Supporting Information

Synthesis and Immunological Study of α-2,9-Oligosialic Acid Conjugates as Anti-Group C Meningitis Vaccines

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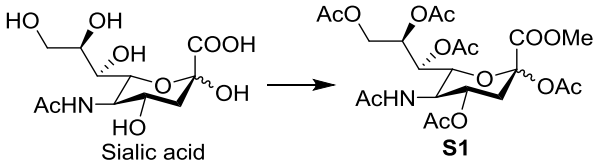
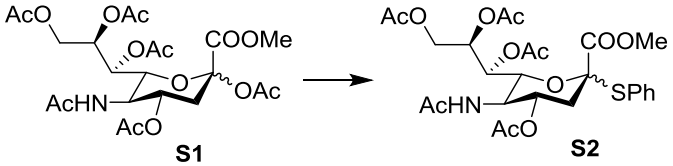
I. Synthesis of Conjugates 1-8

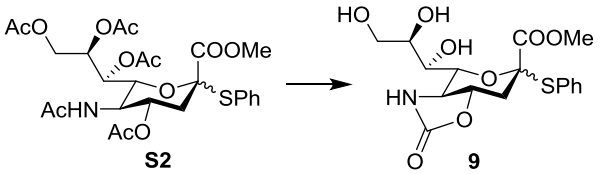
General Methods. Chemicals and materials were obtained from commercial sources and were used as received without further purification unless otherwise noted. MS 4 Å was flame-dried under high vacuum and used immediately after cooling under a N_2 atmosphere. Analytical TLC was carried out on silica gel 60 Å F_{254} plates with detection by a UV detector and/or by charring with 15% (v/v) H_2SO_4 in EtOH. NMR spectra were recorded on a 400, 500, or 600 MHz machine with chemical shifts reported in ppm (δ) downfield from tetramethylsilane (TMS), that was used as an internal reference. Mass spectrometry (MS) was performed using either a Bruker Daltonics Ultraflex MALDI TOF MS or Waters LCT Premier XE high resolution ESI MS.



Methyl 5-Acetamido-2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-D-glycero-α,β-D-galacto-non-2-ulopyranosylonate (S1).[1] The mixture of sialic acid (10.0 g, 32.33 mmol) and Amberlyst 15 resin (40.0 g) in MeOH (300 mL) was stirred at 45 °C for 6 h. The resin was filtered off and the filtrate was concentrated under vacuum to afford the intermediate methyl ester of sialic acid (9.22 g). To a solution of this crude intermediate and DMAP (24.4 mg, 0.10 mmol) in pyridine (100 mL) was slowly added acetic anhydride (58.2 mL, 0.62 mol) at 0 °C. The reaction mixture was stirred overnight at rt. After removing pyridine under vacuum, the residue was dissolved in dichloromethane (DCM). The organic layer was washed with saturated aq. $NaHCO_3$, brine, dried over anhydrous Na_2SO_4 and then concentrated under vacuum. The residue was purified by silica gel column chromatography (MeOH/DCM = 1:50) to give the α,β-mixture of **S1** (10.51 g, 61%). Its spectroscopic data were the same as reported.[1]

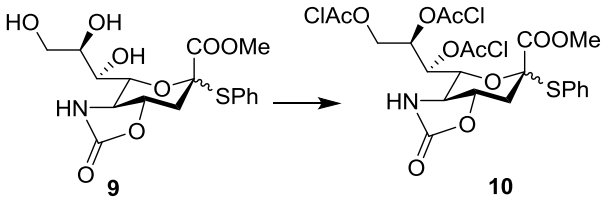


Methyl (Phenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α,β-D-galacto-non-2-ulopyranoside)onate (S2).[1, 2] To a stirred mixture of **S1** (14.0 g, 26.25 mmol), thiophenol (11.60 g, 105.01 mmol) and MS 4 Å (10.0 g) in anhydrous DCM (100 mL) under Ar, $BF_3{\cdot}Et_2O$ (13.3 mL, 105.01 mol) was added dropwise at 0 °C. After stirring at rt for 1 day, the reaction was quenched with saturated aq. $NaHCO_3$ and filtered through a Celite pad. The filtrate was washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (MeOH/DCM = 1:20) to give the α,β-mixture of **S2** (13.51 g, 88%) as white solids. Its spectroscopic data were the same as reported.[1, 2]

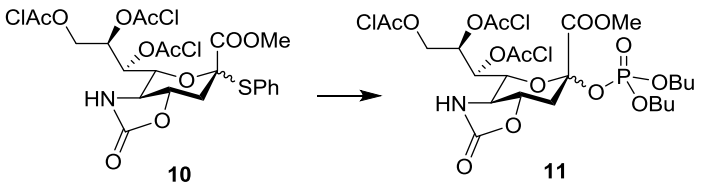


Methyl (Phenyl 5-amido-5-N,4-O-carbonyl-3,5-dideoxy-2-thio-D-glycero-α,β-D-galacto-non-2-ulopyranoside)onate (9).[2] The solution of **S2** (10.0 g, 17.08 mmol) and methanesulfonic acid

(4.4 mL, 68.32 mmol) in methanol (175 ml) was refluxed for 1 day. The reaction was quenched with an excess of Et_3N and concentrated under vacuum. To the stirred mixture of the residue and $NaHCO_3$ (7.20 g, 85.71 mmol) in CH_3CN (60 mL) and water (120 mL) was slowly added 4-nitrophenyl chloroformate (8.64 g, 42.87 mmol) through a dropping funnel at 0 °C. After being stirred for 3 h, the reaction mixture was extracted with AcOEt three times. The combined organic layer was washed with brine, dried over Na_2SO_4, and then concentrated under vacuum. The residue was purified by silica gel column chromatography (MeOH/DCM = 1:50) to give the α,β-mixture of **9** (5.56 g, 82%, α:β = 1:2.5) as white solids. Its spectroscopic data were the same as reported.[2]

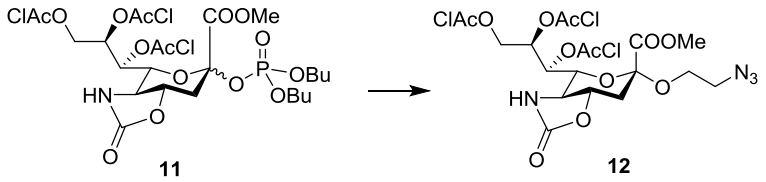


Methyl (Phenyl 5-amido-5-N,4-O-carbonyl-7,8,9-tri-O-chloroacetyl-3,5-dideoxy-2-thio-D-glycero-α,β-D-galacto-non-2-ulopyranoside)onate (10).[2, 3] To a stirred solution of **9** (5.50 g, 13.77 mmol) and pyridine (6.6 mL, 82.62 mmol) in DCM (80 mL) was added dropwise chloroacetyl chloride (3.72 mL, 46.82 mmol) at 0 °C under Ar. After 3 h of stirring, the reaction mixture was diluted with DCM, washed with 10% aq. HCl, water, and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1:3) to afford the α,β-mixture of **10** (7.66 g, 89%,) as a white solids. Its spectroscopic data were the same as reported.[2, 3]



Methyl (Dibutylphosphoryl 5-amido-5-N,4-O-carbonyl-7,8,9-tri-O-chloroacetyl-3,5-dideoxy-α,β-D-glycero-D-galacto-non-2-ulopyranoside)onate (11).[2] A mixture of **10** (6.29 g, 10.0 mmol), dibutyl phosphate (5.0 mL, 25.0 mmol), and activated MS 4 Å (12.0 g) in anhydrous DCM (60 mL) was stirred under an Ar atmosphere at rt for 1 h. After cooling to 0 °C, NIS (3.38 g, 15.0 mmol) and TfOH(180μL) were added, and the reaction was kept being stirred for 12 h. The reaction was quenched with aq. $Na_2S_2O_3$ and then filtered through a Celite pad. The filtrate

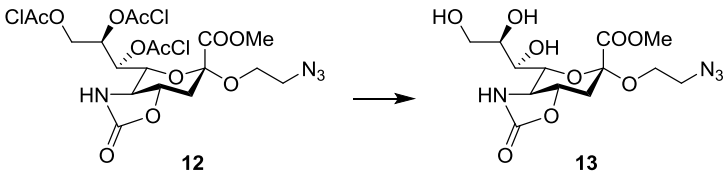
was diluted with DCM and washed with saturated aq. $NaHCO_3$, water, and brine, dried over Na_2SO_4, and then concentrated under vacuum. The residue was purified by silica gel column chromatography (EtOAc/Hexane=1:2) to give the α,β-mixture of **11** (7.05 g, 97%, α:β = 1.6:1) as yellowish solids. Its spectroscopic data were the same as reported.[2]



General procedure for glycosylation reactions using sialyl phosphates as glycosyl donors. A mixture of glycosyl donor (1.1 mmol), accepter (1 mmol), and activated MS 4 Å (1.0 g/mmol) in a mixture of anhydrous CH_3CN and DCM (V/V = 1:2) was stirred under an Ar atmosphere at rt for 3 h. The mixture was cooled to -70 °C, and TMSOTf (180 μL) was added. Then, the reaction solution was slowly warmed to -40 °C and stirred for 1 h. The reaction mixture was diluted with DCM and filtered through a Celite pad. The filtrate was washed with saturated aq. $NaHCO_3$, water, and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography to give the target compound.

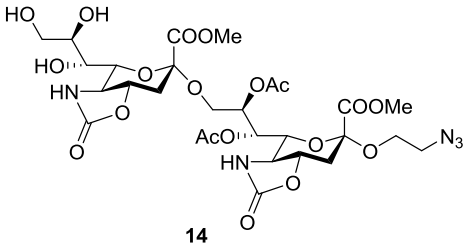
Methyl (2-Azidoethyl 5-amido-5-N,4-O-carbonyl-7,8,9-tri-O-chloroacetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onate (12). Compound **12** (2.26 g, 85%) was prepared from glycosyl donor **11** (3.21 g, 4.39 mmol) and 2-azidoethanol (0.35 g, 4.0 mmol) using the above glycosylation procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 – 5.59 (m, 1H, H-8), 5.38 (s, 1H, NH), 5.22 (dd, *J* = 9.9, 1.8 Hz, 1H, H7), 4.58 – 4.47 (m, 1H, H-9), 4.43 – 4.26 (m, 3H, H-9 and CH_2Cl), 4.24 – 4.13 (m, 3H, H6 and CH_2Cl), 4.08 (s, 2H, CH_2Cl), 4.02 – 3.86 (m, 2H, H4 and ½O$\underline{CH_2}CH_2$), 3.83 (s, 3H, CH_3), 3.50 (m, 1H, ½O$\underline{CH_2}CH_2$), 3.33 (m, 2H, -CH_2N_3), 3.19 – 3.09 (m, 1H, H-5), 2.90 (dd, *J* = 12.2, 3.5 Hz, 1H, H_{3eq}), 2.15 – 2.05 (t, *J* = 12.1 Hz, 1H, H_{3ax}). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 168.1, 167.0, 166.4, 159.1, 100.0, 76.5, 73.6, 70.1, 68.0, 64.8, 63.1, 57.4, 53.4, 50.2, 41.1, 40.5, 40.3, 37.3. HRMS (ESI-TOF, $[M+H]^+$): calcd. For $C_{19}H_{24}C_{l3}N_4O_{12}$, 605.0456; found, 605.0449.

Note: The reaction between glycosyl donor **11** and 2-azidoethanol was also performed under the same conditions using anhydrous DCM as the solvent. Similar workup procedures followed by silica gel column chromatography as described above afford a mixture of α- and β-anomers of **12** in a ratio of 10:1, as proved by 1D and 2D NMR and MS analysis.



12 13

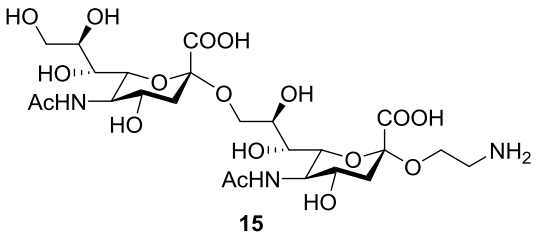
Methyl (2-Azidoethyl 5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onate (13). After **12** (2.43 g, 4.01 mmol) was dissolved in anhydrous MeOH (30 mL), triethylamine (0.3 mL) was added dropwise at rt. The reaction was stirred at rt for 10 min, and was then quenched with 10% aq. HCl. The organic layer was isolated and concentrated, and the residue was subjected to silica gel column chromatography (MeOH/EtOAc/Hexane = 1:5:5) to afford **13** (1.39 g, 92%) as white solids. ^{1}H NMR (500 MHz, CD_3OD): δ 4.17 – 4.06 (m, 2H), 3.99 (m, 1H), 3.87 (s, 3H), 3.82 (m, 2H), 3.73 – 3.67 (m, 1H), 3.67 – 3.58 (m, 3H), 3.45 – 3.29 (m, 3H), 2.97 (dd, *J* = 11.7, 3.7 Hz, 1H, H-3eq), 2.13(t, *J* = 12.3 Hz, 1H, H-3_{ax}). ^{13}C NMR (125 MHz, CD_3OD) δ 168.9, 161.3, 100.2(C), 77.6, 76.0, 70.9, 70.1, 63.7, 63.3, 57.2, 52.3, 50.3, 36. 6. HR MS (ESI-TOF, $[M+Na]^+$): calcd. for $C_{13}H_{20}N_4NaO_9$, 399.1128; found, 399.1127.



14

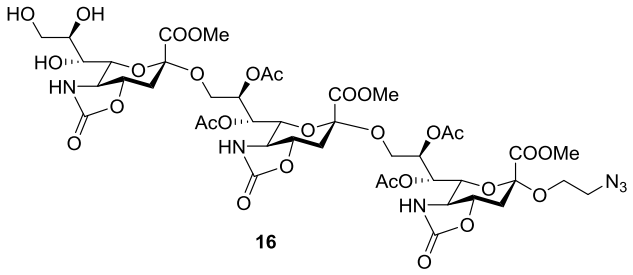
Methyl (Methyl 5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(2-azidoethyl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onate (14). A trichloroacetyl-protected disialoside intermediate was obtained from reaction of **11** (1.02 g, 1.40 mmol) and **13** (0.48 g, 1.28 mmol) by means of the general glycosylation procedure. The intermediate was dissolved in anhydrous DCM (18 mL) and then cooled to 0 °C under N_2. Acetic anhydride (1.2 mL, 12.80 mmol) and TfOH (25 μL) were added and the mixture was stirred for 20 min. The reaction was quenched with saturated aq. $NaHCO_3$, diluted with EtOAc, and washed with water and brine, dried over Na_2SO_4, and then concentrated. The residue was added into a mixture of anhydrous MeOH (10.0 mL) and triethylamine (0.1 ml). The reaction was stirred at rt for 20 min, quenched with 10 % aq. HCl, and concentrated under vacuum. The residue was finally purified by silica gel column chromatography (MeOH/EtOAc/Hexane=1:10:12) to afford **14** (0.79 g, 82%, three steps) as white solids. ^{1}H NMR (400 MHz, CD_3OD): δ 5.42 – 5.34 (m, 1H), 5.29 (dd, *J* = 14.7, 7.2 Hz,

1H), 4.26 (d, *J* = 10.1 Hz, 1H), 4.14 – 4.00 (m, 4H), 3.96 – 3.89 (m, 1H), 3.87 – 3.75 (m, 8H), 3.74 – 3.64 (m, 2H), 3.61 – 3.51 (m, 3H), 3.39 – 3.26 (m, 4H), 2.94 (dd, J = 11.8, 2.6 Hz, 1H, H-3eq), 2.89 (dd, J = 11.8, 3.5 Hz, 1H, H-3eq), 2.26 (s, 3H), 2.15 (s, 3H), 2.09 – 1.99 (m, 2H, H-3ax). ^{13}C NMR (100 MHz, CD_3OD): δ 171.7, 170.4, 168.8, 168.2, 161.1, 160.6, 100.0 (C), 99.7 (C), 77.6, 76.9, 76.2, 73.8, 70.7, 70.1, 68.6, 68.1, 64.2, 63.4, 62.5, 57.3, 57.2, 52.3, 52.2, 50.2, 36.7, 36.6, 19.9, 19.5. HR MS (ESI-TOF, $[M+H]^+$): calcd. for $C_{28}H_{40}N_5O_{19}$, 750.2317; found, 750.2318.



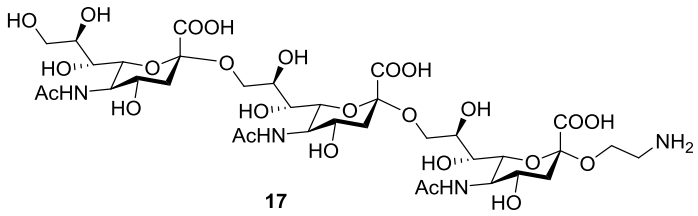
15

(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→9)-(2-aminoethyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onic acid (15). To a stirred solution of **14** (80 mg, 0.11 mmol) in MeOH (5 ml) and H_2O (5 ml) was added LiOH•H_2O (62 mg, 1.47 mmol). After being refluxed for 24 h, the reaction mixture was concentrated under vacuum. The residue was dissolved in H_2O (6 ml), and then $NaHCO_3$ (247 mg, 2.94 mmol) and acetic anhydride (140 μL, 1.47 mmol) were added. After being stirred at rt for 3 h, the reaction mixture was concentrated under vacuum. The residue was dissolved in MeOH (5 mL), and then NaOMe (60 mg) was added. After being stirred at rt for 24 h, the reaction mixture was neutralized with 10% aq. HCl and concentrated under vacuum. After the residue was dissolved H_2O (6 mL), 10% Pd/C (20 mg) was added. The reaction mixture was shaken under a hydrogen atmosphere at 50 psi for 12 h. The solid catalyst was removed by filtration through a Celite pad, and the pad was washed with water. The filtrates were combined and concentrated under vacuum. The residue was purified by Sephadex G-10 column chromatography, using H_2O as the eluent, to give **15** (41 mg, 60%, four steps). ^{1}H NMR (600 MHz, D_2O): δ 3.85 – 3.60 (m, 7H), 3.59 – 3.34 (m, 9H), 3.00 (t, *J* = 11.7 Hz, 2H), 2.54 (t, *J* = 14.9 Hz, 3H), 1.87 (s, 6H), 1.54 (dd, *J* = 25.0, 12.5 Hz, 3H). ^{13}C NMR (150 MHz, D_2O): δ 174.9 (2C), 173.6, 173.4, 100.3, 100.1, 72.4, 72.4, 71.6, 70.2, 68.2, 68.0, 65.1, 62.5, 60.4, 51.8, 51.7, 40.1, 39.7, 39.4, 22.01, 21.96. HR MS (ESI-TOF, $[M-H]^-$): calcd. for $C_{24}H_{40}N_3O_{17}$, 642.2358; found, 642.2361.



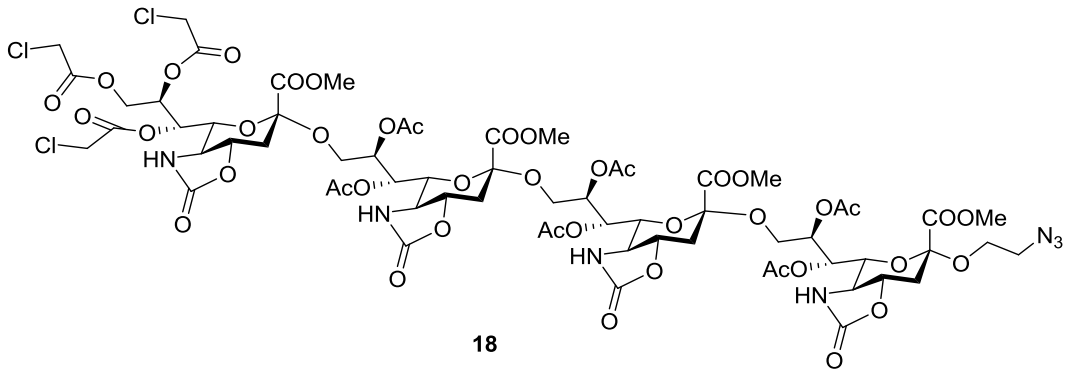
16

Methyl (Methyl 5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(methyl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(2-azidoethyl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onate (16). Compound **16** (0.71 g, 88%, three steps) was prepared from **11** (0.57 g, 0.78 mmol) and **14** (0.54 g, 0.72 mmol) by the procedure described in the synthesis of **14**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.92 (s, 1H), 5.93 (s, 1H), 5.71 (s, 1H), 5.46 (m, 1H), 5.30 – 5.15 (m, 3H), 4.18 (m, 4H), 4.08 – 3.66 (m, 18H), 3.65 – 3.45 (m, 3H), 3.37 (m, 3H), 3.21 – 3.06 (m, 2H), 3.00 (m, 1H, H_{3eq}),), 2.90 (m, 2H, H_{3eq})2.39 – 1.95 (m, 15H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.9, 171.4, 167.0, 169.9, 168.7, 168.3, 167.9, 160.6, 159.8, 159.7, 99.9 (C), 99.7 (2 C), 73.7, 70.8, 69.5, 68.6, 67.3, 67.1, 64.5, 63.2, 62.9, 62.7, 57.7, 57.6, 57.2, 53.6, 53.0, 50.4, 37.3, 37.2, 36.7, 21.1(2C), 20.8, 20.7. HR MS (ESI-TOF, $[M+H]^+$): calcd. for $C_{43}H_{59}N_6O_{29}$, 1123.3326; found, 1123.3369.



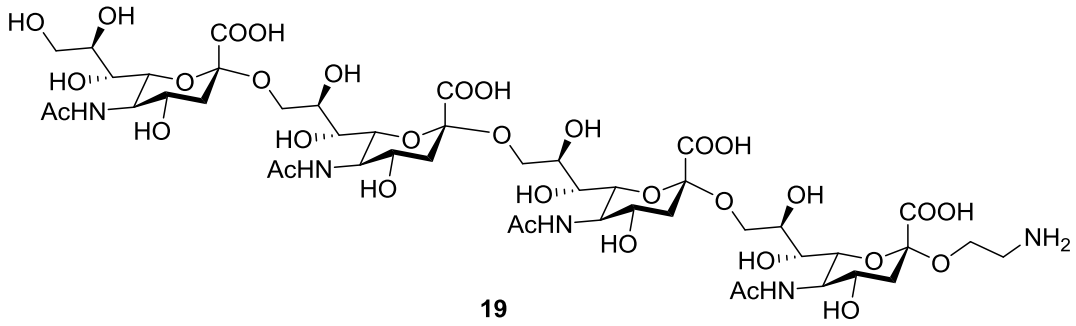
17

(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→9)-(5-acetamido-3,5-dideoxy-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→9)-(2-aminoethyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onic acid (17). Compound **17** (55 mg, 66%, four steps) was prepared from **16** (100 mg, 89 μmol) by the procedure described in the synthesis of **15**. ^{1}H NMR (600 MHz, D_2O): δ 3.87 – 3.62 (m, 10H), 3.61 – 3.37 (m, 13H), 2.99 (m, 2H), 2.60 (m, 3H), 1.88 (s, 9H), 1.55 (m, 3H). ^{13}C NMR (150 MHz, D_2O): δ 174.9 (3C), 173.6, 173.4 (2C), 100.4, 100.2, 100.1, 72.4, 72.3, 72.2, 71.7, 70.2, 68.4, 68.3, 68.0, 65.1, 64.9, 62.6, 51.8, 51.8, 51.7, 40.0(2C), 39.8, 39.5, 22.1(2C), 22.0. HR MS (ESI-TOF, $[M-H]^-$): calcd. for $C_{35}H_{57}N_4O_{25}$, 933.3312; found, 933.3293.



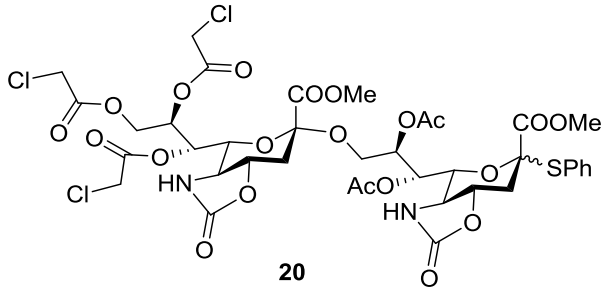
18

Methyl (Methyl 5-amido-5-N,4-O-carbonyl-7,8,9-tri-O-chloroacetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(methyl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(methyl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(2-azidoethyl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-O-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onate (18). A trichloroacetyl-protected trisialoside intermediate was prepared from **11** (107 mg, 0.15 mmol) and **16** (150 mg, 0.13 mmol) by means of the general glycosylation procedure. The resultant intermediate was dissolved in anhydrous DCM (5 mL), and then acetic anhydride (120 μL, 1.28 mmol) and TfOH (2.5 μL) were added at 0 °C. After being stirred for 20 min, the reaction was quenched with saturated aq. $NaHCO_3$, and the mixture was diluted with EtOAc, washed with brine, and dried over Na_2SO_4. Concentration of the solution under vacuum and purification of the residue by silica gel column chromatography (MeOH/EtOAc/Hexane = 1:10:10) afforded **18** (171 mg, 76%, two steps) as a white foamy solid. ^{1}H NMR (600 MHz, $CDCl_3$) : δ 5.72 – 5.10 (m, 12H), 4.53 (d, *J* = 11.9 Hz, 1H), 4.39 – 4.01 (m, 11H), 4.01 – 3.69 (m, 18H), 3.59 (s, 1H), 3.42 (s, 6H), 3.40 – 3.24 (m, 2H), 3.13 – 2.99 (m, 3H), 2.87 (m, 4H, H-3eq), 2.39 – 2.06 (m, 18H), 2.06 – 1.91 (m, 4H, H-3ax). ^{13}C NMR (150 MHz, $CDCl_3$) : δ 171.6, 171.5, 171.3, 169.9, 169.8, 169.7, 168.3 (2C), 168.1, 167.9, 167.6, 167.0, 166.5, 159.6 (2C), 159.3, 158.8, 99.9(2C-2), 99.8(C-2), 99.6(C-2), 76.5, 76.5, 76.3, 73.8, 73.7, 73.6, 73.6, 70.4, 68.3, 68.1, 67.9, 67.3, 67.0, 66.7, 66.5, 64.4, 63.5, 63.4, 62.8, 57.8, 57.7, 57.5, 57.0, 56.8, 53.3, 53.1, 53.0, 52.8, 50.4, 41.2, 40.5, 40.5, 40.4, 37.4, 37.3, 37.1, 37.0, 36.9, 21.1, 21.0, 20.9, 20.8, 20.8, 20.7. HR MS (ESI-TOF, $[M+H]^+$): calcd. for $C_{64}H_{81}Cl_3N_7O_{42}$, 1724.3483; found, 1724.3465.



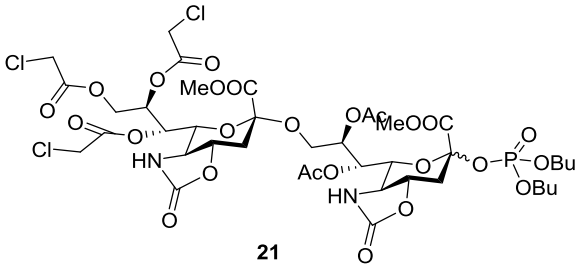
19

(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→9)-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→9)-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→9)-(2-aminoethyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onic acid (19). Compound **19** (22 mg, 62%, four steps) was prepared from **18** (50 mg, 29 μmol) by the procedure described in the synthesis of **15**. ^{1}H NMR (600 MHz, D_2O): δ 3.82 – 3.62 (m, 13H), 3.58 – 3.42 (m, 15H), 3.38 (d, *J* = 11.3 Hz, 2H), 2.95 (m, 2H), 2.60 – 2.50 (m, 4H), 1.88 (dd, *J* = 14.5, 6.2 Hz, 12H), 1.54 (dd, *J* = 23.4, 11.6 Hz, 4H). ^{13}C NMR (151 MHz, D_2O): δ 174.9 (2C), 174.84, 174.81, 173.63, 173.59, 173.58, 173.4, 100.35, 100.14 (2C), 100.08, 72.4, 72.3, 72.2, 71.6, 70.2, 70.19, 70.1, 68.5, 68.3, 68.27, 68.25, 68.1, 68.03, 67.99, 65.1, 64.8, 62.6, 60.7, 51.82, 51.79, 51.74, 51.73, 40.0, 39.7, 39.4, 23.2, 22.1 (2C), 21.98. HR MS (ESI-TOF, $[M-H]^-$): calcd. for $C_{46}H_{74}N_5O_{33}$, 1224.4266; found, 1224.4288.



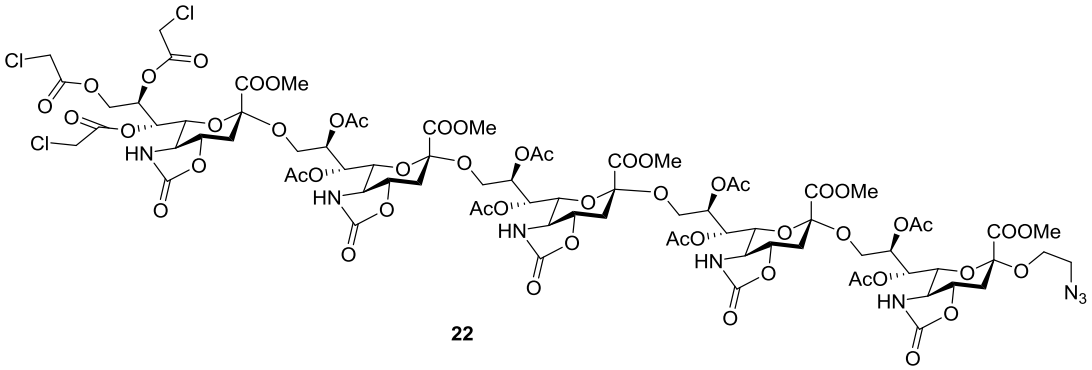
20

Methyl (Methyl 5-amido-5-N,4-O-carbonyl-7,8,9-tri-O-chloroacetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(phenyl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-3,5-dideoxy-2-thio-D-glycero-α,β-D-galacto-non-2-ulopyranoside)onate (20).[2] The α,β-mixture of **20** (780 mg, 91%, two steps) was prepared from **11** (623 mg, 0.85 mmol) and **9** (354 mg, 0.85 mmol) by the procedure described in the synthesis of **18**. Its spectroscopic data were the same as reported.[2]



21

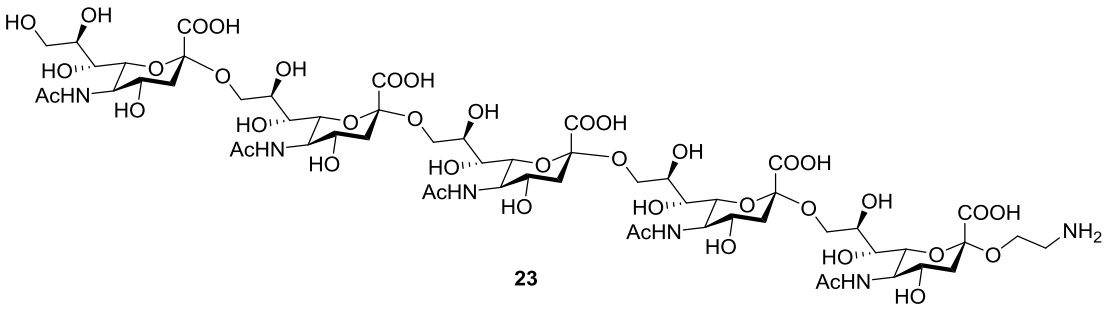
Methyl (Methyl 5-amido-5-N,4-O-carbonyl-7,8,9-tri-O-chloroacetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(dibutylphosphoryl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α,β-D-galacto-non-2-ulopyranoside)onate (21).[2] The α,β-mixture of **21** (543 mg, 87%, α/β = 1.4/1) was prepared from **14** (565 mg, 0.85 mmol) by the procedure described in the synthesis of **11**. Its spectroscopic data were the same as reported.[2]



22

Methyl (Methyl 5-amido-5-N,4-O-carbonyl-7,8,9-tri-O-chloroacetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(methyl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(methyl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(methyl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2→9)-(2-azidoethyl 7,8-di-O-acetyl-5-amido-5-N,4-O-carbonyl-O-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onate (22). Compound **19** (206 mg, 70%) was prepared from **21** (200 mg, 0.18 mmol) and **16** (157 mg, 0.14 mmol) by the procedure described in the synthesis of **18**. ^{1}H NMR (600 MHz, $CDCl_3$) : δ 5.78 (bs, 2H), 5.63 (bs, 1H), 5.54 (bs, 2H), 5.53 – 5.27 (m, 7H), 5.25 -4.91 (m, 3H), 4.47 – 3.72 (m, 38H), 3.61 (m, 2H), 3.50 – 3.37 (m, 3H), 3.37 – 3.26 (m, 4H), 3.23 – 3.09 (m, 3H), 3.00 – 2.78 (m, 5H), 2.53 – 1.89 (m, 29H). ^{13}C NMR (150 MHz, $CDCl_3$) : δ 172.4, 171.5, 171.3, 170.0(2C), 169.9(2C), 169.8, 169.7, 168.3 (2C), 168.0, 167.7 (2C), 167.3, 166.7, 156.0, 159.6, 159.5 (3C), 100.0, 99.9, 99.4(2C),

99.0, 77.7, 76.5, 74.6, 74.2, 74.1, 73.8, 73.4, 69.8, 69.5, 69.3, 68.9, 68.1, 67.7, 67.5, 67.1, 66.8, 66.6, 66.4, 66.0, 65.5, 64.4, 63.9, 63.2, 62.8, 62.6, 61.4, 60.0, 59.7, 57.7, 57.3, 57.2, 56.7, 54.9, 54.3, 53.9, 53.5, 53.4, 53.3, 52.9, 52.9, 51.1, 50.5, 50.0, 48.9, 48.7, 47.3, 46.4, 46.2, 45. 6, 45.0, 43.6, 43.2, 42.6, 42.1, 41.4, 41.0, 40.7, 40.5, 40.0, 39.7, 39.4, 38.3, 37.5, 37.2, 36.8, 36.5, 21.1, 21.1, 21.0, 21.0(2C), 20.8, 20.7, 20.6. HR MS (ESI-TOF, $[M+2H]^{2+}$): calcd. for $C_{79}H_{101}Cl_3N_8O_{52}$, 1049.2285; found, 1049.2229.



