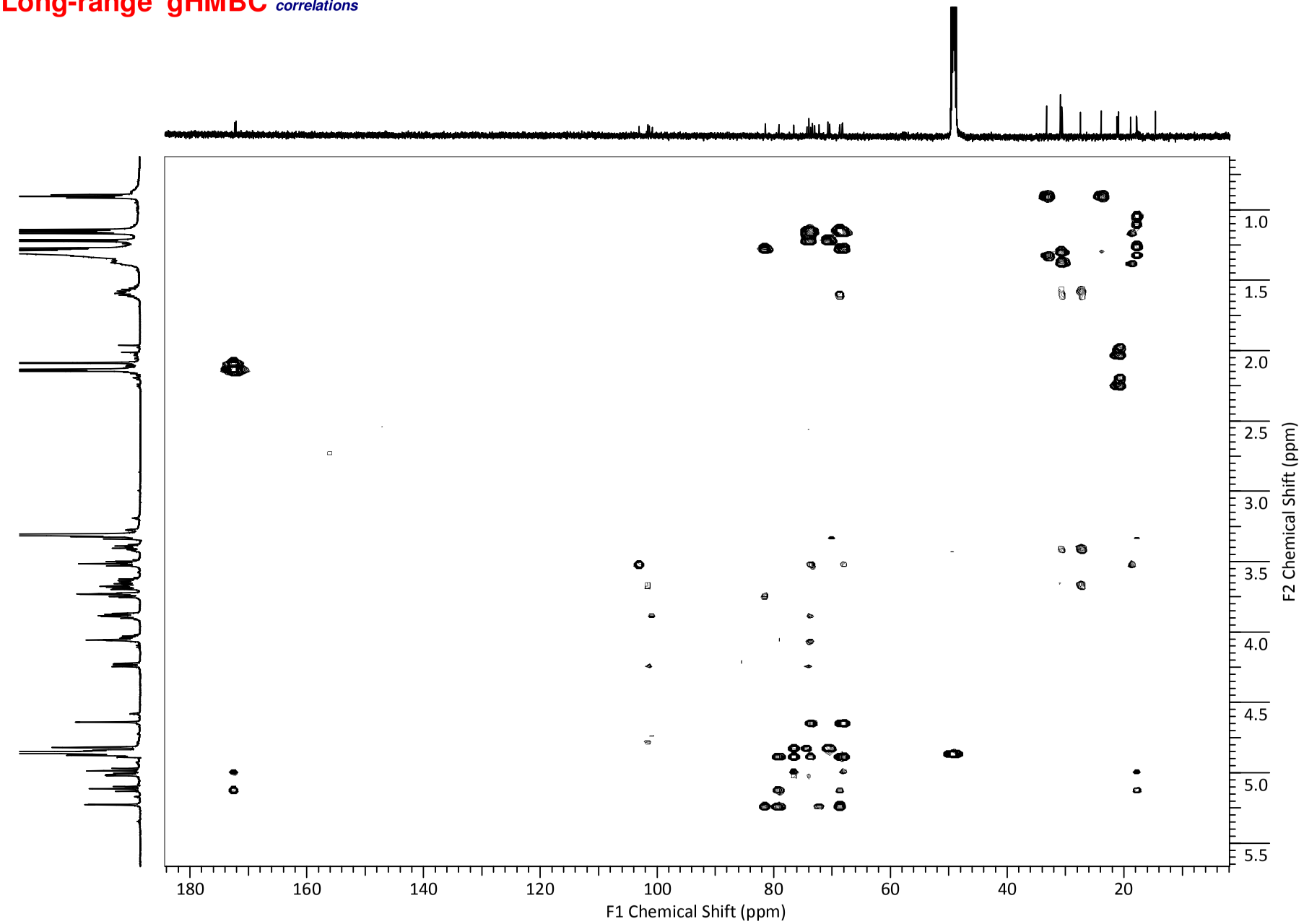


Expansion of the contour plot of the gHMQC spectrum:

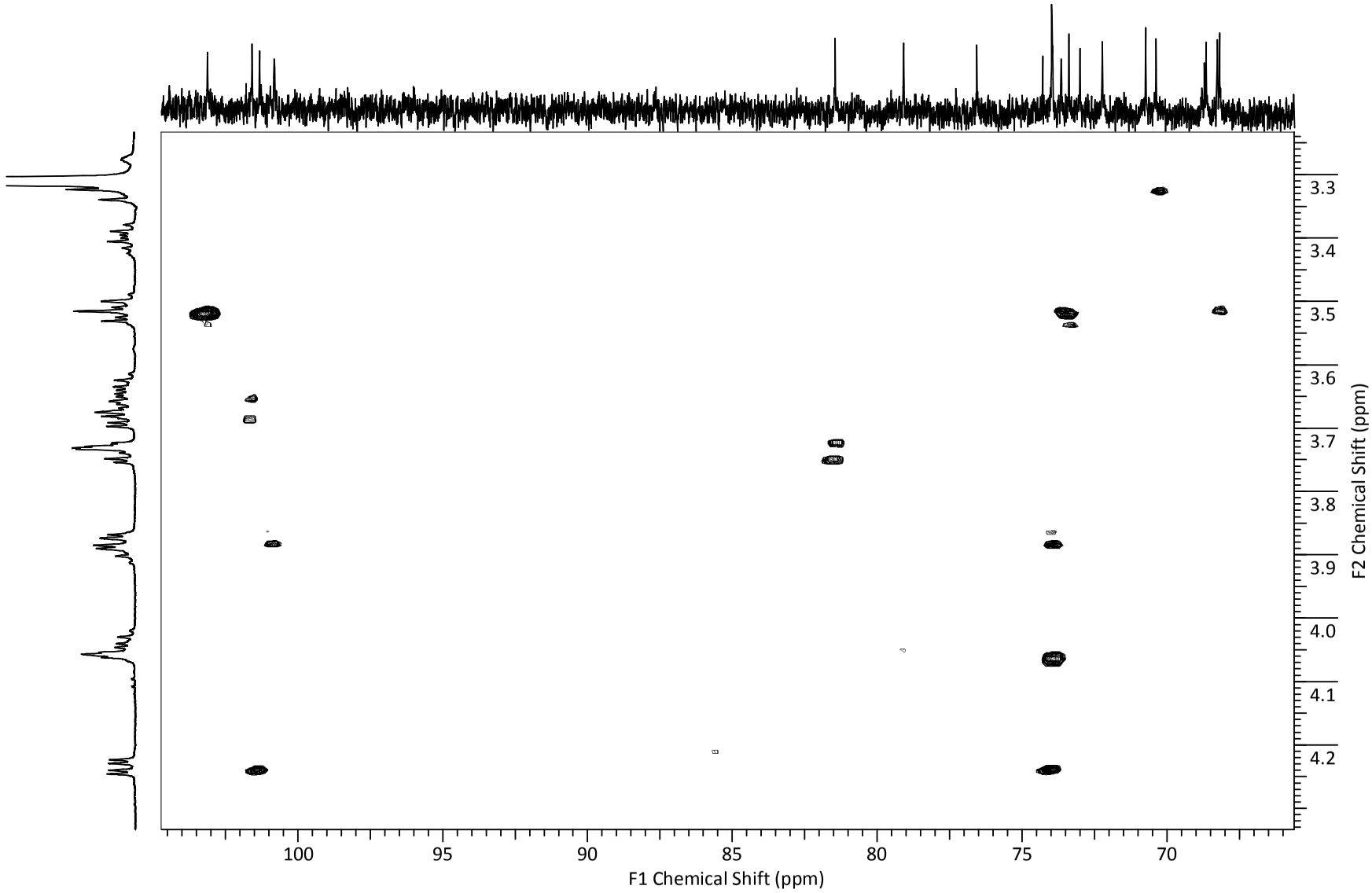
One-bond gHMQC ***correlations***



Long-range gHMBC *correlations*

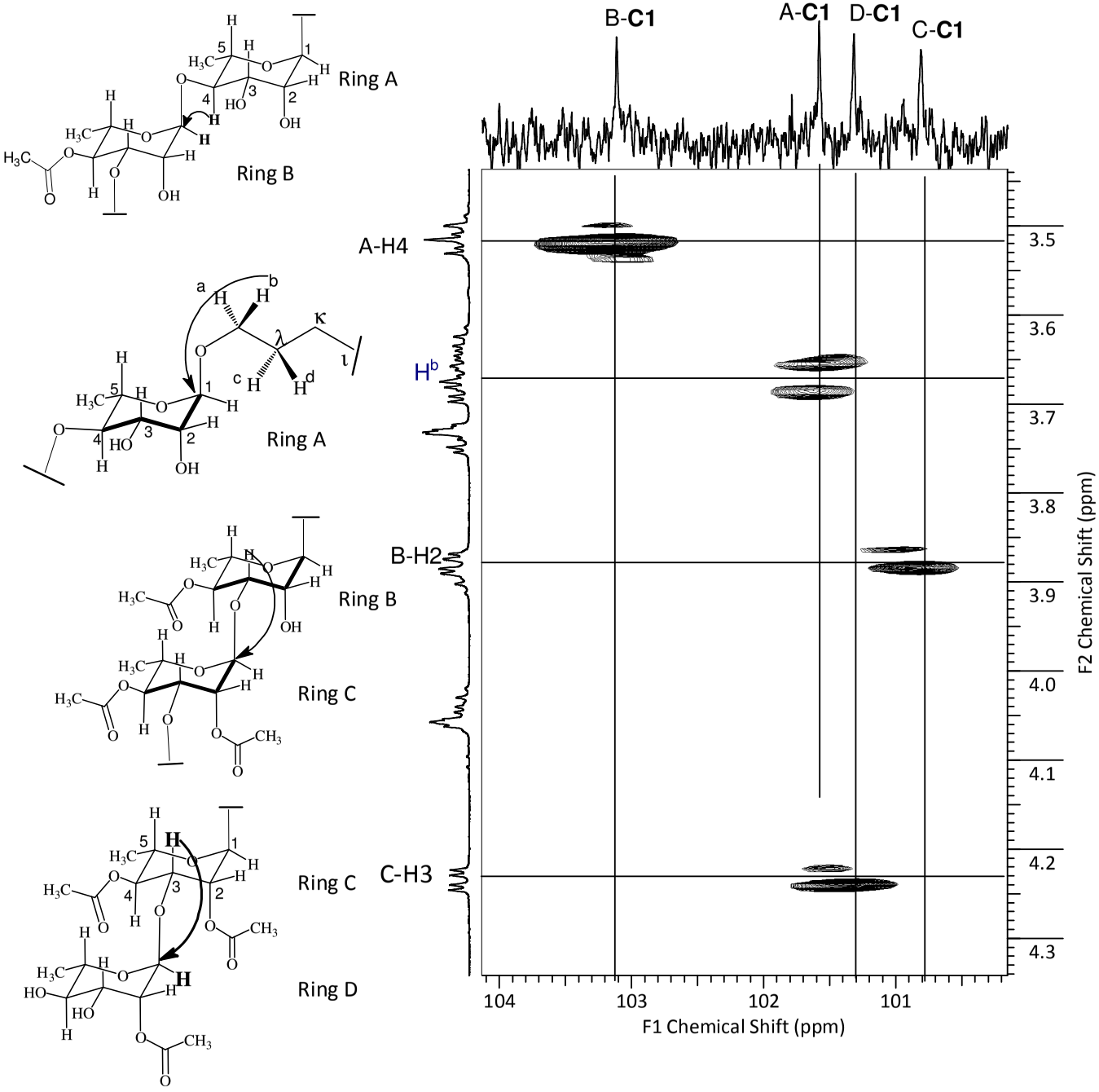


Expansion of the contour plot of the gHMBC spectrum:
Long-range gHMBC *correlations*