23

(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→9)-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→9)-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→9)-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→9)-(2-aminoethyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onic acid (23). Compound **23** (25 mg, 69%, four steps) was prepared from **19** (50 mg, 24 μmol) by the procedure described in the synthesis of **20**.^{1}H NMR (600 MHz, D_2O): δ 3.85 – 3.59 (m, 16H), 3.58 – 3.32 (m, 21H), 2.98 (s, 2H), 2.59 – 2.47 (m, 5H), 1.97 – 1.74 (m, 15H), 1.53 (dd, J = 22.7, 10.9 Hz, 5H). ^{13}C NMR (150 MHz, D_2O): δ 174.8 (4C), 174.8, 173.62, 173.58, 173.6, 173.4, 100.3, 100.1 (3C), 100.0, 72.4, 72.3, 72.2, 71.6, 70.2, 70.2, 70.1, 68.4, 68.3, 68.2, 68.1, 68.99, 67.96, 65.1, 65.0, 64.8, 62.5, 60.6, 51.78, 51.7, 40.0, 39.7 (3C), 39.4, 22.1 (4C), 22.0. HR MS (ESI-TOF, $[M-H]^-$): calcd. for $C_{57}H_{91}N_6O_{41}$, 1515.5220; found, 1515.5216.

Procedure for the activation of oligosialic acids: A mixture of free oligosialic acids **15**, **17**, **19** or **23** (6.0 mg) and disuccinimidal glutarate (15 eq.) in DMF and PBS buffer (0.1 M) (4:1, 0.5 mL) was stirred at rt for 4 h, and the solvents was then removed under vacuum. The activated oligosaccharides **25-28** were separated from the excessive reagents by precipitation with 9 volumes of EtOAc, followed by washing of the precipitates 10 times with EtOAc and drying under vacuum, and were directly used for the next step without further purification.

Procedure for the conjugation of 25-28 with HSA and KLH: The activated oligosaccharides **25-28** were mixed with HSA or KLH at a molar ratio of 30:1 in 0.1 M PBS buffer (0.4 mL). The solution was stirred at rt for 3 days and then applied to a Biogel A 0.5 column using 0.1 M PBS buffer (I = 0.1, pH =7.8) as the eluent to remove free sugars. Fractions containing glycoconjugate, which were characterized by the bicinchoninic acid (BCA) assay for proteins and charring with 15% (v/v) H_2SO_4 in EtOH for oligosialic acids, were combined and dialyzed against distilled water for 2 days, and then lyophilized to afford the desirable glycoconjugates as white solids.

Analysis of the carbohydrate loading of glycoconjugates:[4] The mixture of an accurately weighted glycoconjugate sample (0.3-0.6 mg) dissolved in distilled water (1 mL) and resorcinol reagent (2.0 mL) was heated in a boiling water bath for 30 min. After being cooled to rt, an extraction solution (3 mL of 1-butanol acetate and 1-butanol, v/v = 85/15) was added. The mixture was shaken vigorously and subjected to standing for 10 min. The organic layer was transferred to a 1.0-cm cuvette, and its absorbance was determined at *A*580 nm by an UV-Vis spectrometer, using a blank extraction solution as the control. The sialic acid content of the glycoconjugate is determined by comparing the analyzed sample with a calibration curve created with the solution of standard sialic acid (NeuNAc) samples analyzed under the same condition. The sialic acid loading of each glycoconjugate was calculated according to the following equation, and the results are shown in Table 1.

$$\text{sialic acid loading (\%)} = \frac{sialic\ acid\ content(mg) in\ the\ sample}{weight\ of\ the\ glycocojugate\ sample(mg)} \times 100\%$$

Table 1. Carbohydrate loadings of glycoconjugates **1-8**

Sample	KLH conjugates				HSA conjugates			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Loading (%)	7.5	11.5	7.9	6.8	8.9	11.5	10.9	7.8

Analysis of the HSA and KLH conjugates by MS and SDS-PAGE. HSA and its oligosialic acid conjugates obtained above were subjected to MALDI TOF MS study, and the carbohydrate loadings of the conjugates were calculated according to the following equation:

sialic acid loading (%) = (conjugate MS mass - HSA MS mass)/conjugate MS mass x 100%

Due to high molecular mass of KLH, its conjugates are not feasible for MS analysis, so they were subjected to SDS-PAGE analysis. The mixture of a sample, KLH protein or glycoconjugates **2**, **3**, **4** (1μg), in 0.1 M PSB (1 μl), NuPAGE® LDS NuPAGE® sample buffer (4 ×, 2.5 μl), reducing agent (10 ×, 1 μl) and deionized water (5.5 μl) was heated at 70 °C for 10 min, and loaded upon the SDS-PAGE gel. The upper buffer chamber was filled with 200 ml of SDS running buffer (1 ×) containing 500 μl of NuPAGE® antioxidant and the lower buffer chamber was filled with 600 ml SDS running buffer (1 ×). The gel was run at 200 V for 2 h, and then stained with Coomassie brilliant blue R-250 for 8 h (*Ref.* the NuPAGE® technical instructions of Invitrogen). The results are shown in Figure S1.

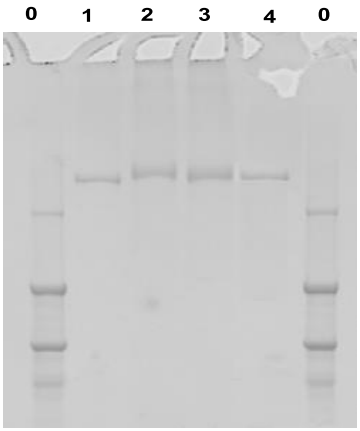


Figure S1. Analysis of KLH and its glycoconjugates on SDS-PAGE. Lane 0: molecular marker; Lane 1: KLH protein; Lane 2: conjugate **2**; Lane 3: conjugate **3**; Lane 4: conjugate **4**.

II. Protocols for Immunological Studies

Preparation of Emulsion of KLH Conjugates: Each conjugate (4.66×10^{-9} mol carbohydrate antigen per dose and 30 doses, that is, 1.19 mg of **1**, 1.14 mg of **2**, 2.17 mg of **3**, and 3.10 mg of **4**, respectively) was dissolved in 1.5 ml of 1 × PBS buffer. The solution was thoroughly mixed with 1.5 ml of Titermax Gold adjuvant (1:1, v/v) to form an emulsion according to the protocol provided by the manufacturer.

Immunization of Mouse: Each female C57BL/6J mouse in a group of six was immunized on day 1 by intramuscular (i.m.) injection of 0.1 mL of the emulsion of the conjugate vaccine and adjuvant prepared above. Following the initial immunization, mice were boosted 3 times on day

14, day 21, and day 28 by subcutaneous (sc.) injection of the same conjugate preparation. Blood samples of each mouse were collected through the leg veins prior to the initial immunization on day 0 and after immunization on day 28 and day 38 and were clotted to obtain antisera that were stored at -80 ^{o}C before use.

Protocols for ELISA: The protocol of ELISA was similar to our previous work.[5] ELISA plates were treated with 100 ul of the solution of a HSA conjugate (2 ug/ml) dissolved in coating buffer (0.1 M bicarbonate, pH 9.6) at 4 ^{o}C overnight, and then at 37 ^{o}C for 1 h, which was followed by treatment with blocking buffer (10% BSA in PBS solution with NaN_3) and washing 3 times with PBS containing 0.05% Tween-20 (PBST). Thereafter, a pooled or an individual mouse antiserum with serial half-log dilutions from 1:300 to 1:656100 in PBS was added to the coated ELISA plates (100 uL/well), which was followed by incubation at 37 ^{o}C for 2 h. The plates were then washed with PBS and incubated at rt for another 1 h with a 1:1000 diluted solution of an alkaline phosphatase linked goat anti-mouse kappa, IgM, IgG2a and IgG2b antibody or with a 1:2000 diluted solution of an alkaline phosphatase linked goat anti-mouse IgG1 and IgG3 antibody (100 uL/well), respectively. Finally, these plates were washed with PBS and developed with 100 uL of *p*-nitrophenylphosphate (PNPP) solution (1.67 mg/mL in buffer) for 30 min at rt, followed by colorimetric readout using a BIO-TEK EL800 plate reader at 405 nm wavelength. The optical density (OD) values were plotted against antiserum dilution values, and a best-fit line was obtained. The equation of the line was employed to calculate the dilution value at which an OD of 0.2 was achieved, and the antibody titer was calculated at the inverse of the dilution value. ELISA results of individual antisera or pooled antisera are shown in Tables 2 and 3.

Table 2. The antibody titers of antigen-specific antibodies in day 38 antisera of individual mouse immunized with **1**, **2**, **3**, and **4**.

Conjugate **1**:

Mouse#	1	2	3	4	5	6	Mean
kappa	84725	113227	91618	117345	118853	84051	101637
IgG1	28560	35596	57259	93663	97963	96375	68236
IgG2a	5333	21765	22061	32436	18356	0	16658
IgG2b	130602	153353	103885	115555	96496	101498	116898
IgG2c	55506	78165	54468	71068	71381	23249	58973
IgG3	0	82	23	4297	13788	3744	3656
IgM	4774	0	2195	1192	473	4369	2167

Conjugate **2**:

Mouse#	1	2	3	4	5	6	Mean
kappa	170585	127693	106385	130147	75376	91676	116977
IgG1	231104	167778	30712	80780	93562	88007	115324
IgG2a	60	1201	3939	4957	2	343	1750
IgG2b	133943	110998	112320	127610	79293	110718	112480
IgG2c	63736	69456	68860	71698	32964	64917	61938
IgG3	8943	1053	6	137	110	133	1730
IgM	129	6505	24092	1840	13916	0	7747

Conjugate **3**:

Mouse #	1	2	3	4	5	Mean
kappa	53415	69193	69574	78676	81212	70414
IgG1	17341	29733	28445	32383	38669	29314
IgG2a	14623	21923	12485	16836	39531	21080
IgG2b	101198	105364	107568	112639	66495	98653
IgG2c	64615	67665	42153	42471	53674	54116
IgG3	80	45	2570	328	555	716
IgM	389	1629	6	10441	420	2577

Conjugate **4**:

Mouse#	1	2	3	4	5	6	Mean
kappa	55385	78929	36419	52056	51534	60265	55765
IgG1	37178	37037	1102	30083	58756	58751	37151
IgG2a	0	3040	2663	0	0	0	951
IgG2b	47322	89322	67211	46158	39249	45161	55737
IgG2c	653	50934	61332	20387	3	1450	22460
IgG3	0	0	0	0	0	11	2
IgM	14141	23180	3	4230	0	0	6926

Table 3. Average titers of antigen-specific total IgG in the day 38 antisera of individual mice inoculated with **1**, **2**, **3**, and **4**.

Conjugate	Antibody titer Mean ± standard error of the mean
1	264421 ± 17076
2	293223 ± 35916
3	203878 ± 5521
4	116300 ± 14338

Cross-reactivity of the antisera with other sialic glycans and the detection of linker-specific antibodies: The same ELISA protocol described above was also utilized to detect cross-reactive antibodies using the HSA conjugates of hexaglucan, GM2 (sialylated), GM3 (sialylated) and sTn (sialylated), all of which had the same linker as that of α-2,9-disialic acid-KLH conjugates, with the corresponding α-2,9-oligosialic acid-HSA conjugates as positive controls. Results are showed in Figure S2. Obviously, no antibody against the linker or GM2, GM3, and sTn was detected.

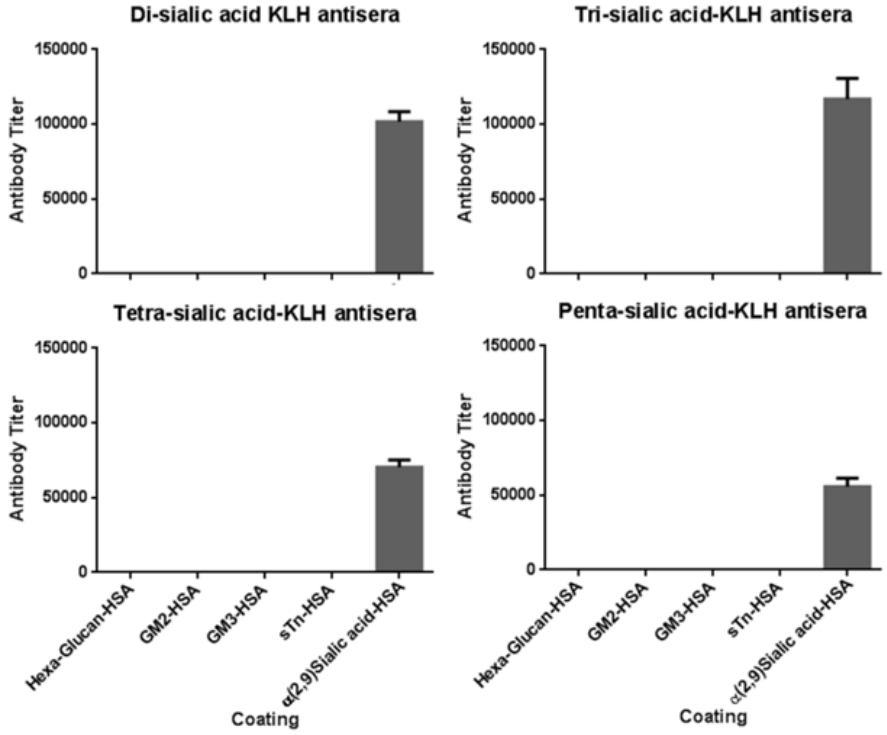


Figure S2. The cross-reaction between antisera obtained with conjugates **1**-**4** and various HSA conjugates. For antibody detection, the ELISA plates were coated with hexaglucan-HSA, GM2-HSA, GM3-HSA, sTn-HSA and corresponding α-2,9-oligosialic acid-HSA conjugate, respectively. The remaining procedures were identical as that described above.

Similarly, the cross-reactions between antisera obtained with various α-2,9-oligosialic acid-KLH conjugates **1**-**4** and the commercially available α-2,8-polysialic acid (Colominic acid derived from *Escherichia coli*, Cat NO: C5762, Sigma Aldrich) was also detected with α-2,9-disialic acid-HSA as a positive control. The results are shown in Figure S3. Obviously, the antibodies induced by conjugate **1**-**4** have nearly no reaction with α-2,8-polysialic acid.

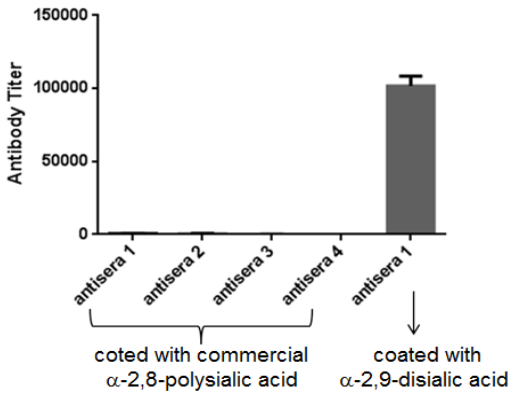


Figure S3. The cross reaction between antisera and α-2,8-polysialic acid, using α-2,9-disialic acid-specific antibody titer as the positive control. Antisera against **1**-**4** are pooled from each group of immunized mice.

III. Protocols for Cell Binding Assays

The binding between serotype C *N. meningitidis* cells and antisera derived from mice immunized with conjugates **1**, **2**, **3** and **4** was assessed with the Bio-Dot microfiltration apparatus. The PVDF membrane was pre-treated with blocking buffer (1% BSA in PBST) and set in the microfiltration apparatus as support for the bacteria cells. *N. meningitidis* (ATCC® 31275™) cells were killed and fixed in formalin solution, and then re-suspended in PBS buffer. The bacterial solution (50 µL, OD 0.2 at 600 nm) was added to each well of the microfiltration apparatus. After removing PBS buffer by filtration, the bacterial cells were remained in the well. Then, 200 µL of blocking buffer was added to each well and kept at rt for 1 h to block the non-specific binding sites on the surface of bacterial cells. The blocking buffer was filtered through the PVDF membrane under vacuum, and each well was washed with 350 µL of PBST three times. The bacterial cells were further incubated with 100 µL of normal mouse serum or pooled antiserum (1:100 diluted in PBS) obtained from mice immunized with **1**-**4** at 37 °C for 2 h, respectively. After incubation, each well was washed six times with 350 µL of PBST. Thereafter, 100 µL of goat anti-mouse kappa AP as the secondary antibody (1:1000 diluted in PBS) was added to each well and incubated at rt for 1 h. Finally, each well was washed again and developed by *p*-nitrophenylphosphate (PNPP,) solution (200 µL, 1.67 mg/mL in buffer) at rt for 30 min. A portion of the solution (100 µL) in each well was transferred to a clear round-bottom 96-well plate for colorimetric reading at 405 nm wavelength by microplate reader ELX800 (Bio-Tek instruments Inc.). The results are shown in Table 4.

Table 4. The OD (405 nm) value of binding assay

Antisera	Normal sera	**1***	**2***	**3***	**4***
OD value (405 nm)	0.435	2.462	2.116	2.079	1.897
	0.448	1.653	2.297	1.707	1.313
	0.442	1.835	2.458	1.806	1.775

* **1**-**4**: Pooled antisera induced by glycoconjugates **1**-**4**.

IV. Additional Information – Stability of *N*-hydroxysuccinimide ester in PBS buffer:

N-Hydroxysuccinimide ester DSG (1.0 mg) was dissolved in 0.1 M PBS buffer (*I* = 0.1, pH = 7.8, 10 ml), and the mixture was stirred at rt. The UV absorptions of the sample was monitored at 212 nm wavelength (λ_{max} for DSG is 212 nm and it is 258 nm for the hydrolysis product *N*-hydroxysuccinimide, Figures S4A). As clearly shown in Figure S4B, there was no obvious change of the UV absorption of the sample after 24 h of stirring at rt. The solution was extract with ethyl acetate, and the product was examined with NMR to be identified as DSG. These results showed that the ester DSG was stable to the PBS buffer used in the coupling and conjugation reactions.

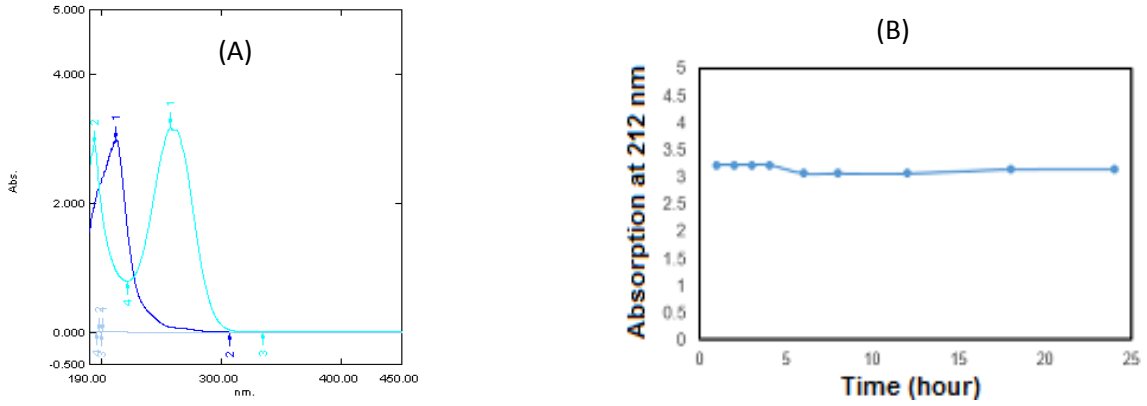
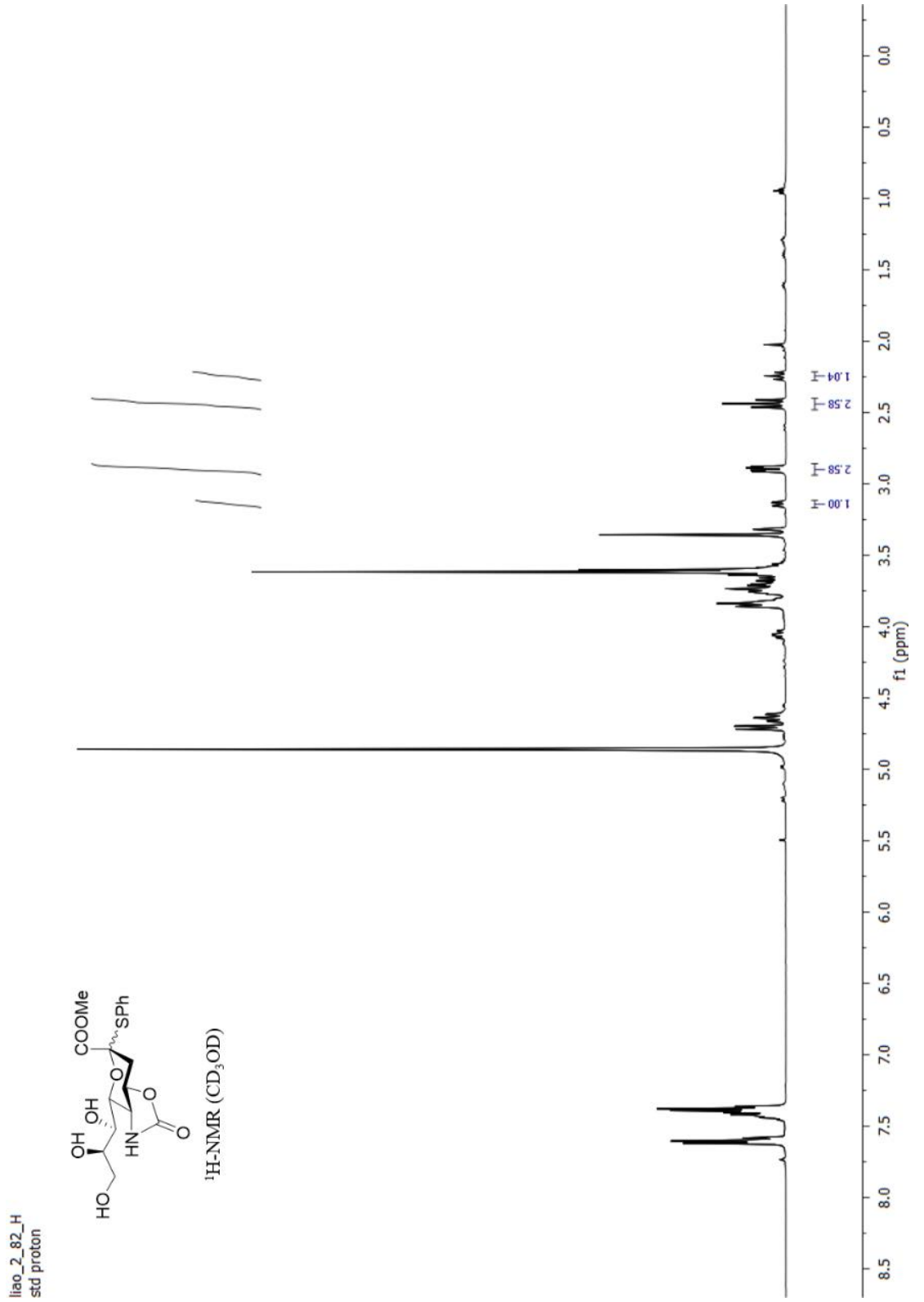


Figure S4. (A) The UV spectra of DSG (blue, λ_{max} = 212 nm) and *N*-hydroxysuccinimide (cyan, λ_{max} = 258 nm), and (B) the UV absorptions (at 212 nm) of a DSG solution in PBS buffer stirred at room temperature

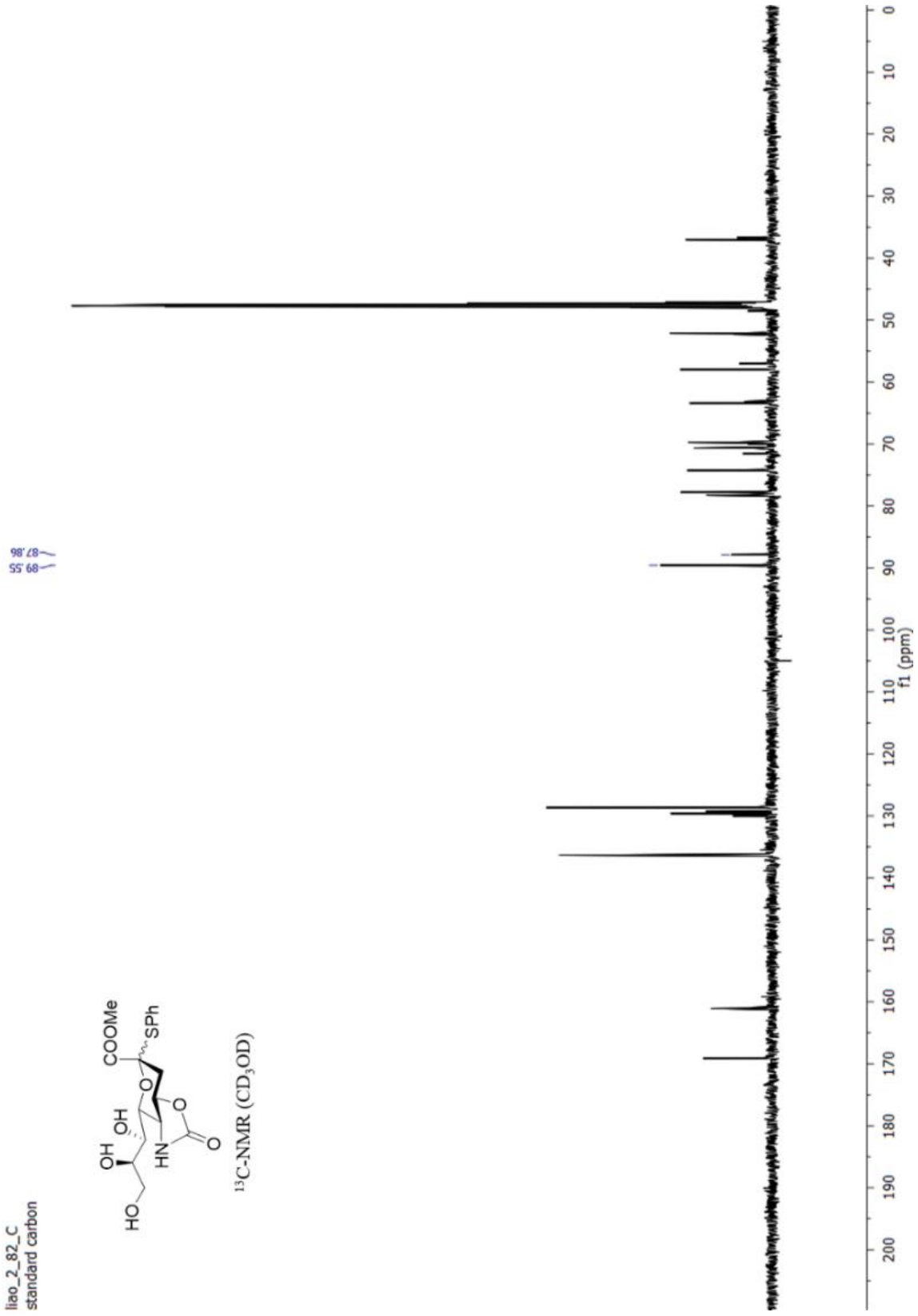
V. References

1. Y. Pan, P. Chefalo, N. Nagy, C. Harding and Z. Guo, *J. Med. Chem.*, 2005, **48**, 875-883.
2. K. C. Chu, C. T. Ren, C. P. Lu, C. H. Hsu, T. H. Sun, J. L. Han, B. Pal, T. A. Chao, Y. F. Lin, S. H. Wu, C. H. Wong and C. Y. Wu, *Angew. Chem. Int. Edit.*, 2011, **50**, 9391-9395.
3. C. C. Lin, N. P. Lin, L. S. Sahabuddin, V. R. Reddy, L. D. Huang, K. C. Hwang and C. C. Lin, *J. Org. Chem.*, 2010, **75**, 4921-4928.
4. L. Svennerholm, *Biochim. Biophys. Acta*, 1957, **24**, 604-611.
5. Z. Zhou, M. Mondal, G. Liao and Z. Guo, *Org. Biomol. Chem.*, 2014, **12**, 3238-3245.