HMBC

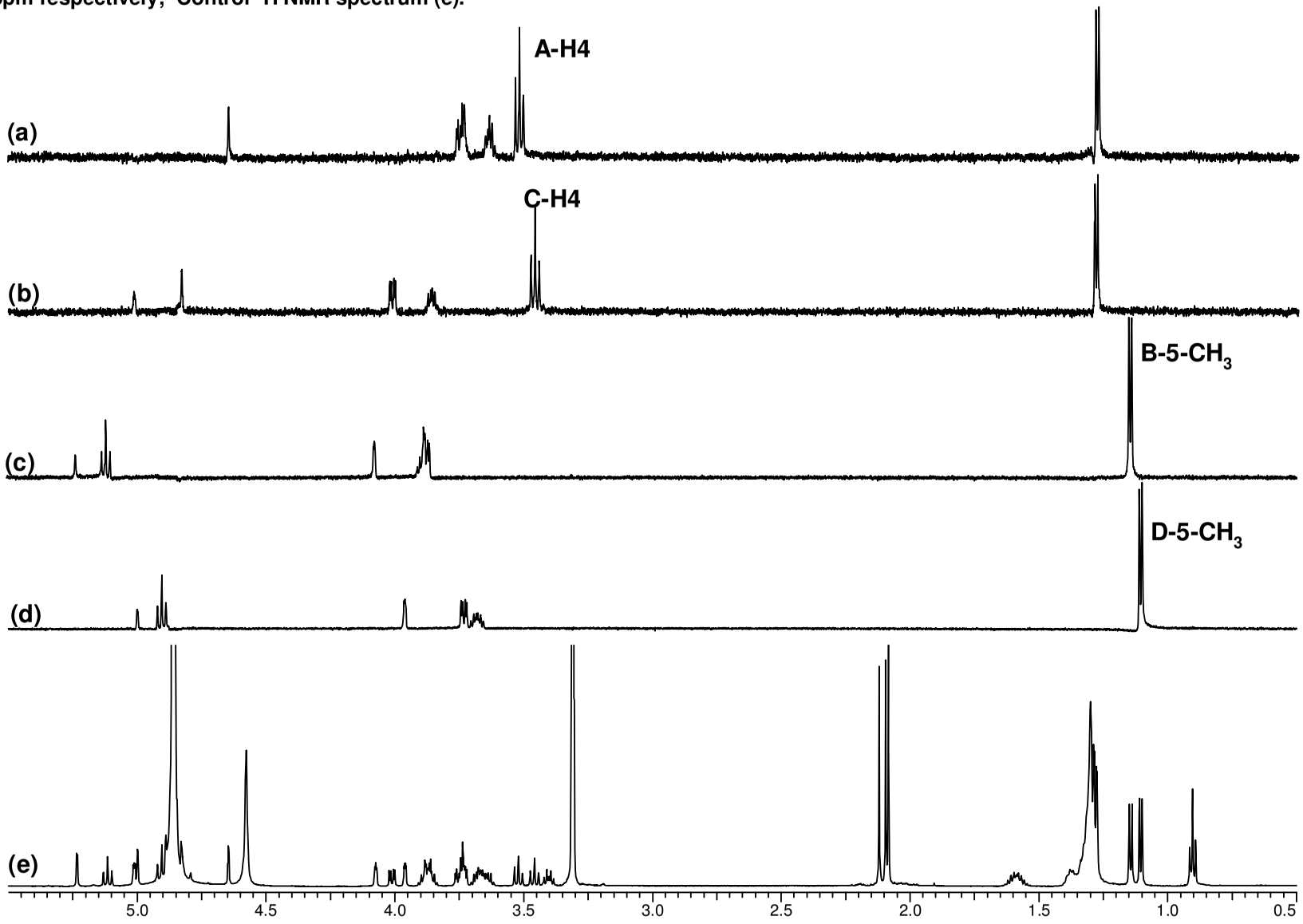
correlations (trough three bond)
confirming connectivities
of four sugar units



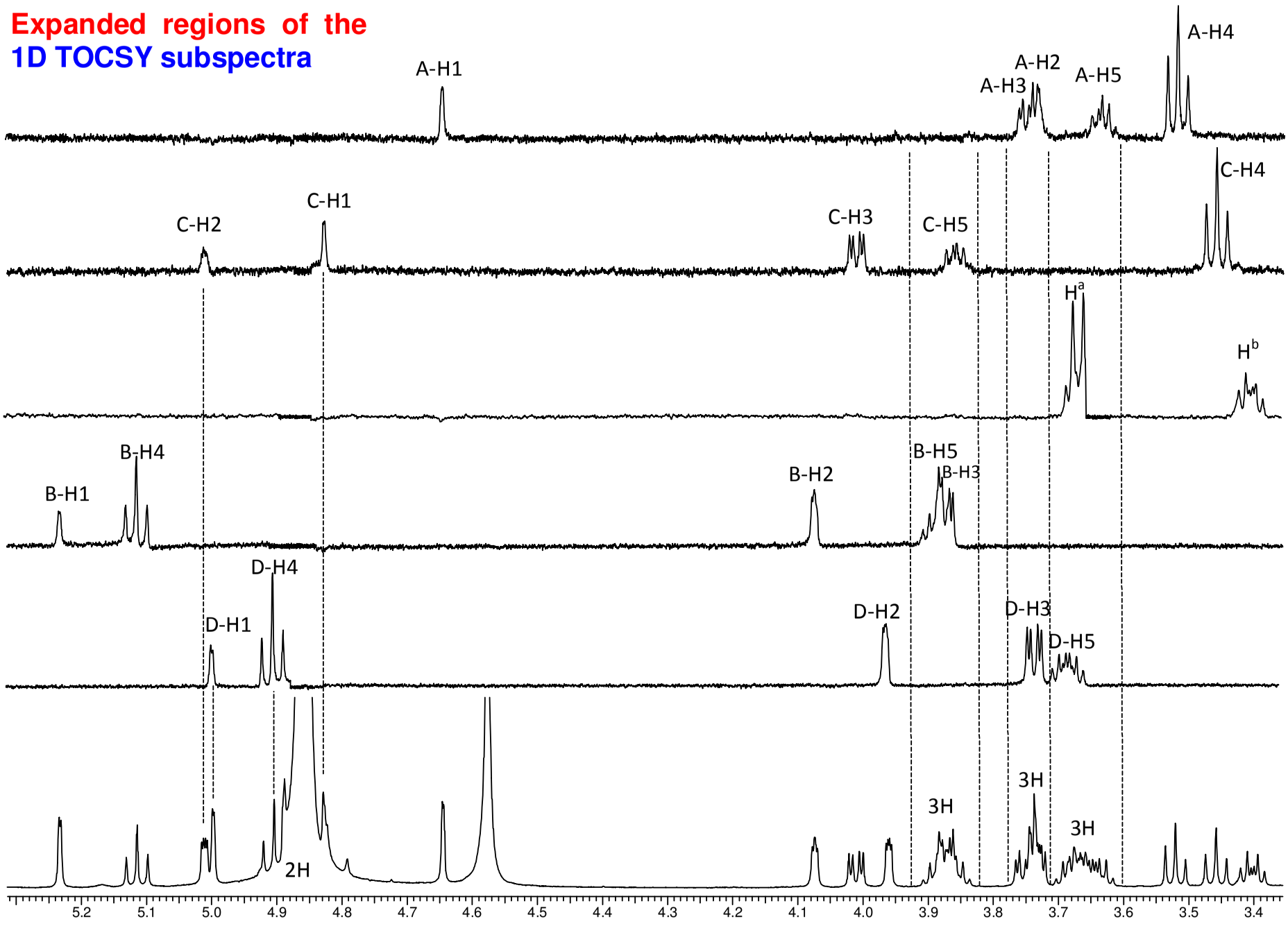
Spin system identification *for four sugar units (A-D)*

1D TOCSY experimental subspectra (a-d);