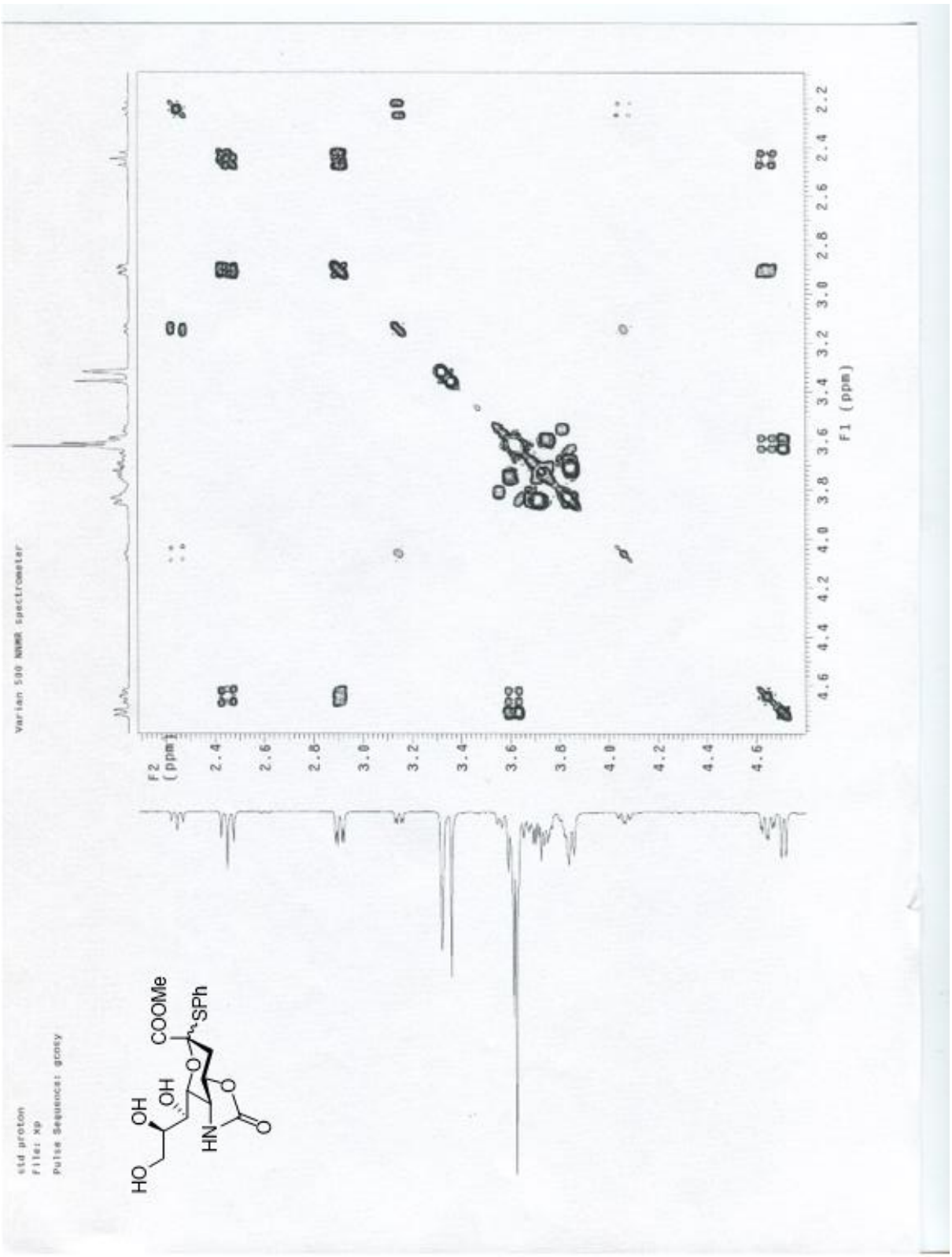
VI. NMR Spectra of Synthesized Compound



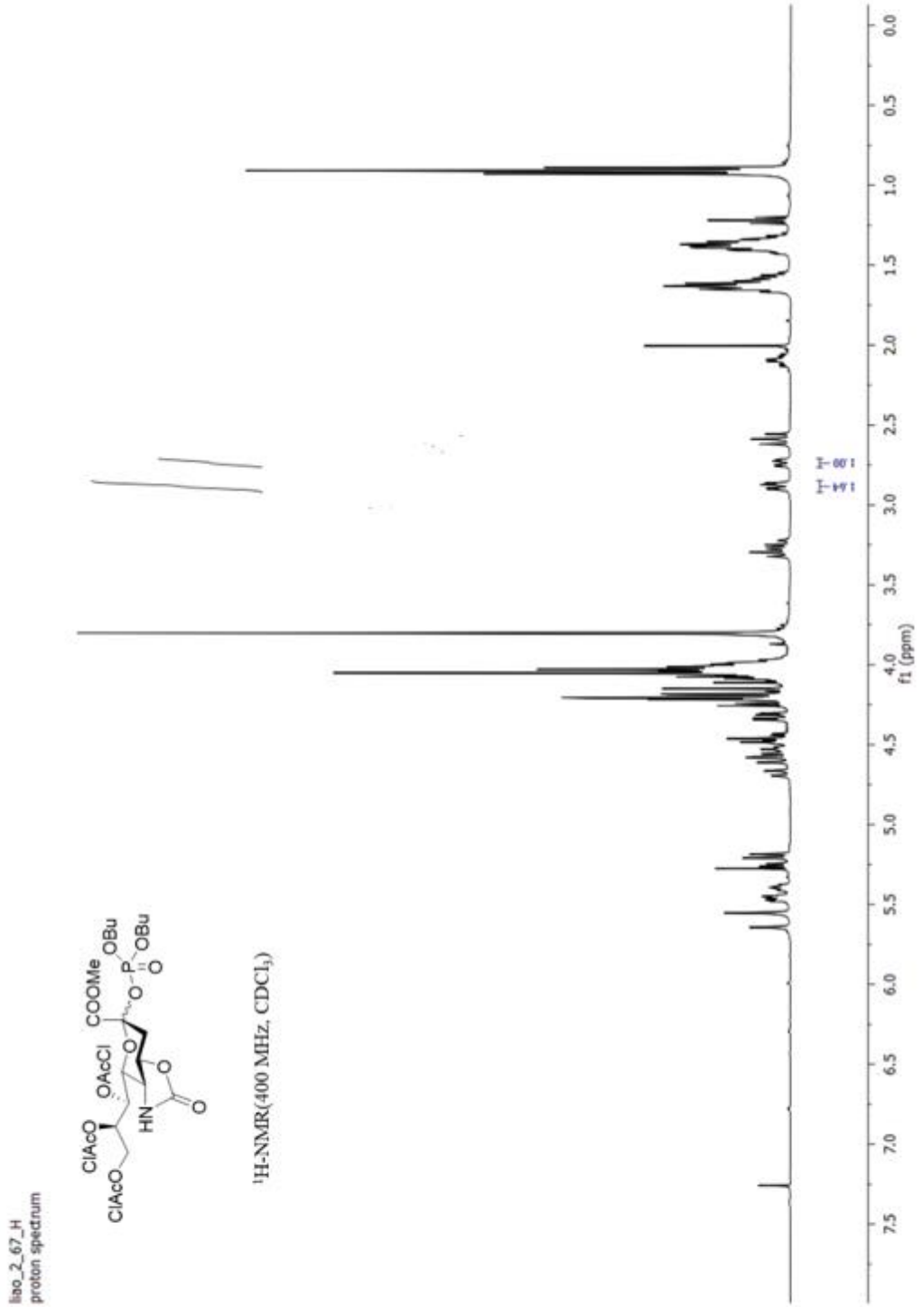
^{1}H NMR Spectrum of compound **9** (CD_3OD, 500 MHz)



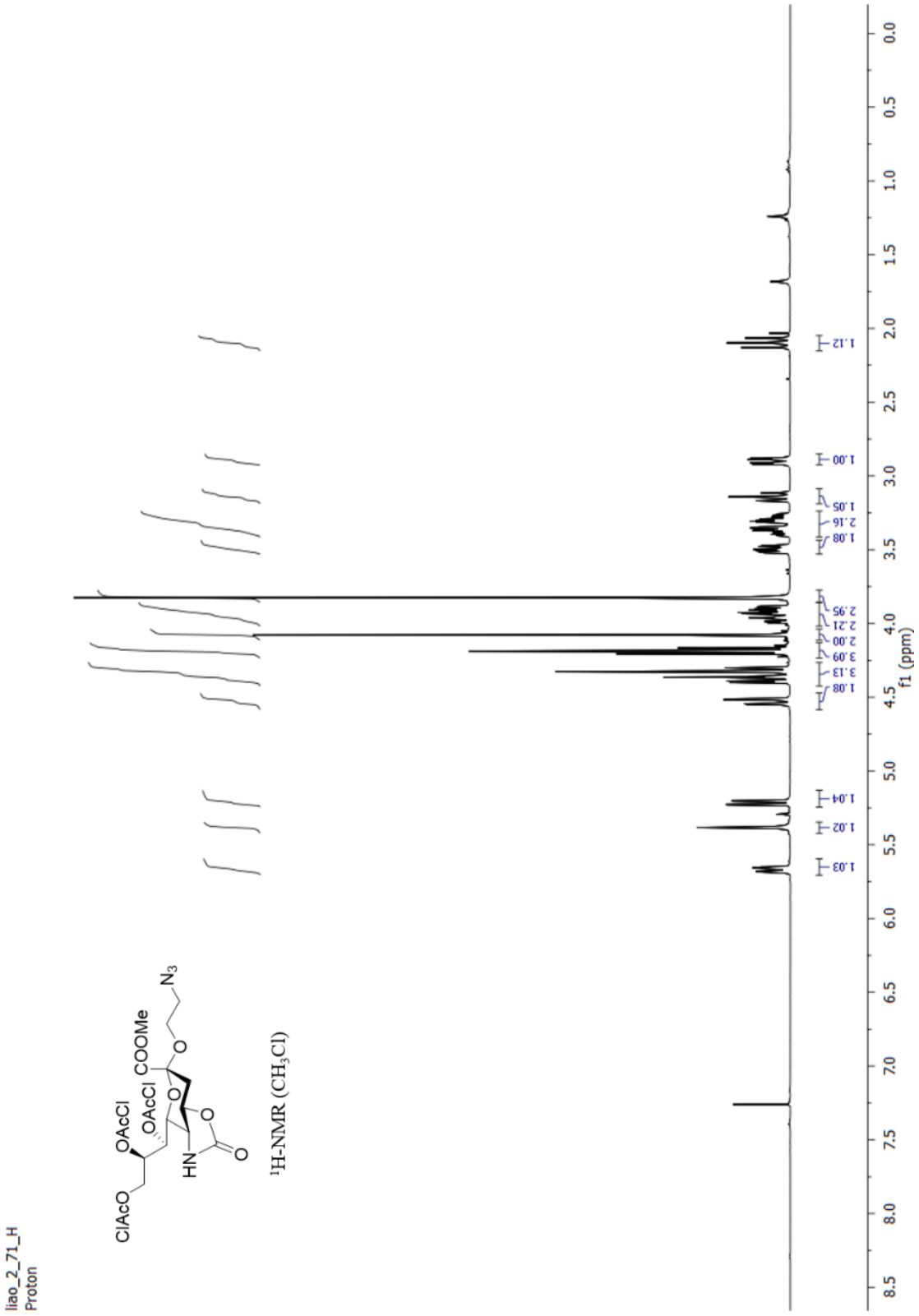
^{13}C NMR Spectrum of compound **9** (CD_3OD, 500 MHz)



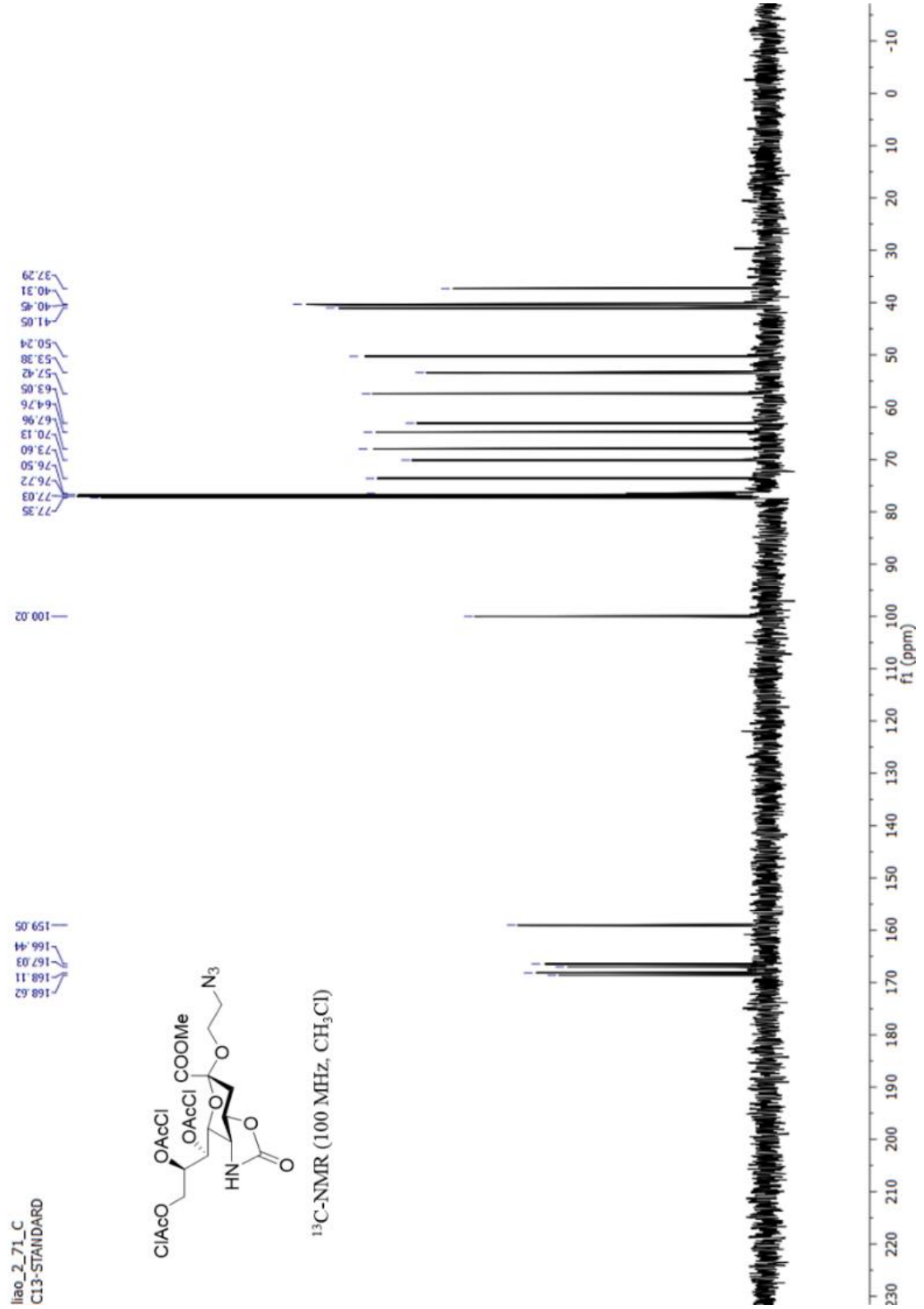
^{1}H-^{1}H COSY Spectrum of compound **9** (CD_3OD, 500 MHz)



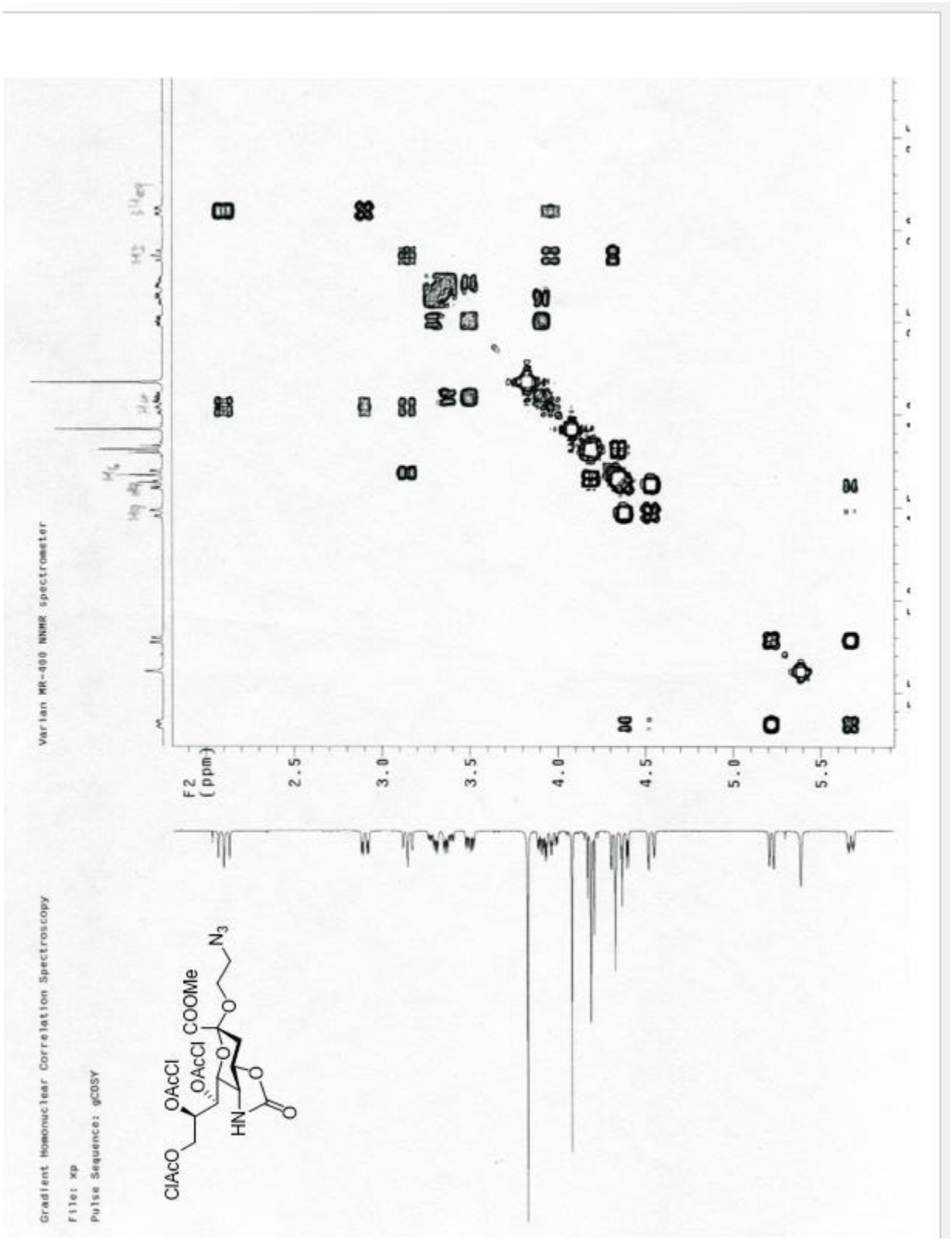
^{1}H NMR Spectrum of compound **11** ($CDCl_3$, 400 MHz)



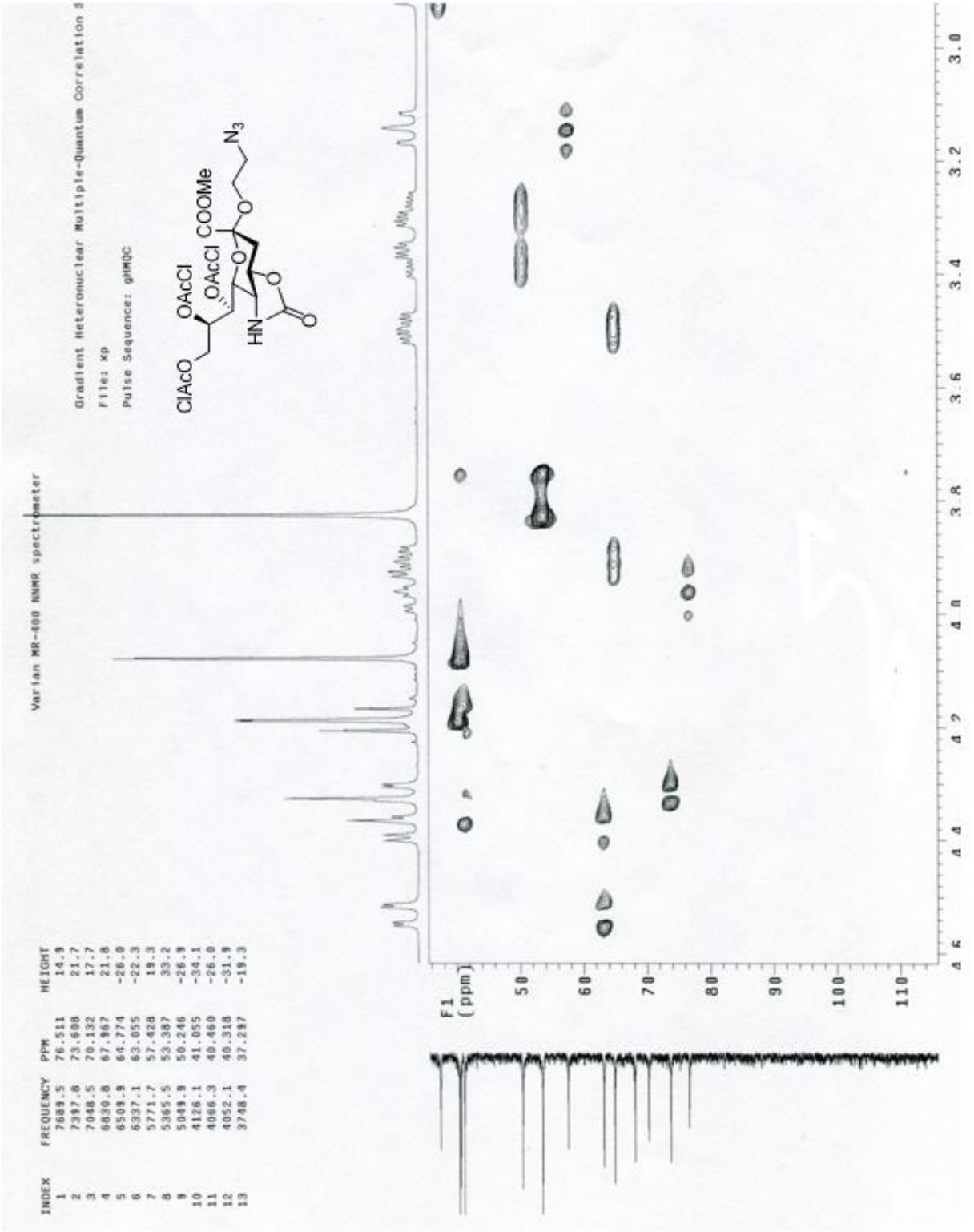
^{1}H NMR Spectrum of compound **12** ($CDCl_3$, 400 MHz)



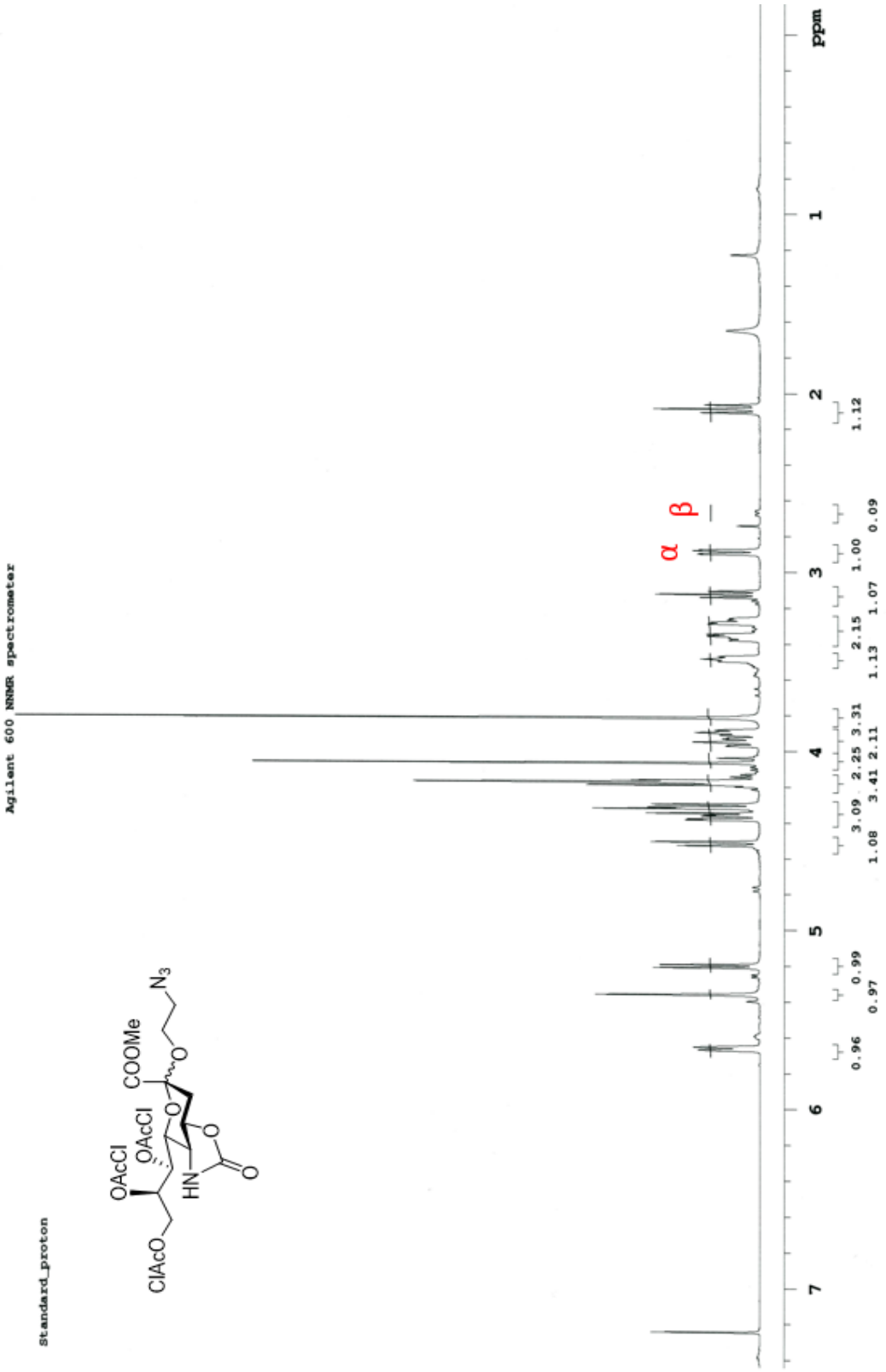
^{13}C NMR Spectrum of compound **12** ($CDCl_3$, 100 MHz)



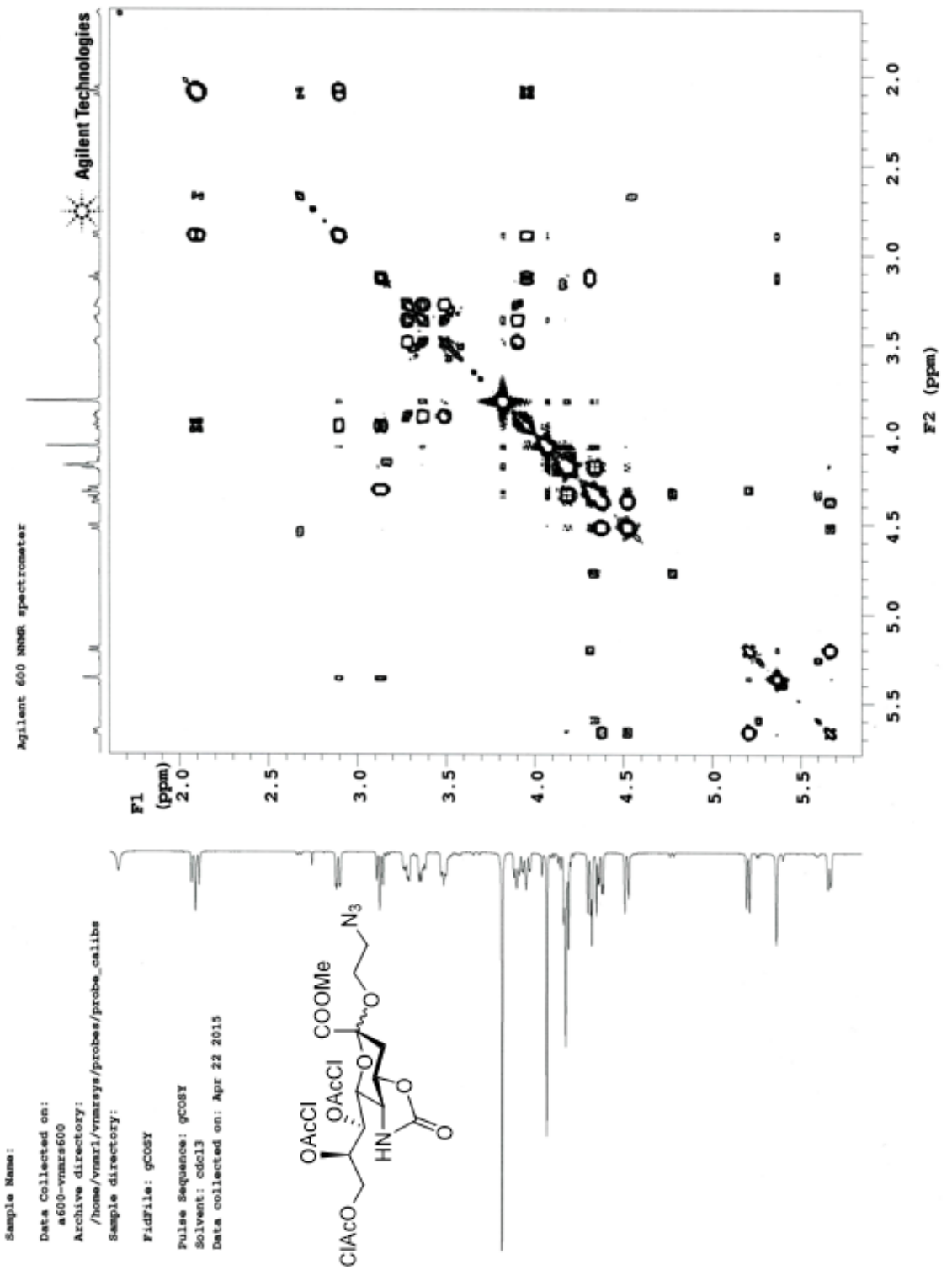
^{1}H-^{1}H COSY Spectrum of compound **12** ($CDCl_3$, 400 MHz)



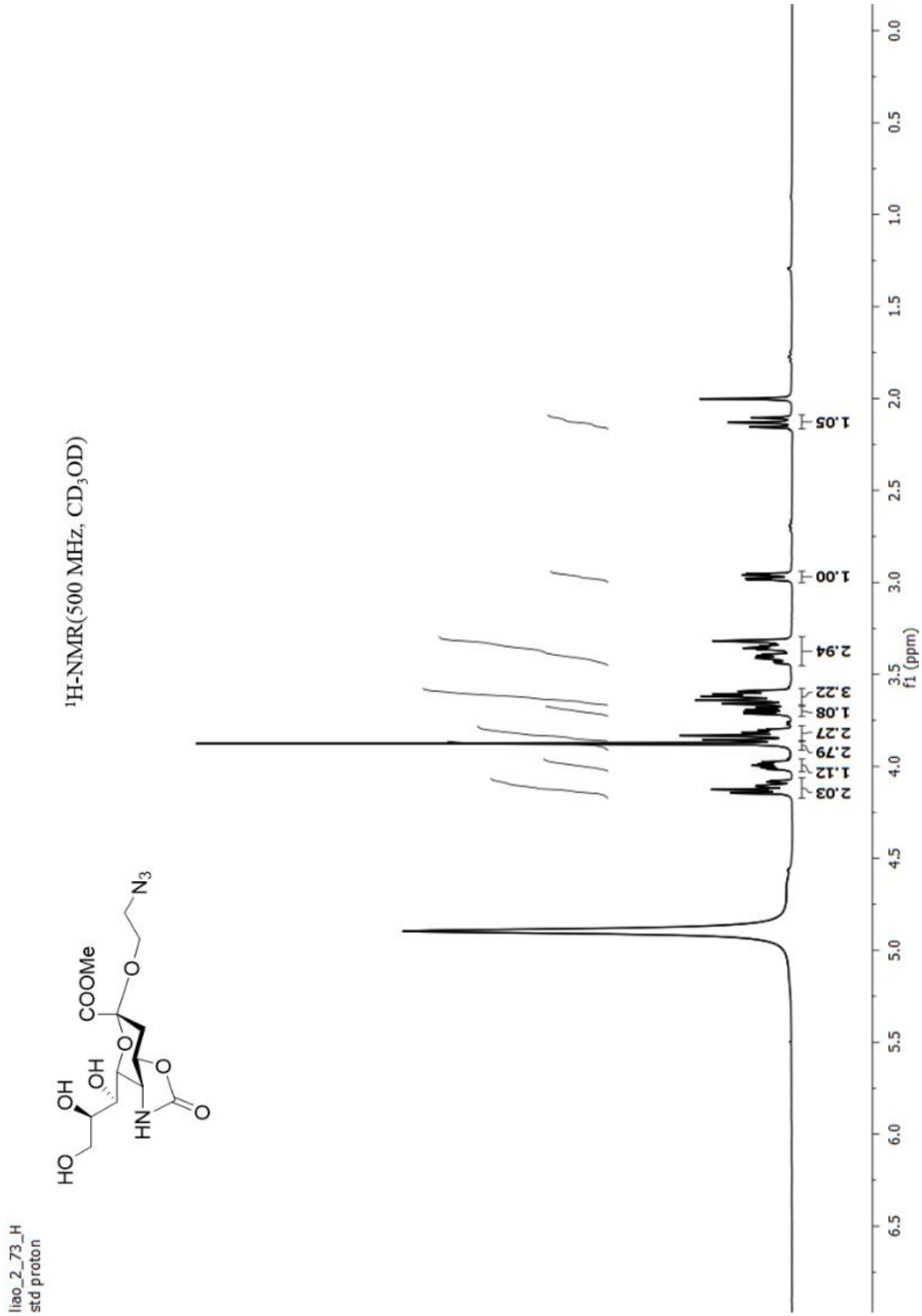
^{1}H-^{13}C HMQC Spectrum of compound **12** ($CDCl_3$, 400 MHz)