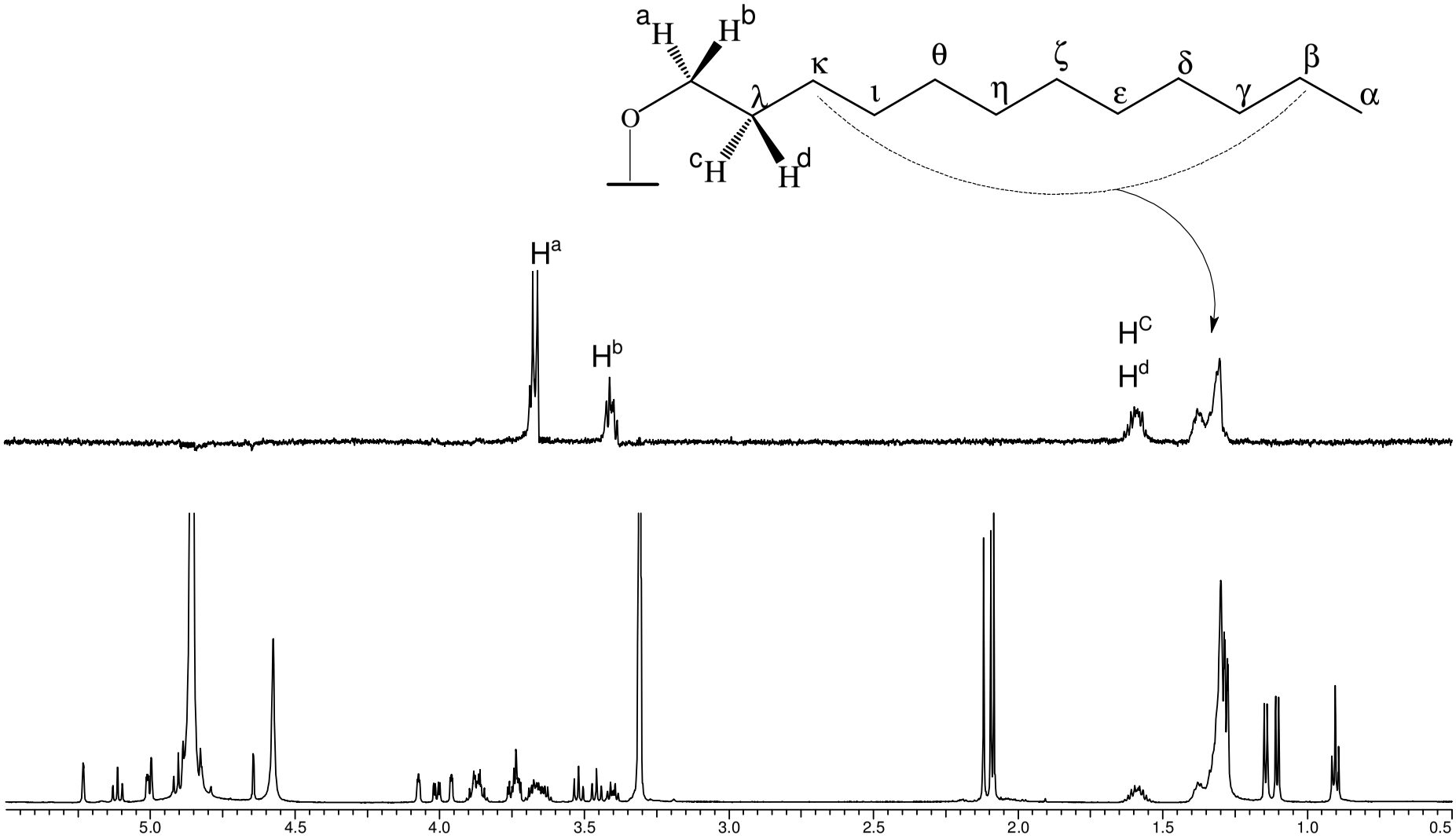
selective excitation of A-H4 (a) and C-H4 (b) at 3.52 and 3.46 ppm respectively, mixing time = 260 ms; (c) and (d) spectra were obtained with mix time of 260 ms: selective excitation of 5-CH_3's at 1.10 and 1.14 ppm respectively; Control ^{1}H NMR spectrum (e).



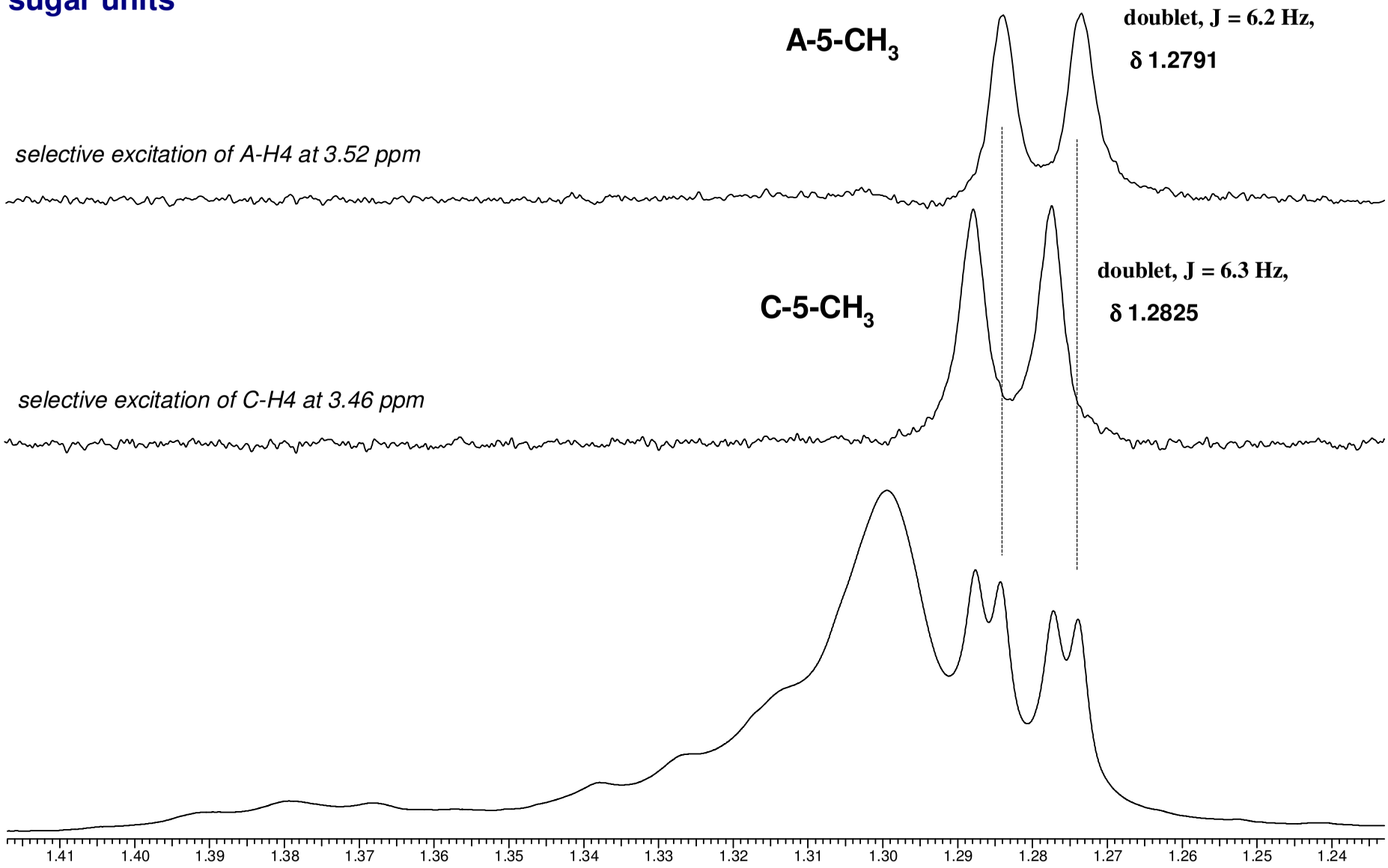
Expanded regions of the 1D TOCSY subspectra



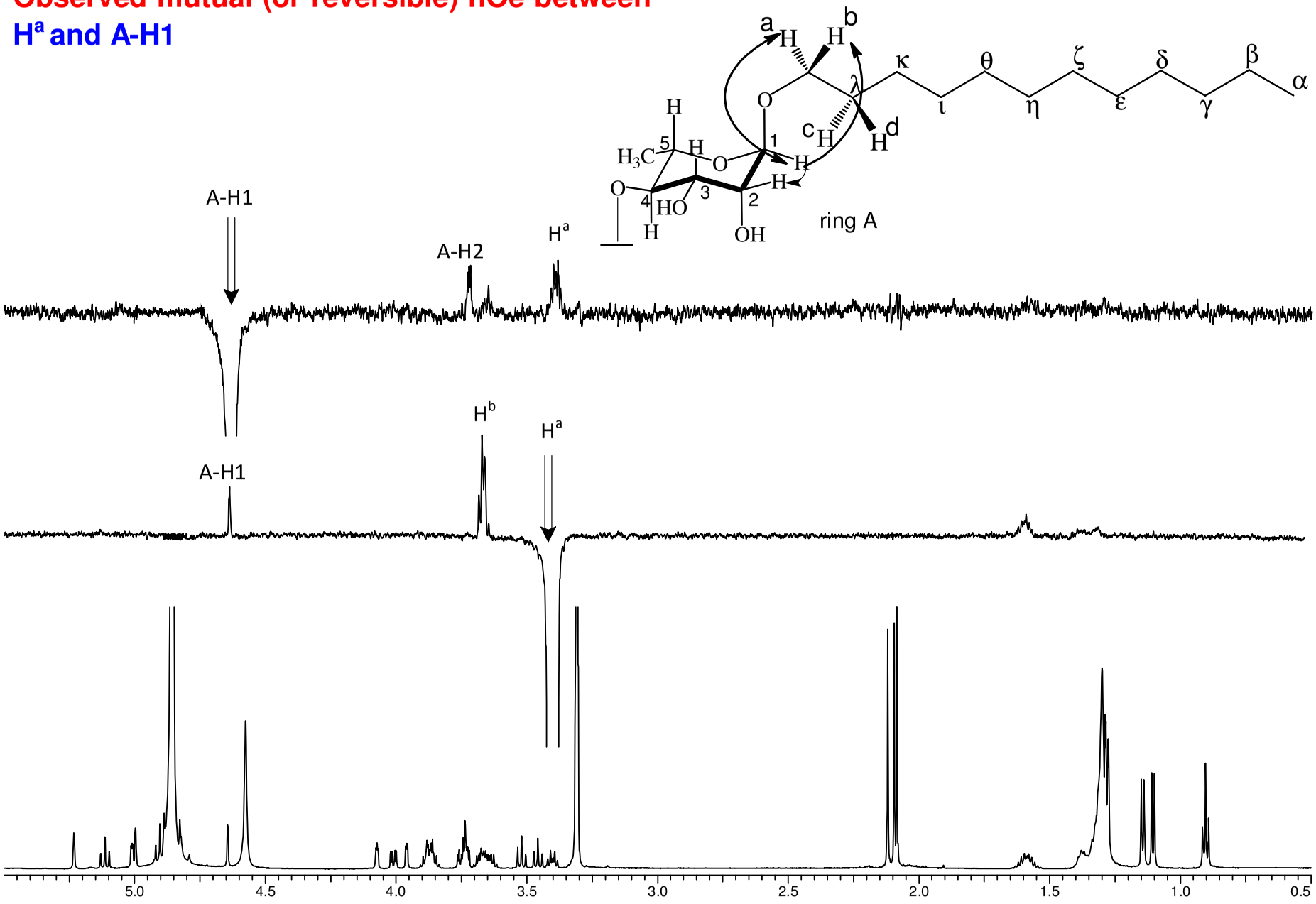
Selective excitation of H^a at 3.39 ppm (mix = 160 ms)



Chemical shifts of 5-CH_3 's of A and C sugar units

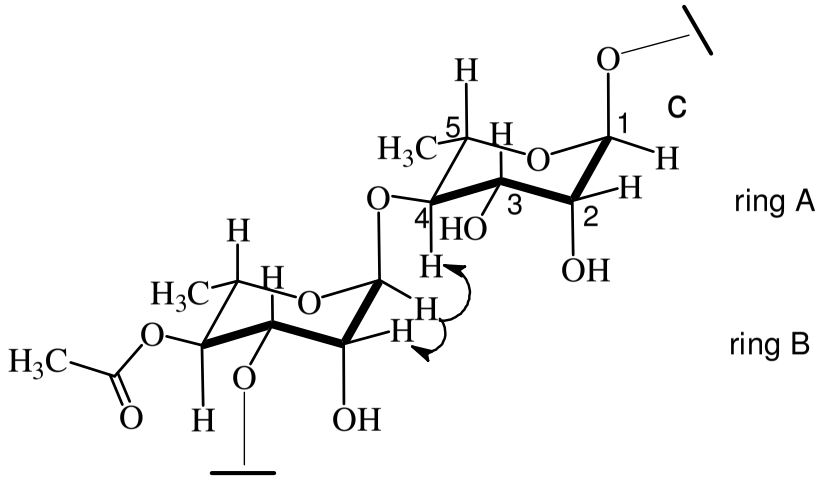


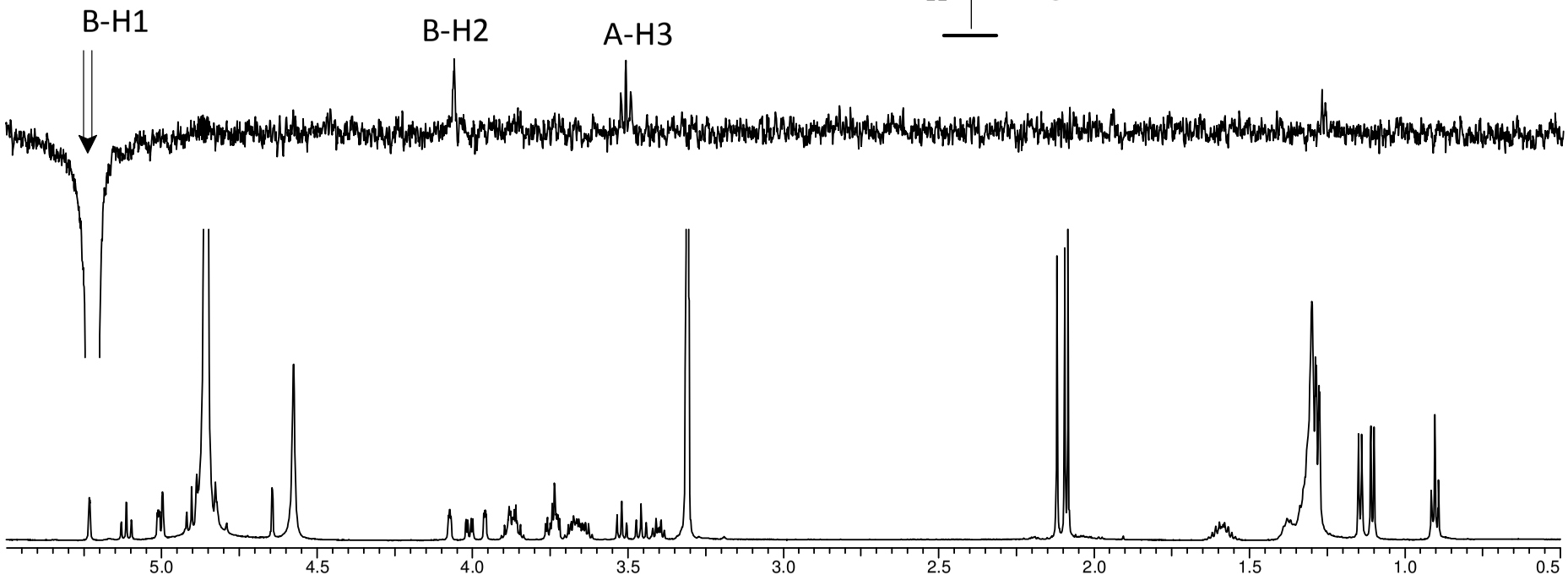
Observed mutual (or reversible) nOe between
H^a and A-H1



DPFGSENOE *spectrum*

Through space connectivity between two rings: A (H1, 3.5198 ppm) and B (H1, 5.2302 ppm)

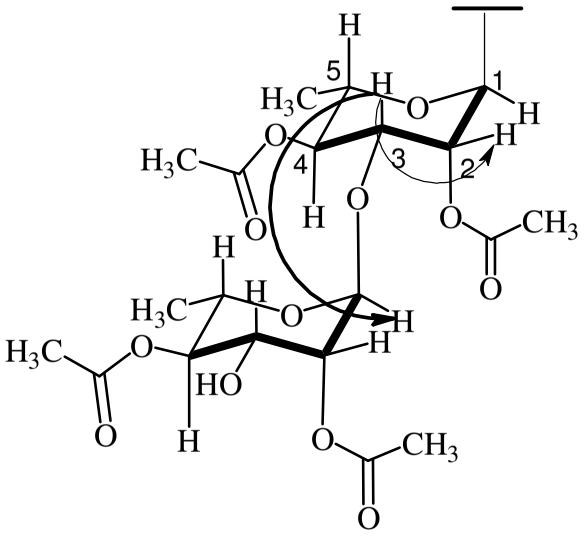




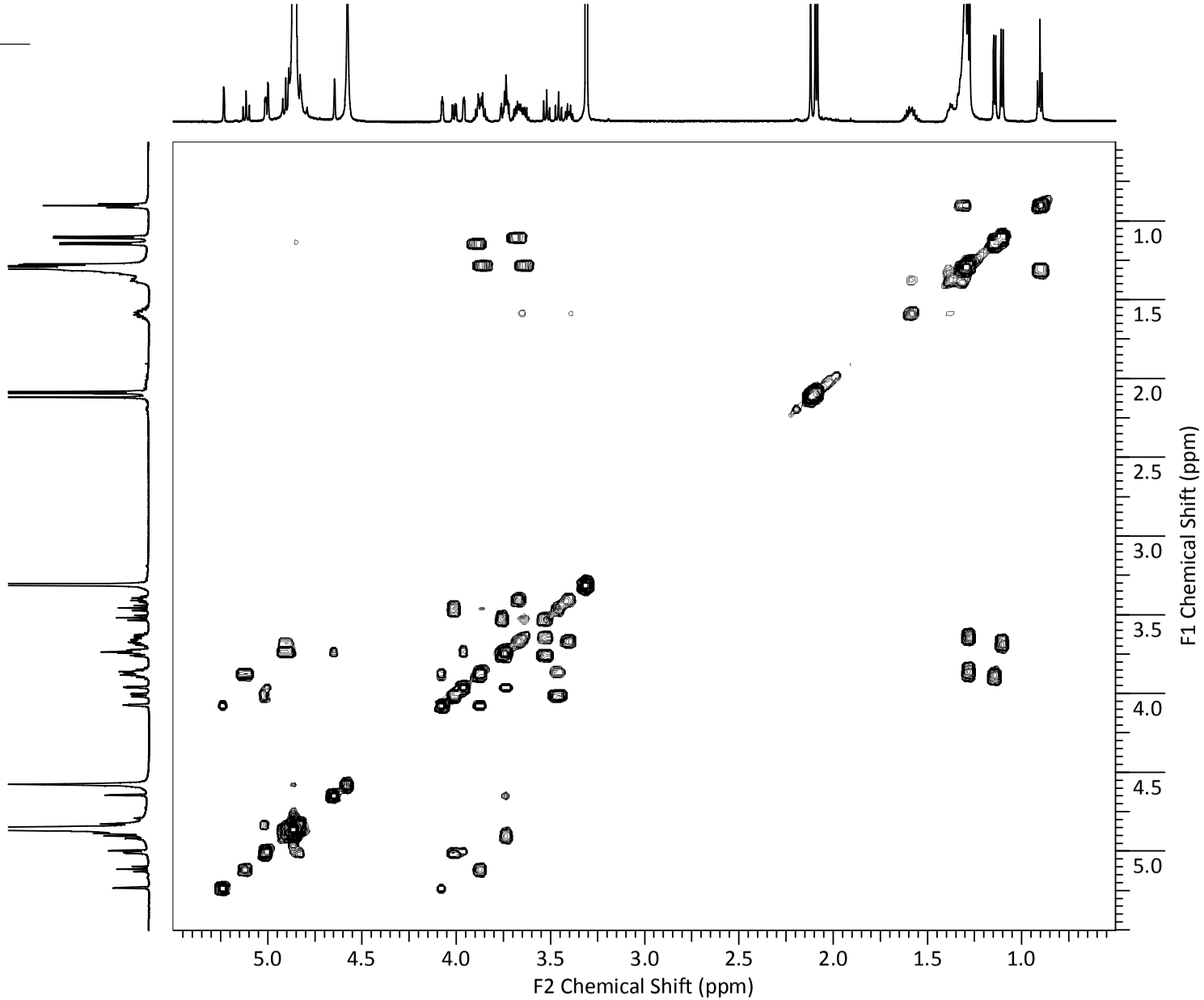
DPFGSENOE *spectrum*

Through space connectivity between two rings:

C (H3, 4.088 ppm) and D (H1, 4.9943 ppm)