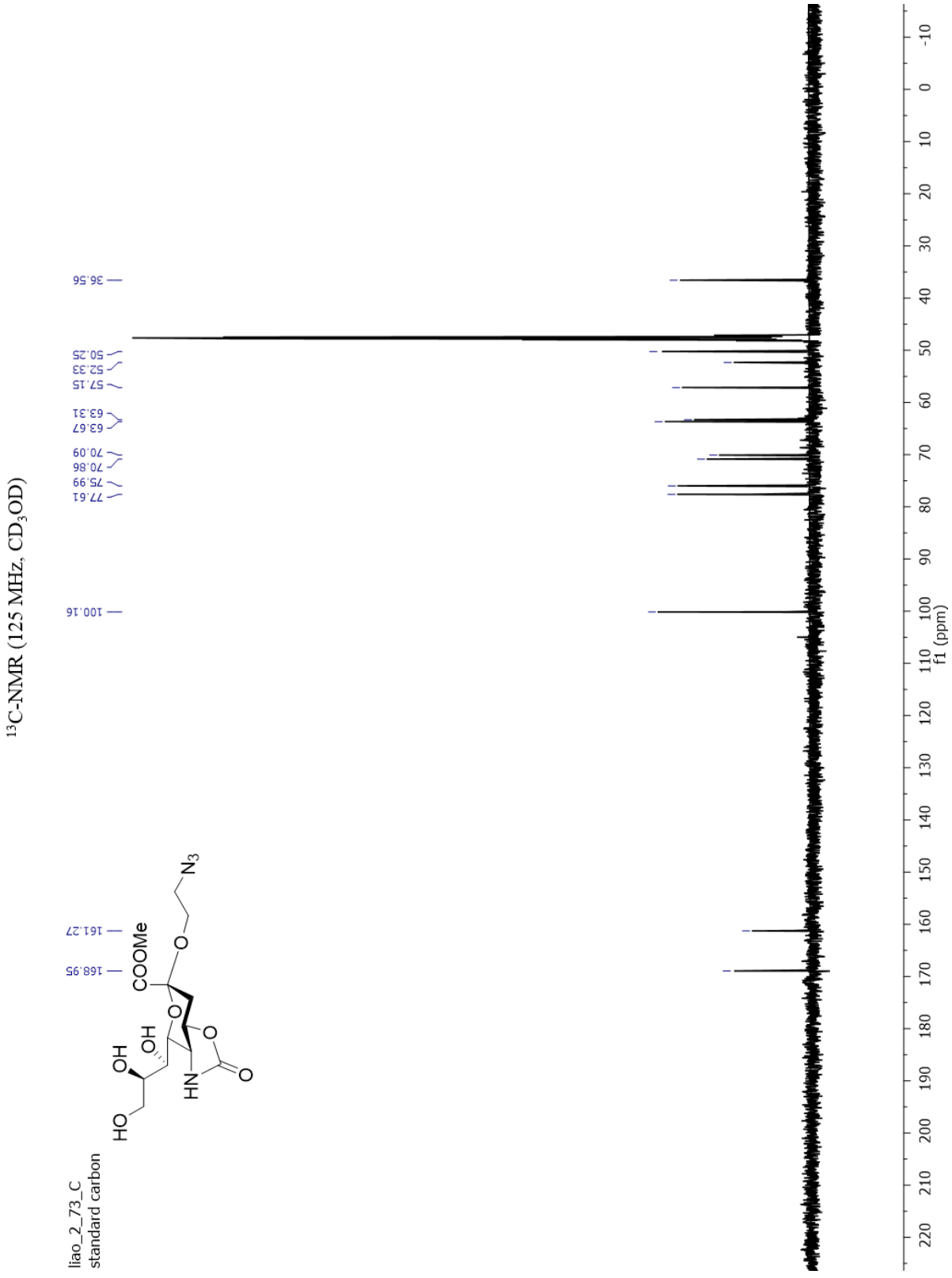
^{1}H NMR spectrum of the α/β-mixture of compound **12** ($CDCl_3$, 600 MHz)



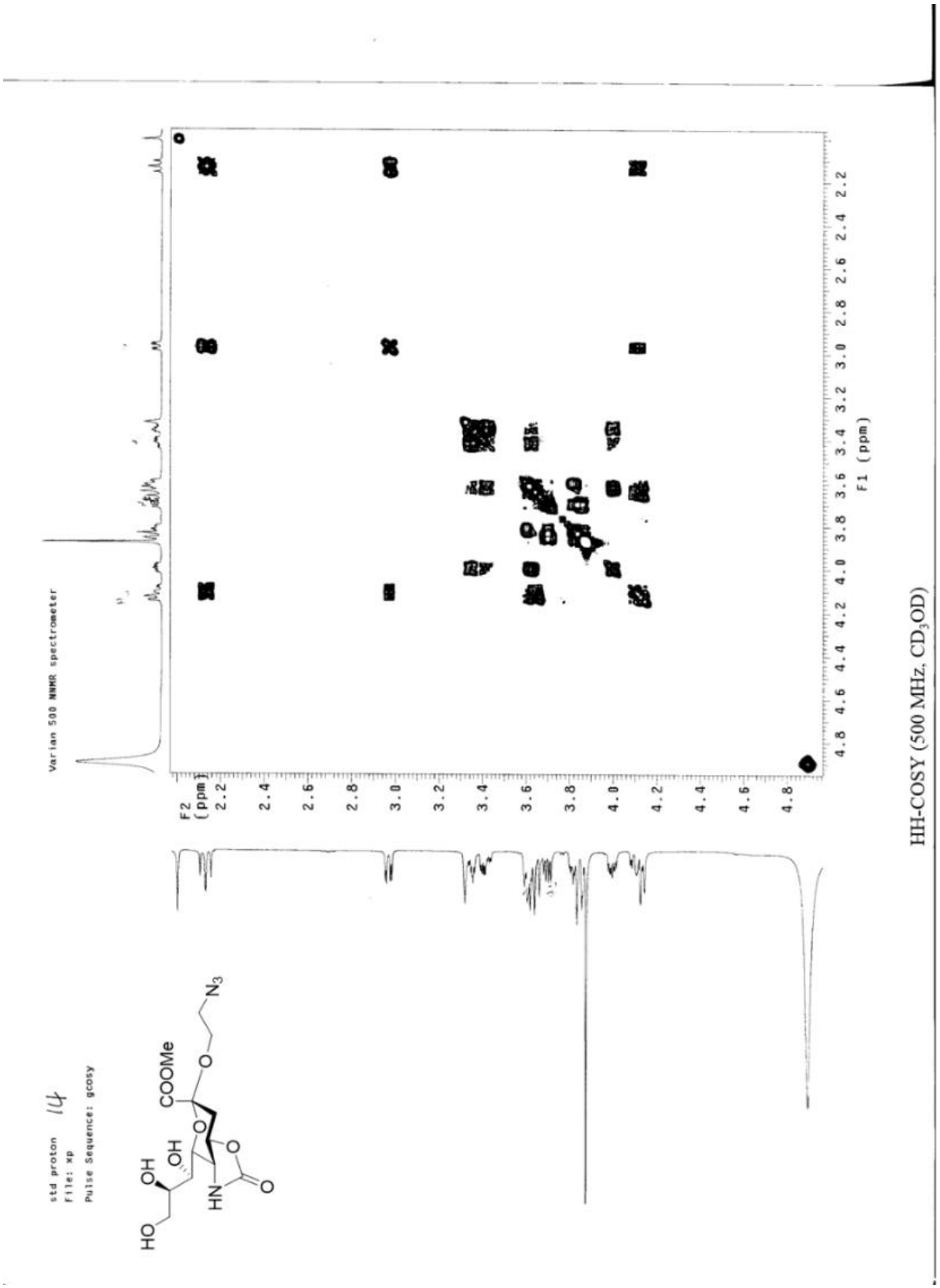
^{1}H-^{13}C HMQC spectrum of the α/β-mixture of compound **12** ($CDCl_3$, 600 MHz)



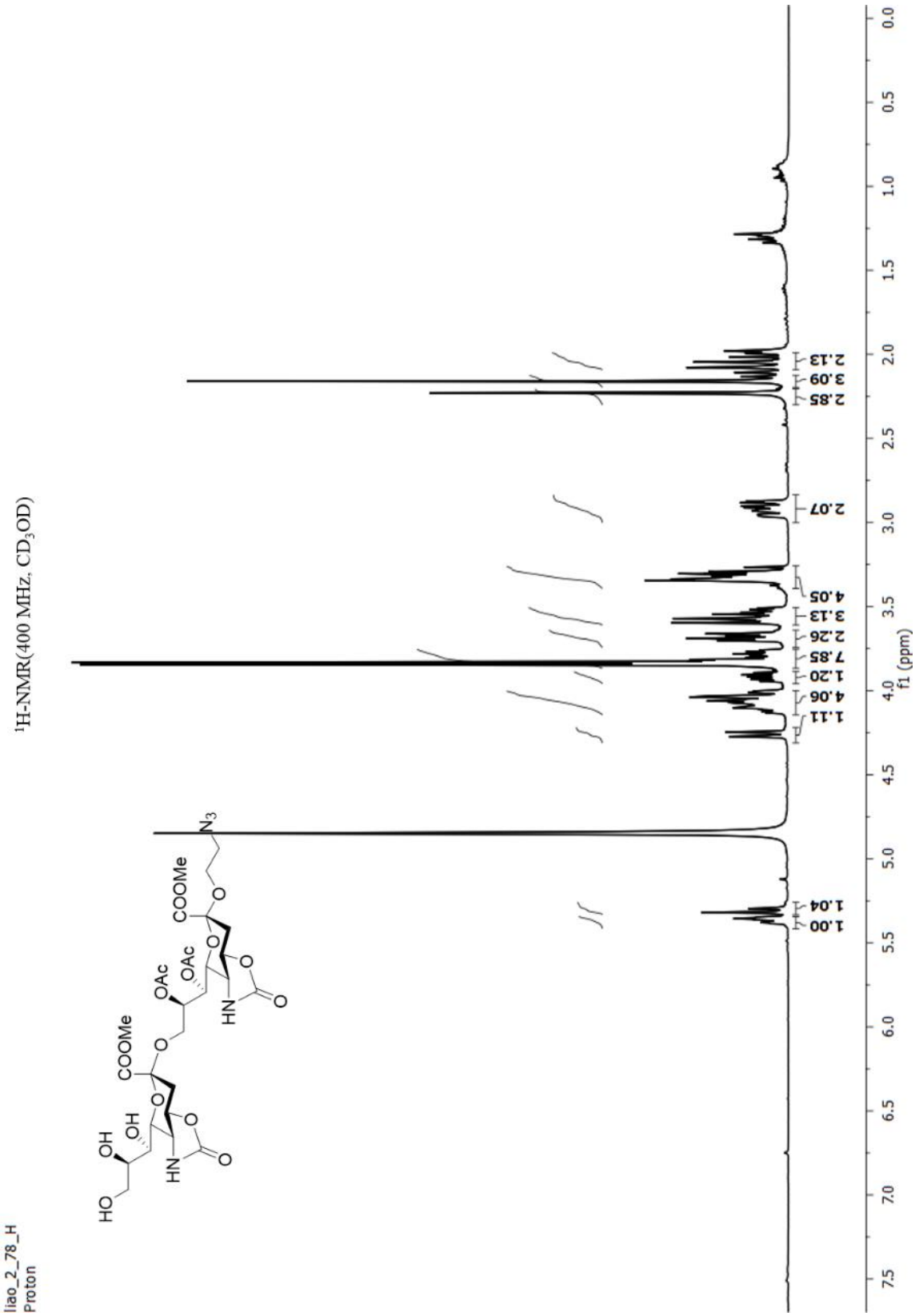
^{1}H NMR Spectrum of compound **13** (CD_3OD, 500 MHz)



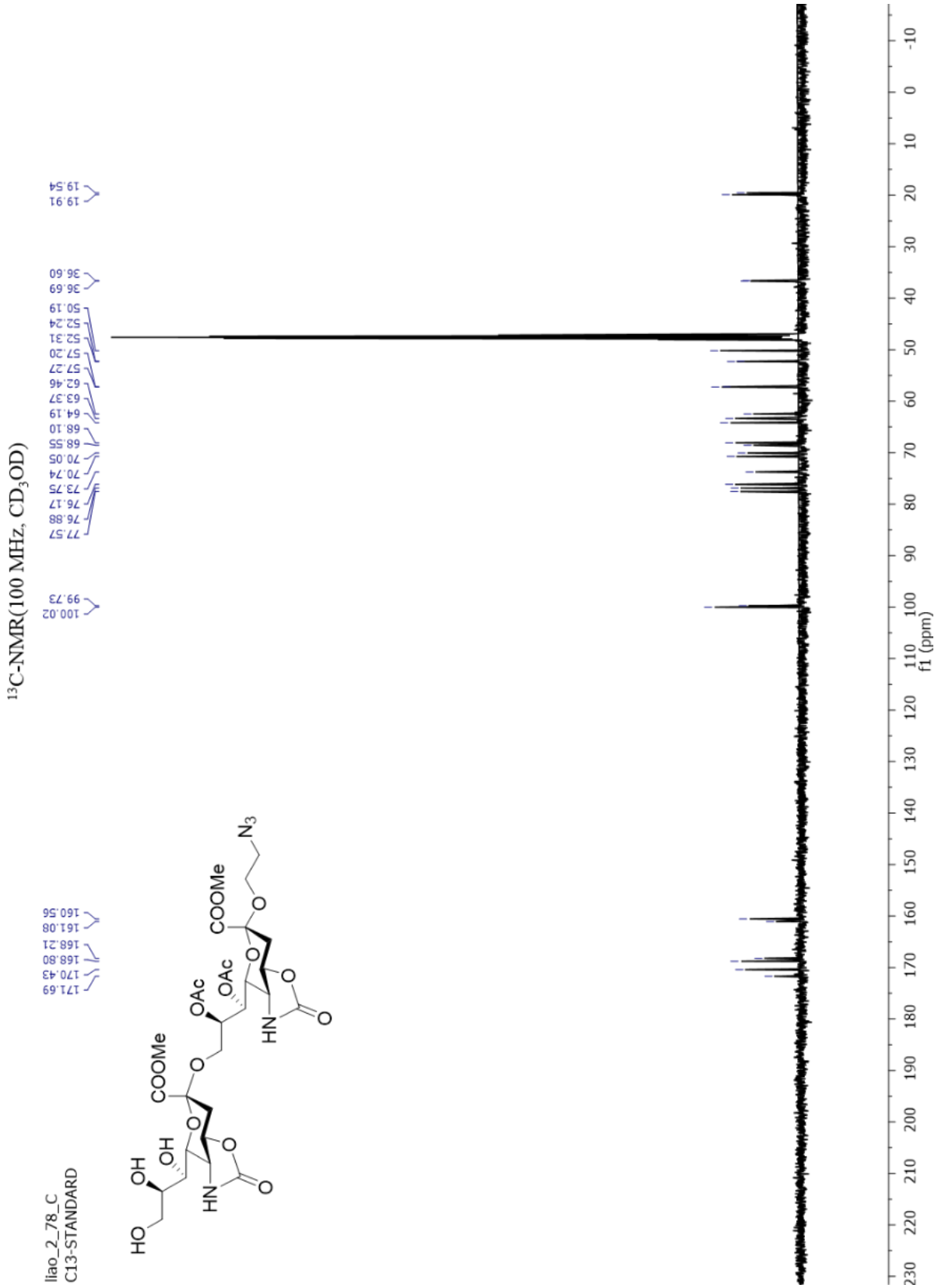
^{13}C NMR Spectrum of compound **13** (CD_3OD, 125 MHz)



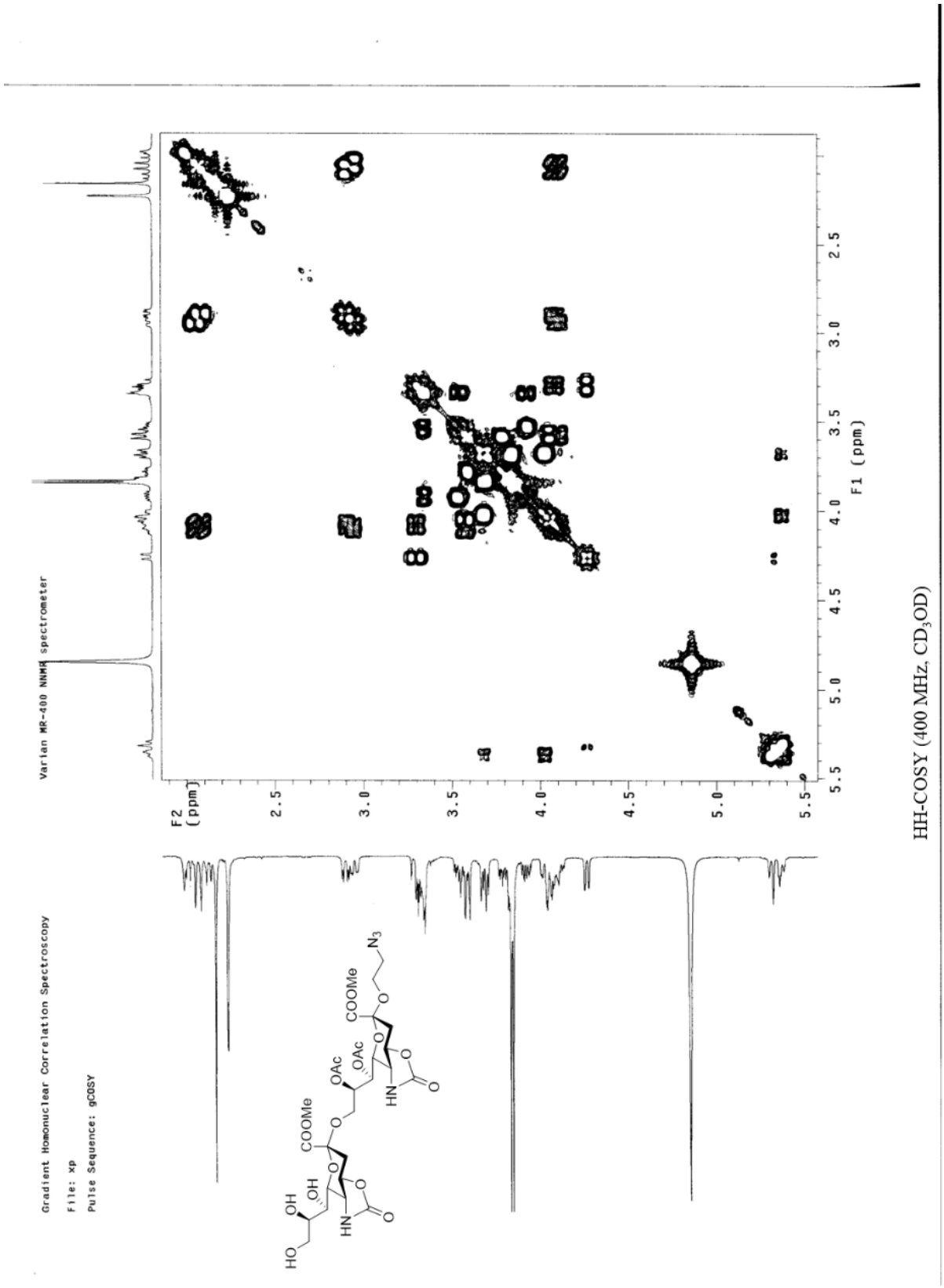
^{1}H-^{1}H COSY Spectrum of compound **13** (CD_3OD, 500 MHz)



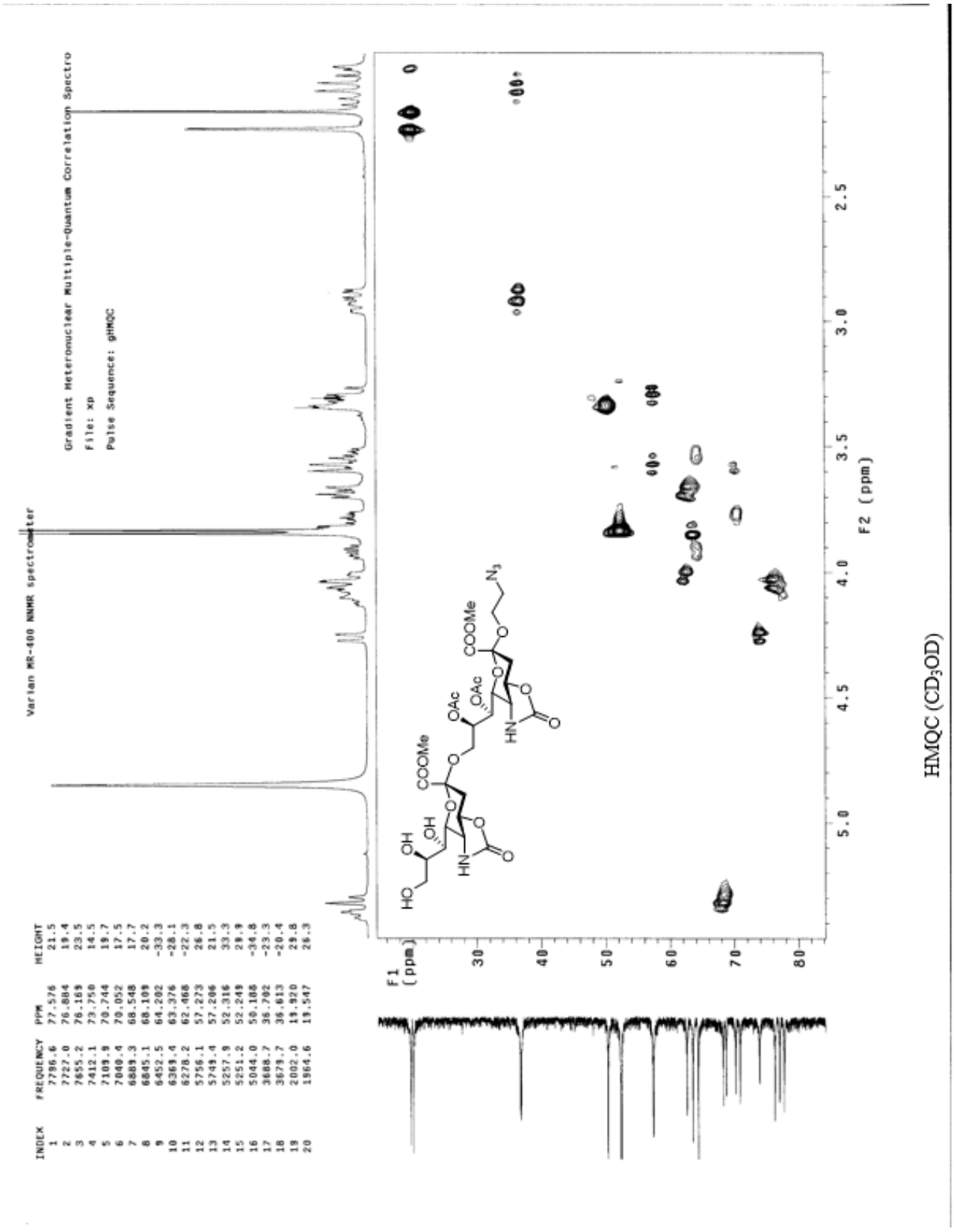
^{1}H NMR Spectrum of compound **14** (CD_3OD, 400 MHz)



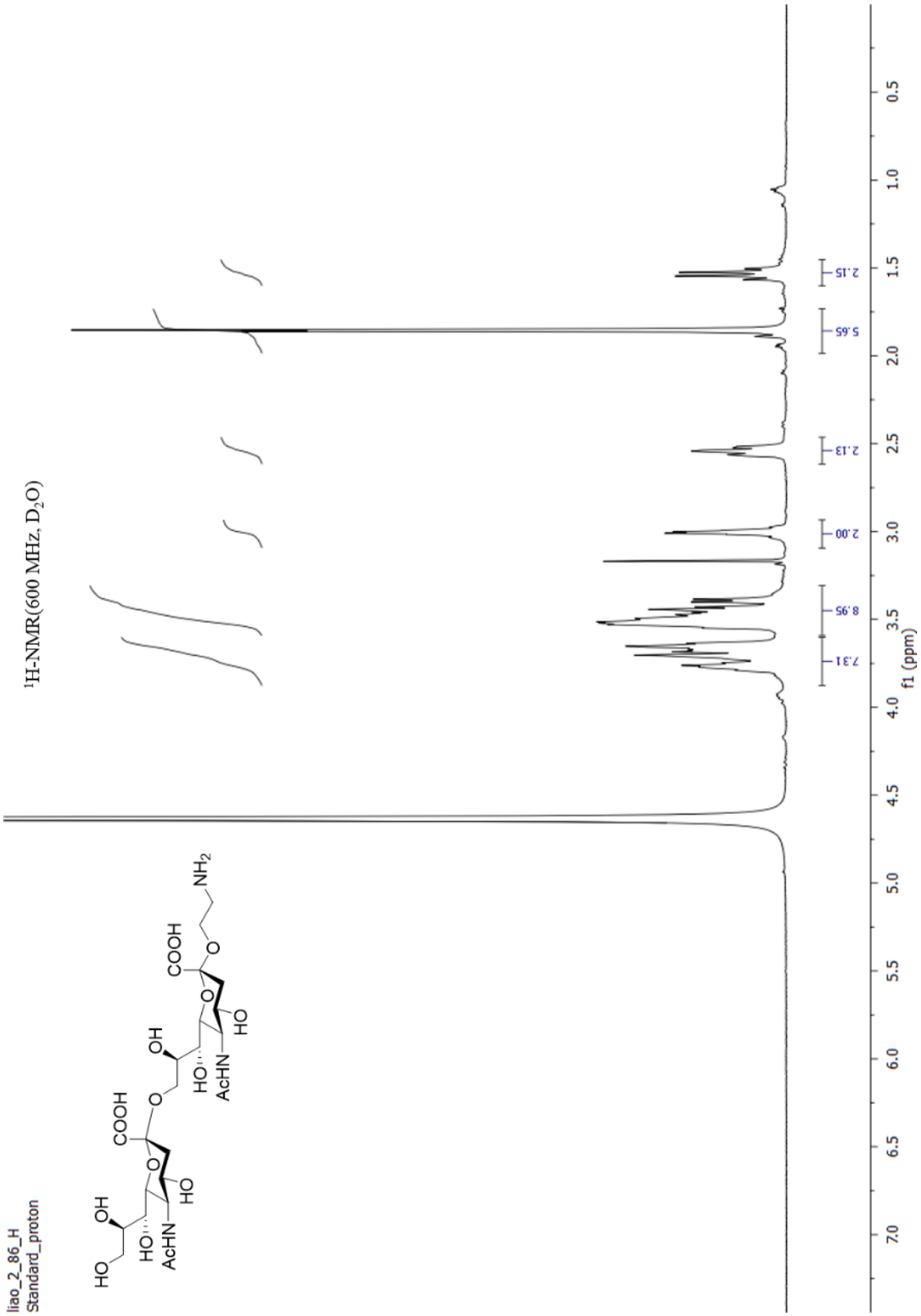
^{1}H NMR Spectrum of compound **14** (CD_3OD, 100 MHz)



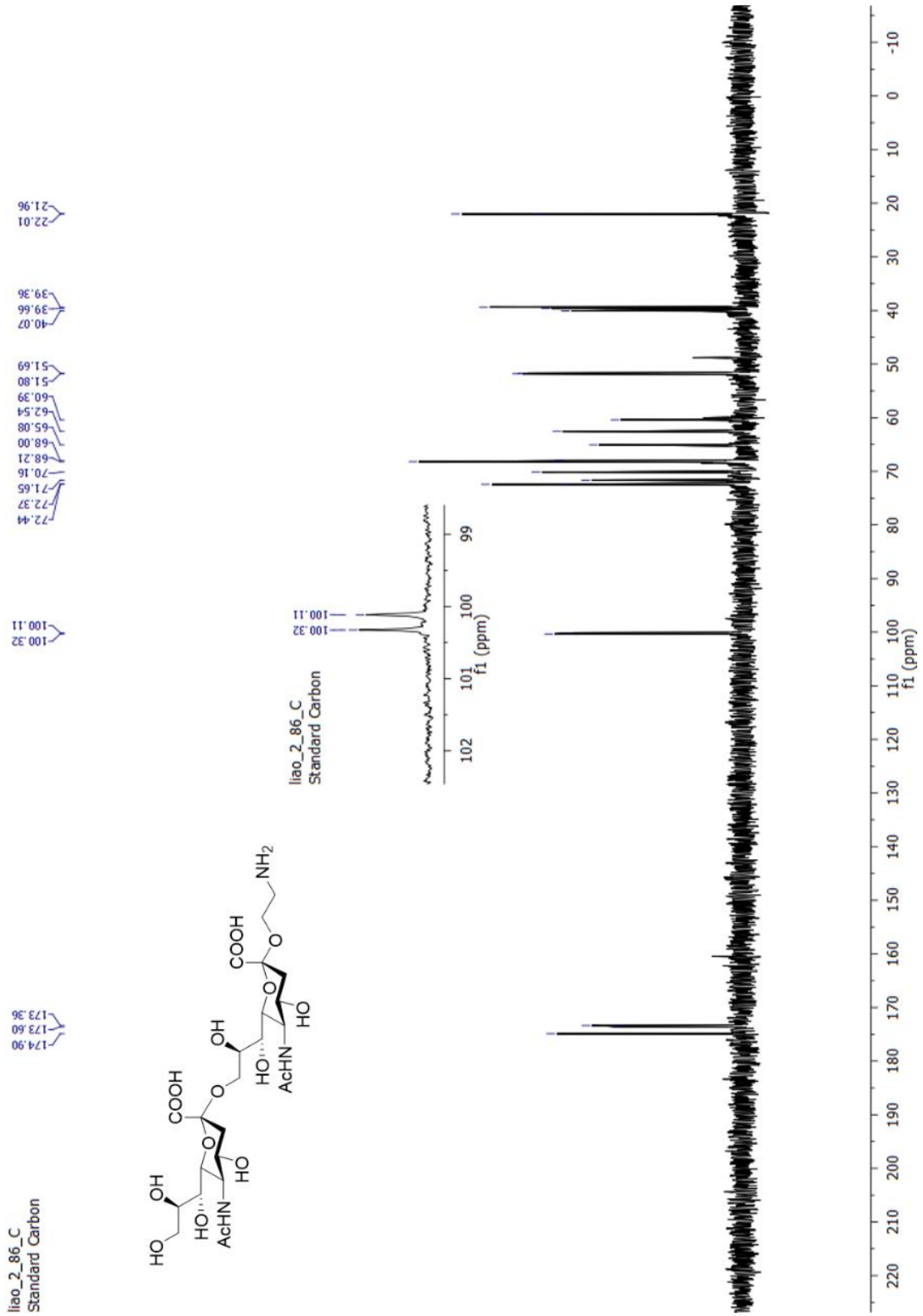
^{1}H-^{1}H COSY Spectrum of compound **14** (CD_3OD, 400 MHz)