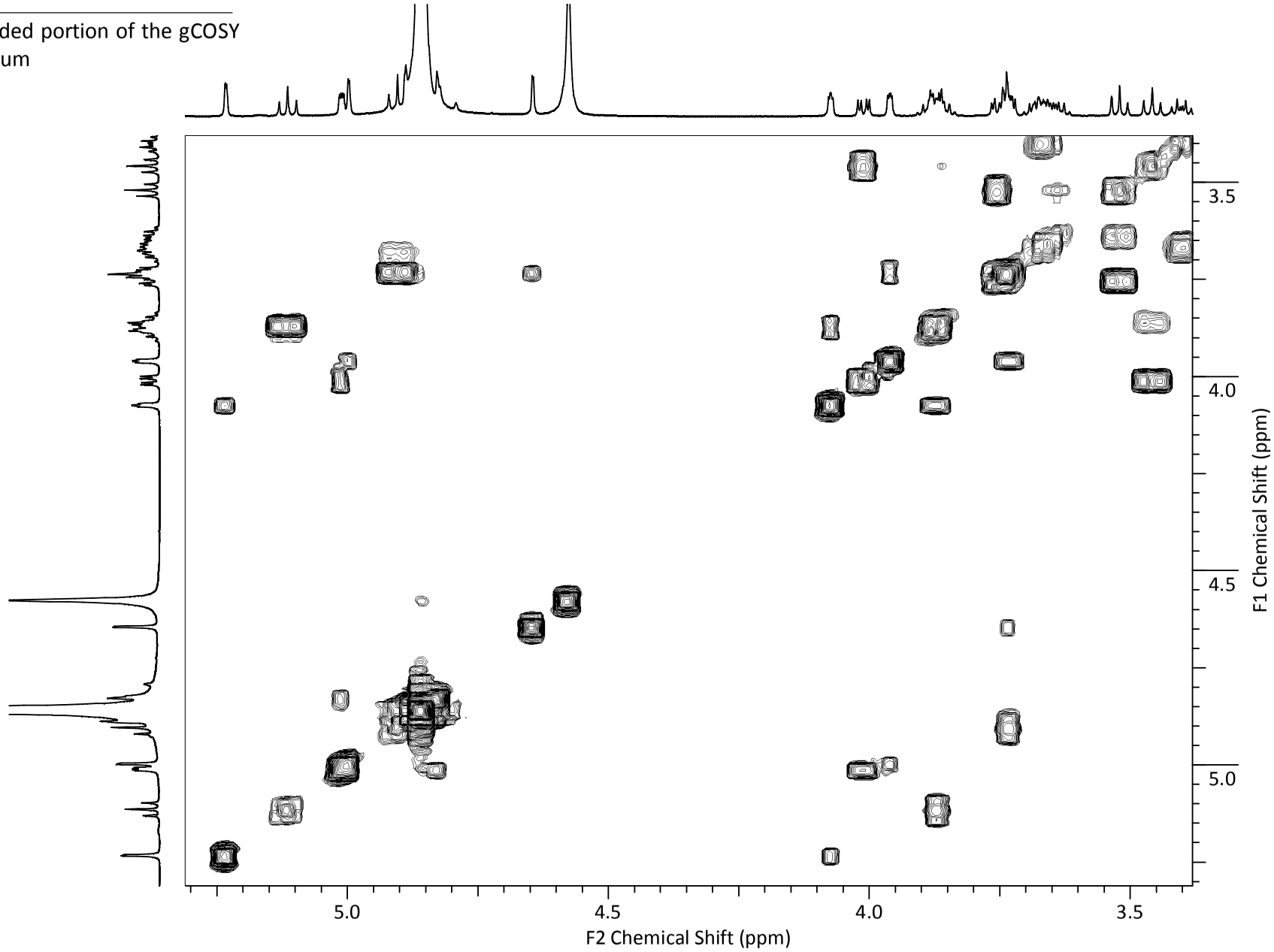


gCOSY


5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
F2 Chemical Shift (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
F1 Chemical Shift (ppm)