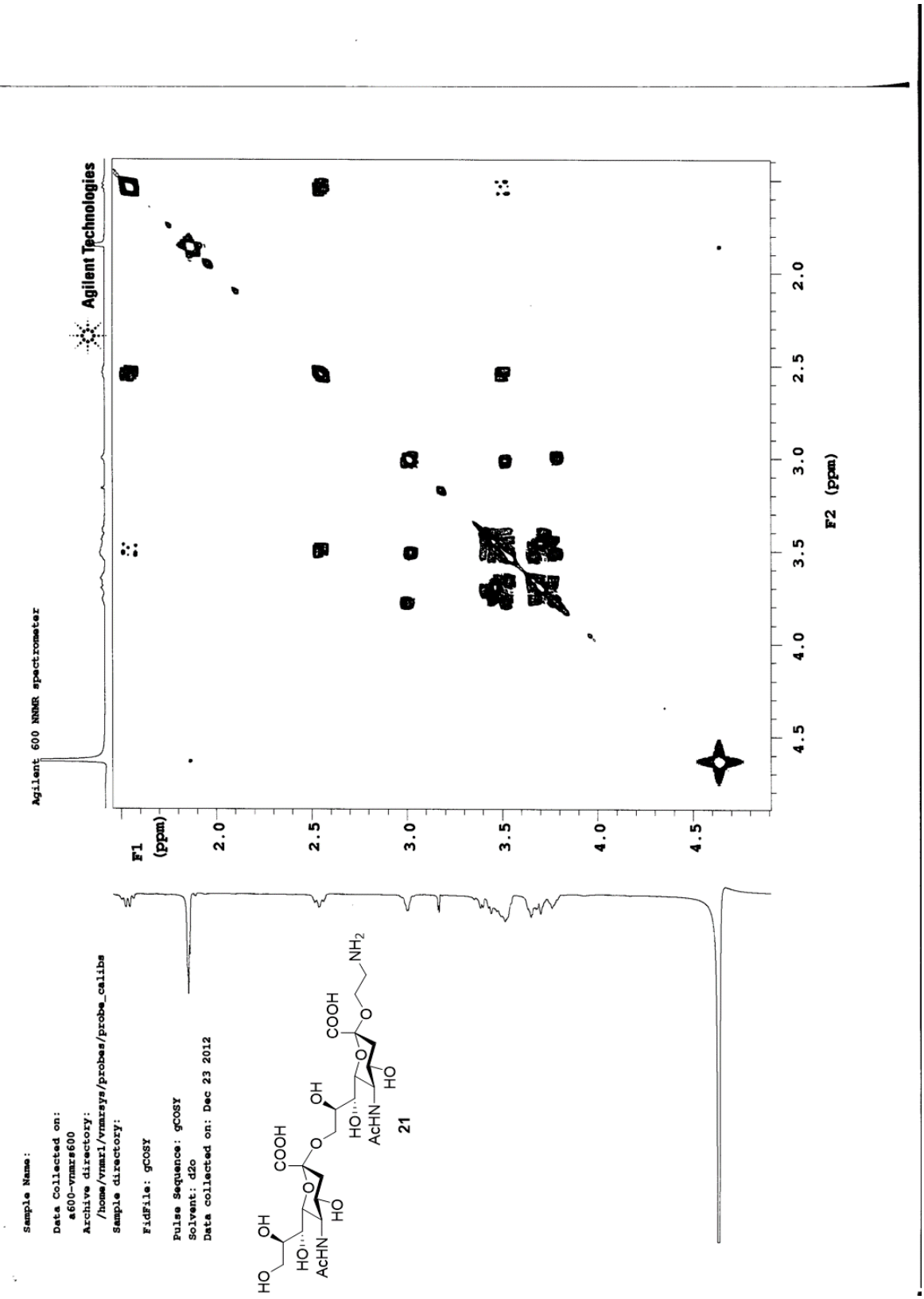
^{1}H-^{13}C HMQC Spectrum of compound **14** (CD_3OD, 400 MHz)



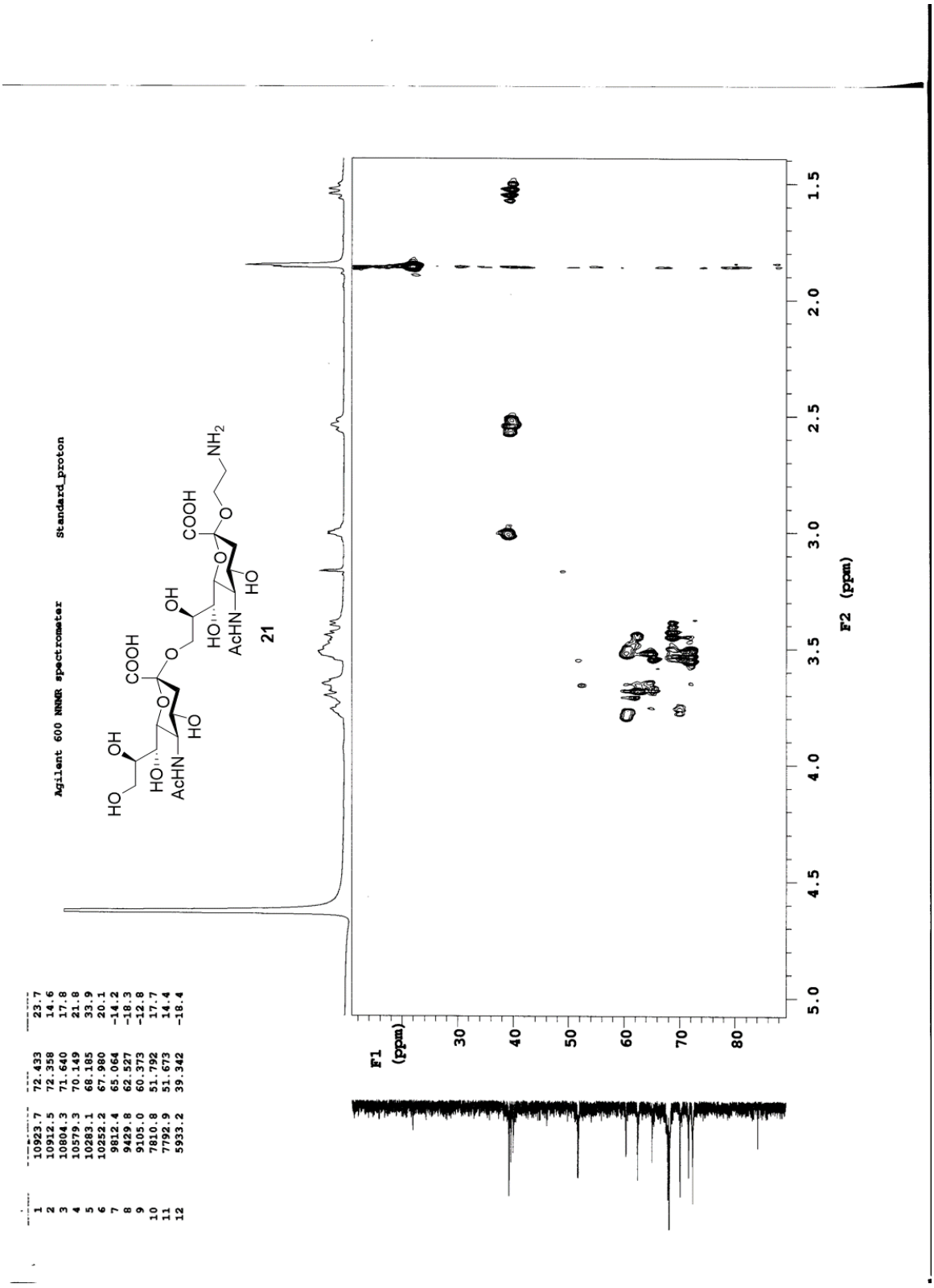
^{1}H NMR Spectrum of compound **15** (D_2O, 600 MHz)



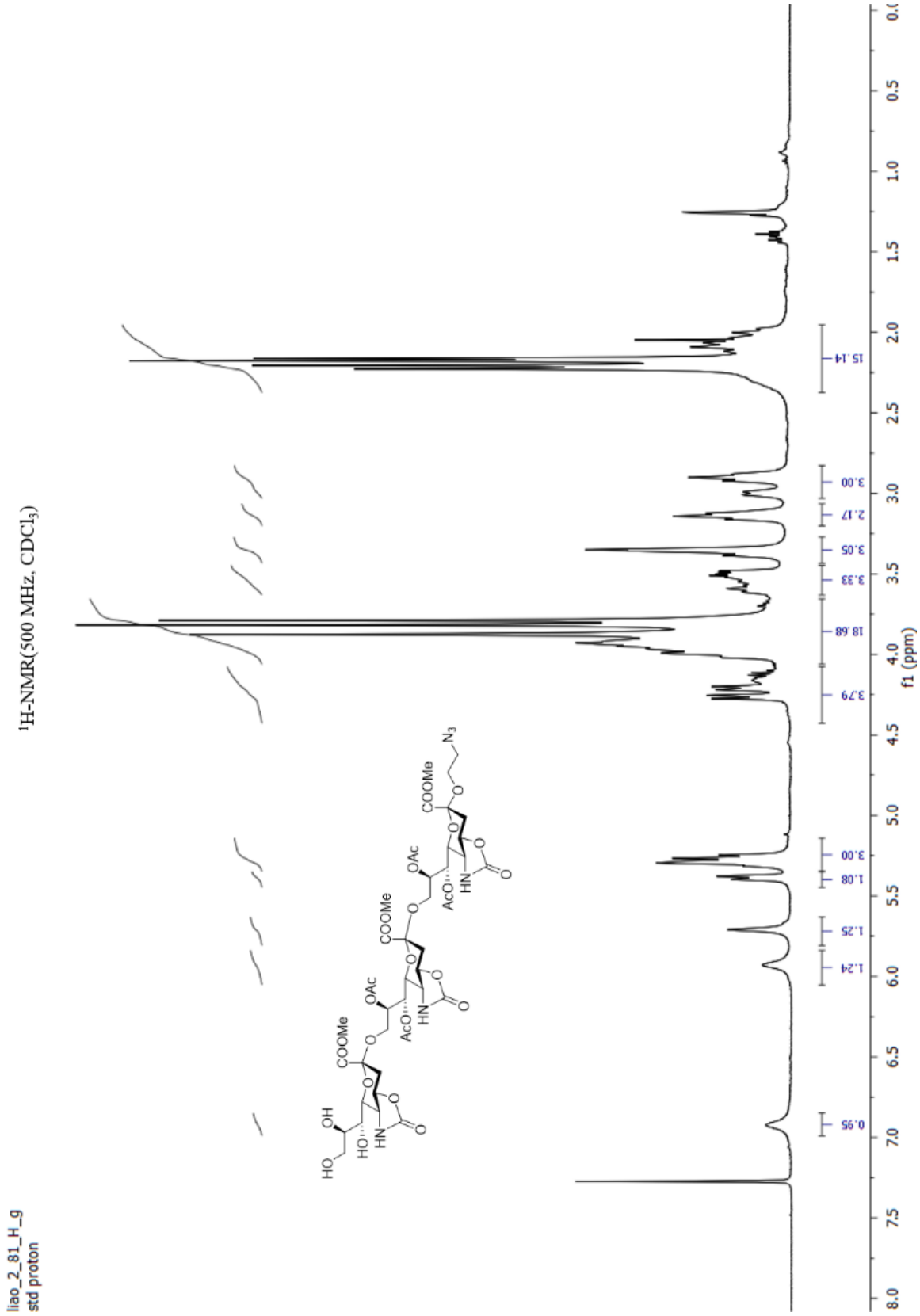
^{13}C NMR Spectrum of compound **15** (D_2O, 600 MHz)



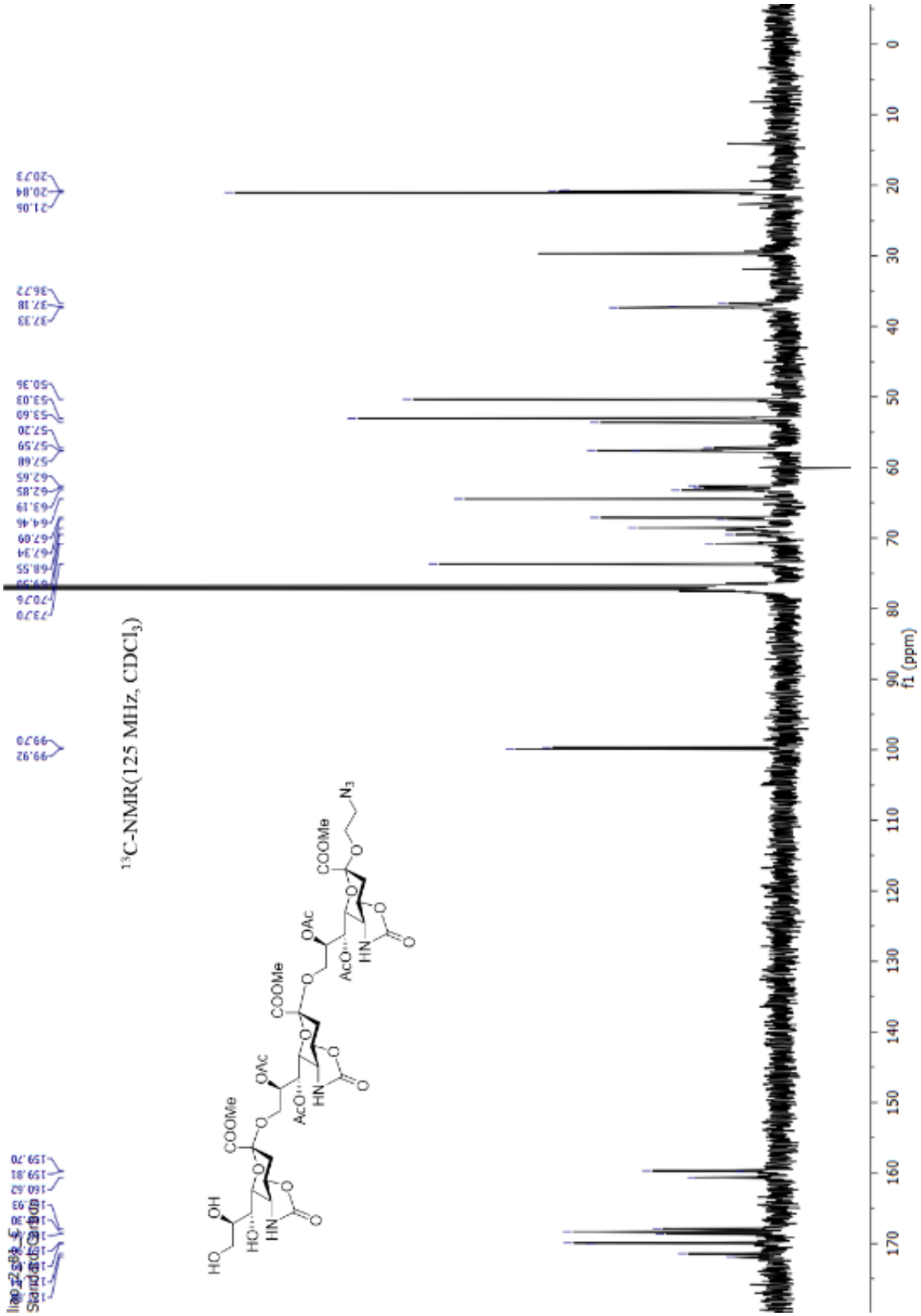
^{1}H-^{1}H COSY Spectrum of compound **15** (D_2O, 600 MHz)



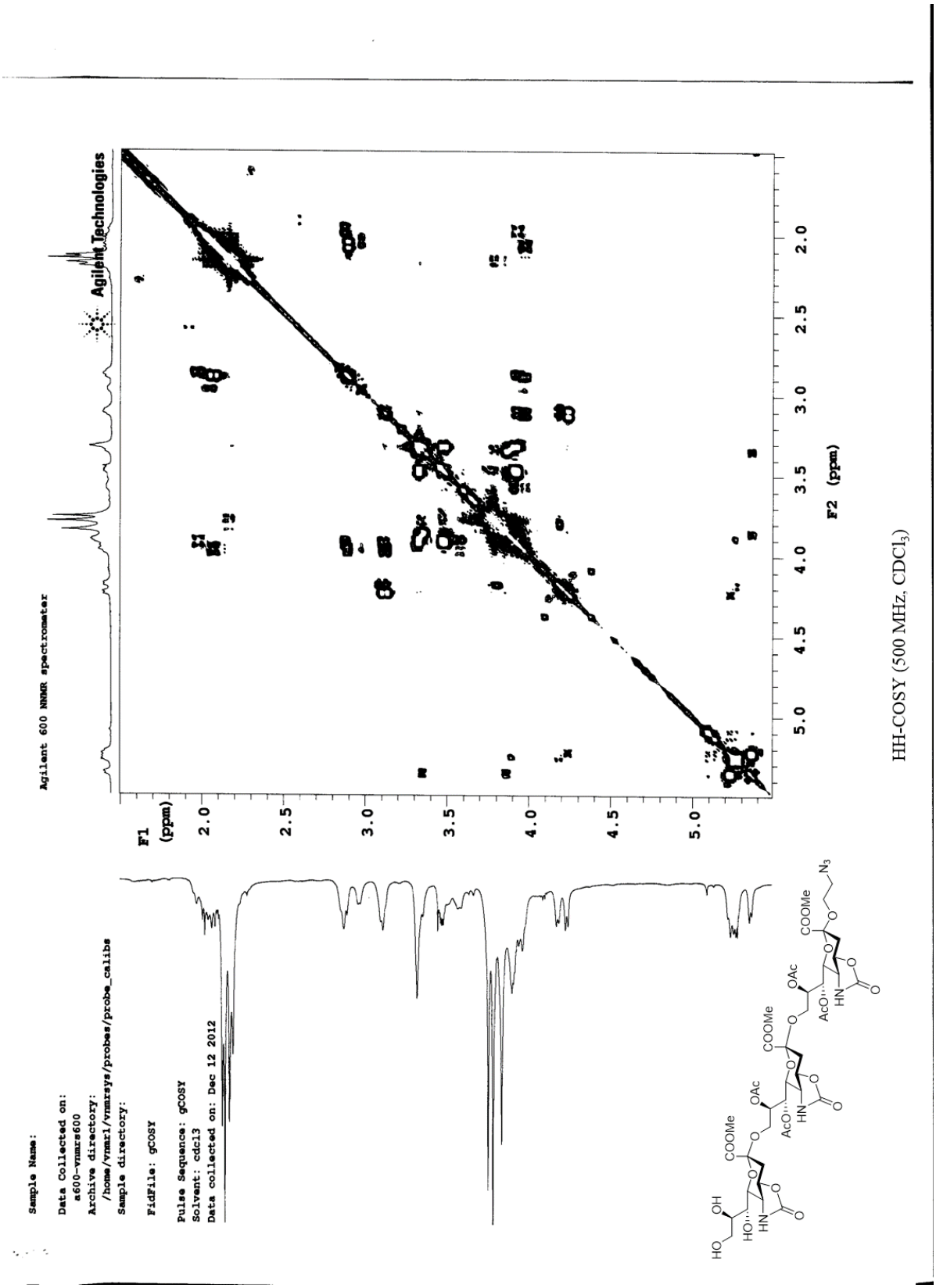
^{1}H-^{13}C HMQC Spectrum of compound **15** (D_2O, 600 MHz)



^{1}H NMR Spectrum of compound **16** ($CDCl_3$, 500 MHz)

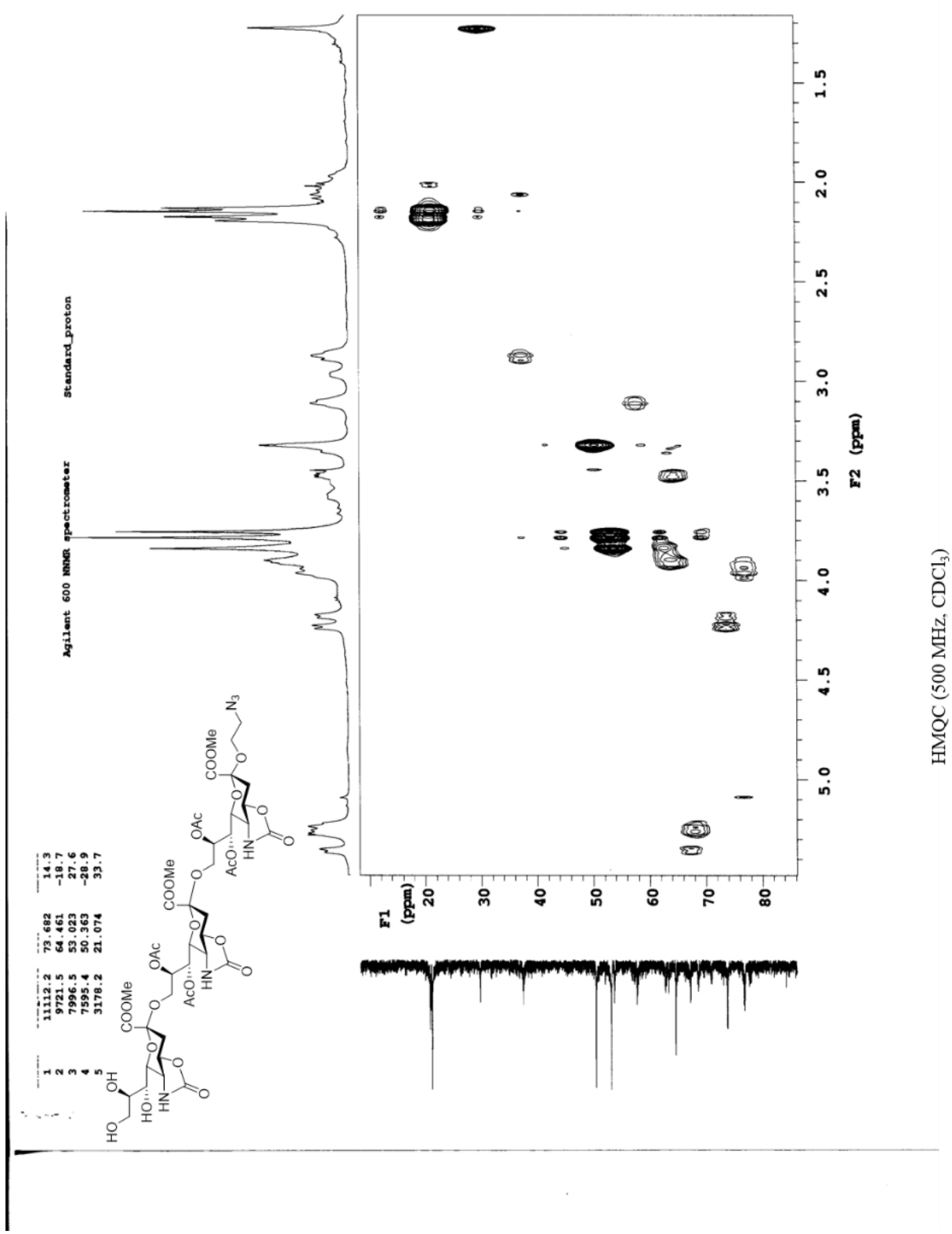


^{13}H NMR Spectrum of compound **16** ($CDCl_3$, 125 MHz)

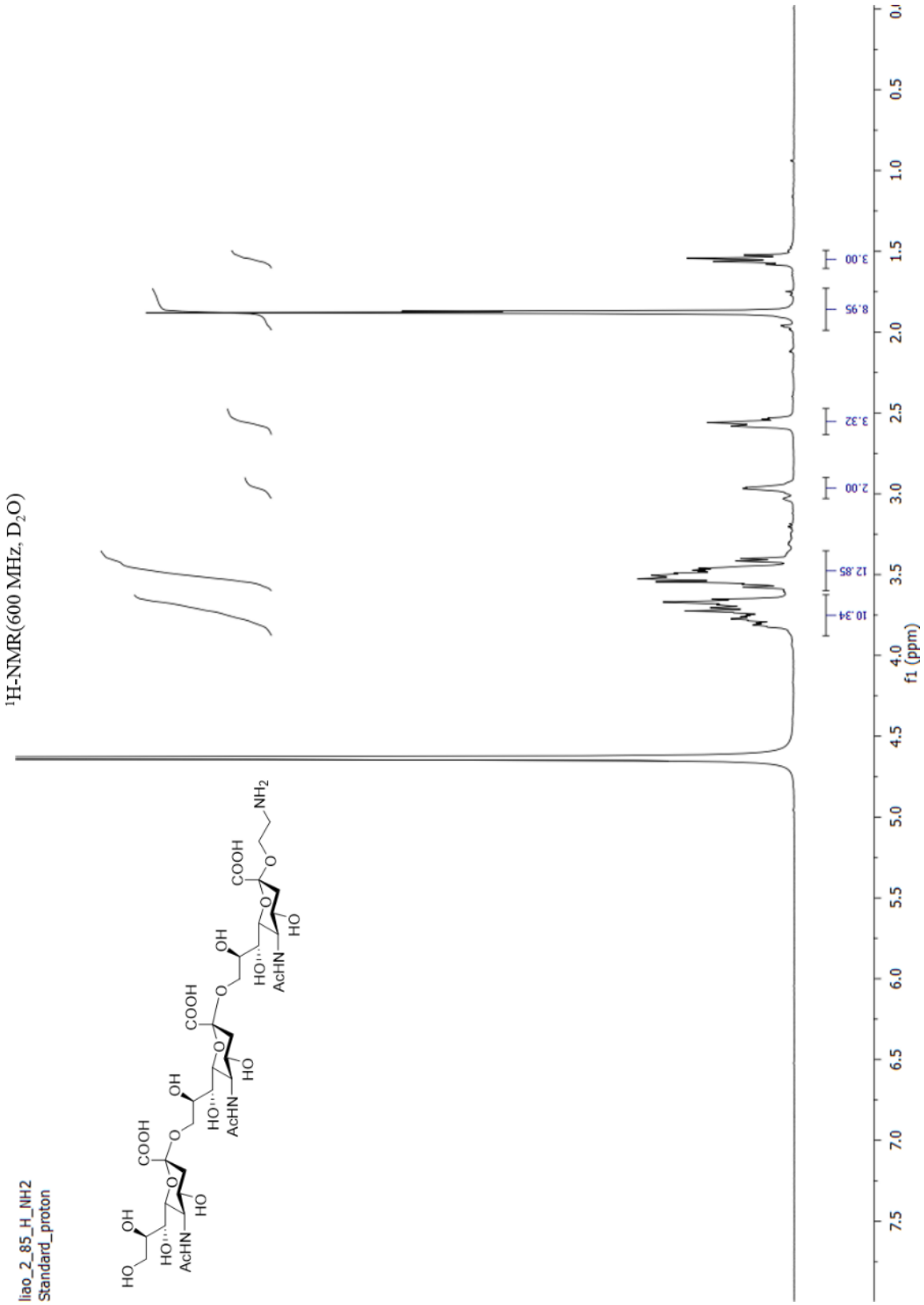


HH-COSY (500 MHz, $CDCl_3$)

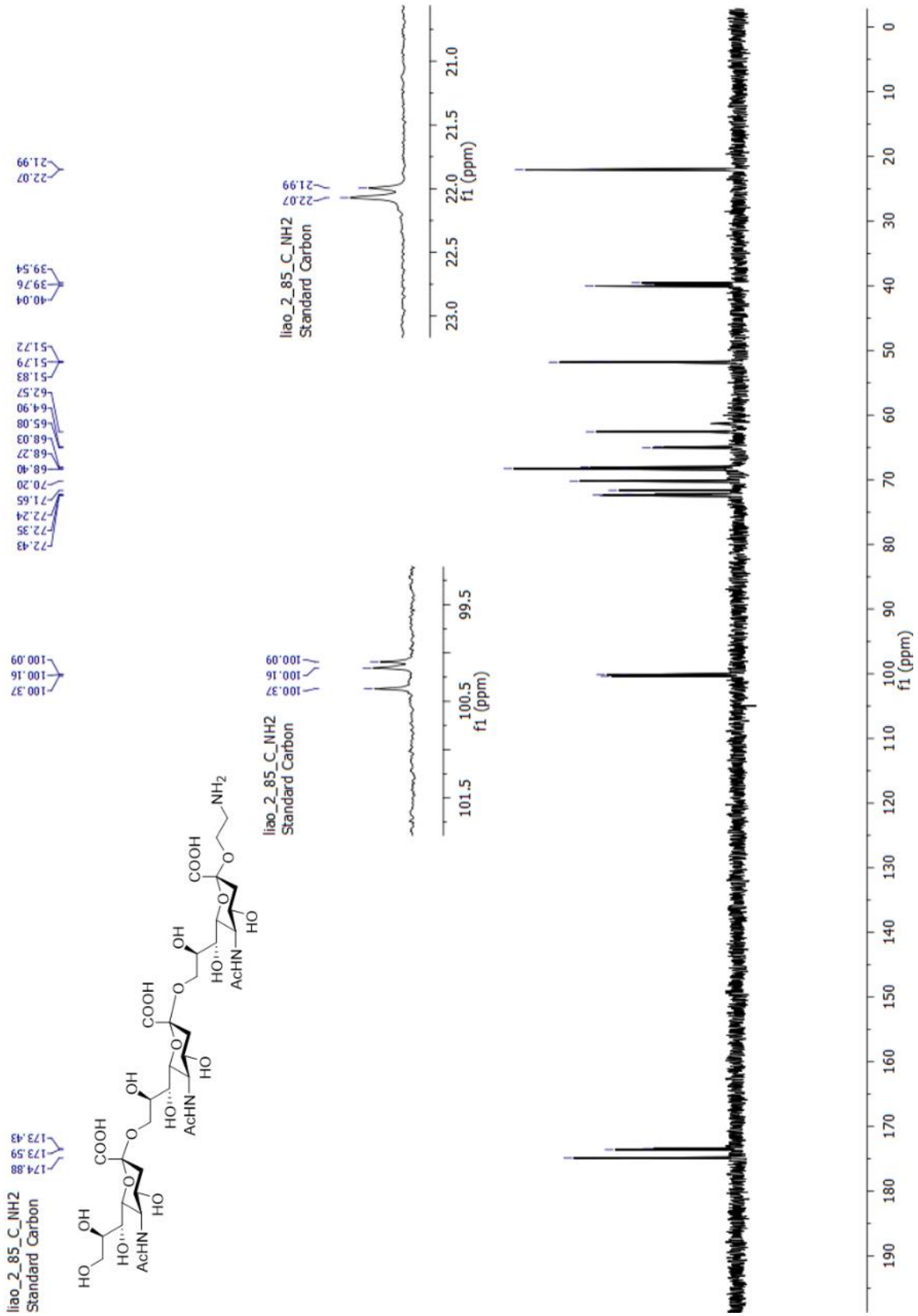
^1H-^1H COSY Spectrum of compound **16** ($CDCl_3$, 500 MHz)