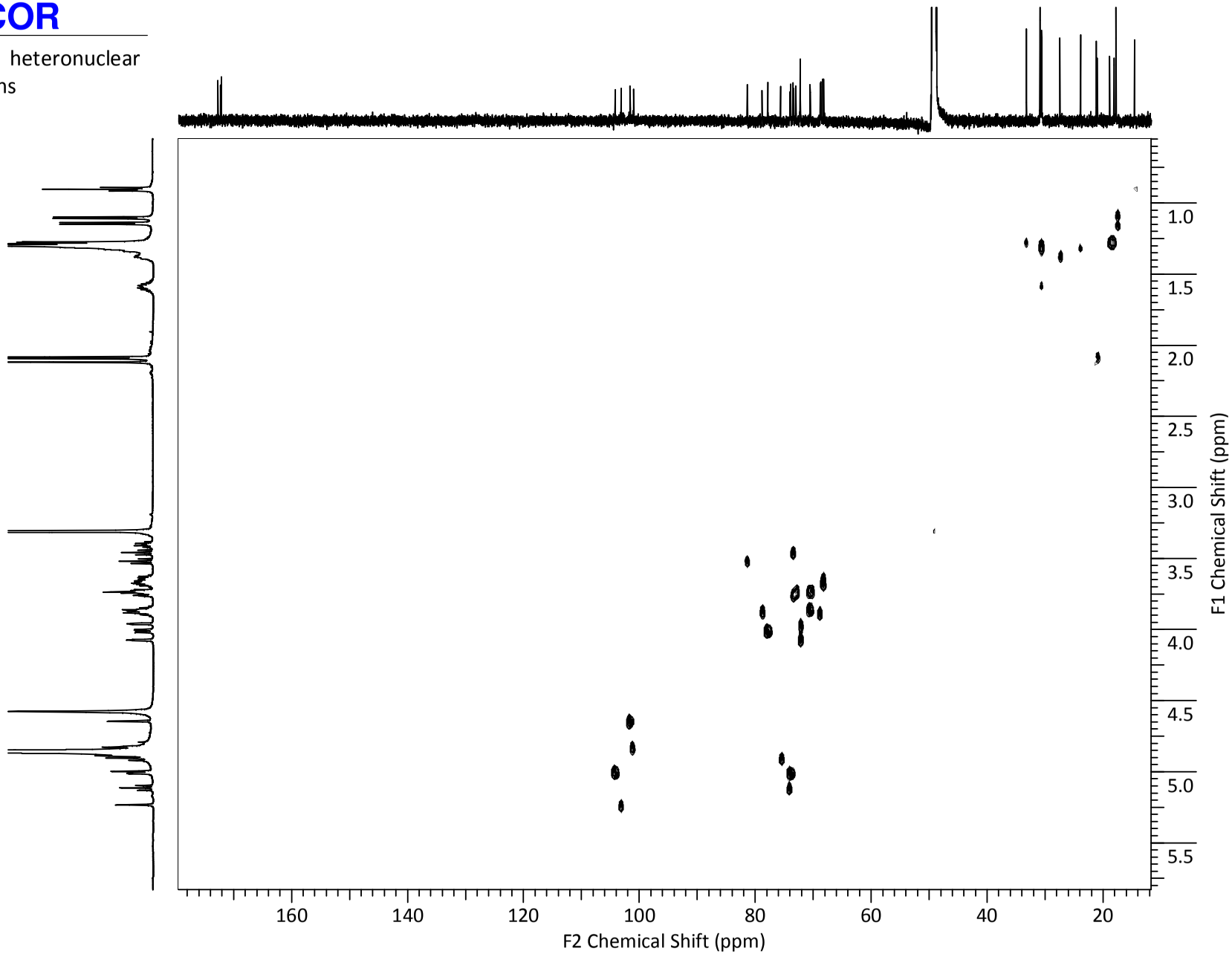
gCOSY

Expanded portion of the gCOSY spectrum



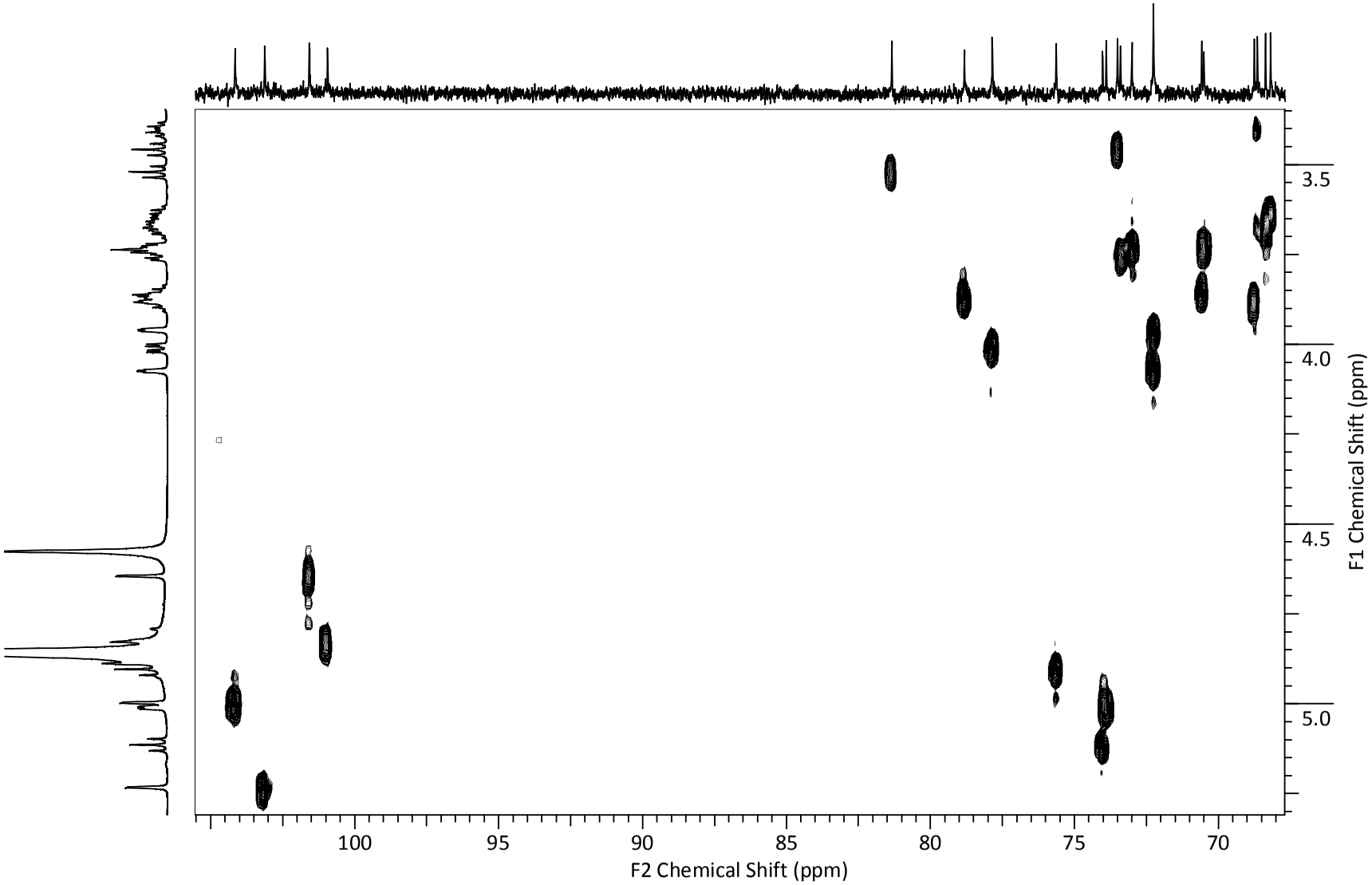
HETCOR

One-bond heteronuclear correlations



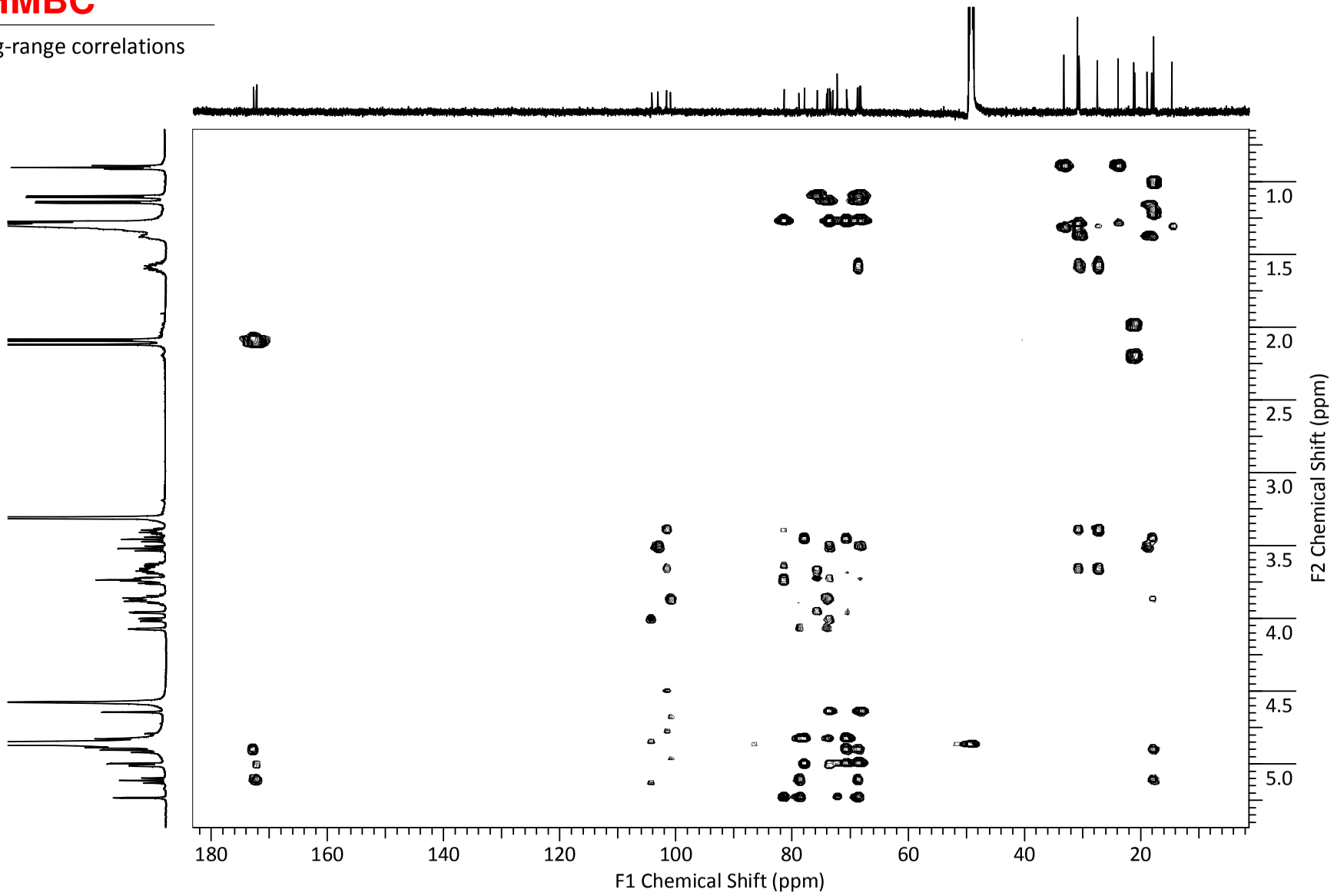
HETCOR

Expanded portion of the HETCOR spectrum



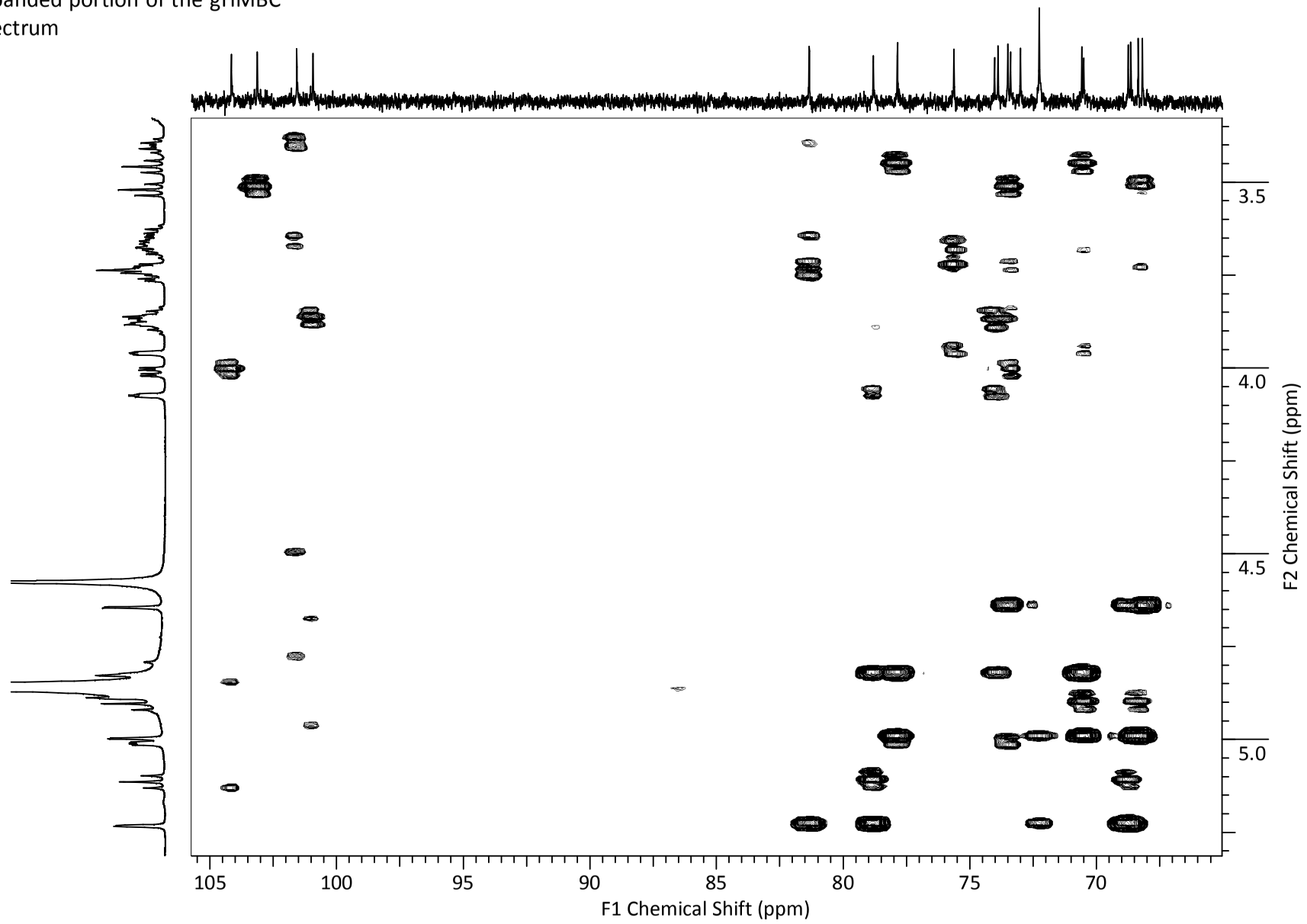
gHMBC

Long-range correlations



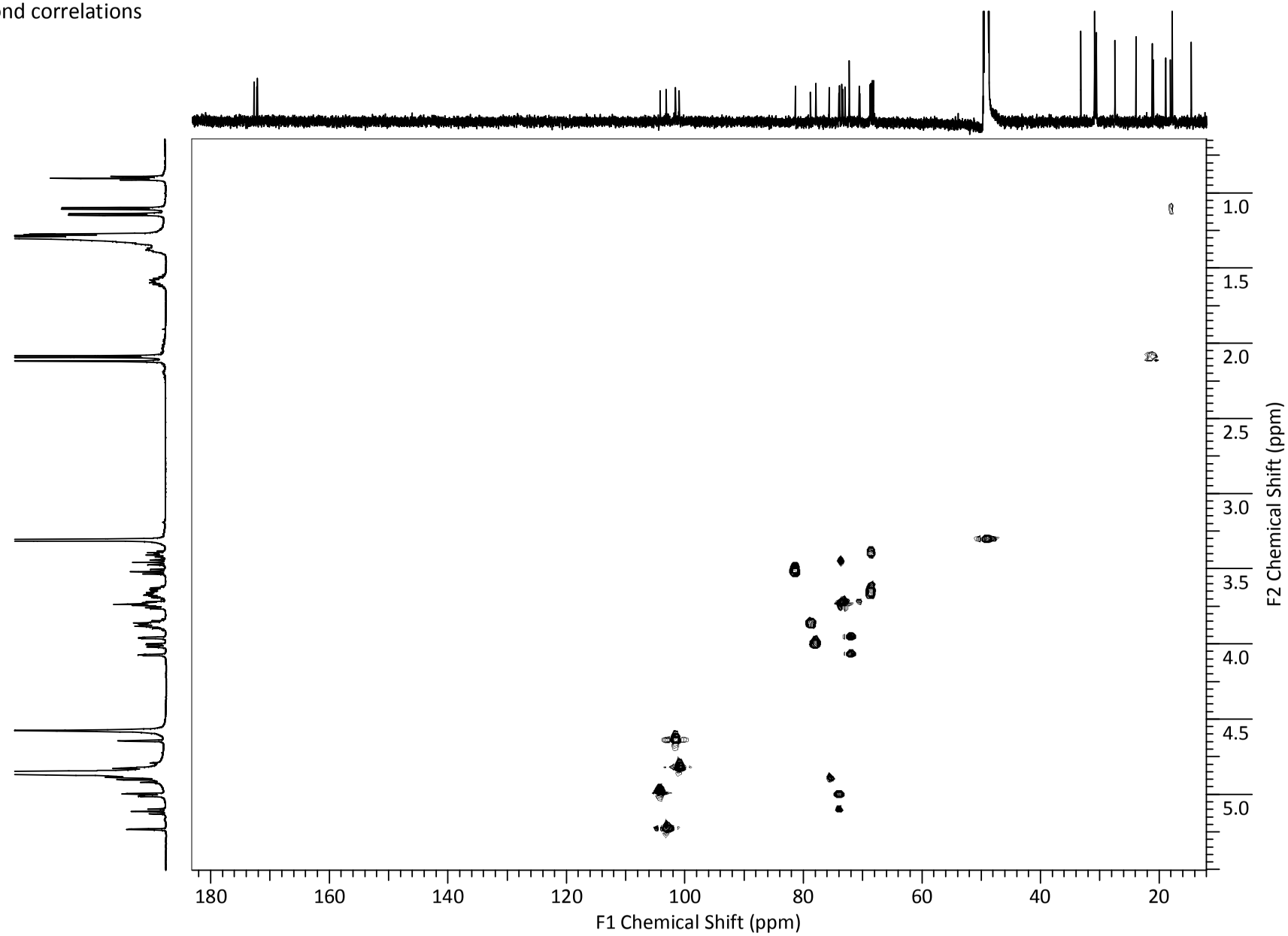
gHMBC

Expanded portion of the gHMBC spectrum



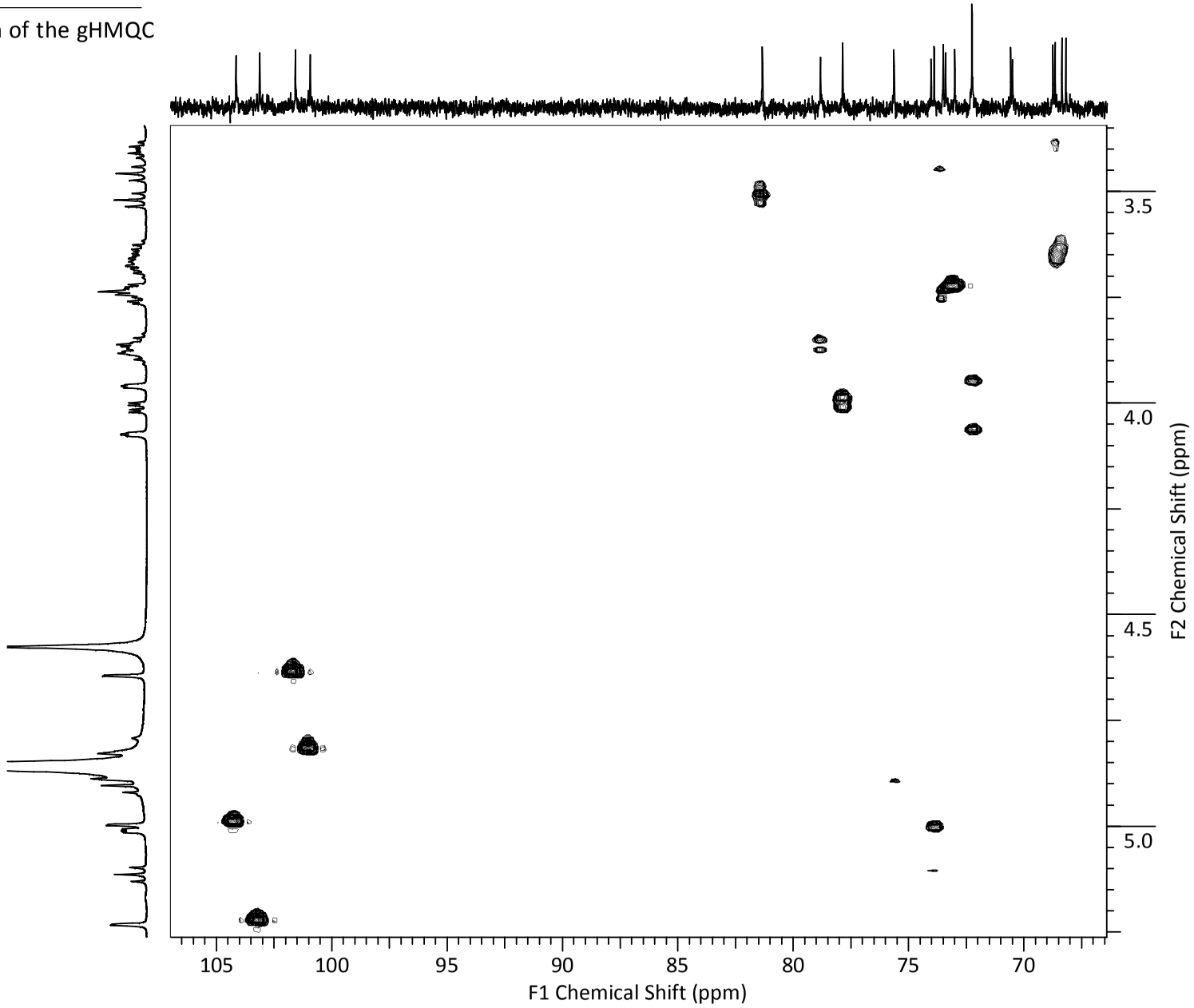
gHMQC

One-bond correlations

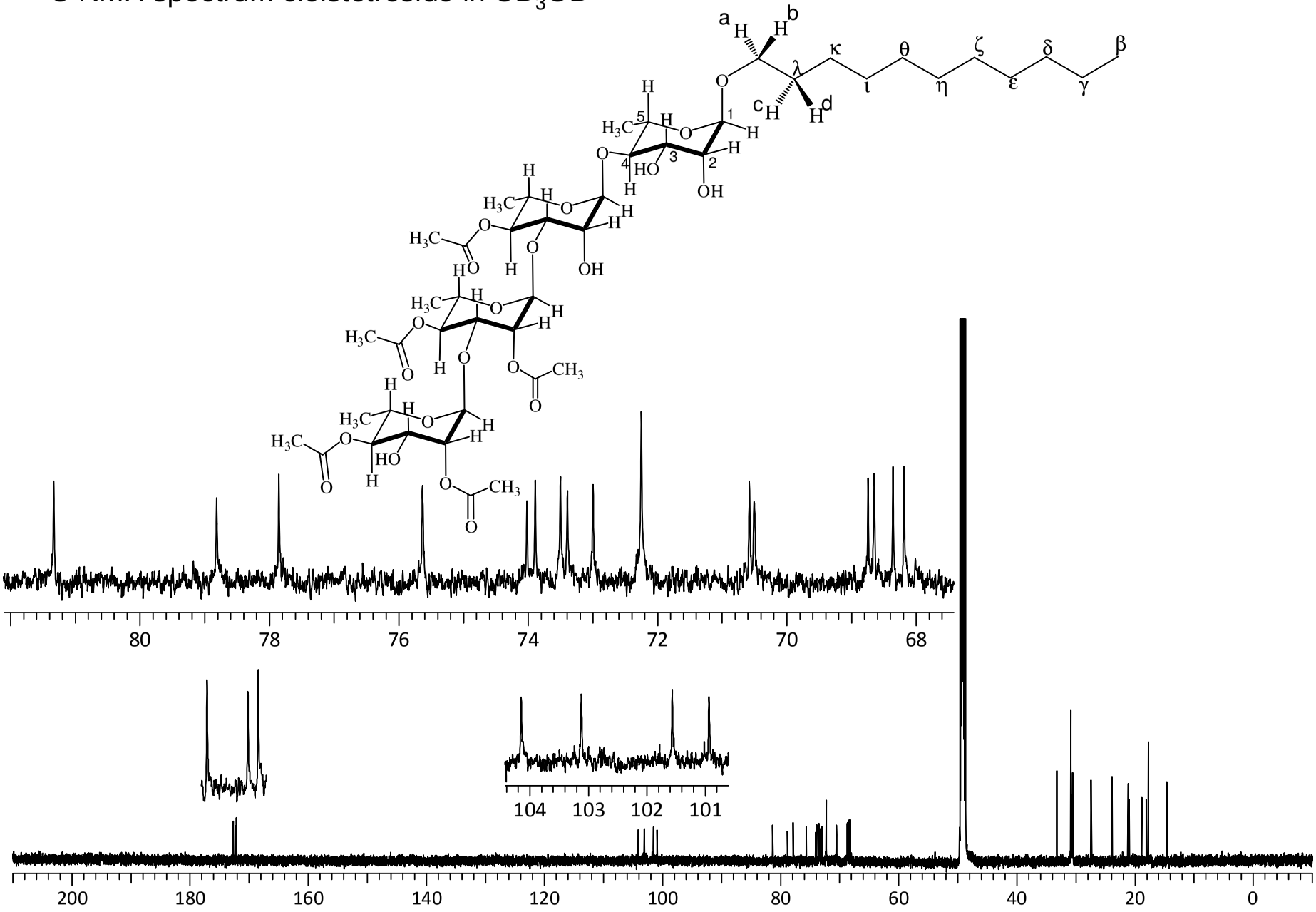


gHMQC

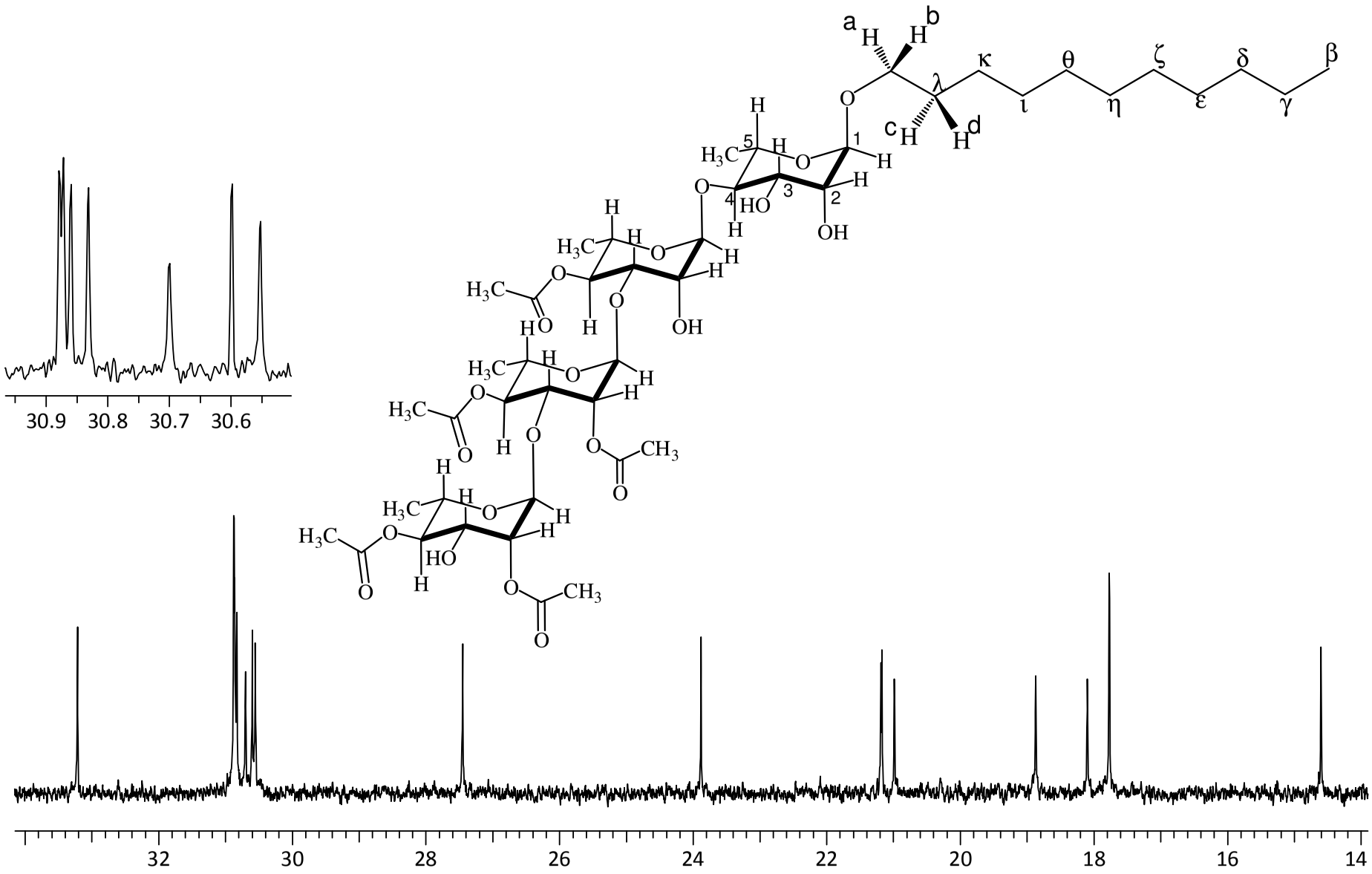
Expanded portion of the gHMQC spectrum



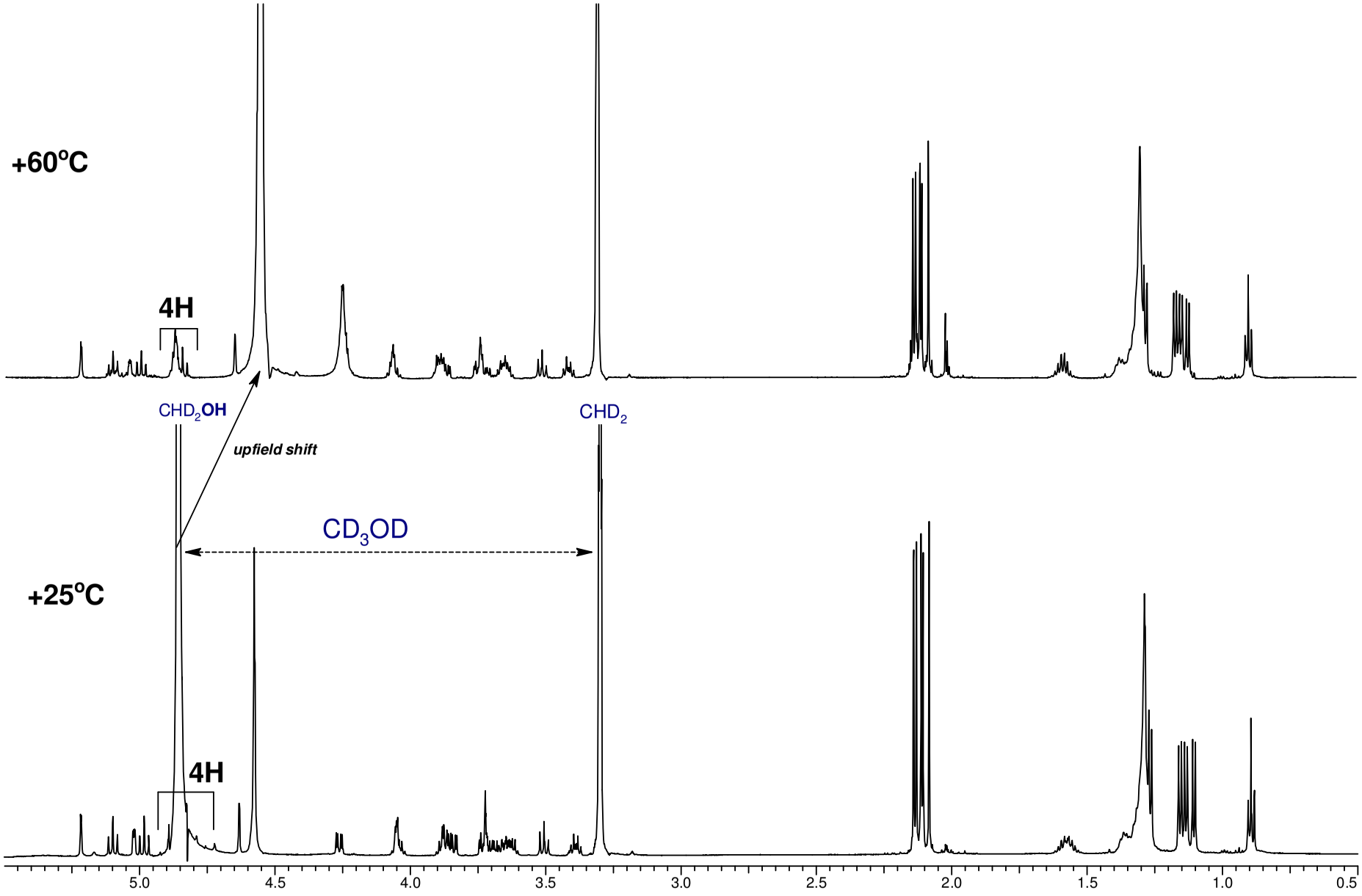
^{13}C NMR spectrum cleistetroside in CD_3OD



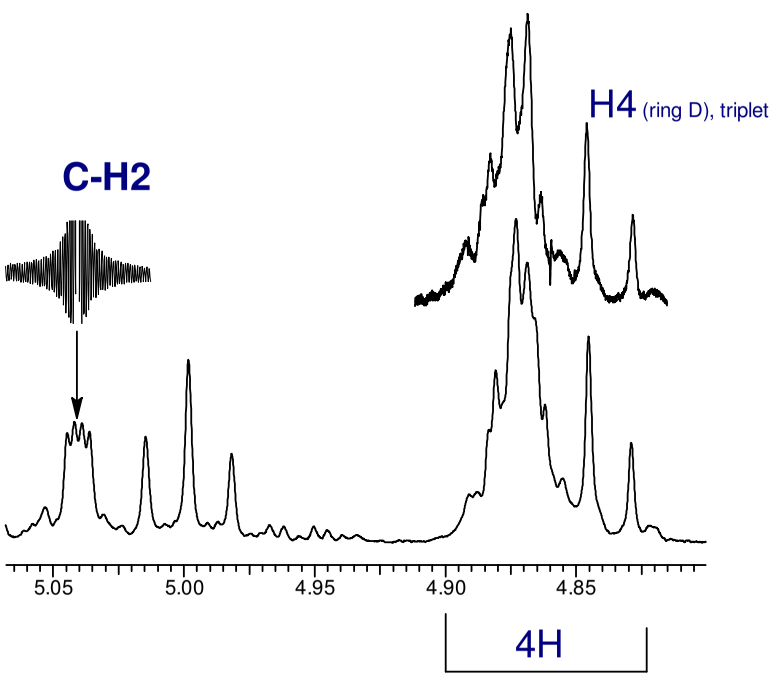
^{13}C NMR spectrum cleistetroside in CD_3OD



Three proton peaks (H1, H2, and H4) of the Ring-D and one proton peak (H1) of the Ring C were hidden by the residual proton peak of solvent (CD_3OD). The ^1H NMR spectrum (a) recorded at +60°C exibit separation of these peaks



Selective decoupled ^{1}H NMR spectra at +60°C



H1, H2, and H4 (Ring D) and H1 (Ring C)

Chemical shift assigments of H1, H2, and H4 (Ring D) and H1 (Ring C)