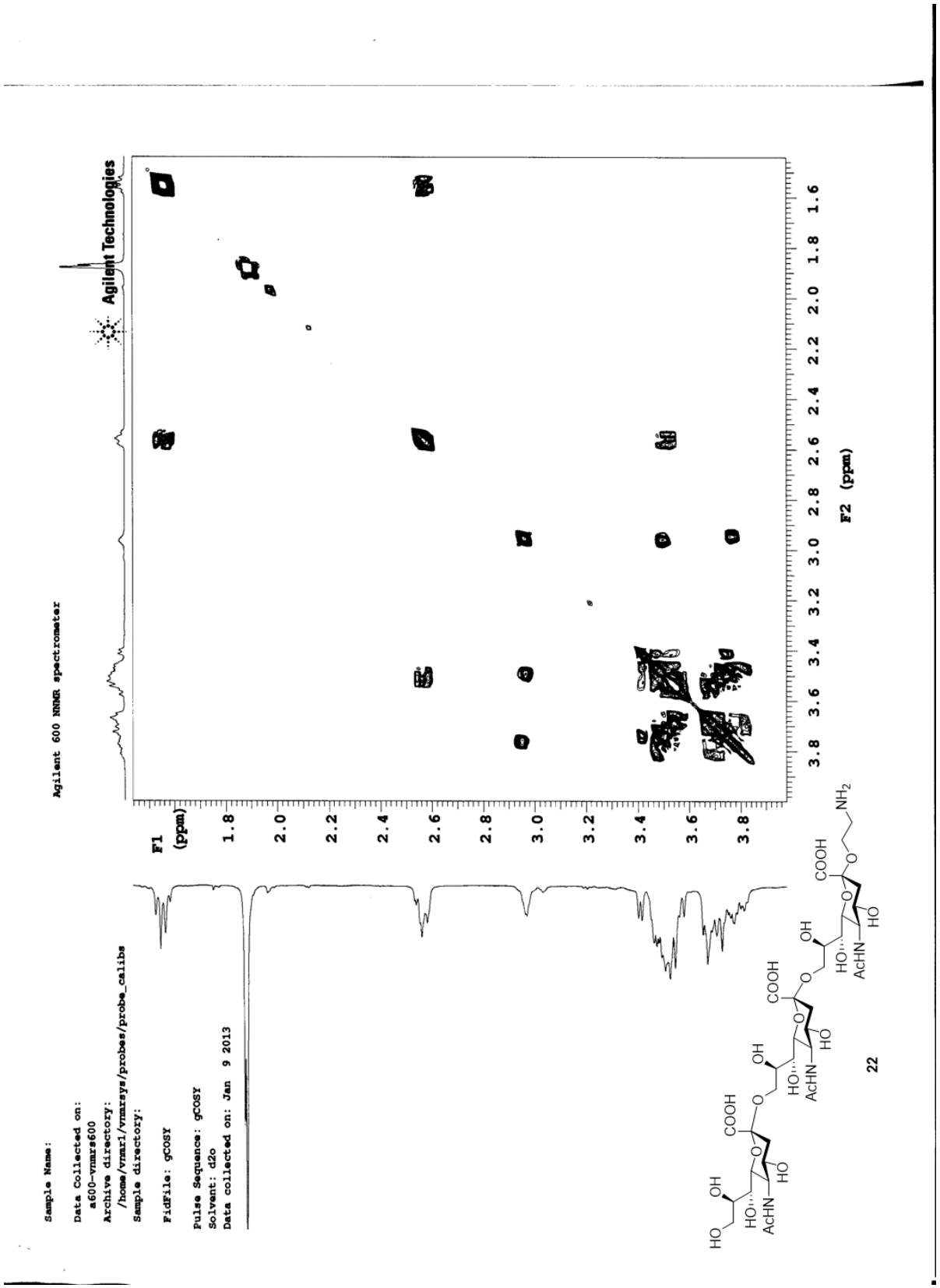
^{1}H-^{13}C HMQC Spectrum of compound **16** ($CDCl_3$, 500 MHz)



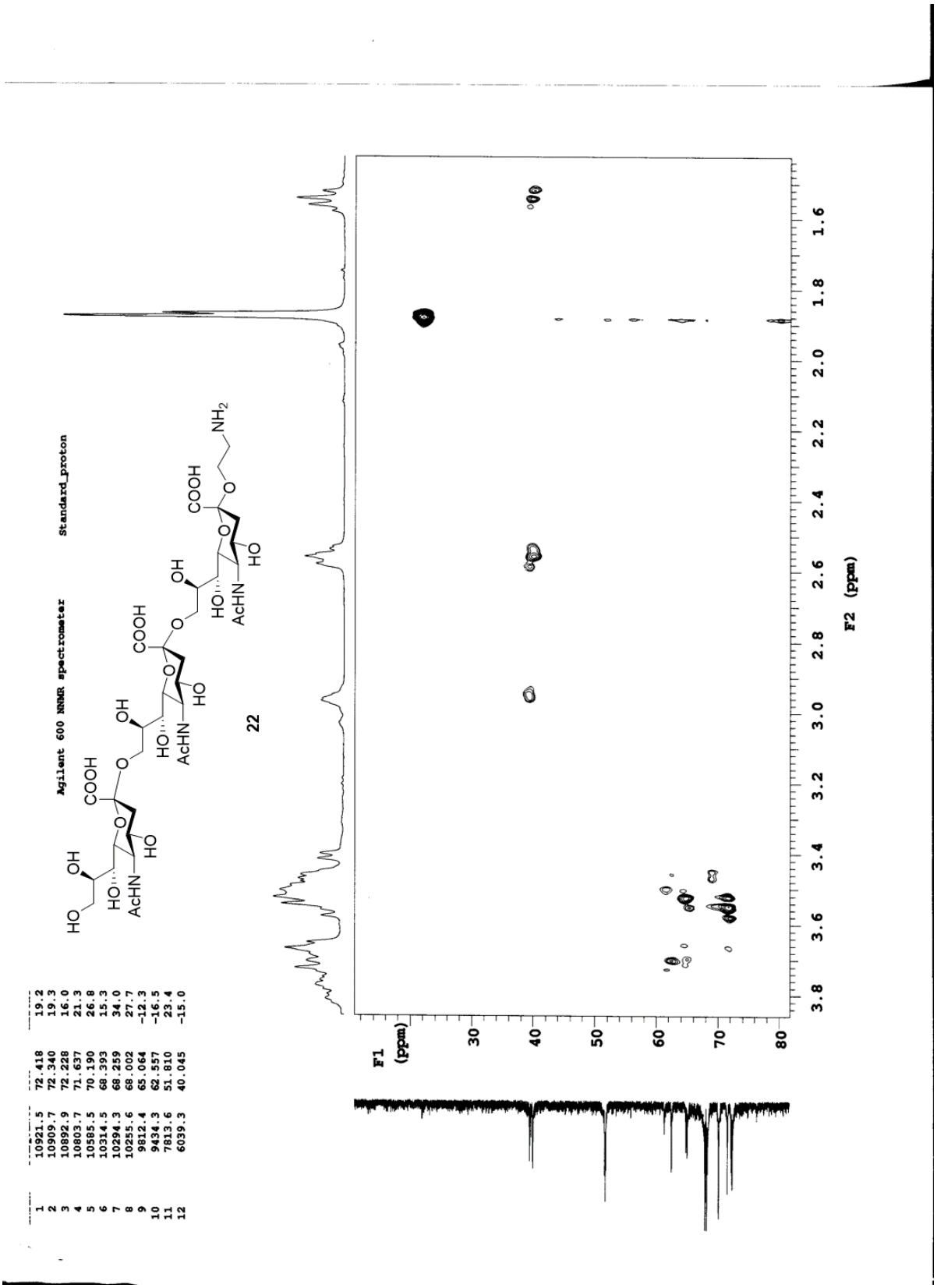
^{1}H NMR Spectrum of compound **17** (D_2O, 600 MHz)



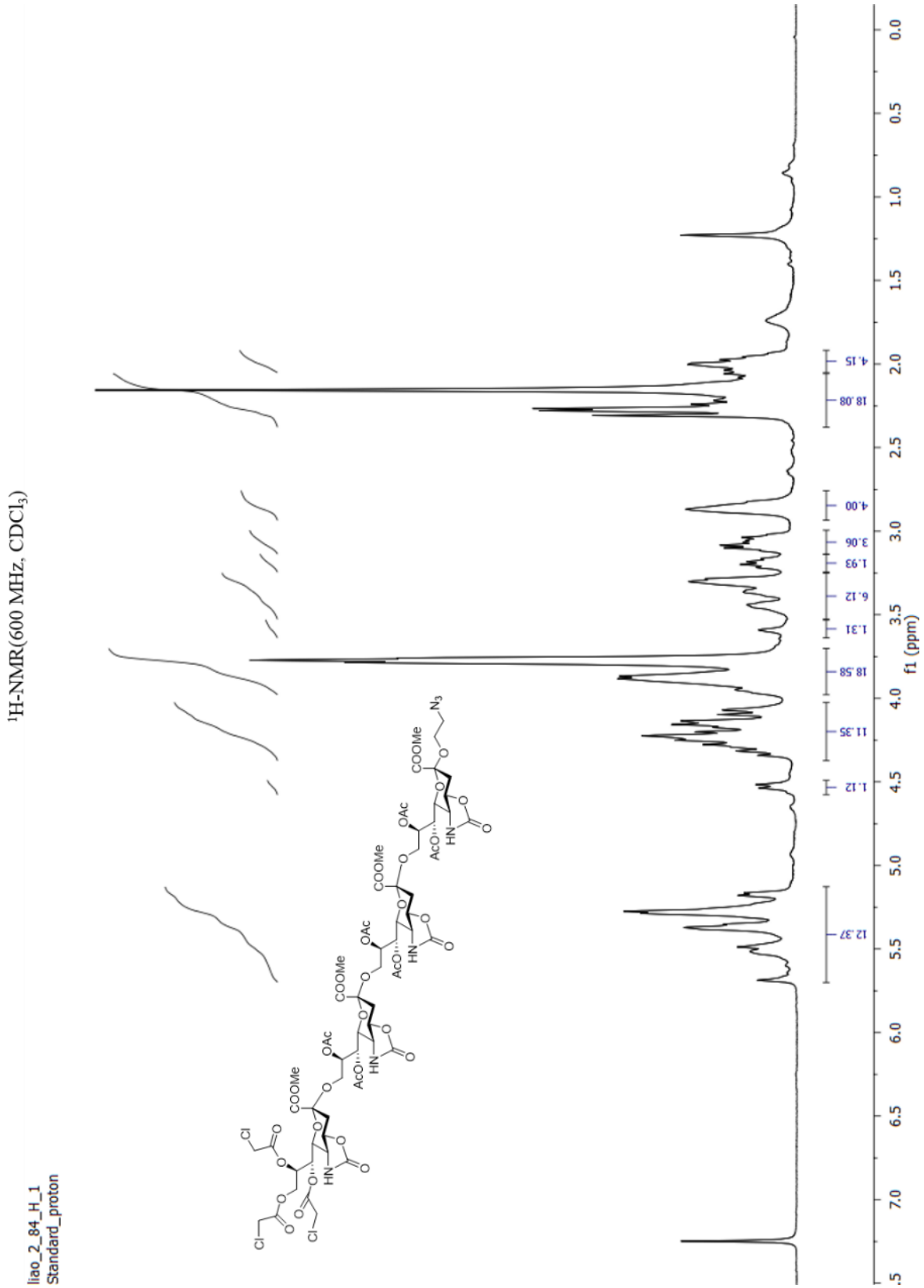
^{13}C NMR Spectrum of compound **17** (D_2O, 150 MHz)



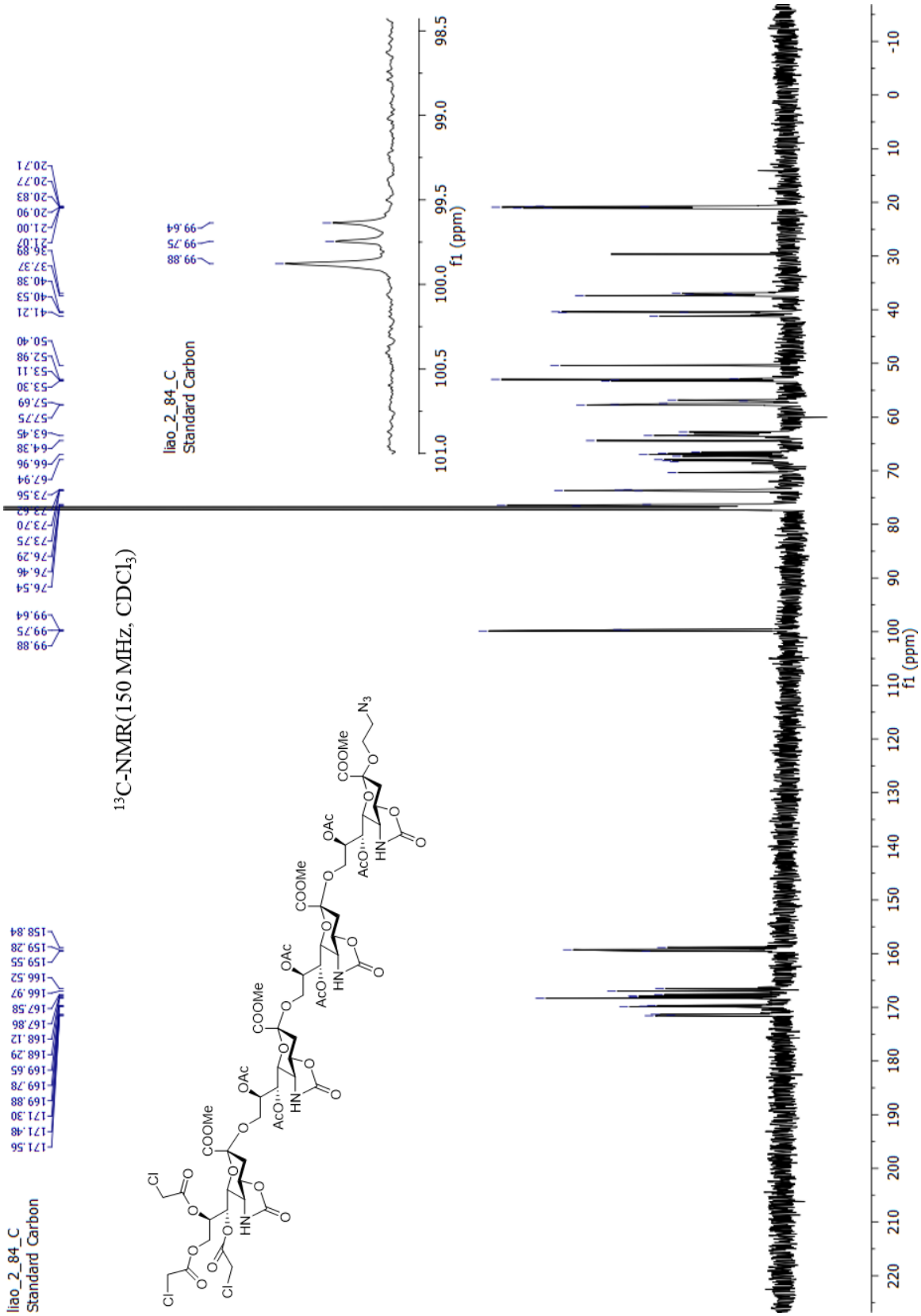
^{1}H-^{1}H COSY Spectrum of compound **17** (D_2O, 600 MHz)



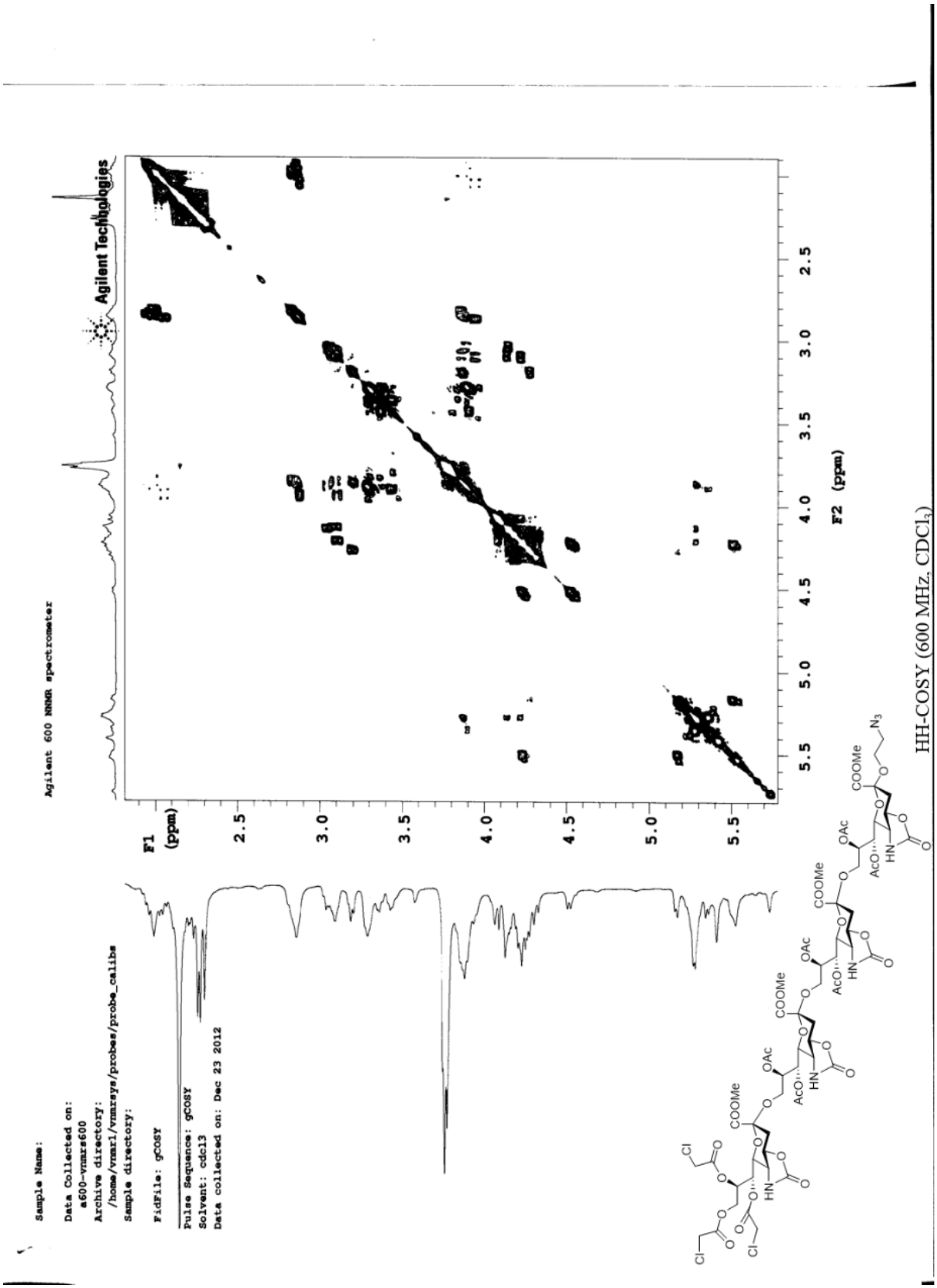
^{1}H-^{13}C HMQC Spectrum of compound **17** (D_2O, 600 MHz)



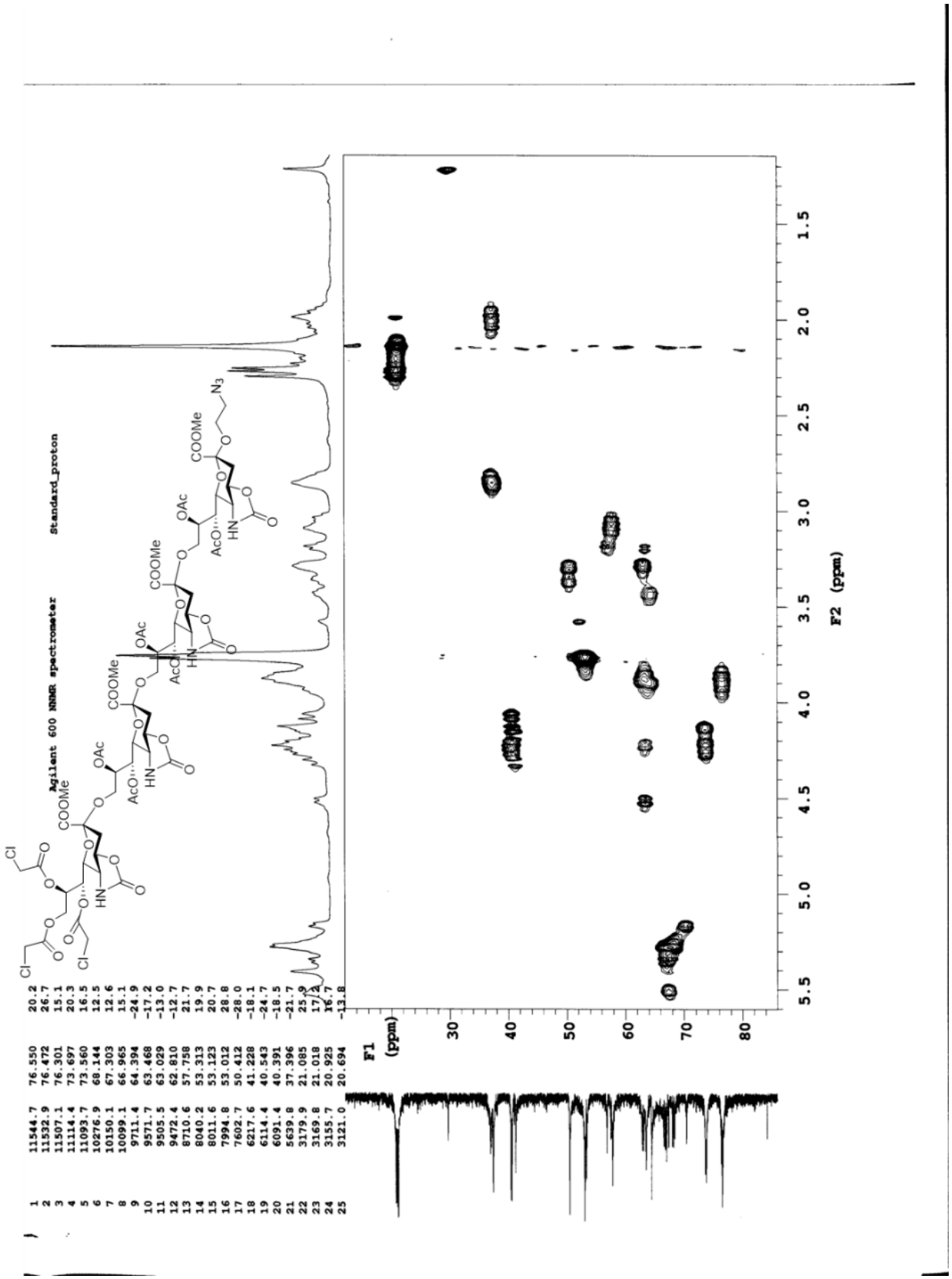
^{1}H NMR Spectrum of compound **18** ($CDCl_3$, 600 MHz)



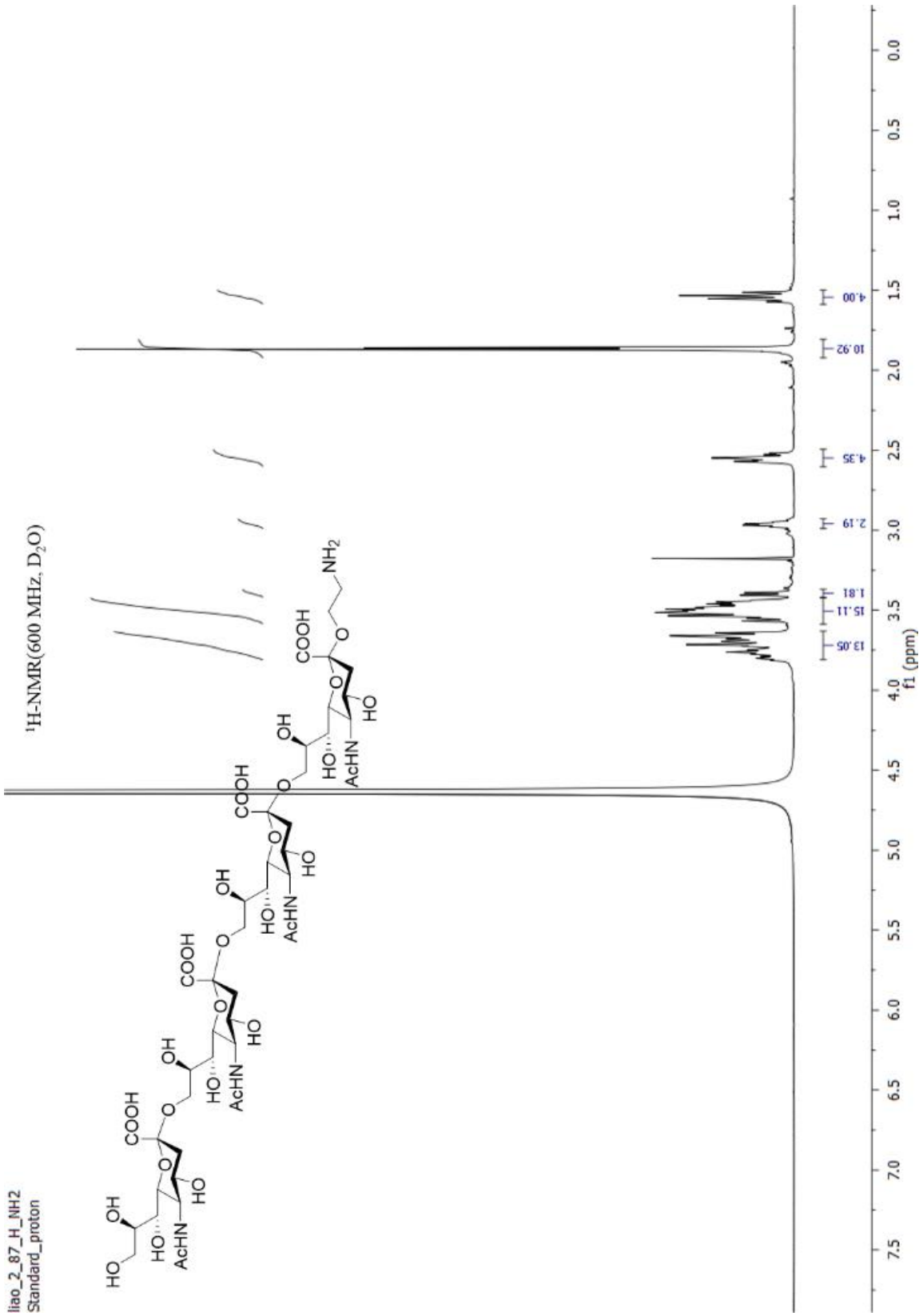
^{13}H NMR Spectrum of compound **18** ($CDCl_3$, 150 MHz)



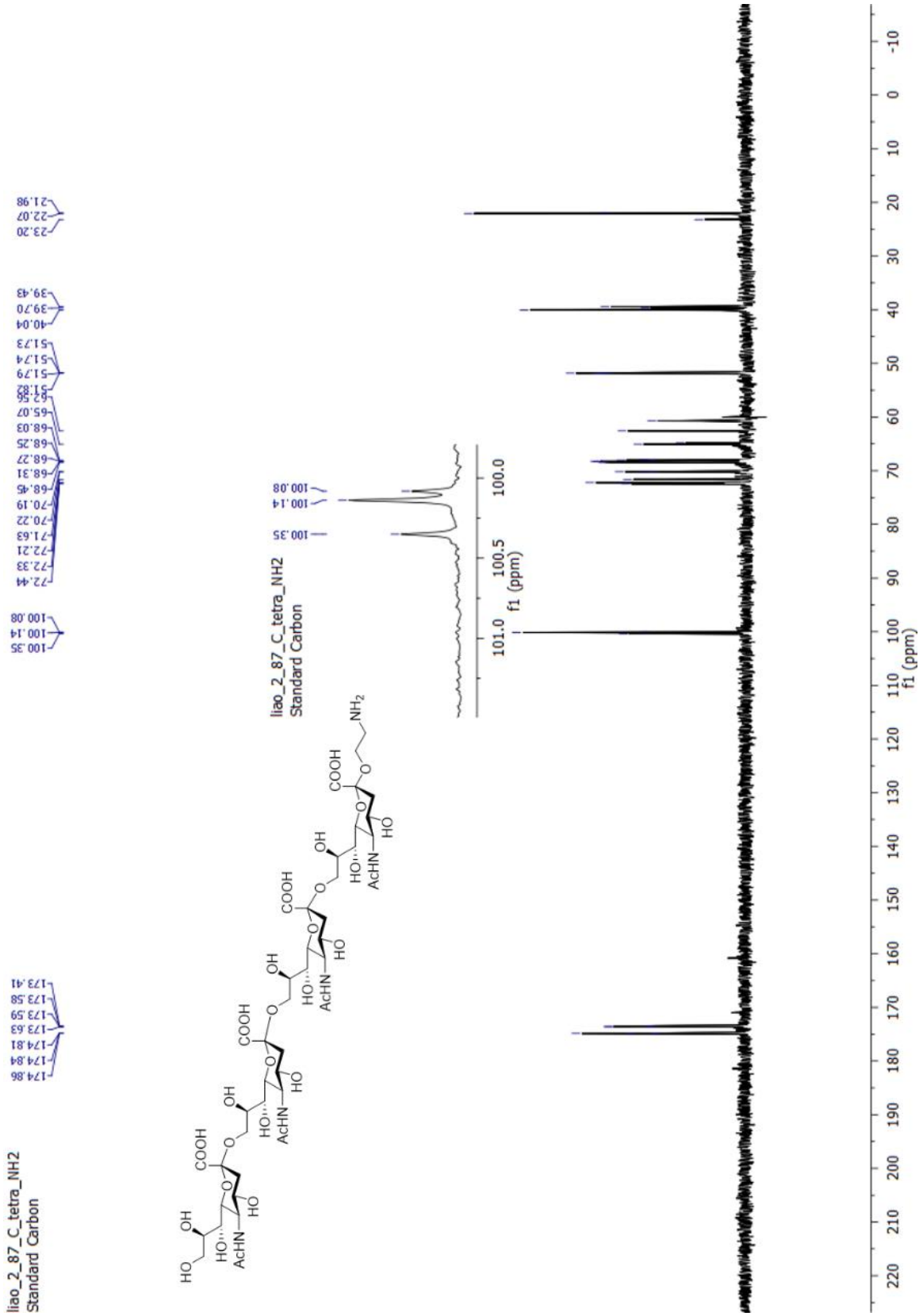
^{1}H-^{1}H COSY Spectrum of compound **18** ($CDCl_3$, 600 MHz)



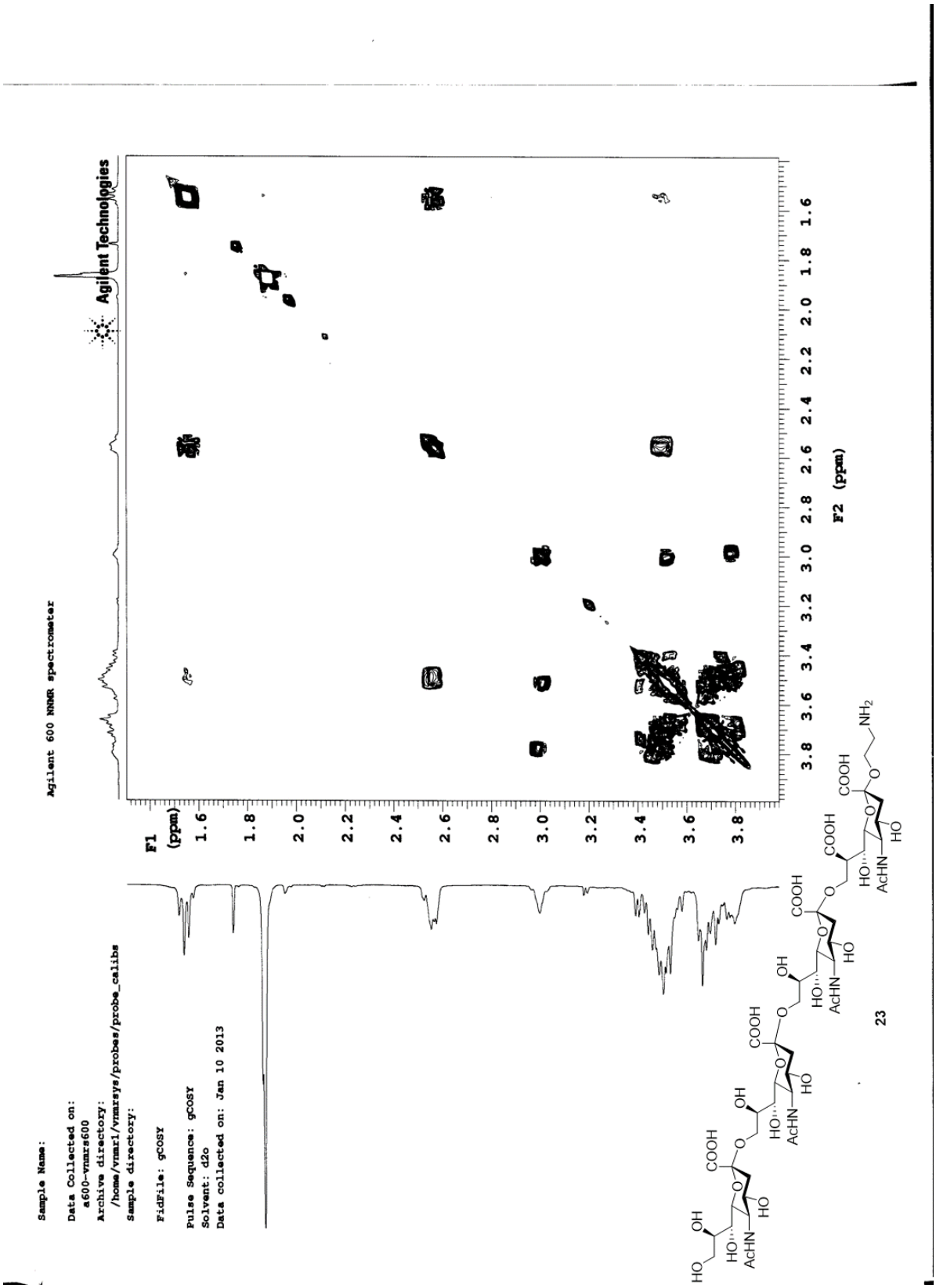
^{1}H-^{13}C HMQC Spectrum of compound **18** ($CDCl_3$, 600 MHz)



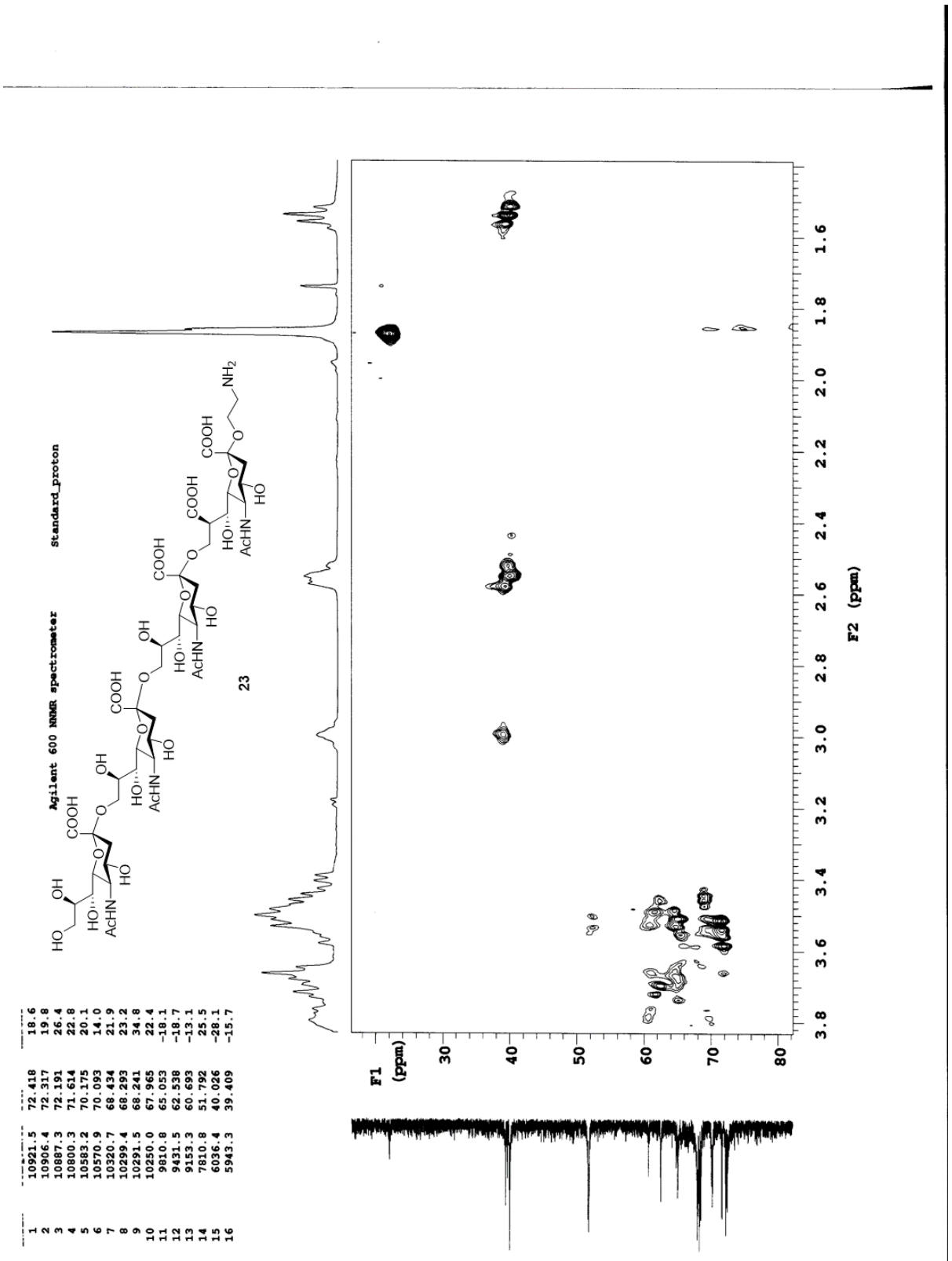
^{1}H NMR Spectrum of compound **19** (D_2O, 600 MHz)



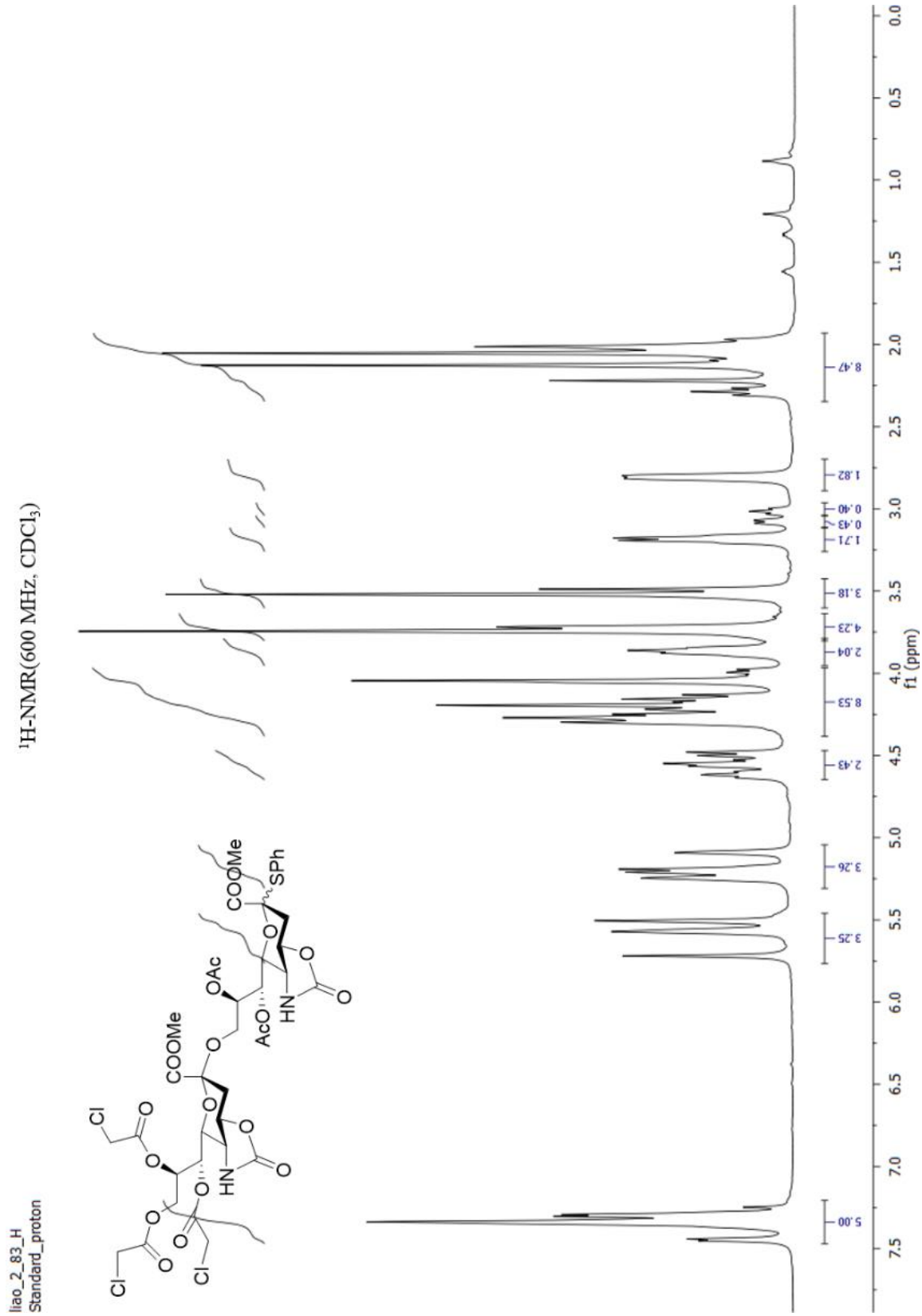
^{13}C NMR Spectrum of compound **19** (D_2O, 150 MHz)



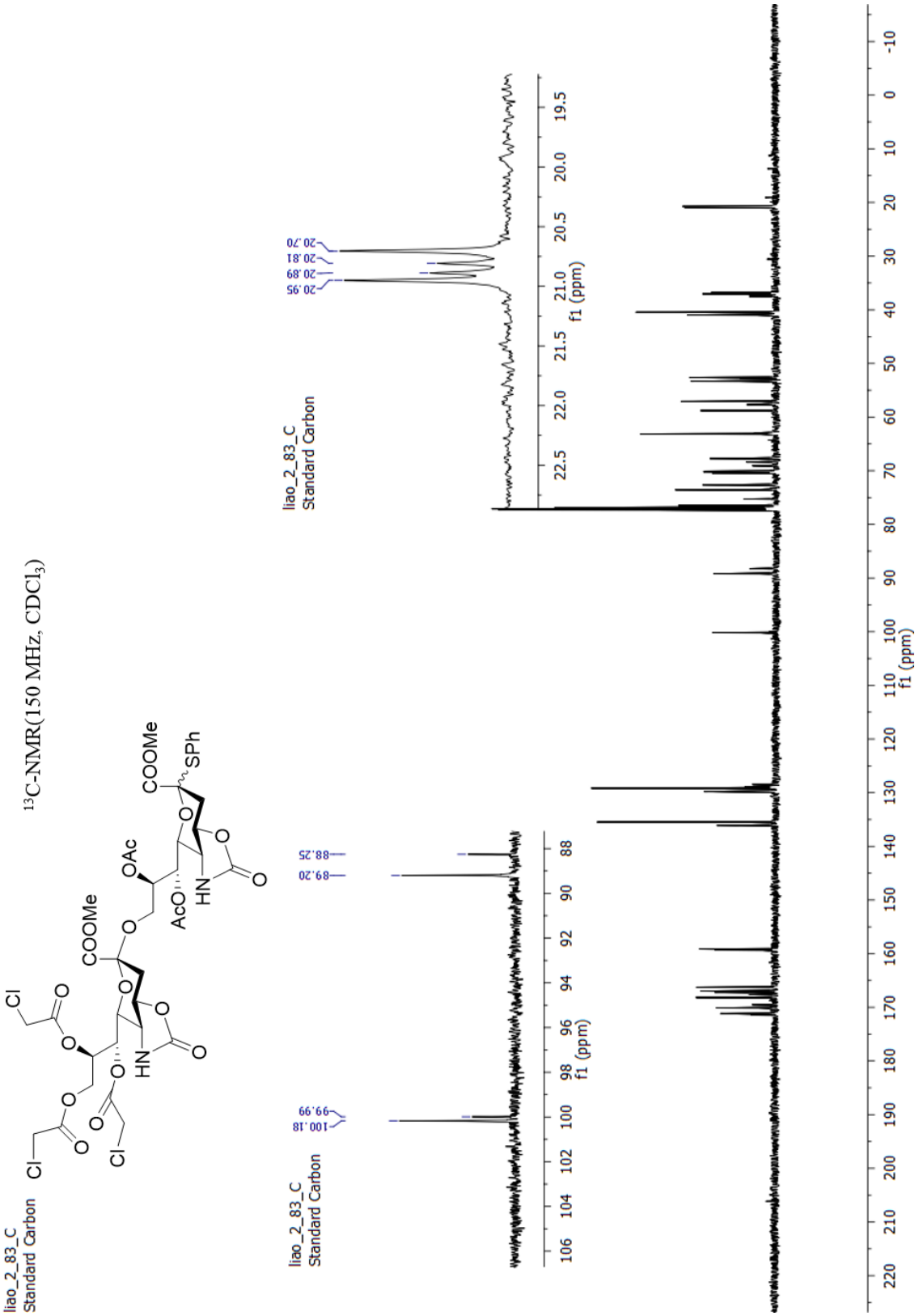
^{1}H-^{1}H COSY Spectrum of compound **19** (D_2O, 600 MHz)



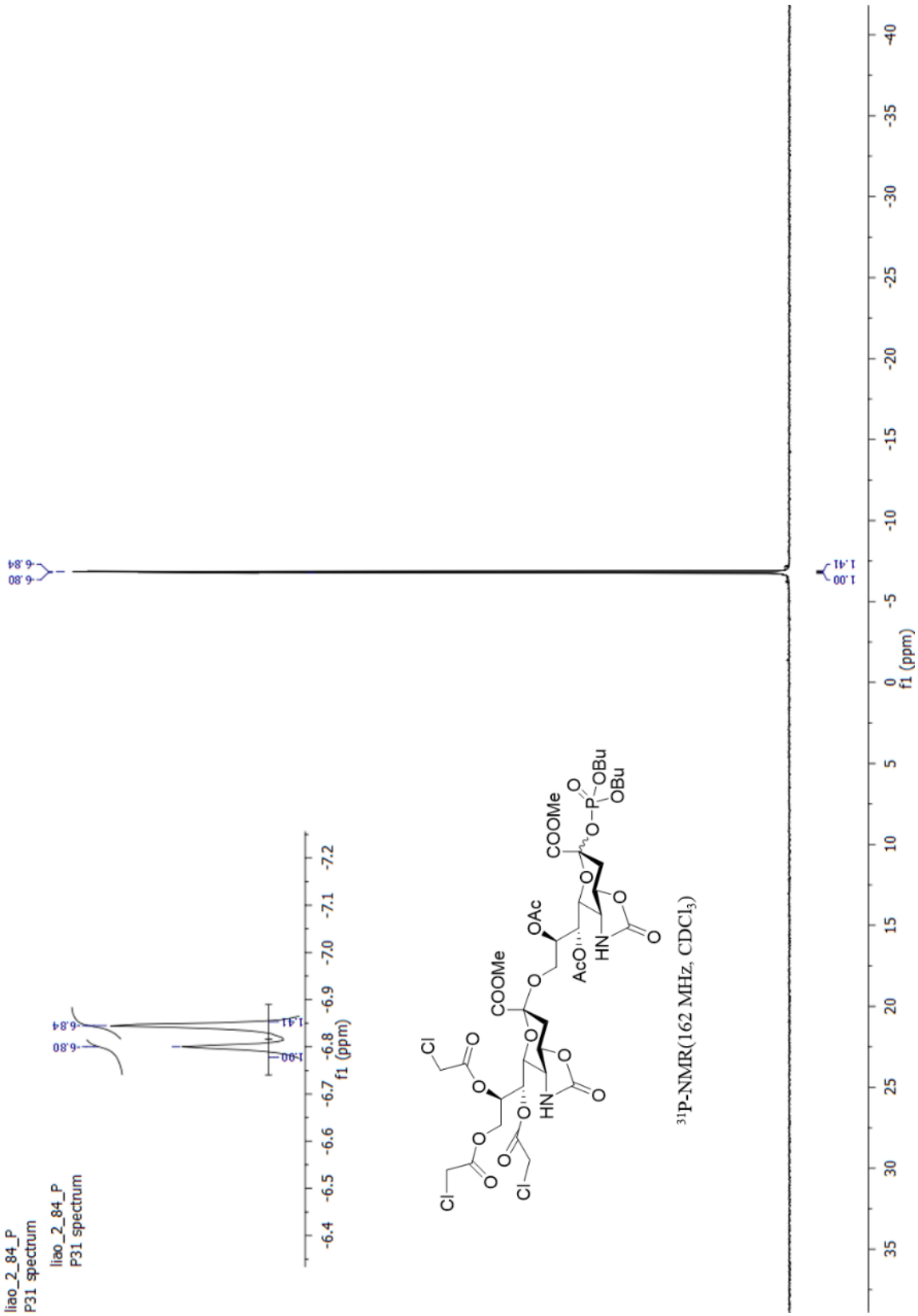
^{1}H-^{13}C HMQC Spectrum of compound **19** (D_2O, 600 MHz)



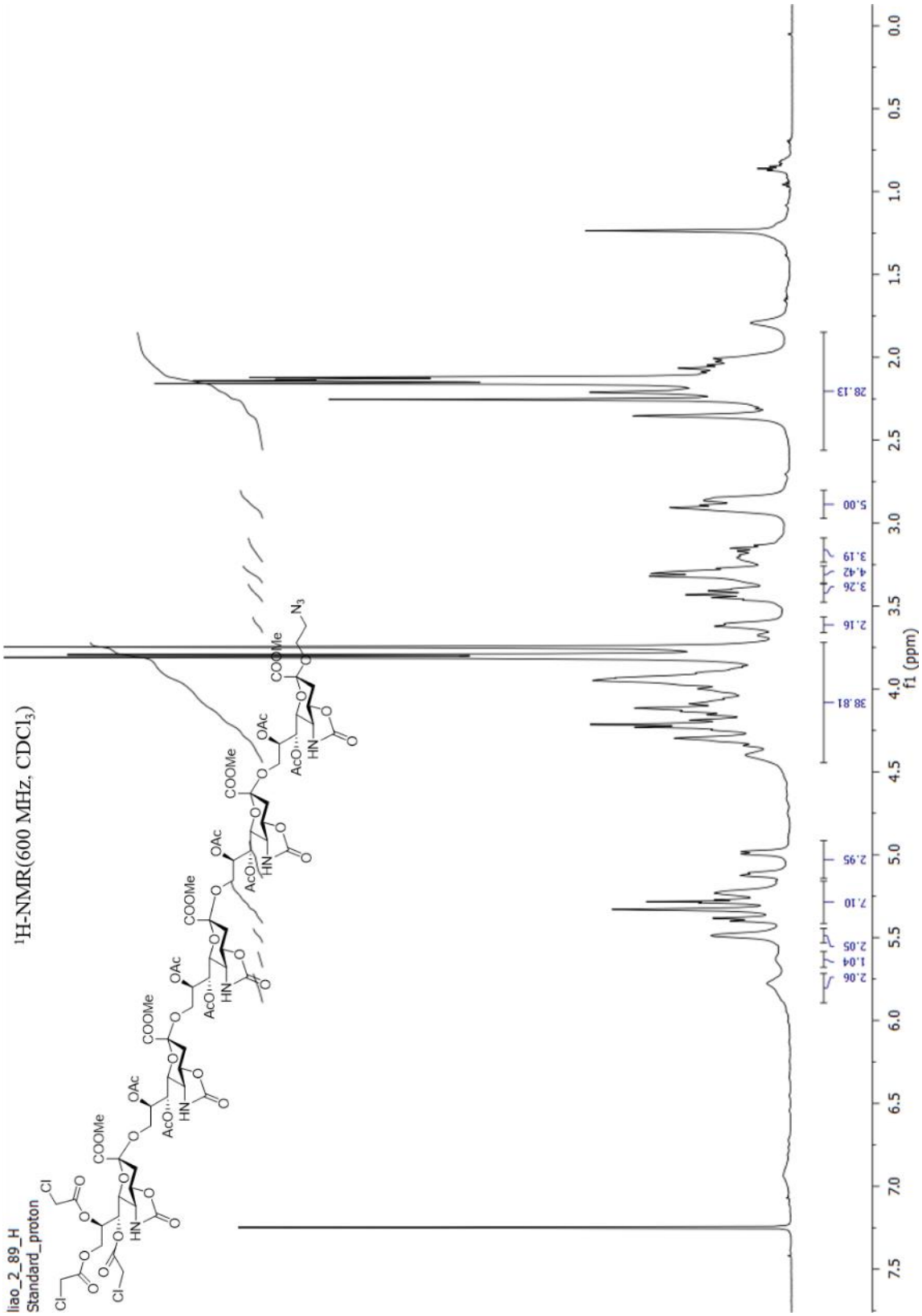
^{1}H NMR Spectrum of compound **20** ($CDCl_3$, 600 MHz)



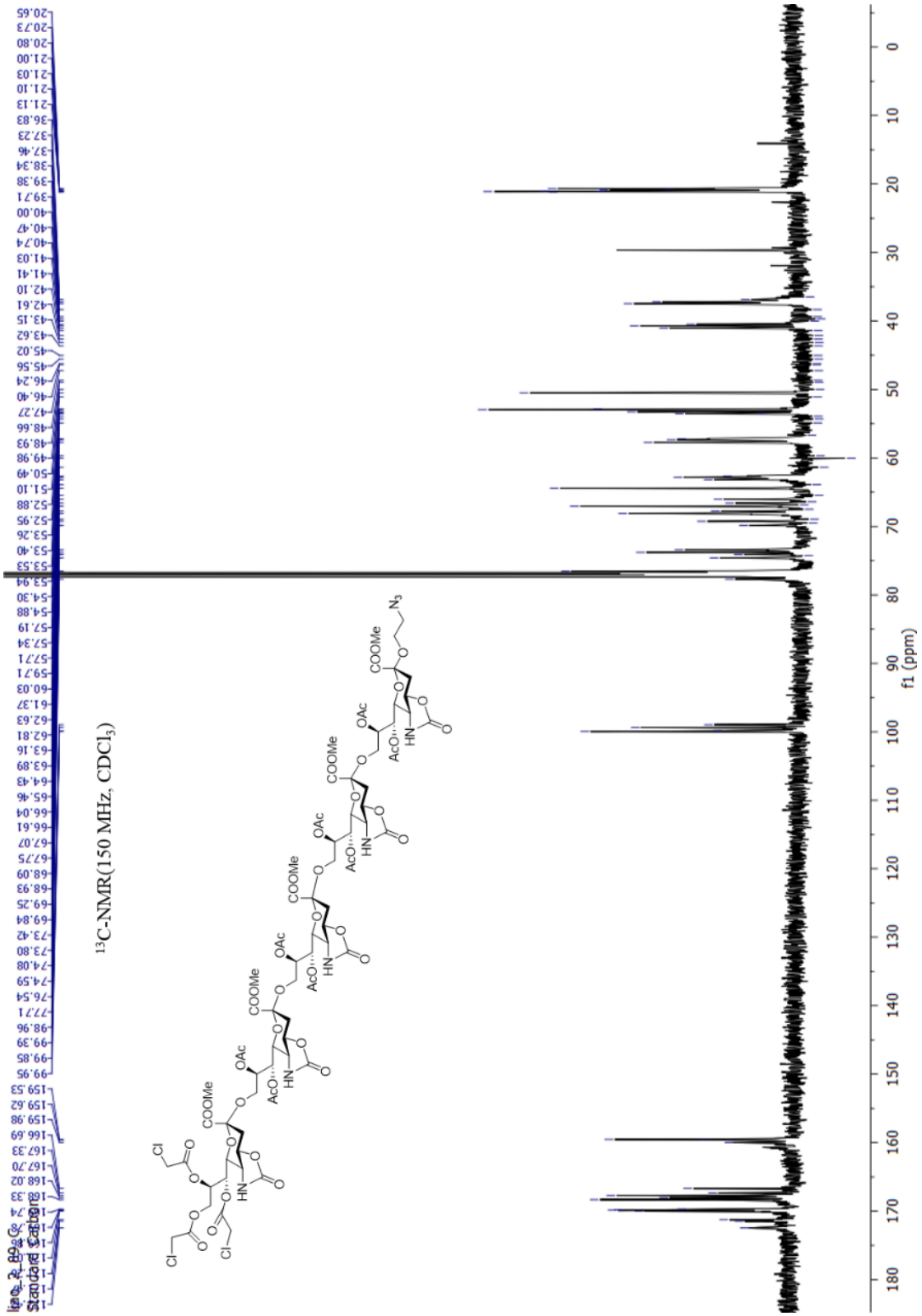
^{13}C NMR Spectrum of compound **20** ($CDCl_3$, 150 MHz)



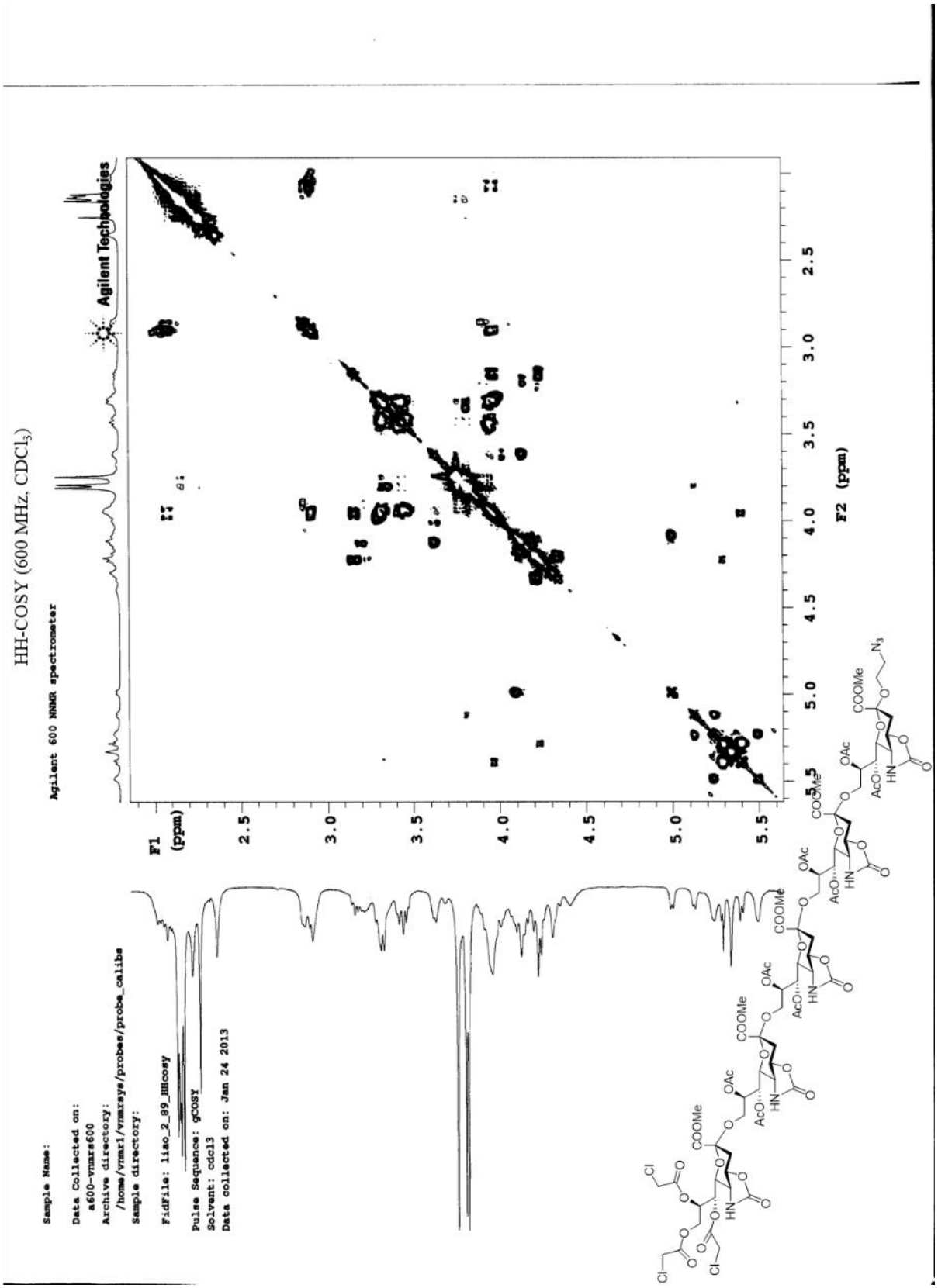
^{31}P NMR Spectrum of compound **21** ($CDCl_3$, 162 MHz)



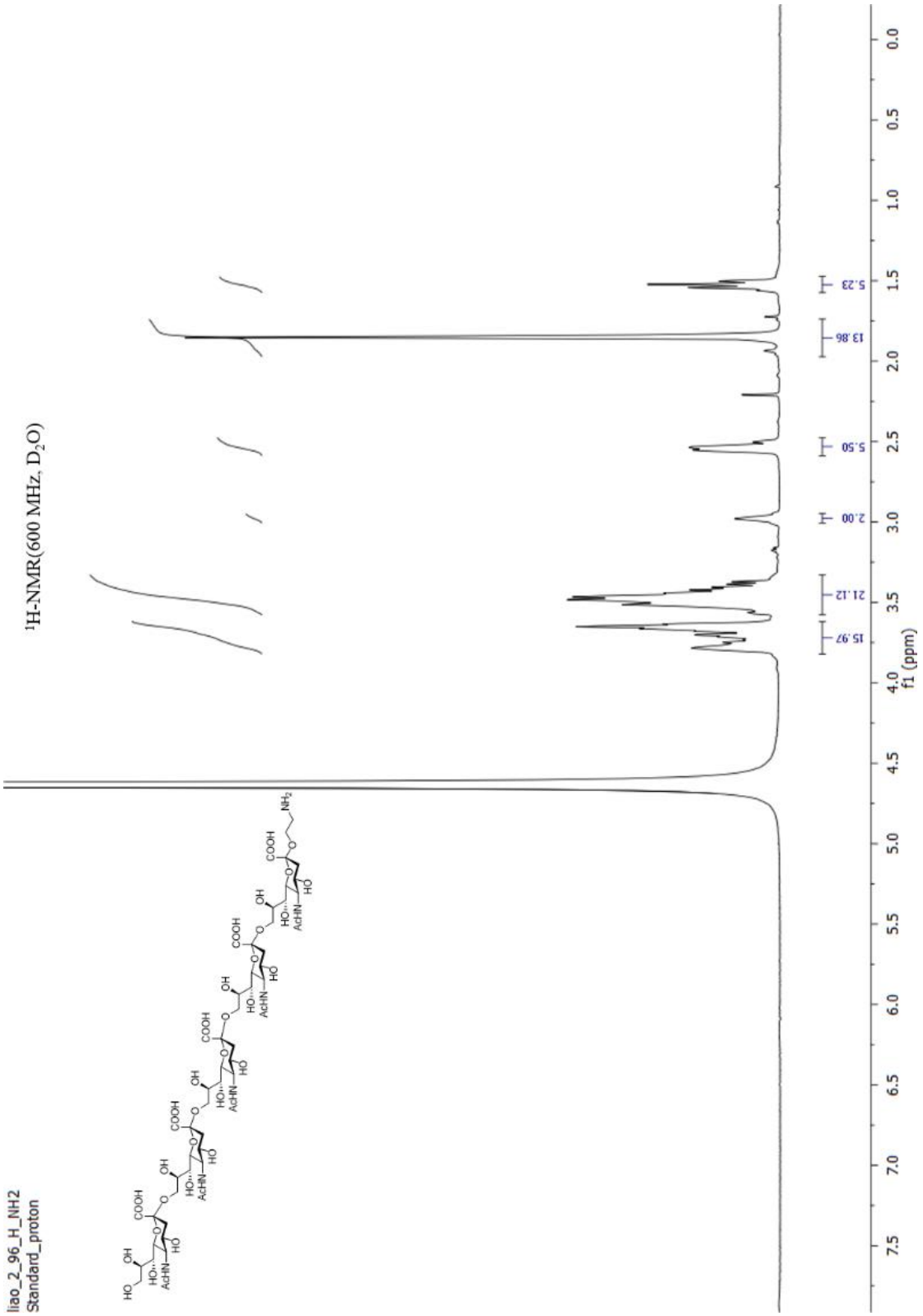
^{1}H NMR Spectrum of compound **22** ($CDCl_3$, 600 MHz)



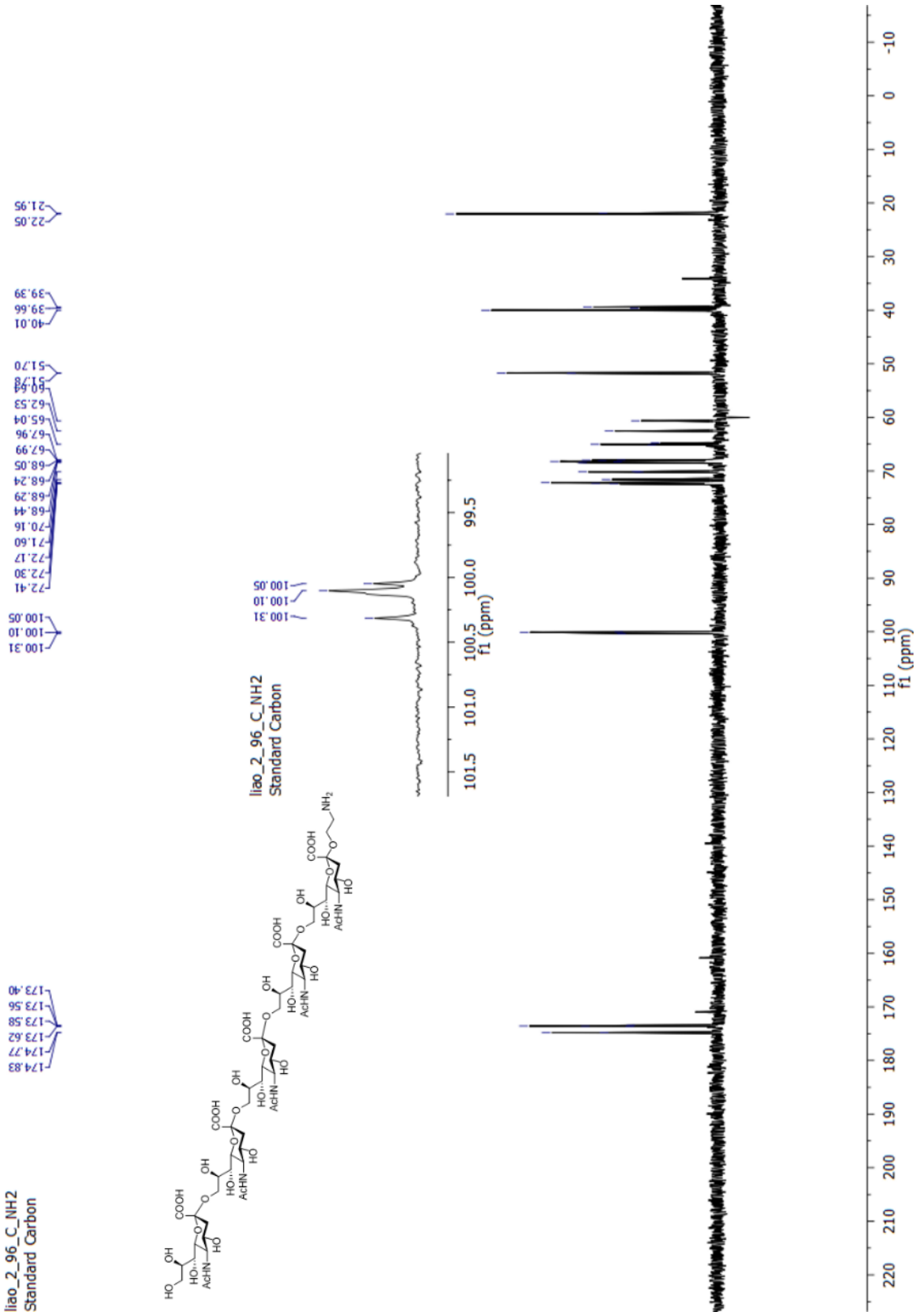
^{13}C NMR Spectrum of compound **22** ($CDCl_3$, 150 MHz)



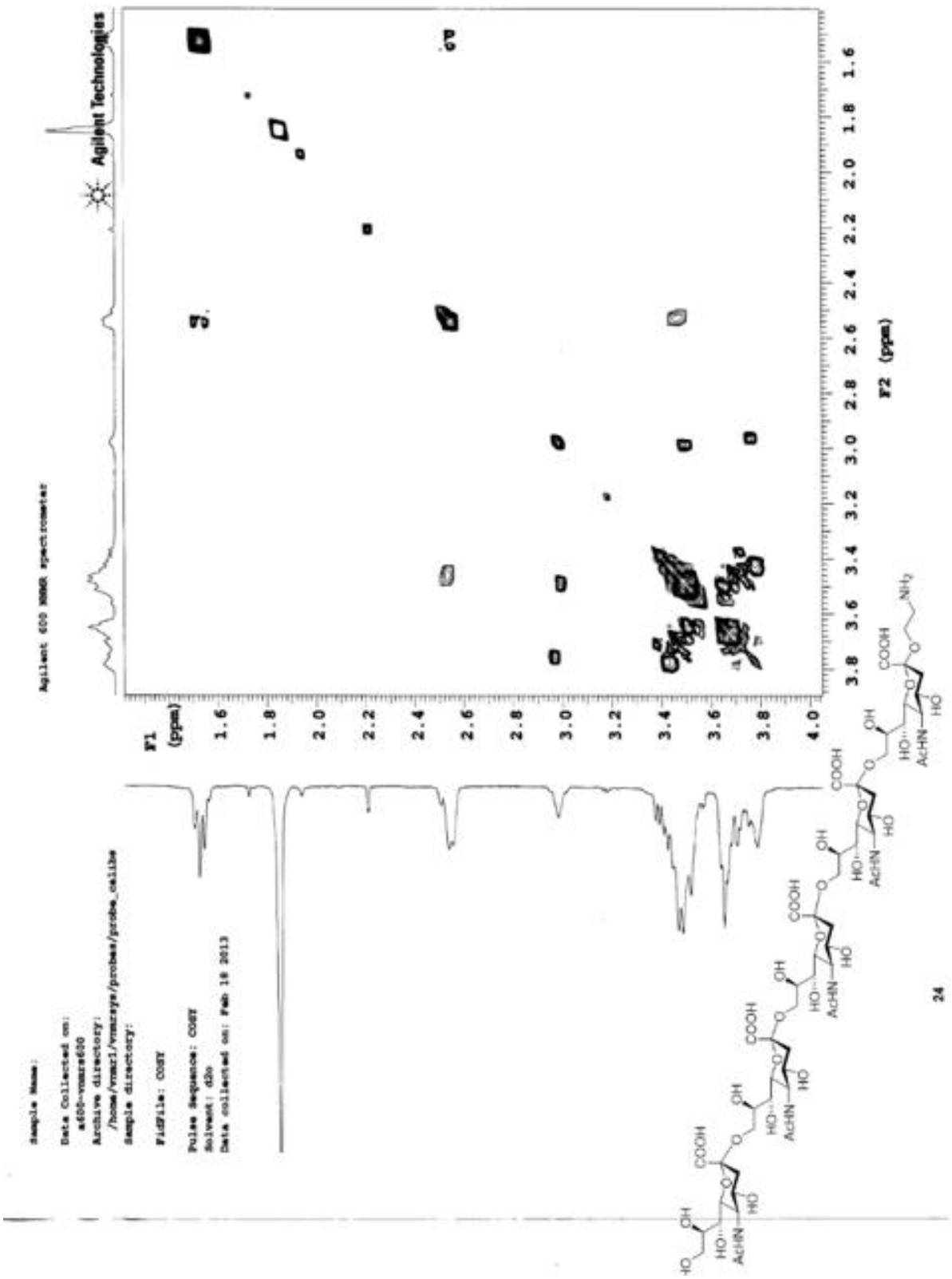
^{1}H-^{1}H COSY Spectrum of compound **22** ($CDCl_3$, 600 MHz)



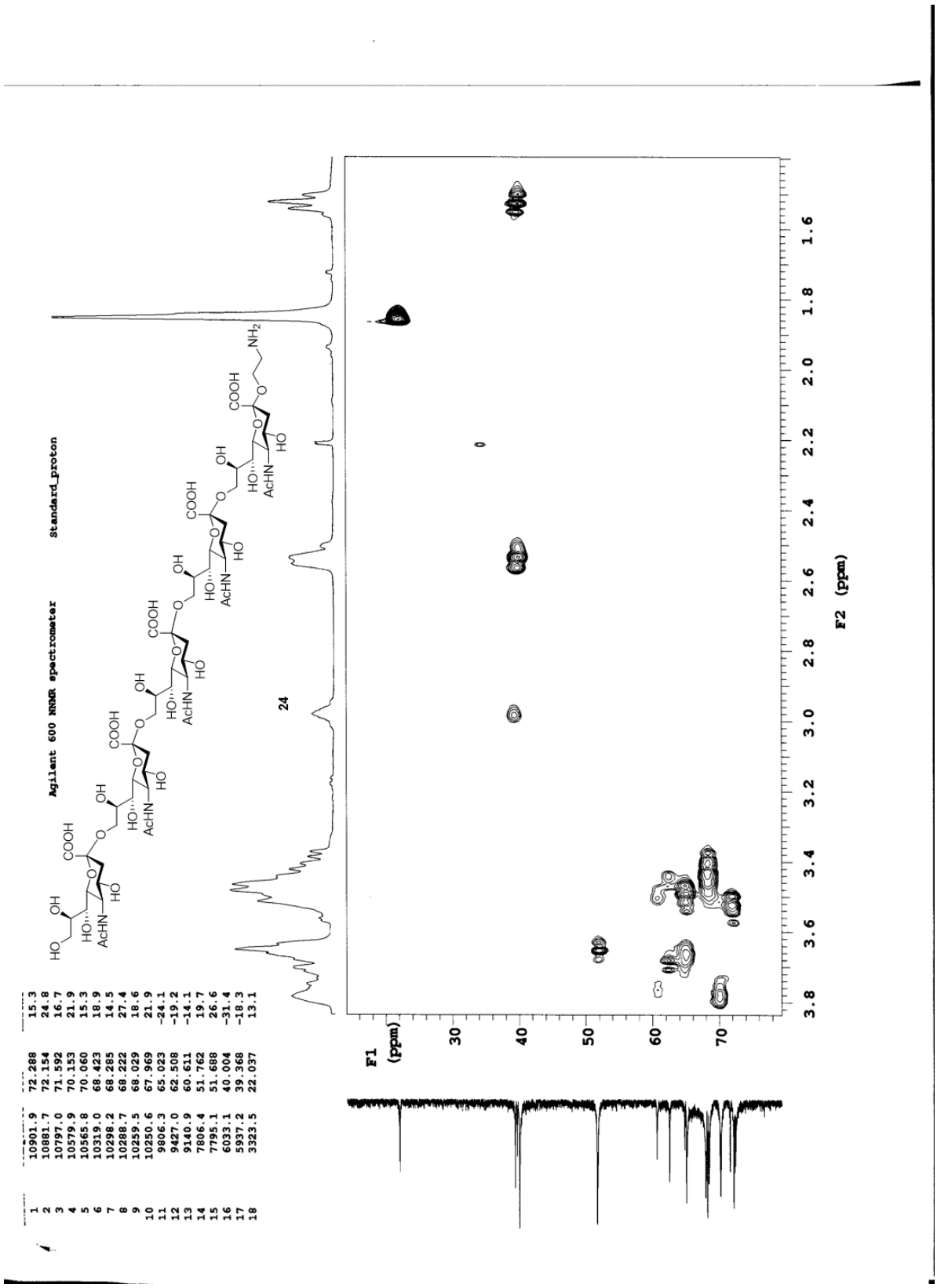
^{1}H NMR Spectrum of compound **23** (D_2O, 600 MHz)



^{13}C NMR Spectrum of compound **23** (D_2O, 150 MHz)

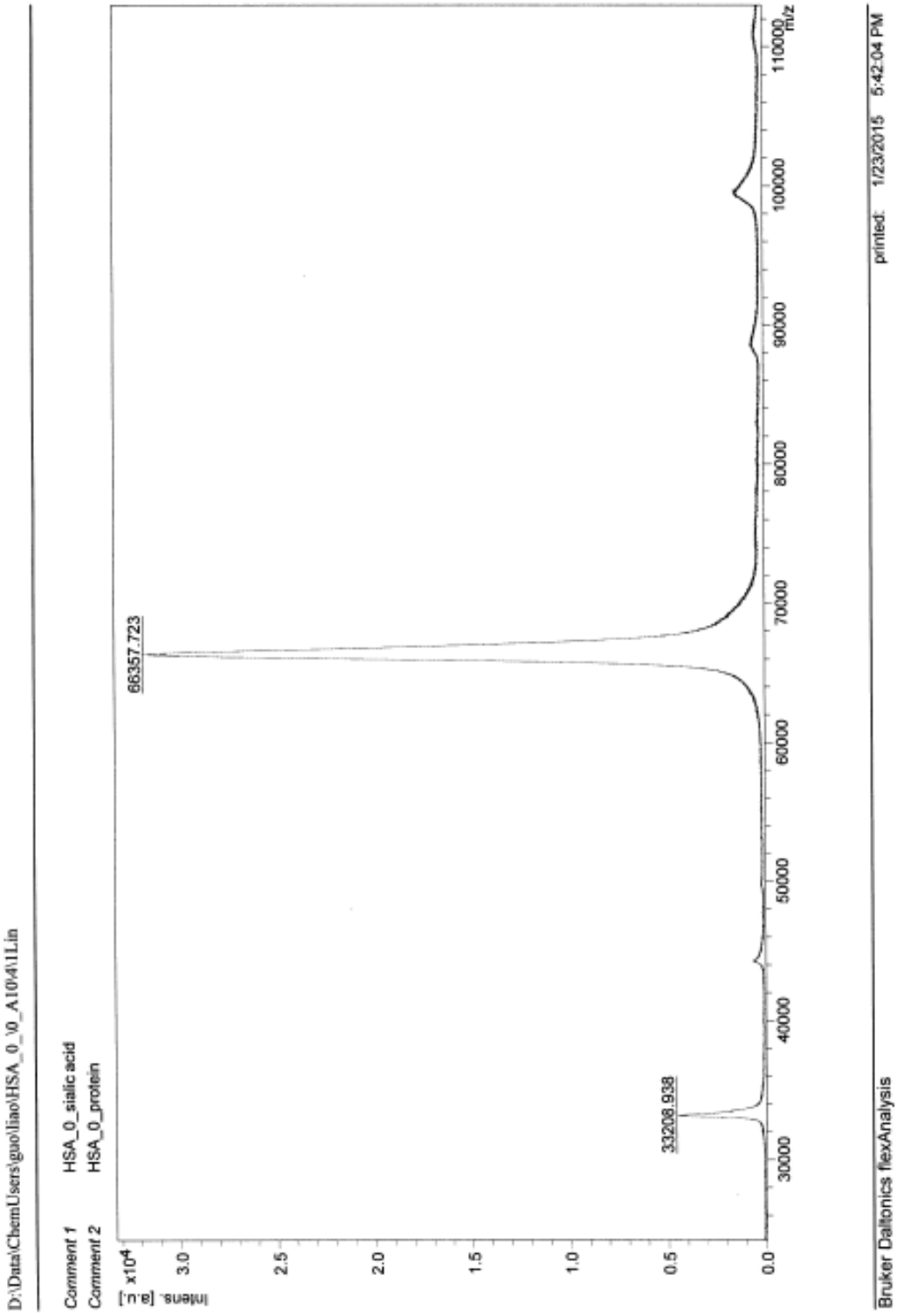


^{1}H-^{1}H COSY Spectrum of compound **23** (D_2O, 600 MHz)

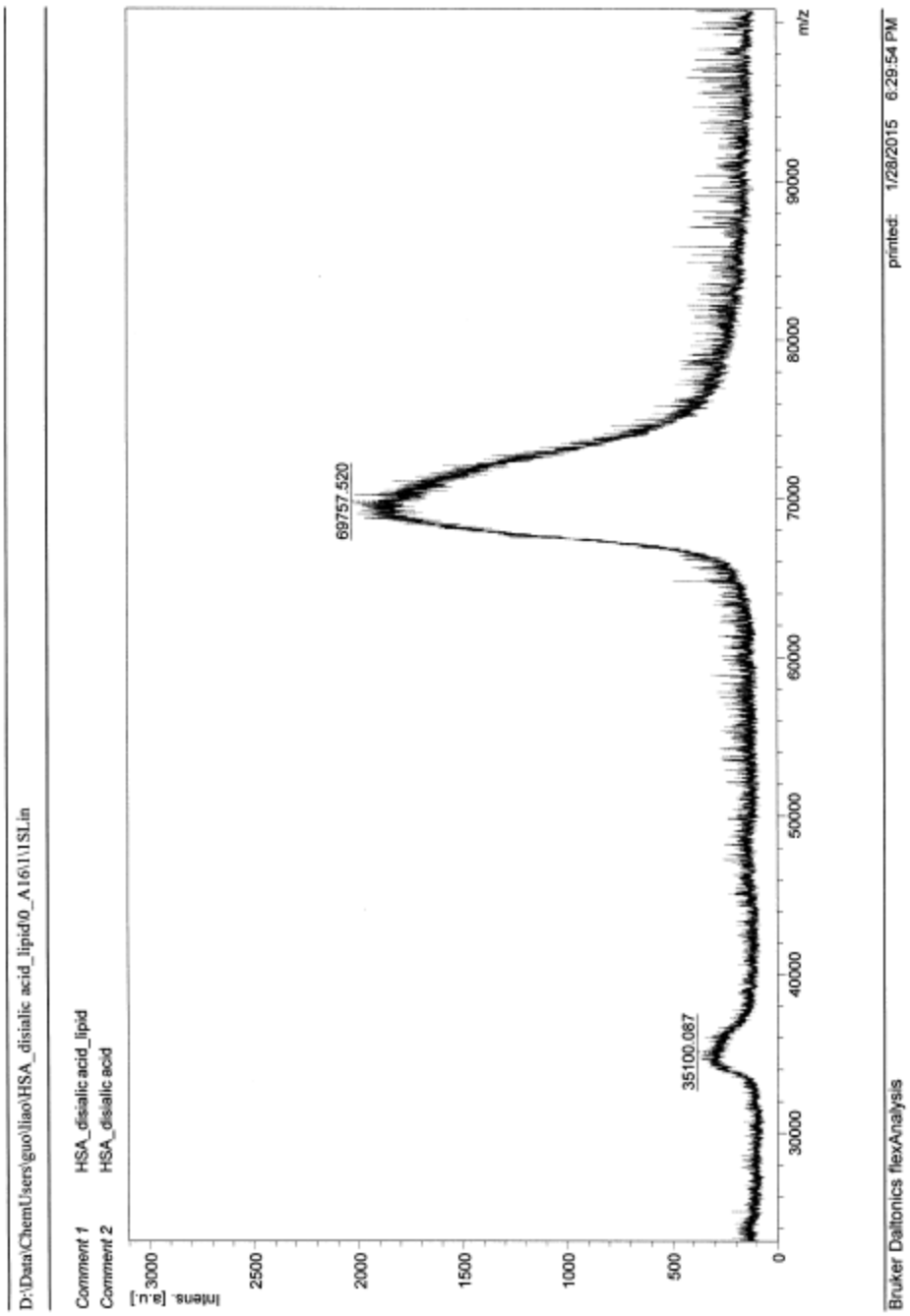


^{1}H-^{13}C HMQC Spectrum of compound **23** (D_2O, 600 MHz)

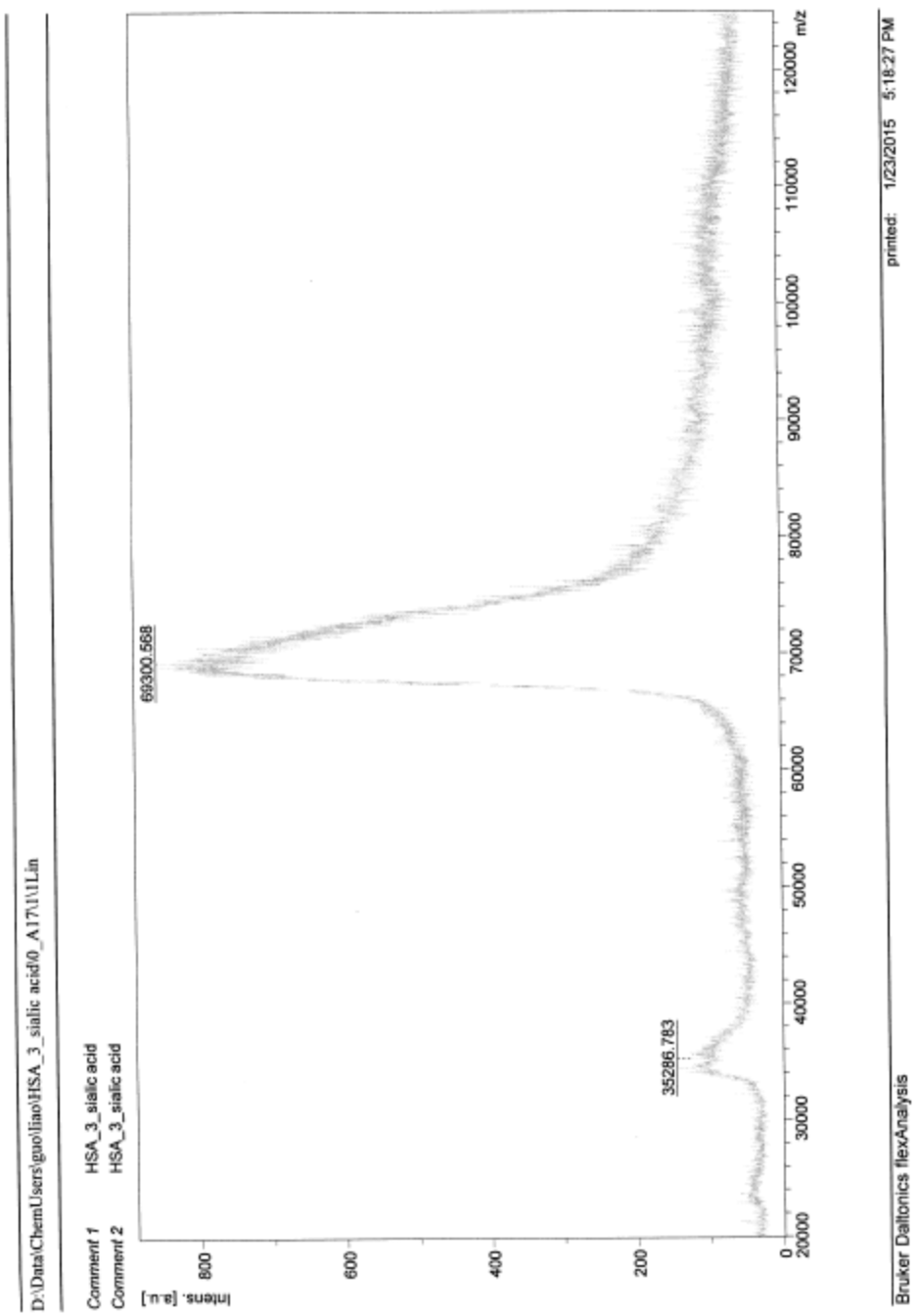
VII. MS Spectra of Conjugates 5-8



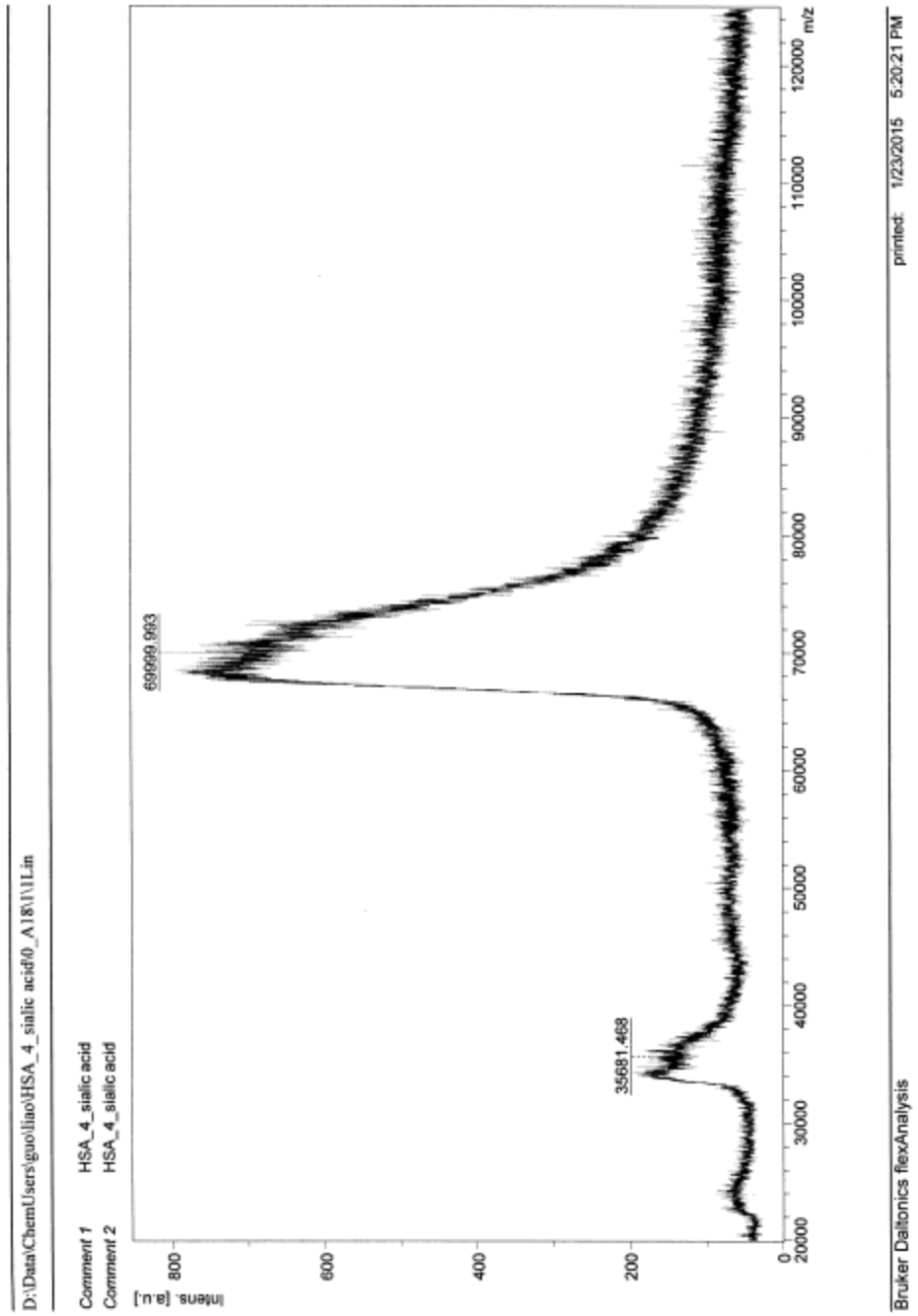
MALDI TOF MS spectrum of HSA protein



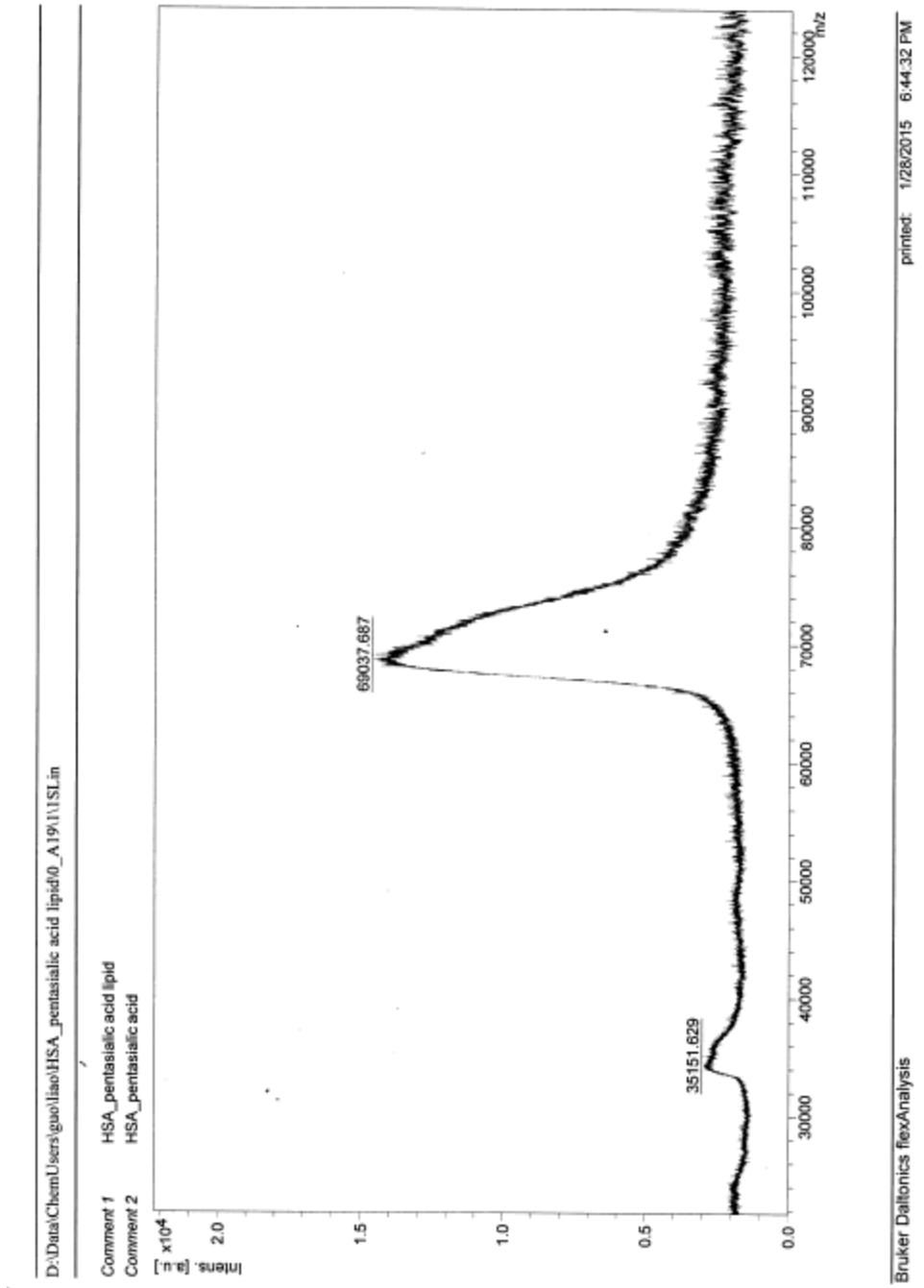
MALDI TOF MS spectrum of conjugate **5**



MALDI TOF MS spectrum of conjugate **6**



MALDI TOF MS spectrum of conjugate **7**



MALDI TOF MS spectrum of conjugate **8**